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An initial investigation of associations between dopamine-linked genetic variation and smoking motives in African Americans

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Abstract

Nicotine dependence (ND) is a heterogeneous phenotype with complex genetic influences that may vary across ethnicities. The use of intermediate phenotypes may clarify genetic influences and reveal specific etiological pathways. Prior work in European Americans has found that the four Primary Dependence Motives (PDM) subscales (Automaticity, Craving, Loss of Control, and Tolerance) of the Wisconsin Inventory of Smoking Motives represent core features of nicotine dependence and are promising intermediate phenotypes for understanding genetic pathways to ND. However, no studies have examined PDM as an intermediate phenotype in African American smokers, an ethnic population that displays unique patterns of smoking and genetic variation. In the current study, 268 African American daily smokers completed a phenotypic assessment and provided a sample of DNA. Associations among haplotypes in the *NCAM1-TTC12-ANKK1-DRD2* gene cluster, a dopamine-related gene region associated with ND, PDM intermediate phenotypes, and ND were examined. Dopamine-related genetic variation in the *DBH* and *COMT* genes was also considered on an exploratory basis. Mediation analysis was used to test the indirect pathway from genetic variation to smoking motives to nicotine dependence. *NCAM1-TTC12-ANKK1-DRD2* region variation was significantly associated with the Automaticity subscale and, further,

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Automaticity significantly mediated associations among *NCAM1-TTC12-ANKK1-DRD2* cluster variants and ND. *DBH* was also significantly associated with Automaticity, Craving, and Tolerance; Automaticity and Tolerance also served as mediators of the *DBH*-ND relationship. These results suggest that PDM, Automaticity in particular, may be a viable intermediate phenotype for understanding dopamine-related genetic influences on ND in African American smokers. Findings support a model in which putatively dopaminergic variants exert influence on ND through an effect on patterns of automatic routinized smoking.

Keywords

nicotine; intermediate phenotype; dopamine; haplotype; SNP; motives; African American

INTRODUCTION

Although African Americans (AAs) initiate smoking later than European Americans (EAs), AA smokers display a greater persistence of smoking into mid-adulthood, lower cessation rates, and higher mortality rates from tobacco-associated diseases (Kandel *et al.*, 2011, Safford *et al.*, 2012). Genetic factors contribute substantially to nicotine dependence (ND; Goldman *et al.*, 2005, MacKillop *et al.*, 2010), with the preponderance of molecular genetic evidence coming from studies focusing on EAs (Munafò *et al.*, 2004, Munafò *et al.*, 2009). Although many non-genetic factors contribute to observed EA/AA differences in smoking (e.g. Primack *et al.*, 2007), examinations of genetic influences in AA smokers are critical due to the distinct phenotypic and genotypic patterns in this population. In addition to the potential for unique etiological influences within AA smokers, AA smokers represent an ethnic subgroup known for its greater polymorphic variation and associated shorter haplotypes (Gabriel *et al.*, 2002). Thus, investigating genetic influences on smoking phenotypes in AAs may broadly inform etiological studies of smoking by providing data that narrows genetic findings from large regions of interest to more specific susceptibility loci.

NCAM1-TTC12-ANKK1-DRD2 variants became a focus of smoking molecular genetics due to the critical role of dopamine D2 receptors (*DRD2*) in nicotine pharmacodynamics (Benowitz, 2010). This chromosome 11q23 gene cluster has been associated with ND in studies using both genome-wide and candidate approaches (Bergen *et al.*, 2009, Ducci *et al.*, 2011, Laucht *et al.*, 2008, Morley *et al.*, 2006, Saccone *et al.*, 2007), including a handful in AAs (David *et al.*, 2010, Gelernter *et al.*, 2006, Huang *et al.*, 2009). Although meta-analyses support a role for *DRD2* in smoking risk (Li *et al.*, 2004, Munafò *et al.*, 2004), heterogeneity of effects exists across studies and meta-analytic reports call for additional studies using non-EA participants. Due to their role in dopamine pathway, also of interest are catechol-O-methyltransferase (*COMT*; located on chromosome 22q11) (Akil *et al.*, 2003), involved in dopamine degradation, and dopamine beta hydroxylase (*DBH*; located on chromosome 9q34), involved in converting dopamine to norepinephrine (Cubells and Zabetian, 2004). Although effects of variation of these genes on smoking have been inconsistent (Han *et al.*, 2008, McKinney *et al.*, 2000, Shiels *et al.*, 2008, Ton *et al.*, 2007), studies in AAs are

limited and there is suggestion of ethnic-specificity of effects (Beuten *et al.*, 2006, Colilla *et al.*, 2005).

Further, genetic influences may be clarified by using intermediate/mechanistic phenotypes that are putatively more narrow and more proximal to the differences in genetic variation (NCI, 2009, MacKillop and Munafò, 2013). This strategy is intended to reveal larger effect sizes, clarify mechanisms of genetic risk and/or protection, and identify a more homogeneous group of smokers who may share a particular genetically-mediated vulnerability to ND. Preliminary work in EAs has shown the four primary dependence motives (PDM) subscales of the Wisconsin Inventory of Smoking Motives (WISDM-68) Automaticity, Craving, Loss of Control, and Tolerance (Piasecki 2010 and Piper et al 2008) are viable intermediate phenotypes that can explicate genetic mechanisms of dependence. For example, neuronal cholinergic receptor (*CHRNA5-A3-B5*) haplotypes were associated with PDM subscales in early onset smokers (Baker *et al.*, 2009). Our recent work in EAs has shown that the PDM subscales are a mediator of the association with *NCAM1-TTC12-ANKK1-DRD2* haplotype variation and ND, and thus support their role as viable intermediate phenotypes that can explicate pathways between genetic risk and dependence (Bidwell *et al.*, 2015). This work suggested that, rather than PDM and FTND being alternative manifestations of the clinical ND phenotype without evidence an indirect effect, the PDM motivational intermediate phenotypes serve as a mediator along an etiological pathway that explains the association between these risk loci and ND in EAs. Within the context of the aforementioned differences in smoking topography of AAs and unique genetic variability, it is important to attempt to replicate our previous findings on EAs to AAs. In this way, studies that employ formal mediation analyses can connect established genotype–ND relationships empirically as credible mechanisms by which genetic variation exerts influence on clinical dependence phenotypes across ethnic groups.

Current Study

Thus, given the need for studies that examine the intersection of smoking intermediate phenotypes, biologically-implicated candidate genes, and nicotine dependence in AAs, we examined WISDM motivational profiles as intermediate phenotypes for ND in an AA sample. Based on limited prior work on the WISDM in AAs, we did not expect phenotypic motivational differences based on race. Modeling our approach after our prior work in EAs (Bidwell *et al.*, 2015), we tested *DRD2-ANKK1-TTC12-NCAM1* haplotypes in association with both clinical dependence and PDM phenotypes. Single nucleotide variation in *COMT* and *DBH* were also considered on an exploratory basis. We then used mediation to evaluate mechanistic pathways testing whether PDM subscales were significant mediators of the genotype-ND relationship.

MATERIALS & METHODS

Sample Description

Participants (N=268; 57% [N=153] males) were recruited via newspaper, Internet, and flyer advertising as part of a larger study of behavioral economics and smoking (MacKillop *et al.*, 2012). Inclusion criteria were 18 years old, 5 cigarettes/day, and 8th grade education.

The full sample consisted of 1,124 participants enrolled across three sites: Providence, RI; Athens, GA; and Aiken, SC. All study procedures were approved by the Brown University and University of Georgia Institutional Review Boards. The current study examines data from the subset of 268 African American (AA) daily smokers who provided both DNA and self-report smoking data. As expected, FTND was significantly higher in AA than the 734 EA participants ($t = 5.13$, $p < .0001$; e.g. Luo *et al.*, 2008), but there were no significant differences on PDM subscales by ethnicity. Of the AA sample, 74% ($n = 198$) stated a preference for menthol cigarettes, compared to 29% ($n = 212$) of the EA sample ($\chi^2 = 168.1$, $p < .0001$). Demographic, smoking, and alcohol use characteristics are listed in Table 1.

Phenotypic Measures

Fagerström Test of Nicotine Dependence (FTND; Heatherton *et al.*, 1991)—The FTND measures the severity of ND using six-items. Two of the items are reversed scored and higher FTND reflects greater dependence on nicotine.

WISDM PDM—The 68-item WISDM-68 (Piper *et al.*, 2004) was used to assess the four primary dependence motives (PDM; Automaticity, Craving, Locus of Control, and Tolerance) reflecting heavy, pervasive smoking (Piasecki *et al.*, 2010, Piper *et al.*, 2008). An index score for each of the four PDM was created by averaging the items within each subscale: Automaticity (e.g. “I often smoke without thinking about it.”), Craving (e.g. “I frequently crave cigarettes.”), Loss of Control (LOC) (e.g. “Cigarettes control me.”), and Tolerance (e.g. “I can only go a couple hours between cigarettes.”). The means and distributions for each subscale are listed in Table 2.

Marker Information and Haplotype Derivation

Genotyping and SNP selection—This study examined 13 markers across the *NCAM1-TTC12-ANKK1-DRD2* candidate gene region and two exploratory markers in the *COMT* and *DBH* genes. HapMap was used to determine the tag SNPs required to capture >80% of the variance within the *NCAM1-TTC12-ANKK1-DRD2* gene cluster and these tag SNPs were augmented with loci that had been implicated in prior studies of this region and nicotine dependence (e.g. Gelernter *et al.*, 2006). Ethanol precipitation was used to extract DNA from collected saliva samples. Samples were genotyped using a MassEXTEND Sequenom assay based on the annealing of an oligonucleotide primer adjacent to the SNP of interest. The assay was performed in multiplex with 20 reactions in a single well; 20% of all samples were randomly run in duplicate resulting in a genotyping error rate of 0.02%. Primer sequences are available in Supplementary Table 1. Genotypes were determined by investigators blinded to phenotypic data.

Frequencies of genotypes/alleles and Hardy-Weinberg Equilibrium (HWE) p -values for each marker are listed in Table 3. One marker (rs4938012) was excluded from subsequent analyses due to greater than 15% missing genotypes and another was excluded due to HW failure (rs1799732).

Haplotype derivation—Haploview was used to visualize haplotype blocks (i.e., the combinations of SNP/InDel markers that are statistically associated) using all available

polymorphic data (Barrett, 2009, Barrett *et al.*, 2005). As expected, greater variation with more, but shorter, haplotype blocks were observed than in our prior paper of EAs (Bidwell *et al.*, 2015). Three haplotype blocks were observed (Figure 1): Block 1 was based on rs2282511 (*TTC12*) and rs877138 (*ANKKI*); Block 2 on rs17115439 (*ANKKI*), rs4938013 (*ANKKI*); and Block 3 on rs4938015 (*ANKKI*); rs11604671 (*ANKKI*).

Haplotype blocks were then confirmed and extracted using PHASE [Version 2.1] (Stephens and Donnelly, 2003, Stephens and Scheet, 2005, Stephens *et al.*, 2001), with the requirement that the probability of a phased haplotype had to be greater than or equal to 0.80 (Oroszi *et al.*, 2009). Diplotypes (i.e., combination of haplotypes across the pair of homologous chromosomes) were then constructed for use in the regression analyses. Table 4 describes the haplotypes and their frequencies in the sample population. Because of the limited and inconsistent literature indicating a putative risk allele at our candidate loci, haplotype, and thus diplotype, scores were created assuming an additive effect for each of the identified haplotypes (Lu *et al.*, 2006, Pajewski *et al.*, 2011). This additive scoring scheme where an individual may possess 0, 1, or 2 copies of each haplotype observed was utilized for every haplotype that was more frequent than .20 in the study sample. Less frequent haplotypes were excluded from further analysis.

Statistical Analyses

Analyses were executed in PLINK v1.07 (Purcell, <http://pngu.mgh.harvard.edu/purcell/plink/>, Purcell *et al.*, 2007) and SPSS 19.0 (IBM, Released 2010.). Smoking phenotypes were initially examined for outliers (using standard scores, criterion $Z=3.29$) and for distribution normality with no observed violations (Tabachnick and Fidell, 2001). Bivariate correlations among smoking phenotypes were examined. Univariate linear regressions were used to test the main effects of genetic variation on smoking phenotypes, using the observed haplotypes as the primary tests and following up with test of the individual loci that did not fall into haplotype blocks. Separate models were run for each smoking measure. Finally, where initial regression analyses determined a significant main effect of a genetic variant on a PDM subscale, we subsequently used selected mediation to test the extent to which the genetic effect (IV) exerts its influence on FTND (DV) through the PDM (mediator). Testing of indirect effects was not conditional on the presence of a statistically significant direct effect from the genotype/haplotype to FTND (i.e., the direct A→C path) because it is not required for the indirect test and several scenarios can give rise to a nonsignificant A→C relationship (for a review, see MacKinnon & Fairchild, 2009). Mediation was tested using the products of the coefficients method in SPSS 19.0 (Preacher and Hayes, 2008). Bias corrected 95% confidence intervals of the indirect effect were estimated using bootstrapping methods (Preacher and Hayes, 2008). Given the relatively large number of phenotypes tested in this preliminary investigation, we adopted a significance threshold $p < .01$ for all analyses; p values of $< .05$ were considered significant at trend level.

RESULTS

Correlations among Smoking Measures

As expected, the smoking motives were each significantly correlated with each other and FTND (all p 's < .001; Table 2).

Main Effects of Genetic Variation on Smoking Phenotypes

Table 5 presents the results of linear regression models testing association among the genetic variants (3 *NCAM1-TTC12-ANKK1-DRD2* haplotype blocks and 7 individual polymorphisms) and smoking phenotypes (FTND and PDM). The direction of the regression coefficient represents the effect of each extra minor allele (i.e., a negative regression coefficient means that the minor allele decreases risk/phenotype mean). For Block 1, the CA haplotype was significantly associated with higher FTND ($R^2 = .02$, $p < .01$) and higher Automaticity ($R^2 = .02$, $p < .01$). In the same block, the AG haplotype was associated at trend level with lower FTND ($R^2 = .02$, $p < .05$) and lower Automaticity ($R^2 = .02$, $p < .05$). For Block 2, the TA haplotype was associated at trend level with lower FTND ($R^2 = .02$, $p < .05$) and lower Automaticity ($R^2 = .02$, $p < .05$). None of the Block 3 haplotypes were associated with the smoking phenotypes. None of the *NCAM1-TTC12-ANKK1-DRD2* loci were significantly associated with FTND or PDM phenotypes, but two were associated at trend level with lower Automaticity, including loci from *TTC12* [rs2303380 (G)] and *DRD2* [rs1079597 (A)] (p 's < .05). In addition, the T allele of rs1611115 in *DBH* was associated with higher levels of each of the three of the four PDM intermediate phenotypes, Automaticity, Craving, and Tolerance (p 's < .01) and at trend level with FTND and the LOC subscale (p 's < .05). Each of the significantly associated haplotype or loci variant accounted for 2-3% of the variance in the phenotype (R^2 's range from .018 to .032).

Indirect Effects of Genetic Variation through Smoking Motives

Table 6 reports the results of the indirect effects for the selected mediation tests. When mediation was formally tested, nearly all of the genetic variants that were significantly associated with a PDM subscale exerted a significant indirect effect on FTND through the requisite PDM intermediate phenotype. Specifically, significant mediation was found for rs1611115 of *DBH* on higher FTND through increasing scores on both Automaticity and Tolerance (p 's < .01) and a trend level mediation effect on higher FTND was found for Craving ($p = .02$). With regard to *NCAM1-TTC12-ANKK1-DRD2* gene cluster, there was a significant indirect effect in the direction of increased risk of the Block 1 AC haplotype on FTND through Automaticity.

DISCUSSION

Albeit preliminary, these data provide novel support for associations among smoking motives and common genetic variation within the *NCAM1-TTC12-ANKK1-DRD2* gene-cluster and the *DBH* gene in an AA sample. With regard to the *NCAM1-TTC12-ANKK1-DRD2* gene-cluster, analyses revealed significant associations between variation in haplotype block 1 (consisting of rs2282511 in *TTC12* and rs877138 in *ANKK1*) and Automaticity motives. Trend level findings were present for block 2 (consisting of

rs17115439 in *ANKK1* and rs4938013 in *ANKK1*) with FTND and Automaticity as well as two additional independent SNPs (rs2303380 in *TTC12* and rs1079597 in *DRD2*) with Automaticity. Each of these *NCAM1-TTC12-ANKK1-DRD2* cluster variants demonstrated significant association with Automaticity in our prior study in a large sample of EAs. In contrast, block 3 (consisting of rs4938015 and rs11604671 both in *ANKK1*) was not associated with any smoking phenotype in the current study, whereas rs4938015 had been significantly associated with Automaticity in our prior study of EA smokers (significant both at an independent SNP level and as part of a five SNP haplotype block). Thus, the strategy of combining haplotype with individual loci analyses sheds light on the potential role of specific individual variants, while simultaneously accounting for LD and genetic background across the region. Further, examining associations and variation across the region specifically in a subsample of AA smokers revealed shorter haplotype blocks and more specific associations that may allow for narrowing of the susceptibility loci in the region. *DBH* and *COMT* SNPs were considered on an exploratory basis. The T allele at rs161115 of the *DBH* gene was significantly associated with three of the four PDM subscales, Automaticity, Craving, and Tolerance, and at trend level with ND and the Locus of Control subscale. There were no associations with any smoking phenotypes and *COMT*.

Mediation analyses supported a significant indirect effect via PDM of both *NCAM1-TTC12-ANKK1-DRD2* cluster (Automaticity subscale) and *DBH* (Automaticity and Tolerance subscales) genetic effects on ND. Findings of a significant indirect effect via PDM is suggestive that, rather than these smoking motives being an alternative manifestation of the clinical ND phenotype, PDM serve as a mechanism along the causal pathway from genetic risk to clinical dependence. Thus, *DBH* variation, linked to ND via the correlated Automaticity, Craving, and Tolerance subscales, may increase the likelihood that a person will become dependent via increased craving, tolerance, and automatic smoking. *NCAM1-TTC12-ANKK1-DRD2* variants showed a particular risk pathway related to Automaticity, potentially exerting their effect through a more narrow pattern of automatic smoking behaviors that can be elicited with little awareness. These findings are consistent with our data in EA smokers demonstrating associations among *NCAM1-TTC12-ANKK1-DRD2* variation and the PDM intermediate phenotypes, as well as a mechanistic role for PDM phenotypes in risk for ND (Bidwell *et al.*, 2015). Taken together, findings support the use of smoking motives as viable intermediate phenotypes for ND in AA populations and suggest that genetic studies of the complex ND phenotype may benefit from focusing on more discrete smoking behaviors like motivational profiles.

Our results are consistent with prior AA studies associating variation in the *NCAM1-TTC12-ANKK1-DRD2* gene cluster with ND (David *et al.*, 2010, Gelernter *et al.*, 2006, Huang *et al.*, 2009). Several of the SNPs suggested here to be part of a pathway to ND via Automaticity motives have been associated with ND in prior studies using AA samples, including rs2303380 (*TTC12*), rs2282511 (*TTC12*), rs877138 (*ANKK1*) and rs4938013 (*ANKK1*) (David *et al.*, 2010, Gelernter *et al.*, 2006). *DRD2*'s rs1079597 has previously been associated with ND and the severity of self-reported smoking withdrawal (Gelernter *et al.*, 2006, Robinson *et al.*, 2007); however, these prior associations were present in primarily EA or pooled samples. Thus, across studies using both EA and AA samples data are broadly

supportive that a substantive susceptibility locus for ND is located in this region, although the specific location or structure of this vulnerability may differ across ethnicities.

As far as we know, this is the first report of an association with rs161115 *DBH* SNP and smoking phenotypes in an exclusively AA sample. Prior studies demonstrating associations among *DBH* variants and smoking phenotypes have almost exclusively focused on EA samples (e.g. Tobacco and Genetics Consortium, 2010, Freire *et al.*, 2006, Johnstone *et al.*, 2002, Siedlinski *et al.*, 2011). In our study, the low activity T allele at this locus was associated significantly or at trend level with increased levels of FTND and each of the four PDM; further, Automaticity and Tolerance intermediate phenotypes were a risk mechanism linking the T allele to increased ND severity. Given these associations and the associations among the specific PDM, the most cogent interpretation appears to be that this SNP confers risk via higher PDM for smoking, not a particular subset of motives. This putatively functional variant, located upstream of the *DBH* translational start site, accounts for between 1/3 to 1/2 of the variation in plasma-DBH activity in both EA and AA samples (Zabetian *et al.*, 2001). Although in that study genotypic relationships with *DBH* enzyme activity values were broadly similar across ethnicities, some profile differences between EA and AA subsamples were reported, particularly in the prevalence of very low activity individuals. Thus, studies should continue to examine the role of functional *DBH* variants in ND and test intermediate phenotypic mechanisms in samples stratified by ethnicity in order to understand if risk mechanisms are operating similarly across various populations.

Our results are consistent with some prior work indicating a lack of association among smoking cessation phenotypes and *COMT* in AA smokers (Colilla *et al.*, 2005). However, these findings are inconsistent with prior family-based work supporting an association among the Val/Met *COMT* polymorphism and FTND in AAs (Beuten *et al.*, 2006) and laboratory work indicating associations with withdrawal severity in a pooled EA/AA sample (Herman *et al.*, 2013). The mixed literature suggests that results may be specific to particular phenotypic features of ND and may vary depending on the ethnic make-up of the sample. Despite these inconsistencies, given the prominent role of *COMT* in dopamine metabolism, it remains an attractive candidate for molecular genetic studies of smoking.

A strength of our study is the focus on a restricted sample of AA smokers. When there are phenotypic and genotypic differences by ethnicity, the use of mixed samples can increase the rate of false discoveries and/or reduce power (Clayton *et al.*, 2005). Further, AA subpopulations tend have greater polymorphic variation, resulting in shorter haplotypes which may aid (as available samples get larger) in narrowing genetic findings from large regions of interest and/or long haplotype blocks to specific susceptibility loci. As with all studies using a candidate based approach, our findings need to be substantiated through replication with more and larger samples. This is especially the case given that, due to the relatively small sample size and the number of SNPs examined, some of the associations reported here would not survive stringent type I error rate correction. In addition, the genotype and analytic approach focused on characterizing common variation in the *NCAM1-TTC12-ANKK1-DRD2* gene region. However, there is additional genotypic variance within this region that was not measured (e.g. both common and rare variants) and additional

mechanisms involved that may act in concert with putatively dopamine-related variants in order to influence risk for smoking.

In sum, this investigation, using individual genotype, haplotype, and mediation analyses, extends the literature and preliminarily implicates *NCAM1-TTC12-ANKK1-DRD2* gene cluster variants in risk for ND in AAs. Our findings, which are consistent with our prior study of EAs, suggest that the effects of genetic variation within the *NCAM1-TTC12-ANKK1-DRD2* gene region on ND may exert itself through a heavy and pervasive smoking ritual that can be elicited with little awareness. In addition, it was found that a variant in *DBH* appeared to increase risk of greater ND by way of PDM subscales more broadly. Together, these findings suggest that smoking motives, particularly Automaticity, are viable intermediate phenotypes for understanding genetic contributions to ND in AA smokers that may shed light on the genetic pathways to dependence and clinical outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Dopaminergic (DA) genetic variation is important in nicotine dependence (ND) etiology
- DA genetic risk is understudied in African American (AA) smokers
- This study examined DA genes and nicotine dependence (ND) phenotypes in AA smokers
- DA genes and haplotypes were associated with both ND and automatic smoking behaviors
- DA genes may increase ND risk through effects on automatic, habitual smoking behaviors

Figure 1. Marker-to-marker D' values for the *NCAM1-TTC12-ANKK1-DRD2* region polymorphisms. D' varies between 0 and 1 describes the extent of linkage disequilibrium, a measure of interdependency between genetic loci. A value of 0 for D' suggests that the examined polymorphisms are independent of one another, while a value of 1 suggests that the polymorphisms provide redundant information. Numbers in the boxes are shown as Haploview output.as whole numbers, but reflect D' correlations that do not exceed 1 (e.g., 91 = .91); an empty box with no numerical value represents D' of 1.

Table 1

Sample demographics, smoking, and other substance use ($N = 268$)

Sample characteristics	Mean	SD
Age	37.3	12.6
Years education	11.8	2.0
Number of daily cigarettes	16.9	12.9
Age smoked first cigarette	14.8	3.9
AUDIT Total	9.0	8.0

Note. AUDIT = Alcohol Use Disorders Test

Table 2

Means, standard deviations, and bivariate correlations for smoking phenotypes.

	Mean	SD	Correlations among smoking measures (<i>r</i>)			
			FTND	Automaticity	Craving	Locus of Control
FTND	4.9	2.1	-			
WISDM-Automaticity	4.0	1.6	0.40	-		
WISDM-Craving	4.1	1.6	0.39	0.69	-	
WISDM-Locus of Control	3.5	1.6	0.41	0.63	0.70	-
WISDM-Tolerance	4.4	1.6	0.55	0.69	0.74	0.62

Note. FTND= Fagerström Test of Nicotine Dependence; WISDM = Wisconsin Inventory of Smoking Motives; all correlations significant at $p < .001$.

Table 3

Genotype and minor allele frequencies of dopamine-related loci.

Polymorphism	Genotypes N (%)		Allele Frequency		HWE <i>p</i> -value
<i>NCAM1-TTC12-ANKK1-DRD2 region loci</i>					
<i>NCAM1</i>					
rs618114 Frequency	GG 156 (58.2)	GA / AG 85 (31.7)	AA 8 (3.0)	G 0.81	A 0.19
<i>TTC12</i>					
rs2303380 Frequency	GG 24 (9.0)	GA / AG 109 (40.7)	AA 124 (46.3)	G 0.29	A 0.71
rs2282511 Frequency	CC 155 (57.8)	CA / AC 78 (29.1)	AA 21 (7.8)	C 0.78	A 0.22
<i>ANKK1</i>					
rs877138 Frequency	GG 18 (6.7)	GA / AG 77 (28.7)	AA 164 (61.2)	G 0.21	A 0.79
rs17115439 Frequency	TT 99 (36.9)	TC / CT 107 (39.9)	CC 51 (19.0)	T 0.61	C 0.39
rs4938013 Frequency	CC 165 (61.6)	CA / AC 78 (29.1)	AA 18 (6.7)	C 0.79	A 0.21
rs4938015 Frequency	TT 101 (37.7)	TC / CT 107 (39.9)	CC 51 (19.0)	T 0.61	C 0.39
rs11604671 Frequency	GG 203 (75.7)	GA / AG 51 (19.0)	AA 51 (19.0)	G 0.90	A 0.10
<i>DRD2</i>					
rs1079597 Frequency	GG 190 (70.9)	GA / AG 63 (23.5)	AA 8 (3.0)	G 0.85	A 0.15
rs6277 Frequency	TT 3 (1.1)	TC / CT 51 (19.0)	CC 207 (77.2)	T 0.11	C 0.89
rs1800497 Frequency	TT 34 (12.7)	TC / CT 112 (41.8)	CC 111 (41.4)	T 0.34	C 0.66
rs1799732 Frequency	Del/Del 56 (20.9)	C.Del/Del.C 86 (32.1)	CC 94 (35.1)	Del 0.37	C 0.63
<i>Explanatory loci</i>					
<i>COMT</i>					
rs4680 Frequency	GG 121 (45.1)	GA / AG 110 (41.0)	AA 25 (9.3)	G 0.70	A 0.30
<i>DBH</i>					
					3.59 E-05

Polymorphism	Genotypes N (%)			Allele Frequency		HWE <i>p</i> -value
rs1611115 Frequency	TT 5 (1.9)	TC / CT 76 (28.4)	CC 178 (66.4)	T 0.16	C 0.84	.39

Note. Table shows the genotypes and frequencies for each marker (in order of chromosomal location). All loci are single nucleotide polymorphisms except for rs1799732 which is an InDel polymorphism. Proportions do not sum to 100 to allow for the accurate depiction of missingness in the data.

Table 4

NCAM1-TTC12-ANKK1-DRD2 region haplotypes and frequencies.

Block 1		Population Frequency	N(%) of Carrying 0, 1, or 2 Copies		
rs2282511		rs877138	0	1	2
C		A	0.77	22 (8.2)	78 (29.1)
A		G	0.21	164 (61.2)	78 (29.1)
Block 2					
rs17115439		rs4938013			
T		C	0.38	108 (40.3)	105 (39.2)
T		A	0.21	162 (60.4)	76 (28.4)
C		C	0.41	98 (36.6)	107 (39.9)
Block 3					
rs4938015		rs11604671			
T		G	0.59	50 (18.7)	104 (38.8)
C		G	0.31	133 (49.6)	93 (34.7)

Note. Table shows haplotype blocks and estimated population frequencies for haplotypes extracted from participants at a probability of at least 80% using PHASE. Proportions do not sum to 100 to allow for the accurate depiction of missingness in the data.

Table 5

Unstandardized estimates from main effects models predicting FTND and WISDM intermediate phenotypes from genetic variants

		WISDM Subscales											
		FTND			Automaticity			Craving			Locus of Control		
		β	SE		β	SE		β	SE		β	SE	
<i>Haplotype Block 1</i> [rs2282511 (<i>TTC12</i>), rs877138 (<i>ANKK1</i>)]													
CA		0.49*	0.20		0.38*	0.01		0.22	0.16		0.23	0.15	
AG		-0.50 [†]	0.21		-0.34 [†]	0.16		-0.23	0.17		-0.23	0.16	
<i>Haplotype Block 2</i> [rs17115439 (<i>ANKK1</i>) and rs4938013 (<i>ANKK1</i>)]													
TC		0.23	0.18		0.07	0.14		0.02	0.14		0.10	0.14	
TA		-0.45 [†]	0.21		-0.34 [†]	0.16		-0.20	0.17		-0.22	0.16	
CC		0.10	0.18		0.17	0.13		0.12	0.14		0.07	0.13	
<i>Haplotype Block 3</i> [rs4938015 (<i>ANKK1</i>) and rs11604671 (<i>ANKK1</i>)]													
TG		-0.16	0.18		-0.19	0.13		-0.14	0.14		-0.06	0.13	
CG		0.09	0.19		0.17	0.15		0.14	0.15		0.01	0.14	
<i>Polymorphism</i>													
	<i>Gene</i>			<i>MA</i>									
rs618114	<i>NCAMI</i>	-0.07	0.00		-0.16	0.18		0.03	0.19		0.04	0.18	
rs2303380	<i>TTC12</i>	-0.30	0.01		-0.32 [†]	0.15		-0.16	0.16		-0.05	0.15	
rs1079597	<i>DRD2</i>	-0.07	0.00		-0.39 [†]	0.19		-0.25	0.20		-0.13	0.19	
rs6277	<i>DRD2</i>	0.02	0.00		-0.12	0.22		-0.18	0.23		-0.12	0.22	
rs1800497	<i>DRD2</i>	0.30	0.01		-0.19	0.14		-0.18	0.15		-0.04	0.14	
rs4680	<i>COMT</i>	-0.02	0.20		-0.17	0.15		-0.13	0.16		-0.04	0.15	
rs1611115	<i>DBH</i>	0.59 [†]	0.25		0.55*	0.19		0.52*	0.20		0.41 [†]	0.19	

Note. FTND=Fagerstrom Test of Nicotine Dependence; WISDM = Wisconsin Inventory of Smoking Motives; *COMT* = Catechol-O-methyltransferase gene; *DBH* = Dopamine beta-hydroxylase gene; *ANKK1* = Ankyrin repeat and kinase domain containing 1 gene; *DRD2* = Dopamine D2 receptor gene; *NCAMI* = Neural cell adhesion molecule 1 gene; *TTC12* = Tetra-trico-peptide Repeat Domain 12 gene. Block 1 = rs2282511 (*TTC12*), rs877138 (*ANKK1*); Block 2 = rs17115439 (*ANKK1*) and rs4938013 (*ANKK1*); Block 3 = rs4938015 (*ANKK1*) and rs11604671 (*ANKK1*).

* $p < .01$,

[†] $p < .05$.

Table 6

Tests of the indirect effects of the genotypes/haplotypes associated with smoking motives in relation to nicotine dependence.

Genetic Variant	Mediator	β	95% BC CI	Z	p
<i>Polymorphism</i>					
rs1611115	Automaticity	0.29	0.10-0.51	2.71	<.01
rs1611115	Craving	0.24	0.06-0.46	2.40	0.02
rs1611115	Tolerance	0.36	0.08-0.64	2.51	0.01
<i>Haplotype</i>					
Block 1 CA	Automaticity	0.21	0.06- 0.39	2.45	0.01

Note. BC CI = Bias Corrected Confidence Intervals. Block 1 = rs2282511 (*TTC12*) and rs877138 (*ANKK1*).