

Mutational Analysis of the MEFV Gene in Egyptian Patients with Familial Mediterranean Fever

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Aim: Familial Mediterranean fever is an autosomal recessive disease affecting primarily populations surrounding the Mediterranean basin. The aim of this study was to find the distribution of MEFV gene mutations in a group of Egyptian patients with FMF and to evaluate any predictive genotype-phenotype correlation in this group of patients.

Materials and Methods: The study included 112 patients (59 males and 53 females). Sequencing of the exon 10, exon 3, and exon 5 and PCR/RFLP analysis of E148Q and R202Q mutations of exon 2 of the MEFV gene were performed for all the patients according to a previously described technique.

Results: Mutations in the MEFV gene were identified in 63 patients (56.25%). The most common mutation was M694I, which was detected in 9.8%, followed by V726A in 7.1%, E148Q in 5.8%, R202Q in 4.9%, M694V in 4.5%, M680I(G/C) in 3.1%, M680I(G/A) in 3.1%, and P706 in 2.6% of studied chromosomes.

Conclusions: The mutation spectrum in Egyptian patients with FMF is heterogeneous. R202Q and P706 might be disease-causing mutations and should be further investigated in more patients in different populations.

Key Words: Familial Mediterranean fever (FMF), MEFV gene, Egypt

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Mısırlı Ailevi Akdeniz Ateşi Hastalarında MEFV Geni Mutasyon Analizi

Amaç: Ailevi Akdeniz Ateşi, daha çok Akdeniz kökenli toplumlara etkileyen, otozomal resesif bir hastalıktır. Bu çalışmanın amacı, bir grup Mısırlı AAA hastasında MEFV gen mutasyonlarının dağılımını göstermek ve genotip-fenotip ilişkilendirmesini yapmaktır.

Yöntem ve Gereç: Çalışma, 112 hastayı (59 erkek, 53 kadın) kapsamaktadır. 10, 3 ve 5. ekzonlar dizi analizi yöntemi ile, 2. ekzon E148Q ve R202Q mutasyonları için PCR/RFLP yöntemi ile daha önce tanımlanan tekniklere göre tüm hastalar taranmıştır.

Bulgular: MEFV gen mutasyonları 63 hastada (% 56,25) tanımlanmıştır. Çalışılan kromozomlarda en yaygın olan M694I mutasyonu % 9,8 oranında bulunurken, V726A % 7,1, E148Q % 5,8, R202Q % 4,9, M694V % 4,5, M680I(G/C) % 3,1, M680I(G/A) % 3,1 ve P706 % 2,6 oranlarında bulunmuştur.

Sonuç: Mısır AAA hastalarında mutasyon dağılımı heterojendir. R202Q ve P706 hastalıkla ilişkili mutasyonlar olabilir ve farklı toplumlarda daha çok hastada incelenmelidir.

Anahtar Sözcükler: Ailevi Akdeniz Ateşi (AAA), MEFV geni, Mısır

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Introduction

Familial Mediterranean fever (FMF, OMIM 249100) is an autosomal recessive disorder characterised by recurrent acute attacks of fever accompanied by abdominal pain, arthritis, and pleurisy. The most severe complication is the development of renal amyloidosis, which 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. The gene responsible for FMF, *MEFV*, was identified in 1997. It is located on chromosome 16p13.3 and comprises 10 exons and 781 codons (3,4) The product of the *MEFV* gene (pyrin or marenostrin) is expressed in polymorphonuclear cells and monocytes and it is

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proposed that it regulates inflammatory responses at the level of leucocyte cytoskeletal organisation (5). More than 90 *MEFV* gene alterations (polymorphisms/mutations) have been reported, mostly in exon 10 and exon 2 (1,6,7).

Many Arab populations originating from North African populations have been investigated for *MEFV* gene mutations; however, data on Egyptian FMF patients are still lacking (8-12). The aim of this study was to find the distribution of *MEFV* gene mutations in a group of Egyptian patients with FMF and to evaluate any predictive genotype- phenotype correlation in this group of patients.

Patients and Methods

Patients:

The study included 112 Egyptian patients from 110 independent families with symptoms suggestive of FMF. Patients were referred to the Medical Genetics Centre in Cairo from different governorates and districts all over Egypt. As patients had been referred from a number of medical centres and physicians of different specialities throughout Egypt, all of them were interviewed directly by one of the Egyptian clinicians. Variables that were determined in all patients were age, consanguinity, family history, age of onset of inflammatory attacks, organs involved during the attacks (resulting in peritonitis, arthritis, pleurisy, or the presence of fever only), previous surgery, number of attacks before taking colchicine, and the response to colchicine.

Methods:

Blood was collected from patients and sent to the Paediatric Molecular Genetics Department of Ankara University. Mutation analysis of the exon 10, exon 3, and exon 5 of the *MEFV* gene was performed for all the patients according to a previously described sequencing technique using an automatic DNA sequencer (Beckman Coulter, USA) (13). E148Q and R202Q mutations of exon 2 were analysed with previously described PCR/RFLP analysis (14).

Results

Clinical data:

The study included 112 patients (59 males and 53 females); their ages ranged between 3.5 and 62 years

with a median of 16.85 years. Their age of onset ranged between 2.5 and 48 years with a median of 11 years. Parental consanguinity was present in 29.8% of patients and a positive family history of FMF was present in 22.5% of patients. The most common clinical feature was abdominal pain, followed by fever, joint pain, and lastly chest pain. None of the patients had amyloidosis. Table 1 shows the clinical data of the studied group.

Mutation analysis:

Mutations in the *MEFV* gene were identified in 63 cases (56.25%). Homozygous mutations were present in 7 cases (3 had M694I/M694I, 3 had V726A/V726A, and 1 had M680I/M680I). Twenty-one patients had compound-heterozygote mutations and 35 patients had only 1 mutation (Table 2).

The most common mutation was M694I, which was detected in 9.8%, followed by V726A in 7.1%, E148Q in 5.8%, R202Q in 4.9%, M694V in 4.5%, M680I (G/C) in 3.1%, M680I (G/A) in 3.1%, and P706 in 2.6% of the studied chromosomes (Table 3).

Table 1. Clinical data of the studied group.

Age of onset	Range Median	2.5-48 years 11 years
Consanguinity		29.8%
Positive family history of FMF		22.5%
Fever		75%
Abdominal pain		90.27%
Chest pain		15.27%
Joint pains		23.6%
Diarrhoea		16.66%
Vomiting		16.38%
Appendectomy		6.94%
Frequency of attacks	≥ 24 attacks/year 12-24 attacks/year ≤ 12 attacks/year	57.5% 27.5% 35%
Colchicine	Was not used yet Complete response Partial response No response	56.5% 13.3% 13.3% 73.3%

Table 2. FMF genotype in the studied group.

Genotype	Patients (n)
V726A/V726A	3
M694I/M694I	3
M680I(G-C)/M680I(G-C)	1
M680I(G/A)/M694I	3
M694I/E148Q	3
M694I/M694V	3
E148Q/V726A	2
M694I/M680I(G-C)	2
M694I/V726A	1
M680I(G/A)/M680I(G-C)	1
M694V/E148Q	1
M694V/R202Q	1
M680I(G/A)/V726A	1
M694V/V726A	1
P706/R202Q	2
P706/E148Q	1
R202Q/-	8
E148Q/-	6
M694I/-	5
V726A/-	5
M694V/-	4
P706/-	3
M680I(G/A)/-	2
M680I(G/C)/-	2

Table 3. Mutations detected in the studied group.

Mutation	Chromosomes (n = 224)	%
M694I	22	9.8
V726A	16	7.1
E148Q	13	5.8
R202Q	11	4.9
M694V	10	4.5
M680I(G/C)	7	3.1
M680I(G/A)	7	3.1
P706	6	2.6
Unknown	132	58.9

Parental consanguinity was present in 71.4% of patients with homozygous mutations and in 48.5% of patients with compound heterozygous mutations.

Genotype-phenotype correlation:

Table 4 shows the clinical data associated with different mutations in the studied group. Patients carrying the M680I mutation had an earlier age of onset (4.5 years), and had no arthritis or pleurisy. Fever was present in all patients carrying the V726A mutation, and chest and joint pains were observed in 75% of patients carrying the M694V mutation. The least number of attacks was present in patients carrying the R202Q and P706 mutations (50% of patients had less than 12 attacks/year). Complete response to colchicines was obtained in all patients carrying the R202Q and M680I mutations and only in 40% of patients carrying the E148Q mutation.

Discussion

In our study, mutations were detected in 56.25 % of patients, which is similar to what was previously found in a mixed Arab population and Arabs in Jordan with FMF (53.4% and 59%, respectively) (11,15). On the other hand, this percentage is less than that reported by Settin et al. (63.6%). This can be explained by the fact that our study included individuals from various governorates and districts all over Egypt and not only 1 or 2 governorates in the Delta region as done by Settin et al. (16).

Unlike the Jewish, Armenian, and Turkish populations, we did not find a single predominant mutation in Egyptian patients with FMF. The diversity of mutations among Arabs was reported before (17) and could be related to the heterogeneous origin of the Egyptian population and the effect of different civilization marks (such as Romans, Byzantines, and Ottomans beside the original inhabitants, the Arabs) left on this country since ancient times because of its unique location at the crossroads between Africa, Europe, and Asia.

The most common mutation detected in our study was M694I (9.8%), which is a common MEFV mutation found among the Arab population with a frequency varying between 13% and 21% in different studies (1,8). Indeed, M694I was considered a specific mutation to the Arab population from Maghreeb (10).

Table 4. Clinical data associated with mutations detected in this study.

		M694I	V726A	E148Q	R202Q	M694V	M680I	P706
Median age of onset		9.7 years	9.13 years	9.5 years	9 years	10 years	4.5 years	9 years
Consanguinity (%)		30	50	57	0	25	50	0
Fever (%)		84.6	100	85.7	50	75	85.7	50
Abdominal pain (%)		67.9	87.5	100	100	75	100	100
Chest pain (%)		23	25	28.57	33.3	75	0	16.6
Joint pains (%)		15.4	37.5	28.57	33.3	66.6	0	33.3
Diarrhoea (%)		23	0	0	33.3	50	0	33.3
Vomiting (%)		30.7	12.5	40	33.3	50	20	33.3
Appendectomy (%)		0	12.5	14.3	0	0	16.6	0
Number of attacks per year (%)	≥ 24	50	60	33.3	25	50	50	25
	12-24	12.5	20	33.3	25	50	25	25
	≤ 12	37.5	20	33.3	50	0	25	50
Response to colchicine (%)	No	14.3	0	40	0	33.3	0	50
	Partial	14.3	25	20	0	0	0	0
	Complete	71.4	75	40	100	66.6	100	50

The second common mutation detected was V726A (7%), which is similar to that found in a Tunisian study (5%) and less than that found in other studies on Arab populations (15%-31%). It is interesting that the 2 mutations at M680I (G to A and G to C) were present in our study group. M680I is common among Arab populations (10%), with a higher frequency in the Tunisian population (32%).

On the other hand, M694V was detected in only 4.5% of the studied chromosomes compared to the high frequencies found by other authors in Arab populations (20%-32%) (1,8). The E148Q mutation, which is recognised as the least penetrant FMF mutation, was the third most common mutation found in this study (5.8%) (18).

The previous data are different from those in El-Shanti et al.'s review on a healthy Egyptian population in which they stated that E148Q was the most common, followed by V726A and M694V (1). This difference necessitates a larger scale study of Egyptian FMF patients to be representative of the large Egyptian population.

P706 is a polymorphism located at exon 10 of the gene. We found 6 FMF patients carrying this polymorphism with a frequency of 2.6%. It is interesting

that 3 had compound status (2 with E148Q and 1 with R202Q mutation). Abdominal pain was detected in all 6 patients, with joint pains in 2 patients and chest pains in only 1 patient. P706 was first reported by Touitou et al. with a frequency of 1.6% in healthy populations and they stated that it is absent in FMF patients and present in 10.5% of the probable Behçet's disease patients (19). Arthralgia, abdominal pain, vasculitis, and uveitis were the main symptoms in those cases. They suggested that as the P706 polymorphism is located in the critical region of the *MEFV* gene it could reduce mRNA stability and/or impair gene regulation through a change in the 3-dimensional conformation of the molecule (7). Recently, in Turkish FMF patients we detected the P706 polymorphism in a heterozygous state in 6 patients (0.53%) of the 1115 FMF patients with a frequency of 0.27. Two of these patients had a heterozygous E148Q mutation at the same time, and 1 patient carried the M694V mutation in a heterozygous form (20). As some of the previously reported intraexonic polymorphisms in other disease states were found to increase the genetic susceptibility, our data in this study show that P706 has at least a role in FMF patients. P706 could have a role in inflammation because of its location in the critical region of the *MEFV* gene.

R202Q (605 G>A) is stated in the FMF database as a frequent polymorphism and G allele to be in linkage disequilibrium with M694V (7). However, during our *MEFV* gene mutation screening among Egyptian FMF patients, we found this mutation in 11 patients (4.9%) and that G allele is not linked to M694V in 10 patients indicating another haplotype. This raises the question of whether R202Q is a disease causing mutation. Recently, in Greek and Turkish FMF patients, R202Q alteration was detected homozygote state without linking to M694V (21,22).

Although genotype-phenotype correlation was difficult to establish in this study due to the diversity of mutation detected found, it was noted that the onset of the disease was very early in patients carrying M680I mutations (4.5 years) and none of these patients had arthritis or pleurisy. This mutation is commonly seen in Armenians and was suggested to be associated with a milder phenotype and lower frequency of amyloidosis (23). The absence of arthritis in patients homozygous to M680I was previously reported by Yalcinkaya et al. (24). However, this very early onset in our patient group was not reported previously.

Furthermore, arthritis and pleurisy were more common in patients carrying the M694V mutation (66%), which is consistent with previous findings reported by other investigators (25). Patients carrying the R202Q and P706 mutations had the least number of attacks per year while all patients carrying the R202Q or M680I mutation had a complete response to colchicines.

In our study, parental consanguinity was present in 29.8% of patients, which is within the range found in the Egyptian population (29%-50%) (26). Parental consanguinity was found in 71.4% of patients with homozygous mutations and in 48.5% with compound heterozygous mutations. This finding may indicate a high carrier rate and a very ancient admixture of mutations from interactions of different ethnic groups that lived in Egypt.

In conclusion, the mutation spectrum in Egyptian patients with FMF is heterogeneous and necessitates a larger scale population screening and sequencing of the whole *MEFV* gene searching for other disease causing mutations. The M680I mutation could be related to earlier age of onset and absence of arthritis and pleurisy. R202Q and P706 might be disease-causing mutations and should be further investigated in more patients in different populations.

References

1. El-Shanti H, Majeed HA, El-Khateeb M. Familial Mediterranean fever in Arabs. *Lancet* 2006; 367: 1016-24.
2. Zemer D, Pras M, Sohar E, Modan M, Cabili S, Gafni J. Colchicine in the prevention and treatment of the amyloidosis of familial Mediterranean fever. *N Eng J Med* 1986; 314: 1001-1005.
3. French FMF Consortium. A candidate gene for Familial Mediterranean fever. *Nat Genet* 1997; 17: 25-31.
4. International FMF Consortium. Ancient missense mutations in a new member of the Roret gene family are likely to cause familial Mediterranean fever. *Cell* 1997; 19: 797-807.
5. Mansfield E, Chae JJ, Komarow HD, Brotz TM, Frucht DM, Askentjevich I et al. The familial Mediterranean fever protein, pyrin, associates with microtubules and colocalizes with actin filaments. *Blood* 2001; 98: 851-859.
6. Centola M, Aksentjevich I, Kastner DL. The hereditary periodic fever syndromes: molecular analysis of a new family of inflammatory diseases. *Hum Mol Genet* 1998; 7: 1581-1588.
7. <http://fmf.igh.cnrs.fr/ISSAID/infevers/search.php>.
8. Chaabouni HB, Ksantini M, M'rad R, Kharrat M, Chaabouni M, Maazoul F et al. MEFV Mutations in Tunisian Patients Suffering from Familial Mediterranean Fever. *Semin Arthritis Rheum* 2007; 36: 397-401.
9. Mattit H, Joma M, Al-Cheikh S, El-Khateeb M, Medlej-Hashim M, Salem N, Delague V et al. Familial Mediterranean fever in the Syrian population: gene mutation frequencies, carrier rates and phenotype-genotype correlation. *Eur J Med Genet* 2006; 49: 481-486.
10. Belmahi L, Sefiani A, Fouveau C, Feingold J, Delpech M, Grateau G et al. Prevalence and distribution of MEFV mutations among Arabs from the Maghreb patients suffering from familial Mediterranean fever. *C R Biol* 2006; 329: 71-74.
11. Majeed HA, El-Khateeb M, El-Shanti H, Rabaiha ZA, Tayeh M, Najib D. The spectrum of familial Mediterranean fever gene mutations in Arabs: report of a large series. *Semin Arthritis Rheum* 2005; 34: 813-818.
12. Medlej-Hashim M, Rawashdeh M, Chouery E, Mansour I, Delague V, Lefranc G et al. Genetic screening of fourteen mutations in Jordanian familial Mediterranean fever patients. *Hum Mutat* 2000; 15: 384.

13. Akar N, Akar E, Özel D, Tekin M, Ekim M, Yalçınkaya F. A note on mutation analysis in familial Mediterranean fever. *Pediatr Nephrol* 2003; 18: 196-197.
14. Akar N, Misirlioglu M, Yalcinkaya F, Akar E, Cakar N, Tumer N et al. MEFV mutations in Turkish patients suffering from familial Mediterranean fever. *Human Mutat* 2000; 15: 118-119.
15. Al-Alami JR, Tayeh MK, Najib DA, Abu Rubaiha ZH, Majeed HA, Al-Khateeb MS et al. Familial Mediterranean fever mutation frequencies and carrier rates among a mixed Arabic population. *Saudi Med J* 2003; 24: 1055-1059.
16. Settin A, El-Baz R, AbdRasool M, El-Khalegy H, Elsayed O, El-Bendary M et al. Clinical and molecular diagnosis of familial Mediterranean fever in Egyptian children. *J Gastrointest Liver Dis*. 2007; 16: 141-145.
17. Brik R, Shinawi M, Kepten I, Berant M, Gershoni-Baruch R. Familial Mediterranean fever: clinical and genetic characterization in a mixed pediatric population of Jewish and Arab patients. *Pediatrics* 1999; 103: e70.
18. Touitou I. The spectrum of Familial Mediterranean Fever (FMF) mutations. *Eur J Hum Genet* 2001; 9: 473-483.
19. Touitou I, Touitou I, Magne X, Molinari N, Navarro A, Quellec AL et al. MEFV mutations in Behcet's disease. *Hum Mutat* 2000; 16: 271-272.
20. Akar N, Ozturk A, Ozel D, Akar E, Ekim M. Is *MEFV* P706 polymorphism important in familial Mediterranean fever patients? *J Nephrol* 2006; 19: 119-120.
21. Giaglis S, Papadopoulos V, Kambas K, Doulas M, Tsironidou V, Raffail S, Kartalis G, Speletas M, Ritis K. MEFV alterations and population genetics analysis in a large cohort of Greek patients with familial Mediterranean fever. *Clin Genet* 2007; 71: 458-467.
22. Öztürk A, Ocakar B, Ekim M, Akar N. Is *MEFV* gene Arg202Gln (605G>A) a disease-causing mutation? *Turk J Med Sci* 2008; 38: 205-208.
23. Pras M. Familial Mediterranean fever: from the clinical syndrome to the cloning of the Pylrin gene. *Scand J Rheumatol* 1998; 27: 92-97.
24. Yalcinkaya F, Cakar N, Misirlioglu M, Tumer N, Akar N, Tekin M et al. Genotype-phenotype correlation in a large group of Turkish patients with familial Mediterranean fever: evidence for mutation-independent amyloidosis. *Rheumatology* 2000; 39: 67-72.
25. Mimouni A, Magal N, Stoffman N, Shohat T, Minasian A, Krasnov M et al. Familial Mediterranean Fever: Effect of genotype and ethnicity on inflammatory attacks and amyloidosis. *Pediatrics* 2000; 105: E70.
26. Mokhtar MM, Abdel-Fattah MM. Consanguinity and advanced maternal age as risk factors for reproductive losses in Alexandria, Egypt. *Eur J Epidemiol* 200; 17: 559-565.