Association of Urinary Biomarkers of Smoking-Related Toxicants with Lung Cancer Incidence in Smokers: The Multiethnic Cohort Study

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ABSTRACT

Background: While cigarette smoking is the leading cause of lung cancer, the majority of smokers do not develop the disease over their lifetime. The inter-individual differences in risk among smokers may in part be due to variations in exposure to smoking-related toxicants.

Methods: Using data from a subcohort of 2,309 current smokers at the time of urine collection from the Multiethnic Cohort Study, we prospectively evaluated the association of ten urinary biomarkers of smoking-related toxicants [total nicotine equivalents (TNE), a ratio of total *trans*-3′-hydroxycotinine (3-HCOT)/cotinine (a phenotypic measure of CYP2A6 enzymatic activity), 4-(methylnitrosamino)-1–3-(pyridyl)-1-butanol (NNAL), *S*-phenylmercapturic acid (SPMA), 3-hydroxypropyl mercapturic acid (3-HPMA), phenanthrene tetraol (PheT), 3-hydroxyphenanthrene (PheOH), the ratio of PheT/PheOH, cadmium (Cd), and (*Z*)-7-(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(*E*,3*S*)-3-hydroxyoct-1-enyl]cyclopenyl]hept-5-enoic acid (8-*iso*-$PGF_{2\alpha}$)] with lung cancer risk ($n = 140$ incident lung cancer cases over an average of 13.4 years of follow-up). Lung cancer risk was estimated using Cox proportional hazards models.

Results: After adjusting for decade of birth, sex, race/ethnicity, body mass index, self-reported pack-years, creatinine, and urinary TNE (a biomarker of internal smoking dose), a one SD increase in log total 3-HCOT/cotinine (HR, 1.33; 95% CI, 1.06–1.66), 3-HPMA (HR, 1.41; 95% CI, 1.07–1.85), and Cd (HR, 1.45; 95% CI, 1.18–1.79) were each associated with increased lung cancer risk.

Conclusions: Our study demonstrates that urinary total 3-HCOT/cotinine, 3-HPMA, and Cd are positively associated with lung cancer risk. These findings warrant replication and consideration as potential biomarkers for smoking-related lung cancer risk.

Impact: These biomarkers may provide additional information on lung cancer risk that is not captured by self-reported smoking history or TNE.

See related commentary by Etemadi et al., p. 289

Introduction

Lung cancer is the second most common cancer and the leading cause of cancer-related death in both men and women in the United States (1). It is well established that cigarette smoking is the primary risk factor for lung cancer. However, disease risk is not equal among individuals, as it is estimated that 11% to 24% of smokers will develop the malignancy over their lifetime (2). Along with nicotine, each cigarette delivers a complex mixture of tobacco-related carcinogens and toxicants. There are 80 established carcinogenic constituents in cigarette smoke, including several lung carcinogens such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), multiple polycyclic aromatic hydrocarbons (PAH), and volatiles such as 1,2-butadiene and acrolein (3). Inter-individual differences in the risk of smoking-related lung cancer may be, in part, attributable to the variation in harmful constituent exposure. Moreover, the investigation of the association of these biomarkers with lung cancer risk may improve our understanding of the mechanisms by which tobacco exposure causes lung cancer (4, 5).

Biomarkers of smoking, such as metabolites of nicotine, and those of tobacco carcinogens and toxicants, have been shown to better reflect the internal dose of these harmful constituents and in some studies, their levels correlate with lung cancer risk. For example, prior epidemiological studies have shown that internal smoking dose, as determined by either cotinine (6–15) or total nicotine equivalents (TNE; the molar sum of nicotine and five or six metabolites in urine; refs. 12, 13, 16), was associated with lung cancer risk, independent of self-reported smoking history. In addition, studies have shown that 4-(methylnitrosamino)-1–3-pyridyl)-1-butanol (NNAL; a biomarker for NNK; refs. 8–13) and phenanthrene tetraol (PheT; a biomarker for PAH; refs. 9–11, 17) are associated with lung cancer, even after adjusting for cotinine or TNE. However, these prior studies were conducted in single populations (e.g., only Whites or Chinese from Asia), and do not address the variation in smoking behaviors and lung cancer risk across populations. In addition, cotinine, along with self-reported smoking history does not fully account for individual variability in nicotine metabolism, which influences smoking dose and exposure to tobacco toxicants (12, 18–21). Cytochrome P450 2A6 (CYP2A6) is the primary catalyst of nicotine metabolism and among current smokers in the Multiethnic Cohort (MEC) study, smoking behavior and dose were influenced by a smoker's urinary nicotine metabolite ratio [total *trans*-3′-hydroxycotinine (3-HCOT)/cotinine; ref. 22]. This ratio is a phenotypic measure of CYP2A6 enzymatic activity and in MEC current smokers we previously reported that it was

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associated with lung cancer risk even after adjustment for self-reported smoking history and TNE (16). Acrolein, a well-known lung irritant, was recently reclassified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans (23). However, the contribution of acrolein exposure to lung cancer risk in smokers has not been well studied. Therefore, a systematic examination of common biomarkers of tobacco toxicant exposure and metabolism is needed and may provide information on lung cancer risk beyond that of self-reported smoking history.

To better understand the individual and joint effects of smoking dose and specific smoking-related toxicant exposures on lung cancer risk, we evaluated the associations of a number of tobacco exposure biomarkers with the risk of smoking-related lung cancer among smokers in the MEC Study ($n = 140$ incident lung cancer cases over an average of 13.4 years of follow-up). These biomarkers included NNAL, *S*-phenylmercapturic acid (SPMA, a biomarker of benzene uptake), 3-hydroxypropyl mercapturic acid (3-HPMA, a biomarker of acrolein uptake), PheT, 3-hydroxyphenanthrene (PheOH, a biomarker of PAH detoxification), the ratio of PheT/PheOH (proposed as a biomarker of metabolic activation of polycyclic aromatic hydrocarbons), cadmium (Cd), and (*Z*)-7-[1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(*E*,3*S*)-3-hydroxyoct-1-enyl]cyclopentyl]hept-5-enoic acid (8-*iso*-$PGF_{2\alpha}$, a biomarker of oxidative stress).

Materials and Methods

Study population

Details of the MEC have been previously described (24). Briefly, the cohort consists of 215,251 men and women recruited from Hawaii and California (primarily Los Angeles) between 1993 and 1996. Participants were between the ages of 45 and 75 years old at recruitment and primarily belonged to five ethnic/racial groups: African American, Japanese American, Latino, Native Hawaiian, and White. Approximately ten years after cohort entry, a subset of participants was asked to participate in a biorepository project by providing a blood and overnight urine sample (Hawaii) or a first-morning urine sample (California).

The present study includes a subcohort of MEC participants ($N = 2,309$) who were lung cancer-free current smokers at the time of urine collection and who have complete urinary biomarkers of both TNE and ratio of total 3-HCOT/cotinine measured (16). Approval for this study, including the consent procedure, was obtained from the Institutional Review Boards of the University of Minnesota, University of Hawaii, and University of Southern California. All study participants provided written informed consent.

Epidemiologic data

All participants completed an epidemiologic questionnaire at the time of MEC study enrollment and at the time of urine collection. These questionnaires included detailed information on demographic characteristics, medical history, average daily cigarettes smoked, cigarette smoking during the past two weeks (questionnaire at urine collection only), smoking duration (years), and a medication record. Measures of body-mass index (BMI) and smoking duration that were missing at the time of urine collection for a few subjects were imputed from other MEC questionnaires, as described previously (16, 20).

Urinary metabolites of smoking-related toxicants

Details of the analytic methods used to determine the levels of urinary biomarkers of nicotine metabolism and tobacco smoking toxicants and data for these biomarkers have been previously published (20, 25–29). Briefly, overnight or first-morning urine was used to measure TNE (the molar sum of nicotine *N*-oxide, total nicotine, total cotinine, and total 3-HCOT; ref. 20), total NNAL (25), SPMA (26), 3-HPMA (27), PheT, and PheOH (28) using LC/MS-MS [where "total" refers to the compound and its glucuronide conjugate(s)]. Urinary Cd was measured using inductively-coupled plasma mass spectrometry (ICP-MS; ref. 29). Urinary 8-*iso*-$PGF_{2\alpha}$ levels were measured as described previously (30). The detection limits were 13 ng/mL for nicotine, 20 ng/mL for cotinine, 18 ng/mL for 3-HCOT, 0.14 pmol/mL for total NNAL, 0.01 pmol/mL for SPMA, 4.5 pmol/mL for 3-HPMA, 0.005 pmol/mL for PheT, 0.05 pmol/mL for PheOH, 0.02 ng/mL for Cd, and 0.03 pmol/mL for 8-*iso*-$PGF_{2\alpha}$. The coefficient of variation (CV) for the assays across runs were 16.7% for nicotine, 10.1% for cotinine, 11.4% for 3-HCOT, 16.2% for total NNAL, 15.0% for SPMA, 9.1% for 3-HPMA, 12.2% for PheT, 19.7% for PheOH, 3.1% for Cd, and 7.0% for 8-*iso*-$PGF_{2\alpha}$. The proportion of 2,309 participants with missing biomarkers or those < LOD can be found in Supplementary Table S1. Biomarker levels below the limit of detection (LOD) were replaced with LOD/2. Creatinine was quantified using a colorimetric microplate assay (CRE34-K01) purchased from Eagle Bioscience (https://eaglebio.com/product/creatinine-microplate-assay-kit/; ref. 20). A urinary biomarker of CYP2A6 enzymatic activity was computed as the ratio of total 3-HCOT/cotinine. A urinary biomarker of metabolic activation of PAH was computed as the ratio of PheT/PheOH.

Follow-up and identification of lung cancer cases and deaths

Participants' follow-up began at the age of urine collection, for which urinary biomarkers were measured, and ended when one of the following events occurred: (i) diagnosis of lung cancer; (ii) death; or (iii) end of follow-up, December 31, 2017. Incident lung cancer cases were identified through linkages to two state-wide NCI Surveillance, Epidemiology and End Results (SEER) Program registries: the Hawaii Tumor Registry and the California State Cancer Registry. The International Classification of Diseases for Oncology (ICD-O-3; ref. 31) and ICD-10 (32) code C34 for malignant neoplasm of bronchus and lung were used for this purpose. Deaths were identified by linkages to the state death certificate files in Hawaii and California and to the National Death Index (NDI) for deaths occurring in other states. By the end of follow-up (average 13.4 years), 140 incident primary lung cancer cases were identified. ICD-O-3 codes were obtained from the tumor registry and classified into four common lung cancer histologic cell types [adenocarcinoma (ADC: 8140, 8250, 8481, and 8490), squamous cell carcinoma (SCC: 8070 and 8071), small-cell lung cancer (SCLC: 8041 and 8045), and large-cell carcinoma (8012 and 8013)] and unspecified malignant neoplasm (8000, 8010, 8020, 8033, 8046, and 8246; ref. 33)

Statistical analysis

The covariate-adjusted geometric mean values with estimated 95% confidence intervals (95% CI) were computed for each metabolite, adjusted for age, sex (male/female), race/ethnicity (African American, Native Hawaiian, White, Latino, Japanese American), body mass index (BMI, kg/m^2; log), creatinine (mg/dL; log). Values of all metabolites were transformed by taking the natural log to better meet model assumptions. Geometric mean values presented in the tables are back-transformed to their natural scale for ease of interpretation. The risk of lung cancer was estimated using HRs and 95% CIs from Cox proportional hazards models, where age was used as the time metric, and follow-up began at the age of urine collection. TNE was used to confirm current smoking status. Subjects with TNE < 1.27 nmol/mL (four times the limit of quantitation) were excluded, as this would indicate that

these participants were not current smokers at the time of urine collection. To compare our results across the different biomarkers with variation in ranges, we standardized each log-transformed biomarker by dividing an individual's value by the SD of the respective log-transformed biomarker for the overall population (Supplementary Table S1). Therefore, we present the HR for incident lung cancer associated with a one-unit SD change of the log urinary biomarker levels. Distribution plots for all biomarkers can be found in Supplementary Fig. S1. All analyses were adjusted for decade of birth (categorical:1910 to <1920, 1920 to <1930, 1930 to <1940, 1940 to <1950, and 1950 to <1960), sex (male/female), self-reported race/ethnicity (African American, Japanese American, Latino, Native Hawaiian, and White), BMI (kg/m^2, log), smoking history (pack-years of smoking), and urinary creatinine (mg/dL, log; Model 1). Model 1 was further adjusted for urinary TNE (Model 2) to assess the independent effects of internal smoking dose beyond that provided by self-reported measures of smoking. For TNE, Model 2 was adjusted for the ratio of urinary total 3-HCOT/cotinine. The proportional hazards assumption underlying Cox regression were checked using the "proportionality test" for the SAS procedure PHREG which tests proportionality among groups by creating interaction terms between log-time and covariates. Assumptions were met for all the variables of interest. Associations by tumor histologic cell-types were conducted when $n >$ 20 and specific race/ethnicity were explored using Model 2 but we note the small sample size in these analyses (n's < 47). For associations by histologic cell-type, we performed a competing risk analysis using cause-specific models for time to lung cancer histologic cell-type outcomes, with censoring at diagnosis for any lung cancer case with a histologic cell-type other than that being considered. To compare the parameters by histologic cell-type, an augmented data approach as described in Lunn and McNeil was implemented that computes simultaneous models for lung cancer of each histologic cell-type (34). Heterogeneity across lung cancer histologic cell-type is assessed by a Wald test comparing the interaction by cell-type and smoking metabolites using robust variance estimates. Lung cancer incidence rates (overall and by race/ethnicity) were left truncated at age 45 and age-standardized using the United States 2000 standard population. All statistical analyses were performed using SAS version 9.4 (Statistical Analysis System, RRID:SCR_008567; http://www.sas.com). Supplementary Figure S2 was generated using Python (IPython, RRID: SCR_001658; http://ipython.org).

Data availability

Restrictions apply to the availability of these data. Data were obtained via an approved proposal by the MEC Study Research Committee. Data requests should be made to the MEC Study (see "Data Sharing" on the MEC website: https://www.uhcancercenter.org/mec). Investigators need to submit a formal application that will be evaluated internally by the MEC Research Committee before any data are released. Documentation of IRB approval is required for all projects requesting to use MEC data.

Results

Baseline characteristics of this cohort of MEC smokers ($N = 2,309$) and the 140 incident lung cancer cases are presented in **Table 1**. Overall, the eligible population was comprised of men (46%) and women (54%) from five racial/ethnic groups: African Americans (16%), Native Hawaiians (14%), Whites (19%), Latinos (20%), and Japanese Americans (31%). Participants' median age was 63 years, and their smoking history was a reported median of 23 pack-years. After an average of 13.4 years of follow-up from biospecimen collection, 140 incident lung cancer cases were identified. Cases were more likely to be male (55%) and there was a slightly greater population of African Americans (21%) and Native Hawaiians (22%) compared with the eligible population. Consistent with the analysis in the overall MEC study (35), age-standardized incidence rates (ASIR) were highest in Native Hawaiians and African Americans, followed by Whites, Japanese Americans, and Latinos (Supplementary Table S2). Here, ASIRs were estimated only among this smoking population ($N = 2,309$). Cases compared to the MEC eligible cohort were slightly leaner (median BMI:24.4 vs. 25.7 kg/m^2) and reported a greater number of pack-years (median:34.3 vs. 23.3). The majority of lung cancer cases were diagnosed with adenocarcinoma (34%), followed by squamous cell carcinoma (29%), other/unspecified/nonspecific (NOS; 17%), small-cell lung cancer (16%), and large-cell carcinoma (4%).

The adjusted geometric means and 95% CIs of all urinary biomarkers evaluated in this analysis are presented in **Table 2** (minimally

Table 1. Characteristics of the MEC smokers and the lung cancer cases identified during study follow-up.

	MEC eligible population ($N = 2,309$) *n* (%)	Incident lung cancer cases ($N = 140$) *n* (%)
Sex		
Males	1,068 (46)	77 (55)
Females	1,241 (54)	63 (45)
Race/ethnicity		
African Americans	368 (16)	29 (21)
Native Hawaiians	331 (14)	31 (22)
Whites	445 (19)	26 (19)
Latinos	456 (20)	15 (11)
Japanese Americans	709 (31)	39 (28)
Age at urine collection		
45–<55 years	105 (5)	2 (1)
55–<65 years	1,271 (55)	71 (51)
65–<75 years	706 (31)	48 (34)
≥75 years	227 (10)	19 (14)
	Median (25–75% IQR)	**Median (25–75% IQR)**
Follow-up time, years	13.4 (11.7–14.3)	8.4 (5.7–10.9)
Body mass index (BMI) at time of urine collection (kg/m^2)	25.7 (22.7–28.8)	24.4 (22.1–27.3)
Urinary creatinine, mg/dL	64 (40–103)	65 (33–107.5)
Pack-years	23.3 (11.6–37.5)	34.3 (20.8–50.6)
		***n* (%)**
Histologic cell-type (case only)		
Adenocarcinoma		47 (34)
Squamous cell carcinoma		40 (29)
Large cell or non-small cell, unspecified		6 (4)
Small-cell lung cancer		23 (16)
Other/unspecified/not specific		24 (17)

Abbreviations: N, number of participants in each category; IQR, interquartile range.

Table 2. Geometric mean (GM) and 95% CI for urinary biomarkers of smoking-related toxicants among the MEC subcohort of current smokers and incident lung cancer cases.

Urinary biomarkers of smokers	MEC eligible population		Incident lung cancer cases	
	N	GM (95% CI)[b]	*N*	GM (95% CI)[a]
TNE (nmol/mL)[b]	2,309	30.8 (30.0–31.6)	140	38.6 (35.3–42.3)
Total 3-HCOT/cotinine	2,307	3.20 (3.10–3.30)	140	3.85 (3.42–4.29)
Total NNAL (pmol/mL)	2,251	1.15 (1.13–1.18)	140	1.42 (1.32–1.54)
SPMA (pmol/mL)	2,169	2.52 (2.42–2.62)	132	2.97 (2.52–3.55)
3-HPMA (nmol/mL)	2,281	3.00 (2.91–3.10)	140	3.93 (3.47–4.44)
PheT (pmol/mL)	2,295	0.95 (0.93–0.98)	139	1.05 (0.95–1.16)
PheOH (pmol/mL)	2,253	0.71 (0.69–0.73)	136	0.71 (0.65–0.77)
PheT/PheOH	2,239	1.35 (1.32–1.39)	135	1.50 (1.33–1.68)
Cd (ng/mL)	1,976	0.60 (0.58–0.61)	125	0.77 (0.70–0.84)
8-iso-$PGF_{2\alpha}$ (pmol/mL)	1,913	0.80 (0.78–0.82)	122	0.83 (0.71–0.92)

Abbreviations: N, number of events; GM, geometric mean; CI, confidence intervals; TNE, total nicotine equivalents; 3-HCOT, *trans* 3′-hydroxycotinine; NNAL, 4-(methylnitrosamino)-1–3-pyridyl)-1-butanol; SPMA, *S*-phenylmercapturic acid; 3-HPMA, 3-hydroxypropyl mercapturic acid; PheT, phenanthrene tetraol; PheOH, 3-hydroxyphenanthrene; PheT/PheOH, a proposed biomarker of metabolic activation of polycyclic aromatic hydrocarbons (PAH); Cd, cadmium; 8-iso-$PGF_{2\alpha}$, (*Z*)-7-[1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(*E*,3*S*)-3-hydroxyoct-1-enyl]cyclopentyl]hept-5-enoic acid.
[a]Geometric means and corresponding 95% CI are adjusted for age, sex (male/female), race/ethnicity (African American, Native Hawaiian, White, Latino, Japanese American), body mass index (BMI, kg/m2; log), creatinine (mg/dL; log), cigarettes per day (CPD), and TNE (nmol/mL; where appropriate).
[b]For TNE, the model does not include TNE.

adjusted for just race/ethnicity in Supplementary Table S3). Compared with the overall subcohort of current smokers at the time of urine collection, incident lung cancer cases had higher geometric mean levels of all urinary biomarkers, except for PheOH, which was the same across groups. All urinary biomarkers were positively correlated with TNE levels (Supplementary Fig. S2).

The associations of known risk factors with lung cancer risk were confirmed (Supplementary Table S4). The association between smoking-related toxicant and carcinogen biomarkers and lung cancer risk in this multiethnic population of current smokers is presented in **Table 3**. After adjusting for decade of birth, sex, race/ethnicity, BMI, and creatinine, a one-SD increase in log-pack-years was associated with a 90% increase in lung cancer risk in current smokers (**Table 3**, Model 1). After adjustment for birth, sex, race/ethnicity, BMI, and creatinine and pack-years of smoking, we found that both a one SD increase of log TNE and a one SD increase of log total 3-HCOT/cotinine ratio were associated with lung cancer risk (HR, 1.36; 95% CI, 1.00–1.84 and HR, 1.37; 95% CI:1.11–1.71, respectively; **Table 3**, Model 1). When TNE was further adjusted for total 3-HCOT/cotinine, the association was attenuated and no longer remained statistically significant (HR per SD increase in log-TNE = 1.22; 95% CI, 0.91–1.64; **Table 3**, Model 2). Whereas the ratio of total 3-HCOT/cotinine remained significantly associated with lung cancer when adjusted for TNE (HR per SD increase in log-total 3-HCOT/cotinine = 1.33; 95% CI, 1.06–1.66; **Table 3**, Model 2).

Table 3. Association of pack-years and urinary biomarkers of smoking-related toxicants with lung cancer incidence in the MEC subcohort of current smokers at time of urine collection.

Urinary biomarkers of smoking-related toxicants[a]		Model 1[b]		Model 2[c]	
	N	HR (95% CI)	*P*	HR (95% CI)	*P*
Pack-years[d]	140	1.90 (1.50–2.41)	<0.0001	1.70 (1.31–2.21)	<0.0001
TNE[e]	140	1.36 (1.00–1.84)	0.047	1.22 (0.91–1.64)	0.192
Total 3-HCOT/cotinine	140	1.37 (1.11–1.71)	0.004	1.33 (1.06–1.66)	0.014
Total NNAL	140	1.20 (0.95–1.52)	0.120	1.10 (0.82–1.47)	0.541
SPMA	132	1.20 (0.96–1.49)	0.104	1.12 (0.89–1.41)	0.325
3-HPMA	138	1.46 (1.14–1.88)	0.003	1.41 (1.07–1.85)	0.016
PheT	139	1.08 (0.86–1.35)	0.500	0.98 (0.76–1.25)	0.842
PheOH	136	1.00 (0.80–1.27)	0.976	0.87 (0.67–1.13)	0.308
PheT/PheOH	135	1.05 (0.88–1.26)	0.579	1.03 (0.86–1.23)	0.758
Cd	125	1.48 (1.21–1.82)	0.0002	1.45 (1.18–1.79)	0.0004
8-iso-$PGF_{2\alpha}$	122	1.17 (0.91–1.49)	0.217	1.14 (0.89–1.46)	0.305

Abbreviations: N, number of events; pack-years, Number of packs of cigarettes smoked per day × number of years the person has smoked.
[a]Pack-years and all urinary biomarkers were standardized using log transformation and dividing the individual value by the overall population SD of the log biomarker and therefore the HR corresponds to a per one-unit SD change in log biomarker level. The SD of the log-biomarker can be found in Supplementary Table S1.
[b]Model 1 (base model): adjusted for decade of birth, sex (male/female), race/ethnicity (African American, Native Hawaiian, White, Latino, Japanese American), body mass index (BMI, kg/m^2; log), creatinine (mg/dL; log), and pack-years of smoking.
[c]Model 2: Model 1 + TNE.
[d]For pack-years, Model 1 adjusted for decade of birth, sex, race/ethnicity, BMI (kg/m^2; log), creatinine (mg/dL; log); Model 2 additionally adjusted for TNE.
[e]For TNE, Model 1 adjusted for decade of birth, sex, race/ethnicity, BMI (kg/m^2; log), creatinine (mg/dL; log), and smoking history (pack-years); Model 2 additional adjusts for total 3-HCOT/cotinine.

Smoking-related toxicant levels of urinary 3-HPMA and Cd were individually associated with lung cancer risk after adjusting for decade of birth, sex, race/ethnicity, BMI, urinary creatinine, and self-reported pack-years (HR per SD increase in log-3-HPMA, 1.46; 95% CI, 1.14–1.88 and HR per SD increase in log-Cd, 1.48; 95% CI, 1.21–1.82; **Table 3**, Model 1). These associations remained even after adjustment for TNE (HR per SD increase in log-3-HPMA = 1.41; 95% CI, 1.07–1.85 and HR per SD increase in log-Cd, 1.45; 95% CI, 1.18–1.79). 3-HPMA association was driven primarily by those who smoked less intensely (TNE < median 32.4 nmol/mL HR, 1.78; 95% CI, 1.17–2.69 vs. TNE ≥ median 32.4 nmol/mL HR, 1.16; 95% CI, 0.80–1.70; Supplementary Table S5) and in a population who smoked less on average (Japanese Americans, $n = 29$; HR, 1.96; 95% CI, 1.14–3.36; $P_{interaction}$ race/ethnicity = 0.029; **Table 4**). The association for Cd was primarily driven among those who smoked more intensely (TNE < median 32.4 nmol/mL HR, 1.42; 95% CI, 1.07–1.89 vs. TNE ≥ median 32.4 nmol/mL HR, 1.44; 95% CI, 1.01–2.04; Supplementary Table S5) and by the results in Whites ($n = 23$; HR Cd, 1.91; 95% CI, 1.12–3.24; $P_{interaction} = 0.383$).

We also explored the association by histologic cell-type (**Table 5**) and found that the association between incident lung cancer risk and pack-years was strongest for adenocarcinoma (ADC; $P < 0.0001$; **Table 5**). The association with TNE was strongest for risk of SCC ($P = 0.038$). The association with 3-HPMA was strongest for risk of lung cancer of other/unspecified histologic cell types ($P = 0.008$). The association with urinary Cd was strongest for risk of ADC ($P = 0.001$). These associations were not found heterogeneous across histologic cell types. Associations of race/ethnicity and by histologic cell-type were not conducted due to small sample size (Supplementary Table S6).

Discussion

This is the first study to examine the individual and joint effects of urinary biomarkers of multiple classes of smoking-related toxicants with the risk of smoking-related lung cancer in a multiethnic population. We demonstrated that urinary biomarkers of total 3-HCOT/cotinine, 3-HPMA (a metabolite of acrolein), and Cd (a biomarker of long-term Cd exposure) were significantly associated with lung cancer risk, independent of self-reported smoking history (pack-years) and internal smoking dose (TNE).

CYP2A6 catalyzed 5′-oxidation, the primary pathway of nicotine metabolism in most smokers produces cotinine (21), the most commonly used biomarker of tobacco exposure (21, 36). However, cotinine levels are influenced by individual variability in nicotine metabolism, which is known to influence exposure to tobacco toxicants (12, 18–21, 37). TNE, which includes metabolites from three pathways of nicotine metabolism accounts for approximately 85% of the nicotine dose and better reflects nicotine uptake, internal smoking dose, and lung cancer risk in some populations (4, 21). Because CYP2A6 also catalyzes the oxidation of cotinine to 3-HCOT, the ratio of 3-HCOT/cotinine is a phenotypic measure of CYP2A6 enzymatic activity. Variation in CYP2A6 activity can influence smoking intensity (30, 37, 38) and the ability to quit smoking over time (39) and therefore the urinary ratio of 3HCOT/cotinine may reflect longer-term smoking behaviors that are not captured by the short-term biomarker of dose (e.g., TNE).

Previously, we reported, in the same population studied here that a one log unit increase in the ratio of 3-HCOT/cotinine was associated with a 52% increase in lung cancer risk ($n = 92$ cases), independent of CPD, self-reported smoking duration, and TNE (16). In the current analysis with an additional 48 cases, the ratio

Table 4. Association of pack-years and urinary biomarkers of smoking-related toxicants with lung cancer incidence by race/ethnicity—MEC.

Urinary biomarkers of smoking-related toxicants[a]	African Americans			Native Hawaiians			Whites			Latinos			Japanese Americans			$P_{interaction}$[c]
	n	HR (95% CI)[b]	*P*	*n*	HR (95% CI)[b]	*P*	*n*	HR (95% CI)[b]	*P*	*n*	HR (95% CI)[b]	*P*	*n*	HR (95% CI)[b]	*P*	
Pack-years[d]	29	1.17 (0.75–1.84)	0.494	31	1.79 (1.06–3.03)	0.029	26	4.19 (2.07–8.48)	<0.0001	15	1.54 (0.58–4.09)	0.387	39	1.60 (0.93–2.75)	0.087	0.011
TNE[e]	29	0.86 (0.51–1.44)	0.564	31	0.98 (0.51–1.88)	0.955	26	1.81 (0.77–4.29)	0.177	15	1.52 (0.57–4.10)	0.407	39	1.71 (0.91–3.21)	0.096	0.072
Total 3-HCOT/ cotinine	29	1.42 (0.84–2.40)	0.195	31	1.21 (0.75–1.95)	0.437	26	1.99 (1.03–3.81)	0.039	15	1.05 (0.47–2.34)	0.899	39	1.19 (0.81–1.75)	0.382	0.318
Total NNAL	29	0.81 (0.44–1.51)	0.511	31	1.02 (0.52–2.00)	0.946	26	0.77 (0.33–1.78)	0.539	15	1.15 (0.39–3.38)	0.799	39	1.45 (0.84–2.51)	0.187	0.044
SPMA	28	1.41 (0.90–2.23)	0.138	30	0.84 (0.52–1.36)	0.478	25	1.19 (0.66–2.14)	0.555	15	0.64 (0.35–1.17)	0.145	34	1.27 (0.83–1.95)	0.276	0.318
3-HPMA	28	0.96 (0.57–1.62)	0.882	30	1.26 (0.62–2.57)	0.521	26	0.95 (0.47–1.91)	0.877	15	2.02 (0.85–4.80)	0.110	39	1.96 (1.14–3.36)	0.015	0.029
PheT	29	1.59 (1.11–2.28)	0.011	30	0.72 (0.37–1.37)	0.315	26	0.75 (0.38–1.49)	0.407	15	0.49 (0.20–1.20)	0.117	39	0.78 (0.44–1.36)	0.376	0.056
PheOH	27	1.03 (0.66–1.60)	0.912	29	0.87 (0.46–1.65)	0.675	26	0.68 (0.31–1.52)	0.346	15	0.29 (0.11–0.76)	0.012	39	0.99 (0.57–1.71)	0.963	0.461
PheT/PheOH	27	0.29 (0.11–0.76)	0.012	28	0.87 (0.57–1.34)	0.529	26	0.29 (0.11–0.76)	0.012	15	1.12 (0.65–1.94)	0.688	39	0.84 (0.57–1.24)	0.375	0.261
Cd	26	1.72 (0.96–3.07)	0.067	29	1.29 (0.88–1.89)	0.197	22	1.91 (1.12–3.24)	0.017	14	1.38 (0.56–3.43)	0.482	34	1.05 (0.67–1.66)	0.821	0.383
8-iso-$PGF_{2\alpha}$	22	1.26 (0.70–2.28)	0.435	29	0.90 (0.58–1.42)	0.660	23	1.38 (0.68–2.80)	0.366	14	1.28 (0.52–3.16)	0.593	34	1.06 (0.64–1.76)	0.819	0.429

Abbreviations: N, number of events; pack-years, number of packs of cigarettes smoked per day × number of years the person has smoked.

[a]Pack-years and all urinary biomarkers were standardized using log transformation and dividing the individual value by the overall population SD of the log biomarker and therefore the HR corresponds to a per one-unit SD change in log biomarker level. The SD of the log-biomarker can be found in Supplementary Table S1.

[b]Model adjusted for decade of birth, sex (male/female), body mass index (BMI, kg/m^2; log), creatinine (mg/dL, log), pack-years (where appropriate), and TNE (nmol/mL; where appropriate).

[c]*P* value across race/ethnicity.

[d]For pack-years, the model does not adjust for pack-years.

[e]For TNE, the model does not include TNE and is additional adjusted for total 3-HCOT/cotinine.

Table 5. Association of pack-years and urinary biomarkers of smoking-related toxicants with lung cancer incidence – the MEC subcohort of current smokers at time of urine collection, stratified by histologic cell-type.

Urinary biomarkers of smoking-related toxicants[a]	ADC			SCC			SCLC			Unspecified			
	n	HR (95% CI)[b]	*P*	*n*	HR (95% CI)b	*P*	*n*	HR (95% CI)[b]	*P*	*n*	HR (95% CI)[b]	*P*	$P_{heterogeneity}$[c]
Pack-years[d]	47	2.15 (1.41–3.27)	0.0004	40	1.65 (1.09–2.51)	0.019	23	1.87 (1.04–3.36)	0.037	27	1.96 (1.13–3.37)	0.016	0.841
TNE[e]	47	0.94 (0.58–1.53)	0.806	40	1.82 (1.03–3.20)	0.038	23	1.27 (0.62–2.61)	0.511	27	1.03 (0.54–1.97)	0.931	0.365
Total 3-HCOT/cotinine	47	1.12 (0.77–1.64)	0.559	40	1.41 (0.91–2.16)	0.121	23	1.72 (0.95–3.09)	0.071	27	1.30 (0.80–2.13)	0.293	0.652
Total NNAL	47	1.38 (0.92–2.08)	0.120	40	1.05 (0.61–1.80)	0.869	23	1.08 (0.50–2.35)	0.844	27	0.63 (0.30–1.34)	0.233	0.172
SPMA	46	1.06 (0.72–1.56)	0.766	36	1.37 (0.88–2.14)	0.166	22	1.04 (0.60–1.81)	0.891	25	0.94 (0.55–1.60)	0.812	0.587
3-HPMA	46	1.27 (0.80–2.04)	0.312	40	1.28 (0.78–2.12)	0.333	22	1.03 (0.50–2.14)	0.930	27	2.21 (1.21–4.04)	0.010	0.762
PheT	46	0.86 (0.55–1.34)	0.510	40	1.18 (0.75–1.84)	0.475	23	0.81 (0.42–1.55)	0.522	27	0.90 (0.50–1.62)	0.718	0.501
PheOH	45	0.77 (0.48–1.25)	0.293	39	0.85 (0.52–1.38)	0.516	22	1.05 (0.56–1.96)	0.883	27	0.97 (0.56–1.67)	0.910	0.418
PheT/PheOH	44	0.97 (0.71–1.33)	0.859	39	1.22 (0.88–1.68)	0.235	22	0.81 (0.51–1.29)	0.377	27	0.95 (0.65–1.39)	0.791	0.544
Cd	42	1.75 (1.25–2.46)	0.001	38	0.96 (0.62–1.49)	0.870	21	1.54 (0.92–2.57)	0.101	22	1.64 (1.05–2.56)	0.030	0.338
8-iso-$PGF_{2\alpha}$	40	1.42 (0.92–2.21)	0.115	37	0.99 (0.63–1.54)	0.948	19	1.49 (0.76–2.92)	0.243	24	0.86 (0.51–1.43)	0.557	0.371

Abbreviations: ADC, adenocarcinoma; SCC, squamous cell carcinoma; SCLC, small-cell lung cancer; unspecified, unspecified malignant neoplasm; N, number of events; pack-years, number of packs of cigarettes smoked per day x number of years the person has smoked.

[a]Pack-years and all urinary biomarkers were standardized using log transformation and dividing the individual value by the overall population SD of the log biomarker and therefore the HR corresponds to a per one-unit SD change in log biomarker level. The SD of the log-biomarker can be found in Supplementary Table S1.

[b]Model adjusted for decade of birth, sex (male/female), race/ethnicity (African American, Native Hawaiian, White, Latino, Japanese American), body mass index (BMI, kg/m^2; log), creatinine (mg/dL, log), pack-years (where appropriate), and TNE (nmol/mL; where appropriate).

[c]$P_{heterogeneity}$ across histologic cell-type in a competing risk model.

[d]For pack-years, the model does not adjust for pack-years.

[e]For TNE, the model does not include TNE and is additional adjusted for total 3-HCOT/cotinine.

of total 3-HCOT/cotinine was also significantly associated with lung cancer risk after adjustment for similar covariates (this analysis adjusted for the measure pack-years instead of CPD and self-reported smoking duration). These data support our hypothesis that this biomarker provides additional information regarding smoking history that may not be captured by self-reported data (16). In the Singapore Chinese Health Study, a positive association between total 3-HCOT/cotinine and lung cancer was observed; however, after adjustment for TNE, the association was attenuated (13). It is not surprising that in different populations with different genetics of nicotine metabolism and tobacco use, the ability of TNE and/or the ratio of total 3-HCOT/cotinine to reflect long- term smoking behavior varies.

Acrolein, an α,β-unsaturated aldehyde, is a ubiquitous environmental and dietary constituent and is produced endogenously as a product of lipid peroxidation, amino acid metabolism, and polyamine metabolism (40). Among current smokers, active tobacco smoking is the major source of exposure to acrolein (23, 41). One recent analysis reported a range of acrolein levels from 30.8–82.6 μg per unit in the mainstream smoke [under International Organization for Standardization (ISO) conditions] of 35 commercial cigarette tobacco products sold in the United States (42). Acrolein is a known lung irritant that produces inflammation and various other effects involved in carcinogenesis. As such, in 2020, IARC reclassified acrolein into Group 2A as "probably carcinogenic to humans" (23). Aligned with this reclassification, we found that 3-HPMA (the major metabolite of acrolein) was associated with lung cancer risk in our MEC smoker population after adjusting for lung cancer risk factors, the short-term biomarker of dose (TNE), and common urinary biomarkers of smoking. This association was primarily driven by those that smoked less intensely and populations who smoked less on average (e.g., Japanese Americans and Latinos). The Shanghai Cohort Study of male current smokers ($n = 343$ lung cancer cases; ref. 43) and never-smokers only ($n = 82$ lung cancer cases; ref. 17) and the Golestan Cohort Study in Iran of male exclusive cigarette smokers ($n = 31$ lung cancer cases; ref. 44) found an association with acrolein (measured by 3-HPMA) and lung cancer risk. However, these studies found that the association was no longer statistically significant after adjusting for total cotinine or smoking history, respectively, suggesting that the observed effect between 3-HPMA and lung cancer risk partly reflected an added measure of smoking dose.

Cd is a known human carcinogen (IARC group 1), and the primary source of exposure in most cases (e.g., leaving aside occupational exposure) is cigarette smoke (45, 46). While toxicologic studies in rodents and occupational studies in humans have shown that Cd is a respiratory toxicant associated with lung cancer, occupational studies cannot be extrapolated to the general population, and few studies have properly adjusted for smoking in their risk estimates (45, 47–49). Three non-occupationally based prospective cohort studies in the U.S. (NHANES III and Strong Heart Study) and Belgium (CadmiBel Study) have shown a positive association between urinary Cd levels, a biomarker of long-term Cd exposure, and lung cancer risk after adjusting for smoking status and/or pack-years (50–52). In two of these studies, after removing current smokers at baseline (Strong Heart Study; ref. 52) or including only never-smokers (U.S. NHANES III; ref. 51) in the analysis, the association remained positive but weaker. In the current study, we further demonstrated a positive association between Cd and lung cancer incidence, independent of measures of self-reported smoking history. Interestingly, when we explored associations by histologic cell-type, we demonstrated a strong positive association between urinary Cd and adenocarcinoma in our population, which is in agreement with previous evidence that has shown chronic Cd inhalation in rodents causes pulmonary adenocarcinomas (53). These results, in combination with those of other studies, suggest that urinary Cd may provide additional information regarding smoking-related lung cancer risk.

Prior studies have also detected associations with NNAL (8–10), PheT (10, 11, 17), PheOH (17), SPMA (43), and 8-*iso*-$PGF_{2\alpha}$ (54, 55) with lung cancer risk, even after accounting for self-reported smoking and/or smoking dose (cotinine and/or TNE). In this study, we did not detect an association between these biomarkers and the risk of lung cancer with adjustment for pack-years and TNE. These reported differences across studies may be a result of differences in sample size (MEC with only 140 lung cancer cases), smoking behaviors, and study population (e.g., the Shanghai population smoked mainly Chinese cigarettes in the 1980s vs. the multiethnic U.S. population smoked mainly domestic cigarettes in the 2000s). While all lung cancer histologic cell types are attributed to tobacco smoking, the cigarette quantity and composition have been shown to influence lung cancer subtypes. For instance, cigarette smoking dose was associated with higher relative risks for squamous cell carcinoma and small-cell lung cancer than adenocarcinoma (56). In addition, increased filtration ventilation of cigarettes has been suggested as a potential contributor to the relative rise of adenocarcinoma (57). Our exploratory analyses stratified by race/ethnicity and by histologic cell-type further supports the possibility that variation in exposure to smoke toxicants may contribute to etiologic differences in lung cancer risk.

The main strength of this study is the use of a well-characterized multiethnic population of current smokers with a range of smoking intensity and our ability to evaluate the effects of nine urinary biomarkers of smoking-related toxicants with lung cancer risk independent of self-reported smoking history and urinary TNE (a biomarker for internal smoking dose). However, our study has some limitations. First, this study included a modest number of lung cancer cases, limiting the power of our racial/ethnic and histologic cell-type–specific analyses. Second, the biomarker levels were only measured from a single urine collection; therefore, we did not account for variations in smoking behavior and exposure to tobacco carcinogens over time. However, adult smoking behavior is relatively stable over time (58). Third, we did not have information on quitting attempts or quitting during follow-up or information on cigarette brands, which could contribute to differences in biomarker levels of smoking-related toxicants.

In conclusion, our findings suggest that urinary total 3-HCOT/cotinine, 3-HPMA, and Cd levels may provide additional information on smoking-related lung cancer risk that is not captured by self-reported smoking history or the short-term biomarker of internal smoking dose (TNE). Our findings also suggest that these biomarkers may provide distinct information for lung cancer risk prediction by population or histologic cell-type. Replication in large prospective studies is warranted.

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Disclaimer

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Authors' Contributions

S.S. Cigan: Conceptualization, formal analysis, methodology, writing–original draft, writing–review and editing. **S.E. Murphy:** Conceptualization, data curation, funding acquisition, investigation, writing–review and editing. **D.O. Stram:** Funding acquisition, methodology, writing–review and editing. **S.S. Hecht:** Conceptualization, resources, data curation, funding acquisition, investigation, project administration, writing–review and editing. **L. Le Marchand:** Conceptualization, resources, data curation, supervision, funding acquisition, investigation, project administration, writing–review and editing. **I. Stepanov:** Conceptualization, resources, funding acquisition, writing–review and editing. **S.L. Park:** Conceptualization, data curation, formal analysis, supervision, funding acquisition, investigation, methodology, writing–original draft, project administration, writing–review and editing.

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