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Received for publication: 20.10.09; Accepted in revised form: 20.1.10

Nephrol Dial Transplant (2010) 25: 2520–2523
doi: 10.1093/ndt/gfp632
Advance Access publication 23 November 2009

MEFV gene compound heterozygous mutations in familial Mediterranean fever phenotype: a retrospective clinical and molecular study

Ahmet Okay Caglayan[1], Fatma Demiryilmaz[1], Isilay Ozyazgan[1] and Hakan Gumus[2]

[1]Department of Medical Genetics, Kayseri Education and Research Hospital, Kayseri, Turkey and [2]Department of Pediatrics, Kayseri Education and Research Hospital, Kayseri, Turkey

Correspondence and offprint requests to: Ahmet Okay Caglayan; E-mail: aocaglayan@erciyes.edu.tr

Abstract

Background. Familial Mediterranean fever (FMF) is an autosomal-recessive inherited inflammatory disease caused by mutations in the *MEFV* gene that encodes pyrin/marenostrin. It is characterized by recurrent short episodes of fever, abdominal pain and serositis affecting mainly Mediterranean and Middle Eastern populations. We determined the frequency of the compound heterozygous mutations which has been rarely reported. The present study not only investigated clinical features of child-onset FMF patients with compound heterozygous mutations but also determined whether there is a phenotype–genotype correlation in the same patient population.

Methods. The medical records of 66 heterozygous patients with FMF were retrospectively reviewed and assessed. Patients were investigated regarding the mutation type, clinical characteristics at the time of inflammatory attacks such as fever, abdominal pain, arthritis, chest pain, erysipelas-like erythema and oedema, epidemiological data, consanguinity, severity score and family history of FMF and amyloidosis.

Results. The most frequent mutation was M694V, identified in 32% of the alleles examined, followed by E148Q in 20.6%, V726A in 17% and M680I in 14.5%, respectively. Consequently, we determined that P369S ($n = 10$; 8%) was the most frequent rare mutation in Turkish FMF patients. Frequency of the other rare mutations were R761H (3%), F479L (3%), A744S (1.5%) and K695R (0.7%). Fever was seen in 96.5%, abdominal pain in 98.5%, arthralgia in 85%, chest pain in 45.5% and erysipelas-like lesions in 23%. None of these patients had amyloidosis, but 16 had a family history of chronic renal failure, 44% had vomiting and 35% had diarrhoea during the attack. Although regular colchicine treatment was effective in 83% of the patients, the percentage of patients that did not start colchicine therapy was 18%. In addition, the patients were divided into four groups according to the presence of the mutation types and we compared genotype–phenotype correlations.

Conclusions. We suggest that regular colchicine therapy may be administered to symptomatic patients with *MEVF* gene compound heterozygous mutations, regardless of the mutation type.

Keywords: compound heterozygous; genetics; *MEFV* gene

Introduction

Familial Mediterranean fever (FMF) is an autosomal-recessive inherited disease. It affects mainly Mediterranean and Middle Eastern populations, including Turks, Jews, Armenians and Arabs. It is characterized by recurring short episodes of fever, abdominal pain and serositis. Reactive AA amyloidosis frequently occurs in patients with this disease and the prognosis is determined by the complication of AA amyloidosis. There is ethnic variability in the prevalence of amyloidosis; the latter occurs in 37% of Sephardic Jews, 27% of non-Ashkenazi Jews, 12% of Turks, 24% of Armenians and 1–2% of Armenians living in the United States [1]. In untreated Jewish patients of North Af-

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rican origin, the frequency of amyloidosis increases progressively with age, occurring in up to 75% of those over the age of 43 years [2]. Colchicine has been shown to be effective in preventing the attacks of FMF as well as the development of amyloidosis [3–5].

Mediterranean fever (MEFV) is caused by mutations in the *MEFV* gene which is localized on 16p13.3 and more than 70 mutations have been described to date, mostly in exon 10, followed by exons 2, 3, 5, 1 and 9. Four of five founder mutations—M694V, V726A, M680I and M694I—are located in exon 10 and E148Q in exon 2 [6]. These founder mutations account for 74% of FMF chromosomes in typical patients from the Mediterranean Basin [6]. FMF carrier frequency in the US Ashkenazi Jewish population is approximately 21% [7]. An Israelian population survey indicated a carrier frequency of 22% in North African Jews, 39% in Iraqi Jews, 21% in Ashkenazi Jews and 6% in Iranian Jews [1]. A previous epidemiological study has shown that the prevalence of FMF is 1:20 in the Turkish population [8].

Allele frequencies in Turkey have been published by two independent centres [8,9]. Yilmaz *et al*. [8] reported the five most common founder mutation frequencies as follows: M694V 51.5%, M680I 9.2%, E148Q 3.6%, V726A 2.9% and M694I 0.4%. Yalcinkaya *et al*. [9] reported the allele frequencies in patients as follows: M694V 43.5%, M680I 13.0%, V726A 11.1% and M694I 2.8% for the four founder mutations.

There are differences in the gene mutations of FMF in the various ethnic groups. The aim of this study is to determine the frequency of the compound heterozygous mutations which has rarely been reported in a comparative way; in addition, we evaluated the phenotypic features of Turkish FMF patients with the compound heterozygous mutation.

Materials and methods

Subjects

The patients with FMF diagnosed at our department and in whom MEFV mutation analysis was available between the years 2007 and 2008 were screened retrospectively. Written informed consent for genetic studies was obtained from all the patients. A total of 66 of the 1123 patients screened were compound heterozygous for the FMF mutations and these were enrolled in the current evaluation. Forty-one patients were compound heterozygous for M694V, 11 were compound heterozygote for M680I(G/C), 10 were compound heterozygous for P369S/E148Q and four were compound heterozygous for F479L/V726A. Only 12 of the 66 patients who had compound heterozygous mutations in the four groups detected through family history were asymptomatic at the time of evaluation. None had received colchicine prior to testing. The diagnosis of FMF was established according to the Tel Hashomer criteria [10]. These patients were seen again and the patient file was reviewed and updated by the same physician. Clinical information included age of onset, frequency of attacks before colchicine treatment, the presence of fever, abdominal pain, pleurisy, arthralgia and arthritis, response to colchicines, family history of amyloidosis and development of amyloidosis.

Mutation analysis

Molecular diagnosis of FMF was carried out in our department. DNA samples were obtained from peripheral venous blood samples of the subjects. We investigated the patients with reverse-hybridization test by using the FMF Strip Assay (ViennaLab Labordiagnostika Gmbh, Austria). Twelve mutations located in exons 2 (E148Q), 3 (P369S), 5 (F479L) and 10 [M680I(G>C), M680I(G>A), I692del, M694V, M694I, K695R, V726A, A744S and R761H] can be simultaneously screened for by a reverse-hybridization procedure. The assay includes four successive steps for which reagents are provided: (i) DNA isolation from blood samples, (ii) *in vitro* multiplex amplification reaction, (iii) hybridization of amplification products to a test strip comprising a staining control, eight wild-type- and 12 mutant-specific immobilized oligonucleotide probes and (iv) detection of bound biotinylated sequences by streptavidin–alkaline phosphatase and colour substrate. Amplifications were conducted on an Applied Biosystems Thermocycler 9700 using the protocol recommended by the manufacturer and hybridizations were performed manually or in an AutoLIPATM automated incubator (Innogenetics), which can process up to 30 strips simultaneously.

Statistical analysis

Results are given as median (range) for age and as percentages for the phenotypic features. Data were analysed using the SPSS programme (SPSS, Chicago, IL, USA).

Results

Compound heterozygous mutations in 66 Turkish FMF patients with typical phenotypes from the central Anatolia region of Turkey were evaluated. These patients were analysed for 12 *MEFV* gene mutations by the FMF strip test. The most frequent mutation was M694V, identified in 32% of the alleles examined, followed by E148Q with 20.6%, V726A with 17% and M680I with 14.5%, respectively. Consequently, we determined that P369S (n = 10; 8%) was the most frequent rare mutations in Turkish FMF patients. Frequency of the other rare mutations were R761H (3%), F479L (3%), A744S (1.5%), K695R (0.7%) and M694I (0.7%) (Tables 1 and 2). All of these mutations were seen in the compound heterozygote state. The median age of onset was 16 years for compound heterozygous, while the median time interval between onset and diagnosis was 9.3 years for FMF mutations. The mean severity score was 7 and the male to female ratio was 1.2 (Table 3). Fever was seen in 96.5%, abdominal pain in 98.5%, arthralgia in 85%, chest pain in 45.5% and erysipelas-like lesions in 23%. None of these patients had amyloidosis, although 16 had a family history of chronic renal failure, 44% had vomiting and 35% had diarrhoea during the attack. Although regular colchicine treatment was effective in 83% of the patients, the percentage of patients that did not start colchicine therapy was 18%. In addition, consanguinity was detected in 15 patients (23%) and a family history of amyloidosis and FMF was detected in 44%. The

Table 1. Mutation distribution of 66 patients (a total of 133 alleles)

Mutation	*n*	%
Met694Val	42	32
E148Q	27	20.7
Val726Ala	22	17
Met680Ile	20	14.5
P369S	10	7.6
F479L	4	3
R761H	4	3
A744S	2	1.5
K695R	1	0.7
M694I	1	0.7

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Table 2. Genotype distribution of 66 patients with compound heterozygous mutation in the *MEFV* gene

Genotype	Patients (*n*)	
	Male	Female
R761H/M694V	2	1
E148Q/M694V	10	6
V726A/M694V	7	5
A744S/M694V		1
M680I(G/C)/M694V	8	1
R761H/M680I(G/C)	1	
E148Q/M680I(G/C)		1
V726A/M680I(G/C)	4	2
A744S/M680I(G/C)		1
K695R/M680I(G/C)		1
M694I/M680I(G/C)		1
F479L/V726A	1	3
P369S/E148Q	3	7

Table 3. Phenotypic features of the patients

n	66
Male/female	1.2
Age at onset (years ± SD)	15.6 ± 2.53
Age at diagnosis (years ± SD)	25 ± 3.154
Time interval between onset and diagnosis (years ± SD)	9 ± 1.723
Abdominal pain (%)	98.5
Fever (%)	96.5
Arthralgia (%)	85
Chest pain (%)	45.5
Erysipelas-like erythema (%)	23
Amyloidosis (%)	–
Disease severity score (mean ± SD)	6.75 ± 0.392
Vomiting (%)	44
Diarrhoea (%)	35
Family history of amyloidosis (%)	44
Family history of FMF (%)	71

patients were divided into four groups according to the presence of the mutation types (Table 4). The frequency of arthralgia was significantly lower in the patients who were heterozygous for the M694V mutation when compared with the other three groups. The frequency of chest pain was significantly higher in the patients who were compound heterozygous for the F479L/V726A mutation when compared with the other three groups. Interestingly, the age of disease onset was lower in patients with the F479L/V726A mutations.

Discussion

The majority of patients with FMF have identifiable mutations in both alleles of the *MEFV* gene, while some individuals with two or three MEFV mutations in one allele do not have the clinical symptoms of the disease. Studies comparing disease phenotype with MEFV genotype [11] indicate that some mutations are more pathogenic than others, but that clinical expression of the disease can vary widely among patients with the same mutations. This study is important, as it is the first one presenting the results of the *MEFV* gene mutation analysis in compound heterozygous patients who had their clinical files carefully screened.

Our study showed that E148Q and V726A mutations were higher than previously reported and were mainly associated with M694V, similar to the literature data. We suggest that these mutations should be included in the mutation scanning analysis research or should be considered if a patient has M694V/? mutation, especially in Turkish FMF patients. Also, the frequencies of fever, abdominal pain, chest pain, arthralgia, arthritis and myalgia were found to be higher in compound heterozygous than their homozygous state, in accordance with the literature. Regular colchicine treatment was seen to be more effective in the majority of our patients (83%) for completely controlling the attacks related to mutations in the homozygous state. However, 20–30% of FMF patients have partial resistance and 10% of the patients have complete resistance to colchicine in homozygous *MEFV* gene-mutated patients.

The most common long-term complication of FMF is amyloidosis, which may cause renal pathology. The renal manifestation of amyloidosis includes persistent or increasing proteinuria, nephrotic syndrome and chronic renal

Table 4. Phenotypic features according to the main mutation types

Mutation type	M694V/+	M680I(G/C)/+	F479L/V726A	P369S/E148Q
n	41	11	4	10
Male/female	27/14	6/5	1/3	3/7
Age at onset (years ± SD)	16.2 ± 5.8	17.2 ± 10.6	6 ± 2.1	15 ± 6.7
Age at diagnosis (years ± SD)	28.8 ± 9.5	24.1 ± 11.5	15 ± 4.8	22 ± 8.5
Time interval between onset and diagnosis (years ± SD)	12 ± 6	6.9 ± 5.4	9 ± 3.5	7 ± 2.8
Abdominal pain (%)	96	100	100	100
Fever (%)	96	90	100	100
Arthralgia (%)	46	94	100	100
Chest pain (%)	51	11	100	20
Erysipelas-like erythema (%)	16	36	0	40
Amyloidosis (%)	–	–	–	–
Disease severity score (mean)	7.1	6.9	7.1	5.9
Vomiting (%)	44	54	50	30
Diarrhoea (%)	14	45	50	30

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failure. Phenotypic variations between different ethnic groups have already been documented. Several authors have emphasized that the Turkish population has a more severe disease with a relatively higher incidence of amyloidosis compared with other ethnic groups. The most common cause of AA type amyloidosis is FMF in Turkey [12]. Saatci *et al*. [13] showed that the family history of amyloidosis was significantly lower in patients without amyloidosis compared to those with amyloidosis. However, the family history of FMF was found to be similar in our two groups. None of our 66 patients had amyloidosis at the time of evaluation, but family history of amyloidosis and FMF was 44% and 71%, respectively. Additionally, it should be emphasized that our study population included relatively young people and it may be speculated that amyloidosis may develop later in life. Colchicine therapy might be valuable to prevent amyloidosis. Further studies are required to clarify this issue.

Our findings may have important clinical implications for the treatment of FMF families. Since colchicine has been shown to prevent the development of amyloidosis, lifelong treatment is recommended for all FMF patients. The findings of our study also show that the M694V mutation is likely to be a risk factor for AA type amyloidosis because it can manifest itself not only in the homozygote but also in genetically compound states. In mildly affected patients (those with infrequent inflammatory attacks) who do not carry this mutation, further studies are required to determine whether the patients should be either treated or tested every 6 months for the presence of proteinuria. Furthermore, the severity of the disease course does not consistently parallel the development of amyloidosis. This is particularly the case for those patients with mutations E148Q, V726A and M680I.

We investigated the patients with the FMF Strip Assay (ViennaLab Labordiagnostika). Our panel includes the five most common founder mutations [E148Q, M680I (G/C), M694V, M694I and V726A], which, according to Touitou [6], should account for an average of 74% of FMF chromosomes within the four most frequently affected ethnic groups (Turks, Arabs, Armenians and Jews), as well as seven less common mutations.

One of the known limitations of reverse hybridization is the difficulty to correctly identify and distinguish between two closely spaced mutations. The other unidentified alleles may be carrying rare mutations in the unscreened exons responsible for 25–30% of FMF disease. We suggest that, if the symptomatic patient has one mutation detected by FMF Strip Assay, screening must be combined with a second, more comprehensive technique (e.g. DNA sequencing) and very efficient genotyping should be performed. Furthermore, in some patients, the disease may not be linked to the known chromosome locus (16p13.3), as indicated in some previous studies [14,15].

How amyloidosis develops during the disease course has yet to be established. As we observed clinical heterogeneity and a high frequency of symptoms, our view is that symptomatic and compound heterozygous patients may require colchicine treatment, regardless of the mutation type, at least until as yet undetermined genetic or environmental factors are found.

Conflict of interest statement. None declared.

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Received for publication: 19.6.09; Accepted in revised form: 27.10.09

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