

Newborn screening for T-cell deficiency

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Purpose of review

Newborn screening for T-cell deficiency is ongoing in two states, and the published results of 1 year of screening in Wisconsin are favorable. In this review, the history, methodology, results, challenges, and future direction of screening are discussed.

Recent findings

As a concept, newborn blood screening (NBS) has evolved from the detection of elevated levels of phenylalanine on filter paper to the application of DNA-based technologies to identify T-cell lymphopenia in infants with severe combined immunodeficiency (SCID). After diagnosis by NBS, one infant with SCID received a hematopoietic stem cell transplant (HSCT), and several others are currently undergoing evaluation for HSCT. The utility of NBS for T-cell deficiency is dependent on the NBS program to define a rigorous screening protocol, ensure seamless follow-up of all identified infants, and demand a renewed emphasis on the training of future clinical immunologists to care for these patients. DNA-based NBS assays are currently being evaluated to identify infants with other serious diseases.

Summary

Newborn screening for T-cell deficiency has ushered in a new era in the detection of infants with primary immunodeficiencies.

Keywords

lymphopenia, newborn screening, primary immunodeficiency, severe combined immunodeficiency, T-cell receptor excision circle

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Introduction

In 1963, Guthrie [1] demonstrated that postnatal testing of dried blood spots (DBS) on filter paper led to the identification and treatment of infants prior to the development of cognitive deficits associated with phenylketonuria (PKU). This newborn blood screening (NBS) test was easy to perform, inexpensive, and was eventually performed in all states.

The application of tandem mass spectrometry led to the ability to simultaneously screen for multiple disorders [2]. Expanded screening programs developed, with some states screening for almost 50 conditions [3]. Recently, the advent of DNA-based technologies has again shifted the paradigm of NBS, resulting in the potential to concurrently screen for numerous disorders.

Criteria for inclusion of newborn screening tests

Although the opportunity to screen for an ever-increasing number of conditions exists, recommendations for NBS have continually emphasized the importance of objec-

tives similar to those in Guthrie's initial PKU screen. The disorder of interest must be well characterized by defined confirmatory tests; affect a sufficient population of infants; be undetectable by routine examination at birth; result in devastating effects if not detected and treated early; and have a well defined, disease-altering treatment. The screening test must have high sensitivity and specificity, demonstrate a positive, population-based economic cost-benefit analysis, pose a low risk to the infant, and include resources and provisions for patient follow-up [4–6]. To date, 51 NBS programs exist independently, in all states and the District of Columbia, and there is wide variation in the practices of each program [7].

Development of the T-cell receptor excision circle assay for the detection of severe combined immunodeficiency

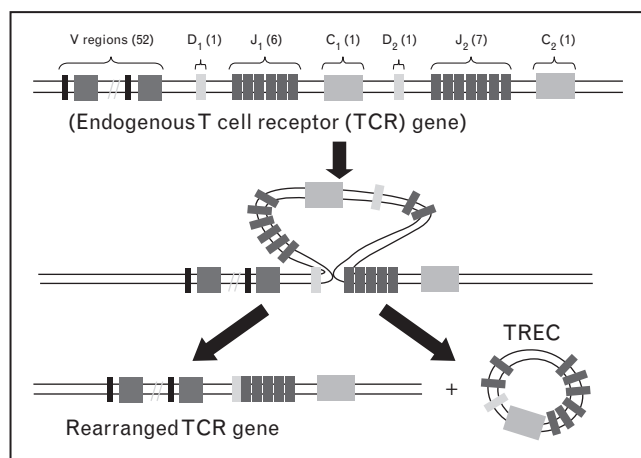
Severe combined immunodeficiency (SCID) is a disorder caused by a variety of genetic defects that manifest with extreme susceptibility to life threatening infections in infancy due to severe defects in both cellular and humoral immunity. Regardless of the underlying genetic defect,

all subtypes of SCID are defined by a deficiency of T cells. Studies show that early diagnosis and treatment of infants with SCID prior to the development of infections are critical to survival. Effective treatments for SCID include hematopoietic stem cell transplant (HSCT), gene therapy, or enzyme replacement [8,9]. In an attempt to facilitate the early diagnosis and treatment of affected infants, newborn screening for SCID became an attractive option.

Initial screening tests for SCID were based on the detection of peripheral blood lymphopenia. However, quantification of peripheral blood lymphocyte counts may fail to identify some infants with SCID due to the presence of normal B-cell or natural killer (NK)-cell numbers, maternal engraftment of T cells, and/or expansion of few T-cell clonotypes [10,11]. In addition, complete blood counts are expensive and do not utilize preexisting NBS cards, making this test unsuitable for mass screening. Enumeration of naïve T-cells by the detection of T-cell receptor excision circles (TRECs) by real-time quantitative polymerase chain reaction (RT-qPCR) overcomes these obstacles (Fig. 1). TRECs are episomal, circular DNA fragments generated during the sequential rearrangement of V, D, and J segments of T-cell receptor (TCR) genes. These circular DNA pieces do not replicate and are therefore only present in T cells that have recently emigrated from the thymus. A specific delta-deleted segment of the TCR gene, $\delta\text{Rec-}\psi\text{J}\alpha$, was identified as a reliable surrogate marker for the numbers of naïve recent thymic T-cell emigrants, as it is rearranged in over 70% of α/β T cells [12].

In 2005, the TREC assay was adapted to detect SCID via RT-qPCR analysis of DNA extracted from DBS on NBS

Figure 1 Schematic representation of T-cell receptor excision circle formation



Rearrangement of the T-cell receptor (TCR) gene yields the formation of a circular piece of DNA, the T-cell receptor excision circle.

cards [13]. Morinishi *et al.* [14•] demonstrated that the TREC assay would detect SCID regardless of its genetic cause. Investigators in Wisconsin further refined the TREC assay and demonstrated that it was highly sensitive and specific for SCID, cost-effective, reproducible, and amenable to high-throughput population-based testing [15•]. In 2008, Wisconsin became the first state to implement mandatory newborn screening for T-cell deficiency. Massachusetts followed in February 2009, offering an optional, pilot newborn screening assay for SCID [16].

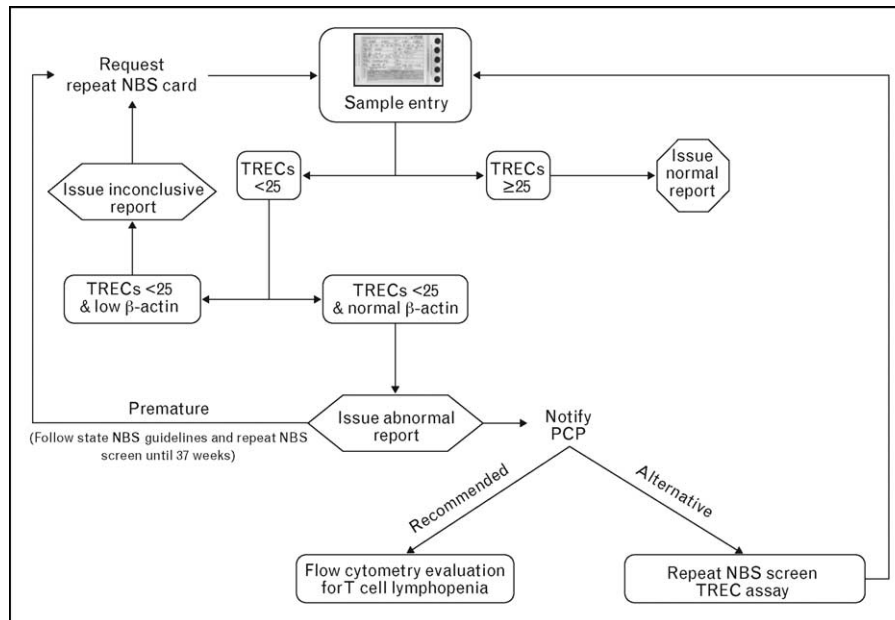
Newborn screening algorithm for T-cell deficiency

The Wisconsin NBS program uses an algorithm based on data from the large-scale pilot study. The algorithm involves three tiers of screening: detection of TRECs in DBS from NBS cards; confirmatory SCID flow cytometry assay for lymphocyte subset analysis; and referral to a clinical immunologist (Fig. 2) [17•]. It should be stressed that an abnormal TREC assay leads to flow cytometry screening only in infants with a gestational age of [or a corrected gestational age (CGA) equivalent to] 37 weeks or more. Premature infants continue to undergo periodic screening until the CGA is equivalent to 37 weeks or more; after that time, follow-up for an abnormal result occurs as per the algorithm for full-term infants. This practice is in accordance with the standard operating procedure for NBS in the state of Wisconsin, and significantly reduces the number of false positive tests.

Following an abnormal TREC result, the physician of record is notified and a confirmatory SCID flow cytometry assay is arranged. This assay is limited in scope to reduce costs and enumerates the number of T cells, NK cells, and B cells. In addition, since maternally engrafted T cells or expansion of few T-cell clonotypes can increase T-cell numbers within the normal range, the percentages of naïve and memory T cells are determined by the expression of CD45RA and CD45RO, markers of naïve and memory T cells, respectively. If the flow cytometry assay reveals normal lymphocyte subset numbers and percentages, a normal report is issued and no further testing is required. If the assay is abnormal, arrangements are made to have the infant referred to a clinical immunologist for further evaluation.

Initial results of screening in Wisconsin

During the first year of statewide screening for T-cell deficiency (2008), a total of 71 000 neonates were screened. Seventeen neonates with a CGA equivalent to 37 weeks or more were ultimately diagnosed with an abnormal TREC assay. In four infants, the physicians elected to repeat the TREC assay, which was normal; one

Figure 2 Wisconsin newborn screening algorithm for T-cell deficiency

All infants born in Wisconsin are screened first by the T-cell receptor excision circle assay. If indicated, a confirmatory severe combined immunodeficiency (SCID) flow cytometry assay is used to determine lymphocyte subset numbers. Referral to a clinical immunologist (not shown) is arranged if the SCID flow cytometry assay is abnormal. NBS indicates newborn blood screening; TRECs, T-cell receptor excision circles; PCP, primary care physician. Reproduced with permission from [17*].

infant died of a disease unrelated to SCID; and one infant's parents refused further testing. Thus, the second-tier SCID flow cytometry assay was performed on a surprisingly small number of infants ($n = 11$). The flow cytometry results were normal in three infants and revealed significant T-cell lymphopenia in the remainder of the infants (22q11.2 deletion syndrome, $n = 2$; third-spacing of lymphocytes secondary to anatomic lymphatic abnormality, $n = 3$; idiopathic T-cell lymphopenia, $n = 3$).

Two points of these results should be emphasized: despite the paucity of abnormal TREC and SCID flow cytometry assays, no known case of SCID was missed by NBS during 2008, and the test was highly specific for T-cell lymphopenia regardless of etiology.

Of the three cases of idiopathic T-cell lymphopenia, one infant was clinically suspicious for 22q11.2 deletion syndrome due to the presence of a cleft palate. Testing was ultimately negative for a 22q microdeletion, and this infant's T-cell counts improved over time. A second infant had an isolated T-cell deficiency, with normal B-cell and NK-cell subsets. Interestingly, this male patient's older sister (born prior to the TREC NBS assay) also had T-cell lymphopenia as determined by flow cytometry. This girl has had both recurrent and opportunistic infections, and failure to thrive due to an inflammatory enteropathy. The boy has been treated with

prophylactic gammaglobulin replacement and antibiotics and has not had opportunistic infections. However, both siblings continue to have significant T-cell lymphopenia with virtually no TRECs in the peripheral blood and are undergoing evaluation for HSCT. The third child with idiopathic T-cell lymphopenia was ultimately diagnosed with a dominant negative mutation in the *Rac2* protein after developing omphalitis and a paratracheal abscess, and underwent a successful HSCT. These cases illustrate the inherent power of the TREC assay to detect other combined immunodeficiencies that have associated low T-cell numbers, in addition to SCID [17*].

Clinical and diagnostic dilemmas of newborn screening for T-cell deficiency

Although the results-to-date of NBS for T-cell deficiency are promising, several issues remain. As noted previously, determination of the NBS algorithm requires special consideration for the screening of premature infants. Premature infants (<37 weeks CGA) naturally have fewer naïve T-cells at birth than their full-term counterparts [18]. Additionally, interventions to decrease morbidity in premature infants, such as the administration of maternal steroids to promote fetal lung maturation, result in lower numbers of naïve T-cells [19]. However, no data have been published on TREC reference ranges for premature infants. Until further information is available,

it is important that all NBS programs have safeguards to ensure repeat testing for premature infants with inconclusive or abnormal results, at least until they reach a full-term CGA.

Even more challenging are cases such as the infants with profound T-cell lymphopenia, where persistent severe T-cell deficiency is detected in the absence of an identifiable underlying genetic disorder or characteristic phenotype. Historically, the detection of SCID and other severe forms of immunodeficiency has been guided by the presence or absence of certain types of infection. In identifying infants prior to the development of these sequelae, the etiology of some cases of T-cell lymphopenia is difficult to interpret, particularly when the infant's laboratory phenotype does not clinically resemble a specific diagnosis. For example, if a child has undetectable T cells on flow cytometry, and does not have complete 22q11.2 deletion syndrome, the diagnosis is relatively straightforward. However, in a child with severely depressed but detectable naïve T-cell counts without a history of infection, it is considerably more difficult to recommend HSCT, particularly when a genetic diagnosis is not available. It is also unclear how long patients should be monitored for regression of developmental lymphopenia, or what absolute T-cell count is necessary to prevent life-threatening infections. The powerful ability of NBS to detect infants prior to the development of a characteristic clinical phenotype necessitates the need for accurate, efficient ways to determine an underlying genetic diagnosis. Accordingly, an efficient algorithm must be developed to identify the genetic defect in infants that fail NBS for T-cell deficiency. The classic candidate gene approach can take months or years, and this approach only identifies known causes of T-cell lymphopenia, and does not account for unexpected presentations of known genetic defects. Previously cost-prohibitive technologies, such as genomic sequencing, are becoming realistic alternatives to traditional diagnostic methods; these technologies, however, present an entirely separate set of ethical questions that warrant full consideration [20,21].

Systematic challenges to newborn screening for T-cell deficiency

NBS for T-cell deficiency is currently underway in Wisconsin and Massachusetts, and the results to date are encouraging. Successful implementation of these screening programs is largely a result of the NBS infrastructure in these states, and this raises key issues for states not currently performing DNA-based screening. The positive outcomes of detecting infants with T-cell deficiency are necessarily dependent on two resource-driven factors: the ability for each state to implement the screening assay on a population basis and the assurance that adequate

clinical care can be provided once the diagnosis of T-cell deficiency is made [22•].

Presently, individual states bear the financial burden of each NBS test performed. This burden includes not only the costs of developing, running, and maintaining a NBS laboratory, but also the personnel costs in reporting the test results and securing follow-up as needed. A feasible solution to this problem would be the use of a regional, or even nationwide, NBS consortium. States in a specific geographic area could send NBS samples to a centralized testing facility and results could be reported back to the state of origination. This would alleviate a great deal of the individual state burden, and facilitate NBS for conditions that might otherwise be superseded by other funding priorities. Indeed, other countries regularly utilize regional and even nationwide NBS programs for specific conditions, often with improved results compared with the decentralized programs previously in place. For example, France's implementation of nationwide screening for cystic fibrosis (CF) in 2002 facilitated the formation of nationwide CF care centers, and allowed further optimization of their screening test [23].

No issue seems more relevant to the fundamental goal of NBS than ensuring there are sufficient numbers of trained clinical immunologists to evaluate and follow all infants identified as having T-cell deficiency. Based on data from the Wisconsin NBS program in 2008, 11/71 000 infants (0.00015% of those screened) had abnormal results requiring formal evaluation by a clinical immunologist; this may not be feasible in all states. For this reason, protocols for NBS for T-cell deficiency require an inherent referral strategy, which might include consultation with clinicians outside the referring state. With monitoring of outcomes over time, this process could potentially lead to the designation of specific 'Centers of Excellence' in the treatment of primary immunodeficiencies, a title that would benefit the care of patients of all ages. Furthermore, the low prevalence and phenotypic variability of patients with T-cell deficiency necessitates collaboration to ensure proper diagnosis and management. De-identified patient data registries have the power of effectively increasing each individual clinician's reference population, among their other beneficial uses, and can facilitate the continual development of detailed best-practice guidelines [24].

Future directions of newborn screening for primary immune deficiency diseases

In January 2010, the Advisory Committee on Heritable Disorders in Newborns and Children, a group established to advise the Secretary of Health and Human Services, unanimously voted to recommend universal newborn

screening for T-cell deficiency/SCID, their first recommendation since the committee's inception in 2005 [25]. On 21 May 2010, Secretary Kathleen Sebelius accepted this recommendation, adding NBS for SCID to the national core panel of newborn screening tests [26]. Looking forward, DNA-based NBS using multiplexed assays could theoretically screen for hundreds of additional conditions with a similar base cost and time-frame as the TREC assay. NBS for 22q11.2 deletion syndrome is currently under investigation, and a recent paper showed that relative Gene Copy Number (rGCN) detection had 100% sensitivity and specificity for the detection of 22q11.2 deletions in 361 blinded samples tested [27]. If multiplexed to the TREC assay, the 22q11.2 assay screening for SCID and 22q11.2 deletion syndrome could be performed concomitantly, screening for both conditions with a single sample. Whether NBS is the appropriate approach for any given disorder, however, is a discussion that requires input from a wide variety of sources, such as bioethicists, families of affected children, and members of the medical team.

Conclusion

NBS for T-cell deficiency is a revolutionary DNA-based approach to the diagnosis of SCID and other primary immunodeficiencies. Further applications of this technology will require careful logistic and ethical considerations; however, the possibility of detecting infants with serious conditions prior to the development of adverse outcomes is strongly promising, and compels further investigation.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

- 1 Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics* 1963; 32:318–343.
- 2 Wilcken B. Recent advances in newborn screening. *J Inher Metab Dis* 2007; 30:129–133.
- 3 Newborn Screening Status Report (updated 3/01/10). National Newborn Screening and Genetics Resource Center. <http://genes-r-us.uthscsa.edu/nbsdisorders.pdf>. [Accessed 6 May 2010]
- 4 Watson MS, Lloyd-Puryear MA, Mann MY, *et al.* Newborn screening: toward a uniform screening panel and system. *Genet Med* 2006; 8 (Suppl.):12S–252S.
- 5 Executive Summary, Committee on Assessing Genetic Risks. In: Andrews LB, Fullerton JE, Holtzman NA, Motulsky AG, editors. *Assessing genetic risks: implications for health and social policy*. Washington, DC: National Academy Press; 1994. pp. 1–28.
- 6 Therrell BL, Panny SR, Davidson A, *et al.* U.S. newborn screening system guidelines: statement of the council of regional networks for genetic services. *Screening* 1992; 1:135–147.
- 7 Mandl KD, Feit S, Larson C, Kohane IS. Newborn screening program practices in the United States: notification, research, and consent. *Pediatrics* 2002; 109:269–273.
- 8 Buckley RH. Advances in the understanding and treatment of human severe combined immunodeficiency. *Immunol Res* 2000; 22:237–251.
- 9 Myers LA, Patel DD, Puck JM, Buckley RH. Hematopoietic stem cell transplantation for severe combined immunodeficiency in the neonatal period leads to superior thymic output and improved survival. *Blood* 2002; 99:872–878.
- 10 Muller SM, Ege M, Pottharst A, *et al.* Transplacentally acquired maternal T lymphocytes in severe combined immunodeficiency: a study of 121 patients. *Blood* 2001; 98:1847–1851.
- 11 Palmer K, Green TD, Roberts JL, *et al.* Unusual clinical and immunologic manifestations of transplacentally acquired maternal T cells in severe combined immunodeficiency. *J Allergy Clin Immunol* 2007; 120:423–428.
- 12 Verschuren MC, Wolvers-Tettero IL, Breit TM, *et al.* Preferential rearrangements of the T cell receptor-delta-deleting elements in human T-cells. *J Immunol* 1997; 158:1208–1216.
- 13 Chan K, Puck JM. Development of population based newborn screening for severe combined immunodeficiency. *J Allergy Clin Immunol* 2005; 115:391–398.
- 14 Morinishi Y, Imai K, Nakagawa N, *et al.* Identification of severe combined immunodeficiency by T-cell receptor excision circles quantification using neonatal Guthrie cards. *J Pediatr* 2009; 155:829–833.
- This paper demonstrates that the T-cell receptor excision circle assay can identify different molecular causes of SCID.
- 15 Baker MW, Grossman WJ, Laessig RH, *et al.* Development of a routine newborn screening protocol for severe combined immunodeficiency. *J Allergy Clin Immunol* 2009; 124:522–527.
- This paper illustrates how the T-cell receptor excision circle assay was refined for use in NBS for SCID, including the adaptations made to enable high-throughput testing.
- 16 Massachusetts to screen babies at birth for immune deficiency. University of Massachusetts Medical School, New England Newborn Screening Program, 13 February 2009. <http://www.umassmed.edu/Content.aspx?id=67542>. [Accessed 8 May 2010]
- 17 Routes JM, Grossman WJ, Verbsky J, *et al.* Statewide newborn screening for severe T-cell lymphopenia. *JAMA* 2009; 302:2465–2470.
- This paper details the results of 1 year of screening for T-cell deficiency/SCID in the state of Wisconsin.
- 18 Peoples JD, Cheung S, Nesin M, *et al.* Neonatal cord blood subsets and response to bacterial antigens. *Am J Perinatol* 2009; 26:647–657.
- 19 Chabra S, Cottrill C, Rayens MK, *et al.* Lymphocyte subsets in cord blood of preterm infants: effect of antenatal steroids. *Biol Neonate* 1998; 74:200–207.
- 20 Samani NJ, Tomaszewski M, Schunkert H, *et al.* The personal genome: the future of personalised medicine? *Lancet* 2010; 375:1497–1498.
- 21 Dhondt JL. Neonatal screening: from the 'Guthrie Age' to the 'genetic Age'. *J Inher Metab Dis* 2007; 30:418–422.
- 22 Lipstein EA, Voroño S, Browning MF, *et al.* Systematic evidence review of newborn screening and treatment of severe combined immunodeficiency. *Pediatrics* 2010; 125:e1226–e1235.
- This review summarizes evidence and potential obstacles to the use of NBS for SCID.
- 23 Munck A, Dhondt JL, Sahier C, Roussey M. Implementation of the French nationwide cystic fibrosis newborn screening program. *J Pediatr* 2008; 153:228–233.
- 24 Howell RR, Engelson G. Structures for clinical follow-up: newborn screening. *J Inher Metab Dis* 2007; 30:600–605.
- 25 US Department of Health and Human Services, Health Resources and Services Administration, Advisory Committee on Heritable Disorders in Newborns and Children. Letter to the Secretary, U.S. Department of Health and Human Services, 25 February 2010. <http://www.hrsa.gov/heritabledisorderscommittee/correspondence/feb2010letter.htm>. [Accessed 5 June 2010].
- 26 US Department of Health and Human Services, Health Resources and Services Administration, Advisory Committee on Heritable Disorders in Newborns and Children. Response from the Secretary, U.S. Department of Health and Human Services, 21 May 2010. http://www.hrsa.gov/heritabledisorderscommittee/correspondence/response5_21_2010.pdf. [Accessed 5 June 2010]
- 27 Tomita-Mitchell A, Mahnke DK, Larson JM, *et al.* Multiplexed quantitative real-time PCR to detect 22q11.2 deletion in patients with congenital heart disease. *Physiol Genomics* (in press).