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# Is there any difference between M694V heterozygote and non-exon 10 mutations on symptoms onset and response to colchicine treatment?

## *M694V heterozigot ve non-ekzon 10 mutasyonları arasında semptomların başlangıcı ve kolşisin tedavisine yanıt açısından fark var mıdır?*

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**Abstract**

**Purpose:** Familial Mediterranean fever (FMF) is the most common inherited autoinflammatory syndrome throughout the world. The most frequent genotype-phenotype correlation is in a certain part of exon 10, especially M694V mutation. There are also a group of patients with non-exon 10 mutations, who have a similar clinical spectrum of the disease. We aim to investigate the genotype-phenotype differences between M694V heterozygote mutations and non-exon 10 mutations.

**Materials and methods:** Data charts of children (n=431) with FMF from two tertiary hospitals were reviewed. Patients were divided into two groups with regard to having M694V heterozygote or non-exon 10 mutations. Genotype-phenotype features and response to treatment were compared.

**Results:** There were M694V heterozygote mutations in 128 (29.7%) patients and non-exon 10 mutations in 303 (70.3%) patients. The follow-up period was 54.5 (33-105) months. There was no difference between the age of symptoms onset, the age of diagnosis, and the diagnosis delay time. The family history in patients with M694V heterozygote mutation was statistically positive compared to non-exon 10 mutation group ($p$=0.001). The symptoms of joint involvement as arthritis and PRAS scores were significantly higher in the M694V heterozygote group ($p$=0.026 and $p$=0.001). Additionally, biological agent need due to colchicine unresponsiveness was statistically higher in M694V heterozygote group than group with non-exon 10 mutation ($p$=0.004).

**Conclusion:** There is a significant difference between children with M694V and non-exon 10 mutations, even when the M694V mutation is present in one allele only. Family history with FMF, musculoskeletal symptoms, and unresponsiveness to colchicine are main parameters.

**Key words:** Familial Mediterranean Fever, M694V, MEFV mutation, colchicine**.**

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**Öz**

**Amaç:** Ailesel Akdeniz Ateşi (AAA), tüm dünyada en sık görülen kalıtsal otoinflamatuvar sendromdur. En sık genotip-fenotip korelasyonu ekzon 10'un belirli bir kısmında, özellikle M694V mutasyonundadır. Ekzon 10 mutasyonu olmayan ve benzer klinik spektruma sahip bir grup hasta da bulunmaktadır. Amacımız M694V heterozigot mutasyonları ve ekzon 10 dışı mutasyonlar arasındaki genotip-fenotip farklılıklarını araştırmaktır.

**Gereç ve yöntem:** İki üçüncü basamak hastaneden AAA'lı çocukların (n=431) veri dosyaları incelendi. Hastalar M694V heterozigot veya non-ekson 10 mutasyonuna sahip olma açısından iki gruba ayrıldı. Genotip-fenotip özellikleri ve tedaviye yanıt karşılaştırıldı.

**Bulgular:** 128 (%29,7) hastada M694V heterozigot mutasyonu ve 303 (%70,3) hastada non-ekson 10 mutasyonu vardı. Takip süresi 54,5 (33-105) aydı. Semptomların başlama yaşı, tanı yaşı ve tanı gecikme süresi arasında fark yoktu. M694V heterozigot mutasyonu olan hastalarda aile öyküsü, ekzon 10 mutasyonu olmayan gruba kıyasla istatistiksel olarak pozitifti ($p$=0,001). Artrit olarak eklem tutulumu semptomları ve PRAS skorları M694V heterozigot grubunda anlamlı olarak daha yüksekti ($p$=0,026 ve $p$=0,001). Ayrıca, kolşisin yanıtsızlığı nedeniyle biyolojik ajan ihtiyacı M694V heterozigot grupta ekzon 10 mutasyonu olmayan gruba göre istatistiksel olarak daha yüksekti ($p$=0,004).

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**Sonuç:** M694V mutasyonu sadece bir alelde mevcut olsa bile, M694V ve non-ekson 10 mutasyonu olan çocuklar arasında anlamlı bir fark vardır. AAA ile aile öyküsü, kas-iskelet sistemi semptomları ve kolşisine yanıtsızlık ana parametrelerdir.

**Anahtar kelimeler:** Ailevi Akdeniz Ateşi, M694V, MEFV mutasyonu, kolşisin.

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## Introduction

Familial Mediterranean Fever (FMF) is the most prevalent hereditary autoinflammatory syndrome globally. It is characterized by recurrent episodes of fever and inflammation affecting serous membranes, joints, and skin [1, 2]. The underlying cause of FMF is mutations in the MEFV gene, which encodes the pyrin protein. Pyrin is crucial for regulating inflammation, particularly in the activation of caspase-1 and the subsequent production of interleukin-1β [3, 4]. The MEFV gene is located on chromosome 16p13.3 and comprises 10 exons separated by 9 introns. Mutations are primarily identified in exons 2, 3, 5, and 10. The Infevers online database documents over 398 alleles classified as mutations or polymorphisms in the MEFV gene [5]. Although FMF is traditionally considered an autosomal recessive disorder, the inheritance pattern is more complex. Heterozygotes can exhibit disease phenotypes, likely due to the influence of other modifying genes, environmental factors, or epigenetic changes affecting the MEFV gene's function [6-8].

Among the documented mutations in the MEFV gene, particularly those in exon 10 like the M694V mutation, there is a clear genotype-phenotype correlation. This correlation is marked by a severe disease phenotype with high inflammatory responses and a predisposition to complications such as amyloidosis [9, 10]. Some FMF patients do not carry exon 10 mutations but still exhibit clinical findings similar to those with exon 10 mutations. Despite genetic diversity, these patients show a similar clinical disease spectrum, highlighting the complexity of FMF pathogenesis and its multifactorial clinical presentation [11, 12].

The genotype-phenotype correlation in FMF remains an area of ongoing research and debate. Many studies have emphasized the clinical significance of exon 10 mutations, particularly the homozygote M694V mutation [13-15]. While some mutations, such as M694V, are linked to a more severe disease course, others like V726A and E148Q tend to present with milder symptoms [8, 16]. In a study investigating amyloidosis cases associated with FMF, 10 patients (8.4%) were reported to have the M694V heterozygote mutation, and 4 patients (3.4%) had non-exon 10 mutations [17]. Kandur et al. [18] found that arthritis was more common in patients with the M694V heterozygote and exon 2 mutation, and these children had higher severity scores compared to those with the M694V heterozygote mutation, underscoring the significant impact of non-exon 10 mutations on FMF clinical manifestations. However, the precise relationship between genotype and phenotype, especially in cases without exon 10 mutations, remains unclear, necessitating further research [19].

In addition to genetic heterogeneity, therapeutic management of FMF can be challenging, particularly in colchicine-resistant cases. While colchicine is the gold standard treatment, a subset of patients does not respond adequately and requires alternative therapeutic approaches, such as anti-interleukin-1 therapies [20, 21].

The aim of this study was to comprehensively characterise the clinical features and genetic profiles of a cohort of patients with non-exon 10 mutation and to elucidate differences in disease manifestations and response to treatment compared to patients with heterozygote M694V mutation. By elucidating these differences, we aimed to advance our understanding of FMF pathogenesis and pave the way for more specialised approaches to diagnosis and treatment in the future.

## Materials and methods

### Study group

Pediatric patients diagnosed with FMF at two tertiary pediatric rheumatology clinic (Dokuz Eylul University childrens' hospital and Dr.B.Uz childrens' hospital) were retrospectively evaluated. Patients were included if they fulfilled at least two of the Ankara clinical diagnostic criteria, including typical fever lasting 6-72 hours, abdominal pain, chest pain, and arthritis attacks, positive family history of AAA, were included [22]. A total of 431 patients previously subjected to MEFV gene analysis were enrolled, encompassing those with heterozygote M694V mutation and heterozygote, homozygote, or compound heterozygote mutations outside of exon 10. The patients were divided into two groups based on the presence of M694V heterozygote mutation and non-exon 10 mutations, allowing for the evaluation of their relationship.

### Clinical and demographic data

Demographic and clinical information such as age, gender, body weight, and height at the last visit, age at the first attack, age of colchicine initiation, delay in diagnosis, follow-up period, presence of FMF in the family, and findings during attacks were recorded. Body weight and height values were adjusted for age according to the data of Nevzi et al [23]. Additionally, the presence of joint complaints between attacks, presence of concomitant rheumatological disease, FMF severity scores calculated using the scoring system of Pras et al. [24], and daily colchicine doses were documented. According to the PRAS score, a score of 3-5 was classified as mild, 6-8 as moderate, and >9 as severe disease.

The most recent hemogram parameters, including hemoglobin (Hb), white blood cell (WBC), and platelet (Plt) counts, as well as neutrophil/lymphocyte ratios (NLR), of all patients during the inter-attack period were recorded using data obtained from the electronic patient record system. Concurrent acute phase responses, such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels, liver and kidney function tests (creatinine (Cr) and alanine aminotransferase (ALT)), and urinary protein excretion were also noted.

Colchicine resistance was defined as experiencing more than one typical episode per three months or an increase in at least two out of three acute phase reactants (CRP, ESR, and serum amyloid A) between attacks despite maximal colchicine intake [25, 26].

### Genetic analysis

Genetic analyses were performed on DNA samples extracted from peripheral blood using the real-time polymerase chain reaction (RT-PCR) method with the Cobas z480 RT-PCR instrument from Roche, Germany, and the LightSNiP assay kit from TIB Molbiol, Germany. This method identified 20 common mutations associated with AAA: E148Q (exon 2), R202Q (exon 2), M680I (exon 10), M694V (exon 10), M694I (exon 10), K695R (exon 10), V726A (exon 10), R761H (exon 10), A744S (exon 10), P369S (exon 3), D510D (exon 5), F479L (exon 5), R314H (exon 3), E230K (exon 2), R408Q (exon 3), R314R (exon 3), G304R (exon 2), R241K (exon 2), S339F (exon 3) and E167D (exon 2).

The study included only patients with a heterozygote M694V mutation among those with exon 10 mutations and all patients with mutations in exons 2, 3 or 5, as well as patients with negative MEFV gene analysis (including those with negative autoinflammatory panel results). Patients with non-exon 10 mutations who were resistant to colchicine were also tested with the autoinflammatory panel. The autoinflammatory panel was used to screen for mutations in genes for MVK, IL1RN, LPIN2, NLRP3, NOD2, NLRP12, ADA2, PSTPIP1, TNFRSF11A, ELANE and TNFRSF1A.

Patients were divided into two groups based on the presence of the heterozygote M694V mutation and non-exon 10 mutations (including exon 2, 3 and 5 mutations and MEFV-negative cases). These groups were analysed to evaluate their demographic, clinical and laboratory characteristics, PRAS scores and response to treatment.

Ethics committee approval was obtained for the study.

### Statistical analysis

Descriptive statistics were used to summarise quantitative process evaluation data: frequency and percentage for categorical variables, and mean, standard deviation, median, minimum and maximum for continuously distributed variables. Normality tests (Kolmogorov-Smirnov test) were performed. The chi-squared test was used to compare categorical variables, and the independent samples t-test was used for continuously distributed variables between two groups. Statistical analyses were performed with IBM SPSS software version 25. A *p*-value of <0.05 was considered statistically significant.

### Results

The study included 431 FMF patients with the M694V heterozygote/non-exon 10 mutation. Among these patients, 45.9% were female and the mean age was 128.4±52.3 months. The most common symptoms at disease onset were abdominal pain, fever and arthralgia. Colchicine resistance was observed in 12 (2.8%) patients who subsequently received anti-IL-1 treatment (Table 1). Among the patients, 128 (29.7%) had the heterozygote M694V mutation, 252 (58.4%) had mutations in exons 2, 3 or 5 (36.8% heterozygote, 21.6% compound heterozygote/homozygote) and 51 (11.8%) had a negative MEFV gene analysis. Detailed MEFV gene mutation results for patients with the M694V heterozygote and non-exon 10 mutations are shown in Table 2.

**Table 1.** Demographic findings of the patients with familial Mediterranean fever (n=461)

| | |
|---|---|
| **Female/Male (n) (%)** | 198/233 (45.9/54.1) |
| **Age** | 128.4±52.3 (20-223) |
| **BMI*** | 18.0±5.1 (10.9-29.5) |
| **Age of symptoms onset*** | 69.7±48.7 (2-200) |
| **Age of diagnosis*** | 95.7±50.1 (13-210) |
| **Diagnosis delay time*** | 25.5±28.0 (3-159) |
| **Follow-up time*** | 26.6±21.8 (6-144) |
| **Family history, n (%)** | 154 (35.7) |
| **Symptoms, n (%)** | |
| Fever | 275 (63.8) |
| Abdominal pain | 292 (67.7) |
| Chest pain | 26 (6.0) |
| Erysipelas like rash | 12 (2.8) |
| Arthralgia | 165 (38.3) |
| Arthritis | 77 (17.9) |
| Myalgia | 49 (11.4) |
| Emesis | 14 (0.9) |
| **Laboratory*** | |
| WBC ($10^3$/uL) | 7550 (6375-9300) |
| Hemoglobin (g/dL) | 12.8 (12.1-13.5) |
| Platelet ($10^3$/uL) | 285.5(247.0-335.0) |
| Neutrophil/Lymphocyte | 1.4 (1.0-2.1) |
| CRP (mg/L) | 0.7 (0.3-2.9) |
| ESR (mm/sa) | 6.0 (3.0-12) |
| **PRAS score*** | 5.8±1.6 (3-11) |
| **PRAS degree*** | 1.6±0.6 (1-3) |
| **Unresponsiveness to colchicine treatment, n (%)** | 12 (2.8) |

*Mean±standard deviation (minimum-maximum), #Median (25-75%), BMI; body mass index, CRP; C-reaktive protein ESR; erythrocyte sedimentation rate, PRAS; projected retained ability score, WBC; white blood cell. All of ages are months

**Table 2.** Distribution of patients according to MEFV gene mutation result (n=431)

| Non-exon 10 mutation | n (%) 303 (100%) | Exon-10 mutation | n (%) 128 (100) |
|---|---|---|---|
| R202Q | 74 (24.4) | M694V | 128 (100) |
| E148Q | 72 (23.7) | | |
| R202Q/R202Q | 24 (7.9) | | |
| E148Q/P369S | 22 (7.2) | | |
| E148Q/R202Q | 21 (6.9) | | |
| P369S | 7 (2.3) | | |
| E148Q/E148Q | 6 (2.0) | | |
| R202Q/P369S | 4 (1.3) | | |
| R314H | 3 (0.9) | | |
| D510D | 2 (0.6) | | |
| R314H/ R314H | 2 (0.6) | | |
| R202Q/P369S/R408Q | 2 (0.6) | | |
| G304R | 1 (0.3) | | |
| D510D/R314R | 1 (0.3) | | |
| E148Q/E230K | 1 (0.3) | | |
| E148Q/F479L | 1 (0.3) | | |
| R202Q/S339F | 1 (0.3) | | |
| R241K/E148Q | 1 (0.3) | | |
| E148Q/E230K | 1 (0.3) | | |
| E148Q/R202Q/P369S | 1 (0.3) | | |
| E148Q/P369S/R408Q | 1 (0.3) | | |
| E167D/ E167D/F479L/ F479L | 1 (0.3) | | |
| E148Q/ E148Q/P369S | 1 (0.3) | | |
| R314R/ R314R/E148Q | 1 (0.3) | | |
| R314R/R314R/R408Q/P369S | 1 (0.3) | | |
| Negative | 51 (16.8) | | |

When comparing the M694V heterozygote and non-exon 10 mutation patient groups, no significant differences were observed with respect to sex distribution, age, age at symptom onset, age at diagnosis, delay in diagnosis, and follow-up periods. However, family history was significantly more common in the M694V heterozygote group ($p$=0.001). Analysis of symptoms during attacks showed that arthritis and myalgia were significantly more frequent in the M694V heterozygote mutation group ($p$=0.026 and $p$=0.005, respectively). There were no significant differences in laboratory findings between the groups, except during attacks. The PRAS score was significantly higher in the M694V heterozygote group ($p$=0.001),

with severe PRAS scores observed in 18.1% of this group compared to 4.3% in the non-exon 10 mutation group. Colchicine resistance was observed in 6.3% of the M694V heterozygote group compared to 1.3% of the non-exon 10 mutation group. No significant difference was found between the 2 groups regarding concomitant rheumatological diseases (Table 3).

**Table 3.** Comparison of demographics of patients with heterozygous M694V mutation and patients with non-exon 10 mutation

| | **Group 1 Non-exon 10 mutation n=303** | **Group 2 M694V heterozygote mutation n=128** | ***p*¥** |
|---|---|---|---|
| **Female/Male (n) (%)** | 141/162 (46.5/53.5) | 57/71 (44.5/55.5) | 0.703 |
| **BMI*** | 17.7±3.1(10.9-29.5) | 19.1± 4.2 (12.0-29.3) | 0.083 |
| **Age of symptoms onset*** | 70.9±50.2 (2-200) | 65.7±43.4 (6-196) | 0.675 |
| **Age of diagnosis*** | 95.9±50.3 (13-210) | 95.1±50.1(21-206) | 0.886 |
| **Diagnosis delay time*** | 24.9±26.0 (3-133) | 27.8±34.3 (4-159) | 0.976 |
| **Follow-up time*** | 24.1±19.4 (6-116) | 35.1±30.8 (8-144) | 0.112 |
| **Family history, n (%)** | 88 (29.2) | 66 (51.6) | 0.001 |
| **Symptoms, n (%)** | | | |
| Fever | 193 (64.3) | 82 (64.6) | 0.963 |
| Abdominal pain | 206 (68.9) | 86 (67.7) | 0.810 |
| Chest pain | 18 (6) | 8 (6.3) | 0.906 |
| Erysipelas like rash | 10 (3.4) | 2 (1.6) | 0.310 |
| Arthralgia | 109 (36.6) | 56 (44.1) | 0.146 |
| Arthritis | 46 (15.3) | 31 (24.4) | 0.026 |
| Myalgia | 26 (8.7) | 23 (18.3) | 0.005 |
| Emesis | 12 (4) | 2 (1.6) | 0.198 |
| **Laboratory#** | | | |
| WBC ($10^3$/uL) | 7600 (6300-9750) | 7300 (6550-8600) | 0.468 |
| Hemoglobin (g/dL) | 12.8 (12-13.5) | 13.1 (12.3-13.7) | 0.116 |
| Platelet ($10^3$/uL) | 288.0 (247.0-341.0) | 274.0 (244.5-306.5) | 0.217 |
| Neutrophil/Lymphocyte | 1.4 (1.0-2.1) | 1.5 (1.1-2.0) | 0.943 |
| CRP (mg/L) | 0.7 (0.3-2.7) | 1.4 (0.4-3.9) | 0.187 |
| ESR (mm/sa) | 6.0 (3.0-13.0) | 6.0 (3.0-20.0) | 0.664 |
| PRAS score* | 5.6±1.6 (3.0-13.0) | 6.4±2.0(3.0-13.0) | 0.001 |
| **PRAS degree n(%)** | | | |
| Mild | 149 (49.2) | 46 (36.2) | |
| Moderate | 141 (46.5) | 58 (45.7) | 0.001 |
| Severe | 13 (4.3) | 23 (18.1) | |
| **Unresponsiveness to colchicine treatment, n (%)** | 4 (1.3) | 8 (6.3) | 0.004 |
| **Presence of concomitant rheumatological disease n (%)** | 26 (8.6) | 13 (10.2) | 0.610 |

*Mean±standard deviation (minimum-maximum), #Median (25-75%), BMI; body mass index, ESR; erythrocyte sedimentation rate
¥Chi-square test (categorical variables) and, Independent simple test (continuously distributed variables)
CRP; C-reaktive protein, PRAS; projected retained ability score, WBC; white blood cell. All of ages are months

Patients with negative MEFV gene mutations and those with non-exon 10 mutations who had colchicine resistance had negative results on the autoinflammatory panel. All patients identified with colchicine resistance who received anti-IL1 treatment responded positively to therapy.

## Discussion

This study analysed 431 patients with FMF to elucidate genotype-phenotype differences between heterozygote M694V mutations and non-exon 10 mutations in FMF patients. The results show significant differences in clinical characteristics and response to treatment, contributing to a more detailed understanding of the pathogenesis and management of FMF.

In our study, no significant differences were found between the groups in terms of sex distribution, age, age at symptom onset, age at diagnosis, delay in diagnosis and follow-up periods. This finding is consistent with other studies [27, 28]. However, a study by Turkucar et al. [29] comparing patients with homozygote M694V mutations with those with exon 10 and exon 2 mutations showed that only the exon 10 mutation group had a significantly earlier onset of attacks.

The heterozygote M694V mutation is associated with more severe clinical symptoms compared to those without exon 10 mutations. This finding is consistent with numerous studies showing a strong genotype-phenotype correlation for exon 10 mutations, particularly M694V. The M694V mutation is often associated with severe disease manifestations, including a higher risk of amyloidosis and frequent, intense inflammatory episodes [30-33]. Our results also showed that patients with the heterozygote M694V mutation had higher PRAS scores and more pronounced musculoskeletal symptoms such as arthritis and myalgia compared to those without exon 10 mutations. Specifically, arthritis and myalgia were significantly higher in the M694V heterozygote group (24.4% and 18.3%, respectively) compared to the other group (15.3% and 8.7%, respectively). Similarly, in the largest cohort study from Turkey, 22.9% of patients with heterozygote M694V mutations had arthritis and 22.2% had myalgia [28].

A review of studies on patients with exon 2, 3 and 5 mutations found more mutation-specific studies. In the study by Kilic et al. [34], arthritis/arthralgia was observed in 32% of the R202Q heterozygote group, while myalgia was present in 16%. In the study by Turkucar et al. [27], arthralgia was observed in 37.5% and arthritis in 20% of 40 patients with heterozygote and homozygote R202Q mutations. In the study by Kilic et al. [34], arthralgia was observed in 49% and arthritis in 3% of 290 patients with heterozygote M694V mutations, while 93.5% and 66.6% of 171 patients with heterozygote R202Q and E148Q mutations had arthralgia and arthritis, respectively. In our study, arthralgia and arthritis were observed in 36.6% and 15.3% of patients with non-exon 10 mutations, respectively. In another study comparing patients with exon 2 mutations to those with exon 10 mutations, no significant difference in clinical findings during attacks was found [29]. In a study of patients with homozygote E148Q mutations, arthralgia was observed in 50%, arthritis in 6.7% and myalgia in 3.3% [35]. Our study found no significant differences in laboratory findings between the attack periods, which is consistent with the literature [27, 29, 36].

In our study, a severe PRAS score was observed in 18.1% of the M694V heterozygote group and 4.3% of the non-exon 10 mutation group. Similarly, in a study of 30 patients with the E148Q homozygote mutation, a severe PRAS score of 3.3% was found [36]. In a study by Kilic et al. [34] analysing genotype-phenotype characteristics in patients diagnosed with FMF, a severe PRAS score was observed in 18.3% of 290 patients with the heterozygote M694V mutation, while severe PRAS scores of 24% and 41.6% were found in 171 patients with non-exon 10 mutations.

Colchicine resistance was found in 8 patients (6.3%) in the M694V heterozygote group and in 4 patients (1.3%) in the non-exon 10 mutation group. In the study by Kilic et al. [34], 5.1% of 290 patients with the heterozygote M694V mutation were unresponsive to colchicine, whereas 6.4% of 171 patients with the heterozygote E148Q and R202Q mutations were unresponsive to treatment. Topaloglu et al. [36] studied 30 patients with the E148Q homozygote mutation and found a 3.3% rate of non-response to colchicine. In a study reported from Japan, colchicine resistance was found in

7 of 27 FMF patients, including those with exon 10 mutations, exon 2 in 2 patients, exon 3 in 2 patients and MEFV negative in 3 patients [35].

Several studies in the literature have suggested that FMF may predispose individuals to other inflammatory diseases such as PFAPA (periodic fever, aphthous stomatitis, pharyngitis, and adenitis), inflammatory bowel disease (IBD), and vasculitis [37-39]. In our study, we observed that 8.6% of patients witho non-exon 10 mutation and 10.2% of patients with the heterozygote M694V mutation had concomitant rheumatological diseases. Consistent with our study, Otar Yener et al. [38], reported a rate of 13.8% of rheumatological comorbidities in patients with heterozygote M694V mutation. Aktay Ayaz et al. [40]. reported comorbidity in 11.8% of 268 patients with M694V heterozygote mutation.

One of the limitations of this study is that we could not screen for rare mutations. We do not know if the rare mutations are associated with FMF as we were not able to screen all exons. In addition, there are studies suggesting that not only MEFV mutations but also environmental factors, miRNA expressions and microbiota influence the FMF phenotype [41-43]. In this study, only genotypic differences were evaluated.

This study shows that the M694V mutation, the clinical significance of which has been established by numerous previous studies, results in more severe PRAS scores and more pronounced musculoskeletal involvement compared to non-exon 10 mutations, regardless of the mode of inheritance, even in cases of heterozygote inheritance. In addition, the presence of severe PRAS scores and colchicine resistance, although less frequent, in non-exon 10 mutations indicates that these mutations should be taken seriously in clinical practice. Future studies investigating the clinical impact of non-exon 10 mutations will help to clarify these findings.

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

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**Authors' contributions to the article**

H.A.D, S.T., C.A., O.A.G., B.M. and E.U. have constructed the main idea and hypothesis of the study. They developed the theory and arranged the material and method section. H.A.D. has done the evaluation of the data in the Results section. Discussion section of the article written by H.A.D. and E.U. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.