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ORIGINAL ARTICLE

The Effect of Gene Mutations on Disease Severity Scores in Pediatric Familial Mediterranean Fever Patients

Ailevi Akdeniz Ateşi Tanılı Çocuk Hastalarda Gen Mutasyonlarının Hastalık Ciddiyet Skorları Üzerine Etkisi

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ABSTRACT

Objectives: Familial Mediterranean Fever (FMF) is a self-limiting autoinflammatory disease. In order to better understand the prognosis of diseases, disease severity scores are used. The aim of this study is to determine the effect of genetic mutations on disease severity scores in children with FMF.
Methods: Patients aged 0-18 years who were diagnosed with FMF according to Yalçınkaya-Özen diagnostic criteria and whose gene analysis was performed were evaluated retrospectively. Pras et al's scoring system, Mor et al's scoring system and International severity score of FMF (ISSF) scoring system were applied to all patients. Genotypes were compared according to disease severity scores.
Results: When the patients were divided into 4 groups as M694V homozygous, heterozygous, M694V/ other allele combined heterozygous and other mutations, according to the score of Pras et al., the frequency of mild disease tended to be less in the M694V homozygous group. When the patients divided as homozygous M694V, heterozygous M694V, heterozygous E148Q, heterozygous M694V/ M680I combined mutations, according to the score of Pras et al., mild disease was found to be less common in the homozygous M694V group. When patients were divided into homozygous and heterozygous M694V (combined with other allele or single) groups, the disease was more severe in the homozygous M694V group according to the three scoring systems. In the concordance analysis between scoring systems, while a good agreement was found between Mor et al.'s scoring system and ISSF, the agreement with Pras et al.'s scoring system was weak
Conclusions: Based on the scoring system described by Pras et al., the rate of severe disease was higher in patients with homozygous M694V allele, whereas the rate of mild disease was statistically significantly higher in the heterozygous group (combined with other allele or single) compared with homozygous group. From this, we can conclude that the M694V homozygous mutation causes more severe disease than the M694V heterozygous mutation, and even more severe disease than its combination with another pathogenic mutation, one of which is M694V.

Keywords: Familial Mediterranean Fever, genotype, disease severity scores, clinic, treatment

ÖZ

Amaç: Ailevi Akdeniz Ateşi (AAA) kendi kendini sınırlayan otoinflamatuar bir hastalıktır. Hastalıklarınprognozunu daha iyi anlamak için çeşitli klinik kriterlerin bir kombinasyonuna göre puanlanan hastalık şiddeti skorları kullanılır. Bu çalışmanın amacı, AAA'lı çocuklarda genetik mutasyonların hastalık şiddeti skorlarına etkisini belirlemektir.
Gereç ve Yöntemler: Yalçınkaya-Özen tanı kriterlerine göre AAA tanısı konan ve gen analizi yapılan 0-18 yaş arası hastalar retrospektif olarak değerlendirildi. Tüm hastalara Pras ve arkadaşlarının skorlama sistemi, Mor ve arkadaşlarının skorlama sistemi ve Uluslararası AAA şiddet skoru (ISSF) skorlama sistemi uygulandı. Hastalar genetik mutasyonlarına göre sınıflandırılarak hastalık şiddeti skorları karşılaştırıldı.
Bulgular: Hastalar M694V homozigot, heterozigot, M694V/diğer alel birleşikheterozigot ve diğer mutasyonlar olarak 4 gruba ayrıldığında Pras ve arkadaşlarının hastalık ağırlık skoruna göre M694V homozigot olanlarda hafif hastalık sıklığı daha az bulunduHastalar en sık bulunan homozigot M694V, heterozigot M694V, heterozigot E148Q ve birleşik heterozigot M694V/M680I mutasyonlar olarak 4 gruba ayrıldığında, Pras ve ark.'ın skorlama sistemine göre homozigot M694V grubunda hafif hastalık sıklığı daha az saptandı. Hastalar homozigot M694V grubu ve heterozigot M694V (diğer bir alelle birleşik ya da tek) grubu olarak ikiye ayrıldığında, üç skorlama sistemine göre homozigot M694V grubunda hastalık daha şiddetliydi. Skorlama sistemleri arasındaki uyum analizinde Mor ve ark. ve ISSF skorlama sistemleri arasında güçlü bir uyum saptanırken, Pras ve ark. skorlama sistemi ile diğer skorlar arası uyum zayıf bulundu.
Sonuçlar: According to the scoring system defined by Pras et al., patients with homozygous M694V allele had a higher rate of severe disease than all other groups. . Buradan M694V homozigot mutasyonun, M694V heterozigot mutasyondan daha şiddetli hastalık yaptığı ve hatta biri M694V olmak üzere diğer bir patojenik mutasyon ile kombinasyonundan da daha şiddetli hastalık yaptığı sonucuna varabiliriz.

Anahtar Kelimeler: Ailevi Akdeniz Ateşi, genotip, hastalık şiddet skorları, klinik, tedavi

Introduction

Familial Mediterranean Fever (FMF) is a self-limiting autoinflammatory disease characterized by inflammation of the serosal surfaces and recurrent episodes of fever along with abdominal pain, arthralgia, arthritis, serositis, and erysipelas-like rash, typically lasting 6–72 hours during attacks (1-3). Although it is seen all over the world, its incidence is higher in ethnic groups living in the Eastern Mediterranean geography, especially Turks, Arabs, Armenians and Jews, compared to other countries. Its prevalence in these geographies varies between 1 in 500- 1000 (1). The most serious complication of the disease is amyloidosis, which can cause long-term morbidity and mortality (4). The Mediterranean fever (MEFV) gene is an autosomal gene that follows the autosomal recessive mode of inheritance and is present on the short arm of the sixteenth chromosome (5). Although only five mutations were found in 85% of the patients, 385 mutations were detected according to the latest data from the Infevers website (6, 7). The most common mutations were M694V, M680I, M694I, and V726A mutations in exon 10, which are believed to be associated with disease severity.

The scoring systems described by Pras et al. (8) and Mor et al. (9) and the International Severity Score of Familial Mediterranean Fever (ISSF) (10) are the most commonly used scoring systems to determine disease severity in FMF. Thanks to these scoring systems, it is possible to classify FMF patients according to disease severity and to try to predict their prognosis. Various demographic, clinical and laboratory findings are used in scoring systems. The scoring systems used in FMF are shown in Table 1.

To date, there are a limited number of studies evaluating the relationship between disease severity scores and gene mutations. For this purpose, we aimed to examine the relationship between three different disease severity scores and gene mutations in patients followed up at our clinic.

Methods

Patients

Patients aged 0-18 years, who were diagnosed with FMF and underwent gene analysis between 2017-2020 in the Pediatric Rheumatology outpatient clinic of Selcuk University Faculty of Medicine, were included in the study.According to the diagnostic criteria by Yalçınkaya and Özen, the presence of at least two of the criteria among fever >38C° measured in the axillary area, abdominal pain, chest pain, arthritis, and a positive family history, lasting 6–72 hours in at least three attacks, were considered sufficient for the diagnosis (11). Patients whose genetic analysis was not performed and patients who did not comply with their follow-up and treatment were not included in the study.

Demographic, clinical, genetic, and laboratory characteristics of these patients were recorded retrospectively from their records and the computer information system. In light of this information, the patients' disease was scored according to the disease severity scoring system by Pras et al. and Mor et al. as well as the ISSF, and the disease severity was classified as mild, moderate, and severe. FMF disease severity scoring systems are summarized in Table 1.

Genetic Analysis of MEFV gene

DNA isolation was performed from the peripheral blood samples of the patients and 22 mutations (M694V, M694I, M680I, V722M, V726A, E148Q, R202Q, R761H, P369S, A744S, E230K, K695R, L110P, U148Q, F479L, R761U, M694 del, I692 del, T681I, A408G, T267I, and E167D) in the 2nd, 3rd, 5th and 10th exons of the MEFV gene were analyzed by fragment analysis. In cases where no mutations could be detected in these, the whole gene sequencing technique, which is an advanced genetic analysis technique, was used. Gene analysis was performed from the mother and father for the detection of combined heterozygous mutations..

The relationship between common genotypes and alleles and disease severity scores were investigated. Mutations were divided into four groups, as homozygous, heterozygous, heterozygous combined, and no mutation, for comparison and evaluated both in terms of disease severity scores.

In addition, patients were divided into four groups as homozygous M694V, heterozygous M694V, heterozygous M694V/other allele combined, and other mutations, and the groups were compared with each other in terms of disease severity scores. Homozygous M694V, heterozygous M694V, heterozygous E148Q, and heterozygous M694V/M680I combined mutations, which were the most common four genetic analysis results in our study, were similarly compared in terms of disease severity scores.

The study was approved by the local ethics committee on June 17, 2020 with the decision number 2020/258.

Statistical Analysis

IBM SPSS version 21 and SDATA version 15.1 were used for the statistical analyses. Descriptive statistics were described as mean±SD, minimum, and maximum. Frequency analysis was performed for categorical data. Chi-Square and Fisher's Exact tests were used to compare categorical variables. Categorical data, which were found to be correlated in the Chi-Square test, were subjected to Chi-Square Trend and Post hoc analyses. Numerical data were evaluated using the Mann-Whitney Utest. A value of $p<0.05$ was considered statistically significant.

Results

Demographic and Clinical Characteristics

Of the patients included in the study, 149 (49.1%) were female and 154 (50.8%) were male. The demographic and clinical characteristics of the patients are summarized in Table 2. The median age of the patients was 10 years (min-max: 10 months-18 years). The

median age at diagnosis was 6 years (min-max: 10 months-18 years). The median time from the onset of the complaints to the diagnosis was calculated as 2 years (min-max: 2 months-13 years). While the number of patients diagnosed under the age of 10 was 220 (72.6%), the number of patients diagnosed over the age of 10 was 83 (27.3%) (Table 2).

When the patients were evaluated in terms of clinical features, abdominal pain was seen in 290 patients (95.7%) and was the most common clinical feature. This was followed by fever in 283 patients (93.3%), joint pain in 164 patients (54.1%), fatigue in 80 patients (26.4%), chest pain in 64 patients (21.1%), and arthritis in 46 patients (15.2%) were followed. Detailed clinical examination is given in Table 2.The diagnosis of the patient with renal involvement was made by kidney biopsy.

Genetic Characteristics

While mutations were detected in 271 (89.4%) of the patients, no mutation was detected in 32 (10.6%) patients. Heterozygous mutations were detected in 136 (44.8%) patients, combined heterozygous mutations in 76 (25%) and homozygous mutations in 59 (19.4%) patients.

When the allele frequencies in the patients included in the study were examined, in total, mutations were detected in 359 alleles. The M694V allele was seen in 157 patients (43.7%) and was found to be the most common allele. The second and third most common alleles were M680I in 61 (16.9%) patients and V726A in 49 (13.6%) patients, respectively. Other common alleles included E148Q, R202Q.

When the mutations of the patients were examined, the most common mutation was M694V heterozygous mutation in 63 (23.2%) patients. This was followed by M694V homozygous mutation detected in 43 patients (15.8%). E148Q heterozygous mutation detected in 27 patients (9.96%) was the third most common genotype. M694V/M680I was detected in 25patients (8.85%), and it was determined as the 4th most common genotype. Other common genotypes were V726A heterozygous mutation, M680I heterozygous mutations. E148Q homozygous, V726A homozygous, and various combined heterozygous mutations were rare genotypes. The mutation frequencies of the patients in our study are shown in Table 3.

Association of Genotype with Disease Severity Scores

We observed that 47 patients belonged to the mild disease group, 198 patients belonged to the

Table 1: Disease Severity Scoring System in FMF

Pras et al. Disease Severity Scoring System			Mor et al. Disease Severity Scoring System	ISSF (International Severity Scoring System for Familial Mediterranean Fever)	
Parameter	Feature	Score	Criteria	Criteria	Score
Age of onset (year)	>31	0	1.≥1 site in a single attack (In at least 25% of the attacks)	1. Chronic sequela (including amyloidosis, growth retardation, anaemia, splenomegaly)	1
	21-31	1			
	11-20	2		2. Organ dysfunction (nephrotic range proteinuria, FMF related)	1
	6-10	3			
	<6	4			
Number of attacks per month	<1	1	2. ≥2 sites in the course of the disease	3. Organ failure (heart, renal, etc, FMF related)	1
	1-2	2			
	>2	3			
Arthritis	Acute	2	3. ≥2 mg/day colchicine to achieve remission	4. A. Frequency of attacks (average number of attacks between 1 and 2 per month)	1
	Persistent	3		B. Frequency of attacks (average number of attacks >2 per month)	2
Erysipelas-Like erythema		2	4. ≥2 pleuritic attacks during the course of the disease	5. Increased acute-phase reactants (any of C-reactive protein, serum amyloid A, erythrocyte sedimentation rate, fibrinogen) during the attack-free period, ≥2 weeks after the last attack (at least two times 1 months apart)	1
Amyloidosis		3	5. ≥2 Erysipelas-like erythema attacks during the courseof the disease	6. Involvement of more than two sites during an individual acute attack (pericarditis, pleuritis, peritonitis, synovitis, ELE, testis involvement, myalgia, and so on)	1
Colchicine Dosage (mg/day)	1	1	6. Age of onset<10 years	7. More than two different types of attack during the course of the disease (isolated fever, pericarditis, pleuritis, peritonitis, synovitis, ELE, testis involvement, myalgia, and so on)	1
	1,5	2			
	2	3			
	>2**	4		8. Duration of attacks (more than 72 h in at least three attacks in a year)	1
**2mg/day unresponsive 3–5 points were classified as mild disease, 6–9 points as moderate disease, and >10 as severe disease.			≥3 points was considered as severe disease, 2 points as moderate disease, and ≤1 points as mild disease.	9. Exertional leg pain (pain following prolonged standings and/or exercising, excluding other causes)	1
				Severe disease ≥6, intermediate disease 3–5, mild disease ≤2. *Criterion 4a/4b can give 0 or 1 or 2 points altogether according to the definition.	

Table 2: Clinical and Demographic Features of The Patients with Familial Mediterranean Fever

Variables	Median	Min-Max
Age (Year)	8	0.83-18
Age of Diagnosis (Year)	7	0.83-18
Time to Diagnosis	2	0.2-13
Attack Duration(Day)	3	0.25-10
Attack Frequency (Week)	4	1-72
Gender	**n=303**	**%**
Female	149	49.1
Male	154	50.8
Clinical Features	**n=303**	
Abdominal Pain	290	95.7
Continuous	262	86.5
Colic	28	9.2
Fever	283	93.4
37 C^0-38 C^0	29	9.6
≥38 C^0	254	83.8
Joint Pain	164	54.1
Fatique	80	26.4
Chest Pain	64	21.1
Arthritis	46	15.2
Ankle	29	9.6
Knee	15	5
Other Joints	8	2.6
Nausea-Vomiting	35	11.6
Constipation	27	8.9
Diarrhea	20	6.6
Headache	14	4.6
Myalgia	11	3.6
Unrest	11	3.6
Erysipelas-like rash	8	2.6

moderate disease group, and 58 patients belonged to the severe disease group according to the scoring system described by Pras et al. Further, we observed that 199 patients belonged to the mild disease group, 43 patients belonged to the moderate disease group, and 61 patients belonged to the severe disease group according to the scoring system described by Mor et al. Additionally, we observed that 176 patients belonged to the mild disease group, 106 patients belonged to the moderate disease group, and 21 patients belonged to the severe disease group according to the ISSF scoring system. Patients with homozygous, heterozygous, heterozygous combined, and no mutations were evaluated according to disease severity scores (Pras et al., Mor et al., and ISSF), and no difference was found between the genotypes in terms of disease severity according to all three scoring

Table 3: Mutation Frequency of Patients

Mutation	n (%)
M694V heterozygous	63 (23.2)
M694V homozygous	43 (15.8)
E148Q heterozygous	27 (9.96)
M694V-M680I compound heterozygous	25 (9.22)
V726A heterozygous	18 (6.64)
M680I heterozygous	14 (5.16)
M694V-V726A compound heterozygous	11 (4.05)
M680I-V726A compound heterozygous	10 (3.69)
R202Q heterozygous	8 (2.95)
M680I homozygous	7 (2.58)
M694V-E148Q compound heterozygous	5 (1.84)
R202Q homozygous	4 (1.47)
V726A-E148Q compound heterozygous	4 (1.47)
E148Q homozygous	3 (1.10)
M694V-R202Q compound heterozygous	3 (1.10)
M694V-M680I-R202Q compound heterozygous	2 (0.73)
V726A homozygous	2 (0.73)
V726A-R202Q compound heterozygous	2 (0.73)
M694V-U148Q compound heterozygous	2 (0.73)
M680I-E148Q compound heterozygous	2 (0.73)
Other*	16 (5.90)
Total	271 (100)

*Rare mutations, one each.(A744S heterozygous, E148Q-P369S compound hererozygous, R202Q-R761H

compound heterozygous, M680I-R202Q compound heterozygous, M694V-R761U compound heterozygous,

V722M heterozygous, R761U heterozygous, M694V-L110P compound heterozygous, M694I heterozygous,

P369S heterozygous, M694V-E230K compound heterozygous, E230K heterozygous, R202Q- A744S compound

heterozygous, V726A-M694I compound heterozygous, M694V-R202Q-E148Q compound heterozygous, M680I-

R761H compound heterozygous)

systems (p=0.571, p=0.630, and p=0.546, respectively). The evaluation of genotypes according to disease severity scores is shown in Table 4.

When patients were grouped as homozygous M694V, heterozygous M694V, heterozygous M694V/other allele and other mutations, a statistical difference was found between the groups in terms of disease severity scores according to Pras et al. (p=0.017). According to the score of Pras et al., the frequency of mild disease tended to be less in the M694V homozygous group. However, no statistically significant difference was found between the groups according to Mor et al. and ISSF (p= 0.608, 0.336, respectively) (Table 4).

When the patients with homozygous M694V, heterozygous M694V, heterozygous E148Q, and heterozygous M694V/M680I combined mutations, which were the most common mutations in our study, were compared in terms of disease severity scores according to the scoring system described by Pras et al., a significant difference was observed between

the groups, and mild disease was found to be less common in the homozygous M694V group (p=0.037). However, there was no difference between them in terms of disease severity accoring to the scoring system described by Mor et al. and the ISSF (Table 4).

When the group with heterozygous M694V and the groups with heterozygous M680I, heterozygous V726A, heterozygous E148Q, and heterozygous R202Q mutations, which were other common mutations, were compared, no difference was found between the mild-moderate and severe groups in terms of disease severity according to the three scoring systems (p=0.609, p=0.697, and p=0.519, respectively).

When the homozygous M694V group and the group with heterozygous M694V (combined with other allele or single) mutation were compared in terms of disease severity, a significant correlation was found indicating that the disease was more severe in the homozygous M694V group based on the three scoring systems (p=0.001, p=0.024, and p=0.050, respectively) (Table 5). When the direction of the correlation in the scores according to the scoring system described by Pras et al. was examined, it was observed that there were more patients with severe disease than those with mild disease in the group with homozygous mutations, and the heterozygous group tended to have more patients with severe disease than those with mild disease. With post hoc analysis, according to Pras et al.'s scoring system, those with mild disease were significantly higher in the heterozygous group compared to the homozygous group (z=2.5, X2=6.25, p= 0.04), while those with severe disease were significantly higher in the homozygous group than those with mild disease. (z=-2.5, X2= 6.25, p=0.04). In the scoring system described by Mor et al., the group with homozygous mutation had a tendency to have severe disease, whereas there was a tendency to have mild disease in the group with heterozygous mutation. In post hoc analysis, the p value was found to be 0.07 in both directions (z=-2.3, X2= 5.9, z=2.3, X2= 5.9, respectively). It was not statistically significant. According to the ISSF classification, mild disease was found to be more common in patients with heterozygous mutation but this was not statistically significant according to p<0.05.

When patients with and without the M694V allele were evaluated in terms of the disease severity scores according to the scoring systems described by Pras et al. and Mor et al. and the ISSF, no difference was found between the groups in terms of disease severity (p=0.453, p=0.657, and 0.336, respectively).

When we grouped and compared the disease severity scores of heterozygous mutations of the common alleles M694V, M680I, V726A, and E148Q as mild-moderate and severe, no difference was found between the groups according to all three disease severity scoring systems (p=0.766, p=0.939, and p=0.964, respectively).

In addition, when we classified the M680I and V726A mutations, which were the second and third most common mutations, into homozygous and heterozygous mutations and compared them based on mild-moderate and severe in terms of disease severity scores, no significant difference was found between the groups in terms of disease severity. When the patients with and without the M680I allele and the patients with and without the V726A allele were compared in terms of disease severity scores, no significant difference was found between the groups.

Similarly, in terms of E148Q and R202Q, which were other common mutations, no difference was found between the groups having homozygous and heterozygous mutations according to all three scoring systems when grouped based on the severity of the disease as mild-moderate and severe. There was no difference in disease severity between patients with and without the E148Q allele. When the presence and absence of the R202Q allele and disease severity scores were compared, no significant correlation was found.

Although there was no homozygous mutation among other mutations, including M694I, R761H, A744S, E130K, P369S, R761U, and V722M, when patients with heterozygous mutations and those without mutations were compared, no difference was found in terms of disease severity according to the three disease severity scoring systems. When the internal consistency of the scoring systems were evaluated with the SDATA statistical program Kappa analysis, a weak agreement was detected among Pras et al. SS and Mor et al. SS with kappa value of 0.357, agreement value of 80.12%, p<0.01. A weak agreement was found between Pras et al. SS and ISSF with a kappa value of 0.297, an agreement value of 81.85%, and p<0.01. However, a strong (good) agreement was found between the Mor et al. SS and ISSF, with a kappa value of 0.661, an agreeement value of 91.17%, and a p<0.01..

DISCUSSION

Due to the developments in the field of molecular genetics, we have been able to gain more knowledge regarding the FMF, which we assumed to have known about for a long time. In fact, genetic examination was added to the EURO Fever/PRINTO diagnostic criteria defined in 2019 for this disease, which was being diagnosed based on clinical criteria only, and the importance of genetic examination was revealed. Many mutations in the MEFV gene that cause FMF have been identified with recent studies on molecular genetics (12).

In the last 20 years, disease severity scores have been developed to determine the severity, prognosis and effective treatment of many diseases, including FMF. In FMF, the disease severity score, which was first developed by Pras et al.in 1997, was used for adult patients (8). In 2005, Mor et al. has been developed to a new scoring system to correct missing conditions such as lack of cause and effect relationship between severity markers and disease severity in Pras disease severity score. (9). Finally, in 2012, the international group of FMF experts developed the ISSF criteria. These criteria are suitable for use in children and adults in

Table 4: Evaluation of Genotypes According to Disease Severity Scores

		Heterozygous n:136 (%)	Homozygous n:59 (%)	Compound heterozygous n:76 (%)	No Mutation n:32 (%)	p
Pras et al. SS	Mild	25 (18.4)	3 (5.1)	12 (15.8)	7 (21.9)	
	Moderate	88 (64.7)	37 (62.7)	50 (65.8)	23 (71.9)	0.571
	Severe	23 (16.9)	19 (32.2)	14 (18.4)	2 (6.3)	
Mor et al. SS	Mild	94 (69.1)	30 (50.8)	50 (65.8)	25 (78.1)	
	Moderate	18 (13.2)	11 (18.6)	9 (11.8)	5 (15.6)	0.63
	Severe	24 (17.6)	18 (30.5)	17 (22.4)	2 (6.3)	
ISSF	Mild	82 (60.3)	26 (44.1)	47 (61.8)	21 (65.6)	
	Moderate	47 (34.6)	24 (40.7)	24 (31.6)	11 (34.4)	0.546
	Severe	7 (5.1)	9 (15.3)	5 (6.6)	0 (0)	
		M694V Heterozygous n: 63 (%)	M694V Homozygous n: 43 (%)	M694V/Other Compound heterozygous n: 52 (%)	Other Mutations n: 110 (%)	
Pras et al. SS	Mild	12 (19)	1 (2.3)	8 (16)	9 (8.1)	
	Moderate	42 (66.6)	27 (62.8)	35 (67.3)	68 (61.8)	0.017
	Severe	9 (14.2)	15 (34.9)	9 (17.3)	33 (30)	
Mor et al. SS	Mild	45 (71.9)	23 (53.5)	33 (63.4)	72 (65.5)	
	Moderate	9 (14.1)	8 (18.6)	7 (13.4)	14 (12.7)	0.608
	Severe	9 (14.1)	12 (27.9)	12(23.0)	24 (21.8)	
ISSF	Mild	40 (64.1)	20 (46.5)	31 (59.6)	62 (56.4)	
	Moderate	20 (31.3)	16 (37.2)	17 (32.6)	41 (37.3)	0.336
	Severe	3 (4.7)	7 (16.3)	4 (7.69)	7 (6.4)	
		M694V Heterozygous n:63 (%)	M694V Homozygous n:43 (%)	E148Q Heterozygous n:27 (%)	M694V/M680I Compound Heterozygous n:25 (%)	
Pras et al. SS	Mild	12 (19)	1 (2.3)	5 (18.5)	5 (20.0)	
	Moderate	42(66.6)	27 (62.8)	17 (63.0)	15 (60.0)	0.037
	Severe	9 (15.3)	15 (34.9)	5 (18.5)	5 (20.0)	
Mor et al. SS	Mild	45 (71.9)	23 (53.5)	18 (66.7)	15 (60.0)	
	Moderate	9 (14.1)	8 (18.6)	5 (18.5)	5 (20.0)	0.466
	Severe	9 (14.1)	12 (27.9)	4 (14.8)	5 (20.0)	
ISSF	Mild	40 (64.1)	20 (46.5)	17 (63.0)	14 (56.0)	
	Moderate	20 (31.3)	16 (37.2)	9 (33.3)	9 (36.0)	0.220
	Severe	3 (4.7)	7 (16.2)	1 (3.7)	2 (8.8)	

both clinical practice and drug trials (10).

Disease severity scores are now used in many diseases to evaluate disease severity more objectively. In previous studies, it was found that the disease severity scores showed more severe disease in the group carrying the homozygous M694V mutation (13-15). However, in a study from Turkey, it was observed that carrying the M694V mutation in one allele or two alleles did not change the severity of the disease (16). In another study, no difference was observed in terms of disease severity scores between common mutations (M694V, V726A, and M680I) and rare mutations (A744S, P369S, K695R, R761H, and F479L)

Table 5: Evaluation of M694V Homozygous and Heterozygous Mutations According to Patient Weight Scores

	M694V Homozygous n=43 (%)	M694V Heterozygous (combined with other allele or single) n=115 (%)	p
Pras et al. SS			
Mild	1 (2.3)	20 (17.3)	
Moderate	27 (62.7)	77 (66.9)	***0.004***
Severe	15 (34.8)	18 (15.6)	
Mor et al. SS			
Mild	23 (53.5)	79 (68.6)	
Moderate	8 (18.6)	14 (12.1)	***0.024***
Severe	18 (27.9)	22 (19.1)	
ISSF			
Mild	20 (46.5)	72 (62.6)	
Moderate	16 (37.2)	36 (31.3)	***0.050***
Severe	7 (16.2)	7 (6.0)	

(17). In a study that only aimed to evaluate the association of E148Q mutation with disease severity and evaluated homozygous M694V mutation with homozygous E148Q, heterozygous E148Q and heterozygous E148Q/Exon 10 combined mutations according to the scoring system described by Pras et al., it was observed that the disease had a more severe course in patients with homozygous M694V mutation (18). In the present study, according to the scoring system described by Pras et al., a correlation was found between homozygous M694V, heterozygous M694V, heterozygous M694V/other combined, and other genotypes, indicating that the rate of mild disease was lower in the M694V homozygous group. Similarly, a relationship was found in terms of disease severity in the comparison of the four most common genotypes: homozygous M694V, heterozygous M694V, heterozygous E148Q, and heterozygous M694V/M680I combined. In addition, when only homozygous M694V and heterozygous M694V groups were compared in pairs, the rate of severe disease tended to be higher in the homozygous group according to all three scoring systems. However, this was not statistically significant according to the scoring system described by Mor et al. and the ISSF. Based on the scoring system described by Pras et al., the rate of severe disease was higher in patients with homozygous M694V allele, whereas the rate of mild disease was statistically significantly higher in the heterozygous group compared with homozygous group. In light of all of these findings, we can say that the homozygous M694V mutation is associated with a more severe disease than other mutations that are common in the population. However, a sound comparison of homozygous M694V and other homozygous mutations could not be made as the incidence of homozygous mutation of other alleles (V726A, E148Q, and M680I) is very low in the population.

Although ISSF scoring was found to be the most appropriate scoring system for children in a recent study conducted in our country, we observed that other scoring systems are used more widely when the literature is examined. (18, 19, 20, 21). In our study, unlike other studies, 3 disease severity scores were used and in fact, the compatibility of these disease severity scores with each other was tried to be seen. According to our study, the results in the scoring system of Pras et al. were different from the scoring system of ISSF and Mor et al. As a matter of fact, in the concordance analysis between scoring systems, while a good agreement was found between Mor et al.'s scoring system and ISSF, the agreement with Pras et al.'s scoring system was weak.

Therefore, although patients with FMF were evaluated across a wide range in terms of genetic, and disease severity scores, the most important limitation of the study was the inability to perform statistical analysis due to the low number of patients for some genotypes.

We believe that further studies are warranted with large samples, multiple centers and even multiple ethnic groups for evaluating the relationship between clinical presentation and genotype and their relationship with disease severity scores in order to better understand FMF. Additionally, this will aid in the understanding of the effects of genetic mutations that have recently been added to the diagnostic criteria on clinical presentation and disease severity.

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