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Effect of *GABRA2* Genotype on Development of Incentive-Motivation Circuitry in a Sample Enriched for Alcoholism Risk

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Heightened reactivity of the incentive-motivation system has been proposed to underlie adolescent-typical risky behaviors, including problem alcohol involvement. However, even in adolescence considerable individual variation in these behaviors exists, which may have genetic underpinnings and be related to variations in risk for later alcohol use disorder (AUD). Variants in *GABRA2* have been associated with adult alcohol dependence as well as phenotypic precursors, including impulsiveness and externalizing behaviors. We investigated the impact of *GABRA2* on the developmental trajectory of nucleus accumbens (NAcc) activation during anticipation of monetary reward from childhood to young adulthood. Functional MRI during a monetary incentive delay task was collected in 175 participants, with the majority ($n = 151$) undergoing repeated scanning at 1- to 2-year intervals. One group entered the study at age 8–13 years ($n = 76$) and another entered at age 18–23 years ($n = 99$). Most participants were children of alcoholics (79%) and thus at heightened risk for AUD. A total of 473 sessions were completed, covering ages 8–27 years. NAcc activation was heightened during adolescence compared with childhood and young adulthood. *GABRA2* genotype (SNP rs279858) was associated with individual differences in NAcc activation specifically during adolescence, with the minor allele (G) associated with greater activation. Furthermore, NAcc activation mediated an effect of genotype on alcohol problems ($n = 104$). This work demonstrates an impact of *GABRA2* genotype on incentive-motivation neurocircuitry in adolescence, with implications for vulnerability to alcoholism. These findings represent an important step toward understanding the genetic and neural basis of individual differences in how risk for addiction unfolds across development.

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INTRODUCTION

Adolescence is a critical period for the initiation and escalation of alcohol use, and the development of alcohol abuse and dependence (Johnston *et al*, 2010). However, even within the framework of normative adolescent behavior there is considerable individual variation in drinking, which is likely to be related to variations in risk for later alcohol use disorder (AUD; Zucker, 2006) and to have genetic underpinnings.

Associations have been found between *GABRA2* and adult alcohol dependence (eg, Edenberg *et al*, 2004). Within the haplotype block showing these associations, there are two common yin-yang haplotypes that account for the majority of the diversity in Caucasians (Enoch, 2008). The less frequent haplotype has been associated with alcoholics

without comorbid drug dependence (Covault *et al*, 2004) and those with a family history of alcoholism (Fehr *et al*, 2006). This haplotype is also associated with childhood and adolescent phenotypic precursors to alcohol dependence, including externalizing problems throughout adolescence (Dick *et al*, 2009) and impulsiveness (Villafuerte *et al*, 2012).

Developmental changes occur in the relative importance of genetic and environmental effects on substance use and problems, with the importance of genetic factors increasing across adolescence into young adulthood (Kendler *et al*, 2008; Rose *et al*, 2001). However, few studies have investigated how risk confered by specific genetic polymorphisms unfolds across development. One exception is the report of a *GABRA2* association with conduct disorder (CD) symptoms in childhood, whereas an association with alcohol dependence symptoms was not evident until the mid-20's (Dick *et al*, 2006). This suggests that genetic influences are expressed differently at different developmental stages. To our knowledge, no studies have explored this within an imaging genetics framework by investigating how genetic risk may influence neural pathways differently across development.

We investigated the neural pathway through which *GABRA2* genetic variation may influence risk for alcoholism from childhood to young adulthood. We focused on the mesolimbic dopamine system, which is centrally involved in the reinforcing properties of drugs of abuse (Robinson and Berridge, 2000), undergoes change during adolescence (Luciana *et al*, 2012; Spear, 2011), and has been implicated in vulnerability to addiction (McBride and Li, 1998; Volkow *et al*, 2002). The nucleus accumbens (NAcc) is of particular importance; it contributes to directing behavior by integrating affective, contextual, and goal-directed information from the limbic system and prefrontal cortex (Grace *et al*, 2007). Inhibitory regulation of NAcc dopaminergic function comes from GABAergic influences (Steffensen *et al*, 1998). The *GABRA2* gene is involved in encoding the α2 subunit of the γ-aminobutyric acid A receptor (GABA$_A$), which is among the predominant receptor subtypes expressed in the NAcc (Schwarzer *et al*, 2001). *GABRA2* gene deletion in a mouse model was found to reduce GABA$_A$-mediated electrophysiological responses in the NAcc (Dixon *et al*, 2010). Therefore, although the specific functional effects of *GABRA2* variation are not understood (Tian *et al*, 2005), one possible neural mechanism through which *GABRA2* may affect vulnerability to AUD is via this incentive-motivation system.

We investigated the developmental trajectory of NAcc function from childhood to young adulthood, the impact of *GABRA2* on NAcc functioning, and whether *GABRA2* effects change over the course of development. A monetary incentive delay (MID) task was used to probe NAcc functioning during the anticipation of monetary rewards in a high-risk sample. We focused on incentive anticipation owing to the evidence that NAcc dopamine functioning is specifically related to anticipatory, appetitive, or preparatory aspects of motivated behavior rather than consummatory aspects (Salamone and Correa, 2012). Functional MRI during the MID was collected at least once in 175 participants, with the majority ($n = 151$) undergoing repeated scanning at 1- to 2-year intervals. The present report is based on 473 scans covering ages 8–27 years. We investigated SNP rs279858; the minor (G) allele, a tag allele for the less frequent *GABRA2* haplotype, has been associated with adult alcohol dependence (Covault *et al*, 2004) as well as adolescent externalizing problems (Dick *et al*, 2009) and impulsiveness (Villafuerte *et al*, 2012). Based on evidence of a positive relationship between NAcc activation to anticipation of reward during the MID and externalizing problems (Yau *et al*, 2012), impulsivity (Hahn *et al*, 2009) and

sensation-seeking (Bjork *et al*, 2008a), we hypothesized that G allele carriers would have heightened NAcc response. Furthermore, because adolescence is a time of increased reward-seeking and risk-taking, and heightened activity in the incentive-motivation system is proposed to be an important contributing factor to this (Casey and Jones, 2010; Galvan, 2010; Luciana *et al*, 2012), we expected that this effect would be most prominent during adolescence. Finally, we hypothesized that heightened NAcc response would mediate a relationship between genotype and problem drinking.

MATERIALS AND METHODS

Participants

At study onset, participants were 76 children/early adolescents aged 8–13 years and 99 late adolescents/young adults aged 18–23 years. The majority ($n = 151$) underwent more than one scan (two to four scans; Table 1). All participants were drawn from the Michigan Longitudinal Study, an ongoing study of families with high levels of parental AUD along with a contrast sample of families without parental AUD (details in Supplementary Materials). Seventy-nine percent of participants ($n = 139$) had at least one parent with an AUD diagnosis (FH +).

Exclusionary criteria included: left-handed or ambidextrous; treatment with centrally active medications within past 6 months; history of psychosis or schizophrenia in first-degree relatives. The presence of most Axis I psychiatric or developmental disorders was exclusionary with the exception of CD, attention deficit/hyperactivity disorder, or substance use disorder (SUD); CD and attention deficit/hyperactivity disorder lie on a developmental spectrum with AUD/SUD and therefore elimination of these participants would eliminate part of the phenomenon of interest. Axis I disorders were assessed by a clinical psychologist based on DSM-IV criteria with the Diagnostic Interview Schedule-Version 4 (Robins *et al*, 2000) at ages 18 + years and the Diagnostic Interview Schedule-Child-Version 4 (Shaffer *et al*, 2000) at younger ages.

All participants were told to abstain from alcohol and illicit substances/recreational drugs for 48 h before scanning. In participants aged 15 years and older, urine drug screens were conducted immediately before the scan; positive results were exclusionary. In participants aged 14 years and younger, we relied on verbal confirmation of drug and alcohol abstinence on the day of the scan. Pregnancy

Table 1 Ages and Number of Scans by Age Group

	Child/early adolescent			Late adolescent/young adult			Total
	Age (years)			**Age (years)**			
	N	**Range**	**Mean (SD)**	**N**	**Range**	**Mean (SD)**	**N**
Scan 1 (baseline)	76	8.1–13.0	10.8 (1.2)	99	18.0–23.0	20.3 (1.4)	175
Scan 2 (T2)	59	10.5–15.1	12.7 (1.0)	92	20.0–25.0	22.5 (1.4)	151
Scan 3 (T3)	33	12.5–16.7	14.7 (1.1)	73	21.1–26.9	24.2 (1.4)	106
Scan 4 (T4)	8	14.2–17.6	15.8 (1.1)	33	22.9–27.1	25.2 (1.2)	41
Total scans	176	8.1–17.6	12.4 (2.0)	297	18.0–27.1	22.5 (2.2)	473

was exclusionary: all female participants were asked if they might be pregnant as part of the standard screening process for MRI. Females aged 15 years and older underwent a urine pregnancy test immediately before the scan; for younger females, we relied on verbal report.

After description of the study to the subjects, written informed consent, approved by the University of Michigan Medical School Institutional Review Board, was obtained. For those under age 18 years, written assent was obtained from the participant and written informed consent was obtained from at least one parent.

Genotyping

We selected SNP rs279858 (exon 5, K132K) from 11 SNPs in linkage disequilibrium that capture the information of a long haplotype block of 109 kb, where previous associations have been reported with alcohol dependence and related traits. For SNP rs279858, TaqMan genotyping was performed using inventoried assays of primers and probes (Applied Biosystems, ABI, Foster City, CA). Twelve duplicates were included and no discrepancies were observed. See Supplementary Material for further details on genotyping methods. Frequency of the minor (G) allele in this sample was 42%; this did not differ significantly across the age groups ($\chi^2 = 2.8$, $p = 0.235$).

The samples were also genotyped for 150 ancestry informative markers (Hodgkinson *et al*, 2008), from which individual ethnic factor scores were computed (Supplementary Materials). The four ethnic factor scores explaining the highest variance (Table 2) were included as covariates in all genetic analyses to control population stratification.

Substance Use

Between ages 6 and 10 years, alcohol and drug use was assessed at 3-year intervals. Beginning at age 11 years, the Drinking and Drug History Form (Zucker *et al*, 1990) was filled out by participants annually. Details of substance use assessment are given in Supplementary Material. Annual drinking data (amount and frequency) are summed across all time points to calculate cumulative lifetime drinking volume (CDV). Participants are also asked about consequences and problems related to alcohol use (see Supplementary Table S1). The number of different drinking-related problems ever reported by the subject is cumulated by age for an annual cumulated alcohol problem measure.

Functional MRI

fMRI paradigm. Brain response during anticipation of incentive stimuli was probed using a modified MID task, as described previously (Yau *et al*, 2012; Supplementary Materials).

fMRI acquisition and data analysis. Whole-brain blood oxygen level-dependent (BOLD) functional images were acquired on a 3.0 Tesla GE Signa scanner (Milwaukee, WI) using a T2*-weighted single-shot combined spiral in/out sequence with the following imaging parameters: repetition time = 2000 ms, echo time = 30 ms, flip angle = 90°; field-of-view = 200 mm; matrix size = 64 × 64; in plane resolution =

$3.12 \times 3.12 \text{ mm}^2$; and slice thickness = 4 mm. A high-resolution anatomical T1 scan was obtained for spatial normalization (three-dimensional spoiled gradient recalled echo, repetition time = 25 ms, min echo time, field-of-view = 25 cm, 256 × 256 matrix, slice thickness = 1.4 mm).

Functional images were reconstructed using an iterative algorithm (Fessler *et al*, 2005). Head motion was corrected using the FSL 5.0.2.2 analysis tools library (Analysis Group, FMRIB, Oxford, UK), slice-acquisition timing was corrected using SPM8 (Wellcome Institute of Cognitive Neurology, London, UK). Runs exceeding 3 mm translation or 3° rotation in any direction were excluded (younger group— baseline: 8.0%; T2: 1.9%; T3&T4: 0%; older group— baseline: 1.4%; T2: 1.5%; T3: 0.7%; T4: 1.8%). Remaining image processing was completed using SPM8 (Wellcome Institute of Cognitive Neurology, London, UK). Functional images were spatially normalized to a standard stereotactic space as defined by the Montreal Neurological Institute. A 6-mm full-width half-maximum Gaussian spatial smoothing kernel was applied to improve signal-to-noise ratio and account for individual differences in anatomy.

Individual analysis was completed using a general linear model. Regressors of interest for all events (cues: win $0.20, win $5.0, lose $0.20, lose $5.0 and neutral $0; anticipation delay for each cue type; positive outcome for each cue type; negative outcome for each cue type) were included and convolved with the canonical hemodynamic response function. Motion parameters and white matter signal intensity were modeled as nuisance regressors to remove residual motion artifacts and capture non-task-related noise, respectively. Scanner drift and other low-frequency noise were removed from the image time series using a 128-s high-pass filter.

Owing to our interest in incentive anticipation, the present report focuses on the contrast between anticipation during monetary gain trials *vs* neutral trials (we refer to this contrast in the remaining text as 'reward anticipation'). To avoid circularity of statistical inference, which can occur by defining volumes of interest (VOI) based on observed contrast activation, and given an *a priori* interest in the NAcc, anatomical masks of NAcc were created as described previously (Bjork *et al*, 2008b; Yau *et al*, 2012; Supplementary Materials) and analyses were restricted to this VOI. Left and right NAcc VOI effect sizes were calculated from parameter estimates using MarsBaR region of interest toolbox (Brett *et al*, 2002) for each incentive amount ($0.20 and $5) compared with neutral.

Effects of hemisphere and incentive amount were investigated using repeated-measures ANOVA (Supplementary Materials), revealing no main effects of, or interactions with hemisphere; therefore, NAcc VOI effect sizes for $0.20 and $5 were linearly combined and averaged across hemispheres.

Intraclass correlation coefficient was calculated to test reliability of NAcc activation across sessions and was found to be acceptable (intraclass correlation coefficient = 0.597; see Supplementary Materials).

Statistical Analysis

Linear mixed-effects models (LMMs). To determine the pattern of NAcc activation across the age-range, a LMM was run in SPSS. LMMs account for correlation between repeated measurements within subjects while also allowing

Table 2 Subject Characteristics by Genotype

GABRA2 genotype	A/A	A/G	G/G	*P*-value[a]
N—Total	55	95	25	
Child/early adolescent	30	38	8	
Late adolescent/young adult	25	57	17	
# Scans—Total	151	249	73	
Child/early adolescent	71	89	16	
Late adolescent/young adult	80	160	57	
Age (years)[b]	18.2 ± 5.7	18.9 ± 5.3	19.7 ± 4.5	0.27
Age range (years)	8.1–26.9	8.5–27.0	9.4–26.4	
Male (%)	60.0	68.4	80.0	0.20
FH + (%)	76.4	82.1	76.0	0.63
IQ	105 ± 14	108 ± 13	108 ± 11	0.53
Ethnic factor score				
Europe[b]	0.74 ± 0.33	0.81 ± 0.26	0.73 ± 0.35	0.15
median	0.918	0.948	0.938	
Africa[b]	0.12 ± 0.27	0.05 ± 0.17	0.03 ± 0.10	0.04
median	0.002	0.000	0.001	
Middle-East[b]	0.05 ± 0.08	0.05 ± 0.10	0.10 ± 0.19	0.10
median	0.025	0.013	0.013	
Asia[b]	0.04 ± 0.09	0.06 ± 0.12	0.10 ± 0.17	0.27
median	0.012	0.013	0.017	
Lifetime depression Dx (%)	7.3	12.6	4.0	0.52
Lifetime GAD Dx (%)	3.6	4.2	0.0	0.58
Lifetime CD or ADD Dx (%)	27.3	25.3	20.0	0.70
Alcohol/drug use and diagnosis: baseline				
Child/early adolescent				
Current tobacco user (%)	0	0	0	NA
# Illicit drugs used[b]	0.07 ± 0.38	0.03 ± 0.17	0	0.72
Binge drinking-days/year	0	0	0	NA
Drink volume-past year[b]	0.64 ± 2.50	0.33 ± 2.00	0	0.73
Lifetime AUD Dx (%)	0	0	0	NA
Lifetime SUD Dx (%)	0	0	0	NA
Late adolescent/young adult				
Current tobacco user (%)	30.8	21.1	27.8	0.57
# Illicit drugs used[b]	1.83 ± 2.24	1.24 ± 1.72	1.00 ± 2.03	0.32
Binge drinking-days/year[b]	50 ± 80	51 ± 76	47 ± 91	0.91
Drink volume-past year[b]	276 ± 451	323 ± 688	241 ± 382	0.62
Lifetime AUD Dx (%)	28.0	14.0	11.1	0.25
Lifetime SUD Dx (%)	36.0	17.5	17.6	0.16
AP (⩾ 3 years post baseline)[b,c]	0.68 ± 1.45	1.81 ± 2.90	2.00 ± 2.47	0.07[d]

Abbreviations: ADD, attention deficit disorder; AP, alcohol problems; AUD, alcohol use disorder; CD, conduct disorder; Dx, diagnosis; FH +, one or both parents have a lifetime diagnosis of alcohol abuse or dependence; GAD, generalized anxiety disorder; NA, not applicable due to zero values; SUD, substance use disorder.
[a]*P*-values represent the results of Pearson χ^2 or ANOVA tests.
[b]Data presented as mean ± standard deviation.
[c]The number of alcohol problems reported over at least 3 years following baseline (range: 3–6 years; mean: 4 years). These data were available in *n* = 104 (AA *n* = 30; AG *n* = 56; GG *n* = 18).
[d]*p* = 0.04 when linear relationship is tested using Pearson correlation with # of minor (G) alleles.

for an unequal number of repeated measurements across subjects (Gueorguieva and Krystal, 2004). The dependent variable was NAcc activation during reward anticipation, as defined above. Time point (baseline, T2, T3, T4) was included as a repeated effect, subject as a random factor, and age-group, linear age, and quadratic age were included

simultaneously as fixed-effect covariates. Additional factors that may impact the developmental trajectory of NAcc activation were also explored (gender, FH, CDV). Schwarz's Bayesian information criteria (Schwarz, 1978) were used to find best-fitting variance-covariance structure; determined to be scaled-identity.

To test the impact of *GABRA2* on the developmental trajectory of NAcc activation, genotype was added as a fixed factor; main effects and genotype by age interactions were investigated.

Repeated-measures analysis. To confirm findings from the LMMs, follow-up analyses were conducted in a subset from each age-group to investigate within-subject effects. In the younger group, individuals had to have a baseline scan conducted before age 13 years and a follow-up scan at 13 years or older ($n = 43$). In the older group, individuals had to have a baseline scan conducted before age 21 years and a follow-up scan at 21 years or older ($n = 58$). Repeated-measure ANCOVAs, with time (baseline, follow-up) as a within-subject factor were used to determine within-subject developmental effects for each group. To test the impact of *GABRA2*, genotype was added as a between-subject factor.

Relationship with alcohol problems. To investigate the hypothesis that *GABRA2* genetic variation is related to problem drinking through its effects on NAcc responding to incentive stimuli, a bias-corrected bootstrapped indirect effect analysis was conducted using an SPSS macro (Preacher *et al*, 2007). *GABRA2* genotype was the independent variable, NAcc activation at baseline was the mediator, and the number of alcohol problems reported over at least 3 years following baseline (AP; range: 3–6 years; mean: 4 years) was the dependent variable. AP was available for 104 participants (see Table 2). Gender, FH, and CDV at baseline were included as covariates. Bootstrapping (10 000 resamples) was performed to determine bias-corrected 95% confidence intervals.

For all genetic analyses, genotype was coded as number of minor alleles (AA = 0, AG = 1, GG = 2), which makes the fewest assumptions and is consistent with literature reporting a dose-dependent allelic effect (Covault *et al*, 2004; Fehr *et al*, 2006), and ethnic factor scores were included as covariates. Drinking variables (CDV and AP) were square-root transformed because of non-normal distribution of raw values.

Supplementary analyses. All analyses were conducted in Caucasians only and no major divergences from the main findings were noted; although *p*-values were reduced, effects trended in the same direction. Analyses were also conducted excluding participants with CD or ADD diagnoses; results indicate these participants were not driving the findings. Further analyses were conducted to explore differences based on FH and gender. Finally, supplementary analyses investigated age-group by linear age interactions to verify that the developmental trajectory identified was not an artifact of investigating age effects across two non-overlapping age-groups. All supplementary analyses are reported completely in Supplementary Material.

RESULTS

Subject Characteristics and Task Performance

There were no genotype differences in gender, age, FH, substance use, or diagnosis of psychopathology (Table 2). In the older group, there was no significant effect of genotype on lifetime diagnosis of AUD/SUD.

Effect of incentive amount on performance is reported in Supplementary Materials. LMMs including age, age^2, genotype, age × genotype, and age^2 × genotype revealed no main effects of, or interactions with, genotype on accuracy to combined reward ($0.20 and $5) compared with neutral trials (p's > 0.14) as expected, given the task is individually calibrated to target 60% accuracy. There were also no genotype effects on accuracy to reward or neutral trials when investigated separately (p's > 0.40).

There were no main effects or interactions of genotype on reaction time during reward trials compared with neutral trials (p's > 0.50). However, when reaction time was investigated for each incentive condition separately, there was a significant effect of genotype during reward trials ($F_{272,2} = 4.8$, $p = 0.009$) but not during neutral trials ($F_{289,2} = 2.4$, $p = 0.092$); G homozygotes responded faster than A allele carriers during reward trials, with a trend for faster responding during neutral trials.

Developmental Effect: LMMs

Both age groups activated the NAcc during reward anticipation (Supplementary Materials). The LMM revealed a significant quadratic effect of age on NAcc activation during reward anticipation ($F_{453,1} = 9.1$, $p = 0.003$) across all 473 scans. Age-group and linear age terms were not significant ($p > 0.4$). Activation of the NAcc to reward anticipation from childhood to early adulthood followed an inverted U-shaped pattern (Figure 1a). This was further supported by exploratory linear regression showing age^2 significantly improved model fit above age-group and linear age (Supplementary Materials). When gender and FH were included, the quadratic age term remained significant (age^2: $F_{452,1} = 9.4$, $p = 0.002$); there were no other significant variables (p's > 0.4). Likewise, the addition of CDV as a covariate did not significantly impact the results (age^2: $F_{456,1} = 9.7$, $p = 0.002$; all other p's > 0.4).

Developmental Effect: Within Subjects

Repeated-measures ANCOVAs found effects of time: in the younger group, activation of the NAcc increased from childhood to adolescence in the same subjects ($F_{42,1} = 7.0$, $p = 0.011$; Figure 2a); in the older group, activation of the NAcc decreased from later adolescence to young adulthood in the same subjects ($F_{57,1} = 8.4$, $p = 0.005$; Figure 1b).

Interaction between Development and *GABRA2*: LMMs

There was a main effect of rs279858 on NAcc activation during reward anticipation (GG > AG&AA; $F_{266,2} = 5.1$, $p = 0.007$), a main effect of age^2 ($F_{460,1} = 10.8$, $p = 0.001$) and an age^2 by genotype interaction ($F_{454,2} = 3.0$, $p = 0.050$; Figure 2a) across all 473 scans. Age-group and linear age were not significant ($p > 0.6$). When gender and FH were
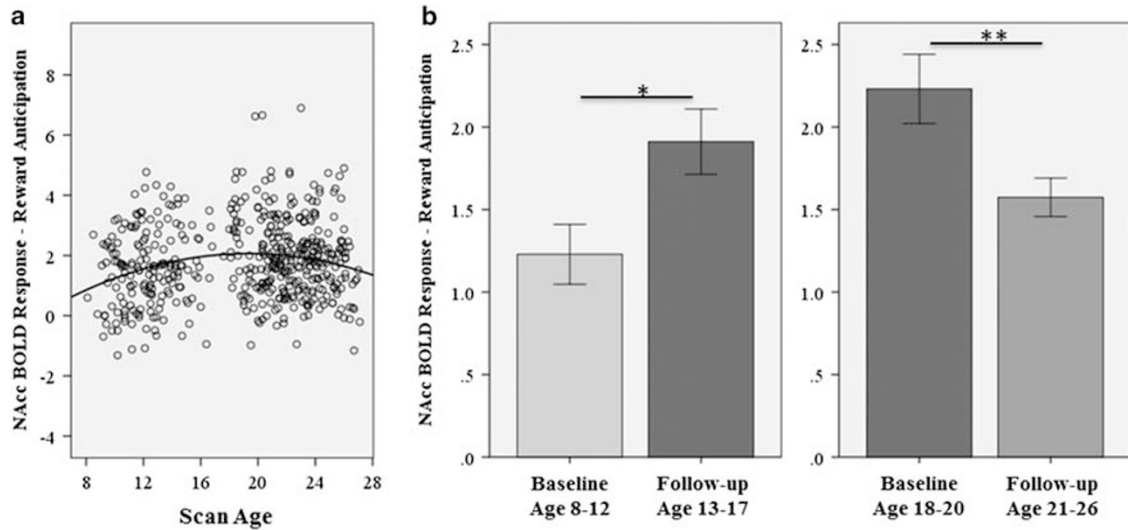
Figure 1 Illustration of significant quadratic age effect on Nacc BOLD activation to reward anticipation. (a) Across all scans covering ages 8–27 years. Note: this figure includes repeated-measures within individuals as described in Table 1 (473 measures on 175 individuals). (b) Within-subject time effect on NAcc BOLD activation to reward anticipation in the child/young adolescent group (left), with baseline scan in childhood and a follow-up scan in early adolescence ($n = 43$); and the late adolescent/young adult group (right), with baseline scan in late adolescence and follow-up scan in young adulthood ($n = 58$). Error bars represent 1 standard error, $*p < 0.05$, $**p < 0.01$.
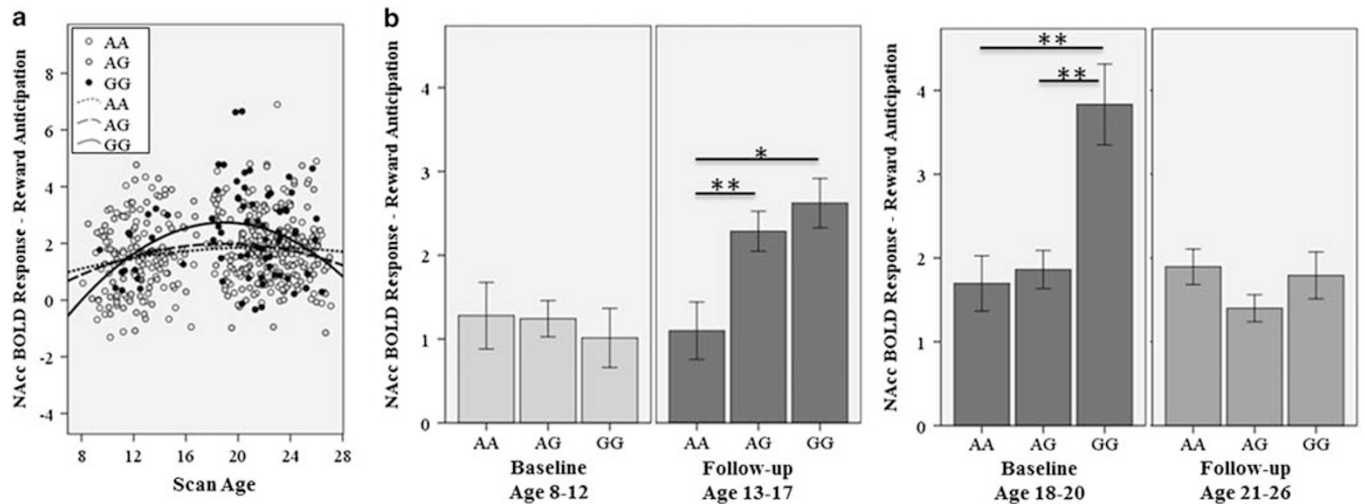

Figure 2 Age by genotype interaction on NAcc BOLD activation to reward anticipation. (a) Age-squared by genotype interaction across the ages 8–27 years. Note: this figure includes repeated measures within individuals as described in Table 1 (473 measures on 175 individuals). (b) Within-subject time by genotype interaction in the child/early adolescent group (left), with baseline scan in childhood and a follow-up scan in adolescence ($n = 43$; AA $n = 15$; AG $n = 23$; GG $n = 5$); and the late adolescent/young adult group (right), with baseline scan in late adolescence and follow-up scan in young adulthood ($n = 58$; AA $n = 14$; AG $n = 31$; GG $n = 13$). Error bars represent 1 standard error, $*p < 0.05$, $**p < 0.01$.

included as covariates, the main effects remained significant (genotype: $F_{265,2} = 5.2$, $p = 0.006$; age^2: $F_{458,1} = 10.8$, $p = 0.001$); genotype $\times age^2$ was reduced to trend-level significance ($F_{452,2} = 2.7$, $p = 0.056$). There were no other significant variables in the model (p's > 0.4). The inclusion of CDV as a covariate did not impact the main effects (genotype: $F_{264,2} = 5.5$, $p = 0.005$; age^2: $F_{457,1} = 11.5$, $p = 0.001$) but further reduced the significance-level of the genotype $\times age^2$ interaction ($F_{451,2} = 2.5$, $p = 0.087$). *Post-hoc* analyses, including all covariates, investigated age effects for each genotype separately; there were significant quadratic effects of age in G allele carriers (GG: $F_{61,1} = 5.5$,

$p = 0.022$; AG: $F_{233,1} = 9.8$, $p = 0.002$) but not A homozygotes ($F_{140,1} = 0.1$, $p = 0.908$).

Interaction between Development and *GABRA2*: Within Subjects

Repeated-measures ANCOVA in the younger group ($n = 43$) including all covariates found no main effects of time ($F_{33,1} = 1.7$, $p = 0.207$) or genotype ($F_{33,2} = 2.8$, $p = 0.077$) but a genotype by time interaction approached significance ($F_{33,2} = 3.1$, $p = 0.057$). *Post-hoc* ANCOVAs investigating genetic effects for
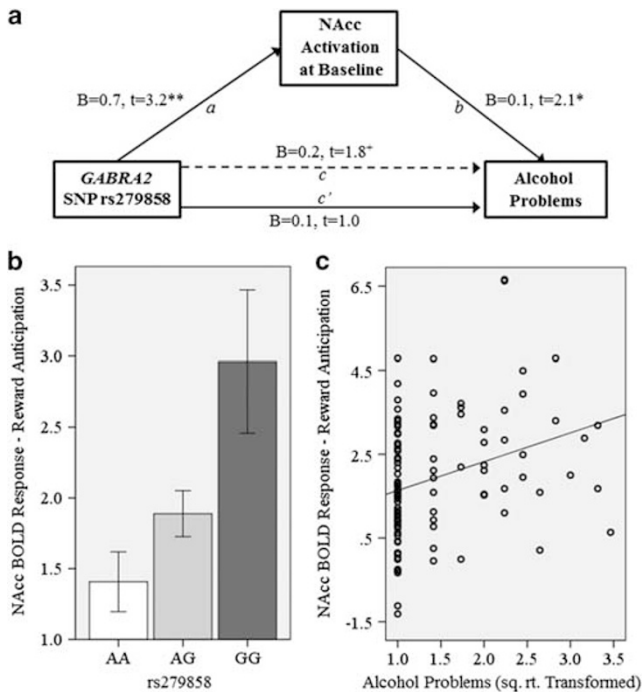
Figure 3 (a) Indirect effect model. Number of minor (G) alleles is independent variable. Nucleus accumbens (NAcc) activation at baseline scan is the mediator. Number of alcohol problems reported over 3–6 years following baseline (average = 4 years) is the dependent variable. The model included the following covariates: gender, family history of alcoholism, four ethnic factors, and cumulative lifetime drinking volume at time of baseline scan. Unstandardized coefficients (b) are given. (b) Illustration of *a* path effect demonstrating increasing NAcc activation with increasing number of G alleles. (c) Illustration of *b* path with increased NAcc activation at baseline associated with increased number of alcohol problems in the following 3–6 years. $^+p < 0.10$, $^*p < 0.05$, $^{**}p < 0.001$.

each time point separately found no effect during childhood (baseline: $F_{33,2} = 0.0$, $p = 0.966$), but a significant genotype effect during adolescence (follow-up: GG&AG > AA; $F_{33,2} = 5.4$, $p = 0.009$) in the same subjects (Figure 2b).

In the older group ($n = 58$), there was a main effect of genotype ($F_{48,2} = 10.0$, $p < 0.001$) and a genotype by time interaction ($F_{48,2} = 6.1$, $p = 0.004$). There was no main effect of time ($F_{48,1} = 0.8$, $p = 0.366$). *Post-hoc* ANCOVAs found a significant effect of genotype during later adolescence (baseline: GG > AG&AA; $F_{48,2} = 10.8$, $p < 0.001$), but not young adulthood (follow-up: $F_{48,2} = 1.0$, $p = 0.372$) in the same subjects (Figure 2b).

Indirect Effect of *GABRA2* on Alcohol Problems via NAcc

Bivariate correlations among the main variables included in the model were significant (rs279858 and NAcc: $r = 0.33$, $p < 0.001$; NAcc and AP: $r = 0.27$, $p = 0.006$; rs279858 and AP: $r = 0.20$, $p = 0.047$). Figure 3 illustrates the results of the indirect model. There was a significant relationship between rs279858 and NAcc activation (*a* path; illustrated in Figure 3b), and a positive relationship between NAcc activation and later alcohol problems (*b* path; illustrated in Figure 3c). The total model was significant ($R^2 = 0.15$, F = 2.3, $p = 0.031$). Bootstrap ($n = 10\,000$) bias-corrected and accelerated 95% confidence intervals (lower limit = 0.007, upper

limit = 0.178) indicated a significant indirect effect of rs279858 on later alcohol problems via NAcc activation during reward anticipation. Confidence intervals remained significant when age at baseline was added to the model as a covariate (lower limit = 0.002, upper limit = 0.167).

DISCUSSION

The goal of this work was to investigate a neural mechanism through which *GABRA2* genetic variation may influence risk for alcoholism and the possible developmental variability in its operation. We found that *GABRA2* genotype is associated with individual differences in NAcc activation during incentive anticipation specifically during adolescence. This was demonstrated across the entire age range as a genotype by age-squared interaction, and also within-subjects as a genotype by time interaction, with an effect of genotype only during adolescence in both age groups. Furthermore, NAcc activation was found to mediate the relationship between genotype and later alcohol problems. These associations are not driven by subjects with ADD or CD diagnoses, as the results are similar when these individuals are excluded.

The G allele of *GABRA2* SNP rs279858 has been associated with adult alcohol dependence (Covault *et al*, 2004), elevated externalizing problems throughout adolescence (Dick *et al*, 2009), impulsiveness (Villafuerte *et al*, 2012), and increased insula activation during incentive anticipation (Villafuerte *et al*, 2012). We show that activation of the NAcc to incentive stimuli is heightened in G carriers during adolescence. Previous studies have reported relationships between increased NAcc activation during reward anticipation and behavioral traits related to vulnerability to addictive disorders, such as impulsiveness (Forbes *et al*, 2009; Hahn *et al*, 2009), sensation-seeking (Bjork *et al*, 2008a), and externalizing behaviors (Yau *et al*, 2012). Consistent with this, we observed a relationship between heightened activation of the NAcc and later alcohol problems. Furthermore, the present study provides an additional link, demonstrating a genetic influence on these relationships. Specifically, *GABRA2* genotype had an indirect effect on alcohol problems via hyperactivation of the NAcc, extending the current knowledge regarding the neural pathway through which *GABRA2* may affect risk for alcoholism.

GABRA2 gene deletion in mice has been shown to reduce electrophysiological responses mediated by GABA_A receptors in the NAcc, abolish behavioral sensitization to cocaine and cocaine's ability to facilitate lever-pressing for a conditioned reinforcer (Dixon *et al*, 2010), and abolish cue-associated responding when no cocaine was available (Dixon *et al*, 2014). This supports a role of *GABRA2* genetic variation in individual differences in incentive motivation, with the NAcc as a critical neural mediator of this effect. Importantly, *GABRA2* gene deletion did not impact alcohol self-administration (Dixon *et al*, 2012), cocaine self-administration, or reinstatement of cocaine-seeking after extinction (Dixon *et al*, 2014), suggesting that the mechanism of this effect does not involve the signaling of drug reward. Rather, Dixon and colleagues conclude that manipulation of the GABA_A α2 subunit has the most consistent impact on the expression of cue-associated behavior—or the

'energizing' aspects of reward-seeking (Dixon *et al*, 2014). The present work is consistent with this, showing that *GABRA2* genetic variation is associated with individual differences in NAcc response during the anticipatory phase of the MID, when participants are preparing to respond for potential reward; specifically, G homozygotes showed heightened NAcc activation compared with A allele carriers during reward anticipation and also had faster responses to reward trials. This is also consistent with evidence that NAcc dopamine is involved in anticipatory, appetitive, or preparatory aspects of motivation, including behavioral activation, approach behavior, and sustained task engagement, as opposed to consummatory aspects (Salamone and Correa, 2012). Therefore, a possible mechanism through which heightened NAcc response to incentives may confer risk for problem drinking is via enhancement of behavioral activation aspects of motivation toward alcohol cues.

An important aspect of this work is the demonstration of developmental effects in NAcc anticipatory response. An inverted U-shaped curve was found to describe the developmental trajectory of NAcc response across the entire age range. This pattern held when lifetime drinking volume was added to the model as a covariate and therefore did not appear to be driven by the amount of substance use in the older group. Furthermore, the pattern was supported within-subjects in each age group. Adolescence is a time of increased reward-seeking and risk-taking, with heightened activity in the incentive-motivation system proposed to be an important contributing factor to this (Casey and Jones, 2010; Galvan, 2010; Luciana *et al*, 2012). Cross-sectional studies using fMRI in conjunction with a variety of reward paradigms have provided support for this view (Ernst *et al*, 2005; Galvan *et al*, 2006; Van Leijenhorst *et al*, 2010). However, others have reported hyporeactivity to reward in adolescents (Bjork *et al*, 2004; Bjork *et al*, 2010; Geier *et al*, 2010). A number of excellent reviews have described possible explanations for these discrepancies, including differences in task design, phases of reward processing, and definitions of adolescence (Galvan, 2010; Spear, 2011). Therefore, we limit this discussion to studies that investigated the anticipation phase during an MID task. In a group comparison between adolescents aged 12–17 years ($n=24$) and adults aged 22–42 years ($n=24$), reduced activation of the NAcc during anticipation of reward was found in adolescents (Bjork *et al*, 2010). However, a comparison of 10- to 17-year olds ($n=24$) with 22- to 48-year olds ($n=30$) found reduced activation in the putamen, but not the ventral striatum (Cho *et al*, 2012). Differences across these reports and the present work may be due in part to definition of age groups, which could introduce confounds (Galvan, 2010; Luna *et al*, 2010). Importantly, the use of distinct age groups separated by 4 years does not allow for a quadratic function of age to be modeled. Indeed, when restricting our data to a group comparison between ages 12–17 and ages 22–27 years in an exploratory analysis, we observed a trend for less activation in the 12- to 17-year-old group ($p=0.12$; data not shown). In contrast, a study of 10- 25-year olds ($n=39$) modeled linear and quadratic age effects across subjects as opposed to using age groups. They found a positive linear relationship between age and ventral striatal activation to anticipation of reward and, in contrast to the present report, the quadratic term did not signifi-

cantly contribute beyond the linear term (Hoogendam *et al*, 2013). It is possible that the more thorough interrogation of the developmental continuum reported on here—with a broader age range (8–27), larger sample ($n=175$), and repeated measures (473 sessions)—is necessary to reveal the inverted U-shaped trajectory of activation to reward anticipation during the MID. Alternatively, given that this was a primarily high-risk sample, the observed trajectory may specifically reflect developmental patterns in those with heightened risk conferred by environmental and genetic influences attributable to a family history of alcoholism. Indeed, supplementary analyses found a significant effect of quadratic age in participants with a family history of alcoholism (79% of sample) but not in those without (presented in Supplementary Material); however, this may be due to the smaller sample size in the latter group. Large-scale imaging studies including repeated-measures in representative samples are required to determine if normative development follows this same trajectory.

Most importantly, age was found to interact with *GABRA2* genotype, resulting in different trajectories of NAcc activation to incentive stimuli; G allele carriers showed an inverted U-shaped developmental curve, whereas AA homozygotes showed no evidence of age-related changes. This is a critical finding; it demonstrates a specific genetic influence on adolescent development as it relates to individual differences in the evolution of risky behavior. Despite the vast literature characterizing adolescence as a period of heightened reward-seeking and risk-taking, not all teens demonstrate these behavioral tendencies and it is important to understand not only group-based profiles, but also individual differences in behavior. The present work suggests that genetic variation in *GABRA2* affects individual differences in adolescent risk-taking via effects on the dopaminergic inventive-motivation system. However, to date, the specific functional effects of *GABRA2* allelic variation have not been identified. Further research is necessary to understand the mechanism through which *GABRA2* variation impacts the functioning of this system throughout development.

Some potential caveats about this work should be noted. First, as noted above, this was a primarily high-risk sample, limiting the generalizability of the findings. Second, although supplementary analyses did not reveal any significant effects of gender, findings were more robust in males than females. A larger sample of female participants is necessary to determine whether there are gender differences in *GABRA2* effects on NAcc responding to incentive stimuli across development. Data collection in this sample is ongoing and this will be an important focus of future work. Third, it should be noted that there is a large amount of variation in BOLD activation even within genotype groups. There are numerous potential sources of variation not accounted for in the current study, including environmental influences such as history of stressful events and past experiences with reward, which can impact functioning of the mesolimbic dopamine system (Pitchers *et al*, 2010). Furthermore, constraining influences from prefrontal regions likely introduce an additional source of variation in the development and functioning of this system. Future work investigating the impact of *GABRA2* genetic variation on the interaction between NAcc and prefrontal cortex

functioning from childhood to adulthood is necessary for a fuller understanding of these relationships. Finally, the developmental trajectory described here was extrapolated from longitudinal data collected in two groups with no age overlap. Although the supplementary analyses investigating age-group by linear age interactions suggest that the developmental trajectory identified was not an artifact of investigating age effects across two non-overlapping age groups, future work is necessary to confirm these findings using longitudinal measures collected from childhood to young adulthood within the same subjects.

We found that GABRA2 affects individual differences in incentive responding during a critical developmental period, leading indirectly to problem drinking. These findings represent an important step toward understanding individual differences in how risk for substance abuse unfolds across development. Understanding individual differences in risk mechanisms may allow a better understanding of differential responsiveness to preventative interventions, leading to more individualized targeting. Furthermore, timing is central to prevention in terms of choosing when to begin intervention, and also determining the specific mechanisms to target. The present work suggests that incentive motivation is a promising target specifically during adolescence for some individuals. Further research is indicated to investigate whether heightened incentive sensitivity is a malleable target for intervention during the adolescent period.

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