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European Journal of Medical Genetics 48 (2005) 412–420
EUROPEAN JOURNAL OF MEDICAL GENETICS
www.elsevier.com/locate/ejmg

Original article

Familial Mediterranean fever (FMF) in Lebanon and Jordan: a population genetics study and report of three novel mutations

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Available online 20 June 2005

Abstract

Familial Mediterranean fever (FMF) is an autosomal recessive disease mostly frequent in Mediterranean populations. Over 50 mutations have been identified in the gene responsible for the disease, *MEFV*. The present study reports the frequencies of *MEFV* mutations in 558 Lebanese and 55 Jordanian FMF patients and points out the severity of the M694V frequently observed mutation among these patients. Three novel mutations, T177I, S108R and E474K were also identified in the Lebanese group. An excess of homozygotes and a deficit of heterozygotes were observed in both samples when compared to the expected number of observed genotypes under the Hardy–Weinberg hypothesis. Homozygotes for M694V and M694I were still in excess in the Lebanese group of patients, even after consanguinous homozygotes were removed, or population structure was considered. This excess is therefore neither due to consanguinity nor to subgroups in the Lebanese population, but rather to more remote consanguinity or to a selection bias favoring the census of these genotypes. The fact that FMF female patients were less censed than male patients may be due to the greater resistance of females to pain and to the possibility of confusing abdominal and gynecological pain. The phenotypic heterogeneity of the FMF could then originate both from genetic causes like allelic heteroge-

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doi:10.1016/j.ejmg.2005.05.010

neity or modulating genes, and cultural background facing the physiological consequences of genotypes at risk.

Keywords: FMF; Genotypes; Mutations; M694V; Severity; Hardy–Weinberg

1. Introduction

Familial Mediterranean fever (FMF) is a recessively inherited disease, mostly frequent among Jews, Armenians, Turks and Arabs [20]. It is characterized by recurrent fever crises, accompanied by serositis affecting the peritoneum, the pleura and/or the synovia. The disease is clinically very heterogeneous and is associated in its most severe form with renal amyloidosis.

The gene responsible for the disease, *MEFV*, localized on 16p13.3 [8,10], encodes for a protein, pyrin/marenostrin, of still unknown function, but belonging to the death domain fold (DDF) superfamily involved in inflammation and apoptosis [22]. Over 50 *MEFV* mutations and polymorphisms have been identified so far in FMF patients, mostly in the 10th exon of the gene [21]. However, 5 mutations are more frequently detected in different ethnical groups: M694V, M680I, V726A, E148Q and M694I.

In the present study, we report the frequencies of the detected *MEFV* mutations in two groups of unrelated genetically tested Lebanese and Jordanian FMF patients, and the results of a population genetics' analysis performed on these samples.

2. Patients and methods

The patients' study groups consisted of 558 Lebanese and 55 Jordanian unrelated FMF patients that have been clinically diagnosed according to Heller et al. [9]. DNA was extracted from these patients, and 14 *MEFV* mutations were tested by restriction enzyme analysis (REA), as previously described [13,14,17].

In patients found heterozygous for one of these mutations, the *MEFV* exons 1, 3–9 were tested by Single Strand Conformation Analysis (SSCA) [18], followed by fluorescent sequencing on an ABI 310 genetic analyzer. Exons 2 and 10 were directly sequenced, because of the frequent polymorphisms found in the first one, and mutations in the second.

Finally, in order to have a preliminary estimation of allelic frequencies in the general Lebanese population, the six most frequently encountered mutations in FMF Lebanese patients, as well as the three novel mutations T177I, S108R and E474K, were tested by REA in 100 Lebanese unrelated healthy individuals. Total Lebanese patients' sex ratio and genotypes' distribution among males and females were also studied.

3. Results

Out of 558 tested Lebanese patients (1116 alleles), 229 (41%) did not carry any mutation, while 105 patients (19%) had only one detected mutation, and 224 patients (40%)

were found to be either homozygous or compound heterozygous. In the latter subgroup, nine patients presented with 3 mutations, but the complex alleles could not be identified, as parents were not available. These patients were not introduced in the genetic population study to avoid complication in allele frequency calculations. The allelic frequencies in the positive groups of patients were, respectively, 30.3% (M694V), 19.4% (V726A), 12.8% (M694I), 7.4% (M680I) and 8.3% (E148Q). The R761H mutation was encountered in 3.1% of alleles and was found at the homozygous state in one patient (Table 1). The A744S, R653H, and I692del rare mutations, originally identified in patients of Arab origin [4,19], were detected at the heterozygous state in eight, three and four patients of the study group, respectively. Noticeably the four patients carrying the I692del mutation had three mutations, one of which happened to be E148Q. Three novel sequence variants were identified for the first time, namely T177I (T530C > T, ACC→ATC) and S108R (C322A > C, AGC→CGC) in exon 2, and E474K (A1420G > A, GAG→AAG) in exon 5. They were not detected in any of the 200 control chromosomes tested for them, which suggests they are pathologic mutations. S108R and E474K were found in the same patient who had also the V726A mutation, therefore defining a new complex allele. S108R was also found in another patient heterozygous for V726A as well. T177I was detected in two patients, one of them presenting the E148Q mutation. The clinical signs of the four patients that have either of these newly identified mutations were not characterized by any specific unusual symptom. Study of the sex ratio showed approximately a 1:3 male to female ratio among the Lebanese cohort of patients. Differences between the various genotypes' distribution among males and females were not significant.

Genotyping of 100 Lebanese control individuals for the six most frequently encountered mutations among Lebanese patients (M694V, M694I, V726A, M680I, E148Q and R761H) showed no carriers for M694V, M694I and R761H, whereas 0.5%, 4% and 5% of tested chromosomes carried the M680I, the V726A and the E148Q mutations, respectively.

Out of 55 Jordanian patients, 39 (71%) were either heterozygous or homozygous for a tested mutation, and 16 (29%) had no identified mutation. Allelic frequencies in the Jordanian positive group were, respectively, 34.6% (M694V), 19.2% (V726A), 2.6% (M694I), 12.8% (M680I) and 6.4% (E148Q), and rare mutations identified included I720M (previously reported) [16], A744S, T267I and F479L, while R761H was not encountered (Table 1).

Table 1
Comparison of the allelic frequencies of mutations in the Lebanese and Jordanian FMF patients groups

n (%)	Lebanese	Jordanian	*P* value (*Z*-test)
M694V	194 (30.3)	27 (34.6)	NS
V726A	124 (19.4)	15 (19.2)	NS
M694I	82 (12.8)	2 (2.6)	P < 0.01
M680I	47 (7.4)	10 (12.8)	NS
E148Q	53 (8.3)	5 (6.4)	NS
R761H	20 (3.1)	0 (0)	NA
Rare mutations [a]	15 (2.3)	5 (6.4)	P < 0.05
Undetermined [a]	105 (16.4)	14 (18)	NS
Total	640	78	

NS: non-significant; NA: not applicable.

[a] Rare mutations include the R653H, K695R, A744S, S108R, E167D, E148V, T177I mutations in the Lebanese FMF patients group and the A744S, T267I, F479L et I720M mutations in the Jordanian one, whereas the undetermined class represents the alleles that were not associated with a mutation in heterozygous patients.

As advocated recently [6], most of the patients with no identified mutations at the *MEFV* locus would have a disease that mimicks FMF. Using their evaluation method, 95% of our 229 Lebanese patients with no identified *MEFV* mutations would be unrelated to this gene. So all these 229 patients were not considered for population genetics' analyses at the *MEFV* locus.

Using the allelic frequencies within the positive groups, the expected numbers of genotypes were calculated assuming that the Hardy–Weinberg panmixia requirements were fulfilled (Table 2, lines 2–3). The large departure in the distribution of genotypes from the panmictic requirement may be either due to inbreeding or to a Walhund effect since observed heterozygotes were less than expected ones while observed homozygotes were in excess. When the expected distribution was calculated after the inbred homozygotes were removed, no significant difference was noted between observed and expected genotypes in the Jordanian group, whereas an excess of homozygotes was still observed, though less important, in the Lebanese group (Table 2, lines 4–5). Inbreeding thus accounts for only a part of this excess.

In order to test the Walhund effect that would result from the hierarchical structure of the Lebanese population, the M694V allele frequency has been estimated within each of the religious communities (Shiites, Sunnites, Druzes, Armenians, Greek Catholics, Greek Orthodoxes and Maronites), for a total of 242 patients including inbred patients and a total of 236, once inbred patients removed. Then the Hardy–Weinberg distributions within each subpopulation were calculated and the mean distribution among all the subpopulations was compared to the observed one (Table 3). An excess of homozygotes was still observed when taking account of the Walhund effect without removing the inbred individuals ($\chi^2 = 16.49$), but also when taking account of both the population structure and consanguinity ($\chi^2 = 11.64$).

From these analyses in the sub-sample without inbred patients, it is possible to calculate the *F*-statistics in order to estimate the remote consanguinity. The total correlation between uniting gametes relative to the total sample (Table 3, line 5) is equal to

$$F_{IT} = 1 - H/2p(1 - p) = 0.277$$

where H is the heterozygote frequency and p is the M694V frequency. The correlation between random gametes within religious communities relative to gametes of the total sample is equal to

$$F_{ST} = V_p/p.q = 0.053$$

Where V_p is the variance of p between communities.

From these values, the remote consanguinity of a patient (all inbred having been removed) is given by the equation

$$F_{IS} = 1 - (1 - F_{IT})/(1 - F_{ST}) = 0.236$$

This value, that roughly equals 0.25 (such as the one issued from a sib-pair mating), seems to be too high to consider that the total correlation would only result from remote consanguinity and population structure.

Table 2
Observed and expected number of genotypes among unrelated Lebanese FMF patients with either removing or not homozygote inbred patients

Genotypes Number	M694V/ M694V	M694V/ V726A	M694V/ M694I	M694V/ other	V726A/ V726A	V726A/ other	M694I/ M694I	M694I/ V726A	M694I/ other	other/ other	total	χ^2 (P) Df [a] = 6
Observed	47	36	12	52	14	45	16	15	23	60	320	52.60 (P < 0.001)
Expected under HW [a]	29.38	37.62	24.82	72.72	12.04	46.56	5.24	15.89	30.72	45.01	320	
Observed once homozygotes inbred removed	39	36	12	52	10	45	14	15	23	59	305	37.34 (P < 0.001)
Expected once homozygotes inbred removed	26.01	33.84	22.80	69.47	11.01	45.2	5.0	14.84	30.45	46.38	305	

Df: Degrees of freedom.
[a] HW: Hardy–Weinberg.

Table 3
Observed and expected samples of M694V homozygotes when taking into account inbreeding or hierarchical structure of the Lebanese population

	M694V/M694V	M694V/others	others/others	Total	Value of χ^2
Total observed	**39**	**70**	**133**	**242**	
Expected assuming Hardy–Weinberg	22.66	102.78	116.56	242	24.55
Expected from subpopulations	25.41	97.28	119.31	242	16.49
Total observed once inbred removed	**33**	**70**	**133**	**236**	
Expected once inbred removed	19.57	96.79	119.64	236	18.12
Expected from sub-populations, once inbred removed	24.78	94.88	116.34	236	11.64

4. Discussion

The various mutations detected in Lebanese and Jordanian FMF patients reflect the allelic heterogeneity that characterizes FMF. The rare mutations encountered, namely the new ones, S108R, E474K and T177I, suggest that heterozygous FMF patients are most likely truly affected patients probably carrying non-identified mutations yet. Once the allelic frequencies between the Lebanese and Jordanian groups of patients were compared, the only mutation that had significantly different frequencies in the two groups was the M694I mutation (Table 1). It was indeed much more frequent among Lebanese patients.

The mutations' frequencies allowed the calculation of the genotypes distribution, according to the Hardy–Weinberg hypothesis. An excess of homozygotes and a deficit of heterozygotes were observed in both Lebanese (Table 2) and Jordanian samples (data not shown). In order to assess whether the observed excess of homozygotes was due to the high consanguinity rates in Lebanon and Jordan [2,11,12], the inbred homozygotes were removed from the data, and a corrected distribution of genotypes was calculated under the Hardy–Weinberg hypothesis (Table 2). Homozygotes for the M694V and M694I mutations were still in excess in the Lebanese group, whereas no such excess was observed in the Jordanian group, probably due to the relatively small sample size. Results obtained within the Lebanese sample had been also observed in a previous study on a smaller mixed group of patients [15]. So the consanguinity cannot explain only by itself the departure between observed and expected genotypes in the Lebanese sample. The stratification of the Lebanese population which is subdivided into various religious communities within which *MEFV* alleles' frequencies differ, could also cause such a departure in the distribution of genotypes in the whole sample, known as the Walhund effect. As reported in Table 3, the departure in the observed genotypes' distribution from the expected one assuming panmixia was reduced, by taking into account either the Walhund effect or consanguinity or both of them. Though the departure was importantly reduced when taking into account both population structure and consanguinity, a significant excess of homozygotes was still observed. An effect of remote consanguinity may be expected, especially within endogamous groups for a long time, but the F_{IS} estimated value seems to be so high that other factors influencing the census of patients are probably involved either with regards to all homozygotes, or especially to M694V homozygotes.

The homozygotes' excess could be due to a more severe phenotypic effect by which these affected people would be more efficiently censed. The M694V and M694I mutations

were indeed previously associated with a severe phenotype [3,13]. They concern the same codon and lead to the substitution of the methionine by a valine and an isoleucine, respectively. As these two amino acids have similar properties, the two mutations may have a similar severe phenotype. Testing of 200 control chromosomes for the most frequent mutations in the Lebanese patients showed that M694V and M694I mutations were very rare, compared to V726A and E148Q mutations that were more observed in the general population. The null frequency of the codon 694 mutations was previously mentioned in other populations' studies [1,4]. This is contradictory with FMF patients' results where M694V and M694I were the most frequent mutations encountered. Moreover, in Lebanese heterozygous patients, whose genotypes cannot be due to consanguinity, the frequent alleles in the general population were expected to be observed while, in actual fact, M694V mutation was most frequently encountered, followed by M694I and V726A mutations. These observations suggest that M694V, and to some extent M694I, are severe mutations, which supports the hypothesis of a selection bias in the census of patients. Moreover, a biochemical hypothesis could be proposed since the coiled-coil domain present in the pyrin/marenostrin structure generally mediates protein dimerization [7]. The M694V/M694V and the M694I/M694I dimers could have a different structure than the M694V/M694I dimers, leading thereby to a less functional protein. This hypothesis could both explain the excess M694V and M694I homozygotes and the deficit in the M694V/M694I heterozygotes within observed genotypes.

Otherwise, the FMF heterogeneity may also be characterized through another phenotypic level, including clinical or even cultural features, together with the drastic severe effect of the M694V mutation. The affected males were more numerous than females within nine out of 10 genotypes and were significantly more numerous within the whole sample (P value at 0.02), a fact which cannot be expected without any systematic cause favoring the male census. It could be hypothetized that females would be more resistant to pain and would sometimes confuse pains of abdominal or gynecological origin, or that hormonal female mechanisms would infer a significant protection to females, and thus decrease their census, or that this sex-biased selection is simply due to cultural reasons whereby parents, in some societies, especially care for boys' pain. The severity of the M694V mutation being pointed out, one would expect M694V female carriers to be as censed as M694V male carriers, which is confirmed by a chi-square test of homogeneity between males and females versus M694V carriers and non-carriers.

In patients without any detected *MEFV* mutation, one can suggest that they either carry new *MEFV* mutations that might be further identified, or they suffer from another autoinflammatory disease with symptoms similar to the FMF ones, but caused by another defected gene. This is why discussions must be supported with these patients' clinicians in order to precise their phenotypes and discriminate any special clinical symptom. On the other side, in patients with only one determined mutation, one can expect either the presence of a still non-identified *MEFV* mutation, or a digenic pattern of inheritance such as the recently detected one in non-syndromic hearing loss whereby affected individuals are double heterozygous for a connexin 26 gene mutation and a connexin 30 gene deletion [5].

This study affords new aspects relative to the heterogeneity in FMF at the allelic level as well as the clinical or even cultural level, and opens up new horizons for the understanding of the phenotypic effects of mutations in various populations.

5. Uncited references

[12,16,19].

Acknowledgments

This work was supported by a grant from the Lebanese Council for Scientific Research and from the Saint Joseph University Research Council.

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