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The Dopamine D₄ Receptor (DRD4) Gene Exon III Polymorphism, Problematic Alcohol Use, and Novelty Seeking: Direct and Mediated Genetic Effects

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Abstract

The present study sought to integrate convergent lines of research on the associations among the dopamine D₄ receptor (DRD4) gene, novelty seeking, and drinking behaviors with the overall goal of elucidating genetic influences on problematic drinking in young adulthood. Specifically, this study tested a model in which novelty seeking mediated the relationship between DRD4 VNTR genotype and problematic alcohol use. Participants ($N = 90$, 40 females) were heavy drinking college students. Analyses using a Structural Equation-Modeling (SEM) framework suggested that the significant direct path between DRD4 VNTR genotype and problematic alcohol use was reduced to a trend level in the context of a model that included novelty seeking as a mediator, thereby suggesting that the effects of DRD4 VNTR genotype on problematic alcohol use among heavy drinking young adults were partially mediated by novelty seeking. Cross-group comparisons indicated that the relationships among the model variables were not significantly different in models for men versus women. These results extend recent findings of the association between this polymorphism of the DRD4 receptor gene, problematic alcohol use, and novelty seeking. These findings may also help elucidate the specific pathways of risk associated with genetic influences on alcohol use and abuse phenotypes.

Keywords

alcohol; DRD4 VNTR; novelty seeking; genetics; mediation; structural equation modeling

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Introduction

Genetic variation in neurotransmitter systems has been implicated in multiple behavioral phenotypes and psychiatric disorders. The dopamine D₄ receptor (DRD4) has been examined as a gene of interest for behavioral and psychiatric phenotypes in part because of its theoretical plausibility and genetic variability. The DRD4 gene contains a 48 base pair variable number of tandem repeats (VNTR) in exon III with lengths varying from 2 to 11 repeats, with 3 with common variants of 2 (D4.2), 4 (D4.4) and 7 repeats (D4.7) (Grady et al 2003; Van Tol et al., 1992). Variations in length of the VNTR have been shown to have functional effects on the receptor (Asghari et al 1995; Oak et al 2000). In vitro, while the D4.7 variant does not appear to bind dopamine antagonists and agonists with greater affinity than the D4.2 or D4.4 variants (Asghari et al., 1994; Van Tol et al., 1992) there is evidence that the D4.7 demonstrates a blunted intracellular response to dopamine (Asghari et al., 1995). This relationship is such that the D4.7 variant is associated with attenuated inhibition of intracellular cyclic AMP (Oak et al 2000). It should be noted that the VNTR is found in the third cytoplasmic loop of the folded protein, which interacts with SH3 domain-binding proteins but does not appear to be a G-protein coupling site (Asghari et al 1994; Oak et al., 2000).

D₄ receptors are structurally very similar to D₂ receptors and are localized in various brain regions, including the cerebral cortex, amygdala, hypothalamus, the pituitary, and other limbic brain structures (Asghari et al 1995; Van Tol et al., 1991). Expression of D₄ receptors in the prefrontal cortex is of particular interest for behavioral phenotypes since these regions are involved in attention and cognition (Oak et al 2000). Results from the animal literature suggest that DRD4 knockout mice display hypersensitivity to drugs of abuse such as ethanol, cocaine and methamphetamine (Rubinstein et al., 1997). Other studies using DRD4 knockout mice show they perform better than their wild-type litter mates on complex motor tasks (Rubinstein et al., 1997) and show enhanced cortical glutamate neuronal activity (Rubinstein, 2001), supporting the notion that DRD4 normally act as inhibitors of neuronal activity. Interestingly, DRD4 knockout mice show decreased behavioral exploration of novel stimuli (Dulawa, 1999).

In the human literature, genetic variation in the DRD4 gene has been examined in the context of personality traits (i.e., novelty seeking and impulsivity) and alcohol-related (i.e., drinking and alcohol craving) phenotypes, among other areas. Novelty seeking (NS) has been conceptualized as a dopamine-mediated heritable tendency towards exploration and excitement in response to novel stimuli (Cloninger et al., 1993). As such, genetic variation in the DRD4 VNTR represents a plausible candidate gene underlying NS behavior. Research on the association between the DRD4 VNTR genotype and NS has produced conflicting results. Some studies have found support for an association between the DRD4 VNTR and NS, such that carriers of the 7-repeat “long” (L; i.e. ≥ 7 repeats) allele of the DRD4 VNTR score higher on measures of novelty seeking as compared to non-carriers (e.g., Laucht et al., 2007; Laucht et al., 2005). However, other studies have failed to replicate these findings (for a review see Kluger et al., 2002).

In the context of alcohol-related phenotypes, the DRD4 VNTR has also produced equivocal findings. Although the direct association between DRD4 and alcohol diagnosis has yielded largely negative results (Tyndale, 2003), the DRD4 VNTR has been significantly associated with alcohol craving in the laboratory, such that carriers of an L allele exhibited higher alcohol craving and consumption, as compared to individuals who were homozygotes for the “Short (S) allele” (i.e., < 7 repeats) (Hutchison et al., 2002, 2003, 2006; MacKillop et al., 2007; McGeary et al., 2006); although a recent study failed to replicate these findings (van den Wildenberg et al., 2007). Novelty seeking and drinking behaviors have in turn been

associated in the literature and the results have generally supported the finding that high levels of novelty seeking predict increased substance use (Etter et al., 2003; Gabel et al., 1999; Rose et al., 1998).

Efforts to explain these equivocal findings regarding the relationships among DRD4, novelty seeking, and drinking behaviors have pointed towards a number of methodological and conceptual issues. Specifically, as noted by Laucht et al. (2007), the role of genetic factors in the etiology of alcoholism may vary at different stages of the trajectory between use initiation and dependence. This notion is consistent with the developmental behavioral-genetic perspective of alcoholism risk proposed by Rose (1998), in which the genetic and environmental influences on alcoholism risk vary across development. For example, the role of genetic factors in alcohol use initiation may be largely negligible but once alcohol use is initiated, differences in quantity and frequency of drinking appear to be strongly influenced by genetic factors (Rose, 1998). Likewise, a twin study of personality traits in later adulthood has suggested that novelty seeking, but not other dimensions of personality, declined in total phenotypic variance across age cohorts (Heiman et al., 2003). Additionally, conflicting results highlight the need to carefully consider the phenotypes under study, given that more refined phenotypic measures, and endophenotypes, may increase sensitivity to detect genetic influences on behavior (e.g., Gottesman & Gould, 2003; Hines et al., 2005). This is especially important when considering that the relative effect of single genetic polymorphisms on complex behaviors, such as alcohol use and abuse, is likely to be small in magnitude (Heath & Phil, 1995).

In summary, there have been a number of studies examining associations among the DRD4 VNTR genotype, novelty seeking, and drinking phenotypes. Yet, very few studies have integrated these converging lines of research. In one such study, however, Laucht and colleagues (2007) found that NS mediated the association between the DRD4 VNTR genotype and heavy drinking in male adolescents from a high-risk community sample. This study seeks to replicate and extend the findings of Laucht et al. (2007) by addressing the following objectives: (1) to examine the interrelationships among DRD4, novelty seeking, and drinking behaviors in order to test the direct effects of DRD4 genotype on novelty seeking and drinking behaviors; and (2) to test a mediational model in which novelty seeking mediates the relationship between genotype and drinking behaviors using a heavy drinking mixed sex college sample. From a developmental perspective this may be an opportune time to assess those relationships among genotype, personality traits, and drinking as the incidence of both alcohol use and novelty seeking behaviors have been shown to peak in early adulthood (e.g., Clements, 1999; Heiman et al., 2003; Wechsler et al., 1994) and the frequency and quantity of alcohol use appears to be under considerable genetic influence at this stage in development (Rose et al., 1998). Consistent with the recent findings of Laucht et al. (2007), we hypothesize that the relationship between genotype and alcohol use will be mediated by novelty seeking.

Materials and Methods

Participants

One hundred and one men and women (44 females) were recruited from a college campus through flyers and announcements in the weekly email bulletin. A total of 90 participants (50 males and 40 females) provided complete data and were included in this study (10 participants did not complete the TPQ-NS and 1 had missing genetic data). The average age was 21.9 ($SD = 1.5$; Range = 21–29) and 93% of the sample was Caucasian. Inclusion criteria were the following: (1) a score of 8 or higher on the Alcohol Use Disorders Identification Test (AUDIT; Allen et al., 1997), indicating a hazardous drinking pattern; and (2) self-reported drinking frequency of 3 or more drinks (2 for women) at least twice per

week. Although there was no exclusionary upper limit of alcohol use for this study, participants who were currently trying to quit drinking or had a history of treatment for a drinking problem were excluded and offered treatment referrals.

Procedure

The study protocol was approved by the Human Research Committee at the University of Colorado and all participants provided written informed consent after receiving a full explanation of the study. Eligibility was determined through telephone interviews and eligible participants were invited to the laboratory for a testing session. Upon arrival at the lab, participants provided informed consent, a saliva sample for DNA analyses, and completed a series of self-report measures of demographics, personality, and drinking behavior. A subset of participants ($n = 38$) was selected based on genotype for the μ -opioid receptor gene (OPRM1) to complete an alcohol challenge session and the results of the experimental study with this genetically selected sub-sample are presented elsewhere (Ray & Hutchison, 2004).

Behavioral Measures

Alcohol use—was evaluated with a variation of the measure used by White and Labouvie (1989). The instructions defined one alcoholic drink as “one beer, one glass of wine, or one serving of hard liquor either by itself or in a mixed drink.” Two items asked “In the last six months, (1) “how often did you consume at least one alcoholic drink?” (answered on a 9-point scale ranging from “never” to “every day”), and (2) “how many drinks did you usually have at one time?” (answered on a 10-point scale ranging from “none” to “more than 20 drinks”). Participants in this study drank an average of 4.9 ($SD = 2.2$) standard drinks per drinking episode and reported an average drinking frequency of slightly over twice weekly. Quantity and frequency items were standardized and averaged to form an alcohol use index, termed *DRINK*. Participants also reported the maximum number of drinks consumed in a single drinking episode during the past year ($M = 13.9$, $SD = 5.9$) and this drinking variable was termed *MAX*.

Rutgers Alcohol Problem Index (RAPI)—The RAPI was used to assess alcohol-related problems. This scale consists of 23-items examining the impact of alcohol on social and health functioning over the past year. In this sample, the average score on the RAPI was 22.2 ($SD = 15.5$). The RAPI has high reliability and validity (White & Labouvie, 1989) and the observed Cronbach's α in this study was 0.93.

Tridimensional Personality Questionnaire, Novelty Seeking Scale (TPQ-NS)—The Novelty Seeking Scale of the TPQ (Cloninger, Svrakic, and Przybeck, 1993) was used in this study. Novelty seeking represents one of the 4 higher order personality dimensions as defined by Cloninger's theory and consists of the sum of the following subscales: Exploratory excitability versus stoic rigidity (NS1), impulsiveness versus reflection (NS2), extravagance versus reserve (NS3), and disorderliness versus regimentation (NS4). The TPQ-NS consists of 35 True/False items, such as “I do things spontaneously” and “I usually think about all of the facts in detail before I make a decision” (reverse scored). The average score on the TPQ-NS in this sample was 21.6 ($SD = 5.2$, Range: 8–32) and the observed Cronbach's α was 0.76.

DNA Analyses

DNA was collected following published procedures (see Freeman et al., 1997; Walker et al., 1999). Participants were asked to swab their cheeks with three cotton swabs, followed by a rinse of the mouth with tap water. Genomic DNA was isolated from buccal cells using a

modification of published procedures (Lench et al., 1988; Spitz et al., 1996). The 48 bp VNTR in the DRD4 was assayed using previously reported methods (Hutchison et al., 2002). The primer sequences used are forward, 5'-AGGACCCTCATGGCCTTG-3' (fluorescently labeled), and reverse, 5'-GCGACTACGTGGTCTACTCG-3' (Lichter et al., 1993). Alleles were visualized using capillary electrophoresis. Based on previous molecular work suggesting that the 7 repeat allele may confer a functional difference in D₄ receptors (Asghari et al., 1995) and molecular work demonstrating that the 7 repeat allele is quite distinct from the 2–6 repeat alleles and likely originated as a rare mutational event that became more frequent as a result of positive selection (Ding et al., 2002), participants were classified as DRD4 L (i.e., homozygous or heterozygous for an allele ≥ 7 repeats; S/L or L/L, coded “1”; $n = 29$) or as DRD4 S (i.e., both alleles < 7 repeats; S/S, coded “0”; $n = 61$). For quality assurance purposes in the event of ambiguity in the genotyping the assay is run in duplicate or triplicate in order to verify the results. The observed genotype frequencies (i.e., approximately 30% of participants were carriers of the L allele) are consistent with previous studies of primarily Caucasian and unselected samples (e.g., Hutchison et al., 2002, 2003; Munafò et al., 2008).

Data Analysis

Pearson Product Moment correlations were calculated to examine the interrelationships among the variables of interest. Mediation analyses were conducted using a Structural Equational Modeling (SEM) framework. The hypothesized model (see Figure 1) examined novelty seeking as a mediator of the effects of DRD4 VNTR genotype on alcohol use. Problematic alcohol use was conceptualized a latent variable with three indicators: (1) RAPI score for the past 12-months; (2) Standardized quantity and frequency of drinking over the past 12-months (DRINK); and (3) Highest number of drinks in a single drinking episode over the past 12-months (MAX). Modeling analyses were conducted using the EQS Version 6.1 for Windows structural equation-modeling program (Bentler, 1995). Model fit was assessed with the comparative fit index (CFI, Bentler, 1990) and the root mean square error of approximation (RMSEA; Browne & Cudeck, 1993). Both the CFI and the RMSEA are sensitive to model misspecification and are minimally affected by sample size (Hu & Bentler, 1995). The CFI ranges from 0 to 1, with .90 indicating acceptable fit (Bentler, 1990). The RMSEA ranges from 0 to ∞ , where fit values less than .05 indicating close fit and values less than .10 indicating reasonable fit (Steiger, 1990). In addition, model fit was assessed using the standardized root mean square residual (SRMR), which represents the standardized difference between the observed covariance and predicted covariance, with a value of zero indicating perfect fit. This measure tends to be smaller as sample size increases and as the number of model parameters increases. A value less than .08 indicates a good fit (Hu & Bentler, 1999).

Results

DRD4 groups differed significantly with regard to gender, such that 20% ($n = 8$) of women had at least one copy of the DRD4-L allele, as compared to 40% ($n = 20$) of men ($\chi^2 = 4.43$, $p < .05$). DRD4 groups, however, did not differ with regard to age or ethnicity ($p > .10$), therefore, it is highly unlikely that population stratification confounded the analyses presented herein. Correlations among the study variables are presented in Table 1.

The estimated mediational model with standardized path coefficients is presented in Figure 1. The model was found to provide an adequate fit of the data, $\chi^2(4, N = 89) = 1.66$, $p = .80$, CFI = 1.00, RMSEA = .00, 90% CI [.00 to .10], SRMR=.02. This model accounted for 23% of the variance in problematic alcohol use. In the proposed model, DRD4 VNTR genotype was found to be significantly associated with novelty seeking, such that carriers of the Long allele scored higher on a trait measure of novelty seeking (i.e., TPQ-NS), $B = .30$, $p < .05$.

Novelty seeking, in turn, was significantly and positively associated with problematic alcohol use, such that individuals reporting higher trait levels of novelty seeking reported higher problematic alcohol use, $B = .34, p < .01$. The following strategy was used to test mediation. First, we estimated a model with only the predictor (i.e., DRD4 VNTR genotype) and outcome variable (i.e., problematic alcohol use) included to assess the direct effect exclusive of any mediator variables. Results indicated a significant direct relationship between the predictor and the criterion, $B = .274, p < .05$. Second, we included a direct path from the DRD4 VNTR to problematic alcohol use in the context of the mediational model to determine whether the significant bivariate relationship between predictor and outcome had been reduced to nonsignificance. As can be seen in Figure 1, the direct effect from DRD4 VNTR genotype to alcohol use was reduced to $B = .245, p = .07$, and the implementation of the Sobel test via EQS suggested that the indirect effect from DRD4 VNTR genotype to alcohol use through novelty seeking showed a trend towards significance, $est = .274, p = .06$. Both of these findings, i.e., a reduction in the size of the direct effect from predictor to criterion in the presence of the mediator, and a close to significant test of the indirect effect, are suggestive of at least partial mediation.

Given that sex was significantly associated with the variables in the hypothesized mediational model and in a recent study (Laucht et al., 2007) we estimated the model in Figure 1 as a cross-groups analysis in EQS to test for moderation of any model relationships by sex. In a cross-group analysis in EQS, Lagrange multiplier tests (LM tests) are calculated for each parameter constrained to be equal across groups (Bentler, 1995). This approach was chosen over testing separate models for males and females given sample size limitations and resulting lower statistical power. This approach allows us to capitalize on the standard errors in the full sample in our tests of cross-group differences, thus reducing the probability of Type II error that would be incurred from conducting two separate tests. A significant LM test suggests a parameter on which the two groups differ. A 1 *df* chi-square test of change ($\chi^2\Delta$) in model fit when a path is constrained to be equal across groups versus when it is allowed to differ across groups provides a test of the equality of a path in the two groups. A significant $\chi^2\Delta$ suggests that the path is indeed different across groups. Results revealed that none of the LM tests were significant, suggesting that the relationships among the variables were not significantly different for men versus women. Moreover, the cross-group model constraining all structural paths and the loadings on the alcohol latent factor to be equal across sex provided an adequate fit to the data, $\chi^2(13, N = 89) = 7.35, p = .88$; CFI = 1.00; RMSEA = .00, 90% CI [.00 to .07]; SRMR = .06. Together, these results provide evidence of partial mediation. Specifically, the effects of DRD4 VNTR genotype on problematic alcohol use in this heavy-drinking college sample are partially accounted for by novelty seeking personality traits, and these effects are not moderated by sex.

Discussion

The objective of the present study was to integrate convergent lines of research on the associations among the DRD4 VNTR genotype, novelty seeking, and drinking behaviors. To that end, we examined a multivariate mediational model in which the effect of the DRD4 VNTR genotype on problematic alcohol use was mediated by novelty seeking. Results of SEM-based analyses provided support for partial mediation, whereby including novelty seeking in the model reduced the significance of the bivariate relationship between the candidate gene and problematic alcohol use in a sample of heavy drinking college students. These results replicate and extend those of Laucht and colleagues (2007) and indicate that the mediational effects of novelty seeking may be present among both males and females. Contrary to Laucht et al. (2007), these results suggest partial mediation of genotype effects on drinking behavior through novelty seeking, as opposed to full mediation. Moreover, in the present study, these effects were not moderated by gender, as reported by Laucht and

colleagues (2007). Notable differences in sample characteristics, such as the fact that the current study focused on heavy-drinking college students whereas Laucht et al. (2007) studied a selected sample of at risk adolescents, may explain the differences in the level of mediation and the gender-specificity of the effects.

As recently articulated by Dick and colleagues (2006), one of the major challenges in the field of psychiatric genetics consists of delineating the risk specifically tied to particular genes across development and in conjunction with environmental risk factors. To that end, studies that take into account developmental factors and multiple theory-driven variables may ultimately lead to more complete models that are also more useful. The present study suggests that in a sample of heavy drinking young adults, the DRD4 VNTR genotype is associated with problematic alcohol use and that these effects are partially accounted for by genotype effects on novelty-seeking. These results may help elucidate the specific pathways of risk associated with genetic influences on alcohol misuse. Importantly, the integration of statistical and behavioral methods provides a promising framework to studying risk pathways for psychiatric phenotypes.

These findings highlight the importance of more accurately characterizing phenotypes, which in turn could help clarify inconsistent findings. Specifically, more refined phenotypic measures, such as endophenotypes, may increase sensitivity to detect genetic influences on behavior (e.g., Gottesman & Gould, 2003; Hines et al., 2005). This is especially important when considering that the relative effect of single genetic polymorphisms on complex behaviors, such as alcohol use and abuse, is likely to be small in magnitude (Heath & Phil, 1995). In terms of characterizing drinking phenotypes, the present study treated problematic alcohol use as a latent variable with three indicators, namely alcohol-related problems, quantity and frequency of drinking, and maximum number of drinks per episode. Similar approaches may be useful in behavior genetics studies of complex disorders as a means of increasing statistical power to detect genotype effects (by decreasing measurement error associated with the phenotype), decreasing multiple comparisons, and ultimately producing more consistent findings.

A number of aspects of the current findings warrant further discussion and raise questions for future research. First, at this point, the underlying molecular processes that account for these effects are unclear. Based on the existing literature, two alternative explanations for these differences exist. From a molecular standpoint, D₄ receptors are widely distributed in the brain, but with notable localization in the ascending corticomesolimbic dopamine axis (Berger et al., 2000; Svingos et al., 2000; Wedzony et al., 2001), running from the ventral tegmental area to the nucleus accumbens and projecting into the prefrontal cortex. In the associated regions, as D₂-like receptors, activation of D₄ receptors serves to inhibit the accumulation of intracellular cAMP. In turn, recent findings suggest that greater cAMP in limbic regions, specifically the nucleus accumbens, results in greater motivation for dopaminergic rewards (Choi et al, 2006; Knapp et al., 2001; Lynch & Taylor, 2005). Therefore, given that the D4.7 variant may be functionally less sensitive (i.e., permits greater cAMP accumulation), it is plausible that greater tonic levels of limbic cAMP may account for both greater novelty seeking and alcohol use in D4.7 carriers. Alternatively, a recent neuroimaging study found that the D4.7 allele is associated with greater activation of in the prefrontal cortex in response to alcohol cues (McClernon et al., 2007), suggesting that D4.7 carriers may engage in greater reward processing through cognitive mechanisms. In the context of the current study, the two possibilities of DRD4 VNTR-mediated alterations to limbic or frontal dopaminergic neurotransmission must remain speculative and await further research.

The second area of the study that warrants further discussion pertains to the behavioral findings. As noted earlier, the construct of novelty seeking is multifarious, including aspects of impulsivity, excitability, and extravagance (Cloninger et al., 1993). As such, the subcomponents of novelty seeking may represent even narrower behavioral phenotypes and it is unclear which subcomponent(s) are most influenced by DRD4 VNTR genotype. Investigating these more fine-grained relationships may further clarify the functional influences of molecular variation at the DRD4 locus. Moreover, consistent with the partial mediation effects, clarifying the connection between DRD4 VNTR and alcohol use, beyond the influence of novelty seeking, remains a priority for future studies.

These results should be interpreted in the context of the study's strengths and limitations. Study strengths include a theory-driven multivariate model of genetic effects and the use of advanced statistical methods. Limitations include the relatively small sample comprised of heavy-drinking college students. The small sample combined with the unequal number of men and women limited our ability to detect significant cross-groups differences (Kaplan & George, 1995), and thus we caution that because of the possibility of increased Type II error, future studies are necessary to fully understand the moderating influence of sex on these relationships. This was a cross sectional study and longitudinal approaches seem warranted. Limitations notwithstanding, these findings advance the understanding of DRD4 VNTR genotype in relation to alcohol use phenotypes and personality traits in heavy-drinking young adults. Future studies that can further dissect these interrelationships will be essential to fully characterize the behavioral effects of variation at the DRD4 locus.

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References

- Allen JP, Litten RZ, Fertig JB, Babor T. A review of research on the Alcohol Use Disorders Identification Test (AUDIT). *Alcohol Clin Exp Res.* 1997; 21(4):613–619. [PubMed: 9194913]
- Asghari V, Sanyal S, Buchwaldt S, Paterson A, Jovanovic V, Van Tol HH. Modulation of intracellular cyclic AMP levels by different human dopamine receptor variants. *J Neurochem.* 1995; 65:1157–1165. [PubMed: 7643093]
- Asghari V, Schoots O, van Kats S, Ohara K, Jovanovic V, Guan HC, Bunzow JR, Petronis A, Van Tol HH. Dopamine D4 receptor repeat: analysis of different native and mutant forms of the human and rat genes. *Mol Pharmacol.* 1994; 46:364–373. [PubMed: 8078498]
- Bentler, PM. EQS: Structural equations program manual [Computer manual]. Multivariate Software; Encino, CA: 1995.
- Bentler PM. Comparative fit indices in structural models. *Psychol Bull.* 1990; 107:588–606.
- Berger MA, Defagot MC, Villar MJ, Antonelli MC. D4 dopamine and metabotropic glutamate receptors in cerebral cortex and striatum in rat brain. *Neurochem Res.* 2001; 26(4):345–52. [PubMed: 11495344]
- Browne, MW.; Cudeck, R. Alternative ways of assessing model fit. In: Bollen, K.; Long, K., editors. *Testing structural models.* Sage; CA: 1993. p. 136–162.
- Choi KH, Whisler DL, Graham DL, Self DW. Antisense-induced reduction in nucleus accumbens cyclic AMP response element binding protein attenuates cocaine reinforcement. *Neurosci.* 2006; 137:373–383.
- Clements R. Prevalence of alcohol-use disorders and alcohol-related problems in a college student sample. *J Am Coll Health.* 1999; 48:111–118. [PubMed: 10584445]

- Cloninger CR, Svrakic DM, Przybeck TR. A psychobiological model of temperament and character. *Arch Gen Psychiatry*. 1993; 50:975–990. [PubMed: 8250684]
- Dick DM, Rose RJ, Kaprio J. The next challenge for psychiatric genetics: Characterizing the risk associated with identified genes. *Ann Clin Psychiatry*. 2006; 18(4):223–231. [PubMed: 17162621]
- Ding Y-C, Chi H-C, Grady DL, Morishima A, Kidd JR, Kidd KK, Flodman P, Spence MA, Schuck S, Swanson JM, Zhang Y-P, Moyzis RK. Evidence of positive selection acting at the human dopamine receptor D4 gene locus. *Proc Natl Acad Sci U S A*. 2002; 99:309–314. [PubMed: 11756666]
- Dulawa SC, Grady DK, Low MJ, Paulus MP, Geyer MA. Dopamine D4 receptor-knock-out mice exhibit reduced exploration of novel stimuli. *J Neurosci*. 1999; 19:9550–9556. [PubMed: 10531457]
- Etter JF, Pelissolo A, Pomerleau C, Saint-Hilaire Z. Associations between smoking and heritable temperament traits. *Nicotine Tob Res*. 2003; 5:401–409. [PubMed: 12791536]
- Freeman B, Powell J, Ball D, Hill L, Craig I, Plomin R. DNA by mail: an inexpensive and noninvasive method for collecting DNA samples from widely dispersed populations. *Behav Genet*. 1997; 27:251–257. [PubMed: 9210796]
- Gabel S, Stallings MC, Scmitz S, Young SE, Fulker DW. Personality dimension and substance misuse: Relationships in adolescents, mothers and fathers. *Am J Addict*. 1999; 8:101–113. [PubMed: 10365190]
- Gottesman II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry*. 2003; 160:636–645. [PubMed: 12668349]
- Grady DL, Chi H-C, Ding Y-C, Smith M, Wang E, Schuck S, Flodman P, Spence MA, Swanson JM, Moyzis RK. High prevalence of rare dopamine receptor D4 alleles in children diagnosed with attention-deficit hyperactivity disorder. *Mol Psychiatry*. 2003; 8:536–545. [PubMed: 12808433]
- Heath AC, Phil D. Genetic influences on alcoholism risk: A review of adoption and twin studies. *Alcohol Health Res World*. 1995; 19(3):166–171.
- Heiman N, Stallings MC, Hofer SM, Hewitt JK. Investigating age differences in the genetic and environmental structure of the tridimensional personality questionnaire in later adulthood. *Behav Genet*. 2003; 33:171–180. [PubMed: 14574150]
- Hines L, Ray LA, Hutchison KE, Tabakoff B. Alcoholism: The dissection for endophenotypes. *Dialogues Clin Neurosci*. 2005; 7(3):153–163. [PubMed: 16262210]
- Hu L, Bentler PM. Cutoff criteria for fit indexes in covariance structure analyses: conventional criteria versus new alternatives. *Structural Equation Modeling*. 1999; 6:1–55.
- Hu, L.; Bentler, PM. Evaluating model fit. In: Hoyle, RH., editor. *Structural equation modeling: Concepts, issues, and applications*. Sage; CA: 1995. p. 76-99.
- Hutchison KE, Ray LA, Sandman E, Rutter MC, Peters A, Swift R. The effect of olanzapine on craving and alcohol consumption. *Neuropsychopharmacology*. 2006; 31:1310–1317. [PubMed: 16237394]
- Hutchison KE, Wooden A, Swift RM, Smolen A, McGeary J, Adler L. Olanzapine reduces craving for alcohol: a DRD4 VNTR polymorphism by pharmacotherapy interaction. *Neuropsychopharmacology*. 2003; 28:1882–1888. [PubMed: 12888781]
- Hutchison KE, McGeary J, Smolen A, Bryan A, Swift RM. The DRD4 VNTR polymorphism moderates craving after alcohol consumption. *Health Psychol*. 2002; 21:139–146. [PubMed: 11950104]
- Kaplan D, George R. A study of the power associated with testing factor mean differences under violations of factorial invariance. *Structural Equation Modeling*. 1995; 2:101–118.
- Kluger AN, Siegfried Z, Ebstein RP. A meta-analysis of the association between DRD4 polymorphism and novelty seeking. *Mol Psychiatry*. 2002; 7:712–717. [PubMed: 12192615]
- Knapp CM, Lee K, Foye M, Ciraulo DA, Kornetsky C. Additive effects of intra accumbens infusion of the cAMP-specific phosphodiesterase inhibitor, rolipam, and cocaine on brain stimulation reward. *Life Sci*. 2001; 69:1673–1682. [PubMed: 11589507]
- Laucht M, Becker K, Blomeyer D, Schmidt M. Novelty seeking involved in mediating the association between the dopamine D4 receptor gene exon III polymorphism and heavy drinking in male

- adolescents: Results from a high-risk community sample. *Biol Psychiatry*. 2007; 61:87–92. [PubMed: 16945348]
- Laucht M, Becker K, El-Faddagh M, Hohm E, Schmidt M. Association of the DRD4 exon III polymorphism with smoking in fifteen-year-olds: A mediating role for novelty seeking? *J Am Acad Child Adolesc Psychiatry*. 2005; 45:477–484. [PubMed: 15843770]
- Lench N, Stanier P, Williamson R. Simple non-invasive method to obtain DNA for gene analysis. *Lancet*. 1988; 1(8599):1356–1358. [PubMed: 2898042]
- Litcher JB, Barr CL, Kennedy JL, Van Tol HH, Kidd KK, Livak KJ. A hypervariable segment in the human dopamine receptor D4 (DRD4) gene. *Hum Mol Genet*. 1993; 2:767–773. [PubMed: 8353495]
- Lynch WJ, Taylor JR. Persistent changes in motivation to self-administer cocaine following modulation of cyclic AMP-dependent protein kinase A (PKA) activity in the nucleus accumbens. *Eur J Neurosci*. 2005; 22:1214–1220. [PubMed: 16176364]
- Mackillop J, Menges DP, McGeary JE, Lisman SA. Effects of craving and DRD4 VNTR genotype of the relative value of alcohol: an initial human laboratory study. *Behav Brain Funct*. 2007; 19(3): 11. [PubMed: 17309802]
- McGeary JE, Monti PM, Rohsenow DJ, Tidey J, Swift R, Miranda R Jr. Genetic moderators of naltrexone's effects on alcohol cue reactivity. *Alcohol Clin Exp Res*. 2006; 30(8):1288–96. [PubMed: 16899031]
- McClernon FJ, Hutchison KE, Rose JE, Kozink RV. DRD4 VNTR polymorphism is associated with transient fMRI-BOLD responses to smoking cues. *Psychopharmacology*. 2007; 194(4):433–41. [PubMed: 17611740]
- Munafò MR, Yalcin B, Willis-Owen SA, Flint J. Association of the dopamine D4 receptor (DRD4) gene and approach-related personality traits: meta-analysis and new data. *Biol Psychiatry*. 2008; 63(2):197–206. [PubMed: 17574217]
- Oak JN, Oldenhof J, Van Tol HH. The dopamine D(4) receptor: one decade of research. *Eur J Pharmacol*. 2000; 405:303–327. [PubMed: 11033337]
- Okuyama Y, Ishiguro H, Nankai M, Shibuya H, Watanabe A, Arinami T. Identification of a polymorphism in the promoter region of DRD4 associated with the human novelty seeking personality trait. *Mol Psychiatry*. 2000; 5:64–69. [PubMed: 10673770]
- Ray LA, Hutchison KE. A polymorphism of the μ -opioid receptor gene and sensitivity to the effects of alcohol in humans. *Alcohol Clin Exp Res*. 2004; 28:1789–95. [PubMed: 15608594]
- Rose R. A developmental behavioral-genetic perspective on alcoholism risk. *Alcohol Health Res World*. 1998; 22:131–143. [PubMed: 15706788]
- Rubinstein M, Cepeda C, Hurst RS, Flores-Hernandez J, Ariano MA, Falzone TL, Kozell LB, Meshul CK, Bunzow JR, Low MJ, Levine MS, Grandy DK. Dopamine D4 receptor-deficient mice display cortical hyperexcitability. *J Neurosci*. 2001; 21:3756–63. [PubMed: 11356863]
- Rubinstein M, Phillips TJ, Bunzow JR, Falzone TL, Dziewczapolski G, Zhang G, Fang Y, Larson JL, McDougall JA, Chester JA, Saez C, Pugsley TA, Gershnik O, Low MJ, Grandy DK. Mice lacking dopamine D4 receptor are supersensitive to ethanol, cocaine, and methamphetamine. *Cell*. 1997; 90:991–1001. [PubMed: 9323127]
- Spitz E, Moutier R, Reed T, Busnel MC, Marchaland C, Roubertoux PL, Carlier M. Comparative diagnoses of twin zygosity by SSLP variant analysis, questionnaire, and dermatoglyphic analysis. *Behav Genet*. 1996; 26:55–63. [PubMed: 8852732]
- Steiger JH. Structural model evaluation and modification: An interval estimation approach. *Multivariate Behav Res*. 1990; 25:173–180.
- Svingos AL, Periasamy S, Pickel VM. Presynaptic dopamine D(4) receptor localization in the rat nucleus accumbens shell. *Synapse*. 2001; 36:222–32. [PubMed: 10819901]
- Tyndale RF. Genetics of alcohol and tobacco use in humans. *Ann Med*. 2003; 35:94–121. [PubMed: 12795339]
- van den Wildenberg E, Janssen RG, Hutchison KE, van Breukelen GJ, Wiers RW. Polymorphisms of the dopamine D4 receptor gene (DRD4 VNTR) and cannabinoid CB1 receptor gene (CNR1) are not strongly related to cue-reactivity after alcohol exposure. *Addict Biol*. 2007; 12(2):210–20. [PubMed: 17508995]

- Van Tol HH, Wu CM, Guan HC, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HB, Jovanovic V. Multiple dopamine D4 receptor variants in the human population. *Nature*. 1992; 358(6382):149–152. [PubMed: 1319557]
- Van Tol HH, Bunzow JR, Guan HC, Sunahara RK, Seeman P, Niznik HB, Civelli O. Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. *Nature*. 1991; 350(6319):610–614. [PubMed: 1840645]
- Walker AH, Najarian D, White DL, Jaffe JF, Kanetsky PA, Rebbbeck TR. Collection of genomic DNA by buccal swabs for polymerase chain reaction-based biomarker assays. *Environ Health Perspect*. 1999; 107:517–520. [PubMed: 10378997]
- Wedzony K, Chocyk A, Maćkowiak M, Fijał K, Czyrak A. Cortical localization of dopamine D4 receptors in the rat brain--immunocytochemical study. *J Physiol Pharmacol*. 2000; 51(2):205–21. [PubMed: 10898094]
- Wechsler H, Davenport A, Dowdall G, Moeykens B, Castillo S. Health and behavioral consequences of binge drinking in college: A national survey of students at 140 campuses. *JAMA*. 1994; 272:1672–1677. [PubMed: 7966895]
- White HR, Labouvie EW. Towards the assessment of adolescent problem drinking. *J Stud Alcohol*. 1989; 50(1):30–37. [PubMed: 2927120]

Figure 1.

Model of problematic alcohol use among hazardous college drinkers, including mediational role of novelty seeking. Coefficients are standardized path coefficients. * $p < .05$; ** $p < .01$; *** $p < .001$; + $p = .07$.

Table 1

Correlations among Model Variables

	DRINK	MAX	RAPI	TPQ-NS	DRD4	Gender
DRINK	1.0					
MAX	0.42***	1.0				
RAPI	0.37***	0.38***	1.0			
TPQ-NS	0.21*	0.24*	0.32**	1.0		
DRD4	0.24*	0.24*	0.18	0.30**	1.0	
Gender	0.22*	0.40***	0.10	0.13	0.22*	1.0

* $p < .05$;
** $p < .01$;
*** $p < .001$