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Plasminogen Activator Inhibitor-1 and Susceptibility to Lung Cancer: A Population Genetics Perspective

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Aim: The aim of this study was to investigate the polymorphism frequency of plasminogen activator inhibitor-1 (*PAI-1*) (rs1799889) 4G/5G in patients with lung cancer. *Methods:* In this study, 286 genomic DNAs (154 lung cancer patients + 132 subjects without lung cancer) were analyzed. Polymorphisms were determined by using the polymerase chain reaction (PCR) method, with 4G and 5G allele-specific primers. PCR products were assessed by a charge-coupled device camera and exposed to 2% agarose gel electrophoresis. *Results:* The frequencies of the *PAI-1* gene 4G/5G genotypes were found to be 21% 4G/4G, 16% 4G/5G, and 62% 5G/5G in the control group and 31.4% 4G/4G, 30.8% 4G/5G, and 37.8% 5G/5G in the patient group. It was determined that the 5G/5G genotype frequency was high in patients in comparison with other genotypes. *Conclusions:* This study found a statistically significant difference between the groups with respect to genotype distribution. Consequently, we can say that the *PAI-1* gene 4G/5G polymorphism is associated with lung cancer in Turkey.

Introduction

Lung cancer, while rare in the early years of the 20th century, has increased over the years, dependent on smoking habits, and has become the most frequently seen type of cancer in the world. Worldwide, lung cancer is responsible for 12.8% of cancer cases and 17.8% of cancer-dependent mortality (Ak *et al.*, 2006). Lung cancer is one of the most frequent cancers and is known to be associated with a very poor prognosis, as the 5-year survival rate in the disseminated disease is only 5%, the highest mortality among other carcinomas (Bayramoglu *et al.*, 2009).

Cancer metastasis is very important. Clinical and experimental studies on the pathogenesis of tumor growth and metastasis showed that the plasminogen activation system is very crucial. Proteolytic degradation of the extracellular matrix and basement membranes is a prerequisite for tumor growth, invasion, and metastasis. Cancer metastasis is dependent on the combined action of several proteolytic enzymes such as collagenases, other metalloproteases, and serine proteases, including plasmin (Offersen *et al.*, 2007). Plasmin is a key enzyme in this process. Plasminogen plays an important role in the behavior of many tumors, including lung cancer. Plasminogen activator inhibitor-1 (*PAI-1*) is a specific inhibitor of plasmin activity and may thereby modulate cell migration and invasion. *PAI-1* is expressed in the invasive areas of many types of cancer. There is clinical evidence implicating *PAI-1* as a key factor in tumor invasion and metastasis. The *PAI-1* gene polymorphism is determined in studies of lung cancer relationship. It has been stated that the *PAI-1* gene polymorphism may contribute to lung cancer (Chorostowska-Wynimko *et al.*, 2004; Di Bernardo *et al.*, 2009).

PAI-1 is a unique member of the serpin (serine proteinase inhibitors) superfamily (Hermans and Hazelzet, 2005), the primary regulator of plasminogen activation, and therefore, an essential factor regulating the physiological thrombotic/fibrinolytic balance *in vivo*. *PAI-1* is also considered one of the key regulators of tumor invasion and metastasis, as well as cancer-related angiogenesis (Duffy and Duggan, 2004; Binder and Mihaly, 2008). Abnormal expression of *PAI-1* has also been reported in various types of human diseases, including atherosclerosis, coronary heart disease, sepsis, renal and lung fibrosis, obesity, and insulin resistance (Chorostowska-Wynimko *et al.*, 2004).

The gene (accession No. P05121) (rs1799889) encoding *PAI-1* (serpin-1) on chromosome 7 is 12 kb (q21.3-q22) (Kokturk *et al.*, 2003; Cosan *et al.*, 2009; Di Bernardo *et al.*, 2009). The 4G/5G polymorphism consisting of the insertion/deletion variation of the guanosine located 675 bases

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upstream from the transcriptional starting point of the relative gene has been determined (Cosan *et al.*, 2009).

The aim of this study was to investigate the role of *PAI-1* gene 4G/5G polymorphism in the causation of lung cancer in the Turkish population.

Materials and Methods

This study included 154 lung cancer patients and 132 controls recruited from the Medical Faculty of Eskisehir Osmangazi University's Department of Chest Disease. Bronchoscopy was performed on all patients under local anesthesia and mild sedation using BF1T60 or Pentax 1830T2 fiberoptic flexible bronchoscopes. Patient blood samples were taken by venipuncture after bronchoscopy. Patients who were histopathologically diagnosed with lung cancer were incorporated in this research. Staging was done for all patients according to the ACCP (American College of Chest Physicians) system (Mountain, 1997). Patients were treated and followed up on by the Department of Chest Diseases as appropriate with their stages. Informed consent was obtained from each patient in accordance with a study protocol approved by the ethics committee of Eskisehir Osmangazi University, Eskisehir, Turkey.

Genomic DNA was extracted using an EZ-10 Spin Column Blood Genomic DNA MiniPreps Kit (Biotechnology Department, Bio Basic, Inc., Markham, Ontario, Canada). DNA samples were amplified using allele-specific primers. Oligonucleotide sequences of the polymerase chain reaction (PCR) primers (Integrated DNA Technologies, Inc., Leuven, Belgium) for 5G/4G alleles were 5′-GTC TGG ACA CGT GGG GG-3′ and 5′-GTC TGG ACA CGT GGG GA-3′. Each in combination with a common downstream primer 5′-TGC AGC CAG CCA CGT GAT TGT CTA G-3′ gives rise to a 139 bp DNA fragment. A control upstream primer 5′-AAG CTT TTA CCA TGG TAA CCC CTG GT-3′ was used as a positive control in the PCR. A 0.5 μL of DNA sample was amplified for 35 cycles, with denaturation at 94°C for 60 s, annealing at 54°C for 30 s, and extension at 72°C for 40 s using a 25 μL PCR mixture containing a 50 pmol allele-specific primer, a 50 pmol common downstream primer, a 2.5 pmol control upstream primer, 1× PCR buffer with magnesium chloride, 0.2 mM dNTPs, and 1.25 U Taq polymerase (Cosan *et al.*, 2009). The PCR products were separated by electrophoresis on 2% agarose gel containing 4 μL ethidium bromide (50 μg/mL) and viewed by a charge-coupled device camera. The results were evaluated using gel analysis software (LabWorks, Cambridge, United Kingdom). The samples were genotyped and classified into one of three possible genotypes 5G/5G, 4G/4G, or heterozygous 4G/5G.

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS ver.15) software package. Some individual features of controls and patients were analyzed by the chi-square and Fisher's exact tests. The distribution of genotypes according to the stage of controls, total patients, and patient subgroups was compared using the chi-square test. Values are given as ± SE and $p < 0.05$.

Results

Individual features of patient and control groups are presented in Table 1. Asbestos exposure ($p < 0.05$) and smoking ($p < 0.05$) rates of patients with lung cancer were significantly higher than the control group. The *PAI-1* 4G/5G genotype counts, percentage of controls, and all of the patients are shown in Table 2. There was a statistically significant difference between the control group and patient group in terms of the *PAI-1* 4G/5G genotype count and percentages ($p < 0.01$).

Table 1. Some Individual Features of Controls and Patients

Individual features	Controls +	Controls −	Patients +	Patients −	Statistics
Asbestos					
n	2	130	37	119	$\chi^2 = 35.063$
%	1.5	98.5	24	76	$^a p < 0.05$
Smoking					
n	42	90	149	7	$\chi^2 = 80.896$
%	32	68	95	5	$^a p < 0.01$
Alcohol use					
n	20	112	32	124	$\chi^2 = 19.497$
%	15	85	80	20	$^a p < 0.05$
Heart disease					
n	3	129	12	143	$p > 0.05^b$
%	2	98	8	92	
Diabetes					
n	10	122	8	148	$p > 0.05^b$
%	8	92	5	95	
Hypertension					
n	20	106	14	142	$\chi^2 = 6.103$
%	16	84	8	92	$^a p > 0.05$
COPD					
n	10	122	6	150	$p > 0.05^b$
%	8	92	4	96	
Asthma					
n	2	130	2	154	$\chi^2 = 4.056$
%	1.5	98.5	1	99	$^a p > 0.05$
Pneumonia					
n	0	132	10	146	$\chi^2 = 8.766$
%	100	0	6	94	$^a p < 0.05$
Family history					
n	14	118	22	134	$p > 0.05^b$
%	11	89	15	85	

$^a p$: χ^2 test.
$^b p$: Fisher's exact test.
COPD, chronic obstructive pulmonary disease.

Discussion

As a result of our study, asbestos exposure and smoking were statistically significantly higher in all patient groups compared with the control group, whereas no difference was found statistically for alcohol use, chronic obstructive pulmonary disease, heart disease, diabetes, hypertension, asthma, pneumonia, and family history. In other studies carried out, patients with lung cancer, asbestos exposure, and smoking rates were reported to be higher compared with the control group (Augustine *et al.*, 1989; Zhou *et al.*, 2005; Gazdar and Thun, 2007; Bayramoglu *et al.*, 2009, 2011), which was consistent with our results.

TABLE 2. DISTRIBUTION OF GENOTYPES ACCORDING TO STAGE OF CONTROLS, TOTAL PATIENTS, AND PATIENT SUBGROUPS

		Genotypes					
		4G/4G		*4G/5G*		*5G/5G*	
	n	n	%	n	%	n	%
Controls	132	28	21.2	22	16.7	82	62.1
Total patients	154	49	31.4	48	30.8	59	37.8
Statistics			$\chi^2=17.256$		$p<0.01$[a]		
Nonsmall cell lung cancer							
Squamous							
Stage 4	29	12	41.4	12	41.4	5	17.2
Stage 3B	19	4	21.1	8	42.1	7	36.8
Stage 3A	27	9	33.3	10	37.0	8	29.6
Stage 2	6	2	33.3	2	33.3	2	33.3
Adenocarcinoma							
Stage 4	7	4	57.1	1	14.3	2	28.6
Stage 3B	21	3	14.3	5	23.8	13	61.9
Stage 3A	8	2	25.0	3	37.5	3	37.5
Stage 2	7	1	14.3	0	0	6	85.7
Small cell lung cancer							
Stage 4	15	7	46.66	2	13.33	6	40
Stage 3B	9	2	33.33	1	16.66	3	50
Stage 3A	6	3	33.33	3	33.33	3	33.33
Stage 2	0	0	0	0	0	0	0

[a]*p*: χ^2 test.

In the present study, the frequencies of the *PAI-1* gene 4G/5G genotypes were found to be 21.2% 4G/4G, 16.7% 4G/5G, and 62.1% 5G/5G in the control group and 31.4% 4G/4G, 30.8% 4G/5G, and 37.8% 5G/5G in the patient group. Thus, there was a significant difference between the control and the patient groups by means of genotype.

In a study on deep vein thrombosis (DVT) in patients with lung cancer, Erdis *et al.* (2010) have determined 4G/4G genotype frequency for patients to be 42% and 4G/5G genotype frequency 58%; and 4G/4G genotype frequency to be 14%, 4G/5G genotype frequency 54%, and 5G/5G genotype frequency 32%, for DVT in patients without lung cancer. As a result, they have reported that the *PAI-1* gene polymorphism may be an important risk factor for DVT occurring in patients with lung cancer. Yende *et al.* have determined 4G/4G genotype frequency to be 29.6%, 4G/5G genotype frequency 48.6%, and 5G/5G genotype frequency 21.8% for patients with pneumonia. They concluded that *PAI-1* gene 4G/5G polymorphism was associated with pneumonia (Yende *et al.*, 2007).

Manduz *et al.* have determined the 4G/4G genotype frequency to be 7%, 4G/5G genotype frequency 83%, and 5G/5G genotype frequency 10% for patients with Buerger's disease; and 4G/4G genotype frequency to be 4%, 4G/5G genotype frequency 43%, and 5G/5G genotype frequency 53% for the control group. Manduz *et al.* (2010) have also reported that there is a relationship between Buerger's disease and the *PAI-1* gene 4G/5G polymorphism.

Vairaktaris *et al.* have determined the carrier 4G allele frequency to be 88.5% and mutant 4G allele frequency 69.8% for patients with oral cancer; and carrier 4G allele frequency to be 65.9% and mutant 4G allele frequency 49.5% for the control group. They also reported that 4G/5G polymorphism is associated with increased oral cancer (Vairaktaris *et al.*, 2006).

Trimarchi *et al.* (2008) have determined the 4G/4G genotype frequency to be 19%, 4G/5G genotype frequency 64%, and 5G/5G genotype frequency 17% for hemodialysis patients; and 4G/4G genotype frequency to be 20%, 4G/5G genotype frequency 45%, and 5G/5G genotype frequency 35% for the control group. They also reported that this polymorphism may be associated with hemodialysis (Trimarchi *et al.*, 2008). Emingil *et al.* (2007) have determined the 4G/4G genotype frequency to be 29.1%, 4G/5G genotype frequency 43%, and 5G/5G genotype frequency 27.9% for periodontitis patients; and 4G/4G genotype frequency to be 35.7%, 4G/5G genotype frequency 43.8%, and 5G/5G genotype frequency 20.5% for the control group.

French *et al.* (2008) reported that leukemia in osteonecrosis may constitute a risk for the distribution of PAI-1 genetics. In a study on patients with esophageal squamous cell carcinoma, Sakakibara *et al.* (2004) reported that expression of the *PAI-1* gene is a useful marker in predicting the occurrence of this cancer.

Di Bernardo *et al.* (2009) reported that the *PAI-1* variation in lung cancer may be a prognostic marker, but more work needs to be done on this issue. In an immunohistochemical study of lung cancer patients, Robert *et al.* (1999) have shown that *PAI-1* and uPA (urokinase-type plasminogen activator) are strongly correlated and linked with tumor progression parameters, suggesting a synergistic effect on tumor cell migration. Salden *et al.* (2000) reported that *PAI-1*, *PAI-2*, *uPA*, and *uPAR* expression levels compared to normal tissue are significantly high in patients with lung cancer. Akhter *et al.* (2010) investigated the *PAI-1* 4G/5G gene polymorphism with DVT and lung cancer in Indian patients. Accordingly, they have reported that the frequency of 4G polymorphism has a higher incidence in patients with lung cancer and DVT.

The *PAI* genes were examined in terms of lung cancer and other diseases, and the *PAI-1* gene determined the relationship with the other diseases. This study is the first trial to specify the relationship between the *PAI-1* gene 4G/5G polymorphism and lung cancer in Turkey. As a result of our study, we can say that the *PAI-1* gene 4G/5G polymorphism is associated with lung cancer in Turkey.

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Author Disclosure Statement

No competing financial interests exist.

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