Renal Failure

ISSN: 0886-022X (Print) 1525-6049 (Online) Journal homepage: https://www.tandfonline.com/loi/irnf20

The Relationship between the MEFV Genotype, Clinical Features, and Cytokine-Inflammatory Activities in Patients with Familial Mediterranean Fever

Bulent Colak, Bahar Gurlek, Zeynep Arzu Yegin, Serpil Muge Deger, Sehri Elbek, Hatice Pasaoglu, İbrahim Dogan, Mehmet Akif Ozturk, Selahattin Unal & Galip Guz

To cite this article: Bulent Colak, Bahar Gurlek, Zeynep Arzu Yegin, Serpil Muge Deger, Sehri Elbek, Hatice Pasaoglu, İbrahim Dogan, Mehmet Akif Ozturk, Selahattin Unal & Galip Guz (2008) The Relationship between the MEFV Genotype, Clinical Features, and Cytokine-Inflammatory Activities in Patients with Familial Mediterranean Fever, Renal Failure, 30:2, 187-191, DOI: 10.1080/08860220701810364

To link to this article: https://doi.org/10.1080/08860220701810364

Published online: 07 Jul 2009.

Submit your article to this journal

Article views: 478

View related articles

Citing articles: 10 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=irnf20

Renal Failure, 30:187–191, 2008
Copyright © Informa Healthcare USA, Inc.
ISSN: 0886-022X print / 1525-6049 online
DOI: 10.1080/08860220701810364

informa
healthcare

CLINICAL STUDY

The Relationship between the MEFV Genotype, Clinical Features, and Cytokine-Inflammatory Activities in Patients with Familial Mediterranean Fever

Bulent Colak, Bahar Gurlek, Zeynep Arzu Yegin, and Serpil Muge Deger
Department of Internal Medicine, Gazi University Faculty of Medicine, Ankara, Turkey

Sehri Elbek and Hatice Pasaoglu
Department of Biochemistry, Gazi University Faculty of Medicine, Ankara, Turkey

İbrahim Dogan, Mehmet Akif Ozturk, Selahattin Unal, and Galip Guz
Department of Internal Medicine, Gazi University Faculty of Medicine, Ankara, Turkey

Familial Mediterranean Fever (FMF) is an autosomal recessive disease characterized by periodic attacks of fever and polyserositis. The effects of the MEFV genotype differences on clinical picture and inflammatory activity have not been well documented. The aim of this study was to investigate levels of conventional inflammation markers, procalcitonin, interleukin levels, TNF-alpha, and C5a levels in patients with FMF who had different MEFV genotypes and compare them with those of healthy subjects. The study consisted of 41 patients with FMF (F/M: 23/18), and 31 healthy subjects (F/M: 18/13). Tests were performed during the attack-free period.

White-blood cell count, CRP and IL-8 levels were higher in patients with FMF than in healthy subjects ($p < 0.05$) and also higher in M680I carriers than in the patients with M694V allele carriers. However, ESR, fibrinogen, procalcitonin, IL-6, C5a, TNF-alpha, and IgD levels were not significantly different between patients and healthy subjects ($p > 0.05$). Arthralgia or arthritis was significantly higher in M694V carriers than in non-M694V carriers ($p < 0.05$). It is concluded that the clinical features and inflammatory-cytokine activities were higher in patients with FMF during the attack-free period than in healthy subjects, and the different genotype might be related to different clinical pictures.

Keywords Familial Mediterranean Fever, MEVF genotype, inflammation, cytokine

Address correspondence to Galip Guz, MD, Department of Nephrology, Gazi University Faculty of Medicine, 06510 Besevler, Ankara, Turkey; Tel.: +90-312-2025229; Fax: +90-312 - 124647; E-mail: galip_guz@hotmail.com

INTRODUCTION

Familial Mediterranean Fever (FMF) is an autosomal recessive autoinflammatory disease with unknown etiology; it occurs commonly in Sephardic Jews, Armenians, Arabs, and Turks.[1] FMF is characterized by recurrent attacks of polyserositis and fever; and some cases with skin lesions, vasculitis, and amyloidosis. The trigger of the inflammatory attacks is not clear. Screening of mutations in the causative gene (MEFV) allows for an accurate diagnosis of FMF among other inflammatory diseases. In this disease, the clinical results of subclinical inflammation are not known without amyloidosis. The inflammation parameters show differences in different studies.[1,2] Each cytokine and acute phase reactant plays different roles in the pathway of inflammation.

Erythrocyte sedimentation rate (ESR) is a precipitating rate of erythrocytes in one hour. C-reactive protein (CRP) was named from pneumococcal capsule antigen. It is normally detected in human plasma with normal concentrations and increases within the sixth hour of inflammation. Fibrinogen is important for homeostasis and tissue repair. Because its half life is longer; it takes longer to return to the normal level after inflammation; another disadvantage is its effect on coagulation disorders and fibrinolysis. Interleukin-6 (IL-6) is a polypeptide secreting from lymphocytes, monocytes, fibroblasts, and several tumor cells. IL-6 induces the final maturation of B cells into plasma cells, stimulates multipotent hemapoietic progenitors, and is also a major inducer of acute phase reactions in response to inflammation or tissue injury. Tumor necrosis factor alpha (TNF-alpha) is an important mediator for

neutrophil activation. TNF-alpha has a major pathologic role in cachexia associated with chronic infections or malignant diseases, in septic shock, and in graft versus host disease. Interleukin-8 (IL-8) is a chemokine that is secreted mainly by macrophages. The complement activation product (C5a) is an anaphylatoxin that causes the release of histamine from mast cells and accumulates neutrophils at the site of complement activation. Procalcitonin is a parameter for the diagnosis of bacterial, fungal, and parasitical infections. In healthy people, because all procalcitonin is produced by the thyroid gland and its half life is short, it resolves and cannot reach the blood stream.

The aim of this study was to investigate levels of conventional inflammation markers (ESR, CRP, and fibrinogen), procalcitonin, IL-6, and IL-8 levels, TNF-alpha, and C5a levels in patients with FMF who had different MEFV genotypes and compare them to those of healthy subjects. In this study, we determined the high value of acute phase reactants, cytokines, and mediators independent from attack.

SUBJECTS AND METHODS

Patients and Healthy Subjects

The study included 41 patients diagnosed with FMF who met the clinical criteria and MEFV genotyping for FMF diagnosis.[3] The patient group consisted of 23 males and 18 females with a mean age of 31.6 ± 10.1 followed up by our clinic. All patients were on colchicine treatment 1.5 mg/d, and blood samples were drawn 12 h later after the last dose. The control group consisted of 31 healthy volunteers; 18 males and 13 females with a mean age of 30.8 ± 8.0 years. All patients or healthy subjects with diabetes mellitus, hypertension, atherosclerotic vascular disease, malignancy, amyloidosis, active infectious disease, pregnancy, or inflammatory diseases were excluded. All patients and healthy subjects had a complete history and physical examination. Clinical and laboratory assessment of FMF patients were performed during an attack-free period. The study was approved by the Gazi University Faculty of Medicine ethical committee, and all participants provided informed consent before entering the study.

Measurement of Inflammation Makers, Cytokines, and IgD

The total blood count was measured by STKS auto analyzer, and leukocyte counts between 4500–11000/mm^3 were considered to be within the normal range. ESR was measured by the Westergreen method. The rates of 0–15 mm/hr for males and 0–20 mm/hr for females were considered to be within the normal ranges. CRP levels were measured by using a nephelometric assay (Specific Protein Analyzer; Beckman, Marburg, Germany) with the normal range defined as 0.0–6 mg/L. Fibrinogen was measured using a chronometric assay (Fibrinomat; STA Diagnostica Stago, Bio Merieux, Marcey L.Etoile, France), with the normal range defined as 200–400 mg/L. Procalcitonin was measured with LUMItest PCT kit (B.R.H.A.M.S., Diagnostica, Berlin, Germany) and an Immunol minometric assay with Lumat LB 9507 (EG&G, Berthold, Germany). The other markers, IL-6, IL-8, TNF-alpha, and C5a were measured by ELISA methods using different commercial kits (i.e., IL-6 [normal <8 pg/mL], IL-8, and TNF-alpha levels from BioSource International, USA; C5a levels from Behring Diagnostics, GmbH, Germany). Immunoglobulin D level was tested by immunodiffusion method (normal: 1.4.5 mg/dL) from Biocientifica S.A., Buenos Aires, Argentina. All blood samples were taken during the attack-free period, and all analyses were performed at the end of study period on serum samples, which had been stored at –30°C.

MEFV Genotyping

Genomic DNA was isolated from peripheral blood sample taken into ethylenediamine tetraacetate (EDTA) tubes. The mutations in exons 10 and 2 of MEFV gene (M694V, M680I, V726A, M694I, R761H, E148Q, and A744S) were investigated by amplifying the genomic DNA samples. Genetic analysis data of FMF patients were obtained from hospital file records.

Statistical Analysis

Statistical analyses were performed using SPSS 10.0 for Windows. Data were expressed as mean ± standard deviation (SD). Distributions of the data were analyzed with one sample Kolmogrov.Smirnov test. The comparisons were accomplished by independent sample t-test for data with normal distribution and two-independent samples test (Mann-Whitney U Test) for data without normal distribution. Differences between patients and healthy subjects for categorical variables were analyzed using the chi-square test or Fisher's exact test, according to the size of the population. A statistically significant difference was inferred when p was <0.05.

RESULTS

Patients and Healthy Subjects

The clinical and demographic features of the 41 patients are given in Table 1. The mean age at the onset of the disease was 26.6 ± 11.9 years, and the mean age of the duration of the disease was 5.3 ± 5.2 years. The symptoms of the disease began during childhood in 26.8% of patients. Abdominal pain (82.9%) was the leading symptom, followed by arthralgia or arthritis (26.8%). Interestingly, none of the patients mentioned skin lesions. Twelve of the patients were homozygous for M694V, and 11 were heterozygous for M694V. Thus, M694 allele (69.8%) was the leading frequency. The MEFV genotype and allele frequency are shown in Table 2.

Abdominal pain was the most common symptom in all MEFV genotypes; however, arthralgia or arthritis was significantly higher in the patients carrying M694V allele than the patients without M694V allele (52% vs. 16.6%, $p < 0.05$).

Table 1
Clinical and demographic features of the patients

Features	
Age(year)	31.6 ± 10.1
F/M	18/23
Age at FMF diagnosis (year)	26.6 ± 11.9
Mean duration of disease (year)	5.3 ± 5.3 (1–20)
Onset of disease in childhood	11 (26.8%)
Colchicine use(year)	3.8 ± 4.3(1–19)
Symptom	
Abdominal pain	34 (82.9%)
Arthritis	11 (26.8%)
Fever	9 (21.9%)
Chest pain	2 (4.9%)
Skin lesions	0 (0%)

Table 2
The MEFV genotype and allele frequency of the patients

Genotype	n (%)	Allele	N (%)
M694V/M694V	12 (29.3)	M694V	44 (69.8)
M694V/?	11 (26.8)	M680I	10 (15.9)
M694V/M680I	6 (14.6)	E148Q	6 (9.5)
E148Q/?	4 (9.8)	V726A	3 (4.8)
M694V/E148Q	2 (4.9)	M694I	0 (0)
V726A/?	2 (4.9)	R761H	0 (0)
M680I/?	2 (4.9)	A744S	0 (0)
M680I/M680I	1 (2.4)		
M694V/V726A	1 (2.4)		

Measurement of Inflammation Makers, Cytokines, and IgD

Erythrocyte sedimentation rate, fibrinogen, and procalcitonin levels were not significantly different between the patients and the healthy subjects ($p > 0.05$). However, ESR and fibrinogen were higher than the normal range in 31.3% and 37.5% of the patients, and 29% and 20.6% of healthy subjects, respectively. Neither the patients nor healthy subjects had procalcitonin levels >0.5 ng/mL. However, IgD levels were similar between the patients and healthy subjects ($p > 0.05$), and none of them had elevated IgD levels.

White blood cell count, CRP, and IL-8 levels were significantly higher in patients than in healthy subjects ($p < 0.05$). ESR, fibrinogen, procalcitonin, IL-6, C5a, TNF-alpha, and IgD levels were not significantly different between patients and healthy subjects ($p > 0.05$). The values of WBC, ESR, CRP, fibrinogen, procalcitonin, IL-6, IL-8, C5a, IgD, and TNF-alpha are given in Table 3. WBC, CRP, and IL-8 levels did not show significant differences between the patients with different MEFV alleles ($p > 0.05$). When compared, WBC, CRP, and IL-8 levels were found to be higher in patients with M680I allele than in patients with M694V, but the difference did not reach statistical significance (8676 ± 1801, 17.5 ± 16.5, and 895.7 ± 1350.6 vs. 782 ± 2895, 13.8 ± 21.7, and 500.2 ± 978.3, respectively). E148Q allele and V726A were not included in this statistical analysis because of the insufficient

Table 3
The inflammation makers, cytokines, and IgD levels of the patients and healthy

	FMF, n = 41	Healthy control, n = 31	*p*
Age (year)	31.6 ± 10.1	30.8 ± 8.0	0.704
F/M	23/18	18/13	0.239
WBC (mm^3)	8174 ± 2876	6773 ± 1409	0.017
ESR (mm/h)	16.8 ± 12.9	17.7 ± 12.6	0.773
CRP (mg/L)	13.5 ± 19.0	1.2 ± 3.5	<0.001
Fibrinogen (mg/dL)	372.7 ± 147.1	344.1 ± 174.0	0.342
PCT (ng/mL)	0.04 ± 0.0	0.045 ± 0.0	0.290
IL-6 (pg/mL)	23.0 ± 44.1	7.4 ± 2.5	0.614
IL-8 (pg/mL)	614.7 ± 114.3	171.1 ± 133.6	<0.001
C5-a (ng/mL)	9.6 ± 2.0	7.7 ± 3.5	0.140
TNF-alpha (pg/mL)	82.3 ± 107.7	42.8 ± 32.5	0.634
IgD (mg/dL)	1.17 ± 1.1	1.6 ± 1.7	0.844

number of patients. Additionally, there were no statistically significance between the patients M694V homozygous and M694V heterozygous in terms of these parameters (7546 ± 3553, 12.5 ± 19.5, and 332.4 ± 433.1 vs. 8096 ± 2647, 6.0 ± 8.6, and 892.6 ± 1012.2, respectively).

DISCUSSION

The patients with FMF had higher WBC, CRP, and IL-8 levels than healthy subjects despite colchicine treatment. On the one hand, the main predictors of inflammation such as ESR, fibrinogen, and procalcitonin levels were not significantly different between patients and healthy subjects. On the other hand, abdominal pain was the leading symptom in all MEFV genotypes; arthralgia or arthritis was significantly higher in patients with M694V allele. Inflammation makers and cytokines did not show significant differencez among the patients with different alleles.

The patients with hyperimmunoglobulinemia D syndrome are frequently misdiagnosed as having FMF. Hyper IgD syndrome is a rare autosomal recessive disorder characterized by recurrent attacks of fever with abdominal pain that lasts 3–6 days. The gene that is located in 12q24 is responsible for the mevalonate kinase deficiency that results with Hyper IgD syndrome. The diagnosis can be confirmed by the detection of elevated excretions of mevalonic acid in urine during fever episodes. Unlike FMF, colchicine had no preventive effect against febrile episodes of this syndrome.[4] Livneh et al. found that although whole Ig levels including IgG, IgA, and IgD increased in FMF, IgD values were significantly lower than the prevalence reported for Hyper IgD syndrome.[3] In the present study, to exclude Hyper IgD syndrome, IgD level was tested in all patients and healthy subjects, and none of them had elevated IgD level.

The distributions of genotypes were similar to the Turkish population as reported in the literature,[5,6] and abdominal pain was the most frequent symptom of patients and supports previous studies.[1] This was true for all MEFV genotypes. In addition, our results confirm that arthralgia or arthritis was significantly higher in the patients carrying M694V allele than the patients without M694V allele. Similarly, Olgun et al. analyzed the most frequent mutations (M680I, M694V, E148Q, and V726A) in FMF patients and reported that the homozygosity of M694V mutations in MEFV gene was associated with arthritis.[7]

Colchicine is used in preventing the recurrent attacks of FMF, the diseases that are characterized by neutrophil infiltration such as gout, primary biliary cirrhosis, immune thrombocytopenic purpura, and skin diseases like scleroderma and psoriasis. Because colchicine has an anti-inflammatory effect by altering the addition and chemotaxis of leukocytes and the function of cytokine production, it can be used chiefly in the treatment of FMF, gout, and other inflammatory diseases. Colchicine is effective in preventing triggers and decreasing the frequency of attacks with a dose of 0.5–2 mg/day, but is toxic in doses greater than 0.1 mg/kg/day and lethal in a dose of 0.8 mg/kg/day.[8] Lidar et al. reported that the patients who were non-responsive to colchicine therapy had some similar characteristics, such as unemployment to therapy, lack of education, and having more than three children.[9] In the present study, all of the patients who were on colchicine treatment at a daily dose 1.5 mg (t.i.d) did not stop taking colchicine for ethical reasons. Nevertheless, all of the patients gave the fasting blood sample 12 h after the last colchicine dose.

Procalcitonin, the precursor of calcitonin, is a polypeptide of 116 amino acids (molecular weight 13 kDa).[10] After it was reported to be a marker of bacterial and viral infections,[11] several investigators discovered that PCT also appeared to be a marker of both inflammation and sepsis, and that the elevated PCT in serum was associated with an increased risk of mortality.[12–15] A normal level of procalcitonin is a very low value as <0.5 ng/mL. In order to investigate the possible importance of procalcitonin in the patients with FMF, we tested procalcitonin levels in the patients and compared them to the levels of healthy subjects. There was not a statistically significant difference between the procalcitonin levels of the patients and healthy subjects. Additionally, procalcitonin levels were within the normal range in all patients and healthy subjects. The high procalcitonin levels might be related FMF attack or infectious status, used for the evaluation of patients.

We found that WBC, CRP, and IL-8 were significantly higher in patients with FMF than in healthy subjects. However, ESR, fibrinogen, procalcitonin, IL-6, C5a, TNF-alpha, and IgD levels were similar in both groups. In spite of the limitation of the study due to the small size of the study and control groups, the numbers were similar in the previous studies.[1,2,6] Gang et al. examined the cytokine network during the attacks of FMF and found that circulating concentrations of IL-6 and TNF alpha were significantly higher during attacks than in attack-free periods.[16] Bagci et al. reported that while the inflammatory cytokines IL-1 alpha, IL-6, and TNF alpha levels rose during the attack in FMF, there was no significant increase in the value of IL-10, which is an anti-inflammatory cytokine.[1] It has been reported that IL-8 levels were significantly elevated during the attack; however, it was not different from the levels in healthy subjects during remission.[17]

In conclusion, abdominal pain was the most prominent symptom in all MEFV genotypes, but arthralgia or arthritis was significantly higher in patients with M694V allele. The patients with FMF had higher WBC, serum CRP, and IL-8 levels during the attack-free period than healthy subjects

did. These parameters were high even though the patients were on colchicine treatment. These results found subclinical disease and inflammation activity in the patients with FMF. However, the inflammation activity was not different between the patients who had different MEFV alleles. The patients with FMF should receive not only effective colchicine treatment but also other therapeutic management to suppress the subclinical disease and inflammation activity no matter which MEFV genotype they have.

REFERENCES

1. Bagci S, Toy B, Tuzun A, et al. Continuity of cytokine activation in patients with Familial Mediterranean Fever. *Clin Rheumatol*. 2004;23(4):333–337.
2. Yalçinkaya F, Cakar N, Acar B, et al. The value of the levels of the acute phase reactants for the prediction of Familial Mediterranean Fever associated amyloidosis. *Rheumatol Int*. 2007;27(6):517–522.
3. Livneh A, Drenth JP, Klasen IS, et al. Familial Mediterranean Fever and hyperimmunoglobulinemia D syndrome: Two diseases with distinct clinical, serologic, and genetic features. *J Rheumatol*. 1997;24(8):1558–1563.
4. Frenkel J, Houten SM, Waterham HR, et al. Mevalonate kinase deficiency and Dutch type periodic fever. *Clin Exp Rheumatol*. 2000;18(4):525–532.
5. Turkcapar N, Tuncali T, Kutlay S, et al. The contribution of genotypes at the MICA gene triplet repeat polymorphisms and MEFV mutations to amyloidosis and course of the disease in the patients with Familial Mediterranean Fever. *Rheumatol Int*. 2007;27(6):545–551.
6. Lachmann HJ, Sengül B, Yavuzsen TU, et al. Clinical and subclinical inflammation in patients with Familial Mediterranean Fever and in heterozygous carriers of MEFV mutations. *Rheumatology (Oxford)*. 2006;45(6):746–750.
7. Olgun A, Akman S, Kurt I, Tuzun A, Kutluay T. MEFV mutations in Familial Mediterranean Fever: Association of M694V homozygosity with arthritis. *Rheumatol Int*. 2005;25(4):255–259.
8. Niel E, Scherrmann JM:. Colchicine today. *Joint Bone Spine*. 2006;73(6):672–678.
9. Lidar M, Scherrmann JM, Shinar Y, et al. Colchicine nonresponsiveness in Familial Mediterranean Fever: Clinical, genetic, pharmacokinetic, and socioeconomic characterization. *Semin Arthritis Rheum*. 2004;33(4):273–282.
10. Le Moullec JM, Jullienne A, Chenais J, et al. The complete sequence of human preprocalcitonin. *FEBS Lett*. 1984; 167(1):93–97.
11. Assicot M, Gendrel D, Carsin H, Raymond J, Guilbaud J, Bohuon C. High serum procalcitonin concentrations patients with sepsis and infection. *Lancet*. 1993; 341(8844): 515–518.
12. Al-Nawas B, Shah PM. Procalcitonin in patients with and without immunosuppression and sepsis. *Infection*. 1996; 24(6):434–436.
13. Bernard L, Ferrière F, Casassus P, et al. Procalcitonin as an early marker of bacterial infection in severely neutropenic febrile adults. *Clin Infect Dis*. 1998;27(4):914–915.
14. Schwenger V, Sis J, Breitbart A, Andrassy K. CRP levels in autoimmune disease can be specified by measurement of procalcitonin. *Infection*. 1998;26(5):274–276.
15. Nylen ES, Whang KT, Snider RH, Steinwald PM, White JC, Becker KL. Mortality is increased by procalcitonin and decreased by antiserum reactive to procalcitonin in experimental sepsis. *Crit Care Med*. 1998;26(6):1001–1006.
16. Gang N, Drenth JP, Langevitz P, et al. Activation of the cytokine network in Familial Mediterranean Fever. *J Rheumatol*. 1999;26(4):890–897.
17. Direskeneli H, Ozdogan H, Korkmaz C, Akoglu T, Yazici H. Serum soluble intercellular adhesion molecule 1 and interleukin 8 levels in Familial Mediterranean Fever. *J Rheumatol*. 1999;26(9):1983–1986.