Identification of Severe Combined Immunodeficiency by T-Cell Receptor Excision Circles Quantification Using Neonatal Guthrie Cards

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Objective To assess the feasibility of T-cell receptor excision circles (TRECs) quantification for neonatal mass screening of severe combined immunodeficiency (SCID).

Study design Real-time PCR based quantification of TRECs for 471 healthy control patients and 18 patients with SCID with various genetic abnormalities (*IL2RG*, *JAK3*, *ADA*, *LIG4, RAG1*) were performed, including patients with maternal T-cell engraftment (n = 4) and leaky T cells (n = 3).

Results TRECs were detectable in all normal neonatal Guthrie cards (n = 326) at the levels of 10^4 to 10^5 copies/μg DNA. In contrast, TRECs were extremely low in all neonatal Guthrie cards (n = 15) and peripheral blood (n = 14) from patients with SCID, including those with maternal T-cell engraftment or leaky T cells with hypomorphic RAG1 mutations or LIG4 deficiency. There were no false-positive or negative results in this study.

Conclusion TRECs quantification can be used as a neonatal mass screening for patients with SCID. *(J Pediatr 2009;155:829-33)*.

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Severe combined immunodeficiency (SCID) is a genetic disorder characterized by the absence of T-cells and adaptive immunity.[1,2] Affected infants usually have severe infections due to opportunistic pathogens in the first months of life. Hematopoietic stem cell transplantation can reconstitute immune function, although severe infections before hematopoietic stem cell transplantation can be fatal to the patients within the first year of life.[3,4] Thus, early diagnosis before the occurrence of severe infection is essential.[5-7]

Four different mechanisms have been identified as a cause of SCID, including purine metabolism defects, defective signaling of the common γ-chain–dependent cytokine receptors, defective V(D)J recombination, and defective pre-TCR/TCR signaling.[1,2] Although human SCID is caused by mutations of at least 10 different genes, all patients have a characteristic decreased number of newly

BCG	Bacillus Calmette-Guérin
BMT	Bone marrow transplantation
CMV	Cytomegalovirus
FISH	Fluorescent in situ hybridization
HSCT	Hematopoietic stem cell transplantation
PB	Peripheral blood
PCR	Polymerase chain reaction
sjTRECs	Signal joint TRECs
SCID	Severe combined immunodeficiency
TCR	T-cell receptor
TRECs	T-cell receptor excision circles
UCB	Umbilical cord blood

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Table. Genotype, lymphocyte subset, and TRECs of patients with SCID

Patient	Sex	Genotype	Age at onset of symptoms	Age at SCID diagnosis	Lymphocytes (/μL)	CD3+ (%)	CD3+ (/μL)	CD19+ (%)	CD45RO+ / CD4 + CD3 + (%)	Maternal lymphocyte engraftment	Guthrie cards TRECs (/μg DNA)	PB Pre-HSCT TRECs (/μg DNA)	PB Pre-HSCT Age
1	M	*IL2RG*	3 mo	3 mo	720	0.0	0	-	86.0	-	<10	-	-
2	M	*IL2RG*	-	0 mo	780	0.0	0	-	94.0	-	<10	<10	0 y, 0 mo
3	M	*IL2RG*	-	0 mo	920	0.0	0	-	91.0	-	<10	<10	0 y, 0 mo
4	M	*IL2RG*	4 mo	5 mo	2550	0.2	5	NA	99.4	-	<10	<10	0 y, 5 mo
5	M	*IL2RG*	10 mo	10 mo	1035	0.0	0	-	94.7	-	<10	<10	0 y, 10 mo
6	M	*IL2RG*	4 mo	5 mo	3560	0.0	0	-	95.8	-	<10	<10	0 y, 5 mo
7	M	*IL2RG*	-	0 mo	966	0.7	7	95.3	77.5	-	<10	<10	0 y, 0 mo
8	M	*JAK3*	4 mo	4 mo	3810	0.0	0	-	87.0	-	<10	-	-
9	F	*JAK3*	2 mo	5 mo	2495	0.0	0	-	89.8	-	<10	<10	0 y, 6 mo
10	M	*ADA*	1 mo	4 mo	90	40.0	36	99.5	4.4	-	<10	<10	0 y, 2 mo
11	M	*ADA*	1 mo	2 m	100	6.8	7	89.9	0.9	-	6.2×10^2	<10	0 y, 1 mo
12	M	*IL2RG*	8 mo	8 mo	3250	40.8	1326	89.8	65.5	T + NK+	-	<10	1 y
13	M	*IL2RG*	-	0 mo	950	4.2	40	NA	68.6	T+	<10	-	-
14	M	*IL2RG*	9 mo	10 mo	860	7.0	60	99.6	85.9	T + NK+	<10	<10	0 y, 10 mo
15	M	*IL2RG*	3 mo	3 mo	300	36.5	110	NA	53.5	T+	<10	-	-
16	F	*LIG4*	-	0 mo	550	38.7	213	97.6	0.3	-	-	<10	2 y
17	M	*LIG4*	1 y, 6 mo	4 y	300	44.3	133	25.2	0.1	-	<10	<10	4 y
18	F	*RAG1*	8 mo	1 y 9 mo	550	53.1	292	91.6	12.0	-	-	8.0×10^1	2 y

NA, Not available.

developed T cells.[1,2,8,9] T-cell receptor excision circles (TRECs) are small circular DNA fragments formed through rearrangement of the T-cell receptor (TCR) α locus and do not multiply during cell division.[9-13] Therefore, TRECs quantification is reportedly useful for determining recent thymic emigrants. Two reports of a method for neonatal screening of SCID using TRECs quantification by real-time PCR have been published.[6,7] Both studies quantified TRECs of patients with SCID using peripheral blood and found significantly lower levels of TRECs than those of control neonates. In addition, Guthrie cards from 2 patients with SCID retrospectively had undetectable TRECs.[6] Most control neonates had high amounts of TRECs. However, TRECs were undetectable in some samples. To increase specificity, 1 study[7] proposed a 2-tiered strategy using a combination of quantified TRECs and IL-7.

We have evaluated blood from Guthrie cards and peripheral blood from control patients and patients with SCID for detecting TRECs.

Methods

Peripheral blood samples were obtained from 112 healthy volunteers (median age, 14 years; range, 0.1 to 51 years). Thirty-three umbilical cord blood samples (median gestational age, 38.9 weeks) were collected at the National Defense Medical College Hospital. Dried blood spots of umbilical cord blood were obtained by applying 50 μL of residual blood to the 11-mm circles on filter-paper cards (PKU-S, Toyoroshi, Tokyo, Japan). Twenty-six neonatal Guthrie cards with dried blood spots were donated from surplus routine samples for newborn mass screening from neonates born at National Defense Medical College Hospital during this study period (January 2005 to December 2007). In addition, 300 neonatal Guthrie cards, stored at −20°C for less than 5 years in a neonatal mass screening center at Shimane University, were analyzed.

Eighteen patients with SCID were analyzed for TRECs (Table). All patients were genetically diagnosed using genomic DNA sequencing. Mutations of either *IL2RG* (n = 11), *JAK3* (n = 2), *RAG1* (n = 1), *ADA* (n = 2), or *LIG4* (n = 2) were identified in the patients (Table).

Peripheral blood samples of 14 patients before hematopoietic stem cell transplantation were used. In addition, neonatal Guthrie cards of 15 patients that had been stored in newborn mass screening centers were obtained.

Maternal T and NK lymphocyte engraftment was diagnosed by fluorescent in situ hybridization (FISH) using X and Y chromosome–specific probes after purification of each compartment by specific monoclonal antibodies and immunomagnetic beads.

The study protocol was approved by the National Defense Medical College Institutional Review Board, and informed consent was obtained from the parents of patients with SCID and healthy control patients, as well as adult control patients, in accordance with the Declaration of Helsinki.

Quantification of TRECs by Real-Time PCR

We used 100 μL of whole blood (EDTA anticoagulated peripheral blood and heparinized cord blood) or 2 punches of 6 mm in diameter from Guthrie card to extract genomic DNA.

Concentrations of DNA from peripheral blood, fresh dried blood punches from normal neonates (n = 26), and stored dried blood spots from normal neonates (n = 300) were