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ORIGINAL ARTICLE

Analysis of *FOXP3* gene in children with allergy and autoimmune diseases

R.M. Pacheco-Gonzalez[a], C. Avila[b,c], I. Dávila[c,d,**], A. García-Sánchez[c], L. Hernández-Hernández[c], D. Benito-Pescador[c], R. Torres[e], P. Prieto-Matos[c,e], M. Isidoro-Garcia[c,f,g,*], F. Lorente[c,e], C. Sanz[c,h]

[a] *Department of Paediatrics, Clinic Hospital Virgen de la Arrixaca, Murcia, Spain*
[b] *Department of Statistics, University of Salamanca, Salamanca, Spain*
[c] *Biosanitary Institute of Salamanca IBSAL, Salamanca, Spain*
[d] *Department of Allergy, University Hospital of Salamanca, Salamanca, Spain*
[e] *Department of Paediatrics, University Hospital of Salamanca, Salamanca, Spain*
[f] *Department of Clinical Biochemistry, University Hospital of Salamanca, Salamanca, Spain*
[g] *Department of Medicine, University of Salamanca, Salamanca, Spain*
[h] *Department of Microbiology and Genetics, University of Salamanca, Salamanca, Spain*

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Abstract
Background: Allergy and autoimmunity are important immunological entities underlying chronic diseases in children. In some cases both entities develop simultaneously in the same patient. *FOXP3* gene codes for a transcription factor involved in regulation of the immune system.

Considering that regulatory T cells are involved in controlling immunological disease development, and the relevant role of *FOXP3* in this kind of T cells, the objective of this study was to analyse the *FOXP3* gene in the most prevalent autoimmune diseases and/or allergies in childhood in a European population.

Methods: A total of 255 Caucasian individuals, 95 controls and 160 patients diagnosed with allergic, autoimmune or both diseases were included in this study. The molecular analysis of *FOXP3* was performed by DNA sequencing following the recommendations for quality of the European Molecular Genetics Quality Network. Genomic DNA was extracted from peripheral blood of all participants and was amplified using the polymerase chain reaction. After the visualisation of the amplified fragments by agarose gel-electrophoresis, they were sequenced.

* Corresponding author.
** Corresponding author.
E-mail addresses: idg@usal.es (I. Dávila), misidoro@usal.es (M. Isidoro-Garcia).

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Results: Thirteen different polymorphisms in *FOXP3* gene were found, seven of which had not been previously described. The mutated allele of SNP 7340C>T was observed more frequently in the group of male children suffering from both allergic and autoimmune diseases simultaneously ($p = 0.004$, OR = 16.2 [1.34–195.15]).
Conclusions: In this study we identified for first time genetic variants of *FOXP3* that are significantly more frequent in children who share allergic and autoimmune diseases. These variants mainly affect regulatory sequences that could alter the expression levels of *FOXP3* modifying its function including its role in Treg cells.

Introduction

Chronic diseases affecting children produce a significant impairment in their quality of life, an alteration in familial and social relationships, and an increase in morbidity and mortality. Many of them are of immune origin, such are allergic and autoimmune diseases. Allergies are due to an immunological response to a usually innocuous antigen (allergen) resulting in the proliferation of T-helper type 2 (Th2) lymphocytes. Autoimmune diseases are produced by an immune response with proliferation of Th1 lymphocytes, which react against own antigens recognised as foreign (autoantigens). Genetic and environmental factors participate in their pathogenic mechanisms. Asthma, rhinoconjunctivitis (RC) and atopic dermatitis (AD) are the most prevalent allergic diseases, and type 1 diabetes mellitus (DM1), coeliac disease (CD) and autoimmune thyroiditis (AT) stand out among autoimmune diseases.

The IPEX syndrome (immunodysregulation, polyendocrinopathy, enteropathy, X-linked), also called XLAAD (X-linked autoimmunity-allergic disregulation), encompasses both immune pathologies. Forkhead Box P3 (*FOXP3*) was identified as a gene involved in this syndrome.[1] However, in some patients with IPEX symptoms no mutations have been identified in the *FOXP3* coding region being diagnosed as IPEX-like syndrome.[2,3] Mutations have been described in the regulatory regions in some of them.[2]

FOXP3 gene, also called *JM2*, is located in Xp11.23 and has eleven coding and three non-coding exons (Fig. 1). It is expressed mainly in a subgroup of CD4+ cells, called T regulatory cells (Treg).[4] The FOXP3 protein is the most important marker of this cell subtype. Its function is not completely established, but it is involved in Treg cell activation.[5,6]

Treg cells represent 5–10% of CD4+ T cells.[7] They also express the alpha chain of the IL-2 receptor, CD25. In addition, cytotoxic T-lymphocyte antigen 4 (CTLA-4) and glucocorticoid induced tumour necrosis factor receptor family related gene (GITR) are also important for Treg function.[4,5] These Treg cells regulate the innate and adaptive immunity, thus contributing to the maintenance of immune tolerance.[6,7] When their function is affected, autoimmune and allergic diseases can appear. Moreover, Treg cells regulate the immune system involved in host defence against infection and tumour proliferation.[7,8] The mechanisms involved in these immunosuppressive effects are not well elucidated; some studies suggest they are cell contact-dependent,[9,10] while others report them to be cell contact-independent.[10] FOXP3 is thought to interact to DNA assisted by FOXP3 cofactors orchestrating a transcription network by multimerisation. Thus FOXP3 functions may change depending on the partners forming the so-called FOXP3 interactome. FOXP3 is considered a multifaceted transcription factor that regulates immune homeostasis.[11] It stimulates the production of inhibitory cytokines, like interleukin-10 (IL-10) and tumour growth factor-β (TGF-β), suppressing IL-2 and IL-4. When this regulation is not present, autoimmunity and allergy can emerge.[5,7]

Considering that Treg cells are involved in controlling the development of autoimmune and allergic diseases, and the relevant role of FOXP3 in these cells, our hypothesis was that FOXP3 could be involved in the development of immune diseases and the objective of this study was to analyse the *FOXP3* gene for polymorphisms associated with the most prevalent autoimmune and/or allergic diseases in children.

Materials and methods

Participants

This is an observational, case-control study in which 255 non-related Caucasian individuals were included: 95 controls and 160 patients that were recruited at the Allergy and Paediatric Departments of the University Hospital of Salamanca. The study was performed following the recommendations of the Hospital's Ethics Committee. An informed written consent was obtained from the children's parents or caregivers.

All patients were under 18 years old, and were affected by one or more autoimmune diseases (DM1, AT and/or CD) and/or atopy. Diagnosis was according to World Health Organization and American Diabetes Association criteria for DM1;[12] thyrotropin up to 10 μU/mL plus thyroid autoantibodies for AT;[13] the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) criteria for CD;[14] atopy was defined by a positive skin prick test to at least one of a battery of common aeroallergens previously described;[15] in addition, patients had rhinoconjunctivitis, and/or asthma and/or atopic dermatitis diagnosed by an allergologist.

Controls were selected from adults, confirming the absence of atopy or autoimmune disease during childhood and adulthood.[16] The following inclusion criteria were required: (i) absence of autoimmune diseases; (ii) absence of clinical symptoms compatible with bronchial asthma or other respiratory diseases; (iii) absence of rhinoconjunctivitis or atopic dermatitis; (iv) absence of other allergic