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Neonatal screening test for severe combined immunodeficiency of primary immunodeficiency diseases: TREC assay and its limitations

Abstract

The aim of any screening program in the newborn is the early detection of treatable genetic disorders having a high velocity of morbidity and mortality. T-cell receptor excision circle (TREC), circular byproducts of T-cell receptor V (D) J recombination, function as an indicator for recently produced naïve T cells. Well- known genetic defects resulting in severe combined immunodeficiency (SCID) detected by the TREC assay are as following: IL-7 receptor; ADA; IL-2 receptor ɣ chain; etc. Other than SCID and leaky SCID patients, less important subjects of TREC assay contain infants with non-SCID T-cell lymphopenia. In this group, most frequent ones are syndromes such as DiGeorge, Down, Nijmegen breakage syndrome, ataxia telangiectasia, etc. Although the TREC assay can identify many types of T-cell lymphopenia, it may not be a screening test for every primary immunodeficiency disease. There is a group of combined immunodeficiencies in which lymphopenia is not a finding, but the immune dysfunction is as severe as in typical SCID patients. For instance; chronic granulomatous disease, congenital neutropenia, toll-like receptor defects, ZAP-70 and MHC class II deficiency that may be missed by this assay. Recent reports also confirm that TREC assay is not effective in identifying a subset of rare but deadly immunodeficiencies. Therefore, it is vital that any infant with unusual or serious infections be assessed by an expert clinical immunologist.

Keywords: newborn screening, TREC, severe combined immunodeficiency, primary immunodeficiency disease, SCID

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Abbreviations: TREC: T-cell receptor excision circle; SCID: severe combined immunodeficiency; CID: combined immunodeficiency; NSP: neonatal screening programs; TCR, T cell receptor; PID, primary immunodeficiency diseases; IL, Interleukin; TCL, T-cell lymphopenia

Introduction

The aim of any screening program in the newborn is the early detection of treatable genetic disorders having a high velocity of morbidity and mortality, like outlined by Wilson and Jungner.[1] While the accomplishment of neonatal screening programs (NSP) for metabolic / genetic disorders dates back to 1963, appropriate technologies to recognize severe congenital errors of immune function have recently become known.[2] Previous to NSP, severe combined immunodeficiency (SCID) has been projected to happen at an incidence of 1/50,000-100,000 births.[3] SCID happens approximately 1/50,000 births, as currently evaluated by impartial, population-based NSP.[4] The expected incidence of primary immunodeficiency diseases (PID) that would need urgent treatment varying from 2-8 individuals / 100,000 live births, rendering high burden on the efficacy and accessibility of this kind of screening tests.[5]

Importance of Severe Combined Immunodeficiency in Primary Immunodeficiency diseases

SCID, recognized as the "bubble boy disease", is mixture of serious deficiencies of cellular and humoral immune system making patients sensitive to opportunistic, severe and recurrent infections.[6,7] The most severe type of PID is SCID which is usually mortal during infancy unless detected early and treated.[8] It is known that subjects recognized during the newborn period by a positive family history associated with better survival compared to index or sporadic cases. Optimal survival in SCID is linked with early treatment in infancy earlier than the progression of unmanageable infections.[9] Therefore, early identification and management of SCID during infancy, whether with a positive family history or sporadic, could be probable only upon establishment of population-based NSP for SCID.

T-cell receptor excision circle (TREC) test for neonatal screening program

The expansion of a different inventory of T cells, with their individual T cell receptor (TCR), is crucial for identification of unknown antigens attached to self MHC molecules. To produce many exclusive TCRs, DNA gene reorganization and linear re-assembly is executed in thymocytes, in order that each cell has its particular mixture of a TCR variable (V), diversity (D) and joining (J) series. The cut out DNA remains of the TCR are also bound at their ends, making various rounded DNA byproducts called as TRECs.[10,11] TRECs, circular byproducts of TCR V(D)J recombination, function as an indicator for recently produced naïve T cells. TRECs are constant, but are not multiplied during mitosis, but become diluted when T cells reproduce.[12] TRECs are easily identified in lymphocytes by a PCR test utilizing primers magnifying a fragment spanning the joint of the circle. Hence, the number of TREC copies detected in NSP is served as a biomarker of newly thymic creation of naïve T cells, with a small quantity of TRECs indicating insufficient autologous T cell creation.[13–15]

Genetic defects resulting in severe T-cell lymphopenia or SCID detected by the TREC neonatal screening assay

A standardized characteristic of SCID patients is low T-cell numbers at birth, evidence that is the basis for the TREC analysis in

identifying SCID patients by the NSP. Well- known genetic defects resulting in SCID detected by the TREC assay are as following: interleukin (IL)-7 receptor; ADA; IL-2 receptor ɣ chain; Jak3; RAG1; RMRP1; 22q11 deletion (DiGeorge) syndrome; Rac2 defect; CHARGE syndrome; Ataxia telangiectasia, etc.[13]

Immunologic disorders beyond SCID detected by TREC Test

Neonates with SCID detected by NSP have a bigger percentage of autosomal recessive gene defects and smaller quantity X-linked IL-2 receptor ɣ chain mutations, possibly owing to improved detection of sporadic cases. A larger part of screened cases are because of defects in the recombinase activating genes (i.e. RAG1 and RAG2), half of which are leaky; devoid of NSP, persons with a diverse range of leaky RAG mutations are not identified until late childhood.[16]

Other than SCID and leaky SCID patients, less important subjects of TREC assay contain infants with non-SCID T cell lymphopenia (immunodeficiencies). Non-SCID T-cell lymphopenia disorders[15] consist of genetic syndromes with T cell impairment; a non-immune illness causing T-cell lymphopenia (TCL); born as very low birth weight neonate and prematurity; and children with "idiopathic TCL".[17] In the most frequent one in TCL group, syndromes, the foremost cause is DiGeorge syndrome.[17] Down syndrome is also one of the frequent reasons of low TRECs, with others including CHARGE syndrome, cartilage hair hypoplasia, Nijmegen breakage syndrome, ataxia telangiectasia, Fryns syndrome, Jacobsen syndrome; VACTERL association; TAR syndrome; ectrodactyly ectodermal dysplasia syndrome;[18] as well as non-immune disorders e.g. chylous effusions making T-cells vanished.[19] TCL also comprise congenital heart disease (excluding DiGeorge) in which neonatal surgery or vascular leakage yields third-spacing of fluid and lymphocytes; gastrointestinal malformations e.g. intestinal lymphangiectasia, gastroschisis, ileal atresia; hydrops; and congenital leukemia, causing leukemic cell infiltration of the bone marrow.[13]

Limitations of the TREC for neonatal screening programs

With the widespread accomplishment of NSP with TREC method, physicians also have to recognize the limitations of this test. Although the TREC assay can identify many reasons for T-cell lymphopenia, it may not be a screening test for every PID.[20] There is a group of CID in which lymphopenia is not a finding, but the immune dysfunction is as severe as in typical SCID patients. The terms T+ - SCID, CID, and some leaky SCID have served to explain this group of inborn immune disorders that contribute to a severe T-cell dysfunction but have important numbers of circulating T cells.[13,21]

By itself, these patients will be missed by the cutoff value of TRECs presently in use. For instance; there are numerous severe but treatable CIDs not resulting in T-cell or B-cell lymphopenia (eg, chronic granulomatous disease, congenital neutropenia, toll-like receptor defects) that may be missed by this assay. Other samples also consist of ZAP-70 and MHC class II deficiency (Bare lymphocyte syndrome).[22,23] ZAP-70 defects similarly result in significant CD8 lymphopenia and a CID phenotype, but can have normal TREC levels. MHC class II deficiency results in severe CD4 lymphopenia and significant risk of infections similar to SCID. This syndrome is typically missed by the TREC assay because CD8 cells are present, so the assay can be within the normal range.[24] ADA deficiency is a common cause of SCID and late-onset ADA deficiency can be overlooked by the TREC assay.[25] TREC screening detects less than 50% of cases of ataxia telangiectasia.[26] Several rare disorders, such as Ora1, Stim1, or CD40 ligand deficiency, show an infectious phenotype similar to SCID but have normal numbers of T cells, and it is unknown whether the TREC assay can detect these disorders.[27,28]

Conclusion

Recent reports confirm that TREC assay is not effective in identifying a subset of rare but deadly CIDs. A mounting number of newly reported CIDs are prone to be overlooked by the TREC assay. If the cutoff level in TREC test is increased some of these illnesses may be identified. Thus, it is vital that any infant with unusual or serious infections be assessed by an expert clinical immunologist.

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Conflicts of interest

There are no financial conflicts of interest.

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References

1. Andermann A, Blancquaert I, Beauchamp S, et al. Revisiting Wilson and Jungner in the genomic age:a review of screening criteria over the past 40 years. *Bull World Health Organ*. 2008;86(4):317–319.
2. Kwan A, Puck JM. History and current status of newborn screening for severe combined immunodeficiency. *Semin Perinatol*. 2015;39(3):194–205.
3. Stephan JL, Vlekova V, Le Deist F, et al. Severe combined immunodeficiency:a retrospective single–center study of clinical presentation and outcome in 117 patients. *J Pediatr*. 1993 123(4):564–572.
4. Verbsky J, Thakar M, Routes J. The Wisconsin approach to newborn screening for severe combined immunodeficiency. *J Allergy Clin Immunol*. 2012;129(3):622–627.
5. Borte S, Wang N, Oskarsdóttir S, et al. Newborn screening for primary immunodeficiencies:beyond SCID and XLA. *Ann N Y Acad Sci*. 2011;1246:118–130.
6. Shearer WT, Dunn E, Notarangelo LD, et al. Establishing diagnostic criteria for severe combined immunodeficiency disease (SCID), leaky SCID, and Omenn syndrome:the Primary Immune Deficiency Treatment Consortium experience. *J Allergy Clin Immunol*. 2014;133(4):1092–1098.
7. Felgentreff K, Perez–Becker R, Speckmann C, et al. Clinical and immunological manifestations of patients with atypical severe combined immunodeficiency. *Clin Immunol*. 2011;141(1):73–82.
8. van der Burg M, Gennery AR. Educational paper. The expanding clinical and immunological spectrum of severe combined immunodeficiency. *Eur J Pediatr*. 2011;170(5):561–571.
9. Chan A, Scalchunes C, Boyle M, et al. Early vs. delayed diagnosis of severe combined immunodeficiency:a family perspective survey. *Clin Immunol*. 2011;138(1):3–8.
10. Baker MW, Grossman WJ, Laessig RH, et al. Development of a routine newborn screening protocol for severe combined immunodeficiency. *J Allergy Clin Immunol*. 2009;124(3):522–527.
11. Morinishi Y, Imai K, Nakagawa N, et al. Identification of severe combined immunodeficiency by T–cell receptor excision circles quantification using neonatal Guthrie cards. *J Pediatr*. 2009;155(6):829–833.

12. Hazenberg MD, Otto SA, Cohen Stuart JW, et al. Increased cell division but not thymic dysfunction rapidly affects the T–cell receptor excision circle content of the naive T cell population in HIV–1 infection. *Nat Med*. 2000;6(9):1036–1042.

13. Verbsky J, Routes J. Screening for and Treatments of Congenital Immunodeficiency Diseases. *Clin Perinatol.* 2014;41(4):1001–1015.

14. Chan K, Puck JM. Development of population–based newborn screening for severe combined immunodeficiency. *J Allergy Clin Immunol*. 2005;115(2):391–398.

15. Puck JM. Laboratory technology for population–based screening for severe combined immunodeficiency in neonates:the winner is T–cell receptor excision circles. *J Allergy Clin Immunol*. 2012;129(3):607–616.

16. Felgentreff K, Perez–Becker R, Speckmann C, et al. Clinical and immunological manifestations of patients with atypical severe combined immunodeficiency. *Clin Immunol*. 2011;141(1):73–82.

17. Kwan A, Abraham RS, Currier R, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. *JAMA*. 2014;312(7):729–738.

18. Mallott J, Kwan A, Church J, et al. Newborn screening for SCID identifies patients with ataxia telangiectasia. *J Clin Immunol*. 2013;33(3):540–549.

19. Kwan A, Church JA, Cowan MJ, et al. Newborn screening for severe combined immunodeficiency and T–cell lymphopenia in California:Results of the first 2 years. *J Allergy Clin Immunol*. 2013;132(1):140–150.

20. Comeau AM, Hale JE, Pai SY, et al. Guidelines for implementation of population based newborn screening for severe combined immunodeficiency. *J Inherit Metab Dis*. 2010;33(2):S273–S281.

21. Kwan A, Puck JM. Newborn screening for severe combined immunodeficiency. *Semin Perinatol*. 2015;39(3):194–205.

22. Grazioli S, Bennett M, Hildebrand KJ, et al. Limitation of TREC–based newborn screening for ZAP70 severe combined immunodeficiency. *Clin Immunol*. 2014 153(1):209–210.

23. Kuo CY, Chase J, Lloret MG. Newborn screening for severe combined immunodeficiency does not identify bare lymphocyte syndrome. *J Allergy Clin Immunol*. 2013;131(6):1693–1695.

24. Lev A, Simon AJ, Broides A. Thymic function in MHC class II–deficient patients. *J Allergy Clin Immunol*. 2013;131(3):831–839.

25. La Marca G, Canessa C, Giocaliere E. Tandem mass spectrometry, but not T–cell receptor excision circle analysis, identifies newborns with late–onset adenosine deaminase deficiency *Allergy Clin Immunol.* 2013 131(6):1604–1610.

26. Mallott J, Kwan A, Church J. Newborn screening for SCID identifies patients with ataxia telangiectasia. *J Clin Immunol*. 2013;33(3):540–549.

27. Picard C, McCarl CA, Papolos A. STIM1 mutation associated with a syndrome of immunodeficiency and autoimmunity. *N Engl J Med*. 2009;360(19):1971–1980.

28. Feske S, Gwack Y, Prakriya M. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. Nature. 2006;441(7090):179–185.

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