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Advances in Brief

A Specific African-American *CYP1A1* Polymorphism Is Associated with Adenocarcinoma of the Lung[1]

Emanuela Taioli,[2] Frances Crofts, Julie Trachman, Rita Demopoulos, Paolo Toniolo, and Seymour J. Garte

Institute of Environmental Medicine and Kaplan Comprehensive Cancer Center, New York University Medical Center, New York, New York 10010

Abstract

A case-control study on lung cancer in African-Americans has been conducted to assess whether a novel African-American-specific polymorphism in the *CYP1A1* gene increases the susceptibility to tobacco-related lung cancer. The prevalence of the AA RFLP was 17.1% in the DNA extracted from archived tissue blocks from 76 incident cases of lung cancer, and was 16.3% in peripheral blood lymphocyte DNA of 123 healthy African-American volunteers recruited from a community in the eastern United States. The analysis by histological type showed an association between adenocarcinoma (AC) of the lung and the AA RFLP (odds ratio, 2.6; 95% confidence interval, 1.1–6.3). One homozygous variant subject was present among the AC cases. The risk of AC in subjects who both smoke and carry the AA RFLP was more than double, in comparison to subjects who only smoke (relative interaction magnitude under the additive model, 24%). The mean value of pack-year in AC with the polymorphism was 5.0 ± 2.5 and in AC without the polymorphism was 37.2 ± 6.5 ($P < 0.05$). Our data suggest that a selective association exists between the AA polymorphism and adenocarcinoma of the lung and that a lower dose of tobacco is sufficient to exert carcinogenic effects on the adenomatous tissue of subjects carrying the AA polymorphism.

Introduction

Age-adjusted incidence of lung cancer in African-American males is 50% higher than in Caucasians (1), 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 (2–4). Among the possible susceptibility factors, genetically determined polymorphisms in the genes responsible for the metabolism of tobacco smoke have been hypothesized (5). Genetic polymorphisms in *CYP1A1*, a gene responsible for the first step of metabolism of polycyclic aromatic hydrocarbons, are present with different frequencies in various ethnic groups (6). These polymorphisms have been associated with lung cancer in Asian populations (7) but not in Caucasians (8, 9). The role of *CYP1A1* polymorphisms in African-American lung cancer susceptibility has only recently been investigated (10). We have discovered a novel *Msp*I polymorphism of the *CYP1A1* gene (the AA RFLP), found only in African-Americans and Africans (11). A case-control study was conducted to assess the association between the AA RFLP and lung cancer in African-Americans.

Patients and Methods

Study Population. We analyzed DNA extracted from archived tissue blocks from 80 African-American incident cases of lung cancer collected at NYU Medical Center between 1984 and 1992. Four samples have been excluded because of the poor quality of the DNA obtained. The prevalence of the AA RFLP was compared to that observed in peripheral blood lymphocytes of 123 healthy African-American volunteers recruited from a community in the eastern United States. These subjects are part of a larger pool of volunteers ($n = 330$) recruited in various areas of the United States. Written informed consent was obtained for each participant. 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 packs 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 ($\alpha = 0.05$).

Laboratory Methods. High molecular weight genomic DNA was isolated from lymphocytes, blood clots, or paraffin tissue blocks. Peripheral blood lymphocytes were isolated, and DNA was extracted as described previously (6) using a standard phenol method and precipitated with ethanol. Frozen blood clots were powdered in a mortar and pestle under liquid nitrogen and then digested in 1 N NH_4OH-0.2% Triton-X for 5 h at 37°C before phenol extraction. A mictrotome was used to cut 10-μm sections from paraffin blocks of lung cancers. Sections were extracted twice for 30 min in 1 ml xylene, followed by 100% ethanol twice. After drying by vacuum, samples were digested with protease G and subjected to phenol extraction. DNA samples were amplified using the primers 5′ CTGACTCGCTTCAGCAAGTT and 3′ GGATATGTGCACTCCCTGTG. PCR was performed for 40 cycles with denaturing at 95°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min. PCR products were digested with excess *Msp*I before electrophoresis.

Results and Discussion

The mean age of lung cancer cases was 54.5 ± 11.7 years (range, 34–86) *versus* 46.1 ± 13.7 years (range, 24–71) among controls. The proportion of males among the cancer cases was 78% *versus* 60% among the controls. As expected, the proportion of ever-smokers was significantly higher among the cases (90%) than among the controls (56%). No association between the prevalence of the AA polymorphism and age and sex was observed among the controls included in this study and among a large pool of African-American controls analyzed previously ($n = 330$).

The prevalence of the AA RFLP was 17.1% among lung cancer cases and 16.3% among the controls. The analysis by histological type (Table 1) showed an association between AC[3] of the lung and the AA RFLP; the prevalence among AC cases was 33.3% (odds ratio, 2.6; 95% confidence interval, 1.1–6.3). One homozygous variant subject was present among the AC cases. The presence of the AA RFLP modifies the association between smoking and AC; the risk of AC is more than double in subjects who both smoke and carry the AA RFLP, in comparison to subjects who only smoke (Table 2). The relative interaction magnitude, under the additive model, was 24%.

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[2] To whom requests for reprints should be addressed, at Institute of Environmental Medicine, New York University Medical Center, 341 E. 25th Street, New York, NY 10010.

[3] The abbreviation used is: AC, adenocarcinoma.

No multiplicative interaction was observed. 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 AC cases with and without the polymorphism (Fig. 1). The mean value of pack-year in AC with the polymorphism was 5.0 ± 2.5 and in AC without the polymorphism was 37.2 ± 6.5 ($P < 0.05$). Two of the three nonsmokers were carriers of the AA RFLP.

Our preliminary data are in agreement with a previous publication showing no overall association between the AA polymorphism and lung cancer in African-Americans (12). However, our data suggest a selective association of the AA polymorphism with AC of the lung and a possible additive interaction between smoking and the AA polymorphism in this histological type. Experimental studies (13) have localized cytochrome P4501A isozymes, inducible by polycyclic hydrocarbons, in bronchiolar cuboidal and ciliated cells from human lung tissue. Furthermore, all of the patients with adenocarcinoma, but less than one-half of those with bronchial cancer, showed the inducible cytochrome P450 isozyme in the peripheral lung tissue. These data seem to support a selective activity of cytochrome P4501A isozymes in the lung tissue from which the adenocarcinomas arise.

The observation of a lower lifetime tobacco consumption in AC cases carrying the polymorphism than in AC cases without the polymorphism 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.

If our preliminary results are confirmed in a larger population of lung cancer cases, the AA polymorphism may become a useful tool to identify African-American subjects more susceptible to tobacco-induced AC of the lung and may help in understanding the mechanism of development of AC, a type of lung cancer more frequent in women and in never/passive smokers (15).

Table 1 *Distribution of the AA polymorphism (AA RFLP) among controls and lung cancer cases*[a]

	AA	Aa	aa	OR[b] (95% CI)
Controls	103	20	0	1.0 (Ref)
Lung cancer	63	12	1	1.1 (0.5–2.3)
Adenocarcinoma	20	9	1	2.6 (1.1–6.3)
Squamous cell ca	24	2	0	0.4 (0.1–2.0)
Large cell ca	8	0	0	
Small cell ca	6	0	0	
Others[c]	5	1	0	1.0 (0.1–9.3)

[a] AA, homozygous wild type; Aa, heterozygous AA RFLP; aa, homozygous AA RFLP; ca, carcinoma.
[b] OR, odds ratio calculated after combining subjects carrying either Aa or aa; CI, confidence interval.
[c] This category includes: mixed cell carcinoma ($n = 3$); bronchioalveolar carcinoma ($n = 2$); and nondifferentiated cancer ($n = 1$).

Table 2 *Effect of the AA polymorphism (AA RFLP) and smoking on the risk of AC of the lung*

Smoking status[a]	AA RFLP	Cases	Controls	OR (95% CI)
–	–	1	24	1.0 (Ref)
–	+	2	4	12.0 (0.7–197.4)
+	–	19	31	14.7 (1.8–120.0)
+	+	7	5	33.6* (3.5–321.4)

[a] –, never smokers; +, ever smokers. OR, odds ratio; CI, confidence interval.
[b] χ^2 for trend: 12.5; $P < 0.001$.

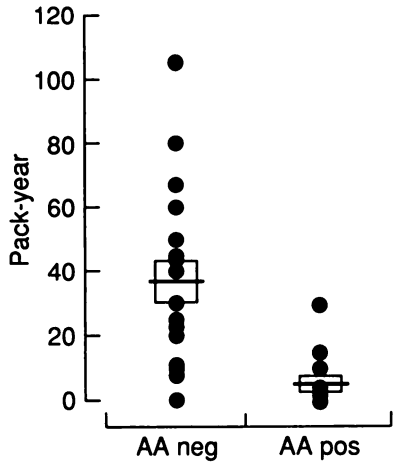

Fig. 1. Distribution of number of packs of cigarettes-year in adenocarcinoma patients with and without the AA polymorphism.

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