대칭성 소두증과 발달지연으로 발현된 *FOXP3* 유전자 변이 1예

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FOXP3 Mutation in a Patient with Proportional Microcephaly and Developmental Delay

Most cases of microcephaly with growth failure and developmental delay have a genetic or metabolic etiology. Whole-exome sequencing (WES) has uncovered many causative genes and has also broadened their phenotypic spectrum. The present study applied WES to a boy with microcephaly, growth failure, developmental delay, seizures and atopic dermatitis, which reveal an unexpected frame-shift mutation (c.1248_1253delinsCT, NM_014009.3; p.Lys416Asnfs, NP_054728.2) in the forkhead box P3 gene (*FOXP3*). Mutations of this gene are known to result in immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. Mutation of *FOXP3* was reverified by Sanger sequencing in the proband and his carrier mother. Flow-cytometry expression study of *FOXP3* in peripheral white blood cells showed that the mean fluorescence intensity of *FOXP3* was lower in the proband than in a normal control. We report a mild form of IPEX syndrome without chronic protracted diarrhea or major infections, instead presenting with proportional microcephaly, growth failure, developmental delay, seizures and atopic dermatitis.

Key Words: Microcephaly, Failure to thrive, Growth and development, Gene, Seizures, Atopic dermatitis

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Introduction

The pathogenesis of microcephaly varies from genetic causes to environmental factors¹⁾. There are no reports of mutations of the forkhead box P3 gene (*FOXP3*) in patients with proportional microcephaly and developmental delay¹⁾. Such mutations have been found in young children with the rare and life-threatening disease of immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, whose clinical triad comprises (i) chronic enteropathy resulting in protracted diarrhea, (ii) eczematous dermatitis, and (iii) autoimmune endocrinopathy such as type 1 diabetes mellitus (DM) and thyroiditis²⁻⁹⁾. However, many patients with *FOXP3* mutations exhibit growth and developmental problems, and some have seizures and hypotonia²⁻⁶⁾.

The present study identified a novel *FOXP3* variant in a boy with mild phenotypes of IPEX syndrome. He presented with microcephaly, growth and

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developmental delay, seizures aggravated by respiratory acidosis, and atopic dermatitis. However, he had no severe diarrhea, major infections, or polyendocrinopathy. This report might broaden the phenotypic spectrum of *FOXP3*, which is a gene that could be considered in patients with similar developmental problems and atopic dermatitis.

Case report

A boy was born at a gestational age of 38 weeks weighing 2.8 kg (corresponding to the 10 percentile) after an uneventful pregnancy to healthy and unrelated parents (Fig. 1A). His growth failure was recognized when he was around 1 year of age. He weighed 7.0 kg, was 66.4 cm tall, and had a head circumference of 41.8 cm; all were below the 3 percentile. However, his parents did not perform further evaluations at that time since he showed normal development and severe atopic dermatitis from 3 month of age was a major concern of his parent. He had experienced recurrent minor infections in the respiratory and gastrointestinal tracts, but no protracted diarrhea. From 3 years, fishes, milk, soy milk, and cheese were restricted on his diet due to severe eczema, which ameliorated his symptoms. At 4 years and 10 months, he visited our hospital due to microcephaly, growth failure, and developmental delay. He weighed 9.1 kg, was 83.0 cm tall, and had a head circumference of 45.0 cm; all were below the 3 percentile. He had no dysmorphic features, problems in sensory organs, or extracranial malformation. He could not run well and was not able to go

stairs up using alternating feet. He could not copy squares or triangles, but he did show appropriate language comprehension and social development. No other abnormal findings were found in neurologic examination. The initial laboratory study showed iron-deficiency anemia, eosinophilia, markedly elevated serum immunoglobulin (Ig)E, and a slightly decreased levels of serum growth hormone, and IGF-binding protein 3. His bone age was 28–30 months. The findings of thyroid-function, chromosome, and chromosomal microarray tests were all normal. Fasting blood glucose, serum electrolytes, protein, and albumin, liver and renal function tests, and urinary analysis showed no abnormalities (Table 1).

After 1 month he was transferred to our hospital due to status epilepticus. He was intubated due to respiratory failure (pH 6.95 and pCO₂ 131 mmHg, PO₂ 83 mmHg). Blood glucose, electrolytes, ammonia, lactate and C-reactive protein were within normal limit, although hemoglobin was quite low (8.4 g/dL). This was his first seizure, and presented as focal to bilateral tonic-clonic seizures with impaired awareness. His seizures were easily controlled by fosphenytoin, levetiracetam, and carbamazepine, and no abnormalities were found in electroencephalography (EEG) or brain magnetic resonance imaging. EEG monitoring showed no subclinical seizures. However, hypercapnia (pCO₂>80 mmHg) recurrently occurred when weaning from mechanical ventilation, which caused the patient to fall into a narcosis and aggravated his seizures. No bacterial, viral, or fungal pathogens were isolated from the blood, urine, stool, or throat swab. A stool examination indicated a pH of 8.0 and no occult blood or pus cells. Before the next status

Fig. 1. Family pedigree (A) and electropherogram showing the sequence of the forkhead box P3 gene (*FOXP3*) variants (B).

Table 1. Comparison of Clinical Features between Our Proband and Patients with Typical Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) Syndrome²⁻⁹⁾

	Typical IPEX syndrome	Our proband
IPEX triad		
Gastrointestinal disease		
Enteropathy	Protracted diarrhea	Intermittent mild gastroenteritis
Early-onset autoimmune endocrinopathies		
Type 1 DM	Yes (related to anti-insulin and anti-GAD antibodies)	No
Thyroiditis	Hashimoto's or Graves' thyroiditis (related to anti-thyroglobulin and anti-microsome-peroxidase antibodies)	No*
Dermatitis		
Atopic dermatitis with eczema	Yes	Severe
Serum immunoglobulin E	Markedly elevated	Markedly elevated [†]
Specific allergen	Yes	Yes [‡]
Others		
Anemia	Yes (frequently Coombs-positive hemolytic anemia)	Chronic iron-deficiency anemia [†]
(no Coombs-positive hemolytic anemia)		
Thrombocytopenia/neutropenia	Possible (autoimmune)	No
Hypogammaglobulinemia	Possible	No
Infection and sepsis	Recurrent major infection	Intermittent mild respiratory and gastrointestinal infection
Nephropathy	Yes (glomerulonephritis, interstitial nephritis, or nephrotic syndrome)	No
Hepatitis	Possible (frequently autoimmune hepatitis)	No
Lymphadenopathy/hepatosplenomegaly	Possible (related to lymphocytic infiltration)	No
Pericarditis	Possible	No
Arthritis	Possible	No
Alopecia	Possible	No
Autoantibodies in connective-tissue diseases	Possible (antinuclear, anti-SCL-70, anti-Jo-1, anti-SS-A, anti-SS-B, anti-RNP, and antimitochondrial antibodies)	No*
Failure to thrive	Yes	Yes [†]
Developmental delay	Possible (hypotonia from birth in some patients)	Motor delay
Seizure	Seizures provoked by metabolic etiology related to DM or enteropathy (e.g., hypoglycemia or metabolic acidosis)	Recurrent late-childhood-onset status epilepticus aggravated by respiratory acidosis
Microcephaly	Possible	Yes

IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; DM, diabetes mellitus; GAD, anti-glutamic-acid decarboxylase; SCL, scleroderma; SS, Sjögren's syndrome; RNP, ribonucleoprotein.

*The antinuclear antibody test was nonspecific, and the antithyroglobulin, anti-microsome-peroxidase, anti-SCL-70, anti-Jo-1, anti-SS-A, anti-SS-B, and antimitochondrial antibodies were not found in our proband. [†]The initial laboratory study at 4 years and 10 months showed eosinophilia (2,100 cells/mm³, 10.8% of the circulating leukocytes), markedly elevated serum immunoglobulin (IgE, iron-deficiency anemia (hemoglobin=10.0 g/dL, serum iron=11 µg/dL, and total iron-binding capacity=395 µg/dL), and a slightly decreased levels of serum growth hormone (0.4 ng/mL, normal=0.7–6 ng/mL), insulin-like growth factor (IGF)-1 (37.76 ng/mL, normal=45–230 ng/mL), and IGF-binding protein 3 (1.375 mg/L, normal=1.4–3.0 mg/L).

[‡]Multiple antigen simultaneous test showed increased levels of IgE specific to house dust mites, soybean, peach, mackerel, crab, and shrimp.

epilepticus, he had been well on levetiracetam. However, his iron-deficiency anemia was refractory to sufficient iron supplementation.

To identify the genetic cause, whole-exome sequencing was performed on the proband and his parents. The Ion AmpliSeq Exome Kit (Life Technologies, Carlsbad, CA, USA) was used on an Ion Torrent platform. The variants were prioritized using our bioinformatics workflow. A potential frame-shift mutation of *FOXP3* (c.1248_1253delinsCT, NM_014009.3; p.Lys416Asnfs, NP_054728.2) was found in exon 10 of the X chromosomes of the proband and his mother, and this was reverified by Sanger sequencing (Fig. 1B). This mutation was not found in any known SNP databases including the Korean Reference Genome Database (622 Korean controls; <http://152.99.75.168/KRGDB/>

menuPages/intro.jsp). A flow-cytometry expression study of FOXP3 in peripheral white blood cells showed that the mean fluorescence intensity of the FOXP3-phycoerythrin channel was decreased in the proband (Fig. 2). Further studies including the blood lymphocyte subset populations and serum IgA, IgG, and IgM showed normal results. Autoantibodies in connective-tissue diseases or autoimmune thyroiditis were not found (Table 1).

At 8 months after the first visit he experienced status epilepticus again accompanied with respiratory failure and sudden cardiac arrest. Although he was transferred to our hospital with spontaneous recovery of systemic circulation, he was in a comatose mental state and died 2 months later due to ongoing multiorgan failure.

Fig. 2. Flow-cytometry expression study of FOXP3 in peripheral white blood cells from the proband (A) and a normal control (B). FOXP3 expression was measured using the mean fluorescence intensity (MFI). The MFI of FOXP3 was decreased in the proband (4.77) compared with that in a normal control (6.54). P, proband; C, control; PE, phycoerythrin.

Discussion

A rare X-linked disorder of IPEX syndrome with dysregulated autoimmunity and antimicrobial immunity is caused by mutations of *FOXP3*²⁻⁹. FOXP3 is an important transcription factor for the development and function of regulatory T cells (Treg), and the expression of this protein in Treg was found to be decreased in patients with *FOXP3* mutations^{3-5,9}. FOXP3 is composed of three domains: (i) the fork-head domain, (ii) the zinc finger and leucine zipper domain, and (iii) the N-terminal domain²⁻⁵. Most *FOXP3* mutations have been found in the fork-head domain as in our case³⁻⁵. However, a genotype-phenotype correlation has not been confirmed yet³⁻⁵.

Phenotypic spectrums of IPEX syndrome are broad, ranging from mild forms with one or two phenotypes of the triad, to typical form without an eventful perinatal history and more severe fetal/neonatal form²⁻⁹. Additional organ dysfunctions include autoimmune hematologic disorders (in about 50% of cases), renal diseases (in about 33%), and autoimmune hepatitis, recurrent infection, serositis, and neurologic abnormalities²⁻⁹. Although our patient had no protracted diarrhea or apparent endocrinopathy, he exhibited severe atopic dermatitis, recurrent minor infection, and chronic iron-deficiency anemia that was a clinical clue for chronic enteropathy²⁻⁹ (Table 1).

Many patients with IPEX syndrome exhibit developmental delay and failure to thrive, which might be due to the devastating course of the disease from infancy²⁻⁹. Some patients already have hypotonia as a fetus or at birth^{2,3,6}. However, the developmental problems have not been well described, being overshadowed by other life-threatening multiorgan dysregu-

lation. Our patient indicates that the developmental problems can be more severe for gross and fine motor functions than for language and cognitive functions, which might be due to gradually progressing muscle weakness caused by a chronic nutritional problem following enteropathy and unfavourable food restriction due to atopic dermatitis. As there is no clear evidence for the expression of *FOXP3* in the human brain, a FOXP3 mutation in our patient might indirectly cause secondary microcephaly through chronic mild enteropathy and diet restriction due to severe atopic dermatitis.

Seizures have been reported in some patients with IPEX syndrome². Rather than a genetic etiology, many seizures occur with a metabolic etiology (e.g., severe diarrhea and DM), which indicates the need to assess the blood glucose level, electrolytes, and metabolic acidosis^{2,3}. However, the seizures in our proband were aggravated by hypercapnic respiratory failure resulting in carbon dioxide narcosis, and progressed to status epilepticus. Since most IPEX patients have weak respiratory muscles and possibly chronic respiratory problems, respiratory acidosis has to be checked frequently and treated promptly in order to avoid life-threatening status epilepticus.

There are few treatment options for IPEX syndrome and they depend on the experiences of a small number of experts due to the rarity of the disease, including supportive treatment, immunosuppressive treatment (e.g., steroid; cyclosporine, tacrolimus, sirolimus, azathioprine, or methotrexate; or monoclonal antibodies), and bone-marrow transplantation^{2,3,5,8}. Although immunosuppressive treatment and bone-marrow transplantation are effective in some patients, there remain critical problems to be solved, including opportunistic

infections, drug toxicity, lymphoproliferative hemophagocytic syndrome, and graft-versus-host disease.

Herein we report a mild form of IPEX syndrome presenting with proportional microcephaly, failure to thrive, developmental delay, seizures, atopic dermatitis, and chronic iron-deficiency anemia, but without protracted diarrhea or recurrent major infections. A particularly notable observation was that his early-childhood-onset seizures were aggravated by hypercapnic respiratory acidosis resulting in carbon dioxide narcosis and had progressed to life-threatening status epilepticus. This case warns clinicians of the hazard of respiratory failure in IPEX patients. In addition, our patient had developmental delays in gross and fine motor functions that need to be investigated further in other IPEX patients. *FOXP3* could be considered in patients with hypotonia or motor delay with atopic dermatitis or recurrent infection.

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Ethics approval

This study was approved by the Human Research Ethics Committee of Chonnam National University Hospital (IRB number CNUH-2014-179). Informed consent to participate was obtained from the parents of the affected proband. The biospecimens were provided by the Chonnam National University Hospital Biomedical Research Institute Biobank with informed consent, under institutional review board-approved protocols.

요약

성장 및 발달 지연을 동반하는 대다수의 소두증은 유전적 원인 또는 대사이상 질환과 연관된 경우가 많다. 전장 유전체 분석은 이러한 질환에서 원인 유전자를 규명하는데 매우 유용하며, 단일 유전자 변이로 유발될 수 있는 임상양상이 얼마나 다양한지에 대한 이해를 넓혀주

고 있다. 본 연구에서는 심한 아토피 피부염과 성장 발달 지연, 경련을 동반한 소두증 환자에서 전장 유전체 분석을 시행하였고, 예기치 않게 forkhead box P3 유전자(*FOXP3*) 변이(c.1248_1253delinsCT, NM_014009.3; p.Lys416Asnfs, NP_054728.2)를 발견하게 되었다. 이 유전자에 변이가 있을 경우, X연관 유전, 면역 조절 이상, 다양한 내분비 기능 이상, 장기능 이상을 보이는 IPEX 증후군이 유발될 수 있다. 상기 *FOXP3* 유전자 변이는 기존 염기 서열 분석을 통해 환자와 보인자 어머니에서 재확인 할 수 있었다. 유세포 분석에서는 환자의 말초혈액 백혈구 *FOXP3* 단백질 발현이 정상인에 비해 감소되어 있었다. 우리는 만성적으로 지속되는 극심한 설사와 반복되는 주요 감염증을 동반하지 않는 경한 IPEX 증후군을 경험하였는데, 환자는 대청성 소두증, 성장 및 발달지연, 경련, 아토피 피부염만을 호소하여 IPEX 질환을 예상하지 못하였으나, 전장 유전체 분석을 통해 *FOXP3* 변이를 발견해 진단할 수 있었다.

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