Table 2. Distribution and quantification of *NLRP3* mutations among sources of genomic DNA (4 cell lineages and 1 tissue type)*

Patient	Sequence variant	Protein variant	Mosaicism, %				
			Neutrophils	Monocytes	T cells	B cells	Buccal mucosa
J1	1709A>G	Y570C	12.6	9.8	8.0	9.5	8.3
J3	919G>A	G307S	9.1	10.8	6.9	10.6	9.0
J4	1699G>A	E567K	3.5	2.3	3.7	3.4	2.2
J5	907G>C	D303H	14.4	8.7	7.7	8.5	13.5

* No significant differences in the level of mosaicism were observed among the sources of genomic DNA.

Phenotype–genotype analysis. Given the previously reported genotype–phenotype association between the *NLRP3* gene and CAPS, the clinical characteristics of NOMID/CINCA syndrome patients with somatic *NLRP3* mutations were compared with those of patients from previous reports who had the same *NLRP3* mutations but with heterozygous germline status (1,4,10–13) (Figure 2) (further information is available at http://web16.kazusa.or.jp/download/). All of the patients in these 2 groups had an early onset of the disease, fever, and urticarial rash. The presence of arthritis, bony overgrowth, contractures, hearing loss, and seizure varied in each group of patients, and no significant difference was detected. However, whereas most patients with heterozygous germline *NLRP3* mutations presented with mental retardation, this was not the case for patients with somatic *NLRP3* mosaicism. A comparison was also made between the clinical data from patients with somatic *NLRP3* mosaicism and the data from patients with neither germline nor somatic *NLRP3* mutations. Again, a lower incidence of mental retardation was observed in patients with somatic *NLRP3* mosaicism

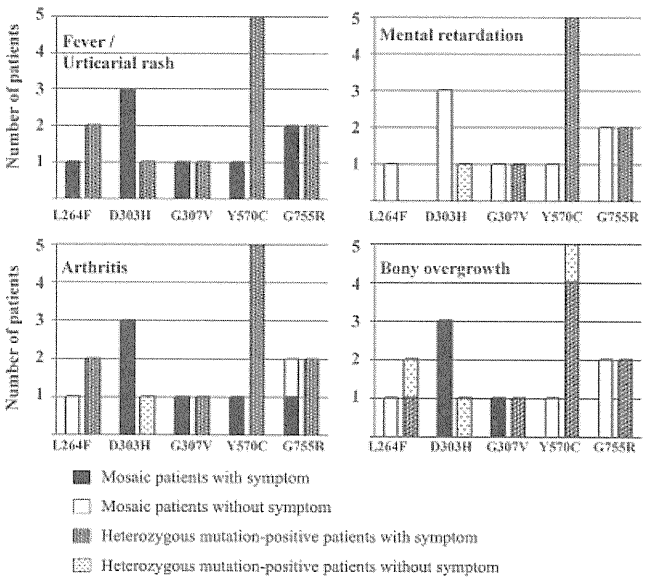

Figure 2. Comparison of the clinical profiles of patients carrying somatic *NLRP3* mutations and patients carrying the same mutation, but with germline status. Clinical profiles of patients with mosaicism and those of patients with heterozygous germline mutations are compared for each protein variant (L264F, D303H, G307V, Y570C, and G755R). The data on 4 typical clinical symptoms are shown. Total numbers of patients with mosaicism and total numbers of patients with heterozygous mutation examined are shown as a bar for each protein variant. Each bar is stratified according to the presence or absence of the symptom. For the protein variant L264F, no data on mental retardation were available for the patient with a heterozygous germline mutation.

Table 3. Clinical profiles of patients with somatic *NLRP3* mosaicism and patients with neither germline nor somatic *NLRP3* mutations*

	Patients with somatic *NLRP3* mosaicism (n = 18)	Patients with neither germline nor somatic *NLRP3* mutations (n = 8)
Age, median (IQR) years	12 (1–30)	10 (3–21)
No. of men/women	10/8	3/5
Age at onset, median (IQR) months	0 (0–24)	0.5 (0–33)
Fever	17/17	7/7
Urticarial rash	14/14	8/8
Mental retardation	4/17	6/8
Meningitis	13/17	5/8
Seizures	2/18	1/7
Hearing loss	10/18	2/7
Arthritis	14/17	7/8
Bony overgrowth	12/17	6/7
Contractures	7/17	4/7
Walking disability	8/18	3/7
Biologic therapy	10/15	3/8

* Except where indicated otherwise, values are the number with the feature/the total number of patients assessed. A lower incidence of mental retardation was observed in patients with somatic *NLRP3* mosaicism ($P = 0.03$). No other significant differences were detected between the groups. IQR = interquartile range.

($P = 0.03$). No other significant differences were detected (Table 3) (further information is available at http://web16.kazusa.or.jp/download/).

DISCUSSION

The present international multicenter study investigated 26 NOMID/CINCA syndrome patients who were mutation negative according to conventional sequencing along with 19 family controls to determine whether low-level mosaicism is a disease-causing genetic mechanism. Following our first report of low-level somatic mosaicism in a NOMID/CINCA syndrome patient (14), we reported a new method of detecting low-level *NLRP3* mosaicism, in which lipopolysaccharide (LPS) induced cell death specifically in *NLRP3* mutation–positive monocytes (8). However, this method requires fresh live monocytes, special equipment such as a cell sorter, and experience in its use due to the rapid time course of LPS-induced necrotic monocytic death. For these reasons, application of this method was not feasible in an international collaborative study. We therefore opted to use genomic DNA, since it is easier to handle and can be stored and shipped. Based on our previous study in Japanese patients showing that the frequency of mutant alleles could be <5%, we designed a subcloning and Sanger-sequencing strategy that could detect this very low allelic mutation frequency.

Presuming that the present cohort is representative of the 40% of NOMID/CINCA syndrome patients who are mutation negative according to conventional sequencing, the results suggest that ~28% of all NOMID/CINCA syndrome patients may carry somatic *NLRP3* mosaicism. CAPS patients present with a continuous spectrum of symptoms, and a degree of genotypic overlap is observed between disease subtypes. Although the present study focused on the most severe NOMID/CINCA syndrome phenotype, it is possible that somatic *NLRP3* mosaicism may also occur in milder forms of CAPS. The presence of somatic mosaicism should also be investigated in patients with other dominantly inherited autoinflammatory diseases caused by gain-of-function mutations and who are mutation negative according to conventional sequencing.

Among the 18 patients with somatic *NLRP3* mosaicism, we found 6 mutations that have previously been identified in NOMID/CINCA syndrome patients as heterozygous germline mutations. We also identified 7 novel mutations, which were confirmed as being functionally active and presumably pathogenic. Functional in vitro assays showed that these novel mutations had greater disease-causing capacity than the previously described mutations. This suggests that the novel mutations may be deleterious and unrecognized if inherited as heterozygous germline mutations.

The present study also addressed the important question of how somatic *NLRP3* mosaicism modifies clinical presentation. Although no statistically significant differences in age at disease onset, skin symptoms, joint involvement, or response to IL-1 blockade were detected, milder neurologic involvement was observed in patients with somatic mosaicism. Comparisons with NOMID/CINCA syndrome patients carrying the same *NLRP3* mutations but with heterozygous germline status made this tendency more prominent. Although the level of somatic mosaicism in blood leukocytes was relatively low, it remains unclear how these low-level mutations influence clinical presentation, including disease severity. One interesting hypothesis is that the difference in the severity of neurologic manifestations is a function of the level of mosaicism. For ethical and technical reasons, it was not possible to evaluate the level of mosaicism in central nervous system (CNS) cells or glial cells in the present study, and this therefore awaits investigation in future studies.

The mechanism through which *NLRP3* somatic mosaicism occurs also requires elucidation. The present study demonstrated that similar proportions of neutrophils, T cells, B cells, monocytes, and buccal cells carried the mutated allele. Therefore, the mutation leading to mosaicism must have arisen before the pluripotent stem cells committed to hematopoietic progenitor stem cells or ectoderm-derived nonhematopoietic cells. Several mechanisms for mosaicism have been proposed, including chimerism due to cell fusion with an aborted dizygotic twin and a mutational event during early embryogenesis (15). The latter mechanism is more likely in the present cohort, since mosaicism at similar frequency was detected in several cell types. To verify the hypothesis of a mutational event during embryogenesis, and to determine the point at which this occurred, it would be helpful to analyze other tissues. However, obtaining such tissues from patients may be ethically problematic.

Approximately 12% of the patients in the present cohort carried neither germline nor somatic *NLRP3* mutations and may therefore be considered to be genuinely mutation negative. However, it is possible that these patients have *NLRP3* mutations that have been overlooked. A recent report described a mutation in the 5′-untranslated region of *NLRP3* in a patient with FCAS

(16), although it remains unclear how this noncoding mutation causes disease. Another possibility is that an extremely low frequency of NLRP3 mosaicism may have been missed. The subcloning and Sanger-sequencing strategy used in this study set the detection limit of mosaicism at 5%. Considering the range of *NLRP3* mosaicism detected (4.2–35.8%), the median (10.2%), and the identification of 2 patients with <5% mosaicism, it is indeed likely that patients with an even lower level of *NLRP3* mosaicism may have been overlooked. Recent advances in next-generation DNA sequencing technology may resolve this technical problem, although the associated error rate could be problematic. Another possibility is that *NLRP3* mutations were present in uninvestigated cell lineages, such as those from CNS tissue, bone tissue, or skin. Future studies of NOMID/CINCA syndrome should investigate these tissues while searching for mutations in other genes.

In conclusion, the present study has clearly demonstrated that a significant proportion of NOMID/CINCA syndrome patients who were mutation negative according to conventional sequencing carried somatic *NLRP3* mutations with a variable degree of mosaicism. Clinicians should therefore consider somatic mosaicism as a possible cause of disease in mutation-negative NOMID/CINCA syndrome patients and implement appropriate therapy. The early diagnosis of NOMID/CINCA syndrome and prompt initiation of therapy would improve clinical outcome. Further goals in this research field are the refinement of genetic screening and the verification of the functional consequences of all detected somatic mutations. Systematic screening for somatic mosaicism will provide new insights into the etiology of human disease.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Drs. Ohara and Nishikomori had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Analysis and interpretation of data. Sakuma, Morimoto, Kawai, Yasumi, Nakahata, Heike.

ROLE OF THE STUDY SPONSOR

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ORIGINAL ARTICLE

Definitive differences in laboratory and radiological characteristics between two subtypes of juvenile idiopathic arthritis: systemic arthritis and polyarthritis

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Abstract We performed this study to investigate the differences in radiological and laboratory findings between systemic juvenile idiopathic arthritis (s-JIA) and polyarthritis (p-JIA). Twenty-two patients with s-JIA and 18 with p-JIA were enrolled. Their laboratory findings and radiographs were examined retrospectively. Plain radiographs were obtained before the induction of biological agents. All radiographs were examined for the presence of soft tissue swelling, juxta-articular osteopenia, joint space narrowing, subchondral bone cyst, erosion, epiphyseal irregularity, and growth abnormalities. Carpal length and bone mineral density of the lumbar spine, an indicator of generalized osteoporosis, were also investigated in all the patients enrolled. Laboratory examinations involved white blood cell counts, platelets, C-reactive protein (CRP), rheumatoid factor (RF), anti-cyclic citrullinated peptide (CCP) antibody, and matrix metalloproteinase (MMP)-3. Comparisons of the laboratory findings between s-JIA and p-JIA indicated that the titers of anti-CCP antibody and RF were significantly increased in p-JIA sera ($P < 0.05$). There was no difference in BMD between the two groups of patients. Carpal length was significantly shorter in p-JIA patients than in s-JIA patients ($P < 0.05$). The most frequent radiological abnormality in s-JIA was juxta-articular osteopenia (93.8%), in comparison to a frequency of 50.0% in p-JIA. Joint space narrowing was shown in 9.8% of the s-JIA patients compared to 35.7% of the p-JIA patients. Subchondral bone cyst and erosion were more frequent in p-JIA than s-JIA. In conclusion, there were differences in radiographic characteristics and laboratory data between s-JIA and p-JIA in this study. In the radiological evaluation, bone-related abnormality was prominent in s-JIA and joint-related abnormality was striking in p-JIA, and these results indicated that the pathogenic bases of arthritis appear to differ between these two subtypes of JIA.

Keywords Pediatric rheumatology · Juvenile idiopathic arthritis · Radiology

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Introduction

Juvenile idiopathic arthritis (JIA) is a chronic inflammatory disease of unknown origin, in which, as a result of prolonged, repetitive inflammation of the joints due to synovitis, destruction of articular cartilage progresses, causing joint contracture and damage [1]. Until now, the disease has been classified into three clinical types, depending on whether systemic symptoms are present, the number of joints affected, and the disease type within 6 weeks of onset: the systemic type; the polyarticular type, in which 5 or more joints are affected; and the oligoarticular type, in which up to 4 joints are affected [2]. Recently, in the classification of the Pediatric Rheumatology Collaborative Study Group (PRCSG), which was set up in 1993 by the International League Against Rheumatism (ILAR) and the

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World Health Organization (WHO), chronic juvenile arthritis was called JIA, and all arthritic conditions that progressed to a chronic state fell into one of seven categories [3]: systemic arthritis, oligoarthritis, rheumatoid factor (RF)-negative polyarthritis, RF-positive polyarthritis, psoriatic arthritis, enthesitis-related arthritis, and other arthritis.

JIA is a heterogeneous disease with subgroups that differ in their clinical patterns. Of these subgroups, systemic JIA (s-JIA) in particular has come to be regarded as a different disease from those in the other subgroups, as it exhibits a wide variety of systemic symptoms and is capable of transformation into macrophage activation syndrome [4]. In addition, it has become clear in recent years that in the treatment of JIA, there are also differences in the effectiveness of drugs, such as biological agents, between s-JIA and the other subgroups [5, 6], suggesting that the causes and pathology differ from one subtype to another.

The purpose of the present study was to detect the differences between s-JIA and polyarticular JIA (p-JIA) through the use of radiological techniques and to analyze differences in the pathology between these two diseases.

Patients and methods

Patients

The medical records of all patients with a diagnosis of JIA who were seen at the Pediatric Rheumatology Clinic of Yokohama City University School of Medicine between March 2003 and November 2006 were reviewed. Patients were included in the study if they met the ILAR criteria and if their radiographs were available for review. In addition, subjects had exhibited resistance to various treatments and had started to be treated with either of the biological agents tocilizumab (an anti-interleukin-6 [IL-6] receptor monoclonal antibody) [7] or etanercept (a human soluble tumor necrosis factor [TNF] α/ lymphotoxin [LT] α receptor drug) [8]. Patients whose disease duration was less than 2 years were excluded from the study. This study was approved by the Ethics Committee of Yokohama City University School of Medicine and was conducted so that individual patients could not be identified. We obtained informed consent from all patients.

Clinical and laboratory data

The following information was extracted from medical records: (1) gender, (2) age at the time of radiographic examination, (3) duration of disease, (4) total dose of prednisolone, and (5) laboratory data on entry to this study [peripheral white blood cell count (WBC), platelets (Plt), serum C-reactive protein (CRP), RF, anti-cyclic citrullinated peptide antibody (anti-CCP antibody), and matrix metalloproteinase 3 (MMP-3)].

Radiological analysis

In all the patients enrolled in the study, conventional film-screen radiographs of the joints (spine and shoulder, elbow, wrist, hand, hip, knee, ankle, and foot joints) had been obtained before the induction of biological agents. The radiological findings were then evaluated.

The radiographs were read by an experienced orthopedist and a pediatric rheumatologist who reached agreement in all cases. The following radiological abnormalities were classified as present or absent: soft tissue swelling, juxta-articular osteoporosis, epiphyseal irregularity, subchondral bone cyst, erosion, joint space narrowing, and growth abnormality [9].

High-intensity light was used to assess soft tissue swelling, and this was scored as present if any evidence of soft tissue swelling was found around a joint. Juxta-articular osteoporosis was defined as present when a localized decrease in bone density was noticed around a joint. Epiphyseal irregularity was defined as a marginal irregularity or an abnormal ossification of the epiphysis and was scored as positive when present in a joint. Localized areas of bone destruction were scored positive for the indication of subchondral bone cysts. The definition of erosion was a discrete area of damage to the cortical surface of the bone. Growth abnormalities were analyzed with regard to the shape, development, and maturation of bone, and were classified as asymmetrical epiphyseal development, premature closure of an epiphysis, or a growth deformity characterized by irregular ossification at an epiphysis resulting in a bony deformity. If any of the above growth abnormalities occurred, the joint/joint group was scored as positive for growth abnormalities. All radiological abnormalities were scored as present or absent.

In addition, to assess joint space narrowing in the hand, we measured carpal length. We used the reported method of calculating the normal length of the carpus from the length of the metacarpals, by the use of standard ratios, and by calculating the standard deviation of the carpal length in JIA patients [10]. The radiometacarpal length (RM) and the maximum length of the second metacarpal (M2) were measured on each radiograph and plotted against each other on the normative chart of Pozanski et al. [10]. The RM is the distance from the base of the third metacarpal bone to the midpoint of the distal growth plate of the radius. The measurement of RM is a method used to estimate the thickness of the cartilage covering the proximal and distal surfaces of the scaphoid and capitate bones, as

well as that covering the proximal end of the third metacarpal bone and the distal end of the radius. This offers a sensitive measure of cartilage loss within the carpus in an incompletely ossified wrist. The RM is estimated by comparison with the M2 rather than by using any relationship to age because the RM is more closely related to stature than to age, and stature correlates well with the length of the second metacarpal bone, which can be determined on the same radiograph. The number of standard deviations between the expected and actual RM for the measured M2 was then calculated for each wrist. For males, the expected RM = 12.97 + (0.4202 × M2). For females, the expected RM = 13.19 + (0.357 × M2) [11]. The number of standard deviations was averaged in the s-JIA and p-JIA groups.

Osteoporosis

In order to check for osteoporosis, we determined bone density. We also determined the presence of compression fractures of any vertebral bodies. To determine bone density, images of the front surface of the second to the fourth lumbar vertebrae were examined by dual-energy X-ray absorptiometry (DXA), and the results were compared with reference data from healthy age- and sex-matched Japanese children [12].

Statistical analysis

The presence of radiological abnormalities was summarized both at the level of the various joints and at the level of the patients. Data are expressed as means ± standard deviation. The Mann–Whitney *U*-test was used to detect differences between results in the subgroups. A *P* value of less than 0.05 was considered to be significant.

Results

Characteristics of patients

Thirty-six patients met the selection criteria. The JIA subtype was systemic in 20 patients and polyarticular in 16. The patients' characteristics and laboratory findings are listed in Table 1. The total dosage of steroids was significantly greater in the s-JIA group ($P = 0.006$). There were no significant inter-group differences in the leukocyte count ($P = 0.45$) or in the CRP value ($P = 0.08$). The p-JIA group had significantly higher titers of anti-CCP antibody than the s-JIA group ($P = 0.0001$).

Radiological findings

In examining the differences in the distribution of abnormal radiographic findings between the two groups, we found no significant difference between the groups in the frequency of abnormal findings for the shoulders, elbows, hands, knees, ankles, or the joints of the feet (Fig. 1). The median frequency of abnormalities of the hip joint was significantly higher in the s-JIA group than that in the p-JIA group (30.8 vs. 0%, $P < 0.01$).

In the s-JIA group, examination of the median frequency of each of the radiological abnormalities revealed that the frequencies of juxta-articular osteoporosis and epiphyseal irregularity were 93.8 and 7.1%, respectively, showing higher frequencies than in the p-JIA group, although the differences were without statistical significance. However, in the p-JIA group, the frequencies of joint space narrowing and subchondral bone cysts were 35.7%, and 7.1%, respectively, values that were significantly higher than those in the s-JIA group (Fig. 2). When the frequency of each of the abnormal findings was reviewed for each joint,

Table 1 Patients' characteristics and laboratory findings in this study

	s-JIA ($n = 20$)	p-JIA ($n = 16$)	*P* value
Male: female	11: 9	13: 3	
Age, years, mean (range)	10.3 (5–14)	12.0 (5–17)	0.08
Disease duration, years, mean (range)	5.5 (2.3–9.7)	6.3 (2.3–11.9)	0.51
Total dose of prednisolone, mg, mean (range)	28,720 (1,691–84,343)	12,602 (1,449–33,141)	0.006
WBC (/μl)[a]	12,0525 ± 4,981	9,855 ± 2,170	0.45
Plt (/μl)[a]	39.3 ± 12.5	38.0 ± 10.0	0.57
CRP (mg/dl)[a]	6.6 ± 7.3	3.3 ± 4.0	0.08
Rheumatoid factor[a]	7 ± 10	91.2 ± 153.7	0.06
Anti-CCP antibody[a]	0.34 ± 0.5	84 ± 150	0.0001
MMP-3 (ng/ml)[a]	338.2 ± 235.1	280.4 ± 363.9	0.09

s-JIA systemic juvenile idiopathic arthritis, *p-JIA* polyarticular JIA, *Plt* platelets, *CRP* C-reactive protein, *CCP* cyclic citrullinated peptide, *MMP* matrix metalloproteinase

[a] Mean ± SD

no particular tendency with regard to the two groups was found for juxta-articular osteoporosis, soft tissue swelling, subchondral bone cysts, erosions, or growth abnormality.

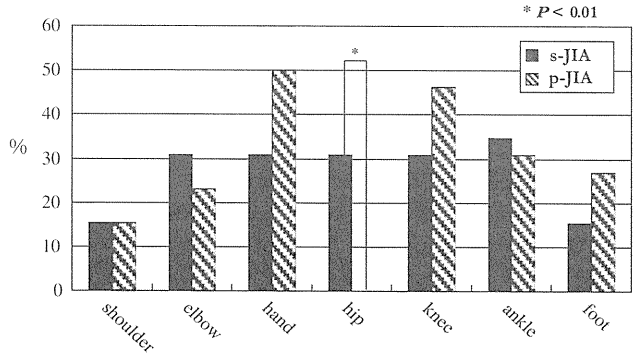


Fig. 1 Comparison of median values of abnormal radiological findings by type of joint between systemic juvenile idiopathic arthritis (*s-JIA*) and polyarticular JIA (*p-JIA*). The median frequency of abnormalities of the hip joint was significantly higher in the s-JIA patients

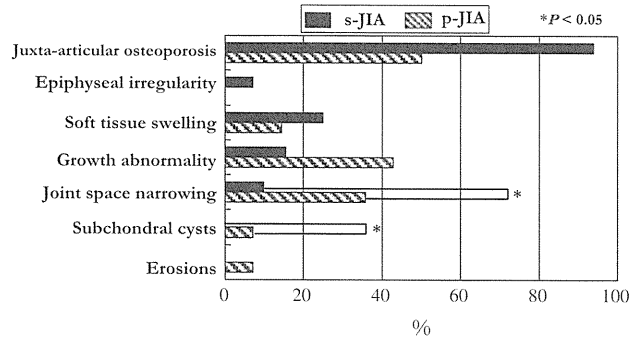


Fig. 2 Comparison of median values of abnormal radiological findings by type of abnormality between s-JIA and p-JIA. In regard to radiological abnormalities in s-JIA and p-JIA, juxta-articular osteoporosis and epiphyseal irregularity were more frequently found in s-JIA patients than in p-JIA patients, but the difference was without statistical significance. Joint space narrowing, subchondral bone cysts, and erosions were frequently found in p-JIA patients

However, in all joints examined in the s-JIA group, epiphyseal irregularities were found at a higher frequency than that in the p-JIA group. In contrast, joint space narrowing was detected more frequently in the p-JIA group than in the s-JIA group in all joints other than the hip joint (Fig. 3).

The standard deviations in mean carpal length were −2.71 and −4.74 in the s-JIA and p-JIA groups, respectively, with the value for p-JIA being significantly lower ($P = 0.01$).

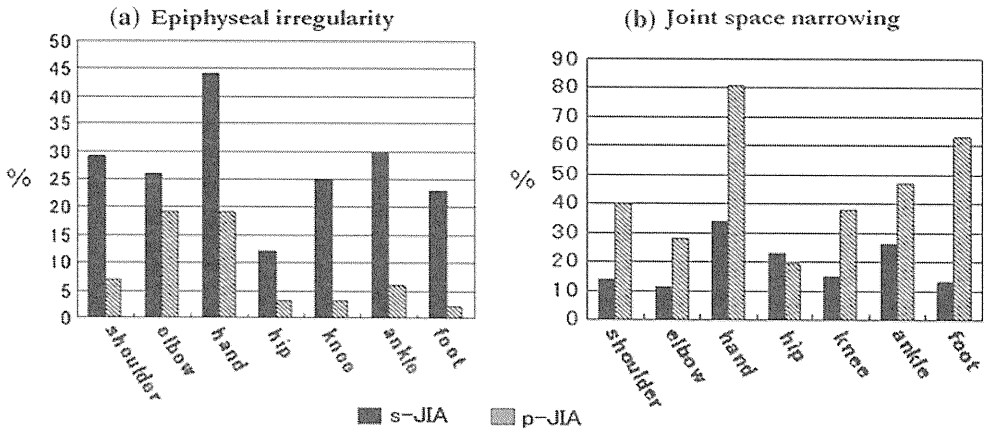
Osteoporosis

Bone density in s-JIA patients was lower than that in p-JIA patients, but the difference was without statistical significance ($P = 0.13$). In contrast, although there was a 25% frequency of vertebral compression fractures in the s-JIA patients, none of the p-JIA patients had a vertebral compression fracture. In each patient with vertebral compression in the s-JIA group, these fractures were present in at least two vertebrae, the average number of vertebral compression fractures per patient being 4.2. It was noted that these fractures were distributed from the thoracic to the lumbar region.

Discussion

Radiographic examination revealed differences in the characteristics of the abnormal radiological findings between the s-JIA and p-JIA groups. First, with regard to distribution, abnormal radiological findings for the hip joint were significantly more frequent in the s-JIA group than in the P-JIA group. In relation to the frequency of each abnormal finding, juxta-articular osteoporosis and epiphyseal irregularity were found to be more common in the s-JIA group than in the p-JIA group, although the difference was without statistical significance. Joint space

Fig. 3 Comparison of radiological abnormalities for two major findings at each joint. Epiphyseal irregularities in all joints examined were found at higher frequencies in the s-JIA group than in the p-JIA group, while joint space narrowing was detected more frequently in the p-JIA group than in the s-JIA group in all joints other than the hip joint



narrowing and subchondral bone cysts occurred significantly more frequently in the p-JIA group than in the s-JIA group, and when joint space narrowing was evaluated, shortening of the carpal length was found to be markedly greater in the p-JIA group than in the s-JIA group. From these findings, it was apparent that inflammation and abnormalities related to the bones without joint space narrowing, such as osteoporosis and epiphyseal irregularities, were more prevalent in the s-JIA group, while in the p-JIA group, the main abnormal findings were due to joint destruction, as with joint space narrowing. These radiological findings also suggested that the locations of lesions differed between the two subtypes.

In s-JIA, various systemic symptoms stand out—in particular, spiking fever. A typical fleeting pink macular rash, hepatosplenomegaly, and pericarditis are common. Generalized enlargement of lymph nodes, especially the axillary nodes, is also typical. Joint involvement, like the rash, may be more marked at the time of temperature elevations and sometimes is entirely absent when the fever has gone. However, p-JIA resembles rheumatoid arthritis (RA) in the adult, consisting mainly of joint symptoms. Different approaches are necessary for the treatment of p-JIA and s-JIA. In s-JIA patients in whom remission has not been achieved upon the administration of non-steroidal anti-inflammatory drugs (NSAIDs), there has hitherto been no alternative to a reliance on steroids. In recent years, however, the anti-interleukin (IL)-6 receptor monoclonal antibody, tocilizumab, has been demonstrated to be very effective in the treatment of these patients. The effectiveness of TNF-α blockers, though, has been limited. In the treatment of p-JIA, NSAIDs are given initially, in the first stage of the disease, and for patients who do not respond to these, low-dose pulse therapy with methotrexate is administered as the main treatment. Inhibition of inflammation by these treatments was shown to occur in 70–75% of pediatric patients. For the other 25–30%, biological agents such as the anti-TNF-α monoclonal antibody, infliximab, the TNF-α receptor blocker, etanercept, and tocilizumab are indicated. These differences in results of treatment strategies between the two groups suggest disparities in the pathogenesis of s-JIA and p-JIA. Results of the laboratory tests performed in the present study also indicated differences between the s-JIA group and the p-JIA group. Because WBC, CRP, and Plt data were obtained during treatment, no differences were seen between them, but it was shown that anti-CCP antibody was significantly higher in the p-JIA group, as shown in Table 1.

Radiological examination of joints is essential for the diagnosis and management of RA, and quantitative methods have been developed to score radiographs. The radiological assessment of RA started with Steinbrocker's staging in 1949 [13]. Then, in 1971, Sharp's method [14], and in 1977, Larsen's technique [15] were announced, and, recently, van der Heijge's modification of Sharp's technique [16] was reported. However, in children, because radiological findings vary with age, these assessment methods for RA are difficult to apply, especially for toddlers. There is no well-established method to evaluate JIA radiologically. In the present study, modification of the radiological assessment for JIA reported by van Rossum et al. [9] was used; these authors used the Dijkstra score in their system for radiological assessment. They scored the following features as present (1 point) or absent (0 point): soft tissue swelling, osteopenia, joint space narrowing, enlargement or other growth disturbances, subchondral bone cysts, erosions, and abnormal joint position, or malalignment. For a further standardized numeric evaluation, they defined the Dijkstra composite scores as follows: the Dijkstra inflammation score (range 0–2) is the summation of scores for swelling (range 0–1) and osteopenia (range 0–1); the Dijkstra damage score (range 0–3) is the summation of scores for joint space narrowing (range 0–1), bone cysts (range 0–1), and erosions (range 0–1); and the Dijkstra growth score (range 0–1) is the score for growth abnormalities (range 0–1). This system is reasonable for evaluation of the main focus of radiological abnormalities. In our modification of that method, juxta-articular osteoporosis and epiphyseal irregularities were considered to represent abnormalities of the bone, and joint space narrowing, subchondral bone cyst, and erosions were considered to represent abnormalities of the joint (cartilage).

In the evaluation system used in the present study, we could only evaluate whether an abnormality was present or absent, but could not evaluate its degree of severity, if present. For instance, even if juxta-articular osteoporosis in the s-JIA group was very severe and that in the p-JIA group was mild, it was evaluated as being present in both groups and the same score was allotted to both groups. In the present study, we found that osteoporosis and epiphyseal irregularities were not only prominent but were also quite severe in s-JIA in comparison with p-JIA (Fig. 4). Recently, the osteoclast has been reported to have an important role in the pathogenesis of bone erosions in RA [17]. Synovial tissues in patients with RA produce a variety of cytokines and growth factors that may increase osteoclast formation and activity. Several studies have demonstrated the expression of the receptor activator of the nuclear factor (NF) κB ligand (RANKL), an essential factor for osteoclast differentiation, in the synovial cells of subjects with RA and in the blood and joint fluid of patients with JIA [18–23]. Severe osteoporosis is thought to be related to osteoclast activity, and our result showing differences in the severity of osteoporosis between s-JIA and p-JIA might indicate a difference in the role of osteoclasts

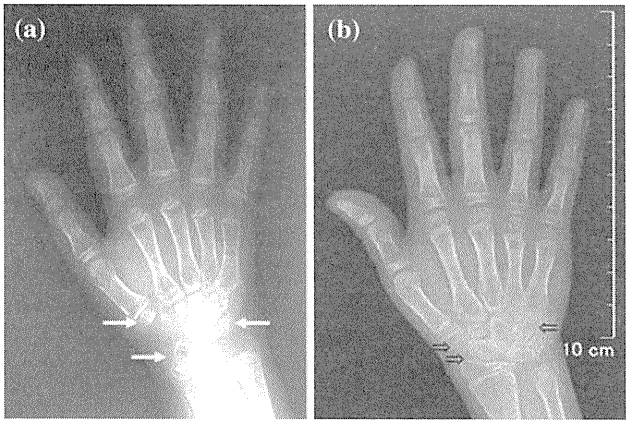


Fig. 4 Radiological features of the hand in s-JIA and p-JIA. **a** Features in a 10-year-old boy with s-JIA. Irregular ossification of the epiphysis without joint space narrowing (*white arrows*) is observed, and shortening of the carpal length is mild. Osteoporosis is very severe. **b** Findings in a 12-year-old male with p-JIA. Joint space narrowing, ankylosis of carpal bones, and shortening of the carpal length are seen (*outlined arrows*). Osteoporosis is moderate

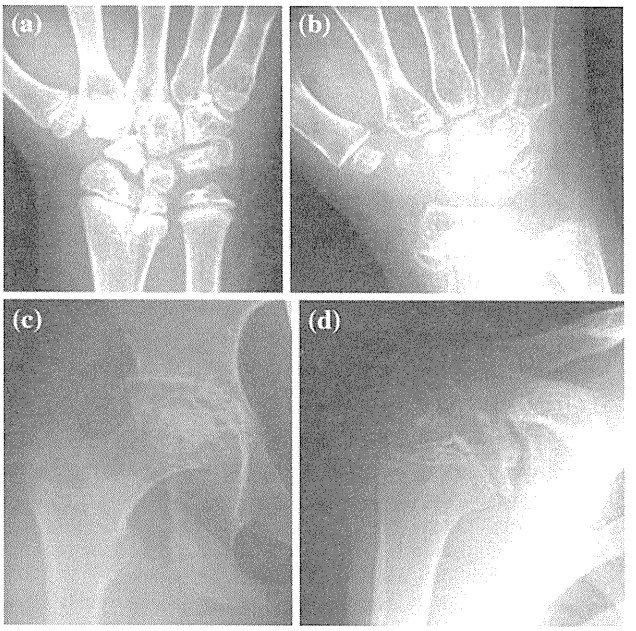


Fig. 5 Epiphyseal dysplasia in s-JIA. Severe irregular ossification of the epiphysis was observed in s-JIA patients. Joint space narrowing was mild in most cases. These findings were seen only in s-JIA, and are thought to be characteristic of s-JIA (**a** a 9-year-old girl, **b** a 10-year-old boy, **c** a 9-year-old girl, **d** a 9-year-old girl)

in the pathogenesis of the two disorders. Even though there was no difference between these groups in BMD of the lumbar spine, multiple compression vertebral fractures were seen only in the s-JIA group, which might also indicate poor bone quality in s-JIA. We believe that epiphyseal irregularity without joint space narrowing or with only mild narrowing is the most characteristic finding in s-JIA. This finding represents irregular ossification of the epiphysis without joint destruction, indicating the possibility of a disorder of ossification of the epiphysis (epiphyseal dysplasia) in the pathogenesis of s-JIA (Fig. 5). However, in terms of the present comparison between the two groups, further consideration is needed because of the large difference in steroid dosage between the two subtypes. The fact that osteoporosis was prominent in s-JIA patients was due to the pathology of s-JIA alone, as well as, undeniably and in a large part, to the strong effect of the steroid dosages used. Aggarwal et al. [24] found decreased BMD in adult patients previously diagnosed with JIA. Likewise, Okumus et al. [25] found lower BMD levels in 30 JIA patients, especially in the polyarticular group. While they did not detect a statistically significant relationship with disease duration, BMD and the *Z* score were associated with lower insulin-like growth factor I (IGF-I) levels. Henderson et al. [26, 27] described low total body BMD (*Z* score lower than −1 SD) in 29.2% of prepubertal and postpubertal JIA patients. Notably, none of the JIA patients or controls had ever received steroid therapy. As noted by these authors, those with greater disease severity and higher levels of inflammatory markers were more likely to have low BMD. Hartman et al. [28] also demonstrated osteoporosis by DXA and by ultrasound in children with chronic rheumatic diseases. Furthermore, it is important to note that IL-6, an important cytokine in JIA, especially systemic JIA, may be an important factor in the development of osteoporosis by stimulating osteoclast activity, as demonstrated in an IL-6 transgenic mouse model by De Benedetti et al. [29].

In relation to the effects of steroids on other radiological abnormalities, further study is needed. More investigation is also necessary because of the small number of subjects in the present study and the effects of steroid dosages on the data.

Conflict of interest None.

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Association of *IRF5* Polymorphisms with Susceptibility to Hemophagocytic Lymphohistiocytosis in Children

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Abstract
Introduction Hemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory syndrome and has a varied genetic background. The polymorphism of *interferon regulatory factor 5* gene (*IRF5*) was reported to be associated with susceptibility to macrophage activation syndrome. IRF5 acts as a master transcription factor in the activation of pro-inflammatory cytokines. We assessed associations of *IRF5* gene polymorphisms with susceptibility to secondary HLH.
Methods Three *IRF5* single nucleotide polymorphisms (rs729302, rs2004640, and rs2280714) were genotyped using TaqMan assays in 82 secondary HLH patients and 188 control subjects.
Results There was a significant association of the GT/TT genotype at rs2004640 with secondary HLH susceptibility ($p<0.01$). The *IRF5* haplotype (rs729302 A, rs2004640 T, and rs2280714 T) was associated with secondary HLH susceptibility ($p<0.01$).
Conclusions These findings indicate that *IRF5* is a genetic factor influencing the susceptibility to secondary HLH and that the IRF5-associated immune response contributes to the pathogenesis of HLH.

Keywords Interferon regulatory factor 5 · polymorphisms · hemophagocytic lymphohistiocytosis

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory syndrome that is accompanied by serious morbidity [1, 2]. The incidence of HLH is estimated to be about 1.2 cases per million individuals per year [3]. HLH is characterized by prolonged fever, cytopenias, hepatosplenomegaly, and hemophagocytosis in reticuloendothelial systems. The characteristic laboratory findings include hypertriglyceridemia, hyperferritinemia, hypofibrinogeneima, and increased soluble CD25 [1–4]. These manifestations and laboratory values are described as the result of hypercytokinemia caused by an

ineffective immunological response mediated by histiocytes (macrophages and dendritic cells), natural killer (NK) cells, and cytotoxic T cells (CTL) [1, 5–7]. Increased levels of several pro-inflammatory cytokines, such as interleukin-6 (IL-6), interferon (IFN)-γ, and tumor necrosis factor (TNF)-α have been demonstrated in patients with HLH [8–10]. HLH is classified into primary (genetic) or secondary (acquired) HLH. There are two subtypes of primary HLH, namely, familial HLH (FHL) and other immunodeficiencies such as Chediak–Higashi syndrome, Griscelli syndrome type 2, Hermansky–Pudlak syndrome type 2, and the X-linked lymphoproliferative syndrome [2, 11]. Mutations of *perforin* (*PRF1*), *UNC13D*, *STX11*, and *STXBP2* genes are responsible for 30–70% of FHLH cases [12–16]. It is thought that other unknown genetic defects remain as causes of FHL. Secondary HLH may occur under conditions of severe infections, malignancies, or autoimmune diseases [1, 2]. Many viruses, bacteria, and other infectious agents have been reported to trigger infection-associated HLH (IHLH) [17]. Epstein–Barr virus (EBV) is the most studied virus that trigger IHLH [18]. EBV-associated HLH (EBV-HLH) has a higher prevalence in East Asian countries [18]. Therefore, there may be a genetic variation in susceptibility to EBV-HLH.

Genetic factors other than *PRF1*, *UNC13D*, *STX11*, and *STXBP2* might influence susceptibility even to secondary HLH. Macrophage activation syndrome (MAS) is one form of secondary HLH [1, 2]. MAS occurs in patients with autoimmune diseases, especially with systemic-onset juvenile idiopathic arthritis (systemic JIA) [19, 20]. We recently reported that the *interferon regulatory factor 5* (*IRF5*) gene polymorphism is associated with susceptibility to MAS in systemic JIA patients [21]. IRF5 is a member of the IRF family of transcription factors and is known to have a crucial role in the Toll-like receptor signaling pathway [22, 23]. The activation of the Toll-like receptor is central to innate and adaptive immunity. IRF5 acts as a master transcription factor in the activation of pro-inflammatory cytokine genes especially in the virus-mediated immunological signaling pathway [23]. In *IRF5* knockout mice, a severely impaired induction of IL-6, IL-12, and TNF-α was observed [22].

In the present study, we hypothesized that polymorphisms in the *IRF5* gene may be associated with susceptibility to secondary HLH. We found a close relationship between the *IRF5* gene polymorphism/haplotype and susceptibility to secondary HLH.

Patients and Methods

Study Population

Patients with secondary HLH except for MAS were diagnosed based on the diagnostic criteria used in the HLH-94 Study (for patients who developed HLH before October 2006) and HLH-2004 Study (after October 2006) [4, 24]. The patients who showed known genetic mutations were excluded as primary HLH in this study. Patients under 1 year were also excluded to reduce the possible inclusion of undiagnosed primary HLH because the onset of FHL is below 1 year of age in 70–80% of the cases [25].

Patients with MAS were diagnosed as having systemic JIA based on the International League of Associations for Rheumatology classification criteria for systemic JIA [26]. Because the HLH-94/2004 diagnostic criteria may not always be appropriate when diagnosing MAS in systemic JIA patients who are under inflammatory conditions, patients with systemic JIA were diagnosed as having MAS based on the preliminary diagnostic guidelines for MAS complicating systemic JIA [27], as follows: (1) clinical criteria including central nervous dysfunction, hemorrhage or hepatomegaly and (2) laboratory criteria including decreased platelet counts ($<26.2\times10^9$/l), elevated levels of asparate aminotransferase (>59 U/l), decreased white blood cell counts ($<4.0\times10^9$/l), and hypofibrinogenemia (<2.5 g/l). The diagnosis of MAS requires the presence of two or more of these criteria.

For the diagnosis of EBV-HLH, EBV load in peripheral blood was quantified by real-time PCR as described in our previous study [28]. Patients were diagnosed as having EBV-HLH if they had EBV loads of over 1,000 genome copies per milliliter in whole blood and fulfilled the diagnostic criteria used in the HLH-94/HLH-2004 Study.

A total of 82 patients, 39 males and 43 females, were enrolled in the present study. Among the 82 patients, 48, including 33 having systemic JIA with MAS, were diagnosed as having secondary HLH at Yokohama City University Hospital between November 2000 and December 2009. The remaining 34 patients, who were diagnosed as having secondary HLH between March 2007 and December 2010, were registered in the HLH-2004 as a study of Japanese Pediatric Leukemia/Lymphoma Study Group. In these patients, 32 were diagnosed as having EBV-HLH. The 188 control subjects were recruited from apparently healthy adult volunteers.

Notably, the 33 MAS patients were identical to those analyzed in our previous study, where the significance of *IRF5* polymorphisms was evaluated among systemic JIA patients with or without MAS. In this study, to evaluate the significance of *IRF5* polymorphisms in the susceptibility to secondary HLH as a whole, data were reanalyzed in comparison with healthy controls using the different study population.

This study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Yokohama City University School of Medicine and each member of the Japan Leukemia/Lymphoma Study Group. Written informed consent was obtained from each patient or his/her guardians as well as the control subjects.

Table I Characteristics entire secondary HLH Study Group and subgroups

	N	Age	Gender
All patients with secondary HLH	82	4.7 (1–16)	39 (47.6%)
Subgroups of HLH patients			
MAS	33	4.8 (1–16)	16 (48.5%)
Non-MAS HLH	49	4.6 (1–15)	23 (46.9%)
EBV-HLH	32	4.3 (1–15)	16 (50.0%)

HLH hemophagocytic lymphohistiocytosis, *MAS* macrophage activation syndrome, *Non-MAS HLH* secondary HLH including EBV-HLH but not MAS, *EBV-HLH* Epstein–Barr virus-associated HLH

Genotyping

Three SNPs—rs729302, rs2004640, and rs2280714—in the *IRF5* gene were selected as described in our previous study [21]. Patients with HLH and control subjects were genotyped using TaqMan SNP Genotyping Assays as described previously [21].

Statistical Analysis

The SNPassoc package using R-language, version 2.8 (The R Foundation for Statistical Computing, http://www.R-project.org) was employed to evaluate the associations between HLH and the SNPs by logistic regression analysis [29]. Haplotype phases and haplotype frequencies were estimated using the Expectation–Maximization algorithm as implemented in the haplostat package (minimum haplotype frequency, >0.05; www.docstoc.com.) [30]. The associations between genotypes under study and laboratory values were analyzed by the Jonckheere–Terpstra test. The following laboratory values were included: levels of hemoglobin, neutrophils, platelets, triglycerides, fibrinogen, ferritin, transaminases, and lactate dehydrogenase. The association between HLH and the *IRF5* haplotypes was evaluated by logistic regression analysis.

Results

Patient characteristics are summarized in Table I. The mean age of the 82 patients with secondary HLH was 4.7 years (1–16 years) at onset. The numbers of patients with MAS and non-MAS HLH were 33 and 49, respectively. In those with non-MAS HLH, 32 with EBV-HLH were included.

The genotype frequencies for the three SNPs of the HLH patients, including their subgroups, and the control subjects were in Hardy–Weinberg equilibrium ($p>0.05$). These results were consistent with the findings of a recent Japanese population study (Table II) [31].

Table II Association of polymorphisms in the *IRF5* gene with susceptibility to secondary HLH

SNP subject subset	*n*	MAF	Allelic association			
			OR	(95% CI)	*p* value	p_c
		rs729302				
All patients with secondary HLH	82	0.20	1.05	0.96–1.15	0.26	n.s.
Subgroups of HLH patients						
MAS	33	0.18	1.04	0.96–1.12	0.32	n.s.
Non-MAS HLH	49	0.20	1.03	0.95–1.12	0.46	n.s.
EBV-HLH	32	0.23	1.00	0.93–1.10	0.90	n.s.
Control subjects	188	0.24	1.0	–	–	
		rs2004640				
All patients with secondary HLH	82	0.49	0.88	0.82–0.95	<0.01	0.006
Subgroups of HLH patients						
MAS	33	0.50	0.92	0.86–0.99	0.02	n.s.
Non-MAS HLH	49	0.49	0.91	0.84–0.98	0.01	0.030
EBV-HLH	32	0.55	0.95	0.88–1.01	0.11	n.s.
Control subjects	188	0.35	1.0	–	–	
		rs2280714				
All patients with secondary HLH	82	0.34	1.1	1.02–1.19	0.02	0.0465
Subgroups of HLH patients						
MAS	33	0.32	1.07	1.00–1.14	0.06	n.s.
Non-MAS HLH	49	0.35	1.07	0.99–1.14	0.09	n.s.
EBV-HLH	32	0.36	1.04	0.98–1.12	0.22	n.s.
Control subjects	188	0.44	1.0	–	–	

IRF5 interferon requlatory factor 5, *SNP* single nucleotide polymorphism, *MAF* minor allele frequency (the C allele at rs729302, T rs2004640, C rs2280714), p_c corrected combined *p* value using the Bonferroni method

Table III Association of polymorphisms in the *IRF5* gene with susceptibility to secondary HLH

SNP	MM/Mm vs. mm			MM vs. Mm/mm		
	OR	(95% CI)	*p* value	OR	(95% CI)	*p* value
rs729302	2.62	0.75–9.19	0.137	1.19	0.69–2.03	0.59
rs2004640	0.43	0.22–0.84	0.18	0.47	0.26–0.83	<0.01
rs2280714	2.54	1.08–5.97	0.03	1.59	0.93–2.71	0.096

Minor allele: the C allele at rs729302, T rs2004640, C rs2280714

SNP single nucleotide polymorphism, *M* major alleles, *m* minor allele

rs2004640 and rs2280714 were associated with susceptibility to secondary HLH as a whole even after Bonferroni correction (Table II). The T allele at rs2004640 was a risk factor for susceptibility to not only secondary HLH as a whole (p_c=0.006, OR=1.13, 95% CI=1.05–1.23) but also to non-MAS HLH (p_c=0.030, OR=1.10, 95% CI=1.02–1.19; Table II). Moreover, the GT/TT genotype at rs2004640 presented a risk for secondary HLH in general (p_c=0.028, OR=2.15, 95% CI=1.21–3.82; Table III). This genotype was also associated with non-MAS HLH (p_c=0.04, OR=2.28, 95% CI=1.12–4.66; Electronic Supplementary Material (ESM) Table 1).

Additionally, a statistically significant association of the ATT haplotype of the *IRF5* gene (rs729302–rs2004640–rs2280714) with susceptibility to secondary HLH was shown (p<0.001, OR=1.92, 95% CI=1.21–3.04; Table IV). This haplotype was also associated with susceptibility to subtypes of the MAS and non-MAS HLH, respectively, but not to EBV-HLH (ESM Table 2).

With regard to the laboratory values in the 34 patients with non-MAS HLH registered in the HLH-2004 Study, the low platelet count was associated with the C allele at rs2280714 (p=0.026, Jonckheere–Terpstra test). Other laboratory values were not associated with the IRF5 gene polymorphisms studied (data not shown).

Discussion

HLH is a clinically heterogeneous syndrome presumably because it is associated with a variety of genetic background. Even in primary HLH, there remain about 30% of FHL patients with unknown responsible genes [13]. With regard to secondary HLH, there may be several HLH-susceptible genes. Although mutations of *PRF1*, *UNC13D*, *STX11*, and *STXBP2* genes can be causable for the pathogenesis of FHL, a particular HLH-susceptible gene may contribute to the pathogenesis of secondary HLH cooperatively with other HLH-susceptible genes and may have the potential of influencing the severity of HLH.

In the present study, we revealed that the T allele at rs2004640 and the ATT haplotype in *IRF5* gene are associated with susceptibility to secondary HLH as well as to MAS in systemic JIA patients. The ATT haplotype in the *IRF5* gene was also associated with an increased risk of SLE [32]. The T alleles at both rs2004640 and rs2280714 were related to higher levels of IRF5 mRNA expression [32]. There seems a potentially important role of the IRF5-associated immune response in the pathogenesis of secondary HLH.

In many cases of HLH, viral infections trigger both primary and secondary HLH [18, 33]. Also, IRF5 has a key role in the induction of the antiviral and inflammatory response and controls the production of pro-inflammatory cytokines [22]. Therefore, the association between gene polymorphisms of *IRF5* and susceptibility to HLH is plausible. In order to assess whether there is an influence of *IRF5* gene polymorphisms on IHLH, we analyzed the association between *IRF5* gene polymorphisms and EBV-HLH. The *IRF5* gene polymorphisms tended to be associated with EBV-HLH, but without statistical significance, presumably because of the small number of patients in this study. Ineffective activation of histiocytes, NK cells, and CTL following viral infections is considered important in the pathogenesis of HLH [5–7]. Recently, several research outcomes were reported about the influence of IRF5 on the function of these immune cells [34–37]. For instance, M1 macrophages, which produce pro-inflammatory cytokines and mediate resistance to pathogens, were characterized by large amounts of IRF5 compared with

Table IV Comparison of IRF5 haplotypes in patients with secondary HLH

The order of SNPs in haplotype is rs729302-rs2004640-rs2280714

Haplotype	Haplotype frequencies in control subjects	Haplotype frequencies in secondary HLH patients	*p* value	OR	95% CI
A-G-C	0.405	0.302	0.02	1.0	–
C-G-T	0.208	0.174	0.37	1.19	0.70–2.04
A-T-T	0.333	0.461	<0.001	1.92	1.21–3.04

M2 macrophages, which produce anti-inflammatory cytokines and promote tissue repair [36]. In addition, IRF5 controls the induction of chemokines, such as IL-8, that mediate recruitment of T lymphocytes [34]. Therefore, IRF5 presumably serves as one of the key factors for the pathogenesis of HLH via influencing the function of these immune cells.

The present study still has some limitations. The first issue is the definition of secondary HLH. The patients with the following criteria were excluded from the study: positive defects of known genes (*PRF1*, *UNC13D*, *STX11*, *STXBP2*, and *SAP*), <1 year old at onset, and low or deficient CTL/NK activity. In male patients who had recurrent HLH episodes or were refractory to treatment, mutations in the *SH2D1A* genes were ruled out [38]. With using these criteria, almost all of the patients can be diagnosed with secondary HLH.

The second issue is that we could not perform a validation study. Although a genetic association study should be validated, the incidence of HLH is too low to validate this association in a single institution and even in a nationwide study. Therefore, it is important that the association between the *IRF5* genotype/haplotype and HLH susceptibility is confirmed by other groups.

We found a close relationship between polymorphisms in the *IRF5* gene and susceptibility to secondary HLH in general and its subtypes (MAS and non-MAS HLH), respectively. This finding suggests a potentially important role of the IRF5-associated immune response in the pathogenesis of HLH.

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REVIEW ARTICLE

Guidance on using tocilizumab for juvenile idiopathic arthritis

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Abstract Medical care for rheumatic disease in children has been supported by advances in rheumatology. In the past few years and based on knowledge about cytokines, particularly marked advances have been made in treatments using biological products. The fact that patients showed a marked response to treatment with biological products also provided uniform direction to treatment choice, which had previously been chaotic. On the other hand, biological products inhibit the action of physiologically essential substances, such as inflammatory cytokines or their receptors. This led to concerns about the risk of fatal or life-threatening adverse reactions, and rheumatologists are now required to take a disciplined approach to the use of these products. Thus, we sincerely hope that this guidance on using tocilizumab for juvenile idiopathic arthritis serves as a desk reference for pediatric rheumatologists and other healthcare professionals treating children with rheumatic diseases by biological drugs.

Keywords Juvenile idiopathic arthritis · Guidance · Tocilizumab (anti-IL-6 receptor antibody) · Corticosteroid

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Introduction

Recent advances in pediatric rheumatology have been brought about by research in two directions—bench to bedside and bedside to bench—successfully working in unison. Bench to bedside is the application of research results to the clinical setting. Bedside to bench is research that is newly inspired by analysis of clinical symptoms and test findings, leading to new topics of research and the eventual application of the results of that research back to the clinical setting. For example, the underlying cause of systemic-onset juvenile idiopathic arthritis (sJIA) is not yet known, but it has been shown that the clinical symptoms and test findings are attributable to dysregulated overproduction of the inflammatory cytokine, interleukin (IL)-6. Meanwhile, it was found that the complex clinical symptoms of JIA improved within a short period when patients were treated with a humanized anti-IL-6 receptor (IL-6R) monoclonal antibody (tocilizumab) developed using genetic engineering techniques. In addition, it was

observed that growth-retarded children with sJIA experienced a marked recovery in growth when dysregulated overactivation of the IL-6/IL-6R signalling was inhibited, giving rise to new topics for research in the bedside-to-bench direction.

Etanercept, a soluble tumor necrosis factor alpha (TNF-α) receptor developed using genetic engineering techniques, is used to treat refractory patients with articular forms of JIA in Europe and the USA, and its efficacy is widely known. Pediatric rheumatologists now have magic bullets, i.e., tocilizumab and etanercept, for children with refractory rheumatic diseases. However, because these treatments suppress physiological inflammation, there is concern that lack of clinical expertise could place patients at great risk. Infliximab, a TNF-α inhibitor similar to etanercept, is associated with increased incidence of tuberculosis. This finding revealed that TNF-α plays an important role in the development of tuberculosis, which needs to be taken into account when applying these drugs for therapeutic purposes. In patients with impaired cardiac function, TNF-α may have a protective effect, giving rise to concerns that inhibition of TNF-α may adversely affect cardiac function. Although various biological activities of IL-6/IL-6R signalling in the immune system and inflammation have been elucidated, its role in cardiac function, the regulatory function of the endocrine and hormonal system (particularly the hypothalamic–pituitary–adrenal axis), and bone and bone growth remains to be elucidated. These biological products therefore need to be used with expertise, and particular attention must be paid to understanding the adverse reactions that may occur with their use as well as to be alert to signals as to their safety during treatment.

There are four biological products, etanercept, tocilizumab, infliximab, and adalimumab, available in Japan. Indication for JIA has been approved only for etanercept and tocilizumab in Japan at the time of release of this guidance. These drugs have led to revolutionary advances in the treatment of JIA. It is, however, very important for us to maximize the effects of these drugs and minimize adverse reactions when using them. We sincerely hope that this guidance serves as a desk reference for pediatric rheumatologists and other healthcare professionals treating children with rheumatic diseases by biological drugs.

How to use this guidance

If a child has been diagnosed and treated according to the "Proposal for Juvenile Idiopathic Arthritis Guidance on Diagnosis and Treatment for Primary Care Pediatricians and Nonpediatric Rheumatologists" [1] but has responded poorly to treatment, treatment at the next level, i.e., using biological products, is required. However, for the time being, the use of these biological products should be initiated and managed by physicians who have been trained in their use, because strict adherence to their indications and exclusion criteria based on clinical expertise and experience is required for their safe and effective use. The aim of this guidance is to create rules to maximize the efficacy of biological products and to minimize risks and adverse reactions associated with these products in treating children with JIA who are refractory to conventional therapies. This guidance describes the indications, exclusion criteria, usage, and criteria for evaluating efficacy of biological products for JIA treatment.

Biological products are all specific receptors or monoclonal antibodies to inflammatory cytokines that have been developed as therapeutics. A characteristic of biological products is that they inhibit only one molecule when administered (one-point-hit drug). It is the first time in the long history of pediatric medicine that we pediatricians have had such drugs available to us. It has become apparent from clinical studies and experience in other countries that the serum concentration of these products needs to be maintained at a certain level if treatment using these products is to be significantly efficacious. This means that these drugs have to be constantly present in the body, which also means that the primary physiological functions of cytokines can also be continuously inhibited (masking effect). It should therefore be kept in mind that inhibition of biologically essential cytokine responses could lead to adverse reactions that risk patient well-being.

To date, pediatricians have had to use long-term high-dose corticosteroids to treat sJIA. With the introduction of tocilizumab, it became possible to reduce inflammation at a single stroke. However, the long-term use of corticosteroids previous to administration of tocilizumab will have extensively undermined the physical function in children, affecting growth, metabolism, and immune function. These problems are not therefore solved at a single stroke, even by tocilizumab, and result a time lag between improvement of inflammation by the biological product and recovery of physical function, which causes a number of problems that must be carefully considered by physicians who prescribe these biological products.

Overview and mechanism of action: tocilizumab

Tocilizumab is a humanized anti-human IL-6 receptor monoclonal antibody produced with Chinese hamster ovary cells using genetic engineering techniques following research and development by Osaka University and Chugai Pharmaceutical Co., Ltd. IL-6 has diverse physiological effects: it induces differentiation of B cells into antibody-producing cells, induces differentiation of various types of cells, regulates immune response, and, as an inflammatory cytokine, causes fever, induces acute-phase proteins, and

increases peripheral white blood cell (WBC) count [2]. IL-6 is not biologically active on its own; after binding with IL-6 receptors (soluble, cell-membrane-bound), it binds to gp130 on the cell surface membrane and transmits signals to the inside of the cell, giving rise to various inflammatory responses [3]. Clinically, it has been shown that IL-6 is involved in diseases such as rheumatoid arthritis [4], Castleman's disease [5], Crohn's disease [6], and JIA [7, 8] and in their pathogenesis. At the same time, tocilizumab is a monoclonal antibody, and the fact that these diseases and disease states improve markedly as a result of IL-6 receptor-blocking therapy with tocilizumab demonstrates in reverse that IL-6 and IL-6R are central to the pathogenesis of these diseases. A summary of the specific methods of treating JIA [9, 10] with tocilizumab were previously reported.

Nonproprietary name: Tocilizumab

Guidance on tocilizumab for systemic juvenile idiopathic arthritis

Eligibility for tocilizumab treatment

Tocilizumab is indicated for patients with refractory sJIA. Patients are considered to have refractory disease if they meet the following criteria despite receiving treatments according to the "Proposal for Juvenile Idiopathic Arthritis Guidance on Diagnosis and Treatment for Primary Care Pediatricians and Nonpediatric Rheumatologists" [1]:

1. Have persistent inflammation and clinical symptoms, such as fever, rash, and arthritis
2. Require prolonged use of high-dose corticosteroids (0.2 mg/kg/day or more of prednisolone) and experience severe adverse reactions induced by corticosteroids
3. Require high-dose corticosteroids in order to suppress inflammation and clinical symptoms, such as fever, rash, and arthritis and to render the disease inactive but which could sooner or later lead to corticosteroid-induced adverse reactions induced

Note: Tocilizumab is not necessarily indicated for all patients with refractory sJIA; it is not indicated in patients who have progressed to macrophage activation syndrome [11]; risk/benefit assessment should be made cautiously when considering this treatment in patients with compromized cardiac function.

Initiation for tocilizumab treatment

For patients with increased disease activity, e.g., persistent fever and continuous excessive inflammatory status evidenced by persistently elevated C-reactive protein (CRP) values, tocilizumab treatment should be initiated after their disease activity is controlled to some extent by intensive therapies, such as corticosteroid pulse therapy.

Contraindications and careful administration

Contraindications

- Patients diagnosed as having macrophage activation syndrome
  - Treatment of macrophage activation syndrome should come first, and tocilizumab should not be started in patients with concomitant macrophage activation syndrome
- Patients with a concurrent serious infection (pneumonia, sepsis, etc.)
- Patients with known hypersensitivity to tocilizumab

Careful administration

Pretreatment evaluation for patient eligibility includes the screening tests described below. If complications such as infections that are considered treatable by medications are identified before starting tocilizumab treatment, such treatment should be a priority. Tocilizumab treatment should be initiated only after confirming recovery from those complications.

Screening tests

1. Infections

Common infections:

- Pneumonia: plain chest X-ray and chest computed tomography (CT) scan
- Blood culture (sepsis or bacteremia)
- Check for dental caries (if necessary): consultation with oral surgery
- Otitis media, sinusitis, etc.
- Urinary tract infection and acute focal bacterial nephritis
- Gallium scintigraphy to exclude deep infection [positron emission tomography (PET) if possible]
- Hepatitis B (HBV) and C (HCV) antibody screening tests
- Epstein Barr virus (EBV) antibody screening tests

Tuberculosis and latent mycosis

- Family history, tuberculin test history, and vaccination history of bacillus Calmette-Guérin (BCG)