Variation in Trans-3′-Hydroxycotinine Glucuronidation Does Not Alter the Nicotine Metabolite Ratio or Nicotine Intake

Andy Z. X. Zhu¹, Qian Zhou¹, Lisa Sanderson Cox², Jasjit S. Ahluwalia³, Neal L. Benowitz⁴, Rachel F. Tyndale¹,⁵*

¹Department of Pharmacology and Toxicology, University of Toronto, Ontario, Canada, ²Department of Preventive Medicine and Public Health, University of Kansas School of Medicine, Kansas City, Kansas, United States of America, ³Department of Medicine and Center for Health Equity, University of Minnesota Medical School, Minneapolis, Minnesota, United States of America, ⁴Division of Clinical Pharmacology and Experimental Therapeutics, Departments of Medicine and Bioengineering & Therapeutic Sciences, University of California San Francisco, San Francisco, California, United States of America, ⁵Campbell Family Mental Health Research Institute, Center for Addiction and Mental Health and Department of Psychiatry, University of Toronto, Ontario, Canada

Abstract

Background: CYP2A6 metabolizes nicotine to its primary metabolic cotinine and also mediates the metabolism of cotinine to trans-3′-hydroxycotinine (3HC). The ratio of 3HC to cotinine (the “nicotine metabolite ratio”, NMR) is an in vivo marker for the rate of CYP2A6 mediated nicotine metabolism, and total nicotine clearance, and has been associated with differences in numerous smoking behaviors. The clearance of 3HC, which affects the NMR, occurs via renal excretion and metabolism by UGT2B17, and possibly UGT2B10, to 3HC-glucuronide. We investigated whether slower 3HC glucuronidation alters NMR, altering its ability to predict CYP2A6 activity and reducing its clinical utility.

Methods: Plasma NMR, three urinary NMRs, three urinary 3HC glucuronidation phenotypes and total nicotine equivalents were examined in 540 African American smokers. The UGT2B17 gene deletion and UGT2B10*2 were genotyped.

Results: The UGT2B17 gene deletion, but not UGT2B10*2 genotype, was associated with slower 3HC glucuronidation (indicated by three 3HC-glucuronidation phenotypes), indicating its role in this glucuronidation pathway. However, neither lower rates of 3HC glucuronidation, nor the presence of a UGT2B17 and UGT2B10 reduced function allele, altered plasma or urinary NMRs or levels of smoking.

Conclusions: Variation in 3HC glucuronidation activity, including these caused by UGT2B17 gene deletions, did not significantly alter NMR and is therefore unlikely to affect the clinical utility of NMR in smoking behavior and cessation studies. This study demonstrates that NMR is not altered by differences in the rate of 3HC glucuronidation, providing further support that NMR is a reliable indicator of CYP2A6 mediated nicotine metabolism.

Introduction

There are currently more than 1.3 billion tobacco smokers worldwide. Nicotine is the primary psychoactive tobacco component responsible for tobacco dependence. The rate of nicotine clearance is variable, and is associated with the level of tobacco consumption [1–3]. In humans, the majority of nicotine is metabolized to cotinine relatively quickly (nicotine’s half-life is around 2 hours and the in vivo total body clearance is 1.0–1.5 L/min) by a genetically polymorphic enzyme CYP2A6 [4,5]. Cotinine is further metabolized by CYP2A6 to trans-3′-hydroxycotinine (3HC) at a relatively slower rate (cotinine’s half-life is around 12–15 hours and the in vivo total body clearance is 0.05 L/min) [5–7]. The disposition of nicotine was previously reviewed in detail with indicative diagrams by [8,9]. The ratio of 3HC/cotinine (also known as the nicotine metabolite ratio or NMR), is used as an in vivo biomarker of CYP2A6 activity, and correlates highly with nicotine clearance [10]. A number of studies have demonstrated that smokers with faster CYP2A6 activity and higher NMR have higher tobacco consumption [11], lower odds of smoking cessation without any active pharmacological intervention [12,13], and lower odds of smoking cessation from...
expressed in the Declaration of Helsinki, and the study protocol written informed consent in accordance with the principles of the University of Kansas Human Subject Committee, the University of Toronto Ethics Review Office, and the University of California San Francisco Human Research Protection Program.

Genotyping

UGTB17 gene deletion and UGT2B10*2 were genotyped using ABI Vii 7 real time PCR machine (Applied Biosystems, Foster City, CA). The UGT2B17 genotyping reaction was performed with 5 μL TaqMan GTXpress master mix and 5 μL of water containing 10 ng of DNA and 0.5 μL of 20x Taqman copy number variant genotyping assay (Hs03183327_cn, specific target UGT2B17 exon 1 which does not amplify the pseudogene UGT2B15, Applied Biosystems, Foster City, CA). The UGT2B10*2 genotyping reaction was performed with 5 μL TaqMan GTXpress master mix and 5 μL of water containing 10 ng of DNA and 0.25 μL of 40x Taqman genotyping assay (rs61750900, Applied Biosystems, Foster City, CA) [22]. The reactions were performed in 96 well plates. The allele discrimination data were analyzed by Vii 7 software version 1.2.

Analytical Chemistry

Plasma cotinine and 3HC levels were determined using liquid chromatography–tandem mass spectrometry as described previously [10]. The urinary analytes were determined using liquid chromatography–tandem mass spectrometry as described previously [30]. Urinary total nicotine equivalents (TNE) was defined as the total urinary level of nicotine and nine of its metabolites (i.e. nicotine, nicotine glucuronide, cotinine, cotinine glucuronide, 3HC, 3HC glucuronide, nicotine-N-oxide, cotinine-N-oxide, nornicotine and norcotinine). The ten analytes account for about 90% of nicotine dose [31], and creatinine adjusted spot urinary TNE correlates with daily tobacco consumption [32].

3HC Glucuronidation Phenotypes

Here, we used 3HC-Gluc over 3HC-free ratio (i.e. the ratio of product over substrate) as the primary 3HC glucuronidation phenotype. The percentage of TNE excreted as 3HC-Gluc and the percentage of total 3HC excreted as 3HC-Gluc were used as secondary 3HC glucuronidation phenotypes.

Plasma and Urinary NMR Definition

Plasma NMR was defined as the ratio of 3HC over cotinine. The primary urinary NMR was defined as the ratio of total 3HC (free and glucuronide conjugated) over free cotinine since only free cotinine is available to be metabolized to 3HC. The ratio of free 3HC over free cotinine (i.e. non-glucuronidated) and the ratio of total 3HC over total cotinine (i.e. total of glucuronidated and non-glucuronidated) were used as secondary urinary NMR phenotypes for comparison to the literature [33,34]. We obtained very similar results between all three versions of urinary NMR.

Statistical Analyses

Comparisons of 3HC-glucuronidation phenotypes or NMRs for UGT2B17 or UGT2B10 genotypes were performed by Kruskal-Wallis, Mann-Whitney or Chi² tests. Post-hoc adjustments were done by Dunn’s multiple comparison tests. The correlations between the primary 3HC glucuronidation phenotype with plasma NMR, urinary NMR, plasma cotinine or urinary TNE were assessed by Spearman’s correlation. Statistical analyses were performed using Stata 11 (StataCorp, College Station, TX).
Results

UGT2B17 and UGT2B10 Genotyping

The UGT2B17 gene deletion and UGT2B10*2 were both in Hardy-Weinberg equilibrium with allele frequencies of 23.4% and 4.3% in this population of African Americans respectively (HWE P = 0.36 and 0.99 respectively) consistent with published frequencies [20,28]. No significant difference in baseline demographics, smoking behaviors, baseline plasma cotinine levels, urinary TNE, and levels of nicotine dependence were observed between UGT2B17 or UGT2B10 genotype groups (Table 1), indicating that neither the UGT2B17 gene deletion nor the UGT2B10*2 were associated with the levels of tobacco consumption or nicotine dependence.

UGT2B17 Gene Deletion was Associated with Slower 3HC-glucuronidation Phenotypes in vivo

As illustrated by Fig. 1A, the UGT2B17 gene deletion was associated with lower urinary 3HC-Gluc over 3HC-free ratio, the primary 3HC glucuronidation phenotype, consistent with an impact on 3HC-glucuronidation (Fig. 1A, P<0.001). Post-hoc analyses revealed that the UGT2B17WT/WT genotype group had a significantly higher urinary 3HC-glucuronidation phenotype compared to the UGT2B17*1/*1 genotype group or the UGT2B17*/* genotype group (medians of 0.23, 0.17, 0.09 respectively, all P<0.01) and the UGT2B17*1/*2 genotype group had a significantly higher urinary 3HC-Gluc over 3HC-free ratio compared to the UGT2B17*/* genotype group (P<0.05). The UGT2B17 gene deletion was also significantly associated with the secondary 3HC-glucuronidation phenotype – the percentage of TNE excreted as 3HC-Gluc (Fig. 1B, P<0.001). The median percentages of TNE excreted as 3HC-Gluc were 4.2%, 3.7% and 0.8% for UGT2B17*/WT, UGT2B17*1/*1, and UGT2B17*/* genotype groups respectively. Post-hoc analyses revealed that the UGT2B17*1/*1 genotype group excreted a significantly higher percentage of their TNE as 3HC-Gluc compared to the UGT2B17*/* genotype group (P<0.05). The UGT2B17*1/*1 genotype group excreted a significantly higher percentage of their TNE as 3HC-Gluc compared to the UGT2B17*/* genotype group (P<0.05). UGT2B17 gene deletion was also significantly associated with another 3HC glucuronidation phenotype – the percentage of total 3HC excreted as 3HC-Gluc (Table 1).

UGT2B10*2 Genotype was not Associated with 3HC-glucuronidation Phenotypes in vivo

As illustrated by Fig. 1C, UGT2B10*2 genotype was not significantly associated with the primary 3HC-glucuronidation phenotype. Furthermore, UGT2B10*2 genotype was not significantly associated with either secondary 3HC-glucuronidation phenotypes (3HC-Gluc/TNE and 3HC-Gluc/3HC-Total). The median percentages of TNE excreted as 3HC-Gluc were 6.8% and 7.3% for UGT2B10*1/*1 and UGT2B10*1/*2 individuals respectively (Fig. 1D, non-significant), and approximately 17.4% of total 3HC was excreted as 3HC-Gluc in UGT2B10*1/*1 individuals compared with the 15.9% in UGT2B10*1/*2 individuals (Table 1. non-significant). Thus, we found no evidence of this UGT2B10 variant altering 3HC glucuronidation in vivo.

UGT2B17 Gene Deletion was not Associated with Altered Plasma or Urinary NMR

As illustrated by Fig. 2A and Fig. 2B, the UGT2B17 gene deletion was not associated with either plasma or urinary NMR. The median plasma NMR were 0.32, 0.33 and 0.35 for UGT2B17WT/WT, UGT2B17*1/*1 and UGT2B17*/* respectively (Fig. 2A, non-significant). The median urinary NMR were 3.0, 3.2 and 3.4 for UGT2B17WT/WT, UGT2B17*1/*1 and UGT2B17*/* respectively (Fig. 2B, non-significant). No significant difference was observed in any of the demographic variables when the UGT2B10*2/*2 group was pooled with the *1/*2 group. Statistical comparison performed by Mann-Whitney or Kruskal-Wallis tests.

Table 1. Impacts of genetic variation in UGT2B17 and UGT2B10.

|                          | UGT2B17       | UGT2B10       | P-value |
|--------------------------|---------------|---------------|---------|
|                          | WT/WT         | WT/*          | */*     | */*2   | */*2   |
| Number of participants   | 315           | 183           | 33      | 490    | 44     | 1       |
| Sex (% Female)           | 67.3%         | 63.4%         | 60.6%   | 0.56   | 66.3%  | 61.4%   | 0.31    |
| Age (Years)              | 46.5          | 46.4          | 46.9    | 0.96   | 46.8   | 43.8    | 54      | 0.19    |
| Menthol (% Smoke mentholated cigarettes) | 83.8%         | 83.6%         | 81.8%   | 0.96   | 82.7%  | 90.9%   | 100%    | 0.35    |
| Baseline cigarettes per day | 8.1           | 7.7           | 7.7     | 0.27   | 8.0    | 7.6     | 1       | 0.10    |
| Plasma Cotinine (mg/mL) | 278           | 271           | 273     | 0.89   | 273    | 281     | 631     | 0.26    |
| Urinary total nicotine equivalent (nmol/mg Cre) | 61.9          | 53.2          | 52.1    | 0.30   | 59.1   | 49.0    | N/A     | 0.54    |
| Fagerstrom Test for Nicotine Dependence (FTND) | 3.2           | 3.1           | 3.0     | 0.51   | 3.2    | 2.9     | 4       | 0.58    |
| 3HC-Glu/Total 3HC (%)     | 19.1%         | 15.3%         | 8.0%    | <0.0001| 17.4%  | 15.9%   | N/A     | 0.53    |

Data presented as Mean (95% Confident Interval).

*1The urinary total nicotine equivalents and urinary 3HC-Glu/total 3HC ratios were only available for 426 individuals.

**No statistically significant difference was observed in any of the demographic variables when the UGT2B10*2/*2 group was pooled with the *1/*2 group. Statistical comparison performed by Mann-Whitney or Kruskal-Wallis tests.
respectively (Fig. 2B, non-significant). UGT2B17 gene deletion was also not associated with the other versions of the urinary NMR, total 3HC/total cotinine or free 3HC/free cotinine ($P = 0.45$ and 0.56 respectively).

**UGT2B10***2 Genotype was not Associated with Altered Plasma or Urinary NMR

As illustrated by Fig. 2C and 2D, UGT2B10*2 genotype was not associated with altered plasma or urinary NMR. UGT2B10*2 was also not associated with the other versions of the urinary NMR such as total 3HC/total cotinine or free 3HC/free cotinine ($P = 0.94$ and 0.72). No significant differences were observed even when the UGT2B10*1/*2 and UGT2B10*2/*2 genotype groups were combined during these analyses.

**3HC Glucuronidation Phenotype did not Correlate with Plasma or Urinary NMR**

The primary 3HC glucuronidation phenotype (the urinary 3HC-Gluc over 3HC-free ratio) did not correlate with either the plasma (Fig. 3A, Spearman’s Rho = 0.05, $P = 0.32$) or the urinary (Fig. 3B, Spearman’s Rho = 0.03, $P = 0.55$) NMR. The primary 3HC glucuronidation phenotype was also not correlated with the alternative versions of urinary NMR, total 3HC/total cotinine or free 3HC/free cotinine (Spearman’s Rho = −0.03 and 0.02;
Furthermore, the urinary 3HC-Gluc over 3HC-Free ratio did not correlate with either plasma cotinine (Fig. 3C, Spearman’s Rho = -0.04, P = 0.42) or the urinary TNE (Fig. 3D, Spearman’s Rho = -0.02, P = 0.63), suggesting variation in 3HC glucuronidation did not change tobacco consumption, consistent with a lack of effect of variation in 3HC glucuronidation on NMR.

**Discussion**

Here we established that among African American smokers the UGT2B17 gene deletion, but not UGT2B10*2 genotype, was associated with slower 3HC glucuronidation in vivo. In human liver microsomes, 3HC can be either O-glucuronidated on its 3'-hydroxyl group to form 3HC-O-Glucuronide or N-glucuronidated to form 3HC-N-Glucuronide [19]. UGT2B17 exhibited highest 3HC-O-glucuronidation activity among all known human UGT1A and UGT2B enzymes [19]. Our observations that UGT2B17 gene deletion was associated with the 3HC-Gluc over 3HC-free ratio, percentage of TNE excreted as 3HC-Gluc, and the percentage of total 3HC excreted as 3HC-Gluc, are consistent with a predominant role of UGT2B17 in the glucuronidation of 3HC [22]. UGT2B10 exhibits the highest 3HC-N-glucuronidation activity among all known human UGT1A and UGT2B enzymes [19]. However 3HC-N-Glucuronide is generally not found in urine [25,35]. Consistent with this we found that UGT2B10*2 genotype was not associated with the 3HC-Gluc over 3HC-free ratio, the percentage of TNE excreted as 3HC-Gluc, or the percentage of total 3HC excreted as 3HC-Gluc suggesting that 3HC-N-glucuronidation, via UGT2B10, does not contribute substantially to 3HC-glucuronidation in vivo.

Our observations also indicate that neither the UGT2B17 gene deletion nor the UGT2B10*2 genotype were associated with plasma or urinary NMR. The NMR is used extensively as an in vivo indicator of nicotine clearance in studies of smoking behaviors and smoking cessation trials [12–14,36–38]. Here we demonstrated that neither UGT2B17 gene deletion nor UGT2B10*2 genotype significantly altered NMR, suggesting variation in 3HC-glucuronidation is unlikely to alter the relationship between NMR and nicotine clearance or to affect the utility of NMR in smoking studies. In this study, each UGT2B17 gene deletion allele was responsible for a roughly 5% reduction in the percentage of total 3HC excreted as 3HC-Gluc (see Table 1). Of note, a large percentage of the individuals who were homozygous for UGT2B17 gene deletion had detectable of 3HC-Gluc levels suggesting other UGTs can also glucuronidate 3HC reducing the likelihood that genetic variation in any one of the UGTs alters this pathway substantially. Together this suggests that genetic variation in UGT2B17 and/or UGT2B10*2 is unlikely to have a substantial effect on 3HC clearance, 3HC plasma levels or NMR.

![Figure 3](https://example.com/figure3.png)

**Figure 3. The urinary 3HC-Gluc over 3HC-free ratio did not correlate NMR or nicotine consumption.** The urinary 3HC-Gluc over 3HC-free ratio did not correlate with either plasma NMR (A) or urinary NMR (B). The urinary 3HC-Gluc over 3HC-free ratio also did not correlate with either plasma cotinine (C) or the urinary TNE (D).

doi:10.1371/journal.pone.0070938.g003
Another notable observation was that the urinary 3HC/Glu over 3HC-free ratio, indicative of 3HC glucuronidation activity (i.e. the total activity of all the enzymes involved in this pathway), was not correlated with plasma or urinary NMR. Here, as seen before [22,31], only 17% of total 3HC was excreted as 3HC-Glu suggesting that the majority (>80%) of 3HC is cleared without further metabolism in humans and the glucuronidation of 3HC is a relatively minor pathway in 3HC clearance. Together this indicates that variation in 3HC glucuronidation has a very minor effect on the clearance of 3HC and does not have a meaningful impact on NMR values. In addition, we found that neither genetic variation in UGT2B17 and UGT2B10, nor the rate of 3HC glucuronidation, had an effect on levels of smoking as indicated by cigarettes per day, plasma cotinine levels or urinary TNE, and on levels of nicotine dependence, consistent with the minimum impact on NMR.

Our observations should be interpreted in the context of some potential limitations. This study was conducted in African Americans. It is possible that the UGT2B17 and UGT2B10 gene variants could have different magnitudes of effect on 3HC glucuronidation in other racial groups. The prevalence of UGTB10*2 was relatively low in African Americans, but a total lack of effect in 540 people would still argue against it contributing meaningfully in other populations. In addition, our study population reported consuming relatively low numbers of cigarettes per day which may limit the generalizability the findings. However, the observed cotinine levels in this study (mean = 240 ng/mL) suggested the findings should be generalizable to both light and heavy smokers since the 240 ng/mL cotinine level was very similar to those previously observed in Caucasian and African American heavy smokers [30]. The high cotinine levels relative to the level of reported smoking may be explained by both the higher nicotine intake per cigarette and the lower ability to clear cotinine (due to genetic variants in CYP1A2 gene, resulting in the accumulation of the cotinine) in African American smokers [7,30].

In conclusion, we demonstrated that the UGT2B17 gene deletion, but not the UGT2B10*2 genotype, significantly altered 3HC glucuronidation. However, variation in 3HC glucuronidation activity, including these caused by UGT2B17 gene deletions, did not significantly alter NMR and is unlikely to affect the clinical utility of NMR in smoking behavior and cessation studies.

Author Contributions
Conceived and designed the experiments: AZ. LSC. JSA. NLB. RFT. Performed the experiments: AZ. QZ. Analyzed the data: AZ. Z. RFT. Wrote the paper: AZ. QZ. LSC. JSA. NLB. RFT.

References
1. Benowitz NL, Jacob P 3rd (1985) Nicotine renal excretion rate influences nicotine intake during cigarette smoking. J Pharmacol Exp Ther 234: 153–155.
2. Benowitz NL, Perez-Stable EJ, Herrera B, Jacob P 3rd (2002) Slower metabolism and reduced intake of nicotine from cigarette smoking in Chinese-Americans. J Natl Cancer Inst 94: 108–115.
3. Piazzella ML, Sellers EM, Tyndale RF (1998) Nicotine metabolism defect reduces smoking. Nature 393: 750.
4. Messina ES, Tyndale RF, Sellers EM (1997) A major role for CYP2A6 in nicotine C-oxidation by human liver microsomes. J Pharmacol Exp Ther 282: 1609–1614.
5. Benowitz NL, Jacob P 3rd (1994) Metabolism of nicotine to cotinine studied by a dual stable isotope method. Clin Pharmacol Ther 56: 483–493.
6. Nakajima M, Yamamoto T, Nunoya K, Yokoi T, Nagashima K, et al. (1996) Characterization of CYP2A6 involved in 3'-hydroxylation of cotinine in human liver microsomes. J Pharmacol Exp Ther 277: 1010–1015.
7. Zhu AZ, Renner CC, Hatsukami DK, Swan GE, Lerman C, et al. (2013) 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 22: 708–718.
8. Hukkanen J, Jacob P 3rd, Benowitz NL (2005) Metabolism and disposition kinetics of nicotine. Pharmacol Rev 57: 79–113.
9. Zhu AZ, Tyndale RF (2012) Nicotine Metabolism and its Implications. Metabolism of Drugs and Other Xenobiotics: Wiley-VCH Verlag GmbH & Co. KGaA. 465–492.
10. Dempsey D, Tutka P, Jacob P 3rd, Allen F, Schoedel K, et al. (2004) Nicotine metabolism ratio as an index of cytochrome P450 2A6 metabolic activity. Clin Pharmacol Ther 76: 64–72.
11. Zhu AZ, Binnington MJ, Renner CC, Lanier AP, Hatsukami DK, et al. (2013) Alaska Native smokers and smokeless tobacco users with slower CYP2A6 activity have lower tobacco consumption, lower tobacco-specific nitrosamine exposure and lower tobacco-specific nitrosamine bioactivation. Carcinogenesis 34: 93–101.
12. Patterson F, Schnoll RA, Wileto EP, Fusto A, Epstein LH, et al. (2006) Toward personalized therapy for smoking cessation: a randomized placebo-controlled trial of bupropion. Clin Pharmacol Ther 84: 320–325.
13. Ho MK, Mwenifumbo JC, Al Koudsi N, Okuyemi KS, Ahluwalia JS, et al. (2009) Association of nicotine metabolite ratio and CYP2A6 genotype with smoking cessation treatment in African-American light smokers. Clin Pharmacol Ther 85: 635–643.
14. Lerman C, Tyndale R, Patterson F, Wileto EP, Shields PG, et al. (2006) Nicotine metabolism ratio predicts efficacy of transdermal nicotine for smoking cessation. Clin Pharmacol Ther 79: 600–608.
15. Benowitz NL, Knut F, Jacob P 3rd, Jones RT, Osman AL (1983) Cotinine disposition and effects. Clinical pharmacology and therapeutics 34: 604–611.
16. Benowitz NL, Jacob P 3rd (2001) Trans-3'-hydroxycotinine: disposition kinetics, effects and plasma levels during cigarette smoking. Br J Clin Pharmacol 51: 53–59.
17. Lea RA, Dickson S, Benowitz NL (2006) Within-subject variation of the salivary 3HC/COT ratio in regular daily smokers: prospects for estimating CYP2A6 enzyme activity in large-scale surveys of nicotine metabolic rate. J Anal Toxicol 30: 396–399.
18. St Helen G, Novalen M, Heitjan DF, Dempsey D, Jacob P 3rd, et al. (2012) Reproducibility of the nicotine metabolite ratio in cigarette smokers. Cancer Epidemiol Biomarkers Prev 21: 1105–1114.
19. Chen G, Giambonne NE, Lazarus P (2012) Glucuronidation of trans-3'-hydroxycotinine by UGT2B17 and UGT2B10. Pharmacogenet Genomics 22: 183–190.
20. Xue Y, Sun D, Daly A, Yang F, Zhou X, et al. (2008) Adaptive evolution of UGT2B17 copy-number variation. American journal of human genetics 83: 337–346.
21. Yang TL, Chen XD, Guo Y, Lei SF, Wang JT, et al. (2006) Genome-wide copy-number-variation study identified a susceptibility gene, UGT2B17, for osteoporosis. American journal of human genetics 83: 663–674.
22. Chen G, Giambonne NE Jr, Dzien DF, Muscat JE, Berg A, et al. (2010) Glucuronidation genotypes and nicotine metabolic phenotypes: importance of functional UGT2B10 and UGT2B17 polymorphisms. Cancer research 70: 7543–7552.
23. Lazarus P, Zheng Y, Aaron Runkle E, Muscat JE, Wiener D (2005) Genotype-phenotype correlation between the polymorphic UGT2B17 gene deletion and NNAL glucuronidation activities in human liver microsomes. Pharmacogenetics and genomics 15: 769–776.
24. Binnington JM, Zhu AZ, Renner CC, Lanier AP, Hatsukami DK, et al. (2012) CYP2A6 and CYP2B6 genetic variation and its association with nicotine metabolism in South Western Alaska Native people. Pharmacogenomics Genomics 22: 429–440.
25. Koshih GE, Murphy SE (2003) N-glucuronidation of trans-3'-hydroxycotinine by human liver microsomes. Chem Res Toxicol 16: 1502–1506.
26. Yamanaka H, Nakajima M, Katoh M, Kanoh A, Tamura O, et al. (2005) Trans-3'-hydroxycotinine O- and N-glucuronidations in human liver microsomes. Drug Metabol Dispos 33: 23–30.
27. Chen G, Belevins-Pinacau AS, Delligner RW, Muscat JE, Lazarus P (2007) Glucuronidation of nicotine and cotinine by UGT2B10: loss of function by the UGT2B10 Codon 67 (Asp>Tyr) polymorphism. Cancer Res 67: 9024–9029.
28. Berg JZ, Mason J, Böttcher AJ, Hatsukami DK, Murphy SE (2010) Nicotine metabolism in African Americans and European Americans: variation in glucuronidation by ethnicity and UGT2B10 haplotype. J Pharmacol Exp Ther 332: 202–209.
29. Cox LS, Nollen NL, Mayo MS, Choi WS, Fauer B, et al. (2012) Bupropion for smoking cessation in African American light smokers: A randomized controlled trial. Journal of the National Cancer Institute 104: 1–9.
30. Benowitz NL, Dains KM, Dempsey D, Wilson M, Jacob P (2011) Racial differences in the relationship between number of cigarettes smoked and nicotine and carcinogen exposure. Nicotine Tob Res 13: 772–785.
31. Benowitz NL, Jacob P 3rd, Fong J, Gupta S (1994) Nicotine metabolic profile in man: comparison of cigarette smoking and transdermal nicotine. J Pharmacol Exp Ther 268: 296–303.
Benowitz NL, Dains KM, Dempsey D, Havel C, Wilson M, et al. (2010) Urine menthol as a biomarker of mentholated cigarette smoking. Cancer Epidemiol Biomarkers Prev 19: 3013–3019.

Swan GE, Lessov-Schlaggar CN, Bergen AW, He Y, Tyndale RF, et al. (2009) Genetic and environmental influences on the ratio of 3'-hydroxycotinine to cotinine in plasma and urine. Pharmacogenet Genomics 19: 388–396.

Derby KS, Cuthrell K, Caberto C, Carmella SG, Franke AA, et al. (2008) Nicotine metabolism in three ethnic/racial groups with different risks of lung cancer. Cancer Epidemiol Biomarkers Prev 17: 3526–3535.

Yamanaka H, Nakajima M, Fukami T, Sakai H, Nakamura A, et al. (2005) CYP2A6 AND CYP2B6 are involved in normocotine formation from nicotine in humans: interindividual differences in these contributions. Drug Metab Dispos 33: 1811–1818.

Lerman C, Jepson C, Wileyto EP, Patterson F, Schnoll R, et al. (2010) Genetic Variation in Nicotine Metabolism Predicts the Efficacy of Extended-Duration Transdermal Nicotine Therapy. Clin Pharmacol Ther 87: 553–557.

Strasser AA, Malaiyandi V, Hoffmann E, Tyndale RF, Lerman C (2007) An association of CYP2A6 genotype and smoking topography. Nicotine Tob Res 9: 511–518.

Strasser AA, Benowitz NL, Pinto AG, Tang KZ, Hecht SS, et al. (2011) Nicotine metabolite ratio predicts smoking topography and carcinogen biomarker level. Cancer Epidemiol Biomarkers Prev 20: 234–238.