MEFV Gene Mutations in a Sample of Turkish Population: A Retrospective Study

Türk Toplumundan Bir Örneklemde MEFV Gen Mutasyonları: Retrospektif Bir Çalışma

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ABSTRACT Objective: Familial Mediterranean fever (FMF) is a hereditary inflammatory disease characterized by recurrent attacks of fever and serositis. FMF is caused by mutations in the protein of MEFV gene (marenostrin). So far 152 mutations and polymorphisms have been identified. The five most common mutations are M694V, M680I, M694I, E148Q, and V726A, most of which are clustered on exon 10 and these mutations account for 74% FMF mutations among Jews, Turks, Arabs and Armenians. In this retrospective study, we aimed to investigate the spectrum of 12 MEFV mutations and genotypes among Turkish FMF patients referred from Medical Genetics Department at Ankara Ataturk Education and Training Hospital located in the central region of Turkey. **Material and Methods:** We performed reverse hybridization (RH) method for detecting MEFV gene mutations. **Results:** We detected different genotypes in 288 (54%) of 532 cases by RH. Forty-six of the cases were homozygote for one mutation, 155 were heterozygote, 87 were compound heterozygote for two different mutations. No mutation was detected in the remaining 244 (46%) cases. The most frequent mutations were M694V, followed by E148Q, M680I(G/C) and V726A. **Conclusion:** Since FMF is an important disease because of its complications, genetic tests must be performed for the patients with FMF criteria and clinicians have to be more careful about this disease.

Key Words: Familial mediterranean fever; marenostrin; mutation

ÖZET Amaç: Ailevi Akdeniz ateşi (FMF, familial Mediterranean fever) tekrarlayıcı ateş ve serozit atakları ile karakterize herediter, inflamatuar bir hastalıktır. FMF'e MEFV geni proteinindeki (marenostrin) mutasyonlar neden olmaktadır. Günümüze dek 152 mutasyon ve polimorfizm tanımlanmıştır. En sık gözlenen mutasyonlar çoğunlukla exon 10'da toplanmış olan ve Yahudiler, Türkler, Araplar ve Ermenilerdeki FMF mutasyonlarının %74'ünden sorumlu olan M694V, M680I, M694I, E148Q ve V726A'dır. Retrospektif olan bu çalışmada Ankara Atatürk Eğitim ve Araştırma Hastanesi Tıbbi Genetik Ana Bilim Dalı'na sevk edilen Türk FMF hastalarında 12 MEFV mutasyonu ve genotiplerinin spektrumunu araştırmak amaçlandı. **Gereç ve Yöntemler:** MEFV gen mutasyonlarını belirlemek için revers hibridizasyon (RH) metodunu kullandık. **Bulgular:** RH yöntemiyle 532 olgunun 288 (%56)'inde değişik genotipler saptandı. Kırk altı olgu bir mutasyon için homozigot, 155'i heterozigot, 87'si iki farklı mutasyon yönünden değişken heterozigottu. Geri kalan 244 (%46) olguda hiç mutasyon saptanmadı. En sık gözlenen mutasyon M694V ve daha seyrek olarak da azalan sırayla E148Q, M680I (G/C), V726A idi. **Sonuç:** Komplikasyonları nedeniyle FMF önemli bir hastalık olduğundan genetik tesler FMF kriterlerinin değerlendirilmesine ek olarak yapılmalıdır ve klinisyenler bu hastalık ile ilgili olarak dikkatli olmalıdırlar.

Anahtar Kelimeler: Ailesel akdeniz ateşi; marenostrin; mutasyon

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Familial Mediterranean fever (FMF) is a hereditary inflammatory disease characterized by recurrent attacks of fever and serositis. It affects more than 100000 patients worldwide. The disease is transmitted in

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an autosomal recessive pattern and predominantly affects people from the Mediterranean region, including Sephardic Jews, Turks, Arabs, and Armenians.[1] The carrier frequency is as high as 1/5 among Turkish and North African Jewish populations and 1/7 among Armenians. It decreases to 1/11 among Ashkenazi Jews. It affects both sexes in a similar ratio, although some studies report a male predominance. Familial Mediterranean fever is characterized by recurrent febrile episodes of sterile peritonitis, pleuritis, and arthritis. Other areas less frequently affected are the skin, the pericardium, and the tunica vaginalis. Most patients (90%) experience their first attack before 20 years of age.[1] FMF attacks unfold suddenly, persist for only a short time (6-96 h), then subside spontaneously.[1,2]

FMF is caused by mutations in MEFV gene. The identification of this gene and its protein called pyrin (or marenostrin) in 1997 have led to further significant progress in FMF investigations.[1] The responsible gene, *MEFV*, has been mapped to chromosome 16p13.3. It consists of 10 exons and encodes a protein of 781 amino acids called pyrin or marenostrin that is expressed mainly in granulocytes and is thought to be a negative regulator of inflammation.[3] The function of pyrin has been identified as a cytoplasmic protein. This is involved in regulation of inflammation, apoptosis and/or cytokine secretion. Abdominal pain is the most frequent symptom in FMF; 50% of the patients cite such an 'abdominal attack' as the first symptom of their disease.[4,5] Homozygosis (i.e. the possession of two alleles with identical mutations of a particular gene) is a common finding when autosomal recessive disorders occur in consanguineous families or inbred populations. In most cases, both parents inherited the same mutation from a common ancestor and passed it on to the affected child. Compound heterozygosis is the possession of two alleles with different mutations of a particular gene.[6] In this retrospective study, we aimed to investigate the spectrum of 12 MEFV mutations and genotypes among Turkish FMF patients referred from Medical Genetics department at Ankara Ataturk Education and Training Hospital located in the central region of Turkey.

MATERIAL AND METHODS

In this study, a total of 532 patients were investigated over a period of three years (October 2005-March 2008). The patients were from the central region of Turkey. All patients were asked to read and sign the informed consent form prior to the genetic testing and ethics committee approval has been obtained from this hospital. DNA was extracted from peripheral blood (with EDTA) using Invisorb® Spin Blood Mini Kit (Invitek Gesellschaft für Biotechnik & Biodesign mbH Berlin, Germany). Twelve FMF mutations (E148Q, P369S, F479L, M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S, R761H) were analyzed by reserve hybridization (RH) assay (FMF StripAssay, Viennalab, Vienna, Austria) kit according to the manufacturer's instruction. For each patient, a multiplex polymerase chain reaction (PCR) was performed to amplify exons 2, 3, 5, and 10 of MEFV gene consisting of denaturation for 2 min at 94ºC followed by 35 cycles of denaturation at 94ºC for 15 s, annealing at 58ºC for 30 s, extension at 72ºC for 30 s, and a final extension step at 72ºC for 15 s. Visualization of amplified PCR products on a 2% agarose gel revealed 206, 236, 295, and 308 bp bands. PCR products were hybridized with test strips in the profiblot T48 (Tecan, Grödig, Austria) system with an appropriate program. The sensitivity of this method in FMF diagnosis is nearly 85%.

In this study, a packet program called STATA is used for data analysis. Descriptive statistics and t-test were used for the analysis. For the t-test, 0.05 was accepted as α significance level.

RESULTS

We detected different genotypes in 288 (54%) of the 532 cases by RH. Genotypes and the number of patients (homozygote, heterozygote and compound heterozygote) are shown at Table 1. Forty-six of the cases were homozygote of one mutation, 155 were heterozygote, 87 were compound heterozygote of two different mutations. No mutations were detected in the remaining 244 (46%) cases. The most frequent mutation was M694V, followed by E148Q, M680I(G/C), V726A, and these findings are in concordance with the literature. Table 2 shows the al-

TABLE 1: MEFV mutations detected by reverse hybridization.

MEFV genotypes	Number of patients N (%)
M694V/M694V	33 (6.2)
M680I(G/C)/M680I(G/C)	09 (1.6)
V726A/V726A	03 (0.5)
F479L/F479L	01 (0.1)
M694V/Wt	72 (13.5)
E148Q/Wt	27 (5.0)
V726/Wt	22 (4.1)
M680I(G/C)/Wt	12 (2.2)
P369S/Wt	06 (1.1)
K695R/Wt	05 (0.9)
M680I(G/A) /Wt	05 (0.9)
R761H/Wt	02 (0.3)
A744S/Wt	02 (0.3)
F479L /Wt	02 (0.3)
E148Q/M694V	18 (3.3)
M694V/V726A	18 (3.3)
M680I(G/C)/M694V	15 (2.8)
V726A/M680I(G/C)	07 (1.3)
M694V/R761H	05 (0.9)
M680I(G/C)/K695R	04 (0.7)
M680I(G/A)/M694V	03 (0.5)
E148Q/V726A	03 (0.5)
E148Q/M680I(G/C)	03 (0.5)
M680I(G/C)/ R761H	02 (0.3)
M680I(G/A)/A744S	01 (0.1)
V726A/F479L	01 (0.1)
M694V/ F479L	01 (0.1)
V726A/P369S	01 (0.1)
A744S/M694V	01 (0.1)
E148Q/R761H	01 (0.1)
E148Q/P369S	01 (0.1)
M694V/K695R	01 (0.1)
M694V/P369S	01 (0.1)
Mutation undetected	244 (45.8)
Total	**532**

lele frequencies of the most frequently seen mutations. Forty two percent of the patients were men, 58% were women. The mutation rate was 54%. The ratio of the women who had mutation to all of the patients was 29%, while the ratio of the men who had mutation to all of the patients was 25%. The most frequently seen mutation type was heterozygosis. The results of the statistical analysis are shown in Table 3. The age of the male patients with the same symptoms was between 5 and 75. The mean age was 31 and the consistence was seen in the ages 17 and 44. The age of the female patients with the same symptoms was between 3 and 72. The mean age was 31 and the consistence was seen in the ages 17 and 45 (Table 4). The difference between the mean ages of women and men with the same symptoms can be seen in Table 5. The mutation rate in the patients with the same symptoms was 54% (Table 6). The mean age of the patients with FMF mutations and with no mutations disregarding the sex was 30 and 31. The consistence was determined in the ages 17 and 46. There was no difference between the mean ages of the patients with and without MEFV gene mutations (Table 7). The mean age of the patients with MEFV gene mutations was 30. The number of the patients with homozygote mutations (mutation 1) was 46 and the mean age in this group was 30. The number of the patients with heterozygote mutations (mutation 2) was 155 and the mean age in this group was 30. The number of the patients with compound heterozygote mutations (mutation 3) was 87 and the mean age in this group was 32 (Table 8). Because of this is a retrospective study, the results had not been confirmed by any other method. In future, we intend to compare the results by another method, for example by DNA sequencing.

DISCUSSION

FMF is the most frequent of all hereditary periodic fever syndromes, Owing to the absence of specific biological tests, diagnosis of FMF has long been exclusively based on phenotypic grounds and a set of

TABLE 2: The allele frequencies of the most frequently seen mutations.

Mutation	Allele frequency
M694V	0.19(201/1062)
M680I (G/C)	0.06 (61/1062)
E148Q	0.05(53/1062)
V726A	0.05 (56/1062)
P369S	0.008 (9/1062)
Wt	0.54 (573/1062)

Wt: Wild type.

TABLE 3: Frequencies of the mutation types and sexes.

	Mutation n (%)				Healthy	Total
	1	2	3	Total		
Men	25 (5)	63 (12)	45 (8)	133 (25)	92 (17)	225 (42)
Women	21 (4)	92 (17)	42 (8)	155 (29)	152 (29)	307 (58)
Total	**46 (9)**	**155 (29)**	**87 (16)**	**288 (54)**	**244 (46)**	**532 (100)**

Mutation 1: Homozygote mutations.

Mutation 2: Heterozygote mutations.

Mutation 3: Compound heterozygote mutations.

TABLE 4: Descriptive statistics of the study sample.

Variable	N	Mean Age	Min	Max	Std. Err.	Std. Dev.	[95% Conf. Interval]	
Male	225	30.55111	5,00	75.00	.9201628	13.80244	28.73783	32.36439
Female	307	30.81433	3,00	72.00	.8219696	14.40207	29.1969	32.43176
Combined	532	30.70301	3,00	75.00	.6130039	14.13901	29.4988	31.90722
Diff		-.2632211			1.241953		-2.702977	2.176534

Diff= mean (male)-mean (female).

currently widely used clinical criteria was proposed in Israel in 1997. The identification in the same year of MEFV, the gene responsible for FMF, allowed the development of a new diagnostic tool.[7] So far, 152 mutations and polymorphisms have been identified. The five most common mutations are M694V, M680I, M694I, E148Q, and V726A, most of which are clustered on exon 10, and these mutations account for 74% FMF chromosomes among Jews, Turks, Arabs and Armenians.[3] The identification of the FMF gene and its various mutations provide a rational basis for medical and genetic counseling for clinical treatment of FMF patients and their families. The prevalence of this disease in Turkey is approximately 0.1%, but it is estimated that the prevalence of the disease may be higher because many patients remain undiagnosed, particularly for mild forms of the disease. Furthermore, in Turkey, especially in Central Anatolia where consanguinity is widespread, FMF prevalence may be higher than observed. Most studies report that FMF affects both genders in a similar ratio. Recent Turkish FMF Study Group records (including both adults and children) document a 1.2:1 (male: female) ratio.[8]

TABLE 5: Two-sample t test results for the means of age in both genders.

diff= mean(male) - mean(female)		t= -0.2119
Ho: diff= 0	degrees of freedom= 530	
Ha: diff< 0	Ha: diff != 0	Ha: diff > 0
Pr (T< t)= 0.4161	Pr (|T|> |t|) = 0.8322	Pr (T> t)= 0.5839

The most serious complication of disease is amyloidosis, caused by tissue deposition of serum amyloid A, most notably in kidneys that ultimately leads to end-stage renal failure.[9]

The type of mutation has been suggested to establish the severity of the disease. Several studies

TABLE 6: Descriptive statistics of the mutation groups.

Variable	N	Mean age	Min	Max	Std. Err.	Std. Dev.	[95% Conf. Interval]	
Mutation	288	30.44792	3,00	72,00	.8147405	13.8266	28.84429	32.05154
No mutation	244	31.0041	3,00	75,00	.9296676	14.52187	29.17286	32.83533
Combined	532	30.70301			.6130039	14.13901	29.4988	31.90722
Diff		-.5561817			1.231146		-2.974706	1.862342

Diff= mean (male)-mean (female).

TABLE 7: Two-sample t test results for the means of mutation groups.

Diff = mean (mutation) _mean (nonmutation)		t= -0.4518
Ho: diff = 0	degrees of freedom= 530	
Ha: diff < 0	Ha: diff =!= 0	Ha: diff > 0
Pr (T < t) = 0.3258	Pr (|T| > |t|) = 0.6516	Pr(T > t) = 0.6742

TABLE 8: Descriptive statistics of the patients with MEFV gene mutations

Factor	Mean Age	Std. Dev.	Freq. (N)
Mut1	30.4	14.600576	46
Mut2	29.8	14.14691	155
Mut3	31.6	12.879689	87
Total	30.4	13.826604	288

have reported that the mutations in codon 694 were associated with severe disease with early onset, high frequency of attacks, the necessity of a high dose of colchicine to control attacks and high frequent occurrence of amyloidosis in untreated patients.[1] Two major studies from Turkey find no association between the development of amyloidosis and M694V homozygosis, but according to study of Düşünsel et al., M694V homozygote genotype clearly forms a predisposition to development of amyloidosis.[8] The associations of M694V homozygosis with a more severe form of the disease and with a higher frequency of amyloidosis is reported although genotype-phenotype correlation is not well established. Nevertheless, in several other studies, the association of M694V mutation seems not prevalent among FMF patients, especially in Turks. A recently reported multicenter Turkish study reveals that the most common genotype is M694V homozygosis, which is associated with an earlier onset and higher frequencies of arthritis and arthralgia but not erysipelas- like erythema and amyloidosis.[10]

The clinical picture varies greatly in FMF. A wide variation in the severity of FMF has been noted in different patients.[11] This variable clinical presentation of the disease has been attributed to the influence of environmental factors, background genes, and different mutations in the disease-causing gene. Generally, there is no strong correlation between genotype and the clinical picture.[12] To understand the correlation between genotypic and phenotypic FMF variants, the existence of complex alleles, modifier loci, genetic heterogeneity, and possible epigenetic factors should be studied extensively in families like those described by using expression profiles.[13]

Recent investigations focused on exploring the pathogenetic pathways and demonstrating the role of pyrin in the inflammatory process. Furthermore, investigators are trying to define the genotype–phenotype correlations and the risk factors for the development of secondary amyloidosis.[1] In several studies, the M694V mutation has been reported to be the most common among the non-Ashkenazi Jews and Turks. In a recent study by the Turkish FMF Study Group, genetic analysis of 1090 patients has confirmed that M694V is the most frequent mutation followed by M680I, and V726A in Turkish patients.[1,3,4,14] These five mutations are located in exon 10 whereas E148Q is located in exon 2. FMF phenotypes vary according to MEFV mutations. Mutations on codon 680 and 694 are associated with severe phenotypes whereas E148Q is the mildest and least penetrable.[14] Although M694V homozygosis is proposed to be an important risk factor for development of amyloidosis, there are amyloidosis patients with mutations other than M694V. Interestingly, previous two major Turkish studies had demonstrated no association between the development of amyloidosis and M694V homozygosity.[1] As FMF is a clinically heterogeneous disease, the underlying genetic cause is important. After MEFV gene was cloned, several methods like PCR-RFLP, ARMS, and DGGE have been used in mutation detection in FMF patients.[15,16] In a study which is held in Turkey, PCR-RFLP and RH results of the patients are compared and RH is found as a recent method used in FMF mutation screening.[16] Briefly, the method involves multiplex PCR amplification of target regions on DNA. Biotin-labeled PCR products are hybridized to oligonucleotide probes on a nylon membrane colored enzymatically afterwards.[15,16] As an advantage of RH, in addition to the frequent mutations, seldom mutations

can be screened at the same time. In different populations, the frequencies of these mutations are reported between 0-5%.[17-21] Thus, RH adds a 5% rise to the mutation ratios of FMF patients. This 5% adds an important percentage to this clinically heterogeneous group. RH kits need to be evaluated about rare FMF mutations in Turkish population in a larger group of patients. The results also need to be confirmed by DNA sequence analyses. No discrepancy has been detected between RH and PCR-RFLP in FMF patient group. RH can be an appropriate method in the first level screening of FMF mutations. Another important point is that the mutation profile of the kit can be modified according to the literature regarding the target population, as rare mutations can be slightly different in each country.[22] In a study by Yiğit et al. in the Black Sea region of Turkey, three different MEFV mutation analysis methods, ARMS (amplification refractory mutation system), PCR-RFLP and RH methods are compared to each other.[23] According to this study, RH method seems a reliable and time-saving method. This study also shows that patients having M694V/M694V carry a risk for amyloidosis.[23] In a study by Delague et al., 100 Lebanese FMF patients were analysed for FMF by using RH and DNA sequencing, and RH method identified 144/149 mutations, and correctly typed all 12 different MEFV mutations covered.[24] Because FMF is autosomal recessive, double mutations can cause the disease. In Mediterranean and Middle Eastern populations, the common genotypes of FMF are M694V/M694V and M694V/V726A.[25] In another study which is held among Jews, Armenians, Arabs, Turks and Iranian Azeri Turks by Esmaeili et al., common mutations of FMF are rare in Arabs.[3] The frequency of E148Q mutation is higher in Arabs than in other Mediterranean ethnic groups and is similar to that observed in European ethnic groups.[3] R761H mutations are quite rare among other FMF mutations. However R761H mutations are mainly together with M694V mutations in the Turkish patients.[26] In our study, we detected a high rate (%0.9) of M694V/ R761H compound heterozygosis compared to other populations. We did not detect E148Q homozigosis. However, M694V homozygosis and heterozygosis were the most frequently seen mutations in Turkish population. In Europe, homozygous M694V is reported to be associated with a higher prevalence of renal amyloidosis or arthritis. In Japan, no case with the same MEFV genotype has been reported, but cases with homozygous M694I are associated with secondary amyloidosis, nodular erythema, and arthritis.[27] According to a Tunisian study, the most common mutation in their population is M680I. The M6801 mutation is frequent in Armenians and Turks but less common in Arabic populations and non-Ashkenazi Jews. M694V is the second most common mutation in the Tunisian population, V726A mutation seems to be rare in the Tunisian population while it can be seen as the second most common mutation in Arabs.[28] Until the identification of the MEFV gene, FMF was a diagnosis of exclusion based on clinical grounds. The diagnostic value of molecular analysis of MEFV has been mainly investigated in selected populations that carry the full clinical criteria or come from classically affected ethnic groups, with genetic confirmation rates ranging from 55% to 89%.[7] Only one MEFV mutation may indeed be conferring a heightened inflammation. The carrier status for MEFV mutations seems to be unique, in that they cause an alteration in the state of "health". In a Turkish study, the presence of febrile episodes more than four per year, arthralgia, past diagnosis for acute rheumatic fever, rheumatoid arthritis and prophylaxis of penicillin, acute rheumatic fever, and rheumatoid arthritis are significantly higher in asymptomatic parents for the MEFV mutations compared to controls.[29]

This study is thought to be helpful for clinicians for indicating the most frequently seen FMF mutations in Turkish population. Despite the current knowledge regarding FMF, prospective clinical studies with large numbers of patients and different ethnic groups will help to clarify this subject in this important disease.

REFERENCES

1. Onen F. Familial Mediterranean fever. Rheumatol Int 2006;26(6):489-96.
2. Zonios D, Mavragani CP, Giaglis S, Ritis K. A novel mutation of the familial Mediterranean fever gene in a Greek family related to a non-classical, variably expressed FMF phenotype. Rheumatol Int 2007;28(2):167-9.
3. Esmaeili M, Bonyadi M, Rafeey M, Sakha K, Somi MH. Common MEFV mutation analysis in Iranian Azeri Turkish patients with familial Mediterranean fever. Semin Arthritis Rheum 2008;37(5):334-8.
4. Simon A, van der Meer JW, Drenth JP. Familial Mediterranean fever--a not so unusual cause of abdominal pain. Best Pract Res Clin Gastroenterol 2005;19(2):199-213.
5. Kuloğlu Z, Ozçakar ZB, Kirsaçlioğlu C, Yüksel S, Kansu A, Girgin N, et al. Is there an association between familial Mediterranean fever and celiac disease? Clin Rheumatol 2008;27(9):1135-9.
6. Seidel H, Steinlein OK. Compound heterozygosity for three common MEFV mutations in a highly consanguineous family with familial Mediterranean fever. Eur J Pediatr 2008;167(7):827-8.
7. Federici L, Rittore-Domingo C, Koné-Paut I, Jorgensen C, Rodière M, Le Quellec A, et al. A decision tree for genetic diagnosis of hereditary periodic fever in unselected patients. Ann Rheum Dis 2006;65(11):1427-32.
8. Duşunsel R, Dursun I, Gündüz Z, Poyrazoğlu MH, Gürgöze MK, Dundar M. Genotype-phenotype correlation in children with familial Mediterranean fever in a Turkish population. Pediatr Int 2008;50(2):208-12.
9. Fragouli E, Eliopoulos E, Petraki E, Sidiropoulos P, Aksentijevich I, Galanakis E, et al. Familial Mediterranean Fever in Crete: a genetic and structural biological approach in a population of 'intermediate risk'. Clin Genet 2008;73(2):152-9.
10. Turkcapar N, Tuncali T, Kutlay S, Burhan BY, Kinikli G, Erturk S, et al. The contribution of genotypes at the MICA gene triplet repeat polymorphisms and MEFV mutations to amyloidosis and course of the disease in the patients with familial Mediterranean fever. Rheumatol Int 2007;27(6):545-51.
11. Gershoni-Baruch R, Shinawi M, Shamaly H, Katsinetz L, Brik R. Familial Mediterranean fever: the segregation of four different mutations in 13 individuals from one inbred family: genotype-phenotype correlation and intrafamilial variability. Am J Med Genet 2002;109 (3):198-201.
12. Koné Paut I, Dubuc M, Sportouch J, Minodier P, Garnier JM, Touitou I. Phenotype-genotype correlation in 91 patients with familial Mediterranean fever reveals a high frequency of cutaneomucous features. Rheumatology 2000; 39(11):1275-9.
13. Chalevelakis G, Apostolakis I, Koliou X, Apessos A, Kyriakopoulou V, Vrakidou E, et al. Different intrafamilial clinical presentation of FMF mutation carriers. Genet Test 2008;12(1):125-7.
14. Gunel-Ozcan A, Sayın DB, Mısırlıoğlu ED, Guliter S, Yakaryilmaz F, Ensari C. The spectrum of FMF mutations and genotypes in the referrals to molecular genetic laboratory at Kirikkale University in Turkey. Mol Biol Rep 2009;36(4):757-60.
15. Tchernitchko D, Legendre M, Delahaye A, Cazeneuve C, Niel F, Goossens M, et al. Clinical evaluation of a reverse hybridization assay for the molecular detection of twelve MEFV gene mutations. Clin Chem 2003;49(11): 1942-5.
16. Gunesacar R, Kasap H, Erken E, Ozer HT. Comparison of amplification refractory mutation system and polymerase chain reaction-restriction fragment length polymorphism techniques used for the investigation of MEFV gene exon 10 point mutations in familial Mediterranean fever patients living in Cukurova region (Turkey). Genet Test 2005; 9(3):220-5.
17. Sarkisian T, Ajrapetyan H, Shahsuvaryan G. Molecular study of FMF patients in Armenia. Curr Drug Targets Inflamm Allergy 2005; 4 (1): 113-6.
18. Giaglis S, Papadopoulos V, Kambas K, Doumas M, Tsironidou V, Rafail S, et al. MEFV alterations and population genetics analysis in a large cohort of Greek patients with familial Mediterranean fever. Clin Genet 2007;71(5):458-67.
19. Dodé C, Pêcheux C, Cazeneuve C, Cattan D, Dervichian M, Goossens M, et al. Mutations in the MEFV gene in a large series of patients with a clinical diagnosis of familial Mediterranean fever. Am J Med Genet 2000;92 (4): 241-6.
20. Medlej-Hashim M, Rawashdeh M, Chouery E, Mansour I, Delague V, Lefranc G, et al. Genetic screening of fourteen mutations in Jordanian familial Mediterranean fever patients. Hum Mutat 2000;15(4):384.
21. Lachmann HJ, Sengül B, Yavuzşen TU, Booth DR, Booth SE, Bybee A, et al. Clinical and subclinical inflammation in patients with familial Mediterranean fever and in heterozygous carriers of MEFV mutations. Rheumatology 2006;45(6):746-50.
22. Sahin FI, Yilmaz Z, Yurtcu E, Baskin E. Comparison of the results of PCR-RFLP and reverse hybridization methods used in molecular diagnosis of FMF. Genet Test 2008;12 (1): 171-4.
23. Yigit S, Bagci H, Ozkaya O, Ozdamar K, Cengiz K, Akpolat T. MEFV mutations in patients with familial Mediterranean fever in the Black Sea region of Turkey: Samsun experience. J Rheumatol 2008;35(1):106-13.
24. Delague V, Kriegshäuser G, Oberkanins C, Mégarbané A. Reverse hybridization vs. DNA sequencing in the molecular diagnosis of familial Mediterranean fever. Genet Test 2004; 8(1):65-8.
25. Sugiura T, Kawaguchi Y, Fujikawa S, Hirano Y, Igarashi T, Kawamoto M, et al. Familial Mediterranean fever in three Japanese patients, and a comparison of the frequency of MEFV gene mutations in Japanese and Mediterranean populations. Mod Rheumatol 2008;18(1):57-9.
26. Demirkaya E, Tunca Y, Gok F, Ozen S, Gul D. A very frequent mutation and remarkable association of R761H with M694V mutations in Turkish familial Mediterranean fever patients. Clin Rheumatol 2008; 27(6): 729-32.
27. Kim S, Ikusaka M, Mikasa G, Basugi A, Ohira Y, Nishizawa S, et al. Clinical study of 7 cases of familial Mediterranean fever with MEFV gene mutation. Intern Med 2007;46(5):221-5.
28. Chaabouni HB, Ksantini M, M'rad R, Kharrat M, Chaabouni M, Maazoul F, et al. MEFV mutations in Tunisian patients suffering from familial Mediterranean fever. Semin Arthritis Rheum 2007;36(6):397-401.
29. Kalyoncu M, Acar BC, Cakar N, Bakkaloglu A, Ozturk S, Dereli E, et al. Are carriers for MEFV mutations "healthy"? Clin Exp Rheumatol 2006;24(5 Suppl 42):120-2.