JOURNAL OF
CONTEMPORARY MEDICINE

DOI:10.16899/jcm.1164970
J Contemp Med 2022;12(5):804-810

Original Article / Orijinal Araştırma

An Alternative Perspective to the FMF Clinic: MCP-1 (A-2518G) and CCR2 (G190A) Polymorphisms and MCP1 Expression

FMF Kliniğine Alternatif Bir Bakış Açısı: MCP-1 (A-2518G) ve CCR2 (G190A) Polimorfizmleri ve MCP1 Ekspresyonu

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Abstract

Background: Familial Mediterranean Fever (FMF) is an autoinflammatory disease and may express as various clinical findings. Chemokines are crucial elements of the inflammatory process. MCP-1 and its' receptor CCR2 are the main chemokines for monocytes/macrophages that may play critical roles in FMF. Thus, it was aimed to investigate the MCP-1 (A-2518G) and CCR2 (G190A) polymorphisms and MCP-1 expression level, which may affect MEFV gene function.

Material and Method: Patients with FMF were identified according to the Tel-Hashomer criteria. DNA and RNA were isolated from the obtained blood samples. Genotyping analysis was performed by PCR-RFLP technique. In addition, expression analyzes were performed by Real-time PCR method. The obtained results were evaluated statistically.

Results: A total of 229 individuals (125 male and 104 female) were included in the study. While 120 individuals had FMF clinic, and 107 individuals did not have. The remaining two individuals had suspicious clinical status. In addition, while 75 individuals were homozygous mutants, 77 individuals were heterozygous mutants, and 77 individuals did not carry mutation in the MEFV gene. No significant relationship was found in between both FMF clinic and MEFV genotypes, and MCP-1 (A-2518G) and CCR2 (G190A) genotypes. In the expression analysis, MCP-1 expression increased in patients with FMF clinic compared to those without. In addition, MCP-1 expression was increased in the heterozygous MEFV group compared to those without mutation, moreover, the expression level was highest in homozygous MEFV group. In addition, according to the MCP-1 (A-2518G) genotyping, MCP-1 expression elevated in the homozygous as well as the heterozygous groups, compared to the Wild type group.

Conclusion: MCP-1 expression is increased in FMF disease, which may explain the clinical differences between FMF patients. MEFV mutations may exacerbate inflammation by increasing MCP-1 transcription. MCP-1 expression is increased in patients with MCP-1(A-2518G) mutations, which aggravates FMF clinic. MCP-1 expression may be assessed as a marker in suspicious cases.

Keywords: Familial Mediterranean Fever, MCP-1, CCR2, expression

Öz

Amaç: Ailevi Akdeniz Ateşi (AAA) otoinflamatuar bir hastalıktır ve çeşitli klinik bulgular olarak kendini gösterebilir. Kemokinler, inflamatuar sürecin önemli unsurlarıdır. MCP-1 ve onun reseptörü CCR2, FMF'de kritik roller oynayabilen monositler/makrofajlar için ana kemokinlerdir. Bundan dolayı MEFV gen fonksiyonunu etkileyebilecek MCP-1 (A-2518G) ve CCR2 (G190A) polimorfizmlerinin ve MCP-1 ekspresyon düzeyinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: FMF'li hastalar Tel-Hashomer kriterlerine göre belirlendi. Elde edilen kan örneklerinden DNA ve RNA izole edildi. Genotipleme analizi, PCR-RFLP tekniği ile yapıldı. Ayrıca Real-time PCR yöntemi ile ekspresyon analizleri yapıldı. Elde edilen sonuçlar istatistiksel olarak değerlendirildi.

Bulgular: Çalışmaya toplam 229 birey (125 erkek ve 104 kadın) dahil edildi. Bunlardan 120 kişide FMF kliniği bulunurken, 107 kişide yoktu. Kalan iki kişi şüpheli klinik duruma sahipti. Çalışmaya alınan bireyler MEFV genotiplemesine göre değerlendirildiğinde ise 75 birey homozigot mutant, 77 birey Heterozigot saptanırken 77 birey ise MEFV geninde mutasyon taşımıyordu. Yapılan analizde Hem FMF kliniği hem de MEFV genotipleri ile MCP-1 (A-2518G) ve CCR2 (G190A) genotipleri arasında anlamlı bir ilişki bulunmadı. Ekspresyon analizinde, FMF kliniği olan hastalarda olmayanlara göre MCP-1 ekspresyonu artmış olarak saptandı. Ayrıca heterozigot MEFV grubunda mutasyonu olmayanlara göre MCP-1 ekspresyonu artmış olarak saptandı, Dahası homozigot MEFV grubunda MCP-1 ekspresyonu en yüksek düzeydeydi. Ek olarak, MCP-1 (A-2518G) genotiplendirmesine göre, MCP-1 ekspresyonu, Wild type gruba kıyasla hem homozigot hem de heterozigot gruplarda yükselmiştir.

Sonuç: FMF hastalığında MCP-1 ekspresyonu artmış olup, bu durum FMF hastaları arasındaki klinik farklılıkları açıklayabilir. MEFV mutasyonları, MCP-1 transkripsiyonunu artırarak inflamasyonu şiddetlendirebilir. MCP-1(A-2518G) mutasyonlu hastalarda MCP-1 ekspresyonu artar, bu da FMF kliniğini ağırlaştırır.

Anahtar Kelimeler: Ailevi Akdeniz Ateşi, MCP-1, CCR2, Expresyon analizi

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Received *(Geliş Tarihi)*: 21.08.2022 **Accepted *(Kabul Tarihi)*:** 06.09.2022

INTRODUCTION

Familial Mediterranean Fever (FMF) is an inherited, chronic autoinflammatory disease characterized by recurrent and self-limiting episodes of fever accompanied by varying degrees of serosal and synovial inflammation causing pain (chest or abdominal pain), arthritis, myalgia, and skin involvement.[1,2] The disease was determined to be associated with the Mediterranean Fever (MEFV) gene, which encodes the pyrin protein, that is thought to play an important role in the regulation of inflammation.[3] Although the MEFV variants, which cause the disease, are quite common in populations of the Eastern Mediterranean and Middle East regions in which FMF is predominantly found.[4] the diagnosis of FMF, atypical FMF and FMF-like disease has been frequently reported from all over the world due to atypical clinical conditions and different modes of inheritance over the years.[5] Currently, 389 nucleotide variants on the MEFV gene are reported in the Infevers database, which is an online registry for autoinflammatory mutations. However, only 28 of these are identified as "pathogenic" or "Likely pathogenic", while the remainder are considered VUS (Variants of Uncertain clinical Significance) or polymorphism.[6] Pyrin is expressed mainly in monocytes and neutrophils, and to a lesser extent in dendritic cells, skin, and synovial fibroblasts. Most of the clinical symptoms of FMF are associated with altered monocyte and neutrophil function.[7] Monocyte/macrophage cells are the main players of the immune system.[8] These cells contribute to the initiation and finish up of inflammation, activation of immunity and regulation of bone metabolism.[9]

Chemokines have an important role in innate and adaptive immunity. They are involved in many physiological and pathological processes such as inflammation, cell proliferation, apoptosis, tumor metastasis and host defense.[10] Monocyte Chemoattractant Protein-1 (MCP-1), a CC chemokine, is encoded by the CCL2 gene[11] and is a potent mononuclear cell chemoattractant which plays a part in a variety of diseases characterized by monocyte-rich leukocyte infiltrates.[12] This molecule activates monocytes and macrophages, by interacting with the membrane CC Chemokine receptor 2 (CCR2), to migrate to areas of inflammation.[13] MCP-1 A-2518G polymorphism and CCR2 190 G/A (Also known as V64I) polymorphism, which are defined in the regulatory region of the MCP-1 gene and are known to affect the transcriptional activity of MCP-1, have been reported to be associated with different inflammatory diseases and cancer.[10,14]

The existence of different clinical findings in FMF disease, which is an inflammatory disease basically, and the variability in the severity of clinical findings are known. Besides, patients without MEFV mutations or with heterozygous mutations are common.[15] The aim of this study is to investigate possible mechanisms that may cause this disease. For this purpose, we focused on chemokines, which are important elements of the inflammatory process and regulate the inflammatory process. We mainly focused on MCP-1 and its receptor CCR2, which is the main effective chemokine on monocytes/macrophages that play critical roles in FMF disease. We aimed to investigate the polymorphisms of MCP-1 (A-2518G) and CCR2 (G190A), which are most frequently studies and found to have an effect on function, and the expression level of MCP-1.

MATERIAL AND METHOD

Study Group

This study was carried out in the laboratories of Cumhuriyet University Faculty of Medicine, Department of Medical Genetics and Selcuk University, Faculty of Medicine, Department of Medical Genetics. A total of 229 individuals, 125 men and 104 women, were included in the study. Among these individuals, 75 had homozygous mutation in MEFV gene, 77 had heterozygous mutation, and 77 had Wild type MEFV. In addition, expression analysis was applied to 18 individuals with homozygous mutation of the MEFV gene, 16 individuals with heterozygous mutation and 14 individuals wild type randomly selected from the groups included in the study. The study was carried out with the permission of Cumhuriyet University Clinical Research Ethics Committee (Decision No: 2011/014) and all individuals included in the study were informed in detail before the study and their written consent was obtained.

Assessment of Patients

The individuals included in the study were classified according to the presence of FMF clinic by filling in the Tel-HaShomer criteria. According to this classification, FMF clinic was detected in 120 individuals, while FMF clinic was not present in 107 individuals. The remaining two individuals had suspicious clinical status. FMF disease was excluded in MEFV Wild type individuals using Tel-HaShomer criteria. This group of individuals without MEFV mutation and FMF clinic was used as a control group to compare with individuals with homozygous and heterozygous mutation for MEFV. The groups were selected from the Central Anatolia region in accordance with age, gender and ethnicity.

Before the study, individuals were questioned in detail in terms of diseases (Behçet, SLE, RA, Inflammatory bowel diseases (crohn, ulcerative colitis)) that may affect the inflammatory process, and individuals with this type of disease were excluded from the study.

DNA Isolation

Peripheral venous blood samples in the amount of 8 ml were taken into tubes containing EDTA from all individuals included in the study. Genomic DNA was isolated from whole blood samples using the genomic DNA extraction kit (GF-1 DNA Extraction Kit, Vivantis) according to the manufacturer's protocol. The concentration and quality of the isolated DNAs

were determined by measuring with spectrophotometry (Thermo Scientific Nanodrop). DNA integrity was controlled using agarose-gel electrophoresis and then stored at +4°C.

Determination of MCP-1 (A–2518G)(rs1024611) and CCR2 (G190A)(rs1799864) Polymorphisms

Genotyping was carried out using the Polimeraz Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. Primers for the promoter region -2518 A>G variant of the MCP-1 gene were designed as Forward: 5'-CCGAGATGTTCCCAGCACAG-3'; Reverse: 5'-CTGCTTTGCTTGTGCCTCTT-3'. Primer sequences for the G>A variant at position 190 of the second exon of the CCR2 gene were designed as forward: 5'-ATTTCCCCAGTACATCCACAAC-3'; reverse: 5'-CCCACAATGGGAGAGTAATAAG-3'. PCR amplification was prepared in a total volume of 25 µL; 2.5 µl genomic DNA (50 ng/µl), 1 µl primer (10 pmol/µl), 1.5 µl dNTP, 1.5 µl MgCl2, 2.5 µL 10xPCR buffer, 0.25 µl Taq polymerase (hot start AT max), and 15.75 µL dH20.

Two-step PCR conditions were established as follows: One cycle of initial denaturation at 94°C for 7 min followed by 10 cycles of amplification and 25 cycles of (denaturation (94°C, 25 h) annealing (58°C, 30 h) and extension (72°C, 30 h)) followed by 7 minutes final extension at 72°C. The PCR conditions for the SNP at position 190 of the second Exon of the CCR2 gene were the same except for the last 3 min extension at 72°C. Samples were stored at +4°C after PCR. The amplified products were electrophoresed for 30 min on a 2% agarose gel pre-stained with 10 µg/ml ethidium bromide (Horizon 11–14, Life Technologies inc., UK) at 160 V and the 930 bp and 317 bp PCR products were visualized under an ultraviolet transilluminator.

MCP-1 (A–2518G)

The restriction endonuclease digestion was prepared using 15 µl PCR products mixed with a 10 µl solution containing 1 µl restriction enzyme, 2 µl restriction buffer and 7 µl sterile deionized H_2O. It was then incubated at 37°C for 1 hour and was cleaved. The Pvu II (New England Biolabs, Beverly, MA, USA) enzyme recognizes and cleaves the restriction site when the mcp-1 gene has the G allele at the -2518 position and it separates into 708 bp and 222 bp fragments (**Image 1**).

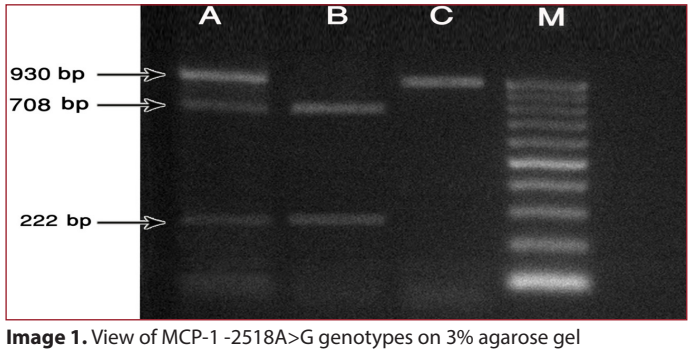

Image 1. View of MCP-1 -2518A>G genotypes on 3% agarose gel
M: 100 bp marker, A: AG genotype, B: GG genotype, C: AA genotype, bp: base pair

CCR2 (G190A)

10 µl of amplified PCR products were cleaved at 65°C for 12 hours using BsaBI (fermantas, USA) restriction endonuclease. The BsaBI recognizes and cleaves the restriction site when the CCR2 gene has the A allele at the 190 position and it separates into 197 bp and 120 bp fragments (**Image 2**). A known genotype was used during digestion to control enzyme function. Restriction products were visualized by ethidium-bromide staining using 3% agarose gel electrophoresis at 160 V for 30 min.

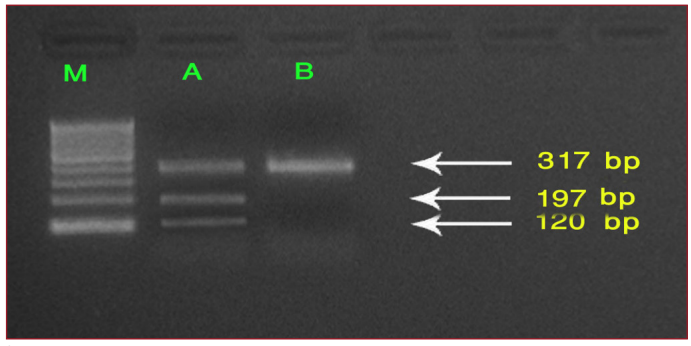

İmage 2. View of CCR2 190 G>A genotypes on 3% agarose gel
M: 100 bp marker, A: GA genotype, B: GG genotype, bp: base pair

MCP-1 Gene Expression Analysis

According to the MCP-1(–2518 A/G) polymorphism, Homozygous GG genotype was detected in only 10 of those with FMF and 4 of those without FMF. In addition, MCP-1 (–2518 A/G) GG genotype was detected in 8 individuals with homozygous mutation in MEFV gene, 2 individuals with heterozygous mutation in MEFV gene and 4 individuals with Wild type MEFV.

Besides expression analysis was applied to a total of 48 individuals, including 18 individuals with homozygous mutation of the MEFV gene, 16 individuals with heterozygous mutation and 14 individuals with wild type MEFV, selected from the groups included in the study in accordance with age, sex and MCP-1 GG genotype. While 24 of 48 individuals had FMF symptoms, 24 did not have FMF symptoms.

In order to determine the effect of MCP-1 (–2518 A/G) genotypes and allele carriage on MCP-1 gene expression, peripheral blood samples were taken from 24 people with FMF clinic and 24 people without FMF clinic, who were age and gender matched with "GG", "AG" and "AA" genotypes. These peripheral blood samples were analyzed for relative mRNA expression.

RNA Isolation and Complementary DNA (cDNA) Synthesizes

2-3 ml of peripheral blood was taken in EDTA-containing tubes from the individuals included in the study in a sterile manner. The volume of blood required to prepare leukocytes was determined by the leukocyte count of each individual. The peripheral blood taken and red blood cell lysis buffer

were mixed at a ratio of 1:2, kept for 7-10 minutes in automatic shaker (Biosan OS-20 Orbital Shaker, Lithuania) and then centrifuged for 15 seconds at 12,000 g. The white pellet and supernatants formed at the bottom were removed. Then, 400 µl of lysis binding buffer was added and was centrifuged for 15 seconds at 8000 g. Finally, Isolated leukocytes were enumerated and stored frozen at -80°C until total RNA isolation. Total RNA was extracted by using RNA Isolation kit (Roche High Pure RNA Isolation kit (Lot no:13064700)) from FMF clinic (+) and FMF clinic (-) groups, which were previously genotyped in terms of MCP-1 (–2518 A/G). The quality of the isolated RNAs was assessed using agarose gel electrophoresis. RNA concentration and purity were measured with NanoDrop and all samples showed an A260/A280 ratio >1.8. A total of 1-10 ng of RNA was translated into cDNA using a cDNA synthesis kit (Roche transcriptor first strand cDNA synthesis kit (lot no:12071632)) according to manufacturer's recommendations. Then, 20 µl of cDNA was aliquoted and stored at -80°C.

The primary efficacy of six different housekeeping genes was evaluated ("GAPDH", "28S", "18S", "RPL32", "UBB" and β- actin). According to the geNorm analysis, GAPDH was determined as the most stable and suitable endogenous gene among the six HKGs to normalize gene expressions and was used in our study.

Quantitative Real Time (qRT)-PCR

mRNA expression was determined by qRT-PCR using SYBR Green Master Mix with the Qiagen rotor gene 5 instrument. The primers used for the GAPDH and MCP-1 genes are shown in **Table 1**. RT-PCR master mix was prepared as follows: 13 µl of SYBR green master mix, 2 µl of cDNA, 2 µl for each primer and up to a total volume of 25 µl of dH20.

Table 1. Primers for housekeeping (GAPDH) and target gene (MCP-1(CCL2))

Gene	Primers	PCR Product	Optimization Temperature
G6PDH	Forward: 5'CATCAAGAAGGTGGTGAAGCAG-3' Reverse: 5'CTGTTGAAGTCAGAGGAGACCA–3'	93 bp	63°C
MCP-1 (CCL2)	Forward: 5' –AGCAGAAGTGGGTTCAGGAT-3' Reverse: 5'-GGTTGTGGAGTGAGTGTTCAAG-3	82 bp	63°C

PCR conditions were optimized as an initial denaturation at 95°C for 10 minutes followed by 45 cycles of denaturation, annealing and amplification. The specificity of the amplification was controlled by melting curve analysis and the temperature was increased from 60°C to 95°C, 1°C per cycle. The whole procedure was performed three times. Results are expressed in relative units determined based on the cycle treshould values obtained from the samples and analyzed by the ΔCt method using GAPDH as an internal control.

Statistical Analysis

Statistical analysis of the data obtained from the patient and control groups was performed using the SPSS 20.0 program. The comparison of genotype distributions and allele frequencies between the groups was made with the chi-square (X2) test. Results with $p<0.05$ were accepted as significant. In addition, quantitative real-time PCR analysis was performed using the relative quantitation method in order to determine the MCP-1 gene expression levels among the groups quantitatively and the fold increase was determined by evaluating the results using the 2-ΔΔct livak method.[16]

RESULTS

Clinical parameters

A total of 229 individuals, 125 men and 104 women, whose MEFV gene analysis was performed, were included in this study. The mean age of men was 22.91, and the mean age of women was 22.96. 120 individuals were classified as FMF (+), and 107 individuals as FMF (-) according to the FMF clinic (Tel hashomer criteria). The remaining two individuals were evaluated as suspicious in terms of FMF. These individuals included in the study were also classified as 75 homozygous mutant individuals, 77 heterozygous mutants and 77 wild type individuals according to MEFV gene analysis.

MCP1 (–2518 A>G) and CCR2 (190 G>A) polymorphisms

First of all, the relationship between FMF clinic and MCP1 (–2518 A>G) and CCR2 (190 G>A) polymorphisms and allele frequencies was evaluated in the individuals included in the study. In the evaluation, no statistically significant relationship was found for either MCP1 (–2518 A>G) polymorphism or CCR2 (190 G>A) polymorphism between individuals with and without an FMF clinic ($p > 0.05$) (**Table 2**). In addition to that, the relationship between the presence of abdominal pain and fever findings and MCP1 (–2518 A>G) and CCR2 (190 G>A) polymorphisms and allele frequencies in individuals with FMF clinic was also evaluated but a significant relationship was not found.

Table 2. MCP1 (–2518 A>G) and CCR2 (190 G>A) genotype distributions and allele frequencies in FMF patients

FMF Clinic			p value	FMF Clinic			p value
MCP-1	(+) (n:120)	(-) (n:107)		CCR2	(+) (n:120)	(-) (n:107)	
Genotype							
AA	61 (50.8%)	49 (45.8%)			1 (0.8%)	2 (1.9%)	
AG	49 (40.8%)	54 (50.5%)	0.184		20 (16.7%)	14 (13.1%)	0.610
GG	10 (8.3%)	4 (3.7%)			99 (82.5%)	91 (85%)	
Allel							
A	171 (71.3%)	152 (71%)	0.958		22 (9.2%)	18 (8.4%)	0.777
G	69 (28.7%)	62 (29%)			218 (90.8%)	196 (91.6%)	

CCR2: CC chemokine receptor 2, MCP: monocyte chemoattractant protein.

In our study, the MEFV gene was analyzed by Whole gene sequence analysis using the Sanger method. As a result of the analysis, the MEFV gene was divided into three subgroups as Homozygous Mutant (Hm Mt), Heterozygous Mutant (Ht Mt) and Wild Type (WT). Then the relationship between these groups and MCP1 (–2518 A>G) and CCR2 (190 G>A) polymorphisms was evaluated. No significant relation was found between both genotypes and allele frequencies ($p > 0.05$) (**Table 3**).

Gene expression

The relationship between the FMF clinic and the MCP1 expression profile was calculated by the livak method. As a result of the calculation, the expression of MCP-1 was found to be increased by 1.93 times in individuals with FMF clinic compared to individuals without FMF clinic (**Figure 1**).

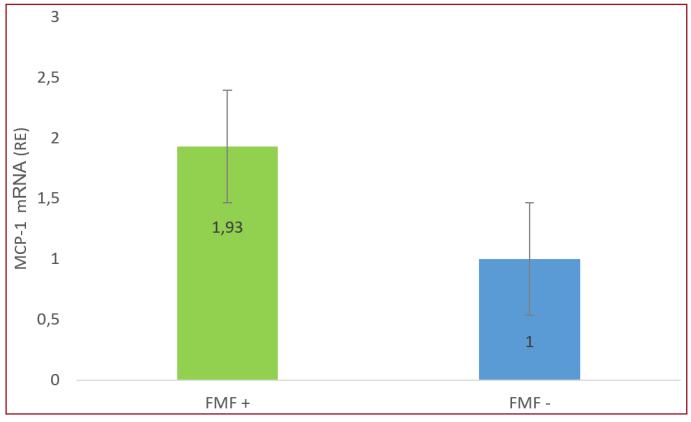

Figure 1. The relative MCP-1 mRNA expression of FMF clinic (+) and FMF clinic (-)

In addition, the relationship between MEFV genotypes and MCP1 expression profile was calculated by the livak method. While it was determined that Mcp-1 was expressed 1.25 times more in individuals with heterozygous mutant MEFV gene compared to individuals with wild type MEFV gene, Mcp-1 was expressed 1.84 times more in individuals with homozygous mutant MEFV compared to individuals with wild type MEFV gene (**Figure 2**).

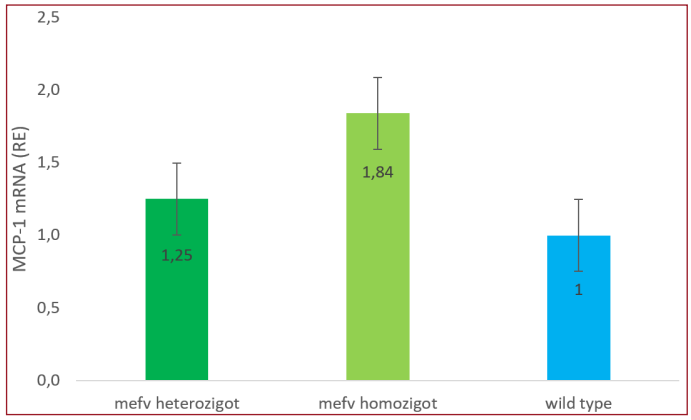

Figure 2. The relative MCP-1 mRNA expression in MEFV genotypes

The relationship between MCP-1 –2518 A>G genotypes and MCP-1 expression profile was also calculated by the livak method in our study. As a result, it was determined that MCP-1 was expressed 1.55 times more in the AG (heterozygous) genotype in the MCP-1 (–2518 A>G) promoter region compared to the AA (wild type) genotype. On the other hand, it was determined that MCP-1 was 3.07 times more expressed in the GG (homozygous mutation) genotype in the MCP-1 (–2518 A>G) promoter region compared to the AA (wild type) genotype (**Figure 3**).

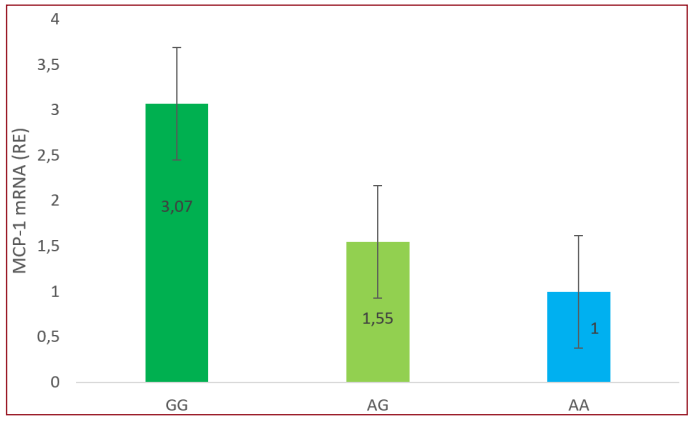

Figure 3. The relative MCP-1 mRNA expression in MCP-1 genotypes

Table 3. MCP1 (–2518 A>G) and CCR2 (190 G>A) genotype distributions and allele frequencies according to the MEFV genotypes

	MEFV Genotypes					MEFV Genotypes			
MCP-1	**HM MT (n:75)**	**HT MT (n:77)**	**WT (n:77)**	**p value**	**CCR2**	**HM MT (n:75)**	**HT MT (n:77)**	**WT (n:77)**	**p value**
Genotype									
AA	37 (49.3%)	42 (54.5%)	31 (40.3%)	0.097		0 (0%)	2 (2.6%)	1 (1.3%)	0.648
AG	30 (40%)	33 (42.9%)	42 (54.5%)			13 (17.3%)	10 (13%)	11 (14.3%)	
GG	8 (10.7%)	2 (2.6%)	4 (5.2%)			62 (82.7%)	65 (84.4%)	65 (84.4%)	
Allel									
A	104 (69.3%)	117 (76%)	104(67.5%)	0.229		13 (8.7%)	14 (9.1%)	13 (8.4%)	0.979
G	46 (30.7%)	37 (24%)	50 (32.5%)			137 (91.3%)	140 (90.9%)	141 (91.6%)	

CCR2: CC chemokine receptor 2, MCP: monocyte chemoattractant protein. HM: Homozygote HT: Heterozygote MT: Mutation WT: Wild Type

DISCUSSION

Familial Mediterranean Fever (FMF), is the most common and best known of the hereditary relapsing fever or periodic fever syndromes.[17] Although FMF is basically defined as an autosomal recessive disease, nearly 25% of patients carry only 1 MEFV mutation.[18] and 10-20% carry no mutation at all.[19] The pathogenesis in FMF patients without MEFV mutations is not clear There are certain considerations opinions about this situation. First of all, a component, which is involved in the same metabolic pathway with pyrin, has been suggested to be associated with upstream or downstream genetic defects that are not yet known.[15] Other possible explanations include; misdiagnosis of other auto-inflammatory diseases clinically similar to FMF, epigenetic changes, such as DNA methylation of the MEFV gene or histone modifications, interactions between genetic polymorphisms and modified genes, environmental factors resulting in FMF attacks and mutations in different as yet unknown genes that cause FMF disease.[15]

Besides, FMF shows a wide spectrum in terms of its clinical presentation (such as the severity of clinical findings, age of onset, frequency and severity of attacks). Monocytes/macrophages have important roles in the inflammation of FMF.[7] Although there are many factors that affect the migration of these cells to the area of inflammation, MCP-1 and its receptor CCR2 are known to exert a strong chemotactic effect on these cells.[20] Despite many studies investigating the relationship between inflammatory systemic diseases and MCP-1/CCR2.[21] no study has been found in literature that investigates the relationship between FMF disease and MCP-1/CCR2 genotype and expression. In a study of Mortensen,S.B et al., it has been suggested that CCL1 and CXCL1 chemokines are potential new biomarkers in the diagnosis of FMF and it has been claimed that the inflammatory activation of pyrin in monocytes may be a future functional diagnostic tool. In addition, an important heterogeneity in the clinical features and genotype-phenotype relationships of FMF was noted and the necessity of additional tools in the diagnosis of FMF was emphasized.[2]

In this direction, our study found no significant relationship between genotype and allele frequencies of MCP-1 -2518A>G and CCR2 190G>A genes between individuals with homozygous mutation and heterozygous mutation for MEFV gene and individuals with wild type MEFV gene. In the expression analysis, it was determined that MCP-1 expression levels were increased 1.25 times in the group carrying MEFV heterozygous mutations compared to the MEFV Wild type group, while MCP-1 expression levels were increased 1.84 times in the group carrying MEFV homozygous mutations compared to the MEFV Wild type group. These findings suggested that two different genes, whose relations with each other have not been determined yet, affect each other at the transcriptional level. In particular, the increase in expression in relation to the number of mutant alleles supports this idea. However, our data should be supported with further functional studies in order to talk about such a relationship. While no significant relationship was found between FMF clinic and MCP-1 -2518A>G and CCR2 190G>A genotypes and allele frequencies, it was observed that MCP-1 expression increased 1.93 times in patients with FMF clinic compared to the group of patients without FMF clinic. This increase was evaluated as a positive relationship between FMF and MCP-1 expression, and it has been thought that this data may be important in the pathogenesis of the disease and in the formation of clinical diversity. However, these data need to be confirmed by more comprehensive further studies.

In our study, MCP-1 -2518A>G genotypes and MCP-1 expression levels were also examined and it was determined that the expression of MCP-1 was increased 1.55 and 3.07 times in AG and GG genotypes, respectively. This increase was interpreted as the possibility of more severe development of the inflammatory process in individuals carrying the MCP-1 mutant genotype. Our observation of a greater increase in expression level as the MCP-1 (-2518A>G) G allele increases, has been interpreted as Carrying the MCP-1 (-2518A>G) GG genotype in FMF patients increased monocyte/macrophage migration to the inflammation site which results in increased MCP-1 release, exacerbating the development of inflammation. When all these findings are considered together, MCP-1 protein is thought to be associated with the pathogenesis of FMF.

This relationship may possibly be related to the role of MCP-1 protein in signaling pathways in triggering inflammatory attacks and clinical manifestations of FMF. Chemotactic factors are released from leukocytes recruited to the serosal regions during the FMF attacks and as a result of this recruitment more leukocytes are drawn to the inflammatory area and as a result, the severity of the inflammatory attack increases.[22] The higher expression of MCP-1 in individuals with MCP-1 (A-2518G) GG genotype in our study suggested that individuals with the same mutation in the MEFV gene may be responsible for the formation of different clinical manifestations. We think that the clinical findings such as fever, abdominal pain and joint pain become more severe and persist longer in patients with increased MCP-1 expression in addition to MEFV gene mutation. In addition, increased mcp-1 expression levels may be explanatory for the clinical findings seen in individuals with wild type MEFV. When the classical inheritance pattern of FMF disease is considered, individuals with heterozygous mutations in the MEFV gene are expected to be carriers. However, most of these individuals show typical symptoms of the disease in the evaluation based on Tel-HaShomer criteria and are considered as patients. In this case, considering the data we obtained from our study, it is possible that heterozygous mutation in the MEFV gene is accompanied by increased mcp-1 levels, and clinical findings of the disease occur with this common mechanism. This hypothesis should be supported by further functional studies.

In addition to monocytes/macrophages, which are the main source and main target of MCP-1 in the inflammatory process in FMF disease, another important inflammatory cell group that MCP-1 does not affect is neutrophils. We accept this as a limitation of our study and therefore, a study targeting neutrophils will provide more findings about the

inflammatory process in FMF. Another limitation of our study is the fact that FMF patients included in this study could not be selected from those in the acute attack period. If MCP-1 expression levels can be measured during the attack, higher level of expression might be encountered. The existence of such a situation may help us to better understand the increased expression levels in FMF patients. Further functional studies are needed to establish such a relationship.

With these findings, we think that MCP-1 expression is important in FMF disease, may explain the clinical differences between FMF patients, and may be an indicator in suspicious cases. Besides, it was thought that there is a relationship between MEFV mutations and MCP-1 expression, and MEFV mutations may exacerbate inflammation by increasing transcription of MCP-1. In addition, the increase in MCP-1 expressions with MCP-1(A-2518G) mutations was interpreted as contributing to FMF disease. As a result, we think that MCP-1 protein can be used as a diagnostic test in individuals with FMF, especially in unexplained situations.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Cumhuriyet University Clinical Research Ethics Committee (Decision No: 2011/014).

Informed Consent: All individuals included in the study were informed in detail before the study and their written consent was obtained

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

Financial Disclosure: This study was supported by Cumhuriyet University Scientific Research Center (Project number T-511).

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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