Individuals differ in their susceptibility to the acquisition of tobacco addiction. Twin studies indicate that 50% or more of the variance in likelihood of developing tobacco dependence is genetic and that genetic factors influence not only who becomes a smoker but also how much they smoke and how hard it is for them to quit.1–3 The genetic determinants of individual differences in vulnerability to nicotine addiction are incompletely understood. One possibility includes individual differences in pharmacologic responses to initial exposure to nicotine.

Smoking prevalence, cigarette consumption among smokers, and the risk of developing smoking-related disease differ among racial groups. Relevant to the present study, Asian Americans are less likely to become smokers, smoke fewer cigarettes per day, and are less likely to develop lung cancer as compared with whites.4,5 Black Americans have a similar prevalence of smoking and smoke fewer cigarettes per day, but are more likely to develop lung cancer as compared with whites.5,6 One factor influencing individual differences in smoking behavior may be the rate of metabolism of nicotine. Nicotine is metabolically inactivated to its major proximate metabolite, cotinine (COT), primarily by the liver enzyme cytochrome P450 (CYP)2A6.7 COT is metabolized to 3-hydroxycotinine by the same enzyme. As compared with whites, Asians and black Americans have a higher prevalence of CYP2A6 gene alleles that are associated with slower metabolism of nicotine.8 Slower metabolism of nicotine is expected to result in the need to smoke fewer cigarettes per day to achieve a desired level of nicotine in the body and has been associated with greater rates of quitting as compared with faster metabolism.9

Differences in pharmacologic response to early nicotine exposure may also be important determinants of differential vulnerability to addiction. Several studies have reported that an initial pleasant nicotine response (for example, feeling high, a pleasurable buzz or rush, or feeling dizzy) among novice smokers is associated with continued smoking and the development of nicotine dependence.10–15 DiFranza et al. also reported that aversive responses to smoking the first cigarette, such as nausea...
and dizziness, were predictors of greater symptoms of nicotine dependence, consistent with the idea that a general sensitivity to nicotine predicts a higher likelihood of development of dependence.\textsuperscript{11} Differences in response to nicotine are difficult to study in people who are already using tobacco because they have developed considerable tolerance to many of the effects of nicotine.\textsuperscript{16} To explore intrinsic individual differences in kinetics and response to nicotine, we conducted a study of transdermal nicotine administration in never-smokers. We also examined variations in CYP2A6 genotype, which cause differences in the rate of nicotine metabolism and which have been reported to influence the likelihood of developing nicotine dependence.\textsuperscript{17-19}

**RESULTS**

**Demography**

Sixty participants (29 females and 31 males) were enrolled in the study. Participants were Asian ($n = 20$), black ($n = 20$), and white ($n = 20$). The average age of the participants was 26.5 years (range: 19–39 years) and did not differ by race ($P = 0.78$). The average body mass index (BMI) was 24.2 kg/m\(^2\) (range: 17.3–30.7 kg/m\(^2\)) and was significantly higher among blacks (mean: 25.9 kg/m\(^2\)) as compared with that among Asians (23.8 kg/m\(^2\)) and whites (22.7 kg/m\(^2\); $P = 0.003$). Four participants did not yield data for the entire 8-h study period: three participants who had their patch removed left the study within 480 min and the respective times of the last sample collection were 180, 240, and 420 min. One other subject with the patch still in place chose to terminate the study after the 420-min blood sample was taken.

**CYP2A6 genotype**

The CYP2A6 genotype was determined in 58 participants; in two subjects (both blacks), genotyping was not conclusive. Thirty-five participants (60%) had the wild-type genotype (*\textsuperscript{1}/*\textsuperscript{1}), and 23 participants (40%) had one or two variant CYP2A6 alleles. The allele frequencies and their distributions by race and gender are presented in Table 1. The proportion of *\textsuperscript{1}/*\textsuperscript{1} vs. variant alleles was significantly different by race ($P = 0.004$); 70% of Asian participants had a variant allele as compared with 28% of blacks and 20% of whites.

**Nicotine toxicity**

Acute nicotine toxicity was evidenced by nausea and/or vomiting, necessitating patch removal in nine subjects. The number of participants who removed their patch is presented by CYP2A6 genotype in Table 1. The times of patch removal by genotype and race were 44 min (*\textsuperscript{10}/*\textsuperscript{10}, Asian), 60 min (*\textsuperscript{1}/*\textsuperscript{9}, white), 89 min (*\textsuperscript{1}/*\textsuperscript{1}, white), 91 min (*\textsuperscript{1}/*\textsuperscript{1}, white), 93 min (*\textsuperscript{1}/*\textsuperscript{9}, Asian), 100 min (*\textsuperscript{4}/*\textsuperscript{10}, Asian), 131 min (*\textsuperscript{1}/*\textsuperscript{1}, black), 372 min (*\textsuperscript{4}/*\textsuperscript{9}, Asian), and 384 min (*\textsuperscript{1}/*\textsuperscript{9}, white). The median time to patch removal was 93 min, and the average time to patch removal was 152 (SD: 131) min.

**Nicotine levels over time**

Figure 1\textsuperscript{a} displays the average plasma nicotine concentration over time for all participants who became ill and for all those who did not, by race. Average peak plasma nicotine concentration was higher ($P = 0.006$) and average time to peak nicotine concentration was shorter ($P = 0.018$) in participants who developed nicotine-induced toxicities as compared with those who did not. Average plasma nicotine concentration over time by CYP2A6 genotype (*\textsuperscript{1}/*\textsuperscript{1} vs. variants) is displayed in Figure 1\textsuperscript{b}. Average peak plasma nicotine concentration did not significantly vary with CYP2A6 genotype. Table 1 displays nicotine pharmacokinetic data (area under the plasma concentration–time curve (AUC), peak plasma concentration ($C_{\text{max}}$), and the time taken to reach $C_{\text{max}}$ ($T_{\text{max}}$)) for all subjects and by race.

**Subjective and cardiovascular responses**

At baseline, there was no significant difference in the 11 individual Visual Analog Nicotine Effects Scale (VANES) parameters between those with variant alleles and those with the *\textsuperscript{1}/*\textsuperscript{1} (wild-type) genotype. At 30 min, two VANES parameters (calmness and dose perception) were significantly different; at 60 min, five parameters (anxiety, concentration, nausea, stimulation, and palpitations) were significantly different; and at 90 min, seven parameters were significantly different in subjects with variants as compared with those with *\textsuperscript{1}/*\textsuperscript{1} genotypes. The group differences were greatest at 120 min, when 8 of 11 VANES parameters were significantly different. The differences in changes in subjective responses to nicotine from baseline to 90 and 120 min comparing subjects with wild-type vs. variant CYP2A6 alleles are presented in Table 2. Those in the variant group had significantly more unpleasant subjective responses than those with the *\textsuperscript{1}/*\textsuperscript{1} genotype. Specifically, those in the variant group reported being more anxious, less calm, less able to concentrate, and more light-headed; experienced more nausea; and reported more palpitations. By 180 min, these differences were no longer significant. The time courses of perceived light-headedness and nausea by genotype group are presented in Figure 2.

For the cardiovascular parameters, at baseline there was no difference between the two genotype groups ($P$-value range: 0.88–0.98). Thereafter, heart rate acceleration was significantly greater (change from baseline) from 30 through 180 min after patch placement for the variant group as compared with the wild-type group (Table 2). Blood pressure did not show consistent differences between the two genotype groups.

**Predictors of nicotine toxicity**

Univariate analyses to investigate the association between demographic data and CYP2A6 genotype and nicotine-induced toxicity (patch removal) found no significant effect for age ($P = 0.6$), gender ($P = 1.0$), BMI ($P = 0.5$), or race ($P = 0.4$). Although six of nine participants who had their patch removed had at least one variant allele, this difference in toxicity was not statistically significant across genotype groups. Table 3 presents further univariate analyses for the association between nicotine toxicity and possible predictors (race, genotype, and pharmacokinetic parameters). AUC and $C_{\text{max}}$ were significantly higher, and $T_{\text{max}}$ was shorter in participants with toxicities as compared with those without toxicities.
Time-to-event analysis (also known as survival analysis) was used to formally test the association between pharmacokinetic parameter or genotype and nicotine-induced toxicity. These results are presented in Table 4. After adjusting for the effects of age, BMI, gender, and race, the hazard ratio of nicotine-induced toxicity in those with a variant genotype as compared with those in the *1/*1 group was 3.81 (0.84–17.2; *P* = 0.083). Although the probability of nicotine-induced toxicity following patch placement over an 8-h time course among individuals with the variant genotype tended to be higher among whites as compared with Asians, followed by blacks, these differences were not statistically significant (*P* = 0.21; Figure 3).

In time-to-event analyses for pharmacokinetic parameters, the adjusted hazard ratio of toxicity among participants was significant for high- as compared with low-toxicity categories based on median split for AUC from 0 to 90 min (AUC<sub>0→90</sub>), AUC from 0 to 360 min (AUC<sub>0→360</sub>), and C<sub>max</sub> (Table 4). The unadjusted hazard ratios were not significant and hence are not presented.

### DISCUSSION

The aim of our study was to examine factors that determine pharmacologic response to nicotine in nonsmokers. We studied nonsmokers because we wanted to look at the effects of nicotine in the absence of tolerance, which is considerable in regular tobacco users. Individual differences in nicotine sensitivity among nonsmokers are thought to influence vulnerability to tobacco addiction.10–15 We used nicotine patches to probe sensitivity because nicotine delivery from patches is controlled as opposed to that from smoking cigarettes, for which individuals...
Articles can alter levels of nicotine delivery through differences in frequency, duration, and depth of inhalation. We hypothesized that variability in the rate of nicotine metabolism, which is determined largely by CYP2A6 activity, would strongly influence responses to nicotine patches, including the risk of toxicity.

We found that pharmacokinetic factors were the strongest predictors of development of nicotine toxicity, which occurred in 15% of our subjects. The peak plasma nicotine, plasma nicotine AUC$_{0\rightarrow90}$ (reflecting rate of rise), and the plasma nicotine AUC$_{0\rightarrow360}$ were all strong predictors of toxicity (hazard ratios: 6.9, 5.9, and 8.4, respectively). Rapid rise of blood concentrations is known to be associated with greater effects of many psychoactive drugs, presumably because higher brain levels are achieved with less time to develop receptor-based tolerance.20,21 Thus, the finding that $C_{\text{max}}$ and rate of rise of plasma nicotine concentration were associated with nicotine toxicity is not surprising. However, it is remarkable that AUC$_{0\rightarrow360}$ is also associated with nicotine toxicity because most of the subjects with toxicity had their patches removed before ~2h. Persistently high nicotine levels despite patch removal indicate slow metabolism, either due to intrinsic metabolic differences or perhaps due to effects of nicotine toxicity on its own clearance (such as by reducing liver blood flow).22

The basis for more rapid absorption of nicotine in some subjects is not entirely clear. Genetic differences in nicotine metabolism appear to play some role, as discussed below, but these do

---

Table 2 Differences in changes in subjective responses (VANES) and heart rate from baseline between participants with CYP2A6 wild-type alleles and those with variant alleles

| Differences in VANES responses between *1/*1 and variants |  |  |
|---|---|---|---|
| At 90 min | $P$ value | At 120 min | $P$ value |
| Alertness | 1.0 | 0.13 | 1.7 | 0.01 |
| Anxiety | $(−0.3, 2.2)$ | 0.04 | $(−1.1, −0.03)$ | 0.04 |
| Calmness | 1.5 | 0.02 | 1.9 | 0.004 |
| Concentration | $(0.2, 2.8)$ | 0.004 | 1.4 | 0.035 |
| Contentment | 1.5 | 0.03 | 1.2 | 0.09 |
| Dose perception | $−0.6$ | 0.26 | $−1.4$ | 0.006 |
| High feeling | $−0.4$ | 0.22 | $−0.2$ | 0.68 |
| Light-headedness | $−1.1$ | 0.02 | $−1.3$ | 0.005 |
| Nausea | $−1.0$ | 0.005 | $−1.1$ | 0.002 |
| Stimulation | $−0.5$ | 0.23 | $−0.4$ | 0.36 |
| Palpitation | $−0.5$ | 0.046 | $−0.6$ | 0.01 |

Cardiovascular parameters, mean (95% CI)

| Heart rate | $−4.0$ | 0.046 | $−4.2$ | 0.035 |
| $−(−8.0, −0.1)$ | $(−8.1, −0.3)$ |

Values presented are the differences in changes in VANES scores between *1/*1 and variants (*1/*1 minus variants); changes in VANES for subjects were computed from baseline to each time point. $P$ values are based on Wilcoxon rank sum tests. CI, confidence interval; VANES, Visual Analog Nicotine Effects Scale.

---

Figure 1 Time course of plasma nicotine following nicotine patch placement. (a) Plasma nicotine concentration over 8h for all participants with toxicity and participants without toxicity by race. Values are geometric means and standard errors. (b) Time course of plasma nicotine concentration by CYP2A6 genotype group (wild-type, *1/*1, vs. those with at least one variant allele, Var).

Figure 2 Time course of perceived light-headedness and nausea by CYP2A6 genotype group (wild-type, *1/*1, vs. those with at least one variant allele, Var).
We hypothesized that differences in CYP2A6 activity would influence response to transdermal nicotine. There is considerable genetic polymorphism in the CYP2A6 gene, including known racial/ethnic differences in allele frequencies.\(^2,3\) As expected, we found that the presence of reduced-function variants was higher in Asian and black subjects as compared with that in white subjects. We did not, however, find a significant effect of CYP2A6 genotype on plasma nicotine \(C_{\text{max}}\) or AUC\(_{0→90}\), perhaps because there is relatively little nicotine metabolism, relative to absorption, during the first 90 min of patch application.

Table 3 Comparison of demographic and pharmacokinetic variables between participants with nicotine toxicity (those who removed patch) and those without

| Variable | No toxicity (n = 51) | Toxicity (n = 9) | \(P\) value\(^a\) |
|----------|---------------------|-----------------|----------------|
| Age      | Mean (range) 26.5 (19–39) 26.6 (19–31) 6.17 |                    |                |
| Sex      | Women (n, %) 25 (41.7%) 4 (6.7%) 1.000 |                    |                |
|          | Men (n, %) 26 (43.3%) 5 (8.3%) |                    |                |
| Race     | Asian (n, %) 16 (26.7%) 4 (6.7%) 0.360 |                    |                |
|          | Black (n, %) 19 (31.7%) 1 (1.7%) |                    |                |
|          | White (n, %) 16 (26.7%) 4 (6.7%) |                    |                |
| BMI      | Mean (range) 24.0 (17.3–30.5) 24.8 (21.0–30.7) 0.49 |                    |                |
| Genotype\(^b\) | *1/*1 (n, %) 32 (55.2%) 3 (5.2%) 0.135 |                    |                |
|          | Variant (n, %) 17 (29.3%) 6 (10.3%) |                    |                |
| AUC\(_{0→90}\) (min-ng/ml) | 216 (182–257) 401 (267–602) 1.9 (1.2–2.9) |                    |                |
| GM (95% CI) | 216 (182–257) 401 (267–602) 1.9 (1.2–2.9) |                    |                |
| AUC\(_{0→360}\) (min-ng/ml) | 1714 (1544–1902) 2535 (1752–3667) 1.5 (1.1–2.0) |                    |                |
| GM (95% CI) | 1714 (1544–1902) 2535 (1752–3667) 1.5 (1.1–2.0) |                    |                |
| \(C_{\text{max}}\) (ng/ml)\(^b\) | 6.6 (6.0–7.3) 9.5 (7.5–12.0) 1.4 (1.1–1.9) |                    |                |
| \(T_{\text{max}}\) (min) | 240 (120–420) 120 (90–420) 0.018 |                    |                |

We hypothesized that differences in CYP2A6 activity would influence response to transdermal nicotine. There is considerable genetic polymorphism in the CYP2A6 gene, including known racial/ethnic differences in allele frequencies.\(^2,3\) As expected, we found that the presence of reduced-function variants was higher in Asian and black subjects as compared with that in white subjects. We did not, however, find a significant effect of CYP2A6 genotype on plasma nicotine \(C_{\text{max}}\) or AUC\(_{0→90}\), perhaps because there is relatively little nicotine metabolism, relative to absorption, during the first 90 min of patch application.

Table 4 Associations of CYP2A6 genotype and nicotine pharmacokinetics with nicotine-induced toxicity (patch removal) obtained using time-to-event analyses

| Predictor\(^a\) | Hazard ratio (95% CI) | \(\chi^2\) | \(P\) value |
|----------------|-----------------------|----------|------------|
| Model 1: genotype |                       |          |            |
| Variants vs. *1/*1 | 3.81 (0.84–17.2) | 3.01     | 0.083     |
| Model 2: AUC\(_{0→90}\) |                       |          |            |
| High vs. low | 5.88 (1.07–32.2) | 4.16     | 0.042     |
| Model 3: AUC\(_{0→360}\) |                       |          |            |
| High vs. low | 8.35 (1.40–49.6) | 5.44     | 0.020     |
| Model 4: \(C_{\text{max}}\) |                       |          |            |
| High vs. low | 6.91 (1.23–38.9) | 4.81     | 0.028     |
| Model 5: \(T_{\text{max}}\) |                       |          |            |
| Long vs. short | 0.22 (0.04–1.15) | 3.24     | 0.070     |

Of the nine subjects who developed nicotine toxicity, 67% had reduced-function CYP2A6 variants, as compared with 35% with reduced-function variants in those who did not develop toxicity. This difference was not statistically significant, but the lack of significance may be a power problem, given relatively few cases of toxicity. Time-to-event analysis indicated a borderline significant effect of genotype (\(P = 0.083\)). There was no association of race, gender, or age with nicotine toxicity. In addition to toxicity, we studied subjective and cardiovascular effects of transdermal nicotine administration in relation to CYP2A6 genotype. Increases in anxiousness, light-headedness, nausea, and palpitations, and decreases in alertness, concentration, and calmness at 120 min, in addition to heart rate acceleration from 30 to 180 min, were significantly greater in subjects with CYP2A6 reduced-function variants. A limitation of our study is that we did not examine genetic variation in other pathways of nicotine metabolism—glucuronidation and \(N\)-oxidation. These are generally minor metabolic pathways but could influence nicotine clearance, particularly when metabolism through CYP2A6 is genetically slow.

In summary, nicotine toxicity (patch removal) in never-smokers is most strongly influenced by rate of rise and peak levels of plasma nicotine. There was a significant association between subjective pharmacological responses and the presence of CYP2A6 reduced-function alleles, presumably related to the slow rate of nicotine metabolism. One implication of our research relates to the administration of nicotine as a medication. Our study of nonsmokers suggests that nicotine toxicity would be more likely to occur in genetically slow metabolizers who receive usual therapeutic doses of transdermal nicotine to aid smoking cessation, although the previous development of tolerance in many smokers is likely to mitigate the problem of toxicity, even among slow metabolizers. Nicotine medications have also been proposed for the treatment of ulcerative colitis, Parkinson's disease, and other disorders.\(^{24,26}\) If these patients are nonsmokers before nicotine therapy, the CYP2A6 genotype...
might be a useful predictor of toxicity, and these patients may need smaller doses at initiation of treatment.

Another implication relates to vulnerability to developing nicotine dependence. Several studies have reported that initial sensitivity to pleasurable effects of nicotine predicts progression to dependent smoking, and some studies report that aversive responses predict a greater level of dependence.10–15 These studies suggest that increased global sensitivity to nicotine is an important determinant of who becomes a regular smoker. Our data indicate that rate of increase in plasma nicotine levels and the presence of reduced-activity CYP2A6 genotype are determinants of initial sensitivity to nicotine. It is unclear how our data regarding rate of rise of plasma nicotine levels from patch use would translate to smoking the first cigarette, from which nicotine absorption is much more rapid. We considered studying more rapid nicotine delivery systems, such as nicotine gum, lozenge, or inhaler, but there is large individual variability in systemic nicotine delivery from such formulations, making comparisons of nicotine effects across subjects difficult. The importance of the rate of nicotine metabolism as a determinant of sensitivity is supported by cohort studies among adolescents, which report that having reduced-function CYP2A6 gene variants is a risk factor for acquisition of dependence and for persistent smoking, and by a cross-sectional study of adolescents indicating that phenotypically slow nicotine metabolism is a risk factor for a higher level of dependence.18,19,27

Our data directly link slow nicotine metabolism with a greater likelihood of experiencing subjective, cardiovascular, and toxic effects of nicotine. The mechanisms by which slow nicotine metabolism and greater sensitivity to nicotine facilitate development of dependence have not been established. We speculate that slower metabolism results in longer persistence of nicotine in the brain, resulting in greater neuroadaptive changes and therefore faster development of dependence.

**METHODS**

**Subjects.** The subjects were 20 whites (11 men and 9 women), 20 blacks (10 men and 10 women), and 20 Asians (10 men and 10 women) who were never regular smokers and had smoked fewer than 100 cigarettes in their lifetime. In all subjects, the screening plasma COT level, measured by gas chromatography, was below the limit of quantitation (10 ng/ml). Twenty-eight (46.7%) of the subjects never smoked even one cigarette, 23 (38.3%) subjects smoked one to five cigarettes, and 9 (15%) subjects smoked more than five but fewer than 100 cigarettes in their lifetime. The time interval between when a subject had last tried a cigarette and study enrollment was not specifically recorded, but the intervals were in years.

Subjects were healthy based on questionnaire, screening blood chemistries, and electrocardiograms and were taking no medications. The criterion for belonging to a particular racial group was having four grandparents of the same race. Subjects were recruited by flyer advertisements at local colleges, cafes, restaurants, and laundromats; by newspaper advertisements; and by a notice on a local website. Subjects were compensated financially for their participation. The study was approved by the Committee on Human Research of the University of California, San Francisco, and the research ethics board for the University of Toronto, and subjects provided signed consent before entering the study.

**Procedures.** Subjects were admitted to the Clinical Research Center at the San Francisco General Hospital Medical Center on the evening before the day of the study. They were asked not to consume any alcoholic beverages for 48 h before admission. Subjects did not eat any food or drink any alcoholic or caffeinated beverages after midnight on the day before the study. A light breakfast was served at 7:30 AM. At ~8:00 AM, a catheter was placed in a forearm vein for blood drawing. Baseline questionnaires and subjects provided signed consent before entering the study.

Subjects were admitted to the Clinical Research Center at the San Francisco General Hospital Medical Center on the evening before the day of the study. They were asked not to consume any alcoholic beverages for 48 h before admission. Subjects did not eat any food or drink any alcoholic or caffeinated beverages after midnight on the day before the study. A light breakfast was served at 7:30 AM. At ~8:00 AM, a catheter was placed in a forearm vein for blood drawing. Baseline questionnaires and subjects provided signed consent before entering the study.

**Subjective responses, blood sampling, and cardiovascular measures.** Subjective responses, blood samples, heart rate, and blood pressure were recorded or obtained before patch placement (baseline) and at 30, 60, 90, 120, 240, 300, 360, 420, and 480 min postdosing. The VANES included 11 subjective ratings: “I feel content”, “I feel alert and awake”, “I feel calm and relaxed”, “I am able to concentrate”, “the strength of the dose is …”, “I feel lightheaded or dizzy”, “I feel high”, “I feel nauseated”, “I feel anxious or tense”, “I feel stimulated”, and “my heart is
beating fast” (palpitations). Each response had a 10-cm line marked off in 0.5-cm intervals. Subjects marked the line to describe how much they rated the particular effect at the moment. The subjective response for the strength of the dose was scored as zero at baseline.

**Analytical chemistry and genotyping.** Plasma was analyzed for nicotine and its metabolite COT. Nicotine and COT analyses were performed by gas chromatography with nitrogen–phosphorus detection.²⁹ CYP2A6 genotyping was performed at the University of Toronto using methods previously described.³⁰–³² The following alleles were genotyped in the study subjects: *1, 2, 4, 7, 8, 9, 10, 12, 14, 17, 20, 23, 24, 25, 26, 27*, and *35. Because the number of people with any one particular variant genotype was small (Table 1), data analysis was performed comparing subjects with normal activity (*1/*1) to those with one or two reduced-function variants.

**Data analysis.** Plasma nicotine concentrations were analyzed as peak concentrations and AUCs. To examine the rate of absorption of nicotine as a predictor of toxicity, we computed the partial AUC from 0 to 90. Overall exposure to nicotine was estimated by the AUC from 0 to 360. AUCs were computed using the trapezoidal rule. VANES scores for each time point were analyzed as the change from baseline (before application of the nicotine patch). We used two-sample t-test to test for differences in log AUC, log Cₘₐₓ, and BMI; the Wilcoxon two-sample test to test for differences in age and Tₘₐₓ between individuals with toxicity and those without; and Fisher’s exact test to test for univariate associations between categorical variables. To investigate the associations between CYP2A6 genotype and median-split nicotine AUC, Cₘₐₓ and Tₘₐₓ on nicotine-induced toxicity, we performed a time-to-event analysis (also known as survival analysis) in which the event modeled was toxicity-induced patch removal. Genotype was entered as a categorical predictor with levels for *1/*1 genotype vs. all variant alleles. All models were adjusted for the covariates age and BMI (as continuous variables), and gender and race. Interactions between race and main predictors were nonsignificant and were omitted from the final models. Based on the model with genotype as a predictor, Kaplan–Meier curves of the probabilities of nicotine-induced toxicity by genotype and race were generated. Statistical analyses were carried out using SAS v. 9.3 (SAS Institute, Cary, NC, USA). All statistical tests were considered significant at α = 0.05.

**ACKNOWLEDGMENTS** This study was supported by US Public Health Service grants DA02277, DA12393, and DA02088 awarded by the National Institute on Drug Abuse, and carried out in part at the General Clinical Research Center at San Francisco General Hospital with the support of the National Institutes of Health/National Center for Research Resources and the University of California, San Francisco UCSF Clinical and Translational Science Institute through grant UL1 RR024131. The genetics work was funded in part by Canadian Institutes for Health Research grant MOP86471. We thank Faith Allen for protocol and data management; Sandra Tinetti for assistance in conducting the clinical study; Lita Ramos and Fredysha McDaniel for performing the analytical chemistry determinations; Bo Xu, Kerri Schoedel, and Eva Hoffmann for performing the CYP2A6 genotyping; and Marc Olmsted and Scott Rostler for editorial assistance.

**AUTHOR CONTRIBUTIONS** N.L.B., G.S.H., D.A.D., P.J.III, and R.F.T. wrote the manuscript; N.L.B, D.A.D., and P.J.III designed the research; and N.L.B., G.S.H., D.A.D., P.J.III, and R.F.T. wrote the manuscript; N.L.B, D.A.D, P.J.III, and R.F.T contributed to the data analysis; O. P. designed the research; and N.L.B., G.S.H., D.A.D., P.J.III, and R.F.T.

**CONFLICT OF INTEREST** R.F.T. has consulted for Novartis and McNeil. As an Associate Editor of Clinical Pharmacology & Therapeutics, R.F.T. was not involved in the review or decision process for this article. N.L.B. has served on smoking cessation advisory boards for Pfizer and has been an occasional consultant to McNeil and GlaxoSmithKline. He has provided paid expert testimony concerning nicotine addiction in litigation against tobacco companies. The other authors declared no conflict of interest.

---

**Study Highlights**

**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**

Difference in pharmacologic response to early nicotine exposure appears to be an important determinant of vulnerability to developing tobacco addiction.

**WHAT QUESTION DOES THIS STUDY ADDRESS?**

Pharmacokinetic and genetic factors underlying individual differences in response to transdermal nicotine administration in never-smokers were characterized.

**WHAT THIS STUDY ADDS TO OUR KNOWLEDGE**

Subjective, cardiovascular, and toxic effects of transdermal nicotine administration in never-smokers are associated with the rate of rise in concentration of nicotine and peak plasma concentration of nicotine and the presence of reduced-function CYP2A6 gene variants.

**HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS**

Our data, in conjunction with other published research, support the idea that global sensitivity to nicotine, mediated in part by genetically slow metabolism of nicotine, is an important determinant of addiction vulnerability in nonsmokers. Our data also suggest that CYP2A6 genotype may be a predictor of nicotine toxicity and the need for dose modification in nonsmokers treated with transdermal nicotine for ulcerative colitis and other disorders.

---

© 2013 American Society for Clinical Pharmacology and Therapeutics

1. Carmelli, D., Swan, G.E., Robineau, D. & Fabritz, R. Genetic influence on smoking—a study of male twins. *N. Engl. J. Med.* **327**, 829–833 (1992).
2. Al Koudsi, N. & Tyndale, R.F. Genetic influences on smoking: a brief review. *Ther. Drug Monit.* **27**, 704–709 (2005).
3. Agrawal, A. et al. The genetics of addiction—a translational perspective. *Transl. Psychiatry* **2**, e140 (2012).
4. Benowitz, N.L., Pérez-Stable, E.J., Herrera, B. & Jacob, P. 3rd. Slower metabolism and reduced intake of cigarette smoking in Chinese-Americans. *J. Natl. Cancer Inst.* **94**, 108–115 (2002).
5. Haiman, C.A. et al. Ethnic and racial differences in the smoking-related risk of lung cancer. *N. Engl. J. Med.* **354**, 333–342 (2006).
6. Trinidad, D.R., Pérez-Stable, E.J., Emery, S.L., White, M.M., Grana, R.A. & Messer, K.S. Intermittent and light daily smoking across racial/ethnic groups in the United States. *Nicotine Tob. Res.* **11**, 203–210 (2009).
7. Hukkanen, J., Jacob, P. 3rd & Benowitz, N.L. Metabolism and disposition kinetics of nicotine. *Pharmacol. Rev.* **57**, 79–115 (2005).
8. McDonagh, E.M. et al. PharmGKB summary: very important pharmacogene information for cytochrome P-450, family 2, subfamily A, polypeptide 6. *Pharmacogenet. Genomics* **22**, 695–708 (2012).
9. Malayiandi, V., Sellers, E.M. & Tyndale, R.F. Implications of CYP2A6 genetic variation for smoking behaviors and nicotine dependence. *Clin. Pharmacol. Ther.* **77**, 145–158 (2005).
10. Pomerleau, O.F., Pomerleau, C.S. & Nameneck, R.J. Early experiences with tobacco among women smokers, ex-smokers, and never-smokers. *Addiction* **93**, 595–599 (1998).
11. DiFranza, J.R. et al. Recollections and repercussions of the first inhaled cigarette. *Addict. Behav.* **29**, 261–272 (2004).
12. Kandel, D.B., Hu, M.C., Griesler, P.C. & Scharffran, C. On the development of nicotine dependence in adolescence. *Drug Alcohol Depend.* **91**, 26–39 (2007).
13. Chen, X. et al. Sensations from initial exposure to nicotine predicting adolescent smoking in China: a potential measure of vulnerability to nicotine. *Nicotine Tob. Res.* **5**, 455–463 (2003).
14. Pomerleau, O.F. Individual differences in sensitivity to nicotine: implications for genetic research on nicotine dependence. *Behav. Genet.* **25**, 161–177 (1995).

15. Pomerleau, O.F., Collins, A.C., Shiffman, S. & Pomerleau, C.S. Why some people smoke and others do not: new perspectives. *J. Consult. Clin. Psychol.* **61**, 723–731 (1993).

16. Fattinger, K., Verotta, D. & Benowitz, N.L. Pharmacodynamics of acute tolerance to multiple nicotinic effects in humans. *J. Pharmacol. Exp. Ther.* **281**, 1238–1246 (1997).

17. Audrain-McGovern, J., Al Koudsi, N., Rodriguez, D., Wileyto, E.P., Shields, P.G. & Tyndale, R.F. The role of CYP2A6 in the emergence of nicotine dependence in adolescents. *Pediatrics* **119**, e264–e274 (2007).

18. O’Loughlin, J. et al. Genetically decreased CYP2A6 and the risk of tobacco dependence: a prospective study of novice smokers. *Tob. Control* **13**, 422–428 (2004).

19. Al Koudsi, N., O’Loughlin, J., Rodriguez, D., Audrain-McGovern, J. & Tyndale, R.F. The genetic aspects of nicotine metabolism and their impact on adolescent dependence. *J Pediatr Biochem* **1**, 105–123 (2010).

20. de Wit, H., Bodker, B. & Ambre, J. Rate of increase of plasma drug level influences subjective response in humans. *Psychopharmacology (Berl.)* **107**, 352–358 (1992).

21. Berridge, M.S., Apana, S.M., Nagano, K.K., Berridge, C.E., Leisure, G.P. & Boswell, M.V. Smoking produces rapid rise of [11C]nicotine in human brain. *Psychopharmacology (Berl.)* **209**, 383–394 (2010).

22. Gries, J.M., Benowitz, N. & Verotta, D. Chronopharmacokinetics of nicotine. *Clin. Pharmacol. Ther.* **60**, 385–395 (1996).

23. Mwenifumbo, J.C. & Tyndale, R.F. Genetic variability in CYP2A6 and the pharmacokinetics of nicotine. *Pharmacogenomics* **8**, 1385–1402 (2007).

24. Lunney, P.C. & Leong, R.W. Review article: Ulcerative colitis, smoking and nicotine therapy. *Aliment. Pharmacol. Ther.* **36**, 997–1008 (2012).

25. Thiriez, C., Villafane, G., Grapin, F., Fenelon, G., Remy, P. & Cesaro, P. Can nicotine be used medicinally in Parkinson’s disease? *Expert Rev. Clin. Pharmacol.* **4**, 429–436 (2011).

26. Silver, A.A., Shytle, R.D., Philipp, M.K., Wilkinson, B.J., McConville, B. & Sanberg, P.R. Transdermal nicotine and haloperidol in Tourette’s disorder: a double-blind placebo-controlled study. *J. Clin. Psychiatry* **62**, 707–714 (2001).

27. Rubinstein, M.L., Shiffman, S., Moscicki, A.B., Rait, M.A., Sen, S. & Benowitz, N.L. Nicotine metabolism and addiction among adolescent smokers. *Addiction* **108**, 406–412 (2013).

28. Stead, L.F. et al. Nicotine replacement therapy for smoking cessation. *Cochrane Database Syst. Rev.* **11**, CD000146 (2012).

29. Jacob, P. 3rd, Wilson, M. & Benowitz, N.L. Improved gas chromatographic method for the determination of nicotine and cotinine in biologic fluids. *J. Chromatogr.* **222**, 61–70 (1981).

30. Schoedel, K.A., Hoffmann, E.B., Rao, Y., Sellers, E.M. & Tyndale, R.F. Ethnic variation in CYP2A6 and association of genetically slow nicotine metabolism and smoking in adult Caucasians. *Pharmacogenetics* **14**, 615–626 (2004).

31. Mwenifumbo, J.C., Myers, M.G., Wall, T.L., Lin, S.K., Sellers, E.M. & Tyndale, R.F. Ethnic variation in CYP2A6*7, CYP2A6*8 and CYP2A6*10 as assessed with a novel haplotyping method. *Pharmacogenet. Genomics* **15**, 189–192 (2005).