

MEFV mutation analysis of familial Mediterranean fever in Japan

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Abstract

Background

Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurrent attacks of fever with serosal inflammation. FMF gene (MEFV) mutations have been identified primarily in patients from Mediterranean populations. Although several clinical cases have been reported in Japan, there have been few reports to date on mutation analysis. We studied FMF patients and their relatives to examine the clinical and genetic features of this disease in the Japanese population.

Methods

Twelve Japanese FMF patients who met the Tel Hashomer criteria and a total of 17 relatives from 5 of 10 families underwent molecular genetic studies to detect MEFV mutations. The characteristics of these Japanese FMF patients and geno-phenotypical correlations were examined.

Results

Almost all of our patients had been suffering for a long time from fever of unknown origin and one patient also had systemic amyloidosis. In our 12 FMF patients, we detected the substitutions E84K, L110P, E148Q, R761H and M694I. We also newly diagnosed 2 relatives as having FMF based on clinical symptoms and the existence of FMF mutations. One patient was homozygous for E148Q, the patient with systemic amyloidosis was a homozygote for M694I and 4 patients from 3 families were compound heterozygotes for E148Q and M694I. Three patients in one family were compound heterozygotes for E148Q, L110P and M694I. There were 3 patients who were heterozygous for E84K, L110P-E148Q or M694I and had no other nucleotide changes in the exons of MEFV. On the other hand, 2 relatives who had never experienced symptoms of FMF were homozygous for L110P-E148Q as well as compound heterozygous for E148Q/E148Q-R761H. E148Q and M694I were the most frequently detected substitutions in our study.

Conclusions

MEFV mutations occur in Japanese FMF patients though FMF is rare in Japan. The identification of MEFV mutations could be a reliable diagnostic test for FMF. The results of genetic analyses on 14 Japanese FMF patients in this study revealed that E148Q and M694I are frequent alleles.

Key words

Familial Mediterranean fever, MEFV, genetic testing.

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Introduction

Familial Mediterranean fever (FMF) is a hereditary inflammatory disease characterized by recurrent attacks of fever and serositis; additionally, a major complication of FMF is secondary amyloidosis. The disease is transmitted in an autosomal recessive pattern, and predominately affects populations surrounding the Mediterranean basin (1).

In 1997, two consortia independently cloned a gene (*MEFV*) on 16p13.1 that is responsible for FMF (2, 3). *MEFV* contains a 2,346-bp coding sequence that spans 10 exons. Normally, the 781-amino acid protein with a predicted molecular weight of 86 kDa, which is known as either “pyrin” or “marennos-trin”, is likely to assist in minimizing inflammation by deactivating the immune response, but an abnormality in this protein will induce an inappropriate full-blown inflammatory reaction, as occurs in attacks of FMF.

To date, approximately 30 mutations associated with FMF have been defined (4). The five most frequent mutations are E148Q in exon 2, and M680I, M694V, M694I and V726A in exon 10, but the spectrum of *MEFV* mutations in FMF patients differs among countries and populations. In North African Jews, M694V is over-represented, while in East European Jews (Ashkenazim), a milder mutation, V726A, is most frequent. The prevalence of these two mutations in Oriental Jews is in between that of North African Jews and Ashkenazim, suggesting that M694V and V726A likely spread from the middle East more than 2500 years ago (4, 5).

The penetrance of M694V homozygosity is very high. It has been demonstrated that this genotype is correlated with a severe disease course. Other mutations at codon 694 and mutation M680I may also be severe. On the other hand, E148Q is the least penetrant FMF mutation and is recognized to have a mild effect on FMF patients.

In Japan, few cases of FMF have been reported and most were sporadic. Accordingly, FMF is thought to be a rare disease, and its diagnosis is relatively difficult in Japan compared to that in Middle Eastern or European countries. Twenty-two cases of FMF (six cases with a family history) had been reported through 2005 (6-11). In the present

study, we examined 12 Japanese FMF patients (2 patients were compatriots and 2 patients were father and son) and relatives from 5 of 10 families who agreed to lineage analysis to examine *MEFV* mutations and the characteristics of FMF in a Japanese population.

Material and methods

Patients

Our subjects were 12 patients (5 males and 7 females) with recurrent fever accompanied by peritonitis, pleuritis or arthritis who had consulted with or been referred to our hospital with a diagnosis of FMF. These patients met the Tel Hashomer criteria for the clinical diagnosis of FMF (12), and other sources of recurrent fever, such as infection or autoimmune, neoplastic or metabolic causes, had been excluded by clinical, laboratory and instrumental examinations.

This study was approved by the relevant institutional ethical review board. We conducted lineage analysis on patients with clinical FMF and relatives from 5 of 10 families after obtaining written informed consent from all subjects.

MEFV mutation analysis

Genomic DNA was extracted from peripheral leukocytes using standard procedures. We analyzed the part of exon 10 in the *MEFV* gene where most of the previously reported mutations were located (hot spot) (13). Polymerase chain reaction (PCR) was performed using 5'-GAGCCTGCAAGACATCCATA-3' and 5'-TGACCACCCACTGGACAGAT-3' as primers. When we failed to detect two mutations in the hot spot region, we continued searching exon 10 and other exons until we detected the gene mutations (3, 14, 15). When we detected a mutation in the sequence from the PCR product, we confirmed the presence of the alteration using the TA cloning method with a commercial kit (pGEM[®]-T Easy Vector; Promega Corporation, Madison, WI, USA).

Results

The clinical features and genotypes of the FMF patients in this study are listed in Table I. All patients met the Tel Hashomer criteria and almost all had been suffering from fever attacks for a long time. Nine patients were sporadic cases (except b-3, 5 and d-3, 4, 5). Four

Competing interests: none declared.

Table I. FMF cases.

Family	Case	Age at onset	Age at study	Sex	Symptoms	Amy	Surg	Analysis	Genotype(s)
a	5	3	40	M	chest and abdominal pain, arthralgia, erythema	yes	no	exon 10	M694I / M694I
b	3	15	60	F	chest and abdominal pain, arthralgia	no	no	exon 2, 10	E148Q / M694I
	5	11	54	F	chest and abdominal pain, arthralgia	no	yes	exon 2, 10	E148Q / M694I
c	4	12	23	F	chest and abdominal pain, arthralgia	no	yes	exon 2, 10	E148Q / M694I
d	1	30s	54	M	chest pain	no	yes	exon 2, 10	E148Q / M694I
	3	15	27	F	chest pain	no	no	exon 2, 10	L110P-E148Q / M694I
	4	2	20	M	chest and abdominal pain, arthralgia	no	no	exon 2, 10	L110P-E148Q / M694I
e	3	12	16	F	chest pain	no	no	exon 2, 10	L110P-E148Q / M694I
	4	15	31	M	laryngeal, chest and abdominal pain	no	yes	exon 2, 10	E148Q / E148Q-R761H
f	3	17	20	F	abdominal pain	no	no	exon 2, 10	L110P-E148Q / L110P-E148Q
g	3	20	27	F	abdominal pain, arthralgia	no	no	exon 2, 10	E148Q / E148Q
h	3	23	28	F	chest pain	no	no	all 10 exons	L110P-E148Q / normal
i	3	22	42	M	abdominal pain, arthralgia	no	no	all 10 exons	M694I / normal
j	3	13	19	F	chest pain	no	no	all 10 exons	E84K / normal

M: male; F: female; Amy: complication of amyloidosis; Surg: past history of abdominal surgery.

patients had a history of abdominal surgery and one patient had systemic amyloidosis (heart, digestive organs and kidneys). The attacks tended to appear during menstruation in Subject b-5.

Mutation analysis was performed on exon 10 for members of Family a; on exons 2 and 10 for Families b, c, d and e and for Subjects f-3 and g-3; and on all 10 exons for Subjects h-3, i-3 and j-3. We found the substitutions E84K, L110P, E148Q, M694I, and R761H in our FMF patients and their relatives (Fig. 1 and Table I). The patient with secondary amyloidosis (a-5) was homozygous for M694I, one patient (g-3) was homozygous for E148Q, and 5 patients (b-3, 5, c-4, d-1, 4) were compound heterozygous for E148Q and M694I. In 3 patients (h-3, i-3, j-3), only one nucleotide change was found. Subject i-3 was heterozygous for M694I, but had no other nucleotide changes in the remaining coding region except for the silent substitutions 1764G→A in exon 9 in both alleles. Subject h-3 was heterozygous for L110P-E148Q, but had no other nucleotide changes in the remaining coding region except for the silent substitutions 942C→T in exon 3, 1422G→A, 1428A→G and 1530T→C in exon 5, and 1764G→A in exon 9.

Figure 1 shows the pedigrees of the 5 FMF families in the present study. Subjects d-3, 4 and 5 were compound heterozygous for L110P-E148Q/M694I. Subjects d-3 and 5 had not been diag-

nosed with FMF but had been suffering from fever and chest pain for many years; their symptoms were not severe enough to have required medical attention. After detecting the mutations in two alleles, we confirmed that they had experienced mild FMF attacks and that their clinical symptoms met the Tel Hashomer criteria. Nevertheless, 2 relatives with mutations had not experienced symptoms of FMF; one was homozygous for E148Q-L110P and the other was compound heterozygous for E148Q/E148Q-R761H. The frequen-

cies of E148Q and M694I were 14/26 and 10/28, respectively.

Discussion

In the present study, many patients had endured more than 10 years from onset to diagnosis of FMF, and 2 sisters (d-3, 5) were relatively asymptomatic although they showed a genotype similar to that of Subject d-4. The factors contributing to this prolonged delay in the diagnosis of FMF are social status (immigrant), female, physician negligence and lack of patient awareness (16). In

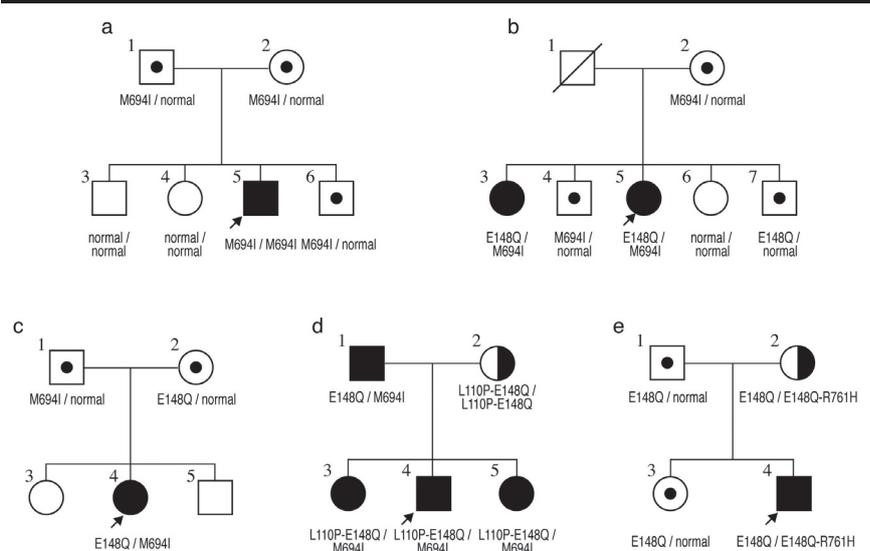


Fig. 1. Pedigrees of 5 FMF families with segregations of *MEFV* mutations. Symbols: square = male; circle = female; filled symbol = affected; empty symbol = unaffected; slashed symbol = deceased; half symbol = unaffected but with nucleotide changes in both alleles; dotted symbol = obligate carrier.

Japan, physician negligence is the most important cause. The authors believe that the gender discrepancy may result from hormonal factors, modifying genes that generate a disease of milder severity in females, differences in fibrositic point sites in males, and socioeconomic factors.

To the best of our knowledge, there have been only 22 other cases of FMF (6 including a family history) reported in Japan since 1976 (Table II), and genetic analyses have been performed on 9 Japanese FMF patients (6-11) other than those in the present study. Based on the results of genetic analyses of Japanese FMF cases including the present results, E148Q and M694I occur most frequently (their respective frequencies in the present study were 21/38 and 18/44).

Two out of 5 subjects who were homozygous for E148Q had not experienced FMF symptoms. The clinical implications of E148Q are a matter of controversy, however, it is recognized to have a mild effect on FMF patients. Touitou found that E148Q was the least penetrant of the five mutations, with the percentage of incomplete penetrance

of E148Q/E148Q being 45% (4). In a study by Topaloglu *et al.*, an analysis of 26 Turkish individuals homozygous for E148Q found that only 22 had experienced FMF attacks (17). In another study of 30 Greek FMF cases, 21 patients carried E148Q, one of whom was homozygous (E148Q/E148Q), while the other 20 showed E148Q in combination with other mutations (compound heterozygotes). It was also found that in a Greek population the E148Q allele was dramatically more frequent in patients than in the control population (18.3% vs. 1.8%) (18). These studies support the view that E148Q is a disease-causing mutation.

On the other hand, E148Q was identified in 15% and 21% of *MEFV* genes from Chinese and Punjabi Indian control subjects, respectively (19). In Japan, the allele frequency of E148Q was reported to be 16% in a small control population (9). In a large study analysis of 233 patients of Sephardic Jewish origin in France and their healthy relatives, the frequency of the E148Q allele was similar (20). These findings support the hypothesis that E148Q is

merely a benign polymorphism and not a disease-causing mutation. We are not prepared to state that E148Q is unequivocally unrelated to the pathogenesis of FMF, however, it is clear that its impact on FMF manifestations is low.

Five patients in the present study had substitutions L110P and E148Q on the same allele as reported case (21). L110P has been reported in 3 FMF cases but not in controls (4, 21). The penetrance of L110P may be as incomplete in FMF as that of E148Q. Since we did not screen Japanese healthy controls for *MEFV* mutations in the present study, it is difficult to interpret the data on E148Q and L110P. We detected R761H, which has been reported in 19 FMF patients but not in controls (4, 13, 14), in Family e. We detected E84K, which to the best of our knowledge has never been reported previously, in Subject j-3. However, we did not assess the allele frequency of E84K in our Japanese study population, so we are unable to comment on the relationship between E84K and FMF.

In 3 patients, only one nucleotide change was found. Although we could not determine if the nucleotide change was transmitted from the patients, there have been reports of FMF patients with only one nucleotide change. Cazeneuve *et al.* report that two FMF alleles were unidentified in 3 patients and only one nucleotide change was identified in 6 patients of 90 sporadic cases of FMF in Armenians (22). Bernot *et al.* failed to detect anomalies in 17 of 120 FMF chromosomes (15). The absence of 1 or 2 nucleotide changes occurs in some patients in nearly every cohort of FMF patients.

There are several explanations for these observations. First, other nucleotide changes, which modify the expression of the *MEFV* product, could lie either in the promoter region, within an intron, in the 3'-untranslated region, or in another gene. Second, it is possible that FMF may be inherited autosomal dominantly. Booth *et al.* report that 3 of 5 families in which FMF appeared to be inherited dominantly were associated with heterozygosity for either M694~~del~~ alone or with compound heterozygosity for E148Q/M694I (23). Recently it has been documented that an H478Y variant alone in a Spanish fam-

Table II. FMF cases in Japan.

Author	(Year)	Age at onset	Sex	FH	Symptoms	Genotype(s)
Hayashi	(1979)	6	M	no	chest and abdominal pain	
Atarashi	(1979)	21	M	no	arthralgia, eruption	
Nishida	(1983)	14	M	no	chest and back pain	
Oimomi	(1985)	30	M	no	eruption	
Kuyama	(1986)	10	M	no	chest pain, arthralgia	
Schwabe	(1987)	16	M	no	chest and abdominal pain, arthralgia	
Ota	(1987)	15	F	no	abdominal pain	
Sugimoto	(1989)	37	M	no	chest and abdominal pain	
Nishitani	(1989)	16	M	no	chest and abdominal pain	
Ide	(1989)	30	M	no	chest and abdominal pain	
Takahashi	(1989)	48	M	yes	chest, back and abdominal pain	
Takenaka	(1994)	19	F	yes	chest and abdominal pain	
Doi	(1996)	21	M	no	abdominal pain, eruption	
Shinozaki	(2002)	1	F	yes	chest and abdominal pain, arthralgia	M694I / M694I*
		9	F	no	chest and back pain	M694I / normal*
Yoshida	(2003)	12	F	yes	chest and abdominal pain	M694I / normal*
Kotone-Miyahara	(2004)	38	M	no	abdominal pain	E148Q / M694I
		42	M	no	abdominal and back pain	E148Q / M694I
Komatsu	(2004)	23	F	yes	chest and abdominal pain	E148Q / M694I
		3	M	no	fever	E148Q-P369S-R408Q / normal
Nakamura	(2005)	20	F	yes	chest and abdominal pain	E148Q / M694I
Suzuki	(2005)	25	M	No	abdominal pain, diarrhea	E148Q / E148Q**

M: male; F: female; FH: family history; *:not analyzed for exon 2; **:other exons were unknown.

ily produced a severe FMF phenotype with amyloidosis (24). The recognition that *MEFV* mutations affecting only a single allele can give rise to FMF suggests that a 50% complement of normal pyrin activity is not sufficient to prevent disease susceptibility.

Several studies have reported that FMF patients who are homozygous for M694V have more severe manifestations, require higher doses of colchicine for treatment (22, 25) and have a higher risk of amyloidosis (26, 27). Ben-Chetrit and Backenroth report a close association between renal amyloidosis and 694 substitutions in the *MEFV* gene (28). In the present study, Subject a-5, who suffered from systemic amyloidosis, was placed on maintenance hemodialysis due to chronic renal failure. To the best of our knowledge, this was the first reported Japanese FMF patient with amyloidosis (29). Additionally, this subject was homozygous for M694I; there may be an association between amyloidosis and homozygosity for M694I among Japanese patients as well.

The *MEFV* mutations that had been reported in the Middle East, Europe, and the United States (13) were also found in Japanese FMF patients and their families in the present study. The origin of the modern Japanese FMF population is considered to be divided into two groups (30): the Jomon people, who immigrated from southeast Asia in the Jomon period (between 12,000 and 2,300 years ago), and the Yayoi people, who immigrated from northern Asia, especially Korea, in the Yayoi period (between 2,300 and 1,700 years ago). Okinawan people are direct descendants of the Jomon people and mainland Japanese are close to the Yayoi people. Japanese FMF patients live both on the mainland and in Okinawa, so the mutational *MEFV* genes may have entered Japan from southeast and northern Asia more than 1,700 years ago.

It is necessary to consider FMF if a patient has recurrent attacks of fever and serositis. The identification of *MEFV* mutations could be meaningful for the diagnosis of FMF in Japan.

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