Underlying IPEX Syndrome in a Patient With Idiopathic Juvenile Arthritis and Vitiligo

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Case report

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Abstract

IPEX syndrome (MIM #304790) also known as immune dysregulation, polyendocrinopathy, enteropathy, X-linked is a monogenic inborn error of immunity due to loss-of-function mutations in the forkhead box 3 (FOXP3) gene. This gene is crucial for the development, maturation and maintenance of $CD4^+$ regulatory T (T-reg) cells. Various phenomenon mainly of autoimmune origin are characteristics of the syndrome such as enteropathy, endocrinopathies, cytopenias, renal disorders and skin manifestations (1).

Introduction

In contrast to the original description of multi organ involvement, the disease seems to have more a single system involvement as a clinical presentation. Immune mediated phenomena such as arthralgia or transient arthritis seems to be rare as it affects 5,1% (2/195 patients) of the already reported FOXP3-IPEX patients. Furthermore, skin involvement in those patients is mainly characterized by eczema or dermatitis in both, single and multiple organ involvement (2).

Here we report the case of a Brazilian patient with juvenile idiopathic arthritis, eczema and vitiligo as the clue for the late diagnosis of IPEX syndrome harboring the A384T mutation in the FOXP3 gene.

Patients And Methods

Clinical Data and Genomic Sequencing

We retrieved clinical data from patient's records, after parents signed a written consent for the publication of any potentially identifiable images or data included in this article. We extracted Genomic DNA from blood samples using a QIAamp® DNA Blood Maxi Kit (Qiagen®, Valencia, CA, USA) and obtained PBMC by density gradient centrifugation (d=1,077g/ml). We designed direct primers to FOXP3 gene exon 12. We performed Sanger sequencing for genetic confirmation and familial segregation using standard procedures.

Quantification of lymphocytic FOXP3 phenotypes by flow cytometry

Peripheral blood mononuclear cells (PBMC) from the patient previously separated by Ficoll density gradient centrifugation and cryopreserved were unfreezed, washed with 1x PBS and stained with titrated mouse anti-human monoclonal antibodies (mAbs). Regulatory T cell subset were characterized by: anti-CD3 FITC, anti- CD4-AmCyan, anti-CD8 Pacific Blue, anti-CD127 PE, FOXP3 PerCp-Cy5.5, CD28 APC and CD25 PE-Cy7. All mAbs were from BD Biosciences, except Foxp3 (eBioscience/Invitrogen). We added a viability marker: FVS 780 (BD Biosciences). The surface staining (mAbs diluted in cold 1x PBS) was performed for 30 minutes at 4°C. For intracellular staining of FOXP3, cells were washed, fixed and permeabilized with Transcription Factor Buffer Set (BD Biosciences) immediately after surface staining, according to the manufacturer's instructions. Fluorescence minus one (FMO) controls were set up for FOXP3 markers.

Results

Case Report

The patient is a 15 years old boy born from non-consanguineous parents from the state of São Paulo, Brazil. He was sent to our evaluation at the age of 4 years due to a recalcitrant atopic dermatitis that, at that time, was associated with a possible milk and egg allergy. In addition, the patient had rhinitis and asthma without sensitization to the most common allergens in Brazil. An oral food challenge with egg and milk ruled out food allergy and the first skin biopsy confirmed the initial suspicion of eczema possible related to atopic dermatitis. Because of the recalcitrant skin condition, the patient was medicated with oral cyclosporine (dose 2,5-4,2 mg/kg/day) for three years and discontinued after remission of the skin condition.

At the age of 7 years during routine laboratory work up a total IgA deficiency was found. Also at seven, the patient begun to complain of diffuse arthralgia with enlargement of knees and the diagnosis of Idiopathic Juvenile Arthritis was done. Anti-TNF (Etarnecept - dose 45 mg/2 weeks) and methotrexate (17,5 mg/week) was initiated. After 5 years of immunosuppression alopecia areata, vitiligo and diarrhea started. A colonoscopy was performed and lymphangiectasia, ileitis, colitis and retitis was found which led to a presumably diagnosis of inflammatory bowel disease. Anti-TNF and methotrexate was discontinued and oral mesalazine was initiated with partial resolution of the diarrhea.

All relevant laboratory analysis, clinical and pathological results are shown in Figure 1 and 2.

Genetic Analysis

Due to a suspicion of an inborn error of immunity a target gene panel of 207 genes (Invitae Primary Immunodeficiency Panel) was requested. An already reported mutation in the FOXP3 gene was found c.1150G>A; p.Ala384Thr, exon 11 in homozygous status. The mutation was therefore confirmed in the index patient and in the mother by Sanger sequencing (Figure 1).

Peripheral quantification of FOXP3 lymphocytes

Both patient and mother expressed normal levels of CD3 and CD4 T cells. FOXP3 expression was observed in lower frequency in the proband when compared to the mother (Figure 2) and to a healthy control.

Discussion

Here we report a case of a late diagnosis of IPEX syndrome in a Brazilian patient harboring an already reported mutation in FOXP3 gene and with a mild phenotype.

Clinical phenotype linked to IPEX syndrome were originally attributed to the acronym of IPEX (immune dysregulation, polyendocrinopathy and enteropathy) inherited in a X-linked pattern (1) . However, many

cases have already been reported lacking the classical severe phenotype. Special attention should be paid to patients like herein reported that initially present with a single symptom, such as eczema, and that progressively accumulate additional findings that culminates with the diagnosis of Inborn Error of Immunity (3, 4). With this report we also expand the clinical findings of IPEX syndrome. Skin manifestations is a common find in IPEX but findings suggestive of vitiligo has not yet been reported. Additionally, immunedysregulatory findings consistent with juvenile idiopathic arthritis is also a novel finding (2) . Such findings are very important not just to expand the constellation of symptoms of IPEX syndrome but also to highlight the necessity to consider genetic screening in patients harboring multiple autoimmune/inflammatory phenomena.

Immunological aspects of IPEX also deserves a special overview. Selective immunoglobulin A deficiency (SIgA-D), as here reported, is not common in IPEX syndrome and usually low levels of all immunoglobulins is observed in patients with severe enteropathy (2). Curiously, SIgA-D is commonly reported in patients with polyendocrinopathy a fact not observed in our patient. This is also of great importance once patients with SIgA-D with many immunedysregulatory phenomena should as well be considered candidates for FOXP3 gene mutations. Peripheral FOXP3 expression is diminished in patients with IPEX syndrome, as here observed, but normal levels of FOXP3 have already been reported (3, 5, 6) . For instance, patients with IPEX syndrome that lack the genetic finding and have low expression of FOXP3 can have a broad spectrum of genetic basis, what demonstrates the genetic variability associated to the same phenotype (3).

Treatment of IPEX vary and seems to be dependent of the severity of the disease manifestation. Data available addressing the treatment regimen for the disease is mainly composed of hematological stem cell transplantation (HSCT), steroids and other immunosuppressive therapies, such as observed in our patient. However, data regarding the specific moment that HSCT should be indicated is not available and the same occurs for the ideal drug to immune modulate mild inflammatory phenotypes (7).

The first description of A384T related to IPEX dates from 2001 in a large family with many premature deaths. At that time, the authors found three patients alive in the same family harboring the mutation A384T without more clinical details (8). Although the residue threonine at the position 384 is similar to the the most common amino acid found in this residue, animal models demonstrated that this mutation leads to IPEX syndrome and therefore impacts the homeostasis of T-cell activation. Later, genotype-phenotype correlations found that mutations localized in the forkhead domain of the gene, as here reported, were more significantly to be associated to alopecia areata as found in our patient. However, many other phenotypes not observed in our patient was also found to be statistically significant in the same domain, such as anemia. Curiously mutations along the poly A seems to be more related to immunedysregulatory findings, wether the mutation herein reported is located in the distal part of the gene and is clinically represented of many immunedysregulatory findings. (2)

Conclusion

IPEX syndrome is a rare but often fatal multi systemic autoimmune disease that manifests with a heterogeneity of phenotypes. Therefore, it is imperative to consider it as a diagnosis in patients affected by multiple autoimmune/inflammatory phenomenas even if those manifestations trespass the classic clinical description of the syndrome and regardless of the onset of those symptoms. Attention shall be paid when screening patients with peripheral FOXP3 expression as normal levels can have a genetic background and many other genes can be implicated in low expression of FOXP3. The widespread use of large panels/exome sequencing has guaranteed the final correct diagnosis and has prompted arrangement of the adequate interventions and treatments resulting in improved quality of life and patient survival.

Abbreviations

IPEX immune dysregulation, polyendocrinopathy, enteropathy, X-linked

FOXP3 Forkhead Box 3

DNA Deoxydribonucleic acid

CD cluster differentiation

S-IgAD Selective Immunoglobulin A Deficiency

Declarations

Ethical Approval and Consent for publication: Written informed consent for publication was obtained from all included in the study.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Ethical Approval: The study is approved at the Hospital das Clínicas, University of São Paulo, School of Medicine. The number of study approval is CAAE 74305817.0.0000.0068.

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Figures

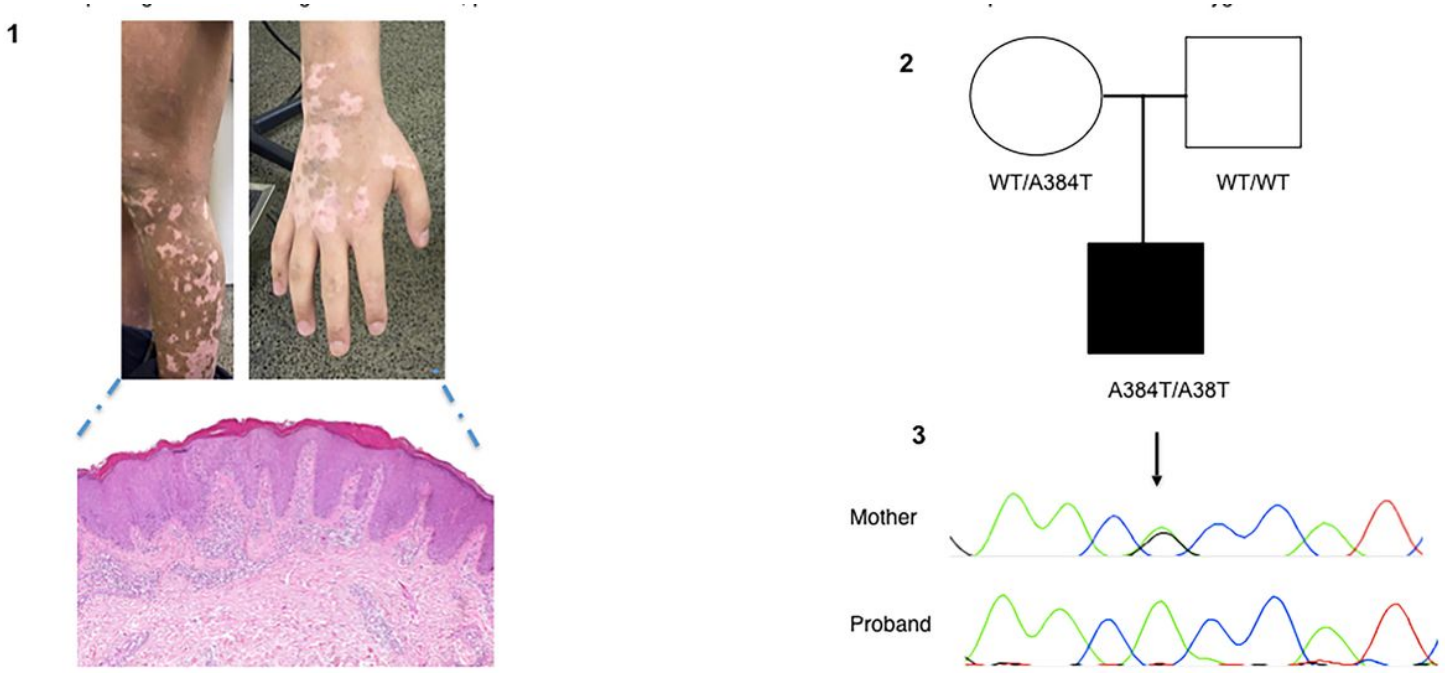

Figure 1

Skin and pathological findings (1), familial pedigree (2) and electropherograms (3). In 1 - Vitiligo-like lesions observed in the legs and hand. Skin biopsy (left forearm), histopathological findings are: the epidermis shows parakeratosis, areas of hypogranulosis, acanthosis and mild spongiosis. The dermis exhibits a perivascular and superficial inflammatory infiltrate of lymphocytes and scattered neutrophils, as well as fibroplasia and verticalized papillary collagen fibers (hematoxylin-eosin, 100x).. In 2 - Familial

pedigree evidencing the affected proband in black quarter. In 3 - Electropherograms evidencing the c.1150G>A; p.Ala38Thr mutation in FOXP3 found in the affected proband and in the mother.

A

Laboratorial analysis	Results	Reference Range
Hemoglobin	13,9 g/dL	14,4 - 16,6 g/dL
Hematocrit	40,6%	43 - 49 %
Leukocytes	10.890/mm³	4.500 - 12.000 /mm³
Neutrophils	7.860/mm³ (72%)	1.800 - 8.000/mm³ (40-75 %)
Eosinophils	10/mm³ (0,1%)	0,00 - 500/mm³(0-6%)
Monocytes	1.040 /mm³ (9,6%)	50 - 800/mm³ (2-10%)
Platelets	216.000 /mm³	150.000 - 400.00/mm³
Immunoglobulin G	1788 mg/dL	716-1711 mg/dL
Immunoglobulin A	**0,00 mg/dL**	47-249 mg/dL
Immunoglobulin M	62,2 mg/dL	15-188 mg/dL
Immunoglobulin E	0,2 UI/ml	until 200 UI/ml
ANCA – C	1/40	Non Reactive
ANA	Non Reactive	Non Reactive
Anti-ENA	Non Reactive	Non Reactive
CD3	1540 cells/mm³ (78%)	1.000 - 2.000 cells/mm³ (56-84%)
CD4	993 cells/mm³ (50%)	530 - 1.300 cells/mm³ (31-52%)
CD8	474 cells/mm³ (24%)	330 - 920 cells/mm³ (18-35%)
CD19	341 cells/mm³ (17%)	110 - 570 cells/mm³ (06-23%)
NK	36 cells/mm³ (2%)	70 - 480 cells/mm³ (03-22%)
DNT cells – CD3	1,62%	< 1,5%
DDNT– T total	1,98%	< 2,5%

B

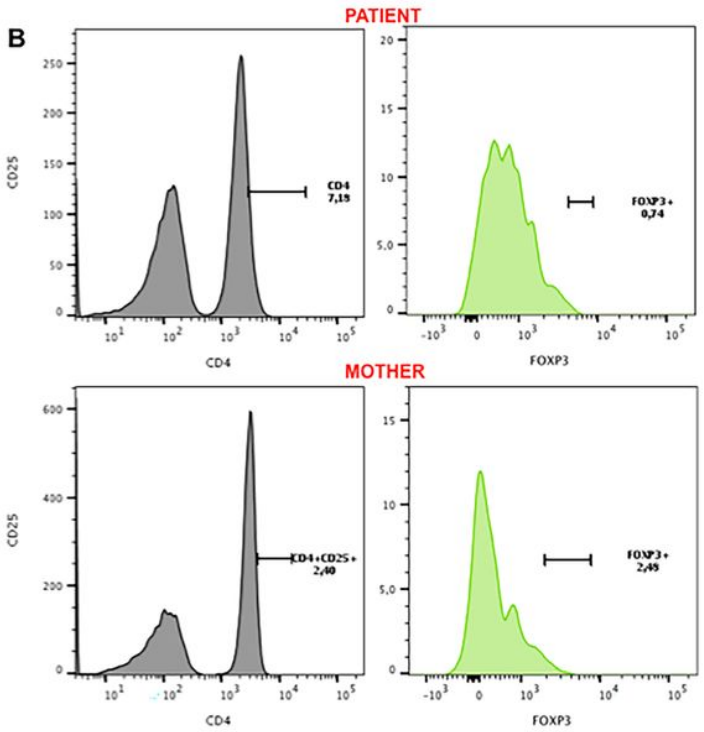

Figure 2

Baseline laboratory findings and peripheral FOXP3 expression. In A - Baseline laboratory findings and in bold absent levels of IgA. In B - Representative histograms of the index patient (upper) and the mother (lower) demonstrating low levels of CD25+CD4+FOXP3 in the index patient.