Association of BER and NER pathway polymorphism haplotypes and micronucleus frequencies with global DNA methylation in benzene-exposed workers of China

Effects of DNA repair genes polymorphisms on genetic damage

Guang-hui Zhang⁎⁎, Jing-chao Ren⁎, Mengkai Luo⁎, Junpeng Cui⁎, Yanqiu Du, Daokun Yang, Shouming Cui, Xiao Wang, Weidong Wu, Jia Cao⁎, Zhao-lin Xia⁎⁎

⁎ Henan Collaborative Innovation Center of Molecular Diagnosis and Laboratory Medicine, School of Public Health, Xinxiang Medical University, 601 Jinsui Road, Xinxiang, 453003, China
⁎⁎ Department of Occupational Health and Toxicology, School of Public Health, Fudan University, 138 Yixueyuan Road, Shanghai, 200032, China

ARTICLE INFO

Keywords:
Benzene
Micronucleus
Base excision repair
Nucleotide excision repair
Single nucleotide polymorphisms
Global DNA methylation

ABSTRACT

Objective: The base excision repair (BER) pathway and nucleotide excision repair (NER) pathway play important roles in the repair of benzene-induced genetic damage, and the effects of polymorphisms in these pathways on genetic damage and global DNA methylation are of great interest.

Methods: Ten single nucleotide polymorphisms (SNPs) in the BER (XRCC1: rs25489, rs25487; APE1: rs1130409) and NER pathways (XPA: rs1800975; XPC: rs2228000, rs2228002; XPD: rs13181, rs1799793; XPG: rs17655; ERCC1: rs3212986) were analyzed by a Kompetitive allele-specific PCR (KASP) assay to find associations with cytokinesis-block micronucleus (MN) frequency and global DNA methylation in 294 shoe factory workers and 102 control participants.

Results: Workers who possessed the following genotypes were associated with high MN frequency: rs25487 AA (FR (95% CI): 1.50 (1.16, 1.9), p = 0.002, reference GG); rs1130409 GG (FR (95% CI): 1.28 (1.05, 1.55), p = 0.010, reference TT); rs17655 GC (FR (95% CI): 1.18 (1.02, 1.38), p = 0.038, reference GG); and rs3212986 TT (FR (95% CI): 1.55 (1.31, 1.83), p < 0.001, reference GG). Workers with four and three mutant alleles showed 3.72-fold (OR (95% CI): 3.72 (1.34, 10.03), p = 0.009) and 2.48-fold (OR (95% CI): 2.48 (1.27, 4.88), p = 0.008) increased risk of genetic damage compared with workers with no or one mutant allele, and a dose-response relationship was found by the trend test (p = 0.006). The rs1130409 variant allele (GG + GT) was associated with low global DNA methylation (β = −0.20, 95% CI: −0.42, 0.03, p = 0.045).

Conclusion: In benzene-exposed workers, BER and NER pathway polymorphism haplotypes are associated with different levels of chromosome damage and had little effect on global DNA methylation.

1. Introduction

Benzene is a ubiquitous environmental and occupational pollutant that has been classified as a group 1 carcinogen by the International Agency for Research on Cancer (IARC 1982). Chronic poisoning by benzene continues to occur in both Western countries and China and is significant because of the high number of recently reported cancer patients, including patients with leukemia, and lung cancer [1,2]. A meta-analysis of 15 studies with clearly defined cumulative exposure doses (CEDs) of benzene that investigated the risk of leukemia (effect size) estimated that the overall effect of benzene, even at a concentration less than 1 ppm, is 1.64 (95% CI: 1.13, 2.39) for a low CED (< 40 ppm-years) compared with the control [3]. However, the mechanism of benzene carcinogenesis remains unclear, and the hallmarks of the early damage caused by benzene are not well understood.

Evidence showing that the carcinogenic and mutagenic effects of
benzene are due to the formation of benzene oxide, hydroquinone and 1,4-benzoquinone during its metabolism is accumulating. These metabolites form DNA adducts or protein adducts, which result in genetic toxicity [4]. In our previous reports, epidemiological evidence indicates that a cytokinesis-block micronucleus (CBMN) assay on peripheral lymphocytes is a highly useful biomarker to assess the genetic damage caused by benzene [5,6]. We found that two endpoints, elevated MN frequency and reduced white blood cell (WBC) count, could be used to estimate a benchmark dose. The results indicated that MN frequency was a more sensitive indicator than WBC count [6].

Genotoxicity is a complex process involving interactions between multiple factors of endogenous and exogenous origin [7,8]. Many studies have confirmed that DNA repair mechanisms also play an important role in the effect of mutagens, such as benzene-induced genetic damage, especially impaired DNA repair capacity, which significantly contributes to mutagen-induced DNA damage [9]. Polymorphisms in genes encoding enzymes involved in benzene activation and detoxification and/or DNA repair may also affect individual susceptibility to benzene toxicity, as shown by the induced aggregated MN frequency [9]. In terms of DNA repair, the base excision repair (BER) pathway and nucleotide excision repair (NER) pathway are the two major methods of DNA excision repair. The BER pathway [9] is facilitated by the scaffold protein XRCC1 via its ability to interact with DNA ligase IIIα, DNA polymerase b, apurinic/apyrimidinic endonucleases (APE1), poly-nucleotide kinase/phosphatase and poly(adenosine diphosphate-ribose) polymerases 1 and 2 (ADPRT-1 and 2). It is generally assumed that BER is quantitatively the most important repair pathway for small oxidative lesions, especially human 8-oxoguanine DNA glycosylase 1 (hOGG1) [10]. The NER pathway is more complex than the BER pathway, and the major proteins involved in NER include XPA, XPC, XPD, XPE, XPF, XPG, and ERCC. The NER plays an important role in the removal of DNA damage caused by 1,4-benzoquinone-induced DNA adducts [10]. The effect of DNA repair polymorphisms on cancer is not a new concept. However, most of the reported endpoints are indicated by hematoxicity, but the micronucleus frequency indicated by chromosome damage can be better associated with polymorphisms in DNA repair genes. Furthermore, a study found no association between polymorphisms in DNA repair genes (APEX1, hOGG1, NBS1, XPD, XRCC1, and XRCC3) and micronucleus frequency in a small sample of 110 volunteers [11]. A large sample set of benzene-exposed workers was necessary to reanalyze the effects of polymorphisms in DNA repair genes on MN formation.

Recently, DNA methylation has provided new insights for cancer research studies because abnormalities in DNA methylation may be responsible for the initiation of tumorigenesis [12]. Cancer is believed to arise from genetic and epigenetic errors induced by environmental pollutants. Furthermore, increasing attention is being paid to mutagens, such as benzene-induced genetic damage. The use of a new concept. However, most of the reported endpoints are indicated by hematoxicity, but the micronucleus frequency indicated by chromosome damage can be better associated with polymorphisms in DNA repair genes. Furthermore, a study found no association between polymorphisms in DNA repair genes (APEX1, hOGG1, NBS1, XPD, XRCC1, and XRCC3) and micronucleus frequency in a small sample of 110 volunteers [11]. A large sample set of benzene-exposed workers was necessary to reanalyze the effects of polymorphisms in DNA repair genes on MN formation.

In the present study, we focused on polymorphisms in the BER (XRCC1: rs25489, rs25487; APE1: rs1130409) and NER pathways (XPA: rs1800975; XPC: rs2228000, rs2228002; XPD: rs13181, 1799793; XPG: rs17655; ERCC1: rs3212986) and the roles they play in susceptibility to genetic damage in benzene-exposed workers. The selected SNPs had a high mutation frequency and were located in the noncoding region (rs1800975 and rs3212986) or coding region (the others). We hypothesized that carriers of variant alleles may undergo genetic damage, as indicated by micronucleus (MN) frequency, and experience global DNA hypomethylation.

2. Materials and methods

2.1. Study population

The study population and design have been previously described [17]. We enrolled 294 benzene-exposed participants from Zhejiang Province in eastern China who were 17–57 years old; 102 control participants between 20 and 71 years old who were indoor workers from the same city were also enrolled. Benzene-exposed workers from shoe factories who used similar materials and production work flows were recruited during routine medical evaluations in Wenzhou. Study subjects were exposed to benzene vapor for a period of at least one year and were selected if they completed a detailed questionnaire and had blood samples collected as part of their routine medical evaluations. After obtaining informed consent, a total of 5 mL of anticoagulated peripheral blood was collected from each subject.

The Research Ethics Review Board of the School of Public Health at Fudan University in China approved this study protocol.

2.2. Assessment of benzene exposure

The assay was performed according to the method described previously [5]. There were three major workshops in the factory: sewing workshop, molding workshop and packing workshop. There were many glue brushing operations in the three workshops, especially in the molding workshop. Briefly, air samples were collected from 26 different worksites of the plant three times during the study with a GAS – IIIB air sampler (Hongyu, Shanghai, China) at 0.1 L/min for 15 min. The samples were analyzed by gas chromatography with a flame ionization detector (GC-FID) after desorption of benzene from the activated carbon with carbon disulfide.

The calculation of the CED for each worker was estimated using a previous method [5,6]. The benzene concentration that was estimated for each worksite and the worker exposure duration based on records of daily work hours, work location and job history at the factory were applied in the analysis. The CED was then calculated as follows: CED (mg/m³-year) = Σ C (mg/m³) × T (year).

2.3. Cytokinesis-block micronucleus assay, white blood cell counts and global DNA methylation

The cytokinesis-block micronucleus (CBMN) assay was performed according to the standard method described by Fenech [18]. Heparin-anticoagulated whole blood (0.5 mL) was added to 4.5 mL of medium (RPMI 1640) and incubated at 37 °C. After 44 h, cytochalasin-B (Sigma-Aldrich, St. Louis, MO) was added to each cell culture to a final concentration of 6 μg/mL to prevent cytokinesis. After 28 h of incubation with cytochalasin-B, the cells were harvested by cytocentrifugation and fixed with methanol and acetic acid at a ratio of 3:1. The slides were stained with Giemsa and air-dried. We microscopically examined 1000 binucleated cells on each slide and identified the number containing the MN according to standard criteria. The MN frequency was expressed as the total number of CBMN cells per 1000 binucleated cells. The WBC count was detected by an automated hematology analyzer (XE-2100, Sysmex, Japan).

2.4. SNP genotyping and methylation detection

DNA was extracted from peripheral blood leukocytes using a DNA blood isolation kit (Life Feng Biotechnology Co., Shanghai, China) according to the manufacturer’s instructions, and the samples were frozen at −80 °C. A Kompetitive allele-specific PCR (KASP) assay was applied to detect the genotypes of rs25489, rs25487, rs1130409, rs1800975, rs2228000, rs2228002, rs13181, 1799793, rs17655, and rs3212986. The primers were designed and supplied by Xiangyin Biotechnology Co. (Shanghai, China). The KASP assays were carried out in 96-well PCR
plates in a 5-μL final volume containing 30 ng of genomic DNA. Fluorescence detection of the PCR products was carried out with a PHERAstar (LGC Genomics, Britain) device according to a previous study [19]. In addition, blank controls were included at the four corners of each plate, and we repeated the analysis of 20% of the samples to confirm the reproducibility of the results.

Global DNA methylation was detected with a MethylFlash Methylated DNA Quantification Kit (EpiGentek, New York, USA). The methods used were reported previously [17].

2.5. Statistical analysis

The SAS software package (version 9.12) was used for the statistical analysis. The significance level (alpha) was set at 5% for all analyses. The genotype distributions in the study population were tested to ensure conformity with Hardy–Weinberg equilibrium by means of Pearson’s χ² test. The global DNA methylation data and WBC counts were analyzed with the normality test, and a linear regression analysis was used to compare the differences between exposed workers and controls after adjusting for gender, age, and smoking or drinking habits. Poisson regression models were used to analyze the MN frequency between exposed workers and controls and determine the differences among genotypes.

3. Results

3.1. Benzene exposure and its correlation with genetic damage and global DNA methylation

In total, 26 worksites in factories were analyzed. The benzene concentrations ranged from 2.6 mg/m³ (lower than the US benzene exposure limits of 1 ppm, equal to 3.23 mg/m³) to 57.0 mg/m³ (median, 6.4 mg/m³) in the air of the shoe factories [6]. The range of CED in the exposed workers was between 3.64 and 364.11 (mg/m³-year), with median 9.71 (mg/m³-year), respectively. Table 1 displays the demographic characteristics of benzene-exposed workers and the controls after adjusting for gender, age, and smoking and drinking habits. Poisson regression models were used to analyze the MN frequency after adjusting for confounding factors to compare the differences between exposed workers and controls and determine the differences among genotypes.

3.2. Distribution of genotypes and risk assessment for gene polymorphisms

The distributions of genotype and allele frequencies among the benzene-exposed workers are summarized in Table 3. Most of the polymorphisms were in Hardy–Weinberg equilibrium, with the exceptions of rs17655 (p = 0.001) and rs3212986 (p = 0.003). In addition, Table 3 presents associations of genotypes with global DNA methylation. The rs1130409 variant alleles GG (β = −0.29, 95% CI: −0.62, 0.04, p = 0.065) and (GG + GT) (β = −0.20, 95% CI: −0.42, 0.03, p = 0.045) were shown to be associated with lower global DNA methylation, although the results were near the threshold of significance.

Table 4 indicates associations of genotypes with MN frequencies and WBC counts in benzene-exposed workers. We found no significant associations between genotypes and WBC counts. A significantly increased MN frequency was observed for variant alleles of rs25487, rs1130409, rs17655, and rs3212986. The MN frequencies in the GA (FR (95% CI): 1.20 (1.06, 1.37), p = 0.006) and AA (FR (95% CI): 1.50 (1.16, 1.90), p = 0.002) alleles of rs25487, the GT (FR (95% CI): 1.20 (1.04, 1.37), p = 0.012) and GG (FR (95% CI): 1.28 (1.05, 1.55), p = 0.010) alleles of rs1130409, the GC (FR (95% CI): 1.18 (1.02, 1.38), p = 0.038) allele of rs17655, and the TT (FR (95% CI): 1.55 (1.31, 1.83), p < 0.001) allele of rs3212986 in each reference genotype were significantly higher than those in the reference genotypes (wild-type).

Supplement Table 1 shows a multiple Poisson analysis of four significant SNPs that were included in the model simultaneously (multiple-SNP model) and adjusted by age, gender, smoking and drinking. The variant alleles of rs25487, rs1130409, and rs3212986 were also shown to be associated with significantly higher MN frequencies than the reference genotype.

3.3. Comprehensive analysis of SNPs and effective biomarkers

To further elucidate the relevance of these variants to MN frequency and global DNA methylation, gene diplotypes should be analyzed. In this study, seven DNA repair genes with ten SNPs were analyzed, but the number of samples could not support the analysis of ten SNPs. Table 5 illuminates the comprehensive analysis of the associations between genetic polymorphism data and effective biomarkers, such as the MN frequency, genetic damage, global DNA methylation and WBC counts of the benzene-exposed workers. The genetic damage was classified as 4%, which was estimated to be in the 95th percentile for MN frequency in the controls, as reported in our previous study [6]. The exposed workers were divided into four groups, according to genotypes of four sites: rs25487 (G→A), rs1130409 (T→G), rs17655 (G→C), and rs3212986 (G→T). Each subject in group 4 contains four sites with mutant allele in homozygote or heterozygote way, as rs25487 (X/A), rs1130409(X/G), rs17655(X/C), and rs3212986(X/T). Subjects in Group 3 contain any three SNPs sites with mutant allele. Group 2 contains any two SNPs sites with mutant allele. The rest of parts were considered as the control group 1, with only one sites contains mutant allele or four sites were all wild type. The MN frequency in samples with two (FR (95% CI): 1.29 (1.07, 1.57), p = 0.004), three (FR (95% CI): 1.42 (1.17, 1.71), p < 0.001) and four (FR (95% CI): 1.73 (1.34, 2.23), p < 0.001) mutant alleles of rs25487 (G→A), rs1130409 (T→G), rs17655 (G→C), and rs3212986 (G→T) was significantly increased and showed a dose-response relationship (p < 0.001). The genetic damage in group 4 (OR (95% CI): 3.72 (1.34, 10.03), p = 0.009) and group 3 (OR (95% CI): 2.48 (1.27, 4.88), p = 0.008) was significantly higher than that in group 1 and showed a dose-response relationship in the trend test (p = 0.006).

The global DNA methylation level in samples with two mutant alleles was significantly lower than that in samples with one or no mutant allele (β = −0.32, 95% CI: −0.60, 0.04, p = 0.026). However, there was

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Exposed workers (%)</th>
<th>Controls (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>136 (46.3)</td>
<td>38 (37.3)</td>
<td>0.132</td>
</tr>
<tr>
<td>Female</td>
<td>158 (53.7)</td>
<td>64 (62.7)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤30</td>
<td>170 (57.8)</td>
<td>49 (48.0)</td>
<td>0.304</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>142 (42.2)</td>
<td>53 (52.0)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>234 (79.6)</td>
<td>92 (90.2)</td>
<td>0.016</td>
</tr>
<tr>
<td>Smoker</td>
<td>60 (20.4)</td>
<td>10 (9.8)</td>
<td></td>
</tr>
<tr>
<td>Drinking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-user</td>
<td>193 (65.6)</td>
<td>64 (62.7)</td>
<td>0.631</td>
</tr>
<tr>
<td>User</td>
<td>101 (34.4)</td>
<td>38 (37.3)</td>
<td></td>
</tr>
</tbody>
</table>

Pearson’s χ² test was applied to analyze the difference.
Global DNA methylation, and MN frequency by various demographic characteristics.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>MN* frequency</th>
<th>DNA methylation level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD(‰)</td>
<td>p</td>
<td>FR ± (95%CI)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>174</td>
<td>2.91 ± 1.82</td>
<td>Reference</td>
</tr>
<tr>
<td>Female</td>
<td>222</td>
<td>2.98 ± 2.06</td>
<td>0.067</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤30</td>
<td>219</td>
<td>2.78 ± 2.02</td>
<td>Reference</td>
</tr>
<tr>
<td>&gt;30</td>
<td>177</td>
<td>3.17 ± 1.85</td>
<td>0.024</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>326</td>
<td>2.93 ± 1.95</td>
<td>Reference</td>
</tr>
<tr>
<td>Smoker</td>
<td>70</td>
<td>3.07 ± 1.95</td>
<td>0.966</td>
</tr>
<tr>
<td><strong>Drinking</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-user</td>
<td>257</td>
<td>2.93 ± 1.92</td>
<td>Reference</td>
</tr>
<tr>
<td>User</td>
<td>139</td>
<td>2.99 ± 2.01</td>
<td>0.159</td>
</tr>
<tr>
<td><strong>Exposure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>102</td>
<td>1.82 ± 1.17</td>
<td>Reference</td>
</tr>
<tr>
<td>Exposed</td>
<td>294</td>
<td>3.34 ± 2.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low exposure</td>
<td>147</td>
<td>3.14 ± 1.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High exposure</td>
<td>147</td>
<td>3.54 ± 2.18</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* MN, micronuclei.

A previous study found that individuals carrying the XRCC1 Arg399Gln variant allele had a significantly higher MN frequency than the controls. Consistent with previous results in 1,3-butadiene-exposed workers, the workers carrying Arg/Gln and Gln/Gln genotypes (FR = 1.26, 95% CI: 1.03,1.53) also had increased MN frequency [27]. However, a previous study found that individuals carrying the XRCC1 280His (rs25489) allele had an increased risk of chronic benzene poisoning compared with those carrying the Arg/Arg allele [21], but we did not find this trend in only one worker carrying the His/His allele.

Apurinic apyrimidinic endonuclease 1 (APE1) is a key protein in the BER pathway, which is responsible for cleaving the phosphodiester backbone 5’ to the AP site and a 3’−OH primer. Rs1130409 is a single transition of the 1349th base pair T allele to a G allele, inducing the substitution of the 148th amino acid aspartate (Asp) to glutamate (Glu); this polymorphism has been extensively investigated in association with lung cancer, breast cancer, and bladder cancer [28–30]. Our results indicated that an rs1130409 variant allele (GG + GT) was also associated with a significantly higher MN frequency than the control genotype and had lower global DNA methylation. This result is consistent with that of a previous study, in which Almutairi et al reported that APE1 (rs1130409) showed a statistically significant association with Asp148Glu and elevated susceptibility to breast cancer [30]. A meta-analysis found a small but significant association between this APE1 polymorphism (rs1130409) and increased lung cancer risk in an Asian population [29], and this polymorphism might also be a genetic risk factor for the development of gastric cancer [28].

The BER pathway plays an important role in DNA demethylation by excising 5-carboxylyctosine to form cytosine [15]. We found no previous reports regarding this genetic polymorphism of APE1 (rs1130409) and its association with global DNA methylation. Not only does APE1 play a key role in the BER pathway, but it is also an important part of DNA demethylation, acting with RING finger protein 4 (RNF4) in active demethylation [16]. The decreased DNA methylation in the GG and GT variant alleles may be because the mutation reduced APE1 activity and then induced a compensatory increase in APE1 expression, resulting in upregulated demethylation.

Xeroderma pigmentosum group G (XPG, or excision repair cross-
complementing group 5, ERCC5), one of the important genes in the NER pathway, can abrogate bulky DNA adducts of endogenous and exogenous origin. A polymorphism at codon 1104 resulting in the amino acid change His1104Asp (rs17655) plays a significant role in the regulation of XPG, in which the C allele at rs17655 downregulates XPG in normal bronchial epithelial cells [31]. Our study indicates that workers carrying the C allele showed increased genetic damage, which is consistent with the report of Li et al, in which Japanese gastric cancer patients with the XPG rs17655 CC genotype showed shorter overall survival (HR = 1.60, 95% CI: 1.08–2.36) than those with GG/GC genotypes [32]. However, a meta-analysis did not indicate that the rs17655 polymorphism contributed to genetic susceptibility for non-small cell lung cancer [33]. In addition, no association between benzene exposure and rs17655 was found. However, one study found a contrasting result, in which individuals with the CC allele had a decreased risk of laryngeal cancer [34].

The ERCC1 rs3212986 variant located in the 3′ untranslated region (3′UTR) of the gene has been associated with the risk for several cancers, but this conclusion is controversial. Our results showed that the TT allele was associated with increased MN frequency, consistent with a report on colorectal cancer, which found that the TT allele was associated with a higher risk (OR: 3.079, 95% CI: 1.192,7.952) of colorectal cancer than the GG genotype [35]. In addition, in non-small cell lung cancer patients, survival of patients with the GT and TT genotypes was 5.7 and 4.7 months, respectively, which is much less than that of patients with the GG genotype (15.6 months) [36]. However,
A meta-analysis found no association between rs25487 at XRCC1, the GG allele of rs1130409 at APE1, the GC + CC alleles of XRCC1, the GG allele of rs1130409 at APE1, the GC + CC alleles of XRCC1, the GG allele of rs1130409 at APE1, and the TT allele of rs3212986 at ERCC1 are associated with elevated MN frequency, and the GG allele of rs1130409 at APE1 was also associated with global DNA hypomethylation, indicating that those SNPs may be potential susceptibility biomarkers for genetic damage due to benzene exposure. This study indicates that BER pathway and NER pathway polymorphism haplotypes are associated with differential levels of chromosome damage and provides further illumination of protective measures for benzene-exposed workers.

### Conflicts of interest

There are no conflicts of interest.

### Acknowledgments

This work was supported by the National Natural Science Foundation of China (NSFC81474929, 81602836), the National Key...
The exposed workers were divided into four groups, according to genotypes of four sites: rs25487 (G→T), rs1130409 (T→G), rs17655 (C→G), and rs3212986 (X→T).

Each subject in group 4 contains four sites with mutant allele in homozygote or heterozygote way, as rs25487 (X/A), rs1130409 (X/G), rs17655 (X/C), and rs3212986 (X/T). Subjects in Group 3 contain any three SNPs sites with mutant allele. Group 2 contains any two SNPs sites with mutant allele. The rest of parts were considered as the control group 1, with only one sites contains mutant allele or four sites were all wild type.

* p < 0.05.
* The genetic damage was adjusted by ≥4%, estimated 95 percentile of MN frequency in the controls.

Research and Development Program "Precision Medicine Initiative" of China (Grant NO: 2016YFC0900803).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://10.1016/j.mrgentox.2019.01.006.

References

nucleotide polymorphisms in nucleotide excision repair pathway genes ERCC1, ERCC2, ERCC3, ERCC4, ERCCS and XPA, Gene 542 (2014) 64–68.

[35] Q. Zhang, X. Zheng, X. Li, D. Sun, P. Xue, G. Zhang, M. Xiao, Y. Cai, C. Jin, J. Yang, The polymorphisms of miRNA binding site in MLH 3 and ERCC 1 were linked to the risk of colorectal cancer in a case–control study, Cancer Med. 7 (2018) 1264–1274.


[38] X. Liu, Z. Zhang, C. Deng, Y. Tian, X. Ma, Meta-analysis showing that ERCC1 polymorphism is predictive of osteosarcoma prognosis, Oncotarget 8 (2017) 62769.