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Genetic Profile of Patients with Familial Mediterranean Fever (FMF): Single Center Experience at King Hussein Medical Center (KHMC)

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ABSTRACT

Objective: To describe the spectrum of genetic mutations in patients with clinical diagnosis of Familial Mediterranean Fever. **Method:** This is a retrospective study of 3359 sera samples for patient with clinical diagnosis of FMF, over a period of 6 years. The samples were tested for 12 mutations of the MEFV gene by PCR& hybridization of the PCR product with Probes immobilized as an array of panel lines. **Results:** A total of 1868 (55.6%) samples were found negative, and one or more mutations were detected in 1491 (44.4%) distributed along the mutations. Of the positive results, the Frequency of the mutations was as follows, the M694V was the most common mutation 30%, followed by E 148Q 21.5%, V 726 A 20%, M680I G/C 9%, M694I 8.3%, P369s 3.7%, A744S 3.1% and 4.2% among the 4 remaining mutations. **Conclusion:** Frequency of common mutations in our study show similar results in comparisons with Mediterranean countries like Egypt, Turkey, and Syria with the most common mutation in our study being M694V followed by E148 Q.

Key words: FMF, genotype, King Hussein Medical Center.

1. INTRODUCTION

Familial Mediterranean Fever (FMF) is an autosomal recessive inherited auto inflammatory disorder (1), most commonly seen in populations of Mediterranean ancestry, like Arabs, Turks, Jews and Armenians (2, 3, 4). This disease is characterized by recurrent episodes of fever 38-40c (5), arthritis and serositis (6). The FMF gene, MEFV was identified by positional cloning in 1997 (French FMF Consortium 1997; International FMF Consortium 1997) (7), it's located on chromosome 16p 13.3 (8), the MEFV gene has a 2,346 bp coding sequence, which spans 10 exons (9) with exon 10 showing the more frequent mutations (10) at two hot spots, one at codon 694 and the other one at codon 680 (2) followed by exon 2, 3, 5 and 9 (3). MFEV gene product is a 781 aa protein known as pyrin or marenostin (8), that has a regulatory effect on inflammation,

and so abnormality in its production would produce an inappropriate inflammatory reaction (9) as seen in FMF manifested as recurrent attacks of fever, arthritis ,pleuritis and sterile peritonitis (11). Amyloidosis is the most sever complication of the disease (4, 12), specially renal amyloidosis leading to an end stage renal disease (5), daily colchicines is the main treatment (13) showed to lower the severity and frequency of attacks and the chance of developing amyloidosis (1).

The most frequent mutations among Arab, Turks, Jews and Iranians are M694V, E148Q, M680I, M694V and V726A (3, 10, 12-15). The order and percentage however is different among each population from the above mentioned ones, and the mutations differ in penertrance and correlation with severity of clinical symptoms, with E148Q having the least penertrance and recog-

nized to have a milder clinical course (2, 9), while M694V recognized as the most severe and with increased risk of amyloidosis (4, 14, 16).

This study aims to identify the spectrum of FMF mutations in patients with clinical picture of FMF at KHMC.

2. METHOD

This retrospective study was conducted on a 3359 sera samples, for patients with clinical diagnosis of FMF over a 6 years period 1/1/2006 - 1/11/2013, the samples were tested for 12 mutations of MEFV gene, using the FMF STRIP ASSAY TM. VIENNA LAB DIAGNOSTICS GmbH. DNA was extracted from whole blood, amplified by PCR, then by hybridization of the amplified product to a test strip that contains allele specific oligonucleotide probes immobilized as an array of panel lines, then the bound biotinylated sequences are detected using streptavidine-alkaline phosphatase and color substrate. The assay covers 12 mutations in MEFV gene which are: E148Q, P369S, F479L, M680I (G/C), 1692 DEL, M694V, M694I, and M680I (G/A), K695R, V726A, A744S, R761H.

3. RESULTS

A total of 1868 (55.6%) samples were found negative, and one or more mutations were detected in 1491 (44.4%) distributed along 57 set of mutations, with heterozygous mutation detected in 872 (58.5%) patients, compound heterozygous mutations detected in 383 (25.6%), homozygous mutation detected in 226 (15.2%), and 10 (0.7%) were rare complex mutations. The Frequency of the mutations was as follows, the M694V was the most common mutation 30%, followed by E 148Q 21.5%, V 726 A 20%, M680I G/C 9%, M694I 8.3%, P369s 3.7%, A744S 3.1%

The Mutation Genotype	Number of patients
A744S/	49
E148Q/ M694I	15
E148Q/ M694V	31
E148Q/ P369S	20
E148Q /V726A	30
E148Q /	283
E148Q / E148Q	27
M680I (G/C) / M680I (G/C)	40
M680I (G/C) , V726A	25
M680I (G/C) , M694V	17
M680I (G/C) /	43
M694 I / M694 I	28
M694I ,/V726A	33
M694I /	43
M694V /	176
M694V/ M694 V	136
M694V / M694I	19
M 694V/ V726A	113
V726A /	154
V726A / V726A	24
P369s /	53
Other 36 mutation which each has less than 15 patients	133

Table 1. The mutations and their frequencies.

and 4.2% among the 4 remaining mutations. Table 1 and Figure 1 show the mutations and their frequencies.

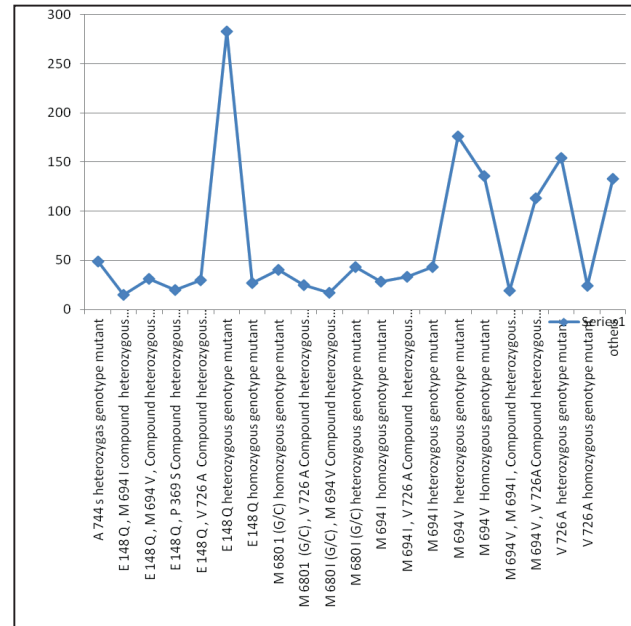


Figure 1. The mutations and their frequencies

4. DISCUSSION

Although clinical symptoms and the course of the illness are still the cornerstone of diagnosing FME, molecular confirmation can help make the diagnosis earlier in suspected cases (3). In our study the heterozygous were the most common mutations with (58.5%), followed by compound heterozygous with (25.6%), homozygous mutation detected in 226 (15.2%) patient, and (0.7%) were rare complex mutations. It is in contrast to previous study done in Jordan in 2006 were the compound heterozygous was the most common (3) but with consistent with a study done in Turkey very recently in 2014 by Oztuzcu S where heterozygous mutations were found to be the most common in (62.3%) (17) and similar to findings by Solak et al where heterozygous mutations were found in (51%), compound heterozygous in (25.8%), homozygous in (22.2%) and (1%) complex alleles (18). The most common mutations in our study were as follows the M694V was the most common mutation 30%, followed by E 148Q 21.5%, V 726 A 20%, M680I G/C 9%, M694I 8.3%, P369s 3.7%, A744S 3.1% and 4.2% among the 4 remaining mutations, as seen in Figure 2.

Distirbution of FMF mutations Jordan/KHMC 2006-2013

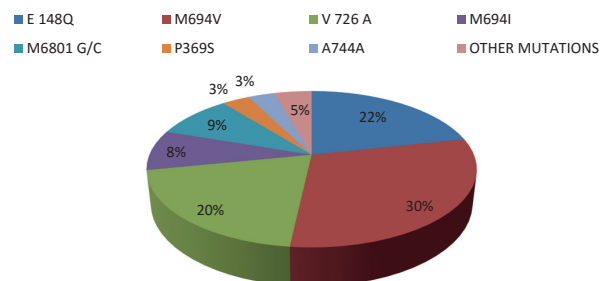


Figure 2. Distribution of FMF mutations in Jordan /KHMC 2006-2013

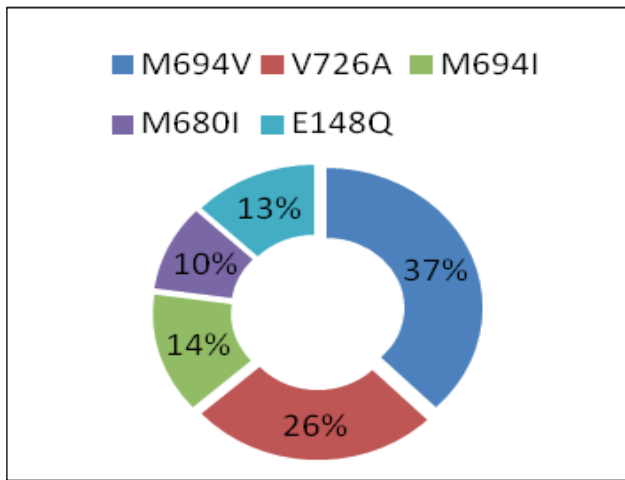


Figure 3. Gene mutation in Arabs

The first 5 mutations are among the most common mutations which are M694V, E148Q, M680I, M694V and V726A (3, 10, 12, 14, 15) and A744S seems to be specific to Arab population (19) which might explain why in Jordan it has such a percentage.

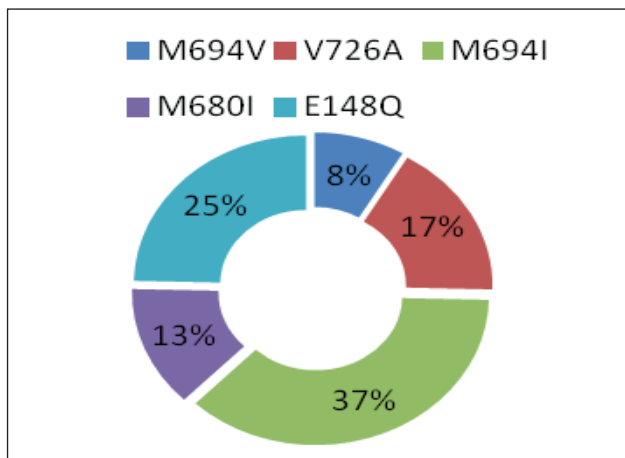


Figure 4. MEFV gene mutations in Egyptian patients

A study conducted in Jordan by HA. Majeed et al found the most common mutations in Arabs to be M694V, as shown in Figure 3 (20), another study done by el Gezery D shown the mutations in Egyptian patients with FMF to be M694I, as shown in Figure 4 (21), Medlej-Hashim et al findings are showed in Figure 5 (22), Matti et al studied the Syrian population for FMF mutations and findings are shown in Figure 6 (23), mutations in Iranian Azeri Turks were shown by Bonyadi to be as in Figure 7 (24).

The mutations among different populations of high prevalence of FMF including the Jordanian patients on which this study was conducted, seem to be similar, but they differ in the order and percentage. In our study there's an increases in percentage of E148Q as compared to previous studies done in Jordan where M694V was the most common followed by V726A (21) and in consistence to other population were again M694V was the most common (20, 22, 23, 24). A possible explanation for the above mentioned results is that the higher frequency of M694V could be due to the fact that this mutation is associated with more severe symptoms that make the

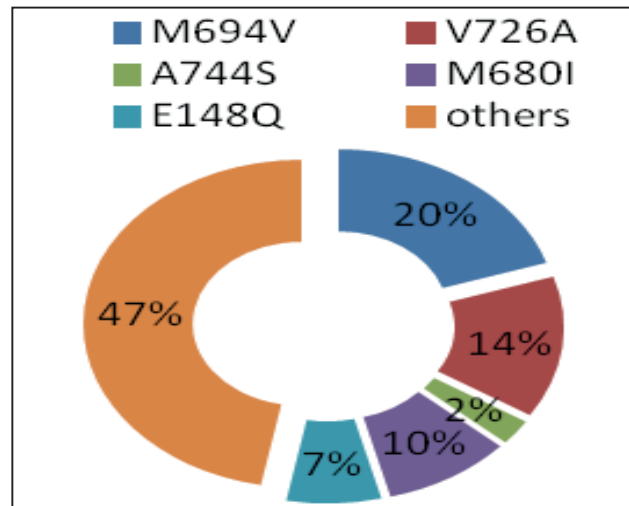


Figure 5. Genetic mutations in Jordanian FMF patients

patient seek medical help, and the physician to have a high index of suspicion of FMF, which leads to sending the patient to make molecular confirmation, while the E148Q mutation has low penertrance (15), and milder clinical course which may not bring the patient to the physician, or if they come, their mild symptoms may not be attributed to FMF. So with that low suspicion index they will not be sent for molecular confirmation, and so a lot of cases might be missed, as the suspicion index increases the detection of this mutation does too. In time and with keeping a higher index of suspicion of FMF our belief is that the E148Q frequency will be on a rise.

Heterozygosity regardless of type of mutations represents the most common finding in our cases, despite the fact that in an autosomal recessive pattern of inheritance, homozygosity should prevail. Different explanations could shed light on such a contradiction in FMF; the kit used for molecular identification of MEFV gene

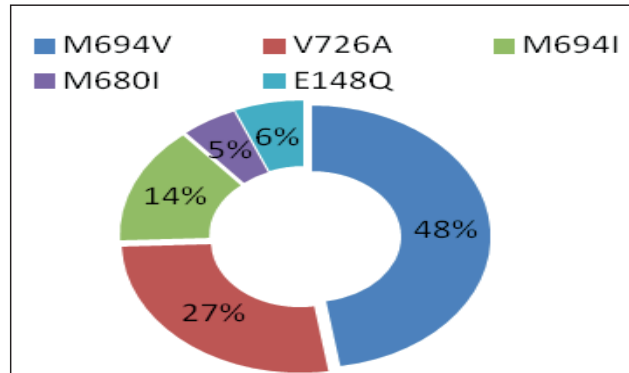


Figure 6. FMF in the Syrian population

mutations span only 12 mutations out of all gene mutations spectrum. Moreover, according to Jeru et al, heterozygosity might be coincidental in many patients due to the very high rate of mutations (25). Furthermore, other genes could be responsible for causing FMF (26, 27). Some authors suggested the hypothesis of having a dominant pattern of inheritance (28, 29). Epigenetics modulations as well as having hidden biomarkers might

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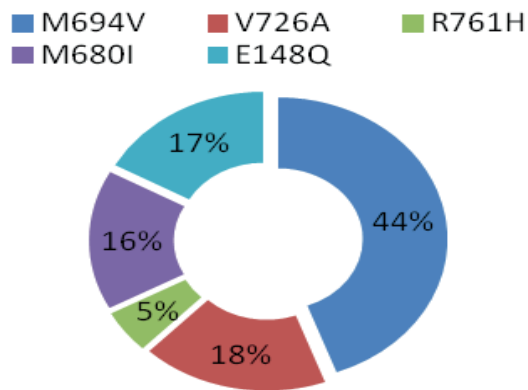


Figure 7. MEFV mutations in Iranian Azeri Turkish patients play a role in heterozygosity as a casualty of FMF in our cases.

5. CONCLUSION

Frequency of common mutations in our study show similar results in comparisons with Mediterranean countries like Egypt, Turkey, and Syria though the most common mutation in our study is M694V followed by E148Q.

CONFLICT OF INTEREST: NONE DECLARED.

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