**MEFV Variants in Patients with PFAPA Syndrome in Japan**

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**Abstract:** Background: The pathogenesis of PFAPA (periodic fever, aphthous stomatitis, pharyngitis, adenitis) syndrome is unknown as yet. In order to understand whether genes implicated in other auto-inflammatory diseases might be involved in the pathogenesis of PFAPA, all variants in the genes causing familial Mediterranean fever (FMF), tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS), and Hyper IgD syndrome were analyzed in children with PFAPA.

Patients and Methods: All variants in MEFV, TNFRSF1A, and MVK were analyzed in 20 patients with PFAPA. PFAPA were diagnosed by previous published criteria. The findings of all analyses in PFAPA patients were compared with those of unaffected normal subjects (n=62).

Results: In the 13 children of 20 with PFAPA, the heterozygous variants of MEFV (5 patients: E148Q-L110P, 2 patients: E148Q, 1 patient: E148Q-L110P/E148Q, 1 patient: E148Q-P369S-R408Q-E84K, 1 patient: E148Q-L110P-P369S-A408G, 1 patient: R202Q, 1 patient: P115R) were found. No variants belonging to TNFRSF1A or MVK were detected in children with PFAPA. The frequency of the E148Q-L110P variants in children with PFAPA was significantly higher than that observed in unaffected normal subjects (7/20 versus 8/62). The duration of the episodes of illness in PFAPA children with MEFV variants was shorter than that of patients without variants.

Conclusion: Genes involved in the development and progression of MEFV may affect the incidence and the phenotype of PFAPA in children.

Keywords: PFAPA, MEFV, FMF, Variant, Japanese.

**INTRODUCTION**

In 1987 [1], Marshall firstly described, the PFAPA (periodic fever, aphthous stomatitis, pharyngitis and adenitis) syndrome, which is characterized by recurrent episodes of fever associated with cervical adenitis, pharyngitis and aphthous stomatitis. The prognosis of this disease has been reported to be better than that of another autoinflammatory diseases [2]. Corticosteroids are effective in controlling episodes of illness in PFAPA, but they do not cure the ailments or prevent recurrence of the symptoms of this syndrome [3]. Interventions like tonsillectomy and administration of H2 blockers have been reported to be partially effective for prophylaxis [3]. However the complete pathogenesis of PFAPA is unknown yet, and hence the therapeutic regimens have not yet been established for PFAPA [4].

Familial Mediterranean fever (FMF) is a recessively inherited disorder characterized by acute attacks of fever, and serositis usually lasting for 1–3 days. FMF is caused by mutations in the ME diterranean FeVer gene (MEFV), which encodes the protein pyrin (marenostrin)[5,6]. Colchicine has been shown to be effective for prophylaxis in only 90% of the patients with FMF [7,8].

Recent studies have described that heterozygous variants of the MEFV gene were found in patients with PFAPA [9–11]. However, it still remains unclear whether these variants are the causative factors of PFAPA.

The purpose of this study was to understand whether heterozygous variants of MEFV may be associated with the onset of PFAPA. We have also tried to understand whether these mutations act as accessory factors and modify the phenotype of patients with PFAPA.

**PATIENTS AND METHODS**

Twenty children with frequent PFAPA episodes who visited our pediatric outpatient clinic were consecutively selected over a 5-year period (from January 2005 to January 2010). The diagnosis of PFAPA syndrome was established using previously established criteria [1,3,9]. These criteria include recurring fevers associated with exudative tonsillitis, negative throat culture, and possibly, aphthous stomatitis and cervical lymphadenopathy. The additional clinical criteria included completely asymptomatic intervals between the episodes, normal growth and development and exclusion of
FMF, Behçet’ s disease, and cyclic neutropenia. Oral low dose of prednisolone (0.3-0.5mg/kg/dose, 1 or 2 doses per day) was effective on all enrolled patients. MEFV, mevalonate kinase (MVK), and tumor necrosis factor receptor superfamily, member 1A (TNFRSF1A) genes of all enrolled patients were sequenced. After obtaining a written informed consent approved by the Institutional Review Board of Kyoto University, peripheral blood was collected from all patients, and, if needed, their family members. Genomic DNA was extracted, and all the exons including exon-intron junctions of the MVK, MEFV, and TNFRSF1A genes were amplified by polymerase chain reaction and then sequenced using the ABI3130.

The results are shown as a mean ± SD or proportion, as appropriate. Differences between the groups in discrete variables were evaluated using Fisher’s exact test at 5% significance.

Two-sided P values were adjusted for multiplicity using Hochberg’s method.

Comparisons of continuous variables were done using unpaired Student’s t-test. All P values given are 2-sided. P values less than 0.05 were considered significant. Statistical calculation was conducted by SAS version 9.1.3.

RESULTS

Twenty patients (9 boys, 11 girls) diagnosed with PFAPA were followed up in our clinic. Thirteen of these patients had a single MEFV (M’ group). No variant of TNFRSF1A and MVK was detected in all patients. The genotypes of the MEFV gene in the 13 patients are seen in Table 1. The most common MEFV variant patterns seen were E148Q-L110P (5 patients) and E148Q (2 patients). One patient was homozygous of E148Q and heterozygous of L110P of MEFV. One patient was heterozygous for E148Q-P369S-R408Q. One patient was heterozygous for E148Q-P369S-R408Q-E84K. One patient had E148Q-L110P-P369S-R408Q. The minor variants, P115R and R202Q, were detected in 2 patients. More than 2 MEFV variants were on 1 allele in all PFAPA patients. In 7 patients, no MEFV mutations were found. The allele frequencies of E148Q, L110P, P369S, R408Q and G304R in 20 PFAPA patients were 0.3, 0.175, 0.075, 0.075 and 0, respectively (Table 3).

Table 1. The Genotypes of MEFV Genes of 13 Patients with PFAPA

| MEFV Variant                | No. of Patients |
|-----------------------------|-----------------|
| E148Q-L110P                 | 5               |
| E148Q                       | 2               |
| E148Q-L110P/E148            | 1               |
| E148Q-P369S-R408Q           | 1               |
| E148Q-P369S-R408Q-E84K      | 1               |
| E148Q-L110P-P369S-R408Q     | 1               |
| R202Q                       | 1               |
| P115R                       | 1               |

Clinical and laboratory data were compared between MEFV positive group (n=13) and negative group (n=7) and are presented in Table 2. Patients carrying an MEFV variant showed shorter duration of episodes of illness than patients without variants (3.6±0.86 days versus 5.3±1.89 days).

Table 2. Clinical Characteristics of PFAPA Patients with Variants in the MEFV Gene Compared with those of PFAPA Patients without MEFV Variants

|                                | Patients with MEFV Variants (n=13) | Patients without MEFV Variants (n=7) | P Value |
|--------------------------------|-----------------------------------|-------------------------------------|---------|
| Age at onset (years)           | 2.8±1.9                           | 3.2±1.9                             | NS      |
| Age at Diagnosis (years)       | 4.3±2.2                           | 4.9±1.9                             | NS      |
| Male: female ratio             | 5/8                               | 4/3                                 | NS      |
| Family history of PFAPA        | 4/9                               | 3/4                                 | NS      |
| Attack duration (days)          | 3.6±0.86                          | 5.3±1.89                            | P=0.0174|
| Interval between attacks (weeks)| 4.9±1.59                          | 5.5±0.96                            | NS      |
| Cyclic periodic attacks        | 5/13                              | 4/7                                 | NS      |
| Pharyngitis                    | 13/13                             | 7/7                                 | NS      |
| Aphthae                        | 7/13                              | 5/7                                 | NS      |
| Enlarged tonsillitis           | 13/13                             | 7/7                                 | NS      |
| Abdominal pains                | 1/13                              | 2/7                                 | NS      |
| Musculoskeletal Pains          | 1/13                              | 2/7                                 | NS      |
| Headaches                      | 4/13                              | 5/7                                 | NS      |
| WBC/µL                         | 143±41                            | 142±41                              | NS      |
| ESR mm/h                       | 87±23                             | 72±14                               | NS      |
| CRP levels mg/dL               | 6.52±3.53                         | 5.87±2.85                           | NS      |

NS: not significant.
**DISCUSSION**

We studied 20 patients with PFAPA who were diagnosed by Marshall criteria [1]. Our aim was to access the roles of the predominant variants in genes that cause other febrile illnesses like FMF, TNF receptor-associated periodic syndrome (TRAPS) and the MKV deficiency. We did not find any incidence of variants of TRAPS and MKV deficiency in PFAPA patients. However several heterogeneous variants of MEFV were detected in 13 out of 20 patients with PFAPA. We analyzed the frequency of the \(E148Q\)-\(L110P\) and \(P369S-R408Q\) variants in PFAPA and control subjects. Our analyses indicate that the incidence of these 2 variants is significantly higher in patients with PFAPA than in normal individuals.

Amongst autoinflammatory disease, only PFAPA syndrome has been described as a non-inherited syndrome, since familial inheritance has not been reported in previous studies [3,12,13]. However some studies have reported familial cases that included siblings or a sibling and the sibling’s mother [14-16]. Therefore, the hereditary nature of this syndrome is still a matter of debate. With respect to the genetic factors that may cause the PFAPA syndrome, one study has strongly argued against the involvement of the MEFV gene [10], but another article [11] has shown that mutations of the MEFV genes were found in 27% of cases diagnosed with PFAPA syndrome on the basis of Marshall’s clinical criteria. Our observations of the high frequency (65%) of MEFV variants are in agreement with that reported by Dagan [11]. The differences in the findings may be attributed to the ethnic differences between the individuals studied, the small sized of the study, and the study population that was selected.

The \(L110P\) variant, which is located in exon 2, was first reported in FMF patients in 2000 [17], and to date, several compound heterozygotes with other variants have been reported even in Japan [18,19]. In contrast, although the role of the \(E148Q\) variant, which is located in exon 2, in FMF patients was controversial, a recent study concluded that the variant is just a benign polymorphism [20]. In a Japanese study [18] of FMF patients, the most frequently observed MEFV variants observed were \(E148Q/M694I\) (25.0%), \(M694I\) (17.5%), and \(L110P/E148Q/M694I\) (17.5%). However, no patients had the \(M694V\) variant. These patterns were quite different from those in Mediterranean patients with FMF. The study also reported that the allele frequencies of \(E148Q\), \(M694I\), and \(L110P\) were 0.44, 0.35, and 0.31, respectively, and that these frequencies were significant difference from those seen in healthy controls. In our study, the allele frequencies of \(E148Q\), \(L110P\), and \(G304\) were 0.3, 0.175, and 0, respectively, and these frequencies did not differ significantly between patients and healthy controls. No mutation at the \(M694I\) was detected in our cohorts of patients and controls. The allele frequency of \(L110P\) is higher in patients with PFAPA than in healthy controls; however, the difference is non-significant.

The frequency of the \(E148Q\)-\(L110P\) variant combination is significantly higher in the PFAPA group than in the healthy control group. If the \(E148Q\) variant is non-functional, the \(L110P\) variant may be associated with the onset of PFAPA syndrome.

In several types of inflammatory such as Behcet’s disease [21], Crohn’s disease [22], ulcerative colitis [23], Henoch-Schönlein purpura [24,25] and the co-incidence of FMF variants has been investigated. These studies show increased incidence of genes involved in FMF in patients with these autoinflammatory diseases as well as the increased severity of the symptoms of each disease. On the other, in the patients with asthma, the incidence of FM mutation was decreased and the lower incidence correlated with reduced severity of symptoms [26]. Thus, FMF variants may affect the transition from Th2 to Th1 polarity in each disease. According to Berkun’s study [27], PFAPA episodes in carriers of MEFV variants were shorter compared to those in patients without variants. In MEFV variant-positive patients, the regular cyclic pattern of attacks and the occurrence of oral aphthae was lower than those in patients without MEFV variants. In the present study, we found that the only affected variable was the duration of PFAPA episodes. Although no significant differences were observed in the regular cyclic pattern of attacks and the occurrence of oral aphthae between the 2 groups, the duration of PFAPA episodes was shorter in the variant-positive group than in the variant-negative group.

Taken together, these results show that the MEFV gene may not affect the onset of several autoinflammatory diseases, but is likely to modify the intensity and the displayed phenotype in terms of disease symptoms.
In conclusion, the MEFV variants, viz. E148Q-L110P, P369-R408Q may be associated with the onset of PFAPA, and some MEFV variants may affect the phenotype of PFAPA.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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