

MEFV mutations in Northwest of Iran: a cross sectional study

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ABSTRACT

Objective(s): Familial Mediterranean Fever (FMF) is an autosomal recessive disorder characterized by recurrent episodes of fever accompanied by peritonitis, pleurisy, and arthritis. FMF affects mainly Mediterranean populations and is caused by mutations in the familial Mediterranean fever (*MEFV*) gene. The aim of this study was to identify the frequency and distribution of *MEFV* mutations in Iranian Azerbaijanis with FMF.

Materials and Methods: Medical records of 1330 Iranian Azerbaijanis who were diagnosed with FMF according to Tel-Hashomer criteria from May 2006 to April 2013 were reviewed and 10 *MEFV* mutations were found in affected individuals.

Results: 243 patients (18.27%) were homozygous, 370 (27.82%) were compound heterozygous and 717 (53.91%) were identified as heterozygous for one of the studied mutations. Of the studied mutations, M694V, E148Q, V726A, M680I, and M694I accounted for 42%, 21%, 19%, 14% and 2% of mutations respectively.

Conclusion: In our study, M694V was found to be the most prevalent mutation. M694I, the most common mutation among Arabs, is rare in this cohort. Allele frequencies of the common mutations in our studied population have some similarities to those of the Turkish population reported previously. However, M680I is less common in our cohort.

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Introduction

Familial Mediterranean Fever (FMF) is an inherited autosomal-recessive disorder expressed by recurrent attacks of febrile peritonitis, pleuritis, and synovitis accompanied by painful manifestations in the abdomen, chest, and joints. The most severe complication of the disease is the development of AA type amyloidosis which affects the kidneys and results in nephrotic syndrome and renal insufficiency (1-3). The *MEFV* gene is responsible for FMF, has been identified on the short arm of chromosome 16p13.3, and includes 10 exons containing a 2,346-bp coding sequence (4, 5). *MEFV* encodes the 781-amino acid protein pyrin/marenostrin which is expressed in granulocytes, cytokine-activated monocytes, and synovial fibro-blasts. The protein has a key role in the regulation of inflammasome activity, prointerleukin-1 β (proIL-1 β) processing, and assists in minimizing inflammation by deactivating the immune response (6, 7).

Up to now, more than 180 gene polymorphisms have been identified in affected persons: 70 with known clinical effects and more than 110 with minimal or no influence on the phenotype (8).

M694V, V726A, M694I, and M680I mutations in exon 10, which is the longest exon in this gene, and E148Q mutation in exon 2 are the most frequently encountered ones (9). These five mutations account for more than 70% of FMF patients and have different frequencies in different ethnic groups of Mediterranean ancestry (10). R761H, P369S, R408Q, A744S, E167D, and R202Q have also been associated with FMF causative rare mutations in Mediterranean populations (11).

FMF is the most common disease in ethnic groups originating from the Mediterranean region. Turks, non-Ashkenazi Jews, North African Jews, Middle Eastern Arabs, and Armenian populations have a high risk of FMF. The frequency of the disease in these populations is about 1 to 4 in a thousand (1-4/1000). The main aim of this study was to evaluate the identification, distribution and frequency of the *MEFV* mutations in a large number of Iranian Azerbaijanis patients with FMF.

Materials and Methods

All patients were Iranian Azerbaijanis from Northwest of Iran which were referred by pediatricians, gastroenterologists, and rheumatologists.

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Table 1. Distribution of the allele frequencies in heterozygote, homozygote, and compound heterozygote groups

Mutation	Heterozygote (%)	Homozygote (%)	Compound heterozygote (%)
M694V	33.05	67.48	35.13
V726A	20.08	10.7	23.37
E148Q	35.28	4.11	18.10
M680I	9.2	15.63	17.83
M694I	1.53	2.05	2.56
R761H	0.28	---	1.62
R408Q	---	---	0.67
P369S	0.14	---	0.54
E167D	---	---	0.13
A744S	0.41	---	---
Total	100	100	100

logists for diagnosis and genetic counseling.

After obtaining written consent of adult patients or parents of children, personal and medical data of patients were recorded. After ethics committee approval, the study was performed in the FMF clinic of Tabriz for about 7 years (from May 2006 through April 2013). All patients were unrelated. The diagnosis of FMF was based on previous published criteria. Definite diagnosis requires 1 or 2 major and 2 minor criteria(12). Major criteria were (a) typical attacks of peritonitis, pleuritis, or pericarditis, (b) fever alone, (c) incomplete abdominal attacks, recurrent febrile episodes accompanied by peritonitis, synovitis, or pleuritis, (d) amyloidosis of the AA type without predisposing disease, and (e) favorable response to continuous colchicines treatment. Minor criteria were (a) recurrent febrile episodes, (b) erysipelas-like erythema, (c) FMF in a first-degree relative, (d) incomplete attacks involving chest, joint, exertional leg pain, and (e) favorable response to colchicines.

For identification of mutations in the *MEFV* gene, blood samples were obtained from patients. Genomic DNA was extracted from peripheral blood leukocytes using standard protocols.

The presence of the M694V, V726A, M680I, M694I, R761H, A744S, and E167D mutations was determined using arms PCR and the accuracy of the PCR was verified by direct sequencing. The E148Q, P369S, and R408Q mutations were detected by PCR-restriction fragment length polymorphism methods.

Descriptive statistics were used to report the findings. All data are presented as number and percentage.

Results

Genotyping for *MEFV* mutations of 1330 FMF Iranian Azerbaijanis patients revealed 717 patients

Table 2. Allele frequencies of the identified 10 mutations in 1330 FMF patients

Mutation(n=1943)	Number	Percent
M694V	825	42.46
E148Q	406	20.94
V726A	369	18.99
M680I	274	14.1
M694I	40	2.05
R761H	14	0.72
P369S	5	0.25
R408Q	5	0.25
A744S	3	0.15
E167D	1	0.051

(53.91%) were with one mutation, 243 patients (18.27%) with a homozygous mutation, and 370 patients (27.82%) with two compound heterozygous mutations.

According to allele, patients were categorized into three groups: homozygous, heterozygous, and compound heterozygous. In the heterozygote group, the most common mutation was E148Q with a percentage of 19% followed by M694V with a percentage of 17.8%. Other common heterozygote mutations were V726A (10.8%) and M680I (5%). In this group, rare mutations were M694I, A744S, R761H, and P369S with less than one percentage. We found M694I mutation in 0.8% of patients, A744S and P369S mutations in 0.2% of patients, and R761H mutation in only 0.1% of patients.

In 243 patients with homozygous mutations, the following genotypes were observed: M694V/M694V (67.49%), M680I/M680I (15.64%), V726A/V726A (10.70%), E148Q/E148Q (4.11%) and M694I/M694I (2.05%).

In 17 observed compound heterozygote groups, four common subgroups had the following genotypes: M694V/V726A, M694V/E148Q, M694V/M680I, and M680I/V726A with 3.7 to 7.8%. Each of the rare subgroups (E148Q/R408Q and V726A/E167D) was observed in only one patient.

Allele frequencies of most common 10 *MEFV* mutations among 1330 patients are given in Table 1. Screening for the five most common mutations M694V, E148Q, V726A, M680I, and M694I showed that the most frequent mutation was M694V (42.46% of the alleles). The E148Q mutation was more frequently found than V726A, M680I, and M694I (20.94%, VS 18.99%, 14.1%, and 2.05% of the alleles respectively). Allele frequency of the rest of the mutations is less than one percent. Of the mutations, R761H, P369S, R408Q, A744S, and E167D accounted for 0.7%, 0.25%, 0.25%, 0.15%, 0.05%, and 0.05%, respectively. Three of the alleles had the A744S mutation and only one allele had the E167D mutation (Table 2).

Table 3. The frequency of *MEFV* gene mutations in FMF patients according to gender

Mutation	Male		Female		Total N
	N	%	N	%	
Homozygote	190	26.72	180	29.07	370
Heterozygote	377	53.02	340	54.92	717
Compound heterozygote	144	20.25	99	15.99	243
Total	711		619		1330

Table 3 displays the genotypes of the patients according to gender. Among the 1330 patients, 711 (53.5%) were males (mean age; 36.6 ± 12.5 years) and 619 (46.5%) were females (mean age; 28.9 ± 14.5 years).

Discussion

Awareness of the incidence, prevalence of disease, and health threats are essential for more effective prevention and treatment of diseases and for elucidating environmental, behavioral, and biological factors associated with health conditions. FMF mostly affects populations of the southeastern Mediterranean region: Arabs, Armenians, non-Ashkenazi Jews, Turks, and Iranian Azerbaijanis. The disease is very rare in other populations, but recent reports suggest it can be found among some European populations including Italian and Greek populations, and to a lesser extent in some other ethnic groups such as the Japanese. It has become a fairly universal disease of the twentieth century due to the extensive population movements. A wide spectrum of mutations has been established in affected populations in various studies. There are reports from Iran and in the present study we have analyzed the frequency of MEFV mutations in Iranian Azerbaijanis populations living in Northwestern Iran. (13–15)

The Iranian Azerbaijanis are a high risk population for developing FMF. The carrier-state frequency of FMF in this population is 25.5% (15), higher than that previously reported in Jews (22%) (16), Arabs (18.5%) (17), Turks (20%) (18) and Armenians (21%) (19). This carrier rate is considerably high, and it places this disease among the highest frequency familial disorders in the Iranian Azerbaijanis population.

The genetic homogeneity/heterogeneity of FMF in Iranian Azerbaijanis patients was approached comprehensively. Data showed that there is a high heterogeneity in allele frequencies of MEFV gene mutations among Iranian Azerbaijanis patients

In this cohort, four of the mutations (M694V, E148Q, V726A, and M680I) accounted for 96.49% of the detected mutant alleles.

Investigating the spectrum distribution of MEFV mutations in FMF patients revealed that M694V is the most common mutation in this ethnic group. This was confirmed to be the same as that reported previously (15). M694V, the most prevalent mutation, accounted for about 42.6% of the identifiable mutations in this cohort. In other classically affected populations such as Jews, Armenians, and Turks, usually THE M694V mutation predominates. Also the M694V seems to be common among Arab patients. In Jordan, Lebanon, and Syria, M694V is the most common mutation in FMF patients (20, 21). M694V was the most common mutation found in a study by Jarjour that accounted for 36.5% of the mutations in Syria (22).

Also in North Africans with FMF, M694V was common among Moroccans (49%) and Tunisians (50%) (23). In Turkey, the frequency of the M694V mutation has been reported to range from 42.05% to 51.4% (24–26).

E148Q is the second most common mutation accounting for about 20.94% of the identifiable mutations in this cohort. E148Q mutation occurred in high percentages in the heterozygous state and accounted for 35.28% of the heterozygotes, but it is less common in the homozygote state. Moreover increased frequency of E148Q mutation is found within Turks. The E148Q mutation is the third most common mutation in Tunisian patients and the fourth most common mutation among the Syrian patients accounting for 6.6% of mutations. It also accounts for 8% of all mutations among the Lebanese and the Jordanians.

V726A was the third most common mutation followed by M680I and M694I. Similar to the findings in Turkey, the V726A was identified as the third most common mutation (24, 27). However, in another study in Turkey, Dundaret *al* have found V726A mutation to be the fourth most common mutation (28). V726A is the second most common mutation in Arabs and non-Ashkenazi Jews (9). Among Egyptian patients with FMF, El-Garfet *al* found that the most frequent gene mutation was V726A which accounted for 41.2% of the mutations (29) while this mutation seems to be rare in the Tunisian population (5% of all alleles). Generally, V726A mutation was prevalent among all patients other than North African Jews.

Furthermore, M680I was the fourth most common mutation in our ethnic group. The M680I mutation is frequent in Armenians and Turks; however, it is less common in Arab populations and non-Ashkenazi Jews (18, 30, 31). M680I is the most common mutation in the Tunisian population with 32% allele frequency, which is the highest percentage that has been reported (32). It has accounted for 10% to 22% of the identifiable mutations in Arabs (20, 33).

The M694I mutation is recognized only in 2.05% of patients from the Iranian Azerbaijanis population. The M694I mutation seems to be rare in the Iranian Azerbaijanis population (1.53% in heterozygote, 2.05% in homozygote and 2.56% compound group alleles) (Table 3), while it is considered the most common mutation in Arabs (23). It was described as specific to North African Arabs (34). In a study among 75 North African Arab patients with FMF, the M694I accounted for 61% of the mutations (35). Studies from Jordan and Lebanon also have shown the high frequency of this mutation in Arabs. M694I accounted for 50% of the MEFV mutations in Algerian Arabs with FMF (36).

To sum up analysis of rare mutations of FMF, we found that 0.72% of cases had R761H. Each of the P369S and R408Q mutations were found only in 0.25% of our examined cases. A744S and E167D were found in 0.15% and 0.051% respectively.

Of the rare mutations, R761H mutation was especially prevalent in Turkish FMF patients (27). It was detected in the Syrian and Lebanese patients but not in the Jordanians (22). The A744S mutation is frequently reported in Arabs and is not frequent in other ethnic groups (9, 37–39).

Conclusion

10 different mutations were investigated in the present study, and 30 different genotypes were found. Of these genotypes, M694V homozygote and compound M694V heterozygotes were the most frequent ones. The *MEFV* gene assessment in the Iranian Azerbaijanis population revealed the molecular epidemiology of the FMF disease in Northwest of Iran. Allele frequencies of the common mutations in our studied population have some similarities to those of the Turkish population reported previously. However, M680I is less common in our cohort. We have shown the diversity and the frequency of the *MEFV* gene mutations in Iranian Azerbaijanis patients. The mutation spectrum in patients with FMF is heterogeneous and for future studies, large scale population screening and sequencing of the whole *MEFV* gene search is necessary.

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Conflict of interest

The authors report no financial or other conflicts of interest relevant to the subject of this article.

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