

Mediterranean Fever Gene Mutations and Messenger Ribonucleic Acid Expressions in Pediatric Patients With Familial Mediterranean Fever in the Trakya Region of Turkey

Hilmi TOZKIR,¹ Hakan GÜRKAN,¹ Neşe ÖZKAYIN,² Necdet SÜT³

¹Department of Medical Genetics, Medical Faculty of Trakya University, Edirne, Turkey
²Department of Pediatric Nephrology, Medical Faculty of Trakya University, Edirne, Turkey
³Department of Biostatistics, Medical Faculty of Trakya University, Edirne, Turkey

Objectives: This study aims to investigate the possible relationship between Mediterranean fever (MEFV) gene mutations and messenger ribonucleic acid (mRNA) expressions and to identify the link between phenotype and genotype of pediatric patients with Familial Mediterranean fever (FMF).

Patients and methods: Seventy-one pediatric FMF patients who were identified with FMF symptoms and diagnosed with FMF according to Tel Hashomer criteria were included at Trakya University, Faculty of Medicine, Department of Paediatric Nephrology. The control group consisted of 73 healthy pediatric participants. Genomic deoxyribonucleic acid was isolated from whole blood samples and the following mutations of MEFV gene were analyzed: E148Q, P369S, H478Y, H479L, S675N, G678E, M680L, M680I (G>A and G>C), T681I, I692del, M694V, M694L, M694I, M695R, M695M, R717S, I720M, V722M, V726A, A744S and R761H. Total RNA isolation from leukocytes was performed and MEFV mRNA expression levels of the patients were compared by using real-time quantitative polymerase chain reaction method. β 2 microglobulin was selected as the control gene. The comparison of mRNA expression levels among the patients was performed using the Δ CT method.

Results: The most common clinical findings were abdominal pain, fever and vomiting. The mutation detection rate in the patient group was OR=4.1 (95% CI: 1.8-9.0) times higher than that of the control group. The MEFV mRNA expression level of the patients with MEFV gene mutations was lower compared to the control group, indicating statistical significance.

Conclusion: Our study results support the findings of previous studies indicating that the MEFV mRNA expression levels of pediatric FMF patients with MEFV gene mutation are lower than the MEFV mRNA expression levels of healthy controls.

Key words: Familial Mediterranean fever; inflammation; messenger ribonucleic acid; mutation.

Familial Mediterranean fever (FMF, OMIM \neq 249100) is an autosomal recessive inherited auto-inflammatory disease that commonly affects people of East Mediterranean ethnic origin; primarily those of Turkish, Greek, Druze, Arab, Armenian and non-Ashkenazi Sephardi (Sephardic) Jewish descent.¹⁻⁶ Familial Mediterranean fever has also been reported with incomplete penetrance in other Mediterranean countries, such as Cyprus, Italy, Spain and Greece.⁷ The disease is characterized by recurrent and self-limiting episodes of fever, serosal inflammation of the membranes (the

synovial membrane, the peritoneum and pleura), abdominal pain attacks, arthritis, skin rashes and serum amyloid A protein deposition in kidneys unless treatment is provided.¹⁻⁶ The prevalence and the carrier rate of FMF in Turkey have been reported as 1/1,000 and 20%, respectively.^{8,9}

Mutations in the Mediterranean fever (MEFV) gene, which is mapped to chromosome 16p13.31-5, play an important role in the pathogenesis of FMF.¹⁻⁵ The MEFV gene codes for a 781 amino acid (aa) marenostriin/pyrin

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Correspondence: Hakan Gürkan, M.D. Trakya Üniversitesi Tıp Fakültesi Tıbbi Genetik Anabilim Dalı, 22030 Edirne, Turkey.
Tel: +90 284 - 235 76 42 / 2330 e-mail: dr_hakangurkan@yahoo.de

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protein, which is expressed in neutrophils, eosinophils, cytokine activated monocytes, dendritic cells, synovial, peritoneal and skin-originated fibroblasts.^{1-3,5} Marenostriin/pyrin proteins have been reported to play regulatory roles in interleukin-1 beta (IL-1 β) maturation, caspase-1 activation (which is responsible for NF- κ B activation), apoptotic mechanisms and the regulation of innate immunity leading to both pro-inflammatory and anti-inflammatory effects.^{2,3,5} Using the green fluorescent protein fusion assay method, it was shown that the full-length (781 aa) marenostriin/pyrin protein is located in cytoplasmic perinuclear region and is associated with microtubules and actin filaments via a B30.2 domain.^{1,2} A shorter form of marenostriin/pyrin is located inside the nucleus. This 570 aa form is translated from the second exon via alternative splicing and lacks the MEFV-d2 transcripts. It has been reported that the MEFV gene is regulated by nonsense-mediated decay, and different isoforms of the MEFV transcript are located at different cellular regions, with different roles in the inflammation process.²

To date, 227 FMF-associated polymorphisms/mutations (221 substitutions, 2 insertions, 2 deletions and 2 duplications) of the marenostriin/pyrin coding MEFV gene have been reported. These MEFV polymorphisms/mutations are located in the following regions of the coding gene: exon 10 (70) > exon 2 (64) > exon 5 (22) > exon 3 (21) > exon 1 (5).¹⁰ According to the results of studies investigating the relationship between MEFV mutations and messenger RNA (mRNA) expression, increasing numbers of MEFV gene mutations are correlated with decreased mRNA expression level. The mRNA expression levels of peripheral blood leukocyte (PBLs) samples from FMF patients have been reported to be lower than those of healthy controls.^{2,3}

In this study, we investigated the relationship between mRNA expression levels and MEFV gene mutations in pediatric patients whose clinical diagnosis of FMF were decisive according to the Tel-Hashomer criteria. We aimed to elucidate the relationship between FMF phenotype and genotype. Furthermore, this is the second study investigating the relationship between mRNA expression levels and MEFV mutations in Turkish pediatric patients.

PATIENTS AND METHODS

Between January 2010 and June 2012, 71 pediatric FMF patients (average age 10.68 \pm 4.4 years) were identified from the Trakya University Faculty of Medicine, Department of Pediatric Nephrology with FMF symptoms and diagnosed as FMF according to the Tel-Hashomer criteria. The healthy control group contained 73 pediatric patients (average age 9.12 \pm 4.9 years) who did not have abdominal pain, fever attacks and any kidney disease. The patients and controls were not related. Local Ethics Committee approval was given by the Trakya University Faculty of Medicine Clinical Research Ethics Committee of Non-Interventional Study Protocol on 07.08.2008 with a 16/20 decision, following inspection of the study protocol and the informed consent form. The informed consent form includes information about the study, and this form was read and signed by the parents of all pediatric patients and all pediatric control subjects.

Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood samples using an EZ1 DNA Isolation Kit (EZ1[®] DNA Blood 200 μ l Kit, Qiagen, Hilden, Germany) with automated EZ1 Advanced XL (Qiagen, Hilden, Germany). The 200 μ l whole blood sample was used for DNA isolation and final dilution was 100 μ l (~40-50 ng/ μ l DNA). Mutation analysis was performed using Pyro Sequencing FMF Primers kit (ATQ Biotech, Ankara, Turkey) with PYRO Sequencing (Qiagen, Hilden, Germany). Exons 2, 3, 5 and 10 were analyzed using this FMF kit, and codons that were examined for mutations included the following: E148Q in exon 2; P369S in exon 3; H478Y, H479L in exon 5; S675N, G678E, M680L, M680I (G>A and G>C), T681I, I692del, M694V, M694L, M694I, M695R, M695M, R717S, I720M, V722M, V726A, A744S and R761H in exon 10. Each sample was studied twice to increase the reliability of the study.

Total RNA isolation from whole leukocytes was performed using a QIAamp[®] RNA Blood Mini Kit (Qiagen, Hilden, Germany). Ribonucleic acid isolation was performed using a 1.5 ml whole blood sample and erythrocytes were lysed via elution buffer provided by a supplier. Leucocytes were recovered selectively by centrifugation according to the supplier's procedure. RNA was isolated from recovered leucocytes.

For each sample, the RNA concentration was measured with a Nanodrop 2000c Spectrophotometer (Thermo Scientific, Wilmington, USA) and diluted to 1000 ng/ μ l to get more significant quantitative results. To prevent gDNA contamination, isolated RNA samples were treated with a gDNA wipeout solution, and complementary DNA was synthesized immediately using a QuantiTect Reverse Transcription cDNA kit, according to the manufacturer's handbook instructions (Qiagen, Hilden, Germany). The cDNA concentration was also diluted to 1000 ng/ μ l.

Quantitative expression analysis was performed with a quantitative polymerase chain reaction (Q-PCR) method using the Corbett RG-6000 real-time (quantitative) PCR machine (Qiagen, Hilden, Germany). The Q-PCR method was applied using a QuantiFast SYBR Green PCR Kit (Qiagen, Hilden, Germany). β 2 microglobulin (B2M) was selected as the control gene, and thus, two reaction tubes were studied for each patient; one reaction was for the MEFV gene, and the other one was for B2M. Each reaction tube contained 12.5 μ l of SYBR Green, 2.5 μ l of forward primer for either the B2M or MEFV gene, 2.5 μ l of reverse primer for either the B2M or MEFV gene and 5 μ l of cDNA, which was diluted to 1000 ng/ μ l. The following primers were designed for the B2M gene: forward primer: 5'-TGC-CGT-GTG-AAC-CAT-GTG-AC-3'; and reverse primer: 5'-ACC-TCC-ATG-ATG-CTG-CTT-ACA-3'. The following primers were designed for exons 2 and 3 of the MEFV gene: forward primer: 5'-GGA-AAA-GAC-AGC-TGC-GAA-TC-3'; and reverse primer: 5'-TCA-ACT-GGG-TCT-CCT-TCC-TG-3'. For each sample, both MEFV gene expression analysis and B2M gene expression analysis were studied twice.

The relative expression level was calculated using the Δ CT method (Δ CT=CT of B2M-CT of MEV, and the $2^{-\Delta$ CT value corresponds to the fold change between target gene, MEFV, and the reference gene, B2M).

Statistical analysis

In this study, the suitability of the quantitative values for a normal distribution was analyzed with a single sampling Kolmogorov-Smirnov test. Normally distributed age values were analyzed with the Student's t-test, while non-normally

distributed expression level values were analyzed using a Mann-Whitney U-test. A Chi-square test was used to analyze the categorical data of patients and controls, odds ratios and 95% confidence intervals (CI) were then calculated. $P < 0.05$ was accepted as a significant statistical value. Statistical analysis was performed using the IBM SPSS Statistics 20.0 software program (IBM Corporation, Armonk, NY, USA).

RESULTS

We compared MEFV allele frequencies between FMF patients and the healthy controls, and found that 38 of the 71 patients (53.5%) had MEFV gene mutations, whereas 33 patients (46.5%) had no mutations. Although 16 controls (21.9%) carried MEFV gene mutations, no mutations were detected in the other 57 healthy controls (78.1%) ($p < 0.001$). The mutation detection rate in the patient group was OR=4.1 (95% CI: 1.8-9.0) times greater than that of the control group. The distribution of MEFV gene mutations detected in the patients and controls and the localizations of the mutations are shown in Table 1. The most frequent mutations detected in patients were E148Q heterozygous mutation (n=8) and M694V heterozygous mutation (n=8). The most frequent mutation detected in the control group was E148Q heterozygous mutation (n=7). Different from the patients, two controls were detected with a G678E heterozygous mutation and a single control was detected with an A744S heterozygous mutation in exon 10. Mutations in exon 5 were not detected neither in patients nor in controls. In healthy controls, the detected MEFV gene mutations affected only one allele (heterozygous), and these patients are carriers of MEFV gene mutations.

The MEFV mRNA expression level of the patients with MEFV gene mutations (n=38) was lower than that of the controls (n=73), these results are statistically significant [mean \pm SD 0.007645 \pm 0.01929 (median 0.00300) vs. mean \pm SD 0.00579 \pm 0.01388 (median 0.00200); $p = 0.009$]. Furthermore, the MEFV mRNA expression level of the patients with MEFV gene mutations (n=38) was also lower than that of controls with no mutations (n=57), and these results are also statistically significant [mean \pm SD

Table 1. The distribution of Mediterranean fever gene mutations detected in patients and controls

Detected mutations and the localization of the mutations	SNP numbers of the mutations detected	Type of mutations	Patients (n=71)		Controls (n=73)	
			n	%	n	%
Exon 2						
E148Q Heterozygous mutant	rs3743930	Missense	8	11.3	7	9.6
E148Q Homozygous mutant	rs3743930	Missense	2	3	-	-
Exon 3						
P369S Heterozygous mutant	rs11466023	Missense	1	1.4	1	1.4
Exon 10						
M680I (G/C) Heterozygous mutant	rs28940580	Missense	3	4.2	-	-
M694V Homozygous mutant	rs61752717	Missense	3	4.2	-	-
M694V Heterozygous mutant	rs61752717	Missense	8	11.3	-	-
K695R Heterozygous mutant	rs104895094	Missense	1	1.4	2	2.7
V726A Heterozygous mutant	rs 28940579	Missense	3	4.2	1	1.4
R761H Heterozygous mutant	rs104895097	Missense	1	1.4	2	2.7
M680I (G/C) Heterozygous mutant (+)	rs28940580	Missense	3	4.2	-	-
M694V Heterozygous mutant	rs61752717	Missense				
M694V Heterozygous mutant (+)	rs61752717	Missense	2	3	-	-
V726A Heterozygous mutant	rs 28940579	Missense				
M694V Heterozygous mutant (+)	rs61752717	Missense	1	1.4	-	-
A744S Heterozygous mutant	rs61732874	Missense				
G678E Heterozygous mutant	rs104895088	Missense	-	-	2	2.7
A744S Heterozygous mutant	rs61732874	Missense	-	-	1	1.4
P369S Heterozygous mutant (+)	rs11466023	Missense	1	1.4	-	-
K695R Heterozygous mutant	rs104895094	Missense				
E148Q Heterozygous mutant (+)	rs3743930	Missense	1	1.4	-	-
M694V Heterozygous mutant	rs61752717	Missense				

SNP: Single-nucleotide polymorphism.

0.00765±0.01929 (median 0.00300) vs. mean±SD 0.00624±0.01541 (median 0.00190); p=0.007]. In the statistical analysis of comparison between patients with no MEFV gene mutations (n=33) and controls with no MEFV gene mutations (n=57), the MEFV mRNA expression levels were not found to be significantly different (p=0.924).

We compared the MEFV mRNA expression levels between controls heterozygous for MEFV mutations (16 controls, all carriers) and controls without detected mutations (n=57), and found no statistically significant difference (p=0.554).

When we compared patients without MEFV mutations (n=33) to the patients with MEFV mutations (n=38), the MEFV mRNA expression level differences were statistically significant, but the values were borderline [mean±SD 0.007645±0.0192912 (median 0.003000) vs. mean±SD 0.003639±0.0048245 (median 0.001800); p=0.052].

Messenger RNA expression levels of patients (n=38) and healthy controls (n=16) with MEFV mutation were compared and the results were

not found to be statistically significant [mean±SD 0.00765±0.019291 (median 0.003000) vs. mean±SD 0.00393±0.004324 (median 0.00200); p=0.191].

The differences in MEFV mRNA expression were not statistically significant between patients with MEFV M694V homozygous mutations (n=3) and those with heterozygous mutations (n=8) (p=0.221). Additionally, significant differences were not detected when we compared the MEFV mRNA expression levels between patients with the E148Q and M694V heterozygous mutations (E148Q (n=8); M694V (n=8); E148Q (n=6); p=0.943).

Differences in the MEFV mRNA expression level were not significant between patients with homozygous or heterozygous E148Q mutations (n=11) and patients with homozygous or heterozygote other MEFV mutations (n=28) (p=0.553).

Seventy-one patients diagnosed as having FMF according to the Tel-Hashomer criteria were evaluated, considering 12 clinical symptoms.

Table 2. Clinical symptoms of Familial Mediterranean fever patients with/without Mediterranean fever gene mutation

Clinical symptoms	Total patients		Mutation positive patients		Mutation negative patients		p	OR	95% CI
	n	%	n	%	n	%			
Abdominal pain	55	77.5	28	73.7	27	81.8	0.413*	0.622	0.199-1.949
Fever	36	50.7	14	36.8	22	66.7	0.012*	0.292	0.110-0.776
Arthralgia	7	9.9	3	7.9	4	12.1	0.697‡	0.621	0.129-3.004
Erythema	5	7	3	7.9	2	6.1	1.000‡	1.329	0.208-8.478
Sicchasia	9	12.7	4	10.5	5	15.2	0.724‡	0.659	0.161-2.690
Vomitus	13	18.3	5	13.2	8	24.2	0.228*	0.473	0.138-1.624
Arthritis	8	11.3	6	15.8	2	6.1	0.270‡	2.906	0.544-15.513
Chronic arthritis	0	0	0	-	-	-	-	-	-
Splenomegaly	0	0	0	-	-	-	-	-	-
Hepatomegaly	0	0	0	-	-	-	-	-	-
Amyloidosis	0	0	0	-	-	-	-	-	-
Proteinuria	2	2.8	1	2.6	1	3	1.000‡	0.865	0.052-14.393

* Chi-square test; ‡ Fisher's Exact test; OR: Odds ratio; CI: Confidence interval.

The prevalence of symptoms, from the highest to the lowest, were abdominal pain (77.5%), fever (50.7%), vomiting (18.3%), nausea (12.7%), arthritis (11.3%), arthralgia (9.9%), erythema (7%) and proteinuria (2.8%). Chronic arthritis, splenomegaly, hepatomegaly and amyloidosis symptoms were not detected in the patients (Table 2).

Patients with MEFV mutation (n=38) and patients with no MEFV mutation (33 patients) were evaluated considering 12 clinical symptoms. The symptom of fever was more frequent in patients who had no MEFV mutation than in patients with MEFV mutation, and the difference

was statistically significant [p=0.0012, odds ratio=0.293, 95% CI= 0.11-0.776], (Table 2). Three patients with M694V/M694V genotype had no proteinuria and serum amyloid A protein deposition.

Patients who had MEFV mutation in exon 2 (n=10) and patients with MEFV mutation in exon 10 (n=25) were evaluated considering 12 clinical symptoms; however, the difference was not statistically significant (p>0.05), (Table 3).

The relationship between some of the MEFV gene mutations found in patients and 12 clinical findings were investigated. However, only

Table 3. Clinical symptoms and genotype correlation in Familial Mediterranean fever patients with Mediterranean fever gene exon 2 and exon 10 mutation

Clinical symptoms	Exon 2 mutation positive patients (n=10)		Exon 10 mutation positive patients (n=25)		p	OR	95% CI
	n	%	n	%			
Abdominal pain	7	70	20	80	0.661‡	1.714	0.323-9.109
Fever	4	40	8	32	0.706‡	0.706	0.155-3.224
Arthralgia	2	20	1	4	0.190‡	0.167	0.013-2.093
Erythema	1	10	1	4	0.496‡	0.375	0.021-6.652
Sicchasia	2	20	1	4	0.190‡	0.167	0.013-2.093
Vomitus	3	30	1	4	0.061‡	0.097	0.009-1.088
Arthritis	0	0	5	20	0.292‡	0.667	0.518-0.859
Chronic arthritis	0	0	0	0	-	-	-
Splenomegaly	0	0	0	0	-	-	-
Hepatomegaly	0	0	0	0	-	-	-
Amyloidosis	0	0	0	0	-	-	-
Proteinuria	1	10	0	0	0.286‡	0.265	0.151-0.464

‡ Fisher's Exact test; OR: Odds ratio; CI: Confidence interval.

Table 4. Clinical symptoms and genotype correlation in Familial Mediterranean fever patients with some Mediterranean fever gene mutations

Clinical symptoms	E148Q heterozygous mutations (n=8)		E148Q homozygous mutations (n=2)		M680I (G/C) heterozygous mutations (n=3)		M694V heterozygous mutations (n=8)		M694V homozygous mutations (n=3)		V726A heterozygous mutations (n=3)		M694V (+) M680I (G/C) heterozygous mutations (n=3)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Abdominal pain	6	75	1	100	3	100	5	62.5	1	33.3	3	100	3	100
Fever	3	37.5	1	100	2	66.6	2	25	2	66.6	0	0	1	33.3
Arthralgia	2	25	0	0	1	33.3	0	0	0	0	0	0	0	0
Erythema	0	0	1	100	0	0	1	33.3	0	0	0	0	0	0
Sicchasia	2	25	0	0	0	0	0	0	0	0	0	0	1	33.3
Vomitus	3	37.5	1	100	1	33.3	0	0	0	0	0	0	0	0
Arthritis	0	0	0	0	0	0	1	12.5	2	66.6	0	0	1	33.3
Chronic arthritis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Splenomegaly	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hepatomegaly	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amyloidosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Proteinuria	1	12.5	0	0	0	0	0	0	0	0	0	0	0	0

frequency calculation could be made since the number of the patient group was low (Table 4).

DISCUSSION

We investigated the distribution of MEFV gene mutations and mRNA expression levels in patients previously diagnosed with FMF and healthy controls living in Turkey's Trakya Area.

According to previous studies investigating the frequency of MEFV gene mutations in different geographic regions of Turkey (specifically, the Aegean, Central Anatolia, Black Sea, and South Eastern Anatolia regions), the most frequent mutation is the M694V mutation.¹¹⁻¹⁶ In our study group, the most frequent MEFV mutation was also M694V, and based on this result, we suggested that the distribution of MEFV gene mutations does not differ greatly between the different geographic areas of Turkey.

It has been reported that the approximate prevalence of FMF is 1/1000, and the rate of carriers (heterozygotes) is 1/5 in Turkey.^{8,9} According to results of our study, the rate of the carriers (heterozygotes) is 21.9%, and this result confirms that the rate of carriers heterozygous for MEFV mutations is high in the Turkish population.

Öztürk et al.¹⁶ analyzed 452 pediatric FMF patients with a mean age of 12.4±4.7 (range, 2-30 years) and indicated that the common

clinical findings were abdominal pain (86.3%), fever (81.9%), myalgia (58.8%) and arthritis/arthralgia (49%). Six (2.9%) and nine (4.3%) of 207 patients carrying two mutant alleles had amyloidosis and proteinuria respectively.

Yigit et al.¹⁴ (Black Sea region/Turkey), Paşa et al.¹³ (Southeastern region/Turkey) and Solak et al.⁴ (Aegean region/Turkey) reported that amyloidosis was more frequent in adult patients with the M694V/M694V genotype. Gürkan et al.⁵ (Trakya region/Turkey) also detected no amyloidosis in two adult patients with the M694V/M694V genotype.

In the present study, abdominal pain and fever were the most frequent symptoms. Since fever was more frequent in patients with no MEFV mutation than in patients carrying the MEFV mutation ($p=0.012$), it can be concluded that diagnosing FMF only with fever-episodes could be deceptive. Amyloidosis and proteinuria were not detected in three patients who had M694V/M694V genotype; this may have resulted from early diagnosis and colchicine treatment.

Phenotype and genotype associations were evaluated and no statistically significant relation was found. We suggest that the small number of patients ($n=71$) and tender age (10.7 ± 4.4) (this may cause the patients to express symptoms of the disease incorrectly and have mix symptoms), and also early diagnosis and colchicine treatment may prevent amyloidosis.

Kireçtepe et al.,² Üstek et al.,³ and Notarnicola et al.¹ investigated the MEFV mRNA expression levels of FMF patients and found that in those

with MEFV mutations, MEFV mRNA expression levels are significantly low. We have also confirmed that the MEFV mRNA expression levels of patients are significantly lower than that of the controls.

Kireçtepe et al.² (pediatric patients) and Üstek et al.³ compared the MEFV mRNA expression levels between FMF patients with MEFV mutations and those without mutations, and they could not detect any significant relationship. However, we found that there was a borderline statistically significant difference between FMF patients with and those without MEFV mutations. This result partly supports the hypothesis of Notarnicola et al.¹ "Inside the possible mechanisms explaining the differences observed between the study groups, MEFV mRNA stability ranging with the presence of MEFV gene mutations".

According to the study of Notarnicola et al.,¹ the MEFV mRNA expression level differences between healthy controls and healthy carriers were statistically significant ($p=0.008$), and they suggested that decreasing MEFV mRNA expression levels may lead to molecular failure, which is independent of FMF symptoms. We did not find significant differences in MEFV mRNA expression between healthy controls and healthy carriers. Notarnicola et al.¹ reported that decreased MEFV mRNA expression in monocytes is more significant than decreased MEFV mRNA expression in polymorphonuclear cells. However, in our study, we investigated the MEFV mRNA expression level only in polymorphonuclear cells. Hence, we suggest that this can explain the differences between the two studies.

Notarnicola et al.¹ reported that the MEFV mRNA expression level is related to the MEFV genotype and the FMF phenotype. M694V mutants have the lowest MEFV mRNA expression levels, while E148Q mutants have the highest MEFV mRNA expression levels. In our study, we compared the MEFV mRNA expression levels between patients homozygous for the M694V mutation ($n=3$) and patients heterozygous for the M694V mutation ($n=8$). We also compared patients and controls heterozygous for the E148Q and M694V mutations (E148Q ($n=8$), M694V ($n=8$), E148Q ($n=6$)). Unfortunately, we could not detect a relationship between the genotype and

the phenotype, unlike in the study of Notarnicola et al.¹ We suggest that this difference is because the number of patients and controls with E148Q and M694V mutations was so small.

In conclusion, this work is only the second study to investigate the relationship between MEFV mutations and the MEFV mRNA expression levels in pediatric FMF patients living in Turkey's Trakya region. Our study is different from the study of Kireçtepe et al.² because we used a higher number of patients ($n=51$) and controls ($n=21$) and the number of MEFV gene mutations investigated ($n=5$ mutation). Our study supports the findings of Kireçtepe et al.,² which revealed that the MEFV mRNA expression levels of infant FMF patients is lower than the MEFV mRNA expression levels of healthy controls.

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Declaration of conflicting interests

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