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FMF Genotype-phenotype correlation in Iranian Azeri Turks: Association between M694V/R761H mutation and amyloidosis

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ABSTRACT

Objective(s): Familial Mediterranean fever (FMF), an inherited autosomal recessive disorder, is frequently present among individuals of Mediterranean origin. Differences in the clinical manifestations of FMF between different ethnic groups have been documented. The aim of the present study was to determine the most common characteristics of FMF and the relationship between clinical findings and the most common mutant alleles of the *MEFV* gene in an Iranian Azeri Turk population.

Materials and Methods: We analyzed clinical and genetic data from 415 patients identified as having FMF clinical symptoms and who were referred to the Molecular Genetics Laboratory of Tabriz/Iran over the last 3 years. The mutation type and clinical characteristics were determined for each patient.

Results: The following primary clinical characteristics of the patients were observed: peritonitis was observed in 378 (93.8%), high-grade fever in 351 (86.88%), arthritis in 215 (54.57%), pleuritis in 207 (53.49%), myalgia in 153 (41.69%), AA amyloidosis in 149 (40.16%), and erysipelas-like erythema in 54 (14.96%) subjects. A positive response to colchicines treatment was noted in 374 (95.1%) patients including 303 patients with two mutated alleles and 71 patients with one identified mutation.

Conclusion: In contrast to previous studies, there was no significant association between M694V mutation and development of amyloidosis. The M680I/M680I, M680I, M694I, and M694V/R761H genotypes were found to be associated with the development of amyloidosis. These results indicate that physicians need to pay careful attention to patients with asymptomatic or mildly symptomatic FMF with these genotypes.

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Introduction

Familial Mediterranean fever (FMF, MIM# 249100) is one of the most common autosomal genetic diseases. FMF primarily affects populations from the Mediterranean basin, especially Turks, Azeri Turks, Armenians, non-Ashkenazi Jews, and Arabs (1-4). It affects more than 100,000 people worldwide. FMF was first described in 1945 and the gene responsible for the disease, MEFV was identified in 1992. MEFV is located on chromosome 16p13.3 and consists of 10 exons, which encode a protein of 781 amino acids named pyrin or marenostrin (5, 6). Pyrin is primarily expressed in granulocytes and is thought to be a negative regulator of inflammation (7, 8). Sets of criteria have been described for diagnosis of FMF. The most famous of these is Tel Hashomer's criteria, which defines diagnosis based on the characterization of major and minor criteria (9).

Definitive diagnosis of FMF requires the fulfillment of two major criteria or one major and two minor criteria. Major criteria are: (a) typical attacks of peritonitis, pericarditis, or pleuritis, (b) fever alone, (c) recurrent febrile episodes accompanied by peritonitis, synovitis, or pleuritis, incomplete abdominal attacks, (d) amyloidosis of the AA type without predisposing disease, and (e) favorable response to continuous colchicines treatment. Minor criteria are: (a) Erysipelas-like erythema, (b) recurrent febrile episodes, (c) FMF in a first-degree relative, and (d) incomplete attacks involving the chest, joint, exertional leg pain, and (e) favorable response to colchicines. Peritonitis is the principal clinical finding of FMF associated with febrile episodes. It is experienced by the majority of patients and is reported as the first symptom in about half of them. The articular pain in FMF episodes is the second-most frequent symptom

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associated with fever and acute arthritis. Usually redness, warmth, tenderness, and swelling are also present. During FMF attacks, not only a developed acute-phase response occurs, but high levels of inflammatory mediators, such as interleukin-6 (IL-6), tumor necrosis factor–alpha (TNF-α), and serum amyloid-A (SAA) can also be found. Between recurrent and self-limited attacks of fever, severe abdominal, articular, and/or chest pain, patients with FMF are usually free of symptoms. Amyloidosis due to chronic inflammation leading to renal failure is the major complication of this disease. Colchicine is an oral drug used for preventing or treating attacks. Daily intake of colchicine (1–2 mg) has been the recommended treatment for FMF since its introduction in 1972 (10). Adherence to treatment results in a significant decrease in the frequency and severity of attacks, or prevents them in about 95% of patients. Since the cloning of the MEFV gene, about 180 mutations have been associated with FMF (11). M694V, V726A, E148Q, M694I, and M680I are the five most frequently encountered mutations and account for 74% of FMF mutations in typical patients (12). The rest of the mutations are very rare in different populations. Differences in clinical presentation of FMF among different ethnic groups have been studied. However, phenotype–genotype correlations in FMF have not been decisively resolved. The aims of this study were to find the most frequent mutation in a cohort of Iranian Azeri Turks with FMF and to investigate the effect of genetic factors on the phenotype, especially in terms of the development of amyloidosis in a population living in the North West of Iran.

Materials and Methods

This study was conducted in the FMF clinic of Medical University of Tabriz between April 2010 and April 2013. A total number of 415 patients were referred by gastroenterologists, rheumatologists, and pediatricians for diagnosis and genetic counseling regarding FMF. After obtaining Informed Consent from patients, personal and medical data were recorded. Diagnosis was done based on the Tel-Hashomer criteria. All patients were of Azeri Turks origin from the North West of Iran. Mean age was 26.0 ± 14.76 years with a range of 3–78 years and a male/female ratio of 1.30. DNA was isolated from peripheral blood lymphocytes by standard procedures and amplified with sequence-specific primers using PCR. Patients were screened for 10 *MEFV* gene mutations including M694V, V726A, E148Q, M680I, M694I, R761H, A744S, P369S, E167D, and R408Q. The presence of the M694V, V726A, M694I, M680I, R761H, E167D, and A744S mutations was determined using arms PCR and the accuracy of the PCR was verified by direct sequencing. The E148Q, R408Q, and P369S mutations were detected by PCR-restriction fragment length polymorphism methods. Genotype–phenotype correlation was investigated in 415 Turkish patients with FMF. The patients were grouped according to their mutations and main clinical parameters (fever, arthritis, peritonitis, and amyloidosis) and comparisons were made among the groups. We analyzed the genotype–phenotype correlation using Chi-square and Fisher's exact tests. The SPSS software (version 16.0), VassarStat, and JavaStat websites statpages.org/ctab2x2.html) were used for data analysis. The odds ratios (OR) and confidence intervals (CI) at 95% significance level were calculated for all data. The significance of the differences of observed genotypes between patients with or without a specific clinical symptom was tested using Chi square test and further comparison of confidence intervals. *P*-values less than 0.01 were considered statistically significant.

Results

In this study, five *MEFV* gene mutations (M694V, V726A, E148Q, M680I, and M694I) accounted for 95.52% of mutations. The most frequent mutation was M694V (42.05% of the alleles). The main clinical characteristics of 235 patients were as follows: peritonitis was observed in 378, fever in 351, arthritis in 215, pleuritis in 207, myalgia in 153, amyloidosis in 149, and erysipelas-like erythema in 54 subjects. A positive response to colchicine treatment was noted in 374 patients, including 303 patients with 2 mutated alleles and 71 patients with 1 mutation. A positive family history of FMF was observed in 103 of the patients, where at least one member of the family had FMF (Table 1). The mean age of onset in the homozygotes group was: 6.96 years for M694V, 10.75 years for E148Q, 22.87 years for V726A, and 14.38 years for M680I. The mean age of onset in the compound heterozygotes group was: 12.73 years for M694V, 13.10 years for E148Q, 11.08 years for V726A, and 12.96 years for M680I. The mean age of onset in the heterozygotes was: 19.37 years for M694V, 16.85 years for E148Q, 18.12 years for V726A, and 27.87 years for M680I. Patients were categorized into three groups according to their allele status: homozygous, heterozygous, and compound heterozygous. In the

Table 1. Phenotypic features of the patients

Features	N=415
Male/Female	235/180
Age at onset	15.59
Peritonitis	93.8
Fever	86.88
Arthritis	54.57
Pleuritis	53.49
Myalgia	41.69
Erysipelaslike erythema	14.96
Amyloidosis	40.16
Family history of FMF	24.88%
Response to colchicine treatment	95.1

Table 2. Phenotype–genotype correlation in homozygote group

	Features n=95	M694V/M694V	V726A/V726A	E148Q/E148Q	M680I/M680I	M694I/M694I
Fever	With (%)	71.11	8.88	2.22	15.55	2.22
	Without (%)	40	0	40	20	0
	P value	<0.001	<0.001	<0.001	0.200	<0.001
Peritonitis	With (%)	70	8.88	3.33	15.55	2.22
	Without (%)	75	0	25	25	0
	P value	0.263	<0.001	<0.001	0.046	<0.001
Arthritis	With (%)	61.53	13.46	3.84	19.23	1.92
	Without (%)	79.06	2.32	4.65	11.62	2.32
	P value	0.002	0.002	0.302	0.052	0.275
Amyloidosis	With (%)	59.09	9.09	4.54	25	2.27
	Without (%)	78.43	7.84	3.92	7.84	1.96
	P value	<0.001	0.301	0.446	<0.001	0.415

Table 3. Phenotype–genotype correlation in compound heterozygote group

	Features n=129	M694V/E148Q	M694V/V726A	M694V/M680I	M694V/R761H	V726A/M680I
Fever	With (%)	22.61	38.26	18.26	4.35	16.52
	Without (%)	28.57	35.71	28.57	0	25
	P value	0.158	0.329	0.050	<0.001	0.068
Peritonitis	With (%)	23.58	36.59	19.51	4.06	16.26
	Without (%)	16.66	66.66	16.66	0	0
	P value	0.106	<0.001	0.280	<0.001	<0.001
Arthritis	With (%)	19.12	35.29	19.12	7.35	19.12
	Without (%)	27.87	40.98	19.67	0	11.47
	P value	0.091	0.222	0.515	<0.001	0.043
Amyloidosis	With (%)	27.12	37.29	22.03	1.69	11.86
	Without (%)	20	38.57	17.14	5.71	18.57
	P value	0.094	0.515	0.138	0.047	0.071

Table 4. Phenotype–genotype correlation in heterozygote group

	Features n=154	M694V	V726A	E148Q	M680I	M694I
Fever	With (%)	30.77	17.95	40.17	11.11	0
	Without (%)	16.2	29.7	46	5.40	2.70
	P value	0.009	0.018	0.230	0.047	0.152
Peritonitis	With (%)	29.85	17.16	42.54	9.70	0.75
	Without (%)	10	45	35	10	0
	P value	<0.001	<0.001	0.139	0.395	<0.001
Arthritis	With (%)	29.49	19.23	38.46	11.54	1.28
	Without (%)	25	22.37	44.73	11.84	0
	P value	0.237	0.249	0.187	0.416	<0.001
Amyloidosis	With (%)	34.29	8.57	37.14	17.14	2.86
	Without (%)	25.21	24.37	42.86	7.56	0
	P value	0.071	<0.001	0.235	0.011	<0.001

homozygote and heterozygote groups, the most common genotypes were M694V/M694V and M694V/- and in the compound heterozygote group the most common genotype was M694V/E148Q. Allele frequencies of ten most common *MEFV* mutations among 415 patients were calculated. The most frequent mutation was M694V (identified in 42.05% of the alleles examined), followed by V726A (19.76%), E148Q (17.23%), and M680I (15.15%). Concurrently, we determined that R761H (1.78%) was the most frequent rare mutation in Iranian Azeri Turks patients. The frequencies of the other rare mutations were: M694I (1.33%), F479L (0.9%), E167D (0.59%), R408Q (0.44%), P369S and A744S (0.29%), and R694I (0.14%).

Discussion

The results of previous mutation studies have led to the hypothesis that phenotypic variation of the disease may be attributable to the existence of some of these mutations. Correlation of the genotypes of patients with FMF with the various phenotypes of the disease, especially amyloidosis, has often been studied. However, there are few published studies conducted with Azeri Turk patients with FMF. To assess the phenotypic variation associated with the existence of particular mutations in Azeri Turk patients with FMF living in Iran, we discussed each genotype with the corresponding patient symptoms.

In our series from Iran, the age of onset in patients in homozygous and compound heterozygotes groups was lower as compared to heterozygotes. In other words, in patients with one mutation, the disease started later. Fever and peritonitis were frequent symptoms in patients with the M694V and E148Q mutations. However, the symptoms were less frequent in patients with the E148Q/E148Q genotype, indicating that two mutations of E148Q may be associated with a

moderate form of the disease. Among the five most common mutations, M694V, V726A, and M694I mutations showed significant association with fever and peritonitis in the homozygote state (P<0.01),while patients with M680I without fever and peritonitis symptoms were significantly associated with amyloidosis. We enrolled 15 homozygous patients for M680I mutation, from which, only four subjects were asymptomatic which were detected through family studies. The remaining 11 patients had amyloidosis. Statistical analysis showed that the association between the presence of M694I and M680I mutations and amyloidosis is the only significant correlation (P<0.01). A number of studies have been published about the association between MEFV mutation and the clinical signs of FMF, especially amyloidosis. Many reports on different ethnic groups revealed that amyloidosis is associated with the M694V mutation, especially in the homozygous state (12-15). However, some studies found that there is no specific correlation between genotype and the development of amyloidosis.

It is hypothesized that amyloidosis occurs in patients with FMF due to differences in patterns of the MEFV genotype. Shohat *et al* found a significant association between amyloidosis and the M694V mutation and observed that patients carrying other mutations did not show amyloidosis (16). Ben-Chetrit found that amyloidosis was more common in FMF patients originating from North Africa who were homozygous for the M694V mutation (17). Shinar *et al* observed that the M694V/M694V genotype is associated with a more severe form of the disease compared to other common genotypes in patients with FMF (18). Majeed *et al* found that the genotypes M694V/M694V and M694V/V726A have a severe clinical course in Arab FMF patients, whereas the M694I/M694I is associated with mild disease (19). Dusunsel *et al* showed that M694V homozygosity is associated with amyloidosis compared to other common genotypes in patients with FMF. The severity of the disease and development of amyloidosis seem to have an association with M694V, the most common mutation in Syrian patients (20). However, the results of this study do not support the view that M694V in the homozygous state has a correlation with amyloidosis. Results of our assessment of genotype and phenotype correlation revealed the association between M694V/R761H mutations and amyloidosis. This finding is in agreement with the conclusions of Demirkaya *et al* in Turkey (21).

In our series from North West of Iran, the most frequent MEFV mutations were M694V, V726A, E148Q, M680I, and M694I. Similar results were observed in studies on the populations of the countries where FMF is prevalent (22-26). However, in a recent study by Gunesacar *et al* on Turkish population, it was shown that the R202Q was the most commonly observed mutation, which was detected in 34.4% of patients (27).

Conclusion

In conclusion, the most frequent mutation in FMF within our study population was M694V and the most frequent genotype was M694V/M694V. Peritonitis and fever were seen in almost all genotypes with common mutations. Renal amyloidosis was observed not only in patients with the M680I/M680I genotype but also in the M680I/- and M694I/- genotypes. Also, M694I and M680I in the heterozygous state showed a significant association with amyloidosis. However, renal amyloidosis was seen more frequently in homozygous genotypes. Despite lots of knowledge on FMF, prospective clinical studies with large numbers of patients from different ethnic groups will help us to further clarify this considerable disease.

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