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History and Current Status of Newborn Screening for Severe Combined Immunodeficiency

Antonia Kwan, MB BS, PhD and Jennifer M. Puck, MD

Department of Pediatrics Box 0519, University of California San Francisco and UCSF Benioff Children's Hospital 513 Parnassus Avenue, HSE 301A, San Francisco, CA 94143-0519, Phone: 415 502 2090, FAX: 415 502 5127

Antonia Kwan: kwana@peds.ucsf.edu; Jennifer M. Puck: Jennifer.Puck@UCSF.edu

Abstract

The development of a T cell receptor excision circle (TREC) assay utilizing dried blood spots in universal newborn screening has allowed the early detection of T cell lymphopenia in newborns. Diagnosis of severe combined immunodeficiency (SCID) in affected infants in the neonatal period while asymptomatic permits early treatment and restoration of a functional immune system. SCID was the first immunodeficiency disease to be added to the Recommended Uniform Screening Panel of Core Conditions in the United States in 2010, and is now implemented in 26 states in the U.S. This review covers the development of newborn screening for SCID, the biology of the TREC test, its current implementation in the U.S., new findings for SCID in the newborn screening era, and future directions.

Keywords

DiGeorge syndrome; hematopoietic cell transplantation; primary immunodeficiency; severe combined immunodeficiency; T cell lymphopenia; T cell receptor excision circle (TREC)

Introduction and Background

Severe combined immunodeficiency (SCID), popularly known as the “bubble boy disease”, is characterized by severe defects of cellular and humoral immunity that render affected infants susceptible to opportunistic and recurrent infections. As the most severe form of primary immunodeficiency (PID), SCID is generally fatal in the first year of life unless recognized and treated.^{1,2} SCID is a collection of individual heritable PID diseases, resulting from defects in genes controlling the maturation of elements of the adaptive immune system. Regardless of the molecular cause, infants born with SCID typically appear normal at birth, are characterized by a severe deficiency of naïve T cells, and are at high risk of serious

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infections after waning of maternal antibody at around 4 to 6 months of age. If untreated, SCID-affected infants are susceptible to recurrent and opportunistic infections, persistent diarrhea, faltered growth, and early demise.

Established treatments for SCID include restoring the faulty immune system by means of allogeneic hematopoietic cell transplant (HCT) from HLA-matched related or unrelated donors, haploidentical parental donors, or cord blood; enzyme replacement therapy for the adenosine deaminase (ADA)-deficient form of SCID; or experimental gene therapy for X-linked SCID and ADA-SCID. By and large, HCTs have been successful over the past decades, and outcomes following HCT for SCID-affected infants are optimized by earlier HCT and by effective prevention and treatment of infections prior to HCT.³⁻⁶

Studies have shown that infants identified in the neonatal period by positive family history have better outcomes compared to index or sporadic cases.^{4,5} Optimal survival and health outcomes for SCID are associated with treatment early in infancy before the development of uncontrollable infections.³⁻⁶ Because SCID-affected infants appear healthy at birth, in the past only those who had a recognized family history of SCID, could have the diagnosis made early, and such infants comprise fewer than 20% of all cases.^{5,7,8} Early diagnosis and treatment for all infants, whether with a positive family history or sporadic, is possible only upon institution of population-based screening for SCID.⁹

Epidemiology of SCID

Prior to newborn screening, SCID had been estimated to occur at an incidence of 1 in 100,000,^{7,10} though specific ethnic populations were recognized to have a higher incidence of certain types of SCID due to founder mutations. For example, ADA-SCID in the Somali population has an incidence of ~1 in 5,000,¹¹ DCLRE1C (Artemis) gene mutations occur in Navajo Americans at a rate of ~1 in 2,000,¹² and RAG1, RAG2, ADA, IL7R, CD3, and ZAP70 mutant alleles have been noted in the Amish and Mennonite populations.^{13,14} However, SCID-affected infants may succumb to opportunistic infections prior to a diagnosis of immune deficiency, and it was widely acknowledged that reported incidence was likely an under-estimate of the true incidence of SCID.¹⁵

Genetic heterogeneity of SCID

Understanding the genetic heterogeneity of SCID is necessary in order to appreciate the development of the various screening tests that were initially proposed for SCID, limitations of tests, and the range of conditions identified.

SCID and combined immunodeficiency (CID) comprise a spectrum of genetic disorders of the immune system in which T and B cell immune responses are impaired.¹⁶ Many proteins are essential for T cell development, and as many as 20 known genes have been associated with SCID due to deleterious mutations that abrogate or alter protein expression and prevent the development and maturation of T cells (Table 1). Impaired development of a diverse repertoire of functional T cells combined with inability to produce specific antibodies, either due to impaired B cell development or lack of T cell help, leads to combined impairment of cellular and humoral immunity.^{1,2}

Previous reports from transplant centers show almost 50% of SCID cases to be caused by *IL2RG* mutations, while all other known SCID defects caused by mutations in autosomal recessive genes.^{7,10} Different gene mutations characteristically give rise to particular phenotypic profiles. All SCID forms have low or absent T cells, but different gene defects are associated with presence or absence of B and NK cells, and in some instances non-immunological manifestations such as radiosensitivity or skeletal, dermatologic or neurologic abnormalities.¹⁶ Hypomorphic mutations of genes can give rise to leaky SCID, in which non-null mutations allow for some T cell development, but cellular immunity remains impaired. Attention to such features can facilitate the search for the causative gene mutations in a given SCID case.

Despite this genetic heterogeneity, the common phenotype of impaired T cell immunity means that infants with SCID present with recurring opportunistic infections, classically described in textbooks to include *Pneumocystis jiroveci* pneumonia, disseminated BCG infection secondary to vaccination, recurrent diarrhea that may be caused by inadvertent administration of live rotavirus vaccine,¹⁷ persistent and severe cytomegalovirus, adenovirus or other viral infections, oral thrush, invasive bacterial, mycobacterial and fungal infections. Without diagnosis of the underlying problem and provision of a functional immune system, SCID-affected infants cannot survive.

Development of screening test for SCID

Criteria for newborn screening

The premise of newborn screening (NBS) is to detect disorders pre-symptomatically, such that effective treatments can be applied. Phenylketonuria (PKU) provided the paradigm for disorders in which pre-symptomatic treatment would be effective.¹⁸ Public, state-based newborn screening programs began in the U.S. over 50 years ago, with the development by Robert Guthrie of the filter paper-based testing technology still currently in use. Guthrie's innovation of heel-stick blood spotted onto a filter and dried facilitated the development of state NBS programs because the samples were easy to obtain and stable, while his assay for phenylalanine was reproducible, inexpensive, and accurate; these are all necessary components for an effective population-based public health program. NBS using biochemical markers to detect certain congenital conditions has become a means for early identification affected newborns in an effort to reduce infant morbidity and mortality. It is a comprehensive system of education, screening, follow-up, diagnosis, treatment/management, and evaluation that must be institutionalized and sustained within state governments often challenged by economic, political, and cultural considerations.^{19,20}

Criteria were developed for screening additional conditions beyond PKU as follows: 1) a sensitive and specific test was available and affordable; 2) the condition evaded clinical recognition early in its course; 3) harmful health consequences could be prevented or reduced by early treatment. Since the advent of tandem mass spectrometry, the number of diseases efficiently screened by this means has expanded greatly, and core disease panels for screening were established, which included PKU and other inborn errors of metabolism, hypothyroidism, hemoglobinopathies, and additional disorders.²¹

SCID as a disease fits screening criteria established by Wilson and Jungner²² – SCID is an important health problem, acceptable diagnosis and treatment are available, there is a recognized latent pre-symptomatic stage, and the natural history of SCID including development from latent to declared disease is well understood. The prospect of preventing death from life-threatening infections by identifying at-risk infants before the onset of such infections makes SCID an excellent target for NBS. What remained was the establishment of a suitable test that is cost effective, acceptable to the population, and economically balanced. If at all possible, newly developed screening tests should take advantage of dried blood spot (DBS) samples to avoid cost of getting a separate sample and to facilitate integration into existing screening programs.

History of laboratory detection of SCID biomarkers

Immunoassays using dried blood spots—There were initial attempts in the 1970's to screen infants for ADA-deficient SCID using a colorimetric ADA enzyme assay on extracts from DBS, but this was unsuccessful due to missed cases and false positives.^{15,23,24} Interleukin-7 (IL-7), produced by stromal cells to induce the proliferation and differentiation of immature thymocytes, was also proposed as a biomarker for SCID, as high levels were observed in a limited number of cases of SCID and acquired lymphopenia.^{25,26} However, this IL-7 immunoassay has not been developed into a robust screening tool for SCID, perhaps relating to limited stability in DBS. Testing for CD3 and CD45 for T cells and total leukocytes, respectively, by a multiplex immunoassay has also been shown to identify immune-deficient newborns,²⁷ though as with the IL-7 immunoassay, this has not been further developed into a robust screening tool.

DNA analysis of dried blood spots—Another approach to SCID screening would be to detect DNA sequence variants in known SCID disease genes; these sequence variations could be either previously defined, or as-yet unreported mutations predicted to be deleterious. Many NBS laboratories that include cystic fibrosis in their screening test panel currently use cystic fibrosis transmembrane conductance regulator (*CFTR*) DNA mutation arrays or genomic sequencing as a second-tier test to detect known gene mutations.²⁸ This method could be adapted for testing multiple gene panels for SCID.²⁹ The main limitations of such arrays are that there are many hundreds of mutations reported in known genes, and there are new SCID-causing mutations that continue to be discovered. Furthermore, infants with SCID who have no mutations identified even after extensive gene sequencing illustrate the extent of genetic heterogeneity in SCID. Thus to rely on a catalogue of mutations on an array would miss genuine cases of SCID.³⁰ Additional problems regarding the sensitivity of detecting sequence variations result from insertions and deletions. Whole exome or whole genome sequencing could be entertained, but the potential for false negatives and high costs as well as longer times required to perform and analyze sequences make sequence detection for SCID impractical for NBS at this time. Because SCID is genetically heterogeneous but characterized by low or absent autologous naïve T cells, it can be more precisely specified by analyte profiles than by DNA variation.

Liquid blood specimens—Determining the absolute lymphocyte count (ALC) from all infants by means of a complete blood count and differential was also proposed as a

screening method, given that a majority of SCID cases could be identified by low total lymphocyte counts, of which CD3 T cells are a majority. However, in forms of SCID in which infants may have high numbers of B cells, maternal engraftment, or Omenn syndrome, ALCs may be normal to high, causing false negative results. In order to capture all cases of SCID, the ALC cutoff would have to be set at a level that would result in unacceptably frequent false positive test results.³⁰ Therefore, the ALC exhibits too much overlap between infants with SCID and healthy infants to be suitable for population-based SCID screening. Moreover, the cost of obtaining a liquid blood sample rather than using the existing DBS sample would be high.

Measuring lymphocyte populations in umbilical cord blood has also been proposed as an assessment for detecting SCID, as demonstrated in one case report.³¹ However, reliance on the obstetric/midwifery team for sample collection and the need for fresh cord blood samples for flow cytometry testing pose significant financial and logistical problems for implementation as a population-based screening method.

TREC test for SCID—The development of a diverse repertoire of T cells, each with its own T cell receptor (TCR), is essential for recognition of foreign antigen bound to self MHC molecules. This diversity enables the immune system to recognize and control a wide variety of invading pathogens. To generate a large number of unique TCRs, DNA gene rearrangement and linear re-assembly is performed in thymocytes, so that each cell has its own combination of a TCR variable (V), diversity (D) and joining (J) sequence. This VDJ recombination is mediated by enzymes that induce double strand breaks at specific sequences that flank each V, D and J segment. Following successful cutting and in frame re-ligation of the DNA, unique productively rearranged VDJ T cell receptors are generated, and T cell precursors expressing these receptors mature and undergo selection, eventually resulting in the diverse population of naïve T cells released from the thymus into peripheral blood.³² The excised DNA fragments of the TCR that are not destined to be incorporated into a recombinant receptor gene are also ligated at their ends, forming a variety of circular DNA byproducts called T cell receptor excision circles (TRECs) (Figure 1). One particular circular species, the $\delta\text{rec-}\psi\text{J}\alpha$ TREC, is produced late in maturation by 70% of all T cells that are destined to express $\alpha\beta$ TCRs.³³ TRECs are stable, but are not replicated during mitosis. Therefore they persist, but become diluted as T cells proliferate.^{33,34} TRECs can be readily detected by a PCR reaction using primers designed to amplify a segment spanning the joint of the circle. Thus, the number of TREC copies can be used as an indicator of thymic production of naïve T cells, with a low TREC number signaling concern for inadequate autologous T cell production.

TRECs were initially used to monitor generation of new T cells in HIV infected individuals receiving effective antiretroviral treatment.³³ TRECs are most abundant in the peripheral blood of young infants, gradually decreasing in number with increasing age, reflecting a successively lower contribution of thymic output of new T cells vs. peripheral expansion of previously made T cells in older children and adults. The TREC assay was adapted to NBS as a real-time polymerase chain reaction (PCR) that amplifies DNA extracted from DBS to detect TRECs as a biomarker of naïve T cells.³⁵ Absence of TRECs identifies low or absent T cell production from any cause. A PCR control consisting of primers amplifying an

unrelated genomic DNA segment (typically from the β -actin or RNaseP gene) can be used as a control for the quality of DNA extracted from DBS. Such a control permits one to distinguish whether low TRECs are due to genuinely low T cells vs. due to insufficient or poor quality DNA.³⁰ Absent or few TRECs are found in infants who have an inadequate number of naïve T cells from any cause.³⁰ Thus the test detects any genetic causes of SCID or impaired T cell production as well as conditions in which there is abnormal loss of T cells from the peripheral circulation. With the development of the TREC assay that is done on DBS samples, SCID became the first immune disorder for which NBS was possible,^{35,36} and at the same time became the first high-throughput DNA-based NBS test.³⁷

The TREC test was predicted by a cost analysis³⁸ and a Markov model³⁹ to be a cost effective strategy to save lives of infants with SCID under a range of assumptions about incidence and cost of early vs. late treatment for SCID.

Conditions detected by TREC test

Primary and secondary targets of the TREC test are summarized in Table 2. Newborns with these conditions are expected to have low TRECs and low T cells. SCID caused by genetic defects that adversely affect T cell generation or maturation prior to and including the formation of TRECs can be identified by the TREC assay.³⁷ Mutations in a number of genes important for T cell development can cause SCID, and mutations that affect developmental stages prior to and including VDJ recombination will exhibit low TRECs.^{30,37} Table 1 includes the different forms of SCID; the more common and typical SCID defects that are regularly associated with abnormal TREC NBS are listed in bold type, along with rarer defects that have been successfully diagnosed by TREC NBS.

It is important to remember that there are genetic forms of combined immunodeficiency (CID) in which mutations occur in genes that affect T cells at developmental stages after the recombination of the TCR. T cells may be produced in normal or near-normal numbers, but they have defects in a variety of effector functions, such as migration, antigen responsiveness, cytokine production, proliferation, survival and generation of immunological memory. Examples include ZAP-70 and MHC class II deficiency. CID disorders are sometimes grouped with SCID because their clinical features can include recurrent and opportunistic infections as well as poor immune regulation or autoimmunity. Although infants affected with CID may be phenotypically similar to those with SCID in their lack of T cell function, TRECs are not expected to be low, and the TREC screening test is therefore not predicted to identify them.^{37,40,41}

Secondary targets of the TREC test are non-SCID immunodeficiencies in which there is a profound decrease in circulating naïve T cells.³⁰ Causes of low T cells that are not SCID include certain syndromes, such as DiGeorge syndrome, trisomy 21 or CHARGE (heart defect, atresia choanae, retarded growth and development, genital abnormality, and ear abnormality) syndrome, as well as non-immune problems such as vascular leakage or chylous effusions, which cause T cells to be lost until the underlying problem is corrected.⁴² Congenital leukemia is also a recognized secondary cause of low T cells.

Infants whose T cell counts are very low for any reason are considered immunologically impaired and may benefit from avoidance of live vaccines, protection from infectious exposures, transfusion precautions, and in some cases administration of prophylactic antibiotics or immunoglobulin infusions.^{17,43}

Findings from current TREC NBS programs to date

Spectrum of SCID genotypes post NBS

The advent of TREC NBS for SCID makes it important to distinguish those gene defects that are proven or expected to be associated with absent or low TRECs at birth (Table 1). Interestingly, leaky SCID due to hypomorphic mutations in the same genes that cause typical SCID may be partially permissive for T cell development, but are regularly identified by NBS because TRECs are well below the normal cutoff value.⁴⁴

In comparison with pre-NBS series of SCID patient genotypes reported by SCID transplant centers, infants with SCID detected by NBS have a larger proportion of autosomal recessive gene defects and fewer X-linked *IL2RG* mutations, possibly due to better ascertainment of sporadic cases with no family history (Figure 2).^{44–46} As X-linked lethal disorders maintain a constant population frequency due to replenishment of the gene pool by new mutations,⁴⁷ the lower proportion of X-linked SCID reflects an actual increase in autosomal recessive SCID cases detected by unbiased screening. Moreover, a larger proportion screened cases are due to defects in the recombinase activating genes *RAG1* and *RAG2*, half of which are leaky⁴⁴; without to NBS, individuals with a heterogeneous spectrum of leaky RAG mutations are not diagnosed until later in childhood^{48,49} and may have autoimmunity or Omenn syndrome rather than typical SCID.^{50–52} Finally, gene defects that were not known previously to cause SCID have been discovered with the advent of SCID NBS, e.g. *TTC7A* mutation resulting in multiple intestinal atresias with associated immune deficiency.^{53–56} A higher proportion of SCID cases detected by NBS have not had known disease genes, even after sequencing multiple typical SCID genes, in contrast to cases reported in the pre-screening era.^{37,44} With high throughput sequencing T lymphopenic infants identified by NBS can be investigated by whole exome or whole genome sequencing, presenting an opportunity to discover new mutations or identify previously unrecognized SCID-causing genes.

Non-SCID T cell lymphopenia detected by NBS

In addition to SCID and leaky SCID, secondary targets of TREC NBS include infants with non-SCID T cell lymphopenia (TCL) (Table 2). These TCL conditions include genetic syndromes with T cell impairment; T cell lymphopenia due to a non-immune illness; certain very low birth weight and premature infants; and infants with “Idiopathic TCL”. Screening programs have detected these non-SCID TCL infants at rates that depend on programmatic selection of TREC and T cell cutoffs.⁴⁴ In the most common TCL category, Syndromes, the predominant condition is DiGeorge/chromosome 22q11 deletion, in which failure of T cell production has been attributed to thymic insufficiency and/or intrinsic T and B cell defects related to haploinsufficiency of the *TBX1* gene in the commonly deleted region. Only a minority of newborns with DiGeorge syndrome have T cells sufficiently low to be identified

by SCID NBS. In complete DiGeorge syndrome, a very rare disorder in which there is aplasia of the thymus, TRECs and T cells are undetectable, and experimental thymus transplantation may be required for survival. The number of partial DiGeorge cases detected by NBS varies according to TREC and T cell cutoffs set by each screening program. For example, in the NBS program of California and other states that define significant TCL as <1500 T cells/ μ L, about 5% of infants with 22q deletion, those with the lowest T cells, are identified by screening.⁴²

Down syndrome/trisomy 21 is also a common cause of low TRECs and low T cells, with other causes including CHARGE syndrome, cartilage hair hypoplasia, Nijmegen breakage syndrome, ataxia telangiectasia, and Fryns syndrome. At least half of the cases of ataxia telangiectasia can be identified by TREC NBS and low T cell numbers.⁵⁷

Secondary causes of TCL include congenital heart disease (apart from DiGeorge syndrome) in which neonatal surgery or vascular leakage cause third-spacing of fluid and lymphocytes; gastrointestinal malformations such as intestinal lymphangiectasia and hydrops; and neonatal leukemia, with low T cells due to leukemic cell infiltration of the bone marrow. Preterm infants with TCL are a small proportion of the infants born at or before 30 weeks' gestation with birth weights of <1500 gm. If they survive their prematurity with its attendant complications, these infants' T cells normalize over time.⁴²

Idiopathic TCL describes infants who have low T cell numbers, but who do not meet diagnostic criteria for SCID or leaky SCID, do not have mutations in SCID-associated genes, and lack a recognized congenital syndromic diagnosis. T or B cell functions may be impaired and there may or may not be normalization over time. This group of infants, whose existence was not recognized prior to institution of population-wide screening for SCID, present an opportunity to discover gene defects not previously known to impair T cell development. Learning the natural history of these infants and defining their underlying disorders is potentially important for immunology and clinical medicine. If a gene diagnosis is proven to cause the low T cells, the infant is moved to the appropriate alternate category in Table 2.

Infants with low T cells in any category are advised to receive follow-up from a pediatric immunologist until their T cell numbers improve, and health-preserving interventions may include prophylactic antibiotics, immunoglobulin infusions, transfusion precautions (they may receive only leukoreduced, CMV-negative, irradiated blood products). Live attenuated rotavirus vaccine and other live vaccines are avoided. Infants whose T cells do not improve may require HCT.

Implementation of SCID screening

The characteristics of SCID that merit its addition to panels of newborn screened disorders, as formulated by Wilson and Jungner,²² are its lack of recognizable features on physical exam, asymptomatic status in early life, high disease burden if untreated, available effective treatment, improved survival and outcome for infants detected early, and availability of a low-cost screening test. NBS programs in the U.S. are under the jurisdiction of the public health departments of individual states and territories. Pilot SCID NBS programs were first

implemented in Wisconsin in 2008, and in Massachusetts and selected Navajo Native American hospitals in 2009. In May, 2010, the U.S. Department of Health and Human Services Secretary's Advisory Committee on Heritable Disorders in Newborns and Children endorsed adding SCID to the recommended uniform screening panel (RUSP) of NBS diseases based on an independent evidence based review.⁵⁸ However, this recommendation was advisory in nature.

States with the largest numbers of births, California, New York, Florida and Texas, as well as a number of smaller states, promptly added SCID screening to their NBS panels, so that by the end of 2013, over half of the births in the U.S. were being screened with a TREC test.⁵⁹ SCID NBS is now being conducted in at least 27 states, the District of Columbia and the Navajo Nation (spanning parts of Arizona and New Mexico), encompassing an estimated two thirds of births in 2014 (Figure 3). In nations beyond the U.S, SCID NBS is occurring in Ontario, Canada, Taiwan, and in pilot phases in several countries in Europe and the Middle East.^{60–62}

All U.S. laboratories that perform TREC NBS have followed the general guidelines published by the Clinical Laboratory Standards Institute.⁶³ In addition to local quality assurance protocols, screening programs participate in the SCID NBS Quality Control Program of the U.S. Centers of Disease Control and Prevention (CDC), which provides sets of unknown samples for quality assessment. In Quarter 3 of 2014, standardized DBS were sent to 22 U.S. and 14 international laboratories to validate TREC testing methods. Each program submitting data receives a report summarizing performance.⁶⁴ However, programs have their own TREC cutoffs and rules for handling testing of ill and preterm infants, reflecting each program's characteristics and population. Thus there is variability in the criteria for recalling infants for additional specimens, implementation of referral to specialists for follow-up, and immunological investigations performed to evaluate infants with non-normal TREC results.⁴⁴ Individual state programs have published their particular SCID NBS technologies and findings to date,^{42,65–67} and a recent publication compared SCID NBS outcomes in 11 programs.⁴⁴ The latter publication, which included over 3 million screened infants, noted that (1) all programs readily detected infants with typical and leaky SCID; (2) no SCID cases were missed by NBS and then detected later; and (3) affected infants were referred for immune restorative treatments in a timely fashion with a high rate of survival.

Clinical impact of newborn screening for SCID

SCID is the primary target for TREC NBS. While historically SCID was defined by growth failure and severe and opportunistic infections, along with low to absent T cells, the pre-symptomatic detection of SCID by NBS has required a new disease definition based on laboratory criteria and not relying on infectious complications. The Primary Immune Deficiency Treatment Consortium (PIDTC), a rare disease network funded by the U.S. National Institutes of Health, now defines typical SCID as infants with <300 autologous T cells/ μ L blood, <10% of control proliferation to the mitogen phytohemagglutinin A (PHA), often with spontaneously engrafted maternal T cells. The diagnosis can also be made or supported by finding deleterious mutations in known SCID-associated genes.^{8,68} If the

underlying gene mutations are not totally null, a diagnosis of leaky SCID can be made.^{48,69} Infants with leaky SCID are defined as having 300 or more autologous T cells/ μ L (expansion of oligoclonal T cells can lead to several thousand T cells/ μ L), no maternal T cell engraftment, and reduced proliferation to PHA. Infants with SCID or leaky SCID may appear totally healthy for the first months of life. Omenn syndrome, which may have early or delayed manifestations, refers to a subset of infants with leaky SCID in whom oligoclonal proliferation of dysregulated autologous T cells leads to generalized erythroderma and desquamation of the skin, lymphadenopathy, splenomegaly, eosinophilia and elevation of immunoglobulin E.

With implementation of SCID NBS in unbiased populations, an accurate measurement of incidence has become available; Kwan et al. reported that 1 in 58,000 infants (95% CI 1/46,000–80,000) are born with SCID or leaky SCID (including Omenn syndrome), nearly twice the previous estimates based on population data or experience of centers performing HCT therapy for SCID.⁴⁴ Where NBS for SCID is not being done, infants remain at risk of succumbing to infectious diseases without the true diagnosis of their immunodeficiency being considered. Cost-benefit arguments and worldwide publicity continue to advocate for universal implementation of SCID NBS.^{70–73}

Immune system restoring therapies for SCID

The premise of SCID NBS is to identify affected infants prior to development of infections so that their treatments can be designed for the most favorable outcomes. The predominant treatment for SCID is allogeneic HCT, with enzyme replacement therapy (ERT) as an option for ADA deficient SCID.^{6,8,74–79} ADA deficient SCID and X-linked γ c deficient SCID can also be treated by experimental *ex vivo* addition of a correct cDNA to autologous hematopoietic stem cells, followed by reinfusion.^{80–84} Current gene therapy trials are reporting good safety and efficiency and may become approved standard care in the future.^{85,86} In addition, advances in genome editing technologies now under development may offer gene correction therapies without the use of viral vectors, reducing the risks of insertional mutagenesis and nonphysiologic regulation of gene expression.^{87,88}

The initial management of a presenting SCID-affected infant is as crucial to the eventual outcome as the choice of conditioning regimen and acute peri-transplant management.⁷⁵ A PIDTC retrospective study of 240 SCID infants showed overall survival in infants >3.5 months of age at treatment who were continuously infection-free (overall survival 90%, 95% CI 67–98) was nearly as high as that of infants treated prior to 3.5 months (overall survival 94%, 95% CI 85–98). Even older SCID infants who had infections that were treated and resolved had very good overall survival (82%, 95% CI 70–90), highlighting the importance of prevention and successful treatment of infection in determining a good transplant outcome.⁶ In addition to the advantages of early diagnosis for SCID-affected infants, when infection prophylaxis can be instigated early prior to onset of infections, elucidation of the causative genetic defect also informs treatment strategies, affecting decisions regarding preferred donor, T cell depletion of donor cells, and conditioning regimen.^{6,75,89,90} Even in the same phenotypic category of VDJ recombination defects giving rise to T-B-NK+ SCID,

susceptibility to toxicity effects of alkylating agents are differentially manifested in RAG-deficient SCID compared to Artemis-SCID.⁹¹

Since NBS for SCID has been implemented, infants have been transplanted at an earlier age when there are suitable donors, and importantly, before opportunistic bacterial infections take place due to precautions that are undertaken prior to transplant. However, viral infections in these immune compromised infants are still problematic, and physicians need to remain vigilant to warn parents regarding exposure to respiratory viruses, potential for transmission of cytomegalovirus in breastmilk, and avoidance of live rotavirus vaccines.^{43,92,93} In cases where HLA-matched related and even unrelated donors are available, studies have shown that chemotherapy conditioning may not be necessary for successful HCT in SCID infants.⁹⁴ However, important considerations for transplantation of young infants include exposure to conditioning chemotherapies and graft vs. host disease (GvHD) prophylaxis in infants with NK+ SCID,⁹⁵ leaky SCID, or those receiving grafts from mismatched donors.⁹⁶ There is a lack of knowledge regarding the pharmacokinetics of chemotherapy regimens in young infants⁹⁷ and careful dosage monitoring is important⁹⁸ in the immediate period as well as in view of late toxicity effects.^{89,91}

The role of public health

The central idea of early detection of disease to facilitate treatment is simple. However successful implementation of newborn screening (on the one hand, bringing to treatment those with previously undetected disease, and, on the other, avoiding harm to those not in need of treatment) is far from simple. Lack of parity between state screening programs is evident from a recent study,⁴⁴ and systemic evaluation of the TREC NBS programs needs to take differences into account.⁹⁹ Fortunately, new programs planning to undertake SCID NBS can now review the results from pilot studies and established programs as they move towards universal SCID NBS for all infants.

Conclusions

NBS has been an effective public health measure and a fertile area for clinical research. Much has been learned about the genetic and clinical heterogeneity of the disorders identified. All newborn screened diseases, SCID among them, have been found to be clinically and genetically more diverse than was originally believed, with an expanded spectrum and new disorders coming to light once screening is instituted.^{100,101} Future research will continue to focus on these areas, and new technologies such as deep sequencing are being applied.

SCID NBS is currently being performed in 27 states, the District of Columbia and the Navajo Nation, and SCID-affected infants are reliably being detected and promptly referred to centers that provide immune system restoring treatments. Population-based screening has determined an unbiased incidence of SCID to be 1 in 58,000 births, higher than previous estimates. As NBS for SCID becomes more widespread, subpopulations with higher incidence due to founder mutations will be further elucidated.^{8,44} Early detection of infants with SCID provides new opportunities to investigate their molecular etiologies and to develop optimal treatment strategies for very small, very young affected infants. Large

multi-center collaborations, such as the Primary Immune Deficiency Treatment Consortium, will define and investigate the impact of the many variables involved in HCT, including SCID genotype, donor selection, donor cell preparation, conditioning regimen, GvHD prophylaxis, to determine optimal transplant protocols for each SCID patient. New protocols will be required to take into account the small size and immaturity of the blood brain barrier of SCID infants detected by NBS so as to minimize toxicity from treatments that to date have been established for larger, older individuals and individuals with malignancy rather than immunodeficiency.⁹⁸

The availability of NBS for SCID requires vigilance from care providers, not only to understand and interpret TREC results, but also to be aware of forms of immune deficiency in which TRECs are not abnormal. These include infants who for whatever reason are not screened or appropriately followed up; infants with defects in late T cell development or function whose ability to make TRECs is preserved; and infants with primary deficiencies of B cells, granulocytes or other immune cells that TREC testing will not reveal. Although screening for B cell immunoglobulin gene rearrangement by measuring immunoglobulin κ -chain excision circles (KRECs) has been suggested, whether this test would on balance improve the effectiveness of immunodeficiency screening is not clear at this time, as additional false positives might outpace gains in detecting pure B cell disorders. With further implementation of SCID NBS, we anticipate improved understanding of primary immune disorders and how best to treat them.

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Figure 1.

Generation of the δ Rec- Ψ J α TREC, showing primers, black arrows, used to amplify and quantitate TREC junction fragment. Excision of the TCRD locus from the TCRA locus results in the excised fragment which circularizes to form the δ Rec- Ψ J α TREC, found in >70% of $\alpha\beta$ T cell receptor expressing T cells. Reprinted from Journal of Allergy and Clinical Immunology, Vol. 129, J.M. Puck, Laboratory technology for population-based screening for severe combined immunodeficiency in neonates: The winner is T-cell receptor excision circles, pp. 607–616.³⁰ Copyright 2012, with permission from Elsevier.

Figure 2.

Distribution of SCID genotypes in the presence of newborn screening. (A) In California, approximately 2,250,000 infants were screened in 4.5 years; SCID incidence was 1 per 59,000 births. (B) A study of 11 SCID NBS programs in the U.S. with 3,030,083 infants screened found an incidence of 1 per 58,000 births; genotype distribution was similar, and included detection of single incidences of additional SCID genotypes CD3D, TTC7A and chromosome 12p duplication.⁴⁴

Figure 3.

Map of the U.S. showing states that have implemented SCID NBS (with year in which this commenced, solid gray), and states that have plans to start NBS for SCID (hatched gray). In Louisiana (LA) and Puerto Rico (PR), SCID NBS was started in 2010 but was discontinued due to lack of funding for the screening programs. There are no known plans for SCID NBS implementation in the unshaded states. Information courtesy of Dr. Amy Brower (Newborn Screening Translational Research Network) and Emily Hovermale (Immune Deficiency Foundation).

Table 1

SCID and CID disorders based on immunologic mechanism, with immune phenotype, gene defect and expectation to have abnormally low TRECs at birth.

Mechanism of disease when defective	Lymphocyte profile	Gene	Protein
<i>Impaired cytokine signaling and early lymphoid progenitor development</i>			
Common γ chain	T– B+ NK–	IL2RG	Common γ chain
JAK3 kinase	T– B+ NK–	JAK3	Janus kinase 3
IL-7Rα receptor	T– B+ NK+	IL7R	IL-7R α
<i>Defects in VDJ recombination</i>			
RAG1	T– B– NK+	RAG1	RAG1
RAG2	T– B– NK+	RAG2	RAG2
RAG1	T– B– NK+	RAG1	RAG1
RAG2	T– B– NK+	RAG2	RAG2
Artemis	T– B– NK+	DCLRE1C	Artemis
DNA-PKcs	T– B– NK+	PRKDC	DNA-PKcs
DNA ligase IV	T– B– NK+	LIG4	DNA ligase IV
(Cerunnos/XLF)	T– B– NK+	NHEJ	Cerunnos/XLF
<i>Impaired signaling through TCR</i>			
CD3δ**	T– B+ NK+	CD3D	CD3 δ
(CD3 ϵ **)	T– B+ NK+	CD3E	CD3 ϵ
(CD3 ζ **)	T– B+ NK+	CD3Z	CD3 ζ
(CD3 γ *)	T– B+ NK+	CD3G	CD3 γ
CD45**	T– B+ NK–/+	PTPRC	CD45
ZAP-70*	T+ B+ NK+ CD4+ CD8–	ZAP70	ZAP-70
(p56lck**)	T– B+ NK+	LCK	p56lck
<i>Decreased lymphocyte survival, increased apoptosis or impaired migration or function</i>			
Reticular dysgenesis T	T– B– NK–	AK2	Adenylate kinase 2
ADA	T– B– NK–	ADA	Adenosine deaminase
(PNP**)	T– B– NK–	PNP	Purine nucleoside phosphorylase
Coronin-1A**	T– B+ NK+	CORO1A	Coronin-1A
MHC Class II*	T+ B+ NK+ CD4– CD8+	CIITA, RFXANK, RFX5, RFXAP	CIITA, RFXANK, RFX5, RFXAP
Cartilage hair hypoplasia**	T– B+ NK+	RMRP	<i>RNA of RNase MRP complex</i>
Ataxia telangiectasia*,**	Tlow	ATM	Ataxia telangiectasia mutated
Nijmegen breakage syndrome*,**	Tlow	NBN	Nibrin

Bold type, proven to be detected by TREC NBS, though hypomorphic gene mutations may result in milder phenotypes that might not be detected by this means.

* CID disorders, often not as severe clinically as typical SCID, in which the defect does not preclude TCR recombination. Therefore TREC formation is not impaired, and SCID NBS with a TREC test would not be expected to identify affected infants.

** CID disorders in which TREC NBS has been diagnostic due to the low numbers of peripheral blood T cells due to impaired T cell survival or release from the thymus, despite intact TCR recombination.

Parentheses indicate expected TREC NBS test performance, though no cases screened at birth have been verified to date. Insufficient data are available to categorize defects of STAT5b, Itk, MHC Class I, FOXP1, or Calcium or Magnesium ion channels.

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Table 2

Classification of infants with low TRECs and low T cells found by SCID NBS.

Category	Definition of Condition ^a
Typical SCID	<300 autologous T cells/ μ L, <10% of normal proliferation to PHA, ^b frequently with maternal T cell engraftment and deleterious defect(s) in a known SCID gene
Leaky SCID	300–1,499 autologous T cells/ μ L (or higher numbers of oligoclonal T cells), reduced proliferation to PHA, no maternal engraftment, generally with incomplete defect(s) in a known SCID gene
Omenn syndrome	Similar to leaky SCID, but also with oligoclonal T cells, erythroderma, hepatosplenomegaly, eosinophilia, and elevated serum IgE levels
Syndrome with low T cells	Recognized genetic syndrome that includes low T cells within its spectrum of clinical findings
Secondary low T cells	Congenital malformation or disease process without intrinsic immunodeficiency that results in low circulating T cells
Preterm birth alone	Preterm infants with low T cells early in life that become normal over time
Idiopathic T cell lymphopenia,	Persistently low T cells (300–1,499/ μ L), functional T and/or B cell impairment, no defect in a typical SCID gene; etiology and clinical course undetermined ^c

^aDefinitions used by Region 4 Stork (R4S) Laboratory Performance Database and Primary Immunodeficiency Treatment Consortium (PIDTC).

^bPHA, phytohaemagglutinin.

^cWhen or if an etiology for low T cells is discovered, the affected individual is moved to the appropriate category.