**UGT1A and UGT2B Genetic Variation Alters Nicotine and Nitrosamine Glucuronidation in European and African American Smokers**


**Abstract**

**Background:** Identifying sources of variation in the nicotine and nitrosamine metabolic inactivation pathways is important to understanding the relationship between smoking and cancer risk. Numerous UGT1A and UGT2B enzymes are implicated in nicotine and nitrosamine metabolism *in vitro*; however, little is known about their roles *in vivo*.

**Methods:** Within UGT1A1, UGT1A4, UGT1A9, UGT2B7, UGT2B10, and UGT2B17, 47 variants were genotyped, including UGT2B10* 2* and UGT2B17* 2*. The association between variation in these UGTs and glucuronidation activity within European and African American current smokers (*n = 128*), quantified as urinary ratios of the glucuronide over unconjugated compound for nicotine, cotinine, trans-3′-hydroxycotinine, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNAL), was investigated in regression models assuming a dominant effect of variant alleles.

**Results:** Correcting for multiple testing, three UGT2B10 variants were associated with cotinine glucuronidation, rs2331559 and rs11726322 in European Americans and rs835309 in African Americans (*P ≤ 0.0002*). Additional variants predominantly in UGT2B10 were nominally associated with nicotine (*P = 0.008–0.04*) and cotinine (*P = <0.001–0.02*) glucuronidation in both ethnicities in addition to UGT2B10* 2* in European Americans (*P = 0.01, P < 0.001*). UGT2B17* 2* (*P = 0.03*) in European Americans and UGT2B7 variants (*P = 0.02–0.04*) in African Americans were nominally associated with NNAL glucuronidation.

**Conclusions:** Findings from this initial *in vivo* study support a role for multiple UGTs in the glucuronidation of tobacco-related compounds *in vivo*, in particular UGT2B10 and cotinine glucuronidation.

**Impact:** Findings also provide insight into ethnic differences in glucuronidation activity, which could be contributing to ethnic disparities in the risk for smoking-related cancers. *Cancer Epidemiol Biomarkers Prev; 24(1): 94–104. ©2014 AACR.*

**Introduction**

Cigarette smoking is the leading risk factor for lung cancer (1, 2), and is also associated with numerous other cancers including those of the respiratory tract, digestive tract, bladder, pancreas, and kidney (3, 4). Genetic factors are associated with differences in the susceptibility to tobacco-related cancers including genes involved in the metabolism of nicotine and tobacco smoke carcinogens (5–7). For instance, CYP2A6 gene variants are associated with reduced lung cancer risk within smokers of diverse ethnicities (8–13), as is a gene variant in CYP2A13 (14), key enzymes in the nicotine inactivation pathway and in the activation pathway of tobacco-specific nitrosamines (TSNA) such as 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NINN), respectively (15, 16).

UDP-glucuronosyltransferases (UGT) represent another group of enzymes with the potential to influence the relationship between smoking and cancer risk via their contribution to the metabolism of both nicotine and nitrosamines, including NNK (Fig. 1). In addition to the major metabolic pathway of nicotine to cotinine and further to trans-3′-hydroxycotinine (3HC) chiefly mediated by CYP2A6, nicotine, cotinine, and 3HC are also substrates of UGTs (17). Glucuronide conjugates account for 25% to 30% of recovered nicotine metabolites in urine (18–20). NNK is extensively metabolized by carbonyl reduction to 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NNAL), which, like NNK, is also carcinogenic (21, 22). NNAL is then metabolically detoxified by glucuronidation, and the noncarcinogenic glucuronide conjugates account for approximately 60% of NNAL detected in urine (23, 24).
UGT gene variants may have utility in cancer risk prediction—deficient activity of some UGT enzymes enhances susceptibility to chemical carcinogenesis in animals (25, 26), and genetic variants in UGT1A7, UGT1A10, and UGT2B17 are associated with the risk for tobacco-related cancers in humans (27–30). In addition, it is important to understand the impact of variable glucuronidation on biomarkers of nitrosamine exposure and on the ratio of NNAL-glucuronide to NNAL, a biomarker of nitrosamine detoxification (31) and a potential marker of cancer risk (32). Variable glucuronidation could also influence smoking through effects on nicotine clearance and could impact the interpretation of biomarkers of nicotine exposure and metabolism (33–35).

Many polymorphisms have been identified in the genes encoding the UGT1A and UGT2B enzymes (36); however, there are limited data on the impact of UGT variants on nicotine and nitrosamine metabolism in vivo, and in current cigarette smokers (37, 38). In vivo investigations have focused on two alleles, UGT2B10*2 and UGT2B17*2. UGT2B10*2 is associated with impaired nicotine and cotinine glucuronidation, whereas UGT2B17*2 is associated with impaired 3HC and NNAL glucuronidation (20, 27, 34, 35, 39).

In vitro studies of human liver microsomes and expressed UGTs implicate additional UGT1A and UGT2B enzymes in the glucuronidation of nicotine, its metabolites, and NNAL. In human liver microsomes, UGT2B10*2 is associated with reduced glucuronidation of nicotine and cotinine, but also 3HC and NNAL (33, 40, 41). UGT2B17*2 is associated with reduced glucuronidation of 3HC and NNAL (33, 42), and UGT1A4*2 and UGT2B7*2 are associated with reduced glucuronidation of NNAL (42, 43). Inhibition studies in human liver microsomes implicate UGT1A1 in nicotine glucuronidation (44), UGT1A4 in nicotine, cotinine, and 3HC glucuronidation (44–46), and UGT1A9 in nicotine and cotinine glucuronidation (44, 46). Recombinant UGTs from baculovirus-infected insect cells or overexpressed UGTs in human cell lines also implicate UGT1A4 in the glucuronidation of nicotine, cotinine, and NNAL (47, 48) and provide evidence for the involvement of UGT1A9 in the glucuronidation of 3HC and NNAL (45, 49) and for UGT2B7 in the glucuronidation of nicotine and 3HC (45, 47).

Given the data suggesting the involvement of multiple UGT1A and UGT2B enzymes in vitro, and the limited knowledge of the contribution of UGT gene variants to nicotine and nitrosamine glucuronidation in vivo, we investigated the association between variation in UGT1A1, UGT1A4, UGT1A9, UGT2B7, UGT2B10, and UGT2B17, and nicotine, cotinine, 3HC, and NNAL glucuronidation in European and African American current smokers. As few UGT gene variants have been characterized with respect to these substrates, and the functional impact of UGT gene variants can be substrate specific (50), we genotyped 43 tag SNPs within the candidate genes. We also genotyped two UGT1A9 insertion/deletion variants and, importantly, UGT2B10*2 and UGT2B17*2, the only alleles previously associated with impaired in vivo glucuronidation in smokers (20, 35, 39). To gauge the suitability of the dataset for genetic association analyses of urinary metabolite ratios, we genotyped participants for altered activity CYP2A6 variants to confirm the well-established association between CYP2A6 and the ratio of 3HC over cotinine, a biomarker for CYP2A6 activity and nicotine clearance (Supplementary Results; ref. 51). In addition to identifying which UGT gene variants influence the glucuronidation of tobacco-related compounds among European Americans, a population in which sources of variation in glucuronidation are better characterized (in vitro studies almost exclusively utilize livers from individuals of European descent), the present study evaluated whether the same UGT variants were associated with glucuronidation among African Americans to provide pilot data focused on understanding the
higher risk of smoking-related lung cancer observed among African Americans compared with European Americans (52, 53).

Materials and Methods

Study description

Participant recruitment and characteristics are detailed elsewhere (54). Briefly, 128 current smokers required to be 18 to 65 years old, healthy, and to have smoked an average of 10 cigarettes per day or more for the past year or longer were recruited for a cross-sectional biomarker study. Subjects had to be self-identified non-Hispanic white (referred to as European American) or African American, with 4 grandparents of the same ethnicity. Smoking measures were collected and the time from smoking the last cigarette before urine sampling was recorded. One European American and one African American participant were excluded from the main genotype–phenotype analysis due to the absence of a urine sample and insufficient UGT genotyping results, respectively. Participant characteristics for the remaining 126 individuals are provided (Table 1). The study was approved by Institutional Review Boards at the University of California San Francisco (San Francisco, CA), the University of Chicago (Chicago, IL), and the University of Toronto (Ontario, Canada).

UGT Genotyping

Tag SNPs were selected within the candidate genes (±10 kb) by determining the minimal set of common SNPs (minor allele frequency ≥5%) that captured the pairwise linkage disequilibrium \( r^2 \) > 0.8) for all common SNPs within the HapMap CEU population. In HapMap2 release 24, the set of SNPs capture (at an \( r^2 \) ≥ 0.8), on average, more than 80% of existing common SNPs within the CEU population, and in the YRI population they capture on average 37% of the existing common SNPs (Supplementary Table S1).

\( UGT2B10^\prime \prime (rs61750900) \) was genotyped using a PCR restriction fragment length polymorphism in which the amplified product was subjected to digestion with HinfI (40). The \( UGT2B17^\prime \) deletion allele was assessed using the TaqMan gene expression assay, HS00854486_sH, with copy number reference assay TaqMan RNase P for the internal control, determined by TaqMan real-time quantitative PCR (55–57). Genotyping for 41 tag SNPs in \( UGT1A4, UGT1A9, UGT2B7, \) and \( UGT2B10 \) was done using the KASP SNP genotyping system by LGC Genomics (formerly KBiosciences, LGC Limited). Genotyping for the \( UGT1A9 \) indels, rs45625337 and rs10538910, was performed using the-in-house GeneScan (PCR-Sizing) assays run on ABI 3700 as described previously (58). Genotyping for \( UGT1A4, 135498 C>A \) (rs6755571) and \( UGT1A4, 135876 T>C \) (rs12468274) was performed using a SNaPshot (Applied Biosystems, Life Technologies) single base extension 2-plex assay as described (59). The extension products were run on an ABI 3130xl (Applied Biosystems), and the data analyzed by the GeneMapper software (Applied Biosystems).

Analytical chemistry

Urine concentrations of nicotine and its metabolites cotinine, 3HC, and their respective glucuronide metabolites were measured by liquid chromatography/tandem mass spectrometry from spot urine with glucuronide conjugates calculated from the difference in total concentration before and after alkaline hydrolysis (nicotine, cotinine) or hydrolysis by \( \beta \)-glucuronidase (3HC, NNAL) as previously described (18, 51, 54, 60). Urine creatinine was measured in the San Francisco General Hospital clinical laboratory using a standard colorimetric assay.

Metabolite phenotypes

To investigate \( UGT \) genotype–phenotype relationships, the urinary ratios of nicotine-glucuronide over nicotine, cotinine-glucuronide over cotinine, 3HC-glucuronide over 3HC, and NNAL-glucuronide over NNAL were used as glucuronidation activity phenotypes (27, 35, 39). The urinary ratio of total 3HC (free and glucuronide conjugated) over free cotinine was used as a phenotype of CYP2A6 oxidative metabolism (51). Nicotine equivalents, a measure of the total intake of nicotine, were calculated as the molar sum of nicotine, cotinine, 3HC, and their glucuronide metabolites in urine corrected for creatinine concentration (61).

Statistical analyses

Comparison of demographic, smoking, and metabolite phenotypes in African versus European Americans was performed by Wilcoxon rank-sum test (continuous variables) or \( x^2 \) (categorical variables). Correlations between metabolite phenotypes were performed on nontransformed values by Spearman. Geometric

### Table 1. Characteristics of participants included in analyses, \( n = 126^{\ast} \)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>European American, ( n = 66 )</th>
<th>African American, ( n = 60 )</th>
<th>( P^{\ast} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, no. (%)</td>
<td>38 (58)</td>
<td>35 (58)</td>
<td>0.93</td>
</tr>
<tr>
<td>Age in years, median (IQR)</td>
<td>34 (25–45)</td>
<td>41 (36–49)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Body mass index, median (IQR)</td>
<td>24 (22–28)</td>
<td>27 (24–33)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Menthol cigarette use, no. (%)</td>
<td>18 (27)</td>
<td>41 (68)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cigarettes/day, median (IQR)</td>
<td>20 (14–20)</td>
<td>15 (10–20)</td>
<td>0.04</td>
</tr>
<tr>
<td>Nicotine equiv. (mg/mg creat)( ^{\ast} ), median (IQR)</td>
<td>63 (54–75)</td>
<td>44 (37–53)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Min from last cig, median (IQR)</td>
<td>80 (50–107)</td>
<td>90 (60–140)</td>
<td>0.08</td>
</tr>
<tr>
<td>Activity ratios (geometric mean, 95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nic-GluCfree Nic</td>
<td>0.33 (0.25–0.44)</td>
<td>0.17 (0.13–0.23)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cot-GluCfree COT</td>
<td>1.03 (0.87–1.23)</td>
<td>0.40 (0.28–0.57)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3HC-GluCfree 3HC</td>
<td>0.19 (0.17–0.22)</td>
<td>0.26 (0.23–0.30)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NNAL-GluCfree NNAL</td>
<td>2.06 (1.87–2.27)</td>
<td>1.85 (1.61–2.12)</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Abbreviation: IQR, interquartile range.

\( ^{\ast} \)128 participants in the original study, 67 European American and 61 African American: One sample dropped due to absence of urinary biomarkers and another due to poor genotyping call rate.

\( ^{\ast} \) \( P \) values: nonparametric Wilcoxon rank-sum test or \( x^2 \).

\( ^{\ast} \) Average of self-reported cigarettes/day in the three days before biomarker collection.

\( ^{\ast} \) Nicotine equivalents: urinary sum of nicotine, cotinine, trans-3-hydroxyxycotinine, and their glucuronide conjugates normalized to creatinine.
mean values are presented for non-normally distributed values unless indicated otherwise. Linkage disequilibrium between the UGT1A and UGT2B variants was assessed using Haploview (62), whereas other statistical analyses were performed using Stata13 (StataCorp). Hardy–Weinberg equilibrium tests were performed using the Stata program hwsnp [Mario A. Cleves, University of Arkansas for Medical Sciences, Little Rock, AR]. Regression association analyses between UGT variants and glucuronidation phenotypes were performed using the Stata program qtlsnp assuming a dominant effect of the variant allele [Mario A. Cleves, University of Arkansas for Medical Sciences, Little Rock, AR]. All regression models were performed separately within each ethnicity and were adjusted for age, gender, and menthol smoking. Because of missing genotypes and/or biomarker data of the individuals, the number of observations within each model ranged from 60 to 66 among European Americans and from 51 to 60 among African Americans. In total 36 UGT variants were tested against four phenotypes in European Americans and 43 UGT variants were tested against four phenotypes in African Americans, hence the significance thresholds were set at \( P < 0.0003 \) and 0.0002, respectively, as determined by a Bonferroni correction for multiple testing. Nominally significant associations are also presented \( (P \leq 0.05). \)

**Results**

**Glucuronidation phenotypes**

The distribution of each of the four glucuronide phenotype ratios by ethnicity was assessed (Fig. 2A–D). African Americans had lower nicotine and cotinine glucuronide ratios compared with European Americans, as reported previously (39, 63). 3HC glucuronide ratios were higher among African Americans compared with European Americans, whereas no ethnic differences in NNAL glucuronide ratios were observed. Within-subject nicotine and cotinine glucuronide ratios were correlated in each ethnic group, but neither was correlated with the 3HC glucuronide ratio (Table 2), consistent with previous studies using 24-hour urine (18, 39). The NNAL glucuronide ratio was correlated with the cotinine glucuronide ratio and with the 3HC glucuronide ratio in each ethnicity (Table 2).

Demographic characteristics and urinary sampling parameters were evaluated as potential covariates of the glucuronide ratios. Demographic characteristics differed by ethnicity—African Americans were older \( (P < 0.01) \), had a higher BMI \( (P < 0.01) \), reported fewer cigarettes smoked per day \( (P = 0.04) \), and a greater prevalence of menthol cigarette use \( (P < 0.01; \text{Table 1}) \). Correlations between glucuronide ratios and time from last cigarette, creatinine

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**Figure 2.** Glucuronidation phenotypes among European Americans and African Americans expressed as the logarithm transformed ratio of glucuronide conjugate over free nicotine in A, cotinine in B, trans-3-hydroxycotinine in C, and NNAL in D. Dotted lines indicate mean values of logarithm transformed phenotypes (refer to Table 1 for nontransformed means). \( P \) values, A–D, from nonparametric Wilcoxon rank-sum test comparing the phenotype distribution by ethnicity.

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concentration, and nicotine equivalents were also assessed, as metabolites were quantified from spot urine. No consistent patterns emerged between glucuronide ratios and age, BMI, creatinine, nicotine equivalents, or time from last cigarette (Supplementary Table S2). No differences in glucuronide ratios by gender were noted among either ethnicity, but 3HC and NNAL glucuronide ratios were lower among African American menthol versus nonmenthol smokers (Supplementary Table S3). Menthol is glucuronidated by enzymes such as UGT2B7 and UGT2B17 (64, 65), which are also capable of glucuronidating 3HC and NNAL (33, 42); thus, menthol could act as a competitive inhibitor, and menthol was included as a covariate in genotype–phenotype analyses. Age and gender were also included as covariates due to a priori evidence that these variables may influence the activity of specific UGTs (37, 66–68).

**UGT genotyping results**

The UGT2B10*2 allele, rs61750900T, had a minor allele frequency of 9% and 4% among European and African Americans, respectively, and the UGT2B17*2 allele had a minor allele frequency of 30% and 21% among European and African Americans, respectively, consistent with published frequencies (27, 35, 39). Among European Americans, rs3771342 and rs835310, and among African Americans, rs12468274 and rs12468543, were not consistent with Hardy–Weinberg Equilibrium and were excluded. Among European Americans, 3 variants in the UGT1A

| Table 2. Spearman correlations between glucuronidation activity measures (nontransformed) |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|
|                               | NIC-Gluc ratio  | COT-Gluc ratio  | 3HC-Gluc ratio  | NNAL-Gluc ratio  |
| EA                            | 0.49, <0.001    | -0.20, 0.13     | 0.19, 0.15      | AA, P = 0.0002  |
| COT-Gluc ratio                | 0.34, 0.005     | -0.05, 0.71     | 0.56, <0.001    |                 |
| 3HC-Gluc ratio                | 0.10, 0.41      | 0.15, 0.31      |                 |                 |
| NNAL-Gluc ratio               | 0.04, 0.76      | 0.34, 0.007     | 0.38, 0.002     |                 |

**NOTE:** Data for EA are in lower left side; AA are in upper right side (shaded).

Abbreviations: EA, European American; AA, African American.

Figure 3. UGT2B10 variants significantly associated with cotinine glucuronidation following correction for multiple testing. Individuals heterozygous or homozygous for the minor alleles of rs2331559 in A and rs11726322 in B had lower cotinine glucuronide ratios, whereas individuals heterozygous or homozygous for the minor allele of rs835309 in C had higher cotinine glucuronide ratios. Genotype–phenotype relationships illustrated with box plots displaying the median value and interquartile range (25th to the 75th percentile) and whiskers displaying the upper and lower values within 1.5 times the interquartile range, and open circles displaying outlying individuals. P values from multivariate logistic regression modeling assuming a dominant effect of the minor allele. EA: European American; AA: African American.
locus and 6 variants in the UGT2B locus displayed high linkage disequilibrium (R² > 0.9; Supplementary Fig. S1A and S2A, respectively). Among African Americans, no variants in the UGT1A locus and only 2 variants in the UGT2B locus displayed high linkage disequilibrium (Supplementary Fig. S1B and S2B, respectively). Variants in high linkage disequilibrium were also excluded leaving 36 and 43 UGT variants in subsequent genotype-phenotype analyses in European and African Americans, respectively (Supplementary Table S4).

**Glucuronidation associations**

Three UGT2B10 gene variants were statistically significantly associated with cotinine glucuronidation (Fig. 3) following correction for multiple testing (outlined in statistical methods). Among European Americans, individuals heterozygous or homozygous for the minor alleles of rs2331559 and rs11726322 had significantly lower cotinine glucuronide ratios compared with those homozygous for the major alleles (Fig. 3A and B). Among African Americans, individuals heterozygous or homozygous for the minor allele of rs835309 had significantly higher cotinine glucuronide ratios compared with those homozygous for the major allele (Fig. 3C). Nominally significant associations are also reported in the following sections, as this was the first investigation of multiple candidate genes chosen based on examination of the contribution of multiple candidate genes in the glucuronidation of nicotine and nitrosamines in smokers.

**Nicotine glucuronidation**

Two UGT2B10 variants were nominally associated with impaired nicotine glucuronidation activity in European Americans, whereas in African Americans, two UGT2B10 variants were nominally associated with enhanced and two with impaired activity (Table 3). A variant in the UGT1A4 locus and two in the UGT1A1 locus were also nominally associated with nicotine glucuronidation in African Americans.

**Cotinine glucuronidation**

In addition to the two UGT2B10 variants significantly associated with impaired cotinine glucuronidation (Fig. 3A and B), UGT2B10*2 was nominally associated with impaired cotinine glucuronidation activity, whereas a single variant in UGT2B7 was nominally associated with enhanced glucuronidation in European Americans (Table 4). Among African Americans, in addition to the UGT2B10 variant significantly associated with enhanced cotinine glucuronidation (Fig. 3C), one other UGT2B10 variant was nominally associated with enhanced and two with impaired activity (Table 4). Two variants in the UGT1A4 locus and single variants in the common UGT1A exons and 3’ flanking region of UGT1A were also associated with cotinine glucuronidation in African Americans (Table 4).

**3HC glucuronidation**

Among European Americans, the UGT2B17 copy number variant was nominally associated with impaired 3HC glucuronidation activity (Table 5). Among African Americans, two variants in the UGT2B7 locus were nominally associated with impaired 3HC glucuronidation activity (Table 5).

**NNAL glucuronidation**

No UGT variants reached nominal significance with NNAL glucuronidation activity among European Americans (Table 6). Among African Americans, two UGT2B7 variants were nominally associated with NNAL glucuronidation, one with enhanced, and one with impaired activity (Table 6). Single variants in UGT1A1, the 3’ flanking region of UGT1A, and UGT2B10 were also associated with NNAL glucuronidation in African Americans (Table 6).

**Discussion**

This is the first study to investigate variation in multiple candidate UGT1A and UGT2B genes and glucuronidation activity within the nicotine and nitrosamine metabolic pathways among European and African American current smokers. Before this investigation, only UGT2B10*2 and UGT2B17*2 had been shown to influence the glucuronidation of tobacco-related compounds in vitro (20, 27, 34, 35, 39); whereas additional UGT1A and UGT2B enzymes were implicated in these pathways in vitro (33, 40–42, 44–49). As few UGT variants have been functionally characterized with respect to our substrates of interest, we focused this initial investigation on tag SNPs to provide a more comprehensive examination of the contribution of UGT genetic variation to nicotine and nitrosamine metabolism.

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**Table 3. UGT variants nominally associated with the nicotine glucuronide ratio**

<table>
<thead>
<tr>
<th>UGT variant</th>
<th>European American smokers</th>
<th></th>
<th>African American smokers</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alleles</td>
<td>European American smokers</td>
<td></td>
<td>Alleles</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UGT1A4 locus</td>
<td>rs13401281</td>
<td>T:G</td>
<td>0.409</td>
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<tr>
<td>UGT1A1 locus</td>
<td>rs6742078</td>
<td>G:T</td>
<td>0.408</td>
<td>G:T</td>
</tr>
<tr>
<td></td>
<td>rs3771342*</td>
<td>C:A</td>
<td>0.112</td>
<td>C:A</td>
</tr>
<tr>
<td>UGT2B10 locus</td>
<td>rs61750900 (‘2)</td>
<td>G:T</td>
<td>0.090</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>rs2331559</td>
<td>G:C</td>
<td>0.119</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>rs835309</td>
<td>G:T</td>
<td>0.106</td>
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</tr>
<tr>
<td></td>
<td>rs11726322</td>
<td>G:C</td>
<td>0.129</td>
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</tr>
<tr>
<td></td>
<td>rs7673996</td>
<td>C:T</td>
<td>0.031</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Abbreviation: MAF, minor allele frequency.

*Variant excluded from analyses in European Americans.

*P value not reported in European Americans as variant in high linkage disequilibrium with rs2331559. Alleles indicated as major:minor with impaired allele in bolded italics and an arrow indicating direction of effect for the minor allele. P** and coefficient with 95% confidence intervals from multivariate logistic regression modeling assuming a dominant effect of the minor allele and reported when P < 0.05. Variants ordered by location along chromosome 2 (UGT1A) and chromosome 4 (UGT2B).
variation in the glucuronidation of tobacco-related compounds in European Americans. We also examined whether these tag SNPs chosen in European Americans were associated with glucuronidation among African American smokers.

Our findings confirm an important contribution of genetic variation in UGT2B10 to nicotine and cotinine glucuronidation with multiple UGT2B10 variants nominally associated with nicotine and with cotinine glucuronidation in both ethnicities. Three variants in UGT2B10 remained statistically significantly associated with cotinine glucuronidation following correction for multiple testing. Of note, the two variants significantly associated with cotinine glucuronidation in European Americans, rs2331559 and rs1726322, had similar effect sizes to nicotine glucuronidation in European Americans, rs2331559 and rs835310, respectively. On the other hand, the variant nominally associated with nicotine glucuronidation in African Americans, rs11726322, had similar effect sizes to cotinine glucuronidation in European Americans, rs2331559 and rs835310.

In addition to UGT2B10, we observed nominal associations between variants in UGT1A1, UGT1A4, and UGT2B7 and nicotine and/or cotinine glucuronidation. Consistent with inhibition studies of UGT1A1 in human liver microsomes, which demonstrate an impact on nicotine but not cotinine glucuronidation (44), UGT1A1 was only associated with the nicotine glucuronide ratio. UGT1A4 is implicated in the glucuronidation of nicotine and cotinine in vitro and is considered to be the second most active UGT in the N-glucuronidation of these compounds after UGT2B10 (44, 46, 47). We observed associations between UGT1A4 variants and both the nicotine and cotinine glucuronide ratios potentially reflecting the minor contribution of this enzyme. UGT2B7 is capable of nicotine glucuronidation in vitro (47); however, the nominal association that we report is between UGT2B7 and cotinine glucuronidation and may represent a chance finding.

As the nicotine and cotinine glucuronide ratios were correlated, we anticipated that the same UGT variants might be associated with both glucuronidation phenotypes. While we observed overlap in variants associated with both pathways, particularly in UGT2B10, more UGT variants were associated with the cotinine glucuronide ratio.
Table 6. UGT variants nominally associated with the NNAL glucuronide ratio

<table>
<thead>
<tr>
<th>UGT1A1 locus</th>
<th>Alleles</th>
<th>MAF</th>
<th>Coef (95% CI)</th>
<th>Alleles</th>
<th>MAF</th>
<th>Coef (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3771342a</td>
<td>C:A</td>
<td>0.112</td>
<td></td>
<td>C:A</td>
<td>0.117</td>
<td>0.012</td>
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<tr>
<td>UGT1A3' flanking</td>
<td>A:T</td>
<td>0.431</td>
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<td>A:T</td>
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<td>0.007</td>
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<tr>
<td>rs10203855</td>
<td>C:G</td>
<td>0.023</td>
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<td>C:G</td>
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</tr>
<tr>
<td>rs835310a</td>
<td>A:G</td>
<td>0.162</td>
<td></td>
<td>A:G</td>
<td>0.275</td>
<td>0.030</td>
</tr>
<tr>
<td>UGT2B7 locus</td>
<td>C:T</td>
<td>0.381</td>
<td></td>
<td>C:T</td>
<td>0.397</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Abbreviation: MAF, minor allele frequency.

*Variant excluded from analyses in European Americans. Alleles indicated as major:minor with impaired allele in bolded italics and an arrow indicating direction of effect for the minor allele. P** and coefficient with 95% CIs from multivariate logistic regression modeling assuming a dominant effect of the minor allele and reported when P < 0.05. Varies ordered by location along chromosome 2 (UGT1A) and chromosome 4 (UGT2B).

in the urine of smokers (free or glucuronidated) compared with cotinine may also have hindered genotype-phenotype associations.

The ratio of NNAL-glucuronide to NNAL is a biomarker of nitrosamine detoxification (31) and a potential marker of cancer risk (32). There is conflicting evidence regarding ethnic differences in this ratio, specifically whether it is lower among African Americans (70, 71). We did not observe a significant difference in NNAL glucuronide ratios by ethnicity, while replicating differences in other ratios (Fig. 2). However, 10% of African Americans had ratios below the lowest ratio observed among European Americans potentially putting these individuals at greater risk for cancer, as Chung and colleagues found that lower NNAL glucuronide ratios were associated with an increased risk of cancer (32).

Variation in the UGT2B10 and UGT2B7 loci may be of particular interest in terms of cancer risk disparities, as these variants were only nominally associated with NNAL glucuronidation activity among African Americans.

In addition to altered carcinogen detoxification, variation in UGTs could influence lung cancer indirectly through altered smoking. Berg and colleagues reported significantly lower nicotine equivalents [a measure of nicotine intake] in smokers with the UGT2B10*1/2 genotype in a study of European Americans (34) and in a mixed analysis of European and African American smokers (39), and speculated that slower nicotine glucuronidation may lead to reduced nicotine consumption. However, in a larger study of African American smokers, Zhu and colleagues did not find a significant association between UGT2B10*2 and lower nicotine equivalents (35). Consistent with glucuronidation as a minor pathway for nicotine inactivation (17), and with Zhu and colleagues, neither UGT2B10*2 genotype [data not shown], nor importantly the actual nicotine glucuronidation ratio [Supplementary Table S2], were associated with nicotine equivalents in either European or African Americans.

Many nominally significant associations between UGT variants and glucuronidation phenotypes were observed despite both the relatively small sample size for a genetic association study and the quantification of metabolites from spot urine with variable time from last cigarette. Biomarker assessment from spot urine is unlikely to have biased findings, since we observed similar correlations between glucuronide ratios as reported for 24-hour urine (18, 39). Furthermore, urinary cotinine, NNAL, and nicotine equivalents, which are all biomarkers of nicotine consumption, were correlated as expected [data not shown] and neither nicotine equivalents nor the glucuronide ratios were systemically associated with time from last cigarette (Supplementary Table S2). Only

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one nominally significant variant, UGT1A1 rs3771342, displayed an inconsistent direction of effect with the nicotine and NNAL glucuronidation phenotypes potentially reflecting an indirect genotype–phenotype association or simply a chance observation. The smaller sample size precluded interactions analyses. In particular, the interaction between menthol and UGT2B7 and UGT2B17 gene variants would be worthwhile exploring in a larger dataset given the conflicting evidence concerning menthol smoking and lung cancer risk (reviewed in ref. 72). More associations were observed among African Americans than among European Americans, as is seen in other genomic regions displaying lower linkage disequilibrium in African populations [e.g., chromosome 15q25 and lung cancer risk (73)]. Of note, the gene variants investigated were initially chosen to provide relatively good coverage of European smokers and provide relatively low coverage of common variation in Africans (Supplementary Table S1) suggesting that even more variation may be identified among this ethnic group. Alternatively, a single untested variant may underlie multiple associations observed in a gene region through linkage disequilibrium.

Overall, study findings confirmed a role for multiple UGTs in the glucuronidation of tobacco-related compounds in vivo and contributed to the understanding of sources of variation in the nicotine and nitrosamine metabolic inactivation pathways. Concurrently examining genetic sources of variation in European and African American smokers also provided insight into ethnic differences in glucuronidation activity, which could be contributing to ethnic disparities in the risk for smoking-related cancers.

Disclosure of Potential Conflicts of Interest
M.J. Ratian has ownership interest (including patents) in provisional patent application related to genomic prescribing and royalties related to UGT1A1 genotyping. N.L. Benowitz is a consultant/advisory board member for Pfizer and GlaxoSmithKline, and has provided expert testimony for litigation against tobacco companies. R.F. Tyndale has received speakers' bureau honoraria from university talks, is a consultant/advisory board member for pharmaceutical companies, and has provided expert testimony for Clinical Pharmacology and Therapeutics journal. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: E.H. Cook, M.J. Ratian, N.L. Benowitz, R.F. Tyndale Development of methodology: S. Das, P. Chen, E.H. Cook, N.L. Benowitz Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.A. Wassenaar, S. Das, P. Chen, N.L. Benowitz, R.F. Tyndale Analysis and interpretation of data (e.g., statistical analysis, biostatistics, Computational analysis): C.A. Wassenaar, D.V. Comi, P. Chen, E.H. Cook, N.L. Benowitz Writing, review, and/or revision of the manuscript: C.A. Wassenaar, P. Chen, M.J. Ratian, N.L. Benowitz, R.F. Tyndale Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): N.L. Benowitz Study supervision: E.H. Cook, N.L. Benowitz, R.F. Tyndale

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Cancer Epidemiology, Biomarkers & Prevention

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