

Clinical and Diagnostic Value of Genetic Testing in 216 Israeli Children with Familial Mediterranean Fever

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ABSTRACT. Objective. Familial Mediterranean fever (FMF) is an autosomal recessive disease with diverse clinical presentation. The FMF gene (*MEFV*) has recently been cloned and 30 point mutations causing the disease have been identified. We appraised the value of mutation analysis as a diagnostic test for FMF in symptomatic pediatric patients, and explored the possible correlations between *MEFV* genotypes and the diverse phenotypic expression of the disease.

Methods. Two hundred sixteen children who met the clinical criteria for FMF underwent molecular genetic studies to detect the 3 most common mutations in the Israeli FMF patient population (*M694V*, *V726A*, *E148Q*). The mutations found were related to clinical presentation and disease severity, using the Tel-Hashomer severity score.

Results. Of the 216 children who fulfilled the diagnostic criteria for FMF, 82 (38.0%) had 2 of the tested mutations, 73 (33.8%) had only one mutation, and 61 (28.2%) had none of the mutations studied. The *M694V* was the most frequent mutation, detected in 174 of 432 *MEFV* alleles (40.0%). The *V726A* mutation was found in 39 alleles (9.0%) and the *E148Q* mutation in 25 (5.8%). The severity score correlated with the number of mutations. Children with no mutations presented at an older age compared to children with one or 2 mutations. Children homozygous for the *M694V* mutation presented at a younger age, had a higher severity score, and more commonly had arthritis.

Conclusion. Limited genetic molecular testing for *MEFV* mutations may explain some of the FMF clinical variability, but is diagnostically ineffective. The use of clinical criteria remains essential in establishing the diagnosis of FMF. (J Rheumatol 2003;30:185-90)

Key Indexing Terms:

FAMILIAL MEDITERRANEAN FEVER
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Familial Mediterranean fever (FMF) is an autosomal recessive disease affecting populations living around the Mediterranean Sea. The disease is characterized by recurrent bouts of fever, associated with one or more of the following: peritonitis, pleuritis, pericarditis, arthritis, acute scrotum, and erysipelas-like erythema (ELE). The attacks are self-limited, lasting between a few hours and several days¹. Amyloidosis, resulting eventually in endstage kidney disease, is the most significant morbidity of FMF. Prophylactic colchicine treatment prevents the attacks, considerably improves the quality of life, and prevents the development of amyloidosis¹⁻³.

The diagnosis of FMF has been based upon clinical criteria alone for many decades. The recent cloning of the FMF gene (*MEFV*) and the identification of the mutations

causing the disease raised hopes for a more rapid and accurate diagnostic test for FMF⁴. However, molecular diagnosis is still not sensitive enough, as it fails to confirm the diagnosis of FMF in a large number of patients with typical presentation, even after the whole gene has been sequenced.

Three common mutations in the *MEFV* gene (*M694V*, *V726A*, and *E148Q*) account for the vast majority of the DNA variations in the Israeli-Jewish FMF patient population⁵. We evaluated the frequency of the different mutations in children with clinically established diagnosis of FMF, and related the severity of the disease, clinical spectrum, ethnicity, and course of the disease to the different mutations in the FMF gene. Given reports showing correlation between the *M694V* mutation, early onset, and grave outcome of FMF⁵⁻⁷, we expected childhood FMF (early onset FMF) to be more severe and display a higher frequency of the *M694V* mutation.

MATERIALS AND METHODS

Patients. All children 16 years old or younger clinically diagnosed as having FMF in the National Center for FMF at the Sheba Medical Center who were examined for the FMF gene mutations until December 2000 were included in the study. The diagnosis was established clinically, according to a set of published criteria formulated for the diagnosis of FMF, with a sensitivity and specificity of > 95%⁸. All children fulfilled the diagnostic criteria for FMF (≥ one major criterion or ≥ 2 minor criteria). There were 216 chil-

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dren, 115 male and 101 female (sex ratio 1.14). The mean age at onset was 7.4 ± 5.7 years. The mean age at study inclusion was 14.4 ± 6.5 years and the mean duration of disease at the time of the study was 10.1 ± 6.1 years. The mean delay in diagnosis was 2.8 ± 3.4 years, and colchicine therapy was administered for a mean of 2.2 ± 0.17 years. The computerized database and clinic charts of all patients were reviewed, focusing on demographic data, laboratory tests, family history, course of the disease, and response to therapy.

Genetic analysis. Genetic analysis of the FMF gene was performed as published. Briefly, DNA was extracted from 100 μ l of blood with a commercial kit (Puregene, Gentra Inc., Minneapolis, MN, USA) and was screened for 3 defined FMF mutations, *M694V*, *V726A*, and *E148Q*, using a commercial kit (Gamidigen, Rehovot, Israel), or polymerase chain reaction amplification and restriction enzyme analysis⁹. The *M680I* and the *M694I* mutations were additionally studied only in non-Jewish patients (less than 2% of our patient population) using a similar technology.

Severity score. To assess the severity of the disease, we used the Tel-Hashomer severity score¹⁰, which accounts for the age of onset, duration and frequency of the attacks, presence of arthritis and ELE, response to colchicine therapy, and presence of amyloidosis. Patients with a score > 10 were considered to have a severe form of the disease, 5–10 a moderate disease, and < 5 a mild disease.

Statistical analysis. Results are given as mean \pm standard deviation or proportion as appropriate. Differences between the groups were evaluated by chi-square for discrete variables, Kruskal-Wallis test for ordinal distribution, and analysis of variance for continuous variables.

RESULTS

Of the 216 children who fulfilled the diagnostic criteria for FMF, 82 (38.0%) had 2 mutations, supporting the diagnosis of FMF. Seventy-three children (33.8%) had only one of the studied mutations. In 61 children (28.2%), none of the studied mutations was found, in spite of typical disease. The number of mutations found correlated with the severity score and the age of disease onset, with a more severe disease and a younger age of onset in children with 2 mutations (Table 1). However, similar delay in diagnosis was found in all groups regardless of the number of mutations found. In 71.2% of patients with 2 mutations there was no family history of FMF, similar to the 68.6% and 71.2% of patients with 1 and 2 mutations, respectively, and there was at least one sibling with FMF in 12.5%, 16.4%, and 17.5% of patients with 0, 1 and 2 mutations respectively.

The distribution of the various forms of the attacks also correlated with the number of detectable mutations, with a higher prevalence of arthritis, pleuritis, ELE, and fever

alone in children with 2 mutations (Table 2). Unexpectedly, a trend for higher rates of peritonitis (Table 2) and a trend for higher rates of appendectomies (8.2% compared with 1.4% and 0% for 0, 1, or 2 mutations, respectively) were found in children with none of the studied mutations. In children with 2 mutations, the FMF attacks involved more sites during the course of the disease than in children with 0–1 mutations; attacks in more than 2 sites were observed in 45.5% of children with 2 mutations, compared with 27.2% and 15.5% in 1 and 0 mutations, respectively ($p < 0.001$).

The doses of colchicine required to control the attacks were also related to the number of mutations. A dose of 1 mg/day was sufficient to control the attacks in 77.8% of the patients with no mutation, in 71.0% of patients with 1 mutation, and only 60.0% of patients with 2 mutations ($p < 0.05$ by analysis of variance). In up to 27.8% of patients with 2 mutations, a dose of 2 mg/day was required, compared to 14.5% and 18.1% of patients with 1 or no mutations, respectively ($p < 0.01$ by analysis of variance). Nonresponders were found only among patients with 2 mutations (3.1%).

Table 3 presents the results of *MEFV* genotype analysis.

Table 2. Number of common mutations is associated with different forms of FMF attacks in 216 children with FMF.

No. of Mutations	Peritonitis*, %	Arthritis*, %	Pleuritis*, %	Fever Alone, %	Acute ELE, Scrotum, %	ELE, %
2	85.4	47.6	26.8	31.4	2.6	26.8
1	78.1	28.8	15.1	19.2	1.4	11.0
No mutation	91.8	18.0	9.8	11.5	0.0	4.9

* p value by chi-square test < 0.05 . ELE: erysipelas-like erythema.

Table 3A. Distribution of genotype in 82 children with FMF, having 2 *MEFV* mutations.

Genotype	n (%)
M694V/M694V	47 (58.0)
M694V/V726A	20 (24.7)
M694V/E148Q	8 (9.9)
V726A/V726A-E148Q	3 (3.7)
V726A/V726A	2 (2.5)
V726A/E148Q	1 (1.2)
Total	82 (100)

Table 3B. Distribution of mutations in 73 children with FMF who have only one of the *MEFV* mutations under study. None of the common mutations were found in the remaining 61 patients.

Mutation	n (%)
M694V	51 (69.9)
E148Q	13 (17.8)
V726A	8 (10.9)
M680I	1 (1.3)
Total	73 (100)

Table 1. Number of common mutations is associated with age of disease onset and severity score in 216 children with FMF.

No. of Mutations	n (%)	Age at Onset*, yrs	Delay in Diagnosis, yrs	Years of Colchicine Treatment	Tel Hashomer Severity Score**
2	82 (38)	6 ± 4.4	2.8 ± 2.3	2.2 ± 0.18	7 ± 2.6
1	73 (33.8)	6 ± 4.2	2.6 ± 3.8	2.2 ± 0.3	4.9 ± 1.9
None	61 (28.2)	10 ± 6.4	3.4 ± 3.8	2.2 ± 0.2	3.8 ± 1.8

* $p < 0.002$ ANOVA and $p < 0.007$ Kruskal-Wallis test. ** $p < 0.0001$ ANOVA and $p < 0.0001$ Kruskal-Wallis test.

As may be inferred, the *M694V* mutation was the most frequent, found in 173 of the 432 FMF chromosomes (40%). The other 2 mutations were found only in 70 alleles (16.2%). Most of the 82 children with 2 mutations (58%) were homozygous for the *M694V* mutation. Other genotypes were much less common (Table 3A). In children with only one of the studied mutations, the *M694V* was again the predominant mutation, found in 51 of the positive 73 alleles (69.9%) (Table 3B).

Homozygotes for the *M694V* mutation were younger at the onset of the disease and had a higher severity score compared to patients with other genotypes (Table 4). Patients carrying only one of the studied mutations, either *V726A* or *E148Q*, had the latest onset and the lowest severity score (data not shown). The prevalence of the various forms of the FMF attacks, as related to the different genotypes, is summarized in Table 5. Arthritis was the only manifestation significantly more frequently associated with *M694V* homozygosity. Pleuritis was found more frequently in association with homozygosity to the *V726A* mutation. Other forms of FMF attacks were equally associated with the different genotypes.

Analysis of the ethnic origin of the patients showed a dominance of Jewish children who originated from North African countries (30.6%). The second largest group was composed of Middle Eastern Jews (17.7%), and the third of Ashkenazi Jews (4.2%) (Table 6). Mutation *V726A* was not found in patients from North African origin. Ashkenazi Jews

Table 4. Age of onset and severity scores of FMF correlate with the *MEFV* genotype.

Genotype	n	Age at Onset*, yrs	Severity Score**
M694V/M694V	47	4 ± 2.7	8 ± 2.3
M694V/V726A	20	7.6 ± 4.4	5.5 ± 0.6
M694V/E148Q	8	10.8 ± 5.1	5.6 ± 2.4
V726A/V726A	6	9.5 ± 5.2	4.3 ± 1.7

* $p < 0.002$ ANOVA and $p < 0.007$ Kruskal-Wallis test. ** $p < 0.0001$ ANOVA and $p < 0.0001$ Kruskal-Wallis test.

Table 5. Only FMF arthritis attacks correlate with the *MEFV* genotype.

Genotype	Peritonitis, %	Arthritis, %	Pleuritis, %	Fever Alone, %	Acute Scrotum, %	ELE, %
M694V/M694V	87.2	61.7	25.5	29.8	2.1	40.4
M694V/V726A	85.0	30.0	25.0	0.0	0.0	15.0
M694V/E148Q	100	12.5	12.5	25	0	37.5
V726A/V726A	83.3	16.7	66.7	16.7	0.0	16.7
p*	0.71	0.006	0.126	0.054	0.8	0.176

* Chi-square.

Table 6. Two mutations were found more frequently among North African than in Middle East and Ashkenazi Jews.

Origin, % of Total	2 Mutations, %	1 Mutation, %	No Mutation, %
North African (NA), 30.6	77.3*	21.2	11.3
Middle East (ME), 17.7	25.7	22.8	51.4
Mixed ME + NA, 29.6	29.7	42.2	28.1
Ashkenazi (A), 4.2	28.6	28.6	42.8
Mixed A + ME, 10.6	15.0	55.0	30.0
Mixed A + NA, 9.3	29.4	47.1	23.6

* $p < 0.01$ ANOVA.

carried all of the 3 common mutations, *M694V*, *V726A*, and *E148Q*. Two mutations were found more frequently among North African (77.3%) than in Ashkenazi (28.6%) and Middle East Jews (25.7%) (Table 6). Since the majority of the children came from mixed marriage of different ethnic groups, the interpretation of the data is limited.

As may be deduced from Table 7, the genotype (homozygosity to *M694V* mutation), rather than the ethnicity, determined the age of disease onset and its severity. No significant differences in age of disease onset, disease severity, or the number of involved sites were found between the sexes (data not shown).

DISCUSSION

The cloning of the FMF gene and identification of missense mutations causing the disease have provided an important laboratory tool that helps in the diagnosis of FMF and perhaps in understanding its heterogeneous clinical presentation. In this study, clinical data and severity scores were subjected to genetic correlation. We found one of the 3 common *MEFV* mutations in 56% of the studied alleles, but only 38% of the children had 2 mutated alleles (Table 1). These 82 children had a more severe disease, compared to children with 1 or 0 common mutations (Table 2). In children with 2 mutations, homozygosity for the *M694V* mutation was associated with the most severe form of the disease, with the youngest age at onset, highest severity score (Table 4), and highest rates of joint involvement (Table 5). The genotype (homozygosity for *M694V*) rather than the ethnic origin determined this phenotypic variability (Table 7).

The finding that the number of the prevailing mutations

Table 7. Age of onset and disease severity are unrelated to ethnic origin in the 47 children homozygous for the *M694V* mutation.

Origin	n	Age at Onset*, yrs	> 2 Sites, n	Severity Score*
North African	30	4.3 ± 2.6	17	8.8 ± 2.1
Other ethnic groups	17	4.0 ± 2.7	16	8.0 ± 2.3

* No significant difference by ANOVA.

is a determinant affecting the clinical picture as detailed above is novel. With respect to genotyping, comparable findings were recently reported in adults⁵ and children^{6,7}, showing that patients homozygous for the *M694V* mutation had an earlier onset, a more severe course, more joint involvement, and more complications. Dewalle, *et al*¹¹ found significantly higher rates of arthritis and pleuritis and an earlier age of onset in patients with the *M694V/M694V* genotype. The rates of peritonitis, response to colchicine, ELE, and amyloidosis were unaffected by the genotypes. Cazeneuve, *et al*¹² reported that patients with the *M694V/M694V* genotype have a higher prevalence of amyloidosis and arthritis, and Shohat, *et al*¹³ found this genotype to be associated with a higher prevalence of amyloidosis. Kone Paut, *et al*⁷ have shown a significant association of *M694V* homozygosity with an earlier age of onset, higher temperature during attacks, and higher prevalence of pleuritis, splenomegaly, arthritis and ELE. Taken together, despite some phenotypic variance detailed above, data in most reports relate increased disease severity to the *M694V/M694V* genotype.

A significant association has been reported between the *M694V* mutation and the development of amyloidosis in adult patients with FMF, especially in patients who are homozygous for this mutation^{9,13}. We were unable to study this association in our series, since only one patient with the *M694V/M694V* genotype developed amyloid nephropathy. The small number of children with amyloidosis in our cohort is in contrast with other reports of FMF pediatric patients^{6,7}. This probably reflects the increased awareness of FMF among Israeli pediatricians and early diagnosis and treatment of these patients. Genetic and environmental factors may also contribute to these discrepancies.

Increased disease severity is related to the presence of the *M694V* mutation, irrespective of the ethnic origin of the patient (Table 7). Similar findings were reported in a study from our center in adults with FMF⁵. This may imply that, compared to the *MEFV* genotype, the role of environmental factors and possibly other genetic factors in phenotype expression is less significant. The contribution of modifier genes such as *MICA* and *SAA1alpha/alpha*^{14,15} recently found to be important in the expression of the disease remains to be determined.

Homozygosity for the *M694V* mutation is almost universally associated with early onset of the disease. Yet childhood FMF is not necessarily nor exclusively due to this genotype alone (Table 3). We did not find enrichment of this genotype and of the *M694V* mutated alleles in our child population with FMF compared to the adult FMF population (Y. Shinar, *et al*, unpublished data) and compared to another Israeli FMF cohort⁶. This is another novel finding, which suggests that the distinct genotype makeup that characterizes late onset FMF¹⁶ has only minor influence on the final genotype proportions of FMF, which remains as established

in childhood. Indeed, the onset of FMF is before 16 years of age (cutoff age in our cohort) in the large majority of FMF patients¹. The finding that only 40% of the FMF chromosomes bear the *M694V* mutation suggests that childhood FMF is not necessarily associated with a grave prognosis.

Since the identification of the *MEFV* gene, 30 point mutations have been reported. Most laboratories providing routine genetic testing of FMF screen for at least the 5 most frequent mutations (*M694V*, *V726A*, *V680I*, *E148Q*, and *M694I*), as the other mutations were found in < 1% of FMF alleles. Our practice is to screen the 3 most frequent mutations found in our patient population (*M694V*, *V726A*, *E148Q*). The other 2 (*M680I* and *M694I*) are studied additionally in non-Jewish patients. With this approach, we were able to identify 2 mutated alleles in only 38% of the children clinically diagnosed with FMF (Table 1). In this cohort, genetic analysis adds valuable information, supporting the clinical diagnosis and reassuring the patients of the necessity of lifelong colchicine prophylaxis. A significant proportion of our patients with a typical clinical presentation of FMF and a favorable response to colchicine had only one mutation or none. Since FMF carries a high risk for the development of kidney amyloidosis leading to chronic renal failure if untreated^{1,3}, nonsupportive mutation analysis should not signify withholding colchicine treatment.

In a recent publication from another center in Israel⁶, molecular testing for the common mutations identified 2 mutations in 48 of 67 (71.6%) patients of Jewish and Arab extraction (46.3% were Arab children). Divergent results reported from different centers may stem from differences in patient population, patient selection for genetic testing, and diagnostic criteria. Indeed, the recent finding from the same center, that 20% of children who have "functional" abdominal pain were misdiagnosed and actually have FMF¹⁷, suggests that the high rates of 2 mutated *MEFV* alleles in that center is due to low clinical suspicion of FMF, causing loss of children with mild FMF to other disease entities, or selection of patients with severe forms of FMF.

The 38% of patients with 2 identified mutations in our cohort is statistically different from the 56% of 450 FMF patients with 2 identified mutations observed among patients of all ages from the FMF clinic in our institution (Y. Shinar, unpublished data). The increased proportion of double mutated *MEFV* in the general FMF population in our institution suggests that, over time, children diagnosed as having FMF and bearing only one of the studied mutations or none are lost to followup. This is consistent with either a prolonged remission or a very mild disease that characterizes this patient population, as discussed elsewhere in this study.

Individuals who meet clinical criteria for FMF, but have only one demonstrable mutation in their FMF gene, may still harbor an unknown mutation, either in the coding region, the promotor, introns, or the 3' untranslated region.

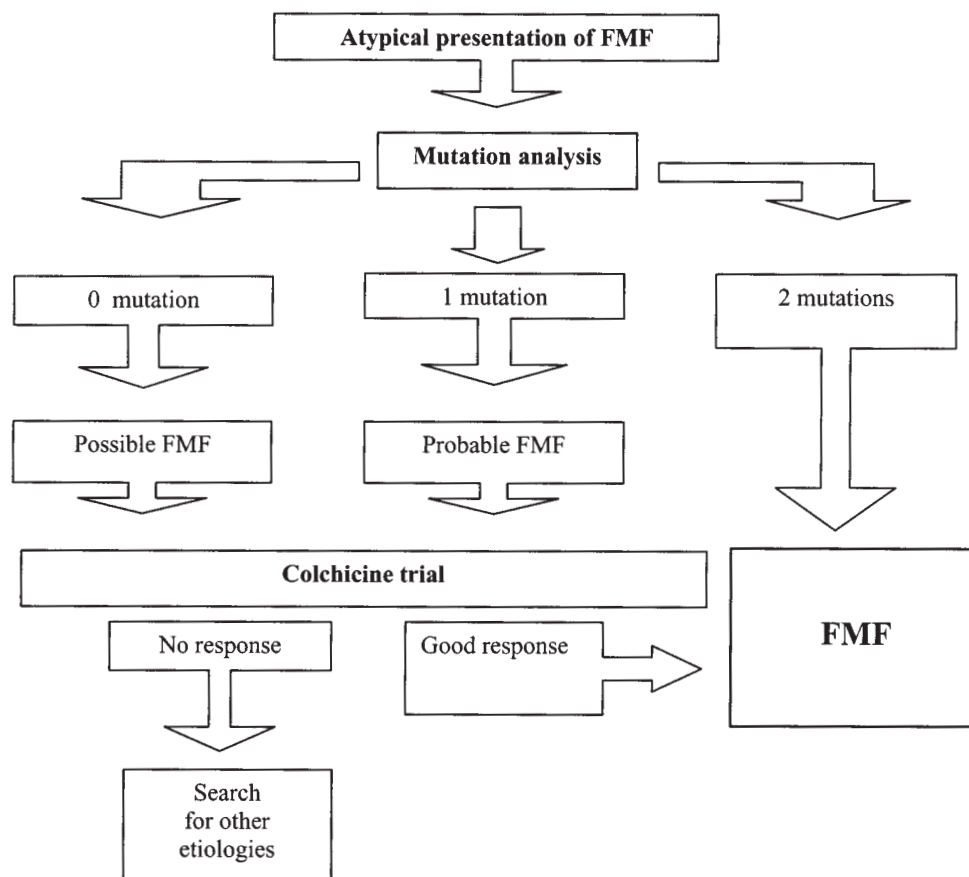


Figure 1. An algorithm for the diagnosis of atypical clinical presentation of FMF. Patients with 2 mutations should be diagnosed with FMF and prescribed prophylactic colchicine therapy. Patients with one mutation should be considered as probable FMF, while patients with no mutations, as possible FMF. Both patient groups should undergo colchicine trial consisting of 6 month treatment, followed by drug discontinuation. Those who have a favorable response (remission with treatment and attacks upon cessation) are then diagnosed as having FMF and should continue prophylactic colchicine. Other etiologies should be sought in those with no response.

Deletions or splicing abnormalities may lead to a loss of one or more *MEFV* exons, which thereby may escape detection. Analysis of the cDNA sequence or assays of the FMF protein may help to resolve these issues. Also, there may be other proteins involved in the FMF inflammatory pathway that when mutated may give rise to the FMF symptoms. It is also possible that a large number of the cases are not *MEFV* linked and suffer “FMF-like” symptoms. In such cases, the favorable response to colchicine treatment is therefore not intriguing. Finally, recent studies assign a possible dominant effect in certain cases of FMF heterozygotes^{18,19}.

Since only 38% of the children in our series had 2 known mutations in their FMF gene, we have resorted to clinical features for the diagnosis of FMF. Genetic testing should be offered to all patients, but it is diagnostically helpful only in atypical cases (Figure 1). We believe that at present the use of clinical criteria is essential in establishing the diagnosis

and determining the need for colchicine treatment. Due to the diminished sensitivity of mutation analysis, the diagnosis of FMF should not be refuted on account of a nonsupportive analysis. It is likely that some new mutations or modifier genes will be discovered in the near future, offering a scientific basis to account for the clinical diversity of the disease.

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