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Molecular Diagnosis of Severe Combined Immunodeficiency—Identification of *IL2RG*, *JAK3*, *IL7R*, *DCLRE1C*, *RAG1*, and *RAG2* Mutations in a Cohort of Chinese and Southeast Asian Children

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Abstract Severe combined immunodeficiencies (SCID) are a group of rare inherited disorders with profound defects in T cell and B cell immunity. From 2005 to 2010, our unit performed testing for *IL2RG*, *JAK3*, *IL7R*, *RAG1*, *RAG2*, *DCLRE1C*, *LIG4*, *AK2*, and *ZAP70* mutations in 42 Chinese and Southeast Asian infants with SCID adopting a candidate gene approach, based on patient's gender, immune phenotype, and inheritance pattern. Mutations were identified in 26 patients, including *IL2RG* (n=19), *IL7R* (n=2), *JAK3* (n=2), *RAG1* (n=1), *RAG2* (n=1), and

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DCLRE1C (n=1). Among 12 patients who underwent hematopoietic stem cell transplantation, eight patients survived. Complications and morbidities during transplant period were significant, especially disseminated bacillus Calmette–Guérin disease which was often difficult to control. This is the first cohort study on SCID in the Chinese and Southeast Asian population, based on a multi-centered collaborative research network. The foremost issue is service provision for early detection, diagnosis, management, and definitive treatment for patients with SCID. National management guidelines for SCID should be established, and research into an efficient platform for genetic diagnosis is needed.

Keyword Severe combined immunodeficiency · SCID · molecular diagnosis · genetics · Chinese · Asian

Introduction

Severe combined immunodeficiencies (SCID) constitute a heterogeneous group of genetic disorders characterized by profound defects in cellular and humoral immunity. The overall incidence of SCID is estimated to be one in 75,000–100,000 live births [1]. Infants with SCID often suffer from fatal opportunistic infections caused by bacteria, *Pneumocystis jiroveci* pneumonia (PCP), cytomegalovirus (CMV), mycobacteria, or fungi. Common respiratory viruses such as adenovirus, respiratory syncytial virus, and parainfluenza virus may lead to severe pneumonia, respiratory failure, and acute lung damage. Patients may also have non-infectious clinical manifestations such as graft-versus-host disease (GVHD) caused by maternal lymphocyte engraftment or non-irradiated blood product transfusion [2]. Some patients with "leaky SCID" caused by hypomorphic mutations may present with atypical manifestations such as immune cytopenia and granulomatous lesions [3].

At present, 16 genes are known to cause SCID with variable clinical and immunophenotypes, with a few others causing abnormal T cell activation or functions [4]. Molecular defects implicated for SCID can be classified into four broad categories: defects in cytokine receptor pathways (γc, Jak3, IL7Rα), T cell receptor signaling defects (CD45, CD3ε/δ/ζ chains), V(D)J recombination defects (Rag1/2, Artemis, Cernunnos, DNA ligase 4), and accumulation of toxic metabolites related to aberrations in basic cellular processes (ADA, PNP) [5]. Coronin-1A, an actin regulator which plays an important role in the egress of T cells from the thymus and lymph nodes, was described in patients with T−B+NK+ SCID [6, 7]. Recently, AK2 was found to be the causative gene for reticular dysgenesis [8, 9]. The challenge is to develop an efficient platform for genetic diagnosis, which would also open new opportunities of discovering novel genes and pathogenetic mechanisms for SCID.

In this study, we described the clinical presentations and molecular diagnosis of 42 infants with SCID, which is the largest collection from Southeast Asia. We sought to identify the active issues in the management of patients with SCID, which would serve as initiation for national, multi-center epidemiological studies and research development.

Methodology

Patients

From 1991 to 2009, nine patients with SCID were diagnosed in Hong Kong and seven received hematopoietic stem cell transplantation (HSCT) in our unit. Since 2001, we received blood samples of 33 patients for molecular confirmation of SCID from 14 hospitals in mainland China, Taiwan, Singapore, Malaysia, Thailand, and the Philippines. Based on research collaboration, mutation detection was performed at no cost to the patients or the referring institutions. Clinical and immunological data were provided by the referring doctors.

Diagnostic Criteria

Diagnosis of SCID was established according to the Pan-American Group for Immunodeficiency/European Society

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for Immunodeficiencies diagnostic criteria for primary immunodeficiencies (PID) [10]. Definitive diagnosis was made if a male or female patient less than 2 years of age had either (a) engraftment of transplacentally acquired maternal T cells or (b) less than 20% CD3+ T cells, an absolute lymphocyte count (ALC) of less than 3,000/mm^3, and confirmed mutation of a causative gene for SCID or adenosine deaminase (ADA) deficiency diagnosed by reduced ADA activity of less than 2% of control. Probable diagnosis of SCID would be made if a male or female patient less than 2 years of age had (a) less than 20% CD3+ T cells, an ALC of less than 3,000/mm^3, and proliferative responses to mitogens less than 10% of control or (b) the presence of maternal lymphocytes in the circulation. Lymphocyte proliferation assay was performed by assessing lymphocyte proliferative response toward mitogens including phytohemagglutinin, concanavalin A, and pokeweed mitogen. ADA deficiency was investigated by measuring red cell lysate enzyme activities, red cell nucleotide profile, and urine deoxyadenosine level (supplementary information) [11, 12].

Genetic analysis for SCID was performed in patients with (1) significant lymphopenia and hypogammaglobulinemia for age; (2) typical clinical presentations such as failure to thrive, recurrent respiration tract infections and diarrhea, Ommen phenotype and "indicator" infections such as PCP, bacillus Calmette–Guérin (BCG) disease, disseminated CMV disease, persistent oromucosal candidiasis, and systemic fungal infections; and (3) lymphocyte subset pattern compatible with SCID. Due to resource limitation in some referral centers, maternal engraftment studies and lymphocyte proliferation test might not be performed.

Data Collection

Demographic data, clinical features, ALC, lymphocyte subset, and serum immunoglobulin levels at the time of diagnosis were recorded. A definite family history of SCID was considered if any sibling or relatives had been diagnosed to have SCID. A family history of SCID was suspected if siblings or relatives suffered from serious recurrent infections in infancy, usually resulted in death.

Details of HSCT for 12 patients from five transplant centers were collected, including stem cell source, degree of matching between donor and recipient, conditioning regimen, stem cell manipulation, GVHD prophylaxis, occurrence of acute and chronic GVHD, other transplant complications, immunoreconstitution, and outcome. Reconstitution of humoral immunity was assessed by serum immunoglobulin levels and the ability to generate antibodies to post-transplant immunization including tetanus and polio vaccines. Reconstitution of cellular immunity was assessed by lymphocyte subsets and lymphocyte proliferation toward mitogen stimulation. Study on thymic function was not performed.

SCID Gene Analysis

A candidate gene approach was adopted to select appropriate genes for PCR direct sequencing, based on the gender, clinical features, inheritance pattern, and lymphocyte subset data. Details of sequencing methodology are provided as supplementary information. *IL2RG* and *JAK3* genes were sequenced for boys and girls with T−B+NK− SCID, respectively. *IL7RA* gene was sequenced for patients with T−B+NK+ SCID. For T−B−NK+ SCID, candidate genes include *RAG1*, *RAG2*, *DCLRE1C*, and *LIG4.* For Omenn syndrome, in addition to those accounting for T−B−NK+ SCID, *IL2RG* and *IL7R* genes were also sequenced. Sequencing of *AK2* and *ZAP70* genes were performed for patients with suspected reticular dysgenesis and CD8 lymphocytopenia, respectively. For patients with X-linked SCID, confirmation of carrier status was performed in patients' mothers, female siblings, or maternally related female family members. In autosomal recessive SCID, carrier status was performed for parents.

Results

Demographics

The cohort consisted of 30 boys and 12 girls (Table 1). Thirty-four patients were ethnic Chinese, while the others were Pakistani (n=3), Malay (n=2), Filipino (n=1), Thai (n=1), and Arab (n=1). The median age of onset was 2 months (range 10 days–11 months), while the median age of diagnosis was 4 months (range day 1–27 months). Presymptomatic investigation was performed, and the diagnosis of SCID was confirmed in a newborn baby girl (P33b) whose brother (P33a) was diagnosed to have T−B−NK+ SCID.

Eight boys with T−B+NK− SCID had family history of early infant deaths related to infections, while two boys with T−B−NK+ SCID had such family history. Parental consanguinity occurred in three kindreds.

Infections

Respiratory tract infections and chronic diarrhea were the most common types of infections seen in patients with SCID. Most patients (83.3%) had recurrent episodes of acute bronchiolitis or pneumonia. Three patients developed bronchiolitis obliterans. PCP was documented in only two patients. Pulmonary aspergillosis was diagnosed in one patient.

Table 1 Clinical features and gene mutations of 42 infants with SCID

Patient	Sex	Ethnicity	Age of onset (months)	Age of diagnosis (months)	Clinical features	ALC ($\times 10^9$/L)	CD3+/μL (%)	CD4+/μL (%)	CD8+/μL (%)	CD19+/μL (%)	CD16/56+/μL (%)
B+ SCID											
T−B+NK−											
P1	M	Chinese	0.5	2	RTI, candidemia	1.84	0%	0%	0%	95%	4%
P2	M	Chinese	2	4	RTI, hepatosplenomegaly, anemia	1.0	5.2%	3.8%	1.6%	89.2%	N/A
P3	M	Chinese	2	4	RTI, anemia	1.16	0.3%	0.2%	0.1%	83.8%	0.2%
P4	M	Chinese	3	5.5	RTI, GE, anemia	1.11	1%	0%	0%	41%	52%
P5	M	Chinese	2	4.5	RTI, oral candidiasis, GE, local BCG abscess, CNS bacteremia, suspected PCP and pulmonary aspergillosis, hepatosplenomegaly	0.7	1 (0.1%)	N/A	N/A	638 (94.7%)	35 (5.2%)
P6	M	Chinese	3	8	RTI, GE, oral candidiasis, *E. coli* bacteremia, hepatosplenomegaly	1.3	0.08%	0.09%	0%	98.6%	0.74%
P7	M	Chinese	1	6	RTI, disseminated BCG	1.1[c]	4 (0.5)	0 (0%)	1 (0.1%)	659 (92.9%)	9 (1.3%)
P8	M	Chinese	0.6	1	RTI	1.1	1%	0%	0%	89%	10%
P9	M	Chinese	3	4	RTI, intestinal candidiasis (*Candida lusitaniae*), anemia, thrombocytopenia, hepatosplenomegaly	1.1	0%	0%	0%	97%	1%
P10	M	Chinese	4	4	RTI, rotavirus GE, BCG-itis, anemia	N/A	0%	0%	0%	89%	0%
P11	M	Chinese	1	2	RTI, oral candidiasis, GE	1.0	2%	0%	1%	89%	1%
P12	M	Chinese	2	4	RTI	1.86	12%	1%	5%	86%	0%
P13	M	Chinese	3	3	RTI, CNS bacteremia	N/A	16%	3%	2%	82%	0%
P14[a]	M	Chinese	1	2	RTI, GE	0.5	N/A	N/A	N/A	N/A	N/A
P15	M	Chinese	0.7	2	RTI	0.31	0%	0%	0%	87%	0%
P16	M	Chinese	0.3	5	RTI, skin abscess	1.41	110 (11%)	25 (6.5%)	76 (6.5%)	779 (84.9%)	12 (1.3%)
P17	M	Chinese	2	3	GE, hepatosplenomegaly	1.4	0%	0%	0%	93%	3%
P18	M	Chinese	4	5	RTI, GE, oral candidiasis	4.94	0 (0%)	0 (0%)	0 (0%)	4,631 (98%)	142 (2%)
P19	M	Chinese	2	4	RTI, GE, local BCG abscess, CMV infection	1.72	0%	0%	0%	68%	29%
P20	M	Chinese	4	5	RTI, candidemia, hepatosplenomegaly	1.84	1%	0%	0%	80%	16%
P21	F	Chinese	1	1	RTI, disseminated CMV	0.49[c]	6 (1.3%)	4 (0.8%)	1 (0.2%)	268 (54.3%)	20 (4.0%)
P22	M	Chinese	2	8	RTI, oral candidiasis, GE, local and axillary BCG abscess, retroperitoneal lymphadenopathy, hepatosplenomegaly	0.2	17.3%	10.3%	7%	80.9%	1.8%
P23	F	Arab	0.2	2	Extensive MRSA cellulitis, RTI, CMV antigenemia	2.1	396	157	187	574	137
P24	F	Chinese	2	3	PCP, CMV antigenemia	1.59[c]	624 (39.3%)	248 (15.6%)	363 (22.8%)	937 (58.9%)	47 (3.0%)
T−B+NK+											
P25	F	Chinese	4	5	Rotavirus GE, disseminated BCG, PCP, rhinovirus infection, bronchiolitis obliterans with organizing pneumonia	1.89[c]	167 (9.7%)	125 (7.3%)	12 (0.7%)	1,010 (58.8%)	501 (29.9%)
P26	M	Filipino	1	6	RTI, recurrent oral candidiasis, local BCG disease	1.21	147 (12.2%)	17 (1.4%)	16 (1.5%)	756 (62.5%)	411 (33.9%)
P27	F	Chinese	2	2	Recurrent diarrhea, multi-organ failure	1.3	2%	2%	0%	80%	10%

B− SCID											
T+B−NK+ (leaky SCID)											
P28	M	Chinese	2	16	Severe eczema, RTI, recurrent *Salmonella* GE, recurrent herpes zoster, eosinophilia	7.64	3,963 (51.9%)	215 (2.8%)	2,900 (38%)	271 (3.5%)	3,704 (48.5%)
T−B−NK+/T^{low}B−NK+											
P29	F	Chinese	4	6	Regional BCG disease	0.74	1%	0%	0%	1%	90%
P30	M	Chinese	2	3	RTI, parainfluenza 3 pneumonitis, GE, regional BCG disease	0.72[c]	7 (1.0%)	2 (0.2%)	1 (0.1%)	1 (0.1%)	674 (93.4%)
P31	M	Chinese	4.5	8	Disseminated BCG with pathological fracture of right humerus, parainfluenza I pneumonitis, *Aspergillus fumigatus* pneumonia, B-lymphoproliferative disease	0.31	86	3	2	2	121
P32	F	Chinese	2	5	Recurrent pneumonia, chronic diarrhea	0.84[c]	343 (40.8%)	211 (25.1%)	234 (27.8%)	208 (24.7%)	198 (23.5%)
P33a[b]	M	Pakistani	2	5	Recurrent oral and perineal candidiasis, diarrhea, lymphopenia, eosinophilia	0.59[c]	12 (2.0%)	3 (0.3%)	5 (0.9%)	16 (2.7%)	479 (80.0%)
P33b[b]	F	Pakistani	–	Newborn	Nil	2.2[c]	25 (1.1%)	3 (0.1%)	3 (0.1%)	30 (1.3%)	1,440 (64.0%)
P34	F	Thai	0.5	8	RTI, chronic diarrhea, oral candidiasis	0.29	244 (24.4%)	132 (13.2%)	100 (10.0%)	158 (15.8%)	520 (52.0%)
P35	M	Malay	2	2	Fulminant *Klebsiella* pneumonia bacteremia, hepatosplenomegaly, pancytopenia and DIC, pulmonary hemorrhage	0.8	2%	1%	0%	0%	96%
P36	F	Chinese	11	27	RTI (RSV, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*), *Staphylococcus warneri* bacteremia, bronchiolitis obliterans, persistent diarrhea, lymphopenia especially CD8 proportion	0.74	582 (78.8%)	520 (70.4%)	50 (6.8%)	18 (2.4%)	96 (13%)
T−B−NK−											
P37	M	Chinese	Newborn	Day 3	Neonatal meningitis, pancytopenia	0.53	311 (58.9%)	189 (35.8%)	124 (23.4%)	73 (13.8%)	20 (3.7%)
Omenn phenotype											
P38	M	Chinese	1	4	RTI, UTI (*Candida*, *E. coli*), *Salmonella* GE, disseminated varicella zoster, candidiasis, influenza B, MRSA infection, eosinophilia	2.06	9.5%	3.9%	7.0%	86.8%	3.7%
P39	M	Chinese	0.5	1	RTI, *Enterococcus* bacteremia, hepatosplenomegaly, generalized erythematous skin rash, eosinophilia, raised serum IgE	10.9	N/A	N/A	N/A	N/A	N/A
P40	F	Malay	0.3	3	RTI, candidiasis, GE, *Klebsiella pneumoniae* bacteremia, generalized erythematous skin rash	0.26	N/A	N/A	N/A	N/A	N/A
P41	F	Pakistani	0.3	3	GE, skin abscess, RTI, erythematous skin rash, deranged liver function, eosinophilia	3.38[c]	3,193 (94.4%)	2,692 (79.6%)	623 (18.4%)	34 (1.0%)	34 (1.0%)

Table 1 (continued)

Patient	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	Gene	Nucleotide change	Codon change or predicted splicing aberration	Parental carrier status	HSCT	Outcome
B+ SCID									
T−B+NK−									
P1	400	28	43	IL2RG	c.849delG	p.V279fsX293	Carrier (M)	No	Died
P2	63	<24	<16	IL2RG	g.IVS6-72 to g.IVS7-11del487	Exon7del	Carrier (M)	No	Died
P3	288	<25	58	IL2RG	c.324-325CA>G	p.H104fsX146	Not done	No	Died
P4	306	<11	76	IL2RG	c.691G>A	p.R226H	Carrier (M)	No	Died
P5	739 (post-IVIG)	<6.7	9	IL2RG	c.385T>C	p.L124P	Not done	No	Lost follow-up
P6	300	11	10	IL2RG	g.IVS6+5G>A	Predicted aberrant splicing	Carrier (M)	No	Lost follow-up
P7	73	<6	17	IL2RG	c.576C>T	p.Q188X	Carrier (M)	Yes	Alive and well
P8	395	4	70	IL2RG	c.684C>T	p.R224W	Carrier (M)	No	Died
P9	1,430 (post-IVIG)	9	33	IL2RG	c.868G>A	Change of exon6/intron6 splice junction from CG/GT to CA/GT, predicted aberrant splicing	Carrier (M)	No	Died
P10	453	13	98	IL2RG	g.IVS6+5G>A	Predicted aberrant splicing	Not done	No	Alive
P11	461	<6.7	44	IL2RG	c.390C>T	p.Q126X	Not done	Not known	Lost follow-up
P12	133	<6.7	36	IL2RG	c.868G>A	Change of exon6/intron6 splice junction from CG/GT to CA/GT, predicted aberrant splicing	Carrier (M)	No	Died
P13	201	<7	55	IL2RG	c.736G>T	p.S241I	Carrier (M)	Not known	Lost follow-up
P14[a]	171	<7	12	IL2RG	c.435delC	p.Q141fsX146	Carrier (M)	No	Died
P15	318	<7	11	IL2RG	c.373-374insA	p.K120fsX167	Carrier (M)	No	Died
P16	51	3	67	IL2RG	c.868G>A	p.R285Q	Carrier (M)	Yes	Died
P17	871 (post-IVIG)	<7	13	IL2RG	c.521delG	Q169fsX170	Carrier (M)	No	Alive
P18	110	2	20	IL2RG	c.868G>A	Change of exon6/intron6 splice junction from CG/GT to CA/GT, predicted aberrant splicing	Not done	Yes	Alive
P19	660 (post-IVIG)	14	19	IL2RG	c.725G>A	p.W237X	Carrier (M)	Pending	Alive
P20	214	7.1	6.7	IL2RG	No mutation found	–	–	No	Died
P21	515	96	53	JAK3	homozygous g.IVS14-11G>A, c.1914-1915insCCCCCTTAG	p.K641X	Carrier (M, F)	No	Died
P22	242	25	17	JAK3	Compound heterozygous c.115-116insC, c.1744C>T	p.Q39fsX51, p.R582W	Carrier (M, F)	No	Died
P23	Reduced	Reduced	Reduced	JAK3, IL7R	No mutation found	–	–	Yes	Died
P24	98	<8	11	JAK3, IL7R	No mutation found	–	–	No	Died
T−B+NK+									
P25	200	15	32	IL7RA	Heterozygous c.65G>T, g.IVS2+2T>A	p.S22I, and predicted aberrant splicing	Not done	Yes	Died 11 months post-transplant
P26	284	68	39	IL7RA	Homozygous c.562delC	p.L188X	Carrier (M, F)	No	Alive
P27	1,060 (post-IVIG)	<6.7	108	IL7RA	No mutation found	–	–	No	Died
B− SCID									
T+B−NK+ (leaky SCID)									
P28	451	<7	30	RAG1	Heterozygous c.1178delG,	p.393fsX402, p.R699W	Not done	Yes	Alive and well

					c.2095C>T				
T−B−NK+/T^{low}B−NK+									
P29	77	<23	<17	RAG2	Compound heterozygous c.-28G>C; c.358delG	Change of exon 1/intron 1 splice site from AG/GT to AC/GT, predicted aberrant splicing; p.V120fsX130	Carrier (M, F)	No	Died
P30	58	<7	10	DCLRE1C	Compound heterozygous g.IVS3-1G>T, c.247-306del60bp c.632G>T	Skipped exon 4, p.I83-E102del p.G211V	Carrier (M, F)	Yes	Alive and well
P31	<140	<25	<76	RAG1, RAG2 DCLRE1C, LIG4	No mutation found	–	–	Yes	Alive and well
P32	69	<12	<7	RAG1, RAG2, DCLRE1C	No mutation found	–	–	Yes	Alive and well
P33a[b]	81	116	7	RAG1, RAG2, DCLRE1C, LIG4	No mutation found	–	–	Yes	Alive and well
P33b[b]	1,050 (newborn)	1.5	<7	Not done[c]	–	–	–	Yes	Alive and well
P34	43	<5.6	<19	RAG1, RAG2, DCLRE1C, LIG4	No mutation found	–	–	No	Died
P35	871 (post-IVIG)	63	43	RAG1, RAG2, DCLRE1C, LIG4	No mutation found	–	–	No	Died
P36	226	<6	21	RAG1, RAG2, DCLRE1C, ZAP70	No mutation found	–	–	No	Died
T−B−NK−									
P37	537	<7	6	AK2	No mutation found	–	–	No	Died
Omenn phenotype						–	–		
P38	<164	<27.5	17	IL2RG, JAK3, RAG1, RAG2	No mutation found	–	–	No	Died
P39	647 (post-IVIG)	50	316	RAG1, RAG2	No mutation found	–	–	No	Died
P40	172	<5	<20	IL2RG, RAG1, RAG2, DCLRE1C, LIG4, IL7RA	No mutation found	–	–	No	Died
P41	37	<7	19	RAG1, RAG2, DCLRE1C, LIG4	No mutation found	–	–	Yes	Died on day +13 post-transplant

Absolute numbers of T and B cells were provided when available, otherwise indicated as percentage of total lymphocytes

ALC absolute lymphocyte count, *CNS* coagulase-negative *Staphylococcus*, *F* father, *GE* gastroenteritis, *M* mother, *RTI* respiratory tract infections, *RSV* respiratory syncytial virus

[a] Patient died and genetic diagnosis was established according to maternal carrier status

[b] P33a and P33b are siblings born to consanguineous parents. Mutations in RAG1, RAG2, DCLRE1C, and LIG4 were not identified in P33a (elder brother) and mutation studies were not performed for P33b

[c] Patients with documented diminished lymphocyte proliferation toward mitogen stimulation less than 10% of control

Persistent oral candidiasis was present in 12 patients (28.6%), and two of them had candidemia. BCG complications occurred in ten patients (23.8%), including abscess formation at the site of inoculation (n=6), regional axillary lymphadenopathy (n=3), and disseminated BCG (n=3). Two patients had disseminated CMV disease.

Immunological Features

The clinical and immunological characteristics of all patients were shown in Table 1. Except patients with Omenn syndrome, all others had lymphopenia $<2.5\times10^9$/L (median=1.1×10^9/L, range 0.26–2.2×10^9/L). All patients had hypogammaglobulinemia. Twenty-seven patients had B+ SCID (T−B+NK−, n=23; T^{low}B+NK−, n=1; T−B+NK+, n=3), 11 had B−SCID (T+B−NK+ leaky SCID, n=1; T−B−NK+, n=6; T^{low}B−NK−, n=3; T−B−NK−, n=1), and four had Omenn phenotype. P37 had suspected reticular dysgenesis characterized by pancytopenia and neutropenia.

Molecular Defects

IL2RG mutations were identified in majority of male infants (19 out of 21) with classical clinical presentations and T−B+NK− phenotype. There were two recurrent mutations among 16 *IL2RG* mutations identified. 868G>A, which led to change of exon 6/intron 6 splice junction, occurred in three unrelated kindreds, while IVS6+5G> A occurred in two unrelated kindreds. A boy without *IL2RG* mutation was subsequently found to have compound heterozygous mutations in *JAK3* (P22).

Three girls had T−B+NK− SCID. One of them (P21) had homozygous G>A substitution mutation of the *JAK3* gene at nucleotide position −11 of intron 14, creating a cryptic splice site 9 bases 5′ to the intron 14/exon 15 splice junction resulting in aberrant splicing and premature stop (K641X) associated with nine-nucleotide insertion at positions 1914–1915. Both of her parents were found to be carriers of this mutation, but they were not consanguineous. *JAK3* and *IL7R* mutation was not identified in the other two female infants with T−B+ SCID.

Two infants had *IL7R* mutations. A Chinese female infant (P25) had compound heterozygous mutations (missense mutation S22I and substitution mutation at intron 2 with predicted aberrant splicing) while a Filipino boy (P26) had homozygous nonsense mutation L188X. Parents of P26 were carriers of the mutation.

P28 had leaky SCID (T+B−NK+) characterized by normal CD3 count which was predominated by CD8+ T cells with low proportions of CD4+ T cells and low numbers of B cells. He was found to have heterozygous p.393fsX402 and p.R699W mutations in *RAG1*, likely to be hypomorphic mutations with residual V(D)J recombination thus explaining his relatively less severe phenotype. P29 and P30 with typical T−B−NK+ SCID were found to have compound heterozygous mutations in *RAG2* and *DCLRE1C*, respectively. For the rest, *RAG1*, *RAG2*, and *DCLRE1C* mutations were ruled out, and *LIG4* mutation was additionally ruled out in P32, P33a, P34, and P35. Gene mutations could not be identified in any of the patients with Omenn phenotype. ADA and PNP deficiencies were ruled out by the absence of deoxyadenosine in the urine, normal red cell nucleotide profile as well as normal ADA and PNP levels in P28, P31, P32, P33a, and P33b as part of the initial investigations.

Hematopoietic Stem Cell Transplantation

From 1992 to 2010, 12 patients from our cohort underwent HSCT (Table 2). Donor source included genoidentical family donors (n=2), phenoidentical family donor (n=1), mismatched related donors (MMRD; n=3), match-unrelated donors (MUD; n=2), and unrelated cord blood (UCB) units (n=4). The median age of diagnosis was 5 months (day 1–16 months), and the median age of transplant was 8 months (2–11 months). The median interval from diagnosis to transplant was 2 months (1–5 months). Busulphan/cyclophosphamide-based myeloablative conditioning regimen was used in four patients. For transplants performed in the recent 3 years, the use of reduced-intensity conditioning (RIC) regimen with fludarabine and melphalan was favored and was given to four patients with history of severe pulmonary infections. Conditioning was not used in four patients.

The total follow-up duration was 76.0 patient-years (mean 6.3 years, 0.25–18 years). None of them required booster stem cell infusion or second transplant. Three patients died in early post-transplant period. P16 and P41 died of severe sepsis and multi-organ failure on D+11 and D+13 respectively. P23 died of idiopathic pneumonia syndrome, characterized by severe respiratory distress and diffuse radiographic infiltrates without identifiable causative pathogens. Severe GVHD occurred in two patients (P25 and P30). P25 died of severe chronic skin, pulmonary, gut, and liver GVHD at 11 months post-transplant. P30 recovered from acute GVHD with mild chronic skin GVHD only, and he was successfully weaned off systemic immunosuppressive therapy.

Severe infections were frequent in post-transplant period, often causing major morbidities. Five patients had bloodstream infections, and three had severe enterocolitis. P30 had bronchiolitis obliterans related to protracted parainfluenza III pneumonitis. BCG disease occurred in six out of 12 patients who underwent HSCT. Three patients had disseminated BCG in the post-transplant period despite continuation of anti-mycobacterial drugs. P7 had resurge of

Table 2 Hematopoietic stem cell transplant in 12 patients with SCID

Patient	Sex	Age of presentation	Age of diagnosis	Pre-transplant complications	Type	Age at transplant (months)	Year of transplant	Donor	stem cell manipulation
P32	F	2 months	5 months	Severe pneumonia requiring mechanical ventilation, chronic diarrhea, failure to thrive	T−B−NK+	10	1992	Haploidentical (father)	T cell depletion by ex vivo campath-1M and autologous complement, 98% T cell depletion
P33a	M	2 months	5 months	Recurrent oral and perineal candidiasis, chronic diarrhea	T−B−NK+	6	1994	Haploidentical (father)	Nil
P33b	F	Pre-symptomatic screen	Day 1	Nil, with prophylactic antibiotics, reverse isolation and IVIG replacement once diagnosed	T−B−NK+	2	1996	Matched sibling (sister)	Nil
P28	M	2 months	16 months	Severe eczema, recurrent *Salmonella* gastroenteritis and pneumonia	T+B−NK+ leaky SCID (RAG1)	18	1997	Matched sibling (sister)	Nil
P41	F	Day 9	3 months	Omenn phenotype, gastroenteritis, multiple skin abscesses, pneumonia, severe failure to thrive requiring parenteral nutrition support	T−B−NK+	8	1999	Match-unrelated donor	Nil
P30	M	2 months	4 months	Protracted diarrhea and severe parainfluenza III pneumonitis, regional BCG disease, severe failure to thrive	T−B−NK+ (DCLRE1C)	6	2005	Unrelated cord blood	Nil
P7	M	1 months	6 months	Recurrent pneumonia, disseminated BCG disease	T−B+NK− (IL2RG)	8	2007	Match-unrelated donor	Nil
P31	M	4.5 months	8 months	BCG osteomyelitis, bronchopneumonia caused by parainfluenza I and *Aspergillus fumigatus* requiring mechanical ventilation, rotavirus gastroenteritis, failure to thrive requiring parenteral nutrition support	T−B−NK+	6	2005	Unrelated cord blood	Nil
P25	F	4 months	5 months	Disseminated BCG disease, rhinovirus and *Pneumocystis jiroveci* pneumonia, bronchiolitis obliterans with organizing pneumonia, rotavirus gastroenteritis	T−B+NK+ (IL7RA)	8	2009	Unrelated cord blood	Nil
P23	F	0.2 months	2 months	Extensive MRSA cellulitis, severe pneumonia requiring mechanical ventilation, CMV antigenemia	T−B+NK−	4	2009	1-Ag mismatched related donor (mother)	Nil
P16	M	3 months	5 months	Recurrent pneumonia requiring prolonged mechanical ventilation, chronic diarrhea	T−B+NK− (IL2RG)	11	2010	Mismatch-related donor (mother)	CD34+ selection
P18	M	4 months	5 months	Recurrent pneumonia, rotavirus gastroenteritis, persistent oral candidiasis, severe failure to thrive	T−B+NK− (IL2RG)	8	2010	Unrelated cord blood	Nil

Table 2 (continued)

Patient	Source	Matching	Conditioning	GVHD prophylaxis	Acute GVHD	Chronic GVHD	Other complications	Immunoreconstitution	Outcome
P32	BM	3 out of 6	Busulphan/ cyclophosphamide/ campath	CycA, MTX	Skin, grade I	Nil	Hemorrhagic cystitis, urinary tract infection, HCV hepatitis, autoimmune hemolytic anemia, MRSA and *Acremonium* bacteremia, *C. difficile* colitis	98% T cells from donor (PHA-stimulated lymphocyte by karyotyping), poor B or NK cell engraftment	Alive, IVIG every 9 weeks
P33a	BM	HLA identical	Nil	CycA, MTX	Skin, grade I	nil	BCG reactivation (axillary lymph node), *Bacillus* bacteremia	All along remains lymphopenic <1, CD4 remains <500/mL, CD20 very low <20/mL at 7 years post-BMT, still poor lymphocyte proliferation towards mitogens	Alive, regular IVIG every 16 weeks
P33b	BM	HLA identical	Nil	CycA, MTX	Nil	Nil	Nil	Successful lymphocyte engraftment 1 month post-BMT, B cells remain low <100/mL at 7 years. Lymphocyte proliferation normal at 4 years	Alive, IVIG stopped at 3 years
P28	BM	HLA identical	Busulphan/ cyclophosphamide/ ATG	CycA, short MTX	Skin	Nil	*Enterococcus* and *Klebsiella* colitis, severe mucositis, *S. mitis* bacteremia, veno-occlusive disease	Complete donor engraftment by bone marrow cytogenetics study, full T and B cell reconstitution	Alive and well, IVIG stopped at 18 months post-BMT
P41	BM	Fully matched MUD	Busulphan/ cyclophosphamide/ ATG	CycA, short MTX	Nil	Nil	Septic shock caused by *Bacillus* bacteremia on day +6, developed acute respiratory distress syndrome and respiratory arrest	–	Died of multi-organ failure on day +13
P30	CBU	1 allelic mismatch at locus A	Nil	cycA (minimal dose)	Grade III, skin, gut, liver	Skin	Bronchiolitis obliterans, prolonged O2 dependence >day +300 BCG reactivation (axillary lymph node)	Selective T cell engraftment, 1 year post-transplant: absent B cells	Alive and well, regular 4-weekly IVIG
P7	BM	10 out of 10	Rituximab, fludarabine, melphalan, ATG	cycA	Nil	Nil	Disseminated BCG (immunoreconstitution inflammatory syndrome), MRCNS and *Klebsiella pneumoniae* bloodstream infection	3 years post-BMT: mixed chimerism by FISH, donor 51.2%, CD4 >500/mL 6 months post-transplant	Alive and well, off regular IVIG at 18 months post-BMT
P31	CBU	1 allelic mismatch	Fludarabine, melphalan	CycA, MMF	Nil	Nil	Periengraftment respiratory distress syndrome, disseminated BCG disease, *Microsporidium* gastroenteritis	Full engraftment with good T and B cell reconstitution	Alive and well
P25	CBU	1 allelic mismatch	Fludarabine, melphalan	CycA, MMF	Grade III, skin, gut, lung and liver	Skin, gut, lung	Autoimmune hemolytic anemia, BCG reactivation (dactylitis), severe chronic GVHD on multiple immunosuppressants	Mixed chimerism, CD3+ T cell count remained low post-transplant	Died of refractory chronic GVHD 11 months post-transplant
P23	PBSC	1 antigen mismatched	Fludarabine, thiotepa, melphalan, alemtuzumab	CycA, short MTX	Nil	Nil	Severe interstitial pneumonitis	–	Died of idiopathic pneumonia syndrome on day +59
P16	PBSC	Haploidentical	Nil	CycA, short MTX	Nil	Nil	Severe infection, engraftment syndrome	–	Died of multi-organ failure on day +11
P18	CBU	2 antigen mismatched	Busulphan/ cyclophosphamide/ ATG	CycA	Skin	Nil	BCG reactivation (identified from cutaneous abscess and nasopharyngeal aspirate with pneumonic changes)	Early post-transplant, not evaluated yet	Alive and well

CBU, cord blood unit; CycA, cyclosporin A; FISH, fluorescent in situ hybridization, MRCNS, methicillin-resistant coagulase-negative Staphylococcus; MRSA, methicillin-resistant Staphylococcus aureus; MTX, methotrexate

Table 3 Post-transplant BCG complications in infants with SCID undergoing hematopoietic stem cell transplant

Reference	SCID type	Extent of BCG disease (pre-transplant)	Treatment	Age of transplant (months)	Donor	Onset of BCG disease post-HSCT	Extent of BCG disease (post-transplant)	Treatment	Complications and outcome
Current study	IL2RG	Disseminated (skin, dactylitis, hepatic and splenic abscess)	Rifampicin, isoniazid, ethambutol	8	MUD	5 days	Local abscess, disseminated skin nodules, hepatic and splenic abscess, intra-abdominal lymphadenopathy	Development of rifampicin-resistant strain, switched to clarithromycin. High disease load with large splenic abscesses and uncontrolled disease, splenectomy performed on day +61	Delayed T cell immunoreconstitution at 4 months. Hypercalcemia and renal impairment, treated with low dose prednisolone. Resolved after 24 months' anti-mycobacterial treatment. In remission
Current study	IL7RA	Disseminated (pelvic, psoas, splenic abscesses)	Rifampicin, isoniazid, ethambutol, amikacin, clarithromycin	8	MUD	70 days	Disseminated BCG disease with multiple dactylitis	Continuation of anti-mycobacterial treatment	Died of severe GVHD 6 months post-transplant
Current study	T−B−NK+	BCG osteomyelitis, liver and splenic abscess	Rifampicin, ethambutol and streptomycin later switched to kanamycin, added on ciprofloxacin	8	MUD	Persistent disease	Disseminated BCG disease	Continuation of anti-mycobacterial treatment	Resolved, in remission
Current study	T−B−NK+	Nil	Nil	5	MRD (father)	1 month	Left axillary lymphadenopathy	Isoniazid and rifampicin	Resolved, in remission
Current study	DCLRE1C	Local disease with ipsilated axillary lymphadenopathy	Isoniazid, rifampicin, ethambutol	6	MUD	30 days	Local abscess, increased size of ipsilateral axillary lymphadenoapthy	Restarted isoniazid and rifampicin	Resolved, in remission
Current study	IL2RG	Nil	Nil	8	UCB	71 days	Cutaneous abscess, pneumonic changes, AFB identified on nasopharyngeal aspirate	Isoniazid, rifampicin, streptomycin, clarithromycin	Continuing treatment
[13]	PNP	Nil	Isoniazid, ciprofloxacin prophylaxis	25	MRD (uncle)	Early post-transplant period	Right preauricular and left axillary lymphadenitis	Added on rifampicin, clofazimine, clarithromycin	Further dissemination with intramuscular and retroperitoneal abscesses requiring surgical drainage, added on streptomycin. Resolved after a total of 30 months' anti-mycobacterial treatment. In remission
[14]	Not mentioned	Local disease	Rifampicin	Not mentioned	Not mentioned	2 months	Disseminated skin changes	Rifampicin, isoniazid, ciprofloxacin	Resolved, in remission
[15]	T−B+	Nil	Isoniazid prophylaxis	7	MSD	6 months	Hepatosplenomegaly, soft tissue abscess	Add on rifampicin, streptomycin, clofazimine for 2 months, isoniazid and rifampicin for 4 more months	BCG disease relapsed in the sixth month of treatment, added on amikacin, ciprofloxacin, clofazimine with response and amikacin stopped at 12 months. Developed multiple pleural and perivertebral abscess at 18 months post-transplant, resumed amikacin, and added on ciprofloxacin. Resolved after 36 months' anti-mycobacterial treatment in total. In remission, residual hearing loss

Table 3 (continued)

Reference	SCID type	Extent of BCG disease (pre-transplant)	Treatment	Age of transplant (months)	Donor	Onset of BCG disease post-HSCT	Extent of BCG disease (post-transplant)	Treatment	Complications and outcome
[16]	IL2RG	Local abscess	Rifampicin, isoniazid, pyrazinamide	8	MSD	6 days	Enlarged local BCG abscess, disseminated skin nodules and erythematous rash, skin biopsy revealed numerous AFB	Add on ethambutol to 3-drug anti-mycobacterial treatment; still persistent disease and add on clarithromycin	Recurrent BCG abscess requiring repeated drainage until 7 months post-HSCT. All BCG abscess resolved, in remission
[17]	T−B+	BCG hepatitis	Rifampicin, isoniazid	7	MMRD (haploidentical)	Persistent BCG hepatitis	Progressive hepatomegaly and deranged liver function, repeat liver biopsy showed abundant AFB	Switched to ciprofloxacin, ethambutol and amikacin	Delayed donor engraftment with loss of donor cells in bone marrow on day +259. Gross splenomegaly and hypersplenism resulting in pancytopenia requiring splenectomy. Donor cells which were present in the peripheral blood were lost after splenectomy. Further, T cell depleted bone marrow graft from father given and led to T and B cell immunoreconstitution. BCG disease resolved
[18]	Omenn syndrome	Neck abscess (suspected retrospectively)	Nil	Not mentioned	MMRD (haploidentical)	90 days	Suppurative lymphadenoapthy at the groin with lymphatic dissemination into pelvic cavity	Isoniazid, rifampicin, amikacin, ansamycin, clofazimine according to in vitro sensitivity pattern	BCG disease resolved, in remission
[19]	Not mentioned	Nil	Nil	Not mentioned	MMRD (haploidentical)	15 weeks	Local disease, distant skin dissemination to neck, chest, abdomen and legs; hematuria	Isoniazid, rifampicin, streptomycin, pyrazinamide	BCG disease resolved, in remission

papular skin lesions, discharge from BCG inoculation site, and hepatosplenomegaly 1 month post-transplant. He also developed fever on day +190 with abrupt onset of hypercalcemia and renal failure coinciding with the increase in ALC, resembling immunoreconstitution inflammatory syndrome (IRIS) in AIDS after commencement of anti-retroviral agents. Hypercalcemia associated with IRIS was believed to be related to the restoration of granulomatous host response toward mycobacteria when CD4+ cell count increased [13–15]. Hypercalcemia resolved with hyper-hydration, diuretics, and low-dose prednisolone.

Detailed information on post-transplant immunoreconstitution was available for seven long-term survivors, all of whom were healthy and without major infections or active autoimmune manifestations. All except one patient (P33a) had good long-term T lymphocyte reconstitution with normal mitogen-induced lymphocyte proliferation. The best immunoreconstitution was seen in the P28 with *RAG1* deficiency who received MSD transplant with myeloablative conditioning and P7 with X-SCID who received ten out of ten matched MUD transplant with RIC regimen. P28 had 100% donor cells by XY-FISH chimerism study on bone marrow, while P7 had 51.2% donor cells by XY-FISH on peripheral blood. IVIG was stopped 18 months post-transplant for both patients, and they were free from significant infections.

P33a who received bone marrow stem cells from his phenoidentical father had persistent lymphopenia and poor mitogen-stimulated lymphocyte proliferation. His B lymphocyte count remained very low (<20/μL) and required regular IVIG replacement. Despite this, he remained healthy without major infections. In contrast, his younger sister had satisfactory T cell reconstitution. Though her B lymphocyte count was all along low (CD19+ B cells remained less than 100/μL), IVIG was stopped 18 months post-transplant and she maintained normal immunoglobulin levels.

P32 received MMRD transplant with myeloablative conditioning had low T cell and B cell counts at 1 year post-transplant. Although T cell count normalized subsequently, her B cells remained low. P30 received MUD transplant without conditioning also had complete absence of B cell reconstitution. Both patients continued to receive IVIG replacement.

Discussion

This study presents the largest cohort of infants with SCID from China and Southeast Asian countries. With the establishment of a referral and collaborative network [16], the number of patients referred to us for molecular diagnosis of SCID in the past 2 years constituted half of the cohort, and we expect a rapid rise in genetically confirmed SCID in China and Southeast Asia with increased awareness and availability of resources.

Most infants with SCID first present to clinicians at the primary or secondary care level, with recurring conditions such as bronchiolitis, pneumonia, mucosal candidiasis, and gastroenteritis. However, the diagnosis of SCID was often considered only when these infants present with severe pneumonia, sepsis, and other "indicator" diseases such as systemic fungal infections, PCP, CMV, and BCG disease, often as life-threatening conditions. Ultimately, many of these infants did not survive long enough to receive HSCT, and some of them did not have access to transplant service because of lack of resources. The key to reduce the lag time from initial presentation to the diagnosis is to promote recognition of these common phenotypes and early immunological investigations. An ALC of less than 2.5×10^9/L in a sick infant with recurrent infections must raise alert. It is well-known that infective morbidities and organ damage have major implications on the outcome of HSCT. Protocols on initial investigations and management should be effectively disseminated to and adopted by all levels of healthcare providers and should be supported by a comprehensive health service policy which ensures that affected individuals are able to gain access to transplant centers and receive such life-saving procedures.

From our data, local and disseminated BCG disease occurred in 23.8% of infants with SCID and caused complications in 50% of patients who underwent HSCT. Three patients (P7, P25, and P31) had distant or disseminated BCG at diagnosis, but despite treatment, they all had BCG dissemination after receiving MUD transplant with RIC causing complications in the post-transplant period. P18 developed BCG reactivation with cutaneous and lung involvement after myeloablative conditioning for UCB transplant. The extent of BCG disease and bacterial load appeared to be an important factor for the occurrence of disseminated disease in the post-transplant period and might be aggravated by immunosuppression imposed by conditioning. There is no large cohort study describing the clinical course and treatment strategy of BCG complications in infants with SCID undergoing transplantation. The manifestations of BCG disease in the post-transplant course of infants with SCID reported in seven articles [17–23] were summarized in Table 3, together with five patients from our cohort. It is obvious that BCG dissemination was common in the early post-transplant course when the immunoreconstitution was not complete, and a systemic inflammatory state often occurred when donor T cells began to engraft. The dissemination was frequently difficult to control with potential to develop drug resistance. Persistent BCG disease might also be implicated for slow immunoreconstitution, as seen in P7 and the patient

reported by Skinner et al. [21]. Therefore, BCG disease does not only contribute to morbidity and mortality prior to transplant but also complicates post-transplant course and adversely affect recovery.

The estimated incidence of disseminated BCG is one to 3.4 per million [24–26]. Approximately one third of vaccinated infants with SCID developed disseminated disease [27], and the incidence of disseminated BCG was as high as 45% in another cohort of 40 Iranian infants with SCID [28]. Our study reported the minimum incidence of 23.8%, as BCG disease might be undiagnosed in other patients, especially in the presence of concomitant systemic infections. In many countries, BCG is incorporated into standard neonatal vaccination schedule, and infants are often vaccinated during the newborn period. Infants with undiagnosed primary immunodeficiencies might have received BCG, which would be contraindicated should their condition been known. It was advocated that careful family history should be taken prior to neonatal vaccination [22]. Practically, the awareness of a family history of vaccine-related complications should be promoted among pregnant women in the antenatal visits, so that sufficient opportunity for detailed evaluation and counseling is possible. However, a positive family history will be present in <10% of SCID cases. Deferring routine BCG vaccination beyond 3 to 4 months of age when infants with PID would manifest themselves was also suggested [21, 23]. Apart from BCG, other live-attenuated vaccine-related complications are increasingly reported in SCID, such as varicella [29] and, recently, rotavirus vaccines [30, 31]. The appropriate strategy to prevent administrating live vaccines to susceptible infants is yet to be determined. In a number of countries, SCID is under consideration for inclusion into the panel conditions for national newborn screening, by means of quantifying the T cell receptor excision circle on dried blood spots [32]. Logically, infants who are screened positive should be excluded from the administration of live vaccines.

A recent large cohort study based on experience from 23 European centers provided important information on prognostic factors for transplant outcome of 699 patients with SCID [33]. Younger age at transplant, absence of respiratory impairment, and viral infection before transplant, B+ SCID, genoidentical and phenoidentical donors, and transplant without T cell depletion were major prognostic indicators for 10-year survival. Early diagnosis and availability of a related, HLA-identical donor provides the best chance of cure and survival of over 90% [34, 35]. P33b had excellent transplant outcome as she had the best of these combinations, together with aggressive infection prophylaxis once she was diagnosed on the first day of life until transplant. Her post-transplant course was uneventful with satisfactory T cell and B cell reconstitution. P28 who received MSD transplant for leaky SCID also had excellent long-term outcome with complete cure of disease. However, the use of myeloablative conditioning led to multiple complications including severe mucositis, veno-occlusive disease, and infective colitis in the early post-transplant period.

Malnutrition and lung damage related to recurrent pneumonia were important background risk factors for P16, P23, and P41 who died in early post-transplant period. In particular, the prolonged time from diagnosis to transplant in P16 (6 months) and P41 (5 months) contributed to increased disease burden. Chemotoxicities from conditioning agents in these severely compromised infants, as in P23 and P41, further aggravated infections and organ failure.

The best overall outcome was achieved in P26 (RAG1 deficiency) and P33b (undefined T−B−NK+ SCID) who received MSD transplant. Unlike P33b, immune reconstitution of her elder brother P33a who received phenoidentical transplant had persistently low CD3 and CD19 cell count and required immunoglobulin replacement. In T−B−NK+ SCID, the presence of normal NK cells is an important barrier to successful engraftment of HLA-mismatched donor stem cells, especially for reconstitution of humoral immunity. P30 with Artemis deficiency received one allelic-mismatched UCB transplant without conditioning and failed to achieve B cell engraftment. B cell reconstitution also failed in P32 who received myeloablative haploidentical transplant for T−B−NK+ SCID. She had documented mixed chimerism state with full donor T cells [36], but B cell numbers remained low. The benefit of conditioning for humoral immunoreconstitution in SCID is controversial. A significant percentage of patients who received myeloablation still require immunoglobulin replacement, implying that the use of conditioning does not guarantee B cell reconstitution [37]. Patients who receive myeloablative conditioning also need to bear the risks of further organ damage and infectious complications, with additional toxicities for patients with radiosensitive SCID. RIC and minimal intensity conditioning is increasingly employed in transplant for patients with PID with success and is especially beneficial to infants and poor-risk patients to minimize toxicities [38]. P7 and P31 who received unrelated donor stem cells with RIC had good T cell and B cell reconstitution.

In this study, 66.7% of patients had B+ SCID, of which γc deficiency constituted 45.2% while JAK3 and IL7Rα deficiency each represented 4.7% of our cohort. RAG1, RAG2, and DCLRE1C deficiencies constituted 7.1% of the cohort, and genetic diagnosis was not identified in the rest of patients with B− SCID. We did not diagnose any patients with ADA or PNP deficiency. Except the absence of patients diagnosed with ADA deficiency in our cohort, the

distribution of genotypes was comparable with the European [33] and North American [39] data. In communities where consanguineous marriage is common, autosomal recessive forms account for a high proportion of SCID [28]. Parental consanguinity was uncommon in our cohort in which majority of patients were ethnic Chinese.

Given the wide genetic heterogeneity of SCID, ascertaining a genetic diagnosis for patients with SCID poses challenge to immunologists and geneticists. For instance, we were not able to identify mutations in *JAK3* and *IL7R* genes for the two female infants P23 and P24 who had T−B + SCID. Other diagnostic possibilities for T−B+ SCID include defects in CD45, CD3δ/CD3ε/CD3ζ, and coronin-1A deficiency, but such defects are extremely rare and have only been described in a few pedigrees in the literature. Similarly, molecular defects underlying Omenn syndrome are broad, most frequently caused by mutations in *RAG1* and *RAG2* but other possibilities include *DCLRE1C*, *LIG4*, *IL2RG*, *IL7R*, *ADA*, and *RMRP*. The extent of investigations is often limited by cost and expertise. Even in expert centers, mutations could not be identified in approximately 15% of infants with SCID [39], as unknown gene defects are yet to be discovered. Molecular diagnosis causing Omenn syndrome remains elusive in half of the patients [40]. In this study, we were not able to establish genetic diagnosis for patients with Omenn phenotype and two thirds of patients with B− SCID. The efficiency of molecular diagnosis can be improved by the use of microarray technology for a panel of known candidate genes [41]. It is expected that the application of next-generation sequencing technology, such as exome sequencing, will lead to discovery of novel genetic basis of PID.

The field of PID is marked by significant clinical and scientific breakthroughs in the past decade. With advancement in supportive care and transplant protocols, infants with SCID have good opportunities for cure and long-term survival if infective morbidities are minimized and transplant is performed at early age. Yet, for most countries, the foremost issue is service provision for early detection, diagnosis, management, and definitive treatment for this group of patients. Studies on the epidemiology, disease burden, and outcome should be initiated, which could be strengthened by multi-center research collaboration and academic network. Data should be disseminated effectively to health care professionals, and awareness campaigns should be organized to promote early recognition and treatment. Referral pathway and practical management protocols should be established [42]. Provision of genetic testing and counseling is needed for patients and their families. In countries where population-based newborn screening programs are established, systematic planning for including SCID as one of the screening conditions should be considered.

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