Polymorphism of the CYP1A1*2A gene and susceptibility to lung cancer in a Brazilian population*, **

O polimorfismo do gene CYP1A1*2A e a suscetibilidade ao câncer de pulmão na população brasileira

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Abstract

Objective: To estimate and compare the frequency of CYP1A1*2A gene polymorphisms in a Brazilian population and determine the possible contribution of these genetic variations to lung cancer risk. **Methods:** The study population included 200 patients with lung cancer, and the control group consisted of 264 blood donors. Genomic DNA was obtained from peripheral blood samples. The PCR-RFLP method was used for analysis of the CYP1A1*2A gene. **Results:** There was no statistically significant difference between the lung cancer patients and the controls in terms of the distribution of CYP1A1*2A polymorphisms (p = 0.49). A multivariate logistic regression model analysis by ethnic group revealed that, within the lung cancer group, the CYP1A1*2A genotype CC plus TC was more common among the African-Brazilian patients than among the White patients (adjusted OR = 3.19; 95% CI: 1.53-6.65). **Conclusions:** The CYP1A1*2A gene cannot be linked with lung cancer risk in Brazilian patients at this time. Larger epidemiologic studies are needed in order to establish whether the CC plus TC polymorphism increases the risk of lung cancer in African-Brazilians.

Keywords: Lung neoplasms; Polymorphism, genetic; Metabolism.

Resumo

Objetivo: Estimar e comparar a frequência do gene polimórfico CYP1A1*2A na população brasileira e determinar uma possível contribuição dessas variações genéticas no risco para câncer de pulmão. **Métodos:** A população estudada incluiu 200 pacientes com câncer de pulmão e o grupo controle consistiu em 264 doadores de sangue. O DNA genômico foi obtido de amostras de sangue periférico. O método usado para a análise do gene CYP1A1*2A foi a PCR-RFLP. **Resultados:** A distribuição do gene CYP1A1*2A polimórfico não foi estatisticamente diferente entre os pacientes com câncer de pulmão e os controles (p = 0,49). Uma análise multivariada utilizando-se o modelo de regressão logística por grupo étnico revelou uma maior frequência do genótipo CC + TC do gene CYP1A1*2A no grupo de pacientes afro-brasileiros do que no grupo de pacientes caucasoides com câncer de pulmão (OR ajustada = 3,19; IC95%: 1,53-6,65). **Conclusões:** O gene CYP1A1*2A não pode ser associado ao risco de câncer de pulmão nesta amostra de pacientes. Um extenso estudo epidemiológico é necessário para estabelecer se os genótipos CC + TC aumentam o risco de câncer de pulmão em afro-brasileiros.

Descritores: Neoplasias pulmonares; Polimorfismo genético; Metabolismo.

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Financial support: This study received financial support in the form of a grant from the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP, Foundation for the Support of Research in the State of São Paulo; grant no. 2004/13519-5).

Submitted: 20 January 2009. Accepted, after review: 5 March 2009.

** A versão completa em português deste artigo está disponível em www.jornaldepneumologia.com.br

Introduction

Worldwide, lung cancer is the leading cause of cancer-related deaths in men and women. Although the primary etiology (smoking) is well established, it is also known that some smokers develop lung cancer, whereas others do not.[1] Therefore, the use of molecular epidemiology techniques in the study of lung cancer has received widespread attention.

The cytochrome P450 1A1, a key enzyme in carcinogen metabolism, is involved in the activation and conjugation of the constituents of tobacco.[2] By virtue of its polymorphic regulation, the CYP1A1 gene has shown promise as a biomarker for susceptibility to certain malignancies, lung cancer in particular.[3]

The first mutation detected (called m1) was a T-to-C transition 1194 bp downstream of exon 7, creating a new MspI cleavage site.[4,5] This mutation was found to be overrepresented among lung cancer patients in Japan.[6]

Protein expression of the variant CYP1A1 genotype can result in increased formation of carcinogenic metabolites due to the hyperactivity of this phase I enzyme. In addition, the subsequent detoxification of reactive metabolites of these carcinogens can be hampered by the absence of functional phase II enzymes such as GSTM1 and GSTT1, which might play a role in the initiation or progression of lung cancer.[7]

Individuals carrying the CYP1A1*2A or CYP1A1*2B allele present increased activity of the respective enzymes isoforms, which might contribute to increasing levels of electrophilic metabolites derived from polycyclic aromatic hydrocarbons (PAHs). Smokers carrying the CYP1A1*2A or CYP1A1*2B allele present increased levels of PAH-DNA adducts and higher rates of p53 mutations.[8,9] Drakoulis et al.[10] showed the prevalence of these alleles to be lower in a population of German lung cancer patients than in a population of Japanese lung cancer patients (7.3% *vs.* 33.2%).[6] The prevalence of the variant genotype is higher among Asians[11] than among Whites[12] and African-Americans.[13]

The aim of the present study was to estimate and compare the frequency of CYP1A1*2A gene polymorphisms in a Brazilian population and to determine the possible contribution of these genetic variations to lung cancer risk.

Methods

The study population included 200 patients with lung cancer (144 men and 56 women; mean age: 64.0 ± 9.7 years) evaluated in the Pulmonology Department of the State University at Campinas *Hospital das Clínicas*, located in Campinas, Brazil, between January of 2004 and December of 2006.

As a control group, 264 blood donors (160 men and 104 women) were recruited from the same hospital. All procedures were carried out according to institutional guidelines, and the study was approved by the Human Research Ethics Committee of the State University at Campinas *Hospital das Clínicas* (CEP 620/2004). All participants gave written informed consent.

In all cases, the diagnosis of lung cancer was confirmed by histological evaluation of tumor biopsies. Clinical data and smoking history data were collected from patient charts.

Genomic DNA was obtained from peripheral blood samples (12 mL) collected into ethylen-

Table 1 - Age, gender, race, smoking status, histology and staging characteristics of the patient and control groups.

Characteristic	Patients (n = 200)	Controls (n = 264)	p
Age, n (%)			
< 64 years	100 (50.0)	250 (94.7)	< 0.0001*
≥ 64 years	100 (50.0)	14 (5.3)	
Gender, n (%)			
Male	144 (72.0)	160 (60.6)	0.0106*
Female	56 (28.0)	104 (39.4)	
Ethnicity, n (%)			
White	158 (79.0)	215 (81.4)	0.5122
African-Brazilian	42 (21.0)	49 (18.6)	
Smoking status, n (%)			
Smoker	179 (89.5)	86 (32.6)	< 0.0001*
Nonsmoker	21 (10.5)	178 (67.4)	
Histology, n (%)			
NSCLC	168 (84.0)		
SCLC	32 (16.0)		
Stage (TNM), n (%)			
I or II	68 (34.0)		
III or IV	132 (66.0)		

NSCLC: non-small cell lung cancer; SCLC: small cell lung cancer; and TNM: tumor-node-metastasis. *chi-square test.

Table 2 - Genotypes of CYP1A1*2A polymorphisms.

CYP1A1*2A genotype	Patients n (%)	Controls n (%)	OR (95% CI)	p
CC	11 (5.5)	9 (3.4)	1.39 (0.40-4.84)	0.69
TC	76 (38.0)	94 (35.6)	1.17 (0.70-1.98)	0.99
CC plus TC	87 (43.5)	103 (39.0)	0.84 (0.51-1.39)	0.49
TT	113 (56.5)	161 (61.0)	1.00 (reference)	

CC: variant of the CY1A1*2A gene; TC: heterozygotic for CYP1A1*2A; CC plus TC: variant plus heterozygotic for CYP1A1*2A; and TT: wild-type variant of the CY1A1*2A gene.

ediaminetetraacetic acid-containing tubes. The DNA was extracted using DNAzol TM reagent (Gibco BRL/Life Technologies, Gaithersburg, MD, USA), proteinase K and lithium chloride.

Each PCR reaction mixture (50 µL) contained 200 ng of each primer—sense (5'-GGC TGA GCA ATC TGA CCC TA-3') and antisense (5'-TAG GAG TCT TGT CTC ATG CCT-3')—100 ng of genomic DNA, 1.5 mM of $MgCl_2$, 100 mM of each dNTP and 1 U of Taq polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA). The reaction involved 35 cycles of incubation at 94°C (30 s), 63°C (1 min) and 72°C (1 min). After an amplified fragment of the expected size (899 bp) had been obtained on an agarose gel, the PCR products were digested overnight with 50 U of the restriction enzyme MspI (New England Biolabs, Beverly, MA, USA) at 37°C, generating smaller fragments when there was a mutation. Fragments were evaluated on a 2.5% agarose gel stained with ethidium bromide.[14]

Statistical analysis

The Hardy-Weinberg equilibrium was tested using the chi-square test for the goodness-of-fit (one degree of freedom) model. Statistical differences between groups were calculated using the chi-square test or Fisher's exact test. Conditional analysis was used to obtain ethnic group, age and gender-adjusted crude odds ratios. All analyses were performed using the statistical package SAS System for Windows, version 8.2 (SAS Institute, Cary, NC, USA).

Results

Patient and control samples were in Hardy-Weinberg equilibrium for CYP1A1*2A gene (χ^2 = 0.148; p = 0.76 and χ^2 = 1.12; p = 0.79, respectively).

As shown in Table 1, the lung cancer patients were older than were the controls (> 64 years: 50.0% *vs.* 5.3%; p < 0.0001), and the proportion of males was greater in the lung cancer group (72.0% *vs.* 28.0%; p = 0.0106), as was the proportion of smokers (89.5% *vs.* 32.6%; p < 0.0001).

The difference between lung cancer patients and controls in terms of the distribution of CYP1A1*2A polymorphisms was not statistically significant (p= 0.49; Table 2). As can be seen in Table 3, there were no statistically significant differences between the two groups for CC plus TC genotype or the TT genotype.

Table 4 shows the unadjusted odds ratios and 95% CIs for all of the studied variables, calculated using a logistic regression model for CYP1A1*2A in patients with lung cancer. When we focused specifically on ethnic groups, the CYP1A1*2A genotype CC plus TC was found to be more common among the African-Brazilian patients than among the White patients (OR = 2.93; p = 0.02).

Table 3 - The CC plus TC genotype and TT genotype in controls and lung cancer patients, by ethnicity.

Ethnicity	Group	CC plus TC	TT	n	OR	95% CI	p
African-Brazilian	Patients	27	15	42	2.03	0.81-5.18	0.14
	Controls	23	26	49			
White	Patients	60	98	158	1.03	0.66-1.61	0.96
	Controls	80	135	215			

CC plus TC: variant of the CY1A1*2A gene plus heterozygotic for CYP1A1*2A; and TT: wild-type variant of the CY1A1*2A gene.

Table 4 - Demographic data and smoking history correlated with CYP1A1*2A genotypes in patients with lung cancer.

Characteristic	CC plus TC	TT	Patients (n)	OR	95% CI	p
Gender						
Male	60	84	144	0.76	0.41-1.42	0.402
Female	27	29	56			
Age						
≥ 64 years	50	61	111	1.15	0.65-2.02	0.623
< 64 years	37	52	89			
Ethnicity						
African-Brazilian	27	15	42	2.93	1.44-5.96	0.02
White	60	98	158			
Smoking history						
≥ 40 pack-years	49	76	125	0.70	0.28-1.79	
< 40 pack-years	28	26	54	1.18	0.43-3.24	0.270
None	10	11	21			

CC plus TC: variant of the CY1A1*2A gene plus heterozygotic for CYP1A1*2A; and TT: wild-type variant of the CY1A1*2A gene.

In the multivariate logistic model, the CC plus TC genotype was more common among the African-Brazilian patients than among the White patients (adjusted OR = 3.19; 95% CI: 1.53-6.65).

We then performed the same univariate logistic regression model analysis for the controls and found no race-related difference in the prevalence of CYP1A1*2A genotypes (Table 5).

Discussion

In the present study, we have investigated whether the presence of CYP1A1*2A polymorphisms is related to increased lung cancer risk, as well as whether such polymorphisms are correlated with age, gender, ethnic group and smoking history.

Patient and control samples were in Hardy-Weinberg equilibrium for the polymorphic gene CYP1A1*2A. Therefore, the controls were considered appropriate for use in evaluating the frequency of that polymorphism in the general population, allowing the comparative analysis with lung cancer patients. The frequencies of the CYP1A1*2A genotypes were similar between the patients and controls. Therefore, a polymor-

Table 5 - Demographic data and smoking correlated with CYP1A1*2A genotypes in controls.

Characteristic	CC plus TC	TT	Controls (n)	OR	95% CI	p
Gender						
Male	57	103	160	0.69	0.46-1.15	0.161
Female	46	58	104			
Age						
≥ 64 years	7	9	16	1.49	0.79-2.79	0.689
< 64 years	96	152	248			
Ethnicity						
African-Brazilian	23	26	49	1.29	0.44-3.41	0.208
White	80	135	215			
Smoking						
Yes	30	56	86	0.77	0.45-1.31	0.339
No	73	105	178			

CC plus TC: variant of the CY1A1*2A gene plus heterozygotic for CYP1A1*2A; and TT: wild-type variant of the CY1A1*2A gene.

phism in this gene does not seem to increase the risk of lung cancer in Brazilians.

Ethnic factors have been shown to correlate with the occurrence of lung cancer in many parts of the world.[15-20] In a study conducted in the United States, Gadgeel et al.[21] showed that the incidence of lung cancer was 37% higher in African-Americans than in Whites.

Homozygosity for CYP1A1 variant alleles has been found to correlate with lung cancer risk among Americans.[22] A similar risk for lung cancer associated with a single mutated allele in CYP1A1 was found in a population in Chile.[23] Sreeja et al.[7] reported the same correlations in a population in India (OR = 3.2; 95% CI: 1.29-7.80), as did Song et al.[24] in a population in China (OR = 2.0; 95% CI: 1.4-2.8). It is possible that ethnically distinct patterns of CYP1A1 genotypes offer a partial explanation for discrepancies in the association between CYP1A1*2A gene polymorphisms and susceptibility to smoking-related lung cancer. It is of note that, among lung cancer patients, CYP1A1 polymorphisms occur in African-Americans at a frequency similar to that observed for Asians.[25] In the present study, we found that, within the lung cancer group, the frequency of the CC plus TC genotype (CYP1A1*2A polymorphism) was higher among the African-Brazilians than among the White Brazilians (OR = 2.93; 95% CI: 1.44-5.96 and adjusted OR = 3.19; 95% CI: 1.53-6.65).

In conclusion, our results suggest that CYP1A1*2A gene polymorphisms have no influence on the risk of lung cancer in our Brazilian population as a whole. However, among lung cancer patients, African-Brazilians carry the CYP1A1*2A genotype CC plus TC more often than do White Brazilians. Our study sample was small, and we therefore could not establish whether carrying this genotype increases the risk of lung cancer in African-Brazilians. Larger studies are needed in order to determine whether the CYP1A1*2A genotype CC plus TC is a cancer-susceptibility allele in individuals of African descent.

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