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Original article

Investigation of MEFV gene polymorphisms (G138G and A165A) in adult patients with familial Mediterranean fever

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ABSTRACT

Aim: Various mutations have been identified in the Mediterranean fever (MEFV) gene which is reported to be responsible from Familial Mediterranean fever (FMF). In our study, we aimed to determine the frequency of the MEFV mutations in our region and to investigate the impact of G138G (rs224224, c.414A>G) and A165A (rs224223, c.495C>A) gene polymorphisms on the clinical findings of the disease.

Methods: One hundred and sixteen patients diagnosed with FMF and 95 control subjects were included in this study. We used the DNA sequence analysis method to identify the most prevailing 10 mutations located in exon 2 and 10 of MEFV gene.

Results: As a result of the MEFV mutation analysis, the most common mutation was the M694V mutation allele with a frequency rate of 41.8%. When the patients group and control group were compared in terms of frequency of both polymorphic alleles (G polymorphic allele, observed in G138G and the A polymorphic allele, observed in A165A), the variation was observed to be statistically significant ($p < 0.001$). It was found that the MEFV mutation types have no relation with clinical findings and amyloidosis ($p > 0.05$).

Conclusions: To our knowledge, our study is the first study in the Southern Marmara region that reports the frequency of MEFV mutations. Our findings imply that the polymorphisms of G138G and A165A may have an impact on progress of the disease. We think that more studies, having higher number of cases and investigating the polymorphisms of MEFV gene, are needed.

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Investigação de polimorfismos no gene MEFV (G138G e A165A) em pacientes adultos com febre mediterrânica familiar

Palavras-chave:
Febre mediterrânica familiar
Gene MEFV
Polimorfismo

RESUMO

Objetivo: Identificaram-se várias mutações no gene da febre mediterrânica (MEFV) que relata-se que são responsáveis pela febre mediterrânica familiar (FMF). Este estudo teve como objetivo determinar a frequência de mutações no MEFV na região sul do mar de Mármara e investigar o impacto dos polimorfismos genéticos G138G (rs224224, c.414A>G) e A165A (rs224223, c.495C>A) nos achados clínicos da doença.

Métodos: Foram incluídos neste estudo 116 pacientes com diagnóstico de FMF e 95 indivíduos no grupo controle. Utilizou-se o método de análise da sequência de DNA para identificar as 10 mutações mais prevalentes localizadas nos éxons 2 e 10 do gene MEFV.

Resultados: Como resultado da análise da mutação MEFV, a mutação mais comum foi a mutação alélica M694V, com uma taxa de frequência de 41,8%. Quando os grupos de pacientes e controles foram comparados em termos de frequência de ambos os alelos polimórficos (alelo polimórfico G, observado no G138G e o alelo polimórfico A, observado no A165A), a variação observada foi estatisticamente significativa ($p < 0{,}001$). Verificou-se que os tipos de mutação no MEFV não tinham relação com os achados clínicos nem com a amiloidose ($p > 0{,}05$).

Conclusões: Que se tem conhecimento, este estudo é o primeiro realizado na região sul do mar de Mármara que relata a frequência de mutações no MEFV. Os achados indicam que os polimorfismos G138G e A165A podem ter um impacto sobre o progresso da doença. Acredita-se que são necessários mais estudos, abrangendo um maior número de casos e investigando os polimorfismos do gene MEFV.

Introduction

Familial Mediterranean fever (FMF) is an autosomal recessively inherited inflammatory disease that affects people from the Mediterranean region, including Turks, Armenians, non-Ashkenazi Jews and Arabs. It is characterized by recurrent episodes of peritonitis, fever, rashes, arthritis and other serosal inflammations.[1–3] Although attacks are self-limited, in some patients FMF causes AA type amyloidosis that leads to renal failure. Amyloidosis commonly causes other organ damages besides renal failure. The prognosis is determined by the complication of AA amyloidosis. The inflammatory episodes and renal amyloidosis can be prevented by colchicine therapy.[2,4–6]

FMF is caused by mutations in MEFV gene. This gene is located on the short arm of chromosome 16 and consists of 10 exons. The protein, encoded by this gene and termed pyrin/marenostrin, is present almost exclusively in neutrophils and their precursors. This protein is involved in regulation of inflammation, apoptosis and/or cytokine secretion.[4,7,8] The most common four mutations, M694V, V726A, M680I, and M694I are found in the exon 10 of the gene. Another mutation E148Q is found in the exon 2. These five mutations are found in more than two thirds of cases.[2,9] A number of studies have shown that the M964V mutation is associated with severe disease characterized with early onset, high attack frequency, need for high doses of colchicine, and high frequency of amyloidosis in untreated patients. E148Q is found in populations in which FMF is rare. E148Q mutation is also common but its role in the disease is controversial. Most FMF experts refer to it as a mutation causing mild disease.[2,11–13] Many polymorphisms like D102D (rs224225, c.306T>C), G138G (rs224224, c.414A>G), A165A (rs224223, c.495C>A), R202Q (rs224222, c.605G>A) are found within the exon 2 of MEFV gene. D102D, G138G and A165A polymorphisms are synonymous variant. In a study, Basarslan et al.[14] reported that allele frequencies of G138G and A165A polymorphisms were found to be higher in patients with FMF. In another study, Akar et al.[15] claimed that 138Gly allele carriers were more prone to the development of amyloidosis and added that there was no statically significant difference in terms of G138G polymorphism between patients and healthy subjects.[14,15]

In the present study, we aimed to determine the frequency of MEFV mutation in the Southern Marmara region and to investigate the impact of G138G and A165A gene polymorphisms on the clinical findings of the disease.

Materials and methods

Study subjects

We retrospectively reviewed the medical records of patients with FMF followed in the department of Internal Medicine at Uludag University Faculty of Medicine, Bursa, Turkey.

The patient group was created by selecting the adults in whom MEFV gene mutation analysis was studied because of presumptive diagnosis of FMF. The patient group consisted of 116 subjects in whom FMF diagnosis was verified clinically (based on Tel-Hashomer criteria) and mutation was identified

in MEFV gene. The control group consisted of 95 subjects in whom the FMF was excluded clinically and no mutation has been identified in MEFV gene. Demographic data were recorded for both patients and control group. The study was approved by the Uludag University Ethics Committee.

DNA isolation and analysis of MEFV gene (exons 2 and 10)

Blood samples were obtained from patient and control groups and collected in EDTA tubes. Genomic DNA was extracted from whole blood using different DNA isolation kit according to the manufacturer's instructions (Dr. Zeydanlı Life Sciences, Ltd., Turkey; RTA Laboratuvarları Biyolojik Ürünler Ilaç ve Makine San. Tic. A.S., Gebze, Kocaeli, Turkey).

The DNA fragments containing two exons of the MEFV gene (exons 2 and 10) were amplified by polymerase chain reaction (PCR) using the specific primers. PCR samples were analyzed by direct sequencing of exons 2 and 10 of the MEFV gene in an ABI-3130 DNA analyzer (Applied Bio systems, USA). After Sequencing, the data were analyzed using DNA sequencing analysis v 5.2 software for most frequent mutations (E148Q, M694V, M680I, V726A, K695R, M694I, R761M, A744S) and polymorphisms of exon 2 (G138G and A165A).

Statistical analysis

Ages were represented as median (minimum–maximum) values and were compared between groups using the Mann–Whitney *U* test. In the comparison of categorical variables, Pearson's chi-square, Yates chi-square test, and Fisher's exact test were used. All statistical analyses were performed with SPSS 13.0 (Chicago, IL). Statistical significance was set at $p < 0.05$.

Results

The study consisted of 116 adults with FMF cases (61 female, 55 male) and 95 control subjects (51 female, 44 male). There was no significant difference in gender or age between the groups ($p = 0.9$ and $p = 0.4$, respectively). The demographic and clinical features of FMF patients are shown in Table 1. In addition, 10 patients (8.6%) had chronic kidney disease and 15 (12.7%) had proteinuria.

The frequency of the five the most common MEFV mutations in this study are shown in Table 2.

The distribution of genotypes of MEFV gene (homozygote, heterozygote, and compound heterozygote) is shown in Table 3. The distribution of genotypes (G138G and A165A) was significantly different between the groups of FMF patients and controls ($p < 0.001$, Table 4). For the G138G gene polymorphism, the frequency of the G allele was 68% in FMF patient group and 46% in control group (Fig. 1). For the A165A gene polymorphism, the frequency of the A allele was 67% in FMF patient group and 45% in control group (Fig. 2). The allelic frequencies of both polymorphisms were statistically significant different between the groups ($p < 0.001$).

There was no significant relation between none of the mutations nor the polymorphisms (G138G and A165A) and clinical findings like fever, abdominal pain, arthritis/arthralgia, erysipelas-like erythema, chronic kidney disease, proteinuria, or amyloidosis ($p > 0.05$ for all).

Table 1 – Clinical characteristics of the FMF patient group and control group.

	FMF patient group $n = 116$ (%)	Control group $n = 95$ (%)	*p*-Value
Gender (female/male)	61 (52.6)/55 (47.4)	51 (53.7)/44 (46.3)	0.9
Age (years)[a]	30 (18–79)	30 (18–71)	0.4
Abdominal pain	114 (98.3)	–	–
Fever	127 (74.1)	–	–
Arthritis/arthralgia	46 (33.6)	–	–
Erysipelas-like erythema	14 (12.1)	–	–
Chest pain	14 (12.1)	–	–
Amyloidosis	7 (6)	–	–

[a] Median (minimum–maximum).

Table 2 – The allele frequencies of the most frequently seen mutations.

Mutation	Allele frequency[a]
M694V	41.8% (97/232)
M680I(G/C)	14.6% (34/232)
V726A	6% (14/232)
K695R	3.4% (8/232)
E148Q	2.1% (5/232)

[a] Total allele = 232.

Discussion

In the present study, we evaluated the frequencies of the MEFV gene mutations, the allele frequency of G138G and A165A

Table 3 – The distribution of MEFV mutations.

MEFV mutations	Number of patients	%
M694V/N	38	32.7
M694V/M694V	20	17.2
M680I(G/C)/N	11	9.4
M694V/M680I(G/C)	10	8.6
V726A/N	7	6
K695R/N	6	5.4
M680I(G/C)/M680I(G/C)	5	4.3
M694V/V726A	5	4.3
E148Q/N	4	3.4
M694V/M694I	2	1.7
M680I(G/C)/V726A	2	1.7
M694V/E148Q	1	0.9
M694V/R761M	1	0.9
M680I(G/C)/R761M	1	0.9
K695R/K695R	1	0.9
A744S/N	1	0.9
R761M/N	1	0.9
Total	116	100

N, normal.

Table 4 – MEFV genotype distribution among FMF group and control group.

	FMF patient group $n=116$	Control group $n=95$	p-Value
G138G gene polymorphism (rs224224, c.414A>G)			
A/A genotype	12 (10.30)	30 (65.70)	<0.001
A/G genotype	50 (43.10)	43 (31.30)	
G/G genotype	54 (46.60)	22 (3)	
A165A gene polymorphism (rs224223, c.495C>A)			
C/C genotype	12 (10.30)	30 (31.60)	<0.001
C/A genotype	53 (45.70)	44 (46.30)	
A/A genotype	51 (44)	21 (22.10)	

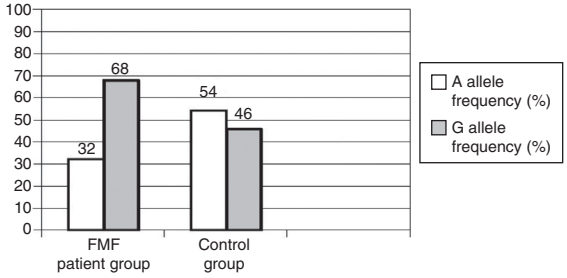

Fig. 1 – Distribution of the allele frequencies of MEFV gene polymorphisms G138G gene polymorphism (rs224224, c.414A>G) in the FMF patient and control groups. The white bars represent the frequency of the A allele, and the gray bars represent the frequency of the G allele in both groups.

polymorphisms and clinical manifestations of the disease in 116 adult FMF patients living in Bursa localized in the Southern Marmara region of Turkey. We found significantly higher frequency of G allele of G138G and A allele of A165A polymorphisms. However, we could not find a significant association between these polymorphisms and clinical features in these patients.

The estimated prevalence of the disease in Turkey is 0.1%. However, because many patients are undiagnosed, it is thought that the prevalence may be higher. A disease-specific laboratory test for the diagnosis of FMF is not yet available. Today, the diagnosis is provided on the basis of patient's

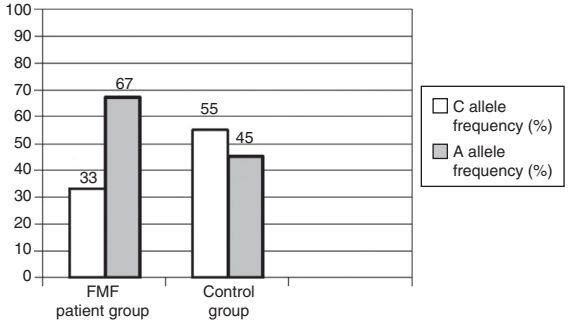

Fig. 2 – Distribution of the allele frequencies of MEFV gene polymorphisms A138A gene polymorphism (rs224223, c.495C>A) in the FMF patient and control groups. The white bars represent the frequency of the C allele, and the gray bars represent the frequency of the A allele in both groups.

clinical features such as clinical findings, ethnicity, family history and colchicine response, etc.[10,16]

By Turkish FMF Study Group, clinical findings in patients older than 18 years are listed as follows; abdominal pain (93.7%), fever (92.5%), arthritis (27.1%) and erysipelas-like rash (10.4%). In another study by Kasifoglu et al., clinical findings were listed as abdominal pain (94.6%), fever (91.9%), arthritis (39.8%) and erysipelas-like rash (23.7%).[17,18] Clinical signs/symptoms other than fever are observed in similar rates in the studies. Because of regular use of colchicine and taking nonsteroidal anti-inflammatory drug with the onset of abdominal pain, frequency of fever may be determined relatively lower.

Despite lack of information in terms of overall mutation frequency in studies conducted in our country, the mutation frequencies of different regions were reported in studies from those regions. Turkish FMF Study Group assessed many patients and healthy people in 2005. According to this report, genetic analysis was performed in 1090 patients and most common mutations were detected in to the following rates, M694V 51.4%, M680I 14.4% and V726A 8.6%.[17] Based on the study conducted by Turkish FMF Study Group, it is considered that the genetic diagnosis of FMF can be made by determination of these three mutations in the majority of patients (about 74%).

In studies conducted in various regions of Turkey, similar results to the rates specified by Turkish FMF Study Group have been reported. Gunesacar et al.[19] reported that the most common mutations in 90 patients around Çukurova region were M694V (51.66%), M680I (17.22%), V726A (10.55%), and M694I (1.66%). In a study made by Akar et al.[20] in Central Anatolia Region, mutation frequencies of M694V, M680I, V726A, and M694I were determined as 43.5%, 12%, 11.1% and 2.8%, respectively. In another study from Erzurum, Ertekin et al.[21] determined M694V (51.3%) as the most common type of mutation. It was followed by M680 (7.3%), V726A (4.9%), E148Q (4.9%) and R761H (2.4%), respectively. According to a study made by Yilmaz et al.[22] around Ankara, the frequencies of mutations in patients were found as follows; 51.55% M694V, 9.22% M680I, 8.8% V726A, 3.55% E148Q, and 0.44% M694I. In recent studies, the mutation frequencies of M694V, M680I, V726A were determined as 33.7%, 15.5%, 5%, respectively by Yigit et al.[23] in Black Sea region; the mutation frequencies of M694V, E148Q, V726A, and M680I were determined as 47.6%, 16.75%, 12.95%, 11.94%, respectively by Akin et al.[2] in the Aegean region. In another recent study from Ankara, Doğan et al.[3] reported the mutation frequencies as follows: 42.05% M694V, 19.27% E148Q, and 16.27% M680I. In our study, mutation frequencies are similar to the results of Turkish FMF Study Group.

FMF is a clinical diagnosis, which can be supported but not excluded by genetic testing. The authors recommended the use of genetic testing only in atypical cases when there are doubts about the clinical diagnosis.[24] Type of mutation is thought to be useful to know for confirmation of diagnosis and determining the management of complications that may occur based on the types of mutation. FMF patients carrying two of the common mutated alleles (homozygotes or compound heterozygotes), especially for M694V mutation or mutations at position 680–694 on exon 10, must be considered at risk of having a more severe disease.[24] Patients

homozygous for M694V mutation are at risk of early onset disease and developing complication of amyloidosis.[18] It is also well known that amyloidosis is a risk for renal failure. It is reported that the initiation of colchicine significantly reduced complications in patients clinically diagnosed at an early stage.[25,26]

In a report of Turkish FMF Study group, joint complaints were significantly more frequent in M694V/M694V homozygous patients while there was no statically significant difference in correlation analysis between mutations and clinical findings such as fever, abdominal pain, and amyloidosis.[17]

In our study, it is was observed that there was no statically significant relation between mutation types and frequencies of chronic kidney disease, amyloidosis, proteinuria, and symptoms like fever, abdominal pain, arthritis, arthralgia, erysipelas-like erythema. To our knowledge, our study is important because of being first study to report the frequency of MEFV mutations in the Southern Marmara region.

Failure to set out exactly the genotype–phenotype relationship with the known classical mutations, has attracted attention to the polymorphisms of which clinical significance is not well-known. In literature, the relationship between polymorphisms of G138G (rs224224), A165A (rs224223), R202Q (rs224222) in exon 2 of MEFV gene and disease and its complications is reported in a small number of publications.[14,15,27]

In a study from our country, Öztürk et al. reported that carriage of R202Q polymorphism did not have any effects in terms of FMF disease, but also it was stated that the mutations in exon 10 of this gene affected the symptoms of the disease.[27]

In a study by Akar et al.,[15] 124 patients with FMF and 81 healthy controls were included and Ala138Gly polymorphism was investigated. Between patient and control groups, there was no statically significant difference in terms of and Ala138Gly polymorphism. However, when 47 patients with amyloidosis were compared with other patients in whom amyloidosis was not detected, significant difference was observed in terms of Ala138Gly polymorphism and polymorphic allele of 138Gly in patients with FMF was reported to be associated with amyloidosis. In the study by Basarslan et al.,[14] the frequencies of polymorphic alleles of G138G (rs224224) and A165A (rs224223) in patients were higher than the control group.

In our study, significant difference was detected in terms of G138G and A165A polymorphisms in comparison of the patients and healthy subjects. In addition, in our study, unlike the other studies, relationship between G138G and A165A polymorphisms and clinical features such as fever, abdominal pain, arthritis/arthralgia, erysipelas-like erythema, chronic renal disease, proteinuria, and amyloidosis was investigated. However, there was no statically significant association. Small number of patients and patients' ages (all of them were over 18) in our group may have affected our results.

In this study there was significant differences between the patient and control groups in terms of G138G and A165A polymorphisms. These findings need to be evaluated in further studies with larger number of cases and follow-up data to determine the relationship between phenotype and the implications for the development of disease complications clearly.

Among the limitations of our study are the cross sectional design, the relatively small sample size, and small number of patients with amyloidosis (7 patients), and exclusion of patients under 18 years old. We believe that further studies with larger sample sizes and longitudinal design are needed to elucidate the exact role of mutations and polymorphisms in the clinical context and the prognosis of FMF patients.

In conclusion, to our knowledge, our study is the first report of the South Marmara region determining frequencies of MEFV mutations, and the frequencies of MEFV mutations in our region were observed at similar rates as in the literature. Besides, polymorphic alleles of G138G and A165A were observed in a higher frequency in FMF patients compared with controls. However, we did not observe any association between these mutations or polymorphisms in these patients.

Conflicts of interest

The authors declare no conflicts of interest.

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