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Distribution and Clinical Aspects of Primary Immunodeficiencies in a Taiwan Pediatric Tertiary Hospital During a 20-Year Period

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Recent advances in immunologic techniques have lead to increased recognition of primary immunodeficiencies. A review of patients with suspected immunodeficiencies in a Taiwan tertiary hospital from January 1985 to October 2004 and molecular/genetic analyses done on some patients were investigated. Of the 403 patients selected based on the *International Classification of Disease, Ninth Revision*, 37 patients with PID (8 females and 29 males) were identified: 17 (46%) with antibody production deficiencies, nine (24%) with defective phagocyte function, four (11%) with combined B and T cell immunodeficiencies, seven (19%) with T cell deficiencies, but none with primary complement deficiencies. Those with secondary immunodeficiencies were excluded from the study. Recurrent sinopulmonary infections (62%) were the most common clinical manifestation, followed by sepsis (57%), severe skin infection (40%), splenomagaly/hepatomegaly (27%), central nervous system dysfunction (22%), chronic diarrhea (22%), and failure to thrive (19%). Seven (19%) patients died, five of infections, one of disseminated intravascular coagulopathy and one of hepatocellular carcinoma. Six novel mutations were found from 11 agreed patients. This is the first report on primary immunodeficiencies in Taiwan covering a 20-year period.

KEY WORDS: Primary immunodeficiency; agammaglobulinemia; recurrent sinopulmonary infections; unrelated umbilical cord stem cell transplantation; Taiwan.

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INTRODUCTION

Primary immunodeficiencies (PID) are a group of diseases characterized by unusual susceptibility to infections. There are 10 warning signs of PID characterized by distinct recurrent patters of infection: [1] eight or more new ear infections within 1 year; [2] two or more serious sinus infections within 1 year; [3] two or more months on antibiotics with little effect; [4] two or more pneumonia within 1 year; [5] deep-seated infections such as meningitis, osteomyelitis, cellulites, or sepsis; [6] recurrent deep skin or organ abscesses; [7] persistent thrush in the mouth or elsewhere on the skin after age 1; [8] need for intravenous antibiotics to clear infections; [9] failure of an infant to gain weight or grow normally, and [10] a family history of PID (1). Since Bruton's first description of a patient with agammaglobulinemia and recurrent sinopulmonary infections in 1952 (2), about 100 different types of PID have been recognized based on the 10 warning signs (1, 3). The increase in the recognition rate of PID is due to advances in our knowledge of the immune system and the novel progress in molecular diagnostic techniques. The estimated occurrence of PID is about 1 per 10,000 live births (excluding asymptomatic IgA deficiency) (1). Epidemiological studies show wide geographical and racial variations in the prevalences and the patterns of PID. Physicians and general practitioners are often poorly informed about the clinical presentation, diagnostic approach, and health impact of PID. Thus, some patients remain untreated for several years and are referred to immunologists when they have critical events; this delay in diagnosis and treatment can result in irreversible sequelae (4, 5).

In order to discover the frequencies of the different types of PID in a Taiwan tertiary children's hospital, we reviewed all referral records during the period of January 1985 to October 2004. Additionally, we performed molecular and/or genetic analyses on PID patients whose families (and patients, as appropriate) consented to testing.

Our goal was to determine the frequencies of these diseases based on World Health Organization (WHO) criteria and classifications (1, 3, 6), to emphasize the importance of early diagnosis and treatment, and finally, to promote research on PID throughout the country and the rest of the world.

PATIENTS AND METHODS

Computer Database Search

All patients with suspected immunodeficiencies, as indicated by the diagnosis numbers of the *International Classification of Disease, Ninth Revision* (ICD-9) were enrolled. Disorders involving the immune mechanism (ICD-9 numbers 279–279.9, 288.1, 288.2, 277.2 and 334.8), including "20 items" of diagnosis codes as shown in Table I were included. Patients with secondary immunodeficiencies were excluded from the study.

Patient Data Collection

The clinical data retrospectively collected from patients' medical records included initial clinical manifestations, age at presentation, family history of immunodeficiencies and/or recurrent infections, autoimmune

Table I. Suspected Patients with Diagnosis Codes Relate to Immunodeficiencies

		Immunodeficiencies (F: female; M: male)					
		Suspected		Primary		Secondary	
ICD code	Description	F	M	F	M	F	M
279.0	Deficiency of humoral immunity						
279.01	Hypogammaglobulinemia; unspecified agammaglobulinemia	36	63[a]			35	59
279.02	Selective IgA immunodeficiency	9	11			9	11
279.03	Selective IgM immunodeficiency	2	2			2	2
279.00	Other selective immunoglobulin deficiencies	10	12			10	12
	Selective deficiency of IgG						
279.04	Congenital hypogammaglobulinemia or agammaglobulinemia	1	3	1	5[b]		
	Bruton's type, X-linked						
279.05	Immunodeficiency with increased IgM		1	1	5[a,b]		
	Autosomal recessive						
	X-linked						
279.06	Common variable immunodeficiency dysgammaglobulinemia (acquired) (conegential) (primary)	6	7[b,c]	1	3	5	
	Hypogammaglobulinemia						
	Acquired primary congenital non-X-linked						
	Sporadic						
279.09	Other						
	Transient hypogammaglobuinemia of infancy	7	10		1	7	9
	Deficiency of cell-mediated immunity						
279.19	Immunodeficiency with predominant T-cell defect, unspecified	1	6		1	1	5
279.11	DiGeorge's syndrome	7	3	1	1	6	2
	Pharyngeal pouch syndrome						
	Thymic hypoplasia						
279.12	Wiskott–Aldrich syndrome	4	4		2	4	2
334.8	Ataxia-Telangiectasia	10	18	1	1	9	17
279.13	Nezelof's syndrome						
279.2	Combined immunity deficiency	6	7	1	1	5	4
	Agammaglobulinemia						
	Autosomal recessive				1[b]		
	Swiss-type						
	X-linked recessive						
	Severe combined immunodeficiency [SCID]				1		
279.3	Unspecified immunity deficiency	45	66			45	66
279.9	Unspecified disorder of immune mechanism	12	14[c]			12	12
288.1	Functional disorders of the polymorphonuclear neutrophils	6	5	2	7[c]	4	2
288.2	Genetic anomalies of leukocytes	4	5			4	5
277.2	Other disorders of purine and pyrimidine metabolism						
Total		166	237	8	29	158	208

[a] Four patients with hypogammaglobulinemia (ICD = 279.01) were reclassified into patients with hyper IgM syndrome (patients 12, 13, and 14).
[b] Patients with mutated Btk (patients 3 and 4) and CD40L (patient 16) gene, and decreased MHC I expression (patient 21) were separated from those patients with the diagnosis code of "CVID" (patients 3, 4, 16, and 21).
[c] Patients with interferon-γ associated immunodeficiency (patients 31 and 32) were isolated from patients from "unspecified disorder of immune mechanism."

diseases, malignancies, basic immunologic laboratory tests, including blood smears, differential counts, immunoglobulin levels, and delayed cutaneous hypersensitivity reactions. Additionally, lymphocyte subpopulations (T, B, and natural killer [NK] cells) enumerated using flow cytometry, immunoglobulin G subclass titers, chemotaxis evaluation, nitro blue tetrazolium (NBT) dye test, chemiluminescence, complement, and hemolytic titration of complement (CH50) were evaluated. B cell function was assessed by paired serum titers before vaccination and 1 month later, along with *in vitro* T cell proliferation by mitogens (phytohemagglutinin, concanavalin-A and/or pokweed mitogen) and/or antigens (*Candida albicans* or bacille Calmette-Guérin [BCG] vaccine) stimulation incubated with ^{3}H-thymidine (7). NK cytotoxicity was measured by the ^{51}Cr release assay or by propidium-iodide based flow cytometry in indicated cases (8, 9).

Sequencing and Molecular Analyses of Candidate Genes

After informed consent was obtained from 11 patients, 10–20 mL of venous blood were collected from each patient into heparin-containing syringes and delivered to our laboratory within 24–72 h. Total RNA was isolated from activated peripheral blood mononuclear cells (4 h of incubation with 10 ng/mL of phorbol 12-myristate 13-acetate and 1 μg/mL of ionomycin) using TRIzol (Invitrogen, Carlsbad, CA). Reverse transcription of mRNA followed by polymerase chain reaction (RT-PCR) was performed as follows: 1–2 μg of total RNA in a volume of 20 μL was reverse-transcribed into cDNA using oligo-dT primers and SuperScript II reverse transcriptase (Invitrogen). Amplification was performed using 1 μL of cDNA in a volume of 20 μL containing 0.5 U High Fidelity Taq DNA polymerase, 1.875 mmol/L $MgSO_4$, 200 μmol/L of dNTP, 500 nmol/L of each pair of oligonucleotide primers and 10$\times$ buffer (Invitrogen). One or two pairs of oligonucleotide primers (Table II) were selected for each gene to cover the entire coding region. The mutations identified from cDNA were confirmed by sequence analysis of genomic DNA. The individual exons, including exon–intron boundaries were amplified using previously designed primers (10–13). Sequencing of the products was performed using the Big Dye Terminator kit (Applied Biosystems, Foster City, CA) and an ABI PRISM 3700 DNA Sequencer (Applied Biosystems) (14).

The deletion of 22q11.2 responsible for DiGeorge syndrome was detected by fluorescence *in situ* hybridization (15). The expressions of candidate molecules and/or proteins were evaluated using flow cytometry or immunostaining for anti-Btk (mouse IgG1 provided by Hans D. Ochs MD, University of Washington Medical Center, Seattle, WA), CD40L (mouse IgG1, Pharmingen, San Diego, CA), IL-2 receptor common gamma chain (IL2RG or CD132, mouse IgG1, Pharmingen), human leukocyte antigen (HLA) class I (HLA-A, B, C; mouse IgG1, Pharmingen) and class II (HLA-DR, DQ, DP; mouse IgG2a, Pharmingen), and anti-Wiskott–Aldrich syndrome protein (WASP) antibodies (rabbit IgG1, a gift from Qili Zhu MD, University of Washington Medical Center, Seattle, WA) as described previously (10–13, 16–19).

RESULTS

The medical records of 166 females and 237 males (403 total patients) were selected based on ICD-9 coding from a total of 214,363 inpatient and outpatient records for the 20-year study period of January 1985 to October 2004. Of the 403 patient records selected, 366 were excluded because the patients had secondary immunodeficiencies caused by malignancies (lymphoma, leukemia and/or chemotherapy in 110 patients), nephrotic syndrome (89 patients), protein-losing enteropathy (49 patients), systemic muscle atrophy (42 patients), ongoing immune-functional development (27 patients), diabetes mellitus (23 patients), systemic lupus erythematosus (19 patients), and short bowel syndrome (7 patients).

Thirty-seven patients (8 females and 29 males; ratio 1:3.6) met the WHO criteria for PID. The age of onset at presentation ranged from 5 days of life to 16 years of age. There were no antenatal diagnoses. Thirty of these

Table II. Primers Used for RT-PCR

	Complementary DNA		Reference Gene Bank
	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	
Btk1-13	CAG TGT CTG CTG CGA TCG AG	CAG TGG AAG GTG CAT TCT TG	NM 000061
Btk11-19	TCA TTG TCA GAG ACT CCA GC	TTG CTC AGA AGC CAC TAT CC	NM 000061
CD40L	GCC AGA AGA TAC CAT TTC AAC	CCG CTG TGC TGT ATT ATG AA	NM 000074
IL2RG	GAA GAG CAA GCG CCA TGT	GGT GAG GTG AGT ATG AGA CG	NM 000206
WASP1-10	GCC TCG CCA GAG AAG ACA AG	GCA ATC CCC AAA GGT ACA GG	NM 000377
WASP9-12	ACG ACT TCA TTG AGG ACC AG	TGA GTG TGA GGA CCA GGC AG	NM 000377

Table III. Born in the 20-Year Period, Thirty Patients with Primary Immunodeficiencies (PID) Diagnosed Per Four Year

Year of diagnosis	Number of patients
1985–1988	1
1989–1992	1
1993–1996	5
1997–2000	13
2001–2004 (at present)	10
Total	30

patients were born in the 20-year study period (Table III). This corresponds to an estimated occurrence of one in 46,000 live births and an incidence of 2.17 per 100,000 live births (20). The immunologic data and clinical features of patients with PID during the study period are shown in Tables IV–VII.

Deficiencies of antibody production were most common (17 patients), followed by phagocyte disorders (nine patients, including two with interferon-γ associated immunodeficiency), four with combined B and T cell immunodeficiencies, and seven with predominantly T cell immunodeficiencies. None had primary complement deficiencies. Seven patients had a family history of PID (from four unrelated families). There was no consanguinity in these families.

Clinical Manifestations and Treatments

Overall, recurrent sinopulmonary infections (otitis media, sinusitis and/or pneumonia) were the most common presentations (23 patients, 62%) and subsequently bronchiectasis (5, 14%) developed. Twenty-one patients (57%) had 33 episodes of septicemia in which *Pseudomonas* spp. predominated (10 episodes), followed by *Salmonella* spp. (8 episodes), *Staphylococcus aureus* (3 episodes), *Enterobacter cloacae* (3 episodes), *Streptococcus pneumoniae* (2 episodes), *Escherichia coli* (2 episodes), *Proteus* spp. (1 episodes), *Candida albicans* (2 episodes), and *Mycobacterium tuberculosis* (2 episodes). Severe skin infections (cellulitis, pustulosis, carbuncles and/or soft tissue abscesses) were found in 15 patients (40%) and extensive varicella infections occurred in two patients with hyper IgE syndrome. Opportunistic infections of *Pneumocystis carinii* pneumonia (PCP) occurred in five patients and *cytomegalovirus* (CMV) in one. Hepatitis occurred in four (*hepatitis B* and *hepatitis C* in two each). Splenomegaly or/and hepatomegaly occurred in 10 (27%) patients, chronic diarrhea, in 8 (22%), central nervous system dysfunction, in 8 (22%), and failure to thrive occurred in 7 (19%).

Seven patients (19%) died, of whom five died of infections, one died of hepatocellular carcinoma (patient 4) and one more died of disseminated intravascular coagulopathy (DIC, patient 24).

Regular intravenous immunoglobulin (IVIG) was infused in patients with hypogammaglobulinemia and recurrent infections. Prophylactic treatment was prescribed to patients with T-cell and phagocytic defects to prevent opportunistic infections. Granulocyte-colony stimulating factor (G-CSF) was intermittently given to a patient (patient 14) with neutropenia. Interferon-gamma (IFN-γ) interestingly relieved patient 32 from refractory recurrent salmonella infections. Unrelated cord blood stem cell transplantation was performed in patient 18 who had severe combined immunodeficiencies because of lack of HLA-matched bone marrow in his relatives.

Molecular and Genetic Analysis

Eleven patients from eight unrelated families agreed to molecular and genetic analyses (Table VIII). Two patients (patients 3 and 4), previously diagnosed with common variable immunodeficiency (CVID), had missense mutations of the *Btk* gene (1694A > T, 1132T > C), resulting in the mutated Btk protein in which each had an amino acid substitution located in the kinase domain (Asp 521 Val, Tyr 334 His) and decreased affinity to anti-Btk antibody. Four patients from two unrelated families (patients 13, 14, 15 and 16) had missense and nonsense mutations of the *CD40L* gene (526T > A, 307A > T), respectively. Monoclonal CD40L antibody did not bind to these two mutated proteins: truncated [Tyr 169 Asn] CD40L protein at exon 5 and pretermination [Lys 86 stop] at exon 2. The mother of patient 16 was the first person that had a mutated X chromosome in one allele, resulting in the first index case in the family. In contrast, patient 18 had a *de novo* mutation of the *IL2RG* gene (234T > G), because of the wild type in all his sisters and biologic mother. A huge deletion of part of the WASP gene, involving the region of the promoter, exon 1 and exon 2, produced such a fragile or few mRNA/protein that did not reach the threshold volume to be amplified by RT-PCR and was not detected by anti-WASP antibody. Two patients (patients 26 and 27) with DiGeorge syndrome who had typical dysmorphic faces, congenital cardiac conotruncal defects and hypoparathyroidism (21, 22) had evidence of deletion of chromosome 22q11.2 by fluorescence *in situ* hybridization.

DISCUSSION

This is the first comprehensive study of Taiwanese distribution and clinical aspects of PID. The percentages and distribution of PID from our study compared similarly to those of other countries for the pediatric population.

Table IV. The Immunoglobulin Levels, Lymphocyte Subsets and Clinical Features in 17 Patients (3 Females, 14 Males)[a] with Predominantly Antibodies Deficiencies

Patient/sex	Age (year) Onset	Now	Immunoglobulins IgM (mg/dL)	IgG (mg/dL)	IgA (mg/dL)	IgE (IU/L)	Absolute count/mm^3 Lymphocyte	Neutrophil	Percentage of lymphocytes (%) CD3	CD4	CD8	CD19	CD16/56	Clinical events: Recurrent sinopulmonary infections	Bronchi ectasis	Chronic diarrhea	Failure to thrive	Sepsis and pathogen(s)	Severe skin infections[d]	Other presentations
Agammaglobulinemia																				
1/M	2	10 (dead)	28	45	<7	<10	4340	6300	92	27	65	0.1	7	+		+		*Pseudomonas* spp. *Proteus*		
2/M	1	6	14	164	<10	15	4251	4711	88	52	41	0.3	—	+				*Pseudomonas aeruginosa*	+ (Neck)	Tinea corpus, central nervous system lesion, including cerebral palsy and developmental delay
3/M[b]	1.5	5	32	320	16	<7	3965	2246	95	59	26	2	—	+		+		*Pseudomonas aeruginosa* *Pseudomonas oryzihabitans*	+ (Face)	Lip gangrene, meningitis, toxic megacolon
4/M[b]	2	27 (dead)	28	228	95	<7	1755	2916	84	28	22	2	15	+	+					Arthritis (*S. pneumonia*, *S. aureus*), appendicitis, chronic hepatitis B, hepatocellular carcinoma, hepatomegaly
5/M	0.3	7	22	224	<7	—	2542	2867	97	51	44	1	—	+	+			*Pseudomonas* spp. *Escherichia coli*		Neutropenia, vesico-ureteral spp. reflex Grade IV, anal fistula
6/F	1	7	18	165	<7	83	1526	5559	85	46	38	0.9	—	+				*Salmonella enteritidis* *Staphylococcus aureus*	+	PCP
Common variable immunodeficiency																				
7/F	6	9	7	48	<6	<7	2631	3242	82	34	29	15	—	+						
8/M	6	10	7	93	<6	25	3524	3685	90	—	—	8	12	+						Alopecia, septic arthritis, abdominal aneurysm vaccine-associated poliomyeloencephalitis
9/M	16	21	<5	59	<6	—	2718	3654	78	47	22	5	—	+	+			*Pseudomonas aeruginosa* *Streptococcus pneumonia*		
10/M	7	22	42	232	18	<7	3459	3258	82	—	—	12	—	+				*Pseudomonas aeruginosa*	+	Anal fistula and colostomy
Hyper IgM syndrome																				
11/F	11	17	109	48	83	<7	6435	9625	82	42	35	10	—	+						Hepatosplenomegaly, cirrhosis, Japanese encephalitis, chronic hepatitis B
12/M	0.5	6	140	51	8	<7	10250	9020	81	61	26	17	—				+	*Pseudomonas aeruginosa* *Candida albicans*		PCP, UGI bleeding
13/M[c]	0.25	0.5	34	190	2	<7	4625	3584	69	58	40	9	11	+		+				PCP
14/M[c]	3	22	187	187	8	<7	3849	3264	75	28	39	16	15	+	+			*Streptococcus pneumonia*	+ (Face)	Recurrent oral ulcer
15/M[c]	3	8	104	12	<6	<7	4902	1720	73	52	22	18	—	+				*Salmonella* spp. *Enterobacter cloacae*	+ (Neck)	Recurrent oral ulcer, spp. splenomegaly, sclerosing cholangitis
16/M	0.5	5	128	18	4	<7	4875	29250	65	45	20	22	1	+				*Pseudomonas aeruginosa*		Splenomegaly
Transient hypogammaglobuliemia of infant																				
17/M	1	5	41	101	22	73	4124	3210	62	44	20	27	—							Croup, recurrent bronchiolitis

Note. PCP, *pneumocystis carinii* pneumonia; UGI, upper gastrointestinal tract.

[a]T cell proliferation tests were performed in seven patients, showing decreased in two patients (patients 8 and 10) and normal in five patients.(patients 3, 6, 7, 12, and 16). Pair-serum of polio-vaccine titers were done in four patients, revealing decreased in three (patients 8, 9, and 16) and normal in one (patient 10). NK activity was not measured in these 17 patients.

[b]From the same family, respectively.

[c]From the same family, respectively.

[d]Severe skin infections include cellulites, pustulosis, carbuncles and deep soft tissue abscesses.

Table V. The Immunoglobulin Levels, Lymphocyte Subsets and Clinical Features 4 Patients (1 Female, 3 Males)[a] with Combined B and T Cell Immunodeficiencies

			Immunoglobulins											Clinical events					
	Age (year)						Absolute count/mm^3		Percentage of lymphocytes (%)					Recurrent sinopulmonary infections	Chronic diarrhea	Failure to thrive	Sepsis and pathogen(s)	Severe skin infections	Other presentations
Patient/sex	Onset	Now	IgM (mg/dL)	IgG (mg/dL)	IgA (mg/dL)	IgE (IU/L)	Lymphocyte	Neutrophil	CD3	CD4	CD8	CD19	CD16/56						
Severe combined immunodeficiency																			
18/M	0.1	0.4	18	45	5	<1	430	7396	0.2	0.2	0.0	77	2	+	+	+	*Escherichia coli* *Mycobacteria bovis*		Urosepsis, PCP, hepatosplenomegaly
19/M	0.2	2 (dead)	31	55	13	2	702	5481	—	28	—	7	—	+	+	+		+	Hepatosplenomegaly, protein-losing enteropathy, alopecia, seborrheic dermatitis
20/F	0.33	1 (dead)	17	94	38	<7	285	3534	15	—	—	17	—	+	+	+	*Enterobacter cloacae*	+	Protein-losing enteropathy, seborrheic dermatitis
MHC Class I deficiency[b]																			
21/M	2	8	46	584	21	—	3845	2628	65	42	0.2	25	10	+				+ (Deep leg ulcer)	polyposis

[a] T cell proliferation tests were measured in all of four patients, showing obviously decreased. Pair-serum of polio-vaccine titers, only performed in patient 21 were low. NK activity was done in patient 21 and normal.

[b] Patient 21 had decreased expression of MHC class I than that of normal control, but normal expression of MHC class II. His family refused further genetic analysis.

Table VI. The Immunoglobulin Levels, Lymphocyte Subsets and Clinical Features in 7 Patients (2 Females, 5 Males)[a] with Predominately T Cell Immunodeficiencies

			Immunoglobulins											Clinical events						
	Age (year)						Absolute count/mm^3		Percentage of lymphocytes (%)					Recurrent						
Patient/sex	Onset	Now	IgM (mg/dL)	IgG (mg/dL)	IgA (mg/dL)	IgE (IU/L)	Lymphocyte	Neutrophil	CD3	CD4	CD8	CD19	CD16/56	sinopulmonary infections	Bronchi ectasis	Chronic diarrhea	Failure to thrive	Sepsis and pathogen(s)	Severe skin infections[d]	Other presentations
Wiskott–Aldrich syndrome																				
22/M	0.2	2 (dead)	—	—	—	—	1445	4318	32	12	25	10	—	+		+				GI bleeding, DIC
23/M	1.5	2 (dead)	146	1540	103	2172	1318	6222	48	28	6.4	3	8	+				*Salmonella Group B* *Staphylococcus aureus*	+	Thrombocytopenia, duodenal ulcer, osteomyelitis, splenomegaly, CMV pneumonitis, GI bleeding, meningits, seizure, HCV hepatitis
Ataxia telangiectasis (DNA breakage associated syndrome)																				
24/F	5	12	410	967	<6	5	10900	8829	67	—	—	12	—	+			+			Hepatosplenomegaly, lymphadenopathy, Cerebellar atrophy, mental retardation, developmental delay, aseptic meningitis, lymphoma at 5 years (family history), alfa-fetoprotein 334 μg/L
25/M	1	15	212	993	<6	—	1254	4620	74	45	22	9	18		+		+			Cerebellar atrophy, intension tremor, horizontal nystigmus, alfa-fetoprotein 354 μg/L
DiGerge syndrome																				
26/F	0.1	4	26	562	30	—	1654	3142	72	42	25	12	6	+		+		*Salmonella spp. Enterobacter cloacae*		Interrupted aortic arch, truncus arteiosus, anemia, thrombocytopenia, GI bleeding
27/M	0.1	3	54	825	24	—	2386	2184	51	31	16	17	8				+			Tetralogy of Fallot
Chronic mucoutaneous candidiasis																				
28/M	2	13	46	1154	45	110	4251	3824	73	10	2	13	11							Onychomycosis, candidiasis, absence antibody to *Candida albicans*

Note. GI, gastro-intestinal; DIC, disseminated intravascular coagulopathy; CMV, cytomagalovirus; HCV, hepatitis C virus.

[a]T cell proliferation tests were performed in four patients, showing decreased in all patients (patients 22, 23, 24, and 28). Pair-serum of polio-vaccine titers were done in two patients, and normal (patients 24 and 28). NK activity was not detected.

[b]Patients with predominately T cell immunodeficiencies had not bronchiectasis and severe skin infections.

Table VII. The Immunoglobulin Levels, Lymphocyte Subsets and Clinical Features in 9 Patients (2 Females, 7 Males)[a] with Defects of Phagocyte Function, Including Interferon-γ Associated Immunodeficiency

	Age (year)		Immunoglobulins				Absolute count/mm^3		Percentage of lymphocytes (%)					Clinical events			
Patient/sex	Onset	Now	IgM (mg/dL)	IgG (mg/dL)	IgA (mg/dL)	IgE (IU/L)	Lymphocyte	Neutrophil	CD3	CD4	CD8	CD19	CD16/56	Recurrent sinopulmonary infections	Sepsis and pathogen(s)	Severe skin infections	Other presentations
Chronic granunomatous disease																	
29/M	2	9 (dead)	251	1568	45	—	4147	30537	84	65	22	10	7	+	*Salmonella* spp. *Candida albicans Mycobacteria tuberculosis*		Hepatosplenomegaly, empyema, meningitis,seizure, PCP pneumonia, asperginosis, respiratory failure
30/F	14	35	165	1450	86	—	2834	28698	82	43	35	12	5	+	*Mycobacteria tuberculosis*		Mycobacteria tuberculosis infection in bone, joint, ankle
Interferon-γ associated immunodeficiency																	
31/M	12	20	601	1580	478	—	2502	3870	79	45	28	16	7		*Salmonella enteritis D*		HCV hepatitis, meningitis, seizure, good response to IFN-γ
32/M	2	5	51	802	43	641	6696	1944	67	40	22	8	9	+	*Salmonella enteritis D Salmonella Group B*	+	Decreased TNF-α production stimulated by IL-12
Hyper IgE syndrome																	
33/M	0.5	4	97	578	18	5260	3376	14559	56	—	—	10	—		*Pseudomonas aeruginosa*	+	Liver abscess, hepatomegaly, ascites, lymph adenitis
34/M	0.25	9	109	1807	223	5960	2368	1885 (AEC)	66	30	31	17	11			+	Severe varicella, atopic-like dermatitis
35/F	9	19	288	3160	226	7920	3247	3552 (AEC)	74	—	—	8	—				Aseptic meningitis, atopic-like dermatitis
36/M	2	10	92	162	136	4940	1953	6417	80	39	33	11	9		*Staphylococcus aureus*	+ (Face)	Lip cellulites, elevated IgE to *S. aureus* and *Candida*
37/M	0.2	9	104	1124	68	6836	4020	3534	84	51	33	12	6			+	Severe varicella, atopic-like dermatitis

Note. AEC, absolute eosinophil count.

[a]T cell proliferation tests were performed in four patients, showing normal all (patients 30, 31, 35, and 36). Pair-serum of polio-vaccine titers were done in three patients, and normal (patients 30, 31, and 35). NK activity was detected in two patients and normal (patients 30 and 35).

[b]Patients with phagocyte dysfunction had not bronchiectasis, chronic diarrhea and failure to thrive in our study.

Table VIII. The Survey of Molecular and Genetic Evidence in 11 Patients[a] from Eight Unrelated Families

Mutation gene patient/family	Genomic DNA mutation (nucleotide[b])	Predicted effect on protein	Affected domain	Protein demonstration		References[b]
				Expression level	Detected method	
Btk gene (Xq21.3)						
3, 4	1694A > T (exon 15)	Asp 521 Val	Kinase	Absence	Flow cytometery	Vetrie D *et al.*[b]
5	1132T > C (exon 12)	Tyr 334 His	Kinase	Decreased	Flow cytometery	Vetrie D *et al.*[b]
CD40L gene (Xq26.3-27)						
13,14,15	526T > A (exon 5)	Tyr 169 Asn	TNF	Absence	Flow cytometery	Hollenbaugh D *et al.*[b]
16	307A > T (exon 2)	Lys 86 Stop	EC	Absence	Flow cytometery	Hollenbaugh D *et al.*[b]
IL2RG gene (Xq13.1)						
18	234T > G (exon 2)	Tyr 74 Gly	Conserved cystein	Decreased	Flow cytometery	Gene Bank NM000206
WASP gene (Xp11.22-23)						
23	Huge deletion, involving promoter, exon 1 and exon 2	Not-detected mRNA	Whole	Absence	Western blot	Zhu Q *et al.*[b]
Deletion of 22q11.2						
26	Deleted by FISH	Deleted	Whole	ND	ND	Gene Bank NM000051
27	Deleted by FISH	Deleted	Whole	ND	ND	Gene Bank NM000051

Note. TNF, Tumor Necrosis Factor Homology domain; EC, extracellular; TM, transmembrane; ND, not done; FISH, fluorescent in situ hybridization.

[a]The eight biologic mothers of these 11 patients are carries except that patient 18 is *de novo*.

[b]Nucleotide number is based on the sequence data on Gene Bank and described by Vetrie D, Vorechovsky I, Sideras P *et al.*: The gene involved in X-linked agammagloulin-emia is a member of the src family of protein-tyrosine kinase. Nature 361: 226–233; 1993; Hollenbaugh D, Grosmaire LS, Kullas CD, *et al.*: The human T cell antigen gp39, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of gp39 with B cell co-stimulatory activity. EMBO J 11: 4313–4321; 1992; Zhu Q, Watanabe C, Liu T, *et al.*: Wiskott–Aldrich syndrome/X-linked thrombocytopenia: WASP gene mutations, protein expression, and phenotype. Blood 90: 2680–2689; 1997.

Table IX. Comparing Distributions of PID in Other Registries According WHO Criteria and Classification

	Chang Gung Children's Hospital ($n = 37$) Female/male	(%)	Singapore ($n = 35$) (%)	Japan, Swiss, Sweden, Norway Spain, Italy, Switzerland, Australia, Latin America, American and Japan ($n = 3369$) (%)
Predominately antibodies deficiencies	3/14	46	51	65
Phagocyte disorders	2/7	24	26	10
Combined immunity deficiencies	1/3	11	14	15
T cell-mediated immunity	2/5	19	9	5
Complement deficiency		0	0	5

Antibody deficiencies were the most common (23–32). However, phagocyte disorders replaced combined immunodeficiencies as the second most common (24–32). Patients with critical combined immunodeficiencies, in our study, may be under-represented because they often die in infancy and go undiagnosed and without referrals to a tertiary care center (33). None of our patient had complement deficiencies, similar to the report from Singapore (23), while 3–5% had complement deficiencies in other nations (Table IX) (24–32). Such cases could possibly have been under reported, although secondary complement deficiencies were almost all attributable to rheumatoid disorders, especially the higher incidence of systemic lupus erythematosus in Taiwan and Singapore (23). The incidence of PID in our study is estimated at 2.17 per 100,000 live births, lower than that for other people of Chinese descent in Singapore, with 2.65 per 100,000 (23), and much lower than in Sweden, with 8.4 per 100,000 live births (24). These statistics reflect that our data under-estimate the disease burden in Taiwan and do not include most patients with adult-onset (over 18 years old) CVID, those with asymptomatic/mild symptomatic IgA, IgG subclass deficiencies, complement deficiencies or some eventful combined immunodeficiencies.

The most common PID presentation among our patients was recurrent bacterial sinopulmonary infection, which is consistent with the finding that antibody deficiency constitutes the majority of PID. In patients with antibody deficiencies, *Pseudomonas* was the most frequently isolated microorganism in septicemia (9/18 episodes in 11 patients). Reasonably, Taiwan pediatric patients who present with recurrent sinopulmonary infections and/or *Pseudomonas* septicemia should be screened for possible antibody deficiency PID. Failure to diagnosis and treat early leads to substantial morbidity and mortality (33–35). Early intervention with regular IVIG in patients with antibody deficiencies decreased the incidence of recurrent infections and irreversible bronchiectasis (36). The deaths of two patients with agammaglobulinemia were due to the inconvenience of IVIG two decades ago in Taiwan (patient 1) and late and irregular IVIG treatment in another patient (patient 4). For patients with severe T-cell and phagocytic defects, the only hope of cure is through stem cell transplantation (37–40) or gene therapy (41, 42). Unfortunately, five patients (patients 19, 20, 22, 23, and 29) died while waiting for appropriate bone marrow donors, even though they received regular IVIG treatment. Patient 18 with *IL2RG* mutation was diagnosed at 3 months of age and received unrelated umbilical cord stem cell transplantation for treatment of *Mycobacterium bovis* infection, caused by vaccination with BCG. Currently, he has 29% donor chimerism in his lymphocyte population and has partial T cell function 3 months after transplantation.

Earlier in the course of the study, we did not have as good knowledge of immunoglobulin development as we do now; our physicians used to interpret pediatric serum immunoglobulin levels according to adult normal values. Thus, pediatric patients with selective IgG, IgA or IgM immunodeficiencies were erroneously suspected and overdiagnosed. Of course, these patients really had normal serum levels of immunoglobulins, according to their age. Additionally, without a set of diagnosis codes for secondary immunodeficiencies comparable to those for PID, our physicians also put other diseases that caused second immunodeficiencies, rather than PID, into the diagnosis codes of PID. Subsequently, the medical records showed an incorrect PID incidence of 188 per 100,000 cases (403 in total 214,363 inpatients and outpatients). This implies a ratio of primary/secondary immunodeficiencies in our cohort of almost 1/10 (37/366). Based on advances in immunological profiling and better definitions of the associated molecular abnormalities, we have recognized more PID since 1997 (as Table III). This progression of knowledge enabled physicians to speculate accurately on whole clinical course of PID and carrier detection, and classified

patients with "overlap" phenotypes into those with different genotypes: patients with mutated *Btk* (patients 3 and 4) and *CD40L* (patient 16) genes, patients with decreased major histocompatibility complex I expression (patient 21) and interferon-γ associated immunodeficiency (patients 31 and 32) were separated from those patients with the diagnosis of CVID (patients 3, 4, 16, and 21) and "unspecified disorders of the immune mechanism (patients 31 and 32)."

Alternatively, some PID patients were concealed in diagnosis codes for recurrent infections. Cunningham-Rundles *et al*. found more PID patients by "sorting on ICD-9 codes for recurrent infections in different organs and systems." They identified 17 more patients with PID, including CVID/IgA deficiency, IgG subclass deficiency, combined immunodeficiencies and DiGeorge syndrome (43). Computer-selected individuals prone to recurrent infections reveal an undiagnosed minority of patients with PID. The current concept of PID will soon begin to reflect the true incidence of PID in Taiwan.

CONCLUSION

Antibody deficiencies are the most frequently diagnosed type of PID in a Taiwan pediatric tertiary care medical center. We emphasis that the number of diagnosed patients with PID reported here does not reflect the actual prevalence of these orders, because some of the patients with mild forms of CVID or/and subclass deficiencies were neglected, and those with severe forms died early in life, before referral to our medical center. This review is important to raise awareness within the medical community to facilitate collection and dissemination of information concerning new immunodeficiency diseases. To keep the knowledge of PID updated, periodic contact must be maintained with all physicians in referral medical centers. We hope that construction of a PID registry system might inspire physicians' awareness of such diseases in the near future.

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