Genes Associated With Alcohol Outcomes Show Enrichment of Effects With Broad Externalizing and Impulsivity Phenotypes in an Independent Sample

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ABSTRACT. Objective: The purpose of this study was to evaluate evidence for association with a panel of genes previously associated with alcohol-related traits in a new sample of adolescent and young adult individuals ($N = 2,128$; 51% female) collected as part of the Collaborative Study on the Genetics of Alcoholism (COGA). We tested for association with phenotypes related to externalizing behavior, including diagnostic symptom counts for disorders on the externalizing spectrum (alcohol dependence, conduct disorder, adult antisocial personality disorder, and illicit drug dependence), and related behavioral/personality traits (Achenbach Externalizing, NEO Extraversion, NEO Conscientiousness, Zuckerman's Sensation Seeking, and the Barratt Impulsivity Scale) based on the substantial literature suggesting that these behaviors may be alternate manifestations of a shared genetic liability. **Method:** We tested for overall enrichment of the set of 215 genotyped single-nucleotide polymorphisms (SNPs) for each of the phenotypes. We conducted secondary analyses comparing results for sensation seeking with results for the other phenotypes. **Results:** For all phenotypes, there was significant enrichment of association results ($p < .05$) compared with chance expectations. The greatest number of significant results was observed with the phenotype Sensation Seeking. Secondary analyses indicated that the number of SNPs yielding $p < .05$ with Sensation Seeking was significantly greater than that observed for each of the other phenotypes. **Conclusions:** We find evidence for enrichment of association results across a spectrum of externalizing phenotypes with a panel of candidate genes/SNPs selected based on previous suggestion of association with alcohol-related outcomes. In particular, we find significant enrichment of effects with sensation seeking, suggesting that this may be a particularly salient behavior associated with risk for alcohol-related problems. (*J. Stud. Alcohol Drugs, 76,* 38–46, 2015)

THE COLLABORATIVE STUDY on the Genetics of Alcoholism (COGA) is a multisite project with the goal of identifying genes that influence alcohol dependence and related traits (Begleiter et al., 1995). COGA has used a variety of strategies to aid in gene identification, including the use of rich phenotyping to characterize genetic effects (Dick et al., 2013b; Kramer et al., 2008), as well as electrophysiological endophenotypes (Begleiter & Porjesz, 1999; Dick et al., 2006b). In addition, the study has used a variety of genetic designs and methodologies. These began with linkage analyses (Reich et al., 1998) and family-based candidate gene studies (Edenberg & Foroud, 2006); more recently, COGA has conducted both case-control (Edenberg et al., 2010) and family-based (Kang et al., 2012; Wang et al., 2012) genome-wide association studies. The most recent data collection phase of the COGA project involves identifying adolescents and young adults who are the younger members of the original extended COGA families. This prospectively followed sample has been assessed with a more extensive phenotype battery that allows us to explore the spectrum of phenotypes associated with genetic variants across developmental stages (Dick et al., 2013b).

In an effort to evaluate the association evidence for identified genes with purported effects on alcohol dependence and related traits, we genotyped a panel of 215 single-nucleotide polymorphisms (SNPs) in 2,128 adolescents and young adults. Genes were nominated by COGA investigators based on putative evidence of association in previous COGA analyses of alcohol dependence or related traits, and a small number were selected from the published literature. We focused on externalizing and impulsivity phenotypes that appear to share common genetic underpinnings with alcohol dependence (Hicks et al., 2004; Kendler et al., 2003; Young et al., 2000) and may represent earlier behavioral manifesta-

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tions of the predisposition to alcohol dependence (Dick et al., 2006a, 2013b).

One of the challenges facing investigators interested in elucidating gene–behavior relationships is how best to report results in the literature, given the large amount of genetic and phenotypic information available in this age of high-throughput genotyping and deep phenotyping. Separate reports for individual genes and/or phenotypes have the potential to lead to artificially inflated estimates of association. In this report, we were interested in testing for genetic associations across a battery of clinical symptoms and impulsivity-related behavioral traits, across two broad developmental stages (adolescence and young adulthood). In addition, we had genotypes across a number of genes, selected using varying criteria, although all were thought to relate broadly to alcohol-related outcomes. Accordingly, we tested for overall enrichment of the set of SNPs for each of the phenotypes of interest.

Method

Sample

COGA is a multisite project with the goal of identifying genes that contribute to alcoholism and related phenotypes. Probands were 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 three or more siblings, 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. Recruitment of adolescents (12- to 17-year-olds) and young adults (18- to 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, from families affected with alcoholism and comparison families. For more than 50% of the subjects, both parents have been personally interviewed. All analyses reported here involve the baseline assessments for participants (N = 2,128 individuals, 51% female) who have been genotyped for the SNPs studied in this report. The self-reported racial/ethnic breakdown of the sample was 62% European American, 26% African American, and 12% other. To maximize power, results are presented for the full sample, with race/ethnicity included as a covariate. However, we repeated analyses in the larger European American sample to ensure consistency. The results—although slightly less significant, as would be expected with the reduced sample size—were similar to those reported here (available on request).

Genotyping

A supplemental table lists the 215 SNPs that were genotyped in the sample and is available on request from the authors. As described above, this set of SNPs was chosen based on the literature, in addition to previous significant COGA results across several samples that included both candidate gene ($p < .05$) and genome-wide association studies ($p < .00001$), to represent top findings. To reduce the scope of multiple testing and financial costs, SNPs in the same gene were chosen such as to not be in linkage disequilibrium with one another.

Genotyping was performed using Sequenom (Sequenom, Inc., San Diego, CA; www.sequenom.com) and OpenArray Technologies (Life Technologies, Grand Island, NY; www.lifetechnologies.com). For Sequenom genotyping, polymerase chain reaction (PCR) primers were designed with Sequenom MassARRAY Assay Designer software. Standard procedures were used to amplify PCR products and then to perform primer extension reactions. The primer extension products were cleaned with resin and spotted onto a SpectroChip (Sequenom, Inc., San Diego, CA). We used a Bruker mass spectrometry workstation (Bruker Corp., Fremont, CA) to scan the chip. The resulting genotype spectra were analyzed with the Sequenom SpectroTYPER software v3.4 (Sequenom, Inc. San Diego, CA). OpenArray genotyping is a multiplex TaqMan assay platform (Life Technologies, Grand Island, NY). We used OpenArray Genotyping Plate Configurator (Life Technologies, Grand Island, NY, www.lifetechnologies.com) to design assays. Reactions were carried out in a Dual Flat Block GeneAmp PCR System 9700 (Applied Biosystems; Life Technologies, Grand Island, NY) with standard PCR cycling conditions.

Arrays were scanned on the OpenArray NT imager (Life Technologies, Grand Island, NY) and genotypes were called using the OpenArray SNP Genotyping analysis software (Life Technologies, Grand Island, NY). Markers that failed in assay design or genotyping process with either Sequenom or OpenArray system were then genotyped with KASPar platform (LGC Limited, Teddington, Middlesex, UK; www.lgcgenomics.com). We used the PrimerPicker software (LGC Limited, Teddington, Middlesex, UK) to design the assays and followed the protocol described in KASPar SNP Genotyping System (LGC Limited, Teddington, Middlesex, UK) manual to run PCR reactions with a GeneAmp PCR System 9700 (Applied Biosystems; Life Technologies, Grand Island, NY). Genotypes were determined using the 7900 HT Fast Real-Time PCR system (Applied Biosystems; Life Technologies Grand Island, NY).

SNPs were genotyped in the entire COGA sample with available DNA but analyzed in the subset with available

phenotype data (N = 2,128). Only founders were used to test for Hardy–Weinberg Equilibrium and to estimate minor allele frequency. SNPs were evaluated for Hardy–Weinberg Equilibrium and minor allele frequency in the European American and African American samples separately. All SNPs were in Hardy–Weinberg Equilibrium (all European American $p > .01$, all African American $p > .0004$). All SNP genotypes were checked for Mendelian inheritance using PEDCHECK (O'Connell and Weeks, 1998). All SNP and sample genotypes had at least a 90% genotyping rate.

Measures

Psychiatric interview. All individuals were interviewed with the Semi-Structured Assessment for the Genetics of Alcoholism (Bucholz et al., 1994; Hesselbrock et al., 1999), using the adult (ages ≥18) or adolescent (ages 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 criteria from the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (American Psychiatric Association, 1994). Symptom counts for the following diagnoses were analyzed: alcohol dependence; illicit drug dependence (sum of symptom counts of marijuana, cocaine, other stimulants, sedatives, and opiates); childhood conduct disorder; and (for individuals > age 18) adult antisocial behavior.

Achenbach Youth/Adult Self-Report. The Externalizing scale of the Youth Self-Report/Adult Self-Report 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. The Barratt Impulsivity Scale 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 1 (*never*), 2 (*occasionally*), 3 (*often*), and 4 (*always*). Total scores are computed by summing subscale items.

Sensation Seeking Scale (SSS). The SSS was developed by Zuckerman and colleagues to measure individual differences in stimulation and arousal (Zuckerman, 1979). 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 30 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 and 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," and "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, M = 14.48, SD = 1.76) and adult sample (ages 18–26; n = 936, M = 19.7, SD = 1.46), as described above. This also avoided potential analytic issues associated with method variance because 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 ns available for each analysis are indicated in Table 2. All analyses were conducted using SAS software Version 8 (SAS Institute Inc., Cary, NC). Association analyses were run using an additive model of SNP effect 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, to account for related individuals and shared environmental variability within families.

Our primary aim was to test whether there was evidence for enrichment of significant association effects across the group of SNPs for each of the phenotypes of interest. Under the null model when phenotype and genotype are independent, p values have uniform distribution in [0, 1], so the expected number of p values ≤ .05 is 215 × .05 = 10.75. To calculate the probability of observing an excess of p values beyond the expected 10.8, we treated each analysis as independent (which is reasonable because most of the SNPs

have different gene locations and are spread across multiple chromosomes) and assigned them binary values (1 if $p \leq .05$, "success"; and 0 if $p > .05$). In this way, we defined a new variable for each SNP/phenotype with a value of 0 or 1 depending on the p value for that SNP/phenotype from the association test. Thus, each group of analyses with $n = 215$ SNPs is converted into a Bernoulli trial with the total number of successes S_n distributed as a Binomial distribution $B(n = 215, p = .05)$. Confidence limits for the total number of successes can be calculated using the Normal approximation of the Binomial distribution. We can interpret $B(n = 215, p = .05)$ as having normal distribution with the expected value $np = 215 \times .05 = 10.75$ and $SD = \sqrt{np(1-p)} = \sqrt{215 \times 0.05 \times 0.95} = 3.2$. The upper bound of the 95% confidence interval for the total number of successes S_{215} (where success means $p \leq .05$) is found to be approximately 17. Calculation of the p value associated with observing $\geq k$ successes corresponds to one-sided Z test with

$$P(S_n \geq k) = P(S_{215} \geq k)$$
$$= P\left(\frac{S_{215} - np}{\sqrt{np(1-p)}} \geq \frac{k}{\sqrt{np(1-p)}}\right)$$
$$\approx P\left(Z \geq \frac{k}{\sqrt{np(1-p)}}\right)$$

Note that for Binomial distribution $P(S_n = k) = C_n^k p^k (1-p)^{n-k}$, for all $k \leq n$. Because the appropriate p value threshold for the analyses cannot be exactly known, we repeated them using more stringent p value thresholds of $p < .01$ and $p < .001$ to examine the consistency of the pattern of results.

Our primary analyses found that one phenotype yielded the most significant results in both the adolescent and adult samples. Accordingly, we conducted secondary analyses to test whether the difference in the number of significant results ("successes") observed with this phenotype was statistically greater than the number of successes with each of the other phenotypes. Because the phenotypes were run for the same SNP, we expected the results to be correlated. Therefore, to compare two correlated binomial samples, we used a paired-samples t test.

Results

Table 1 lists all genotyped genes and indicates for each gene whether any SNP yielded $p < .05$ in either the adolescent or adult sample for each phenotype analyzed. Data are presented in this way for ease of interpretation, but exact SNPs and p values are available from the authors on request. As is obvious from the table, many of the genes yielded $p < .05$ with multiple phenotypes across the samples. Bold in the table indicates SNPs that were significant at a more stringent threshold of $p < .001$. Table 2 indicates the total number of significant SNPs for each phenotype, for each of the samples, across three p value thresholds ($p < .05$, $p < .01$, and $p < .001$). In addition, it lists whether the number of observed p values less than the given threshold ("successes") exceeds that expected by chance. For all phenotypes analyzed, more than 17 successes (the upper bound for the 95% confidence interval representing chance findings using $p < .05$) were observed. P values for the total number of successes observed for each phenotype were highly significant. This was consistent across the range of p value thresholds, with the exception that Externalizing did not show enrichment in either sample at $p < .001$. In the adolescent and adult samples, the greatest number of significant results was observed with the phenotype Sensation Seeking. Our secondary analyses indicated that in both the adolescent and adult samples the number of successes for Sensation Seeking was significantly greater than the number of successes for each of the other phenotypes (Table 2). Although fewer "successes" were observed with more stringent p value thresholds, as would be expected, the pattern of results was consistent across the range of p value thresholds.

Discussion

In this study, we tested whether a set of genes with previous evidence of association with alcohol dependence and related traits was associated with alcohol-related outcomes in a new sample of adolescents and young adults collected as part of COGA. Most SNPs that were genotyped in this sample were selected based on previous evidence for association with various alcohol-related traits or electrophysiological endophenotypes in previous COGA analyses (Bierut et al., 2008; Dick et al., 2007; Edenberg, 2011; Edenberg et al., 2006; Wetherill et al., 2014) or from the literature (Wang et al., 2012). Because these previous analyses focused on several different alcohol-related outcomes, in this study we selected a set of phenotypes in which to test whether evidence of association with alcohol-related outcomes would extend to this new COGA sample. We focused on phenotypes related to externalizing behavior, including both diagnostic symptom counts for disorders on the externalizing spectrum (alcohol dependence, conduct disorder, adult antisocial personality disorder, and illicit drug dependence) and related personality traits (Achenbach Externalizing, NEO Extraversion, NEO Conscientiousness, Zuckerman's Sensation Seeking, and the Barratt Impulsivity Scale) based on the substantial literature suggesting a shared genetic predisposition across these outcomes (Kendler et al., 2003; Krueger et al., 2002; Young et al., 2000). Across all phenotypes, we found an excess of significant results beyond that expected by chance with the set of candidate genes. This provides evidence for enrichment of significant effects among these genes for all tested externalizing phenotypes and further evidence of a common

TABLE 1. Association results for all genes and phenotypes. Cells indicate whether at least one single-nucleotide polymorphism (SNP) with $p < .05$ was observed for that gene/outcome in the adolescent (a) or Adult (A) sample. **Bold** indicates that the result was significant at $p < .001$.

Gene	AD_SX	DD_SX	ASP	CD_SX	EXT	BIS	NEO_C	NEO_E	SS
ABCA8		A					a, A	A	
ACN9	A			A		A	**a,** A	**A**	a
ADAMTS17	A			a					
ADH1B	**a, A**	**a**		A		A	A	a, **A**	**a, A**
ADH1C	a, **A**		A	A	A			A	a, **A**
ADH4	a, **A**	A	A	a, A			A	a, A	**a, A**
ADH7		**a**		a, A		a		a	**a,** A
ALDH1A1	A	a		**a,** A		**A**		A	a, **A**
ALDH1L1					A	a			
ALDH2	a		A	a				a	**a, A**
ANK2	a	a	A	**A**		A		**a**	
ANKK1/TTC12	a	a				A			
ARHGAP28								a	
BCKDHB	A		A	**A**	a	**a**	a	A	**a,** A
C15orf53	A	A			a, A	a		a, **A**	A
C7orf72	a, A	A			a		a		**A**
CACNG2	A				A			A	A
CAPN13		a							a, A
CARS				**a,** A			a	**a,** A	**a, A**
CASZ1			A			**a,** A	A	a	**A**
CAT			A			A			
CHRM2		a, A	A			a	a	a, A	**a, A**
CHRNA3	a		**A**	A	a	a		a	a
CHRNA5			A			a			a
CHRNA6	a, A					A	a	a, A	a, **A**
CHRNB1	A								
CHRNB3	**A**					A	a	a	a, A
CHRNB4	**a**	**a**		A		A		**a**	a, A
COMT			A						A
CSMD1		A							**a, A**
CYYR1				A			a	**a,** A	a, **A**
DAT1	a, A								a
DDX49				a		A			a, **A**
DRD4		a			a	A		A	
DSCAML1						A		**A**	**A**
ELF5									
ENSAP2							a, A	a	**a, A**
ERBB4			A			a			
FAM189A2									
FKBP5	**a**				A			A	A
GABBR2	**a, A**	a			a, A	**A**		a	**A**
GABRA1					A	a, **A**	a, A		a, **A**
GABRA2	A				a, A			a	a, A
GABRA4				A					a
GABRA6									
GABRG1			A			A			a, **A**
GABRG1/A2			**A**					**A**	a, A
GABRG3	**a,** A	a			a	a, A		**a,** A	a, **A**
GABRP		A	**A**	**A**	A	a, A	A		A
GABRR1	a	a			a, A	**a**	**A**		a
GABRR2		A	**A**	**A**	a, A	a, A	A	**a,** A	**a**
GCOM1/GRINL1A	A		A	A		**A**	a, A	a, **A**	a, **A**
GRIA2	A	a		a	A		**A**	a, A	a, **A**
GRID2	A	a	A	**a**			A	a	**a,** A
GRIN2A				A		A	A	A	
GRM8	**a, A**	a, **A**	A	A	a, A			A	a, **A**
HBG2; OR52H1	a						a	A	A
KCNJ6		A	A	a, A	a		A	a	A
KCNQ5	A					a, A		**A**	**a,** A
KIAA0040	**A**			A	A	A		a	
LCN	A			a, A				A	
LINGO2				a, A	a	a			
LOC100128721				A					
LOC151121	a, A	a, A							
LRRK1		A							
LRRN1	a	a, A	**A**	A				a	a, A

Table Continued

Table 1. *Continued*

Gene	AD_SX	DD_SX	ASP	CD_SX	EXT	BIS	NEO_C	NEO_E	SS
LSAMP	**A**		A			**a**	A		**a**
MAL									
MAP6D1							A		A
MARCH1					a	a		**A**	
NA									
NENF					a		A		
NFKB1	A	a	A	A				a, A	A
NID1		a		a, **A**	a	**a**	a		
NKAIN2					a		a		A
NRXN1	A		**A**	a, **A**			**A**		
NTRK3		a							A
OR51L1	A								
OR52S1P		a						a, A	
OSBPL5							A		A
PAH			A	A		a	A		
PAK7	a	A	A	A		A		A	a, **A**
PALLD	A						a, A		
PCDH10	a, A			A	A	A		**a,** A	**a, A**
PCSK2		a							
PDYN		A			a	A		a	**A**
PLCL1						A		A	A
PLSCR4	**a,** A	A						a, A	**a, A**
PM20D2							**A**		A
PPARGC1A					a				
PRKCA	A					a			A
PTPRG		a	**A**					A	**a, A**
RGS2					A			A	
SEC16B							A		
SI	A								
SLC22A18_PHLDA2		a	A	a, A			a	a, A	**a,** A
SLC6A9	**a**		A	a		A		**a,** A	a, **A**
SLC9A9	a					a	A	a, A	a
SNCA	**A**		A			A			
SNX29						a		A	
SOX5		A				A		a	A
STC1	A				A	A			a
TAS2R16	a, A	a, A	A		A	a, A	A	**a**	**a, A**
TAS2R38	**A**			a	A			A	
TEK		a		A			A	a, A	a
TMEM132D	A					A	a, A	A	
TMTC2						A	a		a
TSPAN11	A		A		A	a	a		
TTC12		A			A				a, A
UROC1									
UTP20		**A**			A				A
VAT1L	a			a			a, A	a	
ZNF699					A	a, A		A	a, **A**

Notes: SNP position and function are from human genome build 19, dbSNP 137. AD_SX = alcohol dependence symptoms; DD_SX = drug dependence symptoms; ASP = adult antisocial behavior symptoms; CD_SX = conduct disorder symptoms; EXT = externalizing; BIS = Barrett Impulsivity Scale; NEO_C = NEO Five Factor Inventory conscientiousness; NEO_E = NEO Five Factor Inventory extraversion; SS = sensation seeking.

genetic liability with predispositions to a variety of externalizing outcomes.

The greatest number of significant effects was observed with sensation seeking. This was true in both the adolescent and adult samples, in which 84 and 94 significant findings were observed, respectively (at $p < .05$). Extraversion also showed a large number of significant effects across both samples (55 in the adolescents, 57 in the adults). These findings are of particular interest in light of a recent article showing that sensation seeking and extraversion mediated the relationship between early childhood temperament and increased risk of adolescent alcohol problems in an independent, epidemiological sample followed longitudinally from birth through adolescence (Dick et al., 2013a). Numerous other studies also have found sensation seeking to be associated with elevated risk of adolescent alcohol use and problems (Dick et al., 2010; Smith et al., 2007). Our findings suggest that part of the predisposition to alcohol-related outcomes may be in part through sensation-seeking traits.

Another notable finding was that there was a difference between the number of significant SNPs associated with alcohol dependence symptoms in the adolescent and adult

Table 2. Results for enrichment of significant association findings ($p < .05$) for each phenotype

		$p = .05$			$p = .01$			$p = .001$		
Variable	*N*	no. of successes[&]	*p* of successes	*p* of comparison with sensation seeking	no. of successes[&]	*p* of successes	*p* of comparison with sensation seeking	no. of successes[&]	*p* of successes	*p* of comparison with sensation seeking
Alcohol dependence Sx										
Adolescents	1,140	37	0.0E+00	1.0E-08	18	0.0E+00	1.7E-06	9	0.0E+00	3.9E-03
Adults	899	57	0.0E+00	4.2E-05	31	0.0E+00	2.1E-05	12	0.0E+00	3.2E-06
Drug dependence Sx										
Adolescents	1,140	32	1.5E-11	2.0E-09	14	0.0E+00	1.5E-07	4	0.0E+00	5.8E-05
Adults	897	32	1.5E-11	4.4E-13	20	0.0E+00	7.1E-08	7	0.0E+00	1.3E-07
Adult antisocial behavior Sx										
Adults*	897	46	0.0E+00	1.4E-07	23	0.0E+00	2.0E-07	9	0.0E+00	6.8E-07
Conduct disorder Sx										
Adolescents	1,140	26	9.1E-07	8.5E-11	7	4.4E-04	2.0E-11	3	9.3E-10	1.7E-05
Adults	897	38	0.0E+00	2.2E-09	14	0.0E+00	4.3E-10	8	0.0E+00	6.9E-07
Externalizing										
Adolescents	1,090	26	9.1E-07	8.5E-11	4	1.0E-01	8.2E-12	0	6.8E-01	4.4E-07
Adults	824	34	1.7E-13	2.0E-10	8	3.0E-05	8.1E-13	0	6.8E-01	9.0E-12
Barrett Impulsivity Scale										
Adolescents	1,129	38	0.0E+00	1.8E-07	11	6.6E-10	2.3E-10	7	0.0E+00	2.8E-04
Adults	848	43	0.0E+00	1.7E-08	16	0.0E+00	2.0E-10	4	0.0E+00	1.1E-10
Conscientiousness										
Adolescents	1,117	26	9.1E-07	2.9E-11	10	3.7E-08	5.8E-09	1	4.5E-02	2.3E-06
Adults	873	38	0.0E+00	4.7E-09	11	6.6E-10	2.2E-11	4	0.0E+00	5.7E-09
Extraversion										
Adolescents	1,117	55	0.0E+00	2.5E-04	26	0.0E+00	2.2E-04	9	0.0E+00	2.9E-03
Adults	873	57	0.0E+00	1.5E-05	25	0.0E+00	5.2E-08	9	0.0E+00	2.0E-07
Sensation seeking										
Adolescents	1,124	84	0.0E+00	–	52	0.0E+00	–	23	0.0E+00	–
Adults	856	94	0.0E+00	–	64	0.0E+00	–	41	0.0E+00	–

Notes: No. = number; Sx = symptoms. *Adult antisocial behavior not assessed in adolescents because under age 18.

samples ($n = 37$ and 57, respectively, at $p < .05$). This pattern of results, whereby association of candidate genes with alcohol dependence symptoms becomes more pronounced in young adulthood, has previously been observed with a number of individual genes of interest in both the COGA sample (Dick et al., 2006a) and other independent samples (Guo et al., 2007; Irons et al., 2012). Here, we extend this pattern of findings with a new, diverse set of candidate genes.

The results of this study should be interpreted in the context of the following limitations. Because of the diverse nature of the selection of the SNPs for genotyping, we did not systematically test whether each SNP was associated with the same phenotype from the original association report, which would be the most stringent test of replication (Sullivan et al., 2008). In part, this was because the purpose of the adolescent/young adult COGA sample is not to strictly replicate genetic effects, but rather, to further explore and understand the nature of genetic effects across developmental stages. Further, there was not a systematic criterion used for inclusion of a particular SNP (e.g., a requirement of significance at a particular level in the original report). Accordingly, the nature of the genotypic panel selection led us to the overarching tests for enrichment of association reported here. An important future direction would be to extend these analyses and conduct multivariate phenotypic analyses across the externalizing and impulsivity phenotypes to explicitly test (for example) the extent to which particular genes affect shared variance across the outcomes, or variance specific to a particular aspect of externalizing/impulsivity. Last, the sample consisted of younger individuals from families that were used in the original association study, thus implicating the genes that were genotyped in the present sample leading to the conclusion that the younger individuals are not an entirely independent replication sample.

In conclusion, we find evidence for enrichment of association results across a spectrum of externalizing phenotypes with a panel of candidate genes/SNPs selected based on previous suggestion of association with alcohol-related outcomes. By conducting overarching tests to examine the pattern of results across phenotypes, we find that although any one gene might not be significantly associated with all outcomes, overall there is a clear pattern for genes, originally selected based on association with alcohol-related traits that show association with a wide range of both clinical symptom counts of externalizing disorders and subclinical traits related to behavioral undercontrol and/or reward seeking. In particular, we find significant enrichment of effects with sensation seeking, suggesting that this may be a particu-

larly salient behavior associated with risk for alcohol-related problems.

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