Severe Combined Immunodeficiency (SCID) Screening in Arizona: Lessons Learned from the First 2 Years

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Abstract

Purpose The incidence of severe combined immunodeficiency (SCID) in the USA was reported as 1 in 58,000 live births. In Arizona, it was anticipated that newborn screening would identify two to four cases of SCID per year. This estimate did not consider ethnic nuances in Arizona, with higher percentages of Native American and Hispanic populations compared to national percentages. The true incidence of SCID and non-SCID T cell lymphopenia has not previously been reported in Arizona.

Methods A retrospective chart review was performed on all abnormal SCID newborn screening (NBS) tests in Arizona from January 1, 2018, to December 31, 2019, using data from the Arizona Department of Health Services and the Phoenix Children’s Hospital’s electronic medical record [IRB# 20–025].

Results Seven infants were diagnosed with SCID, yielding an incidence of 1 in 22,819 live births. Four of these infants had Artemis-type SCID. Thirteen infants were identified with an abnormal initial NBS which ultimately did not lead to a diagnosis of SCID. Four of these infants were diagnosed with congenital syndromes associated with T cell lymphopenia. Infants of Hispanic ethnicity were over-represented in this cohort.

Conclusion Over 2 years, NBS in Arizona confirmed an incidence more than 2.5 times that reported nationally. This increased incidence is likely reflective of Arizona’s unique population profile, with a higher percentage of Native American population. The findings in our non-SCID cohort are in alignment with previously published data, except for an increased percentage of infants of Hispanic/Latino ethnicity, possibly reflecting Arizona’s increased percentage of Hispanic/Latino population compared to the general US population.

Keywords Severe combined immunodeficiency · Newborn screening · Founder effect · Arizona · Lymphopenia

Abbreviations SCID · Newborn screening · Founder effect · Arizona · Lymphopenia

TCRs · T cell receptors
TREC · T cell receptor excision circles
PCH · Phoenix Children’s Hospital
CMA · Chromosomal microarray
CBC · Complete blood count
LSP · Lymphocyte subset panel
TCL · T cell lymphopenia
PID · Primary immunodeficiency

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BPD  Bronchopulmonary dysplasia
VUS  Variant of uncertain significance
RUSP Recommended Uniform Screening Panel
CMV  Cytomegalovirus
MSD  Matched sibling donor
MUD  Matched unrelated donor
GVHD  Graft-versus-host disease

Introduction

Severe combined immunodeficiency (SCID) comprises more than 20 identified rare genetic disorders that uniformly result in profound immunodeficiency secondary to absent or very low numbers of T lymphocytes, with or without absent or minimal numbers of B lymphocytes. SCID results in susceptibility to life-threatening infections and was synonymous with death within the first 2 years of life until the advent of hematopoietic stem cell transplantation (HCT). [1] Enzyme replacement therapy became available for adenosine deaminase (ADA)–deficient SCID patients in the mid-1980s [2]. Most recently, gene therapy has proven efficacious and been added to our treatment armory for X-linked, ADA-deficient, and Artemis-deficient SCID [1, 3]. Recognition that most SCID cases occur in families without any family history and that earlier treatment, before infectious complications have occurred, is associated with better outcomes has resulted in newborn screening (NBS) as a tool to aid in timely diagnosis [2, 4, 5]. NBS utilizes T cell excision circle (TREC) copy number as a surrogate marker of thymic production of naïve T cells [6].

The Advisory Committee on Heritable Disorders in Newborns and Children recommended screening for SCID as part of the Recommended Uniform Screening Panel (RUSP) of newborn screened diseases in the USA in 2009 [7]. Arizona was one of the last states to incorporate the TREC assay into newborn screening in mid-2017. The incidence of SCID in the USA was most recently reported as 1 in 58,000 live births based on 11 ongoing NBS programs in the USA [8]. Based on a live birth rate in Arizona of 85,000 per year and SCID incidence rates from the early pilot states, SCID screening was estimated to identify between two and four cases per year [8, 9]. These estimates accounted for the known founder effect present in the Native American population in 2009 in the Native American population in DCLREIC, the gene encoding Artemis SCID [10, 11]. Indeed, implementation of SCID NBS on the Navajo Reservation confirmed an incidence of 1 in 2000 live births [12]. Furthermore, results from the first two years of newborn SCID screening in California had higher than expected numbers of SCID diagnoses with respect to the proportion of total births for Hispanic and Asian populations [13]. These ethnic nuances are of relevance in Arizona, which has higher percentages of Native American/Alaskan Native (5.3%) and Hispanic/Latino (31.7%) populations compared to the general population of the USA (1.3% and 18.5%, respectively) [14]. The effect of this unique population profile has not previously been described with respect to SCID screening.

We present the first natural history report of SCID NBS in Arizona and outcomes of screening 159,730 infants from January 1, 2018, until December 31, 2019, including detection of seven confirmed cases of SCID.

Methods

A retrospective chart review was performed on all Arizona’s abnormal SCID NBS tests from January 1, 2018, to December 31, 2019, using data from the Arizona Department of Health Services (AZDHS), Office of Newborn Screening, and the Phoenix Children’s Hospital (PCH) electronic medical record. We collected data with the intent to have 24 months of follow-up data for the included subjects. A positive NBS for SCID was defined as a TREC < 19 copies/µL, as per Arizona state guidelines. NBS performed on Navajo Reservations within Arizona were processed by a centralized Navajo laboratory in Oregon, and abnormal results were reported to AZDHS and Tuba City Regional Health Care. Navajo infants who were born off reservation had SCID NBS performed by AZDHS, same as infants not of Navajo descent.

Premature infants with an abnormal first NBS but then normalized when the infant reached term-adjusted gestational age and term infants with repeat normal NBS were excluded. Infants with critical abnormal TREC values (defined as < 6 copies/µL) were evaluated by immunology within 24 hours either inpatient or outpatient depending on the infants’ geographical location, social circumstances, and exposure risks. Non-critical TREC screens were evaluated outpatient typically within 72 hours unless the infant was hospitalized for another clinical reason. All infants undergoing evaluation had a complete blood count (CBC) with differential and lymphocyte subset panel (LSP) performed by flow cytometry. T cell lymphopenia (TCL) was defined as having absolute CD3+, CD3+CD4+, or CD3+CD8+ counts below the 10th percentile for age (< 2500 cells/µL, < 1600 cells/µL, and < 560 cells/µL, respectively) [15]. Infants with moderate or severe lymphopenia had naïve/memory T cell phenotyping performed as part of the LSP, markers used included CD4+CD45RA+, and CD4+CD45RO+. Second-tier testing included T cell proliferation and genetic testing for infants with moderate or severe lymphopenia. Genetic testing was not able to be performed for every infant with moderate to severe lymphopenia due to insurance rejection.

Breastfeeding discontinuation is medically recommended to families as soon as there are concerns for a positive NBS for SCID. During the evaluation with
immunology that follows, breastfeeding is resumed when the mothers’ CMV seronegative status is confirmed.

Our cohort is reflective of all Arizona births and positive screens as captured by the AZDHS. Based on geographic catchment areas, PCH is responsible for following up about 85% of all positive NBS for SCID, while the other 15% are followed up by another pediatric referral center in Tucson, Arizona. Our detailed analysis, therefore, was limited to patients followed by PCH. However, the AZDHS was able to provide basic data regarding the infants followed by the referral center in Tucson, Arizona. Data was collected and stored securely utilizing the REDCap database including demographics, confirmatory testing results such as genetic testing, resulting diagnoses, and pre- and post-definitive therapy characteristics for the confirmed SCID patients. The study was approved by the Institutional Review Board of Phoenix Children’s Hospital (IRB# 20–025).

Results

There were 80,423 births in 2018 and 79,307 births in 2019 in Arizona for a total of 159,730 over two years, as reported by the AZDHS. The breakdown of positive NBS for SCID in the state of Arizona is illustrated in Fig. 1. Sensitivity and specificity of Arizona’s TREC NBS were 100% and 99.99%, respectively, for detection of TCL. The positive predictive value and negative predictive values for Arizona’s TREC NBS was 81.5% and 100%, respectively. There were 5 false positive tests, resulting in a false positive rate of 0.00003% for Arizona’s TREC NBS. Of all patients with an abnormal NBS (n = 27), 18.5% of the infants had a false positive test for TCL. There were no false negative tests that the AZDHS or PCH is aware of; thus, the false negative rate was 0%.

SCID Cohort

From January 1, 2018, to December 31, 2019, seven infants were diagnosed with SCID, yielding a SCID incidence in Arizona of 1 in 22,819 live births over two years (Fig. 2, Table 1). One of the SCID infants was evaluated and treated after a positive NBS at a pediatric medical center in Tucson, Arizona; thus, limited clinical information was available (S-7). Four infants with SCID had pathogenic mutations in the DLRECI gene encoding Artemis (S-1, S-5, S-6, and S-7). The other three infants, S-2, S-3, and S-4, were diagnosed with IL7R, ADA-deficient,
and X-linked SCID, respectively. Five infants diagnosed with SCID were of Native American descent (S-1, S-2, S-5, S-6, and S-7). Infant S-3 with ADA-deficient SCID had consanguineous parents and was the only Caucasian infant in the cohort. Three infants (S-3, S-4, and S-5) had a family history of relatives with SCID, including one infant with Artemis SCID whose older sister had Artemis SCID (S-5). Five of the infants (S-1, S-2, S-3, S-4, and S-6) were breastfed prior to knowing the results of their NBS and four of the infants’ mothers were cytomegalovirus (CMV) seropositive (S-1, S-2, S-5, and S-6). One infant (S-2), with IL7R SCID who was breastfed by a CMV-seropositive mother for 4 days prior to the identification of the positive NBS was found to have active CMV viremia and enteritis prior to definitive therapy and ultimately succumbed to disseminated CMV infection within 30 days from their second HCT. This same infant (S-2) also had recurrent campylobacter enteritis prior to HCT.

All infants with SCID (Table 1) received definitive therapy, three received gene therapy (S-1, S-5, and S-6), and four received allogeneic HCT (S-2, S-3, S-4, and S-7). The three infants who received gene therapy had Artemis-type SCID and received their gene therapy as part of an outside institution’s clinical trial. All three infants were followed at PCH prior to gene therapy and upon return, continued to follow with the PCH bone marrow transplant team. The infant with ADA-deficient SCID (S-3) received bridging enzyme replacement therapy from day 36 of life to day 206 of life. The enzyme replacement therapy was discontinued after confirmation of engraftment post-allogeneic HCT. Infant S-3 was the only infant whose HCT was delayed past 3.5 months of life, owing to parental hesitancy to agree on the location and type of definitive therapy. Of the three infants who had HCT at PCH, one (S-4) had a matched sibling donor (MSD), one (S-3) had a matched unrelated donor (MUD) and one infant (S-2) had a haploidentical donor, her mother. Two infants (S-2 and S-3) had their first HCTs without conditioning regimens, but due to primary graft failure, both required second HCTs with conditioning regimens.

One of the Artemis SCID infants (S-5) had severe cutaneous maternal–fetal graft-vs-host disease (GVHD) prior to gene therapy requiring systemic glucocorticoids, sirolimus, mycophenolate mofetil, rabbit anti-thymocyte globulin, and various topical agents to resolve. Of the infants who received allogeneic HCTs at PCH (S-2, S-3, and S-4), all received cyclosporine for GVHD prophylaxis, and none had acute GVHD (Table 1). The infant with X-linked SCID (S-4) had grade 1 cutaneous chronic GVHD upon weaning immunosuppression which resolved with a pause in his weaning of immunosuppression. He was able to successfully continue his immunosuppression wean after resolution of his skin rash. Of the infants followed by PCH, five are clinically well (S-1, S-3, S-4, S-5, and S-6) and two infants are still requiring immunoglobulin supplementation (S-1 and S-5). The infant with Artemis SCID followed by the pediatric medical center in Tucson, Arizona, is also known to be alive after allogeneic HCT (S-7).

Non-SCID Cohort

Thirteen infants were identified with an abnormal initial TREC, ultimately not leading to a diagnosis of SCID (Table 2).

Three infants were premature, with persistently abnormal TREC values on repeat testing, despite reaching a corrected gestational age of greater than 37 weeks. The mean TREC score for preterm infants was 8.52 copies/µL. Two preterm infants died, one (NS-01) with Soto syndrome complicated by bronchopulmonary dysplasia (BPD), the second (NS-02) with Dandy-Walker syndrome with severe cardiopulmonary complications. Infant NS-03 had the lowest initial CD3+T-cell count at 860 K/µL. Their clinical course was complicated by recurrent infection, BPD, patent ductus arteriosus, and patent foramen ovale with ongoing ventilator and G tube dependency. Genetic sequencing (Invitae Primary Immunodeficiency (PID) Panel), demonstrated heterozygous
Table 1  SCID cases from 2018 to 2019 in Arizona

| Case | Sex | Race/ethnicity | Con M | CMV | BF | TREC | Initial CD3+ count (cells/µL) | Lymph Pheno | Genetics | Infect Prior | Tx Type | Age at Tx (mo) | Conditioning | GVHD ppx | Clinical course |
|------|-----|----------------|------|-----|----|------|-----------------------------|------------|----------|-------------|---------|---------------|-------------|---------|----------------|
| S-1  | F   | American Indian/ Naive Alaskan | N    | Y   | Y  | 0.0  | 0.0                          | T− B− NK⁺ | Artemis c.597C>A (p.Tyr199*) DCLRE1C | N          | GT      | 2.5          | LD Bu   | None         | IVIg q4, 28 mo post GT |
| S-2  | F   | American Indian/ Naive Alaskan | N    | Y   | Y  | 0.22 | 0.0                          | T− B⁺ NK⁺ | IL7R c.714G>A (p.Met238Ile) homozygous VUS IL7R | Y          | MMRD HCT | 1ˢᵗ—2.5 2ⁿᵈ—5.0 | 1ˢᵗ—none 2ⁿᵈ—Bu/Flu | 1ˢᵗ—CSA 2ⁿᵈ—CSA | 1ˢᵗ graft failure with 1ˢᵗ HCT, died due to disseminated CMV on D+26 from 2ⁿᵈ HCT |
| S-3  | F   | White           | Y    | N   | Y  | 0.0  | 23                           | T− B− NK⁻ | ADA c.911 T>G (p.Leu304Arg) homozygous | N          | ERT then MUD HCT | ERT—1.2 1ˢᵗ—3.7 2ⁿᵈ—5.8 | 1ˢᵗ—none 2ⁿᵈ—LD Bu/rATG 1ˢᵗ—CSA 2ⁿᵈ—CSA | 1ˢᵗ graft failure with 1ˢᵗ HCT; EBV viremia cleared with ritux, 22 mo post 2ⁿᵈ HCT |
| S-4  | M   | Hispanic/ Latino | N    | N   | Y  | 0.9  | 56                           | T− B⁺ NK⁻ | X-linked c.202G>A (p.Glu68Lys) homozygous IL2RG | N          | MSD HCT | 2.3          | LD Bu/rATG CSA | cGVHD; severe skin, grade 1, 25 mo post HCT |
| S-5  | M   | American Indian/ Naive Alaskan | Y    | N   | Y  | 3.5  | 1.0                          | T− B⁺ NK⁺ | Artemis c.597C>A (p.Tyr199*) homozygous in DCLRE1C | N          | GT      | 2.3          | LD Bu | None         | MSD HCT multiple CVC infections, AIHA, IVIg q5w, 24 mo post GT |
| S-6  | M   | American Indian/ Naive Alaskan | ND   | Y   | Y  | 0.0  | 0.0                          | T− B⁺ NK⁺ | Artemis c.597C>A (p.Tyr199*) homozygous in DCLRE1C | N          | GT      | 2.5          | LD Bu | None         | AIHA, multiple CVC infections, adrenal insufficiency, 44 mo post GT |
| S-7  | M   | American Indian/ Naive Alaskan | -    | -   | -  | -    | -                            | T− B⁻ NK⁺ | Artemis - - - | -          | HCT    | -            | -          | -        | Followed in Tucson, AZ |

*Con consanguineous parents, M maternal, CMV cytomegalovirus, BF breastfed prior to diagnosis, TREC T cell receptor circles/µL, Lymph Pheno lymphocyte phenotype, Infect Prior infection prior to definitive therapy, Tx Type definitive treatment type, Tx definitive treatment, mo months, GVHD graft-versus-host disease, ppx prophylaxis, F female, M male, N no, Y yes, ND not documented, GT gene therapy, HCT hematopoietic cell transplant, MMRD mismatched related donor, MUD matched unrelated donor, MSD matched sibling donor, ERT enzyme replacement therapy, LD low dose, Bu busulfan, Flu fludarabine, rATG rabbit anti-thymocyte globulin, CSA cyclosporine, IVIg intravenous immunoglobulin, 1ˢᵗ primary, D day, EBV Epstein-Barr virus, ritux rituximab, cGVHD chronic graft-versus-host disease, CVC central venous catheter, AIHA autoimmune hemolytic anemia, q3w every 3 weeks*
Table 2  Infants with abnormal SCID NBS not leading to a diagnosis of SCID

| Case # | Sex | Race/Ethn     | Gest | TREC #1 (copies/µL) | TREC #2 (copies/µL) | CD3 + * (K/µL) | # of LSPs | Additional Testing                                                                 | Definitive diagnosis                                      | Clinical course                                           |
|--------|-----|---------------|------|--------------------|--------------------|----------------|----------|-------------------------------------------------------------------------------------|-----------------------------------------------------------|----------------------------------------------------------|
| NS-01  | F   | ND            | 31w6d| 15.14              | 15.14              | 1211           | 1        | n/a                                                                                 | Soto syndrome                                            | NICU death                                               |
| NS-02  | M   | Hispanic      | 29w4d| 0.64               | 10.3               | 1966           | 1        | n/a                                                                                 | Dandy-Walker syndrome                                    | Cardiopulmonary complications, death                     |
| NS-03  | M   | Hispanic      | 30w3d| 0.43               | 0                  | 860            | 6        | Genetics: VUS in FOXL1 and ITGB2                                                    | Idiopathic TCL                                           | Vent and G tube dependency                               |
|        |     |               |      |                    |                    |                |          |                                                                                     |                                                           |                                                          |
| Term Infants                                                                                                                      |
| NS-04  | F   | Caucasian     | 39w0d| 16.9               | 9.15               | ND             | 0        | Chr. analysis                                                                       | 22q11.2 deletion syndrome                                | Lost to f/u                                              |
| NS-05  | F   | Hispanic      | 38w0d| 8.84               | 14.9               | 2987          | 1        | Normal CMA                                                                          | CHD                                                      | No further f/u                                           |
| NS-06  | M   | Caucasian     | FT*  | 14.62              | 13.07              | 2578          | 1        | Chr. analysis                                                                       | Trisomy 21                                               | No further f/u                                           |
| NS-07  | F   | Caucasian     | 39w0d| 12.71              | 3.17               | 2011          | 1        | Genetics: VUS in DCLRE1B, PRKDC, RMRP and LYST BM: non dx                             | Idiopathic TCL                                           | Persistent anemia with suboptimal reticulocytosis        |
|        |     |               |      |                    |                    |                |          |                                                                                     |                                                           |                                                          |
| NS-08  | M   | Hispanic      | 40w0d| 6.43               | 5.02               | 1427          | 2        | n/a                                                                                 | Idiopathic TCL                                           | No further f/u                                           |
| NS-09  | F   | Hispanic      | 40w  | 12.41              | 12.43              | 1787          | 2        | n/a                                                                                 | Idiopathic TCL                                           | Doing well                                               |
| NS-10  | M   | Asian         | 39w0d| 10.76              | 15.08              | 1571          | 6        | Low IgG; normalized by 12 mo                                                        | Idiopathic TCL                                           | Viral infection first 12 mo                              |
| NS-11  | F   | Caucasian     | 38w6d| 11.56              | 1.36               | 943           | 4        | n/a                                                                                 | Idiopathic TCL                                           | Lost to f/u                                              |
| NS-12  | M   | American      | 39w0d| 11.48              | 11.33              | 1098          | 2        | Chr. analysis                                                                       | 22q11.2 deletion syndrome                                | No further f/u                                           |
|        |     | Indian/Alaskan|      |                    |                    |                |          |                                                                                     |                                                           |                                                          |
| NS-13  | M   | Hispanic      | 40w0d| 3.85               | 6.44               | 798           | 6        | Genetics: VUS in TAPI                                                               | Idiopathic TCL                                           | Lost to f/u                                              |

* number, gest gestation, Ethnic ethnicity, TREC s T cell receptor excision circles, LSP s lymphocyte subset panels, ND not documented, F female, M male, n/a not applicable, NICU neonatal intensive care unit, f/u follow-up, vent ventilator, CHD congenital heart disease, FT* full term, specific week and month not recorded, BM bone marrow, TCL T cell lypmphopenia, Chr. analysis chromosomal analysis to include chromosomal microarray, fluorescent in situ hybridization or other quantitative chromosomal testing. CD3 + * Initial CD3 + T cell value
variants of uncertain significance (VUS) in FOXN1 and ITGB2. ITGB2, implicated in autosomal recessive leukocyte adhesion deficiency type 1, was not a plausible explanation for low TREC value or TCL. Heterozygous loss of function variants in FOXN1 are associated with low TREC and TCL at birth and during infancy, predominately affecting CD8+ T cells [16]. The FOXN1 variant identified (c.1036 T > C (p.Trp346Arg)) is not present in gnomAD and has not been reported in the literature. In silico predictive modelling using SIFT and PolyPhen-2 both suggest this variant is likely to be disruptive. Furthermore, CADD score was 26.8, supporting a likely deleterious effect of this variant. Notably, infant NS-03 was not noted to have dystrophic nails, as previously reported in patients with FOXN1 deficiency.

Ten infants with an abnormal NBS TREC were born at term. The mean TREC value for term infants was 10.96 copies/µL. Two term infants were diagnosed with 22q11.2 deletion syndrome and one with Trisomy 21. None of these patients underwent further testing. One infant (NS-07) had initial pancytopenia with persistent anemia and suboptimal reticulocytosis. Bone marrow biopsy and genetic analysis were non-diagnostic. Targeted gene sequencing (Invitae PID Panel) demonstrated heterozygous VUSes in DCLRE1B, PRKDC, RMRP, and LYST.

Ultimately, six term infants were diagnosed with idiopathic TCL.

**Discussion**

**SCID Cohort**

The findings of this retrospective study represent the first analysis of SCID NBS in the state of Arizona since its adoption in mid-2017. Much data already exists in the literature on the utility and importance of newborn SCID screening. The early detection and implementation of appropriate prophylactic measures and definitive treatment of our seven SCID patients is in keeping with the findings of other states’ newborn SCID screening programs [13, 17]. Over 2 years, the state of Arizona’s incidence of SCID is 1 in 22,819 live births which is more than 2.5 times more frequent than the national incidence of SCID.

As previously described, Arizona has a higher percentage of Native American/Native Alaskan and Hispanic/Latino populations compared to the percentages of these populations in the entire USA [14]. While the Native American/Native Alaskan population is approximately 5.3% of the state of Arizona’s population, 71.4% of infants diagnosed with SCID in Arizona over a two-year period were Native American. This large difference is likely due to the previously described higher incidence of SCID (1 in 2000 live births) on the Navajo Reservation and likely the founder effect of the Native American population with DCLRE1C mutations, leading to Artemis-type SCID [10–12]. Gene frequency for the Y192X founder nonsense mutation is reported to be 2.1% in the Navajo nation [11]. However, Arizona is also home to other populations of Native Americans of other tribal origins and their gene frequency is unknown. Given these population differences compared to the rest of the USA, the racial and ethnic composition of Arizona’s unique population has likely contributed to a higher incidence of SCID.

The molecular genotypes of SCID diagnoses also reflect these population differences; the most common being Artemis SCID which is again likely a reflection of a higher proportion of Native American/Native Alaskan within the population.

Consanguinity increases the probability of offspring with autosomal recessive disorders, including SCID. The global prevalence of consanguinity was reported to be 10.4%, with highest prevalence in Northern and sub-Saharan Africa, the Middle East and west, central and south Asia. Less than 1% of all marriages in the United States are consanguineous [18]. However, one infant (S-3) or 16.7% of the infants with SCID followed by PCH, had consanguineous parents. Although our cohort only has seven patients, Arizona is home to communities that support consanguineous marriages and thus is another contributing factor to the state of Arizona’s higher incidence of SCID.

It has been reported by Pai et al. [5] that older age (specifically greater than 3.5 months old) and active infection at the time of HCT were associated with poorer outcomes in SCID patients. Additionally, Heimall et al. [19] showed that infants in the Primary Immune Deficiency Treatment Consortium (PIDTC) SCID cohort who received definitive treatment with HCT after 3.5 months of age but remained infection-free had a two-year survival superior to those infants who received definitive HCT at less than 3.5 months of age who were infection free [19]. Early NBS helps to identify infants with SCID timely, prior to the onset of life-threatening infections, which allows earlier initiation of infection prophylaxis and isolation measures leading to superior clinical outcomes for infants with SCID. Five of the six infants in our SCID cohort (S-1, S-2, S-4, S-5, and S-6) followed by PCH received definitive therapy prior to 3 months of age (Table 1). The infant with ADA-deficient SCID (S-3) received her first HCT at 3.7 months of age due to several psychosocial reasons and debate of GT versus HCT. However, the infant was started on enzyme replacement therapy by six weeks of life with good response in ADA levels and a CD4+ count greater than 50 cells/µL. It is also important to note that only one infant (S-2, Table 1) had an active infection, CMV, at the time of initial SCID diagnosis and sadly had a poor outcome after a second haploidentical HCT with conditioning. This poor outcome aligns with previously well-described results by Pai et al. [5] that there is a 39% probability of survival if an infant had an active
infection at HCT and underwent a mismatched related donor HCT with conditioning, compared with 65% probability of survival with active infection and a mismatched related donor HCT without conditioning [5]. Initially, this infant had a haploidentical HCT without conditioning given her active infection, but unfortunately, she developed primary graft failure, thus necessitating a second allogeneic HCT with conditioning. Hence, it is imperative to assure mothers are CMV seronegative prior to allowing the continuation of breastfeeding for infants with a positive NBS for SCID.

**Non-SCID Cohort**

Our review excluded all infants delivered preterm but with normal TREC after reaching an adjusted gestational age of 37 weeks or greater, a group that often comprises a significant percentage of initial abnormal SCID NBS [20]. Prematurity aside, congenital syndromes have been identified as the most common etiology of abnormal TREC assays [6]. Previous analyses of newborn screening programs in the USA have identified anticipated conditions associated with TCL such as 22q11.2 deletion syndrome and trisomy 21. Implementation of SCID NBS has also led to the early detection of other congenital syndromes including but not limited to trisomy 18, CHARGE syndrome, Jacobsen’s syndrome, Nijmegen syndrome, CLOVES syndrome, and ataxia telangiectasia [6, 17, 21]. Our experience at PCH has mirrored other newborn screening programs in this regard, with three of our 13 non-SCID infants having a congenital syndrome, 22q11.2 deletion syndrome (n = 2) or Trisomy 21 (n = 1) (Table 2). While this 2-year interval did not identify any other congenital syndromes, screening in both late 2017 and 2020 have identified other conditions associated with TCL such as Noonan syndrome (unpublished data). One infant had congenital heart disease, an established cause of secondary TCL [12]. While in utero drug exposure has been associated with abnormal NBS TREC assay and profound TCL, we did not identify any such cases [22].

Although our numbers are small, abnormal initial and repeat TREC values did not normalize on a third assay and did not result in any change in diagnosis or management. This suggests that there is limited, if any, clinical utility to repeating a TREC assay beyond the initial two screens.

Two pediatric institutions in Arizona are designated referral centers for abnormal newborn SCID screening. As previously stated, PCH follows approximately 85% of all NBS with abnormal TREC values concerning for SCID. While it is known that there have been a total of seven confirmed cases of SCID in Arizona (6 followed at PCH, 1 followed in Tucson) during the study period, our detailed data does not include any abnormal initial TREC values that did not result in a diagnosis of SCID that were referred to Tucson, AZ. Therefore, this is the experience of a single center regarding non-SCID diagnoses and TCL over a 2-year period and limited to 13 infants. Furthermore, given the retrospective nature of this study, some details that may have been pertinent to the risk of neonatal TCL were unfortunately not available including maternal drug use during pregnancy of medications such as fingolimod, purine antagonists, and infant birth weight at time of TREC evaluation [20, 22]. The diagnosis of idiopathic TCL requires the absence of genetic defects. However, due to several factors including struggles garnering insurance approval, limited other financial resources to pay for genetic testing, and infants lost to follow-up, only two infants in the non-SCID cohort underwent genetic testing. One of these offered a potential explanation for TCL—heterozygous FOXP1 mutation. It is plausible that genetic testing of all these infants may have resulted in additional diagnoses. In evaluating the efficacy of NBS to detect conditions associated with secondary TCL, it would have been helpful to know how many of these pregnancies were deemed high risk, with known heart disease, chromosomal abnormality or other prior to delivery. Finally, several infants were lost to follow-up between 2018 and the time of this study.

**Conclusions**

The state of Arizona’s incidence of SCID is 1 in 22,819 live births, which is more than double the currently reported incidence of SCID in the USA. This increased incidence is likely due to Arizona’s unique population characteristics including consanguineous communities and a higher percentage of the Native American/Native Alaskan population.

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**Author Contribution** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Natalie Booth and Catherine Freeman. The first draft of the manuscript was written by Natalie Booth and Catherine Freeman, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Data Availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. REDCap was used for data collection and storage.

**Declarations**

**Ethics Approval** This is an observational/retrospective study. The Phoenix Children’s Hospital Research Ethics Committee has confirmed that no ethical approval is required. Phoenix Children’s Hospital Institutional Review Board did approve the study.

**Consent to Participate** As this was a retrospective study and no intervention took place, the study did not require informed consent from study subjects. The Phoenix Children’s Hospital Institutional Review Board approved the study.
Consent to Publish  Not applicable, this is a retrospective study.

Competing Interests  The authors declare no competing interests.

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