Effects of Genetic Polymorphisms of Metabolic Enzymes on Cytokinesis-Block Micronucleus in Peripheral Blood Lymphocyte among Coke-Oven Workers

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Abstract

Exploring the associations between genetic polymorphisms of metabolic enzymes and susceptibility to polycyclic aromatic hydrocarbon (PAH)–induced chromosomal damage is of great significance for understanding PAH carcinogenesis. Cytochrome *P*450, glutathione *S*-transferase, microsomal epoxide hydrolase, NAD(P)H:quinone oxidoreductase, and *N*-acetyltransferase are PAH-metabolizing enzymes. In this study, we genotyped for the polymorphisms of these genes and assessed their effects on cytokinesis-block micronucleus (CBMN) frequencies in peripheral blood lymphocytes among 141 coke-oven workers and 66 non–coke-oven worker controls. The geometric means of urinary 1-hydroxypyrene levels in coke-oven workers and the controls were 12.0 and 0.7 μmol/mol creatinine, respectively ($P < 0.01$). The CBMN frequency (number of micronuclei per 1,000 binucleated lymphocytes) was significantly higher in coke-oven workers (9.5 ± 6.6‰) than in the controls (4.0 ± 3.6‰; $P < 0.01$). Among the coke-oven workers, age was positively associated with CBMN frequency; the *mEH* His^{113} variant genotype exhibited significantly lower CBMN frequency (8.5 ± 6.5‰) than did the Tyr^{113}/Tyr^{113} genotype (11.3 ± 6.4‰; $P < 0.01$); the low mEH activity phenotype exhibited a lower CBMN frequency (8.6 ± 6.8‰) than did the high mEH activity phenotype (13.2 ± 6.7‰; $P = 0.01$); the *GSTP1* Val^{105}/Val^{105} genotype exhibited a higher CBMN frequency (15.0 ± 5.8‰) than did the *GSTP1* Ile^{105}/Ile^{105} or Ile^{105}/Val^{105} genotypes (9.3 ± 6.5‰; $P < 0.01$); the joint effect of high mEH activity phenotype and *GSTM1 null* genotype on CBMN frequencies was also found. Gene-environment interactions between occupational PAH exposure and polymorphisms of *mEH* and/or *GSTM1* were also evident. These results indicate that the *mEH*, *GSTP1*, and *GSTM1* polymorphisms may play a role in sensitivity or genetic susceptibility to the genotoxic effects of PAH exposure in the coke-oven workers. (Cancer Epidemiol Biomarkers Prev 2004;13(10):1631–9)

Introduction

Chemicals present in the coal-coking process has been classified as class I carcinogens by IARC (1), and there has been sufficient epidemiologic evidence suggesting an etiologic link between carcinogenic polycyclic aromatic hydrocarbon (PAH) exposure and lung cancer risk in coke-oven workers (2, 3). Although a high level of PAH exposure may be confined to occupational workers, cigarette smoking and air pollution are important sources of environmental PAHs in the general population. Therefore, the metabolite 1-hydroxypyrene (1-OHP) in urine, a major metabolite of pyrene, has been found to be a golden internal exposure index of recent exposure to PAHs in both occupational and environmental settings (4).

Most PAHs require metabolic activation and detoxification by different enzymes that contribute to their ultimate mutagenic and carcinogenic effects (5). In general, carcinogenic PAHs, such as benzo(*a*)pyrene [B(a)P], need to be activated by the phase I enzymes (e.g., cytochrome *P*450 1A1 and 2E1) to form ultimate carcinogens, such as B(a)P diol epoxide (BPDE), whereas the phase II enzymes (e.g., glutathione *S*-transferases and *N*-acetyltransferases) generally mediate the conjugation of water-soluble moieties, such as glutathione, which are responsible for detoxification of these reactive metabolites (6). The microsomal epoxide hydrolase (mEH) converts *trans*-7,8-diol of B(a)P to more water-soluble *trans*-dihydrodiols, which is further activated by phase I enzymes to form the ultimate carcinogenic diol epoxide (BPDE; ref. 7). NAD(P)H:quinone oxidoreductase 1 could detoxify the quinone-reactive intermediates of PAHs to protect against formation of PAH-DNA adducts (8). However, the genes encoding these enzymes are highly polymorphic and many polymorphisms in these genes with functional significance have been found to modify individual susceptibility to PAH carcinogenesis (e.g., lung cancer; ref. 9).

The cytokinesis-block micronucleus (CBMN) assay could detect chromosome breakage and loss specifically

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in cells that have completed nuclear division once (10, 11) and has emerged as a maturing biomarker of chromosomal damage relevant to cancer in recent years (12, 13). Because chromosome damage is an important early biological event in chemical carcinogenesis, exploring the relationship between genetic variants of metabolic enzymes and PAH-induced chromosomal damages is important for our understanding of PAH carcinogenesis and improvement of risk assessment.

Numerous studies have investigated the effects of polymorphisms in genes involved in activation or detoxification of carcinogens on phenotypic biomarkers of exposure, mainly urinary 1-OHP, DNA or protein adducts, and urinary mutagenicity in populations occupationally exposed to PAHs (14-24). For example, several studies have found consistent associations between the *GSTM1 null* genotype and increased levels of urinary mutagen excretion (25), DNA adducts (15, 17, 19, 20, 22), and BPDE-serum albumin levels (21) and between low mEH activity genotype/phenotype and lower levels of urinary mutagenicity, DNA adducts, and BPDE-serum albumin adducts (16, 26). However, there are few studies addressing the impact of polymorphisms in metabolic genes on biomarkers of early effects in occupationally PAH-exposed populations (23, 27, 28) and some published results are conflicting. No published studies have investigated the relationship between genotypes of metabolic enzymes and the frequencies of CBMN induced by exposure to occupational PAHs that are common in coke-oven workers.

In this study, we genotyped for several metabolic genes involved in PAH metabolism, determined personal PAH exposure (expressed as urinary 1-OHP), and investigated their relationships with the frequencies of CBMN in peripheral blood lymphocyte among 141 coke-oven workers and 66 non–coke-oven workers.

Materials and Methods

Study Population and Sample Collection. All study participants were employed in a steel company in northeast China. Employees (n = 141) working with the same coke-oven, who had been exposed to coke-oven emissions regularly and had been employed for at least 6 months, were recruited into this study as the exposed group. Sixty-six medical staffs, working in the affiliated hospital of the company located ~3 km away from the coking area, were recruited as the nonexposure controls, because no obvious work-related PAH exposure was found for these medical staffs. Exclusion criteria for participation in the study included recent treatment with mutagenic agents (such as X-ray), chronic conditions (such as autoimmune diseases), and recent acute infections that required medications such as antibiotics. After an informed consent was obtained, all participants were interviewed by an occupational physician using a detailed questionnaire including demographic information, smoking history, alcohol consumption, history of occupational exposure, and personal medical history. Individuals who had smoked >100 cigarettes in their lifetime were considered as smokers and asked for information on a variety of smoking-related questions. Among these smokers, individuals who still smoked when interviewed were classified as current smokers; the others were classified as former smokers. Individuals who drank more than twice a week in the last 6 months were classified as alcohol users.

Spot urine samples were obtained from each subject at the end of a work shift after at least 4 consecutive days of work, maintained at −20°C, and used later for the measurement of urinary 1-OHP. Three milliliters of venous blood samples were drawn from each subject, of which 1 mL was treated with heparin as anticoagulant for immediate culture of CBMN assay and 2 mL was anticoagulated with EDTA and stored at −20°C for extraction of DNA.

The research protocol was approved by the Research Ethics Committee of the National Institute for Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention.

PAH Exposure Assessment. Airborne PAHs in the working environment of coke-oven and non–coke-oven workers were sampled ~1.5 month before urine and blood sample collection by the static air sampling method and analyzed with the OSHA method no. 58 (29). The levels of benzene-soluble matter (BSM) were gravimetrically determined and particulate-phase B(a)P levels were analyzed by high-performance liquid chromatography with a fluorescence detector (λ_{ex} = 365 nm, λ_{em} = 405 nm).

The excretion of 1-OHP as the internal dose of personal recent PAH exposure was measured according to the method of Jongeneelen et al. (30) with some modifications (31) described as follows. Briefly, instead of using β-glucuronidase hydrolysis, thawed urine specimen (2 mL) was mixed with 15 mol/L NaOH solution (0.5 mL) and incubated at 100°C for 3.5 hours for hydrolyzation. Carbazole (10 μg/mL, 50 μL), as an internal standard, was added into the urine specimen, and the urine samples were adjusted to pH 3.0 to 5.0 with HCl. Extraction was done with CH_2Cl_2 (4 mL) and the organic solvent was evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 0.2 mL of 75% methanol and 10 μL was injected onto a C18 column. The analyte was eluted at a flow rate of 1 mL/min with a mobile phase of 75% methanol. The 1-OHP was detected by a fluorescence detector ($\lambda_{ex}/_{em}$, 346/386 nm). Figure 1 presents a typical high-performance liquid chromatogram of 1-OHP with carbazole as an internal standard from a top-of-oven worker. Quantification was based on peak height ratios of the 1-OHP to the carbazole. The limit of detection was 0.1 g/L urine (signal-to-noise = 3). Measurements below the limit of detection (for four non–coke-oven workers only) were replaced with LOD/$\sqrt{2}$ prior to statistical analysis, where LOD is limit of detection (32). Quality control samples were assayed together with each batch of measurements. The recovery from the entire procedure was 94.0% at 5 μg/L and 99.5% at 20 μg/L. The intraday relative SD values were 6.2% and 5.8% at 5 and 20 μg/L, respectively, and the interday relative SD values were 7.7% and 8.9% at 5 and 20 μg/L, respectively (n = 6 for each group). The urinary 1-OHP concentrations were corrected by urinary creatinine and presented as μmol/mol creatinine.

CBMN Assay Using Peripheral Blood Lymphocytes. The CBMN assay was done according to the standard method as described previously (10, 11). Duplicate lymphocyte cultures were set up immediately after blood

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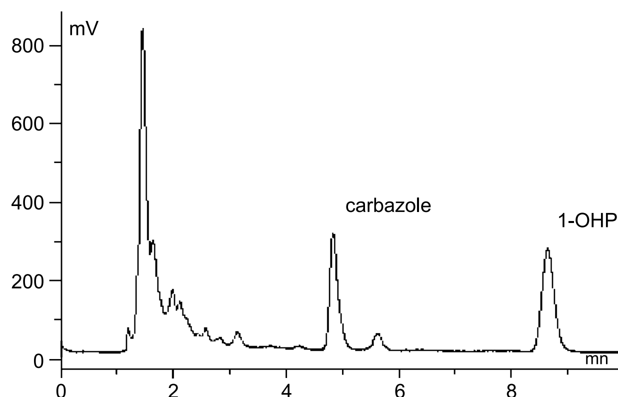

Figure 1. High-performance liquid chromatogram of urinary 1-OHP obtained from an exposed subject (a top-of-oven worker).

collection. Forty-four hours after PHA stimulation, cytokinesis was blocked with 6 μg/mL cytochalasin-B, and 28 hours later, lymphocytes were harvested. Hypotonic treatment was used and cells were fixed with methanol and acetic acid at 4:1 before being transferred to slides. Standard scoring criteria (10, 33) for selecting binucleated cells and identifying a micronucleus were adopted. All slides were coded and scored blindly by an experienced scorer. A total of 1,000 binuleated lymphocytes were examined for detection of micronuclei for each subject.

Genotyping. DNA was isolated from the whole blood using a standard method (34). The *Ile/Val* polymorphism in exon 7 of the *CYP1A1* was analyzed according to the method of Hayashi et al. (35). The *Dra*I polymorphism in intron 6 and *Pst*I polymorphism in the 5′-flanking region of *CYP2E1* were genotyped by the methods of Stephens et al. (36) and Kato et al. ((37), respectively. Analysis of the *GSTP1* polymorphism resulting in an *Ile/Val* substitution at residual 105 in exon 5 was done as described by Saarikoski et al. (38). Detection of the *C/T 609* ($Pro^{187}Ser$) polymorphism of *NQO1* was conducted according to the method of Chan et al. (39). The genotypes of *GSTM1* and *GSTT1* were determined by a modified multiplex PCR method with β-globin as positive control (40, 41). For *NAT2*, *Kpn*I, *Taq*I, and *Bam*HI polymorphisms were determined and classified as fast or slow acetylators according to the method of Hickman and Sim (42). The *Tyr/His* polymorphism at residual 113 in exon 3 and *His/Arg* polymorphism at residual 139 in exon 4 of *mEH* was analyzed as described by Zhou et al. (43). All genotypes were evaluated and agreed upon by at least two persons independently. Ten percent of DNA samples were genotyped a second time and the concordance was 100%.

Statistical Analysis. Student's *t* tests were used to compare age and the ln-transformed urinary 1-OHP levels between two exposure populations and compare the ln-transformed urinary 1-OHP levels between current and noncurrent smokers (former and never smokers). Mann-Whitney *U* tests were used to compare the air levels of PAHs, cigarettes per day, and CBMN frequencies between non–coke-oven workers and coke-oven workers and to compare the CBMN frequencies between the categories of selected variables (age, sex, coking history, and smoking and alcohol status). χ^2 test was used to compare the frequencies of current smokers and alcohol users between two exposure populations. Spearman rank correlation test was used to evaluate the relationship between urinary 1-OHP concentrations and CBMN frequencies. For multivariate analysis, the CBMN data were ln transformed to normalize the variance. The differences in ln-transformed CBMN data between each genotype of single metabolic gene and between each combination of *mEH* phenotypes and *GSTM1* genotypes were analyzed with multivariate analysis of covariance with adjustment for urinary 1-OHP, age, sex, and cigarettes per day in exposed and nonexposed groups separately. For genotypes or genotype combinations with more than two levels in multivariate analysis, dummy variables for genotypes or genotype combinations were designed. To give a convenient summary of the CBMN results in the tables, means and SDs of the nontransformed CBMN data were presented; for urinary 1-OHP, geometric means and their 95% confidence intervals were used. All statistical tests were two sided ($\alpha = 0.05$) and done using Statistical Analysis System software (version 8.0; SAS Institute, Inc., Cary, NC).

Results

The demographic data and environmental exposure information of coke-oven workers and non–coke-oven workers are summarized in Table 1. The distributions of age, sex, and prevalence of drinking were not significantly different between the two exposure groups, but the percentages of current smokers and number of cigarettes smoked per day were higher in coke-oven workers than in non–coke-oven workers ($P < 0.01$). The medians of air BSM and particulate-phase B(a)P were significantly higher in coke-oven (BSM, 328.6 μg/m^3 and B(a)P, 926.9 ng/m^3, $n = 30$) than in non–coke-oven working environment (BSM, 97.8 μg/m^3 and B(a)P, 49.1 ng/m^3, $n = 10$; $P < 0.01$; Table 1) with the highest at the top of oven (BSM, 1,233.3 μg/m^3 and B(a)P, 2,634.9 ng/m^3, $n = 10$). The geometric mean of urinary 1-OHP was significantly higher in coke-oven workers (12.0; 95% confidence interval, 10.4-13.9 μmol/mol creatinine) than in non–coke-oven workers (0.7; 95% confidence interval, 0.6-1.3 μmol/mol creatinine; Table 1) and significantly higher in current smokers than in noncurrent (former and never) smokers in both exposure groups (coke-oven workers, 13.8 versus 9.4 μmol/mol creatinine, $P = 0.01$ and non–coke-oven workers, 1.0 versus 0.5 μmol/mol creatinine, $P < 0.01$, respectively) as well as higher in male workers than in female workers (coke-oven workers, 12.5 versus 8.5 μmol/mol creatinine, $P = 0.09$ and non–coke-oven workers, 0.7 versus 0.5 μmol/mol creatinine, $P = 0.03$).

Table 2 shows the results of CBMN frequencies and the stratification analysis by age, sex, smoking and alcohol status, and coking history. The median CBMN frequency was 9.5 ± 6.6‰ in coke-oven workers, which was significantly higher than that in non–coke-oven workers (4.0 ± 3.6‰; $P < 0.01$). In coke-oven workers, subjects with age ≥44 years had significantly higher

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Table 1. Demographic data of coke-oven and non–coke-oven workers in a Chinese occupational population

Variables	Non–coke-oven workers	Coke-oven workers	*P*
n	66	141	
Age (y, mean ± SD)	38 ± 8	39 ± 7	0.43*
Sex (men/women, % of men)	57/9 (86.4)	128/13 (90.8)	0.34†
Current smokers (yes/no, %)	24/42 (36.4)	90/51 (63.8)	<0.01†
Cigarettes per day (mean ± SD)	5.1 ± 7.7	8.3 ± 7.2	<0.01‡
Alcohol user (yes/no, %)	23/43 (34.8)	60/81 (42.6)	0.29†
Coking history (y, mean ± SD)	—	18.6 ± 6.9	—
BSM in air [μg/m^3, median (range)]	97.8 (2.6-149.1)	328.6 (61.8-2,677.8)	<0.01‡
Particulate-phase B(a)P in air [ng/m^3, median (range)]	49.1 (43.0-51.5)	926.9 (250.7-3,723.1)	<0.01‡
Urinary 1-OHP levels [μmol/mol creatinine, geometric mean (95% confidence interval)]	0.7 (0.6-1.3)	12.0 (10.4-13.9)	<0.01§

*Student's *t* test for difference between coke-oven workers and non–coke-oven workers.
†χ^2 tests for differences in distributions between coke-oven workers and non–coke-oven workers.
‡Mann-Whitney *U* tests for differences between coke-oven workers and non–coke-oven workers.
§Student's *t* test for ln-transformed data between coke-oven workers and non–coke-oven workers.

CBMN frequencies (11.3 ± 6.6‰) than those with age 35 to 44 years (8.8 ± 5.7‰; *P* = 0.05) and those with age <35 years (8.6 ± 7.2‰; *P* = 0.02). We further analyzed the association between age and CBMN frequencies using multivariate analysis of covariance with adjustment for coking history in coke-oven workers and found a similar result (*P* = 0.04 for difference of CBMN frequencies between age >44 and <35 groups). In non–coke-oven workers, the subjects with age >44 years also had higher CBMN frequencies (4.7 ± 5.1‰) than younger groups, but the differences were not significantly. We did not find any statistically significant association between coking history and CBMN frequencies in coke-oven workers. Female subjects had higher CBMN frequencies than male subjects in coke-oven workers, but the difference was not statistically significant even with adjustment of age (*P* = 0.27), and there was no sex difference in the non–coke-oven workers. No differences in CBMN frequencies between current smokers and noncurrent smokers and between alcohol users and nonalcohol users in both exposure groups were found even with adjustment of age and sex (data not shown). The differences in CBMN frequencies between coke-oven workers and non–coke-oven workers in subgroups by age, sex, and smoking and alcohol status remained significantly different by Mann-Whitney *U* test. In all subjects, correlation between urinary 1-OHP concentrations and CBMN frequencies was statistically significant (*n* = 207, Spearman *r* = 0.383; *P* < 0.01); however, this significant correlation disappeared when analyzed for coke-oven workers and non–coke-oven workers separately (data not shown).

The effects of genotypes of phase I and II metabolic enzymes on CBMN frequencies are presented in Tables 3 and 4. Using multivariate analysis of covariance with adjustment for urinary 1-OHP, age, sex, and cigarettes

Table 2. CBMN frequencies (‰) by age, sex, smoking and alcohol status, and coking history in coke-oven and non–coke-oven workers

Variables	Non–coke-oven workers			Coke-oven workers			*P**
	n	Mean ± SD	*P*†	*n*	Mean ± SD	*P*†	
All	66	4.0 ± 3.6		141	9.5 ± 6.6		<0.01
Age (y)							
<35	22	3.5 ± 3.4	Reference‡	48	8.6 ± 7.2	Reference‡	<0.01
35-44	27	3.9 ± 2.5	0.30	47	8.8 ± 5.7	0.32	<0.01
≥44	17	4.7 ± 5.1	0.60	46	11.3 ± 6.6§	0.02	<0.01
Sex							
Men	57	4.0 ± 3.8	Reference‡	128	9.3 ± 6.4	Reference‡	<0.01
Women	9	3.9 ± 2.0	0.53	13	12.4 ± 8.3	0.25	0.02
Smoking status							
Current smokers	24	3.5 ± 3.6	Reference‡	90	9.3 ± 6.2	Reference‡	<0.01
Former and never smokers	42	4.3 ± 3.6	0.27	51	9.9 ± 7.2	0.95	<0.01
Alcohol status							
Alcohol user	23	4.1 ± 3.8	Reference‡	60	10.0 ± 6.4	Reference‡	<0.01
Nonalcohol user	43	3.9 ± 3.5	0.96	81	9.2 ± 6.8	0.32	<0.01
Coking history (y)							
<20				66	9.3 ± 7.2	Reference‡	
≥20				75	9.8 ± 6.0	0.29	

*Mann-Whitney *U* tests for the differences in CBMN frequencies between coke-oven workers and non–coke-oven workers in each subgroup of selected variables.
†Mann-Whitney *U* tests for the differences in CBMN frequencies between the categories of selected variables.
‡Reference group for comparisons of CBMN frequencies between the categories of selected variables.
§*P* = 0.05, compared with those ages 35-44 years.

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Table 3. CBMN frequency (‰) in coke-oven and non–coke-oven workers by genotypes of phase I metabolic enzymes

Genotypes	Non–coke-oven workers			Coke-oven workers			P*
	n	Mean ± SD	P†	*n*	Mean ± SD	P†	
CYP1A1							
Ile/Ile	37	4.7 ± 4.1	Reference‡	67	9.5 ± 7.2	Reference‡	<0.01
Ile/Val	22	2.6 ± 2.0	0.06	61	9.5 ± 6.2	0.90	<0.01
Val/Val	7	4.7 ± 3.7	0.72	13	9.5 ± 5.4	0.67	0.02
Ile/Val-Val/Val	29	3.1 ± 2.6	0.17	74	9.5 ± 6.0	0.81	<0.01
*CYP2E1 Pst*I site							
c1/c1	45	4.1 ± 3.6	Reference‡	92	9.7 ± 6.4	Reference‡	<0.01
c1/c2	21	3.7 ± 3.6	0.52	47	9.4 ± 7.1	0.89	<0.01
c2/c2	0		—	2	7.5 ± 3.5	0.98	
c1/c2-c2/c2	21	3.7 ± 3.6	0.52	49	9.3 ± 7.0	0.90	<0.01
*CYP2E1 Dra*I site							
D/D	38	4.2 ± 3.8	Reference‡	85	9.9 ± 6.5	Reference‡	<0.01
D/C	28	3.8 ± 3.4	0.56	51	8.9 ± 6.9	0.21	<0.01
C/C	0		—	5	10.2 ± 7.0	0.92	
D/C-C/C	28	3.8 ± 3.4	0.56	56	9.0 ± 6.8	0.23	<0.01

*Mann-Whitney *U* tests for the differences in CBMN frequencies between coke-oven workers and non–coke-oven workers by each genotype.
†Multivariate analysis of covariance tests for differences in ln-transformed CBMN data between genotypes with adjustment for urinary 1-OHP, age, sex, and cigarettes per day in coke-oven workers and non–coke-oven workers separately.
‡Reference group for comparisons of ln-transformed CBMN data between genotypes.

per day, coke-oven workers carrying the Tyr^{113}/Tyr^{113} genotype in exon 3 of *mEH* had significantly higher CBMN frequencies than those with the Tyr^{113}/His^{113} genotype or His^{113}/His^{113} genotype (P = 0.01). In coke-oven workers, the subjects with the *GSTP1* gene *Val/Val* genotype had significantly higher CBMN frequencies than those with the *Ile/Ile* (P = 0.05) and *Ile/Val* genotype (P = 0.01) with adjustment for urinary 1-OHP, age, sex,

Table 4. CBMN frequency (‰) in coke-oven and non–coke-oven workers by genotypes of phase II metabolic enzymes

Genotypes	Non–coke-oven workers			Coke-oven workers			P*
	n	Mean ± SD	P†	*n*	Mean ± SD	P†	
mEH exon 3							
Tyr/Tyr	22	4.0 ± 3.4	Reference‡	52	11.3 ± 6.4	Reference‡	<0.01
Tyr/His	25	4.2 ± 4.6	0.62	31	7.9 ± 5.1	0.04	<0.01
His/His	19	3.7 ± 2.1	0.71	58	8.9 ± 7.2	<0.01	<0.01
Tyr/His-His/His	44	4.0 ± 3.7	0.90	89	8.5 ± 6.5	<0.01	<0.01
mEH exon 4							
His/His	57	3.9 ± 2.6	Reference‡	106	9.2 ± 6.6	Reference‡	<0.01
His/Arg	8	4.8 ± 4.0	0.32	34	10.2 ± 6.5	0.57	0.02
Arg/Arg	1	4.0	0.82	1	18.0	0.11	
His/Arg-Arg/Arg	9	4.7 ± 3.7	0.31	35	10.5 ± 6.5	0.42	0.01
GSTM1							
Non-null	41	3.7 ± 3.4	Reference‡	74	8.9 ± 6.8	Reference‡	<0.01
Null	25	4.4 ± 4.0	0.40	67	10.2 ± 6.3	0.12	<0.01
GSTT1							
Non-null	36	3.8 ± 3.1	Reference‡	71	8.9 ± 5.8	Reference‡	<0.01
Null	30	4.2 ± 4.2	0.82	70	10.2 ± 7.3	0.47	<0.01
GSTP1							
Ile/Ile	39	4.7 ± 4.2	Reference‡	84	9.4 ± 6.8	Reference‡	<0.01
Ile/Val	25	3.1 ± 2.1	0.35	51	9.1 ± 6.2	0.56	<0.01
Val/Val	2	0§	0.01	6	15.0 ± 5.8‖	0.01	0.07
Ile/Val-Val/Val	27	2.9 ± 2.2	0.18	57	9.7 ± 6.3	0.85	<0.01
NQO1							
CC	25	3.3 ± 2.7	Reference‡	48	9.4 ± 7.3	Reference‡	<0.01
CT	26	5.0 ± 4.5	0.15	61	9.7 ± 6.5	0.36	<0.01
TT	15	3.4 ± 2.9	0.83	32	9.4 ± 5.7	0.36	<0.01
CT-TT	41	4.4 ± 4.0	0.26	93	9.6 ± 6.2	0.29	<0.01

*Mann-Whitney *U* tests for the differences in CBMN frequencies between coke-oven workers and non–coke-oven workers by each genotype.
†Multivariate analysis of covariance tests for differences in ln-transformed CBMN data between genotypes with adjustment for urinary 1-OHP, age, sex, and cigarettes per day in coke-oven workers and non–coke-oven workers separately.
‡Reference group for comparisons of ln-transformed CBMN data between genotypes.
§P = 0.05, compared with the *GSTP1 Ile/Val* genotype.
‖P = 0.02, compared with the *GSTP1 Ile/Val* genotype.

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Table 5. CBMN frequency (‰) in coke-oven and non–coke-oven workers by phenotypes of *mEH* and NAT_2

Phenotype	Non–coke-oven workers			Coke-oven workers			P*
	n	Mean ± SD	P†	*n*	Mean ± SD	P†	
mEH							
LA	39	4.1 ± 3.9	Reference‡	70	8.6 ± 6.8	Reference‡	<0.01
IA	23	3.3 ± 2.8	0.56	55	9.6 ± 6.0	0.17	<0.01
HA	4	6.8 ± 4.3	0.09	16	13.2 ± 6.7	0.01	0.10
IA-HA	27	3.8 ± 3.2	0.96	71	10.4 ± 6.3	0.04	<0.01
NAT2							
Fast acetylator	27	3.9 ± 3.1	Reference‡	60	8.5 ± 5.5	Reference‡	<0.01
Slow acetylator	39	4.1 ± 3.9	0.92	81	10.4 ± 7.2	0.43	<0.01

*Mann-Whitney *U* tests for the differences in CBMN frequencies between coke-oven workers and non–coke-oven workers by each genotype.
†Multivariate analysis of covariance tests for differences in ln-transformed CBMN data between genotypes with adjustment for urinary 1-OHP, age, sex, and cigarettes per day in coke-oven workers and non–coke-oven workers separately.
‡Reference group for comparisons of ln-transformed CBMN data between genotypes.

and cigarettes per day in the model of multivariate analysis of covariance. Whereas in non–coke-oven workers the *Val* allele of *GSTP1* gene seemed to be associated with lower CBMN frequencies, the sample size for the subjects (n = 2) with the *Val/Val* genotype was too small to have a valid comparison. In coke-oven workers, the CBMN frequency was found to be lower in subjects with the *GSTM1 non-null* genotype than in those with *GSTM1 null* genotype, although the difference did not reach the significance level (P = 0.12). Based on the *in vitro* functional expression study of the variant alleles at residues 113 (exon 3, His^{113} slow allele) and 139 (exon 4, Arg^{139} rapid allele; refs. 44-46), three mEH enzymatic activity phenotypes (low, intermediate, and high) were assigned. Low activity phenotype group (LA phenotype) consisted of subjects homozygous for His^{139} and homozygous for His^{113} or heterozygous for His^{113}, high activity phenotype group (HA phenotype) was homozygous for Tyr^{113} and homozygous for Arg^{139} or heterozygous for Arg^{139}, and the subjects with remaining phenotypes were intermediate mEH activity phenotype group (IA phenotype). The coke-oven workers with LA phenotype of *mEH* had the lowest CBMN frequencies, whereas those with the HA phenotype had the highest CBMN frequencies, and the difference was statistically significant with adjustment for urinary 1-OHP, age, sex, and cigarettes per day in the model of multivariate analysis of covariance (P = 0.01; Table 5). The different effects of *mEH* phenotypes on CBMN frequencies in subjects with different occupational PAH exposure suggested the existence of gene-environment interaction between *mEH* phenotype and occupational PAH exposure. No other significant associations between CBMN frequencies and genetic polymorphisms of metabolic enzymes were found. The differences in CBMN frequencies between coke-oven workers and non–coke-oven workers in subgroups by genotypes or phenotypes remained significantly different.

The joint effect between *mEH* phenotypes and *GSTM1* genotypes on CBMN frequency is shown in Table 6. In coke-oven workers, the difference in CBMN frequencies between subjects with LA phenotype of *mEH* and *GSTM1 non-null* genotype (with the lowest CBMN frequency, 8.2 ± 7.1‰) and those with IA or HA phenotype of *mEH* and *GSTM1 null* genotype (with the highest CBMN frequency, 11.1 ± 6.1‰) was statistically significant (P = 0.02) with adjustment for urinary 1-OHP, age, sex, and cigarettes per day in the model of multivariate analysis of covariance; however, this joint effect between these two polymorphisms was not evident in non–coke-oven workers. The different effects of combined *mEH* and *GSTM1* genotypes on CBMN frequencies in subjects with different occupational PAH exposure also suggested the existence of gene-environment interaction. The CBMN frequencies between coke-oven workers and non–coke-oven workers in subgroups by combined *mEH* and *GSTM1* genotypes remained significantly different.

Table 6. Joint effect of *mEH* phenotypes and *GSTM1* genotypes on CBMN frequency (‰) in coke-oven and non–coke-oven workers

mEH	*GSTM1*	Non–coke-oven workers			Coke-oven workers			P*
		n	Mean ± SD	P†	*n*	Mean ± SD	P†	
LA phenotype	*Non-null*	25	3.7 ± 3.5	Reference‡	39	8.2 ± 7.1	Reference‡	<0.01
LA phenotype	*Null*	14	3.8 ± 3.2	0.54	31	9.7 ± 6.5	0.22	<0.01
IA-HA phenotype	*Non-null*	16	4.9 ± 4.4	0.17	35	9.2 ± 6.5	0.43	0.01
IA-HA phenotype	*Null*	11	3.8 ± 3.4	0.86	36	11.1 ± 6.1	0.02	<0.01

*Mann-Whitney *U* tests for the differences in CBMN frequencies between coke-oven workers and non–coke-oven workers by each genotype.
†Multivariate analysis of covariance tests for differences in ln-transformed CBMN data between genotypes with adjustment for urinary 1-OHP, age, sex, and cigarettes per day in coke-oven workers and non–coke-oven workers separately.
‡Reference group for comparisons of ln-transformed CBMN data between genotypes.

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Discussion

Accurate measurement of PAH exposure levels is important for evaluating PAH-induced early genetic and cytogenetic effects and investigating the modification effect or gene-environment interaction on PAH-induced adverse effects in coke-oven workers. In the present study, the coke-oven workers seemed to have been exposed to a high level of PAHs based on the stationery environmental monitoring, including air BSM and particulate-phase B(a)P, and high level of urinary 1-OHP. The PAH exposure of coke-oven workers in the present study was slightly higher than that in Zhang's study conducted in another steel company in China (13) and much higher than that reported by several other studies using CBMN assay (47-51).

In the present study, the associations between PAH exposure and increase of CBMN frequencies in coke-oven workers suggested that the CBMN assay could be used to detect the PAH-induced chromosomal damage in those who had been exposed to high levels of PAHs. The increase of CBMN frequencies with age, higher values of CBMN frequencies in women, and no effect of smoking and alcohol status on CBMN frequencies in coke-oven workers found in our study were consistent with the results of international collaborative projects aiming to assess the experimental protocol variations and the influence of age, sex, and lifestyle on CBMN frequencies (52-54). However, there were very few workers smoking 30+ cigarettes per day in our population, which exclude us to validate the effect of extreme smoking (30+ cigarettes per day) on CBMN frequencies (53). As the nonexposed controls were medical staff, it could be imagined that their socioeconomic status may differ from the coke-oven workers. However, the nonexposed controls worked in the hospital for occupational diseases affiliated to the steel company, so the average income and education degree of these medical staff were only slightly higher than those of coke-oven workers. At present, no direct evidence suggested that the socioeconomic status could influence CBMN frequencies. Therefore, the socioeconomic status may not be a critical factor influencing the relationship between coke-oven emission exposure and CBMN frequencies.

The distributions of *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1*, *GSTP1*, *mEH*, *NQO1*, and *NAT2* genotypes were similar in non–coke-oven workers and coke-oven workers and consistent with those described in the literature for the Chinese population (55, 56), which suggested no selection bias for the subjects' enrolment in terms of genotypes.

However, coke-oven workers with slow mEH genotype/phenotype had significantly lower CBMN frequencies than those coke-oven workers with high mEH genotype/phenotype with adjustment for other covariates, which was consistent with the reported findings that slow mEH genotype/phenotype was associated with lower levels of DNA adducts, urinary mutagenicity, and BPDE-serum albumin adducts (16, 26) and decreased lung cancer risk in heavy smokers (43). In an *in vitro* study using peripheral blood lymphocytes from 38 healthy donors, Salama et al. (46) found that the His^{113} allele (slow allele) of *mEH* was associated with significantly reduced frequency of B(a)P-induced chromosomal aberration and that the high mEH phenotype had significantly higher B(a)P-induced chromosomal aberration frequency than did all other genotypes. Although additional studies are still needed to provide a clear mechanistic explanation for our findings, it seemed that individuals with the His^{113} allele or low mEH activity phenotype had reduced ability to activate the *trans*-7,8-diol to the ultimate carcinogenic BPDE and hence had a low susceptibility to the genotoxic effects of PAHs.

The superfamily of glutathione *S*-transferase, mainly μ and π, catalyzes transfer of reduced glutathione to PAH-derived epoxides, hydroxides, etc., and homozygous deletion of *GSTM1* causes a loss of GSTM1 enzyme activity (57). In our study, the *GSTM1 null* genotype was associated with an increased CBMN frequencies in coke-oven workers, although the difference was not statistically significant (P = 0.12) but consistent with the findings in an *in vitro* study, in which the *GSTM1 null* genotype was found to be associated with a significantly ($P < 0.02$) increased frequency of chromosomal aberration induced by B(a)P (46). The *GSTM1 null* genotype had been found to be associated with increased levels of DNA adducts (15, 17, 19, 20, 22), BPDE-serum albumin levels (21), urinary mutagen excretion (25), and increased risk of lung cancer (58). These results combined with ours provided evidence to support the influence of *GSTM1* genotypes on biological effects of exposure in occupationally PAH-exposed populations.

GSTP1 is another important glutathione *S*-transferase responsible for the metabolism of PAHs. In our study, the coke-oven workers homozygous for the *GSTP1* Val^{104} allele had significantly higher CBMN frequencies than those with the Ile^{104} allele. This finding is also consistent with previous reports. The *GSTP1* Val^{104} allele had been found to be associated with increased BPDE-DNA adducts in coke-oven workers (16) and increased urinary 8-hydroxydeoxyguanosine levels in pot-room workers (27). These findings combined with ours suggested that, when highly exposed to PAHs as seen in coke-oven workers, the subjects with the *GSTP1* Val^{104}/Val^{104} genotype may be more likely to be prone to genotoxicity than those without this genotype. Interestingly, in our study, the non–coke-oven workers homozygous for the *GSTP1* Val^{104} allele had reduced CBMN frequencies. Because the sample size was too small for the *GSTP1* Val^{104}/Val^{104} genotype in non–coke-oven workers used as the reference group (n = 2), whether this finding was only a chance finding or of mechanistic significance needs to be validated in further studies with larger sample sizes.

CYP1A1 polymorphism is often considered to be associated with enhanced inducibility, leading to higher enzymatic activity that activates precarcinogens. The *CYP1A1* variant genotype has been found to be associated with higher levels of DNA adducts in coke-oven workers (15, 17), increased tail inertias of single cell agarose electrophoresis in pot-room workers (27), and increased sister chromatid exchange frequencies in traffic policemen (28). Although we did not find any relationship between *Ile/Val* polymorphism in exon 7 of the *CYP1A1* and CBMN frequency, we did observed that the PAH exposure level was higher in coke-oven workers (urinary 1-OHP, 12.0 μmol/mol creatinine) than in non–coke-oven workers (0.7 μmol/mol creatinine). In such a

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condition, the metabolic pathway of PAHs catalyzed by CYP1A1 may be saturated and the effect of the *CYP1A1* polymorphism on the CBMN frequency may be insignificant. However, this speculation needs to be validated in larger studies.

We did not find any significant associations between *GSTT1*, *NQO1*, *CYP2E1*, and *NAT2* polymorphisms and CBMN frequencies, which suggested that the formation of CBMN may not be related to the pathways catalyzed by these enzymes. Another possibility is that our study did not have the power to detect small differences in CBMN frequencies among or between genotypes of these metabolic genes in coke-oven workers exposed to high level of PAHs.

The joint effect between the high *mEH* phenotype and the *GSTM1 null* genotype on CBMN frequencies was also found, which suggested that subjects with high ability to transform *trans*-7,8-diol to ultimate carcinogenic BPDE and with low ability to detoxify PAHs would be at a greater risk for chromosomal damage when exposed to a high level of PAHs. In the present study, the gene-environment interactions between occupational PAH exposure and *mEH* phenotypes or combinations of *mEH* phenotypes and *GSTM1* genotypes were also evident, which suggested that the gene-environment interactions between PAH exposure and polymorphism of certain metabolic genes were important mechanism for the PAH-induced chromosomal damage.

In conclusion, our findings indicate that polymorphisms in *mEH*, *GSTP1*, and *GSTM1* may play significant roles in human sensitivity to the genotoxic effects of PAH exposure. However, our study had limitations due to a relatively small sample size that was not big enough to delineate the effect of rare polymorphisms, such as the *GSTP1* Val^{104}/Val^{104} genotype. Several important events involved in the CBMN formation (e.g., cell cycle control and DNA repair) were not a part of this study and should be investigated in further studies.

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