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Original article

Evaluation of the Frequency of R202Q Mutation in Patients with Familial Mediterranean Fever in the Middle Black Sea Region of Turkey

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Abstract. Familial Mediterranean Fever is a chromosomal inherited inflammatory disease, often accompanied by symptoms such as joint pain, abdominal pain and fever. Although Familial Mediterranean Fever is a chronic disease, it can show acute recurrent attacks. These attacks last for a short time, but their frequency is not exactly known. The Mediterranean Fever (MEFV) gene encodes a protein called pyrin that causes FMF disease. Hundreds of mutations have been identified in the MEFV gene, mostly in exon 2 and exon 10. We investigated the mutation prevalence in patients with FMF in the Central Black Sea region of Turkey. We retrospectively examined data from 617 patients tested for molecular genetics. In the study, Light Cycler (Roche) Real-Time PCR system and TIB Molbiol LightSNiP reagents were used for mutation analysis. The mutations detected in the analyzed samples, in descending frequencies were 44.91% for R202Q; 22.46% for M694V; 10.82% for E148Q; and 8.24% for V726A. The frequencies of four mutations were detected in the 1-2% range. R652H and M694I mutations were not detected. The highest allele frequency was detected as 27.86% in R202Q mutation. We found the frequency of the M694V allele, which is common in patients with FMF, was 13.24% and for V726A it was 4.20%. For other mutations detected less frequently, we found allele frequencies as below 1 percent. At least one mutation was detected in most of the patients (71.89%). We identified 16 compound and 3 complex genotypes. As a result, we found a high linkage disequilibrium between the R202Q mutation discussed in our study and the M694V mutation, which is quite common in FMF patients. Comprehensive genotyping studies in larger populations are needed to clarify the genetics of the disease and to contribute to diagnostic testing.

Key words: Familial Mediterranean Fever, DNA mutation analysis, linkage disequilibrium

Introduction

Familial Mediterranean fever- although its etiopathogenesis is not fully known- is a chromosomal disease that develops due to attacks of inflammation and has systemic manifestations. However it is asymptomatic during remission periods. However, during attacks, symptoms such as high fever, arthritis usually involving the lower extremities, abdominal and chest pain due to non-infective inflammation of the serosal membranes can be seen. It is seen in populations from the Mediterranean region, especially Turks, Armenians and Sephardic Jews (1,2). Side effects of colchicine used as a therapeutic agent, pain-fever episodes or complications occurring during the chronic course of FMF negatively affect the patient's life (3). Although not fully explained, a protein called pyrin, which has a regulatory role in inflammatory reactions, is encoded

by the MEFV gene. In the event of a mutation in this gene, the regulatory effect of the miscoded pyrin protein in inflammatory reactions is eliminated and causes disease-specific symptoms. (4). More than 300 mutations have been identified in the MEFV gene, mostly in the exon 2 and exon 10 regions (5). Diagnosis depends on clinical signs and Tel-Hashomer criteria are used, but the genetic basis of the disease is remarkable (6,7). Some of the identified mutations have been found to be associated with emerging clinical symptoms, complications such as amyloidosis, or some accompanying diseases (8,9). The aim of the study is to define both the frequency of genetic mutations in patients whose molecular analysis is performed and whose diagnosis or prediagnosis is FMF, and also frequency of R202Q mutations that are not yet included in the test panels.

Material and Methods

We designed a retrospective study to analyze the data of Gaziosmanpaşa University Medical Faculty Hospital Biochemistry Laboratory in Tokat over a 3-year period. Patients who had been admitted to the Medical Faculty Hospital due to clinical symptoms were evaluated and 617 patients (268 males and 349 females) who had been prediagnosed with FMF and whose MEFV gene mutation analyses had been performed were included in the study. The study group consisted of male (49.3%), and female (50.7%) patients with an overall mean age of 25.9 ±17.02 (min:8, max:68) years. Venous blood samples of the patients (5 ml) were taken into tubes containing ethylenediaminetetraacetate (EDTA). Genomic DNA was extracted with a commercial kit (High purity PCR template preparation kit, Roche).

The PCR method was used to amplify the DNA. MEFV gene mutations A744S, E148Q, F479L, K695R, M680I (G/A), M694I, M694V, R202Q, R652H and V726A were detected using TIB Molbiol LightSNiP reagents and Roche Light Cycler 480II (Real-Time PCR). After the examination, genetic analysis was performed on the patients who had been applied to the polyclinics from different regions of Tokat. All patients or their parents signed an informed consent form prior to genetic testing. Ethical approval was obtained from the Local Ethics Committee for the study (Registration Number 14-KAEK-021).

Results

The detected mutation rates were as follows; R202Q (10.82%-34.09%); M694V (4.04%-18.42%); E148Q (3.55%-7.27%); V726A (0.16%-8.08%), and the frequencies of four mutations (A744S, K695R, F479L, M680I) with only heterozygous genotype were in the range of 0-2%, Two mutations (R652H and M694I) were not detected at all (Table 1).

The allele frequency distributions of the most often detected mutations were as follows; R202Q: 27.86%; M694V:13.24%; E148Q:7.18%; and V726A: 4.20% (Table 2).

Table 1. Frequencies of the MEFV mutations

	HETEROZYGOUS n (%)	HOMOZYGOUS n (%)	TOTAL n (%)
R202Q	211 (34.09)	67 (10.82)	278 (44.91)
M694V	114 (18.42)	25 (4.04)	139 (22.46)
E148Q	45 (7.27)	22 (3.55)	67 (10.82)
V726A	50 (8.08)	1 (0.16)	51 (8.24)
A744S	11 (1.78)	0	11 (1.78)
K695R	3 (0.48)	0	3 (0.48)
F479L	2 (0.32)	0	2 (0.32)
M680I(G/A)	1 (0.16)	0	1 (0.16)
M694I	0	0	0
R652H	0	0	0

Table 2. Frequencies of the alleles

Allele	%	n
R202Q	27.86	345
M694V	13.24	164
E148Q	7.18	89
V726A	4.20	52
A744S	0.88	11
K695R	0.24	3
F479L	0.16	2
M680I(G/A)	0.08	1
M694I	0	0
R652H	0	0

The rate of finding at least one mutation in the analyzed samples was quite high (71.89%). Differently, we identified 16 compound and 3 complex genotypes (Table 3).

High linkage disequilibrium was detected when R202Q and M694V were examined (r^2= 0.3359; D '= 0.922).

The data about the frequency of FMF mutant alleles detected were compared with relevant data obtained from different regions of Turkey and given in Table 4.

Discussion

We reported a more common but less studied MEFV mutation, R202Q, in the Tokat Province. Tokat is one of the provinces where FMF disease is prevalently seen in Turkey. Kısacık et al. found the highest frequency of FMF reported from Turkey in the Tokat Province. Diagnosis of FMF was made in 0.82% of 1095 subjects (10). A patient with arthritis, periodic fever, but with or without abdominal pain leads the clinician to evaluate FMF. There is no specific laboratory test, so diagnosis is made based on clinical symptoms, but ethnicity and family history are also important. Atypical attacks complicate the diagnosis, and molecular genetic testing supports the diagnosis (11,12). The presence of two mutations confirms the diagnosis (13). In addition, these mutations may be related to the complication, severity, and prognosis of the disease (14). Salah et al. compared the genotype in FMF with the cardiac phenotype and found that pericardial effusion in children was associated with E48Q, P369S, V726A mutations (15). Our study population consisted of patients with suspected FMF and 71.89% of them have at least one mutation. This is a high rate, but a mutation (heterozygous) with atypical episodes is not thought to

Table 3. The compound and complex genotypes

The compound genotype			
genotype	**n**	**genotype**	**n**
A744S/R202Q	1	F479L/V726A	1
E148Q/K695R	2	M680I(G/A)/R202Q	1
E148Q/M694V	1	M694V/R202Q	74
E148Q/R202Q	3	M694V/V726A	3
E148Q/V726A	6	R202Q/V726A	6
F479L/M694V	1		
homozygous/heterozygous		**heterozygous/homozygous**	
E148Q/R202Q	5	M694V/R202Q	12
E148Q/F479L	1	**homozygous/homozygous**	
M694V/R202Q	3	M694V/R202Q	24
The complex genotypes			
heterozygous			
E148Q/M694V/R202Q	4		
M694V/R202Q/V726A	12		
homozygous/heterozygous/heterozygous			
E148Q/M694V/R202Q	3		

Table 4. Comparison of the data with results of the other regions of Turkey

Allele	**Tokat** % (n=617)	**İzmir** % (Ülgenalp, 2009) (n=3358)	**Ankara** % (Erden, 2008) (n=196)	**Van** % (Dönder, 2012) (n=1120)	**Malatya** % (Yeşilada, 2005) (n=394)
R202Q	44.91	-	-	-	-
M694V	22.44	16.86	31.63	10.71	14.97
E148Q	10.82	5.96	11.22	9.55	4.56
V726A	8.24	3.57	9.18	3.92	1.77
A744S	1.78	0.36	0.51	0.08	2.28
K695R	0.48	0.6	0	0.08	0.50
F479L	0.32	0.66	1.02	0.17	0.25
M680I(G/A)	0.16	0.68	0	0.53	0.50
M694I	0	0.08	0	0	0
R652H	0	-	-	-	-

support or exclude FMF as a possible diagnosis. The major limitation of the study is that we did not know the exact number of diagnosed FMF patients. In their study, Yılmaz et al. investigated 78 children with FMF in the same region of Turkey. They detected a mutation in the MEFV gene in 96% of the patients and most frequently (55%) found M694V mutation among the detected mutations (16). R202Q has been previously described as a common polymorphism by Bernot (17). Yigit et al. found similar heterozygosity of R202Q both in FMF patients and controls (18). They studied 191 patients and 150 healthy controls. The rates of heterozygosity were nearly similar in patients (42.9%) and controls (42%). However, the rates of homozygosity were significantly different (14.7% in patients, and 2.7% in controls). Therefore, R202Q has been recognized as a risk factor for FMF.

Our results give us a slightly lower percentage relative to these rates (heterozygosity 34.84%; homozygosity, 10.04%). The reason for this discrepancy is that our study group included greater number of patients and healthy control subjects. Their study ignores a linkage disequilibrium we found between the R202Q and the M694V. FMF cannot be diagnosed in patients who are unaware of their disease and have a silent course without resorting to MEFV genotyping. Although hundreds of mutations have been identified in the gene, the most common mutations are usually analyzed for diagnosis. Therefore, if genetic testing does not show any mutations, this cannot rule out FMF. As a result, it would be better to add R202Q to the most common mutation panels of research centers or laboratories in Turkey, especially in our region. We compared our results with those of other regions in Turkey (Table 4).

Our rates are similar to those found by Erden et al. in Capital City of Ankara, in the year 2008. Ankara is a greater metropolitan city closer to Tokat Province, so patients may have gone there for treatment. Kocakap et al also showed that the rate of R202Q mutations in 213 persons from Turkey was 23.7% (19). However, our study population was more numerous, so we obtained higher rates of mutations.

Conclusion

Our study summarizes the spectrum of FMF mutations in Tokat Province and the data of our laboratory center. R202Q mutation has a high rate and shows linkage disequilibrium with M694V. Our study population consisted of suspected FMF patients. Larger studies can be done using the latest diagnostic information of the patients. This will help to see the difference in the mutation frequencies in patients and the healthy population.

Data Availability

Approval was obtained from the regional ethics committee for our study (Registration number: 14-KAEK-021). Due to patient confidentiality, the data of this study can be obtained from the SQL system with the permission of hospital administration of Gaziosmanpaşa University Faculty of Medicine

Conflicts of Interest: The authors declare that they have no conflict of interest.

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