

Angiotensin-Converting Enzyme Gene Polymorphism Is Associated with Anemia in Non–Small-Cell Lung Cancer

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The angiotensin-converting enzyme (ACE) plays an important role not only in the regulation of vascular homeostasis but also in stimulation of hematopoiesis. We aimed to evaluate the association between insertion/deletion (I/D) polymorphism of the ACE gene and anemia at the time of the diagnosis. We enrolled 75 patients with non–small-cell lung cancer (NSCLC) and 85 age- and sex-matched healthy control participants. The I/D polymorphism of ACE was identified by using polymerase chain reaction from peripheral blood samples. Statistical analyses were performed with SPSS for Windows. The distributions of the ACE genotypes and alleles are similar in patients and in healthy participants ($P = 0.29$ and $P = 0.08$, respectively). In patients with NSCLC, 34 (45.3%) had anemia; of whom 3 (8.8%) had genotype II, 24 (70.6%) had genotype ID, and 7 (20.6%) had genotype DD ($P = 0.001$). The patients with the II and ID genotypes had more frequent anemia at the time of the diagnosis (odds ratio = 6.02; $P = 0.001$). Our findings suggest that I/D polymorphism of the ACE gene may influence the development of anemia in patients with NSCLC. *Exp Biol Med* 233:32–37, 2008

Key words: anemia; angiotensin-converting enzyme gene polymorphism; non–small-cell lung cancer.

Introduction

Angiotensin-converting enzyme (ACE) is a zinc metallopeptidase that converts angiotensin I to angiotensin II (Ang II) and degrades bradykinin. The gene that encodes ACE is located on the long arm of chromosome 17 (17q23). It has been found that a polymorphism that involves the presence (insertion [I]) or absence (deletion [D]) of a 287-bp sequence of DNA occurs in intron 16 of the gene.

Mean ACE activity levels in homozygous-for-the-deletion (DD) carriers are approximately twice that found in homozygous-for-the-insertion (II) genotype individuals. Subjects with the heterozygous (ID) genotype have intermediate levels. Because the physiologic importance of I/D polymorphism is discovered through its association with plasma ACE levels (1), most studies focus on an I/D polymorphism in intron 16 of the ACE gene as a marker for a functional polymorphism. Furthermore, increased ACE levels that are observed in the DD genotype stimulate Ang II production, and this causes promitotic, proliferative, and angiogenic effects in tumor biology (2, 3).

Prior studies have shown that the DD genotype has an increased risk for the development of cancer, including breast (4, 5), gastric (6), and prostate cancer (7). In contrast, there is no association between the ACE genotype and a risk for the development of renal (8) and lung cancer (9).

Anemia is commonly observed in various malignancies, including lung cancer. The causes of anemia in cancer patients can be multifactorial. The factors associated with anemia include blood loss or iron deficiency, nutritional deficiencies such as low folic acid or low vitamin B12, a reduced number of erythroid progenitor cells in the bone marrow, increased catabolism, and a relative deficiency of erythropoietin (EPO; Refs. 10, 11). Although anemia is a highly reproducible variable, and although many studies have assessed its prognostic value for outcome, the number of studies that report its predictive value in the treatment of

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cancer is relative small (12, 13). The prevalence of anemia in patients with lung cancer is reported to be 40%–85%, and higher pretreatment hemoglobin levels are favorable prognostic parameters in non-small-cell lung cancer (NSCLC; Refs. 14, 15).

Studies have shown that the renin-angiotensin system (RAS) stimulates hematopoietic progenitor cell proliferation and induces the growth of early erythroid progenitors (16, 17). In addition, it has been shown that the use of ACE inhibitors and Ang II receptor (AT2) antagonists might suppress erythropoiesis and lead to anemia (18, 19). Some pathways possibly are involved in erythropoiesis. One of them, the Jak/STAT pathway, is activated by several cytokines and by growth factors such as interleukin-12 and insulin-like growth factor-1 (20–22). A second, the nuclear factor- κ B (NF- κ B) pathway, is activated by Ang II and controls the expression of genes involved in hematopoiesis (23, 24). Finally, the mitogen-activated protein kinase (MAPK) pathway, which is activated by Ang II, has a mitogenic effect (25) and is another important signal transduction pathway in hematopoiesis. Taken together, these studies suggest that Ang II may influence the dynamic process of red blood cell production *via* different mechanisms.

In the literature, different genotypes of the I/D polymorphism of ACE cause different levels of mean ACE activity; also, different fields of medicine have shown that ACE genotypes are involved in erythropoiesis. For example, in peritoneal dialysis patients, genotype II mostly is associated with lower ACE levels compared with the DD and ID genotypes, and it is associated with higher EPO dose requirements (26, 27). Based on these findings, we aimed in this study to investigate the association between ACE gene polymorphism and anemia at the time of diagnosis in patients with NSCLC.

Materials And Methods

Participants. Seventy-five patients (6 women and 69 men; mean age: 54.9 years; standard deviation: 8.1 years) with NSCLC who were treated at the Department of Medical Oncology, Pamukkale University and at the Dokuz Eylul University, Turkey, between September 2005 and February 2006 were included in this study. The exclusion criteria were usage of ACE inhibitors or of any drug that affected the ACE system (e.g., beta-blockers, AT2 receptor blockers) or erythropoietin synthesis and the existence of a second malignancy in the patients group. A second, control, group consisted of 85 healthy participants (8 women and 77 men; mean age: 52.7 years; standard deviation: 7.6 years) who were age- and sex-matched to the first group and who had no disease history, including no anemia, and who were not using any drug that affects the ACE system. All of the 75 patients in the study population had histologically confirmed NSCLC. Clinical parameters, such as age at onset of disease, histologic type of tumor, stage of disease,

and performance status according to the Eastern Cooperative Oncology Group (ECOG) were recorded. Anemia was defined as hemoglobin level less than 13.0 g/dl in men and less than 12.0 g/dl in women. The pretreatment hemoglobin levels were obtained before surgery, chemotherapy, radiotherapy, or any invasive procedure. Hemoglobin levels were analyzed by automated complete blood cell counting devices on ethylenediamine tetra-acetic acid–anti-coagulated blood. In our laboratory, the normal range of serum hemoglobin level is defined as 13–16 g/dl in men and as 12–14 g/dl in women. Patients with iron, vitamin B12, or folic acid deficiency anemia and hemoglobinopathies were also excluded from the study. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki, as reflected in a prior approval by the institution's human research committee. Additionally, the study was approved by the Ethics Committee of Pamukkale University, and written informed consent was obtained from each participant.

Genetic Analysis. Blood specimens from all participants were obtained with a standard venepuncture technique that used ethylenediamine tetra-acetic acid–containing tubes. DNA was isolated from peripheral blood by a standard phenol/chloroform extraction method, as detailed previously (28). For the detection of the ACE polymorphism, we used polymerase chain reaction methodology, as described previously by Rigat *et al.* (1), and we used the upstream primer 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and the downstream primer 5'-GAT GTG GCC ATC ACA TTC GTC AGAT-3'. Amplification was performed for 35 cycles with denaturation, extension and annealing temperatures of 94 °C, 60 °C and 72 °C, respectively. The resulting polymerase chain reaction products were separated on 2% agarose gels with ethidium bromide staining and were visualized under ultraviolet light. Homozygous-for-the-deletion and -insertion genotypes were described as DD and II, respectively, whereas the heterozygous genotype was reported as ID. A representative sample of a patient with NSCLC is shown in Figure 1.

Statistical Analysis. Analyses of data were performed using the computer software SPSS for Windows (version 10.0; SPSS Inc., Chicago, IL). The frequency of the ACE genotypes in NSCLC and control participants and the association between the gene polymorphism and variables were assessed using the chi-square test or the Fisher exact test. Odds ratios (ORs) were calculated with a corresponding 95% confidence interval (CI). Spearman's correlation analysis was used for correlation analysis between variables. The Mann-Whitney rank sum test and 95% CIs were used to compare the age at onset of disease. The median survival time and the progression-free survival time were estimated by using the Kaplan-Meier method and were compared using the log-rank test. All tests were two-sided, and differences were considered nonsignificant when *P* values were greater than 0.05.

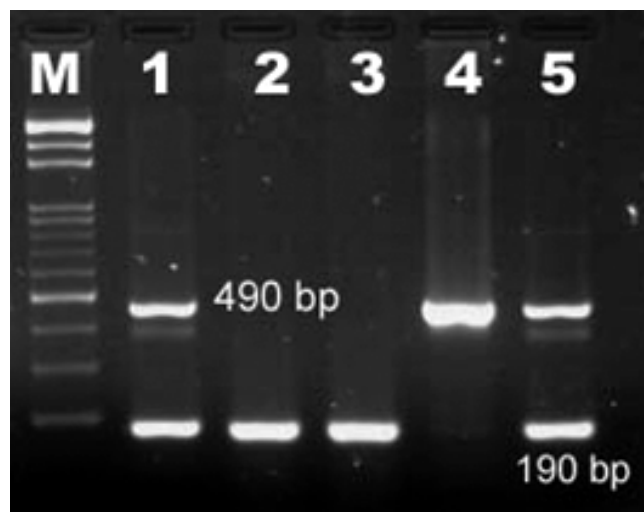


Figure 1. Determination of angiotensin-converting enzyme genotypes by polymerase chain reaction amplification. The deletion (D) and insertion (I) alleles were identified by the presence of 190-bp and 490-bp fragments, respectively. Lanes 2 and 3, DD. Lanes 1 and 5, ID. Lane 4, II. M, Marker (100-bp ladders).

Results

We identified the polymorphism of the ACE gene in 75 patients with NSCLC and in 85 healthy control participants. In the control group, 34 (40.0%) participants had DD, 37 (43.5%) had ID, and 14 (16.5%) had II genotypes. In patients with NSCLC, 32 (42.7%) had DD, 39 (52%) had ID, and 4 (5.3%) had II genotypes. The D and I allele frequencies were 68.7% and 31.3% in patients with NSCLC and 61.8% and 38.2% in control group, respectively (Table 1). The distribution of the genotypes and alleles was similar in the patients and in the healthy control group ($P = 0.08$ and $P = 0.29$, respectively).

The clinical parameters of the 75 patients with NSCLC are listed in Table 2. The median age at disease onset was 52 years (range: 32–72 years); 69 (92%) patients were male, and 6 (8%) were female. Of the 75 patients, 29 (38.7%) had adenocarcinoma, 38 (50.7%) had squamous-cell carcinoma, and 8 (10.7%) had other histologic types of NSCLC (i.e., three had undifferentiated carcinoma, three had large-cell carcinoma, and two had mixed type). Thirteen patients (17.4%) had early-stage (IIB and IIIA) carcinoma, while 65 (82.6%) had locally advanced (stage IIIB) or metastatic (stage IV) disease. Only eight (10.7%) patients with NSCLC had no smoking history. Thirty-four (45.3%) patients had

anemia; the frequency of anemia was 33.3% in women and was 46.4% in men.

The relation between anemia and the ACE genotypes and alleles is shown in Table 3. In patients with anemia, 3 (8.8%) had the II, 24 (70.6%) had the ID, and 7 (20.6%) had the DD genotype. The I and D allele frequencies were 44.1% and 55.9%, respectively, in anemic patients. There was a significant correlation between anemia and the ACE genotypes and alleles ($P = 0.001$ and $P = 0.002$, respectively). We found that the patients with NSCLC who had the II and ID genotypes had a higher rate of anemia at the time of the diagnosis (OR = 6.02; 95 % CI = 2.12–17.07; $P = 0.001$). Furthermore, there was a significant correlation between anemia and stage of disease. Anemia was more common at metastatic disease (OR = 2.8; 95 % CI = 1.06–7.25; $P = 0.04$). When results were adjusted for stage, a significant correlation between the ACE genotypes and anemia persisted (OR = 5.42; 95 % CI = 1.79–12.46; $P = 0.02$). However, anemia was not found to correlate significantly with gender, histologic subtype, age, performance status, or smoking status.

The median survival time and the progression-free survival time of the patients with and without anemia were 8.7 vs. 9.2 months, $P = 0.46$ and 7.5 vs. 8.1 months, $P = 0.82$, respectively. There were no relationships between the ACE genotypes or alleles and the median survival and progression-free survival times in anemic or nonanemic patients.

Discussion

Most of the studies about ACE gene polymorphism in cancer are aimed to determine the cancer risk. Although there is no association between the ACE genotype and renal (8) or lung cancer (9), patients with the DD genotype have an increased cancer risk that is independent of environmental and familial risk factors in breast cancer (4, 5) or gastric cancer (6, 29). In our study, the distribution of the ACE genotypes and alleles was similar in the patients and in the healthy control participants. This means that polymorphism of ACE in cancer patients is not the only mechanism that may cause anemia, because the effect of ACE on red blood cell production is a dynamic process, and many other factors and pathways affect this enzyme in addition to polymorphism of the ACE gene. These results might be related to the fact that the DD genotype had greater ACE activity than the ID and II genotypes. Therefore, it

Table 1. Association Between the Angiotensin-Converting Enzyme (ACE) Genotype/Allele Frequencies in Non–Small Cell Lung Cancer (NSCLC) Patients and Control Participants

	ACE genotypes, No. (%)			ACE alleles, No. (%)	
	II	ID	DD	I	D
NSCLC patients ($n = 75$)	4 (5.3)	39 (52.0)	32 (42.7)	47 (31.3)	103 (68.7)
Control participants ($n = 85$)	14 (16.5)	37 (43.5)	34 (40.0)	65 (38.2)	105 (61.8)

Table 2. Clinical and Histological Characteristics of Non–Small Cell Lung Cancer (NSCLC) Patients

Characteristic	No. (%) of patients (N = 75)
Age of onset (years)	
Mean	54.9
SD ^a	8.1
Gender	
Female	6 (8)
Male	69 (92)
Histological subtype	
Squamous cell carcinoma	38 (50.7)
Adenocarcinoma	29 (38.7)
Other	8 (10.7)
Stage of disease	
IIB	10 (13.3)
III (A/B)	22 (29.4)
IV	43 (57.3)
ECOG performance status ^b	
0–1	55 (73.4)
2–4	20 (26.6)
Patients with anemia	34 (45.3)
Patients without anemia	41 (54.7)
Smoking status	
Never	8 (10.7)
Current/past	67 (89.3)

^a SD, standard deviation.^b ECOG, Eastern Cooperative Oncology Group.

seems that the ACE gene polymorphism may play a role in the development of NSCLC. However, it is impossible to make any clear arguments, as our cases were few in number.

The relations between I/D polymorphism of the ACE gene and the tissue and plasma ACE levels are widely studied in patients who undergo peritoneal dialysis. In these studies, I/D polymorphism of the ACE gene strongly influences ACE tissue and plasma levels, and the II genotype is associated mostly with lower ACE levels compared with the DD and heterozygous genotypes (26, 27). In addition, in one of the studies, the II genotype is associated with higher EPO dose requirements than the DD genotype, whereas the patients with a heterozygous genotype are in between (27). Although this cross-sectional study is limited by the small sample size, it argues for a role of the ACE I/D polymorphism in renal anemia. These results indicate an association between a polymorphism of the ACE gene and anemia; also, a relative EPO resistance could be seen in patients with the II genotype (27, 30).

Because Ang II has promitotic, proliferative, and angiogenic effects in tumor biology (2, 3), ACE inhibitors may have beneficial effects on several aspects of cancer, although the mechanisms are not yet fully understood. There are conflicting studies about the preventive effects of ACE inhibitors in cancer development and about their adverse effects on anemia in chronic renal failure. Many studies have indicated that the use of ACE inhibitors or AT2 receptor antagonists shows an association with anemia (18, 31). Some mechanisms of ACE inhibitor-induced anemia have

Table 3. Correlation Between Anemia and Angiotensin-Converting Enzyme (ACE) Genotypes/Alleles in Non–Small-Cell Lung Cancer (NSCLC) Patients

	Anemic patients (n = 34)	Nonanemic patients (n = 41)	P*
ACE genotypes ^a			
II	3 (8.8)	1 (2.4)	—
ID	24 (70.6)	15 (36.6)	—
DD	7 (20.6)	25 (61.0)	0.001
ACE alleles ^a			
I	30 (44.1)	17 (20.7)	—
D	38 (55.9)	65 (79.3)	0.002

^a Data reported as No. (%) of patients with specified genotype/allele.

* P < 0.05 is statistically significant.

been proposed. ACE inhibitors prevent the stimulatory effect of Ang II on the synthesis of EPO (16) and increase renal plasma flow, which reduces the hypoxic stimulus for EPO formation (32). A tetrapeptide called AcSDKP (N-acetylseryl-aspartyl-lysyl-proline), or goralatide, is degraded by ACE, which is a negative regulator of hematopoietic stem cell differentiation (33, 34); ACE inhibitors have been shown to prevent its degradation and to increase its plasma concentrations (35, 36). However, several studies reported that renin-angiotensin system inhibition with ACE inhibitors and with angiotensin receptor antagonists could induce significant declines in hemoglobin levels within the first month of the treatment at any dosage (37, 38).

Several studies have reported that anemia is more commonly seen in advanced disease, that it is an independent prognostic factor, and that it is associated with shorter survival in patients with NSCLC (14, 15). In our study, it was found that anemia was significantly correlated with advanced-stage disease. Furthermore, anemia may be a poor prognostic factor because of cytokines, such as interleukin-1 and tumor necrosis factor, that are secreted by tumor cells. These cytokines are involved in the inflammatory process, which is more pronounced in advanced-stage NSCLC. It has been reported that chronic inflammation can worsen anemia by reducing iron availability for hematopoiesis (37), and proinflammatory cytokines might both shorten erythrocyte life span and directly inhibit erythrocyte progenitor proliferation (38). Therefore, we can not exclude the effect of the inflammatory process on anemia, because most of our patients had advanced-stage disease. Furthermore, low cardiopulmonary functional capacity resulting from anemia might have contributed to this poor prognosis in these patients.

Although our results indicate that anemia often occurred in patients with NSCLC who had the II and ID genotypes, we can't have clear arguments, as our cases were few in number and consisted of different stages of disease. However, based on these limited data, it can be said that, because NSCLC is observed most commonly in the elderly

population, most of whom have different levels of hypertension, there may be negative effects of ACE inhibitors in this cancer population and that ACE inhibitors can be avoided as an antihypertensive agent in patients with NSCLC who have these genotypes. It would be interesting to see whether these agents contribute to deterioration of the anemia in patients with lung cancer. Before it is shown in clinical trials, it is hard to say that we should avoid ACE inhibitors in cancer patients with anemia. In addition, because of a relative resistance to EPO in these patients, the existence of the II and ID genotypes can also be considered determinants of EPO doses. Confirmation of this possible association with further studies, which would investigate the dose adjustments of EPO according to the genotypes in anemic patients with lung cancer, would create an opportunity to determine roles of these genes on the course of the anemia in patients with NSCLC.

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