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Preliminary Evidence for Associations of *CHRM2* with Substance Use and Disinhibition in Adolescence

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Abstract

Evidence for shared heritable influences across domains of substance use suggests that some genetic variants influence broad risk for externalizing behaviors. Theories of externalizing psychopathology also suggest that genetic liability for substance use manifests as temperamental risk factors, particularly those related to behavioral disinhibition, during adolescence. The cholinergic muscarinic receptor 2 gene (*CHRM2*) is a promising candidate for studying genetic influences on broad-based risk for externalizing traits. This study examined a candidate *CHRM2* polymorphism (rs1455858) in relation to substance use and personality measures of disinhibition in a sample of high-risk adolescents (n = 124). Bivariate analyses and structural equation modeling (SEM) evaluated associations of rs1455858 with measures of drug involvement (alcohol, tobacco and marijuana) and disinhibition (indexed by impulsivity and sensation seeking scores). Bivariate analyses showed significant associations of *CHRM2* with several behavioral phenotypes. In SEM analyses *CHRM2* related significantly to latent measures of substance use and disinhibition; additionally, disinhibition mediated the association of *CHRM2* with substance use. These results suggest that *CHRM2* variants are potentially relevant for adolescent substance use and that temperamental risk factors could contribute to these associations.

Keywords

Disinhibitory psychopathology; drug dependence; response inhibition; risk-taking; SNP

Population variability in most forms of drug use and dependence is explained in part by significant heritable influences (Agrawal & Lynskey, 2008; Kendler et al., 2000; 2007). For alcohol, tobacco and marijuana, the proportion of variability in heavy use or dependence attributable to genetic variation is estimated at roughly 50–70%, 50–75% and 34–78%, respectively (Agrawal & Lynskey, 2006; 2008). While these heritability estimates are derived largely from adult cohorts, evidence also suggests significant additive genetic influences on alcohol, tobacco and marijuana use in adolescence (Hopfer et al., 2003; Rhee et al., 2003; McGue et al., 2000). Characterizing genetic influences on adolescent substance

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use is important for informing questions of etiology and clinical course. For example, relatively earlier initiation and escalation of substance use is a robust predictor of the severity of substance use and related problems in adulthood (Grant & Dawson, 1997; Hingson et al., 2000; Pagan et al., 2006; Prescott & Kendler, 1999; Rose, 1998). Moreover, genetic influences on substance frequency and quantity show substantial overlap with those influencing the risk for dependence (Agrawal et al., 2005; Agrawal & Lynskey, 2008; Kendler, Myers, Dick, & Prescott, 2010a; Sartor et al., 2010). Therefore, variants associated with increased risk for substance use in adolescence are likely to also index risk for the eventual development of drug-related problems and dependence.

Studies of adult (Kendler et al., 2007; Xian et al., 2008) and adolescent (Han et al., 1999; Young et al., 2006) twins provide consistent evidence that common genetic liability factors influence engagement in different types of drug use. In one study of adult twins, over one third of the variance in risk for lifetime alcohol, tobacco and marijuana dependence was attributable to a common heritable factor that largely accounted for lifetime co-occurrence of these disorders (Xian et al., 2008). Similarly, significant overlap in patterns of adolescent alcohol, tobacco and marijuana use is in part reflective of a common genetic diathesis (Han et al., 1999; Young et al., 2006). Furthermore, there is substantial overlap in genetic liability for externalizing disorders—such as conduct disorder (CD) and antisocial behavior (ASPD)—and substance use (Iacono et al., 2008; Krueger et al., 2002; 2007; Young et al., 2000). In sum, genetic vulnerability for broad-based externalizing behaviors comprises a substantial component of genetic risk for substance use (Iacono et al., 2008).

Liability for substance use and externalizing behaviors is attributed generally to temperamental and neurobehavioral risk factors, many of which can be captured under the umbrella term *behavioral disinhibition* (Iacono et al., 2008; Krueger et al., 2002; Young et al., 2000; 2009). Latent measures of behavioral disinhibition, often estimated using a composite index of externalizing symptoms, substance use, and personality risk factors (e.g., impulsivity, behavioral undercontrol, novelty seeking), show notably high heritability (∼80%; Krueger et al., 2002; Young et al., 2000). Recently, increasing emphasis has been placed on temperamental indicators of disinhibition (e.g., impulsivity) and corresponding neurocognitive factors (e.g., response inhibition) as intermediate phenotypes that might partly mediate genetic risk for substance use and other externalizing traits (Congdon & Canli, 2005; 2008; Dick et al., 2010; Iacono et al., 2008; Young et al., 2009).

One promising locus for genetic studies of externalizing disorders is the cholinergic muscarinic receptor 2 gene (*CHRM2*). *CHRM2* was advanced as a candidate marker for substance use in the Collaborative Study on the Genetics of Alcoholism (COGA) when linkage analyses suggested a susceptibility locus for alcohol dependence (AD) on chromosome 7q (Foroud et al., 2000). Further linkage and association analyses suggested associations of *CHRM2* with evoked brain oscillations of the P300 event-related potential, an electrophysiological endophenotype (Jones et al., 2004). In subsequent studies, *CHRM2* variations showed associations with alcohol dependence and affective disorders in the COGA cohort (Wang et al., 2004) and independent samples (Luo et al., 2005). Importantly, evidence suggests that associations of *CHRM2* with AD may in fact reflect associations with broader externalizing behaviors. Follow-up analyses with the COGA sample showed that the

association of *CHRM2* with AD was specific to probands with comorbid drug dependence, a group that also showed significantly higher scores on measures of novelty seeking and externalizing symptoms (CD and ASPD) compared to those without drug dependence (Dick et al., 2007a). In a subsequent study several *CHRM2* polymorphisms showed stronger associations with a composite measure of externalizing behaviors (based on symptom counts for alcohol/drug dependence, CD, ASPD, and scores on novelty seeking and sensation seeking measures) than with these phenotypes individually (Dick et al., 2008). The strongest associations with externalizing factor scores were localized in a linkage disequilibrium (LD) block on intron 3–4 (Dick et al., 2008).

Functional mechanisms underlying associations of *CHRM2* with externalizing behaviors are as yet unknown. Muscarinic cholinergic receptors are a subset of G protein-coupled receptors that show diffuse expression throughout the body and central nervous system, exerting both inhibitory and excitatory effects (Langmead et al., 2008; Volpicelli & Levey, 2004). The muscarinic acetylcholine receptor subtype encoded by *CHRM2* (M_2 receptors) serve diverse functions, including inhibition of adenylate cyclase activity, modulation of potassium channels, and regulation of acetylcholine release and dopamine signaling (Volpicelli & Levey, 2004; Threlfell et al., 2010; Woolf & Butcher, 2010). Further, M_2 receptors are broadly implicated in synaptic plasticity, learning, memory, attention, and motor control (Volpicelli & Levey, 2004). Notably, *CHRM2* is among the genes most frequently studied in relation to intelligence and has been linked to measures of cognitive ability in several reports (e.g., Comings et al., 2003; Dick et al., 2007b; Gosso et al., 2006; 2007). It has also been observed that associations of *CHRM2* with cognitive ability have been reported predominantly samples selected based on inferred genetic vulnerability for substance use (Lind et al., 2009). Therefore, one prospect is that associations of *CHRM2* with cognitive or behavioral phenotypes are more readily detected in samples with elevated risk for substance use or externalizing behaviors (Lind et al., 2009).

The goal of this study was to examine associations of *CHRM2* with substance use (alcohol, tobacco and marijuana) in an adolescent cohort. Because associations of *CHRM2* with cognitive and behavioral phenotypes have been reported largely in samples at putative biological risk for substance use (Lind et al., 2009), we examined these associations in a sample of adjudicated youth, who have relatively higher rates of substance use and externalizing disorders compared to the general adolescent population. Finally, given prior evidence for associations of *CHRM2* with temperamental indicators of behavioral disinhibition (e.g., Dick et al., 2008), we examined a mediation model stipulating that temperamental risk factors (impulsivity and sensation seeking) partly account for associations of *CHRM2* with substance use.

Method

Participants

The present analyses include 124 adolescents who were part of a larger cohort (n=731) recruited for a study of adolescent substance use in Denver, Colorado. Potential participants were identified through their involvement with the criminal justice system and recruited from probation offices in the Denver area. The current analyses include the subset of the

sample who a) completed at least one of the assessments used in the current analyses (6 months and 12 months, described below) and b) had available genetic data. This subsample of adolescents (55% male) averaged 16.07 years of age (SD = .99) at enrollment. Self-reported ethnicity/race included Hispanic (36.3%), African-American (30.6%), Caucasian (10.6%), multi-racial (14.5%), American-Indian/Alaskan Native (1.6%), and Asian/Pacific Islander (1.6%) participants. 2.4% indicated "other" and 2.4% did not report ethnicity/race.

Procedure

Recruitment involved approaching potential participants in waiting rooms of youth probation offices. Research assistants approached adolescents and provided information about a longitudinal research study focusing on health and risk behaviors. Inclusion criteria required that participants be 13–18 years old, currently on probation, able to speak/read English, and to have the informed consent of a parent or legal guardian. Written informed consent was obtained from each participant and verbal consent was obtained from a parent via tape-recorded phone calls (except for participants 18 years or older). Probation and juvenile justice staff had no involvement in recruitment and participation had no impact on adolescents' probation status.

Adolescents who assented to participate completed a baseline assessment at were subsequently contacted for follow-up assessments at 6, 12, 18 and 24 months. Assessments were conducted at various locations depending on the location of the adolescent at a given follow-up point. At each assessment, self-report questionnaires were administered on a laptop computer using Audio Computer-Assisted Self-Interviewing (ACASI). ACASI allows participants to view questions on the computer screen while hearing the recorded questions over headphones, thereby minimizing issues with comprehension while allowing programmable skip patterns. Participants received $50 for completion of each assessment. As noted above, the current analyses include participants who completed at least one of the follow-up assessments to provide behavioral data used in the current analyses (6 and 12 months). DNA collection was added as a study component after initiation of the study and was achieved by collecting a saliva sample, which in most cases occurred at the study endpoint.

Measures

Disinhibition—Measures of impulsivity and sensation seeking, assessed at the 6-month timepoint, were used as personality indicators of disinhibition. Impulsivity was assessed with a 12-item scale assessing impulsive decision-making (Donohew et al., 2000). Example items include "I act on the spur of the moment;" "I do the first thing that comes into my mind;" "I do whatever will work out best in the long run" (reverse scored). Items are assessed on a scale of 1 (Never) to 5 (Always). Internal consistency in the current sample was .81. Sensation seeking was assessed with the Brief Sensation Seeking Scale (BSSS; Hoyle et al., 2002), an eight-item measure adapted from Zuckerman's Sensation Seeking Scale for use in studies with adolescents and young adults. Items (e.g., "I prefer friends who are excitingly unpredictable;" "I would like to explore strange places") were rated on a scale of 1 (Disagree a lot) disagree to 5 (Agree a lot). Internal consistency in the current sample was .70. Neither of these two scales contains items on substance use.

Substance Use—Substance use measures included two indicators each for alcohol (frequency/quantity), marijuana (frequency and endorsement of marijuana-related problems), and tobacco (any recent use and number of cigarettes per day). Individual indicators were selected on the basis of available variables, which varied somewhat across substances. For example, given the lack of a measure for marijuana use quantity, we included marijuana problems as the second index of marijuana involvement. Additionally, whereas data were available for marijuana problems, data on alcohol-related problems were not available. Although the variables included in these analyses were not fully uniform across substances, data from several studies suggests that genetic influences on patterns of substance use (e.g., quantity, frequency) overlap substantially with those influencing heavy/problematic use and dependence symptoms (e.g., Pagan et al., 2006; Kendler et al,. 2010a; Sartor et al., 2010), suggesting that genetic influences on multiple forms of substance use can be examined without full uniformity in these phenotypes across drug classes. The current analyses included substance use variables assessed at the 12-month timepoint (with a recall period of 6 months) to allow a prospective analysis of the hypothesized mediation model.

Alcohol use—Drinking behavior was assessed with a variation of a previously published measure developed for adolescents (White & Labouvie, 1989). Drinking frequency and typical quantity were assessed with the items: “In the last 6 months how often did you consume at least one alcoholic drink?” (1 = “never,” 9 = “every day”) and “How many drinks did you usually have at one time?” (1 = “none,” 10 = “more than 20 drinks”). 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.”

Tobacco use—Tobacco use was assessed with two indicators: participants reported on whether they had smoked cigarettes in the last 6 months, as well as number of cigarettes smoked per day. The latter item was assessed on a scale of 1 (0 cigarettes) to 10 (20 or more per day).

Marijuana use—Marijuana involvement was examined based on two items: frequency of use and number of marijuana-related problems. Frequency of use was assessed with the question “In the last six months, how often did you smoke marijuana?” with answers ranging from 0 (never) to 8 (every day). Marijuana-related problems were assessed using the 23-item Rutgers Marijuana Problem Index, which has been validated as a measure of marijuana-related problems in other research (White et al., 2005). Participants rated how often they experienced various negative occurrences as a result of their smoking marijuana (e.g., “Caused shame or embarrassment to someone”). Ratings were made on a 5-point scale (1=never to 5=more than 10 times). Responses to items were dichotomized to reflect whether a statement was endorsed or not and summed to reflect the number of problems endorsed.

SNP selection and genotyping

A single nucleotide polymorphism (rs1455858) was selected based on published results from the COGA study (Dick et al., 2008). In that study rs1455858 was one of several SNPs

within a linkage disequilibrium (LD) block (intron 3–4) showing associations with externalizing phenotypes. Specifically, rs1455858 significantly predicted a composite measure of externalizing phenotypes (alcohol dependence, drug dependence, CD, ASPD, novelty seeking and sensation seeking; Dick et al., 2008). rs1455858 is in LD with nearby SNPs that also showed significant associations with the composite externalizing factor score (e.g., rs7800170, rs1378646). Given high LD in this region we utilized a tag SNP approach to minimize Type 1 error risk arising from multiple testing, which was noted as a concern in a review of prior studies of *CHRM2* (Lind et al., 2009).

Genotyping was conducted using TaqMan® primer and probe pairs (Applied Biosystems). The probes are conjugated to two different dyes, one for each allelic variant (cytosine [C] or thymine [T]). The PCR reaction mixture consists of 20 ng of genomic DNA, 1× Universal PCR Master Mix, 900 nM of each primer and 200 nM of each probe in a 15 μL reaction volume. Amplification was performed using the TaqMan® Universal Thermal Cycling Protocol and fluorescence intensity was measured using the ABI Prism 7500 Real-Time PCR System. Genotypes were acquired using the 7500 system's allelic discrimination software (SDS v1.2.3).

Preliminary analyses examined descriptive characteristics as well as bivaraite associations of rs1455858 with personality and substance use variables. Primary analyses incorporated structural equation modeling (SEM), using EQS 6.1 with a maximum likelihood estimator, to estimate latent variables for substance use and disinhibition, which were included in a prospective mediation model evaluating the indirect (mediated) effect of *CHRM2* on substance use (assessed at 12 months) through disinhibition (assessed at 6 months).

Results

Genotyping for rs1455858 indicated that 48 participants had the CC genotype, 53 had the CT genotype and 23 had the TT genotype. Allele frequencies did not diverge from Hardy-Weinberg equilibrium ($\chi^2(2) = 1.47$, ns). Most (70.5%) participants reported alcohol use; of these, 64.1% reported consuming an average of 4+ drinks per occasion. 57% and 70.9% of participants endorsed marijuana and cigarette use, respectively. Because descriptive analyses generally suggested increasing risk on several phenotypes as a function of number of copies of the T allele (0, 1, or 2) *CHRM2* was coded as a three-level ordinal variable (0 = CC; 1 = CT; 2 = TT) for subsequent analyses. As depicted in Table 2, correlation analyses showed a significant association of *CHRM2* with several of the behavioral variables, including sensation seeking, alcohol frequency/quantity and tobacco use/quantity. Additionally, the association of *CHRM2* with marijuana problems approached statistical significance ($p = .08$).

We proceeded to test latent variables for substance use and disinhibition for inclusion in the SEM model. Substance use was estimated as a latent factor with indicators for alcohol (drinking frequency, typical drinking quantity), tobacco (any cigarette use, average cigarettes per day) and marijuana (frequency of marijuana use and number of marijuana-related problems). Initial fit of the latent variable was poor (CFI=.76, RMSEA=.23) owing to two residual correlations between indicators, one between the marijuana items and one

between the cigarette items. With these residual correlations estimated the model was an adequate fit to the data, $\chi^2(7, n=124) = 12.87$, $p=.08$, CFI=.97, RMSEA=.09, SRMR=.05). Disinhibition was estimated as a latent factor with two indicators (impulsivity and sensation seeking scores). With only two indicators the model is underidentified so the loadings of the two indicators had to be constrained to equality. Even with this constraint the model was just identified, leaving no degrees of freedom to assess fit. However, the loadings of the two indicators were strong and significant (*p*s<.001), suggesting a solid measurement model for disinhibition.

With convergent validity of the factors established, we began sequential tests of the structural model in Figure 2. To control for potential population stratification, analyses included self-reported ethnicity as an observed covariate by including orthogonal contrasts created for each of the racial/ethnic subgroups that constituted a sizable proportion of the sample. Contrasts consisted of dummy-coded variables (African American vs. other; Hispanic vs. other and Caucasian vs. other). We did not analyze ancestral genetic markers because controlling for ethnicity generally serves as an adequate guard against population stratification (Hutchison, Stallings, McGeary, & Bryan, 2004). Additionally, the current approach controlled for ethnicity while simultaneously examining the paths of interest (i.e., *CHRM2* to disinhibition; disinhibition to substance use). Testing alternative iterations of the model using the different ethnicity contrasts yielded no change to the substantive findings and final model statistics are presented controlling for race/ethnicity. In addition, all analyses controlled for the influence of gender. Results did not differ appreciably with or without gender in the model.

Regressing the latent substance use factor on genotype showed a significant bivariate association of *CHRM2* with the substance use latent variable (unstandardized parameter estimate: .75, $p<.01$). The full meditational model included *CHRM2* as an exogenous predictor of the disinhibition latent factor, which in turn was specified as a predictor of the substance use latent factor. When estimated without a direct path from *CHRM2* to substance use, the model provided an adequate fit to the data, $\chi^2(38, n=124) = 47.38$, $p = .14$, CFI = .96, RMSEA = .05, SRMR=.07. *CHRM2* was significantly associated with disinhibition. Disinhibition, in turn, was significantly, positively associated with substance use. Standardized parameter estimates and significance values for all paths are presented in Figure 2.

The following strategy was used to test mediation. First, we added a direct path from the predictor (*CHRM2*) to the outcome variable (substance use) to determine whether the significant bivariate relationship had indeed been reduced to non-significance (i.e., to test whether mediation was present). This test was accomplished by examining both the significance χ^2 change for the model, the unstandardized parameter estimate, and the significance of that parameter estimate. A non-significant change as well as a non-significant parameter estimate for the direct path is evidence for a mediated effect. Second, we examined the z-test for the adaptation of the Sobel (1982) test of the two-part indirect path implemented in EQS 6.1. A significant z-score indicates a significant indirect (mediated) effect (i.e., whether the mediated effect is significantly different from zero). In this case, the direct path from *CHRM2* to substance use was not significant (*B* = .39, *se*=.36,

ns). Adding this path did not significantly improve model fit (χ^2_Δ (1) = 1.18, ns) so it was removed. Note the reduction in the unstandardized parameter estimate for the direct relationship of *CHRM2* to substance use from .75 in the bivariate model to .39 in the presence of the mediator. Further, the test of the indirect effect was significant, z = 2.796, $p < .01$. Thus, results indicated that the association of rs1455858 with substance use was significantly mediated by the latent disinhibition factor.

Discussion

Based on previously reported associations of *CHRM2* with alcohol and drug dependence in adulthood the current study evaluated *CHRM2* in relation to adolescent substance use. Additionally, evidence that *CHRM2* relates to personality and clinical indicators of behavioral disinhibition (Dick et al., 2007a; 2008) informed our hypothesis that associations of *CHRM2* with substance use would be mediated by a index of temperamental disinhibition. Results showed that a candidate *CHRM2* SNP (rs1455858) was significantly associated with latent measures of substance use and disinhibition, as well as with several individual phenotypes comprising the latent measures. Additionally, the temperamental index of disinhibition significantly mediated the association of *CHRM2* with adolescent substance use. The latter finding is generally consistent with the notion that genetic factors influencing broad risk for substance use are also predictive of temperamental risk factors in adolescence (Iacono et al., 2008).

In interpreting these findings it is important to underscore that functional implications of *CHRM2* variation are generally unknown, although recent evidence suggests that *CHRM2* variation could influence M_2 receptor binding *in vivo* (Cannon et al., 2010). Notably, several reports have linked *CHRM2* to differences in cognitive performance (e.g., Comings et al., 2003; Dick et al., 2007b; Gosso et al., 2006; 2007), with these findings being somewhat specific to cohorts at putatively elevated genetic risk for substance use (Lind et al., 2009). This observation provided a basis for examining *CHRM2* in the current sample of adjudicated youth, who have relatively high rates of substance use compared to the general adolescent population. Taken together, existing findings suggest that cognitive variables serve as potential intermediate phenotypes for future studies of *CHRM2* and that a focus on high-risk samples could be advantageous. Focusing on high-risk adolescents can also be strategic in that variability in substance use (a prerequisite for detecting genetic associations) is more limited in general adolescent samples.

Clinical, personality and behavioral indicators of disinhibition are presumed to reflect underlying neurobiological risk factors (Iacono et al., 2008; Young et al., 2009). For example, relationships between disinhibition and substance use (Nigg et al., 2006) can potentially involve differences in executive cognitive function (Finn et al., 2002a,b; 2004), which would implicate specific brain regions (i.e., the frontal cortex). Previous work suggests altered frontal cortex activity in adolescent substance abusers with conduct problems during attentional control tasks (Banich et al., 2007) and reduced frontal engagement in cocaine and marijuana users during response inhibition tasks (Kaufman et al., 2003; Li et al., 2008). Future studies will be needed to investigate performance-based measures of frontally mediated functions in relation to *CHRM2*. Potentially useful tasks

include those that assess monitoring for and inhibiting responses to task-relevant stimuli (e.g. Go/No-go and Stop Signal task; Aron et al., 2006, 2007; Li et al., 2008); delayed gratification (i.e. delay discounting; Green et al., 1994; Madden et al., 1997; Bickel et al., 1999; Petry et al., 2001); and outcome contingency learning/decision making (e.g. Iowa Gambling Task; Bechara, 1994). Such studies are useful for examining precise neurocognitive mechanisms implicated in behavioral disinhibition. Additionally, neuroimaging of these tasks will provide measurements of activation in candidate brain regions (including the inferior frontal gyrus, dorsolateral prefrontal cortex, orbitofrontal cortex, and anterior cingulate cortex/ventromedial prefrontal cortex) for examination in relation to genetic variation.

Evidence suggests that latent risk for behavioral disinhibition is highly heritable (Krueger et al., 2002; Young et al., 2000; 2009) and efforts to characterize this risk at the molecular genetic level could eventually hold clinical implications. Childhood and adolescence is an opportune time to intervene with youth who evidence early signs of risk. Notably, at-risk children and adolescents who do not receive early intervention appear to show steeper increases in disinhibitory symptoms (e.g., Conduct Problems Prevention Research Group, 2007; Connell et al., 2008). A handful of clinical interventions, particularly family-based interventions, have shown promise in terms of reducing disinhibited behavior in children and adolescents (e.g., Dishion et al., 2008; Eyberg, Nelson, & Boggs, 2008; McMahon, 1994). While efficacious, these psychosocial interventions could potentially be improved by tailoring to individual risk factors, including biological risk factors (e.g., Hutchison, 2010). Assuming eventual advances in characterizing genetic risk, assessments conducted early in development would theoretically allow greater opportunity to enact environmental interventions to offset biological liability (e.g., McGue et al., 2000).

Several study limitations should be considered. First, the current analyses focused exclusively on a sample of justice-involved youth and replication in broader samples will be important. Additionally, while this study sought an initial test of a focused mediation hypothesis, larger studies will be required to provide more stringent tests of potential mediators. For instance, large prospective cohort studies would allow the evaluation of substance use trajectories while controlling for baseline substance use and autoregressive effects across assessment points. It is also important to acknowledge that gene-to-behavior mediational pathways are clearly much more complex than could be accounted for in the current analyses. Given that muscarinic cholinergic receptors serve diverse functions and appear relevant for higher-order processes such as learning, memory and attention (Volpicelli & Levey, 2004; Threlfell et al., 2010; Woolf & Butcher, 2010), any functional pathways are likely to be broad and complex. As noted above, studies using neurocognitive assessments will likely be useful to complement studies that relay on self-report measures of disinhibition (e.g., Congdon & Canli, 2005). Response inhibition could be a particularly relevant construct because behavioral genetic studies show that it relates significantly to latent indices of behavioral disinhibition (e.g., Young et al., 2009) and population variability in response inhibition appears attributable entirely to genetic variation (Friedman et al., 2008).

An additional limitation to this study concerns the lack of diagnostic data on other externalizing disorders (e.g., CD, ODD, ADHD). It is proposed that biological risk for substance use disorders manifests as general disinhibition in childhood and that, during this period, genetic variants associated with behavioral disinhibition may show stronger associations with general externalizing disorders than with specific forms of substance use (Iacono et al., 2008). Because diagnostic indicators (e.g., CD, ODD, ADHD) were not examined, we are unable to clearly evaluate the extent to which associations of *CHRM2* with alcohol, marijuana and tobacco were in part attributable to a genetic association with these broader clinical syndromes. This point is important because research with larger samples suggests that associations of *CHRM2* with alcohol dependence may reflect a general association with disinhbitory psychopathology (Dick et al., 2008). Additionally, recruitment of an adjudicated population in this study results in a sample at greater risk for externalizing behaviors generally, rather than substance use specifically. Thus, associations of *CHRM2* and substance use in this study could partly reflect risk for behavioral disinhibition more broadly. In fact, results of the mediation analyses can be viewed as consistent with this possibility. Evidence further suggests that the relative importance of general versus specific sources of genetic liability for substance use changes across developmental stages. For instance, a recent study of alcohol use demonstrated that the influence of non-specific genetic influences (e.g., those related to general risk for externalizing behavior) on adolescent drinking increased markedly from early to mid-adolescence, peaking at age 15–17 (Kendler, Gardner, & Dick, 2010). In contrast, the influence of alcohol-specific genetic risk factors was relatively weaker in adolescence but increased during the transition to adulthood, during which time the influence of nonspecific genetic risk factors declined (Kendler et al., 2010b).

It is important to consider that this study focused on a single candidate SNP. Dense genotyping of *CHRM2* using larger samples would be important in future studies. Additional work is also needed to characterize effect sizes for associations of *CHRM2* with substance use and related phenotypes, given that associations of *CHRM2* with other phenotypes have varied in magnitude across studies (Lind et al., 2009). Of note, the associations of rs1455858 with personality and substance use variables in this study could be considered large compared to the small effects often observed in genetic association studies. Whereas this finding could reflect an overestimate of this genetic effect, the use of latent variables is expected to increase power by reducing error in phenotype measurement. For example, associations similar in magnitude have been reported in latent variable analyses testing associations of the *DRD4* exon III polymorphism on alcohol use and novelty seeking (Ray et al., 2008). Overall, it will be important to establish reliable effect size estimates in different samples, particularly in larger cohorts of adolescents.

This study extends previous findings by providing preliminary evidence for associations of *CHRM2* with adolescent substance use. Pending replication, these findings may provide a basis for studying *CHRM2* variation in relation to patterns of substance use and homotypic comorbidity across developmental stages. Temperamental factors related to disinhibition (e.g., impulsivity) and their neurocognitive or neurobiological correlates may prove useful as candidate endophenotypes in this context. Prospective cohort studies with multi-method

approaches to characterizing behavioral disinhibition would also useful for evaluating these possibilities.

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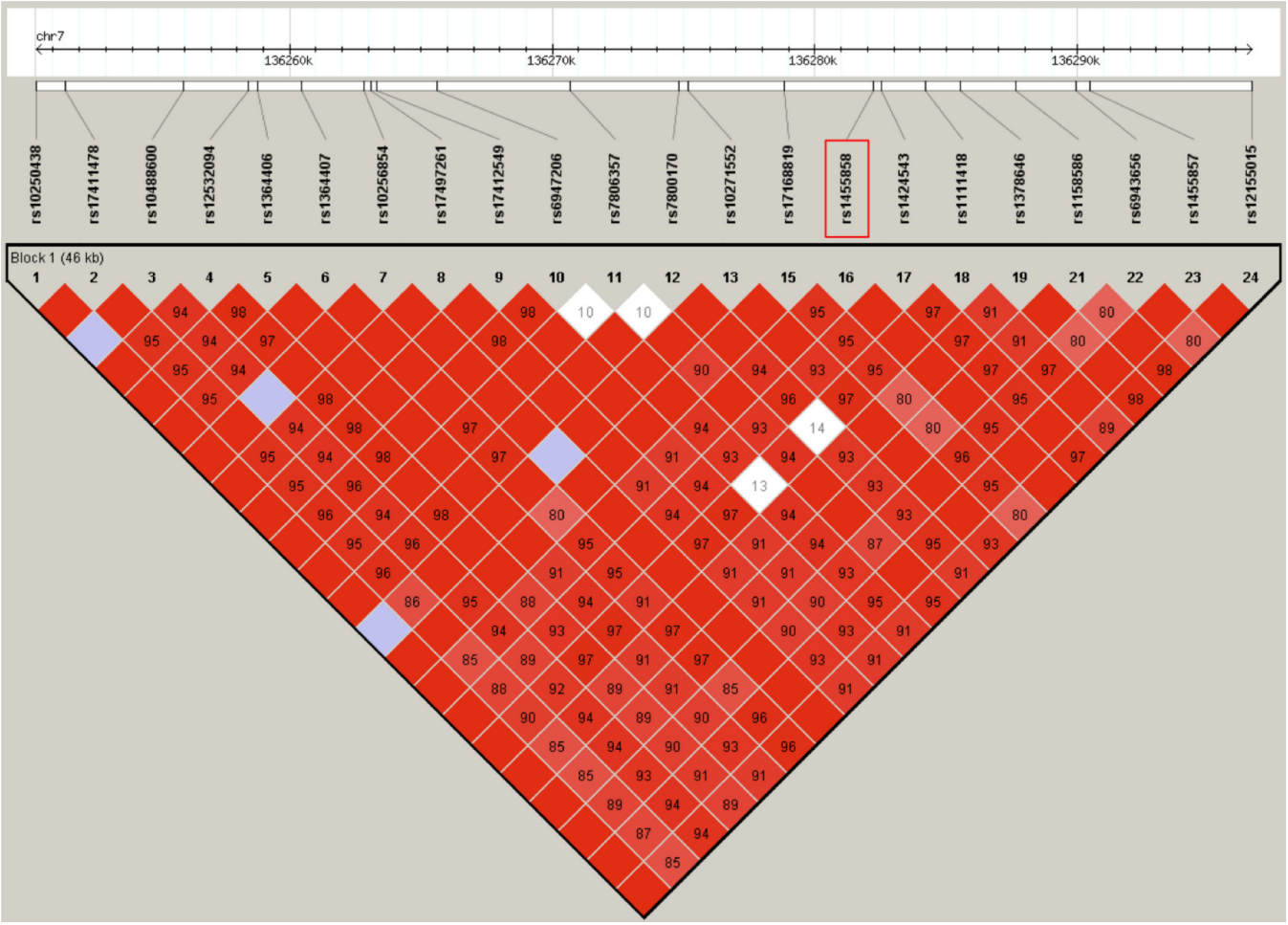

Figure 1.

Location of rs1455858 (chromosome 7) and linkage disequilibrium (D') structure (HapMap3, release 2).

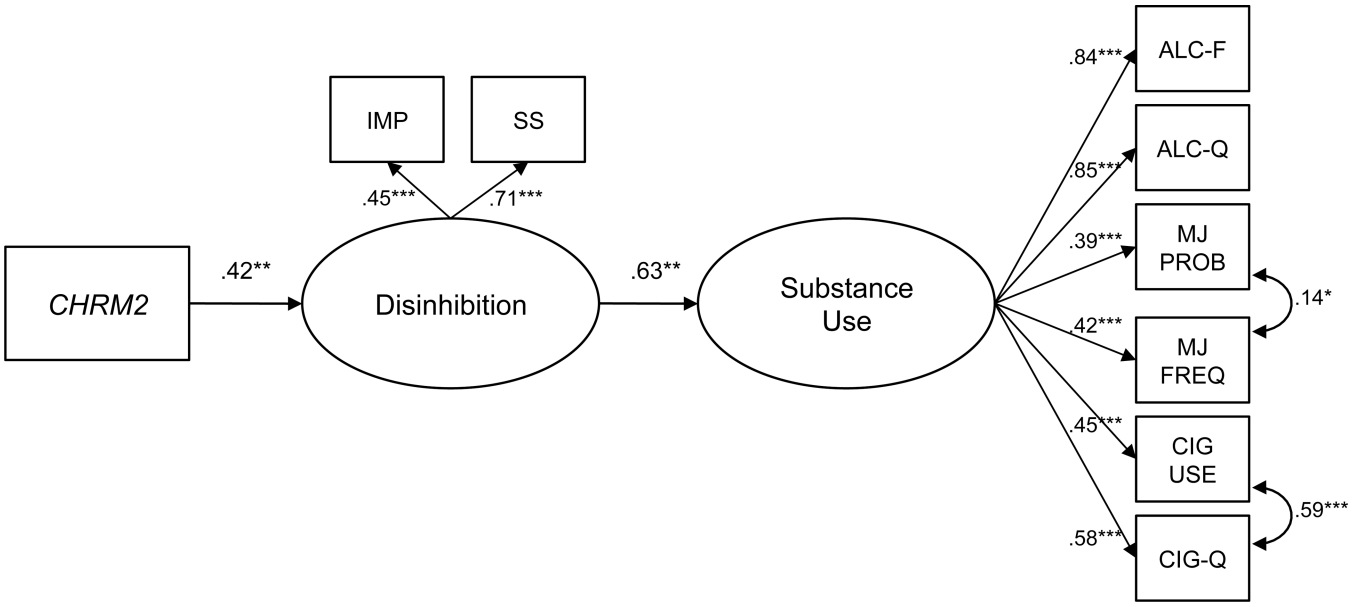

Figure 2.

Structural equation model evaluating a temperamental index of disinhibition (assessed at Time 1) as mediating the association of *CHRM2* with risk for substance use (assessed at Time 2). IMP = impulsivity; SS = sensation seeking; ALC-F = alcohol use frequency; ALC-Q = typical drinking quantity; MJ PROB = number of marijuana-related problems endorsed; MJ FREQ = frequency of marijuana use; CIG USE = smoking status (0 = no, 1 = yes); CIG-Q = typical number of cigarettes per day. Race/ethnicity and gender are controlled for in this analysis. Path coefficients are standardized. * $p < .05$ ** $p < .01$ *** $p < .001$

Table 1

Descriptive statistics for behavioral variables

	M	SD
Impulsivity[a]	2.76	.54
Sensation Seeking[b]	3.32	.75
Alcohol Use Frequency[c]	3.42	2.40
Alcohol Use Quantity[d]	3.30	2.22
Marijuana Problems[e]	8.37	6.39
Marijuana Use Frequency[f]	3.29	3.54
Cigarettes Per day[g]	3.31	2.38
Tobacco Use (% endorsed)[h]	68	

[a] On a scale of 1(never) to 5 (always)

[b] On a scale of 1 (disagree a lot) to 9 (agree a lot)

[c] On a scale of 1 (never) to 9 (every day)

[d] On a scale of 1 (none) to 10 (more than 20 drinks).

[e] Number of problems endorsed (of 23 possible).

[f] On a scale from 0 (never) to 8 (every day).

[g] On a scale of 0 (no cigarettes) to 10 (20 or more cigarettes per day).

[h] Coded 0 (no) or 1 (yes). Substance use variables assessed based on the prior 6 months.

Table 2

Pearson correlations among *CHRM2*, personality and substance use variables

	1	2	3	4	5	6	7	8	9
1. *CHRM2*	-								
2. Impulsivity	.090	-							
3. Sensation seeking	.281**	.348**	-						
4. Drinking frequency	.218*	.134	.321**	-					
5. Drinking quantity	.198*	.183	.391***	.679**	-				
6. Marijuana problems	.167	.247*	.179	.343**	.333***	-			
7. Marijuana use frequency	.004	.114	.324**	.447***	.249**	.295**	-		
8. Cigarette smoker	.230*	.229*	.366***	.333**	.377***	.181	.146	-	
9. Cigarettes per day	.208*	.245*	.297**	.389***	.470***	.124	.073	.673***	-

Note. Correlations for cigarette smoker are point biserial.

* $p < .05$

** $p < .01$

*** $p < .001$