Common *MEFV* Mutation Analysis in Iranian Azeri Turkish Patients with Familial Mediterranean Fever

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Objectives: To identify the frequency and distribution of familial Mediterranean fever (FMF) gene (*MEFV*) mutations among Azeri Turkish patients from northwestern Iran.

Methods: One hundred ninety unrelated patients were referred by specialists to the Molecular-Medical Genetic Center of Tabriz. A clinical diagnosis of FMF was made according to published criteria. Mutation screening of the *MEFV* gene was performed for the 5 most commonly known mutations, namely M694V, V726A, M680I, M694I, and E148Q, by using amplification refractory mutation system for the first 4 and by polymerase chain reaction restriction-digestion testing for E148Q. These methods may also be used as a screening tool within affected families.

Results: Of the unrelated patients investigated, 120 (63%) had 1 or 2 mutations. Of those with mutations, 41 were homozygous, 37 were compound heterozygous, and 42 had only 1 identifiable mutation. Of the studied alleles, the most frequent mutation was M694V (28%), followed by V726A (9%), E148Q (7%), M680I (7%), and M694I (1%) mutations.

Conclusions: Our results indicate that the common Mediterranean mutations are frequent in the Azeri Turkish FMF patients but with some differences in the frequency of individual mutations. The high frequency of E148Q in Azeri Turks compared with Mediterranean ethnic groups is rather interesting. The results open the way for further investigations on patients diagnosed as having FMF and in whom no mutations or only 1 mutated allele were found.

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Familial Mediterranean fever (FMF) is an autosomal-recessive autoinflammatory disorder characterized by self-limited recurrent bouts of fever and painful episodes of sterile serositis that typically involve the peritoneum, pleura, and synovia and sometimes is associated with erysipelas-like erythema. A less frequent but most severe complication is progressive amyloidosis that may affect several organs, especially the kidney, leading to end-stage renal failure (1-4). The disease mainly affects people from the Mediterranean basin, namely Jews, Turks, Arabs, and Armenians with a genetic prevalence of 1 to 6% (5), although recently it has been described in Italians, Greeks, British, French, Cubans, Belgians, Dutch, Spaniards, Indians, Chinese, Afghans, Hungarians, and Portuguese (5,6).

The responsible gene, *MEFV*, has been mapped to chromosome 16p13.3. It consists of 10 exons and encodes a protein of 781 amino acids called pyrin or marenostrin that is expressed mainly in granulocytes and is thought to be a negative regulator of inflammation (7,8). Since the cloning of the *MEFV* gene in 1997 (7,8), about 40 disease-associated mutations have been identified (9). Five founding mutations, E148Q, M680I, M694V, M694I, and V726A, account for more than 70% of deleterious alleles (10). Molecular genetic studies on FMF have not been accomplished on the Iranian population, a population of interest for many reasons. Iran is a large

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Table 1 Distribution of Patients with Common *MEFV* Mutations in Relation to Parental Consanguinity

Consanguinity	Homozygotes	Compound Heterozygotes	Single Mutation	Negative
+	20	6 39 (21%)	13	29 (15%)
−	21	31 81 (43%)	29	41 (22%)
Rate		33%		42%

country with different ethnic groups including Persian (51%), Azeri Turk (24%), Kurd (7%), Arab (3%), and other minorities such as Armenians (11). On the other hand, the location of Ancient Great Iran on the route of Silk Road, and the immigration from the neighboring countries, and also the occurrence of many wars with foreign nations, especially Mediterranean ethnic groups, make the genetic pool of this population highly heterogeneous. Furthermore, the purity of many different races in this country has been highly conserved by geographical borders and by an ancient culture that has always encouraged consanguineous marriages, an important factor in the accumulation of putative recessive mutations (11,12). Therefore, the aim of the present study was to determine the spectrum of the most common *MEFV* mutations in Azeri Turkish FMF patients, most of whom reside in the northwestern part of Iran.

PATIENTS AND METHODS

Over a period of 3 years, a total of 210 unrelated FMF patients were referred by pediatricians, gastroenterologists, and rheumatologists for counseling and genetic testing to the Molecular-Medical Genetic Center of Tabriz. All patients were of Azeri Turk origin. An accurate and detailed family history was obtained for each patient. The diagnosis of FMF was made according to established clinical criteria [Major criteria: attacks involving the abdomen, chest, joints, skin, and scrotum muscle, and typical attacks of fever; minor criteria: exertional dyspnea, response to colchicine, nephropathic amyloidosis, brother or sister having an identical twin with FMF]. Diagnosis requires at least 1 major or 2 minor criteria (13). Every patient was informed about the study and a written consent was signed either by the patient or by his/her parent for blood sampling. Genomic DNA was extracted from peripheral blood leukocytes using standard protocols (14).

Each sample was tested for the 5 common mutations (M694V, M694I, M680I (G to C transversion), V726A, and E148Q) by using amplification refractory mutation system-polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism methods as previously described (7,15,16,17). The appropriate positive and negative controls were employed for each test. The positive results were repeated to ensure reproducibility. PCR products and restriction enzyme-digested fragments were electrophoresed in a 2% agarose gel and visualized by ethidium bromide staining.

RESULTS

Of the 210 referred patients, 20 were excluded from the study due to the study design and incompatibility with established clinical criteria for FMF. The age range of the remaining 190 unrelated patients was 2 to 66 years (mean, 17.5 years) and consanguineous marriages were present in about 36% of the families (Table 1). The male:female ratio was 1.47:1. The main clinical characteristics of the patients were as follows: peritonitis was observed in 186 (98%), fever in 149 (78%), arthritis in 70 (37%), myalgia in 61 (32%), pleuritis in 58 (31%), erysipelas-like erythema in 28 (15%), and amyloidosis in 7 (4%). A positive response to colchicine treatment was noted in 125 (66%) patients including 76 patients with 2 mutated alleles, 33 patients with 1 identified mutation, and 16 patients with none of the studied mutations. Mutation analysis of *MEFV* gene showed that 120 (63%) probands had at least 1 identifiable mutation. Of the 120 patients with mutations, 41 were homozygous, 37 were compound heterozygous, and 42 had only 1 identifiable mutation (Table 2). The most frequent mutation was M694V followed by V726A, E148Q, M680I, and M694I. There was no complex allele in the studied chromosomes regarding the 5 common mutations. The results were conveyed to the families together with their interpretations and counseling advice.

DISCUSSION

The 15 to 20 million Azeri Turks living in northwestern Iran, ethnically identical to Azeris and closely related to Turks, are believed to constitute 25% of the population. This is the first report about the mutation spectrum of the FMF disease in this ethnic group.

The clinical features of FMF in the studied ethnic group differ somewhat from those of the populations of the Mediterranean basin. Fever is the most common symptom, occurring in about 93 to 100% of Turks, Arabs, Jews, and Armenian patients (6), whereas fever was observed only in 78% of Azeri Turkish patients. Peritoneal attacks were present with similar frequency to that noted in other ethnic groups (6). The frequency of pleuritis in our patients was similar to that reported from other

Table 2 Identified Genotypes in Iranian Azeri Turkish Patients with FMF

State of Mutation	Genotype	No (%)
Homozygous	M694V/M694V	34 (28)
	V726A/V726A	3 (3)
	M680I/M680I	2 (2)
	E148Q/E148Q	1 (1)
	M694I/M694I	1 (1)
Compound heterozygous	M694V/M680I	10 (8)
	M694V/V726A	9 (8)
	M694V/E148Q	7 (6)
	M680I/V726A	6 (5)
	M694I/V726A	2 (2)
	M694I/M680I	1 (1)
	M680I/E148Q	1 (1)
	V726A/E148Q	1 (1)
One identified mutation	E148Q	17 (14)
	M694V	13 (11)
	V726A	9 (8)
	M680I	3 (3)
Total		120 (100)

Percentages were round and therefore, may not add up to 100%.

populations except Armenians, and the frequency of arthritis in our FMF cases was only different from that of Jews (6). Erysipelas-like erythema is more frequent in Azeri Turks than in Arabs and Armenians but is less common than in Jews and Turks (6). The low rate of amyloidosis in our series is similar to that reported in Arabs (18). This low rate of FMF-related amyloidosis is probably a result of the fact that these figures were obtained after the establishment of colchicine as the standard of care. Unlike typical FMF, colchicine had no influence on the attacks in the 2 patients with 2 mutated alleles and the 9 patients with only 1 identifiable mutation who received this treatment. In the 16 patients clinically diagnosed as having FMF and who seemed to respond to colchicine, none of the 5 common mutations could be detected. It is probable that these patients carry other, newly described mutations. On the other hand, considering the nonspecificity of the clinical criteria for a definitive diagnosis, these patients might not have FMF but a clinical constellation mimicking the disease (19).

The identification of the causative gene, *MEFV*, has allowed molecular diagnosis of FMF. This report is the first genetic study on the FMF among Iranian Azeri Turkish patients. Our data show that the 5 common mutations accounted for 52% of the studied alleles (Table 3). M694V is the most prevalent mutation, accounting for about 54% of the identifiable mutations in this cohort, where the M694V/M694V was the most common and accounted for 83% of the homozygotes (Table 2), a finding that was observed in the other studies (20-23). Although M694V, especially in the homozygous state, is thought to be an important risk factor for development of amyloidosis, there are amyloidosis patients with mutations other than M694V (4,24). Data from our study point toward an association between homozygosity for M694V and amyloidosis, since 4 of the 7 patients (57%) with amyloidosis were homozygous for the M694V.

The V726A, the second most frequent mutation in Armenians, Arabs, and Jews (Table 3), was found in 17% of the Azeri Turkish alleles studied, and E148Q and M680I were in the next places with 14 and 13% of deleterious alleles, respectively. The current data show the divergence of mutation spectrum in our population in which the V726A and E148Q were more frequent than M680I, while in Turkish patients M680I was the second most common mutation (Table 3).

The M694I mutation, frequently reported in Arabs, seems less common in our population. This mutation is also less common in the other 3 ethnic groups (Table 3).

Our results indicate that the spectrum of common *MEFV* mutations is unique for our population (Table 3), and that the frequency of E148Q mutation is higher than in other Mediterranean ethnic groups and is similar to that observed in European ethnic groups (5).

We did not detect any of the 5 mutations in 182 of the 380 alleles (48%) studied. This could possibly be due to many factors, including the presence of other rare or unknown mutations (in the promoter region, within introns or in the 3′-untranslated region), genetic heterogeneity (25,26), the presence of modifier genes (27), and unknown environmental factors. Finally, overdiagnosis of FMF, or misdiagnosis with other hereditary periodic fever

Table 3 Frequencies of the 5 Most Common *MEFV* Mutations Among Identifiable Mutations in Different Ethnic Groups

Ethnic Group	Mutation Frequencies (%)*					Overall Frequency (%)‡
	M694V	V726A	M680I†	M694I	E148Q	
Jews	77	12.3	0.6	0	10.2	51
Armenians	52	26	20	0.2	1.8	94
Arabs	42.5	23.1	9.6	14.1	10.7	47
Turks	71.3	8.5	15	1.7	3.5	68
Iranian Azeri Turks	54	16.7	12.6	2.5	14.2	52

*Data were compiled from references for Jews (21,28,29), Armenians (23,30), Arabs (22,31-36), Turks (20,37,38), and Iranian Azeri Turks (present study).
†In some references, both G to C and G to A were studied.
‡The fraction of identifiable mutations among all independent alleles regarding the 5 common mutations.

syndromes, may also contribute to the relatively high percentage of mutation-free alleles (20).

Of the 68 families with consanguineous marriages, 29 (43%) had no identifiable mutations (Table 2). Of these 29 patients, 7 have properly responded to colchicine treatment and therefore are candidates for possible rare or new mutations in the *MEFV* gene. Since there is only1 affected member in each of these families, applying linkage analysis to find other possible FMF genes is not feasible.

Since the 68% of the families with mutations belong to nonconsanguineous marriages (Table 1), a relatively high carrier frequency may exist concerning the studied mutations. A population genetics study is necessary to demonstrate the carrier frequency of FMF in our population.

This is the first *MEFV* gene mutation assessment in a large cohort of Iranian Azeri Turkish patients, an ethnic group in which FMF and its molecular basis have not yet been described. Our data show that the common Mediterranean mutations are frequent in the Azeri Turkish FMF patients but with some differences in the frequency of individual mutations. The high frequency of E148Q in Azeri Turks compared with Mediterranean ethnic groups is interesting. These results provide important tools for adapting a molecular diagnostic test for the Iranian Azeri Turks.

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