Addiction
RESEARCH REPORT
doi:10.1111/add.12353

Pharmacotherapy effects on smoking cessation vary with nicotine metabolism gene (*CYP2A6*)

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ABSTRACT

Background and aims Evidence suggests that both the nicotinic receptor α5 subunit (*CHRNA5*) and Cytochrome P450 2A6 (*CYP2A6*) genotypes influence smoking cessation success and response to pharmacotherapy. We examine the effect of *CYP2A6* genotype on smoking cessation success and response to cessation pharmacotherapy, and combine these effects with those of *CHRNA5* genotypes. **Design** Placebo-controlled randomized smoking cessation trial. **Setting** Ambulatory care facility in Wisconsin, USA. **Participants** Smokers ($n = 709$) of European ancestry were randomized to placebo, bupropion, nicotine replacement therapy or combined bupropion and nicotine replacement therapy. **Measurements** Survival analysis was used to model time to relapse using nicotine metabolism derived from *CYP2A6* genotype-based estimates. Slow metabolism is defined as the lowest quartile of estimated metabolic function. **Findings** *CYP2A6*-defined nicotine metabolic function moderated the effect of smoking cessation pharmacotherapy on smoking relapse over 90 days [hazard ratio (HR) = 2.81, 95% confidence interval (CI) = 1.32–5.99, $P = 0.0075$], with pharmacotherapy significantly slowing relapse in fast (HR = 0.39, 95% CI = 0.28–0.55, $P = 1.97 \times 10^{-8}$), but not slow metabolizers (HR = 1.09, 95% CI = 0.55–2.17, $P = 0.80$). Further, only the effect of nicotine replacement, and not bupropion, varies with *CYP2A6*-defined metabolic function. The effect of nicotine replacement on continuous abstinence is moderated by the combined genetic risks from *CYP2A6* and *CHRNA5* (Wald = 7.44, d.f. = 1, $P = 0.0064$). **Conclusions** Nicotine replacement therapy is effective among individuals with fast, but not slow, *CYP2A6*-defined nicotine metabolism. The effect of bupropion on relapse likelihood is unlikely to be affected by nicotine metabolism as estimated from *CYP2A6* genotype. The variation in treatment responses among smokers with genes may guide future personalized smoking cessation interventions.

Keywords Metabolism, nicotine, pharmacogenetics, smoking cessation.

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Submitted 25 April 2013; initial review completed 27 June 2013; final version accepted 3 September 2013

INTRODUCTION

The key goals of genetic studies of smoking behaviors are to identify the genes that confer a vulnerability to nicotine dependence and that guide the development of effective, 'personalized' treatment strategies for smoking cessation. We recently demonstrated that pharmacological treatment affects cessation differently depending on the genotype of the nicotinic receptor subunit gene, *CHRNA5* [1], a locus strongly associated with nicotine dependence [2–5]; a similar association between this locus and cessation has been reported recently [6]. Variation in nicotine metabolism efficiency and variation in the gene that encodes the primary nicotine metabolism enzyme, cytochrome P450 2A6 (*CYP2A6*) are also robustly associated with smoking phenotypes, especially cigarette consumption [5,7–9]. *CYP2A6* is highly polymorphic, with reduced function alleles producing significantly slower rates of nicotine metabolism. Relatively common variants define multiple CYP2A6 haplotypes

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in European populations [10], and the large majority of inter-individual variation in metabolism of nicotine to cotinine can be explained by seven polymorphisms among European Americans [11]. Several studies have reported an influence of nicotine metabolic rate upon cessation [12–14], although the relation between metabolism and different treatment regimens remains unclear.

Previous studies of nicotine metabolism and cessation treatment have examined a proxy for *CYP2A6* activity, nicotine metabolite ratio (NMR), the ratio of two stable nicotine metabolites, cotinine: 3-hydroxycotine, measured in the blood of current smokers [12–16]. We have recently developed another predictive model of nicotine metabolism based on the *CYP2A6* genotype. *CYP2A6* haplotypes included in this model explained 70% ($R^2 = 0.7$) of the variance in metabolism of oral nicotine among European Americans. Metabolism estimates predicted by the model were correlated significantly with self-reported cigarettes smoked per day (CPD) [7,11] and exhaled carbon monoxide (unpublished data).

Previous studies demonstrated that treatment success with nicotine replacement therapy (NRT) is associated with markers of slower nicotine metabolism [12,14]. However, because some of these studies did not include a placebo control group, the interaction between treatment and metabolic rate could not be determined. Another study showed more successful cessation among slow nicotine metabolizers than among fast metabolizers when both received placebo treatment; both groups had equivalent quit rates with bupropion treatment [13]. In the current research, we will determine if the effect of cessation pharmacotherapy varies with nicotine metabolism in the context of different active pharmacotherapy conditions and placebo.

Using data from a multi-armed cessation trial that include NRT, bupropion, combination pharmacotherapies and placebo controls, we tested the hypotheses that: (i) individuals with *CYP2A6* genotype-based fast nicotine metabolism are more likely to relapse sooner than individuals with slow metabolism when given placebo intervention, (ii) the effect of active pharmacotherapy versus placebo will vary (i.e. interact) with *CYP2A6* genotype and (iii) the effects of NRT will differ (interact) with *CYP2A6* genotype but the effects of bupropion will not. In addition, we examined whether the effects of *CYP2A6* on smoking cessation outcome and therapeutic response to NRT are independent from those of *CHRNA5*, another gene associated with cessation outcomes and response to smoking cessation pharmacotherapy [1]. This research was designed to reveal the genetic conditions under which the tested pharmacotherapies exert their optimal effects, a topic of clear relevance to a genetically informed, personalized approach to smoking cessation pharmacotherapy.

METHODS

Participants were from a University of Wisconsin Transdisciplinary Tobacco Use Research Center (UW-TTURC) randomized, placebo-controlled smoking cessation trial [17], aged 18 years or older, smoked 10 or more CPD and were motivated to quit smoking. The University of Wisconsin-Madison institutional review board approved this trial, and all subjects provided written informed consent. Prior to randomization, participants completed baseline assessments of demographics, smoking history (including CPD) and tobacco dependence, including the Fagerström Test for Nicotine Dependence (FTND) [18]. Participants provided a breath sample for alveolar carbon monoxide (CO) analysis to verify their smoking status and to estimate their smoking heaviness. The treatment phase lasted 8 weeks. Participants (European American $n = 709$) were assigned randomly to: placebo ($n = 79$), bupropion SR ($n = 118$), NRT ($n = 377$) or combined bupropion and nicotine replacement therapy ($n = 135$). The pharmacotherapies were: (i) placebo; (ii) bupropion SR (150 mg twice daily for 9 weeks total: 1 week prior to the quit date and 8 weeks post-quit); (iii) nicotine replacement therapy including nicotine lozenge (2 or 4 mg based on the package insert instructions for 12 weeks post-quit), nicotine patch (24-hour patch; 21, 14 and 7 mg; titrated down during 8 weeks post-quit), or nicotine patch + nicotine lozenge combination therapy (dosed as listed above); and (iv) combined bupropion SR and nicotine lozenge therapy (dosed as listed above). In addition, all participants received six brief (10-minute) individual counseling sessions.

Biochemically confirmed 7-day point prevalence abstinence was assessed at end-of-treatment (8 weeks post-quit). All the participants' self-reports of abstinence during study visits were confirmed by an expired CO [abstinence = CO < 10 parts per million (p.p.m)]. Follow-up telephone calls permitted the determination of time of relapse via time-line follow-up assessment [19,20] up to 90 days after the quit date. Relapse was defined as smoking for 7 consecutive days.

Genotyping was performed by the Center for Inherited Disease Research at Johns Hopkins University using the Illumina Omni2.5 microarray (http://www.illumina.com). Data cleaning was led by the GENEVA Coordinating Center at the University of Washington. Additional *CYP2A6* genotyping and application of the predictive model of *CYP2A6* activity were conducted as described previously [7,11]. The predicted nicotine metabolism metric for all subjects was calculated from *CYP2A6*

diplotype. Briefly, all analyses of measured metabolism are performed on a metabolism metric, the ratio of deuterated (D_2) cotinine/($D_{2cotinine} + D_{2nicotine}$), determined 30 minutes following oral administration of $D_{2nicotine}$. The original model parameters were derived from the regression, log (1 − metabolism metric) = $\alpha + \beta_{H1} + \beta_{H2}$, where α is the intercept, H1 represents the first *CYP2A6* haplotype and H2 represents the second *CYP2A6* haplotype for each subject. Slow nicotine metabolism function is defined as the lowest quartile of metabolism function as used in previous research on nicotine metabolism [12–14]. Based on the distribution of the metabolism metric, the cut-point closest to the lowest quartile defines 29.3% of participants with slower metabolism. The frequencies of slow versus fast metabolizers, stratified by treatment group, are shown in Supporting information, Table S1 and Fig. 2.

Analysis

We used Cox proportional hazard regression models to analyze smoking relapse likelihood (smoking on 7 consecutive days) over the 90-day period after the quit date, the primary outcome. Secondary outcomes include: (i) smoking quantity (self-reported CPD) for post-treatment weeks 1–8, analyzed with growth curve mixed models for repeated measures per subject, (ii) biochemically confirmed 7-day abstinence at 8 weeks analyzed with logistic regression and (iii) continuous abstinence (complete abstinence for 90 days post-quit), also analyzed with logistic regression. The primary predictor was *CYP2A6* genotype-based metabolic function, which was examined for interaction with treatment (active pharmacotherapy versus placebo). We tested whether the hazard ratio for relapse associated with treatments differed across predicted metabolic function groups by including a product interaction term in the Cox proportional hazard regression. Covariates included gender, age and CPD (in four levels: ≤10, 11–20, 21–30 and ≥31, while specific cigarette counts were used as the dependent variable for the smoking quantity analysis).

We created a binary variable representing high versus low risk of relapse based on the diplotype of rs16969968 and rs680244 in *CHRNA5* (low-risk: GG_CC, GG_CT, GA_CC; high-risk: GG_TT, GA_CT, AA_CC) and our previous findings [1]. Next, we combined the genetic risks defined by *CYP2A6* and *CHRNA5* into a four-category variable representing the combined genetic risks from *CYP2A6* and *CHRNA5*. We tested whether the hazard ratio for relapse associated with treatments differed across the genetic risk categories by including a product interaction term in the Cox proportional hazard regression.

RESULTS

Subjects of European ancestry enrolled into the UW-TTURC trial with genotype and relapse data were included in this analysis ($n = 709$; Supporting information, Table S1 for demographics). The *CYP2A6* genotype-based metabolism function distribution in the UW-TTURC sample was fast metabolizers (70.7%) and slow metabolizers (29.3%). In this treatment-seeking sample, fast nicotine metabolism was associated with more CPD at baseline, adjusted for age and gender ($\beta = 0.19$, d.f. = 1, $P = 0.0024$).

Metabolism based on *CYP2A6* genotype predicts smoking relapse in the placebo group

In this trial, 49.6% of participants relapsed to smoking during the 90-day post-quit follow-up. In the placebo group, slow metabolism based on *CYP2A6* genotype predicted decreased relapse risk in comparison to fast metabolism [hazard ratio (HR) = 0.40, 95% confidence interval (CI) = 0.19–0.83, $P = 0.013$], adjusted for age and gender. Age and gender did not predict relapse.

Pharmacotherapy effects vary with metabolism based on *CYP2A6* genotype

Active pharmacotherapy decreased the rate of relapse across all participants by almost half in comparison to placebo, adjusted for age, gender and metabolism based on *CYP2A6* genotype (HR = 0.51, 95% CI = 0.38–0.68, $P = 4.3 \times 10^{-6}$). However, the association of metabolism and relapse was significantly moderated by medication status (placebo versus active pharmacotherapy) (interaction effect size = 2.81, 95% CI = 1.32–5.99, $P = 0.0075$; Table 1a). Pharmacotherapy was highly effective in fast metabolizers (HR = 0.39, 95% CI = 0.28–0.55, $P = 1.97 \times 10^{-8}$), but not in slow metabolizers (HR = 1.09, 95% CI = 0.55–2.17, $P = 0.80$). Figure 1 illustrates the effect of pharmacotherapy on relapse in fast and slow metabolizers.

Effects of NRT, but not bupropion, vary with metabolism based on *CYP2A6* genotype

The effect of NRT differed by *CYP2A6*-defined metabolism (interaction effect size = 1.82, 95% CI = 1.07–3.10, $P = 0.028$) while the effect of bupropion did not (interaction effect size = 0.95, 95% CI = 0.58–1.58, $P = 0.85$; Table 1b). NRT was effective in fast (HR = 0.50, 95% CI = 0.38–0.68, $P = 4.41 \times 10^{-6}$), but not slow, metabolizers (HR = 0.93, 95% CI = 0.59–1.46, $P = 0.75$, Fig. 2). Buproprion was effective in both slow and fast metabolizers (HR = 0.76, 95% CI = 0.60–0.96, $P = 0.022$; Table 1b, model 2), its effect not varying with metabolism (Supporting information, Fig. S1).

Table 1 Interaction of *CYP2A6* and pharmacotherapy on time to smoking relapse up to 90 days ($n = 709$).

(a) Interaction of CYP2A6 *and pharmacotherapy (placebo versus active pharmacotherapy)*

	Smoking relapse at 90 days		
Predictors	*HR*	*95% CI*	*P*
CYP2A6 activity[a]			
Fast metabolism	Reference		
Slow metabolism	0.39	(0.19, 0.79)	0.0093
Medication status			
Placebo	Reference		
Active pharmacotherapy	0.39	(0.28, 0.54)	1.4×10^{-8}
Interaction of *CYP2A6* and medication			
Slow metabolism × active pharmacotherapy	2.81	(1.32, 5.99)	0.0075

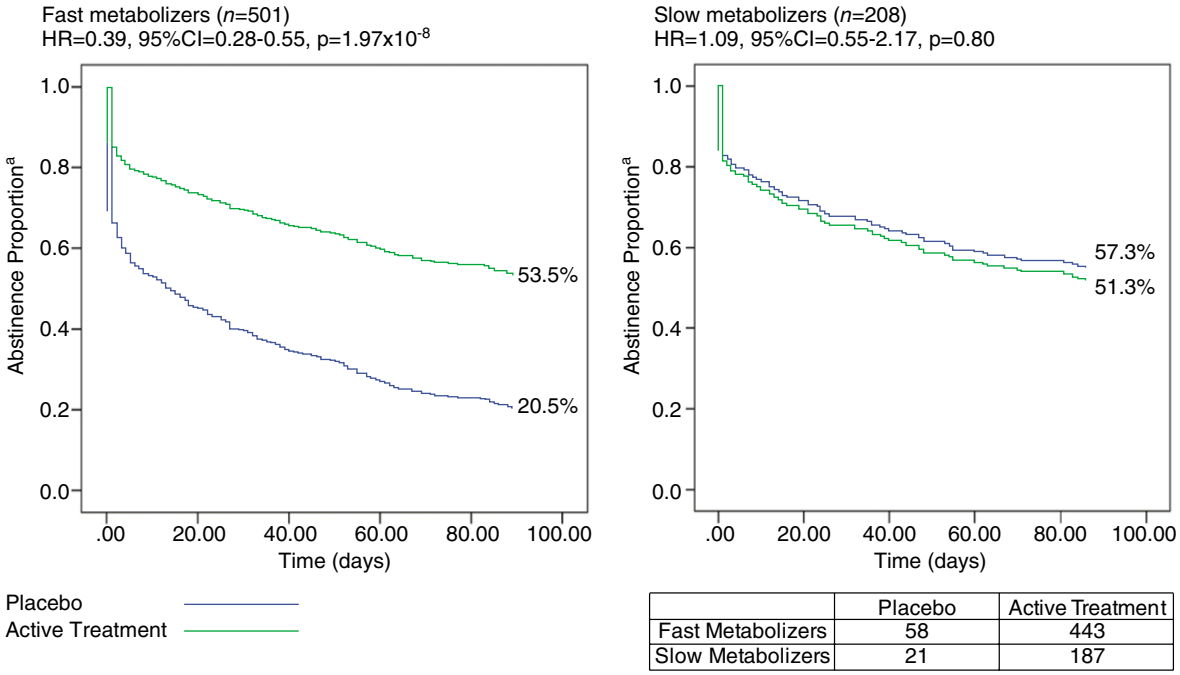
(b) Interaction of CYP2A6 *and pharmacotherapy (placebo versus NRT versus bupropion)*[b]

	Smoking relapse at 90 days		
Predictors	*HR*	*95% CI*	*P*
Model 1: testing interactions			
CYP2A6 activity[a]			
Fast metabolism	Reference		
Slow metabolism	0.67	(0.40, 1.11)	0.12
Use of NRT			
No	Reference		
Yes	0.50	(0.37, 0.67)	2.6×10^{-6}
Use of bupropion			
No	Reference		
Yes	0.77	(0.57, 1.03)	0.082
Interaction of *CYP2A6* and NRT	1.82	(1.07, 3.10)	0.028
Interaction of *CYP2A6* and bupropion	0.95	(0.58, 1.58)	0.85
Model 2: final model			
CYP2A6 activity[a]			
Fast metabolism	Reference		
Slow metabolism	0.65	(0.42, 0.99)	0.045
Use of NRT			
No	Reference		
Yes	0.49	(0.37, 0.66)	9.0×10^{-7}
Use of bupropion			
No	Reference		
Yes	0.76	(0.60, 0.96)	0.022
Interaction of *CYP2A6* and NRT	1.85	(1.11, 3.08)	0.019

All models were adjusted for age and gender. NRT = nicotine replacement therapy. [a]Slow metabolism is defined as the lowest quartile of genotyped-defined *CYP2A6* metabolism; fast metabolism is defined as the higher three quartiles of metabolism. [b]Age and gender are not significant predictors for smoking relapses [hazard ratio (HR) = 1.01, 95% confidence interval (CI) = 0.99–1.02, $P = 0.23$ for age; HR = 1.14, 95% CI = 0.92–1.42, $P = 0.24$ for females].

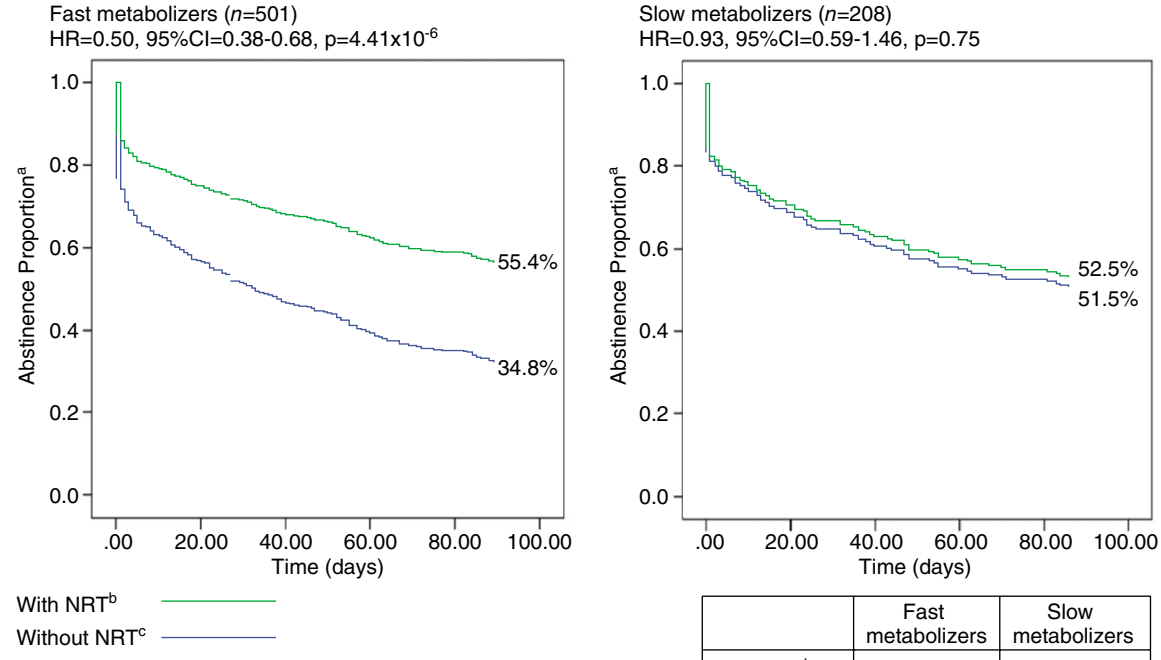
Because metabolism was related to baseline smoking quantity, we examined whether the relations among metabolism, NRT condition and relapse depended on smoking quantity. Heavier smoking at baseline was associated with higher likelihood of relapse (HR = 1.34, 95% CI = 1.17–1.53, $P = 2.5 \times 10^{-5}$), but this relation did not differ by treatment status (interaction effect size = 1.34, 95% CI = 0.94–1.92, $P = 0.11$). The interaction between *CYP2A6*-based metabolism and treatment remained significant (interaction effect size = 2.55, 95% CI = 1.19–5.46, $P = 0.016$; Supporting information, Table S2), after adjusting for CPD. Similar results were found when FTND was used as a covariate.

We found similar results when modeling secondary cessation outcomes (smoking quantity across 8 weeks, point-prevalent abstinence at 8 weeks and continuous abstinence over 90 days). Fast metabolizers receiving placebo escalate their smoking significantly more quickly



	Placebo	Active Treatment
Fast Metabolizers	58	443
Slow Metabolizers	21	187

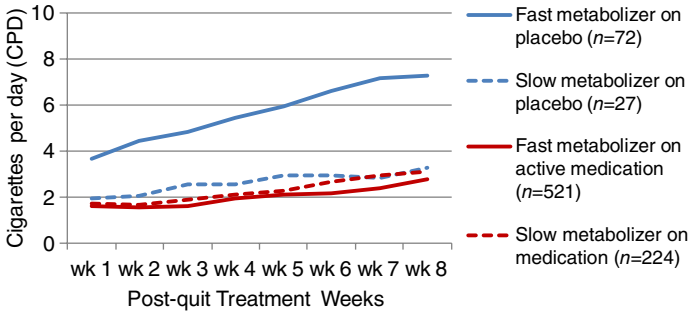
Figure 1 Pharmacotherapy reduces relapse in fast nicotine metabolizers, but not in slow metabolizers defined by *CYP2A6* genotype. [a]Time to relapse over 90 days. There is a significant interaction between medication and *CYP2A6* (interaction effect size = 2.81, 95% CI = 1.32–5.99, $P = 0.0075$).



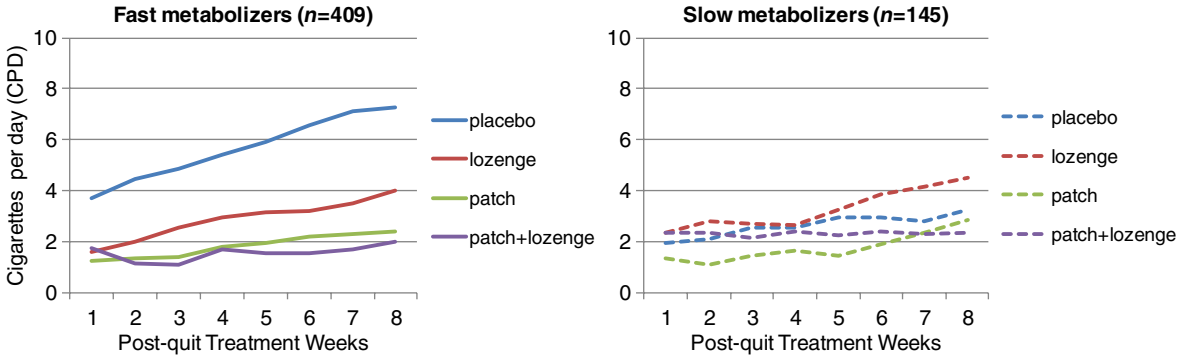
	Fast metabolizers	Slow metabolizers
With NRT[b]	363	149
Without NRT[c]	138	59

Figure 2 Effect of nicotine replacement therapy (NRT) on smoking relapse differs in fast versus slow nicotine metabolizers defined by *CYP2A6* genotype. There is a significant interaction between NRT and *CYP2A6* [interaction effect size = 1.82, 95% confidence interval (CI) = 1.07–3.10, $P = 0.028$). NRT: nicotine replacement therapy. [a]Time to relapse over 90 days. [b]The group with NRT includes all treatment arms with NRT (the NRT arms and the arm receiving both NRT and bupropion. [c]The group without NRT includes all treatment arms without NRT (the placebo arm and the arm receiving bupropion alone).

3A. Active pharmacotherapy vs. placebo



3B. NRT vs. placebo



	Placebo	NRT lozenge	NRT patch	NRT lozenge+patch
Fast Metabolizers	72	98	108	131
Slow Metabolizers	27	42	40	36

Figure 3 Trajectory of post-quit smoking quantity varies by treatment and CYP2A6: fast metabolizers on placebo treatment have a significantly faster escalation into heavy smoking over time [nicotine replacement therapy (NRT) versus placebo]. (a) A significant interaction between active medication and *CYP2A6* on the smoking rate escalation ($t = 3.13$, d.f. = 1, $P = 0.0020$). (b) A trend interaction between NRT combination and *CYP2A6* on smoking quantity over time ($F = 3.75$, d.f. = 1; $P = 0.053$).

than do fast metabolizers on active medication and slow metabolizers on active medication or placebo ($\beta = 0.14$, $t = 3.13$, d.f. = 1, $P = 0.0020$, Fig. 3a). When comparing the four subgroups of subjects formed by crossing NRT versus placebo with *CYP2A6* estimated fast versus slow metabolizer conditions, we found a three-way interaction of NRT condition, *CYP2A6* activity and time (interaction effect size = –0.17, 95% CI = –0.33–0.0025, $P = 0.053$, Fig. 3b) reflecting that smoking escalated especially quickly among fast metabolizers receiving no NRT. The interaction between *CYP2A6* and NRT only approached significance for point-prevalent abstinence at 8 weeks (interaction effect size = 0.69, 95% CI = 0.33–1.46, $P = 0.33$), but was significant for continuous abstinence over 90 days (interaction effect size = 0.37, 95% CI = 0.18–0.78, $P = 0.0091$; Supporting information, Table S3), reflecting that fast metabolizers on placebo were especially unlikely to be abstinent from smoking for the whole 90-day period.

The number needed to treat (NNT) is the average number of patients who need to be treated for one patient to benefit with active treatment versus with placebo treatment. In our study, the NNT for NRT was 2.9 for fast metabolizers (70.7% of the sample) versus >1000 for slow metabolizers (29.3% of the sample). The NNT was 4.2 across all individuals regardless of their genotype status, supporting the established effect of NRT. However, the NNT varied widely depending on the individual's *CYP2A6* genotype (Supporting information, Fig. S2).

Exploratory analyses showed that among fast metabolizers, combination NRT (patch + lozenge) produces lower relapse rates than does NRT monotherapy (HR = 0.61, 95% CI = 0.42–0.89, $P = 0.011$). The lozenge and patch did not differ significantly from one another (HR = 0.69, 95% CI = 0.45–1.05, $P = 0.083$). The effects of the NRT subtypes did not differ among the slow metabolizers.

Combined genetic effects of *CYP2A6* (chromosome 19) and *CHRNA5* (chromosome 15)

Because *CHRNA5* was previously shown to predict smoking cessation and to interact with pharmacotherapy condition, we studied the joint effects of *CYP2A6* and *CHRNA5* on smoking relapse and the interactions between each gene and pharmacotherapy. The interaction of *CYP2A6* and NRT remained significant,

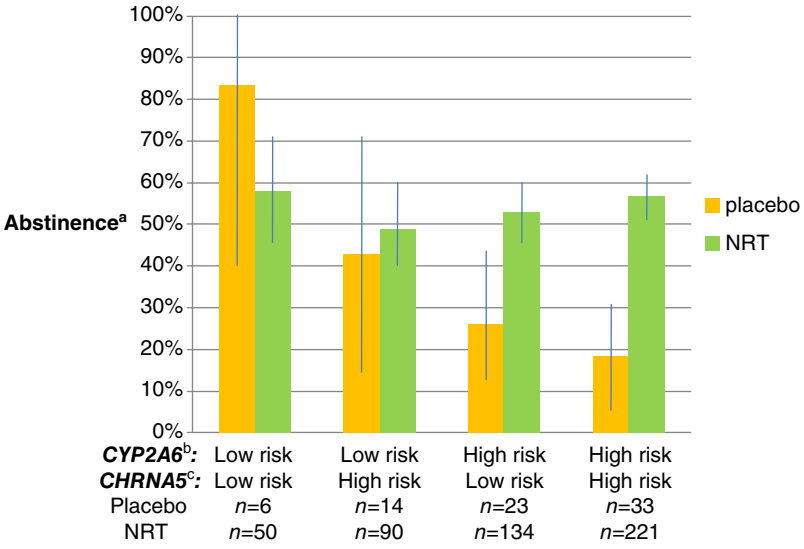


Figure 4 Nicotine replacement therapy (NRT) versus placebo effect on smoking abstinence varies with the combined genetic effects of *CYP2A6* and *CHRNA5*. There is a significant interaction between pharmacotherapy (NRT versus placebo) and four genetic groups (Wald = 7.44, d.f. = 1, $P = 0.0064$). Vertical lines are 95% confidence intervals. [a]Continued abstinence for 3 months. [b]Low versus risk for *CYP2A6* indicates risk for smoking relapse defined by slow versus fast metabolism. [c]Low versus risk for *CHRNA5* indicates risk for smoking relapse defined by CHRNA5 (rs16969968, rs680244) (low-risk diplotypes: GG_CC, GG_CT, GA_CC and high-risk diplotypes: GG_TT, GA_CT, AA_CC).

even after adjusting for the effect of *CHRNA5* (interaction effect size = 1.89, 95% CI = 1.09–3.29, $P = 0.025$; Supporting information, Table S4). Similarly, the interaction effect of *CHRNA5* and pharmacotherapy remained consistent with our previous findings (interaction effect size is 0.49, Wald = 4.59, d.f. = 1, $P = 0.032$, unadjusted for *CYP2A6* and 0.48, Wald = 3.57, d.f. = 1, $P = 0.059$, adjusted for *CYP2A6*). There was no significant interaction between *CHRNA5* and *CYP2A6* on relapse (interaction effect size = 1.15, 95% CI = 0.62–2.17, $P = 0.66$).

Next, we combined the genetic risks defined by *CYP2A6* and *CHRNA5* genotypes into a variable representing the combined genetic risk with four levels. There was a non-linear effect on smoking relapse for NRT as a function of the four-level combined genetic risk (interaction effect size = 0.75, 95% CI = 0.58–0.96, $P = 0.021$), but not for bupropion (interaction effect size = 1.03, 95% CI = 0.82–1.31, $P = 0.79$; Supporting information, Table S5).

To illustrate the interaction between the combined genetic risk and pharmacotherapy (NRT versus placebo), the absolute rates of continuous abstinence over 90 days are shown in Fig. 4. In this study, the NNT for NRT varied with the four combined genetic risk levels: 2.6 for smokers with high-risk status based on *CYP2A6* and *CHRNA5* (44.5% of the sample), 3.7 for smokers with high-risk status based on *CYP2A6* and low-risk status based on *CHRNA5* (27.5% of the sample), 16.6 for smokers with low-risk status based on *CYP2A6* and high-risk status on *CHRNA5* (18.2% of the sample) and >1000 for smokers with low-risk status based on *CYP2A6* and *CHRNA5* (9.81% of the sample). The last very high value indicates the lack of any treatment effect in the lowest risk group. These NNT values may be contrasted with an overall NNT of 4.2 if NRT is given to everyone regardless of the genetic risk.

DISCUSSION

Nicotine metabolism, as estimated from the *CYP2A6* genotype, predicts both smoking cessation success and differential response to cessation pharmacotherapy. Specifically, fast nicotine metabolism is associated with heightened relapse likelihood with placebo and counseling, and this increased genetic risk was 'treated' by cessation pharmacotherapy. Response to NRT differs based on nicotine metabolism. Specifically, active NRT pharmacotherapy is effective among individuals with fast, but not slow estimated nicotine metabolism, thereby reducing the risk of faster metabolism with regard to relapse rate. Conversely, the effect of bupropion on relapse rate does not differ with estimated nicotine metabolism. We also demonstrated that the effect of *CYP2A6* on relapse likelihood remains significant after adjusting statistically for *CHRNA5* (a previously reported genetic predictor of cessation [1]). When both genetic risks are combined, how much an individual benefits from NRT depends upon his/her combined genetic risk levels of both *CYP2A6* and *CHRNA5*. In our study, the wide variation in NNT between smokers with different genetic risks supports the further exploration of pharmacogenetic approaches to smoking treatment.

These findings extend the existing research on *CYP2A6* and the NMR. The NMR is a direct biomarker of nicotine metabolism that reflects both genetic and environmental influences on nicotine metabolism and clearance [21]. In general, faster nicotine metabolism as estimated by NMR has predicted reduced smoking cessation success when individuals were given the nicotine patch, gum or placebo, but not when given nicotine nasal spray or bupropion [14,22]. Using estimated nicotine metabolism based on *CYP2A6* genotypes, we provide additional evidence regarding the effects of specific

pharmacotherapies from a large-scale trial. We confirm that faster nicotine metabolism is associated with a greater relapse likelihood in the placebo condition, and we also find that nicotine metabolism is unrelated to response to bupropion treatment. The latter observation is consistent with existing evidence [13] and the fact that bupropion is primarily metabolized by the CYP2B6 enzyme [23,24]. Instead of using NMR, this study presents the first genotype by NRT interaction with a proper placebo-control arm. Our findings differ from earlier reports primarily because we found that nicotine metabolism did not predict cessation outcome among people given NRT, which appears to neutralize the relapse risk associated with faster nicotine metabolism. At present, it is difficult to resolve the differences between study findings due to differences between subjects and experimental conditions. These differences highlight important methodology considerations: (i) some of the prior studies did not include a placebo arm, which is needed to determine a gene × medication interaction, (ii) NRT and bupropion were often not included in the same trial and (iii) in the current trial the same behavioral counseling was used in all medication conditions, whereas the effects of counseling could vary across trials, which is a possibility based on observed differing abstinence rates in placebo arms of different trials. While future meta-analyses can be helpful, caution should be used, as differences in ascertainment, treatment intensity, assessment and treatment comparisons across trials can result in problematic interpretations [25,26].

Personalized medicine for smoking cessation will require the optimal combination of multiple genetic predictors. We previously reported genetic variants in *CHRNA5* as robust predictors of cessation success and response to pharmacotherapy [1], and similar associations have been reported by a large pharmacogenetic consortium [6] which includes eight trials, including the current study. This current study shows that *CYP2A6* on chromosome 19 has an effect independent of *CHRNA5* on chromosome 15, suggesting that these two genetic markers represent distinct biological pathways that influence smoking behaviors. Our findings extend the recent report of an additive effect of these two genes on smoking quantity (cigarettes smoked per day) and risk for lung cancer [27].

This study has several limitations. First, the sample is limited in several ways. When multiple genetic markers are analyzed, the sample size in certain conditions becomes small, so these effect size estimates should be considered as preliminary. This sample is the same as that used in the Chen *et al*. study [1], thus this research does not provide new evidence to support the relation of *CHRNA5* with cessation outcomes. This study focuses only on European Americans. Secondly, one could surmise that smoking quantity plays a mediating role in our reported association between *CYP2A6* and cessation [28]. We showed that *CYP2A6* remained significantly associated with smoking outcomes, suggesting that it could influence biological processes underlying both smoking quantity and smoking cessation [22,29,30]. However, self-reported smoking rate is an imperfect measure of actual smoking heaviness [31,32], and future research would benefit from the use of sensitive biomarkers of tobacco exposure. Future studies of mechanisms require biomarkers such as cotinine levels. The mechanisms underlying *CYP2A6* and smoking cessation are not entirely clear; the complex genetic architecture in this chromosomal region and other metabolic pathways in addition to nicotine metabolism could play a role. Thirdly, the *CYP2A6* gene is highly polymorphic [33], including many variants in Europeans [10]. Its complex genetic architecture challenges the examination of this gene. We may not have captured other important genetic variation in this region, which could have contributed to the results. We used a *CYP2A6* genotype-based nicotine metabolism estimate derived from an experiment performed in an independent sample [7,11]. Our findings, using a genetic metric of *CYP2A6* activity, provide a complementary research paradigm, but it is certainly possible that different results would have been obtained through use of the NMR. Finally, given such limitations, further work is clearly needed to develop a treatment algorithm that enhances the effectiveness and cost-effectiveness of smoking treatment.

Keeping in mind the above limitations, this study extends previous work on *CHRNA5*, *CYP2A6* and cessation to reach the following conclusions. (i) Nicotine metabolic function estimated via *CYP2A6* genotype predicts relapse likelihood in individuals using placebo medication. (ii) The effect of pharmacotherapy differs as a function of *CYP2A6*-based nicotine metabolism. In particular, NRT significantly benefits smokers with fast but not slow nicotine metabolism. The effect of bupropion on relapse likelihood is largely unrelated to *CYP2A6* status. (iii) The effects of *CYP2A6*-estimated nicotine metabolism on response to NRT are independent from those of the *CHRNA5* genotype, with the effects of the two genes being additive. That is, the likelihood of benefit from NRT may increase as a function of the combined genetic risks from *CYP2A6* and *CHRNA5*. An important clarification of these pharmacogenetic findings will rely upon validation across populations and with different cessation processes, such as natural versus assisted cessation. A larger study or meta-analysis is required to examine the effect of different pharmacotherapies in the combined genetic risk groups. Many other genes have been nominated as predictive of smoking cessation [34], and we anticipate that more genes will be identified as playing a role in smoking

cessation. Risk prediction modeling to incorporate multiple genetic markers and non-genetic predictors will lay the foundation for a personalized treatment algorithm [35].

Declaration of interests

Dr Bierut and Dr Goate are listed as inventors on issued US patent 8,080,371, 'Markers for Addiction', covering the use of certain SNPs in determining the diagnosis, prognosis and treatment of addiction. Dr Saccone is the spouse of Dr S. Saccone, who is also listed as an inventor on the above patent. Dr Smith has served in the past 3 years as a co-investigator on an investigator-initiated research study at the University of Wisconsin-Madison that was supported by Pfizer. Drs Chen, Bloom, Baker, Smith, Piper, Saccone, Hatsukami and Ms Martinez declare no potential conflicts of interest.

Acknowledgements

Glaxo Wellcome provided bupropion at no cost in the UW-TTURC clinical trial. The Wisconsin State Laboratory of Hygiene provided considerable technical assistance in this research effort in the form of DNA extraction, and this research was also aided by the Wisconsin Partnership Program. This research was supported by NIH grants P01 CA089392 (L.J.B.), P50 CA84724 (T.B.B.), and K05CA139871 (T.B.B.) from the National Cancer Institute, P50 DA19706 (T.B.B.), R01 DA026911 (N.L.S.), K02 DA021237 (L.J.B.) and K08 DA030398 (LSC) from the National Institute on Drug Abuse, U01 HG004422 (L.J.B.) from the National Human Genome Research Institute, subaward KL2 RR024994 (L.S.C.) from the National Center for Research Resources, 5T32MH014677-33 from the National Institute of Mental Health (A.J.B.), and the Wisconsin Partnership Program. Genotyping services for the UW-TTURC sample were provided by the Center for Inherited Disease Research (CIDR) and DNA extraction supported by the Wisconsin State Laboratory of Hygiene. Funding support for CIDR was provided by NIH grant U01HG004438 and NIH contract HHSN268200782096C to the Johns Hopkins University. Assistance with genotype cleaning was provided by the Gene Environment Association Studies (GENEVA) Coordinating Center (U01 HG004446).

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Supporting information

Additional Supporting information may be found in the online version of this article at the publisher's website:

Figure S1 Effect of bupropion on smoking relapse does not differ in fast versus slow nicotine metabolizers defined by *CYP2A6 g*enotype[a]

Figure S2 Nicotine replacement therapy (NRT) versus placebo effect on smoking abstinence varies with the combined genetic effects of *CYP2A6*[a]

Table S1 Characteristics of the University of Wisconsin Trans-disciplinary Tobacco Use Research Center (UW-TTURC) sample

Table S2 Interaction of *CYP2A6* metabolism and pharmacotherapy effect on smoking relapse at 90 days, adjusting for smoking heaviness (cigarettes smoked per day) ($n = 709$)*

Table S3 Interaction of *CYP2A6* and pharmacotherapy [placebo versus Nnicotine replacement therapy (NRT) versus bupropion] ($n = 709$) on secondary cessation outcome of 3-month continuous abstinence

Table S4 Testing the effects of both genetic markers (*CYP2A6* and *CHRNA5*) and pharmacotherapy on smoking relapses ($n = 687$)

Table S5 Combined genetic marker (*CYP2A6* and *CHRNA5*) and medication effect [nicotine replacement therapy (NRT), bupropion, placebo] on smoking relapse at 90 days ($n = 687$)