Clinical features and new diagnostic criteria for the syndrome of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis

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Abstract

Aim: The syndrome of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) is a common inflammatory disease that presents with periodic fever. We aimed to establish more specific diagnostic criteria for PFAPA based on the clinical characteristics of PFAPA patients in our directory.

Method: The clinical, laboratory, genetic, and family history details of 257 Japanese PFAPA patients treated at our and other affiliated hospitals between April 2000 and April 2018 were analyzed along with quantitative measurements of the number of CD64 molecules on neutrophils, and the levels of serum inflammatory cytokines. The sensitivity and specificity of the criteria were calculated for several diseases.

Results: Because recurrent fevers were crucial findings, they were defined as the required criterion. Tonsillitis/pharyngitis with white moss were important accompanying signs. Other symptoms associated with febrile episodes were cervical lymphadenitis with tenderness, aphthous stomatitis, sore throat, vomiting, and headache but not cough. A total of 159 (62%) patients had a family history of recurrent fevers, indicating autosomal dominant inheritance. C-reactive protein levels were extremely elevated during febrile attacks but normal in attack-free periods. Serum immunoglobulin D levels were high in 72 of the 199 tested patients. Oral glucocorticoid and cimetidine were extremely effective in all and 51.6% of the patients, respectively. We defined the above as supportive criteria. These criteria were sensitive and specific enough to distinguish PFAPA from other recurrent fever diseases. Raised serum interferon-γ levels and remarkable CD64 expression on neutrophils during flare-ups were recognized, indicating they contributed to diagnosis.

Conclusion: Our new criteria are useful for diagnosing PFAPA.

KEYWORDS

cytokine, diagnostic criteria, periodic fever, PFAPA, tonsillitis

1 | INTRODUCTION

The syndrome of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) is the most common autoinflammatory fever disorder during childhood.[1-3] PFAPA was first described by Drs. Gary Marshall, Alexander Lawton, and colleagues in 1987.[1] This clinical entity is characterized by regular occurrences of high fever (>39°C) that are associated with at least 1 of 3 cardinal clinical signs: aphthous stomatitis, pharyngitis, and cervical adenitis. Additional features, including headache, gastrointestinal symptoms, rash, and arthralgia, may be present[4] but are not consistently noted. Disease onset is generally before the age of 5 years, with attacks lasting 2-8 days (mean: 4 days) and recurring every 3-8 weeks.[4] The episodes recur very regularly with stereotyped clinical characteristics in the majority of patients, giving the impression that a "clockwork" mechanism in the episodes.[5] Patients are asymptomatic between episodes and experience normal growth and development. A Norwegian study of PFAPA reported an incidence of 2.3 per 10,000 children, although worldwide epidemiologic patterns have yet to be explored.[6] Although PFAPA is quite common in childhood, relapse cases have been reported to occur in adults after a temporary remission was reached at a pediatric age.[7]

The discovery of a genetic basis of autoinflammatory disorders has led to the inclusion of syndromes into groups of hereditary systemic autoinflammatory disorders. However, the precise cause of PFAPA is unknown.[2] Di Gioia et al reported that PFAPA appears to have a genetic basis with a pattern indicating autosomal dominance and incomplete penetrance, if a Mendelian disease is considered to have a penetrance factor of almost 50%.[8] Nonetheless, the gene responsible for PFAPA has not yet been identified, and its mode of inheritance remains controversial. Ryan et al observed that patients harboring exon 3 variants (heterozygous P369S and R408Q variants) in the Mediterranean fever (*MEFV*) gene who did not meet the Tel-Hashomer criteria showed PFAPA syndrome-like symptoms.[9] These *MEFV* gene mutations in PFAPA implicated a possible association with PFAPA and familial Mediterranean fever (FMF).[10]

The pathogenesis and diagnostic biomarkers of PFAPA are unknown. Increased levels of several circulating inflammatory cytokines, most notably interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β, IL-18, granulocyte-colony stimulating factor (G-CSF), and IL-12p70, occur during episodes,[11, 12] but not IL-1β levels.[13] In our study of Japanese patients, IL-1β and TNF-α levels were not clearly elevated during PFAPA febrile attacks, while IL-18, IL-6, G-CSF, and IFN-γ were all increased.[14] Remarkable expression of CD64, an Fcγ receptor I family members and neutrophil activation marker, have been observed during PFAPA flares and may also serve as a potential diagnostic biomarker.[14]

The diagnosis of PFAPA is typically made on clinical grounds. Marshall et al proposed diagnostic criteria for PFAPA in 1989[15] that were later modified by Thomas et al in 1999.[2] To increase diagnostic sensitivity and specificity, this study aimed to propose a new criteria for the diagnosis of PFAPA based on clinical characteristics, genetic background, laboratory findings, and therapeutic effects in a cohort of patients with PFAPA who were directly seen at our hospital or related institutions.

2 | PATIENTS AND METHODS

2.1 | Patients

We enrolled 257 consecutive patients with PFAPA who were treated at Shinshu University Hospital or other affiliated hospitals between April 2000 and April 2018. We selected patients who presented with recurrent fever over a long period and high levels of C-reactive protein (CRP) during flare-ups. We excluded patients with infections, malignancies, autoimmune diseases, or hereditary autoinflammatory diseases during long-term observation of the patients and appropriate gene analyses. In particular, hereditary autoinflammatory diseases, such as haploinsufficiency A20, were excluded by clinical findings and genetic analyses when we encountered a case of atypical PFAPA. Among these patients, the diagnosis of PFAPA was made by referring to the diagnostic criteria of Marshall et al[1] and Thomas et al[2] We referred to serum cytokine profiles and neutrophil activation during attacks in diagnostically difficult cases. This study was conducted according to the Declaration of Helsinki and approved by the

Ethics Committee of Shinshu University (No. 540). To compare PFAPA with infectious diseases that have similar clinical courses and symptoms, we examined 104 patients with infectious diseases, including tonsillitis, otitis media, pharyngitis, cystitis, and gastroenterocolitis with fever (over 1 time per year). We also compared PFAPA with a typical type of 67 FMF patients with the *MEFV* M694I mutation, as described by Migita et al[16, 17] We chose typical FMF and infectious diseases as controls because we could collect enough numbers of these diseases to compare them with PFAPA. Since other periodic fever diseases, such as malignancies, autoimmune diseases, or other autoinflammatory diseases, are very rare, we could not collect these diseases as controls for comparing with PFAPA.

2.2 | Mutation analyses

Heparinized blood was collected from patients for genetic analysis after informed consent was obtained. DNA was extracted from the samples using standard methods. Direct sequencing of the *MEFV* gene was performed using primers as previously reported.[17]

2.3 | Quantitative measurement of CD64 expression

Blood samples were collected using ethylenediaminetetraacetic acid-2Na tubes and kept at room temperature. Within 24 hours, 50 μL of whole blood aliquots were stained with anti-human antibodies against the pan-leukocyte marker CD45 (clone H130) conjugated with PerCP-Cy5.5 (BioLegend, San Diego, CA, USA), anti-human CD16 antibody (clone 3G8) conjugated with FITC (BioLegend), and anti-human CD64 antibody (clone 10.1) conjugated with phycoerythrin (PE) (BD Pharmingen). After the samples were incubated for 20 minutes in the dark at room temperature, we added 1 mL of BD Fluorescence-activated Cell Sorter (FACS) Lysing Solution (BD Biosciences). The samples were then incubated for another 20 min or stored at 4°C overnight. Neutrophil and monocyte populations were gated by side scatter and CD45 fluorescence intensity, and the geomean of CD64 was then assessed by flow cytometry using a FACSCalibur

cytometer (BD Biosciences). We constructed a standard curve using QuantiBRITE® PE beads (BD Biosciences) to calculate the number of CD64 molecules per cell.

2.4 | Cytokine assays

Serum samples were collected during patient flare-ups and analyzed to determine the concentrations of the cytokines IL-1β, IL-6, IL-8, TNF-α, IFN-γ, IFN-γ-induced protein 10 (IP-10), and monokine induced by gamma interferon (MIG) using Cytometric Bead Array Flex sets (BD Biosciences) according to the manufacturer's instructions. The lower limit of detection for each cytokine was 5 pg/mL. Serum IL-18 concentrations were determined by enzyme-linked immunosorbent assay using a commercially available kit (Human IL-18 Enzyme-Linked Immunoassay Kit; BML).

2.5 | Statistical Analyses

We constructed receiver operating characteristic (ROC) curves to determine the number of flare-ups in the required criteria as well as how many supportive diagnostic criteria items were needed to obtain the best balance of sensitivity and specificity.

3 | RESULTS

3.1 | Clinical characteristics

The clinical characteristics and laboratory findings of 257 PFAPA cases (143 male and 114 female) are summarized in Table 1. The mean age at onset was 2.7 ± 1.6 years. The vast majority (90.1%) of patients had an onset age of less than 5 years. The mean frequency of febrile episodes was every 1.2 ± 0.8 months (range: 0.5-9.0 months), and the mean duration of fevers was 4.5 ± 1.3 days (range: 2.0-7.9 days). The mean highest temperature reached during fever attacks was 39.7 ± 0.6°C. A total of 159 patients (62%) had at least 1 family member with PFAPA findings, such as recurrent fever, chronic tonsillitis, or tonsillectomy, as described in the genetic background. Tonsillitis or pharyngitis was present in 238 of 251 patients (94.8%). Among these, the tonsils

were coated with white moss in 163 of 226 patients (72.1%). Cervical lymphadenitis or aphthous stomatitis was observed in 147 of 252 patients (58.3%) and 130 of 251 patients (51.8%), respectively. Spontaneous pain and/or tenderness were recognized in the majority of cervical lymphadenitis patients. Other symptoms associated with febrile episodes were sore throat (45.4%), vomiting (21.4%), and headache (33.6%).

Mean white blood cell count, CRP level, and serum amyloid A (SAA) level found in patients during attacks were 12,500 ± 4,700/μL, 6.7 ± 4.6 mg/dL, and 669.2 ± 449.8 μg/mL, respectively, indicating strong inflammation during flare-ups. Serum immunoglobulin D (IgD) levels were elevated in 72 of 199 tested patients (36.2%) to a mean of 23.0 ± 12.2 mg/dL.

Of the 257 PFAPA patients, 95 were treated with cimetidine at a dose of 15-20 mg/kg/day twice daily. After beginning therapy, PFAPA attacks ceased in 49 patients (51.6%). Oral prednisolone (0.5 mg/kg/dose) was administered to 151 patients for febrile episodes and immediately reduced fever in all treated patients (100%). Tonsillectomy was performed in 29 patients and led to the disappearance of recurrent symptoms in 25 (86.2%). As far as we were able to pursue progress, in 21 of the 74 (28.4%) patients without cimetidine and/or tonsillectomy treatment, the recurring PFAPA attacks spontaneously stopped.

3.2 | Genetic background

One hundred and fifty-nine of 257 (62%) patients had a positive family history, that is having relative(s) with recurrent fever, tonsillitis with/without white moss, or tonsillectomy (Table 1). The overall incidence of affected families with PFAPA was 60%. We show the characteristics of the 148 affected families in Table 2. Inheritance from either the father's or mother's side was present in 85.1% of the 148 affected families (Figure 1A). Among these families, 73 PFAPA patients were found in the generation of the parent, aunt or uncle, 33 in three generations, or 20 in one skipped generation of parents, respectively (Figure 1A). Inheritance from both the father’s and the mother’s side were present in 12.8% of the 148 affected families (Figure 1B).

Twenty-one families were found to contain sibling cases (Figure 1C). There were 6 families in which 5 to 6 patients were identified (Figure 1D). Pedigree analysis clearly suggested inheritance compatible with an autosomal dominant with incomplete penetrance model, assuming Mendelian inheritance. On the basis of this model, we searched for pathogenic mutations that could underlie the biological mechanisms of PFAPA in the family shown in Figure 1D (left). However, whole-exome sequencing did not identify a common heterozygous mutation in the five affected family members (data not shown).

MEFV (E148Q-) P369S-R408Q substitutions, also called *MEFV* exon 3 variants, are associated with a highly variable phenotype that includes PFAPA.[9] We examined the *MEFV* gene sequences of patients who presented atypical symptoms, such as abdominal pain, and identified 6 families with members carrying exon 3 variants (Figure 1E), 1 of which was previously reported (Figure 1E, left).[18] Frequent E148Q substitutions in exon 2 have also been reported in PFAPA patients.[19, 20] In our cohort, heterozygous E148Q or R202Q substitutions were detected in exon 2 in 37.1% and 14.3% of 60 randomly selected patients, respectively (E148Q or R202Q were detected in 50 healthy controls at rates of 23% and 2.7%, respectively).

3.3 | Cytokine profiles and neutrophil/monocyte activation

Highly elevated serum levels of a variety of cytokines and chemokines during PFAPA flares have been described,[12, 13] including increases in inflammatory cytokine levels and the numbers of CD64 molecules on neutrophils and monocytes.[14] Analysis of serum IL-1β, IL-6, IL-8, IL-18, TNF-α, IFN-γ, G-CSF, IP-10, MIG and monocyte chemotactic protein-1 (MCP-1) levels showed no elevations in PFAPA remission state (data not shown) and in controls (Table 3). In contrast, IL-6, IL-8, IL-18, IFN-γ, G-CSF, IP-10, MIG, and MCP-1 levels were elevated during PFAPA attack periods (Table 3). In patients with bacterial infections, serum levels of IL-6 and G-CSF were elevated, but G-CSF levels were lower in these patients than in those with PFAPA flare-ups. Elevations

in IFN-γ, G-CSF, IP-10 and MIG were relatively specific to PFAPA flare-ups compared with those of bacterial infections.

The expression of CD64 on neutrophils and monocytes was remarkably increased in PFAPA attacks (Table 3). Interestingly, the number of CD16-positive monocytes, which are considered to be activated, [21] was higher during flare-ups than in healthy controls and patients with bacterial infections. There were large and significant differences in neutrophil CD64 expression between patients experiencing PFAPA flare-ups and those with bacterial infections.

3.4 | Criteria for diagnosis

Table 4 summarizes our diagnostic criteria for PFAPA, which were based on the data obtained in our patients, who were followed up directly for a long period of time. Regarding the required criteria, the majority of patients had recurrent fever of more than 38.0°C once every 0.5-3 months, with many of these fevers reaching 40°C. Recurrent fever was indeed the main finding in PFAPA. Since we considered a differential diagnosis from infections was to be of high importance, we examined the attack frequency of both diseases. The attack frequency was defined according to comparisons of infectious disease (n=104). At an attack frequency of at least 4 (or 3) times per year, the sensitivity and specificity were 98.0% (99.2%) and 80.8% (67.3%), respectively. When the cut-off value was 3, it was difficult to distinguish these diseases. The attack frequency is best at least 4 times to distinguish PFAPA and infectious diseases. We could also differentiate PFAPA from bacterial tonsillitis when using this attack frequency (data not shown).

Regarding the supportive criteria for PFAPA diagnosis, the vast majority (90.1%) of patients had an onset age of less than 5 years, consistent with Thomas' diagnostic criteria for PFAPA. Therefore, we added onset age to the supportive criteria in the new criteria. Of the 94.8% of patients with pharyngitis/tonsillitis, 72.1% were accompanied by white moss. As tonsillitis and/or pharyngitis with white moss appeared to be important characteristic evidence of PFAPA as 1 diagnostic item, we established them as a separate criterion from other concomitant symptoms. Other manifestations, such as

aphthous stomatitis, cervical lymphadenitis, sore throat, vomiting, and severe headache, did not necessarily accompany all cases (Table 1), but these findings were characteristically found in PFAPA patients. Since we considered these as important signs, we set them at least 1 being a criterion. The absence of cough in the majority of patients was deemed important for distinguishing PFAPA from upper respiratory infections. Hence, this was added as an item representing supportive criteria. Since 62% of our subjects had a family history of periodic fever, recurring tonsillitis, or tonsillectomy, a family history examination was considered useful for PFAPA diagnosis. CRP and/or SAA levels were high during febrile attacks but normal in attack-free periods. Since the transitions between these inflammation findings were important, we added these transitions as a supportive criterion. In addition, high levels of serum IgD exhibited relatively high specificity and the number of diseases associated with elevated serum IgD is limited. Thus, this finding represented a diagnostic marker in our criteria and was added as an item of supportive criteria. Finally, given that glucocorticoids were extremely effective in all our patients, a response to glucocorticoids was another key characteristic of PFAPA and was added as an item of supportive criteria.

To exclude FMF and infectious diseases that are similar to PFAPA, ROC plots were constructed to determine the optimal number of supportive criteria using a classification cohort of 104 infectious disease patients and 67 typical-type FMF patients with an *MEFV* M694I mutation. ROC curves showed that the optimal threshold for supportive criteria items was at least 4, which achieved 93.8% sensitivity and 94.2% specificity for PFAPA versus infectious diseases and 93.8% sensitivity and 95.6% specificity for PFAPA versus FMF (Figure 2). When the number was at least 5, specificity was 100% versus both diseases (Figure 2).

4 | DISCUSSION

We analyzed the clinical, laboratory, family history, and therapeutic characteristics of 257 PFAPA patients who were directly explored for a long period of time to propose additional required and supportive criteria for the more specific diagnosis of PFAPA. Recurrent fevers of more than 38.0°C that persist within 8 days are the most important characteristic of PFAPA. The majority of our patients exhibited symptoms of pharyngitis/tonsillitis, as reported by Feder et al[4] Accompanying white moss was also a particularly characteristic finding. Since the presence of white moss is strengthened late during flare-ups, we should be careful not to overlook it. Although we did encounter patients who were older than 5 years old and adult-onset patients, we considered the rate at which PFAPA occurs among older children to be low. Rigante et al reported the differences between PFAPA patients in children and adults revealed that the frequency of flares would seem higher in children, but the duration of inflammatory attacks was longer-lasting in adults.[22] Also, they reported about differences of symptoms between children and adults; pharyngitis was significantly more common in the pediatric age, with exudative features exclusively found among children. Conversely, joint symptoms, myalgia, fatigue, headache, ocular signs, or skin rashes during febrile attacks were significantly more common in adults. These findings may be helpful for diagnosis in adult cases of PFAPA.[22]

Accompanying symptoms of sore throat, vomiting, and/or headache, were not included by Thomas et al[2] but were described by Feder et al[4] and were also important findings in addition to cervical adenitis and aphthous stomatitis. Vanoni et al also found that aphthous stomatitis and cervical adenitis were important findings based on a questionnaire survey.[23] Spontaneous pain/tenderness in cervical adenitis and sore throat, which were not addressed in other studies, may also be useful for distinguishing PFAPA from infectious diseases. In a web-based multicenter cohort study performed in Europe, the incidence ratios of pharyngitis, cervical adenitis, and aphthous stomatitis were 90.0%, 78.4%, and 56.8%, respectively.[24] These data were similar to the incidences found in our Japanese cohort.

Although PFAPA is generally not considered a hereditary disease, several recent reports, including our own, have uncovered family history and potential genetic origins

for this syndrome. Additionally, in our cohort, family history examinations strongly suggested an autosomal dominant with incomplete penetrance model. Cochard et al also reported that nearly half of their PFAPA patients in Romandy and Bordeaux had a positive family history for recurrent fever, including PFAPA.[25] The ratio of PFAPA patients with a positive family history for recurrent fever reported in their study was consistent with our data. However, Hofer et al reported that a positive family history of recurrent fever or recurrent tonsillitis was found in 26.9% of patients in a web-based multicenter European cohort.[24] Manthiram et al reported that 23% of patients had a positive family history among a 260-member PFAPA cohort studied in the USA.[26] Since the presence of a positive family history varies according to racial and ethnic regions, we should consider limitations of this study, which is intended only for application in Japanese cohorts. An exome sequencing study reported by Di Gioia et al revealed the absence of a single mutated gene in all analyzed PFAPA patients, indicating the oligogenic or complex inheritance of variants in multiple disease genes and/or nongenetic factors.[8] Butbul-Aviel et al reported an association between PFAPA and FMF.[27] FMF should also be considered in patients with PFAPA who do not respond to adenotonsillectomy.[28] Although the association between *MEFV* gene mutations, such as E148Q or R202Q, and the development of PFAPA remains unclear, attention should be paid to patients with *MEFV* exon 3 variants with PFAPA symptoms.[9]

Remarkable increases in CRP and SAA levels during flare-ups were important laboratory findings in PFAPA. High CD64 expression on neutrophils indicates that neutrophils are activated during PFAPA attacks.[14] As CD64 expression is not remarkably increased in viral infections (data not shown), bacterial infections, or FMF attacks (data not shown) but is strongly increased in Kawasaki disease,[29] this observation might be useful for the identification of PFAPA flares. Our patients exhibited elevated serum IFN-γ levels during flare-ups, suggesting the production of the factor by T cells or natural killer cells as has been described in other diseases.[30] The striking predilection for T-cell involvement observed in this disease may represent a cause of PFAPA. Two-thirds of PFAPA patients have high levels of IgD.[31] Serum IgD levels are generally elevated in hyper-IgD syndrome and Behçet's disease. It is difficult

to discriminate hyper-IgD syndrome using our criteria, and since it is prevalent in Europe,[2, 32] hyper-IgD syndrome should be distinguished as particularly necessary in this area. Medlej-Hashim et al reported that serum IgD levels were higher in FMF patients with the *MEFV* M694V mutation but only marginally elevated in 2 FMF patients with the *MEFV* M694I mutation.[33] In our cohort of FMF patients with the M694I mutation, serum IgD levels were not elevated (n=13; 5.4 ± 7.6 mg/dL). Since the number of diseases associated with elevated serum IgD levels is limited, this finding may be relatively specific to PFAPA and could therefore aid in its diagnosis.

Several symptoms of PFAPA, including fever, pharyngitis/tonsillitis, and general fatigue, responded rapidly to treatment with prednisolone. In contrast, glucocorticoid was ineffective for fevers in viral infections, such as influenza. Five PFAPA patients in our cohort were mistakenly given prednisolone at the time of influenza and showed no defervescence. Several other patients who were given prednisolone during a bacterial infection exhibited only transient declines in fever, indicating that a remarkable response to steroids is a unique characteristic of PFAPA. Glucocorticoid dramatically improved general status and patient quality of life, but the interval between attacks tended to be shorter in half of our patients who received this medication. Among the cases effectively treated with cimetidine (51.6% of our PFAPA patients), fever attacks disappeared completely after the start of therapy. Given that reports have shown that cimetidine blocks antigen presentation, it may exert its effects by preventing antigen presentation to T cells.[34, 35] Some isolated case reports have suggested that colchicine is partially effective for treating PFAPA, but this medication was not used in our cohort.

It is well known that PFAPA has a clinical diagnosis, for which Thomas et al's diagnostic criteria are useful for clinicians to corroborate its diagnosis. However, these criteria have relatively low specificity; in particular, they cannot distinguish PFAPA from recurrent upper respiratory infections. The exclusion of cyclic neutropenia and normal growth/development described in Thomas' guidelines are consistent with almost all of our cases and was therefore not included in our diagnostic criteria. Since only 1 patient had a cough at the time of flare up in our study, we viewed the absence of cough as an important criterion to exclude acute upper respiratory infections. To compare our

new criteria with Thomas' criteria, we first evaluated their sensitivity and specificity using Thomas' criteria. The sensitivity and specificity for PFAPA versus infectious diseases were 81.7% and 87.0%, respectively. However, the sensitivity and specificity for PFAPA versus FMF were 81.7% and 100%, respectively. Compared to Thomas' criteria, the new criteria had superior sensitivity when at least 4 items of supportive criteria were matched. The specificity was equal or superior to that of Thomas' criteria when at least 5 items of supportive criteria were matched.

It is important to distinguish PFAPA from infectious diseases and FMF. We think that the attack frequency is crucial to distinguishing PFAPA from infectious diseases, such as bacterial pharyngitis/tonsillitis. We concluded that based on sensitivity and specificity, an attack frequency of at least four times is best for distinguishing PFAPA from infectious diseases. Although FMF repeats fever attacks as does PFAPA, clinical symptoms of supportive criteria were greatly different between PFAPA and FMF. The optimal threshold for supportive criteria items to be at least five may be desired to make a securing diagnosis of PFAPA (Figure 2B). However, because not all items in the supportive criteria are present during the early period of PFAPA, clinicians should follow-up those patients with suspected PFAPA in whom only 4 items are matched. Since long-term observation was not uniformly performed in this study, we were unable to evaluate prognoses in more exact detail.

In summary, we propose additional required and supportive diagnostic criteria for PFAPA (Takeuchi criteria) on the basis of clinical, laboratory, and therapeutic characteristics identified in the direct care of patients over 18 years. Overall, our diagnostic criteria are sensitive and specific enough for general physicians to identify PFAPA patients and contribute to the diagnosis of PFAPA. A larger cohort study is needed to assess the accuracy and usefulness of our diagnostic criteria, and the identification of a responsible gene would further aid in the timely diagnosis and management of PFAPA.

CONFLICT OF INTEREST

There are no conflicts of interest in this paper.

AUTHORS' CONTRIBUTIONS

Y Takeuchi and K Ogasawara analyzed the genetic data and cytokine profiles and revised the manuscript. H Nagumo participated in the design of the study and drafted the manuscript. T Ojima and M Furumoto performed the statistical analysis and helped to revise the manuscript. H Fujii, M Takizawa, K Fukushima, T Soga, and Y Umeda conceived the study, participated in its design and coordination, and helped to draft the manuscript. T Shigemura and N Kobayashi collected the clinical data and revised the manuscript. K Agematsu and H Matoba carried out the molecular genetic studies and CD64 analysis and drafted the manuscript. All authors read and approved the final manuscript.

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TABLE 1 Clinical characteristics of PFAPA patients

Clinical characteristics	
Male/female, no.	143 / 114
Age at onset, years	2.7 ± 1.6
Family history, no. (%) †	159 / 257 (62)
Maximum body temperature at attack, °C	39.7 ± 0.6
Interval between episodes (range), months	1.2 ± 0.8 (0.5-9.0)
Duration of fever (range), days	4.5 ± 1.3 (2.0-7.9)
Accompanying symptoms, no. (%) ‡	
Pharyngitis / tonsillitis	238 / 251 (94.8)
White moss	165 / 228 (72.4)
Cervical adenitis	147 / 252 (58.3)
Aphthous stomatitis	130 / 251 (51.8)
Sore throat	93 / 205 (45.4)
Vomiting	47 / 220 (21.4)
Headache	71 / 211 (33.6)
Laboratory findings at onset of fever	
WBC, /μl	12,500 ± 4,700
Seg, % of 100 cells	66.1 ± 12.7
Band, % of 100 cells	3.7 ± 4.8
CRP, mg/dl	6.7 ± 4.6
SAA, μg/ml	669.2 ± 449.8
IgD	
≧10, no. (%)	72 / 199 (36.2)
mean ± SD, mg/dl	23.0 ± 12.2
<10, no. (%)	127 / 199 (63.8)
mean ± SD, mg/dl	3.0 ± 2.7

† Number of patients with family history of recurrent fever, tonsillitis, or tonsillectomy.
‡ As far as we could determine. Band, band neutrophils; CRP, C-reactive protein; PFAPA, the syndrome of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis; Seg, segmented neutrophils; SAA, serum amyloid A.;WBC, white blood cells.

TABLE 2 Characteristics of 148 affected families in 257 PFAPA patients

	Affected family no.
Overall incidence of affected families, no. (%)	148 / 246 (60.2)
Either the father's or mother's side, no. (%)	126 / 148 (85.1)
Generation of the parent, aunt or uncle, no. (%)	73 / 126 (57.9)
Three generations, no. (%)	33 / 126 (26.2)
Skip of one generation, no. (%)	20 / 126 (15.9)
Both the father's and mother's sides, no. (%)	19 / 148 (12.8)
Siblings, no. (%)	21 / 148 (14.2)
Identical twin, no. (%)	1 / 148 (0.7)

PFAPA, the syndrome of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis.

TABLE 3 CD64 expression and serum cytokine profiles

	CD64 expression, molecules		CD16+
	Neutrophils	Monocytes	monocytes, %
Normal individuals† (n=12)	1,384.5 ± 642.9	16,801.3 ± 3,635.3	1.8 ± 0.9
PFAPA at flare up‡ (n=134)	11,421.1 ± 8,421.1	64,472.4 ± 30,902.1	19.5 ± 9.2
Bacterial infection (n=12)	4,455.0 ± 1,304.9	50,817.5 ± 40,183.1	12.3 ± 3.4
	Normal individuals† (n=12)	PFAPA at flare up‡ (n=94)	Bacterial infection (n=12)
IL-1β (pg/ml)	<5	<5	<5
IL-6 (pg/ml)	<5	35.0 ± 89.5	32.5 ± 20.8
IL-8 (pg/ml)	77.8 ± 88.1	223.2 ± 706.4	15.0 ± 5.5
TNF (pg/ml)	<5	0.7 ± 2.1	<5
IFN-γ (pg/ml)	<5	17.8 ± 21.1	<5
G-CSF(pg/ml)	<5	19.1 ± 13.1	11.2 ± 11.7
IP-10 (pg/ml)	105.9 ± 41.1	1,335.8 ± 7,545.5	335.1 ± 419.2
MIG (pg/ml)	125.0 ± 77.0	1,350.8 ± 4,881.3	32.5 ± 28.9
MCP-1(pg/ml)	76.7 ± 41.6	221.0 ± 692.4	23.8 ± 14.0
IL-18 (pg/ml)	166.7 ± 140.2	477.1 ± 226.2	ND

† All subjects were adults.

‡ Whole blood cells and serum were harvested 12-48 hours after appearance of fever.

G-CSF, granulocyte-colony stimulating factor; IFN, interferon; IL, interleukin; IP, interferon gamma-induced protein; MCP, monocyte chemotactic protein; MIG, monokine induced by gamma interferon; ND, not done; PFAPA, the syndrome of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis; TNF, tumor necrosis factor.

TABLE 4 Diagnostic criteria of PFAPA

Required criteria
Body temperature increase to more than 38.0°C that lasts for less than 8 days (mean: 4 days) and recurs at least 4 times.
Supportive criteria
1. Age of onset less than 5 years
2. Tonsillitis or pharyngitis with white moss
3. Concomitant symptoms with at least 1 of the following clinical signs a. Aphthous stomatitis, b. Cervical lymphadenitis†, c. Sore throat, d. Vomiting, e. Severe headache
4. No cough
5. Family history‡
6. Inflammatory laboratory findings, such as CRP and SAA, that are extremely raised during febrile attacks but normal level in attack-free periods
7. High serum IgD level
8. Glucocorticoid medication is highly effective§

Diagnosis of PFAPA: required criteria and at least 5 items of supportive criteria

Possible PFAPA: required criteria and 4 items of supportive criteria

† Tenderness is often seen in swollen lymph nodes.

‡ Including a history of recurrent fever, tonsillitis, or tonsillectomy

§ Fever subsides by as-needed usage of oral prednisolone (0.5 mg/kg) within half a day.

CRP, C-reactive protein; PFAPA, the syndrome of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis; SAA, serum amyloid A.

FIGURE LEGENDS

FIGURE 1 Family tree analysis

(A) Inheritance pattern from either the father's or mother's side (left). PFAPA patients were found in three generations (middle) and in one skipped generation (right). (B) Inheritance patterns from both parents' sides. (C) Families with sibling patients. (D) Families in which 5-6 patients were seen. (E) Families with patients possessing *MEFV* exon 3 variants. PFAPA, the syndrome of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis; ND, not done.

FIGURE 2 Receiver operating characteristic (ROC) curves of supportive criteria number for PFAPA in relation to infectious disease and FMF

(A) ROC curve for PFAPA vs infectious diseases. The optimal threshold for the number of supportive criteria was at least 4 (or 5) items, which provided 93.8% (80.2%) sensitivity and 94.2% (100%) specificity. (B) ROC curve for PFAPA vs FMF. The optimal threshold for the number of supportive criteria was at least 4 (or 5) items, which provided 93.8% (80.2%) sensitivity and 95.6% (100%) specificity. PFAPA, the syndrome of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis; FMF, familial Mediterranean fever.

FIGURE 1

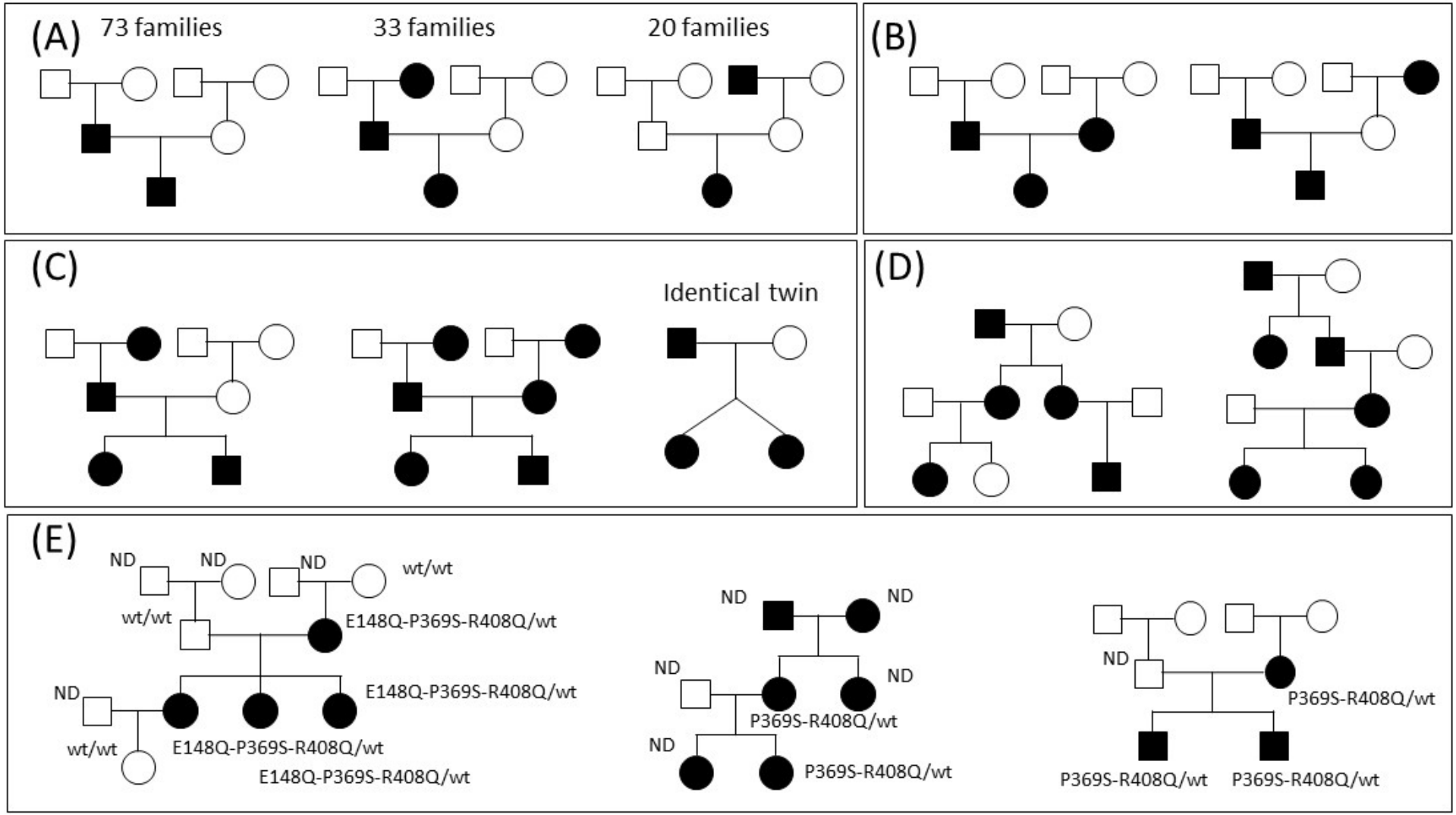
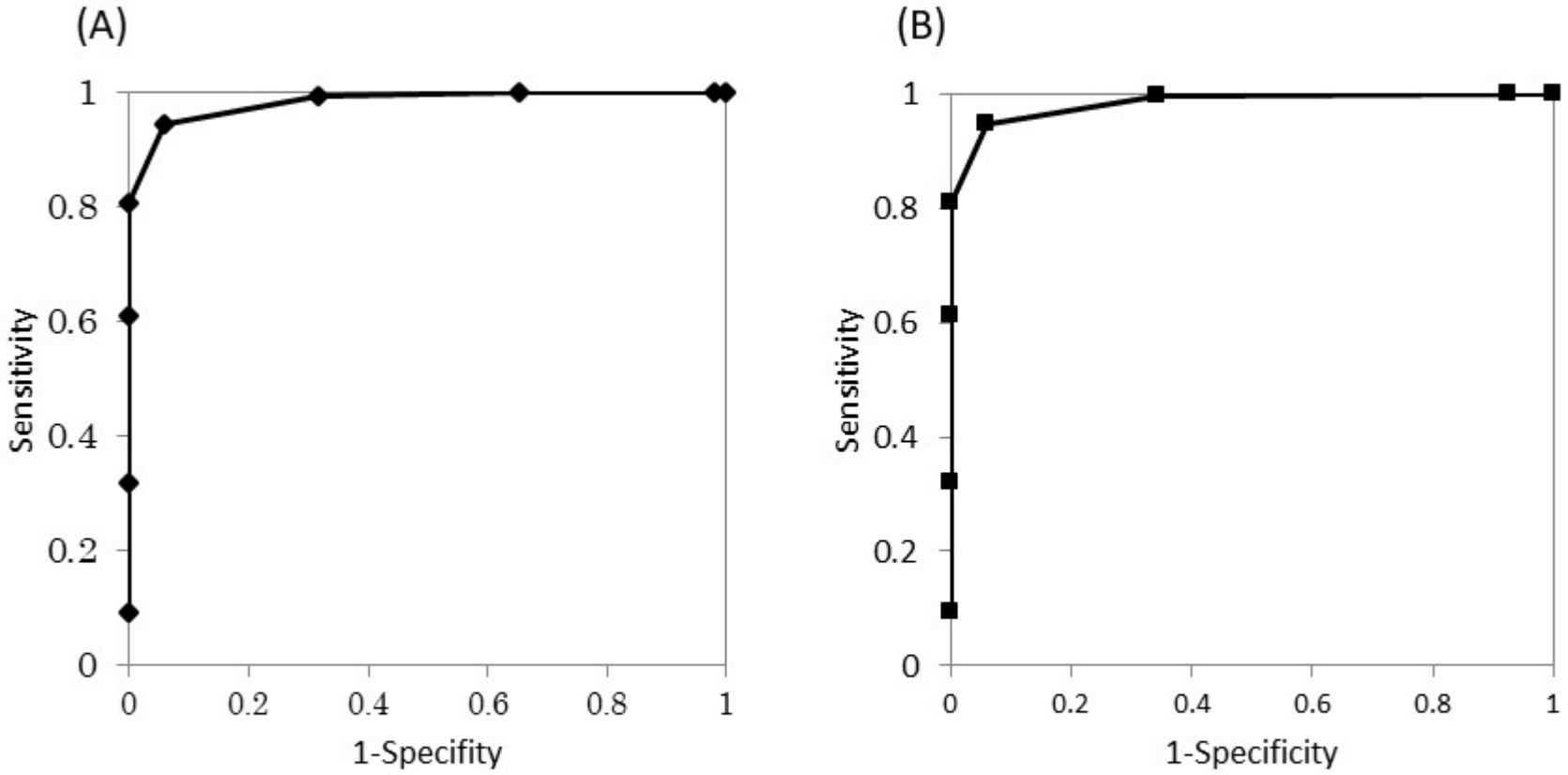

(A)
73 families
33 families
20 families
(B)
(C)
Identical twin
(D)
(E)
ND
ND
ND
wt/wt
wt/wt
E148Q-P369S-R408Q/wt
ND
E148Q-P369S-R408Q/wt
wt/wt
E148Q-P369S-R408Q/wt
E148Q-P369S-R408Q/wt
ND
ND
ND
ND
P369S-R408Q/wt
ND
P369S-R408Q/wt
ND
P369S-R408Q/wt
P369S-R408Q/wt
P369S-R408Q/wt

FIGURE 2


(A)
Sensitivity
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1-Specifity
(B)
Sensitivity
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1-Specificity