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Familial Mediterranean fever in children from the Aegean region of Turkey: gene mutation frequencies and phenotype–genotype correlation

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Background/aim: Familial Mediterranean fever (FMF) is diagnosed by fever episodes with sterile peritonitis, arthritis, pleurisy, and erysipelas-like erythema. The relationship between phenotype and genotype in FMF has not been adequately explained. The aim of this study was to characterize the phenotype and genotype correlation in FMF.

Materials and methods: Clinical diagnosis of FMF was conducted according to the Tel Hashomer criteria. Pras scoring was used to determine clinical severity. FMF strip assay analysis was used, and the hotspot regions were observed with PCR amplification and automatic DNA sequence analysis method.

Results: We showed commonly seen mutations (most frequently M694V) in a study group of 191 patients. The disease severity score of patients with M694V mutation was high on the Pras scoring system. Patients with M694V mutation needed high colchicine dosages to control disease activity. R202Q was the most commonly seen polymorphism in 70 patients. The coexpression of R314R single nucleotide polymorphism on third exon was shown in our study. Moreover, D102D, G138G, and A165A subhaplotypes and E474E, Q476Q, and D510D subhaplotypes were also shown.

Conclusion: DNA sequence analysis should be a commonly used method for progress in the field of molecular genetics and for the better understanding of the FMF phenotype and genotype relationships in all populations.

Key words: Children, familial Mediterranean fever, *MEFV*, genotype, phenotype

1. Introduction

Familial Mediterranean fever (FMF) is the most common autoinflammatory hereditary recurrent fever disease. It affects people from the Mediterranean region, including Turks, Armenians, non-Ashkenazi Jews, Arabs, and, less commonly, Greeks and Italians. FMF is an autosomal recessive hereditary disease linked to a gene named *MEFV*. The identification of the *MEFV* gene on chromosome 16p13.3 initiated the development of genetic diagnostic tests for FMF (1–3). In more than 80% of typical cases, MEFV mutations involve nucleotide substitutions at the last exon (3). The first attack occurs in early childhood before the age of 10 in 65% of cases (4). The clinical manifestations are characterized by recurrent, self-limiting episodes of fever; serositis resulting in pain in the abdomen, chest, joints, and muscles; abdominal and pleuritic pain; arthritis; and cutaneous rashes (5). The clinical diagnosis is made based on typical clinical manifestations, a positive response to colchicine therapy, and genetic testing. The disease is often diagnosed retrospectively on the basis of unexplained recurrent febrile attacks, positive familial history, and the exclusion of other illnesses similar to FMF. Widely used clinical criteria were proposed in Israel in 1997 (5,6). Unfortunately, these criteria were not found to be very useful in other countries.

FMF is considered as an autosomal recessive disease, meaning that the presence of two mutations on different alleles (homozygote or compound heterozygote) makes it possible to confirm the diagnosis. Some patients have only one identifiable mutation and others have no identifiable mutations. When only one mutation exists, the diagnosis cannot be ascertained or excluded. Families with members who present typical FMF in the absence of *MEFV* mutations have been found in Turkey (7). At

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present, FMF is not entirely understood; however, analysis of the sequence variants of the *MEFV* gene in patients with FMF will lead to a greater comprehension of the disease. If we prevent delayed diagnosis, urgent early initiation of colchicine prophylaxis can be started.

The purpose of the present study was to review the clinical and demographic features of child-onset FMF patients and to investigate whether there is a phenotype–genotype correlation.

2. Materials and methods

2.1. Patients

We retrospectively reviewed the medical records of patients with FMF followed in the Department of Pediatric Nephrology at the Ege University Faculty of Medicine, İzmir, Turkey. All patients were of Turkish origin and living in the Aegean region of Anatolia. Tel Hashomer and Livneh criteria were used for the clinical diagnosis of FMF, which is based on major, minor, and supportive criteria. Diagnosis requires the presence of either 1 major or 2 minor criteria, or 1 minor and 5 supportive criteria (5).

A questionnaire was prepared that requested information on the demographic status and clinical manifestations of the patients (fever, abdominal pain, arthritis, chest pain, and erysipelas-like erythema). We noted the age at diagnosis, time interval between disease onset and diagnosis, history of other diseases, and number of attacks per year before and after treatment. In addition, we recorded consanguinity, development of amyloidosis, presence of a family history of FMF and amyloidosis, and treatment regimen. The presence of chronic renal disease was discussed and patients were asked for the type of renal replacement, if present. Response to colchicine treatment was evaluated as complete (attack-free), incomplete (decline of >50% in the frequency of attacks), or unresponsive. The severity score of the disease was calculated based on the Pras scoring system. This scoring has 6 conditions including onset age, colchicine dosages, number of attacks per month, presence of arthritis, erysipelas-like erythema, and amyloidosis (8). Scores are as follows: onset age, 0–3 points; attack frequency, 1–3 points; articular findings, 0–3 points; erysipelas-like erythema, 0–2 points; and occurrence amyloidosis, 0–4 points. Patients were also scored according to the amount of colchicine required to control disease symptoms: no response to colchicum (4 points), 2 mg/day (3 points), 1.5 mg/day (2 points), or 1 mg/day (1 point). Scores of 2–5 indicate mild disease, 6–10 show moderate severity, and 10 or more indicate severe disease. Patients presenting with amyloidosis before the onset of clinical symptoms were phenotype II. For patients with persistent proteinuria or nephrotic syndrome, tissue samples were made from the kidney.

In our study group, none of the patients had immunological abnormality or other rheumatic disease. Active clinical presentation types such as fever, abdominal pain, arthritis, and myalgia were recorded, and laboratory parameters including high levels of serum amyloid A, C-reactive protein, fibrinogen, white blood cell counts, and erythrocyte sedimentation rates were recorded for each patient.

Mutation analysis was initially done using StripAssay. Additionally, for patients in whom no mutation was detected by StripAssay, we carried out bidirectional DNA sequence analysis in all 10 coding exons and exon–intron boundaries for the detection of all coding and noncoding sequence variations along the *MEFV* gene. Patients in whom no mutation was detected by both approaches (StripAssay and sequence analysis) were excluded from the study.

The study was performed according to the Helsinki principles. We received informed consent from all patients or their families. The ethics committee of the Faculty of Medicine of Ege University reviewed and approved this study.

The patients were divided into two groups. Group 1 included patients with common mutations present in strip assay results, and group 2 included patients with coding and noncoding sequence variations of the *MEFV* gene in sequence analysis but no mutation in StripAssay. The two groups were compared by sex, onset age, age of diagnosis, time interval between disease onset and diagnosis, clinical findings of fever, abdominal pain, arthritis, chest pain, erysipelas-like erythema, amyloidosis, number of attacks per year before and after colchicine treatment, severity score, consanguinity, and family history of FMF and amyloidosis.

2.2. Genetic analysis

DNA was extracted using the QIAamp DNA Blood Isolation Kit (QIAGEN, Germany) following the manufacturer's instructions. The extracted DNA concentration was measured using a Thermo Scientific NanoDrop spectrophotometer (Wilmington, DE, USA). For the quality assessment, 2% agarose gel electrophoresis was used. For reverse hybridization assay, FMF StripAssay (ViennaLab, Austria) was used. Multiplex PCR was used with biotinylated primers for exons at 2×, 3×, 5×, and 10× amplification. PCR products were hybridized to a test strip with a parallel array of allele-specific oligonucleotide probes, including 12 *MEFV* mutations (E148Q, P369S, F479L, M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S, R761H). Sequence analysis was performed by SeqScape 2.0 (Applied Biosystems, USA). *MEFV* hot-spot mutations in exons 10, 2, 3, and 5, and when needed in exons 1, 4, 6, 7, 8, and 9, were searched by automated DNA sequence analysis. One microliter of

genomic DNA was added to polymerase chain reaction (PCR) amplification buffer. The PCR program included a hot start denaturation step at 95 °C for 10 min; then 35 amplification cycles of denaturation at 95 °C for 30 s, annealing at 61 °C for exon 10, 58 °C for exons 2 and 3, and 57 °C for exon 5 for 40 s, and elongation at 72 °C for 45 s; and a final extension at 72 °C for 7 min. PCR products were purified using an ExoSAP-IT PCR product clean-up kit. Cycle sequencing PCR products were purified with the BigDyeXT kit (Applied Biosystems). Cycle sequencing reactions were completed with the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). The data were analyzed using an ABI 3130xl Genetic Analyzer (Applied Biosystems). The amplicons of exon 5 were digested by the restriction enzyme Tsp509I. The digestion products were electrophoresed on a 1% agarose gel. The 4 hot spot regions (exons 10, 5, 3, and 2) for *MEFV* mutations were observed using PCR amplification and automatic DNA sequence analysis method. One microliter (100 ng) of genomic DNA was added to amplification buffer, and 10 pmol of each reverse and forward primer synthesized by Invitrogen and 1.0 U of Platinum Taq PCR DNA polymerase (Invitrogen, USA) in a total of 25 µL was used. The cycling conditions comprised a hot start denaturation step at 95 °C for 10 min, then 35 amplification cycles at 95 °C for 30 s and 72 °C for 45 s with a final extension at 72 °C for 7 min. The primers used are shown below.

Exon 2: **sense**: 5-ATC TTG GGC CCT AAA CGT GG-3; **antisense**: 5-GGG TTC TGT TGC CGA GTC-3. Exon 3: **sense**: 5-TAA CTG AGA ACT CGC ACA TCT C-3; **antisense**: 5-CTT GTG TTC CAG GGC GAC CTC-3**.** Exon 5: **sense:** 5-CCA CCT CTT ATC CAC CTC C-3; **antisense**: 5-CTT CAC CCA CTT GTT CC-3. Exon 10: **sense:** 5-GAG GTG GAG GTT GGA GAC AA-3; **antisense:** 5-TGA CCA CCC ACT GGA CAG AT-3.

An ABI 3100 Genetic Analyzer was used for cycle sequencing PCR products after purification with a BigDye XTerminator kit. DNA was sequenced in both directions. Seq-Scape 2.0 sequence analysis software was used.

2.3. Statistical analysis

Data were analyzed using SPSS 11.0 (SPSS, Chicago, IL, USA). Data were categorized as median and range or mean ± standard deviation (SD) according to the normality of distribution. We compared the categorical data and proportions by using the chi-square test or Fisher's exact test. Means were compared with Student's t-test, and medians were compared with the Mann–Whitney U test as indicated. For all tests, $P < 0.05$ was considered significant.

3. Results

The study population consisted of 261 children with FMF (125 boys, 136 girls). The mean age of patients was 104.8 ± 49 months. The presence of one or two mutations was shown by StripAssay in 191 patients. In the remaining 70 patients, the presence of the coding and noncoding sequence variations in the *MEFV* gene was detected by sequence analysis. Mean follow-up time was 32 ± 26.8 months, and the median was 21 months (minimum 8, maximum 160 months) (Table 1).

The age of onset was 79.16 ± 43 months for patients in group 1 and 55.9 ± 31.7 months for patients in group 2. In group 1, the time interval between disease onset and diagnosis was 31.23 ± 29.1 months. There were statistical differences between groups with regard to age of onset, age of diagnosis, and time interval. In group 2, patients had longer periods of complaint and older age at diagnosis.

The consanguinity rate was 27 (10.3%) in our population. A positive family history for FMF was noted in 126 children (48.4%) and for amyloidosis in 8 children (3%). Two patients had amyloidosis (Table 1).

The most common clinical feature was abdominal pain (85.4%), followed by fever (58.2%), arthritis (19.1%), arthralgia (42.5%), chest pain (12.6%), and erysipelas-like erythema (1.5%). No difference was detected between groups with regard to fever, abdominal pain, chest pain, and erysipelas-like erythema, but arthralgia was seen more in group 1 (Table 1).

The number of attacks per year before and after treatment was 8 (range: 1–48) and 0.78 ± 2.2 (range: 0–24), respectively, for 261 patients. Only 2% of the patients had a severe disease score; 56% of the patients had a moderate score (Table 2).

Among the 261 patients, 182 (69.7%) had complete response to colchicine treatment, 67 (25.6%) had some attacks despite colchicine, and 12 (4.59%) were unresponsive. Responses to colchicine treatment were similar in the two groups (Table 2).

None of the patients died or needed renal replacement therapy. Eleven children underwent surgery, and all had an appendectomy. Of patients who had appendectomies, 4 were homozygous for R202Q, M680I, and M694V; 5 were heterozygous for M694V, K695R, M694I, and E148Q; and 2 were compound heterozygous for M694V/V726A and M694V/E148Q.

There was mild or nephrotic proteinuria in 45 patients (17.2%). Renal biopsy indicated amyloidosis in 2 of the 12 patients, and amyloidosis was seen in group 1 (Table 1).

Homozygosity for M694V and M680I was found in 28 (14.6%) and 3 (1.6%) patients, respectively. Four patients (2%) were homozygous for E148Q. The number of heterozygotes for the mutations was 50 (26.1%) for M694V, 6 (3.14%) for M680I, 5 (2.6%) for V726A, and 29 (15.2%) for E148Q. Compound heterozygosity for M694V mutation was present in 30 patients and most of them were E148Q/M694V (12 cases, 6.3%) (Table 3).

Table 1. Summary of phenotypic features.

	All population	Group 1	Group 2	P*
	n: 261	n: 191	n: 70	
Age of onset, (months) mean ± SD	72.9 ± 42.1	79.16 ± 43	55.9 ± 31.7	0.0001
Age of diagnosis, (months) mean ± SD	104.8 ± 49	108 ± 40	95.2 ± 48.6	0.047
Duration of symptoms, (months) mean ± SD	34.8 ± 30.4	31.4 ± 28.09	44.27 ± 34.3	0.005
Time interval between disease onset and diagnosis, (months) mean ± SD	33.5 ± 31.6	31.23 ± 29.1	39.5 ± 37.2	>0.05
The duration of follow-up, (months) mean ± SD	32 ± 26.7			
Fever, number, %	152 (58.2)	110 (57.5)	42 (58.5)	>0.05
Abdominal pain, number, %	223 (85.4)	162 (84.8)	61 (87)	>0.05
Arthritis, number, %	50 (19.1)	40 (20.9)	10 (14.3)	>0.05
Arthralgia, number, %	111 (42.5)	90 (47.1)	21 (30)	0.013*
Chest pain, number, %	33 (12.6)	27 (14.1)	6 (8.6)	>0.05
Erysipelas-like erythema, number, %	4 (1.5)	4 (2.6)	0	>0.05
Amyloidosis, number, %	2	2 (1.04)	0	
Proteinuria, number, %	45 (17.2)	37 (19.3)	8 (11.4)	
Consanguinity, number, %	27	20 (10.4)	7 (10)	>0.05
Family history of FMF, number, %	126 (48.4)	90 (47)	36 (51.4)	>0.05
Family history of amyloidosis, number, %	8 (3.06)	6 (3.14)	2 (2.8)	>0.05
Underwent surgery, number, %	11 (4.21)	9 (4.7)	2 (2.8)	

*Chi-square test.

Table 2. Comparison among disease responses to colchicine therapy, severity score, and colchicine dosage.

Response to colchicine, number, %				P*
Complete	182 (69.7)	129 (67.5)	53 (75.7)	
Incomplete	67 (25.6)	51 (26.7)	16 (22.85)	
Nonresponsive	12 (4.59)	11 (5.75)	1 (1.42)	
Pras disease severity score				
Mild	110 (42.1)	87 (45.5)	23 (32.8)	>0.05
Moderate	146 (55.9)	99 (51.8)	47 (67.1)	0.04
Severe	5 (1.91)	5 (2.6)	0	>0.05
Dosage of colchicine				
0.5 mg/day	45 (17.2)	29 (15.2)	16 (22.8)	>0.05
1 mg/day	187 (71.6)	135 (70.7)	52 (74.3)	>0.05
1.5 mg/day	24 (9.19)	22 (11.5)	2 (2.9)	
2 mg/day	5 (1.91)	5 (2.6)	0	>0.05

*Chi-square test.

Table 3. MEFV mutations in StripAssay analysis.

Mutation	Genotype	Number (%)
Homozygous	M694V	28 (14.6)
	M680I	3 (1.6)
	M694I	1 (0.5)
	E148Q	4 (2.0)
	P369S	1 (0.5)
	F479L	1 (0.5)
Heterozygous	M694V	50 (26.1)
	M680I	6 (3.14)
	M694I	2 (1.0)
	E148Q	29 (15.2)
	P369S	3 (1.6)
	R761H	5 (2.6)
	K695R	5 (2.6)
	V726A	5 (2.6)
	A744S	1 (0.5)
Compound heterozygous	M694V/M680I	9 (4.7)
	M694V/M694I	1 (0.5)
	M694V/V726A	8 (4.2)
	M694V/E148Q	12 (6.3)
	E148Q/M680I	1 (0.5)
	E148Q/M694I	1 (0.5)
	E148Q/P369S	4 (2.09)
	E148Q/K695R	1 (0.5)
	M680I/R761H	3 (1.6)
	M680I/V726A	5 (2.6)
	M694I/V726A	1 (0.5)
	K695R/A744S	1 (0.5)
Total		191

In order to detect the coding and noncoding sequence variations along the *MEFV* gene, we performed bidirectional DNA sequencing analysis in all 10 coding exons and exon–intron boundaries of the respective gene. The reported frequencies of common and rare nucleotide substitutions and synonymous and nonsynonymous single nucleotide polymorphisms (SNPs) found in group 2 are seen in Table 4 and the Figure.

Three patients were homozygous for D102D, G138G, and A165A and heterozygous for R314R, E474E, Q476Q, and D510D. Six patients were heterozygous for D102D, G138G, A165A, R314R, E474E, Q476Q, and D510D. Recently, the R202Q mutation has been increasingly included in discussions. Twenty-two patients (31%) were heterozygous for R202Q and 3 patients (4%) were homozygous. Six patients were homozygous for the R314R mutation located on the third exon and 2 were heterozygous. Heterozygosity for R314R, E474E, Q476Q, and D510D polymorphism was detected in 21 patients (Table 4). Haplotype distribution is shown in the Figure. The most encountered haplotypes in each exon were E474E, Q476A, and D510D in exon 5; R314R in exon 3; and D102D, G138G, and A165A in exon 2.

Table 4. Single nucleotide polymorphisms in group 2.

Single nucleotide polymorphisms	n
D102D, G138G, A165A, R314R, E474E, Q476Q, D510D heterozygote	3
D102D, G138G, A165A homozygote, R314R, E474E, Q476Q, D510D heterozygote	6
R314R, E474E, Q476Q, D510D heterozygote	21
R314R, E474E, Q476Q, D510D homozygote	7
R314R heterozygote	2
R314R homozygote	6
R202Q heterozygote	22
R202Q homozygote	3

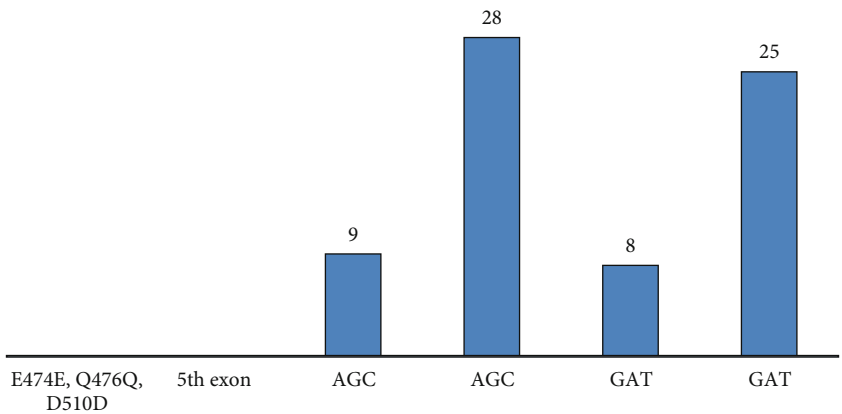

Figure. SNP haplotype distributions.

4. Discussion

FMF is an inherited autosomal recessive disease, diagnosed by the recurrent self-limited episodes of fever and serosal inflammation. FMF is commonly seen in the Middle East and the Mediterranean region (3,9,10). It is rarely encountered in other parts of the world. FMF patients have also been reported from European countries such as France, Germany, Italy, and Spain. Patients diagnosed with FMF have also been encountered in the Unites States, Australia, and Japan (11).

FMF prevalence is 1/500–1/2000 among susceptible races. FMF carrier rate is 16%–22% (12). FMF prevalence is 1:400–1.1:1000 in Anatolia, and the carrier frequency is as high as 1/5 among Turks (11–16). The estimated prevalence of the disease in Turkey is 0.1% (13). We reported the frequencies of the *MEFV* gene mutations, clinical manifestations, and genotype–phenotype correlations in 261 children with FMF from the Aegean region of Turkey.

A male predominance in FMF patients has been documented in some studies. However, most studies report that FMF affects both sexes equally. In our study, the male-to-female ratio showed slight female dominance (M:F ratio: 1:1.08). In another study from Turkey, M:F ratio was documented as 1:1.3 (slight female dominance), although the difference between these figures was not statistically significant (17).

The onset of clinical manifestations begins before the age of 5 in 65% of FMF cases and before 20 years of age in 90% of cases. The onset of the disease may occur as early as 6 months of age (18). In our study, the mean age of onset, mean age at diagnosis, and mean time interval between disease onset and diagnosis were 79.9 ± 42.1, 104.8 ± 49, and 33.5 ± 31.6 months, respectively. The mean age at diagnosis was significantly higher in group 1 than in group 2, and the mean duration of follow-up was similar in both groups. In our patient cohort, the onset of FMF was at 3–5 years in 144 patients and 6–10 years in 81 patients. The onset of clinical manifestations in FMF occurred before the age of 10 in 86% of the cases in our study.

The mean age of onset was 72.9 ± 42.1 months. The early onset age observed in our study may be explained by

the early detection of the disease by clinical suspicion and rapid confirmation by genetic analysis.

Typical clinical findings can vary among different populations and ethnicities. In Jewish patients, fever and abdominal pain were more prevalent, while pleurisy and arthritis were seen more frequently than other findings in Armenian patients.

The main clinical characteristics of our patients were as follows: abdominal pain (n: 223, 85.4%), fever (n: 152, 58.2%), myalgia (n: 8, 3.06%), thoracic pain (n: 32, 12.6%), arthritis (n: 50, 19.1%), arthralgia (n: 111, 42.5%), and erysipelas-like erythema (n: 4, 1.5%). In our study, the most common clinical symptom was abdominal pain, similar to the Turkish FMF study (93.7%). In the Turkish FMF study (14,15), the arthritis rate was reported as 47.4%. In our study, percentages were 19.1% for arthritis and 42.5%. for arthralgia. Two patients developed amyloidosis.

Some patients had typical FMF phenotype, either with only one *MEFV* gene mutation or with no mutation (10,13). Therefore, the disease may be inherited as an autosomal dominant trait with partial penetration. The diagnosis is difficult in mild cases and in atypical forms of the disease.

M694V is encountered in 71% of North African Jews. A mild mutation, V726A, was found in 38% of East European Jews (Ashkenazi). A wide range of mutations were seen in Italians; the most frequently seen mutation is E148Q (18%) (4,6). M694V and E148Q are the first (32%) and second (14%) most frequent mutations in Spain, respectively. In the studies by Touitou et al. (4) and the Turkish FMF study group (15), the most common MEFV mutations in Turkey were M694V (57 and 51.4%, respectively), followed by M680I (16.5 and 14.4%, respectively) and V726A (13.9 and 8.6%, respectively). M694V was the most common mutation in our study, similarly to many previous studies (17,19–24). We found that M694V (41.3%) accounted for the majority of FMF chromosomes, followed by E148Q (19.9%), M680I (10.3%), V726A (7.2%), P369S (3.06%), K695R (2.6%), M694I (2.2%), R761H (3.06%), A744S (0.76%), and F479L (0.38%) (Table 1). The frequencies of E148Q mutation have been reported to range from 3.5% to 18.3% in Turkey (13,20). We also detected a high incidence of E148Q mutation (19.9%). At present, E148Q is considered as a disease-causing mutation, not a benign polymorphism. The most frequent heterozygous carrier mutation was E148Q (6.9%). The carrier rate was 16% with a mutation frequency of 8% in the Turkish population (7).

E148Q is a mild mutation with less penetrance. When E148Q is part of a complex allele, it has been suggested to have a cumulative aggravating effect. Patients homozygous for M694I and M680I, or carrying a combination of both at codons 694 and 680, have as severe a disease as patients homozygous for M694V and V726A.

Amyloidosis is a fatal, devastating complication of FMF causing renal insufficiency and end-stage renal disease. Amyloidosis frequency differs between populations (25). In our study, we found amyloidosis in 2 (0.76%) patients who were M694V homozygous and M694V/M680I compound heterozygous. Because all of our patients were children, they were all informed about the disease and learned the importance of compliance to colchicum treatment.

High rates of consanguineous marriages increase the possibility of transmitting genetic diseases. In our study, 27 patients (10.3%; 15 homozygous, 6 heterozygous, and 5 compound heterozygous) had a family history of consanguinity. A history of FMF was seen in 48.4% of patients. This finding highlights the importance of molecular diagnosis and sequencing, particularly in the ancestral populations of FMF.

The clinical spectrum associated with MEFV mutations ranges from symptom-free to severe complications such as amyloidosis. Highly penetrant M694V homozygosity has been demonstrated to correlate with severe disease in North African Jews, Arabs, and Armenians (99%). In M694V homozygous patients, frequently seen clinical features include amyloidosis, early disease onset, and arthritis. High fever, splenomegaly, and protracted myalgia are seen more frequently in those patients. In our study, the percentages of patients with M694V and with complaints of fever, abdominal pain, and arthritis were 3, 6, and 3 times higher than those in patients with other mutations, respectively. Arthritis was seen in 40% of M694V homozygous and compound heterozygous patients in our cohort ($P = 0.003$; $P < 0.05$). In our study, the presence of the E148Q mutation was significantly associated with increased presence of abdominal pain. In addition, V726A mutation was significantly associated with increased presence of pleurisy.

Pras score was higher in patients with arthritis, arthralgia, and myalgia. Most of our patients had moderate disease (146 cases; 56%). In a study by Pras et al., 80 of 83 patients had M694V mutation in at least one allele. M694V mutation was homozygous in 70 patients, 12 of whom developed amyloidosis (25). Five patients with a severe disease score were M694V homozygous, M694V homozygous, M694V heterozygous, E148Q heterozygous, and M694V/M680I heterozygous. The presence of the M694V mutation increased Pras disease score. Attacks were controlled by higher doses of colchicum in group 1. M694V homozygosity increases disease severity ($P = 0.0001$; $P < 0.05$).

The clinical spectrum changes from the typical findings of the full-blown disease to asymptomatic condition. Therefore, the molecular analysis of the *MEFV* gene is a useful tool in clinical practice in FMF diagnosis.

The diagnosis of FMF is made according to the clinical characteristics and exclusion of other periodic fevers. Thus, a definite diagnosis is difficult in patients with milder or atypical manifestations of the disease. For patients whose *MEFV* gene does not contain mutations of exons 2, 3, 5, and 10, we also performed bidirectional DNA sequencing in exons 1, 4, 6, 7, 8, and 9 (16).

R761H is much more prevalent in Armenians and Turks. K695R is seen more frequently in Jews (non-Ashkenazi and Ashkenazi). A744S is encountered more in Arabs and F479L in Armenians. R761H mutation was seen more frequently in our study than in the literature. R761H mutation was heterozygous in 5 (2.6%) and compound heterozygous M680I/R761H in 3 (1.6%) patients. In a previous study from Turkey, it was found that R761H mutation was heterozygous and compound heterozygous in 330 of 23 patients (3.48%) (26). It is 4.2% in our study.

In healthy populations, R202Q heterozygosity is common and is not associated with disease severity (27). In a study by Giagilis et al., R202Q homozygosity was present in 12 of 25 FMF patients without any *MEFV* gene mutation (27).

We detected additional critical SNPs, of which we have only limited information for Turkish FMF patients. In the same study from Greece, DNA sequencing analysis was made and SNPs were demonstrated. In this study possible recombination regions were A165A and R202Q, R202Q and Q476E, and D510D and Q476E. We found R314R, SNP, D102D, G138G, A165A coexistence and R314R, E474E, Q476Q, D510D recombination on exon 3. R202Q heterozygosity was found in 70 patients with sequencing analysis (Table 4).

We did not observe A165A and R202Q or R202Q and Q476E coexistence. However, D510D and Q476Q were found strongly together (38 cases, 54%). R314R was homozygous in 6 patients. R314R, E474E, Q476Q, and D510D were heterozygous in 21 patients and homozygous in 7 patients. There were 4 haplotypes in our study (Figure). The most commonly seen haplotype was AGCTTAC (E474E, Q476Q, and D510D-R314R, 28R202Q, R314R). The presence of a SNP did not carry statistical difference in terms of clinical findings. The disease severity score was mild in the SNP group. Incomplete colchicum response was seen in 23% of patients with SNP. Fortunately, it was observed that patients in the SNP group needed low doses of colchicum in order to control disease activity. In R202Q homozygous patients, frequency in attacks could not be decreased despite full doses of treatment. In another study from Spain, a place between R202Q and R314R was thought of as a hot spot (28). We thought that R314R may be a hot spot according to our study. Rare mutations and SNPs have great importance for FMF pathogenesis. For FMF, heterogeneity exists in phases of alleles, frequency and critical locations of mutant alleles, and clinical appearance. With sequencing analysis, we prevent missing less common variants that might be restricted to populations by classical methods (16).

Routine screening tests primarily investigate the most prevalent *MEFV* mutations. Therefore, we should primarily research the entire coding sequence of the *MEFV* gene before analyzing the other recurrent fever genes. Eventually, sequence analysis will help prevent rare population-restricted novel sequence variants from being overlooked, which is important for the characterization of typical and atypical FMF (16).

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