CLINICAL TRIALS

Nicotine metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation

Background: Nicotine is metabolized to cotinine, and cotinine is metabolized to 3′-hydroxycotinine (3-HC) by the liver enzyme cytochrome P450 (CYP) 2A6. More rapid metabolism of nicotine may result in lower nicotine blood levels from nicotine replacement products and poorer smoking cessation outcomes. This study evaluated the utility of the 3-HC/cotinine ratio as a predictor of the efficacy of nicotine replacement therapy as an aid for smoking cessation.

Methods: By use of an open-label design, 480 treatment-seeking smokers were randomly assigned to 8 weeks of transdermal nicotine or nicotine nasal spray use, plus behavioral group counseling. Assessments included demographics, smoking history, body mass index, and plasma nicotine, cotinine, and 3-HC concentrations, as well as *CYP2A6* genotypes. Smoking cessation was biochemically verified at the end of treatment and at 6-month follow-up.

Results: The rate of nicotine metabolism, as indicated by pretreatment 3-HC/cotinine ratio derived from cigarette smoking, predicted the effectiveness of transdermal nicotine at both time points. The odds of abstinence were reduced by almost 30% with each increasing quartile of metabolite ratio (odds ratio, 0.72 [95% confidence interval, 0.57-0.90]; $P = .005$). Higher metabolite ratios also predicted lower nicotine concentrations ($\beta = -1.72$, $t_{179} = -3.31$, $P < .001$), as well as more severe cravings for cigarettes after 1 week of treatment ($\beta = 0.32$, $t_{190} = 2.91$, $P = .004$). The metabolite ratio did not predict cessation with use of nicotine nasal spray (odds ratio, 1.05 [95% confidence interval, 0.83-1.33]; $P = .68$).

Conclusion: The nicotine metabolite ratio might be useful in screening smokers to determine likely success with a standard dose of transdermal nicotine. (Clin Pharmacol Ther 2006;79:600-8.)

Caryn Lerman, PhD, Rachel Tyndale, PhD, Freda Patterson, MS, E. Paul Wileyto, PhD, Peter G. Shields, MD, Angela Pinto, BA, and Neal Benowitz, MD *Philadelphia, Pa, Toronto, Ontario, Canada, Washington, DC, and San Francisco, Calif*

Nicotine is metabolized to cotinine, predominantly by the liver enzyme cytochrome P450 (CYP) 2A6.[1,2] Cotinine is metabolized further to 3′-hydroxycotinine (3-HC) by the same enzyme.[1] A number of studies have found that genetic variation in *CYP2A6* predicts cigarette consumption and smoking persistence,[3–5] consistent with the

From the Department of Psychiatry, University of Pennsylvania, Philadelphia; Centre for Addiction and Mental Health and Department of Pharmacology, University of Toronto, Toronto; Department of Oncology, Georgetown University, Washington, DC; and Departments of Medicine, Psychiatry, and Biopharmaceutical Sciences, Division of Clinical Pharmacology, University of California San Francisco, San Francisco.

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Reprint requests: Caryn Lerman, PhD, University of Pennsylvania Transdisciplinary Tobacco Use Research Center, 3535 Market St, Suite 4100, Philadelphia, PA 19104.

E-mail: *clerman@mail.med.upenn.edu*

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premise that faster inactivation and elimination of nicotine require higher levels of smoking to maintain the desired levels of nicotine in the body. Conversely, smokers with *CYP2A6* variant alleles associated with slow nicotine metabolism smoke fewer cigarettes per day and have a lower risk of development of lung cancer.[4] The ratio of 3-HC to its precursor cotinine provides a phenotypic measure of *CYP2A6* activity[6] and, therefore, the rate of nicotine metabolism and also correlates significantly with daily smoking rate.[7,8] A phenotypic measure of CYP2A6 activity is important because there is wide variability in the rate of nicotine metabolism, and relatively little of that variability can be explained by currently identified *CYP2A6* variants.[9]

Given the relationship between the rate of metabolism of nicotine and smoking behavior, the 3-HC/cotinine ratio would be expected to predict systemic exposure to nicotine from a given dose of a nicotine replacement product and was hypothesized to be a useful pretreatment predictor of therapeutic response. Nicotine replacement therapies are a mainstay of smoking cessation therapy around the world. Although nicotine replacement therapies, such as transdermal nicotine, nicotine nasal spray, and nicotine gum, can double a smoker's odds of quitting smoking compared with placebo, these treatments are effective for only 20% to 30% of smokers.[10–12] Thus a clinical pretreatment measure that identifies likely responders and nonresponders to nicotine replacement therapy could guide the choice of treatment for individual patients to improve treatment outcomes. In addition, if rapid metabolizers of nicotine benefit less from a standard dose of nicotine replacement therapy because nicotine blood levels are low, then higher doses of nicotine treatment might be given to these individuals.

We previously reported the outcomes of an open-label randomized clinical trial of transdermal nicotine versus nicotine nasal spray for smoking cessation.[13] This article reports new data supporting the utility of the 3-HC/cotinine ratio as a predictor of treatment outcome in this trial. We predicted that smokers with higher 3-HC/cotinine ratios would have lower rates of success with nicotine replacement therapy. This association should be stronger among smokers treated with transdermal nicotine, which, unlike nicotine nasal spray, does not afford an opportunity for individuals to titrate the dose of nicotine as desired.

METHODS

The University of Pennsylvania Institutional Review Board (Philadelphia, Pa) approved all research procedures, and all study participants provided written informed consent. The procedures for the clinical trial have been presented previously.[13] In brief, smokers responding to advertisements for free smoking cessation treatment were recruited from February 2000 through April 2003. Eligible individuals were aged 18 years or older and smoked at least 10 cigarettes per day for the prior 12 months. Standard exclusion criteria for nicotine replacement therapy were used.[13]

Six hundred fifty-eight smokers met the study eligibility criteria. Of these individuals, 58 (9%) withdrew before treatment, leaving 600 participants. Pretreatment plasma samples were unavailable for 120 of the consenting participants (20%), and these participants were excluded from all analyses. Thus the final sample in the analysis included 480 smokers, 240 of whom were randomized to receive transdermal nicotine and 240 of whom were randomized to receive nicotine nasal spray. Participants for whom a sample was unavailable did not differ significantly from other participants with regard to their smoking histories, 3-HC/cotinine ratios, or abstinence rates (all $P > .2$).

Study design and procedures

The trial was an open-label randomized clinical trial of transdermal nicotine versus nicotine nasal spray for smoking cessation. Nicotine nasal spray (Nicotrol; Pharmacia, Helsingborg, Sweden) was initiated on the target quit date (week 3) and provided over an 8-week period. At the second counseling session (week 2), participants were shown how to self-administer a 1.0-mg dose (0.5-mg spray in each nostril) and were instructed to use nasal spray 8 to 40 times per day (with a maximum of 5 doses per hour) beginning on the target quit date. After 4 weeks of use, participants were instructed to taper their nasal spray dose by one third for a 2-week period and then by another third for the final 2 weeks of treatment. Transdermal nicotine (Nicoderm CQ; GlaxoSmithKline, Research Triangle Park, NC) was used by participants over an 8-week treatment period, beginning on the morning of the target quit date (week 3). A 24-hour tapered-dose formulation was used, as follows: 21 mg for 4 weeks, 14 mg for 2 weeks, and 7 mg for 2 weeks. All participants received 7 sessions of standardized behavioral group counseling.[13]

Assessment procedures

Before treatment, participants completed self-report measures of their smoking habits and provided a breath sample for measurement of the carbon monoxide (CO) level to confirm current smoking status and a blood sample from which plasma nicotine and metabolites

were determined. Although the time of day of the pretreatment blood draw was variable, previous research has shown that plasma cotinine concentrations are fairly stable throughout the day.[8] Because 3-HC has a much more rapid half-life than cotinine, its half-life is determined by the half-life of cotinine, from which it is formed.[8] Therefore the ratio of 3-HC to cotinine will be stable over time and will not depend on time since last cigarette.

After 7 days of nicotine replacement therapy, a second blood sample was collected to reassess plasma nicotine and cotinine levels for the purpose of determining systemic exposure during treatments. These samples were collected in the evening between approximately 4 and 6 PM; at the same time, a CO measurement was taken to biochemically verify abstinence from smoking. To assess smoking cessation, telephone interviews were conducted at the end of treatment (8 weeks after the target quit date) and at 6-month follow-up (6 months after the target quit date) by use of a standard timeline follow-back method.[14] Participants who reported complete abstinence (not even a puff of a cigarette) for at least the 7 days before the assessment were asked to complete an in-person visit at the end of treatment at 6-month follow-up for biochemical verification of abstinence (as described later).

Predictor variables

The primary predictor variable was the 3-HC/cotinine ratio measured in plasma from blood samples collected before treatment (ie, in the baseline smoking state). The 3-HC/cotinine ratio reflects CYP2A6 activity and is correlated with the clearance of nicotine.[8] Plasma concentrations of cotinine and 3-HC were measured by HPLC–tandem mass spectrometry, and concentrations of nicotine were measured by gas chromatography with nitrogen phosphorus detection, as described previously.[8] Participants in this study were previously genotyped for *CYP2A6* alleles.[6]

Covariates

Demographic factors and smoking history were assessed by self-report during the pretreatment assessment visit. The 6-item Fagerström Test for Nicotine Dependence was used to measure tobacco dependence.[15] Height and weight were measured at the medical screening visit. Body mass index was calculated by dividing weight in kilograms by height in square meters, and obesity was defined according to the National Institutes of Health recommendation as a body mass index of 30 kg/m^2 or greater.[16]

Outcome variables

Smoking cessation. Prolonged abstinence at the end of the treatment phase and at 6 months' follow-up was the primary outcome measure.[17] Prolonged abstinence was defined as not reporting 7 consecutive days of smoking at any time during the follow-up period[18] and was biochemically confirmed at each end point by a CO reading of 10 ppm or less.[19] The intent-to-treat sample includes all participants who received even 1 dose of therapy (counseling or nicotine replacement therapy), independent of whether they participated in follow-up surveys. It was presumed that participants who were lost to follow-up (35/658 [5.3%]) and those who self-reported abstinence but failed to provide a CO measure at the end point (41/159 [26%]) had resumed smoking and were coded as such in the primary outcome analyses.[19] Participants lost to follow-up did not differ significantly from other participants in terms of smoking history or 3-HC/cotinine ratio.

Treatment variables

Abstinence-related symptoms. A self-report measure of withdrawal symptoms was administered by a research assistant before each weekly treatment visit. This measure assessed the severity (in the past 7 days) of 18 symptoms, including items such as irritability, difficulty concentrating, anxiety/tension, insomnia, drowsiness, nausea, and general physical complaints (eg, sweating and dizziness).[20,21] Responses to items (ranging from 0 [not at all] to 3 [severe]) were summed to create a withdrawal severity index. Two items assessing cravings for cigarettes were summed to create a craving subscale (ie, "cravings for cigarettes" and "urges to smoke").[22] Analyses focused on symptoms after 1 week of treatment to coincide with the time point for posttreatment plasma nicotine assessments.

Side effects. A side effect checklist was completed by participants to assess the severity of physical complaints potentially associated with nicotine replacement therapy treatment (eg, headache and dizziness). Responses to items after the first week of nicotine replacement therapy (ranging from 0 [none] to 3 [severe]) were summed to create a side effect severity index.

Nicotine replacement therapy usage. Participants assigned to transdermal nicotine use recorded their daily application of patches, and those assigned to nasal spray use recorded the number of doses of nasal spray administered per day. Because usage of nicotine replacement therapy may be confounded by smoking status (ie, participants may discontinue treatment if they resumed smoking), we focused on average usage during the first 2 weeks of treatment.

Table I. Background variables and baseline nicotine metabolic data by treatment group and quitting success

Variable	*All patients (N = 480)*	*Nicotine patch users (n = 240)*	*Nicotine spray users (n = 240)*	*Patients who failed at EOT (n = 316)*	*Patients who succeeded at EOT (n = 164)*
Age (y)	45.5 ± 10.3	45.7 ± 10.6	45.2 ± 10.1	45.8 ± 10.7	44.9 ± 9.6
Female (%)	54.4	53.8	55.4	56.0	51.8
At least some college education (%)	45.4	43.3	47.5	47.5	41.5
White race (%)	65.2	62.5	67.9	67.1	61.6
Body mass index (kg/m^2)	28.2 ± 5.8	27.8 ± 5.6	28.6 ± 6.1	27.0 ± 5.9	28.6 ± 5.8
Cigarettes per day	21.9 ± 9.8	21.4 ± 9.7	22.4 ± 9.8	22.3 ± 9.7	20.9 ± 9.1
Nicotine dependence (Fagerström Test for Nicotine Dependence) score	5.5 ± 2.1	5.4 ± 2.1	5.6 ± 2.1	5.6 ± 2.0	5.3 ± 2.4
Plasma cotinine (ng/mL)*	273.6 ± 131	262.3 ± 112.6	284.9 ± 146.9	279.9 ± 133.2	261.6 ± 126.9
Plasma 3-HC (ng/mL)	96.5 ± 57.5	96.0 ± 51.6	96.9 ± 6309	102.4 ± 61.4	85.0 ± 47.3
Plasma 3-HC/cotinine ratio	0.44 ± 0.9	0.50 ± 1.2	0.38 ± 0.2	0.45 ± 0.9	0.43 ± 0.9
Log of plasma 3-HC/cotinine ratio	−1.09 ± 0.6	−1.04 ± 0.7	−1.14 ± 0.6	−1.05 ± 0.6	−1.15 ± 0.6

Data are given as mean ± SD, unless otherwise specified.
EOT, End of treatment; 3-HC, 3′-hydroxycotinine.
*P = .06 for between-group difference.

Statistical analysis

We calculated that a sample size of at least 200 persons per treatment group was necessary to detect a within–treatment group difference in quit rate of 10% or greater across metabolite ratio groups with .80 power (α set at .025) (PASS, Power and Sample Size; NCSS Software, Kaysville, Utah). Chi-square tests and t tests were used to examine potential differences in pretreatment variables by treatment group assignment. Associations of nicotine metabolite ratio (3-HC/cotinine ratio) with baseline smoking variables were assessed by use of Spearman correlations; because of the skewed distribution of the 3-HC/cotinine ratio measure, we used a logarithmic transformation. Longitudinal logistic regression analysis (generalized estimating equations) was used to test the primary hypothesis of the effects of nicotine metabolite ratio on abstinence rates for the total sample and for each treatment group, controlling for pretreatment levels of nicotine dependence, body mass index, race, and sex. We used regression and ANOVA methods to explore associations of log nicotine metabolite ratio with continuous treatment-related variables (eg, treatment levels of nicotine and cotinine, usage, side effects, and abstinence symptoms). The analyses of treatment variables used complete cases for that time point.

For ease of clinical interpretation in multivariate models, the ratio measure was grouped into quartiles for graphic display and ranked for most regression analyses. Interactions were tested by use of the Wald chi-square test. The assumptions of linearity were examined visually by use of the lowess-smoothed trend in the data, and homoscedasticity was confirmed by use of the Cook-Weisberg test. We also examined colinearity of predictor variables in multiple regressions by examining the correlation matrix resulting from the estimation procedure for high values.

RESULTS

Characteristics of participants

As shown in Table I, there were no significant differences in demographic or baseline smoking history variables or in baseline metabolites by treatment group. The participants' mean age was 45 years (SD, 10.3 years), 55% were women, and 45% had at least some college education. Sixty-five percent of participants were of European ancestry, 28% were of African American ancestry, and 7% were from other

Table II. Longitudinal regression analysis (generalized estimating equations) of smoking cessation by metabolite ratio, time, and treatment, controlled for baseline body mass index, nicotine dependence, race, and sex

Predictor	*OR and 95% CI*	*P*	*Wald test for specific comparisons**
Rank of ratio			
Nicotine patch group	0.72 (0.57-0.91)	.006	$\chi^2_1 = 4.39$, $P = .04$
Nicotine spray group	1.05 (0.83-1.34)	.680	
Treatment group			
EOT†	0.29 (0.14-0.62)	.001	$\chi^2_1 = 5.24$, $P = .02$
6 mo	0.45 (0.20-0.97)	.041	
Time point (6 mo versus EOT)	0.35 (0.26-0.47)	< .0001	

OR, Odds ratio, reflecting change in odds for 1 quartile in rank; CI, confidence interval.
*Wald test contrasts specific pairs of regression coefficients, thereby representing Interaction effects for ratio × Treatment and Time × Treatment.
†Transdermal nicotine group coded as 1 and nicotine nasal spray coded as 0.

ethnic groups. On average, participants smoked about 22 cigarettes per day (SD, 9.8 cigarettes per day), and the mean Fagerström Test for Nicotine Dependence score was 5.5 (SD, 2.1). The mean plasma cotinine level at baseline was 274 ng/mL (SD, 131 ng/mL), and the mean 3-HC level was 96 ng/mL (SD, 58 ng/mL). The mean 3-HC/cotinine ratio was 0.44 (SD, 0.9). The quartile means, medians, and ranges were as follows: (1) 0.17, 0.18, and 0.20 to 0.23; (2) 0.29, 0.29, 0.23 to 0.35, respectively; (3) 0.41, 0.41, and 0.35 to 0.47, respectively; (4) 0.90, 0.61, and 0.47 to 16.1, respectively.

Associations of nicotine metabolite ratio with baseline smoking-related variables

The log 3-HC/cotinine ratio, as determined at the pretreatment assessment, was significantly positively correlated with self-reported number of cigarettes per day ($r = 0.12$, $P = .01$) and with CO levels ($r = 0.12$, $P = .03$) and was negatively correlated with pretreatment plasma nicotine levels ($r = -0.24$, $P < .0001$). The ratio measure was not significantly correlated with nicotine dependence score. The number of cigarettes smoked per day increased in a linear fashion with the 3-HC/cotinine ratio ($\beta = 1.03$, $t_{476} = -2.75$, $P = .01$). Though statistically significant, it should be acknowledged that these correlations are relatively weak.

Effects of nicotine metabolite ratio on smoking abstinence

The 3-HC/cotinine ratio was not significantly associated with smoking abstinence in the total sample (odds ratio [OR], 0.85 [95% confidence interval (CI), 0.71-1.02]; $P = .07$). However, the longitudinal logistic regression analysis (generalized estimating equations) (Table II) indicates that, for those randomized to the transdermal nicotine group, the odds of quitting smoking were significantly lower for those with higher nicotine metabolite ratios (faster metabolizers) at both the end of treatment and 6 months' follow-up (OR, 0.72 [95% CI, 0.57-0.90]; $P = .005$). There was no relationship of nicotine metabolite ratio with smoking cessation in the nicotine spray group (OR, 1.05 [95% CI, 0.83-1.33]; $P = .68$) (Fig 1). Wald tests revealed that the effects of the metabolite ratio on abstinence vary by treatment, with a significantly greater effect of metabolite ratio in the transdermal nicotine group compared with the spray group ($\chi^2_1 = 4.32$, $P = .04$). Among subjects in the transdermal nicotine group, there was almost a 30% reduction in the odds of quitting with each increasing quartile of metabolite ratio; furthermore, smokers in the highest quartile had a 69% reduction in the odds of maintaining abstinence from smoking compared with those in the lowest quartile. The effects of the nicotine metabolite ratio on abstinence did not differ significantly by time point; however, a significant effect of time point indicated that the odds of abstinence were significantly higher at the end of treatment than at 6 months' follow-up. In addition, the model indicated a significant effect of treatment, with higher odds of abstinence with transdermal nicotine versus nicotine nasal spray (Table II). The model was also generated with exclusion of nonresponders to the follow-up surveys, and the results were unchanged.

Effects of nicotine metabolite ratio on treatment levels of plasma nicotine and cotinine

Analyses of the relationship of 3-HC/cotinine ratio with treatment levels of nicotine and cotinine levels (after 1 week of treatment) were stratified by treatment group and were performed with the subset of partici-

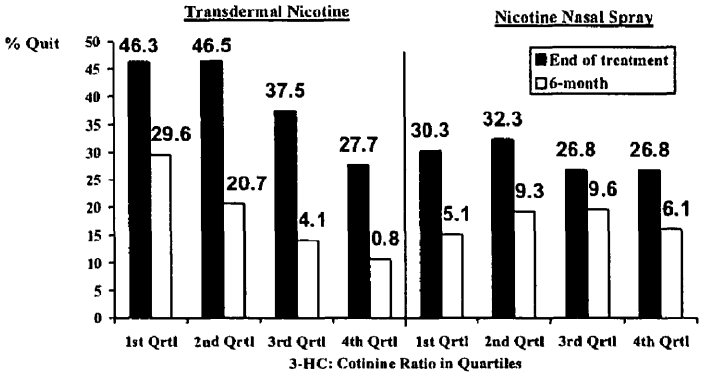

Fig 1. Smoking cessation rates by nicotine metabolite ratio and treatment (N = 480). Among participants randomized to the transdermal nicotine arm, the nicotine metabolite ratio was significantly associated with abstinence at the end of treatment and 6 months' follow-up (P = .006). The association was not significant in the nicotine spray arm (P = .68). Qrtl, Quartile.

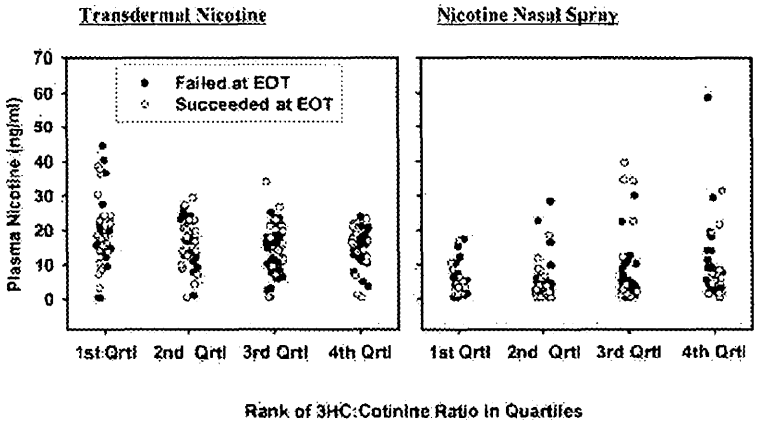

Fig 2. Levels of plasma nicotine after 1 week of treatment by nicotine metabolite ratio and treatment group (abstainers at 1 week after treatment only, n = 333). Among participants randomized to the transdermal nicotine arm, there was a significant linear reduction in plasma nicotine levels for each quartile of the nicotine metabolite ratio (P < .01). Quartiles (x values) were varied randomly to make them visible for the illustration. EOT, End of treatment.

pants who were abstinent from smoking at that time point (biochemically verified by CO level ≤10 ppm) (n = 333). Fig 2 shows plasma nicotine concentrations during nicotine replacement therapy according to the 3-HC/cotinine quartile. In the transdermal nicotine group there was a significant linear reduction in plasma nicotine levels for each increase in quartile of the 3-HC/cotinine ratio ($\beta = -1.72$, $t_{179} = -3.31$, $P <$ .001). In contrast, in the nicotine spray group, in which smokers selected their own dose of nicotine, there was a significant increase in nicotine levels with each quartile ($\beta = 1.63$, $t_{154} = 2.61$, $P <$ .01). In the group of participants with verified abstinence after 1 week of treatment, the log plasma nicotine level during treatment was a significant predictor of abstinence at 6 months' follow-up (OR, 1.97 [95% CI, 1.15-1.39]; $P <$ .02) when we controlled for group, race, and log baseline cotinine level. However, the log plasma nicotine level was not significantly associated with abstinence at the end of treatment (P = .20).

Effects of nicotine metabolite ratio on treatment variables

Among participants with verified abstinence after 1 week of treatment, the log nicotine metabolite ratio was significantly associated with the intensity of cravings for cigarettes in the transdermal nicotine group (β = 0.32, t_{190} = 2.91, P = .004) but not in the nicotine spray group (β = 0.04, t_{169} = 0.37, P = .70). In the transdermal nicotine group cravings increased in a linear fashion with each quartile of the metabolite ratio. However, the ratio did not predict total withdrawal symptoms in either the transdermal group (β = 0.09, t_{190} = 0.17, P = .9) or the nasal spray group (β = 0.19, t_{169} = 0.36, P = .7). There were no associations of the ratio with side effect scores in the population as a whole or in either group (both $P > .10$).

The nicotine metabolite ratio did not predict transdermal nicotine usage among the subset of participants who were abstinent during the first 2 weeks of treatment and who provided usage data (n = 186) ($F_{1,184}$ = 0.04, P = .85); participants in this group reported using transdermal nicotine patches for a mean of 6.5 days (SD, 1.3 days) during this period. Among abstinent participants in the nicotine spray group who provided usage data (n = 153), those with nicotine metabolite ratios above the median used the spray a mean of 11 times per day (SD, 9.4) compared with 8.5 times per day (SD, 7.2) for those with ratios below the median ($F_{1,151}$ = 4.1, P = .04).

We previously genotyped participants in this study for *CYP2A6* alleles. As reported previously,[6] white smokers with genotypes associated with 50% activity or lower (slow metabolizers; genotypes 1/2, 1/4, 9/9, 9/12, and 12/12) have lower 3-HC/cotinine ratios, smoke fewer cigarettes per day, and have higher plasma nicotine levels after 1 week of nicotine patch treatment, as compared with smokers with 2 normal-activity alleles (normal metabolizers, genotype 1/1). For the current analysis, we examined the association of slow metabolizers (n = 15) and normal metabolizers (n = 248). In this sample there were no significant associations of genotype group with abstinence at the end of treatment (abstinence in 27% of slow metabolizers versus 33% of normal metabolizers, χ^2 = 0.23, P = .63) or at 6 months' follow-up (abstinence in 7% of slow metabolizers versus 19% of normal metabolizers, χ^2 = 1.4, P = .23). When the analyses were stratified by treatment condition, the results were unchanged (all $P > .3$). It should be noted that cell sizes in these analyses are very small because of the small number of slow metabolizers. Therefore these *CYP2A6* genotype data should be interpreted cautiously.

DISCUSSION

A novel observation from this clinical trial is that the rate of nicotine metabolism, as indicated by the 3-HC/cotinine ratio, predicts the effectiveness of transdermal nicotine as a treatment for smoking cessation. The difference in smoking cessation rates by the nicotine metabolite ratio is both statistically and clinically significant. At the end of the 8-week treatment phase, 46% of smokers in the lowest quartile of the metabolite ratio had quit successfully, as compared with 28% in the highest quartile, an effect difference that was even greater at 6 months' follow-up.

In previous smoking cessation trials using transdermal nicotine, pretreatment plasma cotinine and nicotine dependence measures have predicted outcome in some studies[23,24] but not others.[25] The plasma cotinine concentration is affected not only by the dose of nicotine taken in but also by the extent of metabolism of nicotine to cotinine, as well as the rate of clearance of cotinine.[26] Therefore plasma cotinine is an imprecise predictor of the dose of nicotine taken in from smoking and, accordingly, an imprecise predictor of the dose of nicotine required to deliver an effective level of nicotine to facilitate smoking cessation.

The predictive value of the 3-HC/cotinine ratio for successful smoking cessation with transdermal nicotine is most likely attributable to differences in systemic exposure to nicotine from this product. Higher metabolite ratios were associated with lower plasma nicotine levels during transdermal nicotine therapy, consistent with more rapid metabolism of nicotine. The findings suggest a nicotine concentration-response relationship, such that higher treatment levels of plasma nicotine result in a higher probability of successful quitting and maintenance of abstinence at 6 months' follow-up; however, treatment levels of plasma nicotine did not predict abstinence at the end of treatment, which is difficult to explain. The results suggest further that smokers with higher nicotine metabolite ratios and lower treatment levels of nicotine have more severe cravings to smoke during the first week of abstinence, a response that can increase relapse liability.[22]

Early clinical trials with transdermal nicotine showed a clear dose-response relationship with smoking cessation comparing 24-hour patches with doses of 7, 14, and 21 mg.[11,27] It has been suggested that doses of transdermal nicotine higher than the standard clinical dose of 21 mg may boost success rates even further.[28] However, some studies have not found better outcomes with a 44-mg dose compared with a 22-mg dose.[23,29] Our study shows that faster metabolizers have a poorer outcome compared with slower metabolizers of nico-

tine with a fixed 21-mg patch dose. Given the wide individual variability in the rate of nicotine metabolism, it makes sense that any future dose-response study using transdermal nicotine would be better interpreted if the rate of nicotine metabolism is also known.

The metabolite ratio did not predict plasma nicotine concentration or smoking cessation outcome with use of nicotine nasal spray. In general, the systemic dose of nicotine taken in from nasal spray is much less than for transdermal nicotine; this may be attributable to either the nature of the pharmacologic reinforcement or the aversive effects of nicotine nasal spray (or both).[13] The primary positive reinforcement from nasal spray is thought to be a result of its rapid absorption and high peak arterial blood concentrations. Because peak blood levels after spray use would be minimally affected by the nicotine metabolic rate, this may explain the lack of association of the metabolite ratio with cessation in this group. In addition, users of nasal spray can titrate their intake of nicotine by taking more or less frequent doses, according to perceived need. This is in contrast to transdermal nicotine, in which the dose is fixed by the product characteristics. Data on nicotine nasal spray usage in this study provide support for this compensation hypothesis and may also explain why the metabolite ratio does not predict nasal spray treatment outcome. Yet, despite the potential for increased dosing with nicotine nasal spray, this product did not outperform transdermal nicotine significantly, even among smokers with the highest rates of nicotine metabolism. Thus it is likely that the difference in smoking cessation response to transdermal nicotine compared with nicotine nasal spray is related to pharmacodynamic differences rather than differences in daily dose or average blood levels of nicotine.

The results of this clinical trial are consistent with growing evidence for the influence of individual variability in the rate of nicotine metabolism on smoking behavior.[3–5] Although several *CYP2A6* gene variants have been associated with slower or faster metabolism of nicotine, smoking rate, and cessation,[30] the currently identified gene variants explain only a small percentage of the variation in nicotine metabolism in white subjects and black subjects.[9] Therefore it is not surprising that the *CYP2A6* genotype did not predict smoking cessation outcomes; however, interpretation of these data is limited by the small number of smokers with slow metabolizer genotypes. Importantly, *CYP2A6* genotype has been associated with the risk of lung cancer, which is attributable to the role of the enzyme in determining cigarette consumption, as well as a role in the activation of tobacco carcinogens.[4]

Because the efficacy of both transdermal nicotine and nicotine nasal spray compared with placebo has been well established,[10] this study used an open-label trial to simulate the usual clinical regimen. Therefore it was not possible to evaluate the relative efficacy of transdermal nicotine versus placebo as a function of 3-HC/cotinine ratio. Nonetheless, our data on the relationship between the metabolite ratio and nicotine concentrations, as well as cigarette cravings during treatment, provide strong support for the hypothesis that this measure predicts the therapeutic response to transdermal nicotine.

The results of this clinical trial may have important applications with regard to the use of nicotine replacement therapy in clinical practice. The 3-HC/cotinine ratio does not depend on time since last cigarette and can be measured reliably in the blood, saliva, or urine,[8] providing a noninvasive measure of CYP2A6 activity that may be used to screen individual smokers to select the type or dosage of treatment that may be most effective. The current findings suggest that smokers having 3-HC/cotinine ratios greater than 0.47 may be better candidates for doses of nicotine higher than 21 mg whereas there may be no benefit of high-dose therapy for smokers with lower ratios. Smokers with high ratios could also be advised to try another form of nicotine replacement therapy or non-nicotine therapy. Because genetic variants associated with faster nicotine metabolism are also associated with an increased risk for lung cancer,[4] the selection of the most effective treatment for these smokers is especially important in reducing morbidity and mortality rates from tobacco use.

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