Association of MEFV Gene Mutations With Rheumatoid Factor Levels in Patients With Rheumatoid Arthritis

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Purpose: Rheumatoid arthritis (RA) is a systemic autoimmune disease primarily affecting the joints. Arthritis disorders are associated with mutations of the Mediterranean fever (*MEFV*) gene. This gene has already been identified as being responsible for familial Mediterranean fever. The aim of this study was to explore the frequency and clinical significance of *MEFV* gene mutations in a cohort of Turkish patients with RA.

Methods: The study included 101 patients with RA and 110 healthy controls. Genomic DNA was isolated and genotyped using polymerase chain reaction and restriction fragment length polymorphism for the 5 *MEFV* gene mutations (M694V, M680I, V726A, E148Q, and P369S).

Results: Carrier rates of *MEFV* gene mutations were 31 (30.7%) of 101 and 26 (23.6%) of 110 in the RA and healthy control groups, respectively ($P > 0.05$; odds ratio, 1.4; 95% CI, 0.77–2.65). Whereas deformed joint count was relatively higher in the mutation carrier group than those of the noncarrier group, the rheumatoid factor levels were significantly higher in the carrier group of patients with RA ($P = 0.001$).

Conclusions: The results of this study suggest that *MEFV* gene mutations are not positively associated with a predisposition to develop RA but might increase the severity of RA. Further research is needed to determine the actual pathogenic role of *MEFV* mutations in this disease.

Key Words: rheumatoid arthritis, *MEFV* gene, mutation, autoimmune disease

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Rheumatoid arthritis (RA) (MIM180300) is a systemic autoimmune disease primarily affecting the joints. It affects approximately 0.5% of the adult population worldwide and occurs in 20 to 50 cases per 100,000 annually, mainly in women after their 40s.[1] It is a complex genetic disease that its onset has important diagnostic, prognostic, and therapeutic implications and is yet to be defined. Genes important for the onset or course of RA have been described mainly by the association of variations in genes coding for proteins that participate in known immune and/or inflammatory events of putative importance for joint inflammation.

Arthritis, or inflammation in joints, is a very common condition in humans. It was demonstrated that arthritis disorders such as inflammatory bowel diseases,[2] juvenile idiopathic arthritis,[3] Behcet disease,[4] intermittent hydrarthrosis,[5] multiple sclerosis,[6] palindromic rheumatism,[7] and ankylosing spondylitis[8] are associated with mutations of the Mediterranean fever (*MEFV*) gene. This gene has already been identified as being responsible for familial Mediterranean fever (FMF).[9] Interestingly, arthritis is a common manifestation in FMF, especially in M694V homozygotes.[10,11] The frequency of arthritis in FMF has been reported to range from 21% to 77% in different ethnic groups.[12–18] Familial Mediterranean fever predominantly affects people living in or originating from areas around the Mediterranean basin, mainly Jews, Armenians, Turks, and Arabs.[19,20] Because *MEFV* mutations are common in general population among Turkish people, it is important to investigate the impact of this genotype on RA.

The *MEFV* gene is located on the short arm of chromosome 16p13.3, comprises 10 exons[21] and encodes a 781–amino acid protein called marenostrin or pyrin. Marenostrin is only expressed in neutrophils and monocytes, which are the cell types involved in innate immune responses. Marenostrin has a key role in the regulation of inflammasome activity and pro–interleukin-1β processing.[22,23] At present, more than 100 different FMF-associated mutations of the *MEFV* gene, which are usually located on exon 10, have been identified. Four of these, called founder mutations (M680I, M694V, M694I, and V726A), are the most prevalent and account for most of the FMF cases worldwide.[9]

The recurrent nature of arthritis in patients with RA and the findings that different recurrent arthritis syndromes associated with the *MEFV* gene suggest that this gene may participate in the pathogenesis of rheumatic diseases characterized by relapsing inflammatory episodes. Therefore, we investigated whether the *MEFV* gene might be implicated in the pathogenesis of RA. We adopted a case-control design to compare the *MEFV* mutation frequency between patients with RA and healthy subjects and to compare disease severity between mutation carriers and noncarriers.

MATERIALS AND METHODS

Patients and Controls

The study population comprised 101 unrelated patients with RA (27 men and 74 women; mean ± SD age, 51.4 ± 13.9 years; mean ± SD disease duration, 6.3 ± 5.8 years) recruited consecutively from those whom were treated and followed up in the physical medicine and rehabilitation department of Gaziosmanpasa University Research Hospital, Tokat, Turkey. Rheumatoid arthritis was diagnosed according to the established criteria.[24] A total of 110 unrelated healthy subjects (30 men and 80 women; mean ± SD age, 53.2 ± 11.2 years) were recruited consecutively. All participants, patients and healthy subjects, were of Turkish origin, from the Central Black Sea region of Turkey. The protocol of this study was approved by the institutional ethics committee, and all participants gave

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TABLE 1. Clinical and Demographic Data on the Patients With RA

	Total (n = 101)	Carrier (n = 31)	Noncarrier (n = 70)	*P*
Sex, no. female/male (%female/% male)	74/27 (73.3/26.7)	23/8 (74.2/25.8)	51/19 (72.9/27.1)	0.917
Age, yrs	51.4 ± 13.9	49.4 ± 12.9	52.3 ± 14.3	0.540
Age at disease onset, yrs	45 ± 13.4	43.8 ± 13.0	45.6 ± 13.7	0.769
Disease duration, yrs	6.3 ± 5.8	5.4 ± 5.6	6.7 ± 5.9	0.769
RF-positive patients, n (%)	91 (90.1)	26 (83.9)	65 (92.9)	0.301
Serum RF levels, IU/mL	134.7 ± 197.7	201.3 ± 254.8	105.2 ± 160.2	0.001*
Swollen/tender joint count	8.2 ± 10.5	7.4 ± 8.8	8.5 ± 10.6	0.261
Deformed joint count	3.8 ± 6.8	4.3 ± 8.1	3.5 ± 6.1	0.054

Data are given as mean ± SD, unless indicated otherwise.
*Statistically significant.

informed consent before entering the study. All participants were evaluated for the clinical findings of FMF, and none of them had symptoms or family history of FMF. The age of disease onset, disease duration, serum rheumatoid factor (RF) levels, and tender/swollen and deformed joint count were obtained.

Genetic Analysis of MEFV Mutations

Genomic DNA was isolated from peripheral blood lymphocytes using a commercial kit (Sigma-Aldrich, Taufkirchen, Germany) according to the manufacturer's instructions. The most frequently observed 4 mutations (E148Q, M694V, M680I, and V726A) and an additional rare mutation (P369S) in the *MEFV* gene were screened in this study. These 5 mutations were detected by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. Polymerase chain reactions of M694V, M680I, and V726A mutations were performed by using previously described protocols.[25] *Hin*fI, *Hph*I, and *Alu*I restriction enzymes were used for restriction fragment length polymorphism of M694V, M680I, and V726A mutations, respectively. The sense oligonucleotide primer for E148Q was 5'-CCTGAAGACTCCAGACCACCCCG-3', and the antisense primer was 5'-GGCCCTCCGAGGCCTTCTCTCTG-3′. The sense oligonucleotide primer for P369S was 5'-TCCCCGAGGCAGTTTCTGGGCACC-3', and the antisense primer was 5'-TGGACCTGCTTCAGGTGGCGCTTAC-3'. Polymerase chain reactions of E148Q and P369S mutations were performed by using previously described protocols.[26] *Bst*NI and *Alu*I restriction enzymes were used for restriction fragment length polymorphism of E148Q and P369S mutations, respectively. The amplified products were separated by electrophoresis on a 2% agarose gel. Ethidium bromide staining was used to detect the amplified fragments.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS version 13.0) and the OpenEpi Info software package version 2.3.1 (www.openepi.com). Results were given as mean ± standard deviation (SD). The relationships between mutation carriers and the clinical features were analyzed by using χ^2 statistics. The Fisher exact test was used to compare categorical variables, and odds ratio (OR) and 95% confidence interval (CI) were used for the assessment of risk factors. All *P* values were 2 tailed, and CIs were set at 95%. $P < 0.05$ was considered significant.

RESULTS

Table 1 shows the demographic and clinical characteristics of the patients with RA according to the presence (carrier) or absence (noncarrier) of *MEFV* mutations. Deformed joint count was relatively higher in the carriers than in the noncarriers of the RA group (4.3 ± 8.1 vs 3.5 ± 6.1, respectively). Serum RF levels were significantly higher in the carriers than in the noncarriers of the RA group ($P = 0.001$). No other significant differences were observed between patients with MEFV mutations and those without MEFV mutations.

In the healthy control (HC) group, mutation analysis showed that 26 subjects (23.6%) were carrying one mutated *MEFV* allele. The frequencies of M694V, M680I, V726A, E148Q, and P369S mutation carriage were 8.2% (with 9/220 allele frequency), 3.6% (4/220), 4.5% (5/220), 6.4% (7/220), and 1% (0.9), respectively (Table 2). Compound heterozygous were not detected in the HC group. In the RA group, mutation analysis showed that 31 patients (30.7%) were carrying at least one mutated *MEFV* allele (Table 2). Compound heterozygous was found for V726A/E148Q (2 patients) and V726A/P369S (one patient) mutations. The frequencies of M694V, M680I, V726A, E148Q, and P369S mutation carriage in the cohort of Turkish patients with RA were 10.9% (with 11/202 allele frequency), 5% (5/202), 6% (6/202), 9% (9/202), and 3% (3/202), respectively. There was no difference of the *MEFV* gene mutation carrier rates between the RA and HC groups (OR, 1.4; 95% CI, 0.77–2.65; $P > 0.05$).

DISCUSSION

In this study, we investigated the presence of genetic variants in the *MEFV* gene, which encodes for pyrin (a putative regulator of inflammasome activity and pro–interleukin-1β

TABLE 2. Distribution of *MEFV* Gene Mutations in the RA and HC Groups

Mutation	RA (n = 101), n (%)	HC (n = 110), n (%)
M694V/WT	10 (9.9)	9 (8.2)
M680I/WT	5 (5.0)	4 (3.6)
V726A/WT	4 (4.0)	5 (4.5)
E148Q/WT	7 (7.0)	7 (6.4)
P369S/WT	2 (2.0)	1 (0.9)
M694V/P369S	1 (1.0)	-
V726A/E148Q	2 (2.0)	-
Total mutations	31 (30.7)	26 (23.6)
Allele frequency	34/202	26/220

WT indicates wild type.

processing), in a cohort of Turkish patients with a clinical diagnosis of RA. Mutation analysis of the *MEFV* gene in this cohort of patients with RA did not show any association between *MEFV* mutations (M694V, M680I, V726A, E148Q, and P369S) and RA. In a previous case-control study, although there were only 2 mutations in common with our study, it was shown that *MEFV* gene mutations (M694I, R408Q, P369S, E148Q, and L110P) was not a genetic risk factor affecting the susceptibility of RA in a Japanese population (126 Japanese patients with RA and 76 Japanese healthy subjects).[27] Again, Koca et al.[28] did not find an association between patients with RA and HCs in a Turkish population from the upper Euphrates region of Turkey for the 5 *MEFV* mutations (E148Q, M694V, M694I, V726A, and P369S). Their analyzed mutations (except one) and number of patients (n = 103) and HCs (n = 103) were similar with ours. In 2 different studies of Turkish population where patients with RA were used as disease control, prevalence of *MEFV* mutations in the patients with RA was found to be similar with healthy subjects.[29,30] Rabinovich et al.[31] had reported that *MEFV* mutations were not positively associated with a predisposition to develop RA in a study of 98 Israeli patients with RA (74 women and 24 men) and 100 healthy subjects. The results of 3 studies in different Turkish populations and other Israeli and Japanese populations are concordant with our results and showed that the prevalence of *MEFV* gene mutations were not different in the patients with RA and in healthy population. However, in another study of Turkish population, it was demonstrated that RA was significantly higher in asymptomatic mutation carrier parents of FMF patients compared to controls.[32]

We also investigated the presence of potential genotype-phenotype relationships in patients with RA with *MEFV* mutations and those without *MEFV* mutations. No differences were identified, with the exception of deformed joint count and RF levels, which were higher in the patients with RA with *MEFV* mutations. Recent analyses with RF levels and *MEFV* mutations (M694V, M694I, M680I, V726A, and E148Q) revealed a significant association between the presence of the E148Q polymorphism with increased RF levels (>15 mg/dL) (χ^2 = 7.358; P = 0.007; OR, 5.41; 95% CI, 1.41-20.64) in an elderly Turkish population free of chronic inflammatory disease (n = 164).[33] Rheumatoid factor levels and *MEFV* mutations were also compared in patients with palindromic rheumatism with *MEFV* mutations and those without *MEFV* mutations in a Spanish population; and on the contrary, no statistically significant associations were found.[7] In another study, Koca et al.[28] found that deformed joint count was significantly higher in the carriers than in the noncarriers of the RA group in a cohort of Turkish population (P = 0.026). Rabinovich et al.[31] have reported that there was a high RF positivity in mutation carriers compared with noncarriers in a cohort of Israeli patients with RA.

Carriers for *MEFV* mutations had an increase in inflammatory symptoms related to the serosal membranes.[32] Thus, even one mutation in the *MEFV* gene may confer a predisposition to inflammation. Inflammation might predispose the carriers to some chronic inflammatory conditions. It was demonstrated that carriers of the *MEFV* mutations compared to the control group had an increased rate of RA.[32] Ozen et al.[3] also suggested that rheumatic diseases were increased in the carriers.

There are several limitations in this study. First, we used a relatively small sample size for a genetic association study, although we used functionally characterized genetic variants. Second, RA is genetically a very complex disease. Besides *MEFV* mutations, numerous other environmental and genetic factors also influence clinical RA characteristics.

CONCLUSIONS

In conclusion, observations reported herein suggest that *MEFV* mutations are not positively associated with a predisposition to develop RA. However, this study shows high RF levels in patients with RA with *MEFV* mutations. This result suggests that mutations in the *MEFV* gene might increase the severity of RA. Our results show that *MEFV* gene mutations may not be a susceptibility factor for the development of RA but might increase the severity of RA. The limitation of the present study is that markers of genetic admixture were not studied, thus introducing the potential for confounding by population stratification. Further research with larger sample size is needed to determine the actual pathogenic role of *MEFV* mutations in this disease.

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