

# Genetic Analysis of Southwestern Iranian Patients with Familial Mediterranean Fever

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## Abstract

**Background:** Familial Mediterranean fever (FMF) is an autosomal recessive genetic disorder characterized by recurrent episodes of self-limited fever and serosal tissues inflammation.

**Methods:** To evaluate clinical symptoms and common genetic mutations in southwestern Iranian patients with FMF, 20 unrelated patients were enrolled in this study based on clinical criteria. A panel of 12 common *MEFV* gene mutations was tested.

**Results:** The most frequent clinical presentations of the patients were fever, colicky abdominal pain and arthritis. Eighteen patients responded completely to colchicine therapy.

*MEFV* gene mutations were detected in only 40% of the patients. The most common mutation was E148Q, detected in five patients (25%). The V726A, M694V and P369S mutations were each observed in one patient.

**Conclusions:** Although none of the 12 mutations we included in our test panel was detected in 60% of our patients, all of them had FMF symptoms and responded well to colchicine. *MEFV* full gene sequencing analysis in these patients may lead to finding new mutations in southwestern Iranian FMF patients which would be helpful in designing a local diagnostic kit.

**Keywords:** Familial Mediterranean fever, *MEFV* gene mutations

## Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive genetic disorder caused by mutations in the *MEFV* gene. The clinical symptoms of this disorder are recurrent and self limited episodes of fever accompanied by short attacks of serosal inflammation (1). The *MEFV* gene on chromosome 16p13.3 contains ten exons encoding a protein named pyrin, which regulates the inflammatory responses (2). Mutations in the *MEFV* gene interfere with the normal role of pyrin, which is responsible for the biosynthesis of a chemotactic factor inactivator; this interference triggers an inflammatory reaction (3). Among more than 150 identified *MEFV* mutations, the most common mutations accumulate in exon 10 and exon 2 (4). Generally, the diagnosis of FMF is based on

Tel Hashomer clinical criteria and the response to colchicine (5), and in fact 80% of the patients carry one or more *MEFV* gene mutations (6). Because the variability in the clinical presentation of FMF makes the diagnosis uncertain, *MEFV* genotyping remains the only reliable diagnostic tool.

Although Armenians, Arabs, Jews and Turks are considered the classically affected populations, FMF is currently distributed worldwide because of widespread migration (3, 6). Different populations display diverse patterns of *MEFV* mutations; accordingly, this study was designed to explore the frequency of 12 common *MEFV* mutations among patients with FMF in southwestern Iran.

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## Materials and Methods

Twenty patients (11 girls and 9 boys) with FMF whose disease was diagnosed based on Tel Hashomer clinical criteria (5) were included in this study. After approval of the study protocol by the Ethics Committee of our university, written informed consent obtained from the children's parents. Then, demographic information and the main clinical data at the time of diagnosis (e.g. duration and frequency of the attacks as well as attack symptoms) were collected.

The results of laboratory tests for hemoglobin, cell blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and urinalysis at the time of the disease was diagnosed were also recorded. The response to colchicine was defined as complete (no attack), partial (>50% decrease in the frequency of attacks) or unresponsive according to clinical condition. For genotyping, DNA was isolated from 200  $\mu$ l of whole blood with the QIAamp Kit (Qiagen, Hilden, Germany). Each DNA sample was tested for a panel of 12 common *MEFV* gene mutations using FMF StripAssay kit (ViennaLab Diagnostics, Vienna, Austria) which were designed based on reverse-hybridization of biotinylated PCR products. Briefly, after amplification of exons 2, 3, 5, and 10 by multiplex PCR using biotinylated primers, PCR products were hybridized to a pre-coated strip with a parallel array of allele-specific probes for *MEFV* mutations including E148Q (in exon 2), P369S (in exon 3), F479L (in exon 5) and M680I, M680I, I692del, M694V, M694I, K695R, V726A, A744S and R761H (in exon 10).

## Results and Discussion

Generally, around 90% of patients with FMF experience their first attack before the age of 20 years. Because of the diversity in the clinical presentation, few patients are diagnosed in infancy or early childhood (7). The mean age of our FMF patients was  $3.4 \pm 2.2$  years at the time of diagnosis. Delay in the diagnosis might be explained by less experienced local physicians due to the low incidence of FMF in southwestern Iran.

The most frequent clinical presentations in our patients were fever in 19 (95%) (fever of 38 °C to 41 °C which continued for approximately 2-3 days), colicky abdominal pain in 18(90%), arthritis

in six (30%) (swelling of the knee joints in four patients, ankle swelling in one, and swelling in both the elbows and knees in one) and erythema in four (20%). Fever and recurrent colicky abdominal pain were common features in our patients. Only one of our patients had no fever and was diagnosed on the basis of recurrent abdominal pain and cutaneous features, supported by increased acute phase reactants and response to colchicine. More than half of our patients had generalized bone pain during disease attacks, and arthritis was detected in 30% of the patients. As in another report, monoarthritis of the large joints (knee, ankle and elbow) was also the main type of arthritis among our patients (6). None of our patients had undergone surgery for peritonitis or any other misdiagnosis before FMF was diagnosed. Three patients had their first attacks during the first year of life, and 17 of the patients were between 1 and 8 years of age at the time of their first attack. The attacks lasted from 24 to 72 hours, and patients were asymptomatic between attacks for about 4 to 6 weeks.

The results of laboratory tests showed elevated levels of acute phase reactants in the FMF attacks. Average hemoglobin during attacks was 11.7 g/dl, WBC count was 9800/ $\mu$ l, ESR was 38.4 mm/h and CRP was 12 mg/dl. The results of urinalysis were normal at the time of diagnosis and follow-up in all patients.

As in other reports, other researchers also showed ESR and CRP levels as well as leukocyte counts were significantly higher than normal ranges in patients during their attacks (1, 8). Although amyloidosis is the most significant complication of FMF, none of our patients had clinical or laboratory evidence of amyloidosis.

Among our patients, 90% responded completely to colchicine, and partial responses were observed in the remaining 10%. No symptoms were observed in the former group during follow-up.

Fifteen patients had good compliance with treatment; in three patients compliance was irregular, and two patients did not continue their treatments.

In this study, *MEFV* gene mutations were detected in only 40% of the patients. The most

common mutation was E148Q, detected in five patients (25%). Four patients were homozygous and one was heterozygous for this mutation. The V726A, M694V and P369S mutations were each observed in one patient. Compound heterozygous mutations in *MEFV* were not detected in any of the patients. The frequency of the E148Q mutation is 55.7% in northwestern Iran (9), 17.5% in Egypt (10) and 22.2% in Lebanese patients (11), and varies from 4% to 31% among different Turkish groups (12). E148Q and M694V have reported the common mutations in Ashkenazi Jews who generally present a milder form of the disease (13). The V726A mutation, which was reported to be the second common mutation in Middle East Arabs (14), was found in one of the patients. Although clinically suspected FMF can be confirmed by genetic analysis, patients with rare or undefined mutations may be overlooked. Therefore, a diagnosis of FMF should never be excluded based solely on the results of genetic testing (5).

The frequency of *MEFV* mutations in people

with FMF varies widely among different populations. According to our results, E148Q was the most frequent mutation in southwestern Iranian FMF patients, while this mutation was the third common mutation among Azeris in southwestern Iran and FMF patients in Turkey (15, 16).

Although none of the 12 mutations we included in our test panel was detected in 60% of our patients, all of them had FMF symptoms and responded well to colchicine. *MEFV* full gene sequencing analysis in these patients may lead to finding new mutations in southwestern Iranian FMF patients which would be helpful in designing a local diagnostic kit.

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