INTRODUCTION

X-linked severe combined immunodeficiency (X-SCID) is a rare, life-threatening disease, caused by mutations in the γc chain gene, which encodes an essential component of the cytokine receptors for interleukin-2 (IL-2), IL-4, IL-7, IL-9, IL-15, and IL-21. A 13-month-old boy with recurrent infections who had reduced serum immunoglobulin levels and decreased numbers of CD3, CD16/56 cells was evaluated for γc chain gene mutation and protein expression. The patient had a C-to-T point mutation at nucleotide position 690, one of the hot spots, resulting in a single amino acid substitution of cysteine for arginine (R226C), as determined by direct sequencing and PCR-RFLP. The patient’s mother was a heterozygous carrier. Percutaneous umbilical cord blood sampling was performed at the 6-month of gestation in a subsequent pregnancy. As the immunophenotype of the fetus showed an identical pattern, the pregnancy was terminated and genetic analysis of the abortus confirmed recurrence. This is the first report of the molecular diagnosis of X-SCID in Korea. Genetic analysis of the γc chain gene is useful for definite diagnosis and genetic counseling for X-SCID.

CASE REPORT

A 13-month-old boy was transferred to the Department of Pediatrics, Chonnam National University Hospital, because...
of refractory, progressive pneumonia, and nodular skin lesions. From the age of 7 months, he frequently visited his primary physician because of recurrent upper respiratory infections. The birth history was not contributory, but there was an infant death history of unknown etiology for his uncle on the mother's side. The pedigree of the family is shown in Fig. 1A. He received routine immunizations until 6 months of age, including BCG.

On admission, he was immediately intubated and maintained with an artificial ventilator because of severe, bilateral pneumonia causing respiratory failure. The blood counts on admission were: white blood cell, 45,100/μL (neutrophil 75.5%, lymphocyte 15.1%); hemoglobin, 14.4 g/dL; platelets 209,000/μL. His serum immunoglobulin profile was: IgG, 27.0 mg/dL (normal range, 345-1,236 mg/dL); IgM, 50.4 mg/dL (41-173 mg/dL); IgA, 22.9 mg/dL (14-159 mg/dL). Flow cytometric analysis of lymphocyte immunophenotype was: CD3 T cells, 2.5% (normal range, 60-85%); CD19 B cells, 95.4% (8-20%); CD16/CD56 natural killer cells, 0.5%. He was thus shown to have T-B+NK- SCID.

On physical examination, he had no BCG scar, and biopsy on the nodular skin lesion was later found to be a tuberculous granuloma. He was treated with broad-spectrum antibiotics, anti-tuberculosis agents, prophylactic antifungal agents, and intravenous globulins. He gradually recovered and was weaned off the ventilator on the 63rd hospital day. The recent blood counts without infection were: 5,300/μL (neutrophils, 58.5%; lymphocytes, 27.4%); hemoglobin, 12.5 g/dL; platelets 265,000/μL.

Blood samples were obtained from the patient and various family members with written informed consent. Heparinized venous blood samples from the patient and his family members were fractionated on a Ficoll-Hypaque gradient to isolate peripheral blood mononuclear cells (PBMCs). PBMCs were washed twice in PBS and 0.02% NaN₃, and analyzed by flow cytometry for γc chain expression using the PE-conjugated rat anti-human γc chain monoclonal antibody TUGh4 (Beckton-Dickinson, Mountain View, CA, U.S.A.). Incubation with unlabeled TUGh4 before the addition of labeled TUGh4 provided the background control staining. The method for immunofluorescence staining was the same as that described previously (16). As shown in Fig. 1B, PBMCs from the mother showed γc chain expression on both CD3- and CD19-positive populations. In contrast, cells from the patient showed a very depressed γc chain expression on CD3- and CD19-positive cells, suggesting both T and B cells from the patient did not express γc chain on the cell surface (Fig. 1B).

The eight exons of the γc chain and surrounding genomic sequences were amplified from genomic DNA as described previously (12). As shown in Fig. 2A, we found a single base substitution (C690T) at exon 5, resulting in an amino acid substitution (R226C) in the patient. His mother was found to have one mutant and one normal allele, indicating the carrier state. male fetus therapeutically aborted after immunophenotyping and genetic analysis. (B) Flow cytometric analysis of the patient and his mother. The level of γc chain expression in PBMCs was significantly reduced in the patient as compared with his mother. The level of γc chain expression was evaluated by flow cytometric analysis following immunostaining with mAb TUGh4.

![Fig. 1. Pedigree and results of flow cytometric analyses of the X-SCID family in this study. (A) Pedigree of the family. The closed square and the closed lozenge indicate the patient and the male fetus with X-SCID, respectively. The dotted circle indicates the carrier state. * male fetus therapeutically aborted after immunophenotyping and genetic analysis. (B) Flow cytometric analysis of the patient and his mother. The level of γc chain expression in PBMCs was significantly reduced in the patient as compared with his mother. The level of γc chain expression was evaluated by flow cytometric analysis following immunostaining with mAb TUGh4.](image-url)
umbilical cord blood was sampled percutaneously, and analyzed for lymphocyte phenotyping. The fetus had the same T-B+NK- phenotype (CD3 T cells, 1.2%; CD19 B cells, 78.6%; CD16/CD56 natural killer cells, 1.2%). Fetal genomic DNA isolated from amniotic cells was analyzed by RFLP for exon 5. As shown in Fig. 2C, the fetal DNA showed the same cleaved DNA pattern as the patient, while the healthy control DNA showed the normal pattern. RFLP analysis demonstrated that the fetus had the same mutation as his brother, and the pregnancy was terminated after lengthy counseling. The patient is now being cared for at our outpatient clinic, awaiting a stem cell transplant from an alternative donor, because he does not have an HLA-matched sibling.

**DISCUSSION**

Here, we report the first mutation analysis of the γc chain gene in a presumed X-SCID patient from a Korean family. The patient had a single point mutation (C690T) at exon 5, resulting in an amino acid change at codon 226 (R226C). Fetal genomic DNA isolated from amniotic cells was analyzed by RFLP for exon 5. As shown in Fig. 2C, the fetal DNA showed the same cleaved DNA pattern as the patient, while the healthy control DNA showed the normal pattern. RFLP analysis demonstrated that the fetus had the same mutation as his brother, and the pregnancy was terminated after lengthy counseling. The patient is now being cared for at our outpatient clinic, awaiting a stem cell transplant from an alternative donor, because he does not have an HLA-matched sibling.

**Fig. 2.** Sequence analysis showing a missense mutation of the common γc chain gene (γc chain) in an X-SCID family. (A) Genomic sequences encompassing a point mutation (C to T) in exon 5 of the γc chain gene from the patient, carrier (mother), and normal control (father) are shown. Arrow, C-to-T transversion. (B) RFLP analysis of the γc chain gene exon 5. Lanes 1 and 2, patient; Lanes 3 and 4, mother; Lanes 5 and 6, normal control. Lanes 1, 3, and 5 show the DNA fragments before restriction enzyme treatment, and Lanes 2, 4, and 6 show the DNA fragments after restriction enzyme treatment.

The male X-SCID patient in the present study had a classical, severe phenotype, and laboratory data showed low numbers of T cells, relatively well-preserved B cells, and reduced NK cell numbers. X-SCID is a disease that is characterized by severe lymphopenia and recurring persistent infections in the first months of life (17). A human Jak3-deficiency disease has similar clinical features to X-SCID (18), and the γc chain and Jak3 signaling pathway have been suggested to be crucial...
for T cell development, and to contribute to the X-SCID phenotype (18). In addition, gene disruption of either IL-7 (19) or the IL-7R subunit (20) has been shown to lead to severe developmental perturbations. Thus, signaling defects in the IL-7/IL-7R system resulting from changes in the \( \gamma c \) chain may account for the developmental anomalies that lead to X-SCID.

We also performed a prenatal diagnosis for this family, based on our genetic findings. Most of the primary immunodeficiency diseases can be diagnosed by means of screening for lymphopenia or for T cell deficiency in cord blood at birth. In addition, fully defining the molecular defects of patients is essential for genetic counseling of family members and prenatal diagnosis.

In conclusion, we described here the molecular and cellular identification of an X-SCID patient in a Korean family. Our data emphasize that flow cytometric analysis is an important tool for prenatal diagnosis for this family, based on our genetic findings. Most of the primary immunodeficiency diseases can be diagnosed by means of screening for lymphopenia or for T cell deficiency in cord blood at birth. In addition, fully defining the molecular defects of patients is essential for genetic counseling of family members and prenatal diagnosis.