COMMON MEFV EXON 10 MUTATIONS ANALYSIS IN EGYPTIAN PATIENTS WITH FAMILIAL MEDITERRANEAN FEVER: GENOTYPE-PHENOTYPE CORRELATION

By

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ABSTRACT

Background: Familial Mediterranean Fever (FMF) is the prototype of a group of inherited inflammatory disorders. FMF is an autosomal recessive condition that primarily affects population of the Mediterranean basin. If undiagnosed effectively and treated with colchicine for life it may lead to serious consequences in terms of renal amyloidosis and renal failure. The gene (MEFV) responsible for this disease comprises 10 exons and 781 codons. Five found mutations, V726A, M694V, M694I, M680I and E148Q account for 74% of FMF chromosomes from typical cases (Armenians, Arabs, Jews, and Turks. **Aim of the work:** The aim of our study was to characterize the *MEFV* gene mutations in exon 10 in clinicaly diagnosed patients, and to examine whether there is a correlation between the different mutations and the clinical symptoms in the affected individuals. **Subjects and Methods:** The present study has been carried out on 62 patients presenting to the Internal Medicine and Tropical Medicine Departments of Zagazig University Hospitals and outpatient clinics of Zagazig University hospitals from May 2007 to April 2010, and 10 healthy volunteers as a control group . We used PCR for amplification of the region that the four common mutations in exon 10 followed by sequencing to detect Exon 10 mutations (M694I, M694V, V726A and M680I). **Results:** Analysis of exon 10 0f *MEFV* gene showed that 42 (67.7%) had 1 or 2 identifiable mutations. Of the 42 patients with mutations, 17 were homozygous, 11 were compound heterozygous, and 14 had only 1 identifiable mutation. The most frequent mutation was M694I in 18 (29%) followed by V726A in 12 (19%), M694V in 8 (13%), M680I (G/C) in 6 (9.6%) and M680I (G/A) in 3 (4.8%). While 20 patients were negative for the studied mutations. Patients carrying the M680I mutation had an earlier age of onset (2.5 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 M680I. Complete response to colchicine therapy was obtained in all patients carrying M680I. **Conclusions:** These findings underscore the importance of performing molecular studies on all suspected FMF patients. In addition to providing accurate diagnosis, these tests allow identification of presymptomatic genetically affected individuals, detection of the carriers and assessment of the risk for amyloidosis in later life. PCR technique provides a rapid, reliable, cost-effective and noninvasive test for establishing a diagnosis of FMF in symptomatic patients and also provides a rational basis for medical and genetic counseling of FMF patients and their families.

INTRODUCTION

Familial Mediterranean fever (FMF) is an autosomal recessive inherited auto-inflammatory disorder, which is frequent in populations originating from the Mediterranean basin[(1)]. The disease is characterised by recurrent short episodes of inflammation and serositis including fever, peritonitis, pleuritis, synovitis and rarely pericarditis. Amyloidosis, similar to that seen in other chronic inflammatory diseases such as rheumatoid arthritis, is the most severe complication of FMF and leads to renal failure[(2)]. FMF peritonitis, the most common

manifestation of this disease, may resembles acute abdomen, leading to laparotomy and appendectomy that reveal only an inflamed peritoneum and neutrophilic exudates. If a surgical procedure is avoided, the attack resolves spontaneously[3]. In these cases, proper genetic consultation may suggest early introduction of colchicine. The MEFV gene locates on the short arm of chromosome 16 and includes 10 exons, and it encodes 781-amino-acid proteins. The protein encoded by the MEFV gene has been named pyrin by an American group for its role in anti-pyrexia. It has been hypothesized that the wild-type pyrin normally regulates inflammation via apoptotic speck-like protein. In FMF, however, the pyrin derived from the mutated gene seems to lose the ability to regulate the normal inflammatory process, particularly that part of the process due to the production of IL-1β and nuclear factor-kB (NF-kB).(3) Pyrin belongs to a class of proteins involved in the regulation of apoptosis and inflammation. N-terminal pyrin domain interacts with the adaptor protein, regulating caspase-1 activation and consequently, IL-1β production. Mutations interfere with the role of the pyrin domain, allowing an uninterrupted inflammatory cascade[4]. Recently 142 mutations have been identified in the MEFV gene, most of which are substitutions. Of these mutations, five account for more than 70% of FMF cases - V726A, M694V, M694I, M680I and E148Q, and have different frequencies in classically affected populations. Forty-eight of the MEFV mutations are found in exon 10[5,6]. Mutation E148Q in exon 2 was found to be the second most common mutation occurring in patients of several ethnicities with different haplotypes[7]. Exons 2 and 10 are the most frequent mutation regions of the MEFV gene. Half of the FMF population carries two mutations, while 30% and 20% carry a single mutation and no identifiable mutations, respectively[8]. Colchicine has been shown to be effective in preventing the attacks of FMF as well as the development of amyloidosis[9,10].

The aim of our study was to characterise the MEFV gene mutations in exon 10 in clinicaly diagnosed patients, and to examine whether there is a correlation between the different mutations and the clinical symptoms in the affected individuals.

SUBJECTS AND METHODS

The present study has been carried out on 62 patients presenting to the Internal Medicine and Tropical Medicine Departments of Zagazig University Hospitals and Outpatient Clinics of Zagazig University hospitals from May 2007 to April 2010, and 10 healthy volunteers as a control group. A written consent had been obtained from patients and controls. All patients were subjected to full analytic history taking and clinical examination either denovo or already diagnosed with irregular use of colchicine, use only during the attacks or not using colchicine therapy at all.

Clinical Scoring was evaluated according to Tel-Hashomer Criteria for diagnosis of FMF[10] :

Major Criteria:

1. Recurrent febrile episodes accompanied by peritonitis, pleuritis, or synovitis.
2. Amyloidosis of A type without predisposing disease.
3. Favorable response to continuous colchicine treatment.

Minor Criteria:

A. Recurrent febrile episodes.
B. Erysipelas - like erythema.
C. positive history of FMF in first degree relatives; Definite Diagnosis= 2 major, Or 1 major + 2 minor, Probable diagnosis = 1 major + 1 minor.

In this study we use PCR for amplification of the region that the four common mutations in exon 10 followed by sequencing to detect Exon 10 mutations (M694V, V726A, M680I and M694I). and to

determine whether the clinical severity of the disease phenotype correlates with the nature of the mutations among the patients.

1- DNA extraction and purification :

Venous blood sample (~3 ml) from each patients were collected on EDTA containing tubes, DNA was extracted promptly using DNA extraction QiAamp Blood kit supplied by Qiagen according to manufacturer's instructions and then stored at -20° C till use.

2- Mutation identifications:

The region that the four common mutations in Exon 10 was amplified with PCR and specific primers:10F1, 5'ccagaagaactaccctgtccc-3' and 10R1, 5'-cagagcagctggcgaatgtat-3'. PCR conditions were denatured at 95°C for 10 min; 30 cycles of 95°C for 15 s, 55 for 30 s and 72 for 3 min, with a final extension at 72 for 10 min. PCR products were purified with Sephadex P100 chromatography and sequenced directly, using specific primers and AmpliTaq FS Dye Termination cycle sequencing kits.

RESULTS

The study included 62 patients. Their ages ranged from 2 to 66 years (median ,17 years) and consanguineous marriages were present in about 36% of the families and positive family history of MFM was present in 17 (28%). The male : female ratio was 1.5:1. The main clinical characteristics of the patients were as shown in Table (1): peritonitis was observed in 62 (100%), fever in 24 (38.7.4%), arthritis in 24 (38.7%), myalgia in 18 (28%), pleuritis in 20 (32%), 14 (22 %) of cases had undergone surgery 10 cases for appendectomy and 4 cases for laparotomy. Proteinuria (suggestive for renal amyloidosis) was found in 2 (3%). Fifty patients were treated with colchicine. A positive response to colchicine treatment was noticed in 37 (74%) patients,while12 patients showed complete response, 7 patients showed partial and 18 patients showed no response.

Mutation analysis:

Analysis of exon 10 0f MEFV gene showed that 42 (67.7%) had 1 or 2 identifiable mutations. Of the 42 patients with mutations, 17 were homozygous, 11 were compound heterozygous, and 14 had only 1 identifiable mutation. The most frequent mutation was M694I in 18 (29%) followed by V726A in 12 (19%), M694V in (13%) , M680I (G/C) in 6 (9.6%) and M680I (G/A)in 3 (4.8%). While 20 patients were negative to the studied mutations (Table 2).

Genotype-phenotype correlation:

Table (3) shows the clinical data associated with different mutations in the studied group. Patients carrying the M680I mutation had an earlier age of onset (2.5 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 and complete response to colchicine therapy were obtained in all patients carrying M680I.

Table (1) : Clinical Data of FMF patients

Clinical Data	No	(%)
Median age of onset (Range)	17 (2-66)	
Consanguinity	22	36%
Family history	17	28%
Fever	24	38.7%
Peritonitis	62	100%
Pleuritis	20	32%
arthritis	24	38.7%
myalgia	18	28%
Vomiting	62	100%
Surgical operations done	14	22%
Number of attacks per year		
≥ 24	28	45%
12-24	12	20%
≤ 12	22	22%
Response to colchicine		
No	18	50%
Partial	7	18%
Complete	12	32%

Table (2) : FMF genotype among the patients

Genotype	Number of cases
M694I/M694I	7
M680I(G-C)/M680I(G-C)	2
V726A/V726A	5
M694V/M694V	3
M694I/M694V	4
M680I(G/A)/M694I	1
M694I/M680I(G-C)	2
M694I/V726A	2
M680I(G/A)/M680I(G-C)	1
M694V/V726A	1
M694I/-	3
V726A/-	4
M694V/-	2
M680I(G/A)/	1
M680I(G/C)/	1

Table (3): Genotypye/ Phenotype correlation among the studied FMF patients

Mutation Clinical data	M694V (n=10)	M694I (n=18)	V726A (n=12)	M680I (n=9)
Median age of onset (Range)	11	10	9	2.5
Consanguinity	5(50%)	6 (34%)	6 (50%)	3 (28%)
Family history	6 (60%)	9 (50%)	3 (25%)	2 (18%)
Fever	10 (100%)	18 (74%)	10(86%)	3 (30%)
Peritonitis	10 (100%)	13 (72%)	11 (90%)	2 (27%)
Pleuritis	6 (60%)	4 (22%)	3 (25%)	0 (0%)
Arthritis	4 (40%)	3 (14%)	3 (23%)	0 (0%)
Myalgia	8 (80%)	8 (44%)	6 (50%)	4 (44%)
Vomiting	4 (40%)	4 (20%)	6 (50%)	2 (22%)
Number of attacks per year				
≥ 24	5 (50%)	10 (56%)	6 (50%)	0 (0%)
12-24	3 (30%)	7 (39%)	4 (33%)	3(33%)
Response to colchicine				
≤ 12	2 (20%)	1 (5%)	2 (17%)	6 (67%)
No	7 (70%)	8 (44%)	5 (42%)	0 (0%)
Partial	3 (30%)	6 (33%)	5 (42%)	2 (22%0
Complete	1 (10%)	4 (23%)	2 (16%)	7 (78%)

DISCUSSION

In our study the most frequent clinical manifestations is peritonitis and vomiting followed by arthritis, fever, pleuritis and myalgia while suspicion of amyloidosis by proteinuria in 2 patients. This is similar to a study on a group of Arab patients; the most common manifestations were peritonitis (93.7%), arthritis (33.7%) and pleurisy (32%). The authors reported a lack of manifestations of amyloidosis, skin lesions, organomegaly and lymphadenopathy[12,13]. And also agree with that noticed by a Turkish study[14] and another study on mixed populations of Sephardic Jews,Armenians, Arabs, Turks, French and others.

In our study, mutations were detected in 67.7% of patients, which is similar to what was previously reported by Settin et al. (63.6%) and less than that in a mixed Arab populations and Arabs in Jordan with FMF (53.4% and 59%, respectively)[15,16]. This may be explained by that our patients were selected from Delta region as that was done by settin et al**.(3)** while the other studies were carried on different regions. The most common mutations detected in our study were M694I in 18 (29%) followed by V726A in 12 (19%), M694V in 8 (13%), M680I (G/C) in 6 (9.6%) and M680I (G/A)in 3 (4.8%). While 20 patients were negative for the studied mutations.

Indeed, M694I was considered a specific mutation to Arab populations from Maghreeb[17]. The second common mutation detected was V726A, which is similar to that found in studies on Arab populations (15%-31%) and less than that of the Tunisian study (5%)[15,18,19].

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 populations (32%)[15]. On the other hand, M694V was detected in (13%) less in frequencies found by other authors in Arab populations (20%-32%)[18].

According to the association between genotype and phenotype, it was noted that the onset of the disease was very early in patients carrying M680I mutations (2.5 ears) and none of these patients had arthritis or pleurisy and (78%) of patients with M680I mutation had a complete response to colchicine. his mutation is commonly seen in Armenians and was suggested to be associated with a milder phenotype and lower incidence of amyloidosis[20]. The absence of arthritis in patients homozygous to M680I was previously reported by Yalcinkaya et al.[21].

In our patients carrying M694V mutation arthritis, pleurisy, fever and myalgia were more common which is consistent with the previous findings reported by other investigators as it is associated with severe phenotype[22].

Based on our findings, it appears that homozygous for M694V are at high risk to amyloidosis as the only 2 patients had proteinuria were homozygous for M694V, and should be treated with colchicines for life[23]. This in agreement with, If further studies confirm these findings, the question of treating asymptomatic individuals homozygous for the Met694Val mutation with colchicine will need to be addressed[24].

In our study, parental consanguinity was present in (36%) of patients, which is within the range found in the Egyptian populations (29% - 50%)[25]. Parental consanguinity was found more in patients with homozygous mutations than that with compound heterozygous mutations. This finding may indicates a high carrier rate .

In this study the genotype-phenotype correlation was difficult to be established due to the diversity of detected mutations and the high percentage of the patients who didn't show mutations as we just studied the common four mutations in exon 10, we need to investigate a large number of patients.

CONCLUSIONS

These findings underscore the importance of performing molecular studies on all suspected FMF patients. In addition to providing accurate diagnosis, these tests allow identification of presymptomatic genetically affected individuals, detection of the carriers and assessment of the risk for amyloidosis in later life. PCR technique provides a rapid, reliable, cost-effective and noninvasive test for establishing a diagnosis of FMF in symptomatic patients and also provides a rational basis for medical and genetic counseling of FMF patients and their families.

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الطفرات الشائعة للإكسون- ١٠ لجين (MEFV) في مرضي حمي البحر الأبيض المتوسط وربطها بالطرز المظهري للمرض

المقدمة :

تعتبر حمي البحر الأبيض المتوسط من الأمراض الالتهابية الوراثية وهي صفه وراثية متنحية وغير مرتبطة بالجنس تنتشر بين سكان حوض البحر الأبيض المتوسط وفي حاله عدم التشخيص المبكر للمرض وإعطاء عقار الكولشيسين قد تسوء الحالة ويحدث أميلويدوزيز بالكلي وتسريب زلال بالبول.ويعتبر جين (MEFV) هو المسئول عن حدوث المرض وللجين عشره إكسونات و(781) كودون. وتوجد خمس طفرات للإكسون-١٠ وهي (E148Q-M680I-M694I-V726A-M694V) وتوجد هذه الطفرات في حوالي (٧٤) من الحالات المصابة.

الهدف من البحث :

دراسة طفرات الإكسون -١٠ لجين(MEFV) بين حالات حمي البحر الأبيض المتوسط وإرتباطها بالطرزالمظهري للمرض.

المرضي وطرق البحث :

لقد أجريت هذه الدراسة علي (٧٢) فرد من بينهم (٦٢) مصاب بحمي البحر الأبيض المتوسط و(١٠) أفراد متطوعه كمجموعه مقارنه في الفتره من مايو(٢٠٠٧) حتي أبريل (٢٠١٠).بقسمي الباطنة العامة وقسم الأمراض المتوطنة وكذلك العيادات الخارجية بمستشفيات جامعه الزقازيق.

ولقد تم إجراء الأتي للمرضي:

١. إقرار كتابي بالموافقة علي المشاركة في البحث .
٢. 2- أخذ التاريخ المرضي والفحص الإكلينيكي الشامل.
٣. تم تشخيص الحالات علي حسب مواصفات تل- هاشومير.
٤. استخلاص (DNA) من الحالات.
٥. التعرف علي الطفرات الجينية وتصنيفها.

النتائج:

بتحليل نتائج طفرات الإكسون – ١٠للجين(MEFV) تبين الأتي:

- (٤٢) مريض (67.7%) لديهم طفرة أو طفرتين.
- الطفرة الأكثر حدوثا هي(M694I) حيث وجدت في (18) مريض (92%).
- الطفرة (V726A) وجدت في(١٢) مريض (19%).
- الطفرة (M694V) وجدت في ثمانية مرضى (13%).
- الطفرة (M680I G|C) وجدت في ستة مرضي (9.6%).
- الطفرة (M680I G|A) وجدت في ثلاثة مرضي (4.8%).
- لم تسجل طفرات في عشرين مريض.
- المرضي الذين لديهم طفرة (M680I) لديهم إصابة بالمرض
- منذ الطفولة وليس لديهم إصابة بالمفاصل ولا الغشاء البلوري.
- المرضى اللذين لديهم طفرة (V726A) يعانون من حمي متكررة.
- المرضى الذين لديهم طفرة (M694V) لديهم معاناة من التهاب بغشاء البلورا والمفاصل.
- الطفرة (M680I) مصحوبة بنوبات نادرة للمرض واستجابة جيده للكولشيسين.

الاستنتاج :

- أهميه التشخيص المبكر للمرض واكتشاف حاملي المرض وتقييم مدي إصابة الكلي بالأميلويدوزيز.
- تحليل PCR للجينات يعتبر اختبار جيد وسريع وصادق لتشخيص الحالات التي لم يظهر عليها المرض ويعطينا المبرر للاستشارات الجينية لمرضي حمي البحر المتوسط وعائلاتهم.