Received: 16 May 2019 | Revised: 12 July 2019 | Accepted: 7 August 2019

DOI: 10.1111/ahg.12347

Annals of human genetics WILEY

ORIGINAL ARTICLE

Mediterranean fever (*MEFV*) gene profile and a novel missense mutation (*P313H*) in Iranian Azari-Turkish patients

Leila Rostamizadeh[1] | **Leila Vahedi**[2] | **Seied Rafi Bahavarnia**[3] | **Shahriar Alipour**[1] | **Somayeh Abolhasani**[4] | **Alireza Khabazi**[5] | **Ebrahim Sakhinia**[6]

[1]Department of Molecular Medicine, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

[2]Liver and Gastrointestinal Disease Research Centre, Tabriz University of Medical Sciences, Tabriz, Iran

[3]Screening laboratory, Tabriz Blood Transfusion Organization, Tabriz, Iran

[4]Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran

[5]Connective Tissue Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[6]Department of Genetics, Faculty of Medical Sciences Tabriz University of Medical Sciences, Tabriz, Iran

Correspondence
Ebrahim Sakhinia Department of Genetics, Faculty of Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran.
Email: tgac1387@yahoo.com

Abstract

Background: Familial Mediterranean fever (FMF) is common in Azari-Turkish people, one of the biggest ethnic groups in Iran. In this study, we sought to investigate the mutation spectrum of the *MEFV* gene and any genotype–phenotype correlations.

Methods and materials: 400 unrelated Azari-Turkish FMF patients were analyzed in this study. Mutations in exons 2, 3, 5, and 10 of the *MEFV* gene were investigated using direct Sanger sequencing, and their correlations with the clinical features of the patients were analyzed.

Results: At least one mutation was detected in 248 (62%) patients. The most common mutations were *M694V* (26.25%) and *E148Q* (24.75%), respectively. Abdominal pain (65.2%) and fever 204 (51%) were the most frequent clinical problems in all subjects. The analysis recognized a novel missense mutation in the coding region of the *MEFV* gene, named *P313H*, which is the first report of a new mutation in exon 2 of the *MEFV* gene in an Azari-Turkish family.

Conclusion: Genotype–phenotype correlations obtained from this study would be helpful in the diagnosis and management of FMF patients in clinical situations. This novel missense mutation may provide useful evidence for further studies of FMF pathogenesis.

KEYWORDS

Familial Mediterranean Fever, *MEFV* gene, mutation, PCR-sequencing

1 | INTRODUCTION

Familial Mediterranean fever ([FMF], MIM# 249100) is the most frequent hereditary monogenic autoinflammatory disease caused by mutations of so-called "autoinflammatory genes," which are translated into an uncontrolled and aimless activation of the inflammatory processes in response to innocuous stimuli (Fonnesu et al., 2009). At first, FMF was described in several ethnic groups arising in the Mediterranean basin, such as Sephardic Jews, Armenians, Turks, North Africans, and Arabs (http://www.clinlabnavigator.com). The carrier rate in these populations varies approximately from 1:4 to 1:8. Researchers speculate that the FMF may have originated more than 3,000 years ago in Mesopotamia. The spread of disease in the modern world is a result of migration and also to weather conditions (https://www.uptodate.com). The prevalence of FMF in the Iranian population has not been well defined but the estimated carrier rate has been reported as 25.5% (Bonyadi, Esmaeili, Karimi, & Dastgiri, 2010). FMF is usually considered an

autosomal-recessive genetic disorder that is characterized by lifelong recurrent and self-limited episodes of paroxysmal attacks of fever and serosal inflammation (Nobakht et al., 2011) without a high-titer of autoantibodies or antigen-specific T cells (Chae, Aksentijevich, & Kastner, 2009). Symptoms and first attack in most FMF patients appear during the first decade of life, and more than 80% of patients experience the disease during childhood and adolescence (Tunca et al., 2005). FMF is 1.1–2.6 times more frequent in males compared to females (Sohar, Gafni, Pras, & Heller, 1967). The most important complication of FMF is the progress of amyloidosis, which in most cases affects the kidneys but may involve other organs as well (Nobakht et al., 2011). At present, colchicine, a neutrophil-suppressive agent (Elshafey et al., 2011), is the only effective therapy to improve the quality of life by contributing to the reduction or abolition of attacks (Fonnesu et al., 2009). This medication is effective in 95% of patients but the rest show resistance (Nobakht et al., 2011).

The *MEFV* (Mediterranean fever) gene, as the underlying gene located on 16p13.3 with 10 exons, encodes a protein named pyrin, or marenostrin, which is expressed predominantly in the cytoplasm of myeloid cell lineage. Pyrin is involved in the inflammatory pathways of the innate immune system against external pathogens and other noxious agents and blunts neutrophil-mediated inflammation. *MEFV* gene mutations in FMF patients result in a mutated pyrin protein that is capable of starting inflammatory reactions even in the absence of any infectious or toxic agents (6)(Chae et al., 2009).

FMF is a disease with incomplete penetrance and variable expression. Severity and range of clinical symptoms are widely variable in different regions, most probably because of the different types of gene mutations, modifier genes, and environmental factors (https://www.uptodate.com). To date, more than 357 disease-associated mutations and polymorphisms in the *MEFV* gene have been reported (https://infevers.umai-montpellier.fr/web), where the majority of mutations are missense changes and more than half of them cluster in exons 2 and 10 (Booty et al., 2009). Most of the cases are caused by four mutations clustered on exon 10 (*M694V*, *V726A*, *M680I*, and *M694I*) and exon 2 (*E148Q*), which represent approximately 70%–86% of mutations (Etem, Deveci, Erol, Yuce, & Elyas, 2010) based on ethnic group (Cazeneuve et al., 1999). The *M694V* mutation is one of the most common mutations (20%–65%) and the corresponding carriers exhibit severe phenotypes and are more likely to develop amyloidosis. Although about 10%–20% of individuals with typical clinical symptoms do not have any mutations in the *MEFV* gene, patients with mutations in exon 10 often demonstrate severe clinical symptoms, while patients with exon 2 and 3 mutations have milder symptoms. In Japan, exon 2–4 mutations are common, and patients have mild symptoms and are treated with lower doses of colchicine (Migita et al., 2012). Early diagnosis of FMF initially avoids unnecessary surgeries such as appendectomy. Second, treatment with colchicine improves disease symptoms and ultimately prevents the occurrence of amyloidosis and renal failure. Molecular investigation of the *MEFV* gene is a useful method in clinical diagnosis, especially in atypical forms of the disease (Etem et al., 2010; Sohar et al., 1967).

The present study aims to investigate the frequency of the *MEFV* gene mutations in clinically suspected Iranian patients in the Northwest of Iran and to compare the results with the studies performed in other countries. The findings will also help establish an early diagnosis of FMF mutations and provide a link that will probably facilitate the clinical interpretation of individualized genetic data. Also, evaluation of phenotypic features of Iranian FMF patients is an important step for family counseling and case management.

2 | MATERIALS AND METHODS

2.1 | Study design, population, and sampling

Our study population was comprised of 400 unrelated FMF patients referred from medical centers with different specialties throughout the Northwest of Iran. Tel-Hashomer criteria were considered as diagnostic criteria (Livneh et al., 1997). The origin of all patients was Azari-Turkish, one of the biggest ethnic groups in Iran who mainly live in East and West Azerbaijan provinces. A questionnaire, including the main clinical information, was registered on a standard form: age, gender, the origin of parents, consanguinity, familial history of FMF, clinical features during attacks (fever, abdominal pain, arthritis, chest pain, or renal disorders), and intake of colchicine. Written informed consent was obtained from all participants in this study.

2.2 | Mutation detection

Peripheral blood samples (~3 ml) were obtained in EDTA tubes from each patient and stored at -20°C for future use. The diagnostic strategy was based on direct Sanger sequencing of exons 2, 3, 5, and 10 of the *MEFV* gene after amplification by polymerase chain reaction (PCR), which allows the detection of any variation on these exons. Genomic DNA was extracted using a previously described standard phenol-chloroform method. PCR reactions were carried out using specific primers for exons 2, 3, 5, and 10: exon 2F: 5′-ATGCGACCTAGAAGCCTTGA-3′ and exon 2R: 5′-GGTGACCGAATGTTCTGGAT-3′; exon 3F: 5′-CAGGAAGGAGACCCAGTTG -3′ and exon 3R: 5′- CAGACTGCAGATGAGGCAGA-3′; exon 5F: 5′-AGGAAGCTGGAGCAGGTGTA-3′ and exon

TABLE 1 Characteristics of patients with familial Mediterranean fever (FMF) in Azari-Turkish in Iran

Variables		F[a] (%)
Gender	Male	194(48.5)
	Female	206(51.5)
Location	West Azarbaijan	67(16.8)
	East Azarbaijan	317(79.2)
	Ardebil	9(2.2)
	Another state	1(0.2)
	Foreign	6(1.5)
Consanguineous marriage	Positive	97(24.2)
	Negative	303(75.8)
	3th degree	66(16.5)
	4th and 5th degree	31(7.8)
Familial history of FMF	Positive	180(45)
	Negative	220(55)
	Near	156(39)
	Far	24(6)
Clinical presentations	Fever	204(51)
	Abdominal pain	261(65.2)
	Joint pain	171(42.8)
	Chest pain	119(29.8)
	Kidney pain	107(26.8)
Receiving drug (Colchicine)	Positive	69(17.2)
	Negative	331(82.8)

[a]Frequency

5R: 5′-TGCAGAAGTTCCCATTCTGA-3′; exon 10F: 5′-AGAATGGCTACTGGGTGGTG-3′ and exon 10R: 5′-AGAGCAGCTGGCGAATGTAT-3′. PCR products were then electrophoresed on a 2% agarose gel. The direct Sanger sequencing method was performed on the PCR products. The sequencing data was analyzed using sequencer software and aligned with reference sequences using online bioinformatics tools.

2.3 | Statistical analysis

Statistical analysis was performed using SPSS software (version 17.0). Chi-square and Fisher exact tests were used to compare genotype–phenotype correlations with regard to the mutation types. For all tests, $P < 0.05$ was considered statistically significant.

3 | RESULT

3.1 | Clinical features

In the present study, 400 cases were reviewed. The demographic characteristics of the patients are shown in Table 1. Out of 400 patients, 194 (48.5%) were males and 206 (51.5%) were female. The mean age of patients was 24.12 years (range, 2–70 years). The most frequent clinical findings in all subjects were abdominal pain (65.2%) and fever (51%) followed by joint pain (42.8%), chest pain (29.8%), and kidney pain (26.8%). Sixty-nine patients (17.2%) were receiving oral colchicine regularly. Sixty-seven individuals were from West Azerbaijan, while 317 individuals were from East Azerbaijan. Consanguinity was reported in 24.2% of the patients (Table 1).

3.2 | *MEFV* gene mutations

MEFV gene mutation spectrum for the investigated patients is shown in Table 2. Out of 400 analyzed subjects, no mutations were found in 152 (38%), while at least one mutation was found in 248 (62%) patients. Fourteen distinct types of mutation were detected in patients. Most of the mutation types were observed on exon 10. The most common mutation was *M694V* with a frequency of 26.25% followed by *E148Q* (24.75%), *V726A* (11.25%), *M680I* (10.75%), and *R761H* (5.75%). Additionally, the *A744S*, *F479L*, *M694I*, *M694L*, *E167D*, *P180R*, *A289V*, and *K695R* mutations were observed as rare mutations of the *MEFV* gene in the present study (Table 2).

The distribution of various genotypes in all patients is shown in Table 3. Thirty-three distinct genotypes were detected. Out of 248 patients with the mutation, 136 (54.83%) were heterozygous, 82 (33.05%) were compound heterozygous, 27 (10.88%) were homozygous, and 3 (1%) had a complex genotype (Table 3). The most common heterozygous and compound heterozygous genotypes were *E148Q/wt* (23.38%) followed by *M694V/wt* (16.12%) and *M694V/E148Q* (7.25%), followed by *M694V/V726A* (6.85%), respectively. The *M694V/M694V* (6.44%) was the prevalent homozygous genotype.

3.3 | Phenotype–genotype correlation

In this study, we analyzed the correlation of the clinical features with common *MEFV* gene mutations and genotypes. The most common symptom associated with mutations was abdominal pain with a frequency of 57% that showed a significant association with *M694V* (*M694V/wt* heterozygous genotype [62%]) and *E148Q* mutations. The second-most common symptom was fever, which had a significant relationship with *M694V*, *E148Q*, *V726A*, and *P313H* mutations. *M694V*, as the most prevalent mutation in this study, showed a significant relationship with the occurrence of fever, abdominal pain, kidney pain, chest pain, and colchicine consumption, while patients harboring the *E148Q* mutation showed only a significant relationship with fever and abdominal pain ($P < 0.05$) (Table 4). Fever and abdominal pain were common phenotypes in almost all distinctive genotypes, whereas joint

TABLE 2 *MEFV* mutations observed in investigated familial Mediterranean fever (FMF) patients

No.	Gene location	Nucleotide mutation	Codon change	Amino acid	Effect of protein	Genotypes		Mutation frequency (out of 400 patients) *F* (%)	Allele frequency (out of 800 alleles) *F* (%)
						Homozygous *F*[a] (%)	Heterozygous *F* (%)		
1	Exon 10	c.2080A > G	GAT→GGT	Met→Val	M694V	17 (4.25)	88 (22.0)	105 (26.25)	A:678 (85) G:122 (15)
2	Exon 2	c.442G > C	CGA→CCA	Glu→Gln	E148Q	3 (0.8)	96 (24.0)	99 (24.75)	G:698 (87) C:102 (13)
4	Exon 10	c.2177T > C	GTT→GCT	Val→Ala	V726A	2 (0.5)	43 (10.75)	45 (11.25)	T:753 (94) C:47 (6)
5	Exon 10	c.2040G > A	TGA→TAA	Met→Ile	M680I	4 (1.00)	39 (9.75)	43 (10.75)	G:753 (94) A:47 (6)
3	**Exon 3**	**c.938C > A**	**CCC→CAC**	**Pro→His**	**P313H**	**1 (4.16)**	**23 (95.83)**	**24 (6)**	**C:775 (97) A:25 (3)**
6	Exon 10	c.2282G > A	CGT→CAT	Arg→His	R761H	1 (0.25)	22 (5.50)	23 (5.75)	G:776 (97) A:24 (3)
7	Exon 10	c.2230G > T	CGC→CTC	Ala→Ser	A744S	0 (0.0)	6 (1.50)	6 (1.5)	G:794 (99) T:6 (1)
8	Exon 5	c.1437C > G	TCT→TGT	Phe→Leu	F479L	0 (0.0)	5 (1.0)	5 (1.25)	C:795 (99.5) G:5 (0.5)
9	Exon 10	c.2082G > A	TGA→TAA	Met→Ile	M694I	0 (0.0)	4 (1.00)	4 (1)	G:796 (99.5) A:4 (0.5)
10	Exon 10	c.2080A > T	GAT→GTT	Met→Leu	M694L	0 (0.0)	2 (0.50)	2 (0.5)	A:798 (99.7) T:2 (0.2)
11	Exon 2	c.501G > C	AGG→ACG	Glu→Asp	E167D	1 (0.3)	1 (0.3)	2 (0.5)	G:798 (99.7) C:2 (0.2)
12	Exon 2	c.539C > G	CCG→CGG	Pro→Arg	P180R	0 (0.0)	1 (0.3)	1 (0.25)	C:799 (99.8) G:1 (0.1)
13	Exon 2	c.866C > T	GCG→GTG	Ala→Val	A289V	0 (0.0)	1 (0.3)	1 (0.25)	C:799 (99.8) T:1 (0.1)
14	Exon 10	c.2084A > G	AAG→AGG	Lys→Arg	K695R	0 (0.0)	1 (0.25)	1 (0.25)	A:799 (99.8) G:1 (0.1)

[a]Frequency.

The bold row represents the new mutation and its features.

TABLE 3 Genotype distribution of FMF Patient in Azari –Turkish in Iran

Mutation Type F (%)	Genotypes	Patient F†	%
Heterozygous 136 (54.83%)	E148Q/wt	58	23.38
	M694V/wt	40	16.12
	M680I/wt	13	5.24
	V726A/wt	11	4.43
	A744S	6	2.41
	R761H/wt	3	1.21
	M694I/wt	3	1.21
	K695R	1	0.4
	A289V	1	0.4
Compound Heterozygous 82 (33.05%)	M694V/E148Q	18	7.25
	M694V/V726A	17	6.85
	E148Q/M680I	8	3.22
	M694V/M680I	7	2.82
	E148Q/ R761H	6	2.41
	M694V/R761H	5	2.01
	M680I/V726A	5	2.01
	E148Q/ V726A	4	1.61
	V726A/E148Q	3	1.21
	V726A/R761H	3	1.21
	M680I/R761H	2	0.8
	M680I/M694L	1	0.4
	M680I/M694I	1	0.4
	V726A/F479L	1	0.4
	M694V/F479L	1	0.4
Complex genotype 3 (1.2%)	M680I/ E148Q/ R761H	2	0.8
(Compound Heterozygous and homozygous)	M694V:M694V/P180R	1	0.4
Homozygous 27(10.88%)	M694V/ M694V	16	6.44
	M680I/ M680I	4	1.61
	E148Q/ E148Q	3	1.21
	V726A/ V726A	2	0.8
	R761H/ R761H	1	0.4
	E167D/E167D	1	0.4
Total patients with mutation	–	248	62
Patients without mutation	–	152	38
Total	–	400	100

†Frequency

pain and chest pain were common only in heterozygous and homozygous genotypes.

The rate of abdominal pain and chest pain were higher in patients with the *M694V/wt* genotype followed by the *E148Q/wt* genotype, while the rate of fever and joint pain were higher in patients with a *E148Q/wt* genotype and were lower in patients with a *M694V/wt* genotype (Table 4). Based on the data, all of the patients with *M694V* mutation were consuming colchicine while the patients with *M680I* and *V726A* mutations received no medication. Our data demonstrate that the patients with the *M694V* mutation had more severe phenotypes than patients with other genotypes.

To investigate the genotype–phenotype correlation, considering the high prevalence of *M694V* mutation in our study population, observed genotypes were divided into four groups based on the presence of the *M694V* mutation (Table 5). Out of 248 patients carrying mutation, most of the patients had no *M694V* mutations (group 4, $N = 143$). Forty-nine patients had *M694V*/others (group 3) followed by *M694V*/wt heterozygous (group 2, $N = 40$) and *M694V*/*M694V* homozygous genotypes (group 1, $n = 16$). Abdominal pain was the most common phenotype in all genotype groups and kidney pain was the least common.

Among patients with *M694V* mutation, individuals with *M694V*/others genotype (group 3) had a higher incidence of abdominal pain (52%), fever (42%), joint pain (41%), chest pain (23%), and kidney pain (22%). Comparing these groups, statistically, significant differences between groups regarding their correlations with specific phenotypes were observed. For fever, when comparing groups 1 and 2, 1 and 4, 2 and 3, and 3 and 4, significant differences were clear ($P < 0.05$). All studied clinical symptoms except chest pain revealed significant differences comparing group 1 and group 4. There were also statistically significant differences between genotype–phenotype correlations (except joint pain) comparing groups 3 and 4 ($P < 0.05$). There were no statistically significant differences among groups 1 and 2, 1 and 3, and 2 and 4 ($P > 0.05$) regarding correlation with abdominal pain; however, these differences were significant when comparing groups 1 and 4 and 2 and 3 ($P < 0.05$). With comparison groups 3 and 4, we did not detect any statistically significant differences between groups regarding their correlations with chest pain. Comparing groups 1 and 4 and 3 and 4, significant differences considering their correlations with kidney pain were interpreted ($P \leq 0.05$). The most frequent drug-consuming patients were individuals with a *M694V*/*M694V* homozygous genotype, approximately 50% of whom were receiving colchicine.

3.4 | Report of a novel variation: *P313H*

Sanger sequencing for exons 2, 3, 5, and 10 of the *MEFV* gene in 400 patients with typical FMF symptoms revealed a new variant: *P313H* missense variation in exon 3, which is caused by a single-nucleotide substitution (C > A) at nucleotide number 938, resulting in a proline–histidine amino acid substitution at the 313th codon (CCC→CAG). Out of 400 patients, 24 (6%) had a *P313H* variation of which 23 were heterozygote and only one patient was homozygote. The most common clinical symptom in these patients was abdominal pain (75%)

TABLE 4 Genotype–phenotype correlations of prevalent genotype

Genotype	No. (%)	Gender		Fever	Abdominal pain	Joint pain	Chest pain	Kidney pain	Receiving colchicine
		Male	Female						
Heterozygous									
E148Q/wt	58 (23.38)	25 (43)	23 (39)	20 (34)	23 (39)	22 (38)	11 (19)	12 (20)	4 (9)
M694V/wt	40 (16.12)	21 (52)	19 (47)	16 (40)	25 (62)	16 (40)	12 (30)	12 (30)	7 (17)
M680I/wt	13 (5.24)	7 (53)	6 (46)	2 (15)	4 (30)	4 (30)	0	1 (7)	0
V726A/wt	11 (4.43)	6 (54)	5 (45)	3 (27)	4 (36)	6 (54)	2 (18)	2 (18)	0
Compound heterozygous									
M694V/V726A	17 (6.85)	10 (59)	7 (41)	14 (82)	15 (88)	8 (47)	9 (53)	8 (47)	6 (35)
M694V/E148Q	18 (7.25)	9 (50)	9 (50)	13 (72)	15 (83)	7 (39)	8 (44)	6 (33)	3 (16)
Homozygous									
M694V/M69V	16 (6.45)	10 (62)	6 (37)	13 (81)	14 (87)	13 (81)	7 (43)	9 (56)	7 (43)
Total	173	88 (59)	75 (43)	81 (47)	100 (57)	76 (43)	49 (28)	63 (36)	27 (15)

TABLE 5 Genotype-phenotype correlations according to *M694V* mutation

Clinical features	Group I N=16	Group II N=14	Group III N=49	Group IV N=143	P (Gr1/2)	P (Gr1/3)	P (Gr1/4)	P (Gr2/3)	P (Gr2/4)	P (Gr3/4)
Fever	13(81%)	16(40%)	39(79%)	60(42%)	0.005	0.88	0.003	<0.001	0.824	<0.001
Abdominal pain	14(87%)	25(62%)	43(87%)	75(52%)	0.066	0.97	0.007	0.005	0.259	<0.001
Joint pain	13(81%)	16(40%)	22(44%)	59(41%)	0.005	0.011	0.002	0.642	0.886	0.656
Chest pain	7(44%)	12(30%)	25(51%)	34(23%)	0.326	0.614	0.109	0.08	0.422	<0.001
Kidney pain	9(56%)	12(30%)	21(42%)	32(22%)	0.067	0.351	0.003	0.212	0.319	0.007
Colchicine	7(44%)	7(17%)	15(30%)	19(13%)	0.04	0.716	0.002	0.154	0.5	0.006

Note: Group I, M694V/M694V; Group II, M694V/wt; Group III, M694V/others; Group IV, Other mutations.

and fever (62.5%). Only the homozygote patient had all the symptoms of the disease. Four out of 24 patients were taking colchicine.

3.5 | Polymorphisms

In this study, we found several genetic variants. We found 26 variations in total, of which five were new. The most common variation was *G138G* with a frequency of 14.2% followed by *E474E* (13.5%), *D510D* (13.3%), *Q476Q* (13%), *A165A* (12.8%), *D102D* (11%), *R202Q* (9.3%), and *R314R* (8.1%). Another variation was also present as rare variants (frequency between 0.1% and 0.6%). Most variants were clustered in exon 2 with variation types.

Out of 400 analyzed patients, 25 individuals demonstrated the *MEFV* gene without any polymorphism, while six of them did not have the mutation. Only *E148Q* and *M694V* mutations were observed in patients without polymorphism. Moreover, we found five new variants, *P313S* and *A310D* in exon 3, *L508Q* and *D510Q* in exon 5, and *L617L* in exon 10.

The distribution of polymorphic genotypes in the 400 patients has also been evaluated in this study and 144 distinct genotypes were detected. Most genotypes were isolated in one or two patients. The most common polymorphic genotypes are listed in Table 6.

TABLE 6 Genotype distribution of polymorphic variants among FMF patients

Polymorphic variants (genotypes)	N.(%)
R314R/D510D/E474E/Q476Q (HET[a])	21(5.2)
D102D/G138G/A165A/R202Q (HET)	20(5)
D102D/G138G/A165A/R202Q (HOM[b])	19(4.75)
D102D/G138G/A165A/R202Q/R314R/D510D /E474E/Q476Q (HET)	15(3.75)
D510D/E474E/Q476Q (HET)	14(3.5)
D102D/G138G/A165A (HET)	14(3.5)
R314R/D510D/E474E/Q476Q (HOM)	13(3.25)
D102D/G138G/A165A/R202Q /D510D/E474E /Q476Q (HET)	11(2.75)
D510D/E474E/Q476Q (HOM)	10(2.5)
Other variants	238(59.5)
Patients without variant	25(6.25)
Total	400(100)

[a]Heterozygote
[b]Homozygote

4 | DISCUSSION

According to the type of mutation, the phenotype of the disease varies greatly. Therefore, the identification of common

mutations in each region and their genotype–phenotype correlation can be helpful in diagnosing the disease quickly and accurately. In the current study, clinical and laboratory results of 400 patients with FMF from Northwestern Iran have been analyzed. Most of the patients were of Turkish origin and living in Northwestern Iran. Although in previous studies the sex distribution is comprised of men as having the major proportion (Meyerhoff, 1980), the distribution of FMF was slightly higher in females than males in this study.

Twenty-five percent of the patients have been observed in consanguineous marriages and more than half of the cases were grade 3. A positive family history of FMF has been observed in 20% of patients, especially close relatives. The diagnosis of FMF is usually based on clinical presentations; however, in children and mild forms, the diagnostic approach becomes more difficult. Therefore, positive family history and ethnic origin confirm a definitive diagnosis (Berkun & Eisenstein, 2014). In a study performed by Coşku et al. on FMF-patients, a positive FMF family history was 43% (Coşku, Kurtgöz, Keskin, Sönmez, & Bozkurt, 2015). The pathogenesis of FMF has not been specified exactly (Berkun & Eisenstein, 2014; Petrushkin, Stanford, Fortune, & Jawad, 2016), although the *MEFV* mutations are the essential factors (Coşku et al., 2015; Sari et al., 2013). So far, approximately 321 types of the mutation have been reported for FMF with various mutations that cause different phenotypes in patients (Cekin, Akyurek, Pinarbasi, & Ozen, 2017; Coşku et al., 2015).

In this study, out of 400 analyzed subjects, no mutation was detected in 152 (38%) patients, whereas at least one mutation was found in 248 (62%) patients. Mutation in another region of *MEFV* gene and other genes may involve in FMF pathogenesis. Of course, it should be considered that incomplete penetrance, changes in the expression of the *MEFV* gene, and the presence of other genetic factors can affect the expression of the *MEFV* gene in FMF patients (Ozen et al., 2014; Topaloglu et al., 2005). The most common mutations in Turkish and Armenian populations are *M694V*, *M680I*, *V726A*, and *E148Q* (Ben-Chetrit & Touitou, 2009). These four major mutations have also been diagnosed in our FMF patients and nearly 90% of patients have at least one of these four mutations. *M694V* has been reported more commonly in the Mediterranean basin, Europe, America, and Africa except for Algeria, while 30% of the Arab populations have been involved with this mutation (Ait-Idir, Bouldjennet, Taha, El-Shanti, & Djerdjouri, 2015; Coşku et al., 2015; Sugiyama et al., 2014). Only the *E148Q* mutation is known as the most common mutation in Japan (Sugiyama et al., 2014). Most of the studies performed on FMF patients in Iran have reported *M694V* as the most common *MEFV* mutation with a frequency ranging from 11% to 30%, while some studies reported *E148Q* as the most common mutation (9% to 25%) (Beheshtian et al., 2016; Hosseini, Dolatshahi, Ebadi, & Zahedi-Shoolami, 2014; Salehzadeh, 2015; Zali et al., 2003). Similar to the other studies, *M694V* mutation was the most common mutation in this study with a frequency of 26.25%; however, *E148Q*, as indicated by several other studies, was the second most common mutation (Cekin et al., 2017; Pasa et al., 2008). After *M694V* and *E148Q*, other frequent mutations observed in this study were *V726A*, *M680I*, *P313H*, and *R761H* with a frequency of 11%, 10%, 6%, and 5%, respectively. *V726A* mutation was reported in several studies as the second most common mutation (Salehzadeh, 2015); however, we report it here as the third most frequent. *A744S*, *F479L*, *M694I*, *M694L*, *E167D*, *P180R*, *A289V*, and *K695R* are rare mutations with a frequency ranging from 0.25% to 1.5% in our study. The difference in the frequency of *MEFV* mutations has been demonstrated in most studies, which can be the result of differences in the method of mutation identification or size of samples, and, more importantly, the different ethnicity of the study population, which strongly affects the frequency of mutations probably because of founder effects. We also reported *P313H* as a novel mutation, which was detected in 6% of our FMF patients.

In the current study, the most common clinical manifestations were related to abdominal pain and fever. Arthritis, chest pain, and renal disorders were other symptoms. Considering the results of the previous studies, the frequency of these clinical presentations varies in different populations; however, the most frequent symptoms are abdominal pain and fever for all ethnics (Cekin et al., 2017). Fever is observed in almost all patients, although it is ignored (Ozen & Bilginer, 2014). The second most common FMF features are articular involvement (Berkun & Eisenstein, 2014; Cekin et al., 2017). Chest pain, joint pain, and myalgia are observed in nearly 50% of patients and skin rashes affect 3% to 46% of patients (Berkun & Eisenstein, 2014; Cekin et al., 2017; Petrushkin et al., 2016).

Overall, the most frequent genotypes in FMF patients are heterozygous, compound heterozygous, and homozygous genotypes, respectively. In previous studies conducted in different regions of Iran, compound heterozygous and homozygous genotypes were the prevalent genotypes (Hosseini et al., 2014; Salehzadeh, 2015). Our results are similar to that of Beheshtian et al. (2016), in which the heterozygous genotype was the most common one. We also evaluated genotype–phenotype correlations of prevalent genotypes. We found that the patients with *E148Q/wt* genotype suffered more from fever and joint pain while the abdominal pain and chest pain were the main symptoms of the patients with the *M694V/wt* genotype.

Because of the high frequency of the *M694V* mutation among the patients and the presence of different genotype forms regarding this mutation, clinical presentations were compared among heterozygous, compound heterozygous, and homozygous genotypes of *M694V* mutation. These

comparisons were more significant in homozygous genotypes of the *M694V* mutation than heterozygous genotypes. Comparing homozygous genotypes of *M694V* with other genotypes, we also found significant differences in all clinical presentations except chest pain. Our results demonstrated that the *M694V* mutation, especially in homozygote form, was associated with severe clinical manifestations, and approximately 50% of the patients received colchicine, similar to other studies (Berkun & Eisenstein, 2014; Coşku et al., 2015).

5 | CONCLUSION

Based on our results, abdominal pain and fever were general phenotypes in all patients. Fifty-seven percent of mutations were in exon 10 and the rest were scattered in exons 2, 3, and 5. In our studied population, *M694V* and *E148Q* mutations were significantly high and patients with *M694V* mutation had more severe phenotypes than other mutations. Therefore, taking the high prevalence of the FMF in this region into account, patients with unexplained abdominal pain and fever should be counseled and genetically tested for the rejection of the FMF. Moreover, the PCR technique provides a rapid, reliable, cost-effective, noninvasive, and sensitive test to establish a diagnostic approach for FMF analysis in symptomatic patients. Additionally, analysis of genotype–phenotype correlation in FMF disease will be helpful in diagnosis and follow-up of patients. Performing multicenter FMF studies with large sample sizes in the regions with high FMF prevalence are also recommended to confirm the results.

ACKNOWLEDGMENTS

We are thankful to all patients who participated in this study. We also greatly appreciate the staff of the Tabriz Genetic Analysis Center.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

INFORMED CONSENT

Informed consent was received from all human participants.

ORCID

Leila Rostamizadeh
https://orcid.org/0000-0001-8770-7188

REFERENCES

Ait-Idir, D., Bouldjennet, F., Taha, R., El-Shanti, H., & Djerdjouri, B. (2015). Prevalence of Mediterranean fever gene mutations in clinically suspected FMF patients in Algeria. *Pediatric Rheumatology*, *13*(1), P94.

Beheshtian, M., Izadi, N., Kriegshauser, G., Kahrizi, K., Mehr, E. P., Rostami, M., & Kariminejad, A. (2016). Prevalence of common *MEFV* mutations and carrier frequencies in a large cohort of Iranian populations. *Journal of Genetics*, *95*(3), 667–674.

Ben-Chetrit, E., & Touitou, I. (2009). Familial Mediterranean fever in the world. *Arthritis Care & Research*, *61*(10), 1447–1453.

Berkun, Y., & Eisenstein, E. M. (2014). Diagnostic criteria of familial Mediterranean fever. *Autoimmunity Reviews*, *13*(4), 388–390.

Bonyadi, M., Esmaeili, M., Karimi, A., & Dastgiri, S. (2010). Common Mediterranean fever gene mutations in the Azeri Turkish population of Iran. *Genetic Testing and Molecular Biomarkers*, *14*(1), 149–151. Retrieved from https://doi.org/10.1089/gtmb.2009.0087

Booty, M. G., Chae, J. J., Masters, S. L., Remmers, E. F., Barham, B., Le, J. M., … Aksentijevich, I. (2009). Familial Mediterranean fever with a single MEFV mutation: Where is the second hit? *Arthritis & Rheumatology*, *60*(6), 1851–1861.

Cazeneuve, C., Sarkisian, T., Pêcheux, C., Dervichian, M., Nédelec, B., Reinert, P., … Delpech, M. (1999). *MEFV*-gene analysis in Armenian patients with familial Mediterranean fever: Diagnostic value and unfavorable renal prognosis of the M694V homozygous genotype—Genetic and therapeutic implications. *The American Journal of Human Genetics*, *65*(1), 88–97.

Cekin, N., Akyurek, M. E., Pinarbasi, E., & Ozen, F. (2017). *MEFV* mutations and their relation to major clinical symptoms of familial Mediterranean fever. *Gene*, *626*, 9–13.

Chae, J. J., Aksentijevich, I., & Kastner, D. L. (2009). Advances in the understanding of familial Mediterranean fever and possibilities for targeted therapy. *British Journal of Haematology*, *146*(5), 467–478.

Coşku, S., Kurtgöz, S., Keskin, E., Sönmez, F., & Bozkurt, G. (2015). Frequency of mutations in Mediterranean fever gene, with gender and genotype–phenotype correlations in a Turkish population. *Journal of Genetics*, *94*(4), 629–635.

Elshafey, A. E., Kader, N. A., Barakat, N., Azab, A., Bastaki, L., & Al-Awadi, S. (2011). Clinical and molecular genetic study of familial Mediterranean fever in a mixed Arab population. *International Journal of Academic Research*, *3*(3), 204–208.

Etem, E., Deveci, S. D., Erol, D., Yuce, H., & Elyas, H. (2010). Familial Mediterranean fever: A retrospective clinical and molecular study in the East of Anatolia region of Turkey. *The Open Rheumatology Journal*, *4*, 1.

Fonnesu, C., Cerquaglia, C., Giovinale, M., Curigliano, V., Verrecchia, E., de Socio, G., … Manna, R. (2009). Familial Mediterranean fever: A review for clinical management. *Joint Bone Spine*, *76*(3), 227–233.

Hosseini, M., Dolatshahi, E., Ebadi, H., & Zahedi-Shoolami, L. (2014). Familial Mediterranean fever in the Iranian population: *MEFV* mutations in different ethnic groups. *Indian Journal of Rheumatology*, *9*(1), 4–8.

Livneh, A., Langevitz, P., Zemer, D., Zaks, N., Kees, S., Lidar, T., … Pras, M. (1997). Criteria for the diagnosis of familial Mediterranean fever. *Arthritis & Rheumatology*, *40*(10), 1879–1885. Retrieved from https://doi.org/10.1002/1529-0131(199710)40:10<1879::Aid-art23>3.0.co;2-m

Meyerhoff, J. (1980). Familial Mediterranean fever: Report of a large family, review of the literature, and discussion of the frequency of amyloidosis. *Medicine*, *59*(1), 66.

Migita, K., Uehara, R., Nakamura, Y., Yasunami, M., Tsuchiya-Suzuki, A., Yazaki, M., … Furukawa, H. (2012). Familial Mediterranean fever in Japan. *Medicine*, *91*(6), 337–343.

Nobakht, H., Zamani, F., Ajdarkosh, H., Mohamadzadeh, Z., Fereshtehnejad, S., & Nassaji, M. (2011). Adult-onset familial Mediterranean fever in Northwestern Iran; clinical feature and treatment outcome. *Middle East Journal of Digestive Diseases*, *3*(1), 50.

Ozen, S., & Bilginer, Y. (2014). A clinical guide to autoinflammatory diseases: Familial Mediterranean fever and next-of-kin. *Nature Reviews Rheumatology*, *10*(3), 135–147.

Ozen, S., Demirkaya, E., Amaryan, G., Koné-Paut, I., Polat, A., Woo, P., … Quartier, P. (2014). Results from a multicentre international registry of familial Mediterranean fever: Impact of environment on the expression of a monogenic disease in children. *Annals of the Rheumatic Diseases*, *73*(4), 662–667.

Pasa, S. R., Altintas, A., Devecioglu, B. L., Cil, T. M. N., Danis, R., Isi, H. L., … Batun, S. (2008). Familial Mediterranean fever gene mutations in the Southeastern region of Turkey and their phenotypical features. *Amyloid*, *15*(1), 49–53.

Petrushkin, H., Stanford, M., Fortune, F., & Jawad, A. S. (2016). Clinical review: Familial mediterranean fever—An overview of pathogenesis, symptoms, ocular manifestations, and treatment. *Ocular Immunology and Inflammation*, *24*(4), 422–430.

Salehzadeh, F. (2015). Familial Mediterranean fever in Iran: A report from FMF registration center. *International Journal of Rheumatology*, *2015*, 912137.

Sari, I., Savran, Y., Kozaci, D., Gunay, N., Onen, F., & Akar, S. (2013). THU0462 increased oxidative stress and macrophage migration inhibitory factor in patients with familial Mediterranean fever. *Annals of the Rheumatic Diseases*, *72*(Suppl 3), A320–A321.

Sohar, E., Gafni, J., Pras, M., & Heller, H. (1967). Familial Mediterranean fever: A survey of 470 cases and review of the literature. *The American Journal of Medicine*, *43*(2), 227–253.

Sugiyama, R., Agematsu, K., Migita, K., Nakayama, J., Mokuda, S., Ogura, F., … Morikawa, S. (2014). Defect of suppression of inflammasome-independent interleukin-8 secretion from SW982 synovial sarcoma cells by familial Mediterranean fever-derived pyrin mutations. *Molecular Biology Reports*, *41*(1), 545–553.

Topaloglu, R., Ozaltin, F., Yilmaz, E., Ozen, S., Balci, B., Besbas, N., & Bakkaloglu, A. (2005). *E148Q* is a disease-causing *MEFV* mutation: A phenotypic evaluation in patients with familial Mediterranean fever. *Annals of the Rheumatic Diseases*, *64*(5), 750–752.

Tunca, M., Akar, S., Onen, F., Ozdogan, H., Kasapcopur, O., Yalcinkaya, F., … Turkish FMF Study Group. (2005). Familial Mediterranean fever (FMF) in Turkey: Results of a nationwide multicenter study. *Medicine*, *84*(1), 1–11.

Zali, M. R., Moghaddam, S. J. M., Taghizadeh, F., Noroozi, N., Narimani, A., Peyman, S., & Derakhshan, F. (2003). *MEFV* gene mutations in Iranian patients with familial Mediterranean fever (FMF). *The American Journal of Gastroenterology*, *98*(9), S72–S73.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Rostamizadeh L, Vahedi L, Bahavarnia SR, et al. Mediterranean fever (*MEFV*) gene profile and a novel missense mutation (*P313H*) in Iranian Azari-Turkish patients. *Ann Hum Genet*. 2019;1–9. https://doi.org/10.1111/ahg.12347