The Clinical Significance of *R202Q* Mutation in the Pyrin Gene

Pyrin Geninde *R202Q* Mutasyonunun Klinik Önemi

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ABSTRACT Objective: Familial Mediterranean fever (FMF) is a chronic autoinflammatory disease which is inherited in an autosomal recessive manner. There are various numbers of mutations detected in Mediterranean fever (*MEFV*) gene, but only some of them are related to this disease. There are differences in *MEFV* gene mutations that are carried by FMF patients who belong to different ethnic groups. The aim of this study was to investigate whether there was a difference regarding the clinical symptoms among the patients with *R202Q* (c.605G>A) mutation and the individuals with one or a few of the common mutations. **Material and Methods:** In our study, we have included 166 individuals [146 patients (89 female; 61% and 57 male; 39%) and 20 healthy controls (10 female; 50% and 10 male; 50%)]. Demographic and clinical symptoms of the patients have been recorded. The mutations in exon 2 and 10 belonging to *MEFV* gene were identified by the DNA Sanger Sequencing method. **Results:** According to our results, the most common mutation detected in our patients was *R202Q* (39.73%) followed by *M694V* (25.34%). The patients were divided into four groups according to the mutations that they carry. There was no difference between the groups in terms of the clinical symptoms (fever, abdominal pain, chest pain, arthralgia, erysipelas-like erythema and family history of FMF) (p=0.812, p=0.149, p=0.502, p=0.685, p=0.782, p=0.104, respectively). **Conclusion:** There was no significant difference regarding the clinical symptoms between the patients carrying *R202Q* mutation and the patients with FMF disease related mutations.

Key Words: Familial Mediterranean Fever; marenostrin

ÖZET Amaç: Ailesel Akdeniz Ateşi [Familial Mediterranean Fever (FMF)], otozomal resesif geçişli kronik otoinflamatuar bir hastalıktır. "Mediterranean Fever" geni (*MEFV*)'nde saptanan çok sayıda mutasyonun sadece bir kısmı hastalıkla ilişkidir. Çeşitli etnik gruplardaki FMF hastalarının taşıdıkları *MEFV* geni mutasyonları arasında farklılıklar vardır. Bu çalışmanın amacı, hasta grubunda *R202Q* (c.605G>A) mutasyonu taşıyanlar ile diğer yaygın mutasyonlardan bir veya birkaçını taşıyanlar arasında klinik semptomların ortaya çıkış şekli açısından fark olup olmadığının araştırılmasıdır. **Gereç ve Yöntemler:** Çalışmaya 146 (89 kadın; %61 ve 57 erkek; %39) hasta ile 20 (10 kadın; %50 ve 10 erkek; %50) sağlıklı kontrol dâhil edildi. Hastaların demografik verileri ve klinik semptomları kaydedildi. Yüz altmış altı örneğin genomik DNA'sından, DNA Sanger dizileme yöntemi ile *MEFV* geni exon 2 ve 10'daki mutasyonlar tanımlandı. **Bulgular:** Hastalarımızda en sık gözlenen mutasyonun %39,73 *R202Q* olduğu bulundu. Bunu %25,34 ile *M694V* izledi. Taşıdıkları mutasyonlara göre hastalar dört gruba ayrıldı. Gruplar arasında klinik semptomların (ateş, karın ağrısı, göğüs ağrısı, eklem ağrısı, eritem benzeri döküntüler ve ailede FMF hikayesi) ortaya çıkış şekli açısından fark bulunamadı (sırasıyla p=0,812, p=0,149, p=0,502, p=0,685, p=0,782, p=0,104). **Sonuç:** Bu çalışma ile *R202Q* mutasyonunun hastalarda bulunan en sık mutasyon olduğu, bu mutasyonu taşıyan hastalar ile diğer hastalık etkeni mutasyonları taşıyan hastalar arasında klinik bulgular açısından fark bulunmadığı görüldü.

Anahtar Kelimeler: Ailesel Akdeniz Ateşi; marenostrin

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Familial Mediterranean Fever (FMF, OMIM 249100) disease is an autosomal recessive autoinflammatory disease, particularly seen in the Mediterranean region. The disease is common in Turks, Armenian,

Sephardic Jewish, and Middle East Arabian whereas it is rare in other societies. Familial Mediterranean Fever is characterized by the recurrent attacks of fever and abdominal, breast and joint pains due to inflammation in serous membranes such as the peritoneum, pleura or synovia and erysipelas-like erythema accompany to these attacks. The highest complication of FMF mortality and morbidity is renal amyloidosis.[1] *Mediterranean fever* gene is localized on chromosome 16p13.3 and contains 10 exons. This gene encodes a long protein called pyrin or Marenostrin which contains 781 amino acids and its genomic DNA is approximately 15 kb.[2,3] Pyrin protein is mainly expressed by neutrophils, eosinophils, monocytes and it is expressed by skin and peritoneal fibroblasts at a lower level. Pyrin protein is localized in the cytoplasm of monocytes whereas it is found in the nucleus of neutrophils and synovial fibroblasts.[4] The diagnosis of FMF is based on clinical criteria. There is no specific laboratory test for this disease and clinical diagnosis is frequently confirmed by the screening of the *MEFV* gene mutations that are considered to be related to FMF disease. Meanwhile, there are also some other alleles with low frequency associated with FMF in the population.[5-7] There can be patients who lack the identified mutations for FMF disease, although they show typical clinical symptoms. It should be considered that there can be undefined mutations related to this disease even some patients are negative for defining FMF related genetic mutations. There are 302 FMF sequence modification records according to hereditary auto-inflammatory disease mutation database called as INFEVERS. 92 of these mutations are related to typical FMF phenotype. Mutations can be in various types such as missense or nonsense mutations, splicing and regulatory region mutations, short deletions and insertions. Mostly, the mutations gathered in 10 exons and they are missense mutations that occur as a result of nucleotide replacement. The most commonly seen five mutations can alter the structure and function of the protein. Four of them are *M694V*, *M680I*, and *V726A* on 10th exon and *E148Q* and they are located on 2nd exon. These mutations are seen in more than two-thirds of the cases.[5,6] In recent years, there have been studies related to patients who do not carry identified mutation for FMF disease, although they show typical clinical symptoms and according to the result of these studies the interpretations on *R202Q* mutations are interesting. When the *R202Q* (c.605G>A) mutation that lead to the replacement of Arginine amino acid by glutamine was first described, it was defined as a benign polymorphism.[7] Furthermore, it was also described as a frequent polymorphism that shows linkage disequilibrium with *M694V* in FMF database (<http://fmf.igh.cnrs.fr/ISSAID/infevers/search.php/>). However, in recent studies, it was revealed that it was a mutation related directly to FMF phenotype.[8,9]

In this study, we have examined the incidence of *R202Q* mutation in FMF patients and its interaction with clinical symptoms as well as we have discussed if this mutation can be a marker for FMF disease.

MATERIAL AND METHODS

146 unrelated FMF patients (89 female; 61% and 57 male; 39%) who applied to Biochemistry Department of İzmir Training and Research Hospital with FMF diagnosis and 20 unrelated healthy controls (10 female; 50%, 10 male; 50%) were included in this study. All participants, patients and healthy controls, were of Turkish origin. Control group consisted of 20 individuals without any inflammatory disease (such as diabetes mellitus, systemic hypertension and liver failure), appendicitis history and chronic abdominal or another pains who are compatible in terms of age and gender.

The mean ages of the patient and control groups were 30.21±13.10 and 37.4±13.2 years, respectively. The study protocol was approved by the Institutional Ethics Committee and written informed consent was obtained from all of the individuals. Typical clinical symptoms of the disease such as recurrent fever attacks, abdominal pain, breast pain, arthralgia and erysipelas-like erythema were queried and the first degree relatives were also questioned about the disease. The patients were divided into four groups in order to indicate the asso-

ciation between *R202Q* mutation and FMF disease: positive individuals for only *M694V* (Group 1; n=37), positive individuals for E148Q, V726A, M680I (G-C), M694I, A744S, R761H, E148Q/L110P mutations except *M694V* mutation (Group 2; n=28), positive for only *R202Q* mutation (Group 3; n=28), and negative individuals for *R202Q* mutation and all of the other frequent and rare mutations (Group 4; n=53) as shown in Table 1.

Genomic DNA was extracted from peripheral blood lymphocytes by using a commercially available DNA extraction kit (RTA Lab, Ltd. Sti, Turkey). Automatic DNA sequencing of *MEFV* gene exon 2 and 10 was performed by using "ABI PRISM Big Dye Terminator v3.1 CycleSequencing Kit (Applied Biosystems, USA)" according to manufacturer's instructions. The electrophoresis of the purified PCR products was performed by using *POP7* polymer by using ABI 3130xl Genetic Analyzer automatic capillary electrophoresis instrument for 45 minutes. DNA sequences were then analyzed by Seqscape v2.6 program.

Median and percentage ratios of demographic data were used for statistical assessments. Chi-square and Fisher's exact tests were performed in order to compare the categorical data. IBM SPSS v20.0 software was used for the analyses and the p values <0.05 were accepted as significant.

RESULTS

Frequent (*M694V*, *E148Q*, *V726A*, *M680I* G-C) and rare (*M694I*, *A744S*, *R761H*) MEFV gene mutations were identified in 65 (44.52%) of the cases. Only 28 (19.18%) patients (14 homozygotes, 14 heterozygotes) were found *R202Q* mutation positive. The allele frequency of all mutations detected in the *MEFV* gene (exon 2 and 10) in both patient and a control group (Table 2). Allele frequencies of silent mutations identified in the patients were *D102D* (36.30%), *G138G* (37.33%), *A165A* (39.73%), *A269A* (0.34%), *G286G* (0.68%), *A287A* (2.40%), *G294G* (0.34%), *P295P* (0.34%), *S747S* (0.34%), *A768A* (0.34%). While *M694V* mutation was detected in 37 of 65 mutation positive patients (56.92%), other common mutations were shown in 28 patients. 29 of 37 patients with the *M694V* mutation had also *R202Q* mutation (78.38%). This high ratio shows the linkage disequilibrium between *R202Q* and *M694V* mutations.

R202Q mutation was found in only one of the 28 patients (3.57%) who carry other common mutations. Genotype distribution of *R202Q* gene replacement (c.605G>A), and the other frequent and rare mutations in 146 FMF patients and control group (Table 3). Two of control group had homozygous *R202Q* mutation, while one of these two

TABLE 1: The relation between clinical symptoms of the patients and mutations.

		Positive *M694V*	Other positive major mutations	Groups only positive *R202Q*	Negative common and *R202Q* mutations	Total	p
Fever	n	17	12	9	25	74	0.812
	%	45.9	42.9	32.1	47.2	43.3	
Abdominal pain	n	21	19	16	36	104	0.149
	%	56.8	67.9	57.1	67.9	60.8	
Chest pain	n	13	11	5	15	52	0.502
	%	35.1	39.3	17.9	28.3	30.4	
Arthralgia	n	15	12	9	23	68	0.685
	%	40.5	42.9	32.1	43.4	39.8	
Erysipelas like erythema	n	7	4	3	9	27	0.782
	%	18.9	14.3	10.7	17.0	15.8	
Family history of FMF	n	18	10	5	13	58	0.104
	%	48.6	35.7	17.9	24.5	33.9	

FMF: Familial Mediterranean Fever.

TABLE 2: Allelic frequencies of MEFV mutations involving major, rare, and novel sequence changes.

	Patients		Controls	
Allele	**Number of alleles**	**Allele frequency (%)**	**Number of alleles**	**Allele frequency (%)**
L110P	4	1.37	0	0
E112D	1	0.34	0	0
T120S	1	0.34	0	0
*E148Q**	14	4.79	1	2.5
S159R	1	0.34	0	0
R162T	2	0.68	0	0
R202Q	86	29.45	6	15.0
T282P	1	0.34	0	0
I612L	11	3.77	0	0
I674L	1	0.34	0	0
*M680I**	14	4.79	1	2.5
*M694V**	45	15.41	1	2.5
*M694I**	2	0.68	0	0
I720L	1	0.34	0	0
*V726A**	10	3.42	0	0
*A744S**	1	0.34	0	0
*R761H**	1	0.34	0	0
D762E	2	0.68	0	0
F756L	8	2.74	0	0
S747S	1	0.34	0	0

*Common Major Mutations.

patients had *M694V* mutation in one allele. When these patients were evaluated in terms of family history and their clinics, they did not have symptoms of FMF disease.

There were no statistically significant relation between four groups, which were generated due to their mutations, according to their symptoms of classical FMF disease (p=0.812, p=0.149, p=0.502, p=0.685, p=0.782, p=0.104, respectively) and the results were given in Table 3.

DISCUSSION

There are differences between the *MEFV* gene mutation frequencies that predominantly affect populations living in the Mediterranean countries. However, the most frequent mutations such as *M694V*, *E148Q*, *V726A*, *M680I* (G-C), and *M694I* constitute 70-80% of the total mutations.[8,10] FMF disease is one of the hereditary auto-inflammatory diseases and when it is tried to be diagnosed, it shows similar clinical symptoms to the other hereditary auto-inflammatory disorders. At this stage, genetic analysis of the *MEFV* gene helps in diagnosis. Besides, since there are mutation-negative cases with typical clinical symptoms, researchers are encouraged to examine the other regions of *MEFV* gene. In recent studies, it has been revealed that *R202Q* mutation in exon 2 of the *MEFV* gene may be a marker for FMF disease. *R202Q* mutation is defined as a frequent polymorphism that shows linkage disequilibrium with *M694V* in Infevers database by the International FMF Consortium but there is no such information whether or not it is a FMF diagnosis marker. In our study, *R202Q* mutation has been the most frequently seen mutation among the patient and control groups (n=58; 39.73% and n=4; 20%, respectively) with the highest allele frequency (29.45% and 20%, respectively). In the study performed with Turkish FMF patients, Yigit et al. and Sayin Kocakap et al. have found that the inci-

TABLE 3: The distribution of major and rare mutations and *R202Q* mutation in *MEFV* gene of control and patient group.

Mutation type	Genotype	Number of patients	Number of controls
Heterozygotes	*E148Q*/wt	8	1
	M680I (G-C)/wt	7	1
	M694V/wt	4	0
	V726A/wt	5	0
	A744S/wt	1	0
	R761H/wt	0	0
	M694 I/wt	1	0
Homozygotes	*E148Q*/*E148Q*	2	0
	M694V/*M694V*	2	0
Compound and complex heterozygotes	*V726A*/*M680I*	2	0
	M694V/*M680I*	1	0
	M694V/*R761H*	1	0
	E148Q/*L110P*	1	0
	R202Q/*M694V*	9	0
	R202Q/*M694V*/*M694I*	1	0
	R202Q/*R202Q*/*M694V*/*M694V*	6	0
	R202Q/*M694V*/*M694V*	0	0
	R202Q/*R202Q*/*M694V*	6	1
	R202Q/*E148Q*/*M694V*	1	0
	R202Q/*V726A*	1	0
	R202Q/*M680I*/*M694V*	2	0
	R202Q/*R202Q*/*M680I*/*M694V*	2	0
	R202Q/*V726A*/*M694V*	2	0
Total		65	3
Heterozygotes	*R202Q*/wt	14	2
Homozygotes	***R202Q/R202Q***	14	1
Total		28	3

wt: wild type.

dences of *R202Q* mutation in patients were 57.5% and control groups was 44.7%.[8,11] Besides, Lobna et al. have detected the incidence of *R202Q* by 32.7% and 22.5% in Egyptian patients and control groups, respectively.[12] Although *R202Q* mutation was the most common mutation in societies, the *R202Q* mutation incidence was different in communities according to geographic regions.[8,11] There was no statistically significant difference between patient and control groups regarding their ratio of heterozygous or homozygous *R202Q* and *M694V* mutations (p=0.231 and p=0.123, respectively). In our study, the mutations that were already known to have the highest incidences in FMF patients (*M694V*, *M680I* (G-C), *E148Q, V726A*, *M694I*, *A744S*, *R761H*, respectively) were determined in our 65 (44.52%) patients. Our results were found to be compatible with the results of other studies conducted in various regions of Turkey.[13-16] Out of 65 patients with the combination of other frequent mutations, 30 of these patients also showed *R202Q* mutation. Furthermore, out of these 65 patients, 37 individuals were detected to carry the *M694V* mutation with the highest ratio (56.92%). *R202Q* mutation was observed in 58 (39.73%) patients by all and out of these 58 patients, *M694V* mutation was also present in 29 (50%) of the patients.The main clinical symptoms such as abdominal pain, fever, arthralgia, breast pain, erysipelas-like erythema were observed in 71.32%, 48.84%, 45.74%, 34.11%, and 17.83% all of the patients, respectively. Additionally, 35.66% of the patients were with a family history of FMF. There was no significant relationship between *R202Q* polymorphism and the clinical symptoms except abdominal pain (p=0.043). The same results were also valid for other common mutations. Our results were consistent with other studies and we have divided our patients into groups in order to understand the association of our results with *R202Q* mutation.[9] We divided the patients into four groups: the individuals only with *R202Q* mutation, only with *M694V* mutation, only with one of the other main mutations and without any mutation. There was no statistically significant difference between the groups with respect to clinical symptom incidences. Abdominal pain was the most frequent symptom in the *R202Q* positive group as it was observed in the other groups (57.1%) whereas other symptoms were also shown to have the same frequency as other groups. Our findings showed that the *R202Q* mutation rate was high in the control group (20%) and even higher in the patient group (39.72%). Besides, the frequencies of clinical symptoms of the *R202Q* positive patient group were similar to three other patient groups. All of these observations were consistent with other findings and they can suggest that *R202Q* mutation can be the reason and a

marker for FMF disease.[9,17-18] Ramirez et al. have revealed that a homozygous *R202Q* mutation positive Crohn's disease patient with recurrent fever attacks gave a response to colchicine therapy.[19] These results indicate that *R202Q* mutation will be continued to be examined considering that it can help the diagnosis of other chronic autoinflammatory disease especially as FMF. Furthermore, we believe that the increase number of studies on *R202Q* mutation will provide awareness for the alterations of pyrin protein function by mutations in other regions of MEFV gene. In conclusion, we need more precise information in order to either provide patient satisfaction or decrease the costs for diagnosis and treatment of FMF disease that is frequently seen in our country and Mediterranean region. This study indicates that *R202Q* mutation may be a reason for FMF as well as the other mutations. However, as described previously, the linkage disequilibrium between *M694V* and *R202Q* mutations which were also observed in our study, limits us to reach clear information about the potential to cause clinical signs of *R202Q* gene alteration in case of a draw with another disease-related mutation.[17] In addition, our groups which were generated according to clinical symptoms included a few number of cases due to the limited number of patients. Therefore, further large scale studies are needed to confirm our findings and the structural, functional, and biochemical effects of this mutation on pyrin protein should be analyzed by further investigations.

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