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Original Article

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Association between diesel exhaust exposure and mitochondrial DNA methylation

Wei Jie Seow[1,2,*], Wei Hu[3], Yufei Dai[4], Roel Vermeulen[5], Hyang-Min Byun[6], Jason Y.Y. Wong[3], Bryan A. Bassig[3], Batel Blechter[3], Huawei Duan[4], Yong Niu[4], George Downward[5], Shuguang Leng[7], Bu-Tian Ji[3], Wei Fu[8], Jun Xu[9], Kees Meliefste[5], Jufang Yang[8], Dianzhi Ren[8], Meng Ye[4], Tao Meng[4], Ping Bin[4], H Dean, Hosgood III[10], Debra T. Silverman[3,†], Nathaniel Rothman[3,†], Yuxin Zheng[4,7,†] and Qing Lan[3,†]

[1]Saw Swee Hock School of Public Health, National University of Singapore and National University Health System, Singapore, Singapore
[2]Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore and National University Health System, Singapore, Singapore
[3]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD, USA
[4]National Institute of Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention, Beijing, China
[5]Division of Environmental Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands
[6]Population Health Sciences Institute, Newcastle University, Newcastle upon Tyne, UK
[7]Cancer Control and Population Sciences, University of New Mexico Comprehensive Cancer Center, Albuquerque, NM, USA
[8]Chaoyang Center for Disease Control and Prevention, Chaoyang, Liaoning, China
[9]School of Public Health, Li Ka Shing (LKS) Faculty of Medicine, The University of Hong Kong, Hong Kong, China
[10]Division of Epidemiology, Albert Einstein College of Medicine, New York, NY, USA

[†]These authors co-supervised the study.
[*]Corresponding author: 12 Science Drive 2, #09-01H, Singapore 117549. Tel: +65 6601 1243; Fax: +65 6779 1489; Email: ephswj@nus.edu.sg

Abstract

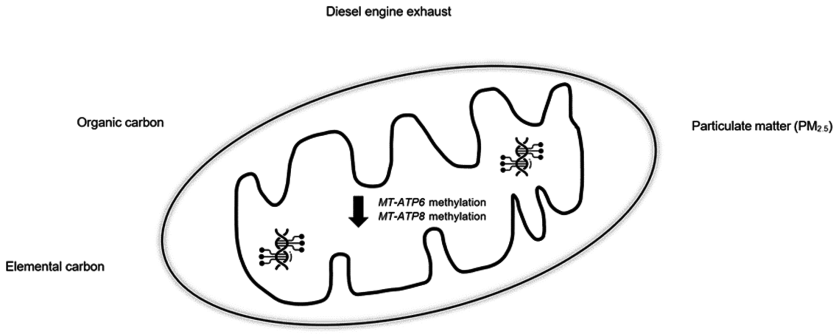
Objectives: Diesel exhaust is an established human carcinogen, however the mechanisms by which it leads to cancer development are not fully understood. Mitochondrial dysfunction is an established contributor to carcinogenesis. Recent studies have improved our understanding of the role played by epigenetic modifications in the mitochondrial genome on tumorigenesis. In this study, we aim to evaluate the association between diesel engine exhaust (DEE) exposure with mitochondrial DNA (mtDNA) methylation levels in workers exposed to DEE.

Methods: The study population consisted of 53 male workers employed at a diesel engine manufacturing facility in Northern China who were routinely exposed to diesel exhaust in their occupational setting, as well as 55 unexposed male control workers from other unrelated factories in the same geographic area. Exposure to DEE, elemental carbon, organic carbon, and particulate matter ($PM_{2.5}$) were assessed. mtDNA methylation for CpG sites (CpGs) from seven mitochondrial genes (*D-Loop*, *MT-RNR1*, *MT-CO2*, *MT-CO3*, *MT-ATP6*, *MT-ATP8*, *MT-ND5*) was measured in blood samples. Linear regression models were used to estimate the associations between DEE, elemental carbon, organic carbon and $PM_{2.5}$ exposures with mtDNA methylation levels, adjusting for potential confounders.

Results: DEE exposure was associated with decreased *MT-ATP6* (difference = −35.6%, *P*-value = 0.019) and *MT-ATP8* methylation (difference = −30%, *P*-value = 0.029) compared to unexposed controls. Exposures to elemental carbon, organic carbon, and $PM_{2.5}$ were also significantly and inversely associated with methylation in *MT-ATP6* and *MT-ATP8* genes (all *P*-values < 0.05).

Conclusions: Our findings suggest that DEE exposure perturbs mtDNA methylation, which may be of importance for tumorigenesis.

Graphical Abstract



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Introduction

Mitochondria are versatile organelles that are involved in many biological processes and serve as central regulators of metabolism and oxidative stress (1,2). Mitochondria also play an important role in the cellular response to environmental exposures such as air pollutants via the generation of reactive oxygen species, and mitochondrial dysfunction has been established as a contributor to carcinogenesis (3). Mitochondrial DNA (mtDNA) can be epigenetically modified via methylation, which affects its transcription (4,5). Recent studies have improved our understanding of the role played by epigenetic modifications in the mitochondrial genome on tumorigenesis (6,7).

Environmental exposures such as air pollution have been reported to induce damage to mtDNA or cause mitochondrial dysfunction, which may increase cancer risk (8,9). Furthermore, exposure to ambient particulate matter has been shown to be significantly associated with changes of mtDNA methylation. Diesel engine exhaust (DEE) is classified as a Group I carcinogen by the International Agency for Research on Cancer (IARC) due to sufficient evidence from human studies of lung cancer (10). Exposure to DEE, which consists of a complex mixture of gaseous and particulate components, most commonly occur in workplaces as a result of incomplete combustion. While there have been *in vivo* and *in vitro* studies on biomarkers showing some evidence for an association between DEE with inflammation and oxidative stress (11,12), the mechanisms by which diesel exhaust causes cancer still remain largely unknown.

We investigated mtDNA methylation level in D-loop and 12s ribosomal RNA (*MT-RNR1*), as well as specific genes associated with ATP synthesis; genes associated with the electron transport chain complex (i.e., *MT-CO2*, and *MT-CO3*); ATP synthase (i.e. *MT-ATP6* and *MT-ATP8*); and NADH dehydrogenase (i.e. *MT-ND5*) in DEE exposed workers in a diesel truck engine testing facility and unexposed controls in a cross-sectional study in Northern China. The purpose of this study was to provide insights into the early biologic effects of DEE exposure.

Methods

Study population

The study population has been described previously (13,14). Briefly, the study population consisted of 53 male workers exposed to DEE while employed in a diesel engine manufacturing facility in Northern China in 2013, and 55 unexposed male control workers from the same local area as the exposed workers who were employed in four separate facilities with no diesel engine exhaust exposure in the same year. Informed consent was obtained from all subjects prior to participation, and the study was approved by the Institutional Review Boards at the US National Cancer Institute and the National Institute of Occupational Health and Poison Control, China CDC.

Exposure assessment

The exposure assessment survey in the diesel factory was conducted from October 2012 to March 2013, as described previously (13). Briefly, repeated full-shift personal air samples for elemental carbon (EC), organic carbon (OC), and fine particulate matter ($PM_{2.5}$) were collected using a cyclone attached to the worker's lapel near the breathing zone, with an aerodynamic cut-off of 2.5 μm ($PM_{2.5}$) at a flow rate of 3.5 L/min using quartz or Teflon filters. There were only five controls with measurements, the rest of the controls were based on modelling and walkthroughs (13).

Isolation of mtDNA and mtDNA methylation measurement

Blood samples were collected from each of the subjects immediately following their work shift, and all plasma samples used for this study were processed and stored consistently in a −80°C freezer within 4 h of collection. mtDNA was isolated from total genomic DNA (gDNA) of 53 exposed and 55 unexposed workers using a modified version of a previously described method (15). Briefly, 500 ng of gDNA from blood was incubated with 1× Plasmid Safe Buffer, Plasmid Safe enzyme (10 U, Cambio, UK), and 25 mM ATP at 37°C for 3 h. Intact mtDNA was captured by the Agencourt AMPure XP beads (Beckman Coulter, USA) and washed with 80% molecular grade ethanol (Sigma-Aldrich, USA) in the Agencourt SPRIPlate 96 Super Magnet Plate (Beckman Coulter, USA). The procedure of enzyme reaction with Plasmid Safe enzyme and washing with ethanol was repeated three times. The final washed mtDNA was quantified with NanoDrop 2000. To ensure the absence of nuclear DNA in the purified mtDNA, we performed an end point PCR with primer sets with one of nuclear DNAs, i.e. *PPARGC-1A*. Extracted mtDNA was stored at −20°C until used. Prior to the bisulfite modification, mtDNA was linearised by endonuclease digestion with BamHI. The linearised mtDNA was then bisulfite treated with EZ DNA Methylation-Gold™ kit (Zymo Research Corp., USA) according to manufacturer's instructions (16,17). After PCR reactions, mtDNA methylation was measured by PyroMark Q96 ID (Qiagen, USA) as previously reported (18,19). PCR primers for D-loop, *MT-RNR1*, *MT-CO2*, and *MT-CO3*, *MT-ATP6*, *MT-ATP8*, and *MT-ND5* were utilised based on previous publications (18,19). The provided mtDNA nucleotide positions are based on NCBI Reference Sequence NC_012920.1. mtDNA methylation was measured in duplicates by pyrosequencing for each subject and the average value of technical replicates was calculated and used in the analysis. The coefficient of variation (CV) was calculated for each mtDNA methylation marker as a quality control (QC) check—MT-ATP6 (28.9), MT-ATP8 (36.1), MT-CO2 (49.7), MT-CO3 (23.6), MT-D-Loop (19.6), MT-ND5 (35.0). CV of the technical replicates of the same subject were calculated. We were unable to calculate the CV for MT-12s-rRNA as we only had one set of run data for MT-12s-rRNA.

Statistical analysis

Characteristics of DEE exposed and unexposed workers were compared using the Wilcoxon rank-sum test for continuous variables, and Fisher's exact test for categorical variables. Linear regression models were used to estimate the associations [betas and 95% confidence intervals (CIs)] between DEE exposure status (exposed versus unexposed) and mtDNA methylation of each marker (log-transformed, continuous). Linear regression models were also used to assess the trend between air pollutant measurements (EC, $PM_{2.5}$, OC) and mtDNA methylation. The mean and standard deviation (SD) of the methylation markers were calculated

for each exposure group. All models were adjusted for age (continuous). Additional covariates were included as potential confounders such as body mass index (BMI; kg/m^2, continuous), recent infection status (categorical; yes/no), current smoking status (categorical; ever/never), and current alcohol consumption (categorical; yes/no) if they were significant at $P < 0.05$ or if there was evidence of confounding (i.e. greater than a 10% change in the regression coefficient).

All statistical analyses were conducted using the SAS software, version 9.4. All *P*-values were reported based on two-sided tests and *P*-values of < 0.05 were considered statistically significant. False discovery rates (FDRs) were calculated using the Benjamini Hochberg method to account for multiple comparisons (20).

Results

Both exposed and unexposed groups have similar characteristics in terms of their age, BMI, smoking status, current alcohol consumption, and recent infection. Exposed workers are generally more likely to be exposed to higher levels of EC (60.1 μg/m^3 in exposed versus 11.1 μg/m^3 in unexposed) ($P < 0.0001$), OC (139.4 μg/m^3 in exposed versus 68.7 μg/m^3 in unexposed) ($P < 0.0001$), and $PM_{2.5}$ (mean of 0.36 mg/m^3 in exposed versus 0.20 mg/m^3 in unexposed) ($P < 0.0001$) (Table 1).

Mitochondrial DNA methylation in *MT-ATP6* (difference in mean = 35.6%, *P*-value = 0.019, FDR = 0.10) and *MT-ATP8* (difference in mean = 29.7%, *P*-value = 0.029, FDR = 0.10) was statistically significantly lower in workers exposed to DEE as compared to controls, after adjusting for potential confounders and multiple comparisons (Table 2). The other mtDNA markers were not statistically different between exposed and unexposed workers.

We also assessed the association between EC, OC, and $PM_{2.5}$ with mtDNA methylation. Elemental carbon was significantly associated with methylation in *MT-ATP6*, *MT-ATP8* and *MT-DLoop* (Table 3). The mean (SD) of *MT-ATP6* methylation (%) for workers unexposed, exposed to < median and ≥ median of ECs levels was 2.83 (1.72), 2.06 (0.48) and 2.11 (0.82), respectively. The mean (SD) of *MT-ATP8* methylation (%) was 2.38 (0.95) for workers exposed to < median and 2.71 (2.44) for workers exposed to ≥ median of EC levels (*P* for trend = 0.034), as compared to unexposed controls (mean = 3.31, SD = 2.20). Elemental carbon was also significantly associated with lower *MT-DLoop* methylation (%) with mean (SD) for < median was 4.10 (2.28) and mean (SD) for ≥ median was 2.82 (1.71), $P_{trend} = 0.030$, as compared to the unexposed controls (mean = 4.18, SD = 3.05).

Similar associations between OC with *MT-ATP6* and *MT-ATP8* methylation were observed. The mean (SD) of *MT-ATP6* methylation (%) was 2.05 (0.48) for workers exposed to < median and 2.12 (0.81) for workers exposed to ≥ median of OC levels ($P_{trend} = 0.038$), as compared to unexposed controls (mean = 2.83, SD = 1.74). The mean (SD) of *MT-ATP8* methylation (%) for workers exposed to < median and ≥ median of OC was 2.28 (0.86) and 2.78 (2.41), respectively ($P_{trend} = 0.039$), as compared to unexposed controls (mean = 3.31, SD = 2.20).

$PM_{2.5}$ was significantly associated with methylation in *MT-ATP6* and *MT-ATP8*. For *MT-ATP6*, the trend across the $PM_{2.5}$ groups was statistically significant ($P_{trend} = 0.049$), with the exposed workers having a mean (SD) methylation of 2.08 (0.77) in the < median and 2.09 (0.56) in the ≥ median

Table 1. Characteristics of study population

Characteristics	Unexposed (*n* = 55)	DEE exposed (*n* = 53)	*P*-value[a]
Age, mean (SD)	42.1 (7.4)	42.0 (6.8)	0.97
BMI, kg/m^2, mean (SD)	25.2 (3.8)	24.6 (3.4)	0.52
Smoking status, *n* (%)			
Current	35 (63.6)	34 (64.2)	0.99
Former	12 (21.8)	11(20.8)	
Never	8 (14.9)	8 (15.0)	
Smoking duration, years, mean (SD)	20.9 (11.1)	19.9 (8.3)	0.59
Smoking intensity, average cigs/day, mean (SD)	7.8 (8.3)	8.4 (8.2)	0.67
Smoking pack-years, mean (SD)	9.6 (11.8)	11.5 (10.4)	0.23
Recent infection, *n* (%)			
No	27 (49.1)	26 (49.1)	1.00
Yes	28 (50.9)	27 (50.9)	
Current alcohol consumption, *n* (%)			
No	8 (14.5)	15 (28.3)	0.08
Yes	47 (85.5)	38 (71.7)	
Work years in diesel factory, mean (SD)	–	19.4 (7.0)	–
EC, μg/m^3, mean (SD)	11.1 (1.3)	60.1 (21.9)	**< 0.0001**
OC, μg/m^3, mean (SD)	68.7 (4.1)	139.4 (25.8)	**< 0.0001**
$PM_{2.5}$, mg/m^3, mean (SD)	0.20 (0.07)	0.36 (0.07)	**< 0.0001**

The bold values refer to statistically significant results with *P*-value <0.05 or FDR < 0.15.
SD, standard deviation; DEE, diesel exhaust exposure; EC, elemental carbon; OC, organic carbon.
[a]Comparison between exposed and controls. *P*-values were obtained from Pearson's chi-squared test for categorical variables and Wilcoxon rank-sum test for continuous variables.

Table 2. Association between diesel exposure status and mtDNA methylation

mtDNA marker	Unexposed (n = 55)[a]	Exposed (n = 53)[a]	Difference in mean levels (%)	P_{crude}	$P_{adjusted}$[b]	FDR
MT-ATP6	2.83 (1.74)	2.08 (0.67)	−35.6	**0.018**	**0.019**	**0.10**
MT-ATP8	3.31 (2.20)	2.56 (1.88)	−29.7	**0.030**	**0.029**	**0.10**
MT-D-loop	4.18 (3.05)	3.46 (2.10)	−20.8	0.20	0.16	0.38
MT-RNR1	0.16 (0.53)	0.23 (0.79)	30.1	0.42	0.52	0.90
MT-CO2	6.88 (4.78)	8.77 (10.45)	21.5	0.93	0.90	0.90
MT-CO3	1.74 (0.69)	1.79 (0.86)	2.9	0.86	0.72	0.90
MT-ND5	3.73 (2.53)	3.41 (1.88)	−9.6	0.60	0.88	0.90

The bold values refer to statistically significant results with P-value <0.05 or FDR <0.15.
FDR, false discovery rate; D-loop, displacement loop; RNR1, 12s ribosomal RNA; CO2, cytochrome C oxidase II; CO3, cytochrome C oxidase III; ATP6, ATP synthase membrane subunit 6; ATP8, ATP synthase membrane subunit 8; ND5, NADH dehydrogenase subunit 5.
[a]Unadjusted mean (± SD).
[b]All multivariable linear regression models were adjusted for age. Additional covariates included in the final models were body mass index (BMI; kg/m^2, continuous), recent infection status (categorical; yes/no), current smoking status (categorical; ever/never), and current alcohol consumption (categorical; yes/no) if they were significant at P < 0.05 or if there was evidence of confounding (i.e. greater than a 10% change in the regression coefficient).

Table 3. Association between elemental carbon (EC), organic carbon (OC), and $PM_{2.5}$ exposures with mtDNA methylation

mtDNA markers	Unexposed workers (n = 55)	DEE exposed workers				P-trend	FDR
		< Median (n = 26)		≥ Median (n = 27)			
	Mean (SD)	Mean (SD)	P-value[a]	Mean (SD)	P-value[a]		
Elemental carbon							
MT-ATP6	2.83 (1.74)	2.06 (0.48)	0.067	2.11 (0.82)	**0.047**	**0.030**	**0.080**
MT-ATP8	3.31 (2.20)	2.38 (0.95)	0.120	2.71 (2.44)	**0.048**	**0.034**	**0.080**
MT-DLoop	4.18 (3.05)	4.10 (2.28)	0.892	2.82 (1.71)	**0.016**	**0.030**	**0.080**
MT-RNR1	0.16 (0.53)	0.31 (1.04)	0.328	0.14 (0.44)	0.956	0.805	0.904
MT-CO2	6.88 (4.78)	8.60 (10.77)	0.886	8.92 (10.34)	0.844	0.877	0.904
MT-CO3	1.74 (0.69)	1.93 (1.11)	0.344	1.64 (0.47)	0.728	0.904	0.904
MT-ND5	3.73 (2.53)	3.27 (1.89)	0.598	3.53 (1.90)	0.770	0.861	0.904
Organic carbon							
MT-ATP6	2.83 (1.74)	2.05 (0.48)	0.051	2.12 (0.81)	0.065	**0.038**	0.135
MT-ATP8	3.31 (2.20)	2.28 (0.86)	0.111	2.78 (2.41)	0.055	**0.039**	0.135
MT-DLoop	4.18 (3.05)	3.91 (2.36)	0.740	3.04 (1.77)	0.062	0.073	0.170
MT-RNR1	0.16 (0.53)	0.13 (0.24)	0.739	0.32 (1.10)	0.483	0.474	0.830
MT-CO2	6.88 (4.78)	11.50 (13.51)	0.402	6.13 (5.31)	0.453	0.593	0.830
MT-CO3	1.74 (0.69)	1.99 (1.14)	0.198	1.59 (0.35)	0.495	0.724	0.845
MT-ND5	3.73 (2.53)	3.35 (1.88)	0.789	3.46 (1.91)	0.976	0.983	0.983
$PM_{2.5}$							
MT-ATP6	2.83 (1.74)	2.08 (0.77)	**0.035**	2.09 (0.56)	0.088	**0.049**	0.171
MT-ATP8	3.31 (2.20)	2.77 (2.46)	0.127	2.35 (1.09)	**0.044**	**0.031**	0.171
MT-DLoop	4.18 (3.05)	3.39 (1.99)	0.228	3.53 (2.25)	0.412	0.310	0.643
MT-RNR1	0.16 (0.53)	0.27 (1.01)	0.582	0.18 (0.48)	0.626	0.572	0.696
MT-CO2	6.88 (4.78)	8.66 (10.76)	0.668	8.88 (10.32)	0.502	0.604	0.696
MT-CO3	1.74 (0.69)	1.64 (0.47)	0.692	1.95 (1.13)	0.281	0.367	0.642
MT-ND5	3.73 (2.53)	3.56 (1.97)	0.829	3.25 (1.81)	0.637	0.696	0.696

P <0.05 or FDR ≤0.10 were in bold.
DEE, diesel exhaust exposure; SD, standard deviation; FDR, false discovery rate; EC, elemental carbon; OC, organic carbon; D-loop, displacement loop; RNR1, 12s ribosomal RNA; CO2, cytochrome C oxidase II; CO3, cytochrome C oxidase III; ATP6, ATP synthase membrane subunit 6; ATP8, ATP synthase membrane subunit 8; ND5, NADH dehydrogenase subunit 5.
[a]All multivariable linear regression models were adjusted for age. Additional covariates included in the final models were body mass index (BMI; kg/m^2, continuous), recent infection status (categorical; yes/no), current smoking status (categorical; ever/never), and current alcohol consumption (categorical; yes/no) if they were significant at P < 0.05 or if there was evidence of confounding (i.e. greater than a 10% change in the regression coefficient).

exposed groups, as compared to unexposed workers (mean = 2.83, SD = 1.74). Similarly, the mean (SD) of *MT-ATP8* methylation (%) for workers exposed to < median and ≥ median of $PM_{2.5}$ was 2.77 (2.46) and 2.35 (1.09) respectively (P_{trend} = 0.031), as compared to unexposed workers (mean = 3.31, SD = 2.20).

Discussion

In our study, we identified several mtDNA methylation markers that were significantly associated with DEE exposure. DEE was inversely associated with *MT-ATP6* and *MT-ATP8* methylation, which was further supported by inverse associations with $PM_{2.5}$, EC and OC, which are components of diesel exhaust. In addition, EC was significantly and inversely associated with methylation in *MT-D-Loop*.

The two significant mtDNA methylation markers are located on genes associated with ATP synthase (*MT-ATP6* and *MT-ATP8*). *MT-ATP6* and *MT-ATP8* are also known as mitochondrially encoded ATP synthase membrane subunits 6 and 8. Mitochondrial ATP synthase plays a pivotal role in catalysing ATP synthesis from ADP by using the energy from the electrochemical gradient of protons across the inner membrane during oxidative phosphorylation (21). Mutations in *MT-ATP6* are found in human carcinomas such as prostate cancer, and promote tumor growth by inhibiting cell death *in vitro* and *in vivo* (22,23). Interestingly, ATP synthase has been demonstrated to be downregulated in cancer (24). Therefore, exposure to diesel exhaust may lead to aberrant methylation of the ATP synthase subunit and mitochondrial dysfunction, promoting tumorigenesis.

Our study found that EC was inversely associated with methylation in *MT-D-Loop*. Decreased methylation of the mitochondrial D-loop region has been shown to be associated with chronic diseases such as colorectal cancer and Alzheimer's disease, likely due to mtDNA copy number changes, increased cell proliferation, and reduced apoptosis (25,26).

Air pollutants such as particulate matter (PM_{10}, $PM_{2.5}$, $PM_{0.2}$), carbon monoxide (CO), nitrogen dioxide (NO_2), and volatile organic compounds (VOCs) have been previously shown to be associated with mitochondrial dysfunction (1). Elemental carbon, a component of $PM_{2.5}$ that is a surrogate for diesel engine exhaust, has been shown to be associated with decreased levels of mtDNA abundance among workers in Beijing, China (27). Furthermore, traffic-related air pollution (TRAP) was found to cause oxidative stress associated with various forms of mitochondrial damage. This may suggest that TRAP induced-effects may be regulated by mtDNA methylation in humans (2), and may be counteracted by mitochondrial-derived peptides (MDPs), which are novel peptides encoded within the mtDNA that serve as signals for cell and organismal protection and energy expenditure, important for regulating metabolism during aging (28). DEE has also been reported to induce mitochondrial dysfunction, dyslipidaemia and liver steatosis in ApoE knockout mice (29). Mitochondrial dysfunction has been shown to result in human diseases such as neurodegeneration and diabetes (30).

A previous study that assessed the effects of traffic-derived EC among truck drivers found *MT-TF* and *MT-RNR1* mtDNA methylation to be associated with metal-rich PM_1 exposure. However, in contrast, they found no significant associations between EC exposure and mtDNA methylation (31). Blood mtDNA methylation levels were found to be inversely associated with $PM_{2.5}$ exposure levels and modified the adverse relationship of $PM_{2.5}$ exposure and heart rate variability outcomes among male healthy welding workers in a boilermaker union (17). To the best of our knowledge, this is the first DEE-mtDNA methylation study to use a novel mtDNA extraction method to isolate mitochondrial DNA from whole genomic DNA in blood. Therefore, compared to previous findings, this study measured a more comprehensive set of mitochondrial genes that are non-biased with nuclear genomes. Additionally, our study population was exposed to very high levels of DEE over a long period of 10 years on average.

Although our study sample size was relatively small, this is the first study to measure mtDNA methylation markers using a novel mtDNA extraction method, and measured samples in duplicates within the same laboratory. In this study, we used a novel mtDNA extraction method (15) to isolate mtDNA from nuclear DNA using readily available total blood DNA. This is superior to previous methods, which are prone to potential cross-measurement of mtDNA methylation from nuclear mitochondrial DNA. This is also one of the few studies to assess the association among a population of workers occupationally exposed to extremely high levels of DEE, as most of the previous studies examined ambient PM exposure among healthy adults. DEE exposure may have affected the proportion of blood cell types and that different cell types may have different levels of mitochondrial gene DNA methylation. We did a sensitivity analysis by further adjusting for basophils, lymphocytes as well as CD3, CD4 and CD8, which were significantly associated with DEE exposure in a previous paper, and the results were similar. Previous studies have demonstrated mitochondrial epigenetic effects from airborne particulate matter rich in metals (31), suggesting that metals could be a potential confounder. It was reported that diesel exhaust particles were typically composed of 75% EC (ranging up to 90%), 20% OC (ranging down to 7%), and small amounts of sulfate, nitrate, trace elements, water, and unidentified compounds (32). Therefore, metal exposures are likely to be low in diesel engine exhaust. However, we did not collect data on metal exposures and were not able to adjust for it in the model. In terms of quality control of the lab assay, most of the mtDNA methylation markers' CVs were < 40%. However, the CV for MT-ATP8 was not ideal (36.1%), therefore, further replication is needed. Other study limitations include potential exposure misclassification for the controls as most of them were based on modelling and walkthroughs, however this is unlikely as no DEE sources were identified based on detailed walk-through surveys. In addition, blood samples were collected at only one time-point, therefore, we were unable to assess temporal trends and changes, if any.

Conclusions

We identified mtDNA methylation markers that were significantly associated with DEE exposure, as well as EC and OC. Our findings suggest that mtDNA methylation may be a potential biomarker of DEE exposure. However, further replication is needed and future large-scale epidemiologic studies are needed to evaluate downstream DEE-associated health effects via mitochondrial dysfunction.

Conflict of Interest Statement

The authors disclose no conflicts of interest.

Data Availability

The data sets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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