Polycyclic Aromatic Compounds, 1996, Vol. 11, pp. 19–26
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ENHANCED RISK OF LUNG AND PROSTATE ADENOCARCINOMA IN AFRICAN-AMERICAN SMOKERS CARRYING A NOVEL CYP1A1 GENE POLYMORPHISM

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Abstract The CYP1A1 gene codes for aromatic hydrocarbon hydroxlase (AHH), a critical enzyme in the metabolism of carcinogenic polycyclic aromatic hydrocarbons. Polymorphisms in this gene have been implicated in aberrant gene function, and in increased risk of cancer from exposure to hydrocarbons such as those found in tobacco smoke. We have discovered a novel restriction polymorphism ("AA RFLP") in this gene that is found only in people of African descent. African Americans suffer a disproportionate high incidence of lung and prostate cancer. This is not due to increased smoking, but may be related to either environmental or genetic factors. We have examined the possibility that the African American specific CYP1A1 RFLP may be associated with higher risk for tobacco related carcinogenesis. The incidence of the AA polymorphism in the healthy African-American population was 14.5 %. In African-Americans with adenocarcinoma of the lung the incidence of the polymorphism was doubled. We observed no difference between cases and controls in the incidence of the exon 7 polymorphism of the CYP1A1 gene. A preliminary study was conducted on tissue blocks from African-American prostate cancer patients. Overall, the AA RFLP was not significantly associated with prostate cancer; however, subjects who both smoke and carry the AA RFLP have significantly higher risk of prostate cancer than subjects who either smoke alone, or carry the AA RFLP alone. This study represents a model for investigation of gene-environment interactions related to PAH induced carcinogenesis.

Key Words: genetic susceptibility, environment, tobacco metabolism, gene-environment interaction

INTRODUCTION

The CYP1A1 gene is a critical component of the inducible Phase I cytochrome P450 supergene family. This gene codes for the aromatic hydrocarbon hydroxylase (AHH)

enzyme system responsible for the oxidative metabolism of polycyclic aromatic hydrocarbons (PAH) such as benzo(a)pyrene and toxicants such as TCDD. The role of CYP1A1 in human carcinogenesis has been intensively studied for many years (1, 2).

Genetic polymorphisms in CYP1A1 are present with different frequencies in various ethnic groups (3). These polymorphisms have been associated with lung cancer and in situ colon cancer in Asian populations (4,5), but not in Caucasians (6,7). We have discovered a novel Msp1 polymorphism of the CYP1A1 gene (the AA RFLP), found only in African-Americans and Africans (8). This novel polymorphism is located in the seventh intron, upstream from the polyadenylation signal, and its role in CYP1A1 gene funtion is not yet known. The role of CYP1A1 polymorphisms in African-American lung cancer susceptibility has only recently been investigated (9,10). We present the distribution of the three known CYP1A1 polymorphisms in a population of Caucasian and African-American healthy subjects, and the role of the novel AA RFLP in lung and prostate cancer susceptibility.

MATERIAL AND METHODS

To study the frequency of the CYP1A1 polymorphisms we enrolled 224 Caucasian and 298 African-Americans healthy volunteers. The case-control study was conducted on 80 African-American incident cases of lung cancer and 42 cases of prostate cancer collected at NYU Medical Center between 1984 and 1992, and 27 lung cancer cases collected at Harlem hospital during the year 1993-1995. DNA was extracted from archived tissue blocks from subjects recruited at NYU, and from fresh whole blood from subjects recruited at Harlem hospital. The prevalence of the AA RFLP in the cases was compared to that observed in peripheral blood lymphocytes of 298 African-American controls. These were healthy volunteers recruited from the community living in the eastern U.S., and patients admitted at Harlem hospital for non-tobacco related diseases other than cancer. All the participants answered a brief questionnaire and signed a written informed consent.

Statistical Analysis

The odds ratio and 95% confidence intervals were computed to assess the relationship between the AA RFLP and lung cancer. Student's t test was used to compare the number of

pack of cigarettes-year between cases with and without the AA RFLP. Data were log transformed to obtain normal distribution. All statistical tests were two-sided (alpha = 0.05).

RFLP Analysis by PCR

DNA was amplified by PCR before digestion with Msp I. DNA was amplified in a total reaction volume of 50 µl containing 1.2 mM dNTP, 1.2 µM oligonucleotide primers, and 2.5 units TAQ polymerase (Amplitaq, Perkin-Elmer). DNA samples were amplified using the following primers: 5'-CTGACTGGCTTCAGCAAGTT and 3' - TAGGAGTCTTGTCTCATGCCT. PCR was performed for 45 cycles with denaturing at 95°C for 1 min, annealing at 56°C for 1 min, and extension at 65°C for 2 min. PCR products were digested with excess Msp I (New England Biolabs) for 3 hr., and then electrophoresed through 1.8% agarose and visualized by ethidium bromide staining. These conditions allow for simultaneous detection of both the Msp1 and AA RFLPs, and also allow for differentiation between the M/A and C/B genotypes, since if both RFLPs are found, it is possible using the fragment size distribution to tell whether the polymorphisms occur on the same or different alleles.

Allele Specific PCR for the exon 7 polymorphism

Genotypes at the exon 7 site were determined by allele specific PCR, essentially as described by Hayashi et.al. (5). Each DNA sample was amplified in two separate reactions using one of two 5' primers: 5'-GAAGTGTAT CGGTGAGACCA-3' or 5'-GAAGTGTATCGGTGAGACCG -3'. All reactions included the 3' primer: 5'-GTAGACAGAGTCTAGGCCTCA-3' Samples were amplified in a Crocodile II thermocycler (Appligene, Pleasanton CA) with an initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of 95 °C for 1 min, 65 °C for 1 min, and 72 °C for 1 min, and a final extension at 72 °C for 10 min. Products were electrophoresed through 1.8% agarose and visualized by ethidium bromide staining.

RESULTS

The frequency of the MspI, exon 7 and AA RFLP polymorphisms in Caucasians and African-Americans is presented in Table 1. None of the Caucasian subjects exhibited the AA RFLP; the exon 7 polymorphism was more frequent in Caucasians than in African-Americans, while the MspI RFLP showed an inverse pattern. In African Americans, we have never detected the exon 7 polymorphism together with the AA polymorphism in the same individual. There also does not appear to be any association between any of the three polymorphisms in African-Americans, unlike the strong association previously found between the MspI and exon 7 polymorphisms in the Asian and Caucasian populations.

TABLE 1
Racial differences in CYP1A1 polymorphisms

Genotype	Caucasians n(%)	African-Americans n(%)
Exon 7		
wild type	180 (82.9)	265 (94.0)
heterozygous	34 (15.7)	17 (6.0)
homozygous	3 (1.4)	0
MspI		
wild type	176 (78.6)	161 (58.8)
heterozygous	43 (19.2)	99 (36.1)
homozygous	5 (2.2)	14 (5.1)
AA		
wild type	224 (100.0)	255 (85.6)
heterozygous	0	42 (14.1)
homozygous	0	1 (0.3)

CYP1A1 polymorphisms in African-American Lung Cancer

The prevalence of the AA RFLP was 20.6% among lung cancer cases, and 16.9% among the controls. The analysis by histologic type (Table 2) showed an association between adenocarcinoma of the lung (AC) and the AA RFLP; the odds ratio for the heterozygous variant was 2.4 (95% CI=1.0-6.0), for the homozygous variant 9.1 (95% CI=0.6-149.6). There was no effect seen in any histologic type of the exon 7 polymorphism in African Americans.

TABLE 2
Distribution of the AA and exon 7 polymorphism among controls and lung cancer cases

	AA RFLP[a]				Exon 7[b]		
	AA	Aa	aa	OR[c] (95%CI)	Ile/Ile	Ile/Val	OR (95%CI)
Controls	255	42	1	1.0 (Ref)	265	17	1.0 (Ref)
Lung Cancer	86	16	1	1.2 (0.6 -2.2)	71	6	1.3 (0.5-3.5)
Adenocarcinoma	28	11	1	2.5 (1.2-5.4)	28	2	1.1 (0.2-5.1)
Squamous CC	32	4	0	0.7 (0.2-2.2)	26	3	1.8 (0.5-6.6)
Large cell ca	8	0	0	–	5	0	–
Small cell ca	7	0	0	–	3	0	–
Others[d]	10	1	0	0.6 (0.1-4.7)	9	0	–

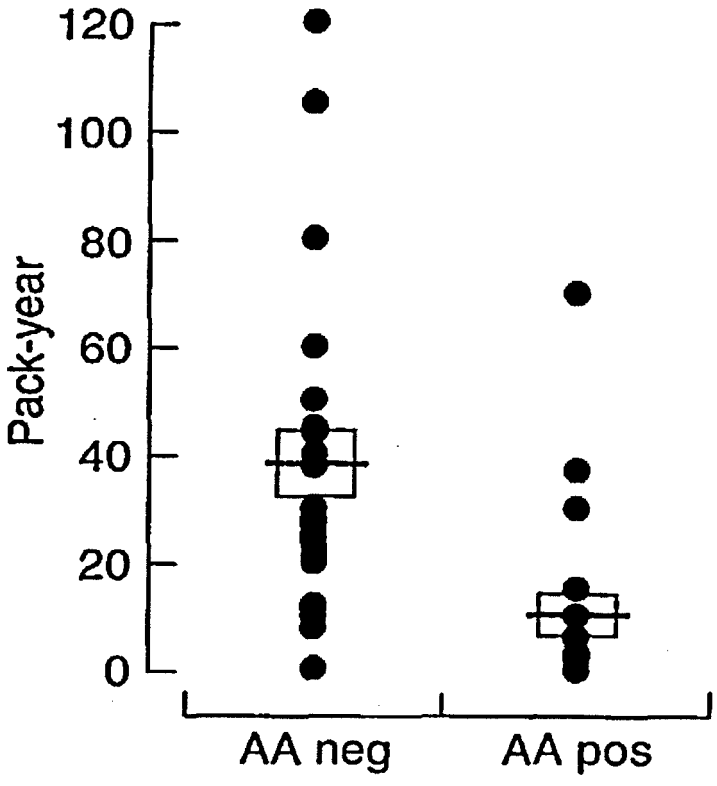
To assess whether the AA RFLP confers an increased susceptibility to the effects of tobacco, we calculated the total amount of tobacco smoked in packs of cigarettes-year among adenocarcinoma cases with and without the polymorphism (Fig 1). The mean value of pack-year in AC with the polymorphism was 10.5±4.1, in AC without the polymorphism was 38.5±5.8 (p=0.0004).

Prostate Adenocarcinoma

Overall, the AA RFLP was not significantly associated with prostate cancer in African-Americans (OR: 1.4; 95% CI: 0.6-3.3); however, subjects who both smoke and carry the AA RFLP have significantly higher risk of prostate cancer than subjects who either smoke alone, or carry the AA RFLP alone (relative interaction magnitude under the additive model assumption: 65%; under the multiplicative model assumption: 44%). The OR of prostate cancer in these subjects was 2.5 (0.7-9.3).

[a] AA = homozygous wild type; Aa = heterozygous AA RFLP; aa = homozygous AA RFLP.
[b] Ile/Ile = wild type; Ile/Val = heterozygous exon 7 polymorphism.
[c] OR calculated after combining subjects carrying either Aa or aa.
[d] This category includes: Mixed cell carcinoma (n = 3), Bronchio-alveolar carcinoma (n = 2), Non differentiated cancer (n = 1).

FIGURE 1
Distribution of number of packs of cigarettes-year in adenocarcinoma patients with and without the AA polymorphism



DISCUSSION

The distribution of CYP1A1 genotypes taking each of the 3 polymorphisms into account, is quite different in African-Americans as compared to Caucasians. Similar results have been previously reported in Asians compared to Caucasians, (3) a fact which may explain the disparate role of these polymorphisms in Japanese relative to Caucasian lung cancer risk. (5,7,8). In other work we have found that the frequency of the AA polymorphism is higher in Africans (from Mali) than in African Americans, suggesting an African origin of this polymorphism. (11).

Age-adjusted incidence of lung cancer in African-American males is 50% higher than in Caucasians (12), although there is no conclusive evidence that African-Americans have a greater exposure to tobacco smoke. Differences in susceptibility to the carcinogenic effects of tobacco smoke may explain why the risk of lung cancer seems to vary among different races, for a given amount of tobacco exposure (13). Our data suggest a selective association of the AA polymorphism with adenocarcinoma of the lung, and a possible additive interaction between smoking and the AA polymorphism in this histologic tumor type. African-Americans with adenocarcinoma of the lung carrying the AA polymorphism have a lower lifetime tobacco consumption than cases without the polymorphism; this is in agreement with previous studies involving other CYP1A1 polymorphisms (14), and indicates that a lower dose of tobacco is sufficient to exert carcinogenic effects on the adenomatous tissue of subjects carrying the AA polymorphism.

African-Americans also have the highest incidence and mortality due to prostate cancer of all ethnic groups (15,16). Although smoking is not considered a risk factor for prostate cancer in Caucasians, its role in African American prostate carcinogenesis has not been fully investigated. It is possible that the AA polymorphism may identify individuals who are highly susceptible to the polycyclic aromatic hydrocarbon components of cigarette smoke. The AA polymorphism may become a useful tool to identify African-American subjects more susceptible to tobacco-induced adenocarcinomas, and may help in understanding the mechanism of development of this histologic type.

REFERENCES

1. Nebert DW. Mutat Res 1991; 247:267-281.
2. Nebert DW, and Gonzalez FJ. Ann Rev Bioch 1987; 56:945-993.
3.Cosma GN, Crofts F, Currie D, Wirgin I, Toniolo P, and Garte SJ.. Canc Epid Biomark Prev 1993; 2:53-57.
4.Hayashi S, Watanabe J, Nakachi K, and Kawajiri K. J Biochem (Japan) 1991; 110:407-411.
5. Sivaraman L, Leatham MP, Yee J, Wilkens LR, Lau AF, Le Marchand L. Cancer Res, 1994; 54:3692-5.
6.Hirvonen A, Pursianen KH, Karjalainen A, Anttila S, and Vainio H. Canc Epid Biomark Prev 1992; 1:485-489.
7.Tefre T, Ryberg D, Haugen A, Nebert DW, Skaug V, Brogger A, and Borresen AL. Pharmacogenetics 1991; 1:20-27.
8.Crofts F, Cosma G, Currie D, Taioli E, Toniolo P, Garte JS Carcinogenesis 1993; 9:1729-31.
9. Shields PG, Caporaso NE, Falk RT, Sugimura H, Trivers GE, Trump BF, Hoover RN, Weston A, Harris CC. Cancer Epidemiol Biom Prev 1993; 2:481-85.
10. Taioli E, Crofts F, Trachman J, Demopoulos R, Toniolo P, Garte SJ. Cancer Res 1995; 55:472-473.
11. Garte SJ, Trachman J, Crofts F, Toniolo P, Buxbaum J, Bayo S, Taioli E, Human Hered. In press.
12.United States Department of Health and Human Services. Cancer Incidence and Mortality in the United States, 1973-87. National Institutes of Health, National Cancer Institute.
13. Marcus, A.C., Shopland, D.R., Crane, L.A., and Lynn, Wr. JNCI 1989; 81:409-414.
14. Nakachi, K., Imai, K., Hayashi, S., and Kawajiri, K. Cancer Res 1993; 53:2994-2999.
15. Mebane C, Gibbs Y, Horm J. JNMA, 1990; 82:782-3.
16. Boring C C, Squires TS, Tong T. CA Cancer J Clin, 1993; 43:7-26.

ACKNOWLEDGMENTS

Supported by NIH Grants ES00260 and CA 70-3-7299. The authors thank Diane Currie and Samantha Garbers for excellent technical assistance, and the Real Love Missionary Baptist Church and Sacred Heart Church of Jersey City, New Jersey for their collaboration.