Familial mediterranean fever mutation frequencies and carrier rates among a mixed Arabic population

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ABSTRACT

Objectives: Familial Mediterranean Fever (FMF) is an autoinflammatory periodic disorder characterized by febrile and painful attacks due to inflammation involving the serosal membranes. The gene implicated in this disorder, *MEFV*, has been cloned and mutations in its coding regions have been identified. We aimed at identifying the frequency of *MEFV* mutations and carrier frequency in a mixed Arabic population.

Methods: We identified 29 probands from 29 unrelated sibships segregating the disorder and representing the affected individual cohort. We screened 200 anonymous deoxyribonucleic acid (DNA) samples, representing a healthy adult cohort, for the mutations found to be common in the affected individual cohort. We also, screened anonymous DNA samples from 4 Arabic countries, namely, Egypt (231), Syria (225), Iraq (176) and the Kingdom of Saudi Arabia (107) thus enlarging our healthy adult cohort. The study was carried out between 1999 and 2002 at Jordan University of Science and Technology, Irbid and the University of Jordan, Amman, Jordan.

Results: Out of the 58 alleles of the 29 probands, only 31 mutations were identified and M694V and V726A are the most common. The mutation E148Q was the most common among the healthy adult cohort, but was not present in affected individuals. The collective mutant allele frequency "q" was 0.101. The expected carrier rate was 18.1% (one in 5.5) while the observed carrier rate was 18.4% (one in 5.4).

Conclusion: E148Q has reduced penetrance and thus, a proportion of the individuals genetically affected with FMF remain asymptomatic. M694I and M680I are more prevalent in the affected individuals cohort, which points to their higher penetrance. The overall carrier rate is one in 5, but the selective heterozygote advantage could not be demonstrated in this study due to the relatively small sample size.

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Familial Mediterranean Fever (FMF) is the prototype of the autoinflammatory periodic disorders characterized by febrile and painful attacks lasting from 12-72 hours and aborting abruptly.[1] The attacks start, most commonly, during childhood or adolescence, with 80% of patients presenting before the age of 20 years and very few after the age of 40 years.[1] One of the significant impacts of the disorder on affected individuals is the occurrence of amyloidosis as a complication.[1] A daily dose of oral Colchicine remains the recommended treatment as it reduces the frequency and severity of the attacks and prevents amyloidosis.[2,3] The FMF gene, *MEFV*, was mapped to the short arm of chromosome 16,[4] then was cloned by 2 consortia independently and simultaneously.[5,6] It consists of 10 exons and encodes a 781 amino-acid protein called Pyrin or Marinostrin.[5,6] With the cloning of the gene, 4 missense mutations, clustered in the 10th exon were

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identified.[5,6] The identification of other mutations in the gene further established *MEFV* as the gene responsible for FMF.[7-13]

Several studies have shown a carrier rate ranging from one in 3 to one in 5 in the major ethnicities affected by the disease.[9,14,15] This high carrier rate suggests a heterozygote advantage in the affected populations. The frequency and distribution of *MEFV* mutations amongst the Arabs have not been adequately studied, although there are few affected individual-based studies.[7,11,16,17-21] In this study, we aimed at finding the spectrum of mutations in patients with FMF in Jordan and then finding the carrier rates for the common *MEFV* mutations among Jordanians. We then further explored the carrier rates in other Arabic populations from Egypt, Syria, Iraq and the Kingdom of Saudi Arabia (KSA).

Methods. We investigated 29 probands from 29 unrelated sibships for mutations in the *MEFV* gene. The probands were all diagnosed with FMF according to the established clinical criteria.[22] An informed consent was obtained from all the probands or the legal guardian. We obtained anonymous (delabeled) deoxyribonucleic acid (DNA) samples from 200 unrelated healthy adults (chosen from a bigger sample for another study) for the detection of the common mutations in the Jordanian population. The subjects in the other study had signed an informed consent regarding the use of the drawn blood samples for the purpose of this study. We then obtained anonymous (delabeled) DNA samples from 231 Egyptians, 225 Syrians, 176 Iraqis and 107 Saudis. All are healthy, unrelated adults (employed or students) resident in Jordan who went through a medical screening as a prerequisite for their residency status. All participants signed a sentence in their medical screening consent denoting their agreement to the use of the extra blood sample for the purpose of this study. All subjects were identified between January 1999 and January 2002 and the work was carried out collaboratively between Jordan University of Science and Technology, Irbid and the University of Jordan, Amman, Jordan.

Mutation analysis. For the cohort of patients, the mutations were analyzed by the sequencing the entire coding sequence of exon 10 and its donor splice site. Exon 10 was amplified in 3 overlapping amplicons using the forward and reverse primers shown in **Figure 1**. Among the exon 2 mutations, only E148Q was analyzed by a naturally occurring restriction site for Ava I restriction endonuclease in the mutated allele. The region harboring the mutation E148Q was amplified using the forward, and reverse primers shown in figure 1. The amplified products were digested overnight with Ava I. We did not test for other mutations in exon 2 or the other exons. In the population samples only 5 mutations were tested, M694V, V726A, M680I, M694I and E148Q. The first 4 mutations were tested by ARMS (amplification refractory mutation system) using 3 primers, a common forward primer and a mutant and a normal reverse primers. The sequence of the primers for each of the 4 mutations using ARMS is shown in **Figure 1**. For each ARMS experiment a homozygous normal, heterozygous and homozygous mutant samples were used as controls. Two observers did the scoring for the amplification in the ARMS, independently. In cases where the 2 observers scoring was different (fewer than

Mutation	Test	Primer Sequence
Ex 10, 1stF	SEQ	CCAGAAGAACTACCCTGTCCCT
Ex 10, 1stR	SEQ	TCCTGGGAGCCTGCAAGACA
Ex 10, 2ndF	SEQ	GAGGTGGAGGTTGGAGACAA
Ex 10, 2ndR	SEQ	ATACATTCGCCAGCTGCT
Ex 10, 3rdF	SEQ	AATGTGACAGCCAGATCCCA
Ex 10, 3rdR	SEQ	GTGCTAGCTGCTATGGGAAA
M694V C	ARMS	TGACAGCTGTATCATTGTTCTGGGCTCTCCG
M694V N	ARMS	TCGGGGGAACGCTGGACGCCTGGTACTCATTTTCCTTCCT
M694V M	ARMS	TCGGGGGAACGCTGGACGCCTGGTACTCATTTTCCTTCCC
V726A C	ARMS	TGGAGGTTGGAGACAAGACAGCATGGATCC
V726A N	ARMS	TGGGATCTGGCTGTCACATTGTAAAAGGAGATGCTTCCTA
V726A M	ARMS	TGGGATCTGGCTGTCACATTGTAAAAGGAGATGCTTCCTG
M680I C	ARMS	TTAGACTTGGAAACAAGTGGGAGAGGCTGC
M680I N	ARMS	ATTATCACCACCCAGTAGCCATTCTCTGGCGACAGAGCC
M680I M	ARMS	ATTATCACCACCCAGTAGCCATTCTCTGGCGACAGAGCG
M694I C	ARMS	TATCATTGTTCTGGGCTC
M694I N	ARMS	CTGGTACTCATTTTCCTTC
M694I M	ARMS	CTGGTACTCATTTTCCTTT
E148Q F	REN	GCCTGAAGACTCCAGACCACCCCG
E148Q R	REN	GTCTCTCTTCCGGAGCCTCCCGGA

Figure 1 - Primer sequences used for exon 10 amplification and for the mutation detection assays. The figure also depicts the type of test for each mutation. The abbreviations used are Ex: exon, SEQ: sequencing, ARMS; amplification refractory mutation system, RREN; restriction endonuclease.

Table 1 - The distribution of the 5 common mutations among the affected individual cohort and among the healthy adult cohort.

Mutation	Affected individuals n	(%)	Healthy adults n	(%)
M694V	11	(35.5)	13	(24.5)
V726A	9	(29)	14	(26.5)
M694I	5	(16)	2	(4)
M680I	3	(9.7)	1	(2)
E148Q	0	(0)	23	(43)
Rare*	3	(9.7)	Not tested	
Total	**31**	**(100)**	**53**	**(100)**

* The rare mutations are M680I (G to A), A744S and R761H

Table 2 - Distribution of the 5 mutations, allele frequencies and carrier rates among the healthy adult cohort from 5 Arabic countries.

Nationality and number	Egyptians (231)	Syrians (225)	Jordanians* (200)	Iraqis (176)	Saudis (107)	Total (939)
n of chromosomes	462	450	400	352	214	**1878**
M694V	2	6	13	0	0	**21**
V726A	8	5	14	9	1	**37**
M694I	4	0	2	0	0	**6**
M680I	0	1	1	0	0	**2**
E148Q	29	30	23	29	12	**123**
Total	**43**	**42**	**53**	**38**	**13**	**189**
Wild type allele frequency 'p'	0.907	0.907	0.8675	0.892	0.939	0.899
Mutant allele frequency 'q'	0.093	0.093	0.1325	0.108	0.061	0.101
Calculated carriers (rate)	39 (16.9)	38 (16.9)	46 (23)	34 (19.3)	12 (11.4)	170
Observed n of carriers	43	42	37	38	13	173

* The calculations of the carrier rate and allele frequency is carried out under the assumption of that there are no complex alleles.

10 scores for all 4 mutations), the whole test was repeated and scored by the 2 observers independently. There was no interobserver variation on the repeated reactions. While the E148Q was not common in the patient cohort, we elected to screen for it, as it is very common in all other populations.[14,15]

Statistical analysis. We used the Hardy-Weinberg equation for the calculation of the distribution of population according to the allele frequencies.

Results. **Table 1** shows the distribution of the observed mutations in the affected individual cohort and the distribution of the 5 common mutations in the healthy adult cohort in Jordanians. Out of the 58 alleles of the 29 probands, only 31 mutations were identified by our mutation detection strategy (53.5%). The rare mutations detected in our patient cohort were one of each of M680I (G to A), A744S and R761H. Out of the 200 anonymous samples, 37 were heterozygous for one of the 5 mutations, 2 were homozygous for E148Q, one was homozygous for V726A and 2 were compound heterozygotes for M694V and V726A. Two samples were compound heterozygote (or a complex allele) for E148Q and M694V, and one was compound heterozygote (or a complex allele) for E148Q and V726A. The collective mutant allele frequency "q"ranges from 0.125 to 0.1325. The range is due to the fact that the 3 samples that are compound heterozygotes for E148Q and an exon 10 mutation could also present complex alleles and thus, the number of mutant allele ranges from 50-53. Thus, the wild type allele frequency "p" ranges from 0.8675-0.875. According to the Hardy-Weinberg equation the expected number of carriers ranges from 43.8-46. The observed number of carriers ranges from 37-40. Accordingly, the expected carrier rate ranges from 22-23% (one in 4.6 to one in 4.35) while the observed carrier rate ranges from 18.5-20% (one in 5 to one in 5.4). **Table 2** shows the distribution of the 5 common mutations in the healthy adults cohort from Egypt, Syria, Iraq and KSA. It also shows the wild type and mutant allele frequencies and the expected and observed carrier rate. There was no homozygotes or compound heterozygotes amongst the Egyptians, Syrians, Iraqis, or Saudis. The overall mutant allele frequency "q" for the mixed Arabic population is 0.1 and thus, the overall carrier rate is 18.1% (one in 5.5). The observed carrier rate is 18.4% (one in 5.4).

Discussion. To the best of our knowledge, this is the first study to examine *MEFV* mutation frequencies in an Arabic population by ascertaining normal healthy adults besides ascertaining affected individuals. It examines an affected individual cohort to observe the frequencies of the symptom-causing mutations. Our results are comparable to almost all the studies carried out on the major affected populations.[20] Our results are also in accordance with almost all the studies that included Arabic populations.[5,7,8,16,23,24] The 2 mutations M694V and V726A are the most frequent among the affected individual cohort and are common in the healthy adult cohort. While the 2 mutations, M694I and M680I constitute slightly less than one third of the

mutant alleles in the affected individual cohort; they constitute 6% of the mutant alleles in the Jordanian healthy adult cohort. This discrepancy is probably due to the near full penetrance of these mutations. Another remarkable discrepancy between the 2 cohorts occurs with the mutation E148Q, which is not present in the affected individual cohort but constitute 2 thirds of the mutations in the overall healthy adult cohort. This clearly demonstrates the reduced penetrance of this mutation and that a considerable proportion of the genetically affected individuals remain asymptomatic. These discrepancies and conclusions are noticeable in 3 other studies that dealt with healthy cohorts, as well as patient cohorts.[9,14,15] It is of note that M694V was not present in Iraqi and Saudi carriers while it is rather common in Jordanians and Syrians.

A previous study looked at the distribution of 14 described mutations among a Jordanian cohort composed of 42 unrelated affected individuals.[17] Approximately 56% of the *MEFV* alleles were mutant alleles with M694V, V726A, M680I, M694V and E148Q being the most common, representing 90% of the identified mutations.[17] Our results from the affected individual cohort are not very different from their results, although the methodology and coverage of mutations are different. The carrier rate in the Jordanian population, 1:5, is higher than has been calculated previously.[25] However, it conforms to the carrier rates in the major ethnic groups affected by this disorder. The overall carrier rate is 1:5.5, which shows that the mutant gene is present in similar proportions in the Arabic populations tested in this study.

It has been suggested that for the maintenance of *MEFV* mutations in the populations for thousands of years, these mutations ought to impart an increase in fitness in the heterozygote; such as selective heterozygote advantage. The carrier rate, as calculated by the Hardy-Weinberg equation was somewhat similar to the observed carrier rate. In 2 other studies, the calculated and observed carrier rates were equal.[14,15] These findings do not support an increase in fitness for heterozygotes, as suggested. However, to detect a minor increase in fitness requires a sample size 10 times the sample size in these studies, as well as screening for all possible mutations. Another alternative to test for this heterozygote advantage is to examine animal models for the increased fitness towards an array of threatening infectious agents.

We suggest that further studies in the Jordanian population, as well as, other Arabic populations are needed to include more patients and to cover more mutations. These studies should pay attention to other periodic autoinflammatory disorders that might be clinically similar to FMF, especially in patients in whom *MEFV* mutations are not identified. Thereafter, newer strategies for screening patients and the normal population should be developed coupled with identification of phenotype and genotype correlations.

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