Whole Genome Expression in Peripheral-Blood Samples of Workers Professionally Exposed to Polycyclic Aromatic Hydrocarbons

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S *Supporting Information*

ABSTRACT: This study aims to examine global gene expression profiles before and after the work-shift among coke-oven workers (COWs). COWs work six consecutive days and then take two days off. Two blood and urine samples in each worker were collected before starting to work after two days off and end-of-shift in the sixth day of work in 2009. Altered gene expressions (ratio of gene expression levels between end-of-shift and preshift work) were performed by a Human OneArray expression system which probes ~30,000-transcription expression profiling of human genes. Sixteen workers, all men, were enrolled in this study. Median urinary 1-hydroxypyrene (1OHP) levels (μmol/mol creatinine) in end-of-shift work were significantly higher than those in preshift work (2.58 vs 0.29, $p = 0.0002$). Among the 20,341 genes which passed experimental quality control, 26 gene expression changes, 7 positive and 19 negative, were highly correlated with across-the-shift urinary 1OHP levels (end-of-shift−preshift 1OHP) (p-value <0.001). The high and low exposure groups of across-the-shift urinary 1OHP levels dichotomized in ~2.00 μmol/mol creatinine were able to be distinguished by these 26 genes. Some of them are known to be involved in apoptosis, chromosome stability/DNA repair, cell cycle control/ tumor suppressor, cell adhesion, development/spermatogenesis, immune function, and neuronal cell function. These findings in COWs will be an ideal model to study the relationship of PAH exposure with acute changes of gene expressions.
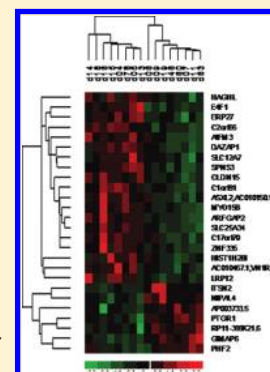
■ INTRODUCTION

Coke-oven workers (COWs) are exposed to high concentrations of polycyclic aromatic hydrocarbons (PAHs), which are also the major hazards from cigarette smoking and traffic pollutants.[1−3] Some PAHs with four or more benzene rings are considered to be human carcinogens.[4] Epidemiologic studies have presented strong evidence that workers with long-term exposure to PAHs have a high incidence of cancer, especially lung and colon cancers.[5,6] Besides their carcinogenesis property, PAHs can affect various organ functions, such as immunologic response and reproductive function.[7−10] PAHs are present in complex mixtures of more than 100 different compounds in the vicinity of coke-oven areas.[3] Our earlier studies have demonstrated that urinary 1-hydroxypyrene (1OHP), a metabolite of pyrene, is a good index of external ambient exposure to PAHs in COWs.[1−3]

Whole genome expression microarray provides a powerful tool for biological research fields due to its ability to scan tens of thousands of genes at one time.[11,12] Although microarray-related studies have increased rapidly in the fields of molecular biology and clinical medicine,[13−16] few studies apply this cutting-edge technique in industrial settings.[17−21] In addition, only one study used a pre- and postexposure study design to study the gene expression changes in workers exposed to metal fumes, which can reduce the error of interindividual variability.[19] Since, to our knowledge, no one has examined the changes of whole genome expression profile before and after occupational exposure in COWs, which is an ideal model to study PAHs exposure, we

evaluated the correlation between urinary PAH biomarkers and the acute changes of gene expressions.

■ MATERIALS AND METHODS

Subject. Sixteen COWs who had worked in one of two coke-oven plants for at least one year in the largest steel company in Taiwan voluntarily participated in this study between July and October, 2009. COWs regularly work 6 days and take two days off.[3] Thus, we collected their blood and urine samples at two different time points: one was during the preshift work on the first day after two days off, and the second one was at the end-of-shift work on the sixth work day. Information about age and smoking status was also collected before the collection of biological specimens. This study was approved by the Institutional Review Boards of KMHU; all study subjects gave written informed consent.

Biomarkers in Urine. All urine samples were stored at −68 °C until analysis. The detailed analytical method is described elsewhere.[1,2,22−24] Briefly, an aliquot amount of thawed urine was hydrolyzed with β-glucuronidase/sulfatase (Roche Diagnostics Ltd.), purified with a Sep-Pack C18 cartridge (2 g/12 mL, BondElut C18 HF, Varian), and condensed by dry N_2 purge to obtain a 2-mL extract. The extract was analyzed by using high performance liquid chromatography (HPLC, Beckman Coulter Module 126, UK) equipped with a fluorescence detector (Jasco FP-920, Japan) to determine 1-naphthol (1NP), 2-naphthol (2NP), 9-phenanthrol (9PHE), and 1OHP levels. The linearity (expressed as R^2), limit of detection (LOD), reproducibility (expressed as coefficient of variation (CV)), and mean recovery rate were 0.9982−0.9998, 1.83−47.56 ng/L, 4.02%−7.27%, and 82.97%−107.85%, respectively. Urinary creatinine was reacted with alkaline picrate, and the creatinine−picrate complex was quantified by spectrophotometry (Hitachi U-2000, Japan) using a wavelength of 520 nm. The concentrations of these four hydroxyl-PAHs were presented in units of μmol/mol creatinine.

RNA Preparation. Five milliliter blood samples were drawn from volunteers. WelPrep RNA Stabilizer (Welgeng Biotech, Taipei, TAIWAN) was immediately added to stabilize the whole-blood total RNA in room temperature for 2 h, and the samples were transferred to our laboratory within one hour. Then, the stabilized blood samples were stored in a −20 °C freezer until extraction within 1 month. Total RNA was isolated with RNeasy Mini Kit (Qiagen, Hilden, Germany) according to manufacturer protocol. The procedure was identical in the preshift and end-of-shift blood samples. The yield and quality of RNA were assessed by spectrophotometry and Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Only samples with an A260/A280 between 1.8 and 2.2 and A260/A230 ratio above 1 were eligible for the subsequent array experiment.

Oligonucleotide DNA Microarrays. Human oligonucleotide DNA microarrays (Human Whole Genome OneArray) from Phalanx Biotech Group (Hsinchu, Taiwan) were used. The Human Whole Genome OneArrayTM (HOAv4.3, Phalanx Biotech Group, Taiwan) contains 32,050 60-mer oligonucleotide probes, including 28,703 probes corresponding to the annotated genes in the Unigene v175 and RefSeq database, 2,265 experimentally defined probes, and 1,082 control probes.[25,26] The detailed descriptions of the gene array list are available at http://www.phalanx.com.tw/tech_support/gene_lists.html.

Microarray Experiment. One-half microgram RNA of each sample was amplified by Illumina TotalPrep RNA Amplification Kit according to the manufacturer's instructions (Ambion, Austin, TX). Then, 10 μg of fragmented biotin-labeled cRNA was hybridized on Phalanx Human OneArray by Phalanx hybridization buffer at 50 °C in an oven for 14−16 h using the bubble-mixing method. Each sample was hybridized in triplicate; thus, a total of 96 chips were used in this study (16 workers × 2 time points × 3 experiments). After nonspecific

binding targets were washed, the hybridization arrays were conjugated with fluorescent detector of Strepavidin-Cy3. Finally, arrays were dried by centrifugation and scanned by DNA Microarray Scanner (Agilent Technologies, Santa Clara, US). Images from the scanned arrays were quantified using GenePix Pro 4.0 (Molecular Devices, Sunnyvale, CA).

Qualification and Normalization of Microarray Chips. Spots in each array with foreground median intensity of wavelength 532 nm greater than or equal to that of background median intensity plus 3-folds standard deviation of wavelength 532 nm were considered as the "Present" flag and included for further analysis. In order to evaluate the quality of each array in the entire array experiment, three evaluation steps were performed: basic, reproducible, and diagram. In the basic step, three parameters, including percentage of "Present" spots among all spots, the average intensity of "Present" spots, and coefficient of variation of intensity for control spots in the entire arrays were all considered. If any two parameters in one array were located outside the 1.5-fold interquartile range (25th−75th) of the same parameters for all arrays, that array was excluded. The remaining arrays were then evaluated in reproducible steps which the repeated arrays of the same sample would pass, when their Pearson's correlation coefficient was larger than 0.95 and their 2-fold percentage was less than 15% (Supporting Information, Figure S1). The 2-fold percentage was the percentage of probes among all probes in which the ratio of the same probe between two arrays exceeded 2-fold. In the final diagram step, the density plot of repeated arrays was used to examine the intensity profile of each array. An array would pass if the profile was similar to that of the rest of arrays in the same phenotype groups. When the arrays passed all three steps, the raw intensity of spots were log-2 transformed for subsequent analysis. To adjust the systematic variation of experiments and dye effects, global Loess normalizations were performed within repeated arrays of the same sample and between the samples. Spot was included for further analysis when it was "Present" in at least one of the qualified arrays.

Validation of Microarray Intensity by Real-Time PCR. To validate the differential expression of genes in the array experiment, we performed the quantitative real-time PCR analysis in the most significant candidate gene (MYO15B) in the 8-paired samples (preshift and end-of-shift samples) randomly from 16 study subjects by ABI StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The primers were 5′-tggagcaactgtgcaacaac-3′ and 5′-agcatctccaccacagcag-3′ for MYO15B and 5′-gca ccg tca agg ctg aga ac-3′ and 5′-atg gtg gtg aag acg cca gt-3′ for GAPDH (internal control). We found a high correlation of MYO15B RNA intensity analyzed by real-time PCR and microarray experiments (Spearman correlation $r = 0.881$, $p = 0.004$, $n = 8$) (Supporting Information, Figure S2).

Statistical Analysis. Mann−Whitney U-test was used to compare the differences between preshift and end-of-shift (across-the-shift) work of urinary biomarkers. Since 1OHP in urine was recognized as the best surrogate to represent for ambient coke-oven emission exposure,[1,3,27] we examined the correlation between urinary across-the-shift 1OHP levels and other biomarkers, including 1NP, 2NP, and 9PHE.

For array analysis, Spearman correlation was used to examine the relationship of across-the-shift urinary 1OHP levels with altered gene expressions (difference of log-transformed gene expression levels between end-of-shift and preshift work) using the Biometric Research Branch statistical program (BRB; http://linus.nci.nih.gov/BRB-ArrayTools.html).[25] Significant p-value was set as <0.001. Hierarchical cluster analysis with average linkage was also used to examine the similarity metric of pairs of samples. Distance metric used in the dendrogram was one minus the Pearson correlation.[28,29] Those differential expressed genes were further analyzed according to their biological process/molecular function by functional annotation clustering of DAVID Bioinformatics Resources 6.7 (http://david.abcc.ncifcrf.gov/).

Table 1. Significance Gene Changes Associated with Across-the-Shift Urinary 1-Hydroxypyrene Levels in 16 Coke-Oven Workers (COWs) and in 5 Nonsmoking COWs

	gene name	gene symbol	location	16 COWs			5 nonsmoking COWs	
				r^a	p-value	FDR[b]	r^a	p-value
1	myosin XVB	MYO15B	17q25.1	−0.862	<1e-07	<1e-07	−0.700	0.188
2	solute carrier family 25, member 34	SLC25A34	1p36.21	−0.885	<1e-07	<1e-07	−0.500	0.391
3	DAZ associated protein 1	DAZAP1	19p13.3	−0.912	<1e-07	<1e-07	**−0.900**	**0.037**
4	GTPase, IMAP family member 6	GIMAP6	7	0.847	0.00001	0.0519	0.600	0.285
5	prostaglandin reductase 1	PTGR1	9q31.3	0.835	0.0001	0.2130	**0.900**	**0.037**
6	zinc finger protein 335	ZNF335	20q13.12	−0.826	0.0001	0.2780	−0.500	0.391
7	spinster homologue 3 (*Drosophila*)	SPNS3	17p13.2	−0.826	0.0001	0.2780	−0.800	0.104
8	*Homo sapiens* genomic DNA, chromosome 11 clone: RP11-810P12, complete sequence	AP003733.5	11	0.818	0.0002	0.3060	0.500	0.391
9	ADP-ribosylation factor GTPase activating protein 2	ARFGAP2	11p11.2-p11.12	−0.818	0.0002	0.3060	−0.700	0.188
10	additional sex combs like 2	ASXL2	2p24.1	−0.818	0.0002	0.3060	−0.600	0.285
11	vomeronasal 1 receptor 4	VN1R4	19q13.42	−0.803	0.0003	0.5070	**−1.000**	**<0.001**
12	PHD finger protein 2	PHF2	9q22.31	0.788	0.0004	0.6630	0.600	0.285
13	apoptosis-inducing factor, mitochondrion-associated, 3	AIFM3	22q11.21	−0.785	0.0005	0.6630	**−0.900**	**0.037**
14	chromosome 1 open reading frame 91	C1orf91	1p36.11-p34.2	−0.785	0.0005	0.6630	−0.600	0.285
15	chromosome 2 open reading frame 66	C2orf66	2q33.1	−0.785	0.0005	0.6630	−0.700	0.188
16	histone cluster 1, H2bi	HIST1H2BI	6p21.3	−0.779	0.0006	0.6750	**−0.900**	**0.037**
17	solute carrier family 12 (potassium/chloride transporters), member 7	SLC12A7	5p15	−0.779	0.0006	0.6750	−0.800	0.104
18	claudin 15	CLDN15	7q11.22	−0.776	0.0006	0.6750	−0.700	0.188
19	endoplasmic reticulum protein 27	ERP27	12p12.3	−0.776	0.0006	0.6750	−0.900	**0.037**
20	intersection 2	ITSN2	2pter-p25.1	0.771	0.0007	0.7070	0.600	0.285
21	E4F transcription factor 1	E4F1	16p13.3	−0.771	0.0007	0.7070	**−1.000**	**<0.001**
22	chromosome 17 open reading frame 70	C17orf70	17q25.3	−0.765	0.0009	0.7070	−0.700	0.188
23	NIPA-like domain containing 4	NIPAL4	5q33.3	0.762	0.0009	0.7070	**0.900**	**0.037**
24	low density lipoprotein receptor-related protein 12	LRP12	8q22.2	−0.759	0.0010	0.7070	−0.800	0.104
25	retinitis pigmentosa 11	RP11−399K21.6	19q13.4	0.759	0.0010	0.7070	0.200	0.747
26	hydroxyacylglutathione hydrolase-like	HAGHL	16p13.3	−0.759	0.0010	0.7070	−0.800	0.104

[a] r: Spearman correlation coefficient. [b] FDR: false discovery rate.

RESULTS

Study Population. All 16 COWs were male, and 11 (68.8%) of them were cigarette smokers. Median urinary 1OHP, 1NP, and 9PHE levels in end-of-shift workers were significantly higher than those in preshift workers ($p < 0.01$) (Supporting Information, Table S1). In contrast, no significant difference was found in 2NP. The high correlation of urinary 1OHP with 1NP (Spearman correlation coefficient $r = 0.68$; $p = 0.0047$) and 9PHE ($r = 0.76$; $p = 0.0006$), but not 2NP ($r = 0.09$; $p = 0.7452$), was noted (Supporting Information, Figure S3).

Array Analyses. In total, 82 (85.4%) out of 96 arrays and 20,341 (70.9%) out of 28,703 genes passed the quality control of array experiments (GEO accession number GSE30504 at http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE30504). Each blood sample had at least two qualified arrays for analyses, and means of their values were analyzed in the subsequent analyses.

Using the cut-point p-value <0.001 of Spearman correlation statistics between across-the-shift 1OHP and altered gene expression, we identified 26 significant gene expression changes, and 7 and 19 of their intensity were positively and negatively correlated with across-the-shift urinary 1OHP levels, respectively

(Table 1). The positive correlations ranged from 0.759−0.847, whereas the negative correlation ranged from −0.759 to −0.912. These 26 significant genes were further classified and displayed two predominant clusters in samples in the high and low exposure groups (the cut-point level of across-the-shift urinary 1OHP levels was ∼2.00 μmol/mol creatinine) (Figure 1). In the low across-the-shift urinary 1OHP group (0.43−1.97 μmol/mol creatinine), 19 genes were positively associated with across-the-shift 1OHP levels and 7 genes were negatively associated with 1OHP across-the-shift 1OHP levels. In contrast, among the high across-the-shift urinary 1OHP group (2.60−8.47 μmol/mol creatinine), 19 genes were negatively associated with across-the-shift 1OHP levels, and 7 genes were positively associated with 1OHP across-the-shift 1OHP levels.

Of the 16 COWs, five were nonsmokers. We also looked at the Spearman correlation between those 26 candidate gene expression changes and across-the-shift urinary 1OHP levels among these five nonsmokers. We found that most values of the Spearman correlation coefficients were close to the original ones in 16 COWs, although only 8 genes reached the significance of p-value at 0.05 (Table 1).

Using the David program analysis, we were able to annotate 24 out of these 26 significant genes (except AP003733.5 and

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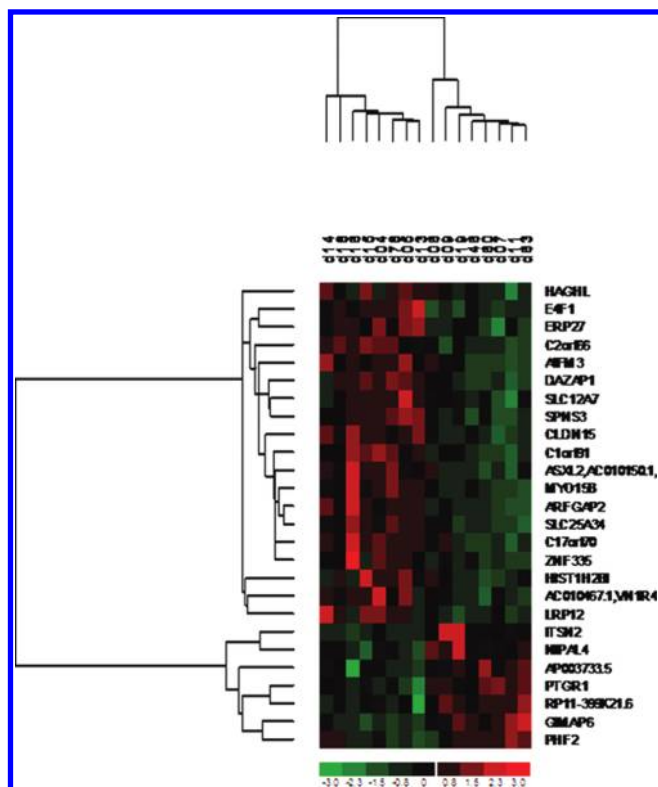
Figure 1. Heatmap and cluster analysis of 16 paired samples using 26 significant gene expression changes identified by the Spearman correlation test with across-the-shift 1-hydroxypyrene (1OHP, μmol/mol creatinine). Each column and row represent one coke-oven worker and gene, respectively.

RP11−399K21.6) (Supporting Information, Table S2). Among these 24 genes, 2 main biological process/molecular functions involved in metal ion binding and transport were identified by David functional annotation clustering (Supporting Information, Table S2). Of these significant genes, 4 had false discovery rates (FDR) ≤ 0.05, including myosin XVB (MYO15B), solute carrier family 25, member 34 (SLC25A34), DAZ-associated protein 1 (DAZAP1), and GTPase, IMAP family member 6 (GIMAP6) (Figure 2).

■ DISCUSSION

1NP and 2NP, 9PHE, and 1OHP in urine are the metabolites of naphthalene, phenanthrene, and pyrene respectively, which belong to 2-, 3-, and 4-benzene rings of PAHs.[30] Urinary 1OHP is commonly used to represent ambient PAH exposures in occupational settings, including that of COWs.[1,3,27] This study found that median urinary 1OHP, 1NP, and 9PHE levels in end-of-shift work were significantly higher than those in their preshift work. In addition, there were the high correlations of across-the-shift urinary 1OHP levels with across-the-shift urinary 1NP and 9PHE levels. These results suggest that the COWs were occupationally exposed to a high amount of different PAHs during work. In contrast, no significant increase of urinary across-the-shift 2NP levels and no significant correlation between across-the-shift urinary 1OHP levels and 2NP levels were found. Possible explanations are as follows: 2NP is not the final metabolite of ambient naphthalene exposure; this may be due to the contributions of smoking greater than coke-oven emissions exposure on

the 2NP metabolite; or the issue of collection of only one-spot urine sample is not representative for continuous coke-oven exposure.[31]

This is, to our knowledge, the first study to use the technique of whole-genome array to investigate the effect of coke-oven emissions, which mainly contain PAHs, on altered gene expressions in humans. Although a few studies have examined the effect of environmental and occupational hazards, such as arsenic or benzene, on whole genome expression changes in humans,[17,19,32] most of the study designs compared gene function differences between exposed and nonexposed subjects, which would potentially introduce interindividual variability bias, especially in the study of evaluating thousands of genes changes at the same time. Our study design, along with the study of Wang et al.[19] to evaluate metal fume exposures, using the same subjects to examine altered gene expressions before and after work shifts, can overcome this bias.

The Human Whole Genome OneArrayTM (Phalanx Biotech Group, Taiwan) used in this study can evaluate ∼30,000 functional gene expressions. Since many genes have unknown functions or their expressions are correlated with each other, we used the p-value significance of <0.001 in Spearman statistics, rather than multiple testing adjustment, to explore the correlation between across-the-shift 1OHP and gene expression changes. Under this approach, we were able to identify 26 significant altered gene expressions, 7 positively and 19 negatively correlated with across-the-shift urinary 1OHP levels (Table 1). These positive and negative Spearman correlations were at least 0.75. Some known gene functions are involved in apoptosis (e.g., AIFM3),[33,34] chromosome stability/DNA repair (e.g., C17orf70, HIST1H2BI),[35,36] cell cycle control/tumor suppressor (e.g., E4F1, LRP12),[37,38] cell adhesion (e.g., CLDN15),[39] development/spermatogenesis (e.g., ASXL2, DAZAP1),[40−42] immune function (e.g., GIMAP6),[43−45] and neuronal cell function (e.g., ITSN2).[46]

The David functional annotation clustering tool clustered two main biological process/molecular functions involved in metal ion binding/cation binding and transport among the 24 out of 26 significant genes (except AP003733.5 and RP11−399K21.6). At this moment, we cannot fully explain how it is that these two molecular functions related to PAH exposures are picked-up by DAVID annotation. The molecular function of 10 candidate proteins involved in metal ion binding/cation binding may interact selectively and noncovalently with any metal ions, including vanadium, manganese, iron, copper, cobalt, nickel, molybdenum, and silver (Supporting Information, Table S2). Two articles have observed that binding of heavy metal ions to cell membrane may affect the sorption of organic pollutants such as PAHs by modulating the structure and chemistry of the cell membrane.[47,48] Another molecular function of 6 candidate proteins associated with transport is involved in the transport of amino acids, calcium ions, trace elements of copper or cobalt (a component of vitamin B12), or electrons probably related to the process of energy conversion. PAHs are well-known carcinogens, but they can also influence intracellular gap-junction communication to act as a tumor promoter in culture cells.[49,50] This is an exploratory study, and further research is needed to elucidate those molecular functions.

Of 26 significant genes, 4, including MYO15B, SLC25A34, DAZAP1, and GIMAP6, had FDR ≤ 0.05. Gene expression changes of MYO15B, SLC25A34, and DAZAP1 were positively correlated with urinary across-the-shift 1-OHP, whereas GI-MAP6 was negatively correlated with urinary across-the-shift 1-OHP. Although these 4 candidate genes were the most significant

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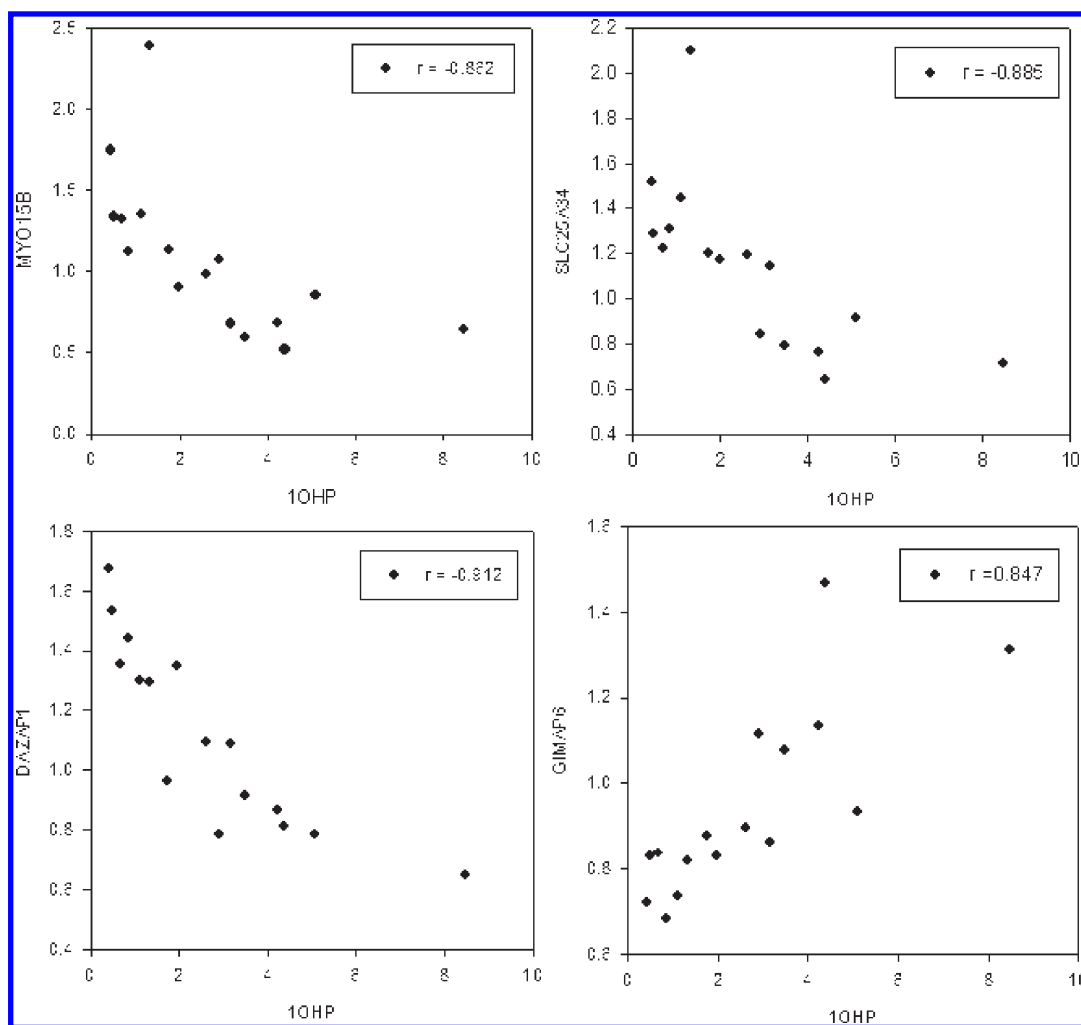
Figure 2. Correlation of urinary across-the-shift 1-hydroxypyrene (1OHP, μmol/mol creatinine) with gene expression changes (folds) between preshift work and end-of-shift work among 16 coke-oven workers. MYO15B, myosin XVB; SLC25A34, solute carrier family 25, member 34; DAZAP1, DAZ associated protein 1; GIMAP6, GTPase, IMAP family member 6.

ones, their gene expression differences between preshift and end-of-shift works were less than 2.5-fold (Figure 2). Since these subjects for array analyses were healthy, their gene expression changes may not be as large as the studies of examining the gene differences between cancer and normal tissues.[17,51]

MYO15B is a transcribed, untranslated pseudogene located at 17q25.[52] On the basis of Bootstrap analysis, MYO15B has the most significant similarity to MYO15A, which is mainly involved in muscular contraction and related to important functions of some specific cells such as melanocytes, kidney and intestinal brush border microvilli, nerve growth cones, or inner ear hair cells associated with hearing impairment.[52−54] SLC25A34 is located at 1p36.21 and belongs to the SLC25 family of mitochondrial carrier proteins whoseh function is to transport molecules over the mitochondrial membrane.[55] The molecules transported by the SLC25 family protein include ATP/ADP, amino acids (glutamate, aspirate, lysine, histidine, and arginine), malate, ornithine, and citrulline.[56] DAZAP1 is an RNA-binding protein located at 19p13.3. Its primary expression is in the testis, and its main function involves spermatogenesis.[40] Recently, Prima et al.[41] also showed that DAZAP1 can translocate and fuse to myocyte enhancer factor 2D to become a fusion protein in

an acute lymphoblastic leukemia cell line (TS-2), suggesting that DAZAP1 may also contribute to human leukemogenesis. GI-MAP6 gene encodes a protein belonging to the GTPases of the immunity-associated protein family and is located in a cluster at 7q36.1.[44] Their predominated mRNA expression sites are in the immune system such as in the spleen and lymph nodes. A few studies have shown that GIMAP GTPases are expressed at very low levels in diverse cancer tissues and cell lines including leukemia and lymphoma.[43] Thus, GIMAP may play a role to decrease cancer growth and probably act as a tumor suppressor gene.

Except for MYO15B, the biological functions of the other three significant genes (SLC25A34, DAZAP1, and GIMAP6) selected by arrays are coincidentally consistent with the findings of our previous epidemiological studies in COWs from the same large steel company.[7,9] Besides the well-known carcinogenic properties of some PAHs, one study of ours has shown that COWs had significantly higher serum IgE and tumor necrosis factor-α levels than those of rolling steel workers as a comparison nonexposed group.[7] In contrast, serum IgA levels were significantly lower in COWs than in rolling steel workers, suggesting that PAH exposure may alter the immune responses in COWs. The mechanism of different immune modulations by PAH

exposure is probably due to the binding activity of the aryl hydrocarbon receptor or oxidative stress induction.[57−59] In addition, another of our studies found a positive correlation between urinary 1OHP concentration and the percentage of abnormal sperm morphology in the same COWs.[9] PAHs such as benzo[a]pyrene and its metabolites can accumulate in the testis and epididymis and may therefore affect androgen-dependent processes by acting as antiandrogens.[51,60] PAHs may also directly influence sperm function through DNA damage to form PAH-DNA adducts or to bind the dioxin receptor in sperm.[61,62] The findings of our genome arrays may add additional information about the biological mechanisms of PAH's effect on systems of carcinogenesis, immune response, and reproductive dysfunction.

In conclusion, this study demonstrates that microarray analysis can become a useful tool to discover the potential known and novel genes or biomarkers in an ideal exposure group such as PAH exposure in COWs, although the sample size is small. Since microarray experiments will generate thousands of items of gene function information, using a repeated measure design, like ours, can reduce interindividual variability bias. Some known gene functions identified by this study and associated with carcinogenesis, immune response, and reproductive dysfunction are consistent with our earlier epidemiological studies in the same group of COWs. Future research is necessary to study the role of other significant genes in COWs professionally exposed to PAHs.

■ ASSOCIATED CONTENT

ⓢ Supporting Information. Significance genes clustered based on biological process/molecular function by DAVID Bioinformatics; scatter plots of one representative coke-oven workers with 3 qualified array assays by Pearson's correlation coefficients; Spearman correlation of MYO15B gene expression change (fold, end-of-shift/pre-shift work) by real-time PCR and microarray analyses in eight coke-oven workers. This material is available free of charge via the Internet at http://pubs.acs.org.

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