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Original Investigation

Dissection of the Phenotypic and Genotypic Associations With Nicotinic Dependence

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Abstract

Introduction: Strong evidence demonstrates that nicotine dependence is associated with 4 genetic variants rs16969968, rs6474412, rs3733829, and rs1329650 in large-scale Genome-Wide Association Studies. We examined how these identified genetic variants relate to nicotine dependence defined by different categorical and dimensional measures.

Methods: Four genetic variants were analyzed in 2,047 subjects of European descent (1,062 cases and 985 controls). Nicotine dependence was assessed with multiple smoking measures, including the Fagerström Test for Nicotine Dependence, the Diagnostic and Statistical Manual for Mental Disorders-IV (*DSM-IV*) nicotine dependence, the Nicotine Dependence Syndrome Scale, and the Wisconsin Inventory of Smoking Dependence Motives. Single-item measures of cigarettes per day (CPD) and time to first cigarette (TTF) in the morning were also examined.

Results: Among the variants, association effect sizes were largest for rs16969968, with measures of craving and heavy smoking, especially cigarettes smoked per day, showing the largest effects. Significant but weaker associations were found for rs6474412 and rs3733729 but not for rs1329650. None of the more comprehensive measures of smoking behaviors yielded stronger genetic associations with these variants than did CPD.

Conclusions: CPD is an important simple measure that captures in part the genetic associations of *CHRNA5* and nicotine dependence, even when other more comprehensive measures of smoking behaviors are examined. The *CHRNA5* gene is associated with heavy compulsive smoking and craving; this should inform the mission to improve the diagnostic validity of *DSM-V*.

Introduction

Modern genetic studies have revolutionized the search for variants that contribute to human disease (Hindorff et al., 2009), and psychiatric genetics have successfully identified genes associated with heavy smoking and nicotine dependence. Recent genome-wide association meta-analyses show strong associations between smoking quantity (cigarettes per day [CPD]) and multiple genetic variants (Liu et al., 2010; TAG, 2010; Thorgeirsson et al., 2010). The most robust genetic finding points to the *CHRNA5-CHRNA3-CHRNB4* gene cluster on chromosome 15 tagged by rs16969968 ($p = 5.57 \times 10^{-72}$) and rs1051730 ($p = 2.75 \times 10^{-73}$). Additional variants that pass the threshold of genome-wide significance include rs6474412 upstream of the *CHRNA6-CHRNB3* gene cluster on chromosome 8p11 ($p = 1.4 \times 10^{-8}$), rs3733829 in *EGLN2* near the *CYP2A6* gene on chromosome 19q13 ($p = 1.0 \times 10^{-8}$), and rs1329650 in an intergenic region on chromosome 10q23 ($p = 5.7 \times 10^{-10}$; Liu et al., 2010; TAG, 2010; Thorgeirsson et al., 2010).

These identified variants may have different biological mechanisms and corresponding phenotypic effects. The variants in *CHRNA5* and *CHRNB3* may be associated with overlapping mechanisms and phenotypes, although existing literature suggests a difference in the phenotypes associated with *CHRNA5* and *CHRNB3* regarding other disorders, such as alcohol consumption and lung cancer (Hoft et al., 2009; Thorgeirsson et al., 2010). The variant in *EGLN2* near *CYP2A6* may affect the metabolic capacity for nicotine and rapid development of tolerance (Ray, Tyndale, & Lerman, 2009).

Though existing meta-analyses use CPD as the primary phenotype, smoking behaviors are complex. Smoking patterns vary, and nicotine dependence is complex with many different

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phenotypic characteristics. Classically, tolerance and withdrawal symptoms define physical dependence (Victor & Adams, 1953), and inability to stop using a substance despite negative physical, mental, and social consequences defines psychological dependence (Keller, 1972).

Most previous research on nicotine dependence has used the Fagerström Test for Nicotine Dependence (FTND) and the Diagnostic and Statistical Manual of Mental Disorders-IV (*DSM-IV*) to index dependence. The FTND scale consists of six questions to measure physical dependence and tolerance processes, and it has been shown to predict cessation (Heatherton, Kozlowski, Frecker, Rickert, & Robinson, 1989). Two FTND items, time to first cigarette (TTF) and the number of cigarettes smoked per day, have been proposed as simpler measures of nicotine dependence that are especially predictive of successful quitting (Baker et al., 2007; Heatherton et al., 1989). In contrast, *DSM-IV* nicotine dependence requires a clustering of at least three of seven symptoms intended to index a maladaptive pattern of substance use leading to significant impairment or distress (APA, 1994). Recently, new diagnostic criteria for *DSM-V* Nicotine Use Disorder have been proposed with changes in threshold and additional symptoms (APA, 2010). The FTND and *DSM-IV* nicotine dependence criteria capture different aspects of dependence (Hughes et al., 2004). The FTND better predicts smoking cessation, whereas the *DSM-IV* nicotine dependence diagnosis correlates more strongly with comorbid psychopathology, such as major depressive disorder (Breslau & Johnson, 2000).

Two multifactorial measures have been developed to assess nicotine dependence. The Nicotine Dependence Syndrome Scale (NDSS; Shiffman, Waters, & Hickcox, 2004) comprises scales reflecting Edward's model of dependence (Edwards, 1986; Edwards & Gross, 1976) in which dependent drug use is compulsive, stereotyped, relatively uninfluenced by external cues, and motivated by strong withdrawal symptoms. The Wisconsin Inventory of Smoking Dependence Motives (WISDM; Piper et al., 2004) assesses multiple distinct motives for dependence that have been supported by extensive research and theory. Both measures target multiple dimensions of dependence, possess good psychometric properties, and can predict important dependence symptoms, such as withdrawal and difficulty with cessation (Piper, Bolt, et al., 2008; Piper, McCarthy, & Baker, 2006; Piper, McCarthy, et al., 2008; Shiffman & Sayette, 2005).

The use of two multifactorial scales permits the comprehensive coverage of the domain of dependence features and allows us to determine whether the same types of dependence features are associated with genetic variants across both scales (i.e., refine the phenotype). Recent research with the WISDM (Piper, Bolt, et al., 2008) has distinguished between core or primary phenotypic features of dependence (Primary Dependence Motives [PDM]) versus secondary features (Secondary Dependence Motives [SDM]). The PDM is especially highly associated with dependence criteria (e.g., relapse back to smoking) and is not frequently endorsed by smokers until they have used nicotine fairly extensively. The SDM taps a variety of dependence motives that involve smoking for some instrumental purpose. The PDM assesses smoking that is heavy, out of control, and associated with strong craving. Therefore, the PDM assesses similar dimensions as those assessed by the NDSS scale. Our working hypothesis was that it would be these core dependence phenotypes that would be most highly associated with rs16969968 and rs3733829; the former being previously associated with the PDM (Baker et al., 2009), and the latter involved in nicotine metabolism, permitting heavier tobacco intake. We did not have strong hypotheses regarding which subscales would be associated with rs6474412 or rs1329650, but the current research allowed for the potential detection of different types of dependence phenotypes with the different risk variants.

This study used data from the Collaborative Genetic Study of Nicotine Dependence (COGEND) to examine the relations among relatively discrete nicotine dependence phenotypes and the four previously identified genetic variants that have passed the threshold of genome-wide significance in the large-scale meta-analysis studies of CPD.

Methods

Subjects

The COGEND sample includes individuals aged 25–44 years, recruited through telephone screening in St. Louis and Detroit. Nicotine-dependent cases (N = 1,062) were defined as current smokers with an FTND score of 4 or more (maximum score of 10; Heatherton, Kozlowski, Frecker, & Fagerström, 1991). Controls (N = 985) were defined as smokers (individuals who smoked at least 100 cigarettes lifetime) who never experienced any symptoms of dependence (lifetime FTND = 0). The threshold of 100 cigarettes smoked over the lifetime is a commonly used threshold for significant smoking exposure (Bondy, Victor, & Diemert, 2009). Analyses focused on subjects who self-identified as European descent, which was confirmed with genetic analysis (N. L. Saccone et al., 2010). The study was approved by the Institutional Review Board at each data collection site, and subjects provided informed consent prior to participating.

Measurements

Phenotypic Data

All subjects were personally interviewed and received comprehensive evaluation of nicotine dependence using the FTND, *DSM-IV* criteria, NDSS, and WISDM during the period in the subject's life when s/he smoked cigarettes the most. Questions covering the proposed *DSM-V* symptoms for nicotine use disorder were available. The NDSS is a 19-item measure comprising five theoretically derived subscales (Drive, Priority, Tolerance, Continuity, and Stereotypy) and is scored using factor loadings (Shiffman et al., 2004). The WISDM comprises 68 items designed to assess 13 theoretically derived motivational domains (PDM and SDM). The PDM subscales include Automaticity, Craving, Loss of Control, and Tolerance. The SDM subscales include Affiliative Attachment, Behavioral Choice, Cognitive Enhancement, Cue Exposure, Negative Reinforcement, Positive Reinforcement, Social Goads, Taste Property, and Weight Control (Baker et al., 2009; Piper et al., 2004).

Genetic Data

Blood samples were collected for genetic analyses, and genotype data were cleaned extensively. Primary genetic associations of this sample have been reported in prior publications (Bierut et al., 2007; S. F. Saccone et al., 2007; N. L. Saccone et al., 2009). We focused on four variants previously shown as associated

with nicotine dependence in the large-scale meta-analyses: rs16969968 (*CHRNA5* on chromosome 15q25), rs6474412 (upstream of *CHRNB3* on chromosome 8p11), rs3733829 (*EGLN2* near *CYP2A6* on chromosome 19q13), and rs1329650 on chromosome 10q23.

Analyses

Association analyses for dichotomous phenotypes and subphenotypes used logistic regression models with age, gender, and the single nucleotide polymorphism (SNP) as covariates. Genotypes were coded additively as the number of nonreference alleles, defined as the minor allele in the European ancestry population. CPD and TTF in the morning were dichotomized with median splits when compared with the other FTND subphenotypes.

In order to capture the subphenotypes of the FTND associated with a specified variant, we tested the associations between the variant (as response variable) and all six FTND subphenotypes (as covariates with age and gender) in stepwise regression models where at each step, an independent variable not in the equation that had the smallest probability of *F* statistics was entered if that probability was sufficiently small. Variables already in the regression equation were removed if their probability of *F* statistics became sufficiently large. The method terminated when no additional variables were eligible for inclusion or removal.

Second, we examined if three FTND dimensional phenotypes (FTND score, CPD score, and TTF score) differed in their level of association with the tested variant. CPD and TTF were tested as quasi-continuous phenotypes with four levels, and *Z*-scores were used to standardize dimensional phenotypes across measures. To test the difference in genetic associations across phenotypes, we modeled the difference in genetic associations between different phenotypes and each genetic variant using mixed models (Andrade, Eaton, & Chilcoat, 1994). The mixed model approach accounted for nonindependence of multiple phenotype measures within individuals. The interaction term between each measure and the genetic variant was a test for a significant differential genetic association.

Third, the genetic associations for other nicotine dependence phenotypes (nicotine dependence defined by *DSM-IV* and *DSM-V* criteria, *DSM-IV* symptom count, *DSM-V* symptom count, NDSS score, and WISDM score) were also compared. We conducted separate analyses to correct for the sampling bias as the sample was recruited based on FTND measures, the primary phenotype. For analyses of these secondary phenotypes, we used statistical methods that reflect the case–control sampling in the analysis of secondary phenotype to provide unbiased estimation of genetic effects and accurate control of false positive rates with software Regression Analysis of Secondary Phenotype Data in Case–Control Association Studies (SPREG; Lin & Zeng, 2009). Results of the regression models were consistent and not materially different from the SPREG models as shown in Supplementary Table 3. We also tested the associations between the SNP (as response variable) and subphenotypes within *DSM* nicotine dependence criteria, NDSS, and WISDM in separate stepwise regression models.

Multiple Test Correction

Our main purpose is not to report novel genetic associations but to characterize the genetic associations across different phenotypes. We made two comparisons of associations across major phenotypes and conducted four tests to characterize the subphenotypes for each of the four SNPs, which resulted in a total of 24 tests. Given the number of tests in this experiment, a *p* value of .001 was selected to control for experiment-wise error ($.001 \times 24 = .024 < .05$).

Results

Table 1 provides the distributions of age, gender, and all phenotypes in cases and controls. Consistent with the study design, the distribution of FTND scores is different between cases and

Table 1. Sample Distributions of Age, Gender, and Dimensional Phenotypes for Nicotine Dependence

	Nicotine-dependent cases		Nondependent smoking controls	
	N = 1,062		*N* = 985	
	M	*SD*	*M*	*SD*
Age	36.9	5.39	35.9	5.53
% Male	46.2	–	30.9	–
FTND score	6.46	1.77	0.0	0.0
Time to first cigarette of the day (TTF)	2.41	.69	0.0	0.0
Cigarettes per day (CPD)	1.93	.89	0.0	0.0
DSM-IV nicotine dependence symptom count	4.38	1.13	2.47	1.45
DSM-V nicotine use disorder symptom count	5.64	1.62	2.76	1.73
NDSS score	−0.11	0.97	−2.22	0.55
WISDM score	54.9	12.4	26.9	10.6

Note. Nicotine-dependent cases: defined by current FTND = 4 or higher. Nondependent smoking controls: defined by smoking 100 cigarettes lifetime and lifetime maximum FTND = 0. FTND: Fagerström Test for Nicotine Dependence score (range 0–10). TTF: time to the first cigarette of the day (0: >60 min, 1: 31–60 min, 2: 6–30 min, and 3: ≤5 min). CPD: number of cigarettes smoked per day (0: ≤10, 1: 11–20, 2: 21–30, and 3: >30). *DSM-IV* nicotine dependence symptom count (range 0–7). *DSM-V* nicotine use disorder symptom count (range 0–11). NDSS: Nicotine Dependence Syndrome Scale (range −3.00 to 2.50). WISDM: Wisconsin Inventory of Smoking Dependence Motives (range 13.0–89.8)

controls. Different phenotypes show high levels of correlation (a positive manifold), suggesting that they are measuring similar underlying constructs (Supplementary Tables 1 and 2).

FTND Phenotypes and rs16969968

The FTND-dichotomized phenotype is strongly associated with rs16969968 (odds ratio [*OR*] = 1.39, 95% *CI* = 1.22–1.58, $p = 9.9 \times 10^{-7}$; Table 2). Each FTND subphenotype is also associated with this variant. When all six dimensional phenotypes are included in the stepwise regression model, CPD is the only subphenotype significantly associated with rs16969968 ($t = 5.2$, $df = 1$, $p = 2.6 \times 10^{-7}$).

Comparing the genetic associations with rs16969968 across the three FTND phenotypes (overall FTND score, CPD score, and TTF score), we found no significant difference in the strength of association among these phenotypes ($F = 2.24$, $df = 2$, $p = .11$; Table 3).

Other Nicotine Dependence Phenotypes (*DSM*, NDSS, and WISDM) and rs16969968

All other nicotine dependence phenotypes are associated with rs16969968 with *p* values ranging from 2.0×10^{-4} (*DSM-IV*) to 2.8×10^{-7} (NDSS; Table 4). There is a trending but not statistically significant difference in the strength of association between these phenotypes ($F = 2.57$, $df = 3$, $p = .053$). The level of statistical significance is lowest for *DSM-IV* symptom count compared with *DSM-V* symptom count, NDSS, and WISDM. Results are similar in analyses adjusted for the sampling bias based on the FTND (Supplementary Table 3).

Next, we characterize the subphenotypes associated with the variant rs16969968 (Table 4 and Supplementary Table 4). In *DSM*, the only criteria significantly associated with rs16969968 are "Craving" ($t = 3.69$, $df = 1$, $p = 2.3 \times 10^{-4}$) and "Withdrawal" ($t = 2.03$, $df = 1$, $p = .042$) among all 11 *DSM* criteria in the stepwise regression. Only "Craving" remains significant after correction for multiple testing. In NDSS, the only subphenotype significantly associated with rs16969968 is "Drive" ($t = 5.29$, $df = 1$, $p = 1.4 \times 10^{-7}$) in the stepwise regression. In WISDM, the only subphenotype significantly associated with rs16969968 is "Loss of control" ($t = 5.47$, $df = 1$, $p = 5.2 \times 10^{-8}$) in the stepwise regression. In summary, the subphenotypes capturing the association with rs16969968 are "Craving" from *DSM*, "Drive" from NDSS, and "Loss of control" from WISDM.

FTND Phenotypes and rs6474412

The FTND-dichotomized phenotype is associated with rs6474412 (*OR* = 0.78, 95% *CI* = 0.67–0.91, *p* = .0014; Table 2). Using stepwise regression with all FTND subphenotypes, "Can't refrain from smoking" is the only subphenotype significantly associated with rs6474412 ($t = -3.40$, $df = 1$, $p = 6.8 \times 10^{-4}$).

Comparing the genetic associations with rs6474412 across the three FTND phenotypes (overall FTND score, CPD score, and TTF score), we found no significant difference in the strength of association among these phenotypes ($F = 0.46$, $df = 2$, $p = .63$; Table 3).

Other Nicotine Dependence Phenotypes (*DSM*, NDSS, and WISDM) and rs6474412

NDSS and WISDM scores are associated with rs6474412, but *DSM-IV* or *DSM-V* symptom counts are not (Table 4). There is a modest not statistically significant difference in the strength of association across these dimensional phenotypes (*DSM-IV*

Table 2. Dichotomous Nicotine Dependence Phenotypes Based on FTND: Association With SNP rs16969968 on Chromosome 15 (*CHRNA5*), SNP rs6474412 on Chromosome 8 (*CHRNB3*), and SNP rs3733829 on Chromosome 19 (*EGLN2*, near *CYP2A6*)

	Chromosome 15			Chromosome 8			Chromosome 19		
	rs16969968			rs6474412			rs3733829		
FTND dichotomous phenotypes	*OR*	95% *CI*	*p* value	*OR*	95% *CI*	*p* value	*OR*	95% *CI*	*p* value
FTND4	1.39	(1.22–1.58)	9.9×10^{-7}	0.78	(0.67–0.91)	1.4×10^{-3}	1.13	(0.99–1.28)	6.8×10^{-2}
FTND items									
Time to first cigarette of the day (TTF)[a]	1.37	(1.20–1.56)	3.2×10^{-6}	0.78	(0.67–0.92)	2.1×10^{-3}	1.12	(0.98–1.27)	9.0×10^{-2}
Can't refrain from smoking	1.23	(1.06–1.42)	6.3×10^{-3}	0.73[b]	(0.61–0.88)	1.1×10^{-3}	1.23[c]	(1.06–1.43)	5.3×10^{-3}
Can't give up first cigarette	1.27	(1.11–1.46)	5.6×10^{-4}	0.89	(0.76–1.05)	1.8×10^{-1}	1.05	(0.92–1.21)	4.5×10^{-1}
Cigarettes per day (CPD)[a]	1.41[b]	(1.23–1.60)	4.6×10^{-7}	0.78	(0.67–0.91)	1.5×10^{-3}	1.11	(0.97–1.26)	1.3×10^{-1}
Smoking more in first hours after waking than rest of the day	1.28	(1.10–1.49)	1.1×10^{-3}	0.91	(0.76–1.10)	3.3×10^{-1}	0.98	(0.84–1.14)	7.5×10^{-1}
Smoking when ill	1.15	(1.00–1.33)	4.6×10^{-2}	0.90	(0.76–1.06)	2.0×10^{-1}	1.14	(0.99–1.31)	6.3×10^{-2}

Note. All models were adjusted for age and gender. All dimensional phenotypes were *Z*-scored. FTND: Fagerström Test for Nicotine Dependence score. FTND4 is a dichotomous phenotype defined by FTND score of 4 or higher. TTF: time to the first cigarette of the day (0: >60 min, 1: 31—60 min, 2: 6–30 min, and 3: ≤5 min). CPD: Number of cigarette smoked per day (0: ≤10, 1: 11–20, 2: 21–30, and 3: >30). *OR* = odds ratio.

[a]TTF and CPD as dichotomous phenotype with median split.

[b]Item significant with $p < .001$ in stepwise regression models that remains significant after correction for multiple testing.

[c]Item significant with $p < .05$ in stepwise regression models.

Table 3 .Dimensional Nicotine Dependence Phenotypes Based on FTND: Association With SNP rs16969968 on Chromosome 15 (*CHRNA5*), SNP rs6474412 on Chromosome 8 (*CHRNB3*), and SNP rs3733829 on Chromosome 19 (*EGLN2*, near *CYP2A6*)

FTND dimensional phenotypes	Chromosome 15 rs16969968			Chromosome 8 rs6474412			Chromosome 19 rs3733829		
	β	95% *CI*	*p* value	β	95% *CI*	*p* value	β	95% *CI*	*p* value
FTND score	.16	(0.10–0.23)	5.8×10^{-7}	−.13	(−0.21 to −0.06)	5.4×10^{-4}	.08	(0.01–0.14)	1.7×10^{-2}
TTF score	.14	(0.07–0.20)	1.5×10^{-5}	−.13	(−0.20 to −0.06)	5.0×10^{-4}	.06	(−0.01 to 0.12)	7.4×10^{-2}
CPD score	.18	(0.11–0.24)	5.2×10^{-8}	−.15	(−0.22 to −0.07)	1.8×10^{-4}	.10	(0.04–0.17)	1.5×10^{-3}

Note. All models were adjusted for age and gender. All dimensional phenotypes were *Z*-scored. FTND: Fagerström Test for Nicotine Dependence score. TTF: time to the first cigarette of the day (0: >60 min, 1: 31–60 min, 2: 6–30 min, and 3: ≤5 min). CPD: number of cigarettes smoked per day (0: ≤10, 1: 11–20, 2: 21–30, and 3: >30). β = linear regression coefficient; SNP = single nucleotide polymorphism.

symptom count, *DSM-V* symptom count, NDSS score, and WISDM score) and rs6474412 ($F = 3.01$, $df = 3$, $p = .029$). Results are similar in analyses adjusted for the sampling bias for these other phenotypes (Supplementary Table 3).

Fewer subphenotypes are associated with rs6474412 than with rs16969968 (Table 4 and Supplementary Table 4). In *DSM*, no subphenotype is significantly associated with rs6474412. In NDSS, the only subphenotypes significantly associated with rs6474412 are "Drive" ($t = -2.35$, $df = 1$, $p = .019$) and "Tolerance" ($t = -2.02$, $df = 1$, $p = .043$) in the stepwise regression. Neither subphenotype remains significant after correction for multiple testing. In WISDM, the only subphenotype significantly associated with rs6474412 is "Tolerance" ($t = -4.14$, $df = 1$, $p = 3.7 \times 10^{-5}$) in the stepwise regression. In summary, the only subphenotype capturing the association with rs6474412 is "Tolerance" from WISDM.

Phenotypic Association of rs3733829

The association between FTND and rs3733829 is modest in our sample (*OR* = 1.13, 95% *CI* = 0.99–1.28, $p = .068$; Table 3). Using stepwise regression with all FTND subphenotypes, "Can't refrain from smoking" is the only subphenotype significantly associated with rs3733829 ($t = 2.80$, $df = 1$, $p = 5.2 \times 10^{-3}$).

Comparing the genetic associations with rs3733829 across the three dimensional FTND phenotypes, we found a trending but not significant difference in the strength of association among these phenotypes ($F = 2.64$, $df = 2$, $p = .07$; Table 3).

The associations between other nicotine dependence phenotypes and rs3733829 are weak ($p > .01$; Table 4 and Supplementary Table 4). Although some subphenotypes emerge as associated with rs3733829, such as "Continued use despite hazards" ($t = 2.59$, $df = 1$, $p = .010$) from *DSM*, "Tolerance" ($t = 2.15$, $df = 1$, $p = .032$) from NDSS, and "Tolerance" ($t = 2.35$, $df = 1$, $p = .019$) from WISDM, none of these subphenotypes remain significant after correction for multiple testing.

Phenotypic Association of rs1329650

Our results examining the association between rs1329650 and the nicotine dependence phenotypes do not support the previous report of association. Most phenotypes show no evidence of association (*OR* = 1.02, $p = .74$ for the FTND dichotomous phenotype; β = .023, $p = .52$ for CPD score; β = .024, $p = .50$ for NDSS score, β = .001, $p = .99$ for WISDM score). Though there is a modest association with *DSM-IV* diagnosis (*OR* = 1.16, $p = .040$), the effect is in the opposite direction from what was reported in previous meta-analyses. These results do not replicate the previously reported association.

Discussion

Comprehensive multidimensional phenotypes provide unique opportunities to distill phenotypic associations and to further validate genetic findings. Our study is one of the first to examine different nicotine dependence phenotypes as a means of clarifying the association between identified genetic variants and the clinical/behavioral features of smoking. We focused on four genetic variants that have passed the threshold of genome-wide significance in large-scale meta-analyses using CPD as the primary phenotype.

We first examined rs16969968, a variant that changes an amino acid in the α5 nicotinic receptor protein. Our results are consistent with the previous finding that this gene cluster is associated with a broad range of nicotine dependence phenotypes (Baker et al., 2009). In addition, consistent with prior findings and our hypotheses, we found that across different dependence instruments, rs16969968 was associated with "primary" dependence: for example, CPD from the FTND, "Craving" from *DSM-V* criteria, "Drive" from the NDSS, and "Loss of control" from the WISDM. That is, rs16969968 is associated with the dependence features that are assessed in the PDM: smoking that is heavy, out of control, and manifests in strong craving. Consistent with this, and as predicted, rs16969968 shares strong relations with the NDSS total score (2.8×10^{-7}) and the PDM composite (3.4×10^{-7}). Craving is a key element of this core dimension, consistent with its correlations ($r = .69$) with CPD, strong genetic associations with *CHRNA5*, and its theoretical basis as one of the fundamental motivational processes for nicotine dependence (Piper, Bolt, et al., 2008).

The key role of smoking heaviness is revealed by the finding that none of the comprehensive nicotine dependence phenotypes including *DSM-IV*, *DSM-V*, NDSS, and WISDM strengthened the genetic association with rs16969968 beyond the CPD measure. In fact, the level of statistical significance was relatively weak for *DSM-IV* symptom count compared with the other measures. This may represent a bias in our sample, given that it

Table 4 .Other Nicotine Dependence Phenotypes: Association With SNP rs16969968 on Chromosome 15 (*CHRNA5*), SNP rs6474412 on Chromosome 8 (*CHRNB3*), and SNP rs3733829 on Chromosome 19 (*EGLN2*, near *CYP2A6*)

	Chromosome 15			Chromosome 8			Chromosome 19		
	rs16969968			rs6474412			rs3733829		
Other dependence phenotypes	β	95% *CI*	*p* value	β	95% *CI*	*p* value	β	95% *CI*	*p* value
DSM-IV symptom count	.13	(0.06–0.19)	2.0×10^{-4}	−.06	(−0.14 to 0.02)	1.3×10^{-1}	.09	(0.02–0.15)	1.1×10^{-2}
DSM-V symptom count	.15	(0.08–0.21)	6.8×10^{-6}	−.05	(−0.13 to 0.03)	2.1×10^{-1}	.08	(0.02–0.15)	1.4×10^{-2}
NDSS score	.17	(0.10–0.23)	2.8×10^{-7}	−.14	(−0.22 to −0.06)	4.8×10^{-4}	.06	(0.00–0.13)	5.3×10^{-2}
WISDM score	.15	(0.09–0.22)	5.1×10^{-6}	−.12	(−0.20 to −0.04)	2.2×10^{-3}	.06	(−0.01 to 0.12)	9.4×10^{-2}
Subphenotypes	*OR*	95% *CI*	*p* value	*OR*	95% *CI*	*p* value	*OR*	95% *CI*	*p* value
DSM-IV nicotine dependence	1.28	(1.12–1.45)	2.0×10^{-4}	0.89	(0.76–1.03)	1.2×10^{-1}	1.07	(0.94–1.21)	3.3×10^{-1}
DSM-IV ND symptoms									
Tolerance	1.22	(1.03–1.43)	1.7×10^{-2}	0.91	(0.76–1.10)	3.2×10^{-1}	1.14	(0.97–1.34)	1.0×10^{-1}
Withdrawal	1.25[a]	(1.10–1.42)	7.0×10^{-4}	0.89	(0.77–1.04)	1.5×10^{-1}	1.04	(0.91–1.18)	5.5×10^{-1}
Smoking more than intended	1.18	(1.00–1.40)	4.8×10^{-2}	0.85	(0.71–1.03)	1.0×10^{-1}	1.10	(0.93–1.30)	2.6×10^{-1}
Can't cut down or quit	1.20	(1.02–1.42)	2.9×10^{-2}	0.93	(0.77–1.13)	4.9×10^{-1}	1.15	(0.98–1.36)	8.5×10^{-2}
Much time spent smoking	1.33	(1.04–1.71)	2.3×10^{-2}	0.88	(0.64–1.20)	4.2×10^{-1}	1.18	(0.91–1.51)	2.0×10^{-1}
Giving up activities to smoke	1.29	(0.96–1.75)	9.5×10^{-2}	1.11	(0.77–1.59)	5.7×10^{-1}	1.33	(0.99–1.80)	6.3×10^{-2}
Continued use despite hazards	1.11	(0.97–1.26)	1.2×10^{-1}	0.96	(0.82–1.12)	6.0×10^{-1}	1.19[a]	(1.04–1.35)	9.3×10^{-3}
DSM-V NUD new symptoms									
Failure in major role obligation	0.97	(0.44–2.15)	9.4×10^{-1}	1.43	(0.60–3.39)	4.2×10^{-1}	1.07	(0.49–2.36)	8.6×10^{-1}
Physically hazardous	1.27	(1.10–1.48)	1.3×10^{-3}	0.95	(0.79–1.13)	5.7×10^{-1}	1.14	(0.98–1.32)	8.6×10^{-2}
Continued use with problems	1.30	(0.94–1.78)	1.1×10^{-1}	1.05	(0.71–1.55)	8.0×10^{-1}	1.35	(0.98–1.85)	6.5×10^{-2}
Craving or a strong urge	1.34[b]	(1.18–1.53)	6.9×10^{-6}	0.97	(0.84–1.13)	7.3×10^{-1}	1.03	(0.91–1.17)	6.1×10^{-1}
NDSS Subphenotypes	β	95% *CI*	*p* value	β	95% *CI*	*p* value	β	95% *CI*	*p* value
Drive	.18[b]	(0.11–0.24)	2.1×10^{-7}	−.12[a]	(−0.20 to −0.04)	2.8×10^{-3}	.04	(−0.03 to 0.11)	2.4×10^{-1}
Stereotypy	.06	(0.00–0.12)	4.9×10^{-2}	−.06	(−0.13 to 0.01)	1.1×10^{-1}	.05	(−0.01 to 0.11)	1.1×10^{-1}
Continuity	.10	(0.03–0.16)	2.5×10^{-3}	−.07	(−0.14 to 0.01)	8.6×10^{-2}	.01	(−0.05 to 0.08)	7.3×10^{-1}
Priority	.04	(−0.02 to 0.10)	1.8×10^{-1}	−.03	(−0.10 to 0.04)	4.2×10^{-1}	.005	(−0.06 to 0.06)	9.2×10^{-1}
Tolerance	.07	(0.01–0.14)	3.3×10^{-2}	−.11[a]	(−0.19 to −0.03)	5.4×10^{-3}	.07[a]	(0.01–0.14)	3.1×10^{-2}
WISDM subphenotypes:PDM									
PDM mean scale	.17	(0.11–0.24)	3.4×10^{-7}	−.14	(−0.22 to −0.06)	3.5×10^{-4}	.07	(0.00–0.14)	3.6×10^{-2}
Automaticity	.13	(0.06–0.19)	1.5×10^{-4}	−.12	(−0.19 to −0.04)	3.1×10^{-3}	.05	(−0.01 to 0.12)	1.3×10^{-1}
Loss of control	.18[b]	(0.11–0.24)	1.0×10^{-7}	−.14	(−0.21 to −0.06)	6.9×10^{-4}	.07	(0.01–0.14)	2.9×10^{-2}
Craving	.15	(0.09–0.22)	6.0×10^{-6}	−.12	(−0.20 to −0.05)	1.8×10^{-3}	.06	(−0.01 to 0.12)	9.0×10^{-2}
Tolerance	.18	(0.11–0.24)	1.1×10^{-7}	−.15[b]	(−0.23 to −0.07)	1.3×10^{-4}	.08[a]	(0.01–0.14)	1.8×10^{-2}

Note. All models were adjusted for age and gender. All dimensional phenotypes were *Z*-scored. β = linear regression coefficient; *DSM-IV* ND symptom = *DSM-IV* nicotine dependence symptom; *DSM-V* NUD new symptom = *DSM-V* nicotine use disorder newly proposed symptom; NDSS = Nicotine Dependence Syndrome Scale; *OR* = odds ratio; PDM = primary dependence motives; SNP = single nucleotide polymorphism; WISDM: Wisconsin Inventory of Smoking Dependence Motives.

[a]Item significant with $p < .05$in stepwise regression models that remains significant after correction for multiple testing.

[b]Item significant with $p < .001$ in stepwise regression models that remains significant after correction for multiple testing.

was selected based on the FTND criteria. However, the strong genetic relations between rs16969968 and the other nicotine dependence phenotypes such as NDSS and WISDM argue against this view. The weaker genetic association with *DSM-IV* symptom count is consistent with twin data of heritability for nicotine dependence. The heritability of nicotine dependence is lower when defined by *DSM-IV* (56%) than when defined by the Heaviness of Smoking Index (71%), an abbreviated version of the FTND that includes only two items, CPD and TTF of the day (Heatherton et al., 1989) or CPD alone (70%; Lessov et al., 2004).

Perhaps the heritability of the different definitions of nicotine dependence is related to the origins of these measures of smoking. The FTND, NDSS, and WISDM were developed specifically for the assessment of nicotine dependence, whereas *DSM-IV* nicotine dependence criteria were developed as part of a general measure for all substance dependence. The application of general dependence criteria in *DSM-IV* is parsimonious but may not capture important characteristics, including genetic features, that are specific to nicotine dependence.

As compared with the chromosome 15 finding, the other variants studied were not as strongly associated with nicotine dependence. The pattern of phenotypic association with rs6474412 was similar to that with rs16969968. Significant associations were seen with FTND, NDSS, and WISDM scores, especially those associated with heavy smoking and craving (e.g., WISDM "Tolerance" and NDSS "Drive"). Associations with *DSM-IV*-defined nicotine dependence or symptom count were not significant. Therefore, rs6474412, like rs16969968, appeared to be significantly associated with compulsive heavy smoking and craving. It is unclear why the FTND item "Can't refrain from smoking" showed the strongest

association with rs6474412, but this could be related to its significant correlation with CPD (r = .59). The strongest association with rs6474412 was with the WISDM "Tolerance" scale, which comprises items, such as "I consider myself a heavy smoker," again affirming that rs16969968 and rs6474412 are associated with overlapping domains of nicotine dependence characterized by heavy smoking.

Before the biological mechanisms are clarified for *CHRNA5* and *CHRNB3*, the possibility of having distinct but related phenotypes with these two variants cannot be ruled out. The third variant, rs3733829 in the *EGLN2* gene about 40 kb from the 3′ end of *CYP2A6*, showed a modest association with nicotine dependence. When the genetic association was modest, we were unable to differentiate the degree of association based on the different phenotypic definitions.

Our data do not support the previously reported association with rs1329650 on chromosome 10. Most nicotine dependence phenotypes showed no evidence of association ($p > .50$). Though *DSM-IV* nicotine dependence demonstrated a weak association, the effect was in the opposite direction from what was previously reported; thus, our results must be interpreted as nonreplication (TAG, 2010). Based on the allele frequency and our sample size, our study has sufficient power to detect an association with *OR* of 1.14 or higher (Gauderman & Morrison, 2006).

There are several limitations to our study. Our sample selection for cases and controls was based on extreme FTND scores, so a comparison of FTND results with the other nicotine dependence phenotypes cannot be directly made. We purposely examined the strength of genetic associations within FTND measures as one set of analyses. In a separate analysis, we examined the level of genetic association among other nicotine dependence phenotypes (*DSM-IV*, *DSM-V*, NDSS, and WISDM). In order to adjust for the ascertainment bias that was built into our study design, we performed additional analyses correcting for the bias (Lin & Zeng, 2009) and obtained similar results.

Second, caution is needed in interpreting these intertwined clinical constructs. There is moderate to high correlation among the examined phenotypes and subphenotypes. As a consequence, the pattern of significant findings can be somewhat misleading. For instance, the *DSM* "Craving" item has a significant stepwise association with rs16969968, but the WISDM "Craving" item did not (instead, the WISDM "Loss of Control" subscale was significant in the stepwise tests). In fact, both craving measures are similarly highly associated with rs16969968 as indicated by their *CI*s (Table 4), suggesting no real inconsistency. Also, because all the dependence measures are meaningfully correlated with one another (given that they measure a common construct), there is limited power to demonstrate significant differences among them in their relations with genetic variants. However, even given that, this study shows that the key rs16969968 and rs6474412 genetic variants were reliably associated only with certain types of dependence measures: those reflecting heavy out-of-control smoking accompanied by strong craving. For none of the genetic variants were the associations of the WISDM SDM scales as strong as those for the PDM scales.

Another limitation is that only selected genetic variants were tested. These variants were identified in several large Genome-Wide Association Studies (GWAS) of smoking, which have used the simple measure of CPD as the primary phenotype (Liu et al., 2010; TAG, 2010; Thorgeirsson et al., 2010). These variants were selected on the basis of their prior associations with CPD, so our finding of CPD as one of the strongest association findings may be the result of a bias based on the original phenotype that identified these variants. The more comprehensive measures of nicotine dependence are not widely available in genetic samples, and no GWAS has been performed on the WISDM or NDSS. Until GWAS using these other phenotypic definitions is performed, we will not be able to determine if there are additional novel variants associated with these other phenotypes to be identified.

Finally, our goal was to examine phenotype–genotype relations by comparing the genetic associations across phenotypes and characterizing the subphenotypes' best capturing known genetic associations. This work informs our understanding of phenotypes and the mission to improve diagnostic validity. For example, a proposed test of the validity of mental disorder diagnosis for *DSM-V* includes identified genetic risk factors (Carpenter et al., 2009). If biological or genetic factors are an important test of the validity of mental disorder diagnosis, this evidence suggests the importance of measuring smoking heaviness and "Craving" to reflect part of the underlying genetic risk. Though the variance explained by these genetic polymorphisms is small (2.4% by four variants for FTND), the current evidence suggests that measures of smoking heaviness, in particular, the number of cigarettes smoked per day, is an important measure of nicotine dependence that captures part of the genetic variance related to nicotine dependence. Adding "Craving" as a symptom criterion in *DSM-V* is supported in our work. Although CPD is a simple measure from a psychiatric or psychological perspective, it is an important measure used in medicine because it assesses the toxic exposure from smoking, which is a determinant of diseases, such as lung cancer and chronic obstructive pulmonary disease. Therefore, we suggest that CPD be considered in the future assessment of nicotine use disorders, including *DSM-V*.

Supplementary Material

Supplementary Tables 1–4 can be found online at http://www.ntr.oxfordjournals.org

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Declaration of Interests

Drs. LJB, AMG, JPR, SS, and JCW are listed as inventors on the patent "Markers for Addiction" (US 20070258898) covering the use of certain SNPs in determining the diagnosis, prognosis, and treatment of addiction. NS is the spouse of SS who is listed on the patent. Dr. LJB acted as a consultant for Pfizer, Inc. in 2008.

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