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Research Article

MEFV Gene Mutations in Familial Mediterranean Fever Patients: Erzincan Experience

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Abstract

Familial Mediterranean Fever is an autosomal recessive autoinflammatory multisystemic genetic disease caused by mutations in the *MEFV* gene. Although it is a common disease in our country, the type and frequency of mutations that cause the disease vary regionally. This study aimed to determine the types and frequencies of *MEFV* gene mutations in patients with a preliminary diagnosis of Familial Mediterranean Fever in our city. 303 patients who were referred to our laboratory with a preliminary diagnosis of Familial Mediterranean Fever were included in the study. The findings of the patients analyzed for common mutations in the *MEFV* gene using the Real-Time PCR method were evaluated retrospectively. While mutations in the *MEFV* gene were detected in 44.8% of the patients included in the study, no mutations related to the investigated regions were detected in 55.2%. In patients with detected mutations; the frequency percentages of the most frequently detected genotypes are M694V/- (36.4%), E148Q/- (18.3%), M694V/M694V (7.3%); the frequency percentages of the alleles were determined as M694V (48.3%), E148Q (22.2%), M680I (10.8%), V726A (10.3%). The results of the study support the heterogeneity in *MEFV* gene mutations seen in Familial Mediterranean Fever patients. Although minor differences are observed in terms of mutation types and frequencies detected in the *MEFV* gene, the findings are compatible with the results of other studies conducted in the Turkish population. Current data on the genotype distribution of Familial Mediterranean Fever patients in our city will contribute to the literature.

Keywords: Familial Mediterranean Fever, *MEFV* Gene, Mutation, Pathogenic, Real-Time PCR

Ailesel Akdeniz Ateşi Hastalarında *MEFV* Gen Mutasyonları: Erzincan Deneyimi

Öz

Ailesel Akdeniz Ateşi, *MEFV* geninde meydana gelen mutasyonların yol açtığı otozomal resesif geçişli otoinflumatuar multisistemik genetik bir hastalıktır. Ülkemizde sık görülen bir hastalık olmakla birlikte hastalığa sebep olan mutasyonların tipi ve sıklıkları bölgesel olarak farklılık göstermektedir. Bu çalışmada, ilimizde Ailesel Akdeniz Ateşi ön tanısı alan hastalarda *MEFV* gen mutasyonlarının tiplerinin ve sıklıklarının belirlenmesi amaçlanmıştır. Ailesel Akdeniz Ateşi ön tanısı ile laboratuvarımıza yönlendirilen 303 hasta çalışmaya dahil edildi. Real-Time PCR yöntemi ile *MEFV* geninde sık görülen mutasyonlar açısından analiz edilen hastaların bulguları retrospektif olarak değerlendirildi. Çalışmaya dahil edilen hastaların %44.8'inde *MEFV* geninde mutasyon saptanırken %55.2'sinde ise araştırılan bölgelere ilişkin mutasyon saptanmadı. Mutasyon tespit edilen hastalarda en sık saptanan genotiplerin frekans yüzdeleri; M694V/- (%36.4), E148Q/- (%18.3), M694V/M694V (%7.3); alellerin frekans yüzdeleri ise M694V (%48.3), E148Q (%22.2), M680I (%10.8), V726A (%10.3) olarak tespit edildi. Çalışmanın sonuçları Ailesel Akdeniz Ateşi hastalarında görülen *MEFV* gen mutasyonlarındaki heterojeniteyi desteklemektedir. *MEFV* geninde saptanan mutasyon tipleri ve sıklıkları bakımından minör farklılıklar gözlense de elde edilen bulgular Türk popülasyonunda gerçekleştirilen diğer çalışmaların sonuçları ile uyumludur. İlimizdeki Ailesel Akdeniz Ateşi hastalarının genotip dağılımlarının güncel verileri literatüre katkı sağlayacaktır.

Anahtar Kelimeler: Ailesel Akdeniz Ateşi, *MEFV* Geni, Mutasyon, Patojenik, Real-Time PCR

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1. Introduction

Familial Mediterranean Fever (FMF) (OMIM #249100) is the earliest identified and most common autosomal recessive hereditary autoinflammatory disease [1-2]. FMF is characterized by recurrent short-term fever, abdominal, chest and joint pain, and erythema-like skin lesions [3]. The period and type of attacks vary according to mutation type, ethnicity and colchicine use, as well as age and gender [4]. Complications of the disease can range from simple skin lesions to life-threatening amyloidosis [5-6].

Although FMF is especially common in populations originating from the Mediterranean basin such as Turks, Jews, Arabs and Armenians, it seen worldwide due to increased travel and migration in recent years [7-8]. It has been reported that the frequency of the disease in our country is approximately 1/1000 and the carrier rate is 1/5 [1]. Due to the high carrier rate in our country, determining Mediterranean Fever *(MEFV)* gene mutations is important in detecting patients as well as other family members carrying the disease, initiating treatment and providing genetic counseling.

The *MEFV* gene responsible for the disease is located on the short arm of chromosome 16 (16p13.3) [9]. This gene, which has 10 exons, encodes the protein called pyrin, which consists of 781 amino acids [10]. This immune regulatory protein functions as an innate immune sensor that triggers inflammation and enables the production of inflammatory mediators [11]. Pyrin is mostly expressed in neutrophils, eosinophils, monocytes, dendritic cells and fibroblasts and plays a role in the regulation of apoptosis, inflammation and cytokine production [12]. Changes in the structure of this protein cause uncontrolled interleukin-1 (IL-1) secretion and an increase in the inflammatory response [12].

The variability in clinical findings and disease severity in FMF patients can be explained by different mutations in the *MEFV* gene. According to the INFEVERS database, 340 different mutations have been identified in the *MEFV* gene [13]. It has been shown that the majority of these nucleotide changes that cause the disease are in exons 2 and 10 [7, 14]. Knowing the mutation types is important in terms of approaching the patient. As far as we know, there is no study on the mutation types of FMF patients in our province. Therefore, our current study aimed to determine the common mutation types in the *MEFV* gene in patients sent to our laboratory with a preliminary diagnosis of FMF.

2. Material and Methods

In our study, the results of 303 patients who were referred from different units of our hospital with a preliminary diagnosis of FMF between 10.10.2022 and 10.10.2023 and were analyzed for *MEFV* gene mutations in our laboratory were evaluated retrospectively. Consent forms were obtained during the outpatient clinic examination. This study was carried out taking into account ethical responsibilities according to the World Medical Association and the Declaration of Helsinki, and ethics committee approval was received from Erzincan Binali Yıldırım

University Non-Interventional Clinical Research Ethics Committee with decision number 2023-18/17.

For *MEFV* gene analysis, 2 ml peripheral venous blood samples were taken from the patients and placed in ethylene diamine tetra acetic acid (EDTA) tubes. Genomic material was isolated with the QIAmp DNA Mini Kit (Qiagen GmbH). The concentration and quality of the obtained DNAs were measured with a Nanodrop spectrophotometer (Thermo Scientific, USA). DNAs with appropriate purity and concentration (OD260/OD280, 1.8-2.0) for analysis were included in the study. The DNA samples obtained were analyzed for mutations common in FMF disease (M694V, E148Q, M680I, V726A, P369S, M694I) with the Real-Time PCR method (Roche Cobas Z 480). Individuals in which mutant and wild type bands were observed together were considered heterozygous, individuals in which only mutant bands were observed were considered homozygous mutant, and individuals in which only wild type peaks were observed were considered normal.

For statistical analysis of the data, SPSS (Statistical Package for Social Sciences for Windows v22.0) program was used. Descriptive statistics; it was calculated as mean ± standard deviation for numerical variables, and as frequency distribution and percentage for heterogenic variables.

3. Results and Discussion

While mutation was detected in 44.8% of 303 patients who were referred to our laboratory with a preliminary diagnosis of FMF disease and included in the study, no mutation was detected in 55.2% of the investigated regions. Detailed molecular and clinical classification of the detected mutations is shown in Table 1.

Table 1: Molecular and clinical information on *MEFV* mutations.

Mutation	Exonic Location	Nucleotide Change	Protein Change	ACMG Classification
E148Q	2	c.442G>C	p.Glu148Gln	Uncertain Significance
P369S	3	c.1105C>T	p.Pro369Ser	Uncertain Significance
M694V	10	c.2080A>G	p.Met694Val	Pathogenic
M680I	10	c.2040G>A	p.Met680Ile	Pathogenic
V726A	10	c.2177T>C	p.Val726Ala	Pathogenic
M694I	10	c.2082G>A	p.Met694Ile	Pathogenic

It was determined that 70.1% of the patients with a mutation were heterozygous, 16.8% were compound heterozygous, and 13.1 were homozygous (Table 2).

Table 2: Distribution of genotypes in patients with detected mutations.

Mutation type	Number of patients (n)	Percentage (%)
Heterozygous	95	70.1
Compound Heterozygous	23	16.8
Homozygous	18	13.1
Total	**136**	**100**

The most frequent genotype in patients with a mutation was M694V/- (36.4%). This is followed by E148Q/- (18.3%) and M694V/M694V (7.3%) genotypes, respectively. Other genotypes observed in the patients are shown in Table 3.

Table 3: Distribution and frequencies of mutations detected in the *MEFV* gene.

Mutation type	Genotype	Number of patients (n)	Percentage (%)
Heterozygous	M694V/-	49	36.4
	E148Q/-	25	18.3
	P369S/-	9	6.6
	V726A/-	8	5.9
	M680I/-	3	2.2
	M694I/-	1	0.7
Total		**95**	**70.1**
Compound Heterozygous	M694V/M680I	6	4.4
	M694V/E148Q	6	4.4
	M694V/V726A	4	2.9
	M680I/V726A	4	2.9
	E148Q/P369S	2	1.5
	V726A/P369S	1	0.7
Total		**23**	**16.8**
Homozygous	M694V	10	7.3
	M680I	3	2.2
	E148Q	3	2.2
	V726A	1	0.7
	P369S	1	0.7
Total		**18**	**13.1**
General Total		**136**	**100**

When the allele frequencies mutations are examined, the most common mutation is M694V with 48.3%. This mutation is followed by E148Q (22.2%) and M680I (10.8%), respectively. The allele frequency distribution of the mutations in the patients is shown in Table 4.

Table 4: Allele frequency distribution of mutations in patients.

Mutation	Number of Alleles	Percentage (%)
M694V	85	48.3
E148Q	39	22.2
M680I	19	10.8
V726A	18	10.3
P369S	14	7.9
M694I	1	0.5
Total	**176**	**100**

FMF is an autosomal recessive systemic inflammatory disease that is more common in the Middle East and Mediterranean countries. The most important symptoms of the disease are fever, abdominal pain, joint pain and skin lesions [15]. The diagnosis of FMF can be made by observing characteristic attacks in patients with typical clinical findings. On the other hand, in atypical cases such as patients with amyloidosis or cases that cannot be distinguished from other periodic fever syndromes, genetic examination comes to the fore as an approach to diagnosis and treatment [11]. It is known that mutations in the *MEFV* gene located on the short arm of chromosome 16 (16p13.3) cause FMF disease [2]. The variability in clinical findings and severity of the disease is explained by different mutations in the *MEFV* gene [15]. Different methods such as sanger sequencing, pyrosequencing, real-time polymerase reaction and next-generation sequencing are used to detect these mutations responsible for the pathogenesis of the disease. In our current study, patients sent to our laboratory with a preliminary diagnosis of FMF were examined using the real-time polymerase reaction method for 5 common mutations in the *MEFV* gene.

Mutations were detected in 136 (44.8%) of the 303 patients included in our study. In studies conducted on the observed mutations in the *MEFV* gene, Çilingir et al. [15] detected mutations in 47.5% of the patients, Yeşilada et al. [3] in 47.21% of the patients, and Dönder et al. [5] in 42.85% of the patients. The results we obtained are similar to the results of these studies.

Among the cases with mutations in our study, the heterozygosity rate was found to be 70.1%, the compound heterozygosity rate was 16.8%, and the homozygosity rate was 13.1%. In a study conducted by Binici et al. in the southeastern region, the *MEFV* gene was analyzed for frequent regions using the pyrosequencing method [1]. In this study, they revealed that 74.13% of the patients were heterozygous, 16.43% were compound heterozygous, and 9.44% were homozygous. In another study by Çilingir et al. in a large patient group using the pyrosequencing method, the heterozygosity rate was found to be 60.77%, the compound heterozygosity rate was 22.56%, and the homozygosity rate was 16.65% [15]. The results we obtained are in agreement with the results of similar studies, especially in terms of the distribution of genotypes. Additionally, the heterozygous mutation rate of 70.1% obtained in our study was found to be higher than most of the studies in our country.

The frequencies of the mutations we detected in our study were determined as M694V/- (36.4%), E148Q/- (18.3%) and M694V/M694V (7.3%), respectively. According to the results

of the study by the Turkish FMF working group in 2005, the most frequently observed mutations were reported as M694V (51.4%), M680I (14.4%), V726A (8.6%), respectively [16]. In the study carried out by Bayrak et al. using the next generation sequencing method, the common mutations were found to be M694V (34.9%), E148Q (26%), V726A (16%) [13]. Taşdemir et al., in their study conducted in the Konya region, stated that they found the mutation frequencies as M694V (22.3%), E148Q (12.9%), and M680I(C) (9.7%) [7]. Teker and Öz, in their study where they examined all coding regions and exon-intron junctions of the *MEFV* gene with the next generation sequencing method, reported the most frequent mutations as M694V (46.6%), V726A (14.5%), and M680I (14%), respectively [12].

The allele frequencies of the mutations in our study were found as M694V (48.3%), E148Q (22.2%), M680I (10.8%) and V726A (10.3%). Özemri et al. found the allele frequencies in 350 patients in which they detected mutations in the *MEFV* gene as M694V (38.1%), E148Q (20.4%), V726A (8.9%) and M680I (6.8%) [17]. In a similar study, Çilingir et al. reported the allele frequencies as M694V (40.13%), E148Q (24.43%), V726A (11.57%) [15]. Similar to studies in the literature, the M694I mutation was found at low frequency in our study [1, 5, 11]. When the data of our study and similar studies in our country are evaluated together, it is concluded that M694V, V726A, M680I(C), E148Q mutations are the most frequently observed mutations in most of the studies and that there are generally differences in terms of *MEFV* gene mutation allele frequencies between regions.

4. Conclusion

In conclusion, the findings this study show that the distribution and allele frequencies of *MEFV* gene mutations in our patients evaluated with a preliminary diagnosis of FMF were compatible with previously reported data in FMF patients in our country. Detection of FMF mutations allows the clinical diagnosis to be confirmed, colchicine treatment to be started without delay, and the patient's relatives to be evaluated in terms of the determined mutation in our country where the carrier rate is high. Apart from the common mutations in FMF disease, it is known that some other mutations may also cause the disease. Therefore, although it has a higher cost compared to other methods, analyzing the *MEFV* gene with the next generation sequencing method, especially in countries with a high prevalence of FMF disease, will contribute to the detection of common, rare or unknown mutations.

The most important limitations of our study are the small number of cases and the fact that it is a single-center study. Another important limitation of the study is that since it was a retrospective study, the relationship between mutation types and the clinic could not be examined.

Ethics in Publishing

Ethics committee approval was received from Erzincan Binali Yıldırım University Non-invasive Clinical Research Ethics Committee on 19.10.2023 with the decision numbered 2023-18/17.

Author Contributions

Main Idea/Planning: AYD, ÖA; Analysis/Comment: AYD, ÖA; Providing Data: ÖA; Writing: AYD; Review and Correction: AYD, ÖA; Confirmation: AYD, ÖA

Conflicts of Interests

There is no conflict of interest between the authors.

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