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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

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*,

PSMB8, *PSMA3*, *PSMB4*, *PSMB9*, *POMP*, *OTULIN*, *HOIP*, *HOIL1*, *LPIN2*, *IL36RN*, *CARD14*, *TREX1*, *RNASEH2A*, *RNASEH2B*, *SAMHD1*, *ADAR* and *IFIH1*) to detect single nucleotide variants and short insertions and deletions via targeted next-generation sequencing (NGS) using the Ion Personal Genome Machine (PGM; Thermo Fisher Scientific, Waltham, Massachusetts, USA) and Ion AmpliSeq Library Kit Plus (Thermo Fisher Scientific). The average depth of coverage of all variants detected by this NGS was 736. The variant allele frequencies in the patients were nearly 50%—or almost 100%—and no chimeras or mosaics were found.

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

TABLE 1 Participant clinical and laboratory characteristics and histopathological type

			Clinical features											
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.

TABLE 2 Participant genetic characteristic of autoinflammatory disease-related gene

	Autoinflammatory disease-related genes	
Case number	***MEFV* gene**	**The other autoinflammatory disease-related genes (*TNFRSF1A*, *NLRP3*, *MVK*, *TNFAIP3*, *CECR1*)**
1	E148Q/P369S/R408Q	*CECR1* H335R/H335R
2	L110P/E148Q	*CECR1* R230Q
3 [5]	I729M	None
4	P369S/R408Q	None
5	R202Q	*TNFRSF1A* V363A
6 [6]	L110P/E148Q	None
7	None	None
8 [12]	None	*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

TABLE 3 Participant characteristic by *MEFV* variants excluding E148Q variants (univariate analysis)

Variables	**All patients (*n* = 14)**	**The group without *MEFV* variants (n = 5)**	**The group with *MEFV* variants (n = 9)**	
MEFV variants (n = 9)	*P*-value			*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. *P*-values were established using Fisher's exact test or the Mann–Whitney *U*-test.
MEFV = Mediterranean fever; PC = plasma cell; CRP = C-reactive protein; ALB = albumin; Hb = hemoglobin.

and significantly lower levels of hemoglobin than those lacking *MEFV* variants ($P = 0.03$, $P = 0.045$, respectively). Other parameters had a tendency (but not significantly) to show higher iMCD disease activity at onset for those patients with *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 *MEFV* gene, and these patients more often exhibited fever and lower levels of hemoglobin than those lacking *MEFV* variants, excluding E148Q variants.

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

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 *CECR1* 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 *CECR1* 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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SUPPORTING INFORMATION

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

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