Research Article

Cancer Epidemiology, Biomarkers & Prevention

Nicotine Metabolite Ratio Predicts Smoking Topography and Carcinogen Biomarker Level

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Abstract

Background: Variability in smoking behavior is partly attributable to heritable individual differences in nicotine clearance rates. This can be assessed as the ratio of the metabolites cotinine and 3′-hydroxycotinine (referred to as the nicotine metabolism ratio; NMR). We hypothesized that faster NMR would be associated with greater cigarette puff volume and higher levels of total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a carcinogen biomarker.

Methods: Current smokers ($n = 109$) smoked one of their preferred brand cigarettes through a smoking topography device and provided specimens for NMR and total NNAL assays.

Results: Faster nicotine metabolizers (third and fourth quartiles versus first quartile) based on the NMR exhibited significantly greater total puff volume and total NNAL; the total puff volume by daily cigarette consumption interaction was a significant predictor of total NNAL level.

Conclusion: A heritable biomarker of nicotine clearance predicts total cigarette puff volume and total NNAL.

Impact: If validated, the NMR could contribute to smoking risk assessment in epidemiologic studies and potentially in clinical practice. *Cancer Epidemiol Biomarkers Prev; 20(2); 234–8. 2011 AACR.*

Introduction

The substantial variability in smoking behavior is attributable, in part, to heritable individual differences in nicotine clearance rates (1). Nicotine, the primary addictive compound in tobacco, is metabolized to cotinine (COT), and then to 3′-hydroxycotinine (3HC), predominantly by the hepatic CYP2A6 enzyme (2, 3). Smokers can extract varying levels of nicotine by altering their smoking topography (e.g., puff volume, number of puffs; refs. 4–6), which in turn can affect level of toxin exposure (6–8).

To provide a noninvasive assessment of CYP2A6 activity, a phenotypic marker has been characterized (9). The ratio of 3HC/COT, referred to as the nicotine metabolite ratio (NMR), reflects *CYP2A6* genetic variation and environmental factors influencing CYP2A6 activity and therefore nicotine clearance *in vivo* (10). The NMR is highly reproducible and independent of time since last cigarette (9, 11). Faster metabolizers of nicotine have higher smoking rates (12), and therefore may have increased risk for lung cancer (13, 14). We hypothesized that smokers with higher NMRs (faster nicotine metabolism) will exhibit (i) increased total puff volume, reflecting efforts to extract more nicotine from their cigarettes and (ii) increased total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) levels, reflecting the effect of total puff volume on toxin exposure. If confirmed, the NMR would be of value in assessment of risk from cigarette smoking.

Materials and Methods

To test this hypothesis, 109 smokers of 10 or more cigarettes per day and ages 18 to 65 years were recruited from participants in a nicotine replacement therapy trial (for exclusion criteria see ref. 15). The study protocol was approved by the Institutional Review Board of the University of Pennsylvania and all other analytical sites.

Participants were recruited from April 2005 to February 2006. Following informed consent and prior to initiating treatment, they completed measures of demographics, smoking history, current cigarette brand, and nicotine dependence level (Fagerstrom Test of Nicotine Dependence; FTND; ref. 16). They provided a 15-mL blood sample for analysis of nicotine metabolites, assayed at

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the University of California, San Francisco via liquid chromatography with tandem mass spectrometry (9). Genotyping for *CYP2A6* variants was done at the Centre for Addiction and Mental Health (Toronto, Canada) as previously described (17). A 30-mL urine sample was provided and assayed for total NNAL, the sum of NNAL, and its glucuronides at the University of Minnesota, per standard procedures (18).

In a smoking-approved ventilated room, participants smoked one of their own brand cigarettes under *ad libitum* conditions, using a smoking topography device (Clinical Research Support System) validated in previous research (19, 20). Participants were asked to refrain from smoking for 1 hour prior to their laboratory smoking session, and were generally compliant (mean = 64.3 minutes; SD = 35.4; range, 42–190). Total puff volume, defined as the sum of all puffs taken, was *a priori* selected as the outcome measure for analysis (21, 22).

NMR values were positively skewed (+1.8) and have positive kurtosis (+4.8) consistent with previously results, and was therefore log-transformed (15, 17). NMR quartiles were created (15, 23) and *CYP2A6* genotypes were coded as described previously (17). NMR quartiles, previously determined from receiver operator characteristic analyses, have been used to characterize smokers' response to transdermal nicotine treatment and bupropion (15, 17, 24), and therefore were used in this study to assist comparisons to previous research that utilized NMR. Hypotheses were tested using analysis of covariance where total puff volume and total NNAL were the outcome measures and NMR quartile was the between group factor. Fisher's post hoc analyses were used to identify quartile differences. Regression analysis was used to examine the association between log-transformed NMR and total puff volume, and with NNAL. Stepwise regression analysis was used to examine the association between smoking behavior (daily cigarette consumption, total puff volume, and their product as an index of daily puff volume) and total NNAL levels, retaining covariates at $P < 0.2$.

Results

The participant sample was 59% men, 96% Caucasian with an average age of 45.4 years (SD = 10.8). On average, they had been smoking for 29.3 years (SD = 11.2), smoked an average of 20.5 cigarettes per day (SD = 8.4), with an average nicotine dependence score of 4.9 (SD = 2.1). Most participants smoked light brand (55%) and nonmenthol (71%) cigarettes. This study sample is similar to the full clinical study sample, ($n = 568$; see ref. 15), but with a significantly greater proportion of Caucasians (84% in the full sample). Of the 142 participants who attended these initial intake sessions, 131 (92%) agreed to complete a smoking topography assessment with their own brand cigarettes; of these 131, 14 withdrew prior to having assays completed, 5 had contaminated urine samples, and 3 had failed topography assessment. Topography session completers did not differ from noncompleters on demographic or smoking variables.

Mean plasma NMR value was 0.395 (SD = 0.20; range, 0.012–1.246). Mean (SD; lower limit and upper limit) for quartiles were as follows (for additional data see Table 1): Quartile 1, 0.192 (0.06; 0.010–0.259); Quartile 2, 0.313 (0.03; 0.260–0.357); Quartile 3, 0.410 (SD0.036; 0.358–0.477); Quartile 4, 0.668 (0.21; 0.478–1.246). Women were more likely to be in the highest NMR (faster metabolism; $P = 0.02$), consistent with previous reports that women metabolize nicotine faster than men (25). NMR was nonsignificantly higher among participants who were older ($P = 0.10$), who had higher nicotine dependence levels ($P = 0.09$), and who smoked nonmenthol cigarettes

Table 1. Descriptive measures for overall study sample and nicotine metabolism ratio quartiles

Measures	Overall ($n = 109$)	Quartile 1 ($n = 26$)	Quartile 2 ($n = 28$)	Quartile 3 ($n = 28$)	Quartile 4 ($n = 27$)
Descriptive					
Nicotine metabolism ratio	0.395 (0.20)	0.192 (0.06)	0.313 (0.03)	0.410 (0.04)	0.668 (0.21)
Cotinine, ng/mL	262.8 (110)	230.4 (122)	308.6 (112)	285.5 (110)	211.4 (62)
Sex, % male	59.0	60.0	67.0	64.0	42.0
Age, y	45.4 (10.8)	41.3 (11.5)	45.6 (11.6)	45.7 (8.2)	48.8 (11.1)
Daily cigarette consumption, *n*	20.5 (8.4)	19.8 (9.9)	18.9 (4.7)	21.7 (10.8)	22.2 (7.8)
FTND	4.9 (2.1)	4.0 (2.1)	5.0 (1.7)	5.3 (2.3)	5.3 (2.1)
Cigarette type (Reg/Lt/U-Lt), %	55/33/12	36/56/8	47/47/6	39/47/14	18/62/20
Menthol/nonmenthol, % nonmenthol	71.0	56.0	67.0	86.0	76.0
Outcome measures					
Total puff volume, mL	785.1 (284.9)	683.4 (250.6)	740.6 (249.1)	840.7 (315.1)	875.0 (290.1)
NNAL, pmol/mg creatinine	1.47 (0.79)	1.09 (0.63)	1.42 (0.70)	1.76 (0.97)	1.61 (0.71)

NOTE: The values are given as mean (SD), unless noted otherwise. Reg, regular cigarette; Lt, light cigarette; U-Lt, ultralight cigarette, based on Federal Trade Commission (FTC) cigarette classifications used at the time data were collected (FTC, 2000).

($P = 0.10$). Thus, these variables were included as covariates.

Mean total puff volume was 785.1 mL (SD = 284.9; range, 247.4–1776.1). There was an overall association of NMR quartiles with total puff volume ($F = 2.62$, $P = 0.05$); smokers in the third quartile ($P = 0.042$) and fourth quartile ($P = 0.016$) exhibited significantly higher total puff volumes than those in the first quartile (Table 1; Fig. 1A). Faster metabolizers by *CYP2A6* (*1/*1, $n = 89$) genotype also had higher puff volumes than slower metabolizers (defined as any of *2, *4, *9, *12 variants, $n = 19$); means were 816.8 mL (SD = 292.0) versus 643.3 mL (SD = 206.9), respectively ($F = 6.04$, $P = 0.02$; results the same when the 5 non-European ancestry subjects were excluded).

Mean total NNAL was 1.47 pmol/mg creatinine (SD = 0.79; range, 0.10–4.2). There was a significant main effect of the NMR ($F = 3.59$, $P = 0.02$); smokers in the third quartile ($P = 0.001$) and fourth quartile ($P = 0.033$) had higher total NNAL levels than those in the first quartile (Table 1; Fig. 1B). A similar, nonsignificant difference was seen in genotypic fast versus slow metabolizers [1.54 pmol/mg creatinine (SD = 0.92) versus 1.34 pmol/mg creatinine (SD = 0.68), $P = 0.35$). Results were unchanged when the nicotine dependence (FTND) covariate was replaced with smoking rate.

Linear regression analysis indicated a positive association between log-transformed NMR and total puff volume (beta = 321.2, $t = 2.35$, $P = 0.024$, $R^2 = 0.051$); and log-transformed NMR and total NNAL (beta = .831, $t = 2.41$, $P = 0.02$, $R^2 = 0.052$). Stepwise regression analysis indicated that the total puff volume by daily cigarette consumption product was positively associated with total NNAL level (beta = 2.538×10^{-5}, $t = 2.94$, $P = 0.004$), controlling for sex ($P = 0.02$), years smoking ($P = 0.04$), and menthol ($P = 0.16$); the overall model was significant [$F(4,104) = 4.85$, $P = 0.001$, $R^2 = 0.16$].

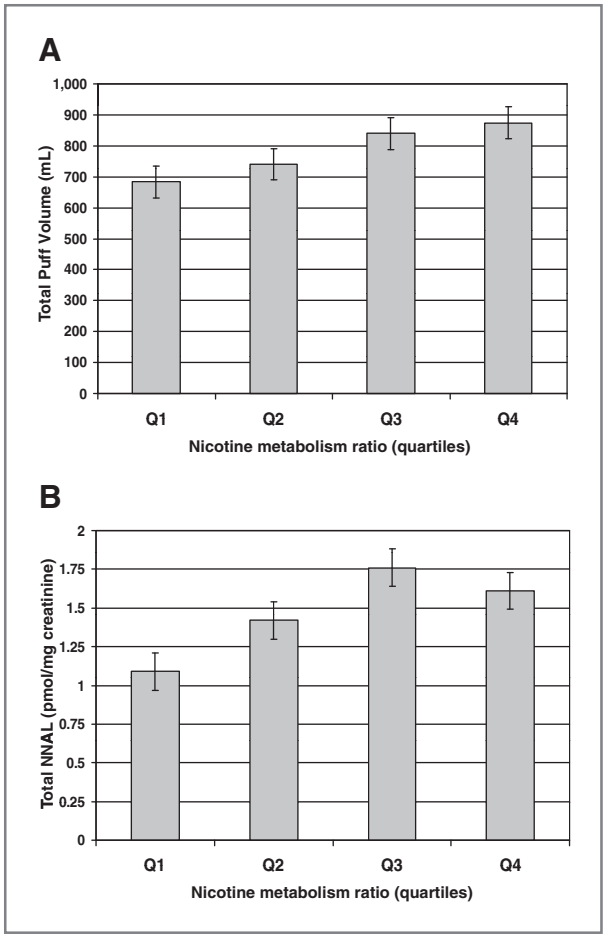

Figure 1. Association of the NMR with total puff volume and NNAL. A, NMR quartiles versus total puff volume (mL). Results are mean ± SD ($F = 2.62$, $P = 0.05$). Post hoc comparisons indicate a significant difference between the first and third quartiles ($P = 0.042$) and the first and fourth quartiles ($P = 0.016$). B, NMR quartiles versus NNAL. Results are mean ± SD ($F = 3.59$, $P = 0.02$). Post hoc comparisons indicate a significant difference between the first and third quartiles ($P = 0.001$) and the first and fourth quartiles ($P = 0.033$).

Discussion

This study is the first to show that a heritable biomarker of nicotine clearance, the NMR, predicts total cigarette puff volume and overall carcinogen exposure based on total NNAL. Compared with the slowest metabolizers (first quartile), smokers in the third and fourth quartiles exhibited 23% and 28% increases in cigarette puff volume, respectively, and total NNAL levels that are 61% and 53% higher, respectively. These results are consistent with our previous study (26) and current results showing increased puff volume among fast metabolizers by *CYP2A6* genotype. Results potentially could be interpreted as faster metabolizers take greater puffs to obtain a desired, greater level of nicotine, or that slow metabolizers smoke less intensely to avoid excessive or toxic nicotine levels. We suggest that it is more likely that faster metabolizers smoke more to obtain a desired level of nicotine, as there is little support for slow metabolizers reporting nausea when learning to smoke (27) and in high-dose transdermal nicotine studies, continued smoking did not lead to signs of nicotine toxicity (28).

The availability of a phenotypic measure of CYP2A6 activity, such as the NMR, is useful as it captures both genetic, such as yet unidentified alleles or other genes, and environmental influences, such as estrogen levels (25), on CYP2A6 activity and nicotine clearance, and NMR can be measured noninvasively (e.g., saliva) without additional drug administration (29, 30).

Cigarette smoking causes the majority of lung cancer cases (31) and total NNAL is a biomarker for one of the most prevalent systemic lung carcinogens in tobacco (32, 33). Yet, there is substantial variability in lung cancer risk at a given level of smoking (34, 35). This may be attributable to individual differences in the amount of toxin exposure per cigarette, and in activation in carcinogens (13). Because lung cancer can take years to develop, the

identification of practical biomarkers to improve risk assessment would be of great value.

There are some limitations of this study. Although puff volume is reliable and stable (19, 20), repeated assessments, rather than the single assessment here, may better reflect daily smoking patterns. Participants in this study were restricted to treatment-seeking smokers who smoked 10 or more cigarettes per day, and may not represent the general smoking population. One might postulate that because slow metabolizers have lower daily cigarette consumption, a high percentage may have not met study inclusion criteria (26). Therefore, these findings should also be replicated in a population-based sample. Lastly, a more comprehensive biomarker panel that includes not only the NMR, but also information on cigarette brand features (e.g., filter ventilation; ref. 6), and diurnal variations in smoking patterns (36) may provide a more refined assessment of risk from smoking. Such measures could potentially enhance risk assessment in epidemiologic studies and, if replicated, could be translated in the future to clinical practice.

Disclosure of Potential Conflicts of Interest

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References

1. Ray R, Tyndale RF, Lerman C. Nicotine dependence pharmacogenetics: role of genetic variation in nicotine-metabolizing enzymes. J Neurogenet 2009;23:252–61.
2. Messina ES, Tyndale RF, Sellers EM. A major role for CYP2A6 in nicotine C-oxidation by human liver microsomes. J Pharmacol Exp Ther 1997;282:1608–14.
3. Nakajima M, Kwon JT, Tanaka N, Zenta T, Yamamoto Y, Yamamoto H., et al. Relationship between interindividual differences in nicotine metabolism and CYP2A6 genetic polymorphism in humans. Clin Pharmacol Ther 2001;69:72–8.
4. Strasser AA, Tang KZ, Sanborn PM, Zhou JY, Kozlowski LT. Behavioral filter vent blocking on the first cigarette of the day predicts which smokers of light cigarettes will increase smoke exposure from blocked vents. Exp Clin Psychopharmacol 2009;17:405–12
5. Benowitz NL, Jacob P III, Herrera B. Nicotine intake and dose response when smoking reduced-nicotine content cigarettes. Clin Pharmacol Ther 2006;80:703–14.
6. Djordjevic MV, Stellman SD, Zang E. Doses of nicotine and lung carcinogens delivered to cigarette smokers. J Natl Cancer Inst 2000;92:106–11.
7. Strasser AA, Pickworth WB, Patterson F, Lerman C. Smoking topography predicts abstinence following treatment with nicotine replacement therapy. Cancer Epidemiol Biomarkers Prev 2004;13:1800–4.
8. Strasser AA, O'Connor RJ, Mooney ME, Wileyto EP. Digital image analysis of cigarette filter stains as an indicator of compensatory smoking. Cancer Epidemiol Biomarkers Prev 2006;15:2565–9.
9. Dempsey D, Tutka P, Jacob P III, Allen F, Schoedel K, Tyndale RF. Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. Clin Pharmacol Ther 2004;76:64–72.
10. Benowitz NL, Hukkanen J, Jacob P III. Nicotine chemistry, metabolism, kinetics and biomarkers. Handb Exp Pharmacol 2009;192: 29–60.
11. Lea RA, Dickson S, Benowitz NL. 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 2006;30:386–9.
12. Benowitz NL, Pomerleau OF, Pomerleau CS, Jacob P III. Nicotine metabolite ratio as a predictor of cigarette consumption. Nicotine Tob Res 2003;5:621–4.
13. Tyndale RF, Sellers EM. Variable CYP2A6-mediated nicotine metabolism alters smoking behavior and risk. Drug Metab Dispos 2001;29:548–52.
14. London S, Idle J, Daly A, Coetzee G. Genetic variation of CYP2A6, smoking, and risk of cancer. Lancet 1999;353:898–9.
15. Schnoll RA, Patterson F, Wileyto EP, Tyndale RF, Benowitz N, Lerman C. Nicotine metabolic rate predicts successful smoking cessation with transdermal nicotine: a validation study. Pharmacol Biochem Behav 2009;92:6–11.
16. Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. Br J Addict 1991;86:1119–27.
17. Lerman C, Jepson C, Wileyto EP, Patterson F, Schnoll R, Mroziewicz M, et al. Genetic variation in nicotine metabolism predicts the efficacy of extended-duration transdermal nicotine therapy. Clin Pharmacol Ther 2010. doi: 10.1038/clpt.2010.3.
18. Carmella SG, Han S, Fristad A, Yang Y, Hecht SS. Analysis of total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in human urine. Cancer Epidemiol Biomarkers Prev 2003;12:1257– 61.
19. Strasser AA, Ashare RL, Kozlowski LT, Pickworth WB. The effect of filter vent blocking and smoking topography on carbon monoxide levels in smokers. Pharmacol Biochem Behav 2005;82:320–9.
20. Lee E, Malson JL, Waters AJ, Moolchan ET, Pickworth WB. Smoking topography: reliability and validity in dependent smokers. Nicotine Tob Res 2003;5:673–9.
21. Strasser AA, Lerman C, Sanborn PM, Pickworth W, Feldman E. New lower nicotine cigarettes produce compensatory smoking and increased carbon monoxide exposure. Drug Alcohol Depend 2007;86: 294–300.
22. Benowitz NL, Jacob P III, Bernert JT, Wilson M, Wang L, Allen F, et al. Carcinogen exposure during short-term switching from regular to "light" cigarettes. Cancer Epidemiol Biomarkers Prev 2005;14: 1376–83.
23. Lerman C, Tyndale RF, Patterson F, Wileyto EP, Shields PG, Pinto A, et al. Nicotine metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation, Clin Pharmacol Ther 2006;79:600–8.
24. Patterson F, Schnoll RA, Wileyto EP, Pinto A, Epstein LH, Shields PG, et al. Toward personalized therapy for smoking cessation: a randomized placebo-controlled trial of bupropion. Clin Pharmacol Ther 2008;84:320–5.
25. Benowitz NL, Lessov-Schlaggar CN, Swan GE, Jacob P III. Female sex and oral contraceptive use accelerate nicotine metabolism. Clin Pharmacol Ther 2006;79:480–8.
26. Strasser AA, Malaiyandi V, Hoffmann E, Tyndale R, Lerman C. An association of CYP2A6 genotype and smoking topography. Nicotine Tob Res 2007;9(4):511–8.
27. Audrain-McGovern J, Al Koudsi N, Rodriguez D, Wileyto EP, Shields PG, Tyndale RF. The role of CYP2A6 in the emergence of nicotine dependence in adolescents. Pediatrics 2007;119:264–74.

28. Benowitz NL, Zevin S, Jacob P III. Suppression of nicotine intake during ad libitum cigarette smoking by high dose transdermal nicotine. J Pharmacol Exp Ther 1998;287:958–62.
29. Swan GE, Benowitz NL, Lessov CN, Jacob P III, Tyndale RF, Wilhelmsen K. Nicotine metabolism: the impact of CYP2A6 on estimates of additive genetic influence. Pharmacogenet Genomics 2005;15: 115–25.
30. Benowitz NL, Dains KM, Dempsey D, Herrera B, Yu L, Jacob P III. Urine nicotine metabolite concentrations in relation to plasma cotinine during low-level nicotine exposure. Nicotine Tob Res 2009;11:954–60.
31. International Agency for Research on Canerer (IARC). Tobacco smoke and involuntary smoking. IARC monographs on the evaluation of carcinogenic risks to humans. Lyon, France: IARC; 2004.
32. Hecht SS. Cigarette smoking and lung cancer: chemical mechanisms and approaches to prevention. Lancet Oncol 2002;3:461–9.
33. Hecht SS. Tobacco smoke carcinogens and lung cancer. J NatlCancer Inst 1999;91:1194–210.
34. United States Department of Health and Human Services. The health consequences of smoking: a report of the surgeon general. Atlanta, GA: Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2004.
35. Godtfredsen NS, Prescott E, Osler M. Effect of smoking reduction on lung cancer risk. JAMA 2005;294:1505–10.
36. Muscat JE, Stellman SD, Caraballo RS, Richie JP Jr. Time to first cigarette after waking predicts cotinine levels. Cancer Epidemiol Biomarkers Prev 2009;18:3415–20.