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Nicotine dependence as a moderator of genetic influences on smoking cessation treatment outcome*

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Abstract

Background—Genetic influences on smoking cessation treatment outcome may be affected by pretreatment patient characteristics. Nicotine dependence is arguably the most salient clinical factor in smoking cessation.

Methods—In this secondary analysis of clinical trial data ($N = 793$), we examined nicotine dependence severity as a moderator of the effects of 1198 single nucleotide polymorphisms (SNPs) in 53 biologically-relevant gene regions on smoking cessation outcomes. P -values were adjusted to account for multiple correlated SNPs within a gene region; corrected system-wide significance was 5×10^{-4} .

Results—SNP $\times$ nicotine dependence interactions reached region-wide significance for several SNPs in the Dopamine Beta Hydroxylase (*DBH*) locus ($0.0005 < \text{Adjusted-}P < 0.05$), including rs1541333, which reached system-wide significance for predicting end of treatment (EOT)

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Contributors

Drs. Lerman, Tyndale, Conit, Swan, and Bergen designed the study. Drs. Lee and Conti undertook the statistical analysis, and Dr. Leventhal wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.drugalcdep.2014.02.016>.

Conflict of interest

Dr. Bergen received prior research support from Medco Health Solutions and Affymetrix. Dr. Swan had a one-day consultation to Pfizer in 2008. Dr. Rachel F. Tyndale has participated in one-day advisory meetings for Novartis and McNeil. Dr. Lerman has received research funding from Pfizer Inc.

abstinence (Adjusted- $P = 0.0004$). A haplotype including 6 *DBH* SNPs predicted abstinence at EOT (OR = 1.7, $P = 0.001$) and 6-month follow-up (OR = 1.6, $P = 0.008$) in those with high nicotine dependence ($n = 526$) but not in those with low dependence ($n = 227$). The *DBH* signal observed here may be distinct from a previously reported genome-wide significant signal for former smoking status and from the principal haplotype associated with plasma dopamine beta-hydroxylase activity. A haplotype within the Chromosome 15 Nicotinic Acetylcholine Receptor gene region predicted abstinence at EOT in those with high (OR = 2.0, $P = 0.0004$) but not low ($P = 0.6$) dependence in *post hoc* analyses.

Conclusions—Considering pre-treatment nicotine dependence level may optimize the prediction of genetic influences on cessation outcomes. If replicated, results like these may inform prognosticative genomic screening panels designed to identify smokers at high risk of relapse when coupled with severe nicotine dependence.

Keywords

Genetics; Smoking cessation; Nicotine dependence; Dopamine

1. Introduction

Several molecular genetic studies of the association between common variants and smoking cessation outcomes have been performed (e.g., Bergen et al., 2009, 2013b; Conti et al., 2008; David et al., 2007a,b, 2008; De Ruyc et al., 2010; King et al., 2012; Lee et al., 2007; Lerman et al., 2006a, 2003; Leventhal et al., 2012; Munafo et al., 2013, 2011; Ray et al., 2010; Rose et al., 2010; Sarginson et al., 2011; Uhl et al., 2008). While some promising findings have been reported, the literature largely identifies variants with relatively modest associations with cessation outcome. Perhaps these findings reflect the reality that common variants typically have small effects on complex behavioral phenotypes, such as smoking cessation. Another possibility is that the strength of effect of common variants on smoking cessation is conditional on important moderator variables. Thus, examining the main effect of common variants on smoking cessation in heterogeneous samples may obscure genetic signals, which may be more robust in certain subpopulations.

Nicotine dependence is an important moderator to study in smoking cessation research for several reasons. Of the universe of possible pretreatment clinical characteristics, self-reported severity of nicotine dependence is one of the most consistent and robust predictors of cessation outcome (Baker et al., 2007; Bolt et al., 2009; Breslau and Johnson, 2000; Fagerstrom et al., 2012). Measures of nicotine dependence that assess smoking frequency and drive to smoke following tobacco abstinence (e.g., Fagerström Test for Nicotine Dependence, FTND; Heatherton et al., 1991) putatively tap an addiction process indicative of compulsive physiologically-driven tobacco use (Piper et al., 2004). Thus, certain genetic sources of smoking relapse risk (e.g., dopaminergic genes associated with poor impulse control; Brewer and Potenza, 2008) may be expressed to a greater extent when coupled with a strong drive to smoke characteristic of severe nicotine dependence. Alternatively, the effect of some genes may be ‘washed out’ when coupled with severe nicotine dependence. For instance, if high-nicotine dependence phenotypes are associated with greater physiological adaptations in the biological pathways involved in the addiction process

(Lesso-Schlaggar et al., 2012), the subtle effect of certain genetic variations in those pathways may be obscured in the presence of pronounced addiction-related biological alterations. By contrast, subtle genetic effects on relapse may be more detectable in smokers with low-nicotine dependence who may lack biological alterations that could mask certain genetic influences. From a practical perspective, self-report indices of nicotine dependence, such as the FTND, are perhaps the most commonly used diagnostic tools in smoking cessation practice (USDHHS, 2008). Thus, if certain combinations of FTND score and genotype can be identified that categorize individuals at very high risk for smoking cessation failure, such combination indices could be readily applied in personalized treatment settings to classify smokers who may require the most intensive intervention.

We are aware of only one study of gene by nicotine dependence severity interactions in predicting smoking cessation (Rose et al., 2010), which utilized an aggregate genetic risk score involving 12,058 SNPs in a sample of 457 smokers. Gene by nicotine dependence two way interactions in predicting cessation outcomes were not reported, but a significant three-way interaction involving the genetic risk score, nicotine dependence, and nicotine replacement therapy (NRT) dose was found. It is important to extend these findings by examining at the individual SNP level to isolate risk carried by particular variants and genes that is moderated by concomitant nicotine dependence.

This secondary analysis study examined nicotine dependence severity as a moderator of the effects of 1198 SNPs in 53 biologically-relevant candidate gene regions on smoking cessation outcomes. Genotyping panels assayed common variants in genes that code for proteins within neurotransmitter systems involved in the brain's reward pathway and the biology of nicotine dependence (e.g., dopamine, serotonin, nicotinic acetylcholine, cannabinoid; for a complete list see supplementary Table¹). Data were pooled from two comparable pharmacogenetic trials of smoking cessation treatment (Conti et al., 2008; Lerman et al., 2004, 2002). We have previously published genetic main effects and gene by treatment interactions using these data, including effects for -141C *Ins/Del* and C957 T in the dopamine D2 receptor gene (*DRD2*; Lerman et al., 2006a), *CYP2A6* and *CYP2B6* (Lee et al., 2007; Lerman et al., 2006b; Malaiyandi et al., 2006; Patterson et al., 2008; Strasser et al., 2007; Yamanaka et al., 2005), a VNTR and SNPs in *SLC6A3* (Conti et al., 2008; Lerman et al., 2003), and a SNP (rs2072661) within *CHRNA2* (Conti et al., 2008). However, no prior paper using these data has examined nicotine dependence as a moderator of genetic effects on cessation outcomes.

2. Methods

2.1. Study sample and design

As in prior publications of these data, we pooled subjects enrolled in two randomized clinical trials conducted by the University of Pennsylvania Transdisciplinary Tobacco Use Research Center—a double-blind randomized clinical trial comparing bupropion and placebo, and an open-label randomized clinical trial comparing transdermal patch versus nasal spray

¹Supplementary Table 1 provides information about the SNPs and gene regions assayed and can be found by accessing the online version of this paper at <http://dx.doi.org> and by entering doi:.

NRT (Conti et al., 2008; Lerman et al., 2004, 2002). Analyses were limited to individuals in each trial who self-identified as white non-Hispanic race/ethnicity ($n = 412$ and 381 , respectively) to avoid differential linkage disequilibrium (LD) across ethnic groups leading to heterogeneity of effect estimates.

In both trials, exclusion criteria (pregnancy, a history of *DSM-IV* Axis-I psychiatric disorder, seizure disorder, current use of any psychotropic medication) were applied to smokers (age 18 years, 10 cigarettes per day over the prior 12 months) who received 7 group cessation counseling sessions plus medication. Participants were instructed to quit smoking on their target quit date (TQD) which occurred 1–2 weeks after pre-quit counseling. Participants in the Bupropion study initiated treatment 2 weeks prior to the TQD and continued for 10 weeks. Those in the NRT study began treatment at TQD and continued for 8 weeks.

2.2. Assessment

2.2.1. Nicotine dependence severity—The FTND (Heatherton et al., 1991), which was administered prior to TQD, is a six-item measure of smoking frequency and compulsion to smoke following tobacco abstinence, in the morning, and during situations in which smoking is constrained. The FTND exhibits good internal consistency, positive relations with key smoking variables and other indices of nicotine dependence, and high test–retest reliability (Heatherton et al., 1991; Pomerleau et al., 1994). Subjects were categorized into low or high nicotine dependence groups (≤ 4 or >4). This cutoff is used clinically to separate those with “minimal to low” nicotine dependence from those with “medium to high” nicotine dependence severity and distinguishes between subgroups of smokers on other important clinical characteristics (Heatherton et al., 1991; John et al., 2003; Patkar et al., 2003). Further, using these clinically-meaningful cutoffs (rather than the continuous FTND score) and reporting marginal effects across groups stratified by high vs. low dependence provides a quantitative estimate of the genetic effects on odds of obtaining for these two types of patients and hence enhances the clinical utility of these results. Post-hoc analyses examining FTND as a continuous variable or at different cutpoints yielded findings that did not meaningfully differ from those using the cutoff score of 4 reported here (results available upon request to the first author, AML).

2.2.2. Smoking abstinence—Smoking abstinence was classified at: (1) End of treatment (EOT) assessed 8 weeks post-TQD and (2) 6-months post-TQD. A timeline follow-back procedure was used to assess self-reported smoking, and both endpoints were biochemically verified via saliva cotinine (Bupropion study) or carbon monoxide (NRT study). Those who reported smoking for the 7 days prior to assessment, failed to provide a saliva sample, had cotinine concentrations > 15 ng/ml, or carbon monoxide levels > 10 ppm were considered non-abstinent. The abstinence definition was the same across the two trials and is consistent with prior work (Conti et al., 2008).

2.2.3. Genotyping—As reported in the original publication of genetic main effects and gene by treatment interactions on cessation outcomes in the bupropion study (Conti et al., 2008), we genotyped SNPs within 53 genes involved in the brain’s reward pathway and

other systems implicated in smoking (see Supplementary Materials Tables 1 and 2² for complete list of genes and SNPs).

Among the 1528 total SNPs, we excluded 41 SNPs with a call rate of zero and 57 SNPs with MAFs < 0.01, leaving 1198 SNPs (118 with putative functional evidence, 1080 to capture underlying LD structure) within the 53 gene regions for association analysis and 232 ancestry informative markers (AIMs). Analysis of these AIMs using STRUCTURE (Pritchard et al., 2000) showed negligible admixture in the current sample. SNPs were genotyped using the Illumina GoldenGate™ assay (Illumina, San Diego, CA, USA). A complete description of the quality control procedures has been previously published (Conti et al., 2008).

2.3. SNP analyses

We used logistic regression to obtain odds ratios for marginal SNP effects within strata of low and high nicotine dependence, and SNP × dependence interaction effects in separate models predicting abstinent (vs. nonabstinent) for EOT and 6-month follow-up. For each SNP we tested either an additive or dominant genetic model consistent with previously reported analyses (Conti et al., 2008). The most common genotype served as the referent. All models were adjusted for gender, age, and treatment. Analyses were performed using the R Statistical Program (R Development Core Team, 2008). We did not examine SNP × FTND × treatment three-way interactions because we lacked sufficient sample size. Haplotypes were estimated using the expectation-maximization (EM) algorithm and expected additive scores using estimated probabilities were used in regression models to estimate risk corresponding to the additional risk from carrying a particular haplotype (Excoffier and Slatkin, 1995; Schaid et al., 2002). The most common haplotype is used as the reference haplotype and all haplotypes with a frequency less than 5% are combined into a single composite haplotype.

P-values for SNP analyses were adjusted to account for the correlation and the number of tests performed across the SNPs within a gene region. Test statistics were modeled as an asymptotically distributed multivariate normal with a covariance structure estimated from the correlation of observed SNPs (Conneely and Boehnke, 2007; Conti et al., 2008). *P*-values are reported for each SNP adjusted for the number of correlated SNPs within each gene region. Adjustments were SNP-specific, such that the *P*-value for each SNP was adjusted based on the level of correlation with all other SNPs in that gene region (lower correlations of a SNP with other SNPs suggest greater independence of the test of that SNP and hence more stringent adjustment). Since these adjusted *P*-values account for the number of effective tests in each gene region, significance within a gene region (i.e., region-wide significance) was determined with an α -level of 0.05. System-wide significance was determined using an additional Bonferroni correction across the 53 gene regions and the two outcome timepoints; $\alpha = .05/(53 \times 2) = 5 \times 10^{-4}$. Hence, adjusted *P*-values < 5×10^{-4} were considered system-wide-significant.

²Supplementary Tables 2a and 2b provide detailed results for each SNP at EOT and 6-month follow up respectively and can be found by accessing the online version of this paper at <http://dx.doi.org> and by entering doi:.

Correlation between SNPs identified in this analysis and the prior literature were performed using the SNP Annotation and Proxy Search and the 1000 Genomes Pilot CEU sample and are described in the Discussion (Johnson et al., 2008).

3. Results

As illustrated in Table 1, subjects categorized as FTND-Low were significantly younger than the FTND-High group. At EOT, 36% of FTND-Low subjects were abstinent compared with 27% of FTND high subjects (OR = 0.6, 95% CI 0.5–0.9). Otherwise, there were no significant differences between FTND categories (Table 1).

Results are presented only for SNPs with adjusted interaction and dependence-stratified marginal *P*-values < 0.05 in Table 2 (analyses for all SNPs are reported in supplementary material Table 2³).

3.1. Dopamine beta hydroxylase (DBH)

3.1.1. rs1541333—At EOT, an interaction achieving system-wide significance was observed for one SNP in *DBH*, rs1541333 (Adjusted *P* = 4×10^{-4}). The SNPs in this region were in moderate LD (Fig. 1). The rs1541333 minor allele is associated with a region-wide significant decreased odds of abstinence in those with low nicotine dependence (OR = 0.4, 95% CI 0.2–0.6, Adjusted *P* = 0.006) and an increase in odds of abstinence in those with high nicotine dependence with an adjusted *P*-value that was not region-wide significant after correcting for correlated tests within the *DBH* locus (OR = 1.6, 95% CI 1.0–2.5, Adjusted *P* = 0.36) but approached uncorrected traditional levels of significance (Unadjusted *P* = .05). As illustrated in Fig. 2, within the FTND-Low group, those with two of the rs1541333 major alleles had a 50% probability of abstinence at EOT, compared to 30% of those carrying the rs1541333 minor allele. Within the FTND-High group, those two major alleles for rs1541333 had a 20% probability of abstinence at EOT, compared to 29% for rs1541333 carriers of the minor allele.

The interaction at 6-month follow-up for rs1541333 achieves region-wide, but not system-wide, significance (Adjusted *P* = 0.017). The direction of interaction effects is similar to those for EOT for rs1541333. There was a decrease in odds of abstinence at the 6-month follow-up associated with the rs1541333 minor allele for those with low nicotine dependence with an adjusted *P*-value that was not region-wide significant after correcting for correlated tests within the *DBH* locus (OR = 0.5, 95% CI 0.3–1.0, Adjusted *P* = 0.5) but approached uncorrected traditional levels of significance (Unadjusted *P* = .05). In those with high nicotine dependence, there was a region-wide significant increase in odds of abstinence associated with the minor allele (OR = 2.2, 95% CI 1.3–3.8, Adjusted *P* = 0.045).

3.1.2. Other DBH SNPs—Associations for additional SNPs in *DBH* do not reach system-wide significance, but at EOT several (rs1076153, rs2797855, rs1541332, rs1108580, rs1076150) have interactions achieving region-wide significance ($0.02 < \text{Adjusted } P \leq 0.04$). rs2797855 is not associated with smoking abstinence at EOT within the FTND-low group

³Supplementary material can be found by accessing the online version of this paper at <http://dx.doi.org> and by entering doi:

(OR = 1.4, 95% CI 0.8–2.4, Adjusted P -value = 0.96) but is associated with a significant decrease in odds of abstinence within the FTND-high group (OR = 0.5, 95% CI 0.3–0.7, Adjusted P = 0.007). The region-wide significant interactions for the other 4 SNPs are caused by non-significant decreases in odds of smoking abstinence within the FTND-low group (ORs = 0.6–0.7, Adjusted P s > 0.05) and non-significant increases in odds within the FTND-high group (ORs = 1.5–1.8, Adjusted P s > 0.05).

At 6-month follow-up, the rs1076153 $\times$ FTND interaction achieves region-wide significance (Adjusted P = 0.046), with non-significant opposing effects within FTND-Low (OR = 0.6, 95% CI 0.3–1.0, Adjusted P = 0.59) and FTND-High (OR = 1.8, 95% CI 1.2–2.9, Adjusted P = 0.12). At 6-month follow up, rs1611123 (OR = 0.6, 95% CI 0.4–0.8, Adjusted P = 0.015), rs279855 (OR = 0.5, 95% CI 0.3–0.8, Adjusted P = 0.036), and rs1108580 (OR = 1.7, 95% CI 1.2–2.4, Adjusted P = 0.03) had marginal effects that achieved region-wide significance within the FTND-High group. Within the FTND-Low group, these 3 SNPs have null effects (ORs = 0.93–1.25, Adjusted P s = 1.0).

3.1.3. DBH haplotypes—Outside of rs1076153, which is in weak LD with all other region-wide significant SNPs in *DBH* ($0.19 < r^2 < 0.30$), LD among region-wide significant SNPs in *DBH* is moderate to strong ($0.40 < r^2 < 0.85$). Therefore, we estimated haplotypes for the 6 SNPs in *DBH* in moderate to strong LD (rs1076150, rs1108580, rs1611123, rs2797855, rs1541333, rs1541332; see Table 3). The most common and referent haplotype (Haplotype 1, 40% frequency) carries the minor allele for only rs1611123 and rs2797855. Haplotype 2 (37% frequency) carries the minor allele for rs1076150, rs1108580, rs1541333 and rs1541332. The *DBH* haplotype $\times$ FTND interaction is significant at EOT (P = 0.002) but is not significant at the 6-month follow up (P = 0.13). Compared to referent haplotype 1, haplotype 2 is not significantly associated with abstinence within the FTND-Low group at EOT (OR = 0.7, 95% CI 0.5–1.0, P = 0.07) or 6-month follow up (OR = 0.9, 95% CI 0.6–1.4, P = 0.63). However, haplotype 1 is associated with significantly increased odds of abstinence within the FTND-high group (EOT: OR = 1.7, 95% CI 1.2–2.3, P = 0.001; 6-month follow up: OR = 1.6, 95% CI 1.1–2.3, P = 0.008). In general, because of the high LD within this block, the haplotype analyses recapitulate the single SNP analyses.

3.2. Other associations

3.2.1. Chromosome 15 nicotinic acetylcholine receptor (nAChR) region—There is strong *a priori* evidence for the role of genes making up the chr15q25.1 nicotinic acetylcholine receptor (nAChR) region (*CHRNA5-CHRNA3-CHRNA4*) in nicotine dependence (Amos et al., 2010; Falcone et al., 2011; Wassenaar et al., 2011), therefore, we investigated their effects in a *post hoc* analysis. At EOT, 7 SNPs (rs684513, rs667282, rs637137, rs3743078, rs938682, rs4887069, rs6495309) in the cluster of genes comprising the chr15q25.1 nAChR region had associations within the FTND-High group that achieved region-wide, but not system-wide, significance (ORs = 1.7–2.2, 0.003–0.03 Adjusted P s < 0.03). Associations for these SNPs within the FTND-Low group were null (ORs = 0.84–0.9, Adjusted P s = 1.0). For each of these SNPs, the SNP $\times$ FTND interactions did not reach region-wide significance (0.08–0.17 Adjusted P s). Associations for these SNPs at 6-month follow-up were null.

Each of the abovementioned 7 SNPs in the chr15q25.1 nAChR region are all in moderate to strong LD ($r^2 = 0.7$) and were examined in haplotype analyses (see Fig. 3 and Table 4). The referent haplotype (Haplotype 1) carries the major allele for all 7 SNPs, the “protective” haplotype (Haplotype 2) carries the minor allele for all 7 SNPs (haplotype frequencies = 0.8 and 0.15, respectively). The haplotype $\times$ FTND interaction is significant at EOT ($P = 0.03$). Haplotype 2 is associated with increased odds of abstinence within the FTND-High group (OR = 2.0, 95% CI 1.4–3.0, $P = 0.0004$) and null odds within the FTND-low group (OR = 0.9, 95% CI 0.5–1.5, $P = 0.6$).

3.2.2. Other findings—Also at 6-month follow-up, six SNPs in guanine nucleotide binding protein (G protein), alpha z polypeptide (*GNAZ*; rs4820537, rs5759593, rs9612234, rs9612237, rs885964, rs13054985) had SNP-nicotine dependence interactions that achieved region-wide significance (0.003 Adjusted P s = 0.04; Table 2).

4. Discussion

In this secondary analysis study of 53 candidate gene regions implicated in the brain’s reward system and smoking, the main finding was that nicotine dependence severity moderated the effects of *DBH* variants on cessation outcomes. The SNP $\times$ FTND interaction achieved system-wide significance for the *DBH* SNP rs1541333 in the prediction of cessation outcomes at EOT and region (but not system) wide significance in the prediction of 6-month outcomes. There was also suggestive evidence that other SNPs in this gene region differentially predicted cessation outcome as a function of nicotine dependence. The patterns of LD across the notable SNPs in this gene region suggest multiple signals within *DBH* whose effects on cessation outcome are modulated by nicotine dependence. When compared to a referent haplotype comprising 40% of the sample, a haplotype (37% frequency) composed of the minor alleles from rs1541333 and other SNPs in *DBH* was associated with significantly increased odds of abstinence at EOT and the 6-month follow up in the subgroup with high nicotine dependence and non-significant decreases in odds of abstinence in those with low nicotine dependence.

DBH is the locus encoding dopamine beta-hydroxylase, the enzyme catalyzing the synthesis of the neurotransmitter norepinephrine from dopamine, which influences the ratio of extracellular dopamine to norepinephrine (Cubells and Zabetian, 2004). Given that both norepinephrine and dopamine are key neurotransmitters involved in mood, cognition, arousal, and behavior (Meyer and Quenzer, 2004), it is not surprising that *DBH* variants have been previously reported to be associated with a variety of behavioral conditions, including psychosis, depression, anxiety, drug and alcohol dependence, and smoking, although these associations are not always consistent (Cubells and Zabetian, 2004). One *DBH* SNP (rs3025343) is associated with former smoking status at genome-wide significance level in two studies (Tobacco and Genetics Consortium, 2010; Siedlinski et al., 2011). rs3025343 is found 23 kbp 5′ of *DBH*, and ~20–33 kbp 5′ of the six *DBH* SNPs that make up the six-SNP haplotype identified in this study. In the 1000 Genomes Pilot CEU sample, rs3025343 is not in significant LD with the six SNPs identified here (r^2 s 0.004–0.033). Thus the six *DBH* SNPs identified here, if replicated, may represent a novel *DBH* locus associated with prospective smoking cessation. However, fine-mapping and functional

studies may be warranted to further investigate this region and elucidate the underlying functional variants to identify if this is an entirely distinct locus. We also did not find any signals for a *DBH* variant (rs1611115) that has been shown to account for 44% of the variance in plasma dopamine beta-hydroxylase activity (Zabetian et al., 2001). In the 1000 Genomes Pilot CEU sample, rs1611115 is in very modest LD with the six SNP haplotype identified in this study (r^2 values from 0.013 to 0.223). While these previously identified variants did not generate signals in this study, some of the notable *DBH* SNPs identified in this analysis have been linked with relevant phenotypes in prior work, including: (1) associations of rs1108580 to schizophrenia (Srivastava et al., 2010), working memory performance (Greenwood et al., 2009; Parasuraman et al., 2005), and plasma dopamine beta-hydroxylase activity (Bhaduri et al., 2010); and (2) associations of rs1108580 and rs1541333 to response variability while completing cognitive performance tasks (Ashley-Koch et al., 2006).

Previous data links *DBH* polymorphisms to attention/deficit hyperactivity disorder and its related phenotypes, such as poor impulse control (Faraone et al., 2005; Kebir et al., 2009), as well as altered dopaminergic and noradrenergic tone (Cubells and Zabetian, 2004). We speculate that individuals with *DBH* variants associated with poorer impulse control may find it particularly difficult to resist the temptation to smoke following a cessation attempt, especially when coupled with the compulsive drive to smoke that characterizes severe nicotine dependence. From a molecular level, adaptations in dopaminergic and noradrenergic systems occur during nicotine dependence (Bruijnzeel, 2012; D'Souza and Markou, 2011). Perhaps when dependence-related neuroadaptations are compounded by inherited alterations in dopaminergic and noradrenergic neurotransmission, there is a synergistic effect that disproportionately increases risk of smoking relapse.

The chr15q25.1 nAChR region (*CHRNA5-CHRNA3-CHRNA4*) has consistently been linked to smoking phenotypes (Amos et al., 2010; Chen et al., 2012; Falcone et al., 2011; Hartz et al., 2012; Thorgeirsson et al., 2010; Wassenaar et al., 2011). *Post hoc* analyses in this study extend past data by showing that the influence of variation in this nAChR gene region on a clinically-important phenotype (i.e., cessation outcome) may be most apparent when coupled with severe nicotine dependence. These findings should be interpreted with caution because individual SNP $\times$ FTND interactions did not reach region -or system-wide significance. In comparison to prior studies that have tagged the architecture of chr15q25.1, the nAChR SNPs identified in this study are in modest to strong LD with the SNP identified by King et al. (2012), but in weak LD with SNPs identified by Sarginson et al. (2011). Furthermore, the current chr15q25.1 nAChR SNPs are in weak LD with candidate variants genotyped in other cessation studies (Bergen et al., 2013a; Chen et al., 2012; Munafo et al., 2011). Hence, the present results add some novel information about the role of variation in the chr15q25.1 nAChR region in smoking cessation outcome.

Region-wide significant effects were found for SNP $\times$ FTND interactions for variants in *GNAZ* in predicting cessation at 6-month follow up. *GNAZ* is locus encoding the G protein, alpha z polypeptide, which a member of a G protein subfamily that mediates signal transduction of dopamine receptors and other pathways (Cardenas-Navia et al., 2010; Wilkie et al., 1992). Given the lack of *a priori* evidence implicating this gene in smoking and that

the SNP $\times$ FTND interactions did not surpass system-wide significance thresholds, this finding should be interpreted with caution.

Limitations of this study have been described previously (Conti et al., 2008; Lerman et al., 2006a). Our significance correction for multiple correlated tests is less conservative than a standard Bonferroni adjustment. Also, we lack a sufficient sample to detect three-way interactions between genes, nicotine dependence, and treatment. Furthermore, while the systems-based genotyping approach was comprehensive for a candidate gene study, interpretation is restricted to those genes selected. Finally, there are several previous publications using this dataset, which may compound chances of type-I error, although none have explored moderation by nicotine dependence.

Limitations notwithstanding, this study provides new evidence that nicotine dependence moderates genetic vulnerability to smoking relapse. If these results are replicated and extended, variants in *DBH* (and eventually other genes) could ultimately be incorporated into prognosticative genomic screening panels to identify smokers who may be at particularly high risk of relapse when coupled with high scores on the FTND. More broadly, our results suggest that taking into account heterogeneity in nicotine dependence may optimize capability to detect genetic effects on smoking cessation outcome that may be expressed in only certain subpopulations of smokers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. *DBH* gene linkage disequilibrium plot. *Note.* The figure displays the pairwise r^2 for each corresponding SNP $r^2 = 1$ is black; $0 < r^2 < 1$ are shades of gray, with darker shades reflecting higher r^2 values; and $r^2 = 0$ is white.

Fig. 2. rs1541333, Nicotine dependence-stratified abstinence probabilities at EOT. *Note.* FTND = Fagerström Test for Nicotine Dependence score. EOT = End of treatment. FTND Low: score 4. FTND High: score 5+. *Difference between those carrying the minor allele and those with two major alleles was significant (Wald P -value < 0.05).

Fig. 3.

Chromosome 15 nAChR linkage disequilibrium plot. *Note.* The figure displays the pairwise r^2 for each corresponding SNP $r^2 = 1$ is black; $0 < r^2 < 1$ are shades of gray, with darker shades reflecting higher r^2 values; and $r^2 = 0$ is white.

Table 1

Study characteristics.

	FTND low <i>N</i> = 267	FTND high <i>N</i> = 526
Sex, n(%)		
Male	119 (44.6%)	271 (51.5%)
Female	148 (55.4%)	255 (48.5%)
Age ^a		
Mean (SD)	43 (11.6)	46.8 (11.3)
Treatment, n(%)		
Placebo	71 (26.6%)	124 (23.6%)
Bupropion	85 (31.8%)	132 (25.1%)
Patch	58 (21.7%)	132 (25.1%)
Spray	53 (19.9%)	138 (26.2%)
FTND Score Distribution, n(%)		
0	8 (3%)	5 135 (25.7%)
1	35 (13.1%)	6 131 (24.9%)
2	46 (17.2%)	7 127 (24.1%)
3	69 (25.8%)	8 82 (15.6%)
4	109 (40.8%)	9 40 (7.6%)
		10 11 (2.1%)
Total Score Mean (SD)	2.9 (1.2)	6.6 (1.3)
Smoking Abstinence, n(%)		
End of Treatment ^a		
Relapsed	172 (64.4%)	385 (73.2%)
Abstinent	95 (35.6%)	141 (26.8%)
6-month Follow-Up		
Relapsed	202 (75.7%)	426 (81%)
Abstinent	65 (24.3%)	100 (19%)

^aSignificant difference between FTND low and high categories (LRT *P*-value < 0.05).

Table 2

FTND-stratified SNP associations at EOT and 6-month follow-up.

SNP	Chromosome	Gene	Minor Allele	MAF	FTND low (< 4)			FTND high (>4)			Interaction	
					OR (95% CI)	LRT p	Adjusted p	OR (95% CI)	LRT p	Adjusted p	LRT p	Adjusted p
End of treatment												
rs1541333	9	DBH	C	0.45	0.36 (0.20-0.62)	2.51E-04	0.006	1.62 (1.04-2.53)	0.031	0.359	1.68 E-05	3.95 E-04
rs1076153	9	DBH	T	0.21	0.60 (0.38-0.97)	0.031	0.401	1.49 (1.06-2.09)	0.023	0.3	0.001	0.027
rs2797855	9	DBH	C	0.43	1.36 (0.78-2.37)	0.272	0.963	0.47 (0.32-0.71)	3.00 E-04	0.007	0.002	0.031
rs1541332	9	DBH	A	0.45	0.58 (0.33-1.01)	0.055	0.566	1.78 (1.12-2.83)	0.012	0.19	0.002	0.038
rs1108580	9	DBH	A	0.46	0.74 (0.50-1.09)	0.128	0.827	1.56 (1.16-2.09)	0.003	0.06	0.002	0.039
rs1076150	9	DBH	T	0.48	0.72 (0.49-1.05)	0.085	0.704	1.46 (1.10-1.93)	0.008	0.129	0.002	0.04
rs3810417	19	KLF16	T	0.17	1.86 (1.16-2.99)	0.01	0.043	0.84 (0.57-1.24)	0.382	0.86	0.015	0.065
rs5871	14	CALM1	C	0.07	1.28 (0.64-2.54)	0.487	0.725	1.97 (1.20-3.24)	0.008	0.045	0.282	0.698
rs4522666	20	CHRNA4	G	0.36	0.89 (0.53-1.52)	0.679	0.99	1.80 (1.19-2.72)	0.005	0.04	0.057	0.315
rs4887069	15	CHRNA3;CHRNA4	G	0.19	0.84 (0.52-1.35)	0.47	0.991	1.67 (1.18-2.36)	0.004	0.038	0.016	0.125
rs3743078	15	CHRNA3;CHRNA5	C	0.19	0.88 (0.51-1.51)	0.638	0.999	1.85 (1.24-2.78)	0.003	0.027	0.025	0.169
rs938682	15	CHRNA3	G	0.18	0.87 (0.50-1.50)	0.611	0.999	1.92 (1.28-2.88)	0.002	0.017	0.018	0.13
rs6495309	15	CHRNA3;CHRNA4	T	0.17	0.89 (0.51-1.55)	0.683	0.999	1.97 (1.31-2.96)	0.001	0.012	0.02	0.143
rs2797855	9	DBH	C	0.43	1.36 (0.78-2.37)	0.272	0.963	0.47 (0.32-0.71)	3.00 E-04	0.007	0.002	0.031
rs667282	15	CHRNA5	C	0.18	0.88 (0.51-1.52)	0.643	0.999	2.07 (1.38-3.10)	4.65 E-04	0.005	0.011	0.086
rs637137	15	CHRNA5	A	0.18	0.88 (0.51-1.52)	0.643	0.999	2.10 (1.40-3.16)	3.49 E-04	0.004	0.009	0.078
rs684513	15	CHRNA5	G	0.16	0.91 (0.52-1.60)	0.733	0.999	2.20 (1.45-3.34)	2.24 E-04	0.003	0.01	0.081
6 month Follow-up												
rs4820537	22	GNAZ	A	0.49	1.63 (1.10-2.42)	0.014	0.149	0.63 (0.46-0.86)	0.003	0.036	2.02 E-04	0.003
rs5759593	22	GNAZ	A	0.39	0.64 (0.43-0.96)	0.029	0.25	1.60 (1.18-2.17)	0.003	0.032	4.50 E-04	0.005
rs9612234	22	GNAZ	C	0.44	0.67 (0.45-0.98)	0.038	0.289	1.58 (1.16-2.16)	0.004	0.041	6.75 E-04	0.008
rs9612237	22	GNAZ	T	0.43	0.67 (0.45-0.99)	0.041	0.303	1.56 (1.15-2.13)	0.004	0.047	8.29 E-04	0.009
rs12280580	11	DRD4	G	0.29	0.58 (0.33-1.03)	0.063	0.416	2.19 (1.38-3.48)	7.29 E-04	0.007	9.63 E-04	0.009
rs885964	22	GNAZ	G	0.44	0.54 (0.30-0.98)	0.043	0.286	1.87 (1.13-3.12)	0.012	0.115	0.002	0.016
rs1541333	9	DBH	C	0.45	0.54 (0.29-0.97)	0.042	0.498	2.22 (1.29-3.83)	0.002	0.045	8.48 E-04	0.017
rs4350602	17	GRB2	C	0.3	1.84 (1.02-3.29)	0.039	0.21	0.62 (0.40-0.97)	0.036	0.187	0.003	0.019

SNP	Chromosome	Gene	Minor Allele	MAF	FTND low (< 4)			FTND high (>4)			Interaction	
					OR (95% CI)	LRT <i>p</i>	Adjusted <i>p</i>	OR (95% CI)	LRT <i>p</i>	Adjusted <i>p</i>	LRT <i>p</i>	Adjusted <i>p</i>
rs3952	22	PRKCABP	G	0.33	1.50 (0.84–2.67)	0.17	0.484	0.52 (0.33–0.81)	0.003	0.028	0.003	0.027
rs737662	22	PRKCABP	G	0.32	1.52 (0.85–2.72)	0.155	0.485	0.53 (0.34–0.83)	0.005	0.037	0.004	0.028
rs4789176	17	GRB2	A	0.18	1.48 (0.83–2.64)	0.185	0.576	0.50 (0.30–0.85)	0.007	0.049	0.005	0.03
rs1369299	17	GRB2	T	0.18	1.48 (0.83–2.64)	0.185	0.576	0.51 (0.30–0.86)	0.008	0.054	0.005	0.032
rs13054985	22	GNAZ	G	0.44	0.58 (0.32–1.04)	0.07	0.416	1.76 (1.06–2.93)	0.025	0.211	0.005	0.044
rs1076153	9	DBH	T	0.21	0.56 (0.30–1.04)	0.063	0.585	1.84 (1.18–2.88)	0.007	0.12	0.003	0.046
rs4789170	17	GRB2	T	0.26	1.59 (0.90–2.81)	0.11	0.463	0.60 (0.38–0.95)	0.027	0.149	0.008	0.047
rs1877032	17	PPP1R1B	C	0.09	0.22 (0.06–0.75)	0.004	0.021	0.57 (0.29–1.13)	0.09	0.285	0.187	0.544
rs2072660	1	CHRNA2	T	0.23	0.41 (0.22–0.76)	0.003	0.027	0.66 (0.41–1.05)	0.076	0.405	0.257	0.86
rs2072661	1	CHRNA2	A	0.24	0.41 (0.22–0.76)	0.003	0.028	0.67 (0.42–1.07)	0.092	0.455	0.232	0.829
rs2271537	4	TDO2	A	0.49	1.99 (1.23–3.24)	0.004	0.034	1.05 (0.75–1.47)	0.766	0.766	0.039	0.231
rs11665625	18	ADCYAP1	A	0.46	1.87 (1.22–2.85)	0.003	0.043	1.12 (0.82–1.54)	0.466	0.994	0.054	0.446
rs1611123	9	DBH	T	0.49	1.14 (0.76–1.72)	0.526	0.999	0.58 (0.42–0.80)	6.67 E–04	0.015	0.015	0.207
rs1108580	9	DBH	A	0.46	0.93 (0.61–1.44)	0.75	1	1.70 (1.22–2.38)	0.002	0.032	0.036	0.39
rs2797855	9	DBH	C	0.43	1.25 (0.68–2.31)	0.472	0.999	0.49 (0.31–0.76)	0.002	0.036	0.017	0.227
rs2238687	19	FOSB	C	0.14	1.65 (0.93–2.92)	0.092	0.399	1.77 (1.18–2.66)	0.007	0.041	0.876	0.876

Note. Only SNPs with marginal effects or SNP × FTND interactions with adjusted *P*-value of .05 are reported. FTND = Fagerström Test for Nicotine Dependence score. EOT = End of treatment. Adjusted *P*-values reflect significance within a gene region (i.e., region-wide significance) at an α -level of 0.05. For system-wide significance, α of adjusted *p*-value $< 5 \times 10^{-4}$.

Table 3

FTND-stratified *DBH* haplotype associations at EOT and 6-month follow-up.

Haplotypes ^{a,b}	Frequency	FTND Low (4)		FTND High (>4)		Interaction	
		OR (95% CI)	Wald <i>p</i>	OR (95% CI)	Wald <i>p</i>	LRT <i>p</i>	
End of Treatment							
(Haplotype 1) CGTCGG	0.40	Ref.	–	Ref.	–		
(Haplotype 2) TACGCA	0.37	0.68 (0.45–1.03)	0.07	1.68 (1.22–2.31)	0.001		0.002
All Other Haplotypes ^c	0.22	0.79 (0.49–1.28)	0.34	1.50 (1.03–2.19)	0.037		
6 month Follow-up							
(Haplotype 1) CGTCGG	0.40	Ref.	–	Ref.	–		
(Haplotype 2) TACGCA	0.37	0.89 (0.56–1.41)	0.63	1.62 (1.114–2.32)	0.008		0.13
All Other Haplotypes ^c	0.22	1.00 (0.58–1.69)	0.98	1.45 (0.94–2.22)	0.09		

Note. FTND = Fagerström Test for Nicotine Dependence score. EOT = End of treatment. *DBH* = Dopamine beta hydroxylase.

^a Haplotypes consist of (in order): rs1076150-rs1108580-rs1611123-rs2797855-rs1541333-rs1541332.

^b Minor Alleles are in bold.

^c Consists of 21 unique haplotypes each with a frequency < 0.05.

Table 4

FTND-stratified Chromosome 15 nAChR haplotype associations at EOT.

Haplotypes ^{a,b}	Frequency	FTND Low (< 4)		FTND High (>4)		Interaction	
		OR (95% CI)	Wald p	OR (95% CI)	Wald p	LRT p	
End or Treatment							
(Haplotype 1) CTTGAAC	0.80	Ref.	–	Ref.	–		
(Haplotype 2) GCACGGT	0.15	0.87 (0.52–1.47)	0.61	2.02 (1.37–2.96)	0.0004	0.03	
All Other Haplotypes ^c	0.06	0.95 (0.45–2.04)	0.9	0.85 (0.43–1.65)	0.63		

Note. FTND = Fagerström Test for Nicotine Dependence score. EOT = End of treatment. nAChr = nicotinic-acetylcholine receptor.

^a Haplotypes consist of (in order): rs684513-rs667282-rs637137-rs3743078-rs938682-rs4887069-rs6495309.

^b Minor Alleles are in bold.

^c Consists of 16 unique haplotypes each with a frequency < 0.05.