

How Phenotype and Developmental Stage Affect the Genes We Find: *GABRA2* and Impulsivity

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Context: The detection and replication of genes involved in psychiatric outcome has been notoriously difficult. Phenotypic measurement has been offered as one explanation, although most of this discussion has focused on problems with binary diagnoses. **Objective:** This article focuses on two additional components of phenotypic measurement that deserve further consideration in evaluating genetic associations: (1) the measure used to reflect the outcome of interest, and (2) the developmental stage of the study population. We focus our discussion of these issues around the construct of impulsivity and externalizing disorders, and the association of these measures with a specific gene, *GABRA2*. **Design, Setting, and Participants:** Data were analyzed from the Collaborative Study on the Genetics of Alcoholism Phase IV assessment of adolescents and young adults (ages 12–26; $N = 2,128$). **Main Outcome Measures:** Alcohol dependence, illicit drug dependence, childhood conduct disorder, and adult antisocial personality disorder symptoms were measured by psychiatric interview; Achenbach youth/adult self-report externalizing scale; Zuckerman Sensation-Seeking scale; Barratt Impulsivity scale; NEO extraversion and conscientiousness. **Results:** *GABRA2* was associated with subclinical levels of externalizing behavior as measured by the Achenbach in both the adolescent and young adult samples. Contrary to previous associations in adult samples, it was not associated with clinical-level DSM symptom counts of any externalizing disorders in these younger samples. There was also association with sensation-seeking and extraversion, but only in the adolescent sample. There was no association with the Barratt impulsivity scale or conscientiousness. **Conclusions:** Our results suggest that the pathway by which *GABRA2* initially confers risk for eventual alcohol problems begins with a predisposition to sensation-seeking early in adolescence. The findings support the heterogeneous nature of impulsivity and demonstrate that both the measure used to assess a construct of interest and the age of the participants can have profound implications for the detection of genetic associations.

■ **Keywords:** *GABRA2*, association, impulsivity, sensation-seeking, externalizing, adolescence

The detection and replication of genes involved in psychiatric outcome has been notoriously difficult (Manolio et al., 2009). Several potential explanations have been offered, including a genetic model involving far more genes than was previously recognized, as well as failure to pay adequate attention to rare variants, copy number variance (CNVs), and gene–environment interaction (Manolio et al., 2009). The importance of the choice of phenotype for genetic studies has also been raised. Much of this discussion has focused on problems associ-

ated with the use of binary diagnostic phenotypes in genetic studies and the utility of analyzing quantitative endophenotypes instead (Cannon & Keller, 2006; Frederick &

RECEIVED 18 February 2013; ACCEPTED 25 February 2013. First published online 8 April 2013.

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Iacono, 2006; Gottesman & Gould, 2003). This article focuses on two additional components of phenotypic measurement that deserve further consideration in evaluating genetic associations: (1) the importance of the measure used to represent the outcome of interest and (2) the importance of developmental stage of the study population. We focus our discussion of these issues around the construct of impulsivity and its association with externalizing disorders (Krueger et al., 2005), where these problems become particularly relevant.

The Role of Impulsivity in Psychiatric Outcome

Twin studies have robustly demonstrated that alcohol dependence, other drug dependence, adult antisocial behavior, and childhood conduct disorder overlap in large part due to a shared genetic factor (Kendler et al., 2003a; Krueger et al., 2002; Young et al., 2000). Electrophysiological endophenotypes, which are strongly genetically influenced (van Beijsterveldt et al., 1996), have been shown to capture the shared susceptibility across these disorders (Iacono et al., 1999; Porjesz et al., 2005), again suggesting a shared etiological connection. What is phenotypically common across these disorders is impaired impulse control, as reflected in two of the criteria for the diagnosis of alcohol dependence: (a) the inability to control the amount of alcohol consumed (or the amount of time spent drinking), and (b) continued drinking despite adverse health-related consequences (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition — DSM-IV; APA, 2000), and one criterion for antisocial personality disorder stating ‘Impulsivity or failure to plan ahead’ (DSM-IV; APA, 2000). Accordingly, it has been hypothesized that the shared element across these disorders comprises a general predisposition to impulsivity. Alternatively, this shared factor has been labeled as behavioral disinhibition (Young et al., 2000) or externalizing (Krueger et al., 2005). Despite these subtle differences in nomenclature, all terms underscore a predisposition toward a lack of impulse control, and this constitutes the unifying construct shared across these common, frequently co-occurring conditions.

However, impulsivity is a loose term, and there is good evidence from other literatures that impulsivity is not a unitary construct (Smith et al., 2007; Whiteside & Lynam, 2001). Depue and Collins (1999) aptly stated: ‘Impulsivity comprises a heterogeneous cluster of lower-order traits that includes terms such as impulsivity, sensation-seeking, risk-taking, novelty-seeking, boldness, adventuresomeness, boredom susceptibility, unreliability, and unorderliness’ (p. 495). Further compounding this problem is the fact that there are many different questionnaires that have been designed to measure these aspects of impulsivity. The constructs assessed in these measures often vary considerably, with some measures of impulsivity reflecting a failure to plan ahead, and others focusing more on engagement in risky behavior. These different measures, referred to as mea-

asures of impulsivity, are not very correlated, and do not load on a single factor; this is true of both questionnaire-based and laboratory-based measures of impulsivity (Dick et al., 2010). Interestingly, recent studies suggest that different aspects of impulsivity appear to be differentially related to different aspects of alcohol use (Smith et al., 2007). Yet, despite growing recognition that impulsivity is a heterogeneous construct (Lejuez et al., 2010), the broad term ‘impulsivity’ (Hamidovic et al., 2009; Racine et al., 2009) continues to be used in the literature in reference to this large variety of scales and dimensions, further contributing to its conceptualization as a unitary phenomenon.

This creates a conundrum in the literature whereby the connection between alcohol dependence, other drug dependence, antisocial adult behavior, and childhood conduct disorder is thought to be explained by a shared unitary factor that is construed as ‘impulsivity’, despite literature suggesting a multidimensional entity. As part of the Collaborative Study on the Genetics of Alcoholism (COGA), we collected multiple measures related to impulsivity, including Barrett’s Impulsivity scale, Zuckerman’s Sensation-Seeking scale, and the NEO Big Five Personality Inventory, in a prospective sample of adolescents and young adults ($N = 2,128$, with phenotypic and genotypic data). In addition, symptom counts for childhood conduct disorder, alcohol and other drug dependence, and adult antisocial behavior (for individuals aged ≥ 18) were obtained through clinical interview, and subclinical levels of externalizing behavior were collected using the Achenbach Child Behavior Checklist. We conducted exploratory analyses to test the extent to which a specific gene, *GABRA2*, was associated with these various clinical, subclinical, and personality measures of impulsive behavior.

These questions are of particular relevance with respect to *GABRA2*, given the previous history of association with this gene. *GABRA2* was originally associated with adult alcohol dependence in the COGA (Edenberg et al., 2004). This association was subsequently replicated by several independent groups (Covault et al., 2004; Enoch et al., 2006; Fehr et al., 2006; Soyka et al., 2008). Further work in the COGA sample found that *GABRA2*’s association was not limited to alcohol dependence, but also included illicit drug dependence (Agrawal et al., 2006; Dick et al., 2006b), childhood conduct disorder (Dick et al., 2006b), and adult antisocial behavior (Dick et al., 2006a). Thus, paralleling the twin literature indicating shared genetic influence across externalizing disorders, *GABRA2* appeared to be a specific gene predisposing to a spectrum of clinical disorders characterized by a lack of impulse control. The association between *GABRA2* and general externalizing behavior has also been extended to a non-clinical, community-based sample, in which individuals carrying the genotype originally associated with adult alcohol dependence in COGA were more likely to evidence an elevated, stable trajectory of externalizing behavior (as measured by the Achenbach Externalizing

scale) across adolescence and into young adulthood as compared with individuals carrying the low-risk genotype (Dick et al., 2009). In sum, this literature suggests that *GABRA2* is involved with multiple outcomes and disorders, all of which reflect problems with impulse control. Because the literature also suggests that impulsivity is not a unitary construct, we explored whether there were particular facets of impulsivity that *GABRA2* is associated with, in an effort to further delineate the risk pathways associated with *GABRA2*.

All analyses were conducted separately for the adolescent sample (individuals between the ages of 12 and 17) and the young adults (18–26 years of age). This division was based on significant developmental changes across this age range that are particularly relevant for substance use outcomes. Most youth leave home after age 18, and this represents a considerable leap in independence, with an associated reduction in parental supervision and monitoring. Although alcohol use remains illegal for individuals aged 18–20, the emotional and logistical shifts associated with reaching adulthood generally offer enhanced opportunity and incentive for engaging in substance use. Previously, independent studies have found that genetic associations between specific genes and alcohol use outcomes are not evident until young adulthood (Dick et al., 2006b; Guo et al., 2007), likely because of the greater importance of environmental factors that influence adolescent experimentation with alcohol (Rose et al., 2001). In contrast, association with conduct problems has been reported earlier in adolescence for genes associated with alcohol dependence in adulthood, suggesting the possibility of heterotypic continuity of genetic effects, that is, the same gene influencing different outcomes at different developmental stages (Dick, 2011). Accordingly, we tested for association with the various impulsivity-related measures separately in adolescents and young adults to allow for the possibility that phenotypic associations with *GABRA2* may vary across developmental stages.

Methods

Sample

The COGA is a multi-site project, with the goal of identifying genes contributing to alcoholism and related phenotypes. Proband was identified through inpatient or outpatient alcohol treatment programs at six sites around the United States and were invited to participate if they had a sufficiently large family (usually sibships >3 with parents available) with two or more members in a COGA catchment area (Begleiter et al., 1995). The institutional review boards of all participating centers approved the study. Written consent was obtained from all study participants. Additional details about the study have been published previously (Edenberg et al., 2004; Foroud et al., 2000; Reich et al., 1998).

The data analyzed here come from the Phase IV Prospective Study of the COGA sample. The recruitment of adolescents (12–17-year-olds) and young adults (18–21-year-olds) into the prospective study began in December 2004. All of these subjects had at least one parent who was interviewed in a previous phase of COGA, including both families affected with alcoholism and comparison families. Both parents have been personally interviewed for over 50% of the subjects. Data collection is ongoing, as individuals who reach their 12th year continue to be recruited and assessed. Follow-up evaluations are being conducted approximately every two years. All analyses reported here involve the initial assessments for participants ($N = 2,128$ individuals, 49% male).

Genotyping was performed at the Washington University MicroArray facility using Illumina's GoldenGate assays. Six single-nucleotide polymorphisms (SNPs) were genotyped across *GABRA2*: rs497068, rs279871, rs279867, rs279858, rs279845, and rs279836. All SNPs were located in the linkage disequilibrium block that previously yielded evidence of association in the COGA sample (average r^2 across the SNPs = 0.82, range 0.71–0.98). All SNPs were in the Hardy-Weinberg equilibrium.

In addition, a panel of 69 population stratification SNPs were genotyped, and plotted using the EIGENSTRAT software for comparison against three HAPMAP populations. Three clusters were identified corresponding to Caucasian (63%), African American (25%), and other (12%; reflecting mixed race ancestry). Age, race, and gender were used as covariates in all genotypic analyses.

Measures

Psychiatric interview. All individuals were interviewed with the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA; Bucholz et al., 1994; Hesselbrock et al., 1999), using the adult (age 18+) or adolescent (age 12–17) version as appropriate. The interviews are nearly identical, with subtle wording changes to make the language age appropriate. All diagnoses were made according to DSM-IV criteria (APA, 2000). Symptom counts for the following diagnoses were analyzed: alcohol dependence, illicit drug dependence (including marijuana, cocaine, other stimulants, sedatives, and opiates), childhood conduct disorder, and (for individuals aged ≥ 18) adult antisocial behavior. These disorders have been previously shown to share a genetic etiology (Kendler et al., 2003b) and have been previously associated with *GABRA2* in the initial adult COGA sample, as reviewed above.

Achenbach Youth/Adult Self-Report (YSR/ASR). The externalizing scale of the YSR/ASR consists of 30 items comprising both rule-breaking (e.g., 'I cut classes or skip school') and aggression items (e.g., 'I am mean to others'), for which the participant indicates whether the behavior is *not true, somewhat or sometimes true, or very or often true*

(Achenbach, 1991, 1997). These measures have been shown to have excellent psychometric properties including high test–retest reliability, content validity, criterion-related validity, and construct validity (Achenbach & Rescorla, 2001, 2003).

Barratt Impulsivity Scale (BIS). The BIS Version 11 was administered. This is a 30-item scale with separate versions for adolescents and adults that measure what the authors characterize as attentional impulsiveness (e.g., ‘I “squirm” at plays or lectures’), motor impulsiveness (e.g., ‘I act “on impulse”’), and nonplanning (e.g., ‘I am a careful thinker’ (reverse coded; Patton et al., 1995). All items are answered as *Never* — 1, *Occasionally* — 2, *Often* — 3 and *Always* — 4. Total scores are computed by summing subscale items.

Sensation-Seeking Scale (SSS). The SSS was developed by Zuckerman (1979) to measure individual differences in stimulation and arousal. The adult version (SSS-V) covers boredom susceptibility (‘I can’t stand watching a movie that I’ve seen before’), thrill- and adventure-seeking (‘I sometimes like to do things that are a little scary’), experience-seeking (‘I have tried marijuana or would like to’), and disinhibition (‘I like wild uninhibited parties’). A version for adolescents (SSS-C) has also been developed (Russo et al., 1993). Total scores are computed by summing all items.

NEO Five-Factor Inventory. We administered the 60-item scale, which measures the personality traits of neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness (Costa & McCrae, 1997). We analyzed the extraversion and conscientiousness subscales, as high extraversion and low conscientiousness were *a priori* hypothesized to be most relevant to the construct of impulsivity under study here. Sample items from the extraversion scale include ‘I like to have a lot of people around me’ and ‘I like to be where the action is’. Sample items from the conscientiousness scale include ‘I’m pretty good about pacing myself so as to get things done on time’; ‘I am not a very methodical person’ (reverse coded).

Analyses

Because the prospective sample spans early adolescence through young adulthood and significant developmental changes are known to occur across this period, we conducted all analyses separately for the adolescent sample (ages 12–17; $N = 1,192$, mean = 14.48, $SD = 1.76$) and adult sample (ages 18–26; $N = 936$, mean = 19.7, $SD = 1.46$), as described above. This also avoided potential analytic issues associated with method variance, since some of the scales had similar but distinct versions for adolescents and young adults. The exact N available for analysis differed slightly across the measures, as the measures were completed at different points in the assessment (e.g., some in person, others through the mail); accordingly, not all participants completed the full assessment battery. All individuals with

data available for a given measure were used in that particular analysis. The N values available for each analysis are indicated in the tables.

All analyses were conducted using the Statistical Analysis System (SAS). Association analyses were run using an additive model with sex, age, and race incorporated as covariates. In addition, the correlated nature of some observations (e.g., children from the same family) was taken into account using the survey option in SAS. We report p -values uncorrected for multiple testing, since the analyses are intended to be an exploratory examination of patterns of association across the impulsivity variables. The *GABRA2* SNPs are in fairly high linkage disequilibrium (LD), and applying a Nyholt correction (Nyholt, 2004) to the data suggests just under two independent signals, which suggests a significance threshold of 0.026. We conducted tests on nine primary phenotypes, and therefore the final Bonferroni-corrected p -value for any single association would be .003, although this is conservative, as it does not take into account the correlated nature of the phenotypes. We regard it as suggestive evidence for association when multiple SNPs yield $p < .05$, and there is a generally consistent pattern across the SNPs. We present all results in order that the reader can evaluate the consistency of evidence across SNPs, phenotypes, and samples.

Results

Table 1 shows the correlations across the variables, with the adult sample shown below the diagonal and the adolescent sample above the diagonal. The pattern of correlations is similar in the adolescent and adult samples; however, the Achenbach Externalizing scale is more strongly correlated with conduct disorder symptoms in adolescents as compared with young adults and less so with alcohol-dependence symptoms in adolescents. The BIS is more strongly correlated with SSS in young adulthood than in adolescence. Extraversion scores showed the lowest correlations with the other measures in both samples.

Table 2 presents p -values from the association tests with the externalizing behavior measures: clinical symptom counts and the Achenbach Externalizing scale. Table 3 presents p -values from the association tests with the various personality scales measuring domains of impulsivity. The pattern of results is striking: there is no association between *GABRA2* and clinical symptoms of any of the externalizing disorders in either the young adult or adolescent sample (there is one $p < .05$ with alcohol-dependence symptoms in the adult sample, but this is not replicated in the other correlated SNPs). There is, however, association with the Achenbach Externalizing scale scores across both samples. A joint analysis of externalizing across both samples yields a minimum $p = .0003$. Furthermore, in the adolescent sample, an association is observed across all SNPs with SSS and NEO Extraversion (Table 3). Two SNPs also yield $p < .05$ in

TABLE 1

Correlations Between Measures; Results Are Shown Above Diagonal for the Adolescent Sample and Below Diagonal for the Adult Sample

	AD symptoms	CD symptoms	ASP symptoms	DD symptoms	Ach_ externalizing	NEO_E	NEO_C	SSS	BIS
AD symptoms	1.00	0.12	–	0.48	0.25	<i>0.03</i>	–0.09	0.23	0.14
CD symptoms	0.24	1.00	–	0.19	0.48	–0.06	–0.20	0.15	0.35
ASP symptoms	0.41	0.57	1.00	–	–	–	–	–	–
DD symptoms	0.47	0.29	0.47	1.00	0.27	–0.06	–0.07	0.17	0.14
Ach_ externalizing	0.33	0.37	0.48	0.32	1.00	–0.07	–0.37	0.37	0.55
NEO_E	–0.04	–0.08	–0.09	–0.05	–0.05	1.00	0.26	0.20	–0.08
NEO_C	–0.13	–0.12	–0.20	–0.14	–0.40	0.30	1.00	–0.17	–0.58
SSS	0.23	0.16	0.22	0.18	0.38	0.22	–0.24	1.00	0.29
BIS	0.22	0.23	0.35	0.25	0.53	–0.05	–0.46	0.35	1.00

Note: All correlations significant at $p < .05$, unless indicated by italics; most correlations significant at $p < .0001$. AD = alcohol dependence; CD = conduct disorder; ASP = Antisocial Personality; DD = illicit drug dependence; Ach = Achenbach scales; NEO_E = Extraversion; NEO_C = Conscientiousness; SSS = Zuckerman's sensation-seeking scale.

TABLE 2

P-Values From Association Tests With Behavioral Measures of Externalizing

SNP	AD_SX	DD_SX	CD_SX	ASP_SX	Ach_EXT
Adult, N	936	935	933	933	857
rs497068	.023	.478	.736	.556	.021
rs279871	.304	.387	.781	.575	.227
rs279867	.209	.507	.816	.635	.050
rs279858	.244	.387	.687	.765	.210
rs279845	.260	.796	.615	.622	.005
rs279836	.415	.404	.621	.469	.095
Adolescent, N	1,192	1,191	1,191		1,141
rs497068	.356	.750	.328		.020
rs279871	.977	.800	.290		.028
rs279867	.923	.728	.733		.150
rs279858	.884	.692	.308		.048
rs279845	.764	.821	.236		.007
rs279836	.831	.579	.335		.064

Note: p values $\leq .05$ are shown in bold.

the adolescent sample with conscientiousness, but no single SNP is less than the Nyholt-corrected p -value of 0.026, suggesting this association should be viewed more tentatively. The association with these personality measures is not observed in the young adult sample. Combining the samples decreases the evidence for association, reinforcing that the association is limited to the adolescent sample.

Discussion

This paper used data from a large sample of adolescents and young adults to explore the association of various behavioral and personality measures of externalizing and impulsivity with *GABRA2*, a gene previously associated with externalizing disorders. Our results illustrate the critical importance of the precision of phenotypic definition for genetic association studies. There are three particularly notable findings that emerge: (1) the association between *GABRA2* and externalizing behavior is limited to subclinical self-reports of externalizing behavior and is not found with diagnostic level DSM symptom counts for any externalizing disorder in either the adolescent or young adult samples; (2)

TABLE 3

P-Values From Association Tests With Various Personality Measures of Impulsivity

SNP	BIS	NEO_C	NEO_E	SSS
Adult, N	884	909	909	892
rs497068	.387	.801	.851	.023
rs279871	.959	.605	.415	.729
rs279867	.238	.972	.559	.320
rs279858	.698	.754	.274	.756
rs279845	.977	.692	.112	.630
rs279836	.778	.646	.054	.752
Adolescent, N	1,179	1,168	1,168	1,175
rs497068	.841	.166	.035	.041
rs279871	.117	.036	.029	.002
rs279867	.152	.042	.038	.003
rs279858	.124	.075	.016	.001
rs279845	.306	.194	.049	.006
rs279836	.105	.157	.019	.000

Note: p values $\leq .05$ are shown in bold.

the association between *GABRA2* and personality measures of impulsivity is observed only with Zuckerman's SSS and NEO Extraversion, not the BIS or NEO Conscientiousness; (3) the association with SSS and extraversion is limited to the adolescent sample, illustrating the developmental shifts that can occur in gene-behavior associations. We discuss each of these primary findings further below.

It is striking that there is no evidence of genetic association with any of the DSM clinical disorder symptom counts for externalizing disorders despite association with these outcomes in the older adult COGA participants. However, we do find evidence of association with nondiagnostic externalizing behavior as measured by the Achenbach self-reports. This is the measure for which association was previously reported with *GABRA2* in an independent, community-based sample of adolescents and young adults spanning the same age range (Dick et al., 2009), but for whom clinical outcomes were not available for comparison. These findings underscore the importance of using developmentally appropriate assessments for genetic studies in individuals of different ages. If only externalizing diagnoses and not subclinical levels of externalizing behavior

had been assessed, one might have erroneously concluded that there was no association with externalizing behavior in this younger COGA sample. Unlike older adult samples previously studied in COGA, there is no association at the level of diagnostic clinical symptoms in this younger sample. Instead, the association is manifest at this stage of development with subclinical levels of externalizing behavior that do not reach the level of diagnostic symptoms until later adulthood. This is in contrast to a previous COGA publication analyzing a smaller sample ($N = 850$) of children/adolescents, in whom we found evidence of association with conduct disorder symptoms (Dick et al., 2006b). This sample differs in two important ways that may have contributed to the discrepant findings with respect to an association between *GABRA2* and clinical level conduct disorder symptoms. In the sample in which an association was found, 77% of children were part of the most densely affected families in COGA; this prospective sample follows all children from both COGA proband and comparison families. That sample also combined information about conduct disorder across multiple assessments and used the maximal symptom count score from any assessment; it is possible that this led to a more reliable and/or more severe conduct disorder symptom count.

Failure to be explicit and comprehensive about the standardized measures under study could have led to very different conclusions about whether *GABRA2* is associated with ‘impulsivity’ in this sample. If only the Barratt Impulsivity scales had been included, one might have concluded that there was no evidence of association with impulsivity. Yet, we do find association with sensation-seeking and extraversion in the adolescent samples. Previous investigators have also noted the importance of distinguishing between various constructs related to impulsivity. A systematic empirical investigation of many existing measures of impulsivity found five different factors describing dispositions to rash action that were only moderately related to each other (Cyders & Smith, 2007; Cyders et al., 2007). Two of the five dispositions were emotion-based: *positive urgency* is the tendency to act rashly when experiencing extremely positive mood and *negative urgency* is the tendency to act rashly when experiencing extremely negative mood. Two were based on deficits in conscientiousness: *lack of planning* is the tendency to act without forethought and *lack of perseverance* reflects a failure to tolerate boredom or to remain focused despite distraction. The fifth was *sensation seeking*, or the tendency to seek out novel or thrilling stimulation (Cyders & Smith, 2007; Smith et al., 2007; Whiteside & Lyman, 2001). Our results support the distinction between these factors and broadly agree with the structure found in that study. Scales that are part of NEO Extraversion and Zuckerman’s Sensation-Seeking scale, the two measures associated with *GABRA2* in this study, both loaded largely onto their empirically derived sensation-seeking factor. In contrast, the other, non-significant measures in the current

investigation (neuroticism, BIS, conscientiousness) loaded primarily onto the urgency and lack of planning factors. There are likely several pathways of risk for the eventual manifestation of alcohol problems (Schuckit et al., 2008; Zucker, 1986); these results suggest that the pathway by which *GABRA2* initially confers risk for eventual alcohol problems begins with a predisposition to sensation-seeking early in adolescence.

It is also striking that the association between *GABRA2* and sensation-seeking scores and extraversion is found in the adolescent sample, but not in the young adult sample. Although personality was once thought to reflect stable trait-like characteristics, recent research has demonstrated personality changes across development, with emerging adulthood being a particularly important period (Littlefield & Sher, 2010; Littlefield et al., 2009). Impulsivity generally decreases across this period, as more individuals take on adult roles and responsibilities (Littlefield et al., 2010). Accordingly, sensation-seeking behavior in emerging adulthood may reflect a different process than sensation-seeking behavior in adolescence. Optimal levels of sensation-seeking likely vary by developmental stage. Some degree of exploratory behavior is an important component of human development, particularly for a developing adolescent. Accordingly, the fact that *GABRA2* is most strongly associated with sensation-seeking behavior in adolescence is an important reminder that this is not a gene for alcohol dependence. Rather, our findings suggest that *GABRA2* may alter eventual risk for alcohol-related problems through developmental processes that start early in development with elevated levels of sensation-seeking. These findings also have implications for prevention and intervention efforts, as some degree of exploratory behavior is likely to be adaptive. Channeling that behavior so as to not result in risky behavior down the line may be an important target for prevention.

In conclusion, we replicate the association between *GABRA2* and externalizing behavior (Dick et al., 2009) in a large sample of adolescents and young adults. However, we find that association is only detected with subclinical levels of externalizing behavior, as indexed by Achenbach’s self-report scales. There was no evidence of association with diagnostic level symptoms of externalizing disorders — alcohol dependence, illicit drug dependence, conduct disorder, or antisocial personality disorder — in these young adult and adolescent samples. Furthermore, we extend this association by exploring whether ‘impulsivity’ may be involved in the association between *GABRA2* and externalizing behavior, and what specific aspects of impulsivity are most relevant. We find evidence for association with sensation-seeking and extraversion in the adolescent sample only. There was not robust association with the BIS or low conscientiousness and *GABRA2*. These results help us understand the pathways by which *GABRA2* conveys risk (and conversely, does not convey risk) to externalizing

behavior. This information will be critical to identifying those individuals most at risk for the eventual development of problems and to intervene and disrupt gene-disorder relationships. Additionally, the findings caution against applying a 'one-size-fits-all' approach to replication of genetic association results; rather, careful attention needs to be paid to the theory surrounding how a particular gene is likely to be involved in clinical outcome, what is known about the developmental progression associated with the clinical disorder under investigation, and how the characteristics of the sample under study fit into that broader picture.

Acknowledgments

The Collaborative Study on the Genetics of Alcoholism (COGA), Principal Investigators B. Porjesz, V. Hesselbrock, H. Edenberg, and L. Bierut, includes 10 different centers: University of Connecticut (V. Hesselbrock); Indiana University (H. J. Edenberg, J. Nurnberger Jr., T. Foroud); University of Iowa (S. Kuperman, J. Kramer); SUNY Downstate (B. Porjesz); Washington University in St. Louis (L. Bierut, A. Goate, J. Rice, K. Bucholz); University of California at San Diego (M. Schuckit); Rutgers University (J. Tischfield); Southwest Foundation (L. Almasy), Howard University (R. Taylor), and Virginia Commonwealth University (D. Dick). Other COGA collaborators contributing to the GWAS data set 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. Rohrbaugh, J.-C. Wang (Washington University in St. Louis); A. Brooks (Rutgers University); and F. Aliev (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). Preparation of this manuscript by DMD was supported by K02AA018755 from NIAAA.

References

- Achenbach, T. M. (1991). *Manual for the youth self-report and 1991 profile*. Burlington, VT: Department of Psychiatry, University of Vermont.
- Achenbach, T. M. (1997). *Manual for the young adult self report and young adult behavior checklist*. Burlington, VT: Department of Psychiatry, University of Vermont.
- Achenbach, T. M., & Rescorla, L. A. (2001). *Manual for ASEBA school-age forms and profiles*. Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families.
- Achenbach, T. M., & Rescorla, L. A. (2003). *Manual for ASEBA adult forms and profiles*. Burlington, VT: University of Vermont, Research Center for Children, Youth, and Families.
- Agrawal, A., Edenberg, H. J., Foroud, T., Bierut, L., Dunne, G., Hinrichs, A. L., . . . Dick, D. M. (2006). Association of GABRA2 with drug dependence in the Collaborative Study of the Genetics of Alcoholism sample. *Behavior Genetics*, 36, 640–650.
- American Psychiatric Association (APA). (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., Text Rev.). Washington, DC: Author.
- Begleiter, H., Reich, T., Hesselbrock, V., Porjesz, B., Li, T. K., Schuckit, M., . . . Rice, J. (1995). The collaborative study on the genetics of alcoholism. *Alcohol Health & Research World*, 19, 228–236.
- Bucholz, K. K., Cadoret, R., Cloninger, C. R., Dinwiddie, S. H., Hesselbrock, V. M., Nurnberger, J. I., Jr., . . . Schuckit, M. A. (1994). 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*, 55, 149–158.
- Cannon, T. D., & Keller, M. C. (2006). Endophenotypes in the genetic analyses of mental disorders. *Annual Review of Clinical Psychology*, 2, 267–290.
- Costa, P. T., Jr., & McCrae, R. R. (1997). Stability and change in personality assessment: The revised NEO Personality inventory in the year 2000. *Journal of Personality Assessment*, 68, 86–94.
- Covault, J., Gelernter, J., Hesselbrock, V., Nellissery, M., & Kranzler, H. R. (2004). Allelic and haplotypic association of GABRA2 with alcohol dependence. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 129, 104–109.
- Cyders, M. A., & Smith, G. T. (2007). Mood-based rash action and its components: Positive and negative urgency. *Personality and Individual Differences*, 43, 839–850.
- Cyders, M. A., Smith, G. T., Spillane, N. S., Fischer, S., Annun, A. M., & Peterson, C. (2007). Integration of impulsivity and positive mood to predict risky behavior: Development and validation of a measure of positive urgency. *Psychological Assessment*, 19, 107–118.
- Depue, R. A., & Collins, P. F. (1999). Neurobiology of the structure of personality: DA, facilitation of incentive motivation, and extraversion. *Behavioral and Brain Sciences*, 22, 491–569.
- Dick, D. M. (2011). Developmental changes in genetic influences on alcohol use and dependence. *Child Development Perspectives*, 5, 223–230.
- Dick, D. M., Agrawal, A., Schuckit, M., Bierut, L., Hinrichs, A. L., Cloninger, C. R., . . . Begleiter, H. (2006a). Marital status, alcohol dependence, and GABRA2: Evidence for gene–environment correlation and interaction. *Journal of Studies on Alcohol*, 67, 185–194.
- Dick, D. M., Bierut, L., Hinrichs, A. L., Fox, L., Bucholz, K. K., Kramer, J. R., . . . Foroud, T. (2006b). The role of GABRA2 in risk for conduct disorder and alcohol and drug

- dependence across developmental stages. *Behavior Genetics*, 36, 577–590.
- Dick, D. M., Latendresse, S. J., Lansford, J. E., Budde, J., Goate, A., Dodge, K. A., . . . Bates, J. E. (2009). The role of *GABRA2* in trajectories of externalizing behavior across development and evidence of moderation by parental monitoring. *Archives of General Psychiatry*, 66, 649–657.
- Dick, D. M., Smith, G. T., Olausson, P., Mitchell, S. H., Leeman, R. F., O'Malley, S. S., & Sher, K. (2010). Understanding the construct of impulsivity and its relationship to alcohol use disorders. *Addiction Biology*, 15, 217–226.
- Edenberg, H. J., Dick, D. M., Xuei, X., Tian, H., Almasy, L., Bauer, L. O., . . . 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. *American Journal of Human Genetics*, 74, 705–714.
- Enoch, M. A., Schwartz, L., Albaugh, B., Virkkunen, M., & Goldman, D. (2006). Dimensional anxiety mediates linkage of *GABRA2* haplotypes with alcoholism. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 141, 599–607.
- Fehr, C., Sander, T., Tadic, A., Lenzen, K. P., Angheliescu, I., Klawe, C., . . . Szegedi, A. (2006). Confirmation of association of the *GABRA2* gene with alcohol dependence by subtype-specific analysis. *Psychiatric Genetics*, 16, 9–17.
- Foroud, T., Edenberg, H. J., Goate, A., Rice, J., Flury, L., Koller, D. L., . . . Reich, T. (2000). Alcoholism susceptibility loci: Confirmation studies in a replicate sample and further mapping. *Alcoholism: Clinical and Experimental Research*, 24, 933–945.
- Frederick, J. A., & Iacono, W. G. (2006). Beyond the DSM: Defining endophenotypes for genetic studies of substance abuse. *Current Psychiatry Reports*, 8, 144–150.
- Gottesman, I. I., & Gould, T. D. (2003). The endophenotype concept in psychiatry: Etymology and strategic intentions. *American Journal of 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. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 144, 417–423.
- Hamidovic, A., Dlugos, A., Skol, A., Palmer, A. A., & de Wit, H. (2009). Evaluation of genetic variability in the dopamine receptor D2 in relation to behavioral inhibition and impulsivity/sensation seeking: An exploratory study with d-amphetamine in healthy participants. *Experimental and Clinical Psychopharmacology*, 17, 374–383.
- Hesselbrock, M., Easton, C., Bucholz, K. K., Schuckit, M., & Hesselbrock, V. (1999). A validity study of the SSAGA — A comparison with the SCAN. *Addiction*, 94, 1361–1370.
- Iacono, W. G., Carlson, S. R., Taylor, J., Elkins, I. J., & McGue, M. (1999). Behavioral disinhibition and the development of substance-use disorders: Findings from the Minnesota Twin Family Study. *Development and Psychopathology*, 11, 869–900.
- Kendler, K. S., Prescott, C., Myers, J., & Neale, M. C. (2003a). The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. *Archives of General Psychiatry*, 60, 929–937.
- Kendler, K. S., Prescott, C. A., Myers, J., & Neale, M. C. (2003b). The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. *Archives of General Psychiatry*, 60, 929–937.
- Krueger, R. F., Hicks, B. M., Patrick, C. J., Carlson, S. R., Iacono, W. G., & McGue, M. (2002). Etiologic connections among substance dependence, antisocial behavior, and personality: Modeling the externalizing spectrum. *Journal of Abnormal Psychology*, 111, 411–424.
- Krueger, R. F., Markon, K. E., Patrick, C. J., & Iacono, W. G. (2005). Externalizing psychopathology in adulthood: A dimensional-spectrum conceptualization and its implications for DSM-V. *Journal of Abnormal Psychology*, 114, 537–550.
- Lejuez, C. W., Magidson, J. F., Mitchell, S. H., Sinha, R., Stevens, M. C., & De Wit, H. (2010). Behavioral and biological indicators of impulsivity in the development of alcohol use, problems, and disorders. *Alcoholism: Clinical and Experimental Research*, 34, 1334–1345.
- Littlefield, A. K., & Sher, K. J. (2010). The multiple, distinct ways that personality contributes to alcohol use disorders. *Social and Personality Psychology Compass*, 4, 767–782.
- Littlefield, A. K., Sher, K. J., & Steinley, D. (2010). Developmental trajectories of impulsivity and their association with alcohol use and related outcomes during emerging and young adulthood I. *Alcoholism: Clinical and Experimental Research*, 34, 1409–1416.
- Littlefield, A. K., Sher, K. J., & Wood, P. K. (2009). Is 'maturing out' of problematic alcohol involvement related to personality change? *Journal of Abnormal Psychology*, 118, 360–374.
- Manolio, T. A., Collins, F. S., Cox, N. J., Goldstein, D. B., Hindorf, L. A., Hunter, D. J., . . . Visscher, P. M. (2009). Finding the missing heritability of complex diseases. *Nature*, 461, 747–753.
- Nyholt, D. R. (2004). A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *American Journal of Human Genetics*, 74, 765–769.
- Patton, J. H., Stanford, M. S., & Barratt, E. S. (1995). Factor structure of the Barratt Impulsiveness Scale. *Journal of Clinical Psychology*, 51, 768–774.
- Porjesz, B., Rangaswamy, M., Kamarajan, C., Jones, K., Padmanabhapillai, A., & Begleiter, H. (2005). The utility of neurophysiological markers in the study of alcoholism. *Clinical Neurophysiology*, 116, 993–1018.
- Racine, S. E., Culbert, K. M., Larson, C. L., & Klump, K. L. (2009). The possible influence of impulsivity and dietary restraint on associations between serotonin genes and binge eating. *Journal of Psychiatric Research*, 43, 1278–1286.
- Reich, T., Edenberg, H., Goate, A., Williams, J., Rice, J., Eerdewegh, P. V., . . . Begleiter, H. (1998). Genome-wide search for genes affecting the risk for alcohol dependence. *American Journal of Medical Genetics*, 81, 207–215.

- Rose, R. J., Dick, D. M., Viken, R. J., & Kaprio, J. (2001). Gene-environment interaction in patterns of adolescent drinking: Regional residency moderates longitudinal influences on alcohol use. *Alcoholism: Clinical and Experimental Research*, *25*, 637–643.
- Russo, M. F., Stokes, G. S., Lahey, B. B., Christ, M. G., McBurnett, K., Loeber, R., Stouthamer-Loeber, M., & Green, S. M. (1993). A sensation scale for children: Further refinement and psychometric development. *Journal of Psychopathology and Behavioral Assessment*, *15*, 69–86.
- Schuckit, M., Smith, T. L., Trim, R., Kreikebaum, S., Hinga, B., & Allen, R. (2008). Testing the level of response to alcohol-based model of heavy drinking and alcohol problems in the offspring from the San Diego Prospective Study. *Journal of Study on Alcohol and Drugs*, *69*, 571–579.
- Smith, G. T., Fischer, S., Cyders, M. A., Annus, A. M., Spillane, N. S., & McCarthy, D. M. (2007). On the validity and utility of discriminating among impulsivity-like traits. *Assessment*, *14*, 155–170.
- Soyka, M., Preuss, U. W., Hesselbrock, V., Zill, P., Koller, G., & Bondy, B. (2008). GABA-A2 receptor subunit gene (GABRA2) polymorphisms and risk for alcohol dependence. *Journal of Psychiatric Research*, *42*, 184–191.
- van Beijsterveldt, C. E., Molenaar, P. C., de Geus, E. J., & Boomsma, D. I. (1996). Heritability of human brain functioning as assessed by electroencephalography. *American Journal of Human Genetics*, *58*, 562–573.
- Whiteside, S. P., & Lynam, D. R. (2001). The five factor model and impulsivity: Using a structural model of personality to understand impulsivity. *Personality and Individual Differences*, *30*, 669–689.
- Young, S. E., Stallings, M. C., Corley, R. P., Krauter, K. S., & Hewitt, J. K. (2000). Genetic and environmental influences on behavioral disinhibition. *American Journal of Medical Genetics*, *96*, 684–695.
- Zucker, R. A. (1986). The four alcoholisms: A developmental account of the etiologic process. In P. C. Rivers (Ed.), *Alcohol and addictive behavior* (pp. 27–83). Lincoln, NE: University of Nebraska Press.
- Zuckerman, M. (1979). *Sensation seeking: Beyond the optimal level of arousal*. Hillsdale, NJ: Erlbaum.
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