

Familial Mediterranean Fever: Clinical and Genetic Characterization in a Mixed Pediatric Population of Jewish and Arab Patients

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ABSTRACT. *Objective.* Familial Mediterranean fever (FMF) is an autosomal recessive hereditary disease which primarily affects non-Ashkenazi Jews, Armenians, Arabs, and Turks. The gene responsible for the disease (*MEFV/FMF*) has been recently identified. Four common mutations in exon 10 of the *MEFV* gene seem to account for 86% of the DNA variations identified in patients with FMF. We conducted a phenotype/genotype correlation study in a mixed population of Jewish and Arab children with FMF.

Study Design. Seventy patients clinically diagnosed as having FMF underwent molecular genetic studies using polymerase chain reaction and restriction endonuclease digestion methods to detect the presence of the four mutations (M694V, M680I, V726A, M694I). We then correlated the presence of each mutation with ethnic origin, age of onset, clinical manifestations, disease severity, and occurrence of amyloidosis.

Results. The M694V mutation, which is predominant in non-Ashkenazi Jews, was found in 92% of our Jewish patients and in only 30% of the Arab patients. All four mutations were identified among 94% of the Arab patients, but with no particular prevalence for any one of them. The presence of a homozygous M694V mutation was significantly associated with a more severe form of the disease: the clinical onset of the disease manifested at an earlier age; the number of attacks per month was higher; the global assessment by the treating physician and the severity of pain scored higher; and arthritis was more frequent. Only patients with the M694V mutation had a family history of amyloidosis. No association was found between the type of mutation and the predominance of fever, abdominal pain, pleuritis, skin eruption, or response to colchicine in the clinical picture.

Conclusions. Homozygosity for the M694V mutation, predominant among North African Jews, is associated with a severe course and prognosis for FMF. This mutation is less common among Arabs and, when present, occurs almost only in heterozygous form. In Arab patients, the disease tends to run a milder course and seems to bear a better prognosis. The phenotype/genotype patterns that are evident from our study of a mixed series of Jewish and Arab children with FMF might provide a rational basis for counseling about the natural history of the disease and for clinical treatment of FMF patients and their families. *Pediatrics* 1999;103(5). URL: <http://www.pediatrics.org/cgi/content/full/103/5/e70>

[pediatrics.org/cgi/content/full/103/5/e70](http://www.pediatrics.org/cgi/content/full/103/5/e70); *FMF*, *MEFV* gene, mutations.

ABBREVIATIONS. FMF, familial Mediterranean fever; *MEFV*, Marenostin-encoding fever (gene); PCR, polymerase chain reaction; bp, base pair.

Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by recurrent attacks of fever and polyserositis. It affects primarily people of Mediterranean extraction, mostly non-Ashkenazi Jews, Armenians, Arabs, and Turks.¹⁻⁴ FMF is very common in the population at risk, with estimated carrier rates of 1:6 in North African Jews. The FMF gene frequency is high in most non-Ashkenazi Jewish ethnic groups and the estimated rates of heterozygotes vary from 1:6.7 in North African Jews to 1:13.3 in Iraqi Jews.⁵ Most patients begin to suffer during childhood—60% before 10 years of age and 90% before 20 years of age.⁶ The disease is characterized mainly by fever with abdominal pain and/or arthritis. Some patients suffer from episodes of pleuritis, erysipelas-like disease, orchitis, and pericarditis.⁷ Before prophylactic treatment with colchicine, FMF amyloidosis constituted a significant cause of renal failure and death among young adult patients.⁸

Because of the array of nonspecific clinical manifestations and the absence of an accurate diagnostic test, young children with FMF may be subjected to extensive investigations, such as exploratory laparotomy, before the correct diagnosis is made and treatment with colchicine is initiated.

The Marenostin-encoding fever gene (*MEFV*) was recently cloned, and four missense mutations (M694V, M680I, M694I, V726A) have been identified in exon 10 in a large proportion of affected patients.^{9,10} The cloning of the gene is of clinical importance, because the detection of mutations in the *MEFV* gene can provide an ultimate diagnosis of FMF. Molecular diagnostic testing for FMF provides a means that is noninvasive, sensitive, specific, and inexpensive for an accurate diagnosis of patients before the full clinical syndrome is present. Moreover, the use of molecular genetic testing can lead to an early detection of at-risk siblings of previously diagnosed patients with FMF for a timely institution of colchicine therapy, whereby morbidity and disability from the disease can be minimized. We therefore recalled for systematic reevaluation our population

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of children who had been clinically diagnosed as having FMF. The patients were screened for the four most common mutations known so far and which seem to account for the majority of mutations identified in patients with FMF from the Middle East.

The purpose of our study was to assess the sensitivity of mutation analysis as a diagnostic test for FMF in symptomatic patients; to explore the possible correlations between the diverse genotypes and the phenotypic expression of the disease; and, to perform a preliminary population genetic study in non-Ashkenazi Jewish and Arab children. This is, to our knowledge, the first such report on a mixed pediatric population of Jewish and Arab patients with FMF.

PATIENTS

The study was approved by the Human Studies Ethics Committee of Rambam Medical Center, and informed consent was obtained from the patients or their legal guardians. All patients who were clinically diagnosed at the Pediatric Rheumatology Clinic of Rambam Medical Center as having FMF were included in the study. The diagnosis of FMF was based on a typical clinical picture of recurrent attacks of fever and pain affecting non-Ashkenazi Jewish or Arab patients, and involving one or two of the following sites at a time: abdomen, chest, joints, muscles, scrotum, and skin. In addition, an increase in acute phase reactants during the attacks, a positive history among siblings or in the extended family, and a good response to colchicine helped to establish the diagnosis. We reviewed the records of 70 patients and looked at the following details: family origin; age of onset; clinical features (fever, abdominal, thoracic, articular, skin, muscular, testicular, and miscellaneous manifestations); severity of the disease by number of attacks per month, duration of attack (days), severity of pain (scale 1–10), and global assessment by the treating physician (scale 1–3); response to colchicine treatment; and, family history of FMF. The pediatric rheumatologist (R.B.) reinterviewed all the patients, and a blood sample was drawn for DNA analysis.

Of the 70 patients, 39 were Jews and 31 were Arabs. Forty-one were males. The mean age was 11.7 ± 5.8 years (range, 2–17 years). Three patients were excluded from the study after scrutiny of the charts and the genetic results: 2 had recurrent attacks of arthritis that responded well to colchicine, but had no fever or other characteristics of FMF; the other was a 2-year-old boy who suffered only from recurrent bouts of fever and had a sister with FMF. No mutations were found in these 3 patients.

METHODS

Samples were obtained from 70 unrelated FMF patients who attended our rheumatology clinic at Rambam Medical Center. DNA was isolated from peripheral blood lymphocytes by standard procedures.

Polymerase Chain Reaction (PCR) and Restriction Endonuclease Digestion

To detect mutations M694V and V726A, primers (F 5'-GAATGGCTACTGGGTGGAGAT-3') and (R 5'-GGCTGTACATTGTA-AAAGGAG-3')—designated FMF7 and FMF8, respectively—were used (unpublished data). The amplification conditions were

94°C for 4 minutes followed by 30 cycles of: 45 seconds at 94°C, 30 seconds at 55°C, and 1 minute at 72°C, followed by a 10-minute incubation at 72°C.

The M694V variant creates a Hph-I restriction site in the PCR product of the mutant allele but not of the normal allele. The FMF7 primer was designed (mismatch) to abolish another constitutive Hph-I site proximal to the mutation. After Hph-I restriction the mutant allele yields one 118-base pair (bp) and one 36-bp fragment; the normal allele gives a 154-bp uncut fragment.

The V726A variant creates an Alu-I restriction site in the PCR product of the mutant allele. The Alu-I restriction site yields a 122-bp and a 32-bp fragment for the mutant allele, whereas the normal allele gives a 154-bp uncut fragment.

To detect the M694I variant, a mismatch is introduced into a primer (FMF9) that anneals adjacent to the mutation locus and thus creates a BspH-I site in the normal allele restriction.

Primers FMF9 (5'-GCTACTGGGTGGTGATAATCAT-3') and FMF8 are used for PCR amplification using the same conditions described above. The BspH-I restriction site yields a 130-bp and a 19-bp fragment for the normal allele whereas the mutant allele gives a 149-bp uncut fragment.

The M680I variant abolishes a native Hinf-I restriction site. The mutation is distinguished by primers p12.2 (5'-TATCATTGTCTGGGCTC-3') and met1 (5'-CTGGTACTCATTTTCCTTC-3').¹⁰ The Hinf-I restriction site yields a 124-bp and a 60-bp fragment for the normal allele whereas the mutant allele gives a 184-bp uncut fragment. The amplification conditions were 94°C for 4 minutes followed by 30 cycles of: 60 seconds at 94°C, 90 seconds at 55°C, 60 seconds at 72°C, followed by a 10-minute incubation at 72°C.

The PCR products were separated on 8% nondenaturing polyacrylamide gel, stained by ethidium bromide, and visualized under an ultraviolet lamp.

Statistical Analysis

Data were analyzed using the SPSS program (SPSS Inc, Chicago, IL). Comparisons among three groups of patients with different combination of mutations were done by analysis of variance. The duration of attacks and number of attacks per month were assessed by the Kruskal-Wallis test. Categorical variables were compared by using the χ^2 test.

RESULTS

Of the 70 patients who were clinically diagnosed as having FMF, 67 were included in the study. Sixty-two (92.7%) had one or two mutation-bearing DNA samples (Table 1). Twenty-six were homozygotes for the M694V mutation, 12 were homozygotes for other mutations, and 11 were compound heterozygotes. Thirteen patients were found to be heterozygous for one of the mutations. The M694V mutation was detected in all non-Ashkenazi Jews, but only in 28% of Moslem Arab patients ($P < .0001$). All four mutations were found in the Arab population but with no particular prevalence for any one of them. In view of the small numbers of patients featuring the other possible allelic combinations, we labeled all non-M694V mutations as other and divided the patients

TABLE 1. Distribution of Founder Mutations Among the Various Ethnic Groups

Mutations	Jews		Arabs				Total
	North African	Other	Moslems	Christian	Druze	Other	
M694V/M694V	20 (74.1%)	5 (83.3%)	1 (4%)	—	—	—	26
M694V/other or none	7 (25.9%)	1 (16.7%)	6 (24%)	—	—	—	14
M680I/M680I	—	—	7 (28%)	—	—	—	7
V726A/other or none	—	—	5 (20%)	—	—	1	6
M694I/M694I	—	—	—	2	1	—	3
V726A/V726A	—	—	2 (8%)	—	—	—	2
M680I/V726A	—	—	4 (16%)	—	—	—	4
Total	27	6	25	2	1	1	62

into three groups: M694V/M694V, M694V/other or none, and other/other or none.

In 5 patients, no mutations were found. They had a clinical picture compatible with FMF and responded well to treatment. Of these, 3 were of Arab origin, 1 was a Kurdish-Iraqi Jew with a family history of FMF, and 1 was a recently arrived immigrant girl from Russia (Caucasus).

Of the 62 mutation-bearing patients, 41 were male. The patients' mean age was 11.7 ± 5.8 years (range, 2–17 years). Mean age of onset of clinical disease was 4.8 ± 2.9 years,¹⁻¹² mean duration of the attacks was 2.7 ± 2.1 days (range, 0.5–10 days), and the mean number of attacks per month was 1.7 ± 1.3 (range, 0.1–6). The clinical characteristics for the three mutant combinations is shown in Table 2.

Presence of the homozygous M694V mutation was associated with a more severe form of the disease, an earlier onset of the disease ($P < .05$), and a higher number of attacks per month ($P < .02$). The severity of pain as judged by the children was significantly greater ($P < .001$) as well as the global assessment for severity by the treating physician ($P < .0001$). Arthritis was more frequent in the presence of the M694V mutation ($P < .02$), but no association was found with fever, abdominal pain, pleuritis, skin reaction, or response to colchicine. However, when the number of symptoms were counted in individual patients, children with the M694V mutation had more organs involved ($P < .05$).

All patients with severe complications of FMF, such as chronic spondyloarthropathy and vasculitis, were homozygotes for the M694V mutation, as were 3 patients in whom a history of amyloidosis, confirmed by renal biopsy, was elicited among affected relatives.

When the homozygous patients for M694V were compared with any other homozygote or compound heterozygote combination of the other three mutations, the same results were obtained: the patients who were homozygous for M694V had more attacks per month ($P < .001$) and more episodes of arthritis ($P < .015$), and the global assessment by the treating physician and the severity of pain scored higher ($P < .0001$).

DISCUSSION

The cloning of the FMF gene by the International and the French Consortium provided a most important tool to address questions ranging from population genetics to structural biology. The pediatric popu-

lation cared for in our clinic is an ethnically mixed population of Jewish and Arab extraction; therefore, the availability of a direct test to detect the four founder mutations in the *MEFV* gene enabled us to screen these patients for their genetic pattern, and allowed us to gain a better understanding regarding the clinical picture and prognosis of FMF. Molecular testing for the four common mutations was able to achieve an ultimate diagnosis of FMF in 62 of 67 (92.6%) children with an appropriate clinical constellation, which reflects a good degree of sensitivity for this test. Ninety-two percent of the Jewish patients (North African and Iraqi extraction) in our population were found to have at least one M694V mutation, which suggests that our patients should be studied first for this mutation on initial diagnostic screening. Similar to findings reported by other groups from Israel and France,¹¹⁻¹³ our patients who were homozygotes for the M694V mutation had an earlier disease onset, a more severe course of the disease, more joint involvement, and more complications. Whereas a family history of amyloidosis was found in 3 of our cases (2 grandfathers and a maternal uncle), there was no instance of amyloidosis in any of our children, because colchicine treatment was instituted as soon as their FMF was diagnosed, and it is well recognized that colchicine can prevent amyloidosis in all compliant patients.⁸ Notably, 2 of our patients bearing the M694V mutation had a grandfather who died from renal failure because of amyloidosis. Thirty-one patients were Arab children, and we could show that the disease had a milder course in this ethnic group. None of these patients had severe complications, including amyloidosis. Only 30% of them had the M694V mutation, and the four studied mutations were equally dispersed among this group. Most of the Arab patients were homozygous to mutations other than M694V, or were compound heterozygotes. When the severity of the disease was compared between homozygosity for M694V and homozygosity for other combinations, it was clear that the presence of the M694V mutation alone is responsible for the worse prognosis rather than homozygosity for any of the other mutations. Because the Arab group was quite heterogeneous (Moslems, Christians, and Druze), more population screening studies are needed to characterize the disease among patients of Arab extraction.

In 5 patients who had been clinically diagnosed as having FMF and seemed to respond to colchicine, none of the four mutations could be detected. It is

TABLE 2. Phenotype-Genotype Correlation

Mutation	Age of Onset (Years)	Duration of Attack (Days)	No. of Attacks/Month	Fever (%)	Abdominal Pain (%)	Pleuritis (%)	Arthritis (%)	Pain (Maximum Score)	Global Assessment (Maximum Score)
M694V/M694V (n* = 26)	4 ± 2.6	3.5 ± 2.7	2.3 ± 1.5	92	77	46	81	69.2%	65.4%
M694V/other or none (n = 14)	4.4 ± 2.6	2.4 ± 1.5	0.8 ± 1.1	93	100	57	50	21.4%	7.1%
Other/other or none (n = 22)	6 ± 2.7	2.1 ± 1.1	1.4 ± 0.9	95	86	41	41	22.7%	22.7%
P value	<.05	NS	<.02	NS	NS	NS	<.02	<.001	<.0001

* n, number of patients.

probable that these patients carry other, newly described mutations.¹⁴ On the other hand, considering the nonspecificity of clinical criteria for a certain diagnosis, these patients might not have FMF but a clinical constellation mimicking the disease.

The M694V mutation was detected in a heterozygous form in 14 of the children with FMF. Their disease was of a lesser severity, but they did suffer from recurrent attacks that responded to colchicine. We feel that these patients should continue receiving colchicine therapy until a better understanding of the effects of alterations in the FMF gene product will be achieved.

CONCLUSIONS

In summary, the identification of the FMF gene and its various mutations has led to the application of an accessible, noninvasive, and sensitive molecular genetic test for an accurate diagnosis of this intriguing disease. This diagnostic tool provides a rational basis for medical and genetic counseling and for clinical treatment of FMF patients and their families.

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