Labour and Welfare of Japan and the Research Committee on the Epidemiology of Intractable Diseases conducted a nationwide survey to elucidate the prevalence of FMF in Japan. We conducted the present study to further estimate the prevalence of FMF and elucidate the clinical features in Japanese patients. Genotype/phenotype correlations were previously reported in Jewish, Turkish, and Armenian patients with FMF.[25,29,48] In the current study we evaluated the genotype/phenotype correlations in Japanese patients with FMF and compared them to those of other ethnic groups.

METHODS

A nationwide survey for FMF was conducted in cooperation with the Japan Research Committee on the Epidemiology of Intractable Diseases in 2009. The target populations were patients with FMF who visited hospitals in 2009. According to the Nationwide Epidemiologic Survey Manual issued by the Research Committee on the Epidemiology of Intractable Disease,[19] we selected 3 types of departments for the targeted survey: pediatrics, internal medicine, and rheumatology/allergy. The hospitals used in the study were selected randomly from a list of all hospitals in Japan. The selection rate was determined according to a stratification based on the number of beds in the hospital. Thus, hospitals with a high number of beds had a greater probability of being selected. The selection rate was 100% for hospitals with 500 beds or more or university hospitals, whereas only 5% of hospitals with fewer than 100 beds were selected at random. In addition, specialized hospitals that had previously reported FMF patients were all selected for the study. After selection, we sent a questionnaire describing the diagnostic criteria for FMF. The primary survey only inquired as to the numbers of patients with FMF who visited the hospital in 2009. Patients were diagnosed clinically according to the modified and simplified diagnostic criteria of Tel-Hashomer,[24] provided with the primary survey. The diagnosis was divided into 1 major criterion: recurrent febrile episodes (3 or more episodes lasting 12 h to 3 d with a fever of 38°C or more) and 8 minor criteria (a febrile attack with 1 of 7 accompanying symptoms including abdominal pain due to peritonitis; chest pain due to pleuritis; monoarthritis of hip, knee, or ankle; pericarditis; scrotum pain due to orchitis; headache due to aseptic meningitis; or a favorable response to colchicine treatment). A diagnosis of FMF was determined if the patient exhibited the major criterion and 1 or more minor criteria. When a suspected FMF patient was identified, a second questionnaire regarding the detailed clinical features for each patient was sent. The present study was approved by the ethical committees of Jichi Medical University (No. 09–20, September 7, 2009).

Using the selection and response rate to the surveys, we estimated the total number of patients with FMF and the 95% confidence intervals (CIs) as described previously.[20,23] The estimate was based on the assumption that department responses were independent of the frequency of patients. The point estimation of prevalence was calculated using the following equation, where SRTk, RRTk, NSk, nk, Nk, and Nki denote the sampling rate, response rate, number of sampling departments, total number of departments, number of responding departments, and the number of departments with i patients in stratum k, respectively.

$$\alpha_k = \frac{1}{SRT_k RRT_k}\sum_i iN_{ki} = \frac{1}{\frac{NS_k}{n_k}\frac{N_k}{NS_k}}\sum_i iN_{ki} = \frac{nk}{Nk}\sum_i iN_{ki}$$

Age and sex distributions of the disease were estimated based on data obtained from the second survey.

Mutation Analysis

Two mL of blood were collected from each subject. Genomic DNA was extracted from whole blood using the Promega Wizard Genomic DNA Purification Kit (Promega, Madison, WI). Mutation analysis was performed by genomic sequencing. Mutations in exon 1–10 of the MEFV gene were tested. Polymerase chain reaction (PCR) was performed using forward and reverse primers for each exon as described previously.[39] PCR products were purified with the ExoSAP-IT (GE Healthcare Japan, Tokyo, Japan) and sequenced directly, using specific primers and BigDye Terminator v1.1 (Applied Biosystems, Tokyo, Japan). Genetic analysis of the MEFV gene was approved by the Ethics Committee of Nagasaki Medical Center (No. 21003, May 11, 2009).

Determination of SAA1 Alleles

SAA1 genotyping by PCR-RFLP was performed, as previously described.[1] Briefly, a portion specific to SAA1 was amplified using the following primer set: 5′-ATGATGCTGCCAAAA GGGGA-3′ (forward) and 5′-TGGCCAAAGAATCTCTGGAT-3′ (reverse). PCR was carried out with 35 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. The products were digested by Ban I and Bcl I, and genotypes were determined by agarose gel electrophoresis.

Statistical Analyses

Data were analyzed using SPSS (SPSS Inc., Chicago, IL). Results were expressed as the mean ± standard deviation (SD) for continuous variables. For quantitative data, analysis was performed using a Mann–Whitney U rank-sum test for comparison of 2 independent groups. Comparisons for categorical variables were evaluated using the chi-square test. $P < 0.05$ was accepted as significant.

RESULTS

Prevalence

In the primary survey, 2251 hospitals or departments of pediatrics, internal medicine, or rheumatology/allergy were selected. Of them, 1380 (61.3%) responded and 170 patients met the diagnostic criteria for FMF. The results of the questionnaire survey are shown in Table 1. The numbers of patients reported from departments of pediatrics, internal medicine, and rheumatology/allergy were 85 (50.0%), 67 (39.4%) and 18 (10.6%), respectively. The total number of patients was estimated to be 292 (95% CI, 187–398). The estimated numbers from pediatrics, internal medicine, and rheumatology/allergy were 118, 129, and 45, respectively (Table 2).

Demographic Features of Japanese FMF Patients

From 170 FMF patients recruited in the first nationwide survey, detailed clinical data were obtained from 122 patients and from another 12 patients who were diagnosed after the first survey in 2009. We analyzed the clinical and demographic features of these 134 patients. The male to female ratio was 1:1.3. The mean (±SD) age at the time of diagnosis was 28.7 ± 18.5 years, and the mean age at onset of symptoms was 19.6 ± 15.3 years. Thirty-four patients (25.4%) experienced their first attack before the age of 10 years, 50 patients (37.3%) in their teens, and 50 patients (37.3%) after 20 years of age. The mean period from disease onset to diagnosis was 9.1 ± 9.3 years, suggesting a delay in diagnosis.

Clinical Features

Clinical data from 134 patients in the second nationwide survey showed 99 patients (76.2%) with no family history suggestive of FMF. The main clinical findings were present at

TABLE 1. Response Rates and Reported Numbers of FMF Patients

		No. of Departments	Subjects for 1st Survey (n)	(%)	Response Rates (n)	(%)	No. of Departments Reporting FMF Patients	No. of Patients Reported
Internal Medicine	University hospitals	154	154	100.0	83	53.9	9	28
General hospitals	≧500 beds	210	210	100.0	118	56.2	10	14
	400−499 beds	166	133	80.1	74	55.6	1	1
	300−399 beds	327	131	40.1	74	56.5	3	5
	200−299 beds	426	85	20.0	48	56.5	0	0
	100−199 beds	1112	111	10.0	67	60.4	0	0
	≦99 beds	3199	160	5.0	82	51.3	0	0
	Specialized hospitals	26	26	100.0	18	69.2	10	19
	Total	5620	1010	18.0	564	55.8	10	67
Pediatrics	University hospitals	108	108	100.0	82	75.9	17	62
General hospitals	≧500 beds	195	195	100.0	154	79.0	6	6
	400−499 beds	155	123	79.4	93	75.6	4	5
	300−399 beds	298	120	40.3	87	72.5	2	6
	200−299 beds	329	66	20.1	38	57.6	0	0
	100−199 beds	604	60	9.9	37	61.7	0	0
	≦99 beds	1062	53	5.0	35	66.0	0	0
	Specialized hospitals	17	17	100.0	10	58.8	4	6
	Total	2768	742	26.8	536	72.2	33	85
Rheumatology/ Allergy	University hospitals	96	96	100.0	51	53.1	9	9
General hospitals	≧500 beds	46	46	100.0	27	58.7	0	0
	400−499 beds	34	34	100.0	23	67.6	1	4
	300−399 beds	59	59	100.0	32	54.2	1	1
	200−299 beds	80	80	100.0	45	56.3	1	2
	100−199 beds	213	85	39.9	43	50.6	1	0
	≦99 beds	422	85	20.1	49	57.6	1	2
	Specialized hospitals	14	14	100.0	10	71.4	0	0
	Total	964	499	51.8	280	56.1	14	18
Total		9352	2251	24.1	1380	61.3	80	170

the following frequencies: fever (128 patients, 95.5%), abdominal pain (84, 62.7%), chest pain (48, 35.8%), arthritis (42, 31.3%), erysipelas-like erythema (10, 7.5%) and amyloidosis (5, 3.7%). The remaining minor symptoms were pericarditis (3, 2.2%) and headache (17, 12.7%). Febrile attacks, chest pain, and arthritis were comparable between Japanese FMF patients and Mediterranean patients, while abdominal pain and amyloidosis were less prevalent among Japanese FMF patients (Table 3).[3,37,47] AA amyloidosis was confirmed in 5 patients (3.7%) whose genotypes were M694I/M694I, E148Q/E148Q, E148Q/R202Q/P369S/R408Q, and M694I/E148Q/L110P (2 patients) (Table 4).

Colchicine was administered orally to 132 patients, and a favorable therapeutic effect was seen in 122 patients (91.8%). Treatment efficacy was not obtained from the questionnaire survey of 5 patients, and 2 patients had not yet been treated with colchicine. The mean dose of colchicine required to control attacks (0.89 ± 0.45 mg/d) was lower for Japanese FMF patients compared with previous reports of Mediterranean patients.[5]

Mutational Analysis

Among 134 Japanese FMF patients with detailed clinical data, 126 patients underwent MEFV mutation analysis. The MEFV gene mutation was not identified in 17 of 126 patients (13.5%). Of the remaining 109 patients (86.5%), 14 were homozygotes, 66 were compound heterozygotes or had complex alleles, and 29 were heterozygotes. The distribution of the MEFV genotypes in the study group is presented in Table 5. The major detected mutations were homozygous, heterozygous, and compound heterozygous for E148Q, E148Q-L110P, P369S-R408Q, and/or M694I. The most frequent genotype was M694I/E148Q, followed by M694I/normal and M694I/M694I. In consideration of the allelic frequencies, the most common MEFV mutations and polymorphisms among Japanese FMF patients were M694I (29.4%), E148Q (31.3%), L110P (11.5%), P369S (5.6%), and R408Q (5.6%). Moreover, the rare mutations M680I, G304R, R202Q, and E84K were detected in the heterozygous state. It is noteworthy that no

TABLE 2. Estimated Number of FMF Patients in Japan*

Department	No. of Reported Patients	Estimated Patient Number, SE	(95% CI)†
Internal medicine	33	129 ± 45	(40–218)
Pediatrics	33	118 ± 27	(65–172)
Rheumatology/Allergy	14	45 ± 10	(27–64)
Total	80	292 ± 54	(187–398)

Abbreviations: SE = standard error.

*The estimated total number of patients = number of reported patients/(number of responding hospitals/number of target hospitals).

†Ninety-five percent confidence intervals were calculated with an assumption of multinomial hypergeometric distribution.

TABLE 3. Main Clinical Features of Japanese and Mediterranean FMF Patients

Feature	Patients From Japan	Patients From Turkey	Patients From Israel	Arab Patients
No. of patients	134	2838	470	175
Fever, (%)	95.5	92	100	100
Abdominal pain (peritonitis), (%)	62.7	93	95	94
Chest pain (pleuritis), (%)	35.8	31	43	32
Arthritis, (%)	31.3	47	75	33
Skin rash (erysipelas-like erythema), (%)	7.5	21	4	3
Amyloidosis, (%)	3.7	13	27	3
Reference	PR	47	37	3

Abbreviations: PR = present report.

homozygous or heterozygous M694V or V726A mutations were observed in Japanese FMF patients. Mutations of the MEFV gene in exon 10 (M694I, M680I) were detected in 67/126 (53.2%) of FMF patients. These patients showed a significantly higher prevalence of chest and abdominal pain and a lower prevalence of arthritis compared with those without mutations in exon 10. In addition, these patients had a more frequent family history of FMF compared to those without mutations (Table 6). Analysis of the frequency of clinical manifestations between FMF patients without MEFV mutations and those with mutations showed no statistical difference: peritonitis (58.8% vs. 63.3%), pleuritis (23.5% vs. 40.4%) and arthritis (52.9% vs. 29.4%), respectively. Similarly, there was no statistical difference in the dose of colchicine (1.02 ± 0.71 mg/d vs. 0.85 ± 0.40 mg/d) and age at onset (17.3 ± 15.9 yr vs. 19.2 ± 14.7 yr) between FMF patients without MEFV mutations and those with mutations.

DISCUSSION

FMF is considered a common hereditary autoinflammatory disease among Mediterranean populations;[2] however, the true prevalence of FMF has not been elucidated in East Asia. To our knowledge, this was the first nationwide survey of the prevalence of FMF in East Asia. The estimated number of Japanese patients with FMF was approximately 300 (95% CI, 187–398 people). In a second survey, we obtained clinical information from 134 FMF patients, currently the largest survey of Japanese FMF patients. Based on these data, we further identified the spectrum of clinical features, MEFV mutations, and genotype/phenotype correlations in Japanese FMF. The age of onset among Japanese FMF patients was significantly different from Mediterranean populations, where 90% of patients developed FMF before 20 years of age compared with 63% of Japanese patients.[4] The incidences of clinical symptoms during febrile attack were relatively similar between Japanese and Mediterranean FMF patients except for peritonitis and amyloidosis. The prevalence of abdominal pain (62.7%) and amyloidosis (3.7%) was lower in Japanese patients than in Mediterranean FMF patients.[3,37] Genetic factors (ethnicity and spectrum of MEFV mutations) and related disease severity could contribute to the differences in the age at onset and incidence of abdominal pain and amyloidosis between Japanese and Mediterranean FMF patients.

Two patients with AA amyloidosis (40%) exclusively carried MEFV gene mutations in exon 2 or 3, which are considered low-risk mutations for FMF-related AA amyloidosis.[43] This supports the concept that the phenotype or genotype of FMF does not necessarily predict the development of amyloidosis.[45] However, recurrent or long-standing subclinical inflammation may have contributed to the association of amyloidosis in these patients. The Turkish FMF Study Group reported that the mean period from disease onset to diagnosis was 6.9 ± 7.7 years.[47] The mean delay from disease onset to diagnosis of Japanese FMF was 9.0 years, thus putting undiagnosed patients at risk of secondary amyloidosis due to recurrent inflammation, which is a significant public health issue. The mainstay of treatment for FMF is daily oral colchicine, which decreases the frequency and intensity of attacks and prevents the development

TABLE 4. Demographic Features of Patients With AA Amyloidosis

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex	M	F	M	M	M
Current age (yr)	46	34	51	50	44
Age at onset (yr)	23	20	20	30	15
Age at diagnosis of FMF (yr)	43	28	51	50	43
Delay in diagnosis of FMF (yr)	20	8	31	20	28
ESRD	(+)	(−)	(−)	(−)	(−)
Current treatment	Colchicine (1 mg/d)	Prednisolone (colchicine was discontinued due to AE)	Colchicine (1 mg/d)	Colchicine (1 mg/d)	Colchicine (1 mg/d)
MEFV genotype	M694I/E148Q/L110P	E148Q/E148Q	E148Q/R202Q/P369S/R408Q	M694I/M694I	M694I/E148Q/L110P
SAA1 genotype	1.3/1.5	NT	1.3/1.5	1.5/1.5	1.1/1.1

Abbreviations: AE = adverse effect, ESRD = end-stage renal disease, NT = not tested.

TABLE 5. Genotype of 126 Japanese FMF Patients

Mutation	Patients No.	Patients (%)
M694I/M694I	8	6.3
M694I/normal	16	12.7
M694I/E148Q	25	19.8
M694I/L110P	2	1.6
M694I/E148Q/L110P	14	11.1
M694I/E148Q/E148Q/L110P/L110P	1	0.8
M680I/E148Q/L110P	1	0.8
E148Q/E148Q	1	0.8
E148Q/E148Q/L110P	2	1.6
E148Q/E148Q/P369S/R408Q	2	1.6
E148Q/normal	8	6.3
E148Q/L110P	7	5.6
E148Q/R202Q	2	1.6
E148Q/G304R	1	0.8
E148Q/S503C	1	0.8
E148Q/L110P/R202Q	1	0.8
E148Q/P369S/R408Q	5	4.0
E148Q/R202Q/P369S/R408Q	1	0.8
E148Q/G304R/P369S/R408Q	1	0.8
R202Q/normal	1	0.8
S503C/normal	1	0.8
E84K/normal	3	2.4
P369S/R408Q	5	4.0
(−)	17	13.5
Total	126	

of amyloidosis. The normal adult dose of colchicine is 1.2–1.8 mg/d and leads to clinical improvement in more than 90% of patients.[12] Pras et al[33] reported that 30% of North African Jewish patients required a minimum colchicine dose of 2 mg/d to control symptoms. In the current study, Japanese patients with FMF were treated with a relatively low dose of colchicine (mean, 0.89 ± 0.45 mg/d), which had a therapeutic efficacy of 91.8% of Japanese FMF patients.

In the current study we investigated the spectrum of MEFV mutations and the genotype/phenotype correlation in Japanese FMF patients registered in the first nationwide survey. The mutations associated with the most severe phenotype were located in exon 10 of the MEFV gene. This encodes the C-terminal pyrin domain, B30-2/SPRY, the binding site of caspase-1.[8] The mutations in exon 10 of the MEFV gene, M694V, V726A, and M694I, are predominant in Mediterranean FMF patients.[15] Our data showed heterogeneous MEFV genotypes consisting of M694I, E148Q, L110P/E148Q, and P369S/R408Q in Japanese FMF patients. M694V and V726A are the most common mutations of Mediterranean FMF patients.[45] However, these mutations were not found in Japanese FMF patients. The founder effect is likely to be related to the biased MEFV mutation spectrum of Japanese FMF patients. An intriguing finding in FMF is the differing penetrance associated with certain mutations or polymorphisms. The E148Q mutation has low penetrance and is described as a polymorphism due to its high carrier rate, but demonstrates a lack of phenotype in some homozygous patients.[40] However, in our nationwide survey, patients carrying the E148Q mutation exhibited the typical FMF phenotype. The incomplete penetrance of mutations in exon 2 or exon 3 of the MEFV gene suggests the presence of other genetic factors or environmental factors that could influence the disease expression. Although the classical MEFV mutations in exon 10 are characterized by high penetrance, the carrier rate of MEFV exon 10 mutations among Japanese healthy controls is extremely rare.[38] Therefore, the diagnostic significance of their presence, even when heterozygous, is very pronounced in Japanese patients.

In the present study we also attempted to evaluate the genotype/phenotype correlation in Japanese FMF patients. FMF patients with exon 1, 2, or 3 mutations or no mutations comprised a genetically distinct phenotype compared to those with MEFV exon 10 mutations. These patients were positive for various mutated alleles, such as E84K, E148Q, P369S, R202Q, and were more likely to have nonspecific musculoskeletal symptoms including arthralgia. In contrast, the frequency of serositis (chest pain and abdominal pain) was significantly lower compared to those carrying MEFV exon 10 mutations. MEFV gene mutations located in exon 3 have been shown to be responsible for a variant form of FMF or atypical clinical manifestations of FMF.[6,34] These findings may partly explain the observation that FMF patients with mutations in other exons of the MEFV gene present with diverse clinical manifestations compared to FMF patients with exon 10 mutations. Previous genotype/phenotype correlation studies have suggested that mutations located within exon 10, that is, M694V, are associated with severe disease and the frequent occurrence of amyloidosis.[14,16] In contrast, mutations in exon 2, such as E148Q, were associated with milder disease with no amyloidosis.[43] The frequencies of exon 2 (E148Q, E148Q/L110P) mutations are relatively high in Japanese patients compared to white patients,[27] and may reflect the milder form of phenotype observed. We also demonstrated a relationship between the M694I mutation and a higher prevalence of serositis and familial aggregation in Japanese patients with FMF.

TABLE 6. Genotype-Phenotype Correlation in Japanese FMF Patients

Clinical Feature	Total (n = 126)	Patients With *MEFV* Exon 10 Mutations (n = 67) No. (%)	Patients Without *MEFV* Exon 10 Mutations (n = 59) No. (%)	P
Abdominal pain	79 (62.7)	50 (74.6)	29 (49.2)	0.001
Chest pain	48 (38.1)	40 (59.7)	8 (13.6)	0.0001
Arthritis	41 (32.5)	15 (22.4)	26 (44.1)	0.021
Myalgia	15 (11.9)	7 (10.4)	8 (13.6)	0.641
Amyloidosis	5 (4.0)	3 (4.5)	2 (3.4)	0.560
Age at onset (yr)	19.1 ± 15.1	17.9 ± 11.6	20.6 ± 18.3	0.915
Male/female	53/73	34/33	19/40	0.035
Family history	32 (25.4)	24 (35.8)	8 (13.6)	0.004

Conclusion

In conclusion, our nationwide survey estimated the prevalence of FMF in Japan and allowed us to establish the spectrum of MEFV gene mutations among Japanese FMF patients. Our data indicated that Japanese FMF patients are clinically or genetically distinct from Mediterranean FMF patients, suggesting a genotype/phenotype relationship. Although Japanese FMF disease may be less severe, patients should be treated earlier to prevent recurrent attacks and subsequent development of AA amyloidosis. Further ethnic-based studies are needed to elucidate the clinical and genetic profiles of FMF in East Asia.

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INTERNAL MEDICINE

CASE REPORT

Familial Mediterranean Fever with Onset at 66 Years of Age

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Abstract

The patient was a 68-year-old woman who had experienced recurrent febrile episodes since 66 years of age. Despite various examinations and treatments, the etiology remained unclear. Further examinations following another referral failed to uncover the cause. Therefore, despite her age, it was presumed that she had familial Mediterranean fever. An analysis of the familial Mediterranean fever (MEFV) gene detected heterozygous L110P, E148Q, and R202Q mutations. No further febrile episodes occurred after colchicine treatment was initiated. Familial Mediterranean fever presenting in patients in their sixties is extremely rare.

Key words: abdominal pain, familial Mediterranean fever, febrile episodes, MEFV gene

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Introduction

Familial Mediterranean fever (FMF) is the most common, genetic, autoinflammatory disease, with a predominantly autosomal recessive pattern of inheritance. It is characterized by periodic fever and symptoms of serositis, such as abdominal pain, chest pain, and joint pain, and occurs mostly at a young age. The case of a patient who presented with FMF for the first time at 66 years of age is herein reported. An investigation of the association between genetic mutations and the age of onset reported in the Japanese literature is also presented.

Case Report

A 68-year-old woman was referred to our department for further evaluation and treatment with a 2-year history of febrile episodes ranging from 37℃ to 40℃, which lasted for a few days. The febrile episodes occurred with a cycle of between one and three months. Initially, the patient was treated with antibiotics at another hospital, although the etiology of the fever was unclear despite various examinations. She was subsequently treated with non-steroidal anti-inflammatory drugs (NSAIDs) alone after it was found that her fever subsided without antibiotic therapy. She was admitted to our department for further evaluation and treatment.

The physical examination on admission showed evidence of severe anemia in the palpebral conjunctiva, a mild systolic murmur on chest auscultation, and vague abdominal tenderness. She had no abnormal neurological findings. There were no signs of a skin eruption, swelling of the lymph nodes and tonsils, or swelling or deformity of the joints. She had no joint pain even during febrile attacks. There was no pain on percussion of the spine or costovertebral angles.

At that point, the differential diagnosis of her febrile attacks included infection, malignancy, collagen disease such as seronegative rheumatoid arthritis, seronegative spondyloarthropathy or vasculitis, and arthritis from gout or calcium pyrophosphate deposition (CPPD).

Laboratory tests during febrile episodes showed evidence of increased inflammation, such as an elevated level of C-

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Table 1. Laboratory Findings during a Febrile Episode

[Peripheral blood]			[Biochemistry]			[Serological tests]			[Tumor markers]		
WBC	10200	/μL	T-Bil	0.3	mg/dL	CRP	12.6	mg/dL	CEA	1.1	ng/mL
seg	83	%	AST	14	IU/L	ESR	169	mm/hr	sIL-2R	761	U/mL
lym	12	%	ALT	15	IU/L				SCC antigen	0.9	ng/mL
mono	4	%	LDH	103	IU/L	RF	<9.4	IU/mL			
RBC	229x10⁴	/μL	ALP	314	IU/L	ANA	<20		[Urinalysis]		
Hb	6.1	g/dL	γGTP	87	IU/L	C3	132	mg/dL	occult blood	-	
Hct	20.3	%	Amy	63	IU/L	C4	27.5	mg/dL	protein	-	
Plt	58.8x10⁴	/μL	CK	19	IU/L	CH50	71.4	U/mL	glucose	-	
			UA	4.9	mg/dL	IgG	1840	mg/dL			
			TP	7.2	g/dL	IgM	126	mg/dL			
			Alb	2.9	g/dL	IgA	278	mg/dL			
			BUN	20	mg/dL	ferritin	462	ng/mL			
			Cr	1.17	mg/dL	FT3	1.66	pg/mL			
						FT4	1.14	ng/mL			
						TSH	1.31	IU/mL			

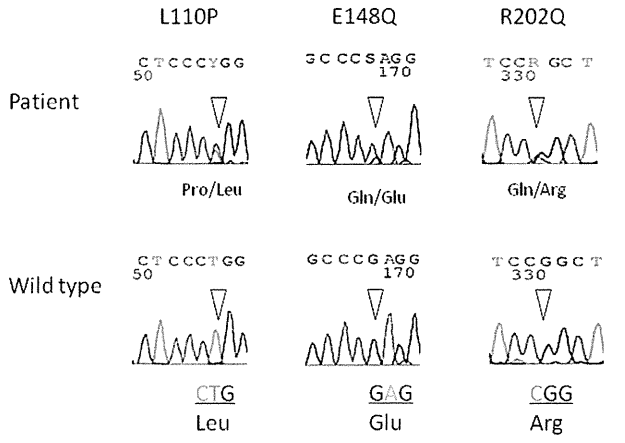

Figure 1. DNA sequencing demonstrating the L110P, E148Q, and R202Q mutations in the patient and a healthy control.

reactive protein (C-reactive protein (CRP); 6.94-28.11 mg/dL), leukocytosis (9,300-31,600/μL, segmented neutrophils 73-95%), and an increased erythrocyte sedimentation rate (ESR; 160 mm/hr). In contrast, during the fever-free period, her inflammatory markers were normal or slightly increased: CRP 0.09-9.47 mg/dL; leukocyte count 3,600-16,000/μL, segmented neutrophils 39-82%; and ESR 66-159 mm/hr (Table 1). The tumor marker levels were within the normal limits, except for the fact that the ferritin level and soluble interleukin 2 receptor level were slightly increased. The plasma uric acid level was not increased. No immunological abnormalities were found. The urinalysis was normal and the fecal occult blood test was negative. X-ray examinations of the chest and abdomen, a computed tomography (CT) scan from the head to the pelvis, ultrasound evaluations of the heart and abdomen, upper and lower gastrointestinal endoscopy, and laryngoscopy were all negative. In addition, positron emission tomography/computed tomography (PET/CT) and bone-marrow aspiration were performed, but they revealed no abnormalities. Repeated blood, urine, and fecal cultures were all negative. Considering the findings of the physical examination, laboratory tests, and imaging, she did not appear to have any infection, malignancy, or arthritis from gout or CPPD.

Treatment with a glucocorticoid (prednisolone 40 mg) was initiated on the assumption that she had had some sort of collagen disease, such as seronegative rheumatoid arthritis, seronegative spondyloarthropathy, or adult Still's disease, because the frequency of febrile episodes increased during hospitalization. However, this did not alleviate her fever, and treatment was stopped. At the same time, a sample specimen was obtained to analyze the familial Mediterranean fever (MEFV) gene to determine whether she had FMF. Exons 1, 2, 3, and 10 of the MEFV gene, where mutations of FMF are often confirmed, were analyzed, and heterozygous L110P, E148Q, and R202Q mutations were identified in exon 2 (Fig. 1). Since FMF is sometimes complicated by AA-type amyloidosis, endoscopic duodenal mucous membrane biopsies were performed, and the serum amyloid A protein (SAA) levels were measured. Amyloid was not detected in the biopsy specimens. The SAA protein level was increased during the febrile episodes, but it did not increase during the fever-free period (Fig. 2).

Colchicine at a dose of 0.5 mg once a day was started. This was insufficient to suppress the febrile episodes, so the dose of colchicine was increased to 1.5 mg three times a day. Therapy was discontinued several times because of abdominal pain, nausea, and diarrhea, which were probably side effects of colchicine or FMF peritonitis. Low-dose colchicine at 0.25 mg once a day was eventually used after one of these discontinuations, followed by an increase to 0.5 mg twice a day. The white blood cell (WBC), CRP, ESR, and SAA protein levels decreased to within the normal limits, and the patient had no further febrile episodes. As of this time, 6 months have passed since her last febrile episode. Her blood inflammatory markers still remain within the normal limits.

Retrospectively, her symptom of vague abdominal pain tended to be seen more frequently during febrile attacks than during the fever-free period. Although there were no apparent physical signs of peritonitis, the pain could have indicated peritonitis. However, this would not be surprising since there are no apparent physical signs of peritonitis in the elderly. The amyloidosis, favorable response to colchicine treatment, and recurrent febrile episodes satisfied the Tel Hashomer criteria for the diagnosis of FMF, which take into account the clinical symptoms and the efficacy of colchicine (Table 2). Therefore, the MEFV gene mutation, the patient's clinical symptoms, and the favorable response to colchicine treatment led to a diagnosis of FMF, though the age at onset of 66 years was very unusual.

Discussion

A case of FMF with an age at onset of 66 years is herein described. FMF is the most common, inherited, autoinflammatory disease. It is inherited in an autosomal recessive pattern, and it is characterized by periodic attacks of fever, aseptic serositis, and synovitis. The Tel Hashomer criteria for FMF have previously relied on clinical signs alone, so it

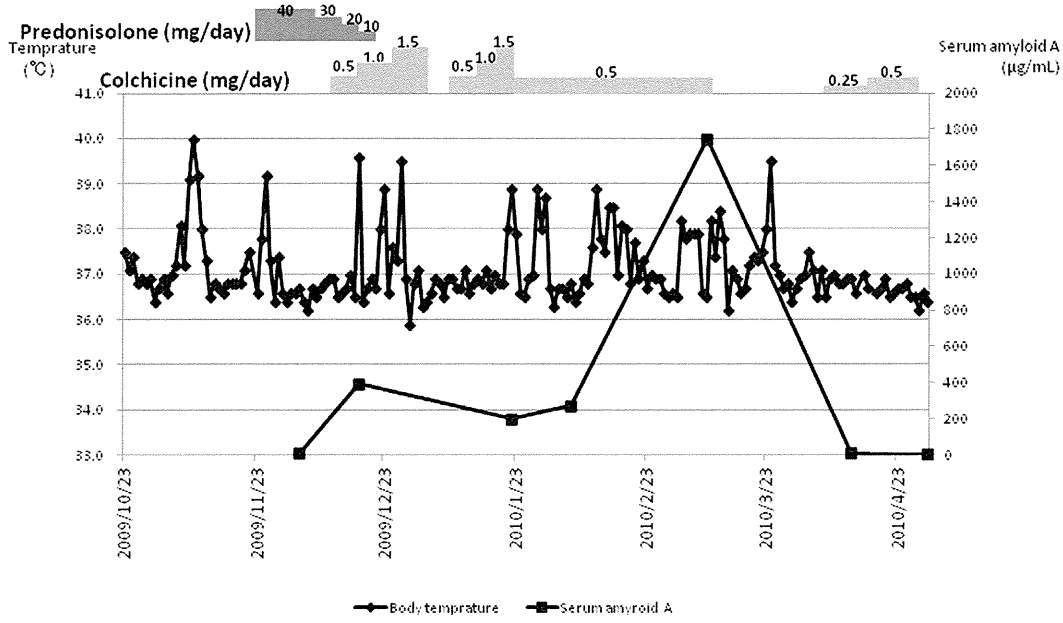

Figure 2. **The patient's clinical course.**

Table 2. **Tel Hashomer Criteria for the Diagnosis of FMF**

Major criteria
1. Recurrent febrile episodes accompanied by peritonitis, synovitis or pleuritis.
2. Amyloidosis of the AA-type without predisposing disease.
3. Favorable response to continuous colchicine treatment.
Minor criteria
1. Recurrent febrile episodes.
2. Erysipelas-like erythema.
3. FMF in a first-degree relative.
Definitive diagnosis: 2 major or 1 major and 2 minor.
Probable diagnosis: 1 major and 1 minor.

Table 3. **53 Cases of FMF in the Japanese Literature**

Sex	Male 26 Female 27	
Age of onset (y)	19.7 ± 12.8 y (1-66 y) 0-10 y: 9(16%) 11-20 y: 29(56%) 21-30: 7(13%) 31-40y: 4(8%) 41-50y: 2(3%) 51-60y: 1(2%) 61y-: 1(2%)	
MEFV mutations (cases)	M694I/E148Q	20
	M694I	11
	M694I/E148Q/L110P	4
	M694I(homo)	3
	E148Q	2
	E148Q(homo)	2
	E148Q/P369S	2
	E148Q/L110P/R202Q	1
	E148Q/R408Q/P369S	1
	E148Q/R202Q	1
	E148Q/S503C	1
	R408Q/P369S	1
	E148Q/L110P	1
	Uncertain	3

was difficult to make a correct diagnosis in patients with mild or atypical symptoms. In 1997, the MEFV gene, which is responsible for the development of FMF, was cloned. It is located on the short arm of chromosome 16 (1). The detection rate of MEFV gene mutations in FMF patients remains low, at only approximately 60% (2). Nevertheless, analyzing the MEFV gene is used as an adjunct diagnostic examination, especially when the clinical features are not distinctive, or when there is no family history of FMF.

The MEFV gene encodes a protein called pyrin, which suppress cryopyrin, which is involved in the induction of an inflammatory reaction. MEFV gene mutations depress pyrin function, which increases the inflammatory reaction. MEFV mutations are found mostly on exons 2 and 10. L110P and E148Q on exon 2 and M680I, M694I, M694V, and V726A on exon 10 are the most common mutations. In the present patient, there was no evidence of a mutation on exon 10, such as M694I, which is the most common mutation related to MEFV in Japan. However, there were heterozygous L110P, E148Q, and R202Q mutations on exon 2. In addition to the MEFV gene mutations, the characteristic clinical symptoms and the efficacy of colchicine, which met the Tel-Hashomer criteria, led to the diagnosis of FMF.

It is well known that FMF usually occurs at a young age. The majority of patients develop FMF before 20 years of age (3). According to Sohar et al. (4), the age at onset of FMF in 755 patients was 0-10 years in 65.5%, 11-20 years in 24%, 21-30 years in 8.2%, 31-40 years in 1.5%, 41-50 years in 0.3%, and unknown in 36 patients. As of October 2010, only 53 cases of FMF have been reported in Japan, including the present case (5-18). Of these, the age at onset was 0-10 years in 9 patients (16%), 11-20 years in 29 patients (56%), 21-30 years in 7 patients (13%), 31-40 years in 4 patients (8%), 41-50 in 2 patients (3%), 51-60 years in 1 patient (2%), and 61 years (2%) in 1 patient (Table 3). The present case had the latest age at onset of FMF in Japan.

The reason why FMF develops in the elderly needs to be considered. The E148Q mutation is considered to be the mildest mutation and to result in a milder form of

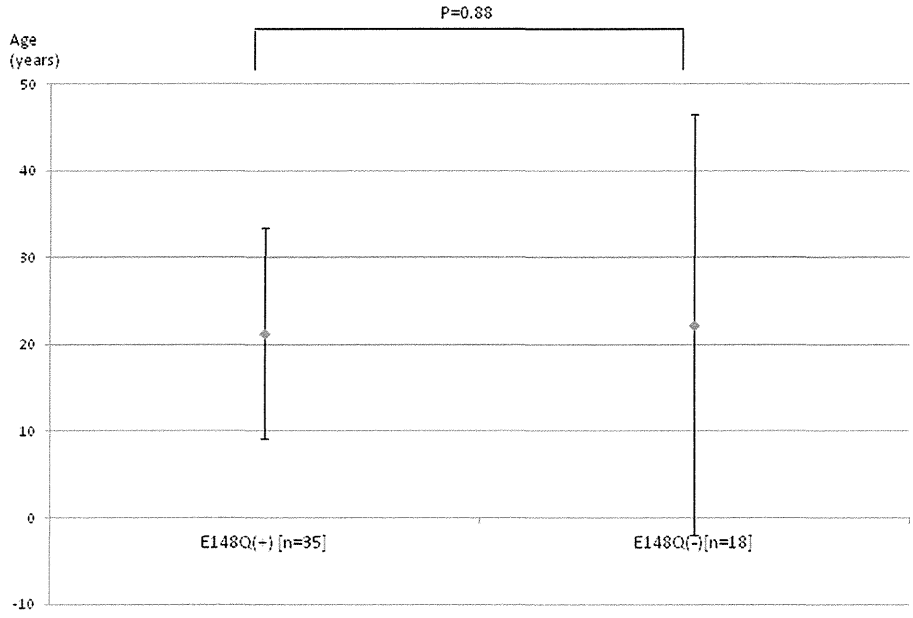

Figure 3. **Age of onset of FMF in the Japanese literature with or without E148Q mutation.**

FMF (19). It has also been reported that patients with homozygosity for the pyrin variant E148Q mutation have less severe symptoms and fewer attacks than those with heterozygosity for the pyrin variant E148Q/M694I mutation (15, 16). The patient in the present case was found to have a heterozygous E148Q mutation. According to our analysis of all cases reported in the Japanese literature (n=53), the age at onset of FMF with the E148Q mutation was 21.31±12.11 years (n=35), while that without the E148Q mutation was 22.23±24.28 years (n=18). There is apparently no significant difference in the age at onset of FMF based on the E148Q mutation (Student's *t*-test, p=0.86) (Fig. 3).

With respect to the R202Q mutation, Giaglis et al. (20) reported that, in 152 Greek FMF patients and 140 Greek healthy controls, homozygosity for the R202Q mutation was detected in 14/152 (9.2%) FMF patients and in 1/140 (0.7%) healthy controls (p=0.001, diagnostic odds ratio = 14.1, 95% CI 2.33-84.72). Heterozygosity of the R202Q mutation was detected in 48/152 (31.6%) FMF patients and in 47/140 (33.6%) healthy controls (p=0.717, diagnostic odds ratio =0.913, 95% CI 0.560-1.49). Yamaguchi et al. (21) reported that R202Q heterozygotes were observed in 7/170 (4.1%) of randomly selected healthy Japanese subjects. They and their families had no episodes of periodic fever similar to FMF. R202Q homozygotes were not observed. In our analysis of all cases of FMF reported in the Japanese literature (n=53), R202Q heterozygotes were observed in 2/53 (3.8%) FMF patients. The rates of R202Q mutation differ between Greek and Japanese, but there is little difference between FMF patients and healthy controls in the rate of heterozygosity of R202Q. It is thus, thought that the heterozygosity of the R202Q mutation does not play a significant role in FMF. On the other hand, homozygosity of the R202Q mutation is strongly associated with FMF. However, few reports have so far analyzed the impact of the L110P mutation.

Therefore, the reasons why FMF develops in the elderly remain unclear. More analysis of the association between the mutations of MEFV, the onset of FMF, and the frequency of febrile episodes is needed. However, the present case suggests that FMF should be considered in cases of unknown fever regardless of the patient's age.

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

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CASE REPORT

Coexistence of familial Mediterranean fever and rheumatoid arthritis

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Abstract Familial Mediterranean fever (FMF) is an autoinflammatory disorder characterized by recurrent febrile polyserositis and arthritis. Although accompanying seronegative spondyloarthropathy has been reported in FMF, coexistence with rheumatoid arthritis (RA) is very rare. This case report describes a Japanese female RA patient who presented with periodic fever. Genetic analysis revealed compound heterozygous mutations in exon 2 and 3 of the *MEFV* gene (E148Q/G304R/P369S/R408Q). The patient was successfully treated with colchicine with 3-year follow-up.

Keywords Familial Mediterranean fever · Rheumatoid arthritis · *MEFV* gene · Colchicine

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Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive disorder prevalent among Mediterranean region inhabitants [1]. It has recently been recognized that FMF can occur in non-Mediterranean populations, including the Japanese population [2]. FMF clinical features include recurrent serositis, fever, arthritis, and myalgia [3]. Arthritis in FMF patients presents as acute attacks of pain and swelling typical of monoarthritis, most frequently affecting the joints of lower extremities [4]. In some cases protracted arthritis develops, mostly in the hips and knees [5, 6]. Some clinical features of FMF mimic those of rheumatic diseases. Although both FMF and rheumatoid arthritis (RA) are systemic inflammatory diseases, coexistence of FMF and RA is very rare [7]. In this report, we describe a patient who fulfilled the definition criteria for RA with periodic fever and compound heterozygous mutations in the *MEFV* gene (E148Q/G304R/P369S/R408Q). Oral colchicine treatment successfully silenced her periodic fever as well as arthritis symptoms. This case study discusses this rare association.

Case report

A 51-year-old Japanese female patient with chronic glomerulonephritis was referred to our department for morning stiffness and tenderness of both wrist joints. She had previously been diagnosed with IgA nephropathy at the age of 40 years, and initially treated with high-dose corticosteroids and antiplatelet agents. She was diagnosed with mild proteinuria and renal dysfunction at the department of nephrology of our hospital. Her first symptoms, present 2 months prior to the first visit, were symmetrical

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polyarthralgia with pain in the wrist and the metacarpophalangeal joints with morning stiffness. In physical examination, cardiovascular and respiratory examinations were unremarkable with no liver or spleen enlargement. However, swelling was observed in both wrists and the metacarpophalangeal joints. As shown in Table 1, blood analysis revealed white blood cell count of 9200 cells/mm^3, hemoglobin of 12.6 g/dl, and creatinine of 1.1 mg/dl. C-reactive protein (CRP) was 0.52 mg/dl, anti-nuclear antibodies (ANA) were positive with a low titer (1:160), and anti-cyclic citrullinated peptide (CCP) antibodies were positive with a high titer (1720 IU/ml). *HLA-DRB1* genotyping demonstrated the presence of a single copy of a shared epitope (0405/0803). Although X-ray analysis of the hand showed no remarkable findings (data not shown), magnetic resonance imaging (MRI) detected synovitis (Fig. 1). The diagnosis of RA was made according to the 2010 American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) classification criteria for RA [8], thus disease-modifying antirheumatic drug (DMARD; sulfasalazine: SASP, 500 mg/day) treatment was started. Joint pain and stiffness were improved following SASP treatment, although proteinuria levels were increased. Therefore, SASP treatment was discontinued. Additionally, 9 years earlier, she had presented with recurrent episodes of high fever (≥38 °C). Recurrent fever occurred approximately once every 2 months and regressed spontaneously within 3 days. A diagnosis of FMF was suspected based on the presence of periodic fever, and thus genetic analysis of the *MEFV* gene was considered. Based on the gene analysis showing compound heterozygous mutations (E148Q/G304R/P369S/R408Q) in exon 2 and 3 of the *MEFV* gene (Fig. 2), we decided to start colchicine treatment. Following colchicine treatment, the periodic fever episodes disappeared and her arthralgia was improved. Her arthritis has since been in remission under maintenance treatment at dosage of 1.0/mg/day colchicine (Fig. 3).

Table 1 Laboratory findings

Peripheral blood		Serological tests	
Red blood cells	383 × 10^4/μl	C-reactive protein	0.52 mg/ml (<0.30)
Hemoglobin	12.6 g/dl	Erythrocyte sedimentation rate	54 mm/h
Hematocrit	36.2 %	IgG	1580 mg/dl (900–2000)
White blood cells	9200/μl	C3	123 mg/dl (70–120)
Neutrophil	74.8 %	C4	30 mg/dl (15–24)
Eosinophil	2.5 %	Anti-nuclear Ab	×160 diffuse
Monocyte	4.5 %	Anti-dsDNA Ab	(−)
Lymphocyte	17.9 %	Anti-CCP Ab	1720 U/ml (<4.5)
Platelets	33.2 × 10^4/μl	RF	120 IU/ml (<20)
Blood chemistry		Anti-RNP Ab	(−)
Total protein	8.0 g/dl	Anti-SSA Ab	(−)
Total bilirubin	1.6 mg/dl	Anti-SSB Ab	(−)
Glutamic-oxaloacetic transaminase	25 IU/l (7–33)	HLA-DRB1	(0405/0803)
Glutamic-pyruvic transaminase	36 IU/l (5–30)	Urinalysis	
Lactate dehydrogenase	187 IU/l (260–480)	pH	6.0
Alkaline phosphatase	319 IU/l (80–250)	Protein	85 mg/dl
Gamma-glutamyl transpeptidase	52 IU/l (5–55)	Glucose	(−)
Creatinine kinase	30 IU/l (60–160)	Occult blood	(±)
Total cholesterol	189 mg/dl	Sediment	np
Blood urea nitrogen	18.7 mg/dl		
Creatinine	1.1 mg/dl		

RF rheumatoid factor, *Anti-CCP Ab* anti-cyclic citrullinated peptide antibody

Discussion

Although an association between FMF and seronegative arthritis has been reported [9], coexistence of FMF and RA has rarely been demonstrated [7]. The most common arthritic attack of FMF is acute large joint monoarthritis, most often affecting the knee and hip, lasting for several days, and rarely protracted arthritis may occur in FMF patients [10–12]. In contrast, RA is an erosive chronic inflammatory disease that affects hand and ankle joints, causing deformity. In early RA the most frequently involved areas are the metacarpophalangeal and proximal interphalangeal joints. The disease often begins with symmetrical involvement of small joints, as seen in the present case, although RA-characteristic findings from radiography analysis, such as bone erosion or joint space narrowing, were not observed in the present case. However, MRI scans demonstrated synovitis, which is a characteristic finding of RA [13]. Additionally, she had high titers of anti-CCP antibodies, RA-specific autoantibody, and HLA-DRB1 shared epitope. In the present case, the patient presented with bilateral and symmetric arthritis in the small joints of the hands. Furthermore, the present case fulfilled the ACR/EULAR 2010 classification criteria for RA, namely presence of tender joints (≥4), swollen joints

(≥4), high titers of anti-CCP antibodies, elevated levels of CRP or erythrocyte sedimentation rate (ESR), and persistent arthritis (≥6 weeks) [8]. However, there are limited studies investigating the relationship between anti-CCP antibodies and FMF patients with arthritis in the literature, reporting conflicting results [14, 15]. Ceri et al. mentioned that anti-CCP Ab positivity rates were significantly higher in FMF patients with arthritis than in patients without arthritis [15]. Furthermore, the clinical manifestation of arthritis in FMF was reported by Majeed and Raushded, who described that 7 patients (5 %) among 133 FMF patients had involvement of small joints of the hands and feet [16]. These findings suggest that anti-CCP antibodies may not be a reliable indicator to differentiate between FMF arthritis and rheumatoid arthritis, even in cases with small joint arthritis. Since the patient also fulfilled the diagnostic criteria for incomplete-type FMF [17], FMF-related arthropathy cannot be completely ruled out. This rare association between FMF and RA seen in the present case suggests a contingent question. This association may be due to chance, but if not: could the occurrence of RA be linked to the deregulated inflammasome activation process [18] seen in FMF patients? *MEFV* gene encodes a protein named pyrin, which is expressed in neutrophils and monocytes [19]. Although the function of pyrin is still unknown, it inhibits the processing of interleukin (IL)-1β to active form and nuclear factor-κB activation [19]. In the presence of *MEFV* mutations, the function of pyrin can be impaired and there is uncontrolled production of active IL-1β [20]. Therefore, it is possible that the mutation of *MEFV* gene may affect the inflammatory process of RA, since efficacy of blocking IL-1 was demonstrated in a subset of RA patients [21].

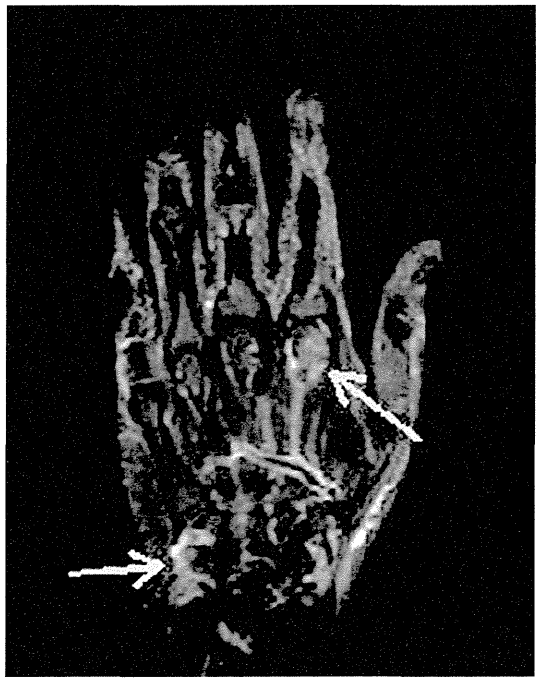


Fig. 1 Magnetic resonance imaging (MRI) of *left hand*. T1-weighted short tau inversion recovery (STIR) image shows synovitis (*arrow*) in second metacarpal bone and carpal bones

Recently, FMF cases with heterozygous *MEFV* exon 2 or exon 3 mutations had atypical clinical features, including protracted arthritis, and were effectively treated with colchicine [22]. Rabinovich et al. [23] suggested a strong association between RA severity and the presence of mutations in *MEFV* exon 2. These findings suggest that mutations of *MEFV* could modulate the disease severity or clinical phenotype of RA. Methotrexate (MTX) is the most

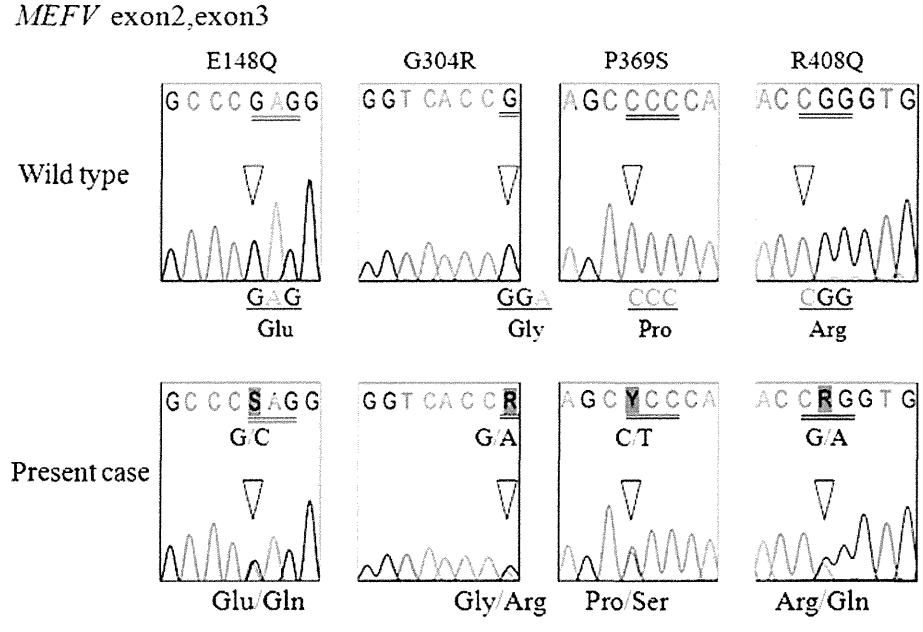
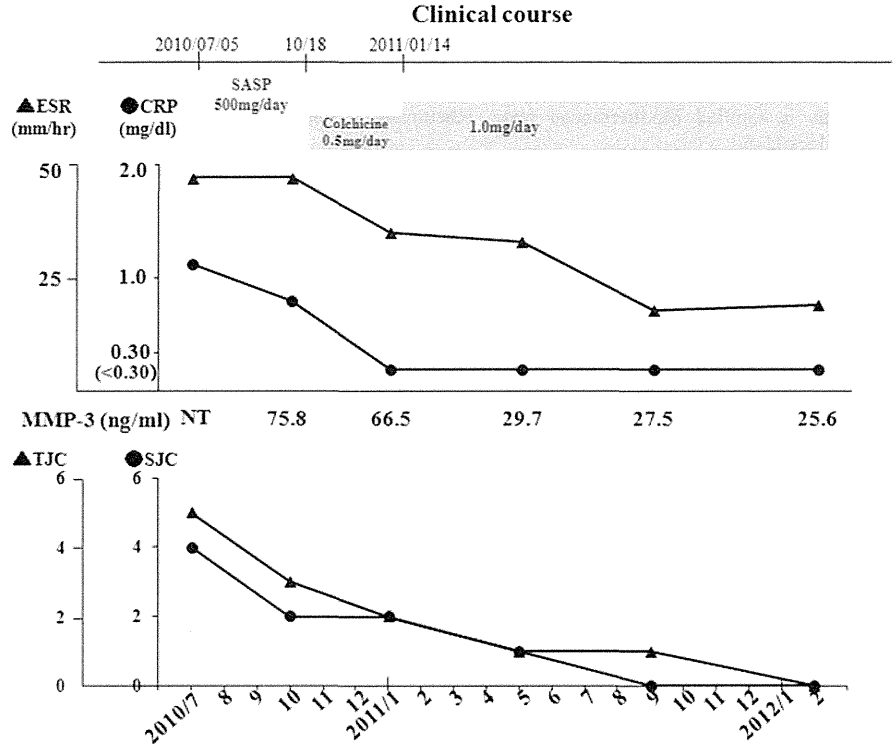


Fig. 2 *MEFV* gene analysis in a healthy control (wild type) and in the present case. In the patient, the G to C transition in codon 148 converting a glutamic acid (E) to glutamine (Q), G to A transition in codon 304 converting a glycine (G) to arginine (R), C to T transition in codon 369 converting a proline (P) to serine (S), and G to A transition in codon 408 converting an arginine (R) to glutamine (Q)



Abbreviations: MMP-3; matrix metalloproteinase-3, NT; not tested, SASP; salazosulfapyridine, SJC; swollen joint count, TJC; tender joint count

Fig. 3 Clinical course of the present case

effective DMARD for treatment of RA [24]. Due to her impaired renal function, we chose SASP for treatment. However, SASP was stopped following the observed exacerbation of proteinuria. Therefore, we administrated colchicine. The patient was sensitive to colchicine treatment, and the periodic fever was eliminated by this treatment. Long-standing protracted arthritis that accounts for 5 % of FMF cases was reported by Sneh et al. [5]. The efficacy of colchicine for synovitis was established in the majority of FMF patients [25, 26]; however, other treatments are required in few patients with protracted synovitis [11]. In general, colchicine is considered to be the treatment of choice in FMF patients with chronic arthritis. In contrast, intervention using colchicine in RA is currently rare and is limited to certain extra-articular manifestations, such as pericarditis [27]. The use of colchicine in our case successfully silenced the arthritis in small joints. Therefore, in the current case, it is possible that the arthropathy appeared to be FMF-related joint involvement, rather than rheumatoid synovitis. It is also plausible that a modifier role of *MEFV* gene mutations could be partly responsible for the colchicine-responsive arthritis seen in the present case, since colchicine-responsive connective tissue diseases with *MEFV* mutations had been reported [28, 29]. Further study is warranted to elucidate the role of *MEFV* mutations in the clinical phenotype of RA.

The present case report has obvious limitations. Since FMF is not a common disease in Japan, with a distribution limited to certain geographic areas, data regarding coexistence of FMF and RA are limited. Since most of the data originate from case reports, case report publication bias is a concern. Increasing the awareness of FMF-related rheumatic symptoms may motivate clinicians to conduct clinical studies concerning this rare association. Such studies may elucidate the associations between FMF and RA.

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Conflict of interest None.

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CASE REPORT

Amyloid A amyloidosis in a Japanese patient with familial Mediterranean fever associated with homozygosity for the pyrin variant M694I/M694I

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Abstract Familial Mediterranean fever (FMF) is an autoinflammatory disease common in eastern Mediterranean populations. The most severe complication is the development of secondary amyloid A (AA) amyloidosis. A 51-year-old Japanese male who had been suffering from periodic fever since in his twenties was referred to our hospital for proteinuria. Histological findings from renal biopsy revealed the deposition of AA amyloid fibrils, suggesting that renal dysfunction was due to AA amyloidosis. Gene analysis of the patient and his mother showed that both were homozygous for the M694I mutation in the *MEFV* gene. His mother was also a carrier of the SAA1.3 allele, which is not only a univariate predictor of survival but also a risk factor for the association of AA amyloidosis with rheumatoid arthritis in Japanese patients, and the SAA1-13T allele in the 13T/C polymorphism on the 5′-flanking region of the SAA1 gene. The patient was also a carrier of the SAA-13T allele. Colchicine resulted in not only an amelioration of the acute febrile attacks of FMF inflammation, but also an improvement in kidney dysfunction due to AA amyloidosis.

Keywords Familial Mediterranean fever · AA amyloidosis · M694I homozygosity · SAA1.3 allele · Colchicine

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Introduction

Familial Mediterranean fever (FMF) is the most common autoinflammatory syndrome. Patients with FMF typically present with recurrent attacks of fever and serositis with significant acute-phase inflammation. The disease is associated with mutations in the responsible Mediterranean fever gene (*MEFV*) that codes for pyrin expressed mainly in neutrophils and monocytes [1] and is transmitted in an autosomal-recessive manner [2]. The clinical symptoms of FMF are believed to vary according to the specific *MEFV* mutation. Secondary amyloid A (AA) amyloidosis is the most severe form of disease course in FMF [3]. Although FMF is a major cause of AA amyloidosis in Mediterranean areas, in Japan FMF is an extremely rare disease because of the low allele frequencies of the *MEFV* gene mutations [4]. Renal involvement with AA amyloidosis secondary to FMF may cause end-stage renal disease. Here, we describe the case of a Japanese patient with FMF who was homozygous for the M694I mutation presenting with proteinuria due to renal AA amyloidosis.

Case report

A 51-year-old Japanese male was referred to our clinic in February, 2010 with complaints of proteinuria that had

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persisted for 2 years. He had had periodic fever arising once or twice a month and abdominal pain attacks since he was 20 years old. His attacks were self-limiting and lasted for 2–3 days. When he was 29 years old, he had severe left knee joint swelling and tenderness, and the etiologies were screened with a focus on the orthopedic aspects, but in vain. His medical history was otherwise unremarkable. His mother had similar febrile episodes after labor following the birth of the patient. The two children of the patient had no febrile history at presentation of the patient. The parents had a non-consanguineous marriage and had been born in different towns.

On physical examination, the patient had some bilateral pitting edema. Laboratory studies showed proteinuria (182 mg/dl) with normal albuminemia (4.1 g/dl) and renal functions; serum creatinine, 0.87 mg/dl; serum blood urea nitrogen, 11.8 mg/ml; estimated glomerular filtration rate (eGFR), 74 ml/min/1.73 m^2; elevated C-reactive protein (CRP), 7.1 mg/dl. Histopathological findings of the specimens obtained by renal needle biopsy revealed the presence of amorphous depositions in both mesangium and arteriole regions by hematoxylin–eosin staining (Fig. 1a) and periodic acid–Schiff staining (Fig. 1b); these depositions were positive for Congo Red staining (Fig. 1c, d). The presence of AA amyloid deposits was confirmed histologically based on positive Congo Red staining, potassium permanganate oxidation susceptibility, and the green birefringence seen by polarization microscopy after Congo Red staining, as well as by the immunohistochemical analysis using anti-AA antibody and anti-immunoglobulin light-chain (AL) antibody to differentiate AL amyloidosis.

After receiving the patient's informed consent, we performed a genetic analysis of all exons (from exon 1 to exon 10) of the *MEFV* gene by direct sequencing. For this analysis, we collected 2 ml of peripheral blood samples. Genomic DNA was extracted from whole blood using the Promega Wizard® Genomic DNA Purification kit (Promega, Madison, WI). The PCR was performed using forward and reverse primers for each exon as described previously [5]. PCR products were purified with the ExoSAP-IT (GE Healthcare Japan, Tokyo, Japan) and sequenced directly, using specific primers and BigDye Terminator v1.1 (Applied Biosystems, Foster City, CA).

The analysis revealed that the patient and his mother carried the homozygous *MEFV* (M694I/M694I) mutation

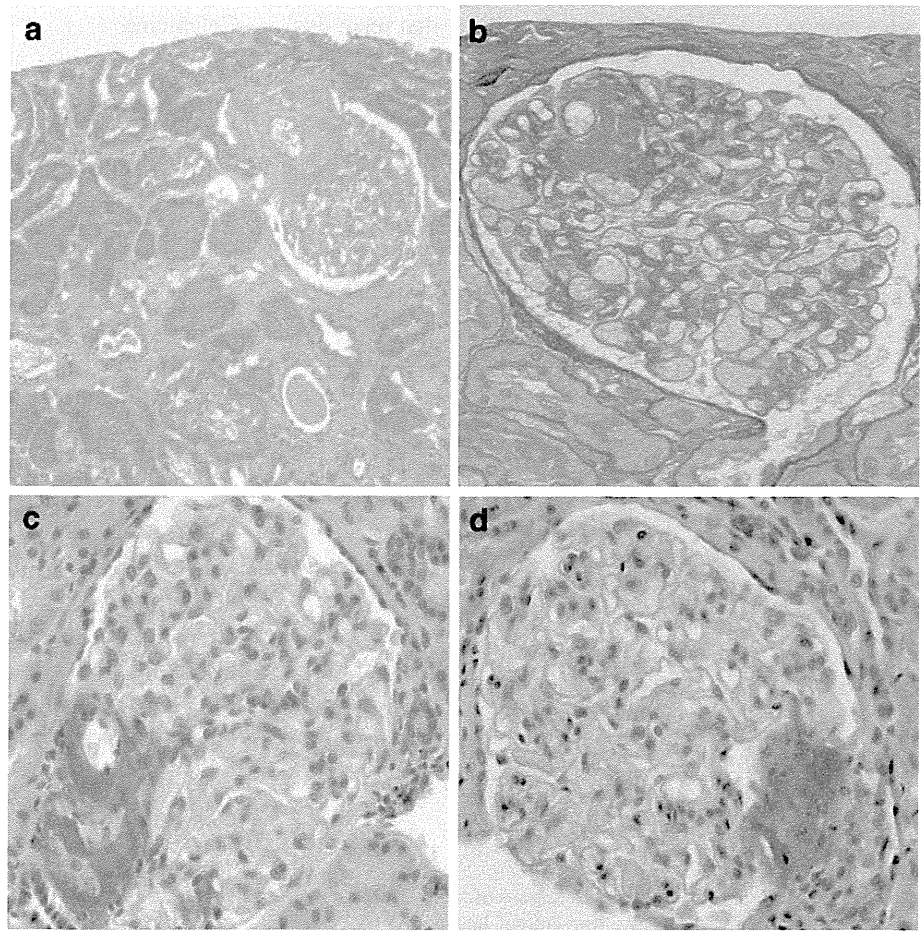

Fig. 1 Histopathological findings of the specimen obtained by needle renal biopsy. Both hematoxylin–eosin staining (**a**) and periodic acid–Schiff (**b**) staining revealed amorphous deposits in the areas of glomerulus tissues. **c**, **d** Congo Red staining showed positivity of the amorphous substances, which could suggest amyloid fibril deposits

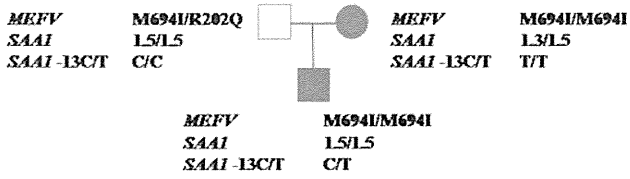

Fig. 2 Pedigree of the patient's family and genetic analysis of the *MEFV* mutation, SAA1 polymorphism, and SAA1-13T polymorphism. *Black symbols* denote individuals who are clinically symptomatic, *circle* patient's mother, *squares* male family members. *Black square* Our patient, who carries the homozygous M694I *MEFV* mutation, the homozygous SAA1.5 allele, and the SAA1-13T polymorphism

Table 1 Genetic analysis of *MEFV* in the patient and his immediate family

Family relationship	Gene polymorphism
Patient (present case)	M694I/M694I/P588P/P588P
Father	D102D/G138G/A165A/R202Q/R314R/M694I
Mother	M694I/M694I

(G → A transversion of nucleotide that results in substitution of isoleucine for methionine). Genetic analysis on the family, including other gene polymorphisms associated with AA amyloidosis, was carried out as previously described [6, 7] and the results are shown in Fig. 2 and Table 1.

The patient not only met the criteria of FMF [8], but the results of the genomic search for the *MEFV* gene (Fig. 2) confirmed the diagnosis of FMF, which was attributable to AA amyloidosis, resulting in proteinuria. The episodes were successfully prevented by the administration of colchicine (1.0 mg/day), and various surrogate markers ameliorated in the clinical course. Changes in the values in these markers in the chronological order February, June, August, and November, 2011 were: CRP, 7.1 , 2.5, 0.7, 0.6 mg/dl; erythrocyte sedimentation rate, 34, 29, 26, 23 mm/h; eGFR, 63.1, 70.8, 70.8. 72.6 ml/min/1.73 m^2; proteinuria, 0.94, 0.98, 0.61, 0.52 g/day; serum AA protein 638, 489, 39.9, 16.0 μg/ml, respectively.

The patient and his parents provided written consent to participate in the study as a requirement for ethical approval. The study design and protocols were approved by the Ethical Committee of the Kumamoto Shinto General Hospital, Kumamoto, Japan.

Discussion

Familial Mediterranean fever is a hereditary autosomal recessive disease characterized by recurrent attacks of fever and polyserositis [9]. In most patients with FMF, the onset of the first attack occurs before the age of 20 years, the attacks of FMF last 1–3 days, and they are self-limiting; therefore, patients are asymptomatic in the attack-free intervals. The severity and frequency of the attacks vary among patients and even sometimes in the same patient. As the dominant manifestation is abdominal pain, it is often difficult to distinguish abdominal pain attacks in FMF from acute abdomen, and an urgent exploratory laparotomy seems to be necessary in 30–40 % of cases. Pleuritic chest pain or arthritis due to pleuritis or synovitis may be other manifestations of FMF [10].

The clinical profile in FMF, including its major manifestation, AA amyloidosis, is influenced by *MEFV* allelic heterogeneity and other genetic and/or environmental factors. Homozygosity for the M694V allele and the presence of the SAA1.1/1.1 genotype are significantly and independently associated with renal AA amyloidosis in eastern Mediterranean patients with FMF [11]. In this population, disease severity was found to be mainly influenced by *MEFV* mutations and not to be associated with genotypes at the SAA1 locus. The SAA1-13T allele was rare, associated mainly with the SAA1.3 isoform, and not related to renal AA amyloidosis. These observations led the authors to conclude that disease severity and the development of AA amyloidosis in eastern Mediterranean patients with FMF are differentially affected by genetic variations within and outside the *MEFV* gene. Renal involvement with AA amyloidosis secondary to FMF is the most important long-term complication, varying from asymptomatic proteinuria to nephrotic syndrome and possibly progressing to end-stage renal disease. In most cases with renal AA amyloidosis, chronic renal failure may develop within 5 years after the onset of proteinuria [12, 13]. Our patient was homozygous for both the M694I mutation and SAA1.5 allele (Fig. 2). In the light of the association of AA amyloidosis secondary to FMF, the *MEFV* mutation might have a more strong effect on the pathogenesis of AA amyloidosis than SAA1 allelic polymorphisms.

A meta-analysis study on the founder populations (Jews, Armenians, Arabs, and Turks) for *MEFV* mutations revealed that the most frequent mutations detected in FMF patients are the M694V (39.6 %), V726A (13.9 %), M680I (11.4 %), E148Q (3.4 %), and M694I (2.9 %) mutations [14]. The four major disease-causing mutations (M694V, M694I, M680I, and V726A) in exon 10 of *MEFV* have low allele frequencies in normal Japanese individuals [15]. Our patient carried only the M694I homo-variant in exon 10 (Table 1). Even for M694I, which seems to be the most common mutation in Japanese FMF patients [4], allele frequency is <0.001 [15]. The multiple variables governing the development of AA amyloidosis in FMF have not yet been fully elucidated. Susceptibility to AA amyloidosis has been reported to be related to sex and genotype at the

MEFV and the SAA1 loci [11, 16]. Further, the risk factor for renal AA amyloidosis has been found to be higher among those carrying the SAA1.1/1.1 genotype in Caucasian populations; in contrast, the SAA1.3 allele is the most predominant genotype among Japanese patients with renal AA amyloidosis [6]. It has been suggested that the -13C/T polymorphism in the 5′-flanking region of the SAA1 gene is presumably associated with susceptibility to AA amyloidosis in both Caucasian and Japanese populations [17]. However, the difference in the prevalence of AA amyloidosis among the different ethnic groups of patients, not only with FMF but also with rheumatoid arthritis (RA), remains as yet unexplained [18]. The mechanisms by which the protein polymorphisms derived from SAA1 genotypes predispose to renal AA amyloidosis remain to be unraveled. One possible explanation is that the SAA protein polymorphism is associated with more severe inflammation compared with other SAA products. Another possibility is that the AA derived from different SAA alleles is either more easily deposited or less easily metabolized after deposition. The only established fact is that more severe inflammation is associated with higher levels of SAA and that higher levels of SAA are amyloidogenic [19, 20]. Whether this may explain the association of AA amyloidosis with arthritis attacks presumably associated with higher SAA levels remains to be explored. The factors governing disease activity and amyloidogenesis are complex and multiple. In an earlier publication, we reported a significant association between the SAA1.3 allele and AA amyloidosis in Japanese patients with RA [6]. The contribution of genotypes at the *MEFV* and SAA 1 loci is paramount; therefore, many other factors still remain to be further elucidated.

Colchicine prevents episodic inflammatory attacks and the development of AA amyloidosis in almost all FMF patients compliant to therapy and also reverses the clinical manifestations of organ involvement in established AA amyloid cases [21]. However, it is generally believed that although AA amyloidosis seems to be clinically cured by colchicine therapy, a large amount of AA amyloid deposits still remains in the involved organs. In our patient, colchicine not only improved the clinical disease activity of FMF but also ameliorated AA amyloidosis, based on the better renal function following treatment, as evidenced by the biological markers. Colchicine is very important in preventing AA amyloidosis secondary to FMF, and it may also arrest the progression of AA amyloidosis in those who already have the complication. However, the specific mode of action of colchicine in AA amyloidosis secondary to FMF has not been determined despite the reported effectiveness of this treatment in a number of anecdotic cases [22–24]. Colchicine is believed to inhibit the enhanced chemotactic activity for polymorphonuclear leukocytes through interleukin 8 and leukotriene B4, which would lead to a suppression of SAA synthesis. The actual mode of action of colchicine in the mechanisms in FMF await further investigation and more conclusive evidence. To ensure clinical improvement in our patient's disease course, if this is possible, a sequential biopsy of the upper gastrointestine and/or kidney is advisable. The aim of specific treatments of AA amyloidosis caused by FMF is to slow down and halt SAA production because AA amyloidosis is caused by the deposition of AA amyloid fibrils in various organs, which in turn derives from the circulatory acute-phase reactant SAA. Thus, the levels of SAA should be monitored and kept to <10 μg/ml [25] in the long-term follow-up, which in turn requires frequent assessment of SAA concentrations. With respect to our patient, bone marrow suppression and other adverse effects have not emerged to date, but careful observation is needed.

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Conflict of interest None.

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