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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

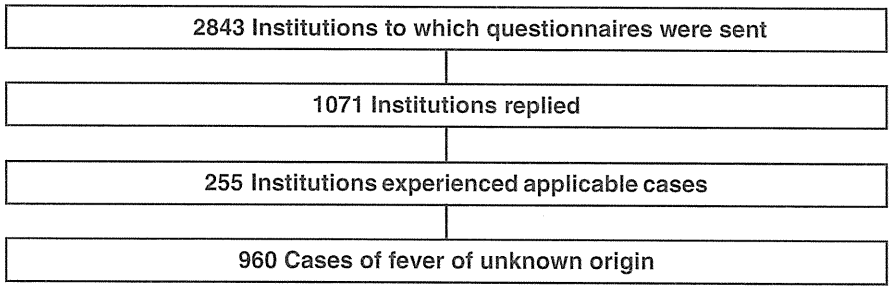


**Fig. 1** Enrollment of primary survey.

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

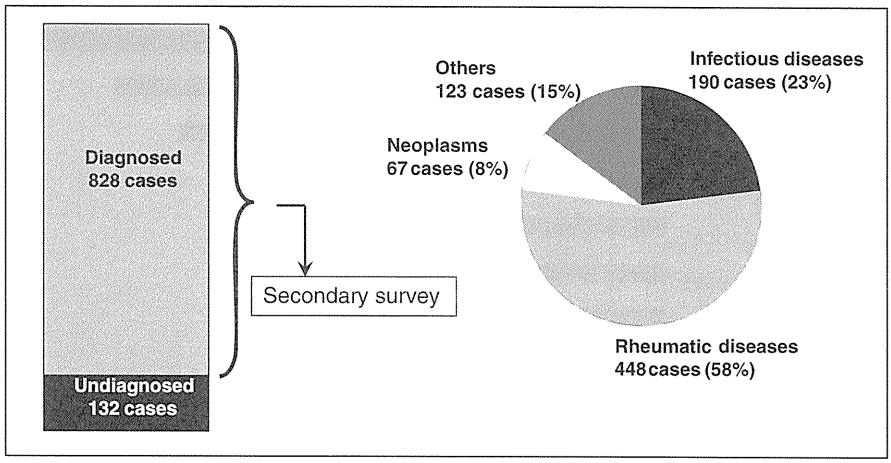


**Fig. 2** Result of primary survey.

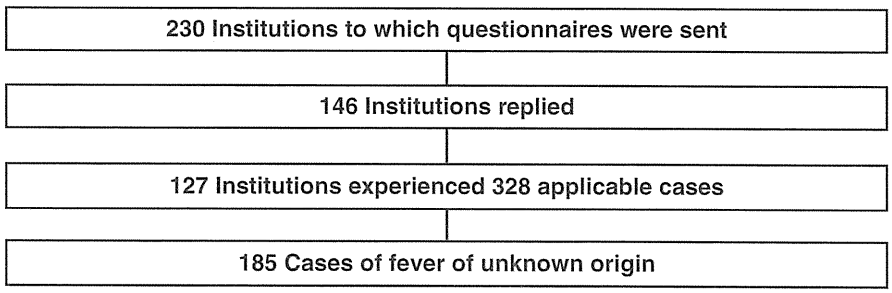


**Fig. 3** Enrollment of secondary survey.

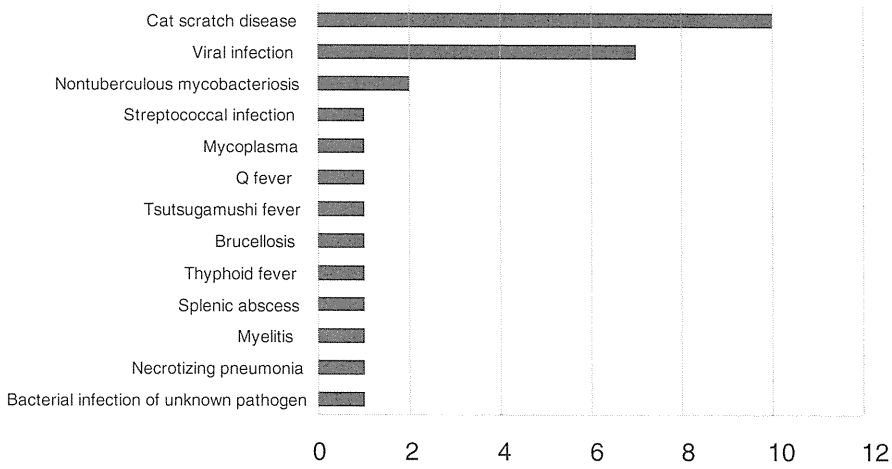


**Fig. 4** Classification of infectious diseases.

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

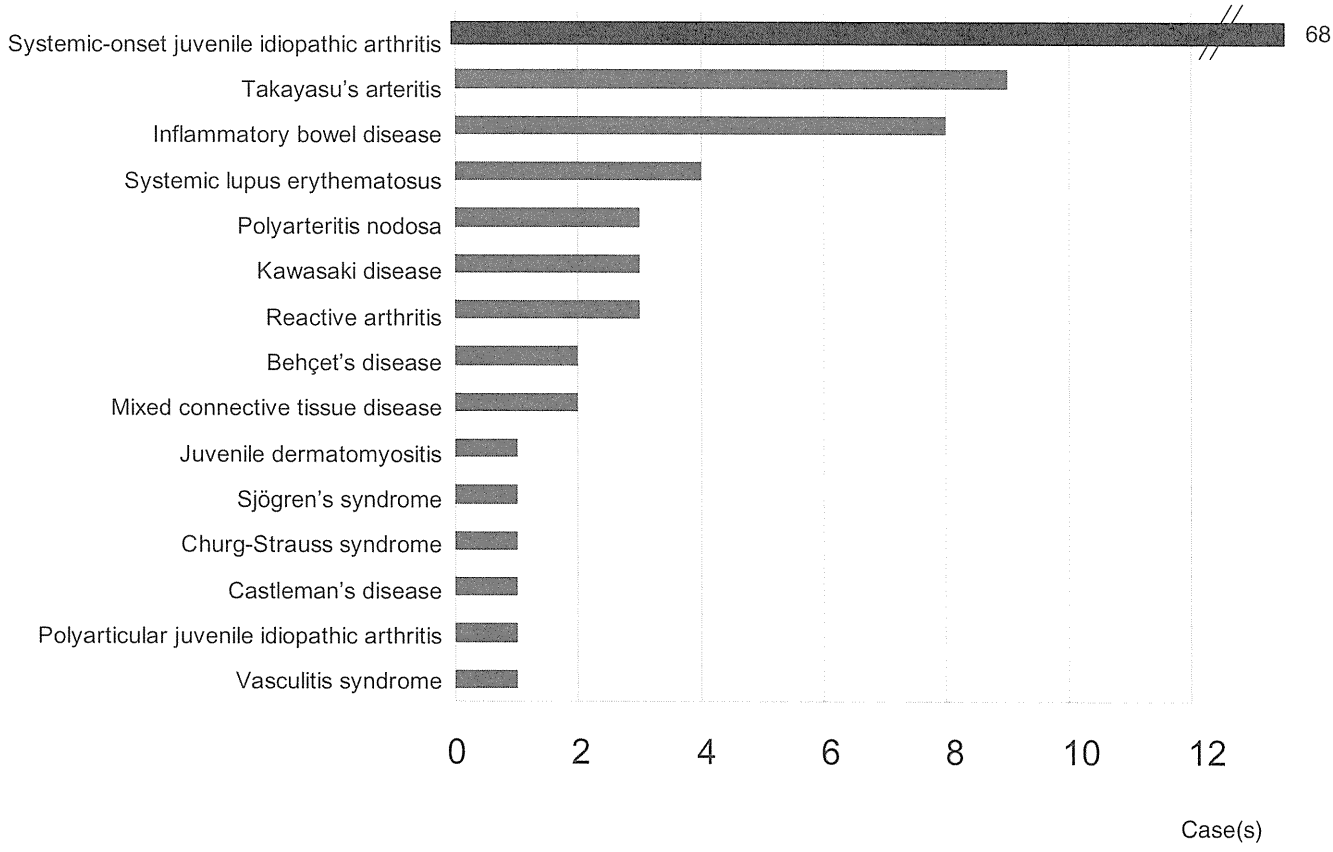


**Fig. 5** Classification of rheumatic diseases.

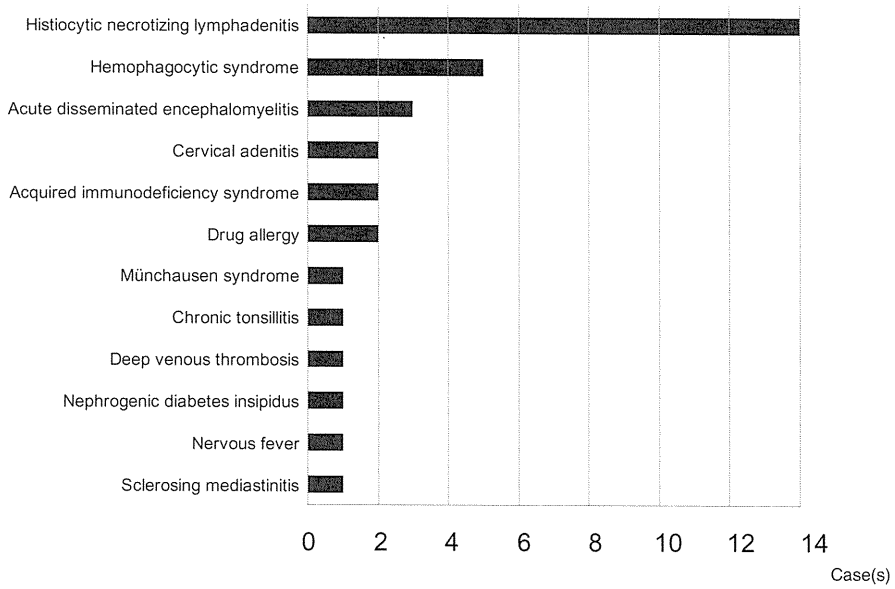


**Fig. 6** Classification of other diseases.

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.

## Acknowledgments

We are deeply indebted to pediatricians all over Japan who kindly contributed to this FUO survey.

## References

1 Petersdorf RG, Beeson P. Fever of unexplained origin: report on 100 cases. *Medicine* 1961; **40**: 1–30.
2 Dechovitz AB, Moffet HL. Classification of acute febrile illness in childhood. *Clin. Pediatr.* 1968; **7**: 649–53.
3 Chantada G, Casak S, Plata JD, Pociecha J, Bologna R. Children with fever of unknown origin in Argentina: An analysis of 113 cases. *Pediatr. Infect. Dis. J.* 1994; **13**: 260–3.
4 Miller ML, Szer I, Yogev R, Bernstein B. Fever of unknown origin. *Pediatr. Clin. North Am.* 1995; **42**: 999–1015.
5 Iikuni Y, Okada J, Kondo H, Kashiwazaki S. Current fever of unknown origin 1982–1992. *Intern. Med.* 1994; **33**: 67–73.
6 Goto M, Koyama H, Takahashi O, Fukui T. A retrospective review of 226 hospitalized patients with fever. *Intern. Med.* 2007; **46**: 17–22.
7 Jasper N, Däbritz J, Frosch M, Loeffler M, Weckesser M, Foell D. Diagnostic value of [18F]-FDG PET/CT in children with fever of unknown origin or unexplained signs of inflammation. *Eur. J. Nucl. Med. Mol. Imaging* 2010; **37**: 136–45.

# External Validation of a Risk Score to Predict Intravenous Immunoglobulin Resistance in Patients With Kawasaki Disease

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**Background:** We previously developed a new risk score to predict intravenous immunoglobulin (IVIG) resistance in Kawasaki disease. However, the IVIG dosage used in that study (1 g/kg/d for 2 consecutive days) differs from the single infusion of 2 g/kg recommended in the United States and elsewhere. Our aim was to assess the validity and applicability of our risk score in patients treated with a single infusion.

**Methods:** We used a database of 1626 patients with Kawasaki disease given initial IVIG treatment at a dose of 1 g/kg/d for 2 consecutive days (n = 990; IVIG- 1 g/kg × 2) or 2 g/kg/d for 1 day (n = 636; IVIG- 2 g/kg × 1) across 17 hospitals in Japan. Patients received the total IVIG dose within 36 hours in IVIG- 1 g/kg × 2 and 24 hours in IVIG- 2 g/kg × 1. We stratified the patients according to a risk scoring system developed to predict IVIG unresponsiveness, based on scores of ≥5 points. We compared the accuracy of prediction between the 2 groups using receiver operating characteristic analysis.

**Results:** Baseline characteristics and clinical outcomes were similar between both groups. The areas under the receiver operating characteristic curve in IVIG- 2 g/kg × 1 were similar to those of IVIG- 1 g/kg × 2. Using a cut-off risk score of ≥5 points, we could identify IVIG resistance in terms of coronary artery abnormalities within 1 month and coronary artery abnormalities at 1 month with equivalent sensitivity and specificity in both groups.

**Conclusion:** Our risk score can be used to predict IVIG unresponsiveness to a regimen based on a single infusion of 2 g/kg IVIG.

**Key Words:** coronary artery abnormalities, relapse, risk score, treatment failure

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Kawasaki disease (KD) is an acute febrile illness of children characterized by clinical, biochemical, and histopathologic manifestations of systemic vasculitis. Echocardiographic and cardiac angiographic data indicate that up to 25% of untreated KD patients develop coronary artery abnormalities (CAA).[1] High-dose intravenous immunoglobulin (IVIG) together with aspirin is effective for reducing the occurrence of CAA.[2] However, approximately 20% of patients KD did not become afebrile or developed recurrent fever,[3,4] and IVIG resistance is thought to increase the risk for CAA. Early identification of IVIG resistance requiring additional therapy might reduce the risk for coronary artery injury.

We previously developed a new risk score to predict IVIG resistance in patients with KD.[5] However, the IVIG dosage used in that study (1 g/kg/d for 2 consecutive days) differs from the single infusion of 2 g/kg over 1 day recommended in the United States and elsewhere. Thus, it is unclear whether our risk score is applicable to patients with KD who are treated with a single infusion of IVIG at 2 g/kg over 1 day. Therefore, the aim of this study was to assess the validity and applicability of our risk score for patients with KD who are treated with a single infusion of IVIG at 2 g/kg relative to those treated with 1 g/kg/d IVIG for 2 consecutive days.

## PATIENTS AND METHODS

### Patients

We reviewed retrospectively the clinical records of KD patients who were referred and admitted to 17 hospitals in Gunma, Saitama, Nagano, and Shimane prefectures, and metropolitan Tokyo, and confirmed diagnosis of KD between August 2000 and July 2009. This database of 1626 patients with KD was divided into 2 groups based on initial treatment, as follows: IVIG at a dose of 1 g/kg/d for 2 consecutive days (n = 990; IVIG- 1 g/kg × 2) or 2 g/kg/d for 1 day (n = 636; IVIG- 2 g/kg × 1). KD was diagnosed using the Diagnostic Guidelines for Kawasaki Disease (5th revised edition).[6] For KD, IVIG is administered via infusion at an initial rate of 30 to 60 mg/kg/h, which can be increased to 90 mg/kg/h if the patient shows no adverse effects. All patients received their total IVIG dose within 36 hours with IVIG- 1 g/kg × 2 and within 24 hours with IVIG- 2 g/kg × 1. Patients also received aspirin (30 mg/kg/d). The dose of aspirin was decreased to 5 mg/kg/d after normalization of C-reactive protein (CRP) levels. We excluded patients who presented with CAA before the initial treatment or who received steroids as part of the initial therapy.

IVIG resistance was defined as patients who were given additional rescue therapy because of persistent fever lasting for more than 24 hours after the end of the IVIG infusion, or recrudescent fever associated with KD symptoms despite an afebrile period after treatment. CAA was diagnosed when ultrasonographic examinations showed an internal lumen diameter of ≥3 mm in a child <5 years old or ≥4 mm in a child ≥5 years old, or if the internal diameter of a segment was at least 1.5 times as large as that of an adjacent segment.

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**TABLE 1.** Risk Score for Prediction of IVIG Resistance

| | Cutoff Value | Score Points |
|---|---|---|
| AST | ≥100 IU/L | 2 |
| Sodium | ≤133 mmol/L | 2 |
| Duration of illness before initial treatment | ≤4 (d) | 2 |
| Neutrophils | ≥80% | 2 |
| CRP | ≥10 mg/dL | 1 |
| Age | ≤12 mo | 1 |
| Platelet count | $\leq 30.0 \times 10^4/mm^3$ | 1 |

IVIG indicates intravenous immunoglobulin; AST, aspartate aminotransferase; CRP, C-reactive protein.

**TABLE 2.** Baseline Characteristics

| | IVIG-1 g/kg × 2 (n = 990) | IVIG-2 g/kg × 1 (n = 636) | *P* |
|---|---|---|---|
| Male | 564 (57.0) | 351 (55.2) | 0.086 |
| Age (mo) | 25 (1–137) | 22 (1–118) | 0.111 |
| Duration of illness before initial treatment (d) | 5 (1–13) | 5 (1–16) | 0.000 |
| White blood cell count ($\times 10^3/mm^3$) | 14.1 (4.1–38.1) | 14.7 (4.1–41.2) | 0.013 |
| Neutrophils (%) | 70 (14–95) | 70 (9–96) | 0.470 |
| Platelet count ($\times 10^4/mm^3$) | 32.7 (3.4–79.6) | 34.5 (4.6–365.2) | 0.002 |
| AST (IU/L) | 37 (13–1517) | 37 (5–1776) | 0.908 |
| Sodium, (mmol/L) | 135 (126–146) | 135 (122–143) | 0.568 |
| CRP (mg/dL) | 7.4 (0.5–40.3) | 7.0 (0.1–32.3) | 0.029 |
| Risk score (points) | 3 (1–10) | 3 (1–10) | 0.467 |

IVIG indicates intravenous immunoglobulin; AST, aspartate aminotransferase; CRP, C-reactive protein.

## Stratification Using a Risk Score

We stratified KD patients according to a risk scoring system developed to predict IVIG resistance.[5] This risk score was determined based on multiple logistic regression analysis of 750 consecutive patients with KD treated with IVIG. Seven variables were included in the risk score (Table 1). If a laboratory test was performed 2 or more times before primary therapy, the highest value was chosen for % neutrophils, aspartate aminotransferase (AST), and CRP, while the lowest value was chosen for platelet count and sodium. We considered risk scores of ≥5 points as high risk for IVIG resistance.

## Statistical Analyses

All analyses were carried out using SPSS software version 16.0J (SPSS Japan Inc, Tokyo). Data are presented as median (range) for continuous variables or as the percentage of patients for categorical variables. Categorical data were compared between IVIG- 1 g/kg × 2 and IVIG- 2 g/kg × 1 using Fisher exact test. Normally distributed continuous variables were analyzed using 2-sample *t* tests, whereas non-normally distributed variables were analyzed using Mann-Whitney *U* tests. Normality was determined with the Kolmogorov-Smirnov algorithm. Differences with 2-tailed *P* values <0.05 were considered significant. We compared the accuracy of prediction by receiver operating characteristic analysis.

# RESULTS

Table 2 shows the baseline characteristics of patients in both groups. The baseline characteristics and clinical outcomes of both groups were similar, except for the duration of illness at initial treatment, white blood cell count, platelet count, and CRP. The median risk scores were similar in both groups, being 3 (range: 1–10) in both groups. The percentage of patients with IVIG resistance, CAA within 1 month, and CAA at 1 month was also similar in both groups (Table 3).

**TABLE 3.** Clinical and Coronary Outcomes Among Both Groups

| | IVIG-1 g/kg × 2 (n = 990) | IVIG-2 g/kg × 1 (n = 636) | *P* |
|---|---|---|---|
| Treatment failure, n (%) | 227 (22.9) | 143 (22.5) | 0.86 |
| No response to initial treatment, n (%) | 197 (19.9) | 120 (18.9) | 0.65 |
| Relapse, n (%) | 30 (3.0) | 23 (3.6) | 0.57 |
| CAA within 1 month, n (%) | 68 (6.9) | 42 (6.6) | 0.92 |
| CAA at 1 month, n (%) | 32 (3.2) | 20 (3.1) | 1.00 |

IVIG indicates intravenous immunoglobulin; CAA, coronary artery abnormalities.

**TABLE 4.** Comparison of the Predictive Values in Both Groups

| | Area Under the ROC Curve (95% Confidence Interval) | Sensitivity (%) | Specificity (%) |
|---|---|---|---|
| IVIG-1 g/kg × 2 | | | |
| Resistance | 0.82 (0.78–0.85) | 73.6 | 79.1 |
| CAA within 1 month | 0.77 (0.71–0.83) | 75.0 | 70.1 |
| CAA at 1 month | 0.81 (0.73–0.89) | 81.3 | 68.3 |
| IVIG-2 g/kg × 1 | | | |
| Resistance | 0.80 (0.76–0.85) | 69.8 | 80.0 |
| CAA within 1 month | 0.78 (0.72–0.84) | 67.5 | 72.3 |
| CAA at 1 month | 0.81 (0.74–0.89) | 80.0 | 71.2 |

ROC indicates receiver operating characteristic; IVIG, intravenous immunoglobulin; CAA, coronary artery abnormalities.

Table 4 shows the predictive values for both groups. The area under the receiver operating characteristic curve for IVIG- 2 g/kg × 1 was similar to that of IVIG- 1 g/kg × 2 for each outcome. Using a cutoff risk score of ≥5 points, we can identify IVIG resistance, CAA within 1 month, and CAA at 1 month with equivalent sensitivity and specificity in IVIG- 1 g/kg × 2 and IVIG- 2 g/kg × 1.

# DISCUSSION

These findings indicate that our risk score can be applied to a regimen based on a single infusion of IVIG at 2 g/kg. The treatment regimen might affect the outcome, particularly the incidence of relapse, because the 1 g/kg regimen delays the time to complete treatment, as compared with the 2 g/kg regimen. However, our results are acceptable because the total dose of IVIG is equal in both groups, and the total IVIG dose was delivered within 36 hours in IVIG- 1 g/kg × 2 and 24 hours in IVIG- 2 g/kg × 1.

About 10% to 20% of patients have persistent or recurrent fever after completing IVIG plus aspirin therapy, while CAA, including transient dilation, occurs in about 10% of patients with KD, despite this therapy.[7] KD is the most common pediatric cause of acquired heart disease in developed countries. IVIG resistance is associated with a higher frequency of CAA compared with that in IVIG responders. Thus, predicting IVIG resistance may help predict the development of CAA. Accordingly, predicting IVIG resistance before initial therapy might help to prevent CAA. Our predictive models may also be valuable to guide decision-making in terms of initial therapy for patients with KD. Since our risk score can be calculated using the patient's background characteristics and laboratory results before starting initial therapy, this

clinical tool could help clinicians to identify high-risk KD patients, and thus reduce severe complications by early intervention. In addition, we can also use this information to predict which patients should be more closely followed up in terms of clinical signs and coronary changes on ultrasonography.

It is important to evaluate the treatment strategy after early identification of IVIG resistance using our risk score. Although IVIG infusion is the gold standard treatment for KD, other therapies have been proposed for patients with IVIG resistance, including repeated IVIG, corticosteroids, high-dose pulse methylprednisolone, cyclophosphamide, and infliximab.[3,8–10] Because IVIG resistance is usually identified 24 to 48 hours after completing the initial course of IVIG, rescue therapies are generally initiated 2 to 3 days after diagnosis of KD. Delaying the administration of rescue therapies may enable the development of CAA. Patients who need rescue therapy are at increased risk for CAA even if they have received effective additional therapy. Therefore, severe coronary complications might be avoided in high-risk patients by early initiation of intensive therapy.[11]

It is also important to verify the efficacy of more intensive first-line therapies, including the additional therapies used to prevent CAA in patients at high risk for IVIG resistance. Among patients with high risk scores for failure of IVIG monotherapy, those treated with IVIG in combination with corticosteroids, high-dose pulse methylprednisolone, cyclophosphamide, or infliximab were more likely to respond to therapy and avoid CAA. On the other hand, among patients at low risk for IVIG failure, the coronary and clinical outcomes were similar between patients treated with IVIG monotherapy and those treated with IVIG plus combination therapies.

There are several limitations that should be discussed. First, our initial dose of aspirin, although common in Japan, is lower than that used in the United States. Second, the Japanese Ministry of Health criteria used to diagnose CAA might underestimate the true incidence of CAA in patients with KD. Third, there were statistically significantly differences between the 2 groups in for some baseline characteristics. Thus, it is possible that these differences influenced our study outcomes.

It must also be acknowledged that this study was conducted in Japan and only included Japanese patients with KD. Tremoulet et al reported that another predictive model created in Japan by Egami et al showed poor performance to predict IVIG resistance in US population.[12] They also speculated that genetics play an important role in determining disease severity and outcome. Other background factors such as earlier diagnosis of KD compared with that in other countries, and the Japanese universal health insurance system might influence the assessment of reproducibility. Thus, further studies, including polymorphic allele analysis, are needed to confirm whether this risk score is applicable for patients in other countries.

In conclusion, we were able to apply our risk score to predict IVIG resistance to a regimen comprising a single infusion of 2 g/kg IVIG. We suggest that the treatment strategy for patients with KD should be evaluated after considering disease severity to prevent CAA. This risk score should encourage the early initiation of appropriate and effective therapy for high-risk KD.

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## REFERENCES

1. Kato H, Sugimura T, Akagi T, et al. Long-term consequences of Kawasaki disease. A 10- to 21-year follow-up study of 594 patients. *Circulation*. 1996;94:1379–1385.
2. Newburger JW, Takahashi M, Beiser AS, et al. A single intravenous infusion of gamma globulin as compared with four infusions in the treatment of acute Kawasaki syndrome. *N Engl J Med*. 1991;324:1633–1639.
3. Wallace CA, French JW, Kahn SJ, et al. Initial intravenous gammaglobulin treatment failure in Kawasaki disease. *Pediatrics*. 2000;105:E78.
4. Burns JC, Capparelli EV, Brown JA, et al. Intravenous gamma-globulin treatment and retreatment in Kawasaki disease. US/Canadian Kawasaki Syndrome Study Group. *Pediatr Infect Dis J*. 1998;17:1144–1148.
5. Kobayashi T, Inoue Y, Takeuchi K, et al. Prediction of intravenous immunoglobulin unresponsiveness in patients with Kawasaki disease. *Circulation*. 2006;113:2606–2612.
6. Ayusawa M, Sonobe T, Uemura S, et al. Revision of diagnostic guidelines for Kawasaki disease (5th revised edition). *Pediatr Int*. 2005;47:232–234.
7. Burns JC, Glode MP. Kawasaki syndrome. *Lancet*. 2004;364:533–544.
8. Sundel RP, Baker AL, Fulton DR, et al. Corticosteroids in the initial treatment of Kawasaki disease: report of a randomized trial. *J Pediatr*. 2003;142:611–616.
9. Shinohara M, Sone K, Tomomasa T, et al. Corticosteroids in the treatment of the acute phase of Kawasaki disease. *J Pediatr*. 1999;135:465–469.
10. Burns JC, Mason WH, Hauger SB, et al. Infliximab treatment for refractory Kawasaki syndrome. *J Pediatr*. 2005;146:662–667.
11. Kobayashi T, Inoue Y, Otani T, et al. Risk stratification in the decision to include prednisolone with intravenous immunoglobulin in primary therapy of Kawasaki disease. *Pediatr Infect Dis J*. 2009;28:498–502.
12. Tremoulet AH, Best BM, Song S, et al. Resistance to intravenous immunoglobulin in children with Kawasaki disease. *J Pediatr*. 2008;153:117–121.

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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 ($\times 10^5$) | 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) |

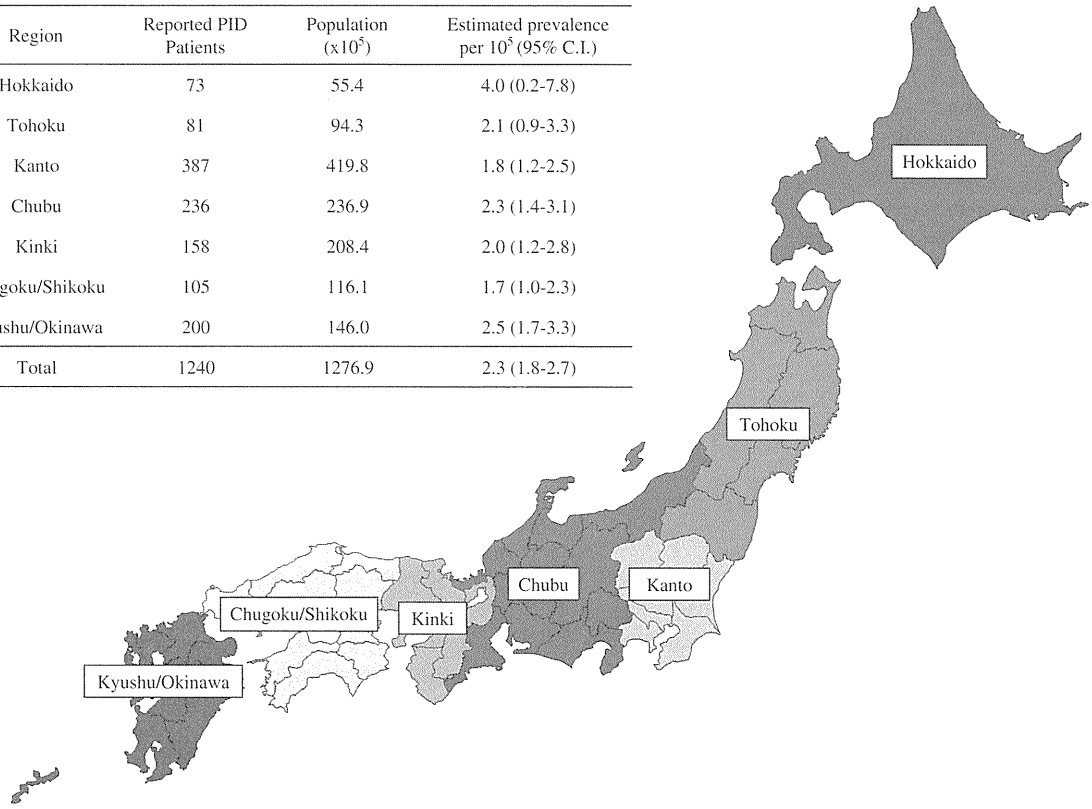


**Fig. 1** Regional distribution of PID patients. *CI* Confidence interval

**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

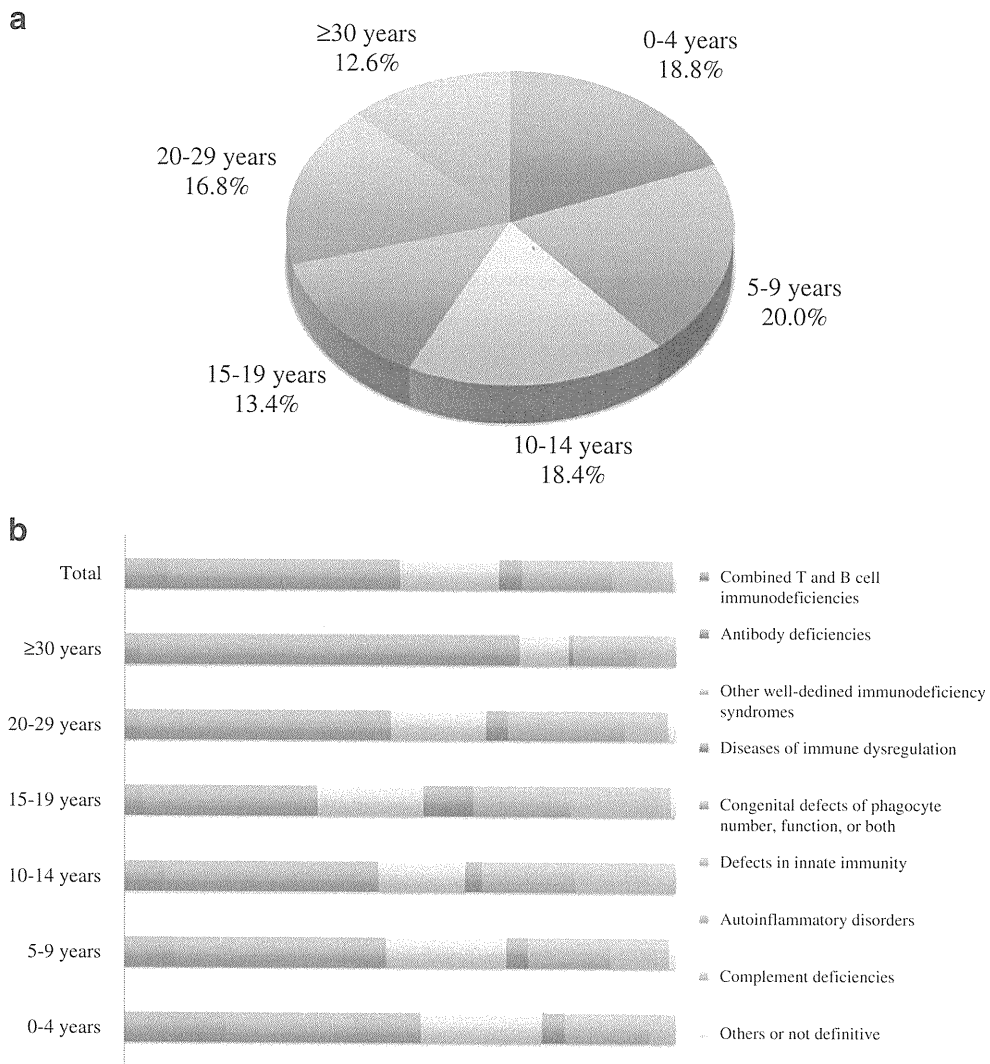


**Fig. 2** **a** Age distribution of PID patients. **b** Distribution of PID in each age group

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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## References

1. Notarangelo LD. Primary immunodeficiencies. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S182–94.
2. Geha RS, Notarangelo LD, Casanova JL, Chapel H, Conley ME, Fischer A, et al. Primary immunodeficiency diseases: an update from the international union of immunological societies primary immunodeficiency diseases classification committee. J Allergy Clin Immunol. 2007;120(4):776–94.
3. Hayakawa H, Iwata T, Yata J, Kobayashi N. Primary immunodeficiency syndrome in Japan. I. overview of a nationwide survey on primary immunodeficiency syndrome. J Clin Immunol. 1981;1 (1):31–9.
4. Primary Immunodeficiency Database in Japan (PIDJ). http://pidj.rcai.riken.jp/ (in Japanese).
5. CEREDIH. The French PID study group. The French national registry of primary immunodeficiency diseases. Clin Immunol. 2010;135(2):264–72.
6. Al-Herz W. Primary immunodeficiency disorders in Kuwait: first report from Kuwait national primary immunodeficiency registry (2004–2006). J Clin Immunol. 2008;28(2):186–93.
7. Stray-Pedersen A, Abrahamsen TG, Froland SS. Primary immunodeficiency diseases in Norway. J Clin Immunol. 2000;20(6):477–85.
8. Nakamura Y, Matsumoto T, Tamakoshi A, Kawamura T, Seino Y, Kasuga M, et al. Prevalence of idiopathic hypoparathyroidism and pseudohypoparathyroidism in Japan. J Epidemiol. 2000;10(1):29–33.
9. Toyoda H, Ido M, Nakanishi K, Nakano T, Kamiya H, Matsumine A, et al. Multiple cutaneous squamous cell carcinomas in a patient with interferon gamma receptor 2 (IFN gamma R2) deficiency. J Med Genet. 2010;47(9):631–4.
10. Takaya J, Fujii Y, Higashino H, Taniuchi S, Nakamura M, Kaneko K. A case of WHIM syndrome associated with diabetes and hypothyroidism. Pediatr Diabetes. 2009;10(7):484–6.
11. Ida H, Kawasaki E, Miyashita T, Tanaka F, Kamachi M, Izumi Y, et al. A novel mutation (T61I) in the gene encoding tumour necrosis factor receptor superfamily 1A (TNFRSF1A) in a Japanese patient with tumour necrosis factor receptor-associated periodic syndrome (TRAPS) associated with systemic lupus erythematosus. Rheumatology (Oxford). 2004;43(10):1292–9.
12. Lim DL, Thong BY, Ho SY, Shek LP, Lou J, Leong KP, et al. Primary immunodeficiency diseases in Singapore—the last 11 years. Singapore Med J. 2003;44(11):579–86.
13. Lee WI, Kuo ML, Huang JL, Lin SJ, Wu CJ. Distribution and clinical aspects of primary immunodeficiencies in a Taiwan pediatric tertiary hospital during a 20-year period. J Clin Immunol. 2005;25(2):162–73.
14. Lee WI, Huang JL, Jaing TH, Shyur SD, Yang KD, Chien YH, et al. Distribution, clinical features and treatment in Taiwanese patients with symptomatic primary immunodeficiency diseases (PIDs) in a nationwide population-based study during 1985–2010. Immunobiology. 2011 Jun 21 [Epub ahead of print].
15. Shabestari MS, Maljaei SH, Baradaran R, Barzegar M, Hashemi F, Mesri A, et al. Distribution of primary immunodeficiency diseases in the Turk ethnic group, living in the northwestern Iran. J Clin Immunol. 2007;27(5):510–6.
16. Matamoros Flori N, Mila Llambi J, Espanol Boren T, Raga Borja S, Fontan Casariego G. Primary immunodeficiency syndrome in Spain: first report of the national registry in children and adults. J Clin Immunol. 1997;17(4):333–9.
17. Gathmann B, Grimbacher B, Beaute J, Dudoit Y, Mahlaoui N, Fischer A, et al. The European internet-based patient and research database for primary immunodeficiencies: results 2006–2008. Clin Exp Immunol. 2009;157 Suppl 1:3–11.
18. Toth B, Volokha A, Mihas A, Pac M, Bernatowska E, Kondratenko I, et al. Genetic and demographic features of X-linked agammaglobulinemia in Eastern and Central Europe: a cohort study. Mol Immunol. 2009;46(10):2140–6.
19. Burrows PD, Fischer A. Building networks for immunodeficiency diseases and immunology training. Nat Immunol. 2008;9(9):1005–7.
20. Valiaho J, Smith CI, Vihinen M. BTKbase: the mutation database for X-linked agammaglobulinemia. Hum Mutat. 2006;27 (12):1209–17.
21. van den Berg JM, van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L, et al. Chronic granulomatous disease: the European experience. PLoS One. 2009;4(4):e5234.
22. Hasui M. Chronic granulomatous disease in Japan: incidence and natural history. The study group of phagocyte disorders of Japan. Pediatr Int. 1999;41(5):589–93.
23. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. Blood. 2008;112(2):277–86.
24. Kanoh T, Mizumoto T, Yasuda N, Koya M, Ohno Y, Uchino H, et al. Selective IgA deficiency in Japanese blood donors: frequency and statistical analysis. Vox Sang. 1986;50(2):81–6.
25. Aghamohammadi A, Moin M, Farhoudi A, Rezaei N, Pourpak Z, Movahedi M, et al. Efficacy of intravenous immunoglobulin on the prevention of pneumonia in patients with agammaglobulinemia. FEMS Immunol Med Microbiol. 2004;40(2):113–8.
26. Quartier P, Debre M, De Blic J, de Sauverzac R, Sayegh N, Jabado N, et al. Early and prolonged intravenous immunoglobulin replacement therapy in childhood agammaglobulinemia: a retrospective survey of 31 patients. J Pediatr. 1999;134(5):589–96.
27. Sakata N, Kawa K, Kato K, Yabe H, Yabe M, Nagasawa M, et al. Unrelated donor marrow transplantation for congenital immunodeficiency and metabolic disease: an update of the experience of the Japan marrow donor program. Int J Hematol. 2004;80(2):174–82.
28. Morio T, Atsuta Y, Tomizawa D, Nagamura-Inoue T, Kato K, Ariga T, et al. Outcome of unrelated umbilical cord blood transplantation in 88 patients with primary immunodeficiency in Japan. Br J Haematol. 2011;154(3):363–72.
29. Vajdic CM, Mao L, van Leeuwen MT, Kirkpatrick P, Grulich AE, Riminton S. Are antibody deficiency disorders associated with a narrower range of cancers than other forms of immunodeficiency? Blood. 2010;116(8):1228–34.
30. Bussone G, Mouthon L. Autoimmune manifestations in primary immune deficiencies. Autoimmun Rev. 2009;8(4):332–6.
31. Arason GJ, Jorgensen GH, Ludviksson BR. Primary immunodeficiency and autoimmunity: lessons from human diseases. Scand J Immunol. 2010;71(5):317–28.
32. Resource of Asian primary immunodeficiency diseases (RAPID). http://rapid.rcai.riken.jp/RAPID/.

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# Defective IL-10 signaling in hyper-IgE syndrome results in impaired generation of tolerogenic dendritic cells and induced regulatory T cells

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**Hyper-IgE syndrome (HIES) is a primary immunodeficiency characterized by recurrent staphylococcal infections and atopic dermatitis associated with elevated serum IgE levels. Although defective differentiation of IL-17–producing CD4$^+$ T cells (Th17) partly accounts for the susceptibility to staphylococcal skin abscesses and pneumonia, the pathogenesis of atopic manifestations in HIES still remains an enigma. In this study, we examined the differentiation and function of Th1, Th2, regulatory T cells ($T_{reg}$ cells), and dendritic cells (DCs) in HIES patients carrying either *STAT3* or *TYK2* mutations. Although the in vitro differentiation of Th1 and Th2 cells and the number and function of $T_{reg}$ cells in the peripheral blood were normal in HIES patients with *STAT3* mutations, primary and monocyte-derived DCs showed defective responses to IL-10 and thus failed to become tolerogenic. When treated with IL-10, patient DCs showed impaired up-regulation of inhibitory molecules on their surface, including PD-L1 and ILT-4, compared with control DCs. Moreover, IL-10–treated DCs from patients displayed impaired ability to induce the differentiation of naive CD4$^+$ T cells to FOXP3$^+$ induced $T_{reg}$ cells ($iT_{reg}$ cells). These results suggest that the defective generation of IL-10–induced tolerogenic DCs and $iT_{reg}$ cells may contribute to inflammatory changes in HIES.**

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Abbreviations used: cDC, conventional DC; DN, dominant-negative; HIES, hyper-IgE syndrome; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked; $iT_{reg}$ cell, induced $T_{reg}$ cell; MoDC, monocyte-derived DC; mRNA, messenger RNA; $nT_{reg}$ cell, natural $T_{reg}$ cell; pDC, plasmacytoid DC; Q-PCR, quantitative RT-PCR.

Hyper-IgE syndrome (HIES) is a rare complex primary immunodeficiency, characterized by atopic dermatitis, extremely high serum IgE levels, staphylococcal skin abscesses, and pneumonia associated with disproportionately mild inflammatory responses (Grimbacher et al., 2005; Minegishi, 2009). Treatments so far are symptomatic, including the prevention of bacterial and fungal infections and management of eczema. Previous studies suggested the benefit from bone marrow transplantation, Ig replacement, and IFN and G-CSF administration (Grimbacher et al., 2005), but a general role for immune reconstitution and modulation in HIES is unproven. To improve the long-term quality of life of HIES patients, it is necessary to develop a new treatment strategy based on a better understanding of molecular mechanisms of this syndrome. We recently demonstrated that



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most cases of HIES are caused by dominant-negative (DN) mutations of the *STAT3* gene (Holland et al., 2007; Minegishi et al., 2007). However, the pathogenesis of this syndrome remains unclear. In particular, the molecular mechanisms underlying the allergic manifestations, including atopic dermatitis and extremely high serum IgE levels, remain one of the great enigmas in the pathogenesis of this syndrome.

STAT3 is a transcription factor that binds to the promoter regions of various genes, including those encoding acute-phase proteins. STAT3 plays a critical role in signal transduction for many cytokines, including those of the γc family (IL-2, IL-7, IL-9, IL-15, and IL-21), the gp130 family (IL-6, IL-11, IL-27, and IL-31), the IL-10 family (IL-10 and IL-22), and receptor-type tyrosine kinases. The systemic deletion of STAT3 in mice is lethal, but studies involving the tissue-specific deletion of STAT3 have demonstrated that STAT3 plays a critical role in cell migration, survival, proliferation, apoptosis, inflammation, and tumorigenesis in many tissues (Akira, 2000). Furthermore, recent data unanimously demonstrated that STAT3 plays an essential role for Th17 cell development in humans (de Beaucoudrey et al., 2008; Ma et al., 2008; Milner et al., 2008; Renner et al., 2008; Minegishi et al., 2009), which could explain, at least in part, why HIES patients suffer from recurrent staphylococcal infections confined to the skin and lung (Minegishi et al., 2009).

Allergic diseases may result from an inappropriate balance between effector Th2 cells and $T_{reg}$ cells (Umetsu and DeKruyff, 2006; Akdis and Akdis, 2009; Lloyd and Hawrylowicz, 2009). Th2 cells respond to allergens and produce IL-4, IL-5, IL-9, and IL-13. Th2 cytokines induce changes in blood vessels that lead to the up-regulation of intercellular adhesion molecule 1 and vascular cell-adhesion molecule 1, in turn leading to the recruitment of very late antigen 4–expressing eosinophils. These factors also induce the survival and activation of eosinophils. In addition, IL-4 and IL-13 are responsible for promoting Ig class switching to IgE (Hammad and Lambrecht, 2008). Newly identified cytokines such as IL-25, IL-31, and IL-33 also participate in Th2 cell–mediated inflammation (Dillon et al., 2004; Wang et al., 2007; Kakkar and Lee, 2008). Th1 cells may also contribute to allergic inflammation by inducing the apoptosis of epithelial cells in atopic dermatitis (Trautmann et al., 2000).

$T_{reg}$ cells are key mediators of peripheral tolerance that actively suppress effector T cells and inhibit immune response–mediated tissue damage. Both $FOXP3^+$ $T_{reg}$ cells and IL-10–producing $FOXP3^-$ $T_{reg}$ cells play an essential role in the regulation of allergic inflammation (Curotto de Lafaille et al., 2001; Zheng and Rudensky, 2007; Sakaguchi et al., 2008). There are two types of $FOXP3^+$ $T_{reg}$ cells: natural $T_{reg}$ cells ($nT_{reg}$ cells) and induced $T_{reg}$ cells ($iT_{reg}$ cells). $nT_{reg}$ cells develop in the thymus, whereas $iT_{reg}$ cells develop in the periphery. In the presence of TGF-β1, naive $FOXP3^-$ $CD4^+$ T cells are converted into $FOXP3^+$ $iT_{reg}$ cells (Chen et al., 2003; Coombes et al., 2007; Rubtsov and Rudensky, 2007; Zheng et al., 2007). Mutations in the human *FOXP3* gene result in immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome (Bennett et al., 2001; Wildin et al., 2001). Patients with IPEX syndrome suffer from enteropathy, autoimmune diabetes and thyroiditis, food allergy, and atopic dermatitis with extremely high serum IgE levels. FOXP3 deficiency in mice also leads to atopic manifestations (Fontenot et al., 2003; Lin et al., 2005).

DCs are central to the orchestration of the various types of immunity and tolerance (Banchereau et al., 2000; Kapsenberg, 2003; Steinman et al., 2003). Immature DCs function as sentinels in the periphery, undergoing terminal differentiation in response to various danger signals. Maturing DCs migrate to the lymph nodes, where they acquire potent antigen-presenting capacity and induce vigorous T cell responses by expressing co-stimulatory molecules and secreting large amounts of proinflammatory cytokines. The interaction between DCs and naive $CD4^+$ T cells is considered to determine the fate of $CD4^+$ T cells. Cytokines produced by DCs, such as IL-12 and IFN-α, may bias $CD4^+$ T cell priming toward the Th1 pathway (Schulz et al., 2000). Notch ligands, such as Jagged 1, expressed by DCs may promote $CD4^+$ T cells toward the Th2 pathway (Amsen et al., 2009). In addition, DCs play a key role in the induction and maintenance of peripheral T cell tolerance (Steinman et al., 2003; Rutella et al., 2006).

We investigated the molecular mechanism underlying the atopic manifestations in HIES by studying Th1–Th2–$T_{reg}$ cell balance and the development and function of primary and monocyte-derived DCs (MoDCs). The results suggest that IL-10 signaling by DCs may be crucial for the generation of tolerogenic DCs and $iT_{reg}$ cells for the maintenance of an appropriate Th1–Th2–$T_{reg}$ cell balance in vivo in humans.

## RESULTS

### Normal Th1 and Th2 differentiation from naive $CD4^+$ T cells but increased Th2 cytokine production from activated T cells in PBMCs of *STAT3* patients

We first evaluated Th1 and Th2 cell development of naive $CD4^+$ T cells in *STAT3* patients. Naive $CD4^+$ T cells were unstimulated or stimulated with anti-CD3 and anti-CD28 (anti-CD3/CD28) mAbs under neutral, Th1, and Th2 differentiation conditions, and the development of IFN-γ– and IL-4–producing cells was evaluated by cytoplasmic staining and flow cytometry. The development of Th1 and Th2 cells was similar in control subjects and *STAT3* patients (Fig. S1 A). This observation was confirmed by ELISA of the culture supernatants of naive $CD4^+$ T cells, showing similar levels of IFN-γ, IL-5, and IL-13 secretion for control subjects and *STAT3* patients (Fig. S1 B). We next evaluated Th1 and Th2 cytokine production from PBMCs after stimulation with anti-CD3/CD28 mAbs. The production of IFN-γ was equivalent between control subjects and *STAT3* patients, but the production of IL-5 and IL-13 was increased in *STAT3* patients compared with control subjects (Fig. S1 C). These results suggest that cells in PBMCs other than naive $CD4^+$ T cells are likely to be responsible for increased Th2 cytokine production in *STAT3* patients.

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### The number and suppressive activity of $T_{reg}$ cells in the peripheral blood are normal in *STAT3* patients

We next evaluated the number of FOXP3$^+$ $T_{reg}$ cells among PBMCs because STAT3 is involved in the transduction of IL-6 and IL-21 signals, which may influence the balance between $nT_{reg}$ cell and Th17 cell differentiation (Harrington et al., 2005; Bettelli et al., 2006; Ivanov et al., 2006; Veldhoen et al., 2006). Similar numbers of PBMCs were obtained from control subjects and *STAT3* patients, and these cells were stained for extracellular CD4 and CD25 and intracellular FOXP3 and evaluated by flow cytometry. The percentages of CD4$^+$CD25$^+$ cells and CD4$^+$FOXP3$^+$ cells did not differ significantly between control subjects and *STAT3* patients (Fig. 1 A).

We then investigated the function of $T_{reg}$ cells in the peripheral blood ex vivo. CD4$^+$CD25$^+$CD62L$^+$ $T_{reg}$ cells were obtained from the peripheral blood of control subjects and *STAT3* patients at a purity of >99% and were co-cultured with autologous CD4$^+$CD25$^-$CD62L$^+$ responder T cells in the presence or absence of anti-CD3/CD28 mAbs. The addition of $1.25 \times 10^3$ control $T_{reg}$ cells to the $1.25 \times 10^4$ control responder T cells resulted in levels of [$^3$H]thymidine incorporation 55% lower than those obtained after the addition of $1.25 \times 10^3$ control responder T cells. Levels of [$^3$H]thymidine incorporation were similarly lowered by the addition of $1.25 \times 10^3$ patient $T_{reg}$ cells to the $1.25 \times 10^4$ patient responder T cells (Fig. 1 B). Modification of the ratio of $T_{reg}$ cells to responder T cells from 1:1 ($T_{reg}$ cell/responder T cell) to 1:100 resulted in no significant difference in the percent suppression of [$^3$H]thymidine incorporation between control subjects and *STAT3* patients (Fig. 1 C). These results indicate that the in vivo generation and ex vivo function of $T_{reg}$ cells were normal in *STAT3* patients.

### Defective IL-10 signaling in MoDCs from *STAT3* patients

We next evaluated the generation of MoDCs in vitro. Isolated CD14$^+$ monocytes from the PBMCs of control subjects and *STAT3* patients were cultured with GM-CSF and IL-4 for 5 d and then allowed to mature in the presence of LPS for 2 d. MoDC differentiation was normal, as shown by evaluations of the forward and side light scatter of the cells (Fig. S2 A), the expression levels of CD1a (Fig. S2 B), CD80, CD83, and CD86 (Fig. S2 C), and FITC-dextran uptake (Fig. S2 D). Of note, levels of CD86 expression before LPS stimulation were slightly higher in *STAT3* patients than in control subjects (Fig. S2 C), suggesting that autocrine IL-10 may regulate the expression of DC maturation markers in control subjects but not in *STAT3* patients (Corinti et al., 2001).

We have previously demonstrated that *STAT3* plays an important role in IL-10 signal transduction in human monocyte-derived macrophages (Minegishi et al., 2007). We therefore investigated IL-10 signal transduction in MoDCs. Consistent with our previous findings, the transcriptional

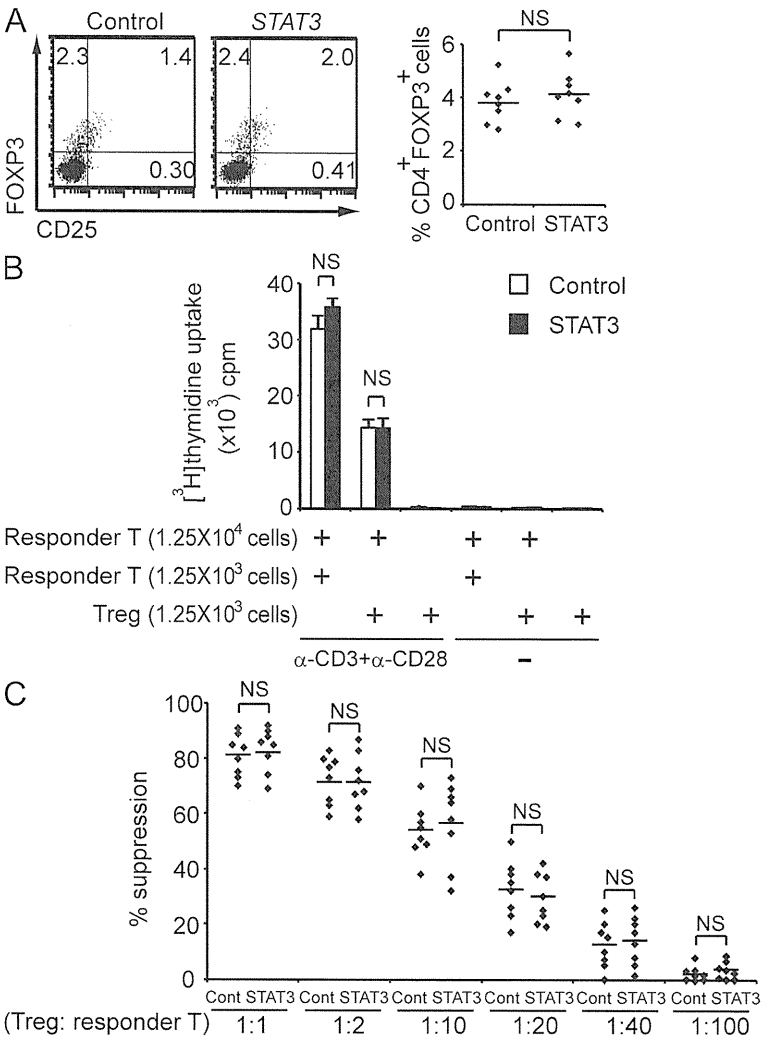


**Figure 1. The number and suppressive activity of $T_{reg}$ cells in the peripheral blood are normal in *STAT3* patients.** (A, left) Representative dot plots gated on CD4$^+$ peripheral blood T cells from a control subject and a *STAT3* patient. (right) Summary data from eight control subjects and eight *STAT3* patients showing percentages of CD4$^+$FOXP3$^+$ cells. Data are representative of at least two independent experiments. (B) CD4$^+$CD25$^-$CD62L$^+$ responder T cells and CD4$^+$CD25$^+$CD62L$^+$ $T_{reg}$ cells were isolated by cell sorting. Responder T cells and $T_{reg}$ cells were co-cultured as indicated for 5 d with or without a 1:100 (vol/vol) dilution of anti-CD3 + anti-CD28 mAb-coated beads. For the evaluation of proliferation, 1 µCi (37 kBq) [$^3$H]thymidine was added to the culture medium for the last 18 h. Graph shows mean ± SD. (C) CD4$^+$CD25$^-$CD62L$^+$ responder T cells and CD4$^+$CD25$^+$CD62L$^+$ $T_{reg}$ cells were isolated and cultured as in B at the indicated ratio. Summary data ($n$ = 8 each) are shown. Data are representative of at least two independent experiments performed in triplicate. (A and C) Horizontal bars indicate mean values.