CASE REPORT
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A New *IL-2RG* Gene Mutation in an X-linked SCID Identified through TREC/KREC Screening: a Case Report

Maryam Nourizadeh[1], Stephan Borte[2,3], Mohammad Reza Fazlollahi[1], Lennart Hammarström[2], and Zahra Pourpak[1]

[1] Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran
[2] Division of Clinical Immunology, Department of Laboratory Medicine, Karolinska University Hospital Huddinge, Stockholm, Sweden
[3] Jeffrey Modell Diagnostic and Research Center for Primary Immunodeficiencies, Municipal Hospital St. Georg, Leipzig, Germany

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ABSTRACT

Severe combined immunodeficiency (SCID) represents a rare group of primary immunodeficiency disorders (PIDs), with known or unknown genetic alterations. Here, we report a new interleukin 2 receptor, gamma chain (IL-2RG) mutation in an Iranian SCID newborn.

The patient was a 6-day old boy with a family history of PID. The child was screened using a molecular-based analysis for the assessment of T cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KRECs). Moreover, a complete immunological evaluation and gene sequencing was performed.

Results showed undetectable TREC but a high level of KREC copy numbers. Flow cytometric data indicated low numbers of T and NK cells, but elevated number of B cells. A novel substitution in IL2RG: c.675 C>A, leading to p.225 Ser>Arg was found. Based on the functional analysis, the mutation is predicted to be damaging. The patient was diagnosed as a T B^+ NK X-linked SCID.

Keywords: Interleukin-2 Receptor gamma Chain; Severe Combined Immune Deficiency; T-cell receptor excision circles; kappa-deleting recombination excision circles

INTRODUCTION

Severe Combined Immunodeficiency (SCID) is a heterogeneous group of rare but serious, potentially fatal, inherited immune disorders in which T lymphocytes fail to develop and B cells or Natural Killer cells (NK cells) are either absent or compromised.[1,2] Many infants with severe T cell deficiencies, especially SCID are often unknown at birth and they are not identified until life-threatening infections occur.[2,3] There are different types of SCID based on protein or genetic defects. The most

Corresponding Authors: Zahra Pourpak, MD, PhD;
Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran, Tel: (+98 21) 6691 9587, Fax: (+98 21) 6642 8995, Email: pourpakz@sina.tums.ac.ir

Lennart Hammarström, MD, PhD;
Department of Laboratory Medicine (LABMED), H5, Division of Clinical Immunology, Karolinska Universitetssjukhuset, Huddinge F79 14186 Stockholm, Sweden. Tel: (+46 0) 8524 83586, E-mail: Lennart.Hammarstrom@ki.se

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common form of SCID is X-linked common γ-chain deficiency affecting nearly 45% of all cases.[4,5] Early diagnosis of SCID infants before the onset of serious infections and subsequent treatment by hematopoetic stem cell transplantation (HSCT) within the first 3-3.5 months of life can establish a normal immune function.[1,3,6] Previously proposed screening methods for SCID included absolute lymphocyte count, IL-7 immunoassay, antibody-based detection of T cell proteins and gene sequencing microarrays.[7,8] However, to date the only assays with adequate sensitivity and specificity for use in regular dried blood spot samples is the T cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KREC) assay using real-time quantitative polymerase chain reaction (RT-qPCR), first published in 2005 by Chan and Puck.[9] TREC and KREC are by products of T cell and B cell differentiations in the thymus and bone marrow, respectively. Recently, improved molecular methods to measure the TREC/KREC copy numbers by multiplex RT-qPCR on DNA extracted from routine newborn blood screening cards (Guthrie cards) for enumerating the naïve T and B cells in peripheral blood have been developed.[1,2,6,8-14] Therefore, precise molecular techniques like TREC/KREC assays can be used both for screening of newborns and for confirming the diagnosis of T/B lymphopenia.

CASE REPORT

The patient was a 6-day old boy; third child of healthy parents. The first child of this family was a boy with SCID who expired five months after birth following BCG vaccination. Vaccination was not performed in the second SCID boy and he received bone marrow transplant one year after birth, but he died at the age of four following Graft versus host disease (GVHD) complications. These two children did not receive a genetic diagnosis. Therefore, the third child was suspected to be a SCID and was referred to the Immunology, Asthma and Allergy Research Institute (IAARI) for immunological evaluation.

Informed consent from newborn's parents was taken for all sampling in this study. Heel-stick blood sample was obtained and stored dry on a Guthrie card immediately after being referred to the IAARI. Although the ideal time for screening the newborn would be up to 72 hours after birth, [9,13] for this neonate, it was done at day 6 when referred for immunological evaluation. Molecular assessment of TREC/KREC was performed on Guthrie cards from the patient and healthy newborns as control (beta-actin (ACTB) was also considered as a housekeeping gene). DNA purification from 3.2-mm punches of Guthrie cards and subsequent triplex quantitative Real-Time PCR were performed using a 7500 Real time PCR (Applied Biosystems®, USA) as previously described.[13]

EDTA-Blood sample was drawn at the first visit for further immunological evaluations including: the assessment of lymphocyte subsets (CD3, CD4, CD8, CD19 and CD16/CD56) and serum immunoglobulin classes. CD markers were measured using a flow cytometer (FACSCalibur™, Becton Dickinson, USA) and serum was derived after centrifugation (3000RPM, 5min).

To find out the exact mutation causing the immunodeficiency, gene sequencing analysis was performed on some target genes of blood-derived DNA of the patient and his parents. As the two previous children of the family were boys, we assessed the exons of *IL-2RG* gene. TREC/KREC assay and gene sequencing analysis were performed at the Jeffrey Modell Diagnostic and Research Center for Primary Immunodeficiencies, Municipal Hospital St. Georg, Leipzig, Germany.

This project has been approved by the ethics committee of IAARI.

We first screened the patient for TRECs and KRECs but could not find any copies of TREC (0 vs. 78±5 for 77 control samples), while the copy numbers of KREC was noticeably higher than those in the controls (1297 vs. 61±4 for 77 samples), suggesting a T-B+ SCID in the patient The ACTB, TREC and KREC copy numbers for paper punches of the T/B cell depleted (TBCD) and no template (NTC) controls were 3304, 0, 0 and 1.416, 0, 1.6 respectively.

The immunologic panel confirmed the diagnosis of SCID with low numbers of circulating T cells and NK cells. Although a decrease in peripheral blood CD4, CD8 and NK cells was observed, an increased frequency of B cells pointed to a T^- B^+ NK^- SCID phenotype. Flow cytometric results indicated a high percentage of B cells (63.57%) but low to zero percent T cells (1.43%) which is consistent with the lack of TRECs, but considerable numbers of KRECs in the PCR assessment. The levels of IgM and IgA were within the age-related normal ranges while it was slightly below the normal limit for IgG. All molecular

and immunological findings of the patient have been shown in table1.

As a result of gene sequencing analysis, we found a novel mutation in *IL2RG* gene. This point mutation has been resulted to a cytosine to alanine substitution (c.675 C>A) in the exon 5, leading to a serine-to-arginine replacement (in the position of p.225) (Figure 1). This change is predicted to be highly damaging using PolyPhen analysis (Polymorphism Phenotyping). The same genetic alteration was found in the patient's mother as a heterozygous mutation (data not shown).

Severe combined immunodeficiency (SCID), a type of PID, comprises a collection of over 20 distinct genetic disorders characterized by profound defects in both cellular immunity and specific antibody production. It is estimated to occur in 1 per 50,000 to 100,000 births, although true population incidence has been unknown prior to screening.[10] It may also be higher than previously reported, especially in Iran due to high overall incidence of consanguineous marriages,[15] particularly in families with congenital disorders like PIDs.[16,22]

More than 300 mutations in the IL2RG gene, located on the long (q) arm of the X chromosome at position 13.1, have been identified in x-SCID patients. Most of these mutations lead to the production of a non-functional version of the common gamma chain or prevent the protein from being produced.[4,17] Mutations in this gene result in very low T- and NK-lymphocyte counts, but the B-lymphocyte count is high (a so-called T-, B+, NK- phenotype).[18]

Table1. Laboratory findings of the newborn SCID patient

Lab tests		Results	Normal range*
WBC (per µl)		5720	11400(5000-20000)
Lymphocyte		1670	5500(2000-17000)
T cell subsets	CD3 count (%)	81.8 (1.43)	2500-5500 (53-84)
	CD4 count (%)	22.3 (0.39)	1600-4000(35-64)
	CD8 count (%)	64.6 (1.13)	560-1700(12-28)
B cells (%)	CD19 count (%)	3636.2 (63.57)	120-2100(14-76)
NK cell subset (%)	CD16/56 count (%)	85.8 (1.50)	170-1100 (3-17)
Immunoglobulins	IgM	20	13(6.3-25)
(mg/dl)	IgG	584	1121(636-1606)
	IgA	5	2.3(1.4-3.6)
triplex quantitative	ACTB/µl	7508	
Real-Time PCR	TRECs/µl	0	
(copy numbers)	KRECs/µl	1297	

*Normal ranges were deduced from Harriet Lane Handbook.[23]

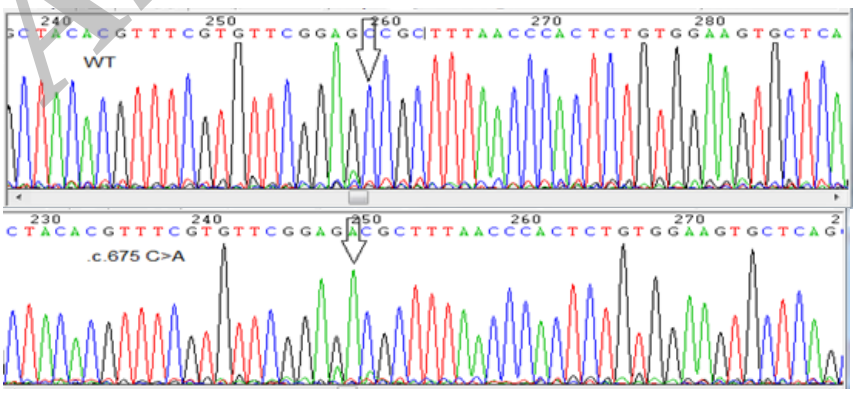

Figure 1. Sequencing analysis of genomic DNA representing the corresponding substitution of cytosine to alanine leading to a serine-to-arginine replacement.

Despite the high number of B-lymphocytes, there is no function since the B cells have abnormal receptors for growth factors on their cell surfaces.[4,19] In this case, we could screen the patient as a T^- B^+ NK^- SCID according to the TREC/KREC assessment which was in keeping with the percentage of lymphocytes. According to the significance of finding the accurate location of existing mutation, gene sequencing analysis was performed. Then, we surprisingly found a novel mutation in the *IL2RG gene* leading to an amino acid change (serine to arginine). It would make sense as there are two surrounding mutations reported in the IL2RG database, which result in an X-SCID patient. Lack of T cells and elevated numbers of B cells were also confirmed by the TREC/KREC assay. The TREC is the only assay currently performed in several countries[2,20,21] whereas the extended assay (incorporating KREC into the assay) is still at a pilot stage.

The SCID patient in this study was subsequently referred for hematopoietic stem cell transplantation (HSCT). Therefore, screening of newborns for PIDs, subsequent genetic counseling and educational programs are essential.

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