Tobacco smoking is the largest preventable cause of premature death in the United States. There are currently 45 million smokers in the United States, and only 3% of them are able to quit smoking each year. Twin studies have estimated that the heritability of smoking cessation is roughly 50%, suggesting that genetic factors play an important role in determining smoking cessation outcomes. A number of genes have been significantly associated with smoking cessation outcomes among Caucasians. However, relatively few studies have focused on African-American smokers despite their comparatively higher risks of smoking-related morbidity and mortality.

Nicotine exerts its pharmacological effects by acting on nicotinic cholinergic receptors in the brain. In Caucasians, multiple independent single-nucleotide polymorphisms (SNPs) in the CHRNA5-A3-B4 gene cluster, which encodes for the α5, α3, and β4 subunits of the nicotinic acetylcholine receptor (nAChR), have been shown to be associated with smoking quantity and smoking cessation outcomes. These loci include rs16969968 and correlated SNPs (sometimes referred to as "Bin A" or "Locus 1"), rs588765 and correlated SNPs (sometimes referred to as "Bin B" or "Locus 3"), and rs578776 and correlated SNPs (sometimes referred to as "Bin C" or "Locus 2"). However, it is not clear whether the influence of CHRNA5-A3-B4 gene variants on smoking cessation is a general effect on smoking cessation (i.e., would alter cessation in placebo treatment), is independent of the specific type of pharmacological treatment (i.e., would alter all active treatments), or alters cessation for specific pharmacological treatments (i.e., nicotine patch). For example, one study suggested that the association between the two CHRNA5-A3-B4 variants rs16969968 (Bin A) and rs680244 (Bin B) and smoking cessation is primarily observed among smokers treated with placebo and is not observed among those who receive active pharmacotherapy.

Associations between CHRNA5-A3-B4 variants and smoking behaviors exist; however, the association with smoking abstinence is less understood, particularly that among African Americans. In 1,295 African Americans enrolled in two clinical trials, we investigated the association between CHRNA5-A3-B4 and smoking abstinence. The rs2056527(A) allele was associated with lower abstinence with active pharmacotherapy (during treatment: odds ratio (OR) = 0.42, \( P < 0.001 \); end of treatment (EOT): OR = 0.55, \( P = 0.004 \)), or with nicotine gum alone (during treatment: OR = 0.31, \( P < 0.001 \); EOT: OR = 0.51, \( P = 0.02 \)), but not significantly with bupropion, although similar directions and magnitudes were observed (during treatment: OR = 0.54, \( P = 0.05 \); EOT: OR = 0.59, \( P = 0.08 \)). In addition, the rs588765(T) allele was associated with abstinence with gum during treatment (OR = 2.31, \( P < 0.01 \)). The SNP rs16969968 occurred at a low frequency and was not consistently associated with abstinence. CHRNA5-A3-B4 variants were not associated with tobacco consumption, and adjustments for smoking behaviors did not alter the associations with smoking abstinence. Together, our data suggest that among African Americans, CHRNA5-A3-B4 variants are not associated with baseline smoking but can influence smoking abstinence during active pharmacotherapy.
variant rs1051730 (which is in high linkage disequilibrium with rs16969968) and smoking cessation outcomes in smokers who were receiving nicotine replacement therapy, with little effect observed in the placebo arm. A recent study also observed a treatment-by-genotype interaction on smoking cessation outcomes (i.e., the genotype effects are in opposite directions for placebo vs. nicotine replacement therapy). Among African Americans, the associations between CHRNA5-A3-B4 gene variants and smoking behaviors are not as well understood. Although some studies have reported positive associations between rs16969968 and smoking behaviors among African-American smokers, other studies were not able to replicate this finding. The low allele frequency of rs16969968 (0–8% in African Americans, compared with 38–40% in Caucasians) could contribute to this discrepancy. A very large genome-wide meta-analysis among African-American smokers (N = 32,389) identified a significant association between rs2036527 within CHRNA5-A3-B4 and number of self-reported cigarettes per day (CPD) but no significant associations between rs16969968 or rs1051730 and CPD. We are not aware of any studies that have investigated the association between CHRNA5-A3-B4 gene variants and smoking cessation among African-American smokers.

Here we used two placebo-controlled clinical trials to compare the influence of CHRNA5-A3-B4 variants on smoking cessation outcomes among African Americans treated with placebo and active pharmacological treatments. We hypothesized that there would be a significant association between CHRNA5-A3-B4 gene variants and smoking cessation outcomes in the active pharmacological treatment arms, as previously reported by Munafò et al. Because rs16969968 occurs at a relatively low allele frequency in African Americans, we focused initially on rs2036527, which has previously been significantly associated with CPD and lung cancer risk among African Americans; we then extended the investigation to rs16969968 ("Bin A"), rs588765 ("Bin B"), and rs578776 ("Bin C").

RESULTS
Descriptive data of the participants
DNA samples from 1,143 participants among the 1,295 participants enrolled in the two studies were extracted from blood and genotyped (609 in Study 1 and 534 in Study 2). All four of the genotyped SNPs were in Hardy–Weinberg equilibrium (all P > 0.05, Supplementary Table S1 online). The SNP rs2036527 was selected for its role in influencing smoking behaviors among African Americans, whereas rs16969968 ("Bin A"), rs588765 ("Bin B"), and rs578776 ("Bin C") were selected to represent the three different haplotype bins that were previously shown to be associated with smoking behaviors. The SNPs rs2036527, rs16969968, rs588765, and rs578776 had minor allele frequencies of 22.3, 5.7, 31.7, and 47.9%, respectively. These four SNPs were in low linkage disequilibrium (all R^2 = 0.02–0.39), suggesting that they were independent of each other (Supplementary Table S1 online). None of the four genotyped SNPs were significantly associated with baseline demographics, CPD, percentage smoking mentholated cigarettes, baseline plasma cotinine levels, baseline levels of total nicotine equivalents, levels of nicotine dependence, or treatment group assignment (Table 1 and Supplementary Table S2 online).

Associations of CHRNA5-A3-B4 variants with smoking abstinence
Figure 1 summarizes the association between the four CHRNA5-A3-B4 variants and verified smoking abstinence at the different time points assessed among the participants who received the active nicotine gum treatment (Study 1, Figure 1a), the active bupropion treatment (Study 2, Figure 1b), any active pharmacological treatment (Studies 1 and 2, Figure 1c), or the placebo treatment (Studies 1 and 2, Figure 1d). For additive models, see Supplementary Figure S1 online. Some notable impacts of the variants on abstinence are highlighted below.

Association between rs2036527 and smoking abstinence
Overall, we found that the “A” allele of rs2036527 was associated with lower abstinence rates during, and at the end of, the treatment period in participants who received active pharmacotherapy but not in those who received placebo (see Figure 2c for statistical comparisons). Specifically, among the participants who received the active nicotine gum treatment (Study 1), those with the “A” allele of rs2036527 had a similar magnitude of reduced abstinence throughout treatment, which diminished during follow-up (during-treatment OR = 0.31 and end-of-treatment (EOT) OR = 0.51, Figure 2a); adjusting for age, sex, baseline CPD, menthol status, and type of counseling sessions did not meaningfully alter this (during-treatment OR = 0.29 and EOT OR = 0.48, Figure 2a). Likewise, adjusting for
CYP2A6 genotype, encoding the main nicotine-metabolizing enzyme, did not alter the ORs for the association between rs2036527 and smoking abstinence (during-treatment OR = 0.31 and EOT OR = 0.51). In the second study, a similar direction and magnitude of the rs2036527 effect was observed in those receiving the active bupropion treatment; this was not statistically significant (during-treatment OR = 0.54 and EOT OR = 0.59, Figure 2b). Adjusting for age, sex, baseline CPD, menthol status, type of counseling sessions, and type of pharmacological treatment did not alter the association between rs2036527 and smoking abstinence (during-treatment OR = 0.42 and EOT OR = 0.55, Figure 2c). rs2036527 was not associated with smoking abstinence at 6-month follow-up. The number needed to treat was 8.9 for the GG genotype group during active treatment (active treatment had no benefit in the GA/AA genotype group). At EOT, the numbers needed to treat were 9.3 and 22.8 for the GG and the GA/AA groups, respectively. No statistically significant association between rs2036527 and smoking abstinence was observed at any time point in the placebo arm, including after adjustment for age, sex, baseline CPD, menthol status, and type of counseling sessions (Figures 1d and 2c).

The association of rs588765 with smoking abstinence

Overall, and in contrast to rs2036527, the “T” allele of rs588765 was associated with short-term smoking abstinence.
pharmacological treatment, the “T” allele of rs588765 was associated with increased smoking abstinence during treatment (OR = 1.81, Figure 3c).

The associations (dominant model) between CHRNA5-A3-B4 variants and smoking abstinence among African-American smokers. (a) Participants who received active nicotine gum treatment (Study 1). (b) Participants who received active bupropion treatment (Study 2). (c) Participants who received active pharmacological treatments (combined analysis). (d) Participants who received placebo (combined analysis). $P$ values <0.0125 were considered statistically significant due to multiple-comparison adjustments. Statistically significant values are shown in bold.

When analyzed together to investigate the role of active pharmacological treatment, the “T” allele of rs588765 was associated with increased smoking abstinence during treatment (OR = 1.85, Figures 1c and 3c). Adjusting for age, sex, baseline CPD, menthol status, type of counseling sessions, and type of pharmacological treatment did not meaningfully alter the association between rs588765 and smoking abstinence (OR = 1.81, Figure 3c). Among the participants who received the placebo treatment (Figures 1d and 3c), the “T” allele of rs588765 was associated with reduced abstinence during treatment (OR = 0.49, Figures 1d and 3c); adjusting for age, sex, baseline CPD, menthol status, and type of counseling sessions did not meaningfully alter the ORs for the association between rs588765 and smoking abstinence during treatment (OR = 0.47; Figure 3c). There was also a significant treatment (combined active vs. combined placebo)-by-rs588765-genotype interaction during treatment (OR = 3.75; $P < 0.001$).

rs588765 was not associated with smoking abstinence at EOT or at 6-month follow-up.

The association of rs16969968 or rs578776 with smoking abstinence

In these two clinical trials of African-American smokers, although some trends were apparent, there was no consistent association between rs16969968 or rs578776 and smoking abstinence with active or placebo treatment, at any time point, with or without baseline demographic adjustments (Figures 1 and 4).

DISCUSSION

We reported novel findings of an association between a CHRNA5-A3-B4 gene variant and smoking abstinence among
African Americans. We identified a consistent association between the "A" allele of rs2036527 and lower rates of smoking abstinence during active pharmacotherapy treatment among African-American light smokers.

**CHRNA5-A3-B4 and smoking behaviors**

Gene variants in *CHRNA5-A3-B4*, particularly rs16969968 and correlated SNPs, have been consistently associated with heaviness of smoking and the levels of nicotine dependence among Caucasians. In contrast to a recent meta-analysis among African-American smokers, in the current study, we did not observe any significant association between *CHRNA5-A3-B4* variants, particularly rs2036527, and baseline smoking behaviors, including self-reported CPD, plasma cotinine levels, or urinary total nicotine equivalents. This was unexpected because our study, although smaller in size as compared with the meta-analysis, had objective biomarker data. Because it is well known that self-reported CPD does not account for the depth of inhalation and is a relatively weak marker of tobacco exposure, we expected the accuracy of biomarker data to compensate for the smaller sample size. One reason for the lack of association between tobacco exposure and rs2036527 in our study
may be that the previous meta-analyses included a substantial number of former smokers, whereas our studies included only current smokers. It was previously observed that the influence of CHRNA5-A3-B4 gene variants on self-reported CPD is stronger in former smokers as compared with current smokers (see Supplementary Table S7 of the study by Amos et al.). It is also possible that the lower number of CPD smoked by light smokers in the current study (mean = 7.7), in contrast to the number reported in the previous meta-analysis (mean ranged from 11.5 to 15.7), mitigated the expression of the underlying biological impact of the CHRNA5-A3-B4 gene variants on smoking level. However, it is important to note that CPD is a poor marker of tobacco consumption, susceptible to reporting and recall biases and insensitive to smoking topography. The average cotinine levels, a more objective marker of tobacco consumption, obtained in our study (mean = 240 ng/ml, SD = 138) were comparable to those previously observed among Caucasian and African-American heavy smokers (mean =170 ng/ml). Previous studies in the CHRNA5-A3-B4 field have suggested that objective biomarkers are more sensitive than CPD. We have previously shown that CHRNA5-A3-B4 variants are significantly associated with cotinine levels in as few as 163 smokers.

![Image](https://example.com/image.png)

**Figure 3** The “T” allele of rs588765 was associated with smoking abstinence among African-American smokers receiving active pharmacological treatment. (a) Participants who received nicotine gum or placebo (Study 1). (b) Participants who received active bupropion or placebo (Study 2). (c) Participants who received active pharmacological treatments (combined analysis). ORadj. = unadjusted OR of quitting for the C/T and T/T genotype group as compared with the C/C genotype group after adjusting for age, sex, baseline interaction during treatment (OR = 3.75, P < 0.001). The OR and P values are shown for all comparisons with ORs <0.7 or >1.4. P values <0.0125 were considered statistically significant due to multiple-comparison adjustments. Statistically significant values are shown in bold. N represents the number of participants. CPD, cigarettes per day; OR, odds ratio.

| Study 1 Nicotine gum: rs588765 | Study 2 Bupropion: rs588765 | Combined: rs588765 | Combined: 6-month follow-up |
|--------------------------------|----------------------------|--------------------|-----------------------------|
| Nicotine gum: During treatment | Nicotine gum: End of treatment | Combined: During treatment | Combined: 6-month follow-up |
| Placebo | Gum | Placebo | Bupropion | Placebo | Bupropion | Placebo | Bupropion | Placebo | Bupropion |
| n = 153 | n = 146 | n = 140 | n = 169 | n = 153 | n = 146 | n = 140 | n = 169 | n = 153 | n = 146 | n = 140 | n = 169 |

| Nicotine gum: During treatment | Nicotine gum: End of treatment | Combined: During treatment | Combined: 6-month follow-up |
|--------------------------------|--------------------------------|-----------------------------|-----------------------------|
| Placebo | Gum | Placebo | Bupropion | Placebo | Bupropion | Placebo | Bupropion | Placebo | Bupropion |
| n = 124 | n = 143 | n = 122 | n = 146 | n = 124 | n = 143 | n = 122 | n = 146 | n = 124 | n = 143 | n = 122 | n = 146 |

| Nicotine gum: During treatment | Nicotine gum: End of treatment | Combined: During treatment | Combined: 6-month follow-up |
|--------------------------------|--------------------------------|-----------------------------|-----------------------------|
| Placebo | Gum | Placebo | Bupropion | Placebo | Bupropion | Placebo | Bupropion | Placebo | Bupropion |
| n = 227 | n = 289 | n = 262 | n = 314 | n = 227 | n = 289 | n = 262 | n = 314 | n = 227 | n = 289 | n = 262 | n = 314 |

| Nicotine gum: During treatment | Nicotine gum: End of treatment | Combined: During treatment | Combined: 6-month follow-up |
|--------------------------------|--------------------------------|-----------------------------|-----------------------------|
| Placebo | Gum | Placebo | Bupropion | Placebo | Bupropion | Placebo | Bupropion | Placebo | Bupropion |
| n = 261 | n = 314 | n = 289 | n = 262 | n = 314 | n = 289 | n = 262 | n = 314 | n = 261 | n = 314 | n = 289 | n = 262 |
Therefore, we believe that the accuracy of biomarkers (vs. self-reports) compensates for the smaller size of our study.

**CHRNA5-A3-B4 and smoking abstinence**

Among Caucasians, a number of studies have examined the association between *CHRNA5-A3-B4* gene variants and clinical trial outcomes for quitting smoking. These studies have varied in whether the predominant effect of *CHRNA5-A3-B4* gene variants was in the placebo arm, a specific treatment arm, or in all active treatment arms more generally.\textsuperscript{15,17,20} Our observations regarding rs2036527 and smoking abstinence among African-American smokers are in agreement with a previous report by Munafò \textit{et al.}\textsuperscript{15} in Caucasians with rs1051730. Both studies reported significant associations between *CHRNA5-A3-B4* gene variants and abstinence (i) primarily in the active pharmacological therapy groups, with little effect observable in all active treatment arms more generally.\textsuperscript{15,17,20} Our observations regarding rs2036527 and smoking abstinence among African-American smokers are in agreement with a previous report by Munafò \textit{et al.}\textsuperscript{15} in Caucasians with rs1051730. Both studies reported significant associations between *CHRNA5-A3-B4* gene variants and abstinence (i) primarily in the active pharmacological therapy groups, with little effect observable...
within the placebo arm; and (ii) only during treatment (no association remains at follow-up, after active treatment has stopped). Of note, among Caucasians, rs2036527 is in high linkage disequilibrium with rs1051730 ($r^2 = 0.90$). Therefore, rs2036527 may be a better pharmacogenetic marker for smoking cessation than rs16969968/rs1051730 because it predicts similar relapse risks among both Caucasians and African Americans.

The $\alpha_5$ nAChR subunit mediates an inhibitory effect of nicotine on brain reward systems. Mice with lower $\alpha_5$ nAChR function exhibited increased nicotine intake. Because rs2036527 was previously shown to be associated with increased nicotine intake among African Americans, it is likely that rs2036527 (or SNPs in high linkage disequilibrium) results in lower $\alpha_5$ nAChR function. Both nicotine gum and bupropion could have inhibitory effects on nicotine reward because both of them can bind to nAChR. The inhibitory effects of nicotine gum and bupropion may be weaker in individuals with rs2036527 (due to their lower $\alpha_5$ function), reducing the efficacy of these treatments.

Our findings of an association between rs588765 and smoking abstinence among African-American smokers are very similar to previous findings among Caucasian smokers. Both studies observed that the “T” allele of rs588765 was associated with lower abstinence in the placebo group and higher abstinence when receiving nicotine replacement therapy (with significant interactions). However, the association between rs588765 and smoking abstinence was still observed at 6-month follow-up among Caucasians but not among African-American smokers. This could be partially due to the relatively low smoking abstinence rates among African-American smokers at 6-month follow-up compared with the rates among Caucasian smokers.

We did not observe any consistent association between rs16969968 and smoking abstinence in this study. It is also worth noting that the direction of the association between rs16969968 and smoking abstinence in African Americans was in the opposite direction as compared with the direction of effect observed in Caucasians. The “A” allele of rs16969968 was associated with lower abstinence with nicotine gum treatment and higher abstinence with placebo treatment in our study of African-American smokers. By contrast, the “A” allele of rs16969968 was associated with higher abstinence with nicotine replacement therapy and lower abstinence with placebo treatment among Caucasians. It is possible that different linkage disequilibrium/haplotype structures between African Americans and Caucasians contribute to this discrepancy. Overall, our smoking abstinence data and the relatively low prevalence of rs16969968 among African Americans would suggest that rs16969968 has very little pharmacogenetic importance among African-American smokers.

In contrast to the study published by Chen et al., we did not observe any long-lasting association between CHRNA5-A3-B4 gene variants and smoking abstinence rates in participants who received the placebo treatment. This discrepancy was unlikely to be the results of limited power because the current investigation had a much bigger placebo group as compared with that of the study by Chen et al. (566 in our combined studies vs. 132 in the study by Chen et al.). It is possible that the effects of the “tag” SNPs are different among different ethnic groups due to the distinct haplotype structures.

Interestingly, adjusting for CPD had little influence on the association between the CHRNA5-A3-B4 gene variants and abstinence in our study and in a number of previous studies that evaluated the association between CHRNA5-A3-B4 gene variants and smoking abstinence. This suggests that the influence of CHRNA5-A3-B4 gene variants on smoking abstinence is not related to their modest effect size on altering tobacco consumption. In addition, the impact of rs2036527 remained unaltered when we controlled for CYP2A6 and/or CYP2B6 genotype, suggesting that the impact of rs2036527 on cessation was independent of variation in nicotine or bupropion metabolism.

Finally, our findings suggested that roughly 60% of African Americans (i.e., individuals with rs2036527(G/G) genotype) would benefit greatly from active smoking cessation treatments, whereas the 40% with the rs2036527(A) variant may not. This demonstrates the potential value of pharmacogenetic approaches in improving smoking cessation outcomes among African-American smokers.

Strengths and limitations
A major strength of our study is that the smoking status (and baseline level of smoking) was biochemically verified. Our selection of African-American light smokers can be viewed as both a strength and a limitation. African-American smokers experience disproportionately higher risks of smoking-related morbidity and mortality as compared with Caucasians. However, very little is known about the efficacy of standard smoking cessation treatments in this population, particularly among light smokers, who make up >50% of African-American smokers. However, the inclusion of light smokers may limit the generalizability of our findings to other groups. In addition, the placebo arm in both trials had relatively low smoking abstinence rates; the low number of abstinence events could limit the statistical power to evaluate these associations. The low number of abstinence individuals also limited our ability to evaluate the effects of multiple SNPs in the same model. Another limitation was that we did not have reliable (biochemically verified) time-of-relapse data in this study, which could provide some time-course information about the effect of rs2036527 on smoking cessation.

Population stratification is an important issue in genetic association studies. A limitation of this study was that we did not have ancestry-informative markers to statistically control for population stratification; however, allele frequencies were similar to those reported in previously published studies (rs2036527 was 22% here and in the large meta-analysis). In addition, because improving smoking cessation treatments for African Americans is our goal, we believe that the limitations due to admixture are balanced against the need to improve smoking cessation in this population. Eliminating individuals who are modestly admixed, but would still self-identify as African Americans, could greatly reduce the clinical applicability of our findings.

Overall, our findings indicate that gene variants in CHRNA5-A3-B4 affect smoking abstinence in African Americans during...
the pharmacological treatment phase but not during follow-up, even after adjusting for baseline smoking behaviors. Greater smoking abstinence was observed with active pharmacological treatments in those with the rs2036527(G/G) genotype, whereas those with the rs2036527(A) allele had cessation rates no greater than the rates with placebo treatment and may require alternative approaches to treatment. Further studies should focus on understanding the mechanism(s) underlying this association in order to optimize the efficacy of smoking cessation treatments.

METHODS

Study descriptions. We evaluated the associations between the CHRNA5-A3-B4 variants and smoking cessation outcomes in two independent smoking cessation trials. The two trials were conducted among African-American light smokers (≤10 CPD) at the same community health center, and the enrollment criteria were similar. These trials account for light smokers because (i) more than 50% of African-American smokers are light smokers and (ii) little is known about the pharmacology and pharmacogenetics of smoking cessation treatments in this population.

Nicotine gum study (Study 1). This study was a randomized double-blind, placebo-controlled trial to evaluate the efficacy of nicotine gum (2 mg) among African-American light smokers. Eligible participants self-identified as “African American” or “black,” were at least 18 years old, had smoked ≤10 CPD for at least 6 months before enrollment, and smoked on at least 25 of the previous 30 days. This study consisted of four treatment arms (n = ~190 each): (i) nicotine gum with health education counseling, (ii) nicotine gum with motivational interviewing counseling, (iii) placebo with health education, and (iv) placebo with motivational interviewing. The nicotine gum treatment lasted 8 weeks, and six counseling sessions were provided to each participant. The participants were followed for a total of 26 weeks (6 months).

Bupropion study (Study 2). This study was a randomized double-blind, placebo-controlled trial to evaluate the efficacy of bupropion (300 mg per day, total n = 540) among African-American light smokers. Eligibility criteria were similar to those in Study 1 and included exclusion based on contraindication of bupropion use. Bupropion use lasted 7 weeks and health education counseling was similar to that in Study 1. The participants were followed for a total of 26 weeks (6 months).

Both studies were approved by the University of Kansas Human Subject Committee, the University of Toronto Ethics Review Office, and the University of California, San Francisco Human Research Protection Program. Both studies assessed age, sex, menthol use, baseline CPD, baseline plasma cotinine level, and Fagerstrom Test for Nicotine Dependence score. Study 2 also assessed urinary total nicotine equivalents, which is line plasma cotinine level, and Fagerstrom Test for Nicotine Dependence

Statistical analyses. The comparisons of baseline demographic variables were performed using Mann–Whitney tests or χ² tests. Evaluation of the association between the dichotomized SNPs (rs2036527 (G/G = 0 and G/A and A/A = 1), rs16969968 (G/G = 0 and G/A and A/A = 1), rs588765 (C/C = 0 and C/T and T/T = 1), and rs578776 (A/A = 0 and A/G and G/G = 1)) and smoking abstinence was conducted using logistic regression. Modeling the influence of the variants as “additive” resulted in very similar statistical results as compared with modeling the influence of the variants as a dichotomized variable (results shown in Supplementary Figure S1 online). Logistic regressions were used to evaluate the association between rs2036527 and rs588765 and smoking abstinence after adjusting for age, sex, baseline CPD, menthol status, the type of counseling sessions (Study 1), and other CHRNA5-A3-B4 SNPs. Adjusting for other CHRNA5-A3-B4 variants (such as rs16969968) did not alter the associations between rs2036527 and rs588765 with smoking abstinence (Supplementary Figures S2–S4 online). Adjusting for CYP2A6 (coded as normal activity > intermediate activity > slow activity) and CYP2B6 (coded as normal activity > intermediate activity > slow activity) also did not alter the association between rs2036527 and rs588765 with smoking abstinence. A detailed description of the specific CYP2A6 and CYP2B6 alleles assessed was reported previously and included CYP2A6*2, *4, *9, *17, *20, *23, *24, *25, *26, *27, *31, and *35 and CYP2B6*4, *6, *9, *18, and *22.31 Because there were four independent SNPs, we considered P values <0.0125 (i.e., 0.05/4) to be statistically significant. The number needed to treat represents the average number of patients needed to treat with the active vs. placebo pharmacotherapy to prevent one patient from smoking relapse. Statistical analyses were performed using Stata 12 (StataCorp, College Station, TX).

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

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AUTHOR CONTRIBUTIONS

R.F.T., A.Z.X.Z., L.S.C., S.P.D., J.S.A., and N.L.B. wrote the manuscript. R.F.T., A.Z.X.Z., L.S.C., J.S.A., and N.L.B. designed the research. A.Z.X.Z., Q.Z., L.S.C., J.S.A., and N.L.B. performed the research. R.F.T. and A.Z.X.Z. analyzed the data.

CONFLICT OF INTEREST

N.L.B. serves as a consultant to several pharmaceutical companies that market smoking cessation medications and has been a paid expert witness in litigation against tobacco companies. S.P.D. is a scientific adviser to Genophen. R.F.T. has participated in one-day advisory meetings for Novartis and McNeil. R.F.T. is an Associate Editor for Clinical Pharmacology & Therapeutics but was not involved in the review or decision process for this article. The other authors declared no conflict of interest.
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