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Urinary 1-Hydroxypyrene Level Relative to Vehicle Exhaust Exposure Mediated by Metabolic Enzyme Polymorphisms

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Abstract: Urinary 1-Hydroxypyrene Level Relative to Vehicle Exhaust Exposure Mediated by Metabolic Enzyme Polymorphisms: Chun-Yu CHUANG, *et al*. Department of Biomedical Engineering and Environmental Sciences, National Tsing-Hua University, Taiwan—Polycyclic aromatic hydrocarbons (PAH) are common air pollutants generated from incomplete combustion. The inhalation of exhaust fumes in urban areas has been suggested to be an additional contributing factor. This study investigated the influence of urban traffic exposure, personal lifestyle factors and metabolic enzyme polymorphisms on the urinary 1-hydroxypyrene (1-OHP) level, approximating exposure to PAH. With consents, 95 male taxi drivers exposed to vehicle exhaust in traffic and 75 male office employees received health interviews and provided urine samples. The results showed taxi drivers had higher urinary 1-OHP than the office employees (mean ± standard deviation were 0.17 ± 0.10 vs. 0.10 ± 0.07 μmol/mol creatinine, $p<0.001$). The average urinary 1-OHP level increased from 0.07 μmol/mol creatinine for non-smoking office employees to 0.17 μmol/mol creatinine for those who smoked more than 20 cigarettes daily. The values for taxi drivers with similar smoking statuses were 0.12 and 0.25 μmol/mol creatinine, respectively. Among non-smokers, taxi drivers still had higher 1-OHP level than office employees (0.12 ± 0.05 vs. 0.07 ± 0.03 μmol/mol creatinine). The subjects with the m1/m2 or m2/m2 genotype of *CYP1A1 Msp*I or *GSTM1* deficiency had significantly higher urinary 1-OHP levels than those with other *CYP1A1 Msp*I and *GSTM1* genotypes. Multivariate logistic regression analysis showed that taxi drivers (adjusted odds ratio (OR)=5.1, 95% confidence interval (CI)=1.1–13.6), smokers (OR=5.5, 95% CI=1.6–18.4) and subjects with the m1/m2 or m2/m2 genotype of *CYP1A1 Msp*I (OR=9.7, 95% CI=2.7–35.0) had elevated urinary 1-OHP (greater than the overall median value, 0.11 μmol/mol creatinine). The results of this study suggest smoking contributes to the elevated urinary 1-OHP levels in taxi drivers in addition to taxi driving, and the excess level contributed from traffic exhaust and smoke was regulated by the *CYP1A1 Msp*I genotype. Traffic exhaust exposure, smoking and *CYP1A1 Msp*I genotype contributed to the variation in levels of urinary 1-OHP excretion.
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Key words: Vehicle exhaust, Cigarette smoking, 1-Hydroxypyrene, Taxi drivers, Genetic polymorphism

Polycyclic aromatic hydrocarbons (PAH) include compounds with two or more fused benzene rings and are widely distributed in the environment. Hundreds of PAH originate from incomplete combustion of fuel, such as in vehicle engines, industrial emissions and tobacco smoking, and also from food cooking[1]. Absorbed via the gastrointestinal duct, lungs and skin[2–4], PAH are metabolized into ultimate carcinogenic forms, bay-region diol expoxides, in a cytochrome p450 and epoxide hydrolase sequential reaction[5]. In humans, PAH are bioactivated to reactive metabolites which can bind covalently to DNA and subsequently initiate mutation and carcinogenesis.

Because PAH are distributed in varying levels between gaseous and particulate phases, exposure assessment has been problematic. Pyrene is contained in PAH mixtures in various proportions and its metabolite, 1-hydroxypyrene (1-OHP), is excreted in urine; it currently is indicated as the most relevant biomarker of PAH exposure to evaluate total-body exposure to PAH[6]. The 1-OHP content in urinary excretion is determined not only by the amount of PAH uptake but also by differences in their distribution, metabolism and excretion.

Studies have shown significant correlations between urinary 1-OHP concentrations and occupational and environmental exposure to all PAH or pyrene concentrations[7–11]. Factors that may cause inter-

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individual differences in urinary 1-OHP levels are lifestyle factors such as smoking, alcohol consumption and dietary intake[12]; personal factors such as age, sex and body mass index (BMI)[13]; and airborne PAH concentrations in high-traffic areas[14]. In addition, genetic polymorphisms of enzymes have been suggested to explain inter-individual differences in the rate of metabolism and activation/deactivation of PAH-derived carcinogens[15]. Cytochromes p-450 (CYP) is a family of biotransformation phase I enzymes which catalyze the metabolism of xenobiotics. CYP1A1 catalyzes the oxidation of PAH and the formation of their active oxygen metabolites[16–18]. Glutathione S-transferases (GSTs) are biotransformation phase II enzymes, which have been reported to catalyze the conjugation of PAH diol epoxide metabolites with reduced glutathione. GSTs are detoxification enzymes, which may play a major role in protecting the individual from PAH-induced mutagenesis and carcinogenesis[19, 20].

Drivers may be exposed to air pollution with high concentrations of lead, carbon monoxide, sulfur dioxide, nitrogen oxide, suspended particles, benzene, and PAH during their professional activities[21, 22]. The International Agency for Research on Cancer[23] classified gasoline engine exhaust as a Group 2B carcinogen. Taxi drivers in Taipei City (the most prosperous metropolis in Taiwan) constitute a group of workers on average spending 12 h a day and 6.5 d per wk in city traffic with extensive exposure to urban air pollutants, especially from motor vehicle exhaust. There are around 67,000 taxies in Taipei, 66% of all the taxies in Taiwan, and there is an average of 11.3 taxies per one thousand persons. Because some occupational drivers are also heavy cigarette smokers, it is not clear whether the combined effect of smoking and occupational exposures accounts for the excess health risks for professional drivers[24–28]. Hansen *et al*.[29] found that the risk of lung cancer, after the effects of smoking was controlled, were significantly higher for drivers than for the total employed population in Denmark. Urban PAH exposure is suspected of being a factor in elevated risk for lung cancer[30–32]. This study determined the levels of urinary 1-OHP for taxi drivers and office employees, and assessed whether taxi driving, lifestyle factors and metabolic enzyme polymorphisms were associated with an increased body burden of 1-OHP implied PAH exposure. The measurement of PAH exposure may be used as an index to classify a cancer risk.

Materials and Methods

Study subjects and data collection

The taxi driver study subjects volunteered to participate in the study after flyer and radio broadcast announcements were disseminated at a taxi rest station, and the office employee study subjects were recruited from residential community with the least air pollution in the Taipei metropolis based on the pollution standards index available in the Taiwan air pollution statistics. One hundred eighty-one male taxi drivers and 92 male office employees were recruited as reference subjects. With informed consent, a physician interviewed the study subjects about their health status and obtained urine samples at the same time. The subjects were asked to complete a self-reported questionnaire including questions on age, height and weight (for body mass index; BMI), exercise, cigarettes smoked per day and 24 h prior to sampling, areca quid chewing, alcohol consumption, incense use and dietary intake. Spot urine and blood specimens from all of the subjects were obtained in the morning after fasting for at least 8 h, transported to our laboratory in cold boxes at 4°C, and respectively stored at –20°C and 4°C until 1-OHP and genotyping analysis.

The taxi drivers who were former smokers (n=41) or had less than 3 yr of taxi driving history (n=45) were excluded from this study. The office employees who were former smokers (n=17) were also excluded from the reference group. We used data from 95 male taxi drivers and 75 male office employees for the statistical analysis.

Determination of 1-hydroxypyrene in urine

The level of 1-OHP in urine was determined using reverse-phase high performance liquid chromatography (HPLC) after a modification of the method of Jongeneelen *et al*.[33]. Ten milliliters of thawed urine were adjusted to pH 5.0 with 1.0 N acetic acid, 10 m*l* of 0.1 M sodium acetate buffer (pH 5.0) was added and the mixture was enzymatically deconjuated by 25 μl of β-glucuronidase/sulfatase (127,300 units/m*l* and 7,500 units/m*l*). After incubation overnight at 37°C in a shaker, the urine sample was centrifuged at 2,000 rpm for 10 min to remove the particulate matter. A LC-18 cartridge (SUPELCO, Supelco Park, Bellefonte, PA, USA) was used to enrich, purify and extract the PAH metabolites in urine. After priming the cartridge with 2 m*l* of methanol, followed by 5 m*l* of distilled water, the sample supernatant was passed through the cartridge at a rate of 5 m*l*/min. The cartridge was then washed with 6 m*l* of 0.1 M sodium acetate buffer (pH 5.0), and the retained solutes were eluted with 1.5 m*l* of isopropanol. The elute was evaporated at 45°C under nitrogen and the residue was then dissolved in 1.0 m*l* of isopropanol. 1-OHP was quantitatively determined using HPLC with a fluorescence detector (Jasco FP-920) with excitation and emission wavelengths of 241 and 388 nm, respectively. Twenty-five microliters of the sample were injected into an ODS C18, 250 × 4.6 mm (5 μm) column (Phenomenex®) (column temperature was 30°C, flow 1.5 m*l*/min). The entire separation runtime using isocratic elution (acetronitrile:water, 65:35) was 8 min. The retention time for 1-OHP was 6.4 min. The concentration range of the standard solutions was 0.49–250 ng/m*l*, with a detection limit of 0.24 ng/m*l*. The recovery rates were

100.8 ± 6.8% at a concentration of 1.95 ng/m*l*, 96.4 ± 3.2% at 7.81 ng/m*l*, and 93.7 ± 5.6% at 31.25 ng/m*l*. The triplicate sample analysis reproducibility was within a coefficient variation of 7.0%. The 1-OHP concentration was expressed as micromole per mole of creatinine to adjust the urine flow for individual variation. The creatinine values in urine samples were determined using an automated analyzer (Hitachi 7250) based on Jaffe's colorimetric method.

Genotyping for CYP1A1, GSTM1 and GSTT1 polymorphisms

Genomic DNA was isolated from 1 m*l* of whole blood from subjects using a DNA extraction kit (Promega, Madison, WI). Two CYP1A1 polymorphisms in the *Msp*I and *Hinc*II restriction sites were tested in this study by the polymerase chain reaction (PCR) - restriction fragment length polymorphism method[34]. *CYP1A1 Msp*I polymorphism is a T to C transition 264 bp downstream from the polyadenylate signal. It was detected by PCR amplification using primers 5'-TAGGAGTCTTGTCTCATGCCT-3' and 5'-CAGTGAAGAGGTGTAGCCGCT-3', followed by digestion with MspI. *CYP1A1 Hinc*II is an A to G transition in exon 7 of the CYP1A1 gene that results in the substitution of valine for isoleucine at residue 462. It was assessed by PCR using primers 5'-GGACTGCCACTTCAGCTGTCT-3' and 5'-GAGAAAGACCTCCAAGCGGTCA-3', followed by digestion with HincII. A multiplex PCR method was used to detect the deletion of the *GSTM1* and *GSTT1* gene loci. We amplified exons 6–7 of the *GSTM1* gene using primers 5'-CTGCCCTACTTGATTGATGGG-3' and 5'-CTGGATTGTAGCAGATCATGC-3', and for the *GSTT1* gene using primers 5'-TTCCTTACTGGTCCTCACATCTC-3' and 5'-TCACCGGATCATGGCCAGCA-3'[35]. We coamplified a 100-bp fragment of the β-globin gene as an internal standard using primers 5'-ACACAACTGTGTTCACTAGC-3' and 5'-CAACTTCATCCACGTTCACC-3'. The digestion and amplification products were separated electrophoretically on a 2% agarose gel.

Statistical methods

Data were analyzed using SPSS for Windows version 13.0. Comparisons of the average urinary 1-OHP levels by demographics, lifestyle factors and metabolic enzyme polymorphisms between taxi drivers or office employees were carried out using Student's t-test or ANOVA. Multiple regression analysis based on the stepwise method was performed to evaluate the relationship between the 1-OHP level and possible modulating factors. The differences in the urinary 1-OHP concentrations between study subjects based on smoking status were investigated using Student's *t*-test, and the 1-OHP trend levels in response to the number of cigarettes smoked was estimated using a general linear model. Multivariate logistic regression was used to estimate the odds ratios (OR) and corresponding 95% confidence intervals (CI) of having an elevated 1-OHP level for the associated variables, using the median level among all participants as the cutoff level. A *p* value less than 5% was considered statistically significant.

Results

Compared with office employees, taxi drivers were approximately 5 yr younger on average. They were more likely to be current smokers, had received less education, and had less exercise. There were no significant differences between the two employee groups with respect to BMI, alcohol drinking, areca quid chewing, incense burning and passive smoke exposure (Table 1).

Table 2 shows that smoking and areca quid chewing were significant factors associated with urinary 1-OHP levels in both groups. Among the office employees, the average 1-OHP level was higher for those with incense use. The urinary 1-OHP level was not associated with diet (data not shown).

The overall average 1-OHP level was significantly higher in taxi drivers than in office employees (0.17 ± 0.10 vs. 0.10 ± 0.07 μmol/mol creatinine) (Table 3). Even among non-smokers in these two groups, taxi drivers still had higher 1-OHP levels than office employees (0.12 ± 0.05 vs. 0.07 ± 0.03 μmol/mol creatinine). The stratified data demonstrated a significant dose-response relationship between the level of 1-OHP by the number of cigarettes smoked daily for both study subjects. The urine 1-OHP level increased from 0.07 μmol/mol creatinine for non-smoking office employees to 0.17 μmol/mol creatinine for those who daily smoked more than 20 cigarettes. The corresponding values for taxi drivers were 0.12 and 0.25 μmol/mol creatinine, respectively.

The median urinary 1-OHP level was 0.11 μmol/mol creatinine for all participants. The cumulative percent graph of urinary 1-OHP levels for taxi drivers and office employees by smoking status showed that approximately 10% of the non-smoking office employees, 45% of the non-smoking taxi drivers, 66% of the smoking office employees and 82% of the smoking taxi drivers had urinary 1-OHP at this median level or higher (Fig. 1). These four cumulative percentage lines were clearly separated. Approximately 27% of the smoking taxi drivers had 1-OHP levels higher than 0.25 μmol/mol creatinine.

With regard to the number of daily driving hours, smoking taxi drivers' urinary 1-OHP level increased by 0.009 μmol/mol creatinine per hour and non-smoking taxi drivers showed a decrease of 0.006 μmol/mol creatinine per hour (Fig. 2). However, the neigher result showed a

Table 1. Demographic and lifestyle characteristics of the taxi drivers and office employees

Variables	Taxi drivers *n*=95 *n* (%)	Office employees *n*=75 *n* (%)	*p*-value
Age (yr)			
30–34	12 (12.6)	9 (12.0)	<0.001
35–39	30 (31.6)	9 (12.0)	
40–44	40 (42.1)	18 (24.0)	
≥45	13 (13.7)	39 (52.0)	
Mean age ± SD	39.7 ± 3.9	44.3 ± 7.2	<0.001
Education (yr)			
≤9	41 (44.6)	23 (31.5)	0.005
10–14	48 (52.2)	37 (50.7)	
≥15	3 (3.3)	13 (17.8)	
BMI (kg/m^2)			
≤24	48 (50.5)	35 (46.7)	0.617
> 24	47 (49.5)	40 (53.3)	
Smoking			
Never	40 (42.1)	43 (57.3)	0.049
Current	55 (57.9)	32 (42.7)	
Passive smoking			
No	45 (47.4)	35 (46.7)	0.927
Yes	50 (52.6)	40 (53.3)	
Regular drinking			
No	78 (82.1)	64 (85.3)	0.573
Yes	17 (17.9)	11 (14.7)	
Areca quid chewing			
No	66 (69.5)	62 (82.7)	0.108
Yes	24 (25.3)	12 (16.0)	
Exercise habit			
No	68 (71.6)	28 (37.3)	<0.001
Yes	26 (27.4)	47 (62.7)	
Incense burning			
No	63 (66.3)	46 (61.3)	0.501
Yes (≥1/wk)	32 (33.7)	29 (38.7)	

significant correlation with the number of daily driving hours.

Among taxi drivers and office employees, the concentrations of urinary 1-OHP were significantly higher in subjects with heterozygote (m1/m2) or variant homozygote (m2/m2) genotype of *CYP1A1 Msp*I than in those with the wild homozygote (m1/m1) genotype (Table 4) as well as the *CYP1A1 Hinc*II polymorphism, especially in non-smoking taxi drivers. No significant association was found between the *GSTM1* and *GSTT1* polymorphisms and urinary 1-OHP levels in taxi drivers. Office employees with a null *GSTM1* genotype showed a higher mean urinary 1-OHP level than those with a non-null *GSTM1* genotype, but no significant association was found using univariate analysis between 1-OHP and the *CYP1A1 Hinc*II and *GSTT1* polymorphisms.

To investigate the effect of possible confounding factors in the urinary 1-OHP excretion, multivariate regression analysis was performed (Table 5). We found that taxi drivers and smokers as well as polymorphisms of *CYP1A1 Msp*I and *GSTM1* were the major contributors with adjustments for other covariates.

Multivariate logistic regression analysis with adjustment for age, education level and exercise habit, showed that taxi drivers were 5.1 times independently (95% CI=1.1–13.6) more likely than office employees to have 1-OHP levels at 0.11 μmol/mol creatinine or above (Table 6). The corresponding odds ratio was 5.5 (95% CI=1.6–18.4) for smokers compared with non-smokers. The subjects with the m1/m2 or m2/m2 genotypes of *CYP1A1 Msp*I had excess risk of higher urinary 1-OHP than those with the m1/m1 genotype (OR=9.7, 95%

Table 2. Average urinary 1-OHP concentrations by demographic characteristics and lifestyle factors of taxi drivers and office employees

Variables	Taxi drivers		Office employees		*p* value[c]
	Mean ± SD (*n*)[a]	*p* value[b]	Mean ± SD (*n*)	*p* value[b]	
Age (yr)					
30–34	0.19 ± 0.11 (12)	0.590	0.08 ± 0.05 (9)	0.114	0.014
35–39	0.18 ± 0.10 (30)		0.10 ± 0.07 (9)		0.004
40–44	0.18 ± 0.11 (40)		0.13 ± 0.08 (18)		0.118
≥45	0.14 ± 0.07 (13)		0.10 ± 0.07 (39)		0.107
Education (yr)					
≤9	0.18 ± 0.11 (41)	0.684	0.14 ± 0.09 (23)	0.142	0.116
10–14	0.17 ± 0.10 (48)		0.09 ± 0.06 (37)		<0.001
≥15	0.16 ± 0.10 (3)		0.08 ± 0.04 (13)		0.055
BMI (kg/m^2)					
≤24	0.16 ± 0.11 (32)	0.487	0.10 ± 0.06 (28)	0.629	0.017
> 24	0.18 ± 0.10 (63)		0.11 ± 0.07 (47)		<0.001
Smoking					
Never	0.12 ± 0.05 (40)	<0.001	0.07 ± 0.03 (43)	<0.001	<0.001
Current	0.21 ± 0.11 (55)		0.15 ± 0.08 (32)		0.004
Passive smoking					
No	0.16 ± 0.09 (45)	0.039	0.09 ± 0.06 (35)	0.231	0.001
Yes	0.19 ± 0.11 (50)		0.11 ± 0.08 (40)		0.001
Regular drinking					
No	0.17 ± 0.10 (78)	0.028	0.10 ± 0.07 (64)	0.410	<0.001
Yes	0.20 ± 0.09 (17)		0.12 ± 0.08 (11)		0.032
Areca quid chewing					
No	0.16 ± 0.10 (66)	0.046	0.09 ± 0.06 (62)	0.002	<0.001
Yes	0.20 ± 0.11 (24)		0.16 ± 0.08 (12)		0.254
Exercise habit					
No	0.17 ± 0.11 (68)	0.544	0.10 ± 0.07 (28)	0.861	0.004
Yes	0.17 ± 0.09 (26)		0.10 ± 0.07 (47)		0.001
Incense burning					
No	0.17 ± 0.10 (63)	0.069	0.09 ± 0.05 (46)	0.020	<0.001
Yes	0.19 ± 0.10 (32)		0.14 ± 0.09 (29)		0.022

[a]Values shown are mean ± standard deviation (number). The unit of 1-OHP is μmol/mol creatinine. [b]Comparison within the taxi drivers or office employees. [c]Comparison between the taxi drivers and office employees.

Table 3. Average urinary 1-OHP levels in taxi drivers and office employees by smoking status

Variable	Taxi drivers	Office employees	*p* value[b]
	Mean ± SD (*n*)[a]	Mean ± SD (*n*)	
Smoking habit			
Current smokers	0.21 ± 0.11 (55)	0.15 ± 0.08 (32)	0.004
No. cigarettes per day			
0 (non-smokers)	0.12 ± 0.05 (40)	0.07 ± 0.03 (43)	<0.001
<10	0.18 ± 0.09 (14)	0.14 ± 0.06 (7)	0.191
10–19	0.19 ± 0.09 (25)	0.14 ± 0.09 (13)	0.046
20+	0.25 ± 0.15 (16)	0.17 ± 0.08 (12)	0.076
p value for linear trend[c]	0.0001	0.0001	
All	0.17 ± 0.10 (95)	0.10 ± 0.07 (75)	<0.001

[a]Values shown are mean ± standard deviation (number). The unit of 1-OHP is μmol/mol creatinine. [b]Comparison between the taxi drivers and office employees. [c]Ordinal scores 0, 1, 2 and 3 were assigned to the four levels of exposure.

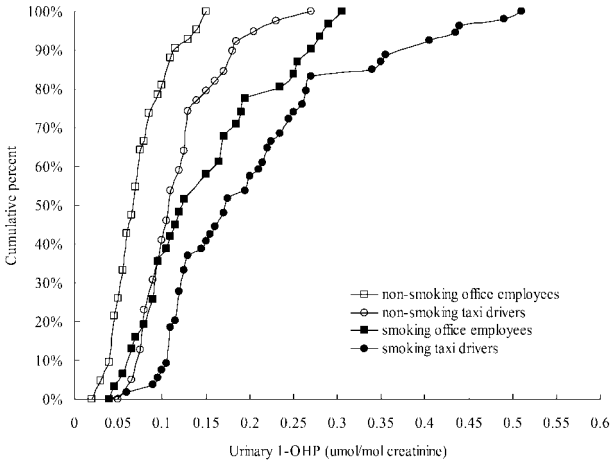

Fig. 1. Cumulative percentage distribution of urinary 1-OHP in taxi drivers and office employees by smoking status.

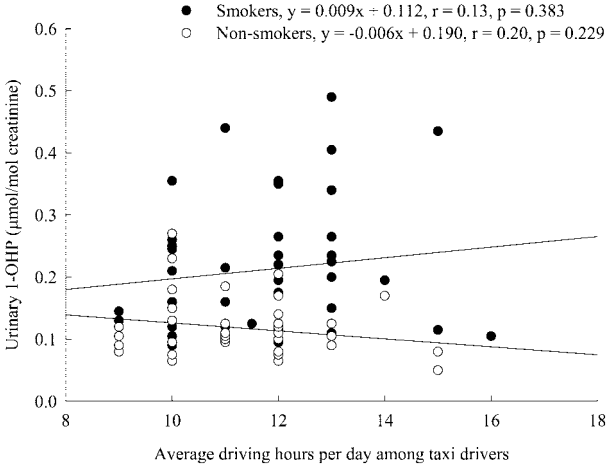

Fig. 2. The correlation between urinary 1-OHP values and the number of daily driving hours of taxi drivers.

Table 4. The level of urinary 1-OHP in taxi drivers and office employees with different *CYP1A1 MspI*, *CYP1A1 Hinc*II, *GSTM1* and *GSTT1* genotypes

	Urinary 1-OHP[a]			
	Taxi drivers		Office employees	
	*CYP1A1 Msp*I			
	m1/m2 or m2/m2	m1/m1	m1/m2 or m2/m2	m1/m1
Total subjects	0.21 ± 0.10 (36)[b]	0.14 ± 0.09 (59)	0.13 ± 0.07 (24)[b]	0.09 ± 0.07 (51)
Non-smokers	0.17 ± 0.04 (12)[b]	0.10 ± 0.03 (28)	0.09 ± 0.04 (15)[b]	0.06 ± 0.03 (28)
Smokers	0.23 ± 0.11 (24)	0.18 ± 0.11 (31)	0.19 ± 0.06 (9)	0.13 ± 0.08 (23)
	*CYP1A1 Hinc*II			
	Ile/Val or Val/Val	Ile/Ile	Ile/Val or Val/Val	Ile/Ile
Total subjects	0.19 ± 0.09 (36)	0.15 ± 0.10 (59)	0.12 ± 0.07 (29)	0.09 ± 0.06 (46)
Non-smokers	0.15 ± 0.05 (16)[b]	0.09 ± 0.03 (24)	0.08 ± 0.04 (17)	0.06 ± 0.03 (26)
Smokers	0.22 ± 0.10 (20)	0.19 ± 0.12 (35)	0.18 ± 0.07 (12)	0.13 ± 0.08 (20)
	GSTM1			
	Non-null	Null	Non-null	Null
Total subjects	0.16 ± 0.07 (44)	0.18 ± 0.12 (51)	0.08 ± 0.05 (35)	0.12 ± 0.07 (40)[b]
Non-smokers	0.11 ± 0.03 (18)	0.12 ± 0.06 (22)	0.06 ± 0.02 (21)	0.08 ± 0.04 (22)[b]
Smokers	0.18 ± 0.08 (26)	0.22 ± 0.13 (29)	0.11 ± 0.07 (14)	0.17 ± 0.07 (18)[b]
	GSTT1			
	Non-null	Null	Non-null	Null
Total subjects	0.16 ± 0.09 (37)	0.17 ± 0.11 (58)	0.11 ± 0.07 (27)	0.10 ± 0.06 (48)
Non-smokers	0.11 ± 0.03 (18)	0.12 ± 0.06 (22)	0.08 ± 0.04 (17)	0.07 ± 0.03 (26)
Smokers	0.20 ± 0.10 (19)	0.20 ± 0.12 (36)	0.18 ± 0.08 (10)	0.13 ± 0.07 (22)

[a]Values shown are mean ± standard deviation (number). The unit of 1-OHP is μmol/mol creatinine.
[b]$p<0.005$ for the comparison with m1/m2 or m2/m2 versus m1/m1, Ile/Val or Val/Val versus Ile/Ile and null versus non-null type group.

Table 5. Multiple regression analysis of 1-OHP level

Independent variables	β[a]	SE[b]	p value
Study subjects			
(taxi drivers vs. office employees)	0.09	0.03	0.001
Age (yr)	–0.001	0.002	0.682
Education (yr)	0.02	0.01	0.187
Smoker (Yes vs. No)	0.18	0.03	<0.001
Areca quid chewing (Yes vs. No)	–0.01	0.04	0.785
Incense burning (Yes vs. No)	0.03	0.03	0.353
*CYP1A1 Msp*I			
(m1/m2 or m2/m2 vs. m1/m1)	0.07	0.03	0.019
*CYP1A1 Hinc*II			
(Ile/Val or Val/Val vs. Ile/Ile)	0.03	0.03	0.287
GSTM1 (Non-null vs. Null)	–0.05	0.03	0.031
GSTT1 (Non-null vs. Null)	0.04	0.03	0.147

[a]Regression coefficient, [b]Standard error.

Table 6. Means, odds ratios (OR) and 95% confidence intervals (CI) for subjects with urinary 1-OHP≥0.11 μmol/mol creatinine by associated variables obtained from multivariate logistic regression

Variables	urinary 1-OHP Mean ± SD (n)[a]	p value	OR (95% CI)[b]
Study subjects			
Office employees	0.10 ± 0.07 (75)	<0.001	1.0
Taxi drivers	0.17 ± 0.10 (95)		5.1 (1.1–13.6)
Smoking status			
No	0.10 ± 0.05 (83)	<0.001	1.0
Yes	0.19 ± 0.11 (87)		5.5 (1.6–18.4)
Areca quid chewing			
No	0.19 ± 0.10 (36)	0.001	1.0
Yes	0.13 ± 0.09 (128)		1.2 (0.3–4.4)
Incense burning			
No	0.13 ± 0.09 (109)	0.018	1.0
Yes	0.16 ± 0.10 (61)		1.5 (0.5–4.3)
*CYP1A1 Msp*I			
m1/m1	0.12 ± 0.08 (110)	<0.001	1.0
m1/m2 or m2/m2	0.17 ± 0.10 (60)		9.7 (2.7–35.0)
GSTM1			
Non-null	0.12 ± 0.07 (79)	0.020	1.0
Null	0.15 ± 0.10 (91)		1.2 (0.4–3.2)

[a]Values shown are mean ± standard deviation (number). [b]Adjusted for age, education, and exercise habits.

CI=2.7–35.0). No significant increase in risk was observed in the subjects with *GSTM1* null type in the model. This regression model also showed that areca quid chewing and incense use non-significantly increased the level of 1-OHP (OR=1.2, 95% CI=0.3–4.4; OR=1.5, 95% CI=0.5–4.3).

Discussion

In this study, we measured the urinary 1-OHP levels of the study subjects as being representative of PAH exposure. There were high correlations between the urinary 1-OHP levels and occupational and environmental exposure to all PAH concentrations. Vaananen *et al.*[36] observed a good correlation between urinary 1-OHP in

paving workers' urine and the airborne concentrations of four- to six-ring PAH as well as total PAH. Merlo *et al.*[37] found in another study that the geometric mean for benzo(a)pyrene (BaP) exposure (an index compound for PAH levels) of traffic police officers (3.67 ng/m^3) could be 70 times higher than that for general office employees, the reference group (0.05 ng/m^3). Urinary 1-OHP could be a suitable biomarker for characterizing workers' PAH exposures. Hansen *et al.*[38] found non-smoking bus drivers excreted more 1-OHP in urine than did mail carriers, indicating that bus drivers were more exposed to PAH than mail carriers. In Taipei City, Chan *et al.*[39] reported the geometric mean for benzene exposure was 160.4 μg/m^3 in bus drivers, 248.2 μg/m^3 in motor vehicle drivers and 370.0 μg/m^3 in motorcycle riders. Tsai *et al.*[40] found that vehicle exhaust significantly affected the total PAH exposure level (11.4 μg/m^3) and 1-OHP levels (3.02 vs. 0.41 μmol/mol creatinine for booth attendants and reference group, respectively) of the booth attendants at a Taiwan highway toll station. The average levels of total hydrocarbons provided by the Taiwan Environmental Protection Administration were detected as 2.27 ppm and 3.37 ppm by general and traffic air monitoring stations, respectively, in the year we performed this study in Taipei City. These data suggest that drivers exposure to traffic exhaust is higher than that of the general population.

Our results show that taxi drivers had higher urinary 1-OHP than office employees (0.17 vs. 0.10 μmol/mol creatinine, $p<0.001$). Previous investigations also indicated that subjects exposed to automobile exhaust fumes in urban areas had a higher risk of PAH exposure and had increased 1-OHP levels in urine[41, 42]. Kuusimaki *et al.*[43] found the concentration of 1-OHP was higher among bus-garage and waste-collection workers exposed to PAH (0.125 μmol/mol creatinine) than in a control group (0.055 μmol/mol creatinine). Autrup *et al.*[44] found that the average urinary 1-OHP value for non-smoking Danish bus drivers in a city center (0.25 μmol/mol creatinine) was similar to that in rural/suburban areas (0.24 μmol/mol creatinine), but higher than that for postal workers (0.15 μmol/mol creatinine). Merlo *et al.*[37] found that the average urinary 1-OHP levels for traffic police officers were 0.102 μmol/mol creatinine for non-smokers and 0.201 μmol/mol creatinine for smokers; office employees had lower average corresponding measurements, 0.067 and 0.179 μmol/mol creatinine, respectively. Ruchirawa *et al.*[45] determined the level of 1-OHP 0.181 μmol/mol creatinine in traffic police officers and 0.173 μmol/mol creatinine in desk-work police officers. Burgaz *et al.*[46] found the values of 1-OHP excretions of taxi drivers and control subjects were 0.57 and 0.32 μmol/mol creatinine, respectively.

These findings of the above researches are similar to the findings of this study for taxi drivers and office employees. The average urinary 1-OHP levels for drivers were 0.12 μmol/mol creatinine for non-smokers and 0.21 μmol/mol creatinine for smokers, higher than the corresponding measurements of 0.07 and 0.15 μmol/mol creatinine for office employees. Taken together, the results indicate non-smoking traffic-related workers have 1-OHP levels elevated about 1.5 folds due to vehicle exhaust. In addition, because of the much smaller cabin volume in a taxi than in a bus, a taxi driver may have higher PAH exposure risk than a bus driver under the same conditions of air pollution[47].

Cigarette smoking has been a significant determinant of urinary 1-OHP concentrations in many previous studies[15, 18, 37, 48–51]. This study confirmed that cigarette smoking significantly increased the urinary 1-OHP concentrations of both taxi drivers and office employees. The urinary 1-OHP concentrations correlated well with the number of cigarettes smoked, suggesting that there is a dose-dependent relationship between smoking and urinary 1-OHP concentration. The increased urinary 1-OHP excretion in smokers could be explained by the fact that cigarette smoke contains a wide variety of toxic xenobiotics including many PAH.

The urinary 1-OHP level was significantly different between taxi drivers and office employees irrespective of smoking status. Smoking taxi drivers had excess urinary 1-OHP of 0.06 μmol/mol creatinine on average compared to office employees who smoked. Non-smoking taxi driving might gain approximately 0.05 μmol/mol creatinine 1-OHP in urine. Motor vehicle exhaust would have contributed to this difference, suggesting that taxi drivers are exposed to PAH in the air during their working activities. Further analysis showed smoking taxi drivers exhibited a positive correlation between the 1-OHP level and taxi driving, however, the number of daily driving hours was not significantly correlated with the 1-OHP values (Fig. 2). It seems that the burden of PAH derived from taxi driving has a limitation.

This study also found that people that using incense were at risk of having higher 1-OHP excretion. Incense burning has been associated with many cultures and religions around the world. Schoental and Gibbard[52] first identified several PAH in incense smoke condensates. When incense is burned at low temperature, much smoke containing PAH is emitted[53, 54], and the emitted particles contain a variety of compounds including PAH and aldehydes[55, 56].

In this study, the non-smoking taxi drivers with m1/m1 genotype of *CYP1A1 Msp*I or Ile/Ile genotype of *CYP1A1 Hinc*II had significantly lower levels of urinary 1-OHP than those with other genotypes of *CYP1A1 Msp*I and *CYP1A1 Hinc*II, which was more obviously than smoking taxi drivers. This result suggests that the increase in urinary 1-OHP arose from traffic exhaust metabolized by the CYP1A1 metabolic pathway. GSTs have been

considered important detoxicants of chemical carcinogens, including PAH, in the diet and tobacco smoke[57]. The *GSTM1* null type increased the number of DNA adducts in coke oven workers with high PAH exposure who smoked[58]. Some previous studies showed the levels of urinary 1-OHP were higher in individuals with the *GSTM1* null genotype than in those with a *GSTM1* non-null genotype[15, 59–61]. In this study, among non-smoking office employees, the level of urinary 1-OHP was significantly higher in subjects with the *GSTM1* null genotype than in those without the *GSTM1* null type. This result can be explained by the fact that a deficiency in *GSTM1* stimulates the glucuronidation pathway, as a result of the accumulation of PAH derivatives that are otherwise conjugated to glutathione[62]. However, among taxi drivers in this study, a significant association was not found between *GSTM1* genotype and urinary 1-OHP concentrations. One possible explanation for this inconsistency is that the taxi drivers had much higher 1-OHP levels than the office employees. There may be a threshold concentration of PAH exposure which results in different activites of the enzymes involved in metabolism and detoxification. During high PAH exposures, only part of the excess PAH can be detoxified by GSTs following the diol epoxide metabolic activation pathway through CYP1A1. At low levels of PAH exposure, possibly complete catalysis and conjugation of diol epoxide metabolites with reduced glutathione takes place protecting individuals from PAH-induced mutagenesis and carcinogenesis. These results were similar to the findings of do Vale Bosso *et al*.[63] They monitored the CYP1A1 polymorphism in sugarcane workers exposed to PAH (harvesting) and the *GSTP1* polymorphism in the non-exposed (non-harvesting) group significantly influenced urinary 1-OHP excretion. Lee *et al*.[64] found CYP1A1 and GSTM1 polymorphisms were statistically significant in analyses of 1-OHP levels among aircraft maintenance workers who smoked. Viezzer *et al*.[58] reported that *GSTT1* non-null individuals had elevated levels of DNA adducts compared with *GSTT1* null subjects. Abnet *et al*.[65] found no effect of deletion in GSTM1 or GSTT1 was associated with urine 1-hydroxypyrene glucuronide concentration. Since previous studies have also reported that urinary 1-OHP levels are not significantly influenced by polymorphic variation of *GSTT1*[59, 66], it is likely that the *GSTT1* polymorphism does not play an important role in the excretion of pyrene.

There are few studies available in the literature which target taxi drivers exposure to PAH and evaluate the individual effects on 1-OHP levels of smoking and vehicle exhaust exposure. This study showed when urinary 1-OHP is used to assess exposure to PAH, taxi driving, smoking and metabolic enzyme polymorphisms independently contributed to the elevated 1-OHP levels. Burgaz *et al*.[46] reported the values of 1-OHP excretions in taxi drivers are higher than control subjects, and the chromosomal aberration frequencies were correspondingly 1.81% and 0.26%. Their results demonstrate that occupational exposure to urban air pollutants leads to significant induction of cytogenetic damage in taxi drivers. Thus, an increased risk of cancer may result in occupations with heavy exposure to traffic-related pollution.

In multivariate logistic regression analysis, our results show that smokers had higher adjusted odds ratio for having elevated urinary 1-OHP than taxi driving, and the excess levels due to traffic exhaust and smoke were regulated by the *CYP1A1 Msp*I genotype. Smoking contributed to the elevated urinary 1-OHP levels in addition to taxi driving. We propose the excess PAH exposure cannot be absolutely detoxified by GSTs, and the PAH-activated diol epoxides from CYP1A1 pathway may cause individual mutagenesis and carcinogenesis. The urinary 1-OHP may be a suitable biomarker for characterizing workers' PAH exposures, and the health effects of PAH exposure from automobile exhaust fumes deserves further attention.

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