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Determining MEFV gene mutations and relationships with disease activity in patients with ankylosing spondylitis

Ankilozan spondilit hastalarında MEFV gen mutasyonları ve hastalık aktivitesi ile ilişkisinin değerlendirilmesi

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SUMMARY

Objective: The aim of this study is to assess presence of MEFV (MEditerranean FeVer) gene mutations and relationship between those mutations and disease activity in ankylosing spondylitis (AS).

Method: Study group was consisted of 34 patients with AS, and 35 healthy volunteers enrolled to study as control group. 12 MEFV gene mutations were screened by Stripe-Assay technique. Patients with AS were divided into 2 subgroups according to whether they are MEFV gene positive or negative. Demographic data of study group were questioned. Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI) and The Bath Ankylosing Spondylitis Metrology Index (BASMI), peripheral joint involvement, and drug use were used as clinical parameters; Visual Analog Scale (VAS) was used to assess pain, and erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were assessed as laboratory parameters.

Results: Among 34 patients with AS, it was found that 5 patients (35.7%) were positive for E148Q, 2 patients (14.2%) for M694V, 2 patients (14.2%) for A744S, 2 patients (14.2%) for V726A and 1 patient (7.1%) for each of the M680I, R761H, P369S. Among 35 individuals in control group, E148Q was detected in two (5.7%) and M694V was detected in another two (5.7%). When MEFV gene mutations were compared between study group and control group, there was no significant difference ($p>0.05$). There was significant difference between MEFV carriers and non-carriers regarding BASDAI, CRP and ESR values and positive family history whereas no difference was found in BASMI, BASFI, VAS, disease duration and biological agent use.

Conclusions: It was thought that MEFV mutation frequency in AS patients was similar with control group but disease should have more progressive course in MEFV positive AS patients. To address this issue, further studies with large series are needed.

Keywords: Anklyosing spondylitis, MEFV gene mutations, disease activity

ÖZET

Amaç: Ankilozan Spondilit (AS) hastalarında MEFV (MEditerranean FeVer) gen mutasyonlarının varlığını ve bunun hastalık aktivitesi ile olan ilişkisini değerlendirmektir.

Yöntem: AS tanısı olan 34 hasta ve 35 sağlıklı gönüllülerden oluşan kontrol gurubu alındı. Strip-assay tekniği ile 12 adet MEFV gen mutasyonuna bakıldı. AS hastaları MEFV mutasyonu pozitif ve negatif olarak iki gruba ayrıldı. Her iki grubun demografik verileri sorgulandı. Klinik parametre olarak, Bath Ankilozan Spondilit Hastalık Aktivite İndeksi (BASDAİ), Bath Ankilozan Spondilit Fonksiyonel İndeksi (BASFİ), Bath Ankilozan Spondilit Metroloji İndeksi (BASMİ), ağrı için

Vizüel Analog Skala (VAS), periferik eklem tutulumu, ilaç kullanımı, labaratuvar olarak eritrosit sedimantayon hızı (ESH) ve C-Reaktif Protein (CRP) değerlendirildi.
Bulgular: AS'li 34 hastanın 7'sinde (%20,6) E148Q, 4'ünde (%11,8) M694V, 2'sinde (%5,9) A744S, 2'sinde (%5,9) V726A, 1'er hastada da (%2,9) M6801, R761H, P369S pozitif olarak bulundu. Kontrol grubundaki 35 bireyden 2'sinde (%5,7) E148Q, 2'sinde (%5,7) M694V tespit edildi. Hasta ve kontrol grubundaki MEFV mutasyonları karşılaştırıldığında farklılık önemsizdi ($p>0,05$). MEFV mutasyonu pozitif olan 14 AS'li hastanın 7'sinde (%50,0) E148Q, 4'ünde (%28,6) M694V tespit edilmiş olup bu oran istatiksel olarak anlamlı bulundu ($p<0,05$). MEFV mutasyonu taşıyan ve taşımayanlar arasında BASDAİ, CRP, ESH ve pozitif aile öyküsü anlamlı bulunurken, BASMİ, BASFİ, VAS, hastalık süresi, biyolojik ajan kullanımı açısından fark bulunamamıştır.
Sonuç: AS hastalarında MEFV mutasyon sıklığının kontrol grubu ile benzer olduğu fakat MEFV mutasyonu pozitif olan AS hastalarında hastalığın daha progresif seyredebileceği düşünülmektedir. Bununla ilgili daha çok olguyla yapılacak çalışmalara ihtiyaç vardır.
Anahtar sözcükler: Ankilozan spondilit, MEFV gen mutasyonları, hastalık aktivitesi

INTRODUCTION

Ankylosing spondylitis (AS) is a common inflammatory rheumatic disease characterized by sacroiliac joint and axial skeletal involvement[1]. It is thought that genetic, environmental, and immunological factors are effective in the pathogenesis of this disease. Best known genetic predisposition factor is HLA-B27 (human leucocyte antigen), but its association with the pathogenesis of this disease is still not exactly known[2]. The risk of occurrence of AS in people who are positive for HLA-B27 is 1.3%[3]. This also suggests that some genes other than HLA-B27 and some environmental factors are also lying under the development of AS. MEFV gene, which is responsible for activations of many rheumatic diseases, may also have a role in the development of AS[4].

MEFV gene, which is responsible for Familial Mediterranean Fever (FMF), is located in the 13.3 region of 16th chromosome (16p), formed of 10 exons and 15 kb of 3505 nucleotides, and expresses a weak transcript of 3.7 kb and encodes a protein of 781 aminoacids[5]. It encodes a protein named pyrine/marenostrine that synthesized in the cytosols of neutrophils, eosinophilia, and activated monocytes. Pyrine protein is thought to play a role in inhibiting the neutrophil activity and inflammation during the FMF episodes[6].

Carrying the heterozygous MEFV mutations in FMF is suspected for increased clinical and subclinical inflammation[7], and disease modification for increased inflammatory response in the development or during the course of the chronic inflammatory diseases. In a study that was conducted for determining the effects of MEFV mutation on disease activity and inflammation, it was found that these parameters were both increased in the presence of MEFV mutations in patients with RA. It is unclear that how the MEFV mutation increases the disease severity in RA, but this was thought to be associated with pyrine functions[8]. Thus, MEFV gene mutations were found to have impact on chronic inflammatory diseases other than RA, such as Ulcerative Colitis (UC), Behcet's disease, multiple sclerosis, Henoch-Schönlein Purpura (HSP), and Chrohn Disease[9-12]. Patients that have the inflammatory load of these diseases are thought to be more vulnerable for more severe disease in the presence of MEFV mutations. We aimed in this study to investigate the presence of MEFV gene mutations in AS, which is also a chronic inflammatory disease, and to determine its associations with the disease activity.

MATERIAL AND METHODS

This study included 34 patients, whom were diagnosed according to the Modified New York Diagnostic Criteria[13], and 35 healthy volunteers as control group. Patients that met criteria for other spondyloarthopathies, having suspected history of FMF, or having FMF in family history were excluded from the study. All patients informed about the study and consents were taken. After inclusion, venous blood samples were taken from the patients in tubes containing 10 mL of

EDTA.

Age, gender, disease onset and duration, medication, family history, and peripheral joint involvement were noted for each patient. Disease activity was evaluated by laboratory parameters as ESR and CRP, and clinical parameters as BASDAI, BASMI and BASFI. Patients and healthy controls were assessed for prevalent 12 MEFV mutations. Patients with AS were separated into two groups according to the presence of MEFV mutation, or not. Disease activity in the preceding 1 week was evaluated by BASDAI. Axial skeletal pain, morning stiffness, peripheral joint pain, non-joint pain and fatigue were evaluated by the patients by a VAS scale of 1 to 10, and arithmetic means were calculated. Mean score of two questions that evaluates morning stiffness was calculated and summed with the other questions. BASDAI values greater than 4/10 were regarded as active disease.

Genetic analysis

Twelve prevalent mutations for MEFV gene were studied from the 10 mL of blood samples. These 12 mutations (E148Q, P369S, F479L, M680I (G/C), M680I (G/A), 1692del, M694V, M694I, K695R, V726A, A744S ve R761H) were analyzed in the Genetics Department by using reverse hybridization stripe assay technique (Vienna Lab) according to the directions of the manufacturer.

Statistical analysis

Data was evaluated by SPSS Ver. 14.0. Chi-square test, Mann-Whitney-U test, and Fisher's Exact Chi-Square test were used for the statistical analyses. Values were presented as arithmetic mean±standard deviation, number of cases and % in the tables. Statistical significance level was considered as 0.05 in all analyses.

RESULTS

All participants were separated into two groups as patients and controls, and patients were then separated into two groups again according to the presence of MEFV mutations. Mean ages of patients were 39.97±11.33 years, and 41.25±9.9 for controls. Difference of the ages of patients and controls was not statistically significant (t=0.50, p=0.618). Female to male ratio were 9 (26.5%) to 25 (73.5%), and 10 (28.6%) to 25 (71.4%) in patient and control groups, respectively. Gender distribution between these two groups was similar (X^2=0.03; p=0.845).

MEFV mutations in 34 patients with AS were as follows: 5 patients (35.7%) with E148Q, 2 patients (14.2%) with M694V, 2 patients (14.2%) with A744S, 2 patients (14.2%) with V726A, 1 patient (7.1%) with M6801, 1 patient (7.1%) with R761H, and 1 patient (7.1%) with P369S. In the control group 2 patients (5.7%) had E148Q, and 2 patients (5.7%) had M694V mutations. When the MEFV mutations were compared between study and control groups, it was found that the difference between the groups was not statistically significant (p>0.05). No homozygous mutations were determined in the patient group, but 4 patients had complex mutations (1 patient M694V/E148Q, 2 patients M694V/A744S, and 1 patient M694V/P369S).

When the BASFI and BASMI values were compared between patients with and without MEFV mutations, no significant differences were found (p=0.186 and p=0.986, respectively). But there was a statistically significant difference in BASDAI (p=0.001). Comparisons of BASDAI, BASFI, and BASMI values between patients with and without MEFV mutations were presented in Table 1. Disease duration and VAS values were found to be similar (p>0.05), but ESR and CRP values were significantly different (p<0.05) between patients with and without MEFV mutations. Comparisons of clinical and laboratory parameters between patients with and without MEFV mutations were presented in Table 2.

Table 1: BASDAI, BASFI, and BASMI values of patients with and without MEFV mutations.

	MEFV positive	**MEFV negative**	**p**
	(n=14)	(n=20)	
	Mean±SD	Mean±SD	
BASDAI	5.12±1.27	3.05±1.34	p=0.001
BASMI	5.35±2.37	4.11±2.45	p=0.186
BASFI	3.85±2.16	3.76±1.78	p=0.986

BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; **BASMI:** Bath Ankylosing Spondylitis Metrology Index; **BASFI:** Bath Ankylosing Spondylitis Functional Index

Table 2: Clinical and laboratory parameters of patients with and without MEFV mutations.

	MEFV positive	MEFV negative	p
	(n=14)	(n=20)	
	Mean±SD	Mean±SD	
ESR	33.21±15.13	21.60±21.13	p=0.020
CRP	16.72±12.67	8.01±11.39	p=0.012
VAS	7.85±1.83	5.85±3.15	p=0.074
Disease duration	10.85±10.97	11.00±9.58	p=0.699

ESR: Erythrocyte Sedimentation Rate; **CRP:** C-reactive protein; **VAS:** Visual Analogue Scale

Table 3: Distribution of MEFV mutations according to peripheral joint involvement.

	Peripheral joint involvement		p
	Positive	Negative	
	n (%)	n (%)	
MEFV (-)	4 (20%)	16 (80%)	0.135
MEFV (+)	7 (50%)	7 (50%)	
Total	**11 (32.4%)**	**23 (67.6%)**	

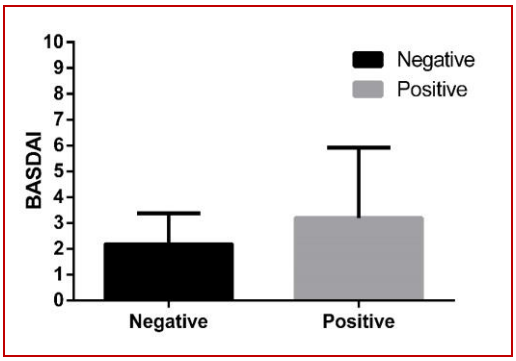

Figure 1. BASDAI values in the patients with and without MEFV mutations.

Peripheral joint involvement was similar between patients with and without MEFV mutations (p>0.05), but involvement rates were higher in MEFV positive patients. Distribution of MEFV mutations according to peripheral joint involvement were presented in Table 3.

When the patients with AS were distributed into two groups according to MEFV positivity, 14 patients constituted the MEFV positive group, and 7 of them (50%) had E148Q, 4 of them (28.6%) had M694V mutations, and this was found to be statistically significant (p=0.001; p=0.022, respectively).

BASDAI values were 5.12 ± 1.27 and 3.05 ± 1.34 in patients with and without MEFV mutations, respectively. The difference between the groups was statistically significant (p<0.05). Mean BASDAI values in these patient groups were presented in Figure 1.

DISCUSSION

Although the etiopathogenesis of AS is not exactly known, generally genetic factors are suspected. Because the majority of the autoimmune diseases are polygenic in nature, and genetic polymorphisms increase the vulnerability to the disease[15], MEFV gene mutations are investigated for AS in this study.

Genetic studies showed that HLA-B27 had a contribution of 30-40%, and MHC region that also includes HLA had a contribution of 50% to the total genetic vulnerability[16]. Genome wide association studies, which were conducted for determining the other genes that contributes to the vulnerability, defined many non-HLA nominate gene regions. Most important non-HLA region that contributes to the AS pathogenesis was reported by these association studies as IL-1 gene family located in 2nd chromosome. This region includes Il-1α, IL-1β and IL-RA genes, and large scaled association studies confirmed the relationships between the polymorphisms of this region and the AS[17].

It was previously reported that mutations of MEFV gene that is responsible for FMF are increased in patients with AS, and carrying the heterozygous mutations of this gene is associated with increased erythrocyte sedimentation rate, peripheral arthritis, and repeated oral ulcers. The association of MEFV gene mutations and increased IL-1β activity also confirms the association between AS and IL-1 polymorphisms[18].

Durmuş et al[19]. found in their study that frequency of MEFV mutations were similar in AS and control groups, but presence of MEFV mutation in patients with AS increased the disease severity. This was explained by the increased inflammatory response could cause more severe disease[19]. Despite of the previous studies, we found the MEFV mutations in a higher frequency. We also checked for laboratory parameters in control group, and both the laboratory parameters and BASDAI values that show the disease

activity were meaningfully higher.

Çınar et al[20]. conducted a longitudinal clinical study in 95 patients with AS, and they could not determine any significant differences for clinical and laboratory parameters between patients that have MEFV mutations or not. They also did not find any E148Q mutations[20]. Despite of their study, we found the most frequent mutation was E148Q (50%) in patients with MEFV mutations and these patients had increased ESR, CRP and BASDAI values. Determination of this mutation in a significantly higher frequency may be related to geographical differences.

The association between MEFV mutations and arthritic diseases were suggested by Booth et al[21]. in their study that reported higher frequencies of E148Q mutations than normal levels in white and Indian patients with inflammatory arthritis and amyloidosis. E148Q is a mutation at 2nd exon and it is seen widely in normal population, and also thought to be a mutation with low penetration. But a previous study reported that combined heterozygosity of V726A-E148Q can cause amyloidosis[22, 23]. Rabinovich et al[8]. also determined E148Q as the most frequent mutation in patients with RA, and reported severe disease in this case. Our patients with AS and MEFV mutations also had higher frequencies of E148Q mutations, and colchicine treatment may be important in these patients in regard of amyloidosis.

As a conclusion, we found that MEFV mutation frequency is similar with control group, but disease severity may increase in patients with AS and MEFV mutations. Further studies are needed when the geographical factors that can affect the MEFV mutation carriers taken into consideration. We think that determination of new gene regions that modifies the severity of AS, which can be a progressive and deteriorating disease, will yield new explanations in the pathophysiology of the disease, and will help develop new treatment protocols.

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