Newborn screening for severe combined immunodeficiency and T-cell lymphopenia in California: Results of the first 2 years

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Background: Assay of T-cell receptor excision circles (TRECs) in dried blood spots obtained at birth permits population-based newborn screening (NBS) for severe combined immunodeficiency (SCID).
Objective: We sought to report the first 2 years of TREC NBS in California.
Methods: Since August 2010, California has conducted SCID NBS. A high-throughput TREC quantitative PCR assay with DNA isolated from routine dried blood spots was developed. Samples with initial low TREC numbers had repeat DNA isolation with quantitative PCR for TRECs and a genomic control, and immunophenotyping was performed within the screening program for infants with incomplete or abnormal results. Outcomes were tracked.
Results: Of 993,724 infants screened, 50 (1/19,900 [0.005%]) had significant T-cell lymphopenia. Fifteen (1/66,250) required hematopoietic cell or thymus transplantation or gene therapy; these infants had typical SCID (n = 11), leaky SCID or Omenn syndrome (n = 3), or complete DiGeorge syndrome (n = 1). Survival to date in this group is 93%. Other T-cell lymphopenic infants had variant SCID or combined immunodeficiency (n = 6), genetic syndromes associated with T-cell impairment (n = 12), secondary T-cell lymphopenia (n = 9), or preterm birth (n = 8). All T-cell lymphopenic infants avoided live vaccines and received appropriate interventions to prevent infections. TREC test specificity was excellent: only 0.08% of infants required a second test, and 0.016% required lymphocyte phenotyping by using flow cytometry.
Conclusions: TREC NBS in California has achieved early diagnosis of SCID and other conditions with T-cell lymphopenia, facilitating management and optimizing outcomes. Furthermore, NBS has revealed the incidence, causes, and follow-up of T-cell lymphopenia in a large diverse population. (J Allergy Clin Immunol 2013;132:140-50.)

Key words: *Severe combined immunodeficiency, newborn screening, T-cell receptor excision circle, T-cell lymphopenia, DiGeorge syndrome*

Severe combined immunodeficiency (SCID) characterized by poor T-cell production and failure of B cells to generate protective antibodies is the most profound primary immunodeficiency and is fatal unless affected infants receive an adaptive immune system through allogeneic hematopoietic cell transplantation (HCT), adenosine deaminase enzyme replacement, or gene therapy. Early detection of SCID is a key to successful treatment. In a retrospective series infants given a diagnosis of and treated with HCT in the first 3.5 months of life had improved survival and reduced morbidity.[1] However, most infants with SCID lack a family history or clinical clues to permit diagnosis before the onset of infection, making this life-threatening condition a candidate for population-based newborn screening (NBS).[2-5] With high burden on affected subjects, effective treatments, and availability of an inexpensive high-throughput assay, SCID fits the accepted criteria for population-based screening. The

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Abbreviations used

CHARGE: Coloboma (ocular), heart anomaly, atresia (choanal), retardation, genital and ear anomalies
CHH: Cartilage-hair hypoplasia
DBS: Dried blood spot
HCT: Hematopoietic cell transplantation
IL-7R: IL-7 receptor
NBS: Newborn screening
NICU: Neonatal intensive care unit
NK: Natural killer
qPCR: Quantitative PCR
RAG: Recombination-activating gene
SCID: Severe combined immunodeficiency
TCL: T-cell lymphopenia
TREC: T-cell receptor excision circle

US Department of Health and Human Services Secretary's Advisory Committee on Heritable Disorders in Newborns and Children recommended adding SCID to the uniform panel of NBS diseases; this recommendation was approved in May 2010.[6] Several states throughout the United States now perform SCID NBS.[7-9] California, the state with the most births and high ethnic diversity, began SCID NBS in August 2010.

Measurement of T-cell receptor excision circles (TRECs) to approximate thymic output was first used in patients with HIV-1 infection[10-12] and hematologic malignancies.[13] Adaptation for dried blood spotted onto filter paper made possible a TREC assay for SCID and other conditions with T-cell lymphopenia (TCL),[8,14] using the dried blood spots (DBS) already universally obtained by means of a heel stick from infants in the first days of life to screen for metabolic diseases, hypothyroidism, hemoglobinopathies, and cystic fibrosis. TREC copy number measured by using quantitative PCR (qPCR) is a biomarker for thymic output of naive T cells.[14,15] Low TREC numbers thus identify infants with SCID and non-SCID immunodeficiencies in which there is a profound decrease in the number of circulating naive T cells.

Wisconsin started SCID NBS in 2008; that state's findings, including 5 cases of SCID in more than 200,000 screened infants, have been published.[9,16] Massachusetts has also instituted SCID NBS and reported cases.[17] Here we summarize California's first 2 years of screening nearly one million newborns. We present TREC data correlated with lymphocyte profiles and characteristics of 50 infants with TCL, including 15 cases of typical and leaky SCID and complete DiGeorge syndrome that required immune-restoring therapy. We established the birth prevalence of SCID compared with other NBS diseases in California.

METHODS

Specimens tested

DBS specimens were collected from all infants born in California, except those whose parents opted out for religious reasons and completed a form accepting responsibility for any harm coming to the child as a result of refusal to test. After completing other tests at regional laboratories, DBSs were sent to the California Department of Public Health (CDPH) Genetic Disease Laboratory (Richmond, Calif) for TREC testing and archiving. An initial pilot was conducted under a waiver of review from the California Department of Public Health Committee for Protection of Human Subjects until SCID NBS was mandated by legislation, effective January 2012. Specimens were tested a mean of 20 days after the infants' birth, and nonnormal results were usually reported when infants were 3 to 4 weeks of age. Follow-up complete and differential blood counts and lymphocyte subset analyses were performed at a single site (Quest Diagnostics, San Juan Capistrano, Calif) as a confirmatory test within the SCID NBS program. Lymphocyte phenotypes were interpreted by California Department of Public Health Immunology Consultants (J.A.C., S.A.M., and J.M.P.).

For details of DNA extraction and determination of TREC and control β-actin gene copy numbers, see the Methods section and Table E1 in this article's Online Repository at www.jacionline.org.

Statistical analysis

Infant ethnicity recorded from parental designation of 19 categories was used for calculations, grouping ethnicities to achieve large enough pools for analysis: Hispanic, white (non-Hispanic), Asian, black, Native American, or other. Rates and projections from current data were performed by using the normal approximation, except where low case numbers required use of the Poisson distribution.

RESULTS

California SCID NBS testing

In 2 years, 993,724 infants were screened (Fig 1 and see Fig E1 in this article's Online Repository at www.jacionline.org). Numbers with each screening result are shown in Fig 1, *A*, whereas proportions from regular nurseries versus neonatal intensive care units (NICUs) are shown in Fig 1, *B*. Only 879 infants (<0.1% of those screened) had an initial TREC number of less than the acceptable cutoff, with a disproportionate contribution of 85% from NICUs. Urgent positive samples (ie, those with undetectable or 1-5 TRECs/μL of blood with normal control β-actin copy numbers) triggered immediate recall of 34 infants for flow cytometry, which was also indicated for 39 more infants with initial positive samples (6-25 TREC and normal β-actin copy numbers).

A second DBS was requested from 806 infants (0.08% of all screened) because of initial samples with low TREC numbers, but also low β-actin (designated "DNA amplification failure") or low TREC numbers in NICU infants (designated "incomplete"; Fig 1, *A*, and see Fig E1). Although providers were instructed to adopt immune system precautions pending further testing, the terms "DNA amplification failure" and "incomplete" were reported to indicate a likely technical problem with the sample or assay rather than to imply that the infant was affected with an immune disorder at this stage of screening. As with initial nonnormal samples, NICU samples predominated, accounting for 90% of requests for a second DBS (Fig 1, *B*). Only 11% of second samples obtained when infants were between 3 and 4 weeks of age were persistently abnormal. Infants with 2 DBS samples with low TREC numbers, irrespective of β-actin copy number, were recalled for venous blood immunophenotyping (Fig 1, *A*, and see Fig E1).

Thirteen infants had flow cytometry after only a single abnormal result because of clinical suspicion of an immune disorder. Eleven NICU infants with initial positive and 3 with repeated low TREC numbers died before flow cytometry could be performed, whereas 1 was lost to follow-up after 2 low TREC measurements; these 15 infants had serious medical conditions that could be associated with low T-cell numbers, as discussed below. The 161 infants who underwent immunophenotyping by means of flow cytometry represented 1 in 6200 births, with 106 (66%) from NICUs.

Lymphocyte profiles

Confirmatory complete and differential blood counts and lymphocyte subset analysis were an integral part of the SCID NBS program. Venous blood was obtained at a mean of 3 to 4

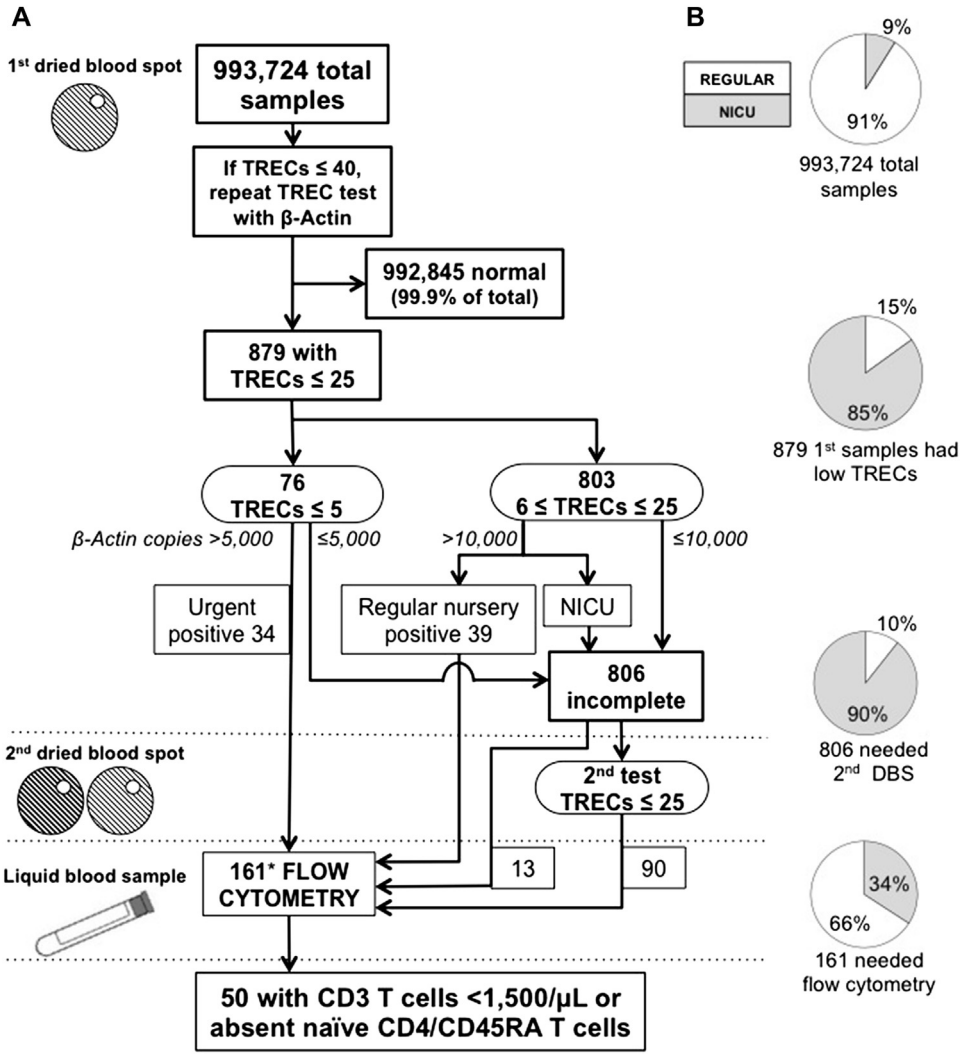


FIG 1. A, California SCID NBS. Sample numbers are shown at each stage *(boxes)*, according to TREC and β-actin values and nursery care as a proxy for clinical status (see also Fig E1). Infants with low TREC numbers on 2 DBSs or positive initial results were recalled to receive flow cytometry. **B,** Distribution of regular nursery versus NICU samples at each NBS stage. *Fourteen infants were deceased, and 1 was lost to follow-up before flow cytometry.

weeks after the initial DBS. TREC numbers increase during early postnatal life,[14,16] and thus this interval allowed for resolution of TCL in unaffected infants with little risk to SCID-affected infants, who are initially protected by transplacentally acquired maternal IgG.

Three pediatric immunologists interpreted all lymphocyte profiles for infants with abnormal TREC numbers. Although the normal range for newborns was 2550 to 5000 CD3 T cells/μL, we focused on those with either TCL of likely clinical significance, which was defined as less than 1500 CD3 T cells/μL, or absence or marked reduction in numbers of CD4 naive T cells (CD4/CD45RA), which was defined as less than 5% of total CD3 T cells. Of infants classified as having "positive" results on the TREC test, 44% had abnormal results determined by means of flow cytometry, and 23% had "incomplete" TREC test results (Table I). False-positive results (ie, samples that were "positive" or "incomplete" by using the TREC test but subsequently normal by using flow cytometry [111 samples]) made up only 0.01% of all the samples tested. Abnormalities in neutrophil, B-cell, and natural killer (NK) cell numbers were noted when present.

Categories of diagnosis among infants with TCL

Of 161 liquid blood samples, 50 (31%) had TCL (T cells <1500/μL) or 1 in 19,900 births. These infants had a spectrum of diagnoses (Table II and see Table E2 in this article's Online Repository at www.jacionline.org).[18-23] Typical SCID, leaky SCID, and variant SCID had a combined incidence of 1 in 49,700 births, with individual incidences of 1 in 90,000, 1 in 331,000, and 1 in 166,000, respectively. Categories of severity were established reflecting the degree of lymphopenia and the extent of immunologic intervention required (Fig 2). As shown, all infants with TCL received immunologic follow-up and instruction to avoid infectious exposures and live vaccines and to exercise transfusion precautions. Infants with multisystem congenital syndromes accompanied by TCL, as well as those

TABLE I. Nonnormal TREC results and their associated flow cytometric results during the first 2 years of SCID NBS in California

	Liquid blood lymphocyte profile		
DBS result	Indicative of a T-cell defect (T cells <1500/μL or absent naive CD4/CD45RA cells)	Not indicative of a significant T-cell defect (T cells ≥1500/μL; naive T cells present)	Total
Positive	27 (includes all SCID cases)	35	62*
Incomplete	23	76	99†
Total	50	111	161

*Eleven of 73 infants initially identified as having positive results by using the TREC test died before flow cytometry (Fig 1).
†Ninety infants had persistently low TREC numbers; 3 died, and 1 was lost to follow-up before flow cytometric confirmation. Also included are 13 infants who proceeded to flow cytometry after only a single abnormal TREC test result for logistical reasons or based on physicians' requests (Fig 1).

with combined immunodeficiency or variant SCID, who had demonstrable T-cell defects but no mutation in typical SCID genes, further required immunoglobulin infusions and antibiotic prophylaxis, initially or on a continuous basis, as dictated by their immunologic status.

The 15 most severely affected infants required immune-restoring therapies, including 11 with typical SCID, 3 with leaky SCID or Omenn syndrome, and 1 with complete DiGeorge syndrome. Diagnostic criteria of the Primary Immune Deficiency Treatment Consortium were used for typical SCID (<300 autologous T cells/μL), often with maternally derived T cells, with *in vitro* responses to PHA of less than 10% of control values, and leaky SCID/Omenn syndrome, with no maternal cells but autologous, oligoclonal, CD45RO memory phenotype T cells with PHA responses that could be greater than 10% but still less than normal values. In both typical and leaky SCID, specific gene mutations provided supporting evidence. Infants suspected of having typical or leaky SCID were immediately hospitalized at primary immunodeficiency centers and, after further evaluation, were treated with HCT, gene therapy, or thymus transplantation (Fig 2 and Table II). Importantly, only 1 infant with typical SCID had a recognized family history that led to prenatal diagnosis with immediate confirmation at birth (CA007, Table II); one other infant, CA027, lost an older brother in infancy, whose retrospective diagnosis of SCID was recognized only after NBS identified our patient.

Specific diagnoses and treatment of infants identified by means of NBS

Table II summarizes information for the 15 infants who required immune-restoring therapies. Within the 11 typical SCID diagnoses, 10 had undetectable TRECs, whereas 1 had 4 TRECs and extensive maternal engraftment with 1736 T cells but no naive CD4/CD45RA T cells. Four male subjects with a $T^-B^+NK^-$ phenotype had defects in the X-linked *IL2RG* gene encoding the cytokine receptor common γ chain. CA007 had a previously reported recurrent mutation (R226C),[18] whereas previously unpublished mutations were found in CA053, CA096, and CA125, a frameshift (M270fs), nonsense (W177X), and predicted damaging missense mutation (L132R), respectively. CA103, who had $T^-B^+NK^-$ SCID, had an in-frame deletion in the Janus-activation kinase gene *JAK3* (L527_G528del) previously noted in an unrelated case (J. Puck, unpublished). Three female subjects with $T^-B^+NK^+$ SCID had homozygous or compound heterozygous mutations in IL-7 receptor *(IL7R)* encoding the IL-7Rα chain, including a frameshift (I94fs) and 3 novel missense mutations. Two male subjects had recombination-activating gene 1 *(RAG1)* defects, 1 previously reported damaging (R404Q),[19] 1 unreported but predicted damaging (R764P), and 1 novel homozygous deletion of a single codon (M661del). Infant CA177 had $T^-B^+NK^+$ SCID with no molecular diagnosis.

Of the 3 infants with leaky SCID and Omenn syndrome, CA174 had undetectable TRECs and a 50% of normal PHA response, whereas CA024 and CA158, the latter of whom also had dwarfism caused by cartilage-hair hypoplasia (CHH), had 20 and 19 TRECs, respectively. CA024 with Omenn syndrome was a compound heterozygote with 1 novel *RAG1* defect and another *RAG1* mutation previously associated with Omenn syndrome (W522C),[20] with less than 5% naive CD4/CD45RA T cells and no maternal engraftment. Infant CA174 had 51 T cells/μL, greater than 10% of control responses to mitogens, and mutations of *RAG1* (R474C and I956T) previously associated with leaky SCID or Omenn syndrome phenotypes.[21,22] Infant CA158 had previously recognized heterozygous single nucleotide mutations in *RMRP* causing CHH[23]; she was the only infant in the severely affected group with any autologous naive CD4/CD45RA T cells, underscoring the utility of combining TREC copy number, total CD3 T-cell count, and naive phenotype markers on CD4 and CD8 T cells to identify infants requiring urgent immune-restoring therapy.

In addition, the female subject CA019 had complete DiGeorge syndrome with undetectable TRECs, absent thymus, and absent T cells. Apart from this infant, who received a thymus transplant, and CA125, a male subject with X-linked SCID who initially received gene therapy, all others received allogeneic HCT according to protocols in place at the individual treatment centers. One infant, CA027, who had RAG1 SCID, died 46 days after transplantation from irreversible hepatic sinusoidal obstruction syndrome, a result of myeloablative conditioning with busulfan. All others are alive, engrafted, and healthy. Four patients, 2 with X-linked SCID, 1 with *IL7R* gene mutation causing IL-7Rα chain deficient SCID, and 1 with RAG1-deficient Omenn syndrome required booster infusions of donor cells, whereas infant CA125 did not engraft autologous gene-corrected HCT and required subsequent conditioning and an allogeneic cord blood transplant. Although some of the patients are still early after transplantation and others continue to require immunoglobulin, 3 have had full B-cell reconstitution and no longer receive immunoglobulin.

Infants assigned to the remaining categories of newborn TCL diagnoses are defined in Table E2 in this article's Online Repository at www.jacionline.org. Six had variant SCID/combined immunodeficiency, which was defined as TCL with functional T-cell impairment for more than 3 months without syndromic features or defects in a known SCID gene. Four had positive and 2 had incomplete TREC test results; their other immunologic features and treatments are listed in Table E2. This category was unanticipated before NBS for SCID but invites a search for new immunodeficiency diagnoses.

Also in Table E2 are 15 infants with congenital syndromes with variable degrees of T-cell impairment; 8 had chromosome 22q11.2 interstitial deletion causing partial DiGeorge syndrome

TABLE II. Infants with low TREC numbers and TCL who required immune-restoring therapy

SCID case no.	Sex	Ethnicity	Nursery	TREC copies	Lymphocyte phenotype	CD3 T cells	CD4 CD45RA T cells	Disease-causing gene (mutations)*	Presenting clinical information†
CA007	Male	Black	NICU	0/μL	$T^-B^+NK^-$	493/μL	<20/μL	*IL2RG* (R226C[18])	Thirty-five-week gestation, prenatal diagnosis (older affected brother); asymptomatic, infection free; maternal engraftment present; PHA <10%
CA053	Male	Hispanic	Regular	0	$T^-B^+NK^-$	<20	<20	*IL2RG* (M270fs)	Asymptomatic, infection free, XY/XO mosaic; no maternal engraftment, PHA <10%
CA096	Male	Asian	NICU	0	$T^- B^+ NK^-$	20	<20	*IL2RG* (W177X)	1 of triplets, 33 week gestation, asymptomatic, infection-free; maternal engraftment present; PHA <10%.
CA125	Male	Hispanic	Regular	0	$T^-B^+NK^-$	78	23	*IL2RG* (L132R)	Asymptomatic, infection free; maternal engraftment present; PHA <10%
CA103	Female	Hispanic	Regular	4	$T^-B^+NK^-$	1736	<20	*JAK3* (L527_G528del)	Rash and cellulitis; maternal engraftment present; PHA not tested
CA004	Female	Hispanic	Regular	0	$T^-B^+NK^+$	60	49	*IL7R* (F71S, H180P)	Asymptomatic, infection free; maternal engraftment present; PHA <10%
CA005	Female	Hispanic	Regular	0	$T^-B^+NK^+$	<20	<20	*IL7R* (I94fs, H180P)	Asymptomatic, infection free but acquired Coxsackie virus at home before HCT; maternal engraftment present, PHA <10%
CA067	Female	Hispanic	Regular	0	$T^-B^+NK^+$	<20	<20	*IL7R* (G215V)	Asymptomatic, infection free; maternal engraftment present, PHA <10%
CA013	Male	Hispanic	Regular	0	$T^-B^-NK^+$	<20	<20	*RAG1* (M661del)	Asymptomatic, infection free, maternal engraftment not tested; PHA not tested
CA027	Male	Hispanic	Regular	0	$T^-B^-NK^+$	28	<20	*RAG1* (R404Q[19] R764P)	Rash coinciding with TREC report, deceased affected brother, infection free; maternal engraftment present; PHA not tested
CA177	Male	Hispanic	NICU	0	$T^-B^+NK^+$	80	3	Unknown‡	Thirty-seven-week gestation, maternal diabetes and hepatitis; low glucose, respiratory distress, hepatomegaly, feeding difficulties; maternal engraftment present; PHA <10%

(Continued)

TABLE II. *(Continued)*

SCID case no.	Sex	Ethnicity	Nursery	TREC copies	Lymphocyte phenotype	CD3 T cells	CD4 CD45RA T cells	Disease-causing gene (mutations)*	Presenting clinical information†
CA024	Female	White	Regular	20	$T^-B^-NK^+$	1583	24	*RAG1* (W522C[20], N968K)	Asymptomatic, infection free; no maternal cells; PHA not done; Omenn syndrome
CA174	Female	Hispanic	Regular	0	$T^-B^-NK^{low}$	51	3	*RAG1* (R474C[21], I956T[22])	Asymptomatic, infection free but acquired rhinovirus at home before HCT; no maternal cells; PHA 50%; leaky SCID
CA158	Female	White	Regular	19	$T^-B^+NK^+$	380	99	*RMRP I* (r.211c>g, 262g>u)[23]	Intrauterine growth retardation, short limbs; no maternal cells; PHA 40%; leaky SCID, CHH
CA019	Female	Hispanic	NICU	0	$T^-B^+NK^+$	<20	<20	22q11 deletion	Complete DiGeorge syndrome with congenital heart disease

SCID case no.	Treatment/donor (match)/cell type	Age at HCT(s)	Conditioning	GvHD prophylaxis	Clinical course§	Current status (age)
CA007	HCT, CD34-selected maternal cryopreserved mobilized peripheral blood	3 wk	None	None	Acute skin GvHD; T-cell engraftment	Alive (28 mo), vitiligo, on IVIG
CA053	HCT, MUD (10/10) bone marrow	2.5 mo	G-CSF, Plerixafor	CsA	Slow T-cell recovery, →boost	Alive (21 mo), on IVIG
		Boost: 4 mo	None	CsA, MTX, tacrolimus	T-cell engraftment	
CA096	HCT, maternal CD34-selected peripheral blood	8 wk	None	None	Slow T-cell recovery, →boost	Alive (15 mo), on scIG
		Boost: 4 mo	None	CsA, methylprednisolone	T-cell engraftment	
CA125	Autologous transduced CD34 cell gene therapy (trial no. NCT01129544)	4 mo	None	None	Failed T-cell engraftment → unrelated donor cord blood HCT, T-cell engraftment	Alive (13 mo), on IVIG
CA103	HCT, MUD (10/10) bone marrow	4 mo	Flu, Bu, rATG, KGF	MTX, tacrolimus, methylprednisolone	T-cell engraftment	Alive (14 mo), on IVIG
CA004	HCT, maternal CD34-selected peripheral blood	2 mo	Plerixafor	None	T-cell engraftment and B-cell function	Alive (28 mo), off IVIG, given live vaccines
CA005	HCT, MUD (10/10) bone marrow	4.5 mo	Flu, Bu, Cytoxan	Tacrolimus, MTX	T-cell engraftment and B-cell function	Alive (28 mo), off IVIG
CA067	HCT, maternal CD34-selected peripheral blood	1.5 mo	None	None	Slow T-cell recovery, →boost	Alive (17 mo), on scIG
		Boost: 5 mo	None	None	T-cell engraftment	
CA013	HCT, matched sibling bone marrow	3.5 mo	rATG ×3	Tacrolimus	Autologous recovery, →2nd HCT	Alive (27 mo), off IVIG
		2nd HCT: 9 mo	Flu, Bu, rATG	Tacrolimus	T-cell engraftment	
CA027	HCT, unrelated (7/10) umbilical cord blood	4 mo	Flu, Bu, rATG	Tacrolimus, methylprednisolone	Veno-occlusive disease (d+28), pulmonary hemorrhage	Died day +46 (at 5 mo) after transplantation
CA177	HCT, MUD (10/10) bone marrow	4 mo	Flu, Bu, rATG, KGF	Tacrolimus, MTX, methylprednisolone	Acute skin and gut GvHD; T-cell engraftment	Alive (7 mo), on IVIG
CA024	HCT, MUD (9/10) T cell–depleted mobilized peripheral blood	3.5 mo	Flu, Bu, Campath	CsA, MMF	Slow T-cell recovery, →boost	Alive (25 mo), on IVIG
		Boost: 7 mo	Flu, ATG	CsA, MMF	T-cell engraftment	

(Continued)

TABLE II. *(Continued)*

SCID case no.	Treatment/donor (match)/cell type	Age at HCT(s)	Conditioning	GvHD prophylaxis	Clinical course§	Current status (age)
CA174	HCT, MUD (9/10) bone marrow	4.5 mo	Flu, Bu, Campath	MTX, CsA	T-cell engraftment	Alive (7 mo), on IVIG
CA158	HCT, MUD (10/10) bone marrow	7 mo	Flu, Bu, Campath	MTX, CsA	T-cell engraftment	Alive (9 mo), on IVIG
CA019	Thymus transplant	12 mo	Bu	None	Immune reconstitution; heart surgery after transplantation	Alive (26 mo), off CsA, on IVIG

Bu, Busulfan; *CsA*, cyclosporine A; *Flu*, fludarabine; *G-CSF*, granulocyte colony-stimulating factor; *GvHD*, graft-versus-host disease; *IVIG*, intravenous immunoglobulin; *KGF*, keratinocyte growth factor; *MMF*, mycophenolate mofetil; *MTX*, methotrexate; *MUD*, matched unrelated donor; *rATG*, rabbit anti-thymocyte globulin; *scIG*, subcutaneous immunoglobulin.

*Single mutation: hemizygous in *IL2RG*, homozygous for other genes, heterozygous for 22q deletion. Two mutations: compound heterozygotes. Previously reported mutations are referenced with superscript numbers; others not published to date.

†Proliferative response *in vitro* to PHA: less than 10% of healthy control range is a criterion for diagnosis of typical SCID, and 10% to 50% is consistent with leaky SCID.

‡No damaging variants found in *ADA* (the gene encoding adenosine deaminase), *AK2*, *CD3D*, *CD3E*, *CD3Z*, *DCLRE1C*, *IL2RG*, *IL7R*, *JAK3*, *LIG4*, *NHEJ1*, *PNP*, *PTPRC*, *RAC2*, *RAG1*, *RAG2*, *RMRP*, and *ZAP70*.

§No major infectious complications.

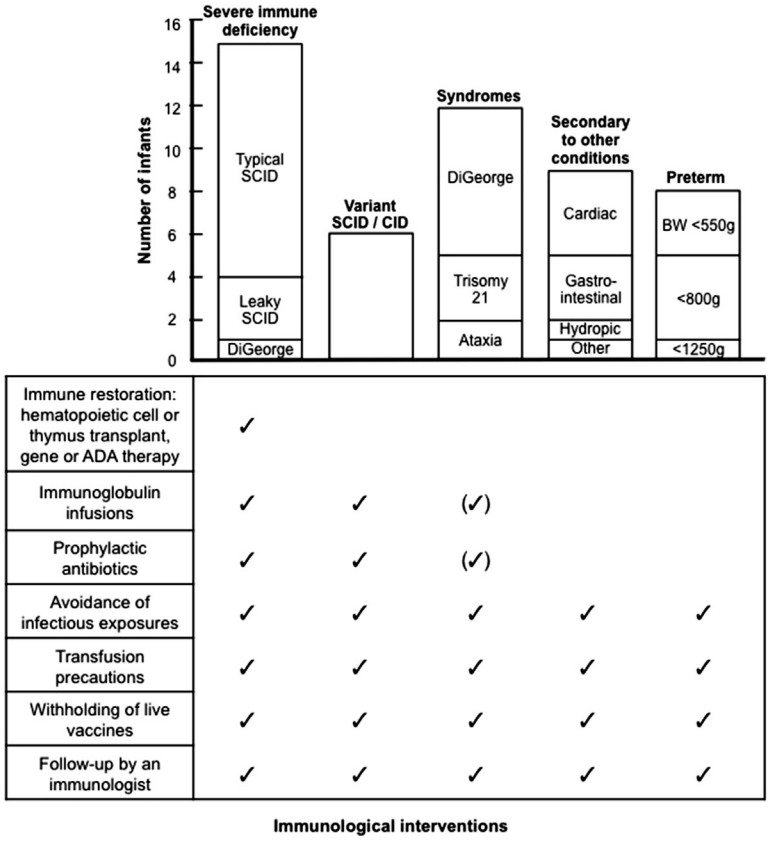


FIG 2. TCL by intervention and underlying condition. Cases requiring immune-restoring therapy included 11 cases of typical and 3 cases of leaky SCID and 1 case of complete DiGeorge syndrome. There were 6 cases of variant SCID, 12 cases of congenital syndromes, 9 cases of secondary TCL, and 8 preterm infants (weight ranges shown). All were advised to avoid live rotavirus and other live vaccines and to receive only cytomegalovirus-negative, irradiated blood products. *BW*, Birth weight; *CID*, combined immunodeficiency.

(with low but detectable T-cell numbers in contrast to the patient with complete DiGeorge syndrome discussed above). Although 4 were recognized immediately after birth by their congenital cardiac malformations, one was given a diagnosis only after SCID NBS revealed his TCL. In addition, 4 infants had TCL associated with trisomy 21, and 1 had ocular coloboma, heart anomaly, choanal atresia, retardation, genital and ear anomalies (CHARGE) syndrome. Although the DiGeorge and CHARGE syndromes and trisomy 21 are often identified by their characteristic facies and clinical features, nonimmunologists might not appreciate the degree of TCL immunodeficiency that could be present; thus NBS has identified the subset of infants with these

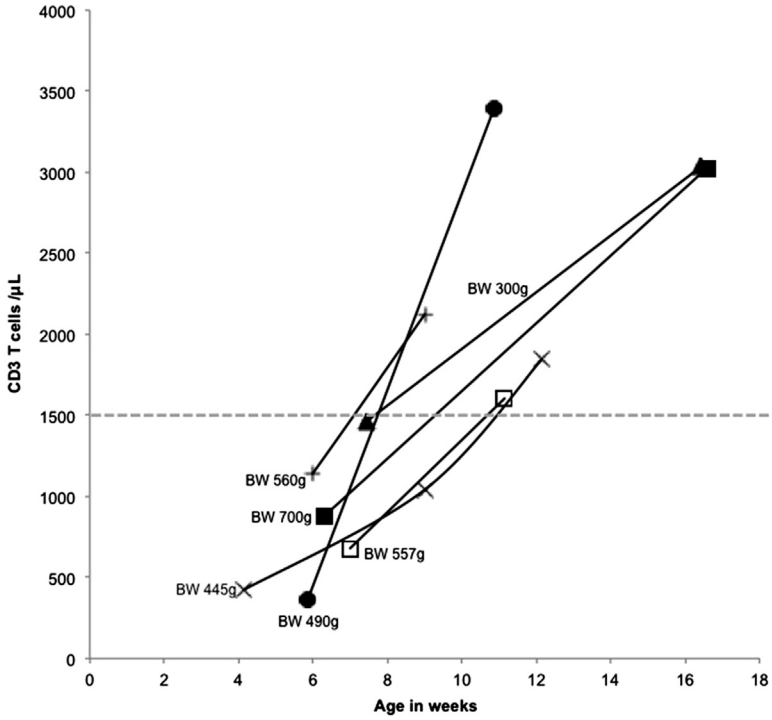


FIG 3. T-cell concentrations in infants with initial TCL associated with preterm birth, obtained at gestational ages on the *x axis*. Birth weights *(BW)* are indicated. Subsequent T-cell concentrations were greater than 1500 cells/μL *(dotted line)*; the threshold was set by using the California Newborn Screening program.

conditions who were particularly affected by TCL and thus at risk for infection. Three of these infants died of complications not associated with the immune system before having lymphocyte enumeration.

NBS also flagged 2 apparently healthy infants with ataxia telangiectasia and newborn TCL, who were initially considered to have variant SCID.[24] A study of newborn DBSs archived from California cases of ataxia telangiectasia showed that greater than 50% would have been identified by means of NBS with the TREC test, as currently performed here.[24] TCL was also found secondary to congenital or postnatal major congenital heart disease (6 cases), gastrointestinal malformations (3 cases), hydrops (2 cases), multiple anomalies without a unifying diagnosis (2 cases), and chylothorax (1 case). See Table E2 for further details.

A final group of 8 infants with TCL were born at 24 to 27 weeks' gestation with birth weights of 300 to 1200 g (see Table E2). All had incomplete TREC results. For 6 surviving infants, subsequent lymphocyte profiles demonstrated improvement in T-cell numbers over time (Fig 3).

Demographics of patients with TCL in California

TREC NBS data combined with self-reported ethnic origins permitted estimates of the incidence of SCID and TCL for California's ethnically diverse population. By using the pre-NBS SCID incidence estimate of 1 in 100,000 from the literature,[14] California Hispanic and Asian populations had higher than expected numbers of SCID diagnoses (including typical, leaky, and variant SCID), with respect to the proportion of total births ($P < .001$ and $P < .05$ for Hispanic and Asian subjects, respectively; Table III). We also considered the incidence of non-SCID TCL secondary to clinical syndromes, congenital malformations, or other nonimmune conditions (excluding preterm birth alone in which TCL resolved with time), estimating incidence at 1 in 50,000 births; Asian, black, and Native American populations had higher numbers of TCL cases than expected with respect to the proportion of total births ($P < .01$, $P < .002$, and $P < .001$, respectively; Table III).

DISCUSSION

NBS programs present a number of ethical and public health questions. Justifications offered in support of NBS include early diagnosis, improved treatments, and lives saved. Decisions to institute a new NBS test are tied to the seriousness of the disorder, incidence, detectability by a sensitive and specific assay, treatment availability and effectiveness, and outcomes and costs of treatment in the presence versus absence of screening. These factors must be judged to outweigh monetary costs, needless anxiety engendered by false-positive results, and workload imposed on medical practices. In 2006, recommendations developed to bring uniformity to NBS led to a core panel of 29 diseases for NBS, which was widely adopted by the states.[25] SCID was the first disease to be added to this federal uniform panel by using evidence-based review and cost-effectiveness analyses.[6,26] At this time, 12 states and the Navajo Nation screen all births for SCID, with several other states planning to add the TREC test to their NBS panels (A. Brower, Newborn Screening Translational Research Network, personal communication). Actual data on the performance of SCID NBS in large populations, such as that presented here, will inform decisions of additional programs regarding adoption of the TREC test.

TABLE III. Ethnic composition of infants with low TREC numbers and TCL

Ethnicity	SCID, leaky SCID, Omenn syndrome, complete DiGeorge syndrome, and variant SCID			Other types of TCL: syndromes with T-cell impairment and secondary TCL, excluding preterm birth alone		
	No. observed	No. expected with incidence 1/100,000	*P* value	No. observed	No. expected with incidence 1/50,000	*P* value
Hispanic	13	4.85	<.001	9	9.70	NS
White	4.5	2.52	NS	8	5.04	NS
Asian	2.5	0.81	<.05	5	1.63	<.01
Black	1	0.53	NS	6	1.06	<.0002
Native American	0	0.09	NS	2	0.18	<.001
Other	0	0.54	NS	1	1.08	NS
Total	21			29		

NS, Not significant.

SCID NBS with the TREC assay in California

In California nearly one million infants were screened over 2 years. Tracking of cases encompassed both SCID disorders, the primary target, and clinically significant TCL with less than 1500 autologous T cells/μL as a secondary target. The 2-year cohort included 50 infants with SCID and TCL (0.5/10,000 births). When compared with data for the 29 other core NBS conditions in California, all TCL disorders would have placed eighth in frequency (see Table E3 in this article's Online Repository at www.jacionline.org). Furthermore, the disorders that require urgent immune-restoring therapies (SCID, leaky SCID, Omenn syndrome, and complete DiGeorge syndrome) would have ranked fifteenth at 0.13 per 10,000 births (see Table E3). Thus SCID and TCL occur more frequently than half of the conditions originally accepted in the uniform federal panel for NBS.

The performance of the TREC test was also excellent in comparison with other NBS tests; less than 0.1% of infants screened had nonnormal results, 0.08% with incomplete results requiring a repeat TREC test, and 0.016% requiring venous blood for immunophenotyping. The positive predictive value was high, with 31% of flow cytometric tests confirming the presence of less than 1500 T cells/μL. Infants in NICUs constituted 9% of total California births but had disproportionately frequent nonnormal TREC results (Fig 1, *B*). The preponderance of nonnormal first test results in NICU infants has been noted by other states using the TREC test[16,27,28] but is also found for other NBS tests. Rather than collect a series of repeated DBS samples from NICU infants, we elected to perform lymphocyte enumeration after 2 nonnormal DBS samples; this was cost-effective because of the timing of the 2 DBS samples roughly 3 weeks apart, the small overall number of venous blood samples needed, and having a single laboratory for flow cytometry.

We believe the designated TCL definition of less than 1500 T cells/μL as a secondary target of TREC screening captures infants with significant immune compromise who should avoid live rotavirus vaccination and receive evaluation and management by pediatric immunologists on an individualized basis. Of the infants with non-SCID TCL confirmed by means of flow cytometry, 76% were cared for in NICUs, which is in contrast to the infants with typical SCID, leaky SCID, and variant SCID, of whom 76% were in regular nurseries. Thus TCL was identified as a significant problem for NICU infants, often accompanying an underlying condition.

Advantages of early SCID diagnosis

Typical SCID, leaky SCID, Omenn syndrome, and complete DiGeorge syndrome are life-threatening disorders that require emergency immune-restoring treatment. However, only 1 infant with typical SCID in our series had a recognized positive family history (Table II), whereas 2 others had presented to a hospital with cellulitis or rash. Thus the early diagnosis of SCID through NBS allowed specific treatments (immunoglobulin and prophylactic antimicrobial agents) to be instituted early to avoid infections and maintain health, which is crucial to the success of all subsequent immune-restoring therapies.

Admission to specialized SCID treatment centers, management of pre-HCT treatment, and timing of HCT depended on family circumstances, identification of an appropriate donor, and center-specific protocols. Through enrollment in current Primary Immune Deficiency Treatment Consortium protocols, our patients' outcomes will help shape future clinical trials to determine optimal treatments for SCID diagnosed by using NBS. HCT treatments ranged from an infusion of cryopreserved maternal CD34-enriched cells at 3 weeks of age with neither conditioning nor prophylaxis for graft-versus-host disease, in the infant with X-linked SCID and an affected brother, to a female infant who received conditioning and graft-versus-host disease prophylaxis for a matched unrelated donor bone marrow transplant at 7 months of age for leaky SCID caused by CHH. One infant received experimental gene therapy for X-linked SCID caused by an *IL2RG* mutation, although he required a subsequent cord blood transplant because of failure of autologous transduced cells to engraft. One infant with complete DiGeorge syndrome received a thymus transplant at 12 months of age.

Fourteen of the 15 infants who required immune restoration are currently alive, engrafted, and healthy at 4 to 27 months after their most recent treatment. Survival of these infants after detection by using NBS has been 93%, which is comparable with retrospective studies reporting increased survival of infants with SCID detected at birth because of a prior positive family history.[3-5] Although the Duke University retrospective data for SCID HCT indicated best survival for infants undergoing transplantation before the age of 3.5 months,[7] our infants identified by means of NBS have excellent survival at older transplantation ages because of prompt institution of measures to avoid infections.

Advantages of diagnosis of TCL

TREC NBS revealed a wide spectrum of non-SCID disorders with TCL as a neonatal feature (Fig 2). The California cutoff values of less than 40 TRECs/μL for having the qPCR rerun and 25 TRECs/μL or greater on a repeat determination with normal β-actin values were selected by testing known positive cases and anonymous DBSs from different nursery settings. Although 1500 T

cells/μL was less than the reference lower limit of 2550 T cells/μL for term healthy newborns, this was chosen as a prudent value to capture significant TCL without incurring a large burden of NICU infants without true immune compromise. These parameters have allowed infants at the most T-cell lymphopenic end of the spectrum of syndromic disorders to be identified, irrespective of whether they had underlying DiGeorge syndrome or an unanticipated number of other syndromes, including trisomy 21, ataxia telangiectasia,[24] CHH, or CHARGE syndrome. Also unexpected were the congenital defects (cardiac and gastrointestinal malformations, hydrops, and chylothorax) that resulted in TCL, although in these cases T-cell numbers improved when the defects were repaired, suggesting either suppression of lymphopoiesis caused by illness or intrinsically normal rates of thymic T-cell production but increased loss or destruction peripherally. Taken together, these cases highlight the importance of TCL as a signal of infection risk and a critical feature of a broad range of genetic syndromes and congenital malformations. Live vaccine avoidance and infection prophylaxis for these infants believed prudent at this time can be provided when they are identified by using TREC NBS.

Furthermore, discovery of previously unsuspected cases of TCL in otherwise healthy infants has made possible investigation of heretofore unknown cases, designated as variant SCID. Eight infants without mutations in known SCID genes were initially allocated to this group, with 2 reclassified when found to have ataxia telangiectasia. Longitudinal follow-up of the remaining 6 infants has revealed persistent TCL with variable degrees of impairment of lymphocyte proliferation to mitogen or antigen stimulation, B-lymphocyte production, and antibody responses to vaccine challenge. Deep sequencing of DNA from patients and unaffected family members might identify their molecular defects.

Limitations of the TREC test

Although TCL is a feature of many immunodeficiencies, screening with the TREC assay cannot identify all infants with serious defects of T-cell function. In patients with disorders such as MHC II deficiency, ZAP70 deficiency, and others in which T-cell receptor rearrangement is intact but more distal pathways in maturation and function are deficient, TREC numbers are not expected to be decreased. Consistent with this expectation, during the 2 years of California's TREC NBS program, 2 cases of MHC II deficiency,[29] 2 cases of Wiskott-Aldrich syndrome, and 1 case of delayed-onset adenosine deaminase SCID were not detected by using TREC screening because their newborn TREC numbers were normal. In addition, defects limited to humoral immunity or neutrophils are not detected. Testing for insufficient TREC numbers, a biomarker of numerically adequate naive T cells, will detect only SCID diseases with impaired T-cell production and secondary conditions that drain naive T cells from peripheral blood. Importantly, a negative TREC test result does not rule out the possibility of primary immunodeficiency. Although SCID NBS with TRECs has raised awareness of SCID among physicians and the public, continued educational efforts are important to make sure immune disorders are sought when clinical features suggest.

Incidence of SCID and related disorders in the diverse California population

The genotypes of patients with typical SCID are consistent with prior reports, with the most frequent genotype being X-linked *IL2RG*. The ethnic variation observed in the incidence of SCID and non-SCID TCL warrant further investigation; data will be forthcoming as cases accumulate. There are reports of differing SCID incidences according to immune deficiency registries from different countries,[30-35] with autosomal recessive SCID being more prevalent in cultures in which consanguineous marriage is common.[36] Because TREC screening continues in the ethnically diverse state of California, underlying ethnic patterns of SCID incidence will be revealed. Furthermore, children from parents of discordant ethnic backgrounds might have a lower than average population incidence of SCID caused by autosomal recessive mutations because they are unlikely to harbor common founder alleles. In contrast, X-linked SCID incidence should be independent of ethnicity caused by the substantial proportion of newly arising mutations.

Incidence of conditions with TCL and proportion of patients with defined syndromes who have significant immune compromise can also be determined in an unbiased and prospective manner. TREC NBS in California combined with retrospective analysis of newborn DBSs from patients with ataxia telangiectasia led to a new incidence estimate of 1 case per 200,000 births.[24] Similarly, by using published incidence estimates of around 1 in 5,000 births for chromosome 22q11 deletion, the most frequent human interstitial deletion syndrome,[37] California should have 100 births each year with this cytogenetic abnormality. In our 2 years of screening, 9 infants, or 5% of the expected number of births with DiGeorge/22q11 deletion, were identified to have less than 1500 T cells/μL. Although our TCL cutoff is somewhat arbitrary, this is the first unbiased estimate of TCL of this degree in this condition.

Conclusion

The California experience lends insight to the logistics, testing algorithms, and results of the TREC test, as applied to NBS for SCID and related TCL disorders. The specificity and sensitivity of the screening test and the advantage of centralized incorporation of confirmatory lymphocyte profiling by means of flow cytometry add to the available experience to date. Furthermore, our large population gives new and accurate estimates of birth prevalence of SCID and non-SCID TCL, justifying inclusion of SCID screening in the uniform core panel. There is great variety in the clinical, genotypic, and demographic characteristics of infants with SCID and TCL in California, and continued screening and monitoring will reveal the full phenotypic spectrum. To complement SCID NBS, we need to continue to improve rapid, high-throughput genotyping to delineate the underlying genetic defects in patients with SCID and TCL to tailor appropriate genotype-specific treatment strategies to optimize their treatments and outcomes. TREC screening will also facilitate understanding of syndromes and conditions that present with TCL, making possible standardization and improvement of their immunologic management.

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Clinical implications: TREC NBS permits early diagnosis and treatment of SCID and other T-lymphopenic disorders, thus revealing new information about their incidence and clinical spectrum while making optimal outcomes possible.

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METHODS

DNA extraction

Genomic DNA was extracted by using Generation DNA purification and elution solution (Qiagen, Hilden, Germany). From the DBS specimens, a 3.2-mm disc was punched into a 96-well plate for DNA extraction. Punches were incubated with 120 μL of DNA Purification Solution at 50°C for 30 minutes. After washing by pipetting the liquid up and down 5 times, the liquid was removed and discarded. Incubation, washing, and aspiration were repeated, after which 200 μL of DNA Elution Solution 1 was added to each sample for 15 minutes at room temperature. This liquid was removed and discarded. Finally, 30 μL of DNA Elution Solution 2 was added to each sample. The plate was sealed (EasySealer; REMP AG, Oberdiessbach, Switzerland), centrifuged briefly, and then incubated at 110°C for 30 minutes. After cooling at 4°C for a minimum of 1 hour, plates were pierced (REMP Universal Plate Piercer), and dissolved DNA was transferred to 384-well plates for PCR.

Real-time PCR and measurement of TREC and human β-actin copy numbers

Reagents, master mix, and plasmid calibrators were prepared in a UV3 Hepa PCR workstation (UVP, Upland, Calif). The master mix was composed of 1× LightCycler 480 Probes Master (Roche Diagnostics, Mannheim, Germany) and forward and reverse primers and probe oligonucleotides (Integrated DNA Technologies, Coralville, Iowa; Table E1). Concentrations of TREC and β-actin plasmids (GenScript, Piscataway, NJ) were determined by using a Nanodrop 2000 Spectrophotometer (Wilmington, Del). Serial dilutions of plasmid formed a calibration curve for each run.

DBS cards simulating TREC copy numbers expected in normal infants were prepared by spotting filter papers with pooled cord blood anticoagulated with acid citrate dextrose. Positive controls to mimic SCID-like TREC copy numbers were made from the blood of selected adult volunteers 50 years or older and from the blood of younger donors that had been depleted of mononuclear cells, as previously described.[E1] Controls in each PCR run also included samples punched from blank filter paper and water blanks.

A LightCycler 480 System (Roche Diagnostics, Mannheim, Germany) was used for qPCR with 20-μL reaction volumes, including 10 μL of extracted DNA and 10 μL of oligonucleotides in master mix. TREC reactions contained 0.5 μmol/L of each primer with a 0.15 μmol/L probe, and β-actin gene reactions contained 0.25 μmol/L of each primer with 0.15 μmol/L probe. The cycling conditions were as follows: 95°C for 10 minutes, 50× (95°C for 10 seconds and 65°C for 1 minute), 40°C for 20 seconds. LightCycler 480 software was used for data analysis. Each crossing point calculated from raw data was converted to TREC or β-actin gene copy numbers per reaction by using a calibration curve. Results were reported as the copy number per microliter of whole blood (0 copies for undetectable PCR and 1 copy/μL being the lowest detectable value), assuming 3 μL of blood per 3.2-mm disc from a DBS.

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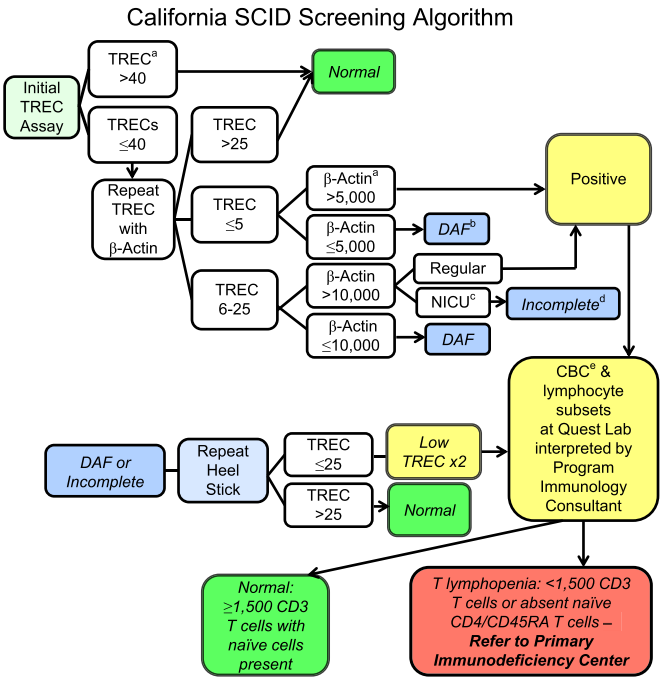


FIG E1. California SCID NBS algorithm. [a]Cutoff values for TREC and β-actin gene copies/μL of blood, assuming each 3.2-mm punch of dried blood filter represents 3 μL of blood. Samples with more than 40 TRECs/μL on initial testing were considered normal. Samples with 40 TRECs/μL or less had repeat TREC with β-actin testing; of these, samples with 25 TRECs/μL or less were categorized as positive if β-actin values were normal, and infants were recalled for lymphocyte subset determination. [b]*DAF,* DNA amplification failure. This requires a repeat heel-stick sample, or if it is already a second sample, recall for lymphocyte subset determination is required. [c]*NICU,* Neonatal intensive care unit. [d]Incomplete, NICU screen result with 6 to 25 TRECs/μL and a β-actin copy number of 10,000 or less. This requires a repeat heel-stick sample. [e]*CBC,* Complete blood count.

TABLE E1. Sequences of primers and probes used in California SCID screening

Name	Sequence
TREC forward primer	5′-ACA CCT CTG GTT TTT GTA A-3′
TREC reverse primer	5′-CAG CTG CAG GGT TAG-3′
TREC probe	5′-(FAM)TAG GCA CCT GCAC CCC(BHQ-1)-3′
β-Actin forward primer	5′-ATG GTG TAT CTC TGC CTT-3′
β-Actin reverse primer	5′-GTC ACC GGA GTC CAT C-3′
β-Actin probe	5′-(FAM)TAC GTT GCT ATC CAG GCT G(BHQ-1)-3′

TABLE E2. Infants with low TREC numbers and TCL not requiring immune-restoring therapy

Diagnosis	Ethnicity	Nursery	TREC copies/μL	Lymphocyte phenotype*	CD3 T cells	CD4 CD45RA T cells	Clinical information	Outcome
Variant SCID								
Unknown CA008	Asian/white	Regular	7	$T^{low}B^{+}NK^{+}$	669/μL	414/μL	Healthy at birth, 1 pneumonia treated with oral antibiotics	Persistent lymphopenias, responded to killed vaccines, no live vaccines
Unknown CA011	Hispanic	Regular	0	$T^{low}B^{+}NK^{+}$	358	136	Healthy at birth, IVIG and prophylactic sulfamethoxazole-trimethoprim for 6 mo	Slow increase in T-cell counts, responded to killed vaccines, no live vaccines, language delay
Unknown CA153	White	Regular	10	$T^{low}B^{+}NK^{+}$	704	99	Bilateral congenital cataracts, bicuspid aortic valve, abnormal facies, hypotonia, normal copy number array	No unifying diagnosis, on sulfamethoxazole-trimethoprim prophylaxis, developmental delay
Unknown CA166	Hispanic	Regular	21	$T^{low}B^{+}NK^{+}$	1296	752	Healthy at birth; persistent TCL; normal proliferation to PHA; and normal IgG, IgA, and IgM levels	Awaiting testing of antibody responses after vaccines
Unknown CA167	Asian	NICU	7	$T^{low}B^{+}NK^{low}$	896	179	Twenty-six-week gestation, persistent low CD4 T-cell numbers, disseminated cytomegalovirus infection	Pulmonary hypertension, died of respiratory failure at 4.5 mo of age
Unknown CA197	White	Regular	8	$T^{-}B^{+}NK^{low}$	248	139	Multiple anomalies, with abnormal cardiac vasculature, low-set ears, bulbous nose, hypocalcemia	Normal copy number array (CGH array), specific gene sequences pending
Syndromes with T-cell impairment								
DiGeorge (partial)	Hispanic	NICU	5	$T^{low}B^{+}NK^{-}$	1060	594	Dysmorphic features, laryngomalacia, right-sided aortic arch	Multispecialty follow-up
DiGeorge (partial)	Black	NICU	15	$T^{low}B^{low}NK^{+}$	960	566	Thirty-five-week gestation	Multispecialty follow-up
DiGeorge (partial)	White	Regular	16	$T^{low}B^{+}NK^{+}$	1381	697	Pharyngeal inadequacy, poor feeding	Multispecialty follow-up
DiGeorge (partial)	White	NICU	21	$T^{low}B^{+}NK^{+}$	1140	650	Tetralogy of Fallot	Multispecialty follow-up
DiGeorge (partial)	Black	Regular	23	$T^{low}B^{+}NK^{+}$	1200	480	Healthy at birth, low T-cell numbers	Multispecialty follow-up
DiGeorge (partial)	Hispanic	NICU	26	$T^{low}B^{+}NK^{low}$	330	158	Tetralogy of Fallot	Multispecialty follow-up
DiGeorge (partial)	Hispanic	NICU	22	$T^{low}B^{+}NK^{+}$	1088	631	Thirty-five-week gestation, truncus arteriosus	Multispecialty follow-up
DiGeorge (partial)	White	NICU	3	Not done	NA	NA	Respiratory distress, cleft palate, heart disease, pulmonary and intracranial hemorrhage	Died on day 6
Trisomy 21 (14q,21) translocation	Hispanic	NICU	19	$T^{low}B^{+}NK^{+}$	858	420	Thirty-three-week gestation, cardiac abnormalities	Died of cardiac complications
Trisomy 21	Asian	NICU	24	$T^{low}B^{low}NK^{+}$	1215	595	Thirty-three-week gestation, hydrops, transient myeloproliferative disorder	Exchange transfusions, T-cell numbers improved
Trisomy 21	Black	Regular	25	$T^{low}B^{+}NK^{+}$	1476	753	Oral thrush	Remains T lymphopenic, no live vaccines
Trisomy 21	Hispanic	NICU	8	Not done	NA	NA	Cardiac atrioventricular canal defect, hydrops	Died at 1 mo

(Continued)

TABLE E2. *(Continued)*

Diagnosis	Ethnicity	Nursery	TREC copies/μL	Lymphocyte phenotype*	CD3 T cells	CD4 CD45RA T cells	Clinical information	Outcome
Ataxia telangiectasia	Asian	Regular	8	$T^{low}B^{-}NK^{+}$	996	456	Healthy at birth, *ATM* mutation found by using whole-exome sequencing	Increased α-fetoprotein levels after 6 mo, absent ATM protein; truncal ataxia at 20 mo
Ataxia telangiectasia	Black/ Hispanic	Regular	21	$T^{low}B^{+}NK^{+}$	1161	418	Healthy at birth, *ATM* mutation found by using whole-exome sequencing	Increased α-fetoprotein levels after 6 mo, absent ATM protein
CHARGE syndrome	Black/ Hispanic	NICU	12	Not done	NA	NA	Multiple cardiac abnormalities	Died at 1 mo
Conditions (other than preterm birth alone) with secondary TCL								
Hypoplastic left heart	Black	NICU	4	$T^{low}B^{-}NK^{+}$	803	450	Second twin, heart surgery, multidrug-resistant pneumonia	Slow T-cell recovery
Hypoplastic left heart	White	NICU	16	$T^{low}B^{+}NK^{+}$	1220	878	Heart surgery, chylothorax	TCL resolved
Congenital heart defects	Asian	NICU	21	$T^{low}B^{low}NK^{low}$	988	514	Thirty-one-week gestation, truncus arteriosus, right-sided aortic arch, hypothyroidism	Died at 3 mo
Congenital heart defects	Hispanic	NICU	2	$T^{low}B^{low}NK^{-}$	553	332	Twenty-seven-week gestation, patent ductus arteriosus, chronic lung disease, hypertrophic cardiomyopathy	Died at 2 mo
Hypoplastic left heart	Black	NICU	20	Not done	NA	NA	Thirty-six-week gestation, failed catheter repair, retroperitoneal hemorrhage, renal failure	Died on day 13
Congenital heart defects	Asian/ Hispanic	NICU	0	Not done	NA	NA	Double-outlet right ventricle, ventriculoseptal defect, necrotizing enterocolitis	Died on day 4
Ileal atresia	White	NICU	0	$T^{low}B^{low}NK^{low}$	576	340	Thirty-one-week gestation, fetal anemia, meconium peritonitis	Surgical repair, TCL resolved
Gastroschisis	Hispanic	NICU	23	$T^{low}B^{+}NK^{-}$	789	437	Thirty-three-week gestation, bowel obstruction	TCL resolved
Gastroschisis	White	NICU	25	$T^{low}B^{+}NK^{+}$	660	420	Thirty-six-week gestation	Postoperative *Staphylococcus hominis* sepsis, TCL resolving
Hydrops	Asian	NICU	8	$T^{low}B^{low}NK^{+}$	1050	567	Thirty-one-week gestation, hydrops fetalis, bilateral pleural effusions, anemia, thrombocytopenia	Lymphocyte count improved at 4 mo
Hydrops	Hispanic	NICU	24	Not done	NA	NA	Thirty-five-week gestation, hydrops, ascites, cardiac myopathy	Died on day 6
Multiple congenital anomalies	White	NICU	22	$T^{low}B^{+}NK^{low}$	1008	393	Thirty-three-week gestation, multiple abnormalities, hypothyroidism	TCL resolved
Multiple congenital anomalies	Other	NICU	5	Not done	NA	NA	Intrauterine growth retardation, renal failure, brain hemorrhage, pulmonary edema, anomalies	Died at 2.5 mo

(Continued)

TABLE E2. *(Continued)*

Diagnosis	Ethnicity	Nursery	TREC copies/μL	Lymphocyte phenotype*	CD3 T cells	CD4 CD45RA T cells	Clinical information	Outcome
Chylothorax	Native American	NICU	0	Not done	NA	NA	Thirty-week gestation, congenital chylothorax with respiratory complications, neutropenic	Received immunoglobulin but died on day 7
Preterm births causing TCL								
Preterm	Hispanic	NICU	6	$T^{low}B^{+}NK^{low}$	876	377	Twenty-four-week gestation, BW of 700 g	TCL resolved
Preterm	Hispanic	NICU	11	$T^{low}B^{low}NK^{low}$	360	169	Twenty-four-week gestation, BW of 490 g	TCL resolved
Preterm	Hispanic	NICU	15	$T^{low}B^{+}NK^{+}$	1210	527	Preterm, BW of 695 g	Unable to follow-up
Preterm	Other	NICU	15	$T^{low}B^{low}NK^{low}$	424	187	Twenty-five-week gestation, BW of 445 g	TCL resolved
Preterm	Hispanic	NICU	28	$T^{low}B^{-}NK^{+}$	1460	599	Twenty-six-week gestation, BW of 300 g	TCL resolved
Preterm	White	NICU	19	$T^{low}B^{low}NK^{+}$	681	293	Twenty-seven-week gestation, BW of 557 g	TCL resolved
Preterm	Black	NICU	10	$T^{low}B^{+}NK^{low}$	1484	876	Twenty-seven-week gestation, BW of 1207 g	Unable to follow-up
Preterm	Hispanic	NICU	9	$T^{low}B^{+}NK^{low}$	1140	353	Twenty-four-week gestation, BW of 560 g	TCL resolved

BW, Birth weight; *NA*, not applicable.
*Included are 8 infants with positive or incomplete TREC results who died before flow cytometry and whose primary diagnosis included a condition associated with low T-cell numbers.

TABLE E3. Birth prevalence of SCID and all cases of TCL in California: comparison with diseases in the Recommended Uniform Screening Panel

ACMG code	Disease	No. of cases*	Cases per 10,000 births	Notes
HEAR	Hearing loss	832 in 1 y†	15	
CH	Congenital hypothyroidism (includes transient and variant)	1390	5.14	Salt wasting: 112 cases; virilizing: 23 cases
CF	Cystic fibrosis	418	1.54	
Hb SS	Sickle cell anemia	248	0.917	
CAH	Congenital adrenal hyperplasia (all forms)	158	0.584	
GALT	Galactosemia (classical/Duarte)	156	0.577	Classical: 29 cases; Duarte: 127 cases
Hb S/C	Hb S/C disease	144	0.532	
TCL	TCL (<1500 T cells/μL and/or absent naive T cells)	50 in 2 y‡	0.503	SCID and non-SCID, including preterm alone (incidence 1/19,900)
MCAD	Medium-chain acyl-CoA dehydrogenase deficiency	126	0.466	
BIOT	Biotinidase deficiency (partial/profound)	84	0.310	Partial: 44 cases; Profound: 39 cases
Hb S/βTh	HbS/β-thalassemia (β^+/β^0)	72	0.266	β^+: 59 cases; β^0: 13 cases
PKU	Phenylketonuria	69	0.255	
3-MCC	3-Methylcrotonyl-CoA carboxylase deficiency	67	0.248	
VLCAD	Very long-chain acyl-CoA dehydrogenase deficiency	41	0.152	
MUT	Methylmalonic academia (mutase deficiency) (mut^-/mut^0)	39	0.144	mut^-: 20 cases; mut^0: 19 cases
SCID disorders	Requiring curative treatment by HCT, enzyme replacement (adenosine deaminase deficiency), gene therapy, or thymus transplantation	15 in 2 y‡	0.133	Typical and leaky SCID, Omenn syndrome, complete DiGeorge syndrome (incidence 1/66,250 births)
CUD	Carnitine uptake defect	34	0.126	
GA 1	Glutaric acidemia type I	32	0.118	
IVA	Isovaleric acidemia	21	0.078	
MSUD	Maple syrup (urine) disease	21	0.078	
CIT	Citrullinemia, type I	9	0.033	
LCHAD	Long-chain L-3-OH acyl-CoA dehydrogenase deficiency	6	0.022	
ASA	Arginosuccinate acidemia	6	0.022	
TYR I	Tyrosinemia type I	6	0.022	
PROP	Propionic acidemia	5	0.018	
MCD	Multiple carboxylase deficiency	3	0.011	
βKT	β-Ketothiolase deficiency	2	0.007	
HMG	3-Hydroxy 3-methyl glutaric aciduria	1	0.004	
TFP	Trifunctional protein deficiency	1	0.004	
HCY	Homocystinuria	1	0.004	
Cbl A,B	Methylmalonic acidemia (Cbl A,B)	1	0.004	

*Cumulative data from 5 years of the California Newborn Screening Program, except as noted within the table.
†Data from the 2008 Newborn Hearing Screening Program for 1 year.
‡Data from 2010-2012, 2 years of SCID Newborn Screening.