

Direct Detection of Common Mutations in the Familial Mediterranean Fever Gene by Using PCR Methods

AİLEVİ AKDENİZ ATEŞİ GENİNDE SIK BULUNAN MUTASYONLARIN PCR YÖNTEMİ İLE DİREKT OLARAK BELİRLENMESİ

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Abstract

Objective : The purpose of this study was to determine the frequencies of the most common Familial Mediterranean Fever (FMF) gene mutations (i.e. M694V, V726A, M680I, M694I) in Turkish FMF patients.

Material and Methods: Four common Familial Mediterranean Fever Gene (MEFV) mutations were investigated in 58 Turkish FMF patients presenting in the pediatrics nephrology departments of Ankara University and Diskapi SSK hospital. Mutation detection was tested by the amplification refractory mutation system (ARMS) and PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis in this investigation.

Result: The distribution of the four common mutations among Turkish FMF patients were determined to be 67.24% for M694V, 15.51% for V726A, 12.06% for M680I, and 5.17% for M694I, respectively. M694V mutation was established to occur in high frequency in Turkish FMF patients ($p < 0.05$).

Conclusion: According to these results, the M694V mutation was the most common among Turkish FMF patients. The study also indicated that FMF is not rare in Turkey. The ARMS technique used in this study is a rapid, effective and accurate method for detecting these four common mutations in the familial Mediterranean fever gene.

Key Words: Familial mediterranean fever, mutation

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Özet

Amaç: Bu çalışmanın amacı, Türk Ailevi Akdeniz Ateşi (FMF) hastalarında sık olarak bulunan, M694V, V726A, M680I, M694I olarak bilinen, FMF gen mutasyonlarının sıklığını belirlemektir.

Gereç ve Yöntemler: Diskapi SSK hastanesi pediatrik nefroloji ve Ankara Üniversitesi pediatrik nefroloji bölümünden elde edilen 58 Türk FMF hastasında, sık bulunan dört Familial Mediterranean Fever Gene (MEFV) mutasyonları araştırıldı. Bu çalışmada, mutasyonların taranması, PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) analizi ve ARMS sistemi test edildi.

Bulgular: Türk FMF hastaları arasında sık bulunan bu dört mutasyonun dağılımı sırasıyla, %67.24 M694V, %15.51 V726A, %12.06 M680I, %5.17 M694I olarak belirlendi. M694V mutasyonunun, Türk FMF hastalarında yüksek sıklıkla olduğu tespit edildi ($p < 0.05$).

Sonuç: Bu sonuçlara göre, M694V mutasyonu, Türk FMF hastaları arasında en sık bulunandı. Çalışmamız göstermiştir ki, Türkiye'de FMF az oranda değildir. Bu çalışmada kullanılan ARMS, FMF geninde bulunan bu dört sık görülen mutasyonların taranması için doğru, etkili ve hızlı bir metodur.

Anahtar Kelimeler : Ailevi akdeniz ateşi, mutasyon

Familial Mediterranean Fever (FMF; #249100) is an autosomal recessive disorder characterized by dramatic episodes of fever

and serosal inflammation. It affects primarily people of Mediterranean extraction, mostly sephadic Jews, Armenians, Arabs and Turks.¹ The disease is characterized by painful, recurrent, self-limited attacks of fever with sterile peritonitis, and/or synovitis.¹ Some patients develop systemic amyloidosis.²

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The clinical diagnosis of FMF, however, leads to the beginning of a daily and life-long administration of colchicine that is an efficient preventive treatment of both the attacks and amyloidosis (3). The gene responsible for FMF (designated

MEFV), located on the short arm of chromosome 16 (16p 13.3),⁴ has been identified by positional cloning.^{5,6} Four missense mutations were found in these study. Some additional mutations have been discovered since then.⁷⁻¹⁰ FMF is very common in the population at risk with estimated carrier rates of 1/6 Armenians, 1/7 in North African Jews and 1/13 in Iraqi Jews.^{11,12} We prospectively investigated a large series of unselected and unrelated patients with clinical suspicion of FMF to determine the spectrum of the mutations and confirm or invalidate the diagnosis of FMF.

Material and Methods

Patients

Peripheral blood samples were obtained from 58 unrelated FMF patients (one patient/family) treated and followed up at the pediatrics nephrology department of Ankara University and the pediatrics nephrology department of Dışkapi SSK hospital. FMF was diagnosed according to established clinical criteria.¹³ Every patient was informed about the procedures and a written approval was signed either by the patient or his /her parent.

DNA Extraction

Genomic DNA was isolated from the patients peripheral-blood leukocytes using standart procedures.¹⁴

Statistical Analysis

Statistical analysis was performed by Chi-square test. Hardy-weinberg's equilibrium and Pearson Chi-square test were used for determination of the Turkish FMF patients genotype and allele frequencies.

Mutation Analysis

Four common mutations (M694V, M680I, V726A, and M694I) were systematically investigated in the Turkish FMF patients.

Exon 10 of the gene was amplified with polymerase chain reaction (PCR) with specific forward and reverse primers (Table 1). For the identification of the M680I mutations, Hinf I (Promega, Madison, USA) restriction endonucleases were used respectively. Hinf I site was destroyed in the first case. The PCR products were analysed on 2% agarose gels.

The mutations were assessed by amplifying the genomic DNA template with three sets of normal and mutant-specific amplification refractory mutation system (ARMS) primers designed to selectively amplify the normal or altered sequence of each of the other FMF gene mutations: M694V, V726A and M694I. Each set of primers consisted of three oligonucleotides, their sequences were shown as Table 1.

Table 1. MEFV primer sequences used in this work for mutation detection.

Nucleotide	Amino acid	Exon	Mutation detection method	Primer name	Sequence 5' → 3'	PCRsize	Reference
				726 Common	TGG AGG TTG GAG ACA AGA CAG CAT GGA TCC		
2040 G → C	M680I	10	Hinf I	726 normal	TGG GAT CTG GCT GTC ACA TTG TAA AAG GAG ATG CTT CCT A	297 bp	Eisenberg et al. 1998
				M694V common M694V normal	TGA CAG CTG TAT CAT TGT TCT GGG CTC TCC G		
2080 A → G	M694V	10	ARMS	M694V mutant	TCG GGG GAA CGC TGG ACG CCT GGT ACT CAT TTT CCT TCC T	212 bp	Eisenberg et al. 1998
				M694I common M694I normal M694I mutant	TAT CAT TGT TCT GGG CTC CTG GTA CTC ATT TTC CTT C CTG GTA CTC ATT TTC CTT T	184 bp	Eisenberg et al. 1998
2177 T → C	V726A	10	ARMS	V726A common V726A normal V726A mutant	TGG AGG TTG GAG ACA AGA CAG CAT GGA TCC TGG GAT CTG GCT GTC ACA TTG TAA AAG GAG ATG CTT CCT A TGG GAT CTG GCT GTC ACA TTG TAA AAG GAG ATG CTT CCT G	247 bp	Eisenberg et al. 1998

PCR amplification was performed in a final volume of 100 µl containing 100 ng, of purified genomic DNA, 0.2 U Taq polymerase (Promega) and its 10xPCR buffer (containing 15 mm MgCl₂), 0.2 mM dNTP mix (Promega) and 1pmol of each primer. The PCR amplification conditions were kept the same for all of the ARMS test and the reaction to 94⁰C for 5 min for denaturation, followed by 35 cycles with denaturation at 94⁰C for 1 min, annealing at 60⁰C for 1 min and extension at 72⁰C for 2 min, followed by 7 min final extension at 72⁰C. The amplified products were separated by electrophoresis on 2% agarose gel was used to detect the amplified fragments.^{5,6,15}

Results

The results of the different genotypes which found in Turkish FMF patients are shown in Table 2. The patients were homozygous or compound heterozygous for MEFV mutations. Four different genotypes were characterized among 58 patients carrying four mutations. Most of them compound heterozygotes. M694V being the most common mutation, found in 67.24% (p < 0.05) of chromosomes studied. The M694I mutation, which was exceedingly rare in the Turkish FMF patients, was found 5.17% (p < 0.05) of the carrier chromosomes in our patient population. The M680I and V726A mutations were found in 12.06% (p < 0.05) and 15.51% (p < 0.05) respectively (Table 3). In the Turkish population, two genotypes were to be strongly overrepresented, the M694V/M694V 34.48% (p < 0.001), the M694V/V726A 31.34% (p < 0.001), M694V/M680I 24.13% (p < 0.001) and M694V/M694I 10.34% (p < 0.001), respectively. In addition, there were no heterozygotes among 58 Turkish FMF patients. Most of cases studied the

Table 2. Frequency of four most common mutations among Turkish FMF patients.

Mutations	n	%	χ ²
M694V/M694V	20	34.48	P < 0.001
M694V/V726A	18	31.34	P < 0.001
M694V/M680I	14	24.13	P < 0.001
M694V/M694I	6	10.34	P < 0.001

Table 3. Frequency of MEFV mutant alleles among Turkish FMF patients

	M694V	V726A	M680I	M694I
Turkish FMF patients	67.24% p < 0.05	15.51% p < 0.05	12.06% p < 0.05	5.17% p < 0.05

genotype analysis of the patients with compound heterozygosity showed that the mutations were situated on different alleles.

Discussion

The FMF gene was recently identified as the target for mutations causing FMF.^{5,6} The first missense mutations found were reported in almost 85 % of the chromosomes studied and each has been linked to a founder haplotype. In the past year, additional mutations, most of them rare, were identified in exons 2.5 and 10 of the FMF gene.⁷

In this study; we presented a mutation analysis of Turkish FMF patients. Our study confirmed the mutational heterogeneity of FMF in a Turkish population. The M694V mutation, which is found in about 67.2 of carrier chromosomes in Turkish FMF patients.¹⁶ The M680I mutation, which is known to be common in Armenians, was found in 12.06% of the carrier chromosomes of our patients.¹⁷ Our data, which included 58 FMF patients along with above finding, confirmed that these four common mutations were used to identify the molecular defect in most Turkish FMF patients.

Two preliminary studies from Israel and France have emphasized the importance of the M694V mutation. Their results indicated that M694V homozygotes generally had more severe disease and higher risk of developing amyloidosis.¹⁸ On the other hand, the Arab-Druze and Iraqi Jewish populations, that present with a milder form had a higher frequency of the V726A mutation.^{6,19}

Although the disease is frequent in Turkey, only a few Turkish FMF patients were previously studied for MEFV mutations.²⁰⁻²³ The distribution of the most common mutation among phenotype II patients was 38% for M694V.²⁰ In contrast; The distribution of the most common mutation among

Turkish FMF patients was 67.24% for M694V. The frequency of the four most common MEFV mutations among healthy individuals (M694V 3%, M680I 5%, V726A 2% and M694I 0%) were significantly different ($p < 0.005$) from that found in our FMF patients (M694V 67.24%, M680I 12.06%, V726A 15.51% and M694I 5.17%).^{21,23} Cakar N et al. Seven MEFV mutations (M694V, M680I, V726A, M694I, K695R, R761H and E148Q) were investigated in 32 patients. Six of these seven studied mutations were found in these patients and clinical diagnosis was confirmed by mutation analysis in 24 patients. Eight patients were found to have mutations on one of the alleles.

However, in our study, four common mutations were systematically investigated in 58 Turkish FMF patients. Four common mutations were found homozygous or compound heterozygous in the FMF gene of the Turkish patients. clinical diagnosis was confirmed by mutation analysis in 58 our FMF patients. All the Turkish FMF patients were found to have mutations on one or two alleles.²² Diagnostic problems persist despite increased understanding of the pathogenesis of FMF.²³ IN Turkish FMF patients, the distribution of the four common MEFV mutations was significantly different from that found in all FMF patients with typical symptoms who do not develop amyloidosis. We therefore suggest that secondary genetic or environmental factors are operative in patients with FMF.²⁰

Our data conclude that diagnosis of FMF should not be done only on the basis of clinical criteria and should include molecular studies. This is a very important point for patients at the beginning of the disease or without family history, to know if a daily long-life colchine therapy should be started or not.

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