SCREENING OF FAMILY MEMBERS OF CHILDREN WITH FAMILIAL MEDITERRANEAN FEVER: TRUE-AUTOSOMAL AND PSEUDO-AUTOSOMAL INHERITANCE

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Abstract

Objectives: Screening of family members of children with Familial Mediterranean Fever (FMF) has been carried out to detect new potential patients and to analyze the type of inheritance other than autosomal recessive.
Methods: Marenostrin encoding fever gene mutational analysis has been performed in 83 subjects – including 19 newly diagnosed children with FMF and their family members.
Results: Fourteen additional patients with FMF were diagnosed by screening family members. Pseudo-dominant and true dominant inheritances were detected in two families respectively, while the rest of the patients exhibited autosomal recessive mode of inheritance.
Conclusion: Screening the family members of newly diagnosed FMF patients provides the opportunity to reveal undiagnosed new cases and to understand the mode of inheritance.

Keywords: Familial Mediterranean Fever; Inheritance; Screening; Mutation; Marenostrin Encoding Fever Gene.

Resumo

Objectivos: Foi efectuado o rastreio dos membros da família de crianças com Febre Mediterrânica Familiar (FMF) para detectar novos doentes potenciais e para verificar outras formas de transmissão, para além da autossómica recessiva.
Métodos: A análise do gene que codifica a Marenostrina (MEFV) foi realizada em 83 indivíduos – 19 crianças com FMF diagnosticada de novo e seus familiares.
Resultados: Foram identificados catorze novos casos de FMF entre os familiares assintomáticos. Duas famílias apresentavam transmissão pseudo-dominante e dominante, respectivamente. Nas restantes registou-se a forma habitual de transmissão autossómica recessiva da doença.
Conclusão: O rastreio de familiares assintomáticos de novos doentes com FMF é uma oportunidade para revelar casos ainda não diagnosticados e para compreender a forma de transmissão da doença.

Palavras-chave: Febre Mediterrânica Familiar; Hereditariedade; Rastreio; Mutação; *Marenostrin Encoding Fever Gene.*

Introduction

Familial Mediterranean fever (FMF), an autosomal recessive disease affecting mainly Mediterranean populations (Jews, Armenians, Arabs, Turks), is the most frequent periodic syndrome.[1] It is characterised by recurrent crises of fever and serosal inflammation, leading to abdominal, thoracic or articular pain. Erysipela-like erythema affecting mainly feet and legs and effort-induced myalgia are less frequently found symptoms. The major complication of FMF is the development of renal amyloidosis. Recent studies documented amyloidosis in 7%–13% of the Turkish patients with FMF.[2] Due to widespread use of colchicine, only a minority of FMF patients now presents with amyloidosis. Therefore, early diagnosis and daily colchicine treatment have a key role in preventing FMF attacks and the development of amyloidosis.[3]

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The disease is caused by mutations affecting both alleles of marenostrin encoding fever gene (MEFV),[4,5] which is apparently expressed only in neutrophils and encodes a protein called pyrin or marenostrin.

There are more than 50 mutations in MEFV,[6] reported in the vast majority of patients with FMF.[1,7-9] The findings are consistent with the autosomal recessive mode of inheritance which has been defined in large population studies.[10-12] There have been occasional reports suggesting that FMF can also be inherited dominantly.[13-17] The occurrence of FMF in more than one generation has been mostly due to a high gene frequency and consanguinity among parents of the affected patients and this situation has been called as pseudo-dominant inheritance.[14-17] By the contrary, true dominant inheritance of FMF is rather rare[13,15,17] and there are few family studies with true autosomal-dominant inheritance proved by MEFV genotyping.

It is our clinical policy to screen all parents and siblings of newly diagnosed children with FMF proved by mutational analysis given that the disease is quite frequent in Turkey.[10,11,18]

Our aim was to find out new patients of newly diagnosed children with FMF and to check if there are different types of inheritance, other than autosomal recessive.

Patients and Methods

Nineteen children were newly diagnosed as having FMF between September 2004 and January 2007. MEFV mutational analysis was performed in eighty-three subjects including patients and their first degree relatives and other relatives of index cases 7 and 12.

Polymorphism parameters were measured (FMF strip assay kits) by polymerase chain reaction (PCR) according to the instructions on the manufacturer (ViennaLab Labordiagnosyika GmbH, Vienna, Austria). The assay for idendification of MEFV gene mutations is based on PCR and reverse-hybridization.[19] The procedure includes three steps: (1) DNA isolation, (2) hybridization of amplification products to a test strip containing allele-specific oligonucleotide probes immobilized as an array of paralel lines. (3) Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates. All samples were tested for 12 mutations in the MEFV gene, which are responsible for approximately 75-80% of all mutations: A761H, A744S, V726A, K695R, M694V, M694I, M694del, M6801 (G —> C), M680I (G —> A) in exon 10; F479L in exon 5; P369S in exon 3, and E148Q in exon 2.

All patients were evaluated according to the Tel Hashomer criteria[20,21] for clinical diagnosis of FMF.

Results

Results of mutation analysis are shown in Table I. Of the 19 children having FMF, nine (47.3%) were compound heterozygotes and six (31.5%) were homozygotes while four (21.2%) have been diagnosed clinically, according to Tel Hashomer criteria. Ten additional patients were identified among parents and siblings of the newly diagnosed children with our clinical policy. Nine of them were compound heterozygotes while only one homozygosity was identified.

Cases 7 and 12 exhibited an autosomal-dominant inheritance (Figure 1 and 2). Pseudo-dominant inheritance has been detected in Cases 6 and 18 (Figure 3 and 4).

Mutations in autosomal-dominant inheritance of Cases 7 and 12 were the same in both families (E148Q/P369S), despite absence of consanguinity.

After a comprehensive analysis of MEFV in these two families, four new patients were added to the additional cluster, which raised the number of additional cases from ten to fourteen. In total, there were 33 patients (25 children and 8 adults) diagnosed with FMF among 83 screened subjects. Two children with FMF had no symptoms and all adult patients had symptoms at the time of diagnosis. None of the heterozygote adults exhibited symptoms.

Discussion

The identification of the FMF gene and its different mutations has led to the application of a noninvasive and sensitive molecular genetic test for an accurate diagnosis of this disease. General approach is that in patients presenting with typical clinical features and with an appropriate ethnic origin, the diagnosis can be made without genetic confirmation.[1,20-22] In patients who are unaware of their illnesses and in the ones who exhibit a silent course, FMF cannot be diagnosed unless MEFV ge-

Table I. Mutations in MEFV gene determined in patients and their family members.

Cases	Mutations	Features
1	M680I (G/C) / M694V	SP
1-Sibling	M680I (G/C) / M694V	SP
1-Father	M680I (G/C) / -	SN
1-Mother	M694V / -	SN
2	M694V / M694V	SP
2-Sibling	M694V / -	SN
2-Sibling	- / -	SN
2-Father	M694V / -	SN
2-Mother	M694V / -	SN
3	M694V / M694V	SP
3-Sibling	- / -	SN
3-Father	M694V / -	SN
3-Mother	M694V / -	SN
4	E148Q / -	Clinical diagnosis, SP
4-Sibling	- / -	SN
4-Father	E148Q / -	SN
4-Mother	- / -	SN
5	M694V / -	Clinical diagnosis, SP
5-Sibling	M694V / -	SN
5-Father	- / -	SN
5-Mother	M694V / -	SN
6	M694V / V726A	Pseudo-dominant inheritance, SP
6-Sibling	E148Q / V726A	SN
6-Sibling	M694V / V726A	SP
6-Sibling	M694V / -	SN
6-Father	E148Q / M694V	SP
6-Mother	V726A / -	SN
7	E148Q / P369S	Dominant inheritance, SP
7-Sibling	E148Q / P369S	SP
7-Father	- / -	SN
7-Mother	E148Q / P369S	SP
7-Grandfather	E148Q / P369S	SP
8	P369S / -	Clinical diagnosis, SP
8-Sibling	P369S / -	SN
8-Father	- / -	SN
8-Mother	P369S / -	SN
9	E148Q / R761H	SP
9-Sibling	- / -	SN
9-Father	R761H / -	SN
9-Mother	E148Q / -	SN
10	R761H / V726A	SP
10-Father	R761H / -	SN
10-Mother	V726A / -	SN
11	M694V / M694V	SP
11-Sibling	M694V / -	SN
11-Sibling	M694V / -	SN

(continue)

notyping has been performed.

There are about 50 mutations of the *MEFV*[6], and in the majority of centers, patients are screened only for the most common mutations. Therefore, the patient may still be diagnosed with FMF even if genetic analysis reveals no mutations or just a single mutation (heterozygous). In this case, if clinical manifestations are compatible with FMF, the patient is put on a trial of colchicine.[20,21] If there is a positive response to the colchicine trial, and symptoms return after cessation of colchicine, it is assumed that there are mutations in other parts of the gene. Since clinical manifestations of cases 4, 5, 8, and 15 were convincing, they have been accepted as FMF patients even though genetic analysis revealed heterozygosity.

Familial Mediterranean fever is an ethnically related disease, with an autosomal recessive inheritance, and an affected population estimated as reaching approximately 120 000 individuals worldwide.[23] It is inherited by a MEFV located on the short arm of chromosome 16.[24] The gene was cloned and the first 4 mutations were reported in 1997.[4,5] Since then, about 50 mutations have been identified so far.[6] Although the common mode of inheritance is autosomal recessive, there are few reports suggesting that FMF can also be inherited dominantly.[13-17] Tel-Hashomer criteria were used for diagnosis of FMF.[20,21] Tel-Hashomer criteria, with major and minor criteria, have been described as a diagnostic criterion of FMF. It is also very valuable in the areas where FMF is common. Major criteria include 1) Recurrent fever together with serositis, 2) Amyloid A (AA) amyloidosis wi-

Table I. ***continuation***

Cases	Mutations	Features
11-Father	M694V / -	SN
11-Mother	M694V / -	SN
12	E148Q / P369S	Dominant inheritance, SP
12-Sibling	E148Q / P369S	SP
12-Sibling	- / -	SN
12-Father	- / -	SN
12-Mother	E148Q / P369S	SP
12-Maternal uncle	E148Q / P369S	SP
12-Maternal uncle's daughter	E148Q / P369S	SP
12-Grand-mother	E148Q / P369S	SP
13	M694V / M694V	SP
13-Sibling	M694V / M694V	SN
13-Father	M694V / -	SN
13-Mother	M694V / -	SN
14	M694V / M694V	SP
14-Sibling	M694V / -	SN
14-Sibling	M694V / -	SN
14-Sibling	M694V / -	SN
14-Father	M694V / -	SN
14-Mother	M694V / -	SN
15	V726A/ -	Clinical diagnosis, SP
15-Father	V726A/ -	SN
15-Mother	- / -	SN
16	E148Q / M680I (G/C)	SP
16-Father	E148Q / -	SN
16-Mother	M680I (G/C) / -	SN
17	M694V / R761H	SP
17-Sibling	- / -	SN
17-Father	M694V / -	SN
17-Mother	R761H / -	SN
18	P369S / M694V	Pseudo-dominant inheritance, SP
18-Father	P369S / -	SN
18-Mother	E148Q / M694V	SP
19	M694V / M694V	SP
19-Sibling	M694V / -	SN
19-Father	M694V / -	SN
19-Mother	M694V / -	SN

thout any other susceptible agent, and 3) Good response to continual treatment with colchicine. Minor criteria also include 1) Recurrent fever, 2) Erysipelas-like erythema, and 3) Positive familial background. Two major criteria or two minor criteria along with one major criterion indicate definite diagnosis of disease. Additionaly, one major criterion with one minor indicates probable diagnosis of disease.

In thirteen children, it is obvious that mode of inheritance is autosomal recessive since all parents are heterozygote. Transmission in four children has been proved to be pseudo-dominant in our two families (index cases 6 and 18 and 2 relatives of case 6). In a recent study, it has been reported that the carrier rate in Turkey is 1 in 5.[18] Therefore, it is not surprising that either the mother or the father carries two mutations while the other parent is simple an heterozygote. In such a case, each of two mutations must have been inherited separately from each parent and this situation is called as pseudo-dominant inheritance.[13-16] This type of transmission is due to a high gene frequency and consanguinity among parents of the patients. A report from Israel found that, among 3 000 patients, the occurrence of FMF in more than one generation was consistent with a recessive mode of inheritance in 75 families.[15].

True dominant inheritance of FMF has been reported very rarely.[13,15,17] The first observation in 1995 preceded the molecular diagnosis era of FMF[15]. The authors found 2 families (one of Ashkenazi and the other of Georgian Iraqi origin), in which FMF occurred in 4 consecutive generations and explained the mode of inheritance only by autosomal-dominant inheritance. In 2000, the first true dominant inheritance of FMF in three families, proved by MEFV gene analysis, has been reported.[13] The results of MEFV analysis in this study provided compelling evidence for autosomal-dominant inheritance. Dominant FMF was associated with E148Q/M694I encoded on a single al-

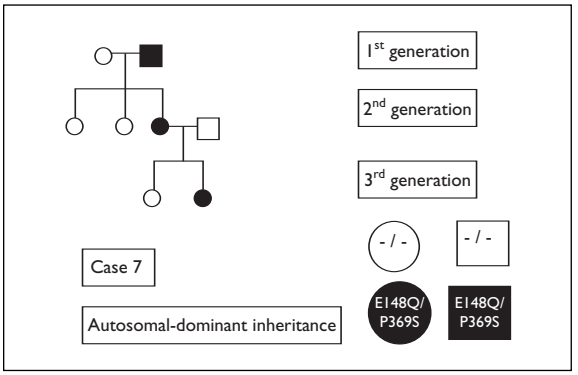

Figure 1. Autosomal-dominant inheritance in family of case 7

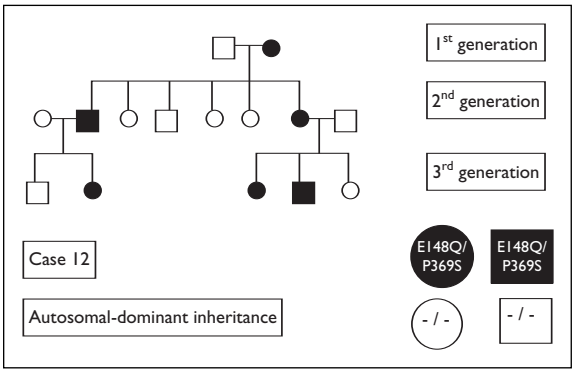

Figure 2. Autosomal-dominant inheritance in family of case 12

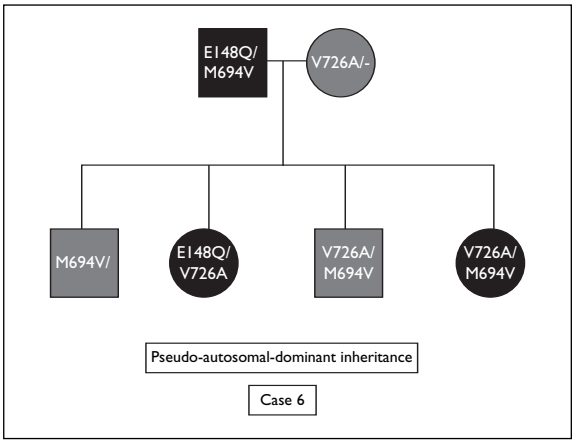

Figure 3. Pseudo-autosomal-dominant inheritance in family of case 6

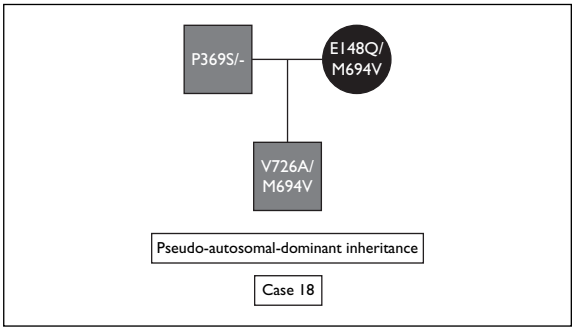

Figure 4. Pseudo-autosomal-dominant inheritance in family of case 18

lele. Sequencing of the complete coding region failed to detect any abnormality in the second MEFV allele in any of the three families (Indian, Turkish and British origin). Another report came from Spain.[17] The authors described a three-generation Spanish kindred with five family members affected by a severe periodic inflammatory disorder associated with renal AA amyloidosis unresponsive to colchicine. However, the diagnosis of FMF in those patients is doubtful. The long fever episodes with a predominant joint involvement and the resistance to colchicine raised the question of whether the periodic syndrome seen in this kindred is a true FMF with unusual manifestations or rather another MEFV-associated periodic syndrome.

Here we described four Turkish children in two families presenting three-generation with true autosomal inheritance associated with E148Q/P369S encoded on a single allele. There was no consanguinity between these two families. The report by Booth *et al*[13] in 2000 also described a Turkish family with E148Q/M694I mutation. Despite the fact that the frequency of E148Q (less than 3.55%) is rather low in Turkey,[10,11,18] the existence of compound heterozygosity with E148Q in three Turkish families without consanguinity is really interesting. Besides, P369S frequency[10,11,18] is even much lower than E148Q. The occurrence of E148Q/P369S is expected to be quite rare under normal conditions. To understand the occurrence of E148Q/P369S in families with true autosomal inheritance, the existence of complex alleles, modifier loci, genetic heterogeneity and possible epigenetic factors should be studied extensively.

Conclusion

Screening the family members of newly diagnosed FMF patients provides the opportunity to reveal undiagnosed new cases and to understand the

mode of inheritance. Newborn screening program might be carried out in Turkey and in other appropriate ethnic origins because of the fact that early diagnosis and daily colchicine treatment have a key role in preventing FMF attacks and the development of amyloidosis.

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