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【 ORIGINAL ARTICLE 】

Clinical and Genetic Analysis of 22 Japanese Patients with Familial Mediterranean Fever: An Examination of *MEFV* and 10 Other Genes Related to Autoinflammatory Syndromes

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Abstract:

Objective Familial Mediterranean Fever (FMF) is the most frequent autoinflammatory syndrome, and its frequency is reported to be increasing in Japan. We studied the clinical features and genetic background of patients with FMF in our hospital.

Methods We analyzed the clinical features and genomic variants of *MEFV*, as well as 10 genes related to other autoinflammatory syndromes, in 22 Japanese patients with FMF. A genetic analysis was performed with a next generation sequencer.

Results The patients were classified into the typical FMF (n=16) and atypical FMF (n=6) groups. Fever, abdominal pain, thoracic pain, and arthralgia were observed in 22, 12, 8, and 10 patients, respectively. *MEFV* variants were found in 19 patients (86.4%). Two cases had no *MEFV* variants and one case only had a variant in the 3' untranslated region (3'-UTR) of *MEFV*. Genomic variants were found in genes other than *MEFV* in 7 patients (31.8%); however, none met the diagnostic criteria for autoinflammatory syndromes with disease-related gene variants, and all were classified as typical FMF. Moreover, none of the 6 patients with atypical FMF had any variants among the 10 disease-related genes. All cases in which the onset occurred before 20 years of age were classified as typical FMF.

Conclusion The clinical features of FMF recorded in our hospital coincided with those from the Japanese national epidemiological survey of FMF in Japan. More than 30% of the patients with FMF had non-*MEFV* genes, related to other autoinflammatory syndromes, thereby suggesting that variants of these genes may act as a disease-modifier in FMF.

Key words: familial mediterranean fever, autoinflammatory syndrome, *MEFV*

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Introduction

Familial Mediterranean Fever (FMF) is a hereditary autoinflammatory syndrome involving a dysfunction of pyrin, which is encoded by the *MEFV* gene (1). The clinical symptoms of FMF are characterized by recurrent fever and serositis. The disease is most prevalent among the four main Mediterranean populations: Arabs, Armenians, Jews, and Turks (2); however, in recent years, FMF has been observed worldwide. In Japan, FMF is the most frequent autoinflammatory syndrome, and there has been a recent increase in the number of reported cases.

FMF can be classified as "typical" or "atypical" based on clinical findings. A typical FMF attack is characterized by episodes of fever lasting from 12 h to 3 days, accompanied by peritonitis, pleuritis, or monoarthritis of the hip, knee, or ankle. In contrast, an atypical FMF attack differs in the fol-

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lowing features: body temperature <38℃, duration longer or shorter than 12 h to 3 days (although not shorter than 6 h or longer than a week), localized abdominal signs, or atypical distribution of arthritis.

Many cases of atypical FMF have been reported in Japanese patients in comparison to Mediterranean patients (3). This could be attributed to the fact that, in addition to variants in exon 10, Japanese patients with FMF have a large number of variants in exon 2, which are often genetic polymorphisms found in healthy individuals. Moreover, variants of other autoinflammatory syndrome-related disease genes may be implicated in pathology and may be associated with the clinical symptoms of atypical FMF, suggesting the necessity to search for other autoinflammatory syndrome-related genes as well.

Due to these reasons, we examined the clinical characteristics and genetic variants of *MEFV* and 10 other genes related to autoinflammatory syndrome in 22 Japanese patients with FMF in our hospital.

Materials and Methods

Patients

Clinical and genetic data were obtained from 22 patients with FMF, who were diagnosed at Kurume University Hospital in Japan between January 2008 and June 2014. FMF was diagnosed if the patient met 1 or more major criteria, or 2 or more minor criteria of the modified Tel-Hashomer criteria (4). On that basis, we divided the patients into the typical FMF and atypical FMF groups. Patients with typical FMF had the typical episode of peritonitis, pleuritis, monoarthritis, or fever alone, as specified in the criteria. Patients with atypical FMF had an "incomplete" attack. An attack was considered incomplete if it differed from a typical attack in only 1 or 2 of the following features: temperature <38°C; attack duration longer or shorter than the specified period [12 h to 3 days (though not shorter than 6 h or longer than a week)]; no sign of peritonitis during an abdominal attack, or localized signs, if any; and atypical distribution of arthritis.

Genetic analysis

Blood from patients with FMF was collected in EDTA-containing tubes, and DNA was extracted using a QIAmp DNA Blood Midi Kit (QIAGEN, Valencia, USA). Each DNA sample was anonymized. We investigated the following 11 genes related to autoinflammatory syndromes: *MEFV, TNFRSF1A, NLRP3, MVK, NOD2, IL1RN, NLRP12, PSTPIP1, PSMB8, NLRC4,* and *PLCG2*. Sequencing of DNA samples, was performed by Kazusa DNA Research Institute (Kisarazu, Chiba, Japan) using next a generation sequencer MiSeq (Illumina) (5). We searched for the allele frequencies of the detected missense variants in East Asia from the Exome Aggregation Consortium (ExAC) Browser and reported mutations from Infevers (http://fmf.igh.cnrs.fr/ISSAID/infevers/). We classified the missense variants in <1% of healthy individuals as rare variants and those not described in the database (Infevers) as novel variants. The study was conducted after obtaining informed consent from the participants, and in accordance with the Declaration of Helsinki. The present study was approved by the ethics committee of Kurume University.

Results

Clinical features

The clinical features and symptoms of patients with FMF are described in Tables 1 and 2. Patients with FMF were classified into the typical FMF (n=16) and atypical FMF (n=6) groups using the modified Tel-Hashomer criteria. There were 9 male patients and 13 female patients (mean age at the disease onset, 30.6±21.1 years; mean age at the diagnosis, 39.1±18.1 years). The onset occurred after 20 years of age in >63.6% of the patients. The onset was after 20 years of age in 8 patients (50%) in the typical FMF group. In contrast, the onset of FMF was after 20 years of age in all patients in the atypical FMF group (Table 1). The average age at onset in the typical and atypical FMF groups was 25.9±21.5 years and 43.0±14.0 years, respectively. In the typical FMF group, only 1 of the 6 patients in whom the onset occurred before 10 years of age was diagnosed before 10 years of age. Seven patients (31.8%) had a family history of periodic fever. Fever, abdominal pain, thoracic pain, and arthralgia were observed in 22 (100%), 12 (54.5%), 8 (36.4%), and 10 (45.5%) patients, respectively (Table 2). There was only one patient with atypical FMF, who showed symptoms of abdominal pain. The administration of colchicine was associated with a good therapeutic effect in all treated patients. Regarding complications, 3 patients had malignant disease (hepatocellular carcinoma, prostate cancer, and malignant lymphoma), 2 had rheumatoid arthritis, and 1 had ulcerative colitis. Neither of the malignant diseases was active at the time of the diagnosis of FMF. One of the patients with rheumatoid arthritis was anti-CCP antibody-positive. No patients had amyloidosis.

Genetic analysis

MEFV variants were found in the coding region of *MEFV* in 19 patients (86.4%) (Table 3). In the atypical FMF group, two patients had no *MEFV* variant, while one patient in the typical FMF group only had a variant in the 3' untranslated region (3'-UTR) of *MEFV*. All patients with pathogenic variants in exon 10 had typical FMF. Variants in exon 2 were the most frequent.

Novel or rare variants, including frame shift of autoinflammatory syndrome-related genes other than *MEFV*, were found in 7 patients (31.8%); the variant genes were *NLRP3* (Q705K, R731W, D750E), *NOD2* (frame shift, R471C), *PSTPIP1* (D289N), *and PSMB8* (R229H) (Table 4). All 7 patients had *MEFV* variants and 2 patients had pathogenic

Table 1. Profile of Patients with FMF.

		Number			Average age±SD or %
		Typical	Atypical	Total	
Age	All	16	6	22	42.5±17.6
	Male	6	3	9	50.4±20.0
	Female	10	3	13	37.0±13.2
Age of onset	All	16	6	22	30.6±21.1
	0-9	6	0	6	27.3%
	10-19	2	0	2	9.1%
	20-39	4	3	7	31.8%
	40-59	2	2	4	18.2%
	60 and above	2	1	3	13.6%
Age of diagnosis	All	16	6	22	39.1±18.1
	0-9	1	0	1	4.5%
	10-19	3	0	3	13.6%
	20-39	6	3	9	40.9%
	40-59	3	2	5	22.7%
	60 and above	3	1	4	18.2%
Familial history of periodic fever		5	2	7	31.8%

FMF: familial Mediterranean fever

Table 2. Clinical Symptoms of Patients with FMF.

		Number			%
		Typical	Atypical	Total	
Clinical episodes	Fever (≥ 38°C)	16	6	22	100
	Abdominal pain	11	1	12	54.5
	Thoracic pain	6	2	8	36.4
	Arthralgia	7	3	10	45.5
	Exanthema	1	2	3	13.6
	Myalgia	5	2	7	31.8
Response to colchicine Administration	Good response	15	6	21	95.5
	No response	0	0	0	0
	Untreated	1	0	1	4.5
Complications	Malignant disease	2	1	3	13.6
	Rheumatoid arthritis	1	1	2	9.1
	Ulcerative colitis	0	1	1	4.5

FMF: familial Mediterranean fever

variants in exon 10. Six cases of atypical FMF had no variants among the 10 genes related to other autoinflammatory syndromes.

Relationship between genetic variants and clinical symptoms

Patients with *NLRP3, NOD2, PSTPIP1,* and *PSMB8* variants did not show respective clinical symptoms, namely cryopyrin-associated periodic syndrome (CAPS), Blau syndrome, pyogenic sterile arthritis, pyoderma gangrenosum, acne (PAPA) syndrome, or Nakajo-Nishimura syndrome (NNS), thus demonstrating that no one met the diagnostic criteria for autoinflammatory syndromes with disease-related gene variants. Pathogenic variants in exon 10 were seen in 2 cases. The remaining 5 cases had less major clinical symptoms, such as thoracic pain and abdominal pain. All patients with *NOD2*, *PSTPIP1*, and *PSMB8* variants had arthralgia (Table 4). All 7 cases were classified as typical FMF.

Regarding the association of complications with *MEFV* variants, one patient with rheumatoid arthritis had major FMF symptoms with pathogenic variants in exon 10 of *MEFV*. On the other hand, a patient with FMF and ulcerative colitis had no *MEFV* variant.

Since it was unique that the onset occurred after 20 years of age in 14 of the patients (63.6%) in our study, the clinical and genetic differences between the patients in whom

Table 3. Genomic Variants of Patients with FMF.

MEFV gene		Number			%
		Typical	Atypical	Total	
Coding region variants		15	4	19	86.4
M694I/E148Q	Exon 10/2	5	0	5	22.7
P369S/R408Q	Exon 3	2	2	4	18.2
L110P/E148Q	Exon 2	3	0	3	13.6
E148Q/P369S/R408Q	Exon 2/3	1	1	2	9.1
E148Q/P369S	Exon 2/3	1	0	1	4.5
E148Q/ E148Q	Exon 2	1	0	1	4.5
R202Q/G304R	Exon 2	0	1	1	4.5
R202Q/normal	Exon 2	1	0	1	4.5
S503C/normal	Exon 5	1	0	1	4.5
3'-UTR variant		1	0	1	4.5
No variant		0	2	2	9.1
Total		16	6	22	100

FMF: familial Mediterranean fever

Table 4. Profile of Patients with FMF and Genomic Variants Other Than *MEFV*.

Age/sex	*MEFV*	FMF	Other genes				Symptoms				
			NLRP3	*NOD2*	*PSTPIP1*	*PSMB8*	F	T	A	J	M
#1 39/M	M694I/E148Q	Ty	-	FS	-	-	+	-	+	+	-
#2 44/F	M694I/E148Q	Ty	-	-	-	R229H	+	+	+	+	-
#3 65/M	S503C/normal	Ty	D750E	-	-	-	+	-	+	-	-
#4 19/F	P369S/E148Q	Ty	Q705K	-	-	-	+	-	-	-	-
#5 48/F	R202Q/normal	Ty	R731W	-	-	-	+	+	-	-	-
#6 33/M	E148Q/L110P	Ty	-	R471C	-	-	+	-	-	+	-
#7 56/F	E148Q/L110P	Ty	-	-	D289N	-	+	-	-	+	+

FMF: familial Mediterranean fever, Ty: typical FMF, FS: frame shift, F: fever, T: thoracic pain, A: abdominal pain, J: joint pain (arthralgia), M: myalgia

the onset occurred at <20 years of age and those in whom the onset occurred at ≥20 years of age were analyzed (Table 5). Interestingly, all patients in whom the onset occurred at <20 years of age and 8 out of the 14 (57.1%) patients in whom the onset occurred at ≥20 years of age were classified into the typical FMF group. Regarding the clinical features, patients in whom the onset occurred at <20 years of age had a higher rate of abdominal pain (75%; n=6), whereas those in whom the onset occurred at ≥20 years of age had higher rates of arthralgia (50%) and myalgia (42.9%). Based on our gene search results, 4 of the 8 patients (50%) in whom the onset occurred at <20 years of age had a pathogenic variant of M694I/E148Q, although this variant only developed in 1 patient (7.1%) in whom the onset occurred at ≥20 years of age. Regarding the 10 gene variants in genes other than *MEFV*, there were 3 variants (37.5%) in patients in whom the onset occurred at <20 years of age, and 4 (28.6%) in patients in whom the onset occurred at ≥20 years of age.

Discussion

In our patients with FMF, the age at the onset was 30.6±21.1 years and the age at the diagnosis was 39.1±18.1 years. The frequency of clinical symptoms was as follows: abdominal pain (54.5%), thoracic pain (36.4%), arthralgia (45.5%), and exanthema (13.6%). In a Japanese nationwide survey of FMF (n=134) in 2009, the age at onset (19.6±15.3 years) and age at diagnosis (28.7±18.5 years) were lower in comparison to our study. One reason could be that our division belongs to the Department of Internal Medicine and therefore manages a smaller proportion of pediatric patients in comparison to the study population of the Japanese nationwide survey of FMF. The time from the onset to the diagnosis was approximately 9 years, which was consistent with that in the Japanese nationwide survey. The frequency of clinical symptoms in the Japanese nationwide survey was similar to that in our study (6). In the typical FMF group, only 1 out of the 6 patients in whom the onset occurred before 10 years of age was diagnosed at <10 years of age,

Table 5. Clinical and Genetic Differences between Patients with Onset before 20 Years of Age and That after 20 Years of Age.

	Onset<20 y/o	Onset≥20 y/o
FMF classifications		
Typical	8 (100)	8 (57.1)
Atypical	0 (0)	6 (42.9)
Clinical episodes		
Fever (≥38°C)	8 (100)	14 (100)
Abdominal pain	6 (75)	6 (42.9)
Thoracic pain	3 (37.5)	5 (35.7)
Arthralgia	3 (37.5)	7 (50)
Exanthema	0 (0)	3 (21.4)
Myalgia	1 (12.5)	6 (42.9)
Genotypes		
Coding region variants (*MEFV*)	7 (87.5)	12 (85.7)
M694I/E148Q	4 (50)	1 (7.1)
3'-UTR variant (*MEFV*)	1 (12.5)	0 (0)
No variant (*MEFV*)	0 (0)	2 (14.2)
Coding region variants (other than *MEFV*)	3 (37.5)	4 (28.6)

Values are shown as number (%). FMF: familial Mediterranean fever

which made it difficult to accurately diagnose FMF. Abdominal pain is reported in >90% of patients with FMF in Middle Eastern regions, such as Turkey; however, the reported frequency of abdominal symptoms is lower in Japanese patients with FMF (7, 8). Patients with atypical FMF had few clinical symptoms other than fever; in particular, abdominal pain was observed only in 1 of 6 cases (3, 9).

Regarding genetic features, 86.5% of patients with FMF had genomic variants of *MEFV*, as per the nationwide survey in Japan (n=126), and 55.4% of them had pathogenic variants in exon 10 while 31.1% had variants in other regions (6). Many patients with FMF in our hospital had *MEFV* variants (86.4%); this rate was almost the same as that in the nationwide survey. Approximately 20% of healthy Japanese people have variants in exon 2 (for example, E148Q); however, whether that is involved in the pathogenesis of FMF, especially in Japan, remains to be resolved. Many heterozygous variants, such as M694I and E148Q, have been reported in Japanese people with typical FMF (3, 6). All pathogenic variants in exon 10 in patients managed in our hospital had M694I/E148Q, and all patients had typical FMF.

One patient with FMF had a variant in the 3'-UTR of *MEFV*. The genetic association between *MEFV* 3'-UTR polymorphism and clinical patients with FMF had been reported previously (10), suggesting that the 3'-UTR sequence plays an important role in regulating the expression of *MEFV*. In the future, we should clarify the significance of non-coding regions, including the 3'-UTR in the *MEFV* gene.

Many reports on autoinflammatory syndromes, including FMF, describe variants of more than one gene (11, 12). In our study, 7 patients (31.8%) had variants of autoinflammatory syndrome-related genes other than *MEFV*. In Japan, many gene variants, other than variants in exon 10 of *MEFV*, have been reported (6); however, the relationship of genetic polymorphism to the pathogenesis of FMF is not yet clear. In 6 cases, there were no variants in exon 10, and 2 of the cases without variants were classified as atypical FMF. Therefore, in atypical FMF, we considered that in addition to *MEFV* variants, gene variants associated with other autoinflammatory syndromes might also play a major role in clinical symptoms. We therefore searched for 10 disease genes other than *MEFV*. However, contrary to our expectations, all 7 patients with variants of 10 disease-related genes other than *MEFV* were classified into the typical FMF group (Table 4). Moreover, none of the 6 patients with atypical FMF had variants of the 10 disease-related genes, thus suggesting the variants of *NLRP3*, *NOD2*, *PSTPIP1*, and *PSMB 8* to possibly act as a disease-modifier in typical FMF. Further studies would be required to analyze the clinical implications of multiple gene variants in patients with FMF.

Interestingly, there was a clinical and genetic difference between the cases in which the onset of FMF occurred before 20 years of age and those in which the onset occurred after 20 years of age. All cases in which the onset occurred before 20 years of age were classified as typical FMF with a typical attack, showing clinical symptoms of abdominal pain; these cases accounted for 4 of the 5 M694I/E148Q pathogenic variants. On the other hand, 42.9% of the 14 cases in which the onset occurred after 20 years of age were classified as atypical FMF, with clinical symptoms of arthralgia and myalgia (9), non-exon 10 variants, and no variants present in 13 of the 14 cases. These clinical tendencies were similar to those reported by Endo et al. (13). In the study by Migita et al. (14), there was no correlation between an onset before 20 years of age and typical FMF; however, the age at the onset of typical FMF was significantly younger in comparison to atypical FMF, which was in line with the results of our study (typical FMF, 25.9±21.5; atypi-

cal FMF, 43.0±14.0). In our study, all cases in which the onset occurred before 20 years of age were classified as typical FMF, while typical and atypical mixed FMF was seen in cases in which the onset occurred after 20 years of age.

The relatively small number of patients with FMF was a limitation of the present study. In future, we would like to investigate whether genes related to autoinflammatory syndromes other than *MEFV* are related to the symptoms and pathogenesis of FMF in a larger cohort of patients.

Conclusion

The clinical and genetic features of FMF in our hospital were similar to those in the Japanese nationwide survey. One-third of the patients with FMF had variants of non-*MEFV* genes related to other autoinflammatory syndromes, suggesting that variants of these genes may act as disease-modifiers in FMF.

The authors state that they have no Conflict of Interest (COI).

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