Cellular Immunology 346 (2019) 103986

Contents lists available at ScienceDirect

Cellular Immunology

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

Short communication

The *AIRE* Ser196Ser synonymous variant is a risk factor for systemic lupus erythematosus

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ARTICLE INFO

Keywords:
Systemic lupus erythematosus
AIRE
Single nucleotide variant Susceptibility

ABSTRACT

The *AIRE* gene influences the expression of a wide array of self-antigens in the thymus, and is essential to the negative selection of self-reactive T cells and establishment of central tolerance. Single nucleotide variants (SNVs) such as rs878081C/T (Ser196Ser) and rs2075876G/T at this locus have been associated with susceptibility to rheumatoid arthritis, mainly in Asian populations, but its role in systemic lupus erythematosus (SLE) has not been documented. We performed a case-control association study with 379 SLE patients and 460 controls from central Mexico. In addition, we replicated our finding in another group of 179 SLE patients and 97 controls from the same region of Mexico. In the first group, we identified that the *AIRE* Ser196Ser synonymous variant was associated with SLE (OR 1.4, $p = 0.009$), meanwhile, in the second group we observed the following: OR 1.7, $p = 0.024$. No association was found between these *AIRE* SNVs and lupus nephritis. Our results suggest that *AIRE* is a risk factor for SLE in our population. This study is the first to document an association between *AIRE* and SLE susceptibility.

1. Introduction

Systemic lupus erythematosus (SLE), the prototype autoimmune disease (AD), is characterized by a loss of tolerance to self-antigens and chronic inflammatory processes generated by the deposition of immune complexes resulting from increased production of autoantibodies [1]. Several proteins are involved in the mechanisms underlying immunological tolerance, but the autoimmune regulator (AIRE), a transcriptional regulator primarily expressed in medullary thymic epithelial cells (mTECs), plays a central role in immune tolerance. AIRE is the main protein related to the negative selection of immature T cells (thymocytes), controlling the ectopic expression of a wide array of peripheral self-antigens in mTECs of the thymus, and is essential to the negative selection of self-reactive T cells and establishment of central tolerance [2,3]. Some ADs, such as autoimmune hepatitis, vitiligo, type 1 diabetes mellitus, myasthenia gravis, rheumatoid arthritis (RA), and systemic sclerosis, may be influenced by genetic variability in the *AIRE* gene [4]. Mutations in this locus cause autoimmune polyendocrinopathy-candidiasis ectodermal dystrophy (APECED), one of the few monogenic ADs that have been described [5]. A recent study showed that the presence of a single nucleotide variant (SNV) in this gene may promote less efficient negative selection and increased susceptibility to ADs [6]. On the other hand, a genome-wide association study (GWAS) in a Japanese population identified an association between the *AIRE* rs2075876G/A and rs760426A/G SNVs and susceptibility to RA [7]. Although the number of studies on *AIRE* has gradually increased for RA and other ADs [4,7–13], its role in susceptibility to SLE has not been clarified. Therefore, considering the crucial role of *AIRE* in the regulation of central tolerance and risk in different ADs, the aim of the present study was to investigate whether the *AIRE* rs878081C/T (Ser196Ser) and rs2075876G/A variants are associated with SLE.

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https://doi.org/10.1016/j.cellimm.2019.103986
Received 5 June 2019; Received in revised form 26 August 2019; Accepted 11 September 2019
Available online 11 September 2019
0008-8749/

2. Materials and methods

2.1. Study subjects

Our study was performed in 379 female patients with SLE from the rheumatology service of Hospital Juárez de México and from the Hospital regional "La Raza" and 460 female healthy controls recruited from central Mexico. A second group consisted of 179 female patients with SLE from the Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán". Additionally, a second group of 97 female controls was included. Cases and controls of this second group also were recruited from the same region of Mexico.

Data regarding lupus nephritis (LN) were available in 224 SLE cases; 88 patients had LN and 136 patients did not. The diagnosis of LN was based on the biopsy. Patients were classified according to the 1997 ACR criteria.

The controls had no family history of chronic inflammation or ADs, this data was obtained by surveys for each individual. All cases and controls were older than 18 years of age. Our protocol was approved by the ethics and research committees (Registry number: HJM 0446/18-I) and informed consent obtained from each participant.

2.2. DNA extraction and genotyping

One blood sample was taken from each subject (cases and controls). The nuclear DNA was isolated using the Invisorb Blood Universal Kit (Stratec molecular GmbH, Berlin, Germany) according to the manufacturer's specifications. An allelic discrimination assay with TaqMan probes was performed (C__2978265_10 for rs878081 and C_15863141_10 for rs2075876) to obtain the *AIRE* genotypes.

3. Statistical analysis

Hardy-Weinberg equilibrium (HWE) and the genetic association between the *AIRE* rs878081C/T and rs2075876G/A variants and SLE susceptibility were analyzed using FINETTI software (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The haplotypes and linkage disequilibrium (LD) were estimated using Haploview V.4.2. A Bonferroni correction test was applied to all *p*-values, an association was considered significant when we identified a $p < 0.025$ (p = 0.05/2 SNVs). An *in silico* analysis about of the possible functional role of the *AIRE* rs878081T allele was evaluated using the SNPinfo web server (https://snpinfo.niehs.nih.gov/cgi-bin/snpinfo/splice.cgi?2_rs878081).

4. Results and discussion

The genotype distribution was in HWE in both patients and controls ($p > 0.05$). The genotypic and allelic frequencies of the two *AIRE* variants and the association analysis are shown in Table 1. The genotype and allele frequencies of *AIRE* rs2075876G/A were similar in patients with SLE and controls; thus, no significant difference was detected with the allelic and codominant models (Table 1). Despite the lack of studies and information about this variant in SLE, some reports have shown a genetic association between *AIRE* rs2075876G/A and RA in Asian populations but not in Caucasians [4,7–12]. On the other hand, our data showed an association between the *AIRE* rs878081T (196Ser) allele and increased risk of SLE (OR 1.4, $p = 0.009$; Table 1). Because this finding has not been previously identified in another population, we decided to evaluate a second group of patients with SLE and controls to replicate our results. Interestingly, we also identified an association between *AIRE* rs878081C/T and SLE (OR = 1.7, $p = 0.024$) (Table 2).

Regarding this variant, García-Lozano et al. [10] and Yang et al. [13] identified *AIRE* rs878081C/T as a risk factor for RA, which is in accordance with our results, despite evaluating different diseases. RA and SLE share an important percentage of the genetic background involved in pathogenesis; for example, the *STAT4* rs7574865G/T polymorphism is a genetic risk factor for both ADs [14].

Regarding the possible functional role of this synonymous variant, as far as we know, no study has reported its biological impact. To determine its possible affect, we conducted an *in silico* analysis, which showed that the rs878081T allele disrupts a binding site for SF2ASF1, protein involved in splicing [15]. Thus, our analysis suggests that this allele (located in exon 5) destroys an enhancer splicing exonic in *AIRE*. Three AIRE isoforms have been described [16,17], it is likely that this allele may affect the splicing of AIRE, however, biochemical studies are necessary to determine its function. The role of common genetic variation of *AIRE* (including the variants analyzed in our study) in SLE patients has been recently evaluated in a GWAS carried out in European individuals, nevertheless no association was identified (http://insidegen.com/insidegen-LUPUS-data.html) [18]. Thus, our study is the first to show an association between *AIRE* rs878081C/T (Ser196Ser) and SLE. The discrepancy of our results and those previously published is the population heterogeneity, European populations are formed mainly by closely related individuals [19], mean, the cases and controls included in our study are from Central Mexico, region formed mainly by an Amerindian (50%), Caucasian (45%) and African (5%) ancestry [20], which favors differences in the genetic background. To rule out the possibility of identify a different frequency of the *AIRE* rs878081T allele in individuals of Mexican origin, we compared our data with those obtained by the 1000 genome project. We identified similar percentages in the 1000 genome project, and in our first and second group of controls (20%, 16% and 13.4%, respectively). Thus, our data showed similar results between these three groups.

On the other hand, the study design, the genetic background and the lack of ancestry-informative markers (AIMs) could be other causes of the differences in the results. Thus, the absence of AIMs is an important

Table 1
Genotypic and allelic frequencies of the *AIRE* SNVs and association analysis in patients with SLE and controls.

SNVs	Model	Genotype or allele	SLE n (%)	Controls n (%)	OR 95% CI	p*
AIRE	Codominant	CC	238 (62.6)	321 (69.8)		
rs878081C/T		CT	125 (32.9)	131 (28.5)	1.3 (0.95–1.73)	NS
(Ser196Ser)		TT	17 (4.5)	8 (1.7)	2.9 (1.22–6.75)	0.012
	Allelic	C	601 (79.1)	773 (84.0)		
		T	159 (20.9)	147 (16.0)	1.4 (1.08–1.78)	0.009
AIRE	Codominant	GG	273 (72.6)	351 (76.3)		
rs2075876G/T		GA	97 (25.8)	96 (20.9)	1.3 (0.94–1.79)	NS
		AA	6 (1.6)	13 (2.8)	0.6 (0.22–1.58)	NS
	Allelic	G	643 (85.5)	798 (86.7)		
		A	109 (14.5)	122 (13.3)	1.1 (0.84–1.47)	NS

SNVs: Single nucleotide variants; OR: odds ratio; CI: Confidence interval.
SLE: Systemic lupus erythematosus; *$p < 0.05$: statistically significant.

Table 2
Genotypic and allelic frequencies of *AIRE* rs878081C/T and association analysis in the second cohort of patients with SLE and controls.

SNVs	Model	Genotype or allele	SLE n (%)	Controls n (%)	OR 95% CI	p*
AIRE	Codominant	CC	110 (61.5)	74 (76.3)		
rs878081C/T		CT	62 (34.6)	20 (20.6)	2.1 (1.16-3.74)	0.013
(Ser196Ser)		TT	7 (3.9)	3 (3.1)		
	Allelic	C	282 (78.8)	168 (86.6)		
		T	76 (21.2)	26 (13.4)	1.7 (1.07-2.83)	0.024

SNVs: Single nucleotide variants; OR: odds ratio; CI: Confidence interval.
SLE: Systemic lupus erythematosus; *$p < 0.05$: statistically significant.

limitation in our study.

Regarding the haplotype analysis, we found four different allele combinations (data no shown); only the TG haplotype was associated with susceptibility to SLE (OR 1.39, p = 0.01, $pc = 0.028$; corrected p-value after 100,000 permutations). This combination was formed by the rs878081T minor allele and rs2075876G major allele, suggesting that the association of the TG haplotype is due to rs878081T minor allele but not by the combination of both *AIRE* SNVs. On the other hand, we did not identify any LD between these two *AIRE* polymorphisms in cases and controls (data not shown). The obtained results in this study showed that these *AIRE* polymorphisms are not susceptibility factors for LN, but other studies should include other populations to determine their role in this clinical manifestation of SLE. In addition, other polymorphisms located in the *AIRE* gene could be in LD and contribute to SLE susceptibility.

AIRE plays a fundamental role in the negative selection of T cells by controlling the promiscuous expression of peripheral self-antigens in mTECs in the thymus [1], so that dysfunction in AIRE due to gene variants causes susceptibility to the development of various ADs, as observed in AR [7]. Regarding our results, these and other *AIRE* polymorphisms should be evaluated in other populations with a genetic background different from ours to validate our results and determine the role in susceptibility to SLE.

Funding

There is no financial support for this work.

Number of Ethics Committee

HJM 0446/18-I.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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