J. Tokyo Med. Univ., **80**(3) : 202-210, 2022

Cytokine profiling and *MEFV* gene analysis of Japanese patients with periodic fever, aphthous stomatitis, pharyngitis, and adenitis syndrome

Junya SAISHO, Shinji SUZUKI, Shigeo NISHIMATA, Gaku YAMANAKA, Yasuyo KASHIWAGI, Hisashi KAWASHIMA

Department of Pediatrics and Adolescent Medicine, Tokyo Medical University

ABSTRACT

Introduction. Periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) syndrome is an autoinflammatory disease of unknown etiology. In recent years, mutations in the *MEFV* gene, which is the causative gene for familial Mediterranean fever (FMF), have been identified in patients with PFAPA, and the efficacy of colchicine as a therapeutic agent has been reported.

Objective. To elucidate the pathology and efficacy of new therapeutic methods, we analyzed the clinical data and genetic analysis data of PFAPA patients, together with the therapeutic effects.

Subjects and Methods. Ten patients (6 boys and 4 girls) from 1 to 8 years of age, who were diagnosed as having PFAPA syndrome were analyzed. Cytokine profiling and genomic study were performed on blood samples from patients with PFAPA and FMF.

Results. Serum levels of Interleukin (IL)-6 and Interferon-γ increased, but the levels of anti-inflammatory cytokines IL-4 did not increase in PFAPA patients. Similar cytokine profiles were found in patients with FMF. There was no statistical difference in the levels of these molecules between patients with FMF and PFAPA. Mutations in exon 2 and 3 of the *MEFV* gene were identified in 4 out of the 7 PFAPA patients. All patients were treated with histamine H2 receptor antagonists, but in 5 of the patients it was necessary to add colchicine, and in 2 patients the treatment was changed to colchicine due to ineffectiveness.

Conclusions. Treatment of colchicine prolongs the afebrile period in patients with PFAPA, irrespective of whether patients have *MEFV* mutations. From reports in the literature and results of our studies, colchicine can be recommended for patients who are resistant to other treatments, irrespective of whether patients have *MEFV* mutations.

Introduction

Periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) syndrome in which patients demonstrate characteristic symptoms of fever, aphthous stomatitis, pharyngitis, and cervical lymphadenitis, is an autoinflammatory diseases with unknown etiology[1,2]. It was first described by Marshall *et al.* in 12 pediatric patients [1]. The fever is accompanied by chills and malaise, begins abruptly and lasts for 3 to 6 days, with temperatures of approximately 40 to 41°C. Similar episodes of clinical symptoms are repeated every 2 to 8 weeks. Treatment with antipyretics and antibiotics are not effective. The administration of oral steroids, cimetidine, and tonsillec-

Received October 4, 2021, Accepted May 9, 2022
Key words : PFAPA syndrome, familial Mediterranean fever, *MEFV*, IL-6, IL-4, colchicine, cimetidine
Corresponding author : Junya Saisho, M.D.
Department of Pediatrics and Adolescent Medicine, Tokyo Medical University
6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan
TEL : +81-3-3342-6111 FAX : +81-3-3344-0643 E-mail : zephyranthes_jsaisho@yahoo.co.jp

tomy have been reported to be partially effective[2-4]. Therefore, the effectiveness and indications of these treatments remain unclear. In recent years, mutations in the *MEFV* gene, which is the causative gene for familial Mediterranean fever (FMF), have been identified in patients with PFAPA syndrome, and the efficacy of colchicine to control the flares in PFAPA patients has been reported[5-7]. Therefore, to elucidate the pathological mechanism and the effectiveness of new therapeutic methods against PFAPA, we retrospectively investigated the clinical data and genetic analysis data of patients, as well as the therapeutic effects of various agents.

Subjects and Methods

The subjects were 10 patients (6 boys and 4 girls) from 1 to 8 years of age, who were diagnosed with PFAPA syndrome (compatible to Thomas's Criteria[8]) and were being treated at the Department of Pediatrics at Tokyo Medical University between 2014 and 2016. Five pediatric patients with FMF were also analyzed. The diagnosis of FMF was performed in accordance with Tel-Hashomer criteria[9] and the published guidelines[10]. The following 27 cytokines and chemokines were measured using the Bio-Plex Multiplex Cytokine Assay System and Bio-Plex Pro Human Cytokine 27-plex Assay : platelet-derived growth factor (PDGF)-BB, interleukin (IL)-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-13, IL-15, and IL-17, eotaxin, fibroblast growth factor (FGF) granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor, interferon (IFN)-, IP-10, monocyte chemoattractant protein-1, macrophage inflammatory protein (MIP)-1a, MIP-1β, RANTES, tumor necrosis factor (TNF)-α, and vascular endothelial growth factor (VEGF) (Bio-Rad Laboratories, Tokyo, Japan). Lymphocytes were collected from whole blood, DNA was extracted, and the *MEFV* gene was analyzed for mutations that have been reported in FMF patients[11,12]. In addition, clinical symptoms were retrospectively analyzed from medical records. Statistical analysis was performed using IBM® SPSS® Statistics Version 25.0 software. A multiple factor analysis was performed.

Results

We retrospectively analyzed the medical records of 10 patients who were followed in the Department of Pediatrics at Tokyo Medical University Hospital because of periodic fevers and/or aphthous stomatitis. Mutations in exons 2 and 3 of the *MEFV* gene were observed in 4 out of 7 patients who underwent genetic testing (L110P, E148Q, R408Q, and P369S). The patients showed periodic fever at intervals of approximately 4 weeks. During most of those periods, their temperature rose to approximately 39°C in the first 2 to 3 days, followed by a lower temperature of 37°C for the next 2 to 3 days. The febrile episodes were accompanied by severe aphthous stomatitis, malaise, and pharyngitis. In addition, some of the patients sometimes complained of headache, abdominal pain, and diarrhea during the febrile episodes (Table 1). Their laboratory data showed no abnormalities during the afebrile period. However, all patients developed leukocytosis and had increased C-reactive protein levels during the febrile period, as shown in Table 1. Levels of interleukin IL-6 and serum amyloid-A also increased during the febrile periods. Autoantibodies were negative in all patients.

Cytokine profiling

We performed cytokine profiling in the sera obtained from 6 patients with PFAPA and 4 patients with *MEFV* gene mutation, and the results are shown in Figure 1. In PFAPA patients during the febrile period, levels of IL-6 and IFN-γ, which are inflammatory cytokines, increased, but not those of the anti-inflammatory cytokines IL-4 and IL-10. In 3 patients, serum cytokines were analyzed on the first febrile day (Figure 2). Serum levels of inflammatory cytokines and chemokines increased, as well as levels of PDGF and VEGF. Similar cytokine profiles were found in patients with FMF, which is shown in Figure 3. There was no statistical difference between the results of patients with FMF and PFAPA, which are shown in Figures 3 and 4. Multivariate analysis identified 4 statistically significant factors. One major factor represented more than 10 cytokines and chemokines (PDGF-bb, IL-1ra, IL-2, IL-10, IL-15, IL-17, FGFb, MIP-1a, RANTES, and TNF-α), and the second factor represented more than 5 cytokines and chemokines (IL-6, IFN-γ, VEGF, and others). The third factor represented mainly IL-9, and the fourth factor represented mainly IL-8. The 2 major detected factors obtained from both patients with PFAPA and FMF were divided into different categories. There were no common characteristics between patients with PFAPA and FMF, as shown in Figure 5. Treatment with antibiotics and nonsteroidal anti-inflammatory drugs did not shorten the duration of the febrile periods in any of the patients. Between the febrile periods, the patients were all healthy and could go to kindergarten or school. All patients were treated with histamine H2 receptor antagonists, but in 5 of the patients it was necessary to add colchicine, and in 2 patients the treatment was changed to colchicine. In the 2 patients treated with colchicine, the antipyretic period became longer than when histamine H2 receptor antagonists alone was administered. However, there was no correlation between patients who showed favorable therapeutic effects of colchicine and positivity of *MEFV* mutations, as shown in Table 2.

Table 1 Characteristics of patients with PFAPA

	Patient no.	1	2	3	4	5	6	7	8	9	10
	Age of onset (years)	2	4	8	4	5	1	5	6	1	2
	Sex	F	M	M	M	F	F	M	M	F	M
	Other diseases	(−)	(※)	(−)	(−)	(−)	(−)	(−)	VATER syndrome (※)	(※)	(※)
Clinical symptoms	Periodic fever	+	+	+	+	+	+	+	+	+	+
	Aphthous stomatitis	+	+	+	+	−	−	+	−	−	+
	Pharyngitis and adenitis	+	+	+	+	+	+	−	+	+	+
	Headache	−	+	−	−	−	−	−	−	−	−
	Cervical lymphadenopathy	−	+	+	−	+	+	+	+	+	+
	Nausea/vomiting	−	−	−	−	−	−	−	−	−	−
	Abdominal pain	−	−	+	−	−	−	−	−	−	+
Laboratory data	IgG (mg/dL)	765	881	1,110	1,329	1,006	1,204	826	1,121	719	1,241
	IgA (mg/dL)	46	109	207	337	103	154	119	160	29	146
	IgM (mg/dL)	93	115	37	35	290	72	174	91	91	98
	IgE (IU/mL)	ND	ND	ND	756.1	ND	ND	ND	ND	ND	344.6
	IgD (mg/dL)	30.9	34	10.7	29.5	ND	5.3	2.5	9.9	5.8	2.1
	CH50 (U/mL)	55	40	70	94	ND	ND	ND	ND	ND	ND
	SAA (μg/mL)	468	≦ 2.5	1,350	ND	45.5	47.4	4.7	53.6	455	38.6
	CRP (mg/dl)	7.7	0.03	3.05	12.11	0.46	0.9	< 0.3	0.71	8.9	0.5

SAA : serum amyloid A ND : not determined ※ : match the FMF classification criteria

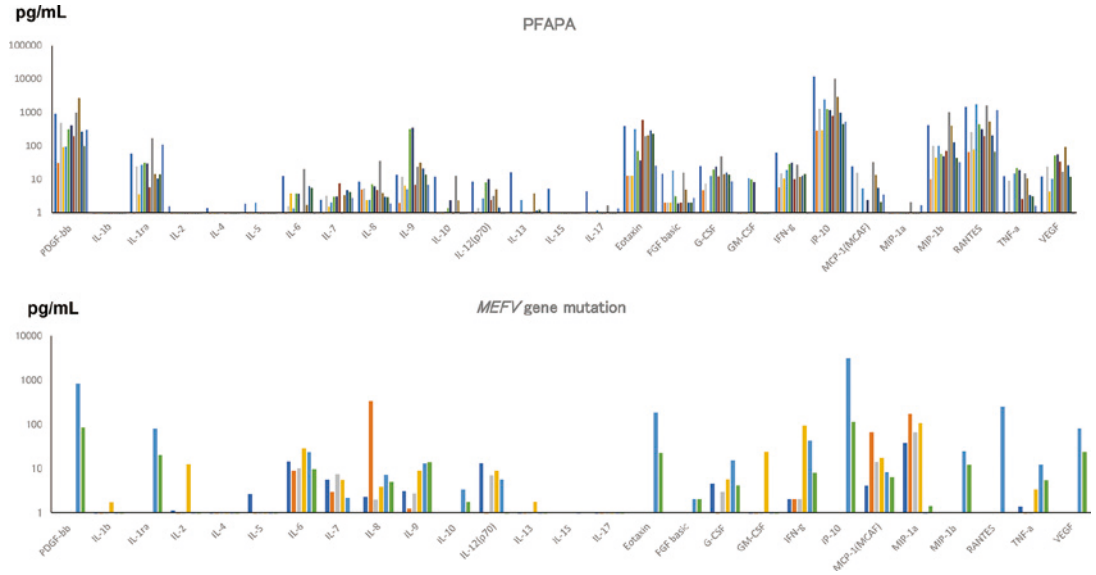

Fig. 1 Serum cytokines in PFAPA patients (13 febrile episodes) on febrile days and *MEFV* gene mutation patients (6 febrile episodes)
Both patients with PFAPA and *MEFV* gene mutation showed similar cytokine profiles.

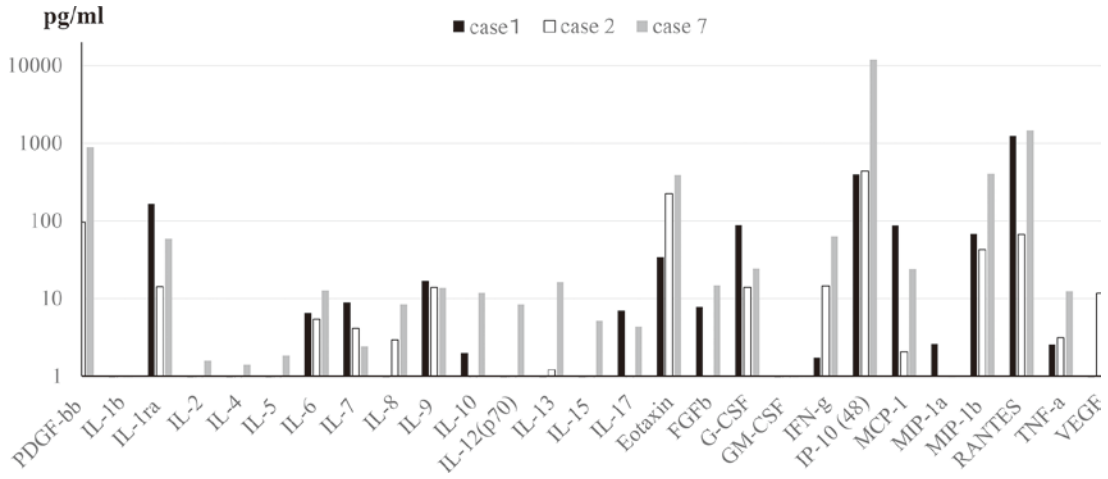

Fig. 2 Serum cytokine profiling in PFAPA patients on the first febrile day
Serum levels of inflammatory cytokines and chemokines increased, as well as the levels of PDGF and VEGF in PFAPA patients on febrile day l. The levels of IL-6 and IFN-γ increased, but not those of anti-inflammatory cytokines IL-4.

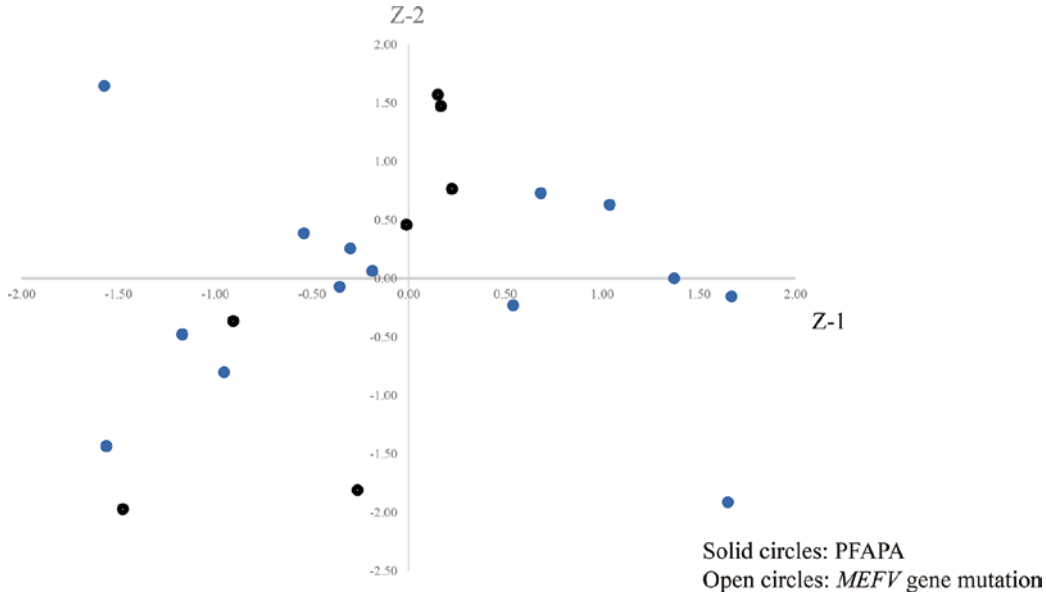

Fig. 3 Comparison of serum cytokines in PFAPA and *MEFV* gene mutation patients
There was no statistical difference between the representative cytokine results (IL-1ra, IL-6, IL-8, IL-10, IFN- γ and TNF-α) of patients with *MEFV* gene mutation and PFAPA.

DISCUSSION

The characteristics of PFAPA syndrome may clinically resemble autoinflammatory diseases, but their etiology is not fully understood. The syndrome is characterized by periodic fevers associated with aphthous stomatitis, pharyngitis and cervical adenitis, which is different from systematic arthritis and Beçhet disease. We previously reported the first Japanese case of PFAPA syndrome[13]. In this previous report, we described the case of an 11-year-old boy who had experienced periodic fevers associated with aphthous stomatitis since the age of 3 months. His symptoms were compatible with those of PFAPA syndrome, and he was diagnosed as having PFAPA syndrome in 2001. After our initial report, many Japanese patients with PFAPA have been reported, and it is no longer a rare disease. Symptoms of PFAPA syndrome usually last an average of 4.5 years. It is crucial to understand the pathophysiology of this disease and methods to treatment these children, who are going through developmental processes both physically and mentally.

Cytokine analysis

We hence performed cytokine analysis in this study towards understanding the pathophysiology. In the present study, the levels of the inflammatory cytokines

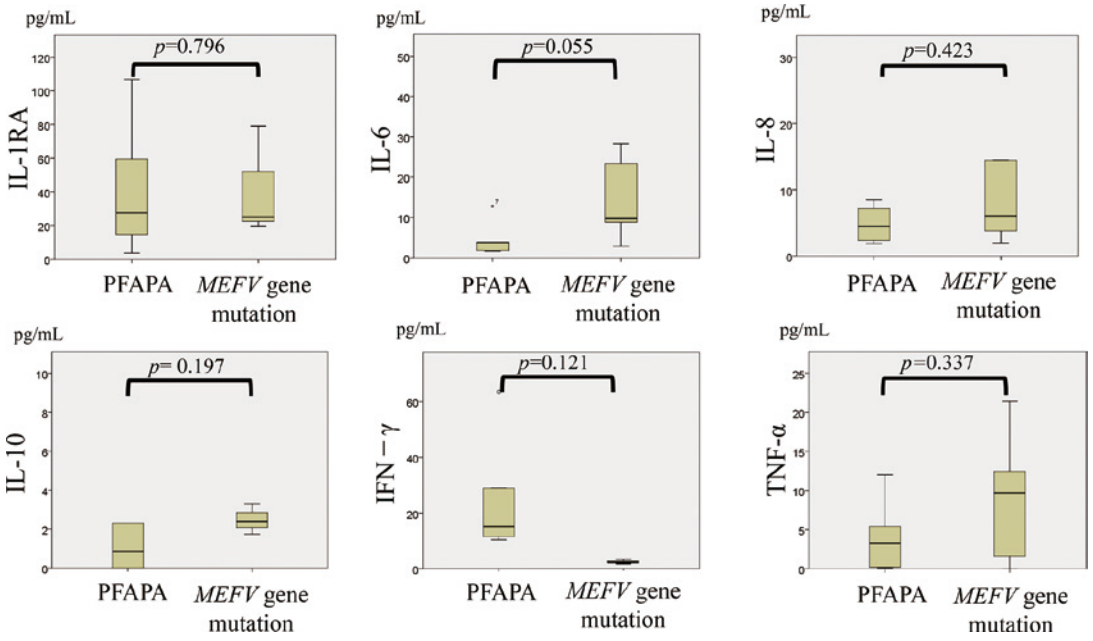

Fig. 4 Comparison of cytokines and chemokine levels between PFAPA and *MEFV* gene mutation patients
By multivariate analysis one first major factor (Z-1) represented more than 10 cytokines and chemokines (PDGF-bb, IL-1ra, IL-2, IL-10, IL-15, IL-17, FGF basic, MIP-1a, RANTES, and TNF-α), and the second factor (Z-2) represented more than 5 cytokines and chemokines (IL-6, IFN-γ, VEGF, and others). The 2 major detected factors (Z-1 and Z-2) obtained from patients with PFAPA and *MEFV* gene mutation were divided into different categories.

Table 2 Characteristics and mutations of PFAPA patients with *MEFV* mutations

	Patient no.	1	2	3	4	5	6	7	8	9	10
Treatment	Acetaminophen, NSAIDs, antibiotics	No effect	No effect	No effect	No effect	No effect	No effect	No effect	No effect	No effect	No effect
	Colchicine	Effective	*1	NT	NT	NT	*2	*3	NT	no effect	NT
	Cimetidine	Effective	Effective	Effective	Effective	Effective	(–)	Effective	Effective	*1	No effect
	Oral steroids	Effective	NT	NT	NT	NT	NT	NT	NT	Effective	NT
MEFV mutation	*Exon 1*	(–)	(–)	(–)	Unknown	Unknown	Unknown	(–)	(–)	(–)	(–)
	Exon 2	(–)	**L110P****	(–)	Unknown	Unknown	Unknown	(–)	**E148Q****	(–)	**E148Q ****
	Exon 3	(–)	(–)	(–)	Unknown	Unknown	Unknown	(–)	(–)	**R408Q**** **P369S****	(–)
	Exon 5	(–)	(–)	(–)	Unknown	Unknown	Unknown	(–)	(–)	(–)	(–)
	Exon 10	(–)	(–)	(–)	Unknown	Unknown	Unknown	(–)	(–)	(–)	(–)

Effective : shortening of febrile period or prolongation of interval
*1 : effective initially but not after, *2 : undetermined, *3 : effective for aphthous stomatitis
NT : not performed, ** : heteromutation

IL-1ra, IL-6, and IFN-γ were increased, whereas those of the anti-inflammatory cytokines IL-4 and IL-10 were not. Stojanov et al. assayed serum and intracellular cytokine levels in 6 patients with PFAPA during the symptom-free period, as well as 6 to 12 hours and 18 to 24 hours after fever onset. Febrile PFAPA attacks led to a significant increase in serum levels of IL-6 and IFN-γ compared with during the symptom-free period and controls, with IL-1*β*, TNF-*α*, and IL-12(p70) levels being significantly higher than in controls[14]. These data were consistent with our present data. They reported that serum anti-inflammatory IL-4 levels were lower at all times in PFAPA patients during the entire periods of PFAPA progression compared with controls, with no differences in serum IL-10 levels. Their data and our data demonstrating inflammatory cytokine activation and a reduced anti-inflammatory response suggest dysregulation of the immune response in patients with PFAPA

syndrome. A similar phenomenon in the tonsils of PFAPA patients has been reported[16]. Valenzuela, et al. reported that IL-4 gene expression in the tonsils was lower in PFAPA patients than in the controls. Three of the 9 patients had recurrent episodes of aphthous stomatitis without fever after tonsillectomy[16]. IL-4 cytokine gene expression in the tonsils was lower in the PFAPA patients. These results suggest a potential pathogenic mechanism based on inhibition of Th2 responses in PFAPA syndrome. Stojanov et al. found that complements, IL-1-associated genes and IFN-induced genes were significantly overexpressed, but T cell-associated transcripts (CD3 and CD8B) were downregulated in the serum of patients during PFAPA attacks. In addition, PFAPA flares were accompanied by significantly increased serum levels of chemokines for activated T-lymphocytes (IP-10/CXCL10 and MIG/CXCL9), G-CSF, and proinflammatory cytokines (IL-18, IL-6). They also treated 5 PFAPA patients with a recombinant IL-1 receptor antagonist. All patients showed a prompt clinical response and an increase in IP-10/CXCL10 level. They hence suggested that particular environments trigger the activation of complements and IL-1β/IL-18 during PFAPA flares, together with the induction of Th1-chemokines and the subsequent retention of activated T-cells in peripheral tissues[15]. IL-6 was found to induce immunological factors. We previously reported that $CD8^+$ T-cells were activated in the aphthous lesions of PFAPA patients[13]. Førsvoll et al. reported that activation of the innate immune response is the initial step in PFAPA, which is followed by a subsequent adaptive response with the activation and redistribution of T-cells. The levels of IL-6, CXCL10, and MIP-1α were significantly increased during febrile episodes, which was similar to our results, and the levels of CXCL10 remained high between febrile episodes. They showed that patients had relative eosinopenia and lymphocytopenia, with reduced numbers of both $CD4^+$ and $CD8^+$ T-cells during the febrile episodes[17]. Serum IL-6 increased in patients. IL-6 is known to be a B-cell-stimulating factor, which is present at high levels in many inflammatory diseases[18]. Cimetidine is known to be as effective as histamine H_2 receptor antagonists, and is an immune modulator of T-cells possessing suppressive functions. PFAPA may hence be caused by abnormalities of suppressor T-cells. Further studies focusing on T-cell differentiation are required to clarify these points. PFAPA syndrome is categorized into the autoinflammatory diseases, which mainly involve the inflammasome. This syndrome might also be correlated with the inflammasome, as patients demonstrated an increase in cytokine levels.

Comparison with FMF

We analyzed serum cytokines obtained from children with FMF, which is a representative autoinflammatory disease. There were no differences in cytokine levels between patients with PFAPA and FMF. *MEFV* is the gene that regulates cryopyrin. The pathophysiology of PFAPA might be associated with activation of the inflammasome, similar to FMF. Family histories have not been investigated in PFAPA syndrome, but hereditary factors may be involved. Kubota et al. performed DNA sequencing analysis of genes associated with autoinflammatory disorders, such as *MEFV, MVK*, *NLRP3*, and *TNFRSF1A* and serum cytokines. Heterozygous *MEFV* variants were detected in 4 out of 7 patients. Serum TNF-α and IL-18 levels were increased during both the attack-free and attack periods compared with the controls. IL-1β, IL-1ra, IL-6, and sTNFR1 only increased during the attack period. Oral prednisolone was administered to these patients, which immediately reduced fever in most patients. They concluded that Japanese PFAPA syndrome patients have cytokine dysregulation as a result of genetic variants of autoinflammatory disorder-associated genes. In recent years, the presence of variants in inflammasome-associated genes have been identified, mostly in *NLRP3* and MEFV, suggesting a possible role of inflammasome-associated genes in the pathogenesis of PFAPA. However, none of these variants appeared to be relevant to PFAPA indicating the high genetic heterogeneity of inflammatory diseases[19].

MEFV mutations FMF is characterized by periodic attacks of high fever, abdominal or chest pain, and intense inflammatory reactions. Colchicine is the drug of first choice for FMF, and shows substantial therapeutic effects in most patients. FMF is caused by mutations in the *MEFV* gene. In Japanese patients with typical symptoms, the M694I mutation within exon 10 is frequently found. In contrast, mutations within exon 2 (E148Q) or exon 3 (P369S and R408Q) are associated with patients who show atypical clinical manifestations[10]. In this study, we were unable to find a correlation between *MEFV* mutations and clinical symptoms, particularly regarding a patient's treatment response to colchicine. Gunes et al. reported that heterozygous mutations in the *MEFV* gene were identified in 57 out of 231 FMF patients (24.7%), and they demonstrated the efficacy of colchicine therapy. In the colchicine group, the interval between episodes was prolonged from 18.8 ± 7.9 days to 49.5 ± 17.6 days by prophylactic colchicine therapy ; furthermore, prophylactic treatment was more effective in reducing episode frequency in patients with *MEFV* gene variants than in those without treatment[5]. It is particularly important to assess and to demonstrate the high response rate to colchicine prophylaxis in PFAPA syndrome patients, particularly those with *MEFV* variants. Dusser et al. also reported the efficacy of col-

chicine as a prophylactic treatment for PFAPA syndrome. Heterozygous *MEFV* gene mutations tended to be more frequent in the responders group. Although not significant, colchicine treatment appeared more effective in patients with less complete PFAPA phenotypes and *MEFV* heterozygosity[6]. However, there has also been a negative report regarding the correlation between *MEFV* mutations and PFAPA. Celiksoy et al. evaluated 64 patients with PFAPA syndrome, and an *MEFV* gene mutation was found in 42 (66.0%) of the children. Heterozygous or compound heterozygous variants of the *MEFV* gene were most frequently found. No significant differences in clinical or laboratory findings were observed between the 2 groups, and there were no significant differences in the period or duration of the fever episodes. Eighteen of the patients using prednisolone received colchicine as a prophylactic treatment, and fever episodes occurred in only 9/18 (50.0%) of the patients, which further decreased within a few months of colchicine treatment[7]. In our present study, we could not find a correlation between positive and negative mutations of *MEFV.*

QOL of PFAPA patients

Thomas et al. described the presentation, clinical course, therapeutic responses, and long-term follow-up of patients with PFAPA syndrome[8]. PFAPA episodes lasted an average of 4.8 days (95% confidence interval [CI] : 4.5 to 5.1 days) and recurred every 28 days (95% CI : 26 to 30 days), with a maximum temperature of 40.5ºC (95% CI : 40.4ºC to 40.6ºC). Although the affected children had no long-term sequelae, febrile episodes reduced their QOL because of their absence from school and inappropriate treatments. Glucocorticoids were highly effective in controlling symptoms. Therapy with cimetidine or prednisone has been reported to be effective in some patients by Marshall *et al.* and Feder[1,4]. However, the effects of these drugs have remained controversial to date. It is hence necessary to collect more data to determine the appropriate therapy for PFAPA syndrome. Recent reports have suggested that PFAPA syndrome does not require any therapy, because all symptoms resolve naturally[21]. However, the patient in our previous report had not received any effective treatment until 11 years of age, and during that period, he was absent from school for approximately 1 week every 2–4 weeks because of fever, malaise, and aphthous stomatitis. From reports in the literature and our studies, colchicine can be recommended for patients who are resistant to other treatments, except patients with mutations in *MEFV*. Finally, other possibilities involved in the etiology of PFAPA include abnormalities of neutrophils or histiocytes and an allergic response[21]. Histopathological analysis of the oral ulcerative lesions demonstrated that the ulcerations were covered by a fibrinopurulent substance, composed of interstitial and perivascular infiltrates of inflammatory cells, predominantly of histiocytes and scattered neutrophils[13]. Moreover, colchicine is known to positively affect the function of neutrophils. Further research on phagocytes may also be useful.

Conclusions

From reports in the literature and result of our studies, colchicine can be recommended for patients who are resistant to other treatments, irrespective of whether patients had *MEFV* mutations.

Limitations

The study has several limitations. First, the data may be unreliable due to the small number of cases considered in this study. Secondly, the number of specimens analyzed in each case varies, and there is no uniformity in testing time, which can lead to errors. We hope to overcome these limitations by conducting future prospective studies.

Acknowledgement

We would like to express our deep gratitude to the pediatric adolescent science laboratory of Tokyo Medical University for their cooperation in the analysis of MEFV genes and cytokines.

Conflict of interest

The authors declare no conflicts of interest.

Ethical considerations

This study was approved by the Ethics Review Board of Tokyo Medical University (study approval no. : SH3842). Blood samples were collected form all patients after obtaining informed consent from the parents at time of patient's admission.

Author contributions

JS and HK designed the study ; SS, SN, and YK performed the experiments, and collected and analyzed the data ; JS wrote the manuscript ; GY provided technical support and conceptual advice. All authors read and approved the final manuscript.

Compliance with ethical standards

The authors declare that they have no conflict of interest.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

Funding

This study was not funded by any grant except for an institutional research funding for our department.

References

1) Marshall GS, Edwards KM, Buler J, Lawton AR : Syndrome of pediatrics fever, pharyngitis, and aphthous stomatitis. J Pediatr **110** : 43-46, 1987
2) Marshall GS, Edwards KM, Lawton AR : PFAPA syndrome. Pediatr Infect Dis J **8** : 658-659, 1989
3) Feder HM, Bialechi CA : Periodic fever associated with aphthous stomatitis, pharyngitis and cervical adenitis. Pediatr. Infect. Dis J. **8** : 186-187, 1989
4) Feder HM : Cimetidine treatment for periodic fever associated with aphthous stomatitis, pharyngitis and cervical adenitis. Pediatr Infect Dis J **11** : 318-321, 1992
5) Gunes M, Cekic S, Kilic SS : Is colchicine more effective to prevent periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis episodes in Mediterranean fever gene variants?. Pediatr Int **59** : 655-660, 2017
6) Perrine Dusser, Véronique Hentgen, Bénédicte Neven, Isabelle Koné-Paut : Is colchicine an effective treatment in periodic fever, aphtous stomatitis, pharyngitis, cervical adenitis (PFAPA) syndrome? Joint Bone Spine **83** : 406-411, 2016
7) Mehmet H Celiksoy, Gonul Ogur, Elif Yaman, Ummet Abur, Semanur Fazla, Recep Sancak, Alisan Yildiran : Could familial Mediterranean fever gene mutations be related to PFAPA syndrome? Pediatr Allergy Immunol. **27** : 78-82, 2016
8) Thomas KT, Feder HM Jr, Lawton AR, Edwards KM : Periodic fever syndrome in children. J Pediatr **135**(1) : 15-21, 1999
9) V P Papadopoulos, S Giaglis, I Mitroulis, K Ritis : The population genetics of familial mediterranean fever : a meta-analysis study. Ann Hum Genet **72**(Pt 6) : 752-761, 2008
10) Yachie A : Familial Mediterranean fever/inflammatory diseases associated with MEFV mutations. Nihon Rinsho 76 : 1763-1769, 2018 (In Japanese)
11) Kiyoshi Migita, Yasumori Izumi, Yuka Jiuchi, Nozomi Iwanaga, Chieko Kawahara, Kazunaga Agematsu, Akihiro Yachie, Junya Masumoto, Keita Fujikawa, Satoshi Yamasaki, Tadashi Nakamura, Yoshifumi Ubara, Tomohiro Koga, Yoshikazu Nakashima, Toshimasa Shimizu, Masataka Umeda, Fumiaki Nonaka, Michio Yasunami, Katsumi Eguchi, Koh-Ichiro Yoshiura, Atsushi Kawakami : Familial Mediterranean fever is no longer a rare disease in Japan. Arthritis Res Ther **18** : 175, 2016
12) Kishida D, Nakamura A, Yazaki M, et al : Genotype-phenotype correlation in Japanese patients with familial Mediterranean fever : differences in genotype and clinical features between Japanese and Mediterranean populations. Arthritis Res Ther **16** : 439, 2014, doi : 10.1186/s13075-014-0439-7.
13) Kawashima H, Nishimata S, Shimizu T, Kashiwagi Y, Takekuma K, Hoshika A : Highly suspected case of FAPA (periodic fever, aphthous stomatitis, pharyngitis and adenitis) syndrome Pediatr Int. **43** : 103-106, 2001
14) Silvia Stojanov, Florian Hoffmann, Anja Kéry, Ellen D Renner, Dominik Hartl, Lohse P, Huss K, Fraunberger P, D Malley J, Zellerer S, Albert MH, Belohradsky BH : Cytokine profile in PFAPA syndrome suggests continuous inflammation and reduced anti-inflammatory response. Eur Cytokine Netw **17** : 90-97, 2006
15) Valenzuela PM, Araya A, Pérez CI, Maul X, Serrano C, Beltrán C, Harris PR, Talesnik E : Profile of inflammatory mediators in tonsils of patients with periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome. Clin Rheumatol **32** : 1743-1749, 2013
16) Stojanov S, Lapidus S, Chitkara P, Feder H, Salazar JC, Fleisher TA, Brown MR, Edwards KM, Ward MM, Colbert RA, Sun H-W, Wood GM, Barham BK, Jones A, Aksentijevich I, Goldbach-Mansky R, Athreya B, Barron KS, Kastner, DL : Periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) is a disorder of innate immunity and Th1 activation responsive to IL-1 blockade. Proc Natl Acad Sci U S A. **108** : 7148-7153, 2011
17) Førsvoll J, Kristoffersen EK, Oymar K : Elevated levels of CXCL10 in the Periodic Fever, Aphthous stomatitis, Pharyngitis and cervical Adenitis syndrome (PFAPA) during and between febrile episodes ; an indication of a persistent activation of the innate immune system. Pediatr Rheumatol Online J **11** : 38, 2013, doi : 10.1186/1546-0096-11-38
18) Kishimoto T : The biology of interleukin-6. Blood 74 : 1-10, 1989
19) Kubota K, Ohnishi H, Teramoto T, Kawamoto N, Kasahara K, Ohara O, Kondo N : Clinical and genetic characterization of Japanese sporadic cases of periodic Fever, aphthous stomatitis, pharyngitis and adenitis syndrome from a single medical center in Japan. J Clin Immunol **34** : 584-593, 2014
20) Theodoropoulou K, Vanoni F, Hofer M : Periodic Fever, Aphthous Stomatitis, Pharyngitis, and Cervical Adenitis (PFAPA) Syndrome : a Review of the Pathogenesis. Curr Rheumatol Rep **18** : 18, 2016, doi : 10.1007/s11926-016-0567-y.
21) Schibler A, Birrer P, Vella S : PFAPA-Syndrome : Periodisches Fieber, adenitis, pharyngitis und aphthose stomatitis. Schweiz. Med. Wochenschr **127** : 1280-1284, 1997

本邦における PFAPA 症候群のサイトカインプロファイリングと MEFV 遺伝子解析

税所純也　鈴木慎二　西亦繁雄
山中岳　柏木保代　河島尚志

東京医科大学小児科・思春期科学分野

【要旨】

【はじめに】 周期的な発熱、アフタ性口内炎、咽頭炎、および頚部リンパ節炎（PFAPA）症候群は、病因が明らかとなっていない自己炎症性疾患である。 近年、家族性地中海熱（FMF）の原因遺伝子である *MEFV* 遺伝子の変異が PFAPA 症候群の患者で確認されており、治療薬としてのコルヒチンの有効性が報告されている。

【対象と方法】 PFAPA 症候群と診断された 1 歳から 8 歳までの 10 人の患者（男児 6 人と女児 4 人）を対象とし、サイトカインプロファイリングと遺伝子解析は、PFAPA 症候群と FMF の患者から採取した血液で実施した。

【結　　果】 インターロイキン（IL）— 6 およびインターフェロン— γ の血清値は上昇したが、PFAPA 症候群の患者では抗炎症性サイトカイン IL-4 の値は上昇を認めなかった。同様のサイトカインプロファイルが FMF の患者に認められた。FMF と PFAPA 症候群の患者間で統計的差異は見られなかった。*MEFV* 遺伝子のエクソン 2 および 3 の変異は、7 人の PFAPA 患者のうち 4 人で同定された。すべての患者でヒスタミン H2 受容体拮抗薬の治療が行われたが、5 人の患者ではコルヒチンを追加する必要があり、2 人の患者では効果がなかったため治療がコルヒチンへ変更された。

【考　　察】 コルヒチンでの治療は、患者が *MEFV* 変異を持っているかどうかに関係なく、PFAPA の患者の無熱期間を延長する。

〈キーワード〉 家族性地中海熱、MEFV、IL-6、IL-4、コルヒチン、シメチジン