The possession of exon 2 variants in the *MEFV* gene promotes inflammasome activation in Japanese patients with familial Mediterranean fever with a heterozygous exon 10 mutation

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ABSTRACT

Objective. *The modification and pathogenesis of MEFV exon 2 or 3 variants in familial Mediterranean fever (FMF) remains unclear. We compared the clinical and laboratory characteristics between the coexistence and non-coexistence of MEFV exon 2 or 3 variants in patients with FMF that had a heterozygous MEFV exon 10 mutation.*

Methods. *We excluded patients with FMF that had two MEFV exon 10 mutations in one or more alleles and/or MEFV mutations in exons other than in exons 2, 3, or 10. Finally, we reviewed 131 Japanese patients with FMF that had a heterozygous MEFV exon 10 mutation, and they were divided into the groups with and without MEFV exon 2 or 3 variants of 97 and 34, respectively.*

Results. *All patients with MEFV exon 2 variants had either E148Q and/or L110P variants, none of patients had exon 3 variants. In the univariate analysis, the group with variants had significantly earlier onset, a higher percentage of thoracic pain with febrile attacks, a higher frequency of attack, and a higher IL-18 level at remission compared to the group without variants (all, $p<0.05$). Importantly, multivariate analyses showed that the coexistence of* MEFV *exon 2 variants was independently and significantly associated with earlier onset of FMF and thoracic pain (both, $p<0.05$).*

Conclusion. *Our results suggested that coexistence of MEFV exon 2 variants have additional effects on manifestations of FMF with MEFV exon 10 mutations. Our findings highlighted the modifications and pathogenesis of such MEFV variants in FMF.*

Introduction

The genetic characteristics of patients with familial Mediterranean fever (FMF) in Japan include a lower percentage of exon 10 mutations and a higher percentage of exon 2 or 3 variants in the Mediterranean fever (*MEFV*) gene compared with patients with FMF in Western countries (1). However, a relatively high proportion of healthy individuals have *MEFV* exon 2 or 3 variants in Japan (2, 3). Therefore, the modification and pathogenesis of the *MEFV* exon 2 or 3 variants in FMF remains unclear.

In the present study, using data from a nationwide multicentre prospective study in Japan, we compared the laboratory and clinical characteristics between the coexisting and non-coexisting *MEFV* exon 2 or 3 variants among patients with FMF who had a heterozygous *MEFV* exon 10 mutation. Moreover, we aimed to elucidate the modifications and pathogenesis of such variants in FMF.

Patients and methods

Study design

This study was part of an ongoing multicentre prospective cohort study that is registered with the University Hospital Medical Information Network Clinical Trials Registry [http://www.umin.ac.jp/ctr/] (#UMIN000015881). The study population consisted of Japanese patients with FMF who were recruited consecutively and prospectively as of January 2009 from 125 related centres of Nagasaki University, Shinshu University, Kanazawa University, and the Nagasaki Medical Center in Japan.

Each patient with FMF fulfilled the Tel Hashomer criteria (4). As described

Table I. Participant characteristic by coexistence of variants among patients with FMF who had a heterozygous *MEFV* exon 10 mutation (univariate analysis).

Variables	All patients (n=131)	The group without variants (n=34)	The group with variants (n=97)	*p*-value
Patient characteristics				
Age at onset (years) *	16.0 (12.0 – 27.0, 130)	20.5 (14.8 – 33.0, 34)	16.0 (11.0 – 23.0, 96)	0.04
Age at diagnosis (years) *	31.5 (20.0 – 44.5, 130)	35.5 (19.0 – 53.5, 34)	31.0 (20.3 – 40.5, 96)	0.43
Male gender (%)	69/131 (52.7%)	18/34 (52.9%)	51/97 (52.6%)	1.00
Family history of FMF (%)	49/131 (37.4%)	11/34 (32.4%)	38/97 (39.2%)	0.54
Comorbidity				
AA amyloidosis (%)	3/131 (2.3%)	0/34 (0.0%)	3/97 (3.1%)	0.57
Autoimmune or autoinflammatory disease (%)	6/131 (4.6%)	1/34 (2.9%)	5/97 (5.2%)	1.00
Clinical characteristics				
Typical FMF (%)	116/131 (88.6%)	33/34 (97.1%)	83/97 (85.6%)	0.11
Frequency of attack (/month) *	1.0 (0.5 – 1.0, 125)	0.5 (0.4 – 1.0, 33)	1.0 (0.5 – 1.5, 92)	0.02
Duration of fever attack *	2.5 (1.5 – 3.0, 125)	2.5 (1.5 – 2.9, 32)	2.5 (1.5 – 3.0, 93)	0.41
Headache (%)	12/131 (9.2%)	5/34 (14.7%)	7/97 (7.2%)	0.30
Thoracic pain (%)	81/131 (61.8%)	15/34 (44.1%)	66/97 (68.0%)	0.02
Abdominal pain (%)	97/131 (74.0%)	26/34 (76.5%)	71/97 (73.2%)	0.82
Pericarditis (%)	0/131 (0.0%)	0/34 (0.0%)	0/97 (0.0%)	NS
Arthritis (%)	30/131 (22.9%)	8/34 (23.5%)	22/97 (22.7%)	1.00
Myalgia (%)	12/131 (9.2%)	5/34 (14.7%)	7/97 (7.2%)	0.30
Erysipelas-like erythema (%)	6/131 (4.6%)	3/34 (8.8%)	3/97 (3.1%)	0.18
Good response to colchicine (%)	97/98 (99.0%)	23/23 (100%)	74/75 (98.7%)	1.00
Laboratory findings at attacks				
WBC ($x10^3/\mu l$) *	10.0 (7.7 – 12.3, 94)	8.7 (7.4 – 12.3, 27)	10.2 (7.9 – 12.3, 67)	0.29
CRP (mg/dl) *	9.7 (5.3 – 13.1, 102)	9.1 (3.0 – 12.7, 27)	10.0 (5.8 – 15.0, 75)	0.18
SAA (μg/ml) *	731 (175 – 1284, 37)	1100 (413 – 1400, 8)	642 (139 – 1277, 26)	0.48
ESR (mm/h) *	32 (22 – 47, 30)	29 (15 – 82, 8)	32 (25 – 47, 22)	0.83
Mutational pattern in the MEFV gene				
Exon 10 mutation, number	M694I/–, 131	M694I/–, 34	M694I/–, 97	NA
Exon 2 or 3 variant, number	E148Q/–, 61; L110P/–, 2; E148Q/E148Q, 1; E148Q/–/L110P/–, 32; E148Q/E148Q/L110P, 1	None	E148Q/–, 61; L110P/–, 2; E148Q/E148Q, 1; E148Q/–/L110P/–, 32; E148Q/E148Q/L110P, 1	NA

*Median (interquartile range, number) or number (percentages) are shown. *p*-values were established using Fisher's exact test or the Mann-Whitney U-test. FMF: familial Mediterranean fever; *MEFV*: Mediterranean fever gene; WBC: white blood cell count; CRP: C-reactive protein; SAA: serum amyloid A; ESR: erythrocyte sedimentation; NS: not significant; NA: not available.

previously (1), we identified typical and atypical FMF cases. All patients gave their signed informed consent to be subjected to the protocol, which was approved by the Institutional Review Board of Nagasaki University and related centres (Approval No. 14092946).

Patients

We reviewed 146 Japanese patients with FMF who had *MEFV* exon 10 mutations. Subsequently, to observe the pure modifications and pathogenesis of *MEFV* exon 2 or 3 variants, we excluded patients with FMF who had two *MEFV* exon 10 mutations in one or more alleles and/or *MEFV* mutations in exons other than exons 2, 3, or 10.

Overall, the outcomes of 131 patients with FMF who had a heterozygous *MEFV* exon 10 mutation were analysed, and patients were divided into groups according to the presence or absence of *MEFV* exon 2 or 3 variants (n = 97 and 34, respectively).

Mutational analysis

Each patient underwent mutational analysis of the *MEFV* gene using the same method at one of the following centres in Japan; Nagasaki University, Shinshu University, Kanazawa University, and the Nagasaki Medical Center. We extracted genomic DNA using the Promega Wizard® Genomic DNA Purification Kit (Promega, Madison, WI). We subsequently performed polymerase chain reaction (PCR) using the forward and reverse primers for each exon of the *MEFV* gene, as described previously (5). We purified the PCR products using the ExoSAP-IT™ reagent (GE Healthcare Japan, Tokyo) and sequenced them directly using specific primers and BigDye Terminator v1.1 Kit (Applied Biosystems, Tokyo).

Assessment of serum IL-18 levels

We measured serum IL-18 levels at remission without febrile attacks in the groups with and without *MEFV* exon 2 or 3 variants (n = 31 and 9), respectively, using a multiplex cytokine bead assay blindly and in parallel using the Bio-plex Pro™ Human Cytokine Assay (Bio-Rad, Hercules, CA), according to the manufacturer's instructions.

Statistical analysis

Discrete variables were compared between the groups using Fisher's exact test, and continuous variables were compared using Wilcoxon's test. In a multivariate analysis, we included clinically important variables in the model. Subsequently, we determined the fac-

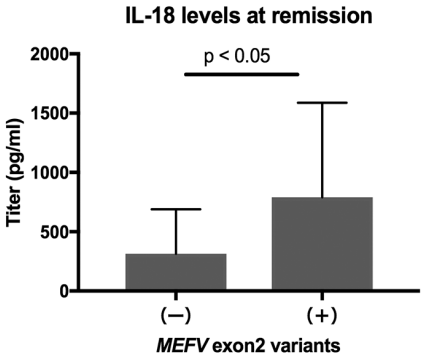

Fig. 1. Serum IL-18 levels at remission by coexistence of *MEFV* exon 2 variants among patients with FMF who had a heterozygous *MEFV* exon 10 mutation.

tors that contributed to age at onset, frequency of febrile attack, or thoracic pain.

All statistical analyses were performed using the JMP Pro 14.0 software (SAS Institute, Cary, NC, USA). *p*-values less than 0.05 (two-tailed) were considered statistically significant in all analyses.

Results

Clinical and laboratory characteristics and mutational pattern

The demographic and clinical characteristics of the enrolled patients are summarised in Table I. All patients with *MEFV* exon 2 variants had either the E148Q and/or L110P variants, while none of the patients had exon 3 variants. In the univariate analysis, the group with variants had a significantly earlier onset ($p<0.05$) and a higher frequency of febrile attack ($p<0.05$) and thoracic pain ($p<0.05$) during febrile attacks compared with the group without variants.

Difference in the serum IL-18 levels at remission

The serum IL-18 levels at remission are shown in Figure 1. The respective median IL-18 levels in groups with and without variants were 606.3 (182.5–1243.0) and 168.4 (35.5–593.2), respectively. The group with variants had a significantly higher level of IL-18 at remission compared with the group without variants ($p<0.05$).

Independent factors associated with age at onset, frequency of febrile attack, and thoracic pain

The results of the multiple regression analysis of age at onset, frequency of attack, and thoracic pain are shown in Table II. Multivariate analyses showed that the coexistence of *MEFV* exon 2 variants was significantly and independently associated with an earlier onset of FMF (coefficient, –2.570; $p<0.05$). Moreover, multivariate analyses showed that the coexistence of *MEFV* exon 2 variants tended to be associated with a higher frequency of febrile attack, albeit without significance.

In addition, multivariate analyses showed the association between two independent factors and thoracic pain: age at onset (odds ratio, 0.964; $p<0.05$) and coexistence of *MEFV* exon 2 variants (odds ratio, 2.561; $p<0.05$).

Discussion

In this study, all patients with *MEFV* exon 2 or 3 variants had either E148Q and/or L110P variants in exon 2, which may be attributed to the association between the M694I mutation and exon 2 variants, as previously speculated (6). The clinical manifestations of FMF differ between populations, which has been attributed mainly to the differences in the mutational pattern of the *MEFV* gene (5, 7, 8). Typical and atypical FMF are associated with *MEFV* exon 10 and non-exon 10 mutations, respectively (6, 8). Furthermore, the M694V mutations in *MEFV* exon 10,

Table II. Multiple regression analysis associated with age at onset, frequency of attack, and thoracic pain among patients with FMF who had a heterozygous *MEFV* exon 10 mutation.

End point		Variables	Odds ratio or coefficient (95% CI)	*p*-value
Age at onset (years)	Model 1	Male gender	1.836 (-0.418 to 4.090)	0.11
		Family history of FMF	-1.626 (-3.950 to 0.697)	0.17
		Coexistence of *MEFV* exon 2 variants	-2.570 (-0.012 to -5.128)	0.049
		Coexistence of autoimmune or autoinflammatory disease	4.247 (-1.125 to 9.619)	0.12
	Model 2	Male gender	1.799 (-0.473 to 4.070)	0.12
		Family history of FMF	-1.628 (-3.969 to 0.713)	0.17
		Coexistence of the E148Q variant	-1.884 (-4.536 to 0.768)	0.14
		Coexistence of the L110P variant	-0.886 (-3.563 to 1.791)	0.16
		Coexistence of autoimmune or autoinflammatory disease	4.106 (-1.316 to 9.529)	0.14
Frequency of attack (/month)		Age at onset (years)	0.0004 (-0.012 to 0.013)	0.95
		Male gender	0.078 (-0.068 to 0.225)	0.29
		Family history of FMF	0.002 (-0.150 to 0.153)	0.98
		Coexistence of *MEFV* exon 2 variants	0.159 (-0.009 to 0.327)	0.06
		Coexistence of autoimmune or autoinflammatory disease	-0.052 -0.427 to 0.323)	0.78
Thoracic pain		Age at onset (years)	0.964 (0.935 to 0.993)	0.02
		Male gender	0.660 (0.303 to 1.436)	0.29
		Family history of FMF	0.883 (0.398 to 1.957)	0.76
		Coexistence of *MEFV* exon 2 variants	2.561 (1.100 to 5.962)	0.03
		Coexistence of autoimmune or autoinflammatory disease	0.275 (0.043 to 1.737)	0.17

Values for age at onset or frequency of febrile attack are the coefficient (95% CI); values for thoracic pain are the odds ratio (95% confidence interval [95% CI]).
FMF: familial Mediterranean fever; *MEFV*: Mediterranean fever gene.

which are known to have high penetrance, are associated with an earlier onset and more severe phenotypes of FMF (9, 10), suggesting an association among high penetrance, earlier onset, and severe phenotypes of FMF.

The clinical manifestations of FMF may differ among carriers of homozygous, heterozygous, and compound heterozygous mutations in the *MEFV* gene. A previous study showed that homozygous *MEFV* mutations contributed to an earlier onset and a more severe disease phenotype compared with heterozygous mutations (11). Although the present study showed that the coexistence of *MEFV* exon 2 variants was associated with an earlier onset and a higher frequency of attack, suggesting a more severe phenotype among patients with FMF who had a heterozygous *MEFV* exon 10 mutation, a total of 11 Japanese patients with FMF with homozygous M694I mutations (based on a nationwide multicentre prospective data used in the present study) tended to have an earlier onset [median (interquartile range), 14.0 (10.0–18.0) years] and a higher frequency of febrile attack [median (interquartile range), 1.0 (1.0–2.0) per month] (not shown) compared with patients with FMF carrying a heterozygous M694I mutation and coexistence of exon 2 variants in the *MEFV*. Considering these results, the sum of the penetrance of each *MEFV* mutation might be associated with an earlier onset, more severe phenotypes of FMF, and overall penetrance. To supplement, our previous study showed that the presence of *MEFV* exon 10 mutations and an earlier onset were significantly associated with serositis (1), which was consistent with the present study showing that not only the coexistence of *MEFV* exon 2 variants but also the earlier onset was independently and significantly associated with thoracic pain.

The serum levels of IL-17 and IL-18 were significantly higher in patients with FMF than they were in healthy controls, not only during the attacks but also during remission, which suggests the contribution of these cytokines to subclinical inflammation (12-14). In particular, the serum IL-18 levels in remission were higher in patients with FMF with a *MEFV* exon 10 mutation than they were in patients with FMF without a *MEFV* exon 10 mutation (13). The present study showed that the coexistence of *MEFV* exon 2 variants boosted serum IL-18 levels in remission among patients with FMF that had a heterozygous *MEFV* exon 10 mutation. Therefore, considering these cytokines, it is suggested that the coexistence of *MEFV* exon 2 variants may have additional effects on more severe phenotypes and subclinical inflammation in patients with FMF with a *MEFV* exon 10 mutation.

This study had several limitations. First, the small size of the cohort data of patients without the coexistence of *MEFV* exon 2 or 3 variants used in the present study suggests the need to analyse a larger number of patients. Second, IL-18 inhibitors have not been shown to be effective in patients with FMF, and the role of IL-18 in the development of FMF has not been elucidated. Thus, the clinical significance of the finding that the coexistence of *MEFV* exon 2 variants increased serum IL-18 levels in remission needs to be investigated further.

Conclusion

Our study suggests that the coexistence of *MEFV* exon 2 variants has additional effects on the manifestations of FMF in patients with *MEFV* exon 10 mutations.

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