

Validation of a Genotype-Based Clinical Prediction Score for Identifying M694V Homozygosity in Pediatric Familial Mediterranean Fever: A Retrospective Single-Center Study

Pediatric Ailesel Akdeniz Ateşinde M694V Homozigotluğunu Öngörmeye Yönelik Genotip Temelli Klinik Skorun Validasyonu: Tek Merkezli Retrospektif Bir Çalışma

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ABSTRACT

Background: The M694V variant of the *MEFV* gene is associated with a more severe phenotype in Familial Mediterranean Fever (FMF). A genotype-based clinical prediction score was recently proposed to identify M694V homozygosity in pediatric FMF; however, validation in independent cohorts remains limited. We aimed to evaluate the predictive performance of this score in a single-center pediatric cohort, to perform a sensitivity analysis in a non-overlapping subset, and to explore genotype–phenotype correlations.

Materials and Methods: This retrospective study included 347 pediatric FMF patients who carried biallelic pathogenic *MEFV* exon 10 variants and were followed between 2010 and 2025. Patients were classified as M694V homozygotes ($n = 155$) or carriers of other exon 10 variants ($n = 192$). Clinical and laboratory data were reviewed to calculate the 40-point M694V prediction score. Diagnostic accuracy was assessed by receiver operating characteristic analysis.

Results: The model demonstrated moderate discriminative ability (area under the curve = 0.69, 95% confidence interval: 0.64–0.75). At the optimal cut-off of ≥ 12 points, sensitivity and specificity were 43.2% and 87.0%, respectively, and the test was beneficial as a rule-in tool in high-risk patients, comparable to the original multicenter findings. Musculoskeletal and cutaneous manifestations were significantly more frequent in M694V homozygotes ($p < 0.01$). Patients with the homozygous genotype exhibited earlier disease onset, higher serum amyloid A levels, and greater use of anti-interleukin-1 therapy.

Conclusion: The genotype-based clinical score retained its diagnostic reliability in this pediatric cohort and may serve as a practical adjunct for identifying high-risk patients, particularly in settings lacking rapid molecular testing. Nevertheless, due to its moderate sensitivity, the score should complement rather than replace genetic confirmation.

Keywords: Familial Mediterranean Fever, M694V, genotype-phenotype association, predictability, scoring system

ÖZ

Amaç: *MEFV* genindeki M694V varyantı, Ailesel Akdeniz Ateşi (AAA) hastalarında daha ağır hastalık fenotipi ile ilişkilidir. Pediatrik AAA'da M694V homozigotluğunu öngörmek amacıyla geliştirilen genotip temelli klinik skor yakın zamanda tanımlanmış olmakla birlikte, dış validasyon çalışmaları sınırlıdır. Bu çalışmada, söz konusu skorun tek merkezli pediatrik bir kohortta tanısal performansının değerlendirilmesi ve genotip–fenotip ilişkilerinin incelenmesi amaçlanmıştır.

Gereç ve Yöntemler: Bu retrospektif çalışmaya 2010–2025 yılları arasında izlenen ve *MEFV* ekzon 10'da bialelik patojenik varyant taşıyan 347 pediatrik AAA hastası dahil edildi. Hastalar M694V homozigot ($n = 155$) ve diğer ekzon 10 varyantlarını taşıyanlar ($n = 192$) olarak sınıflandırıldı. Klinik ve laboratuvar verileri kullanılarak 40 puanlık M694V öngörü skoru hesaplandı. Tanısal doğruluk alıcı işletim karakteristiği analizi ile değerlendirildi.



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Bulgular: Model orta düzeyde ayırt edicilik gösterdi (eğri altında kalan alan = 0,69; %95 güven aralığı: 0,64–0,75). En uygun eşik değeri olan ≥ 12 puanda duyarlılık %43,2, özgüllük %87,0 olarak saptandı. Testin, özellikle yüksek riskli hastalarda “rule-in” aracı olarak faydalı olduğu ve orijinal çok merkezli çalışmayla uyumlu olduğu görüldü. M694V homozigot hastalarda kas-iskelet sistemi ve kutanöz bulgular anlamlı olarak daha sık görüldü ($p < 0,01$). Ayrıca bu hastalarda hastalık başlangıcı daha erken, serum amiloid A düzeyleri daha yüksekti ve anti-interlökin-1 tedavisi kullanımı daha fazlaydı.

Sonuç: Genotip temelli klinik skor, bu pediatrik kohortta tanısız güvenilirliğini korumuş olup, özellikle hızlı moleküler testlere erişimin kısıtlı olduğu durumlarda yüksek riskli hastaların belirlenmesinde pratik bir yardımcı araç olabilir. Bununla birlikte, orta düzey duyarlılığı nedeniyle genetik doğrulamanın yerine geçmemeli, tamamlayıcı olarak kullanılmalıdır.

Anahtar Kelimeler: Ailesel Akdeniz Ateşi, M694V, genotip-fenotip ilişkisi, skorlama sistemi

Introduction

Familial Mediterranean Fever (FMF) is the most prevalent monogenic autoinflammatory disease, showing a notably high frequency among specific Eastern Mediterranean populations, while remaining uncommon in other parts of the world (1). The disease is characterized by recurrent, self-limiting episodes of fever and systemic inflammation, typically accompanied by serositis, including peritonitis, pleuritis, or pericarditis, and arthritis involving large joints (2). These attacks usually begin and end abruptly, last for one to three days, and are followed by symptom-free intervals during which patients experience complete well-being. Although onset most commonly occurs during childhood or adolescence, attack frequency varies widely among individuals, ranging from weekly attacks to intervals of several months in the absence of treatment. The disease results from pathogenic mutations in the *MEFV* gene located on chromosome 16p13.3, which encodes pyrin, a key regulator of the inflammasome pathway that controls interleukin (IL)-1 β production and innate immune activation (3). Variants in *MEFV* disrupt normal inflammasome function, leading to uncontrolled activation and excessive secretion of IL-1 β , thereby triggering recurrent inflammatory episodes. To date, over 350 *MEFV* gene variants have been described, although only a fraction have been definitively established as pathogenic. Variants located within exon 10 of the *MEFV* gene show the strongest association with heightened inflammatory activity, more severe clinical manifestations, and an increased risk of secondary amyloidosis. Among these, the Met694Val (M694V) variant is regarded as the most clinically significant, being consistently linked to earlier disease onset, higher attack frequency, and a markedly increased risk of AA amyloidosis (4). Homozygosity for M694V is particularly associated with more aggressive disease, persistently elevated inflammatory markers, and the frequent need for higher colchicine doses or biologic therapy to achieve adequate disease control (4–6). Although numerous studies have shown that M694V homozygosity

is associated with more severe and treatment-resistant phenotypes, considerable clinical heterogeneity persists among individuals with identical genotypes (7,8). Modifier genes, epigenetic mechanisms, environmental triggers, and variable expression of inflammasome components have all been proposed to account for these differences (9,10). However, genotype alone cannot fully account for the wide spectrum of clinical variability observed among patients carrying identical *MEFV* variants. Thus, while *MEFV* mutations remain the primary determinant of disease risk and severity, genotype alone does not fully predict clinical manifestations. Clarifying these genotype–phenotype relationships has important clinical implications. Early identification of patients with high-risk genotypes, notably M694V homozygosity, could prompt closer monitoring for subclinical inflammation, allow anticipation of a more aggressive disease course, and enable timely therapeutic adjustments, including consideration of IL-1 blockade. Because molecular testing may be unavailable in some settings, the development and use of reliable clinical tools for predicting genotype are particularly valuable.

Tunç et al. (11) previously proposed a genotype-based clinical prediction score to identify M694V homozygosity in pediatric FMF. The present study seeks to externally validate the scoring model in a single-center pediatric FMF cohort and to further investigate genotype–phenotype associations, with particular emphasis on musculoskeletal and cutaneous manifestations that might serve as clinical markers of disease severity.

Materials and Methods

This retrospective study included pediatric patients (aged 0–18 years) diagnosed with FMF who were followed longitudinally at İstanbul University Faculty of Medicine between 2010 and 2025. All patients had been regularly monitored for at least 6 months, with routine clinical evaluations performed every 3 months.

Patients were eligible if they met the Turkish Pediatric FMF diagnostic criteria or the Eurofever/Pediatric Rheumatology

International Trials Organization classification criteria for hereditary recurrent fevers and carried biallelic pathogenic *MEFV* exon 10 variants confirmed by molecular analysis (12,13). Colchicine resistance was defined as ≥ 1 attack per month for at least 6 months despite receiving the maximum tolerated dose of colchicine. Patients were excluded if they had missing genetic data, incomplete clinical information for any predictor variable, or insufficient follow-up.

Demographic, clinical, laboratory, and genetic data were extracted from medical records using a standardized data collection form. The primary outcome was the presence of the M694V homozygous genotype, as confirmed by molecular sequencing. For comparative analysis, patients were classified into two groups: Group 1 included patients homozygous for M694V, whereas Group 2 included patients with other biallelic pathogenic variants in exon 10 of the *MEFV* gene, either as compound heterozygotes or as homozygotes for non-M694V variants. This grouping mirrored the design of the original model development study (11), enabling a direct comparison of the clinical score's predictive performance for identifying M694V homozygotes.

Genetic analyses were performed using Sanger sequencing or next-generation sequencing, covering all coding exons of the *MEFV* gene, with particular emphasis on exon 10. Variant pathogenicity was verified using the Infevers database. Disease severity was assessed using the International Severity Score for Familial Mediterranean Fever (ISSF) (14).

The predictor variables were consistent with those used in the original study and included splenomegaly, erysipelas-like erythema (ELE), arthritis, protracted febrile myalgia syndrome (PFMS), and disease onset at ≤ 3 years of age, myalgia, and a family history of FMF. Each variable was coded dichotomously (0 = absent, 1 = present), and the total score was calculated according to the original scoring model (Supplementary Table 1), yielding a range of 0–40 points. The performance of the scoring system was evaluated at cut-off values of 9, 12, and 15 points.

Statistical Analysis

Statistical analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). The distribution of continuous variables was assessed both visually and with normality tests (Kolmogorov–Smirnov or Shapiro–Wilk). Data were summarized as mean $\pm$ standard deviation or median (Q1–Q3), as appropriate, and categorical variables as frequencies and percentages. Group comparisons were made using the chi-square test or Fisher's exact test for categorical variables, and the Student's t-test or Mann–Whitney U test for continuous variables. The diagnostic performance of the scoring system was evaluated using receiver operating

characteristic (ROC) analysis, and the Youden Index was used to identify the optimal cut-off point. A p value < 0.05 was considered statistically significant. Because our center participated in the multicentre derivation study by Tunc et al. (11), to determine whether cohort overlap influenced the validation, patients previously included in that dataset were identified by matching patient initials and dates of birth ($n = 122$). A sensitivity analysis of the diagnostic performance of the score was then performed on the non-overlapping subset ($n = 225$; 103 M694V homozygotes and 122 carriers of other exon 10 variants).

Ethics

Ethical approval for the study protocol was obtained from the İstanbul University Faculty of Medicine Ethics Committee (decision number: 3767116, date: 06.11.2025). The study was conducted in compliance with the Declaration of Helsinki. Informed consent was obtained from all patients and/or their parents.

Results

The study included 155 patients in group 1 with the homozygous M694V genotype and 192 patients in group 2 with other biallelic pathogenic variants in exon 10. The genotype distribution of Group 2 is detailed in Supplementary Table 2.

Across the entire study population, the most common clinical manifestations were fever (90.2%), abdominal pain (88.2%), arthralgia (59.1%), arthritis (33.1%), and chest pain (27.7%). Baseline demographic characteristics are summarized in Table 1.

The median age at disease onset was significantly lower in group 1 than in group 2 (36 months interquartile range [IQR 18–60] vs. 50 months [IQR 30–80]; $p < 0.001$). The median age at diagnosis was lower in group 1 (50 months [IQR 33–84]) than in group 2 (63 months [IQR 44.2–92], $p = 0.003$). The percentage of patients with symptom onset before age 3 was significantly higher in group 1 than in group 2 (51.6% vs. 37.0%; $p = 0.006$). The duration of disease follow-up was significantly longer in group 1 (90 months [IQR 46–125]) than in group 2 (71 months [IQR 34.5–111]; $p = 0.017$). The median attack duration, annual attack frequency, and diagnostic delay were similar between the groups ($p = 0.146$, $p = 0.090$, and $p = 0.655$, respectively).

When clinical symptoms were compared between genotype groups, the frequencies of fever and abdominal pain did not differ significantly ($p = 0.247$ and $p = 0.117$, respectively). Chest pain was significantly more frequent in group 1 (34.2%) than in group 2 ($p = 0.015$). Arthralgia was significantly more frequent in group 1 than in group 2 (72.9% vs. 47.9%, $p < 0.001$), and arthritis was also more

Table 1. Demographic, clinical, and laboratory features of patients with Familial Mediterranean Fever according to genotype groups.

	Group 1 (n = 155)	Group 2 (n = 192)	Total (n = 347)	p
Demographic features				
Gender				
Female n, (%)	82 (52.9)	99 (51.6)	181 (52.2)	0.804
Male n, (%)	73 (47.1)	93 (48.4)	166 (47.8)	
Age at onset (month) (median) (Q1–Q3)	36 (18-60)	50 (30-80)	46 (24-72)	<0.001*
Age at diagnosis (month) (median) (Q1–Q3)	50 (33-84)	63 (44.2-92)	60 (37-88)	0.003*
Symptom onset before the age of 3 years n, (%)	80 (51.6)	71 (37)	151 (43.5)	0.006*
Time to diagnostic delay (month) (median) (Q1–Q3)	12 (6-22)	11 (6-21.5)	11 (6-22)	0.655
Duration of disease follow-up (month) (median) (Q1–Q3)	90 (46-125)	71 (34.5-111)	80 (36-120)	0.017*
Duration of attacks (day) (median) (Q1–Q3)	2 (2-3)	2 (2-3)	2 (2-3)	0.146
Number of attacks per year (median) (Q1–Q3)	12 (8-18.2)	12 (7.5-12.5)	12 (8-16)	0.090
Family history of FMF n, (%)	112 (72.3)	126 (65.6)	238 (68.6)	0.186
Parental consanguinity n, (%)	43 (27.7)	47 (24.5)	90 (25.9)	0.491
CRP mg/L during attacks (median) (Q1–Q3)	69 (45-112)	67 (38-104)	67.5 (40.9-106)	0.150
SAA mg/L during attacks (median) (Q1–Q3)	104 (30.5-309)	52.4 (21.5-184)	79 (24.4-210.7)	0.041*
ISSF score (median) (Q1–Q3)	3 (2-4)	2 (1-3)	2 (2-4)	<0.001*
Anti-IL1 treatment n, (%)	43 (27.7)	11 (5.7)	54 (15.6)	<0.001*
Clinical features				
Fever n, (%)	143 (92.3)	170 (88.5)	313 (90.2)	0.247
Abdominal pain n, (%)	132 (85.2)	174 (90.6)	306 (88.2)	0.117
Chest pain n, (%)	53 (34.2)	43 (22.4)	96 (27.7)	0.015*
Pleural effusion n, (%)	1 (0.6)	1 (0.5)	2 (0.6)	1.000
Vomiting n, (%)	5 (3.2)	22 (11.5)	27 (7.8)	0.004*
Diarrhea n, (%)	20 (12.9)	31 (16.1)	51 (14.7)	0.396
Arthralgia n, (%)	113 (72.9)	92 (47.9)	205 (59.1)	<0.001*
Arthritis n, (%)	71 (45.8)	44 (22.9)	115 (33.1)	<0.001*
Erysipelas-like erythema n, (%)	45 (29.0)	19 (9.9)	64 (18.4)	<0.001*
Exertional leg pain n, (%)	43 (27.7)	28 (14.6)	71 (20.5)	0.003*
Myalgia n, (%)	60 (38.7)	46 (24)	106 (30.5)	0.003*
Protracted febrile myalgia syndrome n, (%)	1 (0.6)	1 (0.5)	2 (0.6)	1.000
Splenomegaly n, (%)	2 (1.3)	1 (0.5)	3 (0.9)	0.588
Orchitis n, (%)	3 (1.9)	3 (1.6)	6 (1.7)	1.000
Headache n, (%)	6 (3.9)	8 (4.2)	14 (4)	0.889
Amyloidosis n, (%)	2 (1.3)	2 (1)	4 (1.2)	1.000
Data are presented as medians (Q1–Q3) for continuous variables and as counts (percentages) for categorical variables. *p < 0.05 was considered statistically significant.				
FMF, Familial Mediterranean Fever; CRP, C-reactive protein; SAA, serum amyloid A; ISSF, International Severity Score for Familial Mediterranean Fever; IL-1, interleukin-1.				

common in group 1 (45.8% vs. 22.9%, $p < 0.001$). Myalgia, including exertional leg pain, was significantly more common in group 1 (38.7% and 27.7%, respectively) than in group 2 (24% and 14.6%, respectively) ($p = 0.003$ for both). ELE was also observed more frequently in group 1 (29%) than in group 2 (9.9%) ($p < 0.001$).

C-reactive protein levels during attacks did not differ significantly between group 1 and group 2 ($p = 0.150$). The serum amyloid A (SAA) levels were significantly higher in group 1 (104 mg/L [IQR 30.5–309]) than in group 2 (52.4 mg/L [IQR 21.5–184], $p = 0.041$). ISSF was significantly higher in group 1 than in group 2 (median 3 [IQR 2–4] vs.

2 [IQR 1–3]; $p < 0.001$). A higher proportion of patients in group 1 received anti-IL-1 therapy than did those in group 2 (27.7% vs. 5.7%; $p < 0.001$).

ROC curve analysis was performed to assess the diagnostic performance of the clinical scoring system in predicting M694V homozygosity. The continuous score yielded an area under the curve (AUC) of 0.69 (95% CI 0.64–0.75; $p < 0.001$), indicating moderate discrimination. Because the AUC is a threshold-independent measure of the entire ROC curve, a single value is reported for the model; sensitivity and specificity at the predefined cut-offs were 54.2% and 78.6% (≥ 9), 43.2% and 87.0% (≥ 12 ; Figure 1), and 27.7% and 91.7% (≥ 15). Predictive values and likelihood ratios (LRs) for each cut-off are presented in Table 2, all of which refer to this single ROC model.

Diagnostic performance of the clinical scoring system was further assessed using sensitivity, specificity, positive and negative predictive values, and LRs. At a cut-off value of 12 points or greater, the scoring system demonstrated a sensitivity of 43.2% and a specificity of 87.0%, with positive and negative predictive values of 72.8% and 65.5%, respectively. LR analysis yielded $LR^+ = 3.32$ and $LR^- = 0.65$. McNemar's test demonstrated statistically significant differences in patient classification across all cut-off pairs (9 vs. 12, $\chi^2 = 31.03$, $p < 0.001$; 9 vs. 15, $\chi^2 = 64.02$, $p < 0.001$; 12 vs. 15, $\chi^2 = 31.03$, $p < 0.001$). In the non-overlapping subset ($n = 225$), the score retained comparable discrimination (AUC 0.71, 95% CI 0.64–0.77), and its operating characteristics at the ≥ 12 cut-off were essentially identical to the full-cohort estimates (sensitivity 43.7% vs. 43.2%; specificity 85.2% vs. 87.0%; Supplementary Table 3), indicating that the validation findings were not driven by overlap with the derivation cohort.

The study cohort consisted of two subgroups, defined by clinical-genetic classification, with a diagnostic score threshold of 12. The false-negative (FN) group ($n = 88$) comprised patients who were homozygous for the M694V variant but had a score < 12 , i.e., they lacked the specific clinical predictors captured by the score despite their severe genotype. The false-positive (FP) group ($n = 25$) included patients with biallelic (compound heterozygous or homozygous non-M694V) variants who had a score ≥ 12 ,

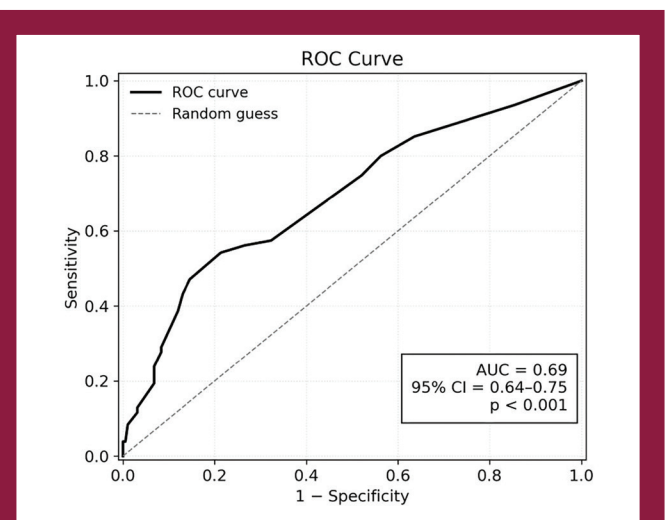


Figure 1. Receiver operating characteristic (ROC) curve of the genotype-based clinical scoring system for predicting the homozygous M694V genotype. The ROC curve demonstrates the diagnostic performance of the clinical prediction score for identifying genetically confirmed M694V homozygosity. The area under the curve (AUC) was 0.69 (95% CI: 0.64–0.75; $p < 0.001$), indicating moderate discriminative ability. At the optimal cut-off value (≥ 12 points), the score showed a sensitivity of 43.2% and a specificity of 87.0%. AUC: area under the curve; CI: confidence interval.

suggesting a more severe clinical presentation despite a less severe genotype.

The median ISSF score was significantly higher in the FN group than in the FP group (FN: 3 [IQR 2–4] vs. FP: 2 [IQR 1–2.5]; $p = 0.01$). The proportion of patients with symptom onset before age 3 years did not differ significantly between the FN and FP groups (46.6% vs. 32%, $p = 0.1$). Musculoskeletal manifestations, such as arthralgia, arthritis, and myalgia, were significantly more frequent in FP patients than in FN patients (88% vs. 59.1%, $p = 0.007$; 84% vs. 19.3%, $p < 0.001$; and 60% vs. 22.7%, $p < 0.001$, respectively). Similarly, ELE was observed more often in FP (64% vs. 3.4%, $p < 0.001$). A positive family history of FMF was also more common in this group (92% vs. 62.5%, $p = 0.005$). Splenomegaly rates did not differ significantly between groups ($p = 0.06$). The

Table 2. Diagnostic performance of the clinical scoring system for predicting the homozygous M694V genotype at different cut-off points.

Cut-off ($\geq$ points)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR–
9	54.2	78.6	67.2	68.0	2.54	0.58
12	43.2	87.0	72.8	65.5	3.32	0.65
15	27.7	91.7	72.9	61.1	3.33	0.79

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and likelihood ratios (LR^+ and LR^-) are presented for each predefined cut-off value of the clinical score. The presence of the homozygous M694V genotype, confirmed by molecular analysis, was used as the reference standard.

frequency of protracted febrile myalgia syndrome (PFMS) did not differ significantly between the FN and FP groups (1.1% vs. 4%, $p = 0.3$).

Discussion

In this study, the genotype-based clinical prediction score proposed by Tunc et al. (11) for identifying M694V homozygosity in pediatric patients with FMF was validated in a single-center cohort. To our knowledge, this is the first single-center validation of the genotype-based M694V prediction model in a pediatric population. However, because our center contributed to the derivation study, this represents a partial, rather than fully independent, external validation and is supported by a sensitivity analysis of the non-overlapping subset (Supplementary Table 3). The score demonstrated moderate discriminative performance in distinguishing M694V homozygotes from patients carrying other pathogenic exon 10 variants in the *MEFV* gene, whether they were non-M694V homozygotes or compound heterozygotes. The AUC was 0.69 (95% CI, 0.64–0.75), closely aligning with the results of the original multicenter derivation study (AUC: 0.65). At an optimal threshold of 12 points or greater, the model achieved sensitivity of 43.2% and specificity of 87%, indicating that its predictive performance was maintained in the single-center validation cohort.

In the original study, the independent predictors of M694V homozygosity included disease onset at ≤ 3 years of age, arthritis, myalgia, PFMS, ELE, splenomegaly, and a positive family history of FMF. Based on the corresponding regression coefficients (β values), a 40-point weighted clinical score was constructed, assigning the highest weights to splenomegaly (10 points), ELE (9 points), and arthritis (8 points). Using ROC curve analysis and the Youden index, the original investigators identified an optimal cut-off value of 12 points or greater, which provided the best balance between sensitivity and specificity (AUC = 0.65; sensitivity = 43%; specificity = 90%).

The present validation demonstrated that the 40-point M694V prediction score retains its reliability and clinical utility. The scoring system was evaluated at three predefined cut-off points (≥ 9 , ≥ 12 , and ≥ 15), consistent with those examined in the derivation study by Tunc et al. (11). At the lowest threshold (≥ 9 points), the model achieved a sensitivity of 54.2% and a specificity of 78.6%, enabling the broader detection of M694V homozygotes, albeit at the cost of reduced specificity. This threshold may therefore be most appropriate for screening purposes, where minimizing false negatives is prioritized. At the intermediate threshold (≥ 12 points), sensitivity was 43.2%, and specificity was 87.0%, which closely matched multicenter findings (sensitivity =

43%; specificity = 90%). This threshold, identified as optimal in the derivation study, reproduced the original estimates most closely. At the highest threshold (≥ 15 points), specificity further increased to 91.7%, while sensitivity decreased to 27.7%, suggesting that this level may be more suitable for confirmatory diagnostic contexts, where minimizing false positives is essential. This finding suggests that higher cut-off values may be preferable in confirmatory diagnostic settings, where maximizing the likelihood of true positives is the priority. The consistent sensitivity and specificity balance across all three thresholds suggests that the model performs reliably in various clinical settings. Notably, two of the highly weighted predictors, splenomegaly (10 points) and PFMS (6 points), were present in fewer than 1% of our cohort (0.9% and 0.6%, respectively). Consequently, the attainable score is driven mainly by the more prevalent features (ELE, arthritis, myalgia, and early disease onset). The rarity of the highest-weighted items partly explains the moderate sensitivity and tempers the everyday utility of the highest cut-offs in populations where such signs are uncommon.

Consistent with previous studies, musculoskeletal and cutaneous manifestations were more frequent and severe among patients with M694V homozygosity (4,5,7,8). In our cohort, arthralgia (72.9% vs. 47.9%), arthritis (45.8% vs. 22.9%), myalgia (38.7% vs. 24.0%), and ELE (29.0% vs. 9.9%) were significantly more prevalent in M694V homozygotes than in patients carrying other biallelic pathogenic exon 10 variants. Furthermore, the higher proportion of patients requiring anti-IL-1 therapy among M694V homozygotes (27.7% vs. 5.7%) underscores the link between these manifestations and a more aggressive inflammatory phenotype.

FN patients, who carried the M694V/M694V genotype but scored below the ≥ 12 cut-off, lacked the specific clinical predictors captured by the score rather than having globally milder disease; consistent with this, their ISSF was not lower than that of the FP group (3 [2–4] vs. 2 [1–2.5]; $p = 0.010$). The FP subgroup, comprising patients with non-M694V biallelic exon 10 variants who reached scores ≥ 12 , more frequently displayed the very features that constitute the score (arthralgia, arthritis, myalgia, ELE, and a positive family history). Because these features define the score itself, the difference is largely expected and presented descriptively rather than as evidence of a distinct or more aggressive biology. Nonetheless, the clinical heterogeneity observed among patients with identical or similar genotypes is compatible with the involvement of additional factors, such as SAA1 polymorphisms, epigenetic regulation of *MEFV* expression, and variable inflammasome activation,

and supports the use of molecular and biomarker-based assessments to complement genotype-based clinical tools, such as the M694V prediction score.

The results of this study highlight the potential clinical value of the M694V predictive score as a complementary diagnostic tool in the management of pediatric FMF. In clinical settings where molecular testing is not readily available or genetic analysis may be delayed, this scoring system may aid in the early identification and prioritization of high-risk patients for confirmatory genotyping. A high clinical score may encourage early initiation of targeted therapy or closer follow-up, particularly in children presenting with early-onset disease, recurrent musculoskeletal symptoms, and cutaneous findings such as ELE. Notably, the clinical variables incorporated into the PREDICT-crFMF score, particularly musculoskeletal involvement, largely overlap with the components of the M694V prediction model validated in our study, supporting the concept that these features represent shared markers of disease severity in FMF (15). The model's high specificity (87%) at a threshold of 12 points or higher supports its use in confirmatory diagnostic settings, minimizing the likelihood of FP results. However, the moderate sensitivity observed in this study suggests that clinical scoring should complement, rather than replace, molecular testing. Integrating clinical assessment with genetic and biochemical data may facilitate early identification of severe genotypes and optimize disease management in FMF.

Study Limitations

The main limitation of this study is its single-center design, which may restrict the generalizability of the findings. Patient populations, disease severity distribution, and management strategies can vary among centers, potentially influencing the external performance of the M694V prediction score. Although our cohort was relatively large and well characterized, multicenter validation, including patients from diverse geographic and ethnic backgrounds, will be essential to confirm these results. In addition, our center contributed to the multicentre derivation study; therefore, the full cohort partially overlaps with the derivation sample. This constitutes a partial rather than a full external validation. To address this, overlapping patients were identified, and a sensitivity analysis restricted to the non-overlapping subset ($n = 225$) confirmed consistent score performance (Supplementary Table 3).

Conclusion

This study validated a genotype-based clinical prediction score for identifying M694V homozygosity in pediatric FMF.

The model demonstrated moderate discriminative ability, with an optimal cut-off of 12 points or greater yielding balanced sensitivity and specificity, consistent with previous multicenter findings. Musculoskeletal and cutaneous manifestations, particularly arthritis, myalgia, and ELE, were significantly more common among M694V homozygotes than among patients carrying other biallelic pathogenic exon 10 variants, highlighting an association with a more severe inflammatory phenotype. The clinical score proved to be a valuable adjunct for identifying high-risk patients, particularly in settings where molecular testing may be limited or delayed. Nevertheless, given its moderate sensitivity, the scoring system should complement, rather than substitute for, genetic confirmation. Integrating such genotype-based clinical tools with biochemical and molecular data may enhance early diagnosis and improve individualized management strategies in children with FMF.

Ethics

Ethics Committee Approval: Ethical approval for the study protocol was obtained from the İstanbul University Faculty of Medicine Ethics Committee (decision number: 3767116, date: 06.11.2025).

Informed Consent: Informed consent was obtained from all patients and/or their parents.

Footnotes

Authorship Contributions

Concept: S.D.A., N.A.A., Design: S.D.A., N.A.A., Data Collection or Processing: S.D.A., A.D.K., B.M., Analysis or Interpretation: S.D.A., N.A.A., Literature Search: S.D.A., A.D.K., B.M., Writing: S.D.A., N.A.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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Supplementary Tables Link: <https://d2v96fxpocvxx.cloudfront.net/a8b2a838-ca73-407f-ba5b-c86eada59dd3/content-images/01ea57be-225e-4f7f-a206-5ce086d13089.pdf>

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