Immunology Letters 175 (2016) 40–43

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

STAT4 confers risk for rheumatoid arthritis and systemic lupus erythematosus in Mexican patients

O. Beltrán Ramírez[a], J.F. Mendoza Rincón[b], R.E. Barbosa Cobos[c], I. Alemán Ávila[a], J. Ramírez Bello[a,*]

[a] Research Unit in Endocrine and Metabolic Diseases, Hospital Juárez de México, Mexico City, Mexico
[b] Laboratorio de Oncología Molecular, Unidad de Diferenciación Celular y Cáncer, FES-Zaragoza, UNAM, Mexico City, Mexico
[c] Rheumatology Department, Hospital Juárez de México, Mexico City, Mexico

ARTICLE INFO

Article history:
Received 6 May 2016
Accepted 9 May 2016
Available online 10 May 2016

Keywords:
Rheumatoid arthritis
Systemic lupus erythematosus
STAT4
Polymorphisms

ABSTRACT

STAT4 has been consistently associated with several autoimmune diseases, including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). The aim of this study was to determine whether the STAT4 rs7574865G/T polymorphism confers susceptibility for RA and SLE in a sample of Mexican patients. This study included 869 individuals: 415 patients with RA, 128 patients with SLE, and 326 controls. Genotyping using TaqMan probes showed an association between the STAT4 rs7574865G/T polymorphism and RA (GG vs. TT: OR 1.99, p = 0.0009; G vs. T: OR 1.42, p = 0.0009) and SLE (GG vs. TT: OR 2.98, 0.0003; G vs. T: OR 1.74, p = 0.0002). Gender stratification showed an association with RA (GG vs. TT: OR 1.99, 95% CI 1.3–3.1, p = 0.002; G vs. T: OR 1.42, 95% CI 1.1–1.8, p = 0.002) and SLE (GG vs. TT: OR 3.3, 95% CI 1.7–6.2, p = 0.0002; G vs. T: OR 1.8, 95% CI 1.3–2.4, p = 0.0002) in women. Thus, the STAT4 rs7574865G/T polymorphism confers risk for RA and SLE in the Mexican population.

1. Introduction

Autoimmune diseases (AIDs) represent a heterogeneous group of pathologies characterized by a loss of immunologic tolerance and the production of autoantibodies against various autoantigens, which mainly affect women [1–3]. Rheumatoid arthritis (RA) is the most common type of rheumatic AID in the general population and represents the prototype chronic inflammatory disease, and systemic lupus erythematosus (SLE) represents the prototype AID [4]. RA is characterized by synovial inflammation, cartilage destruction, and bone erosion [5], whereas SLE is characterized by the loss of immunological tolerance to nucleic acids, the activation of autoreactive lymphocytes, and production of autoantibodies that lead to tissue damage [6]. The etiologies of RA and SLE are multifactorial, and several factors are involved in their development, including environmental factors (e.g., viral, bacterial, hormonal, cigarette smoking), gender, ethnicity, epigenetics, and genetic factors [7,8]. Genetic factors are 60–70% of overall risk factors that contribute to the development of RA and SLE [7,8], suggesting that genetics represent the main risk factor for both AIDs. Genome-wide association studies (GWAS) have identified several *loci* involved in susceptibility to RA and SLE. In 2007, the rs7574865G/T polymorphism located in the third intron of the signal transducer and activator of transcription 4 (STAT4) gene was reported to be associated with susceptibility to RA and SLE [9]. STAT4 is a transcription factor that localizes in the cytoplasm, where it is phosphorylated by membrane-bound receptors. STAT4 then translocates to the nucleus, where it regulates the expression of genes involved in directing helper T cells to pro-inflammatory T-helper type 1 and T-helper type 17 lineages [10,11]. *STAT4* seems to be one of the best examples of a non-HLA gene associated with susceptibility to the development of several AIDs, including RA and SLE. Recently, two meta-analyses showed that the *STAT4* rs7574865G/T polymorphism is a genetic risk factor for RA in the majority of ethnic groups studied [12,13]. On the other hand, two meta-analyses have shown that this polymorphism is also associated with SLE susceptibility [14,15]. However, this polymorphism has not been evaluated in Mexican patients with RA, but this polymorphism has been evaluated in patients with SLE. Thus, the aim of the present study was to determine whether the *STAT4* rs7574865G/T polymorphism confers RA and SLE susceptibility in a sample from Mexico.

* Corresponding author at: Research Unit in Endocrine and Metabolic Diseases, Hospital Juárez de México. Av. Instituto, Politécnico Nacional No. 5160, Delegación Gustavo A. Madero. C.P. 07760, Mexico City, Mexico.
E-mail address: dr.julian.ramirez.hjm@gmail.com (J. Ramírez Bello).

http://dx.doi.org/10.1016/j.imlet.2016.05.003
0165-2478/

Table 1
Demographic characteristics of patients with RA and SLE.

	Controls n = 326(%)	RA n = 415(%)	SLE n = 128 (%)
Age (years)	51.6	51.2	41.0
SD	7.80	14.75	12.88
Gender	(%)	(%)	(%)
Female	280 (85.9)	381 (91.8)	120 (93.8)
Male	46 (14.1)	34 (8.2)	8 (6.2)

SD standard deviation.

2. Patients and methods

2.1. Study population

This study included 869 Mexican mestizo individuals: 415 patients with RA, 128 patients with SLE, and 326 healthy individuals. All cases and controls were unrelated individuals over 18 years of age. The patients with RA and SLE were classified in accordance with the 2010 revised classification criteria of the American College of Rheumatology. Controls were individuals with no antecedent AIDs and chronic inflammatory diseases, including obesity, allergy (to food, drugs, etc.), spontaneous chronic or acute urticaria, coronary artery disease, schizophrenia, cancer, or type 2 diabetes mellitus for three generations. All cases and controls included in the present study were matched for gender and ethnicity. All participants were referred from the Hospital Juárez de México and the SLE foundation —El Despertar de la Mariposa, A.C‖. The Institution Research, Ethics, and Biosecurity committees approved the present study. All participants provided written informed consent.

2.2. DNA extraction

Genomic DNA was isolated from 5 mL of EDTA-treated peripheral blood leukocytes using the Invisorb Blood Universal Kit (Stratec Molecular Gmb H, Berlin, Germany) according to the manufacturerís specifications. The genetic material from each case-control were quantified, diluted, and stored at −20 °C until use.

2.3. Determination of genetic polymorphisms

The *STAT4* rs7574865G/T polymorphism was evaluated using the 5′ nuclease allelic discrimination assay with TaqMan probes on a CFX96 Touch TM Real Time PCR Detection System (Bio-Rad, California, USA) according to the manufacturerís instructions.

2.4. Statistical analysis

The Hardy-Weinberg equilibrium (H-WE) of the *STAT4* rs7574865G/T polymorphism was evaluated in cases versus controls using FINETTI software (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl). EPIDAT 3.1 software was used to estimate whether the alleles and genotype of the *STAT4* rs7574865G/T polymorphism are associated with RA and SLE. This software was used to obtain the odds ratio (OR), 95% confidence interval (CI), and *p*-value (http://www.sergas.es/MostrarContidos_N3_T01.aspx?IdPaxina=62715). Significance was set at $p < 0.05$.

3. Results

3.1. Characteristic of the study population

Our study population had a high proportion of women affected with RA and SLE (91.8%, n = 381 and 93.8%, n = 121, respectively). Our control group consisted mainly of women (85.9%, n = 280; Table 1). The mean (SD) age of the RA, SLE, and healthy control groups was 51.2 (±14.75), 41.0 (±12.88), and 51.6 (±7.80) years, respectively. The demographic characteristics of the RA, SLE, and control groups are described in Table 1.

3.2. H-WE in cases versus controls

The *STAT4* rs7574865G/T polymorphism was in H-WE in patients with SLE and the healthy group ($p = 0.45$ and $p = 0.44$, respectively), but not in patients with RA ($p = 0.005$).

3.3. Allele and genotype frequencies

Comparing the genotype and allele frequencies of the *STAT4* rs7574865G/T polymorphism in patients with RA and controls, we found significant differences, which indicates an association between the *STAT4* rs7574865G/T polymorphism and RA susceptibility (GG vs. TT: OR 1.99, 95% CI 1.32–2.99, $p = 0.0009$; G vs. T: OR 1.42, 95% CI 1.16–1.75, $p = 0.0009$; Table 2). We also found an association between the *STAT4* rs7574865G/T polymorphism and SLE susceptibility (GG vs. GT: OR 2.16, 95% CI 1.3–3.6, $p = 0.003$; GG vs. TT: OR 2.9, 1.6–5.4, $p = 0.0003$; G vs. T: OR 1.7, 95% CI 1.3–2.3, $p = 0.0002$; Table 3). Gender stratification showed an association between this polymorphism and RA and SLE susceptibility in women (Tables 4 and 5).

4. Discussion

In 2007, a GWAS identified a polymorphism (rs7574865G/T) located in the third intron of *STAT4* associated with AR and SLE susceptibility [9]. Different groups immediately replicated this finding

Table 2
Genotype and allelic frequencies of the *STAT4* rs7574865G/T polymorphism and association analysis in patients with RA and controls.

Gene SNP	RA (n = 415) *n* (%)	Controls (n = 326) *n* (%)	OR	95% IC	*p*
STAT 4 rs7574865 Genotype					
GG	126 (30.4)	123 (37.7)	–	–	–
GT	179 (34.1)	149 (45.7)	1.17	0.84–1.63	0.34
TT	110 (26.5)	54 (16.6)	1.99	1.32–2.99	0.0009
Allele	n = 830	n = 652			
G	431 (51.9)	395 (60.6)	–	–	–
T	399 (48.1)	257 (39.4)	1.42	1.16–1.75	0.0009

OR odds ratio, CI confidence intervals, p statistical significant.