ORIGINAL ARTICLE

Mediterranean fever gene variants modify clinical phenotypes of idiopathic multi-centric Castleman disease

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Abstract
Four cases of idiopathic multi-centric Castleman disease (iMCD) reportedly have variants in hereditary autoinflammatory disease-related genes; however, the frequency and role of these variants in iMCD is still unknown. We therefore investigated such gene variants among patients with iMCD and aimed to reveal the relationship between iMCD and autoinflammatory disease-related genes. We reviewed 14 Japanese iMCD patients who were recruited between January 2015 and September 2019. All patients met both the Japanese tentative diagnostic criteria for Castleman disease and the international consensus diagnostic criteria for iMCD. We performed genetic analyses for 31 autoinflammatory disease-related genes by targeted next-generation sequencing. The MEFV gene variants were observed in 10 of 14 patients with iMCD. Although iMCD had a high percentage of exons 2 or 3 variants of MEFV, comparison of data from healthy Japanese subjects indicated that there was no significant difference in the percentage between healthy Japanese subjects and patients with iMCD. Variants of uncertain significance (VUS) in the TNFRSF1A and CECR1 genes were observed in two of the patients, respectively. We divided patients into two groups—those with MEFV variants (excluding E148Q variants) and those without MEFV variants—and compared the clinical characteristics between these two groups. Patients with MEFV variants, excluding E148Q variants, exhibited a significantly higher likelihood of fever and significantly lower levels of hemoglobin than those lacking MEFV variants. Our results indicated that patients with iMCD tended to have a high frequency of MEFV gene variants and the presence of such variants can affect iMCD clinical phenotypes.

KEYWORDS
autoinflammatory disease-related genes, Castleman disease, idiopathic multi-centric Castleman disease, MEFV gene

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INTRODUCTION

Castleman disease (CD) is a rare lymphoproliferative disorder and is described as either unicentric (UCD) or multi-centric (MCD), according to the number of affected lymph nodes. MCD can also be classified, according to etiology, as human herpesvirus 8 (HHV-8) infection-related MCD, which most commonly affects HIV-positive and immunocompromised patients or HHV-8-negative idiopathic MCD (iMCD), which has an unknown etiology. In addition, iMCD is divided into two categories: iMCD, not otherwise specified (iMCD-NOS) and thrombocytopenia, anasarca, myelofibrosis, renal dysfunction and organomegal (TAFRO) syndrome with iMCD (TAFRO-iMCD), both of which have distinct clinical manifestations [1].

Although idiopathic MCD is characterized by systemic inflammatory symptoms that are associated with increased levels of interleukin (IL)-6 [2], increased levels of serum IL-6 are not observed in all patients with iMCD. IL-6 induces differentiation of B cells into plasma cells and promotes vascular growth via expression of vascular endothelial growth factors [2]. While IL-6 inhibitors are recommended as the standard treatment strategy for iMCD, steroids are useful as adjuvant therapy [3].

Although the etiology of increased cytokine production, such as IL-6, in iMCD remains unclear, four cases of previous reports have described iMCD cases with variants in hereditary autoinflammatory disease-related genes such as the Mediterranean Fever (MEFV) genes [4–7]. In addition, one case of previous reports suggested that loss of adenosine deaminase function due to a homozygous CECR1 mutation contributes to a Castleman disease (CD)-like phenotype in pediatric patients and can be successfully treated with IL-6 inhibitors, as with MCD [8,9]. Consequently, the involvement of autoinflammatory mechanisms in the development of iMCD has been suggested. However, there has been only one previous study that investigated the relationship between iMCD and autoinflammatory disease-related genes, which was in a pediatric cohort [7].

To diagnose iMCD, excluding infection-related diseases, autoimmune/autoinflammatory diseases and malignant/lymphoproliferative diseases is essential [10], but the relationships and differences between these diseases and iMCD have not yet been elucidated. We hypothesized that determining the frequency of autoinflammatory disease-associated genes in iMCD may support the diagnosis of iMCD. In the present study, we investigated certain autoinflammatory disease-related gene variants—including those of MEFV and CECR1—among patients with iMCD, with the aim to determine the frequency of these autoinflammatory disease-related genes in iMCD.

PATIENTS AND METHODS

Study design

This study was part of an ongoing multi-center prospective cohort study registered with the University Hospital Medical Information Network Clinical Trials Registry [http://www.umin.ac.jp/ctr/] (#UMIN000015881), and includes patients with familial Mediterranean fever (FMF) or other diseases that require differentiation from FMF, owing to findings of fever and/or chronic inflammation. All patients gave signed informed consent, which was approved by the Institutional Review Board of Nagasaki University and related centers (approval no. 14092946).

Patients

We reviewed 14 Japanese patients with iMCD who were recruited between January 2015 and September 2019 at the Nagasaki University and Toranomon Hospital. We evaluated for the other autoimmune/autoinflammatory conditions and ruled out the patients with a single autoimmune or autoinflammatory disease. We reviewed the lymph node biopsy results of the patients included in our study and confirmed that the pathological findings of all patients were consistent with MCD. Each patient was clinically observed for ≥ 6 months.

Clinical and laboratory assessments

As an objective scale for measuring the disease activity of each iMCD patient at disease onset, we assessed CHAP score, consisting of measurement of C-reactive protein (CRP), albumin, hemoglobin and Eastern Cooperative Oncology Group performance status (each with a subscale range = 0–4; total scale range = 0–16). Higher scores indicate increased disease activity [11]. We defined remission as a CHAP score of 1 or less after 6 months to 1 year of treatment, and we defined improvement as a decrease in the CHAP score of 1 or more, compared with pretreatment.

Genetic analysis

We extracted genomic DNA from the patients’ whole blood using the Promega Wizard® Genomic DNA Purification Kit (Promega, Madison, Wisconsin, USA). We composed a next-generation sequencing panel containing a total of 31 known autoinflammatory disease-related genes (i.e. NLRP3, MVK, PSTPIP1, MEFV, TNFRSF1A, NOD2, IL1RN, NLRP12, NLRC4, PLCG2, HO-1, TNFAIP3, CECR1, COPA,
However, the patient subsequently had prolonged axillary lymphadenopathies, chronic urticaria and inflammatory findings. Finally, the result of lymph node biopsy led to a diagnosis of iMCD. Case 13, with multiple heterozygous MEFV variants, was diagnosed with amyloid A (AA) amyloidosis, as proved by histological findings. For the laboratory findings, the median levels of CRP, albumin and hemoglobin were 7.0 (range = 0.8–21.4) mg/dl, 2.5 (1.2–3.7) g/dl and 9.2 (5.1–11.2) mg/dl, respectively. Within the histological types, three of the iMCD patients were of mixed type, while the other 11 were of the PC type.

**Statistical analyses**

Among the variants detected in the MEFV gene, the E148Q variant was not included as a significant variant in the statistical analysis, because its possession rate was prominently high (the minor allele frequency of E148Q is 22.07%) in Japanese. Accordingly, the enrolled patients with iMCD were divided into two groups: those either with or without MEFV variants, excluding E148Q variants. We compared the clinical characteristics between these two groups. Discrete variables were compared using Fisher’s exact test, and continuous variables were compared using Wilcoxon’s test.

All statistical analyses were performed with JMP Pro version 15.0 software (SAS Institute, Cary, North Carolina, USA). P-values < 0.05 (two-tailed) were considered statistically significant for all analyses.

**RESULTS**

**Clinical and laboratory characteristics and histopathological type**

The demographic clinical characteristics of the enrolled patients, including four cases of iMCD previously reported [5,6,12,13], are summarized in Table 1. All patients met the international consensus diagnostic criteria for iMCD [10] as well as the Japanese tentative diagnostic criteria for CD [11]. All patients were classified into iMCD-NOS. The median age at disease onset was 53.0 years and the percentage of male gender was 71.4%. All patients who were enrolled were treated with tocilizumab and/or prednisolone first, but tocilizumab was switched to rapamycin or canakinumab in two of 14 patients due to poor effectiveness. Six of 14 patients with iMCD exhibited fever (> 38°C), with four of these having recurrent fever. In case 6, with multiple heterozygous MEFV variants, this recurrent fever responded positively to colchicine treatment; FMF was suspected [6]. However, the patient subsequently had prolonged axillary lymphadenopathies, chronic urticaria and inflammatory findings. Finally, the result of lymph node biopsy led to a diagnosis of iMCD. Case 13, with multiple heterozygous MEFV variants, was diagnosed with amyloid A (AA) amyloidosis, as proved by histological findings. For the laboratory findings, the median levels of CRP, albumin and hemoglobin were 7.0 (range = 0.8–21.4) mg/dl, 2.5 (1.2–3.7) g/dl and 9.2 (5.1–11.2) mg/dl, respectively. Within the histological types, three of the iMCD patients were of mixed type, while the other 11 were of the PC type.

**Variants of autoinflammatory disease-related genes**

Here we describe the MEFV, TNFRSF1A, NLRP3, MVK and TNFAIP3 genes, which are relatively common autoinflammatory disease-related genes among various autoinflammatory diseases and are known as responsible genes in the following diseases (Table 2): FMF, tumor necrosis factor (TNF) receptor-associated periodic syndrome, cryopyrin-associated periodic syndrome, mevalonate kinase deficiency and haploinsufficiency of A20. Because adenosine deaminase 2 deficiency caused by a homozygous CECR1 mutation mimicked iMCD in one pediatric patient [9], we also included this gene.

In the genetic analysis, six MEFV variants were observed in 10 of 14 of the included patients. Of the MEFV variants, two were homozygous (P369S and R408Q) in one patient and single or multiple heterozygous in the remaining nine patients. The frequency of each MEFV allele variant in the 14 included patients was as follows: L110P, 14.3%; E148Q, 25.0%; R202Q, 3.6%; P369S, 14.3%; R408Q, 14.3% and I729M, 3.6%. Variants of uncertain significance in the TNFRSF1A and CECR1 genes were observed in two of the patients, respectively. No variants in NLRP3, MVK or TNFAIP3 genes were identified in any of the patients.

In addition, no other variants that were previously reported as being potentially pathogenic in the Infevers database (http://infevers.umai-montpellier.fr) or ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar/) were observed in the remaining 25 of 31 autoinflammatory disease-related genes (not shown).

**Characteristics of iMCD patients with MEFV variants**

The following two groups of iMCD were compared in terms of their clinical characteristics (Table 3); those with MEFV variants (excluding E148Q variants, n = 9) and those without MEFV variants (n = 5). Patients with MEFV variants exhibited a significantly higher presence of fever...
| Case number | Age (years) | Sex | Fever (> 38°C) | Complications of autoimmune or autoinflammatory diseases | Clinical type | Histological type | Initial treatment                                                                 | Serum IL-6 (pg/ml) | CRP (mg/dl) | Albumin (g/dl) | Hemoglobin (g/dl) | PS | CHAP score | Outcome                        |
|------------|-------------|-----|----------------|----------------------------------------------------------|--------------|-------------------|----------------------------------------------------------------------------------|-------------------|-------------|----------------|-----------------------|----|------------|--------------------------------|
| 1          | 73          | Male | −              | None                                                     | iMCD         | PC                | 45 mg/day of PSL, intravenous TCZ                                                  | 14.3              | 0.82        | 1.3           | 7.8                   | 2  | 9          | Death due to recurrent esophageal cancer |
| 2          | 42          | Male | +              | None                                                     | iMCD         | PC                | 20 mg/day of PSL, cyclosporin, intravenous TCZ (switching to rapamycin)          | 267               | 21.36       | 1.2           | 5.1                   | 1  | 13         | Improvement                  |
| 3 [5]      | 66          | Male | +              | None                                                     | iMCD         | PC                | Intravenous TCZ                                                                  | 9.3               | 3.75        | 2.6           | 11.0                  | 0  | 3          | Remission                     |
| 4          | 64          | Male | −              | None                                                     | iMCD         | PC                | 40 mg/day of PSL, intravenous TCZ                                                  | 16.0              | 10.8        | 3.3           | 10.5                  | 0  | 4          | Remission                     |
| 5          | 33          | Female | +          | None                                                     | iMCD         | Mixed             | 15 mg/day of PSL, intravenous TCZ                                                  | 111.7             | 6.6         | 2.5           | 8.7                   | 0  | 5          | Remission                     |
| 6 [6]      | 37          | Female | +          | None                                                     | iMCD         | Mixed             | Colchicine, 10 mg/day of PSL, intravenous TCZ (switching to canakinumab)        | 11.0              | 1.04        | 3.7           | 9.5                   | 1  | 4          | Remission                     |
| 7          | 73          | Male | −              | None                                                     | iMCD         | PC                | 50 mg/day of PSL                                                                  | NA                | 5.75        | 2.2           | 11.2                  | 1  | 6          | Improvement                   |
| 8 [12]     | 41          | Male | −              | IgA vasculitis                                           | iMCD         | Mixed             | 10 mg/day of PSL, intravenous TCZ                                                  | 15.1              | 7.1         | 3.1           | 10.9                  | 0  | 3          | Remission                     |
| 9 [13]     | 56          | Male | −              | None                                                     | iMCD         | PC                | Intravenous TCZ                                                                  | 15.4              | 7.2         | 1.6           | 7.2                   | 1  | 9          | Improvement                   |
| 10         | 50          | Male | −              | None                                                     | iMCD         | PC                | 35 mg/day of PSL, intravenous TCZ                                                  | 11.1              | 3.04        | 2.7           | 10.5                  | 1  | 4          | Remission                     |
| 11         | 65          | Male | −              | None                                                     | iMCD         | PC                | 10 mg/day of PSL, intravenous TCZ                                                  | 99.8              | 14.4        | 2.5           | 10.3                  | 1  | 6          | Improvement                   |
| 12         | 65          | Female | −          | None                                                     | iMCD         | PC                | Intravenous TCZ                                                                  | 31.9              | 6.89        | 2.6           | 8.9                   | 1  | 6          | Improvement                   |
| 13         | 46          | Male | +              | AA amyloidosis                                           | iMCD         | PC                | Intravenous TCZ                                                                  | 83.8              | 8.6         | 1.2           | 8.2                   | 2  | 10         | Improvement                   |
| 14         | 47          | Female | +          | None                                                     | iMCD         | PC                | 50 mg/day of PSL, intravenous TCZ                                                  | 17.6              | 14.15       | 2.3           | 8.0                   | 2  | 9          | Remission                     |

All laboratory findings described in this table were those at active phase before the treatment of MCD. MCD = multi-centric Castleman’s disease; PSL = prednisolone; TCZ = tocilizumab; CRP = C-reactive protein; PC = plasma cell; PS = performance status; NA = not available.
and significantly lower levels of hemoglobin than those lacking MEFV variants \((P = 0.03, P = 0.045, \text{respectively})\). Other parameters had a tendency (but not significantly) to show higher iMCD disease activity at onset for those patients with \textit{versus} without MEFV variants.

**DISCUSSION**

Herein we investigated various autoinflammatory disease-related genes among Japanese patients with iMCD. Our findings showed that those with iMCD tended to have a high frequency of variants in the \textit{MEFV} gene, and these patients more often exhibited fever and lower levels of hemoglobin than those lacking \textit{MEFV} variants, excluding E148Q variants.

FMF is the most frequent autoinflammatory disease caused by variants in the \textit{MEFV} gene and is characterized by recurrent attacks of fever and subclinical inflammation, even during remission. Pyrin protein, which is encoded by the \textit{MEFV} gene, regulates IL-1β activation via regulation of caspase-1 activation. Although FMF is

| Case number | \textit{MEFV} gene | The other autoinflammatory disease-related genes \((\text{TNFRSF1A, NLRP3, MVK, TNFAIP3, CECRI})\) |
|-------------|-------------------|-----------------------------------------------------|
| 1           | E148Q/P369S/R408Q | \textit{CECR1} H335R/H335R                         |
| 2           | L110P/E148Q       | \textit{CECR1} R230Q                                |
| 3 [5]       | I729M             | None                                                |
| 4           | P369S/R408Q       | None                                                |
| 5           | R202Q             | \text{TNFRSF1A V363A}                               |
| 6 [6]       | L110P/E148Q       | None                                                |
| 7           | None              | None                                                |
| 8 [12]      | None              | \text{TNFRSF1A P271L}                               |
| 9 [13]      | E148Q/P369S/P369S/R408Q/R408Q | None                                      |
| 10          | None              | None                                                |
| 11          | None              | None                                                |
| 12          | E148Q             | None                                                |
| 13          | L110P/E148Q       | None                                                |
| 14          | L110P/E148Q       | None                                                |

| Variables | All patients \((n = 14)\) | The group without \textit{MEFV} variants \((n = 5)\) | The group with \textit{MEFV} variants \((n = 9)\) |
|-----------|---------------------------|---------------------|----------------------|
| MEFV variants \((n = 9)\) | \textit{P-value} | \textit{P-value} | \textit{P-value} |
| Age at onset (years)* | 54.1 ± 13.5 | 58.8 ± 13.0 | 51.6 ± 13.9 | 0.35 |
| Male gender (%) | 10 (71.4%) | 4 (80.0%) | 6 (66.7%) | 1.00 |
| Fever (> 38°C) (%) | 6 (42.9%) | 0 (0.0%) | 6 (66.7%) | 0.03 |
| PC type (%) | 11 (78.6%) | 4 (80.0%) | 7 (77.8%) | 1.00 |
| Serum IL-6 (pg/dl)* | 54.2 ± 73.7 | 39.5 ± 41.2 | 60.7 ± 85.8 | 1.00 |
| CRP (mg/dl)* | 8.0 ± 5.7 | 7.4 ± 4.2 | 8.3 ± 6.6 | 0.84 |
| ALB (g/dl)* | 2.3 ± 0.8 | 2.6 ± 0.3 | 2.2 ± 0.9 | 0.32 |
| Hb (g/dl)* | 9.1 ± 1.8 | 10.4 ± 0.9 | 8.4 ± 1.8 | 0.045 |
| Performance status* | 0.9 ± 0.7 | 0.8 ± 0.4 | 1.0 ± 0.9 | 0.66 |
| CHAP score | 6.5 ± 3.0 | 5.0 ± 1.4 | 7.3 ± 3.4 | 0.28 |

*Mean ± standard deviations or number (percentages) are shown. \textit{P-values} were established using Fisher’s exact test or the Mann–Whitney \textit{U}-test. MEFV = Mediterranean fever; PC = plasma cell; CRP = C-reactive protein; ALB = albumin; Hb = hemoglobin.
classically transmitted by autosomal recessive transmission, rare cases of dominant transmission have been reported [14–17]; there are many patients with only a single MEFV variant [18]. Previous results from a pyrin knock-in mouse suggested that autoinflammatory conditions were caused by a gain-of-function of pyrin [19]. Furthermore, MEFV variants confer susceptibility to other inflammatory diseases—including adult-onset Still’s disease, Henoch–Schönlein purpura, polyarteritis nodosa and Behçet’s disease—and modify clinical phenotypes of yet other inflammatory diseases, including Crohn’s disease, rheumatoid arthritis and systemic lupus erythematosus [20]. Therefore, deregulation of the innate immune system and subsequent activation of the innate response, owing to these MEFV variants, may promote the development of various inflammatory diseases or modify their pathologies. IL-6 plays an important role in the pathogenesis of both FMF [21] and MCD. Two cases of MCD with fever were previously reported to have a heterozygous variant in exon 10 of the MEFV gene [4, 7]. Furthermore, we previously reported the rare case of a Japanese iMCD patient (who is also included in the present study) with a novel heterozygous I729M variant in exon 10 of the MEFV gene which had not been reported in any previous reports or worldwide databases, and was suggested to have a potential pathological function based on the results of molecular dynamics simulations and activated inflammasome [5]. Therefore, variants in the MEFV gene may possibly be associated with the development of iMCD as well as the other inflammatory diseases, as described above.

In the present study, patients with iMCD carried L110P, E148Q, R202Q, P369S and R408Q variants in exons 2 or 3 of the MEFV gene. Although considered less pathogenic than variants in exon 10 of the MEFV gene, a previous report showed that these variants were present in a relatively large number of Japanese patients with FMF [18]. The genetic characteristics of patients with FMF in Japan include a lower percentage of variants in MEFV exon 10 and a higher percentage of variants in MEFV exons 2 or 3 when compared with FMF patients in western countries [22]. However, a relatively high proportion of healthy individuals have MEFV exons 2 or 3 variants (especially E148Q variants in MEFV exon 2) in Japan. Based on a database search of 4773 healthy subjects in Japan (https://jmorp.megabank.tohoku.ac.jp/202001/), the frequency of each allele variant in exons 2 or 3 of the MEFV gene was as follows: L110P, 6.96%; E148Q, 22.07%; R202Q, 3.34%; P369S, 5.29%; and R408Q, 4.51%. Although iMCD had a high percentage of exons 2 or 3 variants of MEFV, comparison of data from healthy Japanese subjects indicates that there is no significant difference in the percentage between healthy Japanese subjects and patients with iMCD; however, this study was conducted in a small number of cases, and further investigation is needed. We speculate that other potential factors, including variants in other autoinflammatory disease-related genes, epigenetic modifications, post-translational modification and environmental factors may be involved in the development of iMCD.

Because fever resulting in activated inflammasome is a very common symptom within autoinflammatory diseases and presents in most patients with FMF attributed to activated inflammasome with MEFV variants, carriers of MEFV variants may be affected in terms of iMCD clinical phenotype, including fever. In general, the inflammatory anemia observed in patients with CD is believed to be due to impaired iron utilization caused by excessive production of hepcidin, which is an iron metabolism-regulating hormone, due to high levels of IL-6 [23]. However, our results here showed that there was no significant difference in serum IL-6 levels between patients with and without MEFV variants. Anemia is observed in patients with FMF [24], suggesting that inflammatory cytokines, including TNF, inhibit erythropoiesis [25–27]. Therefore, although there may be an entirely different cause for fever and/or anemia, not directly related to the MEFV gene, we speculated that various inflammatory cytokines, associated with inflammasome activated by MEFV variants, may more often exhibit fever and lower levels of hemoglobin than those lacking MEFV variants.

Patients with FMF have enhanced inflammasome signaling and better response to colchicine for fever. Case 6 had the L110P/E148Q variant, which may have had a facilitative effect on the inflammasome, resulting in a favorable response to colchicine. Regarding amyloidosis, although the presence of the MEFV gene variant L110P/E148Q (case 13) may have had a facilitatory effect, further verification is required to determine whether this is significant because iMCD alone causes amyloidosis due to chronic inflammation.

In the present study, a single heterozygous VUS in the TNFRSF1A gene was observed in two of the patients with iMCD. In addition, a homozygous and a single heterozygous VUS in the CECR1 gene was observed in each of the patients with iMCD. V363A and P271L variants in exons 9 and 10 of the TNFRSF1A gene have not been reported so far without registration of the rs number. None of these variants were observed in a database search of 4773 healthy subjects in Japan (https://jmorp.megabank.tohoku.ac.jp/202001/). Given that TNF receptor-associated periodic syndrome causal variants have been found exclusively in the extracellular domain, encoded in exons 2–6 of the TNFRSF1A gene [28], the clinical significance of those variants remains unclear. In addition, previous reports suggested that loss of function owing to a homozygous CECR1 mutation (i.e. adenosine deaminase 2 deficiency)
contributes to a CD-like phenotype in pediatric patients [8,9]. Although a homozygous H335R variant in the CECRI gene was observed in one of the patients with iMCD, the frequency of this allele variant among 4773 healthy subjects in Japan was 17.6%; it is a polymorphism observed in the general Japanese population. In addition, the clinical significance of the heterozygous R230Q variant in the CECRI gene (case 2), which is a rare variant in healthy subjects (the frequency of this allele variant among 4773 healthy subjects in Japan was 0.09%), remains unclear.

Our study has several limitations. First, it is possible that the allele frequencies in this study were over- or underestimated because of the small sample size. Secondly, we could not investigate autoinflammatory disease-related gene variants among patients with forms of CD other than iMCD-NOS. In addition, we investigated only 31 known autoinflammatory disease-related genes. Further nationwide multi-center investigation on the relationship between CD and autoinflammatory-related genes, including MEFV, is desirable. Thirdly, we investigated genomic DNA from the peripheral blood of patients, but genomic DNA from affected lymph nodes of iMCD patients should also be studied, in order to determine whether these patients have somatic mutations of autoinflammatory-related genes in such affected nodes. Fourthly, nearly all the MEFV gene variants detected in this study are polymorphisms possessed by 5% or more of the general Japanese population, and their pathogenicity is not clear. Although our results suggested the association between carriers of MEFV variants and iMCD, functional studies to determine the significance of these variants are needed. Despite these limitations, our data suggest that carriers of MEFV variants could affect clinical phenotypes of iMCD.

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CONFLICTS OF INTEREST
All the authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS
Tomohiro Koga had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study design: Yushiro Endo, Tomohiro Koga, Atsushi Kawakami. Acquisition of data: Yushiro Endo, Tomohiro Koga, Yoshihumi Ubara, Remi Sumiyoshi. Analysis and interpretation of data: Yushiro Endo, Tomohiro Koga, Kaori Furukawa. Manuscript preparation: Yushiro Endo, Tomohiro Koga. Statistical analysis: Yushiro Endo, Tomohiro Koga.

DATA AVAILABILITY STATEMENT
All data sets generated and/or analysed during this study are available from the corresponding author on reasonable request.

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REFERENCE
1. Iwaki N, Fajgenbaum DC, Nabel CS, Gion Y, Kondo E, Kawano M, et al. Clinicopathologic analysis of TAFRO syndrome demonstrates a distinct subtype of HHV-8-negative multicentric Castleman disease. Am J Hematol. 2016;91:220–6.
2. Yoshizaki K, Matsuda T, Nishimoto N, Kuritani T, Taeho L, Aozasa K, et al. Pathogenic significance of interleukin-6 (IL-6/BSF-2) in Castleman's disease. Blood 1989;74:1360–7.
3. van Rhee F, Voorhees P, Dispenzieri A, Fossà A, Srlakovic G, Ile M, et al. International, evidence-based consensus treatment guidelines for idiopathic multicentric Castleman disease. Blood 2018;132:2115–24.
4. Kone-Paut I, Hentgen V, Guillame-Citrom S, Comperot-Lacassagne S, Tran TA, Touitou I. The clinical spectrum of 94 patients carrying a single mutated MEFV allele. Rheumatology 2009;48:840–2.
5. Endo Y, Koga T, Otaki H, Sasaki D, Sumiyoshi R, Furukawa K, et al. Idiopathic multicentric Castleman disease with novel heterozygous Ile729Met mutation in exon 10 of familial Mediterranean fever gene. Rheumatology 2021;60:445–50.
6. Endo Y, Koga T, Umeda M, Furukawa K, Takenaka M, Kawakami A. Successful canakinumab treatment for activated innate response in idiopathic Castleman’s disease with multiple heterozygous MEFV exon 2 variants. Clin Immunol. 2020;219:108547.
7. Borocco C, Ballot-Schmit C, Ackermann O, Aladjidi N, Delaleu J, Giacobbi-Milet V, et al. The French paediatric cohort of Castleman disease: a retrospective report of 23 patients. Orphanet J Rare Dis. 2020;15:95.
8. Van Eyck L, Liston A, Meyts I. Mutant ADA2 in vasculopathies. N Engl J Med. 2014;371:478–9.
9. Van Niehuwense H, Humblet-Baron S, Van Eyck L, De Somer L, Dooley J, Touray T, et al. ADA2 deficiency mimicking idiopathic multicentric Castleman disease. Pediatrics 2018;142:e20172266.
10. Fajgenbaum DC, Uldrick TS, Bagg A, Frank D, Wu D, Srlakovic G, et al. International, evidence-based consensus diagnostic criteria for HHV-8-negative/idiopathic multicentric Castleman disease. Blood 2017;129:1646–57.
11. Fujimoto S, Koga T, Kawakami A, Kawabata H, Okamoto S, Mizuki M, et al. Tentative diagnostic criteria and disease severity classification for Castleman disease: a report of the research
group on Castleman disease in Japan. Mod Rheumatol. 2018;28:161–7.
12. Oshima Y, Hoshino J, Suwabe T, Hayami N, Yamanouchi M, Sekine A, et al. Multicentric Castleman's disease associated with IgA vasculitis (Henoch–Schönlein purpura) responding well to tocilizumab: a case report. Clin Rheumatol. 2017;36:729–33.
13. Imafuku A, Suwabe T, Hasegawa E, Mise K, Sumida K, Hiramatsu R, et al. Castleman's disease accompanied by hyperlipidemic cerebral hemorrhage and nephrosclerosis. Intern Med. 2013;52:1611–6.
14. Stoffels M, Szperl A, Simon A, Netea MG, Plantinga TS, van Deuren M, et al. MEFV mutations affecting pyrin amino acid 577 cause autosomal dominant autoinflammatory disease. Ann Rheum Dis. 2014;73:455–61.
15. Rowczenio DM, Iancu DS, Trojer H, Gilbertson JA, Gillmore JD, Wechalekar AD, et al. Autosomal dominant familial Mediterranean fever: a case series and genetic exploration. Rheumatology 2017;56:209–13.
16. Nakaseko H, Iwata N, Izawa K, Shibata H, Yasuoka R, Kohagura T, et al. Expanding clinical spectrum of autosomal dominant pyrin-associated autoinflammatory disorder caused by the heterozygous MEFV p.Thr577Asn variant. Rheumatology 2019;58:182–4.
17. Endo Y, Koga T, Nakashima M, Mishima H, Yoshiura KI, Kawakami A. Atypical phenotype without fever in a Japanese family with an autosomal dominant transmission of familial Mediterranean fever due to heterozygous MEFV Thr577Asn mutations. Clin Exp Rheumatol. 2019;37:161–2.
18. Migita K, Agematsu K, Yazaki M, Nonaka F, Nakamura A, Toma T, et al. Familial Mediterranean fever: genotype–phenotype correlations in Japanese patients. Medicine 2014;93:158–64.
19. Chae JJ, Cho YH, Lee GS, Cheng J, Liu PP, Feigenbaum L, et al. Gain-of-function Pyrin mutations induce NLRP3 protein-independent interleukin-1beta activation and severe autoinflammation in mice. Immunity 2011;34:755–68.
20. Westwell-Roper C, Niemietz I, Tucker LB, Brown KL. Periodic fever syndromes: beyond the single gene paradigm. Pediatr Rheumatol Online J. 2019;17:22.
21. Koga T, Migita K, Sato S, Umeda M, Nonaka F, Kawashiri S-Y, et al. Multiple serum cytokine profiling to identify combinational diagnostic biomarkers in attacks of familial Mediterranean fever. Medicine 2016;95:e3449.
22. Endo Y, Koga T, Ishida M, Fujita Y, Tsuji S, Takatani A, et al. Musculoskeletal manifestations occur predominantly in patients with later-onset familial Mediterranean fever: data from a multicenter, prospective national cohort study in Japan. Arthritis Res Ther. 2018;20:257.
23. Song SN, Tomosugi N, Kawabata H, Ishikawa T, Nishikawa T, Yoshizaki K. Down-regulation of hepcidin resulting from long-term treatment with an anti-IL-6 receptor antibody (tocilizumab) improves anemia of inflammation in multicentric Castleman disease. Blood 2010;116:3627–34.
24. Celkan T, Çelik M, Kasapçopur Ö, Özkhan A, Apak H, Oçak S, et al. The anemia of familial Mediterranean fever disease. Pediatr Hematol Oncol. 2005;22:657–65.
25. Moldawer LL, Marano MA, Wei H, Fong Y, Silen ML, Kuo G, et al. Cachectin/tumor necrosis factor-alpha alters red blood cell kinetics and induces anemia in vivo. FASEB J. 1989;3:1637–43.
26. Rusten LS, Jacobsen SE. Tumor necrosis factor (TNF)-alpha directly inhibits human erythropoiesis in vitro: role of p55 and p75 TNF receptors. Blood 1995;85:989–96.
27. Voulgari PV, Kolios G, Papadopoulos GK, Katsaraki A, Sferiadis K, Drosos AA. Role of cytokines in the pathogenesis of anemia of chronic disease in rheumatoid arthritis. Clin Immunol. 1999;92:153–60.
28. Caminero A, Comabella M, Montalban X. Role of tumour necrosis factor (TNF)-alpha and TNFRSF1A R92Q mutation in the pathogenesis of TNF receptor-associated periodic syndrome and multiple sclerosis. Clin Exp Immunol. 2011;166:338–45.

SUPPORTING INFORMATION
Additional Supporting Information may be found online in the Supporting Information section.