

# Rule breaking mediates the developmental association between *GABRA2* and adolescent substance abuse

Elisa M. Trucco,<sup>1</sup> Sandra Villafuerte,<sup>2</sup> Mary M. Heitzeg,<sup>1</sup> Margit Burmeister,<sup>2</sup> and Robert A. Zucker<sup>1</sup>

<sup>1</sup>Addiction Research Center, University of Michigan, Ann Arbor, MI, USA; <sup>2</sup>Molecular & Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, MI, USA

**Background:** This study's primary aim was to examine age-specific associations between *GABRA2*, rule breaking, problematic alcohol use, and substance abuse symptomatology. The secondary aim was to examine the extent to which rule breaking mediates the *GABRA2*-substance abuse relationship. **Methods:** A sample ( $n = 518$ ) of primarily male (70.9%) and White (88.8%) adolescents from the Michigan Longitudinal Study was assessed from ages 11–18. Age-specific effects of *GABRA2* on rule breaking, problematic alcohol use, and substance abuse symptomatology were examined using nested path models. The role of rule breaking as a mediator in the association between *GABRA2* and substance abuse outcomes was tested using prospective cross-lagged path models. **Results:** *GABRA2* is significantly ( $p < 0.05$ ) associated with rule breaking in mid- to late-adolescence, but not substance abuse symptomatology across adolescence. *GABRA2* effects on problematic alcohol use and substance abuse symptomatology operate largely (45.3% and 71.1%, respectively,  $p < 0.05$ ) via rule breaking in midadolescence. **Conclusions:** *GABRA2* represents an early risk factor for an externalizing pathway to the development of problematic alcohol and drug use. **Keywords:** *GABRA2*, rule breaking, substance abuse, adolescence, mediation.

## Introduction

*GABRA2* is a gene encoding the  $\alpha 2$  subunit of the  $\gamma$ -aminobutyric acid A receptor ( $GABA_A$ ). Research identifies allelic variations in *GABRA2* as being associated with alcohol and drug use disorders in adulthood (e.g. Agrawal et al., 2006; Bauer et al., 2007; Edenberg et al., 2004; Enoch, Schwartz, Albaugh, Virkkunen, & Goldman, 2006). A critical next step is to understand mechanistic pathways in the development of these endpoints. Integral to this issue is understanding the mechanisms associated with susceptibility genes and how risk changes across age. *GABRA2* studies are largely cross-sectional and focused on adult samples, without reflecting upon mechanisms of risk (e.g. Agrawal et al., 2006; Edenberg et al., 2004). Studies examining adolescents have not demonstrated consistent effects of *GABRA2* on alcohol and drug use and abuse (Dick et al., 2006; Matthews, Hoffman, Zezza, Stiffler, & Hill, 2007; Sakai et al., 2010). By taking a developmental approach, this paper examines age-specific relations between *GABRA2* and three distinct risk behaviors (rule breaking, problematic alcohol use, substance abuse symptomatology) prospectively across adolescence, and the role of rule breaking as a mediator in the *GABRA2*-substance abuse relationship.

Variants in *GABRA2* have been found to be associated with adult alcohol dependence (e.g. Bauer et al., 2007; Edenberg et al., 2004; Enoch et al., 2006), and extended to drug and nicotine

dependence (Agrawal et al., 2006; Cui, Seneviratne, Gu, & Li, 2012). Yet, efforts to replicate findings among younger samples are equivocal (Dick et al., 2006; Matthews et al., 2007; Sakai et al., 2010). This may be due to the relative effect of genetic influences compared to environmental influences during this age. For example, genes may be less relevant to substance use initiation compared to social contexts such as peers during early adolescence, whereas the extent of genetic influences tends to have a stronger impact on substance use disorders in adulthood (Maes et al., 1999). One study demonstrated that at age 14 genes accounted for 18% of the variance in drinking, compared to nearly 50% at age 18 (Rose, Dick, Viken, Pulkkinen, & Kaprio, 2001). This underscores the importance of prospectively examining developmental differences in the impact of candidate genes on problem behaviors associated with alcohol and drug dependence risk.

Cascade models posit that there is a sequential progression from delinquency in childhood, to later riskier and more problematic behaviors, such as illicit drug use (Dodge et al., 2009). Across childhood and adolescence, delinquency is characterized as rule breaking. During this interval, it reflects normative transgressions to authority figures (e.g. lying, stealing). Research generally finds higher levels of rule breaking behaviors among males compared to females (Lahey et al., 2006). Rule breaking begins during early childhood and declines in adulthood (Shaw, Hyde, & Brennan, 2012), while alcohol use tends to increase throughout adolescence, peaking in early adulthood (Kim-Cohen et al., 2003). Research does not generally support the role of

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problematic alcohol and drug use in childhood as a predictor of rule breaking in adolescence (Windle, 2000). Accordingly, this suggests that rather than focusing on alcohol and drug dependence, there may be utility in examining risk behaviors preceding use in adolescence. Yet, rule breaking is largely overlooked in genetics research. Using a prospective design, a primary aim of this study was to examine age-specific effects of *GABRA2* on rule breaking and substance abuse outcomes.

Exploring genetic pathways to substance abuse may be fruitful since substance abuse often develops gradually and is typically predicted by risk-related behaviors (Dick et al., 2006). One study demonstrated that impulsivity partially mediated the association between *GABRA2* and lifetime alcohol problems in adulthood (Villafuerte, Strumba, Stoltenberg, Zucker, & Burmeister, 2013). There is strong evidence that behavior problems, such as rule breaking, are associated with impulsivity (Burt & Donnellan, 2008). *GABRA2* is also associated with conduct disorder (Dick et al., 2006) reflecting impulsivity and rule breaking behavior; these symptoms in turn, predict rates of later substance dependence (Swendsen et al., 2010). These findings suggest that behaviors associated with impulsivity such as rule breaking may be adolescent manifestations of *GABRA2* effects, which may impact the emergence of problematic substance use. Therefore, our secondary aim was to examine rule breaking as a mediator between *GABRA2* and problematic alcohol use and substance abuse symptomatology using a cross-lagged mediational path model. We are unaware of studies investigating rule breaking pathways from *GABRA2* to substance abuse. It was hypothesized that: (a) those carrying the minor G allele would have higher levels of rule breaking compared to those with the nonrisk allele, (b) *GABRA2* would have weak direct effects on problematic alcohol use and substance abuse symptomatology, (c) rule breaking would mediate the effects of *GABRA2* on problematic alcohol use and substance abuse symptomatology, and (d) problematic alcohol use and substance abuse symptomatology would not predict rule breaking.

## Methods

### Sample

The sample consisted of adolescents from the Michigan Longitudinal Study (MLS), an ongoing, prospective, multiwave study (Zucker, Ellis, Fitzgerald, Bingham, & Sander, 1996). The MLS follows a community sample of high-risk families comprised of men convicted of drunk driving who met criteria for an alcohol use disorder (AUD) diagnosis, their son, and their son's biological mother. A control sample of low-risk families from the same neighborhoods without a substance abuse history was also recruited. Community-identified AUD-diagnosed men and their families were recruited as an intermediate-risk group. Full biological siblings were also included. See Zucker et al. (1996) for a full description of the sample.

### Procedure

Parents and children completed assessments following initial recruitment (Wave 1, ages 3–5) with subsequent assessments occurring every 3 years (e.g. Wave 2, ages 6–8). Children were also assessed annually beginning when the child turned 11. For this study, only self-report measures were examined given that adolescents report more problem behaviors compared to collaterals, and the consensus view is that nonreporting is more of a constraint on validity than manufactured reporting (Moffitt, Caspi, Rutter, & Silva, 2001). Families were asked to provide blood or saliva for genotyping. Written informed consent and assent was obtained from the parents and adolescents. The Institutional Review Board at the University of Michigan approved the study.

This sample included 518 children from 304 families. Approximately one-third (29.9%) of children came from a single-child family, 186 children (35.9%) had another sibling participate, 144 (27.8%) had two siblings participate, and 33 (6.4%) had three or four siblings participate in the study. Participants came from the following alcohol risk categories: low (37.4%), intermediate (26.2%), and high (36.4%). Due to recruitment strategies (females and non-White families included after the initial wave), the sample was predominantly male (70.9%) and White (88.8%). This is important, as delinquency and substance use rates tend to be higher among males. Participants included in analyses did not differ significantly from those without available genetic or annual data ( $n = 135$ ) on sex, race, rule breaking, problematic alcohol use, or substance abuse symptomatology at the larger wave-levels (Waves 4 and 5). Accordingly, missing data likely had minimal impact on the results. Given that we were interested in age-related effects, rather than using wave-level data (3-year span), we calculated values reflecting the maximum value of problem behavior specific to a 2-year span (e.g. 11–12 years of age) to balance the availability of longitudinal data with age specificity.

### Measures

**Rule breaking.** Rule breaking was assessed using the delinquency subscale of the Youth Self Report (YSR; Achenbach, 1991). Items are rated on a 3-point Likert scale (0 = *not true* to 2 = *very true or often true*). Sample items included: *I destroy things belonging to others, I set fires, and I steal from places other than home*. The YSR has been used extensively and had good internal consistency across the larger wave-level assessments (Cronbach's  $\alpha = 0.88$  and  $0.87$  at Wave 4 and 5, respectively).

**Substance use.** To assess substance use outcomes more germane to adolescence, rather than including a binary measure of dependence, we examined multiple markers of risk using the Drinking and Drug History form (Zucker, Fitzgerald, & Noll, 1990). Dimensional outcomes increase reliability and power (Kraemer, Noda, & O'Hara, 2004). Problematic alcohol use was assessed with an item reflecting past year maximum alcoholic beverages consumed in 24 hr. Past year substance abuse symptomatology was assessed with the stem, *Have you ever had any of the following things happen because of your [alcohol] [drug] use?* Participants rated problems related to alcohol use (37-items) and a variety of illicit drugs (22-items) such as marijuana, cocaine, and hallucinogens. Sample items included: *being absent from school, and experiencing physical or medical problems ... because of your alcohol (your drug) use*. The internal consistency for problems related to alcohol use and illicit drugs was good (Cronbach's  $\alpha = 0.99$  across Waves 4 and 5). The combined number of endorsed drinking- and endorsed drug-related problems was summed to create a substance abuse symptomatology

ogy composite, which also had good internal consistency (Cronbach's  $\alpha = 0.98$  across Waves 4 and 5). This questionnaire has been used extensively in a variety of research and clinical settings (e.g. Buu et al., 2012; Nigg et al., 2006).

**Genotyping.** Genetic variation in *GABRA2* is distinguished by numerous single nucleotide polymorphisms (SNPs) that are in linkage disequilibrium (LD), resulting in two common forms of the *GABRA2* gene 'Ying-Yan' haplotypes. This means that it largely does not matter which SNP is analyzed as any will tag the haplotypes. For this study, three SNPs were selected: rs279826, rs279827, and rs279858. All SNPs were in strong LD ( $>0.77$ ) and in a region previously implicated in impulsivity, alcohol, and drug dependence (e.g. Edenberg et al., 2004; Enoch et al., 2006). SNPs rs279826 (intron 4) and rs279858 (exon 5, K132K) were genotyped by Taqman (Villafuerte et al., 2012). SNP rs279827 was included in the Illumina Addiction biology SNP array designed by Hodgkinson et al. (2008) using the Illumina GoldenGate platform (Illumina Inc., San Diego, CA). We included duplicates (78 for the array and 12 for the Taqman assay) and no discrepancies were observed. LD between markers was calculated with Haploview (Barrett, Fry, Maller, & Daly, 2005). Sample demographics by SNP are presented in Table 1. All SNPs were in Hardy-Weinberg equilibrium. Findings focus on SNP rs279826 for simplicity and clarity although results were largely consistent across SNPs. Approximately, half of the sample carried the heterozygous genotype (AG,  $n = 261$ , 50.39%), while a quarter were homozygous for the major allele (AA,  $n = 133$ , 25.68%) and the minor allele (GG,  $n = 124$ , 23.94%). Genotype data were dichotomized (0 = AA, 1 = G-allele carriers) consistent with previous studies (e.g. Pierucci-Lagha et al., 2005) and given that most studies found that the minor (but still common) haplotype increases risk (Bauer et al., 2007; Edenberg et al., 2004; Enoch et al., 2006).

### Data analysis

A series of nested models were run in Mplus version 7.1 (Muthén & Muthén, 1998-2010) to examine age-specific effects of *GABRA2* on rule breaking, problematic alcohol use, and substance abuse symptomatology. Freely estimated paths between *GABRA2* predicting problem behaviors at four different time points (i.e. age 11–12, 13–14, 15–16, and 17–18) were estimated first. A nested model was then evaluated to assess if *GABRA2* equally predicts problem behaviors at different ages by constraining all paths to be equal. The change in chi-square and the Comparative Fit Index (CFI) between the two models was assessed. Research using simulated data demonstrates that a change in CFI greater than  $-0.01$  represents a significant decrement in model fit (Cheung & Rensvold, 2002). In

cases demonstrating a significant change in chi-square and CFI, modification indices were examined to identify which paths should be freed. Once age-specific effects of *GABRA2* were identified, two cross-lagged mediation path models including rule breaking and each substance use outcome at two time points were tested. Sex (0 = females, 1 = males) and race<sup>1</sup> (0 = non-Whites, 1 = Whites) were included as covariates. CFI, Root Mean Square Error of Approximation (RMSEA), and Tucker-Lewis Index (TLI) were used to determine model fit following recommended cutoff values (Hu & Bentler, 1999).

As noted, the sample included siblings from the same nuclear family. Ignoring nested multilevel data structure can bias results. Therefore, an intercept only model estimated family clustering. Results indicated that a significant amount of variance in outcomes was accounted for by family clustering. Thus, multilevel models were estimated.

A prospective design establishes temporal precedence between the mediator and outcome, which is key for testing mediation (Kraemer, Kiernan, Essex, & Kupfer, 2008). In Mplus, it is not possible to control for clustering while using resampling approaches. Therefore, indirect effects when controlling for cluster-effects were compared to bias-corrected bootstrap confidence intervals. Maximum likelihood estimation with robust standard errors was used to accommodate non-normality in substance use outcomes. The full information maximum likelihood estimation was used to handle missing values.

### Results

Table 2 presents descriptive statistics for study variables. Rule breaking, problematic alcohol use, and substance abuse symptomatology significantly ( $p < 0.001$ ) increased over time, except for the interval between rule breaking behavior at age 15–16 to age 17–18. *GABRA2* had few significant associations with other study variables. There was evidence for stability effects and significant associations between rule breaking, problematic alcohol use, and substance abuse symptomatology, except for substance abuse symptomatology at age 11–12.

### Nested model analyses

G-allele carriers had high rates ( $p < 0.01$ ) of rule breaking at ages 13–14 and 15–16 compared to those with the AA genotype. A scaling correction to better approximate chi-square under non-normality

**Table 1** Summary of sample demographics by *GABRA2* SNPs

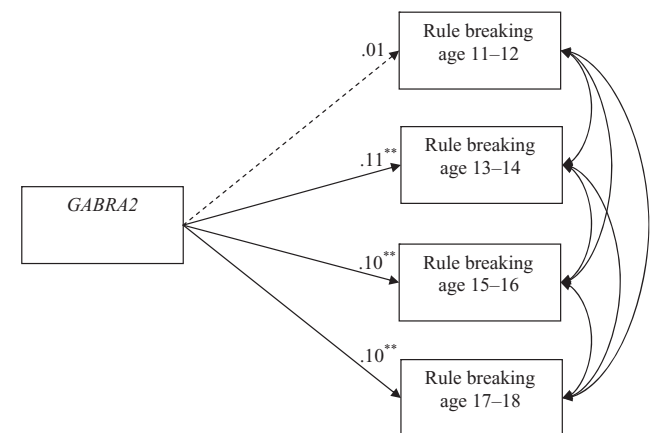
|   | Race $n$ (% within SNP) |             | Sex $n$ (% within SNP) |             |
|---|-------------------------|-------------|------------------------|-------------|
|   | Minority                | White       | Female                 | Male        |
| rs279826 ( $n = 518$ )                  |                         |             |                        |             |
| AA ( $n = 133$ , 25.68%)                | 11 (2.12)               | 122 (23.55) | 47 (9.07)              | 86 (16.60)  |
| G-allele carriers ( $n = 385$ , 74.32%) | 47 (9.07)               | 338 (65.25) | 104 (20.08)            | 281 (54.25) |
| rs279827 ( $n = 445$ )                  |                         |             |                        |             |
| AA ( $n = 120$ , 26.97%)                | 14 (3.15)               | 106 (23.82) | 40 (8.99)              | 80 (17.98)  |
| G-allele carriers ( $n = 325$ , 73.03%) | 33 (7.42)               | 292 (65.62) | 86 (19.33)             | 239 (53.71) |
| rs279858 ( $n = 509$ )                  |                         |             |                        |             |
| AA ( $n = 161$ , 31.63%)                | 23 (4.52)               | 138 (27.11) | 58 (11.39)             | 103 (20.24) |
| G-allele carriers ( $n = 348$ , 68.37%) | 30 (5.89)               | 318 (62.48) | 92 (18.07)             | 256 (50.29) |

SNPs, single nucleotide polymorphisms.

**Table 2** Means, standard deviations, and correlations for study variables

|   | Correlations |      |             |             |             |             |             |             |             |             |             |             |             |       |             |             |
|---|--------------|------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------|-------------|-------------|
|   | Mean         | SD   | 1           | 2           | 3           | 4           | 5           | 6           | 7           | 8           | 9           | 10          | 11          | 12    | 13          | 14          |
| 1. <i>GABRA2</i> (0 = AA, 1 = G-allele) | 0.74         | 0.44 | -           |             |             |             |             |             |             |             |             |             |             |       |             |             |
| 2. Race (0 = female, 1 = male)          | 0.89         | 0.32 | -0.05       | -           |             |             |             |             |             |             |             |             |             |       |             |             |
| 3. Sex (0 = non-White, 1 = White)       | 0.71         | 0.45 | 0.08        | <b>0.11</b> | -           |             |             |             |             |             |             |             |             |       |             |             |
| Rule Breaking                           |              |      |             |             |             |             |             |             |             |             |             |             |             |       |             |             |
| 4. Age 11-12                            | 1.98         | 1.96 | 0.02        | -0.09       | 0.04        | -           |             |             |             |             |             |             |             |       |             |             |
| 5. Age 13-14                            | 2.89         | 2.38 | <b>0.15</b> | -0.00       | <b>0.11</b> | <b>0.49</b> | -           |             |             |             |             |             |             |       |             |             |
| 6. Age 15-16                            | 3.66         | 2.72 | <b>0.13</b> | -0.04       | 0.04        | <b>0.38</b> | <b>0.59</b> | -           |             |             |             |             |             |       |             |             |
| 7. Age 16-18                            | 3.88         | 2.74 | 0.05        | -0.04       | 0.04        | <b>0.42</b> | <b>0.57</b> | <b>0.64</b> | -           |             |             |             |             |       |             |             |
| Problem Alc Use                         |              |      |             |             |             |             |             |             |             |             |             |             |             |       |             |             |
| 8. Age 11-12                            | 0.05         | 0.36 | 0.03        | 0.04        | -0.05       | <b>0.15</b> | <b>0.36</b> | 0.12        | <b>0.23</b> | -           |             |             |             |       |             |             |
| 9. Age 13-14                            | 0.65         | 2.16 | 0.00        | 0.06        | 0.02        | <b>0.28</b> | <b>0.37</b> | <b>0.24</b> | <b>0.32</b> | <b>0.41</b> | -           |             |             |       |             |             |
| 10. Age 15-16                           | 2.93         | 5.58 | 0.00        | 0.05        | -0.02       | <b>0.37</b> | <b>0.40</b> | <b>0.48</b> | <b>0.34</b> | 0.13        | <b>0.40</b> | -           |             |       |             |             |
| 11. Age 17-18                           | 6.24         | 7.54 | 0.09        | -0.02       | <b>0.15</b> | <b>0.32</b> | <b>0.38</b> | <b>0.44</b> | <b>0.46</b> | <b>0.18</b> | <b>0.30</b> | <b>0.56</b> | -           |       |             |             |
| SA Symptom                              |              |      |             |             |             |             |             |             |             |             |             |             |             |       |             |             |
| 12. Age 11-12                           | 0.01         | 0.14 | -0.01       | 0.03        | 0.06        | 0.11        | <b>0.16</b> | 0.14        | 0.13        | -0.01       | <b>0.20</b> | 0.09        | 0.07        | -     |             |             |
| 13. Age 13-14                           | 0.27         | 1.07 | -0.02       | 0.07        | -0.04       | <b>0.22</b> | <b>0.37</b> | <b>0.15</b> | <b>0.27</b> | <b>0.47</b> | <b>0.70</b> | <b>0.31</b> | <b>0.32</b> | 0.02  | -           |             |
| 14. Age 15-16                           | 1.58         | 4.01 | 0.00        | 0.02        | -0.09       | <b>0.18</b> | <b>0.27</b> | <b>0.45</b> | <b>0.32</b> | 0.03        | <b>0.27</b> | <b>0.65</b> | <b>0.35</b> | 0.03  | <b>0.21</b> | -           |
| 15. Age 17-18                           | 2.72         | 4.23 | 0.04        | -0.01       | 0.11        | <b>0.19</b> | <b>0.32</b> | <b>0.44</b> | <b>0.51</b> | -0.04       | <b>0.19</b> | <b>0.42</b> | <b>0.65</b> | -0.05 | <b>0.23</b> | <b>0.37</b> |

SD, standard deviation; Alc, alcohol; SA symptom, substance abuse symptomatology; bold values represent significant ( $p < 0.05$ ) effects.

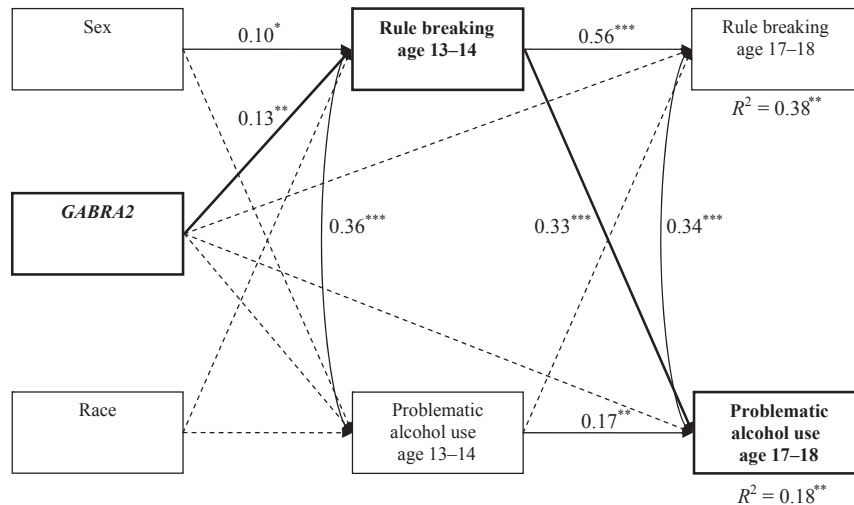


**Figure 1** Nested chi-square model. Path to rule breaking age 11-12 is freely estimated and the other paths are constrained to be equal. Values represent standardized path coefficients. Dashed line represents a nonsignificant path; \*\* $p < 0.01$

was employed as recommended (Satorra, 2000). Constraining all paths to be equal resulted in a significant change in chi-square ( $\chi^2\Delta = 8.20$  (3),  $p < 0.05$ ) and CFI ( $\Delta CFI = -0.02$ ) indicating a decrement in model fit. Modification indices suggested that rule breaking at age 11-12 should be freely estimated. After freeing this path, a significant ( $p < 0.01$ ) effect from *GABRA2* to rule breaking was observed at ages 13-14, 15-16, and 17-18, but not for rule breaking at age 11-12 ( $p = 0.94$ ; see Figure 1). This indicates that G-allele carriers have higher rates of rule breaking compared to those with the AA genotype, this association is not significant in early adolescence, and it is undifferentiated in mid- to late-adolescence. *GABRA2* did not predict substance use outcomes across adolescence.

**Cross-lagged mediation models**

Two separate cross-lagged mediation path models were tested for each outcome. We tested the youngest age (13-14) of rule breaking that was significant in the nested model along with the oldest time period assessed (age 17-18) in order to examine the effect of early indicators of risk on more distal outcomes. The problematic alcohol use model provided a good fit to the data and accounted for 37.8% and 18.1% of the variance in rule breaking and problematic alcohol use at age 17-18, respectively (see Figure 2). Males and G-allele carriers reported more rule breaking at age 13-14. As expected, rule breaking at age 13-14 prospectively predicted problematic alcohol use at age 17-18 above and beyond previous rates of use; however, problematic alcohol use did not predict rule breaking. The indirect effect from *GABRA2* to problematic alcohol use was significant (estimate = 0.69,  $p = 0.02$ ; 95% bias-corrected bootstrap confidence interval (BCBCI) = 0.22-1.37) and 45.3% of the total effect of *GABRA2* on problematic alcohol use operated through rule breaking.

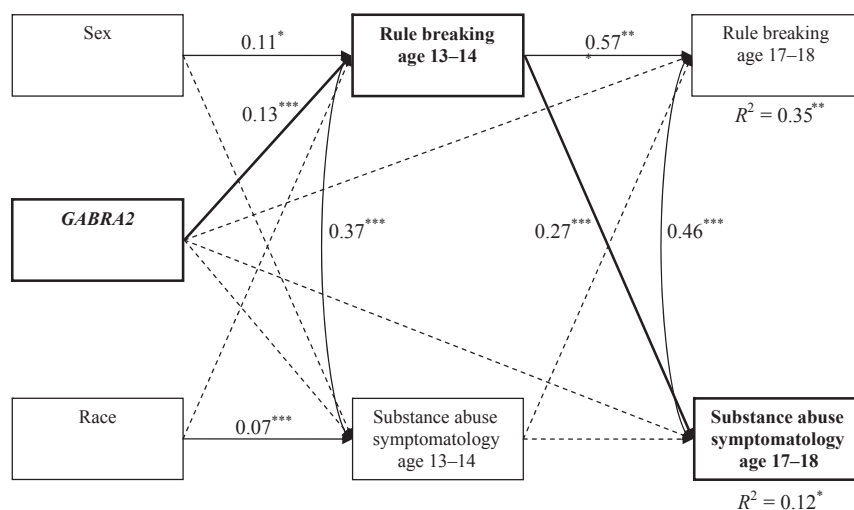


**Figure 2** Cross-lagged model for problematic alcohol use. Model fit: RMSEA = 0.019, CFI = 0.996, TLI = 0.985. Values represent standardized path coefficients. Dashed lines represent nonsignificant paths (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ). Bold lines represent a significant mediated path

The substance abuse symptomatology model provided a good fit to the data and accounted for 34.7% and 11.7% of the variance in rule breaking and substance abuse symptomatology at age 17–18, respectively (see Figure 3). Males and G-allele carriers reported more rule breaking at age 13–14, and White adolescents reported more substance abuse symptomatology at age 13–14. As expected, rule breaking at age 13–14 prospectively predicted substance abuse symptomatology at age 17–18 above and beyond prior rates of substance abuse, but substance abuse symptomatology did not predict rule breaking. The indirect effect from GABRA2 to substance abuse symptomatology was significant (estimate = 0.34,  $p = 0.03$ ; 95% BCBCI = 0.09–0.76) and 71.1% of the total effect of GABRA2 on substance abuse symptomatology operated through rule breaking.

### Discussion

Substance abuse has a complex etiology and considerable individual differences in susceptibility. Advances in genetics research have identified GABRA2 as predicting alcohol and drug dependence in adulthood (e.g. Covault, Gelernter, Hesselbrock, Nellissery, & Kranzler, 2004; Edenberg et al., 2004; Enoch et al., 2006); yet, findings are mixed in younger samples (Dick et al., 2006; Matthews et al., 2007; Sakai et al., 2010). This may be due to the relative importance of genetic factors in the etiology of substance dependence across development (Rhee & Waldman, 2002). Weak direct effects of GABRA2 on alcohol and drug dependence in adolescence highlight the need to understand developmental pathways to psychopathology. This study examined age-specific effects of GABRA2 across adolescence on one nonspecific



**Figure 3** Cross-lagged model for substance abuse symptomatology. Model fit: RMSEA = 0.022, CFI = 0.995, TLI = 0.979. Values represent standardized path coefficients. Dashed lines represent nonsignificant paths (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ). Bold lines represent a significant mediated path

risk behavior and two substance use-specific behaviors in a predominantly White male sample. Rule breaking as a mediator in the relation between *GABRA2* and problematic alcohol use and substance abuse symptomatology was also examined. Four study hypotheses were tested: (a) those carrying the minor allele would have higher levels of rule breaking, (b) *GABRA2* would have weak direct effects on substance use outcomes, (c) rule breaking would mediate the effects of *GABRA2* on substance use outcomes, and (d) substance use would not predict rule breaking. All hypotheses were supported. Namely, G-allele carriers reported higher levels of rule breaking in mid- to late-adolescence, while *GABRA2* did not predict substance use outcomes across adolescence. Rule breaking in mid adolescence mediated the association between *GABRA2* on substance use outcomes in late-adolescence. Lastly, substance use did not predict rule breaking.

### *Age-specific effects of GABRA2*

*GABRA2* had a stronger impact on rule breaking in mid- to late-adolescence versus early adolescence. Namely, G-allele carriers endorsed higher rates of rule breaking compared to those homozygous for the A-allele. This is consistent with work demonstrating associations between *GABRA2* and problem behaviors (Dick et al., 2006). Animal research demonstrates that *GABRA2* receptors are expressed primarily in the amygdala and areas activated from the striatum, such as the substantia nigra (Schwarzer et al., 2001), which is associated with individual differences in impulsivity and reward (Brody, Chen, & Beach, 2013). It is likely that G-allele carriers are more impulsive or find rule breaking more rewarding. It may be that during earlier developmental periods *GABRA2* has a more pronounced effect on precursors to behavioral problems such as difficult temperament (e.g. disinhibition; Edenberg et al., 2004).

*GABRA2* did not predict problematic alcohol use or substance abuse symptomatology across adolescence. Previous research suggests that genetic effects on substance use outcomes increase as individuals age, with some reporting a peak effect at ages 30–33 (Kendler, Gardner, & Dick, 2011). Conversely, the effects of genetic risk in adolescence may be nonspecific and impact more general problem behaviors (Moffitt et al., 2001). Genetic risk for problematic alcohol and drug use may become more relevant in early to midadulthood during typical onset of abuse and dependence (Kendler et al., 2011). Effects at these ages can be studied in the MLS when the sample gets older.

### *Genetic pathways*

Findings demonstrate that the pathway from *GABRA2* to substance abuse operates largely via

rule breaking. G-allele carriers endorsed greater levels of rule breaking behaviors in midadolescence, which in turn predicted problematic alcohol and drug use in late-adolescence. This is consistent with work indicating that impulsivity partially mediates the association between *GABRA2* and alcohol abuse in adulthood (Villafuerte et al., 2013). It is likely that G-allele carriers are less likely to ‘mature out’ of rule breaking and engage in increasingly risky behaviors associated with greater rewards such as alcohol and illicit drug use eventually resulting in problematic use. Clinically, detection of early risk factors and pathways to the abuse endpoint provides information regarding who may be especially susceptible and benefit the most from prevention efforts. The externalizing pathway posits a developmental trajectory to substance use disorders that begins with a genetically driven underlying liability for behavioral disinhibition, which emerges as difficult temperament in infancy, followed by rule breaking behavior, and the eventual onset of problematic alcohol and drug use in later adolescence and early adulthood (Zucker, 2006). Findings provide evidence that carrying the *GABRA2* G-allele may represent this early genetic risk for an externalizing pathway to the development of alcohol and drug abuse.

### *Limitations and future directions*

Although this work provides a greater understanding of genetic pathways to substance abuse, several limitations should be noted. Our study was predominantly comprised of White males. Rates of delinquency and substance abuse tend to be higher among males (Lahey et al., 2006). Accordingly, our findings may not generalize to mixed-sex or primarily female samples. A future direction is to examine whether sex moderates this pathway using moderated mediation models. Rates of problem behaviors also vary across race and ethnicity (Esbensen, 2010). We did not have an adequate number of racial minorities to test these differences. This sample was enriched for alcoholism, limiting generalizability of results. Study variables were based on youth self-report. Although research suggests that adolescent-report of behavior problems is more accurate compared to collaterals (Moffitt et al., 2001), this may increase shared-method variance.

Nevertheless, this work provides evidence for the utility of a genetically informed developmental perspective of *GABRA2* effects on substance abuse. Understanding the role of *GABRA2* as a risk factor for substance use disorders warrants prospective designs that assess behavior more germane to youth and more proximal to genes. Future work should test whether this externalizing pathway predicts nicotine dependence. Investigating additional mediating mechanisms between *GABRA2* and substance

dependence is another important future direction. For example, research demonstrates that a positive subjective effect of alcohol is likely enhanced in individuals with the *GABRA2* variant (Arias et al., 2014). Other work suggests that *GABRA2* impacts temperamental factors such as disinhibition (Edenberg et al., 2004). Given that difficult temperament typically precedes rule breaking, temperament is likely to be a mediator of *GABRA2* in childhood. Future work involving reward-processing areas as potential mediators, such as differential activation in the striatum and the nucleus accumbens, also seems warranted given prior work (Sieghart & Sperk, 2002).

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## Correspondence

Elisa M. Trucco, Addiction Research Center, University of Michigan, 4250 Plymouth Road, Ann Arbor, MI 48109-2700, USA; Email: etrucco@med.umich.edu

## Key points

- *GABRA2* variants indirectly predict the development of alcohol and drug dependence in adulthood.
- There is weak support for the role of *GABRA2* on alcohol and drug dependence in adolescence.
- *GABRA2* may represent an early risk factor for an externalizing pathway to the development of alcohol and substance use problems.
- Cross-lagged path models support the role of rule breaking as a mediator in the association between *GABRA2* and substance abuse outcomes.
- *GABRA2* is relevant in younger developmental periods, but its impact on substance abuse outcomes operates largely via rule breaking behavior.

## Note

1. Given a largely White sample, analyses were also conducted on White participants only. Findings were comparable and frequency genotypes did not differ by race ( $\chi^2(1) = 1.54, p = 0.21$ ). Accordingly, results include consideration of the full sample.

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