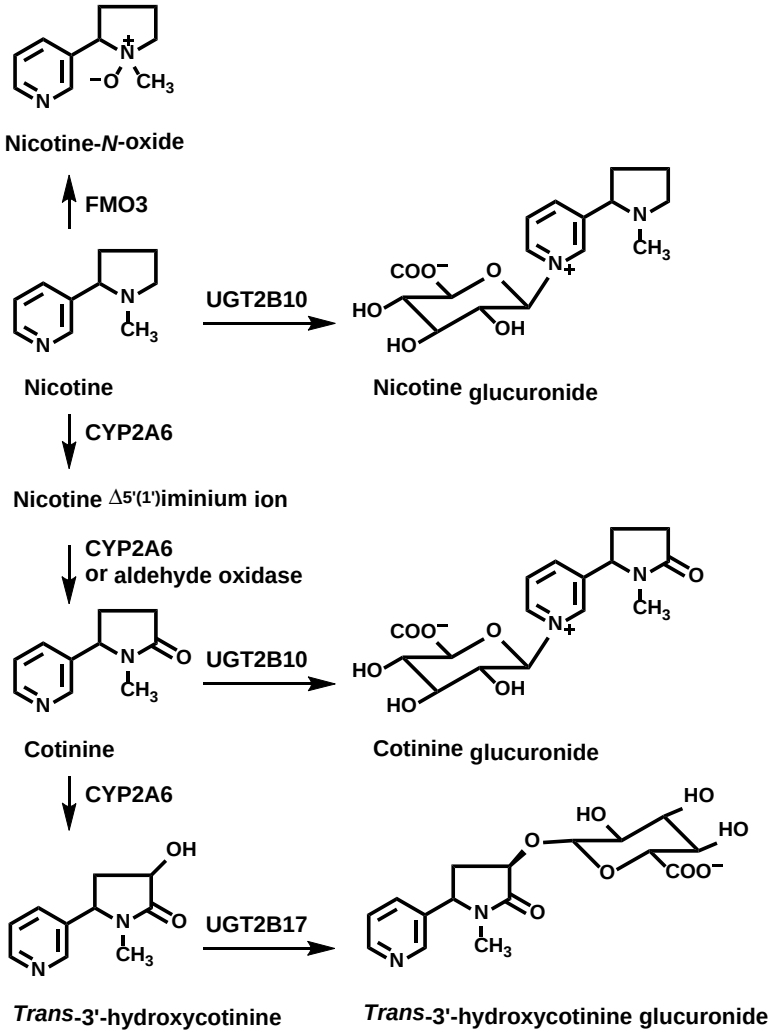
Web Materials

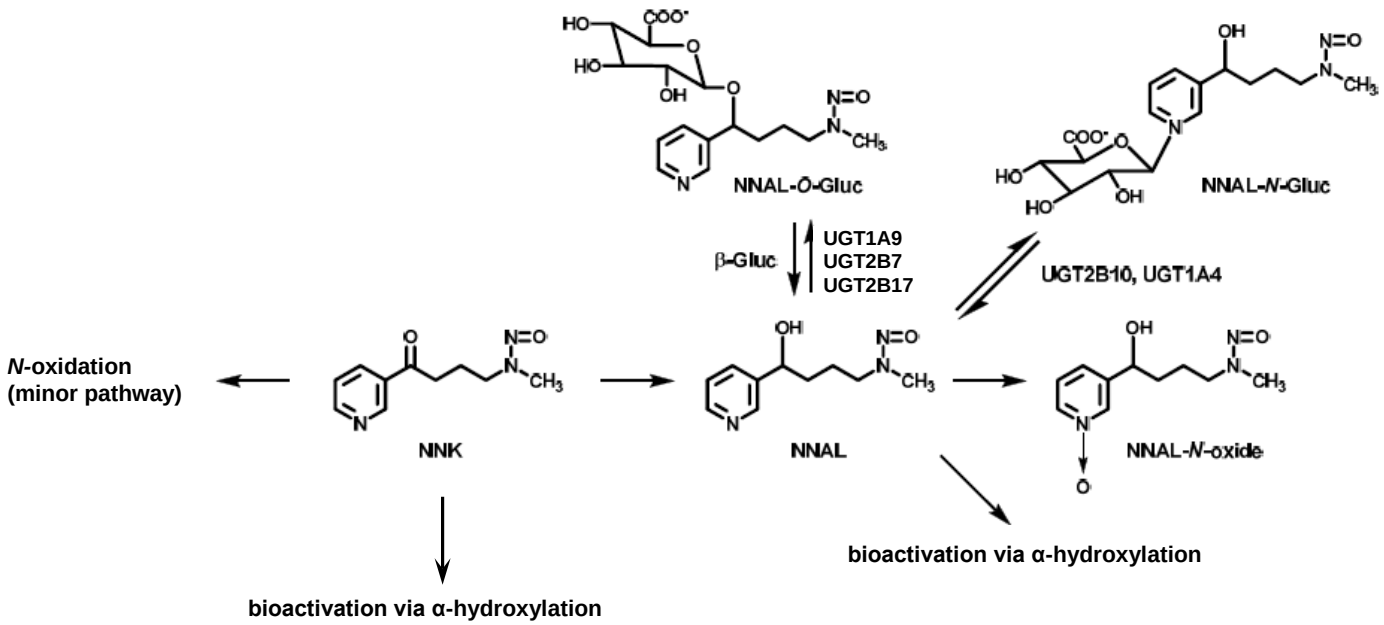
Web Figure 1. Primary pathways of nicotine metabolism (1).



Reference

1. Murphy SE, Park SS, Thompson EF, et al. Nicotine N-glucuronidation relative to N-oxidation and C-oxidation and UGT2B10 genotype in five ethnic/racial groups. *Carcinogenesis*. 2014;35(11): 2526-2533.

Web Figure 2. Primary pathways of NNK metabolism



Web Table 1. 19 analyzed biomarkers (calculation and any transformation applied in analysis).

Biomarker (*Y*)	**Calculation (from measured, if not directly measured)**	**Transformation**
Total nicotine equivalents (TNE) (nmol/ml)	total cotinine + total 3HC + total nicotine + nicotine *N*-oxide	Log (*Y*)
Free nicotine (nmol/ml)		Log (*Y*)
Free cotinine (nmol/ml)		$\sqrt[3]{Y}$
Free 3HC (nmol/ml)		Log (*Y*)
Nicotine *N*-oxide (nmol/ml)		Log (*Y*)
% free nicotine among TNE	free nicotine/TNE	Log (*Y*)
% nicotine glucuronidation among TNE	(total nicotine – free nicotine)/TNE	$\sqrt[3]{Y}$
% nicotine *C*-oxidation among TNE	(total cotinine + total 3HC)/TNE	
% nicotine *N*-oxide among TNE	nicotine *N*-oxide/TNE	Log (*Y*)
CYP2A6 activity 1	total 3HC/free cotinine	Log(*Y*)
CYP2A6 activity 2	total 3HC/total cotinine	Log(*Y*)
% nicotine glucuronidation	(total nicotine – free nicotine)/total nicotine	
% cotinine glucuronidation	(total cotinine – free cotinine)/total cotinine	
% 3HC glucuronidation	(total 3HC – free 3HC)/total 3HC	
% cotinine glucuronidation among nicotine *C*-oxidation products	(total cotinine – free cotinine)/(total cotinine + total 3HC)	
% free cotinine among nicotine *C*-oxidation products	Free cotinine/(total cotinine + total 3HC)	Log(*Y*)
% total 3HC among nicotine *C*-oxidation products	total 3HC /(total 3HC + total cotinine)	Log(*Y*)
Total NNAL (pmol/ml)		Log(*Y*)
% NNAL glucuronidation	(Total NNAL – free NNAL)/total NNAL	

Web Table 2. Distribution of the 19 biomarkers in three admixed groups in the Multiethnic Cohort (1993-now).

	African Americans (n= 362)	**Native Hawaiians (n=300)**	**Latinos (n=437)**
TNE (nmol/ml)	44.4 (26.8, 74.1)	30.2 (19.6, 46.7)	32.6 (20.9, 53.6)
Free nicotine (nmol/ml)	3.91 (1.76, 8.54)	4.05 (2.06, 8.01)	2.58 (0.90, 5.04)
Free cotinine (nmol/ml)	5.57 (3.27, 7.97)	4.24 (2.62, 6.12)	3.66 (1.80, 6.13)
Free 3HC (nmol/ml)	16.1 (8.34, 31.5)	8.6 (4.4, 17.1)	12.5 (5.8, 21.3)
Nicotine *N*-oxide (nmol/mL)	2.65 (1.36, 4.86)	1.87 (0.94, 3.54)	1.49 (0.64, 3.04)
% free nicotine among TNE	8.7 (4.8, 14.9)	13.5 (8.7, 20.5)	7.8 (4.0, 13.2)
% nicotine glucuronidation among TNE	3.18 (1.16, 5.22)	5.57 (3.37, 8.55)	4.54 (2.78, 7.39)
% nicotine *C*-oxidation among TNE	81.0 (71.9, 87.3)	72.7 (63.5, 81.0)	81.1 (72.4, 88.0)
% nicotine *N*-Oxide among TNE	5.84 (3.56, 9.07)	5.8 (4.07, 9.09)	4.41 (2.66, 7.02)
CYP2A6 activity 1: Total 3HC/free cotinine	4.41 (2.80, 7.40)	2.92 (1.78, 4.23)	4.71 (2.94, 7.33)
CYP2A6 activity 2: Total 3HC/total cotinine	2.10 (1.37, 3.45)	1.26 (0.79, 1.93)	1.8 (1.22, 2.63)
nicotine glucuronidation (%)	25.4 (12.1, 41.7)	30.4 (18.8, 41.9)	36.9 (23.6, 53.6)
cotinine glucuronidation (%)	48.0 (27.6, 61.2)	54.9 (45.3, 64.4)	60.2 (49.5, 69.3)
3HC glucuronidation (%)	26.8 (21.2, 35.1)	18.7 (13.6, 24.7)	22.5 (17.4, 29.4)
% cotinine glucuronidation among nicotine *C*-oxidation products	14.4 (7.1, 21.8)	23.4 (16.7, 32.0)	20.2 (14.8, 27.7)
% free cotinine among nicotine *C*-oxidation products	15.3 (9.9, 21.7)	19.4 (14.7, 25.5)	13.7 (9.6, 19.5)
% total 3HC among nicotine *C*-oxidation products	67.7 (57.8, 77.5)	55.7 (44.2, 65.9)	64.3 (55.0, 72.4)
Total NNAL (pmol/mL)	1.80 (1.01, 2.88)	1.06 (0.66, 1.61)	1.22 (0.73, 2.11)
NNAL glucuronidation (%)	74.2 (67.1, 80.5)	68.4 (62.4, 73.9)	71.2 (64.3, 77.3)

Data are medians (interquartile range). All P-values from Kruskal-Wallis test among ethnic groups were < 0.0001.

Web Table 3. Predicted differences in metabolic traits (Y) per 20% increase in ancestries, for relations with a P-value <0.05 between Y and any of the four ancestries in the Multiethnic Cohort (1993-now), from the basic and additional adjustment models

	African Ancestry				Amerindian Ancestry				Asian Ancestry				Hawaiian Ancestry			
	Basic adjustment *		Additional adjustment#		Basic adjustment *		Additional adjustment#		Basic adjustment *		Additional adjustment#		Basic adjustment *		Additional adjustment#	
Trait	Differences (95% CI)	P	Differences (95% CI)	P	Differences (95% CI)	P	Differences (95% CI)	P	Differences (95% CI)	P	Differences (95% CI)	P	Differences (95% CI)	P	Differences (95% CI)	P
Free cotinine (nmol/ml)[$]	.057 (.021, .094)	2.3E-03	.076 (.037, .115)	1.8E-04	-.038 (-.067, -.009)	0.01	-.026 (-.058, .006)	0.12	.033 (.015, .052)	0.0004	.033 (.014, .052)	8.8E-04	.015 (-.004, .034)	0.12	.02 (-.001, .041)	0.07
% cotinine glucuronidation [cotinine-Gluc/total cotinine]	-.074 (-.11, -.041)	1.3E-05	-.086 (-.121, -.05)	4.5E-06	.0008 (-.019, .021)	0.93	-.0083 (-.03, .014)	0.46	-.0197 (-.033, -.007)	0.0031	-.0196 (-.033, -.007)	0.00	-.0107 (-.024, .003)	0.11	-.0153 (-.03, -.001)	0.04
% cotinine-Gluc among C-oxidation products [cotinine-Gluc/(total cotinine + total 3HC)]	-.025 (-.042, -.009)	0.003	-.031 (-.049, -.013)	1.1E-03	-.007 (-.021, .007)	0.33	-.008 (-.024, .008)	0.31	-.003 (-.014, .008)	0.62	-.002 (-.013, .01)	0.76	-.001 (-.012, .01)	0.83	-.001 (-.014, .011)	0.82
% 3HC glucuronidation [3HC-Gluc/total 3HC]	-.0014 (-.019, .016)	0.88	-.0079 (-.027, .011)	0.42	-.0102 (-.024, .004)	0.15	-.0102 (-.026, .005)	0.19	-.0135 (-.022, -.005)	0.0020	-.0128 (-.022, -.004)	5.3E-03	-.0109 (-.02, -.002)	0.013	-.011 (-.021, -.001)	0.03
CYP2A6 activity: [total 3HC/free cotinine][$]	-.089 (-.19, .017)	0.10	-.11 (-.22, .009)	0.07	.092 (0, .184)	0.049	.06 (-.04, .167)	0.24	-.1 (-.17, -.034)	0.0034	-.1 (-.17, -.026)	0.008	-.04 (-.11, .027)	0.24	-.06 (-.13, .023)	0.17
% free nicotine among TNE[$]	.032 (-.098, .16)	0.63	.013 (-.13, .15)	0.86	-.008 (-.14, .12)	0.90	.055 (-.09, .2)	0.45	.09 (.02, .15)	0.0089	.081 (.01, .15)	0.020	.116 (.05, .18)	5.4E-04	.116 (.04, .19)	0.0023
Free nicotine (nmol/ml)[$]	.031 (-.098, .16)	0.64	.012 (-.13, .15)	0.87	-.016 (-.15, .11)	0.81	.055 (-.09, .2)	0.45	.09 (.03, .15)	0.006	.084 (.02, .15)	0.015	.11 (.05, .17)	9.6E-04	.106 (.03, .18)	0.00
Total NNAL (pmol/mL)[$]	.054 (-.025, .13)	0.18	.048 (-.036, .13)	0.26	-.013 (-.083, .06)	0.71	0 (-.079, .08)	0.99	-.064 (-.114, -.01)	0.01	-.051 (-.1, 0)	0.04	0 (-.051, .05)	0.99	-.007 (-.059, .05)	0.79
Nicotine N-oxide (nmol/mL)[$]	.13 (.02, .24)	0.022	.09 (-.03, .2)	0.14	-.09 (-.2, .03)	0.14	-.03 (-.16, .1)	0.65	.04 (-.02, .11)	0.18	.04 (-.03, .11)	2.4E-01	-.01 (-.07, .06)	0.86	.02 (-.06, .09)	0.63
% free cotinine among C-oxidation products [free cotinine/(total cotinine + total 3HC)][$]	.11 (.037, .19)	0.004	.13 (.053, .21)	1.2E-03	-.07 (-.13, 0)	0.05	-.04 (-.113, .04)	0.31	.08 (.04, .12)	1.3E-04	.08 (.034, .12)	5.0E-04	.04 (.001, .08)	0.05	.05 (.006, .1)	0.03
% nicotine N-Oxide among TNE[$]	.13 (.018, .24)	0.02	.08 (-.03, .2)	0.15	-.1 (-.22, .01)	0.08	-.04 (-.163, .09)	0.58	.04 (-.02, .11)	0.18	.04 (-.028, .11)	0.26	0 (-.069, .06)	0.91	.02 (-.052, .1)	0.56
% nicotine glucuronidation [nicotine-Gluc/total nicotine]	-.022 (-.052, .0084)	0.16	-.024 (-.057, .0095)	0.16	-.007 (-.039, .0243)	0.66	-.014 (-.049, .0214)	0.44	-.008 (-.026, .0092)	0.35	-.008 (-.026, .0102)	0.39	-.019 (-.036, -.0009)	0.041	-.017 (-.036, .0033)	0.10
% NNAL glucuronidation [(total - free NNAL)/total NNAL]	-.0079 (-.023, .0073)	0.31	-.012 (-.029, .0045)	0.16	-.01 (-.023, .0024)	0.11	-.004 (-.018, .01)	0.56	-.012 (-.02, -.0029)	0.010	-.009 (-.018, .0001)	0.0551	-.012 (-.021, -.0034)	0.0072	-.01 (-.02, -.0004)	0.04

The P-values are shown in red if <0.00156 (0.05/8/4) and in green if between 0.00156 and 0.05.

Percentages are shown as numbers between 0 and 1.

[$] For these Y's, differences shown are for log-transformed values, except for free cotinine where cubic root transformation was used.

* Basic adjustment for age at urine collection, sex, and creatinine (log-transformed) and TNE (log-transformed), and self-reported numbers of cigarettes smoked per day (CPD) during the last two weeks, where appropriate. Analysis of total NNAL and %NNAL-Gluc was also adjusted for batch of NNAL analysis.

Additional adjustment for BMI and physical activity, ethanol drinking and caffeine intake, education .

Web Table 4. Direction and P-values for suggestive associations (P<.05) between lifestyle factors and tobacco-derived biomarkers (Y)* in the Multiethnic Cohort (1993-now)

Population	Y	lifestyle factor	P-value	Direction of relation
African Americans (n=362)	TNE	Physical activity	0.05	+
		caffeine intake	0.01	+
		education	0.007	–
	% free nicotine among TNE	BMI	0.02	+
		caffeine intake	0.01	+
	% nicotine *N*-Oxide among TNE	BMI	0.02	+
		ethanol intake	0.0004	–
	% nicotine-Gluc among TNE	BMI	0.02	+
	Total NNAL	caffeine intake	0.03	+
	% cotinine glucuronidation	BMI	0.02	+
	% 3HC glucuronidation	education	0.04	–
	CYP2A6 activity3: total 3HC/free cotinine	ethanol intake	0.03	+
	Free nicotine	BMI	0.02	+
		caffeine intake	0.02	+
	Free 3HC	BMI	0.01	–
		caffeine intake	0.05	–
	Nicotine *N*-oxide	BMI	0.03	+
		ethanol intake	0.0004	–
	Free cotinine among *C*-oxidation	ethanol intake	0.04	–
	% cotinine-Gluc among *C*-oxidation	BMI	0.003	+
	Free cotinine	BMI	0.04	–
		ethanol intake	0.04	–
Native Hawaiians (n=300)	TNE	caffeine intake	0.03	+
	% nicotine-Gluc among TNE	education	0.004	+
	% nicotine glucuronidation	caffeine intake	0.03	+
		education	0.008	+
	% cotinine glucuronidation	BMI	0.009	+
	% cotinine-Gluc among *C*-oxidation	BMI	0.006	+
Latinos (n=437)	TNE	BMI	0.00003	–
		Physical activity	0.004	–
		ethanol intake	0.0005	–
		caffeine intake	0.002	+
		education	0.02	–
	CYP2A6 activity: % *C*-oxidation	Physical activity	0.00009	–
		education	0.02	–
	% NNAL glucuronidation	Physical activity	0.02	+
		education	0.03	+
	% 3HC glucuronidation	ethanol intake	0.04	+
	Free cotinine among *C*-oxidation	BMI	0.05	–
	Free cotinine	BMI	0.02	–
		Physical activity	0.02	–
		ethanol intake	0.01	–

*Results were adjusted for age at urine collection, sex, and creatinine (log-transformed) and TNE (log-transformed), self-reported numbers of cigarettes smoked per day (CPD) during the last two weeks, and other lifestyle factors (i.e. BMI, physical activity, ethanol intake, caffeine intake and education), where appropriate. For total NNAL and the percentage of NNAL-Glucs, batch of NNAL assays was adjusted. African ancestry was included for analysis in African Americans. African and Amerindian ancestries were included in analysis in Latinos and Asian and Hawaiian ancestries in analysis Native Hawaiians.

Web Table 5. Predicted differences in metabolic traits per 20% increase in ancestries for statistically significant relations ($p < 0.00156$) from linear regression with adjustment for lifestyle factors including estrogen use and history of birth control pill use in the Multiethnic Cohort (1993-now)

		Additional adjustment [c]	
Ancestry	**Trait**	**Differences (95% CI)**	***P***
African	Free cotinine (nmol/ml) [a]	0.079 (0.040, 0.12)	7.9×10^{-5}
	free cotinine among *C*-oxidation products [Free cotinine/(total cotinine + total 3HC)] [b]	0.14 (0.060, 0.22)	6.2×10^{-4}
	% cotinine glucuronidation [cotinine-Gluc/total cotinine]	-8.4 (-12.0, -4.8)	7.3×10^{-6}
	% cotinine glucuronidation among *C*-oxidation products [Cotinine-Gluc/(total cotinine + total 3HC)]	-3.1 (-4.9, -1.3)	9.5×10^{-4}
Asian	Free cotinine (nmol/ml) [a]	0.037 (0.018, 0.057)	2.3×10^{-4}
	free cotinine among *C*-oxidation products [Free cotinine/(total cotinine + total 3HC)] [b]	0.079 (0.036, 0.12)	3.8×10^{-4}

[a] Differences shown are for cube root-transformed values

[b] Differences shown are for log-transformed values

[c] Adjusted for age at urine collection, sex, creatinine (log-transformed), TNE (log-transformed), numbers of cigarettes smoked per day at urine collection, BMI, physical activity, ethanol drinking, caffeine intake, education, and use of estrogen in any form in women at urine collection (yes/no) and ever use of birth control pills at least a month (yes/no).

Web Figure 3. *P*-values From Admixture Mapping of Cotinine Glucuronidation in African Americans. The reference line indicates the significance level after adjusting for the number of selected independent ancestry informative markers ($P = 2.7\times10^{-7}$).