Short Communication

Cytochrome *P450 1A1 Msp*I Polymorphism and Urinary 1-Hydroxypyrene Concentrations in Coke-Oven Workers[1]

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Abstract

Coke-oven workers are regularly exposed to a high concentration of the benzene-soluble fraction (BSF) of total particulates, which are comprised mainly of polycyclic aromatic hydrocarbons (PAHs). A metabolite of pyrene, 1-hydroxpyrene (1-OHP), is readily measured in the urine of exposed individuals. Epidemiological studies have shown that *P4501A1* (*CYP1A1*) genotypes are associated with PAH-related lung cancer risk. The purpose of this study was to investigate whether *CYP1A1 Msp*I genotypes modulate the relationship of individual occupational exposure to air BSF to urinary 1-OHP concentrations among coke-oven workers. We monitored individual breathing zone air BSF over 3 consecutive days in 80 coke-oven workers in Taiwan from August 1995 to February 1996. Exposure was also dichotomized by work area (topside oven workers and sideoven workers). Preshift urine on the morning of day 1 and postshift urine on the afternoon of day 3 were measured by fluorescent spectrophotometry, and blood samples were analyzed to determine the relative distributions of *CYP1A1 Msp*I polymorphisms. The frequency of the *Msp*I homozygous variant genotypes of *CYP1A1* was 15%. Multiple linear regression showed significant effects of individual occupational exposure to air BSF and preshift 1-OHP on postshift urinary 1-OHP concentrations ($P = 0.002$ and $P < 0.001$, respectively). After adjusting for preshift 1-OHP concentrations and air BSF, subjects with the homozygous variant genotype have a 2-fold higher postshift 1-OHP levels than the combined wild-type and heterozygous ($P = 0.04$). In addition, a positive trend was found in postshift 1-OHP and across-shift change of 1-OHP (postshift 1-OHP − preshift 1-OHP) in decreasing order, as follows: topside oven workers with the homozygous variant trait, topside oven workers with the heterozygous variant trait, sideoven workers with the homozygous variant trait, and sideoven workers with the heterozygous trait ($P < 0.001$). We conclude that *CYP1A1 Msp*I variant genotype can modify the metabolism of PAHs in coke-oven workers.

Introduction

COE[3] are comprised mainly of PAHs. Given the large number of constituent chemical agents in COE, the Occupational Safety and Health Administration chose to use the BSF of total particulates as representative of COE and set a permissible exposure limit (8-h time-weighted average) of 150 $\mu g/m^3$ (1). Coke-oven workers are regularly exposed to PAHs in the coking process. Although PAHs are present in complex mixtures of >100 different compounds in the vicinity of coke-oven areas (2), our earlier study has demonstrated that urinary 1-OHP, a metabolite of pyrene, is a good index of external ambient exposure to BSF in coke-oven workers (3).

To date, researchers have presumed that pyrene is metabolized to 1-OHP by two broad categories of enzymes: phase I enzymes, such as P4501A1, which catalyzes oxidative reactions (4, 5); and phase II enzymes, such as uridine diphosphoglucuronosyltransferase, which catalyzes conjugative reactions of oxidative pyrene (5, 6). The *CYP1A1* gene codes for the AHH enzyme that is closely associated with the metabolism of PAH carcinogens (7). Few studies have examined the effect of *CYP1A1* polymorphisms on modification of the metabolism of PAHs (8). Merlo *et al.* (8) studied traffic police officers who were exposed to ambient PAHs in an urban area. They found that police officers who smoked <15 cigarettes/day and who had the combined homozygous and heterozygous variant traits of *CYP1A1 Msp*I had relatively higher postshift urinary 1-OHP levels than did those with wild-type genotype. However, there was no effect of genotype was found in nonsmokers and smokers who smoked >15 cigarettes/day. In this study, we investigated whether *CYP1A1 Msp*I polymorphism influences the metabolism of pyrene by measuring 1-OHP in urine in coke-oven workers who were exposed to relatively high concentrations of COE.

Materials and Methods

Subjects. The study was approved by the Institute Review Board of the Harvard School of Public Health, and the study population has been described elsewhere (3, 9). The subjects consisted of 80 coke-oven workers who were used in the older of two coke-oven plants of a large steel company in southern Taiwan.

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[3] The abbreviations used are: COE, coke-oven emissions; PAH, polycyclic aromatic hydrocarbon; 1-OHP, 1-hydroxypyrene; AHH, aryl hydrocarbon hydroxylase; ALT, alanine aminotransferase.

Table 1 Demographic data by *CYP1A1 Msp*I polymorphism among coke-oven workers

	No. of *AA/Aa*[a] (%) (n = 68)	No. of *aa* (%) (n = 12)
Age (yr)		
<38	24 (35)	4 (33)
38–43	23 (34)	3 (25)
>43	21 (31)	5 (42)
Quetelet's index (kg/m^2)		
<22	19 (28)	2 (17)
22–25	26 (38)	6 (50)
>25	23 (34)	4 (33)
Residence distance from company (km)		
≤5	23 (34)	3 (25)
>5	45 (66)	9 (75)
Alcohol consumption (g/week)		
Never and former	45 (66)	7 (58)
Current		
≤120	11 (16)	4 (33)
>120	12 (18)	1 (9)
Viral infection[b]		
No	57 (84)	8 (67)
Yes	11 (16)	4 (33)
Serum ALT (units/liter)[c]		
≤39	54 (79)	6 (50)
>39	14 (21)	6 (50)
Smoking status[d]		
No	33 (49)	3 (25)
≤3 packs	11 (16)	2 (17)
>3 packs	24 (35)	7 (58)
Consumption of roasted meat[d]		
No	64 (94)	11 (92)
Yes	4 (6)	1 (8)
Dermal exposure to coal tar (days)[d]		
No	6 (9)	3 (25)
1–3	11 (16)	1 (8)
>3	51 (75)	8 (67)
Regular use of respirators		
No	21 (31)	7 (58)
Yes	47 (69)	5 (42)

[a] *AA*, homozygous wild type; *Aa*, heterozygous; *aa*, homozygous variant.
[b] No viral infection: both hepatitis B antigens (−) and anti-hepatitis C antibodies (−).
[c] Reference from the clinic of the company.
[d] Information was reported in the week prior to urine specimen collection.

Ambient BSF, Questionnaire Information, Urinary 1-OHP, and Laboratory Tests. Personal breathing-zone air samples for BSF were collected by battery-operated personal air sampling pumps for 3 consecutive days between August 1995 and February 1996 (9). Sample collection began after 1.5 or 2 days away from the workplace, and samples were analyzed using Occupational Safety and Health Administration analytical methods (10). Exposure was also categorized by work area (topside oven workers and sideoven workers; Ref. 11). The lowest detection limits and reliable quantitation limits of the overall procedure were 2.1 and 10.9 μg, respectively. The results below the detection limit were assigned a value of 1.0 μg, which was half the detection limit (9).

A self-administered questionnaire was given during day 2 or 3 of the personal air samplings. Subjects reported age, height, weight, distance of residence from the company, alcohol consumption, smoking status, consumption of roasted meat, dermal exposure to coal tar, and regular use of respirators. Given that the half-life of urine excretion of 1-OHP in workers ranges from 6 to 35 h (12), information on smoking status, consumption of roasted meat, and dermal exposure to coal tar were reported for the week prior to urine specimen collection.

Spot urine samples were collected just before the shift in the morning of day 1 and just after the shift in the afternoon of day 3 during the air sampling (3). Urine samples were measured for 1-OHP by high-performance liquid chromatography-fluorescent detector, described in detail elsewhere (11). The limit of detection of urinary 1-OHP was 0.42 ng/ml, determined as the mean + 3 SD of 10 urine samples from unexposed urban residents. The measurements below a concentration of 0.42 ng/ml were set at 0.21 ng/ml, which is half the detection limit (3). The concentration of urine 1-OHP was presented in units of μg/g creatinine.

Blood samples were obtained for profiles of liver function, hepatitis B surface antigen, and anti-hepatitis C antibody from the antecubital vein in the morning following 3 consecutive days of exposure measurements. Subjects were instructed not to eat for at least 6 h prior to blood sampling.

Liver function profiles including aspartate aminotransferase, ALT, *r*-glutamyl transpeptidase, alkaline phosphatase, and total bilirubin were determined by a Hitachi Autoanalyzer 7050 (Tokyo, Japan) on the same day. Hepatitis B surface antigen and anti-hepatitis C were evaluated by commercial ELISAs from Behringwerke AG (Marburg, Germany) and General Biologicals Corp. (Taipei, Taiwan), respectively, within 2 weeks following blood drawings (9).

***CYP1A1 Msp*I Genotyping.** DNA was extracted from whole blood samples using phenol-chloroform extraction. The *Msp*I polymorphism in *CYP1A1* 3′ flanking region was identified by the method of Hayashi *et al.* (13) with modification and determined by PCR and RFLP. DNA sample was amplified with two primers: 5′-CAGTGAAGAGGTGTAGCCGC-3′ (upstream) and 5′-TAGGAGTCTTGTCTCATGCC-3′ (downstream; Perkin-Elmer, Taipei, Taiwan). PCR amplification was carried out with 1 μg of DNA in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM $MgCl_2$, 0.3 mM dNTPs (Boehringer Mannheim GmbH, Mannheim, Germany), 0.2 μM each primer, and 1.5 units of Taq polymerase (AmpliTaq; Perkin-Elmer) in a total volume of 50 μl. Amplification was performed with an initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 61°C for 1 min, and 72°C for 1 min and a final extension at 72°C for 7 min.

Ten-μl amplification product was digested with *Msp*I (New England Biolabs, Beverly, MA) and analyzed on a 2.5% agarose gel. When a *Msp*I restriction site was present, the 340-bp fragment was digested into two lengths: 140 and 200 bp. Homozygous wild-type individuals lacked the 140- and 200-bp fragments (*AA*), heterozygous individuals had three bands (*Aa*), and homozygous rare allele (variant) individuals lacked the large parent band and had the smaller bands (*aa*).

Statistical Methods. Gene frequencies and tests for Hardy-Weinberg equilibrium were calculated first. The distribution of demographic data by *CYP1A1* polymorphisms (*AA* and *Aa versus aa*) was examined. Multiple linear regression models were performed to assess the association between the *CYP1A1* polymorphisms (*AA* and *Aa versus aa*) and postshift 1-OHP levels after adjusting for ambient average BSF exposures, preshift 1-OHP concentrations, and other covariates (3). Covariates in the models included age (tertiles), Quetelet's index (tertiles), residence distance from the company, alcohol consumption, viral infection, serum ALT, smoking status, consumption of roasted meat, dermal exposure, and regular use of respirators. Ambient average BSF, preshift and postshift uri-

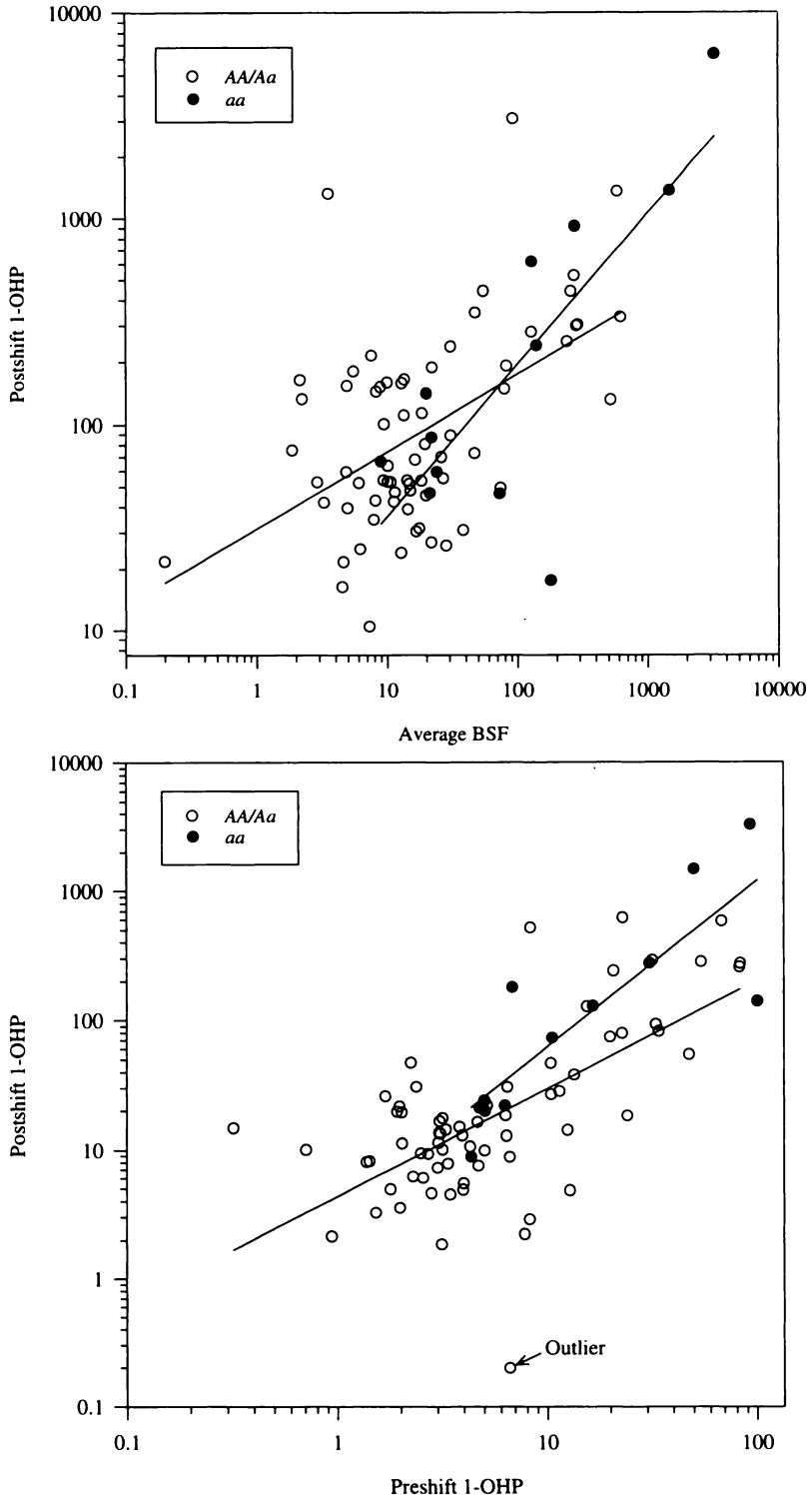

Fig. 1. The relationship of urinary postshift 1-OHP (μg/g creatinine) with the ambient average BSF of total particulates ($\mu g/m^3$) and preshift 1-OHP (μg/g creatinine) by genotypes in the 80 coke-oven workers.

nary 1-OHP concentrations were $\log_{10}$-transformed to normalize their distributions before regression analysis.

Postshift 1-OHP and across-shift change of 1-OHP (postshift in the afternoon of day 3 − preshift in the morning of day 1) were also categorized by genotype (*AA/Aa versus aa*) and work area (topside oven *versus* sideoven workers) to evaluate the gene-environment interaction. The regression models included interaction factors between genotype and work area, using sideoven workers with *AA/Aa* genotypes as the reference group, and other covariates. Postshift 1-OHP and across-shift

Table 2 Predictors of $\log_{10}$-transformed postshift 1-OHP concentrations by multiple linear regressions among 80 coke-oven workers

Model	Determinants	β (SE β)[a]	*P*	r^2
A	Genotype[b]	0.72 (0.21)	0.001	0.13
B	BSF[c]	0.39 (0.13)	0.002	0.56
	Preshift 1-OHP[d]	0.74 (0.13)	<0.001	
C	Genotype	0.33 (0.16)	0.04	0.58
	BSF	0.37 (0.12)	0.003	
	Preshift 1-OHP	0.69 (0.13)	<0.001	
D	Genotype	0.29 (0.16)	0.07	0.59
	BSF	0.36 (0.12)	0.005	
	Preshift 1-OHP	0.74 (0.13)	<0.001	
	Alcohol-1[e]	0.03 (0.14)	0.85	
	Alcohol-2[e]	−0.17 (0.16)	0.27	

[a] SE β, SE of β coefficient.
[b] Homozygous variant *vs.* wild-type/heterozygous.
[c] $\log_{10}$-transformed ambient average BSF of total particulates.
[d] $\log_{10}$-transformed preshift 1-OHP.
[e] Alcohol-1, current drinkers reporting ≤120 g/week *vs.* never and former drinkers; Alcohol-2: current drinkers reporting >120 g/week *vs.* never and former drinkers.

Table 3 Relationship of $\log_{10}$-transformed postshift 1-OHP and across-shift change of 1-OHP with genotype and work area by multiple linear regressions among 80 coke-oven workers, using sideoven workers with the *AA/Aa* genotype as the reference group[a]

Dependent variables	Crude analyses		Adjusted analyses[c]	
	β (SE β)[b]	*P*	β (SE β)[b]	*P*
Postshift 1-OHP				
Sideoven workers, *AA/Aa*				
Sideoven workers, *aa*	0.44 (0.20)	0.03	0.48 (0.20)	0.02
Topside oven workers, *AA/Aa*	1.11 (0.15)	<0.001	1.13 (0.15)	<0.001
Topside oven workers, *aa*	1.63 (0.23)	<0.001	1.70 (0.24)	<0.001
Across-shift change of 1-OHP[d]				
Sideoven workers, *AA/Aa*				
Sideoven workers, *aa*	0.45 (0.22)	0.05	0.47 (0.23)	0.05
Topside oven workers, *AA/Aa*	1.08 (0.17)	<0.001	1.10 (0.18)	<0.001
Topside oven workers, *aa*	1.62 (0.26)	<0.001	1.67 (0.27)	<0.001

[a] Genotypes are defined in Table 1.
[b] SE β, SE of β coefficient.
[c] Adjusting for serum ALT and regular use of respirators.
[d] Six negative values were excluded in the models.

change of 1-OHP were also $\log_{10}$-transformed before regression analysis. Trend tests were used to test postshift 1-OHP and across-shift change of 1-OHP in topside oven workers with the *aa* trait, in topside oven workers with the *AA/Aa* trait, in sideoven workers with the *aa* trait, and in sideoven workers with the *AA/Aa* trait. The data were analyzed using the SAS statistical package (14). All *P*s reported are two-sided.

Results

The prevalences of the homozygous wild-type (*AA*), the heterozygous variant (*Aa*), and the homozygous variant (*aa*) were 42.5% (34 of 80), 42.5% (34 of 80), and 15.0% (12 of 80), respectively. The gene frequency of the *Msp*I RFLP was 36%. The distribution of the different genotypes among the 80 coke-oven workers closely conformed to Hardy-Weinberg expected frequencies ($\chi^2 = 0.47$; $P = 0.79$).

Demographic data categorized by genotypes are shown in Table 1. The homozygous variant genotype subjects had a significantly higher prevalence of serum ALT abnormality ($\chi^2 = 4.71$; $P = 0.03$) and reported a lower frequency of regular respirator use ($\chi^2 = 3.38$; $P = 0.07$) than the combined wild-type and heterozygous variant genotypes. The distribution of other covariates did not differ significantly between these two groups.

We first investigated the effect of various genotypes on postshift 1-OHP. The heterozygous variant group had a slight but not statistically significant increase in postshift 1-OHP compared with the wild-type group. Therefore, we combined the wild-type and heterozygous variant for the remaining analysis.

The association of urinary postshift 1-OHP with ambient average BSF and preshift 1-OHP by genotype is shown in Fig. 1. The homozygous variant genotype group had significantly higher postshift 1-OHP than did the combined (wild-type and heterozygous variant) group after adjusting for ambient BSF or preshift 1-OHP ($P = 0.008$ or 0.03, respectively). When we excluded one outlier in the combined wild-type and heterozygous, the association of genotypes with urinary postshift 1-OHP was slightly strengthened (data not shown).

Multiple regression analysis indicated that occupational exposures to ambient BSF and preshift 1-OHP were significant predictors of postshift urinary 1-OHP concentrations (Table 2, model B). After adjusting for ambient BSF and preshift 1-OHP concentrations, subjects with the homozygous variant genotype had significantly higher postshift 1-OHP levels than did those with the combined genotypes ($P = 0.04$). The homozygous variant genotype resulted in an ~2-fold increase in postshift 1-OHP concentrations (Table 2, model C). Fig. 1 indicates that interactions between ambient BSF and genotype, as well as preshift 1-OHP and genotype, may exist, so we examined interactions in the model C. However, neither interaction variables showed any significant effect on postshift 1-OHP. Alcohol consumption decreased the effect of genotype on postshift 1-OHP ($P = 0.07$; Table 2, model D), although alcohol consumption itself was not a significant predictor of postshift 1-OHP. Other covariates, including age, Quetelet's index, residence distance from the company, hepatic viral infection, serum ALT abnormality, regular use of respirators, smoking status, consumption of roasted meat, and dermal exposure to coal tar, slightly increased the effect of genotype on postshift 1-OHP.

To evaluate possible gene-environment interaction, we used sideoven workers with the combined wild-type and heterozygous variant as the reference group. We found that postshift 1-OHP concentrations were significantly elevated both in sideoven workers with *aa* ($P = 0.02$) and topside oven workers with *AA/Aa* ($P < 0.001$) and *aa* ($P < 0.001$) genotypes after adjusting for serum ALT and regular use of respirators (Table 3). The results were similar even after further adjustment for smoking status and alcohol consumption (data not shown). We excluded six negative values in across-shift change of urinary 1-OHP concentrations in sideoven workers with *AA/Aa* genotypes (range, −1.3 to −8 μg/g creatinine) and assessed the gene-environment interaction. Similar results were found in across-shift change of 1-OHP concentrations (Table 3). Fig. 2 shows positive trends in postshift and across-shift change of 1-OHP in decreasing order from topside oven workers with the *aa* genotype, topside oven workers with *AA/Aa* genotypes, sideoven workers with the *aa* genotype, and sideoven workers with the *AA/Aa* genotype ($P < 0.001$).

Discussion

We observed that the *CYP1A1 Msp*I polymorphism has a modulating effect on the metabolism of 1-OHP in coke-oven work-

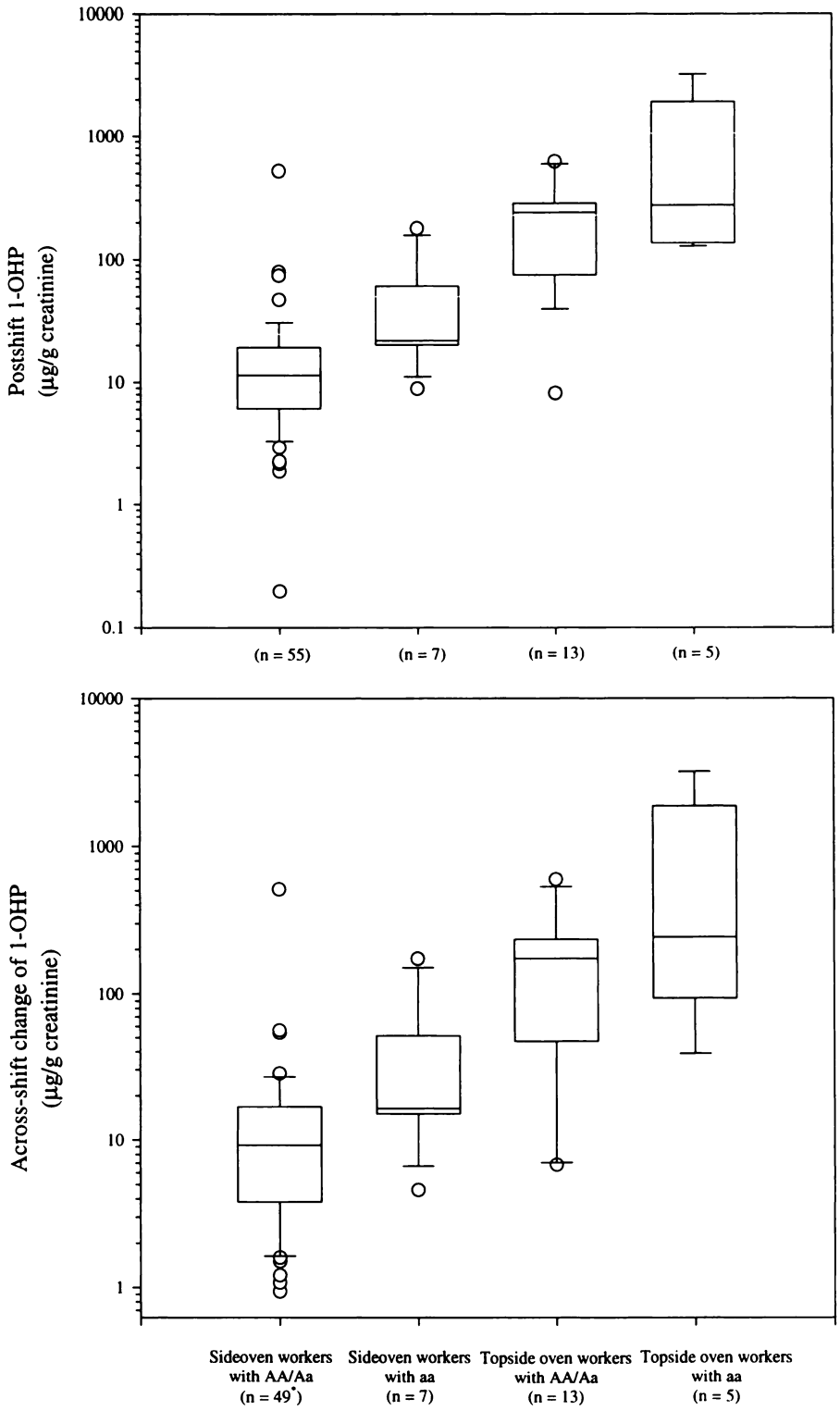

Fig. 2. Boxplot of postshift and across-shift change of 1-OHP by work area and genotype among 80 coke-oven workers. *Bars*, 10th–90th percentiles; *boxes*, 25th–75th percentiles; *horizontal lines*, medians. *, six negative values were excluded.

ers. Individuals with the homozygous variant genotype had significantly higher postshift urinary 1-OHP than did wild-type and heterozygous genotypes combined, after adjusting for ambient BSF and preshift 1-OHP concentrations.

The gene frequency of the *Msp*I RFLP in our study was 36%, which was close to the 30% reported in Japanese (15) but quite different from the 12% reported in the Finnish populations (16) and the 13% reported in African-Americans (17). Although different allele frequencies were observed in different populations, this gene had been demonstrated to be associated

with lung cancer in both Japanese and Caucasian populations (18, 19). Xu *et al.* found that the homozygous *Msp*I variant trait (1% in both lung cancer patients and controls) is infrequent in Caucasians. They combined the heterozygous and homozygous variants to examine the lung cancer risk and found a significant association between the combined ones and lung cancer (18). Nakachi *et al.* (19) reported that the *CYP1A1 Msp*I homozygous variant genotype was significantly associated with squamous cell lung cancer in Japanese populations. These data may indicate interethnic differences in the prevalence and function of *CYP1A1* polymorphisms; hence, caution is warranted in extrapolating the association of genetic polymorphisms with the occurrence of a particular form of cancer from one ethnic group to another (20).

Kiyohara *et al.* (21) examined the relationship between AHH inducibility (3-methylcholanthrene-induced AHH activity/noninduced AHH activity) and the frequency of *CYP1A1 Msp*I and exon 7 polymorphisms in peripheral lymphocytes in 84 healthy Japanese male subjects *in vitro*. They found that age-adjusted AHH inducibilities (mean ± SE) of the wild-type, heterozygous, and homozygous variants of the *CYP1A1 Msp*I gene were 4.89 ± 0.36, 4.82 ± 0.29, and 13.61 ± 1.44, respectively, and the homozygous variants had significantly higher AHH inducibility than did the combined wild-type and heterozygous genotypes. However, no association was found between AHH inducibility and CYP1A1 exon 7 polymorphisms. In contrast, Crofts *et al.* (7) measured gene expression levels and AHH enzymatic activity levels of the *Msp*I and exon 7 polymorphisms in mitogen-stimulated lymphocytes in 51 healthy subjects including 22 Asians, 23 Caucasians, and 6 African-Americans. They reported that subjects with the exon 7 polymorphism (variant alleles; $n = 12$) had a relative level of *CYP1A1* mRNA inducibility (induced/basal) of 9.0 ± 1.7 *versus* 5.9 ± 0.6 in people with the wild-type alleles ($n = 39$); however, variant genotypes at the *Msp*I site had no effect on *CYP1A1* gene induction. In this study, we found the heterozygous variant had a slight but nonsignificant increase in postshift 1-OHP compared with wild type. Our result is consistent with the findings in Japanese populations (20, 22).

Although total PAHs include particulate and gaseous phases and, in our study, we only measured individual ambient BSF (22), the distribution of air PAHs had been found to be relatively stable in individual workplaces (23, 24). Ny *et al.* (25) found correlations of 0.84, 0.81, and 0.80 between air BSF and air total PAHs, pyrene, and benzo(*a*)pyrene, respectively, among 38 workers in a Soderberg Netherlands potroom. These high correlations suggest that air sampling of BSF can be representative for individual and total PAH profiles. If any misclassification exists in the exposure assessment, it will dilute our findings and bias toward to the null.

1-OHP is a major metabolite of pyrene (26), but 1-OHP can be further metabolized to 1,6- and 1,8-dihydroxypyrene in the urine or excreted via bile of both rats and rabbits treated with pyrene (4, 27). In our study, we did not measure other metabolites of pyrene in urine and, hence, could not examine the possible association between genotype and these metabolites.

The slopes of the homozygous variant genotype subjects and the combined wild-type and heterozygous subjects in the association of postshift 1-OHP with ambient BSF and preshift 1-OHP suggested a gene-environment interaction (Fig. 1). This pattern was also evident when we categorized postshift 1-OHP and across-shift change of 1-OHP by genotype (the combined wild-type and heterozygous variant traits *versus* homozygous variant trait) and work area (topside oven workers *versus* sideoven workers; Fig. 2).

We conclude that the *CYP1A1 Msp*I polymorphism can modify the metabolism of 1-OHP in coke-oven workers. The homozygous variant genotype resulted in an ~2-fold increase in postshift 1-OHP concentrations over the combined wild-type and heterozygous genotypes. Further studies should address the effect of this polymorphism on the association of exposure to COE with biologically effective dose measures, such as DNA adducts in the peripheral blood of coke-oven workers. In addition, a larger population should be examined to assess potential interactions of polymorphisms in both phase I and phase II metabolic genes with either preshift 1-OHP or ambient BSF on postshift 1-OHP.

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