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The spectrum of *MEFV* gene mutations and genotypes in Van province, the eastern region of Turkey, and report of a novel mutation (R361T)

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ABSTRACT

Familial Mediterranean fever (FMF) is the most common hereditary inflammatory periodic disease, characterized by recurrent episodes of fever and abdominal pain, synovitis, and pleuritis. The aim of this study was to determine the frequency and distribution of *Mediterranean fever* (*MEFV*) gene mutations in Van province of Eastern Anatolia and to compare them with the other studies from various regions of Turkey. Therefore, we retrospectively evaluated *MEFV* gene mutations in 1058 pediatric patients with suspected FMF. The *MEFV* gene mutations were investigated using Sanger sequencing and the multiplex minisequencing technique. We identified 37 different genotypes and 16 different mutations. The four most common mutations and allelic frequencies were M694V (36.50%), E148Q (32.77%), V726A (14.09%), and M694I (4.41%). M694V was the most common mutation, and the M694I frequency was found to be higher compared to studies from other regions of Turkey. In addition, we identified a novel missense mutation (R361T, c.1082G>C) in exon 3 of the *MEFV* gene in a 12-year-old boy, who had a typical FMF phenotype. In conclusion, this study evaluated the distribution of *MEFV* gene mutations in children with FMF as the first study conducted in Van province, Eastern Anatolia.

1. Introduction

Familial Mediterranean fever (FMF) is one of the most common autosomal recessively inherited inflammatory diseases, characterized by recurrent attacks of fever and abdominal pain, rashes, and arthritis. The most serious complication of FMF is renal failure secondary to renal amyloidosis (Ben-Chetrit and Touitou, 2011). The disease is primarily observed in eastern Mediterranean populations and is particularly prevalent among Turks, Armenians, non-Ashkenazi Jews, and Arabs (Touitou, 2001). The estimated prevalence of FMF is 1/1000, and the carrier rate is 1/5 in Turkey (Tunca et al., 2005). The disease onset is usually in the first decade of life in 60% of patients and under 20 years in 90% of patients (Berkun et al., 2011). Therefore, FMF is usually diagnosed in childhood.

The *Mediterranean fever* (*MEFV*) gene, responsible for FMF disease due to mutations, is located on chromosome 16p13.3. The gene consists of 10 exons and encodes a pyrin protein (also called marenostrin) that regulates neutrophil activity (Consortium, 1997). To date, about 299 mutations associated with FMF have been identified (Touitou et al., 2004). The four missense mutations in exon 10 (M694V, M680I, M694I, V726A) account for 85% of *MEFV* gene mutations in geographic areas where FMF is common (Consortium, 1997; Touitou, 2001). Recently, *MEFV* mutation frequency studies conducted in various regions of Turkey have been reported, but there was no report from Van province in Eastern Anatolia. One of the aims of this study is to contribute to Turkish *MEFV* mutation spectrum data by adding a larger regional study.

In the present retrospective study, 1058 unrelated pediatric patients with FMF were evaluated to analyze the mutations of the *MEFV* gene over a period of three years. We aimed to determine the frequency and spectrum of *MEFV* gene mutations as a first time, in Van province of East Anatolia, and to compare them with *MEFV* mutation frequencies of other geographic regions of Turkey and the country overall.

2. Subjects and methods

In this study, molecular test results for 1058 children (498 boys, 560 girls), who were referred to the genetic laboratory for *MEFV* mutations

Abbreviations: FMF, familial Mediterranean fever; *MEFV*, *Mediterranean fever gene*; SD, standard deviation; wt, wild type; LD, linkage disequilibrium.

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between January 2011 and May 2014 were evaluated. All patients were from Van province in the Eastern Anatolia region. The study was approved by the local Ethical Committee. The data were given as means plus/minus standard deviations (SD) and frequencies.

Genomic DNA was extracted from peripheral blood samples using the DNA isolation kit according to the manufacturer's instructions (Qiagen, Germany). Two hundred fifty-three patients were tested for *MEFV* gene mutations with Sanger sequencing of exons 2, 3, 5 and 10 (Applied Biosystems, BigDye Terminator v3.1). Another 805 patients were tested with a mutation panel for the most common *MEFV* mutations (M694V, V726A, E148Q, P369S, R202Q, F479L, M694L, M694I, M680I, R761H, K695R, K695M, A744S, and 1692del) with the multiplex minisequencing technique (Applied Biosystems, SNaPshot Multiplex, USA).

3. Results

Among the 1058 patients, 560 (52.9%) were girls, and 498 (47.1%) were boys. The mean age of the patients was 8.26 ± 3.55 years. No mutations were found in 605 patients (57.2%). At least one mutation was found in 453 (42.8%) patients. Thirty seven distinct genotypes and 16 distinct mutations were detected in these patients. Out of the 453 patients, 321 (70.86%) had heterozygous mutations, 73 (16.11%) had compound heterozygous mutations, 56 (12.36%) had homozygous mutations, and 3 patients (0.66%) carried three or more mutations, which was named the complex genotype (Table 1).

The most common mutation was M694V with a frequency of 36.5%. In about 90% of our cohort, five mutations were found: M694V (36.50%), E148Q (32.77%), V726A (14.09%), M694I (4.41%) and R761H (4.07%) (Table 2). In addition, eight silent polymorphic variants including G138G, A165A, and D102D (exon 2); R314R (exon 3); E474E, Q476Q, and D105D (exon 5); and P706P (exon 10) were detected in the patients. On the other hand, we identified R202Q variation in 452 patients (42.6%). None of the patients had the 1692del mutation.

We identified a novel missense mutation (R361T) in the *MEFV* gene in a 12 year-old boy and his 18 year-old sister. He had had recurrent episodes of fever, abdominal pain, and arthralgia over the previous two years. His sister had recurrent episodes of fever and abdominal pain and received colchicine throughout the last four years. We screened in the first-degree relatives of the proband, and his mother was heterozygous for this mutation. There was no mutation in two healthy sisters and the father. This mutation caused the substitution of an arginine by a threonine (AGG>ACG, p.Arg361Thr) as a result of a G to C transition at nucleotide position 1082 (c.1082G>C) in exon 3 (Fig. 1). We obtained the rs number for the novel mutation (rs190405488). This mutation was heterozygous in the patients. In addition, five silent variants (A165A (c.495C>A), R314R (c.942C>T), E474E (c.1422G>A), Q476Q (c.1428A>G), D510D (c.1530T>C)) were detected with this mutation in the *MEFV* gene.

Table 1
Genotype distribution of patients.

Mutation (n, %)	Genotype	Patients	
		n	%
Heterozygous (n = 321, 70.9%)	E148Q/wt	153	33.77
	M694V/wt	74	16.34
	V726A/wt	42	9.27
	M694I/wt	16	3.53
	R761H/wt	12	2.65
	M680I/wt	9	1.99
	P369S/wt	7	1.55
	A744S/wt	3	0.66
	K695M/wt	1	0.22
	K695R/wt	1	0.22
	F479L/wt	1	0.22
	G304R/wt	1	0.22
	R361T[a]/wt	1	0.22
	Subtotal	321	70.86
Compound heterozygous (n = 73, 16.1%)	M694V/V726A	19	4.19
	M694V/E148Q	11	2.43
	V726A/E148Q	9	1.99
	M694V/M680I	9	1.99
	E148Q/M694I	4	0.88
	M694V/R761H	4	0.88
	E148Q/R761H	3	0.66
	R761H/V726A	2	0.44
	V726A/M680I	2	0.44
	V726A/A744S	2	0.44
	M694V/P369S	2	0.44
	M694V/M694L	1	0.22
	V726A/T267I	1	0.22
	R761H/M694I	1	0.22
	M680I/M694I	1	0.22
	P369S/R408Q	1	0.22
	E148Q/P369S	1	0.22
	Subtotal	73	16.11
Homozygous (n = 56, 12.4%)	M694V/M694V	47	10.38
	E148Q/E148Q	4	0.88
	V726A/V726A	2	0.44
	M694I/M694I	2	0.44
	M680I/M680I	1	0.22
	Subtotal	56	12.36
Complex (n = 3, 0.7%)	V726A/E148Q/R761H	2	0.44
	M694V/P369S/E148Q/E148Q	1	0.22
	Subtotal	3	0.66
Total patients with mutation		453	100.00
Patients without mutation		605	
Grand total		1058	

[a] Novel mutation.

4. Discussion

In this study, we reported the distributions of *MEFV* mutations and genotypes in 1058 pediatric patients with suspected FMF living in Van province of the Eastern Anatolia region in Turkey. All patients were referred from the pediatric departments of Yüzüncü Yıl University Hospital and Van-Training and Research Hospital. Van is located in eastern Turkey and borders Iran, and does not attract much immigration. Thus, patients with FMF from other regions of Turkey are never referred to our hospital. The results of our study represent the population living in the Eastern Anatolia region of Turkey. The major findings of our study were that (i) the most frequently observed mutation was M694V, (ii) the high frequency of M694I was detected in our study population, and (iii) a novel missense mutation was detected.

FMF and *MEFV* mutation carriers were significantly prevalent; 20% of the Turkish population and Turks are believed to be at high risk of developing FMF (Tunca et al., 2005). *MEFV* gene mutations have high allelic heterogeneity in Turkish patients. Analyzing the distribution of the patients' genotypes, we found that the most common genotypes were E148Q/wt, M694V/wt, and M694V/M694V, found in 153 (33.77%), 74 (16.34%), and 47 (10.38%) patients, respectively.

Recent studies conducted in Turkey have shown the most common four *MEFV* mutations with similar order (M694V, E148Q, V726A, M680I) (Tunca et al., 2005; Akin et al., 2010; Dundar et al., 2011;

Table 2
Allele frequencies of *MEFV* mutations among 453 patients.

Allele	Number of alleles	Frequency (%)
M694V	215	36.50
E148Q	193	32.77
V726A	83	14.09
M694I	26	4.41
R761H	24	4.07
M680I	23	3.90
P369S	12	2.04
A744S	5	0.85
Others	8	1.36
Total	589	100.00