***Short Communication***

# Nicotine Metabolism and CYP2D6 Phenotype in Smokers

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## Abstract

**We tested the hypothesis that the polymorphic enzyme *CYP2D6* is related to nicotine metabolism in 261 healthy subjects enrolling in a smoking cessation clinic. Subjects completed a questionnaire, were given dextromethorphan, and contributed a urine and blood sample. The *CYP2D6* phenotype (based on a determination of dextromethorphan and metabolites in an aliquot of overnight urine) and genotype (based on characterization of *CYP2D6* variant alleles by a PCR-based method on a subset) were determined. Seventeen poor metabolizers (6.5%) were observed among 261 phenotyped smokers. Nicotine and it chief metabolites, cotinine and *trans*-3′-hydroxycotinine were measured in the urine and adjusted for pH. All of the nicotine metabolite levels were significantly related to usual and recent smoking. Neither levels of smoking nor nicotine metabolites overall exhibited a relationship to the *CYP2D6*-deficient metabolizer phenotype. The ratio of nicotine:cotinine + *trans*-3′-hydroxycotinine, stratified by time since the last cigarette, was unrelated to gender, age, education, race (white/African American), recent alcohol or caffeine consumption, or smoking practices. Subjects in either the lowest quintile or decile metabolic ratio (ultrametabolizers) exhibited a significantly lower nicotine:cotinine + *trans*-3′-hydroxycotinine ratio after adjustment for recent smoking, pH, and other factors. These data suggest that the polymorphic *CYP2D6* gene is not a major contributor to nicotine metabolism in tobacco smokers but may influence the disposition of nicotine in the small subset of the population who are CYP2D6 ultrametabolizers.**

## Introduction

We report a study to test the hypothesis that CYP2D6 contributes significantly to the disposition of nicotine in smokers. *CYP2D6* (debrisoquine hydroxylase) has been studied as a putative lung cancer susceptibility factor, and it has been proposed that this enzyme may contribute to the metabolism of nicotine, thereby influencing the amount a person smokes and the delivery of carcinogens. Some *in vitro* (1) and population (2) studies have suggested a role for CYP2D6 in nicotine metabolism, although recent work indicates CYP2A6 is the most important P-450 in nicotine metabolism (3, 4). The CYP2D6 MP[2] is determined by administering DM (5) and determining the ratio of urinary metabolites. A low ratio of unchanged drug:metabolite identifies extensive metabolizers, whereas PMs exhibit a high ratio. The hypothesis that a polymorphic gene influences nicotine disposition is important because addicted smokers engage in smoking behavior in such a way as to maintain plasma nicotine levels in a constant range (6), and the disposition of nicotine will affect smoking behavior and therefore affect the delivery of carcinogens. We chose to characterize *CYP2D6* and nicotine metabolic profile in a large free-living clinic population to determine whether the *CYP2D6* genotype was associated with differences in nicotine metabolite disposition in a group of healthy smokers.

## Materials and Methods

Healthy smokers (age, ≥18 years) with a smoking history of >1 year who smoked >5 cigarettes/day were recruited from Philadelphia and Washington, D.C. by varied newspaper advertisements and flyers for smoking cessation clinics. Subjects with major medical illness, cancer, pregnancy, or psychiatric illness that precluded informed consent or who required certain medications known to interfere with *CYP2D6* phenotyping (neuroleptics, antidepressants, quinidine, and narcotics) were excluded. Subjects completed a self-administered questionnaire on recent and remote smoking, previous quitting attempts, use of other tobacco, passive exposure, brand, and caffeine use; two standard personality questionnaires; and a specific inventory measuring tobacco addiction (7, 8). DM phenotyping, nicotine and metabolite assays and pH adjustment (9, 10), CYP2D6 genotyping (11), and DNA extraction from peripheral blood were conducted by standard methods. DM and DR were assayed, and the metabolic ratio (DM:DR) was calculated. PMs, corresponding to homozygous mutations in CYP2D6, were defined as DM:DR > 0.30 (12). Spearman's rank correlation coefficients and standard regression methods were used to test for associations between the nicotine:cotinine ratio, the debrisoqiune metabolic ratio, and phenotype/genotype, demographic factors, and smoking characteristics.

## Results

Among 261 phenotyped subjects with complete data, there were 17 PMs (6.5%). Among a smaller subset of 31 African

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[2] The abbreviations used are: MP, metabolic phenotype; N:C, nicotine:cotinine + *trans*-3′-hydroxycotinine; DM, dextromethorphan; DR, dextrorphan; PM, poor metabolizer; MR, metabolic ratio; BMI, body mass index.

*Table 1* *P* values for predictors of N:C ratio among *CYP2D6* phenotypic ultrametabolizers

| | Ultrametabolizers[a] | | |
|---|---|---|---|
| | 5% | 10% | 20% |
| N | 14 | 25 | 50 |
| Predictors | | | |
| CYP2D6 MR | 0.002 | 0.001 | 0.02 |
| Sex | 0.25 | 0.23 | 0.47 |
| Race | 0.62 | 0.66 | 0.70 |
| BMI (continuous) | 0.07 | 0.07 | 0.09 |
| Ph (urine) | 0.001 | 0.001 | 0.003 |
| Age | 0.08 | 0.05 | 0.09 |
| No. of cigarettes overnight | 0.30 | 0.37 | 0.40 |
| Nicotine | 0.64 | 0.57 | 0.59 |

[a] Ultrametabolizers defined as those in lowest 5th, 10th and 20th percentile of the CYP2D6 MR distribution.

Americans, the percentage of PMs was similar (2 of 31 subjects, 6.5%). Over half of the participants were women (63%), the mean age was 44.8 years (SD = 11.2 years). Participants smoked 21.0 cigarettes/day (SD = 11.1 cigarettes/day) and had an average age of smoking initiation of 16.6 years (SD = 3.7 years).

For validation purposes, *CYP2D6* genotyping was performed on a subset of 141 individuals (119 Caucasians and 22 African Americans). The *CYP2D6*3* (A2637 deletion) and *CYP2D6*4* (splicing defect) mutations were determined. The genotype distribution (homozygous normal:heterozygous:homozygous deficient) was 74:37:8 in Caucasians and 14:6:2 in African Americans. The 10 individuals homozygous for *CYP2D6* variant alleles (specifically, the *CYP2D6*4* mutation) all had MR $>$ 0.30, corresponding to 7% of the sample as PM. This is consistent with other population studies in healthy subjects. Hardy-Weinberg conditions are met in both the Caucasian and African American samples. One of 43 heterozygous subjects exhibited a MP in the PM range, suggesting that this individual had an additional rare mutation. All 88 subjects in whom no mutations were detected had MPs in the normal range.

Nicotine, cotinine, and *trans*-3′-hydroxycotinine and total metabolites recovered in the urine were all significantly related to recent smoking. Nicotine was significantly associated with the number of cigarettes smoked in the previous 24 h ($r = 0.16$; $P = 0.01$), overnight ($r = 0.17$; $P = 0.006$), and since waking ($r = 0.18$; $P = 0.005$). Similar associations were observed for all of the other urinary metabolites with the indices of recent smoking; for example, a cigarette smoked in the past 24 h was significantly related to *trans*-3′-hydroxycotinine ($r = 0.24$; $P = 0.0002$), cotinine ($r = 0.26$; $P = 0.0001$), and total metabolites ($r = 0.26$; $P = 0.001$). There were no significant associations of the metabolites with gender, age, race, nicotine content of cigarette brand, caffeine or alcohol intake, BMI, or education level. The ratio of nicotine to its chief metabolites cotinine and *trans*-3′-hydroxycotinine (N:C) was used as a measure of the metabolic capacity to eliminate nicotine. The ratio was unrelated to measures of smoking (cigarettes smoked yesterday, overnight, or since awakening; nicotine level of brand; usual number of cigarettes/day), other exposures (alcohol, caffeine), or age, race, or gender.

The *CYP2D6* phenotype was analyzed in relation to nicotine metabolism as both a continuous (*i.e.*, as the MR) and a categorical variable (as the MP). There was no relationship between the MR and questionnaire measures of recent or remote smoking or between the MR and nicotine metabolites. Overall, there was no association of the adjusted (pH, recent smoking, BMI) N:C ratio to the metabolic ratio ($P = 0.34$). Further adjustment for race, age, gender, and nicotine content of cigarettes did not alter these findings. The half-life of nicotine is shorter than the half-life of other metabolites; therefore, this ratio might be affected by recent smoking. We examined the relationship of the nicotine ratio to the MP within smoking categories (*i.e.*, adjusting for recent smoking), but we did not observe any association with the MR.

The distribution of CYP2D6 phenotypes considering either three categories (*i.e.*, PM, IM, and extensive metabolizer) or two categories (PM *versus* all others) was unrelated to demographic variables (age, gender, race, BMI, and education), smoking (recent or usual), the Fagerstrom score (an index of nicotine dependency), or nicotine metabolites (individual, total, or the N:C ratio). The phenotype distribution was unrelated to marital status, BMI tertiles, or overnight, usual, or heavy smoking. Ultrametabolizer subjects (corresponding to individuals with gene amplification/duplication and very small metabolic ratios) comprise 1–8% of Caucasians (13, 14) and have recently been reported to exhibit increased prevalence among heavy smokers (15). We therefore examined subjects who ranked in the lowest 5%, 10%, and 20% of metabolic ratios to see if they exhibited an altered N:C after adjustment for potential confounders. The groups with the lowest 5%, 10%, and 20% metabolic ratios did exhibit a significantly lower N:C ratio after adjustment for age, sex, BMI, pH, race, nicotine content of the reported cigarette brand, and smoking during overnight urine collection (Table 1).

## Discussion

In summary, in this group of tobacco smokers, *CYP2D6* does not influence the disposition of nicotine or nicotine dependency; therefore, this gene is not likely to be a major influence on tobacco addiction. However, the findings are consistent with some effect of CYP2D6 in subjects with the lowest metabolic ratios, corresponding to the ultrametabolizer subjects. Caution is warranted in the interpretation because some misclassification is present with both the DM phenotype (minimized by selecting three different thresholds for the ratio used to identify ultametabolizers) and the nicotine ratio (the ratios studied are imperfect measures of nicotine metabolism because the half-life is different for diverse metabolites; this drawback is minimized by a long urine collection and stratification/adjustment by recent cigarette smoking). Other genes are likely to be important, and continued research is warranted to elucidate specific genetic factors that influence dependency on a drug that causes a major burden of human disease.

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