Genetic polymorphisms of metabolic enzyme genes associated with leukocyte mitochondrial DNA copy number in PAHs exposure workers

Xinling Li1,2 | Xiaoran Duan1 | Hui Zhang1 | Mingcui Ding1 | Yanbin Wang3 | Yongli Yang4 | Wu Yao1 | Xiaoshan Zhou1,2 | Wei Wang1,2

1Department of Occupational Health and Occupational Diseases, College of Public Health, Zhengzhou University, Zhengzhou, China
2The Key Laboratory of Nanomedicine and Health Inspection of Zhengzhou, Zhengzhou, China
3Safety Management Department of Anyang Iron and Steel Group Company, Anyang, China
4Department of Epidemiology and Biostatistics, College of Public Health, Zhengzhou University, Zhengzhou, China

Correspondence
Xiaoshan Zhou and Wei Wang, Department of Occupational Health and Occupational Diseases, College of Public Health, Zhengzhou University, Zhengzhou, China. Email: zhouchaozhou2017@126.com (X. Z.) and wu375@zzu.edu.cn (W. W.)

Funding information
National Natural Science Foundation of China, Grant/Award Number: 81872597; The Outstanding Youth Grant of Zhengzhou University, Grant/Award Number: 1521329035; The Programs for Science and Technology Development of Zhengzhou, Grant/Award Number: 12IPPTG503-2

Abstract

Background: Polycyclic aromatic hydrocarbons (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.

Aim: The aim of the study was to explore the effect of metabolic enzymes’ genetic polymorphisms on mtDNAcn in PAHs-Exposure.

Methods and Results: 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 the Real-time quantitative polymerase chain reaction (PCR) method. PCR and restriction fragment length was used to detect five polymorphisms in GSTT1, GSTM1, GSTP1 rs1695, CYP2E1 rs6413432, and CYP2E1 rs3813867. The mtDNAcn in peripheral blood leukocytes was significantly lower in the exposure group than that in the control group (p < .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 [β (95% CI) = −0.420 (−0.469, −0.372), p < .001], male [β (95% CI) = −0.058 (−0.103, −0.012), p = .013], and AA genotype for GSTP1 rs1695 [β (95% CI) = −0.051 (−0.095, −0.008), p = .020].

Conclusion: The individuals carrying the AA genotype of GSTP1 rs1695 may have a lower mtDNAcn due to their weaker detoxification of PAHs.

KEYWORDS
1-OHPYR, COEs, gene polymorphisms, GSTP1 rs1695, mtDNAcn

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 has 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 mtDNA.2 Several studies have found that PAHs may
Mitochondria play an important role in multiple cellular functions including oxidative phosphorylation, reactive oxygen species generation, calcium homeostasis, and apoptosis. Each mitochondrion contains 2–10 copies of mitochondrial DNA. Due to the lack of protective histones and DNA repair machinery, mtDNA is particularly more vulnerable to various kinds of environmental toxins. The mtDNA copy number (mtDNAcn) will increase as compensation to early poison exposure, however, when the increased mtDNAcn cannot maintain the mitochondria normal function, mitophagy will occur to remove dysfunctional ones, and the mtDNAcn will be reduced. 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, and the decreased mtDNAcn may accelerate the aging process and causes age-related disorders.

After PAHs enter the human body, they are firstly metabolized to electrophilic active intermediates by phase I metabolic 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. Subsequently, the intermediates are converted into more polar and water-soluble products by phase II metabolic enzymes and then excreted from the body. 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. Studies have shown that metabolic enzymes' genetic polymorphisms were associated with many health effects in PAHs exposure population. Whyatt et al. found that the CYP1A1 MspI restriction site had higher DNA adduct levels among newborns with PAHs exposure. One study suggested that the GSTM1 (−) was inversely associated with the DNA integrity in the men occupationally exposed to PAHs. Our earlier study showed that GSTT1 (+) and GSTM1 (+) are the risk factors for oxidative stress in coke oven workers. 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 random cluster sampling method was adopted to enroll the subjects in this study. A total of 544 workers exposed to COEs for more than 1 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. Exposure-group subjects were selected based on the following inclusion criteria: (a) age from 18 to 60, (b) who worked in the coke plant and were occupationally exposed to COEs for more than 1 year, and (c) provided informed consent. Subjects were excluded if they had major organ function failure or a tumor, were pregnant, or were lactating. For the history of occupational exposure, the inclusion and exclusion criteria for the control group were similar to those in the exposure 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 Na2EDTA 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 is described in our previous study.

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.

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

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 the 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'-CCTATAGCTT ACCGAACGA –3' and reverse, 5'-G GTGTAGTGTAGATGTCG-3'. The β-globin gene was forward, 5'-GCTTCTGACACACTGTG-3' and
2.4 | Detection of genetic polymorphisms

The GSTT1 and GSTM1 genotype were determined as previously described.\(^6\) PCR-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. To ensure the accuracy of the experiment, the positive control, and the negative control were set both in the process of PCR and restriction enzyme digestion. What's more, 10% of each gene polymorphic loci samples were selected randomly for repeat and the concordance was 100%. The representative gel pictures for PCR-RFLP were shown in Figure 1.

2.5 | Statistical analysis

Baseline survey data were entered using EpiData 3.1 software. All analyses were performed using SPSS 25.0 software (SPSS, Inc., Chicago, Illinois). 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 \(\alpha = .05\).

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, and 54.4%) were higher than that in the control group (58.4, 17.2, and 42.0%; \(p < .05\)). The age in the exposure group (40.10 ± 6.30) was significantly older than that in the control group (38.39 ± 8.43; \(p = .005\)). The BMI had no significant difference between the exposure group and the control group (\(p = .376\)). The COEs cumulative exposure dose and the four OH-PAHs in the exposure group were higher than those in the control group (\(p < .05\)). 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; \(t = 18.931, p < .001\)). The basic characteristics have been reported in our previous research\(^{22}\) (Table 2).

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 (\(p > .05\)). However, the mtDNAcn of all layers had significant differences between the two groups (\(p < .001\)). The COEs cumulative exposure dose and the four OH-PAHs in the exposure group were higher than those in the control group (\(p < .05\)). 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; \(t = 18.931, p < .001\)). The basic characteristics have been reported in our previous research\(^{22}\) (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 (\(p > .05\); Table 4), 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 5. 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 \( p = .024 \), the 1-OHPYR in AA for GSTP1 rs1695
was significantly higher than that in AG genotype in the control group
\( p = .044 \). 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 \( p = .013 \).

### 3.4 Effects of genetic polymorphisms on mtDNAcn

As shown in Table 6, there were no statistically significant differ-
ences in mtDNAcn among different genotypes in loci of the meta-
bolic enzyme genes. The mtDNAcn in AG + GG for GSTP1 rs1695
was slightly higher than that in the AA genotype in the exposure
group \( p = .077 \).

### 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
predictors, and smoking status, drinking status, and BMI as
covariates. The variables kept in the model included PAHs-exposure
\( b = −0.436, p < .001 \), male \( b = −0.058, p = .013 \) and genotype AA
for GSTP1 rs1695 \( b = −0.051, p = .020 \) (Table 7).

### 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 mitochon-
dria. Increasing evidence indicates that PAHs-exposure may relate
to mtDNAcn decrease. Ling et al. observed that decreased sperm mtDNAcn was associated with PAHs-exposure in the male population in Chongqing, China.5 Pieters et al. 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. Though the covariance analysis showed the mtDNAcn was not significantly different in gender, the male had lower mtDNAcn than the female in the controls and exposure group. What’s more, the GLM indicated that the male may be a risk factor of the decreased mtDNAcn \[\beta (95\% CI) = -0.058 (-0.103, -0.012), \ p = .013\]. Vyas et al.25 found that female has higher mtDNAcn compared to male in a diverse cohort of mid-life and older adults. Yu et al.26 observed the same gender-specific phenomenon in a cross-sectional analysis. The gender difference in mtDNAcn may be due to the mitochondrial maternal inheritance, which enables females to better maintain mitochondrial functions and control over mtDNAcn.27

**TABLE 2** The general characteristics of control and exposure groups

| Variables | Control | Exposure | \(\chi^2/t/Z\) | \(p\) |
|-----------|---------|----------|----------------|------|
| Gender    |         |          |                |      |
| Male      | 139 (58.4) | 390 (71.7) | 13.357<sup>b</sup> | <.001 |
| Female    | 99 (41.6)  | 154 (28.3) |                |      |
| Age grouping |        |          |                |      |
| ≤40       | 142 (59.7) | 273 (50.2) | 5.974<sup>b</sup> | .015 |
| >40       | 96 (40.3)  | 271 (49.8) |                |      |
| Age (years) | 38.39 ± 8.43 | 40.10 ± 6.30 | 2.800<sup>c</sup> | .005 |
| Smoking status |      |          |                |      |
| No        | 197 (82.8) | 321 (59.0) | 41.817<sup>b</sup> | <.001 |
| Yes       | 41 (17.2)  | 223 (41.0) |                |      |
| Drinking status |       |          |                |      |
| No        | 138 (58.0) | 248 (45.6) | 10.176<sup>b</sup> | .001 |
| Yes       | 100 (42.0) | 296 (54.4) |                |      |
| BMI (kg/m²) |         |          |                |      |
| Low weight| 5 (2.1)  | 12 (2.2)  | 3.101<sup>b</sup> | .376 |
| Normal weight | 108 (45.4) | 224 (41.2) |                |      |
| Overweight | 101 (42.4) | 230 (42.3) |                |      |
| Obesity   | 24 (10.1)  | 78 (14.3)  |                |      |
| Cumulative exposure dose | 0.07 (0.06,0.09) | 1.12 (0.34,2.14) | 22.093<sup>d</sup> | <.001 |
| 1-OHPYR   | 1.78 ± 0.98 | 4.44 ± 1.15 | 19.953<sup>a</sup> | <.001 |
| 1-OHNAP   | 3.02 ± 0.92 | 4.08 ± 1.20 | 3.151<sup>a</sup> | .002 |
| 2-OHNAP   | 3.31 ± 0.10 | 4.49 ± 1.03 | 9.792<sup>a</sup> | <.001 |
| 3-OHPHE   | 0.99 ± 1.12 | 2.96 ± 1.06 | 16.828<sup>a</sup> | <.001 |
| mtDNAcn   | 1.03 ± 0.31 | 0.60 ± 0.29 | 18.931<sup>a</sup> | <.001 |

<sup>a</sup>Each variable is represented by the number of samples and percentage or mean ± SD.
<sup>b</sup>\(\chi^2\) test.
<sup>c</sup>t-test.
<sup>d</sup>Rank sum test.

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 activity2829 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, and GSTP1, 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 is 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. Therefore, polymorphism of CYP2E1 may affect the activity of the enzyme express leading to individual differences in PAHs metabolism. Nan et al. observed that CYP2E1 polymorphism was an important factor influencing the levels of 1-OHPYR and 2-OHNAP in urinary from coke oven workers. 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.

Polymorphism of CYP2E1 may also influence the genotoxic of environmental toxins. Guang et al. found that the CYP2E1 rs3813867 mutant allele was associated with higher micronuclei among benzene-exposed shoe workers. Jheneffer et al. reported that alcoholics who heterozygous in the CYP2E1 rs3813867 showed higher DNA damage (tail length and olive tail moment). Jing et al. 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

### TABLE 3 The effect of general characteristics on mtDNAcn

| Variable          | Control         | Exposure        |       |       |
|-------------------|-----------------|-----------------|-------|-------|
|                   | n   | mtDNAcn (x ± s) | n   | mtDNAcn (x ± s) | F     | p     |
| Gender            |     |                |     |                |       |       |
| Male              | 139 | 1.00 ± 0.28    | 390 | 0.59 ± 0.30    | 201.939 | <0.001 |
| Female            | 99  | 1.08 ± 0.34    | 154 | 0.62 ± 0.29    | 133.108 | <0.001 |
| F                 | 3.085|                | 0.231|                |       |       |
| P                 | 0.080|                | 0.631|                |       |       |
| Age grouping      |     |                |     |                |       |       |
| ≤40               | 142 | 1.05 ± 0.31    | 273 | 0.60 ± 0.28    | 223.722 | <0.001 |
| >40               | 96  | 1.01 ± 0.30    | 271 | 0.60 ± 0.31    | 114.037 | <0.001 |
| F                 | 0.816|                | 0.062|                |       |       |
| P                 | 0.367|                | 0.804|                |       |       |
| Smoking status    |     |                |     |                |       |       |
| No                | 197 | 1.04 ± 0.32    | 321 | 0.60 ± 0.29    | 257.887 | <0.001 |
| Yes               | 41  | 0.99 ± 0.27    | 223 | 0.59 ± 0.29    | 64.900  | <0.001 |
| F                 | 0.030|                | 0.201|                |       |       |
| P                 | 0.863|                | 0.654|                |       |       |
| Drinking status   |     |                |     |                |       |       |
| No                | 138 | 1.05 ± 0.33    | 248 | 0.61 ± 0.30    | 168.387 | <0.001 |
| Yes               | 100 | 1.01 ± 0.27    | 296 | 0.58 ± 0.29    | 164.549 | <0.001 |
| F                 | 0.065|                | 0.650|                |       |       |
| P                 | 0.800|                | 0.420|                |       |       |
| BMI (kg/m²)       |     |                |     |                |       |       |
| <24.0             | 113 | 1.01 ± 0.33    | 236 | 0.62 ± 0.30    | 115.357 | <0.001 |
| 24.0-27.9         | 101 | 1.06 ± 0.29    | 230 | 0.58 ± 0.28    | 194.732 | <0.001 |
| ≥28               | 24  | 1.03 ± 0.30    | 78  | 0.58 ± 0.33    | 32.382  | <0.001 |
| F                 | 1.530|                | 0.783|                |       |       |
| P                 | 0.219|                | 0.458|                |       |       |

Note: Covariance analysis was used to analyze the effects of the general characteristics on mtDNAcn with the appropriate adjustment of gender, age (years), smoking index, drinking status, and BMI.
**TABLE 4** Distribution of SNP loci and Hardy–Weinberg equilibrium test

| Gene/SNPs   | Genotype | Number | Genotype Frequency | Allele Frequency | Hardy–Weinberg $\chi^2$ (P)$^a$ |
|-------------|----------|--------|--------------------|------------------|---------------------------------|
| GSTT1       | −        | 101    | 0.424              | −                | −                               |
|             | +        | 137    | 0.576              | −                | −                               |
| GSTM1       | −        | 130    | 0.546              | −                | −                               |
|             | +        | 108    | 0.454              | −                | −                               |
| GSTP1 rs1695| AA       | 148    | 0.622              | A: 0.790         | 0.039 (0.844)                   |
|             | AG       | 80     | 0.336              | G: 0.210         |                                 |
|             | GG       | 10     | 0.042              |                  |                                 |
| CYP2E1 rs6413432 | TT   | 144    | 0.605              | T: 0.780         | 0.048 (0.827)                   |
|             | AT       | 83     | 0.349              | A: 0.220         |                                 |
|             | AA       | 11     | 0.046              |                  |                                 |
| CYP2E1 rs3813867 | GG   | 133    | 0.559              | G: 0.742         | 0.509 (0.476)                   |
|             | CG       | 87     | 0.366              | C: 0.258         |                                 |
|             | CC       | 18     | 0.076              |                  |                                 |

$^a$The Hardy–Weinberg test was performed using the control group.

**TABLE 5** The effect of metabolic enzyme gene polymorphism on 1-OHPYR

| Gene/SNPs   | Control | Exposure |
|-------------|---------|----------|
|             | n       | 1-OHPYR (x ± s) | p | n       | 1-OHPYR (x ± s) | p |
| GSTT1       |         |           |   |         |           |   |
| −           | 35      | 1.79 ± 0.83 | Ref | 150    | 4.49 ± 1.09 | Ref |
| +           | 53      | 1.76 ± 1.08 | .768 | 205    | 4.44 ± 1.20 | .936 |
| GSTM1       |         |           |   |         |           |   |
| −           | 51      | 1.71 ± 0.77 | Ref | 205    | 4.32 ± 1.10 | Ref |
| +           | 37      | 1.86 ± 1.22 | .452 | 150    | 4.61 ± 1.21 | .024 |
| GSTP1 rs1695| AA      | 53       | 1.62 ± 0.89 | Ref | 239    | 4.41 ± 1.11 | Ref |
|             | AG      | 32       | 1.95 ± 1.07 | .044 | 107    | 4.46 ± 1.26 | .582 |
|             | GG      | 3        | 2.66 ± 1.04 | .077 | 9      | 5.17 ± 1.04 | .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 | .260 | 127    | 4.29 ± 1.22 | .067 |
|             | AA      | 3       | 1.87 ± 0.37 | .774 | 18     | 4.82 ± 1.16 | .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 | .473 | 119    | 4.53 ± 1.01 | .761 |
|             | CC      | 3       | 2.60 ± 1.09 | .187 | 20     | 4.32 ± 1.37 | .517 |
|             | $P_{trend}$ | 0.219  |       |       | 0.830 |

Note: 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.
workers may not be suitable for all world populations in this study. Forth, increasing studies have proved that particulate matter exposure may alter mitochondrial dynamics leading to the change of mtDNAcn. In 63 male healthy steel workers, Hou et al.\(^{36}\) found that particulate matter exposure was positively associated with mtDNAcn. However, Hou et al.\(^{37}\) observed decreased mtDNAcn in association with increased exposure to particulate matter in a repeated-measure study. Wong et al.\(^{38}\) and Wang et al.\(^{39}\) also found that particulate matter exposure was linked with decreased mtDNAcn. Therefore, the relationship between particulate matter exposure and mtDNAcn needs to be further studied. Due to lack of data on particulate matter exposure, we did not analysis the effect of particulate matter on mtDNAcn and we would consider it in further study. Hence, further research is needed to address these questions.

In conclusion, the individuals carrying the AA genotype of GSTP1\(^{rs1695}\) may have a lower mtDNAcn due to their weaker detoxification of PAHs.

**ACKNOWLEDGMENTS**

The authors express their gratitude to all the individuals who volunteered to participate in this study.

**AUTHOR CONTRIBUTIONS**

Xinling Li: Formal analysis; writing-original draft. Xiaoran Duan: Investigation. Hui Zhang: Formal analysis. Yanbin Wang: Investigation. Xiaoshan Zhou: Formal analysis; writing-review and editing. Wei Wang: Formal analysis; funding acquisition; writing-review and editing.

**TABLE 6** The effect of metabolic enzyme gene polymorphism on mtDNAcn

| SNPs       | Control | Exposure |
|------------|---------|----------|
|            | n       | mtDNAcn (x ± s) | p   | n       | mtDNAcn (x ± s) | p   |
| GSTT1      |         |           |     |         |           |     |
| –          | 101     | 1.03 ± 0.33 | Ref | 236     | 0.57 ± 0.30 | Ref |
| +          | 137     | 1.04 ± 0.30 | .969| 308     | 0.61 ± 0.29 | .113|
| GSTM1      |         |           |     |         |           |     |
| –          | 130     | 1.04 ± 0.29 | Ref | 313     | 0.60 ± 0.30 | Ref |
| +          | 108     | 1.02 ± 0.33 | .689| 231     | 0.60 ± 0.28 | .929|
| GSTP1 rs1695 |        |           |     |         |           |     |
| AA         | 148     | 1.02 ± 0.29 | Ref | 361     | 0.58 ± 0.29 | Ref |
| AG         | 80      | 1.04 ± 0.33 | .585| 172     | 0.63 ± 0.31 | .062|
| GG         | 10      | 1.17 ± 0.38 | .057| 11      | 0.56 ± 0.36 | .917|
| P\(_{\text{trend}}\) | 0.132 |           |     | 0.126 |           |     |
| GSTP1 rs1695 |        |           |     |         |           |     |
| AA + GG    | 90      | 1.06 ± 0.34 | .308| 183     | 0.63 ± 0.31 | .077|
| CYP2E1 rs6413432 |   |           |     |         |           |     |
| TT         | 144     | 1.04 ± 0.31 | Ref | 315     | 0.60 ± 0.29 | Ref |
| AT         | 83      | 1.03 ± 0.31 | .867| 199     | 0.59 ± 0.30 | .748|
| AA         | 11      | 0.96 ± 0.32 | .568| 30      | 0.57 ± 0.27 | .578|
| P\(_{\text{trend}}\) | 0.828 |           |     | 0.573 |           |     |
| CYP2E1 rs3813867 |   |           |     |         |           |     |
| GG         | 133     | 1.01 ± 0.29 | Ref | 340     | 0.61 ± 0.30 | Ref |
| CG         | 87      | 1.07 ± 0.33 | .231| 167     | 0.59 ± 0.28 | .644|
| CC         | 18      | 1.06 ± 0.34 | .546| 37      | 0.52 ± 0.29 | .112|
| P\(_{\text{trend}}\) | 0.261 |           |     | 0.168 |           |     |

Note: 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.

**TABLE 7** The influencing factors of mtDNAcn

| Influencing factors | β (95% CI) | \(\chi^2\) | p    |
|---------------------|------------|------------|------|
| Constant            | 1.552 (1.221, 1.884) | 84.278 | <.001|
| PAHs-exposure       | −0.420 (−0.469, −0.372) | 289.770 | <.001|
| Male                | −0.058 (−0.103, −0.012) | 6.127 | .013|
| GSTP1 rs1695 AA     | −0.051 (−0.095, −0.008) | 5.395 | .020|

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

Abbreviation: PAHs, polycyclic aromatic hydrocarbons.
CONFLICT OF INTEREST
The authors declare there is no conflict of interest.

ETHICAL STATEMENT
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. The study protocol and consent form from all subjects were subjected to approval by the Ethics Committee of Zhengzhou University, China.

DATA AVAILABILITY STATEMENT
The data will be available if requested from the corresponding author.

ORCID
Yongli Yang https://orcid.org/0000-0002-5361-5733
Wei Wang https://orcid.org/0000-0001-7492-6795

REFERENCES
1. Yang P, Wang YX, Sun L, et al. 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, 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, Hoxa 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, et al. Polycyclic aromatic hydrocarbons exposure decreased sperm mitochondrial DNA copy number: a cross-sectional study (MARHCS) in Chongqing, China. Environ Pollut. 2017;220:680-687.

6. Duan X, Yang Y, Zhang H, 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, et al. 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, 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, et al. Telomere dysfunction induces metabolic and mitochondrial compromise. Nature. 2011;470(7334):359-365.

12. Yang P, Ma J, Zhang B, 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. Saengtienvchai A, Ikenaka Y, Nakayama SM, 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, et al. 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, 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, 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, et al. 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, et al. 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, 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, et al. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ Health Perspect. 2003;111(4):455-460.

24. Duan X, Yang Y, Zhang H, 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. Vyas CM, Ogata S, Reynolds CF 3rd, et al. Lifestyle and behavioral factors and mitochondrial DNA copy number in a diverse cohort of mid-life and older adults. PLoS One. 2020;15(8):e0237235.

26. Ju Y, Liu H, He S, et al. Sex-specific negative association between iron intake and cellular aging markers: mediation models involving TNFalpha. Oxid Med Cell Longev. 2019;2019:4935237.

27. Tower J. Sex-specific gene expression and life span regulation. Trends Endocrinol Metab. 2017;28(10):735-747.

28. 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.

29. 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.
variants. Evidence for differential catalytic activity of the encoded proteins. J Biol Chem. 1997;272(15):10004-10012.
30. 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.
31. Nan HM, Kim H, Lim HS, et al. 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.
32. Walsh AA, Szklarz GD, Scott EE. Human cytochrome P450 1A1 structure and utility in understanding drug and xenобiotic metabolism. J Biol Chem. 2013;288(18):12932-12943.
33. Zhang GH, Ye LL, Wang JW, 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.
34. Ramos JS, Alves AA, Lopes MP, 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.
35. Ren JC, Liu H, Zhang GH, et al. Interaction effects of environmental response gene polymorphisms and benzene exposure on telomere length in shoe-making workers. Chemosphere. 2020;255:126841.
36. Hou L, Zhu ZZ, Zhang X, et al. Airborne particulate matter and mitochondrial damage: a cross-sectional study. Environ Health. 2010;9:48.
37. Hou L, Zhang X, Dioni L, et al. Inhalable particulate matter and mitochondrial DNA copy number in highly exposed individuals in Beijing, China: a repeated-measure study. Part Fibre Toxicol. 2013;10:17.
38. Wong JYY, Hu W, Downward GS, et al. Personal exposure to fine particulate matter and benzo[a]pyrene from indoor air pollution and leukocyte mitochondrial DNA copy number in rural China. Carcinogenesis. 2017;38(9):893-899.
39. Wang X, Hart JE, Liu Q, Wu S, Nan H, Laden F. Association of particulate matter air pollution with leukocyte mitochondrial DNA copy number. Environ Int. 2020;141:105761.

How to cite this article: Li X, Duan X, Zhang H, et al. Genetic polymorphisms of metabolic enzyme genes associated with leukocyte mitochondrial DNA copy number in PAHs exposure workers. Cancer Reports. 2021;e1361. https://doi.org/10.1002/cnr2.1361