Nicotine & Tobacco Research, 2017, 417–425
doi:10.1093/ntr/ntw200
Original investigation
Advance Access publication August 19, 2016

Original investigation

Examination of the Involvement of Cholinergic-Associated Genes in Nicotine Behaviors in European and African Americans

Whitney E. Melroy-Greif PhD[1], Matthew A. Simonson PhD[2], Robin P. Corley PhD[3], Sharon M. Lutz PhD[4], John E. Hokanson PhD[5], Marissa A. Ehringer PhD[3,6]

[1]Department of Molecular and Cellular Neuroscience, The Scripps Research Institute, La Jolla, CA; [2]Massachusetts Institute of Technology, Cambridge, MA; [3]Institute for Behavioral Genetics, University of Colorado Boulder, Boulder, CO; [4]Department of Biostatistics and Informatics, University of Colorado Anschutz Medical Campus, Aurora, CO; [5]Department of Epidemiology, University of Colorado Anschutz Medical Campus, Aurora, CO; [6]Department of Integrative Physiology, University of Colorado Boulder, Boulder, CO

Corresponding Author: Marissa Ehringer, PhD, Department of Integrative Physiology, Institute for Behavioral Genetics, University of Colorado Boulder, 447 UCB Boulder, CO 80309–0447, USA. Telephone: 303-492-1463; Fax: 303-492-8063; E-mail: marissa.ehringer@colorado.edu

Abstract

Introduction: Cigarette smoking is a physiologically harmful habit. Nicotinic acetylcholine receptors (nAChRs) are bound by nicotine and upregulated in response to chronic exposure to nicotine. It is known that upregulation of these receptors is not due to a change in mRNA of these genes, however, more precise details on the process are still uncertain, with several plausible hypotheses describing how nAChRs are upregulated. We have manually curated a set of genes believed to play a role in nicotine-induced nAChR upregulation. Here, we test the hypothesis that these genes are associated with and contribute risk for nicotine dependence (ND) and the number of cigarettes smoked per day (CPD).

Methods: Studies with genotypic data on European and African Americans (EAs and AAs, respectively) were collected and a gene-based test was run to test for an association between each gene and ND and CPD.

Results: Although several novel genes were associated with CPD and ND at $P < 0.05$ in EAs and AAs, these associations did not survive correction for multiple testing. Previous associations between *CHRNA3, CHRNA5, CHRNB4* and CPD in EAs were replicated.

Conclusions: Our hypothesis-driven approach avoided many of the limitations inherent in pathway analyses and provided nominal evidence for association between cholinergic-related genes and nicotine behaviors.

Implications: We evaluated the evidence for association between a manually curated set of genes and nicotine behaviors in European and African Americans. Although no genes were associated after multiple testing correction, this study has several strengths: by manually curating a set of genes we circumvented the limitations inherent in many pathway analyses and tested several genes that had not yet been examined in a human genetic study; gene-based tests are a useful way to test for association with a set of genes; and these genes were collected based on literature review and conversations with experts, highlighting the importance of scientific collaboration.

Introduction

Cigarette smoking is a personally harmful and societally detrimental habit. Despite substantial progress since the first Surgeon General's report was released 50 years ago, smoking is still the largest cause of preventable disease and death in the United States. An estimated half a million Americans die prematurely from smoking each year, and more than 16 million Americans suffer from smoking-related diseases. The economic costs from smoking and exposure to tobacco smoke are estimated at $300 billion annually, with productivity losses of $150 billion per year.[1]

It is widely accepted that nicotine is the major addictive component in tobacco smoke[2,3] and exerts its effect by binding to nicotinic acetylcholine receptors (nAChRs, encoded by the *CHRN* genes) in the peripheral and central nervous systems.[4] Accordingly, numerous studies have identified polymorphisms in several *CHRN* genes associated with nicotine dependence (ND) and cigarettes per day (CPD). The most well-replicated of these associations lies within a cluster of three *CHRN* genes on chromosome 15q25 (*CHRNA3/A5/B4*),[5] although genes for other subunits have been associated with these phenotypes as well. Most notably, *CHRNB3/A6* on chromosome 8p11 has been consistently associated with CPD[6] and ND.[7–13] Other *CHRN* genes associated with CPD and ND include *CHRND/G*, *CHRNB1*, *CHRNA10*, *CHRNA4*, and *CHRNB2*.[10,11,14–19]

Twin studies have estimated that ND and smoking quantity are roughly 56%–72% and 51%–61% heritable in men and women, respectively,[20–24] yet associations between the *CHRN* genes and ND and CPD account for a very small proportion of the variance in smoking (roughly 1 CPD).[5,11] Although this is small percentage, 1 additional CPD over a person's lifetime will accumulate, often to greater than 22 000 additional cigarettes in one's lifetime in the case of the Genetic Epidemiology of Chronic Obstructive Pulmonary Disease[25] study (COPDGene, described in the Samples section). In addition, studies have shown an association between higher levels of adolescent ND and heavier smoking trajectory patterns.[26,27] Based on work providing evidence that common single nucleotide polymorphisms (SNPs) explain a large proportion of the heritability in height, Crohn's disease, bipolar disorder, and type I diabetes,[28,29] we hypothesize that common SNPs also explain a considerable proportion of the variation in smoking quantity and ND. Although it is highly likely rare variants play an equally important role in these behaviors, the purpose of this study was to examine common variants from available datasets. The goal of this study was to utilize set-based approaches to test for association between SNPs within selected genes and nicotine behaviors. We applied SNP-set based methods as a way to increase power to detect associations, and organize associations in a biologically meaningful way.[30,31] Associations can be more easily detected by grouping SNPs into sets, and insights into their combined effects on biological function are more apparent.

Upon chronic exposure to nicotine, nAChRs undergo an upregulation of receptor number[32–34] that is independent of upregulation of *CHRN* mRNA.[35] Although there are many theories describing how this upregulation occurs, including increased receptor trafficking,[36] decreased subunit degradation,[37,38] increased nAChR subunit maturation and folding,[39–41] pharmacological chaperoning by nicotine,[42] and increased translation and second messenger signaling,[43] researchers have yet to test several of the proteins suggested to impact upregulation in concert. Studies have demonstrated that nicotine-induced nAChR upregulation leads to nicotine sensitization in brain regions known to play a role in addiction and reward.[44] Furthermore, nAChR upregulation has been well-documented in rodents exposed to nicotine during prenatal development[45–51] and the long term and widespread consequences of prenatal nicotine exposure have been proposed to play a role in various neurobehavioral and physiological disorders.[52] We posit that gene products are involved in nAChR upregulation through their interaction with nAChRs, and thus play a role in the development of ND.

We have assembled a list of genes that encode proteins known to interact with nAChRs or are known to play a role in their downstream signaling, by curating the literature, and conversing with experts (listed in the acknowledgements) in the field (Figure 1). The goal of this study was to assess the evidence for association with each gene and CPD and ND in African and European Americans (AAs and EAs, respectively). These analyses were carried out in several studies in which genome-wide genotyping was available and results were later combined as a meta-analysis. Although many

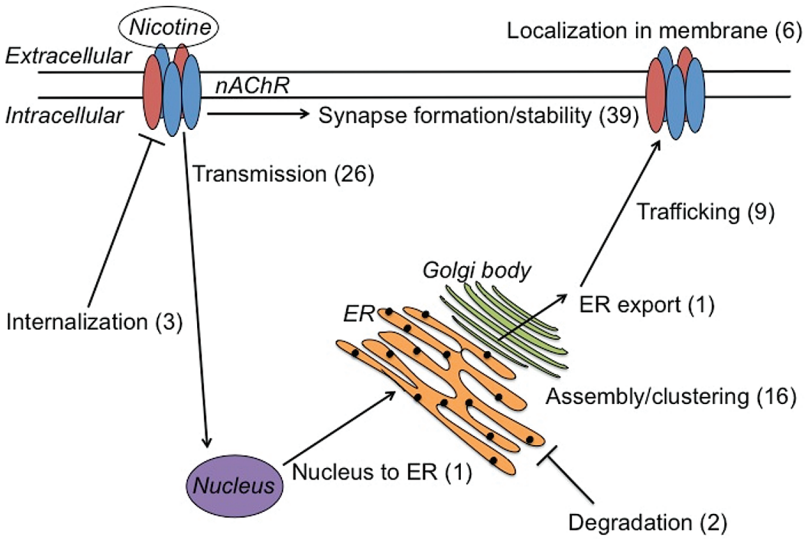

Figure 1. Cellular processes proposed to play a role in nAChR upregulation; the number of genes from our curated list of 96 that play a role in each process is shown in parentheses (several genes were proposed to be involved in more than one cellular process). ER = endoplasmic reticulum. Modified from Melroy-Greif et al.[53]

studies use smoking quantity as a proxy for ND, research has demonstrated unique genetic effects on CPD, suggesting that a smoking quantity measure may not serve as a simple proxy for the genetic influences on ND.[54] Thus, while these phenotypes may be correlated, they represent different aspects of smoking behavior and exposure. Based on these findings, both CPD and ND were used as phenotypes in the present study.

Methods

Samples

Four independent samples with genome-wide genotype data and smoking phenotypes were included in the study (Table 1): (1) the Study of Addiction: Genetics and Environment (SAGE),[55] (2) COPDGene,[25] (3) the Multi-Ethnic Study of Atherosclerosis (MESA),[56] and (4) an in-house sample comprised of unrelated Caucasian subjects from the Colorado Center on Antisocial Drug Dependence and the Genetics of Antisocial Drug Dependence (CADD-GADD).[57] The SAGE sample is comprised of three substudies recruited via studies on cocaine, alcohol, or ND.[55] The COPDGene study consists of smokers who have smoked at least 10 pack-years.[25] MESA is a population-based study designed to investigate the characteristics of subclinical cardiovascular disease.[56] Finally, the CADD-GADD sample is a mixture of various samples including probands targeted for drug use, their families and matched control families, as well as community twin samples. Additional details about the CADD-GADD sample recruitment and assessments are provided in Derringer et al.[57] Study-level principal components (PCs) were supplied along with the SAGE, COPDGene, and CADD-GADD genotype data. PCs were computed in the MESA sample using shellfish (http://www.stats.ox.ac.uk/~davison/software/shellfish/shellfish.php). All genotype data were imputed to Phase 1 of the 1000 Genomes dataset[58] and cleaned using standard quality control procedures: SNPs with low imputation accuracy (<0.9), low minor allele frequency (MAF) (<0.01), and out of Hardy–Weinberg equilibrium (HWE, $P < .001$) were excluded.

We analyzed two phenotypes when available from each study: quantitative ND symptoms as assessed by the Fagerström Test for Nicotine Dependence (FTND),[59] and CPD, binned as follows: 0–10, 11–20, 21–30, 31–40, and 41 or more CPD. CPD was only assessed in two of the substudies from SAGE whereas FTND was collected in all substudies. Likewise, FTND was only assessed in current smokers in the COPDGene sample. FTND was not available in the MESA or CADD-GADD samples. Only subjects who had smoked 100 cigarettes or more in their lifetime were included in the analysis.

Analyses

SNPs were annotated to our genes of interest using the hg19 build. SNPs 20 kb up- and down-stream of each gene were included, given reports that the majority of genetic variants that influence expression are located within 20 kb of a gene.[60] Only SNPs common across all datasets were evaluated. Among the EAs, this was limited to imputed SNPs from the SAGE, COPDGene, MESA, and CADD-GADD samples. Similarly, for the AA analyses, only SNPs common across the AA SAGE, MESA, and COPDGene samples were included. Age, sex, and the first five PCs from each study were included as covariates in each analysis. Additionally, substudy was used as a covariate in the SAGE analysis.

Joint Association of Genetic Variants

JAG[61] (available at http://ctglab.nl/software/jag/) uses raw data and invokes PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml)[62] to run a genome-wide association on each SNP included in the gene. A multivariate SNP test statistic is calculated by summing the $-\log_{10}$ of each SNP *P*-value and the empirical *P*-value for each gene is calculated by summing the $-\log_{10}$(*P*-value) for each permutation of the phenotype. The empirical *P*-value (P_{emp}) represents the number of times the $\Sigma-\log_{10}$(*P*-value) exceeds or equals the $-\log_{10}$(*P*-value) from the genome-wide association. The P_{emp} based on the $\Sigma-\log_{10}$(*P*-value) test statistic tests the hypothesis that, given the linkage disequilibrium (LD) structure of the included SNPs, the multivariate pattern of *P*-values of all SNPs in a gene is significantly different than what is expected under the null hypothesis of no association. Ten thousand permutations were performed for each test. This analysis was applied to our data in order to test the association between individual genes and CPD and FTND.

Meta-analysis

The P_{emp} for each gene was combined across studies using the weighted *Z*-score method.[63] False discovery rate (FDR) corrected *P*-values were generated for each meta-analyzed *P*-value using the stats package in R.[64]

Replication

Summary *P*-values for CPD were used from the Tobacco and Genetics (TAG) Consortium[65] as a replication. These data are freely available at http://www.med.unc.edu/pgc/downloads. MAGMA,[66] a tool that can perform a gene-based test on summary SNP *P*-values, was used to test for association between genes trending toward association with CPD in EAs after meta-analysis. Briefly, SNPs in the 1000 Genomes CEU data were annotated to genes using the hg19 build, including SNPs 20 kb up- and down-stream of each gene. A gene-based test was run on 17 genes: *CHRNA3*, *CHRNA5*, *CHRNB4*, *NCAM1*, *CHRNE*, *DNAJA3*, *IQGAP1*, *MAPRE1*, *UNC50*, *CHRNB1*, *EPHA4*, *NRXN1*, *LRP4*, *CRELD2*, *CAMK2A*, *ITGA7*, and *APC*. The 1000 Genomes CEU data was used as a

Table 1. Study and Sample Characteristics

	Study	N_{CPD}	Age[a]	Sex (% male)	CPD[a]	N_{FTND}	Age[a]	Sex (% male)	FTND[a]
EA	COPDGene	6670	62.09 ± 8.84	52.37	25.84 ± 11.44	2568	57.50 ± 7.86	53.35	4.83 ± 2.42
	SAGE	1255	35.74 ± 6.92	37.83	25.90 ± 19.94	1673	37.53 ± 8.65	43.93	2.99 ± 3.29
	CADD-GADD	588	23.44 ± 4.40	73.98	17.96 ± 11.54	NA	NA	NA	NA
	MESA	628	63.03 ± 9.95	56.53	20.59 ± 21.66	NA	NA	NA	NA
AA	COPDGene	3300	54.68 ± 7.21	55.94	21.30 ± 10.41	2567	53.34 ± 6.04	57.78	4.93 ± 2.34
	SAGE	594	39.30 ± 6.81	47.47	23.64 ± 17.39	779	40.03 ± 7.13	49.81	3.92 ± 2.87
	MESA	53	63.64 ± 9.91	49.06	12.77 ± 11.10	NA	NA	NA	NA

NA = not applicable.

[a]Mean ± standard deviation.

reference panel for LD as evaluation of the gene test-statistic requires LD estimation between SNPs in the gene. Similar to the JAG analysis, MAGMA uses a SNP-wise model in which the SNP *P*-values are converted by taking the $-\log_{10}$ of the *P*-value and combined by computing their sum to obtain a gene-based *P*-value.

Results

Study and sample characteristics for the COPDGene, SAGE, MESA, and CADD-GADD samples are given in Table 1. Subjects in the COPDGene and MESA samples were the oldest and the CADD-GADD the youngest. EAs in the COPDGene and SAGE had similar smoking patterns, as did AAs in the COPDGene and SAGE samples. The AAs had somewhat lower CPD and higher FTND than their EA counterparts. CPD and FTND were correlated at 0.74 and 0.58 in the SAGE EA and AA samples, respectively. CPD and FTND were less correlated in the COPDGene EA and AA samples (0.37 and 0.35, respectively), likely due to FTND assessment in current smokers only.

Of the 112 total genes in our list (96 genes collected from the literature and 16 nAChR subunit genes), 100 genes had SNPs annotated and were used in the analysis in AAs, and 101 genes had SNPs annotated and were used in the EA analysis.

CPD and ND in EAs

Thirty-three thousand four hundred eighty-three SNPs from our gene set were tested for association with CPD in EAs from the COPDGene, SAGE, MESA, and CADD-GADD samples. The gene *P*-values were combined using the weighted *Z*-score method. Although 17 genes were trending at $P < .1$ (and used in the subsequent replication analysis), only *CHRNA3*, *CHRNA5*, and *CHRNB4* were associated with CPD with controlling FDR at $P < .05$ (Table 2).

Thirty-three thousand four hundred eighty-three SNPs were tested for association with FTND in EAs from the COPDGene and SAGE samples. The gene *P*-values were combined using the weighted *Z*-score method. Similar to the results with CPD, while several genes were associated at $P < .05$, only the association with *CHRNA3* survived correction for multiple testing at FDR $P < .05$ (Table 2).

CPD and ND in AAs

Thirty-eight thousand seven hundred eighty-seven SNPs from our set of genes were tested for association with CPD in AAs from the COPDGene, SAGE, and MESA samples and the gene *P*-values combined using the weighted *Z*-score method. Five genes were associated with CPD at $P < .1$ but none of these associations survived correction for multiple testing at FDR $P < .05$ (Table 3).

The same set of SNPs was tested for association with FTND and the gene *P*-values combined in AAs from the SAGE and COPDGene samples. More genes were nominally associated with FTND at $P < .1$ than CPD, but none survived correction for multiple testing at FDR $P < .05$ (Table 3).

Replication

Gene-based tests on summary *P*-values for CPD from the TAG data were run on 17 genes (*CHRNA3*, *CHRNA5*, *CHRNB4*, *NCAM1*, *CHRNE*, *DNAJA3*, *IQGAP1*, *MAPRE1*, *UNC50*, *CHRNB1*, *EPHA4*, *NRXN1*, *LRP4*, *CRELD2*, *CAMK2A*, *ITGA7*, and *APC*). No gene was trending toward association at $P < .1$, with the exception of *CHRNA3, CHRNA5*, and *CHRNB4*, which were highly associated at $P < 5E^{-14}$ (results not shown).

Discussion

Given the highly replicated associations with *CHRN* genes and smoking behaviors, we examined 96 other genes, known to interact with nAChRs, to test for association in four existing samples for which genome-wide data and phenotypic measures were available. As expected, the *CHRN* gene cluster on chromosome 15q25 comprised of *CHRNA3*, *CHRNB4*, and *CHRNA5* showed the strongest associations with nicotine behaviors in EAs in this study, replicating previous associations with these genes and CPD[6,65,67–76] and ND.[7,10,13,18,77–87] Although there was some overlap between the genes nominally associated with CPD and FTND (*CHRNE*, *DNAJA3*, *CRELD2*, *CAMK2A*, and *EPHA4),* no genes were associated after correction for multiple testing at FDR $P < .05$. Similarly, while primarily driven by the largest sample, COPDGene, two genes were trending toward association in more than one study: *CRELD2* and *CAMK2A*.

The TAG results mirror our own study in that only the gene-wise *P*-values for *CHRNA3/A5/B4* were highly significant. Although no other genes survived correction for multiple testing in our study, we would hypothesize that some of the genes would have been trending toward association with CPD in the TAG data. However, there are some notable differences between the original and replication analyses. First, we limited the analyses to SNPs annotated on all datasets used in our study, while additional signals were likely used in the replication sample, and this may have washed out associated SNPs. Second, there likely exist differences in sample ascertainment and phenotypic variables between the four samples used in our meta-analysis and those in TAG.

Unlike in EAs, no genes, particularly *CHRNA3/A5/B4*, were associated with CPD or FTND after controlling FDR at $P < .05$ in AAs. However, two genes showed consistent results across studies and bear future investigation: *PICK1* and *IQGAP1*. None of the cluster genes were associated with CPD in AAs from the SAGE, MESA, or COPDGene samples. This result is perhaps not surprising given previous results with CPD in AAs in the COPDGene sample.[76] However, a previous study using over 32 000 AAs found an association between *CHRNA3/A5/B4* with CPD. In that study, only one SNP, residing in the 5′-distal enhancer region of *CHRNA5*, exceeded genome-wide significance.[88] It is possible that since our analyses sum individual SNP statistics to get an overall gene *P*-value this SNP was overshadowed by SNPs of lower significance. In our study, *CHRNA5* was nominally associated with FTND in the SAGE sample using an alternative set-based test implemented in PLINK[62] at $P = .094$, but this association was not replicated in the COPDGene sample. Although this suggests heterogeneity between the two samples, it aligns with what has been previously seen in the literature. Saccone et al[82] reported an association between SNPs in *CHRNA5* and ND in AAs alone in COGEND, yet no association between *CHRNA3*, *CHRNB4*, or *CHRNA5* with ND was seen in the COPDGene sample.[76]

Although not significant after correction for multiple testing, several genes previously associated with substance use phenotypes in the literature were nominally associated with nicotine behaviors in the present study and will be discussed briefly here in an attempt to guide future replication efforts. *EPHA4*, nominally associated with CPD and FTND in EAs, has been associated with opioid dependence

Table 2. Results From the JAG Test in EAs

Gene	No. of SNPs	No. of effective SNPs[a]	COPDGene P_{emp}	SAGE P_{emp}	MESA P_{emp}	CADD-GADD P_{emp}	Combined P_{emp}	FDR corrected *P*
CPD								
CHRNA3	102	2	.0001	.128	.209	.434	.0001	.013
CHRNA5	29	1	.0001	.304	.348	.620	.0001	.013
CHRNB4	17	3[b]	.0001	.639	.742	.212	.0001	.013
NCAM1	402	5[c]	.006	.154	.721	.337	.004	.193
CHRNE	79	3	.004	.182	.924	.800	.005	.193
DNAJA3	111	2	.004	.527	.900	.472	.007	.258
IQGAP1	246	2	.010	.898	.268	.813	.022	.556
MAPRE1	148	5	.015	.829	.784	.196	.025	.601
UNC50	53	1	.043	.181	.514	.249	.029	.644
CHRNB1	63	3	.038	.216	.878	.531	.039	.729
EPHA4	212	5	.133	.073	.238	.105	.064	.782
NRXN1	2433	22[d]	.022	.992	.516	.493	.064	.782
LRP4	106	3[e]	.089	.178	.599	.441	.070	.782
CRELD2	127	1[f]	.097	.009	.638	.991	.071	.782
CAMK2A	89	5	.178	.059	.034	.174	.075	.782
ITGA7	41	1	.075	.451	.725	.304	.078	.782
APC	231	2	.061	.245	.876	.920	.079	.782
FTND								
CHRNA3	102	2	.003	.028	NA	NA	.00042	.042
CHRNA5	29	1	.012	.060	NA	NA	.003	.193
CHRNB4	17	2	.012	.080	NA	NA	.004	.193
CHRNA9	239	4	.009	.221	NA	NA	.008	.280
DNAJA3	111	2	.008	.338	NA	NA	.012	.381
CHRNE	79	3	.218	.004	NA	NA	.017	.482
EPHA4	212	5	.116	.112	NA	NA	.048	.782
CAMK2G	61	2	.008	.794	NA	NA	.056	.782
SVEP1	598	13	.263	.035	NA	NA	.064	.782
CAMK2A	89	5	.368	.013	NA	NA	.067	.782
ERBB2	64	1	.049	.458	NA	NA	.075	.782
RAPSN	65	2	.253	.056	NA	NA	.077	.782
CRELD2	127	1	.237	.073	NA	NA	.081	.782
LRP4	106	3[g]	.106	.269	NA	NA	.084	.782

The *P*-value is shown for each study as well as the combined *P*-value for genes associated with CPD or FTND at $P < .1$.

[a]The number of signals tested in the gene in each study (unless otherwise noted) as calculated by JAG.

[b]Only two signals calculated in the COPDGene sample.

[c]Six signals calculated in the COPDGene sample.

[d]Twenty-three signals calculated in the CADD-GADD and MESA samples.

[e]Two signals calculated in the SAGE sample.

[f]Two signals calculated in the MESA sample.

[g]Two signals calculated in SAGE.

in subjects with a history of cocaine dependence.[89] *CHRNA9* was nominally associated with FTND in EAs and CPD in AAs and has been previously associated with ND in female Israeli students,[90] as well as with lung cancer in Caucasians.[91] Recently, rare and common variation in *CHRNA9* has been associated with smoking status in EAs and AAs from the Mid-South Tobacco Case-Control study.[92]

It is interesting there was very little overlap in the genes associated with CPD and FTND between EAs and AAs. These findings might indicate underlying genetic differences in the development of smoking behaviors in EAs and AAs. AAs tend to start smoking later in life[93–96] and have a lower lifetime prevalence of ND compared to EAs.[97] Additionally, AAs report higher cravings and more pleasurable sensations after smoking,[96,98] lower rates of regular smoking,[94–97,99–103] higher nicotine intake per cigarette,[104] and slower metabolism rates of nicotine[103–107] compared to EAs. Finally, EAs have higher smoking cessation rates than AAs.[99] Together with our data, this suggests differences in smoking patterns between EAs and AAs may be partially genetically driven.

Although there was significant overlap in the genes implicated in ND and CPD in EAs, our results show little overlap in the genes associated with CPD and FTND in AAs. Given previous studies demonstrating both unique and common genetic influences on CPD and ND,[54] we expected to see some overlap between the two phenotypes. This discrepancy in AAs may be due to the fact that FTND was only measured in current smokers in the COPDGene sample and our results could reflect differences in smoking cessation as well. The AA sample was also considerably smaller than the EA sample; thus these analyses had lower power to detect genetic effects and a higher likelihood of false negatives.

While not without limitations, several strengths of the present study help mitigate these limitations. JAG has higher power than several other pathway algorithms. Specifically, using 1694 cases and 2917 controls from the Wellcome Trust Case Control Consortium the gene-based test in JAG had better power than other algorithms to detect known genes associated with Crohn's Disease.[61] SNP-set based approaches can increase power to detect genetic loci with

Table 3. Results From the JAG Test in AAs

Gene	No. of SNPs	No. of effective SNPs[a]	COPDGene P_{emp}	SAGE P_{emp}	MESA P_{emp}	Combined P_{emp}	FDR corrected *P*
CPD							
CHRNA9	247	12[b]	.005	.321	.424	.004	.193
PICK1	29	4[c]	.067	.082	.043	.040	.729
NTRK2	443	19[d]	.031	.824	.667	.048	.782
DLG4	7	2	.080	.458	.841	.083	.782
CAMK2B	50	3[e]	.092	.504	.877	.098	.799
FTND							
IQGAP1	400	7	.020	.016	NA	.005	.193
PDIA3	120	5	.011	.701	NA	.021	.556
UNC50	56	2	.019	.649	NA	.031	.653
UBXN2A	271	5	.012	.918	NA	.040	.729
EPHA4	221	10	.028	.791	NA	.055	.782
NLGN1	1668	36	.062	.493	NA	.070	.782
NCAM1	427	10[f]	.106	.170	NA	.071	.782
FYN	635	15	.085	.406	NA	.084	.782
PPP3CA	408	12	.068	.607	NA	.089	.799
LAMB1	187	10	.156	.111	NA	.093	.799
CACNA2D1	1325	40[g]	.077	.567	NA	.094	.799

The *P*-value for each gene is shown for each study as well as the combined *P*-value for genes associated with CPD or FTND at $P < .1$.

[a]The number of signals tested in the gene in each study (unless otherwise noted) as calculated by JAG.

[b]Eleven signals in the MESA data.

[c]Three signals in the MESA data.

[d]Eighteen signals in the MESA data.

[e]Two signals in the MESA data.

[f]Nine signals in the SAGE data.

[g]Thirty-nine signals in the SAGE data.

individually small effects by consolidating SNP associations and can help to prioritize associations based on biological relevance.[30,31] A meta-analysis approach was used as opposed to a mega-analysis so associations with genes across studies could be compared, although differences in age and study ascertainment may have clouded these comparisons. By curating a unique list of genes to test, we avoided many potential limitations when examining already curated gene sets from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO); for example, when examining gene sets from KEGG or GO, genes that have been well studied are more thoroughly annotated. As of 2011, only about 5000 human genes had been annotated to KEGG pathways.[31] However, despite being able to curate a unique set of genes, this study was limited to testing SNPs both annotated on the hg19 build and imputed within each sample. In addition, trans effects were ignored by necessity. Similarly, while both JAG and MAGMA take LD within a gene into account when computing the test statistic,[61,66] LD between genes was not taken into account. Finally, gene-based tests do not provide an effect size of the gene so the weighted Z-score method was used to meta-analyze the results. The direction of effect of each individual SNP is thus ignored and the resulting *P*-value differs from that of a traditional meta-analysis where small effects of opposite direction cancel each other out. To summarize, the direction of effect for each signal in an associated gene would be unknown as well as which signals are driving the association.

In conclusion, we tested several genes believed to be involved in nAChR upregulation with FTND and CPD and performed a meta-analysis over four independent samples. The list of genes tested is by no means an exhaustive list, and simply served to test the hypothesis that SNPs in the identified genes are associated with nicotine behaviors by altering nAChR function and/or expression through protein–protein interactions. Although no genes were associated after correction for multiple testing, except for the reliably replicated *CHRNA3/A5/B4* association, it is possible these genes may nevertheless be affecting nAChR upregulation in a manner not directly driven by SNPs; for example, increased transcription factor binding, or enhancer effects. Future studies could assess trans effects in these genes, perform functional bioinformatics, or examine gene-by-gene interactions among this list. This work represents collaboration between neuroscience experts and statistical geneticists and although it did not bear fruit with respect to the current study, future collaborations will continue to be important to identify and characterize novel genetic associations.

Funding

This work was supported by grants from the National Institute on Alcohol Abuse and Alcoholism (R01 AA017889 and T32 AA013525); the National Institute on Drug Abuse (T32 DA017637); and the National Heart, Lung, and Blood Institute of the National Institutes of Health (NIH) (K01 HL125858). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The authors have no conflicts of interest to report. Funding sources for the datasets used in this study are listed as follows. *CADD-GADD*: Supported by NIH grants from the National Institute on Drug Abuse (DA011015 and DA012845). *COPDGene*: Supported by NIH grants (U01HL089897 and U01HL089856) from the National Heart, Lung, and Blood Institute. Use of this data was approved by the COPDGene Ancillary Studies and Publications Committee. *MESA*: Supported by contracts N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168 and N01-HC-95169, from the National Heart, Lung, and Blood Institute, and by grants (UL1-TR-000040, and UL1-TR-001079) from the National Center for Research Resources. These data were obtained through dbGaP. *SAGE*: Supported by NIH grants from the National Institute on Alcohol abuse and Alcoholism (AA008401); the National Institute on Drug Abuse (DA013423); and the National Cancer Institute (CA089392). These data were obtained through dbGaP.

Declaration of Interests

None declared.

Acknowledgments

The authors would like to thank Drs. Millet Treinin, Henry Lester, Marina Picciotto, Roger Papke, and Jon Lindstrom for their suggestions and expertise regarding our nAChR-associated gene set. Additionally, the authors would like to thank Dr Jaime Derringer for her work on the CADD-GADD dataset, as well as Dr Esther Lips for her invaluable assistance running JAG and performing the meta-analyses.

References

1. U.S. Department of Health and Human Services. *The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General*. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2014.
2. Gunby P. Surgeon General emphasizes nicotine addiction in annual report on tobacco use, consequences. *JAMA*. 1988;259(19):2811.
3. Stolerman IP, Jarvis MJ. The scientific case that nicotine is addictive. *Psychopharmacology (Berl)*. 1995;117(1):2–10; discussion 14–20.
4. Gotti C, Clementi F, Fornari A, et al. Structural and functional diversity of native brain neuronal nicotinic receptors. *Biochem Pharmacol*. 2009;78(7):703–711.
5. Berrettini WH, Doyle GA. The CHRNA5-A3-B4 gene cluster in nicotine addiction. *Mol Psychiatry*. 2012;17(9):856–866.
6. Thorgeirsson TE, Gudbjartsson DF, Surakka I, et al. Sequence variants at CHRNB3-CHRNA6 and CYP2A6 affect smoking behavior. *Nat Genet*. 2010;42(5):448–453.
7. Bierut LJ, Madden PA, Breslau N, et al. Novel genes identified in a high-density genome wide association study for nicotine dependence. *Hum Mol Genet*. 2007;16(1):24–35.
8. Saccone SF, Hinrichs AL, Saccone NL, et al. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Hum Mol Genet*. 2007;16(1):36–49.
9. Hoft NR, Corley RP, McQueen MB, Schlaepfer IR, Huizinga D, Ehringer MA. Genetic association of the CHRNA6 and CHRNB3 genes with tobacco dependence in a nationally representative sample. *Neuropsychopharmacology*. 2009;34(3):698–706.
10. Saccone NL, Saccone SF, Hinrichs AL, et al. Multiple distinct risk loci for nicotine dependence identified by dense coverage of the complete family of nicotinic receptor subunit (CHRN) genes. *Am J Med Genet B Neuropsychiatr Genet*. 2009;150B(4):453–466.
11. Saccone NL, Schwantes-An TH, Wang JC, et al. Multiple cholinergic nicotinic receptor genes affect nicotine dependence risk in African and European Americans. *Genes Brain Behav*. 2010;9(7):741–750.
12. Rice JP, Hartz SM, Agrawal A, et al. CHRNB3 is more strongly associated with Fagerstrom test for cigarette dependence-based nicotine dependence than cigarettes per day: phenotype definition changes genome-wide association studies results. *Addiction*. 2012;107(11):2019–2028.
13. Haller G, Druley T, Vallania FL, et al. Rare missense variants in CHRNB4 are associated with reduced risk of nicotine dependence. *Hum Mol Genet*. 2012;21(3):647–655.
14. Han S, Yang BZ, Kranzler HR, Oslin D, Anton R, Gelernter J. Association of CHRNA4 polymorphisms with smoking behavior in two populations. *Am J Med Genet B Neuropsychiatr Genet*. 2011;156B(4):421–429.
15. Chen HI, Shinkai T, Utsunomiya K, et al. Possible association of nicotinic acetylcholine receptor gene (CHRNA4 and CHRNB2) polymorphisms with nicotine dependence in Japanese males: an exploratory study. *Pharmacopsychiatry*. 2013;46(2):77–82.
16. Kamens HM, Corley RP, McQueen MB, et al. Nominal association with CHRNA4 variants and nicotine dependence. *Genes Brain Behav*. 2013;12(3):297–304.
17. Li MD, Beuten J, Ma JZ, et al. Ethnic- and gender-specific association of the nicotinic acetylcholine receptor alpha4 subunit gene (CHRNA4) with nicotine dependence. *Hum Mol Genet*. 2005;14(9):1211–1219.
18. Wessel J, McDonald SM, Hinds DA, et al. Resequencing of nicotinic acetylcholine receptor genes and association of common and rare variants with the Fagerström test for nicotine dependence. *Neuropsychopharmacology*. 2010;35(12):2392–2402.
19. Xie P, Kranzler HR, Krauthammer M, et al. Rare nonsynonymous variants in alpha-4 nicotinic acetylcholine receptor gene protect against nicotine dependence. *Biol Psychiatry*. 2011;70(6):528–536.
20. Kendler KS, Neale MC, Sullivan P, Corey LA, Gardner CO, Prescott CA. A population-based twin study in women of smoking initiation and nicotine dependence. *Psychol Med*. 1999;29(2):299–308.
21. True WR, Xian H, Scherrer JF, et al. Common genetic vulnerability for nicotine and alcohol dependence in men. *Arch Gen Psychiatry*. 1999;56(7):655–661.
22. Lessov CN, Martin NG, Statham DJ, et al. Defining nicotine dependence for genetic research: evidence from Australian twins. *Psychol Med*. 2004;34(5):865–879.
23. Vink JM, Beem AL, Posthuma D, et al. Linkage analysis of smoking initiation and quantity in Dutch sibling pairs. *Pharmacogenomics J*. 2004;4(4):274–282.
24. Broms U, Silventoinen K, Madden PA, Heath AC, Kaprio J. Genetic architecture of smoking behavior: a study of Finnish adult twins. *Twin Res Hum Genet*. 2006;9(1):64–72.
25. Regan EA, Hokanson JE, Murphy JR, et al. Genetic epidemiology of COPD (COPDGene) study design. *COPD*. 2010;7(1):32–43.
26. Karp I, O'Loughlin J, Paradis G, Hanley J, Difranza J. Smoking trajectories of adolescent novice smokers in a longitudinal study of tobacco use. *Ann Epidemiol*. 2005;15(6):445–452.
27. Lessov-Schlaggar CN, Hops H, Brigham J, et al. Adolescent smoking trajectories and nicotine dependence. *Nicotine Tob Res*. 2008;10(2):341–351.
28. Yang J, Benyamin B, McEvoy BP, et al. Common SNPs explain a large proportion of the heritability for human height. *Nat Genet*. 2010;42(7):565–569.
29. Lee SH, Wray NR, Goddard ME, Visscher PM. Estimating missing heritability for disease from genome-wide association studies. *Am J Hum Genet*. 2011;88(3):294–305.
30. Purcell SM, Wray NR, Stone JL, et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*. 2009;460(7256):748–752.
31. Wang L, Jia P, Wolfinger RD, Chen X, Zhao Z. Gene set analysis of genome-wide association studies: methodological issues and perspectives. *Genomics*. 2011;98(1):1–8.
32. Marks MJ, Burch JB, Collins AC. Effects of chronic nicotine infusion on tolerance development and nicotinic receptors. *J Pharmacol Exp Ther*. 1983;226(3):817–825.
33. Schwartz RD, Kellar KJ. Nicotinic cholinergic receptor binding sites in the brain: regulation in vivo. *Science*. 1983;220(4593):214–216.
34. Benwell ME, Balfour DJ, Anderson JM. Evidence that tobacco smoking increases the density of (–)-[3H]nicotine binding sites in human brain. *J Neurochem*. 1988;50(4):1243–1247.
35. Marks MJ, Pauly JR, Gross SD, et al. Nicotine binding and nicotinic receptor subunit RNA after chronic nicotine treatment. *J Neurosci*. 1992;12(7):2765–2784.
36. Darsow T, Booker TK, Piña-Crespo JC, Heinemann SF. Exocytic trafficking is required for nicotine-induced up-regulation of alpha 4 beta 2 nicotinic acetylcholine receptors. *J Biol Chem*. 2005;280(18):18311–18320.
37. Rezvani K, Teng Y, Shim D, De Biasi M. Nicotine regulates multiple synaptic proteins by inhibiting proteasomal activity. *J Neurosci*. 2007;27(39):10508–10519.
38. Rezvani K, Teng Y, Pan Y, et al. UBXD4, a UBX-containing protein, regulates the cell surface number and stability of alpha3-containing nicotinic acetylcholine receptors. *J Neurosci*. 2009;29(21):6883–6896.
39. Harkness PC, Millar NS. Changes in conformation and subcellular distribution of alpha4beta2 nicotinic acetylcholine receptors revealed by

chronic nicotine treatment and expression of subunit chimeras. *J Neurosci*. 2002;22(23):10172–10181.
40. Nashmi R, Dickinson ME, McKinney S, et al. Assembly of alpha4beta2 nicotinic acetylcholine receptors assessed with functional fluorescently labeled subunits: effects of localization, trafficking, and nicotine-induced upregulation in clonal mammalian cells and in cultured midbrain neurons. *J Neurosci*. 2003;23(37):11554–11567.
41. Sallette J, Pons S, Devillers-Thiery A, et al. Nicotine upregulates its own receptors through enhanced intracellular maturation. *Neuron*. 2005;46(4):595–607.
42. Henderson BJ, Lester HA. Inside-out neuropharmacology of nicotinic drugs. *Neuropharmacology*. 2015;96(Pt B):178–193.
43. Gopalakrishnan M, Molinari EJ, Sullivan JP. Regulation of human alpha4beta2 neuronal nicotinic acetylcholine receptors by cholinergic channel ligands and second messenger pathways. *Mol Pharmacol*. 1997;52(3):524–534.
44. Govind AP, Vezina P, Green WN. Nicotine-induced upregulation of nicotinic receptors: underlying mechanisms and relevance to nicotine addiction. *Biochem Pharmacol*. 2009;78(7):756–765.
45. Hagino N, Lee JW. Effect of maternal nicotine on the development of sites for [(3)H]nicotine binding in the fetal brain. *Int J Dev Neurosci*. 1985;3(5):567–571.
46. Navarro HA, Seidler FJ, Eylers JP, et al. Effects of prenatal nicotine exposure on development of central and peripheral cholinergic neurotransmitter systems. Evidence for cholinergic trophic influences in developing brain. *J Pharmacol Exp Ther*. 1989;251(3):894–900.
47. Popke EJ, Tizabi Y, Rahman MA, Nespor SM, Grunberg NE. Prenatal exposure to nicotine: effects on prepulse inhibition and central nicotinic receptors. *Pharmacol Biochem Behav*. 1997;58(4):843–849.
48. Slotkin TA, Orband-Miller L, Queen KL. Development of [3H]nicotine binding sites in brain regions of rats exposed to nicotine prenatally via maternal injections or infusions. *J Pharmacol Exp Ther*. 1987;242(1):232–237.
49. Tizabi Y, Perry DC. Prenatal nicotine exposure is associated with an increase in [125I]epibatidine binding in discrete cortical regions in rats. *Pharmacol Biochem Behav*. 2000;67(2):319–323.
50. Tizabi Y, Popke EJ, Rahman MA, Nespor SM, Grunberg NE. Hyperactivity induced by prenatal nicotine exposure is associated with an increase in cortical nicotinic receptors. *Pharmacol Biochem Behav*. 1997;58(1):141–146.
51. van de Kamp JL, Collins AC. Prenatal nicotine alters nicotinic receptor development in the mouse brain. *Pharmacol Biochem Behav*. 1994;47(4):889–900.
52. Abbott LC, Winzer-Serhan UH. Smoking during pregnancy: lessons learned from epidemiological studies and experimental studies using animal models. *Crit Rev Toxicol*. 2012;42(4):279–303.
53. Melroy-Greif WE, Stitzel JA, Ehringer MA. Nicotinic acetylcholine receptors: upregulation, age-related effects and associations with drug use. *Genes Brain Behav*. 2016;15(1):89–107.
54. Haberstick BC, Timberlake D, Ehringer MA, et al. Genes, time to first cigarette and nicotine dependence in a general population sample of young adults. *Addiction*. 2007;102(4):655–665.
55. Bierut LJ, Agrawal A, Bucholz KK, et al. A genome-wide association study of alcohol dependence. *Proc Natl Acad Sci U S A*. 2010;107(11):5082–5087.
56. Bild DE, Bluemke DA, Burke GL, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. *Am J Epidemiol*. 2002;156(9):871–881.
57. Derringer J, Corley RP, Haberstick BC, et al. Genome-wide association study of behavioral disinhibition in a selected adolescent sample. *Behav Genet*. 2015;45(4):375–381.
58. Abecasis GR, Auton A, Brooks LD, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature*. 2012;491(7422):56–65.
59. Heatherton TF, Kozlowski LT, Frecker RC, Fagerström KO. The Fagerström Test for Nicotine Dependence: a revision of the Fagerström Tolerance Questionnaire. *Br J Addict*. 1991;86(9):1119–1127.
60. Veyrieras JB, Kudaravalli S, Kim SY, et al. High-resolution mapping of expression-QTLs yields insight into human gene regulation. *PLoS Genet*. 2008;4(10):e1000214.
61. Lips ES, Kooyman M, de Leeuw C, Posthuma D. JAG: a computational tool to evaluate the role of gene-sets in complex traits. *Genes (Basel)*. 2015;6(2):238–251.
62. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81(3):559–575.
63. Whitlock MC. Combining probability from independent tests: the weighted Z-method is superior to Fisher's approach. *J Evol Biol*. 2005;18(5):1368–1373.
64. R Development Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2012. http://www.R-project.org.
65. Tobacco and Genetics Consortium. Genome-wide meta-analyses identify multiple loci associated with smoking behavior. *Nat Genet*. 2010;42(5):441–447.
66. de Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: generalized gene-set analysis of GWAS data. *PLoS Comput Biol*. 2015;11(4):e1004219.
67. Berrettini W, Yuan X, Tozzi F, et al. Alpha-5/alpha-3 nicotinic receptor subunit alleles increase risk for heavy smoking. *Mol Psychiatry*. 2008;13(4):368–373.
68. Stevens VL, Bierut LJ, Talbot JT, et al. Nicotinic receptor gene variants influence susceptibility to heavy smoking. *Cancer Epidemiol Biomarkers Prev*. 2008;17(12):3517–3525.
69. Caporaso N, Gu F, Chatterjee N, et al. Genome-wide and candidate gene association study of cigarette smoking behaviors. *PLoS One*. 2009;4(2):e4653.
70. Liu JZ, Tozzi F, Waterworth DM, et al. Meta-analysis and imputation refines the association of 15q25 with smoking quantity. *Nat Genet*. 2010;42(5):436–440.
71. Saccone NL, Culverhouse RC, Schwantes-An TH, et al. Multiple independent loci at chromosome 15q25.1 affect smoking quantity: a meta-analysis and comparison with lung cancer and COPD. *PLoS Genet*. 2010;6(8):e1001053.
72. Sarginson JE, Killen JD, Lazzeroni LC, et al. Markers in the 15q24 nicotinic receptor subunit gene cluster (CHRNA5-A3-B4) predict severity of nicotine addiction and response to smoking cessation therapy. *Am J Med Genet B Neuropsychiatr Genet*. 2011;156B(3):275–284.
73. Sorice R, Bione S, Sansanelli S, et al. Association of a variant in the CHRNA5-A3-B4 gene cluster region to heavy smoking in the Italian population. *Eur J Hum Genet*. 2011;19(5):593–596.
74. Cannon DS, Mermelstein RJ, Hedeker D, et al. Effect of neuronal nicotinic acetylcholine receptor genes (CHRN) on longitudinal cigarettes per day in adolescents and young adults. *Nicotine Tob Res*. 2014;16(2):137–144.
75. Gabrielsen ME, Romundstad P, Langhammer A, Krokan HE, Skorpen F. Association between a 15q25 gene variant, nicotine-related habits, lung cancer and COPD among 56,307 individuals from the HUNT study in Norway. *Eur J Hum Genet*. 2013;21(11):1293–1299.
76. Frederiksen B, Lutz S, Cho MH, et al., for the COPDGene Investigators. Genome-wide association study of nicotine dependence in COPDGene. In: Society for Research on Nicotine and Tobacco's 19th Annual Meeting; March 13–16; Boston, MA.
77. Thorgeirsson TE, Geller F, Sulem P, et al. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. *Nature*. 2008;452(7187):638–642.
78. Bierut LJ, Stitzel JA, Wang JC, et al. Variants in nicotinic receptors and risk for nicotine dependence. *Am J Psychiatry*. 2008;165(9):1163–1171.
79. Weiss RB, Baker TB, Cannon DS, et al. A candidate gene approach identifies the CHRNA5-A3-B4 region as a risk factor for age-dependent nicotine addiction. *PLoS Genet*. 2008;4(7):e1000125.
80. Spitz MR, Amos CI, Dong Q, Lin J, Wu X. The CHRNA5-A3 region on chromosome 15q24-25.1 is a risk factor both for nicotine dependence and for lung cancer. *J Natl Cancer Inst*. 2008;100(21):1552–1556.
81. Baker TB, Weiss RB, Bolt D, et al. Human neuronal acetylcholine receptor A5-A3-B4 haplotypes are associated with multiple nicotine dependence phenotypes. *Nicotine Tob Res*. 2009;11(7):785–796.
82. Saccone NL, Wang JC, Breslau N, et al. The CHRNA5-CHRNA3-CHRNB4 nicotinic receptor subunit gene cluster affects risk for nicotine dependence in African-Americans and in European-Americans. *Cancer Res*. 2009;69(17):6848–6856.

83. Chen X, Chen J, Williamson VS, et al. Variants in nicotinic acetylcholine receptors alpha5 and alpha3 increase risks to nicotine dependence. *Am J Med Genet B Neuropsychiatr Genet*. 2009;150B(7):926–933.
84. Sherva R, Kranzler HR, Yu Y, et al. Variation in nicotinic acetylcholine receptor genes is associated with multiple substance dependence phenotypes. *Neuropsychopharmacology*. 2010;35(9):1921–1931.
85. Erlich PM, Hoffman SN, Rukstalis M, et al. Nicotinic acetylcholine receptor genes on chromosome 15q25.1 are associated with nicotine and opioid dependence severity. *Hum Genet*. 2010;128(5):491–499.
86. Maes HH, Neale MC, Chen X, Chen J, Prescott CA, Kendler KS. A twin association study of nicotine dependence with markers in the CHRNA3 and CHRNA5 genes. *Behav Genet*. 2011;41(5):680–690.
87. Broms U, Wedenoja J, Largeau MR, et al. Analysis of detailed phenotype profiles reveals CHRNA5-CHRNA3-CHRNB4 gene cluster association with several nicotine dependence traits. *Nicotine Tob Res*. 2012;14(6):720–733.
88. David SP, Hamidovic A, Chen GK, et al. Genome-wide meta-analyses of smoking behaviors in African Americans. *Transl Psychiatry*. 2012;2:e119.
89. Levran O, Peles E, Randesi M, et al. Synaptic plasticity and signal transduction gene polymorphisms and vulnerability to drug addictions in populations of European or African ancestry. *CNS Neurosci Ther*. 2015;21(11):898–904.
90. Greenbaum L, Kanyas K, Karni O, et al. Why do young women smoke? I. Direct and interactive effects of environment, psychological characteristics and nicotinic cholinergic receptor genes. *Mol Psychiatry*. 2006;11(3):312–322, 223.
91. Chikova A, Bernard HU, Shchepotin IB, Grando SA. New associations of the genetic polymorphisms in nicotinic receptor genes with the risk of lung cancer. *Life Sci*. 2012;91(21–22):1103–1108.
92. Yang J, Wang S, Yang Z, et al. The contribution of rare and common variants in 30 genes to risk nicotine dependence. *Mol Psychiatry*. 2015;20(11):1467–1478.
93. Duncan AE, Lessov-Schlaggar CN, Sartor CE, Bucholz KK. Differences in time to onset of smoking and nicotine dependence by race/ethnicity in a Midwestern sample of adolescents and young adults from a high risk family study. *Drug Alcohol Depend*. 2012;125(1–2):140–145.
94. Chatila WM, Wynkoop WA, Vance G, Criner GJ. Smoking patterns in African Americans and whites with advanced COPD. *Chest*. 2004;125(1):15–21.
95. White HR, Nagin D, Replogle E, Stouthamer-Loeber M. Racial differences in trajectories of cigarette use. *Drug Alcohol Depend*. 2004;76(3):219–227.
96. Finkenauer R, Pomerleau CS, Snedecor SM, Pomerleau OF. Race differences in factors relating to smoking initiation. *Addict Behav*. 2009;34(12):1056–1059.
97. Edens EL, Glowinski AL, Pergadia ML, Lessov-Schlaggar CN, Bucholz KK. Nicotine addiction in light smoking African American mothers. *J Addict Med*. 2010;4(1):55–60.
98. Carter BL, Paris MM, Lam CY, et al. Real-time craving differences between black and white smokers. *Am J Addict*. 2010;19(2):136–140.
99. Hahn LP, Folsom AR, Sprafka JM, Norsted SW. Cigarette smoking and cessation behaviors among urban blacks and whites. *Public Health Rep*. 1990;105(3):290–295.
100. Kabat GC, Morabia A, Wynder EL. Comparison of smoking habits of blacks and whites in a case-control study. *Am J Public Health*. 1991;81(11):1483–1486.
101. Ellickson PL, Orlando M, Tucker JS, Klein DJ. From adolescence to young adulthood: racial/ethnic disparities in smoking. *Am J Public Health*. 2004;94(2):293–299.
102. Moolchan ET, Franken FH, Jaszyna-Gasior M. Adolescent nicotine metabolism: ethnoracial differences among dependent smokers. *Ethn Dis*. 2006;16(1):239–243.
103. Signorello LB, Cai Q, Tarone RE, McLaughlin JK, Blot WJ. Racial differences in serum cotinine levels of smokers. *Dis Markers*. 2009;27(5):187–192.
104. Pérez-Stable EJ, Herrera B, Jacob P III, Benowitz NL. Nicotine metabolism and intake in black and white smokers. *JAMA*. 1998;280(2):152–156.
105. St Helen G, Dempsey D, Wilson M, Jacob P III, Benowitz NL. Racial differences in the relationship between tobacco dependence and nicotine and carcinogen exposure. *Addiction*. 2013;108(3):607–617.
106. Benowitz NL, Dains KM, Dempsey D, Wilson M, Jacob P. Racial differences in the relationship between number of cigarettes smoked and nicotine and carcinogen exposure. *Nicotine Tob Res*. 2011;13(9):772–783.
107. Rubinstein ML, Shiffman S, Rait MA, Benowitz NL. Race, gender, and nicotine metabolism in adolescent smokers. *Nicotine Tob Res*. 2013;15(7):1311–1315.