Genetic effects on urinary 1-hydroxypyrene levels in a Korean population

Mihi Yang1, Jae-Yeon Jang2,3, Soyeon Kim2, Su-Man Lee1, Seong-Sil Chang2, Hae-Kwan Cheong3, Eunil Lee5, Dahee Kang1, Ho Kim6, Toshihiro Kawamoto7 and Hyong Doo Shin8

1Department of Preventive Medicine/Cancer Research Institute, Seoul National University, Seoul, Korea, 2Department of Preventive Medicine and Public Health, Ajou University, Suwon, Korea, 3Department of Preventive Medicine and Public Health, Chungnam National University, Korea, 4Department of Preventive Medicine, College of Medicine, Dongguk University, Korea, 5Department of Preventive Medicine, Korea University, Seoul, Korea, 6School of Public Health, Seoul National University, Seoul, Korea, 7Department of Environmental Health, University of Occupational and Environmental Health, Kitakyushu, Japan and 8Department of Genetic Epidemiology, SNP Genetics, Seoul, Korea

Urinary 1-hydroxypyrene (1-OHP) has been used as a biomarker for assessing the level of exposure to environmental carcinogenic polycyclic aromatic hydrocarbons (PAHs). In order to perform the appropriate biological monitoring for examining the level of exposure to PAHs, this study investigated whether or not genetic polymorphisms of the metabolic enzymes, which might be involved in the metabolism of pyrene, affected the urinary 1-OHP levels in a population of 661 Koreans (male, 63%; female, 37%; mean age, 36.5 ± 11.1 years) who were not occupationally exposed to PAHs. Urinary 1-OHP was detected in 76% of the subjects (range 0.001–3.8 µg/l). Among the physical and lifestyle factors, cigarette-smoking was found to be associated with the urinary 1-OHP levels (P < 0.05). After adjusting for these factors, we found that the GSTT1 genotypes affected the urinary 1-OHP levels, i.e. the GSTT1 present subjects had ~1.5 times the urinary 1-OHP level than the GSTT1 null subjects (P < 0.05). In the case of the subjects who were also GSTM1 null, this trend became stronger, i.e. the GSTT1 present subjects had ~2 times the urinary 1-OHP level (P < 0.01). However, the genetic polymorphism of the other metabolic enzymes, cytochrome P-450 (CYP)1A1, CYP1B1 and GSTM1 alone, did not affect the urinary 1-OHP level. Therefore, this study suggests that the GSTT1 genetic polymorphism has the potential to affect the biological monitoring of PAHs with urinary 1-OHP, and might act as a genetic factor in PAH-related toxicity.

Introduction

A number of polycyclic aromatic hydrocarbons (PAHs), such as benzo(a)pyrene, are carcinogenic and believed to contribute to the overall burden of human cancer (1). PAHs are ubiquitous in the environment and humans are exposed to them via numerous pathways, e.g. air or soil in urban areas, exposure directly or indirectly to tobacco smoke, and ingestion of food or water polluted by combustion effluents (2,3).

Because 1-hydroxypyrene (1-OHP) is the major metabolite of pyrene, one of the PAHs, urinary 1-OHP has been used as a PAH-exposure biomarker (3). In order to conduct appropriate biological monitoring to assess the exposure to PAHs, individual differences in biotransformation of pyrene to 1-OHP should be considered. If the metabolic enzymes involved in the biotransformation of 1-OHP are genetically polymorphic, their activities would be expected to vary between individuals. There have been reports examining the association between the urinary 1-OHP and genetic polymorphisms in cytochrome P-450 (CYP)1A1 and glutathione S-transferase (GST) M1, but only in small populations (4–11). However, these studies have reported inconsistent results. Therefore, it is unclear as to whether CYP1A1 and GSTM1 genetic polymorphisms affect the biotransformation of urinary 1-OHP. On the other hand, CYP1B1 is an important contributor to the activation of PAHs (12), and a relationship between cancer and CYP1B1 genetic polymorphisms has been reported (13). In addition, GSTM1-deficient people may have alternative metabolic pathways using GSTT1 (14). As a result, the effects of genetic polymorphisms in the CYP1A1, CYP1B1, GSTM1 and GSTT1 on urinary 1-OHP were examined in a population of 661 Koreans.

Materials and methods

Subjects

661 Koreans who were recruited from people who visited five hospitals for a regular health examination, i.e. Seoul National University Hospital and Korea University Hospital at Seoul, Dongguk University Hospital at Gyeongju, Chunnam National University Hospital at Daejeon and Konkuk University Hospital at Chungju, Korea during 2001, were enrolled in this study. They were given an explanation for the study. People who were suspected of being occupationally exposed to PAHs and whose cases were diagnosed with diseases were excluded. The subjects were required to fill out questionnaires on their occupation, education and lifestyle habits, such as tobacco smoking and alcohol consumption (age, 36.5 ± 11.1 years; 63.3% of the subjects, males; 38.9%, smokers; 74.5%, alcohol drinkers). Blood and a spot urine sample were collected from each subject prior to breakfast.

Analysis of urinary 1-OHP and creatinine

The urinary 1-OHP levels were examined using a reverse phase HPLC method (15) and for creatinine using an ion pair reverse-phase HPLC method (16). The HPLC system consisted of a Waters 515 HPLC Pump, a Waters Automated Gradient Controller, and 717plus Autosampler, and a TOSOH TSK-gel ODS-80TM column (4.5 × 150 mm). The 1-OHP was detected using a fluorescence detector (Waters™ 474 Scanning) and the creatinine using a UV-detector (Waters™ 486 Tunable Absorbance Detector). The coefficient of variation in the 1-OHP analyses was 6.5–19.5% (n = 5). The detection limit for the 1-OHP was 0.001 ng/ml.

Determination of genotypes

Genomic DNA was isolated from the buffy coat fraction of each blood sample using a DNA isolation kit provided by Promega (Madison, WI). The CYP1A1 genetic polymorphism was based on single nucleotide polymorphism (SNP) that results in amino acid substitutions at 462 in exon 7 (I462V) (17). The CYP1B1 genotypes were determined according to seven SNPs (17), i.e. R48G and...
and A119S in exon 2, and D374N, E387K, D374N and R469W in exon 3. Genotyping of the CYP1A1 and the CYP1B1 was achieved using the following single base extension methods.

**PCR (polymerase chain reaction) procedure.** PCR was performed in a mixture of 1.25 pmol of each primer (Table I), 50 ng genomic DNA, 250 mM dNTPs and 0.15 U Taq DNA polymerase (Applied Biosystems, Foster City, CA) in the buffer provided by the manufacturer. Amplification was performed in a GenAmp PCR System 9700 thermal cycler (Applied Biosystems) under touchdown conditions (18). To clean the PCR reaction for the primer extension reaction, one unit of SAP (Shrimp Alkaline Phosphatase, Amersham Life Sciences) was added to the PCR products. The mixture was incubated at 37°C for 1 h, followed by 15 min at 72°C to inactivate the enzyme.

**Electrophoresis.** The DNA samples, containing the extension products, and Genescan 120 Liz size standard solution were added to the Hi-Di formamide (Applied Biosystems) according to the manufacturer’s instructions. The mixture was incubated at 95°C for 5 min, then by 5 min on ice, which was followed by electrophoresis using an ABI Prism 3100 DNA Analyzer. The results were analyzed using the ABI Prism GeneScan and Genotyper programs (Applied Biosystems). A multiple PCR method with three sets of primers for GSTM1, GSTT1 and an internal reference gene (\( \beta \)-globin) was used to determine the GSTM1 and GSTT1 genotypes because of presence of either GSTM1 or GSTT1 in genomic DNA samples (14,20). Positive and negative control samples were analyzed in each reaction and the GSTM1 and GSTT1 genotypes were not scored unless the PCR product from the internal reference gene was evident.

To confirm the genotyped results, 200 ID blind samples were regenotyped and reliable reproducibility was obtained for each genotype (coefficient variation < 1%).

**Table I. Sequences of the amplifying and extension primers for CYP1A1 and CYP1B1 SNP genotyping by single base extension method**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1 I462V A&gt;G</td>
<td>Forward</td>
<td>5'-GACGGTTTCTCACCCTCCTAT-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5'-GATGCGATTTGGAAGATCTCA-3'</td>
</tr>
<tr>
<td></td>
<td>Extension</td>
<td>5'-GTGTAAGATAGCTCATATGCT-3'</td>
</tr>
<tr>
<td>CYP1B1 R48G C&gt;G</td>
<td>Forward</td>
<td>5'-GTCACCGCTCCTCTCCTCG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5'-TGAAGATGTCCTCAGTCAGTCT-3'</td>
</tr>
<tr>
<td></td>
<td>Extension</td>
<td>5'-GTAACCGGAGGGCCAGCTGCT-3'</td>
</tr>
<tr>
<td>CYP1B1 A119S G&gt;T</td>
<td>Forward</td>
<td>5'-TGAAGTATTTGCCTCCTTG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5'-ATATAGTTATATAATCATGATTATAATCAATGATAGGAGTG-3'</td>
</tr>
<tr>
<td></td>
<td>Extension</td>
<td>5'-CATAATCATGATGATTATAATCAATGATAGTACGATGCTGAGGCAGATGCTT-3'</td>
</tr>
<tr>
<td>CYP1B1 D374N G&gt;A</td>
<td>Forward</td>
<td>5'-TGAAGTATTTGCTCCTTG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5'-ATATAGTTATATAATCATGATTATAATCAATGATAGGAGTG-3'</td>
</tr>
<tr>
<td></td>
<td>Extension</td>
<td>5'-CATAATCATGATGATTATAATCAATGATAGTACGATGCTGAGGCAGATGCTT-3'</td>
</tr>
<tr>
<td>CYP1B1 E387K G&gt;A</td>
<td>Forward</td>
<td>5'-GTCCTTTTCGTTACCGAGG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5'-GTCCTTTTCGTTACCGAGG-3'</td>
</tr>
<tr>
<td></td>
<td>Extension</td>
<td>5'-GTCCTTTTCGTTACCGAGG-3'</td>
</tr>
<tr>
<td>CYP1B1 L432V C&gt;G</td>
<td>Forward</td>
<td>5'-GTCCTTTTCGTTACCGAGG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5'-GTCCTTTTCGTTACCGAGG-3'</td>
</tr>
<tr>
<td></td>
<td>Extension</td>
<td>5'-GTCCTTTTCGTTACCGAGG-3'</td>
</tr>
<tr>
<td>CYP1B1 N453S A&gt;G</td>
<td>Forward</td>
<td>5'-GTCCTTTTCGTTACCGAGG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5'-GTCCTTTTCGTTACCGAGG-3'</td>
</tr>
<tr>
<td></td>
<td>Extension</td>
<td>5'-GTCCTTTTCGTTACCGAGG-3'</td>
</tr>
<tr>
<td>CYP1B1 R469W C&gt;T</td>
<td>Forward</td>
<td>5'-GTCCTTTTCGTTACCGAGG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5'-GTCCTTTTCGTTACCGAGG-3'</td>
</tr>
<tr>
<td></td>
<td>Extension</td>
<td>5'-GTCCTTTTCGTTACCGAGG-3'</td>
</tr>
</tbody>
</table>

Statistical analysis

The following non-continuous genetic parameter values were categorized for statistical analysis: CYP1A1 codon 462 polymorphism, Ile/Ile = 1, Ile/Val or Val/Val = 0; CYP1B1 codon 48 polymorphism, Arg/Arg = 1, Arg/Gly or Gly/Arg = 0; CYP1B1 codon 119 polymorphism, Ala/Ala = 1, Ala/Ser or Ser/Ala = 0; CYP1B1 codon 432 polymorphism, Leu/Leu = 1, Leu/Val or Val/Val = 0; CYP1B1 codon 453 polymorphism, Asn/Asn = 1, Asn/Ser or Ser/Asn = 0; GSTT1 Null = 0, Present = 1; GSTM1 Null = 0, Present = 1; Standard Least Squares-multiple regression analysis was applied to adjust for age, sex, BMI (body mass index), and smoking. Two-tailed Fisher’s exact tests were used to determine the linkage of each SNP. The P-values used to evaluate the significance of the above analyses were computed using JMP7 (SAS Institute, Cary, NC).

**Results**

**Distribution of genetic polymorphisms in metabolic enzymes**

CYP1B1 has several SNPs, but there are no reports of the distribution of CYP1B1 genotypes in Koreans. Therefore, the CYP1B1 genotypes were determined based on 7 SNPs. The CYP1B1 codon 48 polymorphism was linked to the codon 119 polymorphism \( (P < 0.0001) \), and was associated with the codon 432 polymorphism \( (P < 0.05) \). There were no genetic polymorphisms for CYP1B1 codon 374, 387 nor 469. As a result of the SNPs on the CYP1B1 codons 48, 119, 432 and 453, 8 haplotypes of CYP1B1 were found (Table II). Table III shows the distribution of genetic polymorphisms in the metabolic enzymes, CYP1B1, CYP1A1, GSTM1 and GSTT1. The distribution of the genotypes of the CYP1A1 and the 1B1 followed Hardy–Weinberg’s law (21). Ethnic differences were found in these genotypes: for example, the distribution of the CYP1B1 codon 432 polymorphism in our population (Leu versus Val: 0.90 versus 0.10) was different from that of

---

M. Yang
Africans (0.25 versus 0.75), Caucasians (0.57 versus 0.43) and Chinese (0.83 versus 0.17) (22).

**Distribution of urinary 1-OHP**

Urinary 1-OHP was detected in 74% of the study population (range 0.001–3.79 μg/l). The distribution of the 1-OHP levels was almost logarithmically normal with or without creatinine modification. Therefore, in order to conduct further statistical analyses, the quantified 1-OHP levels were geometrically transformed. Disregarding the subjects with undetectable 1-OHP levels, the geometric mean (GM) of the urinary 1-OHP concentrations were 0.0724 μg/l [geometric standard deviation (GSD), 0.0003] and 0.025 μmol/mol creatinine (GSD, 0.0001) without and with creatinine correction, respectively. For statistical analyses, a value of 0.0005 μg/l urinary 1-OHP was assigned for the undetectable levels, this being half the minimum value of the detectable urinary 1-OHP levels.

**Effects of genetic polymorphisms on urinary 1-OHP**

Of all the two-way interactions between the physical and lifestyle factors, only the interaction between age and number of cigarettes smoked was found to be significant. Therefore, we included all the main effects and the interaction between age and the number of cigarettes smoked in the regression analysis model for the urinary 1-OHP levels. As the CYP1B1 codon 432 polymorphism was associated with the codon 48 and 119 polymorphisms, this study considered the CYP1B1 codons 432 and 453 polymorphisms in CYP1B1 SNPs. As a result, the GSTT1 in genetic factors and the 'cigarette smoking-related factor' from the lifestyle factors appeared to affect urinary 1-OHP levels (Table IV). When the gene–gene interaction was examined, it was found that GSTT1 and GSTM1 interacted with the 1-OHP levels (Table V). Specifically, the GSTT1 present subjects had ~1.5 times the urinary 1-OHP levels found in the GSTT1 null subjects [GM 0.015 (GSD 0.001) versus G.M. 0.016 (GSD 0.001), P < 0.05]. In the case of subjects who were also GSTM1 null, the above trend was stronger, i.e. the GSTT1 present subjects had ~2 times the urinary 1-OHP levels found in the GSTT1 null subjects [GM 0.033 (GSD 0.001) versus G.M. 0.015 μg/l (GSD 0.001), P < 0.01], compared with the cases without consideration of the GSTM1 genotype (P < 0.05).

**Discussion**

**Effects of CYP1A1 and CYP1B1 genetic polymorphisms on urinary 1-OHP**

As a result of the broad substrate-specificity of CYPs, no pyrene-specific CYPs have been clarified. In addition, previous studies examining the association between the urinary 1-OHP and genetic polymorphism of CYP 1A1 have reported inconsistent results (4–11). Therefore, it is unclear as to whether CYP1A1 genetic polymorphisms affect the biotransformation of urinary 1-OHP. This study found no association
between the CYP1A1 genetic polymorphism and the urinary 1-OHP levels (Table IV), which concurs with a previous negative report based on a large Japanese population (n = 527) (9). Recently, several in vitro kinetic studies were undertaken to confirm the epidemiological reports on the association between the CYP1A1 genotype and phenotype, which showed no association (23,24). This study appears to suggest the I462V polymorphism in human CYP1A1 does not play an important role in the 1-OHP biotransformation.

As CYP1B1 is involved in estrogen metabolism and is induced by endocrine disruptors, such as dioxin, the CYP1B1 genetic polymorphism is believed to be an important potential factor in the susceptibility to breast and lung cancers (13). Recently, Hanna et al. reported that the estrogen hydroxylation activities were different among CYP1B1 genetic polymorphisms (25). CYP1B1 is also known to be an activator of PAHs (13). However, it is unclear as to what extent CYP1B1 is involved in the 1-OHP biotransformation, and there are no reports concerning the effect of CYP1B1 on the urinary 1-OHP levels. This study found no association between the CYP1B1 genetic polymorphism and the urinary 1-OHP levels (Table IV). Thus, even though CYP1B1 activates PAHs, it is not thought to play an important role in 1-OHP biotransformation in this study population.

**Effects of GSTM1 genetic polymorphism on urinary 1-OHP**
The genetic polymorphism of GSTM1 is thought to cause susceptibility to cancer due to a distinct enzyme activity change, which is followed by gene loss. As GSTM1 functions to detoxify carcinogens, the GSTM1 null subjects have been thought as being a higher cancer risk group than the GSTM1 present subjects (14). For example, the GSTM1 null genotype correlated with an enhanced mutagenicity of the urine in both smokers and subjects from polluted regions (26). However, GSTM1 is one of the diverse isozymes of GSTs, and its distribution throughout the organs is highly variable in GSTM1-present subjects: Rowe et al. (27) showed GSTM1 to be the second most important GST isozyme in the liver following GSTA1, but was only minor in other organs. Even individual GST subunits may be localized in different cell types of a single organ. Therefore, the functional consequences of a lack of GSTM1 subunits are uncertain (27).

Lee et al. (4) and Alexandrie et al. (8) reported an association between the urinary 1-OHP levels and GSTM1 genetic polymorphisms. However, this association was observed in pot room workers, or smokers, who were highly exposed to PAHs. Furthermore, their studies were based on small populations (n = 97 and 218, respectively). This study found no association between urinary 1-OHP and GSTM1 genetic polymorphism (Table IV). As the degree of exposure to PAHs is suspected of inducing different metabolic enzymes (28), the effects of GSTM1 genetic polymorphisms on the urinary 1-OHP levels of subjects with high 1-OHP concentrations (>0.07 μg/l) and of the smokers were investigated in this study. However, no significant association was found (data not shown). As a result, it is suggested that GSTM1 does not play an important role in the 1-OHP biotransformation.

**Effects of GSTT1 genetic polymorphism on urinary 1-OHP**
GSTT1 detoxifies monohalomethanes and some epoxides, but bioactivates methylene chloride and some bifunctional alkylating agents (29). Namely, GSTT1 expression may be harmful or beneficial depending on the class of chemical exposure. In addition, the bioactivation of carcinogens by GSTT1 is suggested to be a reason why the GSTM1 null and GSTT1 present individuals show a higher cancer risk (14). In the present and huge non-occupationally PAH-exposed subjects (n = 661), i.e. relatively low PAH-exposure population, the fact that the GSTT1 present individuals have higher urinary 1-OHP levels than the GSTT1-deficient individuals is significant. Some reports (8,11,30) based on small populations (n < 200) found a similar trend among high PAH exposed subjects, such as coke oven workers or smokers. However, they did not find the trend to be significant in their whole subjects. With the smokers presenting this study, we also found higher urinary 1-OHP levels in the GSTT1 present subjects than in the GSTT1 null subjects. Therefore, our results suggest that the genetic polymorphism of GSTT1 affects the urinary 1-OHP levels regardless of the PAH-exposure levels.

Even though the role of GSTT1 on pyrene metabolism should be clarified by in vitro kinetic studies, our epidemiological study suggests that GSTT1 plays an important role in the biotransformation of pyrene. In addition, genetic polymorphisms in other main phase II metabolic enzymes, sulfotransferases, uridine diphosphate glucuronosyltransferases and N-acetyltransferases, should be examined to determine if they are involved in the 1-OHP biotransformation from pyrene.

In conclusion, this study based on a reliable sample size and genotyping suggests that genetic polymorphisms among CYP1A1, CYP1B1, GSTM1 and GSTT1 affect urinary 1-OHP levels in a non-occupationally PAH-exposed population. Considering their environment, lifestyle and genetic polymorphisms in the above four enzymes, this study found that the genetic polymorphism of GSTT1 affects urinary 1-OHP levels rather than the other genetic polymorphisms. Therefore, the GSTT1 genetic polymorphism has the potential to affect the biological monitoring of PAHs with urinary 1-OHP, and might act as a genetic factor in PAH-related toxicity.

**Acknowledgements**
This study was funded by a grant from the Ministry of Environment of the Republic of Korea. We would like to thank Dr Brian F. Coles, Division of Molecular Epidemiology, the National Center for Toxicological Research, USA, for his kind comments.

**References**
Genetic effects of urinary 1-hydroxypyrene levels


Received November 8, 2002; revised February 14, 2003; accepted March 20, 2003