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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. Mediational 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, 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.

1. 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

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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 narrower 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 et al., 2010; 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.

1.1. 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.

2. Materials & methods

2.1. 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 1124 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.

2.2. Phenotypic measures

2.2.1. 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.

2.2.2. 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.

2.3. Marker information and haplotype derivation

2.3.1. 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).

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.