Tec Empresarial
P-ISSN: 1659-2395; E-ISSN: 1659-3359

HIGH INCIDENCE OF SEVERE COMBINED IMMUNODEFICIENCY DISEASE IN SAUDI ARABIA DETECTED THROUGH COMBINED T CELL RECEPTOR EXCISION CIRCLE AND NEXT GENERATION SEQUENCING OF NEWBORN.

Amn Owaid Alshammari[1], Noor Asaad Aljasham[2], Ghadah Omar Owaidh Almuzaini[3], Eman Ahmed Ameen Sunyur[4], Ahlam Abdulrahman Alharbi[5], Sultan Hamad B Alkhaibari[6]and Bassam Mushabbab A Alshahrani[7]

[1] Corresponding Author, Medical Technologist, Aoalshammari@kfmc.med.sa, PCLMA, KFMC, Riyadh, SA

[2] Lab technician, Naljasham@kfmc.med.sa, PCLMA, KFMC, Riyadh, SA

[3] Lab technician, Galmuzaini@kfmc.med.sa, PCLMA, KFMC, Riyadh, SA

[4] Phlebotomy specialist, esunyur@kfmc.med.sa, PCLMA, KFMC, Riyadh, SA

[5] Phlebotomy specialist, aabdulrahmanalharbi@kfmc.med.sa, PCLMA, KFMC, Riyadh, SA

[6] Specialist-Laboratory, Alkhaibary.s@gmail.com, PCLMA, KFMC, Riyadh, SA

[7] Specialist-Laboratory, bassam.alshahrani96@gmail.com, PCLMA, KFMC, Riyadh, SA

Abstract

The rare inherited disorder referred to as severe combined immunodeficiency is characterized by anaemia of the autologous T lymphocytes. An early and accurate detection of mutations is critical for ensuring timely illness care and diagnosis. Additionally, it facilitates the identification of relatives who are carriers and serves to enhance family planning. This investigation centred on the initial laboratory and clinical evaluations of an infant suspected of having SCID. Consanguinity, family history, infections, and pedigree analysis were all intended to be included in the directed history. A novel primary immunodeficiency disorder resulting from the initial mutation in the human IL2RB gene was identified through our research. This finding unveiled novel insights into the biology of NK cells and the signaling of IL-2/15. Inducing the deletion of the WSXWS motif, the IL2RB mutation resulted in decreased IL-2R production and dysregulated downstream signaling. The study alerted to the gravity of SCID, emphasizing its potentially fatal consequences for neonates without treatment and the economic burden it imposes on healthcare infrastructures. SCID children are now able to lead normal lives due to the revolutionary advancements in perinatal screening tests for SCID and associated T-cell lymphopenia. SCID and its mechanism of action are now better understood due to the discovery of the IL2RB mutation. The significance of genetic testing in optimizing healthcare resource utilization and improving patient outcomes cannot be overemphasized, given that effective management requires early detection through comprehensive screening.

Keywords: Severe Combined Immunodeficiency, Newborn Screening, T cell receptor, SCID in Saudi Arabia.

1. Introduction

A rare group of hereditary diseases called Severe Combined Immunodeficiency (SCID) renders afflicted individuals more susceptible to potentially fatal infections as a result of inadequate levels of autologous T lymphocytes and deficits in B and natural killer cells [1]. Some individuals with SCID, which is unexpected, are incapable of rejecting maternal T cells that traverse the placenta due to a severe impairment of the immune system. Three subcategories are encompassed within this classification: (1) Omenn syndrome, characterized by autoreactive or hyperinflammatory T cells but no TME; (2) SCID, characterized by an absence of autologous T cells, which is frequently associated with TME; and (3) SCID, exhibiting a scarcity of T cells but lacking TME [2].

Intrinsic Errors of Immunity (IEI), which are linked to mistakes in DNA repair and methylation, may impair the adaptive immunity of certain individuals. As a consequence, their immune repertoire is restricted, and they become more vulnerable to infections [3].

An effective immune response requires a varied repertoire of antigen receptors; this diversity is generated through the recombination of segments from the V, D, and J genes. Junctional diversity, which encompasses arbitrary deletions and non-templated additions of nucleotides, serves to augment the variety [4]. Antigen exposure and thymus development promote T cell receptor (TCR) maturation. The RAG1 and RAG2 complexes are crucial for inducing DNA double-strand breaks during V(D)J recombination; mutations in these loci may lead to SCID [5].

The objective of this research endeavor is to examine the intricate etiology of SCID through an investigation of genetic components, the progression of immune repertoires, and the significance of specific gene mutations. As a result, a more comprehensive understanding of this immunodeficiency disease is achieved, paving the way for the development of more targeted therapeutic approaches.

2. Literature review

A prevalence exceeding 1 in 1000 individuals in the general population is associated with over 450 inherited diseases referred to as primary immunodeficiencies (PIDs), all of which impact the innate or adaptive immune systems [6]. These disorders heighten susceptibility to infections, immunological dysregulation, and malignancy, which places a significant burden on patients and their families. Diagnosis, treatment, and prevention of PIDs, the majority of which are caused by monogenic factors, have been significantly enhanced by the implementation of genetic testing [7]. As an illustration of a life-saving application, consider the capability of screening infants for Severe Combined Immune Deficiency (SCID) prior to the onset of infections [8].

In order to facilitate improved malady treatment and early detection, disease-causing mutations must be precisely identified through genetic testing. Additional advantages encompass enhanced family planning capabilities and the capability to detect carriers among the patient's relatives [7].

The differentiation between pathogenic and non-pathogenic variations poses a challenge for genetic testing, owing to the considerable quantity of uncommon variants that are exposed through whole-genome and whole-exome sequencing [9]. The task of identifying a limited number of detrimental variants amidst the vast majority of hundreds of thousands remains arduous. Ensuring precision in genetic testing is dependent on this boundary.

Environmental factors, infections, pharmaceuticals, and metabolic disorders are non-inherited contributors to secondary immunodeficiency diseases (SIDs). SIDs are significantly more prevalent than PIDs, occurring thirty times more frequently [10]. This can be attributed to various contributing factors.

Severe combined immunodeficiency (SCID) is a genetically diverse group of PIDs characterized by a variety of abnormalities, including dysfunctions in T cell differentiation and function, as well as abnormalities in the development of B and natural killer (NK) cells [11]. In contrast to typical or atypical SCID, newborns presenting with T cell lymphopenia often exhibit specific syndromes, including but not limited to DiGeorge syndrome, trisomy 21, ataxia telangiectasia, CHARGE syndrome, diabetic embryopathy, CLOVES syndrome, EXTL3 deficiency, Fryns syndrome, Nijmegen syndrome, Noonan syndrome, and RAC2 deficiency [11].

Table 1: SCID-related genes and variations, as well as athymia manifesting as SCID

Genes affected	**Immunophenotype**			
	B+NK-	B+NK+	B-NK+	B-NK-
	IL2RG	IL7R	RAG1	AK2
	JAK3	IL2RB	RAG2	ADA
	TBX1	PTPRC	DCLRE1C (Artemis)	PNP
		CD3D	PRKDC	
		CD3E	NHEJ1	
		CD3Z	LIG4	
		CORO1A LAT NFKB1 ZAP70 del22q11		

Infants with SCID do not develop a varied repertoire of mature T cells and, as a result, have no or extremely few T-cell receptor excision circles (TRECs), which are DNA leftovers of T-cell receptor gene rearrangement [12].

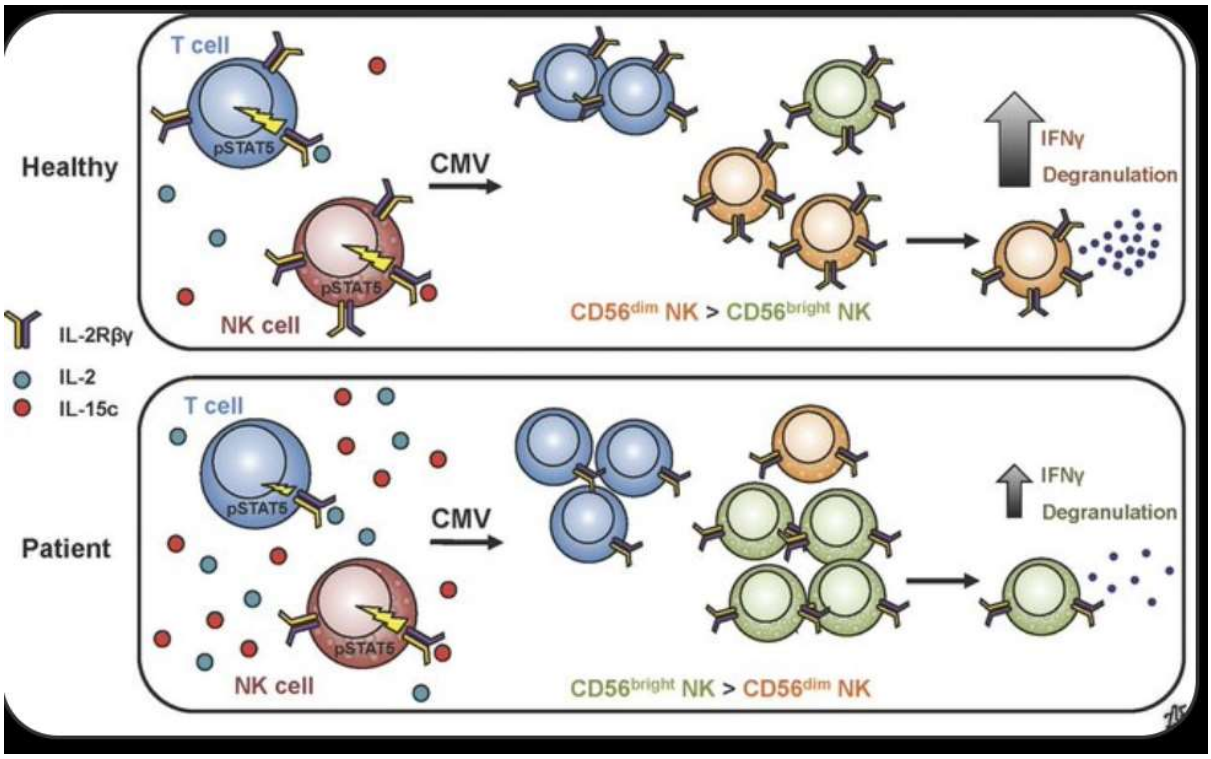

Figure 1: Changes in IL-2R expression and signaling affect T and NK cell function.

Late-onset adenosine deaminase (ADA)-a pronounced pattern of gradual T cell count decline characterizes deficient severe combined immunodeficiency. Newborn screening (NBS) for insufficient T cell receptor excision circles (TRECs) enables the prompt initiation of optimal treatment strategies for neonates afflicted with SCID, which is made possible by early diagnosis. The initiation of the Primary Immune Deficiency Consortium (PIDTC), which is financially supported by the National Institutes of Health and the Office of Rare Diseases at the National Centre for Advancing Translational Sciences, and the increasing use of NBS for SCID in infants who appear to be healthy have prompted the development of new SCID criteria [13].

2.1. Prophylactic drugs for SCID infants

Intravenous immunoglobulin (IVIG) is a vital therapeutic tool in the field of severe combined immunodeficiency (SCID) therapy. A loading dose of 1 gm/kg is prescribed initially, and then within a week or two, you should check your IgG trough levels. A maintenance regimen of 0.5 gm/kg every 3-4 weeks using 2.5 or 5 g full vials is then recommended. The usual dosage is 0.5 gm/kg given every three weeks in order to keep the IgG trough over 800 mg/dL.

A prophylactic dosage of 2.5 mg/kg of trimethoprim/sulfamethoxazole (TMP/SMX) taken orally twice daily for two days a week is recommended for the prevention of Pneumocystis jirovecii pneumonia (PJP). At least 30 days of age is the suggested starting point, with continuous monitoring of bilirubin and complete blood count (CBC). To reduce the risk of neutropenia caused

by TMP/SMX, it is recommended to take leucovorin (folinic acid) at a dosage of 1.25 mg twice weekly with the medication.

As an alternative to TMP/SMX, dapsone may be taken orally once weekly at a dose of 4 mg/kg. Patients weighing 12 kg or more should take 12.5 mg/kg orally twice daily or 8 mg/kg intravenously every 12 hours for herpesvirus prophylaxis, whereas patients weighing more than 12 kg should take 400 mg/m2 orally twice daily or 250 mg/m2 intravenously every 12 hours.

An effective method for preventing fungal infections is to take fluconazole at a dosage of 6 mg/kg every 48 hours for a month. Be cautious to check your liver function tests before starting. Institutional policy mandates every four weeks administration of palivizumab for preventative purposes, whereas PEG-ADA (Adagen) is used during the respiratory syncytial virus (RSV) season. The recommended dosage of PEG-ADA is 30 units/kg (equivalent to up to half a vial) given twice weekly; a complete dose is provided during the first therapy. This regimen is started while the patient is waiting for gene therapy or hematopoietic cell transplantation (HCT) [14].

2.2. Prenatal diagnosis and treatment

A paradigm shift has occurred in the prenatal diagnostic and treatment domains for SCID since the identification of underlying genetic variables. Molecular techniques permit prenatal diagnosis through chorionic villous biopsy between weeks 8 and 10 for patients with a high risk of complications during pregnancy. An alternative approach is to employ immunological techniques, which can be achieved through the use of echography during the 20th and 22nd weeks of pregnancy, in order to ascertain the fetal lymphocyte populations, present in cord blood. Recent significant accomplishments include the effective in-utero treatment of two X-linked SCID fetuses with haploidentical CD34+ cells. While this approach is novel, it should be implemented cautiously due to the possibility of inducing abortions (which is equivalent to treating the fetus at birth) and potential inefficacy in individuals with NK (+) SCID. These advancements are supported by an extensive collection of literature [15].

3. Methods

A methodology is presented in this study for the early clinical and laboratory evaluation of neonates who are suspected of having Severe Combined Immunodeficiency (SCID). Our approach integrates results from significant studies [16-18] to ensure a comprehensive and systematic evaluation of neonates who are at risk for SCID.

The directed history encompasses various components, including but not limited to infections, family history, kinship, and pedigree analysis. Conducting this comprehensive examination is crucial in informing subsequent treatment decisions. The acquired data enables a more informed approach to patient treatment by providing insights into potential risk factors and genetic predispositions.

A comprehensive assessment is conducted throughout the physical examination in order to identify indications of infection, skeletal characteristics, and congenital anomalies associated with specific SCID variants. Oliphant syndrome-associated rashes, and graft-versus-host disease (GVHD) rashes are two potential symptoms that require additional investigation. Desaturation associated with pulmonary alveolar proteinosis should be noted during a comprehensive evaluation of patients with ADA deficiency.

Flow cytometry is employed to validate the outcomes of the initial lymphocyte subset screening, with particular emphasis on the T cell CD45RA/RO ratios. The implementation of validated methodologies enhances the accuracy of diagnostics through the guarantee of consistent and exact flow cytometry analysis.

Vital laboratory assessments comprise blood chemistries, total bilirubin, albumin, liver function tests, and total bilirubin in order to ascertain the general health of the infant. Antigen or polymerase chain reaction (PCR) assays are conducted to detect various infectious agents, including but not limited to respiratory viruses, herpes simplex virus (HSV), eBV, and hepatitis B19. The objective of this comprehensive testing methodology is to detect potential infections and facilitate the development of individualized treatment regimens [17].

It is of the utmost importance when caring for neonates diagnosed with SCID or leaky SCID to immediately isolate children in a specialized SCID treatment facility. To mitigate the probability of complications, rigorous infection control protocols are implemented. In addition, it is critical to expeditiously detect and manage any irregularities in the patient's clinical manifestation that could potentially signify a viral or autoimmune disorder. In addition to providing assistance to families and attending to their physical and mental requirements, social workers are indispensable members of healthcare teams.

The determination of CMV serostatus through CMV IgG serology, which is utilized for infection prevention and surveillance, informs nursing recommendations. When clinical suspicion arises, it is imperative to perform ongoing monitoring of neonatal blood CMV PCR, particularly for four weeks, followed by periodic monitoring after that [18].

Medical assistance includes both establishing intravenous access for gamma globulin replacement and coordinating blood samples to reduce the frequency of venipuncture. Dietary counselling and an iron deficiency screening are integral components of the treatment strategy. Transfusions are administered when necessary, using irradiated packed red cells that are negative for CMV. The use of supplemental immunoglobulins permits the maintenance of IgG concentrations in excess of 800 mg/dL.

Acyclovir, fluconazole, and TMP/SMX should be administered after 30 days of age in order to prevent respiratory syncytial virus. Palivizumab should be administered throughout the season.

4. Results

Our research identified a distinct T cell oligoclonality among children who had prolonged cytomegalovirus (CMV) infection and possessed hypomorphic IL2RB mutations. We redirected our focus from NK cell control of persistent CMV infection to T cell responses to the virus after discovering issues with the former. This study elucidates the biology of natural killer (NK) cells and the signaling pathways involving IL-2 and 15 by casting light on a previously unknown primary immunodeficiency disorder caused by the first known human IL2RB mutation.

The IL2RB mutation identified in this study resulted in defective downstream signaling pathways and decreased synthesis of the IL-2 receptor (IL-2R); the deletion of the WSXWS motif was the cause of these effects. The anticipated consequences of diminished IL-2 signaling were corroborated by the clinical resemblance of this mutation to an IPEX-like syndrome. IL-2RB-mutated patients exhibited comparable symptoms to those of IL-2R deficiency, such as lymphocytic infiltration into multiple organs, an increase in memory T cells, and a reduction in the frequency of regulatory T cells (Tregs).

Clinical failure to suppress CMV infection was observed in patients deficient in both IL-2R and IL-2RB. Nonetheless, the distinctive cytokine signaling implications of these abnormalities suggested that mature NK cells may play a crucial role in mediating this regulation. Significantly, aberrant IL-2/15 signaling led to a selective expansion of the immature CD56bright NK cell fraction in IL-2R-deficient individuals, just as it did in IL-2RB-deficient patients and those administered anti-IL-2R medication. As a consequence of this proliferation, the frequency of terminally developed CD57+NK cells declined [19].

These findings underscore the intricate interplay among NK cell subsets, IL-2/15 signaling, and CMV regulation in the context of IL2RB mutations. The observed decrease in IL-2R production and dysregulation of downstream signaling may be molecularly explicable via the WSXWS motif deletion that was identified. Furthermore, the manifestation of symptoms resembling those of an IPEX-like illness offers valuable insights into the broader ramifications of disruptions in IL-2 signaling that affect immunological homeostasis. Primary immunodeficiency disorders impact immunological responses; this research contributes to the existing body of knowledge on this subject, particularly as it pertains to viral infections such as CMV [19].

5. Discussion

Our results highlight the critical role that IL-2/15 signaling plays in the maturation of T and NK cells and the regulation of antiviral immunity. Our research contributes to the existing body of knowledge regarding the intricate mechanisms that regulate the connection between host immunity and self-tolerance.

Infants born with severe combined immunodeficiency disorders (SCIDs) face an increased susceptibility to infections upon arrival due to the substantial deficiency or absence of protective T, B, and occasionally NK cells in their immune systems. This susceptibility emerges due to compromised immune reactions to infections. Consistent with our findings, Christopher et al.

(2018) discovered a substantial shift in the relative prevalence of certain SCID genetic subtypes in a prospective cohort of SCID patients as opposed to a retrospective cohort [1].

In addition to decreased T-cell function (less than 10% of the lower limit of normal) in response to phytohemagglutinin (PHA), SCID is distinguished by the presence of either no T cells or an extremely low number of them (less than 300 CD3 T cells/mm3). The potential presence of maternal T lymphocytes is an additional feature that may be indicative of SCID [20].

Our results emphasize, in line with prior research on SCID, the significance of T and NK cells in stimulating immune responses that are protective against infections. The study conducted by Christopher et al. revealed a temporal evolution in the distribution of SCID genetic subgroups, suggesting a dynamic nature of primary immunodeficiency diseases. Alterations in the occurrence rate of specific genetic mutations, advancements in genetic screening, or improved diagnostic instruments could all contribute to this development.

Since IL-2/15 signaling is a crucial factor in the maturation of T and NK cells, therapeutic options for individuals with SCID and related immunodeficiency diseases may be better understood. A more thorough understanding of immune system regulation is attained as the mechanisms that govern the delicate equilibrium between self-tolerance and host immunity are elucidated.

In conclusion, the findings of our research underscore the significance of IL-2/15 signaling in SCID and the diverse manners in which it influences immune responses. The recently identified modifications in SCID genetic subgroups and the newly established diagnostic criteria for SCID contribute to the ongoing discourse on primary immunodeficiency diseases. Clinical management and the development of targeted remedies for individuals affected by SCID and related disorders are both significantly impacted by these findings [1, 20].

6. Conclusions

Finally, severe combined immunodeficiency disease (SCID) is an expensive and sometimes fatal infant ailment that requires immediate medical attention. Newborn screening for SCID and associated T-cell lymphopenia is a crucial strategy that may save lives and improve the quality of life for children with SCID diagnoses [21].

Early identification and management are of the utmost importance due to the severe nature of SCID. The implications of SCID, including impaired immune function and vulnerability to potentially fatal infections, may be severe without prompt identification and proper treatment. In this setting, newborn screening is crucial since it allows for the early and preventative identification of afflicted individuals.

Our research supports previous research on SCID and highlights the life-altering effects of neonatal screening on impacted children's futures. Newborn screening changes the disease's natural course by allowing early detection, which opens the window of opportunity for prompt treatment measures.

The introduction of newborn screening has important ramifications for both clinical practice and the economy as a whole since it reduces the expenses of treating serious infections and their consequences over time. Therefore, it is cost-effective to invest in newborn screening as a means of early identification and management to guarantee good health outcomes for people with SCID.

References

1. Dvorak, C.C., et al., *The genetic landscape of severe combined immunodeficiency in the United States and Canada in the current era (2010-2018).* Journal of Allergy and Clinical Immunology, 2019. **143**(1): p. 405-407.
2. Dvorak, C.C., et al., *The natural history of children with severe combined immunodeficiency: baseline features of the first fifty patients of the primary immune deficiency treatment consortium prospective study 6901.* Journal of clinical immunology, 2013. **33**: p. 1156-1164.
3. Tangye, S.G., et al., *Human inborn errors of immunity: 2019 update on the classification from the International Union of Immunological Societies Expert Committee.* Journal of clinical immunology, 2020. **40**: p. 24-64.
4. Weitering, T.J., et al., *ATM: translating the DNA damage response to adaptive immunity.* Trends in immunology, 2021. **42**(4): p. 350-365.
5. Wong, G.K., et al., *Immune dysregulation in immunodeficiency disorders: the role of T-cell receptor sequencing.* Journal of autoimmunity, 2017. **80**: p. 1-9.
6. Fang, M., et al., *T cell repertoire abnormality in immunodeficiency patients with DNA repair and methylation defects.* Journal of Clinical Immunology, 2022. **42**(2): p. 375-393.
7. Heimall, J.R., et al., *Use of genetic testing for primary immunodeficiency patients.* Journal of clinical immunology, 2018. **38**: p. 320-329.
8. Abolhassani, H., et al., *Clinical implications of systematic phenotyping and exome sequencing in patients with primary antibody deficiency.* Genetics in Medicine, 2019. **21**(1): p. 243-251.
9. Findlay, G.M., et al., *Accurate classification of BRCA1 variants with saturation genome editing.* Nature, 2018. **562**(7726): p. 217-222.
10. Dorsey, M.J., et al., *Treatment of infants identified as having severe combined immunodeficiency by means of newborn screening.* Journal of Allergy and Clinical Immunology, 2017. **139**(3): p. 733-742.
11. Fernandez, I.Z., et al., *A novel human IL2RB mutation results in T and NK cell–driven immune dysregulation.* Journal of Experimental Medicine, 2019. **216**(6): p. 1255-1267.
12. Patel, S.Y., J. Carbone, and S. Jolles, *The expanding field of secondary antibody deficiency: causes, diagnosis, and management.* Frontiers in immunology, 2019. **10**: p. 33.
13. Barmettler, S., et al., *Association of immunoglobulin levels, infectious risk, and mortality with rituximab and hypogammaglobulinemia.* JAMA network open, 2018. **1**(7): p. e184169-e184169.

14. Dvorak, C.C., et al., *Comparison of outcomes of hematopoietic stem cell transplantation without chemotherapy conditioning by using matched sibling and unrelated donors for treatment of severe combined immunodeficiency.* Journal of allergy and clinical immunology, 2014. **134**(4): p. 935-943. e15.
15. Cinetto, F., et al., *Subcutaneous immunoglobulins replacement therapy in secondary antibody deficiencies: Real life evidence as compared to primary antibody deficiencies.* Plos one, 2021. **16**(3): p. e0247717.
16. Amatuni, G.S., et al., *Newborn screening for severe combined immunodeficiency and T-cell lymphopenia in California, 2010–2017.* Pediatrics, 2019. **143**(2).
17. Chinn, I.K. and W.T. Shearer, *Severe combined immunodeficiency disorders.* Immunology and Allergy Clinics, 2015. **35**(4): p. 671-694.
18. Kubiak, C., et al., *Fiscal implications of newborn screening in the diagnosis of severe combined immunodeficiency.* The Journal of Allergy and Clinical Immunology: In Practice, 2014. **2**(6): p. 697-702.
19. Thakar, M.S., et al., *A practical approach to newborn screening for severe combined immunodeficiency using the T cell receptor excision circle assay.* Frontiers in immunology, 2017. **8**: p. 1470.
20. Rechavi, E., et al., *Newborn screening for severe combined immunodeficiency in Israel.* International Journal of Neonatal Screening, 2017. **3**(2): p. 13.
21. Bausch-Jurken, M.T., J.W. Verbsky, and J.M. Routes, *Newborn screening for severe combined immunodeficiency-a history of the TREC assay.* International Journal of Neonatal Screening, 2017. **3**(2): p. 14.