

# Unresponsiveness to Colchicine Therapy in Patients with Familial Mediterranean Fever Homozygous for the M694V Mutation

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**ABSTRACT. Objective.** More than 50 disease-associated mutations of the Mediterranean fever gene (MEFV) have been identified in familial Mediterranean fever (FMF), some of which were shown to have different clinical, diagnostic, prognostic, and therapeutic implications. The aim of our study was to define the frequency of mutation type, genotype-phenotype correlation, and response to colchicine treatment in patients with FMF.

**Methods.** This study included 222 pediatric FMF patients. All patients were investigated for 6 MEFV mutations. Then patients were divided into 3 groups according to the presence of M694V mutation on both of the alleles (homozygotes), on only 1 allele (heterozygotes), and on none of the alleles, and compared according to their phenotypic characteristics and response to treatment. M694V/M694V was denoted Group A, M694V/Other Group B, and Other/Other, Group C.

**Results.** Complete colchicine response was significantly lower while the rate of unresponsiveness was significantly higher in Group A compared to Groups B and C ( $p = 0.031$ ,  $p < 0.001$  and  $p = 0.005$ ,  $p = 0.029$ , respectively). No differences except proteinuria were found between the phenotypic features of 3 groups. Group C had the lowest rate of proteinuria development ( $p = 0.024$ ). All the amyloidosis patients were in Group A.

**Conclusion.** Our results indicate that the M694V/M694V mutation is associated with lower response to colchicine treatment. Therefore, patients homozygous for M694V/M694V may be carrying an increased risk for development of amyloidosis. (J Rheumatol First Release Dec 15 2009; doi:10.3899/jrheum.090273)

## Key Indexing Terms:

CHILDHOOD

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RESPONSE TO COLCHICINE

Familial Mediterranean fever (FMF) is the most frequent periodic syndrome characterized by self-limited recurrent attacks of fever and serositis. It is an autosomal-recessive disorder that predominantly affects people from the Mediterranean region, including Sephardic Jews, Turks, Armenians, and Arabs<sup>1</sup>. Studies with large series from Turkey reported that the carrier rate and prevalence of FMF was 1/5 and 1/1073, respectively<sup>2</sup>.

Although the gene (MEFV) resulting in FMF, located on chromosome 16p13.3, has been known for more than 10 years, the molecular pathophysiology of FMF is still

obscure. In several studies, the M694V mutation has been reported to be more common in Armenians<sup>3</sup>, Jews<sup>4</sup>, Arabs<sup>5</sup>, and Turks<sup>6</sup> than in other groups.

The most devastating complication of FMF is AA-type amyloidosis with prominent renal involvement. Colchicine is the mainstay of FMF treatment; it can prevent attacks, amyloid deposition, and mortality from this complication. Prior to the discovery of colchicine in 1972, up to 60% of patients with FMF died of amyloidosis. However, this ratio has been reported as 12.9% in a study that included a large number of FMF patients from Turkey<sup>7,8</sup>.

Several studies have demonstrated that the predisposing factors affecting the development of amyloidosis are ethnicity, heredity, and environment<sup>9-13</sup>. Although the correlation between genotype and phenotype has not been defined completely, it is possible that FMF patients possessing the M694V mutation are under increased risk for developing amyloidosis<sup>14,15</sup>.

The aim of our study was to define the frequency of mutation type and genotype-phenotype correlation, and the response to colchicine treatment in patients with FMF.

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## MATERIALS AND METHODS

This pediatric cohort study included 222 FMF patients who have been treated and followed at the Department of Pediatric Nephrology in Gazi University School of Medicine, Ankara, Turkey. All patients were interviewed by one of the clinicians and a standard clinical data file was compiled including demographic status (sex, age of onset, age at diagnosis, delay in diagnosis of FMF), clinical manifestations (fever, peritonitis, pleuritis, arthralgia/arthritis, erysipelas-like erythema, amyloidosis), history of diseases associated with FMF [polyarteritis nodosa (PAN), inflammatory bowel disease, Behçet syndrome, Henoch-Schönlein purpura (HSP)], misdiagnosis [appendectomy, acute rheumatic fever], family history of FMF, and amyloidosis history. All patients fulfilled the clinical criteria for FMF<sup>16</sup>. The severity score of the disease was calculated according to the Tel Hashomer Severity Score before initiation of treatment<sup>17</sup>. All patients had started colchicine treatment with an initial dose of 0.03 mg/kg/day (minimum 0.5 mg/day, maximum 2 mg/day) in compliance with recommendations<sup>18</sup>. Clinical status of patients was evaluated every 6 months. Response to colchicine treatment was classified into 3 groups: complete response (attack-free), incomplete response (decline > 50% in the frequency and severity of attacks), and unresponsiveness. The colchicine doses of patients who manifested incomplete response or unresponsiveness were gradually increased to 0.05 mg/kg/day and 0.07 mg/kg/day, respectively (minimum 0.5, maximum 2 mg/day). Compliance was assessed by questioning the parents and counting the number of colchicine tablets in every clinical visit. The acceptable criterion for compliance to colchicine treatment was not missing more than 2 doses per month. The colchicine dose of noncompliant patients was not increased. After maintaining their compliance to colchicine treatment, they were reevaluated at the end of another 6 months.

Our clinical cohort included 15 patients with proteinuria. Two of them were phenotype II patients and the other patients developed proteinuria during their clinical followup. The phenotype II patients and 3 of the 13 proteinuric patients who manifested progressive proteinuria underwent renal biopsy. Renal biopsy was not performed in the remaining 10 proteinuric patients because they had mild proteinuria that was not progressive. The histological diagnosis of amyloidosis was proved by presence of amyloid deposits on Congo red staining under polarized light microscopy.

The 6 MEFV mutations (M694V, M694I, M680I, V726A, E148Q, and R761H) were investigated in all patients. DNA was extracted from peripheral blood lymphocytes according to standard procedures<sup>19</sup>. Five mutations on exon 10 and 1 mutation on exon 2 of the MEFV gene were analyzed by polymerase chain reaction (PCR), using primers for exon 10 (forward, 5'-Biotin-*ACT GGG AGG TGG AGG TTG GAG ACA A-3'*, reverse 5'-*GAT ACA AGG CCA GAA GCA GG 3'*), and for exon 2 (forward, 5'-Biotin-*CCG CAG CGT CCA GCT CCC TG 3'*, reverse 5'-*GCA GGT ACA CTT CGA AGG GC*). PCR conditions were similar for all mutations: 12 min at 94°C for a first denaturation, then 35 cycles at repeated denaturation at 94°C (60 s), annealing at 60°C (60 s), and elongation at 72°C (60 s), followed by 10 min at 72°C for a final elongation step. The reaction was carried out in 25 µl containing 500 ng genomic DNA, 1 U Ampli Tag (AB gene, Epsom, Surrey, UK), 1× buffer, 1.5 µl deoxynucleoside triphosphate, and 5 µM primers. Samples were amplified by PCR. Amplicons were purified on a multiscreen filtration system (Millipore, Schwalbach, Germany) and resuspended in 70 µl of 50 mM histidine buffer. Each amplicon was electronically addressed for 120 s to a specific test site, where it was bound to the permeation layer through a biotin-streptavidin interaction. For single-nucleotide polymorphism, 1 stabilizer, 1 wild-type reporter, and a mutant reporter were chosen, following the manufacturer's recommendations (Nanogen Inc., San Diego, CA, USA). Samples were comparatively analyzed using the original Nanogen reporter mix and the universal reporter hybridization mix for MEFV mutation detection. The 6 mutations (M694V, M680I, V726A, M694I, E148, and R761H) were detected with a NanoChip molecular biology workstation (Nanogen) as described<sup>20</sup>.

For evaluation of the mutation-specific difference in the phenotypic expression of the disease and response to therapy, 222 patients were divid-

ed into 3 groups according to the presence of the M694V mutation on both the alleles (homozygotes), on only one of the alleles (heterozygotes), and on none of the alleles.

*Statistical analysis.* Data were analyzed using SPSS program 12.0 (SPSS Inc., Chicago, IL, USA). Mean ± SD were calculated for continuous variables and frequencies were measured for discrete variables. Differences between the patient groups for categorical variables were analyzed by chi-square test or Fisher's exact test, according to the size of the population. One-way analysis of variance was used for comparison of continuous variables between the patient groups. For all tests a 2-tailed  $p < 0.05$  was considered significant.

## RESULTS

*Clinical data.* The clinical characteristics of 222 patients with FMF (119 female, 103 male) are shown in Table 1. The mean age at onset and at diagnosis was  $5.95 \pm 3.43$  years and  $8.38 \pm 3.65$  years, respectively. The mean delay in diagnosis of FMF was  $2.42 \pm 2.11$  years.

Abdominal pain was the most common form of presentation, accounting for 205 patients (92.3%), followed by fever (90.0%). The less frequent manifestations were arthritis in 97 (43.7%), chest pain in 43 (19.4%), erysipelas-like erythema in 16 (7.2%), protracted febrile myalgia in 3 (1.4%), and protracted arthritis in 2 (0.9%). The mean severity score of the patients was  $8.59 \pm 1.93$ .

The study population included 220 phenotype I and 2 phenotype II patients. The 2 phenotype II patients with the chief complaint of edema at presentation were diagnosed

Table 1. Demographic and clinical information for patients with FMF.

Characteristics	Patient, n (%)
Demographic status	
Male/female	103/119
Age of onset (yrs), mean ± SD	$5.95 \pm 3.43$
Age at diagnosis (yrs), mean ± SD	$8.38 \pm 3.65$
Delay in diagnosis of FMF (yrs), mean SD	$2.42 \pm 2.11$
Clinical feature	
Abdominal pain	205 (92.3)
Fever	202 (90.9)
Arthritis	97 (43.7)
Chest pain	43 (19.4)
Myalgia	38 (17.1)
Erysipelas-like erythema	16 (7.2)
Protracted febrile myalgia	3 (1.4)
Protracted arthritis	2 (0.9)
Phenotype II	2 (0.9)
Mean severity score	$8.59 \pm 1.93$
Diseases associated with FMF	
Henoch-Schönlein purpura	9 (4.1)
Polyarteritis nodosa	3 (1.4)
Behçet syndrome	2 (0.9)
Inflammatory bowel disease	1 (0.5)
Misdiagnosis	
Appendectomy	22 (10.0)
Acute rheumatic fever	10 (4.5)
Consanguinity	61 (27.5)
Family history of FMF	113 (50.9)
Family history of amyloidosis	22 (9.9)

with amyloidosis after renal biopsy. Three amyloid patients had renal transplant because of chronic renal failure.

FMF-associated diseases were observed in 15 patients. Nine of them were HSP, 3 PAN, 2 Behçet syndrome, and 1 inflammatory bowel disease. Before the diagnosis of FMF, 22 patients underwent appendectomy and 10 patients were misdiagnosed as having acute rheumatic fever. Family history of FMF, amyloidosis, and consanguinity were detected in 113 (50.9%), 22 (9.9%), and 61 patients (27.5%), respectively.

**Genetic data.** Table 2 shows the MEFV mutations that were detected in 222 FMF patients, of whom 195 (87.2%) had mutations and 27 (12.2%) had none of the studied mutations. Of the 195 patients with mutations, 73 (32.8%) were homozygote, of whom 63 were M694V/M694V, 9 were M680I/M680I, 1 was E148Q/E148Q, and 41 (18.5%) compound heterozygote; 81 (36.5%) patients had only a single mutation. Mutation analysis showed that 6 identified missense mutations accounted for 69.5% of the 444 FMF alleles. The most frequent mutation in our FMF patients was M694V (46.1%). M680I, V726A, E148Q, M694I, and R761H mutations were found in 13.5%, 6.1%, 2.4%, 0.9%, and 0.5%, respectively.

**Genotype-phenotype correlation.** Table 3 summarizes the distribution of phenotypic features according to existence of the M694V mutation among groups (M694V/M694V was denoted Group A, M694V/Other Group B, and Other/Other, Group C). Demographic status, diseases associated with FMF, family history of FMF and amyloidosis, consanguinity, and misdiagnosis did not differ significantly among these groups. Comparing Groups A, B, and C with each

other, no significant difference was found except proteinuria. During followup, 6 patients in Group A, 6 patients in Group B, and 1 patient in Group C developed proteinuria. Group C had the lowest rate of proteinuria ( $p = 0.024$ ). Three patients with progressive proteinuria and amyloidosis and 2 phenotype II patients were present in Group A. Endstage renal failure developed in all 5 patients with amyloidosis. Three of them had renal transplants and the other 2 have undergone peritoneal dialysis.

None of the 27 patients without mutation had detectable proteinuria or amyloidosis, which was not different from the patients with mutation ( $p = 0.136$ ,  $p = 0.520$ , respectively). When we compared these patients without mutation to 193 patients having mutations, no significant differences were detected in clinical or in other features such as demographic status, diseases associated with FMF, family history of FMF and amyloidosis, consanguinity, and misdiagnosis ( $p > 0.05$  for each measure).

**Response to colchicine treatment.** The mean followup of patients under colchicine treatment was  $2.59 \pm 1.48$  years. The mean colchicine dose of all patients was  $1.36 \pm 0.41$  mg/day. When we evaluated the treatment response of 220 patients (excluding 2 phenotype II patients) to colchicine, complete and incomplete responses were observed in 54.5% and 36%, respectively, while 9.5% showed no response at all (Table 4).

A complete colchicine response was significantly lower, while the rate of unresponsiveness was significantly higher, in Group A compared to Groups B and C ( $p = 0.031$ ,  $p < 0.001$  and  $p = 0.005$ ,  $p = 0.029$ , respectively). The frequency of incomplete response did not differ significantly between Groups A and B ( $p = 0.625$ ). Group C had the lowest rate of incomplete response among all groups (Table 4).

The mean colchicine dose was highest among patients in Group A and lowest among patients in Group C (Table 4). No significant difference was found between groups according to mean colchicine dose ( $p = 0.186$ ). During clinical followup, diarrhea was observed in 8 and vomiting in 5 cases as side effects of colchicine treatment. All these patients were receiving colchicine doses of 1.5 mg/day or more. All the side effects were improved by reducing colchicine dose. When side effects were considered, neither the groups classified according to colchicine response nor those classified according to mutations differed.

Twenty-one (77.7%) of the 27 patients without mutation manifested a complete response; incomplete responses were observed in 5 patients (18.5%) and unresponsiveness in 1 patient (3.8%). When we compared these patients with 193 patients having mutations according to colchicine response, the frequencies of complete and incomplete response were significantly higher in patients without mutation ( $p = 0.020$ ,  $p = 0.040$ , respectively). There was no difference between these 2 groups in terms of unresponsiveness ( $p = 0.330$ ).

Table 2. Mutation distribution of 222 FMF patients (a total of 444 alleles).

Mutation	Genotype	Patients, n (%)
Homozygote		73 (32.8)
	M694V/M694V	63
	M680I/M680I	9
	E148Q/E148Q	1
Compound heterozygote		41 (18.5)
	M694V/M680I	20
	M694V/V726A	14
	M680I/V726A	4
	M694V/R761H	1
	M694V/E148Q	1
	M680I/E148Q	1
One identified mutation		81 (36.5)
	M694V/-	43
	M680I/-	17
	V726A/-	9
	E148Q/-	7
	M694I/-	4
Unidentified mutation	R761H/-	1
	NA	27 (12.2)

NA: not applicable.

Table 3. Phenotypic features according to the M694V mutation.

Features	M694V/M694V Group A: n = 63 (%)	M694V/Other Group B; n = 79 (%)	Other/Other Group C; n = 80 (%)	p
<b>Demographic status</b>				
Male/female	35/28	36/43	32/48	
Age of onset yrs, mean ± SD	6.2 ± 3.6	5.6 ± 3.3	6.1 ± 3.4	> 0.05**
Age at diagnosis, yrs, mean + SD	8.6 ± 3.8	7.9 ± 3.2	8.8 ± 3.9	> 0.05**
Delay in diagnosis of FMF, yrs, mean ± SD	2.3 ± 2.5	2.3 ± 1.7	2.7 ± 2.1	> 0.05**
Duration of followup, mo	2.6 ± 1.5	2.5 ± 1.3	2.7 ± 1.6	> 0.05**
<b>Clinical feature</b>				
Abdominal pain	60 (95.2)	73 (92.4)	72 (90.0)	> 0.05*
Fever	60 (95.2)	70 (88.6)	72 (90.0)	> 0.05*
Arthritis	24 (38.1)	36 (45.6)	37 (46.3)	> 0.05*
Chest pain	14 (22.2)	12 (15.2)	17 (21.3)	> 0.05*
Myalgia	11 (17.5)	16 (20.3)	11 (13.8)	> 0.05*
Erysipelas-like erythema	8 (12.7)	5 (6.3)	3 (3.8)	> 0.05*
Protracted febrile myalgia	2 (3.2)	—	—	
Protracted arthritis	1 (1.6)	1 (1.2)	—	
Phenotype II	2 (3.2)	—	—	
Mean severity score	9.07 ± 2.54	8.56 ± 2.42	8.31 ± 2.01	> 0.05**
Proteinuria***	6 (9.8)	6 (7.6)	1 (1.3)	0.024*
Amyloidosis	5 (7.9)	—	—	
Renal transplant	3	—	—	
Endstage renal failure	2	—	—	
Association of other diseases with FMF	5 (7.9)	6 (6.7)	4 (5.0)	> 0.05*
<b>Misdiagnosis</b>				
Appendectomy	7 (11.1)	4 (5.1)	11 (13.8)	> 0.05*
Acute rheumatic fever	3 (4.8)	3 (3.8)	4 (5.0)	> 0.05*
Consanguinity	18 (28.6)	23 (29.1)	20 (25.0)	> 0.05*
Family history of FMF	25 (39.7)	44 (55.7)	44 (55.0)	> 0.05*
Family history of amyloidosis	6 (9.5)	9 (11.4)	7 (8.8)	> 0.05*

\* Chi-square test. \*\* One-way analysis. \*\*\* Excluding phenotype II.

Table 4. Response to colchicine treatment according to the M694V mutation.

	M694V/M694V* Group A: n = 61 (%)	M694V/Other Group B; n = 79 (%)	Other/Other Group C; n = 80 (%)	p
Complete response***	22 (36.1)	43 (54.4)	56 (70.0)	0.031 <sup>1</sup> , < 0.001 <sup>2</sup> , 0.063 <sup>3</sup>
Incomplete response***	28 (45.9)	33 (41.7)	19 (23.7)	0.625 <sup>1</sup> , 0.010 <sup>2</sup> , 0.024 <sup>3</sup>
Unresponsiveness***	11 (18.0)	3 (3.9)	5 (6.3)	0.005 <sup>1</sup> , 0.029 <sup>2</sup> , 0.479 <sup>3</sup>
Mean colchicine dose, mg/day**	1.42 ± 0.33	1.35 ± 0.48	1.31 ± 0.67	0.186

\* Excluding phenotype II. \*\* One-way analysis. \*\*\* Chi-square test. The difference (p value) <sup>1</sup> between Group A and B; <sup>2</sup> between Group A and C; <sup>3</sup> between Group B and C.

## DISCUSSION

Studies conducted after the discovery of the gene (MEFV) related to FMF focused particularly on phenotypic-genotypic associations of this clinically heterogeneous disease. Although results of these studies were sometimes confusing, they clarified that phenotypic-genotypic association of FMF is multifactorial and fairly complex. Ethnicity<sup>9</sup>, MEFV mutations<sup>10</sup>, and other genetic<sup>11,12</sup> and environmental factors<sup>13</sup> contribute to this association.

Studies pointed out that patients who are carriers of the M694V mutation have more severe phenotypic characteristics<sup>3,14,15,21</sup>. To our knowledge, our study is the first to establish the relationship between M694V homozygosity and unresponsiveness to colchicine treatment. In our study, upon evaluating the patients in 3 groups according to the presence of M694V mutation, a complete colchicine response of M694V homozygous patients was determined to be significantly lower, while unresponsiveness to colchicine



treatment was significantly higher in comparison to patients with heterozygote or no mutations.

In the previous studies, 10%–15% of FMF patients were reported to be unresponsive<sup>22–24</sup>. Noncompliance with and side effects of colchicine treatment owing to high-dose colchicine were considered the leading factors in colchicine unresponsiveness. However, these factors did not affect the results of our study because of the higher compliance of our patients to colchicine treatment. We monitored compliance in routine clinical control visits and excluded patients if they missed more than 2 doses of colchicine per month. The dose of colchicine had to be reduced owing to side effects in only 13 patients, who were distributed homogeneously in both classifications, according to colchicine response and mutation groups.

Lidar, *et al* suggested that 2 new findings could be associated with colchicine treatment failure<sup>25</sup>. The first was the colchicine concentration difference in mononuclear cells (MNC) of the FMF patients. Colchicine concentration in responders' MNC was more than twice that in nonresponders. Lidar, *et al* attributed this finding to the difference of activity of the P-glycoprotein (P-gp) efflux pump that is responsible for colchicine transport encoded by the MDR-1 gene. In another study, the colchicine concentration in polymorphonuclear cells (PMNC) was found to be 3 times that of MNC in FMF patients<sup>26</sup>. A difference of P-gp efflux pump activity was suggested as the cause of this difference of colchicine concentration between PMNC and MNC. It is known that prophylactic colchicine treatment prevents chemotaxis of PMNC, which may suggest that MDR-1 gene polymorphism is an important modifier genetic factor that affects colchicine responsiveness, and as a result the course of the disease. However, our study evaluated the response to colchicine treatment only by clinical measurements. Future studies of this issue should include analysis of PMNC-MNC functional interactions, investigating particularly MDR-1 gene polymorphism and other modifier genes, together with clinical evaluation of the colchicine response.

The second finding of Lidar, *et al* was the presence of higher rates of unemployment and lower education in colchicine nonresponders<sup>25</sup>. In addition, a multicenter FMF study of patients without colchicine treatment and from different ethnic groups proposed that the most important risk factor for amyloidosis was country of recruitment<sup>27</sup>. It is difficult to know how different environmental factors affect the prognosis of FMF by influencing either the clinical course of the disease or the response to colchicine treatment. Ozen, *et al* found that the country of residence influences the phenotype of FMF in children and adolescents<sup>28</sup>. However, the authors stated that the only difference in these children was the environment in which they have spent their early years of childhood, a time when they are in contact with the common microbes. In order to evaluate the probable effects of environmental factors on FMF patients, birth-

cohort studies among children of patients living in different environments and having similar genetic and ethnic characteristics may be more useful.

The chief genetic risk factor for development of amyloidosis is the presence of the M694V mutation<sup>11,29,30</sup>. The rare occurrence of amyloidosis among Ashkenazi Jews who had this mutation at a lower rate supports this strong relationship between amyloidosis and M694V<sup>31</sup>. In our study, detection of the M694V mutation in 12 of 13 proteinuric patients and all patients who developed amyloidosis significantly favors this finding. Some studies in Turkey<sup>32–34</sup> reported the M694V mutation as a risk factor for development of amyloidosis, but others did not find any association between M694V and amyloidosis<sup>35,36</sup>. Heterogeneity of the cohorts, absence of genetic modifier risk factors in most of the patients, variety in environmental factors, and diversity of ethnic groups in Anatolia may lead to these contradictory results from Turkey. Nevertheless, an FMF study about MEFV gene mutation types and serum amyloid A-1 (SAA1) polymorphism conducted by Delibas, *et al* reported that the presence of M694V homozygosity and the SAA1a/a genotype increased the frequency of development of amyloidosis by 1.2 and 2.4 times, respectively<sup>33</sup>. Therefore, in order to exactly define the relationship between amyloidosis and genetic factors in Turkey, well designed studies are required involving similar environmental factors and investigating MEFV gene mutations together with other modifier genetic factors in ethnically homogenous groups.

Besides environmental factors, male gender<sup>10,37</sup> and delay in diagnosis of FMF have been proposed as nongenetic factors for the development of amyloidosis<sup>29,38</sup>. Three male and 2 female patients had amyloidosis in our study. This frequency of amyloidosis was insufficient to evaluate the gender risk. Reviewing the FMF studies from Turkey chronologically, the decrease in prevalence of amyloidosis can be clearly observed<sup>33,36,37–40</sup>. In a multicenter Turkish study, the interval between the onset of symptoms and diagnosis of FMF was found to be shortened significantly in those in whom the diagnosis was made after 1992 compared to before 1991<sup>7</sup>. Moreover, Düsünsel, *et al* reported that the delay in diagnosis of FMF shortened to 2.12 years<sup>32</sup>. Similarly, we also found this time lag to be  $2.42 \pm 2.11$  years. Reasons for this shortening in the time lag could be the establishment of clinical diagnostic criteria for FMF through the efforts of international studies in the last 15 years, and more precise understanding of diagnostic approaches to FMF by primary care physicians. Moreover, early diagnosis of FMF patients with atypical clinical features may be possible by determining genes and mutations related to FMF.

Demographic and initial clinical presentation characteristics of our study were similar to those of previous pediatric cohort studies<sup>32,33,35,39</sup>. There was no significant difference between mutation groups in our study and the most common

clinical presentations were abdominal pain<sup>35,39</sup> and fever<sup>32,33</sup>, as in the previous studies. Similar to our study, in the previous studies the frequency of abdominal pain and fever was 70.0% to 96.2% and 80.8 to 96.0%, respectively. Arthritis was the third common manifestation in both our study and previous studies (34.6%–54.0%). Myalgia and erysipelas-like rash were less frequently observed in this study<sup>33,39</sup>. In the previous studies the frequency of pleuritis (4.9%–45%) was found to be considerably different<sup>32,35</sup>. This may be related to the inability of the children to characterize such a manifestation, because the frequency of pleuritis was observed to be higher (39.0%–53.0%) in the adult cohort studies<sup>38,40,41</sup>. In a study in which the clinical symptom frequency was evaluated according to age of onset, adult patients manifested arthritis and erysipelas-like rash less commonly in comparison to the pediatric cohort<sup>41</sup>. However, the largest cohort study in Turkey did not reveal a significant association between age of onset and the frequency of arthritis, myalgia, and erysipelas-like rash<sup>7</sup>. The presence of different ethnic groups in Turkey and the variation of frequency of typical clinical symptoms among different ethnic groups explain these differences between the studies.

As suggested in previous studies, determination of high ratios of consanguinity, family history of FMF, and/or history of amyloidosis supports the recessive hereditary transmission characteristic of FMF<sup>42,43</sup>. Our frequency results were in line with others in terms of consanguinity (27.5%), family history of FMF (50.9%), and family history of amyloidosis (9.9%) as well.

The discovery of the gene responsible for FMF in 1997 has aided diagnosis of FMF in atypical cases. Thus far 151 different polymorphisms of the MEFV gene have been described and 51 of them were shown to be related to pathological phenotypes<sup>44</sup>. M694V, M680I, V726A, and M694I localized in exon 10 and E148Q in exon 2 are the most frequently observed mutations in FMF patients<sup>45</sup>.

Frequency of mutation types varies among different ethnic groups. The most frequent mutation type in large series of different ethnic groups is reported to be M694V<sup>2,3,5-7</sup>. On analyzing 6 mutations of FMF, we found that 87.8% of cases had a mutation distribution of 32.4% homozygous, 18.5% compound heterozygous, and 36.9% simple heterozygous. Among the mutations, M694V (46.1%) was the most common mutation, followed by M680I (13.5%), V726A (6.1%), E148Q (2.4%), M694I (0.9%), and R761H (0.5%).

The results of both Turkish studies and ours were similar in terms of commonly observed mutation rates<sup>2,7</sup>. Frequency of less common mutations showed diversity between the studies from Turkey<sup>2,32</sup>. The most commonly observed mutation in the healthy Turkish population was E148Q (8.4% to 12%), but its rarity in Turkish FMF patients<sup>2,6,32</sup>, and confirming this rarity in different ethnic groups<sup>46</sup>, favors our belief that this polymorphism is a non-

pathogenic one and/or is related to a mild disease. However, the similarity of clinical manifestations of E148Q carriers to those of other patients, as in our study, makes it hard to estimate the phenotypic penetration of E148Q<sup>47</sup>. Thus, further prospective studies investigating detailed clinical features, prevalence of E148Q mutation, and modifier genetic and nongenetic factors are required to determine whether this mutation is related to FMF or not.

The frequency of amyloidosis has decreased to 5.9%–15% from 60% by regular and lifelong use of colchicine by patients with FMF<sup>8,32</sup>. This frequency was 2.3% in our study. Although the frequency of amyloidosis in our study was low in comparison to the other Turkish reports, it rises to 6.7% if it is assumed that proteinuric patients without biopsy have developed probable amyloidosis. In light of the data, colchicine appears to be the most effective treatment to prevent the progression of FMF and the development of amyloidosis. Certain case reports and studies discuss the effectiveness of tumor necrosis factor- $\alpha$  antagonists (etanercept, thalidomide, infliximab), interleukin 1 receptor blockers (anakinra), and selective serotonin reuptake inhibitors in patients who are unresponsive to colchicine treatment<sup>48-50</sup>. However, further largescale studies investigating the effectiveness of these novel treatments are required.

Our study was the first to demonstrate that the presence of the M694V homozygote mutation affects unresponsiveness of colchicine treatment in FMF. However, we discuss this issue only from a clinical viewpoint. Other genetic and nongenetic factors were absent from our study. Our results should be supported with further studies describing other factors affecting colchicine unresponsiveness in FMF, which may help to develop novel therapeutic options for disease management and the improvement of prognosis.

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