Original investigation

Variants in the *CHRNA5–CHRNA3–CHRNA4* Region of Chromosome 15 Predict Gastrointestinal Adverse Events in the Transdisciplinary Tobacco Use Research Center Smoking Cessation Trial

Robert C. Culverhouse PhD^{1,2,*}, Li-Shiun Chen MD, MPH, ScD³,
Nancy L. Saccone PhD⁴, Yinjiao Ma MS, MPH³, Megan E. Piper PhD^{5,*},
Timothy B. Baker PhD⁵, Laura J. Bierut MD³

¹John T. Milliken Department of Medicine, Washington University School of Medicine, St. Louis, MO; ²Division of Biostatistics, Washington University School of Medicine, St. Louis, MO; ³Department of Psychiatry, Washington University School of Medicine, St. Louis, MO; ⁴Department of Genetics, Washington University School of Medicine, St. Louis, MO; ⁵Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI

Corresponding Author: Robert C. Culverhouse, PhD, Department of Medicine, Washington University School of Medicine, Box 8005, 4523 Clayton Avenue, St. Louis, MO 63110, USA. Telephone: 314-454-8712; Fax: 314-454-5113; E-mail: rculverh@wustl.edu

Abstract

Introduction: Reducing adverse events from pharmacologic treatment is an important goal of precision medicine and identifying genetic predictors of adverse events is a step toward this goal. In 2012, King et al. reported associations between genetic variants and adverse events in a placebo-controlled smoking cessation trial of varenicline and bupropion. Strong associations were found between gastrointestinal adverse events and 11 variants in the *CHRNA5–CHRNA3–CHRNA4* region of chromosome 15, a region repeatedly associated with smoking-related phenotypes. Our goal was to replicate, in an independent sample, the impact of variants in the *CHRNA5–CHRNA3–CHRNA4* region on gastrointestinal adverse events and to extend the analyses to adherence and smoking cessation.

Methods: The University of Wisconsin Transdisciplinary Tobacco Use Research Center (TTURC) conducted a multiarmed, placebo-controlled smoking cessation trial of bupropion and nicotine replacement therapy that included 985 genotyped European-ancestry participants. We evaluated relationships between our key variables using logistic regression.

Results: Gastrointestinal adverse events were experienced by 31.6% TTURC participants. Each of the *CHRNA5–CHRNA3–CHRNA4* associations from the King et al. study was found in TTURC, with the same direction of effect. Neither these variants nor the gastrointestinal adverse events themselves were associated with adherence to medication or successful smoking cessation.

Conclusions: Variants in the *CHRNA5–CHRNA3–CHRNA4* region of chromosome 15 are associated with gastrointestinal adverse events in smoking cessation. Additional independent variants in this region strengthen the association. The consistency between the results of these two independent studies supports the conclusion that these findings reflect biological response to the use of smoking cessation medication.

Implications: The fact that our findings from the TTURC smoking cessation trial support the independent findings of King et al. suggest that associations of variants in the *CHRNA5–CHRNA3–CHRNA4* region of chromosome 15 with gastrointestinal adverse events while taking medications

for smoking cessation reflect biology. However, although adherence to medication was a strong predictor of successful smoking cessation in TTURC, neither adverse events nor the genetic variants associated with them predicted either adherence or successful cessation in this study. Thus, although we should strive to minimize adverse events during treatment, we should not expect that to increase successful smoking cessation substantially.

Introduction

Cigarette smoking results in more than 480 000 premature deaths and \$300 billion in total economic cost in the United States each year, with nearly \$170 billion being spent on direct medical care for adults.¹⁻³ Tobacco use disorder is a chronic condition, with many smokers making repeated quit attempts⁴⁻¹⁰ before they achieve long-term success. In 2015, 68.0% adult smokers wanted to stop smoking and 55.4% made a quit attempt in the past year, but only 7.4% successfully quit.¹¹ Although smoking cessation pharmacotherapies are efficacious,¹² adverse events cause distress and discomfort. Reducing adverse events from pharmacological treatment is an important goal of precision medicine, and identifying genetic predictors of adverse events is a step toward this goal. In some cases, adverse events may also contribute to medication nonadherence. Reduced treatment adherence, in turn, is associated with decreased cessation success.¹³⁻¹⁶ Thus, it would be ideal to develop personalized treatment plans that minimize adverse medication experiences, reducing suffering and potentially making it easier for patients to use their medication as prescribed.^{17,18}

It is clear that genetic variants in nicotinic receptor subunits play a substantial role in the development of tobacco use disorder.¹⁹⁻²⁶ Growing evidence demonstrates that variants in these same genes play a role in smoking cessation.²⁷⁻³¹ It has been hypothesized that these variants may also be related to adverse events from smoking cessation medications, which could affect adherence and consequently treatment effectiveness. King et al.³² analyzed a Pfizer smoking cessation trial of varenicline and bupropion based on 1175 smokers of European ancestry and identified a chromosome 15q25 region with 11 variants associated with gastrointestinal (GI) adverse events. None of these variants were found to be associated with smoking cessation. This region contains the genes coding the nicotinic acetylcholine receptor subunits $\alpha 5$, $\alpha 3$, and $\beta 4$, the *CHRNA5-CHRNA3-CHRNA4* gene cluster. Genetic variants in this region have been robustly associated with a variety of smoking phenotypes including nicotine addiction, lung cancer, number of cigarettes smoked per day, delayed cessation, and response to cessation medications.^{19,20,27,33-36} Our goal was to determine if the association between adverse events and variants in the chromosome 15 region could be replicated in an independent dataset. In addition, we examined the role of adverse events and medication nonadherence in smoking cessation success.

Methods

The University of Wisconsin Transdisciplinary Tobacco Use Research Center (TTURC) conducted a multiarmed, placebo-controlled smoking cessation trial of nicotine replacement therapy (NRT) and bupropion as part of a 3-year smoking cessation and health outcomes study. The study recruited adult smokers from January 2005 to June 2007.³⁷ The trial used nicotine patches, nicotine lozenges, and bupropion pills, alone and in combination, along with placebo patches, lozenges, and pills as different arms of the trial. Our

analyses focused on the TTURC participants of European ancestry who were genotyped ($N = 985$).

Adherence Phenotypes

Adherence information was carefully collected during the period from medication initiation through 2 weeks past the target quit date using ecological momentary assessments, which queried participants multiple times a day, asking whether they had taken any medication during the day and, for NRT lozenge users, how many lozenges they had used. Our analyses focused on medication (active or placebo) use starting on the target quit date. A participant was considered adherent on any day that he or she reported taking any assigned medication. We examined a dichotomous adherence phenotype “2-week adherence” (coded as adherent if the participant reported having taken any assigned medication for at least 8 of the first 14 days after the target quit date).

Systematic Coding of Adverse Events

During the period when they were taking study medication, the participants had bi-weekly interviews with the study staff. Adverse events data were collected during these interviews. The adverse event forms included a free-text box for descriptions of the adverse events and checkboxes for severity information. This resulted in 3385 free-text reports of adverse events. Two trained reviewers independently coded each reported event based on a predefined classification system developed by our team. Whenever a report received discordant classifications, it underwent additional reviews culminating in confirmation by Dr Li-Shiun Chen, who made the final classification.

Cessation Outcomes

Two cessation outcomes were studied: short term (14 days) and end of treatment. The short-term cessation was coded as successful if the participant quit and did not relapse within the first 2 weeks after the target quit date. The date of a relapse was defined as the beginning of 7 consecutive days of a return to smoking. End-of-treatment abstinence was defined as 7-day point-prevalence abstinence 8 weeks after the target quit date, biochemically confirmed by exhaled carbon monoxide less than 10 parts per million.

Genotyping

Genotyping was performed by the Center for Inherited Disease Research at Johns Hopkins University using the Illumina Omni2.5 microarray (www.illumina.com). Data cleaning was led by the Gene Environment Association Studies Consortium Coordinating Center at the University of Washington (<https://www.biostat.washington.edu/research/centers/gac>). Information on key steps of the QC procedures for the genotypes is provided in [Supplementary Appendix](#). Imputed genotypes were generated using IMPUTE2³⁸ and the 1000 Genomes Phase 1 integrated variant set (https://mathgen.stats.ox.ac.uk/impute/data_download_1000G_phase1_integrated_SHAPEIT2.html). Of the 11 single nucleotide polymorphisms (SNPs) in the

15q25 region highlighted in the King et al. study, rs17406522, rs578776, and rs12443170 were genotyped in the TTURC study. The rest were imputed with high quality (minimum INFO score = 0.982).

The chromosome 15q25 region encompasses the *CHRNA5-CHRNA3-CHRNA4* gene cluster coding the nicotinic acetylcholine receptor subunits $\alpha 5$, $\alpha 3$, and $\beta 4$. To understand the relationships between variants in this region and smoking phenotypes, we tested each of the six possible pairwise associations among genotype, adverse events, adherence, and cessation.

The statistical analyses for this article were generated using the SAS/STAT® software, version [9] of the SAS System for Linux.³⁹

Results

Table 1 gives demographic and phenotypic information for the sample. The participants were 58.4% ($n = 575$) female and had a mean age of approximately 45 years (range: 18–79). NRT alone was assigned to 53% ($n = 522$) of the participants (lozenge only $n = 167$; patch only $n = 176$; combined lozenge and patch $n = 179$), bupropion alone was assigned to 17% ($n = 167$) participants, and combination therapy of bupropion and NRT was assigned to 18% ($n = 179$) participants. The remaining 12% ($n = 117$) received placebo medication.

GI adverse events were reported by 31.6% ($n = 311$) of the 985 participants, with participants in the bupropion-only arm the least likely to report an event (**Table 1**). Of those participants reporting GI adverse events, 74.0% ($n = 230$) reported only upper GI adverse events (eg, nausea, vomiting, and loss of appetite), 11.9% ($n = 37$) reported only lower GI adverse events, and 14.1% ($n = 44$) reported both upper and lower GI adverse events. Reports of GI adverse events varied by treatment group: NRT-only 36.8% (lozenge 48.5%, patch 17.6%, patch + lozenge 44.7%), bupropion-only 13.8%, combination NRT + bupropion 40.2%, and placebo 20.5%. The difference between placebo and active treatment (20.5% vs. 33.1%, respectively) was significant ($\chi^2_1 = 7.5$, $p = 6.1e - 3$) as were the differences across the active treatment arms ($\chi^2_2 = 42.5$, $p = 5.9e - 10$).

Approximately one-third of participants (33.2%, $n = 327$) were adherent to their assigned medication during the first 2 weeks after

their quit date, defined as taking the assigned medication on at least 8 of the first 14 days (**Table 1**). Adherence varied by assigned medication as follows: NRT-only 31.6% (lozenge 27.0%, patch 28.4%, patch + lozenge 39.1%), bupropion-only 37.1%, combination NRT + bupropion 38.6%, and placebo 26.5%. Although the placebo group was slightly less adherent than the combined active treatment groups (26.5% vs. 34.1%), the difference was not statistically significant ($\chi^2_1 = 2.7$, $p = .10$). However, the differences among the active treatment groups were statistically significant ($\chi^2_2 = 7.2$, $p = .03$).

Most of the participants successfully quit for the first 2 weeks after their target quit date (74.6%, $n = 735$; **Table 1**). The proportion of participants who successfully quit for 2 weeks without relapse in each treatment group were as follows: NRT-only 75.3% (lozenge 70.7%, patch 75.6%, patch + lozenge 79.3%), bupropion-only 74.3%, combination NRT + bupropion 79.9%, and placebo 59.0%. The placebo group was significantly less likely than the active treatment group to successfully quit for 2 weeks (59.0% vs. 76.0%; $\chi^2_1 = 15.6$, $p = 7.8e - 5$). In contrast, there were no statistically significant differences between the active treatment groups ($\chi^2_2 = 3.5$, $p = .17$) at the 2-week timepoint. However, by the end of 8 weeks of treatment, significant differences by treatment did emerge. Overall, 49.4% ($n = 487$) participants were abstinent at the end of treatment (ie, 7-day point-prevalence abstinence at 8 weeks post-target quit date, biochemically confirmed by exhaled carbon monoxide less than 10 parts per million). The proportion of participants who were abstinent after 8 weeks of treatment were as follows: NRT-only 50.2% [lozenge 41.9%, patch 47.2%, patch + lozenge 60.9%], bupropion-only 48.5%, combination NRT + bupropion 58.1%, and placebo 34.2%. Again, the placebo group had significantly less success than the active treatment arms (34.2% vs. 51.5%; $\chi^2_1 = 12.4$, $p = 4.4e - 4$). In contrast to the 2-week endpoint, for this timepoint, the active treatment groups also displayed significant differences ($\chi^2_2 = 13.4$, $p = 1.2e - 3$; **Table 1**).

An examination of the NRT-only subarms found significant differences in the likelihood of GI adverse events ($\chi^2_2 = 49.5$, $p = 3.2e - 9$),

Table 1. Demographic Information for the Transdisciplinary Tobacco Use Research Center (TTURC) Genotyped European American sample ($N = 985$)

Sex	N	Percent						
Female	575	58.4%						
Male	410	41.6%						
Age								
Mean (SD)	Min	Max						
44.8 (11.3)	18.0	79.0						
Days adherent (2 weeks post-TQD)								
Mean (SD)	Min	Max						
5.56 (3.94)	0	14						
Distribution of assigned medication (NRT includes patch, lozenge, or both)								
Bupropion only	NRT only	NRT+B	Neither (placebo)					
167 (17%)	522 (53%)	179 (18%)	117 (12%)					
Key phenotypes			N	Overall%	% by treatment			
Adverse events					NRT	Bupropion	NRT+B	Placebo
Experienced GI adverse events			311	31.6	36.8	13.8	40.2	20.5
Adherence								
Adherent ≥ 8 of first 14 days post-TQD			327	33.2	31.6	37.1	38.6	26.5
Cessation								
14-day no relapse			735	74.6	75.3	74.3	79.9	59.0
Abstinence at end of treatment ^a			487	49.4	50.2	48.5	58.1	34.2

NRT = nicotine replacement therapy; NRT+B = treatment with both NRT and bupropion; TQD = target quit date.

^a7-day point-prevalence biochemically confirmed abstinence at 8 weeks post-TQD

adherence during the first 2 weeks after the target quit date ($\chi^2_2 = 7.2$, $p = .028$), and abstinence at the end of treatment ($\chi^2_2 = 13.4$, $p = 3.8e-3$) but not in the likelihood of relapse during the first 2 weeks after the target quit date ($\chi^2_2 = 3.5$, $p = .17$). Of the NRT treatment arms, patch + lozenge had the highest adherence, intermediate likelihood of GI adverse events, the highest proportion of individuals with no relapse in the first 2 weeks of quitting, and by far the highest abstinence at the end of 8 weeks of treatment.

Genetic Variants in Chr15q25 Are Associated With GI Adverse Events

Results for the TTURC placebo-controlled study were remarkably consistent with those from the King et al. paper, even though the two studies examined different active medications (TTURC: NRT, bupropion; King: varenicline, bupropion). Table 2 provides the odds ratios (ORs) and p values for the SNP genetic variants reported in the TTURC study and the King et al. paper, both based on all treatment groups, including placebo. The two SNPs with the strongest associations are rs684513 and rs12443170. For both, the minor allele is protective with an OR = 0.62 (p values = $7.4e-4$ and $3.9e-3$, respectively). We also note that all 11 SNPs in Table 2 displayed the same direction of effect as found in King et al. study; the probability that this consistency of direction would occur by chance alone is only $4.9e-4$. Meta-analysis results across the two studies, using the conservative random effects model, are listed in the final column of Table 2. Of the eleven variants, eight displayed meta-analysis p values less than $4.5e-3$, the threshold for statistical significance after a Bonferroni correction for 11 tests.

In addition, after examining analyses stratified by treatment, including placebo, we found the genetic associations were consistent across treatment groups, suggesting no evidence for gene by treatment interactions. Overall, the results were similar across

the strata—although the placebo group was underpowered to detect statistical significance because of the much smaller sample size. The different treatment groups are associated with differences in the likelihood of GI adverse events, but we found no statistical evidence of a genotype by treatment group interaction in our analysis.

The coding SNP rs16969968 in *CHRNA5* is of particular interest. It is known to be associated with multiple smoking phenotypes and has previously been shown to be associated with cigarettes smoked per day and smoking cessation in the TTURC data.²⁷ Surprisingly, it is not associated with adverse GI events in these data (OR = 0.97, 95% confidence interval [CI] = 0.79% to 1.20%, $p = .77$). Supplementary Figure 1 is a triangle plot representing the disequilibrium among these SNPs in terms of r^2 values. This figure illustrates that although rs16969968 lies in the region of interest, it is distinct from the other SNPs under consideration because of low correlation with those SNPs.

Because the King et al. paper was based on a limited number of genotyped SNPs in this region, we expanded our study to assess all genotyped common and low frequency SNPs (minor allele frequency > 0.01) in the surrounding region, from 50 kb upstream of gene *IREB2* to 50 kb downstream of gene *ADAMT57* (bp 7690618-79153773), to determine if they might jointly explain more of the variability in GI adverse events. In this analysis, we examined all 233 low frequency and common variants the region genotyped in the TTURC sample using forward regression. Because forward regression requires that no data are missing, we used our imputed data to complete the 233 genotypes for the TTURC subjects (with minimum INFO score = 0.982). This analysis identified seven SNPs additional SNPs in the region that each independently contributed to the total association between this region and GI adverse events (ie, the addition of any one of the seven SNPs to a model that

Table 2. Associations of Chromosome 15 SNPs With Gastrointestinal Adverse Events in the European-American TTURC Data ($N = 985$, N Affected = 311) and King et al.³² data

SNP	Gene	Minor (major)	TTURC results				King et al. results			Meta-analysis (random effects)	
			MAF TTURC	OR SNP	95% CI for OR	p SNP	MAF Pfizer	OR SNP	p SNP	OR SNP	p SNP
rs12899425	Near <i>IREB2</i>	A (G)	0.267	1.28	1.04 to 1.58	.022	0.274	1.39	$8.7e-3$	1.33	$2.8e-4$
rs17406522	<i>IREB2</i>	G (A)	0.068	1.16	0.81 to 1.67	.409	0.072	1.67	.012	1.37	.039
rs4243083	<i>PSMA4</i>	C (G)	0.431	1.37	1.12 to 1.68	$2.6e-3$	0.416	1.40	$4.9e-3$	1.38	$1.8e-5$
rs684513	<i>CHRNA5</i>	G (C)	0.179	0.62	0.48 to 0.83	$7.0e-4$	0.197	0.65	$7.6e-3$	0.63	$1.5e-5$
rs578776	<i>CHRNA3</i>	A (G)	0.254	0.72	0.57 to 0.91	$5.0e-3$	0.273	0.67	$3.9e-3$	0.70	$3.6e-5$
rs2869546	<i>CHRNA3</i>	C (T)	0.390	1.32	1.08 to 1.63	$8.9e-3$	0.370	1.37	$8.8e-3$	1.34	$1.2e-4$
rs12443170	<i>CHRNA3</i>	A (G)	0.099	0.62	0.45 to 0.86	$3.9e-3$	0.126	0.62	.013	0.62	$7.5e-5$
rs1878399	<i>CHRNA3</i>	G (C)	0.436	1.33	1.09 to 1.63	$5.7e-3$	0.424	1.48	$1.2e-3$	1.39	$1.4e-5$
rs6495309	Between <i>CHRNA3-B4</i>	T (C)	0.196	0.74	0.57 to 0.95	.017	0.205	0.50	$4.0e-5$	0.62	$6.9e-3$
rs4887072	<i>CHRNA4</i>	G (A)	0.200	0.74	0.58 to 0.96	.018	0.216	0.55	$2.0e-4$	0.65	$1.6e-3$
rs12441998	<i>CHRNA4</i>	G (A)	0.176	0.86	0.67 to 1.11	.250	0.199	0.68	.015	0.78	.015

Model: adverse event^a = sex^b + bupropion^{c,d} + NRT^{c,e} + SNP^f

MAF = minor allele frequency; OR = odds ratio; SNPs = single nucleotide polymorphisms; TTURC = Transdisciplinary Tobacco Use Research Center.

^aAdverse event outcome = 1 if a participant reported any treatment-related adverse event, 0 otherwise.

^bSex coding: male = 0; female = 1. Being female was protective (maximum OR = 0.66, maximum $p = 3.6e-3$).

^cCoding for medication variables (bupropion, nicotine replacement therapy (NRT)): each was coded 1 if it was assigned to the participant, 0 if it was not assigned. Thus, these analyses are based on intention to treat.

^dBupropion was never significant (minimum $p = .88$)

^eNRT consistently increases risk for gastrointestinal adverse events. Minimum OR = 3.03, maximum $p = 2.8e-9$.

^fSNPs were coded additively in the number of minor alleles carried by an individual.

already included the other six SNPs was nominally significant [$p < .05$]). These SNPs add substantially to the fit of the model that includes the only statistically significant nongenetic factors tested (sex, NRT treatment), increasing the area under the curve from 0.632 for the model without the SNPs to 0.696 when the SNPs are included ($p = 1.4\text{e-}7$; Table 3). In terms of linkage disequilibrium, the maximum R^2 between any two was less than 0.1. In addition, when these seven SNPs are added to the model including sex, NRT, and the King et al. SNPs, the expanded model provides a statistically significant further improvement in the prediction of GI adverse events (area under the curve = 0.717, $p = 2.4\text{e-}5$; Table 3). Receiver operator characteristic (ROC) curves representing these results are provided in Supplementary Figure 2.

Neither Genetic Predictors of Adverse Events Nor GI Adverse Events Themselves Were Associated With Adherence in the TTURC Study

We found no statistically significant evidence of association between any of the adverse event SNPs identified in the King et al. paper and adherence to medication assessed over the first 2 weeks in the TTURC data (Supplementary Table 1). Although one of the eleven SNPs was nominally significant ($p = .01$), this does not pass a Bonferroni correction for the 11 tests. Similarly, none of the seven SNPs in this region identified by forward selection as associated with GI adverse events was significantly associated with 2-week adherence to medication. Moreover, we found no association in these data between reports of GI adverse events and 2-week adherence (OR = 1.01, 95% CI = 0.75% to 1.35%, $p = .95$). In contrast, being female (OR = 1.42, 95% CI = 1.08% to 1.87%, $p = .01$), being older ($\beta = .014$, 95% CI = 0.002% to 0.026%, $p = .03$), and being assigned bupropion (OR = 1.43, 95% CI = 1.07% to 1.91%, $p = .02$) were all associated with increased adherence. NRT was not significantly

associated with adherence (OR = 1.14, 95% CI = 0.83% to 1.57%, $p = .42$).

Adherence Was Strongly Associated With Successful Smoking Cessation

As has been found in previous studies, adherence to assigned medication was associated with successful cessation. Adherence during the first 2 weeks (defined as using assigned medication on at least 8 of the first 14 days) was strongly associated with successful 2-week cessation (OR = 2.05, 95% CI = 1.46% to 2.87%, $p = 3.3\text{e-}5$) and remained strongly associated with abstinence at the end of treatment (OR = 1.93, 95% CI = 1.47% to 2.54%, $p = 2.5\text{e-}6$; Supplementary Table 2).

Occurrence of GI Adverse Events Was Not Associated With Cessation Outcomes

Neither the 2-week nor the 8-week cessation endpoint was significantly associated with GI adverse events (minimum $p = .09$; Supplementary Table 3).

Genetic Variants Associated With GI Adverse Events Were Not Associated With Smoking Cessation Outcomes

The SNPs in this region associated with GI adverse events in our data, including those identified in King et al. study, were not associated with medication adherence or successful cessation (minimum $p = .15$; Supplementary Table 4). Our previous work has shown that rs16969968, a coding SNP in this region associated with multiple nicotine use disorder phenotypes, is associated with smoking cessation.³⁶ Although rs16969968 lies in physical proximity to the adverse event SNPs, it is statistically independent from them.

Table 3. Additional SNPs in the *CHRNA5-CHRNA3-CHRNA4* Gene Cluster Region of Chromosome 15, Identified Through Forward Selection (FS) Regression, Increase the Association to Gastrointestinal Adverse Events Beyond the Association Because of the King et al.³⁰ SNPs Alone

Model	AUC	Comparison model	p Value improved fit
Sex + NRT ^a	0.632		
King SNPs + sex + NRT	0.681	Sex + NRT	.002
FS SNPs + sex + NRT	0.696	Sex + NRT	1.4e-7
FS SNPs + King SNPs + sex + NRT	0.717	King SNPs + sex + NRT	2.4e-5

Effect estimates for factors other than the King SNPs					
Factor	Minor ^b (major)	MAF	OR	95% CI for OR	P^c
Sex			1.47	1.10 to 1.97	.0097
NRT			3.04	2.12 to 4.34	<.0001
SNPs					
rs28526814	T (C)	0.24	0.75	0.59 to 0.96	.0197
rs10519198	C (A)	0.43	1.33	1.07 to 1.66	.0117
rs3813570	T (C)	0.19	0.66	0.49 to 0.89	.0063
rs73467256	C (T)	0.02	2.78	1.34 to 5.74	.0059
rs8041377	A (G)	0.02	2.54	1.21 to 5.32	.0137
rs11634042	C (T)	0.44	1.70	1.25 to 2.32	.0008
rs7168915	A (G)	0.47	0.58	0.43 to 0.78	.0004

AUC = area under the receiver-operator characteristics Curve estimated from the c -statistic; CI = confidence interval; MAF = minor allele frequency; OR = odds ratio; SNPs = single nucleotide polymorphisms.

^aNicotine replacement therapy was highly significant; bupropion was not and so was not included in these models.

^bSNPs in the model were coded additively in the number of minor alleles carried by an individual.

^cListed p values represent the significance when the term is added to the model containing all the other variables.

Summary of Results

Figure 1 summarizes all of our results for the relationships between SNPs in the *CHRNA5* region of chromosome 15q25, GI adverse events in the TTURC smoking cessation trial, adherence to medication in the trial, and successful smoking cessation. The TTURC trial results reproduced the associations identified in the earlier, independent King et al. paper while identifying additional independent SNPs that increase the association between this region and GI adverse events. In analyses stratified by treatment, the genetic associations for GI adverse events were similar across all treatment groups, including placebo. We do not see evidence of gene by treatment interactions impacting the likelihood of GI adverse events. As expected, adherence to medication was highly associated with successful cessation, with use of medication for at least 8 of the first 14 days after the target quit date predicting 7-day abstinence after 8 weeks ($p = 2.3 \times 10^{-13}$). However, we do not find evidence in the TTURC data that these genetic variants or the associated GI adverse events are associated with either adherence or smoking cessation.

Discussion

Smoking cessation is a complex behavior influenced by multiple factors including pharmacologic treatment, the degree of adherence to treatment, and genetic factors. In our analysis of data from a large randomized, placebo-controlled smoking cessation trial, we tease apart some of these interrelated factors. GI adverse events are common, have been associated with the use of both NRT and bupropion, and were the adverse events most strongly associated with genetic variation in the King et al. study. Our analysis began

with previously identified genetic predictors of adverse GI events, and we confirmed this association between genetic variants in the *CHRNA5-CHRNA3-CHRNA4* region with adverse GI events in the current smoking cessation trial. The gut, which is extensively innervated, expresses the nicotinic acetylcholine receptor subunits $\alpha 5$, $\alpha 3$, and $\beta 4$ that are encoded in this region of chromosome 15 (c.f. the Genotype-Tissue Expression project website www.gtexportal.org). These results replicated findings from King et al.,³² and the results for these two studies were remarkably consistent: for each of the 11 SNPs examined, the point-estimate direction of effect is the same for both studies, and for many of the SNPs the OR estimates are very similar between the two studies.

The comprehensive assessments obtained in the TTURC study provided the opportunity to study the relationships among adverse GI events, adherence, smoking cessation, and genetic variants. An unexpected finding was that the GI adverse events reported by the TTURC participants did not predict either adherence (Supplementary Table 2) or successful smoking cessation (Supplementary Table 3). Although we cannot be certain of the reasons, we speculate that those who experienced adverse events may have been a mixed group composed of some individuals who were discouraged by the adverse events, leading to nonadherence, and some individuals who experienced the adverse event because they were taking medication more adherently. This does not rule out a weaker association between these variants and smoking cessation, for which the TTURC sample is underpowered. In fact, although a recent meta-analysis of up to 622 409 individuals did not find genome-wide association studies (GWAS) significant association between this region and smoking cessation,²⁶ another meta-analysis of up to 1.2 million individuals did.²⁵

Figure 1. Results for relationships among factors examined in the Transdisciplinary Tobacco Use Research Center placebo-controlled smoking cessation trial.

As expected, results of the TTURC study show that adherence to medication during the first 2 weeks of a quit attempt was strongly associated with abstinence during those 2 weeks (OR = 2.05; 95% CI = 1.46% to 2.87%, $p = 3.3 \times 10^{-5}$) and with successful 7-day point-prevalence abstinence at the end of treatment (OR = 1.93; 95% CI = 1.47% to 2.54%, $p = 2.5 \times 10^{-6}$; Supplementary Table 2). This provides further support for the accepted wisdom that medications to assist smoking cessation can be highly effective, but only if taken as prescribed.

We also examined the association between genetic variants in the chromosome 15q25 region and adherence and smoking cessation. The SNPs associated with adverse GI events demonstrated no significant association with adherence or successful smoking cessation (Supplementary Table 4). Although the lack of significance could be due to insufficient power, a recent meta-analysis of smoking behaviors for 622 409 individuals did not find GWAS association between any of these SNPs and smoking cessation.²⁶ Given the observed disconnect between adverse events and adherence as well as smoking cessation in this sample, this was not surprising.

Although none of the chromosome15q25 “adverse event” SNPs was associated with cessation, we previously found an association between cessation and a nearby, but statistically independent, SNP having a strong association to nicotine use disorder (rs16969968).²⁷ The distinct phenotypic associations arising from SNPs that are physically near each other reflect the complex genetic architecture of this region. In spite of the physical proximity of the genetic variants, rs16969968 lies on a haplotype that is distinct from the haplotypes containing the SNPs associated with GI adverse events.

We also extended our examination of the region beyond the limited set of SNPs highlighted in King et al. paper. Using an agnostic approach (forward selection), we identified seven additional statistically independent SNPs in the region that by themselves provide a better discriminatory model for predicting GI adverse events than the model using only the King et al. SNPs. Adding the seven new SNPs to the SNPs from the King et al. paper results in a significant improvement in the model ($p = 2.41 \times 10^{-5}$). This suggests that the association between this region and GI adverse events is not fully explained by the variants identified in King et al. study. The functional variants underlying this signal remain to be elucidated.

Both the active medication (NRT, bupropion), including delivery mode for NRT (lozenge, patch, patch + lozenge), and multiple genetic variants in the *CHRNA5-CHRNA3-CHRNA4* region of chromosome 15 were associated with the likelihood of GI adverse events in the TTURC data. We examined the possibility of a treatment by genetic interaction using both analyses stratified by treatment and using explicit modeling of interaction terms. We did not see evidence of a genotype by treatment interaction in these analyses.

The major limitation of this study is that we are unable to determine how these findings might generalize to other ancestry groups. Allele frequencies in this region differ dramatically across ancestral populations. Given the effect sizes seen for these phenotypes, the TTURC sample only had power to examine the European ancestry subsample. Though we expect that genetic variation in this region contributes to GI side effects across ancestral groups, we cannot identify which specific variants. Another limitation is that we examined adverse events from medications that differ from the previous study by King et al. (King: varenicline, bupropion; TTURC: NRT, bupropion). Nonetheless, we find the same set of SNPs highlighted for adverse events in our study and in King et al. The association between the *CHRNA5-CHRNA3-CHRNA4* region of chromosome 15 and GI adverse events in two independent studies supports the thesis that

these associations represent a true biological effect. In contrast, the data from the TTURC study did not replicate the finding of Ware et al.⁴⁰ of an association between adherence to NRT during a smoking cessation attempt and rs1051730, an SNP in our region of interest that is highly correlated with rs16969968. Finally, neither the King et al. trial nor the TTURC study was designed to study adverse events as a primary outcome. As a result, the number of reported adverse events of any specific type was modest and this study is underpowered.

Conclusion

This study found that there are replicable associations between genetic variants near the gene cluster on chromosome 15 encoding nicotinic acetylcholine receptor subunits $\alpha 5$, $\alpha 3$, and $\beta 4$ and GI adverse events during smoking cessation attempts. However, these “adverse event” variants were not associated with either 2-week or 8-week cessation in the TTURC data. This finding is less surprising in light of the fact that although adherence to medication is strongly associated with successful smoking cessation, neither GI adverse events nor the genetic variants associated with them were associated with adherence. However, we note that a recent meta-analysis of up to 1.2 million individuals did report association between our region of interest and smoking cessation.²⁵ These SNPs are statistically independent from the $\alpha 5$ amino acid change, rs16969968, in the region that is associated with heaviness of smoking, smoking cessation, lung cancer, and chronic obstructive pulmonary disorder. To understand the biology underlying these genetic associations to GI adverse events will require examination of the function of the identified SNPs, but the current findings already provide considerable power to predict GI adverse events in this setting (area under the curve = 0.717).

This work supports an association between genetic variants in the *CHRNA5-CHRNA3-CHRNA4* region of chromosome 15 and GI adverse events during a smoking cessation attempt. Reducing adverse events is an important goal of precision medicine, and better understanding of how genetic variation in this region affects individual response to smoking cessation will be a step toward this goal.

Supplementary Material

Supplementary data are available at *Nicotine and Tobacco Research* online.

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Declaration of Interests

LJ Bierut is listed as inventor on Issued U.S. Patent 8,080,371, “Markers for Addiction” covering the use of certain single nucleotide polymorphisms in determining the diagnosis, prognosis, and treatment of addiction. The spouse of NL Saccone is also listed as an inventor on the patent.

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