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국내 최초 T-Cell Receptor Excision Circle과 κ-Deleting Recombination Excision Circle 신생아 선별검사에 관한 연구

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The First Newborn Screening Study of T-Cell Receptor Excision Circle and κ-Deleting Recombination Excision Circle for Severe Combined Immunodeficiency in Korea: A Pilot Study

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Purpose: Severe combined immunodeficiency (SCID) is the most serious form of primary immunodeficiency. Infants with SCID are susceptible to life-threatening infections. To establish newborn screening for SCID in Korea, we performed a screening test for T-cell receptor excision circle (TREC) and κ-deleting recombination excision circle (KREC) in neonates and investigated the awareness of SCID among their parents.
Methods: Collections of dried blood spots from neonates and parent surveys were performed at the Samsung Medical Center and Cheil General Hospital & Women's Healthcare Center in Korea. The amplification crossing point (Cp) value <37.0 was defined as TREC/KREC-positive based on cutoff values from measuring multiplex real-time polymerase chain reaction. A Cp value >39.0 was defined as negative.
Results: For TREC/KREC screening, 141 neonates were enrolled; 63 (44.7%) were male. One hundred forty neonates (99.3%) had positive TREC/KREC results at the time of the initial test; 82.3% and 75.9% were positive and 17.0% and 23.4% were weakly positive for TREC and KREC, respectively. In one neonate (0.7%), the initial TREC/KREC test result was negative. However, repeated tests obtained and confirmed a positive result. For an awareness survey, 168 parents were engaged. Only 2% of parents (3/168) knew that the newborn screening test for SCID had been introduced and performed in other countries. Eighty-four percent of parents (141/168) replied that nationwide newborn SCID screening should be performed in Korean newborns.
Conclusions: In this study, newborn SCID screening was performed along with assessment of public awareness of the SCID test in Korea. The study results showed that newborn SCID screening can be readily applied for clinical use at a relatively low cost in Korea.

Key Words: Severe combined immunodeficiency; Neonatal screening; Surveys and questionnaires; Awareness

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Introduction

Severe combined immunodeficiency (SCID) is the most serious form of primary immunodeficiency (PID) and defects of SCID are mainly seen in the T lymphocyte system. Infants with SCID are susceptible to life-threatening infections; the mortality without proper treatment has reached almost 100% in the past.

Patient with SCID can be cured mainly by hematopoietic cell transplantation (HCT)[1]. It is now well-known that if patients are diagnosed earlier before they develop severe infection, mortality is lower and the HCT success rate is over 90%[2,3].

Several reports showed the importance and the availability of newborn screening for SCID. Newborn screening is now performed in the United States (US)[4] and United Kingdom (UK)[4,5].

It is known that T-cell receptor excision circle (TREC) is an episomal gene formed during T-cell receptor gene rearrangement in the thymus and κ-deleting recombination excision circle (KREC) is formed randomly from differentiating B-cell progenitors during immunoglobulin light chain recombination[6]. Therefore, TREC has been applied to assess thymic output and KREC to measure B-cell replication. TREC/KREC assays have been used to detect SCID and other B-cell immunodeficiencies[7,8]. The aim of this study was to establish a newborn SCID screening system by TREC/KREC assay. In addition, a survey was performed to assess parent awareness of PID and SCID.

Materials and Methods

1. Guthrie card collection

Blood collection on a suitable filter paper (903 Proteinsaver Snap-Apart Card, GE Healthcare, Westborough, MA, USA) was performed from full-term neonates using a heel stick technique after obtaining parental consent (Fig. 1). Blank filter papers spotted with peripheral blood from patients with SCID were used as a negative control for the TREC and KREC assay. The collected blood samples were dried at room temperature, protected from light for up to 3 hours, and then stored at 4℃ for up to 7 days.

2. DNA extraction from dried blood spots

DNA was extracted from dried blood spot (DBS) by using QIAamp DNA Blood Mini Kits (QIAGEN, Hilden, Germany) (Fig. 1). Briefly, three disks from each DBS were punched into a micro-centrifuge tube and the samples were lysed at 56℃ for 1 hour in the presence of 180 μL buffer ATL (QIAGEN) and 20 μL proteinase K (QIAGEN). To allow DNA binding to the column membrane, 200 μL buffer AL (QIAGEN) was added to the lysate and the mixture was incubated at 70℃ for 10 minutes. After incubation, this mixture was transferred onto a QIAamp MinElute column (QIAGEN), and centrifuged at 8,000 rpm. To wash residual contamination, 500 μL buffer AW1 (QIAGEN) and 500 μL buffer AW2 (QIAGEN) were added sequentially and centrifuged in a clean micro-centrifuge tube. Finally, 100 μL buffer AE (QIAGEN) was added and centrifuged at full speed for 1 minute to obtain pure DNA.

3. Real-time polymerase chain reaction for TREC and KREC quantification

The real-time polymerase chain reaction (PCR) reactions were carried out in a final volume of 20 μL containing 5 μL of patient samples, 3.5 μL of PCR-grade water, 10 μL of Roche Master (Roche diagnostics, Risch-Rotkreuz, Switzerland) and 1.5 μL of TREC, KREC, and myostatin (MSTN) specific primers and probes (0.5 μL of LightMix Modular MSTN, 0.5 μL of Light Modular TREC and 0.5 μL of Light Modular KREC, Roche diagnostics) (Fig. 1).

The 96-well plate reactions were performed on COBAS Z480 system (Roche diagnostics), with an initial cycle at 50℃ for 5 minutes (1 cycle), a denaturation cycle at 95℃ for 5 minutes (1 cycle), a heating cycle at 95℃, 60°C, and 72℃ for 15, 30, and 2 seconds respectively (45 cycles) and a cooling cycle at 40℃ for 30 seconds (1 cycle).

The presence of amplifiable TREC/KREC DNA was verified by running an extraction control MSTN as a reference gene. To avoid false negative results due to

PCR failure, sample contamination and unsuccessful extraction of DNA, we obtained results only when MSTN was detected and the negative control (PCR water) was not detected at the same time for multiplex real-time PCR.

The cutoff value for TREC and KREC were decided according to the manufacturer's recommendation, by repeated testing of SCID patients and all 141 samples. Therefore, optimized cutoff values were established by calculated PCR efficacy with serially diluted TREC/KREC controls (10, 100, and 1,000 copies; n=3) (data not shown). A crossing point (Cp) value less than 37.0 was defined as TREC/KREC positive. A Cp value more than 37.0 and less than 39.0 was defined as TREC/KREC weak positive. A Cp value more than 39.0 was defined as TREC/KREC negative (Fig. 2).

4. Survey for awareness

From August 2015 to September 2016, we conducted a questionnaire survey in parents at Samsung Medical Center or Cheil General Hospital & Women's Healthcare Center, Korea.

5. Institutional review and approval

Informed consent was obtained from the all study participants' parents. This study's research protocols were approved by the Institutional Review Board of Samsung Medical Center and Cheil General Hospital & Women's Healthcare Center (IRB File no. 2015-01-115).

Results

1. Participants for SCID screening

From August 10, 2015 to December 19, 2015, a total of 141 Korean newborns were enrolled and their freshly collected Guthrie cards were obtained at Samsung Medical Center and Cheil General Hospital & Women's Healthcare Center after parental consent (Fig. 2). The median age of the screened newborns was 2 days (range, 0 to 4 days) and 63 participants (44.7%) were male.

2. Analysis of TREC and KREC Cp values

Of 141 neonates, 140 neonates (99.3%) had positive TREC/KREC results at initial test; positives were 82.3%

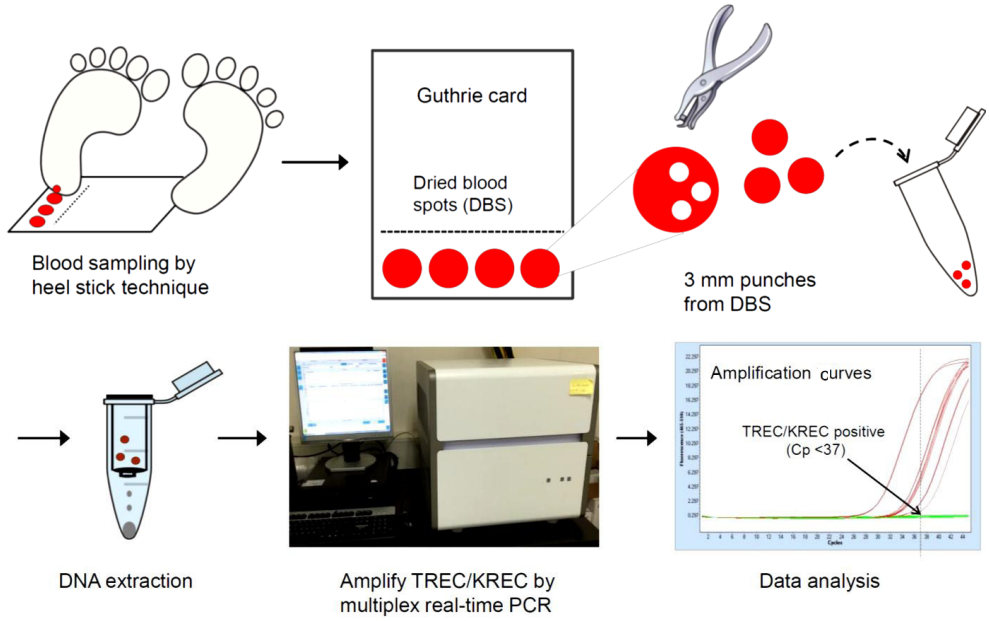

Fig. 1. Schematic illustration for newborn TREC/KREC screening using multiplex real-time polymerase chain reaction (PCR). Abbrevaitions: TREC, T-cell receptor excision circle; KREC, κ-deleting recombination excision circle; Cp, crossing point.

and 75.9% and weak positives were 17.0% and 23.4% for TREC/KREC, respectively (Table 1, Fig. 3). In one neonate (0.7%), the initial TREC/KREC test result was negative. The patient's initial Cp values for TREC/KREC were 39.0 and 40.0 which were considered negative. However, repeated tests confirmed positive values of 37.8 and 37.2 (TREC/KREC weak positive) (Fig. 3). The baby was followed up at the clinic and was found to be healthy with no clinical issues.

3. Survey for awareness on PID and SCID among parents

A total of 168 parents were engaged. Parent characteristics were obtained from 50 participants. There were 86% (n=43) females, and median age was 33 years. Of these, education level for high school, college, and graduate school was 2% (n=1), 82% (n=41), and 14% (n=8), respectively. Eighteen percent (n=9) had an older child.

Table 1. Analysis of TREC and KREC Cp Values

	Positive	Weak positive	Negative
TREC	83.0 (117/141)	17.0 (24/141)	0 (0/141)*
KREC	76.6 (108/141)	23.4 (33/141)	0 (0/141)*

Values are presented as percentage (number).
*In one neonate, initial TREC/KREC test result was negative (Cp=39.9/40.0). However, repeated test confirmed positive (Cp=37.8/37.2, TREC/KREC weak positive).
Abbreviations: TREC, T-cell receptor excision circle; KREC, κ-deleting recombination excision circle; Cp, crossing point.

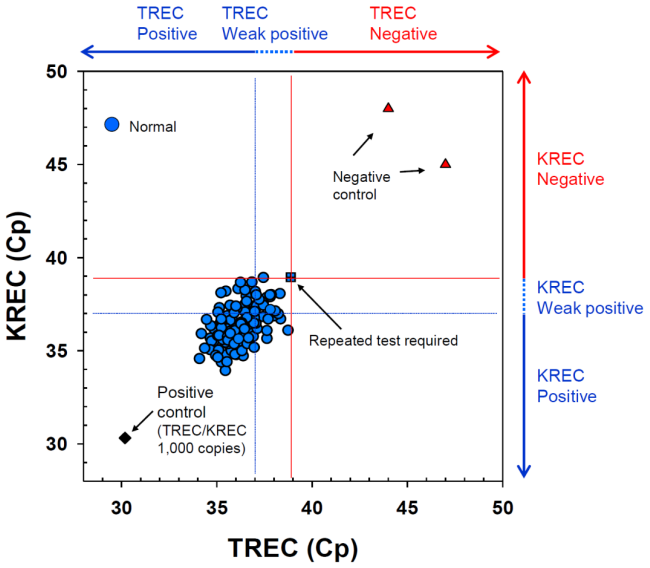

Fig. 3. T-cell receptor excision circle (TREC) and κ-deleting recombination excision circle (KREC) crossing point (Cp) values in dried blood spot samples from 141 neonates and negative controls.

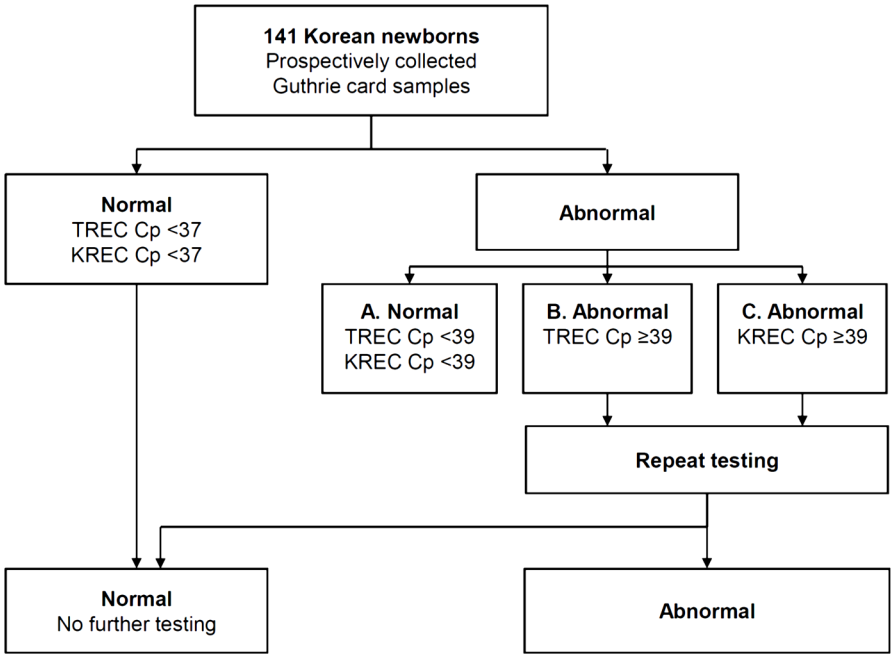

Fig. 2. Flow chart of the multiplex real-time polymerase chain reaction for 141 freshly collected Guthrie cards from Korean newborns. Abbreviations: TREC, T-cell receptor excision circle; KREC, κ-deleting recombination excision circle; Cp, crossing point.

Forty-nine percent of parents (82/168) replied that they had heard of newborn screening for congenital metabolic disease performed free of charge after birth in South Korea (Fig. 4A). Twenty percent of parents (33/168) replied that they had heard of PID (Fig. 4B) and 6% of parents (10/168) had heard of SCID (Fig. 4C). Only 2% of parents (3/168) replied that they knew the newborn screening test for SCID had been introduced and performed in other developed countries (Fig. 4D). Eighty-four percent of parents (141/168) answered that nationwide newborn SCID screening should be performed in Korean newborns if technically possible (Fig. 4E).

Discussion

In this study, TREC/KREC assay was performed in neonates to screen for SCID for the first time in Korea. We also observed low public awareness on PID and SCID screening programs for neonates.

PID is composed of almost 300 genetically defined inborn errors of immunity caused by a single gene mutation. SCID is the most serious form of PID. Without prompt diagnosis and intervention, mortality is significantly high during infancy[9].

From a large scale SCID screening project in US (3,030,083 newborns), the incidence of SCID was estimated to be 1/58,000 births[8]. This incidence is expected to be similar in other countries in Asia. Taiwanese data showed that the incidence of SCID was more than 1/53,196[10]. However, the prevalence of SCID in Korea was reported to be 0.41/1,000,000 which appears to have been an underestimation[11]. Recently, the survival of SCID babies has improved to over 90% through early detection and intervention such as HCT in Western countries[2,3]. However, it is speculated that most SCID patients in Korea may have not had the chance to be diagnosed in time. Furthermore, even after SCID diagnosis, it is hard to expect that they would have a good outcome due to pre-existing infectious complications.

Therefore, SCID screening during the neonatal period has become important. The newborn screening test using a filter paper-based technology was developed by Robert Guthrie over 50 years ago in the US. This innovation of heel stick blood spotting onto a filter

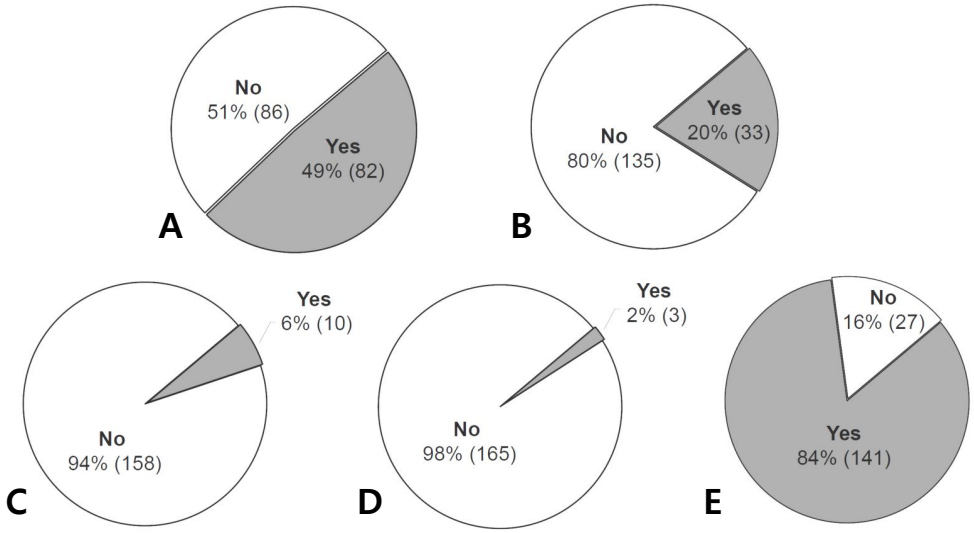

Fig. 4. Questionnaire survey in expecting parents. A total of 168 parents answered. (A) Have you ever heard of newborn screening test for congenital metabolic diseases performed free of charge after birth in Korea? (B) Have you ever heard of primary immunodeficiencies? (C) Have you ever heard of severe combined immunodeficiency (SCID)? (D) Have you ever heard of newborn screening test for SCID that has been introduced and performed in other developed countries? (E) Do you think that newborn SCID screening is needed in Korean newborns?

paper (Guthrie card) facilitated the development of newborn screening for various disorders[12]. TREC and KREC measurement using blood collected onto Guthrie cards made it possible to screen for SCID and other B-cell immunodeficiences[7,8,13]. Newborn screening for SCID using assays to detect TREC began in Wisconsin, US in 2008, and SCID was added to the national recommended uniform panel for newborn screening disorders in 2010. As of 2014, 23 states in the US, the District of Columbia, and the Navajo Nation conduct population-wide newborn screening for SCID[8]. In the UK, national newborn screening was started in 2013[5].

This newborn screening for SCID should be considered when the screening program is cost-effective. It is known that cost for newborn SCID screening is less than $5 in the US[14]. Based on data from the US, it has been shown that assuming society is willing to pay $50,000 for every quality-adjusted life-year saved, a SCID screening test with a cost of less than $5 would be considered cost-effective (a false-negative rate of 0.9% and a false-positive rate of 0.4%)[14]. Korea spent approximately $7,500,000 on a national screening program for six inborn errors resulting in metabolic diseases, including very rare diseases such as homocystinuria (Korean incidence of 1/201,479) and maple syrup urine disease (Korean incidence of 1/238,075) in 438,420 newborns in 2015[15]. However, screening for SCID which has an incidence expected to be less than 1/60,000 is not currently performed in this country. Additional data are needed to evaluate the cost-effectiveness of newborn SCID screening in Korea. Although our study could not provide the incidence of SCID in Korean neonates, it showed the feasibility of SCID screening in the Korean setting. A larger scale newborn SCID screening project is needed to estimate the true incidence of SCID in Korean neonates.

In conclusion, this study was the first to perform newborn SCID screening and to examine the public awareness of SCID in Korea. Our study results will provide valuable information for SCID screening policy and public education in Korea.

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요약

목적: 중증복합면역결핍증(severe combined immunodeficiency [SCID])은 일차면역결핍증 중 가장 심각한 형태의 질병이다. 국내에서 SCID 선별검사를 확립하기 위하여, 신생아에게 T-cell receptor excision circle (TREC)/κ-deleting recombination excision circle (KREC) 선별검사를 시행하고, 부모에게 인식도를 조사하였다.
방법: 혈액 검체에서 TREC/KREC을 multiplex 실시간중합효소연쇄반응으로 분석하였다. 부모에게 설문조사를 실시하였다.
결과: 141명의 신생아가 선별검사에 참여하였고, 140명(99.3%)이 첫 검사에서 TREC/KREC 양성이었으며, 한 명(0.7%)은 처음에 음성이었으나, 추후 양성으로 확인되었다. 인식도 조사에는 84% (141/168)는 SCID 선별검사가 국내에서도 시행되어야 한다고 답변했다.
결론: 본 연구에서는 국내에서 처음으로 신생아 SCID 선별검사와 인식도 시행되었으며, SCID 선별검사가 국내 임상에 적용될 수 있을 것으로 보인다.