Analysis of the Three Most Common *MEFV* Mutations in 412 Patients with Familial Mediterranean Fever

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Abstract

Background: Familial Mediterranean fever is an autosomal recessive disease characterized by recurrent attacks of fever and serositis. The disease is caused by mutations in the *MEFV* gene, presumed to act as a down-regulator of inflammation within the polymorphonuclear cells.

Objectives: To present the results of 412 FMF patients genotyped for three *MEFV* mutations, M694V, V726A and E148Q.

Results: The most frequent mutation, M694V, was detected in 47% of the carrier chromosomes. This mutation, especially common among North African Jewish FMF patients, was not found in any of the Ashkenazi (East European origin) patients. Overall, one of the three mutations was detected in 70% of the carrier chromosomes. M694V/M694V was the most common genotype (27%), followed by M694V/V726A (16%). The full genotype could be assessed in 57% of the patients, and one disease-causing mutation in an additional 26%. Only one patient with the E148Q/E148Q genotype was detected despite a high carrier rate for this mutation in the Jewish population, a finding consistent with a low penetrance of this genotype. The M694V/M694V genotype was observed in 15 patients with amyloidosis compared to 4 amyloidosis patients with other genotypes ($P < 0.0001$).

Conclusions: Because of low penetrance and as yet other undetermined reasons, mutation analysis of the most common *MEFV* mutations supports a clinical diagnosis in only about 60% of patients with definite FMF.

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Familial Mediterranean fever is an autosomal recessive disease characterized by recurrent episodes of fever accompanied by sterile peritonitis, pleuritis, arthritis, and a typical rash termed erysipelas-like erythema [1]. Renal amyloidosis type AA is the most devastating manifestation of the disease, and in the past was a major cause of morbidity and mortality among FMF patients [1]. In some patients, amyloidosis is the only manifestation of the disease (phenotype II). Prophylactic daily colchicine, a treatment modality introduced in the mid 1970s, has altered the natural history of this disease, preventing the occurrence of attacks and the development of amyloidosis [2,3]. FMF is highly prevalent among Middle Eastern populations, especially in North African Jews and Iraqi Jews [4], Armenians [5], Middle Eastern Arabs [6] and Anatolian Turks [7]. The disease is also found in Ashkenazi Jews (East European origin), although it is less common [8].

In 1997, using a positional cloning approach, two groups cloned the FMF gene (*MEFV*). The gene, which lies in chromosome 16p [9], is composed of 10 exons and encodes a 781 amino acid protein. This protein – named pyrin by the International FMF Consortium [10] and marinostrin by the French FMF Consortium [11] – is expressed in polymorphonuclear cells and in activated macrophages. Pyrin's mode of action is still unclear, but it is presumed to act as a down-regulator of inflammation. Thirty disease-associated mutations have been identified so far in *MEFV* [12].

Screening of a control population for *MEFV* mutations revealed an extremely high gene frequency among North African Jews, Iraqi and Ashkenazi Jews, with carrier rates of 20–40% [13–15]. This high carrier rate has highlighted the possibility of a selective advantage for FMF heterozygotes.

In the last 3 years we have routinely screened patients diagnosed with FMF for three of the most common mutations, M694V, V726A and E148Q. In this report we present the results of mutation analysis in 412 patients clinically diagnosed as suffering from definite FMF.

Materials and Methods

Patients and DNA samples

Since 1998, we offer *MEFV* genetic analysis to all patients arriving at our FMF clinic for diagnosis of FMF. This report includes the first 412 patients with definite FMF, diagnosed according to established criteria [16], who undertook this test. Three milliliters of blood were drawn from each patient and DNA was extracted according to standard procedures.

Mutation analysis

The E148Q, M694V and V726A mutations create restriction sites for the enzymes BSTN I, Hph I and Alu I, respectively. Three segments containing these sites were amplified using standard polymerase

FMF = familial Mediterranean fever

chain reaction procedure and the forward and reverse primers: 5'-GCCTGAAGACTCCAGACCACCCCG-3' and 5'-AGGCCCTCC-GAGGCCTTCTCTCTG-3', 5'-ACTCTGTCGCCAGAGAATGGCTACTGGGTGGAGATAAATG-3' and 5'-GTCAGGCCCCTGACCACCCACTGGACAG-3', 5'-ACC,CGCCTGCTAATAAAGGAGCCTCCCAAGCG-3' and 5'-GAAGATAGGTTGAAGGGGCCCAGAGAAAGAGCAGCTGAC-3', respectively.

PCR products were digested with the appropriate enzymes from the above, electrophoresed on a gradient (4–20%) polyacrylamide gel and stained with ethidium bromide.

Statistical analysis

Results were analyzed with the chi-square test.

Results

The ethnic composition of the patients analyzed is shown in Figure 1. The largest ethnic group was North African Jews, followed by Iraqi Jews. Most of the patients who appear under "Others" are of mixed origin; a minority comprises Arab and Druze. The results of the mutation analysis in the 824 carrier chromosomes are shown in Table 1. M694V was by far the most common mutation found in this cohort of patients, followed by V726A. Two mutations on the same carrier chromosome (complex allele) were found in seven carrier chromosomes (1%) of Ashkenazi and Druze patients. The distribution of genotypes in the 412 patients is shown in Table 2. The most common genotype was the M694V/M694V (27%), followed by M694V/V726A (16%). Despite the high carrier rate of the E148Q mutation in the control Jewish population, only one patient with the E148Q/E148Q genotype was detected. The full genotype could be assessed in 57% of the patients. In an additional 26% of the patients, one disease-causing mutation could be determined. Only in 16% of the patients were none of the three mutations identified. Table 3 stratifies the patients in whom the complete genotype could be determined according to their ethnic origin. The largest ethnic group screened for *MEFV* mutations was the North African Jewish patients (n=140). Among them, the M694V/M694V genotype was found in 50% of the patients and M694V/E148Q in an additional 10%. Overall, the complete genotype could be assessed in 63% of the North African Jewish patients. The V726A mutation was relatively rare among patients of this ethnic group. Among Iraqi Jews, the second largest ethnic group consisting of 43 patients, the M694V/V726A genotype was the most common (28%), followed by M694V/M694V (9%). The V726A/V726A was the most common genotype found in the 21 Ashkenazi Jewish patients (29%), followed by the E148Q-V726A/V726A genotype (14%). Interestingly, no M694V alleles were detected in this population group.

Of the 412 patients analyzed in this study 19 were diagnosed with amyloidosis. Fifteen amyloidosis patients were detected among 110 patients with the M694V/M694V genotype, compared to only 4 patients with other genotypes ($P < 0.0001$). Genotypes in these patients included M694V/V726A, M694V/Unknown, V726A-E148Q/V726A, and V726A-E148Q/ V726A-E148Q.

PCR = polymerase chain reaction

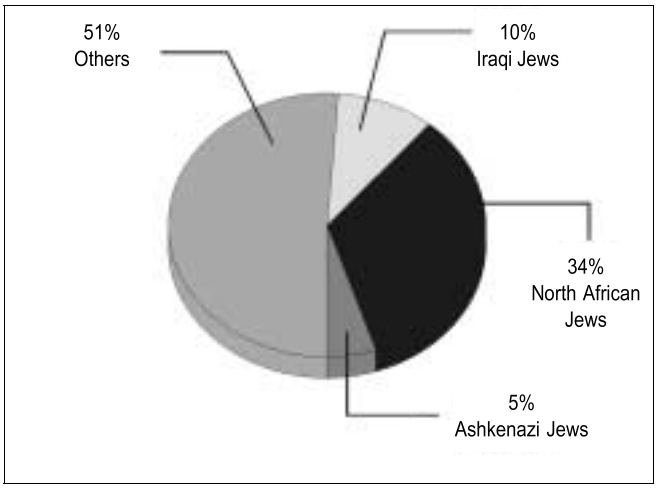

Figure 1. Ethnic distribution of the 412 FMF patients

Table 1. Distribution of MEFV mutations in 412 FMF patients

Mutation	No. of chromosomes	%
M694V	391	47%
V726A	122	15%
E148Q	58	7%
V726A-E148Q	7	1%
?	246	30%
Total	824	100%

Table 2. Genotype distribution in 412 FMF patients

Genotype	No. of patients	%	No. of mutations	No. of patients
M694V/M694V	110	27		
V726A/V726A	14	3		
E148Q/E148Q	1	0.2		
M694V/V726A	64	16		
M694V/E148Q	29	7	2	234
V726A/E148Q	10	2.4		
V726A-E148Q/V726A	6	1.5		
M694V/?	80	19		
V726A/?	18	4	1	110
E148Q/?	12	3		
?/?	68	16	0	68
Total	412	100	2, 1, or 0	412

? = undetermined

Table 3. Fully characterized genotypes stratified according to ethnic distribution

Genotype	North African Jews (n=140)		Iraqi Jews (n=43)		Ashkenazi Jews (n=21)	
	No.	%	No.	%	No.	%
M694V/M694V	69	50%	4	9%	0	0%
V726A/V726A	0	0%	2	5%	6	29%
E148Q/E148Q	0	0%	0	0%	0	0%
M694V/V726A	4	3%	12	28%	0	0%
M694V/E148Q	14	10%	1	2%	0	0%
V726A/E148Q	0	0%	1	2%	1	5%
V726-E148Q/V726A	0	0%	0	0%	3	14%
Total	87	63%	20	46%	10	48%

Discussion

In this report we present the largest series of mutation analysis in FMF patients. The patients were screened for three mutations, which account for 70% of the carrier chromosomes. M694V was the most common mutation found in this series, accounting for 47% of the carrier chromosomes. M694V/M694V, the most prevalent genotype, was especially common among the North African Jewish patients. This genotype has been previously linked to severe disease [17–21] and therefore it is not surprising to find it in the North African Jewish FMF population, which suffers from a severe disease. Conversely, the M694V mutation is very rare in Ashkenazi FMF patients [21,22], contributing to a milder phenotype in this population.

The ethnic distribution of the patients is highly reflective of the prevalence of FMF in the Israeli population, common in North African and Iraqi Jews, and rare in Ashkenazi Jews. This distribution is in striking contrast to the distribution of *MEFV* mutations found in normal controls. Recent studies have shown *MEFV* carrier rates of 20–40% in North African, Iraqi and Ashkenazi Jews [13–15,22]. The low prevalence of FMF in Ashkenazi Jews can be explained by the low penetrance of most of the mutations common in this ethnic group. For example, the carrier rate for E148Q in Ashkenazi controls was 7–12% [13–15,22], yet in this study no Ashkenazi patients with the E148Q/E148Q genotype were detected, a finding consistent with the low penetrance of this mutation, or with the suggestion that E148Q is a single nucleotide polymorphism and not a disease-causing mutation [23]. Of note, however, is the finding that patients with the E148Q/E148Q genotype do exist and actually express a severe phenotype [24], and that a combination of E148Q with other mutations can cause symptomatic disease, as indicated by the 29 patients bearing the M694V/E148Q genotype. Sometimes E148Q can appear on the same chromosome with another mutation, V726A. This rare allele, associated with a more severe phenotype and a relatively high penetrance [21,22,25], was found in Ashkenazi and in Druze patients. Previously, we found another rare mutation common to Ashkenazi Jews and Druze in *SLC3A1*, a gene associated with cystinuria [26]. These common mutations raise the possibility of a common ancestor to the two populations.

Fifteen of the 19 patients with amyloidosis were M694V homozygotes, confirming the association previously shown between this genotype and amyloidosis [18–20]. The finding of four other patients with amyloidosis emphasizes the fact that this dreadful complication can potentially develop with almost any genotype. The only known factor that can prevent amyloidosis is daily prophylactic colchicine, and patients with the M694V/M694V genotype are therefore strongly encouraged to adhere to the treatment protocol.

The finding in our study of FMF patients who carry only one or none of the common mutations is probably mainly related to the limited genetic evaluation conducted, which includes only 3 of the 30 mutations already identified. Yet, a thorough genotyping, using sequence analysis, of the whole *MEFV* still leaves us with a large population (15–20%) of FMF patients with unmutated *MEFV* or with only one mutated allele. This suggests that other genes may be involved in FMF expression.

A third population, not included in the study but which does deserve mentioning, consists of patients who carry two FMF alleles yet do not express FMF manifestations (phenotype III) [15]. Based on the 20–40% carrier rate and direct screening of the Israeli population for double *MEFV* mutations, this forms the largest FMF group in Israel, estimated at around 50,000 individuals. For comparison, the number of patients with overt FMF is around 10,000. Since the results of the present study reflect only symptomatic patients, the subsequent diagnostic, clinical, therapeutic and epidemiologic conclusions may be misleading.

The exact role of molecular analysis in the diagnosis of FMF remains to be established, even in the symptomatic patient group [27]. Genetic testing will probably turn out to be most effective in patients with an uncertain diagnosis, where two mutations in *MEFV* would highly support the diagnosis of FMF [27]. In patients with typical symptoms and a good response to colchicine, genetic testing may confirm the diagnosis, but a negative result will not change the clinical decision and management [27].

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