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Is Turkish MEFV Mutations Spectrum Different Among Regions?

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Background: Familial Mediterranean fever (FMF) is an autosomal recessive inherited inflammatory disease. The gene responsible for the disease, called MEFV, encodes a protein called pyrin or marenostrin. According to recent data, MEFV mutations are not the only cause of FMF, but genetic analysis of MEFV gene is needed for confirming the diagnosis of FMF. In the present study, we aimed to evaluate the molecular testing results of MEFV mutations. *Methods:* Molecular testing results of 1,435 patients were retrospectively evaluated over the last 4 years. These patients were identified as having FMF clinical symptoms. Patients were tested for 12 common mutations in the MEFV gene using a strip assay technique. *Results:* From all 1,435 patients, MEFV mutations were found in 776 patients (54.08%) and 659 patients (45.92%) did not carry any mutations. Patients with mutations were classified as homozygotes ($n = 148$), compound heterozygotes ($n = 197$), heterozygous ($n = 427$), and complex genotypes ($n = 4$, patients with three mutations). Allelic frequencies for the four most common mutations in the mutation-positive groups were 48.79% (M694V), 14.86% (M680I G/C), 13.70% (E148Q), and 12.35% (V726A). The remaining alleles (10.3%) showed rare mutations that were R761H, P369S, A744S, K695R, F479L, and M694I. No patient showed a I692del mutation that is sometimes evident in other Mediterranean populations. *Conclusion:* It was found that the most common four mutations (M694V, M680I [G/C], E148Q, V726A) were similar to those previously reported from different regions of Turkey and this study might add some knowledge to the mutational spectrum data on FMF. J. Clin. Lab. Anal. **30**: 641–644, 2016.

Key words: familial Mediterranean fever; MEFV mutations; allelic frequencies; mutational analysis; molecular testing

Familial Mediterranean fever (FMF) is the most prevalent periodic fever syndrome affecting more than 100,000 patients worldwide (1). It is characterized by recurrent episodes of fever and serositis, resulting in pain in the abdomen, chest, joints, and muscles. The most devastating complication of FMF is systemic amyloidosis. The disease is transmitted in an autosomal recessive pattern and affects mainly Jews, Armenians, Turks, and Arabs. FMF is caused by mutations in MEFV gene that encodes pyrin (2, 3).

The estimated prevalence of FMF in Turkey is 1/1,000 and the estimated carrier rate is 1/5 (4). The Turkish population with more than 75 million inhabitants has a large proportion of all the FMF cases in the world. Therefore, for more effective public health services to be maintained, it is essential to have more accurate FMF data. Recent FMF studies from various regions of Turkey have been reported, but their study populations were relatively small (5–7). Because the identification of MEFV can lead to early diagnosis, which may prevent the occurrence of attacks and renal amyloidosis, MEFV spectrum data become more important. In this study, it is aimed to contribute to the Turkish MEFV mutation spectrum data by adding a larger regional study and compare the other larger studies from different centers in Turkey (8, 9).

In the present study, molecular test results of 1.435 patients referred to our department for MEFV molecular analysis were evaluated and these results were compared with other two large studies from other regions of Turkey in which was used the same genetic analysis technique.

One thousand four hundred thirty-five patients with the suspicion of FMF (635 male, 800 female aged between

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2 and 83) were enrolled in the present study. Their molecular test results, referred to our laboratory for FMF mutation analysis, over 4 years (2006–2010) were evaluated.

The assay was based on polymerase chain reaction (PCR) and reverse hybridization method that allow the detection of the most common 12 mutations identified in all at-risk populations (10). These mutations were E148Q, P369S, F479L, M680I (G/A), M680I (G/C), I692del, M694V, M694I, K695R, V726A, A744S, R761H. Blood samples were obtained in lavender-top tubes with Ethylenediaminetetraacetic acid (EDTA). Genomic DNA was extracted from blood samples according to standard procedures. After DNA isolation, multiplex PCR was performed using biotinylated primers for the exons 2, 3, 5, 10 amplification. Hybridization of PCR products with the immobilized wild-type and mutated oligonucleotide probes was performed on a strip (FMF StripAssay®, ViennaLab, Vienna, Austria). Hybridization was performed in an automated incubator [AutoLIPA, Fujirebio (formerly Innogenetics), Belgium]. Hybridizations were illuminated by the reaction of streptavidin–alkaline phosphatase and a color substrate. The results were interpreted by a coding table.

From all 1,435 patients, MEFV mutations were found in 776 patients (54.08%) and 659 patients (45.92%) did not carry any mutations (Table 1). Four hundred twenty-seven patients (55.03%) of 776 patients were heterozygous, 197 (25.39%) were compound heterozygous, 148 (19.07%) were homozygous, and four patients (0.52%) carried three mutations. These findings were similar to previous reports (8, 9). The patients carrying complex alleles were not included in the calculations. Allelic frequencies for the four most common mutations in the mutation-positive groups were 48.79% (M694V), 14.86% (M680I G/C), 13.70% (E148Q), 12.35% (V726A). The remaining alleles (10.3%) showed rare mutations that were R761H, P369S, A744S, K695R, F479L, and M694I. The most common mutations (M694V, M680I [G/C], E148Q, V726A, R761H, P369S) were similar to the study by Dundar et al. (8). Results were not directly compared with the study of Akin et al. (9) because of absent data.

Diagnosis of FMF is based on Tel-Hashomer criteria (11). The major criteria were as follows: (a) recurrent fever together with serositis, (b) an amyloidosis without any other causative event, and (c) good response to continued treatment with colchicines. Minor criteria: (a) recurrent fever, (b) erysipelas-like erythema, and (c) positive familial background. Two major criteria or two minor criteria along with one major criteria indicate a definitive diagnosis of disease. Because these criteria depend on clinical manifestation, in atypical clinical situations such as a late onset beginning, atypical clinical signs, and absence of family history or ethnic background, genetic analysis of MEFV gene is needed for confirming the diagnosis of FMF (11–13). Moreover, FMF amyloidosis constituted a significant cause of renal failure and death, especially among young adult patients. Because of the array of nonspecific clinical manifestations and the absence of an accurate diagnostic test, young children with FMF may be subjected to extensive investigations, such as exploratory laparotomy, before the correct diagnosis is made and treatment with colchicine is initiated. The cloning of the gene is of clinical importance, because the detection of mutations in the MEFV gene can provide an ultimate diagnosis of FMF.

A number of methods are used in the diagnosis of FMF. In the literature, there are studies that compare the results of one study to another. And the genetical analyses in these studies were made with by different techniques. Many of which have a limited spectrum of mutations and are time consuming. Because the analyses were made from percent rate of mutations, the limitation in spectrum becomes important. To overcome this barrier, we compared our results to other studies in which similar techniques were used (PCR and reverse hybridization method). Also, the comparison studies have had similar study groups, but they were from different regions of Turkey. Different allele frequencies were determined in the literature (14). Owing to the fact that comparative studies were conducted among patients with FMF diagnosis based on clinical signs, penetrance might be more important in this situation. A mutation with low penetrance could show low frequency among FMF patients, for example, E148Q. E148Q is often associated with a mild phenotype, and whether it is even a polymorphism has been questioned (15).

The present study aims to evaluate the distribution of 12 MEFV mutations in a large number of groups of patients living in the central Anatolia region of Turkey and compares the other large studies from different centers. It is a regional study, but Ankara is the capital city of Turkey and takes migration from other regions of Turkey. So its population consists of people from different parts of Turkey. Additionally, our hospital is a central hospital that receives patients from every region of Turkey. So the results of the present study might reflect whole Turkey.

In this study, the most common mutation was M694V as found in the other studies from Turkey. The M694V mutation is the most frequently found mutation evident in Turks, Jordanians, North Africans, and Lebanese in contrast to changing ratios in the studies conducted on Arabs (16–18). The allelic frequency of M694V mutation detected in this study was similar to the frequencies found in other comparative Turkish studies. None of the patients tested showed the mutation of I692del, which was included in the strip test. M694I mutation that is not rare in some populations was extremely rare in our study group (0.54%).

TABLE 1. Genotype Distribution of Patients

		Present study		Dundar et al. (8)		Akin et al. (9)	
Mutation	Genotype	Number of patients (*n*)	Percentage	Number of patients (*n*)	Percentage	Number of patients (*n*)	Percentage
	M694V/-	177	22.81	197	18.87	107	19.56
	E148Q/-	101	13.02	127	12.16	83	15.17
	V726A/-	54	6.96	60	5.75	34	6.22
	M680I(G/C)/-	40	5.15	80	7.66	28	5.12
	A744S/-	15	1.93	19	1.82	12	2.19
	P369S/-	15	1.93	28	2.68	12	2.19
	R761H/-	10	1.29	18	1.72	10	1.83
	K695R/-	10	1.29	6	0.57	7	1.28
	F479L/-	3	0.39	3	0.29	2	0.37
	M694I/-	2	0.26	8	0.77	0	0.00
	M680I (G/A)/-	0	0.00	0	0.00	1	0.18
	1692del	0	0.00	0	0.00	0	0.00
Heterozygotes		427	55.03	546	52.30	296	54.11
	M694V	116	14.95	100	9.58	79	14.44
	M680I(G/C)	21	2.71	45	4.31	11	2.01
	F479L	5	0.64	2	0.19	0	0.00
	E148Q	3	0.39	14	1.34	4	0.73
	V726A	2	0.26	9	0.86	5	0.91
	M680I (G/A)	1	0.13	0	0.00	0	0.00
	M694I	0	0.00	3	0.00	1	0.18
	K695R	0	0.00	1	0.00	0	0.00
	A744S	0	0.00	0	0.00	0	0.00
	R761H	0	0.00	2	0.00	0	0.00
	P369S	0	0.00	0	0.00	1	0.18
	1692del	0	0.00	0	0.00	0	0.00
Homozygotes		148	19.07	176	16.86	101	18.46
	M694V/M680I(G/C)	53	6.83	81	7.76	31	5.67
	M69V/V726A	41	5.28	56	5.36	36	6.58
	M694V/E148Q	28	3.61	47	4.50	27	4.94
	M680I(G/C)/V726A	19	2.45	41	3.93	9	1.65
	M680I(G/C)/R761H	10	1.29	9	0.86	3	0.55
	F749L/V726A	6	0.77	15	1.44	3	0.55
	E148Q/P369S	5	0.64	11	1.05	6	1.10
	M694V/R761H	4	0.52	17	1.63	11	2.01
	Others	31	3.99	37	3.54	19	3.47
Compound heterozygotes		197	25.39	314	30.08	145	26.51
Complex heterozygotes		4	0.52	8	0.77	5	0.91
Total patient with mutations		776	100.00	1044	100.00	547	100.00
Patients with no identified mutations		659		1023		654	
Total number of patients		1435		2067		1201	

No mutations were detected in 45.92% of the 1,435 patients in our study population. This percentage seems higher than other studies in which mutations were determined in study groups consisted of patients with FMF diagnosis. But this percentage was similar to those reported by Dündar et al. (49.49%; 1023/2067) and Akin et al. (54.5%; 654/1201) and these studies were made in unrelated patients with the suspicion of FMF not with the exact diagnosis of FMF (8,9). The difference between the rates of unidentified mutations among studies may be due to the heterogeneity of the selected patient group and also the presence of other rare mutations or unknown mutations. In a study it was shown that in patients with high or low clinical suspicion of the diagnosis of FMF according to Tel Hashomer criteria, the frequency of homozygote patients was significantly higher than the frequency of patients with no mutation, but it was not higher than the frequency of heterozygote patients (19). The technique used in the studies may be a factor for the discrepancy. In some studies, the patients were screened for the four most common mutations known so far and which seem to account for the majority of mutations identified in patients with FMF from the Middle East. So, the rate of unidentified mutations might be higher than the other studies analyzing other eight mutations. In the present study, these mutations were 10.21% of total mutated alleles.

Molecular diagnostic testing for FMF provides a means that is noninvasive, sensitive, and specific for an accurate diagnosis of patients before the full clinical syndrome is present. Moreover, the use of molecular genetic testing can lead to an early detection of individuals with atypical clinical situations and pediatric patients. The colchicine therapy, which is the most effective treatment for FMF patients, might be more effective in early diagnosis.

In conclusion, it is found that the most common four mutations (M694V, M680I [G/C], E148Q, and V726A) were similar to those previously reported in the literature and this study may add some knowledge to the mutational spectrum data on FMF.

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