CYPIA1, GSTM1, and GSTP1 Genetic Polymorphisms and Urinary 1-Hydroxypyrene Excretion in Non-Occupationally Exposed Individuals

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Abstract
The CYPIA1 and glutathione S-transferase enzymes (e.g., GSTM1 and GSTP1) are involved in the activation and conjugation of polycyclic aromatic hydrocarbons (PAHs), respectively, and are controlled by genes that are polymorphic. The CYPIA1*2 allele variant has been associated with elevated urinary 1-hydroxypyrene (1-OHP), a proposed marker for internal dose of activated PAHs, in coke-oven workers. We investigated whether this association could be observed at low exposure levels, such as those experienced by the general population. We conducted a cross-sectional study among 188 individuals (106 Japanese, 60 Caucasians, and 22 Hawaiians) who were selected as controls in a population-based case-control study and provided lifestyle information, a 12-h control study and provided lifestyle information, a 12-h data add to the growing evidence suggesting that individual variability in PAH activation (7, 11, 12).

Introduction
PAHs are generated by the incomplete combustion of organic materials. The general population is exposed to low levels of these chemicals, mostly through smoking and diet (1, 2). PAHs undergo metabolic activation, mainly by cytochrome P4501A1 (3), to diol epoxides capable of binding covalently to DNA, potentially initiating the carcinogenic process. Activated PAH metabolites can be detoxified by phase II enzymes, such as GST and uridine diphosphoglucuronosyltransferase, which catalyze conjugative reactions of oxidative products (4, 5). 1-OHP, a major oxidative metabolite of pyrene that can be measured in urine, is commonly used as a biomarker of internal dose for PAHs (6). Increased urinary levels of 1-OHP have been observed after ingestion of broiled meats or application of coal tar-based ointment, as well as among smokers and workers exposed to coal tar fumes, mineral oil, or bitumen (7–10). When 1-OHP is measured after treating the urine with deconjugating enzymes (e.g., β-glucuronidase), the sum of conjugated and deconjugated species are quantified, reflecting the total amount of hydroxylated metabolite. Although 1-OHP itself is not a carcinogen, it can be used as a marker for a major activating step in the metabolism of PAHs. Past studies have indicated that there is wide variability in urinary 1-OHP excretion among similarly exposed individuals, suggesting interindividual variability in PAH activation (7, 11, 12).

The CYPIA1 gene is polymorphically expressed in humans, and variant alleles at this locus have been associated with an increased risk for lung cancer in smokers (13–15). In a population-based case-control study, we have shown recently that the presence of the CYPIA1*2 variant allele was associated with a 2.4-fold (95% CI, 1.2–4.7) increased risk of squamous cell carcinoma of the lung, a tumor thought to be specifically related to PAHs (15). A number of in vitro studies have suggested that individuals with the CYPIA1*2 or the closely linked CYPIA1*3 variant allele may have a greater capacity to activate PAHs from environmental sources (16). However, few in vivo confirmatory data are available. Two recent occupational epidemiology studies have explored the association between CYPIA1 and urinary 1-OHP among workers exposed to relatively high levels of PAHs (17, 18). Both found higher postshift 1-OHP levels for individuals with the variant CYPIA1*2 allele, compared with those with the common allele (17, 18). The present study investigated whether the same association could be observed in the general population, which is typically exposed to only low levels of PAHs.

1 The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; GST, glutathione S-transferase; 1-OHP, 1-hydroxypyrene; CI, confidence interval; HPLC, high-performance liquid chromatography.
Table 1  Percent distribution of subjects by genotype and main characteristics

<table>
<thead>
<tr>
<th>Genotype</th>
<th>GSTM1</th>
<th>GSTP1</th>
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<tbody>
<tr>
<td>A/A</td>
<td></td>
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<tr>
<td>A/B</td>
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<td>B/B</td>
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Materials and Methods

**Chemicals.** 1-OHP standard was obtained from Aldrich (Milwaukee, WI). β-Glucuronidase and aroylsulfatase was obtained from Boehringer Mannheim (Mannheim, Germany). HPLC grade methanol was from Fisher Scientific (Houston, TX), whereas all other chemicals were of analytical grade.

**Study Subjects.** Twelve-h urine samples were collected overnight from 112 males and 76 females consecutively interviewed as controls for a case-control study of colorectal cancer in Hawaii. Subjects were identified among Oahu residents interviewed by the Hawaii State Department of Health as part of a health survey of a 2% random sample of state households or among Health Care Financing Administration participants on Oahu. These subjects were matched on sex, age (±2.5 years), and ethnicity to the colorectal cancer cases identified during the accrual period. In-person interviews conducted at the subjects’ homes provided information about lifetime smoking history and other lifestyle risk factors. Subjects were requested to collect their urine overnight, during a specified 12-h period. The urine samples were stored on ice during collection and until delivery to the lab, where they were aliquoted and frozen at −80°C. Blood samples were processed within 2 h of collection, and the buffy coat was frozen in the same way. The laboratory analyses for 1-OHP was performed blinded to the genotype and exposure status of the subjects.

**Laboratory Analyses.** 1-OHP was extracted from the urine and measured with a modification of the HPLC method described by Jongeneelen et al. (7). Acetate buffer (0.1 M; pH 5.0) was added to a 10-ml aliquot of urine, to a total volume of 30 ml, and adjusted to pH 5.0 with 2 M hydrochloric acid. The mixture was incubated overnight with 12.5 μl of β-glucuronidase/arylsulfatase (100,000 Fisherman units/800,000 Roy units) at 37°C. The samples were then centrifuged, and the metabolites were extracted and purified on Sep-Pac C18 reverse-phase cartridges. The cartridges were activated by 5 ml of methanol, followed by 10 ml of water, before applying the hydrolyzed urine sample. The samples were subsequently washed with 5 ml each of water and 50% methanol and finally eluted with 10 ml of methanol. The final products were dried and dissolved in 50 μl of methanol before HPLC analysis.

Chromatography was performed on a reversed phase Supelcosil LC-18 column (150 × 4.6 mm; 3 μm pore size) with a linear gradient of methanol in water from 46 to 96% (flow rate, 0.6 ml/min) in 63 min. The column was maintained at a constant temperature of 25°C. 1-OHP was detected by fluorometry (excitation, 239 nm; emission, 389 nm).

Samples of hydrolyzed urine (that did not contain 1-OHP; blank urine) were spiked with the given analyte. These calibration samples were treated as described above. A minimum of five concentrations across the observed range were used to determine the recoveries. Reagent blanks were used to monitor interference. External standard curve was generated using six standard concentrations ranging from 1.0 to 0.01 pmol. Peak areas were used for quantification.

A set of 26 samples, selected randomly, were analyzed in duplicate on different days. The values were then used to calculate the interassay variability. All of the 188 samples were analyzed in duplicate, and the individual values were used to calculate the intraassay coefficient of variation for urinary 1-OHP. The inter- and intraassay variation of coefficient for urinary 1-OHP was 7 and 3%, respectively. Concentrations of 1-OHP were expressed as nmol/12 h.

DNA was extracted from blood lymphocytes, and the samples were genotyped for CYP1A1*2, CYP1A1*3, and GSTM1 as described previously (15). The polymorphisms at the GSTP1 locus were identified using the primers P105F (5′-ACC-CAGGGCTCTATGGGAA-3′) and P105R (5′-TGAGGGCAACAAAGGCCCT-3′) as described by Harries et al. (19).

**Statistical Analysis.** To compare the percent distributions between groups, we used the χ2 test of association. We used analysis of covariance to compare mean 1-OHP levels between groups (e.g., CYP1A1 genotype) while adjusting for sex, race, and age. The number of cigarettes smoked per day was also used as an adjustment variable in models including smokers. This variable was set to 0 for nonsmokers. The distribution of 1-OHP was skewed to the right. Therefore, it was transformed as log(X + 1) to make its distribution normal (tested by the Shapiro-Wilk test), thereby ensuring that the model assumptions were met. The Ps are based on the log-transformed values.

**Results**

The subjects included 106 Japanese, 60 Caucasians, and 22 Hawaiians. The age of the subjects ranged from 39 to 84 years, with a mean of 67.6 ± 10.3 years. Table 1 shows the percent distributions of the subjects by CYP1A1, GSTM1, and GSTP1 genotypes and sex, ethnicity, and smoking status. No significant differences were found in the genotype distributions of men and women and of (current) smokers and nonsmokers by the χ2 test. The frequency for CYP1A1*2 and CYP1A1*3 alleles was higher for Japanese (33.5% and 22.2%, respectively) and Hawaiians (45.5% and 25.0%, respectively) than for...
Caucasians (12.5% and 5.0%, respectively). Similarly, the GSTM1 null genotype was more common in Hawaiians (63.6%) than in Caucasians (58.3%) and Japanese (52.8%). These frequencies are very similar to those found in our previous study (15). The GSTP1 exon 5 polymorphism appears to be less common in Japanese (13.7%) than in Caucasians (27.5%) and Hawaiians (25.0%).

Urinary 1-OHP concentrations for the subjects ranged from 0.02 to 3.9 nmol/12 h. The geometric mean urinary 1-OHP (in nmol/12 h) was 0.32 (95% CI, 0.25–0.38) for males, 0.27 (95% CI, 0.19–0.35) for females, 0.27 (95% CI, 0.21–0.34) for Japanese, 0.34 (95% CI, 0.25–0.45) for Caucasians, and 0.30 (95% CI, 0.16–0.46) for Hawaiians. These means were not significantly different between sexes (P = 0.40) or ethnic groups (P = 0.47). However, 1-OHP urinary levels were found to differ by smoking status. After adjustment for age and ethnicity, current smokers (n = 23) had significantly higher levels of 1-OHP (0.51 nmol/12 h; 95% CI, 0.34–0.69) as compared with nonsmokers (n = 165; 0.27 nmol/12 h; 95% CI, 0.22–0.32; P = 0.006).

The effects of the genotypes on urinary 1-hydroxypyrene are shown in Table 2. Overall and in nonsmokers, urinary 1-OHP was not associated with either the CYP1A1*2 or CYP1A1*3 alleles (Table 2). In contrast, smokers carrying at least one CYP1A1*2 variant allele excreted significantly higher levels of urinary 1-OHP (0.64 nmol/12 h; 95% CI, 0.00–3.60) than smokers with the wild-type genotype (*1/*1; 0.32 nmol/12 h; 95% CI, 0.16–0.50; P = 0.02). Similarly, smokers with at least one CYP1A1*3 variant allele excreted higher levels of 1-OHP than those with the wild-type genotype, and the difference was of borderline significance (P = 0.10). In contrast, the GSTM1 and GSTP1 polymorphisms were not associated with urinary 1-OHP levels in smokers or nonsmokers.

We also looked at the combined effects of CYP1A1*2 with GSTM1 or GSTP1 on urinary 1-OHP. For current smokers, as well as for nonsmokers, individuals with both the CYP1A1*2 allele and GSTM1 or GSTP1 polymorphism had slightly higher levels of urinary 1-OHP but not significantly so (data not shown). However, no interaction was detected, and GSTM1 and GSTP1 had only minor effects on 1-OHP levels.

### Discussion

In this population-based cross-sectional study, we observed an association between linked polymorphisms in CYP1A1 and urinary 1-OHP levels in smokers, after adjusting for smoking dose. No association was found among nonsmokers. Also, no associations were found between 1-OHP levels and polymorphisms in GSTM1 or GSTP1, among smokers and nonsmokers alike.

A relatively large number of statistical tests were performed in the present study, increasing the possibility that any significant results were found by chance. Thus, it is important to interpret our results within the context of past studies on PAH metabolism in humans.

Humans are exposed to PAHs from various occupational, environmental, lifestyle, and therapeutic sources (20, 21). It is known that occupational and therapeutic sources of PAHs result in the highest levels of individual exposure (1, 22). However, these high exposures occur in relatively small groups of individuals, whereas the general population is exposed to lower levels, mainly through smoking and diet (1, 2). 1-OHP is a major metabolite of pyrene that has been shown to be a good marker for total PAH exposure (23) and is thought to reflect PAH activation when both conjugated and deconjugated compounds are measured (17, 18).

Few human studies have used this marker to examine the effect of CYP1A1 polymorphisms on the metabolism of PAHs. Wu et al. (18) observed that coke-oven workers with the CYP1A1*2/*2 genotype had higher postshift urinary 1-OHP than did those with the wild-type and heterozygous genotypes combined, after adjusting for exposure level. Merlo et al. (17) studied traffic police officers exposed to ambient air PAHs in an urban environment. They found that officers who smoked ≥15 cigarettes/day and who had at least one CYP1A1*2 allele had higher postshift urinary 1-OHP levels than those with the wild-type genotype. No differences in 1-OHP levels were found by genotype in nonsmokers or smokers of >15 cigarettes/day. Our data in non-occupationally exposed individuals are consistent with these previous findings in suggesting that the CYP1A1*2 allele modulates urinary 1-OHP excretion in smokers but not in nonsmokers. Our data were also suggestive of a greater effect of the CYP1A1*2 allele at lower levels of smoking (Table 3) and the number of smokers was too small for this study to be conclusive on this issue.

CYP1A1*2 has been associated with an increased risk of squamous cell carcinoma of the lung (13–15, 24) and of early-stage colorectal cancer (25). These associations could at least partially be explained if individuals with the variant CYP1A1 allele had a greater capacity to activate PAHs and experienced, as a result, a greater amount of DNA adduction than individuals with the wild-type allele. However, the evidence for such a relationship has as yet been limited, especially at low levels of exposure. A number of in vitro studies have observed higher gene expression levels and an increased aryl hydrocarbon hydroxylase activity and/or inducibility associated with the CYP1A1 variant alleles (16, 26–28). The urinary 1-OHP studies described above, as well as the present study, provide important

<table>
<thead>
<tr>
<th>CYP1A1</th>
<th>No. of cigarettes/day</th>
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<tr>
<td>≤12 (n = 10)</td>
<td>&gt;12 (n = 9)</td>
</tr>
<tr>
<td>*1/*1</td>
<td>0.22 (0.00–0.55)</td>
</tr>
<tr>
<td>*1/*2 or *2/*2</td>
<td>0.56 (0.30–0.87)</td>
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Adjusted for age, sex, and ethnicity by covariance analysis.
in vivo data supporting the association of an increased PAH activation with the CYP1A1 variant alleles at the levels of exposure observed in occupationally exposed workers and in smokers. Despite a relatively large sample size in the present study and in agreement with the results from Merlo et al. (17), no difference in 1-OHP levels were detected among CYP1A1 genotypes in nonsmokers. This may be attributable to the fact that, at least in an environment with no or negligible air pollution like in Hawaii, exposure of the general population to PAHs may be too intermittent to be detectable with a cross-sectional design. Thus, a better test of the modulating effect of these polymorphisms on PAH activation at low or intermittent exposure, such as those from diet, may require an intervention study with careful control of CYP1A1 inducers and inhibitors. The few studies that have explored the relationship between PAH DNA adduct levels and CYP1A1 genotypes have also been supportive of a greater activation for subjects with the variant alleles. A study of foundry workers found elevated levels of PAH DNA adducts in circulating lymphocytes of individuals with both CYP1A1*2 and CYP1A1*3 variant alleles (29). Similarly, Mooney et al. (30) found that smokers with the CYP1A1*3 allele had a 2-fold higher level of PAH DNA adducts in blood lymphocytes than those with the wild-type genotype.

GST activity is not thought to be directly linked to the metabolism of 1-OHP because it is mainly excreted as glucuronide conjugate (5). Consistent with this view and in agreement with most past studies (19, 31), we failed to detect direct influences of GSTM1 and GSTPI polymorphisms on urinary 1-OHP excretion. In summary, the present data add to the growing evidence suggesting that individuals with the CYP1A1*2 or *3 variant allele have a greater capacity to metabolically activate PAHs from tobacco smoke and occupational exposure and, as a result, are at greater risk for PAH-related cancers, especially squamous cell carcinomas of the respiratory tract.

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References


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