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Nicotine metabolism and smoking: Ethnic differences in the role of P450 2A6

Sharon E. Murphy[†,*]

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

Abstract

Nicotine is the primary addictive agent in tobacco and P450 2A6 (gene name *CYP2A6*) is the primary catalyst of nicotine metabolism. It was proposed more than 20 years ago that individuals who metabolize nicotine poorly would smoke less, either fewer cigarettes per day or less intensely per cigarette, compared to smokers who metabolize nicotine more efficiently. These poor metabolizers would then be less likely to develop lung cancer due to their lower exposure to the many carcinogens delivered with nicotine in each puff of smoke. Numerous studies have reported that smokers who carry reduced activity or null *CYP2A6* alleles do smoke less. Yet only in Asian populations, both Japanese and Chinese, which have a high prevalence of genetic variants has a link between *CYP2A6*, smoking dose, and lung cancer been established. In other ethnic groups, it has been challenging to confirm a direct link between P450 2A6–mediated nicotine metabolism and the risk of lung cancer. This challenge is due in part to the difficulty in accurately quantifying smoking dose and accurately predicting or measuring *P450 2A6*-mediated nicotine metabolism. Biomarkers of nicotine metabolism and smoking exposure, including the ratio of *trans* 3-hydroxycotine to cotinine, a measure of *P450 2A6* activity and plasma cotinine or urinary total nicotine equivalents (TNE, the sum of nicotine and 6 metabolites) as measures of exposure are useful in addressing this challenge. However, to take full advantage of these biomarkers in the study of ethnic/racial differences in the risk of lung cancer requires the complete characterization of nicotine metabolism across ethnic/racial groups. Variation in metabolism pathways, other than those catalyzed by P450 2A6, can impact biomarkers of both nicotine metabolism and dose. This is clearly important for smokers with low levels of UGT2B10-catalyzed nicotine and cotinine glucuronidation, since UGT2B10 genotype influences plasma cotinine levels. Cotinine is not glucuronidated in 15% of African American smokers (compared to 1% of Whites) due to the prevalence of a UGT2B10 splice variant. This variant contributes significantly to the higher plasma cotinine levels per cigarette in this group and may also influence the accuracy of the 3HCOT to cotinine ratio as a measure of P4502A6 activity.

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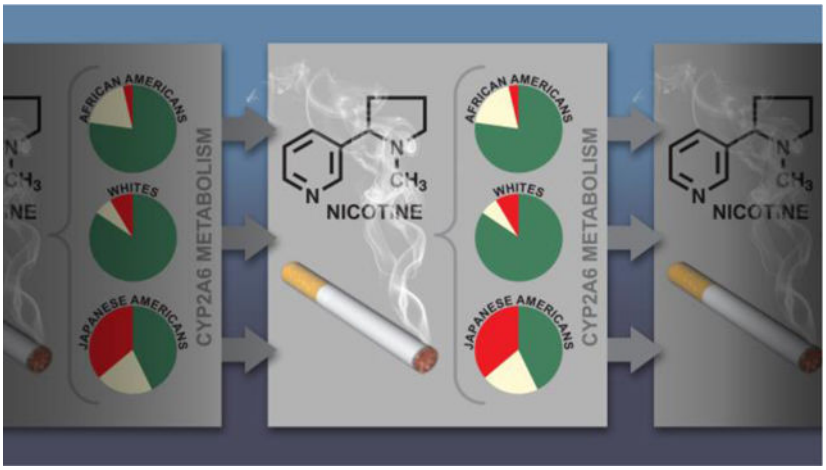
[*]**Corresponding author** Sharon E. Murphy, Ph.D. Masonic Cancer Center, University of Minnesota, 2231 6th Street SE - 2-127 CCRB, Minneapolis, MN 55455, USA, Phone: (612) 624-7633, Fax: (612) 624-3869 murph062@umn.edu.



Introduction

Lung cancer deaths in the United States are estimated to be 158,080 in 2016 and the number worldwide will be more than 1.3 million.[1, 2] Cigarette smoking is the cause of as much as 90% of this death toll.[3] Yet despite a smoker's overwhelming increased risk of lung cancer only 11–24% of smokers will develop the disease.[3] In addition, for the same self-reported number of cigarettes per day (CPD), lung cancer risk differs across ethnic/racial groups.[4] These data may lead one to conclude that susceptibility to the many lung carcinogens in each cigarette varies by ethnicity. But equally or likely more important may be differences in a smoker's response to the nicotine present in each puff of smoke. Nicotine is essential to sustaining tobacco addiction[5] and the activity of both the enzymes that metabolize it and the receptors that mediate its effects will vary across smokers.

It was proposed more than 20 years ago that individuals who metabolize nicotine poorly would smoke less, either fewer CPD or less intensely per cigarette, compared to those smokers who metabolize nicotine more efficiently.[6, 7] These poor metabolizers would then be less likely to develop lung cancer due to their lower exposure to the many carcinogens delivered with nicotine in each puff of smoke. There are at least two challenges to establishing a relationship between nicotine metabolism, tobacco carcinogen exposure, and the risk of lung cancer. The first is the accurate quantitation of tobacco smoke exposure, which requires a better measure than CPD. The second is the accurate quantitation and characterization of nicotine metabolism.

This perspective summarizes nicotine metabolism, the effect of *CYP2A6* genotype on smoking levels and lung cancer risk with a focus on ethnic/racial differences. The influence of ethnic/racial differences in nicotine metabolism on biomarkers of smoking exposure and on the biomarkers used to phenotype *CYP2A6* is discussed. In addition, the association of *CYP2A6* genotype with smoking dose is discussed with a specific emphasis on the effect of UGT2B10 genotype in African American smokers.

Urinary and plasma nicotine metabolites as biomarkers of smoking exposure

Nicotine is metabolized by three pathways; P450 2A6 catalyzed C-oxidation, UGT2B10-catalyzed *N*-glucuronidation, and FMO3-catalyzed *N*-oxidation (Figure 1).[8–11] In the vast majority of smokers the primary pathway of metabolism is P450 2A6-catalyzed 5'-oxidation, which after a second oxidation generates cotinine, the predominant nicotine metabolite in smokers' plasma. Cotinine is further metabolized to *trans* 3'-hydroxycotinine (3HCOT), the principal urinary metabolite of nicotine. Typically 10 to 30% of the 3HCOT excreted is an O-glucuronide conjugate.[8, 9, 11] Cotinine and nicotine are excreted both free and as *N*-glucuronide conjugates[8–10]. The sum of the urinary concentrations of nicotine, cotinine, 3HCOT, nicotine N-glucuronide, cotinine *N*-glucuronide, 3HCOT-glucuronide, and nicotine *N*-oxide is referred to as total nicotine equivalents (TNE) and accounts for 85–90% of the nicotine dose.[12, 13] The most abundant metabolite not included in TNE is 4-hydroxy-4-(3-pyridyl)-butanoic acid (7–9% of the nicotine dose).[14] This metabolite may form by way of P450 2A6 catalyzed 2'-nicotine oxidation or by further metabolism of cotinine [15]. Other minor metabolites in smokers urine include nornicotine (<1%), cotinine N-oxide (2–5%) and 5'-hydroxycotinine (~1.5%)[8]. Nornicotine is a product of P450 2A6-catalyzed nicotine metabolism but is also present in tobacco and tobacco smoke.[16]

Cotinine and total cotinine (the sum of cotinine and its glucuronide conjugate) are used as biomarkers of smoking and nicotine exposure.[17–19] However, variability in the urinary concentrations of cotinine and total cotinine across smokers will occur due to individual differences in metabolism of nicotine and cotinine.[10, 17, 20] For many studies, TNE are a better biomarker of smoking exposure since the level will not be significantly affected by individual differences in metabolism.

There are ethnic/racial differences in the activity of the enzymes that catalyze nicotine metabolism, and therefore the relative distribution of the metabolites excreted may vary across different populations.[9] The unique pattern of urinary nicotine metabolism across five ethnic/racial groups is presented in Figure 2. These data are from a single study in which we quantified nicotine and the metabolites that make up TNE in over 2000 smokers in the Multiethnic Cohort study.[9] The sections in the pie graphs represent the percentage of TNE excreted as unchanged nicotine and the products of the three nicotine metabolism pathways. In all groups, nicotine C-oxidation is by far the major pathway of metabolism, however the mean level for Japanese Americans and to a lesser extent Native Hawaiians is significantly lower. In addition, the amount of nicotine excreted unchanged is higher in these two groups compared to Whites, African Americans, and Latinos, among whom there was no significant difference in the proportion of nicotine metabolized by C-oxidation. Also of note is the lower level of nicotine *N*-glucuronidation in African Americans (4.2%). Characterizing and recognizing the ethnic specific difference in metabolism is critical to the correct interpretation of biomarkers of nicotine exposure and metabolism.

The same compounds that make up the urinary TNE are present in smokers' plasma. However, due to differences in their pharmacokinetic parameters the relative distribution is quite different. Nicotine has a relatively short half-life (100–150 min) and plasma levels are dependent on the time of the last cigarette smoked.[8] Cotinine has a much longer half-life (770–1130 min) and in daily smokers plasma concentrations are relatively constant.[8]

Therefore, plasma cotinine is routinely used as a biomarker of tobacco exposure.[17] The next most abundant metabolite is typically 3HCOT. Nicotine *N*-oxide, 3-HCOT glucuronide, nicotine *N*-glucuronide, and cotinine *N*-glucuronide are present in plasma, but at low levels (1–4% of the cotinine concentration).[10, 21, 22] Despite the low levels of cotinine *N*-glucuronide in smokers' plasma, the concentration of plasma cotinine is significantly influenced by the activity of UGT2B10, which is the sole catalyst of cotinine *N*-glucuronidation in smokers.[10] Several years ago we reported that plasma cotinine levels were 20% higher in smokers who were heterozygous for a non-functional UGT2B10 variant, compared to those who did not carry this variant.[10] In that study the majority of the smokers were White; low cotinine glucuronidation occurs much more frequently in African Americans.[9, 23, 24] More recently, in a study of both African American and White smokers we observed that the mean serum cotinine concentration per TNE in smokers with no UGT2B10 activity was 50% higher than the mean level in smokers who have UGT2B10 activity. [25]

P450 2A6 catalyzed nicotine metabolism

As noted above, P450 2A6 catalyzes the 5'-oxidation of nicotine. The iminium ion product of this reaction is further oxidized to cotinine, either by cytosolic aldehyde oxidase or P450 2A6.[8, 26] Aldehyde oxidase is not required to form cotinine from nicotine, P450 2A6 alone may catalyze two oxidation of nicotine to generate cotinine.[27] The oxidation of nicotine to the iminium ion and the subsequent oxidation of the iminium ion to cotinine by P450 2A6 may occur sequentially without the iminium ion leaving the active site of the enzyme.[26] P450 2A6 also catalyzes the oxidation of cotinine to 3HCOT, but less efficiently than the 5'-oxidation of nicotine.[16, 27, 28] Interestingly, *in vitro* the primary product of cotinine metabolism by either human liver microsomes or heterologously expressed P450 2A6 is *N*-(hydroxymethyl)norcotinine, which decomposes to norcotinine.[28] In addition, 5'-hydroxycotinine is formed at 1.5 times the rate of 3HCOT. However, norcotinine and 5'-hydroxycotinine are minor nicotine metabolite in smokers.[8] We have shown that P450 2A6 catalyzes the sequential oxidation of the nicotine $^{\Delta 5'(1')}$iminium ion to 3HCOT, and that no 5'-hydroxycotinine and little norcotinine is formed by this pathway.[26] Together these data have led us to suggest that a significant portion of the 3HCOT present in smokers may be generated by P450 2A6-catalyzed sequential oxidations of nicotine compared to direct metabolism of cotinine. Regardless of the pathway of 3HCOT formation, P450 2A6 is the most efficient catalyst of cotinine oxidation to 3HCOT and smokers null for the P450 2A6 gene (*CYP2A6*) excrete little 3HCOT.[8, 29, 30] Based on these data and the relatively long half-life of cotinine compared to nicotine the plasma ratio of 3HCOT to cotinine has been used as a measure of P450 2A6.[17] Likewise the ratio of total 3HCOT to total cotinine ("total" refers to the sum of the metabolite and its glucuronide conjugate), total 3HCOT/cotinine, or 3HCOT to cotinine in urine may be used to characterize P450 2A6 activity.[9, 18, 31–33] Several studies have demonstrated the predicted relationship between these ratios and *CYP2A6* genetic variants.[31–35]

UGT2B10 and nicotine and cotinine metabolism

In smokers, UGT2B10 is the catalyst of nicotine and cotinine *N*-glucuronidation. *In vitro*, heterologously expressed UGT1A4 also catalyzes the *N*-glucuronidation of both

compounds.[36, 37] However, UGT2B10 is a much more efficient catalyst of these reactions and UGT1A4 does not play a significant role in either nicotine or cotinine glucuronidation in smokers.[9, 24, 38, 39] Essentially no nicotine or cotinine glucuronide conjugates are excreted by individuals who are homozygous for either of two UGT2B10 genetic variants (Table 1), the Asp67Tyr variant which has no catalytic activity and a splice variant which does not produce full length mRNA.[9, 39, 40] When the genotypes for both variants are taken into account (risk score 2 in Table 1), heterozygous individuals excrete approximately 50% less cotinine and nicotine as glucuronide conjugates. The splice variant has an allele frequency of 37% in individuals of African descent, and therefore at least 14% of smokers with this ancestral background do not glucuronidate nicotine or cotinine.[41] The exclusive role of UGT2B10 in the glucuronidation of cotinine and nicotine, allows one to phenotype a smoker's UGT2B10 activity by quantifying either of these glucuronides relative to their aglycon in urine. The cotinine glucuronidation ratio is a more robust measure of UGT2B10 activity due to less variability in urinary cotinine concentrations compared to nicotine, which is more dependent on the time of last cigarette.

FMO3 and nicotine metabolism

Nicotine *N*-oxidation is catalyzed by FMOs and the *trans* nicotine *N*-oxide excreted by smokers is the product of FMO3 metabolism.[8] Only the *trans* isomer is found in smokers' urine and human FMO3 is highly selective for the formation of this isomer. In a recent study of White smokers we reported a significant effect of FMO3 haplotype on plasma nicotine *N*-oxide concentration.[22] Yet, by far the main contributor to nicotine *N*-oxide levels was *CYP2A6* genotype due to its dominant effect on total nicotine metabolism in this population.[22] Nicotine *N*-oxidation only accounts for a small percentage of hepatic nicotine metabolism but FMO3, unlike *CYP2A6,* is expressed in the brain and we recently measured nicotine *N*-oxidation activity in microsomal preparations from human brain tissue.[22, 42, 43] In the same study, we reported that an FMO3 variant was associated with nicotine dependence, leading us to hypothesize that FMO3 activity in the brain affects tissue nicotine levels, which then influence a smoker's level of addiction.[22]

Ethnic/racial differences in P450 2A6 and UGT2B10 activity, and nicotine metabolism and exposure biomarkers

The three primary enzymes that catalyze nicotine metabolism are polymorphic and, in the case of P450 2A6 and UGT2B10, the frequency of the variant alleles across ethnic/racial groups is quite different.[31, 44] There are numerous genetic variants of *CYP2A6* and several of these, including the deletion, *CYP2A6**4, and *CYP2A6**2, *CYP2A6**7 and *CYP2A6**12 produce little or no functional enzyme.[44] Across ethnic/racial groups *CYP2A6**4 is most prevalent in Japanese, with an allele frequency of 21%. In contrast, in people of European descent, the frequency of *CYP2A6**4 is <2%, approximately the same frequency as *CYP2A6**2. The CYP2A6 *7 variant is common in Asian (frequency 6–13%) but it is rare in individuals of European or African descent. [31, 44]. The allele frequency of CYP2A6*12 ranges from 0.8 to 3.5% across racial/ethnic groups.[31, 44]. CYP2A6*9, a variant that results in decreased enzyme expression is present in all ethnic groups but its frequency varies from 6–8% in Africans and Europeans to 21% in Asian populations.[31, 44]. Whereas CYP2A6*17 is found in African Americans at a frequency of ~11%, but has not been found in individuals

of African or Asian ancestry. [31, 44]. There are fewer polymorphisms of UGT2B10, but two of these result in no functional enzyme; the Asp67Tyr variant and a splice variant, which is common in individuals of African descent.[9, 39, 40] The splice variant is rare in individuals of European descent occurring at a frequency of about 0.3%. [25, 41] However, Latinos, Native Hawaiins and Japanese Americans have frequencies of 4.8 to 7.3%.[9]

It has been recognized for many years that smokers homozygous for *CYP2A6* alleles that code for little or no functional enzyme excrete little cotinine and have lower levels of plasma cotinine for the same nicotine dose.[44–48] Therefore, while plasma cotinine levels are clearly correlated with CPD,[49] the plasma cotinine concentration of individual smokers will also depend on *CYP2A6* genotype. This results in something of a catch 22 when trying to establish the relationship between P450 2A6 enzyme activity and smoking intensity using either plasma or urinary cotinine as a biomarker of tobacco exposure. The hypothesis is that reduced enzyme activity will result in reduced smoking and therefore lower cotinine levels, but a lack of P450 2A6 enzyme activity will also lead to lower cotinine concentrations (at the same level of nicotine exposure). The relationship between *CYP2A6* genotype and cotinine is even more complicated since both the formation and the metabolism of cotinine are dependent on P450 2A6 activity. Smokers who have reduced activity *CYP2A6* alleles or carry only a single null allele were found to have higher, not lower, plasma cotinine levels per TNE compared to smokers with no variant alleles.[50] In contrast, individuals who were homozygous null for *CYP2A6* (*4/*4) had much lower plasma cotinine per TNE.[50] These data suggest that when some P450 2A6 activity is present then any variation in that activity has a greater effect on the metabolism of cotinine than on its formation. However, if a smoker has no active P450 2A6 enzyme, nicotine metabolism to cotinine will be reduced so significantly that cotinine can be easily cleared by enzymes other than P450 2A6. Therefore, among Japanese, a group with a high frequency of null or non-functional *CYP2A6* alleles [31, 51, 52], plasma cotinine levels may on average be lower for the same level of smoking compared to other ethnic groups with much lower prevalence of these alleles. The use of TNE as a biomarker of nicotine dose and smoking exposure will minimize these ethnic specific difference inherent in the use of plasma cotinine.

In smokers, UGT2B10 genotype has not been shown to significantly affect plasma nicotine levels, nor is there any association with *UGT2B10* genotype and TNE.[20] However, as noted above plasma cotinine levels are influenced by *UGT2B10* genotype. Recognizing the effect of *UGT2B10* genotype on plasma cotinine levels is critical to establishing the correct relationship of smoking dose to cotinine levels. It has been long recognized that on average plasma cotinine concentrations are higher in African Americans compared to Whites with the same level of smoking or for similar environmental tobacco exposure exposure.[53–55] After identifying lower levels of nicotine and cotinine glucuronidation in African Americans, Benowitz *et al*[23] proposed that both lower cotinine glucuronidation and lower C-oxidation are likely to contribute to higher plasma cotinine in African Americans, but more recently it is often accepted that low P450 2A6 activity is responsible.[50, 56] However, UGT2B10 genotype does affect plasma cotinine concentration[10] and based on a 37% frequency of the *UGT2B10* splice variant and 5% frequency of the Asp67Tyr variant, 16% of African Americans have no UGT2B10 activity and only 35% carry neither of these non-functional alleles.[41] Therefore, it is likely that *UGT2B10* genotype contributes significantly

to the average higher plasma cotinine observed in African Americans relative to smokers of European descent.

The 3HCOT/cotinine ratio is a measure of *CYP2A6* activity and there are some advantages to using this ratio over genotype (for example, the challenge of complete and accurate genotyping of this complex gene, and the inability of genotype to capture other influences on metabolism). The urinary total 3HCOT/cotinine or total 3HCOT/total cotinine ratios and the 3HCOT/cotinine plasma ratio do vary with *CYP2A6* genotype across ethnic/racial groups as predicted.[31–35] However, these ratios may also be affected by *UGT2B10* genotype. Carriers of either the *UGT2B10* splice variant or an Asp67Tyr variant were found to have higher total 3HCOT/total cotinine urine ratios due to decreased excretion of cotinine glucuronide by these smokers (Table 1).[9] The total 3HCOT/cotinine ratio was not significantly affected by *UGT2B10* genotype. In addition, smokers who do not glucuronidate cotinine (i.e., have no UGT2B10 activity) have significantly lower plasma 3HCOT/cotinine ratios due to the lower level of plasma cotinine in these individuals per TNE.[25] Therefore, it is necessary that *UGT2B10* genotype be taken into account when using the 3HCOT/cotinine ratio as a measure of *CYP2A6* activity. This is particularly important for smokers of African descent due to the frequency of the *UGT2B10* splice variant in this population.

The 3HCOT/cotinine ratio might also be influenced by genetic variants of the enzymes that glucuronidate 3HCOT, but unlike the glucuronidation of cotinine multiple UGTs catalyze this reaction.[57] Among these is UGT2B17, which is polymorphic for a gene deletion. The frequency of the null *UGT2B17* allele ranges from ~20% in some African populations to 90% in Japanese.[58] However, despite the high frequency of the *UGT2B17* null allele, the percentage of 3HCOT excreted as a glucuronide by Japanese Americans was only 30% lower than that by African American smokers.[9] Therefore, the impact of the variant on the 3HCOT/cotinine ratio is expected to be minimal. In support of this conclusion, a study of over 500 African Americans smokers found that the *UGT2B17* deletion did not significantly alter the plasma 3HCOT/cotinine ratio or the total 3HCOT/cotinine ratio in urine.[59]

Several recent studies in African Americans that investigate the effect of P450 2A6 activity (quantified by the 3HCOT/cotinine ratio) on smoking behavior have not included UGT2B10 genotype in their analysis. One pharmacokinetic study of nicotine metabolism grouped African Americans subjects into quartiles by plasma 3HCOT/cotinine ratio and found that the half-life of cotinine decreased from lowest to highest quartile. These data led the authors to conclude that higher plasma cotinine in African Americans compared to Whites is due to lower P450 2A6 activity.[60] They stated that cotinine glucuronidation was not significantly correlated with the 3HCOT/cotinine ratio “suggesting no interaction” between the two. However, a significant positive correlation between the percentage of cotinine excreted as a glucuronide and the plasma 3HCOT/cotinine ratio was found for the data in the paper. That is, when the 3HCOT/cotinine ratio was low, the percentage of cotinine glucuronidated was low. Even more striking was the positive correlation observed between urinary cotinine glucuronide concentrations and the plasma 3HCOT/cotinine ratio. As the ratio decreased the amount of cotinine glucuronide excreted decreased. This is exactly the reverse of what one would expect if the ratio was only reflecting P450 2A6 activity, since as cotinine metabolism

by C-oxidation decreases the amount of cotinine metabolized by *N*-glucuronidation would increase, not decrease. Taken together these data suggest that the 3HCOT/cotinine ratio is affected by the extent of cotinine glucuronidation. Low glucuronidation in African Americans will increase the plasma concentration of cotinine, leading to a lower 3HCOT/cotinine ratio, potentially resulting in misclassification of African American smokers for *CYP2A6* activity. Some of the smokers in the lowest 3HCOT/cotinine quartile in this study may be UGT2B10 null. Data to support this conclusion is in a second publication by these authors[56] and is discussed below.

In another publication looking at nicotine uptake and metabolism in African Americans the authors conclude from their data on plasma 3HCOT/cotinine ratios and the sum of cotinine and 3HCOT in plasma (as a measure of nicotine dose) that African Americans compared to Whites are “less likely to titrate their nicotine content” when they metabolize nicotine less efficiently.[56] This conclusion conflicts with our data on *CYP2A6* genotype and TNE in African Americans (Figure 3) and may be a misinterpretation of their data. Comparing plasma cotinine levels and the 3HCOT/cotinine ratio between African Americans and Whites and not considering UGT2B10 genotype is problematic. An unusual observation noted by the authors was that African Americans but not Whites in the lowest quartile of the 3HCOT/cotinine ratio had higher cotinine levels. The simplest explanation for this is that a significant number of the African American smokers in this quartile do not glucuronidate cotinine and therefore have higher plasma cotinine levels. The relatively higher cotinine would result in a lower 3HCOT/cotinine ratio, and some of these smokers may be misclassified as having low *CYP2A6* activity. These data led us to question the conclusion of the paper, that African Americans do not alter their smoking as a function of *CYP2A6* activity.

CYP2A6 genetic variants and smoking

In both Japanese and White populations, several studies have shown that self-reported CPD are lower for smokers who carry *CYP2A6* variant alleles that code for little or no active P450 2A6 compared to smokers who carry none of these variant alleles.[51, 61–65] Fujieda *et al* reported 2-fold lower CPD for Japanese smokers homozygous for *CYP2A6**4 compared to smokers with no *4, *7, *9, *10 or *11 alleles.[51] In addition, a GWAS in Japanese reported an association between the *CYP2A6* deletion and CPD.[65] Similarly, a GWAS of over 60,000 smokers of European descent found an association between CPD and a SNP in *CYP2A6* that is linked to *CYP2A6**2.[64] However, in these as well as other populations, it has often not been possible to detect an effect of *CYP2A6* genotype on CPD. In some populations this may be due to the low frequency of the *CYP2A6* variants studied, or their modest effect on metabolism, but it is also due to the fact that CPD is a relatively crude measure of smoking dose. *CYP2A6* genotype has been shown to influence smoking intensity (mean and total puff volume)[66] and we have reported a significant association between TNE and *CYP2A6* diplotype for African Americans, Latinos, Whites and Japanese Americans.[31] That is, TNE values decreased with the predicted P450 2A6 activity for the diplotypes categories, across these ethnic/racial groups (Figure 3). Also, in a recent study of Shanghai Chinese we found a significant difference in mean TNE levels of smoker predicted by their genotype to be poor *CYP2A6* metabolizers compared to predicted normal metabolizers.[67] In the same study

there was no significant difference in CPD across *CYP2A6* predicted metabolizer groups. These data support TNE as a useful biomarker for the study of *CYP2A6* and smoking dose. The use of TNE as a biomarker of smoking dose in future studies may allow a better assessment of the contribution of *CYP2A6* genotype to smoking and lung cancer.

CYP2A6 genetic variants and lung cancer

An association of CYP2A6 genotype and lung cancer has been found consistently in Japanese smokers, a group with a relatively high frequency of CYP2A6 null alleles.[51, 52, 68–70] However, a lack of statistical power in ethnic/racial groups with lower frequencies of CYP2A6 null alleles has made establishing an association in these groups more challenging. In addition, the robust association of genetic variation in the nicotinic receptor subunit CHRNA5 with lung cancer risk in is a potential confounding variable in some populations. The risk allele of CHRNA5, which is associated with lung cancer and smoking dose is common in smoker of European descent but rare in Asians and Africans.[71, 72]

The direct link between CYP2A6 and smoking dose and cancer has been established in a handful of studies in Asian populations. In Japanese smokers, studies have found a significant association between lung cancer, smoking and the *CYP2A6* deletion variant, either alone or in combination with other reduced function variants, including *CYP2A6* *7 and *9.[51, 69]. Also, the GWAS that found a significant association between the *CYP2A6* deletion and CPD reported a modest association with lung cancer.[65] Early studies in Chinese populations found no association between *CYP2A6**4 and lung cancer, but several of these studies included non-smokers, and did not take into account other *CYP2A6* variants and the lower frequency of *CYP2A6**4 among Chinese compared to Japanese.[73–75] Recently, we reported in a nested case control study in Shanghai Chinese that *CYP2A6* genetic variants (*4,*7,*9,*1A) were associated with a reduced risk of lung cancer.[67] In the same study the various diplotypes of these variants were confirmed by nicotine metabolism phenotype to confer reduced enzyme activity. The association between *CYP2A6* genotype and lung cancer was no longer significant after adjusting for TNE, supporting the hypothesis that the association was the result of the relationship between nicotine metabolism and smoking dose.

In smokers of African and European ancestry studies that support a role of CYP2A6 in lung cancer risk are limited. A relatively large nested case control study in a European cohort reported a significant association between lung cancer and *CYP2A6* *2, which codes for non-functional enzyme. An interaction with CPD was observed in that study.[62] Another study that found an association of CYP2A6 with lung cancer in smokers of European ancestry was carried out with cases and controls selected from a GWAS that previously found an association with lung cancer and the CHRNA5-A3-B4 gene cluster.[63, 76] An additive association of CYP2A6 genotype and a tag single nucleotide polymorphism in CHRNA5 with lung cancer risk and reported CPD was reported.[63] An independent association with CYP2A6 genotype (*2,*4,*9,*12) was reported only in smokers of <20 CPD.[63] Recently, in a study of two African American cohorts a reduced lung cancer risk was reported for carriers of reduced activity *CYP2A6* alleles.[77] Independently, we reported that

CYP 2A6 genotype, including CYP*17 the most frequent variant in African Americans and most of the other alleles included in the lung cancer study are associated with a reduced level of TNE (Figure 3).[31]

Conclusions

As the primary catalyst of nicotine metabolism P450 2A6 clearly influences smoking dose and this in turn is likely the mechanism by which CYP2A6 genotype is associated with lung cancer. The use of quantitative measures of smoking dose, such as TNE, as well as improved measurement and interpretation of nicotine metabolism phenotypes should allow one to determine the contribution of CYP2A6 genotype to lung cancer risk across different ethnic/racial groups.

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Abbreviations

3HCOT	*trans* 3’-hydroxycotinine
CPD	cigarettes per day
TNE	total nicotine equivalents

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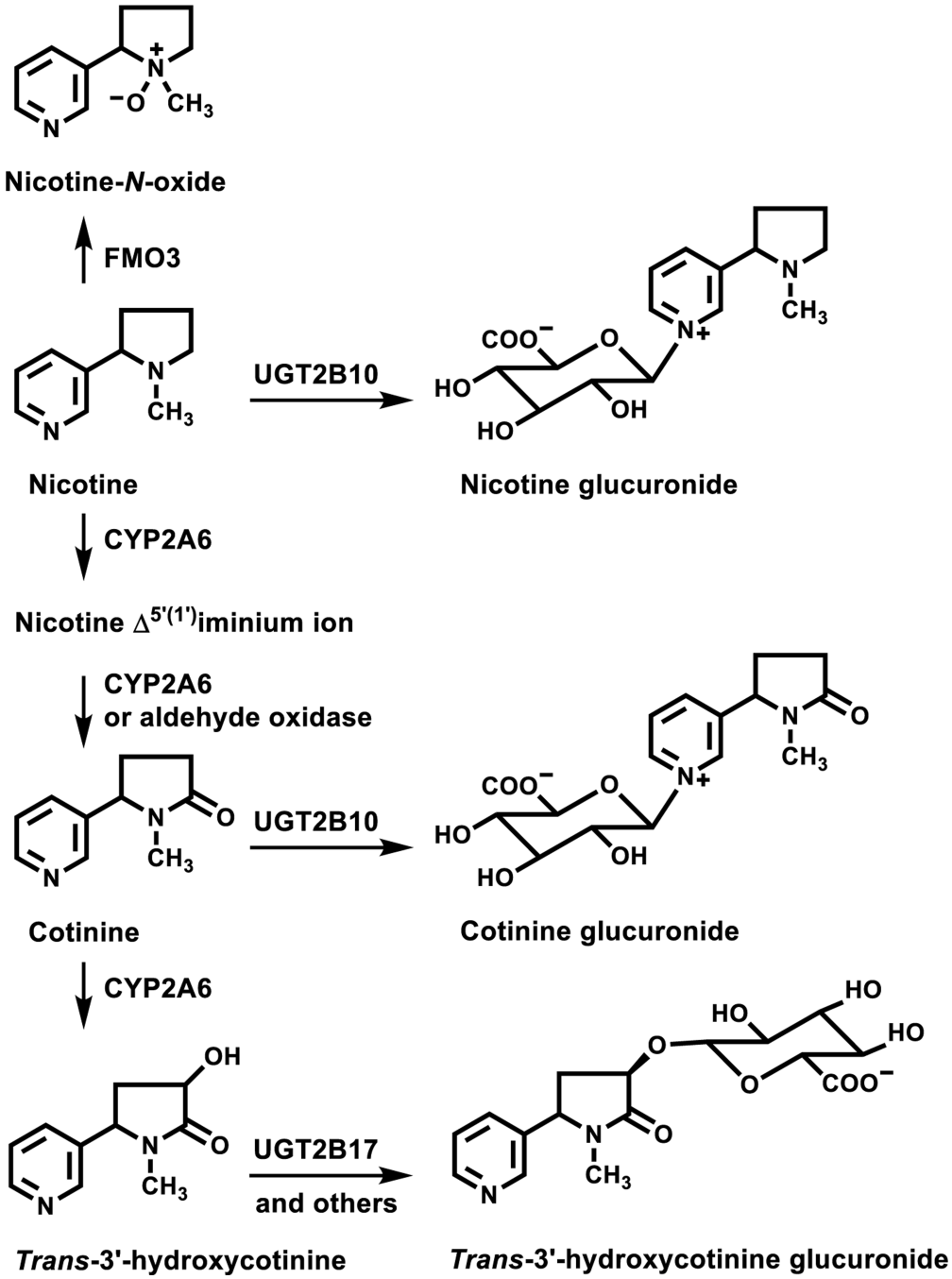

Figure 1.
Nicotine metabolic pathways. Adapted from Murphy *et al*, Carcinogenesis (2014) 35

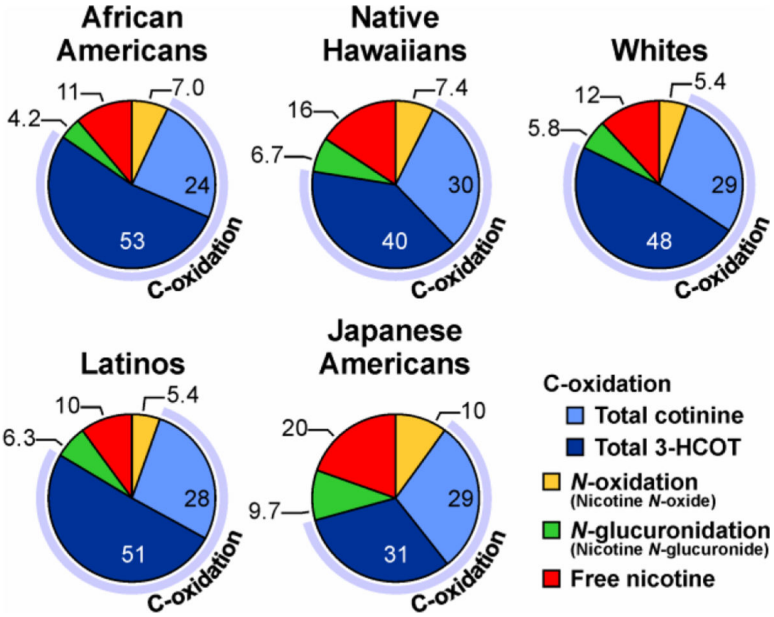

Figure 2.

Proportion of nicotine metabolized by C-oxidation, *N*-glucuronidation and *N*-oxidation in five ethnic/racial groups (n=2239 from a subset of the Multiethnic Cohort). The values are the molar percent of nicotine and six metabolites excreted in urine, and each slice of the pie is the mean percentage of the compound relative to TNE. From Murphy *et al*, Carcinogenesis (2014) 35 (11): 2526–2533. Used by permission of Oxford University Press.

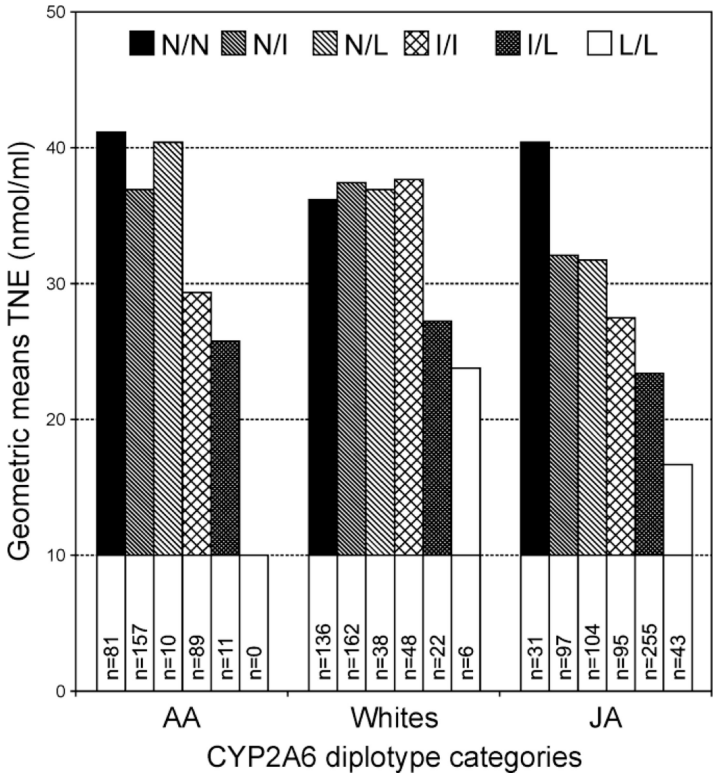

Figure 3.

TNE by CYP2A6 diplotype. Diplotypes categories are defined by the functional activity of each allele as follows: N (no variant allele or **1A +*14*); I (intermediate activity), **1H, *1A, *9, *17, *23*; L (little or no activity), **4, *1A+2, *1H+2, *12, *1H+*7, *7*. Alleles as described http://www.cypalleles.ki.se/cyp2a6.htm. From Murphy *et al*, Carcinogenesis (2016) 37 (3): 269–279. Used by permission of Oxford University Press.

Table 1.

Geometric means of nicotine metabolite ratios stratified by UGT2B10 genotype[9].

	UGT 2B10 Genotype													
	Asp67Tyr Variant (rs6175900)				Splice variant (rs116294140)						Both variants			
	ALL*				ALL*			African Americans			All			
Measures of Enzyme Activity		N	means	(95% CI)		N	means	(95% CI)	N	means	(95% CI)	Genetic Score[T]	N	means	(95% CI)
UGT2B10															
Nicotine glucuronide/ nicotine	GG	2028	0.377	(0.344–0.414)	AA	1853	0.39	(0.352–0.429)	156	0.31	(0.225–0.421)	0	1664	0.45	(0.402–0.496)
	GT	204	0.126	(0.08–0.201)	CA	333	0.23	(0.128–0.411)	166	0.19	(0.143–0.264)	1	503	0.19	(0.155–0.224)
	TT[§]	2	0.00		CC[§]	48	0.01	(0.008–0.027)	41	0.02	(0.01–0.036)	2[§]	67	0.00	(0–0.001)
	p-value**			<.0001				<0.0001			<.0001				<0.0001
Cotinine glucuronide/ cotinine	GG	2031	1.12	(1.07–1.17)	AA	1857	1.28	(1.22–1.38)	157	1.10	(0.97–1.24)[†]	0	1668	1.38	(1.33–1.44)
	GT	205	0.54	(0.43–0.68)	CA	333	0.63	(0.5–0.80)	166	0.48	(0.42–0.54)	1	504	0.68	(0.63–0.73)
	TT[§]	1	0.01		CC[§]	47	0.02	(0.02–0.03)	40	0.02	(0.02–0.03)	2[§]	65	0.01	(0.005–0.013)
	p-value**			<0.0001				<0.0001			<.0001				<0.0001
CYP2A6															
Total 3-HCOT/ Total Cotinine	GG	2032	1.38	(1.34–1.43)	AA	1858	1.33	(1.29–1.38)	157	1.65	(1.469–1.853)	0	1668	1.29	(1.24–1.34)
	GT	205	1.84	(1.56–2.18)	CA	333	1.63	(1.31–2.02)	166	2.24	(2.003–2.513)	1	504	1.78	(1.66–1.90)
	TT[§]	2	4.06	(2.82–5.30)	CC[§]	48	3.76	(3.03–4.67)	41	4.75	(3.794–5.952)	2[§]	67	3.09	(2.04–4.69)
	p-value**			0.0011				0.0006			<.0001				<0.0001
Total 3-HCOT/ Cotinine	GG	2031	3.21	(3.10–3.32)	AA	1857	3.23	(3.11–3.35)	157	3.78	(3.35–4.26)	0	1668	3.23	(3.10–3.37)

	UGT 2B10 Genotype												
	Asp67Tyr Variant (rs6175900)				Splice variant (rs116294140)						Both variants			
	ALL*				ALL*			African Americans			All			
Measures of Enzyme Activity		N	means	(95% CI)		N	means	(95% CI)	N	means	(95% CI)	Genetic Score†	N	means	(95% CI)
	GT	205	3.35	(2.82–3.98)	CA	333	2.90	(2.32–3.63)	166	3.64	(3.23–4.09)	1	504	3.19	(2.97–3.43)
	TT§	1	5.35		CC§	47	3.72	(2.97–4.66)	40	4.52	(3.57–5.72)	2§	65	3.11	(2.01–4.81)
	p-value**			0.631				0.6264			0.2706				0.9452

*Incudes all 5 ethnic groups, values are adjusted for age, sex, creatinine (natural log), BMI (natural log), and race

**Adjusted for age, sex, creatinine (natural log), BMI (natural log)

†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

§if n<10 the mean (minimum-maximum) are presented, not the geometric means and 95% Cis