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CLINICAL CASE

New mutation in FOXP3 gene identified in an infant with chronic diarrhea as manifestation of autoinmune enteropathy - IPEX syndrome

Nueva mutación identificada en el gen FOXP3 en lactante menor con diarrea crónica como manifestación de enteropatía autoinmune - Síndrome IPEX

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What do we know about the subject matter of this study?

IPEX syndrome is a genetic disease caused by FOXP3 gene mutations, which produces immune dysregulation and affects several organs and systems. It has no specific treatment and presents a high mortality rate.

What does this study contribute to what is already known?

We describe a new mutation of the FOXP3 gene in a patient with severe disease expression.

Abstract

Introduction: The IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome is caused by the mutations of the FOXP3 gene, characterized by persistent diarrhea, endocrine disorders, and dermatitis. The treatment is the administration of immunosuppressive drugs, where hematopoietic stem cell transplantation is the only potential cure. **Objective:** To describe a new FOXP3 gene mutation, as well as the findings and evolution of a patient with IPEX syndrome. **Clinical Case:** Male infant presenting at one month of age with chronic diarrhea, intestinal failure, and recurrent infections. Lab tests and intestinal biopsy suggested autoimmune enteropathy. During follow-up, the patient presented resistance to immunosuppressive treatment with corticosteroids, cyclosporine, and tacrolimus, dying at 7 months of age due to vascular complications. He had a maternal family history of multiple deaths of men under 1 year of age. IPEX syndrome was suspected therefore a trio whole-exome sequencing was performed that showed a probably pathogenic FOXP3 gene mutation. **Conclusion:** A new FOXP3 gene mutation is reported in a patient with IPEX syndrome. Despite the low prevalence of this disease, it is important to recognize non-specific but suggestive symptoms for its diagnosis.

Keywords:
IPEX Syndrome; FOXP3; Autoimmune Enteropathy; Diarrhea

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Introduction

IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) is an X-linked recessive syndrome that affects almost exclusively males. Affected men inherit the variant to all their daughters who will be asymptomatic carriers, but not to all their sons.

This pathology results from FOXP3 gene mutations (forkhead box P3), located in Xp11.23. This gene encodes a transcription factor (*FOXP3 protein or scurfin*) consisting of 431 amino acids. The encoded protein is mainly expressed in lymphoid tissue and CD4+ - CD25+ cells, and it regulates the transcription of other genes involved in the development and function of regulatory T cells, which are responsible for mediating the immune response and tolerance to autoantigens[1,2].

The main manifestations of this syndrome are severe chronic diarrhea due to autoimmune enteropathy and endocrine and dermatological alterations secondary to an immune system dysregulation. Hemolytic anemia, thrombocytopenia, and glomerulonephritis may appear as coexisting diseases[3]. The main objective of management is the nutritional recovery and administration of immunosuppressants for disease control. To date, bone marrow transplantation is considered as the only potentially curative therapy of the entity.

It presents a poor prognosis with a high morbidity and mortality rate between the first and second year of life. There are reports of resistance to immunosuppressive therapy and adverse effects such as hepatotoxicity, nephrotoxicity, and increased infections[4], therefore, patients require a multidisciplinary approach to their management.

IPEX syndrome is an ultra-orphan hereditary disease (OMIM 304790)[5] with less than 300 reported cases worldwide in the reviewed literature. Due to its low prevalence, diagnosis is a challenge for health professionals.

The main objective of this article is to describe a new mutation of the FOXP3 gene, as well as the findings and evolution of a patient with IPEX syndrome.

Clinical Case

Male patient, second child of non-blood-related parents, no significant perinatal history, and family history of multiple deceased male infants on the maternal side, including an older brother with no clear cause of death and no autopsy (Figure 1). He was born at 38 weeks of gestation, weighing 2840 g and 50 cm of length, both in the normal range. At one month of age, he started presenting with chronic high-output diarrhea and progressive deterioration of his nutritional status presenting growth retardation, respiratory and urinary tract infections, and catheter-associated bacteremias.

Since the onset of symptoms, the patient was hospitalized in another institution, where it was suspected allergy to cow's milk protein, receiving management with hydrolyzed formula, free amino acids, and no carbohydrates, showing no clinical response, thus starting nutritional support with total parenteral nutrition. The following laboratory tests were performed: Calprotectin (489.9 ug/g, non-specific value in children under 1 year), negative stool reducing substances test, low stool pancreatic elastase (15 mcg/g, value possibly altered due to high-output diarrhea), pilocarpine iontophoresis (22 mmol/L), and immune profile which presented increased IgE (7524 mg/dl).

At 3 months and 15 days of age, he was referred to the San Ignacio University Hospital in Bogotá for management by pediatric gastroenterology, weighing 2340 g and 54 cm of length (weight/length, length/age and weight/age < -3 SD, WHO).

An endoscopic study showed marked flattened mucosa of the duodenum and erythema and hyperemia of the colonic mucosa (Figure 2). Light microscopy in the biopsies identified severe villous atrophy, absence of Paneth cells and goblet cells, increased lymphocytic infiltrate of apoptotic cells in the lamina propria, and findings suggestive of autoimmune enteropathy (Figure 3). Complementary tests were performed ruling out other pathologies (Table 1)[6].

A family history of multiple deceased male infants on the maternal side, along with chronic diarrhea, multiple infections, and increased IgE (4131 mg/dl), led to the suspicion of autoimmune enteropathy secondary to X-linked primary immunodeficiency. We requested a trio exome sequencing study required for patient admission to the hematopoietic cell transplant center.

The initial objective of the management was to improve his nutritional status. We adjusted the parameters of parenteral nutrition for patients with intestinal failure and started administering low volumes of free amino acid-based formula, achieving gradual weight gain, despite the high stool output. We added first-line drug therapy using methylprednisolone, however, after four weeks of treatment the patient persisted with high fecal output. In addition, we administered cyclosporine which caused hepatotoxicity and persistence of diarrhea, requiring changing treatment to tacrolimus, if sirolimus was not available.

At 7 months of age, while under immunosuppressive treatment and mixed nutritional support, the patient presented extensive thrombosis involving multiple blood vessels, making impossible to achieve new

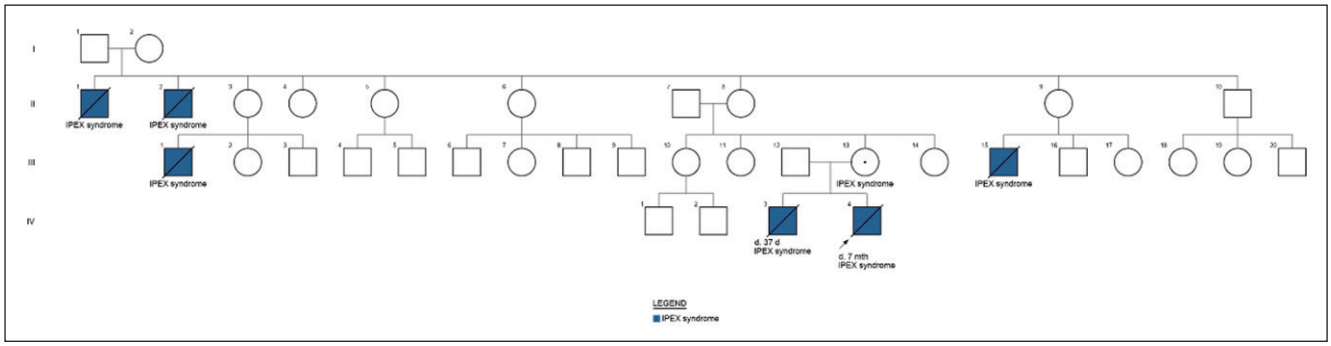

Figure 1. Index case genealogical tree in which a molecular diagnosis was made, identifying a mutation at the FOXP3 gene in him and his mother.

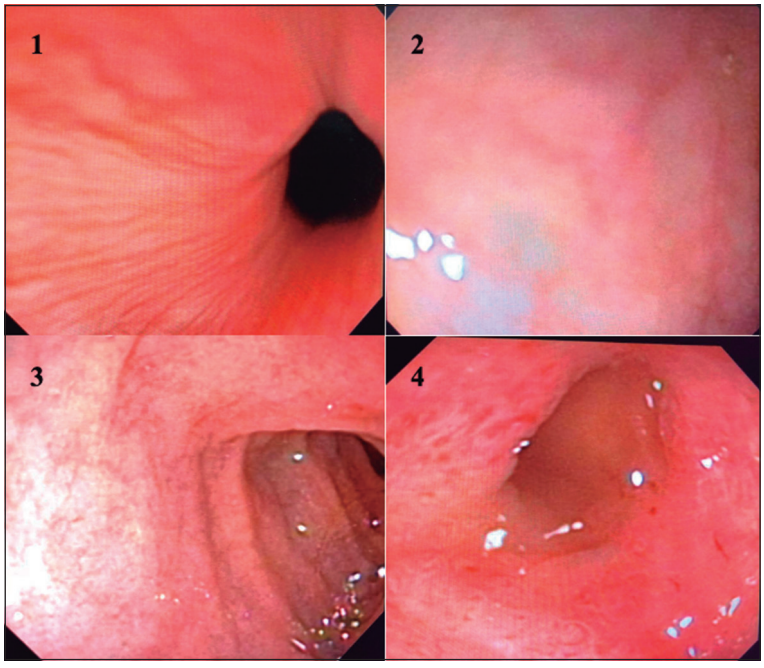

Figure 2. Diagnostic upper endoscopy and colonoscopy: **1.** Esophagus; **2.** Duodenal bulb; **3.** Second portion of the duodenum; **4.** Colon. Upper endoscopy with normal esophagus. Duodenum findings with mucosal congestion and severe villous flattening were observed. Colon with erythema, diffuse friability of the mucosa and multiple erosions less than 5 millimeters.

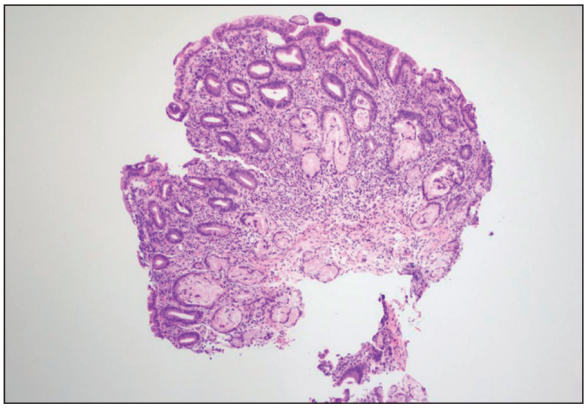

Figure 3. Duodenum biopsy showing villous atrophy, absence of Goblet and Paneth cells, and pseudopyloric metaplasia, with thickening of the lamina propria due to lymphoplasmacytic and neutrophilic infiltrate. Courtesy of doctor Rocío del Pilar López Panqueva.

venous access, thus parenteral nutrition was suspended, leading to the patient's death.

After death, the sequencing report was received which showed a variant in hemizygous state in the FOXP3 gene, *c.2T>C (p.Met1Thr)*, identified in the patient and his mother. This variant had not been previously reported in the literature or the databases reviewed.

Discussion

IPEX syndrome is caused by FOXP3 gene mutations. The loss of function in FOXP3 leads to immune dysregulation, characterized by an exaggerated response to stimuli, through the activation of T-cell receptors (TCR) and the release of inflammatory mediators,

Table 1. Laboratories

Parameter	Result	Normal values (3 months of age)
Immunoglobulin A	49.41 mg/dl	4.6 - 46 mg/dl
Immunoglobulin G	664.68 mg/dl	176 - 581 mg/dl
Immunoglobulin M	41.3 mg/dl	24 - 89 mg/dl
Immunoglobulin E	4131 mg/dl	0.18 - 3.76 mg/dl
CD3	63.2 %	51 - 77%
CD4	51.9 %	35 - 56%
CD8	11.7 %	12 - 23%
CD19	12.88 %	11 - 41%
CD4/CD8 ratio	4.4 %	
Albumin	1.8 g/dl	2.2 - 4.8 g/dl
TSH (Thyroid stimulating hormone)	0.99 mU/l	0.58 - 5.57 mU/l
Free thyroxine	0.94 ng/dl	1.04 - 2.86 ng/dl
Thyroperoxidase antibodies	18.4 IU/ml	
Thyroglobulin antibodies	4.11 IU/ml	
Direct Coombs test	Negativo	
Ferritin	3737 ng/ml	50-200 ng/ml
Vitamin B12	3539 pg/ml	200-835 pg/ml
Antinuclear antibody	Negative	
Extractable nuclear antigen antibodies Antineutrophil cytoplasmic antibodies	Negative Negative	
C4 (Complement component 4)	21.20 mg/dl	8.7 - 27 mg/dl
C3 (Complement component 3)	88 mg/dl	64 - 131 mg/dl

Data from Hughes H, Kahl L. The Harriet Lane Handbook. 21st ed. Elsevier; 2018. 1244p.

causing tissue damage and production of autoantibodies[3,7].

There are no accurate data on the prevalence of this entity. It is estimated that it affects 1 in every 1,600,000 people worldwide[8], however, epidemiological data are not available for Latin America.

The classic symptom triad of this entity consists of enteropathy, diabetes mellitus type I, and dermatological alterations (diffuse eczema and/or alopecia). Other manifestations of autoimmune character have been described such as hypothyroidism, hepatitis, hematological alterations (anemia, thrombocytopenia, and/or neutropenia), and alterations in kidney function[3]. However, endocrine and skin alterations can occur late and are not essential for diagnosis, as in our patient.

Enteropathy is characteristic in all cases, so the first symptom is usually diarrhea. Its relevance lies in the severity of it, which leads to intestinal failure and severe protein-energy malnutrition. Diarrhea is of early-onset, before 3 months of age, lasts longer than 6 weeks, watery, and may occur along with steatorrhea. The concomitant presence of bloody stools is unusual[9].

The diagnosis of this entity lies in the clinical suspicion when a male patient presents the described clinical findings and the identification of a FOXP3 gene mutation. An appropriate clinical approach, examining the patient's family history, is essential. In non-specific diagnostic tests, the lymphocyte count is usually normal in contrast to the increased eosinophil count in peripheral blood.

In relation to cellular immunity, a slight increase in CD4+ count can be observed, and regarding humoral immunity, IgG, IgM, and IgA titers are normal, except for IgE levels which are often highly elevated. Also, anti-enterocyte and anti-colonocyte IgG antibodies may be present[3].

More frequent pathologies such as allergy to cow's milk protein are often overdiagnosed, delaying diagnosis. Other diseases that should be considered in the differential diagnosis of early-onset chronic diarrhea are infectious diseases and cystic fibrosis, among others (Table 2)[10]. Biopsy by endoscopy and colonoscopy is mandatory when autoimmune enteropathy is suspected.

Duclaux-Loras et al. conducted a retrospective multicenter study with 30 patients with IPEX Syndrome. Out of those patients, 19 underwent diagnostic endoscopy, finding severe inflammation of the upper gastrointestinal tract and colon[11]. The classic histological findings are severe villous atrophy, hyperplasia of

Table 2. Causes of chronic diarrhea in the first year of life

Infectious	*Bacterias*	*Parasites*
	- *Escherichia Coli*	- *Giardia lamblia*
	- *Clostridium difficile*	- *Cryptosporidium*
	- *Salmonella*	- *Entamoeba histolytica*
	- *Campylobacter*	- *Strongyloides stercolaris*
	- Small intestinal bacterial overgrowth	
	Virus	*Postenteritis syndrome*
	- Rotavirus	
	- Human noroviruses	
	- Human astroviruses	
	- HIV	
Inflammatory	- Eosinophilic gastroenteritis	
	- Celiac disease	
	- Very early onset inflammatory bowel disease	
	- Primary and secondary immunodeficiencies	
	- Food allergies (Cow milk protein allergy)	
Malabsorption	*Carbohydrate Malabsorption*	
	- Lactose intolerance	
	- Fructose intolerance	
	- Postenteritis syndrome	
	Fat Malabsorption	
	- Cholestasis: Biliary atresia, progressive familial intrahepatic cholestasis, inborn errors of bile acid metabolism	
	- Exocrine pancreatic insufficiency: Cystic fibrosis, Shwachman-Diamond syndrome, chronic pancreatitis, hereditary pancreatitis	
	- Decreased enterohepatic circulation of bile acids: Necrotizing enterocolitis, intestinal volvulus, intestinal ischemia	
Congenital	- Congenital chloride diarrhea	
	- Congenital sodium diarrhea	
	- Congenital glucose-galactose malabsorption	
	- Microvillous inclusion disease	
	- Tufting enteropathy	
	- Autoimmune enteropathy	
	- IPEX/APECED syndrome (Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy)	
Endocrinological disorders	- Hyperthyroidism	
	- Gastrinoma	
	- VIPoma	
	- Neuroblastoma	
	- APUDoma	
	- Ganglioneuroma	

Adapted from Rishi Gupta. Diarrhea. Wyllie, Robert, MD; Hyams, Jeffrey S., MD; Kay, Marsha, MD. Pediatric Gastrointestinal and Liver Disease. 5th edition. Elsevier, 2016. p. 104-114.

the lamina propria with mononuclear inflammatory infiltrate, and crypt hyperplasia with evidence of apoptotic cells, as occurred in our patient. These findings are not pathognomonic of IPEX syndrome and can be found in autoimmune enteropathy of different etiology[12].

The population frequency of the variant in the FOXP3 gene reported in our patient is unknown. According to the American College of Medical Genetics' criteria, it is classified as probably pathogenic. This change is a frameshift mutation that affects the first amino acid of the protein in a highly conserved position. This variant was analyzed with 13 bioinformatic predictors (PolyPhen, MutationTaster, SIFT, among others), of which 12 classified it as pathogenic. The bioinformatic analysis along with the report of other variants in the same position in the literature indicates that it causes the loss of the first amino acid methionine due to a displacement of the reading frame, changing the sequence of the protein with loss of several functional domains of it.

This case describes the importance of genetic studies in very low prevalence pathologies, where the etiological diagnosis is complex due to the diversity and overlapping of symptoms that they share with other clinical entities. Complete exome sequencing is a key tool in monogenic diseases, where diagnosis through other tests is not possible. Its importance lies in establishing prognosis, treatment, and offering genetic counseling to the family, advising the risks in family planning, and presenting the available options to decrease reoccurrence in the offspring. This reduces future costs and the emotional impact on the family and social environment of another child with the disease.

The approach to the patient requires a multidisciplinary team, establishing timely support measures in order to correct secondary metabolic and electrolyte disorders. The administration of glucocorticoids to reduce the inflammatory response is proposed as first-line management, although recent studies suggest the use of Sirolimus (rapamycin) as a first-line treatment with promising results[13]. This drug is not yet available in our country.

In addition to the use of corticosteroids, other immunotherapies have been evaluated such as Cyclosporine, Tacrolimus, and Cyclophosphamide, and less frequently the use of Azathioprine and Mycophenolate Mofetil. Co-administration of these drugs has shown to be effective as maintenance therapy and to decrease the long-term complications of chronic corticosteroid use[9].

Patients who are refractory to immunosuppressive management are candidates for biological therapy with Infliximab, Adalimumab, and Abatacept. To date,

bone marrow transplantation has proven to be the only curative therapy in patients with IPEX syndrome[9].

The survival rate at 15 years in patients undergoing bone marrow transplantation was 73.2%, compared to 65.1% in those who only received immunosuppressive treatment, so far it is the best therapeutic option in patients with this disease[14].

Conclusions

In the context of IPEX Syndrome, early-onset chronic diarrhea is of interest since it is a rare disease. Clinical suspicion may be based on a good anamnesis, interpretation of family history, and laboratory and anatomopathological findings. It is imperative to confirm the diagnosis through molecular study, which allows the description of known and new mutations, as in our case. This will better characterize the disease in order to timely provide comprehensive management and genetic counseling to families.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

Financial Disclosure

Authors state that no economic support has been associated with the present study.

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