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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Original Article

National survey of childhood febrile illness cases with fever of unknown origin in Japan

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Abstract ***Background***: In Japan, an actual condition survey on childhood febrile diseases with fever of unknown origin has never been performed. We carried out a national survey on childhood febrile illnesses in order to collect useful information for the differential diagnosis.

Methods: A nationwide survey using questionnaires was performed on febrile illness cases with fever of unknown origin (FUO) experienced by 2843 pediatrics institutions with sick beds during a 5-year period before 2007. FUO was defined as a febrile illness of at least 2 weeks' duration with a temperature ≥38°C, and failure to establish a diagnosis in spite of intensive evaluation during seven days' hospitalization.

Results: Two hundred fifty-five of 2843 questionnaire-surveyed institutions had 960 FUO cases, of which 132 could not be diagnosed, and 828 could be diagnosed after detailed medical examinations. The diagnoses they clarified included infectious diseases in 190 cases (23%), rheumatic diseases in 448 cases (54%), neoplasms in 67 cases (8%), and others in 123 cases (15%).

Conclusion: Clarification of illnesses that ought to be differentiated in the diagnostic approach to an FUO case is essential for arriving at its definitive diagnosis by exclusion.

Key words child, febrile diseases, fever of unknown origin, final diagnosis, national survey.

We often experience cases with fever of unknown origin (FUO) in a clinical setting, yet research on the actual state of childhood febrile illnesses has rarely been done in our country, even though such research would be useful for the differential diagnosis of FUO. It is unclear in many aspects what diagnoses are made for FUO cases and how their differential diagnosis is made. Making a definite diagnosis of an FUO case is considered important for determination of therapeutic indication for an FUO case that really needs treatment. Therefore, we made a nationwide survey on childhood febrile illnesses on this occasion in order to acquire useful information for the differential diagnosis of FUO.

Methods

Survey institutions were 2843 nationwide children's institutions with sick beds. They were asked to answer primary and secondary retrospective inquiries about FUO cases they had experienced during a 5-year period before 2007. FUO was defined as a febrile illness of at least 2 weeks' duration with a temperature ≥38°C and failure to establish a diagnosis in spite of evaluation during seven days' hospitalization.

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In the primary survey, the number of FUO cases, final diagnosis, sex, and age were investigated. The secondary survey was made to investigate in detail the symptoms and signs as well as differential diagnostic approaches taken in the cases that had been reported to have final diagnoses in the primary survey.

This study protocol was approved by the Ethics Committee of Yokohama City University Hospital (approval no. 042, approval date: 27 July 2007).

Results

Data of the primary and secondary surveys

In the primary survey, of 2843 institutions to which questionnaires were sent, 1071 (37.7%) returned the questionnaire sheets. Valid answers were acquired from 1045 institutions, excluding 26 where pediatrics departments were closed. A total of 255 institutions experienced 960 applicable cases (Fig. 1), of which 132 could not be diagnosed, and 828 were diagnosed after detailed examinations: infectious diseases in 190 (23%), rheumatic diseases in 448 (58%), neoplasms in 67 (8%), and others in 123 (15%) (Fig. 2).

In the secondary survey, we made a more detailed investigation on 828 cases that had been reported in the primary survey with their established diagnoses. We sent questionnaire sheets to 230 institutions, 146 of which returned valid answered sheets. Eighteen institutions replied but their data were invalid for

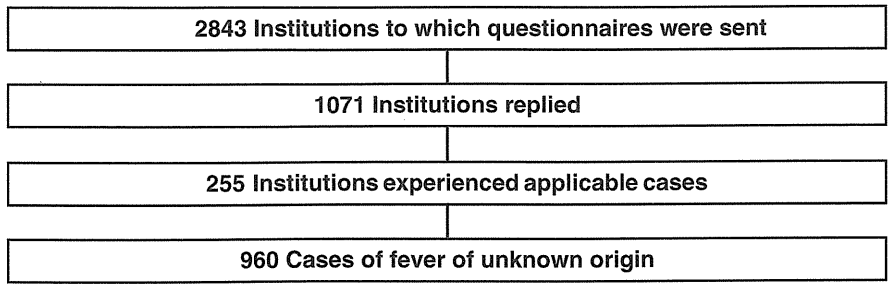

Fig. 1 Enrollment of primary survey.

analysis because their pediatrics departments were closed in some and case records that had been returned in the primary survey were incomplete in others. Of the above 146 institutions that answered properly, 127 reported 328 applicable cases (Fig. 3). These 127 institutions included 53 special hospitals, 60 municipal hospitals, and 14 non-specified facilities. Among the above 328 cases, only 185 met the above definitions.

Patients' backgrounds

A total of 101 patients were boys, and the male/female ratio was 1.2. Symptoms appeared at the age of 2 months to 18 years (mean, 7 years and 0 months) and diagnoses were made at the age of 2 months to 22 years (mean, 7 years and 3 months). Time from fever onset to diagnosis was 86.1 days on average. Diagnosis was established after close examinations in 153 out of 185 cases.

Classification of illnesses

There were 29 cases (15.7%) of infectious diseases, 108 (58.4%) of rheumatic diseases, 14 (7.6%) of neoplasms, and 34 (18.4%) of other diseases.

1 Infectious diseases (Fig. 4)
Cat scratch disease was most frequent in 10 cases, followed by seven cases of infectious diseases affected by viruses such as Epstein–Barr virus, coxsackie virus, adenovirus and others. Next

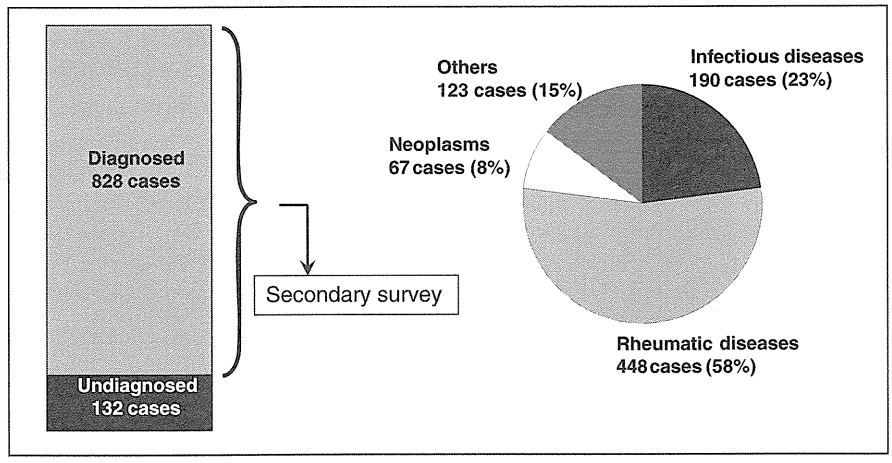

Fig. 2 Result of primary survey.

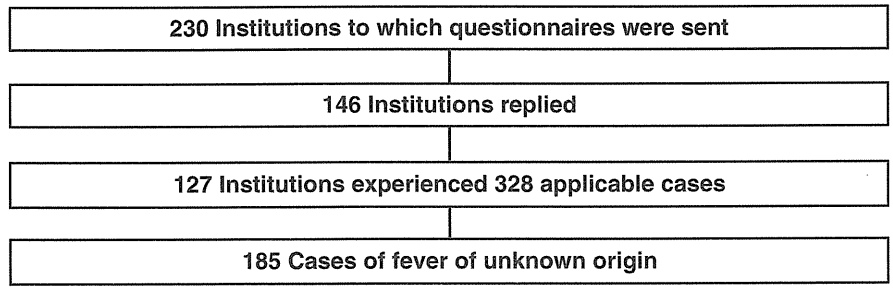

Fig. 3 Enrollment of secondary survey.

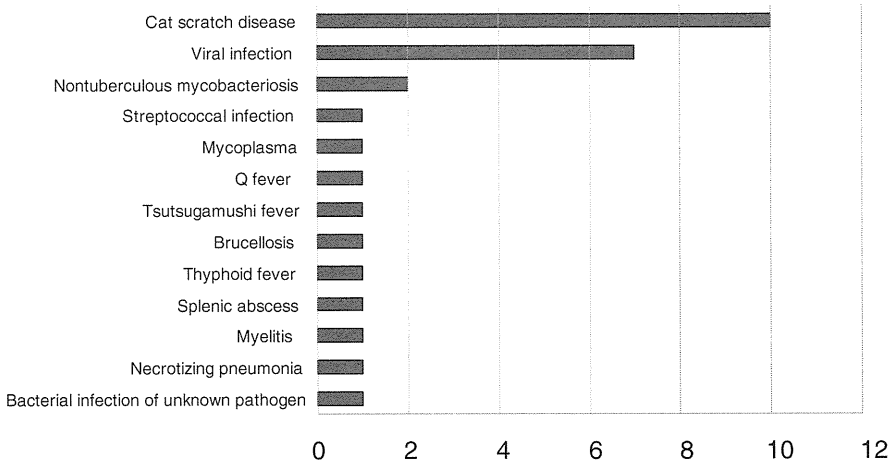

Fig. 4 Classification of infectious diseases.

came two cases of non-tuberculous mycobacteriosis. Rare cases of Q fever, tsutsugamushi fever, brucellosis, typhoid fever, and splenic abscess were included.

2 Rheumatic diseases (Fig. 5)

The most frequent illness was systemic-onset juvenile idiopathic arthritis, composing about 60% (68 cases). Others were nine cases of Takayasu's arteritis, eight cases of inflammatory bowel disease, and four cases of systemic lupus erythematosus.

3 Neoplasms

This category included five cases of Langerhans-cell histiocytosis, four cases of acute lymphocytic leukemia, two cases of

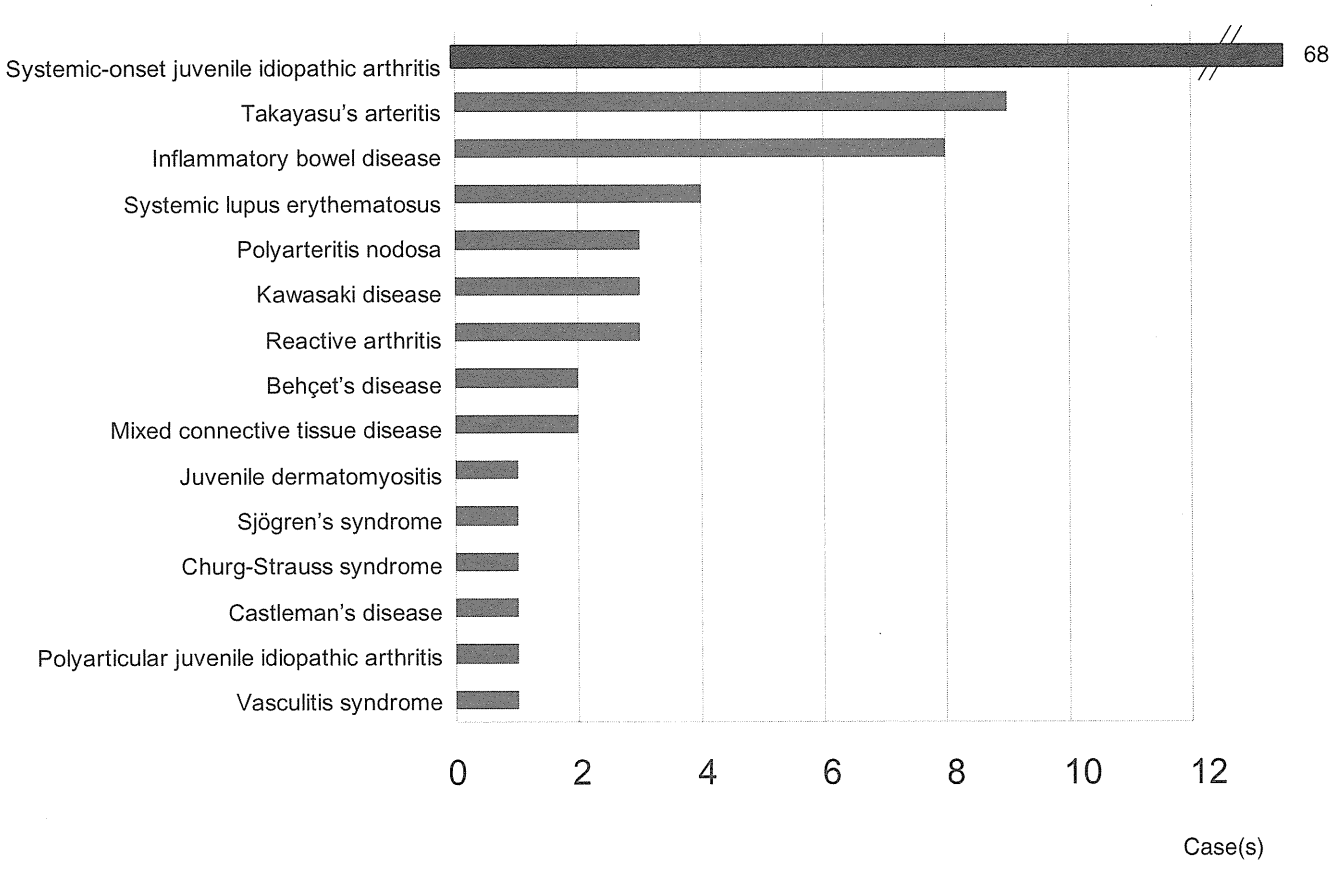

Fig. 5 Classification of rheumatic diseases.

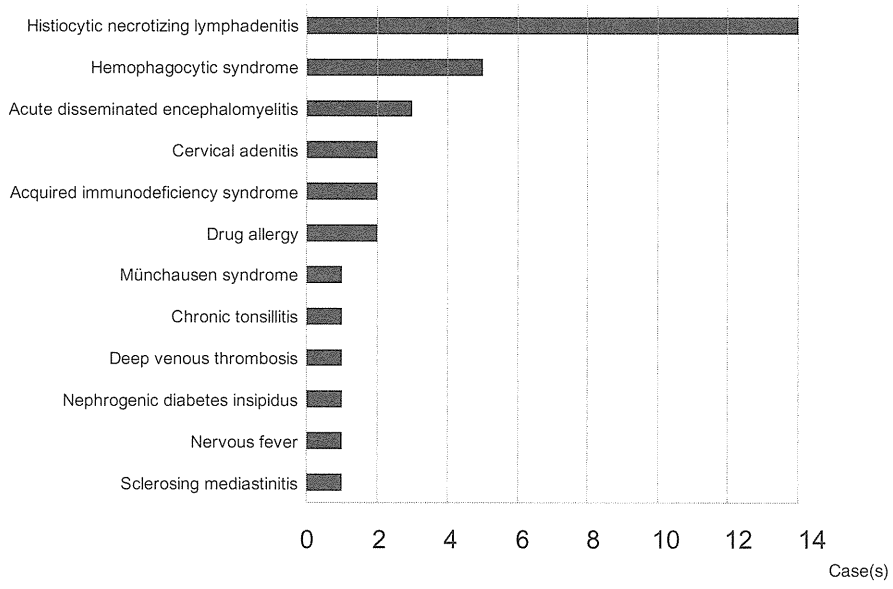

Fig. 6 Classification of other diseases.

malignant lymphoma, two cases of neuroblastoma, and one myofibromatosis case.

4 Others (Fig. 6)

Thirty-four other cases included 14 cases of histiocytic necrotizing lymphadenitis, which was most frequent, five cases of hemophagocytic syndrome, and three cases of acute disseminated encephalomyelitis.

Discussion

We surveyed the reality of cases with FUO that developed during 2003–2007 in order to study what illnesses were differentiated and to utilize it for the diagnostic approach of new FUO cases.

FUO was defined by Petersdorf *et al.* for the first time in 1961 as a febrile illness of at least three weeks' duration, with a temperature ≥38.3°C and failure to establish a diagnosis in spite of 1-week intensive inpatient evaluation.[1] The body temperature of 38.3°C was that measured in the oral cavity and could have been lower by 0.3–0.5 degrees if measured in the axilla. In 1968, Dechovitz *et al.* reported 155 cases of childhood FUO defined as a febrile illness of at least 2 weeks' duration with failure to identify a cause.[2]

In this study, in accordance with these reports, we defined a group of illnesses as a febrile illness with a temperature ≥38°C lasting for 2 weeks or longer and failure to establish a diagnosis in spite of evaluation during 1 weeks' hospitalization. After the report by Petersdorf *et al.*, several papers on FUO concerning pathogenetic classification in particular were published. However, papers on pediatric cases are very scarce, so that the present nationwide study performed in Japan is considered significant in this context.

Most of the papers roughly grouped the causes of FUO into infectious diseases, rheumatic diseases, neoplasms, others, and unknown in descending order of frequency (Pizzo *et al.* reported 100 prolonged fever cases in children: 52 were infectious, 20 collagen-inflammatory, six malignant, 10 miscellaneous, and 12 undiagnosed). Chantada *et al.* reported that 113 childhood FUO cases included 41 cases of infectious diseases, 15 of rheumatic diseases, 11 of neoplasms, and 22 of unknown cause.[3] As mentioned above, most studies concerning childhood FUO reported that infection was the most frequent cause of FUO.[4] In contrast, our present survey revealed that rheumatic diseases comprised the causes in 54%, which exceeded greatly 23% for infectious diseases. A similar trend was observed in a report concerning adults by Iikuni *et al.*[5]

In their report, among 79 adult FUO cases, 29.4% of them had rheumatic diseases, 28.8% infectious diseases, and 14.4% neoplasms, indicating a decrease in the rate of infectious diseases or neoplasms and an increase in that of rheumatic diseases as compared to previous reports. One of the reasons why rheumatic disease was the most common cause of FUO in the present study, as in the above report, was that it took a long time to make a diagnosis of illnesses associated with major conditions of systemic inflammation or vasculitis that had no specific markers. Systemic-onset juvenile idiopathic arthritis has no specific markers helpful for its diagnosis, so that symptoms such as skin rash and arthritis, are a determinant of reaching a diagnosis after all.

However, its diagnosis can be hard to make in the initial phase because of lack of pathognomonic symptoms or signs, including arthritis. This situation allowed the disease to fulfill the definition of FUO in many cases and the disease thus comprised the rheumatic disease group in around 60% in the present study.

Similarly, in an investigation of adult FUO by Goto *et al.*, adult-onset Still's disease, which simulated systemic-onset juvenile idiopathic arthritis in clinical conditions, comprised nearly 40% of the non-infectious inflammatory disease group, including rheumatic diseases.[6] Whereas it is still difficult to diagnose these

rheumatic diseases, the rate of the correct diagnosis of infectious diseases or neoplasms seems to be better than before. The spread of rapid diagnostic methods, the progress of antibody as well as culture examinations, and the expanded use of anti-bacterial agents may have resulted in alleviation of symptoms and signs. This situation may have thus reduced infectious disease cases that meet the FUO definition, while the development of imaging examinations may have improved the diagnosis rate of neoplasms.

When an affirmative diagnosis is difficult with the help of markers, etc., exclusion of other illnesses plays an important role for diagnosis. Fluorodeoxyglucose positron emission tomography has been increasingly reported to be useful for the diagnosis of FUO.[7] Although it is evident that the device is a powerful tool for the establishment of inflammatory pathological conditions, its applicability is currently limited to special facilities because of problems involving equipment investment and indication for children.

When an "FUO" case is presented, it is tempting to give priority to the establishment of a diagnosis by way of differentiating illnesses listed in the present survey. However, we consider it more important to evaluate the "severity" of the case on the basis of available information since the severity suggests the "morbid state" which in turn determines whether further appropriate examinations are required.

The present study has a limitation. Because this is a retrospective and multicenter study, there is a possibility of a recall bias about whether all data in all patients were included.

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Nationwide Survey of Patients with Primary Immunodeficiency Diseases in Japan

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Abstract To determine the prevalence and clinical characteristics of patients with in Japan, we conducted a nationwide survey of primary immunodeficiency disease (PID) patients for the first time in 30 years. Questionnaires were sent to 1,224 pediatric departments and 1,670 internal medicine departments of Japanese hospitals. A total of 1,240 patients were registered. The estimated number of patients with PID was 2,900 with a prevalence of 2.3 per 100,000 people and homogenous regional distribution in Japan. The male-to-female ratio was 2.3:1 with a median age of 12.8 years. Adolescents or adults constituted 42.8% of the patients. A number of 25 (2.7%) and 78 (8.5%) patients developed malignant disorders and immune-related diseases, respectively, as complications of primary immunodeficiency disease. Close monitoring and appropriate management for these complications in addition to prevention of infectious diseases is important for improving the quality of life of PID patients.

Keywords Primary immunodeficiency disease · epidemiology · nationwide survey · Japan

Abbreviations

APECED	Autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy
BTK	Bruton's tyrosine kinase
CGD	Chronic granulomatous disease
CID	Combined T and B cell immunodeficiency
CVID	Common variable immunodeficiency disease
FMF	Familial Mediterranean fever
IPEX	Immune dysregulation polyendocrinopathy enteropathy X-linked
NEMO	Nuclear factor kappa B essential modulator
PID	Primary immunodeficiency disease
SIgAD	Selective IgA deficiency
SLE	Systemic lupus erythematosus

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TRAPS	Tumor necrosis factor receptor-associated periodic syndrome
WAS	Wiskott–Aldrich syndrome
WHIM	Warts hypogammaglobulinemia, infections, and myelokathexis

Introduction

Patients with primary immunodeficiency disease (PID) show susceptibility to infections due to congenital immune system defects. These patients are also associated with noninfectious complications including autoimmune diseases and malignant disorders. Recent studies have revealed the causes of many PIDs to be mutations in various genes encoding molecules involved in the host defense mechanisms [1]. In addition, various new PIDs including defects in innate immunity and autoinflammatory disorders were identified under the recent progress in immunology and molecular genetics [2]. PID classification has been revised according to the identification of new PIDs and on the basis of new findings in PID pathophysiology. For a more precise clinical analysis, data should be obtained in accordance with the latest PID classifications.

The first nationwide survey of patients with PID in Japan was conducted between 1974 and 1979, which included 497 registered cases [3]. By 2007, a total of 1,297 patients were cataloged by a small number of PID specialists into a registration system [4]. The approximate prevalence of PID patients in Japan in the first nationwide survey was 1.0 in 100,000 people, which was much lower than that in other countries [5–7]. This difference in PID prevalence between Japan and other countries suggested that some PID patients in Japan remained unregistered. To determine the prevalence and clinical characteristics of patients with PID in Japan on the basis of the recent international classification system for PID, we conducted a nationwide survey of PID for the first time in 30 years.

Methods

This study was performed according to the nationwide epidemiological survey manual of patients with intractable diseases (2nd edition 2006, Ministry of Health, Labour, and Welfare of Japan) as described previously [8]. PID classification was based on the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee in 2007 [2]. Patients with chronic benign neutropenia and syndrome of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis were excluded because these were considered to be acquired diseases. The survey was conducted on PID patients who were alive on December 1, 2008 and those who were newly diagnosed and dead between December 1, 2007 and November 30, 2008 in Japan. Among the 2,291 pediatric departments and 8,026 internal medicine departments in Japan, hospitals participating in the survey were randomly selected after setting the selection ratio according to the number of beds (overall selection rate: 53.4% for pediatric departments, 20.8% for internal medicine departments; Table I). University hospitals and pediatric training hospitals, where many PID patients were considered to be treated, were stratified separately (Table I). Primary questionnaires regarding the number of patients and disease names based on PID classification were sent to the selected hospitals. Secondary questionnaires regarding age, gender, clinical manifestations, and complications of individual PID patients were sent to respondents who answered that they observed at least one PID patient with characteristics listed in the primary questionnaires.

Results

Questionnaires were distributed to 1,224 pediatric departments and 1,670 internal medicine departments of hospitals in Japan, and the response rate was 55.0% and 20.1%, respectively (Table I). A total of 1,240 patients (1,146 patients from pediatric departments and 94 patients from internal medicine departments) were registered (Table I). The estimated number of patients with PIDs in Japan was 2,900 (95% confidence interval: 2,300–3,500), and the prevalence was 2.3 per 100,000 inhabitants. We also determined the regional distribution on the basis of the patients' addresses. The estimated regional prevalence ranged from 1.7 to 4.0 per 100,000 inhabitants, and no significant differences were observed between different regions in Japan (Fig. 1). The most common form of PID was predominantly antibody deficiencies (40%), followed by congenital defects of phagocyte number, function, or both (19%) and other well-defined immunodeficiency syndromes (16%; Table II). Autoinflammatory disorders were observed in 108 cases (9%). The most common PID was Bruton's tyrosine kinase (BTK) deficiency (182 cases, 14.7%), followed by chronic granulomatous disease (CGD; 147 cases, 11.9%). However, common variable immunodeficiency disease (CVID) and selective IgA deficiency (SIgAD) were observed only in 136 (11.0%) and 49 cases (4.0%), respectively. Among patients registered from internal medicine departments, antibody deficiencies were the most common disorder (71%).

In the secondary survey, 923 cases were registered. The male-to-female ratio was 2.3:1 (n=914, unanswered: 9 cases) with a median age of 12.8 years (range: 0 to 75 years; n= 897, unanswered: 26 cases). The number of adolescent or

Table I Stratification and selection of hospitals and the survey results

	Stratification	Departments in Japan	Departments selected	Selection rate (%)	Return[a]	Response	Response rate (%)	PID Patient	Patients per department	Patients estimated
Pediatrics	University hospital	118	118	100	0	80	67.8	661	8.3	975
	Training hospital	402	402	100	4	242	60.8	376	1.6	618
	≥500 beds	92	92	100	5	48	55.2	24	0.5	44
	400–499 beds	118	118	100	3	63	54.8	42	0.7	77
	300–399 beds	287	230	80.1	4	122	54.0	31	0.3	72
	200–299 beds	289	116	40.1	4	53	47.3	6	0.1	32
	100–199 beds	486	98	20.2	0	44	44.9	4	0.1	44
	<99 beds	499	50	10.0	1	10	20.4	2	0.2	100
	Subtotal	2,291	1,224	53.4	21	662	55.0	1,146	1.7	1,961
Internal medicine	University hospital	156	156	100	1	47	30.3	37	0.8	122
	≥500 beds	374	374	100	1	86	23.1	35	0.4	152
	400–499 beds	328	263	80	1	54	20.6	6	0.1	36
	300–399 beds	692	278	40.2	6	49	18.0	10	0.2	140
	200–299 beds	1,008	202	20.0	0	36	17.8	2	0.1	56
	100–199 beds	2,460	246	10.0	1	36	14.7	1	0.0	68
	<99 beds	3,008	151	5.0	6	24	16.6	3	0.1	375
	Subtotal	8,026	1,670	20.8	16	332	20.1	94	0.3	950
Total		10,317	2,894	28.1	37	994	34.8	1,240		2,911

[a] Due to the closure of departments

adult cases (≥15 years) was 384 (42.8%; Fig. 2a). The male-to-female ratio of the younger generation (<15 years) was 2.7:1, while that of the older generation (≥15 years) was 2.0:1. Combined T and B cell immunodeficiencies (CIDs) were predominantly observed in the younger generation, while antibody deficiencies were more common with

Region	Reported PID Patients	Population ($x10^5$)	Estimated prevalence per 10^5 (95% C.I.)
Hokkaido	73	55.4	4.0 (0.2-7.8)
Tohoku	81	94.3	2.1 (0.9-3.3)
Kanto	387	419.8	1.8 (1.2-2.5)
Chubu	236	236.9	2.3 (1.4-3.1)
Kinki	158	208.4	2.0 (1.2-2.8)
Chugoku/Shikoku	105	116.1	1.7 (1.0-2.3)
Kyushu/Okinawa	200	146.0	2.5 (1.7-3.3)
Total	1240	1276.9	2.3 (1.8-2.7)

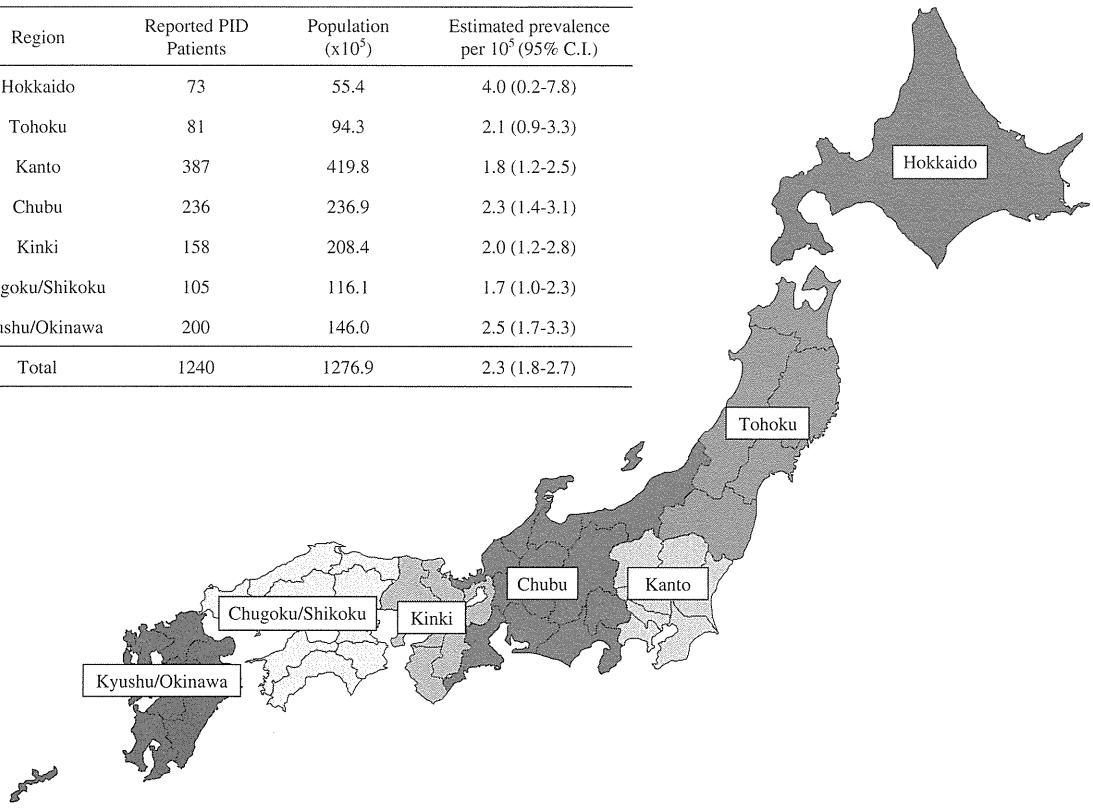

Fig. 1 Regional distribution of PID patients. *CI* Confidence interval

Table II Reported number of PID

Category	Total number	Pediatric department	Internal medicine department
I. Combined T and B cell immunodeficiencies	93 (7%)	93 (8%)	0 (0%)
γc deficiency	47	47	0
Adenosine deaminase deficiency	9	9	0
Omenn syndrome	4	4	0
Others	23	23	0
Untested or undetermined	10	10	0
II. Predominantly antibody deficiencies	501 (40%)	434 (38%)	67 (71%)
BTK deficiency	182	173	9
Common variable immunodeficiency disorders	136	107	29
Selective IgG subclass deficiency	66	58	8
Selective IgA deficiency	49	34	15
Hyper IgM syndrome	34	34	0
Transient hypogammaglobulinemia of infancy	7	7	0
Others	11	7	4
Untested or undetermined	16	14	2
III. Other well-defined immunodeficiency syndromes	194 (16%)	189 (17%)	5 (5%)
Wiskott–Aldrich syndrome	60	60	0
DNA repair defects (other than those in category I)	15	15	0
DiGeorge anomaly	38	38	0
Hyper-IgE syndrome	56	52	4
Chronic mucocutaneous candidiasis	17	16	1
Others	5	5	0
Untested or undetermined	3	3	0
IV. Diseases of immune dysregulation	49 (4%)	48 (4%)	1 (1%)
Chediak–Higashi syndrome	9	8	1
Familial hemophagocytic lymphohistiocytosis syndrome	5	5	0
X-linked lymphoproliferative syndrome	8	8	0
Autoimmune lymphoproliferative syndrome	8	8	0
APECED	4	4	0
IPEX syndrome	7	7	0
Others	2	2	0
Untested or undetermined	6	6	0
V. Congenital defects of phagocyte number, function, or both	230 (19%)	223 (19%)	7 (8%)
Severe congenital neutropenia	44	42	2
Cyclic neutropenia	19	17	2
Chronic granulomatous disease	147	144	3
Mendelian susceptibility to mycobacterial disease	5	5	0
Others	9	9	0
Untested or undetermined	6	6	0
VI. Defects in innate immunity	15 (1%)	15 (1%)	0
Anhidrotic ectodermal dysplasia with immunodeficiency	7	7	0
Interleukin-1 receptor-associated kinase 4 deficiency	2	2	0
Others	5	5	0
Untested or undetermined	1	1	0
VII. Autoinflammatory disorders	108 (9%)	101 (9%)	7 (8%)
Familial Mediterranean fever	44	40	4
TNF receptor-associated periodic syndrome	13	12	1
Hyper IgD syndrome	4	4	0
Cryopyrin-associated periodic syndrome	22	22	0

Table II (continued)

Category	Total number	Pediatric department	Internal medicine department
Others	3	3	0
Untested or undetermined	22	20	2
VIII. Complement deficiencies	32 (3%)	29 (3%)	3 (3%)
IX. Undetermined	18 (1%)	14 (1%)	4 (4%)
Total	1,240	1,146	94

APECED Autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy, *IPEX* immune dysregulation, polyendocrinopathy, enteropathy, X-linked

increasing age (Fig. 2b). The median age of CID, BTK deficiency, CVID, and CGD patients was 5.2, 12.8, 25.1, and 14.7 years, respectively.

It is well known that PID patients are susceptible to many pathogens and experience community-acquired or opportunistic infections. In this study, we focused on noninfectious complications of PID because they have been less well studied on a large scale and may provide important information for improving the quality of life of PID patients. Twenty-five PID patients developed malignant disorders (2.7%; Table III). Lymphoma, in particular, Epstein–Barr virus-related, and leukemia were dominant, while there were no patients with gastric carcinoma. CVID, Wiskott–Aldrich syndrome (WAS), and ataxia telangiectasia were more frequently associated with malignant diseases among PID patients. A case of Mendelian susceptibility

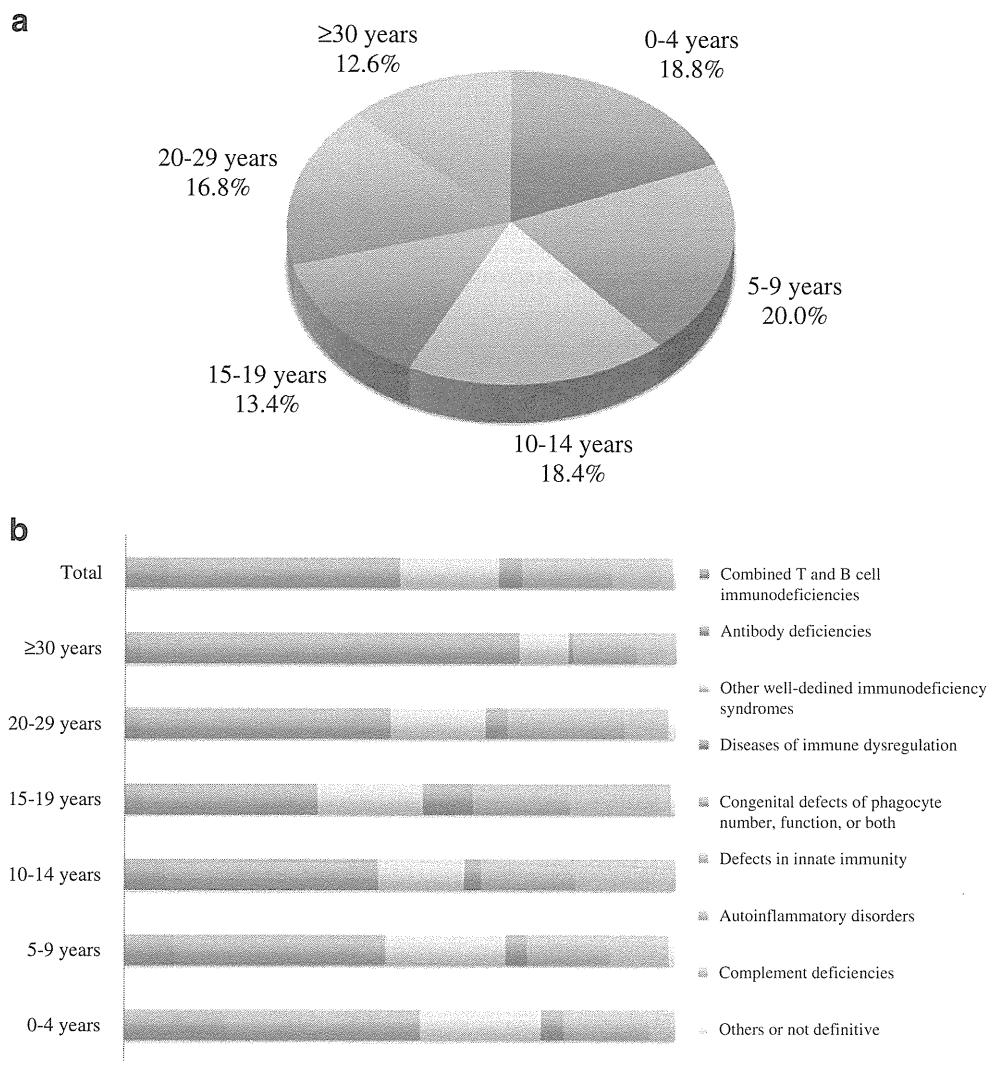

Fig. 2 **a** Age distribution of PID patients. **b** Distribution of PID in each age group

to mycobacterial disease with squamous cell carcinoma was also observed [9] (Table III).

Seventy-eight PID patients had immune-related (autoimmune) diseases (8.5%; Table IVa). Autoimmune lymphoproliferative syndrome, immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome, and nuclear factor kappa B essential modulator (NEMO) deficiency were associated with immune-related diseases at a very high incidence. In addition, immune-related diseases were relatively common in CGD and CVID patients (Table IVa). The most commonly observed immune-related disease was inflammatory bowel disease (33 cases), which was most frequently observed in CGD patients, followed by immune thrombocytopenic purpura (13 cases), autoimmune hemolytic anemia (8 cases), and systemic lupus erythematosus (SLE; 8 cases; Table IVa and b). Kawasaki disease occurred in WAS and CGD patients. In addition, this is the first report of Kawasaki disease in patients with complement deficiency (C9) and familial Mediterranean fever (FMF). A patient with warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome and a patient with tumor necrosis factor receptor-associated periodic syndrome (TRAPS) were first reported as cases of type 1 diabetes mellitus and SLE, respectively [10, 11].

Discussion

We conducted a nationwide survey of PID for the first time in 30 years and report the prevalence of PID in Japan. We registered 1,240 PID patients and found that the estimated prevalence of PID (2.3/100,000) is higher than that previously reported (1.0/100,000) in Japan. Our results are equivalent to those reported in Singapore (2.7/100,000) and Taiwan (0.77–2.17/100,000) [12–14]. However, our values are lower than those reported in Middle Eastern countries such as Kuwait (11.98/100,000) or in European countries such as France (4.4/100,000) [5–7, 15]. The high rate of consanguinity may be a cause of the high prevalence rate of PID reported in Middle Eastern countries [6, 15]. There may has been sample selection bias in this study because some asymptomatic cases (SIgAD, etc.), clinically recovered cases (transient hypogammaglobulinemia of infancy, etc.), and cases in which patients were deceased were not registered. In addition, lack of recognition of PID in internal medicine departments, not just the low response rate, might also have influenced the estimated prevalence of PID as well as the age and disease distribution. The regional prevalence of PIDs in Japan was homogenous, unlike in other countries in which a higher prevalence was

Table III Malignancies in PID patients

Primary immunodeficiency	Total	*n*	Malignancy
I. Combined T and B cell immunodeficiencies	75	2	(2.7%)
Ommen syndrome	3	1	NHL (EBV+) 1[a]
Adenosine deaminase deficiency	4	1	Breast carcinoma 1
II. Predominantly antibody deficiencies	378	8	(2.1%)
Common variable immunodeficiency disorders	93	7	HL 2, ML 2, ALL 1, Basal cell carcinoma 1, Cervical carcinoma 1
Good syndrome	4	1	Double primary carcinoma of breast and colon 1
III. Other well-defined immunodeficiency syndromes	165	7	(4.2%)
Wiskott–Aldrich syndrome	57	5	NHL 3, NHL/HL 1, LPD (EBV-) 1
Ataxia telangiectasia	13	2	T-ALL 1, MDS 1
IV. Diseases of immune dysregulation	38	4	(10.5%)
X-linked lymphoproliferative syndrome	5	2	Burkitt lymphoma 2
Autoimmune lymphoproliferative syndrome	6	2	HL (EBV+) 1, Brain tumor 1
V. Congenital defects of phagocyte number, function, or both	153	4	(2.6%)
Severe congenital neutropenia	35	3	MDS 3 (including 2 cases with monosomy 7)
MSMD	7	1	Squamous cell carcinoma of finger 1
VI. Defects in innate immunity	12	0	(0%)
VII. Autoinflammatory disorders	74	0	(0%)
VIII. Complement deficiencies	23	0	(0%)
IX. Undetermined	5	0	(0%)
Total	923	25	(2.7%)

n Number of PID patients who had malignant disorders, *ALL* acute lymphoblastic leukemia, *EBV* Epstein-Barr virus, *HL* Hodgkin lymphoma, *LPD* lymphoproliferative disease, *MDS* myelodysplastic syndrome, *ML* malignant lymphoma, *MSMD* Mendelian susceptibility to mycobacterial disease, *NHL* non-Hodgkin lymphoma

[a] The number of patients

Table IV Immune-related diseases in PID patients

(a) Immune-related diseases with each PID

Primary immunodeficiency	Total	*n*	Immune-related disease
I. Combined T and B cell immunodeficiencies	75	2	(2.6%)
MHC class II deficiency (suspected)	1	1	ITP with AIHA 1[a]
CD4 deficiency	1	1	Hashimoto disease 1
II. Predominantly antibody deficiencies	378	24	(6.3%)
Common variable immunodeficiency disorders	93	16	ITP 3, RA 2, AIHA 2, Hashimoto's disease 2, IBD 2, SLE 1, MG 1, ADEM 1, Autoimmune hepatitis 1, Uveitis 1
Hyper-IgM syndrome	32	3	JIA 1, SLE (complicated with C1q deficiency) 1, IBD 1
Selective IgA deficiency	28	3	SLE 1, SLE with Kikuchi disease 1, RA 1
IgG subclass deficiency	50	2	ITP with AIHA 1, ITP with MS 1
III. Other well-defined immunodeficiency syndromes	165	5	(3.0%)
Wiskott–Aldrich syndrome	57	3	AIHA 2, Kawasaki disease 1
DiGeorge syndrome	33	2	AIHA 1, ITP 1
IV. Diseases of immune dysregulation	38	10	(26.3%)
X-linked lymphoproliferative syndrome	5	1	IBD 1
Autoimmune lymphoproliferative syndrome	6	4	ITP 3, Graves' disease with IBD 1
APECED	5	1	T1DM with Hashimoto's disease and Vogt–Koyanagi–Harada disease 1
IPEX syndrome	6	4	T1DM 1, T1DM with ITP, AIN and IBD 1, Autoimmune enteritis 1, AIHA with Autoimmune enteritis and Hashimoto's disease 1
V. Congenital defects of phagocyte number, function, or both	153	25	(16.3%)
Chronic granulomatous disease	87	25	IBD 20, ITP 2, JIA 1, MCTD 1, Kawasaki disease 1
VI. Defects in innate immunity	12	5	(41.7%)
NEMO deficiency	7	4	IBD 3, IBD with JIA 1
WHIM syndrome	3	1	T1DM 1
VII. Autoinflammatory disorders	74	3	(4.0%)
Familial Mediterranean fever	36	2	SLE 1, Kawasaki disease 1
TNF receptor associated periodic syndrome	9	1	SLE 1
VIII. Complement deficiencies	23	3	(13.0%)
C4 deficiency	1	1	SLE with RA 1
C6 deficiency	1	1	IBD 1
C9 deficiency	11	1	Kawasaki disease 1
IX. Undetermined	5	1	(20%)
Nakajo syndrome	1	1	SLE 1
Total	923	78	(8.5 %)

(b) Immune-related manifestations associated with PID

Immune-related diseases	*n*
IBD (including autoimmune enteritis)	33
ITP	13
AIHA	8
SLE	8
RA/JIA	6
Hashimoto's disease/Graves' disease	5
Kawasaki disease	4
T1DM	4
Uveitis (including Vogt–Koyanagi–Harada disease)	2
ADEM/MS	2
Others	5

n Number of PID patients who had immune-related disorders, *ADEM* acute disseminated encephalomyelitis, *AIHA* autoimmune hemolytic anemia, *AIN* autoimmune neutropenia, *APECED* autoimmune polyendocrinopathy candidiasis ectodermal dystrophy, *IBD* inflammatory bowel disease, *IPEX* immunodysregulation, polyendocrinopathy, enteropathy X-linked, *ITP* immune thrombocytopenic purpura, *JIA* juvenile idiopathic arthritis, *MCTD* mixed connective tissue disease, *MG* myasthenia gravis, *MS* multiple sclerosis, *RA* rheumatoid arthritis, *SLE* systemic lupus erythematosus, *T1DM* type 1 diabetes mellitus, *WHIM* warts, hypogammaglobulinemia, infections, and myelokathexis

[a] The number of patients

observed in urban areas [5, 7, 16]. This may be because many PID patients were treated or followed by PID specialists distributed nationwide in Japan; this is assumed by the location of hospitals with which they were affiliated.

The distribution ratios of BTK deficiency (14.7%) and CGD (11.9%) in Japan were higher than those in a previous report from Europe (5.87% and 4.33%, respectively), while those of CIDs and other well-defined immunodeficiency syndromes were comparable [17]. The prevalence of BTK deficiency was previously reported to be 1/900,000–1,400,000 in a European cohort study [18]. In contrast, this value was estimated to be 1/300,000 in Japan in our study. BTK deficiency appears to be common in Japan, although this may be partially because more patients, including those showing atypical clinical manifestations, were diagnosed more accurately by the recently established genetic diagnostic network in Japan [19]. This is supported by the highest proportion of Japanese patients in the international mutation database for X-linked agammaglobulinemia (BTKbase) [20]. The reason for the low number of registered CGD patients in Europe in a recent report (1/620,000) [17] is unknown; the prevalence of CGD was 1 in 250,000 in a previous European survey [21], which was similar to our results (1 in 380,000 in this study and 1 in 280,000 in our previous study [22]). The percentage of BTK deficiency and CGD would be lower if more adult cases were registered because the prevalence of these disorders is low in adults. CVID was the most commonly reported PID (20.7%) in Europe, and the onset of symptoms was observed most commonly in the third decade of life in these patients [17, 23]. In this study, CVID constituted 11.0% (136 cases) of PID cases, and only 29 cases were reported from internal medicine departments (Table II). A lower number of registered CVID patients may have led to a lower number of reported patients with antibody deficiency and a lower prevalence of PID, although it is still possible that CVID is not as common in Japan as in European countries. There was no significant difference in the distribution rate of SIgAD between Japanese and Europeans, although SIgAD is rare in Japanese (1/18,500) compared with Caucasians (1/330–2,200) according to seroepidemiologic studies [24]. This may be because most SIgAD patients lack clinical manifestations. The distribution ratio of autoinflammatory disorders in Japan (9%) was much higher than that in Europe (1.02%) [17] (Table II). Considering the disease type of the autoinflammatory disorders was not specified in 22 cases (20%), it is possible that many other patients with autoinflammatory disorders remain undiagnosed in Japan as well as in other countries.

The percentage of men (69.7%) with PID is higher in Japan than in Europe (60.8%) or Kuwait (61.8%), but is equivalent to that in Taiwan (70.2%) [6, 13, 17]. The higher ratio of men, particularly in younger generation (<15 years), appears to be due to the larger number of X-linked PID patients (BTK deficiency, X-CGD, γc deficiency, etc.) in this study compared to that in Europe or Kuwait. Adolescents or adults (≥15 years) constituted 42.8% of the patients in this study, which is equivalent to the number in the European study (≥16 years: 46.6%), while those >16 years constituted only 10.9% in the previous survey [3, 17]. In this study, it was found that CVID and SIgAD are common in adults (Table II) and that antibody deficiencies are more common with increasing age (Fig. 2b). A reason for the increased number of adult PID patients may be long-term survival of PID patients due to improved treatments such as immunoglobulin replacement therapy. In addition, an increased likelihood of patients being diagnosed by internists as having late-onset PID, e.g., CVID and SIgAD, may have contributed to these values [17, 25, 26]. Therefore, it is important for internists to be well-informed regarding PID. In contrast, CIDs are fatal during infancy without hematopoietic stem cell transplantation or gene therapy. Because hematopoietic stem cell transplantation has been widely performed in Japan since the 1990s, surviving patients with CID are limited to the younger generation, similar to French patients (Fig. 2b) [5, 27, 28].

It has been reported that PID patients are at increased risk of developing malignant diseases, in particular, non-Hodgkin lymphoma, leukemia, and stomach cancer [29]. Although lymphoma and leukemia were relatively common, stomach cancer was not observed in our study. In the previous survey in Japan, eight of nine PID patients with malignant disorders (including one gastric cancer patient) died [3]. It is possible that some PID patients with malignant disorders were not registered because they were deceased. PID is also associated with immune-related diseases because of a defect in the mechanisms to control self-reactive B and T cells. The frequency of immune-related manifestations varied among individual PID patients, as reported previously [30, 31]. Four PID patients who had developed Kawasaki disease, one patient with WHIM syndrome and type 1 diabetes mellitus, and one patient with TRAPS and SLE in our study may provide new pathophysiological insights of these diseases and the association between PID and autoimmune diseases.

Conclusions

We report the prevalence and clinical characteristics of PIDs in Japan. Although the advances in diagnostic technologies and treatments have improved the prognoses of PID, many patients continue to experience severe complications such as malignancy and immune-related diseases as well as infections. To improve the quality of life of PID patients, it is necessary to pay attention to

complications and treat them appropriately. Web-based PID databases and consultation systems have been created in Japan (Primary Immunodeficiency Database in Japan [4] and Resource of Asian Primary Immunodeficiency Diseases in Asian countries [32]) to reveal precise information regarding PID and to promote cooperation between doctors and researchers [19].

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Clinical and Host Genetic Characteristics of Mendelian Susceptibility to Mycobacterial Diseases in Japan

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Abstract
Purpose The aim of this study is to investigate clinical characteristics and genetic backgrounds of Mendelian susceptibility to mycobacterial diseases (MSMD) in Japan.
Methods Forty-six patients diagnosed as having MSMD were enrolled in this study. All patients were analyzed for the *IFNGR1*, *IFNGR2*, *IL12B*, *IL12RB1*, *STAT1*, and *NEMO* gene mutations known to be associated with MSMD.
Results Six patients and one patient were diagnosed as having partial interferon-γ receptor 1 deficiency and nuclear factor-κB-essential modulator deficiency, respectively. Six of the seven patients had recurrent disseminated mycobacterial infections, while 93% of the patients without these mutations had only one episode of infection.
Conclusions The patients with a genetic mutation were more susceptible to developing recurrent disseminated mycobacterial infections. Recurrent disseminated mycobacterial infections occurred in a small number of patients even without these mutations, suggesting the presence of as yet undetermined genetic factors underlying the development and progression of this disease.

Keywords Disseminated mycobacterial infection · IFN-γR1 deficiency · NEMO deficiency · flow cytometric analysis

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Introduction

Although the outcome of mycobacterial infection is influenced by many factors, including the virulence of the pathogen and the environment of the host, it has been demonstrated that host genetic factors play important roles in the defense against mycobacteria [1]. Mendelian susceptibility to mycobacterial diseases (MSMD, MIM 209950) is a rare primary immunodeficiency syndrome characterized by a predisposition to develop infections caused by weakly virulent mycobacteria, such as *Mycobacterium bovis* bacille Calmette-Guerin (BCG) and environmental non-tuberculous mycobacteria (NTM) [2–4]. These patients are vulnerable to systemic salmonellosis and infections with *Mycobacterium tuberculosis*, the virulent mycobacterial species, to a lesser extent [5, 6]. Diseases caused by other intracellular pathogens, such as *Nocardia*, *Listeria*, *Paracoccidioides*, *Histoplasma*, and *Leishmania*, and some viruses, such as human herpes virus-8, have only rarely been reported, mostly in single patients [7–12].

To date, interferon (IFN)-γ receptor 1 (*IFNGR1*) [13–15], IFN-γ receptor 2 (*IFNGR2*) [16], interleukin (IL)-12 p40 subunit (*IL12B*) [17], IL-12 receptor β subunit (*IL12RB1*) [18–20], signal transducer and activator of transcription-1 (*STAT1*) [21], and nuclear factor-κB-essential modulator (*NEMO*) [22] mutations were identified as the causes of this primary immunodeficiency. On the other hand, no genetic etiology has yet been reported to be identified for about half of all patients with MSMD [3]. In addition, there have been no precise reports on the clinical characteristics and genetic backgrounds of MSMD in Asian countries, including Japan, which has a high prevalence of tuberculosis.

In this study, we analyzed patients who had a recurrent or disseminated infection with intracellular pathogens to clarify the clinical manifestations and host genetic backgrounds of MSMD in Japan.

Materials and Methods

Subjects

We studied 46 patients (30 males and 16 females) diagnosed as having MSMD because of recurrent infections, or blood-borne infections such as osteomyelitis/arthritis, and multiple infections at different anatomic sites by intracellular bacteria including BCG, NTM, *Salmonella* species, *Listeria monocytogenes*, or *M. tuberculosis* in 34 hospitals in Japan from 1999 to 2009. There was no consanguinity in these families. The clinical information on each patient was collected using a standardized case report form. Informed consent was obtained from the parents of the subjects before the study. This study was approved by the Ethics Committee of Kyushu University.

Flow Cytometric Analysis

Two-color flow cytometric analysis was performed to investigate IFN-γ receptor 1 (IFN-γR1) expression levels on the patients' monocytes by using an EPICS XL instrument (Beckman Coulter, Miami, FL, USA). Peripheral blood mononuclear cells (PBMCs) were stained with mouse anti-IFN-γR1 monoclonal antibody (MAb) (Genzyme, Cambridge, MA, USA), followed by rat phycoerythrin anti-mouse immunoglobulin antibody (BD Bioscience Pharmingen, San Diego, CA, USA). Cells were washed twice and stained with a phycoerythrin 5.1 (PC5)-anti-CD14 MAb (Beckman Coulter). IFN-γR1 expression was analyzed on monocytes determined by their side scatter and CD14 positivity.

Genomic DNA and cDNA Sequence Analysis

The *IFNGR1*, *IFNGR2*, *IL12B*, *IL12RB1*, *STAT1*, and *NEMO* genes were analyzed for coding exons and flanking intronic sequences. These genes were amplified by polymerase chain reaction (PCR) after whole genome amplification with a GenomiPhi V2 DNA Amplification Kit (GE Healthcare, Little Chalfont, UK). The PCR products were treated with an Exo-SAP-IT kit (GE Healthcare, Amersham, UK) and then were analyzed by direct sequencing with an ABI 3130 DNA sequencer (Perkin-Elmer, Foster City, CA, USA). Detected mutations were confirmed by sequencing the PCR product using cDNA as a template.

Statistical Analysis

Comparisons of the proportions were analyzed by the χ^2 test. The Mann–Whitney *U* test was used to compare differences between quantitative variables. A *P* value less than 0.05 was considered to be statistically significant.

Results

The median age of the patients was 8 years (range, 6 months–41 years), and the median age at the onset of infection was 1 year and 4 months (range, 4 months–6 years). The male to female ratio was 1.9:1. Only one patient had not received a BCG vaccination. There were 59 episodes of disseminated mycobacterial infections in the 46 patients. Nine (19%) of 46 patients had two or more episodes of these infections. Two of the patients had three episodes, and one had four episodes of these infections. In all episodes, BCG was the most common pathogen (82.6%, Table I). The *Mycobacterium avium* complex (MAC) was isolated during eight episodes of these infections. *M. tuberculosis* was also confirmed in two episodes of infection. No severe *Salmonella* species, *L. monocytogenes*, or viral infections were observed.

The common clinical manifestations were osteomyelitis/arthritis, lymphadenitis, and subcutaneous abscess/dermatitis (Table I and Fig. 1a). Only one patient was diagnosed as having arthritis, and the lesion spread to the adjacent bone. Two patients showed hepatosplenomegaly during the BCG infection, and two patients with the MAC infection developed pulmonary abscess. Among the BCG infections, the median intervals of time between BCG vaccination and the development of primary BCG infection were 3 (1–10 months), 4 (2–36 months), and 11 months (5–46 months) for the subcutaneous abscess/dermatitis, lymphadenitis, and osteomyelitis/arthritis, respectively (Fig. 1b).

We performed the genetic analysis on these patients for the *IFNGR1*, *IFNGR2*, *IL12B*, *IL12RB1*, *STAT1*, and *NEMO* genes. Six patients (five families) and one patient had mutations in the *IFNGR1* and *NEMO* genes, respectively (Table II). Five of the seven patients who had a mutation in the *IFNGR1* gene were the patients that we

Table I The clinical manifestations of the patients with MSMD

	Patients with genetic mutation, *n* (%)	Patients without a genetic mutation, *n* (%)	Total *n* (%)
Causative pathogen[a]			
BCG	3 (42.9)	35 (89.7)	38 (82.6)
M. avium complex	1 (14.3)	3 (10.2)	4 (8.7)
BCG+*M. avium* complex	2 (28.5)	0 (0)	2 (4.3)
M. avium complex+*M. tuberculosis*	1 (14.3)	1 (2.6)	2 (4.3)
Sites of infection[b]			
Osteomyelitis/arthritis	7 (43.8)	24 (55.8)	31 (52.5)
Lymphadenitis	8 (50.0)	8 (18.6)	16 (27.1)
Dermatitis/subcutaneous	3 (18.8)	11 (25.6)	14 (23.7)
Pulmonary abscess	0 (0)	2 (4.7)	2 (3.4)

The total number exceeds 59 because some patients had multiple lesions at the same time

[a] $n=7$ for patients with a genetic mutation and $n=39$ for patients without a genetic mutation

[b] $n=16$ for patients with a genetic mutation and $n=43$ for patients without a genetic mutation

reported previously [14, 15], and the other two patients were newly identified. All of the IFN-γR1-deficient patients were heterozygotes, and the mutation was in the transmembrane domain in one patient (774del4: patient 5) and in the intracellular domain in five patients (811del4: patient 1, 818del4: patients 2–4, and 832 G>T, E278X: patient 6), which led to the expression of a truncated protein with a dominant negative effect on the IFN-γR1 signaling (Table II and Fig. 2a). The IFN-γR1 expression levels were significantly increased in all six patients with IFN-γR1 deficiency (Fig. 2b). Patient 7 had a missense mutation in *NEMO* (943 G>C, E315Q). The CD14-positive cells from this patient produced a lower level of TNF in response to LPS stimulation (data not shown), which was consistent with the defect in NF-κB signaling.

The proportions of the patients with recurrent mycobacterial infection or multiple osteomyelitis/arthritis were

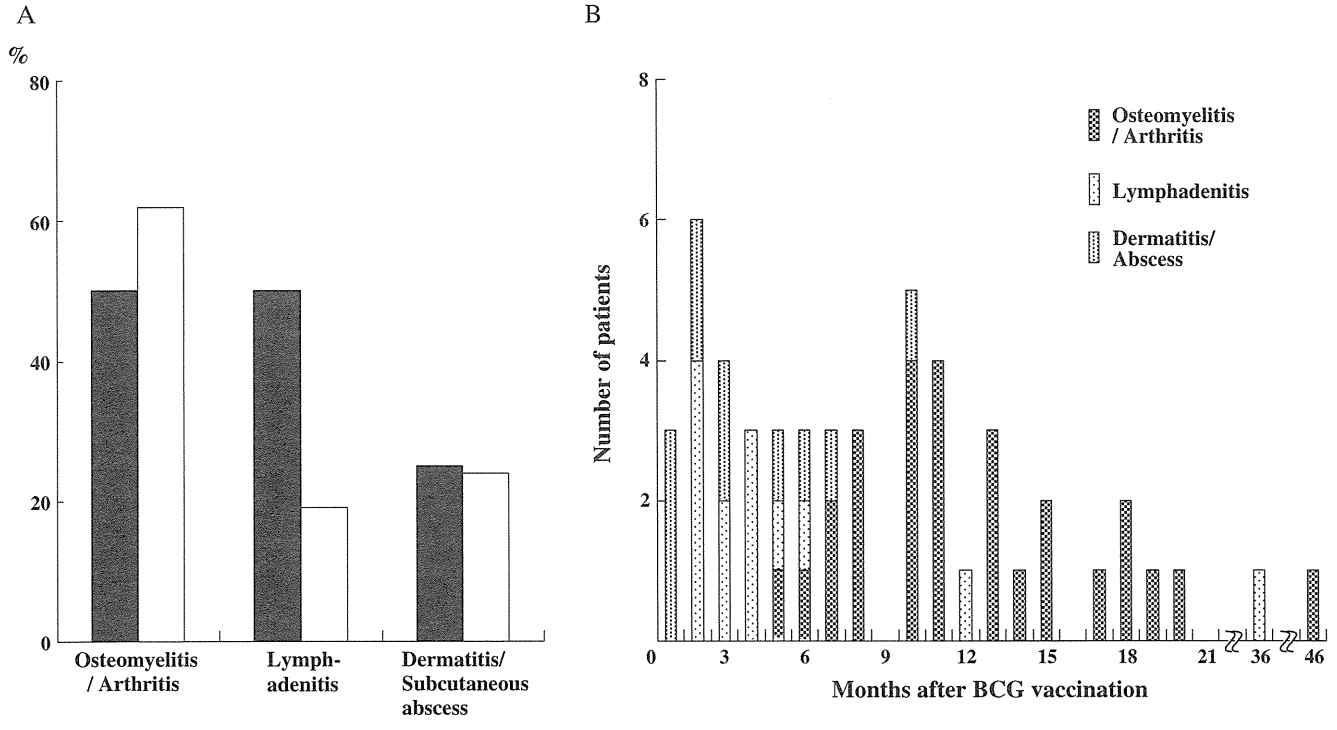

Fig. 1 The clinical features of the patients with BCG infection. The distribution of the sites of infections (**a**) and the intervals between BCG vaccination and the first onset of BCG infection (**b**) are shown. The *black bar* and the *white bar* represent the proportion of the patients with and without genetic mutations, respectively