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Original Article

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Evaluation of Genotypic and Phenotypic Characteristics of Children with Familial Mediterranean Fever in Eastern Turkey

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Abstract

Objective: Familial Mediterranean fever (FMF) is a hereditary disease. It usually affects countries in the Mediterranean region and is common in Turks. This retrospective study was conducted to evaluate phenotype-genotype characteristics of children with FMF in Malatya district and surrounding areas in eastern Turkey.
Materials and methods: A total of 427 patients who had been diagnosed with clinical FMF between 2006 and 2015 were included in the study.
Results: Of the patients, 207 (48.5%) were female, and 220 (51.5%) were male. The mean age of diagnosis was 7.7±3.7 years, and the age of onset of complaints was 5.7±3.5 years. The delay of diagnosis was 1.9±1.8 years. The most common complaint was abdominal pain (95.1%). The most commonly detected mutant allele was M694V (26.9%) mutation. We detected heterozygous mutations in 203 (52%) patients, homozygous mutations in 71 (18%) patients, compound heterozygous mutations in 81 (22%) patients, and no mutation in 8% of the patients. The most common homozygous mutation was M694V (57.7%), the most common heterozygous mutation was E148Q (38.4%), and the most common compound heterozygous mutation was M694V/M680I (17.1%).
Conclusion: In our study, we found that the frequency of mutations was similar to that of the whole population of Turkey, and the severity of the disease was lower.
Keywords: Children, eastern Anatolia, familial Mediterranean fever, phenotype-genotype, MEVF gene

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INTRODUCTION

Familial Mediterranean fever (FMF) is a hereditary disease with autosomal recessive transmission, which usually affects countries in the Mediterranean region and is common in Turks, Armenians, Arabs, and Sephardic Jews (1, 2). Currently, it is the most common and well-known example of hereditary periodic fever syndromes with approximately 1/1000 frequency (3).

Familial Mediterranean fever disease involves mutations in the Mediterranean fever (MEFV) gene. The *MEFV* gene encoding the pyrin/marenostrin protein was first described in 1997 (4, 5). Although the role of the pyrin protein is not fully understood, the main role is thought to control inflammation (6). Clinical findings are the results of inflammation of the synovial and serous membranes. In Turkey, the most common mutation in the *MEFV* gene is M694V, followed by M680I and V726A (7).

Familial Mediterranean fever is a childhood disease; clinical complaints begin before the age of 20 years in 90% of the patients (8). It is a disease characterized by fever with inflammation-related attacks in one or more parts of the body. Inflammation usually occurs in the abdomen, chest, joints, muscles, skin, and scrotum. The phase of the disease when clinical symptoms are seen is called “attack.” The patients feel completely well during the intervals between the attacks, and this feature is important for diagnosis (9, 10).

The aim of the present study was to evaluate phenotype-genotype characteristics of children with FMF in Malatya district and surrounding areas in eastern Turkey.

MATERIALS AND METHODS

This was a retrospective descriptive study. A total of 427 patients from Malatya and surrounding provinces in eastern Turkey who were clinically diagnosed with FMF, who started colchicine treatment, and who were followed up between 2006 and 2015 were included in the study.

The files of the patients were reviewed retrospectively. Clinical findings, physical examination findings, laboratory results, *MEFV* gene mutation analysis results, complaints and findings at the time of control and evaluation, and information about the treatment they received were recorded. Sex, height and weight, birth place, and personal and familial information were also recorded. The age of the patients was noted according to the age of the first attack and the date they were diagnosed. The consanguinity status in the patient's family history information is specified. Laboratory findings at the time of diagnosis or follow-up were recorded. Urine analysis results, proteinuria values, and *MEFV* gene mutation results included in the patients' files were also recorded.

Patients were assessed according to both criteria by Tel Hashomer and Yalçınkaya et al. (11). Two major criteria or one major+two minor criteria were evaluated as definite disease, and one major+one minor criterion was evaluated as possible disease according to the Tel Hashomer criteria. The presence of two or more of the five criteria including ≥3 times >38 °C fever lasting 12-72 h, ≥3 times abdominal pain lasting 12-72 h, ≥3 times chest pain lasting 12-72 h, ≥3 times arthritis episode lasting 12-72 h, and family history for FMF was assessed as the presence of the disease according to the Yalçınkaya criteria.

The severity score of the disease was calculated based on the Pras scoring system (12). This scoring system has six conditions including onset age, colchicine dosages, number of attacks/month, presence of arthritis, erysipelas-like erythema, and amyloidosis. 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 of amyloidosis, 0-4 points.

In addition, the dosage of colchicine treatments they have been receiving and the duration of the treatment in terms of the number of years, as well as their response to colchicine and side effects, were recorded.

The study protocol was approved by the ethics committee of İnönü University School of Medicine (2016/9-30) in accordance with the principles of the Declaration of Helsinki. Informed consent was not required for this type of study.

Genomic DNA Extraction and Pyrosequencing Technique

Genomic DNA was extracted from EDTA anticoagulated venous blood using the EZ1 DNA Blood 200 µL kit and the BioRobot EZ1 Workstation (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A number of methods are used in the diagnosis of FMF. A recently developed simple to use pyrosequencing technique allows for short-read DNA sequencing. The DNA fragments including hot spots within the coding sequences of the *MEFV* gene were amplified by polymerase chain reaction (PCR) using 5 µL genomic DNA and FMF Pyrosequencing kit (ATQ) according to the manufacturer's instructions. Briefly, 10 µL PCR product was added to 40 µL binding buffer (Qiagen) and 2 µL Streptavidin Sepharose High Performance beads (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The PCR products attached to the beads were washed in 70% ethanol, followed by denaturation in 0.2 N NaOH and washing buffer (Qiagen). Purified DNA samples were annealed to the sequencing primer (2.5 µL) and annealing buffer (22.5 µL) (Qiagen) and denaturated for 2 min at 80 °C, followed by cooling down to room temperature for 5 min. The samples were then processed in the PyroMark Q24 Instrument. Set-up of assay and sequence-run, as well as analysis, were performed by the PyroMark Q24 Software (13).

Statistical Analysis

Data were analyzed using the IBM *Statistical* Package for the Social Sciences Statistics program, version 22.0 (IBM SPSS Statistics Corp.; Armonk, NY, USA) for *statistical* analysis. The normal distribution of the data was evaluated by the Shapiro-Wilk test. Data were presented as mean±standard deviation and/or median (min-max). Mann-Whitney U test was used for comparisons of independent two groups, and Kruskal-Wallis test was used for comparisons of more than two independent groups. Multiple comparisons after the Kruskal-Wallis test were made by using Bonferroni corrected Mann-Whitney U test. The relationship between categorical data types was examined by the Pearson chi-square or Yates continuity correction test. A $p<0.05$ was considered statistically significant.

RESULTS

To our knowledge, this is the first study that has been done in eastern Anatolia in Turkey. Data of 427 patients who were examined in the Department of Pediatric Rheumatology and diagnosed with FMF between 2006 and 2015 were evaluated retrospectively. The evaluations were based on the data collected at the time of diagnosis and during follow-up examinations.

Demographic Characteristics of the Patients

The study included 207 (48.5%) female and 220 (51.5%) male patients with FMF. The mean age of diagnosis was 7.7±3.7 (1.0-17.0) years, and the mean age of the onset of complaints was 5.7±3.5 (0.0-16.0) years. The time between the onset of the complaints and the diagnosis was expressed as the delay of diagnosis, and the mean delay of diagnosis was 1.9±1.8 (0.0-15) years. The first episode of complaints occurred between 2 and

10 years old in 68.8% of the patients. The delay of diagnosis was 2 years in 329 (77.2%) patients, between 2 and 5 years in 75 (17.6%) patients, and >5 years in 22 (5.2%) patients. The consanguinity rate was 48 (11.2%) in our population. We found that 182 (42.7%) patients had a family history of the disease. Amyloidosis was not detected in the patients, whereas it was present in the parents of 8 (1.9%) patients. Demographic and clinical characteristics of the patients with FMF are shown Table 1.

Table 1. Demographic and clinical characteristics of patients with FMF

Features	n=427
Male/female (n, %)	207 (48.5)/220 (51.5)
Age at onset (years, mean±SD and min–max)	7.7±3.7 (1.0-17.0)
Age at diagnosis (years, mean±SD and min–max)	5.7±3.5 (0.0-16.0)
Delay at diagnosis (years, mean±SD and min–max)	1.9±1.8 (0.0-15)
Family history of FMF (n, %)	182 (42.7)
Consanguinity of parents (n, %)	24 (5.6)
Family history of amyloidosis (n, %)	8 (1.9)
Abdominal pain (n, %)	405 (95.1)
Fever (n, %)	364 (85.4)
Arthralgia (n, %)	250 (58.7)
Arthritis (n, %)	44 (10.5)
Febrile myalgia (n, %)	43 (10.1)
Erysipelas-like erythema (n, %)	15 (3.5)
Chest pain (n, %)	6 (1.4)
Appendectomy (n, %)	19 (4.5)
Urinary tract infection (n, %)	25 (5.8)
Henoch-Schonlein purpura (n, %)	4 (0.9)
Celiac disease (n, %)	2 (0.4)
Amyloidosis (n, %)	0 (0)

FMF: familial Mediterranian fever

Clinical Characteristics of the Patients

The most common complaints were abdominal pain (95.1%), fever (85.4%), arthralgia (58.7%), arthritis (10.5%), febrile myalgia (10.1%), and chest pain (1.4), respectively. Skin rash/erysipelas-like erythema was observed in 15 (3.5%) patients. Appendectomy was performed in 19 (4.5%) patients. The most common comorbidity accompanying FMF was urinary tract infection, which was observed in 25 patients. Henoch-Schonlein purpura (HSP) was seen in four patients, and celiac disease was seen in two patients.

The cases were grouped according to the disease severity scores developed by Pras et al.; those with <5 points were considered to have mild, those with 6–8 points were moderate, and those with ≥9 points were considered to have serious disease. The disease severity score was calculated for each patient separately. The lowest score was 3, the highest score was 11, and the average severity score was 5.67. According to this classification, 202 (47.3%) were mild, 211 (49.4%) were moderate, and 14 (3.3%) were severe patients with FMF. The mean severity score of the male and female patients was calculated as 6, and no significant difference was found in terms of sex (p=0.865). Regarding the severity scores according to the mutation groups, we found that the severity scores of those carrying the homozygous mutation were statistically significantly higher than those carrying the heterozygous mutation (p=0.028). The homozygous mutation that causes the most severe disease scoring was M694V.

In 2009, Yalçınkaya et al. determined new diagnostic criteria of patients in the childhood age group. They found that two out of

Table 2. The distribution of MEFV genotypes (n=386)

One allele	n (%)	Two allele (homozygous)	n (%)	Two allele (heterozygous)	n (%)
E148Q/–	83 (22.8)	M694V/M694V	41 (11.2)	M694V/M680I	17 (4.6)
M694V/–	63 (17.3)	M680I/M680I	14 (3.8)	E148Q/P369S	15 (4.1)
M680I/–	17 (4.6)	E148Q/E148Q	10 (2.7)	M694V/V726A	14 (3.8)
V726A/–	16 (4.3)	V726A/V726A	3 (0.8)	M694V/E148Q	14 (3.8)
P369S/–	12 (3.2)	P369S/P369S	3 (0.8)	E148Q/V726A	7 (1.9)
A744S/–	12 (3.2)			E148Q/R761H	5 (1.3)
				E148Q/M680I	4 (1.0)
				M694V/R761H	3 (0.8)
				E148Q/A744S	2 (0.5)

Table 3. The most common MEFV mutations reported in different studies from various regions of Turkey

References	Region of Turkey	No. of patients	Median age (years)	Mutations M694V	M680I	V726A	E148Q	P369S	R761H
Tunca et al. (21)	All	1090	23 (2-87)	51.5	8.1	14.4	NR	NR	NR
Yalçınkaya et al. (36)	Central	167	6 (0.1-40)	43.5	11.1	13.0	NR	NR	NR
Öztürk et al. (30)	West	369	12.5 (2-30)	51.9	10.8	10.4	15.5	1.0	5.5
Yılmaz et al. (3)	West	261	8.7 (1-17)	48.7	13.7	9.4	20.7	4.1	4.2
Ece et al. (37)	Southeast	147	9 (2-16)	26.0	13.0	6.3	30.7	13.5	10.5
This study	Eastern	386	7.7 (1-17)	26.4	8.0	4.6	24.0	3.6	2.1

NR: not reported

five of the diagnostic criteria have high sensitivity and specificity in FMF. We evaluated our patients according to the criteria by Yalçınkaya et al., which include fever, abdominal pain, chest pain, arthritis, and FMF story in the family. We found that 261 (61.1%) patients met two criteria, 143 (33.5%) patients met three criteria, and 3 (0.7%) patients met four criteria. There were no patients who met all the criteria in the study, whereas 20 (4.7%) patients did not meet any of the diagnostic criteria. When we evaluated our patients according to the Tel Hashomer diagnostic criteria, we found that 346 patients met the definite diagnosis criteria, 65 patients met the probable diagnostic criteria, and 16 patients did not meet the diagnostic criteria. When we compared the Yalçınkaya criteria with the Tel Hashomer criteria, the number of patients who did not meet the criteria was 14, and the number of patients who met the criteria was 405 in both cases. For patients who met ≥2 of the Yalçınkaya criteria, sensitivity was 98.54% (96.85%-99.46%), and specificity was 87.50% (61.65%-98.45%) according to the Tel Hashomer diagnostic criteria.

Phenotypic-Genotypic Characteristics of the Patients

Mutation results of genetically studied patients are summarized in Table 2. There was no correlation between the most frequent mutations of M694V and E148Q genes and the sex of the patients (p=0.279). There was no statistically significant correlation between M694V and E148Q mutations and mean age at onset, age of diagnosis, and duration of diagnosis (p>0.05). In our study, there were four patients with HSP. Their genetic characteristics were found to be homozygous M694V, heterozygous M694V, homozygous E148Q, and compound heterozygous M694V/V726A. There were two patients with celiac disease, and their genetic characteristics were found to be compound heterozygous E148Q/R761H and M694V/M680I. There were 19 patients who had undergone appendectomy. Of these patients, four had M694V/M680I compound heterozygous, two had homozygous M694V, and two had heterozygous E148Q mutations. There were no mutations in four patients who had undergone appendectomy. In our study, there were a total of nine patients with hepatosplenomegaly, and five had homozygous M694V mutation.

Treatment-Related Features of Events

Colchicine treatment was started with an average initial dose of 1.00 mg/m^2 in all 427 patients. Only six patients developed diarrhea as a side effect while under colchicine treatment. The prevalence of exacerbations in the pre-colchicine treatment was evaluated, and there were no patients who had <3 episodes/year. Up to 6-12 episodes/year were observed with 240 patients before colchicine treatment, followed by 86 patients with 13-24 episodes/year. There were no attacks in 308 patients after colchicine treatment and two attacks per year in 40 patients. After colchicine treatment, 118 (27.7%) patients had an attack, and 307 (72.3%) patients did not. When we examined the mutation types in patients who experienced exacerbations, we found that homozygous and heterozygous mutations were equal in 31% of the patients. In our study, the mean duration of colchicine use of the 427 patients was 2 (0.50-13.00) years. Regarding the patients' responses to colchicine treatment, 72.3% responded fully, 22.7% responded partially, and 5% did not respond (resistant) to colchicine treatment. Homozygous and heterozygous mutations were equally frequent in colchicine-resistant patient (31%). Canakinumab was used in a patient with M694V mutation.

DISCUSSION

Familial Mediterranean fever (FMF) is not universal, but an ethnically based disease. In Turkey, the prevalence of the disease is 1/1000, and the carrier rate is 1/5 (14). Today, diagnosis of FMF is totally based on clinical findings, such as family history, response to colchicine treatment, and ethnicity (15).

In recent years, mutational analyses have started to be used widely to support clinical diagnosis. The *MEFV* gene responsible for FMF disease is defined on the short side of the 16th chromosome by the French FMF Consortium and the International FMF Consortium (16). The *MEFV* gene consists of 10 exons, and DNA alterations occur in the last exon in >80% of the cases. Less common mutations occur in exons 2, 3, and 5 (17). Regardless of the ethnicity of the patients, mutations of M694V, V726A, M680I, and M694I in exon 10 were the most common mutations (18). In the present study, 427 patients with MEFV mutations who were followed up in the pediatric rheumatology department

and who were diagnosed with FMF disease were evaluated retrospectively.

Symptoms usually begin in childhood in patients with FMF. Majeed et al. reported that the disease begins before 10 years old in approximately 80% of patients with FMF and Gedalia et al. reported that the disease begins before in 60% of the patients (19, 20). The mean age at onset of illness was reported to be 9.6 years in 10 large series studies conducted by the FMF study group in Turkey (21). In our study, the mean age of onset of symptoms was 5.7±3.5 years, supporting the majority of the literature. It is known that a certain period has elapsed between the onset of clinical complaints of patients and with FMF diagnosis, and there is a delay in the diagnosis. Yalçınkaya et al. conducted a study in 2009 and found that the duration of the delay was approximately 3 years (11). In our study, there was a mean delay of 1.9±1.8 years of diagnosis in our patients. Although, FMF disease usually occurs between the ages of 5 and 15 years, complaints occur before the age of 20 years in 90% of the patients (21). In our study, the age of diagnosis was found to be 7.7±3.7 (1.0-17.0) years. The diagnosis may be delayed depending on whether the clinical symptoms are mild, or the physician does not consider the diagnosis. A longer duration of delay of diagnosis is very important regarding the complications. Indeed, the gradual decrease in the frequency of amyloidosis, the most important complication of FMF, is associated with a decrease in the duration of delay of diagnosis (22). In the majority of studies, the incidence of FMF is reported to be similar in both sexes, but there are studies reporting that the disease is seen more frequently in girls or boys (23-25). Similar to the majority of the literature, in our study, 207 (48.5%) patients with FMF were females, and 220 (51.5%) were males. Regarding the genetic transmission of FMF disease, it is expected that the disease will be seen more frequently in the relatives. In a previous study, 20.1% of the patients had a close relative who had been diagnosed with FMF, and 18.9% of the patients had a history of consanguineous marriage (26). In our study, 42.7% of our patients had an FMF story in the family, and 5.6% of the patients had a story of consanguineous marriage.

The most common clinical symptom of FMF is recurrent abdominal pain with fever. The incidence of abdominal pain was 93.7%, and fever was 92.5% according to the Turkish FMF study group (21). Tunca et al., in their study conducted over 2000 patients in Turkey, found that abdominal pain is the most common clinical finding, with a rate 93.2% (21). In other studies, the most common complaints of the patients' on admission were abdominal pain and fever (27). In our study, the most common complaint was abdominal pain (95.1%), and fever was seen at the second frequency (85.4%). In the study conducted by the Turkish FMF study group, 19% of the patients had appendectomy, and in our study, the rate was 4.5% (21). The incidence of arthritis in FMF is reported to be between 40% and 70% (10, 28). Arthritis is most prevalent in North African Jews, whereas it has been reported less frequently in Iraqi Jews, Armenians, and Turks (28). In our study, the incidence of arthritis was 10.5%. As a result, we suggest that the incidence of arthritis in patients with FMF in the pediatric age group could be higher than that in the general patient population. In a study of the Turkish FMF study group, the rate of arthralgia was found to be 51.7% in patients <18 years old (21). In our study, similar to the literature, we found the arthralgia ratio as 58.7%. In previous studies, the most common abdominal ultrasonography was splenomegaly (29-31). In the study by Kone et al., the rates of splenomegaly and hepatomegaly were 34% and 3%, respectively, and a relationship between M694V homozygous mutation and splenomegaly was found (32). In our study, the rate of splenomegaly was 2.1%, and it was most often associated with homozygous M694V mutation. In the study by Tunca et al., erysipelas-like erythema is reported to be seen in 20.9% of the patients. Erysipelas-like erythema has been shown to be more common in male sex and in patients <18 years old (21). In our study, 15 (3.5%) patients had erysipelas-like erythema or rash. The most serious complication of FMF disease is amyloidosis, but the frequency of amyloidosis development varies between societies and races. In one study, the development of amyloidosis associated with FMF was reported to be 60% in Turks, 27% in non-Ashkenazi Jews, and 1%-2% in Armenians living in America (1). In a study conducted on Turks, the rate of amyloidosis was reported to be 12.9% (21). In our study, there were no patients with amyloidosis, but the rate of history of amyloidosis in the family was 1.9%. Since amyloid accumulates slowly in the organs and tissues, development of amyloidosis takes a long time. We think that our study was also affected by the fact that our series included only the patients in the childhood age group.

In recent years, mutational analyses have also been widely used to support the clinical diagnosis. In patients with FMF, M694V mutation was the most common mutation in other studies conducted in our country, and the mutation frequency ranges from 43.5% to 70% (33, 34). In a study conducted on a large series in a Turkish society, mutation rates were reported as M694V 51.5%, M680I 9.2%, E148Q 3.5%, V726A 2.8%, and M694I 0.4%, respectively (35). In a study conducted by Yalçınkaya et al., M694V mutation was reported to be 43.5%, M680I was 13%, V726A was 11.1%, and M694I was 2.8%, respectively (36). Ece et al. reported the most common mutations in Diyarbakır region as E148Q, M694V, R761H, and V726A, respectively (37). In the study conducted by Öztürk et al. in İzmir province and its vicinity, the most common mutations in 369 patients among the 452 patients who met the criteria were M694V, E148Q, and V726A, respectively (30). In our study, we detected MEFV mutation in at least one allele in 386 (90.4%) of 427 patients. The remaining 41 (9.6%) patients were considered to have undetected/unknown mutations. In our study, M694V mutation was detected as the most common MEFV mutation with a 26.9% rate, followed by E148Q 24%, M680I 8.0%, V726A 4.6%, P369S 3.6%, and A744S 3.1%, respectively (Table 3).

In our study, we found that the frequency of mutations was similar to that of the whole population of Turkey, and the severity of the disease was lower.

CONCLUSION

Abdominal pain and fever in child patients are common symptoms in primary care physicians. If abdominal pain and fever complaints are recurrent in patients, physicians should consider FMF. Increasing family physicians' awareness of FMF will improve the quality of life of patients with FMF, by allowing early diagnosis and treatment and providing protection against perilous complications, such as amyloidosis.

Ethics Committee Approval: Ethics committee approval was obtained from the ethics committee of İnönü University.
Informed Consent: Written informed consent was not required from patients due to the retrospective nature of the present study.

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