
Molecular diagnosis of FMF: Lessons from a study of 446 unrelated individuals

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ABSTRACT

Background. Traditionally, the diagnosis of familial Mediterranean fever (FMF) has been based on clinical manifestations and the physician's experience. Following the cloning of the gene associated with this disease (MEFV), genetic analysis of its mutations has become available, providing a new tool for the establishment or confirmation of the diagnosis of FMF.

Objectives. We analyzed the results of molecular testing for MEFV mutations in 600 individuals. We wished to determine how many of them bore mutations and what percentage had clinically active FMF. We also compared the rate of genetic confirmation of the FMF diagnosis in referrals with suspected FMF seen by general practitioners with that of persons sent for genetic analysis by FMF experts.

Methods. Of 600 individuals tested for FMF mutations, we analyzed separately 446 unrelated persons for the combination of their mutations, epidemiological data, and clinical manifestations. The five most common mutations in the present cohort were analyzed using the amplification refractory mutation system (ARMS).

Results. Of the 446 subjects analyzed, 249 (55%) bore mutations: 147 of these were homozygotes or compound heterozygotes, all of whom had FMF according to clinical criteria. Of the remaining 102 heterozygotes, 72 had FMF according to clinical criteria. Two patients with none of the five mutations also had FMF. North African Jews bore mainly mutations M694V and E148Q. The M694I mutation was found exclusively in Palestinian Arabs.

The rate of confirmation of FMF diagnosis by mutation analysis in subjects sent by FMF experts was significantly higher than that of persons referred by general practitioners. Analysis of the molecular testing of the multicase fam-

ilies (154 individuals) revealed that 141 of them bore MEFV mutations and that 4 persons homozygous for E148Q were asymptomatic.

Conclusions. Molecular analysis of FMF mutations confirmed the diagnosis in about 60% of the referrals with suspected FMF. Some (33%) of the patients were heterozygotes, and there were also FMF patients with none of the 5 mutations analyzed. A second opinion by an FMF expert may decrease the need for mutation analysis in subjects suspected of having FMF.

Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive hereditary disease manifesting as recurrent attacks of fever, peritonitis, pleuritis, arthritis and less frequently, erysipelas-like skin lesions (1). Several years ago the gene associated with FMF was isolated and four nonsense mutations were identified (2,3). Since then 24 additional mutations have been reported, most of which are very rare (4-8). In Israel, more than 90% of FMF patients bear one of the 5 MEFV mutations: M694V, V726A, E148Q, M680I or M694I (9). In our laboratory we routinely test for these mutations.

Over the last 3 years we have tested more than 600 referrals with suspected FMF. In the present study we report our findings from 446 tested subjects, each of whom represents a single unrelated family. Our goal was to analyze how many of these individuals actually bore FMF mutations, how many of them did have FMF on a clinical basis, what was the distribution of FMF mutations among the tested group, and how many of them bore two mutations, a single mutation or none. We also tried to compare the rate of genetic confirmation of the FMF diagnosis in referrals with suspected FMF seen by general practitioners with the rate for those referred

for testing by physicians who run FMF or human genetic clinics.

Patients and methods

Patients

Our laboratory receives requests for genetic testing for FMF mutations from Jerusalem and its surrounding area. The physicians who request the genetic tests are usually general practitioners, pediatricians, geneticists or physicians who run FMF clinics. Each tested individual provides his or her identity number, ethnic origin and the name of the treating physician. These details allow us to review their charts, to interview them and to contact their physicians in order to collect the necessary demographic and clinical data.

A clinical diagnosis of FMF was made according to previously published criteria (10), e.g., the presence of typical manifestations such as fever and serositis and responsiveness to colchicine treatment. In patients with typical clinical features, a response to colchicine treatment and exacerbation following its discontinuation, a diagnosis of FMF was made even in the absence of MEFV mutations.

MEFV mutation analysis

The mutations M680I, V726A and M694V were analyzed using the amplification refractory mutation system (ARMS) as described by us (11). Mutation M694I was assayed by the same method using, the following primers:

5'- TCGGGGGAACGCTGGACGCCTGGTACTCATTTCCTGT-3' (mutant).

5'- TCGGGGGAACGCTGGACGCCTGGTACTCATTTCCTGC-3' (normal).

5'- TGACAGCTGTATCATTGTTCTGGGCTCTCCG -3' (common).

Mutation E148Q was determined after restriction digestion as described by Aksentijevich *et al.* (8). PCR conditions and separation of the ARMS amplified products were as already described (11).

Statistical analysis

The accuracy of referral for MEFV mutations was compared between "FMF experts" and physicians from other cli-

nics. The percentage of positive FMF mutations identified in each referral group was compared with that of the actual number of FMF patients in this group and the difference between the two groups of referring physicians was calculated by the chi-square test. A p value of < 0.05 was considered as significant.

Results

Of 600 individuals referred to our laboratory for FMF mutation analysis, 446 unrelated subjects were analyzed separately. All were tested for 5 MEFV mutations: M694V, E148Q, V726A, M680I and M694I. As expected, most of the individuals were Jews of North African or Middle Eastern origin and Palestinian Arabs. Nevertheless, about 14% were either Ashkenazi Jews or the offspring of mixed couples (Ashkenazi and non-Ashkenazi Jews) (Table I). Of the 446 individuals, only 249 (55.4%) bore at least one MEFV mutation (Fig. 1). The distribution of the

Table I. Ethnic distribution of the tested individuals.

Ethnic origin	No.	%
North African Jews	146	32.7
Middle Eastern countries*	115	25.7
Others (non-Ashkenazi)**	18	4.0
Ashkenazi Jews	50	11.2
Mixed origin***	16	3.5
Palestinian Arabs	101	22.6

* Jews from Syria, Iraq, Turkey;
 ** Jews from Yemen, Georgia, Iran;
 *** One Ashkenazi and one non-Ashkenazi Jewish parent.

various mutations is presented in Table II. More than 75% of the individuals who bore MEFV mutations carried the M694V variant. Of the 249 individuals with mutation(s) in the MEFV gene, 147 (59%) were homozygotes or compound heterozygotes. Of the remaining 102 heterozygotes, 70% bore the M694V mutation. One patient bore 3

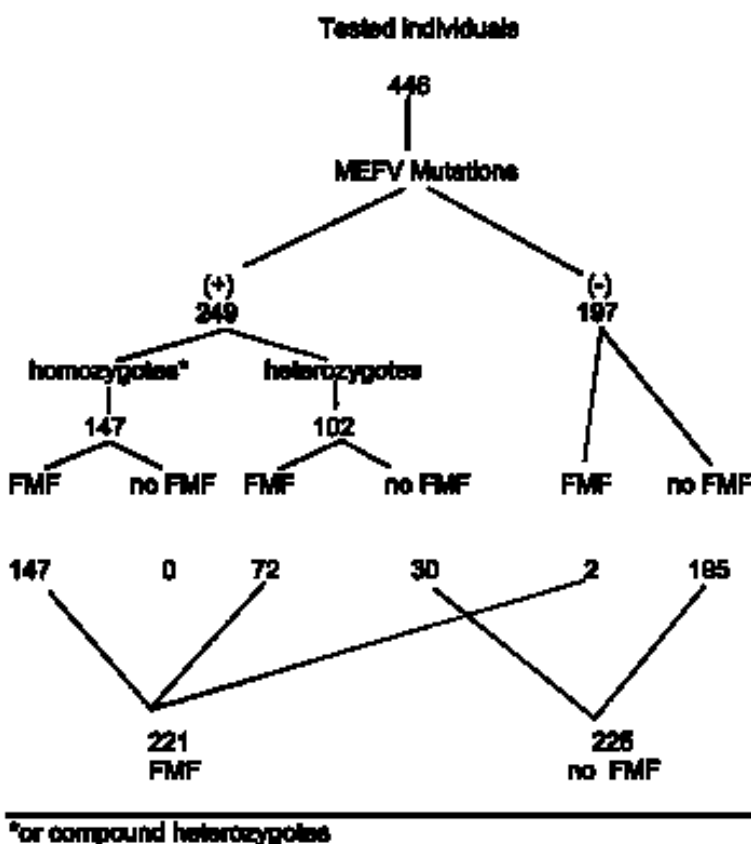


Fig. 1. A summary of the outcome of the 446 tested unrelated individuals.

Table II. Distribution of mutations among the tested individuals.

Mutation	Number	%
M694V/M694V	63	14.1
M694V/V726A	32	7.2
M694V/E148Q	24	5.4
M694I/M694I	8	1.8
V726A/V726A	6	1.3
V726A/M680I	4	0.9
E148Q/E148Q	2	0.4
V726A/E148Q	2	0.4
M694V/N	70	15.7
E148Q/N	15	3.4
V726A/N	14	3.1
M680I/N	3	0.07
N/N	197	44.2

A single case of the following combinations was found: R761H/N [assayed by I. Aksentijevich and D.L. Kastner (8)]. M694V-E148Q/A726V, M680I/E148Q, M694I/E148Q, M694V/M680I, M694V/M694I.
N: normal for the 5 mutations studied.

mutations, two of which were on the same allele (M694V-E148Q/V726A), and another patient had a single rare mutation (R761H) (assayed by Aksentijevich and Kastner). Both patients were Palestinian Arabs.

The distribution of the different mutations according to ethnic origin is shown in Table III. The main findings were the high association between the M694V mutation and North African Jewish origin, the rarity of the V726A mutation in this population and the common combination of M694V with V726A or E148Q among the Jewish offspring of mixed origin. Equally im-

pressive is the high variability of the mutation repertoire among Palestinian Arabs and Middle-Eastern Jews, as compared with that present in the North African Jews who bore mainly the two mutations, M694V and E148Q. Of note is the finding that the M694I mutation was detected only among Palestinian Arabs.

As mentioned above, 197 of the tested individuals did not bear any mutation. After reviewing their charts, or after an interview with the patients or their physicians, we realized that only two of them met the criteria for diagnosis of FMF. On the other hand, of the 249 individuals who bore MEFV mutations, all the 147 homozygotes or compound heterozygotes had clinical manifestations of FMF. Of the remaining 102 heterozygotes, 30 individuals were asymptomatic carriers. Thus, out of all the 446 tested individuals, 221 had FMF (2 with no detected mutations and 72 heterozygotes) and 225 (30 heterozygotes and 195 with none of the tested mutations) did not have FMF. The ethnic distribution of the individuals who did not meet the criteria for the FMF diagnosis is presented in Table IV. More than 50% of them were not North African Jews. About 80% of the Ashkenazi Jews tested did not have FMF, compared with 30% of the suspected Palestinian individuals. A relatively high proportion of the North African individuals (60%) did not meet the criteria for the diagnosis of FMF.

We also analyzed the relationship between the subspecialty of the physicians who referred the individuals for

Table IV. Ethnic distribution of the "healthy" individuals tested for MEFV mutations.

Ethnic origin	No. of cases	% of the whole ethnic group
North African	87	59
Middle Eastern	50	43.4
Others	15	83
Ashkenazi Jews	39	78
Palestinian	34	33.6

genetic testing and the rate of positive results. General practitioners, pediatricians etc. referred 276 individuals for genetic testing, of whom 119 were found to have mutations (43%) and 107 of these (38.7%) had clinical manifestations of FMF. The FMF "specialists" referred 170 individuals for genetic testing of FMF; 130 of these expressed MEFV mutations (76.4%) and 112 (65.8%) had FMF – a highly significant difference ($p < 0.0001$).

Analysis of the remaining 156 individuals (from the multicase families) disclosed the following results (Fig. 2): 141 persons (91.6%) bore MEFV mutations; 83 of them were heterozygotes and 25 (30%) of them had FMF clinically; 58 were either homozygotes or compound heterozygotes and 54 of them had FMF clinically. Four individuals homozygous for the E148Q sequence alteration did not have clinical FMF. None of the 13 persons with no mutation had FMF clinically. The mutation distribution in this cohort resembled that of the 446 unrelated individuals.

Table III. Mutation distribution according to ethnic origin (main combinations).

Ethnic Origin	M694V/ M694V	M694V/N	M694V/ V726A	M694V/ E148Q	V726A/N	E148Q/N	V726A/ V726A	M694I/ M694I
North African Jews	38	40	4	8	-	2	-	-
Middle Eastern Jews	4	20	6	10	7	4	3	-
Ashkenazi Jews	-	1	3	3	2	4	3	-
Mixed origin Jewish	-	-	10	2	2	-	-	-
Others*	-	5	1	-	-	1	-	-
Palestinian Arabs	21	4	8	1	3	4	-	8

*Yemenites, Georgians.
N = no mutation

Discussion

In the present study we describe the results of molecular analysis of 446 unrelated individuals suspected of having FMF and referred by general physicians and FMF experts. Traditionally, the diagnosis of FMF has been based on clinical manifestations and the physician's experience (1, 10, 12). Following the cloning of MEFV, the gene associated with this disease, genetic analysis of its mutations has become a useful adjunct for establishing or confirming the diagnosis of FMF (11). At first, it was believed that this new laboratory test would enable us to solve the problem of diagnosing FMF in cases of atypical presentation. Nevertheless, we realized that there still remain FMF patients in whom none of the known MEFV mutations can be detected. On the other hand, we have encountered individuals homozygous for an MEFV mutation (especially E148Q), who are asymptomatic (some of whom were found in the cohort of the multicase families) (13). Furthermore, we have encountered many cases where FMF patients bear only a single mutation. This problem is especially common among groups with a high frequency of carriers such as the currently screened population.

The present study confirms the previously documented close correlation between the presence of the M694V mutation and North African Jewish patients, the relatively low prevalence of FMF among Ashkenazi Jews, and the correlation of clinically active disease with the presence of two mutations (either homozygotes or compound heterozygotes) (13-15).

In addition, the following conclusions can be drawn from our findings. First, about half of the 446 individuals tested did not have FMF according to published criteria (10). This may suggest a relatively high index of suspicion for FMF by physicians in the community who are seeking quick confirmation or disproof of their diagnosis. Second, the frequency of heterozygotes among the healthy individuals referred for testing in the present cohort was similar to the carrier rate in the same ethnic group in the general population in Israel.

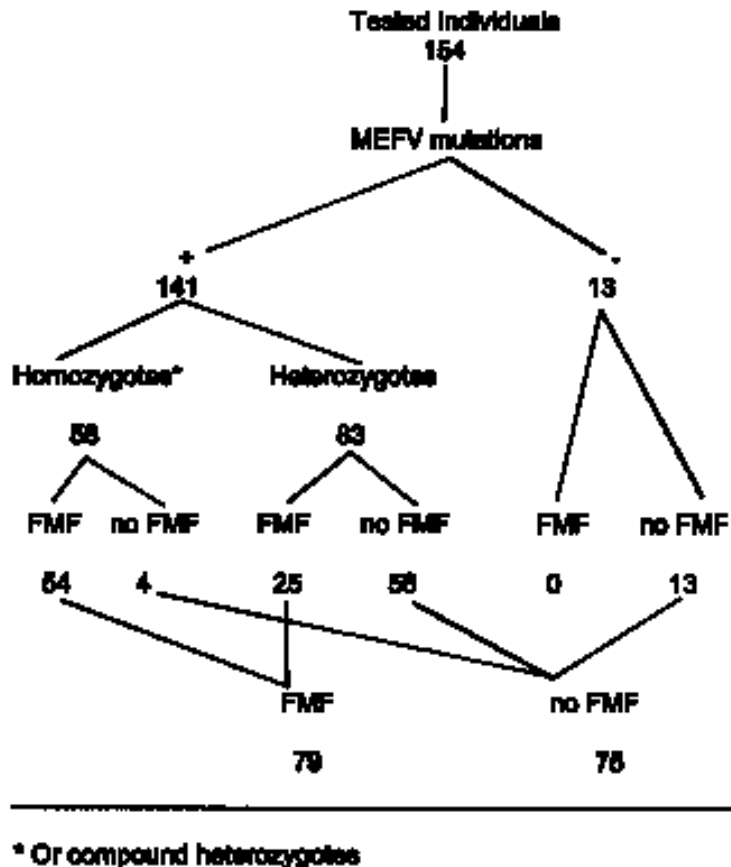


Fig. 2. A summary of the outcome of the 154 individuals.

The finding that almost 60% of the tested individuals of North African origin did not bear any mutation suggests that ethnicity served as a major factor in the decision to look for FMF mutations. It seems that in many of these cases a more careful clinical assessment would obviate the need for expensive genetic testing. On the other hand, 2 patients did present with clinical manifestation of FMF without bearing any of the five common mutations. A possible explanation is that they might bear one of the rare mutations not studied routinely in our laboratory. This possibility is unlikely, since the origin of both patients was North African, a group in which the main mutations are M694V and E148Q, for which they were tested. However, the possibility of genetic heterogeneity in FMF cannot be ruled out (16).

A notable, frequently encountered situation where genetic testing is not helpful is the case of the heterozygous patient. These individuals may either

have FMF or be asymptomatic carriers of the disease. In such cases, clinical assessment is most important and if the patient is symptomatic, a therapeutic trial with colchicine may be required to confirm or refute the diagnosis. In the present study, more than 34% of the FMF patients were either heterozygotes or did not bear any mutation, illustrating the limitation of genetic testing for diagnosis of FMF. Moreover, if we remember that the present study summarizes results from an FMF center located in a region with a high prevalence of this disease and where physicians have much experience of FMF, it seems that molecular testing does not add much to its diagnosis.

Regarding the results from the analysis of the multicase families, it is shown (as expected) that most of them bore MEFV mutations (Fig. 2). Eighty-three of them (56%) were heterozygotes of whom 30% had FMF clinically. An interesting finding was the detection of 4 individuals homozygous for E148Q

with no symptoms of FMF. In the cohort of 446 patients, 2 individuals were also homozygous for E148Q with obvious FMF disease, suggesting that this sequence by itself may not be sufficient to express the disease (13).

Two additional findings emerge from our data. One is the high variability of mutations among Palestinian Arab and Middle Eastern Jewish FMF patients, as compared with North African Jews. This may reflect a founder effect in the latter population and might suggest multiple sources and origins of the Arab and Iraqi Jewish populations. One may speculate about intermarriage between these populations or consider the possibility of a common origin prior to the Islamic period.

The second observation is the difference in the rate of confirmation of the FMF diagnosis by mutation analysis among individuals referred by general practitioners versus physicians running FMF or genetic clinics. The higher rate among those referred by the latter suggests that a second opinion by an FMF specialist might be useful before referring an individual for expensive genetic analysis.

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