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Spectrum of mutations and carrier frequency of familial Mediterranean fever gene in the Algerian population

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Abstract

Objectives. FMF is characterized by recurrent self-limiting episodes of fever and painful polyserositis. We aimed to study the spectrum and distribution of *MEFV* mutations in an Algerian patient cohort using a comprehensive mutation detection method. Using the same methodology, we also studied the carrier rate in an unaffected ethnically matched control cohort.

Methods. We recruited 71 unrelated subjects clinically diagnosed with FMF from various clinics in the central region of Algeria. Two hundred and thirty control subjects were recruited as well. Mutation detection in *MEFV* was performed by re-sequencing the promoter region, the entire coding sequence and all exon–intron boundaries.

Results. We detected eight different mutations located in exons 10 (p.M694I, p.M694V, p.A744S, p.M680I, p.I692Del), 9 (p.I591T), 3 (p.P369S/p.R408Q) and 2 (p.E148Q). Out of the 71 patients, 31 carried at least one mutation. While the 71 patients are expected to have 142 mutant chromosomes, only 50 were identified. p.M694I (17.6%) is the most common mutation, followed by p.M694V (5%), p.E148Q (4.2%), p.A744S (3.5%) and p.M680I (3%). One novel variant was identified in the promoter region in the heterozygous state in three patients and in two controls. The carrier rate of the identifiable mutations is estimated to be 1 : 5.

Conclusion. This study describes the *MEFV* mutational spectrum and distribution in the Algerian population. It shows that p.M694I is the most common *MEFV* mutation in Algerians. It also shows that, similar to other Arabic populations, <50% of mutant chromosomes are identified, even when employing comprehensive strategies.

Key words: Familial Mediterranean fever, Algeria, Carrier rate, Mutation, Amyloidosis, Autoinflammatory.

Introduction

Autoinflammatory diseases are a group of disorders characterized by systemic inflammation without high-titre autoantibodies or antigen-specific T cells and are thought to be due to dysregulation of the innate immunity [1, 2] and include the hereditary periodic fever syndromes. A number of the autoinflammatory diseases are single gene disorders that are clinically characterized by recurrent or persistent systemic inflammation such as fever and elevation of the acute-phase reactants, and organ-specific manifestations such as rashes, osteoarticular, serosal, neurological and ocular manifestations [1–3]. FMF is the archetype of the hereditary periodic fever syndromes and the autoinflammatory diseases. It is characterized by recurrent self-limiting episodes of fever and painful polyserositis and was first described as a distinct disease entity, under the name benign paroxysmal peritonitis in 1945 [4]. FMF is an autosomal recessive disorder [5], with

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considerable prevalence in specific ethnic groups, namely, non-Ashkenazi Jews, Armenians, Turks and Arabs.

The classic clinical picture consists of recurrent febrile episodes that are usually of acute onset, variable frequency, sometimes without a recognized triggering factor but often occurring with menstruation, emotional stress or strenuous physical activity [6]. The episodes are often accompanied by pain due to peritonitis, pleuritis or acute synovitis of large joints. The frequency of the attacks varies and may be associated with long periods of remission. Over the course of the life-long illness, an affected individual will probably experience several forms of the febrile and painful episodes [7]. During the attack there is neutrophilia and a brisk acute-phase reactant response, and histologically there is a massive sterile influx of polymorphonuclear leucocytes (PMNs) into the affected site [7]. The episodes start, most commonly during childhood, with >80% of patients presenting before the age of 20 years and a very few after the age of 40 years [7–9]. The impact of FMF on patients is determined mainly by the presence or absence of its most deleterious complication, amyloidosis [10]. However, the burden of the febrile and painful episodes as manifested in loss of school or work days, repetitive suffering and unnecessary hospitalization and surgery is also substantial. A daily regimen of 1–2 mg of oral colchicine remains the recommended treatment since its introduction in 1972 [11–13]. Adherence to a daily dose of colchicine produces significant decrease in the frequency and severity of the attacks or even cessation of the attacks all together in ~95% of FMF patients [14]. Continuous prophylactic treatment with colchicine in FMF patients inhibits the development of amyloidosis [15], even in non-responders [16].

The gene responsible for FMF, *MEFV*, is located on the short arm of human chromosome 16, and was independently identified by two positional cloning consortia [17, 18]. With the cloning of the gene, four missense mutations in exon 10, namely M694V, V726A, M694I and M680I, were identified [17, 18]. These four mutations and E148Q in exon 2 are the most common *MEFV* mutations among the 59 putative mutations identified to date and are believed to be associated with the disease [19–21]. Exon 10 remains the major site of mutations, with a smaller cluster in exon 2 (available at http://fmf.igh.cnrs.fr/infevers). The FMF carrier rate can be as high as 1 in 3 in the commonly affected ethnic groups, raising the possibility of selective heterozygote advantage [21–26].

There is a paucity of *MEFV* mutational studies in the North African population, and in particular Algeria [27–29]. We report here on the distribution of *MEFV* mutations in Algerian FMF patients, as well as the carrier rate, and also report on a new variant identified in the *MEFV* promoter region.

Subjects, materials and methods

Study subjects

Patients and controls were recruited from the central region of Algeria during 2008 and 2009. Seventy-one unrelated subjects (35 males and 36 females) clinically diagnosed with FMF were recruited from various hospitals and private paediatric clinics after referral by a physician. The diagnosis was based on clinical findings of classical FMF symptoms such as recurrent fever, recurrent abdominal pain, recurrent chest pain and joint involvement. All patients were evaluated for amyloidosis. Despite the absence of rigid consensus among the referring physicians, none of the patients had an atypical form of FMF or the diagnosis was doubtful. Referring physicians collected data, including demographic data, clinical manifestations (age of onset of disease, attack duration, fever, abdominal pain, arthritis, chest pain, erysipelas-like erythema and amyloidosis), detailed family history of FMF and treatment with colchicine. Written informed consent was obtained from all patients or their legal guardians, according to the standards of the Declaration of Helsinki and conforming to the ethical standards currently employed in Algeria. This study was ethically approved by the Agence Nationale pour le développement de la recherche en santé (ANDRS; National Agency for the Development of Health Research), which uses the standards and recommendations of the Declaration of Helsinki. Two hundred and thirty control subjects were recruited from blood transfusion centres in Algiers, Algeria.

Methods

Genomic DNA was extracted from 2–5 ml of peripheral blood samples and stored at −20°C until use. Mutation detection in *MEFV* was performed by re-sequencing the promoter region (~500 bp upstream of the ATG translation start site), the entire coding sequence and all exon–intron boundaries (covering ~50 bp of the exon flanking intronic sequences) in forward and reverse directions after PCR amplification. Amplified products were purified and the sequencing was performed by BigDye chemistry on an ABI Genetic Analyser (3730xl). In controls, the re-sequencing was performed only for amplicons in which variations were detected in the patient cohort (promoter region and exons 1, 2, 3, 9 and 10) and in one direction. The sequencing was repeated for any amplicon in both directions if either the forward or reverse sequence was not clear, until a 100% success rate was obtained. Sequences were analysed by SeqScape 2.5 software (Applied Biosystems, Foster City, CA, USA). The sequences were read by two independent observers and a consensus between the two was achieved for 20% of the reads, chosen at random.

Statistical analysis

For the statistical analysis, the means were compared by the Student's *t*-test and the results were analysed with Statistica 5.1 software (StatSoft Inc., Tulsa, OK, USA). Differences between the means of patients and controls were considered significant when $P < 0.05$. The reported *P*-values are uncorrected.

Results

Patients

The mutation detection protocol revealed in patients, eight different previously reported mutations located in exons 10 (p.M694I, p.M694V, p.A744S, p.M680I, p.I692Del), 9 (p.I591T), 3 (p.P369S/p.R408Q) and 2 (p.E148Q). Out of the 71 patients, 31 (43.66%) carried at least one mutation (15 males and 16 females). While the 71 patients are expected to carry mutant chromosomes, only 50 were identified. Table 1 summarizes the mutation allele frequencies and distribution. The mutation p.M694I (17.6%) is the most common mutation identified in the Algerian population, followed by p.M694V (5%), p.E148Q (4.2%), p.A744S (3.5%) and p.M680I (3%). Other mutations such as p.I692Del, p.P369S/p.R408Q and p.I591T were present at an allele frequency <1%. Eight patients were homozygous, 11 were compound heterozygous and 12 carried only one identifiable mutation. The genotypes of all patients in whom at least one mutation was identified are shown in Table 2. One new variant [single nucleotide polymorphism (SNP)] was identified in the promoter region (c.-170 G > A) in the heterozygous state in three (4.2%) patients and in two (0.87%) controls ($P = 2 \times 10^{-2}$).

The 31 patients who carry at least one mutation have classical clinical symptoms, with fever lasting from few hours to 1 week, which was not different from the 40 patients in whom no mutations were identified. Abdominal pain was sometimes accompanied by vomiting, constipation/diarrhoea or mesenteric lymphadenopathy. In two patients, renal biopsy showed amyloidosis. The clinical picture in the 31 patients is summarized in Table 3 and contrasted with the clinical picture in 40 patients.

Controls

In controls, 44 (19.13%) of the 230 carried one mutation (Table 1). The overall mutant allele frequency in controls was 9.6% (44/460), translating to an Algerian population carrier frequency of about one in five. If we exclude p.E148Q and p.P369S, variants of uncertain clinical significance, then the mutant allele frequency would be 4.4% (19/460), translating to an Algerian population carrier frequency of about 1 in 12. The variant allele frequency in controls conforms with the Hardy–Weinberg equilibrium.

Discussion

To our knowledge, this is the first comprehensive study of FMF in the Algerian population that examines the spectrum and distribution of *MEFV* mutations among affected individuals, as well as a relatively large control cohort. A previous study included 85 Algerian patients living in France among other North African patients, but was limited in mutation detection methods and the control population [27]. This study identified only 35% of mutant alleles in the patient cohort. While 27% of the patient cohort carried two mutations (homozygous or compound heterozygous) and ~17% carried only one mutation, ~19% of the control cohort carried one mutation (carriers).

Table 2 Genotypes of the patient cohorts

Status	Genotype	*n* (%)
Homozygous	WT/WT	40 (56)
	M694I/M694I	7 (9.8)
	M6801/M680I	1 (1.4)
Compound heterozygous	M694I/M694V	4 (5.6)
	M694I/M680I	2 (2.8)
	A744S/E148Q	1 (1.4)
	E148Q; A744S/E148Q	1 (1.4)
	E148Q; I692del/M694I	1 (1.4)
	E148Q/M694V	1 (1.4)
	M694I/I591T	1 (1.4)
Heterozygous	E148Q	3 (4.2)
	A744S	3 (4.2)
	M694V	2 (2.8)
	M694I	3 (4.2)
	P369S;R408Q	1 (1.4)
Total		71 (100)

Table 1 Allele number, frequency and distribution in patients and controls for previously described mutations

		Patients		Controls		$\alpha = 0.05$
	Mutation	*n* (%)	Frequency ($n = 142$), %	*n* (%)	Frequency ($n = 460$), %	*P*-values
1	M694I	25 (50)	17.5	8 (18.1)	1.75	2×10^{-4}
2	M694V	7 (14)	5	3 (6.8)	0.65	2×10^{-4}
3	E148Q	6 (12)	4.2	18 (40.9)	4	0.45
4	A744S	5 (10)	3.5	6 (13.5)	1.3	8×10^{-3}
5	M680I	4 (8)	3	1 (2.2)	0.21	9×10^{-4}
6	I692del	1 (2)	0.7	0	0	10^{-2}
7	I591T	1 (2)	0.7	1 (2.2)	0.21	1.6×10^{-2}
8	P369S	1 (2)	0.7	7 (16)	1.52	0.22
	Total	50 (100)	35	44 (100)	9.6	

Table 3 Comparison between the phenotypic features of FMF patients with at least one mutation ($n = 31$) and those without mutations ($n = 40$)

Clinical symptoms	One mutation, frequency, %	No mutations, frequency, %
Male : female	15 : 16	22 : 18
Fever, %	97	91.5
Age at onset, years	1–20	1–11
Attack duration	A few hours to 1 week	2 days to 2 weeks
Abdominal pain, %	85	80
Arthritis, %	59	64
Chest pain, %	26	8.5
Amyloidosis, %	8	Not observed
Headache, %	1	4.5
Erysiplas-like erythema	Not observed	Not observed
Consanguinity, %	41	33
Family history of FMF, %	61	24

The most common mutant allele in our study is p.M694I, which is consistent with the previous study [27]. However, it only accounts for 50% of identified mutations, as compared with 80% in the previous study. This mutation is responsible for 37 and 25% of the mutations in Morrocan and Tunisian patients, respectively, and is the second most common mutation after p.M694V [27, 28]. On the other hand, p.M694V is the second most common mutation in our study, accounting for ~14% of alleles, in contrast to only 5% in the previous study [27]. The mutation p.M694V is a common mutation in Arabic patients [30–32], Turkish patients [25] and North African Jews [22]. The third, fourth and fifth common mutations in our study were p.E148Q (12%), p.A744S (10%) and p.M680I (8%), respectively. Interestingly, p.V726A was absent in both the patient and control cohorts in our study. Thus, this study confirms that p.M694I is the most common *MEFV* mutation in the Algerian population, but shows a different spectrum and distribution than previously reported [27]. Similar to other Arabic studies, >50% of the mutant chromosomes remain elusive.

Genotypically, the 71 patients were divided into three groups: homozygous or compound heterozygous patients (19), heterozyous patients (12) and patients with no identifiable mutations (40). The clinical picture is fairly similar among the three groups. Within the heterozygous group, three patients carry only p.E148Q and one patient carries p.P369S. All four presented the classical FMF phenotype, probably due to the presence of other unidentifiable mutations or the effect of modifier genes. The indistinguishable picture among the three groups probably reflects that there are other mutations that are unidentifiable by the current methodologies or there are other modifier genes or environmental factors that influence the penetrance of the clinical symptoms.

The *MEFV* mutation carrier frequency in our study is about 1 : 5 (20%), which is very different from the previously reported figure of 1% [27]. This carrier frequency is somewhat similar to what has been reported from other Arabic countries [33]. The frequency of each mutation among the control cohort is different from the patient cohort, with p.E148Q (40%) being the most common, followed by p.M694I (18%), p.P369S (16%), p.A744S (13.5%) and p.M694V (7%). The p.R202Q variant was reported with the same frequency in the patient and control cohorts (results not shown), which confirms its role as a polymorphism rather than a disease-causing mutation [20].

We identified one variant (c.−170G>A) in the promoter region in 3 (4.2%) of the 71 patients and in 2 (0.87 %) of the 230 controls. While these proportions are statistically significant ($P = 2 \times 10^{-2}$), it is difficult to assess the clinical significance without functional studies. Further studies are necessary to calculate the allele frequency in other populations.

In conclusion, our study describes the *MEFV* mutational spectrum and distribution in the Algerian population by studying FMF patients and controls and employing a comprehensive mutation detection strategy. The study confirms that M694I is the most common *MEFV* mutation in Algerians. It also shows that, similar to other Arabic populations, <50% of mutant alleles are identified, even when employing comprehensive strategies. Further studies are needed to identify other mutational mechanisms or genes that play a role in the FMF phenotype in the Arabs.

Rheumatology key messages

- The *MEFV* mutational profile is unique in Algerians and different from North Africans.
- The mutation M694I is most common in Algerians and the carrier rate is about 1 : 5.
- In Algerians, <50% of *MEFV* mutant alleles are identified by the current methodology.

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