ÖZGÜN ARAŞTIRMA ORIGINAL RESEARCH

Med J SDU / SDÜ Tıp Fak Derg ▶ 2019:26(1):000-000 doi: 10.17343/sdutfd.741507

FREQUENCY AND DISTRIBUTION OF MEFV GENE MUTATION IN FAMILIAL MEDITERRANEAN FEVER PATIENTS: A SINGLE CENTER EXPERIENCE

AİLESEL AKDENİZ ATEŞİ HASTALARINDA MEFV GEN MUTASYONUNUN SIKLIĞI VE DAĞILIMI: TEK MERKEZ DENEYİMİ

Şebnem ÖZEMRİ SAĞ[1], Adem ALEMDAR[3], Lamiya ALİYEVA[1], Niyazi KAYA[1], Şehime Gülsün TEMEL[1,2,3]

[1] Bursa Uludag University, Faculty of Medicine, Department of Medical Genetics, Bursa, Turkey.
[2] Bursa Uludag University, Faculty of Medicine, Department of Histology and Embryology, Bursa, Turkey
[3] Bursa Uludag University, Institute of Health Sciences, Department of Translational Medicine, Bursa, Turkey.

ORCID IDs of the authors: Ş.Ö.S. 0000-0002-3948-8889; A.A. 0000-0003-3070-9224; L.A. 0000-0001-9436-9279; N.K. 0000-0002-5122-3824; Ş.G.T. 0000-0002-9802-0880

Cite this article as: Özemri Sağ Ş, Alemdar A, Aliyeva L, Kaya N, Temel ŞG. Frequency and Distribution of MEFV Gene Mutation in Familial Mediterranean Fever Patients: A Single Center Experience. Med J SDU 0000; 00(0): 00-00.

Öz

Amaç
Ailesel Akdeniz Ateşi (FMF) olası tanısı olan hastalarda MEFV gen mutasyon varyantlarının sıklığı ve dağılımını değerlendirmeyi amaçladık.

Gereç ve Yöntem
Eylül 2018 ve Eylül 2019 arasında FMF hedef mutasyon analizi yapılan hastalar retrospektif olarak incelendi. Yirmi altı farklı MEFV gen mutasyon varyantı incelendi. Çalışma katılımcılarının demografik ve klinik verileri hasta listelerinden ve hastane elektronik veri tabanı sisteminden toplanmıştır.

Bulgular
Refere edilen 910 hastanın 350'sinde (%38.5) FMF mutasyonu pozitif bulundu. Toplamda, MEFV geninde 41 farklı genotip ve 26 farklı mutasyon tespit ettik. En yaygın mutasyon ve genotip sırasıyla M694V ve heterozigot M694V idi. İki yüz yetmiş altı hastada (%78.9) tek bir mutasyon vardı. Yetmiş dört hastada bileşik heterozigot mutasyon vardı (%21.1). En yaygın bileşik heterozigot mutasyon P369S/R408Q (%23.3) idi. Beş kurucu mutasyon, tespit edilen tüm mutasyonların yüzde yetmiş beşini oluşturdu. Genel olarak diğer çalışmalarda incelenmeyen nadir mutasyonlar 15 hastada (%4.2) iki farklı bileşik heterozigot genotip formunda mevcuttu. Bu nadir mutasyonların toplam alel sıklığı %5 idi.

Sonuç
Bu çalışmada, MEFV mutasyonlarının genişletilmiş bir panelini inceledik ve literatürde Türk hastalarda yapılan önceki çalışmaların çoğundan daha kompleks genotipler tespit ettik.

Anahtar Kelimeler: Ailesel Akdeniz Ateşi; MEFV; Mutasyon; M694V

Abstract

Objective
We aimed to evaluate frequency and distribution MEFV gene mutation variants in patients with presumptive diagnosis of Familial Mediterranean Fever (FMF).

İletişim kurulacak yazar/Corresponding author: ozemri@uludag.edu.tr
Müracaat tarihi/Application Date: 22.05.2020 • Kabul tarihi/Accepted Date: 18.06.2020
Available online at http://dergipark.gov.tr/sdutfd
Makaleye http://dergipark.gov.tr/sdutfd web sayfasından ulaşılabilir.

Material and Methods
Patients who had undergone FMF targeted mutation analysis between September 2018 and September 2019 were retrospectively analyzed. Twenty-six distinct MEFV gene mutation variants were studied. Demographic and clinical data of study participants were collected from patient charts and hospital electronic database system.

Results
Out of 910 referred patients, 350 (38.5%) were found to have a positive FMF mutation. In total, we detected 41 different genotypes and 26 different mutations in the MEFV gene. The most common mutation and genotype were M694V and heterozygous M694V, respectively. Two hundred and seventy-six patients (78.9%) had a single mutation. Seventy-four patients had compound heterozygous mutation (21.1%). The most common compound heterozygous mutations were P369S/R408Q (23.3%). Five founder mutations constituted the seventy-five percent of the all mutations detected. Rare mutations that generally not examined in other studies were present in 15 patients (%4.2) in the form of two different compound heterozygous genotype. The total allele frequency of these rare mutations was 5%.

Conclusion
In this study, we examined an extended panel of MEFV mutations and detected more complex genotypes than most of the previous studies conducted in Turkish patients in the literature.

Keywords: Familial Mediterranean Fever; MEFV; Mutation; M694V

Introduction

Familial Mediterranean Fever (FMF) is the most common form of the autoinflammatory disorders. The disease is characterized by recurrent self-limited attacks of fever and serositis (1). Turkey has the highest prevalence of the disease reportedly (2). The general prevalence is around 0.1% but as high as 1% in some parts of the Anatolia (3). FMF is also common among other ethnic groups, including Arabs, Jews, and Armenians, among others.

FMF is inherited as autosomal recessive and caused by a mutation in the Mediterranean fever gene (MEFV) which is located on the chromosome 16 and codes pyrin protein. Pyrin takes part in the innate immune system, and the mutated form of the protein leads to an exaggerated inflammatory reaction via increased production of interleukin-1 (4). The diagnosis of FMF is based on the demonstration of two mutations within the MEFV gene in patients with suggestive clinical symptoms. However, the MEFV gene mutations cannot be detected in 10-20% of the patients who have a typical FMF clinical picture and/or response to colchicine. It has been suggested that this might be due to the mutations in upstream or downstream to MEFV associated inflammatory pathways (5).

More than 300 MEFV gene mutations have been reported to date. However, the five founder mutations, M694V, V726A, M680I, M694I, and E148Q, are responsible for approximately 80% of the total FMF cases (6). The most commonly reported genotype is homozygous M694V. Several single-center and multicenter studies have reported relative frequencies of FMF gene mutations in Turkey untill now (7-12). However, almost all of the studies that examined mutation frequency and type have used up to 12 different mutation types.

We think that knowledge about the relative frequencies of less commonly seen MEFV mutation types would facilitate genetic diagnosis efforts and reduce the percentage of patients who have clinically suggestive FMF but a negative genetic analysis. Thus, we aimed to evaluate FMF patients who were referred to our genetic center with a presumptive clinical diagnosis of FMF using genetic analysis that involved 26 MEFV gene mutation variants.

Materials and Methods

Design and Setting
This was a retrospective analysis of patients with a pre-diagnosis of familial Mediterranean fever (FMF) who were referred from various outpatient clinics including pediatrics, and rheumatology, among others. The study included patients who had undergone FMF targeted mutation analysis between September 2018 and September 2019 at the Medical Genetics Department of Uludag University Faculty of Medicine, Bursa. The study protocol was approved by the Uludag University Clinical Studies Ethical Committee (No:2020-5/14).

Data Collection
Demographic and clinical data of study participants were collected from patient charts and hospital electronic database system, including patient sex and age,

family history of FMF, history of appendectomy operation, presence of abdominal pain, recurrent fever, joint pain and swelling, and systemic AA amyloidosis.

Genetic Analysis of FMF

Peripheral venous blood samples were used for mutation analysis. The DNAs were isolated from the peripheral blood. Each area involving particular mutations (E148Q, P369S, F479L, M680I G>C/G>A, M694V, M694I, K695R, V726A, A744S, R761H, E84K, G304R, E148V, E167D, T267I, L110P, P283L, E230K/Q, G632S, R42W, R653H, R408Q, I591T, and R354W) in exons 1, 2, 3, 5, 9 and 10 of the MEFV gene was amplified via reverse transcriptase-polymerase chain reaction (RT-PCR) using special primaries with an internal control (SNP Biotechnology, FMF-26 Multiplex Real Time PCR Kit, Ankara, Türkiye). Mutation analysis of determined amplification was performed simultaneously with RT-PCR. Allele frequencies, mutation types and rates were also analyzed.

Statistical Analysis

Statistical analysis of the study data was performed with Statistical Package for Social Sciences-SPSS 23 (ABD, Chicago, Illinois, USA). Categorical variables were expressed as number and percentage. Continuous numerical variables were expressed as mean +/- standard deviation. Bar chart depicting respective frequencies of genotypes was constructed with Microsoft Excel 2019.

Results

Clinical and Demographic Characteristics

A total of 910 patients (476 (52.3%) females) who were referred to our center with a presumptive diagnosis of FMF were included in the study. The mean age of the entire group was 15.8 ± 13.4 years. Of these, 350 patients (38.5%) were found to have a positive FMF mutation. In the whole group, the most common symptom was recurrent abdominal pain in both sexes. On the other hand, the second most common symptoms were joint pain and recurrent fever in males and females, respectively. Twenty patients (2.2%) had biopsy-proven AA amyloidosis (Table 1).

Among 350 patients who had FMF mutation, 182 patients (48%) were female. The mean age of the patients was 16.9 ± 13.8 years. The most common symptom was recurrent abdominal pain in females, whereas recurrent fever in males. The second most frequent symptoms were recurrent abdominal pain and joint pain in males and females, respectively. In total, 5.4% of the cohort had biopsy-proven AA amyloidosis (Table 2).

FMF Mutation Analysis

In total, we detected 41 different genotypes and 26 different mutations in the MEFV gene. Out of 350 mutation-positive patients, 276 patients (78.9%) had a single mutation. The most prevalent mutation in the genotype analysis was heterozygous M694V (n=97, 27.7%). The second and third most commonly encountered mutations were heterozygous E148Q (16.2%) and homozygous M694V (8.8%), respectively. Overall, 12 different FMF mutations were detected as a single mutation in genotype analysis of mutation-positive patients. Mutations G632S and T267I were the least common of all, seen in only 1 patient for each. Most of the single mutations were heterozygous (83%). Most of the patients who had heterozygous mutation had M694V mutation.

Seventy-four patients had compound heterozygous mutation (21.1%). In total, we found 24 distinct sequence variations of compound heterozygous mutations. The most common compound heterozygous mutation was P369S/R408Q (23.3%). The distribution of studied mutations is shown in Table 3.

Table 1 Clinicodemographic features of the entire study population

Signs and/or symptoms	Female n (%)	Male n (%)	Total n (%)
Recurrent abdominal pain	201 (42.2%)	164 (37.8%)	365 (40.1%)
Recurrent fever	150 (31.5%)	168 (38.7%)	318 (34.9%)
Joint pain	196 (41.2%)	146 (33.6%)	342 (37.6%)
Joint swelling	59 (12.4%)	42 (9.7%)	101 (11.1%)
History of appendectomy	20 (4.2%)	27 (6.2%)	47 (5.2%)
Amyloidosis	12 (2.5%)	8 (1.8%)	20 (2.2%)
Total	476 (100%)	434 (100%)	910

Table 2 Clinicodemographic features of genetically confirmed FMF patients

Signs and/or symptoms	Female n (%)	Male n (%)	Total n (%)
Recurrent abdominal pain	71 (39%)	62 (36.9%)	133 (38%)
Recurrent fever	54 (29.7%)	66 (39.3%)	120 (34.3%)
Joint pain	67 (36.8%)	52 (31%)	119 (34%)
Joint swelling	17 (9.3%)	13 (7.7%)	30 (8.6%)
History of appendectomy	12 (6.6%)	22 (13.1%)	34 (9.7%)
Amyloidosis	11 (6%)	8 (4.8%)	19 (5.4%)
Total	182 (100%)	168 (100%)	350

Table 4 Allele Frequency of founder mutations

Mutation	Number of alleles	Frequency (%)
M694V	185	38.1%
E148Q	99	20.4%
V726A	43	8.9%
M680I	33	6.8%
M694I	6	1.2%

Overall, we studied five founder mutations in our patients. The most common of these was M694V (38.1%), E148Q (20.4%), and V726A (8.9%). Five founder mutations constituted the seventy-five percent of the all mutations detected (Table 4)

We studied rate and allele frequencies of rare mutations that generally have not been examined in the literature, including E167D, E230K, G632S, I591T, L110P, R408Q, R635H, R671H, T267I. The most common of these mutations was R408Q, which was present in 15 patients (%4.2) in two different compound heterozygous genotypes. The total allele frequency of these rare mutations was 5%.

The complex genotype rate among 350 patients with at least one MEFV mutations was 3.7%. The most common complex genotype variant was E148Q/ P369S/ R408Q triplet. We also detected a quartet complex genotype in two patients (Figure 1).

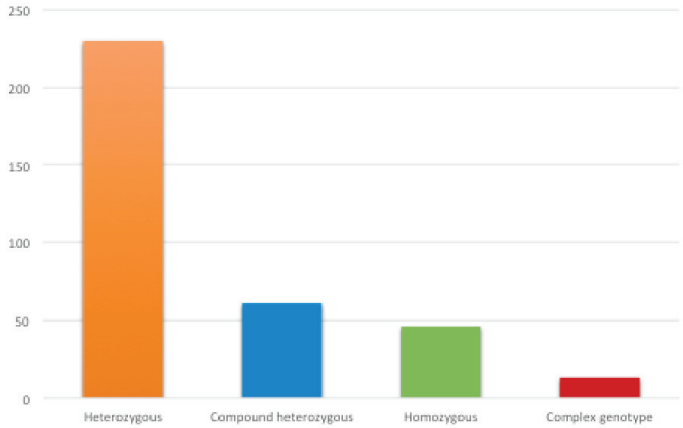

Figure 1
Bar-chart demonstrating frequency of observed genotypes

Table 3 Distribution of simple and compound FMF mutations

Genotype	Mutation type	Patient number n (%)
M694V	Heterozygous (n=97) Homozygous (n=31)	128 (46.4%)
E148Q	Heterozygous (n=57) Homozygous (n=6)	63 (22.8%)
V726A	Heterozygous (n=23) Homozygous (n=6)	29 (10.5%)
M680I	Heterozygous (n=18) Homozygous (n=2)	20 (7.3%)
R761H	Heterozygous (n=10) Homozygous (n=1)	11 (4.0%)
A744S	Heterozygous	8 (2.9%)
K695R	Heterozygous	7 (2.5%)
M694I	Heterozygous	4 (1.5%)
P369S	Heterozygous	2 (0.7%)
I591T	Heterozygous	2 (0.7%)
G632S	Heterozygous	1 (0.4%)
T267I	Heterozygous	1 (0.4%)
Total simple mutation		**276**
P369S/ R408Q	Heterozygous	14 (23%)
M694V/ E148Q	Heterozygous	10 (16.4%)
M694V/ M680I	Heterozygous	6 (9.8%)
V726A/ M694V	Heterozygous	6 (9.8%)
V726A/ M680I	Heterozygous	4 (6.6%)
M694V/ R761H	Heterozygous	3 (4.9%)
M694V/ E148Q	Heterozygous	2 (3.3%)
E148Q/ M680I	Heterozygous	2 (3.3%)
E148Q/ L110P	Heterozygous	2 (3.3%)
P369S/ R635H	Heterozygous	1 (1.6%)
P369S/ I591T	Heterozygous	1 (1.6%)
P369S/ A744S	Heterozygous	1 (1.6%)
M694V/ E230K	Heterozygous	1 (1.6%)
V726A/ R671H	Heterozygous	1 (1.6%)
P369S/ V726A	Heterozygous	1 (1.6%)
M680I/ I591T	Heterozygous	1 (1.6%)
G632S/ R635H	Heterozygous	1 (1.6 %)
E148Q/ V726A	Heterozygous	1 (1.6%)
E148Q/ R408Q	Heterozygous	1 (1.6%)
E148Q/ P369S	Heterozygous	1 ((1.6%)
E148Q/ K695R	Heterozygous	1 (1.6%)
Total compound heterozygotes		**61**
E148Q/ P369S/ R408Q	Heterozygous	10 (76.9%)
M694V/ E148Q/ P369S/ R408Q	Heterozygous	2 (15.4%)
V726A / E167D/ F479L	Heterozygous	1 (7.7%)
Complex mutations		**13**
Total		**350**

Discussion

The salient findings of this current study were as follows; (i) The most common mutation and genotype were M694V and heterozygous M694V, respectively. (ii) Seventy-four patients (21.1%) had compound heterozygous mutation. The most common compound heterozygous mutation was P369S/R408Q (23.3%). (iii) Five founder mutations constituted the seventy-five percent of all mutations detected. (iv) Rare mutations that generally not examined in other studies was present in 15 patients (%4.2) in the form of two different compound heterozygous genotype. The total allele frequency of these rare mutations was 5%.

To the best of our knowledge, this present study is among the few studies that examined rare mutations of FMF. We, in total, investigated the rate and allele frequencies of 26 different MEFV mutations. Twelve of these mutations have rarely been studied in the literature in Turkish patients. Overall, 15 patients (4.2%) have these rare mutations, and the total allele frequency was 5%. If we could not have studied these rare mutations, 15 patients would have been deemed to have one heterozygous mutation erroneously. Thus, the inclusion of these rare mutations in the routine genetic panels of FMF mutations might impact the diagnosis and prospective treatment of several patients.

It is well-known that up to 10-20% of patients who were deemed to have FMF based on the clinical findings do not have any detectible MEFV mutation. For instance, Coskun et al. (9) reported that there was no mutations in 54.3% of their study cohort. The authors only studied 12 different mutations in their patients. The negative MEFV mutation rate was also reported with other studies ranging between 9% and 61% (12-15). In our study, we found that 61.5% of the referred patients did not have the MEFV gene mutation. Although we performed a much larger MEFV gene variant mutation analysis, almost two-thirds of our patients were mutation free. Several explanations account for this; first, the refereed patients might actually not have FMF. Second, some of these patients might have much rarer mutations that we did not study or not currently defined mutations, considering the number of described mutations is now over 300. However, it is clear, when we include more mutations in our standard MEFV variant panels, these negative MEFV mutation ratios inevitably would reduce to some extent.

Several studies in recent years have evaluated the frequency of the MEFV gene mutations in various parts of Turkey (7, 8, 10, 13, 14, 16). In addition, a number of multicenter studies also evaluated the same variables in larger cohorts of FMF patients (12, 17). However, in almost all of these previous studies, 12 MEFV mutations at best were studied. Gumus et al. studied the MEFV gene with a 24 mutation panel in Turkish and Syrian refugee patients (18). In the majority of the studies, the most commonly detected mutation was M694V mutation. However, some studies reported R202Q (7), V726A (10), and R202Q (11) as the most frequent MEFV mutation in their respective cohorts. The M694V mutation was also the most common mutation in our study.

According to the ethnic buildup of the examined area, the genotype of MEFV mutations also shows variation. Yasar Bilge and colleagues (12), in their multicenter study, reported homozygous M694V genotype as the most common, whereas Yildirim et al. found that heterozygous M69V was the most commonly encountered genotype (8). We found that heterozygous MEFV was the most commonly seen genotype.

Complex MEFV genotype means the presence of more than 2 different mutations in the MEFV gene simultaneously. Most of these genotypes involve 3 mutations, while very rarely four mutations can be seen. Complex genotype rates were reported less than 1.5% of all detected genotypes in several Turkish studies (8, 15, 19, 20). Predictably, these studies used more narrow genetic panels consisting of fewer mutations. One study from southeastern Turkey reported the frequency of complex genotype as being 5.1%. The authors used a larger panel that contained more mutations similar to the one that we used in our study (18). Our results showed that 3.7% of the patients had a complex genotype. So, it is plausible to speculate that the more different number of MEFV mutations you use, the more complex genotypes you detect.

Some limitations of this present study should be mentioned. First, since the retrospective nature of the study and patients were referred from various clinics, we did not know how many of the patients fulfilled the FMF diagnostic criteria. Second, we conducted a retrospective single-center study whose results are difficult to generalize to other regions of Turkey. Lastly, we did not evaluate phenotype-genotype association since we did not make sure the referral notes about the patients were complete and comprehensive.

Despite these limitations, we conducted a relatively large single-center study. More importantly, we examined more MEFV mutations more than most of the previous older studies in the literature. Notably,

we detected a greater number of complex genotypes than most previous studies had done.

Conclusion

In conclusion, the most common mutation and genotype were M694V and heterozygous M694V, respectively. Five founder mutations constituted the seventy-five percent of all mutations detected. Rare mutations that generally not examined in other studies was present in 15 patients (%4.2) in the form of two different compound heterozygous genotype. The total allele frequency of these rare mutations was 5%. The complex genotype rate was more than most previous studies reported.

References

1. Alghamdi M. Familial Mediterranean fever, review of the literature. Clin Rheumatol. 2017;36(8):1707-13.
2. Tunca M, Akar S, Onen F, Ozdogan H, Kasapcopur O, Yalcinkaya F, et al. Familial Mediterranean fever (FMF) in Turkey: results of a nationwide multicenter study. Medicine (Baltimore). 2005;84(1):1-11.
3. Kisacik B, Yildirim B, Tasliyurt T, Ozyurt H, Ozyurt B, Yuce S, et al. Increased frequency of familial Mediterranean fever in northern Turkey: a population-based study. Rheumatol Int. 2009;29(11):1307-9.
4. Ozen S, Batu ED. The myths we believed in familial Mediterranean fever: what have we learned in the past years? Semin Immunopathol. 2015;37(4):363-9.
5. Ben-Zvi I, Herskovizh C, Kukuy O, Kassel Y, Grossman C, Livneh A. Familial Mediterranean fever without MEFV mutations: a case-control study. Orphanet J Rare Dis. 2015;10:34.
6. Sari I, Birlik M, Kasifoglu T. Familial Mediterranean fever: An updated review. Eur J Rheumatol. 2014;1(1):21-33.
7. Celep G, Durmaz ZH, Erdogan Y, Akpinar S, Kaya SA, Guckan R. The Spectrum of MEFV Gene Mutations and Genotypes in the Middle Northern Region of Turkey. Eurasian J Med. 2019;51(3):252-6.
8. Yildirim ME, Kurtulgan HK, Ozdemir O, Kilicgun H, Aydemir DS, Baser B, et al. Prevalence of MEFV gene mutations in a large cohort of patients with suspected familial Mediterranean fever in Central Anatolia. Ann Saudi Med. 2019;39(6):382-7.
9. Coskun S, Kurtgoz S, Keskin E, Sonmez F, Bozkurt G. Frequency of mutations in Mediterranean fever gene, with gender and genotype-phenotype correlations in a Turkish population. J Genet. 2015;94(4):629-35.
10. Korkmaz DT, Atak PG, Celik C. Frequencies of the Common MEFV Gene Mutations in Adiyaman, Southeast Anatolia, Turkey. Balkan J Med Genet. 2014;17(2):67-71.
11. Gunesacar R, Celik MM, Arica V, Elmacioglu S, Ozturk OH. Frequency of MEFV gene mutations in Hatay province, Mediterranean region of Turkey and report of a novel missense mutation (I247V). Gene. 2014;546(2):195-9.
12. Yasar Bilge S, Sari I, Solmaz D, Senel S, Emmungil H, Kilic L, et al. The distribution of MEFV mutations in Turkish FMF patients: multicenter study representing results of Anatolia. Turk J Med Sci. 2019;49(2):472-7.
13. Akin H, Onay H, Turker E, Cogulu O, Ozkinay F. MEFV mutations in patients with Familial Mediterranean Fever from the Aegean region of Turkey. Mol Biol Rep. 2010;37(1):93-8.
14. Dundar M, Emirogullari EF, Kiraz A, Taheri S, Baskol M. Common Familial Mediterranean Fever gene mutations in a Turkish cohort. Mol Biol Rep. 2011;38(8):5065-9.
15. Oztuzcu S, Ulasli M, Ergun S, Igci YZ, Igci M, Bayraktar R, et al. Screening of common and novel familial mediterranean fever mutations in south-east part of Turkey. Mol Biol Rep. 2014;41(4):2601-7.
16. Dogan HO, Koca Y, Erden G, Karaaslan Y, Bozat H. Evaluating MEFV mutation frequency in Turkish familial Mediterranean fever suspected patients and gender correlation: a retrospective study. Mol Biol Rep. 2012;39(5):6193-6.
17. Kasifoglu T, Bilge SY, Sari I, Solmaz D, Senel S, Emmungil H, et al. Amyloidosis and its related factors in Turkish patients with familial Mediterranean fever: a multicentre study. Rheumatology (Oxford). 2014;53(4):741-5.
18. Gumus E. The Frequency of MEFV Gene Mutations and Genotypes in Sanliurfa Province, South-Eastern Region of Turkey, after the Syrian Civil War by Using Next Generation Sequencing and Report of a Novel Exon 4 Mutation (I423T). J Clin Med. 2018;7(5).
19. Yilmaz G, Senes M, Kayalp D, Yucel D. Is Turkish MEFV Mutations Spectrum Different Among Regions? J Clin Lab Anal. 2016;30(5):641-4.
20. Cekin N, Akyurek ME, Pinarbasi E, Ozen F. MEFV mutations and their relation to major clinical symptoms of Familial Mediterranean Fever. Gene. 2017;626:9-13.