EPIDEMIOLOGICAL SCIENCE

Clinical characteristics and treatment of 50 cases of Blau syndrome in Japan confirmed by genetic analysis of the *NOD2* mutation

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ABSTRACT

Objectives To collect clinical information and *NOD2* mutation data on patients with Blau syndrome and to evaluate their prognosis.

Methods Fifty patients with *NOD2* mutations were analysed. The activity of each *NOD2* mutant was evaluated in HEK293 cells by reporter assay. Clinical information was collected from medical records through the attending physicians.

Results The study population comprised 26 males and 24 females aged 0–61 years. Thirty-two cases were sporadic, and 18 were familial from 9 unrelated families. Fifteen different mutations in *NOD2* were identified, including 2 novel mutations (p.W490S and D512V); all showed spontaneous nuclear factor kappa B activation, and the most common mutation was p.R334W. Twenty-six patients had fever at relatively early timepoints in the disease course. Forty-three of 47 patients had a skin rash. The onset of disease in 9 patients was recognised after BCG vaccination. Forty-five of 49 patients had joint lesions. Thirty-eight of 50 patients had ocular symptoms, 7 of which resulted in blindness. After the diagnosis of Blau syndrome, 26 patients were treated with biologics; all were antitumour necrosis factor agents. Only 3 patients were treated with biologics alone; the others received a biologic in combination with methotrexate and/or prednisolone. None of the patients who became blind received biologic treatment.

Conclusions In patients with Blau syndrome, severe joint contractures and blindness may occur if diagnosis and appropriate treatment are delayed. Early treatment with a biologic agent may improve the prognosis.

Key messages

What is already known about this subject?

- Blau syndrome is an autoinflammatory granulomatosis disease, characterised by a distinct triad of skin, joint and eye disorders.
- *NOD2* has been identified as the gene responsible for Blau syndrome.

What does this study add?

- p.W490S and D512V are novel *NOD2* mutations associated with Blau syndrome.
- Collected cases in Japan were almost evenly distributed by age through the 40s and decreased after age 50.
- About half of the cases of Blau syndrome had fever from relatively early timepoints in the disease course.
- In some patients, disease onset was recognised after BCG vaccination.
- Most patients who became blind were treated with non-biologics, whereas none of the patients treated with biologics from a young age was blind.

How might this impact on clinical practice or future developments?

- Early biologic treatment for joint involvement may be essential to avoid the irreversible symptoms of joint contracture and to prevent eyesight impairment and blindness.

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INTRODUCTION

Blau syndrome (MIM #186580)[1 2] is an autoinflammatory granulomatosis disease. Its clinical phenotype is characterised by a distinct triad of skin, joint and eye disorders. Skin lesions usually occur in children younger than 4 years of age, in many cases as an initial symptom. The most frequent skin manifestations are scaly erythematous plaques (SE) with multiple lichenoid papules (LP), with no apparent subjective symptoms. Joint lesions become clinically apparent within the first decade of life.[1 3–9] Joint manifestations are chronic, symmetrical and mostly painless polyarthritis conditions.[10] Marked soft-tissue swelling can occur due to granulomatous inflammation in both the intra-articular synovium and the tenosynovium, which can cause camptodactyly and subsequent impairment of physical function. Ocular lesions are responsible for the greatest

morbidity.[11] Most commonly, patients develop a granulomatous panuveitis.[12]

The gene responsible for Blau syndrome is *NOD2*.[13 14] NOD2 is expressed in the cytoplasm of monocytes and can act as an intracellular sensor of bacteria.[15 16] A bacterial cell wall component, muramyl dipeptide (MDP), activates NOD2, resulting in activation of nuclear factor kappa B (NF-κB) and induction of inflammation. *NOD2* mutants associated with Blau syndrome have shown ligand-independent NF-κB activation.[13 17 18]

In 2004, we had the opportunity to care for a Japanese man aged 27 years diagnosed with early onset sarcoidosis (EOS). He had no family history, but he had a p.R334W mutation in *NOD2*, the same as in familial Blau syndrome.[19] Then, we retrospectively collected sporadic Japanese cases, and demonstrated that EOS shared a common genetic aetiology with Blau syndrome.[17] Currently, we have confirmed 50 cases of Blau syndrome in Japan. In this study, we documented their *NOD2* mutations, clinical manifestations and treatment, including follow-up information for previously reported cases.[11 17 20]

METHODS

Patients and clinical information

Among patients diagnosed with Blau syndrome from typical clinical symptoms, 50 patients with *NOD2* mutations confirmed by genetic analysis were included in this study.[12 17 21–23] Since 2009, when we reported the surveillance of 20 cases,[17] genetic testing has been provided as part of the Primary Immunodeficiency Database in Japan (PIDJ) project. Initially, we accepted sporadic patients with the distinct triad of skin, joint and eye disorders and with pathological findings of granuloma. Later, we accepted patients who showed marked soft-tissue swellings on the wrists and ankles, the characteristic signs of Blau syndrome. Recently, we also accepted referrals from ophthalmologists when their patients showed panuveitis. After *NOD2* mutations were identified, we performed a familial analysis if the patients' family members agreed.

Based on information we received from genetic testing, we reconfirmed the information from medical records through attending physicians, and those data were reviewed by authors Matsuda and Kambe, and then the PIDJ members. Information collected included age, sex, family history, triggers of disease onset, including BCG vaccination, fever, skin, joint and eye involvement, soft-tissue swellings on wrists and ankles, ultrasonographic assessment of synovial inflammation, initial diagnosis, treatment and treatment effects. Each clinical symptom was categorised according to our previous study's method,[11] and defined as the month when the symptoms appeared. We categorised fever as intermittent (Int) when temperature was elevated for several hours followed by an interval of normal temperature, whereas fevers lasting >2 weeks were categorised as persistent (Per). Skin eruptions were categorised as multiple LP, SE and erythema nodosum-like lesions (EN). Joint involvement was divided into oligoarticular (oligo, maximum of four affected joints) or polyarticular (poly, five or more affected joints). Eye involvement was divided into anterior (A) and posterior (P), depending on the diagnosis of an ophthalmologist. Other clinical information was collected from a free entry field.

Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our research.

Genetic analysis

Genomic DNA was extracted from peripheral blood, and Sanger sequencing of all exons and exon-intron junctions of *NOD2* was performed. Some recent cases were diagnosed at Kazusa DNA Laboratory (Kisarazu, Japan) based on next-generation sequencing.

The activity of each NOD2 mutant we identified from the patients was evaluated by dual luciferase reporter assay. Briefly, mutants were generated using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, California, USA) and subcloned into the p3xFLAG-CMV vector. The ability of each construct to induce NF-κB activity was assessed in HEK293 cells with/without MDP (5 µg/mL, InvivoGen, San Diego, California, USA).[17]

RESULTS

Patients

Twenty-six of 50 patients were male and 24 were female, showing no difference in disease incidence between sexes (table 1). Eighteen cases in 9 unrelated families were familial, and 32 cases were sporadic. The current age of patients ranged from 0 to 61 years (mean±SD 26.7±15.8 years). Our collected cases were almost evenly distributed in age through the 40s, and the number of cases decreased after age 50 (figure 1).

NOD2 mutations

We identified 15 different mutations in *NOD2*; among them, p.W490S and D512V were novel. The most frequent mutation was p.R334W (c.1000A>T); this was present in 15 patients, among which 7 cases were familial from 3 unrelated family and 8 were sporadic, consistent with our previous report that p.R334W was the most common in Blau syndrome.[11] Nine patients had p.R587C mutation (two familial and five sporadic), five patients had p.R334Q (all sporadic), four patients had p.E383K (two familial and one sporadic), three patients had p.M513T (all sporadic), two patients had p.D382E (two sporadic), two patients had p.E383G (one familial), two patients had p.W490S (one familial) and two patients had p.N670K mutation (sporadic). The p.G481D, C495Y, H496L, L499V, D512V and T605P mutations were each detected in one sporadic case.

As shown in figure 2, with the p.R334W, E383K and R587C mutations and the novel p.W490S and D512V mutations (online supplementary figure 1S), as well as with other mutations in our previous reports,[11 17 24] all showed spontaneous NF-κB activation without MDP stimulation by luciferase assay, suggesting they are disease-causing. Additionally, all of the *NOD2* constructs, including wild-type and p.R311W with a non-functional single-nucleotide polymorphism, can respond to MDP, resulting in NF-κB activation.

Fever

About half of cases (26 of 50 patients) had a fever from relatively early timepoints in the disease course (figure 3A). There were seven cases with the p.R587C mutation, four cases with the p.R334W mutation, two cases with the p.R334Q, E383G, M513T and N670K mutations and one case each with the p.D382E, G481D, C495Y and L499V mutation. Among them, 10 patients had Int fever, and 17 patients had Per fever, including 2 cases that had both fever types.

In contrast, 24 patients had not experienced fever related to Blau syndrome (11 with the p.R334W mutation, 4 with the p.E383K mutation, 2 with the p.W490S mutation and 1 case each with the p.R334Q, D382E, H496L, D512V, M513T, R587C and T605P mutation).

Skin eruption

Excluding 3 patients lacking information on skin symptoms, 43 of 47 patients (91%) had a skin rash; among them, 27 patients

Table 1 Demographic and clinical characteristics of patients with Blau syndrome

Case	Age (yr)	M/F	Family	*NOD2*	Fever		Skin		Joint		Eye	Onset	Ref
					mo	Type	mo	Type	mo	Type	mo	Type		
1†	53	M		R334Q	60	Int, Per	60	LP	60	Poly	60	A, P, blind		
2	32	M		R334Q	60	Int, Per	60	LP	60	Poly	60	A, P		Case 5[11 21]
3	28	M		R334Q	18	Per	?	EN	18	Poly	24	A, P		Case 9[20]
4	20	M		R334Q	20	Int	6	LP, SE	8	Poly	20	A, P		Case 4[11]
5	19	F		R334Q	–	–	–	–	36	Poly	–	–		Case 6[11] Case 7[20]
6	58	F	#1	R334W	–	–	528	LP, SE	96	Poly	36	A, P, blind		Case 15[11]
7†	9	F	#1	R334W	12	Per	12	LP, SE	12	Poly	24	A, P		Case 14[11]
8	44	M	#2	R334W	–	–	24	LP, SE	?	Poly	156	A, P		Case 12, [11]Case 2, [17] Case 10[20]
9	13	M	#2	R334W	–	–	?	?	?	Poly	–	–	BCG	Case 2[20]
10	7	M	#2	R334W	–	–	?	LP, SE	?	Poly	–	–	BCG	Case 1[20]
11	43	F		R334W	–	–	30	SE	120	Poly	95	A		Case 13,[11] Case 4[17]
12	41	M		R334W	24	Int	24	LP, SE	15	Poly	72	A, P, blind		Case 10, [11] Case 1,[17 19]
13	33	F		R334W	23	Per	23	LP, SE	72	Poly	48	A, P, blind		Case 11 [11] Case 3,[17 23]
14	31	M		R334W	–	–	8	?	8	Poly	72	A, P		
15	30	F	#3	R334W	–	–	144	SE	96	Oligo	144	A, P		Case 17,[11 12]
16	28	M	#3	R334W	–	–	72	SE	12	Oligo	72	A, P		Case 16,[11 12]
17	25	M		R334W	8	Per	15	LP, SE, EN	8	Poly	20	A, P		Case 9[11]Case 8[20]
18	21	M		R334W	–	–	24	SE	12	Poly	203	A, P		
19	19	M		R334W	–	–	6	LP, SE	24	Poly	39	A, P	BCG	Case 5,[20 36]
20	7	F		R334W	–	–	24	LP	26	Poly	–	–		
21	30	F		D382E	–	–	40	LP, SE	48	Poly	64	A, P		Case 8, [11]Case 9, [17 22]
22	16	M		D382E	7	Per	7	LP	24	Poly	88	A		Case 3[10 20]
23	61	F	#4	E383G	60	Per	60	EN	132	Poly	132	A, P, blind		Case 2,[11 37 38]
24	28	F	#4	E383G	15	Int	8	EN	36	Poly	132	A	BCG	Case 1[11 37]
25	27	F	#5	E383K	–	–	11	LP, SE	276	Poly	192	A	BCG	[39]
26	3	F	#5	E383K	–	–	21	LP	–	–	–	–		
27	14	F		E383K	–	–	7	LP	108	Oligo	–	–		
28	8	M	#6	E383K	–	–	8	LP, SE	74	Poly	–	–		[24]
29	29	F		G481D	13	Per	3	LP, SE	36	Poly	–	–	BCG	[40]
30	58	F	#7	W490S	–	–	?	?	?	?	324	A		
31	17	M	#7	W490S	–	–	?	LP	?	Poly	?	A		
32	19	M		C495Y	12	Int	12	LP, SE	12	Poly	–	–		Case 20[11 26]
33	47	F		H496L	–	–	12	LP, SE	36	Poly	60	A, P		Case 3,[11] Case 5[17]
34	53	M		L499V*	492	Per	360	LP	180	Poly	60	A, blind	BCG	[33]
35	13	F		D512V	–	–	?	LP	–	–	72	A, P		
36	19	M		M513T	34	Int	32	SE	33	Poly	35	A		Case 18,[11] Case 8[17]
37	5	F		M513T	7	Per	7	LP, EN	40	Poly	–	–		[41]
38	4	M		M513T	–	–	9	EN	31	Poly	–	–		
39	48	F		R587C	360	Per	?	Alopecia	–	–	?	A		
40	44	M	#8	R587C	12	Per	?	?	84	Poly	168	A, P		
41	44	M	#8	R587C	24	Per	–	–	84	Poly	168	A		
42	33	M	#9	R587C	312	Int	–	–	–	–	26	A, P		
43	3	F	#9	R587C	7	Int	?	LP	–	–	7	A, P		
44	30	F		R587C	?	+	156	LP	132	Poly	204	A		
45	27	F		R587C	18	Per	?	EN	6	Poly	24	A, P		
46	26	M		R587C	48	Per	48	LP	48	Poly	51	A		
47	20	F		R587C	–	–	–	–	53	Poly	–	–		Case 4[20]
48	22	M		T605P	–	–	8	LP, SE	18	Poly	39	A, P, blind	BCG	Case 7,[11]Case 6[17]
49	26	F		N670K	20	Int	5	LP, SE, EN	20	Poly	36	A, P		Case 19,[11] Case 7,[17 26]
50	1	M		N670K	6	Per	5	LP	7	Poly	7	A	BCG	

*Six base deletion (E498-L500delinsV).
†Death.
#, familial cases; +, positive but no information on onset or type; –, none
; ?, no information; A, anterior; EN, erythema nodosum-like lesion; Int, intermittent fever; LP, lichenoid papules; mo, months; Oligo, oligoarticular; P, posterior; Per, persistent fever; Poly, polyarticular; SE, scaly erythematous plaques; yr, years.

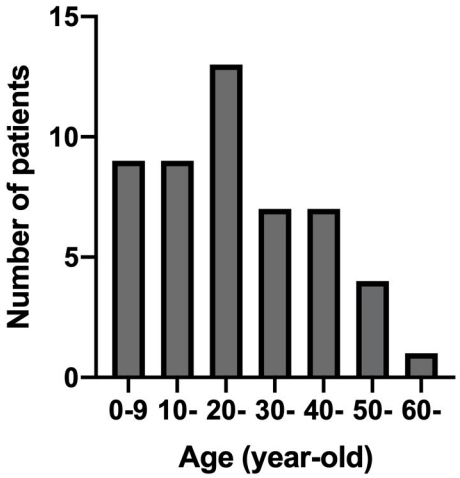

Figure 1 Distribution of the current age of patients with genetically confirmed Blau syndrome in Japan. The oldest patient was 61 years of age.

(63%) had rash as the first symptom of Blau syndrome. In most cases (78%), the rash appeared before the patients were 4 years of age (figure 3B). The most frequent skin manifestations were SE and LP, without pruritus or tenderness. Eight patients (16%) had EN (table 1).

The attending physicians of nine patients stated that BCG vaccination was a trigger for disease onset, because skin eruption appeared after BCG vaccination (table 1).

Joint involvement

Excluding 1 patient lacking information on joint symptoms, 44 of 49 patients (90%) had joint lesions; 41 patients had polyarticular and 3 patients had oligoarticular arthritis (table 1). In seven cases, joint involvement preceded skin lesions, but in most cases, the onset of joint involvement was later than of skin rash but earlier than that of eye involvement (figure 3B).

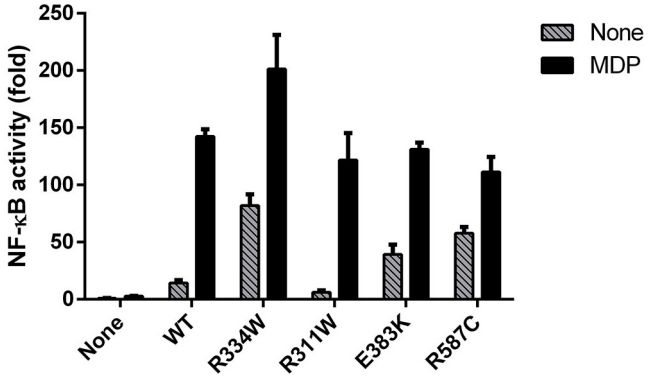

Figure 2 Evaluation of nuclear factor kappa B (NF-κB) autoactivation with identified *NOD2* mutations. HEK293 cells were co-transfected with each NOD2 mutant together with the NF-κB reporter plasmid and internal control plasmid, and NF-κB reporter activity was measured after 12 hours of incubation. In some wells, 5 µg/mL of muramyl dipeptide (MDP) was added. A mock vector (p3xFLAG-CMV, none) and wild-type (WT) *NOD2* were used as controls. Bars show the mean and SD of normalised data (mock sets 1) from triplicate cultures. Results are representative of three independent experiments. p.R311W is a non-functional single-nucleotide polymorphism induced as a negative control, and p.R334W, E383K and R587C are Blau syndrome-associated mutations in *NOD2*.

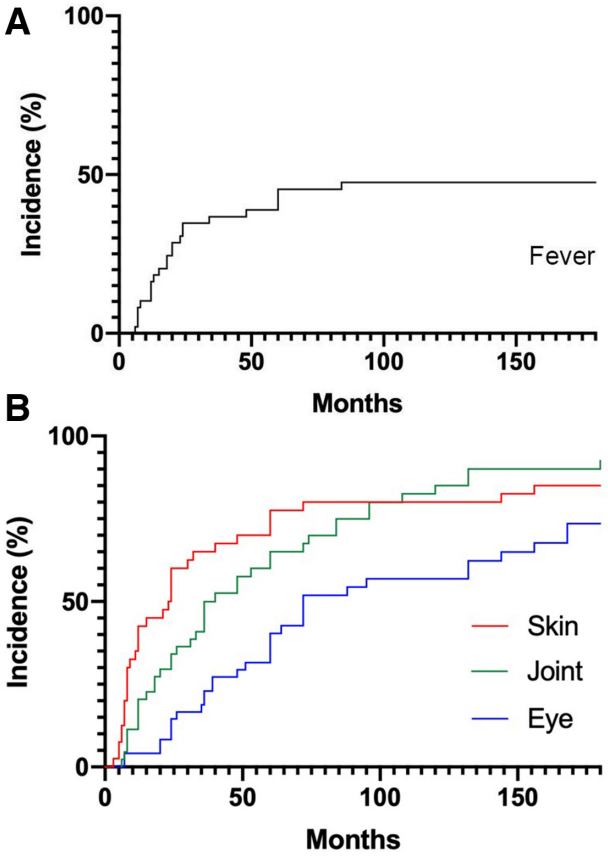

Figure 3 Patient's age at the onset of each symptom in Blau syndrome. (A) Frequency and onset of fever in Japanese patients with Blau syndrome (until 180 months). Among 50 cases, 1 patient who had symptoms but whose time of disease onset was unknown was not included in the figure. (B) Frequency and onset of the triad of Blau syndrome in Japanese patients (until 180 months). Among the 50 patients with Blau syndrome in this study, 3 patients lacked information on skin manifestations and 1 patient lacked information on joint manifestations. Patients who had symptoms but whose time of disease onset was unknown (skin manifestations, seven patients; arthritis, four patients and eye problems, two patients) were not included in the figure.

Eye involvement

Thirty-eight of 50 patients (76%) had ocular symptoms; most had both A and P lesions (table 1), comparable with the previous report on Blau syndrome that both A and P involvements were characteristic.[25] Among our cases, seven patients were blind.

Other clinical symptoms

Case 1 with a p.R334Q mutation died at 53 years of age from renal dysfunction. Case 22 with the p.D382E mutation showed haemorrhagic cerebral infarction, bilateral renal artery stenosis, renal hypertension, aortitis and calcification of the descending aorta. Case 28 with the p.E383K mutation showed hepatosplenomegaly, hypertension and congestive heart failure. Case 35 with the p.D512V mutation showed renal calcification.

Genotype-phenotype relationship

When we focused on the two major mutations in our study, p.R334W and R587C, p.R587C was associated with a relatively high incidence of fever. In patients with the p.R334W mutation, 27% of cases (4 of 15) experienced fever, whereas in those with the p.R587C mutation, about 89% of cases (8 of 9) experienced

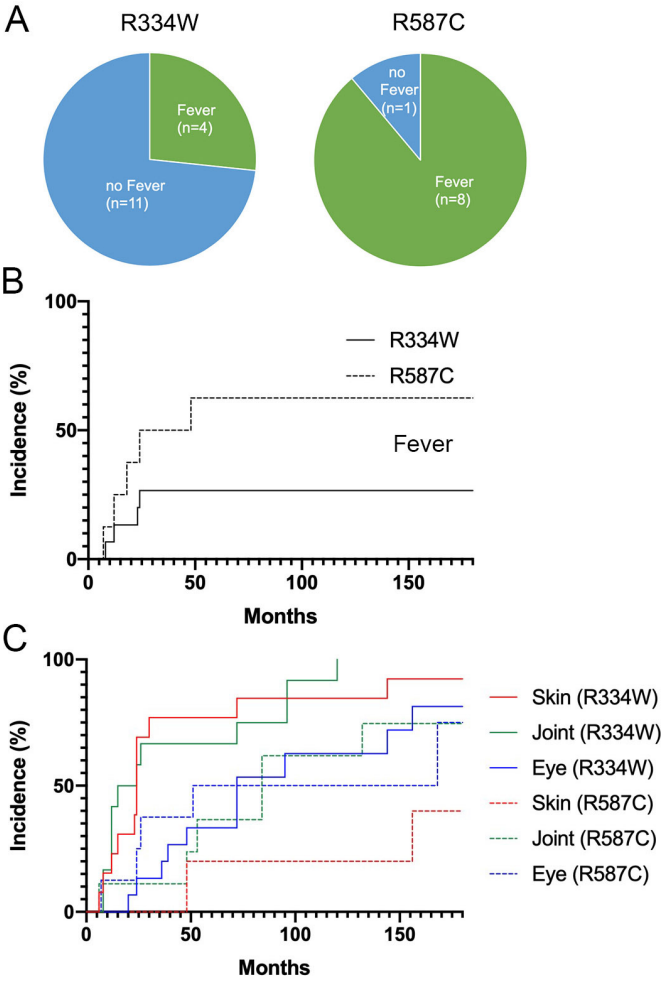

Figure 4 Genotype-phenotype relationship between R334W and R587C. (A) Frequency of fever in patients with the p.R334W and R587C mutations in *NOD2*. (B) Onset of fever in patients with the p.R334W and R587C mutations (until 180 months). (C) Onset of the symptom triad of Blau syndrome in patients with the p.R334W and R587C mutations (until 180 months).

fever (figure 4A and B). In contrast, the onsets of skin and joint involvement were much earlier in patients with the p.R334W mutation compared with those with the p.R587C mutation (figure 4C). The onset of eye involvement did not differ among patients with these mutations.

Initial diagnosis

We asked each patient's attending physician to provide the initial diagnosis before the definitive diagnosis of Blau syndrome, and we received 38 responses. The primary diagnosis other than Blau syndrome was juvenile idiopathic arthritis (JIA) in 16 cases, and most cases (14/16) experienced fever (online supplementary table S1). The other diagnoses were refractory uveitis, Behçet's disease, Takayasu's arteritis, Kawasaki disease, Giannotti's disease, tuberculoid, systemic lupus erythematosus and erythema nodosum.

Treatment

Figure 5A shows the current treatments of 43 patients, excluding 7 patients with no treatment information. Antitumour necrosis factor (TNF) agents were used in 26 patients, including adalimumab in 18 cases, infliximab in 5 cases, golimumab in 2 cases and etanercept in 1 case (figure 5B). Only three patients were treated with biologics alone (figure 5C). Seven patients were treated with methotrexate (MTX), six patients were treated with prednisolone (PSL) and five patients were treated with both PSL and MTX in addition to anti-TNF agent. Cases 32 and 49, both cared for at Okayama University, were treated with combination therapy containing thalidomide[26] (online supplementary table S1).

Seven of the 50 cases included in the study are currently suffering from blindness. With the exception of one patient for whom treatment information was missing, five of the seven patients who were blind were treated with non-biologics (figure 6). In contrast, only one patient who received an anti-TNF agent was blind (case 23, online supplementary table S1); this patient was initially diagnosed with systemic JIA and Behçet's disease and was given PSL 20 mg/day, but ocular and joint symptoms gradually worsened, resulting in left eye blindness. However, since initiation of adalimumab after Blau syndrome diagnosis, her right eye condition has been maintained without deterioration.

DISCUSSION

In this cohort study in Japan, we collected information on 50 patients with Blau syndrome with a confirmed *NOD2* mutation. We have experienced two cases in whom no *NOD2* mutation was identified despite typical clinical and pathological findings of Blau syndrome. One case was reported in our first survey.[17] This female also had hepatosplenomegaly and lymphadenopathy. It is possible that we missed a low frequency *NOD2* mutation[27]

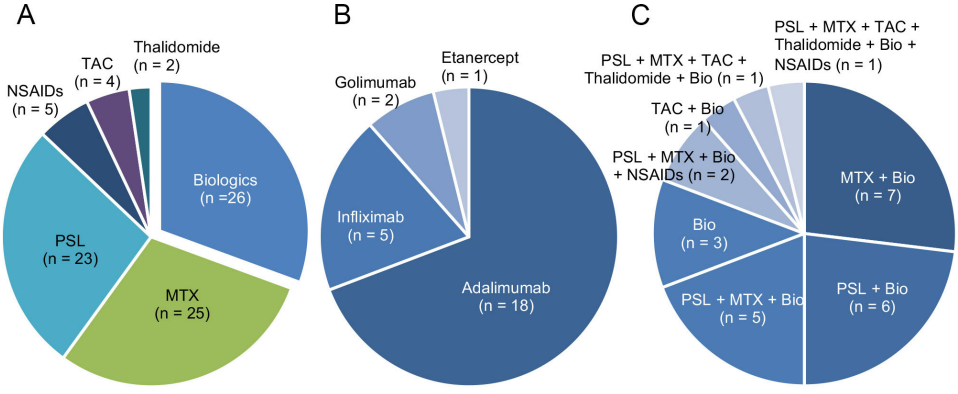

Figure 5 Current treatment for Blau syndrome. (A) Systemic treatment. If a patient had been treated with more than one drug, we counted all of them in this graph. (B) Biologics. All of the biologics were antitumour necrosis factor agents. (C) Combination treatment with a non-biologic plus biologics. Bio, biologicsMTX, methotrexate; NSAIDs, non-steroidal anti-inflammatory drugs; PSL, prednisolone; TAC, tacrolimus.

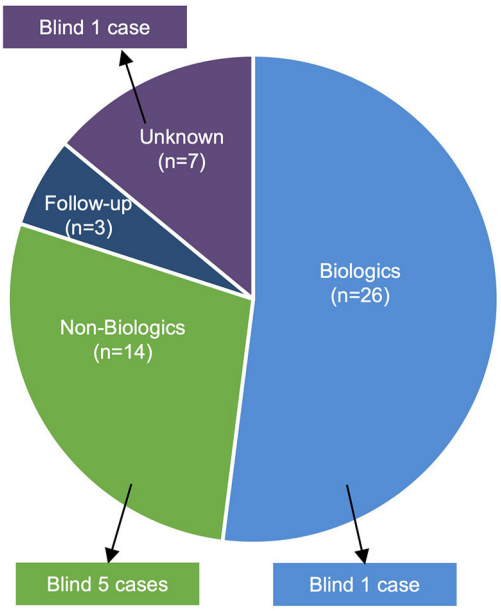

Figure 6 Relationship between treatment type and blindness. In this study, seven cases were blind. With the exception of one patient for whom treatment information was missing, five of seven cases who were blind were treated with non-biologics.

as a somatic mosaic in this case. The other case reported by Oda *et al*[28] was a boy who also had multiple abnormalities since birth, including tricuspid valve insufficiency, aortic coarctation and epilepsy.

The function of Blau syndrome-associated *NOD2* mutations is controversial. A knock-in mouse model carrying the p.R314Q mutation in *Nod2* (corresponding to the p.R334Q mutation in humans) showed reduced cytokine production in response to MDP, suggesting the possibility of loss of function.[29] In contrast, in a model of HEK293 cells overexpressing the mutant *NOD2*[17] as well as our induced pluripotent stem (iPS) cell model established from a patient with the p.R334W mutation,[30] ligand-independent autoactivation of NF-κB was observed. Although it remains unknown how Blau syndrome-associated *NOD2* mutants contribute to granuloma formation, a model of HEK293 cells is suitable for evaluation of whether an identified *NOD2* mutation is a Blau syndrome-associated mutation or a non-functional SNP. In this study, we identified 15 different kinds of mutations in *NOD2* from Japanese patients, and confirmed that all of the mutations, including the novel mutations of p.W490S and D512V, showed spontaneous NF-κB activation in a HEK293 cell model, confirming that they are disease-associated mutations.

Previously, we proposed the concept of basal NF-κB activity to explore the genotype-phenotype correlation of mutated *NOD2*.[11] Basal NF-κB activity was defined as the ratio of NF-κB reporter activities without MDP to those with MDP, using a model of HEK293 cells. However, clinical phenotypes and their onset were varied, even within patients with the same mutation. When comparing the p.R334W and R587C mutations, spontaneous NF-κB activity without MDP in HEK293 cells with the p.R334W mutation was much higher than that of cells with the p.R587C mutation (figure 2), and the basal NF-κB activity calculated from the results was almost the same; however, fever was more commonly observed in patients with the p.R587C mutation (figure 4A). In contrast, the onsets of skin and joint involvement were much earlier in patients with the p.R334W mutation (figure 4C). Thus, we should carefully evaluate the genotype-phenotype relationship in greater numbers of patients or on different genomic backgrounds by establishing an international registry of patients with Blau syndrome. Moreover, we should consider that the HEK293 cell model is quite artificial.

Interestingly, in family 6 (table 1) with the p.E383K mutation, the father of case 26 (not included in table 1) had the same mutation in *NOD2* but no symptoms, suggesting he was an asymptomatic carrier.[24] Although Blau syndrome has been reported as a genetic disease with high penetrance, asymptomatic carrier cases of a family with the same p.E383K mutation have been reported.[31] However, we could not find any peculiar behaviour of the p.E383K mutation in our HEK293 cell model (figure 2), and we are unsure whether other contributing factors are required for development of inflammatory and granulomatous responses in heterozygous carriers of *NOD2* mutations. On the other hand, after Blau syndrome was definitively confirmed by genetic testing, two patients had children. Case 8 has two sons with the same *NOD2* mutation and clinical symptoms (cases 9 and 10, table 1). The other patient's child showed no symptoms and was very young, so we have not yet performed a genetic analysis.

In this cohort study, the current age of the patients was almost evenly distributed through the 40s, but the number of cases decreased after age 50. One possible reason for this is that Blau syndrome is highly recognised by paediatricians, but rheumatologists and other physicians who care for adult patients remain unfamiliar with it, because the clinical phenotype of most monogenic autoinflammatory syndromes presents at younger ages. Another possibility is that Blau syndrome is life-threatening due to continued chronic inflammation. In our study, case 1 died at 53 years of age from renal dysfunction, but we unfortunately could not obtain information on whether his renal dysfunction was due to Blau syndrome. We believe that follow-up surveillance will be important to reveal its prognosis.

Our study revealed that fever is an important clinical feature of Blau syndrome. The concept of autoinflammatory disease was originally established from genetic analyses of periodic fever syndrome. Although fever is not included in the triad of Blau syndrome, about half of the cases had fever from relatively early timepoints in the disease course (figure 3A).

In many cases of Blau syndrome, skin lesions occur as the initial symptom. In this study, except for three cases lacking information on skin symptoms, not all patients (91%) showed skin eruptions, and only 63% had a skin rash as their first symptom. In eight cases, joint involvement preceded skin lesions (table 1). However, we speculate that skin eruptions may be overlooked because they are asymptomatic and often resolve spontaneously.

A possible relationship of BCG vaccination with the onset of Blau syndrome is interesting. There is a case report of juvenile sarcoidosis, in whom eruption had arisen after BCG vaccination.[32] In our study, the onset of disease was observed following BCG vaccination in nine patients (table 1). Especially in case 10, the second son of case 8, the p.R334W mutation was identified just after birth. After obtaining consent from his parents, we administered the BCG vaccination and found that an eruption developed, first at the BCG injection sites, and then gradually expanded to his entire body (online supplementary figure S2). Another interesting case is case 34; at 30 years of age, a skin rash had developed on his extremities after his first BCG vaccination.[33] With these clinical observations, we previously reported that in iPS cell models, interferon (IFN)γ treatment induced NOD2 production.[30] Because IFNγ is a major cytokine associated with BCG-mediated immune responses,[34] BCG vaccination might be involved in providing a NOD2-mediated inflammatory

response. Again, the skin eruption induced after BCG vaccination may be overlooked.

In patients with Blau syndrome, delayed or inappropriate treatment may result in severe joint contracture and blindness. Some cases in our study were initially diagnosed with JIA based on relatively mild symptoms, but joint and eye involvement progressed irreversibly, probably because the amount of glucocorticoid administered was insufficient for Blau syndrome. In contrast, none of the four patients (cases 5, 22, 41 and 46 in online supplementary table S1) who had been treated with biologics from a younger age was blind when they became older.

We previously reported that ultrasonographic assessment of synovial inflammation can be a potent tool for monitoring Blau syndrome activity.[20] Surprisingly, active inflammation was detected in the intra-articular or tenosynovium despite lack of clinical arthralgia or tenderness, which dramatically subsided after treatment with MTX plus a TNF antagonist. In addition to these observations, this study adds the important message that early treatment with a biologic agent for joint involvement may be essential to avoid the irreversible symptoms of Blau syndrome for joint contracture and for preventing eyesight impairment and blindness.[35] However, we should evaluate the effects of treatments for Blau syndrome continuously, including patient quality of life with the Musculoskeletal Health Questionnaire, functional and vocational outcomes.

In conclusion, from our survey of 50 cases of mutation-positive Blau syndrome in Japan, early biologic treatment for joint involvement is essential to avoid the irreversible symptoms of joint contracture and to prevent eyesight impairment and blindness.

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