News Release

Title

TREC/KREC Newborn Screening followed by Next-Generation Sequencing for Severe Combined Immunodeficiency in Japan

Key Points

- Severe combined immunodeficiency (SCID) is a primary immunodeficiency that causes critical immune dysfunction. If not treated immediately, it may be fatal within the first year of life.

- In Aichi Prefecture, we started the first large-scale newborn screening (NBS) programs for SCID in 2017, and have performed NBS tests on about 140,000 newborns to date. As a result, two patients with SCID were diagnosed early and successfully treated with hematopoietic cell transplantation at the appropriate time.

- Our NBS programs followed by the diagnostic NGS analysis for newborns with abnormal TREC and/or KREC values are useful for the early identification and rapid molecular evaluation of not only SCID but also various T- and/or B-cell immunodeficiencies.

Summary

Prof. Yoshiyuki Takahashi, Dr. Hideki Muramatsu, and Dr. Manabu Wakamatsu (First author), at the Department of Pediatrics, Nagoya University Graduate School of Medicine, Prof. Tetsuya Ito, Department of Pediatrics, School of Medicine, Fujita Health University, and Mr. Yoshimi Sakai, Department of Clinical laboratory, Aichi Health Promotion Public Interest Foundation initiated the first large-scale newborn screening (NBS) program for severe combined immunodeficiency (SCID) in 2017 in the Aichi Prefecture, Japan. They have analyzed about 140,000 cases from newborns for the presence of T-cell receptor excision circle (TREC) in the last four years, and we found two patients with SCID who underwent hematopoietic cell transplantation at the appropriate time.

SCID is a rare and fatal disease caused by mutations in one of several genes that play important roles in the development and function of T and B-cells. This disease is fatal without a stem cell transplant within the first year of life. While NBS programs using TREC, which is generated during the production of T lymphocytes, are widely used in foreign regions, SCID has not yet been included in the public NBS program in Japan.

Recently, TREC NBS programs combining the measurement of Kappa-deleting recombination excision circle (KREC), which is generated during the production of B lymphocytes, and comprehensive genetic analysis have been implemented in NBS programs in a few regions. However, the usefulness of these tests has not been fully evaluated.

We measured TREC and/or KREC values in 137,484 newborns in Aichi Prefecture

between April 2017 and December 2021. As a result, 145 newborns had abnormal TREC and/or KREC values, and we performed a comprehensive genetic analysis using next-generation sequencing (NGS) and found 2 patients with SCID (1 in 68,742) (IL2RG-SCID and reticular dysgenesis) and 10 with non-SCID PIDs with T- and/or B-cell deficiencies (1 in 13,748 newborns). Two patients with SCID were successfully treated with hematopoietic cell transplantation at the appropriate time with prophylaxis against infection after diagnosis.

In conclusion, we performed the first large-scale TREC and TREC/KREC NBS programs in Japan. Our NBS programs followed by the diagnostic NGS analysis for newborns with abnormal TREC and/or KREC values are useful for the early identification and rapid molecular evaluation of not only SCID but also different non-SCID PIDs.

Research Background

Newborn screening (NBS) programs are valuable for identifying infants suffering from a range of diseases that are potentially fatal if left untreated because they not only allow targeted identification of affected children early in their life but also enable the prompt provision of appropriate therapeutic interventions, which help them lead a life similar to that of healthy children. Severe combined immunodeficiency (SCID) is a primary immunodeficiency disease in which T lymphocytes do not function appropriately, resulting in severe infections. If the curative treatment, hematopoietic cell transplantation, is not performed at the appropriate time, patients with SCID develop serious infections that result in death within the first year of life.

NBS programs to detect SCID before developing serious infections are widely conducted overseas. In the US state of California, the T-cell receptor excision circle (TREC), which is generated during the production of T lymphocytes, was measured in approximately 3 million newborns. They reported that approximately one newborn per 50,000 could be successfully diagnosed with SCID. However, in Japan, SCID has not been included in public NBS programs until now, and except for cases with family members, most SCID cases were diagnosed after the onset of serious infections, with insufficient clinical outcomes.

Recently, the widespread use of comprehensive genetic analysis using next-generation sequencing (NGS) has improved the diagnostic ability of primary immunodeficiency diseases (PIDS). The use of NGS analysis associated with PIDs integrated with NBS programs for SCID has not been well evaluated. Additionally, the clinical usefulness of measuring Kappa-deleting recombination excision circle (KREC) has not been fully evaluated because of its high false-positive rate and increased cost.

We performed the first large-scale TREC and TREC/KREC NBS programs as an optional screening in Japan. Our NBS programs followed by the diagnostic NGS analysis for newborns with abnormal TREC and/or KREC values are useful for the early identification and rapid molecular evaluation of not only SCID but also different non-SCID PIDs. This NBS program was conducted in cooperation with the Department of Pediatrics, Nagoya University Graduate School of Medicine, the Department of Pediatrics, Fujita Medical College School of

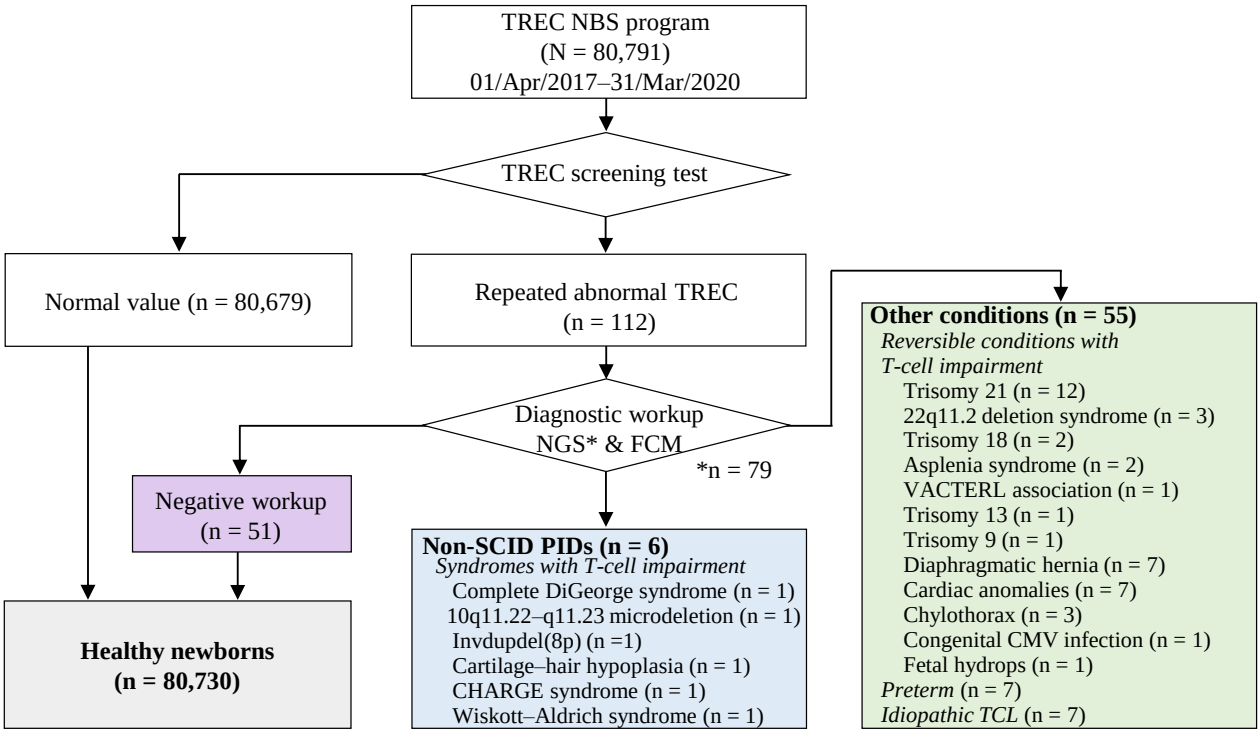
Medicine, and Aichi Health Promotion Public Interest Foundation.

Research Results

TREC and TREC/KREC NBS programs

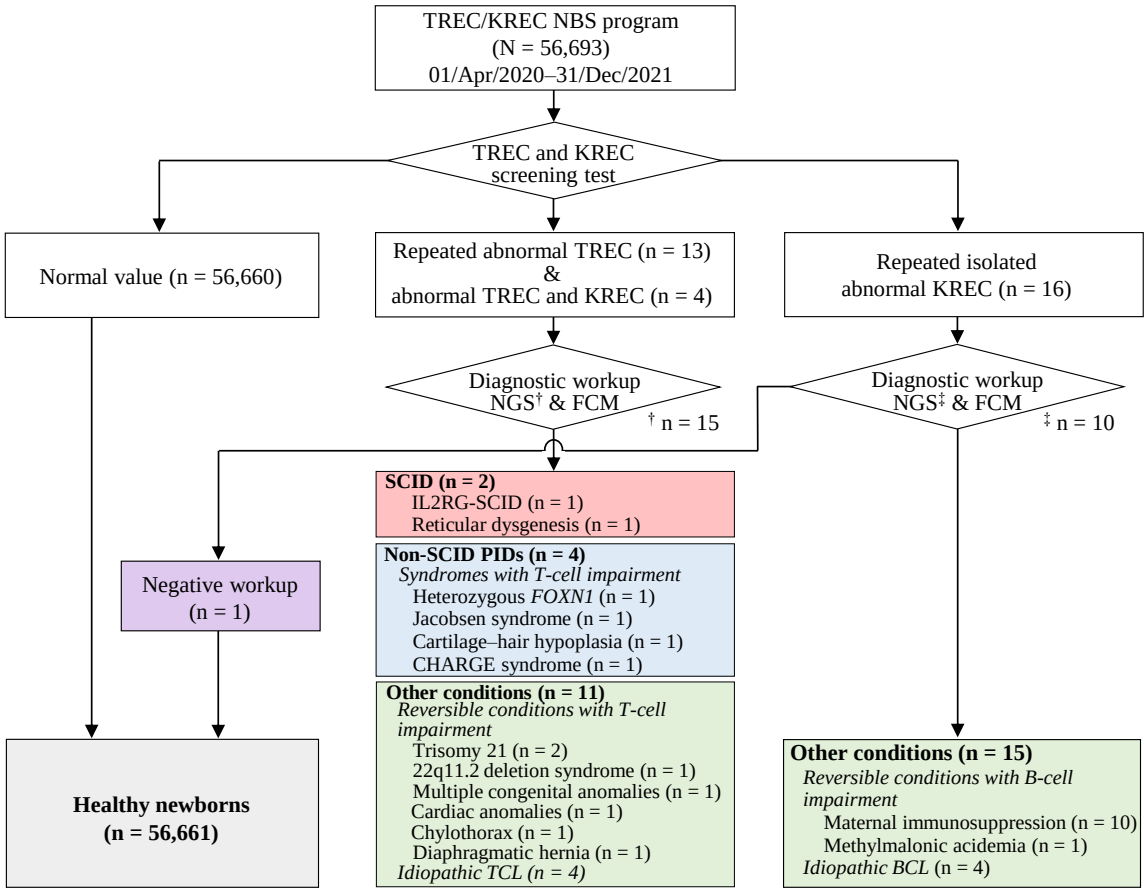
From April 2017 to March 2020, we measured TREC values in 80,791 newborns using PerkinElmer's EnLite TREC kit; of the 80,791 neonates, 112 (0.14%) had abnormal TREC values. Of the 112 cases, we performed the diagnostic NGS analysis of 79 cases, and diagnosed 6 patients with immunodeficiency other than SCID (non-SCID PIDs); complete DiGeorge syndrome, 10q11.22-q11.23 microdeletion, 8p inversion duplication deletion syndrome, cartilage-hair hypoplasia syndrome, CHARGE syndrome, and Wiskott-Aldrich syndrome were detected in one case each (**Figure 1A**).

Fig.1A TREC newborn screening program



In April 2020, we changed from their EnLite TREC kit to the TREC/KREC kit, and performed the simultaneous measurement of KREC and TREC values to assess not only T lymphocytes but also B lymphocytes. As a result, TREC and KREC were measured in 56,693 newborns by December 2021, and 33 (0.058%) had abnormal TREC and/or KREC values. The 33 newborns included 4 newborns with abnormal TREC and KREC, 13 with abnormal TREC, and 16 with abnormal KREC. Of the 33 cases, 25 (76%) underwent the diagnostic NGS analysis, and we identified 2 SCIDs (IL2RG-SCID and reticular dysgenesis) and 4 non-SCID PIDs (thymic hypoplasia associated with heterozygous *FOXN1* gene mutations, cartilage-hair hypoplasia, CHARGE syndrome, and Jacobsen syndrome) (**Figure 1B**). None of the 16 patients with abnormal KREC values had been diagnosed with PIDs with abnormalities only in B lymphocytes.

Fig.1B TREC/KREC newborn screening program



SCID

In total, 2 patients (1 in 68,742 newborns) were diagnosed with SCID (IL2RG-SCID and reticular dysgenesis) and 10 patients (1 in 13,748 newborns) were diagnosed with non-SCID PIDs with T- and/or B-cell deficiencies in our entire NBS program cohort of 137,484 newborns.

UPN5017 had abnormal TREC (0 copy/μL) but normal KREC (307 copies/μL) values. Lymphocyte subset analysis demonstrated that low $CD3^+$, $CD4^+CD45RA^+$ T-cell, $CD19^+$ B cells, and $CD16^+CD56^+$ NK-cell levels were consistent with a $T^-B^+NK^-$ SCID phenotype. Diagnostic NGS identified a hemizygous *IL2RG* mutation, diagnosed with IL2RG-SCID. At 4 months old, the patient was successfully treated with umbilical cord blood transplantation (CBT) without any infections and an increased number of donor-derived naïve T lymphocytes.

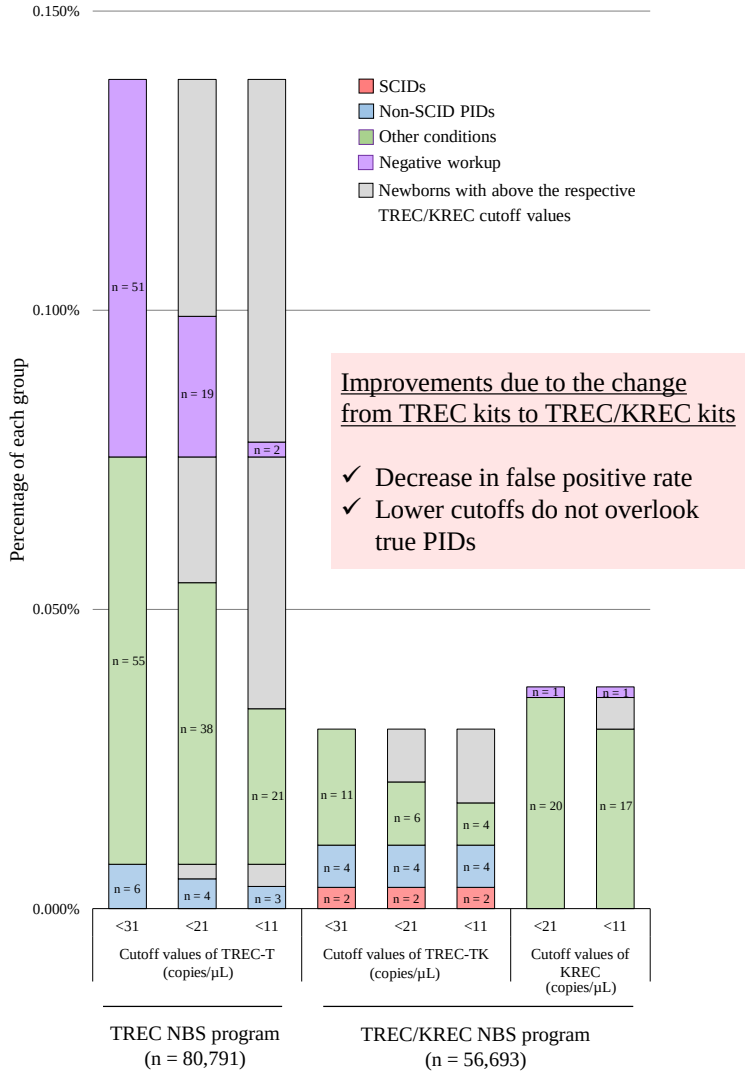
UPN5024 had abnormal TREC (0 copy/μL) and KREC (0 copy/μL) values at 4 days of age. She was referred to our hospital at 6 days of age. The extremely low $CD3^+$ T-cell, $CD4^+$ T-cell, $CD4^+CD45RA^+$ T-cell, $CD19^+$ B-cell, and $CD16^+CD56^+$ NK-cell counts were consistent with a $T^-B^-NK^-$ SCID phenotype. She presented leukopenia, the absence of neutrophils, and profound hearing loss. Diagnostic NGS revealed compound heterozygous *AK2* mutations, diagnosed with reticular dysgenesis. At 5 months of age, we performed CBT using busulfan and cyclophosphamide as a conditioning regimen, and she showed sustained complete donor chimerism of granulocyte, T-cell, and B-cell subsets.

Performance comparison between TREC and TREC/KREC kits

We compared the performance of TREC and TREC/KREC kits. We measured TREC values using these two kits ("TREC-T" by TREC kit and "TREC-TK" by TREC/KREC kit) in the identical 2,849 dried blood spots from 2,841 healthy newborns, 6 non-SCID PIDs, and 2 SCIDs. TREC-TK values were significantly correlated with TREC-T values ($P < 0.001$). Both TREC-T and TREC-TK values were moderately correlated with $CD4^+CD45RA^+$ naïve T-cell counts in 48 patients.

A comparison of the TREC measurements of the two kits utilized in the current NBS programs showed no difference in the diagnostic rates of PID (SCID + non-SCID PIDs) patients between the TREC kit (TREC-T; 80,791 patients) and the TREC/KREC kit (TREC-TK; 56,693 patients) (0.007% [n = 6] vs. 0.010% [n = 6], $P = 0.54$). On the other hand, the TREC kit (TREC-T) showed a significantly higher referral rate (0.138% [n = 112] vs. 0.058% [n = 33], $P < 0.001$) and false-positive rate (0.131% [n = 106] vs. 0.019% [n = 11], $P < 0.001$) compared to the TREC/KREC kit (TREC-TK), respectively (**Figure 2**).

Fig.2 Performance analysis of TREC kit and TREC/KREC kit



Research Summary and Future Perspective

We conducted the first large-scale NBS program in Japan, leading to early diagnosis and therapeutic intervention for patients with SCID. The integration of diagnostic NGS analysis with NBS programs has been very useful in the diagnosis of SCID as well as non-SCID PIDs. Our NBS programs for SCID initiated in Aichi Prefecture are now expanding to other prefectures. In the future, SCID will be included in public NBS programs so that all newborns throughout Japan can benefit from this NBS program.

Publication

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