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A novel CYP1A1 gene polymorphism in African-Americans

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A new MspI RFLP in the CYP1A1 gene has been found in genomic DNA from African-Americans. The polymorphism results from a single A-T to G-C transition in the 3′ non-coding region ~300 bp upstream from the polyadenylation site. This mutation leads to cleavage of the normal 2.3 kb *Msp*I restriction fragment into 1.3 and 1.0 kb fragments. The heterozygous mutation has been seen in 8 of 47 African-Americans, but was not detected in 191 Caucasians or 30 Asians. No linkage was observed with either of the two previously described polymorphisms in this gene.

Introduction

The CYP1A1 gene is of critical importance for metabolism of polycyclic aromatic hydrocarbons. The gene product, aromatic hydrocarbon hydroxylase (AHH*), catalyses the first step in the conversion of many environmental carcinogens (such as benzo[*a*]pyrene) to their ultimate DNA-binding, carcinogenic form. The human gene is polymorphic, and two linked mutation sites, one in exon 7 (codon 462) and the other producing an *Msp*I RFLP in the 3′ non-coding region have been associated with increased risk for lung cancer in Asian but not in European populations (1–4). The homozygous variant of this genotype is found in 13% of Asians but in only 2% of Caucasians (5), which might explain the discrepancies between case control studies using relatively small populations of predominantly one racial group. We have found the frequency of the homozygous *Msp*I RFLP genotype in African-Americans (AA) to be 6–7%, intermediate between Caucasians and Asians (5).

Racial differences in frequencies of genetic polymorphisms are common (6–8) and several alleles have been reported to be unique to AAs (9,10). In a study of the related cytochrome P450 gene CYP2EI, Kato *et al.* demonstrated significant differences in genotypes between ethnic groups, although no association with lung cancer was seen (11). However, a case control study examining three genes did show a significant association of rare H-*ras* alleles with lung cancer risk in AAs (12). Ethnic differences in susceptibility to the carcinogenic effects of tobacco smoke may explain why the age-adjusted lung cancer incidence is higher in AAs than in any other racial group (13), even though there is no evidence that AAs have a greater exposure to tobacco smoke (14,15). Polymorphisms in genes involved in the metabolism of carcinogens found in cigarette smoke are among the possible environmental or genetic susceptibility factors that might explain the higher rate of lung cancer in AAs.

We now report a novel *Msp*I RFLP in the CYP1A1 gene found only in AAs and not detected in Caucasians or Asians.

*Abbreviations: AHH, aromatic hydrocarbon hydroxylase; AA, African-American.

Materials and methods

Genotype analyses

High mol. wt genomic DNA was isolated from blood clots, lymphocytes and placental tissue samples. Lymphocyte DNA was extracted as previously described (5) using the standard phenol method and precipitated with ethanol. Frozen blood clots and placenta tissue were powdered in a mortar and pestle under liquid nitrogen, then digested in 1 N NH_4OH, 0.2% Triton-X for 5 h at 37°C before phenol extraction. Ten μg DNA samples were digested with 20 units of *Msp*I (Boehringer-Mannheim, Indianapolis, IN) for 16 h at 37°C following manufacturer's instructions. The resultant DNA fragments were electrophoretically separated on 1.2% agarose slab gels, transferred onto nylon membrane filters (Nytran, Schleicher & Schuell, Keene, NH) and hybridized to the human CYP1A1 probe (phP1–450–3′) and the human b-actin probe (pHFbA-1), (obtained from ATCC, Bethesda, MD) as previously described (5).

Polymerase chain reaction

Amplimers used for PCR were 5′-CTGACTGGCTTCAGCAAGTT-3′, (upstream) and 5′-GGATATGTGCACTCCCTGTG-3′ (downstream). Amplification was performed in a thermal cycler with initial denaturation at 95°C for 5 min, followed by 30 cycles of 95°C 1 min, 56°C 1 min, and 72°C 1 min, and final extension at 72°C for 10 min.

DNA sequence analysis

The 317 bp PCR products were purified by passage through Microcon-100 filtration units (Amicon). Purified DNA (200 ng) was used for direct cycle sequencing using the Cyclist Taq DNA sequencing kit obtained from Stratagene, La Jolla, CA. One microliter of primer (10 pmol) was mixed with heat denatured template DNA, 3.5 μl reaction buffer, 1 μl of [^{32}P]dATP (NEN, Wilmington, DE) and 2 units of Taq DNA polymerase in a total vol of 20 μl. Then 5 μl of this labeling mixture were transferred to each of the four termination tubes containing 5 μl of the termination mixture (A, G, C and T). The termination reactions were incubated in a DNA thermal cycler using the same conditions as described in the Cyclist Sequencing Kit and stopped with 5 μl of stop solution. All samples were denatured at 95°C and 4 μl of each sample were loaded onto a 6% polyacrylamide sequencing gel.

Statistical analysis

Differences in proportions were tested by means of the Fisher's exact test.

Results and discussion

The novel *Msp*I RFLP is illustrated by the Southern blot shown in Figure 1. A new restriction site results in cleavage of the wild type 2.3 kb fragment into 1.3 and 1.0 kb bands as shown in lanes a,b and h. We refer to this as the AA RFLP (for AAs) in order to distinguish it from the more common *Msp*I RFLP which produces 1.9 and 0.4 kb fragments as seen in lanes c, d and f of Figure 1.

Table I shows the frequency of the AA genotype in three racial groups. In the small population of AAs studied so far the prevalence of the AA mutation is 17% (8/47); we have not observed any homozygous variants. However, one individual with the AA mutation was also heterozygous for the original *Msp*I RFLP so that each allele contained a mutation. None of the 191 Caucasian or 30 Asian samples examined exhibited the AA RFLP. Table I shows that there is no apparent linkage between the two *Msp*I RFLPs in CYP1A1, since seven of the eight individuals who were heterozygous for the AA mutation had the normal other allele, and no examples of double mutations on a single allele have as yet been observed. Furthermore, as shown in Table II, there was no concordance between the AA RFLP and the isoleucine to valine mutation in exon 7 in the 23 subjects tested for both mutations. In this group, all six individuals who

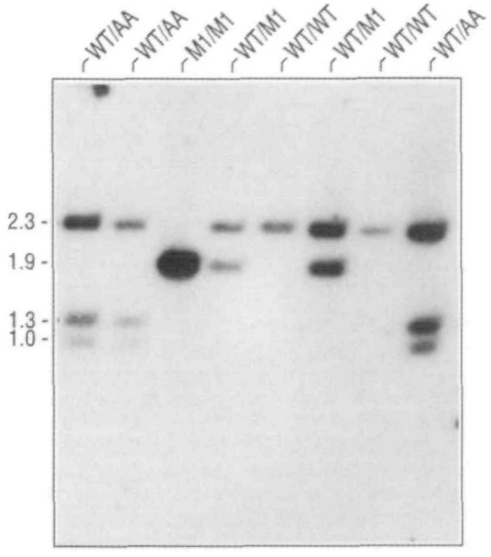

Fig. 1. Southern blot hybridization of *Msp*I digested DNA from several representative AA subjects. Molecular weights of the bands are as shown. Wt = wild type (most common genotype); M1 = the original *Msp*I RFLP; AA = African American *Msp*I RFLP.

Table I. Frequency of AA genotypes in different racial groups

Race	N	AA/Wt[a]	AA/M1[b]	Total
Caucasian	191	0	0	0
Oriental	30	0	0	0
AA	47	7	1	8

[a]AA/Wt, heterozygous AA.
[b]M1, *Msp*I RFLP previously described (1–5).

Table II. Concordance between the two *Msp*I RFLPs and exon 7 mutation in CYP1A1 in 23 AAs

	*Msp*I			
	Wt/Wt	Wt/M1	M1/M1	Wt/AA
Exon 7				
Wt/Wt	10	3	1	6
Wt/M	0	3	0	0
M/M	0	0	0	0

exhibited the AA RFLP were negative for the exon 7 mutation, while the original *Msp*I RFLP was associated with the exon 7 mutation ($P = 0.035$ by Fisher exact test), as previously reported (2,4,16).

In order to determine the specific site of the AA RFLP, we sequenced the appropriate region of the CYP1A1 gene (as determined by restriction mapping) after PCR amplification. Figure 2 shows a A-T to G-C transition mutation at base no. 5996 (2), in the 3′ non-coding region, 300 bp upstream from the polyadenylation signal. This mutation produces a new *Msp*I site which results in the normal 2.3 kb fragment being cleaved into 1.3 and 1.0 kb fragments.

Several laboratories as well as our own (2,4,16) have demonstrated linkage between the *Msp*I RFLP and a mutation in the coding region (exon 7) of CYP1A1. The AA RFLP is not linked to either of these mutations, but may be linked to another mutation in the coding or regulatory domains. The functional

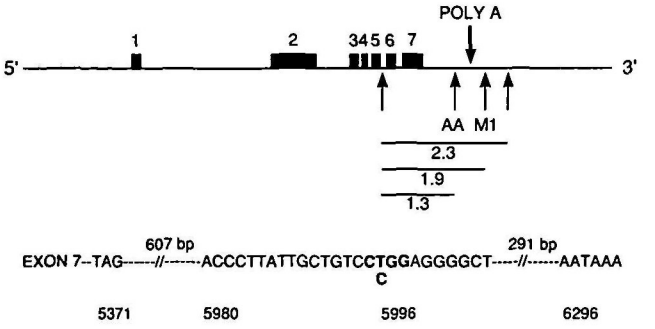

Fig. 2. Location of the new CYP1A1 mutation. (**A**) A map of the human CYP1A1 gene showing the new *Msp*I restriction site in relation to other sites and coding regions. The black boxes represent the exons, arrows represent the *Msp*I sites. M1 indicates the original *Msp*I RFLP and AA shows the new mutation. (**B**) DNA sequence of the mutation showing position of the mutated T to C in relation to the stop (TAG) codon at the end of exon 7, and the polyadenylation signal. The new *Msp*I restriction site is indicated in bold type.

significance of this RFLP for AHH catalytic activity or gene transcription is also not yet known, however we have recently found that enhanced enzymatic activity is associated with *Msp*I RFLP homozygosity and exon 7 mutation heterozygosity in lymphocytes from unrelated individuals (16,17) confirming an earlier study in a single family (18).

A mutant CYP1A1 genotype has been associated with lung cancer risk in Asians (1,2). The finding of a novel CYP1A1 mutation unique to AAs, who suffer a high rate of lung cancer, makes this gene a strong candidate for case control studies in this population. It is not likely, given the complexities of tobacco induced carcinogenesis, including the multitude of carcinogens found in tobacco smoke, that a polymorphism in a single metabolic gene will completely explain racial differences in lung cancer incidence. However, if future experiments do point to a role for the novel AA RFLP in lung cancer susceptibility among AAs, the implications for public health and cancer prevention in minorities as well as for enhanced mechanistic understanding of the carcinogenic process in humans would be significant.

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