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Between and within-family association test of the dopamine receptor D2 TaqIA polymorphism and alcohol abuse and dependence in a general population sample of adults

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Abstract

Objective—Dopaminergic dysfunction has been hypothesized to play an important role in the etiology of alcohol use disorders. A restriction fragment length polymorphism (RFLP) in the 3' untranslated region (3'UTR) of the DRD2 gene affects gene expression and has been implicated as a risk factor for alcohol dependence. This polymorphism (TaqIA) has been reported as positively associated with alcohol use disorders in case-control samples, but these results have not been replicated in family-based association studies. These mixed results of association between the DRD2 TaqIA polymorphism and alcohol use disorders may be due to differences in sample size, phenotype definition, heterogeneity of the samples and genetic admixture.

Method—We conducted tests of association in a sample of 838 adults participating in the National Youth Survey Family Study (NYSFS). We examined whether the DRD2 TaqIA polymorphism was associated with a symptom counts measure of alcohol abuse and dependence derived from the DSM-IV and Craving Withdrawal models.

Results—Tests of association were non-significant across each classification system examined. Power calculations suggested these results were despite the ability to detect an effect size of 1%.

Conclusions—This study supports other family-based association tests that have reported no association between the DRD2 TaqIA polymorphism and alcohol abuse and dependence.

Keywords

DRD2; DSM-IV; alcohol abuse; dependence; TaqIA; Craving Withdrawal Model

Introduction

Since the observation by Olds et al (1954), extensive research has implicated dopamine as an etiological factor in the rewarding and motivating effects of alcohol. Findings from human and

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animal studies have refined this observation further suggesting that it is the increased release of dopamine within the mesolimbic pathway that constitutes the primary "reward pathway" which influences alcohol intake and alcohol use disorders (Boileau et al, 2003). The mesolimbic pathway begins in the ventral tegmental area and includes the nucleus accumbens and striatum; brain regions that mediate the reinforcing effects of ethanol. Dysregulated dopaminergic transmission within this pathway is thought to underlie many of the cognitive, emotional and locomotor behaviors observed with alcohol and drug intake (Koob and Le Moal, 1997) and its etiology has therefore been a major research priority for the past decade.

Synaptic levels of dopamine are regulated through an interconnected process involving two pre-synaptic receptors, the dopamine transporter (DAT) and D2 auto-receptor (DRD2). While the transporter determines the magnitude and duration of dopaminergic transmission, the D2 receptor inhibits the rate-limiting enzyme of dopamine synthesis, tyrosine hydroxylase. Reduced levels of the DRD2 receptor are a widely observed risk factor associated with increased alcohol consumption (Heinz et al, 2004; Tupala et al, 2003; Volkow et al, 1996) and have been correlated with greater levels of alcohol craving (Self, 1998). Heightened brain activation within the orbital-frontal and medial prefrontal cortices and anterior cingulate has been associated with low D2 receptor expression (Volkow and Fowler et al, 2000) with differences in alcohol-cue processing, and compulsive, uncontrolled drug use (Heinz et al, 2004). Experimental studies have implicated the D2 receptor in goal-directed behavior (Goto and Grace, 2005) and over-expression of D2 receptor levels reduces alcohol intake (Thanos et al, 2001). Taken together, these findings implicate differences in pre-synaptic receptor functioning and expression in the etiology of alcohol use disorders.

Family, twin and adoption studies suggest strong genetic contributions to alcohol consumption and misuse as well as the possibility of sex-specific risks (Heath et al, 1997; Nurnberger et al, 2004; Prescott et al, 1999a; 1999b; Whitfield et al, 2004). The dopamine D2 receptor is encoded by a single, alternatively spliced gene (Giros et al, 1989; Monstma et al, 1989), mapped to the short-arm of chromosome 11. This receptor has a polymorphic TaqIA restriction endonuclease site 2500 base pairs (bp) downstream (3' untranslated region) from the coding region of the gene. This site is designated the TaqIA site to distinguish it from a second restriction site (TaqIB) located in intron 2. Functionally, TaqIA has been associated with reduced D2 expression *in vivo* within both clinical and epidemiological samples (Laakso et al, 2005; Laruelle et al, 1998; Thompson et al, 1997), suggesting that this polymorphism may be an important candidate locus.

Association studies of the DRD2 TaqIA polymorphism with alcohol use disorders have been conducted using either a case-control or family-based framework. Case-control studies are mixed in their support of a positive association, with differences in phenotypic definition, sample sizes and heterogeneity, and genetic admixture potentially contributing to differences between studies (Connor et al, 2002; Nobel et al, 2003; Young et al, 2004). These meta-analyses, however, do find evidence for an association between Al allele of the DRD2 TaqIA polymorphism and more severe alcohol use problems. Similar associations were observed in ethnically homogeneous samples and in samples where the concurrent use of other drugs was controlled. These clinically based case-control associations, however, were not supported by most within-family association studies of alcoholic parents and their children (Edenberg et al, 1998; Neiswanger et al, 1995; Parsian et al, 1991). Finally, the Al allele was found to be associated with greater alcohol consumption (Hopfer et al, 2005) and dependence (Limosin et al, 2002) in two general population samples of healthy volunteers. As alcohol use is thought to be a multifaceted and complex behavior, this pattern of results is not surprising but does encourage further study of the TaqA1 locus and alcohol use disorders.

Alcohol use disorders are predominantly assessed using the Diagnostic and Statistical Manual of Mental Disorders (DSM). Currently, DSM-IV recognizes two types: abuse and dependence. Alcohol abuse is assessed using four criteria characterized by adverse social and legal outcomes. Dependence is assessed using seven criteria characterized by physiological and behavioral symptoms that emphasize compulsive use and a diminished ability to abstain. A diagnosis of abuse is given in the presence of one or more criteria while a dependence diagnosis requires three or more criteria. Although evidence supports the distinction between abuse and dependence (Hasin et al, 1994; Schuckit et al, 2001), others contend that the two disorders share a common underlying liability and should be viewed along a severity continuum (Bucholz et al, 1996; Proudfoot et al, 2006). Concern, however, has been raised about a diagnosis of alcohol dependence in absence of the physiological symptoms of tolerance and withdrawal (Langenbucher et al, 2000). A diagnosis in absence of withdrawal symptoms, in particular, has raised concern because withdrawal problems provide a clinically useful marker for more severe dependence and is often the focus of brain-imaging and pharmacological investigations (Buonopane and Petrakis, 2005; de Bruijn et al, 2004).

Given the above concerns about assessment using the DSM-IV, two alternative models of alcohol use disorders have been proposed. They include the Withdrawal-Gate Model (Langenbucher et al, 2000) and Craving Withdrawal Model (de Brujin et al, 2004). Both models are rearrangements of DSM-IV abuse and dependence criteria into different syndromes in order to reflect the importance of withdrawal symptoms in alcohol dependence. Clinical levels of abuse in these models are indicated by the presence of two or more of the remaining 10 DSM-IV abuse-dependence symptoms. Each of these models have been validated in general population samples and demonstrate an improved predictive and discriminant validity (de Bruijn et al, 2006; Langenbucher et al, 2000). An important distinction between these two models, however, is the inclusion of craving or the strong desire to consume alcohol in a diagnosis of dependence. For candidate gene studies of the DRD2 TaqIA locus, including craving as a dependence criterion may represent a more severe problem alcohol use phenotype. Furthermore, it is consistent with evidence implicating genotypic effects on alcohol craving in treatment-based samples (Heinz et al 2004, 2005; Self, 1998)

In the current report we summarize findings from an association study of the DRD2 TaqIA polymorphism and problem alcohol use. Abuse and dependence symptoms were collected from a general population sample of adults participating in the National Youth Survey Family Study (NYSFS). As the tobacco use has been shown to influence alcohol consumption as well as share a common underlying genetic etiology with a variety of alcohol related phenotypes (Bierut et al, 2000; Tyndale, 2003), we included a measure of cigarette smoking as a covariate in our analyses. Since prior evidence implicates the Al allele of the DRD2 gene with both alcohol consumption and severe problem use, we utilized two measures that varied as a function of severity. Our analyses were designed to investigate two questions. First, was the Al allele of the DRD2 TaqIA polymorphism associated with the observed variation in problem alcohol use? Second, was the Al allele associated with greater symptom severity as conceptualized by the Craving Withdrawal Model?

Method

Sample

The National Youth Survey Family Study (NYSFS; Institute of Behavioral Science and Institute for Behavioral Genetics, University of Colorado) is a prospective longitudinal study of problem behavior from adolescence into adulthood. The NYSFS is based on a nationally representative multi-stage probability sample of households in the continental United States (Elliott et al., 1989). This sample was drawn in 1976 and contained 2360 eligible youth respondents ages 11-17 at the time of the initial interview. Of these, 1725 (73%) agreed to

participate and the participating youth are proportionally representative of the age, sex, and ethnic composition of the 11-17-age population as determined by the U.S. Census Bureau. In 2002, a follow-up interview was conducted (age range: 35-43) during which behavioral data and DNA samples were collected. As of 2002, 24 of the original respondents were deceased, 15 declined further participation, and 117 had not been interviewed over the last several waves. These 156 cases were subsequently excluded from our analyses; leaving an available NYSFS sample size of 1571. Of this 1571 sample, 1340 respondents (85.2%) completed interviews in 2002, and of these, 1007 (75%) provided buccal swab DNA samples. Of these 1007, there were a total of 838 individuals for which we had both phenotypic and genotypic information.

Within the NYSFS there was no statistically significant disproportionate loss by alcohol use for the total adolescent sample (age 11-17) or for either early adolescent alcohol users (ages 11-14) or late adolescent users (ages 15-17). In fact, the nature of the non-significant disproportionality is for a greater percentage of drinkers to remain in the study through 2002.

Measures

Alcohol use behaviors were assessed with informed consent during a face-to-face structured interview including an adaptation of the Composite International Diagnostic Interview – Substance Abuse Module (CIDI-SAM; Robbins, Cottler and Babor, 1990). Alcohol abuse, dependence, and craving symptoms were assessed in those participants who indicated they had drunk alcohol six or more times in their lifetime. Craving was assessed by two questions that measured whether they had "ever felt a strong desire or urge to drink" and if they ever "needed a drink so badly that they could not think of anything else". For descriptive analyses, based on previous work (Heath et al, 1991a, b; Hopfer et al, 2005), we identified three groups of drinkers in this sample: 1) current, 2) former, and 3) abstainers. Current drinkers were identified by positive responses to whether they had consumed six or more drinks within the past-year. Former drinkers were those who had not drank in the past-year but had consumed six or more drinks during an earlier period of their life. Abstainers were those who indicated they had not had six or more drinks in their lifetime.

Alcohol abuse and dependence symptoms were measured using two classification systems: the DSM-IV and the Craving Withdrawal Model. Alcohol abuse and dependence symptoms for each of the two models are shown in Table 1. Each abuse and dependence symptom was scored as a dichotomous variable with a positive response (Yes) being given a score of one. Quantitative scores based on past-year information were constructed by summing across the dichotomous items within measure and across diagnostic classification. Total abuse scores on the DSM-IV could range between 0 and 4 while total dependence scores could range from 0 to 7. Total scores on the Craving Withdrawal Model could range between 0 and 10 for abuse and 0 to 12 for dependence.

Past-year tobacco use was measured using nicotine dependence symptoms as assessed by the CIDI-SAM. Each of the seven criteria was scored in a similar manner to that done for the DSM-IV alcohol abuse and dependence criteria, with a symptom count score representing the sum of all items.

Genotyping

Buccal cell DNA was collected with written informed consent. Isolation, extraction, and PCR protocols were performed as described elsewhere (Anchordoquy et al, 2003). For these analyses we utilized a redesigned assay for genotyping the DRD2 TaqIA polymorphism as described previously (Haberstick and Smolen, 2004). Primer sequences were: forward, 5'-GTGCAGCTCACTCCATCCT-3' (fluorescently labeled), and reverse, 5'-GCAACACAGCCATCCTCAAAG-3'. Probe sequences were: VIC-

CCTGCCTTGACCAGC-NFQMGB and FAM-CTGCCTCGACCAGC-NFQMGB. All reactions were performed in an ABI Prism® 7000 Sequence Detection System using the allelic discrimination mode as described by Livak (1999) and in the accompanying instrument documentation.

Genotype error checking

To ensure genotyping accuracy, error checking was conducted in two ways. First, errors were determined by comparing allele calls made by two individuals independently. In situations where there were disagreements, a third investigator reviewed the calls and the samples were rerun if necessary. Second, 10% of all samples were run a second time without knowledge of previous results. Two individuals reviewed the results from the second run independently, with disagreements resolved by a third investigator. Final allele calls from the first and second runs were subsequently compared with discordant calls between the two runs genotyped one final time.

Statistical Analyses

Association analyses were conducted using the publicly available statistical package QTDT (Version 3.15, http: sph.umich.edu/csg/abecasis/QTDT). Candidate gene association tests between our alcohol use measures and the DRD2 TaqIA polymorphism were conducted using the total association and orthogonal models (Abecasis et al, 2000). The orthogonal model is based on a model proposed by Fulker et al (1999) that extends sib-pair analysis methods for quantitative traits to test for association in the absence of parental genotypes. Alcohol abuse and dependence scores (y_{ij}) in the orthogonal model are a function of an overall mean, μ, and a genotypic score that is decomposed into its between- and within-family components (equation: $\hat{y}_{ij} = \mu + \beta_b b_i + \beta_w w_{ij}$), where y_{ij} is the observed score for the jth individual in the ith family, b_i is the mean genotype score for the ith family, w_{ij} is the within family deviation of the jth individual's genotype score from the family mean, and $\beta_b$and β_w are the estimated regression coefficients. The between-family component, β_b, may be sensitive to the effects of population admixture or stratification. Siblings discordant for genotype contribute information to the within-family component, β_w, which is robust to confounding effects of admixture and detects the presence of linkage-disequilibrium (LD) between the measured marker and a polymorphism affecting the observed phenotype. In the absence of population stratification, tests of total association are based on both concordant and discordant siblings for the TaqIA genotype and result in a greater number of informative individuals. Tests of association were conducted by calculating the likelihood chi-square ratio between a model where β_w was freely estimated and one with β_w fixed at zero, with β_b free in both (Fulker et al, 1999). The effects of population stratification were examined by equating the between-and within-effects and comparing the difference in fit with a model in which they were freely estimated (Abecasis et al, 2000). These tests were conducted within a variance-component framework that models the background genetic and random environmental effects at the DRD2 TaqIA locus. In the presence of non-normal distributions, permutation testing was conducted in order to control of their effects on association tests.

Tests of Hardy-Weinberg equilibrium (HWE) were conducted using PEDSTATS (Wigginton and Abecasis, 2005). Power calculations were based on a variance components model of total association for sib-ships using the Genetic Power Calculator (Purcell et al, 2003). The total number of participants from households with more than one respondent determined our sample size for these calculations. Based on our data, a sibling correlation for DSM-IV abuse-dependence symptom count scores of 0.38 and a minor allele frequency (MAF) of 0.23 were also included in these calculations.

We adopted a significance level of $p < 0.05$ (2-tailed) for these analyses. Allele and genotypic frequencies, and descriptive and predictive statistics were estimated using SPSS (Version 14.0). As the frequency of self-reported ethnicities for groups other than Caucasian and African-Americans were small to zero, we created a mixed ethnicity group (*Other*, n = 64). Prior to association testing, symptom count scores were adjusted for sex, the linear regression of age, and dummy coded ethnicity.

Results

Count scores of abuse and dependence symptoms were determined using responses from 872 current and former drinkers of whom 49.4% were male. Of these, 32.7% (n = 286) had one other sibling participant, 12.7% had two additional sibling participants, and 2.7% had three other sibling participants. A total of 462 (52.9%) participants had no other sibling participants. A total of 38 participants reported not having drunk alcohol at any point in their lifetime and were therefore excluded. Nearly two-thirds of this sample had begun to drink by the age of 21, with roughly 10% reporting the age at which they began to drink as 30 or older. Therefore, the mean age of drinking onset in this sample was 21.58 (range 10-42). Demographic characteristics, drinking status, and tobacco use within the entire sample and for males and females are summarized in Table 2. As shown, males and females did not differ substantially for any of the examined variables.

Alcohol abuse and dependence status across males and females were determined using the DSM-IV hierarchical decision rule for each of the two classification systems examined. As shown in Table 3, the frequency of abuse for both sexes was greatest using the Craving Withdrawal Model. The frequency of dependence, on the other hand, was highest using DSM criteria. Regardless of classification system, males in our sample met diagnostic threshold for alcohol abuse more frequently then females. For dependence, however, statistically significant differences between males and females were observed only when the DSM-IV criteria were examined. The distinction between abuse and dependence, however, is somewhat artificial as many who met criteria for dependence also met criteria for abuse. In this sample, only 6% of those classified as alcohol dependent on the DSM-IV did not meet threshold for abuse. A similar percentage (8%) was observed when participants were classified using the Craving Withdrawal Model. This suggested that, within this general population sample, abuse and dependence symptoms often co-occurred within individuals.

A total of 34 DRD2 TaqIA genotypes could not be obtained after multiple genotyping runs due to poor signal strength or failed PCR reactions. Excluding these 34 resulted in an analysis sample size of 838 for which we had both phenotypic and genotypic information. Genotypic and allelic frequencies as a function of gender and ethnicity are presented in Table 4. Genotypic distributions for the DRD2 TaqIA polymorphism were in HWE and no significant differences were observed as a function of sex ($\chi^2 = 1.39$, df = 2, $p = .500$). Frequency differences in TaqIA genotype were, however, observed between Caucasians and each of our two other ethnic groups ($p < .001$).

Candidate gene association tests included both current and former drinkers and were conducted on square-root transformed symptom count scores. Prior to transformation, symptom count scores on the DSM-IV and Craving Withdrawal Model were positively skewed (1.39 and 1.49, respectively). There was no evidence of kurtosis in either distribution of scores. Following transformation, skewness was estimated at 0.40 and 0.45, respectively. Residual scores that controlled for the effects of age, sex, and ethnicity were subsequently calculated on transformed variables.

Across all tests conducted, estimates of population stratification were non-significant for both the DSM-IV ($p < 0.578$) and Craving Withdrawal Model ($p < 0.623$). As shown in Table 5, association tests of the DRD2 TaqIA polymorphism using the total or orthogonal models were not significant (Test 1). Similar results were observed for association tests controlling for the effects of concurrent tobacco use (Test 2). This suggested the DRD2 TaqIA locus was not a susceptibility factor for alcohol abuse-dependence in this sample of adults. Results from power calculations (not shown) indicated that these analyses had 80% power to detect an effect size as small as 1% assuming the TaqIA polymorphism was the putative locus. This suggested that our null results were not due to insufficient power.

Discussion

In the current study, we examined the previously reported association between the DRD2 TaqIA locus and alcohol abuse-dependence symptoms. Results from association tests using both the DSM-IV and Craving Withdrawal Model systems of classification could not support this hypothesis. Power calculations suggested that these results were obtained despite having enough power to detect an effect size of 1%.

To test the association between the TaqIA polymorphism and a quantitative measure of alcohol abuse and dependence symptoms, we adopted a combined between- and within-family approach. Simulation studies have shown that partitioning the genotypic effect into its between- and within-family effects enhances the power of a traditional family-based association study (Fulker et al, 1999; Abecasis et al, 2000). Recently, Sakai et al (in press) adopted a similar method in a clinical sample of adolescents, finding no evidence for a positive association. Similar findings were reported from four previous studies of affected family-based samples (Bolos et al, 1990; Edenberg et al, 1998; Neiswanger et al, 1995; Parsian et al, 1991). Increased rates of allele sharing for the TaqI polymorphism have, however, been reported from two sib-pair linkage studies (Cook et al, 1996; Hill et al, 1999), although this findings was not replicated in another study (Reich et al, 1998). Taken together, our results are congruent with the notion that the A1 allele is not a generalized risk factor for alcohol abuse and dependence symptoms in the general population.

One contributing factor for non-replication in this and other candidate gene studies could be allelic and genetic heterogeneity. Allelic heterogeneity refers to other variation within a given gene, while genetic heterogeneity is other variation within the genome. Different types of genetic variation have been identified within the DRD2 gene and include two functional polymorphisms; the C957T SNP in exon 7 (Hirvonen et al, 2004) and the promoter insertion/deletion polymorphism (Arinami et al, 1997). Both of these affect D2 receptor expression and densities, while the C957T SNP only has been shown to decrease mRNA stability and translation in the striatum (Duan et al, 2003). Linkage disequilibrium between these two markers and the TaqIA polymorphism varies with ethnicity, though haplotype blocks that include these three polymorphisms are common (Duan et al, 2003; Gelernter et al, 1998). Haplotype association studies that include these and other DRD2 genetic variants, however, have offered only limited support for the role of allelic heterogeneity in alcohol dependence (Bloomqvist et al, 2000; Gelernter and Kranzler, 1999; Noble et al, 2000). As DRD2 haplotypes have only been examined in clinical samples, an important extension of this and other general population studies of alcohol use disorders would be the inclusion of multiple DRD2 SNPs in future analyses.

A second factor that could be important in non-replication studies is the role of trait heterogeneity. Similar to other general population samples, alcohol abuse symptoms in this adult sample were frequently reported with dependence symptoms. For example, 67.8% of similarly-age Caucasian men and 54.7% of Caucasian women who evidenced dependence also

evidenced abuse in the National Epidemiology Survey on Alcohol and Related Conditions (NESARC) sample (Hsain et al, 2005). Similar finding have been reported from other large general population samples (Harford et al, 2005; Proudfoot et al, 2006) and suggest that among those dependent on alcohol, abuse symptoms are commonly present. To this end, the NYSFS sample appears to be similar to other samples currently being investigated. With respect to candidate gene studies of the A1 allele, larger sample sizes than examined here and in Hopfer et al (2005) may be needed in order to maximize the range of alcohol use disorder symptoms.

Findings from this study should be considered in light of a number of limitations. First, previous positive association studies were reported with severe alcoholism, as defined by the presence of alcohol-related medical problems. In this sample, few participants indicated medical problems due to drinking and as such we could not replicate a severe alcohol phenotype in this sample. To this end, however, we did examine symptom count scores on the Craving Withdrawal Model, which has been shown to identify a more severe form of alcohol dependence (de Bruijn et al, 2006). A related limitation is that craving was not self-reported by many adults in this sample. We would expect that in samples where craving was more highly endorsed, dependence as classified by the Craving Withdrawal Model would result in a more severe phenotype and information for candidate gene association studies. Second, associations between the DRD2 TaqIA polymorphism and alcohol abuse and dependence could differ as a function of assessing past-year symptoms, rather than lifetime symptoms. Third, the NYSFS is a predominantly Caucasian sample and therefore findings from this study are not necessarily applicable to other ethnic groups. Fourth, we did not include behavioral measures such as antisocial personality disorder (ASPD) or aggression in our analyses. Though their presence has been shown to be related to increased alcohol consumption, such problems were not commonly self-reported in the NYSFS sample at this age (Huizinga et al, 2006). Finally, we did not examine the potential role for DRD2 TaqIA genotype-environment interaction and therefore cannot exclude that possibility in these data.

Despite these limitations, the current analyses were able to address many of the methodological problems present in previous studies. First, we adopted an analytic approach that controlled for differences in allele frequency among ethnic groups. This allowed us to include all participants in the sample and avoid the reduction in statistical power that results from stratifying along ethnic group membership. Second, we examined a continuous measure of alcohol use and dependence, which retained more trait information and resulted in greater statistical power than would result from dichotomizing a trait (MacCallum et al, 2002). Third, TaqIA genotype status was ascertained using a method with greater sensitivity and less error than methods utilized previously. Lastly, we controlled for the effect of co-occurring tobacco use. There is some evidence that that tobacco use shares a common genetic etiology and has been shown to influence the quantity of alcohol consumption (Bierut et al, 2000; Tyndale, 2003).

In conclusion, our analyses agree with previous family-based association studies that the DRD2 TaqIA polymorphism is not associated with alcohol use disorders within the general population. In light of these agreements and non-replication results from case-control studies, recent efforts to provide wide-scale genetic testing for alcohol use disorders using the DRD2 TaqIA A1 allele (New York Daily News, 2006) should be considered premature. In this sample, such a test would have resulted in 76.8% of non-dependent alcohol users being identified as “at-risk”.

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Table 1

Diagnostic criteria for alcohol abuse and dependence on the DSM-IV and Craving Withdrawal Model.

	DSM-IV		CWM
	Dependence ≥ 3		**Dependence**
1.	Tolerance	1.	Craving *and*
2.	Withdrawal **a.** Characteristic withdrawal syndrome *or* **b.** drinking to avoid or relieve withdrawal	2.	Withdrawal **a.** Characteristic withdrawal syndrome *or* **b.** drinking to avoid or relieve withdrawal
3.	Persistent desire or unsuccessful effort to cut down		
			Abuse ≥ 2
4.	More or longer use than intended	1.	Failure to fulfill major role obligations
5.	Much time spent obtaining, using or recovering	2.	Recurrent use in hazardous situations
6.	Continuing use despite physical or psychological harm	3.	Recurrent legal problems
7.	Reduction or giving up of important activities	4.	Continuing use despite social or interpersonal harm
		5.	Tolerance
		6.	Persistent desire or unsuccessful effort to cut down
	Abuse ≥ 1		
1.	Failure to fulfill major role obligations	7.	More or longer use than intended
2.	Recurrent use in hazardous situations	8.	Much time spent obtaining, using or recovering
3.	Recurrent legal problems	9.	Continuing use despite physical or psychological harm
4.	Continuing use despite social or interpersonal harm	10.	Reduction or giving up of important activities

Note: CWM: Craving Withdrawal Model.

Table 2

Descriptive statistics.

Measure	Full sample (%)	Males (%)	Females (%)
Age	39.0	39.1	38.9
Range	35 - 44	35 - 43	35 – 43
Ethnicity			
Caucasian	719 (82.5)	348 (80.9)	371 (84.1)
African-American	86 (9.9)	50 (11.6)	36 (8.2)
Other	64 (7.3)	32 (7.4)	32 (7.3)
Education			
≤ 12	380 (43.8)	192 (44.8)	188 (42.8)
13 - 16	399 (45.9)	187 (43.6)	212 (48.3)
≥ 17	89 (10.3)	50 (11.7)	39 (8.9)
Drinking Status			
Current	638 (73.2)	325 (75.2)	313 (71.0)
Previous	229 (26.3)	105 (24.3)	124 (28.1)
Tobacco Use[†]	2.59	2.50	2.69
Range	0 - 7	0 – 7	0 – 7

[†] Mean number of nicotine dependence symptoms.

Table 3

Alcohol abuse and dependence status as a function of the DSM-IV and Craving Withdrawal Model classification systems.

Measures	Males (%)	Females (%)	$\chi^2(1)$
	Abuse[†]		
DSM-IV	143 (33.4)	97 (22.1)	13.71, p <.000
Craving Withdrawal	204 (47.4)	111 (25.3)	45.83, p <.000
	Dependence[‡]		
DSM-IV	104 (24.3)	56 (12.8)	19.05, p <.000
Craving Withdrawal	28 (6.5)	23 (5.3)	0.62, p = .430

[†] Includes only current and previous drinkers who met the threshold for alcohol abuse only.

[‡] Includes those current and previous drinkers who met the alcohol dependence threshold with and without abuse.

Table 4

DRD2 TaqIA genotypic distribution and allelic frequencies.

	Genotypic Distribution (%)†			Allele Frequency (%)	
Group	**A1A1**	**A1A2**	**A2A2**	**A1**	**A2**
Full (n = 838)‡	50 (6.0)	281 (33.5)	508 (60.5)	379 (22.6)	1297 (77.4)
Males (n = 414)	25 (6.0)	131 (31.6)	259 (62.4)	179 (21.6)	649 (78.4)
Females (n = 424)	25 (5.9)	150 (35.4)	249 (58.7)	200 (23.6)	646 (76.2)
Caucasian (n = 692)	26 (3.9)	204(29.9)	457 (66.2)	261 (18.9)	1123 (81.1)
African-American (n = 83)	15 (18.1)	40 (48.2)	28 (29.9)	70 (42.2)	96 (57.8)
Other (n = 61)	8 (13.1)	32 (54.1)	20 (32.8)	49 (40.2)	73 (59.8)

† Genotype-wise comparison between males and females: $\chi^2 = 4.56$, df = 2, p = .102.

‡ Full: includes both males and females.

Table 5

Association test results and model fit statistics.

Test	Measure	DRD2 Marker	Covariate	Model	$\chi^2(1)$	Theoretical p-value	No. of informative individuals
1.	DSM-IV	TaqIA	--	Total	0.09	0.762	832
				Orthogonal	0.09	0.768	143
	CWM	TaqIA	--	Total	0.03	0.865	836
				Orthogonal	0.02	0.884	143
2.	DSM-IV	TaqIA	ND	Total	0.62	0.434	480
				Orthogonal	0.00	1.000	80
	CWM	TaqIA	ND	Total	0.75	0.387	482
				Orthogonal	0.00	1.000	80

Note: CWM: Craving Withdrawal Model; ND: Nicotine dependence.