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Effect of MEFV gene variants and treatment modalities on attack-free period acute phase reactants of patients with familial Mediterranean fever

Ailevi Akdeniz ateşi hastalarında MEFV gen varyantları ve tedavi yöntemlerinin ataksız dönemdeki akut faz reaktanları üzerindeki etkisi

Ege Sinan Torun[1], Duygu Birinci[2], Ezgi Fındık[2], Elif Ertaş[3]

[1]University of Health Sciences Türkiye, Prof. Dr. Cemil Taşcıoğlu City Hospital, Department of Internal Medicine, Division of Rheumatology, İstanbul, Türkiye
[2]University of Health Sciences Türkiye, Prof. Dr. Cemil Taşcıoğlu City Hospital, Department of Internal Medicine, İstanbul, Türkiye
[3]Selçuk University Faculty of Medicine, Department of Biostatistics, Konya, Türkiye

Abstract

Objective: Attack-free period C-reactive protein (CRP) and serum amyloid A (SAA) are reliable indicators of subclinical inflammation in familial Mediterranean fever (FMF). We aimed to compare the acute phase reactants during the attack-free period, and the presence of subclinical inflammation in FMF patients with different gene variants and different treatment modalities.

Methods: CRP and SAA levels during a symptom-free period of at least 2 weeks were obtained, and the median CRP and SAA levels were calculated during the attack-free period. "Subclinical inflammation" was defined as "median attack-free CRP >10 mg/L or median attack-free SAA >10 mg/L." Patients were classified according to MEFV variants (two, one, or zero exon 10 variants) and treatments (colchicine-only or colchicine+interleukin 1 inhibitors).

Results: Seventy-six patients had two exon 10 variants, 79 had one exon 10 variant, and 17 had non-exon 10 variants. Most patients used colchicine (n=155), and 17 patients used colchicine + interleukin-1 inhibitors. Attack-free CRP, SAA, and rate of subclinical inflammation were significantly different among variant groups, higher among patients with 2 exon 10 variants. Patients receiving combination treatment had higher levels of attack-free CRP and SAA compared to the colchicine-only group. CRP and SAA were strongly correlated.

Conclusion: Patients with two exon 10 variants had higher attack-free acute phase reactants and more frequent subclinical inflammation, which reflects the pathogenicity of exon 10 variants. Patients receiving interleukin 1+colchicine continue to have higher attack-free acute phase reactants, which reflects their higher inflammatory burden and severe clinical features.

Keywords: C-reactive protein, familial Mediterranean fever, serum amyloid A, subclinical inflammation

Özet

Amaç: Ataksız dönemdeki C-reaktif protein (CRP) ve serum amiloid A (SAA), ailevi Akdeniz ateşi (AAA) hastalarında subklinik enflamasyonun güvenilir belirteçleri arasındadır. Bu çalışmada farklı genetik varyantlar taşıyan ve farklı yöntemlerle tedavi edilen AAA hastalarında ataksız dönemdeki akut faz reaktanlarını ve subklinik enflamasyonu karşılaştırmayı amaçladık.

Yöntem: Hastaların en az 2 haftadır semptomsuz olduğu dönemlerde bakılan CRP ve SAA düzeyleri kaydedilerek ataksız dönemdeki medyan CRP ve SAA değerleri hesaplanmıştır. "Subklinik enflamasyon," "ataksız dönemde medyan CRP'nin >10 mg/L olması veya medyan SAA'nın >10 mg/L olması şeklinde tanımlanmıştır. Hastalar MEFV varyantlarına göre (iki, bir ya da sıfır 10. ekzon varyantı olan hastalar) ve tedavilerine göre (yalnızca kolşisin alanlar veya kolşisin+interlökin 1 inhibitörü alanlar) şeklinde sınıflandırılmıştır.

Bulgular: Yetmiş altı hastada iki 10. ekzon varyantı, 79 hastada bir 10. ekzon varyantı, 17 hastadaysa 10. ekzon dışı varyantlar mevcuttu. Hastaların çoğu yalnızca kolşisin (n=155), 17 hasta ise kolşisin+interlökin 1 inhibitörü kullanmaktaydı. Ataksız dönem CRP, SAA ve subklinik enflamasyon oranı iki 10. ekzon varyantı taşıyan hastalarda anlamlı olarak daha yüksekti. Kombinasyon tedavisi alan hastalarda ataksız dönem CRP ve SAA, yalnızca kolşisin kullananlara göre daha yüksekti. CRP ve SAA arasında güçlü korelasyon mevcuttu.

Sonuç: İki 10. ekzon varyantlı hastalardaki daha yüksek ataksız dönem akut faz reaktanı düzeyleri ve subklinik inflamasyon oranı, 10. ekzon varyantlarının patojenitesini yansıtmaktadır. Kolşisin+interlökin 1 inhibitörü alan hastalarda ataksız dönem akut faz reaktanlarının daha yüksek olması bu hastaların daha yoğun enflamasyon yükünü ve daha ağır klinik özelliklerini ortaya koymaktadır.

Anahtar Kelimeler: C-reaktif protein, ailevi Akdeniz ateşi, serum amiloid A, subklinik inflamasyon

Correspondence / İletişim: Ege Sinan Torun MD,
University of Health Sciences Türkiye, Prof. Dr. Cemil Taşcıoğlu City Hospital, Department of Internal Medicine, Division of Rheumatology, İstanbul, Türkiye
E-mail: egesinantorun@hotmail.com **ORCID ID:** orcid.org/0000-0002-4842-0683
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Introduction

Familial Mediterranean fever (FMF) is recognized as the most prevalent monogenic autoinflammatory disorder, with Turkey exhibiting the highest rates of incidence and prevalence globally.[1,2] The condition arises from variants in the *MEFV* gene, which is responsible for encoding the protein pyrin. Among these variants, those found in exon 10, particularly the widely studied M694V variant, have been identified as the most pathogenic and thoroughly characterized.[2,3]

FMF is marked by recurring episodes of fever and serositis, accompanied by a significantly elevated acute phase response.[4] Between these episodes, acute phase reactants typically return to normal levels. However, some patients may experience persistently elevated levels of these reactants even during periods without acute symptoms.[5] Various studies have utilized markers to indicate subclinical inflammation, one of which is serum amyloid A (SAA). The activation pattern of SAA closely resembles that of C-reactive protein (CRP), and studies have indicated that the sensitivity of SAA is comparable to or even exceeds that of CRP.[4] If not addressed, subclinical inflammation heightens the risk of complications, including AA amyloidosis.[6] AA amyloidosis initially manifests as proteinuria, which can escalate to nephrotic levels and ultimately lead to end-stage renal disease.[7]

The objectives of treatment for FMF involve preventing the recurrence of attacks, normalizing acute phase reactants, and effectively managing subclinical inflammation during periods without attacks to avert complications.[8] Colchicine is the primary medication used for FMF management. In cases that respond inadequately to colchicine or in patients who cannot tolerate colchicine, interleukin-1 inhibitors such as anakinra and canakinumab are employed.[9] Colchicine therapy may help reduce levels of markers that are indicative of subclinical inflammation.[6] For patients who do not achieve sufficient control of inflammation, interleukin-1 inhibitors are introduced. A recent study conducted by Atalar et al.[10] revealed that even interleukin-1 inhibitors might not sufficiently suppress subclinical inflammation in FMF patients suffering from AA amyloidosis. A Phase III trial of canakinumab in FMF also demonstrated that, in patients treated with this agent, even though median CRP concentrations were always normal, median SAA concentrations remained over the limit of normal (10 mg/L).[11]

Persistent subclinical inflammation is a significant factor in FMF management and should be taken into account in the long-term care of FMF patients because of the increased risk of AA amyloidosis, which can negatively impact long-term prognosis. We hypothesize that FMF patients with two exon 10 variants will have higher attack-free CRP and SAA levels, and therefore, a higher rate of subclinical inflammation compared to patients with one or zero exon 10 variants, because the literature clearly demonstrates that the most pathogenic variants of the *MEFV* gene are found in exon 10 and these variants are associated with a more severe phenotype characterized by earlier disease onset and more frequent attacks.[12] We also hypothesize that, in accordance with the preexisting studies, patients who require interleukin-1 inhibitors in addition to colchicine will have higher attack-free CRP and SAA levels and more frequent subclinical inflammation compared to patients who only receive colchicine. Therefore, this study aims to compare the levels of acute phase reactants and the presence of subclinical inflammation among FMF patients with different MEFV gene variants (categorized by the number of exon 10 variants) who are receiving different treatment modalities.

Materials and Methods

Patient Selection and Data Collection

This research was granted approval by the Non-Interventional Clinical Research Ethics Committee of Prof. Dr. Cemil Taşcıoğu City Hospital, University of Health Sciences, Türkiye, with the decision number 230, dated 22.10.2024. Informed consent was acquired from the patients involved.

In this cohort study, we conducted a retrospective analysis of the medical records of patients diagnosed with FMF based on the Tel-Hashomer criteria, who visited our Rheumatology Outpatient Clinic at Prof. Dr. Cemil Taşcıoğlu City Hospital from March 2022 to August 2023, without accompanying vasculitis (including Behçet's disease) or spondylarthritis. FMF patients with concomitant spondylarthritis or vasculitis would have additional reasons for elevated attack-free acute phase reactants and were therefore excluded. The study included patients for whom MEFV gene variants were identifiable. Those without any detectable MEFV gene variants were excluded from the analysis. Patients with MEFV gene variants that were classified as "pathogenic," "likely pathogenic", or "variants of uncertain significance" according to the Infevers database were included. Conversely, patients with variants deemed "likely benign" or "benign", such as R202Q, were excluded. Additionally, patients who did not have at least one measurement of CRP or SAA that indicated an "attack-free" status were also excluded.

Patients were classified into three groups according to their MEFV gene variants. Group 1 consisted of patients with two variants in exon 10 of the *MEFV* gene, while Group 2 included those with a single variant in the same exon. Group 3 was made up of patients who did not have any variants in exon 10. Additionally, patients were categorized into two treatment groups: Group A included those who were treated solely with colchicine, whereas Group B contained patients who received both colchicine and interleukin-1 inhibitors during the specified 18-month period.

The medical records of the patients included in the study were analyzed for various biomarkers, including serum CRP, SAA, serum creatinine, estimated glomerular filtration rate (eGFR), serum albumin, proteinuria, the presence of end-stage renal disease, and the occurrence of AA amyloidosis, which was confirmed through kidney biopsy samples. Kidney biopsies were performed on FMF patients who exhibited proteinuria exceeding 1 gram per day and/or a progressive rise in creatinine levels, provided that no other causes of renal failure were identified. The attack-free period for acute phase reactants was defined by CRP and/or SAA levels measured during a symptom-free interval of at least two weeks. Blood samples collected during FMF attacks, within two weeks following an attack, or during infections (with both FMF attacks and infections ruled out based on the patient's history and clinical assessment) were excluded, as they would not accurately represent the "attack-free" period. Subsequently, the median values for CRP, SAA, serum creatinine, eGFR, serum albumin, and proteinuria were calculated. Human leukocyte antigen (HLA)-B27 results were also recorded, if present.

To establish a definition for "subclinical inflammation" in patients, we examined existing literature on the topic. Our definition draws from the review by Ben-Zvi and Livneh[6], which indicated that an increase in colchicine dosage was necessary to effectively manage subclinical inflammation when SAA levels surpassed 10 mg/L. Based on this literature, subclinical inflammation is identified when the median attack-free CRP exceeds 10 mg/L or the median attack-free SAA is greater than 10 mg/L. Additionally, we recorded the patients who were classified as having "subclinical inflammation".

Statistical Analysis

For continuous variables, the mean ± standard deviation and median (Q1-Q3) were employed. For categorical variables, frequency and percentage were calculated.

In assessing the variations in attack-free median CRP, attack-free mean SAA, median serum creatinine, median eGFR, median serum albumin, and median proteinuria across various MEFV gene variant groups and treatment groups, non-parametric tests were employed.

In assessing the differences in attack-free median CRP, attack-free median SAA, median serum creatinine, median eGFR, median serum albumin, and median proteinuria, across various MEFV gene variant groups, the Kruskal-Wallis H test was employed. If a statistical difference was identified among the groups, a post-hoc analysis using the Tukey test was conducted. For comparisons of the median attack-free CRP and SAA among genetic subgroups in Group 2, the Mann-Whitney U test was applied. Similarly, when examining the median attack-free CRP and SAA of genetic subgroups in Group 3, the Kruskal-Wallis H test was utilized, followed by a post-hoc Tukey test if significant differences were found.

The Mann-Whitney U test was employed to assess the differences in attack-free median CRP, attack-free median SAA, median serum creatinine, median eGFR, median serum albumin, and median proteinuria across various treatment groups.

The chi-square test was employed to evaluate the association between genotype—encompassing various variant groups and subgroups, the count of "pathogenic" alleles, "likely pathogenic" alleles, "variants of uncertain significance", and the quantity of each specific allele—and the occurrence of subclinical inflammation, end-stage renal disease, and AA amyloidosis. Additionally, the chi-square test was utilized to examine the relationship between treatment groups and the presence of subclinical inflammation, end-stage renal disease, and AA amyloidosis.

Patients with subclinical inflammation and the risk factors identified for subclinical inflammation (age, sex, number of exon 10 variants, number of non-exon 10 variants, mean colchicine dose, and HLA-B27 positivity) were reported with odds ratios and 95% confidence intervals. Univariate binary logistic regression analysis was utilized to assess the effect of these risk factors on the presence of subclinical inflammation.

The Spearman's correlation test was employed to examine the relationship between the median attack-free values of CRP and SAA.

A statistical significance level of $p<0.05$ was applied during the evaluation. The analysis was conducted using IBM SPSS version 25.

Results

Between March 1, 2022, and August 31, 2023, a total of 253 patients with FMF (without accompanying spondylarthritis or vasculitis, including Behçet's disease)

attended the Rheumatology Outpatient Clinic. The medical records of 56 patients were found to be missing information on the MEFV gene variants, leading to their exclusion from the study. Upon reviewing the MEFV variants of the remaining patients, we identified that 18 individuals were either homozygous or heterozygous for the R202Q variant. While some research suggests that R202Q variants may have clinical significance, we opted to exclude these patients from our analysis, as the "Infevers" database classifies this variant as benign. The MEFV gene variants of the remaining 179 patients were classified as "pathogenic", "likely pathogenic", or "variants of uncertain significance" based on the Infevers database. Among these, 7 patients did not have acute phase reactants measured during the attack-free period and were therefore excluded. Consequently, a total of 172 patients were included in the study. A flowchart illustrating the patient selection process is presented in Figure 1.

Demographic Characteristics

A total of fifty patients (30.2%) were male, while 122 patients (69.8%) were female. The average age of the patients was 38.1±12.3 years, with a median age of 39 years (ranging from a minimum of 17 to a maximum of 66 years). The mean duration of follow-up for the patients was 8.8±5.7 months, with a minimum of 1 month and a maximum of 18 months.

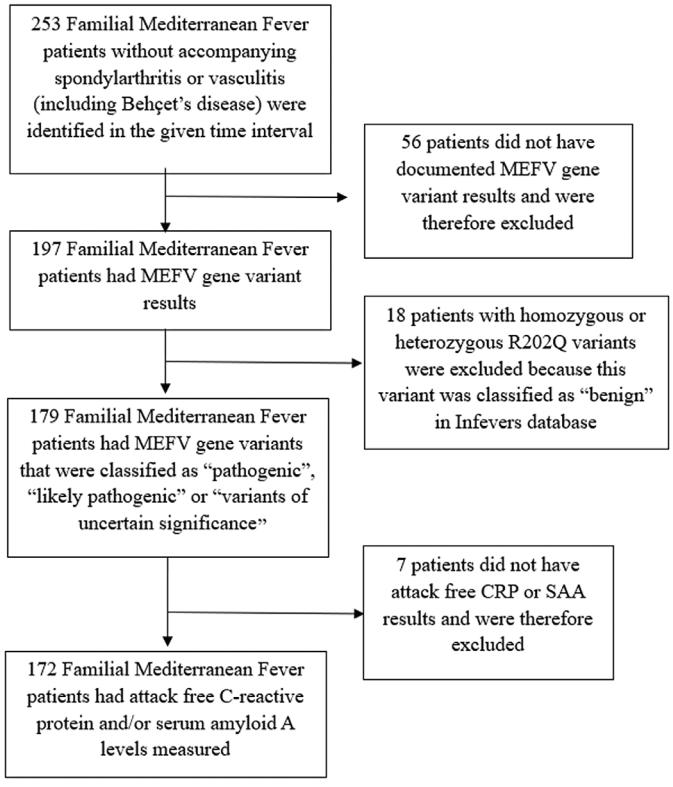

Figure 1. Flowchart of patient selection for the study
CRP: C-reactive protein, SAA: Serum amyloid A

MEFV Gene Variants of the Patients and Different Treatment Groups

Table 1 presents the variants of the *MEFV* gene observed in the patients. Among the participants, 76 patients (44.2%) exhibited two variants in exon 10 (Group 1), 79 patients (45.9%) had one variant in exon 10 (Group 2), and 17 patients (9.9%) showed no variants in exon 10 (Group 3). The predominant variant in Group 1 was the homozygous M694V variant, found in 38 patients, while the most frequent variant in Group 2 was the heterozygous M694V variant, identified in 34 patients. In Group 3, the most common variant was the heterozygous E148Q variant, which was present in 9 patients.

Table 1. MEFV gene variant analysis of the patients

Variants	Number (percent)
Patients with 2 exon 10 variants	**76 (44.2%)**
M694V homozygote	38 (22.1%)
M694V/V726A compound heterozygote	9 (5.2%)
M694V/M680I compound heterozygote	7 (4.1%)
M694V/R761H compound heterozygote	2 (1.2%)
M694V/A744S compound heterozygote	2 (1.2%)
M694I homozygote	2 (1.2%)
M694I/V726A compound heterozygote	1 (0.6%)
M680I homozygote	3 (1.7%)
M680I/V726A compound heterozygote	6 (3.5%)
M680I/R761H compound heterozygote	1 (0.6%)
V726A homozygote	3 (1.7%)
V726A/R761H compound heterozygote	1 (0.6%)
R761H homozygote	1 (0.6%)
Patients with 1 exon 10 variant	**79 (45.9%)**
M694V heterozygote	34 (19.8%)
M694V heterozygote, E148Q heterozygote	7 (4.1%)
M694I heterozygote, E148Q heterozygote	2 (1.2%)
M680I heterozygote	7 (4.1%)
M680I heterozygote, E148Q heterozygote	3 (1.7%)
V726A heterozygote	15 (8.7%)
V726A heterozygote, E148Q heterozygote	1 (0.6%)
R761H heterozygote	5 (2.9%)
R761H heterozygote, F479L heterozygote	1 (0.6%)
A744S heterozygote	2 (1.2%)
K695R heterozygote	2 (1.2%)
Patients with no exon 10 variants	**17 (9.9%)**
E148Q homozygote	1 (0.6%)
E148Q heterozygote	9 (5.2%)
E148Q heterozygote, T309M heterozygote	2 (1.2%)
E148Q heterozygote, P369S heterozygote	1 (0.6%)
E148Q heterozygote, P369S heterozygote, R408 heterozygote	2 (1.2%)
T267I heterozygote	1 (0.6%)
P369S heterozygote, R408 heterozygote	1 (0.6%)

The majority of patients (n=155, 90.1%) were treated only with colchicine (Group A), while 17 patients (9.9%) received a combination of colchicine and interleukin-1 inhibitors (anakinra or canakinumab) (Group B). Mean dose of colchicine was 1.27±0.34 mg/day, median colchicine dose was 1.15 (1-1.5) mg/day; where the minimum dose was 0.5 mg/day and the maximum dose was 2 mg/day. The addition of interleukin-1 inhibitors was necessitated by an insufficient response to colchicine in all 17 patients. Among them, ten patients were administered anakinra, with a mean duration of use of 4.55±4.8 months, (ranging from a minimum of 1 month to a maximum of 14 months). The average dosage of anakinra was 100±40.8 mg/day, with a minimum of 50 mg/day and a maximum of 200 mg/day. Three patients transitioned from anakinra to canakinumab, with one patient citing skin reactions following anakinra injections and two patients reporting inadequate responses to anakinra. Additionally, ten patients were treated with canakinumab, all receiving a consistent dose of 150 mg per month. The mean duration of canakinumab treatment was 5.7±3.4 months, and none of the patients discontinued canakinumab during the 18-month observation period.

Comparison of Attack-free Period C-reactive Protein and Serum Amyloid A in Patients in Different Groups

CRP values were recorded for 171 patients. The median CRP level during attack-free periods was 5.2±7.8 mg/L, with values ranging from a minimum of 0.10 mg/L to a maximum of 53 mg/L. SAA values were available for 156 patients, with a median attack-free SAA value of 20±43 mg/L, ranging from a minimum of 1.1 mg/L to a maximum of 332 mg/L.

Table 2 presents the mean and median values of attack-free CRP and SAA for patients categorized into Group 1 (two exon 10 variants), Group 2 (one exon 10 variant), and Group 3 (no exon 10 variants). The findings indicate a statistically significant difference in the median attack-free mean CRP across the groups (p<0.001). Specifically, Group 1 exhibited a significantly higher median attack-free CRP than Group 2 (p=0.01) and Group 3 (p=0.006). Additionally, the analysis revealed a significant difference in attack-free SAA levels among the groups (p=0.02), with Group 1 showing a notably higher median attack-free SAA than Group 2 (p=0.009).

In Group 2, 65 patients exhibited a single exon 10 variant, while 14 patients presented with one exon 10 variant and one non-exon 10 variant. The subgroup analysis revealed that the levels of attack-free CRP and SAA were not significantly different between patients with only one exon 10 variant and those with one exon 10/one non-exon 10 variant (p=0.44 for CRP, p=0.24 for SAA).

In Group 3, there were 10 patients with a single non-exon 10 variant, 5 patients with two non-exon 10 variants, and 2 patients with three non-exon 10 variants. The analysis revealed no statistically significant differences among the three groups concerning attack-free median CRP or attack-free median SAA levels (p=0.82 for CRP, p=0.91 for SAA).

Table 3 illustrates the mean and median values of attack-free CRP and SAA for patients in Group A (colchicine) and Group B (colchicine combined with interleukin 1 inhibitors). The median attack-free CRP in Group B was notably greater than that in Group A [p=0.004], and the median attack-free SAA in Group B also exceeded that of Group A significantly [p=0.03].

Presence of Subclinical Inflammation According to Different Groups

As detailed in the Methods section, we documented patients exhibiting subclinical inflammation. Based on our criteria, 37 out of 76 patients in Group 1, 21 out of 79 patients in Group 2, and 4 out of 17 patients in Group 3 were identified as having subclinical inflammation. The chi-square test indicated a statistically significant difference among the three groups (p=0.009) regarding the occurrence of subclinical inflammation.

In the comparison of the two subgroups within Group 2 regarding the presence of subclinical inflammation, no statistically significant difference was observed between patients with one exon 10 variant/one non-exon 10 variant

Table 2. Evaluation of the attack free CRP and SAA according to *MEFV* gene variant groups of the patients

	Group 1-two exon 10 variants n=76	Group 2-one exon 10 variant n=79	Group 3-no exon 10 variants n=17	p-value*	p-value**
	Mean ± SD Median (Q1-Q3)	Mean ± SD Median (Q1-Q3)	Mean ± SD Median (Q1-Q3)		1 vs. 2 1 vs. 3 2 vs. 3
Mean and median attack free CRP (mg/L)	8.08±10.11 3.31 (1.69-11)	3.27±4.88 1.94 (0.72-4.19)	1.74±1.97 0.85 (0.5-3.16)	**<0.001**	**0.001 0.006** 0.73
Mean and median attack free SAA (mg/L)	31.2±60.3 9.2 (4.1-27.7)	9.9±13.1 5 (2-12.1)	10.9±11 8.1 (2.9-15.9)	**0.02**	**0.009 0.19** 0.99

*CRP: C-reactive protein, SAA: Serum amyloid A, SD: Standard deviation, Q1-Q3: First and third quartile, *Kruskal-Wallis H test, **Pos-hoc Tukey test, 1: Two exon 10 variants, 2: One exon 10 variant, 3: No exon 10 variants*

and those who had only one exon 10 variant (p=0.18). Similarly, when the three subgroups of Group 3 were analyzed, the chi-square test did not reveal any statistical significance in relation to the presence of subclinical inflammation among patients with one, two, or three non-exon 10 variants (p=0.64).

The investigation into the correlation between subclinical inflammation and the quantity of specific alleles revealed that only the V726A alleles showed statistical significance (p=0.03). Furthermore, when examining the relationship between subclinical inflammation and the numbers of "pathogenic" variants, "likely pathogenic", and "variants of uncertain significance," it was found that only the number of "pathogenic" alleles reached statistical significance (Supplementary Table 1).

When patients were classified based on their treatment modalities, 53 individuals in Group A and 9 individuals in Group B exhibited subclinical inflammation. The chi-square test indicated that there was no significant difference between the two groups regarding the presence of subclinical inflammation (p=0.18).

Comparison of Renal Parameters, AA Amyloidosis, and End Stage Renal Disease in Different Groups

No notable differences were observed among the three variant groups regarding median serum creatinine, median eGFR, median serum albumin, and median proteinuria. In Group 1, seven patients were diagnosed with AA amyloidosis, while neither Group 2 nor Group 3 had any cases of this condition. The chi-square test indicated that this disparity among the three variant groups was statistically significant (p=0.02). Additionally, two patients in Group 1 had end-stage renal disease, whereas there were no cases of end-stage renal disease in either Group 2 or Group 3. The chi-square test revealed that the difference in this regard among the three variant groups was not statistically significant (Supplementary Table 2).

There was no notable difference in the median values of serum creatinine and eGFR between the two treatment groups. However, the median serum albumin level in Group A was significantly greater than that in Group B (p=0.04). Additionally, median proteinuria was significantly higher in Group B compared to Group A (p<0.001). In Group A, two patients were diagnosed with AA amyloidosis, while in Group B, five patients had the same condition. The chi-square test revealed a significantly higher prevalence of AA amyloidosis in Group B (p<0.001). Additionally, none of the patients in Group A experienced end-stage renal disease, whereas two patients in Group B did. This difference was statistically significant, as indicated by the chi-square test (p=0.009) (Supplementary Table 3).

Risk Factors for Subclinical Inflammation

According to our definition of subclinical inflammation, 62 patients (36%) had subclinical inflammation. Supplementary Table 4 demonstrates the risk factors for the presence of subclinical inflammation and the results of the univariate logistic regression analysis. In this analysis, presence of subclinical inflammation was not associated with age, sex, number of exon 10 variants, number of non-exon 10 variants, mean colchicine dose, and HLA-B27 positivity.

Correlation of C-reactive Protein and Serum Amyloid A

The Spearman's correlation test revealed a strong positive correlation (correlation coefficient: 0.70) between the median levels of CRP and SAA levels without attacks, with a significance level of p<0.001.

Discussion

Our research categorized patients based on the presence and quantity of variants in exon 10. The identified exon 10 variants among our patients included M694V, M694I, M680I, V726A, R761H, A744S, and K695R. The variants M680I, M694V, M694I, K695R, V726A, and A744S, which are situated within the B30.2 domain of the pyrin protein, are associated with the most prevalent and severe manifestations of FMF.[13] In the investigation conducted by Van Gorp et al.[14], a functional assay was established, revealing that the

Table 3. Evaluation of the attack free CRP and SAA according to treatment groups of the patients

	Group A- Only colchicine n=155	Group B- Colchicine+anti interleukin-1 n=17	p-value
	Mean ± SD Median (Q1-Q3)	Mean ± SD Median (Q1-Q3)	
Mean and median attack free CRP (mg/L)	4.65±7.33 2.1 (0.76-4.98)	10.09±10.14 7.15 (2.44-13.5)	**0.004**
Mean and median attack free SAA (mg/L)	16.4±34.1 6.4 (3.3-15.9)	55.4±89.7 16.8 (6.1-79.5)	**0.03**

CRP: C-reactive protein, SAA: Serum amyloid A, SD: Standard deviation, first and third quartile, Mann-Whitney U test

R761H variant in exon 10 also contributes to pyrin activation independently of microtubule dynamics, akin to the disease-associated FMF variants M680I, M694V, and M694I. Nevertheless, this study indicated that the mechanism of pyrin inflammasome activation in patients carrying K695R alleles may differ from that observed in FMF patients with the classical exon 10 variants.

Research comparing phenotype-genotype correlations in children with FMF has shown that those with homozygous or compound heterozygous variants in the *MEFV* gene experienced an earlier onset of the disease, more frequent attack episodes, and a higher incidence of fever, serositis, arthritis, and erysipelas-like erythema.[15,16] Our study provided a unique classification of FMF patients based on the number of variants in exon 10 and examined the state of subclinical inflammation by assessing CRP and SAA levels during attack-free periods. Patients with two variants in exon 10 exhibited significantly elevated median levels of CRP and SAA compared to other variant categories. Consistent with our findings, Kelesoglu et al.[17] reported that CRP levels during attack-free periods were higher in homozygous M694V patients than in other groups, despite these patients generally having normal CRP levels. Additionally, a study indicated that individuals with homozygous or compound heterozygous exon 10 MEFV variants had increased SAA levels during attack-free periods.[5]

Effectively managing subclinical inflammation is a key goal in the treatment of FMF to avert complications such as AA amyloidosis. Nonetheless, a universally accepted definition of "subclinical inflammation" in FMF patients is lacking. In this study, we examined the levels of CRP and SAA in FMF patients during periods free from attacks, and based on existing literature,[6] we proposed a definition for subclinical inflammation. Our findings indicated a significant difference in the prevalence of subclinical inflammation among the three variant groups. Specifically, as per our proposed definition, patients with two variants in exon 10 exhibited notably higher rates of subclinical inflammation compared to those with a single variant.

This study primarily examines variants in exon 10; however, subgroup analyses were conducted to assess the potential impact of non-exon 10 variants on baseline inflammation in FMF patients. The findings indicated no statistically significant difference in the levels of attack-free acute phase reactants and the presence of subclinical inflammation between patients with one exon 10 variant and a non-exon 10 variant, compared to those with only one exon 10 variant. Additionally, subgroup analysis of Group 3 did not reveal significant differences in attack-free CRP, attack-free SAA levels, or subclinical inflammation among patients with one, two, or three non-exon 10 variants. Consequently, non-exon 10 variants do not appear to significantly influence baseline inflammatory markers in FMF patients. This aligns with existing literature, suggesting that individuals with non-exon 10 variants exhibit a "milder" phenotype, characterized by reduced disease severity, fewer joint symptoms, a lower requirement for biologic treatments, and greater responsiveness to colchicine, in contrast to patients with the homozygous M694V variant.[18,19]

The analysis of the correlation between subclinical inflammation and the quantity of specific alleles, as well as the counts of "pathogenic variants", "likely pathogenic variants", and "variants of uncertain significance", revealed a statistically significant association between subclinical inflammation and both the number of "pathogenic variants" and the count of V726A alleles. While the link between pathogenic variants and subclinical inflammation is expected, the notable association between V726A alleles and subclinical inflammation is particularly intriguing. The research conducted by Lofty et al.[20] indicated that children with FMF who possessed the V726A allele experienced a higher frequency of attack-free SAA, which corroborates our findings.

AA amyloidosis, recognized as the most serious complication of FMF, was found to be more prevalent in patients carrying two variants in exon 10. This increase was associated with heightened levels of subclinical inflammation within this patient cohort. Additional research has shown that individuals with homozygous M694V variants also exhibited elevated rates of subclinical inflammation and AA amyloidosis, corroborating the findings of our study.[21,22]

Our research also categorized FMF patients based on their treatment approaches. Those who were treated with both interleukin-1 inhibitors and colchicine (Group B) exhibited significantly higher median values of attack-free CRP and SAA, compared to those who received only colchicine (Group A). This observation may be attributed to the more severe phenotype of FMF in these patients, as they were prescribed interleukin-1 inhibitors due to an insufficient response to colchicine. Nevertheless, no significant difference was observed between the two treatment groups regarding the presence of subclinical inflammation. The relatively small sample size in Group B (only 17 patients) may have hindered the ability to achieve statistical significance. Consequently, future research involving a larger cohort may enhance our understanding of subclinical inflammation among patients receiving different treatment regimens.

Patients treated with interleukin-1 inhibitors alongside

colchicine also exhibited lower median albumin levels, increased median proteinuria, and a higher incidence of AA amyloidosis and end-stage renal disease. Although they are receiving a more potent treatment, subclinical inflammation remains inadequately managed in this group, potentially leading to the development of AA amyloidosis. This situation indicates a greater inflammatory burden among these patients. While some studies have indicated that both anakinra and canakinumab can effectively manage subclinical inflammation,[23] our findings did not support this outcome. A Phase III trial of canakinumab in FMF also demonstrated that even though the CRP of patients remained below 10 mg/L under canakinumab treatment, SAA levels remained above 10 mg/L.[11] Despite significant clinical improvements and partial management of subclinical inflammation in these severe FMF patients receiving a combination of interleukin-1 inhibitors and colchicine, there remains a critical need for more effective strategies to control subclinical inflammation and further mitigate the risk of AA amyloidosis.

In order to better comprehend the factors associated with the presence of subclinical inflammation, we performed univariate binary logistic regression analysis for certain demographic, laboratory, and treatment parameters. In our analysis, none of the parameters (age, sex, number of exon 10 variants, number of non-exon 10 variants, mean colchicine dose, and HLA B27 positivity) were associated with the presence of subclinical inflammation. Due to the incompleteness of our retrospective data, we were unable to incorporate different clinical features (such as presence and frequency of pleuritis, peritonitis, arthritis, erysipelas-like erythema, smoking status, body mass index) into our analysis. Our cohort had a relatively high percentage of patients with subclinical inflammation (36%). In a similar study, Babaoglu et al.[24] detected a lower percentage of subclinical inflammation (15%) among their 917 FMF patients. Their analysis demonstrated that male sex, history of exertional leg pain, inflammatory comorbidities, M694V homozygosity, colchicine resistance, lower education levels, and musculoskeletal attack dominance were the independent predictors of persistent inflammation.

Evidence for the superiority of monitoring FMF with one acute phase reactant over another is scarce.[25] Nonetheless, several earlier studies have indicated that SAA levels might be a more sensitive indicator of subclinical inflammation compared to CRP levels.[26,27] Our findings revealed a significant correlation between CRP and SAA. Consequently, we propose that in healthcare environments where SAA is not accessible, CRP can be utilized independently to identify subclinical inflammation, as long as taking thorough histories and performing detailed physical examinations exclude the possibility of an FMF attack or infection.

Study Limitations

The retrospective design of the study, along with the absence of data regarding the frequency of FMF attacks within a specified timeframe, the lack of information about the number of patients with and the frequency of serositis, musculoskeletal symptoms, or presence of family history of FMF are notable limitations of the study.

During the study's design phase, we chose not to include the number of attacks, as we believed that this information was not consistently documented in the patients' medical records. Additionally, the patients themselves reported the attacks, which raises the possibility of incorrectly identifying symptoms related to other conditions as FMF attacks. Furthermore, patients did not always seek medical attention during these episodes, which would have allowed trained medical professionals to utilize patient history, physical examinations, and laboratory results to objectively confirm the attacks. Consequently, our findings should be approached with caution, as the variations observed in inflammatory markers may be influenced by the frequency of attacks rather than indicating genuine subclinical inflammation. Data concerning the number of patients with serositis and frequency of serositis and musculoskeletal symptoms, and number of patients with a positive family history were unfortunately incomplete for most of our patients. Therefore, these parameters were not reported. Long-term prospective studies, performed in a larger number of patients, which report more optimally on the clinical features relevant to this topic, are necessary to address the limitations identified in this study.

Conclusion

In summary, this research highlights the elevated levels of attack-free CRP and SAA, increased instances of subclinical inflammation, and a greater prevalence of amyloidosis among patients who are either homozygous or compound heterozygous for variants in exon 10. Furthermore, it reveals that in individuals with a significant inflammatory burden necessitating the use of interleukin-1 inhibitors, subclinical inflammation may remain insufficiently controlled. There is a strong correlation between attack-free CRP and SAA levels and in situations where SAA testing is not available, CRP can be effectively utilized to evaluate subclinical inflammation.

Ethics

Ethics Committee Approval: This research was granted approval by the Non-Interventional Clinical Research Ethics Committee of Prof.Dr. Cemil Taşcıoğu City Hospital, University of Health Sciences, Türkiye, with the decision number 230, dated 22.10.2024.

Informed Consent: Informed consent was acquired from the patients involved.

Footnotes

Authorship Contributions

Concept: E.S.T., D.B., E.F., E.E., Design: E.S.T., D.B., E.F., E.E., Data Collection and Processing: E.S.T., D.B., E.F., E.E., Analysis or Interpretation: E.S.T., D.B., E.F., E.E., Literature Search: E.S.T., D.B., E.F., E.E., Writing: E.S.T., D.B., E.F., E.E.

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Supplementary Table 1. Relationship of presence of subclinical inflammation and the number of specific alleles and the number of "pathogenic variants", "likely pathogenic variants" and "variants of uncertain significance"

	Subclinical inflammation		
	Present n=62	Absent n=110	
Number of M694V alleles	**n (%)**	**n (%)**	**p-value**
0	20 (32.3)	50 (45.5)	0.11
1	23 (37.1)	40 (36.4)	
2	19 (30.6)	20 (18.2)	
Number of V726A alleles			
0	51 (82.3)	88 (80)	**0.03**
1	8 (12.9)	22 (20)	
2	3 (4.8)	0 (0)	
Number of M694I alleles			
0	61 (98.4)	106 (96.4)	0.12
1	0 (0)	4 (3.6)	
2	1 (1.6)	0 (0)	
Number of M680I alleles			
0	54 (87.1)	92 (83.6)	0.49
1	8 (12.9)	15 (13.6)	
2	0 (0)	3 (2.7)	
Number of R761H alleles			
0	58 (93.5)	103 (93.6)	0.38
1	3 (4.8)	7 (6.4)	
2	1 (1.6)	0 (0)	
Number of A744S alleles			
0	60 (96.8)	109 (99.1)	0.30
1	2 (3.2)	1 (0.9)	
Number of K695R Alleles			
0	61 (98.4)	109 (99.1)	0.99
1	1 (1.6)	1 (0.9)	
Number of E148Q alleles			
0	53 (85.5)	91 (82.7)	0.71
1	9 (14.5)	17 (15.5)	
2	0 (0)	2 (1.8)	
Number of F479L alleles			
0	61 (98.4)	110 (100)	0.36
1	1 (1.6)	0 (0)	
Number of T309M alleles			
0	62 (100)	108 (98.2)	0.41
1	0 (0)	2 (1.8)	
Number of P369S alleles			
0	60 (96.8)	108 (98.2)	0.46
1	2 (3.2)	2 (1.8)	
Number of R408Q alleles			
0	61 (98.4)	108 (98.2)	0.99
1	1 (1.6)	2 (1.8)	
Number of T267I alleles			
0	62 (100)	109 (99.1)	0.99
1	0 (0)	1 (0.9)	
Number of pathogenic variants			
0	9 (14.5)	19 (17.3)	**0.03**
1	20 (32.2)	55 (50)	

2	33 (53.2)	36 (32.7)	
Number of likely pathogenic variants			
0	57 (91.9)	102 (92.7)	0.14
1	3 (4.8)	8 (7.3)	
2	2 (3.2)	0 (0)	
Number of variants of uncertain significance			
0	50 (80.6)	88 (80)	0.85
1	10 (16.1)	16 (14.5)	
2	1 (1.6)	5 (4.5)	
3	1 (1.6)	1 (0.9)	

Supplementary Table 2. Evaluation of the renal parameters, AA amyloidosis and end stage renal disease according to ***MEFV*** gene variant groups of the patients

	Group 1-two exon 10 variants n=76	Group 2-one exon 10 variant n=79	Group 3-no exon 10 variants n=17	p-value*	p-value**
	Mean ± SD Median (Q1-Q3)	Mean ± SD Median (Q1-Q3)	Mean ± SD Median (Q1-Q3)		1 vs. 2 1 vs. 3 2 vs. 3
Mean and median serum creatinine (mg/dL)	0.88±0.82 0.7 (0.62-0.84)	0.70±0.14 0.67 (0.61-0.78)	0.68±1.13 0.68 (0.61-0.75)	0.57	-
Mean and median estimated glomerular filtration rate (mL/min)	86.4±13.5 90 (90-90)	88.9±2.9 90 (90-90)	89.7±1.4 90 (90-90)	0.38	-
Mean and median serum albumin (g/dL)	4.51±0.42 4.5 (4.4-4.7)	4.53±0.34 4.5 (4.3-4.78)	4.49±0.29 4.5 (4.25-4.65)	0.87	-
Mean and median proteinuria (mg/day)	309.39±480.01 104 (70.25-181.5)	142.82±223.38 94 (70-125)	112.26±64.25 100 (65-139)	0.59	-
AA amyloidosis absent	69 (90.8)	79 (100)	17 (100)	**0.02**	
AA amyloidosis present	7 (9.2)	0 (0)	0 (0)		
End stage renal disease absent	74 (97.4)	79 (100)	17 (100)	0.38	
End stage renal disease present	2 (2.6)	0 (0)	0 (0)		

*AA: Amyloid A, SD: Standard deviation, Q1-Q3: First and third quartile *Kruskal-Wallis H test, **Post-hoc Tukey test, 1: Two exon 10 variants, 2: One exon 10 variant, 3: No exon 10 variants*

Supplementary Table 3. Evaluation of the renal parameters, AA amyloidosis and end stage renal disease according to treatment groups of the patients

	Group A-only colchicine n=155	Group B-colchicine+anti interleukin-1 n=17	p-value
	Mean ± SD Median (Q1-Q3)	Mean ± SD Median (Q1-Q3)	
Mean and median serum creatinine (mg/dL)	0.71±0.14 0.69 (0.62-0.77)	0.94±0.71 0.65 (0.59-0.85)	0.87
Mean and median estimated glomerular filtration rate (mL/min)	89±2.48 90 (90-90)	74.43±23.75 90 (85.7-90)	0.10
Mean and median serum albumin (g/dL)	4.54±0.35 4.5 (4.35-4.70)	4.29±0.49 4.4 (4.08-4.62)	**0.04**
Mean and median proteinuria (mg/day)	155.83±341.77 93 (67-129)	822.64±1665.81 184 (147.5-533.75)	**<0.001**
AA amylodosis absent	153 (98.7)	12 (70.6)	**<0.001**
AA amyloidosis present	2 (1.3)	5 (29.4)	
End stage renal disease absent	155 (100)	15 (88.2)	**0.009**
End stage renal disease present	0 (0)	2 (11.8)	

AA: Amyloid A, SD: Standard deviation, Q1-Q3: First and third quartile, Mann-Whitney U test

Supplementary Table 4. Assessment of presence of subclinical inflammation according to different risk factors

	Odds ratio	%95 CI	p-value
Age	0.99	0.97-1.02	0.61
Sex	1.31	0.67-2.5	0.44
Number of exon 10 variants	0.77	0.40-1.47	0.43
Number of non-exon 10 variants	0.41	0.04-4.01	0.44
Mean colchicine dose	1.04	0.39-2.81	0.94
HLA-B27	0.62	0.12-3.12	0.56

CI: Confidence interval, HLA: Human leukocyte antigen, univariate binary logistic regression