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## Newborn Screening for SCID and T Cell Lymphopenia in California: Results of the First Two Years

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### Abstract

**Background**—Assay of T cell receptor excision circles (TRECs) in dried blood spots (DBS) obtained at birth permits population-based newborn screening (NBS) for severe combined immunodeficiency (SCID).

**Objective**—To report the first 2 years of TREC NBS in California.

**Methods**—Since August 2010, California has conducted SCID newborn screening. A high-throughput TREC qPCR assay using DNA isolated from routine DBS was developed. Samples with initial low TREC values had repeat DNA isolation with qPCR 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 (11), leaky SCID or Omenn syndrome (3), or complete DiGeorge syndrome (1). Survival to date in this group is 93%. Other T lymphopenic infants had variant SCID or combined immunodeficiency (6), genetic syndromes associated with T cell impairment (12), secondary T lymphopenia (9) or preterm birth (8). All T lymphopenic infants

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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 flow cytometry.

**Conclusions**—TREC NBS in California has achieved early diagnosis of SCID and other conditions with T lymphopenia, facilitating management and optimizing outcomes. Furthermore, NBS has revealed the incidence, causes and follow-up of T lymphopenia in a large, diverse population.

### Keywords

Severe combined immunodeficiency (SCID); newborn screening (NBS); T cell receptor excision circle (TREC); T cell lymphopenia (TCL); DiGeorge syndrome

## Introduction

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 (ADA) enzyme replacement, or gene therapy. Early detection of SCID is a key to successful treatment. In a retrospective series, infants diagnosed and treated with HCT in the first 3.5 months of life had improved survival and reduced morbidity.<sup>1</sup> However, most infants with SCID lack a family history or clinical clues to permit diagnosis prior to the onset of infections, making this life-threatening condition a candidate for population-based newborn screening (NBS).<sup>2–5</sup> With high burden on affected individuals, effective treatments, and availability of inexpensive high-throughput assay, SCID fits accepted criteria for population-based screening. The United States 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.<sup>6</sup> Several states throughout the US now perform SCID NBS.<sup>7–9</sup> 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 HIV-1 infection<sup>10–12</sup> and hematological malignancies.<sup>13</sup> Adaptation for dried blood spotted onto filter paper made possible a TREC assay for SCID and other conditions with T cell lymphopenia (TCL),<sup>8,14</sup> utilizing the dried blood spots (DBS) already universally obtained by 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 a quantitative polymerase chain reaction (qPCR) is a biomarker for thymic output of naïve T cells.<sup>14,15</sup> Low TRECs thus identify infants with SCID as well as other non-SCID immunodeficiencies in which there is a profound decrease in circulating naïve T cells.

Wisconsin started SCID NBS in 2008; that state's findings, including 5 cases of SCID in >200,000 screened infants, have been published.<sup>9,16</sup> Massachusetts has also instituted SCID NBS and reported cases.<sup>17</sup> 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 T cell lymphopenia, 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, DBS were sent to the California Department of Public Health (CDPH) Genetic Disease Laboratory (GDL) (Richmond, CA) for TREC testing and archiving. An initial pilot was conducted under a waiver of review from the CDPH 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 non-normal results were usually reported when infants were 3–4 weeks of age. Follow-up complete and differential blood counts (CBC) and lymphocyte subset analyses were performed at a single site (Quest Diagnostics, Inc., San Juan Capistrano, CA) as a confirmatory test within the SCID NBS program. Lymphocyte phenotypes were interpreted by CDPH Immunology Consultants (JAC, SAM and JMP).

For details of DNA extraction and determination of TREC and control  $\beta$ -Actin gene copy number see the JACI Online Repository.

### 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 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; also see Figure E1 in the Online Repository). Numbers with each screening result are in Fig. 1A, while proportions from regular nurseries vs. neonatal intensive care units (NICUs) are in Fig. 1B. Only 879 infants (<0.1% of those screened) had an initial TREC value below the acceptable cutoff, with a disproportionate contribution of 85% from NICUs. Urgent positive samples, those with undetectable or 1–5 TRECs/ $\mu$ L of blood with normal control  $\beta$ -Actin copies, triggered immediate recall of 34 infants for flow cytometry, also indicated for 39 more infants with initial positive samples (6–25 TREC copies, normal  $\beta$ -Actin).

A second DBS was requested from 806 infants (0.08% of all screened) because of initial samples with low TRECs, but also low  $\beta$ -Actin (designated “DNA amplification failure”), or low TRECs in NICU infants (designated “incomplete”, Fig. 1A, 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 non-normal samples, NICU samples predominated, accounting for 90% of requests for a second DBS (Fig. 1B). Only 11% of second samples, obtained when infants were between 3–4 weeks of age, were persistently abnormal. Infants with 2 DBS samples with low TRECs, irrespective of  $\beta$ -Actin copy number, were recalled for venous blood immunophenotyping (Fig. 1A, Fig. E1).

Thirteen infants had flow cytometry following only a single abnormal result due to clinical suspicion of an immune disorder. Eleven NICU infants with initial positive and 3 with repeated low TREC results died before flow cytometry could be performed, while one was lost to follow-up after 2 low TREC results; 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 flow cytometry represented 1/6,200 births, with 106 (66%) from NICUs.

### Lymphocyte profiles

Confirmatory CBC and lymphocyte subset analysis were an integral part of the SCID NBS program. Venous blood was obtained at a mean of 3–4 weeks after the initial DBS. TRECs increase during early postnatal life;<sup>14,16</sup> 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 TRECs. Although the normal range for newborns was 2,550–5,000 CD3 T cells/ $\mu$ L, we focused on those with either TCL of likely clinical significance, defined as <1,500 CD3 T cells/ $\mu$ L, or absence or marked reduction in CD4 naïve T cells (CD4/CD45RA), defined as <5% total CD3 T cells. Of infants classified “positive” by TREC test, 44% were abnormal by flow cytometry, as were 23% with “incomplete” TREC tests (Table I). False positives, samples that were “positive” or “incomplete” by TREC test but subsequently normal by flow cytometry (111 samples) made up only 0.01% of all the samples tested. Abnormalities in neutrophils, B cells and NK cells were noted when present.

### Categories of diagnosis among infants with T lymphopenia

Of 161 liquid blood samples, 50 (31%) had TCL (T cells <1,500/ $\mu$ L), or 1/19,900 births. These infants had a spectrum of diagnoses (Table II, Table E2 in Online Repository). Typical SCID, leaky SCID and variant SCID had a combined incidence of 1 in 49,700 births, with individual incidences of 1/90,000, 1/331,000 and 1/166,000, respectively. Categories of severity were established reflecting the degree of lymphopenia and the extent of immunological intervention required (Fig. 2). As shown, all infants with TCL received immunological follow-up and instruction to avoid infectious exposures and live vaccines and to exercise transfusion precautions. Infants with multi-system congenital syndromes accompanied by TCL, as well as those with combined immunodeficiency (CID) 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 one with complete DiGeorge syndrome. Diagnostic criteria of the Primary Immune Deficiency Treatment Consortium (PIDTC) were used for typical SCID, <300 autologous T cells/ $\mu$ L, often with maternally derived T cells, with in vitro responses to phytohemagglutinin (PHA) <10% of control; and leaky SCID/Omenn syndrome, no maternal cells, but autologous, oligoclonal, CD45RO memory phenotype T cells with PHA responses that could be >10%, but still below normal. In both typical and leaky SCID, specific gene mutations provided supporting evidence. Infants suspected to have 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, Table II). Importantly, only one infant with typical SCID had a recognized family history that had led to prenatal diagnosis with immediate confirmation at birth (CA007, Table II); one other infant, CA027, had lost an older brother

in infancy, whose retrospective diagnosis of SCID was recognized only after newborn screening identified our patient.

### Specific diagnoses and treatment of infants identified by NBS

Table II summarizes information for the 15 infants who required immune restoring therapies. Within the 11 typical SCID diagnoses, 10 had undetectable TRECs, while one had 4 TRECs and extensive maternal engraftment with 1,736 T cells, but no naïve CD4/CD45RA T cells. Four males with a T<sup>-</sup>B<sup>+</sup>NK<sup>-</sup> phenotype had defects in the X-linked *IL2RG* gene encoding the cytokine receptor common  $\gamma$  chain. CA007 had a previously reported recurrent mutation (R226C),<sup>18</sup> while previously unpublished mutations were found in CA053, CA096 and CA125, a frameshift (M270fs), nonsense (W177X), and predicted damaging missense mutation (L132R), respectively. CA103 with T<sup>-</sup>B<sup>+</sup>NK<sup>-</sup> SCID had an in-frame *JAK3* deletion (L527\_G528del) previously noted in an unrelated case (J. Puck, unpublished). Three females with T<sup>-</sup>B<sup>+</sup>NK<sup>+</sup> SCID had homozygous or compound heterozygous mutations in *IL7R* encoding the IL-7 receptor  $\alpha$  chain, including a frameshift (I94fs), and 3 novel missense mutations. Two males had *RAG1* defects, one previously reported damaging (R404Q),<sup>19</sup> one unreported but predicted damaging (R764P), and one novel homozygous deletion of a single codon (M661del). Infant CA177 had T<sup>-</sup>B<sup>+</sup>NK<sup>+</sup> SCID with no molecular diagnosis.

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

In addition, female 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 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, with *RAG1* SCID, died 46 days post transplant of 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 IL-7R $\alpha$  SCID and 1 with *RAG1*-deficient Omenn syndrome required booster infusions of donor cells, while infant CA125 did not engraft autologous gene-corrected HCT and required subsequent conditioning and an allogeneic cord blood transplant. While some of the patients are still early post-transplant 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 in Table E2. Six had variant SCID/combined immunodeficiency (CID), defined as TCL with functional T cell impairment for >3 months, without syndromic features or defects in a known SCID gene. Four had positive and 2 had incomplete TREC tests; their other immunologic features and treatments are listed. This category was unanticipated prior to 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 (with low but detectable T cells, in contrast to the complete DiGeorge patient discussed above). While 4 were recognized immediately after birth by their congenital cardiac malformations, one was diagnosed only after SCID NBS revealed his TCL. In addition, 4 infants had TCL associated with trisomy 21 and one had CHARGE (ocular coloboma, heart anomaly, choanal atresia, retardation, genital and ear anomalies) syndrome. While DiGeorge and CHARGE syndromes and trisomy 21 are often identified by their characteristic facies and clinical features, non-immunologists may fail to appreciate the degree of TCL immunodeficiency that may be present; thus NBS has identified the subset of infants with these conditions who were particularly affected by TCL and thus at risk for infections. Three of these infants died of complications not associated with the immune system prior to having lymphocyte enumeration.

NBS also flagged 2 apparently healthy infants with ataxia telangiectasia (AT) and newborn TCL, who were initially considered to have variant SCID.<sup>24</sup> A study of newborn DBS archived from California cases of AT showed that >50% would have been identified by NBS with the TREC test as currently performed here.<sup>24</sup> 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 Online Table E2 for further details.

A final group of 8 infants with TCL were born at 24–27 weeks' gestation with birth weights of 300–1200g (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 T lymphopenias in California

TREC NBS data combined with self-reported ethnic origins permitted estimates of incidence of SCID and TCL for California's ethnically diverse population. Using the pre-NBS SCID incidence estimate of 1/100,000 from the literature,<sup>14</sup> California Hispanic and Asian populations had higher than expected numbers of SCID diagnoses (including typical, leaky and variant SCID), with respect to proportion of total births ( $p < 0.001$  and  $p < 0.05$  for Hispanics and Asians, respectively) (Table III). We also considered the incidence of non-SCID TCL secondary to clinical syndromes, congenital malformations or other non-immune 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 than expected, with respect to proportion of total births ( $p < 0.01$ ,  $p < 0.002$ , and  $p < 0.001$ , respectively) (Table III).

### Discussion

Newborn screening programs present a number of ethical and public health questions. Justifications offered in support of newborn screening include early diagnosis, improved treatments, and lives saved. Decisions to institute a new NBS test are tied to 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 vs. 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, widely adopted by the states.<sup>25</sup> SCID was the first disease to be added to this federal uniform panel by evidence-based review and cost effectiveness analyses.<sup>6,26</sup> At this time 10 states, Puerto Rico 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, NBSTRN, 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.

### 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  $<1,500$  autologous T cells/ $\mu\text{L}$  as a secondary target. The 2-year cohort included 50 infants with SCID and TCL, or 0.5/10,000 births. When compared to data for the 29 other core NBS conditions in California, all TCL disorders would have placed 8<sup>th</sup> in frequency (Table E3). Furthermore, the disorders that require urgent immune restorative therapies (SCID, leaky SCID, Omenn syndrome and complete DiGeorge syndrome) would have ranked 15<sup>th</sup> at 0.13/10,000 births (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;  $<0.1\%$  of infants screened were non-normal, 0.08% with incomplete results needing a repeat TREC test, and 0.016% needing venous blood for immunophenotyping. Positive predictive value was high, with 31% of flow cytometry tests confirming  $<1,500$  T cells/ $\mu\text{L}$ . Infants in NICUs constituted 9% of total California births, but had disproportionately frequent non-normal TREC results (Fig. 1B). The preponderance of non-normal first tests in NICU infants has been noted by other states using the TREC test,<sup>16,27,28</sup> 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 non-normal 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  $<1,500$  T cells/ $\mu\text{L}$  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 flow cytometry, 76% were cared for in NICUs, 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 emergent immune restorative treatment. However, only one infant with typical SCID in our series had a recognized positive family history (Table II), while 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, 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 PIDTC protocols, our patients' outcomes will help shape future clinical trials to determine optimal treatments for SCID diagnosed by newborn screening. 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 (GvHD) in the infant with X-linked SCID and an affected brother, to a female infant who received conditioning and GvHD prophylaxis for a matched unrelated donor bone marrow transplant at 7 months of age for leaky SCID secondary to cartilage hair hypoplasia. One infant received experimental gene therapy for X-linked SCID due to an *IL2RG* mutation, though he required a subsequent cord blood transplant due to 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 NBS has been 93%, comparable to retrospective studies reporting increased survival of SCID infants detected at birth due to a prior positive family history.<sup>3-5</sup> Although the Duke University retrospective data for SCID HCT indicated best survival for infants transplanted before the age of 3.5 months,<sup>7</sup> our infants identified by newborn screening have excellent survival at older transplant ages because of prompt institution of measures to avoid infections.

### Advantages of diagnosis of T cell lymphopenias

TREC newborn screening revealed a wide spectrum of non-SCID disorders with TCL as a neonatal feature (Fig. 2). The California cutoff values of <40 TRECs/ $\mu$ L for having the qPCR re-run, and 25 TRECs/ $\mu$ L on a repeat determination with normal  $\beta$ -Actin were selected by testing known positive cases and anonymous DBS from different nursery settings. Similarly, 1,500 T cells/ $\mu$ L was below the reference lower limit of 2,550 T cells/ $\mu$ L for term healthy newborns, but 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 lymphopenic end of the spectrum of syndromic disorders to be identified, whether they had underlying DiGeorge syndrome or an unanticipated number of other syndromes including trisomy 21, ataxia telangiectasia,<sup>24</sup> cartilage hair hypoplasia, or CHARGE syndrome. Also unexpected were the congenital defects (cardiac and gastrointestinal malformations, hydrops, chylothorax) that resulted in T cell lymphopenias, though in these cases the T cell numbers improved when the defects were repaired, suggesting either suppression of lymphopoiesis due to 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 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 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 may 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 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, TRECs 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,<sup>29</sup> 2 of Wiskott Aldrich syndrome, and 1 of delayed-onset adenosine deaminase (ADA) SCID were not detected by TREC screening because their newborn TREC numbers were normal. In addition, defects limited to humoral immunity or neutrophils are not detected. Testing for insufficient TRECs, a biomarker of numerically adequate naïve T cells, will detect only SCID diseases with impaired T cell production and secondary conditions that drain naïve T cells from peripheral blood. Importantly, a negative TREC test does not rule out the possibility of primary immunodeficiency. While 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,<sup>30–35</sup> with autosomal recessive SCID being more prevalent in cultures in which consanguineous marriage is common.<sup>36</sup> As 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 may have a lower than average population incidence of SCID caused by autosomal recessive mutations, as they are unlikely to harbor common founder alleles. In contrast, X-linked SCID incidence should be independent of ethnicity due to 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, prospective manner. TREC NBS in California combined with retrospective analysis of newborn dried blood spots from patients with ataxia telangiectasia led to a new incidence estimate of one case per 200,000 births.<sup>24</sup> Similarly, using published incidence estimates of around 1/5,000 births for chromosome 22q11 deletion, the most frequent human interstitial deletion syndrome,<sup>37</sup> 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 <1,500 T cells/ $\mu$ L. While our TCL cutoff is somewhat arbitrary, this is the first unbiased estimate of T lymphopenia 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 newborn screening 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 flow cytometry add to available experience to date. Furthermore, our large population gives the new, 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 SCID and TCL infants 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 SCID and TCL patients, in order to tailor appropriate genotype-specific treatment strategies to optimize their treatment 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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## Abbreviations

<b>ADA</b>	adenosine deaminase
<b>AT</b>	ataxia telangiectasia
<b>CDPH GDL</b>	California Department of Public Health Genetic Disease Laboratory
<b>CHARGE</b>	coloboma (ocular), heart anomaly, atresia (choanal), retardation, genital and ear anomalies
<b>CHH</b>	cartilage hair hypoplasia
<b>CID</b>	combined immunodeficiency
<b>CMV</b>	cytomegalovirus
<b>DAF</b>	DNA amplification failure
<b>DBS</b>	dried blood spot
<b>HCT</b>	hematopoietic cell transplant
<b>NBS</b>	newborn screening
<b>NBSTRN</b>	Newborn Screening Translational Research Network
<b>NICU</b>	neonatal intensive care unit
<b>PCR</b>	polymerase chain reaction
<b>qPCR</b>	quantitative PCR
<b>PHA</b>	phytohemagglutinin
<b>PIDTC</b>	Primary Immune Deficiency Treatment Consortium
<b>TCL</b>	T cell lymphopenia
<b>TREC</b>	T cell receptor excision circle
<b>SCID</b>	severe combined immunodeficiency

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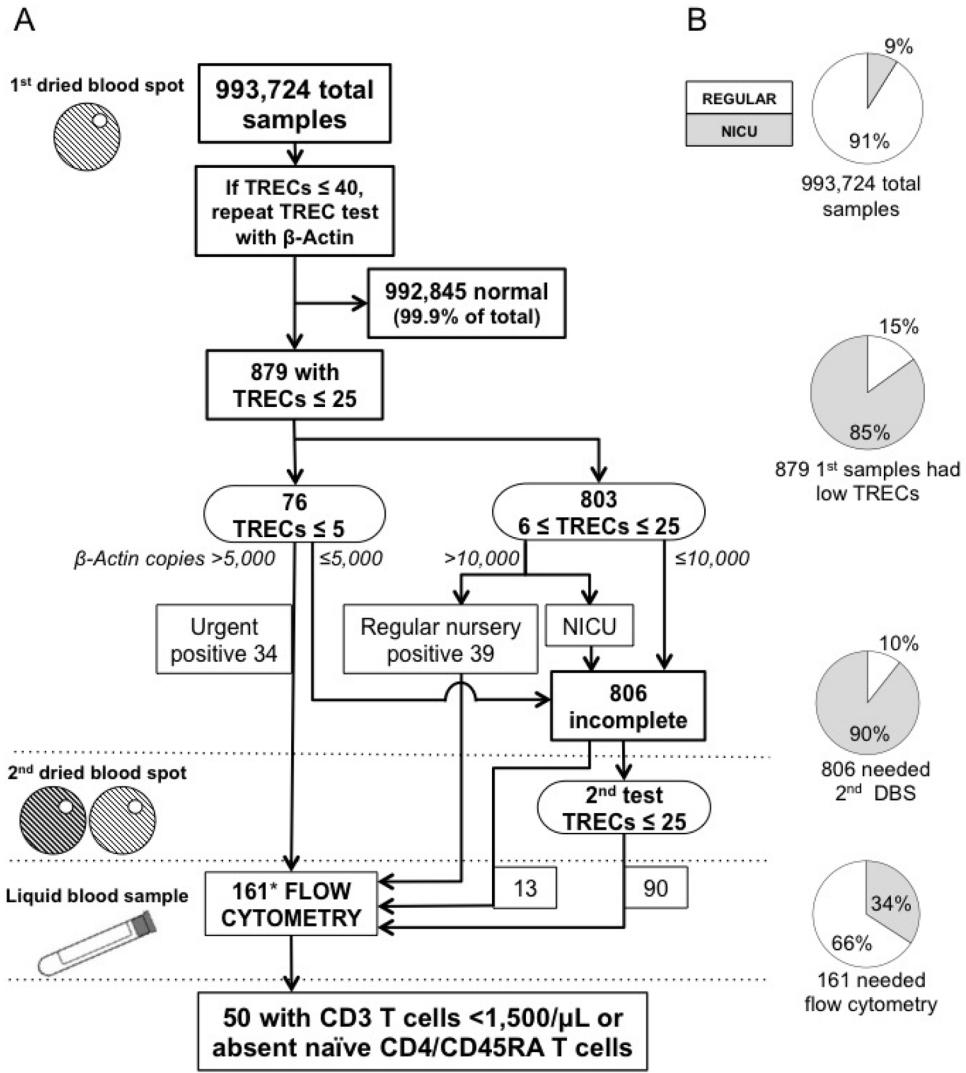
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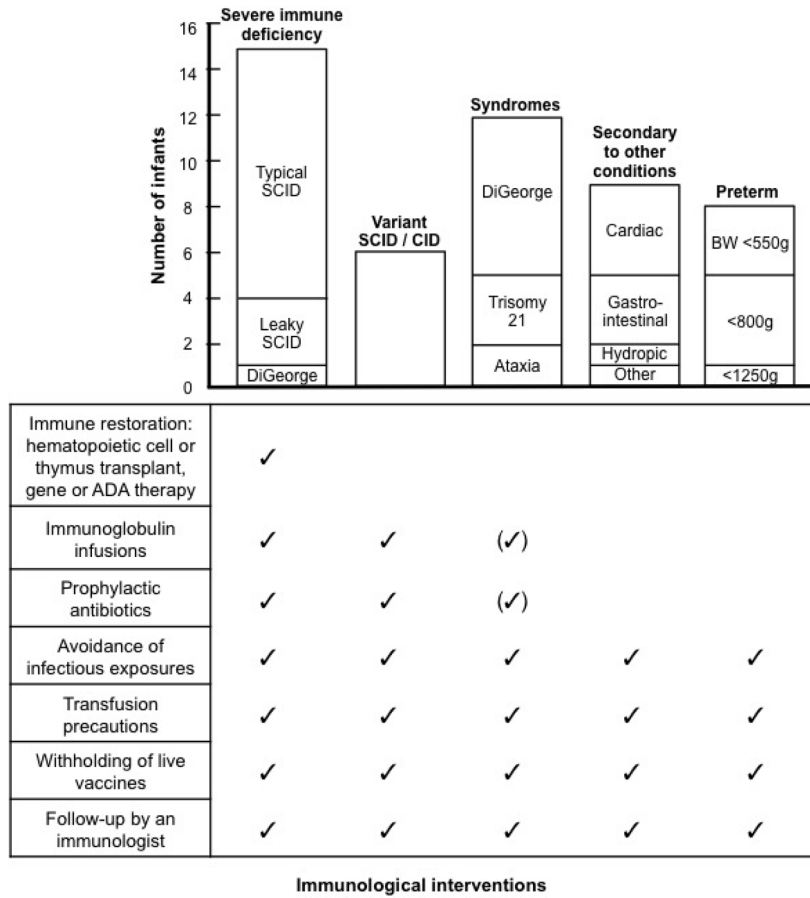
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### **Clinical Implications**

TREC newborn screening 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.

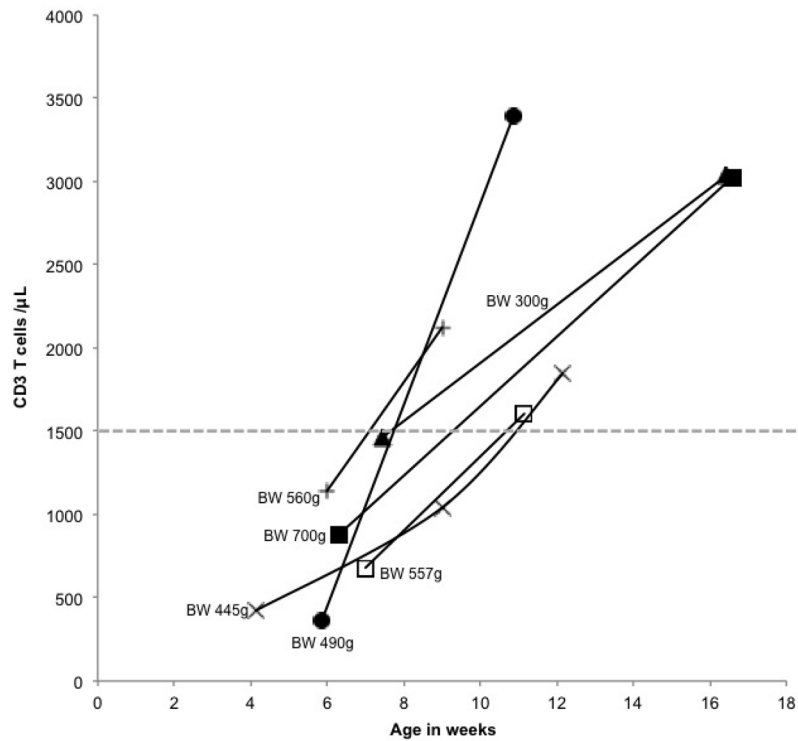


**Figure 1.** A) California SCID NBS. Sample numbers are shown at each stage (boxes) according to TREC and  $\beta$ -Actin and nursery care as proxy for clinical status. [Also see Online Repository Fig. E1]. Infants with low TRECs on 2 DBS or positive initial results were recalled to receive flow cytometry. B) Distribution of regular nursery vs. neonatal intensive care unit (NICU) samples at each NBS stage.  
 \*14 infants deceased, 1 lost to follow-up before flow cytometry.



**Figure 2.** T cell lymphopenia (TCL) by intervention and underlying condition. Cases requiring immune restoring therapy included 11 typical and 3 leaky SCID and 1 complete DiGeorge syndrome. There were 6 variant SCID, 12 congenital syndromes, 9 secondary TCL, and 8 preterm infants (weight ranges shown). All were advised to avoid live rotavirus and other live vaccines and to receive only CMV negative, irradiated blood products.





**Figure 3.** T cell concentrations in infants with initial T cell lymphopenia associated with preterm birth, obtained at gestational ages on the X-axis. Birth weights are indicated. Subsequent T cell concentrations were  $>1,500$  cells/ $\mu\text{L}$ , dotted line, the threshold set by California NBS program.

Non-normal TREC results and their associated flow cytometry results during the first 2 years of SCID NBS in California.

**Table 1**

Dried blood spot result	Liquid blood lymphocyte profile		Total
	Indicative of a T cell defect (T cells <1,500/ $\mu$ L or absent naïve CD4/CD45RA cells)	Not indicative of a significant T cell defect (T cells 1,500/ $\mu$ L; naïve T cells present)	
Positive	27 (includes all SCID cases)	35	62 <sup>a</sup>
Incomplete	23	76	99 <sup>b</sup>
<b>Total</b>	<b>50</b>	<b>111</b>	<b>161</b>

<sup>a</sup> 11 infants died prior to flow cytometry, out of 73 infants initially identified as positive by TREC test (Fig. 1)

<sup>b</sup> 90 infants had persistent low TRECs; 3 died and 1 was lost to follow up prior to flow cytometry confirmation. Also included are 13 infants who proceeded to flow cytometry after only a single abnormal TREC test result, due to logistical reasons or physician requests (Fig. 1).

**Table II**

Infants with low TRECs and T cell lymphopenia who required immune restoring therapy.

SCID case #	Sex	Ethnicity	Nursery <sup>a</sup>	TREC copies	Lymphocyte phenotype	CD3 T cells	CD4 CD45RA T cells	Disease causing gene (mutations) <sup>b</sup>	Presenting clinical information <sup>c</sup>
CA007	M	Black	NICU	0/μL	T <sup>-</sup> B <sup>+</sup> NK <sup>-</sup>	493/μL	<20/μL	<i>IL2RG</i> (R226C <sup>18</sup> )	35 week gestation, prenatal diagnosis (older affected brother). Asymptomatic, infection-free; maternal engraftment present; PHA <10%.
CA053	M	Hispanic	Regular	0	T <sup>-</sup> B <sup>+</sup> NK <sup>-</sup>	<20	<20	<i>IL2RG</i> (M270fs)	Asymptomatic, infection-free, XY/XO mosaic; no maternal engraftment, PHA <10%.
CA096	M	Asian	NICU	0	T <sup>-</sup> B <sup>+</sup> NK <sup>-</sup>	20	<20	<i>IL2RG</i> (W177X)	1 of triplets, 33 week gestation, asymptomatic, infection-free; maternal engraftment present; PHA <10%.
CA125	M	Hispanic	Regular	0	T <sup>-</sup> B <sup>+</sup> NK <sup>-</sup>	78	23	<i>IL2RG</i> (L132R)	Asymptomatic, infection-free; maternal engraftment present; PHA <10%.
CA103	F	Hispanic	Regular	4	T <sup>-</sup> B <sup>+</sup> NK <sup>-</sup>	1736	<20	<i>JAK3</i> (L527_G528del)	Rash and cellulitis; maternal engraftment present; PHA not tested.
CA004	F	Hispanic	Regular	0	T <sup>-</sup> B <sup>+</sup> NK <sup>+</sup>	60	49	<i>IL7R</i> (F71S, H180P)	Asymptomatic, infection-free; maternal engraftment present; PHA <10%.
CA005	F	Hispanic	Regular	0	T <sup>-</sup> B <sup>+</sup> NK <sup>+</sup>	<20	<20	<i>IL7R</i> (I94fs, H180P)	Asymptomatic, infection-free, but acquired coxsackie virus at home pre-HCT; maternal engraftment present, PHA <10%.
CA067	F	Hispanic	Regular	0	T <sup>-</sup> B <sup>+</sup> NK <sup>+</sup>	<20	<20	<i>IL7R</i> (G215V)	Asymptomatic, infection-free; maternal engraftment present, PHA <10%.
CA013	M	Hispanic	Regular	0	T <sup>-</sup> B <sup>+</sup> NK <sup>+</sup>	<20	<20	<i>RAG1</i> (M661del)	Asymptomatic, infection-free, maternal engraftment not tested; PHA not tested.
CA027	M	Hispanic	Regular	0	T <sup>-</sup> B <sup>+</sup> NK <sup>+</sup>	28	<20	<i>RAG1</i> (R404Q <sup>19</sup> R764P)	Rash coinciding with TREC report, deceased affected brother, infection-free; maternal engraftment present; PHA not tested.
CA177	M	Hispanic	NICU	0	T <sup>-</sup> B <sup>+</sup> NK <sup>+</sup>	80	3	<i>unknown<sup>d</sup></i>	37 week gestation, maternal diabetes & hepatitis. Low glucose, respiratory distress, hepatomegaly, feeding difficulties; maternal engraftment present; PHA <10%.
CA024	F	White	Regular	20	T <sup>-</sup> B <sup>+</sup> NK <sup>+</sup>	1583	24	<i>RAG1</i> (W522C <sup>20</sup> , N968K)	Asymptomatic, infection-free; no maternal cells; PHA not done. Omenn syndrome.

SCID case #	Sex	Ethnicity	Nursery <sup>a</sup>	TREC copies	Lymphocyte phenotype	CD3 T cells	CD4 CD45RA T cells	Disease causing gene (mutations) <sup>b</sup>	Presenting clinical information <sup>c</sup>
CA174	F	Hispanic	Regular	0	T <sup>-</sup> B <sup>-</sup> NK <sup>low</sup>	51	3	<i>RAG1</i> (R474C <sup>21</sup> , 1956T <sup>22</sup> )	Asymptomatic, infection-free, but acquired rhinovirus at home pre-HCT; no maternal cells; PHA 50%. Leaky SCID.
CA158	F	White	Regular	19	T <sup>-</sup> B <sup>+</sup> NK <sup>+</sup>	380	99	<i>RMRP1</i> (r.211c>g, 262g>u) <sup>23</sup>	Intrauterine growth retardation, short limbs; no maternal cells; PHA 40%. Leaky SCID, cartilage hair hypoplasia.
CA019	F	Hispanic	NICU	0	T <sup>-</sup> B <sup>+</sup> NK <sup>+</sup>	<20	<20	22q11 deletion	Complete DiGeorge syndrome with congenital heart disease.

SCID case #	Treatment <sup>e</sup> / donor (match) / cell type	Age at HCT (s)	Conditioning <sup>f</sup>	Graft vs. host disease prophylaxis <sup>g</sup>	Clinical course <sup>h</sup>	Current status (age) <sup>i</sup>
CA007	HCT, CD34-selected maternal cryopreserved mobilized peripheral blood	3 wk	None	None	Acute skin graft vs. host disease (GvHD); T cell engraftment	Alive (28 mo), vitiligo, on IVIG
CA053	HCT, MUD (10/10) bone marrow	2.5 mo Boost: 4 mo	G-CSF, Plerixafor None	CsA CsA, MTX, tacrolimus	Slow T cell recovery, →boost T cell engraftment	Alive (21 mo), on IVIG
CA096	HCT, maternal CD34-selected peripheral blood	8 wk Boost: 4 mo	None None	None CsA, methylprednisolone	Slow T cell recovery, →boost T cell engraftment	Alive (15 mo), on scIG
CA125	Autologous transduced CD34 cell gene therapy (trial #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, Cytosan	Tacrolimus, MTX	T cell engraftment and B cell function	Alive (28 mo), off IVIG
CA067	HCT, maternal CD34-selected peripheral blood	1.5 mo Boost: 5 mo	None None	None None	Slow T cell recovery, →boost T cell engraftment	Alive (17 mo), on scIG
CA013	HCT, matched sibling bone marrow	3.5 mo 2 <sup>nd</sup> HCT: 9 mo	rATG x3 Flu, Bu, rATG	Tacrolimus Tacrolimus	Autologous recovery, →2 <sup>nd</sup> HCT T cell engraftment	Alive (27 mo), off IVIG

SCID case #	Treatment <sup>e</sup> / donor (match) / cell type	Age at HCT (s)	Conditioning <sup>f</sup>	Graft vs. host disease prophylaxis <sup>g</sup>	Clinical course <sup>h</sup>	Current status (age) <sup>i</sup>
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) post transplant
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	
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 transplant	Alive (26 mo), off CsA, on IVIG

<sup>a</sup>NICU: neonatal intensive care unit.

<sup>b</sup>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.

<sup>c</sup>Proliferative response in vitro to phytohemagglutinin, <10% of healthy control range is a criterion for diagnosis of typical SCID; 10–50% consistent with leaky SCID.

<sup>d</sup>No damaging variants found in *ADA*, *AK2*, *CD3D*, *CD3E*, *CD3E*, *DCLRE1C*, *IL2RG*, *IL7R*, *JAK3*, *LIG4*, *NHEH1*, *PNP*, *PTPRC*, *RAC2*, *RAG1*, *RAG2*, *RMRP*, *ZAP70*.

<sup>e</sup>HCT, hematopoietic cell transplant; MUD, matched unrelated donor.

<sup>f</sup>G-CSF, granulocyte colony stimulating factor; Flu, Fludarabine; Bu, Busulfan; rATG, rabbit anti-thymocyte globulin; KGF, keratinocyte growth factor.

<sup>g</sup>CsA, cyclosporine A; MTX, methotrexate; MMF, mycophenolate mofetil.

<sup>h</sup>No major infectious complications.

<sup>i</sup>IVIG, intravenous immunoglobulin; scIG, subcutaneous immunoglobulin.

Table III

Ethnic composition of infants with low TRECs and T cell lymphopenias.

Ethnicity	SCID, Leaky SCID, Omenn Syndrome, Complete DiGeorge, and Variant SCID		Other T cell lymphopenias: Syndromes with T cell impairment and Secondary T cell lymphopenia excluding preterm birth alone			
	Number observed	Number expected with incidence 1/100,000	<i>p</i> -value <sup>a</sup>	Number observed	Number expected with incidence 1/50,000	<i>p</i> -value <sup>a</sup>
Hispanic	13	4.85	<0.001	9	9.70	NS
White	4.5	2.52	NS	8	5.04	NS
Asian	2.5	0.81	<0.05	5	1.63	<0.01
Black	1	0.53	NS	6	1.06	<0.0002
Native American	0	0.09	NS	2	0.18	<0.001
Other	0	0.54	NS	1	1.08	NS
<b>Total</b>	21			29		

<sup>a</sup>NS, not significant.