Genetic Influences on Individual Differences in Nicotine Glucuronidation

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Nicotine and its primary oxidative metabolites are metabolized in part by glucuronidation. Genetic variation in UGT isoenzymes that catalyze glucuronidation activity suggests that variation in glucuronidation rate is in part genetically determined. The relative contribution of genetic and environmental sources to individual differences in the rate of glucuronidation of nicotine, cotinine, and *trans*-3′-hydroxycotinine was estimated in a twin study of nicotine pharmacokinetics. Glucuronidation rate was defined using measures that either accounted for variability in renal clearance or assumed the same relative renal clearance of parent drug and glucuronide conjugate across individuals. The former definition resulted in highly correlated nicotine and cotinine glucuronidation measures that were substantially influenced by the combined effect of additive (heritable) and non-additive (dominant and epistatic) genetic effects. These findings suggest that genetic variation in UGT isoenzymes that act in additive and interactive ways is an important determinant of individual variability in nicotine and cotinine metabolism via glucuronidation pathways.

Keywords: nicotine metabolism, glucuronidation, twins, heritability

Nicotine and its primary metabolites cotinine and *trans*-3′-hydroxycotinine (3HC) are metabolized in part by glucuronidation by UDP-glucuronosyltransferase (UGT) isoenzymes (Figure 1) and are excreted in urine as glucuronide conjugates (Hukkanen et al., 2005). Glucuronide metabolites of nicotine, cotinine, and 3HC account for on average 25%–30% of total nicotine metabolites in urine (Benowitz et al., 1994; Byrd et al., 1992; Ghosheh & Hawes, 2002). To investigate the source of interindividual variability in the metabolism and clearance of nicotine we conducted a twin study to elucidate the relative contributions of genetic and environmental influences. Study subjects underwent a 30-minute infusion of stable isotope-labeled nicotine and its major metabolite, cotinine, followed by an 8-hour in-hospital stay. Blood and urine samples were taken at regular intervals for analysis of nicotine, cotinine, and metabolites. In prior publications we have presented similar analyses for total and renal clearance of nicotine and cotinine, as well as the metabolic clearance of nicotine to cotinine (primarily mediated by CYP2A6) (Benowitz et al., 2008; Swan et al., 2005). In the present study we examine the heritability of nicotine, cotinine and 3HC metabolism via glucuronidation. To the best of our knowledge no prior published twin studies have examined the metabolism of any drug by glucuronidation or other conjugation pathways.

Materials and Methods

The disposition kinetics of nicotine was measured in 110 monozygotic (MZ) and 29 dizygotic (DZ) twin pairs recruited from the Northern California Twin Registry (NCTR). The NCTR is a volunteer registry created in 1995 through an extensive advertising campaign that included advertising in 19 newspapers, San Francisco Bay area-wide movie theaters, and AM/FM radio stations. Within 2.5 years, this campaign resulted in the enrollment of a total of 1,054 twins, from which twins for the present study were recruited. Contact is maintained with NCTR twins via annual newsletters and birthday cards. A 5-year NCTR anniversary-celebration party held in July 2000 increased enrollment to 1,765 individual twins. Enrollment continues to this day and is done mostly through referrals by registered twins and through the registry website. Currently, there are over 2,700 twins registered with the NCTR.

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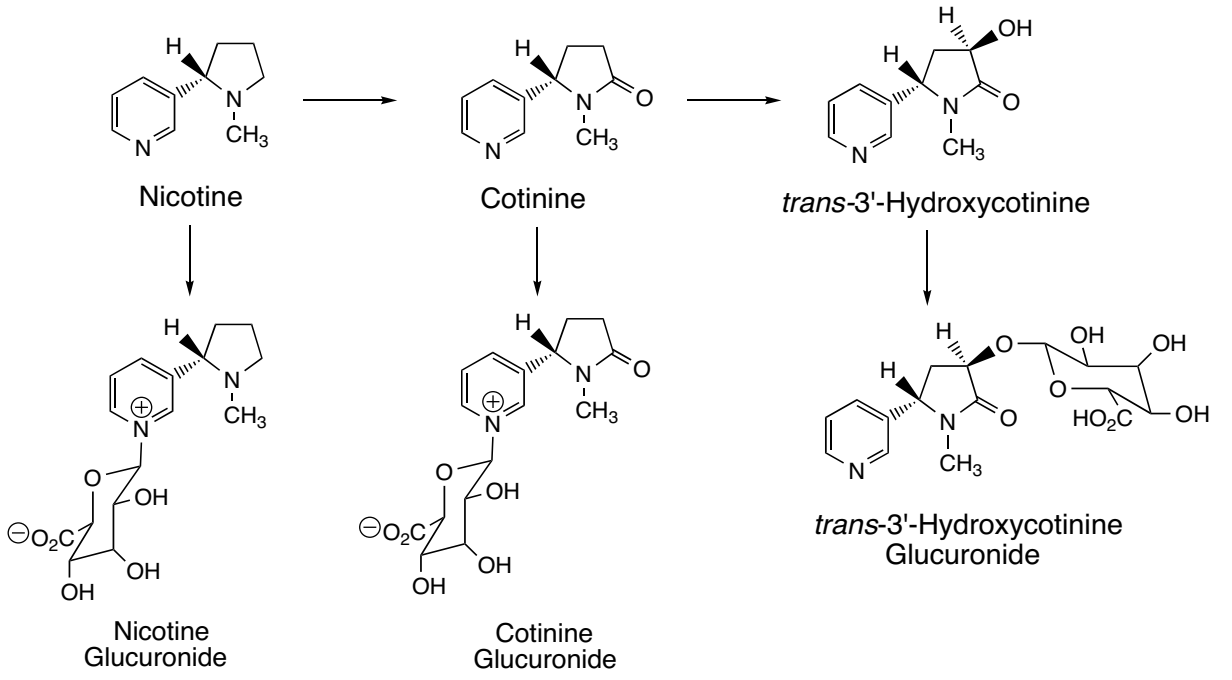

Figure 1

Metabolism of nicotine to cotinine and to trans-3 -hydroxycotinine and their respective glucuronide conjugates.

Stringent exclusion criteria for study participation resulted in a healthy sample. Study methodology has been described in detail (Swan et al., 2004). Briefly, exclusion criteria included ages younger than 18 or older than 65; obesity; pregnancy in women; use of medications known to affect drug metabolism (anticonvulsants or barbiturates); past year history of a host of medical conditions (e.g., uncontrolled hypertension or diabetes, liver or kidney disease, cancer, heart disease, psychiatric illness); and discomfort with venipuncture procedures.

After overnight fasting and overnight smoking abstinence (for smokers), subjects were administered a 30-minute intravenous infusion of deuterium-labeled nicotine (nicotine-d2) and its proximate metabolite cotinine (cotinine-d4), which are isotopic variants used to study kinetics and metabolism. Labeled compounds are necessary because individuals who use tobacco already have considerable levels of nicotine and cotinine in their bodies that would make measurement of clearance of unlabeled nicotine or cotinine impossible. Doses ranged from 0.5 to 2.0 μg/kg/min depending on smoking status and if a smoker, on the screening plasma cotinine concentration. Participants received 0.5 μg/kg/min if plasma cotinine levels were 50 ng/ml or lower (levels consistent with not smoking or smoking five or fewer cigarettes per day), 1.0 μg/kg/min if plasma cotinine levels were 50–150 ng/ml (levels consistent with smoking 5–15 cigarettes per day); and 2.0 μg/kg/min if plasma cotinine levels were > 150 ng/ml (levels consistent with smoking 15 or more cigarettes per day). The dose was always based on the lower plasma cotinine level within a twin pair so that both twins of pairs discordant for smoking received the same dose.

Details on synthesis of labeled compounds, experimental procedures and analytical chemistry have been described (Swan et al., 2004). Blood samples were taken at frequent intervals during and after infusion. Subjects voided prior to the start of the infusion and then urine was collected over the ensuing 8 hours.

Pharmacokinetic analysis: The rates of glucuronidation of nicotine, cotinine and 3HC were defined in two ways. One definition was based on the ratio of the glucuronide to parent compound in 8 hour urine samples (designated Nic-G-d2/Nic-d2, Cot-G-d4/Cot-d4, and 3HC-G/3HC-d4), which specifically captures the glucuronidation pathway. The use of these ratios assumes that everyone has the same relative renal clearance of parent drug and glucuronide conjugate. The second definition was the ratio of the amount of nicotine and cotinine glucuronide excreted in the urine to area under the plasma concentration time curve (AUC) of nicotine and cotinine over 8 hours of urine collection (designated Nic-G-d2/AUC Nic-d2 and Cot-G-d4/ AUC Cot-d4). This definition provides a broader measure of glucuronidation rate that accounts, at least in part, for individual variability in renal clearance. Plasma concentrations of 3HC were not measured therefore the broader definition of glucuronidation rate for 3HC could not be computed.

Statistical Analysis

Twin method. In twin pairs reared together, trait phenotypic variance can be decomposed into the additive

effects of genes (A), which contribute to twin pair similarity, to shared environmental effects common to twin siblings (C), which also contribute to twin pair similarity, and to nonshared environmental effects (E), which index experiences unique to each twin as well as measurement error and contribute to twin pair dissimilarity. Nonadditive genetic effects (D) also contribute to twin pair similarity and index interaction of gene alleles at the same locus (dominance) or at different loci (epistasis). Nonadditive genetic and shared environmental effects are confounded in twin studies and cannot be modeled simultaneously. In models without nonadditive genetic effects, the correlation between MZ twins is r_{MZ} = A + C, and that between DZ twins is r_{DZ} = 0.5A + C. A r_{MZ}/r_{DZ} ratio of 2 indicates a heritable trait and little to no importance of shared environment. A contribution of shared environment results in greater similarity of DZ cotwins relative to MZ cotwins and r_{MZ}/r_{DZ} < 2. The r_{MZ}/r_{DZ} is close to 1 if shared environmental influences are primary contributors to twin pair resemblance and additive genetic effects are not important. In the case of non-additive genetic effects, the MZ twin pair correlation is r_{MZ} = A + D and that of DZ twin pairs is *r*DZ = 0.5A+0.25D resulting in r_{MZ}/r_{DZ} > 2.

Biometric models. To identify the best models that fit the data, we compared model fit between general and nested models. The general model estimated all three A, C (or D), and E variance components ('ACE' or 'ADE' models). The fit of nested genetic ('AE' or 'AD'), shared environmental ('CE') or non-shared environmental ('E') models was compared to that of the general model using the likelihood ratio chi-square difference test. A significant difference (p < .05) in the –2 log likelihood (–2LL) statistics between the general and nested models at the difference degrees of freedom suggested deterioration of model fit of the nested model, thus favoring the general model. Best model fit was evaluated based on the chi-square difference test and on model parsimony indicated by the Akaike Information Criterion (Akaike, 1987) favoring the fit of a model with fewer estimated parameters; smaller or negative AIC indicates better model parsimony. Models were fit to MZ and DZ twin pair raw data, unadjusted, and adjusted for age, sex, body mass index (BMI), race/ethnicity, current cigarette smoking, and oral contraceptive use in women. The choice of covariates was based on exploratory analysis and earlier work showing a relationship between these measures and nicotine metabolism variables (Benowitz, Lessov-Schlaggar et al., 2006; Benowitz, Swan et al., 2006). Biometric analyses were conducted using Mx software (Neale et al., 2002).

Results

Of a total of 278 twins who underwent the infusion protocol, one MZ pair had missing data on all glucuronidation measures. An additional five twins from different MZ pairs and two twins from the same MZ pair had missing data on one of the variables due to urine analyte levels below the limits of quantitation. Sample demographics have been reported (Swan et al., 2005). Here, sample demographics are shown by zygosity (MZ vs. DZ) (Table 1). Overall, glucuronidation data were available from 109 MZ and 29 DZ twin pairs comprising 74 MZ and 19 DZ female same sex pairs, 35 MZ and 4 DZ male same sex pairs, and 6 DZ opposite sex pairs. MZ and DZ twins did not significantly differ on demographic characteristic. Group comparisons were conducted using logistic regression (zygosity group was the dependent variable) in STATA software (StataCorp, 2005). A robust estimator for standard errors was applied to adjust estimates for data non-independence.

Geometric mean values for the metabolite ratios are shown in Table 2 before and after removal of outliers > 3 *SD*s beyond sample means. Normal distributions were achieved using either logarithmic or square root transformation and the transformation with the best skewness and kurtosis statistics for the total sample as well as in MZ and DZ zygosity groups were selected for biometric analysis.

Phenotypic correlations between metabolite ratios are shown in Table 3. The ratio of 3HC-gluc-d4/3HC-d4 was not significantly correlated with Nic-G-d2/ Nic-d2 and had low, though statistically significant, correlations with the other three metabolite ratios (r = 0.17–0.19). Nic-G-d2/Nic-d2 was moderately correlated to Cot-G-d4/Cot-d4, Nic-G-d2/AUC Nic-d2, and Cot-G-d4/AUC Cot-d4 (r = 0.42–0.57) and these latter three ratios were strongly correlated with each other (r = 0.62–0.78).

MZ and DZ twin pair correlations and their ratios are shown in Table 4. Three metabolite ratios had r_{MZ}/r_{DZ} >2 suggesting the influence of non-additive genetic factors and the other two twin pair correlation ratios were close to 1, suggesting an important influ-

Table 1

Sample Demographic Characteristics

	MZ twins		DZ twins	
	n	Mean (*SD*) or %	*n*	Mean (*SD*) or %
Age	218	37.7 (12.0)	58	37.8 (11.8)
Body weight (kg)	218	70.4 (14.0)	58	72.7 (11.9)
BMI (kg/m^2)	218	24.9 (4.0)	58	25.4 (3.9)
Sex (% female)	218	67.9	58	75.9
Race/ethnicity (% Caucasian)	218	76.2	58	75.9
Current smoker	218	17.4	58	29.3
Former smoker		24.3		24.1
Never smoker		58.3		46.6
OC use in women (%)	148	27.7	44	22.7
OC use in women from DZ female–male pairs (%)			6	50.0

Note: BMI = body mass index; OC = oral contraceptives.

Table 2

Nicotine and Cotinine Glucuronide Metabolite Ratios

	n	Mean	*SD*	Min	Max
Nic-G-d2/Nic-d2	274	0.63	0.70	0	3.99
Cot-G-d4/Cot-d4	276	0.36	0.28	0	1.56
3HC-G-d4/3HC-d4	272	0.16	0.10	0	0.60
Nic-G-d2/AUC Nic-d2	275	13.92	10.52	0	67.35
Cot-G-d4/AUC Cot-d4	276	3.00	2.52	0	17.19
No Outliers					
Nic-G-d2/Nic-d2	266	0.55	0.52	0	2.38
Cot-G-d4/Cot-d4	269	0.34	0.23	0	1.19
3HC-G-d4/3HC-d4	269	0.15	0.09	0	0.42
Nic-G-d2/AUC Nic-d2	272	13.41	9.36	0	44.22
Cot-G-d4/AUC Cot-d4	271	2.80	2.07	0	10.22

Note: Nic-G-d2/Nic-d2, Cot-G-d4/Cot-d4, and 3HC-G/3HC-d4=ratio of nicotine, cotinine, or 3HC glucuronide to parent compound in urine; Nic-G-d2/AUC Nic-d2 and Cot-G-d4/AUC Cot-d4 = ratio of nicotine or cotinine amount excreted in urine to area under the plasma concentration time curve.

ence of shared environmental factors. All MZ twin pair correlations were significant. Due to the small DZ sample size (n = 26–29 pairs), three of five DZ twin pair correlations were not statistically significantly different from zero. Based on the twin pair correlation ratios, 'ADE' models were fit to data for Nic-gluc-d2/Nic-d2, Nic-gluc-d2/AUC Nic-d2, and Cot-gluc-d4/AUC Cot-d4, and 'ACE' models were fit to data for Cot-gluc-d4/Cot-d4 and 3HC-gluc-d4/3HC-d4. Because the sole influence of non-additive genetic effects cannot exist without the background of overall additive genetic effects, nested nonadditive 'DE' models were not considered.

Full and reduced biometric models are shown in Tables 5 and 6, before and after covariate adjustment, respectively. In unadjusted models, consistent with the pattern of twin pair correlations, variation in Cot-G-d4/Cot-d4 and 3HC–G/3HC-d4 was primarily attributable to shared environmental factors. The 'CE' model was best fitting for Cot-G-d4/Cot-d4 since dropping the shared environmental factor contributed to significant deterioration of model fit (p = .042), while dropping the additive genetic component did not significantly change model fit (in fact a p value for the difference –2LL between the 'ACE' and 'CE' models could not be estimated due to identical –2LL estimates in the two models). For Nic-G-d2/Nic-d2 and Nic-G-d2/AUC Nic-d2, variance components estimates in the full 'ADE' models showed a relatively greater contribution of nonadditive compared to additive genetic variance on phenotypic variability, while additive genetic effects appeared more important for Cot-G-d4/AUC Cot-d4. In every case, however, dropping the nonadditive component did not result in significant deterioration of model fit, but dropping both genetic components resulted in worsening of model fit. This pattern of results suggests that, overall, genetic effects are important contributors to individual differences in Nic-G-d2/Nic-d2, Nic-G-d2/AUC Nic-d2, and Cot-G-d4/AUC Cot-d4 metabolite ratios.

Biometric results did not change substantially after covariate adjustment. Shared environmental factors remained more important contributors to phenotypic variance of Cot-G-d4/Cot-d4 and 3HC-G/3HC-d4 metabolite ratios, and additive and non-additive genetic factors played a significant role on variance in Nic-G-d2/Nic-d2, Nic-G-d2/AUC Nic-d2, and Cot-G-d4/AUC Cot-d4 metabolite ratios.

Table 3

Correlations Between Metabolite Ratios

	Nic-G-d2/Nic-d2 (log)	Cot-G-d4/Cot-d4 (log)	3HC-G-d4/3HC-d4 (log)	Nic-G-d2/AUC Nic-d2 (sqrt)
Nic-G-d2/Nic-d2 (log)	—			
Cot-G-d4/Cot-d4 (log)	0.45 (0.35, 0.55)**	—		
3HC-G-d4/3HC-d4 (log)	0.08 (-0.04, 0.20)	0.19 (0.08, 0.31)**	—	
Nic-G-d2/AUC Nic-d2 (sqrt)	0.57 (0.48, 0.64)**	0.62 (0.54, 0.69)**	0.17 (0.05, 0.28)**	—
Cot-G-d4/AUC Cot-d4 (log)	0.42 (0.31, 0.51)**	0.78 (0.73, 0.82)**	0.19 (0.07, 0.30)**	0.78 (0.73, 0.82)**

Note: log = natural logarithm transformation; sqrt = square root transformation; numbers in parentheses are 95% confidence intervals; ** $p < .01$.

Table 4

Twin Pair Correlations

	n pairs	r_{MZ} (95% CI)	*n* pairs	r_{DZ} (95% CI)	r_{MZ}/r_{DZ}
Nic-G-d2/Nic-d2 (log)	100	0.32 (0.14, 0.49)	28	0.12 (–0.26, 0.47)	2.7
Cot-G-d4/Cot-d4 (log)	104	0.53 (0.47, 0.71)	26	0.45 (0.07, 0.71)	1.2
3HC-G-d4/3HC-d4 (log)	103	0.41 (0.24, 0.56)	28	0.52 (0.18, 0.75)	0.8
Nic-G-d2/AUC Nic-d2 (sqrt)	105	0.51 (0.36, 0.64)	29	0.05 (–0.33, 0.41)	10.2
Cot-G-d4/AUC Cot-d4 (log)	104	0.52 (0.36, 0.65)	29	0.20 (–0.18, 0.53)	2.6

Table 5

Model Fitting Results for Nicotine Metabolite Ratios in Unadjusted Models

	Model fit statistics					Model parameter estimates		
Metabolite Ratios	Model	–2LL	*df*	AIC	*p* diff	A (95% CI)	C or D (95% CI)	E (95% CI)
Nic-G-d2/Nic-d2 (log)	ADE	89.64	262	–434.36		2.24 (0, 47.6)	30.0 (0, 48.2)	67.8 (51.9, 86.3)
	AE	89.74	263	–436.26	.754	31.8 (13.4, 47.7)	—	68.3 (52.3, 86.6)
	E	100.72	264	–427.28	.004	—	—	100
Cot-G-d4/Cot-d4 (log)	ACE	–281.42	265	–811.42		0 (0, 57.3)	61.8 (4.0, 71.5)	38.2 (28.5, 50.6)
	CE	*–281.42*	*266*	*–811.42*	*uncalc*	—	*61.8 (49.4, 71.6)*	*38.2 (28.5, 50.6)*
	AE	–277.29	266	–809.29	.042	60.8 (47.9, 70.7)	—	39.3 (29.3, 52.1)
	E	–225.20	267	–759.20	< .001	—	—	100
3HC-G-d4/3HC-d4 (log)	ACE	–634.51	265	–1164.51		0 (0, 54.1)	43.9 (0, 56.9)	56.1 (42.1, 71.2)
	CE	–634.51	266	–1166.51	uncalc	—	44.0 (28.8, 56.9)	56.1 (43.1, 71.2)
	AE	–631.94	266	–1163.94	.109	45.9 (29.5, 59.4)	—	54.1 (40.6, 70.5)
	E	–607.69	267	–1141.69	< .001	—	—	100
Nic-G-d2/AUC Nic-d2 (sqrt)	ADE	855.73	268	320.68		0 (0, 59.9)	49.5 (0, 61.8)	50.5 (38.2, 65.5)
	AE	856.68	269	318.68	.329	48.8 (33.5, 61.4)	—	51.3 (38.6, 66.5)
	E	887.73	270	347.73	< .001	—	—	100
Cot-G-d4/AUC Cot-d4 (log)	ADE	378.31	267	–155.69		49.1 (0, 63.5)	2.4 (0, 63.1)	48.6 (36.5, 63.5)
	AE	378.31	268	–157.69	.976	51.4 (36.5, 63.5)	—	48.6 (36.5, 63.5)
	E	412.70	269	–125.30	< .001	—	—	100

Note: –2LL = –2 times log likelihood; df=degrees of freedom; AIC = Akaike Information Criterion; *p* diff = *p* value for the chi-square difference test between the full model and submodels; A, C, D, E = variance components estimates of additive genetic, shared environmental, nonadditive genetic, and individual-specific environmental factors; uncalc = uncalculable *p* value due to zero or near zero –2LL difference between models. Model in italics provides the best fit to the data.

Table 6

Model Fitting Results for Nicotine Metabolite Ratios in Adjusted Models*

	Model fit statistics					Model parameter estimates		
Metabolite Ratios	Model	–2LL	*df*	AIC	*p* diff	A (95% CI)	C or D (95% CI)	E (95% CI)
Nic-G-d2/Nic-d2 (log)	ADE	67.24	256	–444.76		0 (0, 42.4)	26.6 (0, 43.4)	73.4 (56.6, 92.3)
	AE	67.48	257	–446.52	.626	26.0 (7.1, 42.8)	—	74.0 (57.2, 92.9)
	E	74.65	258	–441.35	.025	—	—	100
Cot-G-d4/Cot-d4 (log)	ACE	–316.48	259	–834.48		11.8 (0, 68.7)	48.8 (0, 69.7)	39.4 (29.0, 52.6)
	CE	–316.31	260	–836.31	.677	—	39.8 (47.1, 69.9)	40.2 (30.1, 52.9)
	AE	–314.76	260	–834.76	.190	61.0 (48.1, 71.0)	—	39.0 (29.0, 51.9)
	E	–263.18	261	–785.18	< .001			100
3HC-G-d4/3HC-d4 (log)	ACE	–641.40	259	–1159.40		1.7 (1.6, 49.7)	39.8 (1.5, 53.5)	58.6 (44.6, 74.3)
	CE	–641.45	260	–1161.45	uncalc	—	41.2 (25.5, 54.8)	58.8 (45.2, 74.5)
	AE	–638.73	260	–1158.73	.102	42.6 (25.5, 56.8)	—	57.4 (43.2, 74.5)
	E	–618.60	261	–1140.60	< .001	—	—	100
Nic-G-d2/AUC Nic-d2 (sqrt)	ADE	835.34	262	311.34		0 (0, 56.2)	45.5 (0, 58.8)	54.5 (41.2, 70.5)
	AE	836.38	263	310.38	.306	44.4 (28.2, 58.0)	—	55.6 (42.0, 71.9)
	E	860.66	264	332.66	< .001	—	—	100
Cot-G-d4/AUC Cot-d4 (log)	ADE	357.41	261	–164.59		30.1 (0, 59.9)	16.7 (0, 60.0)	53.1 (39.8, 69.5)
	AE	357.46	262	–166.54	.821	46.6 (30.4, 59.9)	—	53.4 (40.1, 69.6)
	E	383.61	263	–142.39	< .001	—	—	100

Note: See Table 5 footnote for explanations.
*Covariates = age, sex, BMI, race/ethnicity, current smoking, and oral contraceptive use in women.

Discussion

Prior research has identified a high correlation in the ratios of glucuronide to parent drug between nicotine and cotinine. Therefore, nicotine and cotinine glucuronidation is thought to occur via the same pathway (Benowitz et al., 1994). Nicotine and cotinine glucuronidation appears to be catalyzed primarily by UGT2B10 and UGT1A4 and to a minor extent by UGT1A9 and UGT2B7 isoenzymes (Chen et al., 2007; Kaivosaari et al., 2007; Kuehl & Murphy, 2003; Nakajima et al., 1996). We expected, therefore, to observe high correlations between nicotine and cotinine glucuronide metabolite ratios and similarity in results in terms of genetic and environmental contribution to phenotypic variance.

Genetic variation in the *UGT2B10* gene is associated with reduced nicotine and cotinine glucuronidation (Chen et al., 2007). Functional genetic variation has also been identified in the *UGT1A4*, *UGT1A9*, and

UGT2B7 genes (Argikar et al., 2008), and variant *UGT1A4* genotypes have been associated with reduction in glucuronidation of the tobacco-specific nitrosamine NNAL (Ehmer et al., 2004). We expected, therefore, that nicotine and cotinine glucuronide metabolite ratios would be, at least in part, heritable phenotypes.

Both expectations were met with respect to the ratios of nicotine and cotinine excreted in the urine relative to respective areas under the plasma concentration-time curve. These two ratio measures were highly correlated (r = 0.78) and showed overall strong genetic contribution (combined additive and non-additive genetic variance) even after controlling for a number of covariates. The high estimate for non-additive genetic effects for Nic-G-d2/AUC Nic-d2 is due to the very large r_{MZ}/r_{DZ} ratio resulting from a near zero DZ twin pair correlation and a significant MZ twin pair correlation. The small sample of DZ twin pairs likely contributes to unreliable DZ twin pair correlation estimates, as also evidenced by the wide 95% confidence intervals. The Northern California Twin Registry from which twins for this study were recruited, has about three times more MZ than DZ twins, consistent with the zygosity distribution in this study. DZ twin pairs have been much more difficult to recruit to the registry. It is possible that because this is a volunteer-based registry, DZ twin pairs do not feel more alike than any two simblings and thus are less willing to participate in a twin registry compared to MZ twin pairs.

Because the relatively small number of DZ twin pairs reduced power to detect nonadditive genetic effects, 'ACE' models were also fit to Nic-G-d2/Nic-d2, Nic-G-d2/AUC Nic-d2, and Cot-G-d4/AUC Cot-d4 metabolite ratio data. The shared environmental estimate was zero in every case, and the additive genetic estimate was 26%, 44.4%, and 46.5% in adjusted models, respectively. The additive genetic component could be dropped from all models without deterioration of model fit, but the non-shared environmental models significantly worsened model fit. Taken together, biometric analyses suggest a substantial genetic contribution to individual variation in Nic-G-d2/AUC Nic-d2, and Cot-G-d4/AUC Cot-d4 (around 45% to 50% after combining the effects of additive and nonadditive genetic effects), more modest genetic contribution to variation in Nic-G-d2/Nic-d2 (around 30%), and largely shared environmental contribution to variation in Cot-G-d4/Cot-d4 and 3HC-G-d4/3HC-d4 metabolite ratios. Because renal clearances of nicotine and cotinine are pH-dependent, it is likely that variation in the metabolite ratios of Cot-G-d4/Cot-d4 and 3HC-G-d4/3HC-d4 is affected by urine pH, which in turn can be affected by diet and fluid intake. Thus the shared environmental influences could indicate commonality in diet in twin siblings. We do not have data on diet or on twin sibling cohabitation history, which could give some clues on likelihood of sharing dietary habits around the time of study participation.

The moderate correlation between Nic-G-d2/Nic-d2 and Cot-G-d4/Cot-d4 (r = 0.45) and differences in biometric results are most likely related to individual differences in the clearance of nicotine and cotinine in relation to their glucuronide metabolites, based on differences in urine pH and perhaps urine flow. Identification of enzymes that catalyze nicotine and cotinine glucuronidation is an active area of research and it is also possible that there are as yet unidentified proteins involved in glucuronidation of nicotine but not cotinine or vice versa.

Glucuronidation of 3HC is primarily catalyzed by UGT2B7 and to a lesser extent by UGT1A9 (Yamanaka et al., 2005). 3HC glucuronidation represents a different glucuronidation pathway from that of nicotine and cotinine as previously suggested (Benowitz et al., 1994) and as supported by the results in this paper, which show little to no correlation between the 3HC glucuronide ratio and either the nicotine or cotinine glucuronide ratios. No association between functional variants in either the *UGT2B7* or *UGT1A9* genes and nicotine-related metabolism has been reported to date.

Glucuronidation is for most people a minor nicotine metabolism pathway, but plays a larger role in nicotine metabolism in those who carry a slow metabolism variant allele of the polymorphic *CYP2A6* gene (Benowitz, Swan et al., 2006). Because 80% of nicotine is metabolized via the CYP2A6 pathway in those with normal CYP2A6 activity (Hukkanen et al., 2005) even a small decrease in metabolic rate via this major pathway could have significant impact on the contribution of minor metabolic pathways.

In summary, our findings suggest that genetic variation in UGT isoenzymes that act in additive and interactive ways is an important determinant of individual variability in nicotine and cotinine metabolism via glucuronidation pathways, as assessed by the Nic-G-d2/AUC Nic-d2 and Cot-G-d4/AUC Cot-d4 ratios.

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