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Urinary metabolites of a polycyclic aromatic hydrocarbon and volatile organic compounds in relation to lung cancer development in lifelong never smokers in the Shanghai Cohort Study

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Exposures to polycyclic aromatic hydrocarbons (PAHs) from various environmental and occupational sources are considered a primary risk factor for lung cancer among lifelong never smokers, based largely on results from epidemiologic studies utilizing self-reported exposure information. Prospective, biomarker-based human studies on the role of PAH and other airborne carcinogens in the development of lung cancer among lifelong non-smokers have been lacking. We prospectively investigated levels of urinary metabolites of a PAH and volatile organic compounds in relation to lung cancer risk in a nested case–control study of 82 cases and 83 controls among lifelong never smokers of the Shanghai Cohort Study, a prospective cohort of 18 244 Chinese men aged 45–64 years at enrollment. We quantified three PAH metabolites: *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT), 3-hydroxyphenanthrene (3-OH-Phe) and total hydroxyphenanthrenes (total OH-Phe, the sum of 1-, 2-, 3- and 4-OH-Phe), as well as metabolites of the volatile organic compounds acrolein (3-hydroxypropyl mercapturic acid), benzene (*S*-phenyl mercapturic acid), crotonaldehyde (3-hydroxy-1-methylpropylmercapturic acid) and ethylene oxide (2-hydroxyethyl mercapturic acid). Urinary cotinine was also quantified to confirm non-smoking status. Compared with the lowest quartile, odds ratios (95% confidence intervals) for lung cancer risk for the highest quartile levels of PheT, 3-OH-Phe and total OH-Phe were 2.98 (1.13–7.87), 3.10 (1.12–7.75) and 2.59 (1.01–6.65) (all $P_{trend} < 0.05$), respectively. None of the metabolites of the volatile organic compounds were associated with overall lung cancer risk. This study demonstrates a potentially important role of exposure to PAH in the development of lung cancer among lifelong never smokers.

Introduction

Although cigarette smoking is the primary cause of lung cancer (1), it is estimated that 16 000–24 000 lung cancer deaths (i.e. 10–15% of total lung cancer deaths) in the USA occur among lifelong never smokers (2). The causes of lung cancer in never smokers are not well understood. Exposures to environmental tobacco smoke and radon in indoor air are accepted causal factors for non-smoking-related lung cancer, and together these factors may account for approximately one third of lung cancer deaths among never smokers in the USA (2). Multiple epidemiologic studies have also provided evidence for various other factors in relation to risk of lung cancer, including exposures to indoor and outdoor air pollution (3,4), occupational exposure to asbestos and silica dust (5,6), infectious agents (7,8), history of lung disease (9,10) and family history of lung cancer (11). The role of these factors in contributing to the overall risk of non-smoking-related lung cancer in the general population is poorly defined.

Ambient air contains a myriad of compounds including polycyclic aromatic hydrocarbons (PAH) and volatile organic compounds, such as acrolein, crotonaldehyde, benzene and ethylene oxide. PAHs are ubiquitous environmental contaminants formed in all processes involving incomplete combustion of organic matter (12–14). PAHs always occur in mixtures that include highly carcinogenic compounds, such as benzo[*a*]pyrene, and weakly or non-carcinogenic compounds, such as phenanthrene (12–14). Acrolein and crotonaldehyde are also combustion products that are present in the general environment (15). Benzene is present in the atmosphere, with major sources including automobile exhaust, fuel evaporation from gasoline-filling stations and various industrial sources (16). Human exposure to these compounds occurs through inhalation of contaminated ambient air and/or consumption of contaminated food (17). Benzene, ethylene oxide and benzo[*a*]pyrene, a representative PAH, are considered carcinogenic to humans by the International Agency for Research on Cancer (16).

Numerous epidemiologic studies have presented data that support modest associations with lung cancer among lifelong never smokers for air pollutant exposure from sources, such as environmental tobacco smoke (18), use of coal for household heating and cooking (19), fumes from high-temperature cooking (20) and occupational exposures to silica dust and diesel exhaust (21). However, we are aware of no epidemiologic study to date which has provided evidence for a specific chemical compound(s) present in these sources that contributes to an increased risk of non-smoking-related lung cancer. A biomarker-based approach can quantify specific metabolites of PAH and volatile organic compounds that represent their *in vivo* dose and metabolism. We have used this approach to demonstrate that urinary *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT), a validated biomarker of PAH uptake and metabolism, independently predicts the risk of developing lung cancer among smokers (22). To our knowledge, no similar biomarker study has been done among lifelong never smokers.

Therefore, in this study, we used a biomarker-based approach to examine the relationship between risk of lung cancer among lifelong never smokers and specific metabolites of phenanthrene, a representative PAH, and of volatile organic compounds. Specifically, we quantified urinary levels of PAH metabolites, including PheT, 3-hydroxyphenanthrene (3-OH-Phe) and total hydroxyphenanthrenes (total OH-Phe, the sum of 1-, 2-, 3- and 4-OH-Phe), and metabolites of volatile organic compounds, including acrolein, benzene, crotonaldehyde and ethylene oxide, on 82 incident lung cancer cases and 83 matched controls among lifelong never smokers who participated in the Shanghai Cohort Study. Findings of this study fill a major gap in our knowledge concerning the role of PAH and volatile organic compounds in the development of lung cancer in lifelong never smokers.

Abbreviations: 3-OH-Phe, 3-hydroxyphenanthrene; CI, confidence interval; HEMA, 2-hydroxyethyl mercapturic acid; HMPMA, 3-hydroxy-1-methylpropylmercapturic acid; HPMA, 3-hydroxypropyl mercapturic acid; OR, odds ratio; PAH, polycyclic aromatic hydrocarbon; PheT, r-1,t-2,3,c-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene; SPMA, S-phenyl mercapturic acid; total OH-Phe, total hydroxyphenanthrenes.

Methods

Subjects

Details of the Shanghai Cohort Study have been published previously (23,24). In brief, the cohort consisted of 18 244 men (constituting 80% of eligible subjects) enrolled from 1 January 1986 through 30 September 1989 who were between 45 and 64 years of age and resided in one of four small geographically

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defined communities in Shanghai, China. In addition to in-person interviews eliciting information on use of tobacco and alcohol, usual diet and medical history, we collected a 10 ml blood sample and one single void urine sample from each participant at baseline. The Shanghai Cohort Study has been approved by the Institutional Review Boards at the University of Pittsburgh, the University of Minnesota and the Shanghai Cancer Institute.

Identification of incident lung cancer cases and deaths was accomplished through annual in-person follow-up interviews of all surviving cohort members and routine review of reports from the population-based Shanghai Cancer Registry and from the Shanghai Municipal Vital Statistics Office. As of 31 December 2008, losses to follow-up totaled 985 individuals (5.4%) after 22 years of study.

As of 31 December 2008, lung cancer developed in 795 cohort participants. Among them, 647 reported currently smoking, 49 quit smoking and 99 reported never smoking at baseline. This study focused on the 99 cases who self-reported that they were never smokers. For each of these 99 patients with lung cancer, we randomly selected one control subject from all cohort members who self-reported never smoking at enrollment and were free of cancer and alive at the time of cancer diagnosis of the index case. The control subject was matched to the index case by age at enrollment (±2 years), year and month of biologic specimen collection (±1 month) and neighborhood of residence at recruitment.

Laboratory measurements

Urine samples of all study subjects were retrieved from the biorepository of the Shanghai Cohort Study that are stored at –70°C. Specimens from matched control subjects and their index cases were always assayed in the same batch. All urine aliquots were identified only by unique codes and randomly placed in any given batch by laboratory personnel who had no knowledge of the case–control status of the test samples.

Analyses of PheT, 3-OH-Phe and total OH-Phe were carried out essentially as described (25,26) except that electron impact gas chromatography-mass spectrometry was used for 3-OH-Phe and total OH-Phe. We measured the following urinary mercapturic acid metabolites of volatile organic compounds: 3-hydroxypropyl mercapturic acid (HPMA), a stable metabolite of acrolein; *S*-phenyl mercapturic acid (SPMA) for benzene; 3-hydroxy-1-methylpropylmercapturic acid (HMPMA), also called 4-hydroxybut-2-yl mercapturic acid, for crotonaldehyde and (*N*-acetylcysteinyl)ethanol, also called 2-hydroxyethyl mercapturic acid (HEMA), for ethylene oxide. These mercapturic acids are accepted specific validated biomarkers of exposure to the volatile organic toxicants acrolein, benzene, crotonaldehyde and ethylene oxide (27). The analyses for the mercapturic acids were carried out essentially as described previously (28). Interday precision values for the analyses reported here were as follows (coefficient of variation, %): PheT (2.1), 3-OH-Phe (7.2), total OH-Phe (9.5), HPMA (17.0), SPMA (20.0), HMPMA (11.0) and HEMA (19.0). Approximate detection limits for the assays were as follows (pmol/ml): PheT (0.1), OH-Phe (0.05), HPMA (2.3), SPMA (0.01), HMPMA (0.2) and HEMA (0.2). Although data on long-term stability of these metabolites are not specifically available, our unpublished results indicate that they are all stable indefinitely when samples are stored at –20°C or below.

Quantification of cotinine in urine was carried out using a liquid chromatography–tandem mass spectrometry method as described previously (29). We measured urinary cotinine instead of total cotinine (cotinine plus its *N*-glucuronide) to reduce the sample processing time and cost. Urine samples were depleted for five subjects (two cases and three controls) after we performed assays for urinary biomarkers of PAH and volatile organic compounds. The limit of detection for cotinine was 0.5 ng/ml (2.8 pmol/ml). The intraday and interday precision values (coefficients of variation) for the assay were 1.8% and 2.8%, respectively. Urinary creatinine was assayed by the Fairview Medical Center Diagnostic Laboratories (Minneapolis) with a Kodak Ektachem 500 chemistry analyzer.

Statistical analysis

In our earlier analysis, we defined subjects with <35 ng/ml of urinary total cotinine as lifelong never smokers (22). In our previous study in the same cohort (22), we found that cotinine accounted for 51% of urinary total cotinine (S.E.Murphy, unpublished results). Therefore, any subjects with urinary cotinine above 18 ng/ml (equivalent to 35 ng/ml total cotinine) (15 cases and 13 controls) or missing urinary cotinine values due to the depletion of urine samples after measurement of other urinary biomarkers (two cases and three controls) were excluded from the present analysis. Thus, results for PAH metabolites are presented for 82 lung cancer cases and 83 controls, whereas results for mercapturic acid metabolites are for 80 lung cancer cases and 82 controls due to missing values on additional two cases and one control subject.

All urinary biomarkers for final statistical analysis were expressed per mg creatinine (Cr) to correct for varying water content of individual spot urine samples. The distributions of all urinary biomarkers measured were markedly skewed toward high values, which were corrected to a large extent by transformation to logarithmic values. Therefore, formal statistical testing was performed on logarithmically transformed values, and geometric (as opposed to arithmetic) means are presented.

We used the analysis of covariance method (30) to examine the difference in the levels of urinary biomarkers between cases and controls with adjustment for age, neighborhood of residence, duration of biospecimen storage before laboratory analysis and urinary levels of cotinine (a biomarker for environmental tobacco smoke). Standard statistical methods were used for case–control studies (31). The original matched case–control pairs were broken to maximize the number of subjects to be included in the present analysis. Unconditional logistic regression models were used to calculate odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) and *P* values. For each urinary biomarker, study subjects were grouped into quartiles according to the distribution among control subjects. The linear trend test for the association between levels of biomarkers and lung cancer risk was based on ordinal values of quartile categories.

Statistical analyses were carried out using SAS software version 9.2 (SAS Institute, Cary, NC). All *P* values reported are two-sided, and those that were <0.05 were considered to be statistically significant.

Results

Of the 193 urine samples (97 cases and 96 controls) tested for cotinine, all but one had detectable cotinine (i.e. >0.5 ng/ml). Among them, 165 samples (82 cases and 83 controls) had ≤18 ng/ml cotinine, 16 (six cases and 10 controls) had 19–100 ng/ml and 12 (nine cases and three controls) had >100 ng/ml cotinine. The 28 subjects (15 cases and 13 controls) whose urinary cotinine levels >18 ng/ml were excluded from further analysis for their possible use of cigarettes. The geometric means of urinary cotinine were comparable between cases and controls (3.4 versus 3.7 ng/mg creatinine; $P = 0.50$) included in the present analysis. The most likely source of nicotine, the parent compound of cotinine, in the study population was secondhand smoke given the rare use of nicotine replacement products in Shanghai, China, in 1986–89 when urine samples were collected from study subjects.

Of the 82 lung cancer cases included in the present analysis, 61 (74%) were histopathologically confirmed, whereas the remaining 21 (26%) were based on clinical diagnosis including radiography or computer-assisted tomography. Among the histopathologically confirmed cases, 16 (26%) were squamous cell carcinomas, 34 (56%) adenocarcinomas, two (3%) small cell cancers and nine (15%) other cell types. The mean age (±SD) of all patients at cancer diagnosis was 70.9 (±7.6) years. The average interval between baseline biospecimen collection and cancer diagnosis was 12.3 (±5.4) years, ranging from 8 months to 22.5 years.

Age at recruitment, level of education and history of tuberculosis infection were comparable for lung cancer cases and controls, whereas body mass index was slightly lower in cases than in controls (Table I). The percentage of regular drinkers of alcohol and the amount of alcohol consumed per day among drinkers was comparable between cases and controls (Table I).

The three urinary PAH biomarkers measured in this study were highly correlated with each other. Among control subjects, Spearman's correlation coefficients were 0.88 ($P < 0.001$) between 3-OH-Phe and total OH-Phe, 0.62 ($P < 0.001$) between 3-OH-Phe and PheT and 0.55 ($P < 0.001$) between total OH-Phe and PheT. The correlation coefficients for urinary cotinine with 3-OH-Phe, total Phe and PheT were 0.32 ($P = 0.003$), 0.24 ($P = 0.028$) and 0.21 ($P = 0.056$), respectively. We also examined the associations between the districts of subject's residence at urine collection and urinary levels of PAH biomarkers. Individuals who were living at or near districts with more industrial manufacturing or shipping yards showed elevated urinary levels of all three PAH biomarkers. The geometric means (95% CIs) of 3-OH-Phe, total OH-Phe and PheT in control subjects living at or near industrial districts were 8.26 (6.32–10.80), 24.74 (19.60–31.24) and 23.74 (16.66–33.82) pmol/mg creatinine, respectively. The corresponding figures in those living at shopping districts were 4.74 (4.18–5.36), 14.12 (12.68–15.72) and 15.92 (13.52–18.74) pmol/mg creatinine; the differences between districts of residence were all statistically

significant (*P*s < 0.0001 for 3-OH-Phe and total Phe) or borderline significant (*P* = 0.053 for PheT). We also examined and found no association between a subject's occupation at the time of urine collection and urinary PAH levels (data not shown).

Urinary levels of all three PAH biomarkers—PheT, 3-OH-Phe and total OH-Phe—in patients with lung cancer were higher than those in control subjects (Table II). Similarly, high levels of all three PAH biomarkers were associated with statistically significantly increased risk of lung cancer (Table III). Compared with the lowest quartile, men with the highest quartile of PheT, 3-OH-Phe and total OH-Phe had ORs (95% CIs) of 2.98 (1.13–7.87), 3.10 (1.24–7.75) and 2.59 (1.01–6.65), respectively, for the development of lung cancer after adjustment for urinary cotinine (a biomarker of environmental tobacco smoke) and all matching factors including neighborhood of residence and age (all *P* for trend < 0.05).

The urinary levels of individual mercapturic acid metabolites of volatile organic compounds were very different from each other. Among controls, the highest levels were seen for HPMA (a metabolite

Table I. Baseline demographic and lifestyle characteristics in lung cancer cases and control subjects among lifelong never smokers, the Shanghai Cohort Study, 1986–2008

	Cases	Controls	*P**
No. of subjects	82	83	—
Age (years), mean ± SD	58.1 ± 5.2	58.0 ± 5.4	0.87
Time (years) between blood draw and cancer diagnosis, mean ± SD	12.3 ± 5.4	12.3 ± 5.5	0.99
Body mass index (kg/m^2), mean ± SD	21.9 ± 2.7	22.7 ± 2.9	0.09
Level of education, %			
No formal education or primary (1–6 years)	29.3	30.1	0.91
Secondary and above	70.7	69.9	
Self-reported history of physician-diagnosed tuberculosis, %			
No	76.8	81.9	
Yes	23.2	18.1	0.42
Alcohol drinking, %			
Non-drinkers	68.3	62.6	0.45
Regular drinkers	31.7	37.4	
No. of drinks/day, mean ± SD	2.0 ± 2.1[a]	1.9 ± 1.5[a]	0.90
Urinary cotinine (ng/mg creatinine), geometric mean (95% CI)	3.4 (2.5–4.6)	3.7 (2.7–5.2)	0.50

[a]Among alcohol drinkers only.
*Two-sided *Ps* were based on *t* test for continuous variables or chi-square test for categorical variables.

Table II. Geometric means of urinary PAH metabolites in lung cancer cases and control subjects among lifelong never smokers, the Shanghai Cohort Study 1986–2008

Urinary PAH metabolite	Geometric mean (95% CI) (pmol/mg creatinine)[a]		*P*
	Cases (*n* = 82)	Controls (*n* = 83)	
PheT[b]	19.84 (16.16–24.34)	16.10 (13.02–19.88)	0.03
3-OH-Phe	6.64 (5.64–7.80)	5.44 (4.60–6.42)	0.01
Total OH-Phe	19.10 (16.46–22.16)	16.08 (13.78–18.76)	0.02

[a]Adjusted for age at baseline, neighborhood of residence at enrollment, years of sample storage and urinary cotinine level.
[b]One case with missing PheT was excluded from this analysis.

Table III. Urinary levels of PAH metabolites in relation to lung cancer risk among lifelong never smokers, the Shanghai Cohort Study 1986–2008

Urinary PAH metabolites	Quartile of biomarker				*P* for trend
	First (lowest)	Second	Third	Fourth (highest)	
PheT					
Cases[a]	13	24	20	24	
Controls	24	21	19	19	
OR (95% CI)[b]	1.00	2.32 (0.91–5.86)	2.01 (0.78–5.17)	2.98 (1.13–7.87)	0.049
3-OH-Phe					
Cases	18	12	18	34	
Controls	24	19	21	19	
OR (95% CI)[b]	1.00	0.93 (0.35–2.45)	1.28 (0.50–3.28)	3.10 (1.24–7.75)	0.010
Total OH-Phe					
Cases	15	18	22	27	
Controls	23	20	20	20	
OR (95% CI)[b]	1.00	1.49 (0.59–3.77)	1.95 (0.76–4.98)	2.59 (1.01–6.65)	0.042

[a]One case with missing PheT was excluded from this analysis.
[b]Adjusted for age at baseline, neighborhood of residence at enrollment, years of sample storage and urinary cotinine level.

of acrolein) and HMPMA (a metabolite of crotonaldehyde). Levels of both SPMA (a metabolite of benzene) and HEMA (a metabolite of ethylene oxide) were relatively low (Table IV). The correlation among the four mercapturic acid metabolites was weak or moderate. Among control subjects, Spearman's correlation coefficients ranged from 0.16 to 0.33 ($0.002 < P < 0.14$). These mercapturic acid metabolites also were weakly associated with urinary cotinine (Spearman's correlation coefficients 0.06–0.23; $0.037 < P < 0.56$) or any PAH biomarkers (Spearman's correlation coefficients 0.05–0.21; $0.055 < P < 0.64$). No statistically significant difference in urinary levels of mercapturic acid metabolites was observed between different districts of residence at recruitment (all *P*s > 0.29) (data not shown).

Lifelong never smokers who developed lung cancer had urinary levels of these mercapturic acid metabolites of volatile organic compounds that were comparable to their counterparts who did not have cancer (Table IV). Similarly, the increasing levels of urinary mercapturic acid metabolites were not associated with an increased risk of lung cancer (Table V).

We examined the association between urinary biomarkers of PAH or metabolites of volatile organic compounds and risk of lung cancer by histology. Elevated urinary levels of all three PAH biomarkers were associated with elevated risk of both squamous cell carcinoma and adenocarcinoma of the lung, although none of them reached statistical significance given the small sample size. Compared with the lowest quartile, ORs (95% CIs) of squamous cell carcinoma for the highest quartile of PheT, 3-OH-Phe and total Phe were 3.78 (0.57–25.09), 3.41 (0.80–14.46) and 3.44 (0.74–15.99), respectively. The corresponding figures for lung adenocarcinoma were 2.74 (0.84–8.92), 2.23 (0.64–7.70) and 1.84 (0.48–7.12). Among metabolites of volatile organic compounds, elevated urinary SPMA (metabolite of benzene) was associated with a statistically significantly increased risk of lung squamous cell carcinoma (only 16 cases). ORs for the second and third tertiles of SPMA were 1.97 (95% CI = 0.31–12.65) and 5.76 (95% CI = 1.11–28.96), respectively, compared with the lowest tertile (*P* for trend = 0.023). This positive association remained after adjustment for urinary PAH biomarkers (data not shown). There was no statistically significant association between urinary levels of SPMA and risk of lung adenocarcinoma (*P* for trend = 0.34). No association was observed for any of the other three mercapturic acids (HPMA, HEMA and HMPMA) with risk of either squamous cell carcinoma or adenocarcinoma of the lung (all *Ps* for trend ≥ 0.10).

Discussion

This study provided novel findings that demonstrated a statistically significant, positive association between urinary levels of PAH biomarkers, namely PheT, 3-OH-Phe and total OH-Phe, and risk of developing lung cancer among lifelong never smokers, although based on a relatively small sample size. Lifelong never smokers account for ~10–15% of all lung cancer diagnoses in the USA (2). Our data provide direct evidence that PAH may play an important role in the development of lung cancer among never smokers, which accounted for 12.5% of total lung cancer in the Shanghai Cohort study population. The results of this study also confirm our previous findings of PAH exposure (e.g. urinary PheT) on lung cancer risk among smokers after taking into account smoking intensity and duration and uptake of nicotine and the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (22).

Table IV. Geometric means of urinary mercapturic acid metabolites of volatile organic compounds in lung cancer cases and control subjects among lifelong never smokers, the Shanghai Cohort Study 1986–2008

Urinary mercapturic acid metabolite	Source	Geometric mean (95% CI) (pmol/mg creatinine)[a]		*P*
		Cases ($n = 80$)[b]	Controls ($n = 82$)[b]	
HPMA (pmol/mg Cr)	Acrolein	2184.0 (1627.0–2931.6)	1974.8 (1454.5–2681.3)	0.48
SPMA (pmol/mg Cr)	Benzene	1.18 (0.90–1.52)	1.02 (0.78–1.34)	0.27
HMPMA (pmol/mg Cr)	Crotonaldehyde	1750.5 (1425.0–2150.4)	1714.2 (1384.3–2122.7)	0.83
HEMA (pmol/mg Cr)	Ethylene oxide	9.28 (7.24–11.86)	9.74 (7.54–12.60)	0.67

[a]Adjusted for age at baseline, neighborhood of residence at enrollment, years of sample storage and urinary cotinine level.
[b]Two cases and one control subjects with missing value of urinary mercapturic acids were excluded from the analysis.

Table V. Urinary levels of mercapturic acid metabolites of volatile organic compounds in relation to lung cancer risk among lifelong never smokers, the Shanghai Cohort Study 1986–2008

Urinary mercapturic acid metabolites	Quartile of biomarker				*P* for trend
	First (lowest)	Second	Third	Fourth (highest)	
HPMA					
Cases[a]	21	19	19	21	
Controls[a]	21	20	21	20	
OR (95% CI)[b]	1.00	0.97 (0.40–2.34)	0.98 (0.40–2.36)	1.13 (0.47–2.75)	0.79
SPMA					
Cases[a]	17	18	19	26	
Controls[a]	21	20	21	20	
OR (95% CI)[b]	1.00	1.03 (0.39–2.69)	1.10 (0.44–2.78)	1.57 (0.65–3.80)	0.31
HMPMA					
Cases[a]	24	17	19	20	
Controls[a]	21	20	21	20	
OR (95% CI)[b]	1.00	0.75 (0.31–1.83)	0.80 (0.33–1.97)	1.00 (0.41–2.41)	0.99
HEMA					
Cases[a]	24	21	19	16	
Controls[a]	21	20	21	20	
OR (95% CI)[b]	1.00	1.02 (0.43–2.43)	0.86 (0.36–2.06)	0.75 (0.31–1.85)	0.49

[a]Two cases and one control subjects with missing value of urinary mercapturic acids were excluded from the analysis.
[b]Adjusted for age at baseline, neighborhood of residence at enrollment, years of sample storage and urinary cotinine level.

PAHs such as phenanthrene are ubiquitous in the general environment and are released into ambient air by tobacco smoke, vehicle exhaust and incomplete combustion of coal and other organic materials. PAH commonly enter the human body through inhalation of cigarette smoke or polluted ambient air or consumption of contaminated food (17). PheT and other PAH biomarkers are significantly elevated in smokers (14,32). In the present study population, we also observed significantly higher urinary PheT levels in smokers (geometric mean = 28.1, 95% CI = 26.7–29.5) (22) than lifelong never smokers (geometric mean = 16.1, 95% CI = 13.0–19.9). Furthermore, the level of PheT in lifelong never smokers in the Shanghai Cohort Study was approximately twice the levels seen in their counterparts in Qidong, a less urban area in China, six times the levels in Singapore and >10 times the levels in Minneapolis, MN, USA (33).

Although no data on specific PAH sources could explain the large variation in urinary PAH biomarker levels across the different populations, it is likely that the higher PAH levels present in the general environment in Shanghai in the mid-1980s when the urine specimens were collected were responsible for the elevated level of urinary PAH biomarkers in the Shanghai Cohort Study participants. Until the late 1980s, Shanghai was a major industrial city in China, where coal burning was the principal source of energy and electricity generation and the means of domestic cooking. Air monitoring data were scarce in Shanghai before the 1990s; thus, we were unable to correlate urinary PAH levels to their levels in ambient air. However, our analysis of urinary PAH biomarkers among control subjects according to their residence by districts within Shanghai at the time of urine sample collection revealed that individuals who were living at or near districts with more industrial manufacturing or shipping yards showed statistically significantly elevated urinary levels of all three PAH biomarkers. These results suggest that environmental exposure to PAH derived from coal burning in industrial manufacturing could directly contribute to the observed elevation of urinary PAH, thus increased risk of lung cancer among never smokers.

High occupational exposure to PAH also can occur during the conversion of coal to coke and coal tar and during the processing and use of products derived from coal tar (14). Many previous studies demonstrated significantly elevated levels of urinary 1-hydroxypyrene, a widely used biomarker of PAH exposure, in the urine of coke-oven workers and others with occupational exposures to PAH (14,34). Lung cancer mortality was approximately double in coke-oven workers as compared with non-oven workers, and the risk increased with increasing years of coke-oven employment and the cumulative exposure to coal tar pitch volatiles (35,36).

Domestic use of coal for cooking and heating was probably a major source of PAH exposure in China. Analysis of the organic extract of indoor air particles from homes in Yunnan Province, China, indicated phenanthrene to be the most abundant PAH. Non-smokers who were regularly exposed to smoke from burning 'smoky' coal for heating and cooking at home exhibited elevated urinary levels of specific PAH biomarkers (37). Given that coal was commonly used as a cooking fuel in Shanghai before the mid-1980s, this could be one of the reasons for the high levels of urinary PAH biomarkers in Shanghai relative to other populations (33). Significantly elevated lung cancer mortality was observed among people who used smoky coal, as opposed to wood or smokeless coal (38,39), implicating the role of PAH exposure in the development of lung cancer. It should be noted that the coal used in household cooking and manufacturing industry in Shanghai was not the smoky type.

Given the diffuse, ubiquitous sources of PAH in the general environment worldwide, the estimation of human exposure from individual sources is challenging. In this study, we used a biomarker-based approach quantifying PheT, 3-OH-Phe and total OH-Phe in urine with validated assays (26,40), offering an objective measure of PAH exposure from all sources, including those that await identification. These measurements are well suited for large-scale epidemiologic studies. Phenanthrene is the simplest PAH with a bay region, a feature closely associated with carcinogenicity (14), although phenanthrene is generally regarded as non-carcinogenic (41). The metabolism of phenanthrene by the diol epoxide pathway closely parallels that of benzo[*a*]pyrene (42), an accepted human carcinogen which is likely involved in lung cancer etiology (14). We have previously reported a positive association between urinary PheT and lung cancer risk among smokers, which was independent of smoking intensity and duration, as well as the uptake of the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and nicotine (22). In this study, the statistically significant positive association between urinary biomarkers of PAH and lung cancer risk among lifelong never smokers pinpoints the role of PAH in the development of non-smoking-related lung cancer. Given a positive association between urinary PheT and lung cancer in both smokers and non-smokers, further studies should determine if PheT, its parent compound and/or those PAH with similar metabolism pathways as phenanthrene play a role in the development of lung cancer.

This study demonstrates a statistically positive association between urinary levels of SPMA, a mercapturic acid metabolite of benzene, and risk of lung squamous cell carcinoma. Previous studies showed that benzene causes tumors at multiple organ sites including the lung when administered to mice by gavage and in some cases by inhalation (43,44). Occupational studies reported increased risks of mortality from hematopoietic malignancies and sometimes lung cancer in workers who were exposed to benzene (35,45–47). Benzene is considered to be causally related to various types of leukemia in humans (48). In our previous analysis, urinary levels of SPMA were not significantly associated with risk of either squamous cell carcinoma or adenocarcinoma of the lung among current smokers after adjustment for urinary total cotinine and smoking intensity and duration (49). Given the relatively small number of cancer cases ($n = 16$), this study could not rule out the adverse effect of benzene, or its sources including benzene, on non-smoking lung cancer risk. Future studies with larger sample sizes are warranted to confirm or refute our findings of a positive association between urinary SPMA and non-smoking-related lung squamous cell carcinoma risk.

Our study did not demonstrate statistically significant, positive associations between the urinary mercapturic acid metabolites HPMA, HMPMA and HEMA and lung cancer risk among lifelong never smokers. Our previous study showed that the differences in these urinary mercapturic acid metabolites between cases and controls among smokers could be explained by smoking intensity and duration and urinary total cotinine levels (49). Thus, the null associations between these mercapturic acid metabolites and lung cancer risk in lifelong never smokers in the present study are consistent with our findings in current smokers from the same study population, as reported previously (49). The lack of statistically significant association between these mercapturic acid metabolites and lung cancer suggests that their parent compounds—acrolein, crotonaldehyde and ethylene oxide—may not play an important role in the development of lung cancer in humans.

There are several strengths of this study. The analysis included lifelong never smokers based on both self-reported smoking history and urinary levels of cotinine. The metabolites of PAH and volatile organic compounds were measured in urine samples collected before cancer diagnosis, thereby ruling out the possibility of spurious associations resulted from changes in exposure due to cancer diagnosis and/or treatment for lung cancer. Another strength is the simultaneous measurement of multiple urinary metabolite biomarkers, thus allowing for examining the differential effects of PAH versus volatile organic compounds on lung cancer risk. The 20 plus years of follow-up of the cohort study provided an opportunity to evaluate a relatively long-term latent effect of PAH on lung cancer risk. A limitation of the present study is that we did not collect information on the type of fuel, a known source of PAH, used for home cooking at the time of urine collection. Therefore, we were unable to control for this source of PAH in the statistical analysis. Another potential limitation is that urine samples were collected only once, at baseline, from all subjects. Thus, single measurement might not adequately represent an individual's true exposure given the intraindividual variation in urinary biomarkers measured over a long time period. Other limitation of this study is the relatively small sample size given the relatively low

incidence of lung cancer among lifelong never smokers, thus limiting our capability to examine the differential associations between biomarkers of PAH and risk of lung cancer by histological type. Future studies with prospectively collected urine samples and larger sample sizes are warranted to confirm our findings.

In summary, using prospectively collected urine samples from participants of the Shanghai Cohort Study, we demonstrated a statistically significant, dose-dependent relationship of urinary concentrations of PheT, 3-OH-Phe, total OH-Phe, biomarkers of PAH uptake and metabolism, to lung cancer risk among lifelong never smokers. These results along with our previous findings in smokers strongly support an important role for PAH in the development of lung cancer. The null associations between urinary HPMA, HMPMA and HEMA and lung cancer risk in lifelong never smokers and in smokers in our previous study suggest a limited role of acrolein, crotonaldehyde and ethylene oxide in the development of lung cancer. The finding of a statistically significant positive association between urinary levels of SPMA and risk of lung squamous cell carcinoma is intriguing and warrants further investigation in future studies.

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References

1. U.S. Public Health Service, Office of the Surgeon General, National Clearinghouse for Smoking Health (1972) *The Health Consequences of Smoking*. U.S. Public Health Service, Washington, DC. http://profiles.nlm.nih.gov/ps/access/NNBBKM.pdf (1 May 2012, date last accessed)
2. Samet,J.M. *et al.* (2009) Lung cancer in never smokers: clinical epidemiology and environmental risk factors. *Clin. Cancer Res.*, **15**, 5626–5645.
3. Turner,M.C. *et al.* (2011) Long-term ambient fine particulate matter air pollution and lung cancer in a large cohort of never-smokers. *Am. J. Respir. Crit. Care Med.*, **184**, 1374–1381.
4. IARC (2010) Household use of solid fuels and high temperature frying. *IARC Monographs on the Evaluation of Carcinogenic Risk to Humans,* **Vol. 95**. International Agency for Research on Cancer, Lyon, 430pp.
5. Siemiatycki,J. *et al.* (1990) Silica and cancer associations from a multicancer occupational exposure case-referent study. *IARC Sci Publ.*, **97**, 29–42.
6. Ahrens,W. *et al.* (1998) A standard tool for the analysis of occupational lung cancer in epidemiologic studies. *Int. J. Occup. Environ. Health*, **4**, 236–240.
7. Cheng,Y.W. *et al.* (2004) Gender difference in human papillomarvirus infection for non-small cell lung cancer in Taiwan. *Lung Cancer*, **46**, 165–170.
8. Littman,A.J. *et al.* (2004) Chlamydia pneumoniae infection and risk of lung cancer. *Cancer Epidemiol. Biomarkers Prev.*, **13**, 1624–1630.
9. Hinds,M.W. *et al.* (1982) Tuberculosis and lung cancer risk in nonsmoking women. *Am. Rev. Respir. Dis.*, **125**, 776–778.
10. Santillan,A.A. *et al.* (2003) A meta-analysis of asthma and risk of lung cancer (United States). *Cancer Causes Control*, **14**, 327–334.
11. Matakidou,A. *et al.* (2005) Systematic review of the relationship between family history and lung cancer risk. *Br. J. Cancer*, **93**, 825–833.
12. Boström,C.E. *et al.* (2002) Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environ. Health Perspect.*, **110** (suppl. 3), 451–488.
13. ECSCF (2002) *Polycyclic Aromatic Hydrocarbons-Occurrence in Foods, Dietary Exposure and Health Effects.* European Commission Scientific Committee on Food, Brussels. http://ec.europa.eu/food/fs/sc/scf/out154_en.pdf (13 May 2013, date last accessed).
14. IARC (2010) Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. **Vol. 92.** International Agency for Research on Cancer, Lyon, 853pp.
15. Stevens,J.F. *et al.* (2008) Acrolein: sources, metabolism, and biomolecular interactions relevant to human health and disease. *Mol. Nutr. Food Res.*, **52**, 7–25.
16. IARC (2012) Chemical agents and related occupations. *IARC Monographs on the Evaluation of Carcinogenic Risk to Humans,* **Vol. 100 F**. International Agency for Research on Cancer, Lyon, 599pp.
17. Ramesh,A. *et al.* (2004) Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. *Int. J. Toxicol.*, **23**, 301–333.
18. Tse,L.A. *et al.* (2009) Environmental tobacco smoke and lung cancer among Chinese nonsmoking males: might adenocarcinoma be the culprit? *Am. J. Epidemiol.*, **169**, 533–541.
19. Hosgood,H.D. 3rd *et al.* (2010) In-home coal and wood use and lung cancer risk: a pooled analysis of the International Lung Cancer Consortium. *Environ. Health Perspect.*, **118**, 1743–1747.
20. Shields,P.G. *et al.* (1995) Mutagens from heated Chinese and U.S. cooking oils. *J. Natl. Cancer Inst.*, **87**, 836–841.
21. Tse,L.A. *et al.* (2011) Silica dust, diesel exhaust, and painting work are the significant occupational risk factors for lung cancer in nonsmoking Chinese men. *Br. J. Cancer*, **104**, 208–213.
22. Yuan,J.M. *et al.* (2011) Urinary levels of cigarette smoke constituent metabolites are prospectively associated with lung cancer development in smokers. *Cancer Res.*, **71**, 6749–6757.
23. Ross,R.K. *et al.* (1992) Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. *Lancet*, **339**, 943–946.
24. Yuan,J.M. *et al.* (1996) Morbidity and mortality in relation to cigarette smoking in Shanghai, China. A prospective male cohort study. *JAMA*, **275**, 1646–1650.
25. Hecht,S.S. *et al.* (2006) Comparison of polymorphisms in genes involved in polycyclic aromatic hydrocarbon metabolism with urinary phenanthrene metabolite ratios in smokers. *Cancer Epidemiol. Biomarkers Prev.*, **15**, 1805–1811.
26. Carmella,S.G. *et al.* (2004) Analysis of phenanthrols in human urine by gas chromatography-mass spectrometry: potential use in carcinogen metabolite phenotyping. *Cancer Epidemiol. Biomarkers Prev.*, **13**, 2167–2174.
27. Hecht,S.S. *et al.* (2010) Applying tobacco carcinogen and toxicant biomarkers in product regulation and cancer prevention. *Chem. Res. Toxicol.*, **23**, 1001–1008.
28. Carmella,S.G. *et al.* (2009) Effects of smoking cessation on eight urinary tobacco carcinogen and toxicant biomarkers. *Chem. Res. Toxicol.*, **22**, 734–741.
29. Murphy,S.E. *et al.* (2013) Cotinine and trans 3'-hydroxycotinine in dried blood spots as biomarkers of tobacco exposure and nicotine metabolism. *J. Expo. Sci. Environ. Epidemiol.*, **23**, 513–518.
30. Winer,B.J. (1971) *Statistical Principles in Experimental Design*. McGraw-Hill, New York, NY.
31. Breslow,N.E. *et al.* (1980) *Statistical Methods in Cancer Research. Volume 1. The Analysis of Case-Control Studies*. IARC Scientific Publication, Lyon.
32. Hecht,S.S. *et al.* (2005) Longitudinal study of urinary phenanthrene metabolite ratios: effect of smoking on the diol epoxide pathway. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 2969–2974.
33. World Cancer Research Fund/American Institute for Cancer Research (2007) *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective*. AICR, Washington, DC, pp. 280–288.
34. Wu,M.T. *et al.* (2002) Relationship of exposure to coke-oven emissions and urinary metabolites of benzo(a)pyrene and pyrene in coke-oven workers. *Cancer Epidemiol. Biomarkers Prev.*, **11**, 311–314.
35. Costantino,J.P. *et al.* (1995) Occupationally related cancer risk among coke oven workers: 30 years of follow-up. *J. Occup. Environ. Med.*, **37**, 597–604.
36. Fannick,N. *et al.* (1972) Exposure to coal tar pitch volatiles at coke ovens. *Am. Ind. Hyg. Assoc. J.*, **33**, 461–468.
37. Mumford,J.L. *et al.* (1995) Human exposure and dosimetry of polycyclic aromatic hydrocarbons in urine from Xuan Wei, China with high lung cancer mortality associated with exposure to unvented coal smoke. *Carcinogenesis*, **16**, 3031–3036.
38. Mumford,J.L. *et al.* (1987) Lung cancer and indoor air pollution in Xuan Wei, China. *Science*, **235**, 217–220.
39. Barone-Adesi,F. *et al.* (2012) Risk of lung cancer associated with domestic use of coal in Xuanwei, China: retrospective cohort study. *BMJ*, **345**, e5414.
40. Hecht,S.S. *et al.* (2003) *r*-1,*t*-2,3,*c*-4-Tetrahydroxy-1,2,3,4-tetrahydrophenanthrene in human urine: a potential biomarker for assessing

polycyclic aromatic hydrocarbon metabolic activation. *Cancer Epidemiol. Biomarkers Prev*., **12**, 1501–1508.
41. LaVoie,E.J. *et al.* (1988) Structure-activity relationships among tricyclic polynuclear aromatic hydrocarbons. In Yang,S.K. *et al.*. (eds) *Polycyclic Aromatic Hydrocarbon Carcinogenesis: Structure-Activity Relationships*. **Vol. 1**. CRC Press, Boca Raton, FL, pp. 151–175.
42. Shou,M. *et al.* (1994) Regio- and stereo-selective metabolism of phenanthrene by twelve cDNA-expressed human, rodent, and rabbit cytochromes P-450. *Cancer Lett*., **83**, 305–313.
43. Farris,G.M. *et al.* (1993) Carcinogenicity of inhaled benzene in CBA mice. *Fundam. Appl. Toxicol*., **20**, 503–507.
44. U.S. Department of Health and Human Services (2004) *Report on Carcinogens*. U.S. Department of Health and Human Services, Research Triangle Park, NC, pp. III-37–III-39.
45. Yin,S.N. *et al.* (1989) A retrospective cohort study of leukemia and other cancers in benzene workers. *Environ. Health Perspect*., **82**, 207–213.
46. Hayes,R.B. *et al.* (1996) Mortality among benzene-exposed workers in China. *Environ. Health Perspect*., **104** (suppl. 6), 1349–1352.
47. Sorahan,T. *et al.* (2005) Cancer risks in a historical UK cohort of benzene exposed workers. *Occup. Environ. Med*., **62**, 231–236.
48. IARC (1987) *Overall Evaluation of Carcinogenicity: An Updating of IARC Monographs the Evaluation of Carcinogenic Risk of Chemicals to Humans.* **Vol. 1–42**. IARC Scientific Publications, Lyon.
49. Yuan,J.M. *et al.* (2012) Urinary levels of volatile organic carcinogen and toxicant biomarkers in relation to lung cancer development in smokers. *Carcinogenesis*, **33**, 804–809.

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