Table 5 | Continued

Disease	Affected cells	Affected function	Associated features	Inheritance	Genetic defect/presumed pathogenesis	OMIM number
GATA2 deficiency (Mono MAC Syndrome)	Monocytes peripheral DC + NK + B	Multilineage cytopenias	Susceptibility to *Mycobacteria*, Papilloma Viruses, Histoplasmosis, Alveolar proteinosis, MDS/AML/CMML	AD	*GATA2*: loss of stem cells	137295
Pulmonary alveolar proteinosis*	Alveolar macrophages	GM-CSF signaling	Alveolar proteinosis	Biallelic mutations in pseudoautosomal gene	*CSF2RA*	306250

XL, X-linked inheritance; AR, autosomal recessive inheritance; AD, autosomal dominant inheritance; ACTB, actin beta; B, B-lymphocytes; CEBPE, CCAAT/enhancer-binding protein epsilon; CMML, chronic myelomonocytic leukaemia; CTSC, cathepsin C; CYBA, cytochrome b alpha subunit; CYBB, cytochrome b beta subunit; DC, dendritic cells; ELANE, elastase neutrophil-expressed; GATA2, GATA binding protein 2; IFN, interferon; IFNGR1, interferon-gamma receptor subunit 1; IFNGR2, interferon-gamma receptor subunit 2; IL12B, interleukin-12 beta subunit; IL12RB1, interleukin-12 receptor beta 1; IFR8, interferon regulatory factor 8; F, fibroblasts; FPR1, formyl peptide receptor 1; FUCT1, fucose transporter 1; GFI1, growth factor independent 1; HAX1, HLCS1-associated protein X1; ITGB2, integrin beta-2; L, lymphocytes; M, monocytes–macrophages; MDC, myeloid dendritic cells; MDS, myelodysplasia; Mel, melanocytes; Mϕ, macrophages; MSMD, Mendelian susceptibility to mycobacterial disease; N, neutrophils; NCF1, neutrophil cytosolic factor 1; NCF2, neutrophil cytosolic factor 2; NCF4, neutrophil cytosolic factor 4; NK, natural killer cells; ROBLD3, roadblock domain containing 3; SBDS, Shwachman–Bodian–Diamond syndrome; STAT, signal transducer and activator of transcription.

**Ten or fewer unrelated cases reported in the literature.*

***Table 5** includes seven newly described genetic defects of phagocyte number and/or function including Barth syndrome, Cohen syndrome and Poikiloderma with neutropenia. In these three clinically well-known diseases the genetic defects have been elucidated, although their molecular pathogenesis remains ill-defined. A new cause of autosomal recessive chronic granulomatous disease, namely a deficiency of the cytosolic activating protein p40 phox, has now been found in two CGD patients and is included under defects of respiratory burst. Under the heading of Mendelian susceptibility of mycobacterial disease (MSMD) two new entities were added: a) a subgroup of X-linked gp91 phox deficiency with isolated susceptibility to mycobacteria and a defect of the respiratory burst in macrophages only; b) an autosomal dominant form of IRF8 deficiency, resulting from a lack of CD1c+ myeloid dendritic cells that would normally secrete IL12. The clinical phenotype of MSMD may vary, depending on the nature of the genetic defect. Finally GATA2 deficiency was recently identified as the cause of the Mono MAC syndrome, with multilineage cytopenias (of monocytes, peripheral dendritic cells, NK- and B-lymphocytes) resulting in opportunistic infections (including mycobacteria), alveolar proteinosis and malignancy.*

Table 6 | Defects in innate immunity.

Disease	Affected cell	Functional defect	Associated features	Inheritance	Genetic defect/presumed pathogenesis	OMIM number
ANHIDROTIC ECTODERMAL DYSPLASIA WITH IMMUNODEFICIENCY (EDA-ID)						
EDA-ID, X-linked (NEMO deficiency)	Lymphocytes + monocytes	NFκB signaling pathway	Anhidrotic ectodermal dysplasia + specific antibody deficiency (lack of Ab response to polysaccharides) + various infections (mycobacteria and pyogenes)	XL	Mutations of NEMO (*IKBKG*), a modulator of NF-κB activation	300291, 300584, 300301
EDA-ID, autosomal dominant*	Lymphocytes + monocytes	NFκB signaling pathway	Anhidrotic ectodermal dysplasia + T cell defect + various infections	AD	Gain-of-function mutation of *IKBA*, resulting in impaired activation of NF-κB	612132
IRAK4 deficiency	Lymphocytes + monocytes	TIR-IRAK signaling pathway	Bacterial infections (pyogenes)	AR	Mutation of *IRAK4*, a component of TLR- and IL-1R-signaling pathway	607676
MyD88 deficiency	Lymphocytes + monocytes	TIR-MyD88 signaling pathway	Bacterial infections (pyogenes)	AR	Mutation of *MYD88*, a component of the TLR and IL-1R-signaling pathway	612260
WHIM (warts, hypogammaglobulinemia, infections, myelokathexis) syndrome	Granulocytes + lymphocytes	Increased response of the CXCR4 chemokine receptor to its ligand CXCL12 (SDF-1)	Hypogammaglobulinemia, reduced B cell number, severe reduction of neutrophil count, warts/HPV infection	AD	Gain-of-function mutations of *CXCR4*, the receptor for CXCL12	193670
Epidermodysplasia verruciformis	Keratinocytes and leukocytes		Human Papilloma virus (group B1) infections and cancer of the skin	AR	Mutations of *EVER1*, *EVER2*	226400
HERPES SIMPLEX ENCEPHALITIS (HSE)*						
TLR3 deficiency*	Central nervous system (CNS) resident cells and fibroblasts	TLR3-dependent IFN-α, -β, and -λ induction	Herpes simplex virus 1 encephalitis	AD	Mutations of *TLR3*	613002
UNC93B1 deficiency	CNS resident cells and fibroblasts	UNC-93B-dependent IFN-α, -β, and -λ induction	Herpes simplex virus 1 encephalitis	AR	Mutations of *UNC93B1*	610551
TRAF3 deficiency	CNS resident cells and fibroblasts	TRAF3-dependent IFN-α, -β, and -λ induction	Herpes simplex virus 1 encephalitis	AD	Mutation of *TRAF3*	
Predisposition to fungal diseases*	Mononuclear phagocytes	CARD9 signaling pathway	Invasive candidiasis and peripheral dermatophytosis	AR	Mutations of *CARD9*	212050
CHRONIC MUCOCUTANEOUS CANDIDIASIS (CMC)						
IL-17RA deficiency*	Epithelial cells, fibroblasts, mononuclear phagocytes	IL-17RA signaling pathway	CMC	AR	Mutation in *IL-17RA*	605461
IL-17F deficiency*	T cells	IL-17F-containing dimers	CMC	AD	Mutation in *IL-17F*	606496

(Continued)

Table 6 | Continued

Disease	Affected cell	Functional defect	Associated features	Inheritance	Genetic defect/presumed pathogenesis	OMIM number
STAT1 gain-of-function	T cells	Gain-of-function STAT1 mutations that impair the development of IL-17-producing T cells	CMC	AD	Mutations in *STAT1*	Not in OMIM yet
Trypanosomiasis*		APOL-I	Trypanosomiasis	AD	Mutation in *APOL-I*	603743

XL, X-linked inheritance; AR, autosomal recessive inheritance; AD, autosomal dominant inheritance; NF-κB, nuclear factor κ B; TIR, toll and interleukin 1 receptor; IFN, interferon; HP, human papilloma virus; TLR, toll-like receptor; IL: interleukin.

**Ten or fewer unrelated cases reported in the literature.*

*Four new disorders have been added to **Table 6**. AD TRAF3 deficiency is a new genetic etiology of HSE that has been diagnosed in a single patient. A new entry in the Table is CMC, for which three genetic etiologies have been discovered. AR IL-17RA deficiency and AD IL-17F deficiency have been found in one kindred each. Gain-of-function mutations in STAT1 have been found in over 50 patients with AD CMC. The mechanism of CMC in these patients involves impaired development of IL-17-producing T cells, due to the hyperactivity of STAT1-dependent signals.*

Table 7 | Autoinflammatory disorders.

Disease	Affected cells	Functional defects	Associated Features	Inheritance	Genetic defect/ presumed pathogenesis	OMIM number
DEFECTS EFFECTING THE INFLAMMASOME						
Familial Mediterranean fever	Mature granulocytes, cytokine-activated monocytes	Decreased production of pyrin permits ASC-induced IL-1 processing and inflammation following subclinical serosal injury; macrophage apoptosis decreased	Recurrent fever, serositis and inflammation responsive to colchicine. Predisposes to vasculitis and inflammatory bowel disease	AR	Mutations of *MEFV*	249100
Hyper IgD syndrome		Mevalonate kinase deficiency affecting cholesterol synthesis; pathogenesis of disease unclear	Periodic fever and leukocytosis with high IgD levels	AR	Mutations of *MVK*	260920
Muckle–Wells syndrome	PMNs monocytes	Defect in cryopyrin, involved in leukocyte apoptosis and NFkB signaling and IL-1 processing	Urticaria, SNHL, amyloidosis	AD	Mutations of *CIAS1* (also called *PYPAF1 or NALP3*)	191900
Familial cold autoinflammatory syndrome	PMNs, monocytes	same as above	Non-pruritic urticaria, arthritis, chills, fever, and leukocytosis after cold exposure	AD	Mutations of *CIAS1* Mutations of *NLRP12*	120100
Neonatal onset multisystem inflammatory disease (NOMID) or chronic infantile neurologic cutaneous and articular syndrome (CINCA)	PMNs, chondrocytes	same as above	Neonatal onset rash, chronic meningitis, and arthropathy with fever and inflammation	AD	Mutations of *CIAS1*	607115
NON-INFLAMMASOME-RELATED CONDITIONS						
TNF receptor-associated periodic syndrome (TRAPS)	PMNs, monocytes	Mutations of 55-kD TNF receptor leading to intracellular receptor retention or diminished soluble cytokine receptor available to bind TNF	Recurrent fever, serositis, rash, and ocular or joint inflammation	AD	Mutations of *TNFRSF1A*	142680
Early onset inflammatory bowel disease	Monocyte/ macrophage, activated T cells	Mutation in IL-10 or IL-10 receptor leads to increase of TNFγ and other proinflammatory cytokines	Early onset enterocolitis enteric fistulas, perianal abscesses, chronic folliculitis	AR	Mutations in *IL-10*, *IL10RA*, or *IL10RB*	146933
Pyogenic sterile arthritis, pyoderma gangrenosum, acne (PAPA) syndrome	Hematopoietic tissues, upregulated in activated T cells	Disordered actin reorganization leading to compromised physiologic signaling during inflammatory response	Destructive arthritis, inflammatory skin rash, myositis	AD	Mutations of *PSTPIP1* (also called C2BP1)	604416
Blau syndrome	Monocytes	Mutations in nucleotide binding site of CARD15, possibly disrupting interactions with lipopolysaccharides and NF-κB signaling	Uveitis, granulomatous synovitis, camptodactyly, rash, and cranial neuropathies, 30% develop Crohn's disease	AD	Mutations of *NOD2* (also called CARD15)	186580

(Continued)

Table 7 | Continued

Disease	Affected cells	Functional defects	Associated Features	Inheritance	Genetic defect/ presumed pathogenesis	OMIM number
Chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anemia (Majeed syndrome)*	Neutrophils, bone marrow cells	Undefined	Chronic recurrent multifocal osteomyelitis, transfusion-dependent anemia, cutaneous inflammatory disorders	AR	Mutations of *LPIN2*	609628
DIRA (Deficiency of the interleukin 1 receptor antagonist)*	PMNs, monocytes	Mutations in the IL1 receptor antagonist allows unopposed action of interleukin 1	Neonatal onset of sterile multifocal osteomyelitis, periostitis, and pustulosis	AR	Mutations of *IL1RN*	612852

AR, autosomal recessive inheritance; AD, autosomal dominant inheritance; PMN, polymorphonuclear cells; ASC, apoptosis-associated speck-like protein with a caspase recruitment domain; CARD, caspase recruitment domain; CD2BP1, CD2 binding protein 1; PSTPIP1, proline/serine/threonine phosphatase-interacting protein 1; SNHL, sensorineural hearing loss; CIAS1, cold-induced autoinflammatory syndrome 1.

**Ten or fewer unrelated cases reported in the literature.*

Autoinflammatory diseases are clinical disorders marked by abnormally increased inflammation, mediated predominantly by the cells and molecules of the innate immune system, with a significant host predisposition. While the genetic defect of one of the most common autoinflammatory conditions, PFAPA, is not known, recent studies suggest that it is associated with activation of IL-1 pathway and response to IL-1 beta antagonists.

Table 8 | Complement deficiencies.

Disease	Functional defect	Associated features	Inheritance	Genetic defect/ presumed pathogenesis	OMIM number
C1q deficiency	Absent CH50 hemolytic activity, defective MAC faulty dissolution of immune complexes faulty clearance of apoptotic cells	SLE-like syndrome, rheumatoid disease, infections	AR	Mutations in *C1QA*, *C1QB*, *C1QC*, and loss of early complement activation	120550; 601269; 120575
C1r deficiency	Absent CH50 hemolytic activity, defective MAC faulty dissolution of immune complexes	SLE-like syndrome, rheumatoid disease, multiple autoimmune diseases, infections	AR	Mutations in C1r and loss of early complement activation	216950
C1s deficiency	Absent CH50 hemolytic activity	SLE-like syndrome; multiple autoimmune diseases	AR	Mutations in C1s and loss of early complement activation	120580
C4 deficiency	Absent CH50 hemolytic activity, defective MAC faulty dissolution of immune complexes defective humoral immune response to carbohydrate antigens in some patients	SLE-like syndrome, rheumatoid disease, infections *C4A*; homozygous; SLE, type I diabetes *C4B:* homozygous: bacterial meningitis	AR	Mutations in *C4A* and *C4B* and loss of early complement activation	120810; 120820
C2 deficiency	Absent CH50 hemolytic activity, defective MAC faulty dissolution of immune complexes	SLE-like syndrome, vasculitis, atherosclerosis, polymyositis, pyogenic infections; glomerulonephritis	AR	Mutations in *C2* and loss of early complement activation	217000
C3 deficiency	Absent CH50 and AP50 hemolytic activity, defective MAC defective bactericidal activity defective humoral immune response	Life threatening pyogenic infections; SLE-like disease; glomerulonephritis; atypical hemolytic–uremic syndrome; selected SNPs with age related macular degeneration	AR	Mutations in *C3* and loss of complement activation by classical and alternative pathways	120700
C5 deficiency	Absent CH50 and AP50 hemolytic activity, defective MAC defective bactericidal activity	Neisserial infections, SLE	AR	Mutations in *C5α*? or *C5β*? and loss of complement activation	120900
C6 deficiency	Absent CH50 and AP50 hemolytic activity, defective MAC defective bactericidal activity	Neisserial infections, SLE	AR	Mutations in *C6* and loss of complement activation	217050
C7 deficiency	Absent CH50 and AP50 hemolytic activity, defective MAC defective bactericidal activity	Neisserial infections, SLE, vasculitis	AR	Mutations in *C7* and loss of terminal complement activation	217070
C8a deficiency	Absent CH50 and AP50 hemolytic activity, defective MAC defective bactericidal activity	Neisserial infections, SLE	AR	Mutations in *C8α* and loss of terminal complement activation	120950
C8b deficiency	Absent CH50 and AP50 hemolytic activity, defective MAC defective bactericidal activity	Neisserial infections, SLE	AR	Mutations in *C8β* and loss of terminal complement activation	120960
C9 deficiency	Reduced CH50 and AP50 hemolytic activity, defective MAC defective bactericidal activity	Neisserial infections, weaker association than in C5, C6, C7, or C8 deficiency	AR	Mutations in *C9* and loss of terminal complement activation	

(Continued)

Table 8 | Continued

Disease	Functional defect	Associated features	Inheritance	Genetic defect/ presumed pathogenesis	OMIM number
C1 inhibitor deficiency	Spontaneous activation of the complement pathway with consumption of C4/C2 spontaneous activation of the contact system with generation of bradykinin from high molecular weight kininogen	Hereditary angioedema	AD	Mutations in *C1* inhibitor and loss of regulation of proteolytic activities of complement C1	138470
Factor D deficiency	Absent AP50 hemolytic activity	Severe neisserial infection	AR	Mutations in factor D (*CFD*), impairing alternative complement activation	134350
Properdin deficiency	Absent AP50 hemolytic activity	Severe neisserial infection	XL	Mutations in properdin (*PFC*), impairing alternative complement activation	312060
Factor I deficiency	Spontaneous activation of the alternative complement pathway with consumption of C3	Recurrent pyogenic infections, glomerulonephritis, SLE; hemolytic–uremic syndrome; selected SNPS: severe pre-eclampsia	AR	Mutations in factor I(*CFI*), leading to accelerated catabolism of C3	610984
Factor H deficiency	Spontaneous activation of the alternative complement pathway with consumption of C3	Hemolytic–uremic syndrome, membranoproliferative glomerulonephritis; neisserial infections; selected SNPS: severe pre-eclampsia	AR	Mutations in factor H(*CFH*), leading to continuous activation of the alternative complement pathway and C3 deposition in tissues	609814
MASP1 deficiency	Potential loss of embryonic cell migration signals	A developmental syndrome of facial dysmorphism, cleft lip, and/or palate, craniosynostosis, learning disability and genital, limb and vesicorenal anomalies	AR	Mutations in *MASP1* leading to impaired complement pathway through the mannan-binding lectin serine proteases.	600521
3MC syndrome COLEC11 deficiency	Potential loss of embryonic cell migration signals	A developmental syndrome of facial dysmorphism, cleft lip and/or palate, craniosynostosis, learning disability and genital, limb and vesicorenal anomalies	AR	Gene product CL-K1, a C-type lectin that may serve as a chemoattractant	612502
MASP2 deficiency*	Absent hemolytic activity by the lectin pathway	Pyogenic infections; inflammatory lung disease	AR	Mutations in *MASP2* leading to impaired complement pathway through the mannan-binding lectin serine proteases	605102
Complement receptor 3 (CR3) deficiency	See LAD1 in **Table 5**		AR	Mutations in *INTGB2*	116920
Membrane cofactor protein (CD46) deficiency	Inhibitor of complement alternate pathway, decreased C3b binding	Glomerulonephritis, atypical hemolytic–uremic syndrome; selected SNPS: severe pre-eclampsia	AD	Mutations in *MCP* leading to loss of the cofactor activity needed for the factor I-dependent cleavage of C3B and C4B	120920
Membrane attack complex inhibitor (CD59) deficiency	Erythrocytes highly susceptible to complement-mediated lysis	Hemolytic anemia, thrombosis	AR	Mutations in *CD59* leading to loss of this membrane inhibitor of the membrane attack complexes	107271

(Continued)

Table 8 | Continued

Disease	Functional defect	Associated features	Inheritance	Genetic defect/ presumed pathogenesis	OMIM number
Paroxysmal nocturnal hemoglobinuria	Complement-mediated hemolysis	Recurrent hemolysis; hemoglobinuria, abdominal pain, smooth muscle dystonias, fatigue, and thrombosis	Acquired X-linked mutation	Disease results from the expansion of hematopoietic stem cells bearing mutations in PIGA and subsequent loss of biosynthesis of glycosylphosphatidylinositol (GPI) a moiety that attaches proteins to the cell surface.	300818
Immunodeficiency associated with Ficolin 3 deficiency*	Absence of complement activation by the Ficolin 3 pathway.	Recurrent severe pyogenic infections mainly in the lungs; necrotizing enterocolitis in infancy; selective antibody defect to pneumococcal polysaccharides	AR	Mutations in *FCN3*, leading to impaired complement deposition	604973

XL, X-linked inheritance; AR, autosomal recessive inheritance; AD, autosomal dominant inheritance; MAC, membrane attack complex; SLE, systemic lupus erythematosus; MBP, Mannose binding Protein; MASP2, MBP associated serine protease 2.

**Ten or fewer unrelated cases reported in the literature.*

*New entities added to **Table 8** demonstrate the important role of complement regulators in a group of well-described inflammatory disorders. In particular, we have added mutations in membrane bound as well as surface attached soluble complement regulatory proteins recognized in hemolytic–uremic syndrome, age related macular degeneration and pre-eclampsia. The connecting theme of these otherwise unrelated clinical events is excessive activation or insufficient regulation of C3; these events lead to recruitment of leukocytes and permit secretion of inflammatory and anti-angiogenic mediators that disrupt the vascular bed of the target organ. Alterations in the genes for factor B (CFB), factor I (CFI), factor H (CFH), and CD46 act as susceptibility genes rather than disease causing mutations. Population studies reveal no detectable increase in infections in MBP (also known at mannose binding lectin – MBL) deficient adults. The 3MC syndrome, a developmental syndrome, has been variously called Carnevale, Mingarelli, Malpuech, and Michels syndrome.*

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

Received: 25 August 2011; accepted: 20 September 2011; published online: xx October 2011.

Citation: Al-Herz W, Bousfiha A, Casanova J-L, Chapel H, Conley ME, Cunningham-Rundles C, Etzioni A, Fischer A, Franco JL, Geha RS, Hammarström L, Nonoyama S, Notarangelo LD, Ochs HD, Puck JM, Roifman CM, Seger R and Tang MLK (2011) Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency. Front. Immun. 2:54. doi: 10.3389/fimmu.2011.00054

This article was submitted to Frontiers in Primary Immunodeficiencies, a specialty of Frontiers in Immunology.

Copyright © 2011 Al-Herz, Bousfiha, Casanova, Chapel, Conley, Cunningham-Rundles, Etzioni, Fischer, Franco, Geha, Hammarström, Nonoyama, Notarangelo, Ochs, Puck, Roifman, Seger and Tang . This is an open-access article subject to a nonexclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.

Author Queries Form

Queries

Query No.	Details required	Author's Reponse
Q1	Please provide short running title with maximum of five words.	
Q2	Please confirm the edit made in the Table 1.	
Q3	Please cite Table 7 inside the text.	

J Clin Immunol (2011) 31:968–976
DOI 10.1007/s10875-011-9594-7

Nationwide Survey of Patients with Primary Immunodeficiency Diseases in Japan

Masataka Ishimura · Hidetoshi Takada · Takehiko Doi · Kousuke Imai · Yoji Sasahara · Hirokazu Kanegane · Ryuta Nishikomori · Tomohiro Morio · Toshio Heike · Masao Kobayashi · Tadashi Ariga · Shigeru Tsuchiya · Shigeaki Nonoyama · Toshio Miyawaki · Toshiro Hara

Received: 7 August 2011 / Accepted: 11 September 2011 / Published online: 29 September 2011
© Springer Science+Business Media, LLC 2011

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

M. Ishimura (✉) · H. Takada · T. Doi · T. Hara
Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University,
3-1-1 Maidashi, Higashi-ku,
Fukuoka 812-8582, Japan
e-mail: ischii@pediatr.med.kyushu-u.ac.jp

K. Imai · S. Nonoyama
Department of Pediatrics, National Defense Medical College, Tokorozawa, Japan

Y. Sasahara · S. Tsuchiya
Department of Pediatrics, Tohoku University School of Medicine, Sendai, Japan

H. Kanegane · T. Miyawaki
Department of Pediatrics, Graduate School of Medicine and Pharmaceutical Science, University of Toyama, Toyama, Japan

R. Nishikomori · T. Heike
Department of Pediatrics, Kyoto University Graduate School of Medicine, Kyoto, Japan

T. Morio
Department of Pediatrics, Tokyo Medical and Dental University Graduate School, Bunkyo-ku, Tokyo, Japan

M. Kobayashi
Department of Pediatrics, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan

T. Ariga
Department of Pediatrics, Graduate School of Medicine, Hokkaido University, Sapporo, Japan

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)

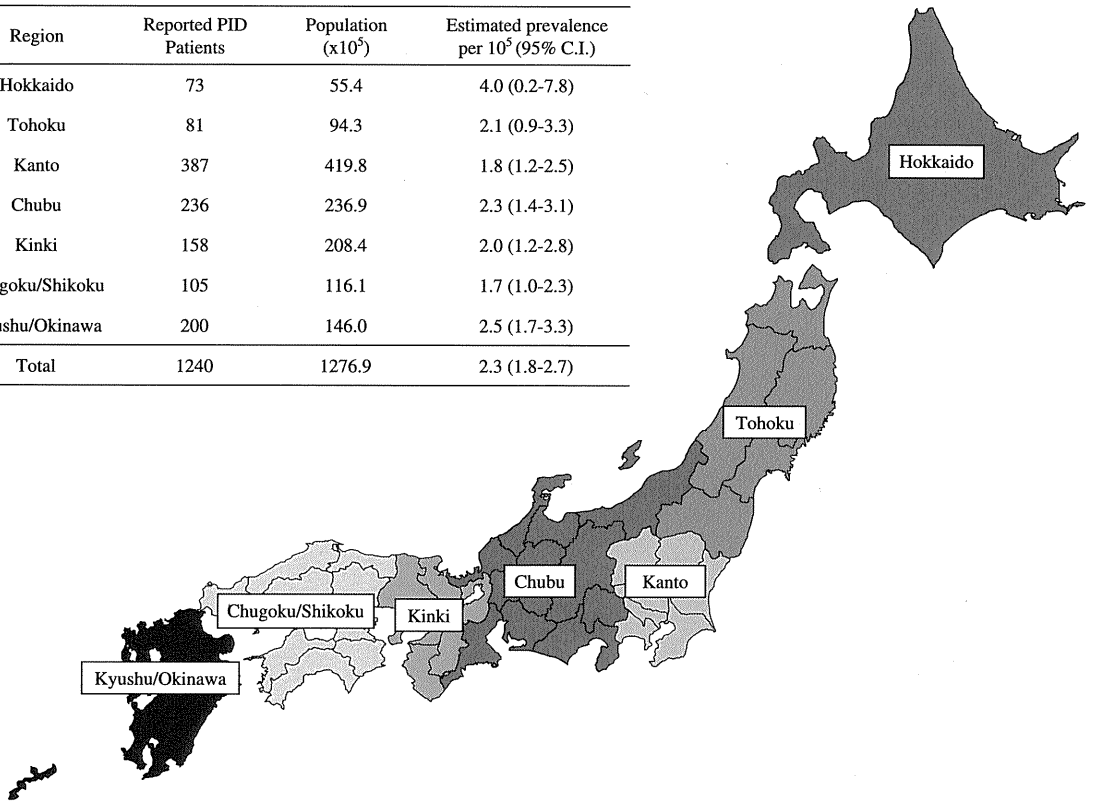

Fig. 1 Regional distribution of PID patients. *CI* Confidence interval

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

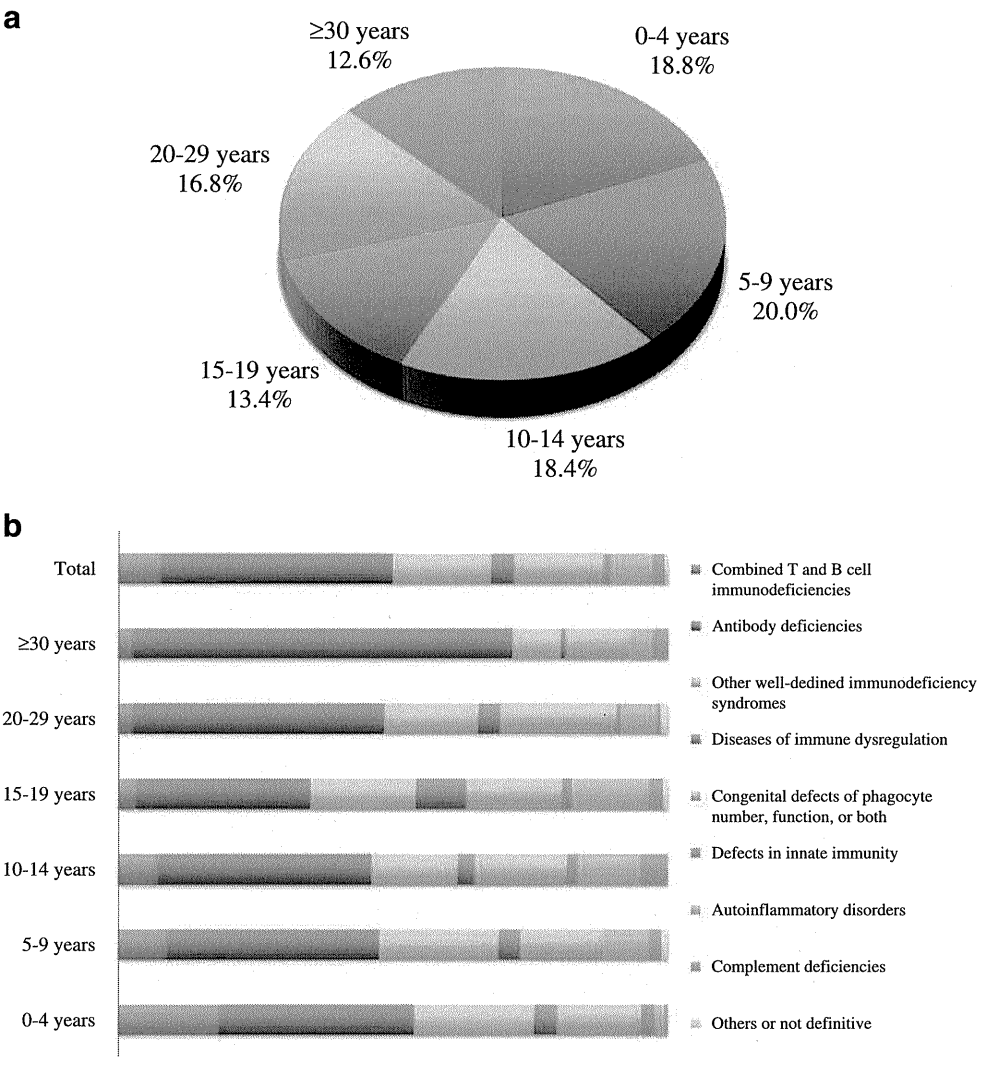

Fig. 2 **a** Age distribution of PID patients. **b** Distribution of PID in each age group

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].

Acknowledgments The authors would like to thank the support of the Japanese Research Group on Primary Immunodeficiency Diseases, which is supported by Japan's Ministry of Health, Labour and Welfare.

Conflict of Interest There is no actual or potential conflict of interest in relation to the study.

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/.

In conclusion, the associations among asthma, biofilm-forming bacteria, and revision ESS are strong and robust after adjusting for other factors in patients with CRS from a tertiary medical center. Despite its limitations, this study may improve our understanding of refractory CRS pathogenesis, possibly leading to more effective treatment strategies, such as incorporating the treatments of asthma and biofilm infection into conventional CRS therapies. Prospective cohort studies in diverse populations are needed to assess the causality of these associations.

We thank Alexander Chiu for providing the clinical samples, Andrew Cucchiara for helping with the data cleansing and analysis, and Jennifer Kofonow, Anthony Prince, Jacob Steiger, Michael Cohen, Edwin Tamashiro, and Natalia Goldstein for performing the Calgary biofilm assay and organizing the data.

Zi Zhang, MD[a]
Darren R. Linkin, MD, MSCE[b]
Brian S. Finkelman, BS[a]
Bert W. O'Malley, Jr, MD[c]
Erica R. Thaler, MD[c]
Laurel Doghramji, RN, BSN[c]
David W. Kennedy, MD[c]
Noam A. Cohen, MD, PhD[c]
James N. Palmer, MD[c]

From [a]the Center for Clinical Epidemiology and Biostatistics, [b]the Department of Medicine, Division of Infectious Diseases, and [c]the Department of Otorhinolaryngology–Head and Neck Surgery, University of Pennsylvania School of Medicine, Philadelphia, Pa. E-mail: james.palmer@uphs.upenn.edu.

Supported by the Flight Attendant Medical Research Institute Clinical Investigator Award (053367 to N.A.C. and 052414 to J.N.P.).

Disclosure of potential conflict of interest: J. N. Palmer receives research support from the Flight Attendant Medical Research Institute, has provided legal consultation/expert witness testimony in cases related to complications in sinus surgery, and is a member of the board of directors for the American Rhinologic Society. D. W. Kennedy is the medical director of ENT Care and a member of RhinActive. N. A. Cohen receives research support from the Flight Attendant Medical Research Institute. The rest of the authors have declared that they have no conflict of interest.

REFERENCES

1. Fokkens W, Lund V, Mullol J. EP3OS 2007: European position paper on rhinosinusitis and nasal polyps 2007: a summary for otorhinolaryngologists. Rhinology 2007; 45:97-101.
2. Newman LJ, Platts-Mills TA, Phillips CD, Hazen KC, Gross CW. Chronic sinusitis: relationship of computed tomographic findings to allergy, asthma, and eosinophilia. JAMA 1994;271:363-7.
3. Psaltis AJ, Weitzel EK, Ha KR, Wormald PJ. The effect of bacterial biofilms on post-sinus surgical outcomes. Am J Rhinol 2008;22:1-6.
4. Prince AA, Steiger JD, Khalid AN, Dogrhamji L, Reger C, Eau Claire S, et al. Prevalence of biofilm-forming bacteria in chronic rhinosinusitis. Am J Rhinol 2008;22: 239-45.
5. Meltzer EO, Hamilos DL, Hadley JA, Lanza DC, Marple BF, Nicklas RA, et al. Rhinosinusitis: establishing definitions for clinical research and patient care. J Allergy Clin Immunol 2004;114:155-212.
6. National Asthma Education and Prevention Program. Expert Panel Report 3 (EPR-3): guidelines for the diagnosis and management of asthma-summary report 2007. J Allergy Clin Immunol 2007;120:S94-S138.
7. Banerji A, Piccirillo JF, Thawley SE, Levitt RG, Schechtman KB, Kramper MA, et al. Chronic rhinosinusitis patients with polyps or polypoid mucosa have a greater burden of illness. Am J Rhinol 2007;21:19-26.
8. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999;284:1318-22.
9. Richtsmeier WJ. Top 10 reasons for endoscopic maxillary sinus surgery failure. Laryngoscope 2001;111:1952-6.

Available online March 24, 2011.
doi:10.1016/j.jaci.2011.02.022

Quantification of κ-deleting recombination excision circles in Guthrie cards for the identification of early B-cell maturation defects

To the Editor:

X-linked agammaglobulinemia (XLA) is a primary immunodeficiency caused by severely decreased numbers of mature peripheral B lymphocytes as a result of a mutation in the *BTK* gene. Non-XLA is characterized by hypogammaglobulinemia with decreased B-cell counts (less than 2% of mature B cells) in the absence of the *BTK* gene mutation. Both XLA and non-XLA are caused by an early B-cell maturation defect.[1] In patients with XLA and non-XLA, recurrent infections appear between 3 and 18 months of age, whereas the mean age at diagnosis is 3 years.[2] This delayed diagnosis results in frequent hospitalization because of pneumonia, sepsis, meningitis, and other bacterial infections, which frequently require intravenous administration of antibiotics and can be fatal. Frequent pneumonia results in a high incidence of chronic lung diseases.[3] Thus, early diagnosis and early treatment, including periodical intravenous immunoglobulin replacement therapy, is essential to improve the prognosis and the quality of life of patients with XLA and non-XLA.

In the process of B-cell maturation, immunoglobulin κ-deleting recombination excision circles (KRECs) are produced during κ-deleting recombination allelic exclusion and isotypic exclusion of the λ chain.[4] Coding joint (cj) KRECs reside within the chromosome, whereas signal joint (sj) KRECs are excised from genomic DNA. cjKREC levels remain the same after B-cell division, whereas sjKREC levels decrease, because sjKRECs are not replicated during cell division.[5] Because the B-cell maturation defects in XLA and non-XLA occur before κ-deleting recombination, KRECs are not supposed to be produced. Therefore, measurements of KRECs have the potential to be applied to the identification of these types of B-cell deficiencies in patients, which consist of around 20% of all B-cell defects.[6] In addition, some types of combined immunodeficiencies show an arrest in B-cell maturation and can also be identified by this method. The success of newborn screening for T-cell deficiencies by measuring T-cell–receptor excision circles[7] prompted us to develop a newborn screening method for XLA and non-XLA by measuring KRECs derived from neonatal Guthrie cards.

The study protocol was approved by the National Defense Medical College institutional review board, and written informed consent was obtained from the parents of normal controls, the affected children, and adult patients, in accordance with the Declaration of Helsinki.

First, we determined the sensitivity of detection levels of cjKRECs and sjKRECs in Guthrie cards using real-time quantitative PCR.[5] Normal B cells from a healthy adult were isolated from peripheral blood (PB; mean purity, 88.5%). PB was also obtained from 1 patient with XLA (P20) whose B-cell number was 0.09 in 1 μL whole blood and who was negative for sjKRECs ($<1.0 \times 10^2$ copies/μg DNA). Various numbers of normal B cells were serially added to 1 mL whole PB obtained from this patient with XLA. The B-cell–added XLA whole blood was then applied to filter papers, and 3 punches (3 mm in diameter) of dried blood spots were used for DNA extraction. At least 3 DNA samples containing the same B-cell concentrations (0.09-400 B cells/μL) were used for the real-time quantitative PCR of cjKRECs and sjKRECs. The percentages of the positive samples ($>1.0 \times 10^2$ copies/μg DNA) of cjKRECs and sjKRECs increased constantly

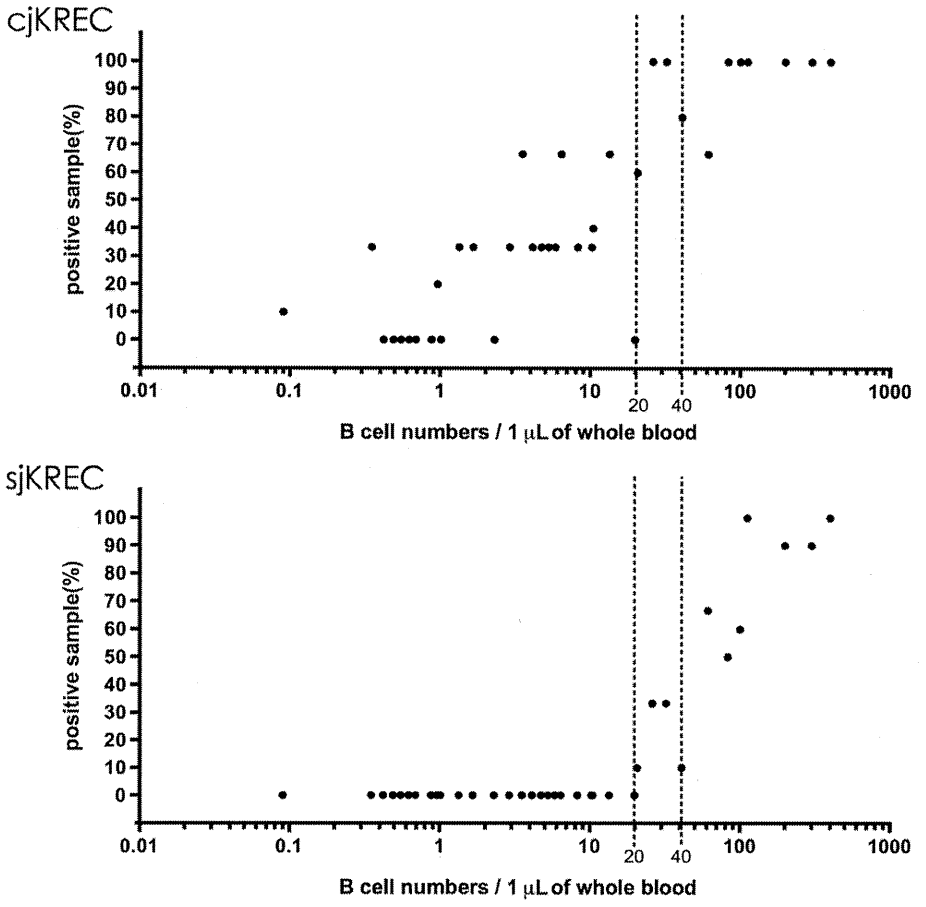

FIG 1. Sensitivity levels of cjKRECs and sjKRECs. Various numbers of purified normal B cells were serially added to whole PB from a patient with XLA (P20) to obtain B-cell–added XLA whole blood. cjKRECs and sjKRECs were measured in 3 to 10 samples of each concentration in triplicate. In all analyses, RNaseP (internal control) was positive ($2.3 \pm 0.2 \times 10^5$ copies/μg DNA). *X-axis,* B-cell numbers in 1 μL whole blood from a patient with XLA. *Y-axis,* Percentages of the KREC-positive results in the tests.

as the B-cell concentrations increased (Fig 1). None of the samples were positive for sjKRECs when the B-cell numbers were less than 20/μL, but cjKRECs were often positive. It has been reported that 90% of patients with XLA have less than 0.2% B cells in the PB at diagnosis.[1] Because peripheral lymphocyte numbers in neonates range from 1200 to 9800/μL,[8] the absolute B-cell numbers of 90% of patients with XLA are estimated to be 2.4 to 19.6/μL at the time of blood collection for Guthrie cards, although exact B-cell numbers of XLA in neonatal periods are not known at this moment. Because neonates are known to have fewer B cells than infants,[9] and we observed that B-cell numbers are constantly low in patients with XLA throughout infancy (Nakagawa, unpublished data, June 2010), which is consistent with the fact that BTK plays an essential role in B-cell maturation. It is likely that neonates with XLA also have severely decreased B cells. On the other hand, all samples obtained from 400 B cells/μL were positive for both cjKRECs and sjKRECs. We also observed that all healthy infants (1-11 months old; n = 15) were sjKREC-positive (Nakagawa, unpublished data, June 2010) and might have at least 600 B cells/μL whole blood.[9] From these data, it is assumed that at least 90% of patients with XLA are sjKREC-negative, and healthy neonates are positive for sjKRECs on neonatal Guthrie cards.

Next, we measured cjKRECs and sjKRECs in dried blood spots in filter papers or Guthrie cards from 30 patients with XLA and 5 patients with non-XLA and from 133 neonates born at the National Defense Medical College Hospital during this study period (August 2008 to October 2009) and 138 healthy subjects of various ages (1 month to 35 years old) to investigate the validity of this method. The levels of B cells of the patients ranged from 0.0% to 1.1% of total lymphocytes and 0.0 to 35.78/μL. IgG levels were 10 to 462 mg/dL (see this article's Tables E1 and E2 in the Online Repository at www.jacionline.org). Patients with leaky phenotypes[1,10] were included; 1 patient (P30) had more than 1% B cells and 34.22/μL total B cells, and 4 patients had more than 300 mg/dL serum IgG (P12, P30, P31, P33). All of the normal neonatal Guthrie cards were positive for both cjKRECs and sjKRECs ($7.2 \pm 0.7 \times 10^3$ and $4.8 \pm 0.6 \times 10^3$ copies/μg DNA, respectively). All healthy subjects of various ages were also positive for both cjKRECs and sjKRECs (Nakagawa, unpublished data, June 2010). In contrast, specimens from all 35 B-cell–deficient patients were sjKREC-negative ($<1.0 \times 10^2$ copies/μg DNA; Fig 2). All 5 patients with leaky phenotypes were also sjKREC-negative, which might be explained by the hypothesis that leaky B cells of patients with XLA are long-lived B cells that divided several times and have fewer sjKRECs than naive B cells.