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Toward Personalized Therapy for Smoking Cessation: A Randomized Placebo-controlled Trial of Bupropion

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We examined whether a pretreatment phenotypic marker of nicotine metabolism rate (NMR) predicts successful smoking cessation with bupropion. Smokers ($N = 414$) were tested for pretreatment NMR, based on the ratio of 3′-hydroxycotinine/cotinine derived during smoking, before entering a placebo-controlled randomized trial of bupropion plus counseling. At the end of the 10-week treatment phase, slow metabolizers (1st NMR quartile) had equivalent quit rates with placebo or bupropion (32%). Fast metabolizers (4th NMR quartile) had low quit rates with placebo (10%), and these were enhanced significantly by bupropion (34%). Smokers in the 2nd quartile (placebo: 25%, bupropion: 30%) and the 3rd quartile (placebo: 20%, bupropion: 30%) did not benefit significantly from bupropion. At the 6-month follow-up, the relationship between the NMR and quitting remained similar, but was no longer statistically significant. A pretreatment assessment of NMR may identify smokers who are most and least likely to benefit from treatment with bupropion for smoking cessation.

Personalized medicine, in which therapies are delivered to individual patients on the basis of pretreatment biological markers, is emerging as a new model of healthcare delivery.[1,2] In the area of tobacco dependence treatment, genetic variants in nicotine-metabolizing enzymes are plausible candidates for prediction of therapeutic response.[3,4] Nicotine is metabolized to cotinine (COT), predominantly by the liver enzyme cytochrome P450 (CYP) 2A6;[5,6] COT is further metabolized to *trans*-3′-hydroxycotinine (3HC) by the same enzyme.[5] Consistent with the premise that faster inactivation and elimination of nicotine lead to higher rates of smoking so as to maintain nicotine levels in the system, reproducible associations of *CYP2A6* genotype with smoking behavior have been reported.[3,7–9]

The ratio of the levels of 3HC/COT arising from cigarette smoking, as measured in plasma, saliva, or urine, is associated with the *CYP2A6* genotype.[3,10,11] This phenotype measure is highly reproducible and independent of time elapsed since the last cigarette.[12–14] Further, the ratio is strongly correlated with plasma nicotine levels and nicotine clearance.[10,14–16] Consistent with these genetic and pharmacokinetic data, we have reported that the nicotine metabolite ratio predicts successful quitting of smoking with the use of transdermal nicotine.[16] In this earlier investigation, the odds of achieving smoking cessation with transdermal nicotine therapy were reduced by 30% with each increasing quartile of the nicotine metabolite ratio. Faster metabolizers of nicotine were less successful in quitting than slower metabolizers and also had lower plasma levels of nicotine during treatment and stronger cravings to smoke.

In order to extend this line of research on the role of NMR in achieving success in smoking cessation, we examined the predictive clinical validity of the 3HC/COT ratio within a double-blind placebo-controlled pharmacogenetic clinical trial of bupropion efficacy.[17–19] On the basis of the retrospective study of Gu *et al.*,[9] we hypothesized that, within the placebo condition, slower metabolizers would have significantly higher quit rates than faster metabolizers. Given that bupropion is a non-nicotine, antidepressant medication with proven efficacy in the treatment of tobacco dependence,[20] we also predicted that faster metabolizers would achieve significant benefit from bupropion, while slower metabolizers would have similar quit rates with bupropion and placebo.

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Characteristics of the participants

The mean age of the participants was 44.3 years (SD = 11.3); 57% of the sample consisted of women; 40% had graduated from college; and 82% were of European ancestry. The mean number of cigarettes smoked per day was 21.5 (SD = 9.7), and the mean Fagerström test for nicotine dependence score was 5.4 (SD = 2.0). The mean values of the metabolite variables were as follows: pretreatment COT (259 ng/ml, SD = 112), 3HC (104 ng/ml, SD = 56), and nicotine metabolite ratio (0.43, SD = 0.23). The means, medians, and lower and upper limits of the nicotine metabolite quartiles were: (i) 0.19, 0.20, 0.046, and 0.261; (ii) 0.33, 0.33, 0.262, and 0.382; (iii) 0.45, 0.46, 0.383, and 0.540; and (iv) 0.75, 0.69, 0.541, and 1.416.

A total of 210 participants received bupropion and 204 received placebo. Marital status (χ^2 (1) = 3.80, P = 0.05) and education (χ^2 (1) = 4.48, P = 0.04) differed significantly by treatment arm; participants receiving bupropion were less likely to be married and more likely to have a college education. These variables were therefore controlled in the regression models. No other baseline variables differed significantly by treatment arm.

Participants lost to the study at the 6-month follow-up point (n = 14 for placebo, n = 15 for bupropion) were compared with participants who completed the 6-month assessment in terms of demographic factors, smoking history, and nicotine metabolite ratio. Noncompleters were significantly younger ($\overline{X}$ = 40.0 vs. 44.7; F[1,412] = 4.63, P = 0.03) and tended to smoke more cigarettes per day at enrollment ($\overline{X}$ = 25.2 vs. 21.2; F[1,412] = 4.60, P = 0.03). Nicotine dependence level (which includes cigarettes per day)[21] and age were also controlled in all models. The nicotine metabolite ratio did not differ between participants who completed the 6-month follow-up time point and those who did not (F[1,412] = 1.01, P = 0.31).

Associations of nicotine metabolite ratio with baseline smoking-related variables

The nicotine metabolite ratio was significantly higher among women (t(410) = −5.03, P < 0.01), individuals of European ancestry (t(412) = 3.79, P < 0.01), and older participants (r = 0.16, P < 0.01), but was not related to other demographic variables. As expected, the nicotine metabolite ratio was negatively associated with pretreatment levels of plasma nicotine (r = −0.21, P < 0.01), but unrelated to cigarette smoking rate or baseline nicotine dependence level (P > 0.10 for both). Similarly, nicotine metabolite quartiles were associated with pretreatment levels of plasma nicotine (F(3,400) = 5.75, P < 0.01), but unrelated to cigarette smoking rate or baseline nicotine dependence level.

Association of nicotine metabolite ratio with smoking cessation

As shown in **Table 1**, in a logistic regression model of abstinence (quit = 1), there was a significant main effect of treatment assignment both at the end of treatment (bupropion = 1; odds ratio (OR) = 4.22 (95% confidence interval (CI): 1.59–11.20) P < 0.01) and at 6-month follow-up (OR = 3.26 (95% CI: 1.14–9.36) P = 0.03). The interaction between the continuous log-transformed ratio and treatment was significant at the end of treatment (OR = 2.46 (95% CI: 1.07–5.66) P = 0.035), but not at the 6-month follow-up.

Table 1 Multivariate logistic regression of smoking abstinence by continuous 3HC/COT ratio and treatment

	Abstinence at EOT			Abstinence at 6 months		
	OR	95% CI	*P*	OR	95% CI	*P*
Treatment	4.22	1.59–11.20	0.004	3.26	1.14–9.36	0.03
NMR	0.55	0.30–1.03	0.06	0.72	0.36–1.47	0.37
Treatment × NMR	2.46	1.07–5.66	0.04	1.59	0.64–3.99	0.32
Gender	0.58	0.36–0.94	0.03	0.90	0.53–1.52	0.69

The effects of treatment site, race, age, nicotine dependence, education, and marital status were controlled in both models and were nonsignificant.

3HC/COT, *trans*-3′-hydroxycotinine/cotinine; CI, confidence interval; EOT, end of treatment; gender (males = 1, females = 0); NMR, continuous log-transformed nicotine metabolite ratio; OR, odds ratio; Treatment (bupropion = 1, placebo = 0).

When we examined the relationship between nicotine metabolism quartiles and abstinence, a dose–response relationship was observed within the placebo group at the end of treatment and at the 6-month follow-up (**Figure 1**). Among the fastest metabolizers, end-of-treatment abstinence rates were 34% and 10% for bupropion and placebo, respectively. Among the slowest metabolizers (1st quartile), end-of-treatment abstinence rates were equivalent in both treatment groups (32%).

In order to evaluate the significance of treatment effects within nicotine metabolite ratio groups, a dummy coded quartile variable was tested in a logistic regression model of abstinence that also included terms for treatment assignment within each quartile group (**Table 2**). There was a significant effect of treatment among smokers within the 4th quartile of the nicotine metabolite ratio (fastest metabolizers) at the end of treatment (OR = 4.84 (95% CI: 1.61–14.55), P = 0.005) and at the 6-month follow-up (OR = 4.48 (95% CI: 1.34–14.97); P = 0.02). Treatment effects were nonsignificant in the 1st–3rd quartile groups.

Association of the nicotine metabolite ratio with treatment-related variables

There were no significant associations of the nicotine metabolite ratio (quartile measure) with changes in withdrawal symptoms, average side effects, or pill counts (P values > 0.10).

Discussion: this study contributes new evidence that rapid nicotine metabolism, as determined on the basis of the ratio of 3HC/COT derived from cigarette smoking, is a risk factor for smoking relapse. Further, we find that bupropion is an efficacious smoking cessation medication for rapid metabolizers of nicotine, who are at increased risk of relapse. The observed inverse relationship between NMR and quitting success is consistent with prior results from a nicotine replacement therapy trial.[16] New data from the present trial indicate that the slowest metabolizers (1st quartile) respond well with counseling alone and achieve no additional benefit from bupropion (quit rates of 32% in bupropion and placebo arms). In contrast, the fastest metabolizers (4th quartile) respond poorly with counseling-only, and achieve significant benefits from bupropion that are maintained at

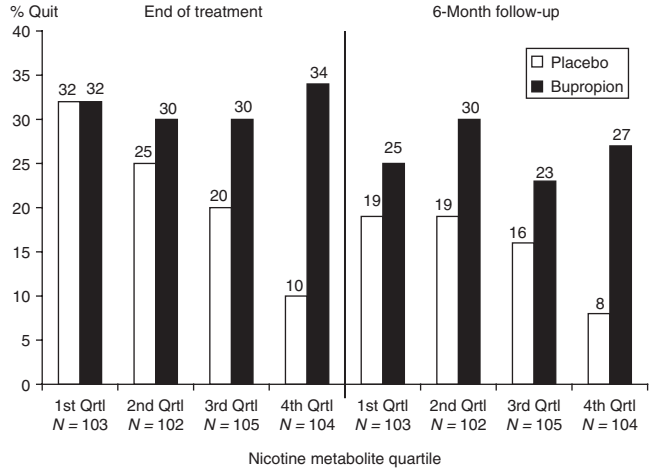

Figure 1 Smoking cessation rates by nicotine metabolite ratio and at the end of treatment and at 6-month follow-up ($N = 414$). Qrtl, quartile.

the 6-month follow-up. Specifically, bupropion boosted end-of-treatment quit rates from 10 to 34% in this group.

These results add to a growing body of evidence that individual variability in the rate of nicotine metabolism influences smoking behavior. *CYP2A6* genotype is associated with the rate of nicotine metabolism;[3] however, for a given *CYP2A6* genotype, there is considerable interindividual variability in the rate of nicotine metabolism.[22] In part, this is because of unidentified CYP2A6 genetic variants[23] and because CYP2A6 activity is also influenced by environmental factors such as steroid hormone exposure,[24] smoking status,[25] ethnicity,[25] and age.[10] Therefore, the phenotypic measure of NMR used in this study (reflecting both genetics and environmental influences) may have greater current utility for predicting relapse risk and tailoring treatment than genetic assays, which do not account for environmental influences. Also, the use of a simple blood test to determine the 3HC/COT ratio may be more easily translated to clinical practice than genetic analyses of CYP2A6 polymorphisms.

Although the higher risk of relapse observed among faster metabolizers in the placebo-/counseling-only arms is consistent with the previous data from a nicotine replacement therapy trial,[16] the mechanisms that underlie this effect remain unknown. One might speculate that those who metabolize nicotine faster are more nicotine dependent; however, this hypothesis is not supported by the data from this study or by the data from our earlier nicotine replacement therapy trial.[16] Moreover, the models of smoking abstinence in this study include nicotine dependence as a covariate, suggesting that effects of the metabolite ratio on abstinence are independent of nicotine dependence level. Yet, nicotine dependence is a complex, multidimensional construct, and other measures may better capture specific elements of dependence. Recent data indicate that the nicotine metabolite ratio correlates significantly with a particular subcomponent of nicotine dependence among current smokers, namely the propensity to experience cravings to smoke.[26] Human laboratory and naturalistic investigations of slow and rapid metabolizers that elucidate the mechanisms of the effect of the NMR on quitting success are warranted. Such research could identify novel targets for smoking cessation treatment in smokers at high risk for relapse.

Table 2 Multivariate logistic regression analysis of smoking abstinence by quartiles of 3HC/COT ratio, treatment, and treatment effects within quartiles ($N = 414$)

	Abstinence at EOT			Abstinence at 6 months		
	OR	95% CI	*P*	OR	95% CI	*P*
1st NMR quartile (reference)	1.00	—	—	1.00	—	—
2nd NMR quartile[a]	0.74	0.31–1.76	0.50	0.97	0.36–2.61	0.96
3rd NMR quartile[a]	0.57	0.23–1.42	0.23	0.72	0.26–2.03	0.54
4th NMR quartile[a]	0.27	0.09–0.80	0.02	0.36	0.10–1.24	0.10
Treatment: 1st NMR quartile[b]	1.01	0.43–2.38	0.98	1.50	0.57–3.92	0.41
Treatment: 2nd NMR quartile[b]	1.26	0.52–3.06	0.62	1.87	0.73–4.82	0.19
Treatment: 3rd NMR quartile[b]	1.82	0.72–4.58	0.20	1.71	0.63–4.68	0.29
Treatment: 4th NMR quartile[b]	4.84	1.61–14.55	0.005	4.48	1.34–14.97	0.02
Gender	0.60	0.37–0.97	0.04	0.97	0.57–1.63	0.91

The effects of treatment site, race, age, nicotine dependence, education, and marital status were controlled in both models and were nonsignificant.

3HC/COT, *trans*-3′-hydroxycotinine/cotinine; CI, confidence interval; EOT, end of treatment; gender (males = 1, females = 0); NMR = quartiles of nicotine metabolite ratio; OR, odds ratio; treatment (bupropion = 1, placebo = 0; within each quartile group).

[a]Odds of abstinence in NMR quartile relative to 1st quartile (slow metabolizers) reference group. [b]Effect of bupropion vs. placebo on abstinence within each of the four quartile groups.

In addition to providing novel evidence for the effects of NMR on relapse risk, this study provides strong support for the benefits of using bupropion treatment for the fastest metabolizers. These data also indicate that slow metabolizers perform well with counseling-only and achieve no further benefit from bupropion. The *CYP2A6* genotype is unrelated to bupropion metabolism,[27] thereby suggesting that the observed difference in bupropion response in relation to the nicotine metabolite ratio does not reflect a direct biological interaction with the medication. Because fast metabolizers of nicotine do not benefit from transdermal nicotine or nicotine nasal spray,[16] the significant benefits of bupropion in this group are most likely attributable to the fact that bupropion is an efficacious non-nicotine medication.

This study has some limitations that should be noted. First, blood samples for analysis of the nicotine metabolite ratio were not available for 135 of the trial participants, most of whom were from one study site. However, there was no evidence for bias in treatment assignment or outcome based on sample availability. Second, the study population was not sufficiently large to test whether the effects of the metabolite ratio on abstinence are modified by gender or race, two factors known to influence the rate of nicotine metabolism.[10,24,28]

This study provides new evidence that the nicotine metabolite ratio can provide a simple pretreatment assay to identify

smokers who are most and least likely to benefit from different treatments for tobacco dependence. The translation of these findings into practice will be aided by data supporting the cost-effectiveness of tailoring the treatment on the basis of the nicotine metabolite ratio. Data from future prospective trials, comparing conventional (randomized) treatment with personalized treatment based on this pretreatment biomarker, would also help in the practical application of these findings. Such research has the potential to improve the outcomes of treatment for individual smokers, thereby reducing avoidable mortality from tobacco use.

METHODS

Design overview. The study was a placebo-controlled, randomized trial that examined the efficacy of standard course of bupropion for smoking cessation.

Setting and participants. Participants were enrolled at Georgetown University (Washington, DC), and the State University of New York at Buffalo (Buffalo, NY) between May 1999 and September 2001. Study procedures for both the institutions were approved by Institutional Review Board, and the trial is registered at http://www.clinicaltrials.gov. All eligible participants provided written informed consent before participation. Eligible smokers were aged 18–65 and smoked at least 10 cigarettes per day in the last year. Standard exclusion criteria for bupropion treatment were used. Additional information about the methods and procedures used in this trial can be found in previous publications.[17–19,29]

The flow of study participants is shown in **Figure 2**. Initially, 729 people were screened for medical and psychiatric eligibility, and from this group, 670 people were randomized (341 bupropion, 329 placebo). A total of 327 participants were enrolled at Georgetown and 343 at the University of Buffalo. A total of 115 participants withdrew before provision of a blood sample (56 bupropion, 59 placebo), resulting in a sample of 555 who initiated treatment. Of the 555 who entered treatment, pretreatment blood samples were unavailable for 135 subjects (70 bupropion, 65 placebo), precluding assessment of the 3HC/COT ratio. In addition, six participants had missing data on covariates of interest (5 bupropion, 1 placebo). The resulting sample included 414 participants (210 bupropion, 204 placebo) with data for analysis of the nicotine metabolite ratio. Participants who dropped out during the treatment period are included in the analysis (52 bupropion, 54 placebo).

To determine whether sample availability biased the study results, missing samples of participants ($n = 135$) were compared with those for whom samples were available ($N = 414$). Analyses were stratified using study site, because the majority of missing samples were from one study site. There were no significant differences in treatment assignment, demographic variables, baseline smoking rate, or quitting success between the two participant groups ($P > 0.10$ for both).

Randomization and interventions. Participants were randomly assigned to receive 10 weeks of placebo or bupropion, which was initiated at week 1 and delivered according to standard therapeutic dose (150 mg/day for the first 3 days, followed by 150 mg/day b.i.d.). The target quit date for all participants was week 3 of treatment. Masters' level smoking cessation counselors provided seven 1-h sessions of standardized group behavioral counseling on a weekly basis from study week 1 until study week 5 and then biweekly in study weeks 7 and 9. Counseling initially focused on planning for cessation (e.g., identification of triggers to smoke) and participants were instructed to reduce smoking rate in the first 2 weeks before the target quit date. Later sessions focused on stress management and relapse prevention. Smoking status was assessed and biochemically confirmed (saliva COT <15 ng/ml) at all counseling sessions, at the end of treatment (week 11), and at 6-month follow-up.

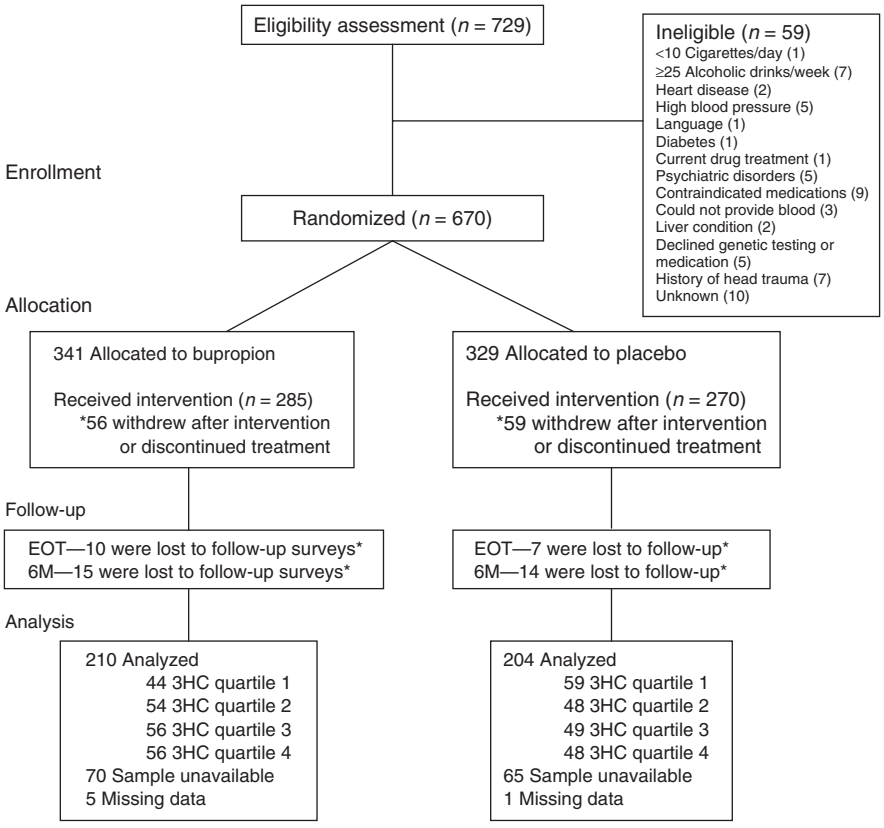

Figure 2 Participant flow. *Participants included in analysis.

The study database manager generated the allocation sequence and separate randomizations were performed at each study site. Participants and research staff were blinded to treatment assignment, NMR, and study hypotheses. Following the pretreatment assessment, participants provided a blood sample for analysis of nicotine metabolites (see "Assessments" below).

Assessments.
Covariates: Participants self-reported their demographic characteristics (e.g., age and race) and smoking history at baseline. The Fagerström test of nicotine dependence, a six-item self-report measure of nicotine dependence,[21] was also completed.

Predictor: The primary predictor variable was the 3HC/COT ratio measured in plasma from blood samples collected before treatment (i.e., baseline smoking state). The 3HC/COT ratio reflects CYP2A6 activity,[5,6] is correlated with nicotine metabolism and clearance,[3,14] and is independent of time since last cigarette.[12–14] Plasma concentrations of COT and 3HC were measured by high-performance liquid chromatography–tandem mass spectrometry; nicotine concentrations were assessed using gas chromatography with nitrogen phosphorous detection.[14]

Outcome: Self-reported 7-day point prevalence abstinence at the end of treatment and at 6 months following the quit date was biochemically verified with saliva COT.[30] Participants with a COT ≥15 ng/ml at the end of treatment and 6 months were deemed smokers.[30] As per convention, participants were assumed to be smoking if they self-reported to be smoking or could not be reached for biochemical confirmation of smoking status.[30]

Treatment-related variables: An 18-item withdrawal symptom checklist (e.g., cravings, irritability, difficulty in concentration, and increased hunger) was administered at baseline and at all study visits.[31] Two items validated in previous research[32] were used to assess urges to smoke at each time point. Side effects were assessed using a 17-item list of physical complaints possibly related to bupropion (e.g., headache and dry mouth) that was administered at sessions two through seven. A summary score for each time point was generated by totaling the responses to all items.[17] To evaluate treatment adherence, a self-reported pill count was obtained at all study visits.

Statistical analysis. The detectable difference was determined using Power and Sample Size (NCSS Software, East Kaysville, UT). To provide 80% power to detect an OR ≥ 3.5 for the interaction of the continuous nicotine metabolite ratio with treatment assignment, a sample size of 402 would be required.

Participant characteristics were evaluated (e.g., demographics, smoking history, and metabolite ratio) using descriptive statistics. Because the nicotine metabolite ratio distribution was skewed, a log-transformed measure of the nicotine metabolite ratio was used to assess associations of the continuous measure with baseline smoking variables.

The effect of the nicotine metabolite ratio on abstinence and response to therapy was assessed in two steps. First, we tested for the effects of the continuous log-transformed metabolite ratio using logistic regression; terms for treatment and the ratio by treatment interaction were included. Second, to facilitate interpretation of ratio effects and to identify subgroups of responders and nonresponders based on the ratio, a model was generated using a quartile variable for the ratio. Terms for age and nicotine dependence (which includes cigarettes per day) were included in the models as both these variables predicted study retention (see results above). Marital status and education were also included because these variables differed by treatment assignment (see results above). Study site was included because of site differences in sample availability. Gender and race were also included as covariates of *a priori* interest, on the basis of gender and race differences in NMR.[10,24,28] Separate logistic regression models were run for abstinence at the end of treatment and 6-month follow-up.

Potential mechanisms of effects of the nicotine metabolite ratio were examined using one-way analysis of variance in which the nicotine metabolite quartile was the independent variable. Outcome measures included changes in withdrawal and smoking urges from 1-week prequit to 1-week post quit, average side effects for the first 2 weeks of treatment (prequit), and average pill counts for the duration of treatment.

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CONFLICT OF INTEREST

GlaxoSmithKline markets the study medication, bupropion. Dr Lerman has worked as a consultant for pharmaceutical companies that market smoking cessation medications, including GlaxoSmithKline. Dr Benowitz has been a paid consultant for pharmaceutical companies that market smoking cessation medications. He has also provided expert testimony in litigation against tobacco companies. Dr Tyndale holds shares in Nicogen (Toronto, Ontario, Canada), a company focused on creating novel smoking cessation treatments. Dr Epstein has worked as a consultant with Kraft foods. No funding was received from GlaxoSmithKline, Nicogen, or Kraft foods for this study. The other authors have no potential conflicts to report.

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