Three Japanese patients (mother and two children) with familial Mediterranean fever associated with compound heterozygosity for L110P/E148Q/M694I and an autosomal true dominant inheritance pattern

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Summary

Familial Mediterranean fever (FMF) is characterized by repeated episodes of fever, peritonitis, pleuritis, and synovitis. We describe here 3 Japanese patients (a mother and 2 children) in whom FMF was diagnosed on analysis of *MEFV*. A 40-year-old woman presented with fever and abdominal pain. The patient had had these symptoms on and off since childhood and consulted many hospitals. A 38-year-old man had abdominal pain and fever since the age of 30 years. A 59-year-old woman had had episodes of fever, abdominal pain, and chest pain for more than 20 years. *MEFV* gene analysis showed compound heterozygosity for L110P, E148Q, and M694I in all three patients. In Japanese patients with FMF, this mode of autosomal true dominant inheritance has not yet been reported. FMF is difficult to diagnose unless it is included in the differential diagnosis by physicians. We hope that our valuable experience will promote increased awareness and understanding of FMF. *(Asian Pac J Allergy Immunol 2013;31:325-9)*

Key words: *autoinflammatory disease, familial Mediterranean fever, FMF, MEFV gene*

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Introduction

Familial Mediterranean fever (FMF), a representative autoinflammatory disease, is an autosomal recessive genetic disease characterized by periodic episodes of fever with nonbacterial serositis, which repeatedly occur and resolve. FMF primarily affects certain ethnic groups, such as Sephardic Jews, Armenians, Turks, and Arabs, but is rare in Japan.[1] We describe our experience with 3 Japanese patients (a mother and 2 children) with repeated bouts of fever and serositis in whom FMF was diagnosed on genetic analysis of *MEFV*, the causative gene of FMF.

Patient 1

A 40-year-old woman presented with fever, abdominal pain, and arthralgia (Figure 1A). She experienced "3 to 4 episodes of fever and abdominal pain per year" since elementary school. At the age of 30 years, she was admitted to a university hospital to undergo a detailed work-up for fever of unknown origin, but a definitive diagnosis could not be made. At the age of 34 years, the patient had abdominal pain and underwent surgery (at another hospital) for a suspected diagnosis of pancreatic disease. However, the postoperative pathological examination failed to yield a definitive diagnosis. The physical findings were as follows: body temperature, 38.4°C; mild tenderness in the lower abdomen; arthralgia in left finger joints without swelling; no lymphadenopathy; no skin erythema; no myalgia; and no edema in the extremities. The neurologic findings were normal. Symptoms such as fever, abdominal pain, and arthralgia resolved spontaneously within 1 week and the patient was symptom-free between attacks. The results of laboratory tests were as follows: erythrocyte sedimentation rate (ESR), 73 mm/hour; C-reactive protein (CRP), 13.5 mg/dl; white blood cell count (WBC), 7100/μl (neutrophils, 59%; eosinophils, 1%; basophils, 0%; monocytes, 4%; and lymphocytes, 35%), hemoglobin, 12.3 g/dl; hematocrit, 36%; platelet, 475 x 10^3/μl; serum

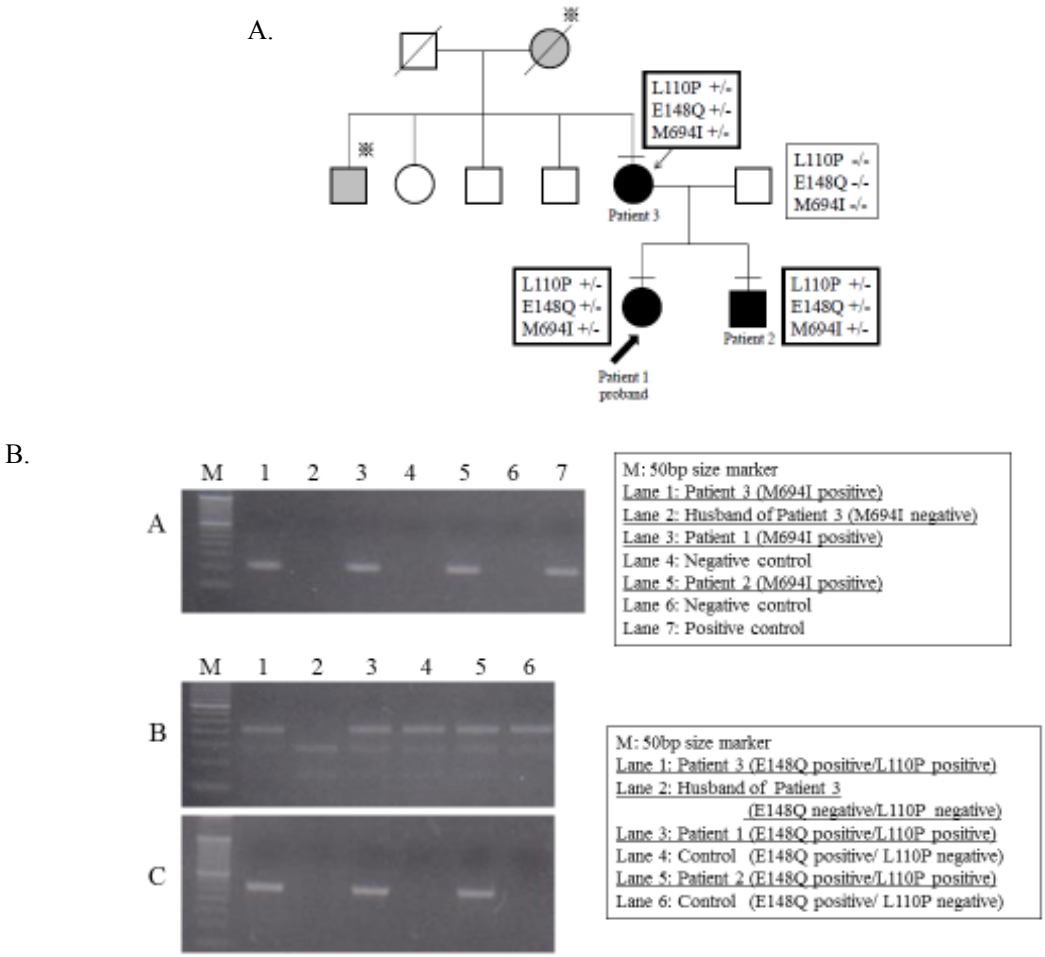

Figure 1. A. Family tree of our patients with FMF and *MEFV* gene mutations. Open boxes are asymptomatic family members. Black boxes are FMF patients with compound heterozygous mutations of L110P, E148Q, and M694I. *Shaded boxes are symptomatic individuals (reportedly had fever and abdominal pain) but details are unknown.
B. Results of *MEFV* gene analysis. A. Detection of M694I mutation by amplification refractory mutation screening system (ARMS). B. Detection of E148Q mutation by restriction fragment length polymorphism (RFLP) method. C. Detection of L110P mutation by ARMS. Lane 1: Patient 3, Lane 2: Husband of Patient 3, Lane 3: Patient 1, Lane 5: Patient 2. Patients 1, 2 and 3 showed compound heterozygosity for L110P, E148Q, and M694I.

aspartate aminotransferase (AST), 18 IU/l; serum alanine aminotransferase (ALT), 10 IU/l; serum lactate dehydrogenase (LDH), 186 IU/l; serum creatine kinase (CK), 28 IU/l; serum total protein (TP), 7.9 g/dl; blood urea nitrogen (BUN), 8.6 mg/dl, serum creatinine, 0.6 mg/dl; serum uric acid (UA), 7.2 mg/dl; serum amylase, 42 IU/l; Ferritin 77.7 ng/ml; serum amyloid A protein (SAA), 2.5 μg/ml (less than 8 μg/ml); Rheumatoid factor, 8 U/ml; antinuclear antibodies (ANA), negative; serum anti-DNA antibody, 2.0 U/ml; proteinase 3-antineutrophil cytoplasmic antibodies <10 EU; myeloperoxidase-antineutrophil cytoplasmic antibodies <10 EU; urinary protein (±); urinary glucose (+); and urine sediment, within normal limits. Chest and abdominal X-ray films showed no abnormal findings. All bacteriologic cultures of the pharynx, blood, and urine were negative. In association with the spontaneous remission of the symptoms, the CRP also normalized within 2 weeks. These episodic symptoms and laboratory findings were inconsistent with collagen vascular disease or

infectious disease. Because she had "3 to 4 episodes of fever and abdominal pain per year" since a young age, a diagnosis of autoinflammatory disease such as FMF was suspected, and *MEFV* gene analysis was performed. As for the family history, the parents were not consanguineous.

Patient 2

A 38-year-old man, the younger brother of Patient 1, had fever and abdominal pain (Figure 1A). Since the age of 30 years, the patient had 1 or 2 episodes of abdominal fullness and dull abdominal pain per year. During the past several years, episodes of fever (38.0°C to 38.9°C) with abdominal pain occurred about once per month. These symptoms spontaneously improved within 1 week. He visited to our hospital for examination of the *MEFV* gene. The physical findings were as follows: body temperature, 36.8°C; no tenderness in the abdomen; no arthralgia; no lymphadenopathy; no skin erythema; and no myalgia. The neurological findings were normal. The results of laboratory tests were as follows: ESR, 2 mm/hour; CRP, 0.1 mg/dl; WBC, 8800/μl (neutrophils, 45%; eosinophils; 16%; basophils, 1%; monocytes, 5%; and lymphocytes, 33%); hemoglobin, 15.0 g/dl; hematocrit, 43.4%; platelet, 302 x 10^3/μl; AST, 25 IU/l; ALT, 23 IU/l; LDH, 207 IU/l; CK, 131 IU/l; TP, 7.6 g/dl; BUN, 15.8 mg/dl; serum creatinine, 0.8 mg/dl; UA, 7.9 mg/dl; serum amylase, 76 U/l; SAA, 2.5 μg/ml; ANA, negative; urinary protein (-); urinary glucose (-); urinary occult blood (-); and urine sediment, within normal limits.

Patient 3

A 59-year-old woman, the mother of Patients 1 and 2, had episodes of fever, abdominal pain, chest pain, and arthralgia (Figure 1A). For more than 20 years (since the age of about 30 years), she had had periodic episodes of fever (38.0°C to 38.9°C) and abdominal pain. Fever, abdominal pain, and upper precordial pain occurred about once a month. She visited to our hospital for examination of the *MEFV* gene. The physical findings were as follows: body temperature, 38.2°C; mild tenderness in right lateral abdomen and upper precordial chest pain; arthralgia in the right shoulder joint; no lymphadenopathy; no skin erythema; and no myalgia. The neurological findings were normal. The results of laboratory tests were as follows: ESR, 57 mm/hour; CRP, 3.4 mg/dl; WBC, 7800/μl (neutrophils, 51%; eosinophils; 1%; basophils, 0%; monocytes, 4%; and lymphocytes, 44%); hemoglobin, 11.4 g/dl; hematocrit, 34.9%; platelet, 299 x 10^3/μl; AST, 18 IU/l; ALT, 16 IU/l; LDH, 218 IU/l; CK, 107 IU/l; TP, 7.7 g/dl; BUN, 12.5 mg/dl; serum creatinine, 0.6 mg/dl; UA, 4.4 mg/dl; serum amylase, 92 U/l; SAA, 4.2 μg/ml; ANA, x40; urinary protein (-); urinary glucose (-); urinary occult blood (2+); and 1 to 4 red blood cells/high power field (hpf) and less than 1 white blood cell/hpf in urine sediment.

MEFV gene analysis

Figure 1B shows the results of *MEFV* gene analysis. After obtaining informed consent from the 3 patients and the husband of Patient 3, gene analysis was performed. Genomic DNA was extracted from peripheral blood. Subsequently, sequence analysis of the *MEFV* gene showed a heterogeneous mutation (ATG [Met] to ATA [Ile]) at codon 694 in exon 10 in all 3 patients, but not in the husband of Patient 3. Next, L110P, M694I, and R202Q mutations were screened with the use of an amplification refractory mutation screening system (ARMS). The E148Q mutation was screened with a restriction fragment length polymorphism (RFLP) method. Consequently, all patients were negative for R202Q and positive for L110P, E148Q, and M694I, with the exeption of the husband of Patient 3. With the use of RFLP analysis, these *MEFV* gene mutations were confirmed to be compound heterozygous and encoded on a single allele.

Clinical course

On the basis of the clinical diagnostic criteria for FMF,[2] Patient 1 satisfied 1 of the major criteria (peritonitis) and 2 of the minor criteria (joint, favorable response to colchicine), Patient 2 satisfied 2 of the minor criteria (abdomen, favorable response to colchicine), and Patient 3 satisfied 2 of the major criteria (peritonitis, pleuritis) and 2 of the minor criteria (joint, favorable response to colchicine) (Table 1). All 3 patients therefore met the clinical diagnostic criteria for FMF. Treatment with oral colchicine (0.5 mg/day) was started. Symptoms resolved in Patient 2 and Patient 3. Because fever and abdominal pain recurred in Patient 1, the dose of colchicine was increased to 0.75 mg/day, and her condition stabilized.

Discussion

FMF is a representative autoinflammatory disease with an autosomal recessive pattern of inheritance. In Japan, FMF is relatively rare and about 50 cases have been reported.[3] The causative gene of FMF resides on chromosome 16p13.3 and was identified to be *MEFV* in 1997.[4] This gene

Table 1. Summary of 3 patients. SAA denotes serum amyloid A protein

		Patient 1	Patient 2	Patient 3
	Sex	W	M	W
	Age at diagnosis (yr)	40	38	59
	Age at disease onset (yr)	7	30	30
	Delay of diagnosis (yr)	33	8	over 20
Mutations	*MEFV* gene	L110P E148Q M694I	L110P E148Q M694I	L110P E148Q M694I
Symptoms	Fever	+	+	+
	Abdominal pain	+	+	+
	Chest pain	−	−	+
	Arthralgia	+	−	+
	Frequency	1 /month	0.5-1 /month	1 /month
Examination on 1st visit	ESR	73 mm/hr	2 mm/hr	57 mm/hr
	CRP	13.5 mg/dl	0.1 mg/dl	3.4 mg/dl
	SAA	2.5 μg/ml	2.5 μg/ml	4.2 μg/ml
	WBC	7100 /μl	8800 /μl	7800 /μl
	Urinary protein	±	−	−
Treatment	Colchicine	0.75 mg/day	0.5 mg/day	0.5 mg/day

encodes an 86-kDa pyrin (marenostrin) protein consisting of 781 amino acids.[5,6] Mutations of pyrin can lead to loss of control of interleukin-1β production and nuclear factor-kappa B activation,[7,8] causing invasion of activated neutrophils into the serosa and the onset of inflammatory responses. The most common mutations occur in M694V, M680I, M694I, and V726A located on exon 10 of *MEFV* gene and in E148Q on exon 2.[9] In Japanese patients, *MEFV* gene mutations are characterized by the involvement of either E148Q or M694I and no mutations of M694V, M680I, or V726A have been reported.[3,10-14] All 3 of our patients had compound heterozygous mutations of L110P, E148Q and M694I. Although detailed results of gene analysis are not available, the mother and an elder brother of Patient 3 had had similar periodic episodes of symptoms. The husband of Patient 3 had no *MEFV* gene mutations (Figure1A and 1B). These gene mutations reside on the same chromosome and are inherited from one parent by their offspring in an autosomal true dominant mode. In Japanese patients with FMF, this mode of inheritance has not been reported in detail so far. Some Japanese patients with FMF have compound heterozygous mutations involving E148Q and M694I. However, M694I mutations are rarely detected in healthy subjects.[15] This mutation is therefore considered diagnostically significant. E148Q mutations are found in 16.3% to 44.7% of healthy Japanese subjects.[10,15] FMF has been associated with homozygosity for the E148Q mutation,[13] but asymptomatic patients who are homozygous for E148Q mutation have also been reported.[15] Whether E148Q mutation causes FMF thus remains to be determined. Reports on L110P mutations in FMF are rare, and its clinical significance remains unknown.

The clinical characteristics of our patients were as follows (Table 1). The mean age at disease onset ranged from 7 to 30 years. Episodes of fever, abdominal pain, chest pain, and arthralgia occurred periodically. These symptoms were well controlled by treatment with colchicine. There was no erysipelas-like eruption and no evidence of secondary amyloidosis. FMF may be incorrectly diagnosed as collagen vascular disease or psychosomatic disease, and some patients may needlessly undergo surgery. Some patients seek

medical attention at different hospitals over the course of many years. When patients present with periodic episodes of fever and serositis, FMF and autoinflammatory disease should therefore be included in the differential diagnosis.

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