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Extremely Early Onset IPEX Syndrome Caused by a Novel Small Exonic Deletion in *FOXP3*

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Case Report

A 37-week small for gestational age (birth weight 1.8 kg) white male infant was referred for evaluation at two weeks of age with a history of hyperglycemia and diarrhea. The pregnancy was remarkable for oligohydramnios and intrauterine growth restriction. There was no relevant family history. On day of life two, the patient developed hyperglycemia (536 mg/dL) necessitating an insulin drip. Initial genetic testing was normal, including chromosomal microarray analysis, as well as negative sequencing results for neonatal diabetes associated genes: *KCNJ11*, *INS*, *ABCC8*, and *PDX1*. Starting on day of life thirteen, the baby developed watery diarrhea. Laboratory testing showed a non-anion gap metabolic acidosis, eosinophilia, and low insulin levels. The diarrhea improved with cessation of feeds and stool electrolytes were consistent with an osmotic diarrhea. He was fed various different formulas (high MCT oil content and carbohydrate free formulas) without stool improvement. His fecal elastase was < 50 mcg/g (normal > 200 mcg/g). Abdominal ultrasound demonstrated a normal sized pancreas, although diffusely echogenic consistent with medical pancreatic disease. Pancreatic enzyme replacement did not result in clinical improvement. On repeat testing, fecal elastase was normal (449 mcg/g). At three weeks of age he developed a diffuse dry erythematous rash. As stool output increased independent of oral intake, transformation from an osmotic to a secretory diarrhea was suspected. At five weeks of age, an upper endoscopy was performed. Biopsies showed villous atrophy and increased cellularity in the lamina propria consistent with autoimmune enteropathy (1) (Figure 1A and 1B). Shortly thereafter, thrombocytopenia and Coombs positive hemolytic anemia were noted. With these additional findings, the diagnosis of Immune dysregulation, Polyendocrinopathy, Enteropathy X-linked (IPEX) syndrome was considered (2). Sequencing of the *FOXP3* gene revealed a novel mutation in the C-terminal

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portion of the forkhead DNA-binding domain resulting in a deletion of 9 nucleotides within the coding region (c.1227_1235delTGAGCTGGA) that is near other known causative mutations (Figure 2). This deletion causes an amino acid substitution of glutamic acid for aspartic acid at position 409 (p.Asp409Glu) followed by in-frame deletion of three additional amino acids (p.Glu410_Glu412del). As a result, few CD25⁺FOXP3⁺ regulatory T cells (Tregs) were observed in the CD4⁺ T cell population (Figure 3).

After the diagnosis of IPEX was confirmed, immunosuppressive therapy with cyclosporine A was initiated and within one week transient improvements in stooling, blood counts, and rash were noted. While waiting for the nutritional status to improve and for identification of a bone marrow donor, he developed cellulitis, recurrent bacteremia and worsening rash. At five months of age he underwent a reduced intensity allogeneic matched unrelated bone marrow transplant. He had several complications, including veno-occlusive disease, pulmonary hemorrhage, and candidemia, and died on day nine post-transplant.

Discussion

IPEX is a rare condition with a variable clinical phenotype, likely related to the specific mutation and degree of functional FOXP3 protein expression (2). FOXP3 is a transcription factor whose expression is primarily confined to a subset of T cells (Tregs) that play a primary role in regulation of immune responses (3). Mutation of the *FOXP3* gene (with subsequent alterations in protein expression) leads to an unchecked autoimmune response. This results in inflammation affecting multiple organ systems. While immunosuppressive therapy can alleviate symptoms, hematopoietic stem cell transplantation remains the only known cure for IPEX (4).

Our patient developed symptoms early in the postnatal period. In addition to insulin-dependent diabetes from the second day of life, his disease course progressed to multi-organ autoimmunity including enteropathy, cytopenias, and eczema. Unfortunately, he developed severe and recurrent infections, which contributed significantly to his ultimate demise.

FOXP3 is a member of a large family of transcriptional regulators that have a highly conserved forkhead DNA-binding domain. To date, approximately 55 gene mutations associated with IPEX have been described with many of these occurring in the forkhead domain (5). Our patient had a new, previously undescribed *FOXP3* mutation affecting the forkhead DNA-binding domain of the protein. The virtual absence of CD25⁺FOXP3⁺ Tregs in the CD4⁺ T cell population suggests that this mutation leads to a FOXP3 protein that is functionally defective and unable to sustain Treg development. The *FOXP3* gene sequences of both parents were normal, suggesting a *de novo* mutation in our patient.

Onset of insulin-dependent diabetes on the second day of life in our patient implies that an autoimmune attack of the pancreas began in utero, well before birth. This suggests that usual physiologic immunoregulatory mechanisms exerted by the placenta to prevent rejection of the fetus by the mother are insufficient to prevent autoimmunity in the baby in the absence of the infant's own Tregs. Diabetes is often, although not always, the first feature to develop in IPEX. Since there are now well over 20 gene causes for neonatal diabetes, the traditional

approach has been to proceed with testing of genes based on suggestive symptoms (6). However, as cost of gene sequencing continues to fall and comprehensive testing can be done more efficiently, it will become increasingly possible to make an early genetic diagnosis that will allow for prompt recognition and treatment of all syndromic features. While our patient had severe, early onset disease characterized by the classic phenotype of IPEX including enteropathy, endocrinopathy, dermatitis and other autoimmunity, it is important to remember that a significant proportion of IPEX patients have *FOXP3* mutations that lead to less severe disease that may not present with the full clinical spectrum of disease (7). We recommend that a clinical suspicion for IPEX be raised in any male patient with diabetes, particularly if they exhibit signs of enteropathy or other organ-specific autoimmunity. In all cases, the gold standard for confirming a diagnosis of IPEX is *FOXP3* gene sequencing.

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Figure 1.

1A Hematoxylin and Eosin staining at 33x magnification of a biopsied section of small intestine demonstrating villous blunting. 1B depicts a magnified image (132x) of the duodenal lamina propria demonstrating increased mononuclear and neutrophil infiltrates.

Figure 2.

Schematic representation of the FOXP3 protein. The bold arrow indicates the location of the novel mutation in the forkhead DNA-binding domain at amino acid position 409. The narrow arrows represent several previously identified mutations. RD, repressor domain; ZF, zinc finger; LZ, leucine zipper.

Figure 3.

Flow cytometry showing $CD25^{+}FOXP3^{+}$ regulatory cells in the CD4 T cell population in peripheral blood. The area within the dashed box shows the difference in expression of FOXP3 in the cells and the decreased percentage of FOXP3-expressing regulatory T cells in the patient compared to the control.