

## Original Investigation

# Newborn Screening for Severe Combined Immunodeficiency in 11 Screening Programs in the United States

Antonia Kwan, PhD, MRCPCH; Roshini S. Abraham, PhD; Robert Currier, PhD; Amy Brower, PhD; Karen Andruszewski, BS; Jordan K. Abbott, MD; Mei Baker, MD; Mark Ballow, MD; Louis E. Bartoszesky, MD; Vincent R. Bonagura, MD; Francisco A. Bonilla, MD, PhD; Charles Brokopp, DrPH; Edward Brooks, MD; Michele Caggana, ScD; Jocelyn Celestin, MD; Joseph A. Church, MD; Anne Marie Comeau, PhD; James A. Connelly, MD; Morton J. Cowan, MD; Charlotte Cunningham-Rundles, MD; Trivikram Dasu, PhD; Nina Dave, MD; Maria T. De La Morena, MD; Ulrich Duffner, MD; Chin-To Fong, MD; Lisa Forbes, MD; Debra Freedenberg, MD; Erwin W. Gelfand, MD; Jaime E. Hale, BS; I. Celine Hanson, MD; Beverly N. Hay, MD; Diana Hu, MD; Anthony Infante, MD, PhD; Daisy Johnson, BSN; Neena Kapoor, MD; Denise M. Kay, PhD; Donald B. Kohn, MD; Rachel Lee, PhD; Heather Lehman, MD; Zhili Lin, PhD; Fred Lorey, PhD; Aly Abdel-Mageed, MD, MBA; Adrienne Manning, BS; Sean McGhee, MD; Theodore B. Moore, MD; Stanley J. Naides, MD; Luigi D. Notarangelo, MD; Jordan S. Orange, MD; Sung-Yun Pai, MD; Matthew Porteus, MD, PhD; Ray Rodriguez, MD, JD, MPH, MBA; Neil Romberg, MD; John Routes, MD; Mary Ruehle, MS; Arye Rubenstein, MD; Carlos A. Saavedra-Matiz, MD; Ginger Scott, RN; Patricia M. Scott, MT; Elizabeth Secord, MD; Christine Seroogy, MD; William T. Shearer, MD, PhD; Subhadra Siegel, MD; Stacy K. Silvers, MD; E. Richard Stiehm, MD; Robert W. Sugerman, MD; John L. Sullivan, MD; Susan Tanksley, PhD; Millard L. Tierce IV, DO; James Verbsky, MD, PhD; Beth Vogel, MS; Rosalyn Walker, MD; Kelly Walkovich, MD; Jolan E. Walter, MD, PhD; Richard L. Wasserman, MD, PhD; Michael S. Watson, MS, PhD; Geoffrey A. Weinberg, MD; Leonard B. Weiner, MD; Heather Wood, MS; Anne B. Yates, MD; Jennifer M. Puck, MD

**IMPORTANCE** Newborn screening for severe combined immunodeficiency (SCID) using assays to detect T-cell receptor excision circles (TRECs) began in Wisconsin in 2008, and SCID was added to the national recommended uniform panel for newborn screened disorders in 2010. Currently 23 states, the District of Columbia, and the Navajo Nation conduct population-wide newborn screening for SCID. The incidence of SCID is estimated at 1 in 100 000 births.

**OBJECTIVES** To present data from a spectrum of SCID newborn screening programs, establish population-based incidence for SCID and other conditions with T-cell lymphopenia, and document early institution of effective treatments.

**DESIGN** Epidemiological and retrospective observational study.

**SETTING** Representatives in states conducting SCID newborn screening were invited to submit their SCID screening algorithms, test performance data, and deidentified clinical and laboratory information regarding infants screened and cases with nonnormal results. Infants born from the start of each participating program from January 2008 through the most recent evaluable date prior to July 2013 were included. Representatives from 10 states plus the Navajo Area Indian Health Service contributed data from 3 030 083 newborns screened with a TREC test.

**MAIN OUTCOMES AND MEASURES** Infants with SCID and other diagnoses of T-cell lymphopenia were classified. Incidence and, where possible, etiologies were determined. Interventions and survival were tracked.

**RESULTS** Screening detected 52 cases of typical SCID, leaky SCID, and Omenn syndrome, affecting 1 in 58 000 infants (95% CI, 1/46 000-1/80 000). Survival of SCID-affected infants through their diagnosis and immune reconstitution was 87% (45/52), 92% (45/49) for infants who received transplantation, enzyme replacement, and/or gene therapy. Additional interventions for SCID and non-SCID T-cell lymphopenia included immunoglobulin infusions, preventive antibiotics, and avoidance of live vaccines. Variations in definitions and follow-up practices influenced the rates of detection of non-SCID T-cell lymphopenia.

**CONCLUSIONS AND RELEVANCE** Newborn screening in 11 programs in the United States identified SCID in 1 in 58 000 infants, with high survival. The usefulness of detection of non-SCID T-cell lymphopenias by the same screening remains to be determined.

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**Author Affiliations:** Author affiliations are listed at the end of this article.

**Corresponding Author:** Jennifer M. Puck, MD, Department of Pediatrics, HSE-301A, University of California, San Francisco, 513 Parnassus Ave, San Francisco, CA 94143 (puckj@peds.ucsf.edu).

The purpose of newborn screening is early detection of inborn conditions for which prompt treatments mitigate mortality or irreversible damage. The first heritable immune disorders to which newborn screening has been applied are those that together comprise severe combined immunodeficiency (SCID), caused by defects in any of a diverse group of gene products essential for development of adaptive immunity provided by T and B lymphocytes.<sup>1,2</sup> A feature of all SCID is defective production of T cells. In most SCID, B cells are also defective, but even normal B cells cannot produce antibodies without T-cell help. Thus, infants with SCID are susceptible to life-threatening infections. Early detection and treatment optimize survival.<sup>3-5</sup> Provided that SCID is diagnosed before infections become overwhelming, affected infants can be rescued with hematopoietic stem cell transplantation; gene therapy; or, for adenosine deaminase deficiency, enzyme replacement therapy.<sup>2,5-8</sup>

Population-based screening is the only means to detect SCID prior to the onset of infections in most cases, as more than 80% lack a positive family history.<sup>9,10</sup> T-cell receptor excision circles (TRECs), a biomarker for T lymphopoiesis,<sup>11</sup> can be measured by polymerase chain reaction (PCR) using DNA isolated from infant dried blood spots collected for newborn screening.<sup>9</sup> Dried blood spots from apparently healthy newborns who were later diagnosed with SCID lacked TRECs.<sup>9</sup> Confirmation of the utility of the TREC test,<sup>12</sup> adaptation for pilot newborn screening programs in Wisconsin<sup>13</sup> and Massachusetts,<sup>14</sup> and an evidence-based review led to the recommendation by the US Department of Health and Human Services Secretary in 2010 that SCID be added to the Uniform Screening Panel for all newborns, with related T-cell deficiencies added to the list of secondary targets.<sup>15</sup> Currently, 23 states, the District of Columbia, and the Navajo Nation screen approximately two-thirds of all infants born in the United States for SCID. Individual states have confirmed detection of SCID as well as additional disorders with low T-cell numbers, which also may benefit from further assessment of immune dysfunction and from protective treatments.<sup>13,16-18</sup> Here we present the first combined analysis of more than 3 million infants screened for SCID in 10 states and the Navajo Nation, providing a population-based overview of SCID and non-SCID T-cell lymphopenia.

## Methods

All SCID newborn screening programs active as of July 31, 2013, were invited, and 11 provided data for this study with the following accrual dates: California (August 16, 2010, to May 31, 2013), Colorado (February 1, 2012, to March 31, 2013), Connecticut (October 1, 2011, to May 1, 2013), Delaware (July 6, 2012, to June 30, 2013), Massachusetts (February 1, 2009, to January 31, 2013), Michigan (October 1, 2011, to March 31, 2013), Mississippi (January 1, 2012, to December 31, 2012), New York (September 29, 2010, to September 28, 2012), Texas (December 1, 2012, to May 31, 2013), Wisconsin (January 1, 2008, to December 31, 2012), and the Navajo Nation spanning parts of Arizona, New Mexico, and Utah, where health care is provided through the Navajo Area Indian Health Service

(February 1, 2012, to June 30, 2013). Five states had insufficient data due to short SCID screening program duration: Iowa began June 3, 2013, and had fewer than 3000 births screened by the close of our study, based on published summaries of national vital statistics<sup>19</sup>; Pennsylvania, Utah, and Wyoming began July 1, 2013; and Ohio began July 29, 2013; thus, these states had no screened births prior to the close of our study. Florida started screening October 1, 2012, and screened an estimated 160 000 infants for SCID during the study period while Minnesota started January 7, 2013, accruing data for around 33 000 infants during the study period. In both states an administrative decision not to participate was made based on programmatic constraints. An estimated maximum of 196 000 screened births could have been included in the study if all programs had participated (a 6.5% increase above the total included in the 11 participating programs).<sup>19</sup> All programs, whether participating in the study or not, conformed to the approved guidelines for implementation of SCID screening developed by the Clinical and Laboratory Standards Institute.<sup>20</sup>

Institutional review board approvals for research with human subjects or waivers for submitting data for this study were obtained in accord with requirements of each participating program. Deidentified SCID screening information was captured either via the R4S database,<sup>21</sup> a tool for quality improvement of newborn screening supported by the Newborn Screening Translational Research Network, or via electronic spreadsheets. As defined in **Table 1**, typical SCID, leaky SCID, and Omenn syndrome, which require immune system restoration for survival, were the primary targets of SCID screening, while additional diagnoses were detected as secondary targets.<sup>5,20,22</sup> Infants with abnormal TREC results had flow cytometry to enumerate lymphocyte subsets; HIV PCR or maternal serodiagnosis; and further evaluation to establish a diagnosis. To ensure follow-up and ascertainment of SCID cases, public health programs engaged as advisors the immunologists and transplant clinicians who have diagnosed and cared for infants with SCID in each state. Regular reviews were conducted between public health personnel and clinical experts in each program to uncover any missed (false-negative) cases and monitor screening test performance and follow-up.

### Aggregate Population Data and Case Data

Programs provided accrual dates, numbers of newborns screened, and data about infants with nonnormal TREC results (after 1 or multiple dried blood spot samples) in each diagnosis category. State-designated immunologists provided deidentified data in consultation with screening program officials to ensure compliance with privacy policies. Gene and syndrome diagnoses were requested. Numbers of infants with T cells within designated ranges and interventions and outcomes were reported by public health programs and by participating immunologists who evaluated and followed up or referred infants for treatment.

### TREC Newborn Screening

See the eMethods and eTable in the Supplement for individual program details beyond those published.<sup>13,14,17,18,20</sup> All

**Table 1. Classification of Conditions With Low T-Cell Receptor Excision Circles and Low T-Cell Numbers Found by Newborn Screening**

	Definition of Condition		
	CD3 T Cells/ $\mu$ L	Proliferation to PHA	Other Supporting Features
<b>Primary Targets of Newborn Screening</b>			
Typical SCID <sup>a</sup>	<300 (autologous)	<10% of normal	Detectable maternal T cells in peripheral blood; proven deleterious defect(s) in a known SCID gene
Leaky SCID <sup>a</sup>	300-1500, few naive T cells	Reduced (10%-50% of normal)	No maternal T cells detectable; incomplete defect(s) in a known SCID gene
Omenn syndrome	Oligoclonal T cells	Reduced (10%-50% of normal)	Erythroderma, hepatosplenomegaly, eosinophilia, and elevated levels of serum IgE antibody
<b>Secondary Targets of Newborn Screening</b>			
Syndrome with low T-cell numbers	Recognized genetic syndrome that includes low T-cell numbers within its spectrum of clinical findings		
Secondary T-cell lymphopenia	Congenital malformation or disease process without an intrinsic defect in production of circulating T cells		
Preterm birth alone	Preterm birth and low birth weight, with low T-cell numbers early in life that normalize over time		
Idiopathic T-cell lymphopenia, also called variant SCID	Low T-cell numbers without recognized cause; 6 programs used 300-1500 autologous T cells/ $\mu$ L plus evidence of functional immune cell impairment, while other programs included infants with higher T-cell numbers (see Table 4). <sup>b</sup>		

Abbreviations:  
PHA, phytohemagglutinin;  
SCID, severe combined immunodeficiency.

<sup>a</sup> As adopted by the Primary Immune Deficiency Treatment Consortium and R4S Laboratory Performance Database, SCID and leaky SCID were defined by laboratory criteria rather than infectious complications.

<sup>b</sup> On discovery of an etiology for low T cells, the affected individual was moved to the appropriate alternative category.

programs conformed to the guidelines that included reporting any nonnormal TREC test results within the first 3 weeks of life and performing flow cytometry, where indicated, by 4 to 5 weeks of age. In addition, all programs participated in the TREC Proficiency Quality Assurance Program, cosponsored by the Centers for Disease Control and Prevention and the Association of Public Health Laboratories.<sup>23</sup>

### Statistical Analyses

Statistical analyses were conducted in SAS version 9.3 (SAS Institute). Confidence intervals were derived from normal approximation of binomial data or from inversion of cumulative binomial distribution, as appropriate, but not calculated where numbers were too small. Confidence intervals were 2-sided, except that when the number of cases or noncases was 5 or fewer, 1-sided intervals were calculated. *P* values less than .05 were considered statistically significant.

## Results

This study included data for 3 030 083 infants from 11 programs (Table 2). Nonparticipating programs cited insufficient data, lack of personnel to assemble data, or privacy concerns. California, with nearly 3 years of screening and 12.5% of all US births,<sup>23</sup> contributed 46%, followed by New York with 16% from 2 years. Wisconsin and Massachusetts, with fewer annual births but longer program durations, contributed 11% and 10%, respectively.

### Detection of SCID

There were 52 SCID cases (42 with typical SCID, 9 with leaky SCID, and 1 with Omenn Syndrome), an overall incidence of 1 in 58 000 births (95% CI, 1/46 000-1/80 000) (Table 2). The incidence was not significantly different in any state program but as expected was higher in the Navajo Nation (1/3500; 95% CI, 1/630-1/4000), where a frequent founder mutation in *DCLRE1C*, encoding a DNA repair protein, causes SCID in an estimated 1

in 2000 births.<sup>24,25</sup> No cases of SCID as defined in Table 1 were initially missed by TREC screening but detected later, and overdiagnosis of SCID when not clinically present was avoided by having flow cytometric determination of T-cell numbers, a definitive test, mandated for all infants with very low or undetectable TRECs (eTable in the Supplement).

Genetic causes and outcomes of the 52 infants with conditions that were primary targets of TREC newborn screening are shown in Table 3 and included 42 infants (81%) with typical and 10 (19%) with leaky SCID. Mutations in the X chromosome-linked *IL2RG* gene, encoding the cytokine receptor common  $\gamma$  chain, accounted for only 19% of cases. Recombinase activating gene 1 (*RAG1*) defects, causing impairment of V(D)J lymphocyte antigen receptor recombination, were detected in 4 typical and 4 leaky SCID cases, 1 of the latter with Omenn syndrome, accounting for 15% of all 52 cases. Interleukin-7 defects and adenosine deaminase deficiency contributed 12% and 11%, respectively. New SCID gene defects included mutations of tetratricopeptide repeat domain 7A (*TTC7A*) that disrupted not only T-cell development, but also intestinal epithelial polarity, leading to multiple bowel atresias.<sup>26,27</sup> In addition, typical SCID was diagnosed in a case of Pallister-Killian syndrome, in which congenital diaphragmatic defects associated with tetrasomy 12p are frequently incompatible with life, as in this case. Although not previously recognized as an immune deficiency, Pallister-Killian syndrome has been known for poor lymphocyte proliferation in the context of cytogenetic analysis.<sup>28</sup>

Of the 12 infants without a molecular diagnosis, no gene test results were available for 2, and 2 males with T-B+NK- phenotype died prior to testing (Table 3). However, in 6 typical and 2 leaky SCID cases (15% of all typical and leaky SCID cases found), no molecular defects were identified in known SCID genes: the common  $\gamma$  chain or interleukin-7 receptors, adenosine deaminase or purine nucleoside phosphorylase enzymes, janus kinase-3, recombinase activating genes, the DNA repair enzyme Artemis, or components of the CD3 receptor complex.

Table 2. Infants Screened and Incidence of SCID (Including Leaky SCID) in 11 Contributing Programs

	California	Colorado	Connecticut	Delaware	Massachusetts	Michigan	Mississippi	Navajo Nation	New York	Texas	Wisconsin	Total
Duration of screening included, mo	34	13	19	12	48	18	12	17	24	6	60	
Infants screened, No. <sup>a</sup>	1 384 606	70 989	57 136	11 202	293 371	162 528	37 613	3 498	485 912	183 191	340 037	3 030 083
Flow cytometry referrals, No. (%) [95% CI] <sup>c</sup>	206 (14.9) [12-17]	10 (14.1) [5.4-23]	22 (38.5) [22-55]	9 (80.3) [28-133]	63 (21.5) [16-27]	114 (70.1) [57-83]	5 (13.3) [1.6-25]	1 (28.6)	478 (98.4) [90-107]	249 (135.9) [119-153]	108 (31.8) [26-38]	1265 (41.8) [39-44]
SCID cases	23	1	3	1	4	2	1	1	10	2	4	52
SCID incidence	1/60 000	1/71 000	1/19 000 <sup>d</sup>	1/11 000 <sup>d</sup>	1/73 000	1/81 000	1/38 000	1/3500 <sup>d</sup>	1/49 000	1/92 000	1/85 000	1/58 000 [1/46 000-1/80 000]
SCID cases per 100 000 screened, No. [95% CI] <sup>c</sup>	1.7 [1.0-2.3]	1.4 [0.3-5.2]	5.2 [1.9-15]	8.9 [2.2-49]	1.4 [0.4-3.5]	1.2 [0.4-4.4]	2.7 [0.6-5]	29 [6.9-159]	2.0 [0.8-3.3]	1.1 [0.3-3.9]	1.2 [0.3-3.0]	1.72 [1.3-2.2]
SCID infant survival, No./Total No. (%) [95% CI] <sup>c-e</sup>	21/23 (91) [83-100]	1/1 (100)	3/3 (100)	1/1 (100)	4/4 (100)	1/2 (50)	0/1	1/1 (100)	9/10 (90) [70-100]	0/2	4/4 (100)	45/52 <sup>f</sup> (86) [79-98]

Abbreviation: SCID, severe combined immunodeficiency.

<sup>a</sup> Includes 175 nonviable infants (<0.006% from all programs) who had a nonnormal TREC result, but died before further testing could be done; although causes of these deaths were not available and it is theoretically possible that some of these infants had SCID, SCID is not known to lead to death in the newborn period.

<sup>b</sup> Per 100 000 screened. Referral criteria for flow cytometry varied according to individual program algorithms (see the eTable in the Supplement).

<sup>c</sup> Confidence intervals derived from normal approximation of binomial data or inversion of cumulative binomial distribution, as appropriate. No CI given where numbers were too small.

<sup>d</sup> Navajo SCID incidence was an outlier, consistent with the known founder mutation of this population. Because of the low numbers screened, apparent higher incidences in Connecticut and Delaware were not significantly different ( $P > .05$ ) from the other states.

<sup>e</sup> Survival percentage CI for California and New York is the exact 1-sided binomial CI; total survival percentage CI is 2-sided.

<sup>f</sup> Of the 7 deceased infants, 1 each in Mississippi, New York, and Texas died prior to receiving immune restoring therapy because of complications present before referral for hematopoietic cell transplantation; the remaining 4 died after transplantation.

### Definitions and Incidence of Abnormal TRECs and Low T Cells

Although all programs identified SCID cases with undetectable or very low TRECs, differences in intermediate steps for arriving at a SCID diagnosis influenced rates of follow-up testing and capture of non-SCID conditions (Table 1 and Table 4).<sup>20</sup> After an abnormal TREC screen, flow cytometry to enumerate T, B, and NK cells, as well as naive and memory phenotype T cells, was standard for all programs. However, different TREC cutoffs resulted in different referral rates for flow cytometry; therefore, neither aggregate analysis nor interprogram comparison of incidences of infants with particular TREC cutoff values was possible. Rates of referral for flow cytometry were less than 15 per 100 000 in California, Colorado, and Mississippi but 7- to 9-fold higher in New York and Texas (Table 4).

Furthermore, definitions of T-cell lymphopenia varied. Healthy newborns have abundant T cells (mean, 3100/ $\mu$ L; range, 2500-5500).<sup>29</sup> While 6 screening programs defined significant T-cell lymphopenia as T-cell count less than 1500/ $\mu$ L and opted not to recall infants with higher T-cell numbers as long as the proportion of naive cells was adequate, 4 programs used T-cell cutoffs of 2500/ $\mu$ L or more, and New York left it to individual immunologists to define T-cell

lymphopenia.<sup>30</sup> Different TREC and T-cell lymphopenia cutoffs thus resulted in variable false-positive rates, defined here as nonnormal TREC results that require a follow-up flow cytometry test, which when performed shows T cells above the program cutoff for T-cell lymphopenia (Table 4). These false-positive rates ranged from 0 in Mississippi and the Navajo Nation, where all infants referred to flow cytometry had T-cell lymphopenia by program definitions (<2500/ $\mu$ L and <1500/ $\mu$ L, respectively), to 82% in New York, where 478 infants were referred for flow cytometry, but only 84 (18%) had T-cell lymphopenia as determined by treating physicians (Table 4). A subgroup analysis for the 6 programs defining T-cell lymphopenia as a T-cell count less than 1500/ $\mu$ L showed a positive predictive value of 36% (95% CI, 32%-41%) for a nonnormal TREC test to indicate this degree of T-cell lymphopenia.

Regardless of selected T-cell lymphopenia cutoff, all programs identified predominantly male infants; the 6-program subgroup had 66% of males with T-cell lymphopenia (95% CI, 59%-73%). Programs did not report preterm infants with low T cells in a uniform manner, partly due to automatically repeated TREC testing of preterm infants in neonatal intensive care units in some screening programs (eMethods in the Supplement). However, 13% (95% CI, 8.4%-18%) of infants with

Table 3. Diagnosis and Course of 52 Infants With Primary Target Conditions: SCID and Leaky SCID

Gene Diagnosis	No. of Infants	Treatments <sup>a</sup>			Outcomes (to Age ≥11 Months)
		Hematopoietic Cell Transplant	Gene Therapy	Adenosine Deaminase Enzyme Replacement Therapy	
Typical SCID	42				
<i>IL2RG</i> (NP_000197.1)	9	9	1	0	Surviving, engrafted
<i>IL7RA</i> (NP_002176.2)	6	6	0	0	Surviving, engrafted
<i>ADA</i> (NP_000013.2)	5	0	3	2	Surviving, 3 with gene-modified T cells and 2 with T cells after having received enzyme replacement
<i>RAG1</i> (NP_000439.1)	4	4	0	0	3 surviving, engrafted; 1 died posttransplant due to busulfan toxicity <sup>b</sup>
<i>JAK3</i> (NP_000206.2)	3	3	0	0	Surviving, engrafted
<i>DCLRE1C</i> (NP_001029027.1)	1	1	0	0	Surviving, engrafted
<i>RAG2</i> (NP_000527.2)	1	1	0	0	Surviving, engrafted
<i>CD3D</i> (NP_000723.1)	1	1	0	0	Surviving, engrafted
<i>TC7A</i> (NP_001275880.1)	1	1	0	0	Surviving, engrafted
Pallister-Killian syndrome with tetrasomy 12p	1	0	0	0	Died due to severe diaphragmatic defect
No mutation found, with known SCID genes excluded	6	5	0	0	4 surviving, engrafted; 1 died posttransplant due to cytomegalovirus present at diagnosis; 1 not treated, died due to severe congenital anomalies
Genetic testing not completed	4	3	0	0	2 surviving, engrafted; 1 died posttransplant due to respiratory illness present at diagnosis; 1 not treated, died before transplant
Leaky SCID	10				
<i>RAG1</i> (NP_000439.1)	4	4 (1 with Omenn syndrome)	0	0	Surviving, engrafted
<i>RMRP</i> (NR_003051.3)	2	2	0	0	1 surviving, engrafted; 1 died posttransplant due to busulfan toxicity <sup>b</sup>
<i>IL2RG</i> (NP_000197.1)	1	1	0	0	Surviving, engrafted
<i>DCLRE1C</i> (NP_001029027.1)	1	1	0	0	Surviving, engrafted
No mutation found, with known SCID genes excluded	2	2	0	0	Surviving, engrafted

Abbreviation: SCID, severe combined immunodeficiency.

<sup>a</sup> Some infants had >1 treatment modality.

<sup>b</sup> Hepatic sinusoidal obstructive syndrome, a known complication of busulfan chemotherapy.

T-cell lymphopenia in the 6-state subgroup had prematurity or low birth weight as the only identified cause. As previously reported, T-cell lymphopenia of prematurity resolved to normal over time.<sup>13,18</sup> After excluding infants with SCID and prematurity, the rate of non-SCID T-cell lymphopenia in the subgroup was 1 in 14 000 infants (95% CI, 1/11 600-1/16 400), whereas more inclusive definitions led to 1 in 2100 in Michigan, 1 in 6500 in Massachusetts and New York, and 1 in 8100 in Wisconsin (Table 4).

### Causes of Non-SCID T-Cell Lymphopenia

Of 411 infants with non-SCID T-cell lymphopenia (Table 1 and Table 5), 136 (33%) were reported to have a recognized con-

genital syndrome associated with T-cell impairment. Of these syndromic infants, 78 (57%) had DiGeorge syndrome/chromosome 22q11.2 deletion, followed by 21 (15%) with trisomy 21. The remaining specified syndrome diagnoses included repeated instances of ataxia telangiectasia<sup>31</sup> and trisomy 18 (each 3%), CHARGE (coloboma, heart defect, atresia choanae, retarded growth and development, genital and ear abnormalities) syndrome (2%), and other rare entities as listed (Table 5).<sup>32</sup>

There were 117 cases of T-cell lymphopenia attributed to other medical conditions (28% of all non-SCID T-cell lymphopenia cases) (Table 5), the most predominant being congenital heart disease in 30 cases (26%), followed by other congen-

Table 4. Infants With Non-SCID T-Cell Lymphopenia Followed Up in Each Program After Nonnormal TREC Results

TCL <sup>a</sup>	<1500 T Cells/μL Cutoff							<2500 T Cells/μL Cutoff			<3505 T Cells/μL Cutoff	No Defined Cutoff
	California	Colorado	Connecticut	Delaware	Navajo Nation	Texas	Subgroup Summary	Massachusetts	Mississippi	Wisconsin	Michigan	New York
Infants with TCL/Total No. referred to flow cytometry <sup>b</sup> (%) [95% CI] <sup>c</sup>	80/206 (39) [28-50]	4/10 (40) [12-65]	9/22 (41) [8.8-73]	4/9 (44) [14-70]	1/1 (100)	82/249 (33) [23-43]	180/479 (36) [32-41]	51/63 (81) [62-86]	5/5 (100) [55-100]	49/108 (45) [31-60]	78/114 (68) [58-79]	84/478 (18) [8-24]
False-positive rate <sup>d</sup> [95% CI] <sup>e</sup>	61 [50-72]	60 [35-88]	59 [27-91]	56 [30-86]	0	67 [57-77]	64 [59-68]	19 [14-38]	0 [0-45]	55 [40-69]	32 [21-42]	82 [76-92]
Males with TCL/Total No. with TCL (%) [95% CI] <sup>c</sup>	47/80 (59) [48-70]	3/4 (75)	6/9 (67) [36-98]	3/4 (75)	0/1	60/82 (73) [64-83]	119/180 (66) [59-73]	35/51 (69) [54-80]	3/5 (60) [15-85]	37/49 (76) [64-88]	52/78 (67) [56-77]	57/97 (59) [49-70]
Preterm alone infants with TCL/Total No. with TCL (%) [95% CI] <sup>c</sup>	14/80 (18) [9.2-26]	0/4	1/9 (11) [2.8-34]	0/4	0/1	9/82 (11) [4.2-18]	24/180 (13) [8.4-18]	1/51 (2) [0.5-7.0]	1/5 (20) [5.3-52]	3/49 (6) [2.3-14]	0/78 [0-3.8]	0/84 [0-3.5]
Incidence of all non-SCID TCL	1/32 000	1/26 000	1/11 000	1/3700	1/3500	1/2600	1/14 000 <sup>e</sup>	1/6400	1/13 000	1/8100	1/2100	1/6600
Non-SCID TCL cases per 100 000 screened [95% CI] <sup>c</sup>	3.1 [2.2-4.0]	4.2 [1.5-10]	8.8 [1.1-16]	26 [9.7-64]	29 [6.9-105]	39 [30-48]	7.4 [6.1-8.6]	16 [11-20]	8.0 [2.9-19]	12 [8.6-16]	47 [36-57]	15 [12-19]

Abbreviations: SCID, severe combined immunodeficiency; TCL, T-cell lymphopenia; TREC, T-cell receptor excision circle.

<sup>a</sup> T-cell lymphopenia also included lack of naive T cells (Table 1, eTable). Texas data are from Texas Children's Hospital, Houston, and University of Texas Southwestern, Dallas. In New York, immunology experts diagnosed TCL at their own discretion, without a predefined cutoff.

<sup>b</sup> See Table 2 for rates of referral to flow cytometry.

<sup>c</sup> Confidence intervals derived from normal approximation of binomial data or inversion of cumulative binomial distribution, as appropriate. One-sided CI used when frequency = 0. No CI given where numbers were too small.

<sup>d</sup> False-positive rate defined as infants referred to flow cytometry due to nonnormal TREC screen but having T cells by flow cytometry that were above the program cutoff for TCL.

<sup>e</sup> 95% CI, 1/11 600-1/16 400.

ital anomalies, vascular leakage and hydrops (grouped as loss into third space), gastrointestinal anomalies including gastroschisis, and 4 neonatal leukemias. No cases of HIV infection were detected.

Idiopathic T-cell lymphopenia, also termed variant SCID, was found in only 3% of non-SCID T-cell lymphopenia cases (12/411, or 1/250 000 births); these infants did not meet the diagnostic criteria for leaky SCID but had persistent T-cell lymphopenia and immune dysfunction without defects in known SCID genes (Table 1).<sup>18,30</sup> One of these 12 infants eventually required hematopoietic cell transplantation. The screening program in New York identified 30 further cases as having idiopathic T-cell lymphopenia,<sup>30</sup> included in Table 5 among the unspecified T-cell lymphopenia cases because their T-cell counts were not available.

### Interventions for Infants With Deficient T Cells Identified Through SCID Newborn Screening

Of the 52 infants detected with SCID in the first weeks of life, 49 received immunity restoring therapies. Forty-four had hematopoietic cell transplants, 4 had gene correction of *IL2RG*

and *ADA* defects by ex vivo transduction of a normal gene sequence into autologous hematopoietic stem cells (1 of whom required subsequent hematopoietic cell transplant due to inadequate correction), and 2 had adenosine deaminase enzyme injection therapy. In addition, non-SCID cases requiring immune restorative treatment included 1 infant with *Rac2* deficiency (a syndrome of defective neutrophil adhesion) and 1 with variant SCID who received hematopoietic cell transplantation, and 2 infants with complete DiGeorge syndrome who received thymus transplantation (Table 3 and Table 5). Of 7 deaths among the 52 infants with typical SCID and leaky SCID, 3 were due to perinatal complications, including 1 with Pallister-Killian syndrome, 1 with intestinal malrotation and severe respiratory distress,<sup>30</sup> and 1 with undescribed medical problems that precluded transport to a center where hematopoietic cell transplant could be done. Four infants with SCID died after transplant. Thus, overall SCID survival was 45 of 52 (87%), while 45 of 49 treated infants (92%) survived, comparable with experience from transplant centers for uninfected SCID patients treated early in life.<sup>4-7</sup> Posttreatment deaths were due to cytomegalovirus infection acquired early postnatally in 1,

pretransplant respiratory compromise in 1, and hepatic sinusoidal obstructive disease secondary to pretransplant busulfan chemotherapy in 2 (Table 3).

All infants with T-cell lymphopenia were directed to avoid infectious exposures, transfusions (except with cytomegalovirus-negative, irradiated blood products), and live rotavirus vaccines until such time as immune compromise was no longer present. Prophylactic antimicrobials and immunoglobulin infusions were given as indicated by immunology specialists.

## Discussion

To our knowledge, this is the first multistate report of results of newborn screening for SCID, a core condition in the US Recommended Uniform Screening Panel. Our experience has demonstrated the feasibility of assaying for TRECs, a biomarker for naive T-cell lymphopoiesis, followed by confirmatory flow cytometry, as a means to identify SCID. Newborn screening has provided a new, population-based incidence of SCID of 1 in 58 000 births, higher than the incidence of 1 in 100 000 suggested from retrospective clinical diagnoses.<sup>33-35</sup> Furthermore, the proportion of *IL2RG* deficient X-linked SCID in our study (19%) is in contrast to nearly half of cases in published cohorts from referral centers that treat SCID.<sup>5-8</sup> Because X-linked disorders with severe phenotype maintain constant frequency due to replenishment in the gene pool by new mutations,<sup>36</sup> our lower proportion of *IL2RG*-deficient SCID is likely to reflect increased ascertainment of autosomal-recessive SCID cases by population-based screening. Moreover, compared with series from large transplant centers, in which less than 10% of cases lacked a molecular diagnosis,<sup>5,8</sup> our newborn screened cases had a higher proportion of leaky SCID and more than 15% of typical and leaky SCID without a proven molecular diagnosis despite extensive gene sequencing (Table 3). These findings support the view that SCID has previously been underdiagnosed in infants with fatal infections. Furthermore, proportions of typical SCID, leaky SCID, and Omenn syndrome in our cohort appear distinct from those previously reported for older infants; features of Omenn syndrome develop over months after birth, and the clinical diagnosis of leaky SCID can be delayed for years.<sup>37</sup>

Additional data collection may reveal new demographic patterns, such as the known high Navajo incidence of SCID due to a *DCLRE1C* founder mutation and Amish and Mennonite founder mutations in *ADA*, *IL7R*, and *RAG1*.<sup>38,39</sup> Inclusion of data from more SCID screening programs in additional states would be required to know if the results from the 11 participating programs included here are fully generalizable. Whether the excess of males with abnormal SCID newborn screens is explained by the known higher rate of male preterm births as well as the common X-linked SCID gene *IL2RG* also needs to be explored. The unanticipated high proportion of SCID without a defined genotype and new discovery of non-SCID T-cell lymphopenias illustrate how unbiased population screening reveals a wide phenotypic spectrum and affords opportunities to discover previously unknown genes essential to human T-cell development.

**Table 5. Diagnoses of 411 Infants With Non-SCID T-Cell Lymphopenia Identified by Newborn Screening**

Condition	No. of Infants
Syndromes with T-cell impairment <sup>a</sup>	136
DiGeorge	78 <sup>b</sup>
Trisomy 21	21
Ataxia telangiectasia	4
Trisomy 18	4
CHARGE	3
Jacobsen	2
CLOVES	1
ECC	1
Fryns	1
Nijmegen breakage	1
Noonan	1
Rac2 defect	1 <sup>c</sup>
Renpenning	1
TAR	1
Not specified	10
Cytogenetic abnormalities <sup>d</sup>	6
Secondary T-cell impairment	117
Cardiac anomalies	30
Multiple congenital anomalies	23
Loss into third space	15
Gastrointestinal anomalies	15
Neonatal leukemia	4
Not specified	30
Preterm birth alone	29
Variant SCID	12 <sup>e</sup>
Unspecified T-cell lymphopenia <sup>f</sup>	117

Abbreviations: CHARGE, coloboma, heart defect, atresia choanae, retarded growth and development, genital and ear abnormality; CLOVES, congenital lipomatous overgrowth, vascular malformations, epidermal nevi, and spinal/skeletal anomalies; ECC, ectodermal dysplasia, ectrodactyly, and clefting; SCID, severe combined immunodeficiency; TAR, thrombocytopenia and absent radius.

<sup>a</sup> Eponymous syndromes: DiGeorge, cardiac defects, hypocalcemia, thymus dysplasia, and other anomalies, most often with chromosome 22q11.2 interstitial deletion; Jacobsen, growth and psychomotor retardation and congenital anomalies with chromosome 11qter deletion; Fryns, diaphragmatic hernia and other congenital anomalies; Noonan, multiple congenital anomalies; Renpenning, X chromosome-linked mental retardation with distinctive facies.

<sup>b</sup> Included 3 infants with complete DiGeorge syndrome and absent T cells, 2 of whom received a thymus transplant.

<sup>c</sup> Eventual hematopoietic cell transplant performed.<sup>17</sup>

<sup>d</sup> Included chromosome 6p deletion, ring chromosome 14, ring chromosome 17, chromosome 17q duplication, and 2 siblings with unspecified chromosome abnormalities.

<sup>e</sup> Eventual hematopoietic cell transplant performed for 1 case.

<sup>f</sup> Includes infants from Michigan (46), New York (30), Massachusetts (25), Wisconsin (13), Connecticut (2), and Delaware (1); further information was not available for these infants, although those from New York were reported to require ongoing monitoring or treatment for a deficiency of T cells.<sup>30</sup>

Now that infants with SCID are being detected at a very young age in diverse medical settings, it is imperative to tailor protocols for their treatment, including choice and pharmacokinetic monitoring of drugs administered to facilitate hematopoietic cell engraftment. Busulfan chemotherapy led to

fatal hepatic sinusoidal obstruction, also known as veno-occlusive disease, in 2 infants diagnosed with SCID by newborn screening. Prospective studies conducted by the Primary Immune Deficiency Treatment Consortium will address whether dose adjustments based on age or alternate regimens will provide enhanced safety while still affording long-lasting immune reconstitution.<sup>5,8,21,40</sup>

A major limitation of this study was the lack of uniformity of assay methodology and rules for retesting among the individual newborn screening programs, despite general adherence to the Clinical and Laboratory Standards Institute guidelines.<sup>20</sup> Use of different TREC assays and test algorithms resulted in a variety of rates both for recall for additional testing and for having T cells by flow cytometry in a range defined as normal. Specific information about the ages at which samples for TREC screening and for flow cytometry were obtained were not available. No program identified a false-negative test for SCID, the primary target condition. Furthermore, although the definitive flow cytometry test was universally used as follow-up for infants whose TRECs were not normal, different cutoffs were used to define non-SCID secondary targets of screening. Therefore, the incidence of T-cell lymphopenia cases referred for follow-up varied from 3 to 47 cases per 100 000 infants (Table 4).

While unsuspected non-SCID immunodeficiency syndromes were identified and 4 infants had immune defects sufficiently serious to require hematopoietic cell or thymus transplantation, these benefits must be weighed against the burdens of heightened parental anxiety and costs of further testing in infants with less profound T-cell lymphopenias. As with development of each newborn screening test since the original one for phenylketonuria,<sup>41</sup> different initial approaches for SCID screening are anticipated to evolve and become standardized over time, as evident in adjustments to TREC screening algorithms that have already occurred.<sup>17,30</sup> Specific data regarding persistence of non-SCID T-cell lymphopenia over time and

functional T-cell abnormalities were not available for our analysis but should in the future be collected to clarify which infants require interventions, such as avoidance of live rotavirus vaccination, which can cause serious diarrheal disease in infants with immunodeficiency.<sup>42,43</sup>

Differences in cutoffs between the SCID screening programs in this study may prove helpful for public health programs in other states and countries considering instituting SCID newborn screening. In addition, the R4S SCID database will permit future analytical and clinical correlations to optimize cutoffs for key markers, such as T-cell numbers, to inform best practices.<sup>19,44</sup>

The TREC assay has proven excellent for detecting disorders with poor T-cell production or inadequate numbers of circulating T cells, but finding additional immune defects prior to onset of recurrent or life-threatening infections will require further methods. A few more entities may be captured by screening for the circular by-products of B-cell immunoglobulin gene rearrangement,<sup>45</sup> and mild as well as severe cases of adenosine deaminase deficiency may be identified by a modification of the current mass spectrometry already widely used for newborn screening.<sup>46</sup> However, infants with defects affecting T cells beyond the developmental stage of recombination of T-cell receptors (eg, major histocompatibility complex class II deficiency<sup>47</sup>) have normal TRECs but impaired T-cell function. Genomic sequencing may be required to detect deleterious mutations in primary immune defects, of which nearly 200 are known.<sup>1</sup>

## Conclusions

Newborn screening in 11 programs in the United States identified SCID in 1 in 58 000 infants, with high survival. The usefulness of detection of non-SCID T-cell lymphopenias by the same screening remains to be determined.

### ARTICLE INFORMATION

**Author Affiliations:** Department of Pediatrics, University of California, San Francisco, San Francisco (Kwan, Cowan, Puck); UCSF Benioff Children's Hospital, San Francisco, California (Kwan, Cowan, Puck); Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota (Abraham); Genetic Disease Screening Program, California Department of Public Health, Richmond (Currier, Lorey); Newborn Screening Translational Research Network, American College of Medical Genetics and Genomics, Bethesda, Maryland (Brower, Watson); Michigan Department of Community Health, Lansing (Andruszewski, Wood); Division of Allergy and Immunology, Department of Pediatrics, National Jewish Health, Denver, Colorado (Abbott, Gelfand); Newborn Screening Laboratory, Wisconsin State Laboratory of Hygiene, Madison (Baker); Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison (Baker); Women and Children's Hospital of Buffalo, Buffalo, New York (Ballow, Lehman); Department of Pediatrics, Christiana Care Health System, Wilmington, Delaware (Bartoszesky); Department of Medicine,

Boston Children's Hospital, Boston, Massachusetts (Bonilla, Notarangelo, Pai); Harvard Medical School, Boston, Massachusetts (Bonilla, Notarangelo, Pai); Department of Population Health Sciences, University of Wisconsin School of Medicine and Public Health, Madison (Brokopp); Department of Pediatrics, University of Texas Health Science Center at San Antonio (Brooks, Infante); Newborn Screening Program, Wadsworth Center, New York State Department of Health, Albany (Caggana, Kay, Saavedra-Matz, Vogel); Division of Allergy and Immunology, Albany Medical College, Albany, New York (Celestin); Department of Pediatrics, University of Southern California, Los Angeles (Church, Kapoor); Children's Hospital Los Angeles, Los Angeles, California (Church, Kapoor); New England Newborn Screening Program, University of Massachusetts Medical School, Jamaica Plain (Comeau, Hale); University of Michigan C. S. Mott Children's Hospital, Ann Arbor (Connely, Walkovich); Mount Sinai Medical Center, New York, New York (Cunningham-Rundles); Clinical Immunodiagnostic and Research Laboratory, Medical College of Wisconsin, Milwaukee (Dasu); Department of Pediatrics, University of Mississippi Medical Center, Jackson (Dave, Rodriguez, Walker,

Yates); Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas (De La Morena); Division of Blood and Bone Marrow Transplantation, Helen DeVos Children's Hospital, Grand Rapids, Michigan (Duffner, Abdel-Mageed); University of Rochester School of Medicine and Dentistry, Rochester, New York (Fong, Weinberg); Department of Pediatrics, Baylor College of Medicine, Houston, Texas (Forbes, Hanson, Orange, Shearer); Texas Children's Hospital, Houston (Forbes, Hanson, Orange, Shearer); Texas Department of State Health Services, Austin (Freedenberg, Johnson, Lee, G. Scott, Tanksley); Department of Pediatrics, University of Massachusetts Medical School, Worcester (Comeau, Hay, Sullivan); Tuba City Regional Health Care, Tuba City, Arizona (Hu); Department of Pediatrics, University of California, Los Angeles, Los Angeles (Kohn, Moore, Stiehm); PerkinElmer Genetics, Bridgeville, Pennsylvania (Lin); Connecticut Department of Public Health Laboratory, Rocky Hill (Manning); Department of Pediatrics, Stanford University School of Medicine, Palo Alto, California (McGhee, Porteus); Lucille Packard Children's Hospital, Palo Alto, California (McGhee, Porteus); Immunology Department,



Quest Diagnostics Nichols Institute, San Juan Capistrano, California (Naides); Division of Allergy and Clinical Immunology, Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut (Romberg); Department of Pediatrics, Children's Research Institute, Medical College of Wisconsin, Milwaukee (Routes, Verbsky); Children's Hospital of Michigan, Detroit (Ruehle, Secord, Tierce); Division of Allergy and Immunology, Montefiore Medical Park, Bronx, New York (Rubenstein); Newborn Screening Program, Delaware Public Health Laboratory, Smyrna (P.M. Scott); Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison (Seroogy); New York Medical College, Westchester Medical Center, Valhalla, New York (Siegel); Medical City Children's Hospital, Dallas, Texas (Silvers, Sugerman, Wasserman); Department of Pediatrics, Massachusetts General Hospital, Boston (Walter); Harvard Medical School, Boston, Massachusetts (Walter); Department of Pediatrics, State University of New York Upstate Medical University, Syracuse (Weiner); Steven and Alexandra Cohen Children's Medical Center, North Shore-LIJ Health System, Great Neck, New York (Bonagura).

**Author Contributions:** Drs Kwan and Puck had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Kwan and Abraham contributed equally to this work.

**Study concept and design:** Kwan, Abraham, Brower, Bonagura, Church, Comeau, Cowan, Lee, Lorey, Naides, Routes, Saavedra-Matiz, Secord, Shearer, Stiehm, Tanksley, Watson, Puck.

**Acquisition, analysis, or interpretation of data:** Kwan, Abraham, Currier, Brower, Andruszewski, Abbott, Baker, Ballow, Bartoszesky, Bonagura, Bonilla, Brokopp, Brooks, Caggana, Celestin, Church, Connelly, Cowan, Cunningham-Rundles, Dasu, Dave, De La Morena, Duffner, Fong, Forbes, Freedenberg, Gelfand, Hale, Hanson, Hay, Hu, Infante, Johnson, Kapoor, Kay, Kohn, Lehman, Lin, Lorey, Abdel-Mageed, Manning, McGhee, Moore, Naides, Notarangelo, Orange, Pai, Porteus, Rodriguez, Romberg, Routes, Ruehle, Rubenstein, Saavedra-Matiz, G. Scott, P. Scott, Secord, Seroogy, Shearer, Siegel, Silvers, Stiehm, Sugerman, Sullivan, Tierce, Verbsky, Vogel, Walker, Walkovich, Walter, Wasserman, Watson, Weinberg, Weiner, Wood, Yates, Puck.

**Drafting of the manuscript:** Kwan, Abraham, Baker, Bonagura, Gelfand, Kapoor, Abdel-Mageed, Manning, Routes, Rubenstein, P. Scott, Shearer, Sullivan, Walker, Puck.

**Critical revision of the manuscript for important intellectual content:** Kwan, Abraham, Currier, Brower, Andruszewski, Abbott, Ballow, Bartoszesky, Bonagura, Bonilla, Brokopp, Brooks, Caggana, Celestin, Church, Comeau, Connelly, Cowan, Cunningham-Rundles, Dasu, Dave, De La Morena, Duffner, Fong, Forbes, Freedenberg, Gelfand, Hale, Hanson, Hay, Hu, Infante, Johnson, Kay, Kohn, Lee, Lehman, Lin, Lorey, Abdel-Mageed, Manning, McGhee, Moore, Naides, Notarangelo, Orange, Pai, Porteus, Rodriguez, Romberg, Routes, Ruehle, Saavedra-Matiz, G. Scott, Secord, Seroogy, Shearer, Siegel, Silvers, Stiehm, Sugerman, Tanksley, Tierce, Verbsky, Vogel, Walkovich, Walter, Wasserman, Watson, Weinberg, Weiner, Wood, Yates, Puck.

**Statistical analysis:** Kwan, Abraham, Currier, Abbott, Hanson, Johnson, Kay, Lee, Lorey, Routes, G. Scott, P. Scott, Puck.

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**Study supervision:** Abraham, Baker, Bonagura, Caggana, Celestin, Comeau, Cowan, Gelfand, Infante, Lee, Lehman, Abdel-Mageed, Naides, Routes, Rubenstein, Saavedra-Matiz, Stiehm, Sullivan, Watson, Puck.

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