MUTATION IN BRIEF

The E148Q *MEFV* Allele Is Not Implicated in the Development of Familial Mediterranean Fever

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Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by recurrent attacks of fever and serositis, common in populations of Armenian, Arab, Sephardic Jewish and Turkish origin. Early diagnosis is crucial to start colchicine therapy that prevents the occurrence of attacks and renal amyloidosis. In the absence of functional test for FMF, the diagnosis remains clinical and is generally confirmed by molecular analysis of the *MEFV* gene. More than 40 missense mutations and two in-frame deletions have been reported, most of them being located in exon 10 of the gene. The M694V (c.2080A>G) mutation, the most frequent defect, is responsible for a severe phenotype when present in the homozygous state. The E148Q (c.442G>C) sequence variant, which is situated in exon 2, is also common, but its role in FMF is controversial. In order to assess the implication of the E148Q variation in FMF, we investigated 233 patients of Sephardic Jewish origin living in France and 213 disease-free relatives of these patients. The frequency of the E148Q allele was found to be similar in the two groups (3.62% and 3.75%, respectively, p=0.93). Most importantly, the frequency of the M694V/E148Q compound heterozygous genotype was comparable between the patients group (3.9%) and the healthy relatives group (4.2%, p=0.85). This population-based study, therefore, strongly supports the hypothesis that E148Q is a just a benign polymorphism and not a disease-causing mutation. Considering this variant as a mutation may lead to set false positive diagnoses and to neglect the likely existence of genetic heterogeneity in FMF © 2003 Wiley-Liss, Inc.

KEY WORDS: FMF, *MEFV*; Jewish; French

INTRODUCTION

Familial Mediterranean fever (FMF; MIM# 249100), the most frequent hereditary recurrent fever, is an autosomal recessive disease that affects commonly populations of Armenian, Arab, Sephardic Jewish or Turkish origin. The diagnosis of the disease, which is based on various sets of clinical criteria (Livneh et al., 1997; Pras, 1998), can be confirmed by molecular means that provides the only objective diagnostic criterion in the absence of functional test. To date, more than 40 missense mutations and two in-frame deletions of the *MEFV* gene have been

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identified, most of them are situated in exon 10 that codes for the C-terminal end of pyrin/marenostrin protein. If the link between FMF and mutations such as M694V, M694I (c.2082G>A) or M680I (c.2040G>C) has been clearly established by population genetics and genotype-phenotype correlation studies (The International FMF consortium, 1997; The French FMF Consortium, 1997; Cazeneuve et al., 1999; Cazeneuve et al., 2000; Shinawi et al., 2000; Mansour et al., 2001; Gershoni-Baruch et al., 2002), the clinical implication of the E148Q sequence variant - situated in exon 2 - remains controversial. Several authors suggest that this variant would be a disease-causing mutation with reduced penetrance (Bernot et al., 1998; Aksentijevich et al., 1999). Although the frequency of the E148Q allele was found to be similar in control populations and among patients (Ben-Chetrit et al., 2000; Stoffman et al., 2000), it was also proposed that the E148Q variation is a functional polymorphism whose phenotypic effect is restricted to compound heterozygotes bearing a severe mutation such as M694V (Ben-Chetrit et al., 2000).

In order to assess the implication of the E148Q variation in the course of FMF, we analyzed allele and genotype frequencies among 233 patients of Sephardic Jewish descent living in France and their 213 asymptomatic relatives.

MATERIALS AND METHODS

Mutation analysis

The mutation analysis was performed as described previously (Cazeneuve et al., 1999). Briefly, exons 2, 3, 5 and 10 of the *MEFV* gene (GenBank AJ003147) were analyzed by denaturing gradient gel electrophoresis combined with restriction enzyme analysis or direct sequencing. In the samples displaying a mobility shift, the mutations were identified by enzyme restriction analysis or direct sequencing.

Population analysis

We investigated 233 consecutive French patients of Sephardic Jewish descent with FMF and 213 of their unaffected relatives belonging to 165 unrelated families. The FMF diagnosis was established according to accepted clinical criteria (Livneh et al., 1997) and phenotypic features were collected through a standardized form by physicians from different medical specialities including paediatrics, rheumatology, nephrology, gastroenterology and internal medicine. Informed consent was given by all patients or their parents. Allele counting was done on independent chromosomes from affected and healthy individuals of each family. Genotype and allele frequencies were compared using Chi-square test.

RESULTS

DNA samples of 233 FMF patients and 213 relatives were scanned for most common *MEFV* gene mutations. Distributions of alleles and genotypes are shown in Tables 1 and 2, respectively. Two mutated alleles were identified in 101 (43.3%) patients; in 67 (28.8%) patients, only one mutated allele was detected, whereas no mutation was detected in 65 (27.9%) patients. These proportions of patients with a single or no identified mutation are similar to those reported in other independent studies including FMF patients of Sephardic Jewish origin (Dodé et al., 2000; Ben-Chetrit et al., 2002; Padeh et al., 2003); such results may suggest the existence of a FMF-like disorder mimicking FMF.

The M694V mutation was the most frequent one (48.3%). No mutation was found in 44.4% of alleles.

We compared allele and genotype frequencies among patients and unaffected relatives. Given the recessive mode of inheritance of FMF, the rate of disease-causing alleles should be higher in obligatory carriers than in the general Jewish Sephardic population, but lower than in patients. As expected, the frequency of the M694V mutation was found to be higher among patients (48.3%) than among healthy individuals (29.2%) ($p=10^{-6}$). In contrast, both groups showed the same proportion for the E148Q variant (3.6% vs. 3.8%, p=0.93) (Table 1). In addition, the frequency of the M694V/E148Q compound heterozygous genotype among patients (3.9%) was found to be similar to that observed among healthy relatives (4.2%, p=0.85). As the mean age of the nine M694V/E148Q healthy relatives was 39.2 years (eight of them were over 35 and a single individual was 9), we can consider that they have a low risk to develop the disease. We also observed similar proportions of the E148Q/N heterozygous genotype among patients and in the group of relatives (2.6% vs. 3.8%, p=0.48).

Table 1: The Distribution of Independent Mutated *MEFV* Alleles among Sephardic Jewish Patients and their Non-affected Relatives

Mutation	Patients	Healthy relatives	p
M694V (c.2080A>G)	187 (48.32%)	78 (29.21%)	10-8
E148Q (c.442G>C)	14 (3.62%)	10 (3.75%)	0.93
V726A (c.2177T>C)	6 (1.55%)	2 (0.75%)	NT(1)
M694I (c.2082G>A)	4 (1.03%)	3 (1.12%)	NT
A744S (c.2230G>T)	1 (0.26%)	1 (0.37%)	NT
K695R (c.2084A>G)	3 (0.78%)	0 (0.00%)	NT
No mutation	172 (44.44%)	173 (64.79%)	NT
Total allele number	387 (100%)	267 (100%)	

(1)NT: non tested

Table 2: The Distribution of *MEFV* Genotypes among Sephardic Jewish Patients and their Non-affected Relatives

Genotype	Patients	Healthy relatives	p
M694V/M694V	82 (35.2%)	5 (2.3%)(3)	10^{-15}
M694V/E148Q(1)	9 (3.9%)	9 (4.2%)	0.85
M694V/V726A	5 (2.1%)	0 (0.0%)	
M694V/M694I	4 (1.7%)	1 (0.5%)	
M694I/M694I	1 (0.4%)	0 (0.0%)	
M694V/N (2)	56 (24.0%)	96 (45.1%)	10^{-6}
E148Q/N	6 (2.6%)	9 (4.2%)	0.33
K695R/N	3 (1.3%)	0 (0.0%)	
V726A/N	1 (0.4%)	2 (0.9%)	
A744S/N	1 (0.4%)	1 (0.5%)	
M694V-E148Q/N	0 (0.0%)	1 (0.5%)	
N/N	65 (27.9%)	89 (41.8%)	
Total	233 (100%)	213 (100%)	

(1) All healthy relatives were shown to carry trans-allelic mutations; five patients could not be cis or trans genotyped.
(2) N: no mutation was detected by DGGE analysis of at least exons 2, 5 and 10.
(3) One parent (55-year-old mother of a patient) was asymptomatic today, but had a history of febrile abdominal pain during childhood.

DISCUSSION

To date, in the absence of any biochemical testing for FMF, the diagnosis of this disease remains clinical; however, the molecular analysis of the *MEFV* gene is the only objective test that confirms the diagnosis. It is, therefore, crucial to make a distinction between sequence variations associated with a disease phenotype and those that represent benign polymorphisms. The E148Q sequence variant is concerned by this issue. This sequence variation was first reported as a disease-causing mutation by Bernot et al. (1998) who found it in 24% of the chromosomes with no exon 10 mutations from FMF patients from different origins; it was found in 2.3% of the chromosomes from a group consisting of healthy relatives of the previous patients and of unrelated individuals from CEPH panel (various Caucasoid origins).

Several authors have considered E148Q as a mild mutation (Bernot et al., 1998; Aksentijevich et al., 1999). In accordance with this hypothesis, healthy individuals have been found to be homozygous for the E148Q variation (Aksentijevich et al., 1999; Ben-Chetrit et al., 2000; Stoffman et al., 2000). Similar examples of mild mutations are described in the literature, such as the H63D variant in the *HFE* gene involved on hemochromatosis. Homozygotes for this mutation are in general disease free and individuals carrying the C282Y/H63D compound heterozygous genotype do not have a severe phenotype. However, the H63D allele frequency and C282Y/H63D genotype rate are considerably higher in patients with hemochromatosis than in healthy populations (Feder et al., 1996; Risch,

1997). In contrast, the present study shows that the E148Q allele frequency is comparable among patients and asymptomatic relatives (3.6% vs. 3.8% respectively, p=0.93). This result is in keeping with the allele frequencies found in the previously investigated North African Jewish populations: 7.8% in patients vs. 6.4% in healthy controls (Ben-Chetrit et al., 2000), 5% vs. 4.1% (Stoffman et al., 2000) and 4.2% vs. 5.5% (Gershoni-Baruch et al., 2001).

Most importantly, we observed the same rate of the M694V/E148Q genotype among patients with FMF and their healthy relatives (p=0.85). This data strongly argues against the hypothesis that E148Q is a disease-causing mutation when present in the compound heterozygous state in combination with a severe mutation like M694V (Ben-Chetrit et al., 2000).

Taken together, our results strongly suggest that the E148Q allele is just a polymorphic variant and not a deleterious mutation. However, it leaves open the possibility that clinical severity of the complex V726A(c.2177T>C)-E148Q allele outweighs that associated with the V726A allele (Gershoni-Baruch et al., 2002). From a diagnostic viewpoint, the main consequence of our study is that considering the E148Q variation to be of clinical relevance may lead to establish false positive diagnoses and to miss necessary further molecular investigations within the *MEFV* gene or other potentially implicated genes.

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