The role of CYP1A1 Msp1 gene polymorphisms on lung cancer development in Turkey

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ÖZET

Türk popülasyonunda CYP1A1 Msp1 gen polimorfizminin akciğer kanseri gelişimindeki rolü

Sitokrom P450 1A1 (CYP1A1) metabolik enzimlerini kodlayan genlerdeki polimorfizm, kişilerin akciğer kanserine olan duyarlılığındaki değişikliklere katkıda bulunuyor olabilir. CYP1A1'in akciğer karsinogenezindeki rolü, düşük düzeyli karsinojen maruziyetinde daha da önemli olabilir. Çalışmamızda CYP1A1 gen polimorfizmi, sağlıklı ve akciğer kanserli Türk popülasyonunda araştırıldı. Bu vaka kontrol çalışmasına 31 akciğer kanserli hasta ve randomize olarak seçilen 37 sağlıklı birey kontrol grubu olarak alındı. DNA örnekleri tam kandan alındı ve polimeraz zincir reaksiyonu (PCR) ile çoğaltıldı. CYP1A1 Msp1 (-/+ veya +/+) polimorfizm prevalansı akciğer kanserli hastalarda %19.4; buna karşın kontrol grubunda %16.2 idi ve fark istatistiksel olarak anlamlı bulunmadı (OR= 1.24, 95%CI= 0.36-4.32, p= 0.74). Akciğer kanserinin histopatolojik analizinde histopatolojik tip ile CYP1A1 Msp1 polimorfizmi arasında bir ilişki saptanmadı (p= 0.6). Spesifik biyomarkerlar kullanılarak yapılan genetik çalışmaların akciğer kanser riskinin izlenmesinde yararlı olacağı umut edilir. Güvenli ve doğru istatistiksel bilgiler için çok merkezli kohort çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: *Akciğer kanseri, genetik polimorfizm, moleküler epidemiyoloji, CYP1A1 gen polimorfizmi.*

SUMMARY

The role of CYP1A1 Msp1 gene polymorphisms on lung cancer development in Turkey

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Polymorphisms for genes encoding the metabolic enzymes cytochrome P450 1A1 (CYP1A1) might contribute to the variability in individual susceptibility to lung cancer. The role of CYP1A1 in lung carcinogenesis might be more important at low levels of exposure to carcinogens. In our study, CYP1A1 gene polymorphisms were investigated in healthy subjects and lung cancer patients in Turkish population. This case-control study encompassed 31 lung cancer patients and randomly selected 37 healthy individuals in control group. DNA samples, extracted from the whole blood were amplified using polymerase chain reaction (PCR) method. The prevalence of CYP1A1 Msp1 (-/+ or +/+) polymorphism in the lung cancer patients was 19.4%, compared to 16.2% in control group but the result was not statistically significant (OR= 1.24, 95% CI= 0.36-4.32, p= 0.74). Another important result obtained in this study is that 16.2% of Turkish population carries a CYP1A1 Msp1 polymorphism.

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The analysis of patients by histological type of lung cancer showed no association between histopathologic type of lung cancer and CYP1A1 Msp1 polymorphism (p= 0.6). Genetic researches using specific biomarkers are expected to be helpful in monitorizing the risk of lung cancer. Multicenter cohort studies are necessary to be able to obtain reliable and correct statistical information.

Key Words: *Lung cancer, genetic polymorphism, molecular epidemiology, CYP1A1 gene polymorphism.*

Lung cancer is the most common malignancy and the leading cause of cancer death in men world wide and also the second most lethal cancer in women after breast cancer (1,2). Active and passive smoking, various occupational exposures, and carcinogens in heavily polluted air are causally related to lung cancer in humans. These environmental carcinogens are strongly influenced by individual susceptibility factors (3). Only one of ten lifetime smokers develops lung cancers implying that the differential risk for lung cancer may be explained by genetic susceptibility factors (3). Several of the genes of the enzymes involved in metabolic activation and detoxification of pulmonary carcinogens such as polycyclic aromatic hydrocarbons (PAHs) and aromatic amines are known to be polymorphic in humans. Interindividual differences in the ability to activate and detoxify carcinogens are expected to affect the risk of developing lung cancer (4).

Environmental exposures, especially cigarette smoke contains several thousands chemicals, of which about 50 compounds are known carcinogens, including PAHs, aromatic amines and N-nitroso compounds. Some of these compounds are reactive carcinogens, but most are procarcinogens, which need to be activated by phase I enzymes such as those encoded by the CYP super gene family, and converted into reactive carcinogens. All these reactive carcinogens can bind to DNA and form DNA adducts capable of inducing mutation and initiating carcinogenesis. CYPs are a multigene superfamily of mixed function monooxygenases (5). Cytocrome P4501A1 (CYP1A1) metabolizes a range of PAHs, including benzo pyrene, and is induced by various PAHs through transcriptional activation (6). A Msp1 polymorphic site at the 3' noncoding region of CYP1A1 gene was identified and this allele was called CYP1A1*2A (6). The association between the CYP1A1*2A allele and lung cancer has been reported in Caucasian, Japanese, Korean, Taiwanese and Hawainan populations but not in studies of such populations as Finnish, Norwegian, German and North American (7-10).

The risk of lung cancer is expected to be monitorized by genetic studies using specific biomarkers. Thus, if we can obtain satisfactory results, meaning that some specific gene polymorphisms have significant roles in susceptibility to lung cancer, it will be possible to inform the patients in potential risk not to be exposed to some environmental factors (smoking, air pollution, food, drug... etc.) which are important at least as much as our genetic structure in cancer pathogenesis. Moreover, there will be a great advantage of taking necessary medical precautions for physicians in earlier stages.

The aim of the present study was to evaluate whether genetic polymorphisms in CYP1A1 Msp1 RFLP influence individual susceptibility lung cancer in Turkish population.

MATERIALS and METHODS

Study Subjects and Sample Collection

The study population consisted of 31 lung cancer patients who attended the Yedikule Teaching Hospital for Chest Diseases and Thoracic Surgery, Istanbul, Turkey. The mean ages of patients were 55 ± 10 (range 30-72) years old. There were composed of 29 males and 2 females. Twenty-eight patients were smokers and 3 patients were nonsmokers. Randomly selected 37 healthy individuals in control group, composed of 24 males and 13 females. The mean ages of controls were 34 ± 11 (range 20-65) years old. Nine patients were smokers and 28 patients we-

re nonsmokers. There did not have any one of cancers or chronic diseases. This study was approved by local hospital's medical ethical committee on human research. All patients gave informed consent.

Peripheral blood samples were collected from lung cancer patients and control subjects. DNA was isolated from peripheral blood samples using a DNA isolation kit (Qiagen, Hilden, Germany).

Genotyping of CYP1A1*2A (Msp1 RFLP) Polymorphism

The CYP1A1 Msp1 in the 3' flanking region was determined by polymerase chain reaction (PCR) and RFLP according to the method described by Wu et al (11). The DNA samples were amplified with the primers: 5'-CAGTGAAGAGGTGTAGCCGC-3' (upstream) and 5'-TAGGAGTCTTGTCTCATGCC-3' (downstream). The PCR amplification was carried out 1 µg DNA in 10 mM Tris-HCL, pH 8.3, 50 mM KCl, 3 mM $MgCl_2$, 0.3 mM deoxyribonucleotide triphosphates (Fermentas), 0.2 µM of each primer and 1.5 U of Taq polymerase (Fermentas) in a total volume of 50 µL. Amplification was performed with initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 61°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 10 min.

The amplification product (10 µL) was digested with 3 U Msp1 restriction enzyme (Fermentas) at 37°C incubation for 5 h. Then fragment lengths were analyzed on a 3% agarose gel. When an Msp1 restriction site was present, the fragment of 340 base pairs (bp) was digested into two lengths: 140 and 200 bp. Homozygous wild type subjects lacked the 140 and 200 bp fragment, and heterozygous people had three bands: 340, 200 and 140 bp fragments, homozygous mutant allele people lacked the large parent band and had the smaller bands (Figure 1).

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences Program (SPSS, Version 10). Data were evaluated using the Pearson's x^2 test, χ^2 test and multiple logistic regression test.

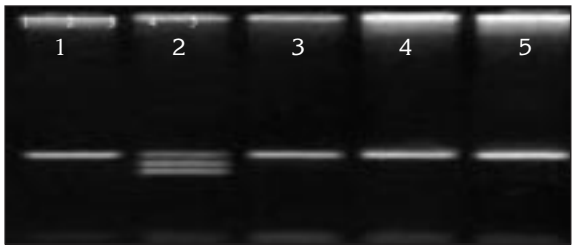

Figure 1. CYP1A1 Msp1 RFLP genotyping. 1, 3, 4 and 5 patients: wild type; 2 patient: heterozygous patient.

RESULTS

The results of this study were summarized in Table 1 and Table 2. Of the patients (n= 31), previously diagnosed lung cancer, it was determined that 25 (80.6%) patients and 6 (19.4%) patients have CYP1A1 Msp1 (-/-) and CYP1A1 Msp1 (+/-) polymorphisms respectively. In the control group composed of the healthy individuals (n= 37), it was observed that 31 (83.7%) and 6 (16.2%) subjects carry CYP1A1 Msp1 (-/-) and CYP1A1 Msp1 (+/-) polymorphisms respectively (Table 1,2). CYP1A1 Msp1 (+/+) polymorphism was determined in none of the groups. In our study, CYP1A1 polymorphism was found to be 16.2% and 19.4% in control group and in patient group respectively but the result was not statistically significant (OR= 1.24, 95% CI= 0.36-4.32, p= 0.74) (Table 2).

The analysis of patients by histopathologic type of lung cancer [48.4% epidermoid carcinoma (EPCA), 12.9% adeno carcinoma (ACA), 9.7%

Table 1. CYP1A1 Msp1 genotype in controls and patients.

	All	CYP1A1 Msp1		
		-/-	+/-	+/+
Patients	31	25	6	0
Male	29	24	5	0
Female	2	1	1	0
Controls	37	31	6	0
Male	24	20	4	0
Female	13	11	2	0

Table 2. CYP1A1 Msp1 genotyping percentages in controls and patients.

Gene	Control (%)	Patients (%)	OR (95%) CL	p
CYP1A1 Msp1 (-/-)	83.7	80.6		
CYP1A1 Msp1 (-/+)	16.2	19.4	1.24 (0.36-4.32)	0.74
CYP1A1 Msp1 (+/+)	-	-		

small cell carcinoma (SMCC), 6.4% large cell carcinoma (LCC) and 22.6% non small cell carcinoma (NSCC)] showed no association with CYP1A1 Msp1 polymorphism (p= 0.6).

DISCUSSION

Polymorphisms of the genes encoding phase I and phase II xenobiotic metabolizing enzymes have been shown to be associated with susceptibility to lung cancer in a number of epidemiologic studies (12). However most of these studies are limited by lack of adequate statistical power. To overcome this limitation, the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (GSEC) was begun and is on-going to pool raw data of studies on metabolic genetic polymorphisms and cancer risk (13).

CYP1A1 plays an important role in the metabolism of PAHs, an important group of lung carcinogens (14). Numerous studies have investigated whether the CYP1A1 polymorphisms modify the risk of lung cancer and they have reached conflicting conclusions. The significance of the CYP1A1 Msp1 polymorphism has also been questioned since it is located outside the coding region of the gene and consequently does not effect the amino acid sequence of the enzyme. Studies examining the significance of the CYP1A1 polymorphisms have not been conclusive (13).

CYP1A1 variants have been associated with increased risk of lung cancer among smokers in the Japanese and a few Caucasian populations (8). In Caucasian populations, in which the frequency of the polymorphisms is lower than the Japanese; studies of CYP1A1 polymorphisms largely yielded negative results (9). Lung tumors of Japanese smokers with the CYP1A1 genotype were more likely to have p53 mutations than those of Japanese patients without the genotype (15). Variations in genes coding for other cytochrome P450 enzymes may also modulate lung cancer risk, although the evidence is less compelling (16). CYP1A1 polymorphisms with high enzymatic activity were demonstrated in 10 to 20% of various Caucasian populations and 35-55% of American Blacks, Japanese, Chinese, Koreans, Chilians and Hawaians (7,17-19).

Ozturk et al found similar frequencies of CYP1A1 mutant variant (Ile/Val) in both control subjects (43.1%) and patients with lung cancer (32.7%) (20). In our study, CYP1A1 polymorphism was found to be 16.2% and 19.4% in control group and in patient group respectively but the result was not statistically significant.

Studies on Asians have in general showed a stronger association than studies on Caucasians, possible due to higher prevalence of the variant CYP1A1 alleles in Asians (6,12). The Turkish population is mainly Caucasian. Although Ozturk et al found similar frequencies of CYP1A1 Ile/Val variant as in non-Caucasians, Ozbek et al and Aynacioglu et al found similar results of CYP1A1 Msp1 polymorphism as in Caucasian (respectively 13% and 18.1%) (20-22). Our results were similar as in Caucasians.

Numerous studies have investigated whether the CYP1A1 polymorphisms modify the risk of lung cancer and they have reached conflicting conclusions. Studies on Asians have in general showed a stronger association than studies on Caucasians and African-Americans, possible due to higher prevalence of the variant CYP1A1 alleles in Asians (6,23). The significance of the CYP1A1 Msp1 polymorphism has also been questioned

since it is located outside the coding region of the gene and consequently does not effect the amino acid sequence of the enzyme. Studies examining the significance of the CYP1A1 polymorphisms have not been conclusive (24).

In conclusion, genetic researchs using spesific biomarkers are expected to be helpful in monitorizing the risk of lung cancer. Multicenter cohort studies are necessary to be able to obtain reliable and correct statistical information.

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