Effect of race and glucuronidation rates on the relationship between nicotine metabolite ratio and nicotine clearance

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Objectives **To investigate if the nicotine metabolite ratio (NMR, the ratio of nicotine metabolites 3′-hydroxycotinine/cotinine) is a reliable phenotypic biomarker for nicotine clearance across races, and as a function of differences in the rate of nicotine, cotinine and 3′-hydroxycotinine glucuronidation and *UGT* genotypes.**

Methods **Participants [Caucasians (Whites), African Americans (Blacks) and Asian-Americans (Asians)] received an oral solution of deuterium-labeled nicotine and its metabolite cotinine. Plasma and saliva concentrations of nicotine and cotinine were used to determine oral clearances. Rates of glucuronidation were assessed from urine glucuronide/parent ratios, and *UGT2B10* and *UGT2B17* genotypes from DNA.**

Results **Among the 227 participants, 96 (42%) were White, 67 (30%) Asian and 64 (28%) Black. Compared to the other two races, Whites had higher nicotine and cotinine total oral clearance, Blacks had lower nicotine and cotinine glucuronidation rates and Asians had lower 3′-hydroxycotinine glucuronidation rates. A strong positive correlation (correlations coefficients 0.77–0.84; $P<0.001$) between NMR and nicotine oral clearance was found for all three races, and NMR remained a strong predictor for the nicotine oral clearance while adjusting for race, sex and age. Neither the metabolite glucuronidation ratios nor the *UGT* genotypes had significant effects on the ability of NMR to predict nicotine oral clearance.**

Conclusions **NMR appears to be a reliable phenotypic biomarker for nicotine clearance across races, glucuronidation phenotypes and genotypes. Racial differences in the relationships between NMR, smoking behaviors and addiction are unlikely to be related to an inadequate estimation of nicotine clearance on the basis of NMR. *Pharmacogenetics and Genomics* XXX: 000–000**

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Introduction

Nicotine is metabolized primarily by the hepatic cytochrome P450 (CYP) enzyme CYP2A6, with approximately 70–80% of nicotine converted to its inactive metabolite cotinine, which is in turn further metabolized exclusively by CYP2A6 to 3′-hydroxycotinine (3HC) [1,2]. The *CYP2A6* gene is genetically polymorphic, with a number of variants associated with slower metabolism [2]. The ratio of 3HC/cotinine (usually based on unconjugated levels), also called the nicotine metabolite ratio (NMR), is a phenotypic biomarker of CYP2A6 activity that can be measured in plasma, urine and saliva of users of nicotine products and has been shown to be correlated with the rate of nicotine clearance [3]. The NMR accounts for both genetic and nongenetic influences on CYP2A6 activity, is reproducible within subjects and is generally independent of the time since last cigarette [4–6].

In addition to CYP2A6, nicotine is also metabolized by the flavin monooxygenase 3 (FMO3) to nicotine-*N*-oxide (NNO), with FMO3 variations not shown to substantially influence nicotine metabolism in previous studies [7], and by the uridine diphosphate-glucuronosyltransferase UGT2B10 to nicotine-glucuronide [2,8–10] (Fig. 1).

Glucuronidation of nicotine is usually a minor metabolic pathway (<10%) [9], but can be a larger determinant of nicotine clearance in people with reduced CYP2A6 activity [1,11]. UGT2B10 also catalyzes the glucuronidation of cotinine (as well as nicotine), whereas UGT2B17 catalyzes the formation of 3HC-glucuronide [1,2] (Fig. 1). It is, therefore, possible that differences in the rate of glucuronidation might affect cotinine and 3HC levels and thus also the NMR. Furthermore, due to the larger impact on cotinine than on nicotine (Fig. 1), *UGT2B10* variants might affect the NMR more than nicotine clearance,

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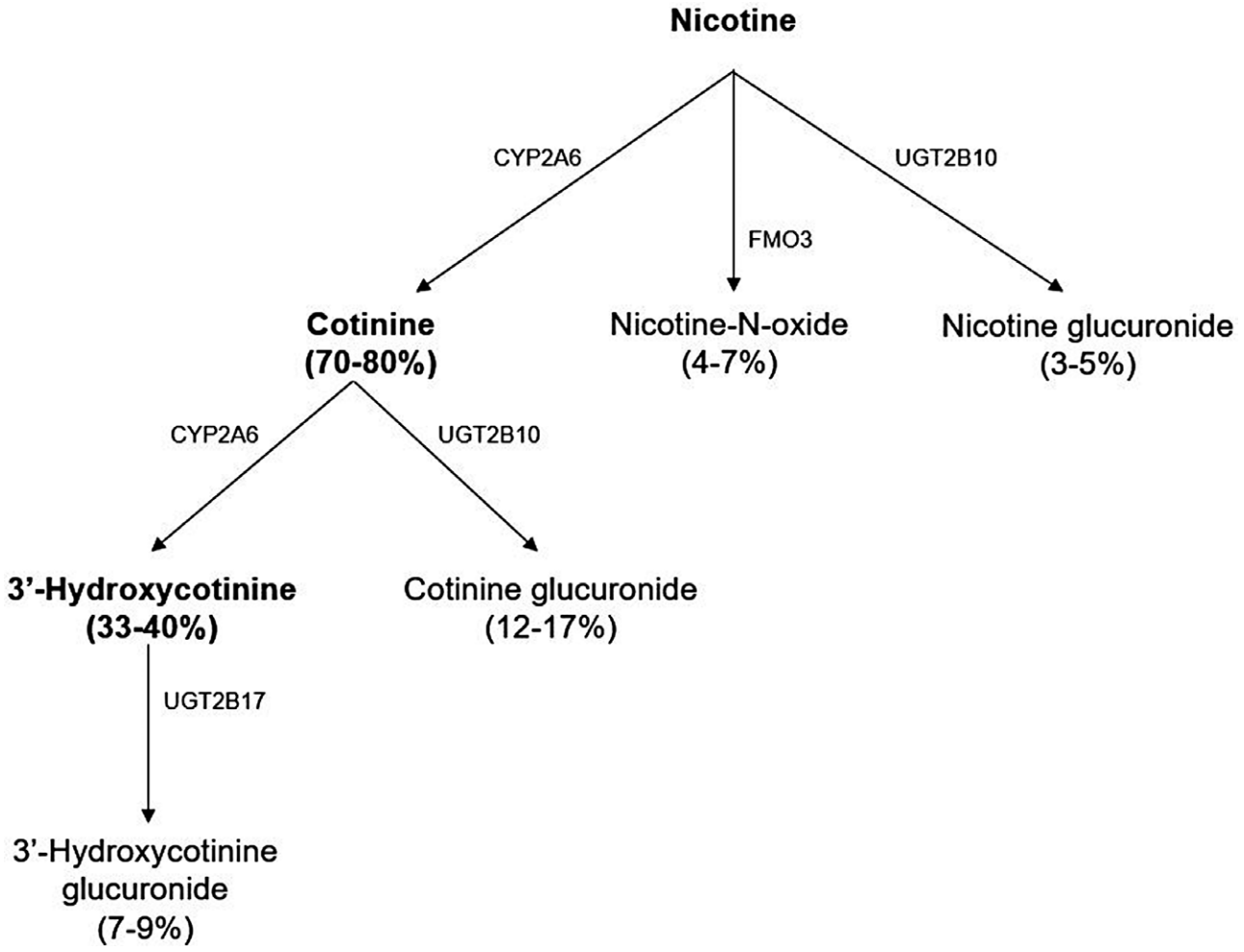
Fig. 1



Nicotine metabolism pathways (adapted from [2,10], data from [2,8,10]).

whereas, due to the effect on 3HC, *UGT2B17* variants might affect the NMR with no impact on nicotine clearance. Thus, the reliability of the NMR as a phenotypic marker of total nicotine clearance might be impacted by variation in the glucuronidation of cotinine or 3HC and variation in the genes for these UGT enzymes, which differs among races [12–14].

Individual differences in the rate of nicotine metabolism (proxied by the NMR) have been shown to influence cigarette smoking behavior and biomarkers of toxicant exposure and inflammation in smokers [15]. Rapid metabolism of nicotine is associated with smoking more cigarettes per day, greater nicotine dependence and lower rates of quitting smoking in the absence of pharmacotherapy and with nicotine transdermal patches compared to slower metabolizers [16–21]. However, it is possible that racial differences, such as the slower glucuronidation of nicotine and cotinine in African Americans (referred to here as Blacks) due to higher frequencies of slow metabolism variants in *UGT2B10* [12–14], might affect the NMR and its correlation with the nicotine clearance. Previously racial differences in the relationship between NMR and nicotine exposure have been observed, with a greater influence of NMR on nicotine intake in Caucasians (referred to here as Whites) compared to Blacks, suggesting the potential for NMR to predict nicotine clearance less accurately across racial groups [22]. On the other hand, although previous studies in Black smokers found no significant impact of *UGT2B17* reduced function alleles on NMR [23,24], a potential association between *UGT2B17* and NMR was found in a Genome-wide association meta-analysis in European smokers, indicating a possible inter-ethnic variation regarding this relationship [25].

The main aim of the present study was to investigate the reliability of NMR as a phenotypic biomarker to predict nicotine clearance among different races and as a function of differences in the glucuronidation rates of nicotine, cotinine and 3HC and variation in *UGT* genotypes by comparing Whites, Blacks and Asian-Americans (referred to here as Asians), using oral doses of nicotine and cotinine as metabolic probes. Due to the larger impact on cotinine than on nicotine, *UGT2B10* variants might affect the NMR more than nicotine clearance. Slower cotinine glucuronidation could be associated with higher cotinine levels, but without an effect on NMR, because more cotinine should generate proportionately more 3HC. However, others have hypothesized that slower cotinine glucuronidation would be associated with higher cotinine levels and thus lower NMR [9], so we tested that hypothesis. Although no impact of 3HC glucuronidation on NMR was found in previous studies with Black participants [23], we retested the hypothesis that slower 3HC glucuronidation, which may result in higher 3HC levels, could alter NMR, while also including Asians, a racial group with higher prevalence of *UGT2B17* deletion alleles than Whites or Blacks [23,26].

Methods

Participants

The study included male and female Whites, Blacks and Asians (four grandparents of the same race), aged 18–70 years. Participants were selected as healthy by medical history and taking no regular medication other than vitamins. Women of reproductive capacity had to have a negative pregnancy test. Participants with current alcoholism or illicit drug use were excluded. Potential participants were recruited by advertisements in San Francisco newspapers and campuses in the Bay Area, and postings on *Craigslist*, the *Research-Online* and our own website. The study was approved by the Committees on Human Research at the University of California, San Francisco and at the University of Toronto.

Screening and consenting

Potential participants were initially screened in a telephone interview. If suitable for the study, they were invited to the General Clinic Research Center or the Tobacco Research Clinic of the Zuckerberg San Francisco General Hospital for a screening visit. At this visit, they were asked to sign the consent form, and then fill out questionnaires inquiring about demographic, smoking, alcohol, caffeine, drug and medication, and general medical histories. A simple physical examination was performed (height and weight, vital signs and electrocardiogram), a saliva sample was collected for determination of cotinine level (to confirm smoking status), and a urine specimen was requested from females of childbearing potential for pregnancy testing.

Study procedures

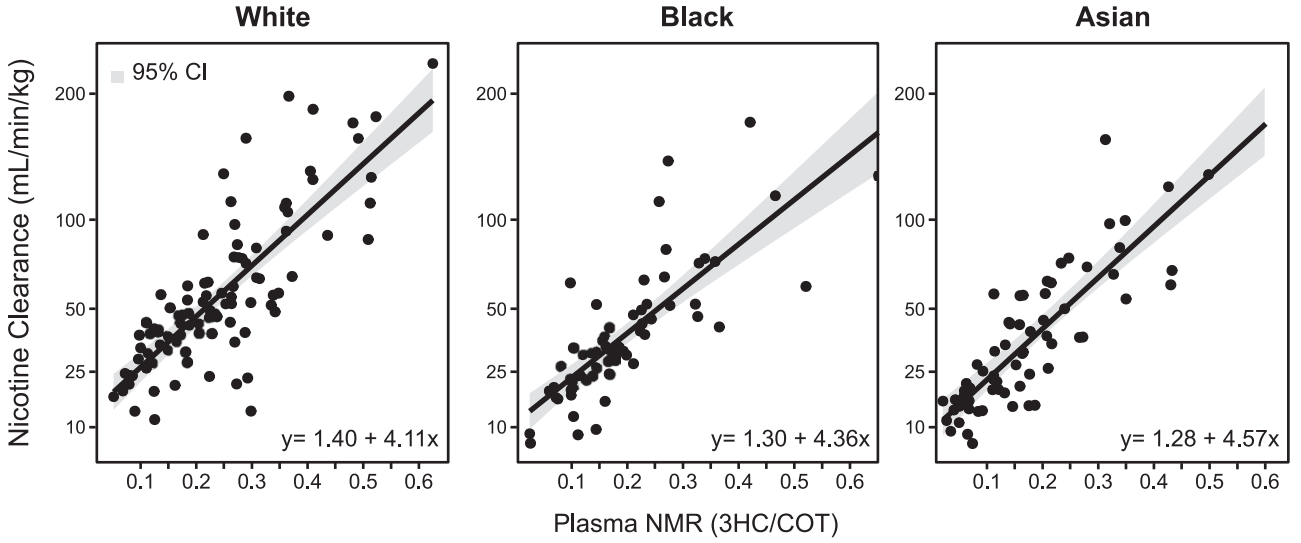
The study was conducted at the Clinical Study Center at Zuckerberg San Francisco General Hospital. Participants were asked not to eat or use tobacco starting at 10 p.m. on the previous night and to refrain from grapefruit or grapefruit juice for 48 h prior to and for the duration of the study. At 8 a.m. on study day, participants were given in solution a 2 mg oral dose of deuterium-labeled nicotine (nicotine-d2) and a 5 or 10 mg dose of either deuterium-labeled cotinine (cotinine-d4) for smokers or

Table 1 Participants' demographics and pharmacokinetic parameters [shown as median (range) unless otherwise indicated]

Measure	All (n=227)	Whites (n=96)	Blacks (n=64)	Asians (n=67)
Demographics				
Age, mean (SD) [a,b,c]	33.2 (11.2)	33.4 (11.2)	38.1 (11.7)	28.3 (8.3)
Male, *n* (%)	128 (56.4)	54 (56.2)	38 (59.4)	36 (53.7)
BMI, kg/m^2, mean (SD)[c]	25.1 (3.7)	25.2 (3.8)	26.1 (4.0)	24.0 (3.0)
Nonsmoker, *n* (%)	133 (58.6)	50 (52.1)	36 (56.2)	47 (70.1)
Nicotine pharmacokinetics				
Nicotine clearance, mL/min/kg[a,b]	39.7 (7.0–230.3)	48.9 (11.8–230.3)	32.3 (7.0–174.3)	27.4 (7.0–159.6)
Nicotine half-life, min[b]	122.2 (31.9–623.4)	113.2 (31.8–569.1)	133.2 (38.2–585.6)	144.3 (61.7–623.4)
Cotinine pharmacokinetics				
Cotinine clearance, mL/h/kg[a,c]	54.9 (6.8–341.4)	58.9 (21.4–341.4)	36.4 (6.8–146.8)	54.6 (19.7–245.9)
Cotinine half-life, h[a,b]	14.2 (6.9–59.9)	13.5 (8.0–33.9)	16.0 (8.2–59.9)	14.8 (6.9–30.4)
Urinary glucuronidation ratios				
Nicotine glucuronide/total nicotine[a,c]	0.25 (0.00–0.77)	0.29 (0.00–0.77)	0.14 (0.00–0.69)	0.29 (0.00–0.68)
Nicotine glucuronide/free nicotine[a,c]	0.33 (0.00–3.37)	0.40 (0.00–3.37)	0.16 (0.00–2.11)	0.40 (0.00–2.14)
Cotinine glucuronide/total cotinine[a,c]	0.19 (0.00–0.76)	0.23 (0.00–0.76)	0.13 (0.00–0.60)	0.20 (0.00–0.57)
Cotinine glucuronide/free cotinine[a,c]	0.24 (0.00–3.20)	0.29 (0.00–3.20)	0.15 (0.00–1.52)	0.25 (0.00–1.30)
3HC glucuronide/total 3HC[b,c]	0.13 (0.00–0.68)	0.14 (0.00–0.30)	0.14 (0.00–0.68)	0.10 (0.00–0.46)
3HC glucuronide/free 3HC[b,c]	0.15 (0.00–2.11)	0.16 (0.00–0.44)	0.16 (0.00–2.11)	0.11 (0.00–0.86)
Phenotypic biomarker of nicotine clearance				
Plasma NMR (6 h postcotinine dosage)[a,b]	0.18 (0.02–0.65)	0.23 (0.05–0.63)	0.17 (0.02–0.65)	0.15 (0.02–0.50)

NMR, nicotine metabolite ratio; 3HC, 3′-hydroxycotinine.
[a]Significant differences detected between Blacks and Whites.
[b]Significant differences detected between Asians and Whites.
[c]Significant differences detected between Blacks and Asians.

Fig. 2



Correlation of plasma nicotine metabolite ratio (NMR) to oral plasma nicotine clearance for all three races (Whites, *n*=96; Blacks, *n*=64; Asians, *n*=67; clearance values on square-root transformed scales; CI, confidence interval; COT, cotinine; 3HC, 3′-hydroxycotinine).

Table 2 Investigation of predictor effects on oral plasma nicotine clearance (ml/min/kg)

	Multivariate unadjusted model	Multivariate model adjusted for age, sex and smoking status[a]
Variable	β (SE)	β (SE)
NMR	4.48 (0.20)***	4.27 (0.23)***
Age	0.07 (0.05)	0.01 (0.03)
Sex (male)	−0.24 (0.01)*	−0.04 (0.06)
Race – Black[b]	−0.41 (0.11)***	−0.17 (0.07)**
Race – Asian[b]	−0.52 (0.11)***	−0.15 (0.07)*
Smoking status (positive for 'smoker')	−0.13 (0.09)	−0.06 (0.05)

B, beta coefficient; NMR, nicotine metabolite ratio; SE, standard error.
[a]F (6.220) =83.4, $P<0.001$ Adj. $R^2=0.69$; *$P<0.05$, **$P<0.01$, ***$P<0.001$.
[b]White (largest population) used as reference.

unlabeled cotinine (cotinine-d0) for nonsmokers. These labeled compounds were synthetized as described previously [27,28]. The 2 mg dose of nicotine was selected as a dose that is well tolerated by nonsmokers but results in plasma nicotine concentrations that are easily measurable. The dose of cotinine was selected as a dose that would result in adequate saliva concentrations of cotinine over a 60-h period, to allow us to determine the terminal elimination half-life of cotinine. The first 116 participants received 10 mg cotinine; afterwards the dose was reduced to 5 mg because pharmacokinetic analyses showed that this would not affect the ability to calculate the desired data. Deuterium-labeled cotinine was given to smokers because smokers already have unlabeled cotinine in their bodies which makes it impossible to do a kinetic study without a label. Blood samples were collected at 0 (i.e. before), 0.5, 1, 1.5, 2, 3, 4, 6 and 8 h after dosing. One blood sample was also collected for genotyping. Samples of saliva (3–5 ml) were collected at 0, 6, 12, 24, 36, 48 and 60 h following dosing. Participants could choose between staying overnight and have only three saliva samples taken at home, or get discharged after 8 h, in which case they were requested to provide five additional saliva samples taken at home. Urine samples were collected for 8 h after dosing and assayed for concentrations of nicotine, cotinine and 3HC and their glucuronides.

Analytical chemistry

Measurement of nicotine and cotinine in blood and saliva for the pharmacokinetic analysis was performed by gas chromatography–mass spectrometry, as described previously [29], modified for tandem mass spectrometry (MS/MS) for improved sensitivity. The limit of quantitation (LOQ) for nicotine was 0.5 ng/mL and for cotinine 1 ng/mL. For the calculation of plasma NMR, 3HC and cotinine were determined by liquid chromatography–tandem mass spectrometry (LC-MS/MS), as described previously [30]. The LOQ for 3HC and cotinine was 1 ng/mL. Urine concentrations were measured by LC-MS/MS. The LOQ for the urine analytes was 10 ng/mL. Nicotine, cotinine and 3HC glucuronides were generated by subtraction of the free form (measured without adding the beta-glucuronidase enzyme) from total form (measured after adding beta-glucuronidase enzyme type IXA from *Escherichia coli* and incubated overnight to deconjugate the molecule).

Genotyping was performed at the University of Toronto. Four single nucleotide polymorphisms (SNPs) within the *UGT2B10* gene, that is rs2331559, rs11726322, rs835309, and the splice site variant rs2942857 (merged with rs116294140), that have been previously shown to be associated with nicotine or cotinine glucuronidation in at least one of the racial groups examined here [9,31],

were genotyped. The *UGT2B17* copy number variant assay for the *UGT2B17*2* deletion allele, that has been associated with impaired 3HC glucuronidation in smokers [12,23,32], was also genotyped. Individuals with a *UGT2B17* duplication of intron 1 (i.e. *UGT2B17 *1/*1x2*, $n=3$), detected by the copy number variant assay, were merged with the *UGT2B17 *1/*1* genotype, because there is currently no data indicating that this results in a gain of function. The *CYP2A6* genotyping data are provided as supplementary material (Supplementary Document S1, Supplemental digital content 1, *http:///links.lww.com/FPC/B389*).

Data analysis

The main measure of the rate of nicotine metabolism was the oral plasma clearance of nicotine-d_2, determined as the dose divided by the area under the plasma nicotine concentration–time curve extrapolated to infinity. The oral saliva clearance of cotinine (high correlation of plasma and saliva clearance of cotinine shown in previous studies [33]) was computed in a similar manner by use of the area under the saliva cotinine concentration–time curve. Both nicotine and cotinine clearances were normalized by subject body weights (kg). Elimination half-lives were determined by nonlinear least squares fitting of the log concentration versus time using Phoenix WinNonlin (Pharsight Corporation, Mountain View, California,USA). The plasma NMR, based on the ratio of free (unconjugated) cotinine and 3HC, was determined from the 6 h postdose plasma sample of dosed labeled (for smokers) or unlabeled (for nonsmokers) cotinine. A high correlation between the plasma NMR ratio derived at this time point and oral nicotine clearance has been shown in previous studies [$r=0.9$; $P<0.01$ for the unlabeled (3HC-d0/cotinine-d0) and $r=0.79$; $P<0.01$ for the deuterium-labeled (3HC- d_4/cotinine-d_4) ratios] [3]. Urine data were used to estimate the glucuronidation activity as the ratio of glucuronide (ng/ml)/free (ng/ml) analyte and glucuronide/total (i.e. free and glucuronide) analyte. These were computed for nicotine and cotinine, phenotypic markers of UGT2B10 activity, and for 3HC, a phenotypic marker of UGT2B17 activity.

Numerical data are presented as arithmetic mean and SD if normally distributed or median and range if not normally distributed, and nominal data as a proportion (%). Missing data were not imputed and not available glucuronidation ratios due to values below LOQ were not included in the analysis. Spearman's correlations were used to describe variable associations between NMR, clearances and glucuronidation ratios. Between-group differences were tested using the chi-square or Fisher's exact test for categorical variables, one-way analysis of variance (ANOVA) for continuous normally distributed variables, and the Kruskal–Wallis test for nonnormally distributed variables. Additional analyses were performed using general linear model (GLM) where the log-transformed nicotine or cotinine clearance were predicted as a function of plasma NMR, sex [34,35], age [2,20], race, BMI [20] and smoking status [36]. Because glucuronidation might affect the nicotine clearance and NMR, but has a different role in this relationship than the *CYP2A6* genotype, which is an established contributor to NMR and nicotine clearance (e.g. [3,37]), the *CYP2A6* genotype was not included in the analyses regarding the relationship between clearance and NMR. Analyses were conducted using SPSS statistical software (IBM SPSS Statistics 25.0) or R (version 3.5). A $P<0.05$ was considered statistically significant.

Results

A total of 227 participants were included in the study. The NMR and pharmacokinetic data for nicotine were available for all participants ($N=227$), whereas cotinine pharmacokinetic data were missing (e.g. due to missing saliva samples) in eight cases. Due to a missing sample in one case and values below LOQ in the rest of the cases, the glucuronidation ratios were not available in 11, 1 and 1 cases for nicotine, cotinine and 3HC, respectively. The glucuronidation ratios were zero (i.e. no glucuronide present) in 17, 17 and 29 cases for cotinine, nicotine and 3HC, respectively. The *UGT2B10* genotyping was available for all participants, whereas *UGT2B17* genotype was missing in one case.

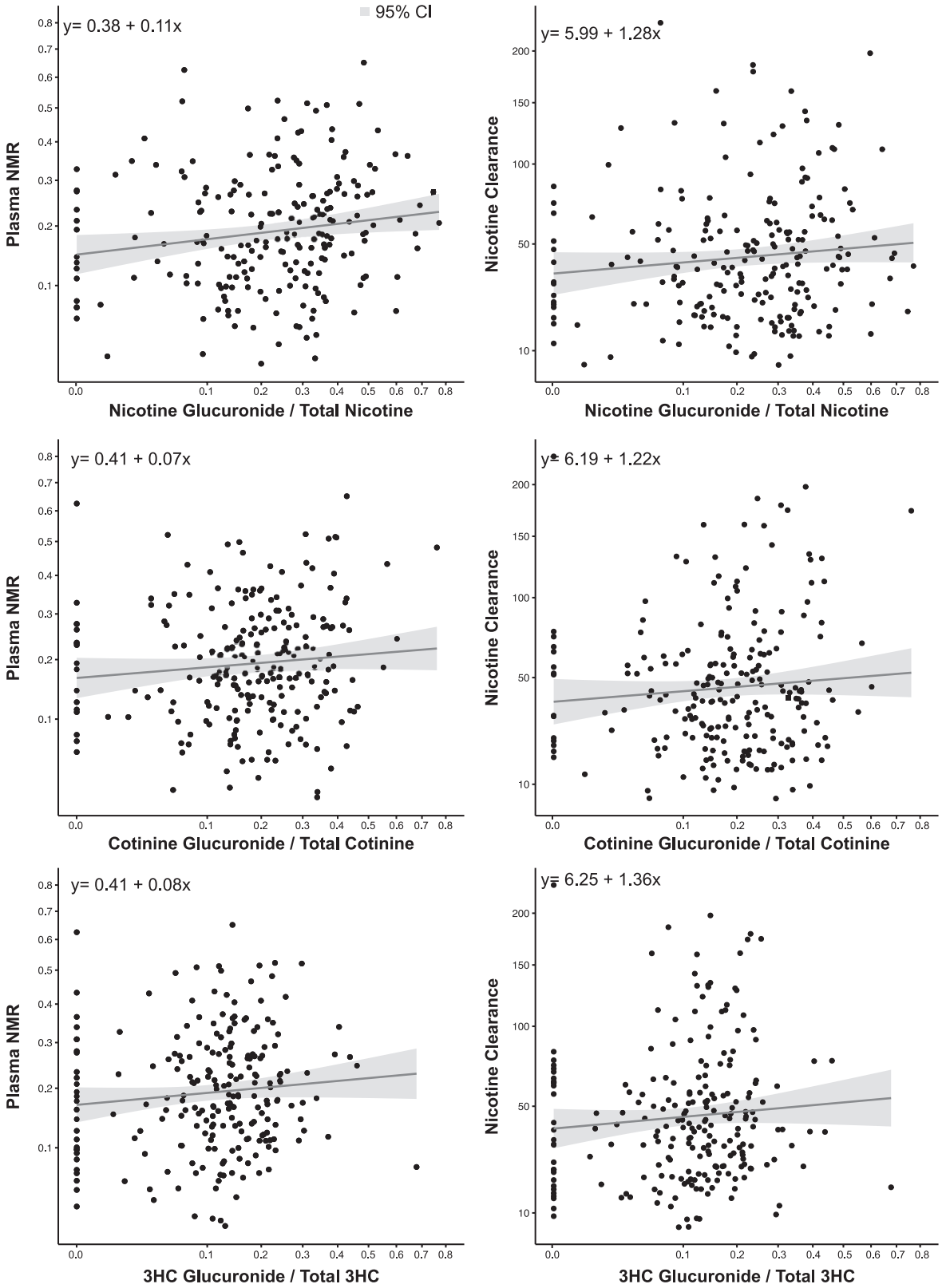
Among the 227 participants, 96 (42%) were White, 67 (30%) Asian and 64 (28%) Black. The participants' demographics and pharmacokinetic data are shown in Table 1; compared to the other two races, Whites had higher nicotine and cotinine total oral clearance, Blacks had lower nicotine and cotinine glucuronidation rates, and Asians had lower 3HC glucuronidation rates.

Plasma NMR was strongly correlated with nicotine clearance within each of the three race categories (White: correlation coefficient $\rho=0.77$; Black: $\rho=0.83$; Asian: $\rho=0.84$; all $P<0.001$) (Fig. 2); the correlation remained significant among all three race categories after adjusting for age and sex.

In the multivariate GLM, the NMR's correlation with nicotine clearance [$F\ (1.220)=380.1$; $P<0.001$] continued after adjustment for age, sex, race and smoking status (Table 2). In the unadjusted model, NMR, sex and race were predictors of nicotine clearance, whereas in the adjusted model, only NMR and race remained significant predictors (Table 2). A stronger relationship to nicotine clearance was found for NMR compared to race in both models.

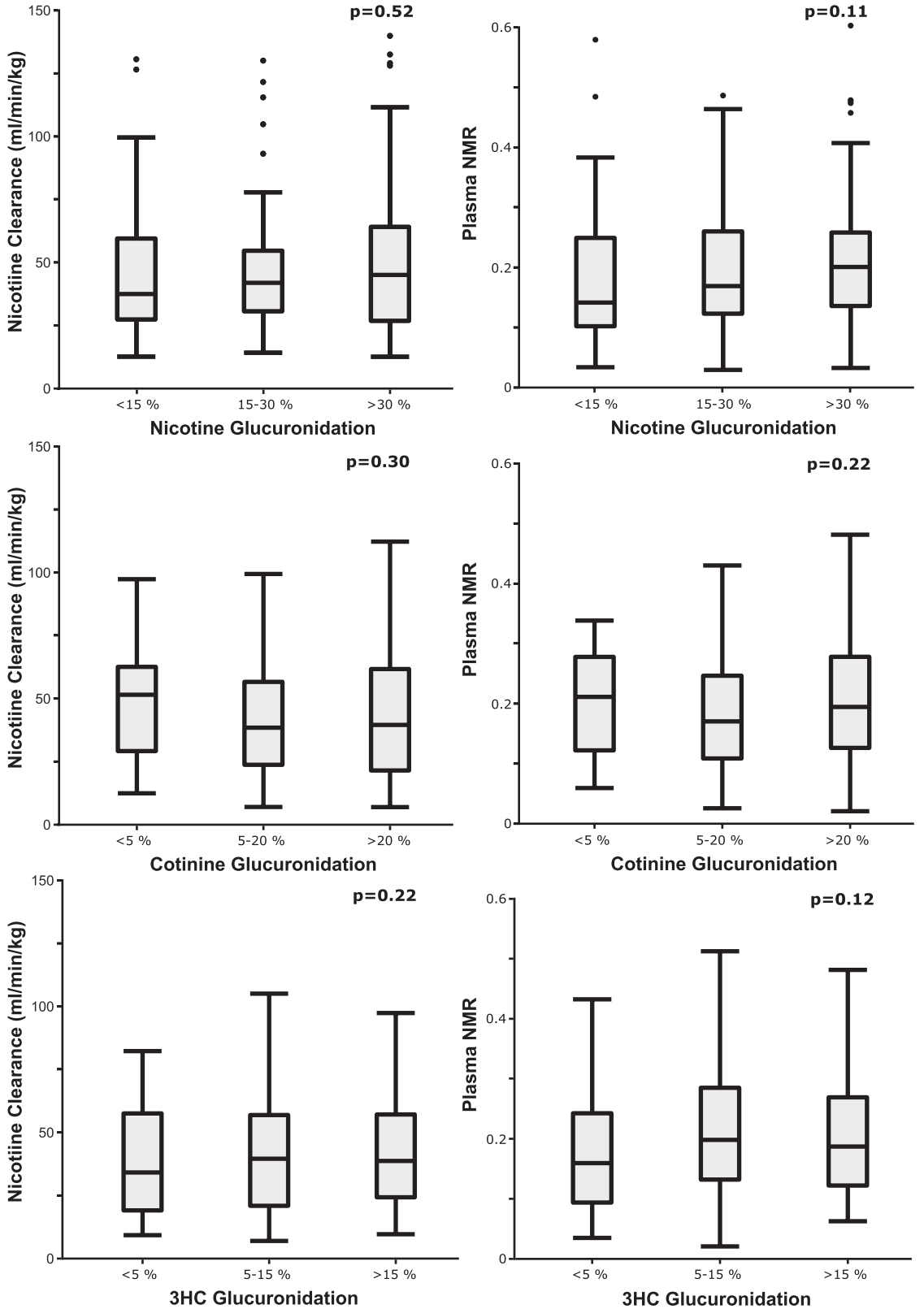
Investigation of correlations of the nicotine, cotinine and 3HC glucuronidation activity showed little evidence of a relationship with nicotine clearance (ρ value = 0.12; $P=0.09$ for nicotine; ρ value = 0.06; $P=0.38$

Fig. 3



Correlations between nicotine (n=216), cotinine (n=226) and 3′-hydroxycotinine (3HC; n=226) glucuronidation activity (assessed from urine glucuronide/parent ratios) and plasma nicotine metabolite ratio (NMR) or oral plasma nicotine clearance (values on square-root transformed scales). CI, confidence interval.

Fig. 4



Relationship between nicotine (n=216), cotinine (n=226) and 3′-hydroxycotinine (3HC; n=226) glucuronidation activity (assessed from urine glucuronide/parent ratios), and oral plasma nicotine clearance and plasma nicotine metabolite ratio (NMR), based on the percentage of the measured glucuronidation activity.

for cotinine; ρ value = 0.11; $P = 0.09$ for 3HC) or NMR (ρ value = 0.19; $P < 0.001$ for nicotine; ρ value = 0.11; $P = 0.10$ for cotinine; ρ value = 0.12; $P = 0.07$ for 3HC) (Fig. 3, Table S1).

The correlations remained very weak or weak (all ρ values <0.4) also when investigating each race separately (Supplementary Table S1, Supplemental digital content 1, *http://links.lww.com/FPC/B389*).

When examining the relationships in Fig. 3, some individuals demonstrated very low rates of glucuronidation, most likely due to reduced activity or null UGTs. A categorical analysis based on the percent of the measured nicotine, cotinine and 3HC glucuronidation activity, to also address the cases with very low or no glucuronidation activity, showed little evidence of a relationship with either nicotine clearance or NMR (Fig. 4); the results remained insignificant also when analyzed by race (analysis not shown).

Table 3 shows the number of individuals with no glucuronidation activity for the total study population and also by race.

As a secondary approach to examining the impact of variation in glucuronidation on NMR's ability to predict nicotine clearance, the impact of *UGT2B10* and *UGT2B17* genotype groups was examined. As the *UGT* genotypes variant frequencies vary by race, these are provided in Table 4; the allele frequencies were in Hardy–Weinberg equilibrium and similar to those found in *1000 Genomes* and previously published [23,24,31].

Table 3 Number of participants with no glucuronidation activity by race

	All (n=227)	Whites (n=96)	Blacks (n=64)	Asians (n=67)
Nicotine (n, %)	16 (7.0)	4 (4.2)	10 (15.6)	2 (3.0)
Cotinine (n, %)	17 (7.5)	5 (5.2)	9 (14.1)	3 (4.5)
3′-hydroxycotinine (3HC; n, %)	29 (12.8)	8 (8.3)	10 (15.6)	11 (16.4)

The *UGT* variants had different SNP frequencies, linkage disequilibrium structures and impact on nicotine and cotinine glucuronidation ratios between races (Supplementary Table S2, Supplemental digital content 1, *http://links.lww.com/FPC/B389*), as expected and published previously (e.g. [9,23]).

The *UGT* variants, as well as the glucuronidation ratios, were examined for their impact on NMR's ability to predict nicotine clearance using regression analyses while adjusting for age, sex and race (Table 5). None of the glucuronidation activities, nor any *UGT* genotype, affected the ability of NMR to predict nicotine clearance. The proportion of variation in nicotine clearance predicted by NMR vary less than 2% among the models.

Discussion

We present novel data showing a significant and strong correlation between NMR and nicotine oral clearance in three major racial groups, thus confirming that NMR is a reliable phenotypic biomarker for nicotine clearance across races. Further, none of the metabolite glucuronidation ratios had a significant effect on NMR and its ability to predict nicotine oral clearance. The *UGT* genotype frequencies and linkage disequilibrium structure varied between races as expected, but none of the *UGT* variants altered the ability of NMR to predict nicotine clearance (Table 5). On the basis of our findings, reports showing racial differences in the relationship between NMR, smoking behavior, levels of addiction and response to smoking cessation treatment are not likely to be related to inadequate estimation of nicotine clearance based on NMR.

Table 4 Prevalence of *UGT* variants by race (shown as *n* (%) within race; *p*-values: comparisons by race; bold: values with adjusted residuals of <–2 or >2 for comparisons by race; Whites, *n*=96; Blacks, *n*=64; Asians, *n*=67)

			Race			
		N	White (n=96)	Black (n=64)	Asian (n=67)	*P* value
UGT2B10 rs2331559	G/G[a]	118	**73 (61.9)**	**10 (8.5)**	35 (29.7)	<0.001
	C/G	78	**21 (26.9)**	**30 (38.5)**	27 (34.6)	
	C/C	31	**2 (6.5)**	**24 (77.4)**	5 (16.1)	
UGT2B10 rs835309	G/G[a]	146	**74 (50.7)**	**15 (10.3)**	**57 (39.0)**	<0.001
	T/G	60	21 (35.0)	**29 (48.3)**	**10 (16.7)**	
	T/T	21	**1 (4.8)**	**20 (95.2)**	**0 (0.0)**	
UGT2B10 rs11726322	G/G[a]	158	**77 (48.7)**	**33 (20.9)**	48 (30.4)	<0.001
	G/C	57	18 (31.6)	22 (38.6)	17 (29.8)	
	C/C	12	**1 (8.3)**	**9 (75.0)**	2 (16.7)	
UGT2B10 rs2942857	A/A[a]	175	**89 (50.9)**	**30 (17.1)**	56 (32.0)	<0.001
(splice variant, previously rs116294140)	A/C	47	**7 (14.9)**	**29 (61.7)**	11 (23.4)	
	C/C	5	–	**5 (100)**	–	
UGT2B17	*1/*1[b]	77	**43 (55.8)**	**30 (39.0)**	**4 (5.2)**	<0.001
	*1/*2	89	**45 (50.6)**	27 (30.3)	**17 (19.1)**	
	*2/*2	60	**7 (11.7)**	**7 (11.7)**	**46 (76.7)**	
	Missing	1	1 (100)	–	–	

[a]Faster cotinine glucuronidation based on previous publications [9, 31]
[b]Faster 3'-hydroxycotinine glucuronidation based on previous publications [23, 31]

Table 5 Regression analyses of plasma nicotine metabolite ratio's ability to predict oral plasma nicotine clearance, adjusted for an individual glucuronidation rate (assessed from urine glucuronide/parent ratios), an individual *UGT* variant, as well as age, sex and race (Whites, *n*=95; Blacks, *n*=64 and Asians, *n*=67)

GLMs (models a–g) predicting nicotine clearance from plasma NMR	β	95 % CI	Scaled β	ANOVA model parameters (also includes adjustment for age and sex)
a: R^2=0.69; P<0.001				
Plasma NMR	4.32	3.88–4.75	0.80	F (1.219)=385.2; **P<0.001**
Cot-Gluc/total ratio				F (1.219)=0.69; P=0.41
Race				F (2.219)=4.15; **P=0.02**
b: R^2=0.69; P<0.001				
Plasma NMR	4.29	3.86–4.72	0.79	F (1.219)=383.4; **P<0.001**
3HC-Gluc/total ratio				F (1.219)=0.08; P=0.77
Race				F (2.219)=3.88; **P=0.02**
c: R^2 =0.69; P<0.001				
Plasma NMR	4.31	3.87–4.74	0.79	F (1.219)=383.8; **P<0.001**
UGT2B10 rs2331559 genotype				F (2.219)=0.75; P=0.48
Race				F (2.219)=2.43; P=0.09
d: R^2 =0.69; P<0.001				
Plasma NMR	4.31	3.88–4.73	0.79	F (1.219)=397.2; **P<0.001**
UGT2B10 rs835309 genotype				F (2.219)=2.67; P=0.07
Race				F (2.219)=2.27; P=0.11
e: R^2 =0.68; P<0.001				
Plasma NMR	4.28	3.84–4.71	0.79	F (1.219)=380.3; **P<0.001**
UGT2B10 rs11726322 genotype				F (2,219)=0.35; P=0.70
Race				F (2.219)=3.91; **P=0.02**
f: R^2=0.69; P<0.001				
Plasma NMR	4.34	3.91–4.77	0.80	F (1.219)=399.6; **P<0.001**
UGT2B10 rs2942857 genotype				F (2.219)=2.53; P=0.08
Race				F (2.219)=2.23; P=0.11
g: R^2=0.69; P<0.001				
Plasma NMR	4.31	3.89–4.74	0.80	F (1.219)=392.5; **P<0.001**
UGT2B17 genotype				F (2.218)=1.94; P=0.14
Race				F (2.218)=3.25; **P=0.04**

Nicotine Clearance (ml/min/kg); Not displayed are parameters for age and sex, which were also included in all models.
ANOVA, analysis of variance; CI, confidence interval; GLM, general linear model; NMR: nicotine metabolite ratio.

As also reported by others, we found significant differences by race in pharmacokinetic measures, with higher nicotine and cotinine oral clearance in Whites compared to Blacks or Asians [2,11], and lower nicotine and cotinine glucuronidation rates in Blacks, the latter in line with the higher frequency of slow *UGT2B10* variants in this population [12,38]. In a recent study using cotinine glucuronidation as a phenotypic marker of UGT2B10 activity approximately 15% of the Black participants excreted essentially no glucuronide [38], in line with the higher frequency of the *UGT2B10* splice variant rs2942857 (formerly rs116294140) seen in this population [9], which was included in our study. The lower 3HC glucuronidation rates in Asians are likely related to the higher frequency of low activity *UGT2B17* variants [23,26].

In final regression models, we found no effect of the glucuronidation ratios or of the *UGT2B10* and *UGT2B17* genotypes on the ability of NMR to predict nicotine clearance (Table 5). Similarly to our findings, a previous study investigating the impact of *UGT2B10* and *UGT2B17* variation on nicotine pharmacokinetics in Blacks also found no significant effect on NMR [24]. Lower nicotine and cotinine glucuronidation ratios in Blacks compared to Whites have also been reported previously [31]. However, these studies included either only Blacks [24], or only Whites and Blacks [31], whereas our study addressed these questions while including three different races, thus expanding the previously reported findings and also investigated the impact of race and glucuronidation on the ability of NMR to predict nicotine clearance across races.

In a recent study, smokers with essentially no UGT2B10 activity, as assessed by no quantifiable cotinine glucuronide, had lower NMR values compared to smokers with phenotypic higher UGT2B10 activity, but the difference did not remain significant after adjusting for cigarettes smoked per day, urinary total nicotine equivalents and UGT2B10 activity [38]. A large genotyping study, including five racial groups (Blacks, Whites, Native Hawaiians, Latino and Japanese Americans) investigated the effect of *UGT2B10* variants on nicotine metabolic pathways and found that, as expected, *UGT2B10* variants associated with reduced activity in Blacks had a significant effect on the extent of nicotine glucuronidation [9]. The authors suggested that NMR might be less useful in Blacks, because the slower glucuronidation related to the high frequency of low activity *UGT2B10* variants might impact the NMR and lead to misclassification of the *CYP2A6* genotype. However, even in the case of slower cotinine glucuronidation and higher cotinine levels, no effect on NMR is expected, because more cotinine is expected to generate proportionately more 3HC, thus leaving the 3HC/cotinine ratio (i.e. NMR) unaffected. In line with this theoretical prediction, we found no significant effect of the cotinine glucuronidation rate on NMR.

In contrast to the extent of cotinine glucuronidation, it would be expected that the 3HC glucuronidation might affect the NMR, because slower 3HC glucuronidation could result in higher free 3HC levels thus affecting the ratio. However, neither the 3HC glucuronidation ratios nor the *UGT2B17* genotypes were significant predictors for NMR in our study, or in a previous study [23]. Zhu *et al*. [23] only included Blacks, while a higher prevalence of *UGT2B17* deletion occurs in Asians [26]. We cannot exclude the possibility that a significant effect of 3HC glucuronidation on NMR would have been observed if we had a larger sample size of Asians in our study.

Limitations of our study include the smaller number of Black and Asian participants compared to Whites, the fact that these designations can represent heterogenous populations with variation in enzyme activity levels, as well as some missing values. However, although previous studies have demonstrated effects of race on NMR and nicotine clearance, this study is the first to investigate the relationship between NMR and nicotine oral clearance across races, thus providing a basis for the reliability and use of this phenotypic biomarker in clinical practice in different populations. In addition to the inclusion of three races, another strength was the detailed analyses on the effect of individual differences in rates of glucuronidation, assessed both phenotypically and by *UGT2B17* and *UGT2B10* genotypes, on the relationship between nicotine clearance and NMR.

In summary, our findings show that neither race nor differences in the glucuronidation rates of cotinine and 3HC appear to have a significant effect on NMR or the NMR's ability to predict nicotine clearance, thus confirming that NMR is a reliable phenotypic biomarker for nicotine clearance and supporting its future use in smoking cessation studies and clinical practice.

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Conflicts of interest

R.F.T. has consulted for Quinn Emanuel and Ethismos Research Inc. and as a paid consultant to pharmaceutical companies on unrelated topics. N.L.B. has been a consultant for Pfizer and Achieve Life Sciences, companies that market or are developing smoking cessation medications, and has been an expert witness in litigation against tobacco companies. For the remaining authors, there are no conflicts of interest.

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