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Common MEFV mutations in Egyptian patients with familial Mediterranean fever

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ABSTRACT

Background: Familial Mediterranean fever (FMF) which is an autosomal recessive condition that primarily affect 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.
Objectives: We aim to check for the presence of FMF mutations in clinically suspected Egyptian patients, as an important step for family counseling and case management.
Subjects and Methods: The study is a pilot study to check for the presence of FMF mutations among suspected cases (24 cases) from Sharkia Governorate. The control subjects (24) were selected from healthy volunteers. We examined FMF mutations by PCR technique for MEFV gene analysis in order to establish a diagnosis of FMF by examining two mutations, M694V and E148Q.
Results: We found 58.3% (14/24 cases) of cohort were positive for M694V mutation, and all cohort were negative for E148Q mutation. The normal controls were negative for previous two mutations.
Conclusions: PCR technique provides a rapid, reliable, cost-effective, noninvasive, and sensitive 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.

Key Words: FMF, MEFV, mutations, Egypt.

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INTRODUCTION

Familial Mediterranean fever (FMF) is an autosomal recessive inherited auto-inflammatory disorder, which is frequent in populations originating from the Mediterranean basin.[1] It is a febrile disease characterized by acute, spontaneously resolving episodes of fever and pain caused by serosal inflammation and associated with mutations in the FMF gene, (MEFV).[2] The MEFV gene was independently cloned by American and French groups in 1997: 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. Its N-terminal pyrin domain interacts with the ASC 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]

The MEFV gene locates on short arm of chromosome 16 and includes 10 exons and it encodes 781-amino-acid protein. To date, 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]. Mutation E148Q in exon 2 was found to be the second most common mutation occurring in patients of several ethnicities with different haplotypes[6]. 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 mutation, respectively.[7]

The FMF disorder is characterized by recurrent episodes (Exacerbations and remissions) of unprovoked inflammation involving the joints; the pleural and peritoneal cavities; and less frequently; the skin. FMF peritonitis, the most common manifestation of this disease, may resemble 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[8]. In these cases, proper genetic consultation may suggest early introduction of colchicine.

Many Egyptian populations have been investigated for MEFV gene mutations. However, data from FMF patients from Sharkia region are still lacking. So the purpose of this study is to use PCR technique for MEFV gene analysis in order to establish a diagnosis of FMF in symptomatic patients and to perform a preliminary population genetic study in Sharkia region.[9]

SUBJECTS AND METHODS

The study is a pilot study to check for the presence of FMF mutations among suspected cases (24 cases) from Sharkia Governorate presenting to the Internal Medicine and Tropical Medicine Departments of Zagazig University Hospitals and outpatient clinics of Zagazig university hospitals during the years 2007-2009. The control subjectswere selected from healthy volunteers. The study and the control group were matched in regard of age and sex. Informed consent had been obtained from patients and controls. All patients were subjected to full analytic history and clinical examination, including: age, sex, consanguinity, family history of FMF, attacks of abdominal pain, arthralgia or arthritis, chest pain, bone aches, renal affection, duration of attacks, effect of colchicine on frequency

and duration of attacks, organ involvement. Routine investigations specially Urine analysis were also done.

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[3]
C. positive history of FMF in first degree relative; Definite Diagnosis= 2 major, Or 1 major+2 minor, Probable diagnosis 1 major+1 minor.

In this study we use standard PCR technique for MEFV gene analysis in order to establish a diagnosis of FMF by examining two mutations, M694V and E148Q and to determine whether the clinical severity of the disease phenotype correlates with the nature of the mutation among Egyptians.

1- DNA extraction and purification

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

2- Quantification of genomic DNA

Spectrophotometric optical densities of 260nm and 280nm were used to investigate the DNA quantity. DNA purity was measured using the appropriate ratio of OD260: OD280 (1.65-1.85). Concentrations (ng/µl) and A260/A280 readings were recorded for each sample.

The extracted DNA concentration was measured and adjusted by dilution to conc. 20-25 ng/µl prior to PCR using deionized bi-distilled, sterile water (Fluka, Germany).

3- Oligonucleotide primers

All primers used in this study were synthesized by (Tib Molbiol, Berlin, Germany) and obtained in a lyophilized state[11].

All primers were solved before use to obtain a final concentration of 20 pmol/µl of each. These primers make amplification for mutations that were coding regions of the E148Q and M694V mutation in the MEFV gene with the following sequences; M694V: Forward primer: 5'-GCCTGAAGACTCCAGACCACCCCG-3'

Reverse primer: 5'-AGGCCCTCCGAGGCCTTCTCTCTG-3'

E148Q: Forward primer: 5'-GCCTGAAGACTCCAGACCACCCCG-3'

Reverse primer: 5'-AGGCCCTCCGAGGCCTTCTCTCTG-3'

4- DNA amplification and Mutation analysis

PCR was carried out on PCR system 9700 (Roche, Syngapore). For each series, a master mix was prepared.

Each DNA sample was tested for the

two mutations (M694V and E148Q). All amplicons were stored at 4ºC until separation by gel electrophoresis. The PCR amplification was performed in a final volume of 25µL containing 100 ng of purified genomic DNA, 0.04 U of Ampli Taq Gold (Roche, Germany) and its 1x PCR buffer (contains 15 mmol of MgCl2 per L), 0.2 mmol of deoxynucleoside 5'-triphosphate mix per L (Roche, Germany) and 10 pmol of each primer.

The procedure was carried out as follows: The reaction was heated to 94°C for 10 minutes for denaturation, followed by 35 cycles with denaturation at 94°C for 10 seconds, annealing at 60°C for 10 seconds and extension at 72°C for 30 seconds. Final extension was done for 10 minutes at 72°C.

Then the PCR products were separated by electrophoresis on a 2% agarose gel (Sigma). Ethidium bromide staining of the agarose gel was used to detect the amplified fragments.

5- Agarose gel electrophoresis examination for identification of PCR products

Together with the different amplicons were separated on 2% w/v agarose gel, tris-base/borate buffer stained with 0.01% ethidium bromide solution (0.5 mg/L). 10 µl of all amplicons and DNA marker were added before gel electrophoresis to µl xylenecyanol dye solution (1 mg xylenecyanol, 400 mg sucrose and completed to 1 ml with water) and then subjected to electrophoresis for 45 min. The amplicons were made visible by ethidium bromide. The results from gel electrophoresis were visualized on a UV transillumination (254 nm). Agarose gel preparation as well as electrophoresis were carried out using buffer solution (pH 8.0), containing 45 mmol/L Tris-base/boric acid and 1 mmol/L EDTA adjusted with hydrochloric acid.To determine the size of the DNA fragments, DNA of a known size (100 bp DNA marker, Roche, Germany) was used.

RESULTS

Analysis of the presenting clinical manifestations of studied patients, showed that non of them had a positive family history for FMF. Parental consanguinity was positive in 16.66% of these patients. Recurrent febrile episodes and abdominal pain were reported in 95.8% of patients. Peritonitis affects 87.5% of all patients. Joints affection during attacks were: arthralgia (54.16%), arthritis (20.8%) and bone aches (83.3%). Chest pain was a symptom in 41.6% of patients. Pleuritis in 20.8% of patients. 20.8% from all patients underwent surgical operations; 4.16% of them underwent laparotomy and appendectomy in 8.3% of them and tonsillectomy in 8.3% of patients. No Skin erythema was reported in all patients. Proteinuria suggestive for renal amyloidosis was found in 8.3% of patients.

The results of examined FMF M694V and E148Q mutations showed that 58.3% (14 cases) had positive mutations for M694V mutation, while all our patients were negative for E148Q mutation. The normal controls were negative for previous two mutations.

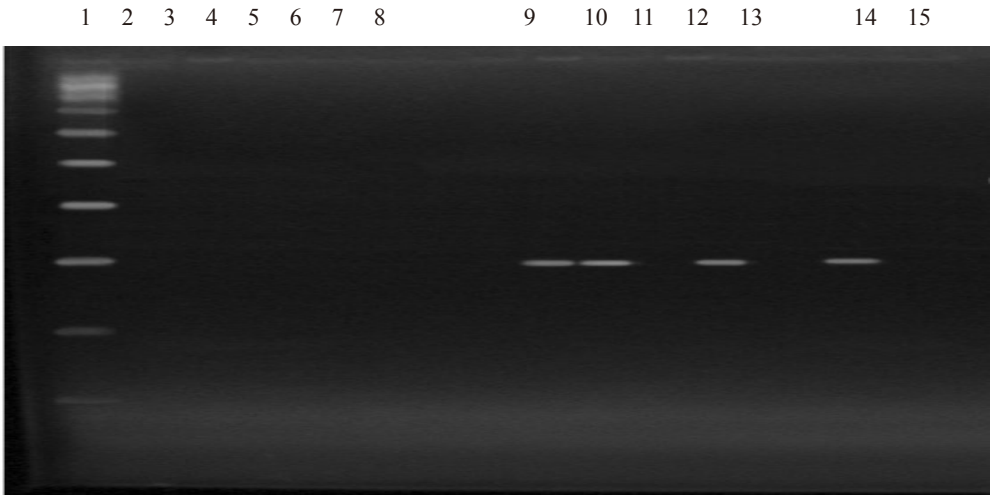

Fig. 1: Detection of the of the MEFV gene in different blood samples. DNA was extracted from different samples and examined by PCR-analysis using primer pair M694V for PCR-analysis. Lane 1: DNA ladder100 bp, lanes from 2- 8 were PCR products of normal controls, Lanes from 9 – 15 were PCR products from patients samples, lanes 9,10,12 and 14 showed positive bands at 300 bp for M694V mutation. Other patients samples were negative.

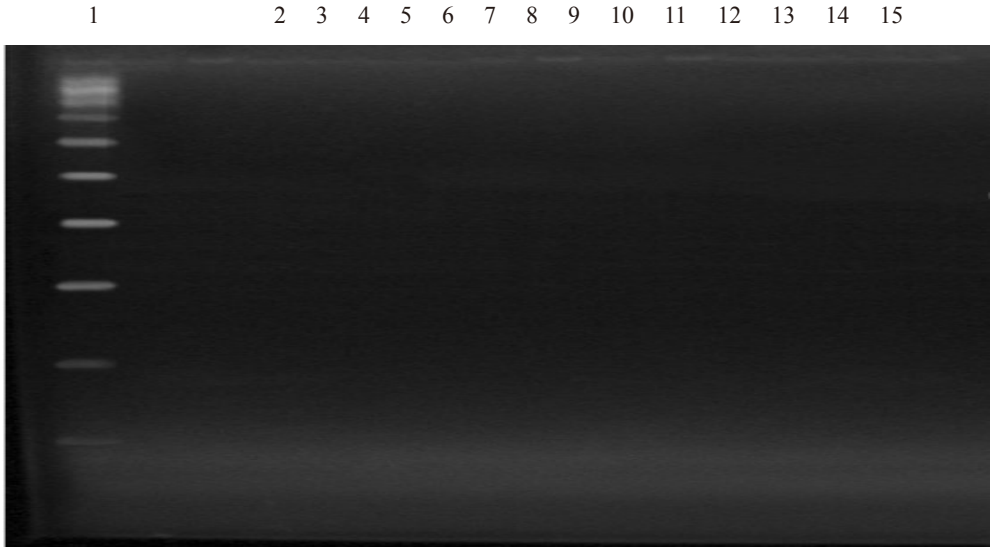

Fig. 2: Detection of the of the MEFV gene in different blood samples. DNA was extracted from different samples and examined by PCR-analysis using primer pair E148Q for PCR-analysis. Lane 1: DNA ladder100 bp, lanes from 2- 8 were PCR products of normal controls, Lanes from 9 – 15 were PCR products from patients samples. All samples were negative for E148Q mutations.

DISCUSSION

Familial Mediterranean fever (FMF) is an autosomal recessively-transmitted disease characterized by attacks of fever and serositis[12] MEFV gene mutations are responsible for the disease.[13] This gene was discovered firstly in 1997 (Mediterranean Fever – MEFV) and this has created possibilities to study the distribution of various mutations in geographically and ethnically different populations.[5]

Traditionally, the diagnosis of FMF has been based on clinical manifestations and the physician's experience. Following the cloning of MEFV, genetic analysis of its mutations has become a useful adjunct for establishing or confirming the diagnosis of FMF.[14]

In this study, recurrent febrile episodes and abdominal pain were the most common features (95.8%) followed by peritonitis (87.5%), joint affection by bone aches (83.3%), arthralgia (54.16%) or arthritis (20.8%) and chest pain (41.6%) or pleuritis (20.8%). Interestingly, 20.8% of cases had undergone surgery either appendectomy (8.3%), tonsillectomy (8.3%) or laparotomy (4.16%). Proteinuria suggestive for amyloidosis was found in (8.3%) of patients. No skin erythema. In comparison with our study, Zekri, et al.[15], reported in his Egyptian patients that the clinical features were: fever (100%), abdominal pain (95%), arthritis (55%), pleurisy (40%) with no skin rash or pericarditis. Also they have reported that 25% of the cases had a past history of appendectomy or laparotomy[15]. Another Egyptian study was done by Settin, et al[8]. who found that abdominal pain was the most common symptom (87.9%) followed by fever (82%), arthritis or arthralgia (56.1%), chest pain (45%) and myalgia (6%). Laparotomy had been done during attacks for exploration or appendectomy in 27% of cases.

In a group of Arab patients, the most common manifestations were peritonitis (93.7%), arthritis (33.7%) and pleurisy (32%). The authors reported lack of manifestations of amyloidosis, skin lesions, organomegaly and lymphadenopathy[16]. On the other hand, in another study on arab children, Rawashdeh and Majeed[17] reported that 82% had recurrent abdominal pain, 43% had pleurisy, 37% had arthritis, 15% had cutaneous manifestations, 12% had splenomegaly and 4% had hepatomegaly[17]. Tunca, et al.[18] studied a large series of Turkish cases and noted that their clinical features included peritonitis (93.7%), fever (92.5%), arthritis (47.4%), pleuritis (31.2%), myalgia (39.6%) and erysipelas-like erythema (20.9%).

We found positive M694V mutation in 58.3% of all studied patients, which consistent with Settin, et al.[8] who found that in their 66 patient, M694V was the most common allelic mutation found followed by V726A then M680I (18.8%, 17.42% 12.1% respectively). The difference in percentage between our result and their may refer to different number of cases. In another study in Egypt, Zekri, et al.[15] reported that the M694V mutation was detected in 100% and V726A mutation in 85% of their cases. El Shanti, et al.[19] found that M694V mutation was the most common MEFV mutation between Arabs, although it is less common than in other ethnic groups. Al Alami, et al.[20] studied Arabic population from Egypt, Syria, Iraq and Saudi Arabia and found

that 53.4% are mutation positive with mutational types M694V and V726A are the most common. Another study from Jordan found that 59% had 1 or 2 mutations, of the studied mutations M694V, V726A, M680I, accounted for 38%, 26%, 10%, respectively[21]. In Syria, 89% were positive either for one, two or three mutations. The allelic frequency of M694V, V726A, M680I mutations was in the form of 45.8%, 26%, 4.8% respectively[22]. On the other hand, Öztürk, et al. reported that M694V was detected in only 4.5% of the studied chromosomes of Egyptian patients.[23] Among north Africa population, the most frequent mutations were M694V and M694I. These mutations account for different proportions of the MEFV mutations in Algeria (5%, 80%), Morocco (49%, 37%) and Tunisia (50%, 25%). They pointed out that M694I mutation is specific to the Arab population from Maghreb.[24]

We found that all studied patients are negative for E148Q Mutation which agree with study by Al-Alami and his colleagues[20] who indicated that E148Q had reduced penetrance in the Arab population and thus a proportion of the genetically affected individuals remain asymptomatic. El Shanti, et al.[19] reported that E148Q mutation was the least penetrant and might be a polymorphism. It has been identified in Arab patients alone and in a complex allele with other exon 10 mutations, but is generally seen in healthy carriers. This may be noteworthy due to the use of the restriction endonuclease digestion test for the detection of the E148Q mutation that can lead to misdiagnosis in the presence of the E148V mutation, which could artificially increase the number of individuals identified as carriers of the E148Q mutation. Al Alami, et al.[20] found that the E148Q mutation was the most common among the healthy adult cohort, but was not present in affected individuals. On the other hand in cases from Tunis 18% were positive for E148Q mutation[25]. Among Lebanese patients, Sabbagh, et al.[26], reported that the most important feature in their study was the relatively high frequency of E148q that allowed them to question it as mutation rather than polymorphism. It was the second most common mutation in their study group (22.2%), while M694V mutation was found to be the most common (26.1%).

In 2007, Lidar and Livneh[4], reported that around 80% of FMF patients have an identifiable MEFV mutation, 57% have two mutations, 26% have a single mutation, while 16% have no identifiable mutation and the majority of cases are caused by four mutations clustered on a single exon: M694V, V726A, M680I and M694I. The prevalence of which varies according to the population studied. They also added that the role of the exon 2, E148Q mutation, as a disease-causing mutation is controversial. This non-founder mutation is found in populations in which FMF is distinctly rare, such as the Japanese, Chinese and Punjabi Indians. Additionally, E148Q homozygotes are rarely found in the FMF population.[4]

FMF is a recessively inherited disease, finding two mutations does not necessarily confirm the disease genetically. Phasing by analysis of the parent's DNAs is mandatory, especially if one of the two mutations is E148Q, a low-penetrance mutation frequent (up to 4%) in the general population.[27]

Yepiskoposyan and Harutyunyan,[5] reported FMF inflammatory attacks can be triggered by stress and extreme physical exercise. In general, the effect of environment on the inflammatory attacks in FMF is not surprising and is also seen in other cyclic conditions such as sickle cell anemia. However, in contrast to the latter, where only one mutation exists, in FMF, predisposition to the influence of environment is dependent on which mutations are present. This is confirmed by stronger predisposition in the M694V homozygotes.

In conclusion FMF is mainly a clinically diagnosed disease with validated diagnostic criteria and it necessitating a high index of suspicion in patients from high-risk ethnic groups. PCR technique provides a rapid, reliable, cost-effective, non invasive and sensitive molecular genetic test for establishing a diagnosis of FMF in symptomatic patients and provides a rational basis for medical and genetic counseling and clinical treatment of FMF patients and their families, but we recommended studying other mutations of MEFV gene in Sharkia region on large scale of population.

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