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GABRA2, alcohol, and illicit drug use: an event-level model of genetic risk for polysubstance use

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Abstract

GABRA2, the gene encoding the α2 subunit of the $GABA_A$ receptor, potentially plays a role in the etiology of problematic drinking, as *GABRA2* genotype has been associated with subjective response to alcohol and other alcohol-related reward processes. The *GABRA2* gene has also been associated with illicit drug use, but the extent to which associations with drug use are independent of associations with alcohol use remains unclear, partly because most previous research has used a cross-sectional design that cannot discriminate comorbidity at the between-person level and co-occurrence within-persons. The present study employed a daily monitoring method that assessed the effects of *GABRA2* variation on substance use as it occurred in the natural environment during emerging adulthood. Non-Hispanic European participants provided DNA samples and completed daily reports of alcohol and drug use for one month per year across four years (N = 28,263 unique observations of N = 318 participants). *GABRA2* variants were associated with illicit drug use in both sober and intoxicated conditions. Moreover, the effect of *GABRA2* variation on drug use was moderated by an individual's degree of intoxication. These findings are consistent with recent genetic and neuroscience research, and they suggest *GABRA2* variation influences drug-seeking behavior through both alcohol-related and alcohol-independent pathways.

Keywords

genetics; substance use; *GABRA2*; longitudinal; multilevel modeling

Introduction

Despite significant prevention and intervention efforts, alcohol and illicit drug use continue to be two of the greatest contributors to preventable morbidity and mortality in the United States (Johnson, Hayes, Brown, Hoo, & Ethier, 2014). Together, they exact more than $400 billion annually in costs related to increased incarceration, lost productivity, and health care (Rehm et al., 2009; Rice, 1999; Sacks, Gonzales, Bouchery, Tomedi, & Brewer, 2015). Problematic alcohol and drug use are often co-morbid, and this co-morbidity is due, in part, to a shared genetic etiology. Twin studies have found that between 46 and 72% of the shared variance between alcohol and drug dependence can be attributed to additive genetic effects on both phenotypes (Vrieze, McGue, Miller, Hicks, & Iacono, 2013). Moreover, prior

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research suggests that liability for comorbid alcohol and drug dependence may be more heritable than alcohol or drug dependence alone (Kendler, Jacobson, Prescott, & Neale, 2003; McGue, Pickens, & Svikis, 1992; Pickens, Svikis, McGue, & LaBuda, 1995). This shared etiology indicates that specific genetic variants associated with alcohol use behaviors might confer risk not just for problematic alcohol consumption, but also for concurrent alcohol and other drug use among polysubstance users.

To date, the majority of genetic research on substance use has employed cross-sectional methods with diagnostic phenotypes (e.g., case-control studies of substance dependent populations versus healthy controls). However, as substance dependence begins with recreational use, and substance use behaviors exist on a continuum of individual differences, it is important to identify genetic factors that influence drug use at the level of everyday behavior. Event-level research is uniquely poised to contribute to the field by identifying genetic variants involved in the complex patterns of polysubstance use behaviors. Here, we demonstrate the benefits of longitudinal event-level designs in genetic research by characterizing the effects of *GABRA2* on concurrent alcohol and drug use as it naturally occurs across 28,263 daily events.

GABRA2 is Associated with Substance Use

Early genome-wide linkage studies of alcohol dependence (AD) identified *GABRA2* as a candidate gene for future research (Reich et al., 1998), and subsequent studies supported its association with various measures of alcohol abuse (Covault, Gelernter, Hesselbrock, Nellissery, & Kranzler, 2004; Edenberg et al., 2004). Although *GABRA2* has not reached statistical significance ($p < 5 \times 10^{-8}$) in genome-wide association (GWA) studies of AD, there is evidence to suggest that *GABRA2* is associated with alcohol-related phenotypes other than the AD diagnosis. In particular, the extant evidence supports an association between *GABRA2* and the physiological response to alcohol, which has not yet been examined in a large-scale GWA study.

GABRA2 encodes for the α2 subunit of the $GABA_A$ receptor, the major inhibitory neurotransmitter receptor in the human brain (Sigel & Steinmann, 2012). Electrophysiological research has found that this receptor is sensitive to ethanol (Glykys et al., 2007; Wallner, Hanchar, & Olsen, 2003), such that exogenous ethanol potentiates the tonic current of the receptor. An investigation of post-mortem human brain tissue found that levels of the α2 subunit mRNA and protein differed as a function of *GABRA2* genotype (Haughey et al., 2008). This suggests that *GABRA2* variation may modify the activity of synaptic $GABA_A$ receptors, perhaps across multiple neural systems.

Several studies reported correlations between *GABRA2* variants and neural processes in humans. An early investigation of *GABRA2* found that genotypic variation was associated with altered brain oscillations in the beta frequency range (Edenberg et al., 2004). Task-based neuroimaging studies have since found that *GABRA2* variation affects activation of numerous brain regions during reward processing paradigms. For instance, a recent fMRI study of healthy adults found that activation of the ventromedial prefrontal cortex and the ventral tegmental area varied by rs279871 genotype in response to olfactory alcohol cues (Kareken et al., 2010). In two fMRI studies of adults with a family history of alcoholism,

researchers found that minor allele carriers of rs279858 exhibited greater activation of the insula (Villafuerte et al., 2012) and the nucleus accumbens (Heitzeg et al., 2014) during a reward anticipation task.

Additionally, laboratory studies have found that *GABRA2* is associated with differential subjective response to alcohol (Pierucci-Lagha et al., 2005; Roh et al., 2011), notably heightened stimulation (Arias et al., 2014) and reduced negative effects of intoxication (Uhart et al., 2013). This pattern of subjective responses is thought to confer greater risk for problematic alcohol use, as evidence suggests that high-risk drinkers experience greater stimulant-like effects from alcohol during the ascending limb of a drinking episode and fewer sedative-like effects during the descending limb (King, Hasin, O'Connor, McNamara, & Cao, 2016; Newlin & Renton, 2010; Quinn & Fromme, 2011b); this theory of psychobiological risk for AD is termed the "differentiator model."

Interestingly, *GABRA2* has also been associated with illicit drug use (Dixon et al., 2010; Enoch, Hodgkinson, Yuan, Shen, & Roy, 2010) and its comorbidity with alcohol use (Agrawal et al., 2006; Dick et al., 2006); however, the means by which *GABRA2* influences illicit drug use are not well understood. One possibility is that the effects of *GABRA2* on drug use are independent of one's experiences with alcohol. That is, a person with an "at-risk" *GABRA2* genotype may be predisposed to use illicit drugs even if they are never exposed to alcohol. Alternatively, *GABRA2* may influence drug use through its effects on alcohol-related processes, such as subjective response. If *GABRA2* genotype does, as has been previously suggested, modulate subjective response to alcohol (Arias et al., 2014; Roh et al., 2011; Uhart et al., 2013), then individuals with *GABRA2* risk alleles might be particularly likely to use drugs when intoxicated. Consistent with this idea, individual differences in subjective intoxication influence an individual's likelihood to use illicit drugs during drinking episodes (Quinn & Fromme, 2012). Based on the hypothesized link between *GABRA2* and subjective intoxication, it may be that *GABRA2* will be associated with illicit drug use during drinking episodes, but not necessarily illicit drug use in the absence of alcohol.

A Within-Subject Approach to Studying Polysubstance Use

Previous studies of genetic contributions to polysubstance use have typically used an exclusively cross-sectional, between-subjects design. In the current paper, we aim to elucidate further the relationship between *GABRA2* polymorphisms and alcohol and illicit drug use by using a longitudinal, event-level design. Specifically, we used a daily self-monitoring protocol that required participants to report their alcohol and drug use each day, during four 30-day periods, across four years, resulting in 28,263 daily observations regarding alcohol and illicit drug use in 318 participants. This event-level approach allows us to disaggregate comorbidity between alcohol and drug use at the between-person level (i.e., are people who drink at any point more likely to also use drugs at any point?) from co-occurrence at the within-person level (i.e., is a person more likely to use drugs when he or she consumes alcohol?). As has been described by previous authors, between-person and within-person relationships are not necessarily synonymous, and, in fact, most psychological processes are expected to be non-ergodic (Molenaar & Campbell, 2009).

A comparison between the event-level design used in the current study and the typical design of a genetic association study can be understood using Cattell's (1952) data box, which represents potential data structures: multiple variables collected from multiple people on multiple occasions. A typical genetic association design maximizes data across the dimensions of people and (genetic) variables, but measurement is limited to a single occasion. In contrast, the current study examines a limited number of genetic variables (eight polymorphisms within the *GABRA2* gene, rather than all measured polymorphisms genome-wide) in a relatively small sample of persons who were measured repeatedly over 120 occasions (four 30-day daily diaries).

We use the combination of genetic information and event-level measurement to test two hypotheses. First, we hypothesize that *GABRA2* variation will be associated with both (a) between-person differences in the overall likelihood to use illicit substances, and (b) within-person co-occurrence between drinking and illicit drug use. Second, as prior research has demonstrated that *GABRA2* is implicated in subjective response to alcohol, we hypothesize that *GABRA2* polymorphisms will moderate the within-person association between blood alcohol content and the likelihood to use illicit drugs, such that people with risk variants will be particularly likely to use drugs as a function of increasing intoxication.

Method

Participants

The present sample was drawn from a larger cohort of subjects who participated in a longitudinal investigation of alcohol abuse and other behavioral risks among college students. Recruitment procedures for the full study have been described in previously published articles (Fromme, Corbin, & Kruze, 2008; Ashenhurst, Harden, Corbin & Fromme, 2015). A subset of the full sample completed a daily monitoring protocol and provided DNA for genotyping procedures (N = 517, 64% non-Hispanic European, 67% female; see Figure S1 for a flow chart of study recruitment). Analyses were limited to the non-Hispanic European portion of this sample (N = 330, 68% female) to avoid potential bias due to population stratification. Prior research with this cohort has demonstrated that non-Hispanic European participants who provided DNA samples engage in largely similar rates of substance use and externalizing behavior compared to non-Hispanic European participants who did not provide DNA samples (Ashenhurst, Harden, Corbin, & Fromme, 2016). An additional 12 participants were excluded following quality control procedures (detailed below). All procedures were approved by the university's Institutional Review Board.

Longitudinal Event-Level Design

As has been previously described (Neal & Fromme, 2007; Quinn & Fromme, 2011a, 2012), participants were invited to complete up to 30 consecutive days of online self-monitoring in each of their first four years of college. Beginning in August of the first year, a random selection of 200 students was initially invited to participate (to ensure sufficient monitoring in the first weeks) and then 40–43 students were invited in each subsequent week throughout the calendar year. Participants completed their daily monitoring during the same 30-day

period each year. Participants were instructed to use the self-monitoring website (maintained by DatStat, Seattle, WA) in order to answer questions about the previous day. Participants were compensated $1 per day of monitoring, and received a $5 bonus for completing all 30 days within a given year of collection.

Each day, participants answered questions about the previous day related to time-varying demographics (e.g., weight), alcohol consumption ("*How many drinks did you consume yesterday?*" and "*Of the times that you drank this day, how long was your heaviest drinking episode?*"), and illicit drug use ("*Did you use illicit drugs yesterday?*"). If participants endorsed using illicit drugs on any given day, they were asked to specify whether the drug use occurred while sober or during a drinking episode. Although this procedure clearly determined whether substance use occurred in the presence of alcohol, it did not permit specific assessment of *when* the drug use occurred (i.e., the ascending limb, peak, or descending limb of the blood alcohol content curve).

Using self-reported data on gender, weight, quantity of drinks consumed, and duration of drinking episode, we calculated event-level estimates of blood alcohol content (eBAC) by following the procedure developed by Matthews and Miller (1979). Sober events had an eBAC of zero. Previous studies have demonstrated the validity of eBAC as a measure of objective alcohol intoxication (Hustad & Carey, 2005), and found that eBAC was significantly associated with multiple facets of subjective intoxication across breath alcohol concentration trajectories (e.g., Piasecki, Wood, Shiffman, Sher, & Heath, 2012). For these reasons, the use of eBAC has been recommended when breath alcohol concentrations are not available (Leeman et al., 2010). Finally, to aid interpretation of results, we multiplied person-average and event-level eBAC by 100, meaning that odds ratios for these variables reflect the increase in odds of illicit drug use associated with a .01 increase in eBAC.

Consistent with previous studies using this sample (e.g., Neal & Fromme, 2007; Quinn & Fromme, 2011, 2012), we took several steps to maximize the reliability and validity of the daily monitoring data. First, to reduce bias due to over-exclusion or inclusion of noncompliant participants, we excluded participants who did not provide at least 14 days of monitoring data (N = 8). Second, we winsorized 60 event-level eBAC values (1.08% of 5,577 estimates) that exceeded 0.40 g/dl, as they were considered to be potentially erroneous estimates of blood alcohol content (final range = 0.000 – 0.400 g/dl). Four additional participants were excluded from analyses as genomic principal component analysis revealed that they were ancestral outliers (discussed below). These quality control procedures resulted in a final sample of 318 participants with 28,263 event-level observations.

Genotyping Procedures

Participants provided 2 mL of saliva in Oragene-Discover (Oragene™, DNAgenotek, Ottawa, Ontario, Canada) collection kits that were distributed and returned via mail. DNA extraction and purification was conducted at the Institute for Behavior Genetics at the University of Colorado, Boulder. The DNA was prepared from 500 μl of the Oragene™ solution with the Beckman-Coulter DNAdvance (Brea, CA) system according to the manufacturer's protocol, with the final elution volume being 150 μl. Samples were diluted 1:20 in TE and the DNA was quantified using Picogreen fluorescence (Invitrogen,

ThermoFisher, Grand Island, NY). Samples were standardized to 50 ng DNA/μl for chip genotyping.

Purified and diluted samples were then sent to the Neuroscience Genomics Core at the University of California, Los Angeles, for single nucleotide polymorphism (SNP) genotyping assay. Samples were run on an Illumina BeadLab platform using an Illumina Infinium PsychArray BeadChip array (San Diego, CA), which assays 265,000 tag-SNPs across the genome—approximately 50,000 of which are markers associated with common psychiatric disorders. Chips were scanned on an Illumina iScan confocal laser, with genotype calls performed using the manufacturer's parameters in GenomeStudio (Illumina, v 2011.1, genotyping module v1.9.5).

For the present study, we tested eight *GABRA2* SNPs: rs534459, rs548583, rs526805, rs1808851, rs62304121, rs279845, rs4695148, and rs9291283 (descriptive information and statistics presented in Table 1). All eight SNPs had a genotyping rate greater than 98% and were in Hardy-Weinberg equilibrium. As Haploview (Barrett, Fry, Maller, & Daly, 2005) indicated that all eight SNPs exhibited high pairwise LD (see Figure 1 for further detail), PLINK (Purcell et al., 2007) was used to phase individual haplotypes. Six haplotypes were identified: AACCCAC (40%), TTGACTC (30.9%), TTGATTC (13.7%), TTGACTT (10.1%), TTGACAC (2.5%), AACCCTC (1.2%). For the present paper, only common haplotypes with a frequency greater than 5% were included in analyses.

Additionally, to determine whether the eight target SNPs adequately captured *GABRA2*, we used the Tagger program within Haploview to assess European White (CEU+TSI) reference panel data from HapMap (Gibbs et al., 2003). The downloaded reference data included 31 SNPs with MAF over 5%, four of which corresponded with our selected SNPs (rs534459, rs548583, rs526805, and rs1808851). The four SNPs captured 26 of 31 alleles at $R^2 > 0.8$. Average pairwise R^2 between the target SNPs and the 31 HapMap SNPs was 0.978, indicating a high degree of coverage across this gene.

Allelic Scoring

In addition to our haplotype- and SNP-based analyses, we used an allelic scoring approach to further interrogate the relationships between *GABRA2*, alcohol use, and illicit drug use. Importantly, this approach leverages effect sizes from an independent GWA study to calculate individual-specific estimates of risk for a disorder. Here, we first obtained summary statistics from a larger case-control GWA study of AD (Bierut et al., 2010), which served as proxy-phenotype (i.e., a correlated phenotype) to condition our effect size estimates. We then extracted the effect sizes for the five SNPs that were present in the GWA study of AD and the present sample (rs534459, rs548583, rs526805, rs1808851, and rs9291283) and used the allelic scoring function in PLINK (Purcell et al., 2007) to calculate individual-specific estimates of risk conferred by *GABRA2*.

Genomic Principal Components Analysis

To further address potential population stratification within our sample, we used EIGENSTRAT (Price et al., 2006) to extract genomic principal components in participants who self-reported non-Hispanic European ancestry. Default parameters were used in

accordance with recommendations for this procedure (Turner et al., 2011), and data was linkage disequilibrium (LD) pruned ($R^2 < 0.5$) to reduce computational burden. Within the final sample, we included the top ten eigenvectors as grand-centered covariates in statistical analyses. Per the results of the genomic principal components analysis, we excluded four participants from analysis for being ancestral outliers (sigma > 6.0).

Analytic Approach

We used two-level hierarchical linear models (HLM) with robust standard errors (Raudenbush & Bryk, 2002) to analyze the relationships between *GABRA2* variation, alcohol consumption, and the likelihood to use illicit drugs. We conducted three sets of analyses examining the effects of (1) *GABRA2* haplotypes, (2) individual *GABRA2* SNPs, and (3) omnibus *GABRA2* risk as calculated from allelic scoring. Events were nested within participants for all statistical analyses. Reporting year (YR2, YR3, and YR4; Year 1 as reference), genomic principal components of ancestry (GPC_1 … GPC_{10}), biological sex (SEX), age at first wave of data collection (AGE_{W1}), and person-average alcohol consumption ($EBAC_{AVG}$) were included as covariates in all models.

The model is illustrated in Figure 2 and described below (for haplotypes):

$$\textbf{LEVEL 1 MODEL}$$
$$\text{Prob}\ (\text{DRUGUSE}=1|\pi)=\varphi$$
$$\text{Log}\left[\frac{\varphi}{(1-\varphi)}\right]=\eta$$
$$\eta=\pi_0+\pi_1\,(\text{EBAC}_{\text{EVENT}})+\pi_2\,(\text{YR2})+\pi_3\,(\text{YR3})+\pi_4\,(\text{YR4})$$
$$\textbf{LEVEL 2 MODEL}$$
$$\pi_0=\beta_{00}+\beta_{01}\,(\text{AACCCAC})+\beta_{02}\,(\text{TTGACTC})+\beta_{03}\,(\text{TTGATTC})+\beta_{04}\,(\text{TTGACTT})+\beta_{05\ldots014}\,(\text{GPC}_1\ldots\text{GPC}_{10})+\beta_{015}\,(\text{SEX}$$
$$\pi_1=\beta_{10}+\beta_{11}\,(\text{AACCCAC})+\beta_{12}\,(\text{TTGACTC})+\beta_{13}\,(\text{TTGATTC})+\beta_{14}\,(\text{TTGACTT})+\beta_{15\ldots114}\,(\text{GPC}_1\ldots\text{GPC}_{10})+\beta$$
$$\pi_2=\beta_{20}+r_2$$
$$\pi_3=\beta_{30}+r_3$$
$$\pi_4=\beta_{40}+r_4$$

Illicit drug use was analyzed with a logit model that estimated a log odds value for each participant, which was subsequently converted to a probability. The Level 1 (event level) equation modeled the likelihood of a participant engaging in substance use at each event. Specifically, the likelihood of engaging in drug use on a given reporting day was modeled as a function of a person-specific random intercept ($\boldsymbol{\pi}_0$), a random slope describing change in the likelihood of using drugs with increasing event-level eBAC ($\boldsymbol{\pi}_1$), and three random slopes describing change in the likelihood of using drugs over the four reporting years ($\boldsymbol{\pi}_2$, $\boldsymbol{\pi}_3$, and $\boldsymbol{\pi}_4$). Event-level eBAC was centered on the person mean, and thus reflects the effect of whether the person was more or less intoxicated than was typical for him or her. (Centering predictors with respect to the person-specific mean is necessary to discriminate the within-person vs. between-person effects of alcohol intoxication on illicit drug use; Raudenbush & Bryk, 2002). The three dummy-coded reporting year variables were uncentered. Overall, the Level 1 equation tested whether extent of alcohol intoxication (eBAC) and reporting year predicted whether a person was more likely to use illicit drugs on some occasions, relative to other occasions.

The Level 2 (person level) equation then modeled between-person variability in the likelihood to use illicit drugs (aggregating across all occasions), and in the relationship between eBAC and drug use. Here, the intercept for illicit drug use (π_0), representing a person's average likelihood to use drugs across all events, was modeled as a function of the main effect of *GABRA2* haplotypes (β_{01}, β_{02}, β_{03}, and β_{04}), as well as the main effects of ancestry (β_{05} … β_{014}), sex (β_{015}), age (β_{016}), and average (i.e., person-mean) eBAC (β_{017}). The random slopes for eBAC, representing how much more likely a person was to use drugs when he or she was intoxicated versus not intoxicated, were modeled as a function of the effects of *GABRA2* haplotypes (β_{11}, β_{12}, β_{13}, and β_{14}), ancestry (β_{15} … β_{114}), sex (β_{115}), and age (β_{116}). Between-person residuals were included for all event-level slopes (r_0, r_1, r_2, r_3, and r_4), allowing for person-to-person heterogeneity in the magnitude of the within-person effects. Overall, the Level 2 model tested whether age, sex, and *GABRA2* haplotype predicted whether people were, aggregating across events, more likely to use illicit drugs overall and to be particularly likely to use drugs with increasing alcohol intoxication.

In the second set of analyses, individual *GABRA2* SNPs replaced the four Level 2 *GABRA2* haplotypes (see Figure 2 for a path diagram). For these models, genotypic variation was analyzed under an additive risk model, coded with respect to the number of minor alleles. Using the web-based software SNPSpD (Nyholt, 2004), we examined the LD structure between target SNPs to determine the correct *p*-value adjustment for multiple comparisons. As SNPSpD indicated that the effective number of independent marker loci was 2, we established our threshold for statistical significance to be a *p*-value of 0.025. Although all SNPs were in high LD, we opted to test all markers, as they may possess different patterns of LD with the causal SNP(s).

Finally, we conducted a third analysis in which allelic scores (i.e., individual-specific estimates of genetic risk conferred by *GABRA2*) replaced the individual *GABRA2* SNPs. In this model, the number of minor alleles (0, 1, or 2) at a given SNP was multiplied by the effect size of that SNP, as derived from a larger GWA study (Bierut et al., 2010). Allelic scores were then calculated by summing across all included SNPs (rs534459, rs548583, rs526805, rs1808851, and rs9291283). This final approach allowed us to leverage effect sizes from a larger GWA study (Bierut et al., 2010) to examine the aggregate influence of *GABRA2* on illicit drug use in the present sample.

Results

Thirty-two percent (n = 103, 30% female) of the sample engaged in illicit drug use during the daily monitoring study. When illicit substances were used, 594 (58.93%) of the 1,008 drug use events occurred while sober and the remaining 414 (41.07%) events occurred during a drinking episode. We present the raw proportion of illicit drug use episodes across the full range of eBAC in Figure S2. Additionally, although we did not explicitly model alcohol use as an outcome in our HLMs, we did test for associations between *GABRA2* variation and alcohol consumption, as indexed by person-average eBAC (Supplementary Table S1). We did not observe any significant effects of *GABRA2* haplotypes, SNPs, or allelic score on person-average eBAC.

Associations between *GABRA2* Haplotypes, Alcohol Use, and Illicit Drug Use

The effects of the four haplotypes on the random intercept and slope for illicit drug use are presented in Table 2. We observed a significant protective effect of the TTGACTC and TTGACTT haplotypes ($B = -0.114$, OR = 0.893, $p = .007$ and $B = -0.166$, OR = 0.847, $p = .001$, respectively), such that both were associated with a lower intercept for drug use. That is, the TTGACTC and TTGACTT haplotypes were associated with a lower overall likelihood to use illicit drugs. Notably, these haplotypes consist almost entirely of major alleles. We did not observe any significant effects of *GABRA2* haplotypes on the slope of illicit drug use on event-level eBAC. Additionally, we did not observe any effect of sex or age on drug use, although person-average eBAC was significantly associated with a greater likelihood to use drugs ($B = 0.125$, OR = 1.133, $p < .001$).

Associations between Individual *GABRA2* SNPs, Alcohol Use, and Illicit Drug Use

The effects of each individual SNP on the random intercept and slope for illicit drug use are presented in Table 3. After correcting for multiple comparisons (Nyholt, 2004), we observed significant effects of rs534459 ($B = 0.072$, OR = 1.075, $p = .004$), rs548583 ($B = 0.069$, OR = 1.072, $p = .004$), rs526805 ($B = 0.069$, OR = 1.072, $p = .004$), rs1808851 ($B = 0.067$, OR = 1.070, $p = .005$), rs62304121 ($B = 0.101$, OR = 1.107, $p = .020$), rs279845 ($B = 0.077$, OR = 1.080, $p = .001$), and rs4695148 ($B = -0.129$, OR = 0.879, $p < .001$) on the intercept. With the exception of rs4695148, the minor alleles for all related SNPs were associated with a greater overall likelihood to use illicit drugs. Similar to the haplotypic model, there was no effect of sex or age on drug use, but person-average eBAC was significantly associated with a greater likelihood to use drugs ($p < .001$ in all models).

Additionally, there was a positive within-person association between event-level eBAC and illicit drug use, and the slope of that relationship varied as a function of genotype. After correcting for multiple comparisons, we observed significant effects of rs62304121 ($B = 0.008$, OR = 1. 008, $p = .023$) and rs4695148 ($B = -0.009$, OR = 0.991, $p = .010$) on the slope of illicit drug use on event-level eBAC. The minor allele of rs62304121 (T) conferred greater risk for drug use as intoxication increased (illustrated in Figure 3), whereas the minor allele of rs4695148 (T) attenuated risk. The divergent effects of the two SNPs may be due, in part, to the negative LD between rs62304121 and rs4695148 (D' = 1.00, $R^2 = .01$). We also observed a nominally significant effect ($p < .05$) of rs279845, such that the minor allele (A) conferred greater risk for drug use as eBAC increased.

Associations between *GABRA2* Allelic Scores, Alcohol Use, and Illicit Drug Use

We observed a significant effect of *GABRA2* allelic score on the intercept of illicit drug use ($B = 0.013$, OR = 1.013, $p = .016$). That is, individual-specific estimates of aggregate risk conferred by *GABRA2* were associated with a significantly greater overall likelihood to use illicit substances. We did not observe any significant effects of *GABRA2* allelic score on the slope of illicit drug use on event-level eBAC ($p = .09$). However, it is important to note that the three SNPs associated with the slope between eBAC and illicit drug use in the present sample were not included in Bierut and colleagues' (2010) GWA study. As a result, those SNPs did not influence the allelic score. Finally, as with all previous models, we did not observe any effect of sex or age on drug use, although person-average eBAC was

significantly associated with a greater likelihood to use drugs ($B = 0.123$, OR = 1.314, $p < .001$).

Sensitivity Analyses

Split-half—To interrogate the robustness of our findings, we conducted a set of split-half analyses to test whether results were consistent across different portions of the data. Specifically, we split the event-level dataset into two equivalent halves by assigning alternating events to separate datasets. This resulted in two event-level datasets, henceforth referred to as First Half ($n = 14,187$ observations, $m = 44.61$ observations per person) and Second Half ($n = 14,189$ observations, $m = 44.62$ observations per person). We then re-estimated all three models in both halves. The results for our haplotype- and SNP-based sensitivity analyses are presented in Tables 4 and 5, respectively, while the results of our allelic scoring sensitivity analysis are described below.

Notably, the results of our sensitivity analyses are largely consistent with those of our primary models. The First Half dataset replicated all statistically significant effects observed in the full dataset except for one (the effect of rs4695148 on the slope). The Second Half dataset yielded largely similar results to the full dataset with a few exceptions. First, the effects of rs548583 and rs526805 on the intercept remained nominally significant, but they did not withstand correction for multiple comparisons. Second, the effect of rs62304121 on the slope between eBAC and illicit drug use was not significant in this half of the data. Third, the *GABRA2* allelic score was not a significant predictor of the overall likelihood to use illicit drugs in the Second Half dataset ($p = .075$). However, despite these minor discrepancies, it is important to note that the 95% confidence intervals for our sensitivity analyses contained the original point estimate in all instances.

Drug-exposed subsample—We conducted an additional set of sensitivity analyses that re-estimated all three models in the subsample who used illicit drugs ($N = 8,383$ observations of $N = 103$ participants). These sensitivity analyses explicitly tested whether *GABRA2* variation influenced the overall likelihood to use illicit drugs among participants who used drugs, and whether *GABRA2* variation moderated the slope between eBAC and illicit drug use for these participants only. Results of these sensitivity analyses are presented in Tables 4 and 5. Here, we see that the effects of *GABRA2* variation are either attenuated or no longer significant. However, this can likely, at least partially, be attributed to the appreciably smaller sample size and larger standard errors. Furthermore, 12 of the 13 significant effects in the original models have 95% confidence intervals that overlap with the original point estimate.

Power Calculations for the Present Within-Person Design

The present study utilized a longitudinal, event-level design, which facilitated more precise measurement of daily alcohol and substance use than traditional retrospective recall. We sought to illustrate these advantages by conducting supplementary power analyses; however, consensus on the proper method to estimate power in two-level HLMs with binomial outcomes has yet to be achieved. We instead present a series of power simulations for two-level HLMs with continuous outcomes under a variety of conditions, illustrating the

advantages of repeated measurement. As such, the resulting power estimates should be interpreted as illustrative and not definitive. Specifically, we used Optimal Design (Spybrook et al., 2011) to complete power analyses that contrasted our intensive longitudinal design (~89 observations per person) with a traditional cross-sectional design (1 observation per person). The results of our power analysis are illustrated in Supplementary Figure S3 and described below.

Statistical power in HLMs is partially dependent on the intraclass correlation (ICC) of the outcome (i.e., how similar events are within person). That is, more statistical power is gained by repeated measurements when the outcome is more state-like or variable from day-to-day (indexed by a low ICC). In random intercept models, the ICC of a binomial outcome is calculated by comparing the variance component of the outcome (τ_{00}) to itself plus the residual variance ($\pi^2/3$), which, in a binomial multilevel model, is simply a constant attribute of the logistic distribution. The equation is shown below.

$$\mathrm{ICC}=\left[\frac{t_{00}}{(t_{00}+\pi^2/3)}\right]$$

In models with random slopes, calculating the ICC of a binomial outcome is more complicated, as the ICC for the outcome will depend on the value(s) of the within-person predictor(s). Although calculating estimates of ICCs in models with random slopes is more complicated and less precise (Goldstein, Browne, & Rasbash, 2002), it is possible to obtain a general estimate by including the variance components of all event-level predictors in the denominator. Here, we use the equation described below.

$$\mathrm{ICC}=\left[\frac{t_{00}}{(t_{00}+t_{01}+t_{02}+t_{03}+t_{04}+\pi^2/3)}\right]$$

This yields an ICC estimate of .18 in our dataset, which we will use for the purposes of the present power analysis. However, given the uncertainty of this estimate, we provide additional estimates of statistical power with ICC parameters of .10 and .30. We present this range of statistical power estimates as it more accurately represents the fluctuating ICC in our HLM with a random intercept and random slopes.

Finally, large-scale GWA studies of complex human traits suggest that effect sizes of individual SNPs are very small (e.g., $r^2 \approx .002$, $d \approx .09$; Okbay et al., 2016). If we assume a very small effect size of $d = .09$ for our *GABRA2* SNPs (all MAF > 5%) and haplotypes, we see that our statistical power most likely ranges between ~.303 to ~.674 to detect a nominally significant association ($p = .05$). Conversely, a cross-sectional study with only one observation per person with the same number of participants only has a power of ~.125 to detect the same effect. Clearly, repeated measurement vastly improves researchers' ability to detect small genetic effects—even with moderate sample sizes.

Discussion

The present study employed a daily monitoring protocol in combination with genotyping procedures to assess the effect of *GABRA2* variation on the likelihood to engage in drug use and polysubstance use. Whereas previous investigations of *GABRA2* and substance use have focused solely on between-person variation, we applied a novel analytic method in which we simultaneously assessed between-person and within-person variation. The results of all three event-level models suggest that *GABRA2* is indeed associated with illicit drug use and polysubstance use, specifically concurrent alcohol and drug use.

Our haplotypic analyses identified two common haplotypes, TTGACTC and TTGACTT, that served as protective factors against illicit drug use. Notably, both haplotypes are largely characterized by major alleles. This finding coalesces with the results of our SNP-based models, which demonstrate that the minor alleles of rs534459, rs548583, rs526805, rs62304121, rs279845, rs9291283, and rs1808851 conferred greater risk for illicit drug use. Furthermore, the *GABRA2* allelic score also indicated that the minor alleles of *GABRA2* confer a greater overall likelihood to use illicit drugs.

Analysis of individual SNPs additionally revealed that two SNPs moderated the association between event-level eBAC and illicit drug use. Specifically, we found that the minor allele of rs62304121 was positively associated with the slope of illicit drug use on event-level eBAC. That is, minor allele carriers were significantly more likely to use illicit drugs with increasing levels of eBAC. Conversely, the minor allele of rs4695148 was negatively associated with the slope between event-level eBAC and drug use, such that carriers of the minor allele experienced a lesser increase in the likelihood to use drugs as eBAC increased. We also observed a nominally significant effect of rs279845, which has previously been reported to moderate subjective response to alcohol (Uhart et al., 2013).

Although we found that several SNPs predicted individual differences in the slope between eBAC and illicit drug use, we did not observe the same for *GABRA2* haplotypes or allelic score. We hypothesize that we did not observe haplotype × eBAC effects because the small individual SNP × eBAC interaction effects were rendered non-significant when aggregating effects across SNPs (i.e., including non-significant SNPs). Similarly, we posit that we did not observe any allelic score × eBAC interaction because the allelic score did not include any of the SNPs (rs62304121, rs279845, and rs4695148) that interacted with eBAC.

Overall, these findings corroborate previous studies that have linked *GABRA2* to illicit drug abuse (Enoch et al., 2010; Dixon et al., 2010) and its co-occurrence with alcohol abuse (Agrawal et al., 2006; Dick et al., 2006), as well as externalizing phenotypes more broadly (Dick et al., 2009; Villafuerte, Strumba, Stoltenberg, Zucker, & Burmeister, 2013). Notably, our results identify *GABRA2* as a contributor to illicit drug use at the event level, which allows us to better characterize how genetic variants influence substance use behaviors. For example, our results illustrate that a .01 increase in eBAC was associated with a 1.1% increase in the likelihood of using drugs among major allele homozygotes of rs62304121. However, this same change in eBAC was related to a 1.9 % increase among heterozygotes and a 2.7% increase in the likelihood to use drugs among minor allele homozygotes.

GABRA2 and Polysubstance Use

Biologically, it is plausible that (a) genotypic variation of *GABRA2* can predispose individuals to use illicit drugs and (b) that predisposition can be exacerbated by the intoxicating effects of alcohol. Recent genetic and neuroscience research suggests that α2-containing $GABA_A$ receptors may be particularly relevant to addiction. For example, an investigation of genetically engineered mice found that activation of α2-containing $GABA_A$ receptors in the nucleus accumbens is necessary to induce behavioral sensitization, a well-supported mechanism of drug-seeking behavior and relapse (Dixon et al., 2010). To emphasize the importance of their results, the investigators also conducted a case-control genetic association study that demonstrated a relationship between *GABRA2* variation and cocaine addiction in humans.

Studies of humans and animals have found that *GABRA2* may modulate the rewarding effects of cocaine (Dixon et al., 2010), benzodiazepines (Reynolds et al., 2012), methylphenidate (Duka et al., 2015), and alcohol (Heitzeg et al., 2014; Kareken et al., 2010; Villafuerte et al., 2012). The rewarding effects of illicit substances directly impact drug-seeking behavior (Koob & Le Moal, 2001), and subjective response to alcohol has also been found to influence drug use in the natural environment (Quinn & Fromme, 2012). Our results coalesce with the existing literature, and indicate that *GABRA2* variation influences an individual's drug-seeking behavior in the natural environment under both sober and intoxicated conditions. Thus, it is possible that the effects of *GABRA2* on concurrent alcohol and drug use are mediated by subjective intoxication, such that individuals who experience greater disinhibition in response to alcohol are more likely to engage in drug-seeking behavior.

It is also intriguing to consider that *GABRA2* may modulate biological and cognitive processes independent of the stimulating and sedating effects of alcohol, which could increase one's propensity to seek illicit substances. Considering the central role of GABA in many neural systems, it is plausible that genetic variation could confer risk through overlapping but distinct mechanisms in both sober and intoxicated states. An in-depth examination of individual differences in subjective response to alcohol was beyond the scope of the current study; however, future examination of such differences could improve understanding of the mechanisms that mediate genetic variation and liability for problematic alcohol and drug use.

Of the SNPs significantly associated with illicit drug use in the present study, rs279845 and rs548583 have been previously reported to be associated with problematic alcohol use (Bierut et al., 2010; Edenberg et al., 2004) and polysubstance use (Agrawal et al., 2006). Laboratory studies have found that rs279845 moderates an individual's subjective response to alcohol (Uhart et al., 2013). Additionally, many of our tagged SNPs exhibit strong LD with other SNPs throughout *GABRA2*. Indeed, average pairwise R^2 between our 8 *GABRA2* SNPs and the 31 *GABRA2* SNPs tagged in the HapMap genomes was 0.978. Prior research shows that the SNPs rs534459, rs548583, and rs526805 are in very strong LD with a high-risk haplotype that has been previously associated with problematic alcohol use and subjective intoxication (e.g., Edenberg et al., 2004; Uhart et al., 2013).

Person-Centered Models of Dynamic Processes

As demonstrated in the present paper, the use of person-centered models can be particularly useful for studying pharmacogenetic effects of alcohol in humans. By collecting daily event-level data that characterizes patterns of within-person variation, we can more accurately assess concurrent pharmacological and/or psychological processes. Proper characterization of the target phenomena is critical for researchers hoping to assess gene × drug or gene × environment interactions. Moreover, the power simulations described above illustrate the advantages of using repeated daily measurements when investigating small effects of individual SNPs or haplotypes. As a result, intensive longitudinal methods, like the event-level method described here, provide a level of insight beneficial to the study of pharmacogenetic effects that is not afforded by traditional methods.

Many longitudinal studies of polysubstance use rely on aggregate data (i.e., retrospective counts and averages) to assess the simultaneous use of alcohol and drugs. This can be problematic when studying concurrent processes because aggregate data can obscure the true relationship between two variables. More specifically, the covariance of two behaviors can differ depending upon the level of measurement. Our event-level approach, which characterized the within-person covariation of alcohol and illicit drug use in daily life, identified several *GABRA2* SNPs associated with substance use that may not have been identified otherwise.

Conclusions

Our results bolster recent genetic and neuroscience research implicating *GABRA2* and the α2 subunit in substance use. The present findings corroborate the current literature that suggests *GABRA2* variation may exert effects on illicit drug-seeking behavior, perhaps through substance-related reward pathways (e.g., Dixon et al., 2010; Duka et al., 2015; Heitzeg et al., 2014). In addition to identifying several *GABRA2* variants associated with substance use and concurrent alcohol and drug use, our analyses demonstrate the benefits of modeling concurrent psychological processes with daily event-level data. Given the dynamic nature of substance use across the lifespan and even daily contexts, the examination of both between-person and within-person variation is necessary to advance our understanding of the genetic etiology of substance use.

Despite the advantages of our novel approach, the findings of the present study should be interpreted in light of several limitations. First, our measure of illicit drug use is not specific to any particular substance. Although the questionnaire adequately assesses whether illicit drugs were used, as well as the general context in which the illicit drug is consumed (i.e., whether it occurred during a drinking episode), the questionnaire did not assess the specific type of illicit substance consumed. As a result, we are unable to comment on the role of *GABRA2* variation in drug-specific patterns of substance use. Nevertheless, given the relative prevalence of drug use in the present sample (e.g., Fromme, Corbin, & Kruse, 2008), and across the U.S. population more broadly, marijuana is likely the most co-used substance.

Second, as the present study only assessed daily covariation between alcohol consumption and illicit drug use, we are unable to comment on specific temporal or causal relationships.

Thus, it is possible that rising eBAC did not causally increase the likelihood to use illicit drugs (i.e., drug use could have occurred at any point during a drinking episode). To address this possibility, future studies would need to integrate genotypic information with even denser longitudinal measurement, such as ecological momentary assessment (e.g., Shiffman et al., 2002, Piasecki et al., 2011; Piasecki, Wood, Shiffman, Sher, & Heath, 2012). Ideally, these studies should also collect additional contextual and situational data to examine more closely the specific environments in which substances are consumed (e.g., house parties, bars, restaurants). Such data may grant a greater understanding of the environmental factors that promote both drug use and heavy drinking.

Third, given the risk of spurious findings caused by population stratification, the analyses reported here are limited to a subset of non-Hispanic European participants that comprises approximately 65% of our total sample with genetic information. Consequently, the generalizability of our findings to other ancestral populations may be limited.

This study exhibited notable strengths, though, as it is the largest longitudinal event-level investigation to date of genetic influences on alcohol and drug use in daily life. Although the final sample size consists of 318 participants, each participant provided approximately 72 longitudinal reports for a total of 28,263 independent events, which substantially increases our power to detect genetic effects, as well as the ecological validity of the study. As a result, the present findings contribute to our understanding of concurrent alcohol and drug use by elucidating the complex relationship between *GABRA2*, alcohol, and illicit drug use. Lastly, our results illustrate the importance of person-centered approaches to the study of polysubstance use and/or pharmacogenetics. As interest in the study of genetic contributions to polysubstance use and addiction liability continues to rise, it will be important to employ person-centered approaches that characterize the true relationship between psychological factors and drugs of abuse. In doing so, future research will be better suited to identify therapeutic targets across multiple modalities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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General Scientific Summary

Genetic factors influence an individual's response to alcohol, which can, in turn, influence their likelihood to engage in other substance use behaviors while intoxicated. Here, we demonstrate that *GABRA2* variation is a specific genetic risk factor for illicit drug use, especially during drinking episodes. As frequent recreational substance use increases an individual's likelihood of substance use disorders, it is important to identify specific risk factors that influence daily behavior.

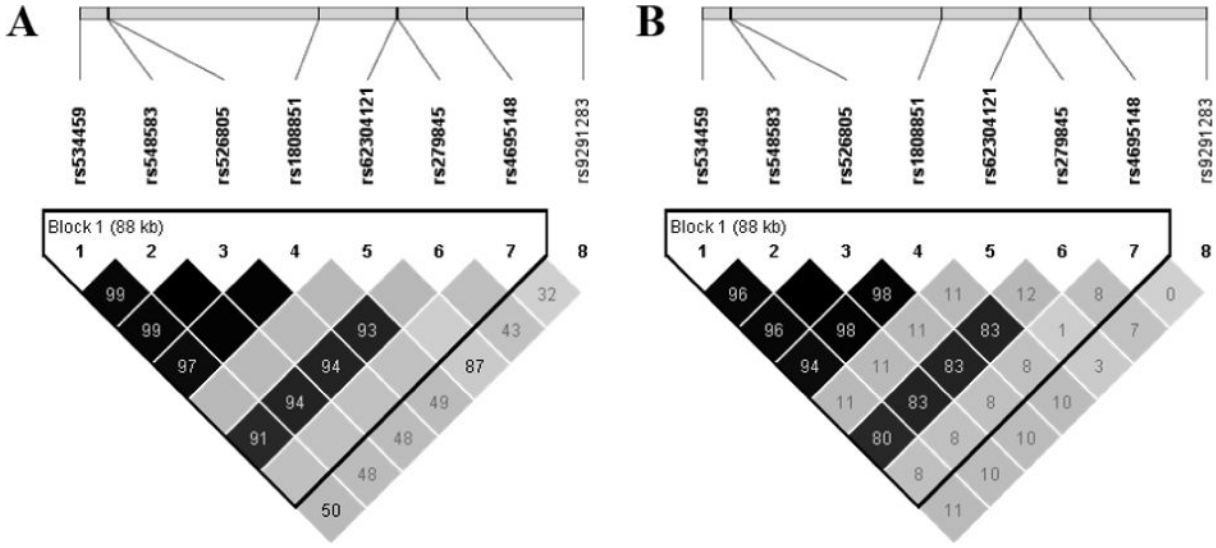

Figure 1.

LD plots generated in Haploview (Barrett, Fry, Maller, & Daly, 2005). Values presented are A) D' and B) R^2. Blank boxes indicate D'=100%.

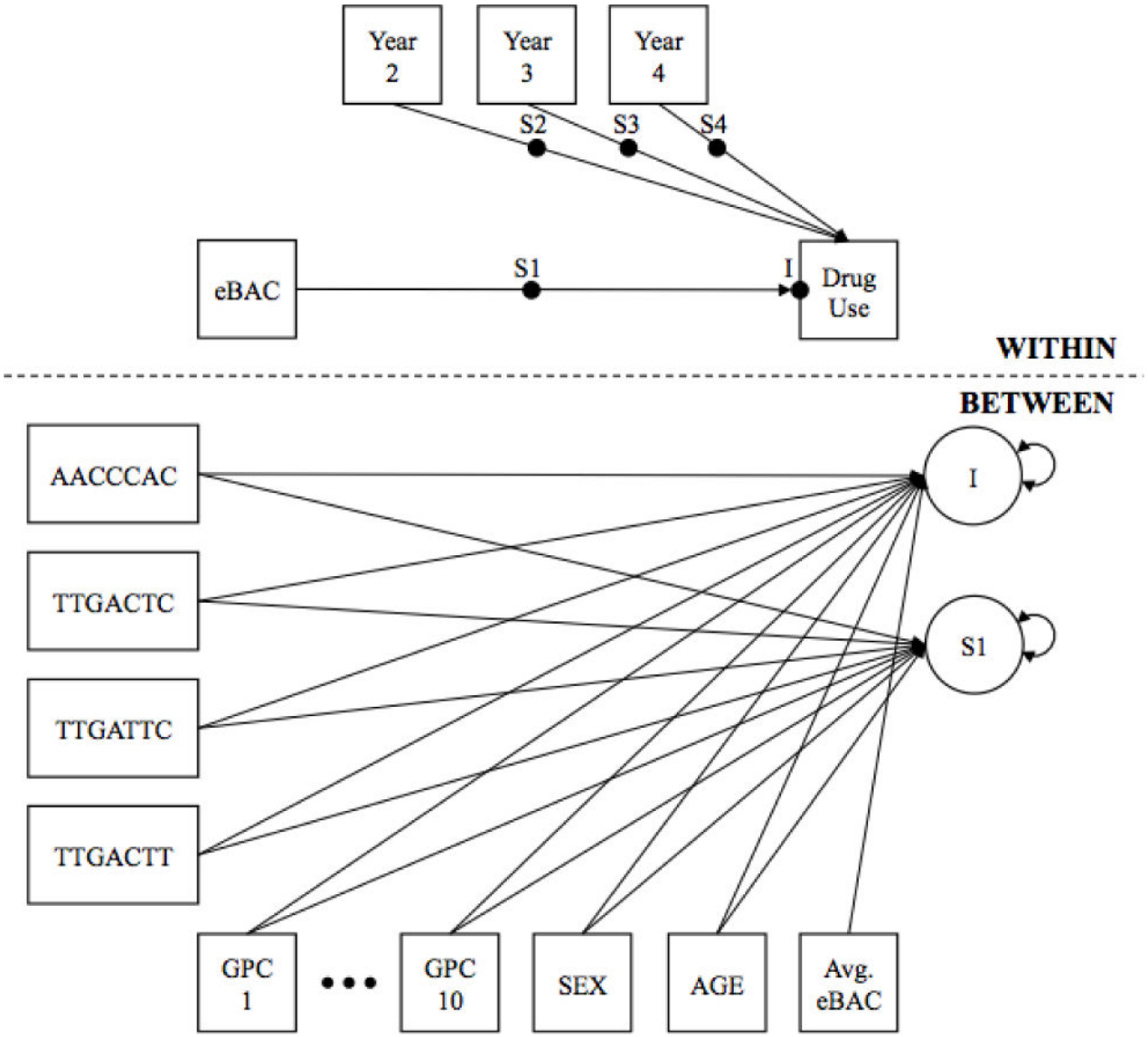

Figure 2.

Path diagram of the multilevel model testing for the effect of *GABRA2* haplotypes. Each haplotype is regressed on the random intercept of drug use and the random slope of drug use on estimated blood alcohol content (eBAC) across all reported events (N=28,263). Three dichotomous dummy variables are included at the within-person level (i.e., event level) to account for the effect of reporting year. S2, S3, and S4 (and their respective error terms) are not displayed at the between-person level (e.g., trait level), as there were no trait predictor × reporting year interactions in the model. Biological sex, age at first wave of data collection, average eBAC, and the top 10 genomic principal components (GPC1 … GPC10) are included as covariates in all models.

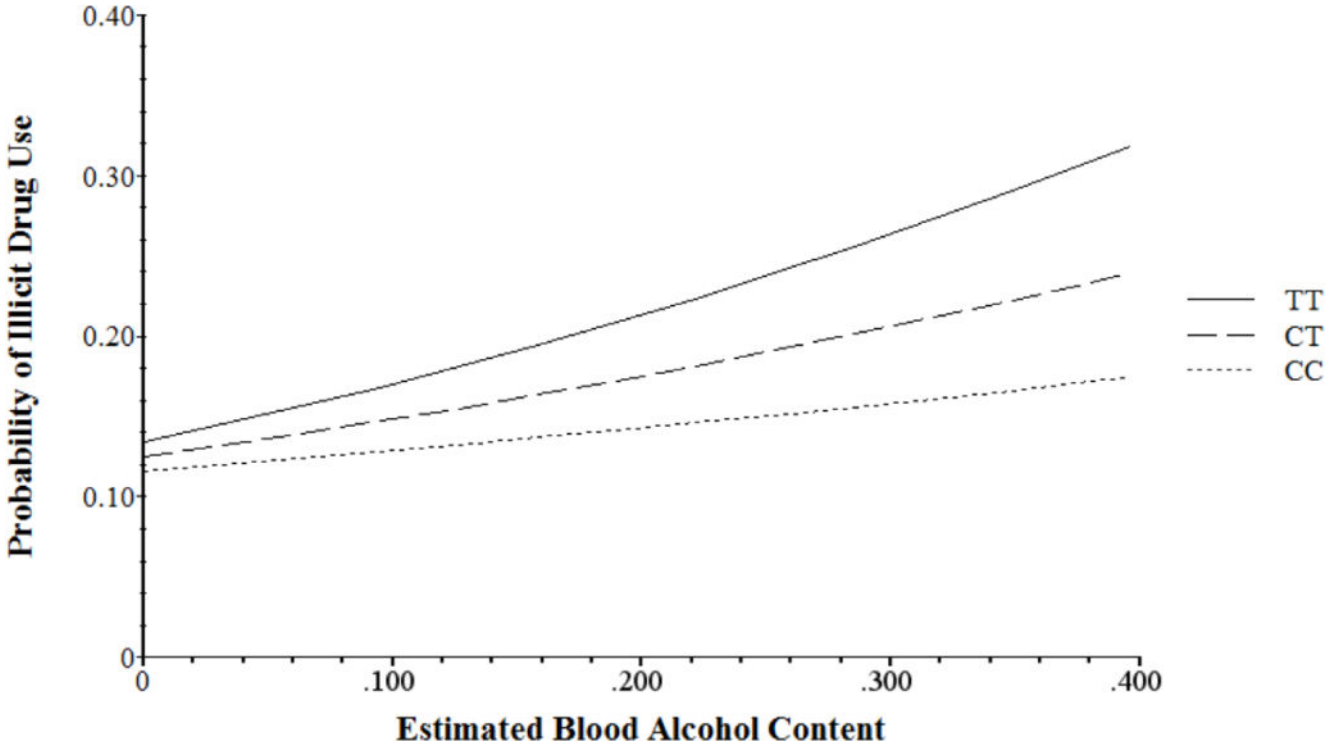

Figure 3.

The effects of rs62304121 on the likelihood to use illicit drugs as a function of increasing eBAC. Results show that the minor allele (T) conferred greater risk for drug use as alcohol intoxication increased (see Table 3).

Table 1

Descriptive statistics for GABRA2 SNPs

SNP	Major allele	Minor allele	Major HO	HE	Minor HO	MAF	HW *p*-value
rs534459	T	A	0.30	0.54	0.15	0.42	0.11
rs548583	T	A	0.31	0.53	0.15	0.42	0.15
rs526805	G	C	0.31	0.53	0.15	0.42	0.15
rs1808851	A	C	0.31	0.53	0.16	0.42	0.21
rs62304121	C	T	0.77	0.22	0.02	0.14	0.43
rs279845	T	A	0.30	0.51	0.18	0.43	0.52
rs4695148	C	T	0.78	0.20	0.01	0.10	0.27
rs9291283	G	A	0.58	0.35	0.06	0.24	0.65

Note. HO = homozygotes. HE = heterozygotes. MAF = minor allele frequency. HW = Hardy-Weinberg.

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Table 2

Effects of GABRA2 haplotypes on the intercept and slope of illicit drug use

Haplotype	B	OR	95% CI
INTERCEPT (π_0)			
AACCCAC	0.013	1.013	(0.928, 1.106)
TTGACTC	−0.114*	0.893	(0.822, 0.970)
TTGATTC	0.054	1.055	(0.931, 1.196)
TTGACTT	−0.166*	0.847	(0.766, 1.936)
EBAC SLOPE (π_1)			
AACCCAC	0.006	1.006	(0.996, 1.017)
TTGACTC	−0.001	0.999	(0.990, 1.009)
TTGATTC	0.010	1.010	(0.998, 1.022)
TTGACTT	−0.005	0.995	(0.983, 1.008)

Note.

*p-values significant after correction for multiple comparisons (Nyholt, 2004).

†p-values nominally significant at α = .05. OR = Odds ratio. CI = Confidence interval.

Table 3

Effects of individual GABRA2 SNPs on the intercept and slope of illicit drug use

SNP	B	OR	95% CI
INTERCEPT (π_0)			
rs534459	0.071*	1.073	(1.010, 1.141)
rs548583	0.068*	1.071	(1.008, 1.137)
rs526805	0.069*	1.072	(1.022, 1.124)
rs62304121	0.101*	1.107	(1.016, 1.205)
rs279845	0.077*	1.080	(1.031, 1.132)
rs4695148	−0.129*	0.879	(0.816, 0.947)
rs9291283	0.012	1.012	(0.934, 1.096)
rs1808851	0.067*	1.070	(1.021, 1.120)
EBAC SLOPE (π_1)			
rs534459	0.005	1.005	(0.999, 1.011)
rs548583	0.006	1.006	(1.000, 1.011)
rs526805	0.005	1.005	(0.999, 1.011)
rs62304121	0.008*	1.008	(1.001, 1.015)
rs279845	0.006†	1.006	(1.000, 1.011)
rs4695148	−0.009*	0.991	(0.984, 0.998)
rs9291283	0.004	1.004	(0.998, 1.010)
rs1808851	0.005	1.005	(0.999, 1.010)

Note.

*p-values significant after correction for multiple comparisons (Nyholt, 2004).

†p-values nominally significant at $\alpha = .05$. OR = Odds ratio. CI = Confidence interval.

Table 4

Sensitivity analyses for the effects of GABRA2 haplotypes on the intercept and slope of illicit drug use

	First Split-Half			Second Split-Half			Drug-Exposed Subsample		
Haplotype	**B**	**OR**	**95% CI**	**B**	**OR**	**95% CI**	**B**	**OR**	**95% CI**
INTERCEPT (π_0)									
AACCCAC	0.073	1.075	(0.980, 1.179)	−0.016	0.984	(0.890, 1.087)	−0.036	0.965	(0.701, 1.329)
TTGACTC	−0.125*	0.882	(0.809, 0.962)	−0.132*	0.876	(0.793, 0.968)	−0.264	0.768	(0.567, 1.040)
TTGATTC	0.132	1.141	(0.996, 1.307)	0.032	1.032	(0.902, 1.181)	−0.007	0.993	(0.687, 1.436)
TTGACTT	−0.165*	0.848	(0.767, 0.938)	−0.174*	0.840	(0.747, 0.945)	0.246	1.279	(0.835, 1.959)
EBAC SLOPE (π_1)									
AACCCAC	0.005	1.005	(0.994, 1.016)	0.002	1.002	(0.989, 1.015)	0.008	1.008	(0.991, 1.024)
TTGACTC	−0.005	0.995	(0.984, 1.006)	−0.002	0.998	(0.985, 1.011)	0.000	1.000	(0.984, 1.016)
TTGATTC	0.010	1.010	(0.998, 1.022)	0.005	1.005	(0.991, 1.011)	0.014	1.014	(0.997, 1.031)
TTGACTT	−0.003	0.997	(0.983, 1.011)	−0.010	0.990	(0.975, 1.006)	0.010	1.010	(0.987, 1.033)

Note.

*p-values significant after correction for multiple comparisons (Nyholt, 2004).

†p-values nominally significant at α = .05. OR = Odds ratio. CI = Confidence interval.

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Table 5

Sensitivity analyses for the effects of GABRA2 SNPs on the intercept and slope of illicit drug use

	First Split-Half			Second Split-Half			Drug-Exposed Subsample		
SNP	**B**	**OR**	**95% CI**	**B**	**OR**	**95% CI**	**B**	**OR**	**95% CI**
INTERCEPT (π_0)									
rs534459	0.112*	1.119	(1.058, 1.182)	0.067*	1.069	(1.011, 1.131)	0.118	1.126	(0.992,1.277)
rs548583	0.107*	1.112	(1.053, 1.175)	0.062†	1.064	(1.007, 1.124)	0.114	1.121	(0.990, 1.270)
rs526805	0.107*	1.112	(1.053, 1.175)	0.062†	1.064	(1.007, 1.124)	0.114	1.121	(0.990, 1.270)
rs62304121	0.143*	1.154	(1.050, 1.268)	0.106*	1.112	(1.020, 1.212)	0.085	1.088	(0.863, 1.372)
rs279845	0.114*	1.121	(1.062, 1.184)	0.070*	1.073	(1.017, 1.132)	0.123†	1.131	(1.002, 1.277)
rs4695148	−0.154*	0.857	(0.797, 0.921)	−0.119*	0.888	(0.818, 0.965)	0.372	1.450	(0.992,2.119)
rs9291283	0.034	1.035	(0.960, 1.116)	−0.022	0.978	(0.906, 1.055)	0.057	1.059	(0.906, 1.238)
rs1808851	0.100*	1.105	(1.048, 1.165)	0.065*	1.067	(1.012, 1.126)	0.109	1.115	(0.986, 1.261)
EBAC SLOPE (π_1)									
rs534459	0.006	1.006	(1.000, 1.013)	0.002	1.002	(0.995, 1.010)	0.003	1.004	(0.996, 1.011)
rs548583	0.006	1.006	(1.000, 1.013)	0.003	1.003	(0.995, 1.010)	0.004	1.004	(0.997, 1.012)
rs526805	0.006	1.006	(1.000, 1.013)	0.003	1.003	(0.995, 1.010)	0.004	1.004	(0.997, 1.012)
rs62304121	0.010*	1.010	(1.002, 1.017)	0.006	1.006	(0.998, 1.014)	0.009†	1.009	(1.000, 1.018)
rs279845	0.006†	1.006	(1.000, 1.012)	0.005	1.005	(0.998, 1.012)	0.004	1.004	(0.998, 1.011)
rs4695148	−0.007	0.993	(0.984, 1.002)	−0.011*	0.989	(0.980, 0.998)	0.004	1.004	(0.986, 1.022)
rs9291283	0.006	1.006	(0.999, 1.012)	0.001	1.001	(0.993, 1.008)	0.001	1.001	(0.994, 1.009)
rs1808851	0.006†	1.006	(1.000, 1.013)	0.002	1.002	(0.995, 1.010)	0.004	1.004	(0.997, 1.011)

Note.

*p-values significant after correction for multiple comparisons (Nyholt, 2004).

†p-values nominally significant at $\alpha = .05$. OR = Odds ratio. CI = Confidence interval.