Analysis of common *MEFV* mutations in Egyptian patients with familial Mediterranean fever: molecular characterisation of the disease

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ABSTRACT

Familial Mediterranean fever (FMF) is a hereditary inflammatory disorder transmitted as an autosomal recessive trait. It predominantly affects people living in, or originating from, areas around the Mediterranean and was difficult to diagnose until mutations in the *MEFV* gene were identified. This study aims to analyse the five most common *MEFV* mutations in Egyptian patients diagnosed clinically as FMF. Thirty-eight unrelated patients were tested for the presence of the *MEFV* gene mutations V726A, M694V, M694I, M680I and E148Q, using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and the amplification refractory mutation system (ARMS). Twenty-three patients (60.5%) had one or more mutations, whereas no mutation was found in the remaining 15 patients (39.5%). The most common mutation was M694I (42.5%), followed by V726A (22.5%), M680I (17.5%) and E148Q (17.5%). The M694V mutation was not detected. The profile of *MEFV* gene mutations in this study suggests that the origin of FMF in Egypt is heterogeneous, a finding in concordance with that for other Arab populations; however, some differences were observed as M694V, the most common mutation reported in Arabs, was not detected in this study.

KEY WORDS: Familial Mediterranean fever.
Frequency.
MEFV gene.
Mutation.

Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive hereditary disease characterised by short, recurrent bouts of fever, accompanied by pain in the abdomen, chest and joints or with erysipelas-like erythema.[1] The disease is prevalent among populations surrounding the Mediterranean, and is an ethnically restricted genetic disease commonly found among Jews originating in North African countries, Armenians, Turks and Arabs.[2] Despite its Mediterranean origin, FMF cases have been reported in countries such as the United States and Japan.[3] Familial Mediterranean fever is the first auto-inflammatory syndrome for which a gene has been identified;[4] the gene responsible being MEFV,[5,6] which codes for the 781 amino acids pyrin protein 'marenostrin'.[7]

Mutations in the *MEFV* gene are mostly seen in exon 10.[8] At least 50 *MEFV* mutations have been associated with FMF,[9] including four conservative missense mutations (M694V, V726A, M694I and M680I) clustered in exon 10, which, together with mutation E148Q (in exon 2), account for the vast majority of FMF chromosomes in populations with a high prevelance of the disease.[10–12] In the Middle East, it is reported that these five mutations are detected in more than 85% of FMF patients.[2]

So far, the diagnosis of FMF has been based on established clinical criteria;[13] however, genetic diagnosis of FMF is essential to confirm the diagnosis in ~70% of FMF patients.[14] Moreover, genetic analysis may be very useful in preventing the occurrence of renal amyloidosis, the main complication of FMF, the occurrence of which is influenced by *MEFV* genotype.[15]

Mutation analysis study of Egyptians with FMF is limited.[16–18] Thus, the present study examines the prevalence of the five common *MEFV* Arab mutations in a total of 38 Egyptian FMF patients from an area of the Suez Canal located in north-east Egypt, a region that survived invasion, migration and settlement throughout history. In addition, the detected mutation spectrum is correlated to phenotypic features in Egyptian FMF patients.

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Materials and methods

Patients

A total of 38 unrelated Egyptian patients were referred by physicians in internal medicine clinics to the Oncology Diagnostic Unit, Suez Canal University, for FMF mutation detection during the period from May 2007 to August 2009. Clinical diagnosis of FMF was made according to the international FMF criteria.[13] An accurate detailed history of the disease was obtained including the presence of parental consanguinity and a positive family history of FMF. Clinical findings were evaluated and disease severity was scored according to previously described criteria.[19] Written

Table 1. List of the primers used for the diagnosis of common *MEFV* mutations by RFLP and ARMS.

Mutation	Primer*	Restriction enzyme	Amplification product
M694I	Common: 5'-TATCATTGTTCTGGGCTC-3'	No digestion	184 bp
	Normal: 5'-CTGGTACTCATTTTCCTTC-3		
	Mutant: 5'-CTGGTACTCATTTTCCTTT-3'		
V726A/M680I	Forward: 5'-TATCATTGTTCTGGGCTC-3'	*Alu* I/*Hinf* I	664 bp
	Reverse: 5'-CTCCGTACTTCCTCCTCT-3'		
E148Q	Forward: 5'-AACTTTAATATCCAAGGGGATTC-3'	*Ava* I	771 bp
	Reverse: 5'-TTCTCTGCAGCCGATATAAAGTA-3'		
M694V	Forward: 5'-GAGGTGGAGGTTGGAGACAA-3'	*Hph* I	275 bp
	Reverse: 5'-AGAGCAGCTGGCGAATGTAT-3'		

*Mansour *et al*.[20]

informed consent was obtained from adult patients or the legal guardians of children for blood sampling.

Mutations analysis

Anticoagulated blood (EDTA) was collected and genomic DNA was extracted using the Wizard Genomic DNA purification kit (Promega, Madison WI). Mutation analysis was performed by polymerase chain reaction (PCR) amplification, followed by restriction enzyme analysis as a rapid technique to identify the M694V, M680I, V726A and E148Q mutations. The PCR amplification was performed in a 25 µL reaction volume containing 250 ng DNA in 1x buffer containing 10 mM Tris-HCl (pH 8.3) and 1.5 mmol/L $MgCl_2$ (Promega), 2 mmol/L each dNTP, forward and reverse primers (10 pmol each; as described by Mansour *et al*.,[20] Table 1) and 2.5 units *Thermus aquaticus* (*Taq*) polymerase (Promega). Amplification was performed using the following cycling conditions: denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 58°C (for M694V and E148Q) or 52°C (for M680I and V726A) for 30 sec, and 72°C for 30 sec, followed by a final extension at 70°C for 10 min. The size of the PCR product was checked in 2% agarose gel.

Products (10 µL) were subjected to digestion at 37°C overnight with 5 units *HpH*1, *Alu*I, *Hinf* I, and *Ava*I restriction enzymes (Fermentas, USA) for M694V, V726A, M680I and E148Q, respectively. The digested PCR products were then submitted to electrophoresis in a 2.5% agarose gel to identify normal and mutant alleles (Fig. 1).

The M694I mutation was detected by the amplification refractory mutation system (ARMS). Briefly, PCR amplification was carried out in a total volume of 25 µL with 250 ng genomic DNA, 2.5 units *Taq* polymerase in 1x *Taq* polymerase buffer (Promega), 1.5 mmol/L $MgCl_2$, 2 mmol each dNTP and 50 pmol each primer. Two allele-specific sense primers, one for the normal and the other for the mutant allele, and a common antisense primer were used as previously described (Table 1),[20] generating a PCR product of 184 bp. Amplification was performed using the following cycling conditions; denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 52°C for 30 sec, and 72°C for 30 sec, and a final extension at 72°C for 10 min. Amplified products were visualised by electrophoresis in 2% agarose gel (Fig. 1).

Table 2. Genotype distribution of *FMF* gene in Egyptian patients studied.

Mutation status	Genotype	Number of patients (%)
Homozygous	M694I/M694I	3 (7.9)
	M680I/M680I	1 (2.6)
	E148Q/E148Q	1 (2.6)
Compound heterozygous	M694I/M680I	3 (7.9)
	M694I/V726A	3 (7.9)
Complex alleles	E148Q/E148Q/M694I	2 (5.3)
	M694I/M694I/M680I	1 (2.6)
	Subtotal	**14 (36.8)**
One mutation identified	V726A	6 (15.9)
	E148Q	1 (2.6)
	M680I	1 (2.6)
	M694I	1 (2.6)
	Subtotal	**9 (23.7)**
Patients with mutations		23 (60.5)
Patients without identified mutation		15 (39.5)
Total		**38 (100)**

Statistical analysis

Clinical symptoms and detected mutations were analysed statistically according to age, gender and the age of onset of the disease. Results are given as mean for age and age of disease onset and as percentage for genotype and allele frequencies.

Results

Of the 38 patients screened for *MEFV* mutations, 26 (68.4%) were males and 12 (31.6%) were females (ratio: 2.16:1). Mean age was 27.1±12.1 years (range: 6–50 years). Eleven (29%) patients were diagnosed clinically before the age of 18 (mean age of onset: 10.3±3.5 years [range: 5–15]). Mean age of the disease onset in the 27 adults (71%) was 27.2±5.6 years (range: 18–38).

A family history of the disease was found in 13% of patients, but no paternal consanguinity was recorded.

Presenting clinical features included abdominal pain (78.9%), low-grade fever (57.8%), high-grade fever (42.2%), pleuritis (21%), arthritis (15.7%) and myalgia (18.4%). None showed signs of skin involvement or amyloidosis.

Analysis of the five common *MEFV* mutations showed that 23 patients (60.5%) had at least one mutation, while no mutation was detected in the other 15 patients (39.5%) (Table 2). Of those patients with mutations, five were homozygous, six were compound heterozygous, three had complex alleles and nine had only one identified mutation (Table 2).

Distribution of the five *MEFV* mutations is shown in Table 3. The most common mutation was M694I (42.5%), followed by V726A (22.5%), M680I (17.5%) and E148Q (17.5%). No patient in the study carried M694V, a mutation reported to be one of the most frequent in Arab populations. Thus, the M694I mutation was more frequent than previously reported in Egypt.[16–18] Absence of the M694V mutation and the higher frequency of M694I indicate that the study population is genetically different from those reported earlier.

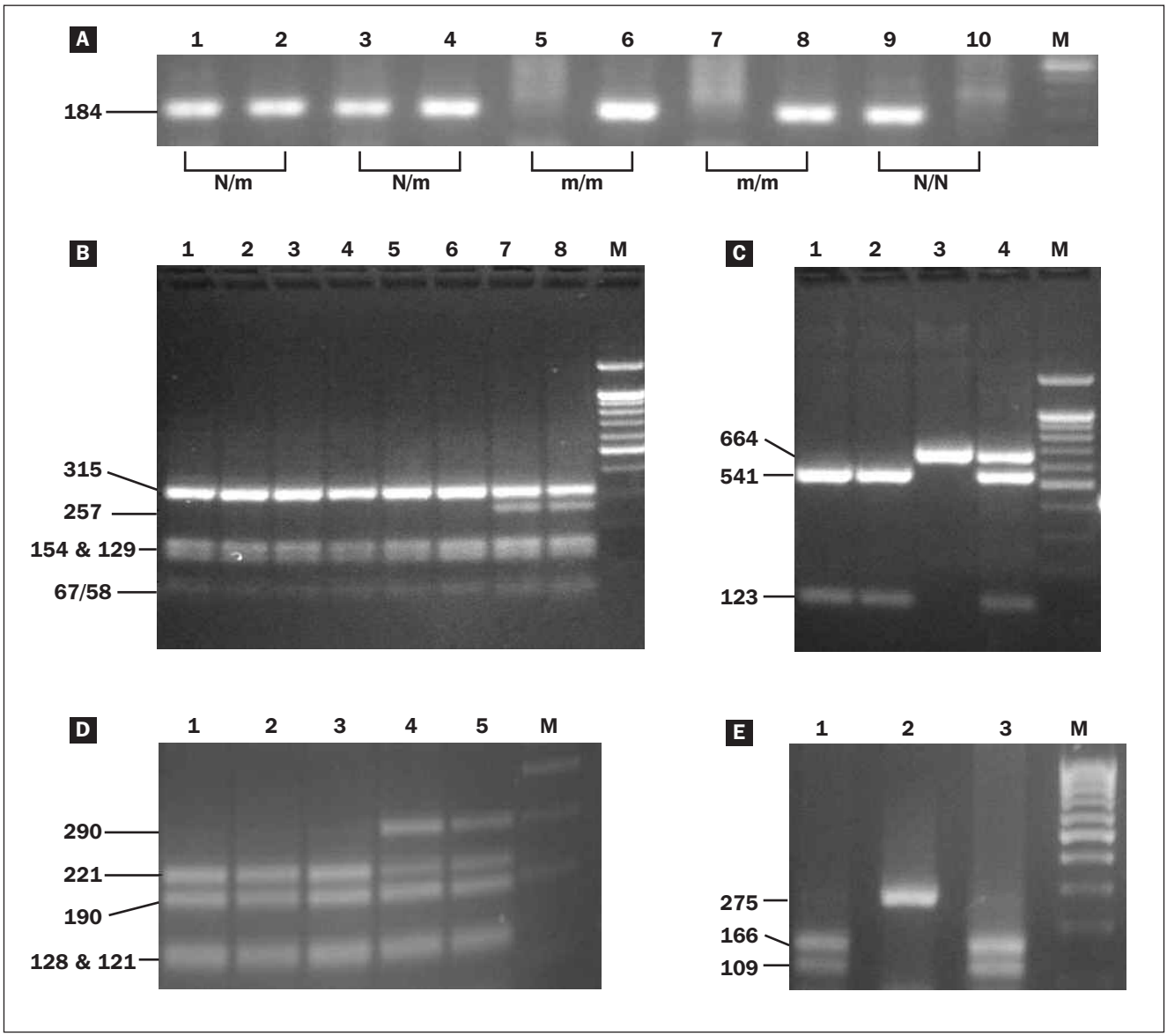

Fig. 1. Detection of *MEFV* mutations studied by restriction enzyme analysis and ARMS. (A) Electrophoresis pattern of M694I mutation detected by ARMS. Each patient is represented by two lanes (normal and mutant). N/N: normal homozygous allele, N/m: mutation in a heterozygous form, m/m: homozygous mutation. (B) Electrophoresis pattern of the V726A mutation. Lanes 1b–6b: normal alleles (the 664-bp PCR product digested into 315, 154, 129 and 67 bp fragments); lanes 7b and 8b: heterozygotes showing the 257-bp fragment characteristic of the mutant allele, together with the four fragments of the normal allele and a 58 bp fragment (not well separated from the 67-bp fragment). (C) Electrophoresis pattern of the M680I mutation. Lanes 1c and 2c: normal alleles where the amplified product (664 bp) is digested by the *Hinf* I restriction enzyme into two fragments (541 and 123 bp), lane 3c: homozygous mutant allele (664 bp), lane 4c: heterozygote showing the three fragments. (D) Electrophoresis pattern of the E148Q mutation. Lanes 1d–3d: homozygous normal alleles (the 771 bp fragment digested by the *Ava* I enzyme into 221, 190, 128, 121, 69 and 42 bp fragments – last two fragments are not shown), lanes 4d and 5d: heterozygous for the mutation showing the 290-bp fragment of the mutant allele and the other six fragments of the normal allele. (E) Electrophoresis pattern of the M694V mutation. Lanes 1e and 3e: homozygous normal allele (the 275 bp amplified product digested by the *Hph* I enzyme into 166 and 109 bp fragments), lane 2e: undigested amplified product (275bp). M: 100-bp molecular weight marker.

Severity of disease phenotype and the genotype were correlated in all patients (Table 4). Phenotype severity was evaluated using a score of 26 points (≥14: severe, 8–13: moderate, 2–7: mild).[19] One patient showed a mild phenotype (2.6%), 28 (73.7%) showed a moderate phenotype, and nine (23.7%) showed a severe phenotype, including one patient homozygous for M694I, two patients with the M694I/V726A genotype, one patient with a complex genotype (M680I/M694I/M694I) and one patient heterozygous for V726A. The remaining four severe phenotype patients showed no mutation.

Table 3. Distribution of the five MEFV mutations among the 23 patients with identified mutations

Mutation	Number of mutations (%)
M694I	17 (42.5)
V726A	9 (22.5)
M680I	7 (17.5)
E148Q	7 (17.5)
M694V	0 (0)
Total	40 (100)

Discussion

Familial Mediterranean fever is an established common genetic disease in Arabs, with a prevalence of about one in 25 and a carrier rate of about one in five.[21] The results of the *MEFV* gene mutation assessment are in concordance with the findings of Öztürk *et al.*,[17] who showed that the most common mutations in Egyptian patients referred from across Egypt were M694I (9.8%), V726A (7.1%), E148Q (5.8%), M694V (4.5%), M680I (G/C; 3.1%) and M680I (G/A; 3.1%).

The M694V allele was not detected in the present study but was recorded to be the most frequent mutation by Settin *et al.*,[18] who studied FMF patients from the Nile Delta region in Egypt. M694V was also the second most frequent allele reported by El-Garf *et al.*[16] This heterogeneous mutation spectrum could be attributed to the historical and geographical background of Egypt that was controlled by a succession of powerful empires. This heterogeneity is also a reflection of the diversity of the mutation pattern among Arabs, as most of the Egyptian population is now of Arab origin. Oriental Arabs show a higher prevalence of V726A than Arabs from North Africa, while M694I seems to be relatively specific to Maghrebins.[22]

The most common mutation in this study was M694I, which is known to be the third most common mutation in Arabs.[23] It is found mainly in Armenians, Turks and Arabs,[23–25] which explains how this mutation was carried to Egypt. It is described as specific to North African Arabs[26] and accounts for 10–17% of the identified mutations in different studies.[20,23]

The V726A mutation was the second most common in the present study, as it is in Armenians, Arabs, Turks, and Jews.[19,24,25,27] The M680I and E148Q mutations each represented 17.5% of those identified. This is consistent with data published on FMF patients from Arab and Turkish populations where these mutations represent the third, fourth or fifth most common.[23,28–30]

No mutations were detected in 39.5% of the patients tested, and is consistent with the results of other studies conducted on Arab populations.[21,31,32] However, some patients in the present study may have mutations not covered by the analysis spectrum or have mutations in other FMF-causing genes.[3] Furthermore, similarities in FMF symptoms with other diseases could be misleading.

In the present study, abdominal pain was the most common symptom (78.9%), with similar frequencies observed in Turks, Jews and Arabs.[2,21,33,34] Fever was the second most common symptom, followed by pleuritis, arthritis and myalgia. Skin involvement and amyloidosis were not reported. Different manifestations of FMF have been observed among populations of varying ethnicity. Fever and peritonitis were present in more than 90% of patients, joint involvement is the next most common symptom of FMF, being more common among non-Ashkenazi Jews than in Turks, Arabs or Armenians.[3]

Table 4. Mean severity score by genotype and the clinical picture in function of the genotypes.

Genotype	Number of patients	Severity score (mean)	Thoracic pain	Arthritis	Myalgia	Amyloidosis
M694I/M694I	3	13.3	–	—	–	–
M680I/M680I	1	9	1	–	–	–
E148Q/E148Q	1	13	–	–	–	–
M694I/M680I	3	13	–	–	–	–
M694I/V726A	3	13.7	–	–	–	–
E148Q/E148Q/M694I	2	12.5	–	–	–	–
M694I/M694I/M680I	1	14	1	1	–	–
V726A/–	6	11.2	1	–	–	–
E148Q/–	1	13	–	–	–	–
M680I/–	1	12	–	–	–	–
M694I/–	1	10	–	–	–	–
–/–	15	11.6±2.5	3	3	5	–
Total	38		6	4	5	0

The absence of amyloidosis in the present study may be related to the absence of the M694V allele, which is known to be associated with more severe disease.[19,35] It may also reflect the importance of the early introduction of colchicine in controlling the disease.[36]

In the current study, seven genotypes were identified, six carrying compound heterozygous alleles, five had homozygous alleles and three carried complex alleles. No parental consanguinity was reported in this study despite the high rate of consanguineous marriage (58.8%) reported in the Egyptian population.[37] Two cases of complex allele had an E148Q/E148Q/M694I genotype and one had an M694I homozygous/M680I genotype, the latter showing the highest severity score and presenting with abdominal pain, fever, chest pain and arthralgia.

Interestingly, all patients who carried the M694I mutation in homozygous or compound heterozygous form had the highest mean severity score. This mutation located within codon 694 is associated with severe disease and early onset, high frequency of attack, the need for high-dose colchicine to control attacks, and a high frequency of amyloidosis in untreated patients.[22,38]

The least penetrant mutation is E148Q and has only mild effect in FMF,[22] and has been suggested to be a polymorphism.[39,40] However, when E148Q is part of a complex allele it is thought to have an aggravating effect, with dominant transmission when allelic to M694I with a second wild-type allele.[41] This was seen in two of the current patients who carried the complex alleles E148Q/E148Q/M694I and showed a mean severity score of 12.5. Moreover, several studies have indicated a role for E148Q in the diagnosis of FMF, and it is suggested to have an important role in disease pathophysiology.[34,42]

The mechanism by which common *MEFV* gene mutations and/or the involvement of other effector gene mutations affect the development of FMF is unknown, and whether the co-inheritance of one or more specific variants may increase the risk of developing FMF. Clearly, further study of *MEFV* gene mutations is warranted.

In conclusion, this preliminary study highlights the value of simple molecular biology tests in the detection of common mutations in the *MEFV* gene in order to establish a genetic diagnosis. Demonstration of the most common mutations can establish a definite diagnosis in suspected patients, and this is especially helpful to clinicians treating patients with FMF. As few research studies have been conducted on *MEFV* gene mutations causing FMF in Egypt,[16–18] the present study may add knowledge to the mutational data on the disease in this population. The spectrum of FMF mutations in Egyptians is somewhat different from that reported previously in Arab populations, and the current data will be valuable in the implementation of a counselling service for FMF patients and their families. □

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