

## Angiotensin-Converting Enzyme Gene Insertion/Deletion Polymorphism in Patients with Pulmonary Thromboembolism

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**KEYWORDS** Pulmonary Embolism. Angiotensin-Converting Enzyme. Renin-Angiotensin System. Gene Polymorphisms. Venous Thromboembolism

**ABSTRACT** The aim of the present study is to investigate the relationship between angiotensin-converting enzyme (ACE) gene polymorphism and pulmonary embolism by comparing the frequency of ACE gene polymorphism between cases diagnosed with pulmonary embolism with that of the control group. The study included 73 patients and 73 healthy subjects as the control group. Isolated DNAs were genotyped using the polymerase chain reaction (PCR) method for the identification of the ACE insertion/deletion (I/D) polymorphism. The genotypes were determined according to the bands observed in the agarose gel electrophoresis. The frequency of ID genotype was 39.7 percent, the frequency of insertion/insertion (II) genotype was 17.8 percent, and the frequency of the deletion/deletion (DD) genotype was 42.5 percent in the patient group. In the control group, the frequency of the II genotype was 21.9 percent, the frequency of the ID genotype was 38.4 percent, and the frequency of the DD genotype was 39.7 percent. There were no statistically significant differences between the patient group and the control group in terms of the frequencies of II, ID, and DD genotypes ( $p > 0.05$ ). The findings of the present study showed no association between ACE gene polymorphism and the risk of developing the pulmonary embolism. Due to the limited number of patients however, these results must be confirmed by further studies incorporating larger series of patients.

### INTRODUCTION

The term venous thromboembolism (VTE) is used to express deep vein thrombosis (DVT) and pulmonary embolism (PE). Approximately ten percent of patients who suffer from deep venous thrombosis develop PE later in their life, and ten percent of these patients die (Moser 1990). Despite the improvements in diagnostic methods, pulmonary embolism continues to be associated with high morbidity and mortality rates due to delays in diagnosis. It was reported that two-thirds of cases with pulmonary thromboembolism cases are misdiagnosed, and mortality

rate is as high as thirty percent in these patients, whereas the rate of mortality decreases to three percent if appropriate treatment and prophylactic therapies are instituted following accurate diagnosis (Carson et al. 1992; Goldhaber et al. 2000).

Angiotensin-converting enzyme (ACE) is a dipeptidase produced in the pulmonary vascular area and it is found at various levels in several diseases of the lungs. The ACE gene plays an important role in the regulation of serum ACE levels. This observable difference in ACE between individuals is due to the polymorphisms of the ACE gene. Although polymorphisms do not cause damage to the DNA, they are predisposing factors for the disease. The most frequently studied polymorphism in the renin-angiotensin system is the ACE insertion/deletion (ID) polymorphism. This polymorphism is defined by the presence (insertion, I) or absence (deletion, D) of the 287<sup>th</sup> base pair (bp) of the

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Alu repeat sequence within intron 16 of the ACE gene located on chromosome 17 (Hooper et al. 2002). Therefore, there are three genotypes of this polymorphism: deletion/deletion (DD), insertion/insertion (II), and insertion/deletion (ID) (Della et al. 2001; Tseng et al. 2002; Plati et al. 2004). One of these three genotypes is present in diseases. The distributions of the ACE I and D alleles are almost equal in healthy people. The distribution of these genotypes in Caucasian individuals is as follows: twenty-five percent II, fifty percent ID, and twenty-five percent DD genotypes (Hessner et al. 2001). Plasma ACE activity, while being stable in one person, shows considerable differences between individuals. This observable variance in ACE levels between individuals is due in particular, to the genetic polymorphism of the ACE gene. The ID polymorphism of the ACE gene is responsible for fifty percent of the changes in serum ACE concentrations (Hessner et al. 2001).

Although most studies on the pathophysiological effects of the renin-angiotensin system are limited to arterial vascular pathologies, there are accumulating studies showing that the venous system may also be affected (Chae et al. 2014; Mogielnicki et al. 2014). In addition to the disturbances in fibrinolysis, inhibition of the fibrinolytic system caused by the changes in the renin-angiotensin system can also lead to venous thromboembolism. In previous studies, research groups included only patients with DVT or VTE (DVT+PE) (Gohil et al. 2009; Kaya et al. 2013). The goal of the present study is to investigate the presence of the ACE gene polymorphism in patients with pulmonary embolism. The present study aimed to investigate whether this genetic difference creates a predisposition for the disease.

## MATERIAL AND METHODS

The study included 73 patients with pulmonary embolism, who underwent testing and received treatment at Uludag University Medical Faculty Pulmonary Medicine Clinic, and 73 healthy volunteers as the control subjects. Particular care was given to the inclusion of patients who were diagnosed with pulmonary embolism based on the clinical findings, and laboratory and/or imaging methods, and the control group did not have any known systemic diseases. Age, gender, body mass index, comorbidities,

predisposing factors, symptoms, and findings of the patients with pulmonary embolism together with the results of high-contrast thoracic tomography, lung perfusion scintigraphy, lower limb venous Doppler ultrasonography, D-dimer levels, and ECHO findings were recorded.

The approval of the Uludag University Faculty of Medicine Medical Research Ethics Committee was obtained, and the subjects in the study were requested to sign an informed consent form. The patients with diabetic nephropathy, cardiomyopathy, and coronary and carotid atherosclerosis were excluded from the study.

## DNA Isolation and Identification of ACE Genotype

Blood samples of the patients and control subjects were collected in tubes containing EDTA. DNA was isolated according to the D. Z. DNA isolation kit (Dr. Zeydanli Life Sciences Ltd., Turkey) procedures and stored at -20° C until the polymerase chain reaction (PCR) assay.

The ACE gene I/D polymorphism in the DNA samples was identified using the PCR method. The F: 5'-CTG GAG ACC ACT CCC ATC CTT TCT 3', R 5'GAT GTG GCC ATC ACA TTC GTC AG T-3' primers were used for the ACE ID polymorphism and the insertion site-specific primers F: 5'GCC TGG GAC AGC CAC CGC CCA CTA CCG C-3', R: 5'-TCG GCC CTC CCA CCA CCA TGC TAA-3' were used to verify the DD genotype (Lee and Tsai 2002).

The samples were photographed after they were run through the two percent agarose gel electrophoresis and then stained with ethidium bromide for the examination of PCR amplification. As a result of amplification, 190 bp amplification bands in samples with the DD genotype, 490 and 190 bp bands in the ID genotypes and 490 bp bands in the II genotype samples were observed. In the second PCR assay performed for the confirmation of the DD genotype, a 335 bp amplification was observed in samples with insertion bands.

## Statistical Analysis

The statistical analysis of the study data was performed at the Department of Biostatistics, Uludag University Faculty of Medicine. SPSS for Windows 13.0 (Chicago, IL) software pack-

age was used in statistical analysis. The continuous variables were expressed as mean, standard deviation, and range of minimum and maximum values. The Kruskal-Wallis test was used for the comparison of continuous variables showing normal distribution between the three groups. The Mann-Whitney U test was used to compare variables between the two groups that showed a significant difference.

Pearson's chi-square and Fisher's exact tests were used to examine the distribution of categorical variables across the groups. A  $p$ -value < 0.05 was considered statistically significant.

## RESULTS

General characteristics of the patients and control subjects are shown in Table 1. Age and body mass index of the patients were significantly higher compared to the control group ( $p < 0.001$  and  $p = 0.021$ , respectively). The II genotype was detected in 13 patients in the study group (17.8%), 29 patients had the ID genotype (39.7%), and 31 patients had the DD genotype (42.5%). In the control group, 16 subjects (21.9%) had the II genotype, 28 (38.4%) had the ID genotype,

and 29 (39.7%) had the DD genotype. There was no significant difference between the patient and control group in terms of the frequencies of II, ID, and DD genotypes ( $p = 0.821$ ) (Table 2).

In the present study, patients with pulmonary embolism were divided into three groups: non-massive, massive, and sub-massive. These groups were created based on the hemodynamic parameters, echocardiography findings, radiographic findings, and scintigraphic findings. The patients were classified in the non-massive group if they had no hypotension and no signs of right ventricular overload on ECHO, as sub-massive if ECHO findings were present, but hemodynamics was normal and there was no hypotension, and as massive if hemodynamic instability and hypotension were present, with or without echo findings. No differences were found when comparing the II, ID and DD genotypes between the non-massive, massive/sub-massive and control groups according to this classification ( $p > 0.05$ ) (Table 3).

The presence of risk factors for the development of pulmonary embolism was evaluated in association with II, ID, and DD genotypes. Of the patients, four were pregnant (5.5%), 13 patients had malignancy (17.8%), eight patients had postoperative complications (11%), seven patients had genetic factors (9.6%), five patients had a history of DVT and/or PE (6.8%) and 20 patients had other risk factors (27.4%), while 16 patients did not have any risk factor (21.9%). II, ID, and DD genotypes did not significantly differ between the risk/non-risk groups and the control group ( $p > 0.05$ ) (Table 4).

## DISCUSSION

Although significant improvement has been achieved in the prevention and treatment of VTE,

**Table 1: Characteristics of the patient and control groups**

|                                    | Patient      | Control      | <i>p</i> -value |
|------------------------------------|--------------|--------------|-----------------|
| Total (number)                     | 73           | 73           | $p > 0.05$      |
| Female (number-%)                  | 35-47.9      | 42-57.5      |                 |
| Male (number-%)                    | 38-52.1      | 31-42.5      |                 |
| Mean Age                           | 54.8 ± 16.5  | 34.6 ± 10.9  | $p < 0.001$     |
| Body mass index                    | 28.43 ± 5.49 | 24.06 ± 4.23 | $p = 0.021$     |
| Smoking status (smoker/non-smoker) | 28/45        | 20/          | $p = 0.021$     |

**Table 2: Comparison of the ACE genotype of the patient and control groups**

| ACE genotype | PE     |      | Control |      | Total |            |
|--------------|--------|------|---------|------|-------|------------|
|              | Number | %    | Number  | %    |       |            |
| II           | 13     | 17.8 | 16      | 21.9 | 29    | $p > 0.05$ |
| ID           | 29     | 39.7 | 28      | 38.4 | 57    |            |
| DD           | 31     | 42.5 | 29      | 39.7 | 60    |            |
| Total        | 73     | 100  | 73      | 100  | 146   |            |

ACE: Angiotensin Converting Enzyme, PE: Pulmonary Embolism, II: Insertion/Insertion, ID: Insertion/Deletion, DD: Deletion/Deletion.

**Table 3: Comparison of ACE genotypes based on the type of embolism**

| ACE genotype | Embolism type |                     |            |            |        |
|--------------|---------------|---------------------|------------|------------|--------|
|              | Non-massive   | Massive+Sub-massive | Control    | Total      |        |
| II           | 6 (15%)       | 7 (21.2%)           | 16 (21.9%) | 29 (19.9%) | p>0.05 |
| ID           | 17 (42.5%)    | 12 (36.4%)          | 28 (38.4%) | 57 (39%)   |        |
| DD           | 17 (42.5%)    | 14 (42.4%)          | 29 (39.7%) | 60 (41.1%) |        |
| Total        | 40 (100%)     | 33 (100%)           | 73 (100%)  | 146 (100%) |        |

ACE: Angiotensin Converting Enzyme, PE: Pulmonary Embolism, II: Insertion/Insertion, ID: Insertion/Deletion, DD: Deletion/Deletion.

**Table 4: Comparison of ACE genotypes based on the presence of risk factors**

| ACE genotype | PE risk factor |            |            |       |        |
|--------------|----------------|------------|------------|-------|--------|
|              | Present        | Absent     | Control    | Total |        |
| II           | 2 (12.5%)      | 11 (19.3%) | 16 (21.9%) | 29    | p>0.05 |
| ID           | 8 (50%)        | 21 (36.8%) | 28 (38.4%) | 57    |        |
| DD           | 6 (37.5%)      | 25 (43.9%) | 29 (39.7%) | 60    |        |
| Total        | 16             | 57         | 73         | 146   |        |

ACE: Angiotensin Converting Enzyme, PE: Pulmonary Embolism, II: Insertion/Insertion, ID: Insertion/Deletion, DD: Deletion/Deletion

pulmonary embolism continues to be one of the common causes of in-hospital deaths in the early period. As a result of difficulties in identifying patients who are at increased risk due to both acquired and congenital factors, VTE continues to be associated with high morbidity and high mortality. Most genetic mutations and polymorphisms associated with VTE are found in the coagulant and anticoagulant proteins. Recent studies suggest that genetic changes in the renin-angiotensin system might play a role in the development of VTE. The aim of the present study was to investigate the presence of ACE gene polymorphisms in patients, who were monitored with the diagnosis of pulmonary embolism. Based on study results, no statistically significant difference was found between patients with pulmonary embolism and control subjects in terms of the ACE ID gene polymorphism. When patients with pulmonary embolism were classified as non-massive and massive/sub-massive, the comparison of these subgroups with the control group did not show a significant difference. Additionally, the comparison of patients with and without risk factors for the development of pulmonary embolism with the control group did not show statistically significant difference. The cases diagnosed with VTE should be evaluated in a broader context taking hereditary and acquired risk factors into consideration.

The family history and medications used should be questioned, and the etiology of this condition must be sought in the medical history. The hereditary factors account for twenty percent of patients with pulmonary embolism. Testing for inherited risk factors should be performed in a timely and appropriate manner, and the results should be interpreted with precaution. The identification of hereditary risk factors will enable the early recognition of family members who have not yet been diagnosed with VTE and early administration of prophylaxis for a sufficient duration in high-risk cases. It must also be kept in mind that some acquired factors can also contribute to the development of thrombosis in people who possess these factors.

In the literature, 15 patient/control studies investigated the role of the ACE ID polymorphism in patients with VTE. The findings of the present study are consistent with six research studies, which showed no association with VTE (Dilley et al. 1998; Jackson et al. 2000; Della et al. 2001; Ekim et al. 2004; Koppel et al. 2004; Buddingh et al. 2005; Kaya et al. 2013). However, the remaining seven studies (Philipp et al. 1998; Dilley et al. 2000; Gonzales et al. 2000; Lu et al. 2001; Fatini et al. 2003; von Depka et al. 2003; Wells et al. 2003; Gohil et al. 2009) reported an association between gene polymorphism and VTE. These seven studies are inconsistent with

each other in terms of ACE genotypes that were associated with VTE. In four of these studies, the DD genotype was shown to be associated with high or moderately increased risk of VTE (Philipp et al. 1998; Lu et al. 2001; Fatini et al. 2003; von Depka et al. 2003); whereas two studies (Gonzales et al. 2000; Wells et al. 2003) reported a decreased risk of VTE. In another study (Buddingh et al. 2005), the DD genotype was reported to have a protective effect on a female-only group. The present study found no gender-specific association with ACE genotypes in the two groups. The selection criteria for the patients vary widely in the published studies. The inclusion criteria also vary between the studies such as the history of trauma and surgery, pregnancy, cancer, or ethnicity, which act as a predisposing factor for VTE.

In the study by Ay et al. (2007), 100 patients, and 125 control subjects were compared and no statistical difference was found between the two groups. The same study also compared serum ACE levels and did not find a difference between the two groups. Both genetics and serum ACE levels were reported not to pose a risk for VTE. Serum ACE levels were low in the acute phase of PE and these levels returned to normal within six months (Munoz et al. 1997). In another study, serum ACE levels were measured at rest and following stimulation with venous stasis as a marker for endothelial abnormalities. Patients with recurrent VTE had lower basal serum ACE levels and weaker serum ACE activity responses to venous stasis compared to the control group (Drouet et al. 1988).

ACE gene polymorphism is associated with various diseases related to the renin-angiotensin system (Okumus and Arseven 1988; Daimod et al. 2003). The common characteristic of these diseases associated with ACE polymorphism is the presence of vascular endothelial injury. Endothelial damage is involved in the pathogenesis of many vascular diseases. Angiotensin II leads to endothelial damage through several mechanisms (Ekim et al. 2004). Plasma ACE levels of patients with the DD genotype are approximately two times higher than the levels in patients with the II genotype (Della et al. 2001). Therefore, the DD genotype is associated with increased level of angiotensin II, which in turn increases tissue damage. ID polymorphism of the ACE gene is associated with structural changes in the artery (Cambien et al. 1992).

ACE leads to thromboembolism through its strong vasoconstrictive effects and as a result of a decrease in fibrinolysis, platelet activation and aggregation (Wiwanitkit 2004). ACE gene polymorphisms are associated with an increase in vascular tone, decreased fibrinolysis, and increased platelet aggregation. Hypercoagulable states are associated with increased symptomatic thromboembolic events. To date, known cases with a hypercoagulable state consisted of a few genetic defects related to the coagulation cascade constituting only a small percentage of patients with venous thrombosis. These conditions included antithrombin III, protein C, and protein S deficiencies. ACE polymorphisms are also considered to be associated with hypercoagulable states (Fatini et al. 2003).

Although the ACE ID polymorphism has been suggested to play a role in the pathogenesis of thrombosis through various mechanisms, there are conflicting opinions regarding the involvement of ACE ID polymorphism in the pathogenesis of VTE. The studies that evaluated the role of gene polymorphisms in VTE reported different results regarding the association between ACE DD genotype and VTE. The ACE DD genotype was suggested to be a sensitive marker of thrombosis in subjects who do not exhibit significant thrombophilic changes or possess predisposing factors. The attempts should be made to determine different genetic polymorphisms in order to elucidate the etiology in patients who do not possess a significant risk factor and who are deemed to have an idiopathic VTE. In the study by Wells et al. (2003) conducted on patients with idiopathic VTE and age-, gender-, and ethnicity-matched control subjects, the frequency of ACE DD genotype was lower in the patient group, and the ACE DD genotype was therefore suggested to have a protective effect against idiopathic VTE.

The limitations of the present study include a small number of patients compared to other studies, the absence of an age-matched control group, and a lack of analysis for serum ACE levels that was performed in some studies in addition to genetic investigation.

## CONCLUSION

There was no significant association between ACE gene polymorphism and PE. However, further studies are required to investigate dif-

ferent gene polymorphisms in patients with PE that do not have a significant risk factor.

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