Potential Benefits of TNF Targeting Therapy in Blau Syndrome, a NOD2-Associated Systemic Autoinflammatory Granulomatosis

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Blau syndrome is a systemic autoinflammatory granulomatous disease caused by mutations in the nucleotide-binding oligomerization domain 2 (*NOD2*) gene. NOD2 is an intracellular pathogen recognition receptor. Upon binding to muramyl dipeptide (MDP), NOD2 activates the NF-κB pathway, leading to the upregulation of proinflammatory cytokines. Clinical manifestations of Blau syndrome appear in patients before the age of four. Skin manifestations resolve spontaneously in some cases; however, joint and eye manifestations are progressive, and lead to serious complications, such as joint contracture and blindness. Currently, there is no specific curative treatment for the disease. Administration of high-dose oral steroids can improve clinical manifestations; however, treatments is difficult to maintain due to the severity of the side effects, especially in children. While several new therapies have been reported, including JAK inhibitors, anti-IL-6 and anti-IL-1 therapies, anti-TNF therapy plays a central role in the treatment of Blau syndrome. We recently performed an *ex vivo* study, using peripheral blood and induced pluripotent stem cells from patients. This study demonstrated that abnormal cytokine expression in macrophages from untreated patients requires IFNγ stimulation, and that anti-TNF treatment corrects the abnormalities associated with Blau syndrome, even in the presence of IFNγ. Therefore, although the molecular mechanisms by which the genetic mutations in *NOD2* lead to granuloma formation remain unclear, it is possible that prior exposure to TNFα combined with IFNγ stimulation may provide the impetus for the clinical manifestations of Blau syndrome.

Keywords: Blau syndrome, NOD2, granuloma, IFNγ, TNF

INTRODUCTION

Blau syndrome (MIM #186580) is a rare, systemic granulomatous disease caused by mutations in the nucleotide-binding oligomerization domain 2 (NOD2) gene and is inherited in an autosomal dominant manner. It is classified as an autoinflammatory syndrome, a concept that has received increasing attention in recent years.

In 1985, Blau (1) reported a family that caused granulomas in the skin, eyes, and joints over four generations. In 1990, Pastores et al. (2) reported a mother and daughter with similar symptoms and considered them to be from the same category as the diseases reported by Blau; thus, they named the disease Blau syndrome. Subsequently, a linkage analyses of a large pedigree were presumed, and discovered that Blau syndrome and Crohn's disease have mutations in the same gene (3). In 2001, when mutations in the NOD2 gene were identified in Crohn's disease (4, 5), Miceli-Richard et al. (6) examined the NOD2 gene in four families with Blau syndrome, and identified three gene mutations.

Idiopathic sarcoidosis pediatric cases are rare, yet it is known that in a small subset of patients, peak onset occurs before the age of four years (less than 0.5% of all sarcoidosis cases) (7). The clinical features in these cases were the absence of bilateral hilar lymphadenopathy, and the presence of joint symptoms. Hence, these cases were occasionally referred to as early-onset sarcoidosis (EOS), and the difference from idiopathic sarcoidosis debated (8). Initially no gene abnormalities were found in two solitary cases of EOS (6). We collected ten EOS cases in Japan and identified the same NOD2 mutations in these patients as those reported for Blau syndrome (9), and now Blau syndrome and EOS are considered to be the same disease (10, 11).

MOLECULAR MECHANISMS

NOD2 consists of 1,040 amino acids and has a three domain structure. The N-terminus contains two caspase activation and recruitment domain s (CARD), which are important as in signal transduction; the centrally located NOD region is involved in polymerization; and the C-terminus contains a leucine-rich repeat (LRR). The LRR recognizes muramyl dipeptide (MDP), a component of bacterial cell walls. In Blau syndrome, most patients have mutations in exon 4, the NOD region. Most of these mutations are missense, with single amino acid substitutions. The 334th amino acid of NOD2 is a hot spot for mutations. Typically, arginine (R) is mutated to glutamine (Q) or tryptophan (W) in this region (p.R334Q and p.R334W). Among the 50 patients with NOD2 mutations currently identified in Japan, the p.R334W mutation was most common (fifteen cases), followed by the p.R587C (nine cases), and then the p.R334Q (five cases) (12).

It is still unclear how the mutations identified in Blau syndrome are involved in the formation of granulomas. When NOD2 was shown to recognize MDP, an experimental system to overexpress the NOD2 genes into HEK293 cells was used, in which the mutant with a frameshift in the LRR frequently identified in Crohn's disease was shown to be hyporesponsive to MDP (13). In contrast, mutants identified in Blau syndrome spontaneously promoted NF-κB transcription in the same experimental system, even without the addition of MDP (14). This system of assessing NF-κB transcriptional capacity using luciferase as an indicator is still today a very useful system for confirming whether identified mutations are those associated with Blau syndrome, and indeed all 15 mutants we identified showed spontaneous NF-κB transcriptional enhancement in the absence of MDP (9, 15). This ligand-independent NF-κB activation induced by mutations has been observed in another group of hereditary autoinflammatory syndromes known as cryopyrin-associated periodic syndromes (CAPS). CAPS is caused by mutations in the NLRP3 gene, another NOD family receptor (16). Interestingly, the p.R334W mutation in the NOD2 gene frequently found in Blau syndrome, corresponds to a missense mutation in an analogous position of the NLRP3 gene, the p.R260W mutation in CAPS. These mutations suggest a common molecular mechanism, inducing the autoactivation of NOD family receptors, between these two inflammatory diseases (17).

Nevertheless, we observe an overproduction of IL-1β in peripheral blood monocytes extracted from CAPS patients and the macrophages established from CAPS patients-derived induced pluripotent stem (iPS) cells compared to healthy controls (18, 19), whereas a reduced response to MDP to produce inflammatory cytokines in the peripheral blood of patients with Blau syndrome were reported (20, 21). This lower responsiveness to MDP at the cellular level was also observed in studies using iPS cells differentiated into macrophages established from our patients (22), or knock-in mice with a corresponding Nod2 mutation identified in Blau syndrome (23). Since granulomas may arise from the inability to eliminate foreign substances and pathogens, it is possible that a reduced response to MDP at the cellular level may induce chronic inflammation, leading to granuloma formation in Blau syndrome.

CLINICAL MANIFESTATIONS

The clinical phenotype of Blau syndrome is characterized by a distinct triad of skin, joint and eye disorders.

Skin Rash

Skin lesions often present as a first symptom (12). The most frequent skin manifestations are scaly erythematous plaques with multiple lichenoid papules and no apparent subjective symptoms. Occasionally, a BCG vaccination is the trigger for a skin rash that closely resembles lichen scrofulous, a tuberculous rash. Histological findings are characterized by the presence of epithelioid granulomas, with giant cells in the dermis. Cutaneous manifestations may disappear spontaneously and are often overlooked without a proper diagnosis, due to the lack of subjective symptoms. Cases of erythema nodosum have also been reported (15).

Joint Symptoms

The skin rash is followed by joint symptoms (12). Symmetrical polyarthritis occurs in small joints, such as the fingers and toes; and large joints, such as the hands, elbows, knees, feet; and rarely in the shoulders (24, 25). Painless, cystic swelling of the dorsal surfaces of the wrist and ankle, sausage-like swelling of the fingers and toes especially toward the base, and camptodactyly are characteristic findings.

The absence of arthralgia in the presence of joint swelling, the lack of an initial limitation of movement, and the presence of cystic swelling on the dorsum of the hands and feet, without pain, are of high diagnostic value and are important in differentiating the disease from juvenile idiopathic arthritis (JIA). In Blau syndrome, inflammation of the joint synovium is rare, and in the early stages of the disease, the tendon sheath synovium is damaged, leading to edema around the synovium and a limitation of movement. Joint sonography (26, 27) and MRI are useful to identify the main site of inflammation. However, in children, it is difficult to distinguish synovial thickening due to the lack of ossification in the cartilage on the surface of the bone, and a large area of low-density echoes.

Ocular Manifestations

Ocular manifestations appear later than skin and joint symptoms (12). The most common ocular manifestation is a bilateral uveitis (28–30). Other symptoms include posterior iris adhesion, conjunctivitis, retinitis, and optic nerve atrophy, all of which affect the entire eye. If the lesions persist for an extended time, secondary cataracts and glaucoma may develop, leading to blindness, which greatly affects the prognosis.

Fever

Although fever is not included in the triad of symptoms, fever is an important clinical feature of Blau syndrome. Analysis of the clinical manifestations from fifty patients in Japan showed that in about half of the cases (26 out of 50), fever occurred relatively early in the course of the disease (12). Among these patients, ten had intermittent fever, while seventeen had persistent, which includes two patients that exhibited both types. The frequency of the fever was reported to be once every few days to once a month (31, 32), and the duration varied from a few hours to ten days (31, 33). The range of the fever was mostly high (38-40°C) (31, 32, 34–36), with one case of a mild fever (37°C) reported (34).

Fever is important in Blau syndrome diagnosis. De Rose et al. (37) reported that it is essential to keep Blau syndrome in mind as a cause of unknown fever in infants up to four years old. Interestingly, Rosé et al. (38) demonstrated that all three Blau syndrome patients with the NOD2 p.R587C mutation presented with fever, consistent with our study (12), where eight of nine patients with the p.R587C mutation had fever, compared to four out of 14 patients with p.R334W mutation had fever. In CAPS, there is a clear genotype-phenotype relationship depending on the intensity of the NLRP3 mutation. However, for NOD2 mutations, differential enhancement of NF-κB transcription was dependent on the site of the NOD2 mutation when evaluated in HEK293 cells (15) and the differences did not necessarily correlate with the severity of clinical symptoms in Blau syndrome. Considering that the frequency of cases that present with fever are higher in the p.R587C mutation than in the other mutations, fever may be the clinical symptom that best reflects the ability of NOD2 variants.

TREATMENT

Blau syndrome is currently treated with various therapies, with multiple reports on their effectiveness (**Table 1**). However, there is no specific cure for the disease. Currently, the relationship between the variant of the genetic mutation and the response to treatment is not clear.

Steroids

High doses of steroids can improve clinical manifestations when there is a rapid worsening of joint or ocular symptoms (43, 65); however, prolonged treatment, especially in children, is difficult due to the side effects, including hypertension, growth retardation (48), iatrogenic Cushing syndrome, and elevated intraocular pressure (51). The most common initial diagnosis in our study (12) was JIA with 16 patients. Some of these cases were treated with oral steroids and because these cases were considered relatively mild for JIA, small doses of steroids were continued for an extended time, resulting in a delay in the appropriate therapeutic intervention, and the progression to more severe symptoms, such as joint contractures and blindness.

Topical steroids are often used for skin lesions but are not effective. One patient (12), initially diagnosed with atopic dermatitis, had been treated with topical steroids for an extended time, despite a poor therapeutic response. After a skin biopsy was performed, and granulomas were detected, the patient was subsequently diagnosed with Blau syndrome. Therefore, if a skin rash is not responsive to topical steroids, the possibility of granulomatous diseases, including Blau syndrome, needs to be considered. However, since rashes tends to disappear spontaneously, it can be difficult to evaluate the effectiveness of treatment. In contrast, topical steroid injections and steroid eye drops improved ocular symptoms (45).

Methotrexate and Other Oral Compounds

Methotrexate (MTX) is effective for joint symptoms (33), and useful for steroid sparing (39), although it often needs to be combined with other treatments (12, 43, 65). In our study (12), excluding the seven patients with no treatment information, out of 43 patients, 25 patients were treated with MTX (seven were in combination with biologics, seven in combination with prednisolone [PSL] and biologics, five in combination with PSL, four with MTX alone, and two in combination with PSL, tacrolimus, and biologics).

Other reports suggest that thalidomide may be effective in the treatment of Blau syndrome (36, 48), but the number of cases is limited, and further studies are needed to evaluate the long-term efficacy and side effects.

TABLE 1 | Characteristics results of different treatment methods for Blau syndrome.

Treatment	Effective	Not Effective	Side effect
Steroids	• Relieves symptoms (39–42) • Higher doses are required during inflammation, and can be maintained with lower doses during stable phase (40, 43, 44) • Local injection is effective for ocular symptoms (45) • Eye drops is effective for uveitis (43) • Local therapy is effective for uveitis (46) • Effective for skin lesions, joint symptoms, and uveitis (40)	• No effect on uveitis (47) • Uveitis cannot be controlled with steroids alone and requires immunosuppressive agents and biologics (48–50) • Recurrence of ocular symptoms due to steroid reduction (46, 51)	• Growth retardation (48) • Hypertension (39, 48) • Cataract (39) • Diabetes (39) • Avascular necrosis of hip (39) • Iatrogenic Cushing syndrome (51) • Elevated intraocular pressure (51) • Macular edema (52) • Retinal detachment (52)
MTX	• Used with steroids to help reduce steroid use (39, 41, 53) • Effective for skin lesions (43) • Effective for joint symptoms (33) • Effective for mild joint symptoms (42) • Steroid combinations are effective for joint symptoms (47) • Relieves skin and joint symptoms (48) • Effective for uveitis (46) • Good visual prognosis with steroid combinations (54)	• Often needs to be combined with other treatments (51) • Steroid combinations are not effective for joint symptoms (49, 55) • NSAIDs combination is not effective for joint symptoms (55) • No effect on uveitis (47, 50) • Steroid combinations are not effective for uveitis (47, 48, 50, 51) • Steroid combinations are not effective for skin lesions, joint symptoms, and uveitis (48)	
Thalidomide	• Effective for skin lesions, joint symptoms, and uveitis (48)		
NSAIDs	• Effective for pain control on demand (40) • Effective for fever of mild case (42)	• Cannot prevent progression (40) • No effect on joint symptoms (55)	
Cyclosporine	• Effective for uveitis (41)	• No effect on skin lesions, joint symptoms, and uveitis (41)	• Renal dysfunction (41)
Anakinra	• Improves inflammatory symptoms (42) • Normalizes cytokines (42)	• Steroids and cyclosporine combinations are not effective for uveitis (20) • MTX combination is not effective for joint symptoms and uveitis (20)	
Canakinumab	• Effective for severe uveitis (52) • Effective for skin lesions, joint symptoms, uveitis, and fever (56)	• No effect on joint symptoms (56)	
Tofacitinib	• Effective for joint symptoms (55) • Inhibits the production of IL-1, IL-6, IFNγ, and TNF production (55)		
Tocilizumab	• Effective for uveitis (32) • Effective in cases of fever, lymphadenopathy, and hepatosplenomegaly (32)	• No effect on joint symptoms (55, 57) • MTX combination is not effective for joint symptoms (55)	• Anti-tocilizumab antibody appears (57)
Anti-TNF agents *Infliximab*	• Effective for joint symptoms (33, 58, 59) • MTX combination is effective for joint symptoms (58) • High quality of life can be achieved with a single agent (41) • Mildly effective for joint symptoms (58) • MTX combination is effective for joint symptoms (33, 58) • Steroid and MTX combinations are effective for joint symptoms (59) • MTX combination is effective for uveitis (60) • Effective for joint symptoms and uveitis (20) • MTX combination is effective for joint symptoms and uveitis (43, 57) • Steroid and MTX combinations are effective for joint symptoms and uveitis (41) • CRP and other laboratory values improve with MTX (57)	• Steroid combinations are not effective for skin lesions, joint symptoms, and uveitis (48) • Relapses with MTX reduction/discontinuation (43) • No effect on uveitis (20, 32, 50) • Steroid and MTX combinations are not effective for uveitis (50) • Uveitis relapses (48)	• Fever (48) • Autoimmune encephalitis while treatment with MTX and steroids (61) • Occurs resistance (20)
Adalimumab	• Indicated for the treatment of non-infectious uveitis • Effective for joint symptoms (43, 62) • MTX combination is effective for joint symptoms (57) • Effective for uveitis (43, 50, 51, 62) • MTX combination is effective for uveitis (51, 60) • Steroid and MTX combinations are effective for joint symptoms (59)	• No effect on skin lesions (43) • No effect on joint symptoms (56) • No effect on uveitis (47)	• Facial redness (60)

(Continued)

TABLE 1 | Continued

Treatment	Effective	Not Effective	Side effect
Etanercept	• Steroid and cyclosporine combinations are effective for uveitis (20) • CRP and other laboratory values improve with MTX (57) • Effective for joint symptoms (63) • Steroid and MTX combinations are effective for joint symptoms (64)	• Single agent or MTX combination does not work for joint symptoms (20, 41, 63) • NSAIDs combination is not effective for joint symptoms (55) • No effect on uveitis (41) • MTX combination is not effective for skin lesions, joint symptoms, and uveitis (48) • No effect (63)	• Infection (41) • Myelopathy (64)

Other treatments used for Blau syndrome include mofetil mycophenolate (39, 42, 54), azathioprine (11), tacrolimus (12, 39), and cyclophosphamide (39), which are sometimes used in combination.

In 2018, there was an encouraging report of a significant response to the use of tofacitinib, a Janus kinase inhibitor (JAKi), in patients with cutaneous idiopathic sarcoidosis (66). The patient achieved clinical and histological remission, suggesting that JAK- signal transducer and activation of transcription (STAT) signaling plays a role in the pathogenesis of sarcoidosis and other granuloma diseases. In skin lesion samples taken before treatment, phosphorylated STAT1 (pSTAT1) was identified consistently in granulomas composed of CD68-positive cells, and pSTAT3 was found within inflammatory cells surrounding the lesion; however, these signals disappeared from the granulomas after ten months of treatment with tofacitinib. RNA sequencing results showed that not only interferon-γ (IFNγ), which is dependent on the JAK-STAT pathway, but also tumor necrosis factor-α (TNFα), which is not directly mediated by the JAK-STAT pathway, was elevated in skin lesions before treatment. Expression of these factors was also reduced upon treatment. Based on the successful treatment of sarcoidosis with a JAKi, this treatment has been applied in Blau syndrome and has been reported to be effective. Zhang et al. (55) reported that a single dose of tofacitinib suppressed TNFα and IL-6 production, along with the production of other inflammatory cytokines. Substantial improvements in clinical symptoms and laboratory parameters in the tofacitinib-treated patients were observed.

Biologics

Interleukin (IL)-1, a downstream product of NOD2 through NF-κB transcription, is anecdotally reported to be elevated in some patients with Blau syndrome, and anti-IL-1β therapy was shown to be effective (52, 56). In general, however, IL-1β cannot be detected in serum from patients with Blau syndrome or in cultures from MDP-stimulated MNC (20, 67). Martin et al. (20) showed that IL-1β is not overexpressed in patients of Blau syndrome and the patients were unresponsive to IL-1β therapy.

Similarly, IL-6, another downstream product of NOD2 activation, increases in some patients with Blau syndrome, and Lu et al. (32) mentioned good responses to tocilizumab, especially for patients with fever, lymphadenopathy, and hepatosplenomegaly. Conversely, Nagakura et al. (57) reported that tocilizumab treatment was discontinued because of recurrent arthritis and the development of anti-tocilizumab IgE antibodies due to the absence of co-therapy with MTX.

TNF-Targeting Therapy

When the disease is not controlled by steroids or MTX, biologic agents may be useful and anti-TNF agents are primarily used. Infliximab is effective in treating joint symptoms and may prevent the onset of ocular symptoms (68). Adalimumab is indicated for the treatment of non-infectious uveitis and effective for ocular symptoms, joint symptoms, and systemic symptoms (60, 62, 69, 70). However, etanercept-induced myelopathy has been reported in pediatric patients with Blau syndrome (64).

From the patient cohort in Japan (12), 26 out of 43 patients were treated with biologics, all of which were anti-TNF agents (18 patients were treated with adalimumab, five patients with infliximab, two patients with golimumab, and one patient with etanercept). Focusing on the prognosis of ocular symptoms, of the 26 patients that were treated with anti-TNF therapies, only one was blind. In this case, since adalimumab treatment began, the condition in the right eye did not deteriorate. Conversely, of the 14 patients that did not use biologics, five were blind. Of the remaining patients, three were under observation without treatment, seven had unknown treatment details and one was blind. Four patients who had been treated with biologics for JIA prior to their Blau syndrome diagnosis maintained their ocular status despite a relatively advanced age. Thus, we conclude that early treatment targeting TNF is necessary to avoid irreversible ocular symptoms.

DISCUSSION

An investigation into the cellular phenotypes of Blau syndrome may be necessary to evaluate the efficacy of anti-TNF therapy since the pharmacological mechanism behind their effectiveness in Blau syndrome is unknown.
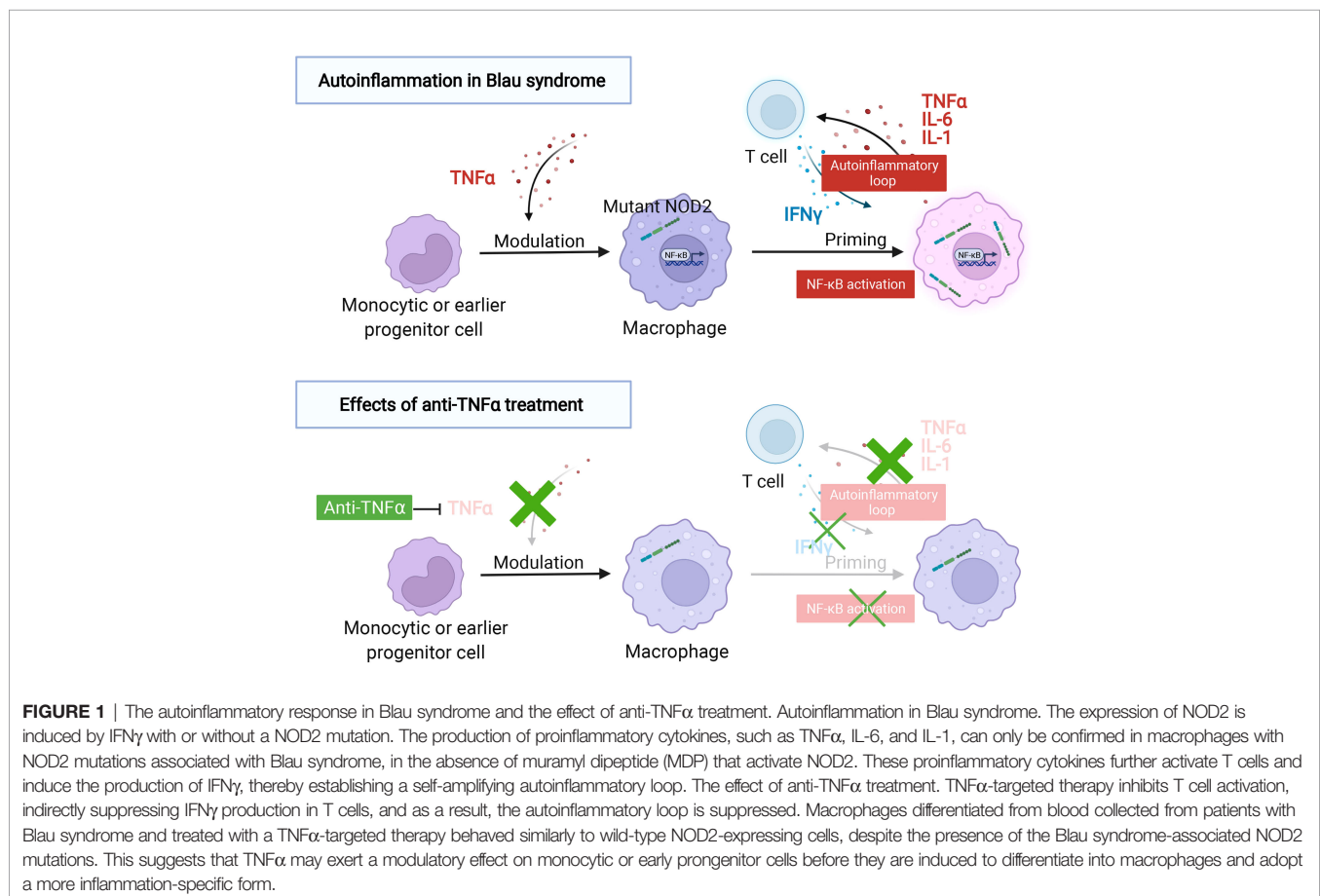
The IFNγ signal has been reported to be increased in localized skin lesions of idiopathic sarcoidosis and Blau syndrome (71) and IFNγ upregulates the expression of NOD2, acting as a priming signal, as previously reported (22). This was established through iPS cells from patients with Blau syndrome that were differentiated into macrophages and confirmed that IFNγ treatment enhanced the expression of NOD2. The enhanced expression of NOD2 through IFNγ

priming was observed regardless of the presence or absence of the NOD2 mutations. However, the spontaneous transcriptional enhancement of NF-κB and the production of proinflammatory cytokines were observed only in cells that contained the NOD2 mutation associated with Blau syndrome. These effects were not found in cells where the NOD2 mutation was corrected to the wild-type amino acid using the CRISPR-Cas9 system. Of interest, skin lesions have appeared following BCG vaccination in some patients with Blau syndrome. Upon review of fifty patients in the cohort from Japan, the attending physician provided information indicating that BCG vaccination may have induced symptoms in nine patients (12). Since IFNγ is a cytokine that is highly associated with BCG-mediated immune responses, BCG vaccination may be involved in NOD2-mediated inflammatory responses through the induction of NOD2 expression.

While verifying the studies using Blau syndrome patient-derived iPS cells, additional, surprising results were obtained (72). Macrophages, differentiated from the peripheral blood of Blau syndrome who did not receive anti-TNF treatment, released inflammatory cytokines after being primed with IFNγ, as described above. In contrast, macrophages differentiated from patients treated with anti-TNF, did not produce proinflammatory cytokines, similar to macrophages differentiated from healthy subjects. This was despite anti-TNF patient macrophages being primed with IFNγ and being differentiated *ex vivo* for one week with unchanged culture conditions. A comprehensive gene expression analysis of all three groups indicated that the cells from the anti-TNF treatment patient group had a nearly identical gene expression pattern as the cells differentiated from healthy individuals, without the *NOD2* mutation. Furthermore, when primed with IFNγ, cells from these two groups (healthy individuals and anti-TNF treated patients) behaved in the same manner and had gene expression patterns that differed from macrophages differentiated from patients not receiving an anti-TNF agent. This study suggests that prior exposure to a proinflammatory cytokine, such as TNFα, at the monocytic or earlier progenitor cell stage is an important determinant for the macrophage response to IFNγ. Thus, the use of anti-TNF antibodies for Blau syndrome treatment is appropriate in the sense that long-term administration of anti-TNF antibodies may correct the abnormalities that occur in the early progenitor stage by blocking the autoinflammatory loop and restoring the threshold for which IFNγ stimulation triggers an inflammatory response in macrophages (**Figure 1**).

At present, no specific treatment for Blau syndrome exists, based on its etiology, and the disease is treated empirically. However, early diagnosis will allow for the prediction of future

FIGURE 1 | The autoinflammatory response in Blau syndrome and the effect of anti-TNFα treatment. Autoinflammation in Blau syndrome. The expression of NOD2 is induced by IFNγ with or without a NOD2 mutation. The production of proinflammatory cytokines, such as TNFα, IL-6, and IL-1, can only be confirmed in macrophages with NOD2 mutations associated with Blau syndrome, in the absence of muramyl dipeptide (MDP) that activate NOD2. These proinflammatory cytokines further activate T cells and induce the production of IFNγ, thereby establishing a self-amplifying autoinflammatory loop. The effect of anti-TNFα treatment. TNFα-targeted therapy inhibits T cell activation, indirectly suppressing IFNγ production in T cells, and as a result, the autoinflammatory loop is suppressed. Macrophages differentiated from blood collected from patients with Blau syndrome and treated with a TNFα-targeted therapy behaved similarly to wild-type NOD2-expressing cells, despite the presence of the Blau syndrome-associated NOD2 mutations. This suggests that TNFα may exert a modulatory effect on monocytic or early progenitor cells before they are induced to differentiate into macrophages and adopt a more inflammation-specific form.

symptoms that are likely to appear, and prompt treatment will prevent or delay the onset of severe symptoms (joint contractures and blindness), which significantly impair the patient's quality of life. Future accumulation of a large number of patients, with detailed information on disease progression, treatment, and prognosis, would be advantageous to analyze disease pathogenesis and establish a specific treatment based on disease etiology.

AUTHOR CONTRIBUTIONS

TM, NaK, RT-I, YU, SN, MS, ST, and NoK contributed to conception and design of the study. TM and NaK wrote the first draft of the manuscript. NaK and MS created the first draft of the figure, while RT-I created the final figure. All authors have read, contributed to revisions of the manuscript, and approved the submitted version.

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Assessment of type I interferon signatures in undifferentiated inflammatory diseases: A Japanese multicenter experience

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Purpose: Upregulation of type I interferon (IFN) signaling has been increasingly detected in inflammatory diseases. Recently, upregulation of the IFN signature has been suggested as a potential biomarker of IFN-driven inflammatory diseases. Yet, it remains unclear to what extent type I IFN is involved in the pathogenesis of undifferentiated inflammatory diseases. This study aimed to quantify the type I IFN signature in clinically undiagnosed patients and assess clinical characteristics in those with a high IFN signature.

Methods: The type I IFN signature was measured in patients' whole blood cells. Clinical and biological data were collected retrospectively, and an intensive genetic analysis was performed in undiagnosed patients with a high IFN signature.

Results: A total of 117 samples from 94 patients with inflammatory diseases, including 37 undiagnosed cases, were analyzed. Increased IFN signaling was observed in 19 undiagnosed patients, with 10 exhibiting clinical features commonly found in type I interferonopathies. Skin manifestations, observed in eight patients, were macroscopically and histologically similar to those found in proteasome-associated autoinflammatory syndrome. Genetic analysis identified novel mutations in the *PSMB8* gene of one patient, and rare variants of unknown significance in genes linked to type I IFN signaling in four patients. A JAK inhibitor effectively treated the patient with the *PSMB8* mutations. Patients with clinically quiescent idiopathic pulmonary hemosiderosis and A20 haploinsufficiency showed enhanced IFN signaling.

Conclusions: Half of the patients examined in this study, with undifferentiated inflammatory diseases, clinically quiescent A20 haploinsufficiency, or idiopathic pulmonary hemosiderosis, had an elevated type I IFN signature.

Introduction

A critical role for type I interferons (IFNs) in the pathogenesis of inflammatory diseases has been increasingly recognized in recent years. IFNs are a group of cytokines that play an important role in host defense against viruses. IFNs consist of three distinct families, namely, type I (IFNα/β/ε/τ/κ/ω/δ/ζ), II (IFNγ), and III (IFNλ).IFNα and IFNβ are the most understood, and broadly expressed type I IFNs. They are produced by most cell types in response to stimulation from pattern recognition receptors through intracellular and endosomal nucleic acids. Once secreted extracellularly, they bind to type I IFN receptors and activate hundreds of IFN stimulated genes (ISGs), which affect the innate and adaptive immune response (1, 2).

In 2003, multiple investigators reported that peripheral blood cells from systemic lupus erythematosus (SLE) patients demonstrated an overexpression of a characteristic pattern of ISGs, termed an IFN signature (3–5). Although there is a large overlap between the ISGs induced by all three IFN families, the primary IFN signature is most consistent with induction from type I IFNs (6, 7). Since detection of type I IFN in human serum by conventional enzyme-linked immunosorbent assay (ELISA) is complicated by low reproducibility and poor correlation with functional assay (8), expression of an IFN signature has been widely used to assess type I IFN activity. An increased IFN signature has been identified in many autoimmune diseases, including SLE, rheumatic arthritis (RA), systemic sclerosis (SSc) and dermatomyositis (DM), and its utility as a biomarker to predict disease severity or to assess disease activity is readily studied (9–16).

Type I IFNs are also involved in the pathogenesis of autoinflammatory diseases. In 2011, Crow et al. proposed the concept of type I interferonopathy, which refers to a group of Mendelian inflammatory disorders where chronic and autonomous enhancement of type I IFN production was posited as directly relevant to pathogenesis (17). Since then, numerous Mendelian genotypes were found to be associated with enhanced type I IFN signaling (18). Several investigators have suggested the utility of a type I IFN signature as a biomarker to distinguish patients with type I interferonopathy from those with other inflammatory diseases (19–22). Moreover, several groups reported that a type I IFN signature correlates with disease activity and treatment from JAK inhibitors, suggesting that an IFN signature may serve as a biomarker to monitor treatment response (23).

Recent studies have examined the efficacy of JAK inhibitors for the treatment of various autoimmune and autoinflammatory diseases (23–29), suggesting a causal relationship between enhanced type I IFN signaling and disease pathogenesis. Clinically, it is becoming more important to diagnose type I IFN-driven inflammatory diseases rapidly and accurately for personalized medical treatment. In this report, the expression of a type I IFN signature in patients with various inflammatory diseases was investigated. An increased IFN signature was detected in disease states, whose etiologies have not previously been associated with type I IFNs, and in some patients with clinically and genetically undiagnosed inflammatory diseases. In some patients where enhanced IFN signaling was observed, a retrospective assessment of clinical phenotypes revealed characteristics similar to monogenic, type I interferonopathies. In particular, skin manifestations in these cases were macroscopically and histologically similar. These findings may be indicative of a pathogenetic role for type I IFNs in these diseases and suggest the existence of unknown genotypes, which may lead to the upregulation of type I IFN signaling.

Material and methods

Patients and healthy controls

Patients, suspected of having autoinflammatory or undifferentiated autoimmune diseases, were selected for this study based on the recommendation of their attending physician. Samples were collected from a total of 117 individuals, comprising 57 patients with diagnosed inflammatory diseases and 37 patients with undifferentiated inflammatory diseases who had no genetic or clinical diagnosis upon referral. Eleven Japanese adults who self-reported to have no known medical conditions or infection symptoms were recruited as healthy controls (HCs). Asymptomatic pediatric patients with noninflammatory diseases, such as congenital heart disease and hydronephrosis, who attended a hospital for a routine examination were recruited as the pediatric controls. IFN signatures were measured in 140 samples collected between 2016 and 2020 (Table S1).

Study approval

The ethics committee of Kyoto University approved this study, which was conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from all of the subjects or legally authorized representative

Clinical and genetic evaluation

Clinical and biological data from the 37 patients with undifferentiated inflammatory diseases was collected retrospectively from their medical records or from interviews with the attending physician.

An in-depth genetic analysis was performed on all 19 patients with undifferentiated inflammatory diseases whose type I IFN signature was elevated. Trio-based, whole exome sequencing

(WES) was conducted on ten of the patient samples, while targeted genomic sequencing (TS), that analyzed a panel of 533 genes associated with immunodeficiency and autoinflammatory diseases, including monogenic type I interferonopathies, was completed on the other nine patient samples.

IFN score (IS)

The IFN signature was measured using quantitative reverse transcription polymerase chain reaction (RT-qPCR) as described previously (19). Briefly, total RNA was extracted from whole blood using the PAXgene Blood RNA kit (PreAnalytix, Hombrechtikon, Switzerland). Gene expression of six ISGs (IFI27, IFI44L, IFIT1, ISG15, RSAD2, and SIGLEC1) was then determined by RT-qPCR using cDNA derived from 40 ng of total RNA and the TaqMan™ Gene Expression Master Mix (Thermo Fisher Scientific, Waltham, MA). PCR was performed using the StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific). TaqMan probes for IFI27 (Hs01086370_m1), IFI44L (Hs00199115_m1), IFIT1 (Hs00356631_g1), ISG15 (Hs00192713_m1), RSAD2 (Hs01057264_m1), SIGLEC1 (Hs00988063_m1), and β actin (HS01060665_g1) were used. The relative abundance of each target gene transcript was measured using the ΔΔCT method. The expression of each ISG in each patient was normalized to the β actin expression level and then calculated relative to the median expression level of the 11 HCs. The IS was defined as the median relative expression level of the six ISGs. An abnormal IS was defined as that greater than two standard deviations from the mean IS in the control group (i.e., 5.04).

Proteasome activity detection

For the detection of chymotrypsin activity, monocytes isolated using the autoMACS® cell separator (Miltenyi Biotec, Bergisch Gladbach, Germany) from peripheral blood mononuclear cells, obtained by Lymphoprep™ (STEMCELL Technologies, Vancouver, Canada) separation, were seeded at a concentration of 1×10^4 cells/well in a white 96-well plate. DMSO or 1 µM ONX-0914 (Adooq Bioscience, Irvine, CA) was added, and 3 hours later, TNF-α and IFN-γ (100 ng/mL) were added to stimulate the cells. After culturing the cells for 21 hours, 100 µL Proteasome-Glo™ Chymotrypsin-Like Cell-Based Assay (Promega, Madison, WI) was added for 1 hour at 37°C. Then, chemiluminescence was detected using the 2104 EnVision Multilabel Plate Reader (PerkinElmer, Waltham, MA).

To detect β5 and β5i subunit activity, 3×10^4 cells were seeded into each well of white 96-well plates. DMSO or 1 µM ONX-0914 was added to the wells, and 3 hours later, the cells were stimulated with TNF-α and IFN-γ (100 ng/mL). After

culturing the cells for 21 hours, Ac-Trp-Leu-Ala-AMC (R&D Systems, Inc., Minneapolis, MN) to detect β5 activity, or Ac-Ala-Asn-Trp-AMC (R&D Systems, Inc.) to detect β5i activity, was added to a final concentration of 50 µM, followed by incubation at 37°C for the indicated times (Figure 3B). AMC fluorescence was detected using the 2104 EnVision Multilabel Plate Reader.

Immunohistochemistry

Tissue sections (4 µm thick) from archived paraffin-embedded tissue blocks were prepared for immunohistochemical and hematoxylin and eosin (H&E) staining. Immunohistochemistry was performed using antibodies against CD3 (2GV6; Roche, Basel, Switzerland), CD20 (L26; DAKO, Santa Clara, CA), CD15 (Carb-3; DAKO), CD123 (6H6; Thermo Fischer Scientific), CD163 (10D6; Leica Microsystems, Wetzlar, Germany), or MPO (polyclonal; DAKO). All staining procedures were performed using an autoimmunostainer (Bond III [Leica Microsystems] or BenchMark Ultra [Ventana Medical Systems, Oro Valley, AZ]).

Statistical analysis

Descriptive statistical analyses were performed and differences in proportions between the groups in Table 1 were evaluated by a Fisher's exact test. Results for Figure S2 were analyzed using a one-way ANOVA with a Dunnett's multiple comparisons test. All statistical analyses, described above, were performed using the GraphPad Prism software version 8.00 (GraphPad Software, La Jolla, California, USA, www. graphpad.com).

Results

The type I IFN signature in clinically or genetically defined cases

Patients ISs were plotted, according to disease diagnosis, as shown in Figure 1. The relative expression levels of each ISG are presented as a heatmap in Figure S1. All patients with monogenic type I interferonopathies, including Aicardi-Goutières syndrome (AGS); proteasome-associated autoinflammatory syndrome (PRAAS); STING-associated vasculopathy with onset in infancy (SAVI); COPA syndrome; spondyloenchondrodysplasia with immune dysregulation (SPENCDI); and other monogenic and polygenic diseases, which are associated with the upregulation of type I IFN signaling, including chronic granulomatous disease (CGD); SLE; and DM; demonstrated high ISs.

A20 haploinsufficiency (HA20) is a systemic autoinflammatory disease caused by a heterozygous loss-of-function mutation in

TABLE 1 Comparison of clinical phenotypes in patients with and without enhanced IFN signaling.

	Patients with high IS Number of patients affected/evaluated (%)(n=19)		Patients without high IS Number of patients affected/evaluated (%)(n=18)		Fisher's exact test p-value a
Age at onset (median [IQ range]), months	8	(1–96)	180	(78-286)	0.007
Male	7/19	(36.8)	6/13	(35.3)	>0.99
Fever	14/19	(73.7)	14/18	(77.8)	>0.99
Skin involvement	14/19	(73.7)	10/18	(55.6)	0.31
- chilblain	6/19	(31.6)	0/18	(0)	0.02
- nodular erythema	9/19	(47.4)	3/18	(16.7)	0.08
Panniculitis	2/19	(10.5)	0/18	(0)	0.49
Myositis	2/19	(10.5)	0/18	(0)	0.49
Arthritis/Arthralgia	4/19	(21.0)	3/18	(16.7)	>0.99
Interstitial pneumoniae	1/19	(5.3)	0/18	(0)	>0.99
CNS manifestation					
- headache	2/19	(10.5)	4/18	(22.2)	0.40
- intracranial calcification	1/14	(7.1)	0/7	(0)	>0.99
- aseptic meningitis	0/19	(0)	1/18	(5.6)	0.49
Transaminitis	7/19	(36.8)	1/18	(5.6)	0.042
Autoantibody	7/19	(36.8)	2/17	(11.8)	0.13
- including ANA (1:40 and 1:80)	13/19	(68.4)	3/17	(17.6)	0.003

a; Difference in age at onset was analyzed using a Mann-Whitney test. Two patients from each group whose age of disease onset was ambiguous were excluded from the statistical analysis for this category. All four of these patients self-reported that they had symptoms since early childhood. ANA, antinuclear antibody.

the TNF-α-induced protein 3 (TNFAIP3). Recent reports have demonstrated the elevation of a type I IFN signature and potential therapeutic benefits of JAK inhibitors in HA20 patients (30, 31). Since nine patients (from six unrelated families) in the study were diagnosed with HA20, their type I interferon signature was analyzed. Determination of the patients' genotypes and TNFAIP3 variants revealed several previously reported variants (32) and as well as two variants (p.Lys329Asn*1 and p.Gly583*) newly confirmed to be pathogenic using a NF-κB reporter gene activity assay (see the method of NF-κB reporter gene activity assay an Figure S2 in the electronic supplemental material). All patients had elevated ISs (Figure 1). Intriguingly, when the samples were collected, seven patients were assessed as clinically inactive by the attending physician and the patients reported very few symptoms between hospital visits (Table S2).

Two out of the three patients with idiopathic pulmonary hemosiderosis (IPH) also demonstrated an upregulation of type I IFN signaling (Table S3), despite being in treatment-induced remission for several years. Genetic analyses ruled out COPA syndrome, which is known to be associated with an elevated type I IFN signature and alveolar hemorrhage (33) in two of the patients. Interestingly, one patient with subsequent development of anti-citrullinated protein (ACPA)-positive RA did not have enhanced IFN signaling, while two patients without any autoimmune-related manifestations, other than pulmonary symptoms, demonstrated high ISs.

One patient with chronic active Epstein-Barr virus infection (CAEBV) exhibited moderately enhanced IFN signaling. Expression of the type I IFN signature in one patient with hypersensitivity to mosquito bites was upregulated slightly and was not correlated with other clinical symptoms or viral loads. Interestingly, this patient had a normal type I IFN signature at first, despite having a whole blood viral DNA load higher than that of the patient with CAEBV (32000 vs. 2400 copy/μg DNA). This demonstrates that the presentation of a normal type I IFN signature cannot exclusively rule out the presence of a chronic viral infection. (Table S4).

Clinical characteristics of patients with undifferentiated inflammatory diseases that exhibited an elevated type I IFN signature

Of the 37 patients with undifferentiated inflammatory diseases, just over half (19 patients, 51.4%) demonstrated high ISs. A comparison of the clinical features in patients, with and without elevated ISs, is shown in Table 1. Disease onset was earlier in the patient group with high ISs. The clinical and laboratory features that were more frequently found in the high IS group include, chilblain (31.6% vs. 0%, p = 0.0197) and transaminitis (elevation of liver enzymes) (36.8% vs. 5.6%, p = 0.0422). Nodular erythema (47.4% vs. 16.7%) and the presence
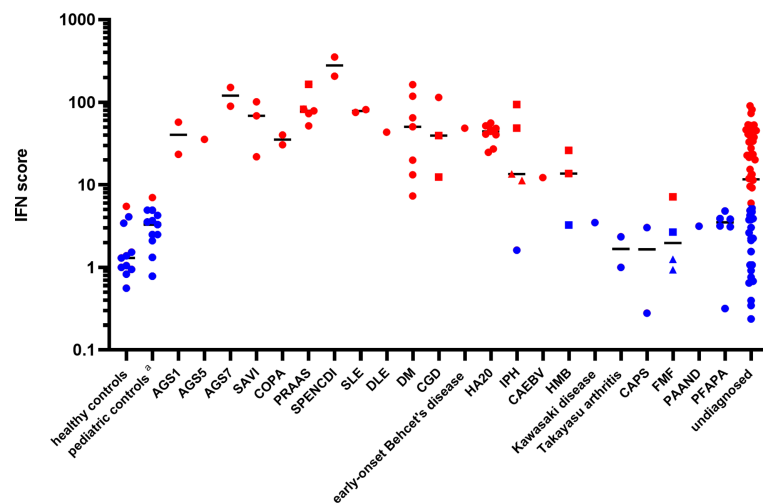
FIGURE 1
Patient interferon scores according to disease diagnosis. Red dots represent ISs greater than 5.04, while blue dots represent ISs below 5.04, based on two standard deviations from the mean score found in healthy controls. The circles represent the ISs of the different subjects, except for those of the "undiagnosed" patients with a high IS. The squares and triangles represent repeat samples from the same subjects, respectively. Details of the ISs of the "undiagnosed" patients with a high IS are described in Table 2. Black horizontal lines represent the median for each patient group. DLE, discoid lupus erythematosus; CAEBV, chronic active Epstein-Barr virus infection; HMB, hypersensitivity to mosquito bites; FMF, familial Mediterranean fever; PAAND, pyrin-associated autoinflammation with neutrophilic dermatosis; PFAPA, periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis. The pediatric controls include asymptomatic pediatric patients with noninflammatory diseases such as congenital heart disease and hydronephrosis who attended a hospital for a routine examination.

of autoantibodies (36.8% vs. 11.8%) were also observed more frequently in patients with high ISs. Of note, low titer antinuclear antibody (ANA) expression (i.e., 1:40 or 1:80), which is usually considered clinically insignificant, was frequently detected in patients with high ISs. Thus, the presence of all autoantibodies, including low titer ANAs, was observed more frequently in patients with high ISs (68.4% vs. 17.6%, p = 0.0031).

Amongst the 19 patients with high ISs, ten very early onset cases (< 2 years old, Table 2, P1-10) presented with some symptoms that led to the suspicion they had monogenic type I interferonopathies, namely, nodular erythema, chilblain-like erythema, panniculitis, myositis, basal ganglia calcification, and interstitial pneumoniae (30) (31). With the exception of P8, all of these patients had similar nodular erythema with post-inflammatory hyperpigmentation, which persisted for weeks to months after resolution, half of which were associated with pain (Figure 2A). Skin biopsy results for all ten patients were available and are shown in Figure 2B. The H&E-stained sections for these patients, with the exception of P7 and P8, showed similar features, consisting of perivascular and periadnexal mononuclear dermal infiltrates with variable positivity for MPO, CD163, and CD3 expression. Most of the MPO-positive infiltrates lacked nuclear segmentation and showed faint CD15 expression, which is usually expressed by mature neutrophils (Figure S3 and Table S5). These

findings resembled skin manifestations seen in PRAAS (36). Patient P3 had two available biopsy results; one specimen (3–1) was taken from a red papule on an upper limb, and the other (3–2) was taken from a painful nodular erythema on a lower limb two years after the first biopsy. Although the immunohistochemistry results were limited, the H&E-stained sections of sample 3-1 shared similar characteristics with the other patients, except for P7 and P8. However, sample 3-2 showed more severe inflammation with dermal infiltration of matured neutrophils, leukocytoclastic vasculitis and septal panniculitis (Figure S3). The microscopic features of the skin specimens from P7 and P8 resembled those seen in SLE (Figure S3). The H&E sample for patient P7 exhibited lobular panniculitis with MPO+/CD15- mononuclear cell infiltration, in addition to superficial and deep perivascular dermatitis with interface vacuolar degeneration. Patient P8's sample showed superficial, dermal CD3-positive T cell infiltration and vacuolar degeneration of the basal layer. MPO +/CD15- mononuclear cells were not identified in P8. These ten early onset patients were strongly suspected of having monogenic type I interferonopathy. Indeed, one patient was found to have compound heterozygous mutations in *PSMB8*, which were proven to be pathogenic, and the other four patients were found to have rare variants, of unknown significance, in genes linked to type I IFN signaling using a trio-based WES functional assay (manuscript in preparation).

TABLE 2 Genotypes and clinical phenotypes of patients with high ISs.

Patient	Gender	Age at IS analysis	Disease-onset age	IS	Clinical manifestations							CNS lesion	Autoantibody	Number of interferonopathy-like symptoms[a]	Genotype	Treatment	Efficacy
					Recurrent fever	Chilblain	Nodular erythema	Panniculitis	Myositis	Transaminitis	Other symptoms						
1	M	1y3m	7m	81.1/ 39.4/ 27.7/ 52.8/ 46.3	+	+	+	–	+	+	None	–	Anti-DNA Anti-ssDNA Anti-dsDNA	3	Compound heterozygous mutations in PSMB8	1. PSL 2. Baricitinib	1. Partial 2. Effective
2[b]	M	6y	11m	32.9	+	+	+	–	–	–	None	–	–	3	Possibly pathogenic variant found in WES	Topical TAC	N/D
3[b]	M	42y	Early childhood	53.3	–	–	+	+	–	–	None	–	ANA 1:40	2	Possibly pathogenic variant found in WES	Topical steroid	N/D
4	F	3m	At birth	20.1	–	–	+	–	–	–	Hepatosplenomegaly, anemia	–	ANA 1:40	1	Possibly pathogenic variant found in WES	No treatment	–
5	M	4y	6m	13/ 2.6/ 11.9	+	–	+	–	–	–	None	–	–	1	No known pathogenic mutations found in WES	No treatment	–
6	F	1y6m	10d	34.4	+	+	+	–	+	+	None	–	ANA 1:40	3	Possibly pathogenic variant found in WES	Topical steroid	partial
7[c]	F	16y	3m	23.3	+	+	+	+	–	+	Arthritis, iritis	Basal ganglia calcification	–	4	No known pathogenic mutations found in WES	1.PSL, 2.CyA 3.MTX, 4.AZA 5.Adalimumab 6.Tocilizumab	1,3,4,5. Partial 2.effective 6.ineffective
8	F	7y	1y	72.9/ 45.2	+	+	–	–	–	+	Livedo reticularis, ulcers, partial necrosis of liver, recurrent pneumoniae, arthritis	–	Anti-cardiolipin Anti-smooth muscle	1	No known pathogenic mutations found in WES	Colchicine	Partial
9	M	4y	10d	22.7/ 90.3	–	+	+	–	–	+	None	–	Anti-smooth muscle	2	No known pathogenic mutations found in TS	Topical steroid	Partial
10	F	1y10m	1m	9.5	+	–	+	–	–	–	Interstitial pneumoniae, fever	–	–	1	No known pathogenic mutations found in TS	1.PSL 2.hydroxychloroquine	Both were effective to IP

(Continued)

TABLE 2 Continued

Patient	Gender	Age at IS analysis	Disease-onset age	IS	Clinical manifestations							CNS lesion	Autoantibody	Number of interferonopathy-like symptoms[a]	Genotype	Treatment	Efficacy
					Recurrent fever	Chilblain	Nodular erythema	Panniculitis	Myositis	Transaminitis	Other symptoms						
11[c]	F	7y	6y4m	45.7	+	–	–	–	–	+	Thrombocytopenia, anemia, anasarca, rash, parotitis, hepatosplenomegaly, hypocomplementemia, acute renal insufficiency	White matter hyperintensity	ANA 1:160 Anti-SS-A Anti-SS-B	0	No known pathogenic mutations found in TS	1.mPSL pulse 2.IVCY, 3.AZA 4.MMF, 5.Rituximab	Partial
12	F	15y	Early childhood	35.9	–	–	–	–	–	–	Anemia, polyclonal hypergammaglobulinemia, systemic lymphadenopathy, hepatosplenomegaly	N/D	ANA 1:80 Anti-cardiolipin Anti-ssDNA RF	0	No known pathogenic mutations found in TS	Tocilizumab	Effective
13[d]	F	44y	41y	41/ 11.3/ 33.7	+	–	–	–	–	–	Headache, abdominal pain, arthralgia, weight loss, refractory asthma	–	Anti-sm	0	No known pathogenic mutations found in WES	1.PSL (for asthma) 2.Tofacitinib	1.Ineffective 2.Partial
14[d]	F	44y	41y	21.4	+	–	–	–	–	–	Recurrent pneumoniae, persistent cough	N/D	ANA 1:40	0	No known pathogenic mutations found in WES	No Treatment	–
15	F	18y	14y	53.3/ 23.4	+	–	–	–	–	–	Myalgia, conjunctivitis, pharyngitis, abdominal pain, lymphadenopathy, pleural effusion, lytic lesion in a thoracic vertebra with soft tissue swelling.	–	ANA 1:40	0	No known pathogenic mutations found in TS	PSL	Effective
16	M	1y10m	8m	37.6/ 15.4	–	–	–	–	–	+	Craniosynostosis, elongation of APTT	–	ANA 1:40 Anti-dsDNA Anti-cardiolipin	0	No known pathogenic mutations found in TS	No Treatment	–
17	F	22y	13y	13.3/ 6.0 10.0	+	–	–	–	–	–	None	–	ANA 1:40	0	No known pathogenic mutations found in TS	Colchicine	Ineffective
18	M	6y	4m	9.2/ 1.1	+	–	–	–	–	–	Cold-induced urticaria associated with fever and arthritis, recurrent conjunctivitis	N/D	–	0	No known pathogenic mutations found in WES	Antihistamine	Ineffective
19	F	14y	6y4m	5.2	+	–	–	–	–	–	Headache without meningitis, optic disc swelling	–	–	0	No known pathogenic mutations found in TS	No Treatment	–

a: Interferonopathy-like symptoms include nodular erythema, chilblain-like erythema, panniculitis, myositis, basal ganglia calcification, and interstitial pneumoniae. b: Patient 3 is the father of patient 2. c: Patient 7 and 11 have been previously studied (34, 35). d, Patient 13 is an elder sister of patient 14.

M, male; F, female; TAC, tacrolimus; CyA, cyclosporine; MTX, methotrexate; AZA, azathioprine; mPSL, methylprednisolone; IVCY, intravenous cyclophosphamide; MMF, mycophenolate mofetil ; N/D, no data.
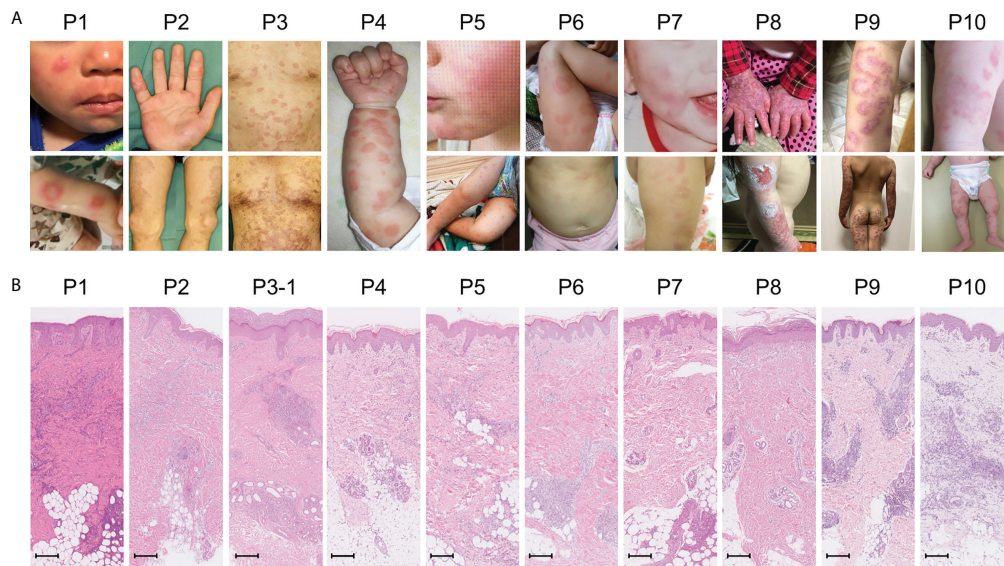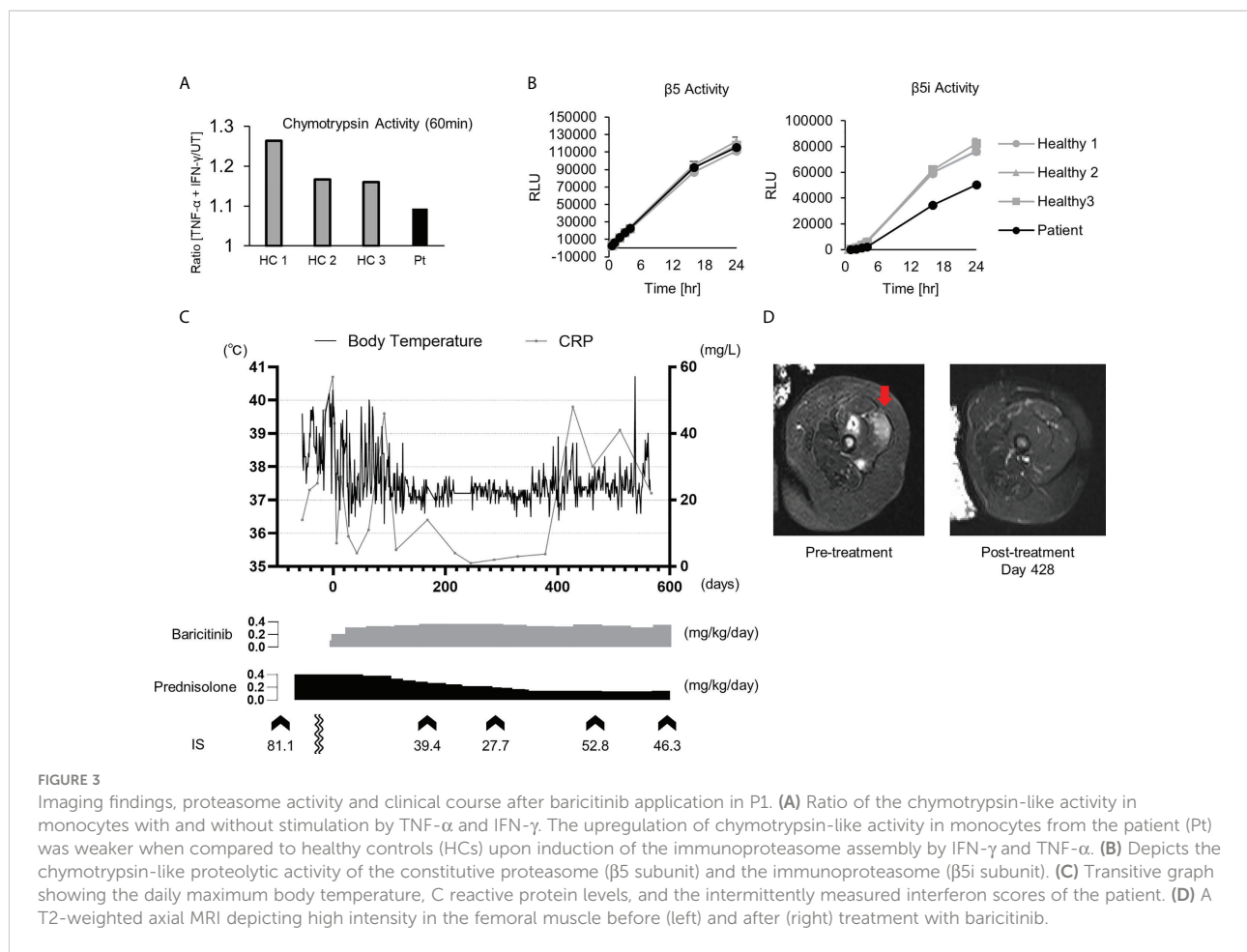
FIGURE 2
Macroscopic skin manifestations in patients with monogenic interferonopathy-like symptoms. Images of the macroscopic skin manifestations for each patient are shown in panel **(A)**. All patients, with the exception of P8, displayed nodular erythema. These lesions were palpable, sometimes annular, erythematous or violaceous plaques that healed with residual purpura (refer to the lower pictures for P2 and 3). P8 presented with livedo reticularis and skin ulcers. Panel **(B)** shows H&E-stained sections from the skin lesions. In all patients, with the exception of P7 and P8, mononuclear infiltrates in the perivascular and periadnexal dermis were seen. The mononuclear infiltrates in P7 were more intense in deep adipose tissues. Epidermal and superficial dermal infiltrates were observed in P8. The scale bar shown represents 200 μm.

The other nine patients with high ISs, but relatively late disease onset, displayed heterogenous clinical phenotypes (Table 2, P11-19). Patients 11 and 12 had similar clinical features to TAFRO syndrome and idiopathic multicentric Castleman's disease – not otherwise specified (iMCD-NOS), respectively, although their diagnosis was not established since their histopathologic findings were not typical of these diseases. Patient 11 could have been diagnosed with SLE under the 2019 EULAR/ACR classification criteria; however, the patient's symptoms were resistant to intensive immunosuppressive treatments targeting SLE, and the patient subsequently died from massive gastrointestinal bleeding at the age of 9 (34). The other seven patients remained clinically and genetically undiagnosed. Three of these patients (P13, 14 and 16) had robust family histories with autoimmune diseases, indicating a highly susceptible genetic background towards autoimmunity or an enhanced IFN response. Also of note, three patients (P13, 14, and 17) suffered from recurrent fever and various accompanying symptoms; however, aside from their high ISs, no other obvious abnormalities were detected. This was despite a thorough work up for fever of unknown origin, and included several imaging studies (CT, MRI, PET-CT), gastrointestinal endoscopy, and bone marrow examination. Interestingly, these three patients showed no significant elevation of inflammatory reactants, such as C reactive protein (CRP) and erythrocyte sedimentation rate (ESR), even during febrile episodes; yet, they continued to show constitutively elevated levels of ISs even without apparent symptoms.

The therapeutic effect of a JAK inhibitor on a pediatric case of PRAAS

Patient 1 in Table 2 suffered from a cyclic fever and chilblains-like erythema on the extremities and cheeks, both of which started in early infancy. TS analysis identified a previously reported pathogenic variant (c602G>T, p.Gly201Val) and a novel frame-shift variant (c.389delT, p.129Argfs*27) within the PSMB8 gene. Compound heterozygosity was confirmed by Sanger sequencing in the patient's parents. Analysis of the cDNA derived from the patient's whole blood cells indicated that the mRNA containing the c.389delT variant was eliminated probably by nonsense-mediated mRNA decay. Moreover, decreased catalytic activity of the immunoproteasome subunit β5i in monocytes of the patient compared with healthy controls confirmed the pathogenicity of the novel frameshift variant (Figures 3A, B). These results in addition to the patient's clinical manifestations and elevated type I IFN signature confirmed a diagnosis of PRAAS.

Although oral prednisolone (PSL; 1 mg/kg/day) quickly induced remission, the dosage could not be reduced below 0.5 mg/kg/day due to relapse. As the patient became steroid-dependent, baricitinib, a selective JAK1/2 inhibitor, was administered. Oral baricitinib was started at 0.1 mg/kg/day and titrated to 0.38 mg/kg/day. The patient's spiking fever and myositis resolved and CRP levels decreased to a normal level,

FIGURE 3
Imaging findings, proteasome activity and clinical course after baricitinib application in P1. **(A)** Ratio of the chymotrypsin-like activity in monocytes with and without stimulation by TNF-α and IFN-γ. The upregulation of chymotrypsin-like activity in monocytes from the patient (Pt) was weaker when compared to healthy controls (HCs) upon induction of the immunoproteasome assembly by IFN-γ and TNF-α. **(B)** Depicts the chymotrypsin-like proteolytic activity of the constitutive proteasome (β5 subunit) and the immunoproteasome (β5i subunit). **(C)** Transitive graph showing the daily maximum body temperature, C reactive protein levels, and the intermittently measured interferon scores of the patient. **(D)** A T2-weighted axial MRI depicting high intensity in the femoral muscle before (left) and after (right) treatment with baricitinib.

corresponding with the dose escalation (Figures 3C, D). The patient's daily PSL dose was reduced from 0.4 mg/kg to 0.15 mg/kg. However, discontinuation of the PSL was difficult due to a relapse of occasional low-grade fever and mild erythema, despite that the patient was treated with a higher dose of baricitinib than a typical therapeutic dose for RA (Figure 3C) (37).

Discussion

This study identified several patients with clinically and genetically undifferentiated inflammatory disease that had a demonstrated, enhanced IFN signature. Upregulated IFN signaling was also observed in diseases where the association between the etiology and type I IFN has not been completely established, i.e., clinically quiescent HA20 and IPH.

The relatively large number of clinically and genetically undiagnosed patients with enhanced IFN signatures was surprising. Notably, the clinical and laboratory features found more frequently in patients with high ISs potentially might have resulted from enhanced type I IFN signaling. For example, type I IFN is important for promoting the survival and activation of B

cells and thus is involved in tolerance breach and autoantibody production (38). In addition, impaired liver function is a common side effect of IFNβ, which is used for multiple sclerosis treatment (39).

Especially, 10 undiagnosed patients with upregulated type I IFN signaling presented with very early disease onset (an average of 4.5 months) and possessed some clinical characteristics indicative of monogenic type I interferonopathies. Several shared, unique characteristics were observed in the microscopic features of the patients' nodular erythema, which resembled histological findings in PRAAS. These findings support the possibility that these patients have some Mendelian genetic defects associated with genes related to type I IFN signaling. Consistent with this theory, a causal mutation was identified in the PSMB8 gene of one patient, and four others were found to have rare variants, of unknown significance, in genes that may be associated with type I IFN signaling. Measuring the IFN signature, in these cases, was useful to narrow down the candidate variants found through genetic analysis. In addition, considering the similarities in type I interferonopathy-like clinical manifestations that may be induced by upregulated type I IFN, it is possible that treatment with JAK inhibitors may be effective in patients without

confirmed pathogenic mutations. The possibility of utilizing personalized medicine in patients with undifferentiated inflammatory diseases, based on clinical phenotypes and IFN signatures, to identify patients who will respond to treatment with JAK inhibitors, will be important to determine in future studies.

Several patients with undiagnosed inflammatory diseases that had no symptoms indicative of type I interferonopathies were also identified as having enhanced type I IFN signaling, likely for heterogenous reasons. The diseases in two of these patients were clinically, but not histopathologically, compatible with iMCD-TAFRO and iMCD-NOS respectively. MCD clinical manifestations are believed to be driven by excessive expression of proinflammatory cytokines, particularly IL-6; however, the effectiveness of an IL-6 blockade or other immunosuppressants varies between patients, implying that this syndrome is a heterogenous disease (40, 41). Several recent reports have indicated that IFN signaling was upregulated in some patients with TAFRO syndrome (42, 43), and an inhibitor of mTOR, a molecule downstream of type I IFN signaling, was effective (44). Thus, determining whether an IFN signature can be utilized as a biomarker to classify and predict treatment responses in the patients with iMCD is of interest. Some of the patients in the study cohort had a strong family history of autoimmune disease. This is interesting considering the fact that enhanced production of type I IFN has been frequently reported in healthy relatives of SLE patients (45) and in patients with a phase of subclinical autoimmunity (46). Thus, these patients may be at risk for progression to full blown autoimmune disease in future.

Baricitinib was effective for the treatment of PRAAS, but a rather high dose was required to suppress inflammation, as reported previously (37). Interestingly, although the patient's IS was decreased considerably by a high dosage of baricitinib, it remained abnormally elevated even during the clinically quiescent phase. As the IFN signature is reportedly correlated more strongly with tapering of corticosteroid doses compared with acute-phase reactants such as CRP (23), his symptoms relapsed after reduction of the corticosteroid dose. Although a higher dosage of JAK inhibitors may further suppress IFN signaling and reduce the corticosteroid dose requirement, it could increase the risk of severe adverse effects such as infection and venous thromboembolism (47). Further studies are necessary to determine the optimal dosage of JAK inhibitors for the treatment of type I interferonopathies.

An association between IFN and the etiology of HA20 was first proposed in 2019 (48). Contrary to a previous report (49), an enhanced IFN signature was observed in clinically inactive patients in this study. However, one must consider that these findings are dependent on self-reporting from the patients as well as an assessment of disease activity from the attending physicians, differing from the previous report (49), in which an autoinflammatory disease activity index was used. The results obtained in this study indicate that patients with HA20 may possess a constitutive elevation of ISGs, rather than temporary

elevation during flares. It is important from both an etiological and clinical standpoint, to determine whether all patients with HA20 have a constitutive upregulation of type I IFN signaling, as in other monogenic type I interferonopathies. If so, an assessment of the IFN signature in patients will be helpful for diagnosis; for example, when variants of unknown significance are found in the TNFAIP3 gene, as in the present study. A larger study cohort will be necessary to answer this question.

IPH is a rare disorder characterized by diffuse alveolar hemorrhage. Although its etiology remains unknown, the involvement of immunological abnormalities has been suggested based on the presence of autoimmune antibodies (50–52) and the subsequent development of other autoimmune disorders, which have been observed in number of patients with IPH during follow-up (52–55). Some investigators have suggested that circulating immune complexes deposited into the pulmonary capillaries were involved in the disease pathogenesis (50), which may provoke the upregulation of type I IFN signaling. In this study, two out of three patients diagnosed with IPH had elevated IFN signatures, suggesting a possible link between type I IFN and the etiology of IPH. Further assessment of the type I IFN signature in a cohort of IPH patients may help to characterize this heterogenous disease and provide insight into its etiology. In addition, the IFN signaling pathway could provide a potential target for the treatment of this potentially fatal disorder.

There were some limitations in this study. First, the study cohort was recruited based on the recommendation of the attending physician; therefore, patients with clinical findings indicative of interferonopathies were more likely to be recruited. Second, since the clinical information was collected retrospectively, and not all patients were systematically assessed, limited information was available for some patients. For example, three patients in the study cohort had normal IFN signatures and nodular erythema; however, neither skin images nor histological results were available for these patients. Therefore, no comparison could be made with regard to their skin manifestations and the nodular erythema observed in patients with high IFN signatures. Third, ISs were measured only once in 10 of the 19 patients with high IFN signatures. IFN signatures are known to be elevated during infection; while no evidence of infection was observed during blood sampling, it may be more accurate to repeat the assessment in order to rule out a temporary elevation in IS, especially in patients where a moderate elevation of IFN signature was measured. Fourth, 8 of 18 patients with undifferentiated inflammatory diseases and ISs within normal range did not undergo in-depth genetic analysis. Therefore, we cannot conclude that a normal IFN signature can rule out a diagnosis of type I interferonopathy.

Overall, this study demonstrated that a subset of patients exist that have an upregulation of type I IFN signaling without any confirmed disease-causing mutations. Some of these patients may have unknown pathogenic genotypes in genes associated

with an upregulation of type I IFN signaling. In some patients, an assessment of the type I IFN signature was useful to narrow down candidate gene variants identified by genetic analysis. The type I IFN signature, in combination with other clinical findings, has the potential to become a useful biomarker for disease diagnosis and treatment choice in the care of patients with inflammatory diseases, although further longitudinal and intervention studies are necessary.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Kyoto University Hospital ethics committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

TakM, YH, and KIz designed the experiments and authored the manuscript. The first draft of the manuscript was written by TakM. TakM and YH performed the experiments, and TakM analyzed the data. TakM, YH, KIz, NKan., HO, TS, YNa, SA, KM, MB, YNi, AI, TF, DN, NI, YOt, SI, MN, KT, TN, TU, YOh, YT, MS, TE, KIw, AK, TKaw, TadM, TT, SO, and TY collected the clinical data and provided samples (from patients and relatives) for the analyses. MI-N, HN, JA, EH, JT, RN, and TY provided critical conceptual input and helped author the manuscript. SK and HO performed the NF-kB reporter gene activity assay. NKas and MKS analyzed proteasome activity. MF, TatM, and TKan prepared the microscope slides. MF and NKam performed the microscopic analysis of the skin samples. OO conducted the genetic analysis. All authors contributed to the article and approved the submitted version.

Funding

Acknowledgments

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.905960/full#supplementary-material

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LETTER TO THE EDITOR

A case of VEXAS syndrome with myositis possibly associated with macrophage activation syndrome

Dear Editor,

VEXAS (vacuoles, E1 enzyme, X- linked, autoinflammatory, somatic) syndrome initiated by the somatic mutation of methionine-41 (p.Met41) in the *UBA1* gene leading to abnormal ubiquitination and activation of the innate immune system is a newfound disorder, first reported in 2020 by Beck et al. as a late-onset adult autoinflammatory syndrome with various autoimmune and hematologic manifestations.[1] Here, we report a case of VEXAS syndrome with myositis.

A 62-year-old man presented with a 4-month history of recurring erythema (Figure 1a) accompanied by fever and elevated C-reactive protein (CRP) levels (3–10 mg/dL). Histopathology of the erythema demonstrated leukocytoclastic vasculitis. The patient also complained of bilateral ear lobe edema, which resolved within a month without medication (Figure 1b). Schnitzler syndrome was suspected and the patient was given prednisolone (15 mg/d). However, >9 mg/d of prednisolone were required to control disease activity. Over time, he also developed progressive, idiopathic macrocytic anemia, until now.

Two years later, he exhibited erythema, purpura, pain, edema of the left leg, and high-grade fever for 2 weeks, refractory to antibiotic therapy (Figure 1c,d). Serum tests showed the following: creatine kinase, 443 U/L; ferritin, 2394.97 ng/mL; white blood cell count, 25 200/μL (neutrophil 94%, lymphocyte 5.0%, monophil 1.0%); hemoglobin, 9.5 g/dL, mean corpuscular volume, 110 fL; CRP, 28.94 mg/dL; soluble interleukin 2 receptor, 3710 U/mL; autoantibodies, negative; and blood cultures, negative. Magnetic resonance imaging indicated iso- and high-intensity areas in T1- and T2-weighted images, respectively, in the adductor magnus and vastus intermedius muscles (Figure 1e), suggesting myositis. Histopathology displayed infiltration of CD68-positive macrophages in intermuscular fibers, with small numbers of CD4-positive cells and neutrophils (Figure 1f). Prednisolone (70 mg/d) was initiated but 3 days later he developed erythema on his entire body, which showed leukocytoclastic vasculitis (Figure 1g,h). Three-day methylpredonisolone pulse therapy alleviated the symptoms, while pancytopenia caused by hemophagocytic lymphohistiocytosis[2] found by bone marrow aspiration (Figure 1i①) developed and lasted for a month. Thus, both myositis and hemophagocytosis may be linked to macrophage activation syndrome. Three weeks after starting predonisolone 70 mg/d, prednisolone was tapered to 9 mg/d without recurrence, after methotrexate (6 mg/wk) addition. Sanger sequencing revealed the somatic mutation c.122T>C (p.Met41Thr) in *UBA1* in the patient's blood and skin, but not muscle, biopsy specimens (Figure 1j). Histopathology showed characteristic vacuoles in myeloid precursor cells in bone marrow aspirate (Figure 1i②), leading to the diagnosis of VEXAS syndrome.

VEXAS syndrome has the following characteristic features: fever (92%), skin involvement (88%) manifesting as neutrophilic dermatoses or leukocytoclastic vasculitis, macrocytic anemia (96%), ear and nose chondritis (64%), pulmonary infiltrates (72%), and bone marrow vacuoles (100%); all of these were found in our case, except pulmonary infiltrates.[1] This is the first reported case of VEXAS syndrome with myositis in association with macrophage activation syndrome,[2] which has already been reported as a complication of VEXAS syndrome.[3] Myositis in this case mimicked inflammatory myopathy with abundant macrophages, which is frequently associated with macrophage activation syndrome.[4] Dermatologists should consider VEXAS syndrome in cases presenting with atypical erythema, macrocytic anemia, and fever, requiring continuous oral steroids, especially in older men.

CONFLICT OF INTEREST

None declared.

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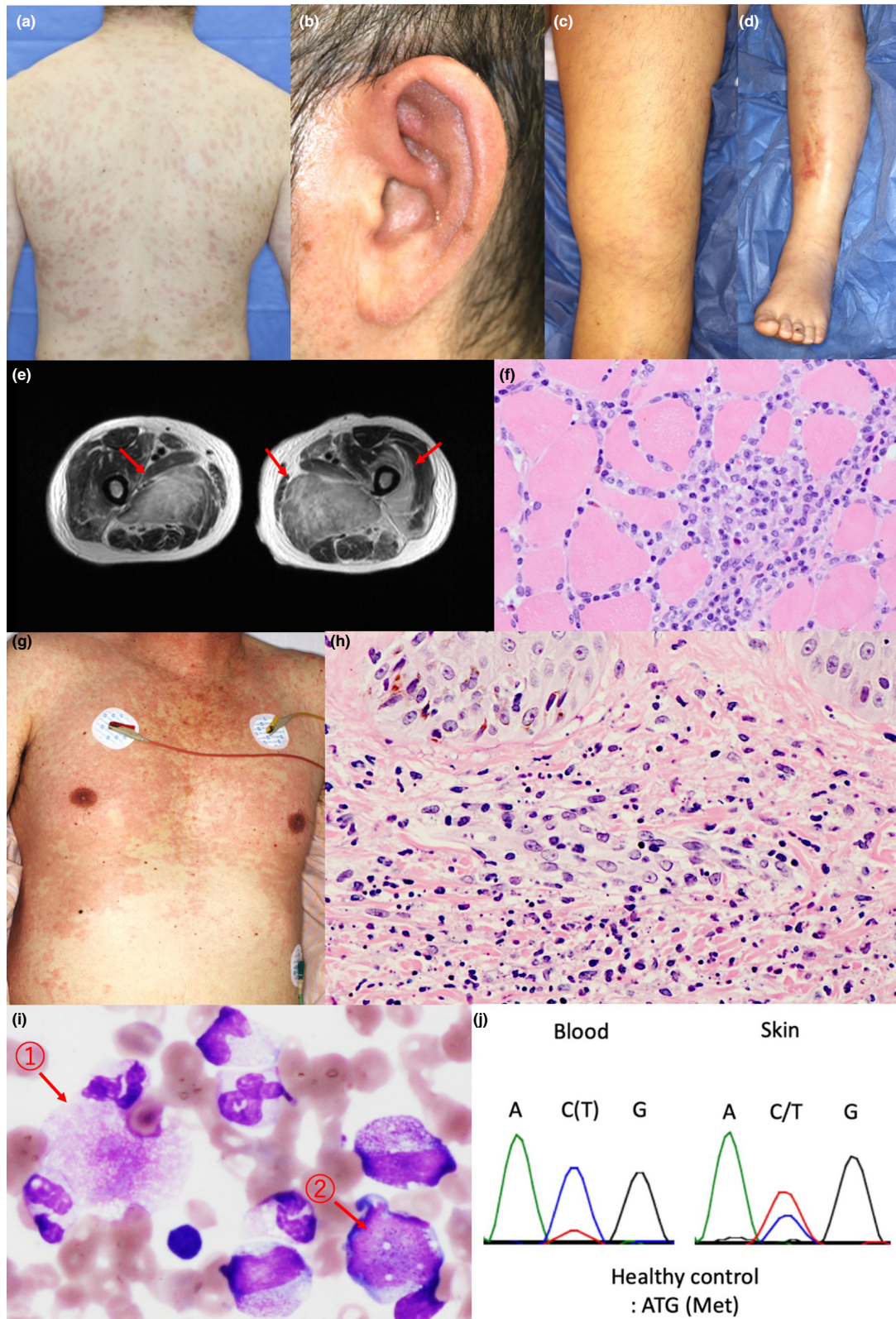Email: matsu_yasu00@yahoo.co.jp

FIGURE 1 (a) Diffuse erythema on the patient's trunk and upper limbs on first consult. (b) Swelling of the upper part of the left ear lobe. (c) Swelling of the left thigh with pain on applied pressure with mild livedo reticularis. (d) Erythema and purpura on the left lower leg. (e) High signal intensity areas seen in T2-weighted images of the adductor magnus muscles and left vastus intermedius muscle. (f) Histopathological images of the muscle with histiocytes infiltration (HE ×400). (g) Diffuse erythema on the patient's entire body, 3 days after 70 mg/d prednisolone administration. (h) Histopathological images of the skin with leukocytoclastic vasculitis (HE ×400). (i) ① blood cell phagocytosis of macrophage, ② vacuoles of myeloid precursor cell. (j) Somatic mutation at c.122T>C (p.Met41Thr) in *UBA1* gene from the patient's blood and skin biopsy specimen. Healthy control shows only ATG (met) (not shown).

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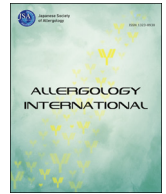
Original Article

Summary of the current status of clinically diagnosed cases of Schnitzler syndrome in Japan

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A R T I C L E   I N F O

A B S T R A C T

Background: Schnitzler syndrome is a rare disorder with chronic urticaria, and there is no report summarizing the current status in Japan.
Methods: A nationwide survey of major dermatology departments in Japan was conducted in 2019. We further performed a systematic search of PubMed and Ichushi-Web, using the keywords "Schnitzler syndrome" and "Japan" then contacted the corresponding authors or physicians for further information.

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Results: Excluding duplicates, a total of 36 clinically diagnosed cases were identified from 1994 through the spring of 2022, with a male to female ratio of 1:1. The median age of onset was 56.5 years. It took 3.3 years from the first symptom, mostly urticaria, to reach the final diagnosis. The current status of 30 cases was ascertained; two patients developed B-cell lymphoma. SchS treatment was generally effective with high doses of corticosteroids, but symptoms sometimes recurred after tapering. Colchicine was administered in 17 cases and was effective in 8, but showed no effect in the others. Tocilizumab, used in six cases, improved laboratory abnormalities and symptoms, but lost its efficacy after several years. Rituximab, used in five cases, was effective in reducing serum IgM levels or lymphoma mass, but not in inflammatory symptoms. Four cases were treated with IL-1 targeting therapy, either anakinra or canakinumab, and achieved complete remission, except one case with diffuse large B-cell lymphoma.
Conclusions: Since Schnitzler syndrome is a rare disease, the continuous collection and long-term follow-up of clinical information is essential for its appropriate treatment and further understanding of its pathophysiology.

Abbreviations

ALP	alkaline phosphatase
CT	computed tomography
CLC	colchicine
CRP	C-reactive protein
CsA	cyclosporine
DFPP	double filtration plasmapheresis
DLBCL	diffuse large B-cell lymphoma
DMARDs	disease-modified anti-rheumatic drugs
ESR	erythrocyte sedimentation rate
KU	Kyoto University
MRI	magnetic resonance imaging
PET	positron emission tomography
PGA	physician global assessment
PSL	prednisolone
PUVA	ultraviolet radiation
RTX	rituximab
SchS	Schnitzler syndrome
SDC	Strasbourg Diagnostic Criteria
WBC	white blood cell
WM	Waldenström macroglobulinemia

Introduction

Schnitzler syndrome (SchS) is a rare disorder characterized by chronic urticarial rash and monoclonal IgM or, rarely, IgG gammopathy. This syndrome was first reported in 1972 by French dermatologist Lilian Schnitzler. Since then, over 200 cases have been reported in 25 countries; most patients were Caucasian.[1,2]

In 2010, Lipsker *et al.*[3] proposed SchS diagnostic criteria. In 2013, Lipsker, Schnitzler, and other experts compiled the current diagnostic criteria (Strasbourg Diagnostic Criteria; SDC),[1] which defined chronic urticarial rash and monoclonal IgM or, rarely, IgG gammopathy as obligate criteria and the following symptoms as minor criteria: recurrent fever, abnormal bone remodeling with or without bone pain, a neutrophilic dermal infiltrate upon skin biopsy, and leukocytosis and/or elevated C-reactive protein (CRP). The major complications are lymphoproliferative disorders,[3] including Waldenström macroglobulinemia (WM), lymphoplasmacytic lymphoma with IgM monoclonal protein.

The first case in Japan was reported in 1995 by Nagoya City University.[4] We recently experienced five cases at Kyoto University (KU),[5] and became aware that there is no established treatment for this syndrome, though some clinical trials have been conducted for anakinra,[6] rilonacept,[7] canakinumab,[8,9] and tocilizumab.[10] To address this situation, we first collected cases of this acquired autoinflammatory disorder in Japan to understand its current status and needs.

Methods

Collecting patient data in Japan

In 2019, Dr. Nobuo Kanazawa conducted a nationwide survey to identify domestic cases of SchS in the dermatology departments of 122 university hospitals and 233 large (more than 500 beds) hospitals. The project was led by Dr. Takashi Hashimoto of the Osaka Metropolitan University and funded by the Science Research Grant for Policy Research on Intractable Diseases from the Ministry of Health, Labor and Welfare, Japan.

Collecting patient data from the literature

Next, we conducted a search of PubMed (National Library of Medicine) and Ichushi-Web (Japan Medical Abstracts Society) using the keywords "Schnitzler syndrome" and "Japan" and collected published manuscripts and conference reports from 1994 to the spring of 2022.

Clinical information collection

From the literature, we extracted the age at onset, symptoms, blood sampling data, imaging data, and treatments performed. We then contacted the authors for more detailed information and a physician global assessment (PGA) for the five primary SchS symptoms (urticarial rash, fatigue, fever/chills, myalgia, and arthralgia/bone pain), which were each graded on a 5-point Likert scale: 0 = no, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe disease activity.[7,8,10] From the authors' responses, we supplemented the existing clinical and laboratory information with the long-term course of treatment and its effects. The study was approved by the ethics committee of KU (R2559).

Results

Patient data collection

The nationwide survey conducted in 2019 identified seven cases (cases 1–6 and 28 in Table 1). We received responses from 109 (31%) of the 355 facilities, including 60 (49%) of the 122 university hospitals and 49 (21%) of the 233 large hospitals. A literature search

Table 1
Demographic and characteristics per the Strasbourg diagnostic criteria.

Case[†]	Age[‡]	M/F	Obligate criteria		Minor criteria				Ref
			Rash[§]	IgM or IgG[¶]	Fever[††]	Bone[‡‡]	Biopsy[§§]	WBC & CRP[¶¶]	
Definitive cases									
1	51	F	+	IgMκ	+	N/A	+	+	11
2	51	F	+	IgMκ	+	N/A	+	+	20
3	45	F	+	IgMκ	+	N/A	+	+	22
4	57	M	+	IgMκ	+	N/A	+	+	23
5	35	M	+	IgMκ	+	N/A	+	+	N/A
6	40	F	+	IgM	+	N/A	+	+	24
7	75	F	+	IgMκ	+	N/A	N/A	+	13
8	46	M	+	IgMκ	+	+	N/A	N/A	16
9	39	M	+	IgMκ	+	N/A	+	+	17
10	68	F	+	IgMκ	+	N/A	+	−	18
11	42	F	+	IgMκ	+	N/A	+	+	4
12[†]	76	M	+	IgMκ	+	N/A	+	+	25
13	73	M	+	IgMκ	+	−	+	+	27
14	62	F	+	IgMκ	+	N/A	+	+	28
15[†]	71	F	+	IgMκ	+	N/A	+	−	29
16	72	M	+	IgM	+	+	+	N/A	30
17[†]	63	F	+	IgM	+	N/A	+	N/A	32
18	64	M	+	IgM	+	N/A	+	+	33
19	63	F	+	IgM	+	N/A	+	−	35
20	45	M	+	IgMκ	+	+	+	+	36
21	61	F	+	IgMκ	+	−	+	+	5,56
22	60	M	+	IgMλ	+	N/A	+	+	5
23	43	F	+	IgMκ	+	+	+	+	5,39
24	56	F	+	IgMκ	+	+	+	+	5
25	41	M	+	IgMκ	+	N/A	+	+	38
26	54	M	+	IgMκ and IgGκ	+	N/A	−	+	40
27	49	M	+	IgMκ	+	N/A	−	+	N/A
Probable cases									
28	67	F	+	IgMκ	−	N/A	+	−	21
29	75	M	+	IgMκ	−	N/A	+	−	12
30	68	M	+	IgGκ	+	N/A	+	N/A	14
31	62	M	+	IgMκ	+	N/A	N/A	−	15
32	65	M	+	IgMκ	+	N/A	N/A	N/A	26
33	57	F	+	IgM	−	N/A	+	N/A	34
34	65	M	+	IgMκ	−	N/A	N/A	+	37
Unmet Cases									
35	39	F	+	−	+	+	+	+	19
36	42	F	+	IgAλ	+	−	N/A	+	31

N/A, Not performed or not mentioned in the literature.

[†] Case with: Indicates that the patient is already dead.

[‡] Age = the age (year) of onset.

[§] Rash = urticarial rash.

[¶] IgM or IgG = the monoclonal IgM or IgG and subtype of κ or λ, where available.

[††] Fever = recurrent fever over 38 °C, and otherwise unexplained.

[‡‡] Bone = the presence or absence of abnormal bone remodeling with or without bone pain as assessed by bone scintigraphy, MRI, or elevated bone alkaline phosphatase.

[§§] Biopsy = the presence or absence of neutrophilic dermal infiltrate without fibrinoid necrosis and significant dermal edema in the biopsy specimen.

[¶¶] WBC & CRP = the presence or absence of leukocytosis (neutrophils >10,000/mm³) and/or elevated CRP (>30 mg/L).

revealed 10 cases published in English,[4,11–19] 21 cases published or presented in Japanese,[20–40] and our previous 5 cases at KU Hospital.[5] Excluding duplicates, a total of 36 clinically diagnosed cases were identified in Japan from 1994 to the spring of 2022, of which 27 were definitive cases when checked with the SDC, 7 were probable, and 2 did not meet the SDC (Table 1). An additional case was reported as SchS,[41] but we excluded it since the reporters themselves revised the diagnosis to polyarteritis nodosa with secondary amyloidosis.

Epidemiology

The mean age of onset for the 27 definitive cases is 55.6 years, with a male-to-female ratio of about 1:1. Among the 36 total cases,

the mean age of onset is 56.5 years, and the gender composition remained the same. On average, based on available data for 35 patients, it took 3.3 years from the first symptom, mostly urticaria, to reach a final diagnosis of SchS. None of the cases presented with a family history of SchS symptoms nor was there any identifiable, common patient history.

Among the 36 cases, we were able to contact the paper authors or the patients' physicians for 30 cases; three definitive cases were reported to have died (Table 1). Two cases of SchS-related lymphomas were reported: a mature B-cell lymphoma of uncertain histologic subtype from a probable case[18] and a diffuse large B-cell lymphoma (DLBCL) from a definitive case.[32] The former survived following treatment, but the latter patient died; an autopsy revealed that the intima of the great vessels and heart valves were covered with IgM-positive deposits. The remaining two definitive patients died of diseases unrelated to SchS, interstitial pneumonia[25] and non-occlusive mesenteric ischemia.[29] Eight cases (cases 5, 7, 10, 11, 13, 18, 31 and 33) have not recently followed up with their physicians. However, 19 patients are still attending their care physicians, 7 of whom (cases 1, 2, 6, 9, 14, 21 and 24) are alive more than 10 years after the symptom onset, and all are definitive cases. The mean follow-up period was 6.1 years.

Obligate criteria

All 36 patients had a chronic urticarial rash (Fig. 1A, Supplementary Fig. 1A), an obligate criterion of the SDC.[1] The frequency of urticaria ranged from daily to several times a year, and the extent of skin lesions varied over the trunk and extremities, sparing the head, palms, and soles. Usually, the lesions were non-pruritic and lasted more than 24 h.

Gammopathy was present in all, except one unmet case[19] for the SDC, and 34 cases were monoclonal. One patient presented with IgGκ and IgMκ biclonal gammopathy[40] (Fig. 1B, C), and we counted this case as definitive. Among the 27 definitive cases, there was no mention of the light chain subtype for 5 patients but all patients, except the biclonal gammopathy case, had monoclonal IgM gammopathy, 20 (74%) had the IgMκ subtype, and 1 (3.7%) IgMλ.[5] In contrast, one probable case of IgGκ subtype[14] and an IgAλ subtype unmet case[31] were reported (Supplementary Fig. 1C). Based on the available data from 27 definitive patients, the average IgM serum level at first visit was 792.4 mg/dl (normal range; male: 33–190 mg/dl, female: 46–260 mg/dl) (Fig. 2A, B). Even in definitive cases, there were cases with IgM levels close to the upper limit of normal in both men and women (Fig. 2A, B), but over time, many cases were observed to have elevated IgM levels (Fig. 2C). The outliers (*1 in Fig. 2A and *2 in 2B) are cases 9[17] and 19,[35] respectively; case 9 presented 10 years after the onset of urticaria; detailed information for case 19 was unavailable. Bence-Jones proteins in the urine were only mentioned for 11 cases of SchS, out of which 6 definitive cases (cases 2, 9, 11, 12, 14 and 22) and one probable case (case 30) were positive.

Minor criteria

One of the minor SchS criteria was fever. In definitive cases, all patients had recurrent fevers (Fig. 1D), mostly up to 38–39 °C, sometimes accompanied by a rash. Febrile events ranged in frequency from daily to several times per year. On the other hand, among the 36 cases for which information could be collected, 4 probable cases did not develop fever (Supplementary Fig. 1D).

In Japan, magnetic resonance imaging (MRI) and bone scintigraphy were not performed routinely but occasionally, when bone pain or arthralgia was observed. Only 9 patients were examined using these techniques, and 5 definitive cases[5,16,30,36,39] and one
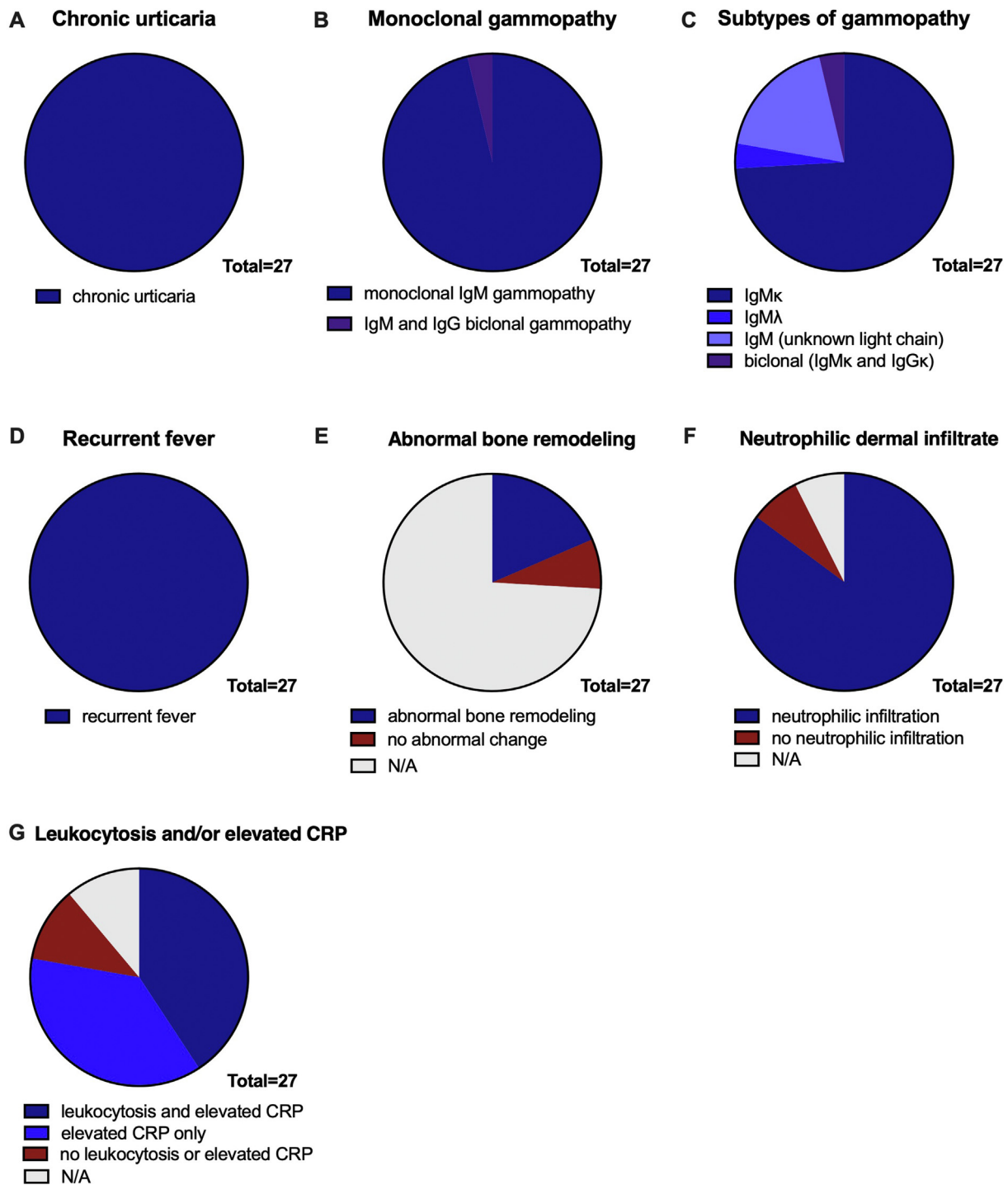
Fig. 1. The ratio of definitve patients with clinical and laboratory features defined in the Strasbourg diagnostic criteria (n = 27). **(A)** Chronic urticaria was seen in 100% of patients. **(B)** Monoclonal IgM or IgG gammopathy was seen in 100% of patients. **(C)** Subtypes of monoclonal gammopathy. **(D)** Recurrent fever was seen in 100% of patients. **(E)** Five cases were positive for abnormal bone remodeling (n = 7). **(F)** Skin biopsy revealed 23 cases with neutrophilic dermal infiltrate (n = 25). **(G)** Leukocytosis and/or elevated CRP was seen in 21 cases (11 with both leukocytosis and elevated CRP, and 10 with elevated CRP only); 3 cases had neither leukocytosis nor elevated CRP.

unmet case[19] were positive for abnormal bone remodeling of the lumbar vertebrae, femur, or tibia (Fig. 1E, Supplementary Fig. 1E). In some cases, positron emission tomography (PET)/computed to-mography (CT) revealed fluorodeoxyglucose uptake by the bone marrow,[19,31] or osteosclerosis was discovered by a whole-body CT scan[5] or X-ray.[37] Out of 36 patients, bone pain, mostly of the femur or tibia,[5,17,39] affected 8 out of 27 definitive cases, and 2 probable and one unmet cases, while 10 out of 27 definitive cases, 3 probable

and one unmet cases suffered from joint pain, such as in the knees.[12,14,18]

Skin biopsies were performed in 25 definitive cases, and 23 showed perivascular and/or interstitial infiltrate of neutrophils (Fig. 1F), sometimes with lymphocyte or eosinophil infiltration and nuclear dust. Among probable and unmet cases, all the 5 cases (4 probable and one unmet) in which skin biopsy was performed showed neutrophilic infiltration, and one of the probable cases
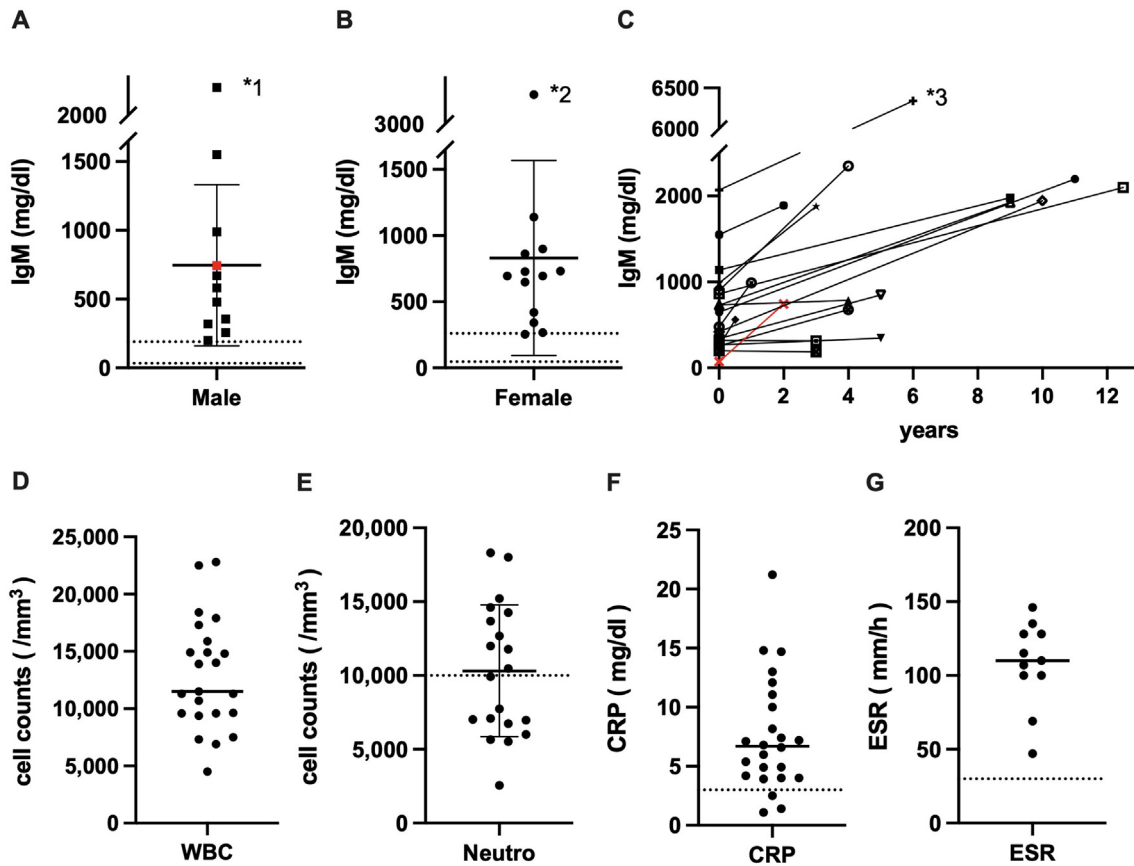
Fig. 2. Laboratory data of definitive patients. **(A, B)** Serum IgM levels at first presentation of male (n = 11) and female (n = 13) patients. The standard value (male: 33—190 mg/dl, female: 46—260 mg/dl) is marked with a dotted line. **(C)** The long-term changes of serum IgM levels (n = 18). In case 12 (red line), the serum IgM level was 71 mg/dl when the patient first noticed urticaria and fever and visited the previous doctor, but at the first presentation to the authors it had risen to 743 mg/dl,[25] which was plotted in **(A)** (red dot). **(D)** White blood cell counts at first presentation (n = 23). **(E)** Neutrophil counts at first presentation (n = 20). The threshold in the Strasbourg diagnostic criteria (neutrophils >10,000/mm^3) is marked with a dotted line. **(F)** Serum CRP levels at first presentation (n = 24). The threshold in the Strasbourg diagnostic criteria (CRP >3.0 mg/dl) is marked with a dotted line. **(G)** ESR levels at first presentation (n = 10). The threshold described as biological findings of SchS patients by Simon et al.[1] (>30 mm/h) is marked with a dotted line.

showed basophilic infiltration.[12] No fibrinoid necrosis of the vessel wall was reported. Immuno-deposits consisting of IgM, C3, and sometimes IgG were seen in a few cases,[4,18,29] but the deposit locations varied from interstitial granular patterns to along vessel walls.

In 27 definitive cases, leukocytosis (neutrophils >10,000/mm^3) and/or elevated C-reactive protein (CRP >3 mg/dl) were observed in 21 cases (Fig. 1G); 11 of these presented with leukocytosis (average = 10,311/mm^3) and all 21 presented with elevated CRP (average = 7.6 mg/dl) (Fig. 2E, F). Erythrocyte sedimentation rate (ESR), another laboratory marker of systemic inflammation, was elevated in most patients (when tested, 10 of 27) for an average of 107 mm/h (Fig. 2G). When probable and unmet cases were included in the analysis, 24 of the 30 cases with data were found to have leukocytosis and/or elevated CRP (Supplementary Fig. 1G), including 12 cases with leukocytosis (average = 10,090/mm3) but all 24 cases had elevated CRP (average = 7.2 mg/dl) (Supplementary Fig. 2E, F). ESR was elevated in 12 of 36 tested for an average of 108 mm/h (Supplementary Fig. 2G).

Other features

Some cases of SchS progress to hematologic tumors,[2] and this evaluation is necessary. Lymphadenopathy was reported in 5 definitive patients (cases 8, 13, 14, 16 and 20)[16,27,28,30,36] and each one probable (case 34)[37] and unmet case (case 36).[31] Only two definitive patients[16,30] had hepatosplenomegaly. Bone-marrow aspiration was

performed in 19 cases, and normal bone marrow was observed in most cases, but one each of definitive (case 2), probable (case 30), and unmet (case 36) cases showed a moderate increase in plasma cells without atypical differentiation. Only in a definitive case 10,[18] which had B-cell lymphoma, did the analysis of bone-marrow aspirates indicate the existence of clonal, mature lymphoma cells.

One definitive patient (case 15)[29] developed membranoproliferative glomerulonephritis due to cryoglobulinemic vasculitis. None were found to develop amyloidosis.

Genetic analysis

Previously, somatic mosaicism of *NLRP3* in the myeloid lineage and the *MYD88* p.L265P mutation were found in a few cases of SchS[42,43]; a gain-of function mutation in *NLRP3* causes overexpression of IL-1β in cryopyrin-associated periodic syndrome (CAPS),[44] and the *MYD88* p.L265P mutation is detectable in more than 90% of WM.[45] In the survey of Japanese hospitals, only a few patients were screened for these mutations, but none presented with either an *NLRP3* or *MYD88* mutation. The somatic mosaicism of *NLRP3*, first detected in CAPS by our group,[44] was not reported either.

Treatment

Supplementary Table 1 shows treatments performed on each case. Since the treatment effects described in the literature for SchS
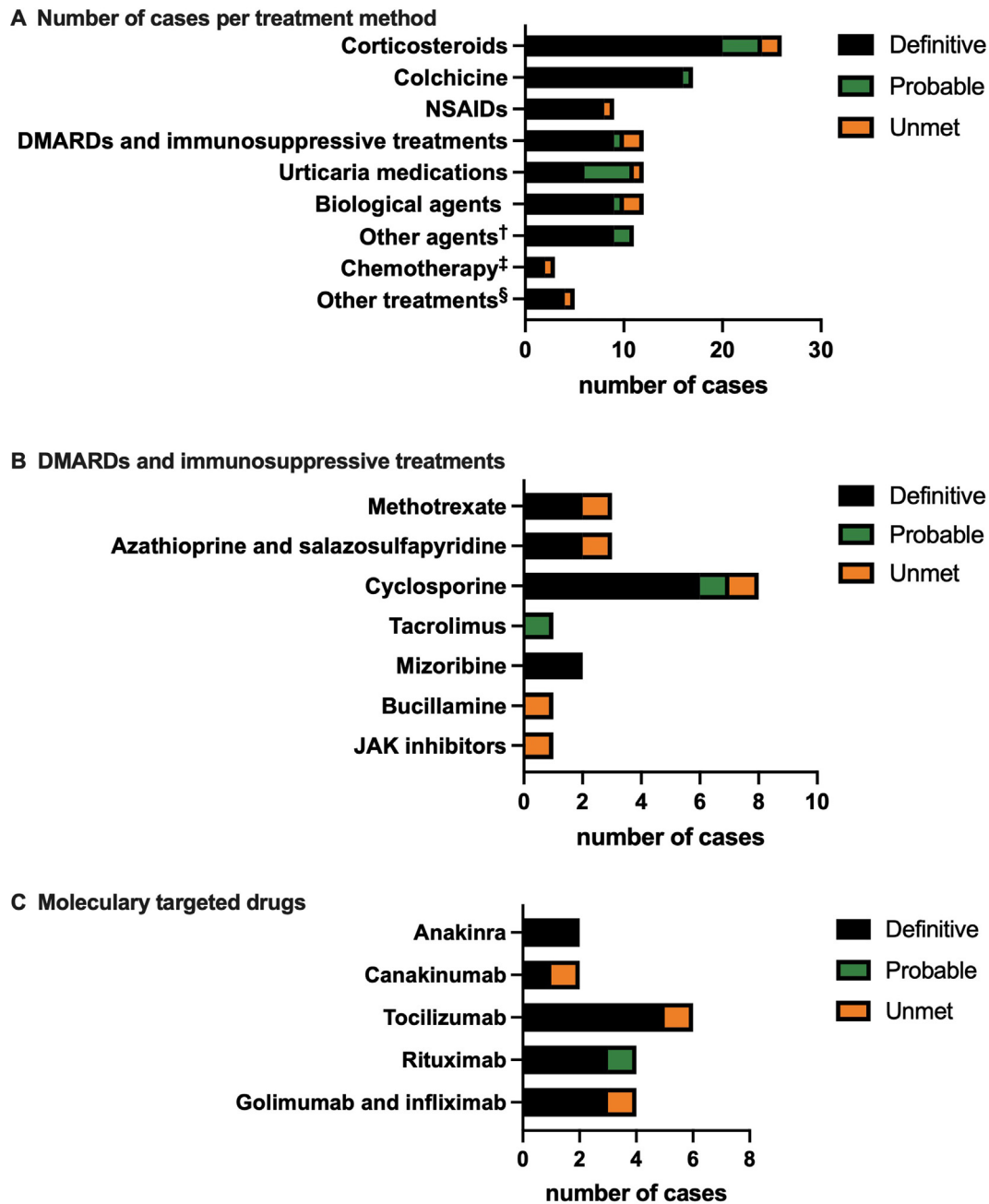
A   Number of cases per treatment method

B   DMARDs and immunosuppressive treatments

C   Molecularly targeted drugs

Fig. 3. Number of cases treated with each treatment method. **(A)** is a summary, while **(B)** or **(C)** is more detailed data. **(B)** Number of patients treated with DMARDs, immuno-suppressive agents and biological agents. **(C)** Number of cases treated with molecularly targeted drugs including IL-1 blocking therapies (Anakinra and Canakinumab), an anti-IL-6 agent, Tocilizumab, and an anti-CD20 antibody, Rituximab. Other agents[†]: KI (potassium iodide), DDS (diaminodiphenyl sulfone), RXM (roxithromaycin), and BP (bisphosphonate). Chemotherapy[‡]: R-EPOCH (rituximab, etoposide, prednisolone, vincristine, cyclophosphamide, and doxorubicin), DMVM-IFNa (dexamethasone, ranimustine, vincristine, melphalan, and interferon-α), and BD (bortezomib and dexamethasone). Other treatments[§]: Radiation, PUVA (psoralen and long-wave ultraviolet radiation), DFFP (double filtration plasma-pheresis), and TKR (total knee replacement).

are short-term and lack standard evaluation criteria, we evaluated the disease status of the 19 cases for whom we had direct contact with their physicians using the provided PGA scores (Supplementary Table 2). Of these, 16 had a definitive diagnosis, one had a probable, and two did not meet SDC.

Corticosteroids were the most common treatment; used in 20 definitive, 4 probable and 2 unmet cases (Fig. 3A, Supplementary Table 1). They were usually started at high doses (over 0.5 mg/kg/ day) and were effective. However, the side effects of corticosteroids limit its use at high doses for extended periods, and symptoms sometimes recurred when corticosteroids were tapered off.

Therefore, most cases were treated with other therapies in addition to steroids, with the exception of a definitive case 14,[28] who was maintained only on prednisolone (PSL) 15 mg/day (Supplementary Table 2).

Colchicine (CLC) was used in 16 definitive and one probable cases (Fig. 3A). Among 19 cases under follow-up (Supplementary Table 2), CLC was administered to 9 definitive cases, and 4 of the 9 patients achieved a PGA of zero. Three of these patients (cases 1, 2 and 5) were treated with CLC alone and one (case 4) with CLC plus 2 mg/day PSL. In three cases (cases 6, 12 and 27), the post-treatment PGA score improved, but did not reach zero. Two cases (cases 3 and

25) showed no clinical response to treatment with CLC. Among eight cases where information could not be confirmed directly with the care physicians and was collected only from the literature, two (cases 7 and 29) reportedly showed improvement following CLC therapy. Briefly, for a probable case 29,[12] who was resistant to antihistamines and omalizumab, and a definitive case 7,[13] who responded to 30 mg PSL but relapsed upon tapering, the initiation or addition of CLC dramatically improved symptoms. The remaining six cases[4,5,17,33] showed little or no response to CLC.

Both in the literature and follow-up data, NSAIDs alone had little therapeutic effect but reduced fever and bone pain when used on demand (Supplementary Table 2).[4] According to the literature we collected, most kinds of disease-modified anti-rheumatic drugs (DMARDs) and immunosuppressive treatments (Fig. 3B) like azathioprine, methotrexate, mizoribine, salazosulfapyridine, and tacrolimus were ineffective,[4,5] while cyclosporine (CsA) was sometimes effective but difficult to continue due to side effect.[11] Common urticaria medications, like antihistamines and omalizumab, were ineffective therapies for SchS.[12,19,21]

Molecularly targeted drugs (Fig. 3C) were also used. IL-1 blocking therapy is regarded as one of the most effective treatments[2] but was tried only in a few cases in Japan. Anakinra, an IL-1 receptor antagonist, was used in two definitive cases,[5,32] one of whom died of DLBCL (case 17). Canakinumab, an anti-IL-1β antibody, was used in two cases, one is definitive and the other is unmet case who does not have monoclonal IgM.[5,19] In the three cases under follow up that received either of the IL-1-targeting therapies, a PGA of zero was achieved, but serum IgM levels in two definitive cases did not improve (Supplementary Table 2).

Tocilizumab, an anti-IL-6 agent, was used in six cases (Fig. 3C). The literature showed that initiating tocilizumab dramatically improved laboratory abnormalities, joint symptoms, and fever.[5,30,39] Two definitive patients (cases 22 and 26) have been on tocilizumab for 1 and 3 years, respectively, and both have achieved a PGA of zero (Supplementary Table 2). However, some patients treated with tocilizumab experienced symptom recurrence within a few years (definitive cases 21, 23, and unmet case 36).

The anti-CD-20 antibody rituximab (RTX) was used in five cases (Fig. 3C). RTX was effective in improving serum IgM levels or lymphoma lesions, but was insufficient for cutaneous symptoms.[18,33] According to follow-up data for a probable case 28, after receiving five doses of RTX, the patient remained asymptomatic for 2 years with PSL, CsA, and diaminodiphenyl sulfone; however, the withdrawal of CsA was accompanied by an urticarial rash. In a definitive case 9, an interview revealed that after their serum IgM level rose to 6342 mg/dl (*3, Fig. 2C), seven RTX injections lowered serum IgM, but they gradually relapsed with fevers and high CRP. In another definitive case 10, where a retroperitoneal tumor suggested to be a B-cell lymphoma was found,[18] the mass disappeared with RTX therapy but the urticarial rash was resistant. The rash gradually improved with post-RTX radiation along with complete remission of the mass.

Chemotherapy regimens for malignant myeloma were performed in three cases (Supplementary Table 1); one definitive case combined with RTX failed against DLBCL,[32] one unmet case achieved a reduction of paraprotein but failed at improving symptoms,[31] and another definitive case was repeated three times before reducing paraprotein levels but still failed to control skin eruptions.[36]

Psoralen and long-wave ultraviolet radiation (PUVA) was tried in one case,[27] and while symptoms improved, it needed to be repeated. In cases 15 and 20,[29,36] double filtration plasmapheresis (DFPP) temporarily improved serum IgM levels and skin symptoms but they relapsed after discontinuation (Fig. 3A).

Discussion

In this survey, 36 clinically diagnosed cases of SchS were identified, which we believe includes all the cases diagnosed in Japan, of which 27 were definitive cases when checked for SDC, 7 were probable, and 2 did not meet the SDC (Table 1). We also followed up with the reporting author and/or physician. Since this disease is rare, with a total of only about 300 cases reported worldwide to date, mainly in Europe and the United States,[2] it is essential to first determine the number of cases, the clinical course and treatment course, also in Asian country, Japan. We believe that this report will raise awareness of potential and under-diagnosed patients.

The mean age of onset and clinical characteristics of the Japanese cases are similar to previous reviews from overseas,[2] except that objective findings of abnormal bone remodeling, arthralgia, bone pain, and/or anemia are less common in Japanese cases (Fig. 1E); comparing 27 definitive cases in this Japanese research with 281 cases in the previous review,[2] abnormal bone remodeling in only 5 out of 9 tested cases vs 85% (82 out of 97 tested cases showed increased uptake by bone scintigraphy), arthralgia in 37% vs 68% (192 out of 281 cases), bone pain in 29.6% vs 55% (155 out of 281 cases), and anemia 0% vs 63% (62 out of 98 cases), respectively. One reason is that SchS is described as an urticaria-related disease in the Japanese urticaria practice guidelines and is highly recognized by dermatologists in Japan. It may be necessary to raise awareness that abnormal bone remodeling is better detected by MRI or bone scintigraphy than PET/CT.[46] Since few patients reported bone pain or arthralgia and a very few cases showed elevated alkaline phosphatase (ALP), it is possible that the number of cases with abnormal bone remodeling is really small in Japan, rather than it being overlooked.

Of note, chronic urticaria often precedes other symptoms, and it may take several years to complete the symptoms. In particular, paraprotein and hyper IgMemia may not present in the first 2–3 years.[25] Some patients had serum IgM levels near the upper border of normal at their first visit (Fig. 2A, B), and IgM levels gradually increased over the course of the disease (Fig. 2C). Therefore, it is necessary to monitor immunoglobulins continuously if SchS is suspected. In fact, even in a definitive case 12,[25] the serum IgM level was 71 mg/dl when the patient first sought care for urticaria and fever, but 2 years later it had risen to 743 mg/dl (red dot, Fig. 2A) when he presented to the authors and was diagnosed with SchS. Similarly, low inflammatory markers at first visits (Fig. 2D, E) do not rule out SchS, and must be reexamined especially when a patient is symptomatic.

Regarding treatment, Japan seems to be unique in that CLC is used more (17/36) than in other countries (51/281),[2] possibly due to difficulty accessing IL-1-targeting therapy. CLC inhibits the formation of NLRP3 inflammasomes by disrupting microtubules, which decreases IL-1β production,[47] and inhibits neutrophil recruitment.[48] Cases 1, 2, and 4 were initially treated with either PSL or CsA, which were successfully tapered off after the addition of CLC. Case 5 has been successfully treated with CLC only since his SchS diagnosis, while case 25 was initially treated unsuccessfully with CsA and high-dose of PSL and switched to CLC, but was still resistant to CLC. Though the number of cases is low for generalizable conclusions, those who respond well to low-dose PSL or CsA are likely to have their symptoms controlled with CLC. Simon et al.[1] recommended that cases without severe symptoms or persistent, elevated inflammation markers should be controlled with CLC.

Patients with severe symptoms may initially respond well to tocilizumab but might develop secondary failure. In addition, in most cases treated with IL-1 targeting therapy, the paraprotein levels increased progressively even when autoinflammatory

symptoms were well-controlled.[5] A similar phenomenon was observed in the long term study of canakinumab on SchS,[8] which suggests that suppressing autoinflammation may not prevent lymphoma development. In our present survey, the detailed clinical course is unknown outside of a postmortem report, but it was reported that anakinra did not prevent the development of DLBCL in case 17.[32] In contrast, only RTX succeeded in lowering serum IgM levels. Since erythema and fever remained or recurred in all RTX-treated patients, we suspect that immune cells such as neutrophils and mature plasma cells that do not express CD20 are responsible for the inflammation. Recently, Masson Regnault et al. reported that the spontaneous release of proinflammatory cytokines by the peripheral blood mononuclear cells of SchS patients was higher than in controls, suggesting myeloid inflammation in SchS.[49]

Thus, the most interesting part of SchS is the association between the pathogenesis of autoinflammatory symptoms and monoclonal IgM gammopathy. One of the reasons why SchS is considered an acquired autoinflammatory syndrome is that it shares clinical manifestations with CAPS, in which mutations in *NLRP3* cause clinical symptoms such as urticaria, fever, and abnormal bone remodeling. NLRP3 is an intracellular pattern recognition receptor, and activation of this molecule leads to the formation of inflammasomes and activation of IL-1β, one of the cytokines that trigger inflammation.[50,51] Therefore, acute-phase inflammatory findings observed in CAPS, such as clinical symptoms and abnormal laboratory values, are clearly mediated by IL-1β. Nevertheless, Louvrier et al. recently reported that no somatic or germline pathogenic variations have been identified in *NLRP3* in their large cohort of 40 SchS patients,[52] indicating that *NLRP3* is not a potential candidate gene for SchS and that previously reported SchS patients with an *NLRP3* mosaic mutation may instead have a late-onset NLRP3-autoinflammatory disease.[53] However, the efficacy of IL-1 targeting therapy for SchS probably indicates that IL-1β is the main actor of SchS inflammation, and thus, if accessible, SchS should be treated with IL-1 targeting therapy, as the therapeutic effect on inflammation lasts for a long observation period. Since there is no difference in the clinical presentation except for fewer findings associated with abnormal bone remodeling than in foreign patients, we expect that IL-1 targeted therapy will be successful in Japanese patients with SchS. When the therapy is introduced in Japan, further observation is needed to determine whether long-term treatments targeting IL-1 can prevent IgM elevation and progression to hematologic malignancies.

Conversely, MYD88, of which gain-of-function mutations were reported in more than 90% of WM,[45] is involved in signal transduction downstream of the IL-1 receptor. Neutrophil infiltration of the skin, which causes an urticaria-like rash, and abnormal bone remodeling in SchS, can be evoked by enhanced IL-1R1/MYD88 signaling.[54,55] Although the mechanism of SchS development remains unclear, we believe that accumulating detailed clinical manifestations is a reliable approach to elucidating its pathogenesis.

In summary, all Japanese cases clinically diagnosed as definitive SchS showed urticarial rash, monoclonal IgM gammopathy and recurrent fever. Most of their clinical features are similar to that of foreign cases, but less showed abnormal bone remodeling and more cases were treated with Colchicine than foreign cases. In contrast, this study has limitations. Although this research covers almost all the SchS cases diagnosed in Japan, statistical analysis was impractical due to the number of cases and the lack of unified treatment plan.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2022.11.004.

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RESEARCH ARTICLE

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Efficacy and safety of baricitinib in Japanese patients with autoinflammatory type I interferonopathies (NNS/CANDLE, SAVI, And AGS)

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Abstract

Background This study evaluated the efficacy and safety of baricitinib (Janus kinase-1/2 inhibitor), in adult and pediatric Japanese patients with Nakajo-Nishimura syndrome/chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (NNS/CANDLE), stimulator of interferon genes-associated vasculopathy with onset during infancy (SAVI), or Aicardi-Goutières syndrome (AGS).

Methods A Phase 2/3, multicenter, open-label study (NCT04517253) was conducted across 52 weeks. Primary efficacy endpoint assessed the change in mean daily diary score (DDS) from baseline to the end of primary treatment period. Other efficacy endpoints included change in mean DDS to the end of maintenance period, daily corticosteroid use, Physician's Global Assessment of Disease Activity (PGA) scores, and daily symptom-specific score (DSSS) from baseline to primary and maintenance treatment periods. All treatment-emergent adverse events (TEAEs) that occurred postdosing were recorded.

Results Overall, 9 patients (5 with NNS, 3 with SAVI, and 1 with AGS) were enrolled; 55.6% were females, mean age was 26 years, and mean corticosteroid use/weight was 0.2 mg/kg. At the end of primary treatment period, mean DDS decreased from baseline in patients with NNS/CANDLE (0.22) and SAVI (0.21) and increased in the patient with AGS (0.07). At the end of maintenance treatment period, mean DDS decreased from baseline in patients with NNS/CAN-DLE (0.18) and SAVI (0.27) and increased in the patient with AGS (0.04). Mean percent corticosteroid use decreased by 18.4% in 3 out of 5 patients with NNS/CANDLE and 62.9% in 1 out of 3 patients with SAVI. Mean PGA score decreased from baseline in patients with NNS/CANDLE (1.60), SAVI (1.33), and AGS (1.0), and mean DSSS improved from baseline. All patients reported ≥ 1 TEAE. Frequently reported AEs included BK polyomavirus detection (3; 33.3%), increased blood creatine phosphokinase (2; 22.2%), anemia (2; 22.2%), and upper respiratory tract infection (2; 22.2%). Three (33.3%) patients reported serious adverse events, 1 of which was related to study drug. One patient with SAVI died due to intracranial hemorrhage, which was not related to study drug.

Conclusion Baricitinib may offer a potential therapeutic option for patients with NNS/CANDLE, SAVI, and AGS, with a positive benefit/risk profile in a vulnerable patient population with multiple comorbidities.

Trial registration NLM clinicaltrials.gov, NCT04517253. Registered 18 August 2020.

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Background

Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE), stimulator of interferon genes (STING)-associated vasculopathy with onset during infancy (SAVI), and Aicardi-Goutières syndrome (AGS) are Mendelian autoinflammatory interferonopathies characterized by early-onset systemic and organ-specific inflammation and a prominent interferon (IFN) response gene signature (IGS) [1].

CANDLE, SAVI, and AGS are monogenic disorders caused by pathogenic genetic defects [1, 2]. Autosomal recessive loss-of-function (LOF) mutations in the proteasome subunit beta type 8 (*PSMB8*) cause Nakajo-Nishimura syndrome (NNS) with nodular erythema, elongated and thickened fingers, and emaciation [1, 3, 4], and CANDLE [1, 4, 5]. Additionally, LOF mutations in other proteasome subunits (*PSMB9, PSMB4, PSMA3*) and the proteasome assembly proteins could contribute to the disease condition [1]. Clinical manifestations commonly associated with CANDLE include fever, nodular or plaque-like violaceous skin rashes, myositis, and joint contractures [1, 5, 6]. SAVI is caused by dominant gain-of-function mutations in *TMEM173* encoding the STING protein [1]. Clinical manifestations commonly associated with SAVI include rash with fever, vasculopathic lesions in cold sensitive acral areas, paratracheal adenopathy, abnormal pulmonary function tests, myositis, and arthritis [1, 7, 8]. Although AGS is a monogenic disorder, it is genetically heterogenous and caused by mutations in seven genes encoding nucleic-acid-processing enzymes and cytosolic nucleic acid sensor. AGS is characterized by unexplained fevers, hepatosplenomegaly, encephalopathy and white matter disease, "chilblains" or cold-induced acral dermatosis, systemic and pulmonary hypertension, and early-onset, monophasic, congenital infection-like syndrome [1, 2, 9, 10]. Moreover, the cerebrospinal fluid shows chronic lymphocytosis and increased IFN-α levels [10].

Considering the dysregulation of IFNs in CANDLE, SAVI, and AGS, an anti-IFN approach could be a possible therapeutic option. Moreover, due to the presence of a high IGS, patients with CANDLE and SAVI respond poorly to disease-modifying antirheumatic drugs and interleukin-1-blocking agents [11]. In patients with AGS, neurological improvement was variable when administered with corticosteroids such as prednisolone [12]. Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway is the principal signal transduction pathway for IFNs [13].

Thus, blocking molecules involved in the JAK–STAT pathway could be a possible therapeutic option for NNS/CANDLE, SAVI, and AGS.

Baricitinib is a selective JAK1/2 inhibitor that reduces the phosphorylation and activation of STATs, consequently modulating inflammation, cellular activation, and proliferation of key immune cells [14]. In Japan, baricitinib is approved for the treatment of rheumatoid arthritis (RA) in patients who have had an inadequate response to conventional treatments (including the prevention of structural joint damage), in patients with moderate to severe atopic dermatitis in patients who have had an inadequate response to topical treatments, alopecia areata for extensive and intractable cases, and pneumonia associated with COVID-19 infection (limited to patients requiring supplemental oxygen) [15]. A population pharmacokinetic (PK) modeling was performed to characterize the PK profile of baricitinib in patients with rare Mendelian autoinflammatory interferonopathies enrolled in the FDA-approved compassionate use program (NCT01724580) [16]. The results indicated a dose-dependent decrease of IFN biomarkers and IGS, confirming an in vivo effect of baricitinib and supporting a weight- and estimated glomerular filtration rate (eGFR)-based baricitinib dosing regimen as a therapeutic option for patients with Mendelian autoinflammatory interferonopathies [16].

This study evaluates the efficacy and safety of baricitinib in pediatric and adult Japanese patients with NNS/CANDLE, SAVI, and AGS. The safety and tolerability data from this study are intended to establish an understanding of the benefit/risk relationship of baricitinib in patients with NNS/CANDLE, SAVI, and AGS.

Methods

Study population

Patients demonstrating ≥ 2 signs and symptoms and with a confirmed genetic diagnosis of NNS/CANDLE, SAVI, or AGS (including familial chilblain lupus) were eligible. Patients with NNS/CANDLE or SAVI ≥ 17.5 months of age and patients with AGS ≥ 6 months of age were included in the trial [17]. All the patients weighed ≥ 5 kg with average daily dairy score (DDS) of

- ≥ 0.5 for patients with NNS/CANDLE at Visit 2,
- ≥ 1.0 for patients with SAVI at Visit 6, and
- ≥ 0.5 for patients with AGS at Visit 6

Study design

This Phase 2/3, multicenter, open-label study (ClinicalTrials.gov Identifier: NCT04517253) evaluated the efficacy and safety of baricitinib in adult and pediatric Japanese patients with autoinflammatory type I interferonopathies including NNS/CANDLE, SAVI, and AGS.

The study was divided into 6 periods including (i) screening, (ii) pre-treatment (only for patients with NNS/CANDLE), (iii) dose adjustment, (iv) primary treatment, (v) maintenance treatment, and (vi) post-treatment follow-up period (Fig. 1).

The screening period varied for patients with NNS/CANDLE, SAVI, and AGS. The screening period ranged from 7 to 35 days prior to baseline (Visit 6) for patients with SAVI and AGS, whereas patients with NNS/CANDLE had a screening period of 1 week (Visit 1) before entering the pre-treatment period.

Patients with NNS/CANDLE entered a 12-week pre-treatment period at the beginning of Visit 1. The data from the pre-treatment period was used for baseline comparison.

Patients who met all eligibility criteria entered the 8-week dose adjustment period (Visit 6 to Visit 9). Patients initially received treatment dose based on weight class and eGFR, which was further escalated to identify tolerable dose. The dose escalation model was based on the results from previous PK studies of baricitinib [11, 16]. Patients who weighed < 40 kg received baricitinib tablets or liquid suspension orally based on patient choice, whereas patients who weighed ≥ 40 kg were recommended to receive only tablets. If the patients' weight changed during the study, dose regimens could be modified. Compliance was assessed by counting returned tablets or weighing a returned bottle for liquid suspension. Patients treated with baricitinib were considered noncompliant if they missed ≥ 20% of the prescribed doses during the study or if they were judged by the investigator to have intentionally or repeatedly taken more than the prescribed amount of study medication.

Patients received the optimized dosage throughout the primary and maintenance treatment periods. The
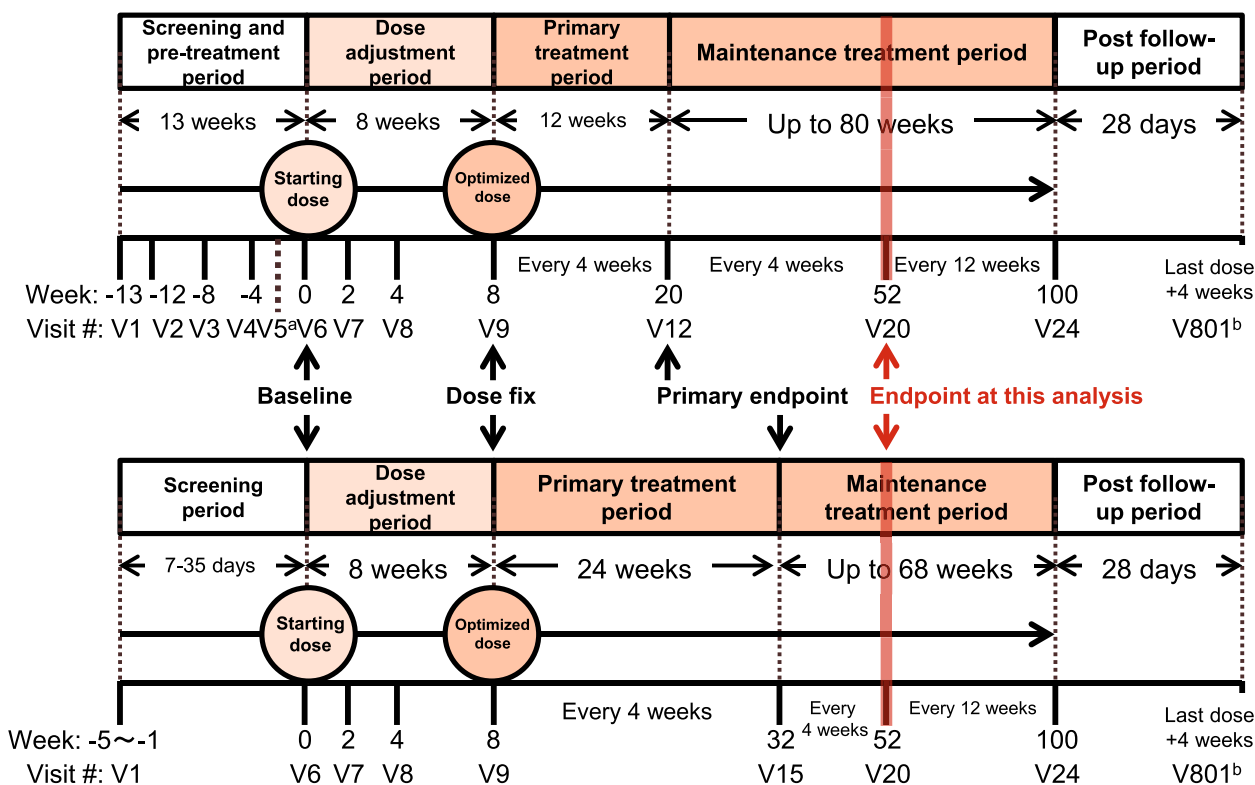
Fig. 1 Study design. AGS = Aicardi-Goutières syndrome; NNS/CANDLE = Nakajo-Nishimura syndrome/chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature; SAVI = STING-associated vasculopathy with onset during infancy; STING = stimulator of interferon genes; V = visit; wk = week. [a]Patient can skip Visit 5 and proceed to Visit 6 if biologic agents are not administered during pre-treatment period or an appropriate washout duration of biologic agents defined in Exclusion Criterion #28 has already passed at Visit 5. [b]Visit 801 should occur approximately 28 days after the last dose of study drug. Patients who will transition from this study to commercial baricitinib are not required to complete Visit 801

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duration of primary treatment period was 12 weeks for patients with NNS/CANDLE and 24 weeks for patients with SAVI and AGS based on the results from the JAGA (NCT01724580) trial [11]. Entry into the maintenance period after completion of the primary treatment period occurred at Visit 12 for patients with NNS/CANDLE and Visit 15 for patients with SAVI and AGS. This study is ongoing to collect long-term efficacy and safety results.

Treatment period was considered from the first dose of baricitinib (Visit 6; Week 0) to the date of final visit or early discontinuation period. Endpoint of post-follow-up period was Visit 801. Primary endpoint assessed the change in mean DDS from baseline (Week 0; Visit 6) to Week 20 in patients with NNS/CANDLE and Week 32 in patients with SAVI and AGS. Other efficacy and safety endpoints were assessed until Week 52.

The study protocol was approved by the institutional review boards prior to patient recruitment, and each patient or their guardian provided written informed consent prior to enrollment. The study was conducted in accordance with consensus ethics principles derived from international ethics guidelines, including the Declaration of Helsinki and the International Ethical Guidelines by the Council for International Organizations of Medical Sciences and the International Council for Harmonization E6 Guidelines for Good Clinical Practice.

Clinical benefit assessment
Decrease in daily diary score
The primary effectiveness measure evaluated the decrease in DDS of patients' signs and symptoms. Daily symptoms, including fever, rash, musculoskeletal pain, headache, and fatigue, were rated with increasing level of severity. Signs and symptoms for NNS/CANDLE, SAVI, and AGS are detailed in Supplementary Table 1. The average score of each symptom was calculated using the data from 7 days preceding the current visit. The calculated average score for each symptom is summed up and divided by the number of assessed symptoms (5 symptoms for NNS/CANDLE, 6 symptoms for SAVI, and 8 symptoms for AGS) to calculate the average score for each patient. DDS does not change linearly; therefore, no settings for "minimal clinically important difference" could be set. However, patients were considered well-controlled if a DDS of < 0.5 for NNS/CANDLE and < 1.0 for SAVI was observed [11].

Decrease in daily dose of corticosteroids
A decrease in daily dose of corticosteroids was defined by a < 0.15 mg/kg/day prednisone equivalent systemic corticosteroid dose or a daily dose percentage decrease of at least 50% from baseline.

Decrease in physician's global assessment of disease activity score
The patients' current disease activities were assessed using the 21-circle visual analog scale ranging from 0 to 10 with increasing level of severity [18].

Other clinical assessments
An improvement in diary symptom-specific score (DSSS) from baseline, Barthel index evaluating the activity of living with intractable diseases [19], mean changes in height and growth from baseline, and laboratory parameters including C-reactive protein (CRP), aspartate transaminase, alanine aminotransferase, gamma glutamyl transferase, and creatine phosphokinase were assessed.

Mean change from baseline in biomarkers of IFN signaling and IGS was assessed throughout the study. The IFN signature score used in IGS assessment, comprised of 6-gene IFN signature assays from 28 IFN Response Genes. The final assay was parameterized to separate IFN-high and IFN-low patients with a cut point of zero where each gene was scaled to have the same range. A change of 1 point in the IFN signature means twofold change in gene concentration.

Safety assessment
All adverse events (AEs) occurring after signing the informed consent form were recorded in the electronic case report form. AEs were classified based on the Medical Dictionary for Regulatory Activities. AE of special interest included infections, myelosuppressive events, thrombocytosis, malignancies, hepatic events, major adverse cardiovascular events, and thrombotic events. Electrocardiograms, physical examinations, vital signs including lung and liver function tests, BK virus plasma and urine screening using quantitative PCR, hepatitis B virus DNA monitoring (only in patients who were positive for HbcAb or HbsAb at Visit 1), and height and weight measurement were performed, and clinically significant findings were reported.

Statistics
In lieu of anticipating that relatively few patients with each condition would be enrolled, no formal statistical tests were planned. Instead, descriptive summaries and data listings were planned to summarize the results.

Continuous data were summarized in terms of the mean, standard deviation (SD), minimum, maximum, median, and number of observations; categorical data were summarized as frequency counts and percentages. Although the efficacy population set included all enrolled patients who had at least 1 dose of baricitinib, efficacy analyses were divided among diagnosis-specific efficacy population subsets.

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Mean DDS was calculated from the average scores of 7 days preceding the current visit; however, if more than 50% symptom scores were missing, the mean DDS was not calculated. A last-observation-carried-forward imputation replaced missing data with the most recent non-missing postbaseline assessment. Proportion of days meeting threshold was calculated by the total number of days meeting the threshold divided by total number of days with non-missing diary scores during the interval. Mean DDS with < 0.5 was used as a threshold for all diseases as an indication of disease control. All corticosteroid doses were standardized to an equivalent prednisone dose.

Results

Study population

A total of 9 patients were enrolled in the study. Five patients were diagnosed with NNS/CANDLE, 3 patients with SAVI, and 1 patient with AGS. One patient with NNS/CANDLE discontinued the study treatment and study due to an AE and one patient with SAVI discontinued the study treatment and study due to death.

Overall, the mean age (SD) of patients was 26 (19.1) years, a higher proportion were female (5; 55.6%), and the mean baseline eGFR was 117.6 mL/min/1.73 m^2 (Table 1). All patients had a history of concomitant medications, with a majority of patients using prednisolone (8; 88.9%), and the mean corticosteroid dose per weight was 0.2 mg/kg (Table 1).

Treatment

The maximum total daily dose of baricitinib ranged from 8 to 12 mg. The median (range) duration of exposure to baricitinib was 52.1 (8.4–53.1) weeks. Among patients with NNS/CANDLE, the minimum and maximum duration of exposure was 13.3 weeks and 52.4 weeks, respectively. In patients with SAVI, the minimum and maximum duration of exposure was 8.4 weeks and 53.0 weeks, respectively; 1 patient discontinued early due to death and 1 patient was enrolled in the study after primary data cutoff and did not complete 52 weeks as of April 2022. In the patient with AGS, the duration of exposure was 53.1 weeks. Patients were deemed compliant if they missed < 20% of the expected number of doses; all patients were compliant in the study.

Efficacy

Primary efficacy endpoint

Mean daily diary score at the end of the primary treatment period   The overall mean DDS decreased from baseline in patients with NNS/CANDLE (0.22) and SAVI (0.21),

Table 1 Summary of demographic and baseline characteristics

Parameter	NNS/CANDLE (*N* = 5)	SAVI (*N* = 3)	AGS (*N* = 1)	Total (*N* = 9)
Age, years				
Mean (SD)	39.2 (13.8)	9.7 (9.8)	9.0 (NA)	26.0 (19.1)
Range (minimum, maximum)	15–48	4–21	9–9	4–48
Sex, n (%)				
Female	1 (20.0)	3 (100.0)	1 (100.0)	5 (55.6)
eGFR (mL/min/1.73m^2)				
Mean (SD)	124.7 (19.3)	109.4 (22.5)	106.7 (NA)	117.6 (19.6)
Weight, kg				
Mean (SD)	45.8 (5.9)	22.7 (17.3)	14.30 (NA)	34.6 (16.6)
Weight, n (%)				
≥ 10 to < 20 kg	0	2 (66.7)	1 (100.0)	3 (33.3)
≥ 40 to < 50 kg	4 (80.0)	1 (33.3)	0	5 (55.6)
≥ 50 to < 60 kg	1 (20.0)	0	0	1 (11.1)
Height (cm)				
Mean (SD)	150.9 (11.2)	112.9 (31.2)	112.2 (NA)	133.9 (26.6)
BMI (kg/m^2)				
Mean (SD)	20.2 (2.3)	15.7 (3.2)	11.4 (NA)	17.7 (4.0)
Corticosteroid use				
n (%)	5 (100.0)	1 (33.3)	0	6 (66.7)
Corticosteroid total daily dose (mg)[a]				
Mean (SD)	8.9 (4.3)	4 (NA)	0	8.08 (4.3)
Corticosteroid dose per weight (mg/kg)[a]				
Mean (SD)	0.2 (0.1)	0.4 (NA)	0	0.2 (0.1)

AGS Aicardi-Goutières syndrome, *BMI* body mass index, *eGFR* estimated glomerular filtration rate, *n* number of patients with non-missing data, *N* number of patients in the safety population within each disease subpopulation, *NA* not applicable, *NNS/CANDLE* Nakajo-Nishimura syndrome/chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature, *SAVI* STING-associated vasculopathy with onset during infancy, *SD* standard deviation, *STING* stimulator of interferon genes

[a] The mean corticosteroid doses are reported as prednisone equivalent doses in patients taking corticosteroids

but slightly increased in the patient with AGS (0.07) (Table 2).

At the end of the primary treatment period (Week 20), patients with NNS/CANDLE met the response criterion of mean DDS < 0.5 with a mean proportion of days of 0.4 and a change from the pre-treatment baseline with a mean proportion of days of 0.04 (Table 2).

Secondary endpoint

Mean daily diary score at the end of the maintenance treatment period   The overall mean DDS decreased from baseline in patients with NNS/CANDLE (0.18) and SAVI (0.27), but slightly increased for the patient with AGS (0.04) (Table 2 and Fig. 2). Three of 5 patients with NNS/CANDLE and all 3 patients with SAVI showed decreased

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Table 2 Summary of efficacy parameters by subgroup

Parameters	Period	Week	NNS/CANDLE (*N* = 5)		SAVI (*N* = 3)		AGS (*N* = 1)	
			Observed	Change	Observed	Change	Observed	Change
Mean DDS, mean (SD)	Pre-treatment[a]		0.64 (0.32)		-		-	
	Baseline	0	0.92 (0.35)		0.79 (0.28)		1.25 (NA)	
	Primary treatment[b]	20/32	0.70 (0.51)	-0.22 (0.59)	0.57 (0.23)	-0.21 (0.25)	1.3 (NA)	0.07 (NA)
	Maintenance treatment	52	0.74 (0.57)	-0.18 (0.64)	0.52 (0.28)	-0.27 (0.34)	1.29 (NA)	0.04 (NA)
Proportion of days with < 0.5 in mean DDS, mean (SD)	Pre-treatment[a]		0.36 (0.24)		-		-	
	Baseline	0	0.0 (0)		0.23 (0.39)		0 (NA)	
	Primary treatment[b]	20/32	0.40 (0.55)	0.40 (0.55)	0.32 (0.56)	0.10 (0.17)	0 (NA)	0.0 (NA)
	Maintenance treatment	52	0.36 (0.50)	0.36 (0.50)	0.63 (0.55)	0.41 (0.47)	0 (NA)	0.0 (NA)
Physician's Global Assessment of disease activity, mean (SD)	Pre-treatment[a]		5.45 (1.33)		-		-	
	Baseline	0	5.80 (1.15)		3.83 (1.26)		2.50 (NA)	
	Primary treatment	20/32	3.70 (1.10)	-2.10 (0.82)	2.50 (1.80)	-1.33 (1.76)	2.0 (NA)	-0.50 (NA)
	Maintenance treatment	52	4.20 (2.80)	-1.60 (1.85)	2.50 (1.80)	-1.33 (1.76)	1.50 (NA)	-1.0 (NA)
Dose of systemic corticosteroid with prednisone equivalent (mg/kg/day)[c]	Baseline, mean (SD)	0	0.19 (0.09)		0.39 (NA)		-	
	Primary treatment, mean (SD)	20/32	0.15 (0.15)	-0.04 (0.10)	0.15 (NA)	-0.24 (NA)	-	-
	Primary treatment, % change			-23.5% (41.5%)		-61.2% (NA)		
	Maintenance treatment, mean (SD)	52	0.17 (0.15)	-0.03 (0.11)	0.14 (NA)	-0.24 (NA)	-	-
	Maintenance treatment, % change			-18.4% (43.4%)		-62.9% (NA)		

AGS Aicardi-Goutières syndrome, *DDS* daily diary score, *N* total number of patients, *NA* not applicable, *NNS/CANDLE* Nakajo-Nishimura syndrome/chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature, *SAVI* STING-associated vasculopathy with onset during infancy, *STING* stimulator of interferon genes

Patients with SAVI and AGS had no pre-treatment scores

[a] Averaged scores collected during pre-treatment period were used

[b] Week 20 for NNS/CANDLE; Week 32 for SAVI and AGS

[c] Patients on systemic corticosteroid at baseline were included

mean DDS at last observation compared with baseline (Fig. 2).

At the end of the maintenance treatment period (Week 52), patients with NNS/CANDLE met the response criteria of mean DDS < 0.5 with a mean proportion of days of 0.36 and a change from the pre-treatment baseline with a mean proportion of days of 0.01 (Table 2 and Fig. 2).

Daily dose of corticosteroid   At the end of the primary treatment period, all 5 patients with NNS/CANDLE reported corticosteroid use at baseline and showed a mean decrease of 23.5% in systemic corticosteroid use with a mean change of -0.04 mg/kg/day from baseline. One patient with SAVI reported corticosteroid use at baseline and showed a clinically significant mean per-
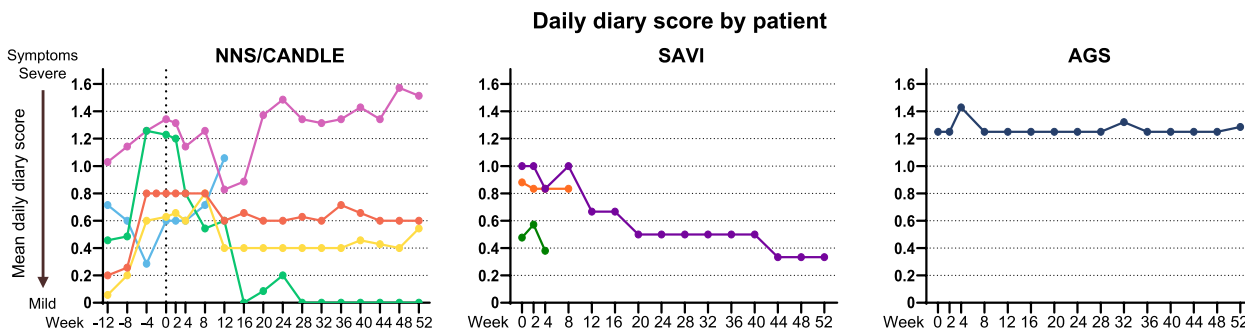

Fig. 2 Mean daily diary score by patients with (**A**) NNS/CANDLE, (**B**) SAVI, and (**C**) AGS. AGS = Aicardi-Goutières syndrome; NNS/CANDLE = Nakajo-Nishimura syndrome/chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature; SAVI = STING-associated vasculopathy with onset during infancy; STING = stimulator of interferon genes

cent decrease of 61.2% in corticosteroid use and a mean change of -0.24 mg/kg/day from baseline (Table 2 and Fig. 3).

At the end of the maintenance period, patients with NNS/CANDLE showed a mean percent decrease of 18.4% in systemic corticosteroids use with a mean change of -0.03 mg/kg/day from baseline. The patient with SAVI showed a clinically significant mean percent decrease of 62.9% in systemic corticosteroids use and a mean change of -0.24 mg/kg/day from baseline (Table 2 and Fig. 3). The patient with AGS did not use any corticosteroid at baseline.

Physician's global assessment of disease activity score   At the end of the primary treatment period, patients with NNS/CANDLE (2.10), SAVI (1.33), and AGS (0.50) showed mean decrease in Physician's Global Assessment of Disease Activity (PGA) score from baseline. Decrease in PGA score (1.75) from pre-treatment baseline was observed in patients with NNS/CANDLE (Table 2).

A similar trend was observed at the end of the maintenance period (Week 52). Patients with NNS/CANDLE (1.60), SAVI (1.33), and AGS (1.0) showed mean decrease in PGA score from baseline. Decrease in PGA score (1.25) from pre-treatment baseline was observed in patients with NNS/CANDLE (Table 2).

Individual patient PGA scores are listed in Fig. 4.

Other efficacy analyses   DSSS across all patient subgroups improved from baseline. Rash, musculoskeletal pain, and fatigue symptom scores improved in patients with NNS/CANDLE. Fever, rash, musculoskeletal pain, fatigue, and respiratory/breathing symptom scores improved in patients with SAVI, and length of uninterrupted sleep increased in the patient with AGS (Supplementary Table 2).

A mean decrease from baseline in disease severity of fever and rash was observed in patients with NNS/CANDLE. Patients with SAVI and AGS were assessed using the Barthel index, with 1 patient with SAVI
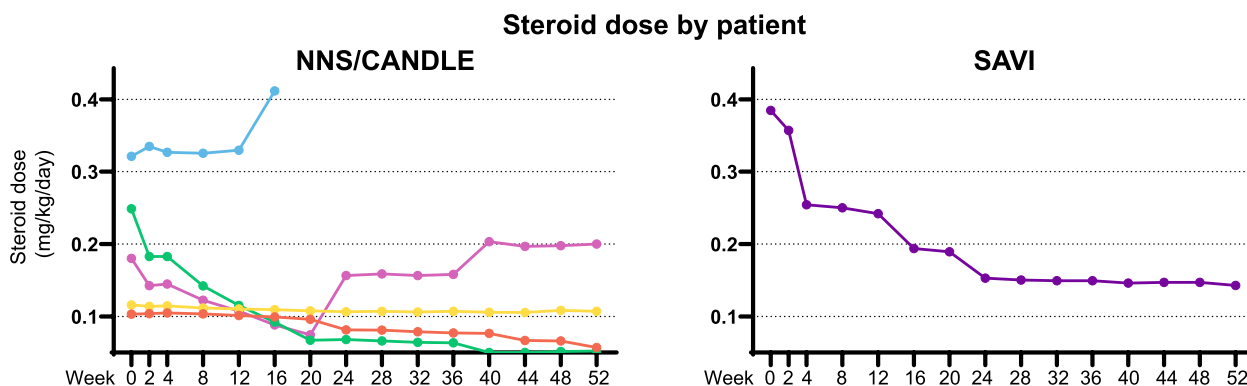

Fig. 3 Corticosteroid use in patients with (**A**) NNS/CANDLE and (**B**) SAVI. NNS/CANDLE = Nakajo-Nishimura syndrome/chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature; SAVI = STING-associated vasculopathy with onset during infancy; STING = stimulator of interferon genes
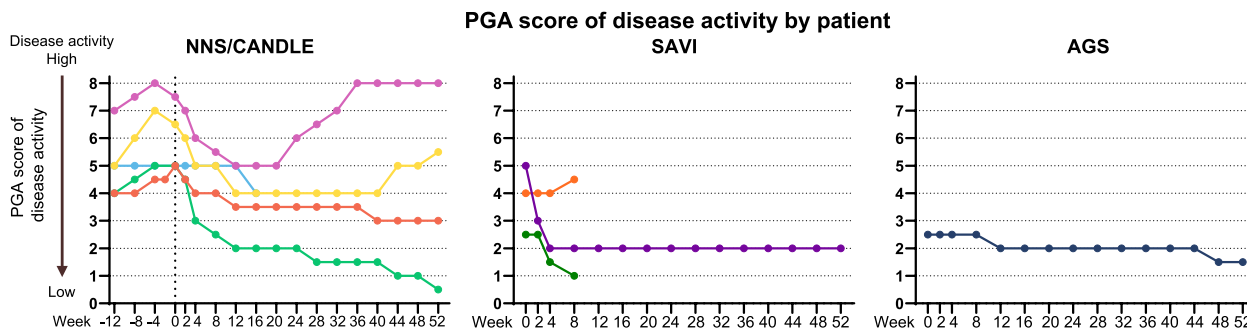

Fig. 4 PGA scores in patients with (**A**) NNS/CANDLE, (**B**) SAVI, and (**C**) AGS. AGS = Aicardi-Goutières syndrome; NNS/CANDLE = Nakajo-Nishimura syndrome/chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature; SAVI = STING-associated vasculopathy with onset during infancy; STING = stimulator of interferon genes

Table 3 Summary of adverse events during the dose adjustment and primary treatment period, and maintenance period

Incidence, n (%)	NNS/CANDLE (N = 5) n (%)	SAVI (N = 3) n (%)	AGS (N = 1) n (%)	Total (N = 9) n (%)
TEAE[a]	5 (100.0)	3 (100.0)	1 (100.0)	9 (100.0)
Severe TEAEs[b]	2 (40.0)	1 (33.3)	0 (0.0)	3 (33.3)
Moderate TEAEs[b]	2 (40.0)	0 (0.0)	0 (0.0)	2 (22.2)
Mild TEAEs[b]	1 (20.0)	2 (66.7)	1 (100.0)	4 (44.4)
Death[c]	0 (0.0)	1 (33.3)[c]	0 (0.0)	1 (11.1)
SAE	2 (40.0)	1 (33.3)[c]	0 (0.0)	3 (33.3)
Discontinuation from study and study treatment due to AE	1 (20.0)	1 (33.3)[c]	0 (0.0)	2 (22.2)

AE adverse event, *AGS* Aicardi-Goutières Syndrome, *n* number of patients with at least one adverse event per event type, *NNS/CANDLE* Nakajo-Nishimura syndrome/chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature, *SAE* serious adverse event, *SAVI* STING-associated vasculopathy with onset during infancy, *STING* stimulator of interferon genes, *TEAE* treatment-emergent adverse event

[a] Patients may be counted in more than one category

[b] Patients with multiple occurrences of the same event are counted under the highest severity

[c] The death event was included in SAE and discontinuations due to AE

demonstrating an increase in index scores for transfers and mobility on level surfaces and stairs.

Safety
Adverse events
All 9 patients across disease subgroups reported at least 1 treatment-emergent adverse event (TEAE). Overall, a higher proportion 4 (44.4%) of patients reported mild TEAEs. At the end of the primary treatment period, 3 (33.3%) patients reported serious adverse events (SAEs) including acute coronary syndrome, pancytopenia, and bronchopulmonary aspergillosis, of which pancytopenia was deemed related to the study drug. One (11.1%) patient with SAVI was reported dead due to intracranial hemorrhage, which was not related to the study drug, and 1 (11.1%) patient with NNS/CANDLE discontinued study treatment due to *Pneumocystis jirovecii* pneumonia, which was related to the study drug (Table 3). No SAEs or deaths were reported during the maintenance treatment period.

The most frequently reported events by preferred term included BK polyomavirus test positive (3; 33.3%), increased blood creatine phosphokinase (2; 22.2%), anemia (2; 22.2%), and upper respiratory tract infection (2; 22.2%).

Adverse events of special interest
A majority (6; 66.7%) of patients had infections. Upper respiratory tract infections were observed in 2 patients (22.2%). All other infections including BK virus infection were observed in 1 patient each, across NNS/CANDLE, SAVI, and AGS groups (Table 4).

Clinical laboratory evaluation
No clinically meaningful changes were observed in systolic blood pressure, diastolic blood pressure, or pulse rate.

Mean increase in growth (height and weight) and growth velocity were observed for all 4 patients younger than 18 years of age during the study, from baseline (Visit 6).

Table 4 Adverse events of special interest – infections

Preferred Term	NNS/CANDLE (N = 5) n (%)	SAVI (N = 3) n (%)	AGS (N = 1) n (%)	Total (N = 9) n (%)
Patients ≥ 1 TEAE	3 (60.0)	2 (66.7)	1 (100.0)	6 (66.7)
Upper respiratory tract infection	1 (20.0)	1 (33.3)	0 (0.0)	2 (22.2)
Atypical mycobacterial infection	0	1 (33.3)	0 (0.0)	1 (11.1)
BK virus infection	1 (20.0)	0	0 (0.0)	1 (11.1)
Bronchopulmonary aspergillosis	0 (0.0)	1 (33.3)	0 (0.0)	1 (11.1)
Conjunctivitis	0 (0.0)	1 (33.3)	0 (0.0)	1 (11.1)
Cytomegalovirus chorioretinitis	1 (20.0)	0 (0.0)	0 (0.0)	1 (11.1)
Folliculitis	1 (20.0)	0 (0.0)	0 (0.0)	1 (11.1)
Localized infection	1 (20.0)	0 (0.0)	0 (0.0)	1 (11.1)
Periodontitis	1 (20.0)	0 (0.0)	0 (0.0)	1 (11.1)
Pharyngitis	0 (0.0)	0 (0.0)	1 (100.0)	1 (11.1)
Pneumocystis jirovecii pneumonia	1 (20.0)	0 (0.0)	0 (0.0)	1 (11.1)
Pneumonia cytomegaloviral	1 (20.0)	0 (0.0)	0 (0.0)	1 (11.1)
Tinea capitis	1 (20.0)	0 (0.0)	0 (0.0)	1 (11.1)

AGS Aicardi-Goutières syndrome, *n* number of patients with at least one adverse event per event type, *NNS/CANDLE* Nakajo-Nishimura syndrome/chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature, *SAVI* STING-associated vasculopathy with onset during infancy, *STING* stimulator of interferon genes, *TEAE* treatment-emergent adverse event

Biomarkers

Interferon-inducible protein 10/C-X-C motif chemokine 10 (IP-10/CXCL10) decreased from baseline in patients with NNS/CANDLE at the end of the primary treatment period (9146.5) and maintenance treatment period (7652.2). Results of IP-10/CXCL10 could not be determined for patients with SAVI and AGS due to limited blood samples. IGS decreased in patients with NNS/CANDLE and AGS consistently throughout the study, with the mean change from baseline at -2.7 and -1.4 at the end of the maintenance period (Week 52), respectively (Fig. 5). CRP level decreased from baseline in patients with NNS/CANDLE (0.83) and in the patient with AGS (0.13) and minor change in patients with SAVI (-0.003) was observed (Fig. 6).

Discussion

Our study was the first that evaluated the efficacy and safety of baricitinib in Japanese patients with autoinflammatory interferonopathies, NNS/CANDLE, SAVI, or AGS. The overall reduction in mean DDS from baseline in patients with NNS/CANDLE or SAVI at the end of the primary treatment period and maintenance period demonstrated consistent efficacy. Decrease in PGA score and DSSS, consequently leading to decrease in corticosteroid use, reinforced the efficacy of baricitinib in patients with these rare autoinflammatory type I interferonopathies. Four (44.4%) of 9 patients had mild TEAEs, and infections were the most frequently reported. SAEs observed at the end of the primary treatment period were not reported at the end of the maintenance period, and 1 SAE was deemed related to the study drug. Two patients with NNS/CANDLE and SAVI discontinued study due to AE and death, respectively. The patient with SAVI had SAEs, including pneumonia due to aspergillus infection and nontuberculous mycobacterium infection, who consequently died. As per the study investigator, the SAEs were related to the study drug, as the drug was immunosuppressive. However, the death due to intracranial hemorrhage was not deemed to be related to the study drug, as the study drug
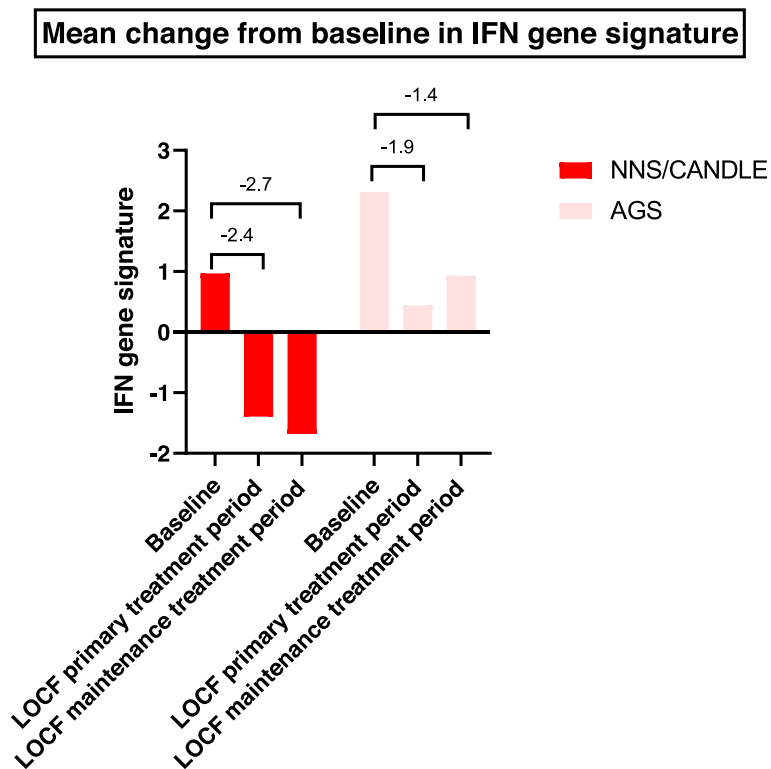

Fig. 5 Mean change from baseline in IFN gene score across patients with NNS/CANDLE and AGS. AGS = Aicardi-Goutières syndrome; IFN = interferon; LOCF = last observation carried forward; NNS/CANDLE = Nakajo-Nishimura syndrome/chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature; SAVI = STING-associated vasculopathy with onset during infancy; SLE = Systemic Lupus Erythematosus; STING = stimulator of interferon genes. Note: Genes including *OAS3*, *IFI44*, *MX1*, *USP18*, *LY6E*, and *DDX60* were used to calculate the IFN gene signature. The IFN signature score is composed of a 6-gene IFN signature assays from 28 IFN Response Genes of the ModaPlex platform. A 6-gene IFN signature assay was developed by Lilly based on data from more than 2000 SLE patients. The final ModaPlex assay is parameterized to separate IFN-high and IFN-low patients with a cut point of 0 where each gene is scaled to have the same range. A difference of 1 point in the IFN signature correlates with twice the difference in gene concentration. LOCF data were used for imputation. For patients with SAVI, samples were not obtained due to young age; therefore, no patients had both baseline and postbaseline measurements of IFN gene signature
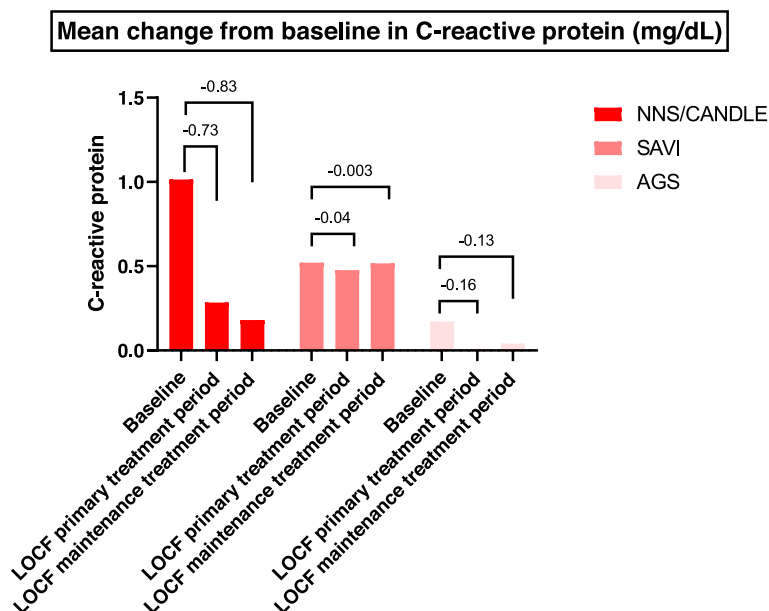
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Fig. 6 Mean change from baseline in C-reactive protein (mg/dL) across patients with NNS/CANDLE, SAVI, or AGS. AGS = Aicardi-Goutières syndrome; IFN = interferon; LOCF = last observation carried forward; NNS/CANDLE = Nakajo-Nishimura syndrome/chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature; SAVI = STING-associated vasculopathy with onset during infancy; STING = stimulator of interferon genes. LOCF data were used for imputation

was discontinued three months prior due to *Pneumocystis jirovecii* pneumonia. The safety results were comparable to an earlier baricitinib trial (NCT01724580) [11]. Mean increases in height and weight was observed in patients younger than 18 years, indicating that this JAK inhibitor potentially does not inhibit growth hormone receptor-induced tyrosine kinase JAK2 phosphorylation [20]. Moreover, biomarkers including IP-10/CXCL10, CRP, and IGS decreased in patients with NNS/CANDLE, indicating a pronounced clinical response.

The results of our study were comparable to the baricitinib trial conducted as an expanded access program in patients with type I IFN-mediated autoinflammatory diseases (JAGA trial; NCT01724580) [11]. Among the individual subgroups of patients, percent improvement in DDS in our study was observed in a lower percent of patients with NNS/CANDLE (60% vs 80%) and higher percent of patients with SAVI (100% vs 75%) compared with the JAGA trial [11]. The variation in the percentage of patients with NNS/CANDLE could be attributed to the mean age difference in the population. In the JAGA trial, the mean age was 12.5 years (range: 1.2–24.1 years), whereas in our study the mean age for patients with NNS/CANDLE was 39.2 years (range: 15–48 years). In concordance with the JAGA trial [11], symptoms and corticosteroid usage decreased across all patient disease subgroups, with the clinical responses being most distinct in patients with NNS/CANDLE. The

safety profile of baricitinib in patients with RA [21] was similar to our study, with upper respiratory tract infection being commonly observed. Based on the wide age group, it can be hypothesized that the pediatric population with shorter disease history could respond effectively to baricitinib. Further studies in this population could be helpful.

Our data showed a smaller proportion of patients with upper respiratory tract infections (22.2% vs 77.8%) and BK polyomavirus infection (33.3% vs 50%) than the JAGA trial [11]. This could be due to the smaller sample size in our study, the varied demographics, ethnicity, and duration of the trial. However, both studies indicated that infections were the most frequently reported, and overall, the drug was well tolerated.

BK polyomavirus positivity in patients treated with baricitinib was consistent between our study and the JAGA trial. Safety was assessed and BK viral load was monitored in 7 additional patients after the JAGA trial. Of the total 23 patients in the trial, 20 (87%) developed BK viruria [22]. Observation of BK viruria and viraemia is unique and monitoring an evaluation of BK viral load in patients on chronic immunosuppressive therapy should be considered.

The study had limitations including small sample size, heterogenous patient population, absence of comparators and randomization, use of immunosuppressive concomitant medications, and presence of clinically significant

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preexisting conditions, leading to potential bias. However, although the enrolled Japanese patient population was small, open label baricitinib consistently decreased mean DDS across 52 weeks. Overall, the drug was well tolerated, but physicians need to be cautious of BK polyomavirus. Continuation of this trial would further provide clarity on the long-term effect and benefit/risk ratio of baricitinib in patients with NNS/CANDLE, SAVI, or AGS.

Conclusion

In conclusion, the findings suggest that baricitinib could be a potential therapeutic agent with a positive benefit/risk profile in a vulnerable patient population with autoinflammatory interferonopathies.

Abbreviations

AE	Adverse event
AGS	Aicardi-Goutières syndrome
CANDLE	Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature
CRP	C-reactive protein
CXCL10	C-X-C motif chemokine 10
DDS	Daily diary score
DSSS	Daily symptom-specific score
eGFR	Estimated glomerular filtration rate
IGS	Interferon gene signature
IFN	Interferon
IP-10	Interferon-inducible protein 10
JAGA	I4V-MC-JAGA (NCT01724580)
JAK	Janus kinase
LOF	Loss of function
NNS	Nakajo-Nishimura syndrome
PGA	Physician's Global Assessment of Disease Activity
PK	Pharmacokinetic
RA	Rheumatoid arthritis
SAE	Serious adverse event
SAVI	Stimulator of interferon genes-associated vasculopathy with onset during infancy
SD	Standard deviation
STAT	Signal transducer and activator of transcription
STING	Stimulator of interferon genes
TEAE	Treatment-emergent adverse event

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12969-023-00817-8.

Additional file 1: Supplementary Table S1. Daily symptoms of patients with NNS/CANDLE, SAVI, and AGS. Supplementary Table S2. Symptom-specific change from baseline in patients with NNS/CANDLE, SAVI, and AGS.

Authors' contributions

NK, TI, YT, AN, RN participated in the interpretation of study results, and in the drafting, critical revision, and approval of the final version of the manuscript. TI was an investigator in the study. YT conducted the statistical analysis and was involved in conception of work and data analysis.

Availability of data and materials

Eli Lilly and Company provides access to all individual participant data collected during the trial, after anonymization, with the exception of PK or genetic data. Data are available to be requested for 6 months after the indication studied has been approved in the United States and the European Union and after primary publication acceptance, whichever is later. No expiration date of data requests is currently set once data are made available. Access is provided after a proposal has been approved by an independent review committee identified for this purpose and after receipt of a signed data sharing agreement. Data and documents, including the study protocol, statistical analysis plan, clinical study report, and blank or annotated case report forms, will be provided in a secure data-sharing environment. For details on submitting a request, see the instructions provided at https://vivli.org/.

Declarations

Ethics approval and consent to participate

The protocol was approved by the institutional review boards prior to patient recruitment, and each patient provided written informed consent prior to enrollment. The study was conducted in accordance with consensus ethics principles derived from international ethics guidelines, including the Declaration of Helsinki and the International Ethical Guidelines by the Council for International Organizations of Medical Sciences and the International Council for Harmonisation E6 Guidelines for Good Clinical Practice.

Consent for publication

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship of this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Competing interests

Taeko Ishii, Yasushi Takita, and Atushi Nishikawa are Lilly's employees and shareholders of Eli Lilly and Company. Nobuo Kanazawa and Ryuta Nishikomori have no conflicting interests.

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