Association of Aryl Hydrocarbon Receptor Gene Polymorphisms and Urinary 1-Hydroxypyrene in Polycyclic Aromatic Hydrocarbon–Exposed Workers

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Abstract

Polycyclic aromatic hydrocarbons (PAH) in coke oven emissions could cause lung cancer in human. Individual's genotype of the metabolic enzymes and early biological changes were known to be associated with the susceptibility of cancer development. Knowledge of metabolic gene polymorphisms, which affect on the urinary 1-hydroxypyrene (1-OHP), could benefit us in understanding the interindividual difference in the mechanism of PAH-induced carcinogenesis. In this study, we investigated the association of aryl hydrocarbon receptor (*AhR*) gene polymorphisms and urinary 1-OHP. One hundred forty-seven workers exposed to PAH and 69 nonexposure workers were recruited. Seven tagging single nucleotide polymorphisms in *AhR* gene were selected by pariwise r^2 method and minor allele frequency cutoff of 0.05 from Chinese genotype data in HapMap project. These seven tagging single nucleotide polymorphisms were genotyped by PCR-based methods. Multivariate analysis of covariance revealed that the levels of 1-OHP in PAH-exposed workers carrying genotype *CT* were lower than workers carrying wild genotype *TT* at loci rs10250822 and rs2282885 of *AhR* gene (P = 0.032 and 0.044, respectively). In PAH-exposed workers, the urinary 1-OHP levels showed a linear correlation (P_{trend} = 0.041) with the genotypes at locus rs2282885, especially in low and moderate exposure groups. In contrast, no significant association was found between urinary 1-OHP level and *AhR* genotypes among nonexposed workers. Our findings indicated that polymorphisms of *AhR* gene were associated with the level of 1-OHP among PAH-exposed workers, suggesting that AhR-mediated signaling might contribute to individual susceptibility to PAH exposure. (Cancer Epidemiol Biomarkers Prev 2008;17(7):1702–8)

Introduction

Polycyclic aromatic hydrocarbons (PAH), as the human carcinogens, are primary compounds in coke oven emissions generated in the coking process (1). Both epidemiologic and experimental studies show that coke oven emissions are causally associated with lung cancer among coke-oven workers (2, 3). PAH are present in complex mixtures of >100 different compounds at workplaces in the vicinity of coke ovens. The pyrene appears at relatively high concentrations in coke oven emission mixtures and is almost exclusively metabolized to 1-hydroxypyrene (1-OHP), which accounts for ~90% of the total urinary excretion of pyrene in humans (4). Earlier study showed that urinary l-OHP, a metabolite of pyrene, is considered as a golden marker of external ambient exposure to benzene-soluble matter in coke-oven workers (5-7). At present, 1-OHP has been validated as a biomarker of occupational exposure to PAH, especially in coke production settings (8). As a biomarker assessing internal exposure to PAH, urinary 1-OHP may be affected by both environmental PAH exposure level and the individual genetic susceptibility to PAH. Previously, our studies have shown that *microsomal epoxide hydrolase* (*mEH*) gene polymorphisms affected urinary 1-OHP levels after PAH exposure, but *CYP1A1* and *GSTM1* gene polymorphisms examined did not correlate with 1-OHP levels (9). Given the fact that *mEH* is not a primary enzyme mediating the pyrene metabolism, thus far, the genetic polymorphisms that associate with the susceptibility variation to PAH remained elusive.

PAH bind to aryl hydrocarbon receptor (AhR) and transfer to target tissue or organ (10). AhR is a ligand-activated transcription factor basic helix-loop-helix protein that heterodimerizes with the basic helix-loop-helix protein aryl hydrocarbon nuclear translocator, forming a complex that binds to xenobiotic regulatory elements in target gene enhancers and involved in reactivation of xenotoxic metabolism (11). In presence of PAH, the expression of CYP1A1, a member of cytochrome *P*450 (CYP450), which catalyses the conversion of PAH into proximate and ultimately into carcinogen, was induced

Received 11/18/07; revised 4/23/08; accepted 5/6/08.

Grant support: National Key Basic Research and Development Program of China 2002CB512903, National Natural Science Foundation of China 30400348 and 30625031, and Doctoral Program of Higher Education of China 20060558024.

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doi:10.1158/1055-9965.EPI-07-2812

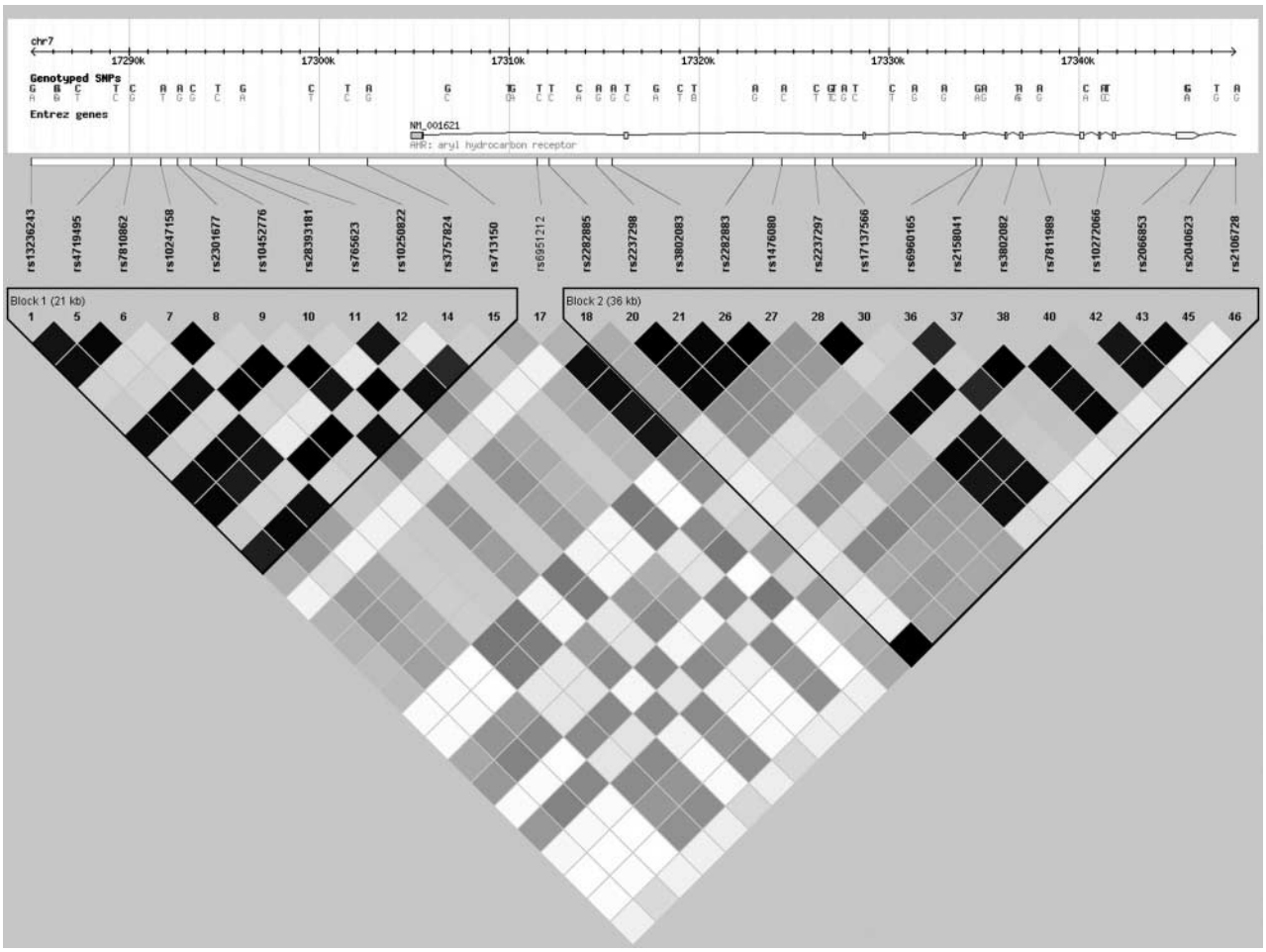

Figure 1. Linkage disequilibrium plot for SNPs in the *AhR* gene. The grade of each diamond is presented as R square between pairs of SNPs, estimated based on 45 unrelated individuals from Beijing in HapMap project. The black-to-white gradient reflects higher-to-lower linkage disequilibrium.

(12, 13). Thus, we hypothesize that the *AhR* gene plays an important role in regulating the PAH metabolism and the urinary 1-OHP level may be affected by variation of the *AhR* gene. To date, there is limited information regarding *AhR* gene polymorphisms and the metabolism of pyrene. The goal of the study is to fill in the gap that has hampered the application of PAH biomarkers in assessing the internal burden of PAH exposure. In this study, we analyzed seven tagging single nucleotide polymorphisms (SNP) in *AhR* gene selected from Chinese genotype data in HapMap Project and their correlations with the levels of urinary l-OHP in coke-oven workers.

Materials and Methods

Subjects and Sample Collection. This study was approved by the Research Ethic Committee of the National Institute for Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention. Details of the study population have been described previously (14, 15). In brief, the exposure group consisted of 147 workers from the same coke oven and the nonexposure control group comprised 69 medical staffs. According to the literature (6, 16), 147 exposure workers were classified into top-oven workers (n = 31), side-oven workers (n = 76), and bottom-oven workers (n = 40), respectively. The information regarding age, gender, smoking and drinking status, charbroiling diet status 2 days before urine collection, and history of occupational exposure were collected from questionnaires. After informed consent was obtained, venous blood and 4-day-shift-end urine were collected for each subject.

PAH Exposure Information and Urinary 1-OHP Detection. The air levels of benzene-soluble matter and particulate-phase benzo(a)pyrene at workplace were measured ~1.5 month before urine and blood sample collection. Details of the PAH exposure information were described previously (9, 14). Urinary 1-OHP was measured by high-performance liquid chromatography with florescence detector as described by Jongeneelen et al. (17) with minor modification (18). The urinary 1-OHP concentrations were normalized by level of urinary creatinine (Cr) and expressed as μmol/mol Cr.

Table 1. Information about the RS numbers, location and function variation, primers sequences, PCR productions, restriction endonucleases, and fragment length for the seven tagging SNP of *AhR* gene

RS no.	SNP	Chromosomal location	Function variation	Forward primer
rs2066853	A/G	7p15	Nonsynonymous ($Arg^{554}Lys$)	The same to the reference (20) G1721A
rs10250822	C/T	7p15	Intron	5′-TGATGCTTGGTATGGGGTCTGAGTG-3′
rs1476080	A/C	7p15	Intron	5′-CCCTAAATGCCACATCTCTTCGTATC-3′
rs10247158	A/T	7p15	Intron	5′-TCCAGCCTTTTATGAGCCTATT-3′
rs7811989	A/G	7p15	Intron	5′-GTTTTCTTGTTACAAAGTCTGAACAC-3′
rs2282885	C/T	7p15	Intron	5′-AACTGCACTTGACTTGGATTACGCT-3′
rs6960165	A/G	7p15	Intron	5′-TTATAAATATTTGATATATTGGAGGG-3′

SNP Selection and Genotyping. Seven tagging SNP in *AhR* gene were selected with $r^2 > 0.8$ and minor allele frequency > 5% in Chinese population from HapMap project (19), including rs2066853, rs10250822, rs1476080, rs10247158, rs7811989, rs2282885, and rs6960165 (Fig. 1).

The genotypes of these seven tagging SNP among 216 subjects were determined by PCR-based methods. The genotype at locus rs2066853 was determined according to the protocol published previously (20); the other six tagging SNP were determined by PCR-RFLP. The primers for analyzing tagging SNP at loci rs10250822, rs1476080, rs10247158, rs7811989, rs2282885, and rs6960165 were designed by program of PCR-primer-introduced restriction analysis (21) and shown in Table 1. The PCR amplifications were done in a 30 μL volume containing 75 mmol/L Tris-HCl (pH 8.8), 200 mmol/L $(NH_4)_2SO_4$, 0.1% Tween 20, 166 mmol/L deoxynucleotide triphosphates, 2 mmol/L $MgCl_2$, 0.25 mmol/L each primer, 0.75 units Taq polymerase (Fermentas MBI), and 50 ng genomic DNA. The reactions were initialized by denaturation at 95°C for 5 min followed by 32 cycles of 30 s at 95°C, 30 s at optimal annealing temperature (60°C for rs10250822, 58°C for rs1476080, 61°C for rs10247158, 57°C for rs7811989, 61°C for rs2282885, and 57°C for rs6960165), 30 s at 72°C for extension, and a final elongation at 72°C for 10 min. The PCR production of these six tagging SNP was 412, 161, 551, 250, 377, and 232 bp, respectively, in length and subjected to restriction enzyme digestion by *Bsm*AI, *Bci*VI, *DrA*I, *BbV*I, *Bsp*HI, and *Bsm*FI (New England Biolabs), respectively (Table 1). For quality control, 10% of samples were genotyped a second-time and the concordance was 100%.

Statistical Analysis. Student's *t* test was used to compare age and the ln-transformed urinary 1-OHP concentrations between exposure workers and control group. The goodness-of-fit χ^2 test was used to examine whether the allele frequencies were in Hardy-Weinberg equilibrium. For multivariate analysis, the values of 1-OHP were ln-transformed to normalize the variance. The differences of urinary 1-OHP between genotypes of *AhR* gene were compared by multivariate analysis of covariance with adjustment for sex, age, cigarettes per day, and PAH external exposure categories in exposed and nonexposed groups separately. Multivariate linear regression was done for the trend test between urinary 1-OHP levels and genotypes with adjustment for the same variables as described. *P* values ≤ 0.05 were considered significant. All statistical analyses are done by two-sided criteria using Statistical Analysis System software (version 8.0; SAS Institute).

Results

General Demographic Data and PAH Exposure. The demographic information and PAH exposure information for this study population was described in detail previously (9, 14). In brief, the distributions of age, gender, alcohol consumption, and charbroiling diet status 2 days before collected urine were not significantly different between exposure workers and control group, except smoking status. Workers exposed to PAH during working time were divided into three groups (top, side, and bottom oven) according to working location. From the measurement of benzene-soluble matter and particulate-phase benzo(*a*)pyrene, it appeared to be top-oven > side-oven > bottom-oven > control in terms of benzo(*a*)pyrene concentration. The urinary 1-OHP of exposure workers was much higher than those of controls (12.22 ± 1.24 versus 0.74 ± 0.32 μmol/mol Cr).

Urinary 1-OHP and *AhR* Genotypes. The variant allele frequencies of *AhR* at loci rs2066853, rs10250822, rs1476080, rs10247158, rs7811989, rs2282885, and rs6960165 were 0.35, 0.43, 0.14, 0.31, 0.33, 0.24, and 0.24 among 216 subjects, respectively. The distributions of the seven polymorphisms were in Hardy-Weinberg equilibrium in exposed workers and controls (data not shown).

Table 2 lists the distribution of *AhR* genotypes and urinary 1-OHP levels of exposure workers and control group. With adjustment for sex, age, cigarettes per day, and external exposure categories, the multivariate analysis of covariance revealed that in exposure workers the *AhR* rs10250822 *CT* genotype exhibited significantly lower urinary 1-OHP levels than did the wild genotype *TT* (9.83 ± 1.05 versus 14.48 ± 1.29 μmol/mol Cr; $P = 0.032$). For the *AhR* rs2282885 polymorphism, *CT* genotype carriers exhibited significantly lower urinary 1-OHP levels than did the wild genotype *TT* (10.37 ± 1.01 versus 13.51 ± 1.23 μmol/mol Cr; $P = 0.044$). In contrast, there was no significant difference in urinary 1-OHP levels between *AhR* genotypes among nonexposure group ($P > 0.05$; Table 2). The results from multivariate linear regression analysis for the correlation of urinary 1-OHP levels with diverse *AhR* genotypes with adjustment for the same variables revealed that only *AhR* locus rs2282885 polymorphism was associated with levels of urinary 1-OHP ($P_{trend} = 0.041$; Table 2).

In addition, with stratification analysis for the locus rs2282885 among exposed workers, we also found that there was a significant difference with urinary 1-OHP levels in side-oven workers. *CT* genotype carriers exhibited significantly lower urinary 1-OHP levels than

Table 1. Information about the RS numbers, location and function variation, primers sequences, PCR productions, restriction endonucleases, and fragment length for the seven tagging SNP of *AhR* gene (Cont'd)

Reverse primer	PCR productions (bp)	Restriction endonucleases	Fragment length (bp)
The same to the reference (20) G1721A	333 and 493	None	*A(A)/G(G)* (333); β-actin (493)
5′-CCTCCGTGGGCTGAAGAATATGTGT-3′	412	*Bsm*AI	*TT* (412); *CT* (412, 219, 193); *CC* (219, 193)
5′-AAAGGGGATATGCTTTCCCTGG-3′	161	*Bci*VI	*AA* (161); *AC* (161, 139, 22); *CC* (139, 22)
5′-AGCGCAAATTTCACTAGTCAGG-3′	551	*Drd*I	*AA* (551); *AT* (551, 410, 141); *TT* (410, 141)
5′-TCCTCTTCAGAAATAAACACATAAAC-3′	250	*Bbv*I	*AA* (250); *AG* (250, 199, 51); *GG* (199, 51)
5′-AAGATAGCATTCAGACTGGCATTGG-3′	377	*Bsp*HI	*TT* (377); *CT* (377, 258, 119); *CC* (258, 119)
5′-GTGAGCTTAATAGAAATGCAAA-3′	232	*Bsm*FI	*GG* (232); *AG* (232, 208, 24); *AA* (208, 24)

did the wild genotype *TT* (P = 0.006). Moreover, multivariate linear regression analysis revealed that there was a decline trend of urinary 1-OHP in different genotype (from wild allele to variant allele) carrier at lower PAH exposure group (P_{trend} = 0.068 for bottom-oven workers and P_{trend} = 0.007 for side-oven workers; Fig. 2). Taken together, these findings showed that *AhR* activity determined by distinct genotypes affected the metabolism of PAH, which might be associated with the individual susceptibility to PAH exposure.

Discussion

PAH are ubiquitous in ambient air and in a certain occupational environment, particularly in the coke production. Epidemiologic studies indicate that occupational exposure to PAH was correlated well with levels of PAH metabolites such as 1-OHP in urine (22). It appeared that urinary l-OHP is the most widely used biomarker and that the analytical method is robust and nonlaborious (23). As a detoxified product of pyrene,

Table 2. Distribution of *AhR* genotypes and urinary 1-OHP levels

Genotypes	Nonexposure workers			Exposure workers		
	n	1-OHP levels (μmol/mol Cr, G ± S_G)	*P**	*n*	1-OHP levels (μmol/mol Cr, G ± S_G)	*P**
rs2066853	69			147		
GG	26	0.84 ± 0.32	Reference†	63	10.91 ± 1.20	Reference†
AG	30	0.71 ± 0.31	0.501	74	13.32 ± 1.14	0.146
AA	13	0.62 ± 0.37	0.288	10	8.97 ± 1.52	0.774
Trend‡			0.212			0.811
rs10250822	69			147		
TT	24	0.77 ± 0.36	Reference†	47	14.48 ± 1.29	Reference†
CT	35	0.72 ± 0.31	0.195	71	9.83 ± 1.05	0.032
CC	10	0.75 ± 0.30	0.976	29	13.79 ± 1.25	0.549
Trend‡			0.768			0.114
rs1476080	69			147		
AA	54	0.74 ± 0.31	Reference†	108	12.16 ± 1.14	Reference†
AC	13	0.81 ± 0.38	0.602	34	11.45 ± 1.48	0.516
CC	2	0.27 ± 0.15	0.019	5	9.86 ± 0.66	0.56
Trend‡			0.271			0.91
rs10247158	69			147		
AA	44	0.72 ± 0.29	Reference†	93	12.37 ± 1.15	Reference†
AT	22	0.84 ± 0.37	0.711	51	11.45 ± 1.30	0.328
TT	3	0.35 ± 0.27	0.226	3	7.18 ± 1.01	0.415
Trend‡			0.732			0.12
rs7811989	69			147		
GG	31	0.63 ± 0.30	Reference†	58	11.36 ± 1.41	Reference†
AG	33	0.87 ± 0.35	0.945	77	11.98 ± 1.03	0.383
AA	5	0.63 ± 0.19	0.352	12	14.48 ± 1.33	0.516
Trend‡			0.775			0.784
rs2282885	69			147		
TT	43	0.73 ± 0.36	Reference†	82	13.51 ± 1.23	Reference†
CT	21	0.80 ± 0.26	0.248	58	10.37 ± 1.01	0.044
CC	5	0.59 ± 0.31	0.49	7	9.41 ± 2.22	0.338
Trend‡			0.923			0.041
rs6960165	69			147		
AA	43	0.72 ± 0.33	Reference†	87	10.93 ± 1.24	Reference†
AG	23	0.77 ± 0.34	0.649	46	13.12 ± 1.20	0.289
GG	3	0.84 ± 0.21	0.363	14	14.73 ± 0.88	0.379
Trend‡			0.628			0.609

*Multiple analysis of covariance tests for differences in ln-transformed 1-OHP between genotypes with adjustment for sex, age, cigarettes per day, and PAH external exposure categories.
†Reference group for comparisons of ln-transformed 1-OHP concentration between genotypes.
‡Multivariate linear regression for the trend of urinary 1-OHP levels with genotypes with adjustment for sex, age, cigarettes per day, and PAH external exposure categories.

the level 1-OHP in urine was determined by both the environmental PAH exposure and the genetic variation of metabolic enzyme. In the present study, the coke-oven workers were exposed to a high level of PAH based on the stationery environmental monitoring, including air benzene-soluble matter and particulate-phase benzo(*a*)pyrene, and high level of urinary 1-OHP. The pyrene, the important component of coke oven emission mixtures, is almost exclusively metabolized to 1-OHP. The metabolic pathway from pyrene to 1-OHP mainly catalyzed by cytochrome *P*450 enzyme and UDP-glucuronosyltransferases (8). In general, in presence of pyrene and other PAH, the AhR can induce the expression of CYP1A1 and CYP2E1 (12, 13), leading to catalyze the conversion of pyrene into 1-OHP. Based on this pathway, it is apparent that *AhR* gene polymorphisms may play a role on interindividual differences in the metabolism of PAH in coke-oven workers.

Haploview is a well-used tool that generates marker quality statistics, linkage disequilibrium information, haplotype blocks, population haplotype frequencies, and single-marker association statistics and also directly accepts genotype data dumped from the Human HapMap Web site (http://www.hapmap.org; ref. 19). On loading a data set, Haploview presents to the user a series of marker genotyping quality metrics. In this study, we selected seven tagging SNP covered >80% variances of *AhR* gene (Fig. 1) from the database of the International HapMap Project of the Chinese population using Haploview. The purpose of the study is to investigate the relationship between polymorphisms of *AhR* and the urinary 1-OHP levels among PAH-exposed workers based on tagging SNP. Here, we found that two loci of *AhR* gene variations seemed to associate with the level of urinary 1-OHP, consisting with the notion that activity of AhR correlated with metabolism of PAH.

In this study, seven tagging SNP of *AhR* gene, rs2066853, rs10250822, rs1476080, rs10247158, rs7811989, rs2282885, and rs6960165 were evaluated. Only two of them, rs10250822 and rs2282885 variants, were associated with urinary 1-OHP levels in the exposed group. Analysis using multivariate linear regression for the trend of urinary 1-OHP levels with genotypes suggested that there was a linear trend between levels of 1-OHP and *AhR* rs2282885 genotypes among exposure workers. In addition, a significant association between rs2282885 polymorphism and urinary 1-OHP level was also found among side-oven workers. Moreover, there was a decline trend in lower PAH-exposed level from wild allele to variant allele. These observations indicated that the variation of *AhR* has an effect on urinary 1-OHP among the population exposed to PAH. The environmental exposure assessment showed that workers in bottom and side ovens are exposed to relatively lower level of PAH than that of top oven. However, the genetic polymorphisms of *AhR* influence the 1-OHP level only in the low and moderate exposure workers, not the case for top-oven workers. We speculated that two possibilities accounted for this result. One is due to only one case carrying *CC* genotype in the top-oven group. The other might be the case that the PAH exposure level was too high to cover up the effect derived from genetic variance.

The results from previous surveys investigating the correlations of PAH metabolites such as 1-OHP with the genetic polymorphisms among coke oven workers were inconsistent. Although several lines of evidence showed that variant genotypes of cytochrome *P*450 such as *CYP1A1* and *CYP2E1* could modify the metabolism of PAH in coke-oven workers (24-26), other studies reported no correlations between cytochrome *P*450 polymorphism and urinary 1-OHP level (9, 27-29). Therefore, it is hard to interpret the relation between cytochrome *P*450 polymorphism and urinary 1-OHP level.

Our findings that variant of *AhR* rs10250822 and rs2282885 significantly associate the modification of urinary 1-OHP show that AhR-mediated signaling might participate in regulation of PAH metabolic activation and contribute to susceptibility of PAH exposure. Similar

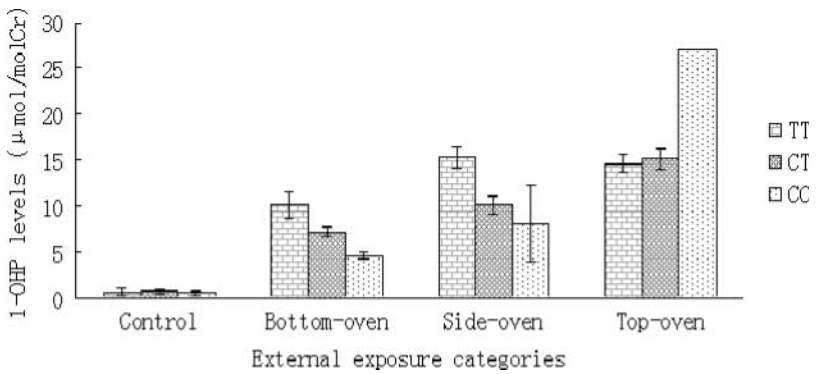

Figure 2. Urinary 1-OHP levels in subjects by genotypes of *AhR* rs2282885 stratified by external exposure categories. Multiple analysis of covariance tests for differences in ln-transformed 1-OHP between genotypes with adjustment for sex, age, cigarettes per day, and PAH external exposure categories. There was a significant difference with urinary 1-OHP levels in side-oven workers ($P < 0.01$). The *CT* genotype carriers exhibited significantly lower 1-OHP levels in urinary than did the wild genotype *TT* ($P = 0.006$). Multivariate linear regression for the trend of urinary 1-OHP levels with genotypes with adjustment for the same variables. There was a significant decline trend of urinary 1-OHP in lower PAH-exposed level ($P_{trend} = 0.068$ for bottom-oven workers and $P_{trend} = 0.007$ for side-oven workers). As there was only one person with *CC* genotype in the top-oven group, we did not show the SD.

result was found when urine 1-OHP glucuronide concentration was used as the metabolite of PAH and another *AhR* locus rs4986826 was analyzed (30). It was reported that genetic variations in the *AhR* gene in rodents could dramatically alter ligand binding function and transcriptional regulation by the receptor. For example, an intronic mutation in the *AhR* gene that altered RNA splicing either 38 or 43 amino acids near the end of the carboxyl terminus resulted in a deletion from the transactivation domain of the receptor. This mutation was responsible for the differences in sensitivity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced toxicity (31). In the early life stages of *Cyprinodon variegatus*, pyrene significantly induced the expression of AhR-regulated gene *CYP1A1* in a time- and dose-dependent manner (32). In humans, the expression change or mutation of *AhR* gene in human tissue has been studied as a potential factor related to risk or survival of cancer in lung or other sites (33, 34). Haplotypes of *AhR* gene play an important role in the development of lung cancer (35). In the present study, the polymorphisms were found in introns of *AhR*; it is conceivable that these variants modify the metabolism of PAH through the regulation of AhR transcription.

When the data of 1-OHP in the urine of coke-oven workers from different coke plants were combined according to the concentrations of PAH in the air, Jongeneelen et al. (36) found that it was possible to establish an indirect relationship between lung cancer mortality risk and the biological exposure indicator for coke-oven workers. In this study, we evaluated the effect of gene-environment interaction on the levels of 1-OHP in coke-oven workers exposed to PAH. Higher level of 1-OHP might indicate higher external exposure or faster detoxification process. Although we could not get direct relation between the levels of 1-OHP with the cancer incidence based on the data, the finding showed that AhR-mediated signaling might contribute to individual susceptibility to PAH exposure.

In conclusion, this study showed the association between *AhR* gene polymorphisms and urinary 1-OHP among coke-oven workers exposed to PAH, suggesting that *AhR* genetic variation may alter the AhR protein activity and then induces the excretion of pyrene. The alteration of PAH metabolic pathway may interact with environmental exposure and contribute to tumorigenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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We thank Prof. Youxin Liang (Fudan University) for critical reading of the article.

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Cancer Epidemiol Biomarkers Prev 2008;17:1702-1708.