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Angiotensin Converting Enzyme Gene Insertion/Deletion Variant and Familial Mediterranean Fever-related Amyloidosis

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Introduction. The most important complication of familial Mediterranean fever (FMF) is secondary amyloidosis, which can lead to kidney failure. Genetic variability in the genes of various components of the renin-angiotensin system may play a role in the pathogenesis of the kidney disorders. The aim of the present study was to investigate the association between angiotensin converting enzyme (*ACE*) gene I/D variant and risk of developing FMF-related amyloidosis in Turkish patients.

Materials and Methods. A total of 240 individuals consisting of 40 patients with FMF-related amyloidosis, 100 FMF patients without amyloidosis, and 100 healthy controls were recruited. For all of the participants, *ACE* I/D variant was detected by the polymerase chain reaction using specific primers.

Results. A significant difference was found between the patients with FMF-related amyloidosis and the control group as for genotype distribution of *ACE* I/D variant ($P < .05$). The *ACE* D/D and I/D genotypes were more frequent in the patients with FMF-related amyloidosis while the I/I genotype was less frequent in the same patients. The FMF patients (with and without amyloidosis) had significantly higher percentages of the D/D and I/D genotypes than the healthy controls ($P < .05$). Comparison between the subgroups of FMF patients, divided into those with and without amyloidosis, yielded a significant correlation according to ID+II versus DD genotypes ($P < .03$, odds ratio, 3.24; 95% confidence interval, 1.05 to 12.01).

Conclusions. Based on these observations, the *ACE* I/D variant D/D genotypes implicate a possible risk in the FMF-related amyloidosis among Turkish population.

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INTRODUCTION

Familial Mediterranean fever (FMF) is one of the most common autosomal diseases that mainly affects ethnic groups living along the Eastern Mediterranean Sea, including the Turks, Sephardic Jews, Armenians, and Arabs.[1] It is characterized by fever with an irregular pattern, abdominal pain, and inflammatory episodes of polyserositis.[2] The Mediterranean fever gene, responsible for FMF, encodes a 781-amino acid protein named *pyrin* (also known as *marenostrin*), which appears to play a pivotal role in the regulation of both inflammation and apoptosis.[1] Recently, more than 300 sequence variants of the Mediterranean fever

gene have been reported in the Infevers Database.[3] The major and potentially fatal complication of the disease is secondary (AA) amyloidosis, which often influences the kidneys. Amyloidosis occurs as a result of tissue accumulation of amyloid, a proteolytic cleavage product of amyloid A, which is an acute-phase reactant.[4] The incidence of FMF-related amyloidosis varies among diverse ethnic groups.[5]

Renin-angiotensin system (RAS) acts as a key regulator of blood pressure and cardiovascular homeostasis. The angiotensin converting enzyme (ACE) is a major modulator in the RAS and kallikrein-kininogen systems acting by hydrolysing angiotensin I into angiotensin II, a potent vasopressor.[6] Angiotensin converting enzyme is found in numerous tissues such as the lungs, vascular endothelium, kidneys, heart, and testicles. It was shown that ACE has a major role in the physiology of the blood vessels and inflammatory process, and its association with several autoimmune disorders have been investigated widely.[6] The *ACE* gene is located on chromosome 17q23, containing an insertion (I) and deletion (D) variant (rs1799752) within intron 16 with the presence or lack of 287 bp repeat sequence.[7] When the three genotypes (II and DD homozygotes and ID heterozygotes) are taken into account, DD genotype carriers have the highest levels of serum ACE, whereas cases with the II genotype have the lowest serum ACE levels.[7] Given the background, investigation of variant in the *ACE* gene could provide information about the development of kidney diseases and could create innovative treatment models. With regard to the significance of RAS and the role of *ACE* gene variation in several cellular functions, the aim of the present study was to investigate the association between *ACE* I/D variant and risk of developing FMF-related amyloidosis in a Turkish population.

MATERIALS AND METHODS

Participants

Forty patients with FMF-related amyloidosis, 100 FMF patients without amyloidosis, and 100 healthy controls were included in present study. Patients were diagnosed and followed at Genetic Clinics of Samsun Training and Research Hospital, Department of Nephrology in Ordu State Hospital and Department of Internal Medicine in Gaziosmanpasa University Medical School. Amyloidosis was suspected clinically (recent proteinuria or nephrotic syndrome) and diagnosis was confirmed by kidney biopsy. For comparison, a control group was formed, consisting of 100 unrelated healthy individuals who had a similar ethnic background and resided in the same geographic area with the patients. All individuals were of Turkish origin. The study protocol was approved by the local ethics committee, and the patients signed an informed consent which was prepared according declaration of Helsinki.

Genotyping Analysis

The total genomic DNA was isolated from the whole blood of the patients and controls using DNA extraction kit (Sigma, USA) according to the manufacturer's protocol, and was stored at -20°C. The *ACE* I/D genotypes were detected by a polymerase chain reaction protocol using previously described methods.[8] Reactions were performed with specific 10 pmol of each primer: F: 5'CTG GAGACCACTCCCATC CTT TCT 3' and R: 5'GAT GTG GCC ATC ACATTC GTC AGAT 3' in a final volume of 50 µL, containing 3 mM of magnesium chloride, 50 mM of potassium chloride, 10 mM of Tris-HCl pH 8.4, 0.1 mg/mL of gelatin, 0.5 mM of each dNTP, and 2.5 U of Taq DNA polymerase. The polymerase chain reaction profiles were as follows: 30 cycles with denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute and 45 seconds, and extension at 72°C for 1 minute and 30 seconds, using a thermal cycler (Techne USA). The polymerase chain reaction products were separated on 2% agorose gels after etidium bromide staining. Two DNA fragments were observed after electrophoresis: one 190-bp fragment indicating the D allele, and one 490-bp fragment indicating the I allele.

Statistical Analysis

The the SPSS software (Statistical Package for the Social Sciences, version 22.0, SPSS Inc, Chicago, IL, USA) and the OpenEpi Info software package program were used for statistical analysis. Genotype distributions were calculated for deviation from the Hardy-Weinberg equilibrium by using the chi-square test. The Fisher exact test (when expected values were less than 5) or the chi-square test was used to compare the allele and genotype frequencies in patients and controls. Odds ratios (ORs) and

95% confidence intervals (CIs) were calculated. *P* values less than .05 were considered significant.

RESULTS

This case-control study included 40 patients with FMF-related amyloidosis (19 men and 21 women; mean age, 29.80 ± 13.70 years), 100 FMF patients without amyloidosis (42 men and 58 women; mean age, 29.19 ± 12.00 years), and 100 ethnically-matched healthy individuals (47 men and 53 women; mean age, 32.40 ± 12.02 years) with no previous family history of renal, vasculitis, allergies, or rheumatologic disease. The baseline characteristics of the participants are summarized in Table 1.

Genotype and allele frequencies for the *ACE* I/D variant in the patients with FMF-related amyloidosis and the control groups are shown in Table 2. A significant difference was found between the patients with FMF-related amyloidosis and the control group as for genotype distribution of *ACE* I/D variant ($P < .05$). The *ACE* I/D variant I/I genotype was lower in patients while D/D and I/D genotypes were higher in patients with FMF-related amyloidosis. There was no significant difference in allele frequencies of the *ACE* I/D between patients with FMF-related amyloidosis and the controls.

Genotype and allele frequencies of the *ACE* I/D variant in the FMF patients (with and without amyloidosis) and the controls are presented in Table 3. The FMF patients as a group had a significantly higher percentage of the D/D and I/D genotype than the controls ($P < .05$). No significant difference was found in the allele frequency of the ACE I/D variant between the FMF patients (with and without amyloidosis) and the control groups.

Furthermore, comparison between the subgroups

Table 1. Baseline Characteristics of Patients With Familial Mediterrenean Fever (FMF) and Control Group*

Characteristics	Controls (n = 100)	FMF Patients Without Amyloidosis (n = 100)	FMF Patients With Amyloidosis (n = 40)
Sex			
Male	47	42	19 (47.5)
Female	53	58	21 (52.5)
Mean age, y	32.40 ± 12.02	29.19 ± 12.00	29.80 ± 13.70
Mean age at onset of first symptoms, y	...	10.82 ± 7.00	12.12 ± 7.17
Mean age at onset, y	...	17.53 ± 9.79	21.12 ± 10.41
Mean frequency of attacks, d	...	25.54 ± 14.66	30.92 ± 18.32
Use of colchicine	...	72	33 (82.5)
Response to colchicine	...	70	3 (93.9)
Family history	...	54	24 (60.0)
Fever	...	82	33 (82.5)
Abdominal pain	...	90	36 (90.0)
Pleural effusion	...	34	19 (47.5)
Joint involvement	...	69	25 (62.5)
Erythema	...	17	35 (87.5)

*Values are mean ± standard deviation for continuous variables and frequency (percentage) for categorical variables.

Table 2. Genotype and Allele Frequencies of *ACE* I/D Variant in Patients With Familial Mediterrenean Fever (FMF)-related Amyloidosis and Controls

ACE I/D	FMF Patients With Amyloidosis	Controls	*P*	Odds Ratio (95% Confidence Interval)
Genotypes				
I/I	4 (10.0)	33 (33.0)		
I/D	20 (50.0)	37 (37.0)		
D/D	16 (40.0)	30 (30.0)	< .05	...
DD+ID:II	36:4	41:9	> .05	1.961 (0.56 to 7.88)
DD:ID+II	16:24	21:29	> .05	0.921 (0.36 to 2.32)
Alleles				
I	28 (35.0)	38 (38.0)		
D	52 (65.0)	62 (62.0)	> .05	0.879 (0.49 to 1.62)

Table 3. Genotype and Allele Frequencies of *ACE* I/D Variant in All Patients With Familial Mediterranean Fever (FMF) Regardless of Amyloidosis and Controls

ACE I/D	FMF Patients	Controls	*P*	Odds Ratio (95% Confidence Interval)
Genotypes				
I/I	13 (9.3)	33 (33.0)		
I/D	66 (47.1)	37 (37.0)		
D/D	61 (43.6)	30 (30.0)	< .05	...
DD+ID:II	127:13	41:9	> .05	2.135 (0.82 to 5.39)
DD:ID+II	61:79	21:29	> .05	1.066 (0.55 to 2.07)
Alleles				
I	92 (32.9)	38 (38.0)		
D	188 (67.1)	62 (62.0)	> .05	0.798 (0.49 to 1.29)

of FMF patients–divided into those with amyloidosis and those without–yielded a significant correlation according to DD versus ID+II genotypes ($P < .03$, odds ratio, 3.24; 95% confidence interval, 1.05 to 12.01). No significant relationship was noted in the *ACE* I/D allele and genotype distributions in each subgroup. The distributions of the *ACE* gene I/D variant in the two FMF groups are given in Table 4.

DISCUSSION

Familial Mediterranean fever belongs to the group of auto-inflammatory diseases in which the innate immune system is primarily affected.[9] With an incidence of 1:400 to 1:1000, Turkey is one of the countries in which FMF is seen most commonly.[10] The most important and potentially lethal complication of FMF is secondary amyloidosis, which is usually seen with renal involvement. Familial Mediterranean fever-related amyloidosis occurs in more than 50% to 60% of untreated patients.[5] Amyloidosis is not necessarily related with the severity of FMF, as it may be seen in patients with in frequent and mild attacks, as well as in asymptomatic individuals who do not have any clinical manifestations of the disease (phenotype II).

It has been well documented for nearly 50 years that RAS modulates many physiological functions. Comprehensive research in the past decade has established the idea that the RAS, aside being a circulating endocrine system, is also an organ- and tissue-based system that executes paracrine/autocrine functions. It is now believed that the dominant effector peptide of the RAS, angiotensin II is a real cytokine that has an impact on the inflammatory response.[11] Angiotensin II is involved in regulating inflammatory cell responses. In this context, angiotensin II acts as a chemotactic factor for mononuclear cells, neutrophils, and lymphocytes T and B.[12,13] Angiotensin II also plays a role in upregulation of the expression of monocyte chemoattractant protein type 1, tumor necrosis factor-α, interleukin-6, and interleukin-8, that are all strong chemoattractants and activators of neutrophils.[14] Moreover, angiotensin II enhances acute inflammation marker C-reactive protein both in mRNA and protein levels in macrophages via angiotensin II type 1A receptor-mediated reactive oxygen species synthesis and nuclear factor-kappa B activation.[15] In addition, inflammatory cells express

Table 4. Genotype and Allele Frequencies of *ACE* I/D Variant in Patients With Familial Mediterranean Fever (FMF) With and Without Amyloidosis

ACE I/D	FMF Patients With Amyloidosis	FMF Patients Without Amyloidosis	*P*	Odds Ratio (95% Confidence Interval)
Genotypes				
I/I	4 (10)	9 (9)		
I/D	20 (50)	46 (46)		
D/D	16 (40)	45 (45)	> .05	
DD+ID:II	36:4	91:9	> .05	0.890 (0.26 to 3.51)
DD:ID+II	16:24	55:45	.03	3.244 (1.05 to 12.01)
Alleles				
I	28 (35)	64 (32)		
D	52 (65)	136 (68)	> .05	1.144 (0.65 to 1.97)

all the components of the RAS, and can synthesize angiotensin II. The monocytes/ macrophages at inflammatory sites possess high ACE activity.[16]

Furthermore, angiotensin II acts as a renal growth factor.[17] Some models of kidney injury, independent of the existence of hypertension, activation of the tissue RAS has been reported.[18] Angiotensin II is involved in the recruitment of infiltrating cells into the kidney; angiotensin II leads to the adhesion of circulating cells to endothelial and mesangial cells, and the migration of inflammatory cells into the kidney. This event is regulated by upregulation of adhesion molecules, cytokines, and chemokines.[19] Intravenous administration of angiotensin II to rats results in inflammatory cell accumulation in the glomeruli and noticeably in the interstitium.[12] These animals also manifested an elevated glomerular expression of the chemokine regulated upon activation of normal T cell expressed/secreted.[12] In human kidney diseases, the activated RAS system has been identified. In diabetic nephropathy, high degree of angiotensin II production was associated with the presence of inflammatory cell infiltration, the activation of nuclear factor-kappa B and proinflammatory gene overexpression.[19]

Moreover, some of the degradation metabolites of angiotensin II, including angiotensin III, also play a role in regulation of cardiovascular and kidney function. New evidence supports that angiotensin III can be involved in the pathogenesis of kidney damage.[20]

Therefore, the current study was designed to establish the association of the *ACE* gene I/D variant in subjects with FMF-related amyloidosis. Previously, Yigit and colleagues reported that *ACE* gene was associated with an increased risk in FMF in a Turkish cohort.[8] But, as far as we know, the association between *ACE* I/D variant and FMF-related amyloidosis has not been investigated previously in a Turkish population. In our multicentre study, we collected the clinical and demographic characteristics of the FMF patients with amyloidosis. The ratio of male to female in patients with amyloidosis was 0.9. We showed that *ACE* D/D and I/D genotype were significantly more frequent in FMF patients (with and without amyloidosis) compared to the control group. Additionally, we found that *ACE* I/D variant I/I genotype was lower in patients while D/D and I/D genotype were higher in patients with FMF-related amyloidosis. It was known that the *ACE* D allele is correlated with increased serum ACE activity in humans. Therefore, in the present study as well, D allele in homozygous condition, ie, DD genotype, is expected to make FMF patients with DD genotype more susceptible to renal involvement. Our results were consistent with this expectation. However, there are some limitations of this study. For instance, lack of assessment of expression levels of ACE is a limitation of this study.

CONCLUSIONS

Although FMF is a single-gene disorder, there are still unclarified aspects in its pathophysiology. In conclusion, we demonstrate for the first time that the presence of *ACE* gene I/D variant constitutes a risk factor for etiopathogenesis of FMF-related amyloidosis. Our results suggest that the DD genotype of the *ACE* gene could be a useful genetic marker with important clinical and prognostic implications in recognizing FMF subjects that are at greater risk of amyloidosis. However, larger prospective studies will be necessary to assess the role of the *ACE* D allele in FMF patients with and without renal involvement.

CONFLİCT OF İNTEREST

None declared.

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