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Title: The Spectrum Of MEFV Gene Mutations And Genotypes In The Middle Northern Region Of Turkey

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ABSTRACT

Objectives: Familial Mediterranean fever (FMF) is a common, inherited, autosomal recessive inflammatory illness in children. The diagnosis of FMF is based on clinical features and positive family history supported with genetic testing. The aim of this study is to determine the frequency and distribution of Mediterranean fever (MEFV) gene alterations of a city in Northern Anatolia.

Methods: We evaluated MEFV gene mutations in 374 children preliminary diagnosed as FMF by a commercial kit based on real time polymerase chain reaction technique in a one year period and screened twelve mutations.

Results: At least one mutation was detected in 213 patients (57%) and 38 genotypes with eleven distinct mutations. One hundred and thirty-seven (64. 3%) of mutation positive children were heterozygous, 45 (21. 1%) were compound heterozygous, 2 (0.9%) were complex heterozygous and 14 (6.4%) patients were homozygous, 6 (2.8%) were compound homozygous, 3 (1.4%) were complex homozygous. R202Q was the most common mutation with a frequency of 50.1%. Also R202Q/M694V was the most common compound heterozygous genotype. In 43 alleles R202Q - M694V mutations were found to be in linkage disequilibrium. M694V, E148Q, V726A, M680I (G/C) were other common mutations whereas F479L, A744S, K695R, P369S, M694I, R761H were the rare mutations of our cohort. None of our patients had M680I (G/A) mutation.

Conclusion: We determined the most common MEFV alteration prevalence in children of our region for the first time. The high R202Q mutation and linkage disequilibrium rates were remarkable results of this study.

KEY WORDS: MEFV mutations, FMF, R202Q, Northern Anatolia

INTRODUCTION:

Familial Mediterranean Fever (FMF) is a common, hereditary autoinflammatory disease in children. It is a monogenic disorder inherited in the autosomal recessive manner [1]. FMF, also named as “recurrent polyserositis” is characterized by spontaneously resolving, self-limited recurrent paroxysms of fever, polyserosal, synovial sterile inflammation, rash, family history and favourable response to colchicine treatment. It is one of the important reasons of fever of unknown origin in children [1, 2]. The most severe complication of the disease is renal amyloidosis, leading to renal failure [1]. The diagnosis of FMF is based on clinical features and positive family history. Genetic testing is used for supporting the clinical diagnose, especially in small children with a family history as they cannot localize pain and express themselves correctly [1, 2].

FMF is the result of mutations in the **ME**diterranean **Fe**V**e**r **(MEFV)** gene located on chromosome 16p13.3 and mainly expressed in granulocytes. It consists of 10 exons and encodes pyrin (marenostrin) protein [1, 3]. Pyrin is the key of caspase-1 and interleukin -1β pathways, leading to inflammation. The role of pyrin in the pathogenesis of FMF is not well known [1, 2]. It may have a regulator role in either suppressing or exacerbating the inflammatory response [2, 4]. Mutations in MEFV are also related with polyarteritis nodosa, Henoch Schönlein purpura (HSP), juvenil idiopathic arthiritis (JIA)

FMF is common in Armenians, Turks, Sephardic Jews and Arabs, groups that comprise the populations of the Eastern Mediterranean and Middle East regions, also in France, Germany, Italy, Spain, Japan and United States; throughout the entire world which is thought to be the result of migrations in our century [1, 2]. The ethnic diversity in Turkey causes a high rate of heterozygosity and homozygosity. The prevalence of FMF in Turkey is reported as 1:1000 and its frequency is similar in girls and boys. The carrier frequency is 1: 5 among Turks [5]. Although FMF is inherited autosomal recessively, some recent studies suggested that heterozygous people may manifest a spectrum of clinical features of mild and late onset FMF. In populations with high carrier rates and high rate of consanguineous marriages it is possible that one or both parents have pathogenic variants or may be affected mildly [1]. Pseudo dominant transmission is also thought be one of the reasons [2]. Heterozygous people usually have a later age of onset (mean age 18 years) and milder disease with fever and abdominal symptoms without frank peritonitis [1, 2].

To date 329 sequence variants for MEFV are defined. The mutations responsible for the disease may be missense, nonsense or deletion type [6]. The four missense mutations in exon 10 (M694V, M680I, M694I, V726A) are responsible for approximately 85% of MEFV gene mutations. E148Q and R202Q mutations are often determined in geographic areas where FMF is common, but the clinical outcome of these alterations is not well defined [6]. Mutation causing R202Q (c.605G>A) change was described as a frequent polymorphism and G allele was found in linkage disequilibrium with M694V [6]. Linkage disequilibrium is a way of genetic diversity so that certain alleles of each gene are inherited together more often than that would be expected by chance [6]. Although clinical symptoms and the course

of the illness are still the cornerstones of diagnosing FMF, molecular confirmation can help make the diagnosis earlier in suspected cases [5].

In this present retrospective study 374 paediatric patients with suspicious FMF were evaluated by analyzing MEFV gene mutations in one year period. Our aim was to determine the frequency and spectrum of MEFV gene mutation alterations in our province to contribute the MEFV mutation data of Turkey. We focused on only genetic tests ignoring clinical findings.

METHODS:

In this retrospective study molecular genetic testing of 374 children who admitted to the paediatrics, paediatric surgery and paediatric emergency outpatient clinics of a tertiary medical centre in the middle Black Sea region, north of Turkey, with the clinical signs of FMF between January 1, 2016 and December 31, 2016; throughout one year, were investigated. FMF referring symptoms were defined as three or more paroxysismal episodes of abdominal pain, pleuretic chest pain, fever, fever of unknown origin, mono-oligoarthiritis; all lasting for 6-72 hours and eryisipelas like erythema (Livneh and Yalçınkaya criteria [2, 7]). Patients suffering from at least two clinical signs of FMF and confirmed family history underwent mutation analyses after three or more attacks. All patients were referred from clinics of our hospital, the only medical centre in our city which is small, located in the middle northern region of Turkey and does not have a high immigration rate. The results of our study represent the prevalance of MEFV mutations in patients with suspicious FMF in a period of one year. Peripheral venous blood samples were collected in tubes with

Ethylenediaminetetraacetic acid (EDTA) and sent to an external laboratory for analyses. Genomic Deoxyribonucleic acid (DNA) was extracted from blood samples according to standard procedures. After DNA isolation a commercially available kit, which is based on real time polymerase chain reaction (PCR) technique with Montania 4896 instrument, was applied for the detection of MEFV mutations. E148Q, R202Q (exon 2), P369S (exon 3), F479L (exon 5), M680I (G/C), M680I (G/A), M694V, M694I, A744S, R761H, V726A, and K695R (exon 10) mutations were screened

Statistical Analyses:

The results were presented with descriptive features. All the descriptive analyses were performed using computer software (SPSS, version 15.0, Chicago, IL) with presentation as mean ± SD or percentages for normally distributed variables and medians with minimum–maximum values for not normally distributed variables when indicated.

Ethical approval:

Our study was approved by the local committee of education and research hospital with decision no: 62949364-000-6221

RESULTS:

Demographic and genetic features of the participants:

Among 374 patients 216 were girls (56. 8%), 158 were boys (41.6%) with the mean age 7.20±4.49 years. One hundred and sixty-one patients (43%) had no mutations where as at least one mutation was detected in 213 patients (57%). Thirty- eight different genotypes and eleven distinct mutations were detected in 213 patients. One hundred and thirty-seven (64.

3%) of these mutation positive children were heterozygous, 45 (21. 1%) were compound heterozygous, 2 (0.9%) were complex heterozygous whereas 14 (6.4%) patients were homozygous, 6 (2.8%) were compound homozygous, 3 (1.4%) were complex homozygous.

MEFV Gene Mutation Analyses:

Mutant genes were detected in 341 alleles in our cohort and R202Q was the most common mutation with a frequency of 50. 1%. The frequencies of heterozygous, compound heterozygous, homozygous, compound homozygous and complex genotypes of the R202Q mutation were 21. 8% (n = 83), 10.1% (n = 38), 2.1% (n =8), 0.5% (n=2) and 3% (n=11), respectively. Five patients were heterozygous with M694V and homozygous with R202Q. One patient had another complex genotype with R202Q/M680I (G/C)/M694V/M694V (Table1). R202Q/wt heterozygosity was found in 83 patients as the most common genotype (21. 8%). R202Q/M694V was the most common compound heterozygous genotype (6. 6%). We found R202Q and M694V were in linkage disequilibrium in 43 alleles. Also R202Q/R202Q was the most frequent homozygous genotype with a frequency of 2.1% (Table1). M694V (16. 7%), E148Q (11. 1%), V726A (9. 9%), M680I (G/C) (6. 4%) were other common detected mutations. F479L, A744S, K695R, P369S, M694I, R761H were the rare mutations of our cohort. None of our patients had M680I (G/A) mutation (Table2).

DISCUSSION:

Here we reported the frequency of MEFV mutations of 374 paediatric patients with preliminary diagnosis of FMF living in our province. Genotypes of 213 mutation positive patients were also included. Eleven different mutations with 38 distinct genotypes were

determined. The major finding of this study is that the most common alteration detected in our province is R202Q mutation with an allele frequency of 50.1% (n=171). The most common five mutations and their frequencies in our study were as follows: R202Q (50. 1%), M694V (16.7%), E148Q (11.1%), V726A (9.9%), M680I (G/C) (6.4%). In previous studies from Turkey M694V, M680I, V726A, M694I, E148Q are reported as common mutations (Table 3). These are also the most frequent mutations among the population of countries where FMF prevalence is high [1]. F479L, A744S, K695R, P369S, M694I, R761H were the rare mutations of our cohort. None of our patients had M680I (G/A) mutation. The data of rare mutations differ between different regions of Turkey [8, 9]. In our study R202Q mutation is determined as the most frequent mutation. R202Q alteration has been known since 1998 [6]. It is defined as a variation observed in symptomatic patients [6]. R202Q (c. 605G>A) is described as a frequent polymorphism, and the G allele is found to be in linkage disequilibrium (LD) with M694V in Infevers Database [6]. There are few studies addressing the R202Q mutation in the literature. Studies in Greek and Turkish patients suggest that R202Q is associated with an FMF phenotype, but limited data are available about the clinical significance of R202Q alteration. Ritis et al.reported R202Q homozygosity in 4 of 26 Greek patients with FMF, compared to 60 healthy controls having no mutation and suggested that R202Q gene alteration may be a mutation more than a polymorphism [10]. Also the same authors detected homozygosity of the R202Q polymorphism in 9.2 % of FMF patients compared to 0.7 % healthy controls in a Greek population [11]. The clinical significance of R202Q alteration in Turkish patients has been published in independent studies. Öztürk et al. reported that R202Q homozygosity might be

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associated with the disease in some FMF patients; although there was no R202Q homozygosity in their control group, but a high frequency of heterozygosity in the control group, concluding that it has no effect when it is in a heterozygous state [12]. Also Yiğit et al. found that although the heterozygosity of R202Q was similar in patients with FMF and healthy controls, the homozygosity was higher in patients with FMF when compared with healthy controls (14.7 -2.7 %) [13]. The authors claimed that R202Q polymorphism can be the cause of illness only in the homozygous form [12, 13]. Another study by Çomak et al. showed that seven patients with R202Q alterations in a cohort of 225 R202Q (+) patients had typical episodes of FMF of which two (3.6 %) had heterozygous R202Q alterations. The authors suggested that R202Q alteration is associated with an inflammatory phenotype and has clinical significance for FMF [14]. Guneşacar et al. reported that R202Q was the most frequently observed mutation in 427 (21.35%) of 2000 alleles in Hatay province, in the Mediterranean region of Turkey [15]. A recent study from İstanbul, reflecting whole Turkey, Barut et al reported R202Q mutation prevalence as 6.9% in FMF diagnosed children [16] Coşkun et al reported 452 (42.6%) R202Q variation in 1058 FMF suspected individuals from Van, but they had no healthy control group, either [17]. The absence of a healthy control group is a limitation of our study, too. Because of that we failed to determine the frequency of R202Q homozygosity or heterozygosity among healthy individuals to compare our data in the preliminary FMF diagnosed group. However, all patients with homozygous or heterozygous R202Q mutation had at least one or more clinical features of FMF with family history.

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We also found that in 43 alleles R202Q and M694V were in linkage disequilibrium. Unknown genetic alterations may cause phenotypical features by linkage-disequilibrium [6]. The study by Sayın Kocakap from Turkey showed high linkage disequilibrium between R202Q and M694V [9]. Kılınç et al from South-eastern Mediterranean region (Kahramanmaraş) of Turkey reported R202Q mutation frequency as 39.13 % in 260 heterozygous subjects of their study group consisting of 831 FMF patients. They attracted attention to frequent M694V/R202Q togetherness in their cohort concluding that clinical investigations must be conducted to identify its role in phenotype [18].

In our study the second most commonly seen mutation was M694V with an allele frequency of 16.7% (n=57). In nearly all regions of Turkey, the most common mutation is M694V. The overall frequency of M694V mutation in FMF suspected and diagnosed patients is reported as 23.5% within 16693 individuals from different parts of Turkey [9]. It is also the most frequent mutation in the populations where FMF is common and related with renal amyloidosis, the worst complication of the disease [1]. Ece et al, from the Southeast of Turkey reported M964V mutation with a frequency of 26% as the second common mutation in their study group consisting of 147 patients and 192 independent alleles [19].

E148Q was determined as the third most frequent mutation with a frequency of 11. 1% (n=38) in our study. E148Q was also detected as the third common mutation in over all Turkish patients with a frequency of 6. 8% [9]. However Evliyaoğlu et al reported this mutation as the most common one in the southern part of Turkey [20]. This mutation is thought to be a functional polymorphism and usually related with atypical FMF presenting a

mild clinical phenotype. It is also related with other recurrent fever and inflammation syndromes [6].

In this present study the frequency of V726A mutation was found as 9. 9% (n=32). In previous studies from Turkey, the allele frequency of the V726A mutation was 1. 9% [15]. It was reported as the fourth most common mutation in many studies from Turkey [9, 15]. It is also one of the most common mutations in Middle East populations and related with classical FMF phenotype [6, 21].

The fifth common mutation in our cohort was M680I (G/C) and its frequency was 6.4% (n=22). It is one of the common mutations in the Turkish population [8, 9]. The frequency was reported as 15.9% in a different study and one of the symptomatic mutations of MEFV [20]. It is also common in Middle East countries and Armenia [6].

In this study group the rare mutations were determined as K695R, A744S, F479L, P369S, R761H and M694I. The frequencies of these rare mutations ranged from 1.1% to 1.9%. The distribution of rate mutations was compatible with the data of other Turkish population studies [15].

In conclusion our study is the first study from our region reflecting the MEFV mutation data of child population suspicious of FMF in just one year. The most common five mutations in decreasing order were R202Q (50. 1%), M694V (16.7%), E148Q (11.1%), V726A (9.9%), M680I (G/C) (6.4%) respectively. Upon the fact that there are many ethnic groups in Turkey, our study group also confirms the mutational heterogeneity of FMF in our region (Table 3; [22-29]). FMF is frequent in the central Anatolia and Black Sea region of Turkey [30]. To our knowledge our data is one of the highest R202Q mutation frequency rates from

Turkey. It has not been identified yet that whether R202Q is a mutation or a polymorphism, more studies referring clinical features are needed for this identification. We had no healthy control group which limited us to conclude that this is either a polymorphism or a mutation significant for clinical phenotype. One of other limitations of our study is that according to the study design we did not mention the clinical features of our cohort and report just one year's data. This is an important shortcoming and in this study we only focused on genetic variations. More time is needed for clinical follow up and certain diagnosis. The discrepancies between the present study and the previous ones that were carried out in populations in different regions of Turkey might have been resulted from the number of participants, genetic heterogeneity, methods and available kits used for detection of the MEFV mutations. These techniques are not sufficient enough to detect rare or undefined alterations; direct sequencing of MEFV gene may be useful for identification of unknown mutations in regions where FMF is prevalent. Further studies with large patient series are needed to define the mutation table of Turkey.

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Table 1: Genotype distribution of our patients

Mutation (n; %)	Genotype	Patients (total)	
		n	%
Heterozygous genotypes (n=137, 64.3 %)			
	A744S/ wt	2	0.5
	E148Q/ wt	24	6.3
	F479L/ wt	1	0.3
	K695R/ wt	1	0.3
	M680I(G/C)/ wt	9	2.4
	P369S/ wt	1	0.3
	R202Q/ wt	83	21.8
	V726A /wt	14	3.7
	M694V/ wt	2	0.5

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Compound Heterozygous Genotypes (n= 45; 21.1%)	E148Q/M680I(G/C)	2	0.5
	E148Q/V726A	1	0.3
	F479L/ M680I(G/C)	1	0.3
	M694V/V276A	2	0.5
	P369S/E148Q	1	0.3
	R202Q/A744S	2	0.5
	R202Q/E148Q	3	0.8
	R202Q/K695R	1	0.3
	R202Q/M680I(G/C)	1	0.3
	R202Q/M694V	25	6.6
	R202Q/P369S	2	0.5
	R202Q/V726A	3	0.8
	M694I/R202Q	1	0.3

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Complex Heterozygous Genotypes (n=2; 0.9%)	E148Q/M694V/R202Q	1	0.3
	R202Q/M694V/M680I(G/C)	1	0.3
Homozygous genotypes (n=14; 6.5%)	E148Q/E148Q	1	0.3
	M680I(G/C)/M680I(G/C)	2	0.5
	R202Q/R202Q	8	2.1
	R761H/R761H	1	0.3
	V726A/V726A	2	0.5
Compound Homozygous (n=6; 2.8%)	E148Q/M694V	1	0.3
	E148Q/V726A	1	0.3
	F479L/V726A	1	0.3

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	R202Q/M694V	2	0.5
	V726A/M680I(G/C)	1	0.3
Complex Homozygous (n=3; 1.4 %)			
	R202Q/M694V/K695R	2	0.5
	R202Q/V726A/M694V	1	0.3
Others (n=6; 2.8 %)			
	M694V/R202Q/R202Q	5	1.3
	R202Q/M680I(G/C)/M694V/M694V	1	0.3
Subtotal	**Patients with mutation**	**213**	**56.9**
No mutation	**Patients without mutation**	**161**	**43.1**

Table2: Allele and mutation frequencies of MEFV mutations among mutant patients (n=213)

Allele	Number of alleles	Allele frequency	Number of	Mutation frequency

		(%)	mutations	(%)
R202Q	171	50.1	142	50.5
M694V	57	16.7	43	15.3
E148Q	38	11.1	35	12.4
A744S	4	1.1	4	1.4
F479L	4	1.1	3	1.0
K695R	6	1.7	4	1.4
M680I(G/C)	22	6.4	18	6.4
P369S	4	1.1	4	1.4
V726A	32	9.9	26	9.2
M694I	1	0.3	1	0.3
R761H	2	0.6	1	0.3
Toplam	**341**	**100**	**281**	**100**

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Table 3: Common MEFV alteration frequencies in FMF suspected patients in paediatric population from different regions of Turkey (%)

Reference	R202Q	M694V	E148Q	V726A	M680I(G/C)	Number of patients	Age of patients	Region
Şahin S et al [22].		15	4.9	2.7	5.1	929	----	Northern Anatolia
Günel Özcan A. et al [23]		6.8	8.3	4.4	4.4	136	9±3, 27±9	Central Anatolia
Ceylan et al [24]		14.2	4.4	5.4	3.9	802	3-75y (range)	Central Anatolia

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Dündar et al [25]		14.7	5.5	4.8	7.6	2067	0-80y (range)	Central Anatolia
Sayın KD et al [9]	23.7	14.8	6.9	3.9	4.1	351	21±14.3	Central Anatolia
Özalkaya E et al [26]		24.4	6.7	5	8.1	308	9.6±3.9	Western Anatolia
Battal F et al [27]	11.7	20	13.3	6.7	11.7	60	3-18Y (range)	Western Anatolia
Coşkun S et al [16]	42.6	36.5	32.7	14	3.9	1058	8.2±3.5	Eastern

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							Anatolia
Doğan H et al [28]		42.8	14.7	16.3	14.1	1620	1-72 (range)	Eastern Anatolia
Öztuzcu S. et al [29]		41.7	26.8	8.3	8.9	3341	1-80y	South-eastern Anatolia
Evliyaoğlu O et al [19]		3.2	9.6	1.9	1.4	332	1-15y (range)	South-eastern Anatolia
Güneşaçar R et al [15]	21.3	7.9	8.8	1.8	2.4	1000	1-70 (range)	Southern Anatolia
Barut K et al. [16]	6.9	41.1	5.7	4.8	5.6	708/617	12.3±	İstanbul

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		4.4	(North-western, reflecting Turkey

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