Expanding the Panel of *MEFV* Mutations for Routine Testing of Patients with a Clinical Diagnosis of Familial Mediterranean Fever

Yael Kilim MSc, Nurit Magal PhD and Mordechai Shohat MD

Recanati Genetic Institute, Rabin Medical Center (Beilinson Campus), Petah Tikva, Israel

ABSTRACT: **Background:** Since the identification of the *MEFV* gene 198 mutations have been identified, not all of which are pathologic. The screening methods used in Israel to test patients suspected of having FMF include a kit that tests for the five main mutations (M694V, V726A, M680Ic/g, M694I, E148Q), and the sequencing of *MEFV* exon 10 in combination with restriction analysis for detecting additional mutations.

Objectives: To determine the contribution of testing for five additional mutations – A744S, K695R, M680Ic/t, R761H and P369S – to the molecular diagnosis of patients clinically suspected of having FMF.

Methods: A total of 1637 patients were tested for FMF mutations by sequencing exon 10 and performing restriction analysis for mutations E148Q and P369S.

Results: Nearly half the patients (812, 49.6%) did not have any detectable mutations, 581 (35.5%) had one mutation, 241 (14.7%) had two mutations, of whom 122 were homozygous and 119 compound heterozygous, and 3 had three mutations. Testing for the additional five mutations enabled us to identify 46 patients who would have been missed by the molecular diagnosis kit and 22 patients in whom only one mutation would have been found. Altogether, 4.3% of the patients would not have been diagnosed correctly had only the kit that tests for the five main mutations been used.

Conclusions: This study suggests that testing for the additional five mutations as well as the five main mutations in patients with a clinical presentation of FMF adds significantly to the molecular diagnosis of FMF in the Israeli population.

IMAJ 2011; 13: 206–208

KEY WORDS: Familial Mediterranean fever, *MEFV* gene, mutations, sequencing

Familial Mediterranean fever is an autoinflammatory disease mainly affecting certain populations living around the Mediterranean basin, especially Arabs, Jews, Armenians and Turks [1-3]. FMF is also found in other Mediterranean countries, including Cyprus, Italy and Spain [4-6]. The disease is characterized by recurrent self-limited attacks of fever accompanied by peritonitis, arthritis or pleuritis [1,7]. The most severe complication of FMF is progressive systemic amyloidosis that may lead to renal failure [8].

In 1997 the gene responsible for FMF (designated *MEFV* for MEditerranean FeVer) was identified on chromosome 16p13.3. *MEFV* consists of 10 exons encoding 781 amino acids, and the protein product has been named pyrin by the International FMF Consortium [9] and marenostrin by the French FMF Consortium [10]. To date, 198 mutations have been detected according to the Infevers Website (http://fmf.igh.cnrs.fr/ISSAID/infevers/), of which 84 have an associated phenotype (i.e., are pathologic), 82 may or may not have an associated phenotype (i.e., may or may not be pathologic), and 32 do not have an associated phenotype (i.e., are not pathologic). The majority of these are missense changes (http://www.hgmd.cf.ac.uk/ac/all.php). Five of the mutations – E148Q in exon 2 and M680I, M694I, M694V and V726A in exon 10 – account for a large percentage of FMF patients [11], even though in some studies only three mutations (M694V, V726A and E148Q) were tested [12].

In Israel, screening for FMF mutations is mostly carried out by two methods. The first is direct detection of up to five main mutations, as detailed above, using a kit or self-prepared restriction enzyme analysis. The second is by sequencing part of exon 10 and examining eight specific mutations. In our laboratory at the Rabin Medical Center, the genetic screening of FMF patients consists of a combination of sequencing 660 base pairs in exon 10 for the detection of eight mutations together with restriction analysis of mutation E148Q in exon 2. The mutation P369S is also tested in a subgroup of patients who underwent previous FMF testing for nine mutations, not including P369S, and in whom only one mutation was found.

In this study we analyzed the results of sequencing and performing restriction analysis in 1637 patients to determine the contribution of testing for an additional five mutations – R761H, A744S, K695R, M680Ic/t and P369S – to the molecular diagnosis of patients clinically suspected of having FMF.

FMF = familial Mediterranean fever

PATIENTS AND METHODS

During the years 2007–2009, 1637 patients visited the FMF clinic at Rabin Medical Center for a consultation for FMF. We offer *MEFV* genetic analysis to all patients; this includes sequencing of exon 10 and restriction analysis of the E148Q mutation in exon 2. In addition, restriction analysis of P369S mutation in exon 3 is also performed in a small subgroup of patients as described above.

MUTATION ANALYSIS

Genomic DNA was extracted using the Magna Pure LC 2.0 instrument (Roche). The E148Q and P369S mutations create restriction sites for the enzymes AvaI and AluI respectively. Genomic DNA was amplified using forward and reverse primers.

E148Q

5'-GGGTTCTGTTGCCGAGTC-3'
5'-GTGGGACAGCTTCATCATTTTG-3'

P369S

5'-TCCCCGAGGCAGTTTCTGGGCACC-3'
5'-TGGACCTGCTTCAGGTGGCGCTTA-3'

Polymerase chain reaction products were digested with the appropriate enzyme (AvaI and AluI) and electrophoresis was performed on a 4% Nusieve gel stained with ethidium bromide.

The mutations detected by performing sequencing of 660 base pairs in *MEFV* exon 10 are: M680I (c/g, c/t), M694V, M694I, K695R, V726A, A744S, and R761H.

Genomic DNA was amplified using forward and reverse primers:
5'-CCAGAAGAACTACCCTGTCCC-3'
5'-CAGTGTTGGGCATTCAGTCAG-3'.

Fluorescence sequencing was performed by using the reverse primer 5'-CAGTGTTGGGCATTCAGTCAG-3' with Big Dye Terminator version 1.1 on an ABI PRISM 3130 Genetic Analyzer (both from Applied Biosystems)

RESULTS

During the years 2007–2009, 1637 patients were screened for FMF mutations by the sequencing of exon 10 and by restriction analysis of the mutations E148Q and P369S in exons 2 and 3 respectively. Within this cohort of patients, 812 (49.6%) were found to have no detectable mutations and were referred back to their physicians; 581 patients (35.5%) were found to have one mutation and 241 patients (14.7%) had two mutations, of whom 122 (7.4%) were homozygous and 119 (7.2%) were compound heterozygous. Three patients (0.2%) were found to have three mutations [Table 1]. Altogether, 1076 mutations were detected; of these, 1004 (92%) consisted of the five main mutations: E148Q, M680I(c/g), M694I, M694V and V726A [Table 2]. The screening for the additional five mutations – R761H, A744S, K695R, M680I(c/t) and P369S – accounted for the detection of 72 (6.7%) extra mutations [Table 3].

Table 1. Number of FMF patients with detected mutations

No. of mutations	Total no. of patients (%)	No. of patients fully diagnosed only after testing for the 5 additional mutations
None	812 (49.6%)	–
One	581 (35.5%)	44
Two	241 (14.7%)	22 compound heterozygotes + 2 homozygotes
Three	3 (0.2%)	2

Table 2. Distribution of the five main mutations in FMF patients

Mutation	No. of chromosomes	%
M694V	554	55%
E148Q	218	21%
V726A	179	18%
M680I(c/g)	41	4%
M694I	12	2%
Total	1004	100%

Table 3. Distribution of the additional five mutations in FMF patients

Mutation	No. of chromosomes	%
A744S	36	50%
K695R	15	20.8%
M680I(c/t)	12	16.7%
R761H	1	1.4%
P369S	8	11.1%
Total	72	100%

The results of our analysis indicate that with our screening method we were able to detect 44 heterozygous and 2 homozygous patients who would not have been detected otherwise. In addition, 22 patients who would have been diagnosed as having only one mutation were found by our screening method to be compound heterozygotes.

DISCUSSION

Molecular diagnosis of FMF patients in Israel is carried out either by direct testing of the five mutations that are the most frequently detected in FMF patients (M694V, M694I, V726A, M680Ic/g and E148Q) or by *MEFV* exon 10 sequencing together with restriction analysis, which jointly

enable the detection of additional mutations that we at the Rabin Medical Center test and specifically report here on 10 (R761H, A744S, V726A, K695R, M694I, M694V, M680Ic/g, M680Ic/t, E148Q and P369S).

During the years 2007–2009 we tested samples from 1637 patients who were suspected of having FMF. We found that the combination of sequencing part of exon 10 together with restriction analysis of the mutations E148Q and P369S had an advantage over the testing method that is commonly used in Israel. By this method we detected mutations in 68 patients: 44 heterozygotes and 2 homozygotes who would not have been detected otherwise; in addition, 22 patients who would have been found to have only one mutation were found in our laboratory to be compound heterozygotes.

It should be noted that FMF is traditionally considered to be an autosomal recessive disease and we would therefore expect to detect two mutations in a patient with this condition. However, it has been observed that a large percentage of patients who are diagnosed clinically as suffering from FMF and respond well to colchicine treatment carry only one mutation [13].

Our sequencing data suggest that in some of these patients a second mutation can be found by testing for these additional five mutations. Another advantage of our sequencing method is the ability to detect the two forms of mutation M680I. Mutation M680I has been found to exist in two forms, one by the transition of c/g and the second by the transition of c/t [14]. We observed that in the Arab population in northern Israel both the c/g and the c/t forms of the mutation are detected. The c/t form cannot be detected by the diagnostic test that is commonly used, but is detected by our method, thereby expanding the percentage of patients who can be diagnosed molecularly.

To conclude, this study suggests that the testing of mutations R761H, A744S, K695R, M680I(c/t) and P369S, in addition to routine analysis in Israeli patients with a clinical presentation of FMF, markedly increases the likelihood of achieving a more accurate molecular diagnosis for this disease. Since performing these additional tests does not increase the cost of the molecular diagnosis, it should be considered as the method of choice by all laboratories that carry out molecular testing for FMF in Israel.

Acknowledgments

The authors thank Dr. Ora Keren and Dr. Gabrielle J. Halpern for their editorial assistance.

Corresponding author:
Dr. M. Shohat
Director, Recanati Genetic Institute, Rabin Medical Center (Beilinson Campus), Petah Tikva 49100, Israel
Phone: (972-3) 937-7659
Fax: (972-3) 937-7660
email: mshohat@post.tau.ac.il

References

1. Sohar E, Gafni J, Pras M, Heller H. Familial Mediterranean fever. A survey of 470 cases and review of the literature. *Am J Med* 1967; 43: 227-53.
2. Livneh A, Langevitz P, Zemer D, et al. Criteria for the diagnosis of familial Mediterranean fever. *Arthritis Rheum* 1997; 40: 1879-85.
3. Odabas AR, Cetinkaya R, Selcuk Y, Bilen H. Familial Mediterranean fever. *South Med J* 2002; 95: 1400-3.
4. Deltas CC, Mean R, Rossou E, et al. Familial Mediterranean fever (FMF) mutations occur frequently in the Greek-Cypriot population of Cyprus. *Genet Test* 2002; 6: 15-21.
5. La Regina M, Nucera G, Diaco M, et al. Familial Mediterranean fever is no longer a rare disease in Italy. *Eur J Hum Genet* 2003; 11: 50-6.
6. Aldea A, Calafell F, Aróstegui JI, et al. The west side story: MEFV haplotype in Spanish FMF patients and controls, and evidence of high LD and a recombination "hotspot" at the MEFV locus. *Hum Mutat* 2004; 23: 399.
7. Ben-Chetrit E, Levy M. Familial Mediterranean fever. *Lancet* 1998; 351: 659-64.
8. Zemer D, Livneh A, Danon YL, Pras M, Sohar E. Long-term colchicine treatment in children with familial Mediterranean fever. *Arthritis Rheum* 1991; 34: 973-7.
9. The International FMF Consortium. Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. *Cell* 1997; 90: 797-807.
10. The French FMF Consortium. A candidate gene for familial Mediterranean fever. *Nat Genet* 1997; 17: 25-31.
11. Touitou I. Standardized testing for mutations in familial Mediterranean fever. *Clin Chem* 2003; 49: 1781-2.
12. Zaks N, Shinar Y, Padeh S, et al. Analysis of the three most common *MEFV* mutations in 412 patients with familial Mediterranean fever. *IMAJ Isr Med Assoc J* 2003; 5: 585-8.
13. Booty MG, Chae JJ, Masters SL, et al. Familial Mediterranean fever with a single MEFV mutation: where is the second hit? *Arthritis Rheum* 2009; 60: 1851-61.
14. Aksentijevich I, Torosyan Y, Samuels J, et al. Mutation and haplotype studies of familial Mediterranean fever reveal new ancestral relationships and evidence for a high carrier frequency with reduced penetrance in the Ashkenazi Jewish population. *Am J Hum Genet* 1999; 64: 949-62.