

Is *MEFV* Gene Arg202Gln (605 G>A) A Disease-Causing Mutation?

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Aim: Familial Mediterranean fever (FMF) is an autosomal recessive disease. Arg202Gln was reported as a frequent polymorphism, and G allele of the mutation was in linkage disequilibrium with M694V. Thus, the aim of this study was to determine the distribution of the R202Q (605G>A) mutation in exon 2 of the *MEFV* gene in Turkish FMF patients and controls.

Materials and Methods: The study included 160 FMF and 41 FMF/amyloid patients and 121 controls. Sequencing of exon 10 and exon 5 and PCR/RFLP analysis of E148Q and R202Q mutations of exon 2 of the *MEFV* gene were performed for all patients according to previously described techniques.

Results: We found that 5 out of 76 M694V homozygote FMF patients carry a different haplotype from the one expected. Eleven of the patients had homozygous GG allele indicating the second haplotype. None of the 121 controls was homozygous for R202Q (605G>A), but 8 controls were heterozygous for M694V mutation and 5 (4.1%) of them were in linkage disequilibrium with R202Q.

Conclusions: It seems that R202Q has no effect when it is in heterozygous state; however, when combined with another disease-causing mutation, the clinical spectrum appears. Thus, R202Q might be a disease-causing mutation at least in some of the FMF patients.

Key Words: Familial Mediterranean fever (FMF), amyloidosis, *MEFV* gene, R202Q

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MEFV Arg202Gln (605 G>A) Taşıma Hastalık Oluşturan Bir Mutasyon mu?

Amaç: Ailevi Akdeniz Ateşi (AAA) otozomal resesif kalıtılan bir hastalıktır. Arg202Gln sık görülen bir polimorfizm olmakla birlikte G allelinin M694V ile birlikte kalıtıldığı bildirilmiştir. Bundan dolayı bu çalışmanın amacı, *MEFV* geni 2. ekzonda yer alan R202Q (605 G>A) mutasyonunun Türk FMF hastalarında ve kontrollerdeki dağılımını göstermektir.

Yöntem ve Gereç: Çalışma, 160 FMF, 41 FMF/amiloid hastalarını ve 121 kontrolü kapsamaktadır. 10 ve 5. ekzonlar dizi analizi yöntemi ile, 2. ekzon E148Q ve R202Q mutasyonları için PCR/RFLP yöntemi ile daha önce tanımlanan tekniklere göre tüm bireyler taranmıştır.

Bulgular: M694V homozigot olan 76 FMF hastasının 5'i beklenenden farklı bir haplotip taşımaktadır. 11 hastada homozigot GG alleli bulunmakta ve ikinci bir haplotipe işaret etmektedir. 121 kontrolde homozigot R202Q (605 G>A) bulunmama fakat 8 kontrolden 5'i (% 4,1) M694V mutasyonu için heterozigot ve R202Q ile birleşik heterozigottur.

Sonuç: R202Q heterozigotluğu durumunda hastalık yapıcı etkisi görülmemektedir. Fakat hastalıkla ilişkili başka bir mutasyonla birlikteliğinde kliniğe etkisi görülmektedir. Bundan dolayı R202Q, en azından bazı FMF hastalarında, hastalığa neden olan bir mutasyon olabilir.

Anahtar Sözcükler: Ailevi Akdeniz Ateşi (AAA), Amiloid, *MEFV* geni, R202Q

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Introduction

Familial Mediterranean fever (FMF, OMIM 249100) is characterized by recurrent acute attacks of fever accompanied by abdominal pain, arthritis and pleurisy. The most severe complication is the development of systemic amyloidosis, ultimately leading to renal failure, but it can be prevented by the daily and life-long administration of colchicine therapy (1-2). FMF primarily affects populations surrounding the Mediterranean basin: Sephardic Jews, Armenians, Turks, Arabs, Greeks, Druze and Ashkenazi Jews. Several mutations in the gene responsible for FMF have been reported (3). Most of the previous reports are confined to exon 2 and exon 10 of the *MEFV* gene,

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as most of the mutations are clustered in these two exons (1,4). More than 150 gene alterations (polymorphisms/mutations) located in the *MEFV* gene have been identified (5).

During our *MEFV* gene mutation screening among Turkish FMF patients for molecular diagnosis, we detected R202Q (605 G>A) at exon 2 of the *MEFV* gene in some of our patients. R202Q was reported as a frequent polymorphism, and G allele of the mutation was in linkage disequilibrium with M694V (5). During our study, in some of our patients, we found that G allele was not linked to M694V.

We thus aimed to study the distribution of the R202Q (605 G>A) mutation in Turkish FMF patients and controls.

Materials and Methods

Patients

All patients were of Turkish origin, and diagnosis of FMF was based upon the published criteria (6). The patient group included 201 unrelated Turkish FMF patients, of whom 76 were homozygous M694V without amyloidosis and 84 were heterozygous for one *MEFV* mutation (M694V [n=58]; E148Q [n=14]; V726A [n=6]; M680I [n=6]). Forty-one biopsy-proven FMF-amyloidosis patients were also included. A written consent was obtained from the parents of the children.

Methods

Mutation analysis of exon 10 and exon 5 of the *MEFV* gene was performed for all patients according to previously described sequencing technique by using automatic DNA sequencer (Beckman Coulter, USA). E148Q mutation of exon 2 was analyzed with previously described polymerase chain reaction (PCR)/restriction endonuclease analysis (7,8). R202Q mutation of exon 2 was analyzed using primers F:5'-ATCTTGGGCCCTAAACGTGG-3' and R:5'-TCCTTCAGG TCCGCAGATGC-3' with an annealing temperature of 58° C. 658 bp PCR product was restricted with Pvu II, which recognizes the mutation. One-hundred twenty-one individuals without any symptoms of FMF were also included in the study as controls.

Results

Distribution of R202Q mutation among Turkish FMF patients is given in Table 1, and Table 2 shows the distribution of R202Q mutation among FMF patients with amyloidosis. We found that 5 of 76 M694V homozygote FMF patients without amyloidosis carry a different haplotype from the one expected. These patients carry G allele instead of A allele at R202Q. In screening of the patient group with only one mutation detected, 15 of these patients had homozygous AA allele. Eleven of them had homozygous GG allele indicating the second haplotype. Three heterozygous patients with

Table 1. Distribution of exon 2 R202Q mutation among Turkish FMF patients.

R202Q	GG	GA	AA
M694V/M694V (n=76)	1	4	71
M694V/- (n=58)	11	32	15
Control (n=107)	71	36	-
E148Q/- (n=14)	11	3	-
V726A/- (n=6)	6	-	-
M680I/- (n=6)	6	-	-

Table 2. Distribution of exon 2 R202Q mutation among FMF amyloidosis patients.

	Exon 2 R202Q		
	GG	GA	AA
FMF+Amyloid (n=41)	14 (34.1%)	13 (31.7%)	14 (34.1%)
No mutation detected	7	2	2
M694V/-	1	3	4
M694V/M694V	1	-	8
M680I/M694V	-	3	-
M680I/-	1	1	-
(G/A) M680I/M694V	-	1	-
M694I/-	1	-	-
M694V/K695R	-	1	-
M694V/V726A	-	1	-
M694V/E148Q	-	1	-
M680I/M680I	1	-	-
M680I/V726A	1	-	-
V726A/-	1	-	-

E148Q had heterozygous R202Q. Analysis of the amyloidosis patients revealed two patients with homozygous R202Q (AA allele) mutation. One patient with homozygous M694V mutation had homozygous GG allele. Four heterozygous patients with M694V had homozygous R202Q. One patient was in compound state for M680I and R202Q.

When we screened 121 individuals without any symptoms of FMF, none was homozygous for R202Q (605 G>A) but 41 (33.8%) were heterozygous for this mutation. It was found that 8 controls (6.6%) had *MEFV* mutations at exon 10 in heterozygous state. Seven controls (5.8%) were heterozygous for M694V mutation, consistent with the study in which M694V mutation frequency was found as 3% (9). Five controls (4.1%) were compound heterozygous with R202Q. A control with M680I heterozygosity (0.8%) was also determined.

Discussion

Debate still continues on phenotype-genotype relationship in FMF since the cloning of the *MEFV* gene (10,11). The main debate centers on the relation between the mutation type and amyloidosis. M694V, V726A and E148Q were subjects of the debates (1,12,13).

Recently, during routine mutation analysis of our FMF patients, we observed that R202Q mutation exists at exon 2 of the *MEFV* gene, which was reported as a prevalent polymorphism with linkage disequilibrium with M694V mutation (5). However, when we analyzed our

patient groups, we found that there is one more haplotype, which was not in linkage disequilibrium with M694V, indicating that this might be a disease-causing mutation at least in some FMF patients. As we found homozygous R202Q in two cases with amyloidosis, this may also confirm our hypothesis. Furthermore, when we analyzed this mutation in the control group, the expected value for R202Q homozygosity was estimated as 3%, but interestingly, R202Q in homozygote state was not found in our control group.

Recently, Giaglis et al. (14) reported *MEFV* mutation distribution in Greek FMF patients. They also stated that R202Q is common in the Greek population. In conclusion, they stated that R202Q is a disease-causing mutation; however, they did not mention the previously reported linkage disequilibrium information concerning R202Q.

One important point should be taken into account. R202Q mutation was also common in our control population. It seems that it has no effect when it is in heterozygous state. However, when combined with another disease-causing mutation, the clinical spectrum appears. Further, it is also important from the diagnostic point of view. If it is a disease-causing mutation, it will be included in the routine mutation screening. Evaluation of this mutation in different ethnic patient groups should be performed as soon as possible.

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