Common Mediterranean fever (MEFV) gene mutations associated with ankylosing spondylitis in Turkish population

Serbulent Yigit\textsuperscript{a}, Ahmet Inanir\textsuperscript{b}, Nevin Karakus\textsuperscript{a,c,*}, Esra Kesici\textsuperscript{b} and Nihan Bozkurt\textsuperscript{a}

\textsuperscript{a}Department of Medical Biology, Faculty of Medicine, Gaziosmanpasa University, Tokat, Turkey
\textsuperscript{b}Department of Physical therapy and Rehabilitation, Faculty of Medicine, Gaziosmanpasa University, Tokat, Turkey
\textsuperscript{c}Department of Medical Biology, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey

Abstract. Ankylosing spondylitis (AS) is a common inflammatory rheumatic disease. Mediterranean fever (MEFV) gene, which has already been identified as being responsible for familial Mediterranean fever (FMF), is also a suspicious gene for AS because of the clinical association of these two diseases. The aim of this study was to explore the frequency and clinical significance of MEFV gene mutations (M694V, M680I, V726A, E148Q and P369S) in a cohort of Turkish patients with AS. Genomic DNAs of 103 AS patients and 120 controls were isolated and genotyped using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. There was a statistically significant difference of the MEFV gene mutation carrier rates between AS patients and healthy controls ($p=0.004$, OR: 2.5, 95% CI: 1.32–4.76). This association was also observed in allele frequencies ($p=0.005$, OR: 2.3, 95% CI: 1.27–4.2). A relatively higher frequency was observed for M694V mutation in AS patients than controls (10.7% versus 4.2%, $p=0.060$). There were no significant differences between MEFV mutation carriers and non-carriers with respect to the clinical and demographic characteristics. The results of this study suggest that MEFV gene mutations are positively associated with a predisposition to develop AS.

Keywords: Ankylosing spondylitis, MEFV gene, mutation, inflammatory rheumatic disease

1. Introduction

Ankylosing spondylitis (AS) is a common inflammatory rheumatic disease characterized by inflammation of the axial joints, peripheral oligoarthritis, and enthesitis as well as extraarticular features including acute anterior uveitis, skin lesions, and gut inflammation. The prevalence of AS was shown to be between 0.12–0.49% in Turkey [1,2]. Genetic factors play an important role in the pathogenesis of AS. For nearly 40 years it has been known that HLA-B27 formed the most significant association with AS. Other than the HLA-B27, a number of genetic influences have been implicated in susceptibility to AS in small cohorts. One of these suspicious genes was Mediterranean fever (MEFV). This gene has already been identified as being responsible for familial Mediterranean fever (FMF) [3]. Association of FMF with AS was described in several previous reports [4–10].

In persons with MEFV gene mutations, it was suggested that upregulation of the inflammatory response most likely favors inflammation in general and as such predisposes MEFV mutant carriers to inflammatory diseases [11]. The up-regulation of the inflammatory response in carriers of MEFV mutations can also affect the severity of the accompanying chronic diseases such as rheumatoid arthritis (RA), multiple sclerosis (MS), Behcet’s disease (BD), palindromic rheumatism (PR) and ulcerative colitis (UC) [12–16]. Patients with the inflammatory burden of these chronic diseases appear...
to be highly susceptible to develop a more severe disease if they also have a mutated MEFV.

The MEFV gene is located on the short arm of chromosome 16p13.3, comprises 10 exons [17] and encodes a 781–amino acid protein called marenostrin or pyrin. Pyrin is only expressed in neutrophils and monocytes, which are the cell types involved in innate immune responses. Pyrin has a key role in the regulation of inflammasome activity and pro–interleukin-1β (proIL-1β) processing [18, 19]. At present, > 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 [3].

It was hypothesized that, MEFV mutations may change the inflammatory response to infectious and inflammatory diseases and may contribute to the development of AS. Considering that FMF is very common in Turkey and that patients with FMF can present with AS as a sole manifestation, it is important to investigate MEFV mutations and impact of these mutations on AS. Therefore, we investigated whether the MEFV gene might be implicated in the pathogenesis of AS. We adopted a case-control design, to compare the MEFV mutation frequency between patients with AS and healthy controls, and to compare disease severity between mutation carriers and non-carriers.

2. Materials and methods

2.1. Study population

The study population comprised 103 unrelated AS patients (mean age 38.7 ± 9.2 years; mean disease duration 6.3 ± 5.2 years; 54 males, 49 females) recruited consecutively from those whom were treated and followed up in the Physical Medicine and Rehabilitation Department of Gaziosmanpasa University Research Hospital, Tokat, Turkey. All patients fulfilled the modified New York criteria for AS [20]. A total of 120 unrelated healthy subjects (mean age 40.9 ± 13.5 years; 53 males, 67 females) were recruited consecutively. All participants, patients and healthy controls, were of Turkish origin, from the inner Central Blacksea region of Turkey. The protocol of this study was approved by the Gaziosmanpasa University, Deanery of Medical Faculty, Medical-Surgical-Drug Research Ethics Committee (Approval no:08-GEKTIP-009), and all participants gave written informed consent before entering the study. All the participants were evaluated for the clinical findings of FMF, and none of them had symptoms or family history of FMF. The AS patients were assigned to two groups as MEFV mutation carriers and non-carriers. Clinical and laboratory findings were compared between two groups.

2.2. Analysis of MEFV gene 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 four mutations (E148Q [rs3743930; c.442G>C], M694V [rs61752717; c.2080A>G], M680I [rs28940580; c.2040G>C or c.2040G>A] and V726A [rs28940579; c.2177T>C]) and an additional rare mutation (P369S [rs11466023; c.1105C>T]) in the MEFV gene were screened in this study. These five mutations were detected by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. PCRs of M694V, M680I, and V726A mutations were performed by using previously described protocols [21]. HinfI, HphI, and AluI restriction enzymes were used for RFLP of M694V, M680I, and V726A mutations, respectively. The sense oligonucleotide primer for E148Q was 5′-CCTGAAGACTCCAGACCACCCCG-3′ and the antisense primer was 5′-GGCCCTCCAGGGCTTCTCTCTCTG-3′. The sense oligonucleotide primer for P369S was 5′-TCCCCGAGCGCTTCTCGAGTCCGAGGCTTTACACTTG-3′ and the antisense primer was 5′-TGGACCTGCCTTCAAGCTGCGCTTAC-3′. PCRs of E148Q and P369S mutations were performed by using previously described protocols [22]. BstNI and AluI restriction enzymes were used for RFLP 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.

2.3. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS 13.0) and the OpenEpi Info software package version 2.2 (www.openepi.com). Results were given as mean ± standard deviation (S.D.). The relationships between mutation carriers and the clinical and demographics features were analyzed by using chi-square test or analysis of variance (ANOVA) statistics. Chi-square test and Fisher’s exact test were used to compare categor-
Table 1 shows the demographic and clinical characteristics of AS patients according to the presence (carrier) or absence (non-carrier) of MEFV mutations. Although so many clinic and demographic features (sex, age, age at disease onset, disease duration, BASDAI, Schober test, chest expansion, ESR, HLA-B27 status, family history of AS, Syndesmophotes, ocular involvement, hip involvement, cardiac involvement, articular pain, cervical pain, sakroiliitis, oral aphthous ulcers, bamboo spine, dorsal kyphosis, training, smoking) were analyzed, no significant differences were observed between patients with and those without MEFV mutations.

In healthy controls group, mutation analysis showed that 19 (15.8%) of the subjects were carrying one mutated MEFV allele. The frequencies of M694V, M680I, V726A, E148Q and P369S mutation carriage were 4.2% (with 5/240 allele frequency), 4.2% (5/240), 2.5% (3/240), 4.2% (5/240) and 0.8% (1/240), respectively (Table 2). Homozygous and compound heterozygous mutations were not detected in healthy controls. In patients group, mutation analysis showed that 33 (32%) of the patients were carrying at least one mutated MEFV allele (Table 2). No compound heterozygous but one homozygous mutation (M680I/M680I) was observed in patients group. The frequencies of M694V, M680I, V726A, E148Q and P369S mutation carriage in patients with AS were 10.7% (with 11/206 allele frequency), 6.8% (8/206), 4.9% (5/206), 8.7% (9/206) and 1% (1/206), respectively. There was a statistically significant difference of the MEFV gene mutation carrier rates between patients and controls groups ($p = 0.004$, OR: 2.5, 95% CI: 1.32–4.76) (Table 2). This association was also observed in allele frequencies ($p = 0.005$, OR: 2.3, 95% CI: 1.27–4.2). When the MEFV gene mutations were separately compared between patients and controls, any association couldn’t be observed between these two groups. However, a relatively higher frequency was observed for M694V mutation in AS patients than controls (10.7% versus 4.2%, $p = 0.060$).

### 4. Discussion

In this study, we investigated the presence of genetic variants in the MEFV gene, which encodes for pyrin (a putative regulator of inflammation activity and proIL-
1)β processing), in a cohort of Turkish patients with a clinical diagnosis of AS. In the present study, we found a statistically significant increased prevalence of MEFV variants in AS patients in comparison with healthy controls. Four independent groups of investigators from Turkey recently reported their studies on MEFV variations in patients with AS [23–26]. The first study, from Central Anatolia, showed an increased frequency of eight MEFV variations (M694V [c.2080A>G, p.Met694Val], V726A [c.2177T>C, p.Val726Ala], E148Q [c.442G>C, p.Glu148Gln], M680I [c.2040G>T, p.Pro369Ser], F479L [c.1437C>T, p.Phe479Leu], and R761H [c.2282G>A, p.Arg761His]) in 95 patients with AS, without any clinical correlation [23]. The second study, from the Central Black Sea region, did not find any significant difference between 80 AS patients and 85 controls in terms of twelve common MEFV gene mutation (E148Q, P369S, F479L, M680I [G/C], M680I [G/A], I692del [c.2074_2076del], p.Ile692delIle], M694V, M694I, K695R [c.2084A>G, p.Lys695Arg], V726A, A744S [c.2230G>T, p.Ala744Ser], and R761H) frequencies [24]. Because the statistical analysis were based on all MEFV gene mutations taken together in the study of Durmus et al., a significantly higher frequency of M694V in the AS patients was escaped from the notice of them (OR: 3.9, 95% CI 1.03–14.75, \( P < 0.045 \)) [27]. In the third study, it was shown that the allele frequency of MEFV variants (M694V, V726A, E148Q, M680I, P760P, K695R) in 62 AS patients was significantly higher than that in the pooled control group of 50 healthy controls plus 46 rheumatoid arthritis (RA) patients from the Aegean region of Turkey (15.3% versus 6.8%; \( P = 0.021 \)). M694V was significantly more common in the AS group than in the combined control groups (\( P = 0.008 \)) [25]. In the fourth study from the Marmara region of Turkey, it was revealed that MEFV variations (M694V, M680I, V726A and E148Q) were significantly more frequent in 193 patients with AS (22.3%) compared with 103 healthy control subjects (9.7%, OR: 2.67, 95% CI 1: 1.28–5.56). This difference was more prominent for exon 10 variations (M694V, V726A, M680I) (OR 3.75, 95% CI 1.41–9.97), especially for the most-penetrant variation M694V (\( p = 0.006, \) OR 4.73, 95% CI 1.39–16.12) [26]. The results of these four studies from different Turkish populations are concordant with our study. These studies can be accepted as confirmation of the association of the penetrant MEFV variations with AS in independent groups of patients from Turkey. However, although we found a relatively high frequency of M694V mutation in patients with AS, we could not reach the statistical significant as the other researchers found.

There was no significant difference between MEFV mutation carriers and non-carriers with respect to the clinical and demographic characteristics which was also shown in the previous studies [23,25]. However, Durmus et al. found significant differences between the carrier and non-carrier groups regarding BASFI, BADI, hip involvement and ESR [24]. In another study, MEFV variations were more frequent in HLA–B27-negative patients with AS, and the difference was statistically significant in patients carrying exon 10 variants (M694V, M680I, V726A) [26].

In conclusion, this study reveals evidence suggesting that MEFV variations are associated with AS. However, this and other studies have low statistical power because

### Table 2

| No. heterozygous | AS patients (\( n = 103 \)) (%) | Healthy subjects (\( n = 120 \)) (%) | \( P \) value | OR (95% CI) |
|------------------|---------------------------------|---------------------------------|----------|------------|
| M694V/WT        | 11 (10.7)                       | 5 (4.2)                        | 0.060    | 2.8 (0.92–8.20) |
| M680I/WT        | 6 (5.8)                         | 5 (4.2)                        | 0.569    | 1.4 (0.42–4.81) |
| V726A/WT        | 5 (4.9)                         | 3 (2.5)                        | 0.569    | 2.0 (0.38–13.09) |
| E148Q/WT        | 9 (8.7)                         | 5 (4.2)                        | 0.161    | 2.2 (0.71–6.80) |
| P369S/WT        | 1 (1.0)                         | 1 (0.8)                        | –        | –          |
| No. homozygous or compound heterozygous | | | | |
| M680I/M680I     | 1 (1.0)                         | 1 (0.8)                        | –        | –          |
| Total mutations (\( n \)) | 33 (32.0)                       | 19 (15.8)                      | 0.004    | 2.5 (1.32–4.76) |
| Total allele frequency (\( n \)) | 34/206 | 19/240 | 0.005 | 2.3 (1.27–4.2) |
| M694V allele    | 11/206                          | 5/240                          | 0.065    | 2.7 (0.91–7.76) |
| M680I allele    | 8/206                           | 5/240                          | 0.261    | 1.9 (0.61–5.90) |

**MEFV**: Mediterranean Fever; AS = ankylosing spondylitis; WT: wild type. The results that are statistically significant are shown in boldface.
of their small sample size and the low frequency of ME芙 variants. Further studies with larger populations will be required to confirm these findings suggesting an association of M694V with AS and to clarify its influence on the disease course. Molecular analysis of ME芙 gene in AS patients should be important in order to offer a genetic counseling.

Acknowledgments

This study was supported by the Gaziosmanpasa University (Project No: 2011/54).

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