Prediction of More Severe MEFV Gene Mutations in Childhood

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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 (6-186) 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, M694I, M680I, V726A and E148Q.

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.12 The attacks are self-limiting and accompanied by a high acute-phase response.1 The disease mostly
affects individuals with Turkish, Armenian, Arabic, and Jewish ancestry.2 Autosomal recessive mutations in the MEFV gene are responsible for the clinical manifestations.1,12 The estimated prevalence of FMF is 1/1000 and the carrier rate is 1/5 to 1/8 in Turkey.1,4

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.3-7 However, Eurofever/PRINTO proposed the new separate sets of criteria based on clinical features only and both clinical and genetic features.1 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.3 The most frequent mutations described in our country are M694V, M694I, M680I, V726A, and E148Q, in order of decreasing frequency.1,4 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.2,10,11

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 criteria6 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 P368S) 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 children14: (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/m²/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.6,16 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 simple heterozygous mutation was also M694V. The variations defined are shown in Table 2. When the allele most common simple heterozygous mutation was also M694V, the 3 most common mutations were M694V, E148Q, and V726A (12%), and P706P (10%), respectively.

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)       | R761I/                 | 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)       | IS91T*                 | 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.,9 (n = 1687) | Barut et al.,9 (n = 708) | Öztürk et al.,9 (n = 452) | Tunca et al.,7 (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 (6-168) 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)             |                          |                          |                          |                          |
| Abdominal pain, n (%)  | 187 (71)            | 1398 (83)                 | 629 (88.8)               | 334 (81.9)               | 2603/2813 (92.5)         |
| Arthritis:610 (36); Arthralgia:909 (54) | 224 (85) | 1436 (85) | 634 (99.2) | 352 (86.3) | 2635/2811 (93.7) |
| Chest pain, n (%)      | 82 (31)             | Arthritis:288 (40.7)     | 200 (49)                 |                          |                          |
| Erysipelas-like erythema, n (%) | 20 (8) | 383 (22.7) | 122 (17.2) | 150 (36.8) | 816/2615 (31.2) |

M, male; F, female; y, year; m, month; *only in subjects with renal involvement.
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. 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 associated disease 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. Colchicine was not discontinued in any of the patients.

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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 3. The Distribution of Comorbid Diseases and the Associated MEVF Gene Mutations (\( n = 63 \))

| Comorbid Diseases                  | \( MEVF \) Mutations | n (%) |
|------------------------------------|----------------------|-------|
| IgAV*                              | M694V/M694V          | 4 (6.1) |
| Enuresis†                          | E148Q/E148Q          | 2 (3)  |
| Recurrent urinary tract infections*| M680I/M680I          | 1 (1.5) |
| Epilepsy†                          | M694V/M680I          | 3 (4.6) |
| Asthma†                            | M694V/V726A          | 2 (3)  |
| Isolated persistent microscopic hematuria† | M694V/E148Q        | 1 (1.5) |
| Behçet’s disease†                   | M694V/V726A          | 1 (1.5) |
| Thalassemia trait†                  | M680I/V726A          | 1 (1.5) |
| Protracted febrile myalgia†         | M680I/E148Q          | 1 (1.5) |
| Juvenile idiopathic arthritis†      | E148Q/P369S          | 1 (1.5) |
| PFAPA†                             | E148Q/R761H          | 1 (1.5) |
| Nephrolithiasis†                    | V726A/K695R          | 1 (1.5) |
| Extrarenal pelvis†                  | P369S/R408Q          | 1 (1.5) |
| Hypertension†                       | M694V/               | 18 (27.7) |
| Renal scarring†                     | E148Q/               | 14 (21.5) |
| Vescicoureteral reflux†             | V726A/               | 6 (9.2) |
| Crohn’s disease†                    | M680I/               | 4 (6.1) |
| Immune deficiency†                  | R653H/               | 1 (1.5) |
| HbH disease†                       | R761H/               | 1 (1.5) |
| Mitral insufficiency†               |                      |       |
| Atrial septal defect†               |                      |       |
| Donnai-Barrow syndrome‡             |                      |       |

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

| Parameter                        | 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 (11–12.8)                                          | 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.

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). Male5,10 or female dominancy5,9 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.
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. 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 MEVF 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. 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, subjects with milder phenotypes and cases with severe phenotypes, and even colchicine resistance or amyloidosis have been reported. 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. 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. 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. In our study and in the previous study from our city, the second most frequent allele was E148Q and the third was V726A. 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. The homozygous and compound heterozygous M694V mutations have the most severe disease activity as well as the risk of renal amyloidosis or colchicine resistance. 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. 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, some others have demonstrated that mean height SDS was significantly lower at the last visit. 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. 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. 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. There are accumulating data on the diseases coexisting with FMF, particularly in recent years, in large series of children and/or adults. It has been suggested that concomitant disorders, especially inflammatory diseases, may influence FMF severity and worsen the course of FMF. 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. Although damage indexes are associated with persistent subclinical inflammation in adult studies, 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. 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.

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

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

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