Table I Clinical manifestations and laboratory findings of HNL patients

Number of HNL patients	24
Gender male:female	11:13
Age at onset (years)	11.7 (3.5–14.3)*
Family history of HNL	0
Fever	24
Cervical lymphadenopathy	24
Biopsy of lymph node	8
Relapse of HNL	7
Association with autoimmune disease	1
WBC (/μL)	2840 (1340–6010)*
Platelet count ($x10^9$/L)	158 (86–308)*
LDH (IU/L)	625 (211–1179)*
CRP (mg/dL)	0.7 (0–3.7)*
Ferritin (ng/mL) (n=17)	262 (74–8123)
ESR (mm/1 h) (n=19)	31 (8–60)

*The variables expressed as median (range)

CRP C-reactive protein, *ESR* erythrocyte sedimentation rate, *LDH* lactate dehydrogenase, *WBC* white blood cell count

two-fold differences in the mean expression levels were selected. The data with low signal-to-noise ratios (S/N<2) were not used for further analysis. The data were analyzed using GeneSpring Software (Agilent Technologies, CA, USA).

Real-Time Quantitative PCR

First-Strand cDNA Synthesis Kit (GE Healthcare) with random hexamers was used to prepare the first-strand cDNA.

Table II The number of subjects in each group for discriminate analysis

Group	Number of subjects
1. Histiocytic necrotizing lymphadenitis (HNL)	24
2. Disease control (DC)	93
Kawasaki disease (KD)	11
Systemic onset juvenile idiopathic arthritis (SoJIA)	14
Systemic lupus erythematosus (SLE)	4
Measles	18
Varicella	5
Infectious mononucleosis (IM)	15
Adenovirus infection (ADV)	5
Influenza type A virus infection (FluA)	5
Purulent lymphadenitis (LNitis)	5
Sepsis	7
Lymphoid malignancy	4
3. Normal control (NC)	34

IFI44L, *CXCL10*, *GBP1*, *EPSTI1*, and *IFI27* mRNA expression levels were analyzed by TaqMan® gene expression assays Hs00199115_m1, Hs00171042_m1, Hs0026671_m1, Hs01566789_m1, and Hs00271467_m1, respectively (Life Technologies), and TaqMan Gene Expression Master Mix (Life Technologies). A TaqMan human *ACTB* (beta actin) endogenous control (Life Technologies) was used as an internal control. The mRNA expression levels of the interested and *ACTB* genes were quantified by a StepOnePlus™ Real Time PCR System and analyzed by using StepOne™ Software Version 2.1 (Life Technologies), as manufacturer's instruction. A comparative threshold cycle (C_T) method was used to determine the gene expression levels [13], by comparing the values of PBMC and lymph nodes from NHL patients with the median gene expression levels in 10 healthy donors and 4 patients with reactive lymphadenopathy, respectively. All experiments were carried out in triplicate.

Serum Concentration of CXCL10

Serum concentrations of CXCL10 (also known as IP-10) were measured by BD™ Human Chemokine Cytometric Bead Array Kit (BD Biosciences, NJ, USA) and an EPICS XL flow cytometer (Beckman Coulter, CA, USA) as manufacturers' instructions.

Statistical Analysis

Computation was carried out by using a statistical software; JMP version 8.0 (SAS Institute), according to JMP 8 Statistics and Graphics Guide, Second Edition. When we analyzed the data obtained by RT-PCR, logarithmically transformed values were used, because they showed log-normal distribution. Correlation coefficient was determined by Pearson's method. Statistical differences of the values of gene expression levels of PBMC and lymph nodes, and serum CXCL10 concentrations between HNL and controls were analyzed by Dunnett's test. Statistical difference of the serum CXCL10 concentrations between symptomatic and convalescent phases of HNL patients was analyzed by paired t-test. Canonical discrimination analysis was performed to distinguish HNL from disease controls and healthy donors by using the log-value of relative gene expression levels. The model was determined by stepwise procedure.

Results

Microarray Analysis

By microarray analysis of the mRNAs from PBMC in HNL patients, disease controls and healthy controls, one hundred and thirty seven up-regulated genes in HNL patients were

Table III Microarray analysis of peripheral blood mononuclear cells from patients with HNL, SoJIA, KD, and a healthy donor

The difference of mean gene expression levels between 2 HNL patients, 5 SoJIA patients, 3 KD patients and a healthy control donor in microarray analysis is given. One hundred and thirty-seven genes that showed more than two-fold expressional differences between HNL patients and SoJIA patients, KD patients as well as a healthy donor were selected, and the top 10 genes are listed

Gene name	Synonyms	Fold differences between a healthy donor		
		HNL	SoJIA	KD
Interferon-induced protein 44-like	*IFI44L*	12.99	3.32	0.39
Chemokine (C-X-C motif) ligand 10	*CXCL10*	12.04	2.21	0.56
Guanylate binding protein 1, interferon-inducible, 67 kDa	*GBP1*	8.30	2.51	1.12
Epithelial stromal interaction 1 (breast)	*EPSTI1*	7.10	3.01	1.05
Interferon, alpha-inducible protein 27	*IFI27*	6.90	2.14	1.05
Tumor necrosis factor (ligand) superfamily, member 10	*TNFSF10*	6.36	2.84	1.87
Immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides	*IGJ*	6.26	2.08	2.29
Interferon-induced protein 44	*IFI44*	5.59	1.97	0.96
Interferon-induced protein with tetratricopeptide repeats 3	*IFIT3*	5.29	2.31	0.40
Proteasome (prosome, macropain) activator subunit 2 (PA28 beta)	*PSME2*	4.87	2.32	0.85

identified (data not shown), and the top 10 genes are shown in Table III. Nine of these genes, other than *IGJ*, were interferon-stimulated genes (ISGs) [14].

Quantitative RT–PCR Analysis

The 5 most up-regulated genes (*IFI44L*, *CXCL10*, *GBP1*, *EPSTI1*, and *IFI27*) in HNL patients were analyzed by RT-PCR to confirm the microarray data. As shown in Fig. 1, the expression levels of the 5 genes in HNL patients were significantly higher than those in normal controls. Gene expression profile of the relapsing patients was not significantly different from that of patients without relapse (data not shown). Interestingly, positive correlations were observed in the expression levels among the 5 genes (r^2=0.28–0.60 : Fig. 2), suggesting the up-regulation of these 5 genes by some common mechanism, possibly through the stimulation of type 1 IFNs. Although the expression levels of these genes in HNL patients were higher than those in normal controls, bacterial infections, and lymphoid malignancy, the expression levels of each gene were not specific for HNL: these were equivalent to or less than those of viral infections and SLE (Fig. 1).

Analysis with Lymph Node and Serum Samples of HNL Patients

We then investigated the expression levels of these ISGs in involved lymph nodes by quantitative PCR. The genes were expressed significantly higher in HNL than in reactive lymphadenopathy (Fig. 3). Next, we analyzed the serum concentration of CXCL10. Although serum CXCL10 concentrations of HNL patients were not significantly different from those of KD, IM, and purulent lymphadenitis patients (Fig. 4a), the CXCL10 levels at symptomatic phase diminished at convalescent phase in HNL patients (Fig. 4b).

Discriminate Analysis

For the purpose of classifying HNL based on the gene expression profile, we performed canonical discrimination analysis by using the log-value of relative gene expression levels of the 5 ISGs. The subjects were separated into 3 groups as shown in Table II for discriminate analysis. In the stepwise procedure, all 5 genes were selected as parameter. The canonical discriminate analysis was able to classify the subjects into 3 groups with 84.2 % accuracy (misclassified number: 24). The area under the curve (AUC) of HNL group was 0.975 (Fig. 5a and Table IV). The scoring coefficients in canonical plot were as follows:

$$\text{Canonical 1} = -0.1947\text{IFI44L} - 0.2058\text{CXCL10} + 0.4870\text{GBP1} - 0.1620\text{EPSTI1} + 0.2197\text{IFI27},$$

$$\text{Canonical 2} = 0.2485\text{IFI44L} + 0.2488\text{CXCL10} - 0.2387\text{GBP1} - 0.2802\text{EPSTI1} + 0.1598\text{IFI27}$$

In the 2 group model (HNL and disease control), top 3 genes (*IFI44L*, *CXCL10*, and *GBP1*) were selected as parameters and the statistical power was equal to 3 group model; the accuracy and AUC were 82.2 % (misclassified number: 21) and 0.942, respectively (Fig. 5b and Table IV). The scoring coefficients in canonical plot were as follows:

$$\text{Canonical 1} = -0.2632\text{IFI44L} - 0.3061\text{CXCL10} + 0.5101\text{GBP1}$$

Discussion

In this study, we observed that ISGs (*IFI44L*, *CXCL10*, *GBP1*, *EPSTI1*, and *IFI27*) were up-regulated in PMBC as well as in involved lymph nodes of HNL patients (Table III, Fig. 1). The discriminate analysis showed that the expression levels of these genes were specific for HNL patients (Fig. 5).

The type I IFN response protects a host against the invasion of viral pathogens. The cellular factors mediating this defense are the products of the ISGs [14]. The involved lymph nodes of HNL are primarily composed of pDCs, histiocytes and T lymphocytes, and pDCs are known to be one of the major producers of type I IFNs [1, 2]. It is reported that CXCL10 and IL-18 were expressed in histiocytes, and CXCR3 and IFN γ were expressed in T lymphocytes by immunohistochemical staining, which suggested that the cytokine and chemokine pathways play important roles in the pathophysiology of HNL [15]. Elevated serum levels of CXCL10 (Figs. 1 and 4), as well as IFN-γ and IL-6 [11] suggest a systemic inflammation in HNL, which possibly contributed to the gene expression profile of PBMC. It is supposed that pDCs were the major producer of the type 1 IFNs which induced ISGs

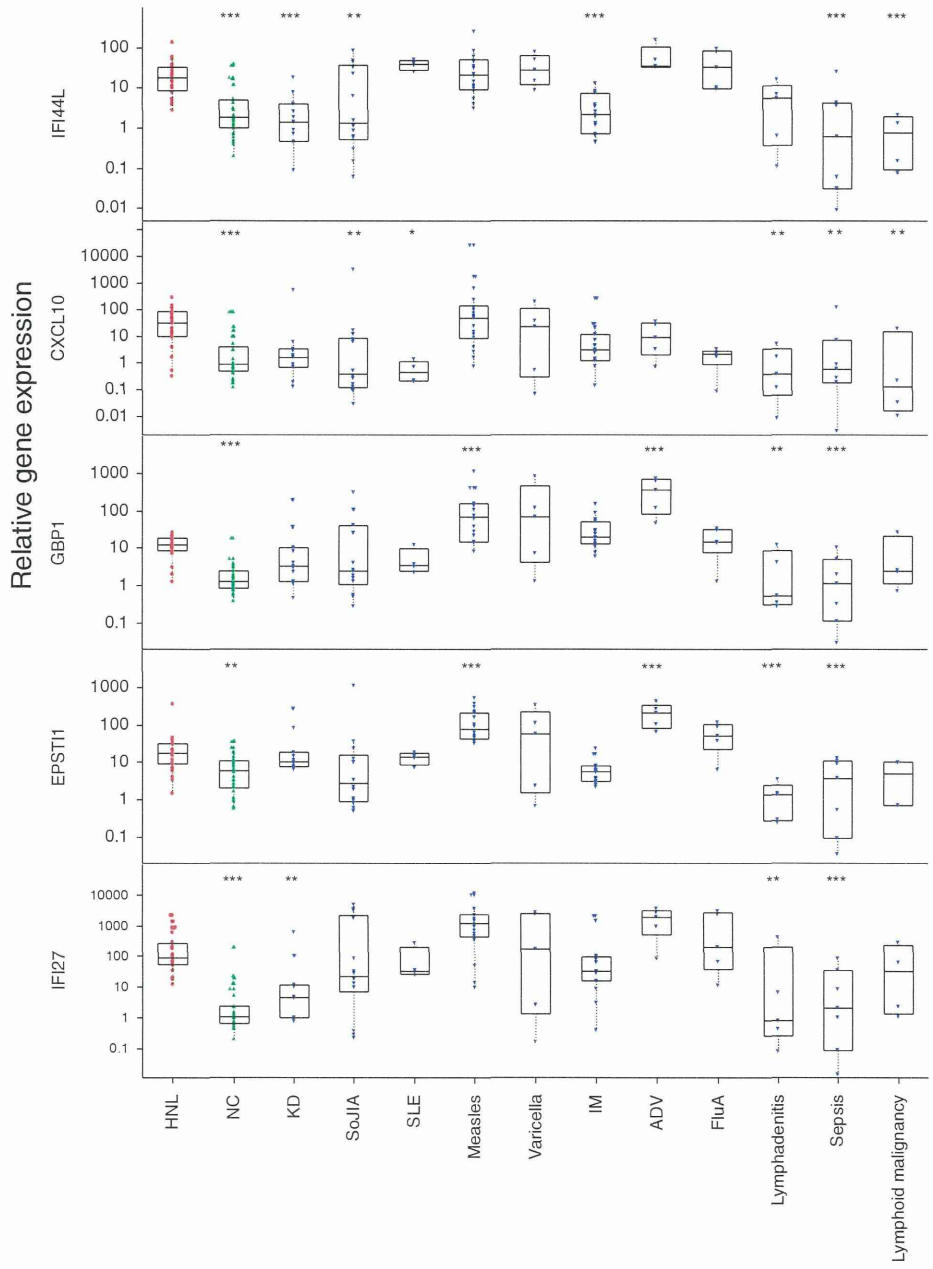

Fig. 1 Relative gene expression levels of mRNA from PBMC. The form of boxplot is as follows. The *bottom* and *top* of the box are the 25th percentile and the 75th percentile points, respectively. The *line* inside the box is the median. Each whisker extends up to 1.5 interquartile ranges from the end of the box. *$p<0.05$, **$p<0.01$, ***$p<0.001$

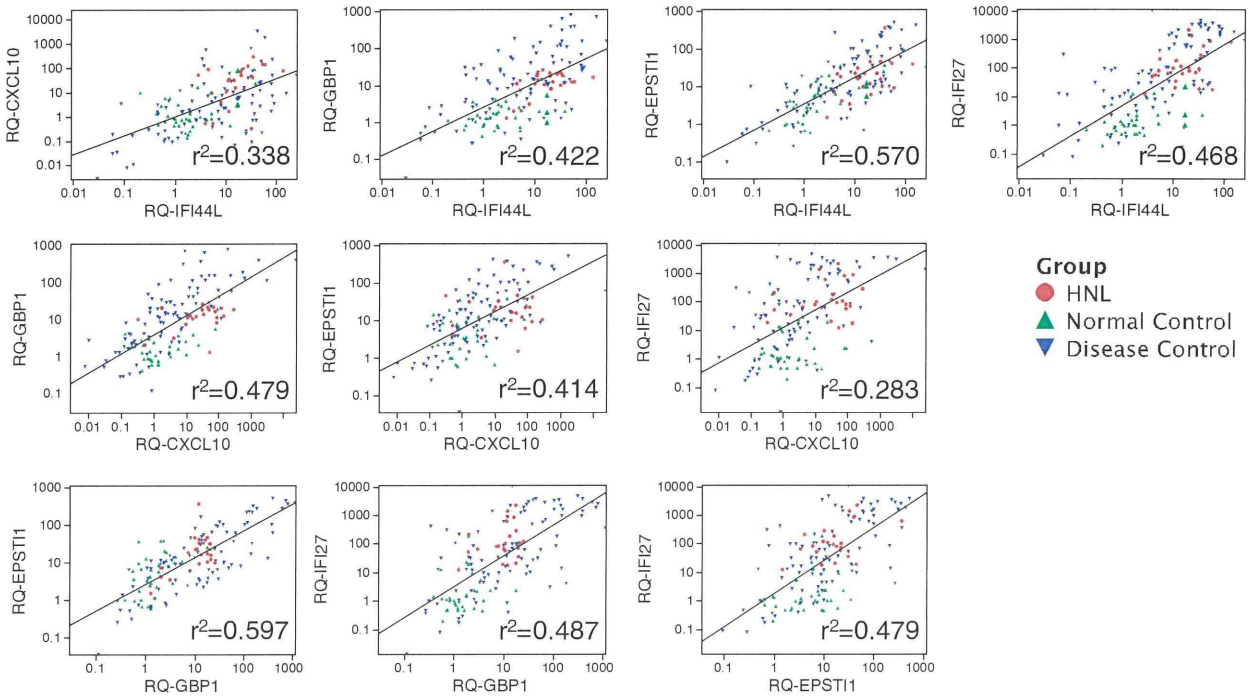

Fig. 2 Scatter plot of gene expression levels of 5 ISGs. The line is least-squares fit to data. *r2* correlation coefficient, *RQ* relative quantitation value

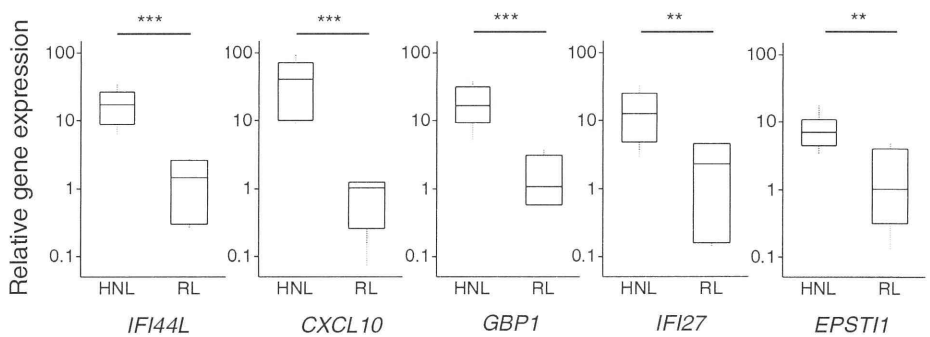

Fig. 3 Relative gene expression levels of mRNA from lymph nodes of patients with HNL and reactive lymphadenopathy. The form of box-plot is the same as Fig. 1. *RL* reactive lymphadenopathy $^{**}p<0.01$, $^{***}p<0.001$

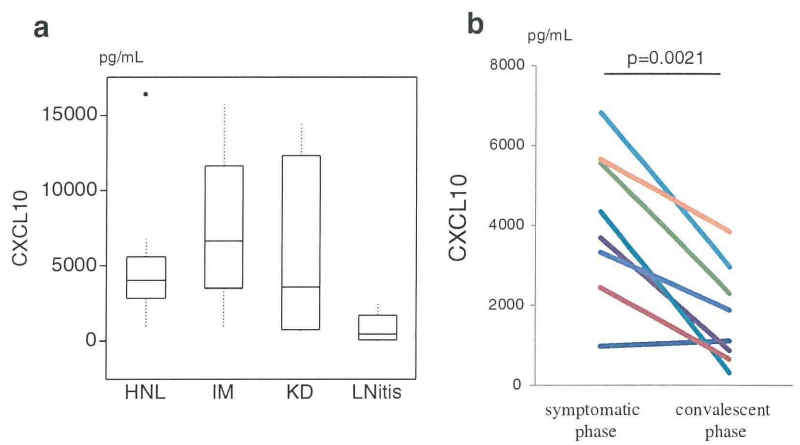

Fig. 4 Serum concentration of CXCL10 in HNL patients. **a** Serum concentration of CXCL10 at acute phase of HNL, IM, KD and LNitis. The form of box-plot is the same as Fig. 1. There was no significant difference between HNL (n=12) and disease control; IM (n=4), KD (n=5), and LNitis (n=5). **b** Diminishing serum CXCL10 concentration at convalescent phase of HNL (n=8)

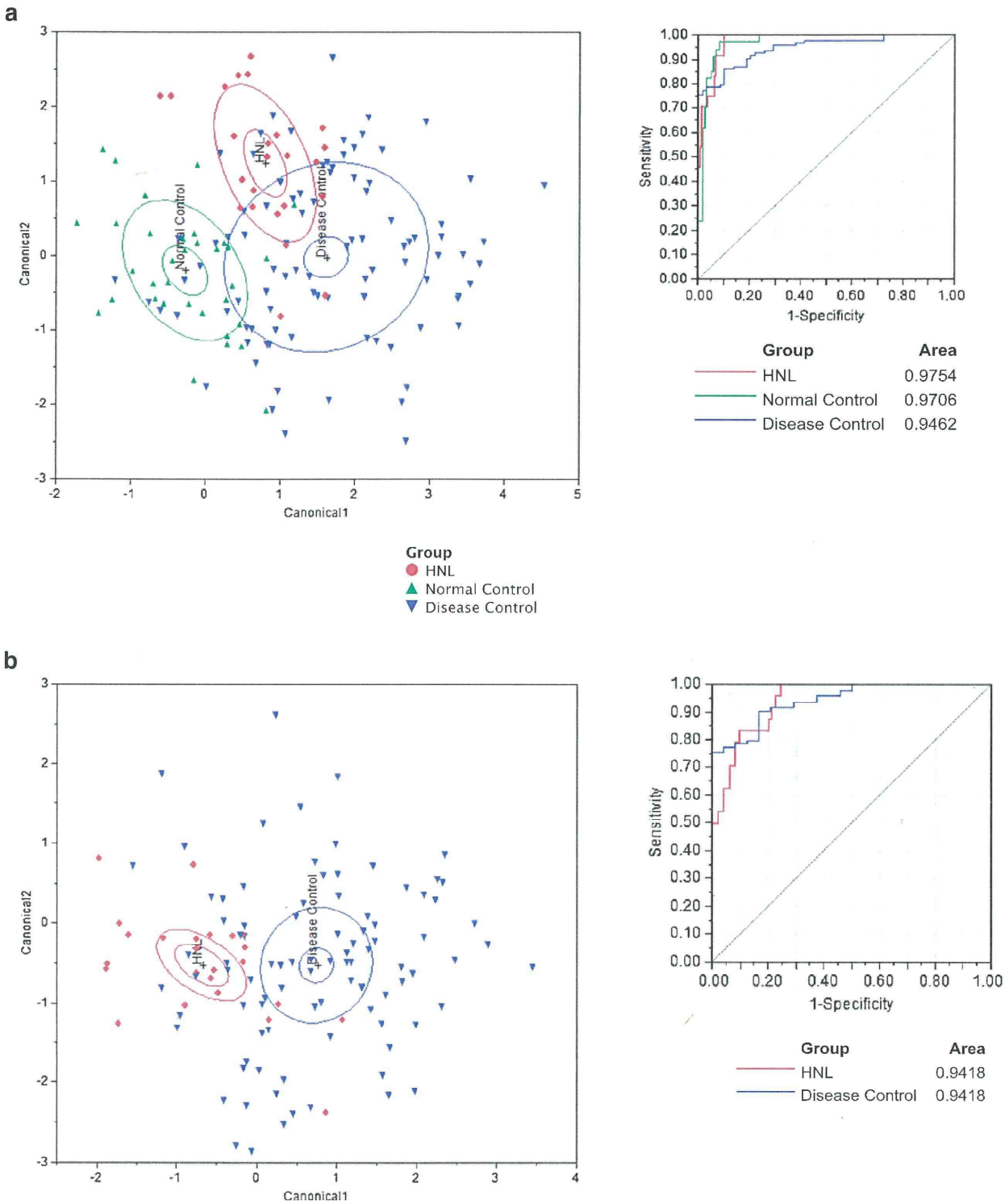

Fig. 5 Canonical plot and ROC curve in 3 groups; HNL (n=24), disease control (n=93), and normal control (n=34) (**a**), and in 2 groups; HNL and disease control (**b**). The inner and outer ellipses show 95 % and 50 % confidence regions, respectively

expression in this disease. On the other hand, these cytokines themselves were not highly up-regulated in PMMC of HNL patients in this study (data not shown). A paucity of pDCs in PBMC would account for it [16].

Table IV Numbers in discriminate analysis: actual rows by predicted columns

Three groups using 5 gene expression levels (Fig.5a)			
	HNL	Disease control	Normal control
HNL	22	2	0
Disease control	11	74	9
Normal control	1	1	32
Misclassified number: 24, accuracy: 84.2 %			
Two groups using 3 gene expression levels (Fig.5b)			
	HNL	Disease control	
HNL	20	4	
Disease control	17	77	
Misclassified number: 21, accuracy: 82.2 %			

Clinically, malignant lymphoma and leukemia are the most important disorders to be distinguished from HNL. A lymph node biopsy is often required for definitive diagnosis. CXCL10 expression may be enhanced in PBMC of the patients with these diseases because it was reported that CXCL10 expression was associated with the progression of leukemia and with the poor prognosis of lymphoma [17, 18]. In this study, we could distinguish HNL from these diseases by analyzing expression levels of other ISGs (Figs. 1 and 5). On the other hand, SLE presents the most challenging differential consideration, and sometimes its histologic presentation is almost identical to HNL [1, 2, 9]. Ishii et al. [16] reported that expression levels of *IFI27* and *EPSTI1* were increased in PBMC of SLE patients. GBP1 was expressed in lesional skin, and *IFI27*, *IFI44* and *IFI44L* were up-regulated in the synovium of patients with SLE [19, 20]. *IFI27* was also increased in PBMC of patients with Sjögren syndrome [21]. In our study, 4 ISGs, other than CXCL10, were up-regulated in SLE patients. Szturz et al. indicates that the pattern of serum cytokine levels in patients with HNL is similar to that of SLE patients [22]. These findings suggest that HNL and SLE are similar in the pathophysiology which includes immune responses mediated by type 1 IFNs.

Hundreds of ISGs were identified and different viruses are targeted by unique sets of ISGs. In addition, combined expression of pairs of ISGs showed additive antiviral effects [14]. We found that the expression levels of five ISGs (*IFI44L*, *CXCL10*, *GBP1*, *EPSTI1*, and *IFI27*) showed log-normal distribution and moderately positive correlation among them (r^2=0.28–0.60) (Figs. 1 and 2). These results suggest that the 5 genes are coordinately-induced in HNL. On the other hand, up-regulation of ISGs was similarly observed in patients with measles, varicella and other viral infections, and it was reported that GBP1, IFI27 and IFI44L could suppress hepatitis C virus replication [14, 23]. These findings indicate that HNL might be related with certain viral infections.

There are two kinds of ISGs, broad-acting effectors like interferon regulatory factor 1 (IRF1), retinoic acid-inducible gene-I (RIG-I), and melanoma differentiation-associated protein 5 (MDA5), and specific effectors which include IFI44L. It is known that unique sets of ISGs are important for specific antiviral effects [14]. The combination of up-regulated ISGs in HNL seemed to be a specific response induced by viral infections or autoantigens, which would be helpful for a non-invasive diagnosis for HNL.

There is a limitation for the availability of this model in daily routine for the diagnosis of HNL. Although it can not give the direct definitive diagnosis of HNL itself, the evaluation of the ISGs mRNA expression levels of peripheral blood seems to be helpful. Further research with more patients would be necessary for the early, non-invasive, and definitive diagnosis for HNL.

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Safety and efficacy of canakinumab in Japanese patients with phenotypes of cryopyrin-associated periodic syndrome as established in the first open-label, phase-3 pivotal study (24-week results)

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Abstract

Objectives

Cryopyrin-associated periodic syndrome (CAPS), a rare hereditary auto-inflammatory disease, is associated with mutations in the NLRP3 gene resulting in elevated interleukin-1β (IL-1 β) release. CAPS generally occurs in early childhood with most patients presenting with periodic fever, skin rash, osteoarthropathy, aseptic meningitis, sensorineural hearing loss and optic neuritis. Canakinumab, a fully human anti-IL-1β monoclonal antibody which binds selectively to IL-1β, has demonstrated good efficacy with CAPS. This is the first study to evaluate the safety and efficacy of canakinumab in Japanese patients with CAPS.

Methods

In this open-label study, 19 Japanese CAPS patients aged ≥2 years received canakinumab either 150 mg s.c. or 2 mg/kg for patients with a body weight ≤ 40 kg every 8 weeks for 24 weeks. The primary objective was to assess the proportion of patients who were free of relapse at week 24.

Results

A complete response was achieved in 18 (94.7%) patients with some requiring a dose and/or a frequency adjustment to attain full clinical response. The majority of patients (14/18; 77.8%) were in remission, i.e. free of relapse at week 24. Auto-inflammatory disease activity as assessed by physician's global assessment declined from baseline to end of the study (score of absent in 10.5% at baseline versus *31.6% at end of the study). Two patients had serious adverse events (SAEs), which resolved with standard treatment. One patient reported a mild injection-site reaction. No malignancies or deaths were reported during the study.*

Conclusion

Canakinumab 150 mg s.c. every 8 weeks was well-tolerated, highly efficacious and offered a convenient dosing regimen for treating Japanese patients with CAPS.

Key words

canakinumab, cryopyrin-associated periodic syndrome, interleukin-1β, auto inflammatory syndromes

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Competing interests: none declared.

Introduction

Cryopyrin-associated periodic syndrome (CAPS) represents a group of rare inherited auto-inflammatory diseases and encompasses phenotypes of varying severity. An increase in severity is evident between phenotypes: familial cold auto-inflammatory syndrome (FCAS) is the mildest, while Muckle-Wells syndrome (MWS) is predominantly of intermediate severity, and neonatal-onset multisystem inflammatory disease (NOMID)/chronic infantile neurological cutaneous and articular syndrome (CINCA) is the most severe phenotype of CAPS. All phenotypes are characterised by urticaria-like rash, fever, variant degree of central nervous system and tissue inflammation, arthropathy, risk of development of amyloidosis (1) and other constitutional symptoms. CAPS is associated with mutations of the *NLRP3* gene encoding cryopyrin (2-6), an important component of inflammasome. Inflammasone activates caspase-1, leading to enhanced production of the cytokine interleukin-1beta (IL-1β) and subsequent inflammation (7, 8). The pathogenic role of IL-1β in CAPS has been demonstrated by the achievement of complete response after treatment with IL-1β inhibitors (9-13). Positive therapeutic effects of the IL-1 receptor antagonist and anakinra have been hampered by the need for frequent injections (14-17) associated with severe pain, which impairs the quality of life of patients, especially the paediatric population. Canakinumab (ACZ885, Ilaris®, Novartis Pharma), a fully human anti-IL-1β monoclonal antibody (18), has shown prolonged selective IL-1β inhibition (19, 20) and has demonstrated rapid (within hours), complete and sustained response in CAPS patients of mainly Caucasian origin without any consistent pattern of side effects (21). Canakinumab is approved by the US Food and Drug Administration (FDA) for FCAS and MWS (22) only and by EMA for treatment of all three phenotypes of CAPS (23).

At present, there are no approved therapies for CAPS in Japan. The present study was therefore conducted to evaluate safety and efficacy of canakinumab in Japanese paediatric and adult patients with CAPS. Herein we report the study data up to 24 weeks.

Materials and methods

Study design, patients and study definitions

This was an open-label, safety and efficacy study of canakinumab administered for 24 weeks (6 months) in Japanese patients diagnosed with FCAS, MWS or NOMID. Molecular diagnosis showed that 17 (89.5%) patients were positive for *NLRP3* mutations and two (10.5%) patients (one each with MWS and NOMID) were negative for the mutation. The study included an extension phase to provide canakinumab treatment to study patients until canakinumab is marketed in Japan. Two NOMID patients aged 2 and 3 years previously treated with anti-IL-1 agents (anakinra) were enrolled.

Patients received canakinumab 150 mg s.c. or 2 mg/kg for those patients with body weight ≤40 kg for every 8 weeks. In case of residual symptoms, stepwise increase of the dose up to 600 mg s.c. or 8 mg/kg s.c. (≤40 kg) and/or increased dosing frequency were allowed.

After a 6-hour washout period for those patients previously treated with anakinra, 19 patients were included. Ten had received anakinra prior to study initiation, of which five patients had reported a complete response, while the remaining had achieved partial response to anakinra. Patients requiring oral steroids, NSAIDs and/or disease-modifying anti-rheumatic drugs (DMARDs) were enrolled if they were on a stable dose (oral steroids: <20 mg/day or ≤0.4 mg/kg prednisone or prednisone equivalent, whichever applies) for at least 4 weeks prior to the screening visit. Steroid therapy was tapered after the first canakinumab treatment cycle (8 weeks between doses), at the discretion of the investigator. TNF-α inhibitors and IL-6 receptor blockers were not allowed during the study. Women of child bearing potential had to use an accepted form of contraception during the study and for at least 3 months after the last dose. Patients receiving live vaccine within 3 months before recruitment were excluded.

Complete response assessed at day 15 and day 29 was defined by (i) physician's global assessment of no or minimal auto-inflammatory disease (on a 5-point Likert scale ranging from absent, minimal, mild, moderate to severe) and assessment of no or minimal skin disease, and (ii) serological remission defined as serum CRP <1 mg/dL, and/or SAA <10 μg/mL. Patients who did not achieve (or maintain) complete response following canakinumab injection in any treatment period could receive a dose escalation (supporting Fig. 1). The possible step-wise up-titration regimens were: 300 mg s.c. (or 4 mg/kg for patients with a body weight ≤40 kg), 450 mg s.c. (or 6 mg/kg for patients with a body weight ≤40 kg), and 600 mg s.c. (or 8 mg/kg for patients with a body weight ≤40 kg).

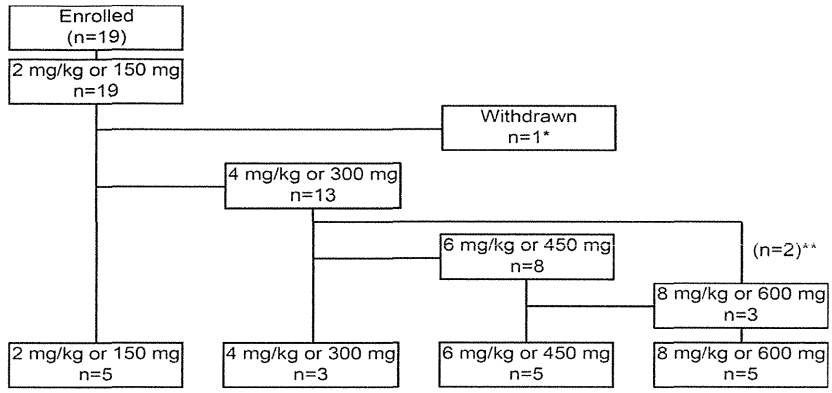
The primary efficacy endpoint was defined as the proportion of patients who did not experience a relapse at week 24. Relapse was defined as clinical relapse (physician's global assessment of both auto-inflammatory disease activity and assessment of skin disease, mild or greater) and serological relapse (serum CRP >3 mg/dL, and/or SAA >30 μg/mL).

Clinical improvement of the central nervous system (CNS) was assessed in NOMID patients only (defined as a mean weekly headache score [from the daily diary] <0.5 and a normal white cell count [≤15 cells/mm^3] in cerebrospinal fluid). Other key secondary endpoints included safety and tolerability of canakinumab, assessed by the occurrence of adverse events (AEs), serious AEs (SAEs) and immunogenicity.

This study was approved by the Independent Ethics Committee for each centre and performed in accordance to the ethical principles of the Declaration of Helsinki. All patients, parents or legal guardians (for patients aged <20 years) provided written informed consent.

Statistical analyses

Safety and full analysis set (efficacy analysis) included all patients who received at least one dose of study treatment. Only 19 patients were enrolled due to the low prevalence of CAPS, hence the estimation of statistical power was not applicable. Descriptive statistics were used to summarise demographics, baseline characteristics, efficacy and safety. Missing values were not imputed.



*One patient withdrew from this study by cancellation of the consent.

**Two patients needed two up titrations till Day 15 due to incomplete response to the first administration of canakinumab. Patients with incomplete response from the standard dosing regimen (2 mg/kg or 150 mg) received step-wise up-titration regimen. Patients who did not achieve complete response or had a relapse before the next planned administration received a dose up-titration.

Fig. 1. Patient disposition and dosing.

Results

Patients, demographic and baseline characteristics

A total of 19 CAPS patients (12 [63.2%] male/7 [36.8%] female) with a diagnosis of MWS (n=7; 36.8%) or NOMID (n=12; 63.2%) were enrolled in this study, of which 18 (94.7%) completed the 24-week study phase. One patient withdrew consent (Fig. 1). At study entry, there were 11 patients (57.9%) aged <16 years and eight patients (42.1%) aged 16 years or older. Median age was 14 years (range 2–48). Of 19 patients, five (26.3%) weighed >40 kg at baseline. Other key demographic and baseline characteristics are summarised in Table I.

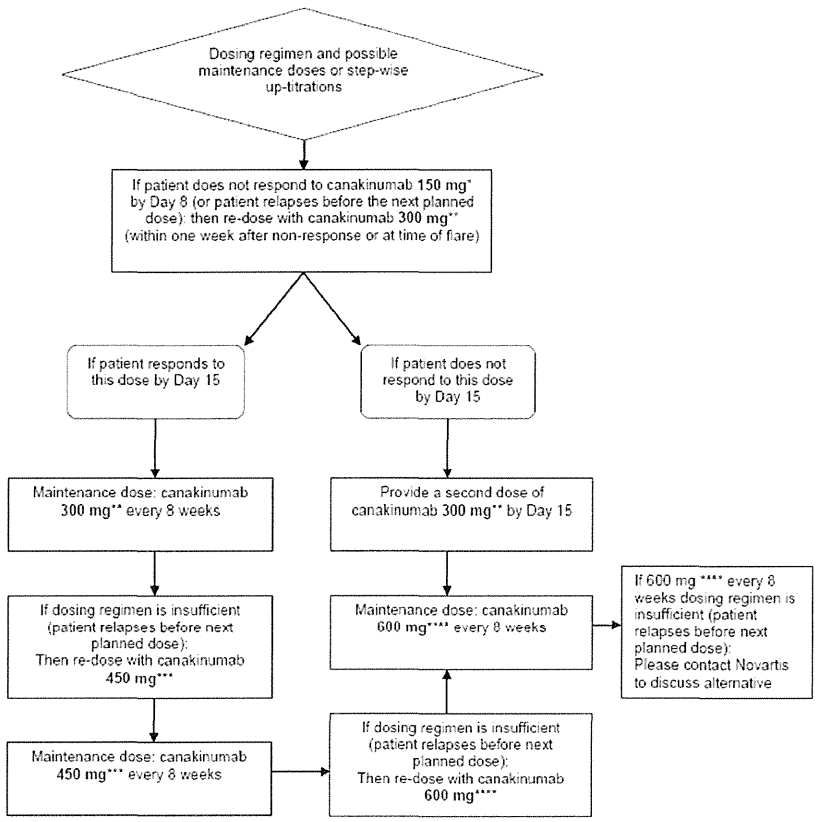
Treatment with canakinumab

At time of the 24-week analysis, the median treatment duration was 168 days (range 59–197 days) and patients received an average of 4.1 injections over 24 weeks of the study; 13 (68%) patients (MWS; n=4 and NOMID, n=9) received an up-titration of their dose, primarily due to absence of a complete response and in one patient the dose frequency was increased to every 6 weeks starting from day 49. In one NOMID patient aged 16 years, the

Table I. Baseline demographics and disease characteristics (safety population).

Characteristics	Canakinumab (n=19)
Sex, n (%)	
Male	12 (63.2)
Female	7 (36.8)
Age (years)	
Mean (SD)	14.8 (11.4)
Median (range)	14.0 (2–48)
≥2– <12 years, n (%)	8.0 (42.1)
≥12– <16 years, n (%)	3 (15.8)
≥16 years, n (%)	8 (42.1)
Weight (kg), n (%)	
≤40	14 (73.7)
>40	5 (26.3)
BMI (kg/m^2)	
Mean (SD)	17.6 (2.2)
Median (range)	17.2 (13.5–21.5)
Diagnosis, n (%)	
FCAS	0
MWS	7 (36.8)
NOMID	12 (63.2)
Molecular diagnosis of	
NLRP3 mutation, n (%)	19 (100.0)
Positive	17 (89.5%)
Negative	2 (10.5%)
Previous use of anakinra, n (%)	10 (52.6)
C-reactive protein (mg/dL) (normal value: <1mg/dL)	
Mean (SD)	4.52 (4.3)
Median (range)	3.3 (0.1–13.2)
Serum Amyloid A (μg/mL) (normal value: <10μg/mL)	
Mean (SD)	324.2 (364)
Median (range)	236 (2.6–1380)

BMI: body mass index; FCAS: familial cold autoinflammatory syndrome; MWS: Muckle-Wells syndrome; NOMID: neonatal-onset multisystem inflammatory disease; NLRP3: NOD-like receptor family, pyrin domain containing3; SD: standard deviation.



* canakinumab 150 mg s.c. for patients whose body weight is > 40 kg (or 2 mg/kg for patients with a body weight ≤ 40 kg)
** canakinumab 300 mg s.c. (or 4 mg/kg for patients with a body weight ≤ 40 kg)
*** canakinumab 450 mg s.c. (or 6 mg/kg for patients with a body weight ≤ 40 kg)
**** canakinumab 600 mg s.c. (or 8 mg/kg for patients with a body weight ≤ 40 kg)
There is currently no long-term safety information for doses greater than 600 mg s.c. available.
The above outlined decision tree may be applied to those patients who either did not achieve a complete response by Day 8 or Day 15 or to those patients who relapse prior to their next scheduled dose.

Supporting Fig. 1. Alternative dosing regimen for CAPS patients who do not experience sufficient symptomatic relief.

canakinumab dose was escalated to the highest dose of 600 mg. Four patients (8–25 years) with baseline body weight ≤40kg received a dose escalation to 8 mg/kg.

Proportionally higher mean last doses of canakinumab were required in patients ≤40 kg (n=12) *versus* >40 kg (n=6) at 6 mg/kg and 250 mg, respectively; in patients weighing >40 kg, the canakinumab dose administered was 350 and 150 mg for NOMID and MWS, respectively.

Efficacy

Relapse assessment. Overall, protocol-defined complete response was achieved in 18 (94.7%) patients. One patient achieved a complete response by day 148. This patient achieved clinical remission by day 29, but the inflammatory markers remained elevated until day 148. One non-responder patient achieved clinical remission, but the patient's CRP and SAA levels remained above normal during the study; however there was a significant decrease by week 24 compared to baseline. Some patients required either a dose escalation and/or a frequency adjustment to attain full clinical response (supporting Fig. 1); 15 (78.9%) patients achieved a complete response within 15 days, 2 patients were up-titrated within 29 days, and 1 patient by day 148. At week 24, the majority of patients (n=14/18 [77.8%]) were in remission, *i.e.* free of relapse (Table II).

Table II. Relapse at week 24 in MWS and NOMID patients (full analysis set).

Characteristics	Canakinumab n=19 n (%)
Number of complete responders by week 24	
Total	18 (94.7)
Day 15*	15 (78.9)
Day 29*	2 (10.5)
Day 148*	1 (5.3)
Relapse at week 24	4 (22.2)
No relapse at week 24	14 (77.8)
MWS patients	6 (85.7)
NOMID patients	8 (72.7)
No clinical/serological relapse at week 24	12 (66.7)
Discontinue prematurely prior to week 24	1 (5.6)

*Patients requiring either a dose and/or a frequency adjustment to attain full clinical response.
MWS: Muckle-Wells syndrome; NOMID: neonatal-onset multisystem inflammatory disease.

Of 12 NOMID patients, 11 achieved complete response by week 24 and nine achieved a complete response by day 15; one achieved complete response with dose adjustment by day 29 and one by day 148. Three (27.3%) out of the 11 complete responders (all NOMID patients) had a relapse at week 24. All patients with MWS (n=7) achieved complete response by week 24, though one patient had a relapse at week 24. All except one patient achieved complete response with canakinumab. All prior responders to anakinra also achieved a complete response with canakinumab.

Auto-inflammatory disease activity

The severity of auto-inflammatory disease activity as assessed by physician's global assessment declined from baseline to the end of the treatment period. This decrease in disease activity was apparent in all the individual symptom components including assessments of skin disease, headache/migraine, conjunctivitis and fatigue/malaise (Fig. 2).

Inflammatory markers

Canakinumab treatment induced a rapid decline in CRP levels within 15 days (Fig. 3a). Overall, mean CRP levels decreased by 2.94±2.99 mg/dL (38% decrease) from baseline to end of the study, day 169 (4.52 mg/dL *vs.* 1.19

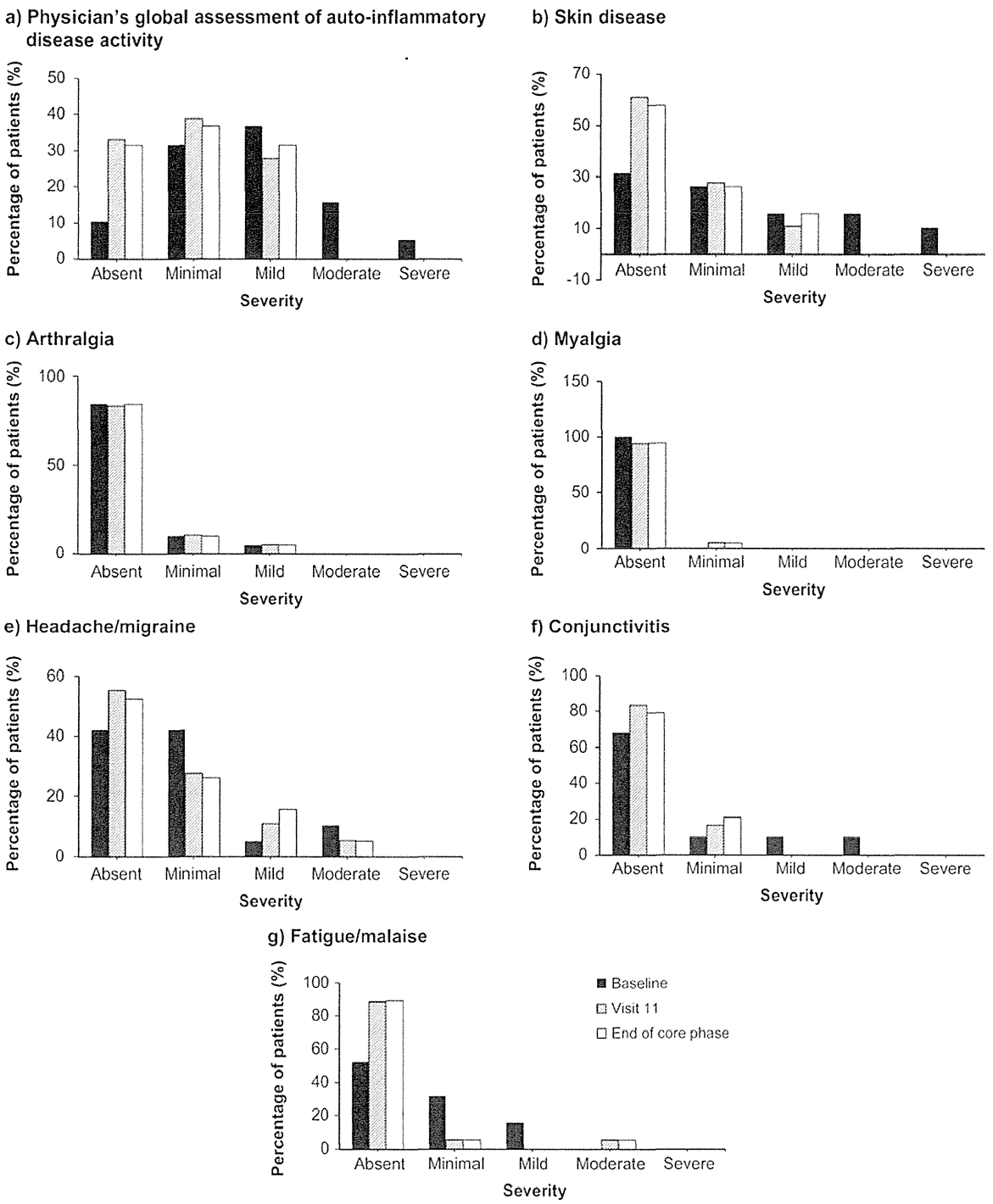


Fig. 2. Summary of assessment of auto-inflammatory disease activity (full analysis set).

mg/dL). A similar trend was observed for mean serum SAA level, which decreased from baseline to end of the study (324.19 μg/mL *vs.* 54.71 μg/mL) (Fig. 3b). On day 57, there was an increase in CRP and SAA levels, however this was driven by measurements from three patients whose mean values were near normal at other time points.

Immunogencity

Of the 19 patients, three were detected with anti-canakinumab binding antibodies during one of the post-dose assessments. However, no anti-canakinumab antibodies were detected afterwards.

Specific assessments in NOMID patients

A protocol-defined CNS remission was achieved in 33.3% (n=4/12) of the NO-

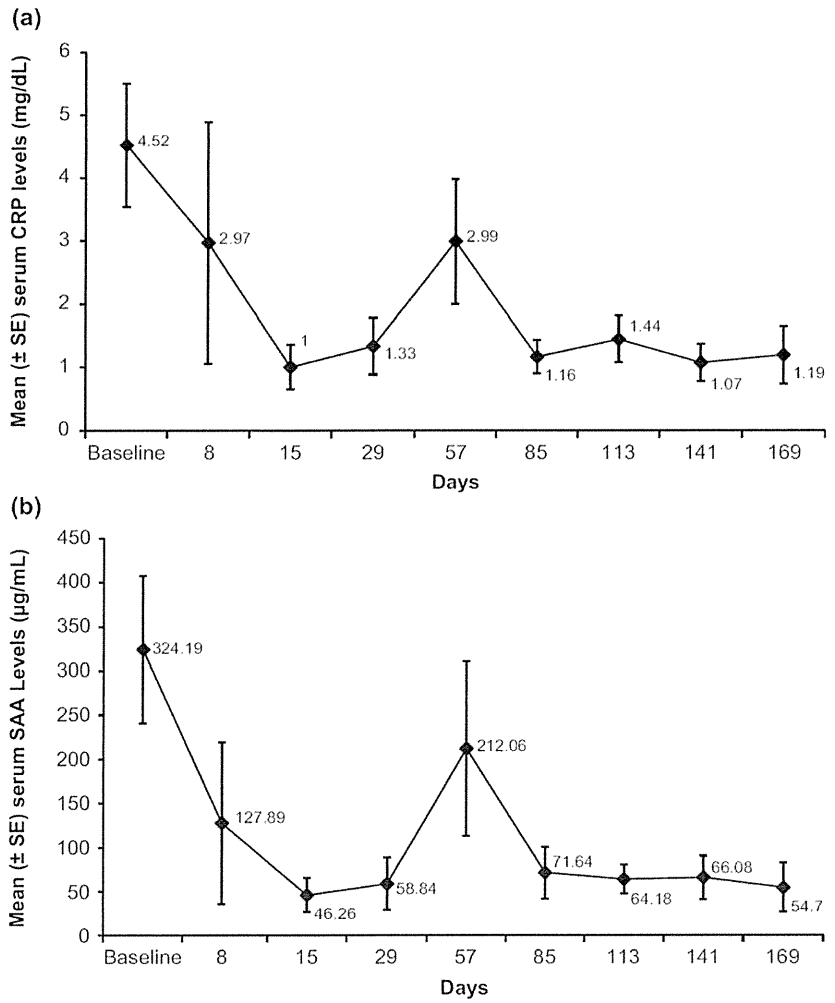


Fig. 3. (a) Serum CRP level across time points (full analysis set); (b) Serum SAA levels across time points.

MID patients by day 8 and in 41.7% (n=5/12) at the end of study; 9/12 patients had CNS remission at week 24 (with just the headache score). Lumbar puncture was only performed in 7/12 patients, of which five were in CNS remission based on the headache score and normal white cell count. A CNS relapse was reported in two (16.7%) patients on day 57 and in one patient (9.1%) on day 113. Of the three patients with a protocol-defined CNS relapse, one was uptitrated from 4mg/kg to 6mg/kg due to a concomitant clinical and serological relapse. In the other two patients, no uptitration was performed for CNS relapse. In these three patients, there was no association between the CNS relapse and clinical flare. The results of key cerebrospinal fluid assessments in NOMID patients were available in only 6 patients, who had both baseline and week 24 values. In these patients (n=6), mononuclear cells (lymphocytes, macrophages, monocytes) remained unchanged or elevated slightly from baseline to week 24 (normal values: adult ≤5 WBC/mm^3, newborns ≤20 WBC/mm^3). Absolute neutrophils which markedly reduced in two NOMID patients remained largely unchanged in the other three patients, even though it was elevated in one patient at week 24 compared with baseline. None of these patients reported headache, but they were noted to have elevated CRP and/or SAA levels. In addition to elevated SAA levels, one patient had physician's global assessment of autoinflammatory disease activity above minimal and had a relapse at week 24.

Table III. Most frequently occurring (>10%) adverse events regardless of study drug relationship (safety population).

Primary system organ class/ preferred term	Canakinumab n=19 n (%)
Total patients with AEs	18 (94.7)
Gastrointestinal disorders	7 (36.8)
Abdominal pain upper	2 (10.5)
Diarrhoea	2 (10.5)
Stomatitis	2 (10.5)
General disorders and administration site conditions	3 (15.8)
Infections and infestations	16 (84.2)
Nasopharyngitis	7 (36.8)
Gastroenteritis	6 (31.6)
Upper respiratory tract infection	3 (15.8)
Nervous system disorders	2 (10.5)
Respiratory, thoracic and mediastinal disorders	5 (26.3)
Rhinorrhoea	3 (15.8)
Cough	2 (10.5)
Skin and subcutaneous tissue disorders	6 (31.6)
Acne	2 (10.5)
Dry skin	2 (10.5)
Urticaria	2 (10.5)
Vascular disorders	2 (10.5)
Hypertension	2 (10.5)

A patient with multiple occurrences of an AE is counted only once in the AE category. A patient with multiple adverse events within a primary system organ class is counted only once in the total row. AE: adverse event.

Safety

Overall, 18 (94.7%) patients experienced at least one AE. The most commonly reported AEs (≥15% of patients) were nasopharyngitis (n=7, 36.8%), gastroenteritis (n=6, 31.6%), upper respiratory tract infection (n=3, 15.8%), and rhinorrhea (n=3, 15.8%). Twelve (63.2%) patients reported AEs, which were suspected to be study drug-related (Table III). The majority of AEs were mild (n=13, 68.4%) or moderate (n=3, 15.8%) in severity. Severe AEs of diffuse vasculitis and pneumonia were each reported in one (5.3%) patient. All but one MWS patient experienced at least one AE. Nasopharyngitis was reported in a higher proportion of NOMID patients (n=6, 50%) compared to MWS patients (n=1, 14.3%). All other AEs in NOMID and MWS patients occurred at similar frequencies or in less than three patients in each group. Two patients had serious AEs, which were suspected to be treat-

ment-related (Parvovirus infection and Epstein-Barr virus infection [n=1] and pneumonia [n=1]), but resolved with standard treatment. Of the 19 patients, only one reported a mild injection-site reaction. No deaths were reported during the study. Higher canakinumab s.c. doses (>150mg or 2mg/kg q8wks) did not appear to be associated with a differential safety profile.

Discussion

The present study confirms the clinical and serological efficacy of canakinumab in a Japanese population of paediatric and adult CAPS patients presenting with the most severe NOMID and MWS-phenotypes. Eighteen (94.7%) out of 19 patients enrolled in this study have achieved a complete response with some patients requiring either a dose and/or a frequency adjustment to attain full clinical response. For most patients (78.9%), irrespective of CAPS phenotype, a complete response was achieved with the standard subcutaneous canakinumab dose (13), *i.e.* 150 mg (>40 kg body weight) or 2 mg/kg (≤40 kg body weight) every 8 weeks.

All clinical symptoms frequently observed in CAPS patients such as inflammation of skin, eyes, bones, joints and meninges accompanied by recurrent fever, severe fatigue, myalgia and headache, showed an improvement during canakinumab treatment. Improvement in clinical outcomes with canakinumab therapy such as autoinflammatory disease activity, and reduction in the levels of acute phase proteins such as CRP and SAA confirms the pivotal role of IL-1β and its inhibition in CAPS.

The sustained effects of canakinumab on patient's clinical symptoms have been associated with its mean terminal half-life of 26 days and a possibly disease-modifying effect through autocrine down-regulation of IL-1β production (19). The canakinumab administration schedule of one injection every 8 weeks and the low incidence of injection-site reactions, as previously observed in other phase II and III canakinumab CAPS studies (21, 25, 26), may be beneficial, especially to paediatric patients.

In the present study, individualised up-titration in patients with an incomplete response proved to be a safe and an efficacious approach for the majority of patients achieving a complete response within one month. Patients with incomplete response, as shown by changes in clinical symptoms (headache, fever or rash according to CAPS) and raised inflammatory marker levels (elevated CRP >3 mg/dL, and/or SAA >30 μg/mL), had initially received canakunimab titrated up to 8 mg/kg. The dosage interval was shortened by up to four weeks if patients failed to achieve a complete response. There was no clear correlation between the genotype, phenotype, and treatment response. The mean dose requirement for patients ≤40 kg was found to be proportionally higher (6 mg/kg) than for those with a body weight >40 kg (250 mg). In the group of patients with a body weight >40kg, the NOMID patient subgroup required a higher mean dose compared to the MWS patient subgroup, in line with the level of severity of the disease.

At baseline, 12 NOMID patients presented with CNS symptoms that included headache and pleocytosis and 9 showed improvement in these symptoms by week 24. Patients showed no significant changes, either worsening or improvement, based on audiogram and neurological or ophthalmic assessments. Two patients showed normalisation in auditory acuity and one patient showed normalisation in visual acuity. There were no organic changes observed on magnetic resonance imaging (MRI). This may be attributed to the fact that the observation period was relatively short and approximately 53% of patients were pre-treated with anakinra at the time of the study entry.

In the present study, no patients discontinued due to unsatisfactory therapeutic effect, suggesting that an effective individual canakinumab dosing regimen was determined. The safety profile was comparable to that observed in previous canakinumab studies (21, 24), with no new or unexpected safety findings. Consistently with previous studies and other biologics, infections were the most frequent AEs and the patients responded well to standard therapy. There were no deaths, discontinuations nor dose adjustments/or interruptions due to AEs. In 3 out of 19 patients, anti-canakinumab binding antibodies were detected in one of the post-dose visits, however these patients showed no evidence of immunogenicity related AEs or impaired efficacy. The overall safety profile observed in previous canakinumab studies in CAPS was confirmed in this Japanese population including the paediatric and NOMID sub-populations.

The present study has limitations, including the small size of the patient population, the non-controlled design and the relatively short-term observation period, each of which were addressed in previous studies. Additionally, the small sample size and short follow-up period did not allow detailed assessment of side effects related to anti-IL-1 therapy such as malignant disease and autoimmunity. Long-term observation with a large population is needed to address these issues (27).

Conclusion

Canakinumab 150 mg s.c. dosed every 8 weeks proved to be efficacious and provided a convenient dosing regimen for treating Japanese patients with CAPS. Higher canakinumab doses in younger patients and in adult patients with more severe CAPS disease were efficacious in achieving a complete response and were well tolerated without any evidence of increased AEs. While these results for the treatment of CAPS with canakinumab for up to 197 days are encouraging, the long-term safety of canakinumab in CAPS patients will be further evaluated in this ongoing study.

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PEDIATRIC RHEUMATOLOGY

CASE REPORT Open Access

The identification of a novel splicing mutation in *C1qB* in a Japanese family with C1q deficiency: a case report

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Abstract

C1q deficiency is a rare disease that is associated with a high probability of developing systemic lupus erythematosus. We report a 4-year-old Japanese girl who presented with fever, facial erythema, joint pain, and oral ulceration. Complement deficiencies were suspected because of her persistent hypocomplementemia and normal levels of the complement proteins C3 and C4. We identified a novel homozygous splicing mutation in the *C1qB* gene, c.187 + 1G > T, which is the first mutation to be confirmed in a Japanese individual. Because treatment with steroids and immunosuppressive drugs was not effective, we commenced use of fresh frozen plasma to provide C1q supplements. Currently, the patient remains almost asymptomatic, and we are attempting to control the drug dosage and administration intervals of fresh frozen plasma.

Keywords: C1q deficiency, Systemic lupus erythematosus, Hypocomplementemia, Novel mutation, Fresh frozen plasma

Background

The complement system involves a group of proteins that function as part of the immune system. Three complement pathways make up the complement system, the classical, alternative, and lectin pathways. The C1q protein is the first component of the classical pathway and is composed of the C1qA chain, C1qB chain, and C1qC chain, which are encoded by *C1qA*, *C1qB*, and *C1qC* genes, respectively [1].

C1q deficiency is a rare disease that is associated with a high probability of developing systemic lupus erythematosus (SLE) [2,3]. It is also complicated by cutaneous disease, glomerulonephritis, central nerve system lupus, and recurrent bacterial infection at an early age [2,4]. A deficiency of other complement components such as C1r, C1s, C2, and C4 is also involved in SLE, with C1q deficiency being the strongest single risk factor for SLE development [5]. The first C1q deficiency-causing mutation was reported by McAdam et al. in 1988 [6]. Today, 14 mutations have been identified, all of which are nonsense or missense mutations [7-9]. Although immunosuppressive therapy is administered to C1q deficiency patients, there is currently no curative therapy.

We report herein a girl with C1q deficiency, and the identification of a novel splice site mutation in the *C1qB* gene, which is the first confirmed genetic mutation in a Japanese individual with C1q deficiency.

Case presentation

A 4-year-old Japanese girl was referred to our hospital with a three month history of fever, facial erythema, joint pain, and oral ulceration. She had been diagnosed with discoid lupus erythematosus following a skin biopsy at another hospital. Her symptoms were alleviated transiently after the oral administration of prednisolone; however, they relapsed after cessation of treatment. She had no past history of recurrent infections or fevers of unknown origin. Her parents are not consanguineous,

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and all other family members, including parents and two brothers, were healthy and had not complained of SLE-like symptoms at that time.

At the first visit to our department, physical examinations revealed low grade fever, scarring facial erythema, oral ulceration, and a chilblain-like rash on the extremities of her limbs (Figure 1). Breath sounds were clear and unlabored. Cardiac examination revealed no murmurs, rubs, or gallops. The abdomen was soft, nontender, and nondistended. Neurological examinations of the cranial nerve, motor strength and coordination, reflexes, gait and sensation were normal. Blood and urine tests for rheumatic fever and other febrile illness revealed elevated inflammatory markers (erythrocyte sedimentation rate (ESR) 65 mm/60 min, C-reactive protein (CRP) 1.32 mg/dl), although blood cell count, electrolytes, blood urea nitrogen, creatinine, liver function, and urinary tests were all normal (Table 1). Total complement activity (CH50) was not detectable but an immunoturbidimetric assay revealed that C3 and C4 levels were within the normal range. Speckled antinuclear antibody and rheumatoid factor were positive, and anti-double stranded DNA IgG antibody levels were negative. A chest computed tomography (CT), echocardiography, magnetic resonance imaging of the brain, and renal biopsy specimen were normal.

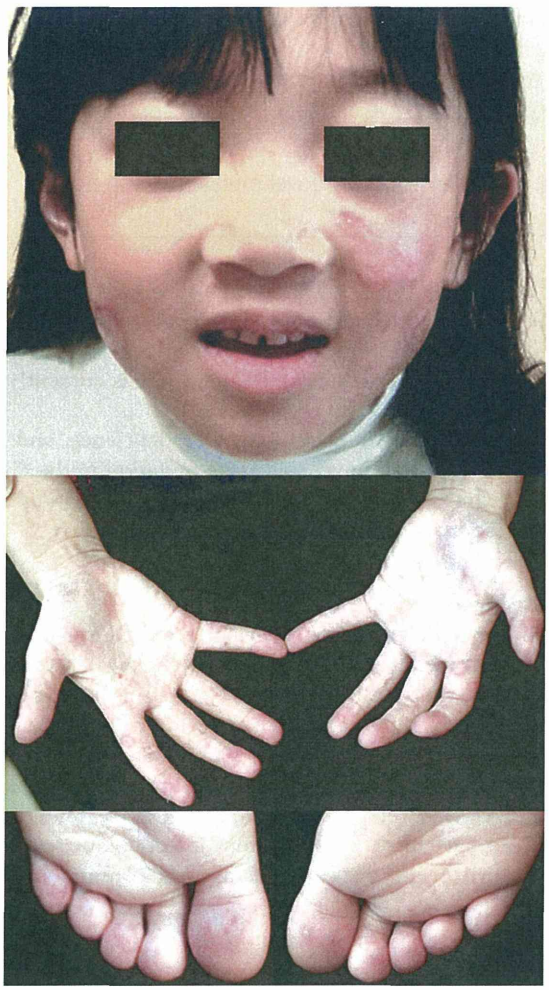

Figure 1 Lupus erythematosus malar skin rash and rash on the hand and foot of our patient.

Initially, the patient had been diagnosed with SLE and had received prednisolone and mizoribine treatment. Although symptoms ameliorated immediately, CH50 remained consistently negative during a period of six months, whereas C3 and C4 were within the normal range. Antinuclear antibody and rheumatoid factor levels gradually elevated, but anti-double stranded DNA IgG antibody levels were still negative. From the above discrepancy between symptoms and laboratory data changes, we suspected complement deficiency so investigated her complement system in detail. Serum C1q levels measured at SRL Inc. (Tokyo, Japan) by nephelometry were 2.8 mg/dl (normal range: 8.8–15.3 mg/dl). Further analysis of the complement systems revealed that whole alternative complement pathway activity (ACH50) and C4 and C2 activities were within the normal range, whereas C1 activity was very low (Table 2) [10-13]. The addition of purified human C1 subcomponent enabled CH50 to be restored to a normal range only when C1q was added, but neither C1r nor C1s was effective (Table 3). For this reason, we strongly suspected a C1q deficiency.

Genomic DNA was extracted from EDTA-blood cells using standard procedures. PCR primers were designed to amplify all exons and exon-intron boundaries of *C1qA*, *C1qB* and *C1qC* genes. Table 4 shows the primer sets and sequencing performed with the BigDye terminator v3.1 Cycle Sequencing Kit. Sequence analysis revealed a novel homozygous splice site mutation in *C1qB*, c.187 + 1G > T (Figure 2), but no other mutations. Reverse transcriptase-polymerase chain reaction amplification of *C1qB* demonstrated the presence of an abnormal single band on gel electrophoresis caused by a splicing error of intron 2/intron 3 (Figure 3). Combined with the complement assay, this DNA sequencing result enabled the molecular diagnosis of C1q deficiency to be confirmed. Mutation analysis and CH50 measurements of family members demonstrated that the patient's parents and one sibling were heterozygous for c.187 + 1G > T with normal CH50 levels. The second sibling (0 years old) with undetectable CH50 levels was also homozygous for the mutation (Figure 4).

After about one year from the first visit, the patient experienced a recurrent fever every few days with no

Table 1 Laboratory findings on first visit of patient

Blood test	Result	Normal range	Blood test	Result	Normal range
WBC	6600/μl	4500–15500	CRP	1.32 mg/dl	<0.1
Seg	5600/μl	1500–8500	ESR 60 min	65 mm/60 min	0–15
Ly	660/μl	1200–8000			
RBC	379×10^4/μl	390–490	CH50	<12.0 CH50/ml	22–40
Hb	10.7 g/dl	11.4–14.2	C3	122 mg/dl	71–159
Ht	30.5%	34–40	C4	45 mg/dl	13–30
Plt	15.2×10^4/μl	14.0–45.0	RF	31 IU/ml	<40
AST	31 IU/l	18–63	ANA(speckled)	1:320	<1:40
ALT	21 IU/l	20–50	Anti-dsDNA IGG	<10 IU/ml	<10
LDH	297 IU/l	142–297	Anti-Sm	1:2	<1:2
Na	141 mEq/l	134–143	Anti-RNP	1:16	<1:2
K	3.3 mEq/l	3.4–4.9	Anti-Ro	(–)	(–)
Cl	104 mEq/l	98–107	Anti-La	(–)	(–)
Ca	9.0 mg/dl	8.8–10.3			
TP	6.3 g/dl	5.6–7.7	Urinalysis		
Alb	3.8 g/dl	3.1–4.8	U-protein	(–)	(–)
BUN	6 mg/dl	5–27	U-glucose	(–)	(–)
Cre	0.40 mg/dl	0.30–0.90	U-occult blood	(–)	(–)

sign of infection. Despite increasing the doses of prednisolone (15 mg/day) and mizoribine (300 mg/every other day), fever and erythematosus rash were aggravated and the ESR elevated to 68 mm/60 min, which is the index that most reflects her condition. We carried out intravenous methylprednisolone pulse therapy, but the effect was only temporary. Accordingly, we initiated fresh frozen plasma (FFP) transfusion for supplementation of C1q. Immediately after transfusion of FFP (10 ml/kg), her CH50 levels recovered to within the normal range but became undetectable 24 h later (Table 5). Nevertheless, she remained afebrile and her rash improved slowly, while ESR declined to 37 mm/60 min following once weekly administration of FFP (10 ml/kg). We are currently attempting to slowly reduce the prednisolone dosage and the frequency of FFP.

Conclusions

We report a girl diagnosed with SLE because of C1q deficiency caused by a novel homozygous splice site mutation in *C1qB*. C1q deficiency is a rare autosomal recessively inherited disease, with only 41 patients from 23 families reported in 1998 [2]. More recently, C1q deficiency has been confirmed in 64 cases within 38 families, 88% of which present with SLE or SLE-like disease [7].

Table 2 Analysis of complement system activity

Hemolytic activity	NHS	Patient serum	Reference value
CH50 (U/ml)	125	0	90–160
(NHS %)	100%	0%	
ACH50 (U/ml)	18.5	18.9	
(NHS %)	100%	102%	70–130%
C1 activity (U/ml)	1450	100	
(NHS %)	100%	7%	70–130%
C2 activity (U/ml)	400	360	
(NHS %)	100%	90%	70–130%
C4 activity (U/ml)	2000	1600	
(NHS %)	100%	80%	70–130%

NHS normal human serum, *ACH50* alternative complement pathway activity.

Table 3 Addition of purified human C1 subcomponent

Hemolytic activity	NHS
CH50 (U/ml)	125
(NHS %)	100%
Patient serum + C1q (U/ml)	140
(NHS %)	112%
Patient serum + C1r (U/ml)	0
(NHS %)	0%
Patient serum + C1s (U/ml)	0
(NHS %)	0%
Patient serum + activated C1s (U/ml)	0
(NHS %)	0%

NHS normal human serum.

Table 4 List of primers used in this work

Primer name	Gene	Exon	F/R	Primer sequence (5′-3′)	Tm (°C)	Annealing Tm (°C)
C1QA-02-F	C1qA	02	F	TTGTGTGCATGGGACTCAAG	56	60
C1QA-02-R	C1qA	02	R	GGCCAAGTCAGGCCAAG	58	
C1QA-03a-F	C1qA	03a	F	TCCCTGAGGACCAGTAGGC	60	60
C1QA-03a-R	C1qA	03a	R	GGACAGGCAGATTTCCCAC	58	
C1QA-03b-F	C1qA	03b	F	TCATCTTCGACACGGTCATC	56	60
C1QA-03b-R	C1qA	03b	R	ATTTTACAGGCGGAGCATGG	56	
C1QB-02-F	C1qB	02	F	GGATGCAGATGGAGGGATAG	58	60
C1QB-02-R	C1qB	02	R	AGGCAACTGTGACTTGGGAG	58	
C1QB-03a-F	C1qB	03a	F	GCAGGCCTCCTTCTTTTGG	58	60
C1QB-03a-R	C1qB	03a	R	TCACGCACAGGTTCCCTC	58	
C1QB-03b-F	C1qB	03b	F	CAGACCATCCGCTTCGAC	58	60
C1QB-03b-R	C1qB	03b	R	GGGGTAGAGTGAGCGTTGC	60	
C1QC-02-F	C1qC	02	F	ATCCATGGTGAGGCTCCTG	58	60
C1QC-02-R	C1qC	02	R	CCCAGACAGACACTCTGATCC	60	
C1QC-03a-F	C1qC	03a	F	GTTCCCTGGAAGACACCCTC	60	60
C1QC-03a-R	C1qC	03a	R	TATGCGACGCGTGGTAGAC	58	
C1QC-03b-F	C1qC	03b	F	AGCCTGATCAGATTCAACGC	56	60
C1QC-03b-R	C1qC	03b	R	TGGCCAGTAAGGTGGGTCC	60	

F forward, *R* reverse, *Tm* temperature.

There are three hypotheses regarding the relationship between C1q deficiency and SLE or SLE-like disease. The first is that C1q deficiency causes autoimmunity by impairing the clearance of apoptotic cells [2], while in the second the absence of C1q affects the negative selection of autoreactive B cells [14]. The third is that the lack of C1q leads to increased interferon-α production and the defective regulation of dendritic cells [15]. It has also been reported that C1q activates canonical Wnt signaling, which regulates T cell development and dendritic cell maturation [16-18]. The suppression of Wnt signaling in association with C1q deficiency may result in the inadequate activation of lymphocytes.

The majority of C1q deficiency patients are European or Middle Eastern, with only four cases reported to

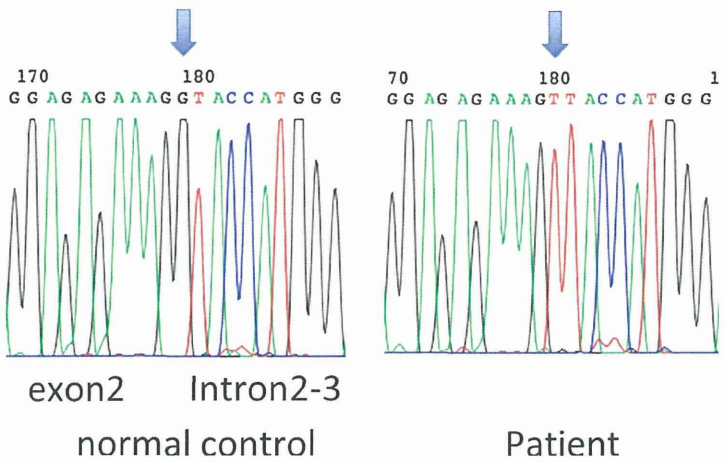

Figure 2 Sequence analysis of the *C1qB* gene. A homozygous mutation was identified at the consensus splicing donor site in intron 2–3 caused by a G-to-T transversion (c.187 + 1G > T).

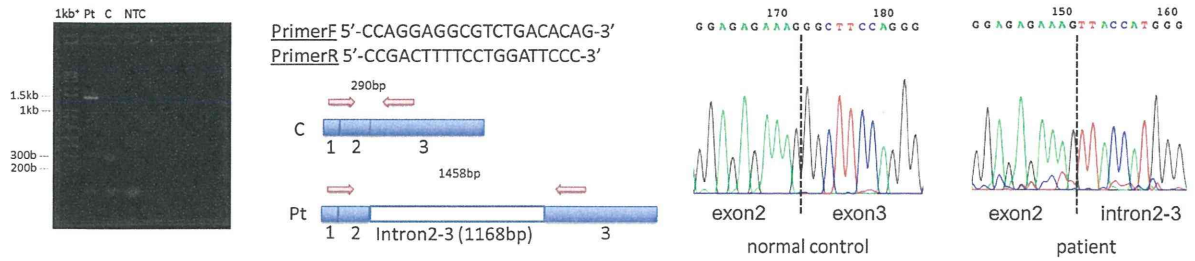

Figure 3 Reverse transcriptase-polymerase chain reaction analysis of *C1qB* mRNA. A single band observed at 1458 bp was caused by a splicing error of intron 2–3. 1 kb+, 1,000 bp DNA-ladder marker; Pt., patient; C., normal control; NTC, no template control.

date in Japan, none of which were confirmed by genetic analysis [7,19-22]. Al-Mayouf et al. reported that C1q deficiency patients tend to develop SLE in less than five years [4]. Our patient also developed SLE at the age of four, with persistent hypocomplementemia and normal C3 and C4 levels. We believe that the possibility of C1q deficiency should therefore always be considered in such cases. To our knowledge, 14 different C1q deficiency-causing mutations have been identified to date, most in European and Middle Eastern patients, with one in an African-American ancestry [7-9]. All are missense or nonsense mutations, so the present mutation is the first report of a splicing error associated with the disorder.

Previously reported complications of C1q deficiency include glomerulonephritis in 30%, central nervous system involvement in 19%, and bacterial infections in 41% of patients [7]. Moreover, symptoms present with varying degrees of severity, even in the same family [23]. It is possible that environmental factors such as exposure to ultraviolet radiation or a history of infection may affect the epigenetic regulation of developing SLE-like syndrome. In our case, the patient may develop complications later in life, and her homozygous sibling may also develop SLE. It is therefore important to carefully follow up our patients to prevent the onset of glomerulonephritis and central nervous system involvements.

At present, no specific therapy is available for C1q deficiency, so steroids and immunosuppressive agents such as hydroxychloroquine (not currently available in Japan) are used as treatments. Bone marrow transplantation is a potential cure, but it has not yet been performed in humans [24,25]. Some reports show that the infusion of FFP restores C1q levels, temporarily complements hemolytic activity, and suppresses SLE symptoms for a long period [8,24]. It is therefore a valid therapy for C1q deficiency patients. However, the long-term administration of FFP increases the risk of infection and the possibility of side effects caused by the development of anti-C1q antibodies [26]. In the light of such risks, alternative treatment strategies should be considered for this intractable disease.

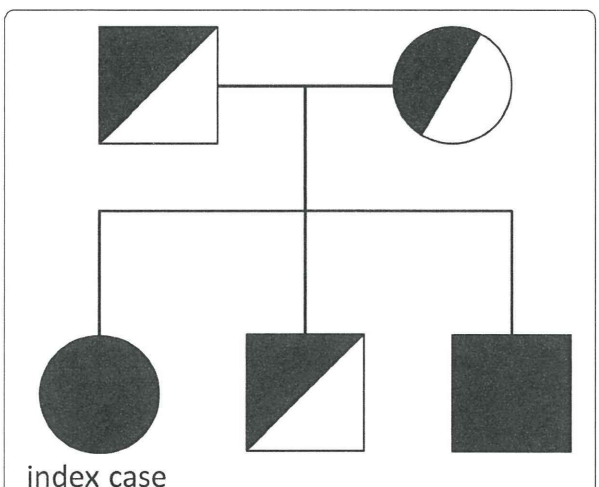

Figure 4 Pedigree showing the heterozygosity of the patient's parents and older brother, and the homozygosity of her younger brother, for c.187 + 1G > T.

Consent

Written informed consent was obtained from the patient for publication of this Case Report and any accompanying images. A copy of the written consent is available for review from the Editor-in-Chief of this journal.

Table 5 Measurement of CH50 following transfusion of FFP in the patient

Time (hr)	0	3 (end of transfusion)	9	24
CH50 (U/ml)	<12.0	25.5	24.6	<12.0