Racial Differences in Restriction Fragment Length Polymorphisms and Messenger RNA Inducibility of the Human *CYP1A1* Gene[1]

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Abstract

Recent studies have examined the relationship between genetic polymorphisms of the human cytochrome P-4501A1 (*CYP1A1*) gene and lung cancer susceptibility. We have quantified genotypic frequencies and measured gene expression in the *CYP1A1* gene within racially diverse groups in order to determine the relationship between genotype and transcriptional regulation of the *CYP1A1* gene. Lymphocytes were obtained from 68 individuals of European-American, African-American, and Asian descent, and *CYP1A1* gene inducibility was measured in mitogen-stimulated cells. *CYP1A1* gene inducibility was significantly lower in African-Americans than in European-Americans or Asians, while several other population parameters were found to have no effect on gene expression levels. Restriction fragment length polymorphism analysis of lymphocyte DNA following *Msp*I restriction enzyme digestion revealed a significant difference in the frequencies of *CYP1A1* genotypes between European-Americans and Asians. The only homozygous variants detected were of Asian descent. The frequencies of *CYP1A1* genotypes in all races conformed to Hardy-Weinberg genotypic equilibrium. When *CYP1A1* gene inducibility was compared to *CYP1A1* genotype, no significant correlations were found. These studies, along with our previous survey of *CYP1A1* gene expression in creosote-exposed workers, add further support to the use of *CYP1A1* gene inducibility as a potential marker of polycyclic aromatic hydrocarbon exposure in human populations.

Introduction

The study of the metabolism of PAHs[3] to mutagenic, DNA-binding metabolites by the mixed function oxidase system has evolved from earlier enzyme studies (1) to the more recent appraisal of the cytochrome P450 family by molecular biological approaches (2). Many members of this gene superfamily have been sequenced and studied at both the evolutionary and gene regulation levels (3). One class of *P450* genes that has been studied extensively is the *P450IA* family (4). The protein products of these genes are responsible for the metabolism of environmental aromatic hydrocarbons, including the human carcinogen benzo[*a*]pyrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. A compelling concept that has emerged from these studies has been the idea that these genes, particularly the *P450IA1* gene, may play a pivotal role in environmental aromatic hydrocarbon-related human cancers. Furthermore, individual susceptibility to certain cancers may be related to genetic and functional differences in cytochrome *P450* genes (5).

The potential use of *CYP1A1* inducibility as a determinant of PAH exposure, and as a marker of genetic susceptibility, in human populations has been actively pursued (for recent reviews, see Refs. 6 and 7). Evidence for the relationship of *CYP1A1* inducibility to environmental PAH exposure has come from studies of aryl hydrocarbon hydroxylase (AHH) activity in peripheral lymphocytes from asbestos-exposed workers (8), as well as increases in PAH-DNA adducts in lymphocytes from foundry workers (9), while studies of lung cancer patients suggested a relationship between lymphocyte AHH inducibility and cancer susceptibility (10). Recently, we conducted a study to determine whether PAH exposure could be detected by the measurement of *CYP1A1* gene inducibility in lymphocytes of creosote-exposed railroad workers (11). We observed an elevation in lymphocyte *CYP1A1* inducibility in workers in the summer months, when creosote exposures were greatest, but not in the winter or fall months. These studies led us to propose that *CYP1A1* inducibility may be a useful biomarker in the detection of PAH exposure in human populations.

There have been a number of recent reports indicating that an *Msp*I RFLP in the 3′ region of the human *CYP1A1* gene may be related to increased susceptibility to tobacco-related lung cancers in Japanese populations (12, 13). A study of a Norwegian population failed to demonstrate a correlation between this RFLP and susceptibility to lung cancer (14); however, there appears to be a difference in the frequency of the RFLP between the two populations (13). In addition, Petersen *et al.* (15) demonstrated an association between the *CYP1A1* genotype, an elevated lymphocyte AHH inducibility, and a Mendelian inheritance of the polymorphism within one European family. Considering the conflicting results from these studies, and the different racial groups in which

Received 4/15/92.
[1] Supported by NIH Grants ES04895, ES00260, and CA13343.
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[3] The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; CYP1A1, cytochrome P-4501A1; RFLP, restriction fragment length polymorphism; AHH, aryl hydrocarbon hydroxylase; cDNA, complementary DNA; SSC, standard saline citrate; SDS, sodium dodecyl sulfate.

Table 1 Comparison of study population characteristics by ethnic group

	European-Americans	Asians	African-Americans
n	26	23	6
Mean age (SD)	34.7 (9.5)	37.2 (6.8)	39.2 (12.4)
Sex			
Males	15	15	5
Females	11	8	1
Mean weight (lbs) (SD)	170.2 (55.1)	139.5 (21.2)	165.0 (21.8)
Cigarette smoking			
Never/past	21	20	5
Current	5	3	1

they were performed, we have investigated the occurrence of this polymorphism in the *CYP1A1* gene in a racially diverse population. To determine the relationship between the polymorphism and transcriptional control of the *CYP1A1* gene, we have measured lymphocyte *CYP1A1* mRNA in these same individuals. These studies were conducted as a further effort to determine the potential utility of *CYP1A1* gene inducibility as a marker of PAH exposure in human populations.

Materials and Methods

Study Subjects and Data Collection. Study subjects were 37 male and 22 female employees or graduate students of New York University Medical Center who are part of a roster of volunteers who are available for research projects requiring human subjects. After informed consent was obtained, all volunteers were briefly interviewed to elicit essential information on age, ethnicity, weight, height, recent and past tobacco consumption, as well as possible occupational or leisure exposure to polycyclic aromatic hydrocarbons. Individuals were assigned into the following racial groups: (*a*) European-Americans born in the United States and descended from European and northern African populations; (*b*) African-Americans born in the United States and descended from sub-Saharan populations; and (c) Asians born in either China or Southeast Asia. Upon completion of the interview, 50 ml of blood were drawn from a peripheral vein into a glass Vacutainer (Becton-Dickinson) collection tubes containing sodium heparin as an anticoagulant. All specimens were blindly coded and delivered to the laboratory for processing within 4 h after collection. Following exclusion of 4 samples because of low cell yield in culture, there remained 55 individuals (35 males and 20 females) for statistical analyses of *CYP1A1* gene expression and individual phenotype. For statistical analyses of the racial distribution of *CYP1A1* genotypes, 13 additional males with occupational exposure to creosote who were part of our previous study of human *CYP1A1* gene expression (11) were included, so that a total of 68 genotypes were available for analysis.

Table 2 Levels of expression of *CYP1A1* gene mRNA in human lymphocytes by ethnic group[a]

	European-Americans	Asians	African-Americans
n	26	23	6
Basal	0.24 (0.17)	0.20 (0.16)	0.18 (0.11)
Induced	1.04 (0.69)	1.04 (0.66)	0.48 (0.15)[b]
Induced/basal	6.12 (4.48)	7.36 (4.69)	3.38 (1.95)

[a] Data shown are means (SD) of densitometry values corrected for actin expression as described in "Materials and Methods."
[b] Significantly less than European-Americans and Asians. $P = 0.005$.

Lymphocyte Gene Expression Assays. The details of *CYP1A1* mRNA analysis in freshly harvested human lymphocytes have been described previously (11). Briefly, peripheral blood lymphocytes were isolated via Ficoll (Histopaque; Sigma, St Louis, MO) separation from whole blood and cultured for 3 days in the presence of the mitogen phytohemagglutinin (M-form; Gibco, Grand Island, NY). Each sample was split into two cultures: induced samples received 1 μM 3-methylcholanthrene and basal samples received vehicle (0.001% acetone). Strict controls of culture conditions and reagents were maintained in all experiments. Total RNA was harvested from lymphocyte cultures by a rapid guanidinium-phenol extraction method (11). Slot-blot analyses were performed on glyoxal-denatured RNA samples, with filter hybridization to nick-translated, ^{32}P-labeled cDNA probes, by previously established procedures (11). The human *CYP1A1* probe (phP1-450-3′) and the human β-actin probe (phFβA-1) were obtained from the American Type Culture Collection (Bethesda, MD). Following final filter washes of 0.4× SSC (1× = 3 M NaCl − 0.3 M Na citrate) + 0.1% SDS, *CYP1A1* and actin mRNA was visualized by film autoradiography, and signal strengths were quantitated by the measurement of optical densities with a scanning laser densitometer (LKB Ultroscan). All *CYP1A1* gene expression results were normalized to actin expression, which served as an internal standard to ensure equal loading of RNA onto filters.

DNA RFLP Analyses. High-molecular-weight genomic DNA from frozen lymphocyte samples was isolated using a previously reported procedure following the incubation of lymphocytes with 1 N NH_4OH + 0.2% Triton X-100 (16). Following standard phenol-chloroform extractions, 10 μg of DNA samples were digested with 20 units of *Msp*I (Boehringer-Mannheim, Indianapolis, IN) for 16 h at 37°C following the manufacturer's instructions. The resultant DNA fragments were electrophoretically separated on 1.2% agarose slab gels and transferred onto nylon membrane filters (Nytran, Schleicher and Schuell, Keene, NH) by previously described methods (11). Hybridization of filter-bound DNA to radiolabeled cDNA probes was performed as described above, and following a high-stringency final wash of 0.1× SSC + 0.1% SDS,

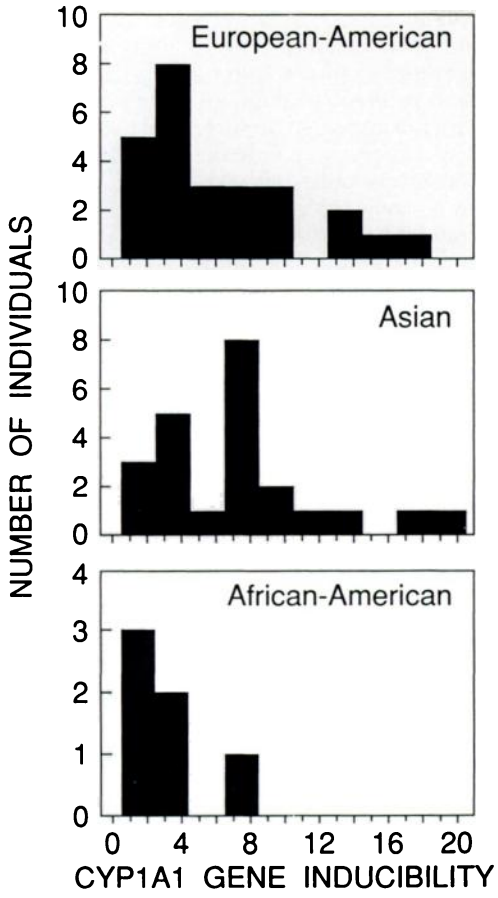

Fig. 1. Distribution of *CYP1A1* gene expression (induced:basal mRNA) values in the study population. *CYP1A1* gene inducibility was determined in mitogen-stimulated lymphocytes following 3 days of treatment with 3-methylcholanthrene (induced) and in control cultures (basal). All *CYP1A1* mRNA values were normalized to actin expression prior to calculation of induced:basal ratios.

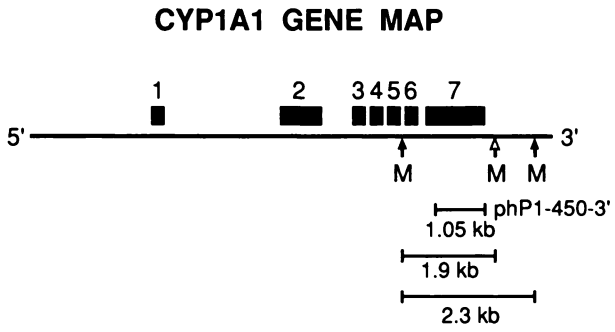

Fig. 2. A simplified map of the human *CYP1A1* gene with pertinent *MspI* restriction sites (*M*) around exon 7. *Filled arrows*, two *MspI* sites that result in a 2.3-kilobase DNA fragment being detected by the phP1-450-3′ cDNA probe following enzyme digestion; *Open arrow*, site of the *MspI* polymorphism that results in the detection of a 1.9-kilobase fragment following DNA digestion.

visualization of hybridized DNA fragments was accomplished by film autoradiography. Mobility of all DNA fragments was measured, and molecular sizes (in kilobases) were determined by comparison to known DNA standards.

Statistical Analyses. Basal, induced, and induced:basal *CYP1A1* gene expression values appeared to better approximate normal distributions after log transformation than when untransformed. Two-sided statistical testing ($\alpha = 0.05$) was therefore performed on transformed values. In univariate analyses, Student's *t* test was used to compare means of gene expression values between groups defined by *CYP1A1* genotype, sex, age, weight, smoking habits, and race. To further examine the relationship between gene expression and race, we utilized multiple linear regression models in which log induced:basal *CYP1A1* mRNA values were the dependent variable and race (2 dummy variables) was the independent variable. Log basal mRNA values were forced in all models. Additional covariates were identified on the basis of potential associations observed in univariate analyses. Stepwise backward elimination was used to select the covariates in the final models by examining the least significant terms ($P > 0.10$) one by one and eliminating them only if their exclusion did not cause an appreciable change (10%) in the regression coefficient for race. Due to the small size of the study, no interaction terms were tested.

The frequencies of *CYP1A1* genotypes were determined within each racial group and compared for significance of differences by χ^2 table analysis (Statview; Abacus Concepts). Observed frequencies of *CYP1A1* genotypes were compared to expected frequencies by Hardy-Weinberg analysis and evaluated for differences.

Results

Table 1 compares selected characteristics of the study population used for phenotypic comparisons between ethnic groups. There were no substantial differences in age, sex, and cigarette smoking habits between the three groups. As expected, Asians weighed appreciably less than the other groups. Basal, induced, and induced:basal values for *CYP1A1* mRNA for the three groups are compared in Table 2. While basal levels of gene expression were similar in all groups, African-Americans exhibited a significantly lower degree of *CYP1A1* inducibility (Table 2). The hypothesis that induced:basal ratios were lower among African-Americans was also tested using a multiple linear regression approach, in which the potential confounding effect of weight (<160, 160+ lbs) and cigarette smoking (current *versus* never/past) was taken into

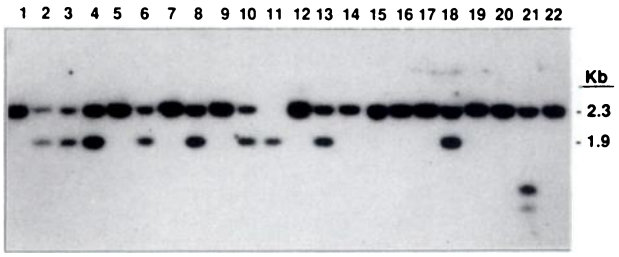

Fig. 3. Restriction fragment length polymorphisms of the *CYP1A1* gene in human lymphocytes from 22 individuals. Genomic DNA was digested with *MspI*, electrophoresed through a 1.2% agarose slab gel, and following Southern transfer to a nylon membrane filter, hybridized with a radiolabeled *CYP1A1* cDNA probe, phP1-450-3′. Final wash stringencies were 0.1× SSC, 0.1% SDS at 65°C. *Right ordinate*, molecular sizes in kilobases.

Table 3 *CYP1A1* genotypic frequencies in 3 racial groups

Race (*n*)	Homozygous normal	Heterozygous	Homozygous variant
European-American (33)	0.70 (23)	0.30 (10)	0
African-American (12)	0.58 (7)	0.42 (5)	0
Asian (23)	0.48 (11)	0.39 (9)	0.13 (3)

account. Current smoking showed an appreciable negative confounding effect and was retained in final models in which log induced:basal (inducibility) was the dependent variable and in which log basal mRNA levels were forced. The adjusted regression coefficient for African-American ethnicity (*versus* all other groups) was −0.5749 (SE = 0.2202; $P = 0.01$). The distributions of *CYP1A1* gene inducibility (induced:basal) values among the three groups are shown in Fig. 1.

Selected features of the human *CYP1A1* gene map are illustrated in Fig. 2, which shows the pertinent restriction sites around exon 7. Normally, the presence of two *Msp*I sites results in detection of a 2.3-kilobase fragment by the phP1-450-3′ cDNA probe following *Msp*I digestion. A point mutation in the noncoding 3′ end of the gene creates a new *Msp*I recognition site which results in a 1.9-kilobase RFLP. Fig. 3 shows an example of *Msp*I RFLP analysis on 22 individuals in which the 3 genotypes corresponding to the *Msp*I polymorphism are represented: individual 1 is homozygous for the normal 2.3-kilobase fragment; individual 2 is heterozygous, with both the 2.3-kilobase and the variant 1.9-kilobase DNA fragments; and individual 11 is a homozygous variant with only the 1.9-kilobase fragment. We have also identified a novel *Msp*I polymorphism, displayed by individual 21, which has not been previously described. *Msp*I digestion of *CYP1A1* DNA from this individual resulted in 1.2- and 1.05-kilobase variant fragments, along with the normal 2.3-kilobase fragment. We presume this polymorphism is the result of a point mutation within exon 7 since both of the cleaved fragments were recognized by the phP1-450-3′ probe and their combined sizes were equivalent to the normal 2.3-kilobase fragment.

Table 3 summarizes the frequencies of the *CYP1A1* genotype in each racial group. A Hardy-Weinberg analysis of genotypic frequencies did not demonstrate significant differences between observed and expected frequencies of the *CYP1A1* genotype for each race or for the entire population, suggesting genetic equilibrium in each group. A comparison of *CYP1A1* allelic frequencies demonstrated significant differences between European-Americans and Asians ($\chi^2 = 4.8$; $P < 0.05$) and between European- and African-Americans versus Asians ($\chi^2 = 4.5$; $P < 0.05$), but not between African-Americans and Asians versus European-Americans. No other population parameter was correlated to RFLP distribution.

The level of *CYP1A1* gene inducibility is shown in Fig. 4 for each of the three genotypes in the whole study population. No correlation was observed between genotype and level of *CYP1A1* gene induction. Genotype also had no effect on basal levels of *CYP1A1* mRNA (data not shown). This lack of association between genotype and *CYP1A1* gene expression was also seen when these values were compared within each of the three racial groups.

Discussion

We have demonstrated in this report that there are significant differences in the frequencies of *CYP1A1* genotypes among three racial groups and that this polymorphism does not appear to be correlated to the inducibility of the *CYP1A1* gene. Our previous study of *CYP1A1* gene expression in creosote-exposed railroad workers demonstrated a significantly higher inducibility of the gene corresponding to the period of highest exposure (11). Our conclusions from that study, although necessarily tempered by the small sample size and incomplete assessment of creosote exposure, were that the measurement of *CYP1A1* gene expression may represent a suitably sensitive biomarker of PAH exposure in human populations. The current study was conducted in part to further determine whether other factors, such as a *CYP1A1* genetic polymorphism, may also affect expression of the gene. Of course, other polymorphisms in the gene, or perhaps mutations in other genetic regulatory elements, including the aryl hydrocarbon (Ah) receptor and negative regulatory element, may affect transcriptional control of the *CYP1A1* gene (17). Nevertheless, the conclusions from our studies have lent further credence to the proposal of *CYP1A1* gene inducibility as a marker of PAH exposure.

In our previous survey of creosote-exposed railroad workers, no population variables that were taken into account (excluding diet) correlated to *CYP1A1* gene inducibility other then creosote exposure (11). In the present study we have found an apparent racial difference in expression levels, with African-Americans having lower induction values. However, this preliminary conclusion must remain speculative due to the inadequate statistical power of the data analyses given the small sample size. Interestingly, African- and European-Americans appeared to have similar *CYP1A1* genotypic frequencies,

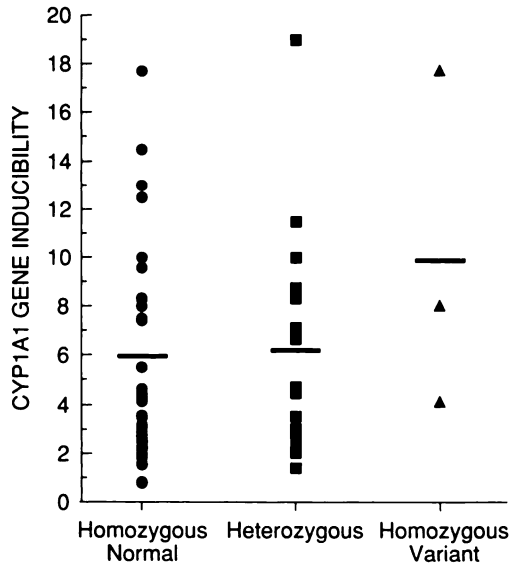

Fig. 4. Comparison of *CYP1A1* gene inducibility to CYP1A1 genotype in the study population. Genotypes and inducibilities were determined as described in Figs. 3 and 1, respectively. *Horizontal bars*, mean values of *CYP1A1* inducibility.

despite their differences in gene inducibility. Again, this conclusion must be tempered due to the small sample size of the study population. Nevertheless, these data further suggest a lack of association between *CYP1A1* genotype and gene expression and warrant a larger study of interracial differences in *CYP1A1* gene expression in order to further develop this potential biomarker of PAH exposure in human populations. Independent verification of exposure must also accompany *CYP1A1* gene inducibility measurements, along with a rigorous survey of population variables, including diet.

The *CYP1A1* allelic frequencies observed in our studies of an Asian population is in agreement with those in a Japanese population (13). Furthermore, the allelic frequencies of European-Americans are similar to those observed in a Norwegian population (14). The most striking difference between the two races with respect to their *CYP1A1* genotypes is the higher number of homozygous variants within the Asian population. This apparent difference in frequencies may provide a partial explanation as to the conflicting conclusions regarding lung cancer risk reached by the two groups, since it was only homozygous variants in the Japanese study that displayed a higher risk for developing lung cancer (13). Interestingly, the Japanese study of lung cancer risk demonstrated an enhanced incidence of cancer only among homozygous individuals who smoked fewer cigarettes, but no differences were noted in cancer incidence among individuals with higher tobacco consumption, regardless of genotype (13). These data suggest that differences in *CYP1A1* activity, with concomitant changes in PAH metabolism, may be associated with genotype. However, as we have demonstrated in this report, there does not appear to be a direct role for the genetic polymorphism in the transcriptional regulation of the *CYP1A1* gene, so the mechanism by which the polymorphism affects smoking-related lung cancer remains obscure. We cannot exclude the possibility, however, that homozygous variants do not have an altered *CYP1A1* gene regulation, given their lower representation in our study population.

A recent report by Hayashi *et al.* (18) has demonstrated a genetic linkage between the *CYP1A1* polymorphism and a point mutation in the coding region in exon 7 of the human *CYP1A1* gene, which results in the substitution of an isoleucine for valine in the critical catalytic site of the protein. It is possible that the nucleotide substitution in exon 7 may change the catalytic activity of the *CYP1A1* protein, thereby affecting tobacco-related PAH metabolism, which may account for observed differences in susceptibility to lung cancer (13). The potential association between genotype and *CYP1A1* protein structure may also explain the association between *CYP1A1* genotype and enhanced AHH enzyme activity that was previously reported (15). We are currently assessing *CYP1A1* enzyme activity in this population to determine whether *CYP1A1* genotypes are associated with differences in catalytic activity of the *CYP1A1* protein.

Acknowledgments

The authors thank Patricia Weideman, R.N., for expert assistance in blood collection.

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