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Mitochondrial DNA Copy Number and Exposure to Polycyclic Aromatic Hydrocarbons

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Abstract

Background—Increased mitochondrial DNA copy number (mtDNAcn) is a biological response to mtDNA damage and dysfunction predictive of lung cancer risk. Polycyclic aromatic hydrocarbons (PAHs) are established lung carcinogens and may cause mitochondrial toxicity. Whether PAH exposure and PAH-related nuclear DNA (nDNA) genotoxic effects are linked with increased mtDNAcn has never been evaluated.

Methods—We investigated the effect of chronic exposure to PAHs on mtDNAcn in peripheral blood lymphocytes (PBLs) of 46 Polish male non-current smoking cokeoven workers and 44 matched controls, who were part of a group of 94 study individuals examined in our previous work. Subjects PAH exposure and genetic alterations were characterized through measures of internal dose (urinary 1-pyrenol), target dose [*anti*-benzo[a]pyrene diolepoxide (*anti*-BPDE)-DNA adduct], genetic instability (micronuclei, MN and telomere length [TL]) and DNA methylation [*p53* promoter] in PBLs. mtDNAcn (MT/S) was measured using a validated real-time PCR method.

Results—Workers with PAH exposure above the median value (>3 μmol 1-pyrenol/mol creatinine) showed higher mtDNAcn [geometric means (GM) of 1.06 (unadjusted) and 1.07 (age-adjusted)] compared to controls [GM 0.89 (unadjusted); 0.89 (age-adjusted)] (p=0.029 and 0.016), as well as higher levels of genetic and chromosomal [i.e. *anti*-BPDE-DNA adducts (p<0.001), MN (p<0.001) and TL (p=0.053)] and epigenetic [i.e., *p53* gene-specific promoter methylation (p<0.001)] alterations in the nDNA. In the whole study population, unadjusted and age-adjusted mtDNAcn was positively correlated with 1-pyrenol (p=0.043 and 0.032) and *anti*-BPDE-DNA adducts (p=0.046 and 0.049).

Conclusions—PAH exposure and PAH-related nDNA genotoxicity are associated with increased mtDNAcn.

Impact—The present study is suggestive of potential roles of mtDNAcn in PAH-induced carcinogenesis.

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Keywords

Polycyclic Aromatic Hydrocarbon; pulmonary carcinogens; mitochondrial DNA copy number; nuclear DNA damage; occupational exposure

Introduction

Growing evidence has shown that DNA alterations in two cellular organelles — the nucleus and the mitochondria — are involved in age-related disorders, including lung cancer, and show inter-related age-related changes (1). Lung cancer is still the most frequent malignancy and leading cause of mortality for cancer-related deaths (2). Investigating new pathogenic pathways and identifying predictive risk markers in individuals at high risk are a priority for public health and preventive medicine (2).

In the present study, we investigated cokeoven workers occupationally exposed to polycyclic aromatic hydrocarbons (PAHs), an established class of lung carcinogens (3,4) and pro-aging compounds (5,6). PAHs are also widespread in the general population due to tobacco smoking, diets, and environmental pollution (3,7). PAHs have been extensively characterized for their capacity to produce damage on the nuclear DNA (nDNA). However, PAH effects on mitochondrial DNA (mtDNA), an independent DNA molecule with central roles in controlling oxidative balance, cellular respiration and apoptosis, are less well established.

Benzo[a]pyrene (BP), a primary tracer of PAH mixtures, has been shown to form genotoxic-mutagenic bulky *anti*-benzo[a]pyrene diolepoxide (*anti*-BPDE)-DNA adduct in the nucleus (8). However, BP has been reported to have even higher damaging potential to the mtDNA — with 40 to 90-fold higher affinity for mtDNA (9–11) than for nDNA. Also, PAH metabolic activation—via aldo-keto reductase and/or manganese superoxide dismutase (MnSOD) (12)—produces reactive oxygen species (ROS) that can form high levels of oxidized guanine in both the nuclear (13) and mtDNA (14). ROS from exogenous sources like PAH metabolites may impair electron chain transport and damage mtDNA. Mitochondria — which have diminished protective histones and DNA repair capacity compared to nDNA — are highly prone to be damaged (15) and compensate for damage by replicating their mtDNA molecules and increasing their cellular mtDNA copy number (mtDNAcn) (16–19). Thus, we hypothesize that mtDNA would be a relevant target in PAH exposure.

Peripheral blood lymphocytes (PBLs), which can be easily and non-invasively obtained from human individuals, have been shown to mirror at least some of the DNA modifications induced by PAHs in the lung. In particular, consistent correlations have been found in lung cancer patients between bulky-DNA adducts in PBLs and lung tissue (for a review see 20) and between DNA methylation levels in PBLs and lung (21). Moreover high levels of mtDNAcn (22), as well as some nDNA alterations, such as DNA adducts (23) micronuclei (MN) (24), and *p53* hypomethylation (25) in PBLs (23,24) and peripheral blood leukocytes (22,25) have all been shown to predict lung cancer risk. Telomere length in blood cells, an additional marker affected by oxidative damage, has been evaluated in relations with lung cancer risk, although results have provided both positive (26) and negative correlations (27,28). However, no studies have yet addressed whether PAH(BP) alters mtDNAcn in PBLs from exposed individuals.

In the present study, we investigated the effects of chronic exposure to PAHs on mtDNAcn measured in PBLs of Polish male non-current smoking cokeoven workers and matched controls. PAH exposure and its effects on nDNA were characterized using measures of

internal dose (urinary 1-pyrenol) and PBL measures of target dose [*anti*-BPDE-DNA adduct], genetic instability [micronuclei, MN and telomere length, TL], and DNA methylation [*p53* promoter].

Materials and methods

Study design

The study participants ($n = 90$) were all males non-current smokers, including 46 cokeoven workers in three polish cokeries and 44 controls matched by gender and ethnicity, who were part of a group of 94 study individuals examined in our previous work (6) with DNA still available (enrolled from January through May 2006). Cokeoven workers all performed tasks (i.e. charging, coking and pushing operations at the cokeoven battery section) involving exposure to high levels of PAHs. We excluded individuals whose work involved exposure to benzene (i.e. workers in by-product operations). Controls were clerks of the Institute of Occupational Medicine and Environmental Health in Sosnowiec recruited during their periodic check-ups at the Preventive Health Services of the Institute. All individuals were non-current smokers, defined as either never-smokers or former smokers who had quit smoking at least 1 year before sample collection. Urine analysis of nicotine and its metabolites (29) confirmed that all individuals were non-exposed to tobacco smoke as they had urine nicotine concentrations <0.01 mg/mmol creatinine.

PAH exposure was assessed by measuring 1-pyrenol in a urine sample (50 ml) collected from each of the workers at the end of their work shift (after at least 3 consecutive working days) and in the late afternoon from controls. At the same time urine was collected, blood samples were taken and placed in EDTA (20 ml) and heparin tubes (10 ml) for adduct and MN analysis, as described previously (29). Samples were brought at the Institute of Occupational Medicine and Environmental Health in Sosnowiec where: (i) PBL cultures for MN detection were prepared and MN analyses performed; (ii) PBLs for adduct analyses were isolated in Ficoll solution (Seromed, Berlin, Germany) within 4 h after blood collection and kept frozen at -80°C until shipment to the Occupational Health Section, Padova, Italy, where DNA was extracted. Data on other possible non-occupational PAH exposure (diet and environment) and consumption of fruit and vegetables were collected by means of a structured-questionnaire, as described previously (6,29). Individuals with high dietary intake of PAHs were defined as those who reported consumption of PAH-rich meals (charcoaled meat and pizza) more than once a week; individuals with indoor exposure to PAHs were those who used wood or coal heating at home; individuals with environmental exposure to PAHs were those with high-exposure index based on the location of their residence in areas with intense local traffic and/or presence of industries. The study was reviewed by the Ethics Committee of the Institute of Occupational Medicine and Environmental Health in Sosnowiec. All participants gave their written informed consent prior to enrolling.

Analysis of exposure to PAHs

Internal dose: 1-pyrenol—Exposure to PAHs was determined as previously described (29) by measuring 1-pyrenol in urine samples by high-performance liquid chromatography–fluorescence. Creatinine levels were used to estimate urinary dilution using a colometric test, based on the Jaffé reaction between creatinine and sodium picrate. Urinary 1-pyrenol was expressed as micromoles per mole of creatinine.

Analysis of nDNA alterations

Target dose: *anti*-BPDE-DNA adduct—*Anti*-BPDE–DNA adduct formation was detected, after DNA isolation with a Promega Wizard genomic DNA purification kit (Promega, Milano, Italy), by high-performance liquid chromatography/fluorescence analysis

of BP-tetrol-I-1 (*r*-7,*c*-10,*t*-8,*t*-9-tetrahydroxy-7,8,9,10-tetrahydro-benzo[*a*]pyrene) released after acid hydrolysis of DNA samples, as described previously (29). The mean coefficient of variation (CV) for analyses of a standard curve repeated five times on five different days was 10%. The highest CV value was 5.70% for those samples ($n = 8$) with >200 μg DNA repeated twice.

Genetic instability: Micronuclei (MN)—MN analysis was performed on coded slides scored by light microscopy at $\times 400$ magnification, as described previously (29). To exclude artefacts, the identification of MN was confirmed at $\times 1000$ magnification in 10% of samples. The scoring of bi-, tri- and tetra-nucleate cells and MN was done and the cytokinesis block proliferation index was calculated as being equal in both groups ($p = 0.60$).

Genetic instability: TL measurement by quantitative PCR—TL was measured in PBL DNA using the real-time quantitative polymerase chain reaction (PCR) method developed by Cawthon (30) and described previously (6). This method measures the relative TL in genomic DNA by determining the ratio of telomere repeat copy number (T) to single copy gene (S) copy number (T:S ratio) in experimental samples relative to the T/S ratio of a reference pooled sample (6). The single-copy gene used in this study was human β -globin (*hbg*). An eight-point standard curve was generated from a serially diluted DNA pool (obtained from 20 DNA samples randomly selected from the samples tested in the present study), ranging from 30 to 0.234 ng in each plate, so that relative quantities of T and S (in nanograms) could be determined from it. All samples and standards were run in triplicate and the average of the 3 T/S ratio measurements was used in the statistical analyses. The PCR runs were performed in triplicate on a 7900HT Fast Real Time PCR System (Applied Biosystems, Foster City, CA) (6). After PCR amplification, the specificity of the product was confirmed by dissociation curve analysis. To test the reproducibility of TL measurements, we amplified telomere (T) and *hbg* (S) in 15 samples that were replicated three times on each of three different days. The within-sample CV for the average T:S ratio over the 3 consecutive days was 8.7%, which was similar to the CV reported for the original protocol (30).

p53 promoter DNA methylation—DNA methylation in *p53* gene was quantified as previously described (31), using bisulphite-PCR and pyrosequencing. In brief, the samples were bisulphite treated using the EZ-96 DNA Methylation-Gold KitTM (Zymo Research, Orange, CA) and PCR amplified. The degree of methylation was expressed as percentage of methylated cytosines divided by the sum of methylated and unmethylated cytosines. Every sample was tested three times for each marker to confirm the reproducibility of our results and their average was used in the statistical analysis. CV in replicate pyrosequencing runs was 1.8%.

Analysis of mtDNAcn

MtDNAcn was measured in PBL DNA using real-time quantitative PCR as previously described (32). This assay measures relative mtDNAcn by determining the ratio of mitochondrial (MT) copy number to single copy gene (S) copy number in experimental samples relative to the MT/S ratio of a reference pooled sample (33). Briefly this method is based on quantification of Mt and S quantities expressed as Cts derived from a standard curve obtained from serial dilutions of a reference DNA. The single copy gene used in this study was human [beta] globin (*hbg*). The Mt PCR mix was: iQ SYBR Green Supermix (Bio-Rad) 1 \times , MtF3212 500 nM, MtR3319 500 nM, EDTA 1 \times . The S (*hbg*) PCR mix was: iQ SYBR Green Supermix (Bio-Rad) 1 \times , *hbg*F 500 nM, *hbg*R 500 nM, EDTA 1 \times . 4 ng DNA was loaded in a 20 μl PCR reaction. As a reference sample, we used pooled DNA from

20 participants randomly selected from this same study (500 ng for each sample) to create at every Mt and S PCR run a fresh standard curve, which ranged from 20 ng/μl to 0.25 ng/μl. The primers for RT Q-PCR analysis of MtDNA and hbg were previously described (32). All PCRs were performed on 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The thermal cycling conditions for mtDNA PCR were: initial 2 minutes at 50 °C, and 3 minutes at 95 °C to activate the hot-start iTaq DNA polymerase, followed by 35 cycles comprised of 15s denaturation at 95 °C and 60s anneal/extend at 60 °C. The thermal cycling conditions for the *hbg* PCR were 3 minutes at 95 °C to activate the hot-start iTaq DNA polymerase, followed by 35 cycles comprised of 15s denaturation at 95 °C and 60s anneal/extend at 58 °C. Each run was completed by melting curve analysis to confirm the amplification specificity and absence of primer dimers. All samples were run in triplicates on 384 plate. The average of the three Mt measurements was divided by the average of the three S measurements to calculate the MT/S ratio for each sample. The coefficient of variation for the MT/S ratio in duplicate samples analyzed on two different days was 7.8%.

Statistical analysis

Statistical comparisons between groups were performed using the non-parametric Mann–Whitney *U*-test for continuous variables or the chi-square test for categorical variables. Linear regression analysis was used on the entire study population to test the association of PAH exposure (urinary 1-pyrenol), *anti*-BPDE–DNA adduct, MN, *p53* DNA methylation levels, or TL (independent variables) with mtDNAcn (dependent variable) in unadjusted or age-adjusted models. Linear regression was also applied to compare mtDNAcn between the first and fourth quartiles of the above explanatory variables. Multiple linear regression analysis was used to assess the influence of urinary 1-pyrenol, *anti*-BPDE–DNA adduct, MN, *p53* DNA methylation levels, TL and age (independent variables) – all fitted in the same model – on mtDNAcn (dependent variable). PAH exposure was fitted in univariate and multivariate analysis as a categorical variable (0=controls; 1 and 2 = workers with 1-pyrenol and >3 μmoles/mol creatinine, respectively). All statistical tests and p-values were two-sided and were performed with Statsdirect Statistical software (Ashwell, Herts, UK).

RESULTS

Participants characteristics, exposure and biomarkers levels

The study population of 46 cokeoven workers and 44 controls (Table 1) included all non-current smoking males. The two groups were similar for age range, dietary behaviors (PAH-rich meals and daily consumption of fruit and vegetables), as well as for their environmental or indoor exposure to PAHs (Table 1). Cokeoven workers had a median of 12 working years and were heavily exposed to PAHs as reflected in their urinary 1-pyrenol with a median value of 3 μmoles/mol creatinine. Workers exhibited significantly higher levels of different genotoxic alterations as reflected in higher *anti*-BPDE–DNA adduct ($p<0.001$) and MN ($p<0.001$), and lower TL ($p=0.053$) and DNA promoter methylation of *p53* ($p<0.001$) compared to controls. Among former workers, no correlation was found between mtDNA and years since quitting smoking.

mtDNA copy number according to PAH-exposure

Coke-oven workers were divided in low and high exposure groups using the median levels of urinary 1-pyrenol (1-pyrenol and >3 μmoles/mol creatinine), taken as a measure of exposure to PAHs. Blood mtDNAcn geometric mean (GM) was significantly higher in the high exposure group of coke oven workers [GM 1.06] compared to controls [GM 0.89 MT/S, $p=0.029$] (Table 2). Age-adjusted analysis confirmed the finding of higher mtDNAcn in highly-exposed workers (1.07 MT/S) compared to controls (0.89 MT/S, $p=0.016$). MtDNAcn in the low-exposure group of workers was not significantly different from

controls suggesting that mtDNAcn levels was similar in low-exposed workers and controls. Tests for trend for increased mtDNAcn across the three exposure categories [controls, low (1-pyrenol <3) and high exposed (1-pyrenol>3) workers] were statistically significant in both unadjusted (p-trend=0.043) and age-adjusted (p-trend=0.032) analyses. As regards long term exposure assessment, no measure were available and the only information was on duration of exposure as a coke-over worker. There was no significant association between mtDNAcn and years worked in the coke-oven factory (linear regression $r=0.081$, $p=0.52$).

Correlation of mtDNA copy number with nDNA alterations

In the whole study population ($n=90$; table 3), mtDNAcn (MT/S) was positively correlated with *anti*-BPDE-DNA adduct levels in both unadjusted ($p=0.046$) and age-adjusted analyses ($p=0.049$). MtDNA was not associated with MN, TL, and *p53* promoter methylation (table 3). A linear regression model applied to compare mtDNAcn between the first and fourth quartiles of the explanatory biomarkers showed higher levels of mtDNA in the highest TL quartile in both unadjusted and age-adjusted analyses ($p=0.044$ and $p=0.036$), but no associations with the other variables (MN and *p53* methylation) (data not shown). When biomarkers were analyzed separately in exposed workers and controls, no significant associations were found. However, although not statistically significant, the positive correlation between number of adducts and mtDNAcn was found also among exposed workers, with a regression coefficient higher than in the whole study population ($\beta=0.03$ vs. $\beta=0.02$). The multiple linear regression analysis of urinary 1-pyrenol, *anti*-BPDE-DNA adduct, MN, *p53* DNA methylation levels, TL and age, all fitted in the same model as determinants of mtDNAcn, revealed that TL, but not the other markers, was the only independent significant determinant of increasing mtDNAcn ($p=0.031$).

Discussion

In the present study, we showed that workers with exposure to PAHs above $3 \mu\text{mol}$ 1-pyrenol/mol creatinine had significantly higher mtDNAcn in PBLs compared to controls. Workers also exhibited higher levels of genetic and chromosomal alterations in *anti*-BPDE-DNA adducts, MN, TL, and epigenetic changes [i.e., *p53* hypomethylation] as previously reported (6,31). Increased levels of mtDNAcn in PBLs have been associated with future risk of lung cancer (22). Our findings extend those observations by demonstrating that increased mtDNAcn may also occur in healthy individuals who are occupationally exposed to considerably higher levels of carcinogenic PAH(BP) than Biological Exposure Index (BEI) proposed by Jongeneelen (34), i.e., 1-pyrenol of $2.28 \mu\text{moles/mol}$ creatinine. At this value, corresponding to the post-shift excretion value at an environmental exposure equal to the airborne threshold limit value (TLV) of coal tar pitch volatiles (CTPV) (i.e. 0.2 mg/m^3 of "benzene soluble matter", ACGIH (35), cokeoven workers have been shown to be at a 30% increased risk of lung cancer (34). Alterations in mtDNA have long been suggested to contribute to the development of lung cancer (for a review see 36). However, whether mtDNAcn has a direct role in lung carcinogenesis is still under investigation. Increased mtDNAcn and a concurrent decline in mitochondrial function of salivary cells has been shown in response to tobacco smoke (18,19), as well as in normal adjacent lung tissues of cancer patients. Individuals with higher blood mtDNAcn at baseline have higher risk of developing lung cancer (22,37). In addition, mtDNAcn alterations are associated with impaired apoptosis and subsequent increased cellular proliferation (38), as well as with nDNA mutations due to aberrant mtDNA insertion into the nuclear genome (39). The findings of the present study are suggestive of potential roles of mtDNAcn in PAH-induced carcinogenesis. However, whether mtDNAcn changes contribute to determining increased risks of malignancies in PAH-exposed individuals remains to be determined. Our findings on high PAH-exposed workers indicate that lymphocyte mtDNAcn may represent a novel

marker specifically associated with levels of PAH exposure above the risk threshold. Increased PBL mtDNAcn has been associated with exposures derived from combustion processes such as benzene (40,41) and particulate matter (32). Our results may indicate a potential role of PAHs, also a product of combustion, in those associations.

In our study, we found that mtDNAcn was correlated with genotoxic *anti*-BPDE-DNA adduct formation that, in addition to offering an assessment of the dose of carcinogens close to the molecular targets, represents a measure of cumulative exposure to carcinogenic PAHs due to the longer life span of PBL DNA compared to urine metabolites. Since adducts detection was performed in DNA aliquots derived from the same DNA samples where mtDNAcn were determined, we cannot exclude that part of *anti*-BPDE-DNA adducts may have mtDNA origin. Therefore, at least part of the correlation observed between *anti*-BPDE-DNA adduct level and mtDNAcn might depend on direct adduct formation to the mtDNA, rather than to interconnections between the nuclear and mitochondrial compartment. However, mtDNA is a small molecule of approximately 15,000 base pairs. Even considering a potential, albeit high, number of 10,000 mtDNA copies in one single cell, the total number of mtDNA base pairs in a cell would amount to just approximately 150 million base pairs, i.e. only 5% of the ~3 billion base pairs in the human nuclear genome. Nonetheless, the lipophilic character of BP and its metabolites, coupled with the very high ratio of lipid/DNA in mitochondria may facilitate the access of *anti*-BPDE to the mtDNA. Also, *anti*-BPDE has 40 to 90-fold higher affinity for mtDNA than for nDNA (9–11). Compared with nDNA, mtDNA has diminished protective histones and DNA repair capacity, and is therefore particularly susceptible to DNA damage. Consequently, the contribution of mtDNA-bound *anti*-BPDE could be a relevant portion of the total cellular burden of DNA adducts.

Mitochondria have been shown to compensate for damage and dysfunction by replicating their mtDNA and increasing mtDNAcn (16). A rise in mtDNA content has been directly associated with DNA damage (42,43) and reduced respiratory chain function secondary to oxidative damage (17, 44). In our study, *anti*-BPDE-related mtDNAcn changes were detected at high PAH-exposure doses. Although PAHs are still considered the primary genotoxic carcinogens produced by coal combustion emissions (for a comprehensive review see ref. 3 and references therein), the presence in coke emissions of toxic metals, or alternatively of reactive oxygen species produced by PAH (12) or metal (45) metabolism, might have contributed – along with BP exposure – in determining the mtDNAcn alteration observed in our study. Also, alterations of mitochondrial lipids and proteins produced by PAHs and/or *anti*-BPDE may have operated as concurrent events contributing to the increased mtDNAcn. However, we cannot exclude that different socio-economic status might have contributed, along with PAH exposure (for which we have supplied a measure of internal dose and biologically effective dose, i.e. the specific promutagenic *anti*-BPDE–DNA), in the increased of mtDNAcn that we observed in PBLs of cokeoven workers.

Interrelationships between the mtDNA and nDNA is however suggested in our study by the finding that individuals with lower mtDNAcn – even after adjusting for age – also had lower TL. This finding specifically suggests a link between nuclear telomere attrition, a marker of biological aging, and mitochondrial alterations. This observation is in line with previous findings by Sahin et al. (46) that showed a potential unifying mechanism connecting the nucleus and mitochondria in cellular aging. In that work, progressive nuclear telomere shortening – mediated by the activation of a *p53*-dependent pathway – was found to determine a reduction of mitochondrial function and mtDNAcn (46).

The present study has several strengths. The enrollment of the study participants was carefully designed to minimize potential confounding and increase the capability to reveal PAH effects by selecting non-current smoking males, all living in the same residential area.

The selection of non-current smokers minimized the probability that the observed associations were dependent on factors other than the occupational PAH exposure. We also evaluated several other potential sources of PAH exposure, including dietary PAHs, indoor PAH exposure and environmental PAH exposure, which showed no differences between cokeoven workers and controls. Our study had reliable measurements of PAH(BP) internal and target doses. Also, we measured in the study participants biomarkers of genetic instability and methylation that allowed for characterizing the intercorrelation between mtDNAcn and nDNA alterations. Finally, the results of this study appear to be biologically plausible and the direction of the effects is consistent with the available literature data on mtDNAcn mechanisms.

We also recognize limitations to our study. This is a small-sized study and its results need to be confirmed in a larger independent investigation. Its cross-sectional design does not allow for investigating the temporal relationship of PAH exposure with mtDNAcn, as well as of the biomarkers of damage, genetic instability, and DNA methylation. The absence of air monitoring, as well as of repeated biological sampling, are also limitations of the study exposure assessment strategy. However, PAH exposure was assessed using biomarkers of internal dose (urinary 1-pyrenol) and target dose (*anti*-BPDE-DNA adduct), which may more appropriately represent the effective exposure dose. To limit confounding, we matched coke-oven workers and controls for their individual characteristics, including age, gender, and ethnicity. In addition, we adjusted the analysis contrasting high exposed workers – as well those based on continues exposure or biomarker variables – for age. Because of the limited number of study subjects, it is possible that the associations observed were due to confounding or chance. The small sample size might have also caused false negative findings. For instance, we did not find any difference in mtDNAcn between low-exposed cokeoven workers and controls. Future studies with augmented sample size are warranted to better characterize the effects of PAH exposure on mtDNAcn at low doses.

In conclusion, cokeoven workers exposed to high levels of PAHs exhibited significantly higher PBL mtDNAcn, as well as genetic alterations in nDNA (i.e. *anti*-BPDE-DNA adduct, MN, shorter TL, *p53* hypomethylation). Individuals with shorter TL showed lower mtDNAcn, thus linking PAH exposure and mitochondrial dysfunction with cellular aging. These features were found in PBLs of individuals chronically exposed to PAHs. As previous investigations have shown that increased mtDNAcn is predictive of future risk of lung cancer, the results of the present study are highly suggestive that mtDNAcn may serve as a biomarker of cancer risk due to PAH exposure. Although these results imply a role of mtDNAcn in PAH carcinogenesis, whether mtDNAcn mediates the risk of lung cancer determined by PAH exposure should be determined in future mechanistic investigations.

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Table 1

Characteristics of Polish noncurrent smoker male cokeoven workers and non-exposed controls

	Cokeoven workers (n=46)	Controls (n=44)	p
Age, mean (range)	36 (20–59)	38 (21–58)	0.95
Diet high in PAH content, ^a n	4	1	0.18
Fruit and vegetables, ^b n	27	21	0.30
Environmental exposure to PAHs, ^c n	15	15	0.88
Indoor exposure to PAHs, ^d n	24	18	0.28
Ex-smokers, n	20	15	0.36
Years of work in the cokery, mean (range)	12 (1–40)	NA	
PAH exposure,			
urinary 1-pyrenol μ moles/mol creatinine ^e , median (range),	3.00 (0.20–7.48)	0.09(0.01–0.40)	<0.001
nDNA alterations			
<i>Anti</i> -BPDE–DNA, adducts per 10 ⁸ nucleotides, ^f median (range)	4.97 (0.90–12.2)	0.21 (0.12–5.56)	<0.001
MN per 1000 binucleate cells, median (range)	3 (1–11)	1 (0–4)	<0.001
Relative TL, median T/S (range)	1.01 (0.31–3.00)	1.20 (0.43–2.12)	0.053
<i>p53</i> , median %mC, ^g (range)	12.2 (5.63–25.1)	18.0 (6.85–46.2)	<0.001

^aNumber of individuals with charcoaled meat consumption more than or equal to once a week.

^bNumber of individuals with daily consumption of fruit or vegetables.

^cNumber of individuals with high environmental exposure from residence in town, intense traffic and presence of industries near home (see Materials and Methods).

^dNumber of individuals with wood- or coal-based heating at home.

^ePAH exposure evaluated by urinary excretion of 1-pyrenol.

^fA value of 0.125 adducts per 10⁸ nucleotides was assigned to individuals with non-detectable adducts.

^gMethylated cytosine percent.

Table 2

MtDNAcn (MT/S) in controls, low and high exposed cokeoven workers: comparison of high-exposed workers with controls, and trend across PAH exposure categories

	Controls n= 44	Coke oven workers		p-value (high exposure vs. controls)	p-value (high exposure vs. low exposure)	p-value (Test for trend)
		low exposure (1-pyrenol $3)$ ^a n =24	high exposure (1-pyrenol>3) ^a n=22			
mtDNAcn (MT/S) geometric mean (95% CI)	0.89 (0.81–0.98)	0.91 (0.81–1.03)	1.06 (0.93–1.18)	0.029	0.08	0.043
mtDNAcn (MT/S) Geometric mean (95% CI), age-adjusted	0.89 (0.82–0.97)	0.90 (0.80–1.01)	1.07 (0.95–1.21)	0.016	0.06	0.032

^aCoke-oven workers were divided in low and high exposure groups using the median levels of urinary 1-pyrenol, taken as a measure of exposure to PAHs

Table 3

Correlation of mtDNAcn (MT/S) with nDNA alterations.

nDNA alterations	mtDNAcn (MT/S) [Model 1] ^a		mtDNAcn (MT/S) [Model 2] ^b	
	b (SE)	p	b (SE)	p
Adducts	0.021 (0.010)	0.046	0.020 (0.010)	0.049
MN	0.013 (0.014)	0.336	0.008 (0.014)	0.52
TL	0.073 (0.066)	0.273	0.090 (0.066)	0.18
<i>p53</i>	-0.006 (0.004)	0.133	-0.005 (0.006)	0.18

^aUnadjusted analysis.^bAnalysis adjusted by age.