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Research Article

FAMILIAL MEDITERRANEAN FEVER MUTATION FREQUENCIES AND CARRIER RATES AMONG SYRIAN POPULATION

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ABSTRACT

Objective: The main objective is to determine the prevalence of MEFV gene mutations in apparently healthy Syrian Population and the mutation frequency in the clinically diagnosed FMF patients.

Subjects and methods: the study included 376 samples (254 healthy individuals and 122 FMF patients). FMF strip assay was used to detect 12 MEFV gene mutations.

Results: We found a high frequency of carriers in the apparently healthy Syrian population (18.1%). The distribution of the MEFV mutations among apparently healthy individuals group was: M694V (8.3%), M680I(G/C) (8.3%), V726A (16.6%), M694I (4.2%) and E148Q (58.4%), F479L (4.2%). Whereas the distribution among FMF patients was: M694V (44.8%), M680I (G/C) (16.2%), V726A (11.1%), M694I (4.9%) and E148Q (16.7%), A744S (3.8%), P369S (2.5%). There was a statistical significant difference between the two groups (P<0.005)

Conclusion: MEFV gene mutations are common among apparently healthy Syrian population. E148Q was the most common mutation among apparently healthy Syrian population, whereas M694V was the most common mutation among FMF patients.

Keywords: FMF, MEFV gene mutations, FMF strip assay.

INTRODUCTION

Familial Mediterranean Fever (FMF) is an autosomal recessive inflammatory disorder predominantly affecting people in areas around the Mediterranean Sea, mainly non-Ashkenazi Jews, Turks, and Arabs [1]. It is the most common of a rare group of disorders collectively termed familial hereditary periodic fever syndromes [2]. The commonest manifestation is a self-limited attack of fever with peritonitis that may resemble acute abdomen and may necessitate surgical intervention [3]. Amyloidosis due to chronic inflammation progressing to renal failure is one of the most serious potential complications of this disease that may develop without overt crises [4]. The International FMF Consortium [5] and the French FMF Consortium independently identified Mediterranean fever (MEFV) gene as a candidate gene defective in FMF. This gene is located on chromosome 16p13.3 and encodes a protein named pyrin or marenostrin that blunts neutrophil-mediated inflammation [6].

Colchicine, a neutrophil suppressive agent, when administered on a long-term basis causes a decrease in both frequency and severity of attacks of FMF. Moreover, colchicine prevents and ameliorates amyloidosis. [7-8]

The most frequent mutations (M680I, M694V, V726A, M694I and E148Q) are found in more than two thirds of cases [9-10]

Several studies have shown a carrier rate ranging from one in 3 to one in 5 in the major ethnicities affected by the disease. [11-12]

The frequency and distribution of MEFV mutations among Syrians have not been adequately studied. In this study, we aim to find the spectrum of mutations in FMF patients, and to find the carrier rates for the 12 MEFV mutations in Syrian populations.

MATERIAS AND METHODS

From January 2010 to December 2012, the study involved 122 Syrian patients 72 males (59%) and 50 females (41%) from different governorates, The diagnosis of FMF in these patients was made according to international criteria [13]. and 254 apparently healthy individuals 152 males (59.8%) and 102 females (40.2%) who were known not to have any consanguinity.

12 mutations were tested by FMF strip assay using kit (ViennaLab Labordiagnostika). The 12 mutations located in exons 2 (E148Q), 3 (P369S), 5 (F479L), and 10]M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S, and R761H] can be simultaneously screened for by a reverse hybridization procedure.The assay includes three successive steps:[14]

1-DNA extraction of whole blood (EDTA).

2-PCR amplification using biotinylated primers. Amplifications were conducted on Roche Light Cycler which is available in the research laboratory at the Faculty of Medicine in Aleppo University.

3-Hybridization of amplification products to a test strip containing alleles -specific immobilized oligonucleotide probes. The bound biotinylated sequences can be detected by streptavidin–alkaline phosphatase and color substrate.

The determination of the genotype of the sample is made by using gradient ruler comprising a staining control, 12 mutant lines, and 8 corresponding wild-type lines. (figure1)

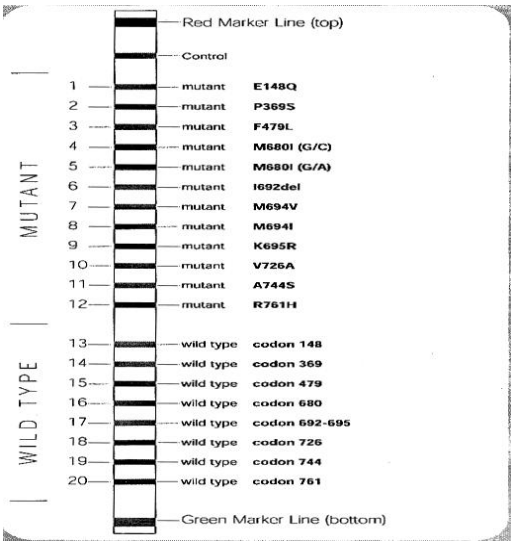


Fig. 1: It shows the gradient ruler

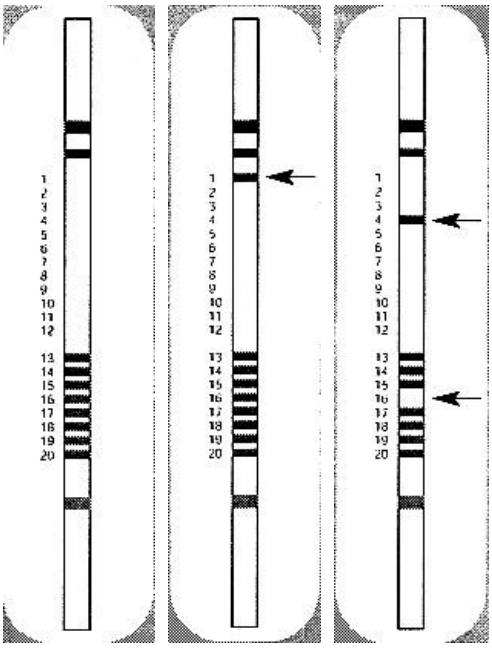
After fixing the sample strip on the gradient ruler correctly the results were read as follows:

• The normal: the presence of only the wild-type lines corresponds to the absence of the 12 mutant lines. (figure 2.A)

• Heterozygous genotypes: the presence of one mutant lines associated with presence of the wild-type lines (i.e. the mutant is present in one allele) (figure 2.B)

• Homozygous mutant: the presence of mutant lines associated with disappearance of the corresponding wild-type lines (i.e. the mutant is present in the two alleles). In this case the person is a patient of FMF.(figure 2.C)

• Compound Heterozygous: the presence of two mutant lines.



A **B** **C**

Fig. 2: it shows different genotypes (A) the normal (B) heterozygous genotypes (C) homozygous mutant

The comparison of the distribution of the MEFV mutations between FMF patients and FMF carriers was done by the χ^2 test.

RESULTS

1- The FMF patients:

1-1- The study of mutations distribution among FMF patients: (table1)

Table 1: Shows the mutations distribution among FMF patients

The mutation	Number of mutations	Frequency of alleles
E148Q	27	11
P369S	4	1.64
F479L	0	0
M680I(G/C)	26	10.65
M680I(G/A)	0	0
I692del	0	0
M694V	72	29.5
M694I	8	3.27
K695R	0	0
V726A	18	7.38
A744S	6	2.45
R761H	0	0
Total mutations	161	65.99
Total alleles	244	

1-2- The study of the genotypes among FMF patients: (table2)

Table 2: shows the genotypes among FMF patients

The genotype	Number of patients (total 122)	Frequency of genotype
E148Q / A744S	1	0.8
E148Q / M694V	4	3.2
E148Q / P369S / M681(G/C)	2	1.6
E148Q / M694V / V726A	2	1.6
M680I(G/C) / M694I	2	1.6
M680I(G/C) / M694V	6	4.9
M694V / M694I	2	1.6
M694V / V726A	6	4.9
M680I(G/C) / M680I(G/C)	4	3.2
M694I / M694I	2	1.6
M694V / M694V	20	16.4
A744S / NK*	5	4
E148Q / NK*	18	14.7
M681(G/C) / NK*	8	6.5
M694V / NK*	12	9.8
p369S / NK*	2	1.6
V726A / NK*	10	8.2
NK* / NK* (Wild Type)	16	13.1

*NK: Not Known

2- The apparently healthy individuals:

2-2-the study of mutations distribution among apparently healthy individuals :(table3)

Table 3: Shows the mutations distribution among healthy individuals

The mutation	Number of mutations	Frequency of alleles	Frequency of carrier
E148Q	28	5.5	11
P369S	0	0	0
F479L	2	0.39	0.7
M680I(G/C)	4	0.78	1.5
M680I(G/A)	0	0	0
I692del	0	0	0
M694V	4	0.78	1.5
M694I	2	0.39	0.7
K695R	0	0	0
V726A	8	1.57	3.1
A744S	0	0	0
R761H	0	0	0
Total mutations	48	9.4	18.1
Total alleles	508	-	-

In the apparently healthy population group (n=254) used for the carrier screening study we found that all the carriers were heterozygous except two individuals were compound heterozygous E148Q / M680I(G/C) and none of them had any symptoms.

DISCUSSION

In the apparently healthy individuals group the prevalence of MEFV mutations using FMF strip assay was 18.1%. The most common mutation was E148Q (5.5 %), Followed in frequency were the mutations V726A (1.57%), [M680I(G/C), M694V] (0.78%), and [M694I, F479L] (0.39%). Whereas the other mutations couldn't be detected among this group. All the carrier were heterozygous except two individuals were compound heterozygous E148Q / M680I(G/C) and none of them had any symptoms.

On the other hand, in the FMF patients group the number of the mutations was 161 and the frequency of the alleles was 65.99%.The most common mutation was M694V (29.5%), followed in frequency were the mutations E148Q (11%), M681 (G/C) (10.65%), V726A (7.38%), M694I (3.27%), A744S (2.45%), and P369S (1.61%),whereas the other mutations couldn't be detected among this group.

For comparison of the distribution of the MEFVgene mutations between FMF patients and FMF carrier we had the following table (table 4).

Table 4 compares the distribution of the different mutations between healthy carriers and FMF patients (P < 0.05). The commonest mutation in patients, M694V, was found in 44.8 % of all patients, but in only 8.3% of carriers. In contrast, mutation E148Q was very frequent among healthy carriers 58.4%, but was found in only 16.7% of FMF patients. This suggests that this mutation may be associated with a high non-penetrance rate.

Previous reports indicated that, individuals who were homozygous or compound heterozygous for E148Q may lack clinical features[15].

Mutations in both alleles were identified in 51(42.9%) patients, 26 (21.4%) were homozygous for the same mutation, 25 (20.5%) were compound heterozygous for different combinations of the mutations. 55(45%) patients were found to be heterozygous for one mutation. In 16 (13.1%) patients no mutation of the 12 mutations detected with the assay could be detected (Wild Type).

The most common genotype was M694V / M694V (16.4%), following in frequency were the genotypes]M694V / V726A, M680I(G/C) / M694V](4.9%),] E148Q / M694V, M680I(G/C) / M680I(G/C)](3.2%),] E148Q/P369S/ M681(G/C), E148Q /M694V/ V726A, M694I / M694I, M680I(G/C) / M694I](1.6%), and E148Q / A744S (0.8%). As we notice there were four patients who had compound alleles, two of them had the genotype E148Q / P369S / M681 (G/C), and the others had the genotype E148Q / M694V / V726A.Anyway, we can't determine the two mutations that exist in the same allele without studying the parents.

Finally, a high carrier rate of familial Mediterranean fever mutations was observed in this study. It is highly recommended that every doctor should think about FMF before abdominal surgery is done. Further studies are needed to define other mutations in the MEFV gene of the FMF patients who didn't have mutations using FMF strip assay.

Table 4: Shows the comparison of the distribution of the MEFV gene mutations between FMF patients and FMF carrier

The mutation	The frequency of mutations according to total mutations	
	FMF patients	**FMF carrier**
E148Q	27/161 (16.7%)	28/48 (58.4%)
P369S	4/161 (2.5%)	0
F479L	0	2/48 (4.2%)
M680I(G/C)	26/161 (16.2%)	4/48 (8.3%)
M680I(G/A)	0	0
I692del	0	0
M694V	72/161 (44.8%)	4/48 (8.3%)
M694I	8/161 (4.9%)	2/48 (4.2%)
K695R	0	0
V726A	18/161 (11.1%)	8/48 (16.6%)
A744S	6/161 (3.8%)	0
R761H	0	0
Total alleles	161	48

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