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Original Investigation

Human neuronal acetylcholine receptor A5-A3-B4 haplotypes are associated with multiple nicotine dependence phenotypes

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Abstract

Introduction: Previous research revealed significant associations between haplotypes in the *CHRNA5-A3-B4* subunit cluster and scores on the Fagerström Test for Nicotine Dependence among individuals reporting daily smoking by age 17. The present study used subsamples of participants from that study to investigate associations between the *CHRNA5-A3-B4* haplotypes and an array of phenotypes not analyzed previously (i.e., withdrawal severity, ability to stop smoking, and specific scales on the Wisconsin Inventory of Smoking Dependence Motives (WISDM-68) that reflect loss of control, strong craving, and heavy smoking.

Methods: Two cohorts of current or former smokers ($N = 886$) provided both self-report data and DNA samples. One sample (Wisconsin) comprised smokers making a quit smoking attempt, which permitted the assessment of withdrawal and relapse during the attempt. The other sample (Utah) comprised participants studied for risk factors for nicotine dependence and chronic obstructive pulmonary disease and included individuals originally recruited in the Lung Health Study.

Results: The *CHRNA5-A3-B4* haplotypes were significantly associated with the targeted WISDM-68 scales (Tolerance, Craving, Loss of Control) in both samples of participants but only among individuals who began smoking early in life. The haplotypes

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were significantly associated with relapse likelihood and withdrawal severity, but these associations showed no evidence of an interaction with age at daily smoking.

Discussion: The *CHRNA5-A3-B4* haplotypes are associated with a broad range of nicotine dependence phenotypes, but these associations are not consistently moderated by age at initial smoking.

Introduction

Increased knowledge of the genetic influences on the development and manifestation of nicotine dependence would have both applied and theoretical benefits. Such knowledge could benefit tobacco control efforts by revealing who is at elevated risk for dependence and also could clarify mechanisms of dependence (e.g., McClure & Swan, 2006).

Genetic mapping of nicotine dependence will likely prove to be quite complex (Lessov, Swan, Ring, Khroyan, & Lerman, 2004). For example, different genes may affect different stages in the development of dependence (e.g., Munafó, Johnstone, Murphy, & Walton, 2001). Further, the mature nicotine dependence phenotype may have multiple correlated but distinct features (Baker, Moffit, Caspi, & Conti, in press; Hudmon et al., 2003; Piper, McCarthy, & Baker, 2006; Piper et al., 2004; Shiffman, Waters, & Hickcox, 2004). Thus, a given genetic variant might be related only to particular features of the complex phenotype (e.g., Pergadia, Heath, Martin, & Madden, 2006).

The complexity and multidimensionality of nicotine dependence argues for relating targeted genetic variants with strategically selected subphenotypes of dependence (Baker et al., in press) to reveal the breadth or specificity of the effects of genetic variants. For instance, research (e.g., Cannon et al., 2005) demonstrated a relationship between the PTC gene and the Wisconsin Inventory of Smoking Dependence Motives (WISDM-68; Piper et al., 2004) Taste/Sensory subscale for nicotine dependence but not other dependence indices.

Baker et al. (in press) proposed a model of the nicotine dependence phenotype that provides an empirical and theoretical basis for organizing dependence phenotypes for genetic studies. The model comprises three core nicotine dependence criteria: (a) a pattern of heavy, pervasive, and automatic tobacco use; (b) the tendency to relapse after a quit attempt; and (c) withdrawal severity. The model is based on analyses of behavioral genetic data and on covariance structure modeling of nicotine dependence phenotype measures. Each core criterion has been linked theoretically and empirically to a global dependence construct (e.g., Baker et al., in press; Piper et al., 2006), but the evidence also shows only modest overlap among these features. For instance, measures of heavy, pervasive tobacco use, and withdrawal severity account for orthogonal variance in relapse likelihood (e.g., Baker et al., in press; Kenford et al., 2002) and tend to load on different factors (e.g., Baker et al., in press; Lessov, Martin, et al., 2004).

Our research group reported major susceptibility and protective haplotypes for nicotine dependence in the *CHRNA5-A3-B4* subunit cluster on chromosome 15 (Weiss et al., 2008). Following a single nucleotide polymorphism (SNP) discovery effort involving resequencing human neuronal acetylcholine receptor (nAChR) genes (*CHRNA7* was only partially surveyed), individual SNPs in nAChR genes were associated with nicotine dependence. Haplotypes located in the *CHRNA5-A3-B4* subunit cluster were characterized and correlated to the Fagerström Test for Nicotine Dependence (FTND; Heatherton, Kozlowski, Frecker, & Fagerström, 1991). Specifically, the major haplotypes A and C (H_A, H_C) were associated with high and low FTND scores, respectively (Weiss et al., 2008). Haplotype A includes the risk alleles T of rs1051730 and A of rs16969968 that have been associated with nicotine dependence in prior studies (Saccone et al., 2007; Thorgeirsson et al., 2008).

Several features of the Weiss et al. (2008) work are noteworthy. First, this research demonstrates the presence of a significant gene × environment interaction. Specifically, strong associations ($p = 2.0 \times 10^{-5}$) between *CHRNA5-A3-B4* variants and nicotine dependence were seen only among smokers who began daily smoking relatively early in life (e.g., at age 16 or younger; early-onset smokers). No associations ($p = .38$) with haplotypes in the *CHRNA5-A3-B4* cluster were seen in smokers initiating daily smoking at age 17 or later. Second, the obtained associations were of substantial magnitude (odds ratio [*OR*] = 1.82, 95% *CI* = 1.39–2.39). Third, the research is notable because the same pattern of associations was observed in three large (n = 2,210) independent samples.

Credence in the Weiss et al. (2008) results is bolstered by other research that found signals for nicotine dependence in the same region. In a small study of Israeli women, Greenbaum et al. (2006) obtained a suggestive association between their measure of dependence and a *CHRNA3* SNP, rs1051730 (see also Thorgeirsson et al., 2008). Sherva et al. (2008) have reported a significant association between rs16969968 and smoking status as well as experiencing a "pleasurable buzz" in response to early experimentation with smoking. Studies examining the *CHRNA5-A3-B4* cluster as candidate genes (e.g., Berrettini et al., 2008; Saccone et al., 2007) found evidence for associations between common variants in the cluster and their respective phenotypes. Three new genome-wide association studies provide strong evidence for an association between *CHRNA5-A3-B4* variants and lung cancer, but they differ on whether the connection is direct or mediated by smoking behavior (Amos et al., 2008; Hung et al., 2008; Thorgeirsson et al., 2008).

Previous *CHRNA5-A3-B4* research has been based on a limited assessment of nicotine dependence. Several of the earlier studies related the *CHRNA5-A3-B4* variants to the FTND, either to the total score or to individual items (e.g., Greenbaum et al., 2006; Saccone et al., 2007; Thorgeirsson et al., 2008; Weiss et al., 2008). Factor analyses typically show that the FTND items reflect only a smoking heaviness or compulsive smoking factor and a secondary morning smoking factor (Baker et al., in press; Breteler, Hilberink, Zeeman, & Lammers, 2004; Nonnemaker & Homsi, 2007; Radzius et al., 2003; Richardson & Ratner, 2005), and FTND items tend not to be highly correlated with other key features of nicotine dependence (i.e., withdrawal severity and the tendency to relapse to tobacco use; Baker et al., in press; Baker et al., 2007; Payne, Smith, McCracken, McSherry, & Antony, 1994; Piper et al., 2004, 2006; Pomerleau, Carton, Lutzke, Flessland, & Pomerleau, 1994; Pomerleau et al., 2005). Thorgeirsson et al. (2008) also related *CHRNA*3 SNP rs1051730 to *DSM-IV*

nicotine dependence criteria (American Psychiatric Association, 1994). Although the *DSM* criteria elicit information about withdrawal severity and relapse likelihood, little evidence indicates that the criteria measure these dimensions accurately (Breslau & Johnson, 2000; Piper et al., 2006). Finally, Thorgeirsson et al. (2008) related the *CHRNA*3 SNP rs1051730 to unconfirmed self-reports of whether a person was a current or an ex-smoker. These authors found that individuals possessing the risk allele were more likely to be current smokers ($OR = 1.07$, $p = .015$). These data do not reveal whether those who possessed the risk alleles were less likely to have made quit attempts or less successful in quit attempts. Therefore, existing data do not clearly show the relationship between the *CHRNA5-A3-B4* variants and two important indices of nicotine dependence: withdrawal severity and the tendency to relapse to tobacco use (Baker et al., in press; Piper et al., 2006).

This paper relates *CHRNA5-A3-B4* cluster haplotypes and diplotypes with three different phenotypic measures. One measure is a rating of withdrawal symptoms made in real time by subjects enrolled in a cessation study (McCarthy, Piasecki, Fiore, & Baker, 2006). The second is biochemically confirmed relapse in a randomized clinical trial. The third is a new measure of nicotine dependence that reflects heavy, automatic, and pervasive tobacco use that appears to capture a core element of nicotine dependence. This new measure was the mean of four scales of the WISDM-68 nicotine dependence measure (Baker et al., 2007; Cannon et al., 2005; Piper et al., 2004, 2006). Studies show that the WISDM-68 Craving, Tolerance, Automaticity, and Loss of Control scales, hereafter labeled as the Primary Dependence Motives (PDM) scale, have especially strong relationships with important dependence criteria and appear to index necessary and sufficient features of nicotine dependence (Baker et al., 2007; Piper et al., in press). We hypothesized that the *CHRNA5-A3-B4* cluster variants would be especially highly related to the PDM scale, compared with the mean of the other nine WISDM-68 scales (labeled the Secondary Dependence Motives [SDM] scale). The SDM scales tap a variety of dependence motives that typically involve smoking for some instrumental purpose (e.g., smoking to reduce negative affect, to enhance positive affect, and to control weight), and factor analyses show that they constitute a different factor than do the PDM scales (Piper et al., in press). To the extent that the *CHRNA5-A3-B4* cluster variants index risk for a central or core element of nicotine dependence, they should show especially strong relationships with the PDM scales.

This research adds to the existing literature by relating *CHRNA5-A3-B4* cluster haplotypes and diplotypes with strategically selected phenotypes that were not used in previous research. The present study sought to determine whether the *CHRNA5-A3-B4* cluster variants interacted with age at onset of daily smoking in the prediction of phenotypic variance, as was found in Weiss et al. (2008). This research uses the same population of smokers that Weiss et al. used and therefore constitutes an extension of that work, not an independent replication.

Methods

Participants

Participants were 886 current or former daily smokers. They were members of the Utah (UT, $n = 486$) and Wisconsin (WI, $n = 400$) cohorts of our previous study (Weiss et al. 2008) who had major haplotypes (i.e., they had only H_A, H_B, H_C, or H_D) of the *CHRNA5-A3-B4* cluster. All participants reported that they were of European descent, and we found no genetic evidence of population stratification (Weiss et al. 2008). A total of 442 participants (48%) were female, and the mean participant age was 51.8 years ($SD = 13.4$, range = 25–86). On average, participants began smoking daily at 17.2 years ($SD = 4.5$), smoked a mean of 25.6 cigarettes/day ($SD = 13.3$), and had a mean FTND score of 5.6 ($SD = 2.3$). The WI cohort comprised current smokers who were participating in smoking cessation trials in which either bupropion or placebo was administered in a double-blind randomized trial (McCarthy et al., 2008; Piper et al., 2007). Further details of accession methods and sample characteristics are given in Weiss et al.

Genotyping

Haplotypes were based on five tagging SNPs (rs680244, rs569207, rs16969968, rs578776, and rs1051730) in the *CHRNA5-A3-B4* region. These five tagging SNPs were chosen from a larger genotyping study that included 11 SNPs from the *CHRNA5-A3-B4* region identified by resequencing a subset of individuals from these study populations (Weiss et al., 2008). Genotyping was performed using the SNPlex method, a multiplexed oligonucleotide ligation, polymerase chain reaction assay (Applied Biosystems, Foster City, CA). Individual haplotype and diplotype assignments were inferred from genotypic data using *fastPHASE* and independently evaluated using the EM algorithm implemented in *SNPHAP* (Weiss et al. 2008).

The haplotype counts and frequencies observed in the 886 individuals were as follows: H_A (CCAGA, 699, 39.4%), H_B (TCGGG, 670, 37.8%), H_C (CTGAG, 337, 19.0%), and H_D (TCGAG, 66, 3.7%). A linkage disequilibrium (LD) plot of the *CHRNA5-A3* region defined by the five tagging SNPs is shown in Figure 1A, along with the observed r^2 LD statistic between pairs of SNP loci. Haplotype sequences reported are from the chromosome 15 (+) strand, and the structure of the haplotypes derived from the five tagging SNPs is shown in Figure 1B.

Phenotypes

Primary and SDM. The 68-item WISDM-68 (Piper et al., 2004) was used to assess both the primary dependence subphenotype of heavy, pervasive smoking and the SDM. The PDM score was the mean of four WISDM-68 scales (i.e., Automaticity, Craving, Loss of Control, and Tolerance). The SDM score was the mean of the other nine WISDM-68 scales: Affiliative Attachment, Behavioral Choice/Melioration, Cognitive Enhancement, Cue Exposure/Associative Processes, Negative Reinforcement, Positive Reinforcement, Social/Environmental Goads, Taste/Sensory Properties, and Weight Control. We report *CHRNA5-A3-B4* relationships with the PDM and SDM scores as well as with the four individual scales that constitute the PDM. Representative contents of the PDM scales are depicted in Table 1.

Withdrawal. Withdrawal was measured only in the WI sample ($n = 400$). Data were gathered with real-time data acquisition with either handheld electronic diaries or cell phones connected to an interactive voice-response system. Withdrawal was a growth curve model coefficient that reflected the linear slope of withdrawal symptoms over the first postquit week (McCarthy, Bolt, & Baker, 2007; Piper et al., 2008).

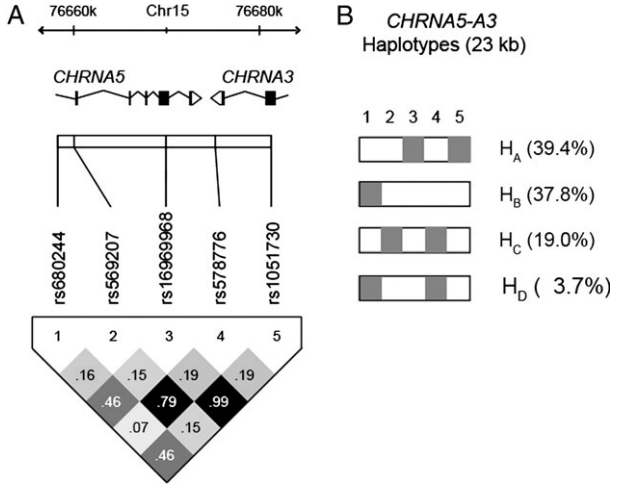

Figure 1. Linkage disequilibrium (LD) structure and haplotype frequency in the *CHRNA5-A3* region using five haplotype tagging single nucleotide polymorphisms (SNPs). (A) The LD plot between pairs of SNP markers from this chromosome 15 region is based on the measured r^2. Pairwise LD values (r^2) are indicated at the intercept of the two SNP markers on the matrix. Each square represents the magnitude of LD for a single pair of markers, with black color indicating high r^2. (B) Haplotypes spanning this LD block are shown along with their frequency in the combined Utah and Wisconsin cohorts. Chromosome 15 coordinates are from NCBI Build 36.1 (hg18).

Ability to quit. The final dependence phenotype, ability to quit smoking, was assessed only in the WI cohort. Ability to quit was assessed using the number of days to relapse, which was defined as any smoking on three consecutive days during the first 90 days postquit. Time of relapse was determined via timeline follow-back assessment (see Piper et al., 2007; Sobell & Sobell, 1992).

Age at onset of daily smoking

Age at onset of daily smoking was assessed retrospectively, and respondents were dichotomized into early onset (i.e., at age 16 or younger) and late onset (i.e., at age 17 or older) (Weiss et al., 2008). Some 49% of the sample fell into the early-onset condition. Data support the validity of such retrospective recall (Brigham, Lessov-Schlaggar, Javitz, McElroy, & Swan, 2008; Huerta, Chodick, Balicer, Davidovitch, & Grotto, 2005).

Table 1. Selected items from the WISDM-68 Automaticity, Craving, Loss of Control, and Tolerance subscales (for the full sets of items, see Piper et al., 2004)

WISDM-68 subscale	Selected items
Automaticity	I often smoke without thinking about it.
	I smoke without deciding to.
	Sometimes I'm not aware that I'm smoking.
Craving	It is hard to ignore an urge to smoke.
	I frequently crave cigarettes.
	When I haven't been able to smoke for a few hours, the craving gets intolerable.
Loss of control	Cigarettes control me.
	Sometimes I feel like cigarettes rule my life.
	My smoking is out of control.
WISDM-68-Tolerance	I can only go a couple hours between cigarettes.
	Other smokers would consider me a heavy smoker.
	I usually want to smoke right after I wake up.

Note. WISDM-68, Wisconsin Inventory of Smoking Dependence Motives.

Data analyses

Genetic variants were analyzed both as haplotypes and as diplotypes. The total diplotype counts for the 886 participants were as follows: AA (141), AB (255), AC (135), AD (27), BB (127), BC (138), BD (23), CC (24), and CD (16). Because of the low frequency of H_D (3.7%), we excluded it from all haplotype analyses, and we excluded diplotypes containing H_D from diplotype analyses. We also excluded diplotype CC from diplotype analyses due to its low frequency (2.7%). Thus, across age-at-onset conditions, haplotype tests were based on 1,706 observations, and diplotype tests were based on 796 observations. Our haplotype tests have greater power, but diplotype tests were included for the information they provide regarding inheritance models.

Quantitative phenotypes (WISDM-68 scales, withdrawal, and days to relapse) were tested as both continuous and dichotomous variables to attain a comprehensive appraisal of their genotype relationships. Categorical scoring of these phenotypes was included because research suggested nonlinear relationships between the individual WISDM-68 PDM scales and dependence criteria (Piper et al., 2004) and inspection of the relationships between dependence scale deciles and the frequency of H_A relative to H_C in early-onset smokers in the present sample indicated nonlinearity for some scales. Categorical coding of WISDM-68 scales and withdrawal was based on median splits of the entire sample across age-at-onset conditions. For the dichotomous 90-day relapse variable (0 = no relapse, 1 = relapse), the 46 participants who were lost to follow-up within the first 90 days after quitting but before they reported a relapse were assumed to have relapsed the day following their last follow-up contact.

Logistic regression was used to test associations between genotypes and dichotomous phenotypes. In logistic regression haplotype analyses, H_A was chosen as the reference condition and the H_A versus H_C contrast was of primary interest; in diplotype analyses, homozygous diplotype AA was chosen as the reference condition.

Given our a priori prediction that *CHRNA5-A3-B4* effects on nicotine dependence severity would be more robust in early-onset smokers (Weiss et al., 2008), all haplotype–nicotine dependence relationships were tested for age-at-onset interactions using logistic regression analyses, since interaction tests can be biased by nonlinearity. If the interaction was significant, associations between the haplotype or diplotype and the phenotype were tested by age-at-onset condition; otherwise, they were tested across age-at-onset conditions.

Continuous WISDM-68 scale scores and withdrawal were analyzed using the general linear model (GLM). Significant haplotype or diplotype effects were followed by Tukey pairwise comparisons of the least-squares haplotype or diplotype means. Although all pairwise genotype comparisons were tested, we made an a priori prediction, based on our previous results with the FTND (Weiss et al., 2008), that H_A would be associated with greater nicotine dependence than H_C.

For the nondichotomous days-to-relapse measure, a nonparametric Cox model survival analysis was tested using Kaplan–Meier estimation of relapse probability and the Mantel–Haenszel method for calculating the log-rank test. If the overall haplotype or diplotype test was significant, pairwise comparisons were made. In survival analyses, the days-to-relapse variable was censored for subjects lost to follow-up within 90 days who had not reported a relapse by the date of their last follow-up assessment. Although follow-up data were available for the first year postquit, survival analyses were computed over the first 90 days postquit because the hazard function was negatively accelerated (the probability of relapse if one remained abstinent for 90 days was only 0.18 over the remainder of the year).

Results

Phenotype relationships

Table 2 presents zero-order and point-biserial correlations among the major phenotype measures. The two smoking heaviness measures (FTND and PDM) were highly correlated with one another ($p < 1 \times 10^{-9}$) and were modestly correlated with 90-day relapse (p's < .008). Withdrawal was not correlated with smoking heaviness measures (p's > .08) or with relapse ($p = .36$). Therefore, the modest to null relationships among these measures of the three core nicotine dependence criteria make it possible that each core criterion may be associated with different *CHRNA5-A3-B4* variants.

Heavy, automatic, and pervasive smoking

Analyses of PDM indicated that H_A was associated with heavier smoking than was H_C in early-onset, but not late-onset, smokers (Table 3). With dichotomous PDM as the dependent variable, the interaction between age-at-onset condition and the H_A versus H_C contrast was significant, $p = .04$, $OR = 0.57$ (95% $CI = 0.34$–0.97). As shown in Figure 2, in the early-onset condition, H_A was associated with a higher percentage of PDM scores above the median than was H_C, and we found a nonsignificant trend for H_A to be associated with more PDM scores above the median than was H_B, $p = .08$ (see Table 3). We found no association between haplotypes and dichotomous PDM scores in late-onset smokers (see Figure 2 and Table 3). These results with dichotomous PDM scoring were consistent with findings obtained with analyses of continuous scores within age-at-onset condition. In the early-onset condition, there was a significant haplotype effect, $F(2, 823) = 3.88$, $p = .02$, and pairwise comparisons were significant only for the H_A versus H_C test, $p = .02$. H_A was associated with higher PDM scores ($M = 5.1$, $SD = 1.3$) than was H_C ($M = 4.7$, $SD = 1.3$). The haplotype effect on continuous PDM scores was not significant in late-onset smokers.

Table 2. Zero-order and point-biserial correlations of major phenotype variables

	FTND	PDM	SDM	WDR
PDM	0.61			
SDM	0.41	0.72		
WDR	0.01	−0.09	−0.07	
90-Day relapse	0.19	0.13	0.05	0.05

Note. PDM, Primary Dependence Motives; SDM, Secondary Dependence Motives; FTND, Fagerström Test for Nicotine Dependence; and WDR, withdrawal. 90-Day relapse was coded dichotomously such that positive correlations indicate that the variable was associated with higher likelihood of relapse (0 = no relapse, 1 = relapse). WDR was dichotomously coded for these correlations via a median split (0 = below median, 1 = at or above median). All other variables were coded as continuous measures. *N* values vary from 883 to 886 for the PDM, SDM, and FTND intercorrelations, which involved both samples and from 395 to 400 for associations involving the FTND and WDR, which were available only in the Wisconsin sample.

Analyses of the relationship between PDM and the diplotypes revealed that diplotype AA was a risk factor relative to diplotype BC in early-onset but not late-onset smokers. With the binary PDM variable, the AA versus BC × age-at-onset interaction was significant, $p = .02$, $OR = 0.32$ (95% $CI = 0.12$–0.83). Within the early-onset condition, there was an AA versus BC effect, $p < .01$, $OR = 0.38$ (95% $CI = 0.19$–0.77), but there was no significant diplotype effect in the late-onset condition (see Figure 2). A post-hoc analysis in the early-onset condition, in which diplotype AB was the reference condition, indicated a significant AB versus BC effect, $p = .001$, $OR = 0.37$ (95% $CI = 0.20$–0.67). With continuous scoring of PDM, there was a diplotype effect in early-onset smokers, $F(4, 376) = 2.36$, $p = .05$. There were nonsignificant trends in the early-onset condition for diplotype BC ($M = 4.6$, $SD = 1.5$) to be associated with lower PDM scores than either AA ($M = 5.2$, $SD = 1.3$), $p = .06$, or AB ($M = 5.1$, $SD = 1.3$), $p = .07$. There was no diplotype effect in late-onset smokers. Overall, these findings suggest that H_A and H_C exert relative risk and protective effects, respectively, on heavy, automatic, and pervasive smoking in early-onset smokers.

We also examined the associations of individual PDM scales with genetic variants to shed light on the features of heavy, pervasive smoking that are most sensitive to the *CHRNA5-A3-B4* genetic signal. We analyzed the associations between dichotomous scoring of each of the four constituent PDM scales and both haplotypes and diplotypes among early-onset smokers. The strongest haplotype signals were obtained with the Tolerance scale (see Figure 3). H_A was associated with higher Tolerance scores than either H_B, $p < .01$, $OR = 0.65$ (95% $CI = 0.48$–0.89), or H_C, $p = 9 \times 10^{-5}$, $OR = 0.47$ (95% $CI = 0.32$–0.68). Similar but slightly weaker signals were obtained with Craving: H_A versus H_B, $p < .04$, $OR = 0.71$ (95% $CI = 0.52$–0.96); and H_A versus H_C, $p < .03$, $OR = 0.64$ (95% $CI = 0.44$–0.93). Although there was a trend for H_A to be associated with higher Automaticity and Loss of Control scores than was H_C, we found no significant haplotype effects for either of these scales. The Tolerance scale also was strongly associated with diplotypes (see Figure 3). Diplotype AA was associated with higher Tolerance scores than either BB, $p = 0.03$, $OR = 0.44$ (95% $CI = 0.21$–0.94), or BC, $p = .001$, $OR = 0.29$ (95% $CI = 0.14$–0.60). For the Craving scale, AA was associated with higher scores than BB, $p = .04$, $OR = 0.46$ (95% $CI = 0.22$–0.95), and there was a nonsignificant trend for AA to be associated with higher scores than BC, $p = .07$, $OR = 52$ (95% $CI = 0.26$–−1.04). Consistent with the findings for Tolerance and Craving, diplotype AA was associated with higher Loss of Control scores than was BB, $p = .004$, $OR = 0.35$ (95% $CI = 0.17$–0.71). Diplotype AA also was associated with higher scores on Loss of Control than was AB, $p = .05$, $OR = 0.53$

Table 3. Logistic regression results for the association between haplotypes and dichotomized PDM, WDR, and relapse during the first 90 days postquit

	PDM-early		PDM-late		WDR		90-Day relapse	
Effect	*OR* (95% *CI*)	*p* value	*OR* (95% *CI*)	*p* value	*OR* (95% *CI*)	*p* value	*OR* (95% *CI*)	*p* value
A–B	0.75 (0.55–1.03)	.08	0.98 (0.73–1.33)	.92	1.20 (0.87–1.64)	.26	0.74 (0.53–1.02)	.07
A–C	0.56 (0.38–0.82)	.003	0.98 (0.68–1.42)	.92	1.57 (1.05–2.34)	.03	0.59 (0.39–0.89)	.01

Note. OR, odds ratio. Primary Dependence Motives (PDM) tests were computed by age at onset of daily smoking condition (PDM-early = daily smoking at 16 or younger; PDM-late = daily smoking at 17 or older), whereas withdrawal (WDR) and 90-day relapse associations were tested across both age-at-onset conditions combined. The PDM tests were based on the Utah and Wisconsin (WI) cohorts combined, whereas WDR and 90-day relapse were based on WI participants only. Effect = the specific haplotype contrast tested, that is, A–B = H_A vs. H_B and A–C = H_A vs. H_C.

(95% *CI* = 0.28–1.00). Automaticity was not associated with the diplotypes.

To assess the robustness of the PDM findings across cohorts, haplotype associations with dichotomized PDM and Tolerance (i.e., the subscale with the strongest signal across cohorts) scales were tested by age-at-onset condition within each cohort (UT; WI) separately. The H_A versus H_C contrast was significant in early-onset smokers in both cohorts for Tolerance: UT, $p < .01$, $OR = 0.50$ (95% $CI = 0.29–0.87$); WI, $p < .002$, $OR = 0.45$ (95% $CI = 0.27–0.75$), and for PDM: UT, $p = .05$, $OR = 0.58$ (95% $CI = 0.33–1.01$); WI, $p < .02$, $OR = 0.54$ (95% $CI = 0.32–0.90$). The H_A versus H_B contrast for Tolerance was significant among early-onset smokers only in the UT cohort, $p < .05$,

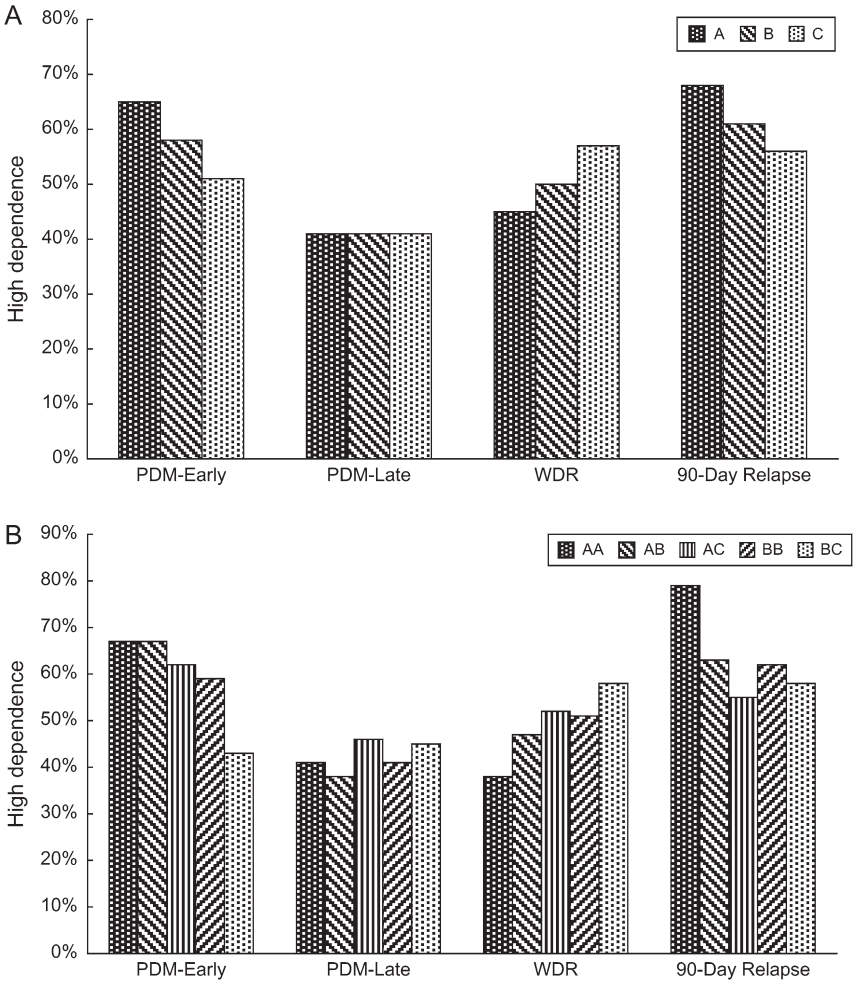

Figure 2. Dichotomous phenotypes by haplotype (top) and diplotype (bottom). PDM, Primary Dependence Motives; Early, daily smoking at age 16 or younger; Late, daily smoking at age 17 or older; and WDR, withdrawal. High Dependence = percentage in the dichotomous category indicative of a higher level of nicotine dependence, that is, percentage above the median for PDM and WDR and the percentage who relapsed for 90-day relapse.

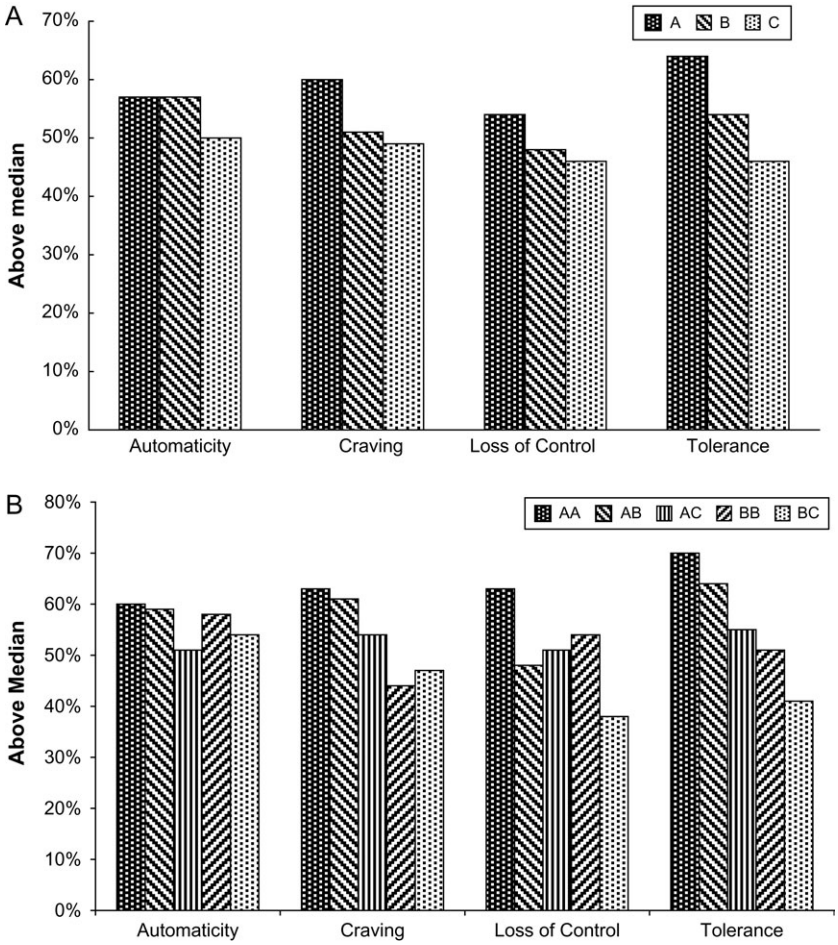

Figure 3. Dichotomous Primary Dependence Motives scales by haplotype (top) and diplotype (bottom) in early-onset smokers. The scales were scored dichotomously based on the median for both age-at-onset conditions.

OR = 0.60 (95% *CI* = 0.38–0.97). No significant haplotype effects were observed among late-onset smokers in either cohort. Thus, the finding in the combined sample that H_A versus H_C effects were stronger in early-onset than late-onset smokers was obtained in the two different cohorts for both Tolerance and the PDM composite.

Secondary smoking motives

CHRNA5-A3-B4 variants were related to dichotomously scored SDM. Age-at-onset condition interacted with the H_A versus H_C contrast, $p < .01$, *OR* = 0.49 (95% *CI* = 0.29–0.83). Within the early-onset condition, 61% of H_A scores were above the SDM median, compared with 48% of H_C scores, $p < .01$, *OR* = 0.60 (95% *CI* = 0.41–0.87). There was no significant haplotype effect on SDM among late-onset smokers. The pattern of results for PDM and SDM was similar in that, for both, there was an interaction of age-at-onset with the H_A versus H_C contrast and a significant H_A versus H_C effect in early-onset but not late-onset smokers.

To determine whether the H_A versus H_C association with SDM was independent of its association with PDM in early-onset smokers, we tested logistic regression models in which one composite (PDM or SDM) was a dichotomous dependent variable (defined by a median split) and the other composite (PDM or SDM), as either a continuous or dichotomous variable, was a covariate. The H_A versus H_C contrast with PDM as the dependent variable was marginally significant with dichotomized SDM as the covariate, $p = .06$, *OR* = 0.66 (95% *CI* = 0.42–1.02), and was statistically significant with continuous SDM as the covariate, $p = .02$, *OR* = 0.56 (95% *CI* = 0.36–0.89). However, neither test of the H_A versus H_C contrast was significant with SDM as the dependent variable and PDM as the covariate, p's $\geq .18$, *OR*'s ≥ 0.74. Because the correlation between PDM and SDM in this sample was high ($r = .72$), approximately half the variance in these scales (r^2) was common to both. However, the unique variance of only PDM was associated with H_A versus H_C effects; therefore, haplotype status had no effect on SDM independent of its relationship with PDM.

Withdrawal severity

We found no haplotype × age-at-onset interaction in the logistic regression analysis in which a median split of the withdrawal slope was the dependent variable. Thus, subsequent analyses of this phenotype were computed across both age-at-onset conditions. H_C was associated with more severe withdrawal relative to H_A (see Figure 2), $p < .04$, *OR* = 1.57 (95% *CI* = 1.05–2.34). In a diplotype analysis, diplotype AA was associated with less

withdrawal severity than diplotype BC (see Figure 2), $p = .05$, $OR = 2.18$ (95% $CI = 1.04–4.59$). In GLM analyses of the continuous withdrawal slope, there was neither a significant haplotype effect nor a significant diplotype effect across age-at-onset conditions.

We examined the possible effect of bupropion treatment on the relationship between haplotype and withdrawal severity. First, bupropion was entered in a logistic regression model along with haplotype coding and a haplotype × bupropion interaction with dichotomous withdrawal as the dependent variable. No significant interaction effects were observed for either the H_A versus H_C, $p = .23$, or the H_A versus H_B, $p = .40$, contrast. In a second model, the interaction term was dropped but bupropion was retained as a covariate. In this second test, the H_A versus H_C contrast was significant, $p = .03$, $OR = 1.56$ (95% $CI = 1.04–2.33$). The bupropion effect did not reach nominal significance, $p = .08$, $OR = 0.76$ (95% $CI = 0.56–1.03$). Thus, the protective effect of H_A relative to H_C on withdrawal severity was independent of any effect bupropion may have had on withdrawal severity.

Smoking cessation

In a logistic regression analysis in which the dependent variable was relapse versus no relapse over the first 90 days postquit, we found no haplotype × age-at-onset interaction. Thus, further tests of smoking cessation were made across the combined age-at-onset conditions. As shown in Figure 2 and Table 3, H_A was associated with a higher relapse rate than H_C, and the H_A versus H_B contrast approached significance. Diplotype analyses, also shown in Figure 2, indicated that diplotype AA had a higher 90-day relapse rate than every other diplotype, p's = .006–.03, OR's < 0.45 (95% CI's = 0.15–0.93).

Abstinence rates, expressed as Kaplan–Meier survival functions, over the first 90 days postquit are shown as a function of haplotype and diplotype in Figure 4. A survival analysis revealed a significant haplotype effect, $\chi^2(2, n = 767) = 7.45$, $p = .02$. Pairwise comparisons were significant for both the H_A versus H_B test, $\chi^2(1, n = 629) = 5.41$, $p = .02$, and the H_A versus H_C test, $\chi^2(1, n = 638) = 4.58$, $p < .04$. The H_B versus H_C comparison was not significant. We also found a significant diplotype effect across all five diplotypes, $\chi^2(4, n = 629) = 5.41$, $p = .02$. Pairwise comparisons indicated that the relapse rate was higher for diplotype AA than for each of the other four diplotypes, p's = .005–.03 (see Figure 4). Entering drug (bupropion vs. placebo) as a covariate in the haplotype or diplotype survival analyses did not alter any of these findings nor were there any significant interaction effects with drug.

Predictive validities of haplotypes and SNPs

To permit comparison of the predictive validities of both the haplotypes and the tagging SNPs, Table 4 displays the association between both the SNPs and haplotypes with the three major phenotypes: PDM, withdrawal, and relapse likelihood. Associations with PDM were determined in early-onset smokers, whereas associations with cessation (90-day relapse) and withdrawal involve all subjects in the WI cohort. The depicted *OR*s show that the relationships between the haplotypes and the phenotype measures are somewhat stronger than those between the constituent SNPs and phenotypes, but the relationships are quite similar, as expected given the LD structure (see Figure 1) determined from this region (Weiss et al., 2008).

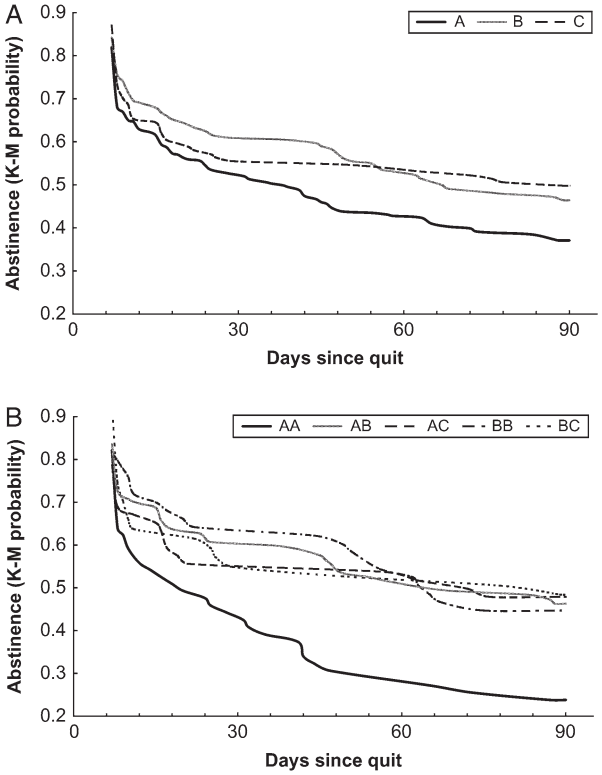

Figure 4. Abstinence following a smoking cessation trial by haplotype (top, $n = 767$) and diplotype (bottom, $n = 356$). Abstinence is shown as the Kaplan–Meier probability of remaining abstinent for the first 90 days postquit. Data are based on the age-at-onset conditions combined.

Discussion

The present results with the PDM expand our previous findings using the FTND in the same samples (Weiss et al., 2008). The analyses reported here suggest that H_A and H_C of the *CHRNA5-A3-B4* cluster are relative risk and protective factors, respectively, for heavy, pervasive, and automatic smoking among individuals who began smoking early in life (i.e., daily smoking at age 16 or younger). The interaction between age-at-onset condition and the H_A versus H_C contrast with PDM as the dependent variable is consistent with our previous finding with FTND as the dependent variable. Further, although the SNPs and haplotypes are associated similarly with the phenotypes (see Table 4), the haplotypes provide information germane to the underlying genetic architecture (see Figure 1). Finally, the present results expand our knowledge by linking *CHRNA5-A3-B4* haplotypes with withdrawal and smoking cessation phenotypes. This set of genetic variants has broad implications for the manifestation of nicotine dependence.

Several findings of this research suggest that the *CHRNA5-A3-B4* haplotypes confer important information about dependence risk. First, the association between these haplotypes and PDM was found in two separate samples. Second, the magnitude of the associations was meaningful. For example, persons with diplotype AA relapsed at about twice the rate of other individuals.

Table 4. Logistic regression results for the association between *CHRNA5-A3-B4* variants and the three major phenotypes: dichotomized PDM, WDR, and relapse during the first 90 days postquit

	PDM		WDR		90-Day relapse	
	OR (95% *CI*)	*p* value	*OR* (95% *CI*)	*p* value	*OR* (95% *CI*)	*p* value
rs1051730 (H_A)	1.49 (1.12–1.97)	.006	1.34 (1.00–1.77)	.05	1.51 (1.12–2.02)	.007
rs16969968 (H_A)	1.47 (1.11–1.97)	.008	1.31 (0.98–1.74)	.07	1.45 (1.07–1.95)	.02
rs680244 (H_B)	1.12 (0.85–1.47)	.42	1.09 (0.82–1.45)	.54	1.22 (0.91–1.63)	.18
rs569207 (H_C)	1.51 (1.08–2.12)	.02	1.40 (0.97–2.03)	.08	1.40 (0.97–2.03)	.08
rs578776 (H_C)	1.53 (1.12–2.09)	.008	1.48 (1.05–2.09)	.02	1.54 (1.09–2.17)	.01
H_A vs. H_B	1.33 (0.97–1.82)	.08	1.20 (0.87–1.64)	.26	1.36 (0.98–1.89)	.07
H_A vs. H_C	1.79 (1.23–2.61)	.003	1.57 (1.05–2.34)	.03	1.69 (1.12–2.55)	.01

Note. OR, odds ratio. Primary Dependence Motives (PDM) tests were computed within the early-onset smoking condition (daily smoking at age 16 or younger), whereas withdrawal (WDR) and 90-day relapse associations were tested across both age-at-onset conditions combined. The PDM tests were based on the Utah and Wisconsin (WI) cohorts combined, but WDR and 90-day relapse were based on WI participants only. The five single nucleotide polymorphisms (SNPs) are the tagging SNPs used to determine the *CHRNA5-A3-B4* haplotypes (the haplotype with which each SNP is associated is shown in parentheses adjacent to the SNP rs number). *OR*s less than 1.00 were inverted to make cross-variant comparisons easier.

Third, the haplotypes were predictive of risk across multiple phenotypes: pervasive, heavy smoking, withdrawal magnitude, and relapse. The relationships suggested that the nature of the genetic risk differed for heavy smoking and relapse on the one hand versus withdrawal magnitude on the other hand (see Discussion). Fourth, the general correspondence in the results of continuous and binary scorings of the PDM suggests that the obtained results are not an artifact of incorrectly modeling the relationship between this dependence index and the genotypes.

We found evidence that H_A is a risk factor and H_C a protective factor for both smoking heaviness and short-term relapse or cessation failure. We also found evidence that, for withdrawal severity, H_A is a relative protective factor and H_C a relative risk factor. The smoking heaviness and withdrawal severity phenotypes were not highly associated with one another in this research (Table 2) or in other studies (e.g., Baker et al., in press; Piper et al., 2006). Therefore, they may reflect different genetic underpinnings. It remains unclear, though, why the same cluster of genes impinges on the phenotypes in such different ways. One possibility is that H_A has a biological effect that fosters or permits smoking heaviness, whereas H_C increases withdrawal severity independently. A second possibility is that H_A, relative to H_C, may decrease the effects of nicotine, resulting in greater smoking but weaker withdrawal. The latter possibility is suggested by a recent functional study that reports diminished response to the nicotine agonist epibatidine associated with a variant in the α5 nAChR subunit (Bierut et al., 2008).

The associations with withdrawal and cessation were not moderated by age at onset of daily smoking. It is possible that, in fact, the age-at-onset moderation of nicotine dependence is specific to the smoking heaviness subphenotype. However, the withdrawal and cessation phenotypes were available in only one cohort, which reduced the power for testing moderation effects. Further research is needed to address the moderation of the *CHRNA5-A3-B4* effect by age.

As noted earlier, the present results suggest a gene × environment interaction (see Caspi et al., 2003; Moffitt, 2005; Swan & Lessov, 2004) such that early exposure to nicotine is needed for the association of the risk for smoking heaviness with H_A. Confidence in this finding is bolstered somewhat by independent evidence that early exposure is associated with either higher levels of nicotine self-administration or greater neurobiological impact of nicotine. Research with smokers suggests that exposure to nicotine relatively early in life is related to heightened consumption of cigarettes in adulthood (Breslau & Peterson, 1996; Broms, Silventoinen, Lahelma, Koskenvuo, & Kaprio, 2004), a relative inability to quit smoking (Breslau & Peterson, 1996; Chen, Stanton, Shankaran, & Li, 2006; Hymowitz et al., 1997; John, Meyer, Hapke, & Rumpf, 2004), and more severe nicotine dependence (Grant, 1998; Pergadia, Heath, Agrawal, et al., 2006; Xian et al., 2007). Research with rodents shows that nicotine exposure during periadolescence induces long-lasting neurochemical, anatomical, physiological, and behavioral changes that differ markedly from those seen with initial exposure in adulthood (Adriani et al., 2003; Cruz, Delucia, & Planeta, 2005; Schochet, Kelley, & Landry, 2004; Slotkin, 2002). Thus, research suggests that early nicotine exposure may produce a more severe level of nicotine dependence, and the present results suggest that in humans this effect depends on *CHRNA5-A3-B4* status.

Analyses in which the WISDM-68 PDM and SDM scales served as covariates for one another suggested that the H_A effect can be linked more directly to the particular motives indexed by PDM versus the smoking motives reflected by SDM. SDM was related to the haplotypes only to the extent that it was correlated with PDM. Recent research suggests that the PDM scales may constitute necessary and sufficient features of dependence (Piper et al., in press). That is, smokers do not show significant dependence on a variety of other measures unless these scales are elevated, and smokers can be significantly dependent if only these scales are elevated. The present findings suggest that the *CHRNA5-A3-B4* variants are related to a core dimension of dependence.

In theory, the present results suggest a causal model in which the *CHRNA5-A3-B4* cluster variants increase the likelihood that a person can or will smoke very heavily, and such

heavy smoking then produces a highly automatic smoking ritual that can be elicited with little awareness, is hard to control, and the interruption of which occasions strong craving (Curtin, McCarthy, Piper, & Baker, 2006; McCarthy, Curtin, Piper, & Baker, in press; Tiffany, 1990). These features of heavy, automatic smoking with accompanying craving could then increase the likelihood of relapse via various routes. For example, strong craving might precipitate relapse, and so on.

Some research suggests why SDM does not reflect a core dimension of dependence. Many of the individual SDM scales elicit information on instrumental uses of smoking, for example, smoking to control weight and negative moods or to experience pleasure. If entrenched smoking reflects habit learning (Everitt & Robbins, 2005), it makes sense that instrumental dependence motives would be less highly associated with important markers of severe dependence than the features tapped by PDM.

In conclusion, the present research shows that major *CHRNA5-A3-B4* haplotypes identify countervailing susceptibility (H_A) and protective (H_C) influences on nicotine dependence as it is indexed by a subphenotype that reflects self-report of heavy, automatic smoking that defies conscious control. The assessed haplotypes showed significant relationships with this subphenotype only among smokers who began daily smoking relatively early in life, suggesting the expression of genetic risk occurs only among those with early tobacco exposure. This effect could have public health implications because interventions that reduce youth tobacco use (e.g., excise taxes) could be used to mitigate the expression of genetic risk. The research also shows that the assessed *CHRNA5-A3-B4* haplotypes were significantly associated with two other major nicotine dependence subphenotypes: withdrawal severity and an inability to stop smoking. Although H_A was associated with a heightened risk of relapse relative to H_C, H_C was associated with more severe withdrawal relative to H_A. The results show that the assessed haplotypes are associated with all three of the major nicotine dependence subphenotypes, and they may confer risk via multiple routes.

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Declaration of Interests

TBB has served as an investigator on research projects sponsored by pharmaceutical companies, including Pfizer, GlaxoSmithKline, Sanofi, and Nabi. Over the past 3 years, MCF has served as an investigator in research studies at the University of Wisconsin that were funded by Pfizer, GlaxoSmithKline, and Nabi Biopharmaceuticals. In 1998, the University of Wisconsin appointed Fiore to a named chair funded by an unrestricted gift to the university from Glaxo Wellcome.

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