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Nicotine metabolite ratio predicts smoking topography: The Pennsylvania Adult Smoking Study

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ABSTRACT

Background: The nicotine metabolite ratio (NMR) as measured by the ratio of 3′hydroxycotinine to cotinine has been examined in relation to tobacco use patterns including cigarettes per day and quit success to determine its role in nicotine dependence. We examined the NMR in relation to smoking topography and tested the hypothesis that normal metabolizers have a greater total daily puff volume than slow metabolizers.

Methods: The Pennsylvania Adult Smoking Study (PASS) is a longitudinal study of 352 adults who smoked, on average, 17 cigarettes per day. Subjects used a portable smoking topography device over a two-day period at home and at work. We measured the ratio of 3′hydroxycotinine to cotinine in the saliva of the subjects.

Results: In multiple linear regression analyses, a higher rate of nicotine metabolism was significantly associated with increased daily puffs and total daily puff volume. In a mediation analysis, a significant, indirect effect of race on the relationship between NMR and puff volume was observed, with 22% of the effect mediated by white race. A higher NMR was also associated with female gender, white race, cigarettes per day and nicotine dependence measures.

Conclusion: The NMR was associated with tobacco use patterns including smoking topography. Faster nicotine metabolism was associated with greater total daily puffs and puff volume.

1. Introduction

Cigarette smokers regulate their nicotine dose or intake by the number of cigarettes smoked daily and the amount of smoke inhaled. Puffing behaviors, or smoking topography, is associated with measures of smoke exposure such as expired carbon dioxide, nicotine intake, and cotinine (Blank et al., 2009; Hammond et al., 2005; Lee et al., 2003; Ross et al., 2016b; Strasser et al., 2005), and much of the relationship between cigarettes per day (CPD) and nicotine intake is mediated through puff volume (Krebs et al., 2016).

When nicotine is absorbed into the body, it is metabolized to cotinine and further into 3′-hydroxycotinine by cytochrome P450 2A6 (CYP2A6). The rate of metabolism and clearance of nicotine metabolites is affected by CYP2A6 variants (Messina et al., 1997; Nakajima et al., 2001) and non-genetic influences such as estrogen levels in female smokers (Benowitz et al., 2006). The nicotine metabolite ratio (3-hydroxycotinine/cotinine) (NMR) is a marker of nicotine metabolism and clearance (Dempsey et al., 2004), and can be measured in blood, urine, and saliva (St. Helen et al., 2012).

The NMR can be used to classify smokers as slow metabolizers versus normal metabolizers (Lerman et al., 2006; Schnoll et al., 2014; Strasser et al., 2011). Slow metabolizers smoke fewer CPD (Benowitz et al., 2003; O'Loughlin et al., 2004; Rao et al., 2000; Schnoll et al., 2014), although results vary (Ross et al., 2016b). Generally, slow metabolizers clear nicotine at a slower rate, reducing their need to smoke more frequently. However, mixed findings have been reported on the relationship between NMR and nicotine dependence measures, such as the Fagerström Test for Nicotine Dependence (West et al., 2011).

The NMR may or may not play a role in nicotine dependence, and research has been conducted to determine if it affects tobacco use behaviors such as quitting success and daily cigarette frequency. Few studies have determined whether NMR affects smoking topography. Compared to slow metabolizers, normal metabolizers may be expected to extract more nicotine per cigarette. In a laboratory session of 119 treatment seeking adult smokers smoking 10 or more CPD, CYP2A6 variants that reduce the rate of CYP2A6 activity were associated with significantly lower puff volume (Strasser et al., 2007, 2011). In a subsequent sample of 109 smokers who had measured levels of nicotine metabolites, the puff volume was significantly lower in subjects with a lower NMR (Strasser et al., 2011). These studies were conducted in a ventilated facility after smoking a single cigarette ad libitum following a one-hour abstention. In contrast, the NMR was not associated with

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smoking topography in a laboratory study of 85 adolescent daily smokers (Moolchan et al., 2009). One previous study involved the use of a smoking topography device at home. In smokers with bipolar disorder, increasing NMR was associated with lower mean interpuff interval but not with other topography measures (Williams et al., 2012).

Cigarette puffing patterns in a laboratory or clinical setting differ from a naturalistic environment (Ossip-Klein et al., 1983). Smokers smoke more intensively when under observation (June et al., 2012). Smoking patterns in a natural environment are also contextual. For example, smokers take more puffs per cigarette during a smoking break at work than in social settings (Chapman et al., 1997). The current study builds on the laboratory-based studies to determine the effect of NMR on smoking topography in a naturalistic-based setting, using multiple longitudinal measures of topography over time. We hypothesized that normal metabolizers have more intense smoking topography measures and higher CPD than slow metabolizers.

2. Methods and materials

2.1. Study population

The Pennsylvania Adult Smoking Study (PASS) is a study of 352 adult cigarette smokers, conducted in central Pennsylvania. The study received approval from the Penn State College of Medicine Institutional Review Board (Hershey, PA, USA). Detailed methods of the study can be found elsewhere (Krebs et al., 2016). In brief, daily smokers were recruited from 2012 to 2014 using a variety of methods. Eligible participants gave written consent and were scheduled for two home study visits. Trained interviewers administered a multiple-domain, structured questionnaire that contained questions on cigarette-use history, measures of nicotine dependence such as the Fagerström Test for Nicotine Dependence (FTND) (Heatherton et al., 1991) and the Hooked on Nicotine Checklist (HONC) (Wellman et al., 2006), and socio-demographic factors. The study incorporated items from the PhenX (Consensus Measures of Phenotypes and Exposures) Toolkit (version March 23, 2012, Ver 5.1). Participants were given instructions on the use of the Smoking Puff Analyzer-Mobile (SPA-M) (SODIM SAS, France). The device was provided on the first study visit to use over a 2-day period and was collected on the second, follow-up visit. Participants were asked to use the device for all cigarettes smoked, and compliance was estimated by comparison against self-reported cigarettes per day. Saliva samples for laboratory analyses of nicotine metabolites were collected. Study data were collected and stored in REDCap (Research Electronic Data Capture), a secure web-based database application (Harris et al., 2009).

2.2. Smoking topography

The SPA-M is a portable touch-screen enabled pre-calibrated device where a cigarette is placed into a mouthpiece, and flow and pressure changes are recorded using pressure sensors. The SPA-M is battery-operated and can be recharged by the subject with a power cord. The readings were downloaded onto a desktop computer with software that calculates the puff flow (ml/s), the number of puffs, puff duration (s), the interval between puffs (s), and puff volume (ml) after each subject's use. A counter that keeps track of each cigarette smoked is reset for the next subject. The devices can be used continuously from subject to subject, pending any mechanical malfunction. The derived variables, total daily puff volume and total daily number of puffs, were the summation of the total cigarette puffs within a 24-hour period. Puff flow parameters that were either beyond the physiological capabilities of the smoker or resulted from movement artifact were excluded, based on previously reported suggestions (Williams et al., 2012). These exclusions included puff volume greater than 150 mL, average flow rate less than 10 mL/second, and peak flow rate less than 10 mL/second. Approximately 2% of the puffs were considered aberrant and removed from the analysis. In addition, smoker-level criteria were applied where if more than 25% of a smoker's cigarettes had aberrant puffs, the individual smoker was removed from the study (n = 20).

2.3. Salivary nicotine metabolites

Participants' saliva samples were analyzed using mass spectrometry for nicotine metabolites (cotinine and 3′hydroxycotinine) as previously described (Chen et al., 2010; Krebs et al., 2016). The NMR (3′hydroxycotinine/cotinine) was derived from these measurements.

2.4. Statistical analysis

The characteristics of the sample were described using descriptive statistics, including means and standard deviations for continuous variables and frequencies and percentages for categorical variables. We determined the median NMR, where the sample was split into normal and slow metabolizers (NMR cut-off = 0.359). Two-sample Wilcoxon-Mann-Whitney tests were used to look at the differences between the normal and slow metabolizers in relation to smokers' characteristics.

The hypothesis that NMR affects smoking topography was analyzed by linear regression. We selected three topography parameters as dependent variables for this analysis including total daily puffs, mean puff volume, and total daily puff volume. The analyses controlled for age and sex.

We further investigated the relationships between the rate of nicotine metabolism and total daily puff volume by statistical mediation analyses. We examined race as a mediator on the pathway between NMR and smoking topography. We used the causal step method proposed by Baron and Kenny (Baron and Kenny, 1986) and the bootstrapping method of Preacher and Hayes (Preacher and Hayes, 2008). The mediation analyses consisted of comparing the direct effect of topography with NMR to the indirect effect of topography with both NMR and race. For all analyses, significance was set at $p < 0.05$.

Table 1
Sample characteristicsof adult smokers.

Variable	Mean (or %)	Standard Deviation
Demographics		
Female Sex (n = 187)	58%	
White Race (n = 287)	88%	
Height (inches)	66.8	4.0
Age (years)	37.6	11.6
Body Mass Index	183.0	48.8
Smoking & Dependence		
Cigarettes per Day	16.5	8.1
Total Daily Puffs	116.0	77.5
Total Daily Puff Volume (mL)	5547.0	3917.0
Mean Puff Volume (mL)	48.3	14.9
Puff Duration (sec)	1.6	0.4
Puffs per Cigarette	7.6	4.6
Puff Volume per Cigarette (mL)	360.0	222.0
Time to first cigarette (minutes)	31.7	59.4
Fagerström Test for Nicotine Dependence	4.4	2.3
Hooked on Nicotine Checklist	7.3	2.1
Biomarkers		
Cotinine (ng/ml)	291.57	162.04
3'-hydroxycotinine (ng/ml)	115.45	86.17
Nicotine Metabolite Ratio	0.4	0.3