

ORIGINAL ARTICLE

Personal exposure to fine particulate matter and benzo[a]pyrene from indoor air pollution and leukocyte mitochondrial DNA copy number in rural China

Jason Y.Y. Wong^{1,*}, Wei Hu¹, George S. Downward², Wei Jie Seow^{1,3,4}, Bryan A. Bassig¹, Bu-Tian Ji¹, Fusheng Wei⁵, Guoping Wu⁵, Jihua Li⁶, Jun He⁶, Chin-San Liu⁷, Wen-Ling Cheng⁸, Yunchao Huang⁹, Kaiyun Yang⁹, Ying Chen⁹, Nathaniel Rothman¹, Roel C. Vermeulen^{2,†} and Qing Lan^{1,†}

¹Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services, Rockville, MD, 20850, USA,

²Division of Environmental Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, 3508 TD, the

Netherlands, ³Saw Swee Hock School of Public Health, National University of Singapore and National University Health

System and ⁴Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore and National

University Health System, 117549, Singapore, ⁵China National Environmental Monitoring Center, Beijing 100012, People's

Republic of China, ⁶Qujing Center for Diseases Control and Prevention, Sanjiangdadao, Qujing, Yunnan 655099, People's

Republic of China, ⁷Department of Neurology and Vascular and Genomic Center, Changhua Christian Hospital, Changhua,

Taiwan, Graduate Institute of Integrated Medicine, College of Chinese Medicine, China Medical University, Taichung 500,

Taiwan, ⁸Laboratory of Mitochondrial Medicine, Chunghua Christian Hospital, Taipei 500, Taiwan and ⁹Department of

Cardiothoracic Surgery, Third Affiliated Hospital of Kunming Medical University (Yunnan Tumor Hospital), Kunming 650118,

People's Republic of China

*To whom correspondence should be addressed. Tel: +1 (240) 276-5149; Email: jason.wong@nih.gov

†Co-senior authors.

Abstract

Households in Xuanwei and Fuyuan, China, possess hazardous levels of fine particulate matter with an aerodynamic diameter <2.5 microns (PM_{2.5}) and polycyclic aromatic hydrocarbons (PAHs) from coal combustion. Previous studies found that increased exposure to PM_{2.5} and benzo[a]pyrene (BaP; a PAH) were associated with decreased mitochondrial DNA copy number (mtDNAcn), a marker of oxidative stress. We further evaluated these associations in a cross-sectional study of 148 healthy non-smoking women from Xuanwei and Fuyuan. Personal exposure to PM_{2.5} and BaP was measured using portable devices. MtDNAcn was measured using qPCR amplification of leukocyte DNA that was collected after air measurements. Linear regression models were used to estimate the associations between personal exposure to PM_{2.5} and BaP, and mtDNAcn adjusted for age, body mass index (BMI) and fuel type. We found inverse associations between exposure to PM_{2.5} and BaP, and mtDNAcn. Each incremental log-μg/m³ increase in PM_{2.5} was associated with a significant decrease in mtDNAcn of -10.3 copies per cell [95% confidence interval (95% CI): -18.6, -2.0; P = 0.02]. Additionally, each log-ng/m³ increase in BaP was associated with a significant decrease in mtDNAcn of -5.4 copies per cell (95% CI: -9.9, -0.8, P = 0.02). Age, BMI, fuel type and coal mine type were not significantly associated with mtDNAcn. Exposure to PM_{2.5} and BaP may alter mitochondrial dynamics in non-smoking Chinese women. MtDNAcn may be a potential mediator of indoor air pollution on chronic disease development.

Received: April 14, 2017; Revised: June 11, 2017; Accepted: June 22, 2017

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Abbreviations

BaP	benzo[a]pyrene
BMI	body mass index
mtDNAcn	mitochondrial DNA copy number
95% CI	95% confidence interval
CV	coefficient of variation
HB	human β -globin
PAH	polycyclic aromatic hydrocarbon
PM _{2.5}	fine particulate matter with an aerodynamic diameter <2.5 microns

Introduction

Indoor air pollution is a substantial global health burden in low- and middle-income nations. Residential combustion of smoky (bituminous) coal for heating and cooking in households of rural Xuanwei and Fuyuan, China, has led to hazardous levels of fine particulate matter with an aerodynamic diameter <2.5 microns (PM_{2.5}) and polycyclic aromatic hydrocarbons (PAHs) (1–3). Primarily formed through combustion, the composition of PM_{2.5} varies depending on physical environment, atmospheric conditions and source of emissions. Inhalation of PM_{2.5} is a substantial health concern because it can be deposited into the alveoli of the lung, where it can cause local inflammation, in addition to systemic inflammation if its components diffuse into the blood stream (4). PM_{2.5} can influence health outcomes through mechanisms involving lipid peroxidation of cell membranes, oxidative damage to the genome and DNA adduct formation (5–8). Indeed, exposure to PM_{2.5} in residential, occupational and public settings has been shown to be associated with increased risk of chronic respiratory conditions, cardiovascular disease and all-cause mortality (9–13). PAHs are organic compounds composed of multiple benzene rings that are emitted from the combustion of fossil fuels. A common type of PAHs is benzo[a]pyrene (BaP), which is often used as a surrogate for overall levels of PAHs. The carcinogenic and genotoxic potential of reactive PAH/ BaP metabolites is well established (14). Upon enzymatic conversion by cytochrome P-450, metabolites such as benzo[a]pyrene diol epoxide can form adducts with guanine, resulting in the disruption of normal DNA replication (14).

The burden of indoor air pollution is compounded for women of Xuanwei and Fuyuan, who because of a traditional agrarian lifestyle, spend considerable time indoors preparing food for their families. Further, despite government sanctioned stove improvement/ventilation programs, many households still use unvented fire pits for cooking (1,15). We previously found that the geometric mean average indoor PM_{2.5} and BaP exposure concentrations for female smoky coal users were 148.0 $\mu\text{g}/\text{m}^3$ and 44.7 ng/m^3 , respectively (1,15). Although outdoor PM_{2.5} levels in rural Xuanwei and Fuyuan are still being characterized, the indoor PAH concentrations exceeded outdoor levels that were reported in a previous study and were beyond the national outdoor criteria of 10.0 ng/m^3 (16).

Mitochondria are the eukaryotic organelles that serve two prominent roles in the cell: (i) energy production through the synthesis of adenosine triphosphate via the Krebs cycle and electron transport chain and (ii) production of reactive oxygen species, which are released in unison with cytochrome c to initiate apoptosis. Human mitochondrial DNA consists of a 16 569 bp circular double-stranded DNA molecule. Mitochondrial DNA copy number (mtDNAcn) is normally in steady state, which is related to the energy demand of the host cells (17). However, due to the lack of DNA repair machinery, mitochondria compensate for mtDNA damage by altering its copy number (18).

Indeed, oxidative stress and mtDNA damage have been found to be associated with increased mtDNAcn in leukocytes (18,19). In various somatic cells, mitochondria possess 2–10 000 copies of their genomes (20–23). Further, population-based estimates of leukocyte mtDNAcn vary from ~50 copies per cell in Danish adults (24), to about 110–120 copies per cell in Sardinian and U.S. adults (25,26).

Given the genotoxic potential of PM_{2.5} and PAHs, these pollutants may influence mitochondrial dynamics. MtDNAcn has been found to be associated with risk of various cancers (27–30), including that of the lung, in case–control and nested case–control studies (20,26,31,32). However, few studies have examined the relations between environmental exposure to PM_{2.5} and PAHs, and mtDNAcn. The dynamics between PM_{2.5} and PAH exposure and mtDNAcn are complex within the current literature. A study of an elderly Flemish population from Genk, Belgium, found that increased exposure to PM_{2.5} was associated with decreased mtDNAcn (33). A study of truck drivers in Beijing did not find a significant association between PM_{2.5} and mtDNAcn (34). Other studies found that PAH exposure was associated with both higher and lower mtDNAcn (35,36). Perturbations in mtDNAcn in either direction may be reflective of cumulative oxidative damage. To unravel these relationships further, we conducted a cross-sectional molecular epidemiological study of healthy non-smoking women in Xuanwei and Fuyuan, China. Our objective was to assess the associations between personal exposure to PM_{2.5} and BaP from household air pollution and leukocyte mtDNAcn.

Materials and methods**Study population and design**

The study population has been described previously in detail (15). In brief, the Xuanwei Exposure Assessment Study characterized household air pollutants and exposures related to the combustion of solid fuels for cooking and heating. We enrolled 148 healthy (disease-free) female heads of household from 30 villages across Xuanwei and Fuyuan during the first visit in August 2008 to February 2009. Up to five households were preferentially selected in each village based on (i) having a stove that used solid fuel; (ii) the residence was more than 10 years old; (iii) use of the same cooking or heating equipment for the past 5 years; and (iv) presence of a non-smoking healthy woman aged 20–80 years, who was primarily responsible for cooking. We conducted two sequential 24 h air measurements and whole blood samples were collected on the second day.

Personal PM_{2.5} and PAH exposure assessment

Personal PM_{2.5} measurements across 24 h were collected on pre-weighed 37 mm Teflon filters using portable devices with an aerodynamic cut-off of 2.5 μm (Model BGI, GK2.05SH) at a flow rate of 3.5 l/min ($\pm 20\%$) as described previously (15). The pump was packed in a hip bag and the portable device was attached near the breathing zone of each participant while they were awake. The sampling bag was placed next to the women's bed while they were sleeping. All exposed filters were individually placed in Petri slides, sealed in plastic bags and stored at -80°C before post-weighing. Particulate mass was determined by pre- and post-weighing of the filters in duplicate. PM_{2.5} concentrations ($\mu\text{g}/\text{m}^3$) were calculated by dividing the weights by the total volume of air drawn through the filters. Approximately, 10% of households were randomly selected to have duplicate PM_{2.5} measurements to assess reproducibility. The coefficient of variation (CV) for the PM_{2.5} measurements was 13%. Particle-bound PAHs were collected with 37 mm Teflon filters in the cyclones, whereas personal gas phase PAHs were measured with XAD-2 sorbent tubes at a median air flow rate of 63 ml/min (1). PAH species (ng/m^3) were identified using gas chromatograph connected to a mass spectrometer (Shimadzu QP2010 plus) (1). The median CV for PAH readings from 13 duplicate samples was 25% (1).

Household interviews and measurement

In-person interviews were conducted by two trained personnel. Information on household, demographic and anthropometric characteristics was collected. Body mass index (BMI; kg/m²) was derived from weight (kg) and height (m). The women's activities during the sampling periods were recorded using an activity questionnaire. This survey collected information on household stove and ventilation type, cooking activities, secondhand smoke exposure, heating practices, coal mine type that supplied household fuel and fuel usage. Coal type was reported as either smoky or smokeless and confirmed via petrochemical analysis of collected coal samples (37). Other categories of fuel included wood, plants, mixed coal (combinations of briquettes, smoky and smokeless coal) and mixed use of fuel (combinations of wood, plant materials and coal). Household stove and ventilation characteristics for this study population were described previously in detail (1,15). Written informed consent was obtained from all eligible participants. This study was approved by the National Cancer Institute Special Studies Institutional Review Board (#06CN092).

Leukocyte mtDNAcn

Total DNA was isolated from the whole blood using phenol-chloroform extraction. A modified version of a previously described SYBR green-based qPCR method was used to determine mtDNAcn on a Roche LC480 System (Roche Molecular Biochemicals, Pleasanton, CA) (19,38). Briefly, the qPCR assay was performed using LightCycle-FastStart DNA Master SYBR Green I at Changhua Christian Hospital. Each reaction used 20 ng of total DNA template and was performed in triplicate. The single copy nuclear gene [human β -globin (HB)] was amplified using the following primers—forward: 5'-GAAGAGCCAAGGACAGGTAC-3' and reverse: 5'-CAACTTCATCCACGTTCCACC-3'. Amplification of the HB gene was performed under the following qPCR cycling conditions: 95°C for 300 s, followed by 40 cycles of 10 s at 95°C, 5 s at 58°C and 18 s at 72°C. The single copy mitochondrial gene (ND1) was amplified using the following primers—forward: 5'-AACATACCCATGGCCAACCT-3' and reverse: 5'-AGCGAAGGGTTGTAGTAGCCC-3', under the follow conditions: 95°C for 300 s, followed by 40 cycles of 10 s at 95°C, 5 s at 58°C and 8 s at 72°C. Standard curves were performed for each batch for both the HB and ND1 reactions to assess qPCR efficiency. Linear correlations (R^2) ≥ 0.98 and qPCR efficiencies between 1.95 and 2.00 were considered acceptable, or else the reactions were repeated. The relative ratio of ND1 and HB threshold cycle numbers, estimated using a linear regression model, is proportional to the mtDNAcn in each cell (26,39). mtDNAcn was interpolated from the linearity of the dosage-dependently constructed standard plasmids of the ND1 and HB genes as described previously (19,38). The average CV from 10 quality control samples for mtDNAcn was 9.8%. The intraclass correlations from the same quality control samples with two to four replicates were 79% [95% confidence interval (95% CI): 58–90].

Analysis

The distribution and normality of continuous variables were assessed using histograms and Shapiro-Wilks tests, respectively. Spearman correlation coefficients were used to assess relations among ordinal and continuous variables. Multivariable-adjusted linear regression models were used to assess the associations between log-transformed personal PM_{2.5} and BaP exposure averaged from the two sequential collection days, and leukocyte mtDNAcn (copies per cell). Separate models were performed for PM_{2.5} and BaP as main exposures due to high collinearity ($\rho = 0.72$, $P < 0.0001$). The models were adjusted for age (continuous), BMI (continuous) and fuel type (smoky coal, smokeless coal, other coal, other fuels and wood/plant) via stepwise inclusion. Since lung cancer risk was found to differ by fuel type (40), we stratified the analyses by smoky coal users and users of all other fuels (non-smoky coal users). Further, we assessed effect modification using interaction terms between PM_{2.5} X fuel type and BaP X fuel type. We also conducted stratified analyses for PM_{2.5} and BaP, dichotomized as high and low by each other's median values (PM_{2.5}: <167 and ≥ 167 $\mu\text{g}/\text{m}^3$; BaP: <39 and ≥ 39 ng/m^3). P-values <0.05 and P-interactions <0.10 were considered statistically significant. All analyses were performed using SAS v9.3 (SAS Institute Inc., Cary, NC).

Results

Personal and household characteristics

With respect to personal characteristics, the average age was 55.4 (14.4 SD) years and the average BMI was 21.8 (3.4 SD) kg/m² (Table 1). Additionally, there was an average of 119 (30 SD) copies of mitochondrial DNA per cell in leukocytes. When examining air pollutants, the median personal PM_{2.5} and BaP exposure averaged from the two sequential 24 h periods were 167 $\mu\text{g}/\text{m}^3$ (IQR: 118, 235) and 40 ng/m^3 (IQR: 18, 77), respectively. During the first visit, half of the women used smoky coal for heating and cooking in their homes (50.7%), whereas 8.8% used smokeless coal, 5.4% used wood/plant biomass, and 35.1% used a mix or other fuel types.

Associations between PM_{2.5} and BaP exposure, personal and fuel characteristics and leukocyte mtDNAcn

The distributions of leukocyte mtDNAcn across quartiles of PM_{2.5} and BaP exposure are presented in Supplementary Table 1, available at *Carcinogenesis* Online. We found significant inverse correlations between mtDNAcn and ordinal quartiles of personal PM_{2.5} ($\rho = -0.21$, $P < 0.01$) and BaP ($\rho = -0.20$, $P = 0.01$) (Figure 1). Stronger trends were observed when analyses were restricted to smoky coal users. The correlations were non-significant in analyses that were restricted to users of other fuel types. Similar trends were observed when categorizing PM_{2.5} and BaP as continuous (Figure 2).

Age ($\beta = 0.2$ copies per cell; 95% CI: -0.1 , 0.1 ; $P = 0.16$) and BMI ($\beta = -1.1$ copies per cell; 95% CI: -2.5 , 0.4 ; $P = 0.15$) were not significantly associated with mtDNAcn. Additionally, the type of coal mine that supplied household fuel was not significantly associated with mtDNAcn, compared with smokeless coal mine (coking coal: $\beta = -8.1$ copies per cell, 95% CI: -22.8 , 6.7 , $P = 0.28$; gas fat coal: $\beta = 13.3$ copies per cell, 95% CI: 4.6 , 31.2 , $P = 0.14$; one-third coking coal: $\beta = 10.2$ copies per cell, 95% CI: -12.5 , 32.9 , $P = 0.37$; other: $\beta = -11.2$ copies per cell, 95% CI: -29.2 , 6.9 ,

Table 1. Characteristics of the Xuanwei Exposure assessment study

Air pollutants/demographic/anthropometric		
Age (years), mean, SD	55.4	(14.4)
BMI (kg/m ²), mean, SD	21.8	(3.4)
Average personal PM _{2.5} exposure ($\mu\text{g}/\text{m}^3$), median (IQR)	167	(118, 235)
Average personal BaP exposure (ng/m^3), median (IQR)	40	(18, 77)
County of residence, n (%)		
Xuanwei	74	(50.0)
Fuyuan	74	(50.0)
Molecular biomarkers		
Leukocyte mtDNA copy number (count per cell), mean, SD	119	30
Fuel type, n (%)		
Smoky coal	75	(50.7)
Smokeless coal	13	(8.8)
Wood	4	(2.7)
Plant	4	(2.7)
Other	52	(35.1)
Type of coal mine which supplied fuel, n (%)		
Coking coal	73	(49.3)
Gas Fat coal	23	(15.5)
Smokeless coal	20	(13.5)
One-third coking coal	10	(6.8)
Other	20	(13.5)
Unknown	2	(1.4)

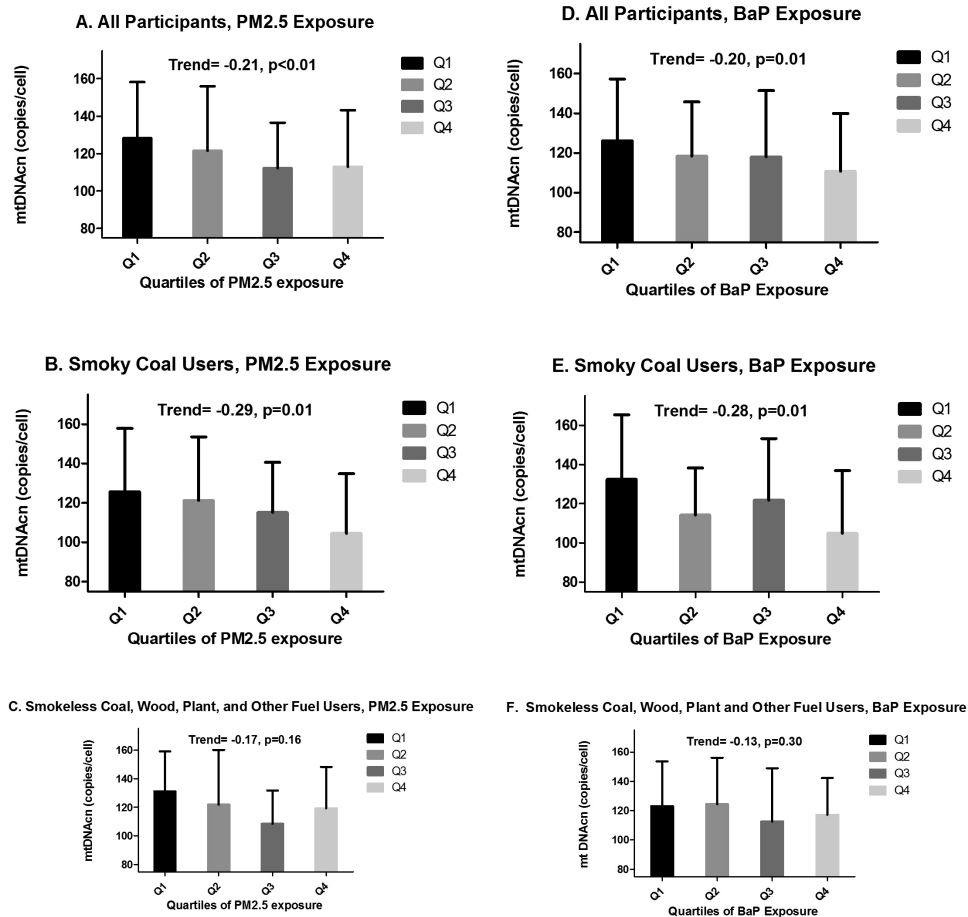


Figure 1. Mean mtDNA copy number by quartiles of personal PM_{2.5} and BaP. (A) In all participants, by quartiles of PM_{2.5}; (B) in smoky coal users, by quartiles of PM_{2.5}; (C) in users of smokeless coal, wood, plant and other fuels, by PM_{2.5}; (D) in all participants, by quartiles of BaP; (E) in smoky coal users, by quartiles of BaP; (F) in users of smokeless coal, wood, plant and other fuels, by BaP. Whiskers represent standard deviations. Trends between quartiles of exposure and mtDNAcn were analyzed with Spearman correlations. For PM_{2.5}: Q1 = 41–117 $\mu\text{g}/\text{m}^3$, Q2 = 118–166 $\mu\text{g}/\text{m}^3$, Q3 = 167–233 $\mu\text{g}/\text{m}^3$, and Q4 = ≥ 234 $\mu\text{g}/\text{m}^3$. For BaP: Q1 = 2–17 ng/m^3 , Q2 = 18–38 ng/m^3 , Q3 = 39–76 ng/m^3 , Q4 = ≥ 77 ng/m^3 . P-values < 0.05 were considered statistically significant.

$P = 0.22$). Furthermore, smoky coal use ($\beta = 0.4$ copies per cell; 95% CI: -10.0, 10.8; $P = 0.95$) and smokeless coal use ($\beta = 13.6$ copies per cell, 95% CI: -4.8, 32.0; $P = 0.15$) were not significantly associated with mtDNAcn compared with other fuel types, adjusted for age and BMI.

Each incremental $\log\text{-}\mu\text{g}/\text{m}^3$ increase in personal PM_{2.5} exposure was associated with a statistically significant decrease in leukocyte mtDNAcn of -10.3 copies per cell (95% CI: -18.6, -2.0; $P = 0.02$), adjusted for age, BMI, and fuel type (Table 2). When stratified by fuel type, similar statistically significant inverse associations with PM_{2.5} were observed when analyses were restricted to smoky coal users but not users of other fuel types. When stratified by median BaP levels, PM_{2.5} was significantly inversely associated with mtDNAcn when BaP was below the median (<39 ng/m^3 : $\beta = -16.2$ copies per cell; 95% CI: -30.6, -1.8; $P = 0.03$) but was not statistically significant when BaP was above the median (≥ 39 ng/m^3 : $\beta = -6.8$ copies per cell; 95% CI: -23.2, 9.7; $P = 0.41$).

Each incremental $\log\text{-}\text{ng}/\text{m}^3$ increase in personal BaP exposure was associated with a statistically significant decrease in leukocyte mtDNAcn of -5.4 copies per cell (95% CI: -9.9, -0.8; $P = 0.02$), adjusted for covariates (Table 2). When stratified by fuel type, similar statistically significant inverse associations with BaP were observed when analyses were restricted to smoky coal users but not users of other fuel types. When stratified by median

PM_{2.5} levels, significant associations between BaP and mtDNAcn were not found in either low or high PM_{2.5} levels (<167 $\mu\text{g}/\text{m}^3$: $\beta = -3.6$ copies per cell, 95% CI: -12.7, 5.5, $P = 0.43$; ≥ 167 $\mu\text{g}/\text{m}^3$: $\beta = -5.7$ copies per cell, 95% CI: -13.5, 2.2, $P = 0.16$). The interaction terms between PM_{2.5} × fuel type and BaP and fuel type were not statistically significant and thus not included in the models.

Discussion

In our cross-sectional molecular epidemiological study, we found that increased personal exposure to PM_{2.5} and BaP was associated with decreased leukocyte mtDNAcn in non-smoking women from Xuanwei and Fuyuan, China. The inverse relationships were observed in smoky coal users but not users of other fuels.

Several factors may explain our results; therefore, sensitivity analyses were conducted to assess the robustness of the findings. Potential confounders such as age, BMI and fuel type may have introduced bias to the results. However, we found that sequential stepwise inclusion of these potential confounders to the models did not appreciably change the estimates. Furthermore, fuel type may be a potential effect modifier given the compositional differences between emissions. When the analyses were stratified by fuel type, similar directions of associations were observed in both smoky coal and non-smoky coal

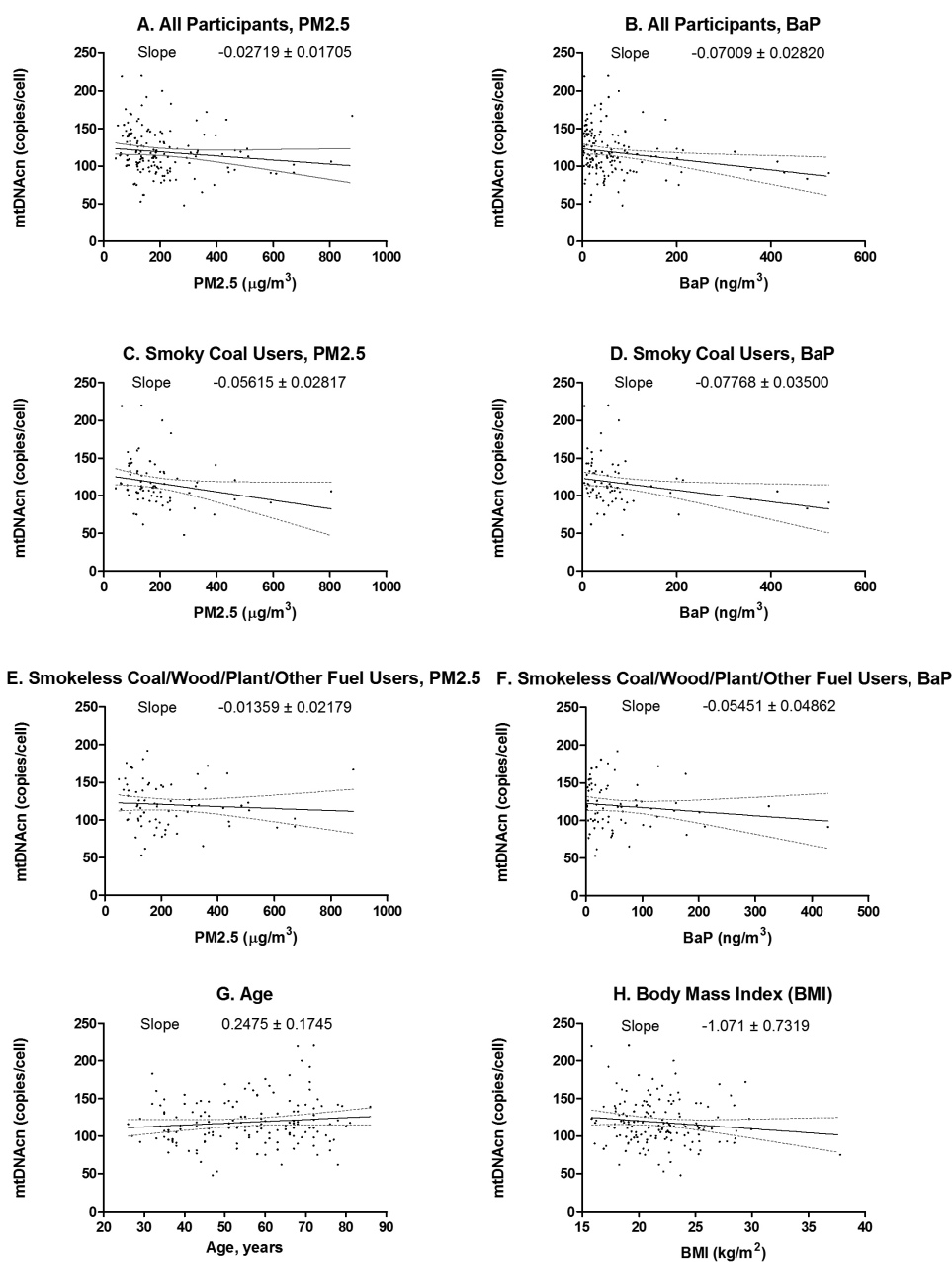


Figure 2. Scatter plots between PM_{2.5}, BaP, age, BMI and continuous leukocyte mtDNA copy number. Red dotted lines represented 95% CIs of the slope.

users. The associations were only significant among smoky coal users, albeit the interaction terms with fuel type were not significant, which may be due to limited statistical power. However, these findings may be attributed to compositional difference in PM_{2.5} between fuel types. For instance, a previous geologic study found elemental differences in transition metals including nickel, silicon, aluminum, titanium, iron, and manganese between smoky and smokeless coal (37). When stratified by median BaP levels, the inverse association between PM_{2.5} and mtDNAcn was more pronounced at low BaP levels; suggesting that the relationship was unlikely to be driven by high BaP levels. Additionally, BaP was not significantly associated with mtDNAcn when stratified by median PM_{2.5} levels.

Few population-based studies have examined the relationships between PM_{2.5} and PAH exposure, and mtDNAcn. Similar to our study, Pieters et al. in 2016 (33) found that increased PM_{2.5}

exposure was associated with decreased mtDNAcn in an elderly Flemish population from Genk, Belgium. Furthermore, a short-term repeated-measures study of 35 Type 2 diabetes patients in Shanghai, China, found non-significant inverse associations between various fractions of PM_{0.25-10} and mtDNAcn (41). However, a repeated-measures study of 60 truck drivers and 60 office workers from Beijing, China, did not find significant associations between average cross-shift shift PM_{2.5} exposure and mtDNAcn; but inverse associations were observed with PM₁₀ (34). A cross-sectional study of 63 male healthy steel workers from Brescia, Italy, found that ultrafine PM₁, which are subsumed by PM_{2.5}, was positively associated with mtDNAcn. The differences in findings between studies may be attributed to variability in the toxicities of PM_{2.5} components and other compounds between geographic regions and seasons (42). For instance, compositional components that may be different between diesel and coal combustion

Table 2. Associations between personal exposure to PM_{2.5} and BaP, and leukocyte mtDNAcn in non-smoking women of the Xuanwei exposure assessment study

	All participants (n = 148)					Smoky coal users (n = 75)					Smokeless coal, wood, plant, coal cakes and other users (n = 73)				
	Estimate	95% CI		P-value	Estimate	95% CI		P-value	Estimate	95% CI		P-value	Estimate	95% CI	
		Lower	Upper			Lower	Upper			Lower	Upper			Lower	Upper
PM _{2.5} , log (µg/m ³)															
Unadjusted main effect	-10.0	-18.2	-1.8	0.02	*	-15.0	-27.7	-2.3	0.02	*	-7.1	-18.2	4.0	0.20	
Adjusted main effect															
Including age	-10.4	-18.6	-2.2	0.01	*	-15.3	-28.1	-2.5	0.02	*	-7.5	-18.4	3.5	0.18	
Including age and BMI	-10.6	-18.8	-2.5	0.01	*	-16.0	-28.2	-3.8	0.01	*	-7.3	-18.2	3.7	0.19	
Including age, BMI, and fuel type	-10.3	-18.6	-2.0	0.02	*	—	—	—	—	—	—	—	—	—	
BaP, log (ng/m ³)															
Unadjusted main effect	-5.8	-10.1	-1.5	0.01	*	-9.6	-16.4	-2.8	0.01	*	-3.3	-9.1	2.5	0.27	
Adjusted main effect															
Including age	-5.8	-10.1	-1.6	0.01	*	-9.7	-16.5	-2.8	0.01	*	-3.4	-9.1	2.4	0.25	
Including age and BMI	-5.8	-10.1	-1.6	0.01	*	-9.3	-15.9	-2.7	0.01	*	-3.2	-8.9	2.6	0.27	
Including age, BMI, and fuel type	-5.4	-9.9	-0.8	0.02	*	—	—	—	—	—	—	—	—	—	

*P-values <0.05 were considered statistically significant.

include organic and elemental carbon, NO₃⁻, SO₄²⁻, NH₄⁺, and heavy and transition metals (43–47). Furthermore, the amount of energy in the combustion process, degree of incomplete combustion and atmospheric conditions can greatly affect the size and composition of the components.

With respect to BaP, results from our study were in the same direction as those reported by Pieters *et al.* in 2013 (36), which found inverse associations with mtDNAcn. However, our results and those of Pieters *et al.* (2013) were in the opposite direction of findings by Pavanello *et al.* in 2013 in 46 Polish non-smoking men (35). These discordances may be attributed to sex and geographic differences between the study populations. Additionally, we cannot discount the contribution of other lifestyle, diet and environmental factors that were unique to each study. Differences in PAH exposure levels and exposure time-windows among studies may also be influential. Given the limited number of studies that examined the associations between exposure to PM_{2.5} and PAHs, and mtDNAcn, firm relationships have yet to be established.

Previous case-control and cohort studies found positive associations of both smoky coal use and mtDNAcn, to risk of lung cancer mortality (20,26,32,40,48). However, a recent pooled analysis did not find associations between mtDNAcn and lung cancer risk (49). Furthermore, higher PM_{2.5} levels were previously reported in smoky coal users compared with smokeless coal users in Xuanwei (15). Given the inverse associations between PM_{2.5} and mtDNAcn found in our study, it was possible that the elevated risks of lung cancer mortality associated with smoky coal use found in previous studies may be due to other components emitted from its combustion or unrelated to mtDNAcn pathways all together.

This study had strengths in addressing the study question. First, the sample population was exclusively non-smoking Chinese women, which removes potential confounding by active cigarette smoking, race/ethnicity and sex. Further, nearly all participants were exposed to secondhand smoke from cohabitating family members. Second, differential misclassification of the exposure and outcome was unlikely because PM_{2.5} and BaP, and mtDNAcn were independently measured and could not possibly affect each other. Moreover, laboratory personnel were blinded to characteristics of the participants. However, there were important assumptions and caveats of note. First, we assumed that the PM_{2.5} measurements were representative of exposure spanning further into the past and that the effect on mtDNAcn would be captured within the study's time frame. Second, the sample size of this study was limited, which hindered our statistical power to detect subtle associations. Third, white blood cell differentials and platelet concentrations, which may be sources of mtDNAcn variability, were not assessed.

In summary, we found that increased personal exposure to indoor PM_{2.5} and BaP produced via smoky coal combustion may be associated with decreased leukocyte mtDNAcn in non-smoking Chinese women. These findings suggest that exposure to PM_{2.5} and BaP may alter mitochondrial dynamics and that mtDNAcn may be a potential mediator of indoor air pollution on chronic disease development. However, given the aforementioned limitations, we cannot discount the possibility of chance findings. Caution is recommended when interpreting the results. Subsequent investigations of air pollution and mtDNAcn should utilize large prospective cohorts with numerous repeated-measurements to characterize their intricate dynamics throughout the lifecourse.

Supplementary material

Supplementary data are available at *Carcinogenesis* online.

Funding

This study was supported by intramural funding from the National Cancer Institute.

Conflict of Interest Statement: None declared.

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