Turkish Archives of Pediatrics

ORIGINAL ARTICLE
DOI: 10.5152/TurkArchPediatr.2021.21147

Prediction of More Severe *MEFV* Gene Mutations in Childhood

Seviye Güneş-Yılmaz[1,*], Belde Kasap-Demir[2,3,*], Eren Soyaltın[3], Gökçen Erfidan[3], Özgür Özdemir-Şimşek[3], Seçil Arslansoyu-Çamlar[4], Demet Alaygut[4], Fatma Mutlubaş[4]

[1]Department of Pediatrics, University of Health Sciences, İzmir Tepecik Training and Research Hospital, İzmir, Turkey
[2]Department of Pediatric Nephrology and Rheumatology, İzmir Katip Çelebi University School of Medicine, İzmir, Turkey
[3]Department of Pediatric Nephrology, University of Health Sciences, İzmir Tepecik Training and Research Hospital, İzmir, Turkey
[4]Department of Pediatric Nephrology, University of Health Sciences School of Medicine, İzmir, Turkey
*These authors have contributed equally to this work.

What is already known on this topic?

- *Familial Mediterranean Fever (FMF) is the most common monogenic autoinflammatory disease characterized by periodic attacks of fever, polyserositis and arthritis.*
- *The most frequent mutations in our country are M694V, M694I, M680I, V726A and E148Q.*
- *M694V mutations are associated with a more severe phenotype although the phenotype-genotype correlations have not been clarified definitively yet.*

What this study adds to this topic?

- *Pras scores were higher and high dose colchicine was needed more frequent in M694V homozygous/compound heterozygous cases.*
- *The presence of FMF in a first-degree relative increases the probability of having homozygous/compound heterozygous M694V mutations by 2.39 times.*
- *Each 1unit increase in Pras score increases this probability 1.43 times. The threshold Pras score for this possibility is 5.5 (AUC: 0.651, 95% CI, 0.545-0.757, P=.006).*

Corresponding author:
Belde Kasap-Demir
✉beldekasap@gmail.com
Received: May 11, 2021
Accepted: July 7, 2021

ABSTRACT

Aim: This study aimed to present the demographic, clinical, and laboratory features of children clinically diagnosed with familial Mediterranean fever (FMF) and to predict more severe mutations by evaluating those findings.

Methods: We enrolled cases diagnosed with FMF with a defined variation in at least one allele. The medical charts of the patients were reviewed retrospectively. The patients were grouped as homozygous, compound heterozygous, and simple heterozygous cases, with and without *M694V* mutation. We compared the data between the subgroups using logistic regression analysis and determined the risk factors for being homozygous or compound heterozygous for *M694V*.

Results: A total of 263 (M/F =109/154) cases were included. The mean age at the onset of symptoms, follow-up duration, and time to diagnosis were 6.75 ± 3.9 (0.25-17) years, 51.78 ± 39.31 (6-166) months, and 9.23 ± 14.44 (1-132) months, respectively. The rates of parental consanguinity, positive family history for FMF, and FMF in a first-degree relative were 15%, 42%, and 31.4% respectively. The most common symptom was abdominal pain (85%). There was no difference between the growth parameters of the cases during the initial and final control periods. The most frequent alleles were *M694V*, *E148Q*, and *V726A*. The most common accompanying disease was IgA vasculitis (20%). Almost 90% of the cases fulfilled all the defined criteria. The rate of patients having a first-degree relative with FMF was higher, Hb values were lower, and the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) values were higher during the attack period; the ESR and CRP values were higher in the attack-free period; and Pras disease severity scores were higher in homozygous or compound heterozygous cases carrying *M694V*. The presence of FMF in a first-degree relative increases the probability of being homozygous and compound heterozygous for *M694V* by a factor of 2.39; and each 1 unit increase in the Pras score increases this probability by a factor of 1.43. The threshold Pras score for this possibility is 5.5 (AUC = 0.651; 95% CI, 0.545-0.757; *P* = .006; sensitivity, 65%; specificity, 55%).

Conclusion: *M694V* was the most common and severe mutation in our cohort. The presence of a first-degree relative with FMF and Pras scores ≥5.5 may predict a homozygous or compound heterozygous mutation for *M694V*.

Keywords: Familial Mediterranean fever, children, genotype, phenotype

INTRODUCTION

Familial Mediterranean Fever (FMF) is the most common monogenic autoinflammatory disease characterized by periodic attacks of fever, polyserositis, and arthritis.[1,2] The attacks are self-limiting and accompanied by a high acute-phase response.[1] The disease mostly

Cite this article as: Güneş-Yılmaz S, Kasap-Demir B, Soyaltın E, et al. Prediction of more severe MEFV gene mutations in childhood. *Turk Arch Pediatr.* 2021; 56(6): 610-617.

affects individuals with Turkish, Armenian, Arabic, and Jewish ancestry.[2] Autosomal recessive mutations in the *MEFV* gene are responsible for the clinical manifestations.[1,2] The estimated prevalence of FMF is 1/1000 and the carrier rate is 1/5 to 1/8 in Turkey.[3,4]

Historically, the Livneh, Tel-Hashomer, and Turkish FMF Pediatric Study Group criteria have been established for the diagnosis, and the genetic analysis was not necessary.[5-7] However, Eurofever/PRINTO proposed the new separate sets of criteria based on clinical features only and both clinical and genetic features.[8] There are over 350 mutations that have been described to date and 12 of them are the most frequent and routinely screened in mutation analysis.[9] The most frequent mutations described in our country are *M694V*, *M694I, M680I, V726A,* and *E148Q*, in order of decreasing frequency.[3,4] Although the genotype–phenotype correlations have not been clarified definitively yet, it is well-known that *M694V* mutations are associated with a more severe phenotype and amyloidosis.[3,10,11]

In this study, we evaluated our cases with a defined variation in at least one allele in terms of genotype–phenotype correlation, growth, and coexisting diseases, and compared the results with the most recent studies published in the past decade on the largest pediatric series. Besides, we aimed to predict more severe mutations including homozygous or compound heterozygous *M694V* mutations by using demographic, clinical, and laboratory data.

METHODS

We enrolled all the pediatric cases diagnosed with FMF between January 2010 and July 2020 according to Tel-Hashomer criteria[6] who were followed-up in the pediatric nephrology and pediatric rheumatology clinics for at least 6 months. We excluded those who had none or only benign variations in their genetic analysis. The demographic, clinical, and laboratory data of the patients were recorded from the medical charts of the patients in a retrospective manner. The demographic data included gender, age at onset of the clinical symptoms, and standard deviation scores (SDS) of body weight, height, and body mass index (BMI), parental consanguinity, presence of FMF patients in the family and among the first-degree relatives, time to diagnosis, frequency of attacks before and after colchicine treatment, and presence of accompanying diseases. Growth parameters including SDS of height, weight, and BMI were determined via the Child Metrics program according to the published normal values.[12,13]

The clinical data included the follow-up period, the clinical findings including fever, abdominal pain, chest pain, joint pain, and erysipelas-like rash at the onset of the disease and in the follow-up, colchicine dose, and Pras disease severity score. The laboratory data included white blood cell (WBC) count, hemoglobin (Hb), thrombocyte count, percentage of neutrophils, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels at the time of the attacks and in the attack-free period. Persistently elevated levels of at least one acute-phase reactant (APR) during the attack-free period was defined as "subclinical inflammation." At least the most frequent 12 mutations (*M694V, M694I, M680I* (G>C), *M680I* (G>A), *V726A, E148Q, K695R, R761H, A744S, F479L, I692del*, and *P369S*) in the *MEFV* gene were screened in all cases.

According to the Tel-Hashomer criteria, 2 major or 1 major+2 minor criteria are needed for a definite diagnosis of FMF. The major criteria include recurrent fever with polyserositis, AA type amyloidosis, and response to colchicine treatment; the minor criteria include recurrent fever, erysipelas-like erythema (ELE), and FMF in a first-degree relative.[6] The disease severity was evaluated with Pras disease severity score adapted for colchicine dose in children[14]: (a) age at onset [11-20 years (2 points), 6-10 years (3 points) and <6 years (4 points)]; (b) the number of attacks per month [<1 (1 point), 1-2 (2 points), >2 (3 points)]; (c) acute or protracted arthritis (2 and 3 points, respectively); (d) presence of ELE (2 points); (e) dose of colchicine [less than the appropriate dose (0 points), appropriate dose (1 point), and more than the appropriate dose (2 points)]; and (f) development of amyloidosis (3 points). The severity score is the sum of the points gained for each parameter. A score of 3-5 is accepted as "mild," 6-9 as "moderate," and >9 as "severe" disease. The mentioned appropriate dose was 0.5 mg/day for children younger than 5 years; 0.5-1 mg/day for children between 5 and 10 years; and 1.5 mg/day for children older than 10 years. The standard dose of colchicine prescribed for children is 0.04 mg/kg or 1.2 mg/m^2/day.[15] In cases with an inadequate response, the dose would be increased up to 1.5 mg/day for children between 5 and 10 years and up to 2 mg/day in children older than 10 years of age.[4,16] The resistance to colchicine treatment was defined as experiencing ≥1 attack per month despite receiving the maximally tolerated dose for ≥ 6 months.[16]

Statistical Analysis

Analyses were performed using Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM SPSS Corp.; Armonk, NY, USA). Descriptive statistics were shown in mean ± standard deviation and frequency format. The Kolmogorov–Smirnov test was used to evaluate the normal distribution of continuous variables between the groups. The parameters with normal distribution were compared by Student's *t*-test, whereas those without normal distribution were evaluated by the Mann–Whitney *U*-test. The categorical variables between the groups were compared using the chi-square test. For comparison of the anthropometric parameters at the time of diagnosis and the last visit, the paired-samples *t*-test was used for parameters with normal distribution and Wilcoxon's signed-rank test for those without normal distribution. The correlation of the Pras scores and the anthropometric and laboratory parameters were evaluated by Spearman's correlation test. Calculation of the area under the curve (AUC) of the receiver-operating characteristic curve was used for assessing the ability of the Pras score to predict homozygous or compound heterozygous *M694V* mutation. Logistic regression was used to assess the probability of being homozygous or compound heterozygous for *M694V*. Variables having a statistical significance of < 0.100 in univariate comparisons were deemed to have clinical importance and were included in the multivariate logistic regression,[17] and odds ratios for independent factors were determined. A *P*-value of <.05 was considered significant in all statistical evaluations.

RESULTS

The study group included 311 subjects diagnosed with FMF according to the Tel-Hashomer criteria. Children without mutations (n = 18) and those with benign variations only [*R202Q* homozygous (n = 11), *R202Q* heterozygous (n = 18), *P706P* (n = 1)] were excluded.

Demographic, Clinical, and Laboratory Findings of the Patients

Finally, 263 cases (M/F =109/154) were involved in the study. The mean age of the cases at the onset of symptoms, the mean follow-up duration, and the duration between the onset of symptoms and diagnosis, family history for FMF, and the rate of cases with a first-degree relative diagnosed with FMF are depicted in Table 1. The rate of parental consanguinity was 15% (n = 39). The most frequent symptom among the cases was abdominal pain (85%), followed by fever, joint pain, and chest pain, and the least frequent symptom was ELE (Table 1). Our patients were diagnosed according to the Tel-Hashomer criteria. Of those, 240 (91.2%) fulfilled the Livneh, 261 (99.2%) fulfilled the Turkish Pediatric FMF Group and 260 (98.8%) fulfilled the Eurofever/PRINTO criteria as well.

In our cohort, 127 patients (48%) had 2 alleles with defined variations, and 136 (52%) had one allele with a defined variation. The most frequent homozygous phenotype in our cohort was *M694V/M694V*. The most common compound heterozygous phenotype was the *M694V* mutation in one allele. The most common simple heterozygous mutation was also *M694V*. The variations defined are shown in Table 2. When the allele frequency was detected, the 3 most common mutations were *M694V* (35.5%), *E148Q* (12%), and *V726A* (10%), respectively.

Only 2 cases had persistent non-orthostatic proteinuria in whom amyloidosis was confirmed by biopsy. One had a renal transplant and both were on anti-IL-1R therapy. Both cases had homozygous *M694V* mutations. Five additional cases were resistant to colchicine and treated with anti-IL-1R therapy. Three of them had homozygous *M694V* and the other 2 cases had *M694V/M680I* compound heterozygous mutations.

The mean Pras disease severity score was 5.64±1.41 (3-10) points. The distribution of the disease severity was mild in 118 (45%), moderate in 137 (52%), and severe in 8 (3%) of the cases. Colchicine was prescribed as 0.5 mg daily in 35 (13.3%), 0.5 mg daily in 2 (0.8%), 1 mg daily in 147 (56.3%), 1.25 mg daily in 3 (1.1%), 1.5 mg daily in 58 (22.1%), and 2 mg daily for 17 (6.5%) of the patients at the last visit. These colchicine doses were

Table 2. The Distribution of the *MEFV* Gene Variations in the Cohort

Variants	n (%)	Variants	n (%)
M694V/M694V	37 (14)	*V726A/K695R*	3 (1.1)
M694V/M680I	17 (6.4)	*P369S*/R408Q**	4 (1.5)
M694V/V726A	17 (6.4)	*E167D/F479L*	1 (0.4)
*M694V/E148Q**	9 (3.4)	*M694V/*	65 (24.7)
M680I/M680I	4 (1.5)	*M680I/*	12 (4.6)
E148Q/E148Q**	6 (2.2)	*E148Q*/*	28 (10.6)
V726A/V726A	3 (1.1)	*V726A/*	19 (7.2)
M694V/M694I	1 (0.4)	*P369S*/*	1 (0.4)
M694V/R761H	4 (1.5)	*M694I/*	1 (0.4)
M680I/V726A	5 (2)	*R761H/*	2 (0.8)
M694I/V726A	1 (0.4)	*K695R/*	3 (1.1)
E148Q/D510N**	1 (0.4)	*R653H*/*	2 (0.8)
E148Q/M680I*	3 (1.1)	*A511V*/*	1 (0.4)
E148Q/P369S**	8 (3)	*I591T*/*	1 (0.4)
E148Q/R761H*	2 (0.8)	*A744S*/*	1 (0.4)
E148Q/V726A*	1 (0.4)	Total	263 (100)

*Variants unknown significance.

Table 1. Comparison of the Demographic and Clinical Findings of Our Cohort With Similar Studies

	Our Study, (n = 263)	Ayaz et al.,[10] (n = 1687)	Barut et al.,[19] (n = 708)	Öztürk et al.,[18] (n = 452)	Tunca et al.,[3] (n = 2838)
Gender (M/F)	109/154 (0.7)	846/841 (1.01)	362(346 (1.05)	180/228 (0.79)	1541/1297 (1.19)
Age at onset	6.75 ± 3.9 y	5.4 ± 4.05 y	4.8 ± 3.4 y	5.3 ± 3.4 y	9.6 (8.55) y
Time to diagnosis	9.23 ± 14.44 (1-132) m	8 ± 4.2 y	7.3 ± 3.8 y	8.4 ± 3.5 y	16.4 (11.57) y
Follow-up period	51.78±39.31 (6-166) m	3 (0.5-18) y		42 (6-174) m	
FMF in the family, n (%)	111 (42)	778 (46)	370 (52.3)	117 (28.7)	818/2436 (3)*
FMF in a first-degree relative, n (%)	82 (31)	433 (25.6)	-	-	-
Consanguinity, n (%)	39 (15)	-	207 (29.2)	107 (26.2)	-
Fever, n (%)	187 (71)	1398 (83)	629 (88.8)	334 (81.9)	2603/2813 (92.5)
Abdominal pain, n (%)	224 (85)	1436 (85)	634 (89.2)	352 (86.3)	2635/2811 (93.7)
Joint pain, n (%)	82 (31)	Arthritis:610 (36); Arthralgia:909 (54)	Arthritis:288 (40.7)	200 (49)	Arthritis:1264/2668 (47.4); Arthralgia:1190/2597 (49.7)
Chest pain, n (%)	20 (8)	383 (22.7)	122 (17.2)	150 (36.8)	816/2615 (31.2)
Erysipelas-like erythema, n (%)	3 (1)	281 (16.6)	213 (30.1)	69 (16.9)	530/2532 (20.9)

M, male; F, female; y, year; m, month; *only in subjects with renal involvement.

Table 3. The Distribution of Comorbid Diseases and the Associated *MEFV* Gene Mutations (n = 63)

Comorbid Diseases	n (%)	*MEFV* Mutations	n (%)
IgAV*	13 (20)	*M694V/M694V*	4 (6.1)
Enuresis#	10 (15.4)	*E148Q/E148Q*	2 (3)
Recurrent urinary tract infections#	9 (13.8)	*M680I/M680I*	1 (1.5)
Epilepsy#	6 (9.2)	*M694V/M680I*	3 (4.6)
Asthma*	5 (7.7)	*M694V/V726A*	2 (3)
Isolated persistent microscopic hematuria#	5 (7.7)	*M694V/E148Q*	1 (1.5)
Behçet's disease*	3 (4.6)	*M694I/V726A*	1 (1.5)
Thalassemia trait#	3 (4.6)	*M680I/V726A*	1 (1.5)
Protracted febrile myalgia*	2 (3)	*M680I /E148Q*	1 (1.5)
Juvenile idiopathic arthritis*	2 (3)	*E148Q/P369S*	1 (1.5)
PFAPA*	2 (3)	*E148Q/R761H*	1 (1.5)
Nephrolithiasis#	2 (3)	*V726A/K695R*	1 (1.5)
Extrarenal pelvis#	1 (1.5)	*P369S/R408Q*	1 (1.5)
Hypertension#	1 (1.5)	*M694V/*	18 (27.7)
Renal scarring#	1 (1.5)	*E148Q/*	14 (21.5)
Vesicoureteral reflux#	1 (1.5)	*V726A/*	6 (9.2)
Crohn's disease*	1 (1.5)	*M680I/*	4 (6.1)
İmmune deficiency#	1 (1.5)	*R653H/*	1 (1.5)
HbH disease#	1 (1.5)	*R761H/*	1 (1.5)
Mitral insufficiency#	1 (1.5)		
Atrial septal defect#	1 (1.5)		
Donnai-Barrow syndrome#	1 (1.5)		

*Associated diseases; #coincidental diseases; PFAPA, periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis syndrome; HbH, Hemoglobin H.

classified as low in 123 (46.7%), appropriate in 118 (44.9%), and high in 22 (8.4%) of the cases according to Pras et al.[14] Colchicine was not discontinued in any of the patients.

When the growth parameters at the onset of the diagnosis and the last visit were compared, bodyweight SDS (−0.21±1.33 vs. −0.25±1.35, P = .521), height SDS (0.02±1.55 vs. −0.06±1.49, P = .237), and body mass index (BMI) SDS (−0.33±1.37 vs. −0.25±0.30, P = .284) levels were similar. Pras disease severity scores were not correlated with body weight SDS, height SDS, or BMI SDS at the onset of the diagnosis or the last visit ($P > .05$). Moreover, the Pras scores were not correlated with WBC, hemoglobin, ESH, or CRP levels in the attack and attack-free periods, either ($P > .05$). Of the patients, 65 (24.7%) had at least one coexisting disease that may have been coincidental or associated with FMF. Five cases had 2 and 1 patient had 3 coexisting disorders. The most frequent accompanying disorder was IgA vasculitis (IgAV) and the most frequent allele associated with accompanying diseases was *M694V* (Table 3).

Genotype–Phenotype Analysis of the Patients

Gender, age at onset, consanguinity of the parents, presence of other patients with FMF in the family and among the first-degree relatives, time to diagnosis, coexisting diseases, fever, abdominal pain, chest pain, joint pain, and erysipelas-like rash at the onset of the disease and in the follow-up, frequency of attacks before and after colchicine treatment, WBC and thrombocyte count, percentage of neutrophils, ESR, Hb, and CRP levels, and having at least 1 high APR at the time of the attacks and in the attack-free period, body weight SDS, height SDS, and BMI SDS at the onset of the disease and the last visit, the Pras scores, and having mild, moderate, and severe Pras scores were compared among the groups separately.

Comparison of the Data Between the Subgroups

- **Patients with homozygous variants:**

When *M694V* homozygous (n = 37) and other homozygous (n = 13) cases were compared, the ESR values during the attack period [45 (31-67) vs. 23.5 (13.75-41.5), P = .004], the ESR and CRP values in the attack-free period [17.5 (12-28) vs. 9.5 (8-11.75), P = .001 and 3.10 (1.5-8.05) vs. 0.9 (0.18-1.63), P =.001, respectively], the ratio of having at least one high APR level in the attack-free period (57% vs. 15%, P = .024) and the Pras scores [6 (5-7) vs. 4 (4-6)] were higher in the *M694V* homozygous group.

- **Patients with heterozygous variants**:

When the combined heterozygous groups with (n = 48) and without (n = 29) *M694V* mutation were compared, those with *M694V* had higher incidence of chest pain at the disease onset [10 (20.8%) vs. 1 (3.4%), P = .045], lower Hb values [11.8 (11-12.4) vs. 12.4 (11.9-13.52), P = .033], and higher ESR [28 (21.5-38) vs. 18.5 (8-38.5), P = .044] and CRP [28.6 (8.9-93.4) vs. 4.75 (1.83-23.8), P = .011] values during the attack period.

- **Patients with simple heterozygous variants:**

When the cases with a heterozygous *M694V* mutation (n = 65) and other simple heterozygous mutations (n = 71) were compared, joint pain during the follow-up was more common (12% vs. 2.8%, P = .048) in the *M694V* heterozygous group, and the height SDS was lower (0.22 ± 1.53 vs. −0.12 ± 1.65) in the last control in the group with other heterozygous mutations, compared to the baseline.

Table 4. Comparison of the Demographic, Clinical, and Laboratory Parameters Between the Patients With Homozygous or Compound Heterozygous Variants for *M694V* (*M694V**) and the Others

	Homozygous or Compound Heterozygous for *M694V*, (n = 85)	Others, (n = 178)	*P*-value
Parental consanguinity (%)	13	16	.682
1 relative with FMF (%)	40	27	.046
FMF in the family (%)	47	40	.333
Abdominal pain (%)	80	88	.148
Chest pain (%)	13	5	.046
Joint pain (%)	34	30	.570
WBC in the attack period	9.1 (6.95-13.30)	8.45 (6.85-10.75)	.078
Hb in the attack period	11.7 (11-12.6)	12.1 (11.2-12.9)	.019
ESR in the attack period	32 (23-46)	23.5 (15-41)	<.001
CRP in the attack period	34.9 (9.1-92.7)	10.65 (2.4-35.2)	<.001
WBC in the attack-free-period	7.5 (6.3-8.8)	7.2 (5.9-8.6)	.037
Hb in the attack-free period	12.3 (11.5-13.1)	12.4 (11.8-13.2)	.342
ESR in the attack-free period	13 (9-19)	11.5 (8-17)	.003
CRP in the attack-free period	2.1 (0.7-3.8)	1.5 (0.5-3.0)	.008
Pras score	6 (5-7)	5.5 (5-6)	.005

Data presented as median percent or (interquartile range). FMF, familial Mediterranean fever; WBC, white blood cell; Hb, hemoglobin; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

- **Two-allele mutated groups:**

The Hb values were lower [11.8 (10.8-12.4) vs. 12.4 (11-11.35), *P* = .024] and the ESR and CRP values were higher [35 (25-50) vs. 19 (11-41.25), *P* = .001 and 33 (9.6-93.3) vs. 10.45 (2.32-22.82), *P* = .007, respectively] during the attack period; ESR and CRP values in the attack-free period [14 (9.3-18) vs. 9 (7.75-11.25), *P* < .001 and 2.25 (0.7-4) vs. 1.25 (0.2-1.85), *P* = .006, respectively], and the rate of having at least one high AFR level in the attack-free period (35% vs. 15%, *P* = .043) were significantly higher in cases that were homozygous or compound heterozygous for *M694V* (n = 85), when compared to those without an *M694V* mutation despite having homozygous or compound heterozygous variations. It was also observed that the Pras score was higher and the need for high-dose colchicine was more frequent in the latter group.

Since having homozygous or compound heterozygous *M694V* mutations raised significant concerns, those patients were compared with the rest of the patients (Table 4). The parameters causing difference with a significance of < 0.100 (Table 4) were included in the binary logistic regression analysis, having a first-degree relative with FMF increased the probability by a factor of 2.39, and each 1 point increase in Pras score increased this probability by a factor of 1.43. Although significant, each 1 mg/L increase in CRP increased the probability by a factor of only 1.007 (Table 5). Besides, despite a low sensitivity (65%) and specificity (55%), the threshold Pras score value showing this possibility was 5.5 with an AUC of 0.651 (95% CI, 0.545-0.757, *P* = .006).

DISCUSSION

In this study, we have evaluated the demographic parameters, genotype–phenotype correlation, laboratory findings, accompanying diseases, and growth, in a large group of children with FMF. Confirming the previous studies, we have demonstrated that the *M694V* genotype is the most frequent mutation and is associated with a more severe disease course. However, the most frequent comorbid disease was IgAV in our cohort. Moreover, we have demonstrated that a family history of FMF in a first-degree relative and Pras disease severity scores >5.5 may predict cases with homozygous or compound heterozygous *M694V* mutations. We have also compared the results of our study with the most recent childhood series published in the past decade with the highest number of cases, and with our nationwide study including both adults and children.

Familial Mediterranean fever is expected to affect both genders equally. In our cohort, the male to female ratio was 0.7 (109/154). Male[5,10] or female dominancy[18,19] has been reported in various childhood series. Besides, a male to female ratio of 1.2 : 1 has been reported in the nationwide Turkish FMF Study Group data (3) (Table 1). The recurrent episodes of FMF usually begin before 10 years of age (2). The mean age at the onset of symptoms was 6.75±3.9 years in our patients, which was slightly higher compared to the pediatric series with the highest number of cases from our country, but lower when compared to the nationwide data. However, the period between the onset of the symptoms and the diagnosis was markedly shorter in our series when compared to the previous reports.

Table 5. Summary of the Multiple Binary Logistic Analysis of Independent Variables to Assess the Probability of Having Homozygous and Compound Heterozygous Variations for *M694V*

	B	S.E.	sig	OR	95% CI for OR
1 relative with FMF	0.873	0.404	0.031	2.394	1.084-5.284
CRP in the attack period	0.007	0.003	0.016	1.007	1.001-1.013
Pras score	0.354	0.135	6.859	0.009	1.093-1.856

SE, standard error; OR, odds ratio; CRP, C-reactive protein; FMF, familial Mediterranean fever.

Since FMF is an autosomal recessive disease, family history plays an important role. The rates of having FMF in the family and a first-degree relative were higher and the parental consanguinity rates were lower in our cohort (Table 1). The consanguinity rate is 20% to 25% in our general population, which is higher in the eastern and lower in the western part of our country.[18] Since our cohort is originated from the Aegean region, the lower rate of consanguinity in our series may be associated with the geographic zone.

Abdominal pain was more frequent than fever among our patients, which is similar to the other series (Table 1). Joint pain was less common in our series (31%). That would be because the other series were reported from rheumatology clinics, where cases with joint pains were admitted and *MEFV* gene analysis may have been performed in the selected cases where FMF needed be considered in the differential diagnosis. However, our cases were diagnosed and followed-up in both rheumatology and nephrology units. In a previous study, 36.1% of the subjects with joint pain had *M694V* homozygous mutation (21), and among our cases, 19% had *M694V* homozygous mutation. The incidence of ELE was distinctly less frequent in our cohort (1%). The ratio was between 16% and 30% in the previous pediatric series.[10,19,20] The frequency of ELE was higher in subjects with *M694V* homozygous mutation. In our cohort, ELE was reported in 3 cases at the time of diagnosis and in 2 cases in the follow-up. Only one of them had homozygous and 2 of them had compound heterozygous *M694V* mutations. Our patients were diagnosed according to the Tel-Hashomer criteria, and at least 90% of them fulfilled the other 3 sets of criteria.

We have excluded the cases without a defined mutation and those with benign variants including *R202Q*. Although *R202Q* is accepted as a benign variant in databases,[9,21] subjects with milder phenotypes and cases with severe phenotypes, and even colchicine resistance or amyloidosis have been reported.[10,22-24] However, this variant has not been reported in many labs for a while. Thus, we preferred to exclude cases with *R202Q* variants. Finally, the leading mutation in our cohort was *M694V*. This was the same in the mentioned pediatric series and the nationwide study.[3,10,19,20] Only in a pediatric series reported from the southeast part of our country, the most frequent mutation was *E148Q* and *M694V* was in the second place.[18] In the nationwide study and the 2 large childhood series from Istanbul, the second most frequent mutation was *M680I*. However, the third frequent mutation was *V726A* in the nationwide study and it was *E148Q* in the latter 2 studies, respectively.[3,10,20] In our study and in the previous study from our city, the second most frequent allele was *E148Q* and the third was *V726A*.[19] These results have shown that there may be clustering of genes in different regions even within the same country.

Patients with 2 mutant alleles had a more severe phenotype and significantly higher disease severity scores when compared to those with a one-allele mutation.[19] The homozygous and compound heterozygous *M694V* mutations have the most severe disease activity[18] as well as the risk of renal amyloidosis or colchicine resistance.[4,18] Similar to those studies, we found that subjects with homozygous and compound heterozygous *M694V* mutations had a more severe disease activity correlated with more severe laboratory responses, both in the attacks and in the attack-free periods, and had higher Pras scores.

Amyloidosis is the most severe complication of FMF; however, with the increased awareness and early treatment of the disease, the incidence is gradually decreasing.[11] Only 2 of our cases had amyloidosis and both had homozygous *M694V* mutations. Indeed, amyloidosis could have been prevented in both of our cases. The family of the first case refused to use anti-IL1 therapy and began to use it just before the transplantation. The second case was an adolescent with non-adherence to colchicine treatment. Besides, 5 cases with colchicine resistance were either homozygous or compound heterozygous for *M694V*, as well. All cases were put on anti-IL1 therapy.

In the previous series, the effect of colchicine treatment on growth has been controversial. Although some studies have demonstrated significantly improved height development after colchicine,[25-27] some others have demonstrated that mean height SDS was significantly lower at the last visit.[28] Another study has demonstrated that there was no change in weight or height SDS levels before and after colchicine, but there was a significant increase in BMI after colchicine.[29] The effect of genotype, mainly *M694V* mutation, and the disease severity scores on growth also have controversial results. In our study, we could not demonstrate any significant change in growth parameters in the whole group or in subjects grouped concerning genotype, and we have found no correlation between Pras disease severity score and growth either.

Comorbidity is defined as the simultaneous existence of one or more additional conditions to the existing disease in a patient.[10,30] The comorbid diseases may be directly related to FMF probably due to increased innate inflammation or may be defined by coincidence. The association of IgAV, polyarteritis nodosa (PAN), Behçet's disease, and sacroiliitis with FMF is well-established.[3,10,31-33] There are accumulating data on the diseases coexisting with FMF, particularly in recent years, in large series of children and/or adults.[3,10,19,30,34-38] It has been suggested that concomitant disorders, especially inflammatory diseases, may influence FMF severity and worsen the course of FMF.[34] The frequency ranges between 7% and 32.8%, and it was 24.7% in our study. The most frequent associated disease is juvenile idiopathic arthritis (JIA) and the second most frequent is IgAV in most of the series, whereas the third most frequent disease differs among them (Table 6). In our series, the most frequent accompanying disease was IgAV, followed by enuresis and recurrent urinary tract infections. This difference was presumably due to the fact that most of our cases were admitted to pediatric nephrology clinics. Although IgAV was related to FMF, we thought that the urological problems were incidental.

Acute-phase response was elevated during the attacks when compared to the attack-free periods in our cases, as expected. In addition, ESR and CRP levels were significantly higher in cases that were homozygous or compound heterozygous for *M694*, both during the attacks and in the attack-free periods. Having high APR levels in the attack-free period is defined as subclinical inflammation, and the presence of *M694V* mutation has been defined as one of the risk factors.[39,40] Although damage indexes are associated with persistent subclinical inflammation in adult studies,[40] we could not demonstrate a correlation between the Pras scores and inflammation markers in the attack-free periods. Twenty-five percent of our cases had at least one high APR level in the

attack-free period, and of those, 32% were homozygous for *M694V* mutations, 12% were *M694V* compound heterozygous, and 25% were heterozygous for *M694V* mutations. In addition to higher APR levels, we have demonstrated that the hemoglobin levels were significantly lower during the attacks in cases with *M694V* homozygous and compound heterozygous mutations. Lower hemoglobin levels have also been reported in previous studies.[10,39]

As the patients with homozygous or compound heterozygous *M694V* mutations represent the most severe clinical and laboratory findings, we have tried to determine the indicators to anticipate the probability of having these genetic variations before performing genetic analysis. We have found that having a first-degree relative with FMF increased this probability by a factor of 2.39 and each 1 point increase in the Pras score increased this probability by a factor of 1.43. Such an analysis has not been performed previously.

The results of this study should be interpreted with its limitations. It has a retrospective design and data were collected from files. In addition, we reported this study from the western part of our country, but we did not consider the parental origins of our cases. Finally, the molecular *MEFV* gene analyses were performed in different centers at different periods.

CONCLUSION

The *M694V* mutation, which is classically known to be the most severe and the most common mutation in our society, was accompanied by more severe clinical and laboratory findings in our cohort and caused higher disease severity scores. In addition, the second and third most frequent mutations, as well as the most common mutations, were in accordance with the data reported from our region. Our data also showed that additional diseases that might be encountered with FMF should be kept in mind. In our study, family history and Pras scores ≥5.5 were important in the prediction of homozygous and compound heterozygous cases carrying *M694V*, which can be considered as the most severe mutation, and it is proposed that they could help determine the priority group for genetic analysis.

Table 6. The Most Frequent Comorbid Diseases Accompanying FMF in Different Cohorts

	Our Study	Balcı-Peynircioğlu et al.[29]	Ayaz et al.[10]	Yıldız et al.[34]	Ataş et al.[35]	Salahzadeh et al.[36]	Özçakar et al.[37]	Kışla-Ekinci et al.[33]	Öztürk et al.[18]	Tunca et al.[3]
	C	C+A	C	C	A	C+A	C	C	C	C+A
Frequency	65/263	656/2000	118/1687	130/686	205/971	57/400	77/600	85/494	ND	377/2716
(%)	(24.7)	(32.8)	(7)	(19)	(21.1)	(14)	(12.8)	(17.2)	(10.7)	(14)
1	IgAV	JIA	JIA	JIA	SpA	PFAPA	JIA	JIA	JIA	ARF
2	Enuresis*	IgAV	IgAV	Asthma	IgAV/BD/IBD	Peptic ulcer*	IgAV	Asthma	IgAV	IgAV
3	Recurrent UTI*	IBD	PFM	IgAV	Psoriasis	JIA/RA	PAN	IgAV	ND	Seronegative SpA

*Incidental diseases. C, children; A, adults; ND, not determined; JIA, juvenile idiopathic arthritis; IgAV, IgA vasculitis (Henoch-Schoenlein Purpura); SpA, spondyloarthritis; PFAPA, periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis syndrome; ARF, acute rheumatoid fever; BD, Behçet's disease; IBD, inflammatory bowel disease; UTI, urinary tract infection; PFM, protracted febrile myalgia; RA, rheumatoid arthritis; PAN, polyarteritis nodosa.

Ethical Committee Approval: Ethics committee approval was received from the Institutional Review Board of Izmir Katip Çelebi University Ethics Committee (17.01.2018/16).

Informed Consent: No informed consent has been planned for the study as it was a retrospective study.

Peer Review: Externally peer-reviewed.

Author Contributions: Concept – S.A.Ç., B.K.D.; Design - S.G.Y., B.K.D.; Supervision - B.K.D., F.M., Resource - B.K.D.; Materials - S.G.Y., E.S., Ö.Ö.Ş.; Data Collection and/or Processing - S.G.Y., G.E., Ö.Ö.Ş.; Analysis and/or Interpretation - E.S., .K.D., S.A.Ç., D.A.; Literature Search - S.G.Y, D.A., B.K.D.; Writing - S.H.Y., B.K.D.; Critical Reviews - B.K.D., F.M.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Ben-Chetrit E, Levy M. Familial Mediterranean fever. *Lancet*. 1998;351(9103):659-664. [CrossRef]
2. Ozen S, Bilginer Y. A clinical guide to autoinflammatory diseases: familial Mediterranean fever and next-of-kin. *Nat Rev Rheumatol*. 2014;10(3):135-147. [CrossRef]
3. Tunca M, Akar S, Onen F, et al. Familial Mediterranean fever (FMF) in Turkey: results of a nationwide multicenter study. *Med*. 2005;84(1):1-11. [CrossRef]
4. Yıldız M, Haşlak F, Adrovic A, Barut K, Kasapçopur Ö. Autoinflammatory diseases in childhood. *Balk Med J*. 2020;37(5):236-246. [CrossRef].
5. Livneh A, Langevitz P, Zemer D, et al. Criteria for the diagnosis of familial Mediterranean fever. *Arthritis Rheum*. 1997;40(10):1879-1885. [CrossRef]
6. Pras M. Familial Mediterranean fever: from the clinical syndrome to the cloning of the pyrin gene. *Scand J Rheumatol*. 1998;27(2):92-97. [CrossRef]
7. Yalçinkaya F, Ozen S, Ozçakar ZB, et al. A new set of criteria for the diagnosis of familial Mediterranean fever in childhood. *Rheumatology*. 2009 ;48(4):395-398. [CrossRef]
8. Gattorno M, Hofer M, Federici S, et al. Classification criteria for autoinflammatory recurrent fevers. *Ann Rheum Dis*. 2019;78(8):1025-1032. [CrossRef]
9. Infevers. *Infevers: An Online Database for Autoinflammatory Mutations*. 2021. Available at: http://fmf.igh.cnrs.fr/ISSAID/infevers/.
10. Ayaz NA, Tanatar A, Karadağ ŞG, et al. Comorbidities and phenotype–genotype correlation in children with familial Mediterranean fever. *Rheumatol Int*. 2021 ;41(1):113-120. [CrossRef]
11. Akse-Onal V, Sağ E, Ozen S, et al. Decrease in the rate of secondary amyloidosis in Turkish children with FMF: are we doing better? *Eur J Pediatr*. 2010;169(8):971-974. [CrossRef]
12. Demir K, Konakçı E, Özkaya G, et al. New features for child metrics: further growth references and blood pressure calculations. *J Clin Res Pediatr Endocrinol*. 2020;12(2):125-129. [CrossRef]
13. Neyzi O, Bundak R, Gökçay G, et al. Reference values for weight, height, head circumference, and body mass index in Turkish children. *J Clin Res Pediatr Endocrinol*. 2015;7(4):280-293. [CrossRef]
14. Pras E, Livneh A, Balow JE Jr, et al. Clinical differences between North African and Iraqi Jews with familial Mediterranean fever. *Am J Med Genet*. 1998;75(2):216-219. [CrossRef]
15. Kallinich T, Haffner D, Niehues T, et al. Colchicine use in children and adolescents with familial Mediterranean fever: literature review and consensus statement. *Pediatrics*. 2007;119(2):e474-e483. [CrossRef]
16. Ozen S, Demirkaya E, Erer B, et al. EULAR recommendations for the management of familial Mediterranean fever. *Ann Rheum Dis*. 2016;75(4):644-651. [CrossRef]
17. Bursac Z, Gauss CH, Williams DK, Hosmer DW. Purposeful selection of variables in logistic regression. *Source Code Biol Med*. 2008;3:17. [CrossRef]
18. Ece A, Çakmak E, Uluca Ü, et al. The *MEFV* mutations and their clinical correlations in children with familial Mediterranean fever in southeast Turkey. *Rheumatol Int*. 2014;34(2):207-212. [CrossRef]
19. Ozturk C, Halicioglu O, Coker I, et al. Association of clinical and genetic features in FMF with focus on MEFV strip assay sensitivity in 452 children from western Anatolia, Turkey. *Clin Rheumatol*. 2012;31(3):493-501. [CrossRef]
20. Barut K, Sahin S, Adrovic A, et al. Familial Mediterranean fever in childhood: a single-center experience. *Rheumatol Int*. 2018;38(1):67-74. [CrossRef]
21. Shinar Y, Obici L, Aksentijevich I, et al. Guidelines for the genetic diagnosis of hereditary recurrent fevers. *Ann Rheum Dis*. 2012;71(10):1599-1605. [CrossRef]
22. Comak E, Akman S, Koyun M, et al. Clinical evaluation of R202Q alteration of *MEFV* genes in Turkish children. *Clin Rheumatol*. 2014;33(12):1765-1771. [CrossRef]
23. Giaglis S, Papadopoulos V, Kambas K, et al. *MEFV* alterations and population genetics analysis in a large cohort of Greek patients with familial Mediterranean fever. *Clin Genet*. 2007;71(5):458-467. [CrossRef]
24. Nursal AF, Tekcan A, Kaya SU, Turkmen E, Yigit S. Mutational spectrum of the *MEFV* gene in AA amyloidosis associated with familial Mediterranean fever. *Iran J Kidney Dis*. 2016;10(3):107-112.
25. Ozçakar ZB, Kadioğlu G, Siklar Z, et al. The effect of colchicine on physical growth in children with familial Mediterranean fever. *Eur J Pediatr*. 2010;169(7):825-828. [CrossRef]
26. Yoldaş TÇ, Çakar N, Başaran Ö, et al. The effect of colchicine and disease severity on physical growth in children with familial Mediterranean fever. *Clin Rheumatol*. 2016;35(6):1603-1607. [CrossRef]
27. Zung A, Barash G, Zadik Z, Barash J. Familial Mediterranean fever and growth: effect of disease severity and colchicine treatment. *J Pediatr Endocrinol Metab*. 2006;19(2):155-160. [CrossRef]
28. Kişla Ekinci RM, Balci S, Akay E, et al. Disease severity and genotype affect physical growth in children with familial Mediterranean fever. *Arch Rheumatol*. 2019;34(3):288-293. [CrossRef]
29. Türkmen M, Soylu OB, Kasap B, et al. Growth in familial Mediterranean fever: effect of attack rate, genotype and colchicine treatment. *J Pediatr Endocrinol Metab*. 2008;21(8):789-792. [CrossRef]
30. Balcı-Peynircioğlu B, Kaya-Akça Ü, Arıcı ZS, et al. Comorbidities in familial Mediterranean fever: analysis of 2000 genetically confirmed patients. *Rheumatology*. 2020;59(6):1372-1380. [CrossRef]
31. Ozer E, Seker D, Taner E, et al. The frequency of juvenile spondyloarthropathies in childhood familial Mediterranean fever. *Clin Exp Rheumatol*. 2018;36(6)(suppl 115):141-145.
32. Ozdogan H, Arisoy N, Kasapçapur O, et al. Vasculitis in familial Mediterranean fever. *J Rheumatol*. 1997;24(2):323-327.
33. Aydin F, Özçakar ZB, Çakar N, et al. Sacroiliitis in children with familial Mediterranean fever. *J Clin Rheumatol*. 2019;25(2):69-73. [CrossRef]
34. Kişla Ekinci RM, Balci S, Ufuk Altintaş D, Yilmaz M. The influence of concomitant disorders on disease severity of familial Mediterranean fever in children. *Arch Rheumatol*. 2018;33(3):282-287. [CrossRef]
35. Yildiz M, Adrovic A, Tasdemir E, et al. Evaluation of co-existing diseases in children with familial Mediterranean fever. *Rheumatol Int*. 2020;40(1):57-64. [CrossRef]
36. Atas N, Armagan B, Bodakci E, et al. Familial Mediterranean fever is associated with a wide spectrum of inflammatory disorders: results from a large cohort study. *Rheumatol Int*. 2020;40(1):41-48. [CrossRef]
37. Salehzadeh F, Moghaddam AE. Coexisting diseases in patients with familial Mediterranean fever. *Open Access Rheumatol*. 2020;12:65-71. [CrossRef]
38. Özçakar ZB, Çakar N, Uncu N, Çelikel BA, Yalçinkaya F. Familial Mediterranean fever-associated diseases in children. *QJM*. 2017;110(5):287-290. [CrossRef]
39. Basaran O, Uncu N, Celikel BA, Aydın F, Cakar N. Assessment of neutrophil to lymphocyte ratio and mean platelet volume in pediatric familial Mediterranean fever patients. *J Res Med Sci*. 2017;22:35. [CrossRef]
40. Babaoglu H, Armagan B, Bodakci E, et al. Predictors of persistent inflammation in familial Mediterranean fever and association with damage. *Rheumatol*. 2021;60(1):333-339. [CrossRef]