

Are *MEFV* mutations susceptibility factors in enthesitis-related arthritis patients in the eastern Mediterranean?

B. Gülhan¹, A. Akkuş¹, L. Özçakar², N. Beşbaş¹, S. Özen¹

¹Department of Paediatric Nephrology and Rheumatology, and

²Department of Physical and Rehabilitation, Hacettepe University Faculty of Medicine, Ankara, Turkey.

Bora Gülhan, MD
Abdulkadir Akkuş, MD
Levent Özçakar, MD
Nesrin Beşbaş, MD
Seza Özen, MD

Please address correspondence to:

Prof. Seza Özen,
Department of Paediatrics,
Faculty of Medicine,
Hacettepe University,
06100 Sıhhiye, Ankara, Turkey.
E-mail: sezaozen@hacettepe.edu.tr

Received on June 17, 2013; accepted in revised form on November 11, 2013.

Clin Exp Rheumatol 2014; 32 (Suppl. 84): S160-S164.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2014.

Key words: enthesitis-related arthritis, familial Mediterranean fever

ABSTRACT

Objective. *Enthesitis-related arthritis (ERA), is a complex genetic disease. Although HLA-B27 is well established, it does not explain all the genetic load in ERA. Familial Mediterranean fever (FMF), caused by mutations in the MEFV gene, is a frequent autoinflammatory disorder in the eastern Mediterranean.*

Methods. *We investigated the clinical and imaging features of 53 ERA patients, as well as the frequency of MEFV gene mutations in those who were HLA-B27 negative.*

Results. *The mean age of the patients was 13.3±2.2 years and 49 were boys. Peripheral arthritis was present in all and sacroileitis in 26 patients. Ultrasonography showed enthesitis in 6 patients of the tendons, whereas these were assessed to be normal by physical examination. Forty patients (75.5%) were positive for HLA-B27. MEFV analysis was performed for patients who were HLA-B27 negative. One patient refused MEFV analysis. 9 patients carried MEFV mutations: 2 patients were homozygous for M694V (both patients were subsequently started colchicine along with ERA treatment), 5 patients were heterozygous for M694V mutation, 1 patient was heterozygous for E148Q, and 1 patient was heterozygous for K695R mutation. None of the patients had features suggesting FMF at diagnosis of ERA; 1 patient subsequently developed typical FMF attacks.*

Conclusion. *Our findings suggest that MEFV mutations may represent a susceptibility factor for ERA in the populations of the eastern Mediterranean.*

Introduction

Enthesitis-related arthritis (ERA) is a subtype of juvenile idiopathic arthritis (JIA). The term ERA and its pertinent classification have been introduced by

ILAR, with an aim to also cover the previously suggested terms of juvenile spondyloarthritis and seronegative enthesitis arthritis syndrome (1, 2). The etiopathogenesis of ERA is not certain however, it is a complex genetic disease like the other subtypes of JIA. Genetic predisposition has a significant impact on ERA, where common single nucleotide polymorphisms in multiple genes are contributory as well, with real but variable environmental components. HLA-B27 accounts for the major genetic load and is positive in approximately 90% of the patients with juvenile ankylosing spondylitis (AS) (3).

Familial Mediterranean fever (FMF) is an autosomal recessive autoinflammatory disorder characterised by recurrent attacks of fever and polyserositis (4). Mutations in the gene for FMF (*MEFV*) are very common and the carrier rate is very high in the eastern Mediterranean (5). Several case reports and cohorts suggest an association between mutations in *MEFV* gene and certain inflammatory disorders, such as rheumatoid arthritis (6), systemic onset JIA (7), seronegative spondyloarthritis (8), Henoch-Schönlein purpura (9), polyarteritis nodosa (10, 11), Behçet's disease (BD) (12, 13), and Crohn disease (14).

The aim of this study was to analyse the characteristics of Turkish children with ERA in a single referral centre, and to determine the frequency of *MEFV* mutations among those who were HLA-B27 negative.

Patients and methods

Patients

Patients followed in Hacettepe University Paediatric Nephrology and Rheumatology Unit were included in this study; the ERA population of our centre consists of children from all around the country, the minority being from

Competing interests: none declared.

the local area. The study group of this cross-sectional study consisted of 53 Caucasian children (92.5% male; M:F ratio was 12.2) who met ERA criteria introduced by ILAR (1). These criteria are the presence of arthritis and enthesitis in the same patient or any two of them that accompany arthritis or enthesitis; sacroiliac joint tenderness, HLA-B27 positivity, history of arthritis after the age of 8, acute anterior uveitis, presence of HLA-B27-related disease history in first degree relatives. All patients also met European Spondyloarthropathy Study Group (ESSG) criteria (15). The study was approved by the ethics committee at the Hacettepe University.

Clinical assessment

During routine outpatient visits, complete physical and rheumatological examinations were performed. Additionally, spinal mobility was measured with modified Schober test. The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) was used to assess disease activity (16). Evaluations, treatment plans and follow-up visits of the patients were performed by the same paediatric rheumatologist. Tel-Hashomer Criteria and recently proposed set of FMF criteria for children were used to evaluate all patients for FMF (17, 18). All patients were treated with non-steroidal anti-inflammatory drugs (NSAIDs). For resistant patients, sulfasalazine or methotrexate was added as disease-modifying drugs (DMARDs). Etanercept was used for patients unresponsive to DMARDs.

Laboratory testing

High acute phase reactants was defined as ESR >20 mm/hour and/or C-reactive >0.5 mg/dl. HLA-B27 was checked in all patients and MEFV mutation analysis was studied in patients who were HLA-B27 negative. One patient who was HLA-B27 negative refused MEFV analysis. MEFV analysis was performed in 12 patients by using restriction fragment length polymorphism (RFLP). The following MEFV mutations were investigated: E148Q in exon 2, P369S in exon 3, F479L in exon 5 and M680I (G/C), M680I (G/A),

I692del, M694V, M694I, K695R, V726A, A744S, R761H in exon 10.

Radiological findings

Sacroileitis was assessed by magnetic resonance imaging (MRI) and/or x-ray. Ultrasonographic imaging was available in 34 patients and performed bilaterally for knee and ankle joints. Quadriceps, patellar and Achilles tendons and plantar fasciae were scanned longitudinally and axially. The same physician (with musculoskeletal ultrasound experience of more than 10 years) who was blinded to the clinical data performed all the evaluations using a 8–16 MHz linear probe (Diasus Dynamic Imaging).

Statistical analysis

The results were analysed by using SPSS for windows 13.0 and descriptive statistics are presented as percentages, means and SDs if they were equally distributed, and as median (IQR, interquartile ratio) if they were unequally distributed.

Results

Patients

In the study cohort, 49 patients (92.5%) were male and 4 patients (7.5%) were female. Clinical features of the subjects are summarized in Table I. The mean age of the patients at time of the study was 13.3 ± 2.2 . The mean age at diagnosis was 12.7 ± 2.3 years, ranging from 8.0 years to 16.9 years. All 53 patients had peripheral disease; the mean number of joints involved was 2.1 ± 1.1 (1–4). Nineteen patients (35.8%) had 1 joint, 19 patients had ≥ 3 joints involved. The most commonly affected joint was the knee (56%), followed by the ankle (32%) and the hip (12%). Mean BASDAI score at diagnosis was 5.7 (IQR: 4.4–6.3). Modified Schober test was used for evaluation of thoracolumbar spinal mobility and mean value was 5.3 ± 1.1 (3–8).

Sixteen patients (30.2%) had HLA-B27-related disease history in first or second degree relatives. 40 patients (75.5%) were HLA B27 positive.

Only 8 patients (15%) responded to the initial NSAID treatment. Sulfasalazine was started in 40 patients (75.5%) and

methotrexate was also combined for the peripheral arthritis in 24 patients (45.3%). Seventeen patients received intraarticular steroids. In 11 patients, short-term steroids were used in active arthritis periods. At the end of the follow-up, all patients improved with a mean BASDAI score of 2.0 (IQR: 0.8–4.0). Five patients who continued to have active arthritis, with high BASDAI scores and acute phase reactants were switched to etanercept. All of those patients were in remission at 6 months of their follow-up. None of the patients showed side effects related to the drugs.

Laboratory parameters

Mean ESR at diagnosis was 47 (IQR: 18–72) mm/hour. Thirty-three patients (62.3%) had elevated acute phase reactants at diagnosis. Forty patients (75.5%) were positive for HLA-B27. MEFV mutation analysis was carried out in 12 children with ERA who were HLA-B27 negative: the study revealed mutations in 9 patients (11 alleles had MEFV mutations among the 24): 2 patients were homozygous for M694V (both patients were put on colchicine treatment along with ERA treatment), 5 patients were heterozygous for M694V mutation, 1 patient was heterozygous for E148Q, and 1 patient was heterozygous for K695R mutation. None of the patients had features suggesting FMF at diagnosis of ERA; 1 patient started to develop typical FMF attacks during follow-up (after the mutation analysis was performed).

Mean age of the patients with a MEFV mutation was 13.4 ± 1.8 (11.1–15.8) and was comparable to the cohort of our patients. The pattern of joint and tendon involvement among the patients who had a MEFV mutation, was again similar to the other patients. All of these nine patients had elevated acute phase reactants at presentation.

Radiological findings

Sacroileitis was diagnosed in 26 patients (49.1%). Twenty-three patients were evaluated only with x-ray, 18 patients only with MRI and 12 both with MRI and x-ray. X-ray showed sacroileitis in 10/35 patients (28.6%). A total

Table I. Clinical features of 53 children with enthesitis-related arthritis.

Features	n (%)
Age at symptom onset (years)	11.1 ± 1.8
Age at diagnosis (years)	12.7 ± 12.3
Disease duration (years)	2.16 ± 1.43
Abnormal Schober test [n (%)]	34 (64.2%)
Enthesitis [n (%)]	26 (49.1%)
Sacroileitis [n (%)]	26 (49.1%)
Uveitis [n (%)]	5 (9.4%)
Morning stiffness [n (%)]	25 (47.2%)

of 30 patients were evaluated with MRI and 17 (56.7%) out of them were diagnosed to have sacroileitis. In patients who were evaluated with both MRI and x-ray (n=12), MRI showed sacroileitis in seven patients although they had normal x-ray ($p<0.05$).

On ultrasound (n=34), 15 and 21 patients had effusions in the right and left knee, respectively. All patients with effusion on right side also had effusion on the left side. None of the patients had effusion on ankles. Pathologic features were detected in the periarticular area in 9 patients: six patients had retrocalcaneal bursitis (2 bilateral, 2 on the right and 2 on the left side).

On USG evaluation (n=34), 19 patients had enthesitis at least in one tendon. On the other hand, 15 did not have any enthesitis findings in any joints. Enthesitis was detected in 1 tendon in 5 patients, 2 tendons in 10 patients, 3 tendons in 3 patients, 4 tendons in 1 patients (Fig. 1). In 25 patients the physical examination agreed with the musculoskeletal ultrasound. However, in six patients ultrasound examination detected entesopathy whereas physical examination was judged to be normal. Only the left plantar fascia was found to be thicker in HLA-B27-positive patients when compared with those of HLA-B27-negative patients. The presence of either sacroileitis or uveitis did not have any impact on tendon thickness measurements.

Discussion

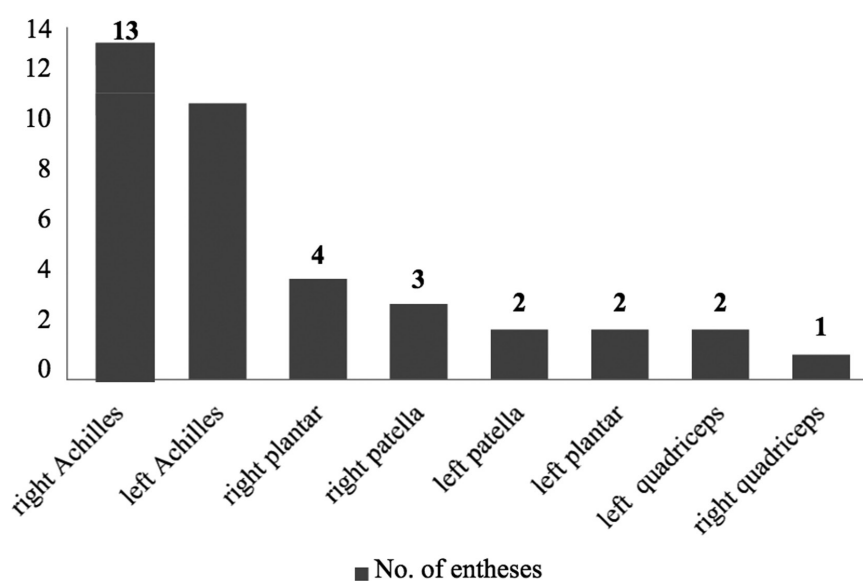
The presented Turkish cohort, presents certain differences from other series. The number of joints involved is fewer and the frequency of uveitis is less compared to series reported

by Ansell *et al.* (27.3%), Häfner *et al.* (14%), and O'Shea (27.7%) (19-21). Axial involvement is less compared to the series reported by O'Shea *et al.*; 49.1% versus 74% (21). The frequency of HLA-B27 is also lower than expected (3). Although this is a small series these features are compatible with previous unpublished observations of the centre. Furthermore, being a referral centre the patient population mirrors the characteristics of the Anatolian population.

In our patient group the response to NSAID and sulfasalazine was very good. This may be attributed to the dominance of peripheral arthritis pattern in our patient population. Only about 10% of the patients required anti-TNF treatment. These five patients had an excellent response to etanercept monotherapy. Actually, around 50% of the patients in our series developed sacroileitis and thus met the Modified New York Criteria for Ankylosing Spondylitis (AS), fulfilling the term juvenile onset AS (JoAS) (22). In the series reported by O'Shea *et al.* the mean age of the patients with JoAS at diagnosis was relatively higher (17.2 years), which is higher as compared to that of our series, and could be a cause of higher axial involvement (21). We cannot predict the final percentage of our patients who will go on to develop ankylosing spondylitis. This raises the

concern on the duration of anti-TNF treatment in these patients who can be foreseen to have a long-lasting disease ahead of them.

Another new aspect of our study group was the identification of MEFV mutations in a substantial number of patients who were HLA-B27 negative. Genetic predisposition has a significant impact on ERA, where common single nucleotide polymorphisms in multiple genes contribute to risk, with real but variable environmental components. The association with an HLA polymorphism, B27, with ankylosing spondylitis and ERA is probably one of the most striking associations in rheumatology. However, HLA-B27 does not account for all the genetic susceptibility. In addition to HLA-B27, non-HLA genes IL23R and ERAP1 (also known as ARTS1) have recently been identified as susceptibility factors (23). This latter gene product also been shown to influence shedding of the IL-1 and IL-6 receptors (24, 25). Being a dominant inflammatory molecule, IL-1 may indeed have an important affect on the pathogenesis of ERA and AS. A number of studies have highlighted the association of the disease with IL-1 polymorphisms (26, 27). Some of these poly-morphisms had a functional effect on the IL1 levels (28). A meta-analysis of 2675 AS cases and 2592 healthy controls for the IL1A, IL1 β , IL1F10

**Fig. 1.** Distribution of enthesitis in various tendons.

and IL1RN polymorphisms revealed a strong association of three single nucleotide polymorphisms in the IL1A gene (rs2856836, rs17561, rs18943999) (29). Since mutations in the *MEFV* gene are also associated with high IL1 levels may serve as a possible explanation of the association that we have shown in our population. In the adult literature, there are studies that investigate the prevalence of *MEFV* gene variants in AS patients with no history of FMF. Cosan *et al.* genotyped 193 AS patients and 103 healthy controls for the most common mutations (M694V, M680I, V726A, E148Q) by RFLP and they also showed that exon 10 variants of the *MEFV* gene were more frequent among HLA B27 (-) patients with AS (30). They further analysed M694V and *MEFV* mutations in their whole AS cohort: *MEFV* mutations were more frequent among the AS patients compared to healthy controls however, exon 10 mutations were significantly more frequent among the HLA B27(-) patients as compared to HLA 27+ ones (30). Two other uncontrolled studies conducted in central Anatolia estimated a high allelic frequency of M694V among AS patients at 6.3% and 12.3% in the general population of the same region (31, 32). The previously reported figure in the general population of the same region was about 1.1–3% (30, 33). This data suggests that *MEFV* mutations may be contributing to the overall pathogenesis of AS as well. Thus *MEFV* mutations may well be one of the susceptibility factors showing a significant association with ERA and AS in at least in certain areas of the world. In fact we know that the carrier rate for the *MEFV* mutations are increased among patients with various rheumatic diseases and complaints in the eastern Mediterranean including polyarteritis nodosa, Henoch Schönlein purpura and some forms of juvenile idiopathic arthritis (7, 9, 11, 12, 34). A simple explanation of this association may be the non-specific effect of increased inflammatory state introduced by the presence of *MEFV* mutations (34–37). *MEFV* mutations may be effective through the upregulation of the innate immune system which serves as

the initial response to the environmental trigger (34, 37). Thus it has been suggested that for the aforementioned clinical associations, mutations/polymorphisms in the *MEFV* gene may be serving as susceptibility factors for the disease or a more severe course (34). The role of *MEFV* mutation in human diseases is not completely understood. Current medical evidence suggest that *MEFV* mutations are gain-of-function mutations which lead to increased responsiveness to bacterial products (38). Mutations of the *MEFV* gene have been previously suggested to be associated with certain rheumatic diseases. Recent paper published by Kirino *et al.*, investigated the role of the *MEFV* gene mutations in the development of BD in Japanese and Turkish populations (39). In Turkish population, M694V mutation (both homozygous and heterozygous) conferred strong risk for BD. On the other hand, this association of M694V with BD was not found in Japanese population. In our study, we saw this association with ERA patients. Thus it is tempting to speculate that certain *MEFV* mutations may be acting as susceptibility factors for ERA/JAS in the eastern Mediterranean. On the other hand, the presence of two patients with homozygous mutations also suggest that ERA should be considered in FMF patients with specific features. In fact spondyloarthropathies have been previously recognised to be more frequent among FMF patients with the existing case reports (8). It is very interesting that two of the presented patients lacked other clinical features of FMF at diagnosis. They were nevertheless started on concomitant colchicine. New technology improves the detection of musculoskeletal features in these patients. MRI showed sacroileitis in 7 seven patients that would be missed with conventional radiography. Ultrasonography was more sensitive for detecting enthesitis, suggesting the need to use these tools in our paediatric practice. Mutations and polymorphisms may represent a further group of SNPs to assess in the etiopathogenesis of ERA. Mutations and polymorphisms of the *MEFV* gene are common to eastern Mediterranean (5). They are very uncommon

in other populations. However, in a British study the E148Q mutation / polymorphism of the *MEFV* gene was surprisingly found in a group of British and Indian chronic arthritis patients who developed secondary amyloidosis (40). *MEFV* mutations may indeed be one of the susceptible genetic factors in ERA/AS in this part of the world.

References

- PETTY RE, SOUTHWOOD TR, BAUM J *et al.*: Revision of proposed classification criteria for juvenile idiopathic arthritis: Durban, 1997. *J Rheumatol* 1998; 25: 1991–4.
- PETTY RE, SOUTHWOOD TR, MANNERS P *et al.*: International League of Associations for Rheumatology. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. *J Rheumatol* 2004; 31: 390–2.
- CASSIDY JT, PETTY RE: Juvenile ankylosing spondylitis. In: CASSIDY JT, PETTY RE, LAXER LM, LINDSLEY (Eds.). *Textbook of Pediatric Rheumatology*. Philadelphia: Elsevier Saunders 2005: 304–323.
- SHOHAT M, HALPERN GJ: Familial Mediterranean fever – a review. *Genet Med* 2011; 13: 487–98.
- YILMAZ E, OZEN S, BALCI B *et al.*: Mutation frequency of familial Mediterranean fever and evidence for a high carrier rate in the Turkish population. *Eur J Hum Genet* 2001; 9: 553–5.
- RABINOVICH E, LIVNEH A, LANGEVITZ P *et al.*: Severe disease in patients with rheumatoid arthritis carrying a mutation in the Mediterranean fever gene. *Ann Rheum Dis* 2005; 64: 1009–14.
- AYAZ NA, OZEN S, BILGINER Y *et al.*: *MEFV* mutations in systemic onset juvenile idiopathic arthritis. *Rheumatology* (Oxford) 2009; 48: 23–5.
- LANGEVITZ P, LIVNEH A, ZEMER D, SHEMER J, PRAS M: Seronegative spondyloarthropathy in familial Mediterranean fever. *Semin Arthritis Rheum* 1997; 27: 67–72.
- GERSHONI-BARUCH R, BROZA Y, BRIK R: Prevalence and significance of mutations in the familial Mediterranean fever gene in Henoch-Schönlein purpura. *J Pediatr* 2003; 143: 658–61.
- OZEN S, BEN-CHETRIT E, BAKKALOGLU A *et al.*: Polyarteritis nodosa in patients with Familial Mediterranean Fever (FMF): a concomitant disease or a feature of FMF? *Semin Arthritis Rheum* 2001; 30: 281–7.
- YALÇINKAYA F, OZÇAKAR ZB, KASAPÇOPUR O *et al.*: Prevalence of the *MEFV* gene mutations in childhood polyarteritis nodosa. *J Pediatr* 2007; 151: 675–8.
- TOUITOU I, MAGNE X, MOLINARI N *et al.*: *MEFV* mutations in Behçet's disease. *Hum Mutat* 2000; 16: 271–2.
- LIVNEH A, AKSENTJEVICH I, LANGEVITZ P *et al.*: A single mutated *MEFV* allele in Israeli patients suffering from familial Medi-

- terranean fever and Behçet's disease. *Eur J Hum Genet* 2001; 9: 191-6.
14. FIDDER HH, CHOWERS Y, LIDAR M *et al.*: Crohn disease in patients with familial Mediterranean fever. *Medicine* (Baltimore) 2002; 81: 411-6.
 15. DOUGADOS M, VAN DER LINDEN S, JUHLIN R *et al.*: The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. *Arthritis Rheum* 1991; 34: 1218-27.
 16. GARRETT S, JENKINSON T, KENNEDY LG *et al.*: A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. *J Rheumatol* 1994; 21: 2286-91.
 17. LIVNEH A, LANGEVITZ P, ZEMER D *et al.*: Criteria for the diagnosis of familial Mediterranean fever. *Arthritis Rheum* 1997; 40: 1879-85.
 18. YALÇINKAYA F, OZEN S, OZÇAKAR ZB *et al.*: A new set of criteria for the diagnosis of familial Mediterranean fever in childhood. *Rheumatology* (Oxford) 2009; 48: 395-8.
 19. ANSELL BM: Juvenile spondylitis and related disorders. In: MOLL JMH (Ed). *Ankylosing Spondylitis*. Edinburgh, Churchill Livingstone, 1980, p.120.
 20. HÄFNER R: Juvenile spondylarthritis. Retrospective study of 71 patients. *Monatsschr Kinderheilkd* 1987; 135: 41-6.
 21. O'SHEA FD, BOYLE E, RIARH R *et al.*: Comparison of clinical and radiographic severity of juvenile-onset versus adult-onset ankylosing spondylitis. *Ann Rheum Dis* 2009; 68: 1407-12.
 22. VAN DER LINDEN S, VALKENBURG HA, CATS A: Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum* 1984; 27: 361-8.
 23. BURTON PR, CLAYTON DG, CARDON LR *et al.*: Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat Genet* 2007; 39: 1329-37.
 24. CUI X, ROUHANI FN, HAWARI F, LEVINE SJ: Shedding of the type II IL-1 decoy receptor requires a multifunctional aminopeptidase, aminopeptidase regulator of TNF receptor type 1 shedding. *J Immunol* 2003; 171: 6814-9.
 25. CUI X, ROUHANI FN, HAWARI F, LEVINE SJ: An aminopeptidase, ARTS-1, is required for interleukin-6 receptor shedding. *J Biol Chem* 2003; 278: 28677-85.
 26. MAKSYMOWYCH WP, RAHMAN P, REEVE JP *et al.*: Association of the IL1 gene cluster with susceptibility to ankylosing spondylitis: an analysis of three Canadian populations. *Arthritis Rheum* 2006; 54: 974-85.
 27. SIMS AM, TIMMS AE, BRUGES-ARMAS J *et al.*: International Genetics of Ankylosing Spondylitis. Prospective meta-analysis of interleukin 1 gene complex polymorphisms confirms associations with ankylosing spondylitis. *Ann Rheum Dis* 2008; 67: 1305-9.
 28. HALL SK, PERREGAUX DG, GABEL CA *et al.*: Correlation of polymorphic variation in the promoter region of the interleukin-1 beta gene with secretion of interleukin-1 beta protein. *Arthritis Rheum* 2004; 50: 1976-83.
 29. SIMS AM, TIMMS AE, BRUGES-ARMAS J *et al.*: Prospective meta-analysis of interleukin 1 gene complex polymorphisms confirms associations with ankylosing spondylitis. *Ann Rheum Dis* 2008; 67: 1305-9.
 30. COSAN F, USTEK D, OKU B *et al.*: Association of familial Mediterranean fever-related MEFV variations with ankylosing spondylitis. *Arthritis Rheum* 2010; 62: 3232-6.
 31. CINAR M, DINC A, SIMSEK I *et al.*: The rate and significance of Mediterranean fever gene mutations in patients with ankylosing spondylitis: a three-month, longitudinal clinical study. *Rheumatol Int* 2008; 29: 37-42.
 32. MARAS Y, CALGUNERI M, AKDOGAN A *et al.*: MEFV mutations frequency and effect on disease severity in ankylosing spondylitis. *EULAR Meeting Abstracts*. 2007; 66: 400.
 33. YILMAZ E, OZEN S, BALCI B *et al.*: Mutation frequency of Familial Mediterranean Fever and evidence for a high carrier rate in the Turkish population. *Eur J Hum Genet* 2001; 9: 553-5.
 34. OZEN S: Mutations/polymorphisms in a monogenetic autoinflammatory disease may be susceptibility markers for certain rheumatic diseases: lessons from the bedside for the benchside. *Clin Exp Rheumatol* 2009; 27 (Suppl. 53): S29-31.
 35. DUZOVA A, BAKKALOGLU A, BESBAS N *et al.*: Role of A-SAA in monitoring subclinical inflammation and in colchicine dosage in familial Mediterranean fever. *Clin Exp Rheumatol* 2003; 21: 509-14.
 36. LACHMANN HJ, SENGÜL B, YAVUZŞEN TU *et al.*: Clinical and subclinical inflammation in patients with familial Mediterranean fever and in heterozygous carriers of MEFV mutations. *Rheumatology* (Oxford) 2006; 45: 746-50.
 37. BEN-CHETRIT E, TOUITOU I: The impact of MEFV gene identification on FMF: an appraisal after 15 years. *Clin Exp Rheumatol* 2012; 30 (Suppl. 72): S3-6.
 38. CHAE JJ, CHO YH, LEE GS *et al.*: Gain-of-function Pyrin mutations induce NLRP3 protein-independent interleukin-1 β activation and severe autoinflammation in mice. *Immunity* 2011; 34: 755-68.
 39. KIRINO Y, ZHOU Q, ISHIGATSUBO Y *et al.*: Targeted resequencing implicates the familial Mediterranean fever gene MEFV and the toll-like receptor 4 gene TLR4 in Behçet disease. *Proc Natl Acad Sci USA* 2013; 110: 8134-9.
 40. BOOTH DR, LACHMANN HJ, GILLMORE JD, BOOTH SE, HAWKINS PN: Prevalence and significance of the familial Mediterranean fever gene mutation encoding pyrin Q148. *QJM* 2001; 94: 527-31.