Genetic Polymorphisms of Metabolic Enzyme Genes Associated with Leukocyte Mitochondrial DNA Copy Number in PAHs Exposure Workers

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Abstract

Background: PAHs exposure had been reported to be a risk factor of mtDNAcn in our early study. However, the effect of metabolic enzymes' genetic polymorphisms on mtDNAcn in PAHs-Exposure workers has not been fully evaluated.

Methods: We investigated the effects of metabolic enzymes' genetic polymorphisms on mtDNAcn among 544 coke oven workers and 238 office staffs. The mtDNAcn of peripheral blood leukocytes was measured using Real-time quantitative polymerase chain reaction method. Polymerase chain reaction and restriction fragment length was used to detect five polymorphisms in GSTT1, GSTM1, GSTP1 rs1695, CYP2E1 rs6413432, and CYP2E1 rs3813867.

Results: The mtDNAcn in peripheral blood leukocytes was significantly lower in the exposure group than that in the control group ($P < 0.001$). The 1-OHPYR had an increasing trend with the genotypes AA→AG→GG of GSTP1 rs1695 in the control group. Generalized linear model indicated that the influencing factors of mtDNAcn were PAHs-exposure [b(95% CI) = -0.420 (-0.469, -0.372), $P < 0.001$], male [β(95% CI) = -0.058 (-0.103, -0.012), $P = 0.013$] ,and AA genotype for GSTP1 rs1695 [β(95% CI) = -0.051 (-0.095, -0.008), $P = 0.020$].

Conclusions: The male was susceptibility to PAHs exposure. The AA genotype of GSTP1 rs1695 may influence the toxicity of PAHs and associated with the decreased of mtDNAcn.

1. Introduction

Coke oven emissions (COEs) are generally derived from the incomplete combustion of organic matter and possess hazardous levels of fine particulate and polycyclic aromatic hydrocarbons (PAHs). Most chemicals of PAHs are classified as human carcinogens and the genotoxic potential of PAHs have been extensively studied. In addition to producing damage on the nuclear DNA [1], PAHs have 40 to 90 fold higher affinity for mitochondrial DNA (mtDNA) than nuclear DNA and cause even higher level damage to with mtDNA [2]. Several studies have found that PAHs may interfere mitochondrial biosynthesis and further alter mitochondrial DNA copy number (mtDNAcn) [3–5]. Our previous study also found that mtDNAcn decreased with environmental PAHs-exposure [6].

Mitochondria play an important role in multiple cellular functions including oxidative phosphorylation, reactive oxygen species (ROS) generation, calcium homeostasis, and apoptosis. Each mitochondrial contains 2 to 10 copies of mitochondrial DNA. Due to lack of protective histones and DNA repair machinery, mtDNA is particularly more vulnerable to various kinds of environmental toxins [7]. The mtDNAcn will increase as compensation to early poison exposure [8], however, when the increased mtDNAcn cannot maintain the mitochondria normal function, mitophagy will occur to remove dysfunctional ones, and the mtDNAcn will be reduced [9]. Therefore, mtDNAcn might be a sensitive and important target to the genotoxic of PAHs. Moreover, the alteration of mtDNAcn has also been found to be associated with various disease development. For example, alterations of mtDNAcn are interrelated

with lung cancer risk [10], and the decreased mtDNAcn may accelerate the aging process and causes age-related disorders [11].

After PAHs enter the human body, they are firstly metabolized to electrophilic active intermediates by phase Ⅰmetabolic enzymes, such as cytochrome P450 (CYP) monooxygenases. The metabolic intermediates may produce DNA adducts, leading to DNA mutations, alteration of gene expression, and even tumorigenesis [12]. Subsequently, the intermediates are converted into more polar and water-soluble products by phase II metabolic enzymes and then excreted from the body [13]. The phase II metabolic enzymes include glutathione S-transferases (GST), UDP glucuronyl transferases, NADPH quinone oxidoreductases, aldo-keto reductases, and epoxide hydrolases. Therefore, extensive polymorphism of metabolism enzymes genes may be one of the main reasons for the individual variable susceptibility to exogenous toxic substances [14]. Studies have shown that metabolic enzymes' genetic polymorphisms were associated with many health effects in PAHs exposure population. Whyatt et al. found that CYP1A1 Mspl restriction site had higher DNA adduct levels among newborns with PAHs exposure [15]. One study suggested that the GSTM1 (-) was inversely associated with the DNA integrity in the men occupationally exposed to PAHs [16]. Our earlier study showed that GSTT1 (+) and GSTM1 (+) are the risk factors for oxidative stress in coke oven workers [17]. However, the influence of metabolic enzymes' genetic polymorphisms on mtDNAcn in PAHs-Exposure workers has not been studied yet.

Therefore, we detected mtDNAcn to investigate the genotoxic of PAHs-exposure and screened GSTT1, GSTM1, GSTP1, and CYP2E1 gene to explore the role of metabolic enzymes genetic polymorphisms on mtDNAcn in PAHs-exposure.

2. Materials And Methods

2.1. Study population and epidemiological data

A total of 544 workers exposed to COEs for more than one year were recruited as the exposure group from the Henan Anyang Iron and Steel Group, Henan, China. Their workplaces were in five representative locations, including auxiliary production, office personnel, oven bottom, oven side, and oven top. Also, 238 healthy workers without a history of exposure to occupational poison were enrolled from the same region as the control group.

Detailed information on general demographic characteristics, professional history, and biological samples from each participant was collected by trained interviewers. The blood was collected using Na_2EDTA and heparin anticoagulants, and the urine (the end-of-work urine of the occupational exposure population and morning urine of the control group) was retained with 50 ml centrifuge tubes. The study protocol and consent form from all subjects were subjected to approval by the Ethics Committee of Zhengzhou University, China. More detailed information as described in our previous study [6].

2.2. Determination of environmental exposure

According to the Sampling specifications for monitoring hazardous substances in the workplace air (GBZ159-2004) and Exhaust for the stable pollution source-Determination of benzene soluble matter-Soxhlet extraction (HJ690-2014), the representative air samples were collected with a medium flow sample and the COE cumulative exposure dose of the exposure group were determined. Based on living environmental concentration and age, the COE cumulative exposure dose of the control group was estimated. Detailed detection methods were described in our previous study [18].

High-performance liquid chromatography (HPLC) was used to detect the concentrations of four OH-PAHs [1-hydroxypyrene (1-OHPYR) and 1-hydroxynathalene (1-OHNAP), 2-hydroxynathalene (2-OHNAP), and 3-hydroxyphenanthrene (3-OHPHE)] in urine, as described in the previous study [19].

2.3. Analysis of mtDNAcn

DNA was extracted from the peripheral blood leukocytes using a Large Amount of Whole Blood Genomic DNA Extraction Kit (Beijing BioTeke Corporation). The mtDNAcn was measured using Real-time quantitative polymerase chain reaction (PCR) method. This assay measures relative mtDNAcn by determining the ratio of the ND-1 mitochondrial gene to the human β-globin gene. The ND-1 mitochondrial gene primers were forward, 5’-CCTAATGCTT ACCGAACGA – 3’ and reverse, 5’-GGGTGATGGTAGATGTGGC-3’. The β-globin gene was forward, 5’-GCTTCTGACACAACTGTGTTCACTAGC-3’ and reverse, 5’-CACCAACTTC ATCCACGTTCACC-3’. Detailed information as described previously [6].

2.4. Detection of genetic polymorphisms

The GSTT1 and GSTM1 genotype were determined as previously described [20]. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect other loci for genotyping, including GSTP1 rs1695, CYP2E1 rs6413432, and CYP2E1 rs3813867 [21]. The primers are shown in Table 1.

Table 1
Primers sequences for gene polymorphism

Polymorphism		Primers sequences
GSTT1	Forward:	5’-TTCCTTACTGGTCCTCACATCTC-3’
	Reverse:	5’-TCACCGGATCATGGCCAGCA-3’
GSTM1	Forward:	5’-GAACTCCCTGAAAAGCTAAAG C-3’
	Reverse:	5’-GTTGGGCTCAAATATACGGTG-3’
ALB*	Forward:	5’-GCCCTCTGCTAACAAGTCCTAC-3’
	Reverse:	5’-GCCCTAAAAAGAAAATCGCCAATC-3’
GSTP1 rs1695	Forward:	5’-CTTCCACGCACATCCTCTTCC-3’
	Reverse:	5’-AAGCCCCTTTCTTTGTTCAGC-3’
CYP2E1 rs6413432	Forward:	5’-TCGTCAGTTCCTGAAAGCAGG-3’
	Reverse:	5’-GAGCTCTGATGCAAGTATCGCA-3’
CYP2E1 rs3813867	Forward:	5’-CCAGTCGAGTCTACATTGTCA-3’
	Reverse:	5’-TTCATTCTGTCTTCTAACTGG-3’
*ALB is a control gene used in multiple PCR for GSTT1 and GSTM1.		
Forward represents the upstream primer and reverse represents the downstream primer.		

2.5. Statistical analysis

Baseline survey data were entered using EpiData 3.1 software. All analyses were performed using SPSS25.0 software (SPSS Inc., Chicago, IL, USA). The data of four OH-PAHs were converted by natural logarithm to satisfy normal distribution. After adjusting appropriate adjustments for gender, age (years), smoking status, drinking status, and BMI, covariance analysis was used to analyze the effects of the general characteristics and gene polymorphism on OH-PAHs or mtDNAcn. Multiple linear regression analyzed the trend of OH-PAHs change with mutant allele loci. The generalized linear model (GLM) analyzed the influencing factors of mtDNAcn by adjusting the smoking index, drinking status, and BMI. All statistical tests were two-sided, and the level of statistical significance was set at $\alpha = 0.05$.

3. Results

3.1. Individual characteristics and mtDNAcn

The cohort consists of 544 COEs-exposed workers as the exposure group, and 238 healthy people. The proportion of male, smoking, and drinking in the exposure group (71.7%, 41.0%, 54.4%) were higher than

that in the control group (58.4%, 17.2%, 42.0%) ($P < 0.05$). The age in the exposure group (40.10 ± 6.30) was significantly older than that in the control group (38.39 ± 8.43) ($P = 0.005$). The BMI had no significant difference between the exposure group and the control group ($P = 0.376$). The COEs cumulative exposure dose and the four OH-PAHs in the exposure group were higher than those in the control group ($P < 0.05$). MtDNAcn in peripheral blood leukocytes was significantly lower in the exposure group (0.60 ± 0.29) than that in the control group (1.03 ± 0.31) ($t = 18.931$, $P < 0.001$). The basic characteristics have been reported in our previous research [22] (Supplementary Table 1).

3.2. The effects of gender, age, smoking, drinking and BMI on mtDNAcn

After adjusting appropriate adjustments of gender, age (years), smoking index, drinking status, and BMI, covariance analysis showed that no factors were related to the mtDNAcn ($P > 0.05$). However, the mtDNAcn of all layers had significant differences between the two groups ($P < 0.001$). As reported in our early study [6], the results are shown in Supplementary Table 2.

3.3. Effects of genetic polymorphisms on 1-OHPYR

The genotype distribution for each genetic polymorphism locus did not deviate from the Hardy-Weinberg balance ($P > 0.05$) (Supplementary Table 3), and the allele frequencies were similar to those of Asians in the International Human Genome HapMap Project, suggesting the control samples had representativeness.

The differences in 1-OHPYR among metabolic enzyme genes polymorphisms are shown in Table 2. With the adjustment of the covariates affecting 1-OHPYR, covariance analysis showed that the 1-OHPYR in non-deletion for GSTM1 was significantly higher than that in deletion in the exposure group ($P = 0.024$), the 1-OHPYR in AA for GSTP1 rs1695 was significantly higher than that in AG genotype in the control group ($P = 0.044$). After adjusting the covariates (gender, age, smoking index, drinking status, and BMI), the trend test of multiple linear regression revealed that the 1-OHPYR had an increasing trend with the genotypes AA→AG→GG in the control group ($P = 0.013$).

Table 2
The effect of metabolic enzyme gene polymorphism on 1-OHPYR

Gene/SNPs	Control			Exposure		
	n	1-OHPYR ($\dot{x} \pm s$)	*P*	n	1-OHPYR ($\dot{x} \pm s$)	*P*
GSTT1						
-	35	1.79 ± 0.83	Ref	150	4.49 ± 1.09	Ref
+	53	1.76 ± 1.08	0.768	205	4.44 ± 1.20	0.936
GSTM1						
-	51	1.71 ± 0.77	Ref	205	4.32 ± 1.10	Ref
+	37	1.86 ± 1.22	0.452	150	4.61 ± 1.21	0.024
GSTP1 rs1695						
AA	53	1.62 ± 0.89	Ref	239	4.41 ± 1.11	Ref
AG	32	1.95 ± 1.07	0.044	107	4.46 ± 1.26	0.582
GG	3	2.66 ± 1.04	0.077	9	5.17 ± 1.04	0.059
P_{trend}		0.013			0.160	
CYP2E1 rs6413432						
TT	53	1.63 ± 0.82	Ref	210	4.50 ± 1.11	Ref
AT	32	2.00 ± 1.21	0.260	127	4.29 ± 1.22	0.067
AA	3	1.87 ± 0.37	0.774	18	4.82 ± 1.16	0.120
P_{trend}		0.307			0.747	
CYP2E1 rs3813867						
GG	49	1.64 ± 0.76	Ref	216	4.41 ± 1.21	Ref
CG	36	1.89 ± 1.20	0.473	119	4.53 ± 1.01	0.761
CC	3	2.60 ± 1.09	0.187	20	4.32 ± 1.37	0.517
P_{trend}		0.219			0.830	

Covariance analysis was used to compare 1-OHPYR among genotypes, adjusted for gender, age (years), smoking index, drinking status, and BMI.

Multiple linear regression analyzed the trend of 1-OHPYR change with mutant allele loci, adjusting gender, age, smoking index, drinking status, BMI.

Ref: The reference group when comparing.

We also analyzed the effect of metabolic enzyme genes polymorphisms on 1-OHNAP, 2-OHNAP, and 3-OHPHE (Supplementary Table 4, Supplementary Table 5, and Supplementary Table 6). With the adjustment of the same covariates, covariance analysis showed that the 2-OHNAP and 3-OHPHE in non-deletion for GSTM1 were significantly higher than that in deletion in the exposure group ($P = 0.005$ and $P = 0.005$, respectively). There were no statistically significant differences in 1-OHPYR, 1-OHNAP, 2-OHNAP, and 3-OHPHE among different genotypes in other loci of the metabolic enzyme genes.

3.4. Effects of genetic polymorphisms on mtDNAcn

As shown in Table 3, there were no statistically significant differences in mtDNAcn among different genotypes in loci of the metabolic enzyme genes. The mtDNAcn in AG + GG for GSTP1 rs1695 was slightly higher than that in the AA genotype in the exposure group ($P = 0.077$).

Table 3
The effect of metabolic enzyme gene polymorphism on mtDNAcn

SNPs	Control			Exposure		
	n	mtDNAcn ($\dot{x} \pm s$)	*P*	n	mtDNAcn ($\dot{x} \pm s$)	*P*
GSTT1						
-	101	1.03 ± 0.33	Ref	236	0.57 ± 0.30	Ref
+	137	1.04 ± 0.30	0.969	308	0.61 ± 0.29	0.113
GSTM1						
-	130	1.04 ± 0.29	Ref	313	0.60 ± 0.30	Ref
+	108	1.02 ± 0.33	0.689	231	0.60 ± 0.28	0.929
GSTP1 rs1695						
AA	148	1.02 ± 0.29	Ref	361	0.58 ± 0.29	Ref
AG	80	1.04 ± 0.33	0.585	172	0.63 ± 0.31	0.062
GG	10	1.17 ± 0.38	0.057	11	0.56 ± 0.36	0.917
P_{trend}		0.132			0.126	
GSTP1 rs1695						
AA	148	1.02 ± 0.29	Ref	361	0.58 ± 0.29	Ref
AG + GG	90	1.06 ± 0.34	0.308	183	0.63 ± 0.31	0.077
CYP2E1 rs6413432						
TT	144	1.04 ± 0.31	Ref	315	0.60 ± 0.29	Ref
AT	83	1.03 ± 0.31	0.867	199	0.59 ± 0.30	0.748
AA	11	0.96 ± 0.32	0.568	30	0.57 ± 0.27	0.578
P_{trend}		0.828			0.573	
CYP2E1 rs3813867						
GG	133	1.01 ± 0.29	Ref	340	0.61 ± 0.30	Ref

Covariance analysis was used to compare mtDNAcn among genotypes, adjusted for gender, age (years), smoking index, drinking status, and BMI.

Multiple linear regression analyzed the trend of mtDNAcn change with mutant allele loci, adjusting gender, age, smoking index, drinking status, BMI.

Ref: The reference group when comparing.

SNPs	Control			Exposure		
	n	mtDNAcn ($\dot{x} \pm s$)	*P*	n	mtDNAcn ($\dot{x} \pm s$)	*P*
CG	87	1.07 ± 0.33	0.231	167	0.59 ± 0.28	0.644
CC	18	1.06 ± 0.34	0.546	37	0.52 ± 0.29	0.112
P_{trend}		0.261			0.168	
Covariance analysis was used to compare mtDNAcn among genotypes, adjusted for gender, age (years), smoking index, drinking status, and BMI.						
Multiple linear regression analyzed the trend of mtDNAcn change with mutant allele loci, adjusting gender, age, smoking index, drinking status, BMI.						
Ref: The reference group when comparing.						

We further analyzed the combined effect of GSTP1 rs1695 and environmental exposure on mtDNAcn (Table 4), and found that the combined effects of GSTP1 rs1695 and PAHs exposure on mtDNAcn were statistically significant ($P < 0.001$). However, the interaction between the AA genotype and PAHs exposure did not affect mtDNAcn (X^2=0.044, $P = 0.834$).

Table 4
The combined effect of GSTP1 rs1695 and environmental exposure on mtDNAcn

rs1695 (AA)	Exposure	mtDNAcn($\dot{x} \pm s$)	β (95% CI)	X^2	P
-	-	1.06 ± 0.34	Ref		
+	-	1.02 ± 0.29	-0.036(-0.114, 0.042)	0.803	0.370
-	+	0.63 ± 0.31	-0.431(-0.506, -0.356)	126.679	< 0.001
+	+	0.58 ± 0.29	-0.476(-0.545, -0.408)	185.066	< 0.001
GLMs was used to analyse the combined effect of GSTP1 rs1695 and environmental exposure on mtDNAcn adjusted for gender, age, smoking index, drinking status, and BMI.					

3.5. The influencing factors on mtDNAcn

The influencing factors were screened by GLMs with mtDNAcn as the dependent variable, PAHs exposure, gender, age, GSTT1, GSTM1, GSTP1 rs1695, CYP2E1 rs6413432 and CYP2E1 rs3813867 gene as predicators, and smoking status, drinking status and BMI as covariates. The variables kept in the model included PAHs-exposure ($b = -0.436$, $P < 0.001$), male ($b = -0.058$, $P = 0.013$) and genotype AA for GSTP1 rs1695 ($b = -0.051$, $P = 0.020$) (Table 5).

Table 5
The influencing factors of mtDNAcn

Influencing factors	β (95% CI)	χ^2	P
Constant	1.552(1.221, 1.884)	84.278	< 0.001
PAHs-exposure	-0.420(-0.469, -0.372)	289.770	< 0.001
Male	-0.058(-0.103, -0.012)	6.127	0.013
GSTP1 rs1695 AA	-0.051(-0. 095, -0.008)	5.395	0.020

GLMs was used to analyze the influencing factors of mtDNAcn adjusted for smoking index, drinking status, and BMI.

4. Discussion

Workers in coke oven plants are exposed to a wide variety of volatile organic compounds and particulates, especially PAHs. The PAHs and their metabolic intermediates might cause damage to the mitochondria [23]. Increasing evidence indicates that PAHs-exposure may relate to mtDNAcn decrease. Ling et al. (2013) observed that decreased sperm mtDNAcn was associated with PAHs-exposure in the male population in Chongqing, China [5]. Pieters et al. (2013) demonstrated that mtDNAcn was inversely associated with indoor PAHs exposure population and their findings were also confirmed in human TK6 cells [3]. Our previous study showed that mtDNAcn had significantly negative correlations with the levels of COE cumulative exposure dose [24]. Moreover, our previous study also found that mtDNAcn had significantly negative correlations with the levels of 1-OHPYR which can be used to estimate the internal exposure of PAHs. In this study, our result showed that male had lower mtDNAcn than female, suggesting that male was susceptibility to PAHs exposure.

GSTT1, GSTM1, and GSTP1 are the important phase II metabolic enzymes in glutathione-*S*-transferases enzymes system and the genetic polymorphisms in these enzyme genes may alter gene expression levels and its enzymes activity, and subsequently, involve toxicity of PAHs.

The GSTP1 rs1695 is located on exon 5 of chromosome 11q13, and contains a wild-type G allele and mutant A allele. The transition of an A allele to G allele in GSTP1 rs1695 confers increased conjugating activity [25] [26] and may also with lower levels of genotoxicity in PAHs exposure. Our study found that the 1-OHPYR had an increasing trend with the genotypes AA→AG→GG of GSTP1 rs1695 in the control group. Moreover, our results firstly showed that AA for GSTP1 rs1695 was a risk factor for mtDNAcn in PAHs-exposure. Therefore, we inferred that the toxicity of PAHs may be influenced by GSTP1 rs1695 polymorphisms resulting in the different alteration of mtDNAcn.

Though we also analyzed the association between mtDNAcn and polymorphisms in GSTT1, GSTM1, there was no significant difference. The possible reason is that mitochondrial copy number is affected by many factors, and the polymorphism of the metabolic enzyme gene has a modest effect on mtDNAcn.

However, our result showed that the 2-OHNAP and 3-OHPHE in non-deletion for GSTM1 were significantly higher than that in deletion in the exposure group. This confirms that metabolic enzyme gene polymorphism may alter the toxicity of PAHs.

CYP2E1 is an important member of CYP450 system and located on homo sapiens chromosome 10q24.3. The CYP2E1 rs3813867 is a G/C polymorphism located at 1259 position in the 5'-flanking region and the CYP2E1 rs6413432 (T > A) located on intron 6, which can affect the CYP2E1 gene expression level and its enzyme activity [27]. Therefore, polymorphism of CYP2E1 may affect the activity of the enzyme express leading to individual differences in PAHs metabolism. Nan et al. (2001) observed that CYP2E1 polymorphism was an important factor influencing the levels of 1-OHPYR and 2-OHNAP in urinary from coke oven workers [28]. In this study, there was no significant difference between OH-PAHs and CYP2E1 polymorphism, that may be because the PAHs are mostly activated by CYP1A and CYP1B [29].

Polymorphism of CYP2E1 may also influence the genotoxic of environmental toxins. Guang et al. [30] found that CYP2E1 rs3813867 mutant allele was associated with higher micronuclei among benzene-exposed shoe workers. Jheneffer et al. [31] reported that alcoholics who heterozygous in the CYP2E1 rs3813867 showed higher DNA damage (tail length and olive tail moment). Jing et al. [32]found heterozygous in the CYP2E1 rs6413432 had shorter telomere lengths among benzene-exposed shoe workers. However, mtDNAcn change was not associated with CYP2E1 polymorphism in the present study.

In the study, we have analyzed a larger number of samples, which is the advantage of the study. However, several limitations of the present study need to be considered. First, due to the cross-sectional design of this study, a causal relationship between PAHs exposure and mitochondria damage cannot be established. Second, White blood cell differentials and platelet concentrations, which are the major sources of mtDNAcn variability, were not assessed. Third, a large number of studies have shown that there are differences in metabolic enzyme gene polymorphism among different populations. The effects of metabolic enzyme gene polymorphism on mtDNAcn of coke oven workers may not be suitable for all world populations in this study. Hence, further research is needed to address these questions.

In conclusion, the male was susceptibility to PAHs exposure. The AA genotype of GSTP1 rs1695 may influence the toxicity of PAHs and associated with the decreased of mtDNAcn.

Declarations

Ethical Approval and Consent to participate

All procedures performed in studies involving human participants were following the ethical standards of the institutional and or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent for publication

We have obtained consents to publish this paper from all the participants of this study.

Availability of supporting data

The datasets used in the current study are available from the corresponding on reasonable request.

Competing interests

The authors declare no conflict of interests.

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Authors' contributions

Dr Wei Wang and Yongli Yang designed the study and applied for Research Ethics Board approval. Xiaoran Duan and Mingcui Ding recruited the subjects and collected the data. Xinling Li analyzed the data and prepared tables. Xinling Li and Dr Xiaoshan Zhou prepared the manuscript with important intellectual input from Dr Wei Wang and Wu Yao. All authors approved the final manuscript. All authors had complete access to the study data.

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References

1. Yang P, Wang YX, Sun L, Chen YJ, Liu C, Huang LL, Lu WQ, Zeng Q: **Urinary metabolites of polycyclic aromatic hydrocarbons, sperm DNA damage and spermatozoa apoptosis**. *J Hazard Mater* 2017, **329**:241-248.
2. Allen JA, Coombs MM: **Covalent binding of polycyclic aromatic compounds to mitochondrial and nuclear DNA**. *Nature* 1980, **287**(5779):244-245.
3. Pieters N, Koppen G, Smeets K, Napierska D, Plusquin M, De Prins S, Van De Weghe H, Nelen V, Cox B, Cuypers A *et al*: **Decreased mitochondrial DNA content in association with exposure to polycyclic aromatic hydrocarbons in house dust during wintertime: from a population enquiry to cell culture**. *PLoS One* 2013, **8**(5):e63208.
4. Pavanello S, Dioni L, Hoxha M, Fedeli U, Mielzynska-Svach D, Baccarelli AA: **Mitochondrial DNA copy number and exposure to polycyclic aromatic hydrocarbons**. *Cancer Epidemiol Biomarkers Prev* 2013, **22**(10):1722-1729.

5. Ling X, Zhang G, Sun L, Wang Z, Zou P, Gao J, Peng K, Chen Q, Yang H, Zhou N *et al*: **Polycyclic aromatic hydrocarbons exposure decreased sperm mitochondrial DNA copy number: A cross-sectional study (MARHCS) in Chongqing, China**. *Environ Pollut* 2017, **220**(Pt A):680-687.
6. Duan X, Yang Y, Zhang H, Liu B, Wei W, Wang L, Sun C, Yao W, Cui L, Zhou X *et al*: **Polycyclic aromatic hydrocarbon exposure, miRNA genetic variations, and associated leukocyte mitochondrial DNA copy number: A cross-sectional study in China**. *Chemosphere* 2019, **246**:125773.
7. Han D, Williams E, Cadenas E: **Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space**. *Biochem J* 2001, **353**(Pt 2):411-416.
8. Liu CS, Tsai CS, Kuo CL, Chen HW, Lii CK, Ma YS, Wei YH: **Oxidative stress-related alteration of the copy number of mitochondrial DNA in human leukocytes**. *Free Radic Res* 2003, **37**(12):1307-1317.
9. Pickles S, Vigie P, Youle RJ: **Mitophagy and Quality Control Mechanisms in Mitochondrial Maintenance**. *Curr Biol* 2018, **28**(4):R170-R185.
10. Hosgood HD, 3rd, Liu CS, Rothman N, Weinstein SJ, Bonner MR, Shen M, Lim U, Virtamo J, Cheng WL, Albanes D *et al*: **Mitochondrial DNA copy number and lung cancer risk in a prospective cohort study**. *Carcinogenesis* 2010, **31**(5):847-849.
11. Sahin E, Colla S, Liesa M, Moslehi J, Muller FL, Guo M, Cooper M, Kotton D, Fabian AJ, Walkey C *et al*: **Telomere dysfunction induces metabolic and mitochondrial compromise**. *Nature* 2011, **470**(7334):359-365.
12. Yang P, Ma J, Zhang B, Duan H, He Z, Zeng J, Zeng X, Li D, Wang Q, Xiao Y *et al*: **CpG site-specific hypermethylation of p16INK4alpha in peripheral blood lymphocytes of PAH-exposed workers**. *Cancer Epidemiol Biomarkers Prev* 2012, **21**(1):182-190.
13. Saengtienchai A, Ikenaka Y, Nakayama SM, Mizukawa H, Kakehi M, Bortey-Sam N, Darwish WS, Tsubota T, Terasaki M, Poapolathep A *et al*: **Identification of interspecific differences in phase II reactions: determination of metabolites in the urine of 16 mammalian species exposed to environmental pyrene**. *Environ Toxicol Chem* 2014, **33**(9):2062-2069.
14. Huang YF, Chen ML, Liou SH, Chen MF, Uang SN, Wu KY: **Association of CYP2E1, GST and mEH genetic polymorphisms with urinary acrylamide metabolites in workers exposed to acrylamide**. *Toxicol Lett* 2011, **203**(2):118-126.
15. Whyatt RM, Perera FP, Jedrychowski W, Santella RM, Garte S, Bell DA: **Association between polycyclic aromatic hydrocarbon-DNA adduct levels in maternal and newborn white blood cells and glutathione S-transferase P1 and CYP1A1 polymorphisms**. *Cancer Epidemiol Biomarkers Prev* 2000, **9**(2):207-212.
16. Recio-Vega R, Olivas-Calderon E, Michel-Ramirez G, Martinez-Salinas RI, Gallegos-Arreola MP, Ocampo-Gomez GL, Perez-Morales R: **Associations between sperm quality, DNA damage, and CYP1A1, GSTT1 and GSTM1 polymorphisms with 1-hydroxypyrene urinary levels in men occupationally exposed to polycyclic aromatic hydrocarbons**. *Int Arch Occup Environ Health* 2018, **91**(6):725-734.

17. Zhang H, Wang S, Duan X, Feng X, Wang T, Wang P, Ding M, Wang W, Zhou X, Yao W *et al*: **The interaction effects of coke oven emissions exposure and metabolic enzyme Gene variants on total antioxidant capacity of workers**. *Environ Toxicol Pharmacol* 2019, **70**:103197.
18. Duan X, Zhang D, Wang S, Feng X, Wang T, Wang P, Ding M, Zhang H, Liu B, Wei W *et al*: **Effects of polycyclic aromatic hydrocarbon exposure and miRNA variations on peripheral blood leukocyte DNA telomere length: A cross-sectional study in Henan Province, China**. *Sci Total Environ* 2020, **703**:135600.
19. Kuang D, Zhang W, Deng Q, Zhang X, Huang K, Guan L, Hu D, Wu T, Guo H: **Dose-response relationships of polycyclic aromatic hydrocarbons exposure and oxidative damage to DNA and lipid in coke oven workers**. *Environ Sci Technol* 2013, **47**(13):7446-7456.
20. Zhu SM, Ren XF, Wan JX, Xia ZL: **Evaluation in vinyl chloride monomer-exposed workers and the relationship between liver lesions and gene polymorphisms of metabolic enzymes**. *World J Gastroenterol* 2005, **11**(37):5821-5827.
21. Wang W, Qiu YL, Ji F, Liu J, Wu F, Miao WB, Li Y, Brandt-Rauf PW, Xia ZL: **Genetic polymorphisms in metabolizing enzymes and susceptibility of chromosomal damage induced by vinyl chloride monomer in a Chinese worker population**. *J Occup Environ Med* 2010, **52**(2):163-168.
22. Duan X, Yang Y, Zhang D, Wang S, Feng X, Wang T, Wang P, Ding M, Zhang H, Liu B *et al*: **Genetic polymorphisms, mRNA expression levels of telomere-binding proteins, and associates with telomere damage in PAHs-Exposure workers**. *Chemosphere* 2019, **231**:442-449.
23. Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, Wang M, Oberley T, Froines J, Nel A: **Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage**. *Environ Health Perspect* 2003, **111**(4):455-460.
24. Duan X, Yang Y, Zhang H, Liu B, Wei W, Wang L, Sun C, Yao W, Cui L, Zhou X *et al*: **Polycyclic aromatic hydrocarbon exposure, miRNA genetic variations, and associated leukocyte mitochondrial DNA copy number: A cross-sectional study in China**. *Chemosphere* 2020, **246**:125773.
25. Harries LW, Stubbins MJ, Forman D, Howard GC, Wolf CR: **Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer**. *Carcinogenesis* 1997, **18**(4):641-644.
26. Ali-Osman F, Akande O, Antoun G, Mao JX, Buolamwini J: **Molecular cloning, characterization, and expression in Escherichia coli of full-length cDNAs of three human glutathione S-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins**. *J Biol Chem* 1997, **272**(15):10004-10012.
27. Haufroid V, Buchet JP, Gardinal S, Lison D: **Cytochrome P4502E1 phenotyping by the measurement of the chlorzoxazone metabolic ratio: assessment of its usefulness in workers exposed to styrene**. *Int Arch Occup Environ Health* 2002, **75**(7):453-458.
28. Nan HM, Kim H, Lim HS, Choi JK, Kawamoto T, Kang JW, Lee CH, Kim YD, Kwon EH: **Effects of occupation, lifestyle and genetic polymorphisms of CYP1A1, CYP2E1, GSTM1 and GSTT1 on urinary 1-hydroxypyrene and 2-naphthol concentrations**. *Carcinogenesis* 2001, **22**(5):787-793.

29. Walsh AA, Szklarz GD, Scott EE: **Human cytochrome P450 1A1 structure and utility in understanding drug and xenobiotic metabolism**. *J Biol Chem* 2013, **288**(18):12932-12943.
30. Zhang GH, Ye LL, Wang JW, Ren JC, Xu XW, Feng NN, Zhou LF, Ru JG, Hao YH, Tian W *et al*: **Effect of polymorphic metabolizing genes on micronucleus frequencies among benzene-exposed shoe workers in China**. *Int J Hyg Environ Health* 2014, **217**(7):726-732.
31. Ramos JS, Alves AA, Lopes MP, Pedroso TM, Felicio LP, Carvalho WF, Franco FC, Araujo Melo CO, Goncalves MW, Soares TN *et al*: **DNA damage in peripheral blood lymphocytes and association with polymorphisms in the promoter region of the CYP2E1 gene in alcoholics from Central Brazil**. *Alcohol* 2016, **57**:35-39.
32. Ren JC, Liu H, Zhang GH, Wang T, Li J, Dong T, Wu H, Xia ZL: **Interaction effects of environmental response gene polymorphisms and benzene exposure on telomere length in shoe-making workers**. *Chemosphere* 2020, **255**:126841.

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