Clin. Lab. 2026;72:XXX-XXX

CASE REPORT

Differential Trends of T-cell Receptor and Kappa-Deleting Recombination Excision Circles in Dichorionic Twins

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SUMMARY

Background: **In April 2023, T-cell receptor excision circles (TREC) and kappa-deleting recombination excision circles (KREC) were added to the expanded neonatal screening panel in Tokyo, Japan.**
Methods: **We observed dichorionic twins with divergent TREC and KREC trends despite no maternal differences. Levels were monitored across screenings.**
Results: **The first-born had normal TREC and KREC, whereas the second-born showed undetectable TREC with low KREC at first screening. Further immunological evaluation found no abnormalities. By the fourth screening on day 190, TREC and KREC levels in the second-born normalized.**
Conclusions: **TREC and KREC levels may be influenced by the in utero environment.**
(Clin. Lab. 2026;72:xx-xx. DOI: 10.7754/Clin.Lab.2025.250562)

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Case Report accepted May 30, 2025

KEYWORDS

neonatal screening, rotavirus vaccine, severe combined immunodeficiency, small-for-gestational age

INTRODUCTION

T-cell receptor excision circles (TREC) and kappa-deleting recombination excision circles (KREC) are established biomarkers of primary immunodeficiency. These markers, which reflect T- and B-lymphocyte production, are valuable indicators of severe combined immunodeficiency (SCID), X-linked agammaglobulinemia (XLA), and autosomal recessive XLA-like disorders [1]. SCID is asymptomatic at birth, and clinical signs only appear when infections have already occurred [2]. As patients with SCID or B cell deficiencies are at a high risk of severe illness following the administration of live vaccines, such as Bacillus Calmette-Guerin [3] and rotavirus [4], early diagnosis of these conditions is crucial. Furthermore, early diagnosis can help prevent severe recurrent infections and optimize the management of primary immunodeficiencies [5]. In April 2023,

TREC and KREC were introduced into the expanded neonatal screening panel in Tokyo, Japan. TREC and KREC levels are reportedly correlated with gestational age, with elevated levels typically observed in later gestational weeks [6]; however, the effect of the intrauterine environment on these levels remains unclear. In the present case, we observed divergent TREC and KREC trends in dichorionic twins despite no differences in maternal factors. Our case highlights that TREC and KREC levels may be influenced by the in utero environment. Consent was obtained from the parents for publication of this case report.

CASE PRESENTATION

The mother, a 37-year-old woman, had a history of three pregnancies, one of which resulted in a live birth. The patient had no history of immunosuppressive drug use and was diagnosed with gestational hypertension at 19 weeks of gestation. The twins were delivered via cesarean section at 32 weeks and 5 days of gestation owing to fetal distress in the second-born twin. The first-born twin was a girl with a weight of 1,389 g (-1.76 standard deviation [SD]), length of 38.0 cm (-1.92 SD), and head circumference of 28 cm (-0.98 SD), indicating that she was small for gestational age (SGA). Her Apgar scores were 8 and 9 at 1 and 5 minutes, respectively. The second-born twin was also a girl, with a weight of 820 g (4.41 SD), length of 31.0 cm (4.30 SD), and head circumference of 25.6 cm (2.2 SD), indicating that she was also SGA. Her Apgar scores were 8 and 9 at 1 and 5 minutes, respectively. Their complete blood counts (CBC) and serum C-reactive protein (CRP) levels were within the normal range; therefore, antibiotics were not administered on day zero. While the firstborn twin continues to show no signs of infection and was discharged 72 days after birth, the second-born twin experienced three catheter-related bloodstream infections on days 12, 46, and 89. Blood cultures were obtained, and antibiotic treatment was initiated for all three infections; however, only the culture obtained on day 46 was positive. At the age of 46 days, routine blood test of the second-born twin showed a very high CRP level (10.69 mg/dL; normal value < 0.2 mg/dL). The investigations showed total leukocyte count of 9,600/μL, absolute neutrophil count 5,900/μL, and lymphocyte count 1,500/μL. We removed the peripherally inserted catheter and initiated antibiotics (vancomycin and meropenem) for a suspected catheter-related bloodstream infection. Blood culture results showed methicillin-susceptible *Staphylococcus aureus* and methicillin-susceptible coagulase-negative *Staphylococcus* as well as sensitivity to sulbactam/ampicillin and vancomycin. Consequently, the antibiotics were downgraded from vancomycin and meropenem to vancomycin and sulbactam/ampicillin, respectively, based on the sensitivity pattern on day 52. The infant's clinical condition gradually improved, and antimicrobial administration was terminated on day 67.

The expanded screening results are presented in Table 1a. At the initial screening (day 5), the first-born twin showed normal TREC (50.9 copies/μL, cutoff 13.0) and KREC (225.9 copies/μL, cutoff 25.0) levels. At the second screening (day 64), her TREC level was 97.4 copies/μL, whereas her KREC levels were 335.8 copies/μL. In contrast, the second-born twin demonstrated undetectable TREC levels (0.0 copies/μL) with low KREC levels (66.8 copies/μL) at the first screening. At the second screening (day 22), TREC levels remained low (40.8 copies/μL), and KREC levels were very low (5.0 copies/μL). By the third screening (day 64), TREC levels became undetectable (0.0 copies/μL), and KREC levels were low (24.6 copies/μL). These abnormal findings prompted further immunological investigation. Lymphocyte subset analysis revealed mildly reduced CD3 (51.52%) and CD8 (16.62%) counts, with other subsets within normal ranges (Table 1b). Lymphocyte function test results, including natural killer cell activity assessed using phytohemagglutinin stimulation, remained within the normal range. Genetic testing for SCID (Panel 1) submitted on day 190 showed no abnormalities, and G-banding chromosome analysis revealed that the second-born twin had a karyotype of 46, XX.

The second-born twin was administered routine vaccination on day 183, although the rotavirus vaccine was withheld until SCID was definitively ruled out. By the fourth screening (day 190), her TREC (26.3 copies/μL) and KREC levels (517.6 copies/μL) were within the normal limits. Throughout the follow-up period, the lymphocyte counts of the second-born twin remained lower than those of the first-born twin, particularly when the TREC values were low (Figure 1). After discharge from the neonatal intensive care unit, both twins remained free of severe infections. To date, neither of the twins has been hospitalization due to infection. The first-born twin received all routine vaccinations, whereas the second-born twin missed only the rotavirus vaccine because of uncertainty regarding SCID. At 1 year and 8 months (corrected age: 1 year and 6 months), the first-born twin showed nearly normal development, whereas the second-born twin experienced moderate psychomotor retardation.

DISCUSSION

Severe combined immunodeficiency syndrome is a combination of both antibody production deficiency and cellular immunodeficiency, causing severe infections in the first six months of life. These infections significantly reduce the success rate of curative therapies such as hematopoietic cell transplantation and gene therapy; diagnosis in the neonatal period is thus essential [7]. B-cell deficiency causes severe infections owing to a lack of vaccine protection. These diseases are often asymptomatic during the neonatal period and early infancy, making their diagnosis difficult. Newborn screening us-

Table 1a. Trends in T-cell receptor and kappa deletion recombination excision circles in the twins.

First-born twin	Day 5		Day 64	
TREC (Copies/μL)	050.9		097.4	
KREC (Copies/μL)	225.9		335.8	
Second-born twin	**Day 5**	**Day 22**	**Day 64**	**Day 190**
TREC (Copies/μL)	000.0	040.8	000.0	026.3
KREC (Copies/μL)	066.8	005.0	024.6	517.6

Cutoff value: TREC 13.0 (Copies/μL), KREC 25.0 (Copies/μL).
KREC kappa-deleting recombination excision circles, TREC T-cell receptor excision circles.

Table 1b. Lymphocyte subsets in the second-born twin.

Components	Results (Normal ranges)
CD3 (%)	51.52 (58.0 - 84.0)
CD19 (%)	9.96 (5.0 - 24.0)
CD4 (%)	27.89 (25.0 - 54.0)
CD8 (%)	16.62 (23.0 - 56.0)
CD4/CD8 ratio	1.68 (0.40 - 2.30)
CD16 + 56 (%)	36.89

CD Cluster of Differentiation.

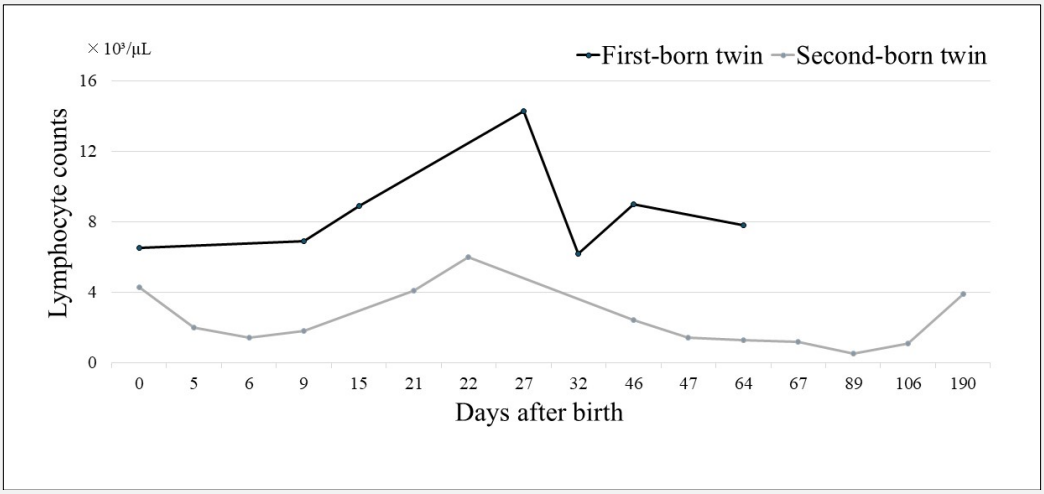

Figure 1. Trends of lymphocyte counts in the twins.

KREC kappa-deleting recombination excision circles, TREC T-cell receptor excision circles.

ing TREC was first reported in the U.S. in 2005 [8], and the first state-wide SCID screening pilot study commenced in Wisconsin in 2008 [9]. Newborn screening for primary immunodeficiency diseases using both TREC and KREC began in Sweden [10] and has spread worldwide. It was introduced to Tokyo in April 2023. Regular administration of rotavirus vaccine was started in October 2020 in Japan. This initiative means that the number of infants receiving the rotavirus vaccine before being diagnosed with SCID would increase. The recommended age for the first dose of the rotavirus vaccine is 8 - 15 weeks, given that late administration should be avoided to minimize infection severity. Live rotavirus vaccines should not be administered until SCID has been ruled out; therefore, early diagnosis is necessary [11].

Preterm infants are known for relative immunodeficiency, which is associated with false-positive newborn screening results for SCID. In a previous study, TREC and KREC levels were correlated not only with birth weight, but also with weeks of gestation in preterm infants [6]. This is because the thymus develops during the gestational period. Therefore, further immune evaluation (flow cytometry, proliferative responses to mitogens, serum immunoglobulin levels, and DNA arrays to reveal genomic copy number variations) is needed [12]. In the present case, the second-born twin exhibited consistently low TREC and KREC levels during expanded screening, raising initial concerns regarding SCID. However, lymphocyte subset analysis and functional and genetic testing revealed no abnormalities. Although both twins had dichorionic pregnancies with identical maternal factors, they exhibited distinct trends in their TREC and KREC levels. Given the significant differences in birth weight and length, the in utero environment potentially influences the levels of these biomarkers. This case represents the first report of its kind, highlighting the potential impact of prenatal environment on TREC and KREC levels.

As a limitation, the first-born twin in this case did not undergo immunological or genetic testing; therefore, whether genetic differences exist between the twins remains unclear. If the twins are dichotomous, their genetic predispositions may be different. Barbaro et al. reported a notable difference in the TREC and KREC levels within twin pairs; however, one in four pairs was monozygotic and genetically identical [10]. It is highly likely that non-genetic factors are hidden. Therefore, further studies are required to explore this relationship thoroughly.

CONCLUSION

TREC and KREC levels may be influenced by the in utero environment and should be carefully monitored, particularly with regard to the timing of live vaccinations.

Acknowledgment:
We express our gratitude to Dr. Nobuyuki Ishige of the Tokyo Metropolitan Preventive Medicine Association for his dedication in conducting the TREC and KREC tests in this case.

Source of Funds:
This study was supported by the Children and Families Agency Program (Grant number: JPCA23DA0801).

Declaration of Interest:
The authors declare no conflict of interest.

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