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SHORT REPORT

Higher than expected carrier rates for familial Mediterranean fever in various Jewish ethnic groups

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Familial Mediterranean fever (FMF) is an autosomal recessive disease characterised by recurrent attacks of inflammation of serosal membranes. Amyloidosis leading to renal failure is the most severe complication in untreated patients. In Israel FMF is most frequent among Jews of North African origin. Recently the causative gene (*MEFV*) has been found and the common mutations characterised. The aim of this study was to investigate the carrier rates of the common *MEFV* mutations among 400 healthy members of four different ethnic groups (100 in each group) in Israel, and to compare the distribution of the different mutations between FMF carriers and patients. We found a high frequency of carriers among Jews from the various ethnic groups. In North African Jews it was 22%, in Iraqi Jews 39%, in Ashkenazi Jews 21%, and in Iranian Jews 6%. The distribution of the four most common *MEFV* mutations among healthy individuals (*M694V* 29%, *V726A* 16%, *M680I* 2% and *E148Q* 53%) was significantly different ($P < 0.003$) from that found in patients (*M694V* 84.4%, *V726A* 9.0%, *M680I* 0% and *E148Q* 6.6%). Six healthy asymptomatic individuals were found to carry mutations in both alleles: two homozygotes for *E148Q* and four compound heterozygotes *E148Q*/other. These results demonstrate a very high carrier rate among all Jewish ethnic groups. They confirm that mutation *E148Q* is associated with a milder phenotype, which explains the lower prevalence of FMF among the Ashkenazi and Iraqi Jews. This study raises the question of the need for molecular screening for *M694V* homozygotes in the Israeli North African Jewish community. *European Journal of Human Genetics* (2000) **8**, 307–310.

Keywords: FMF; mutations; carrier rate; Jews; Israel

Introduction

Familial Mediterranean fever (FMF) is characterised by recurrent short episodes of inflammation and serositis including fever, peritonitis, pleuritis, and synovitis.[1] Colchicine has been shown to be effective in preventing the attacks of FMF as well as the development of amyloidosis.[2]

The gene causing FMF (*MEFV*) has been cloned,[3,4] and three common founder *MEFV* mutations were initially found in exon 10: *M694V, M680I* and *V726A*.[3,4] Additional less common mutations have been found subsequently in exons 10, 3 and 5, and more recently a common mutation has been found in exon 2: *E148Q*.[5] Genotype/phenotype correlation has demonstrated higher morbidity with mutation *M694V*, especially with regard to the risk of developing amyloidosis.[6–11] Based on prevalence patterns, it is clear that this disease is common in the Mediterranean area, mainly among Armenians, Turks, Arabs and Jews. In Israel it has been shown that 53–61% of all the patients are of North African origin. A high percentage (16–24%) of Jews of Iraqi and Kurdish origin are also affected, while 10% of patients are Ashkenazi Jews.[12,13] Recently Aksentijevich *et al*[14] found a carrier rate for *MEFV* mutations of 21% in 200 anonymous Ashkenazi Jewish DNA samples with *E148Q* being the most

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common mutation. This further suggests a heterozygote advantage in the Mediterranean area.

The aim of our study was to find the carrier rates for the different mutations in the *MEFV* gene in different Jewish ethnic groups in Israel. Since amyloidosis correlates with homozygosity for *M694V*,[6–11] and since this complication can be the first and only manifestation of FMF with no inflammatory attacks (phenotype 2),[15] such information could help in determining the need for mutation detection screening in the different ethnic groups in the general population.

Subjects and methods

We studied 400 blood samples from healthy individuals who were referred for cystic fibrosis screening belonging to four different ethnic origins – North African Jews (mainly Moroccan), Iraqi Jews, Iranian Jews, and Ashkenazi Jews. The subjects were pooled from the various prenatal clinics of our genetic institute. The blood samples were tested for the four most frequent FMF mutations, three of which are located on exon 10 (*M694V, V726A, M680I*), and one on exon 2 (*E148Q*).

Mutation analysis was performed by genomic sequencing. Seven mutations in exon 10 were tested by PCR amplification using the forward oligonucleotide 10F1; 5'-ccagaagaactaccctgtccc-3' and the reverse oligonucleotide 10R1; 5'-cagagcagctggcgaatgtat-3'. The PCR amplification was performed under the following conditions: 95°C for 10 min followed by 30 cycles of 95°C for 15 s, 55°C for 30 s and 72°C for 30 s; and final extension at 72°C for 10 min. PCR products were purified with High Pure PCR Product Purification Kit (Boehringer, Mannheim, Germany) and sequenced directly, using specific primers and Thermo Sequenase Kit (Amersham, Buckinghamshire, UK).

Mutation *E148Q* in exon 2 was analysed by restriction of PCR products from genomic DNA. The region harbouring mutation *E148Q* was amplified using the forward oligonucleotide: 5'-gcctgaagactccagaccaccccg-3' and the reverse oligonucleotide 5'-aggccctccgaggccttctct-3'. The amplified products were digested for 3 h with 3 IU of BstN1.

The frequencies and distribution of the mutations found in the carriers were compared with the frequencies and distribution of the mutations found in Israeli FMF probands who were referred randomly to our clinic for confirmation of the clinical diagnosis of FMF. Included in the study were 169 patients of only four ethnic groups: North African Jews (120), Iraqi Jews (20), other non-Ashkenazi Jews (Iran and Levant) (12) and Ashkenazi Jews (17). We considered as FMF patients those found to be homozygotes or compound heterozygotes for the four FMF mutations described above, and patients with diagnostic clinical signs of FMF but molecular findings of only one of the mutations which has been identified up to now. Not all the individuals classified as patients met one of the published sets of clinical criteria for the diagnosis of FMF, but there were some individuals with borderline histories who turned out to be mutation positive. We did not include any individuals with clinical signs of FMF but in whom none of the four mutations was found.

All computations were made with the use of the SAS statistical package. The frequencies of the four most common mutations were calculated with 95% confidence interval, and the comparison of the distribution of the *MEFV* mutations between FMF patients and FMF carriers was done by the χ^2 test.

This study was approved by the Human Subjects Committee at the Rabin Medical Center, Beilinson Campus, Petah Tikva, Israel, and informed consent was obtained from each participant.

Results

Table 1 depicts the frequency of the four most common mutations of the *MEFV* gene in the different ethnic groups

Table 1 Frequency of the four most common MEFV mutations (95% confidence interval) among healthy individuals according to ethnic group

	M694V	*V726A*	*M680I*	*E148Q*	*Mutation frequency*	*Carrier rate*
North African Jews (*n*=200)[a]	16/200 (0.05–0.13)	2/200 (0.00–0.03)	0 (0–0.02)	10/200 (0.02–0.09)	28/200 (0.09–0.19)	22/100 (0.14–0.31)
Iraqi Jews (*n*=200)[b]	11/200 (0.03–0.10)	10/200 (0.02–0.09)	1/200 (0.0001–0.03)	23/200 (0.07–0.17)	45/200 (0.17–0.29)	39/100 (0.29–0.49)
Iranian Jews (*n*=200)	1/200 (0.00–0.03)	0 (0.00–0.02)	0 (0.00–0.02)	5/200 (0.01–0.06)	6/200 (0.01–0.06)	6/100 (0.02–0.13)
Ashkenazi Jews (*n*=200)[c]	1/200 (0.00–0.03)	4/200 (0.01–0.05)	1/200 (0.0001–0.03)	15/200 (0.04–0.08)	21/200 (0.07–0.16)	21/100 (0.13–0.30)
Total (*n*=800)	29/800 (0.024–0.052)	16/800 (0.011–0.03)	2/800 (0.00–0.01)	53/800 (0.05–0.86)	100/800 (0.10–0.15)	88/400 (0.18–0.26)

[a]In this group was one individual who was homozygous for *E148Q* and two others who were compound heterozygotes *E148Q/M694V*; [b]In this group was one individual who was homozygous for *E148Q* and two others who were compound heterozygotes: one *E148Q/M694V* and one *E148Q/V726A*; [c]Of 88 healthy Ashkenazi Jewish patients subsequently tested, seven (7.95%) were carriers of the mutation *P369S*.

within the general population. The carrier rates were calculated after excluding positively identified homozygotes or compound heterozygotes (see below). The mutation *M694V* was the most common in the North African Jews, while in the Ashkenazi, Iranian and Iraqi groups, the mutation *E148Q* was found to be by far the most prevalent. Overall, the mutation *E148Q* was found to be the most frequent – 0.07 (95% CI = 0.05–0.08). In Iraqi Jews it was as high as 0.115 (95% CI = 0.07–0.17), with frequencies of 0.075 (95% CI = 0.04–0.08) in Ashkenazi Jews, 0.05 (95% CI = 0.02–0.09) in North African Jews, and 0.025 (95% CI = 0.01–0.06) in Iranian Jews. Following in frequency was the mutation *M694V*, with an overall frequency of 0.035 (95% CI = 0.024–0.05), and as high as 0.08 (95% CI = 0.05–0.13) in Jews from North Africa (Table 1).

Table 2 depicts the distribution of the mutations found in FMF patients. In North African Jews and Iraqi Jews the most common mutation is *M694V*, and in Ashkenazi Jews the mutation *V726A* is the most prevalent. Mutation *E148Q* is only second or third in frequency in all groups.

Table 3 compares the distribution of the different mutations between healthy carriers and FMF patients ($P < 0.003$). The commonest mutation in patients, *M694V*, was found in 84.4% of all patients from all ethnic groups, but in only 29% of carriers. In contrast, mutation *E148Q* was very frequent (53%) among healthy carriers, but was found in only 6.6% of FMF patients. This difference was found in each ethnic group when analysed separately.

In the healthy population group used for the carrier screening study, we identified six adults who each carried two mutations. In the Iraqi group we found one individual who was homozygous for *E148Q* (aged 30), one compound heterozygote *E148Q/M694V* (aged 30), and one compound heterozygote *E148Q/V726A* (aged 44). In the North African group we found one individual who was homozygous for *E148Q* (aged 26), and two individuals who were compound heterozygotes *E148Q/M694V* (aged 29,30). On enquiry, none of these individuals had any signs or symptoms of FMF attacks or amyloidosis. Among the FMF patients no *E148Q* homozygotes were found, but there were patients with *E148Q/V726A* and *E148Q/M694V*.

Discussion

This study demonstrates a high prevalence of the different *MEFV* mutations in all ethnic groups in Israel. This rate exceeds that expected by the prevalence of the disease, especially in Ashkenazi and Iraqi Jews, and therefore suggests that many patients in these ethnic groups are undiagnosed. Indeed, the carrier rate was previously estimated, based on family studies, to be as high as 1:5–1:7 among some non-Ashkenazi Jewish populations,[16] and 1:7 among Armenians in California.[17] Owing to the high prevalence in Jews, Israeli doctors find themselves including FMF in their differential diagnosis quite often, especially when the patient is of North African origin. The carrier rates that we have found exceed these estimations, and to the best of our knowledge demonstrate the highest carrier rates for an autosomal recessive disease described so far. The discrepancy between the estimation of the carrier rates on the basis of family clinical studies and those found in this study is even greater for Iraqi Jews (39%), and Ashkenazi Jews (22%). This latter is in agreement with the carrier frequency of 21% recently found in Ashkenazi Jews.[14] Thus these data may suggest a greater degree of clinical underdiagnosis, or of non-penetrance, or both, among Ashkenazi and Iraqi Jews. Indeed, the analysis of the different distributions of the mutations between the Jewish groups – Ashkenazi and Iraqi Jews have a higher prevalence of *E148Q*, whereas the North African Jews have a higher prevalence of *M694V* – might explain this phenomenon. Mutation *E148Q* was proportionately more common among the carriers than among the patients, further suggesting that this mutation may be associated with a high non-penetrance rate. We did, in fact, identify two individuals homozygous for *E148Q* and another four compound heterozygotes with *E148Q* who had no clinical evidence of inflammatory attacks or amyloidosis by age 26–44 years.

After we had completed the testing of the subjects described in this paper, we performed additional testing on Ashkenazi Jews for the presence of the mutation *P369S*. Out of 88 healthy individuals, seven were found to carry this mutation (7.95%), whereas out of 58 FMF patients, only two were found to be compound heterozygotes for this and

Table 2 Distribution of the MEFV mutations among FMF patients in different Jewish groups in Israel (mutation frequency for the known mutations)

	M694V	*V726A*	*M680I*	*E148Q*	*M694I*	*A744S*	*K695R*	*Unknown*
MAJ[a]	205 (94.4%)	2 (0.9%)	–	9 (4.1%)	–	–	1 (0.5%)	23 alleles
Iraqi Jews	17 (55%)	8 (26%)	–	5 (16%)	–	1 (3.2%)	–	9 alleles
Other non-Ashkenazi Jews	13 (65%)	6 (30%)	–	1 (5%)	–	–	–	43 alleles
Ashkenazi Jews[b]	10 (38.5%)	10 (38.5%)	–	4 (15.3%)	–	2 (7.7%)	–	8 alleles

[a]NAJ = North African Jews; [b]Of 58 Ashkenazi Jewish patients subsequently tested, one (1.72%) carried the mutation P369S.

Table 3 Comparison of the distribution of the *MEFV* mutations found in FMF patients and in healthy individuals

	M694V	*V726A*	*M680I*	*E148Q*
FMF patients (290 alleles)	245 (84.4%)	26 (9.0%)	–	19 (6.6%)
Asymptomatic adults with MEFV mutations (100 alleles)	29 (29%)	16 (16%)	2 (2%)	53 (53%)

$P<0.003$

E148Q (our data). This would suggest that *P369S* is probably another mild mutation which is common in Ashkenazi Jews, but further studies would be required to confirm this.

Previous studies have demonstrated a significant association between *M694V* and amyloidosis.[6–11] Conversely, in our extensive series of FMF patients with amyloidosis, none of the patients who carried the mutation *E148Q* developed this complication. These findings concur with those of other researchers.[6,10] There have been no documented cases of amyloidosis in homozygotes for *E148Q* and only one in a patient who was a compound heterozygote M694V/E148Q,[18] which suggests that this mutation carries a relatively low risk for FMF related amyloidosis. So far, it would appear that amyloidosis may develop rarely only when this mutation is combined with *M694V* (ie *M694V/E148Q*).

Our findings may have important clinical implications for the treatment of FMF families. Since colchicine has been shown to prevent the development of amyloidosis,[2] lifelong treatment is recommended for all FMF patients. Based on our findings, it appears that homozygotes for *M694V* are at the highest risk, and should be given colchicine treatment for life. In mildly affected patients (those with infrequent inflammatory attacks) who do not carry this mutation, further studies are needed in order to determine whether the patients should be either treated, or tested every 6 months for the presence of proteinuria. This is particularly the case for those patients where one or both mutations are *E148Q*.

The high prevalence of mutation *M694V* in North African Jews (gene frequency of 0.08 [95% CI = 0.05–0.13] and carrier rate of 0.14 [95% CI = 0.09–0.19]), raises the question of the need for screening the newborns in this population, especially when both parents are from the same ethnic origin. Amyloidosis in such patients may appear in untreated individuals even prior to the onset of inflammatory attacks (phenotype 2)[15] in as many as 10% of the North African Jewish patients. Since this is preventable by colchicine therapy, it appears that end-stage renal failure could be prevented in 1:2000 screened individuals. If further confirmed, screening for the mutation *M694V* among the North African Jewish population in Israel may appear to be justified, and should be considered for other at-risk groups in the future.

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