HHS Public Access

Author manuscript

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2021 February 01.

Published in final edited form as:

Cancer Epidemiol Biomarkers Prev. 2020 August ; 29(8): 1673–1678. doi:10.1158/1055-9965.EPI-20-0203.

UGT2B10 genotype influences serum cotinine levels and is a primary determinant of higher cotinine in African American smokers

Christopher J. Sipe[1], **Joseph S. Koopmeiners**[2], **Eric C. Donny**[3], **Dorothy K. Hatsukami**[4], **Sharon E. Murphy**[5,*]

[1]Masonic Cancer Center, University of Minnesota, Minneapolis, MN 55455

[2]Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, MN 55455

[3]Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC 27109

[4]Masonic Cancer Center and Department of Psychiatry, University of Minnesota, Minneapolis, MN 55455

[5]Department of Biochemistry Molecular Biology and Biophysics and Masonic Cancer Center, University of Minnesota, Minneapolis, MN 55455

Abstract

Background: Cotinine is the most widely used biomarker of tobacco exposure. At similar smoking levels African Americans (AAs) have higher serum cotinine than Whites. UGT2B10-catalyzed cotinine glucuronidation impacts these levels, and AAs often have low UGT2B10 activity due to a high prevalence of a *UGT2B10* splice variant (rs2942857).

Methods: Two *UGT2B10* SNPs (rs6175900 and rs2942857) were genotyped in 289 AA and 627 White smokers. Each smoker was assigned a genetic score of 0, 1 or 2 based on the number of variant alleles. Total nicotine equivalents (TNE), the sum of nicotine and 6 metabolites, and serum cotinine and 3′-hydroxycotinine were quantified. The contribution of *UGT2B10* genetic score to cotinine concentration was determined.

Results: Serum cotinine was significantly higher in smokers with *UGT2B10* genetic scores of 2 versus 0 (327 ng/ml versus 221 ng/ml, $p<0.001$); TNE were not different. In a linear regression model adjusted for age, gender, CPD, TNE, race, and CYP2A6 activity, geometric mean cotinine increased 43% between genetic score 2 versus 0 ($p<0.001$). A 0.1 increase in the CYP2A6 activity ratio, 3′-hydroxycotinine/cotinine, resulted in a 6% decrease in cotinine. After adjustment for *UGT2B10* genotype and the other covariants there was no significant difference in serum cotinine by race.

[*]**Corresponding author**: Sharon E. Murphy, Ph.D., Masonic Cancer Center, University of Minnesota, 2231 6th Street SE - 2-127 CCRB, Minneapolis, MN 55455 USA, Phone: (612) 624-7633, Fax: (612) 624-3869, murph062@umn.edu.

Conflict of Interest Statement

The authors declare no potential conflicts of interest.

Conclusion: *UGT2B10* genotype is a major contributor to cotinine levels and explains the majority of high serum cotinine in AA smokers.

Impact: Cotinine levels in smokers may greatly overestimate tobacco exposure and potentially misinform our understanding of ethnic/racial difference in tobacco-related disease if *UGT2B10* genotype is not taken into account.

Introduction

The nicotine metabolite cotinine is the most widely used biomarker of nicotine intake and tobacco exposure (1). However, cotinine levels in blood and urine are influenced by differences in metabolism of nicotine and cotinine (1–4). The effect of racial/ethnic differences in metabolism on the relatively high plasma cotinine levels in African American compared to White smokers has been recognized for many years (5, 6). However, the primary driver of this difference, cotinine glucuronidation, has only recently been appreciated (7).

The major pathways of nicotine metabolism are presented in Figure 1. In most smokers, greater than 75% of the nicotine dose is metabolized by CYP2A6- catalyzed 5′-oxidation (8). The iminium ion product of this reaction is further oxidized to cotinine by either CYP2A6 or aldehyde oxidase (8, 9). Cotinine is then metabolized to *trans* 3′-hydroxycotinine (HCOT) or to cotinine *N*-glucuronide. Nicotine is also metabolized by *N*-glucuronidation as well as *N*-oxidation (Figure 1). The *N*-glucuronidation of both nicotine and cotinine is catalyzed by UGT2B10 (10, 11). CYP2A6 is the catalyst of cotinine oxidation to HCOT and the ratio of HCOT to cotinine in plasma or total HCOT to cotinine in urine are measures of CYP2A6 activity [“total” refers to the sum of the compound and its glucuronide conjugate]. The sum of the urinary concentration of nicotine and its six major metabolites, referred to as total nicotine equivalents (TNE), is an excellent biomarker of nicotine dose and therefore tobacco exposure (1, 12). In contrast to cotinine, TNE is not significantly influenced by individual differences in metabolism.

More than 30 years ago (13), it was reported that average plasma cotinine levels were higher in African Americans compared to White smokers who used a similar number of cigarettes per day (CPD). Some of this difference has been attributed to higher smoking intensity among African Americans than Whites (5). That is, more nicotine is taken up per cigarette. However, even after adjusting for TNE the difference persists (7). In addition, African American smokers administered deuterated cotinine and abstinent African American smokers on the nicotine patch excreted a much lower percentage of cotinine as a glucuronide conjugate than do White smokers under the same conditions (6, 14).

UGT2B10 is the primary if not exclusive catalyst of cotinine glucuronidation in smokers and a UGT2B10 splice variant, present at a high frequency in African Americans, contributes significantly to low levels of cotinine glucuronidation (3, 4, 15, 16). In a GWAS carried out in a subset of current smoker from the multiethnic cohort 33.2% of the variation in the fraction of urinary cotinine excreted as its glucuronide conjugate was explained by 15 independent and globally significant SNPs (16). In African Americans 55% of the variance was explained by these SNPs. The strongest single SNP association was for rs115765562,

which is highly correlated with the UGT2B10 splice variant. The splice variant together with rs6175900 (Asp67Tyr) explains 24.3% of the variation in all 5 ethnic groups and the vast majority of the variation in African Americans. Smokers homozygous for either of these SNPs excreted no cotinine glucuronide (4, 16).

We recently reported that serum cotinine levels are significantly impacted by a smoker's ability to glucuronidate cotinine (7). Smokers who excrete no cotinine glucuronide, that is, those who have no UGT2B10 activity have 48% higher serum cotinine levels than smokers with UGT2B10 activity. In addition, when a smoker's serum cotinine concentration was adjusted for their cotinine glucuronidation activity, there was no longer a significant difference between the serum cotinine of African American smokers compared to White smokers. In the study presented here we investigated directly the effect of *UGT2B10* genotype on serum cotinine levels and determined the contribution of *UGT2B10* genotype to cotinine levels in African American and White smokers.

Materials and Methods

Study population

This study was carried out using participants from a randomized trial of reduced nicotine content cigarettes (17). Only baseline samples were used. The subjects included were those who self-described as non-Hispanic Black or White and for whom urine, serum and DNA was available. At baseline, the participants were smoking their usual brand of cigarette and did not report using any other nicotine containing products in more than 9 of the last 30 days. Eligibility criteria for the trial included an age of 18 years, use of at least 5 CPD and either expired carbon monoxide levels >8ppm or urinary total cotinine >100ng/ml at the screening visit. Among these participants, two *UGT2B10* variants (rs61750900 and rs2942857) were successfully genotyped in 289 of 300 African American smokers and 627 of 650 White smokers. The average CPD was assessed with the use of an interactive voice response system (InterVision Media), which telephoned and asked participants to report the number of cigarettes smoked in the past day. The demographic parameters are presented in Table 1.

Biomarkers

Liquid chromatography with tandem mass spectrometry was used for the analyses of urinary, saliva, and serum biomarkers as previously described (4, 17, 18). In urine (first morning void) total nicotine, total cotinine, total HCOT, nicotine *N*-oxide and cotinine were quantified. Total refers to the sum of the analyte and its glucuronide conjugate and was measured by treating the urine with β-glucuronidase prior to analysis. TNE values were calculated as the sum of total nicotine, total cotinine, total HCOT and nicotine *N*-oxide. UGT2B10 phenotype was determined as the urinary ratio of total cotinine to cotinine. A phenotype ratio of 1 would reflect no UGT2B10 activity. As in a prior study, participants were assigned a *UGT2B10* genetic score based on two SNPs rs61750900 and rs2942857 (4). Specifically, scores were as follows: score 0, rs61750900 GG and rs116294140 AA; score 1, rs61750900 GT or rs116294140 CA; score 2, rs61750900 TT or rs116294140 CC, or both rs61750900 GT and rs116294140 CA. Cotinine and HCOT were quantified in serum and the

ratio of HCOT to cotinine was calculated as a measure of CYP2A6 activity as previously described (23).

Genotype

Saliva samples were collected by Oragene-Discover kits (https://www.dnagenotech.com) and DNA was isolated using Qiagen QIAamp DNA mini kit per manufacturer's instructions (https://www.qiagen.com) . Genotyping for the two *UGT2B10* variants rs61750900 and rs2942857 were carried out in the University of Minnesota Genomic Center using the iPLEX gold method (Agena San Diego, CA). iPLEX reagents and protocols for multiplex PCR, single base primer extension, and generation of mass spectra were carried out per the manufacturer's instructions (https://agenabio.com/products/applications/genotyping-and-mutation-detection/). The design sequences for the amplicons used are provided in Supplemental Figure 1. In the case of rs61750900, the original design sequence resulted in a poor call rate in African Americans. This was likely due to SNPs in the amplified DNA that were present in African Americans and not Whites, therefore, a new longer amplicon was designed and performed well on all samples.

Statistical analysis

Participant characteristics including demographics, baseline CPD, biomarkers, UGT2B10 phenotype, and allele frequencies for *UGT2B10* genotype were summarized and compared by race using the sample mean, sample proportion, or geometric mean, as appropriate. Biomarkers were compared by genotype score and UGTB10 phenotype using ANCOVA in unadjusted analyses and analyses adjusting for covariates. Comparisons of urinary biomarkers by genotype score were adjusted for age, gender, and race, comparisons of serum cotinine by genotype score were adjusted for age, gender, CPD, TNEs, race, and NMR, and comparisons of biomarkers by UGTB10 phenotype were adjusted for CPD, TNE, and total to free cotinine ratio. Throughout, biomarkers were analyzed on the log scale and differences were summarized by the ratio of the geometric mean.

Results

The African Americans in this study smoked 30% fewer CPD than Whites, and their TNE concentration was 10% lower (Table 1). However, despite lower levels of smoking, serum concentration of cotinine was higher in African Americans compared to Whites (Table 1, mean: 302 ng/ml vs 258 ng/ml, $p<0.001$ geometric mean 260 ng/ml vs 229 ng/ml, $p = 0.002$). In addition UGT2B10 phenotype, a measure of cotinine glucuronidation, was significantly lower in African Americans than in whites (2.03 vs 3.04, $p<0.001$). This observed difference in glucuronidation is due to the high prevalence of a *UGT2B10* splice variant (rs2942857) in African Americans (36.2%).

To determine the effect of *UGT2B10* genotype on serum cotinine, participants were assigned a genetic score that has been used previously (4). The score is based on two *UGT2B10* variant alleles, the splice variant (rs2942857) and an Asp 67Tyr variant (rs61750900), both of which code for non-functional enzyme. The splice variant allele frequency was 36.2% in African Americans and 0.4% in Whites (Table 1). The Asp67Tyr variant occurred more

frequently in Whites than in African American (10.5% vs 4.7% allele frequency). Smokers were assigned a genetic score of 0, 1, or 2, depending on the number of variant alleles they carried. These scores were associated with UGT2B10 phenotype, the ratio of total cotinine to cotinine (Table 2). Smokers with a genetic score of 2 excreted no cotinine glucuronide (UGT2B10 phenotype 1.02), whereas those with a score of 0 excreted almost twice as much cotinine glucuronide as unconjugated cotinine (UGT2B10 phenotype 2.88, Table 2). The UGT2B10 phenotype of smokers with a genetic score of 1 excreted approximately equal amounts of cotinine and cotinine glucuronide (UGT2B10 phenotype 1.95). When these data are stratified by race, the relationship of UGT2B10 phenotype to the genetic score is similar. Although, African Americans with scores of 1 and 2 had lower UGT2B10 phenotype values (i.e. less cotinine glucuronide) than did Whites with these scores. All differences in UGT2B10 phenotype by genetic score were significant ($p<0.001$, Supplemental Table 1). TNE concentrations did not vary by *UGT2B10* genetic score, nor did serum nicotine, or HCOT levels. However, serum cotinine was directly related to *UGT2B10* genotype in both White and African American smokers. Smokers with both variant alleles (risk score 2) had significantly higher serum cotinine levels (327 ng/ml) than smokers with one or no variant alleles, 261 ng/ml and 222 ng/ml, respectively (Table 2, $p<0.001$). The same significant effect was observed regardless of race (Table 2, $p<0.001$). However, a much higher percentage of African Americans carry these variant alleles. Eighty percent of Whites carry neither the splice nor Asp67Tyr variant compared to only 34% of African Americans.

The contribution of the covariants, age, gender, CPD, TNE, *UGT2B10* genetic score, race and CYP2A6 activity to serum cotinine levels are reported in Table 3. The major contributor by far was *UGT2B10* genetic score. In an unadjusted regression model, a difference in genetic score of 0 compared to 2 results in a 43% increase in the geometric mean cotinine concentration. This increase is unchanged when the model is adjusted for the other 6 variables. A 1 point difference in *UGT2B10* genotype score results in a 19% increase in the geometric mean of serum cotinine (Supplemental Table 2). In comparison, a difference of 5 CPD results in a 9% increase in the geometric mean. CYP2A6 activity, the ratio of HCOT to cotinine in serum, referred to as the nicotine metabolite ratio (NMR), had a modest effect on the serum cotinine point estimate (a 6% decrease for a difference of 0.1). The geometric mean NMR was 0.25 in African Americans and 0.38 in Whites (Table 1). After adjustment for *UGT2B10* genetic score and the other variables in the model there is no significant difference in serum cotinine by race (Table 3, $p = 0.116$).

As in prior studies, the percentage of TNE excreted in the urine as total cotinine was significantly lower in smokers with a genetic score of 2 versus 0 (Table 4, 10.5% versus 19%, $p<0.001$). In contrast, the percent of free cotinine excreted increases. Total nicotine excreted was also significantly lower in smokers with a genetic score of 2 compared to 0, due to the lack of nicotine glucuronide in smokers who do not produce functional UGT2B10. The observed variation in cotinine and total cotinine with *UGT2B10* genetic score significantly influenced two urinary measures of CYP2A6 activity, the ratio of total HCOT to total cotinine, and the ratio of total HCOT to cotinine. However, if these ratios are adjusted for race, *UGT2B10* genetic score does not significantly affect the ratio of total HCOT to cotinine (Table 4, $p = 0.955$). When the data in Table 4 is stratified by race, the ratio of total HCOT to total cotinine (but not the ratio of total HCOT to cotinine) varies

significantly by *UGT2B10* genetic score (Supplemental Table 3). Similarly, the serum NMR stratified by race was not significantly affected by *UGT2B10* genotype (Table 2 and Supplemental Table 1).

Discussion

Serum cotinine levels are higher in African American smokers, NRT (nicotine replacement therapy) users, and non-smokers exposed to tobacco smoke than they are in Whites with similar exposures to nicotine (13, 14, 19, 20). It was recognized by Benowitz *et al.* more than 20 years ago that a significant subset of African Americans glucuronidate cotinine much less efficiently than do Whites (6). The enzyme responsible for the glucuronidation of cotinine in smokers is UGT2B10 (10, 11), and smokers homozygous for either of two SNPs in the *UGT2B10* gene excrete no cotinine glucuronide (4). In the study reported here we confirm that these two SNPs, an Asp 67Tyr variant and a splice variant are by far the greatest contributors to serum cotinine concentrations, independent of race. The high frequency of the *UGT2B10* splice variant in African Americans explains much of the higher cotinine concentrations in these smokers compared to White smokers. CYP2A6 activity contributes a much smaller amount to serum cotinine levels. A 0.1 unit decrease in the NMR results in a 6% increase in serum cotinine, whereas smokers who carry two variant *UGT2B10* alleles have a 43% higher cotinine level than those smokers who carry no variant allele. The magnitude of the effect of UGT2B10 genotype is striking given that an increase of 5 CPD or 20 nmol/ml TNE results in only a 9% increase on serum cotinine. Race (African American versus White) has no additional influence on geometric mean serum cotinine levels when adjusted for age, gender, CPD, TNE, *UGT2B10* genotype and NMR.

We reported a relationship between the Asp67Tyr *UGT2B10* variant and serum cotinine levels more than 10 years ago (3). The study was carried out prior to the characterization of the *UGT2B10* splice variant, but only 8% of the participants were African American (87% were White). The prevalence of the Asp 67Tyr variant was 10.6%, similar to that reported here. There were no homozygous variant individuals. The mean serum cotinine concentration of *UGT2B10* heterozygous individuals was 20% higher than in smokers who did not carry the variant allele, even though the mean TNE level of the heterozygous smokers was about 15% lower. In a GWAS, plasma/serum cotinine levels were significantly associated with a SNP in the region of *UGT2B10* that was in high linkage with the Asp67Tyr variant (21). The minor allele was associated with an increase in cotinine of 39 ng/ml (mean cotinine levels were about 180 ng/ml). The data in these two studies are consistent with the magnitude of the increase in serum cotinine we attributed to *UGT2 B10* genotype, 19% higher in smokers with one variant allele. In addition more recently, *UGT2B10* genotype was confirmed to have a significant impact on cotinine pharmacokinetics in African American smokers (22).

We observed here, as we have in previous studies, that urinary cotinine levels account for a significantly higher percentage of the total TNE excreted by smokers who carry either the *UGT2B10* splice variant or the Asp67Tyr variant (3, 4). Therefore, urinary cotinine levels, like plasma levels, may overestimate tobacco and nicotine exposure. In contrast, total cotinine levels might underestimate exposures in these smokers, since the percentage of

TNE excreted as total cotinine by these smokers was lower than in smokers who do not carry these *UGT2B10* variants. The percentage of TNE excreted as HCOT was higher in smokers with 2 variant *UGT2B10* alleles (genetic score 2) than in those with either one or no variants, but the difference was not significant. This change in the distribution of cotinine and its metabolites by *UGT2B10* genotype resulted in a significant difference in the ratio of total HCOT to total cotinine, which has been used as a measure of CYP2A6 activity. This difference remains significant after adjustment by race. Using this urinary metabolite ratio might result in smokers with *UGT2B10* variants being misclassified as having high CYP2A6 activity. After adjustment by race or stratification by race the urinary ratio of total HCOT to cotinine was not significantly different by *UGT2B10* genetic score. The plasma NMR ratio was also unaffected by *UGT2B10* genotype when stratified by race.

In many clinical, epidemiological and genome wide association studies serum cotinine levels are used to access the relationship of tobacco use to the outcome of interest, be that tobacco dependence or disease risk (1). However, as reported here for UGT2B10 genotype and previously for cotinine glucuronidation phenotype, the serum concentration of cotinine is significantly impacted by UGT2B10 activity (7). In contrast, there is not a significant association of TNE with either UGT2B10 genotype or activity (7, 16). We and others have reported higher TNE in AA smokers compared to Whites at similar levels of CPD (4, 23). The higher TNE levels, likely a reflection of increased smoking intensity, appear to explain much of the reported increased lung cancer risk in AA compared to Whites at relatively low CPD (24). Serum cotinine has also been related to lung cancer risk, and in many studies is a useful measure of tobacco exposure (25). However, in some populations with a high prevalence of UGT2B10 variants cotinine levels will be confounded by UGT2B10 genotype, which if not controlled for could lead to misinterpretation of the data in these studies.

In summary, the data from this study support the conclusion that *UGT2B10* genotype is a major contributor to cotinine concentrations in smokers, and explains the vast majority of the relatively high cotinine values found in African American smokers. Mean cotinine concentration is predicted to be significantly higher in smokers with no functional UGT2B10. Specifically, we found that smokers with comparable smoking levels who do not metabolize cotinine will have on average 43% higher levels of serum cotinine. Due to the high prevalence of the *UGT2B10* splice variant in African Americans, 15% of African American compared to 1% of White smokers will not glucuronidate cotinine. Therefore, this ethnic/racial group will have higher mean cotinine levels at similar levels of tobacco exposure. Based on this *UGT2B10* genotype study, as well as our prior UGT2B10 phenotype study (7) and a pharmacokinetic study by other investigators (22), we conclude that the tobacco exposure biomarker cotinine will greatly overestimate exposure in African Americans compared to other ethnic/racial groups if *UGT2B10* genotype (or activity) is not taken into account.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Research reported in this publication was supported by the National Institute on Drug Abuse and FDA Center for Tobacco Products (CTP) (U54 DA031659 to E.C. Donny, D.K. Hatsukami, and S.E. Murphy). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or the Food and Drug Administration.

Abbreviations

TNE	Total nicotine equivalents
HCOT	*trans* 3′-hydroxycotinine
CPD	cigarettes per day
NMR	nicotine metabolite ratio

References

1. Benowitz NL, Bernert JT, Foulds J, Hecht SS, Jacob P, Jarvis MJ, et al. Biochemical verification of tobacco use and abstinence: 2019 update. Nicotine Tob Res 2019;doi: 10.1093/ntr/ntz132. [Epub ahead of print].
2. Zhu AZ, Renner CC, Hatsukami DK, Swan GE, Lerman C, Benowitz NL, et al. The ability of plasma cotinine to predict nicotine and carcinogen exposure is altered by differences in CYP2A6: the influence of genetics, race, and sex. Cancer Epidemiol Biomarkers Prev 2013;22:708–18. [PubMed: 23371292]
3. Berg JZ, von Weymarn LB, Thompson ET, Wickham KM, Weisensel NA, Hatsukami DK, et al. UGT2B10 genotype influences nicotine glucuronidation, oxidation and consumption. Cancer Epidemiol Biomarkers Prev 2010;19:1423–31. [PubMed: 20501767]
4. Murphy SE, Park SS, Thompson EF, Wilkens LR, Patel Y, Stram DO, et al. Nicotine N-glucuronidation relative to N-oxidation and C-oxidation and UGT2B10 genotype in five ethnic/racial groups. Carcinogenesis 2014;35:2526–33. [PubMed: 25233931]
5. Perez-Stable EJ, Herrera B, Jacob P III, Benowitz NL. Nicotine metabolism and intake in black and white smokers. JAMA 1998;280:152–6. [PubMed: 9669788]
6. Benowitz NL, Perez-Stable EJ, Fong I, Modin G, Herrera B, Jacob P III. Ethnic differences in N-glucuronidation of nicotine and cotinine. J Pharmacol Exp Ther 1999;291:1196–203. [PubMed: 10565842]
7. Murphy SE, Sipe CJ, Choi K, Raddatz LM, Koopmeiners JS, Donny EC, et al. Low cotinine glucuronidation results in higher serum and saliva cotinine in African American compared to White smokers. Cancer Epidemiol Biomarkers Prev 2017;26:1093–9. [PubMed: 28264876]
8. Hukkanen J, Jacob P III, Benowitz NL. Metabolism and disposition kinetics of nicotine. Pharmacol Rev 2005;57:79–115. [PubMed: 15734728]
9. von Weymarn LB, Retzlaff C, Murphy SE. CYP2A6 and CYP2A13-catalyzed metabolism of the nicotine delta 1′(5′) iminium ion. J Pharmacol Exp Ther 2012;343:307–15. [PubMed: 22869927]
10. Chen G, Blevins-Primeau AS, Dellinger RW, Muscat JE, Lazarus P. Glucuronidation of nicotine and cotinine by UGT2B10: loss of function by the UGT2B10 codon 67 (Asp>Tyr) polymorphism. Cancer Res 2007;67:9024–9. [PubMed: 17909004]
11. Kaivosaari S, Toivonen P, Hesse LM, Koskinen M, MH C, Finel M. Nicotine glucuronidation and the human UDP-glucuronosyltransferase UGT2B10. Mol Pharmacol 2007;72:761–8. [PubMed: 17576790]
12. Scherer G, Engl J, Urban M, Gilch G, Janket D, Riedel K. Relationship between machine-derived smoke yields and biomarkers in cigarette smokers in Germany. Regul Toxicol Pharmacol 2007;47:171–83. [PubMed: 17034917]

13. Wagenknecht LE, Cutter GR, Haley NJ, Sidney S, Manolio TA, Hughes GH, et al. Racial differences in serum cotinine levels among smokers in the Coronary Artery Risk Development in (Young) Adults study. Am J Public Health 1990;80:1053–6. [PubMed: 2382740]
14. Berg JZ, Mason J, Boettcher AJ, Hatsukami DK, Murphy SE. Nicotine metabolism in African Americans and European Americans: variation in glucuronidation by ethnicity and UGT2B10 haplotype. J Pharmacol Exp Ther 2010;332:202–9. [PubMed: 19786624]
15. Fowler S, Kletzl H, Finel M, Manevski N, Schmid P, Tuerck D, et al. A UGT2B10 splicing polymorphism common in African populations may greatly increase drug exposure. J Pharmacol Exp Ther 2015;352:358–67. [PubMed: 25503386]
16. Patel YM, Stram DO, Wilkens LR, Park SS, Henderson BE, Le Marchand L, et al. The contribution of common genetic variation to nicotine and cotinine glucuronidation in multiple ethnic/racial populations. Cancer Epidemiol Biomarkers Prev 2015;24:119–27. [PubMed: 25293881]
17. Hatsukami DK, Luo X, Jensen JA, al'Absi M, Allen SS, Carmella SG, et al. Effect of immediate vs gradual reduction in nicotine content of cigarettes on biomarkers of smoke exposure: A randomized clinical trial. JAMA 2018;320:880–91. [PubMed: 30193275]
18. Murphy SE, Wickham KM, Lindgren BR, Spector LG, Joseph A. Cotinine and trans 3′-hydroxycotinine in dried blood spots as biomarkers of tobacco exposure and nicotine metabolism. J Expo Sci Environ Epidemiol 2013;23:513–8. [PubMed: 23443235]
19. Benowitz NL, Bernert JT, Caraballo RS, Holiday DB, Wang J. Optimal serum cotinine levels for distinguishing cigarette smokers and nonsmokers within different racial/ethnic groups in the United States between 1999 and 2004. Am J Epidemiol 2009;169:236–48. [PubMed: 19019851]
20. Pirkle JL, Bernert JT, Caudill SP, Sosnoff CS, Pechacek TF. Trends in the exposure of nonsmokers in the U.S. population to secondhand smoke: 1988–2002. Environ Health Perspect 2006;114:853–8. [PubMed: 16759984]
21. Ware JJ, Chen X, Vink J, Loukola A, Minica C, Pool R, et al. Genome-wide meta-analysis of cotinine levels in cigarette smokers identifies locus at 4q13.2. Sci Rep 2016;6:20092. [PubMed: 26833182]
22. Taghavi T, St Helen G, Benowitz NL, Tyndale RF. Effect of UGT2B10, UGT2B17, FMO3, and OCT2 genetic variation on nicotine and cotinine pharmacokinetics and smoking in African Americans. Pharmacogenet Genomics 2017;27:143–54. [PubMed: 28178031]
23. Roethig HJ, Munjal S, Feng S, Liang Q, Sarkar M, Walk RA, et al. Population estimates for biomarkers of exposure to cigarette smoke in adult U.S. cigarette smokers. Nicotine Tob Res 2009;11:1216–25. [PubMed: 19700523]
24. Stram DO, Park SL, Haiman CA, Murphy SE, Patel Y, Hecht SS, et al. Racial/ethnic differences in lung cancer incidence in the Multiethnic Cohort Study: An update. J Natl Cancer Inst 2019;111:811–9. [PubMed: 30698722]
25. Boffetta P, Clark S, Shen M, Gislefoss R, Peto R, Andersen A. Serum cotinine level as predictor of lung cancer risk. Cancer Epidemiol Biomarkers Prev 2006;15:1184–8. [PubMed: 16775179]

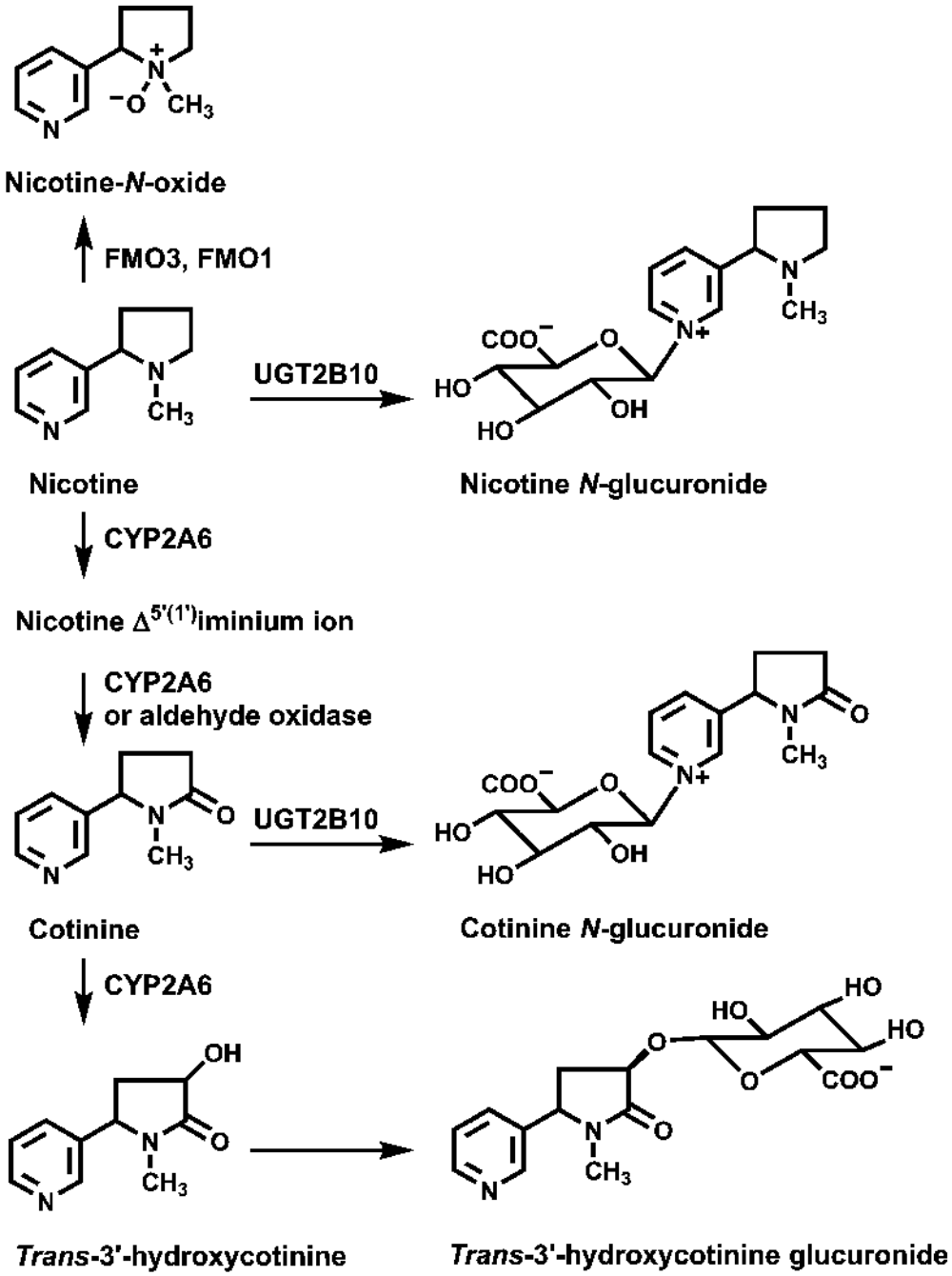

Fig. 1.
Major pathways of nicotine metabolism

Table 1.

Demographics and biomarkers of nicotine exposure and metabolism[a].

Variable		African Americans (n = 289)	Whites (n = 627)
Age (yrs)		47.3 (11.1)	45.2 (14.5)
Sex			
	Women	122 (42%)	280 (45%)
	Men	167 (58%)	347 (55%)
BMI (kg/m^2)		30.9 (7.5)	28.9 (6.3)[c]
CPD		13.6 (6.4)	19.4 (9.1) [c]
TNE (nmol/ml)			
Mean (SD)		74.1 (48.1)	81.7 (54.5)[d]
Geometric mean (min, max)		60.5 (11.7, 298.8)	66.8 (10.7, 329.2)[d]
Serum Cotinine (ng/ml)			
Mean (SD)		302 (157.1)	258 (124.4) [c]
Geometric mean (min, max)		260 (44.2, 1045.4)	229 (26, 854.9)[d]
Serum HCOT/Cotinine[1]			
Mean (SD)		0.3 (0.21)	0.44 (0.24) [c]
Geometric mean (min, max)		0.25 (0, 1.98)	0.38 (0.01, 1.95) [c]
UGT2B10 phenotype[b]		2.03 (1.03)	3.04 (1.53) [c]
UGT2B10 genotype allele frequency	rs61750900 - T allele	4.7%	10.5%
	rs2942857 - C allele	36.2%	0.4%

[a]Values in parentheses are standard deviations unless described otherwise. Abbreviations: BMI, body mass index, CPD, cigarettes per day, TNE, total nicotine equivalents, HCOT, *trans* 3′-hydroxycotinine

[b]The ratio of urinary total cotinine/cotinine

[c]Significantly different between African Americans and Whites p<0.001

[d]Significantly different between African Americans and Whites p<0.05

Table 2.

Geometric means of UGT2B10 phenotype and biomarkers of nicotine exposure stratified by UGT2B10 genetic score[a,b].

	UGT2B10 Phenotype[c]	TNE (nmol/ml)	Serum Nicotine (ng/ml)	Serum Cotinine (ng/ml)	Serum HCOT (ng/ml)	Serum HCOT/cotinine
All Participants (n=916)						
Score = 2 (n = 54)	1.02	61.6	11.0	327	87.1	0.24
Score = 1 (n = 265)	1.95	62.2	11.5	261	85.3	0.3
Score = 0 (n = 597)	2.88	66.2	11.5	222	86.1	0.36
Non-Hispanic Whites (n=627)						
Score = 2 (n = 9)	1.02	75.3	9.45	325	145	0.41
Score = 1 (n = 119)	2.15	64.6	11.8	255	102	0.37
Score = 0 (n = 499)	2.97	67.2	11.8	221	91.5	0.38
African Americans (n=289)						
Score = 2 (n = 45)	1.02	59.2	11.4	327	78.2	0.22
Score = 1 (n = 146)	1.8	60.3	11.3	266	73.5	0.25
Score = 0 (n = 98)	2.44	61.5	9.88	225	62.2	0.25

[a] Genetic score 0, rs61750900 GG and rs116294140 AA; score 1, rs61750900 GT or rs116294140 CA; score 2, rs61750900 TT or rs116294140 CC, or both rs61750900 GT and rs116294140 CA

[b] p values for the comparison of biomarkers between groups is provided in Supplemental Table 1

[c] ratio of total urinary cotinine to cotinine

Table 3.

Determinants of serum cotinine

Covariate	Ratio of Geometric Means: Unadjusted[a] Point Estimate	p-value	Ratio of Geometric Means: Adjusted[b] Point Estimate	p-value	Ratio of Geometric Means: Adjusted[c] Point Estimate	p-value
Age (5 yr difference)	1.03 (1.01, 1.04)[d]	< 0.001	1.04 (1.03, 1.05)	< 0.001	1.03 (1.02, 1.04)	< 0.001
Gender = male	1.07 (0.99, 1.14)	0.086	0.94 (0.89, 1)	0.042	0.99 (0.93, 1.05)	0.718
CPD (5 CPD difference)	1.09 (1.07, 1.11)	< 0.001	1.09 (1.07, 1.11)	< 0.001	1.09 (1.07, 1.11)	< 0.001
‘ (20 nmol difference)	1.09 (1.08, 1.11)	< 0.001	1.1 (1.09, 1.11)	< 0.001	1.09 (1.08, 1.11)	< 0.001
UGT2B10 genetic score (2 point difference)	1.43 (1.27, 1.61)	< 0.001	1.43 (1.29, 1.58)	< 0.001	1.45 (1.3, 1.62)	< 0.001
Race = white	0.88 (0.81, 0.95)	0.001	0.94 (0.88, 1.01)	0.116	0.87 (0.81, 0.94)	< 0.001
NMR (difference of 0.1)	0.95 (0.93, 0.96)	< 0.001	0.94 (0.93, 0.95)	< 0.001		

[a] Covariate is only variable in regression model

[b] Values are adjusted for all other covariates

[c] Adjusted for all other covariates but removing NMR from the model

[d] Values in parenthesis are 95% CI

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2021 February 01.

Table 4.

Urinary nicotine and nicotine metabolites as percentage of total nicotine equivalence and urinary CYP2A6 phenotype stratified by UGT2B10 genotype[a].

	Genetic Score					
	0	1	2	p-value	p-value[b]	p-value[c]
Total Nicotine	7 (6.5, 7.5)	6 (5.4, 6.8)	4.3 (3.3, 5.8)	< 0.001	< 0.001	< 0.001
Nicotine *N*-oxide	3 (2.8, 3.3)	3.2 (2.8, 3.5)	3.6 (2.8, 4.5)	0.203	0.195	0.68
Total cotinine	19 (18, 20.1)	14.4 (13.3, 15.5)	10.5 (9, 12.3)	< 0.001	< 0.001	< 0.001
Free cotinine	6.6 (6.3, 6.9)	7.4 (6.8, 7.9)	10.3 (8.9, 12)	< 0.001	< 0.001	< 0.001
Total 3′-hydroxycotinine	31.5 (29.4, 33.8)	32.2 (28.8, 36)	37.9 (30.5, 47.1)	0.235	0.214	0.009
Total 3′-hydroxycotinine /total cotinine	1.7 (1.6, 1.7)	2.2 (2.1, 2.4)	3.6 (3, 4.3)	< 0.001	< 0.001	< 0.001
Total 3′-hydroxycotinine /cotinine	4.8 (4.5, 5.1)	4.4 (4, 4.8)	3.7 (3.1, 4.4)	0.008	0.009	0.955

[a] Values are geometric means (95% confidence interval), p for trend

[b] Adjusted for age and gender

[c] Adjusted for age, gender, and race