Mutations in the Gene for Familial Mediterranean Fever: Do They Predispose to Inflammation?

SEZA OZEN, AYSIN BAKKALOGLU, ENGIN YILMAZ, ALI DUZOVA, BANU BALCI, REZAN TOPALOGLU, and NESRIN BESBAS

ABSTRACT. ***Objective.*** To analyze 70 individuals who were found to have the Mediterranean fever (MEFV) gene for the presence of definite familial Mediterranean fever (FMF) and to assess if they were prone to clinical and laboratory inflammation. We also prospectively evaluated 72 patients with childhood rheumatic diseases for the presence of MEFV mutations.

Methods. Seventy patients with one MEFV gene mutation were reevaluated for the presence of a clinical FMF phenotype using a new set of criteria. They were also questioned for the presence of musculoskeletal symptoms and rheumatic diseases. They were sampled for erythrocyte sedimentation rates and C-reactive protein levels. A second group with childhood rheumatic diseases were diagnosed according to international criteria.

Results. Median age of the 70 heterozygous individuals was 12 years. About 1/3 (34.3%) were classified with clinical FMF phenotype according to the suggested criteria. Fifteen (21.4%) were classified as normal and 3 (4.3%) had recurrent abdominal pains but did not fulfill all criteria for clinical FMF. Overall, 28 (40.0%) had some form of rheumatic complaint and 15 (21.4%) had developed a rheumatic disease including Behçet's disease, a vasculitis, or acute rheumatic fever. The mean ESR and CRP levels were 45.47 ± 33.05 mm/h and 4.00 ± 6.73 mg/dl, respectively. Among the 72 patients with rheumatic diseases of childhood, 22 (30.5%) carried one or 2 mutations of the MEFV gene. The mutated allele frequency among patients with rheumatic diseases was significantly higher than those in controls ($p < 0.05$). Within this group, among the 59 patients with juvenile idiopathic arthritis 15 had mutations in the heterozygous or homozygous form.

Conclusion. We confirm the acute phase response in the carriers for MEFV mutations. We suggest that these patients may have a tendency to develop certain manifestations due to an increased baseline of inflammation, and the presence of these mutations may affect their disease course when they develop rheumatic disease. (J Rheumatol 2003;30:2014–8)

Key Indexing Terms:
FAMILIAL MEDITERRANEAN FEVER CARRIER FREQUENCY JUVENILE ARTHRITIS

Familial Mediterranean fever (FMF) is an autoinflammatory disease characterized by self-limited attacks of fever and serositis along with an increase in acute phase reactants[1-3]. More than 25 mutations in the Mediterranean fever (MEFV) gene cause the disease[4,5]. Defining the gene has been a milestone in the field but has also introduced new problems.

Since FMF is generally accepted to be an autosomal recessive disease, mutations on both alleles are needed for the genetic confirmation of the disease. However, FMF is accepted as a clinical diagnosis. Physicians practicing in countries where the disease is frequent would accept a patient with recurrent febrile episodes accompanied by abdominal and/or chest pain and/or arthritis along with increased acute phase reactants as definite FMF, regardless of the genetic analysis. Livneh, *et al* introduced sets of criteria for the diagnosis of FMF[6,7]. These criteria need to be validated in multiethnic populations. All patients fulfilling these criteria and/or who are being followed with the diagnosis of FMF in specialized clinics do not have genetic confirmation when screened for the common mutations. On the other hand, all patients with mutations on both alleles do not have symptoms of FMF and it has been suggested that they be designated as phenotype III patients[8].

FMF is the most common periodic fever disease. The carrier rate in certain ethnic groups has been reported to be as high as 1/3 to 1/6[9-12]. Whether carriers of the disease had an advantage in ancient times has been an intriguing debate[4,13]. It has been hypothesized that the heterozygotes had the advantage of mounting a better acute response with their increased C-reactive protein (CRP) levels[14,15]. On the other hand Kogan, *et al*[8] have claimed an absence of a biological advantage for the carrier state and that carriers in their Askhenazi Jewish population had an excess of febrile episodes. Thus, whether one may have a disorder in the

From the Departments of Pediatrics and Molecular Biology, Hacettepe University Faculty of Medicine, Ankara, Turkey.

S. Ozen, MD, Professor; A. Bakkaloglu, MD, Professor; A. Duzova, MD; R. Topaloglu, MD; N. Besbas, MD, Professor, Department of Pediatrics; E. Yilmaz, PhD; B. Balci, MSc, Department of Molecular Biology.

Address reprint requests to Dr. S. Ozen, Department of Pediatrics, Hacettepe University, 06100 Ankara, Turkey.
E-mail: sezaozen@hacettepe.edu.tr

Submitted July 3, 2002; revision accepted February 7, 2003.

inflammation pathway with one MEFV mutation becomes an attractive area of research.

We aimed to evaluate in detail individuals with one mutation only. We analyzed the prevalence of clinical FMF or recurrent abdominal pains only, and whether individuals with one MEFV mutation were prone to increased inflammation in laboratory and clinical terms.

We also took an alternative approach and prospectively screened patients with rheumatic diseases of childhood for mutations in MEFV to see whether they introduced a risk factor for developing an inflammatory disease.

MATERIALS AND METHODS

Patients. Individuals with one mutation in the MEFV gene among the consecutively studied samples in our center were the subjects of this study. Only those whose files were available for review and those who were willing to be reevaluated were included. They were reevaluated mainly to define the percentage who fit within the clinical phenotype of FMF. Further, these individuals were questioned for a history of rheumatic disease confirmed by a pediatrician and the presence of any musculoskeletal complaints such as arthritis and myalgia. The genotype analysis had been carried out in the Department of Molecular Biology of Hacettepe University Faculty of Medicine. Mutation analysis for the MEFV gene had been performed in these patients either because they were close relatives (siblings or cousins, etc.) of a patient with FMF or were referred to our Department of Pediatric Nephrology and Rheumatology because of complaints suggestive of FMF with/without unusual features. The department no longer tests clinically unaffected relatives.

The subjects were reevaluated by the same physician and were classified into 4 categories: (a) Normal: may have had occasional abdominal pains but not more than once a year or coupled with nonspecific infections; (b) Those with clinical FMF [defined by the following: (1) at least 3/year self-limited attacks of fever plus abdominal pain and/or chest pain and/or arthralgia and/or arthritis, (2) symptoms had a typical duration of 12 hours to 3 days and no lymphadenopathy, (3) favorable response to colchicine, (4) return of symptoms after cessation of colchicine, or if the latter is not available, elevation of acute phase reactants during the attack with a decrease afterwards]; (c) Those who have recurrent abdominal pain attacks but do not fulfill all the criteria for the FMF phenotype as defined above; (d) Those with rheumatic or musculoskeletal complaints [Behçet's disease (BD), acute rheumatic fever, polyarteritis nodosa (PAN), Henoch Schonlein purpura (HSP), other rheumatic diseases, reactive arthritis, and disabling symptoms of myalgia-arthralgia].

Among the diagnostic criteria introduced for the definition of clinical FMF, the first and third items fulfill the diagnosis of FMF according to the Tel Hashomer criteria; the second item distinguishes the disease from the other periodic fevers in general. The fourth item strengthens clinical judgment, since we lack genetic analysis for these patients.

At the time of sampling for DNA extraction, at least one acute phase reactant [erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP)] was studied in all individuals.

The second part of the study included pediatric patients with juvenile idiopathic arthritis (JIA) (n = 59), HSP (n = 5), PAN (n = 3), and BD (n = 4). These patients were all followed in the Pediatric Department of Hacettepe University. None of them had clinical features suggestive of FMF at presentation. JIA was diagnosed according to the Durban criteria[16], BD was defined according to international criteria[17].

The frequency of alleles with MEFV mutations was compared to our data obtained from 100 healthy individuals as described[11].

The primer set used to amplify exon 10 is Ex10F 5'$[GC]_{40}$GAG AAG CAG GAA GAG AGA TGC 3' and Ex10R 5'TAT CAT TGT TCT GGG CTC 3'. Polymerase chain reaction (PCR) amplification was performed under the following conditions; 95°C for 5 min followed by 40 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and final extension at 72°C for 5 min. Fifteen µl of the PCR products were subjected to electrophoresis at 160 V and 60°C for 6 h on a 6.5% polyacrylamide gel containing a linearly increasing denaturant concentration of 30–80%. The PCR products that did not display a shift in mobility were systematically mixed with a PCR product from a normal control to detect homozygous mutations in exon 10. M680I and V726A mutations were confirmed by restriction analysis by Hinf I and Alu I after DGGE analysis. Mutation in exon 2 (E148Q) was analyzed by Bst NI restriction enzyme digestion, after amplification of genomic DNA with the Ex2F: 5' GCC TGA AGA CTC CAG ACC ACC CCG 3' and Ex2R: 5' AGG CCC TCC GAG GCC TTC TCT 3' primers.

RESULTS

A total of 70 individuals with mutations in one allele of the MEFV gene were evaluated in the first part of the study. The median age of the patients was 12 years. Female:male ratio was 40:30.

Overall, 24 (34.3%) of these individuals were classified with the typical clinical phenotype for FMF (Table 1). Fifteen (21.4%) were classified as normal as they did not have specific complaints. Three had recurrent abdominal pains but did not fulfill our criteria for FMF.

Twenty-eight (40.0%) had some form of rheumatic complaint and 15 (21.4%) of these heterozygotes had developed a rheumatic disease including BD (n = 3), JIA (n = 2), PAN (n = 2), unclassified small vessel vasculitis (n = 2), HSP (n = 2), or acute rheumatic fever (n = 4). The remaining 13 patients either had reactive arthritis or incapacitating attacks of arthralgia and/or myalgia. In some, myalgia was in the form of exertional pain.

The mean ESR and CRP of this group of patients (n = 28) were 53.87 ± 38.03 mm/h and 4.43 ± 7.87 mg/dl, respectively. The mean ESR and CRP levels of all heterozygotes (n = 70) were 45.47 ± 33.05 mm/h and 4.00 ± 6.73 mg/dl, respectively. When these laboratory variables were assessed separately in the aforementioned clinical groups the mean ESR and CRP levels were significantly higher in those who had definite FMF and those who had rheumatic complaints when compared to those classified as normal ($p < 0.05$ for all).

When the patients were evaluated separately according to their mutation, the distribution of these groups was not significantly different between the carriers for the M694V versus E148Q mutation (Table 1) ($p > 0.05$).

In the second arm of the study patients with pediatric rheumatic diseases were screened for the mutations of the MEFV gene (Table 2). Among the 72 patients screened, 22 had mutations in the MEFV gene in one or both alleles. The majority of these were JIA patients (n = 59). Among these, 6 were homozygous or compound heterozygotes for the MEFV mutation and 16 were carriers for a mutation in one allele only. Most of these patients were carriers for the M694V mutation. This is in contrast to our findings that most of 100 healthy Turkish controls were carriers for

Table 1. Clinical classification of individuals with MEFV mutations in one allele.

Mutations, n (%)	Clinical Classification			
	Normals	FMF*	Musculoskeletal/Rheumatologic Complaints	n
M694V**	8 (23.5)	14 (41.2)	12 (35.3)	34
M680I	1 (20.0)	2 (40.0)	2 (40.0)	5
V726A	1 (10.0)	5 (50.0)	4 (40.0)	10
E148Q	5 (27.8)	3 (16.7)	10 (55.6)	18
Total	15 (22.4)	24 (35.8)	28 (41.8)	67**

* Defined in the text. ** 3 patients, all M694V, with recurrent abdominal pain, were not included in this table.

Table 2. MEFV mutations in 72 patients with childhood rheumatic diseases: The JIA + vasculitides group had significantly more alleles with the MEFV mutation (29/144) compared to healthy controls (22/200) ($p < 0.05$).

Patients	Genotypes				
	M694V Alleles	E148Q Alleles	V726A Alleles	Other	Total
JIA patients (n = 59)	8/118	9/118	2/118	1/118	20/118
Vasculitides (n = 13)	8/26	—	1/26	—	9/26
Total (n = 72)	16/144*	9/144**	3/144	1/144	29/144
Turkish controls (n = 100)[11]	3/200*	12/200**	2/200	5/200	22/200

* Comparison of M694V mutation frequency between patients with (JIA + Vasculitides) (16/144) and healthy controls (3/200); $p < 0.005$. ** Comparison of E148Q mutation frequency between patients with (JIA + Vasculitides) (9/144) and healthy controls (12/200); $p > 0.05$.

E148Q (Table 2). Further, the allele frequency was significantly higher in the patients with rheumatic diseases compared to healthy controls ($p = 0.02$).

One patient homozygous for the M694V mutation had been admitted to the hospital with a diagnosis of classic PAN. After the mutation analysis was obtained, he subsequently developed typical FMF symptoms. Another patient with classic PAN was a carrier, whereas a third did not show any MEFV mutations. Two patients with BD and 2 with HSP were carriers for MEFV mutations.

Among the 2 JIA patients homozygous for E148Q, one denied any symptoms of FMF (Table 2). The other subsequently developed classical attacks of fever and arthritis or abdominal pain. He also admitted to 2 previous brief episodes of arthritis thought to be related to his JIA. This latter patient was diagnosed with FMF plus JIA[18]. Another JIA patient homozygous for M694V denied any symptoms compatible with FMF. However, this patient and one carrying the complex allele M694V-E148Q/E148Q had the most severe polyarticular course among this group of patients with destructive arthritis with a rapid course necessitating orthopedic surgery. The localization and course of arthritis in these patients were not compatible with that of FMF arthritis.

DISCUSSION

This is the first report of a thorough evaluation of patients who are carriers for a mutation of MEFV. We have confirmed a higher level of acute phase reactants in these heterozygous individuals than has been suggested in the relevant literature. Tunca, *et al*[13] have previously shown an acute phase response in individuals considered carriers for the MEFV gene with increased CRP levels. This acute phase response may be reflecting a proinflammatory state by laboratory variables.

Our aim was to study patients who had been confirmed as heterozygotes in our center. The results may be skewed toward overestimating the frequency of both FMF and other rheumatic complaints since they were either siblings of patients who may have been sensitized to symptoms, or had presented to our center with certain complaints. In line with this, E148Q was the most frequent mutation in the healthy Turkish population but not in this study cohort. One may presume that the carriers of the E148Q mutation did not present to our department because they did not have any complaints[10].

We have used a new set of criteria to define FMF that included elevated acute phase reactants. The explanation of a definite phenotype in these heterozygous patients may simply be the presence of mutations for which we have not screened. However, these rare mutations are infrequent in our population. Another explanation may be the presence of a second gene as we had suggested[19]. Mutations in the promoter region or translation defects may also be suggested. On the other hand the presence of modifier genes and reduced penetrance are the norm for Mendelian disorders. Modifying genes and/or epigenetic factors may cause inflammatory outbursts in these individuals who already are prone to inflammation.

An important percentage of the presented carriers had

musculoskeletal complaints. More than 1/5 of those who were heterozygous for the MEFV mutation had been classified with a definite rheumatic disease. Another interesting observation was that none had systemic lupus erythematosus (SLE), although SLE is slightly more frequently seen than PAN in our pediatric department (unpublished result). This might be simply because vasculitis features are within the spectrum of features of FMF[20], or it may be speculated that because of their high CRP levels or the Th1 polarization[21], patients with FMF have protection against the development of SLE.

We also investigated whether the MEFV mutations were increased in patients with vasculitis and other rheumatic diseases. We screened for MEFV mutations in these patients who did not present features of FMF (Table 2). Interestingly, we found that 30.5% of these patients and 25.4% of the JIA patients had one or 2 mutations for the MEFV gene. The high carrier rate may suggest that an elevated baseline of inflammation is a risk factor for developing severe rheumatic disease. The carrier rate among the healthy Turkish population is also high and has been found to be as high as 1/5 in our recent study[10]. However, the allele frequency among our patients with childhood rheumatic diseases is significantly higher. Moreover, the distribution of the alleles is different, since E148Q is the most frequent mutation (12%) in the healthy population, whereas M694V was the most frequent in the rheumatic disease patients. The M694V mutation is known to be associated with the most severe disease in FMF, while E148Q is associated with a milder phenotype and nonpenetrance[4,8].

Among the vasculitis, BD deserves a special comment. Both FMF and BD are frequent diseases of the Eastern Mediterranean[22]. It has been suggested that the MEFV mutations may confer additional disease susceptibility in BD[23], whereas Schwartz, *et al*[24] suggest that FMF phenotype could develop in patients with one MEFV mutation only in BD patients. However, Ben-Chetrit, *et al*[22] suggested that there was no mutual effect of FMF on BD or *vice versa*. Thus the increased frequency of the MEFV mutations among BD and vasculitis patients needs to be confirmed in larger patient groups.

Genotypically, 2 of the JIA patients had FMF, and these FMF patients with JIA had the most severe disease course. Secondary amyloidosis is the most serious complication of JIA, reflecting persistent inflammation. In a recent study Booth, *et al*[14] have analyzed British patients with inflammatory arthritis failing to show any mutation. However, among 25 who developed amyloidosis, 3 were carriers for MEFV mutation, although this mutation has not been defined in their healthy British cohort. Further, they have shown all 5 of their Indian patients with amyloidosis secondary to inflammatory arthritis had E148Q mutations of the MEFV gene.

The Turkish population is known to have a high carrier rate of MEFV mutations. Thus it is tempting to speculate that once juvenile arthritis does develop, the presence of a MEFV mutation may increase the baseline level of inflammation and alter the course of the disease just as certain cytokine polymorphisms alter the course in JIA.

Mutations in MEFV have been confined to certain ethnic groups who can be traced back to ancient times, and this might explain their absence in English JIA patients without amyloidosis. Why this mutation has been so highly selected in certain ethnic groups is still a topic of debate. However, we suggest that this selection has conferred to the carriers increased susceptibility for inflammation in laboratory and clinical terms, and to diseases of inflammation.

REFERENCES

1. Samuels J, Aksentijevich I, Torosyan Y, et al. FMF at the Millennium. Clinical spectrum, ancient mutations, and a survey of 100 American referrals to the National Institutes of Health. Medicine 1998;77:268-97.
2. Ben-Chetrit E, Levy M. Enigmas in FMF. Clin Exp Rheumatol 2001;19:s1-s5.
3. Ozen S. Vasculopathy, Behçet's disease and familial Mediterranean fever. Curr Opin Rheumatol 1999;11:393-8.
4. Kastner DL. FMF: The genetics of inflammation. Hosp Prac 1998;33:131-58.
5. Cazaneuve C, Ajrapetyan H, Papin S, et al. Identification of MEFV-independent modifying genetic factors for FMF. Am J Hum Genet 2000;67:1136-43.
6. Livneh A, Langevitz P, Zemer D, et al. Criteria for the diagnosis of FMF. Arthritis Rheum 1997;40:1879-85.
7. Livneh A, Langevitz P, Zemer D, et al. Establishment of a new set of criteria for the classification of FMF. In: Sohar E, Gafni J, Pras M, editors. Familial Mediterranean fever. London: Freund Publishing House; 1997:203-8.
8. Kogan A, Shinar Y, Lidar M, et al. Common MEFV mutations among Jewish ethnic groups in Israel: high frequency of carrier and phenotype III states and absence of a perceptible biological advantage for the carrier state. Am J Med Genet 2001;102:272-6.
9. Touitou I. The spectrum of FMF mutations. Eur J Hum Genet 2001;9:473-83.
10. Aksentijevich I, Samuels J, Centola M, et al. Mutation and haplotype studies of FMF reveal new ancestral relationships and evidence for a high carrier frequency with reduced penetrance in the Askhenazi Jewish population. Am J Hum Genet 1999;64:949-62.
11. Yilmaz E, Ozen S, Balci B, et al. Mutation frequency of FMF and evidence of a high carrier rate in the Turkish population. Eur J Hum Genet 2001;9:553-5.
12. Daniels M, Shohat T, Brenner-Ullman A, Shohat M. FMF: high gene frequency among the non-Ashkenazic and Ashkenazic Jewish populations in Israel. Am J Med Genet 1995;55:311-4.
13. Ozen S, Balci B, Ozkara S, et al. Is there a heterozygote advantage for familial Mediterranean fever carriers against tuberculosis infections? Speculations remain. Clin Exp Rheumatol 2002;20:S57-8.
14. Tunca M, Kirkali G, Soytuk M, Akar S, Pepys M, Hawkins P. Acute phase response and evolution of FMF [letter]. Lancet 1999;353:1415.
15. Booth DR, Lachmann HJ, Gillmore JD, Booth SE, Hawkins PN. Prevalence and significance of the FMF gene mutation encoding pyrin Q148. QJM 2001;94:527-31.
16. Petty RE, Southwood TR, Baum J, et al. Revision of proposed

classification criteria for juvenile idiopathic arthritis: Durban 1997. J Rheumatol 1998;25:1991-5.
17. International study group for Behçet's disease. Criteria for diagnosis of Behçet's disease. Lancet 1990;335:1070-80.
18. Ozen S, Besbas N, Bakkaloglu A, Yilmaz E. Pyrin 148Q does cause clinical FMF. QJM 2002;95:332-3.
19. Akarsu N, Ozen S, Saatci U, Bakkaloglu A, Besbas N, Sarfarazi M. Genetic linkage study of FMF to 16p13.3 and evidence for genetic heterogeneity in the Turkish population. J Med Genet 1997;34:573-8.
20. Ozen S, Ben-Chetrit E, Bakkaloglu A, et al. PAN in patients with FMF: a concomitant disease or a feature of FMF? Semin Arthritis Rheum 2001;30:281-7.
21. Aypar E, Ozen S, Okur H, et al. TH1 polarization in FMF (submitted for publication).
22. Ben-Chetrit E, Cohen R, Chajek-Shaul T. FMF and Behçet's disease — are they associated? J Rheumatol 2002;29:530-4.
23. Touitou I, Magne X, Molinari N, et al. MEFV mutations in Behçet's diseaae. Hum Mutat 2000;16:271-2.
24. Schwartz T, Langevitz P, Zemer D, Gazit E, Pras M, Livneh A. Behçet's disease in FMF: Characterization of the association between the two diseases. Semin Arthritis Rheum 2000;29:286-95.