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The Relationship Between rs3779084 in the Dopa Decarboxylase (DDC) Gene and Alcohol Consumption is Mediated by Drinking Motives in Regular Smokers

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Abstract

Background—Motivational models of alcohol use propose that the motivation to consume alcohol is the final common pathway to its use. Both alcohol consumption and drinking motives are influenced by latent genetic factors that partially overlap. This study investigated whether drinking motives mediate the associations between alcohol consumption and 2 single-nucleotide polymorphisms (SNPs) from genes involved in serotonin (TPH2; rs1386496) and dopamine synthesis (DDC; rs3779084). Based on earlier work showing that enhancement and coping motives were heritable in regular smokers but not in nonregular smokers, we hypothesized these motives would mediate the relationships between alcohol consumption and these SNPs in regular smokers.

Methods—Drinking motives data were available from 830 young adult female twins ($n = 344$ regular smokers and $n = 486$ never/nonregular smokers). We used confirmatory factor analyses to model enhancement, coping, and alcohol consumption factors and to conduct mediation analyses in the regular smoker and never/nonregular smoker groups.

Results—Our hypothesis was partially supported. The relationship between alcohol consumption and rs1386496 was not mediated by drinking motives in either group. However, in the regular smokers, the relationship between alcohol consumption and rs3779084 was mediated by enhancement and coping motives. Carriers of the rs3779084 minor allele who were regular smokers reported more motivation to consume alcohol. Given this pattern of results was absent in the never/nonregular smokers, our results are consistent with a gene $\times$ smoking status interaction.

Conclusions—In regular smokers, variability at the locus marked by rs3779084 in the DDC gene appears to index biologically based individual differences in the motivation to consume alcohol to attain or improve a positive affective state or to relieve a negative one. These results could be because of increased sensitivity to the reinforcing effects of alcohol among minor allele carriers who smoke, which might be due to structural or functional differences in mesocorticolimbic dopamine “reward” circuitry.

Keywords

Alcohol; Tobacco; Drinking Motives; DDC; TPH2; Mediation

It is well established that alcohol use disorders (AUDs) are genetically influenced. However, less is known about how distal genetic influences operate through more proximal risk factors to affect alcohol consumption outcomes. The Motivational Model of Alcohol Use (Cox and Klinger, 1988) can be used to characterize these relationships. The model states that among the multiple factors known to affect alcohol consumption, the motivation to consume alcohol is the final common pathway to its use. Specifically, alcohol's perceived incentive value—the changes in affective state anticipated to be brought about by consuming alcohol compared with not consuming alcohol, such as drinking for “enhancement” (to attain or improve a positive affective state) or drinking to “cope” (to alleviate a negative affective state)—are involved in the final decision about whether or not to drink (Cooper, 1994). It is through such motives that distal factors such as sociodemographic, personality, psychopathology, or genetic factors are thought to exert their influence on drinking behaviors. Thus, the motivational model provides a useful theoretical and empirical framework for research on the genetic etiology of AUDs—one that facilitates explicit testing of genetic–drinking motive–alcohol consumption pathways.

There is evidence that drinking motives mediate or moderate the genetic liability to alcohol use outcomes. In a study of adult female and male twins, Prescott et al. (2004) reported that the latent genetic influences on mood-related motives and diagnoses of alcohol abuse and dependence partially overlap. Also, Van der Zwaluw et al. (2011) reported that among male and female adolescents, the dopamine D2 receptor gene (DRD2) Taq1A polymorphism (rs1800497) interacted with coping motives to influence binge drinking and alcohol problems. Carriers of the risk allele scored higher on coping motives and reported more binge drinking episodes and more alcohol-related problems compared with carriers of the low-risk genotype.

Our own work raises the question of whether drinking motives might mediate the genetic liability to alcohol use in certain phenotypic groups, but not in others. For example, in a study of young adult female twins that focused on mechanisms of alcohol and tobacco co-use (Kristjansson et al., 2011), we reported evidence that genetic influences on coping and enhancement motives, measured using the Drinking Motives Questionnaire-Revised (DMQ-R; Cooper, 1994), were stronger in regular smokers. Specifically, in regular smokers, the heritability estimates were 0.40 for enhancement and 0.35 for coping; in nonregular smokers, the estimates were 0.00 and 0.14, respectively. Further, in line with results from laboratory research with humans and rodents that suggests nicotine potentiates the rewarding effects of alcohol and increases and/or prolongs the motivation to consume it (Barrett et al., 2006; Blomqvist et al., 1996; Chi and de Wit, 2003; Clark et al., 2001; Kouri et al., 2004; Larsson and Engel, 2004; Le et al., 2000, 2003, 2009; Perkins et al., 1995; Potthoff et al., 1983; Smith et al., 1999), regular smokers also reported being more motivated to drink for coping and enhancement reasons compared with the nonregular smokers. In the present article, we seek to extend this work by examining measured gene–drinking motive–alcohol consumption pathways in groups defined by different smoking phenotypes.

Recent work by our group has identified gene variants (single-nucleotide polymorphisms [SNPs]) that are good candidates for such analyses. Specifically, a candidate gene association study of alcohol consumption in a subset of 827 female twins examined the association of 1,014 SNPs from 130 addiction-related genes (Agrawal et al., 2010). In this study, alcohol consumption was quantified using a latent factor, reported in a previous study to be 50% heritable (Agrawal et al., 2009). The top association signals were found for SNP clusters in the neuronal tryptophan hydroxylase (TPH2) and dopa decarboxylase (DDC) genes, with rs 1386496 from TPH2 and rs3779084 from DDC, showing the strongest

signals. Carriers of the minor allele of rs 1386496 had lower alcohol consumption scores while carriers of the minor allele of rs3779084 had higher scores.

TPH2 is involved in the synthesis of serotonin, and DDC is involved in the synthesis of serotonin and dopamine (Haavik et al., 2008). TPH2 is responsible for the rate-limiting step in the biosynthesis of serotonin, the conversion of dietary L-tryptophan to 5-hydroxytryptophan. DDC encodes a protein that is responsible for the next step in the biosynthesis pathway, the conversion of 5-hydroxytryptophan to serotonin. Further, DDC is also involved in the synthesis of dopamine from L-dopa. A large body of research has implicated the involvement of both serotonergic and dopaminergic systems in reward and motivational system functioning, AUDs, nicotine dependence, and other addictive disorders (see Muller et al., 2010).

In this study, we examine whether in a sample of young adult females, the associations of these 2 SNPs with alcohol consumption are mediated by drinking motives as assessed by the DMQ-R. Although the DMQ-R assesses 4 types of motives, here, we focus only on enhancement and coping. As outlined by Cooper (1994) enhancement and coping motives involve drinking to alter affective states that are because of internal sources, as opposed to those that are because of external sources (e.g., to conform with peers or to facilitate social interaction); we reasoned that motivation to drink to alter affect because of sources within the individual would be particularly relevant to genes that regulate serotonin and dopamine synthesis. We hypothesized that coping and enhancement would mediate the effects of these SNPs on alcohol consumption, but only in those who are regular smokers.

MATERIALS AND METHODS

Participant Recruitment and Characteristics

Participants were from the Missouri Adolescent Female Twin Study (MOAFTS; PI Andrew Heath), which is a longitudinal study of a cohort of female twin pairs born between July 1, 1975 and June 30, 1985. At baseline, twins, who were identified from Missouri birth records, were eligible to participate if both members of the twin pair had survived past infancy and were not adopted at birth and if their biological mother was a resident of the state at the time of their birth. Using a cohort-sequential sampling design for initial recruitment, interviews were attempted with at least 1 biological parent (wherever possible, the biological mother) and both twins during 1994 to 1999, when the twins were 13, 15, 17, or 19 years old. Recruitment of the 13-year-olds continued over a 2-year period as twins became age eligible. After obtaining consent from parents and adult twins and assent from minor twins, a telephone diagnostic interview was administered to the twins and their parents. Details of the study design, recruitment, and baseline assessments (which are not included in this study) are given elsewhere (Heath et al., 1999, 2002; Knopik et al., 2005).

Subjects in this study were those who participated in the MOAFTS wave 4 data collection conducted during 2002 to 2005. This sample includes 3,060 women who had been interviewed at baseline, along with 728 women from the baseline sampling frame, who had not participated previously. Wave 4 data were collected via telephone diagnostic interview and a mailed questionnaire. The diagnostic interview was adapted from the Semi-Structured Interview for the Study of the Genetics of Alcoholism (SSAGA; Bucholz et al., 1994) that assessed lifetime DSM-IV psychopathology, and it also included a DSM-based nicotine dependency assessment adapted from the CIDI (Cottler et al., 1991). The mailed questionnaire included the DMQ-R (Cooper, 1994), as well as the Multidimensional Personality Questionnaire (MPQ; Tellegen, 1982).

Genetic Data—As part of a broader study of addiction genetics, 1,536 SNPs that comprise the custom “Addiction” array were geno-typed on a subset of subjects. Genotyping was completed by Illumina and of the 1,536 SNPs, 1,441 (success rate of 93.8%) were successfully typed on 1,188 MOAFTS subjects. These included 23 of the 186 SNPs included as ancestry informative markers. A list of genes that encompass the Addiction array may be found in another publication (Hodgkinson et al., 2008). The genotyping success rate was >99.5% with 100% reproducibility in 220 plated duplicate samples. A detailed characterization of the SNPs and procedures used for cleaning and quality control of the genetic data are available elsewhere (Agrawal et al., 2010).

The sample in this study included 830 women ages 18 to 27 ($Mn = 22.1$), with telephone interview, questionnaire, and genetic data who reported consuming more than 6 alcoholic beverages lifetime. All participants were of European-American descent. The sample included 236 complete twin pairs and 358 singletons. One hundred and forty-two of the pairs were monozygotic (MZ) twins, and 94 were dizygotic (DZ) twins. Of the singletons, 170 individuals were MZ and 188 were DZ.

Measures

The primary outcome measure used in this study was a quantitative alcohol consumption phenotype. Wave 4 interview respondents who reported consuming more than 6 alcoholic beverages were asked to report, retrospectively, on their lifetime and past 12-month alcohol consumption. From these reports, 4 continuous alcohol consumption variables were created from which a latent alcohol consumption variable was extracted using factor analysis. Further details about the measurement characteristics, heritability, and rationale for this alcohol consumption factor are provided elsewhere (Agrawal et al., 2009).

Heaviest Period Typical Weekly Alcohol Consumption (LQNTFRQ)—This is the product of the typical weekly frequency at which the respondents drank and the typical weekly quantity of alcohol they consumed during the 12-month period of heaviest use. These values were log-transformed to account for skewness. Typical frequency of use was taken from an interview question where respondents were asked on how many days they had any alcoholic drink during their period of heaviest use. The responses were coded into 10 categories ranging from “1 day per year” to “every day.” Typical quantity was coded into 13 response categories ranging from “1 to 2” to “30 or more.”

Maximum Drinks in a 24-Hour Period (LMAXALC)—This is the maximum number of drinks reported during a 24-hour period. This is a lifetime measure and might not have occurred during the 12-month period of heaviest use. These values were also log-transformed to account for skewness.

Frequency of Heavy Drinking (FIVE)—This is the frequency at which respondents drank 5 or more drinks in a 24-hour period in the 12-month period of heaviest use. A 6-level variable was created: “never,” “once per month or less,” “2 to 3 times per month,” “1 to 2 times per week,” and “daily.”

Frequency of Intoxication During Period of Heaviest Use (INTOX)—This was assessed using a 14-point scale ranging from “never” to “1 day per year” to “every day.”

Drinking Motives—The DMQ-R (Cooper, 1994), was used to measure enhancement and coping motives (5 items each). In this study, the DMQ-R items were scored on an ordinal scale with 6 response categories (1 = never, 2 = almost never, 3 = some of the time, 4 = about half of the time, 5 = most of the time, and 6 = almost always).

Smoking Status—Following previous work (Kristjansson et al., 2011; Madden et al., 1997), we defined regular smokers as those who had smoked 100 or more cigarettes lifetime or as having smoked 21 to 99 cigarettes but having smoked at least weekly for a period of 2 months or longer prior to the interview. Never/nonregular smokers were defined as those who had never smoked a single cigarette, or those had smoked up to 99 cigarettes but did not smoke weekly during the 2 months prior to the interview.

Genotypes—We created dichotomous variables for each SNP. Homozygotes for the major allele were coded “0,” and heterozygotes and homozygotes for the minor allele were coded “1.” We did not create trichotomous genotype variables because the frequencies for minor allele homozygotes were low: among regular smokers, 14 individuals (4.1%) and 10 individuals (2.9%) were minor allele homozygotes for rs3779084 and rs1386496, respectively; and among never/nonregular smokers, 17 individuals (3.5%) and 16 individuals (3.3%) were minor allele homozygotes for rs3779084 and rs1386496, respectively.

Data Analysis

The mediation analyses were conducted within a multigroup confirmatory factor analytic (CFA) framework using Mplus 6.1 (Muthén and Muthén, 1998–2010). Prior to testing for mediation, we conducted preliminary analyses to examine whether the 2-factor drinking motive model (with correlated factors) and the single-factor alcohol consumption model fit the data well within the smoker and the never/nonregular smoker groups. In the preliminary CFAs for the drinking motives, we used weighted least squares with mean and variance adjustment estimation as is appropriate for ordered categorical data (Lubke and Muthén, 2004). For the alcohol consumption factor, we used a robust maximum likelihood estimator, because the observed alcohol consumption items were continuous. In these analyses (and all that follow), we adjusted the standard errors for nonindependence because of familial clustering. Adequacy of model fit was determined using the Comparative Fit Index (CFI), Tucker-Lewis Index (TLI), and Root Mean Square Error of Approximation (RMSEA).

Because valid between-group comparisons at the latent level require the measurement structures of factors to be sufficiently invariant across groups (Lubke and Muthén, 2004; Meredith, 1993), after determining that the factor models fit the data within each group reasonably well, we used multigroup models to test for measurement invariance. Specifically, we used multigroup CFAs to test for invariance in the thresholds and factor loadings (drinking motive factors) and to test for invariance in the intercepts and factor loadings (alcohol consumption factor) across the regular and never/nonregular smoker groups. Measurement invariance was determined with chi-square difference tests where the fit of the constrained model was compared with the fit of the unconstrained model (Muthén and Muthén, 1998–2010). A significant χ^2 difference ($p < 0.05$) between the 2 models suggested a poorer fit of the constrained model.

Mediation Analyses—Once we determined that the measurement structures of the factors were sufficiently invariant across the groups, we examined whether the relationships between the SNPs and alcohol consumption were mediated by drinking motives. To do so, we used path analysis where we regressed, simultaneously, the alcohol consumption factor onto the enhancement and coping factors, and the alcohol consumption, enhancement, and coping factors onto the 2 SNPs. Our model allowed the enhancement and coping factors to correlate. Preliminary analyses indicated that the SNPs were not significantly associated; consequently, our model did not allow the SNPs to correlate. We examined mediation effects by testing for statistical significance, the total indirect effects, using the products of coefficients approach (MacKinnon, 2000). The total indirect effects represent the

associations of the SNPs with alcohol consumption via the drinking motives, while controlling for all other variables in the model.

To account for the effects of potential confounding variables, we also regressed the alcohol consumption and drinking motive factors onto several covariates that were significantly associated with smoking status and/or alcohol consumption. These included 2 variables that indexed aspects of lifetime psychopathology, a dichotomous age variable that identified participants at or below legal drinking age (18 to 21, coded 0) from those who were older (22 to 27; coded 1) and an early onset drinking variable that identified those who reported consuming at least 1 full drink of alcohol prior to age 16 (coded 1) from those who reported consuming 1 full drink at age 16 or older (coded 0). The covariates that indexed psychopathology were lifetime DSMIV diagnosis of major depression (0 = no depression, 1 = depression diagnosis) and an “externalizing problems” composite. The externalizing problems composite (also coded 1, 0 in the same manner) identified participants who: (i) met criteria for DSM-IV lifetime diagnoses of Alcohol Dependence (AD), Alcohol Abuse (AA), Nicotine Dependence (ND), or (illicit) Drug Dependence (DD); (ii) reported 3 or more criteria for Conduct Disorder; or (iii) scored low (based on a median split) on behavioral control as assessed by the MPQ control scale. The externalizing problems variable was used because the base rates of the abuse and dependence diagnoses were too low to be included as separate covariates. Additionally, a final covariate, zygosity, identified whether participants were MZ twins (coded 0) or DZ twins (coded 1).

RESULTS

Descriptive statistics for the variables included in this study are shown in Table 1 separately for the regular and never/nonregular smoker groups. Also, shown are the results of statistical tests for between-group differences on each of the variables. The observed scores for the 5 items representing each drinking motive were used to compute the mean enhancement and coping scale scores, and lower MPQ control scores (at or below the median) represent less behavioral control. Compared with the never /nonregular smokers, regular smokers had significantly higher coping and enhancement scores, higher levels of quantity/frequency of drinking, less behavioral control, earlier age of drinking onset, and higher rates of lifetime DSM-IV psychopathology. The groups did not significantly differ with respect to age or to the frequencies of the minor allele for both SNPs. Importantly, because the minor allele frequencies did not differ between the groups, the results described later cannot be explained by gene–environment correlations (Purcell, 2002; Scarr and McCartney, 1983).

Also, shown (Table 2) are the bivariate (unconditional) correlations between the independent and dependent variables and the covariates that were included in the mediation models. Correlations below the diagonal are from the regular smoker group, and those above the diagonal are from the never/nonregular smoker group. The alcohol consumption and drinking motive variables are factor scores from the CFA models described later.

Measurement Invariance of the Drinking Motive and Alcohol Consumption Factors

Drinking Motives

For the drinking motives, constraining all of the factor loadings and thresholds across the regular and never/nonregular smoker groups did not result in significant deterioration in fit compared with the unconstrained model, χ^2 (adj. df = 48) = 53.25, $p > 0.05$. This indicated that the enhancement and coping factors were sufficiently invariant across the 2 groups to ensure the drinking motive items were assessing the same latent drinking motive factors in each group. The CFA model with constrained factor loadings and thresholds (which was used to test our hypotheses) fit the data well (CFI = 0.992, TLI = 0.994, RMSEA = 0.060).

Alcohol Consumption

To obtain an adequate fitting baseline CFA model for the alcohol consumption factor, we allowed the residuals for INTOX and FIVE to correlate in the regular smoker group and the residuals for LMAXALC and LQNTFRQ to correlate in the never/nonregular smoker group. Compared with the unconstrained model, constraining all of the intercepts and factor loadings across the regular and never/nonregular smoker groups resulted in a significant deterioration in model fit, $\chi^2(6) = 21.10$, $p < 0.05$. When the intercept for LMAXALC was freely estimated across groups, the reduction in fit was not significant, $\chi^2(5) = 7.23$, $p > 0.05$. Thus, we used the alcohol consumption model with partial invariance to test our hypotheses. Fit indices suggested this model fit the data well (CFI = 0.995, TLI = 0.991, RMSEA = 0.036).

Mediation of the Associations Between the SNPs and Alcohol Consumption by the Drinking Motives

Multigroup Path Models—The path models used to test for mediation in each group are represented in Fig. 1, and the covariate-adjusted, standardized coefficients for each path are shown. In Table 3, we present the standardized and unstandardized coefficients for each path, their standard errors and results of significance tests. Results indicate that in the regular smoker group, the associations of rs3779084 with enhancement and the associations of rs1386496, enhancement, and coping with alcohol consumption all were statistically significant. However, in the never/nonregular smoker group, only the associations of enhancement and coping with alcohol consumption were statistically significant.

Total Effects and Total Indirect Effects of the SNPs on Alcohol Consumption

—Total effects summarize the association of an SNP with alcohol consumption through all of the paths leading from that SNP to alcohol consumption. In contrast, the total indirect effect summarizes the associations of an SNP with alcohol consumption via the enhancement and the coping motives. The total indirect effect coefficient represents mediation, because it includes all the pathways from an SNP to alcohol consumption via both drinking motives, while controlling for (excluding) the SNP's direct path to alcohol consumption. The covariate-adjusted, standardized total effects and total indirect effects of each SNP on alcohol consumption in the regular and the never/nonregular smoker groups are presented in Table 4. In the never/nonregular smoker group, the total effects and the total indirect effects were not statistically significant; in this group, we found no evidence for a significant association of the SNPs with alcohol consumption and no evidence for mediation by the drinking motives.

In the regular smoker group, the total effects of both SNPs on alcohol consumption were statistically significant. However, the total indirect effect of rs1386496 on alcohol consumption was not statistically significant; similar to the never/nonregular smokers, in regular smokers, there was no evidence that the drinking motives mediated the rs1386496–alcohol consumption association. In contrast, the total indirect effect of rs3779084 on alcohol consumption was statistically significant, suggesting the rs3779084–alcohol consumption association was mediated by the drinking motives.

Test for Moderated Mediation—We directly tested whether the level (magnitude of) mediation by the drinking motives differed between the regular smoker and never/nonregular smoker groups. Specifically, using a test for moderated mediation, we examined whether the total indirect effect coefficient that summarized the rs3779084–drinking motive–alcohol consumption pathways in each group differed between the groups. To do so, we constrained the total indirect effects to be equal across the groups and tested for a statistically significant deterioration in model fit using a chi-square difference test. Results

indicated that the fit of the constrained model was not significantly different from the fit of the unconstrained model, $\chi^2(4) = 2.94, p > 0.05$. However, a Monte Carlo simulation study suggested the power to detect this difference with the current sample size was only about 50%. Thus, the probability of this result being a Type II error is high.

Specific Indirect Effects of rs3779084 on Alcohol Consumption in Regular Smokers—Because the total indirect effect of rs3779084 on alcohol consumption was statistically significant in the regular smoker group (suggesting mediation by both drinking motives), we conducted additional analyses to examine whether or not the association between rs3779084 and alcohol consumption was mediated by enhancement only or by coping only. To do so, we tested the specific indirect effects for statistical significance. Specific indirect effects refer to the association of an SNP with alcohol consumption through 1 and only 1 of the drinking motives, while adjusting for the effects of all other variables in the model. The specific indirect effect for the rs3779084–enhancement–alcohol consumption pathway was not statistically significant, $z = 1.73, p > 0.05$, nor was the specific indirect effect for the rs3779084–coping–alcohol consumption pathway, $z = 1.45, p > 0.05$. This suggests there is not sufficient evidence to attribute the mediation effect to only 1 drinking motive; the results are more in line with the interpretation that both motives mediate the rs3779084–alcohol consumption association.

Follow-Up Analyses—Although our mediation models included covariates to adjust for zygosity, age, and psychopathology, we conducted 1 follow-up analysis to test the robustness of our results. Because the regular smokers tended to consume alcohol more frequently and heavily than did the never/nonregular smokers, we stratified the sample by levels of alcohol consumption and again tested for mediation. Here, we used CFA to compute and output alcohol consumption factor scores for the entire sample. We then used a median split of the factor scores to divide the sample into heavier/more frequent and lighter/less frequent drinker groups and re-ran the multigroup mediation model (including the same covariates) using the factor scores as the dependent variable. Results indicated that the total indirect effects of the SNPs on alcohol consumption in both groups were not statistically significant (all $ps > 0.30$), suggesting that the mediation of rs3779084 on alcohol consumption by the drinking motives has a degree of specificity to the regular smoker phenotype.

DISCUSSION

In the present study, we examined whether 2 drinking motives, enhancement and coping, mediate the effects of 2 measured genotypes on alcohol consumption in young adult female regular and never/nonregular smokers. Based on earlier work, we hypothesized that in regular smokers, enhancement and coping would mediate the relationships between alcohol consumption and 2 SNPs (rs1386496 from TPH2 and rs3779084 from DDC) in genes involved in serotonin and dopamine biosynthesis.

Our results partially support our hypothesis. Unexpectedly, we found no evidence for a motive-mediated association between rs1386496 and alcohol consumption in either group. In line with our hypothesis, we did find that in regular smokers, the association between rs3779084 and alcohol consumption was mediated by enhancement and coping motives, but in never/nonregular smokers, there was no such evidence. Although a statistical test for a between-groups difference in the magnitudes of mediation (i.e., moderated mediation) was not statistically significant, a power analysis indicated with the current sample size, the probability of Type II error associated with this test was unacceptably high. Overall, our results suggest that in the regular smoker group, minor allele carriers reported more motivation to consume alcohol for positive and negative reinforcement reasons, to attain or

improve a positive affective state, and to relieve a negative one. Thus, 1 key finding in the present study is that in regular smokers, variability at the locus marked by rs3779084 appears to index biologically based individual differences in the motivation to consume alcohol. A second key finding is that the rs3779084–drinking motive–alcohol consumption relationship has some degree of specificity to the regular smoker pheno-type; the mediation results were robust to covariate adjustment, and we did not find similar evidence for mediation in the never/nonregular smokers, in those who drank more heavily and more frequently or in those who drank less heavily and less frequently. This pattern of results is consistent with a gene $\times$ smoking status interaction.

The rs3779084 SNP is intronic, and to date, the functional significance of this polymorphism, if any, is not known. Likewise, currently, it is unknown whether this SNP is in linkage disequilibrium with, and serves as a proxy for, another functional DDC SNP. Given these unknowns, we can only speculate on the mechanisms involved in this putative interaction; biologically based individual differences in the motivation to consume alcohol could be because of individual differences in the sensitivity of mesocorticolimbic “reward” circuitry. The DDC gene is involved in the synthesis of dopamine, and dopamine is a major neurotransmitter responsible for mediating reward signaling.

These putative individual differences in reward system sensitivity might be explained by a gene $\times$ environment interaction. Here, smoking status could be considered either a putative exogenous or a “modifying endogenous” environment. In the exogenous case, exposure to a social milieu associated with regular smoking, such as situations where drinking and smoking jointly occur, or having a network of peers who smoke and drink, might modify the association between the SNP and drinking for enhancement and coping reasons (and subsequent alcohol consumption) in regular smokers.

The presence of nicotine within the brain can be considered a modifying endogenous environment. Substantial research suggests the presence of nicotine alters structure and function at the neuronal, neurotransmitter and circuitry levels, particularly within the mesocorticolimbic system (Markou, 2008; Portugal and Gould, 2008). Although nicotine is pharmacologically active at many sites throughout the brain, its primary targets are the nicotinic acetylcholine receptors (nAChRs) on glutamate terminals in the ventral tegmental area (VTA; Markou, 2008). In addition, nicotine binds to nAChRs on GABA-releasing terminals in the VTA. The result is increased dopamine transmission within the VTA and interactions among dopamine, glutamate, GABA, and acetylcholine neurotransmitters. Subsequently, dopamine is released in several mesocorticolimbic structures, which drives the rewarding, pleasurable effects of nicotine (Mansvelder and McGehee, 2002; Mansvelder et al., 2002; Markou, 2008). Studies also suggest nicotine exposure is associated with neuroadaptations within mesocorticolimbic structures. These, for example, include long-term potentiation and long-term depression of neurons within the VTA and nucleus accumbens (Thomas and Malenka, 2003), nAChR desensitization and up-regulation (Mansvelder et al., 2002; Wonnacott, 1990), and change in the number nAChRs (Wonnacott, 1990). Thus, we assume the “neurobiological environment” in which the regular smokers consumed alcohol and developed their enhancement and coping motivations to drink substantially differs from that of the never/nonregular smokers in our sample. This regular smoking neurobiological environment might account for altered gene expression in the regular versus the never/nonregular smokers and in regular smokers who differ on rs3779084.

CONCLUSION

Research in humans (Markou, 2008) and in rodents (Harrison et al., 2002) indicates that acute nicotine lowers reward thresholds for numerous types of stimuli. With respect to alcohol, evidence from rodent models suggests that nicotine potentiates the rewarding effects of alcohol and increases and/or prolongs the motivation to consume it (Blomqvist et al., 1996; Clark et al., 2001; Larsson and Engel, 2004; Le et al., 2000, 2003, 2009; Potthoff et al., 1983; Smith et al., 1999). Conversely, treating rats with the nicotinic receptor antagonist, mecamylamine, decreases alcohol consumption (Blomqvist et al., 1996; Larsson and Engel, 2004; Le et al., 2000). Placebo-controlled co-administration studies with humans have found the same general patterns (Barrett et al., 2006; Chi and de Wit, 2003; Kouri et al., 2004; Perkins et al., 1995). Further, outside of a laboratory setting, Kristjansson et al. (2011) found that compared with young adult, female nonregular smokers, regular smokers reported more motivation to drink for enhancement and coping reasons and also that genetic influences on these motives were stronger. The present study complements and extends this research by identifying a potential biological mechanism indexed by variability at the rs3779084 locus in the DDC gene that is associated with increased motivation to consume alcohol for affect related reasons in young adult females who smoke regularly.

LIMITATIONS

Study limitations might influence the interpretation and generalizability of our results. First, participants were European-American, Missouri-born, young adult, female twins who might not be representative of individuals from other ethnic backgrounds or geographic regions, and our results might not extend to samples of different ages or to males. Comparable data on males are not available. While a data set on a small number of African-American twins is available, it lacks power. Second, we do not account for heterogeneity in regular smokers (e.g., regular smokers who are nicotine dependent), particularly the extent to which nicotine dependence influences the structure of drinking motives or the mediation analysis. However, the size of our sample precludes further refinement of smoking phenotypes. Third, we use the same sample that Agrawal et al. (2010) used in their study that identified the associations between the rs3779084 and rs1386496 SNPs with alcohol consumption. Thus, our results should be considered preliminary until these associations are investigated in an independent and larger sample.

The rs3779084 SNP is intronic, and the functional significance of this polymorphism, if any, is not known. To explain our results, we speculate that the rs3779084 (or some other functional variant in linkage disequilibrium with it) is involved in modulating reward system sensitivity to alcohol in regular smokers. Although speculative, it is not unlikely. The DDC gene is involved in the catalysis of dopa decarboxylase, an enzyme that synthesizes dopamine from L-Dopa (Haavik et al., 2008). In the brain, dopamine primarily is synthesized in 3 areas, the substantia nigra, striatum, and VTA, where it activates 5 types of dopamine receptors, D1 to D5 (Haavik et al., 2008). Phasic dopamine release in the nucleus accumbens, amygdala, dorsal striatum, and prefrontal cortex appears to mark the motivational significance and the value of particular experiences, cues, and action responses (Hyman et al., 2006). In the context of this study, phasic dopamine release in these substrates (via nicotine binding to nAChRs in the VTA) when paired with (and perhaps facilitated by) alcohol, could lead to greater valuation of and motivation to consume alcohol in smokers. It is possible that the rs3779084 SNP in DDC could be involved in even greater valuation and motivation among minor allele carriers who smoke.

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Fig. 1. Model of structural paths from rs3779084 and rs1386496 to drinking motives to alcohol consumption in the regular and never/nonregular smoker groups. Statistically significant paths are represented by solid-lined arrows, and nonsignificant paths are represented by dotted-lined arrows. Correlations between the motives are represented by solid-lined bidirectional arrows. The coefficients are standardized and adjusted for zygosity, age, early drinking onset, DSM-IV depression and externalizing problems. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Descriptive Statistics for Regular and Never/Nonregular Smoker Groups and Results of Statistical Tests for Between-Group Differences

Table 1

Continuous variables	Regular smokers (<i>n</i> = 344)		Never/nonregular smokers (<i>n</i> = 486)		Between-group differences	
	<i>Mn (SD)</i>	range	<i>Mn (SD)</i>	range	<i>z</i>	<i>p</i>
Enhancement scale	3.01 (1.3) ^a	1–6	2.79 (1.2)	1–6	2.38	< 0.05
Coping scale	2.08 (1.05)	1–6	1.78 (0.93)	1–5.8	4.22	< 0.001
Maximum typical consumption (LQNTFRQ)	2.06 (1.14) ^b	0.140–4.40	1.32 (1.00) ^c	0.140–3.98	9.11	< 0.001
Maximum drinks in 24-hours (LMAXALC)	2.49 (0.52) ^a	0.693–4.17	2.09 (0.58)	0.693–3.49	9.80	< 0.001
Frequency of heavy drinking (FIVE)	1.56 (1.70) ^a	0.00–7.00	0.67 (1.11) ^d	0.00–7.00	8.21	< 0.001
Frequency of intoxication (INTOX)	1.23 (1.48) ^e	0.00–7.00	0.54 (0.91) ^f	0.00–7.00	7.39	< 0.001

Dichotomous variables	Frequency (%)		Frequency (%)		Odds ratio (95% CI)	
rs3779084 (minor allele homo/heterozygotes)	143 (41.6)	184 (37.9)	1.17 (0.84–1.62)			
rs1386496 (minor allele homo/heterozygotes)	104 (30.2)	154 (31.7)	0.93 (0.66–1.32)			
Older age (>21)	206 (59.9)	274 (56.4)	1.16 (0.84–1.59)			
Early drinking onset (<16)	190 (55.2)	131 (27)	3.34 (2.47–4.53)			
Major depression	85 (24.7)	71 (14.7)	1.86 (1.29–2.66)			
Multidimensional Personality Questionnaire control Scale (median)	170 (53.3) ^g	177 (38.3) ^h	1.52 (1.15–2.02)			
3 Conduct disorder criteria (CD)	26 (7.8) ⁱ	1 (0.2) ^j	n/a			
Nicotine dependence (ND)	163 (47.4)	–	–			
Alcohol dependence (AD)	42 (12.2)	26 (5.3)	2.46 (1.44–4.19)			
Alcohol abuse (AA)	33 (9.6)	16 (3.3)	3.12 (1.69–5.74)			
Other drug dependence (DD)	26 (7.6)	3 (0.6)	n/a			
Externalizing problems composite	264 (76.7)	198 (40.7)	4.80 (3.51–6.56)			

Based on subjects with complete data; n/a: test of between-group differences not performed because of low frequencies.

^a₃₄₃

^b₃₄₂

^c₄₈₅

^d₄₈₄

e_{337}
 f_{476}
 g_{319}
 h_{462}
 i_{334}
 j_{483}

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Table 2

Bivariate Correlations Among the Alcohol Consumption and Drinking Motive Factors, Single-Nucleotide Polymorphisms, and Covariates in the Regular Smoker (Below Diagonal) and Never/Nonregular Smoker Groups

	1.	2.	3.	4.	5.	6.	7.	8.	9.
1. Alcohol consumption	–	0.436	0.421	0.067	–0.147	0.073	0.270	0.119	0.257
2. Enhancement	0.316	–	0.684	0.036	–0.038	–0.192	0.226	–0.024	0.138
3. Coping	0.325	0.725	–	0.034	–0.080	–0.157	0.242	0.029	0.110
4. rs3779084	0.163	0.159	0.111	–	–0.035	–0.010	0.007	–0.038	0.014
5. rs1386496	–0.152	–0.096	–0.065	0.016	–	–0.129	–0.226	–0.105	0.034
6. Older age (>21)	0.060	–0.215	–0.127	0.007	0.059	–	–0.291	0.132	–0.049
7. Early drinking onset (<16)	0.183	0.058	0.032	0.113	0.096	–0.204	–	0.035	0.199
8. Major depression	0.037	–0.023	–0.021	–0.032	0.034	0.123	0.071	–	–0.040
9. Externalizing composite	0.203	0.170	0.189	–0.018	–0.102	–0.202	0.100	0.517	–

Correlations in bold are statistically significant ($p < 0.05$).

Table 3
Estimates and Results of Significance Tests of Coefficients from the Covariate-Adjusted Path Model

Independent variable–dependent variable	Regular smokers				Never/nonregular smokers			
	Est.	SE	Z	p	Est.	SE	Z	p
rs3779084–enhancement	0.275 (0.265)	0.124	2.22	<0.05	0.053 (0.051)	0.107	0.49	ns
rs3779084–coping	0.194 (0.189)	0.122	1.59	ns	0.041 (0.040)	0.111	0.37	ns
rs1386496–enhancement	−0.143 (−0.137)	0.129	−1.10	ns	−0.060 (−0.058)	0.109	−0.55	ns
rs1386496–coping	−0.117 (−0.115)	0.129	−0.91	ns	−0.133 (−0.129)	0.116	−1.15	ns
rs3779084–alcohol consumption	0.164 (0.175)	0.115	1.43	ns	0.144 (0.166)	0.080	1.79	ns
rs1386496–alcohol consumption	−0.268 (−0.286)	0.122	−2.20	<0.05	−0.161 (−0.186)	0.091	−1.78	ns
Enhancement–alcohol consumption	0.188 (0.208)	0.070	2.69	<0.01	0.255 (0.305)	0.046	5.55	<0.001
Coping–alcohol consumption	0.207 (0.225)	0.072	2.89	<0.01	0.167 (0.199)	0.052	3.21	<0.001

Standardized coefficients are shown in parentheses, and statistically significant coefficients are shown in bold font.

Table 4

Estimates and Results of Significance Tests of the Total Effects and Total Indirect Effects of the Single-Nucleotide Polymorphisms (SNPs) on Alcohol Consumption

Dependent variable: Alcohol consumption									
Total effects (association that includes all paths from the SNP to alcohol consumption)					Total indirect effects (association that includes paths from the SNP to motives and motives to alcohol consumption)				
Group	SNP	Est.	SE	Z	p	Est.	SE	Z	p
Regular smokers	rs3779084	0.256 (0.273)	0.123	2.09	<0.05	0.092 (0.098)	0.045	2.02	<0.05
	rs1386496	-0.319 (-0.340)	0.127	-2.51	<0.05	-0.051 (-0.054)	0.045	-1.14	ns
Never/nonregular smokers	rs3779084	0.164 (0.190)	0.088	1.86	ns	0.020 (0.023)	0.040	0.51	ns
	rs1386496	-0.198 (-0.229)	0.102	-1.95	ns	-0.038 (-0.043)	0.042	-0.89	ns

Standardized coefficients are shown in parentheses, and statistically significant coefficients are shown in bold font.