

MEFV Mutations in Patients with Familial Mediterranean Fever in the Black Sea Region of Turkey

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ABSTRACT. Objective. To investigate MEFV mutations among patients with familial Mediterranean fever (FMF), their relatives, and healthy controls in the Black Sea region of Turkey; to compare 3 different MEFV mutation analysis methods; to evaluate the role of MEFV mutations in the diagnosis of FMF; and to investigate the role of M694V in the development of amyloidosis.

Methods. In total, 890 subjects (625 patients, 165 relatives, 100 healthy controls) were included in this prospective study. MEFV mutations were studied with the amplification refractory mutation system (ARMS; $n = 335$), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP; $n = 335$), and reverse hybridization assay (FMF StripAssay; $n = 693$).

Results. All methods were used in 79 patients. The ratio of false negativity was about 2% using ARMS compared to PCR-RFLP. The FMF StripAssay was used to investigate 9 more mutations and detected 17 mutations in 14 patients. The M694V/M694V genotype was more common in patients with amyloidosis (37%) compared to patients without amyloidosis (18%) ($p = 0.009$). The frequency of MEFV carriers was 27%. The frequency of individuals having 2 mutations among asymptomatic relatives of FMF patients was 6%.

Conclusion. The FMF StripAssay is a reliable and time-saving method. In spite of detection of new mutations and developments in MEFV assay technology, there were patients in whom no mutation was detected. Our data, combined with previous studies, show that patients having M694V/M694V carry a risk for amyloidosis. (First Release Dec 1 2007; J Rheumatol 2008;35:106–13)

Key Indexing Terms:

FAMILIAL MEDITERRANEAN FEVER
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Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by recurrent self-limited attacks of fever with serositis, synovitis, or erysipelas-like skin lesions^{1,2}. The disease primarily affects non-Ashkenazi Jews, Armenians, Arabs, and Turks with phenotypical variations. The gene responsible for the disease, MEFV, which is localized on chromosome 16.123.3, was identified in 1997^{3,4}. To date, over 100 mutations in exons 2, 3, 5, and 10 have been identified⁵. Since the diagnosis of FMF is mainly based on clinical findings^{2,5,6}, identification of genes responsible for FMF led to expectations in the diagnosis of FMF. Through MEFV gene analysis, 3 clinical definitions for diagnosis have

been proposed. Patients with typical clinical features are defined as phenotype I. Patients who present with renal amyloidosis without typical attacks of the disease have been defined as phenotype II⁷⁻⁹. Individuals who do not have any symptoms related to FMF but have at least 2 MEFV mutations are referred to as phenotype III^{10,11}. Genetic analysis has some limitations in the diagnosis of FMF. Another unresolved issue is the relation between genotype and phenotype in FMF. Several investigators have reported that M694V homozygous genotype was associated with the development of amyloidosis, whereas others did not confirm this¹²⁻¹⁶.

The aims of our study were (1) to investigate MEFV mutations among patients with FMF, their relatives, and healthy controls in the Samsun region of Turkey; (2) to compare 3 different MEFV mutation analysis methods; (3) to evaluate the diagnostic value of MEFV mutations in the diagnosis of FMF; and (4) to investigate the role of M694V mutation in the development of amyloidosis.

MATERIALS AND METHODS

Eight hundred ninety subjects (625 patients with diagnosis of definite or probable FMF, 165 relatives of patients with FMF, and 100 healthy controls) were included in this prospective study. Subjects were questioned for the presence of the Tel-Hashomer criteria for diagnosis of FMF¹⁷ and categorized into 3 groups — those with definite or probable FMF and healthy controls. Subjects

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referred as having FMF, but not having definite or probable FMF based on the Tel-Hashomer criteria, were not included in the study.

Demographic characteristics of subjects are shown in Table 1. Most of the patients (87%) as well as relatives and controls were from the middle Black Sea region.

Five ml blood samples were collected from all subjects. DNA extraction was done as described¹⁸. MEFV mutations were studied with 3 different methods: the amplification refractory mutation system (ARMS), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and a reverse hybridization assay (FMF StripAssay; ViennaLab Labordiagnostika GmbH, Vienna, Austria). In order to compare 3 methods, MEFV mutations were studied with more than one method in some cases. All 3 methods were used in 138 subjects (79 patients, 29 relatives, 30 controls). Two methods (ARMS and PCR-RFLP) were used in 335 subjects (205 patients, 100 relatives, 30 controls). Three mutations (M694V, M680I, and V726A) were assessed by ARMS method. Primers were designed as described¹⁹. The same mutations were analyzed in the same 205 patients by PCR-RFLP as previously described²⁰. M680I, M694V, and V726A mutations were detected by PCR-RFLP method using HinfI, HphI, and AluI, respectively. The FMF StripAssay method allows detection of the 12 most frequent MEFV mutations located in exon 2 (E148Q), exon 3 (P369S), exon 5 (F479L), and exon 10 [M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S, and R761H]²¹. Isolated DNA was used for detection of mutations as described by the manufacturer.

The study was conducted with subjects' written informed consent and approved by the local ethics committee.

Statistical analysis. Categorical variables were compared by Fisher exact test.

RESULTS

Table 2 shows MEFV mutations detected by each method. MEFV mutations were studied by ARMS and PCR-RFLP methods in 205 patients. All methods were used in 79 patients. The ARMS method failed to detect 4 mutations (two M694V,

two M680I) in 4 patients. These 4 patients were found to have no mutations by the ARMS method, but had one mutation by PCR-RFLP. The ratio of false negativity was about 2% in ARMS compared to PCR-RFLP. Among relatives of patients with FMF and healthy controls, there was not more mutation detected by PCR-RFLP compared to the ARMS method.

The FMF StripAssay was used to investigate 9 more mutations and detected 17 mutations in 14 patients: previously 0 to 3 mutations (n = 1), 0 to 2 mutations (n = 1), 1 to 2 mutations (n = 8), and 0 to 1 mutation (n = 4). Eight of the 10 patients who did not have 2 mutations by the PCR-RFLP method, but had at least 2 mutations after the FMF StripAssay, had been diagnosed as definite FMF according to the Tel-Hashomer criteria. Genetic analyses of 625 patients are presented in Table 3. Data were obtained mainly from the FMF StripAssay (499) and the remainder from PCR-RFLP (126).

Since the FMF StripAssay was used to investigate 12 mutations, we compared MEFV mutations and the Tel-Hashomer criteria for diagnosis of FMF only in the 499 patients for whom the FMF StripAssay was used (Table 4). The MEFV mutations in patients having amyloidosis are shown in Table 3. None of the patients had any other disease predisposing to amyloidosis. The M694V/M694V genotype frequency was 37% (14/38) in patients with amyloidosis, while it was 18% (105/587) in patients without amyloidosis, the difference of which is statistically significant (p = 0.009, OR 2.67, 95% CI 1.3–5.3).

The frequency of MEFV carriers was 27% among 100 healthy controls (M694V: 8, E148Q: 7, M680I: 5, V726A: 4, K695R: 2, P369S: 1). No control had 2 mutations. One hundred thirteen of the 165 (68%) relatives of FMF patients had at least one mutation and 26 of them had 2 mutations (Table 3). Ten of the 26 subjects (relatives of FMF patients) did not have any symptoms; the frequency of asymptomatic genetically affected individuals (having 2 mutations) was 6% (10/165) among relatives of FMF patients. Eight of the

Table 1. Demographic features of the subjects.

Group	n	Male/Female	Mean Age Range (years)
Patient	625	282/343	20.81 (2–67)
Relative	165	84/81	37.07 (4–67)
Healthy control	100	50/50	28.42 (11–80)

Table 2. Number of mutations detected by each investigation method.

Group	Number of Mutations (n)			Total no. of Mutations
	0 Mutation	1 Mutation	≥ 2 Mutations	
ARMS				
Patients (n = 205)	56	47	102	251
Relatives (n = 100)	40	52	8	68
Healthy controls (n = 30)	23	7	0	7
PCR-RFLP				
Patients (n = 205)	52	51	102	255
Relatives (n = 100)	40	52	8	68
Healthy controls (n = 30)	23	7	0	7
FMF StripAssay				
Patients (n = 499)	126	162	211*	587
Relatives (n = 94)	24	49	21	91
Healthy controls (n = 100)	73	27	0	27

* 3 patients had 3 mutations. ARMS: amplification refractory mutation system; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism.

Table 3. MEFV mutations in 625 FMF patients, patients with amyloidosis, and relatives.

No. of Mutations	Patients, n (%)	Amyloidosis, n (%)	Relatives, n (%)
0 mutation	151 (24.2)	6 (15.8)	52 (31.5)
1 mutation			
M694V/—	93 (14.9)	4 (10.5)	49 (29.6)
M680I/—	41 (6.6)	2 (5.3)	24 (14.5)
V726A/—	13 (2.1)		4 (2.4)
E148Q/—	19 (3)	1 (2.6)	2 (1.2)
A744S/—	5 (0.8)		2 (1.2)
K695R/—	5 (0.8)	1 (2.6)	1 (0.6)
F479L/—	2 (0.3)		1 (0.6)
P369S/—	5 (0.8)		2 (1.2)
R761H/—	3 (0.5)		2 (1.2)
≥ 2 mutations			
M694V/M694V	119 (19)	14 (36.8)	1 (0.6)
M694V/M680I	61 (9.8)	4 (10.5)	4 (2.4)
M694V/V726A	17 (2.7)		1 (0.6)
M694V/E148Q	6 (1)		4 (2.4)
M694V/A744S	1 (0.2)		1 (0.6)
M694V/R761H	2 (0.3)		1 (0.6)
M680I/M680I	30 (4.8)	2 (5.3)	4 (2.4)
M680I/V726A	25 (4)	3 (7.9)	2 (1.2)
M680I/R761H	1 (0.2)		
M680I/E148Q	4 (0.6)		1 (0.6)
V726A/F479L	3 (0.5)		1 (0.6)
V726A/E148Q	2 (0.3)		1 (0.6)
E148Q/P369S	4 (0.6)	1 (2.6)	5 (3)
M694I/E148Q	3 (0.5)		
E148Q/E148Q	1 (0.2)		
E148Q/F479L	1 (0.2)		
M680I(G/C)/M680I(G/A)	1 (0.2)		
M694V/M694I	1 (0.2)		
M694V/E148Q/P369S	1 (0.2)		
E148Q/P369S/K695R	1 (0.2)		
E148Q/M680I/M694V	1 (0.2)		
M694V/P369S	1 (0.2)		
V726A/K695R	1 (0.2)		
V726A/R761H	1 (0.2)		
Total	625	38	165

Table 4. MEFV mutations and Tel-Hashomer criteria¹⁶.

FMF Diagnosis	n	0 Mutation	1 Mutation	≥ 2 Mutations
Definite	241	28	58	155
Probable	258	98	104	56
Total	499	126	162	211

asymptomatic and genetically affected individuals were older than 18 years.

DISCUSSION

The estimated prevalence of FMF in Turkey is 1/1000¹. Since the population of Turkey is around 70 million, a large proportion of all the FMF cases in the world live in Turkey. The results of the nationwide multicenter study showed 70% of the

patients had originated from the central-eastern and Black Sea regions¹. Our study is the first from the Black Sea region reporting genetic analysis of patients with FMF and it includes the highest number of FMF patients in whom 12 MEFV mutations have been studied in Turkey.

To date, over 100 MEFV mutations have been identified, and M694V, M694I, V726A, M680I, and E148Q are the most common^{3,8,12,13,15,16,22-27}. Before the identification of MEFV gene, several criteria had been proposed for the diagnosis of FMF^{2,7,17}. MEFV gene analysis is a valuable test in the diagnosis of FMF, but with some limitations. Table 5 summarizes the major studies investigating the frequency of MEFV mutations and their diagnostic role in FMF. In order to prepare the data in Table 5, we did 2 PubMed searches using the terms (1) familial Mediterranean fever and genotype, and (2) familial Mediterranean fever and MEFV, and selected the reports including at least 50 patients. We also extracted abstracts from conference proceedings^{39,48,50} and other national meetings held in Turkey.

The absence of any MEFV mutation or the presence of only one mutation in patients with clinically diagnosed FMF limits the role of MEFV mutations in the diagnosis of MEFV. The data investigating the relationship between MEFV mutations and diagnosis of FMF is controversial (Table 5). In this study, 64% (155/241) of the patients fulfilling the Tel-Hashomer criteria for the diagnosis of FMF had at least 2 MEFV mutations, while 12% (28/241) of them had no mutation. It is possible that at least some of these individuals might have other mutations that were not included among the 12 examined.

Another problem that limits the use of MEFV mutations in the diagnosis of FMF is the presence of asymptomatic patients having 2 mutations, with no clinical and laboratory findings related to FMF. These patients are classified as phenotype III or genotype X¹¹. We also investigated the frequency of phenotype III individuals using the search method described above, and the frequency of asymptomatic individuals having at least 2 mutations is shown in Table 6. As expected, the frequency was higher in the relatives of FMF patients compared to healthy individuals; 26 (16%) of the 165 relatives had 2 mutations and 10 (6%) of them were asymptomatic. The absence of any symptoms at the time of the MEFV mutation testing does not exclude the appearance of symptoms in the future, as reported by Tunca, *et al*⁶⁴. Since the level of acute-phase proteins is increased in FMF carriers⁴⁹, it is expected that phenotype III individuals are at risk. FMF has a wide clinical spectrum and a long course, and the use of colchicine affects genotype-phenotype correlation. Since the diagnosis of FMF generally precedes the initiation of colchicine treatment, only data from patients having delay in the diagnosis and genetic analysis of patients having sufficient medical information before the start of colchicine treatment can help in investigation of the genotype-phenotype correlation. Most studies, including the current study, evaluating genotype-phenotype

Table 5. The frequency of *MEFV* mutations in FMF patients based on Tel-Hashomer¹⁶, Livneh², other⁶, or unknown diagnostic criteria.

Study	Country	No.	No. of Mutations Studied	Mutations Detected			Diagnostic Criteria
				0	1	≥ 2	
Gershoni-Baruch ²⁸	Israel	146	5	≤ 9	NA	82–90	TH/L
Cazeneuve ¹⁴	Armenia	85	8	4	7	89	TH/L
Konstantopoulos ^{29†}	Greece	33	9	3	9	88	TH/L
Yalcinkaya ¹⁶	Turkey	167	4	2	20	78	TH/L
Sarkisian ³⁰	Armenia	3000	7	6	19	76	TH/L
Günesacar ³¹	Turkey	90	4	9	20	71	TH/L
Mattit ²²	Syria	83	≥ 7	11	19	70	TH/L
Turkcapar ³²	Turkey	105	5	3	30	68	TH/L
Ben-Chetrit ³³	Israel	221	5	1	33	67	TH/L
Grateau ^{34†}	France	126	≥ 7	19	13	67	TH/L
Grateau ^{34††}	France	40	≥ 7	20	15	65	TH/L
Current study [†]	Turkey	241	12	12	24	64	TH/L
Akar ²⁶	Turkey	230	7	16	24	60	TH/L
Yilmaz ²⁴	Turkey	450	5	23	18	58	TH/L
Zaks ³⁵	Israel	412	3	17	27	57	TH/L
Mansour ³⁶	Lebanon	79	15	20	28	53	TH/L
Konstantopoulos ^{29†}	Greece	29	9	28	28	45	TH/L
Tchernitchko ³⁷	France	233	4 exons	28	29	43	TH/L
La Regina ^{38†}	Italy	37	≥ 8	41	16	43	TH/L
Cerquaglia ^{39*}	Italy	144	≥ 18	30	≤ 28	≥ 42	TH/L
Padeh ⁴⁰	Israel	216	3	28	34	38	TH/L
Federici ⁴¹	France	1118	≥ 5	NA	NA	37	TH/L
Majeed ⁴²	Jordan	407	5	42	22	36	TH/L
Samli ^{43*}	Turkey	84	5	36	29	36	TH/L
Chaabouni ⁴⁴	Tunisia	139	8	56	12	32	TH/L
Samuels ⁴⁵	USA	86	8	45	27	28	TH/L
Current study ^{††}	Turkey	258	12	38	40	22	TH/L
Majeed ¹³	Jordan	278	≥ 7	55	27	18	TH/L
La Regina ^{38††}	Italy	21	≥ 8	76	10	14	TH/L
Tchernitchko ⁴⁶	France	208	12	99	1	0	TH/L
Sarkisian ⁴⁷	Armenia	> 5000	12	10	NA	NA	TH/L
Shohat ¹²	Israel	138	4	NA	NA	93	O/U
Tunca ⁴⁸	Turkey	77	7	6	8	86	O/U
Lachmann ⁴⁹	England/Turkey	43	7	5	12	84	O/U
Deltas ⁵⁰	Cyprus	87	≥ 6	≥ 18	NA	63–82	O/U
Mimouni ^{51**}	Israel	314	12	—	25	75	O/U
Brik ²⁵	Israel	67	4	7	19	73	O/U
Altioik ⁵²	Turkey	94	15	18	13	69	O/U
Medlej-Hashim ⁵³	Lebanon	613	14	40	19	41	O/U
Sari ⁵⁴	Turkey	212	≥ 3	11	NA	NA	O/U
Dode ⁵⁵	France	303	≥ 12	41	16	44	SS/R
Ayesh ⁵⁶	Palestine	511	24	42	18	40	SS/R
Oberkanins ⁵⁷	Armenia	199	12	45	15	40	SS/R
Berdeli ⁵⁸	Turkey	190	≥ 6	34	29	37	SS/R
Nucera ⁵⁹	Italy	90	≥ 7	46	19	36	SS/R
Bennetts ⁶⁰	Australia	193	≥ 9	70	12	18	SS/R
Berdeli ⁶¹	Turkey	1653	≥ 4	44	NA	NA	SS/R
Naman ⁶²	Lebanon	317	≤ 5	≤ 41	NA	NA	SS/R

* Includes probable cases. **At least 1 mutation is essential for FMF diagnosis. †Definitive FMF; ††Probable FMF. TH: Tel-Hashomer⁶, L: Livneh², O: other⁶, U: unknown, SS/R: studies including suspicious subjects or relatives. NA: not available.

notype correlation do not differentiate the data regarding effects of colchicine. Amyloidosis is the most severe manifestation of FMF. Identifying the patients who have never been treated with colchicine and who are over the age of 25 years and the percentages of these patients with amyloidosis will be useful to clarify this issue. Although M694V mutation has

been implicated in the development of amyloidosis^{12,14,51}, there is no consensus on the relationship of M694V allele and amyloidosis in Turkey. Some studies^{12,51} of Turkish patients not conducted in Turkey demonstrate the role of M694V allele in the development of amyloidosis. The relationship between M694V allele and amyloidosis has been shown in most of the

Table 6. The frequency of phenotype III patients with FMF among healthy individuals, relatives of FMF patients, and other groups.

Study	Country	n	No. of Mutations Studied	%
Healthy individuals				
Gershoni-Baruch ²⁸	Israel	1173	4	0.8
Shinar ⁶³	Israel	255	3	1.6
Tunca ⁶⁴	Turkey	19	≥ 7	4.1
Mattit ²²	Syria	242	≥ 7	1.2
Berdeli ⁶¹	Turkey	165	≥ 4	0
Yilmaz ²⁴	Turkey	100	5	1.0
Atagunduz ⁶⁵	Turkey	185	3	0
Booth ⁶⁶	England	182	E148Q	2.7
Stoffman ⁶⁷	Israel	400	4	1.5
Lachmann ⁴⁹	England/Turkey	49	7	4.1
Current study	Turkey	100	12	0
Relatives of FMF patients				
Berdeli ⁵⁸	Turkey	111	≥ 6	18.9
Tchernitchko ³⁷	France	213	4 exons	7.0
Gershoni-Baruch ⁶⁸	Israel	13 (a family)	4	61.5
Lachmann ⁴⁹	England/Turkey	73	7	12.3
Tunca ⁶⁴	Turkey	73	≥ 7	9.6
Current study	Turkey	165	3, 12	6.1
Other				
Kogan ¹⁰	Israel	521	3	1.0
Bybee ⁶⁹	England	499	≥ 5	> 0.8
Ben-Chetrit ³³	Israel	225	5	0

studies conducted in Turkey^{32,70,72,73}, but 3 studies^{16,65,71} found no correlation. A recent international study investigated the susceptibility for amyloidosis in 2482 patients (260 patients with amyloidosis) from 14 countries²⁷. This study included 447 patients from 2 centers in Turkey, 56 of whom had amyloidosis. The results indicated that the country of case recruitment was the leading risk factor for amyloidosis, followed by M694V homozygosity, proband status, and disease duration. M694V homozygosity for the MEFV gene was reported to be a risk factor for amyloidosis in patients living in Armenia, Israel, and Arabian countries, whereas association of M694V homozygosity with renal amyloidosis was borderline in Turkey and undetectable in countries defined as “others”²⁷.

Our study demonstrated M694V homozygosity as a risk factor for amyloidosis. Based on these findings, we analyzed the published studies having available data. If more than one study from one center had been published, in order to avoid repetition we included only the study with the highest number of patients with amyloidosis (Table 7). Data for control groups (patients without amyloidosis) were not available in all studies. We compared M694V/M694V mutations in 282 patients having amyloidosis to 1190 patients from a nationwide multi-center study conducted by the Turkish FMF Study Group or a total number of patients (n = 280) from published studies having available data^{16,65,72,73}. We demonstrated that the M694V/M694V genotype is a risk factor for amyloidosis among FMF patients in Turkey (p < 0.001 for both compar-

Table 7. Frequency of M694V/M694V genotype in Turkish FMF patients with amyloidosis.

Study	Total No.	Patients with M694V/M694V, n	Patients not Having M694V/M694V, n	Control Group	Relationship with Amyloidosis and M694V/M694V
Yalcinkaya ¹⁶	25	4	21	Yes	No
Atagündüz ⁶⁵	37	15	22	Yes	No
Yilmaz ⁷⁰	73	37	36	No	Yes
Çakar ⁷¹	32	5	27	No	No
Türkçapar ³²	31	10	21	No	Yes
Sayhan ⁷²	19	14	5	Yes	Yes
Delibas ⁷³	27	16	11	Yes	Yes
Our study	38	14	24	Yes	Yes
Total	282	115	167		

isons). We are aware of problems in comparing these groups, but it seems these problems will not affect the final conclusion that M694V/M694V genotype is a risk factor for amyloidosis. In addition to M694V, relatively rare mutations (P369S, K695R) and E148Q allele are associated with amyloidosis.

Our study shows that (1) the FMF StripAssay is a reliable, cost-effective, sensitive, and time-saving method for investigation of MEFV mutations; (2) in spite of detection of new mutations and developments in MEFV assay technology, there are patients having no mutation; (3) M694V is the most common mutation among FMF patients in Turkey; and (4) although previous studies do not express a consensus on M694V mutation as a risk factor in amyloidosis caused by FMF in Turkey, our data combined with previous studies show that patients having M694V/M694V carry risk for amyloidosis.

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