Clinical Relevance of *MEFV* Gene Mutations in Japanese Patients with Unexplained Fever

To the Editor:

At the beginning of 2007, we investigated the frequencies of *MEFV* gene mutations in Japanese patients with unexplained fever or undifferentiated arthritis to determine their role in phenotypical features of familial Mediterranean fever (FMF)-related diseases. Patients were asked to complete a questionnaire concerning fever, recurrent typical attacks of FMF, including peritonitis, pleuritis, and arthritis, and transient inflammatory response. On the basis of the Tel-Hashomer criteria[1], we divided the study subjects into 3 groups, as follows: Group 1, typical FMF (presence of 1 or more major criteria independent of the presence of minor criteria); Group 2, probable FMF (absence of major criteria and 2 or more minor criteria); Group 3, unlikely (not belonging to either Group 1 or 2). Patients who had previously been diagnosed with typical FMF were not included. All patients were first enrolled as having unexplained fever, and finally diagnosed as FMF based on clinical evidence. We stress that the overall survey for the recent clinical manifestations, including the response to colchicine, was not complete in a few patients.

Up to January 2011, we had enrolled 142 Japanese patients with unexplained fever or undifferentiated arthritis in our genetic analysis. The subjects are 86 women and 56 men, with mean age of 38.2 ± 17.8 years. As shown in Table 1, 72 (50.7%) patients had single-nucleotide polymorphisms (SNP) of exon 2 of *MEFV* gene and 15 (10.6%) patients had SNP of exon 3 of *MEFV* gene. We identified 16 patients carrying a mutation of exon 10 (M694I): 3 were homozygotes, 11 were compound heterozygotes, and 2 were heterozygotes. All patients having the M694I mutation had typical episodes of serositis or monoarthritis in addition to periodic fever, and had been newly diagnosed as typical FMF. In contrast, the prevalence of FMF in patients with *MEFV* exon 1, 2, or 3 SNP was markedly lower (36.0%) than that in carriers of M694I. We compared the allele frequencies among typical or incomplete FMF patients and healthy subjects (35 women, 41 men, mean age 31.5 ± 8.0 yrs). The frequencies of M694I and E84K alleles were increased in patients with typical FMF, and frequencies of E148Q, P369S, and R408Q were increased in patients with probable FMF compared to healthy subjects ($p < 0.05$, Fisher exact test; Table 2).

We identified 12 patients carrying E84K mutation; clinical features of these patients are listed in Table 3. Among these 12, 4 had typical episodes of serositis or synovitis and periodic fever and could be diagnosed as typical FMF (Group 1). There was remittance of clinical symptoms with colchicine therapy in these patients with typical FMF, except for 1 patient (Patient 3) who remitted spontaneously. Another 2 patients carrying E84K mutation were considered to be "probable FMF" (Group 2); one patient remitted spontaneously, and colchicine was beneficial in the other patient. The remaining 3 patients carrying E84K mutation had atypical symptoms and did not fulfill a diagnosis of FMF (Group 3). In the last group, who had been diagnosed as having definite rheumatic diseases (Group 4), E84K mutation may have contributed the modification or sustained musculoskeletal symptoms to concomitant rheumatic diseases despite optimal treatment including steroid and immunosuppressants. We also analyzed the clinical features of the patients carrying the SNP of exon 1, 2, or 3 of the *MEFV* gene. Similarly, a subgroup of these patients were diagnosed as having typical or probable FMF (Table 4).

In our study, all 16 patients with M694I mutation were newly diagnosed as having typical FMF and showing the higher penetration of these mutations compared to that of exon 1 (E84K), exon 2 (L110P, E148Q, R202Q, G304R), or exon 3 (P369S, R408Q) mutations. Interestingly, we found 12 patients carrying a heterozygous E84K mutation who presented heterogeneous clinical phenotypes, in contrast to M694I carriers with typical FMF. Our findings indicated that a portion of the patients carrying E84K fulfilled the diagnostic criteria for typical FMF; however, more than half of these patients had atypical symptoms. We could not find any relevant clinical similarity in patients with E84K mutation, and the clinical phenotype of these E84K carriers might differ from the homogenous FMF phenotype. Our observations suggest that the *MEFV* gene mutations, which are attributed mainly to FMF, may also be responsible for additional clinical manifestations that do not meet the criteria of FMF as described[2,3]. A significant number of patients diagnosed as FMF have only a single mutation despite sequencing of the entire *MEFV* genome region or other autoinflammatory genes, and this has led to a reconsideration of the simple loss of function of the recessive model of FMF inheritance[4,5]. Recently, Chae, *et al*[6] demonstrated that gain-of-function pyrin mutations induce NOD-like receptor family, a pyrin domain containing 3 (NLRP3)-independent interleukin 1ß activation and autoinflammation. A plausible explanation might

Table 1. Genotypes of *MEFV* gene in patients with unexplained fever (n = 142).

MEFV Genotypes	n (%)	FMF Criteria	
		Typical (female 13, male 15)	Probable (female 11, male 6)
M694I/M694I	3 (2.1)	3	
M694I/normal	2 (1.4)	2	
M694I/E148Q	9 (6.3)	9	
M694I/E148Q/L110P	2 (1.4)	2	
P369S/normal	1 (0.7)		
P369S/R408Q	4 (2.8)		1
G304R/P369S/R408Q	1 (0.7)		
E148Q/P369S/R408Q	5 (3.5)		2
E148Q/E148Q/P369S/R408Q	2 (1.4)	1	
E148Q/R202Q/P369S/R408Q	1 (0.7)		1
E148Q/G304R/P369S/R408Q	1 (0.7)		1
E148Q/normal	22 (15.5)	3	3
R202Q/normal	4 (2.8)	1	
G304R/normal	2 (1.4)		
E148Q/E148Q	1 (0.7)		
E148Q/L110P	13 (9.2)	1	3
E148Q/R202Q	1 (0.7)	1	
E148Q/E148Q/L110P	4 (2.8)		2
E148Q/L110P/R202Q	1 (0.7)		1
E84K/normal	9 (6.3)	4	2
E84K/E148Q	1 (0.7)		
E84K/E148Q/L110P	2 (1.4)		
Normal	51 (35.9)	1	1
Total (%)	142	28 (19.7)	17 (12.0)

FMF: familial Mediterranean fever.

Table 2. Allele frequencies of *MEFV* gene mutations in Japanese patients with FMF and healthy subjects.

Alleles	Allele Frequencies (%)		
	Typical FMF, n = 28	Probable FMF, n = 17	Healthy Subjects, n = 75
M694I	19 (33.9)*	0	
P369S	1 (1.8)	5 (14.7)*	6 (4.0)
R408Q	1 (1.8)	5 (14.7)*	5 (3.3)
L110P	3 (5.4)	6 (17.6)	13 (8.7)
E148Q	18 (32.1)	15 (44.1)*	35 (23.3)
R202Q	2 (3.6)	2 (5.9)	5 (3.3)
G304R	0	1 (2.9)	4 (2.7)
E84K	4 (7.1)*	2 (5.9)	2 (1.3)

* $p \leq 0.05$ compared to healthy subjects. FMF: familial Mediterranean fever.

Table 3. Clinical manifestations of patients with E84K heterozygous mutation.

Case	Age, yrs	Sex, Age at Onset, yrs	Fever	Synovitis (arthritis)	Serositis	Other Clinical Symptoms	Response to Colchicine	Additional Mutation	FMF Criteria	Outcome	Concomitant Rheumatic Diseases
Group 1: typical FMF											
Case 1	14	M 14	+	—	Peritonitis	Myalgia	Good	—	Typical	Improved (colchicine)	—
Case 2	44	M 40	+	+	—	Myalgia	Good	—	Typical	Improved (colchicine)	—
Case 3	19	F 17	+	+	Peritonitis	—	Untreated	—	Typical	Remitted	—
Case 4	46	F 18	+	+	—	Osteomyelitis	Good	—	Typical	Improved (colchicine)	—
Group 2: probable FMF											
Case 5	17	F 14	–	+	Peritonitis	—	Good	—	Probable	Improved (colchicine)	—
Case 6	28	M 15	+	+	—	—	Untreated	—	Probable	Remitted	—
Group 3: undifferentiated											
Case 7	19	F 19	+ NT	—	—	—	Untreated	E148Q/–	—	Remitted	—
Case 8	9	M 8	+ NT	—	—	—	No response	E148Q/L110P	—	Sustained	—
Case 9	45	M 41	+ NT	+	—	Myalgia	Untreated	—	—	NA	—
Group 4: other rheumatic disease											
Case 10	59	F 58	+ NT	+	—	Myalgia	Untreated	—	—	Death (infection)	SLE + SSc
Case 11	6	M 6	+	—	—	Stomatitis, cervical lymphadenopathy	Untreated	—	—	Remitted	PFAPA
Case 12	50	F 41	+ NT	+	—	—	Untreated	E148Q/L110P	—	Sustained	AOSD

FMF: familial Mediterranean fever; AOSD: adult-onset Still's disease; NA: not available; NT: not typical; PFAPA: periodic fever with aphthous pharyngitis and adenitis; SLE: systemic lupus erythematosus; SSc: systemic sclerosis.

Table 4. Final diagnosis of patients with *MEFV* exon2 or exon3 single-nucleotide polymorphisms.

Patients	E148Q/–E148Q/ E148Q, n = 24	E148Q/L110P E148Q/E148Q/ L110P, n = 18	P369S/R408Q P369S/–, n = 6	P369S/R408Q/ E148Q, n = 9
Group 1 (typical FMF)	4	1		1
Group 2 (probable FMF)	3	6	1	4
Group 3 (undifferentiated)	13	8	4	4
Group 4 (other rheumatic diseases)	4	3	1	0
	Behçet disease (2) Sjögren syndrome Rheumatoid arthritis	Seronegative arthritis (2) Crohn disease	Behçet disease	

FMF: familial Mediterranean fever.

be that a subject having the *MEFV* single mutation carries a combination of polymorphisms that would favor more inflammation under the influence of a certain environmental factor and cross the threshold of manifesting an FMF phenotype[7]. These polymorphisms would be expected to belong to genes of the innate immune pathway[8]. Possible environmental factors are thought to be the patient's country of origin, with a geographically related, as yet unknown pathogenesis[9].

Our data showed a significant prevalence of FMF in Japanese patients with unexplained fever or undifferentiated arthritis; and we have to be more aware of the presence of a variant type of FMF or modification of other diseases by polymorphisms of the *MEFV* gene.

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