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Review Article

Review of a Decade of International Experiences in Severe Combined Immunodeficiency Newborn Screening Using T-cell Receptor Excision Circle

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Abstract Severe Combined Immunodeficiency Disorders (SCIDs) are a diverse group of monogenic inborn errors of immunity (IEIs) characterised by severe lack of T lymphocyte number and function, with or without affecting B and NK lymphocyte numbers. Severe infections lead to early infant death. SCID infants are seemingly well prior to onset of infections, causing them to miss the golden window for haematopoietic stem cell transplant (HSCT) prior to the age of newborn screening. T cell receptor excision circle is a clinical marker for naive T cell number and can be used to screen for SCID with high sensitivity. International experiences showed that no classical SCIDs have been missed by screening programs so far. Due to extremely favourable outcomes (90% long term survival) for SCID patients picked up by screening and savings incurred by early HSCT, screening for SCID with TREC assay is great value for money. Hong Kong should join the global trend of screening for SCID and save the lives of SCID children.

Key words Newborn screening; Primary immunodeficiencies; Severe combined immunodeficiency; T cell receptor excision circle

Severe Combined Immunodeficiency Disorder

Severe Combined Immunodeficiency Disorders (SCIDs) are a genotypically and phenotypically diverse group of monogenic diseases in which adaptive immunity is profoundly impaired due to a severe lack of T lymphocyte number and function, with or without low B and NK lymphocyte numbers. SCIDs belong to a large class of disorders known as primary immunodeficiency disorders (PIDs), or in a more recent term, inborn errors of immunity (IEIs), which are mostly due to defects of a single gene in the immune system. Collectively, PIDs are not 'rare' and have an estimated prevalence of 1 in 1200;[1] yet SCID per se occurs at around 1 in 50000 - 1 in 65000 in populations with newborn screening and low consanguinity.

SCID infants have grim prognosis due to severe recurrent infections. Serious infections with both common and opportunistic pathogens, such as Candidiasis, and at times by live vaccines such as Bacillus Calmette-Guérin vaccine, form the classic SCID triad together with chronic diarrhoea and failure to thrive. They are often seemingly well prior to onset of infections at about 2 months,[2] due to the protective effect of residual transplacental maternal IgG in the first months of life. In some patients, transplacental maternal T cells cause graft-versus-host disease (GVHD) due to impaired infant immunity.[3] Atypical, or leaky SCID are caused by hypomorphic mutations, and therefore have a less severe clinical picture and may present after 1 year in life.

In the laboratory, SCID infants usually have absolute lymphocyte counts lower than 3×10^9/L.[2] Apparently normal lymphocyte count may be due to maternal engraftment, therefore lymphocyte subset analysis demonstrating low

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amount or proportion of *CD45RA+* (naive) T cells is also preferred. Primary Immunodeficiency Treatment Consortium diagnostic criteria for typical SCID require less than 300 autologous T cells/mm^3 in peripheral blood sample with less than 10% response to PHA when compared against control or demonstration of maternal engraftment. Atypical or leaky SCID, such as Omenn syndrome, is characterised by less severe T lymphopenia.[4] Presence and absence of B cells and NK cells distinguish the type of SCID.

With improving technology and increased use of exome sequencing for SCID patients, in this decade, more than 90% of SCID patients receive a genetic diagnosis; and among the more than 30 causes of SCID, the most common causes of SCID are *IL2RG*, *RAG1/2*, and *ADA*.[5] SCID mostly exhibit autosomal recessive inheritance pattern, except for X-linked *IL2RG* SCID and moesin deficiency[6] and autosomal dominant *BCL11B* SCID,[7] *RAC2* SCID[8] and complete DiGeorge syndrome. Populations with high consanguinity have a lower proportion of *IL2RG* SCID. Phenotypic variations may help clinicians identify the candidate gene for sequencing in some cases (Table 1).

Lymphopenia in SCID mostly originates from abnormal development of T, B and/or NK cells. Impaired **lymphocyte signalling** pathways, such as cytokine signalling (*IL2RG, JAK3, IL7R, BCL11B*),[7] T cell receptor signalling pathway (*CD3D, CD3E, CD3Z, CD45, CORO1A, LAT, ZAP70*), major histocompatibility complex class II antigen presentation pathway (*RFXANK, CIITA, RFXAP, RFX5*) or others (*ORAI1, MSN* and *PGM3*) present intrinsic blocks in lymphocyte development.[15,37-39] Genetic defects impairing **thymus development**, the site for T cell maturation, can cause SCID (*FOXN1,* chromosome 22q11.2 deletion). Inborn errors of **metabolism** may also affect lymphocytes severely due to metabolic stress or the role of metabolites in immune system signalling, such as inside the purine salvage pathway, adenosine deaminase (*ADA*) deficiency and purine nucleoside phosphorylase (*PNP*) deficiency, and outside, adenylate kinase 2 (*AK2*) which creates cellular ATP supply, are well known to cause SCID.[10,40] SCID may also arise from mistakes in **V(D)J recombination** which confers antigen specificity. Null mutations in *RAG1/2* which initiates the process by inducing DNA strand breaks and non-homologous end-joining pathway (NHEJ) effectors, including *NHEJ1 (Cernunnos)*, *DCLRE1C (Artemis), LIG4* and *PRKDC*, which repair the breaks, cause arrest in T and B cell development.[9,29] **Genomic instability** due to *TTC7A, RMRP, RPP25* and *SMARCAL1* mutations increase DNA breaks and may cause early death of lymphocytes.[13,20,41,42] As a unique cause of SCID, *TPP2* is involved in extra-lysosomal peptide degradation; murine models suggest *TPP2* deficiency promotes premature stress-induced **senescence** in and causes apoptosis of T and B cells, leading to severe immunodeficiency and autoimmunity.[33]

Treatment considerations in SCID infants vary according to their genotype. In general, when a diagnosis of SCID is suspected in an infant presenting with acute infection, they should be put in protective isolation and given aggressive antimicrobial treatment. While *Pneumocystis jirovecii* prophylaxis (septrin) and antifungal prophylaxis (itraconazole and fluconazole) must be given, acyclovir is reserved for those with history or risk of herpes infection.[43] Live vaccines such as Bacillus Calmette–Guerin vaccine and rotavirus vaccine, unirradiated blood products, and breastfeeding by CMV IgG positive mothers must be avoided. Infants screened for SCID and have received BCG vaccine may begin antimycobacterial treatment. For cure, allogenic haematopoietic stem cell transplant (HSCT) is well-established, with different conditioning regimens for different types of SCID and donors. Younger age (<3.5 months) and infection-free status at transplant have been shown to improve survival significantly in large cohort studies;[44,45] infants who receive transplant earlier than 3.5 months of age have a 94% 5-year survival, compared to 66% for those later than in one cohort. Gene therapy has been shown to be as effective and safe as HSCT in *ADA*-SCID (Strimvelis®),[46] and is showing great promise for X-SCID and perhaps other forms of SCID.[47]

T Cell Receptor Excision Circle

T Cell Receptor Gene Recombination

The dried blood spot (DBS) high throughput quantitative polymerase chain reaction (qPCR) screening strategy used worldwide nowadays for SCID was first proposed by Chan and Puck in 2005.[48] The invention stems from our knowledge of V(D)J recombination of T cell receptors (TCR). T cell receptors are the cell surface antigen receptors of T cells, and are heterodimers consisting of either α and β, or γ and δ chains, with the α and β combination forming the major TCR type found in thymus and blood. The extracellular portion of the chains fold into the antigen-binding variable region of the receptor, which confers the specificity of the lymphocyte, and the rest constitute the constant region. The diverse repertoire of TCR is created

Table 1 Genetics and phenotype variations in severe combined immunodeficiencies

Gene	Lymphocyte	Special features	Proportion
IL2RG^	T-B+NK-	The most common SCID, X-linked recessive	~30%
RAG1/2	T-B-NK+	Autoimmune haemolytic anaemia; Omenn syndrome.[9]	~20%
ADA^	T-B-NK-	Low IQ, sensorineural hearing loss, non-infectious pulmonary disease, radiologic skeletal abnormalities, renal sclerosis.[10]	~10%
IL7R^	T-B+NK+	Isolated T cell deficiency	~10%
22q11.2*	T-/+B+NK+	Chromosome 22q11.2 deletion syndrome (DiGeorge syndrome): conotruncal cardiac anomalies, hypocalcaemia, thymic hypoplasia / aplasia[11]	~5%
JAK3^	T-B+NK-	Identical to *IL2RG* SCID, but autosomal recessive	~5%
DCLRE1C / Artemis^	T-B-NK+	Sensitivity to ionising radiation[12]	~5%
RMRP*	T-B+/-NK+	Cartilage hair hypoplasia[13]	~5%
CD3D, E&Z^	T-B+NK+	Isolated T cell deficiency	Rare (<5%)
CD45 / PTPRC	T-B+NK+	Isolated T cell deficiency; can be caused by uniparental disomy[14]	Rare
CORO1A	T-B+NK+	Detectable thymus on chest X-ray, Epstein-Barr virus induced lymphoproliferation,[15] attention-deficit and hyperactivity disorder[16]	Rare
LAT	T-B+NK+	Serious autoimmunity, e.g. autoimmune haemolytic anaemia and immune-mediated thrombocytopenia[17]	Rare
ZAP70*	T-B+NK+	Ulcerative colitis, cytopenia, eczema[18]	Rare
FOXN1*	T-B+NK+	'Nude SCID': alopecia universalis and nail dystrophy; CNS defect, Omenn syndrome. Present in carriers as nail disease.[19]	Rare
BCL11B*	T-B+NK+	Craniofacial abnormalities, agenesis of corpus callosum, umbilical hernia, erythematous psoriaform dermatitis[7]	Rare
SMARCAL1*	T-B+NK+	Schimke immunoosseous dysplasia – spondyloepiphyseal dysplasia, renal insufficiency, cerebral ischaemia, pancytopenia[20,21]	Rare
RFXANK, CIITA, RFX5, RFXAP*	T-/+B+NK+	Liver or biliary tract disease, autoimmune cytopenia[22,23]	Rare
ORAI1*	T+B+NK+	Muscular hypotonia, anhidrotic ectodermal dysplasia[24]	Rare
PNP*	T-B+/-NK+	Hypotonia, delayed development, undetectable uric acid in plasma, brain atrophy[25]	Rare
PGM3*	T-B-NK+	Facial dysmorphism, short limbs, atrial septal defect, intestinal malrotation, horseshoe kidney, bilateral exaggerated lesser trochanter[26]	Rare
LIG4	T-B-NK+	Characteristic bird-like face, microcephaly, growth and developmental delay, pancytopenia, dermal anomalies, radiosensitivity[27]	Rare
NHEJ1 / Cernunnos	T-B-NK+	Microcephaly, growth retardation, sensitivity to ionising radiation[28]	Rare
PRKDC / DNAPKcs	T-B-NK+	Granuloma, organ-specific autoimmunity[29]	Rare
TTC7A*	T-B-NK+/-	Multiple intestinal atresias,[30] very early onset inflammatory bowel disease[31]	Rare
RAC2	T-B-NK-	Gain-of-function mutation: myeloid dysfunction[8]	Rare
AK2	T-B-NK-	Agranulocytosis, sensorineural deafness[32]	Rare
MSN*	T-B-NK-	Neutropenia, monocytopenia[6]	Rare
TPP2*	T-B-NK-	Refractory multilineage cytopenia; neurodevelopmental delay[33,34]	Rare
RPP25*	Unclear	To be reported	Rare
12p duplication*	Unclear	Pallister-Killian syndrome: hypotonia, intellectual disability, hearing and vision impairment, facial anomalies[35]	Rare

^Mainly manifests as typical SCID[5]

*Yet to be listed as a cause of severe combined immunodeficiency but may be included in other categories in the International Union of Immunological Societies inborn errors of immunity classification updated in November 2019[36]

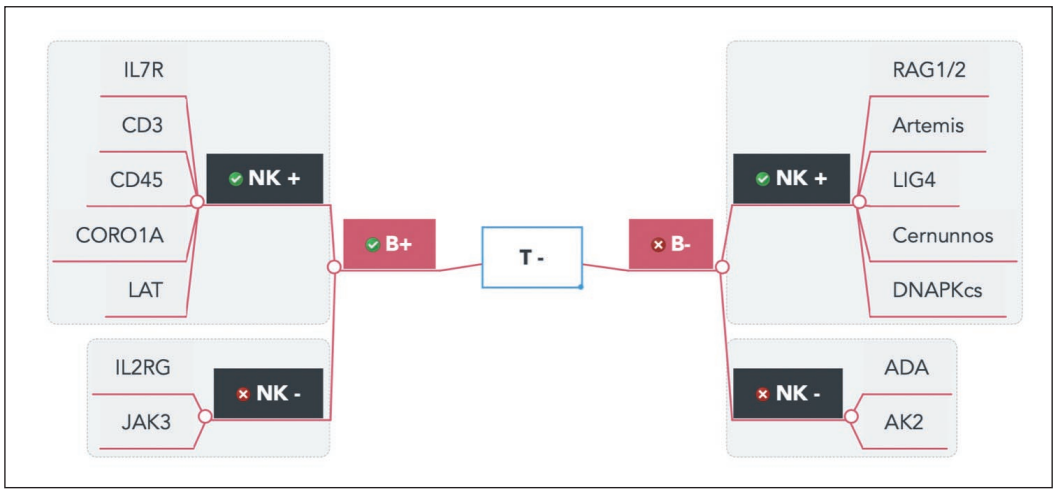


Figure 1 Immunophenotypes of major types of severe combined immunodeficiencies.

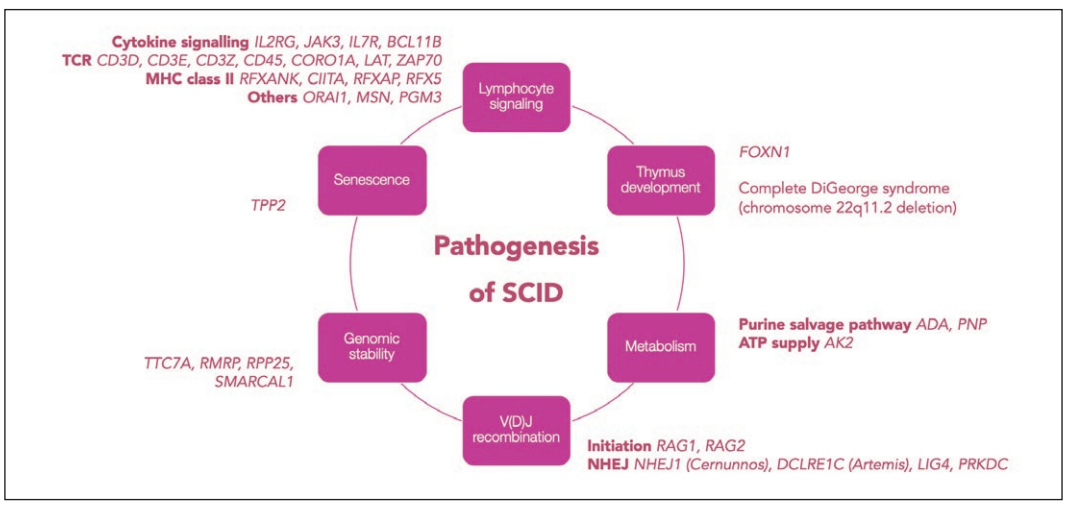


Figure 2 Pathogenesis of SCID.

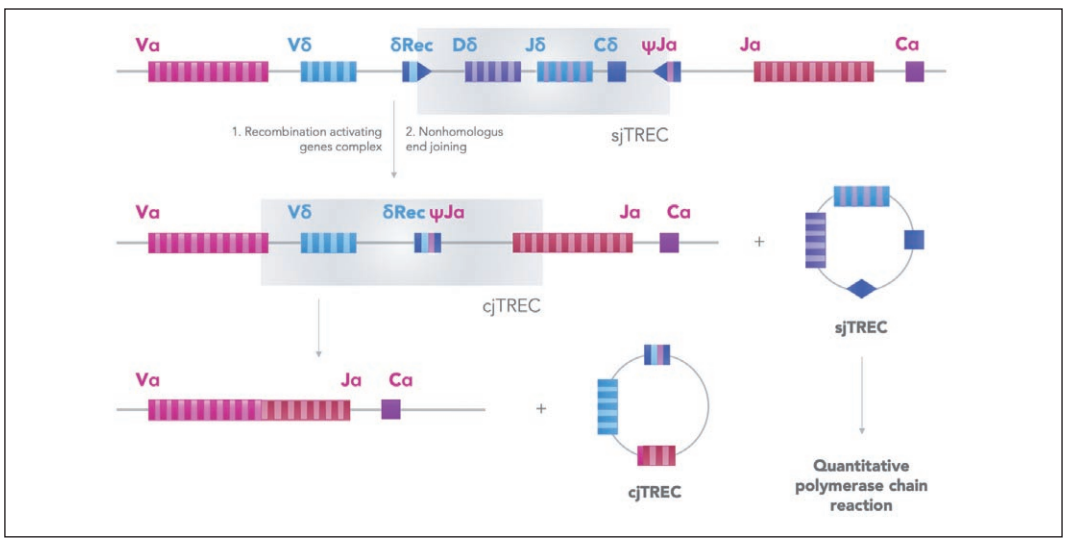


Figure 3 V(D)J recombination at the TCRA/D gene cluster on chromosome 14.

by recombination in the individual segments of the TCR genes – V stands for variable, D for diversity and J for joining, as shown in Figure 3. Each TCR gene contains several alternative versions of these gene segments for diversity. To initiate the process, the recombination-activating genes (RAG1/2) complex recognises the recombination signal sequences that flank the gene segments and creates double-strand DNA breaks which are repaired by nonhomologous end joining (NHEJ),[49] forming a signal joint T-cell receptor excision circle (sjTREC) and a coding joint T-cell receptor excision circle (cjTREC). Quantification of the signal joint TREC formed at the TCRA/D gene cluster on chromosome 14 in blood is therefore an indicator of naive T cells that have completed TCR VDJ recombination.

Measuring TREC

DNA contained in the dried blood spots is isolated; and TREC and a control gene fragment, e.g. beta-actin or RNase P, are quantified by qPCR. The average healthy newborn would have approximately 250 copies of TREC per microliter of blood.[50] SCID infants, who have very low naive T cells, can be identified by very low or zero TREC count regardless of exact genetic aetiology. In the USA, Wisconsin state began the world's first SCID TREC DBS NBS pilot in 2008,[50] and many countries have since then started their own pilots using in-house developed assay or commercial product. To date, only one commercial product, the PerkinElmer EnLite™ Neonatal TREC kit, has been approved by the US Food and Drug Administration for SCID newborn screening.

Alternative screening methodologies have since then also been developed for SCID. Vidal-Folch et al identified the room for improvement in the qPCR method and proposed the use of multiplex droplet digital PCR (ddPCR) instead in 2017,[51] which would allow the measurement of absolute TREC amount without normalisation, repetition nor standardisation. Initial results from a small group of subjects have been encouraging as expected, yet the feasibility and cost-effectiveness of the technology being implemented for large-scale population screening should be further investigated. To increase coverage of the qPCR

Table 2 Findings of published SCID TREC screening pilot services worldwide

	California (2010-17)[55]	**USA° (2008-13)[63]**	**Taiwan (2010-17)[62]**	**France (-2018)[59]**	**Israel (2015-16)[60]**	**Catalonia (2017-18)[56]**	**Sweden (2013-15)[61]**	**Seville (2014-15)[57]**
Assay choice	EnLite™*	Mixed	Mixed	EnLite™	EnLite™	EnLite™	In-house	In-house
Screened	3252156	3030083	920398	190517	177277	130903	58834	5160
Low TREC	562 (0.02%)	1265 (0.04%)	173 (0.02%)	165 (0.09%)	46 (0.03%)	30 (0.02%)	16 (0.03%)	5 (0.1%)
TCL	213	463	136	62	35	21	N/A	5
Total SCID (incidence)	50 (1 in 65000)	52 (1 in 58000)	7 (1 in 130000)	6 (1 in 32000)	8 (1 in 22000)	1 (1 in 131000)	1 (1 in 59000)	0
Typical SCID	39	42	7	3	5	1	N/A	0
Leaky SCID	11	10	0	3	3	0	N/A	0
SCID missed	2 leaky	0	0	0	0	0	0	0
SCID survival	0.94	0.87	1	0.67	0.88	1	N/A	N/A
Non-SCID TCL	163	411[Δ]	125	56	27	20	N/A	4
Syndromes	72	136	34	7	9	6	N/A	1
Preterm	33	29	59	7	9	2	N/A	2
Secondary	25	117	24	15	4	2	N/A	2
Idiopathic^	33	12	8	27	5	10	N/A	0

° Includes newborn screening programmes from 11 states[63]

*California switched from in-house assay to EnLite™ neonatal TREC kit in 2015[55]

[Δ] Information for 117 infants with non-SCID T cell lymphopenia not available[63]

^Idiopathic T cell lymphopenia includes both persistent and transient T cell lymphopenia of unknown causes

to X-linked agammaglobulinemia (XLA), a B cell defect in which mutated Bruton tyrosine kinase leads to near absence of CD19+ B cells, Borte et al developed a triplex qPCR for detection of TREC, kappa-deleting recombination excision circle (KREC), the equivalent of TREC in B cells, and a control gene.[52] Alternatively, TREC assay can be multiplexed to *SMN1* assay, allowing the detection of patients with spinal muscular atrophy (SMA) as well.[53] The practice of including certain conditions in the national screening programmes yet varies among regions, but it is believed that more conditions will be and should be added to newborn screening for the benefit of more rare disease patients at a low added cost as both XLA and SMA can be screened by the same methodology as SCID.

Potential New Methods

Apart from PCR, new screening methodologies are being developed to screen for more primary immunodeficiencies. Collins et al investigated the application of peptide immunoaffinity enrichment coupled with selection reaction monitoring mass spectrometry (immuno-SRM) to assay for Wiskott-Aldrich syndrome protein (WASP), BTK and CD3E proteins on dried blood spots as markers for Wiskott-Aldrich syndrome, an immunodeficiency with neutropenia or thrombocytopenia, XLA and SCID.[54] In the future, with discovery of novel disease markers, lowered price of cutting-edge laboratory assays and new testing methods, more and more primary immunodeficiency patients can benefit from population screening.

International SCID Screening Experience

Ever since the first SCID TREC NBS pilot started in Wisconsin in 2008, numerous feasibility studies and pilot services have been rolled out around the world, screening millions of neonates. Beginning from December 2018, all newborns in the United States are screened for SCID. The biggest and most representative study so far published is perhaps the California TREC screening service, which has screened 3.3 million infants in the period of 6.5 years between 2010 and 2017.[55] Experiences of population screening with TREC have also been published by programs in Catalonia, Seville (Spain), France, Israel, Sweden, Taiwan and other states in the US.[56-64] These pilot studies offer a large amount of useful data and new insights about T cell lymphopenia, demonstrating the effectiveness, feasibility and vital importance of SCID screening in all situations.

Screening Protocol

While in-house assays are occasionally used by population screening programs worldwide, the PerkinElmer EnLite™ Neonatal TREC kit is widely used, including in California (after 2015), Catalonia, France, Israel, Taiwan (Taipei Institute of Pathology) et cetera. Multiple pre-service evaluative studies using the PerkinElmer EnLite™ Neonatal TREC kit on anonymised dried blood spots have also been published, including those by Australian (Victoria), Dutch, and Saudi scientists.[65-67] Taiwan's Chinese Foundation of Health is unique in using the Roche LightMix® Modular TREC assay in their service.[62]

There are various screening algorithms used by these studies as decided locally by the relevant authorities. Prior to screening pilot service, a retrospective study using already collected dried blood spots by pre-existing newborn screening programmes is common to determine the initial cut-off values of the pilot service and to verify the performance of the assay. The cut-off value is set cautiously and will be revisited from time to time to balance sensitivity and recall rate as more data accumulate. Availability of resources is also a factor. Dried blood spots may first be tested in singlicate for TREC only, which if below a higher threshold, triggers re-test in singlicate or duplicate for TREC and control gene against a lower final threshold. An urgent threshold may also be defined to implicate a very high chance of SCID. Special neonate groups, e.g. those in neonatal intensive care or born preterm before 37 weeks may have a different threshold. Whenever the control gene level falls below a pre-defined threshold, the result is invalidated and necessitates a second dried blood spot be taken. In some programmes, a confirmed low TREC leads to immediate evaluation of the patient by immunologists with flow cytometry, but in others a second dried blood spot being taken, if not below the urgent threshold, if any. After flow cytometry, targeted sequencing or next generation sequencing confirm the diagnosis.

Patients with Low TREC

The number of patients detected with low TREC depends highly on the cut-off level of TREC in populations without widespread consanguinity or known founder mutations for SCID. As shown in Table 2, percentage population with low TREC levels or referred to flow cytometry varies between 0.1% and 0.02%. All patients

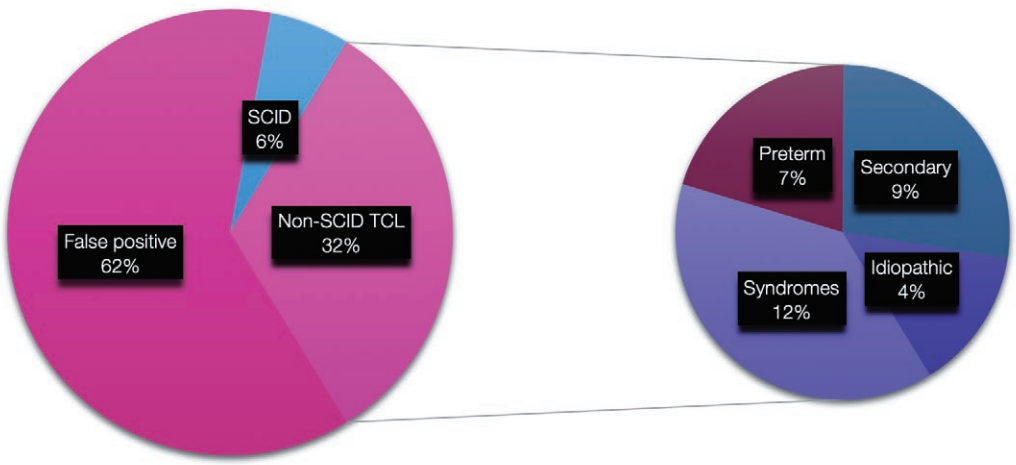
with low TREC would be given flow cytometry, perhaps except in programmes with long delay in result confirmation in which patients may die before given a chance for laboratory testing.

T cell receptor excision circle is a clinical marker for naive T cell lymphopenia (TCL). Incidence of TCL revealed by TREC screening has been found to be between 1 in 1030 (Seville, Spain) and 1 in 15300 (California, US), which may be explained by the threshold for TREC levels that trigger immunological evaluation and different diagnostic thresholds for TCL. Having a higher threshold for TREC level may capture more cases of TCL, in particular non-SCID TCL, yet will inevitably increase the false positive rate. In contrast, SCID incidence is more similar amongst different populations at around 1 in 58000. In the California study, for every SCID newborn found, 3 cases of non-SCID TCL were found, and 6 had low TREC but normal T cell numbers.[55]

Causes of non-SCID TCL can be grouped into syndromic, preterm, secondary or idiopathic. DiGeorge syndrome (DGS), in which 22q11.2 is deleted in the infant is the predominant cause of syndromic non-SCID TCL.[68] The chromosomal microdeletion syndrome causes conotruncal cardiac anomalies, hypocalcaemia and the most relevant in this case, thymic hypoplasia, which leads to variable T cell deficiency. Thymic aplasia happens in 1% of DiGeorge syndrome patients, dubbed complete DGS, causes severe combined immunodeficiency. Infants with DiGeorge syndrome may have very low to normal TREC depending on the level of thymic hypoplasia.[69] Other known causes of non-SCID TCL syndromes include trisomy 21, trisomy 18, ataxia-telangiectasia, CHARGE syndrome, diabetic embryopathy, et cetera.[63,68] These patients may benefit from immune interventions such as IgG infusion. Complete DGS patients may require thymic transplant or haematopoietic cell transplant. In the California study, 10 out of 72 cases of non-SCID TCL syndromes required immune interventions apart from live vaccine avoidance; 9 out of 72 cases died of non-immune causes and 0 of immune causes.[68] Apart from syndromic causes, preterm T cell lymphopenia is an expected secondary target of TREC screening, and secondary causes would include third space displacement, chylothorax, maternal use of immunosuppressive agents during pregnancy, neonatal leukaemia and more.[55,60] T cell deficiency in these scenarios are reversible. Idiopathic T cell lymphopenia is an incidental finding of TREC screening programs. In the New York cohort, patients have variable clinical features.[70] They remain clinically stable but both the causes and outcomes of their conditions are unclear. Continued monitoring is needed to understand the condition, thus their detection by TREC screening programs may be of benefit.

Implications for SCID Patients

As shown by data reported in Table 2, TREC screening is effective in picking up SCID. Except for 2 infants with



Note: False positive cases refer to infants with low TREC but normal lymphocyte subset results. The Swedish study is excluded due to inadequate information.

Figure 4 Results of SCID screening pilots mentioned in Table 2.

leaky SCID missed by the California program, none of the other programs reported missed SCID patients, giving TREC screening programs 100% sensitivity on classical SCID.[55] The 2 cases of leaky SCID missed by the Californian NBS had sufficiently hypomorphic mutations in *IL2RG* and *ADA* genes, causing TREC levels to be significantly above the screening cut-off in the neonatal period, and were successfully treated by haematopoietic cell transplant and gene therapy respectively.[68] For the patients that have not been missed, prognosis is generally good with higher than 85% survival reported in most cohorts. SCID patients that are picked up early have better outcome in haematopoietic stem cell transplant as shown in several large studies as previously mentioned.[44,45] As higher standard of care, better haematopoietic stem cell transplant conditioning regimen and more therapeutic options such as gene therapy, become available for SCID patients, the survival rate for SCID patients may approach 100%.

Live vaccines such as BCG vaccine and rotavirus vaccine form a part of universal childhood vaccination program in many countries, yet SCID patients exposed will be at risk of developing vaccine-related infections. SCID patients identified through newborn screening can avoid those vaccines, but only if the vaccines are not administered before screening results become available. For example, Taiwanese vaccination schedule once included the BCG vaccine on the first day of life, yet it was postponed to 1-4 weeks in 2012 for newborns participating in a new SCID TREC screening pilot at that time, and was further delayed to 5-8 months after it was found that postponing BCG vaccination in the population did not increase the cases of neonatal tuberculosis and did not slow down the downward trend of TB incidence.[62]

Financial and Ethical Justifications

Early SCID HSCT is Cheaper than Late

Cost-effectiveness is one of the considerations when pursuing public health policy. Several papers evaluating the financial aspects of TREC newborn screening have been published, either evaluating real field data of patients with newborn screening or making estimations based on patients with early HSCT. Dutch analyses by Van der Ploeg et al estimated that newborn screening reduces the cost of treating SCID in a population of 100,000 by 187,000 EUR (207,000 USD; 1,620,000 HKD) while the cost of screening the same population is 609,800 EUR (676,000 USD; 5,300,000 HKD), which is estimated to lead to the gain of 11.7 quality-adjusted life years (QALYs) in the same population, resulting in a cost-utility ratio of 33,400 EUR/QALY (37,000 USD; 290,000 HKD) gained and may be considered cost-effective.[71] The UK newborn screening council review conducted in 2017 had an even more favourable cost-utility ratio estimate of 17,600 GBP/QALY gained (23,000 USD; 179,000 HKD).[72]

While the exact costs of screening and treatment vary among localities due to socioeconomic factors, experts generally agree that newborn screening can reduce cost of treating SCID due to shortened diagnostic journey, reduced cost of treating complications which could be prevented by prophylaxis and placing infants in protective isolation, and reduced cost of treating morbidities in the future because of better outcomes from early HSCT.[59,73,74] Costs of screening may be further reduced by controlling turn-around time, which determines the timing of institution of prophylactic measures, thus the chances of infection before HSCT and therefore its cost; and the recall rate for immunological evaluation, reducing the diagnostic costs associated with false positive samples.

Ethical Considerations

The appropriateness of including SCID in any population newborn screening programme is well proven by experiences from all around the world. The epidemiology and natural history of SCID are now more well understood than ever – it is a severe disease that requires early aggressive treatment. There are no primary prevention measures available, other than carrier testing, which can only be done in minority of cases with family history of diagnosed SCID. TREC assay is a well-validated, 100% sensitive assay for typical SCID. The sample required, dried blood spots, can be collected from neonates with minimal risk. Haematopoietic stem cell transplant is a potentially curative treatment for SCID patients, and outcomes such as survival and immune reconstitution improve when HSCT is offered early.

As with any screening programmes, the specificity of the first-tier testing is low as the cut-off is set to increase sensitivity. Half of the newborns with low TREC have normal T cells. Yet, it is a reasonable sacrifice to save the lives of infants with serious conditions. Physicians and other medical professionals involved in the screening process must explain the implication of a positive TREC test to the family accurately. Patients with conditions other than SCID diagnosed by TREC screening should be referred

to appropriate specialties so they may benefit from early detection as well. Studies on idiopathic T cell lymphopenia should continue to better understand the pathogenesis and optimal treatment for such patients.

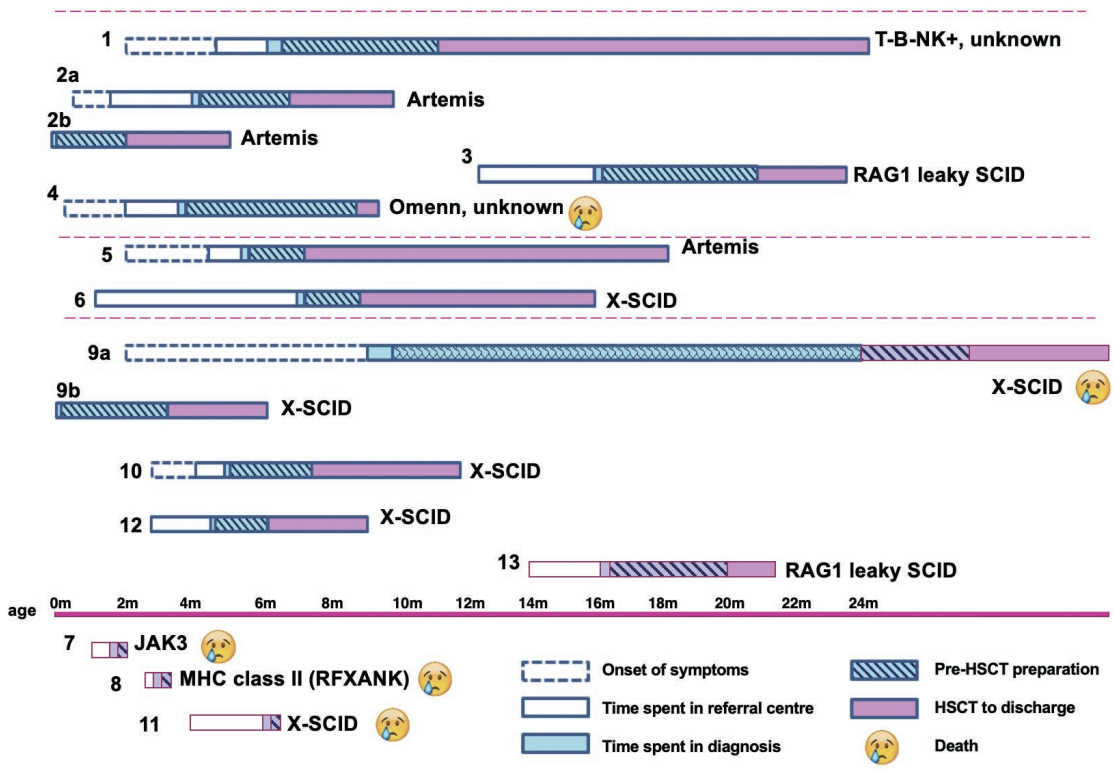
SCID Screening for Hong Kong

Local SCID Care Experience

The Queen Mary Hospital is the tertiary and teaching hospital affiliated with the University of Hong Kong and has been the referral centre for patients diagnosed with primary immunodeficiencies in the region. The Asian Primary Immunodeficiency Network founded by the authors offer free molecular diagnostics for suspected primary immunodeficiency, including SCID.[75,76] We have diagnosed over 100 cases of SCID, and our experience has culminated in several publications.[2,77-80]

Between years 1991 and 2017, 15 infants with SCID have been referred to Queen Mary Hospital, including 13 classical SCIDs and 2 leaky SCIDs. Six were X-SCID (*IL2RG*), 3 classical *Artemis* SCID, 2 leaky *RAG1* SCIDs, and 1 each for *JAK3* deficiency and MHC II deficiency (*RFXANK*). Two infants have perished before molecular diagnosis could be given. Only 12 of the 15 SCID patients lived long enough to receive HSCT, and for those treated we have an 83% long-term survival rate. Among them, patients 2b and 9b were diagnosed early and received HSCT early by 3.5 months of age due to the family history of their siblings 2a and 9a. 2b and 9b are still alive as of last follow up and do not require immunoglobulin therapy.

Based on 1.75 million accumulated births in that same period (1991-2017) in Hong Kong,[81] and on an estimated population incidence of 1 in 60,000, one could calculate that 29 SCID infants have been born in that period, only 15 of them received the diagnosis, and only 10 of them were saved assuming all SCID infants diagnosed in Hong Kong have been diagnosed by us and undiagnosed SCID infants have 100% mortality. The long-term survival for a SCID infant born in Hong Kong is therefore 34%. This figure could be improved significantly with newborn screening and rise to above 90%.



Note: False positive cases refer to infants with low TREC but normal lymphocyte subset results.
The Swedish study is excluded due to inadequate information.

Figure 5 Diagnostic and therapeutic journey of 15 SCID patients diagnosed in 1991-2017 in Hong Kong.

Proposed SCID Pilot

Our data prove that Hong Kong has a great need of screening for SCID, yet currently there is no SCID newborn screening in Hong Kong. Recently, the newborn screening pilot for inborn errors of metabolism (IEM) has been completed in Hong Kong, and it is based on mass spectrometry of dried blood spots.[82] A territory-wide screening service for IEM is expected to begin in all public hospitals. There is a well-developed and mature service model which includes steps in universal newborn screening beginning from informed consent collection and recall and counselling. As SCID TREC screening programs typically use dried blood spots as well, adding severe combined immunodeficiency, and in the future other conditions such as spinal muscular atrophy to the IEM screening programme would be highly economical and add little additional workload to frontline postnatal staff. Follow-up of low TREC infants can be handled centrally in the Hong Kong Children's Hospital by paediatric immunologists and clinical geneticists. The authors estimate that the annual budget for screening the expected birth cohort of 55000 infants will only increase by less than 3 million HKD if SCID TREC screening is included. With the many reports of success in saving lives of SCID patients with newborn screening worldwide, the time has come for Hong Kong to join the world in this respect.

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Declaration of Interests

The authors declare no competing interests.

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