Original investigation

***CHRNA4* and *ANKK1* Polymorphisms Influence Smoking-Induced Nicotinic Acetylcholine Receptor Upregulation**

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Abstract

Introduction: Tobacco smoking leads to increased numbers of β_2^* -containing nicotinic acetylcholine receptors (β_2^* -nAChRs) throughout the brain, which return to nonsmoker levels over extended abstinence. The goal of the current study was to determine whether the degree of tobacco smoking-induced changes in β_2^* -nAChR availability is genetically influenced.

Methods: In this study, 113 European Americans participated in one or two [¹²³I]5-IA-85380 single photon emission computed tomography (SPECT) brain scans. Smokers ($n = 58$) participated in one scan at 7–9 days of abstinence and those who remained abstinent ($n = 27$) were imaged again at 6–8 weeks of abstinence. Age- and sex-matched nonsmokers ($n = 55$) participated in one scan. Blood samples were collected for DNA analysis and genotyped for single nucleotide polymorphisms (SNPs) in the *CHRNA4* and *ANKK1* gene loci. β_2^* -nAChR availability was measured in the thalamus, striatum, cortical regions, and cerebellum.

Results: The *CHRNA4* SNP rs2236196 and *ANKK1* SNP rs4938015 were associated with significantly higher cerebellar and cortical β_2^* -nAChR availability in smokers versus nonsmokers for specific genotypes. There were no significant differences by carrier status in the change in β_2^* -nAChR availability in smokers from 7–9 days to 6–8 weeks of abstinence.

Conclusion: This study provides evidence for genetic regulation of tobacco smoking-induced changes in β_2^* -nAChR availability and suggests that β_2^* -nAChR availability could be an endophenotype mediating influences of *CHRNA4* variants on nicotine dependence. These results highlight individual differences in the neurochemistry of nicotine dependence and may suggest the need for individualized programs for smoking cessation.

Implications: This study demonstrates genetic regulation of smoking-induced changes in β_2^* -nAChRs throughout the brain and highlights the need for personalized programs for smoking cessation.

Introduction

Tobacco smoking remains a leading cause of death. It is estimated that 80% of smokers will die of a smoking-related disease, yet approximately 20% of the adult American population continues to smoke. The majority of smokers express a desire to quit smoking and make yearly cessation attempts; however, less than 6% achieve sustained abstinence in a given year. Nicotine, the primary addictive chemical in cigarettes, enters the brain and activates nicotinic acetylcholine receptors (nAChRs; the most prevalent are those containing the β_2 subunits— β_2^* -nAChRs), which are responsible for the reinforcing effects of nicotine.¹

Preclinical,^{2–5} postmortem human^{6–9} and clinical imaging studies^{10–12} have consistently demonstrated that nicotine and tobacco smoke exposure robustly upregulate, that is, increase the numbers of, β_2^* -nAChRs in the brain. A postmortem study⁷ and a study in our laboratory¹² demonstrated that higher β_2^* -nAChR availability in smokers is persistent, and does not decrease to nonsmoker control levels until 6–12 weeks of abstinence. Additionally, higher β_2^* -nAChR availability in abstinent smokers was associated with increased craving,^{10,12} and more recently, with a poorer response to smoking cessation treatment.¹³ These phenomena—higher β_2^* -nAChR availability and normalization over extended abstinence, and the relationship between receptor availability and behavioral variables—are not uniform across smokers. There is evident inter-individual variability in β_2^* -nAChR availability^{12,13} and some of the variability may be explained genetically. Some genes encoding nAChRs are significantly associated with smoking behaviors and nicotine dependence,^{14,15} but the functional significance of these associations is not clear. Specifically, it is not known whether these genes are associated with changes in β_2^* -nAChR availability in tobacco smokers, such as upregulation as a result of smoking and normalization following abstinence.

Two genes in particular that encode, respectively, a nicotinic receptor subunit and a protein involved in regulation of nAChRs, *CHRNA4* and *ANKK1*, have been associated with nicotine dependence. The *CHRNA4* gene encodes the α_4 subunit of the nAChR, which generally assembles with the β_2 subunit and is a key component of the receptor composition that is upregulated in response to nicotine ($\alpha_4\beta_2^*$ nAChRs). Single nucleotide polymorphisms (SNPs) at *CHRNA4* have been associated to number of cigarettes smoked, severity of nicotine dependence,¹⁶ nicotine dependence,^{16–18} binding of the nonselective nicotinic agonist epibatidine in postmortem tissue,¹⁹ function, trafficking and assembly of nAChRs,²⁰ sensitivity to nicotine in living humans, and tobacco smoking cessation outcomes.¹⁹ These studies suggest that *CHRNA4* genotypes influence the etiology of nicotine dependence and that there may be subgroups of smokers who differ in the effects of chronic nicotine exposure on β_2^* -nAChRs. This is critical since individuals sensitive to the regulatory effects of nicotine on β_2^* -nAChR availability may be more likely to respond to nicotine replacement therapies versus those who are not as sensitive to the regulatory effects of nicotine.

A polymorphic variant now known to map to ankyrin repeat and kinase domain (*ANKK1*), rs1800497, formally known as the *DRD2* *Taq1A* site,²¹ encodes a tyrosine kinase. Tyrosine phosphorylation is a significant post-translational modification that regulates numerous ligand gated ion channels including nAChRs. This variant causes an amino acid substitution in the *ANKK1* protein, which may affect substrate binding specificity.²¹ *ANKK1* is associated with nicotine dependence.²² While the phenotype of nicotine dependence is broad, *ANKK1* has been associated specifically with smoking reward²³ and

smoking initiation and cessation.²⁴ The *ANKK1* protein product may play a role in determining the phosphorylation state of nAChR subunits, which may modulate desensitization and recovery of the nAChR in the presence and absence of nicotine.^{25,26} It is also possible that this variant has a regulatory effect on *DRD2*, which is adjacent.

Thus, the primary goal of the present study was to determine whether SNPs at *CHRNA4* and *ANKK1* genes were associated with differences in β_2^* -nAChR availability in European American smokers versus nonsmokers, measured with the radiotracer [¹²³I]5-IA-85380 ([¹²³I]5-IA) and single photon emission computed tomography (SPECT) brain imaging. Additionally, we examined whether these SNPs were associated with changes in β_2^* -nAChR availability in tobacco smokers who were able to maintain abstinence successfully for 6–8 weeks.

Materials and Methods

Participants and Screening Methods

This study was approved by the Yale University School of Medicine Human Investigation Committee, the West Haven Veterans Administration Human Subjects Subcommittee, and the Radiation Safety Committee. Participants provided written informed consent and were recruited by word of mouth, posters, and television and newspaper advertisements. Eligibility was determined as follows: a medical examination including a physical examination, electrocardiogram, serum chemistries, thyroid function studies, complete blood count, urinalysis, and urine toxicology screening was completed. Participants had no history of significant medical illness or major head trauma. The Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (SCID) was administered to rule out Axis I Disorders including Substance and Alcohol Dependence but not Nicotine Dependence in tobacco smokers.

In total, 113 European American individuals participated in both imaging and genetic components of the study. A subset of subjects in this sample ($n = 23$ smokers, $n = 26$ nonsmokers) has been included in our previous study.¹² Fifty-five individuals were nonsmokers (33.4 ± 11.7 years; 23 men, 32 women) and 58 were smokers (35.4 ± 10.8 years; 28 men, 27 women). All subjects participated in up to two [¹²³I]5-IA SPECT scans and one magnetic resonance imaging (MRI) study. Nonsmokers were imaged once with [¹²³I]5-IA SPECT. Tobacco smokers were imaged at 7–9 days of abstinence, based on previous studies demonstrating that 7–9 days of nicotine abstinence are necessary for nicotine to clear from the brain to prevent competition of nicotine with the radiotracer at the binding site.^{10,12} Tobacco smokers who were able to remain abstinent for 6–8 weeks, which is the time required for β_2^* -nAChR availability to normalize (ie, return to nonsmoker levels)¹² were imaged a second time with [¹²³I]5-IA SPECT ($n = 27$).

Tobacco smokers were required to have smoked at least 10 cigarettes daily for at least 1 year, confirmed by plasma cotinine levels >150 ng/mL, urine cotinine levels >100 ng/mL and carbon monoxide levels >11 at intake. Smokers were helped to quit smoking using Clinical Practice Guidelines and contingency management^{27–29} as described previously.¹² Subjects were not permitted to use any nicotine replacement treatments or medication throughout the study. Nonsmoker status (defined as <100 cigarettes in lifetime, and none in the previous 2 years) was confirmed by plasma cotinine levels <15 ng/mL, urine cotinine levels <100 ng/mL and carbon monoxide levels <8 ppm on intake and scan day. On scan days, abstinent smokers were required to have plasma cotinine levels <15 ng/mL, urine

cotinine levels <100 ng/mL and carbon monoxide levels <8 ppm. Plasma nicotine and cotinine levels were measured as previously described.¹⁰ Urine cotinine levels were measured with NicAlert cotinine test strips (Nymox Pharmaceutical, Hasbrouck Heights, NJ). Women had negative pregnancy tests during screening and prior to each radiotracer injection. Menstrual cycle phase was not controlled and hormonal contraception was not exclusionary. We have previously demonstrated that β_2^* -nAChR availability does not fluctuate over the menstrual cycle.³⁰

Assessments

In smokers, nicotine dependence severity was assessed at intake with the Fagerström Test for Nicotine Dependence (FTND),³¹ and craving and nicotine withdrawal symptoms were assessed with the Urge to Smoke Questionnaire (QSU)³² and the Minnesota Nicotine Withdrawal Scale,³³ respectively, at intake, and on scan days. The QSU yields two factors, the intention/desire to smoke (QSU-Intent) and relief of negative affect and withdrawal (QSU-Relief). In smokers and nonsmokers, symptoms of depression were assessed at intake and on scan days with the Center for Epidemiological Studies Depression Scale (CES-D).³⁴

Magnetic Resonance Imaging

T-1 weighted scans were obtained for each subject on a 1.5 or 3.0 Tesla Siemens camera in a standard orientation (echo time [TE] = 5–7 ms; repetition time [TR] = 24 ms; a 256 × 192 matrix; 1 number of excitations [NEX]; field of view [FOV] 30 cm; 124 contiguous slices with 1.2 mm thickness) and were used for co-registration to the SPECT images.

SPECT Protocol

Subjects received a 0.6 g saturated solution of potassium iodide to protect their thyroid from possible exposure to radioactive iodide prior to radiotracer administration. [¹²³I]5-IA was synthesized as previously described³⁵ and administered as a bolus plus constant infusion at a ratio of 7.0 h for 8 hours. There were no differences in amount of radiotracer injected between smokers at 7–9 days of abstinence (342.1 ± 58 MBq mean ± SD), 6–8 weeks of abstinence (345.9 ± 37 MBq mean ± SD), and nonsmokers (343.5 ± 44 MBq mean ± SD). Three consecutive 30-minute emission scans and one 15-minute simultaneous transmission and emission scan were obtained between hours 6 and 8 of the [¹²³I]5-IA infusion on a Picker PRISM 3000 XP (Cleveland, OH) SPECT camera. The axial resolution (full width at half maximum) was 12.2 mm, measured with a ¹²³I line source in water in a cylindrical phantom. Blood was drawn prior to injection and at the midpoint of the emission scans for analysis of plasma total parent and free fraction of parent tracer in plasma (f_p , free fraction). The chemical fate of [¹²³I]5-IA post injection was assessed in plasma as previously described.³⁵

SPECT Image Analysis and Outcome Measures

Images were reconstructed and analyzed as previously described, including a nonuniform attenuation correction.³⁶ MRIs were coregistered to the SPECT images to provide an anatomical guide for placement of the regions of interest using Medx (version 3.4) software (Medical Numerics, Inc, MD). Regions-of-interest were those known to express β_2^* -nAChRs and included frontal, parietal, anterior cingulate, temporal and occipital cortices, thalamus, striatum (an average of caudate and putamen) and cerebellum. Cortical regions (frontal, parietal, anterior cingulate, temporal, and occipital

cortices), were averaged together due to the high inter-correlation between these regions.

The outcome measure, volume of distribution (V_T/f_p), is defined as the regional radioactivity in the tissue (kBq/cc) divided by free plasma parent (kBq/mL) at equilibrium, that is, 6 to 8 hours after infusion start.³⁷ V_T/f_p was selected as the outcome measure because there is no appropriate [¹²³I]5-IA reference region due to the widespread expression of β_2^* -nAChRs in the brain.³⁶ We refer to V_T/f_p as “ β_2^* -nAChR availability” because subject variations in this measure predominantly reflect differences in the number of receptors that are “available” to the radiotracer for specific binding. Receptors that are already occupied, for example, by residual nicotine, a pharmacologically active metabolite (cotinine or nornicotine) or by endogenous neurotransmitter (acetylcholine), are not available for radioligand binding.

Genotyping and Quality Assurance

DNA extracted from whole blood from each subject was used to determine genotypes for the following genes and SNPs: the *CHRNA4* rs2273502, rs2273504, and rs2236196, and *ANKK1* rs4938015. The ABI PRISM 7900 TaqMan system (ABI, Foster City, CA) was used for SNP genotyping using a fluorogenic 5′ nuclease assay. For quality assurance, in each 96-well DNA set, two blank lanes were included as contamination controls and a CEPH control of known genotype. In addition, at least 8% of genotypes were repeated and no discrepancies were noted.

Statistical Analysis

Statistical analyses were conducted using SAS, version 9.3 (SAS Institute Inc, Cary, NC). Independent *t* tests were used to examine demographic and radiotracer variables between groups. Linear mixed models, one for each of the four SNPs of interest, were used to compare β_2^* -nAChR availability (V_T/f_p). These models included SNP genotype (major allele homozygote, heterozygote, minor allele heterozygote) and smoking status (smoker, nonsmoker) as between-subjects factors, and brain region (average cortex striatum, thalamus, and cerebellum) as a within-subjects factor. All multi-way interactions were modeled and the best-fitting variance-covariance structure was chosen using information criteria. For *CHRNA4**rs2236196, GG genotypes were grouped with AG genotypes (and compared to AA genotypes) since no GG nonsmokers and only 3 GG smokers were observed. Based on our previous findings that β_2^* -nAChR availability is associated with sex, we included sex as a covariate. Least square (LS) means (± standard errors) were compared and plotted post hoc to determine the specific effects of smoking or SNP allele genotype with β_2^* -nAChR availability.

Analyses among smokers were conducted to examine associations between SNP genotype status and change in β_2^* -nAChR availability over abstinence. In smokers who completed two scans, the percent change in β_2^* -nAChR availability from scan one to scan two was calculated, and compared in a linear mixed model with SNP allele genotype status as a between-subjects factor and region as a within-subjects factor. Potential associations between β_2^* -nAChR availability, SNP genotypes, and smoking characteristics (cigarettes per day, FTND, years smoked, QSU [desire/relief], Minnesota Nicotine Withdrawal Scale) on scan day were explored with correlation analyses. A Bonferroni adjusted α threshold of 0.0125 (0.05/4) was used to determine significance for all post hoc comparisons based on the four SNPs tested.

Results

Clinical Population

Fifty-eight tobacco smokers and fifty-five age- and sex-matched nonsmokers were included in the study. Plasma cotinine and nicotine and carbon monoxide levels were negligible in smokers and nonsmokers on scan days. There were no significant differences in age between smokers and nonsmokers at baseline. Smokers smoked 19 ± 7.5 cigarettes per day for 17.4 ± 10.4 years, and were moderately dependent on nicotine, with FTND scores of 5.2 ± 2.4 . Self-reported symptoms of depression did not significantly differ between groups at baseline; depressive symptoms in smokers (CES-D) and smoking craving (QSU-Desire and QSU-Relief) did not change significantly from time 1 to time 2 (Table 1).

Consistent with our previous studies,^{10,12} smokers at 1 week of abstinence had significantly higher β_2^* -nAChR availability compared to nonsmokers in the cerebellum ($F(1,110) = 9.7$, $P = .002$, 17% higher) and cortex ($F(1,110) = 16.0$, $P = .0001$, 22% higher) but not the striatum or thalamus (Figure 1). Also consistent,¹² after 6–8 weeks abstinence in the smokers, β_2^* -nAChR availability in the cerebellum ($P = .14$) and cortex ($P = .57$) reverted to nonsmoking levels. We then examined whether genetic factors play a role in these relationships. Successful genotyping was accomplished for the following genes: *CHRNA4*: (54 of 55 nonsmokers; 53 of 58 smokers; 24 of 27 returners), and *ANKK1* rs4938015 (55 of 55 nonsmokers; 55 of 58 smokers; 25 of 27 returners).

CHRNA4 and *ANKK1* Polymorphisms Impact Differences in β_2^* -nAChR Availability Between Smokers and Nonsmokers

There was a significant SNP genotype by smoking interaction for *CHRNA4**rs2236196; $F(1,102) = 5.0$, $P = .03$, predominantly explained by higher β_2^* -nAChR availability in smokers compared

Table 1. Demographic, Smoking and Mood Characteristics for Nonsmokers and Smokers on Scan Day

	Nonsmokers	Smokers	
		1 week	6–8 weeks
Number of subjects	55	58	27
Age, y	33.4 (11.7)	35.3 (11.0)	35.6 (11.4)
Sex	32 women	27 women	15 women
Carbon monoxide level, ppm	2.3 (2.4)	2.6 (2.3)	2.2 (2.2)
Plasma cotinine, ng/mL	10.4 (6.3)	11.1 (12.4)	18.2 (44.0)
QSU-desire	NA	6.0 (4.2)	3.0 (1.8)
QSU-relief	NA	6.9 (5.2)	3.6 (1.0)
MNWS	NA	4.5 (5.0)	3.4 (5.2)
CES-D	4.3 (4.8)	8.0 (7.3)	4.6 (5.1)

CES-D = Center for Epidemiologic Studies Depression Scale; MNWS = Minnesota Nicotine Withdrawal Scale; QSU-Desire = Urge to Smoke Questionnaire intention/desire to smoke; QSU-Relief = QSU relief of negative affect and withdrawal. Unless otherwise indicated, data are expressed as mean (SD).

Figure 1. β_2^* -containing nicotinic acetylcholine receptor (β_2^* -nAChR) availability in nonsmokers (open symbols) and smokers at 1 week of abstinence (closed squares) and smokers at 6–8 weeks of abstinence (closed diamonds). Significantly greater receptor availability was observed for smokers compared to nonsmokers in cerebellum and cortex. Sample sizes are as follows: Nonsmokers ($n = 55$); Smokers, 1 week abstinence ($n = 58$); Smokers, 6–8 weeks abstinence ($n = 27$).

to nonsmokers with AG but not AA genotypes in the cerebellum ($F(1,102) = 15.7, P = .0001$) and cortex ($F(1,102) = 17.3, P = .0001$, Figure 2). Further, among nonsmokers, AA genotypes had significantly greater β_2^* -nAChR availability than AG genotypes in the cerebellum ($F(1,102) = 4.0, P = .05$). There were no significant effects of genotype status for the rs2273502 or rs2273504 *CHRNA4* SNPs.

There was a trend toward a significant SNP genotype by smoking interaction for *ANKK1**rs4938015 ($F(2,103) = 3.2, P = .04$) owing primarily to significantly higher β_2^* -nAChR availability in smokers compared to nonsmokers in the cerebellum (CC genotype, $F(1,103) = 6.7, P = .01$; CT genotype, $F(1,103) = 9.1, P = .003$) and cortex (CC genotype, $F(1,103) = 9.7, P = .002$; CT genotype, $F(1,103) = 14.8, P = .0002$) among both CC and CT genotypes, but not TT genotypes (Figure 3). Among TT subjects, smokers had lower β_2^* -nAChR availability in the thalamus compared to nonsmokers, but this did not reach significance at the adjusted $\alpha = 0.01$ threshold ($F(1,103) = 5.5, P = .02$).

CHRNA4 Polymorphisms and Change in β_2^* -nAChR Availability Over Abstinence in Smokers

When examining the percent change of β_2^* -nAChR availability in abstinent smokers, a greater percent change, indicating more normalization, was observed in AA carriers compared to AG+GG carriers of the *CHRNA4**rs2236196 allele, but this main effect did not reach significance ($F(1, 21) = 2.3, P = .15$). While there was a significant main effect of region ($F(3,21) = 6.7, P = .003$), the gene by region interaction was not significant. For the *CHRNA4**rs2273504

allele AG carriers had a greater percent change than the GG carriers, but again, this main effect did not reach significance ($F(1, 21) = 2.7, P = .12$); and, no gene by region interaction was observed. Finally, for the *CHRNA4**rs2273502 allele, a trend toward a greater percent change was observed among CC compared to CT carriers ($F(1, 21) = 4.0, P = .06$) but no gene by region interaction was observed. There were no suggestions of trends for carrier group differences for the *ANKK1**rs4938015 allele. These results suggest possible genetic influences by the *CHRNA4* gene on normalization of β_2^* -nAChR availability in smokers, which the current study was not sufficiently powered to detect. The percent changes in β_2^* -nAChR availability are shown by genotype for each region in Table 2.

There were no significant differences by SNP genotype in smokers who did and did not abstain to participate in the second scan. There were no significant relationships between clinical correlates of tobacco smoking (craving, withdrawal, FTND) and the change in β_2^* -nAChR availability over abstinence by SNP genotype in smokers.

Discussion

The main finding of this study is that SNPs in the *CHRNA4* and *ANKK1* genes were associated with differences in β_2^* -nAChR availability in smokers compared to nonsmokers. We have previously shown significantly higher β_2^* -nAChR availability in smokers versus nonsmokers during acute abstinence¹⁰ that normalizes over extended abstinence.¹² The current study extends these findings

Figure 2. β_2^* -containing nicotinic acetylcholine receptor (β_2^* -nAChR) availability in nonsmokers (open symbols) and smokers (closed symbols) for G carriers (AG/GG) and AA subjects at *CHRNA4**rs2236196. Significantly greater receptor availability was observed for smokers compared to nonsmokers in cerebellum and cortex for G carriers (AG/GG) but not AA homozygotes. Sample sizes are as follows: Nonsmoker AG ($n = 26$) and AA ($n = 28$); Smoker GG/AG ($n = 27$) and AA ($n = 26$).

Figure 3. β_2^* -containing nicotinic acetylcholine receptor (β_2^* -nAChR) availability in nonsmokers (open symbols) and smokers (closed symbols) for *ANKK1**rs4938015. Significantly greater receptor availability was observed for smokers compared to nonsmokers in cerebellum and cortex for CC homozygotes and CT heterozygotes, but not TT homozygotes. Conversely, in thalamus nonsmokers had significantly greater availability than smokers among TT subjects. Sample sizes are as follows: Nonsmoker CC ($n = 25$), CT ($n = 22$) and TT ($n = 8$); Smoker CC ($n = 23$), CT ($n = 23$) and TT ($n = 9$).

Table 2. Percent Reductions in β_2^* -nAChR Availability From Time 1 to Time 2 in Smokers for the *CHRNA4* SNPs

Gene SNP	rs2236196		rs2273502 ^a		rs2273504	
	GG/AG ($n = 11$)	AA ($n = 13$)	CT ($n = 8$)	CC ($n = 16$)	AG ($n = 12$)	GG ($n = 12$)
Thalamus	−4% (4)	−14% (4)	−14% (3)	−1% (5)	−15% (4)	−4% (4)
Striatum	1% (7)	−3% (6)	−5% (6)	7% (8)	−7% (6)	5% (6)
Cerebellum	−8% (5)	−18% (5)	−18% (4)	−4% (6)	−18% (5)	−9% (5)
Cortex	−15% (4)	−26% (4)	−23% (4)	−15% (5)	−22% (4)	−20% (4)

β_2^* -nAChRs = β_2^* -containing nicotinic acetylcholine receptors; SNP = single nucleotide polymorphism. Shown is the percent change [mean, (SE)] from scan 1 (7–9 days of abstinence) to scan 2 (6–8 weeks of abstinence).

^aIndicates trend of greater percent change among CC compared to CT carriers ($P = .06$).

by highlighting the role of genetic variation in the neurochemical changes that occur during the recovery from tobacco smoking. Specifically, we found significantly higher β_2^* -nAChR availability in the cortex and cerebellum of smokers compared to nonsmokers for carriers of *CHRNA4* rs2236196*G and *ANKK1* rs4938015*C but not AA or TT homozygotes, respectively. Greater percent decreases in β_2^* -nAChR availability in smokers over prolonged abstinence were found in homozygotes of *CHRNA4* SNP rs2273502 and rs2273504 compared to heterozygotes but we were underpowered to detect significant effects.

These findings extend a large body of literature indicating roles for *CHRNA4* and *ANKK1* genes in nicotine dependence by demonstrating β_2^* -nAChR availability as a potential functional mechanism mediating the gene effects on nicotine dependence. The *ANKK1*

rs4938015*C allele has been repeatedly associated with increased smoking rates in European Americans.^{22,38} Similarly, *CHRNA4* rs2236196 has been consistently shown to influence smoking status in large populations consisting of African Americans,^{16,17} European Americans^{17,19} and Germans.³⁹ In the present study, smokers who carried the alleles associated with increased smoking risk at rs2236196 or rs4938015 exhibited significantly higher β_2^* -nAChR availability relative to matched-nonsmokers. The higher β_2^* -nAChR availability in smokers compared to nonsmokers was not observed in smokers who did not carry the increased risk alleles. Thus, it appears that the genetic vulnerability to smoking may come with a predisposition to a greater sensitivity to nicotine, exhibited as a greater change in β_2^* -nAChR availability (ie, likely a greater degree of upregulation) in response to chronic nicotine use. The present finding provides

a functional intermediary biomarker, β_2^* -nAChR availability, by which genes may impact the phenotype of nicotine dependence.

How these genes alter the availability of β_2^* -nAChRs is not yet clear, but could be a result in differences in receptor assembly, trafficking or stability. We previously showed in two subjects that rare variants in *CHRNA4*, which are protective against nicotine dependence, may increase β_2^* -nAChR availability in nonsmokers.¹⁸ These rare variants in *CHRNA4* result in altered nAChR assembly and function in a number of in vitro assays, providing a potential mechanism for these changes.²⁰ In addition, these SNPs may alter the function of nAChRs⁴⁰ or could affect phosphorylation of nAChRs, which has been shown to influence receptor desensitization,^{41,42} both of which could alter nAChR numbers.

Our group previously demonstrated that sex is one source of variability in the differences between smokers and nonsmokers in β_2^* -nAChR availability.⁴³ The genetic association literature has been mixed with regard to sex differences. In one study, European American men with a specific haplotype block including the *ANKK1* SNP rs4938015 were shown to have a greater likelihood of smoking initiation compared to other groups.³⁸ Conversely, no relationship between *CHRNA4* rs2236196 genotype, sex, and smoking status (defined by scores on the FTND) was established in German individuals.³⁹ In the current study, there was no evidence of an association between sex with *ANKK1* or *CHRNA4* genotype and β_2^* -nAChR availability in smokers versus nonsmokers. Despite the comparatively large sample size for this SPECT imaging study, it may have been underpowered to determine a potential interaction between sex, risk alleles, and β_2^* -nAChR availability. A further demographic consideration is the homogenous European American population recruited for this study. The effect of these genetic influences on β_2^* -nAChR availability and nicotine dependence in other ethnicities remains to be determined. This is of particular interest in African Americans with the rs2236196 SNP, since the allele frequency is reversed in this population¹⁷ compared to EAs (ie, the major allele is G in African Americans with a major allele frequency of 34.1%, while it is A in European Americans with a major allele frequency of 29.2%).

The *CHRNA4* gene encodes the α_4 -nAChR subunit. The α_4 subunit assembles with the β_2 -subunit, providing a potential mechanism for an alteration in β_2^* -nAChR availability, either by altering assembly of $\alpha_4\beta_2^*$ -nAChRs, or by regulating the trafficking or associated proteins with the receptor.²⁰ It has been speculated that rs2236196, which is located in the 3' UTR of *CHRNA4*, may alter α_4 -nAChR protein expression such that $\alpha_4\beta_2^*$ -nAChRs have increased sensitivity to cholinergic stimulation, evidenced by genotype significantly influencing subjective response to a laboratory model of smoking and smoking cessation outcomes.¹⁹ Receptor availability can be affected by a number of factors including the number of receptors present in the tissue, affinity of radioligand for the receptor, and concentration of endogenous neurotransmitter (eg, acetylcholine) or exogenous drug (eg, nicotine). Similarly, the *ANKK1* gene encodes a protein that phosphorylates receptors²¹ including nAChR subunits,⁴⁴ and consequently may influence trafficking of the receptor to the cell surface.⁴⁵ Future studies examining acetylcholine neurotransmission⁴⁶ or response to nicotine administration may be warranted to examine the mechanisms influencing differences in receptor availability.

The genetic influences on β_2^* -nAChR availability in tobacco smokers may have important implications for treatment. For example, male smokers have higher β_2^* -nAChR availability than male nonsmokers but female smokers have similar β_2^* -nAChR availability as female nonsmokers.⁴³ Male smokers also tend to have a better

response to nicotine replacement therapies than female smokers.⁴⁷ Interestingly, G carriers of SNP rs2236196—the same genotypes shown here to result in significantly higher β_2^* -nAChR availability in smokers than nonsmokers—showed improved treatment outcomes with nicotine replacement therapy using a fast-acting nasal spray as opposed to the slow-acting transdermal patch.¹⁹ Taken together, this suggests that genotyping of SNP rs2236196 and rs4938015 may provide improved personalization of therapies for smoking cessation, as G carriers in particular may benefit from fast-acting nicotine replacement therapies. This hypothesis should be tested clinically.

In conclusion, this study establishes the first in vivo evidence of β_2^* -nAChR availability as an endophenotype mediating influences of the *ANKK1* and *CHRNA4* genes on nicotine dependence. These results identify one possible functional mechanism underlying the influence of genes on smoking behaviors, and highlight individual differences in nicotine dependence and the consequent need for individualized programs for smoking cessation.

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Declaration of Interests

None declared.

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