Association of HLA Class II Genes with Systemic Sclerosis in Spanish Patients

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ABSTRACT. ***Objective.*** To examine the role of HLA-DRB1 and HLA-DQB1 alleles in the susceptibility to systemic sclerosis (SSc) and its clinical expression in a Spanish population.

Methods. One hundred Spanish Caucasian patients with SSc and 130 controls were studied. Molecular HLA-DRB1 and HLA-DQB1 typing was performed by polymerase chain reaction (PCR) sequence-based typing and PCR sequence-specific oligonucleotide.

Results. HLA-DRB1*11 was associated with genetic susceptibility to SSc, whereas HLA-DRB1*07 (HLA-DRB1*0701) showed a protective effect. A significant increase in the frequency of the DRB1*1104 allele was observed in patients with anti-topoisomerase I autoantibodies (anti-Topo I) while HLA-DRB1*01 and HLA-DQB1*05 alleles were significantly increased in patients with anticentromere antibodies (ACA). The HLA-DRB1*11 allele was more frequent in patients with pulmonary fibrosis; however, no significant association with any HLA-DRB1 or DQB1 alleles was identified in patients with pulmonary arterial hypertension.

Conclusion. HLA alleles play a role in genetic susceptibility to SSc in Spanish patients. Some alleles are more prevalent in patients with pulmonary fibrosis and in patients with certain SSc-specific autoantibodies (anti-Topo I and ACA). (J Rheumatol First Release Nov 1 2009; doi:10.3899/jrheum.090377)

Key Indexing Terms:
HLA SYSTEMIC SCLEROSIS CLINICAL SUBSETS
AUTOANTIBODIES PULMONARY INVOLVEMENT

Systemic sclerosis (SSc) is an autoimmune systemic disorder of unknown origin characterized by skin fibrosis, vasculopathy, and visceral involvement. Pathogenesis of scleroderma remains unclear, but genetic factors are thought to contribute to the disease. HLA genes have been implicated in the susceptibility to SSc and its clinical and serological manifestations[1-8]. Several case-control studies have identified slight associations between certain HLA alleles and SSc[1-5], including a significant increase in Class II HLA-DR5 (DRB1*1101 and DRB1*1104), HLA-DR3 (DRB1*0301), HLA-DQA1*0501, and HLA-DQB1*0201 in American and European White patients[1,4,5]; HLA-DRB1*1502 and HLA-DQB1*0601 in Japanese[2]; and HLA-DRB1*1602, HLA-DQA1*0501, and HLA-DQB1*0301 in Choctaw Native Americans[3]. The HLA differences among these populations highlight the importance of ethnicity and HLA types in disease expression. Moreover, the HLA-DRB1 allele has been associated with the limited cutaneous form (lcSSc)[1] and DRB1*11 (DRB1*1104) has been found in the diffuse cutaneous form (dcSSc)[1,6]. Similarly, several studies reported a correlation of internal organ involvement of SSc such as pulmonary arterial hypertension (PAH) and pulmonary fibrosis (PF) as well as some of its autoimmune patterns with different specific serological HLA status[7,8]. Molecular studies indicate that these associations differ according to ethnicity[2,8,9-12]. Thus, more information from different ethnic groups is needed to highlight the exact relationship between the immunogenetic background of SSc and its autoantibody response[8,9,11-15]. Currently, no information is available on the Spanish Caucasian population.

The aim of our study was to determine the role of HLA alleles in the susceptibility to develop SSc in a Spanish Caucasian population and to evaluate their involvement in disease expression in a case-control study.

MATERIALS AND METHODS

Patients and controls. We evaluated a cohort of 100 consecutive Spanish Caucasian patients (89 women and 11 men) with SSc attending the outpatient Scleroderma Unit of the Vall d'Hebron Hospital from October 2003

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Accepted for publication July 9, 2009.

to December 2005. The median age at diagnosis was 50 years (range 15–74) and the median disease duration from SSc diagnosis to the study was 10 years (range 5–56). Seventy patients were classified as having lcSSc and 30 as dcSSc. Clinical SSc evaluation criteria were as described[16,17]. One hundred thirty healthy Spanish Caucasians were included as controls. All individuals included in the study gave their written informed consent to provide a DNA sample. The study was approved by the Vall d'Hebron Hospital Research Ethics Committee.

HLA typing. HLA-DRB1 and DRB3/4/5 typing was performed by polymerase chain reaction (PCR) sequence-based typing. HLA-DQB1 typing was performed by PCR sequence-specific oligonucleotide. In both cases, typing ambiguities were resolved by PCR sequence-specific primer.

Statistical analysis. Phenotype frequencies of HLA alleles in patients and controls were analyzed using the chi-square test or Fisher's 2-tailed exact test. P values ≤ 0.05 were considered significant after correction for the number of alleles with a frequency higher than 5% (non-rare allele) and the number of tests assessed (Bonferroni correction).

RESULTS

One hundred consecutive Spanish Caucasian patients (89 women and 11 men) with SSc were compared to 130 controls. The clinical and immunological characteristics of the SSc cohort are summarized in Table 1.

HLA alleles and susceptibility to scleroderma. The HLA-DRB1*11 allele was associated with a susceptibility to develop SSc (19.7% vs 10.2% of alleles in patients and controls, respectively; OR 2.467, p corrected = 0.036). In addition, the HLA-DRB1*1104 allele showed only a trend towards susceptibility to SSc. By contrast, we found a protective effect for HLA-DRB1*07, specifically HLA-DRB1*0701 (8% vs 18% of alleles in patients and controls, respectively; OR 0.345, p corrected = 0.009; Table 2).

Table 1. Clinical and demographic data of 100 Spanish patients with SSc.

Characteristic	n	%
Sex		
Male	11	11
Female	89	89
Skin involvement		
Diffuse	30	30
Limited	70	70
Lung involvement	85	85
FVC < 55%	23	23
PAH	13	13
Digestive involvement	84	84
Esophagus	76	76
Heart involvement	64	64
Scleroderma renal crisis	3	3
Anti-topoisomerase I	21	21
Anticentromere antibodies	47	47
Deaths	23	23
PAH	9	39
Heart disease	5	21.7
Malignancy	5	21.7
Scleroderma renal crisis	2	8.6
Lung fibrosis	1	4.3
Portal hypertension	1	4.3

FVC: forced vital capacity; PAH: pulmonary arterial hypertension.

Table 2. Distribution of HLA-DRB1 and HLA-DQB1 alleles in Spanish patients with SSc and controls. Results are shown as no. (%) of alleles.

	SSc, n = 100	Controls, n = 130	OR	Fisher's p (corrected)
DRB1 alleles				
DRB1*01	24 (12.7)	27 (10.9)	1.205	0.632
DRB1*03	23 (12.2)	32 (13.0)	0.915	0.876
DRB1*04	28 (14.8)	36 (14.6)	1.015	1.000
DRB1*07†	15 (7.9)	44 (17.8)	0.345	0.009
DRB1*08	12 (6.3)	7 (2.8)	2.396	0.810
DRB1*11‡	37 (19.6)	25 (10.1)	2.467	0.036
DRB1*13	15 (7.9)	30 (12.1)	0.588	0.135
DRB1*15	19 (10.1)	26 (10.5)	0.938	1.000
Other	16 (8.5)	20 (8.1)	1.061	1.000
DQB1 alleles				
DQB1*02	33 (34.4)	66 (50.8)	0.508	0.075
DQB1*03	67 (69.8)	70 (53.8)	1.980	0.095
DQB1*04	9 (9.4)	7 (5.4)	0.965	1.000
DQB1*05	31 (32.3)	43 (33.1)	0.659	0.159
DQB1*06	28 (29.2)	50 (38.5)	0.508	0.075

† HLA DRB1*0701: 7.9% of SSc patients vs 17.8% in controls (OR 0.345, p corr = 0.01). ‡ HLA DRB1*1104: 11% SSc patients vs 4.5% in controls (OR 2.705, p corr = 0.180). Other: alleles with frequencies less than 5%, patient or control group.

HLA and disease manifestations in SSc. *Diffuse/limited disease*. Trends towards higher frequency of HLA-DRB1*04 and lower frequency of HLA-DRB1*1104 alleles were found in patients with lcSSc versus patients with dcSSc, but these associations were not statistically significant (Table 3).

Autoantibody specificities. Only the HLA-DRB1*1104 allele was more frequent in anti-Topo I-positive compared to anti-Topo I-negative SSc patients (23.8% vs 5.7%, respectively; OR 6.09; p corrected = 0.01). In patients positive for anticentromere antibodies (ACA), HLA-DRB1*01 and HLA-DQB1*05 alleles were found more frequently than in ACA-negative patients (19% vs 5.7%; OR 4.754, p corrected = 0.018; and 23.4% vs 8.6%; OR 4.667, p corrected = 0.005, respectively; Table 3).

Pulmonary fibrosis. The HLA-DRB1*11 allele was more frequent in patients with PF than in patients without PF (33% vs 15%; OR 4.385, p corrected = 0.045), but the frequency of the HLA-DRB1*1104 allele did not reach statistical significance. In the HLA-DQB1 region, only HLA-DQB1*0301 showed a higher frequency in patients with PF. Interestingly, the HLA-DQB1*0201 allele showed a trend towards a protective effect, as no patients with PF presented this allele compared to 12.5% in patients without PF; however, this association was not significant after Bonferroni correction (data not shown).

Pulmonary arterial hypertension. No association was identified among any HLA-DRB1 and HLA-DQB1 alleles and pulmonary vascular involvement.

Table 3. Association of HLA-DRB1 and DQB1 alleles with different clinical manifestations. Results are shown as no. (%) of alleles.

HLA Alleles	Diffuse SSc, n = 30 (%)	Limited SSc, n = 70 (%)	OR	p Corrected*
DRB1*04	3 (5)	21 (15)	0.190	0.099
DRB1*07	8 (13)	5 (3.5)	3.782	0.270
DRB1*11	14 (23.3)	19 (13.6)	1.750	0.251
DRB1*1104	11 (18.3)	6 (4.2)	4.921	0.090
	Anti-Topo I Positive, n = 21 (%)	Anti-Topo I Negative, n = 78 (%)		
DRB1*11	13 (31)	23 (14.7)	3.886	0.09
DRB1*1104	10 (23.8)	9 (5.7)	6.090	0.01
DQB1*03	19 (45.2)	47 (30.1)	5.457	0.085
	ACA Positive, n = 47 (%)	ACA Negative, n = 53 (%)		
DRB1*01	18 (19)	6 (5.7)	4.754	0.018
DRB1*14	5 (5.3)	0 (0)	2.238	0.18
DRB1*1401	5 (5.3)	0 (0)	2.238	0.21
DRB1*05	22 (23.4)	9 (8.6)	4.667	0.005
DRB1*0501	17 (18)	7 (6.7)	3.958	0.09
DRB1*0503	5 (5.3)	0 (0)	2.308	0.19
	PF, n = 23 (%)	No PF, n = 77 (%)		
DRB1*11	10 (33)	26 (15)	4.385	0.045
DRB1*1104	7 (23)	13 (8)	4.667	0.12
DQB1*03	13 (43)	54 (33)	2.716	0.217
DQB1*0301	12 (40)	34 (21.5)	5.412	0.05
	PAH, n = 13 (%)	No PAH, n = 87 (%)		
DRB1*15	8 (18.2)	5 (6)	4.000	0.432
DRB1*1501	8 (18.2)	4 (5)	5.143	0.19

* p corrected by Bonferroni test. Anti-topo-I: anti-topoisomerase I antibodies; ACA: anticentromere antibodies; PF: pulmonary fibrosis; PAH: pulmonary arterial hypertension.

DISCUSSION

Our study investigated the frequencies of HLA class II alleles in Spanish Caucasian patients with SSc and their relationships with clinical and serological manifestations.

HLA alleles and susceptibility to scleroderma. As expected, HLA-DRB1*11 was associated with susceptibility to SSc, although we did not find the increased frequency of HLA-DRB1*1104 alleles observed in studies with American and European populations[1,4,5]. By contrast, HLA-DRB1*07, specifically DRB1*0701, was found to be protective, in agreement with the results of Gladman, *et al*[1] and Frezza, *et al*[5]. None of the HLA-DQB1 alleles was significantly increased in SSc[7]. So our study with Spanish Caucasian SSc patients confirms previous HLA findings in other SSc populations, which showed a role for HLA genes in SSc susceptibility[1-5,7,8,12]. Our study also highlights the importance of taking into account the stratification of patient populations according to ethnicity in studies of SSc.

HLA and disease manifestation in SSc. *Diffuse/limited disease*. HLA-DRB1*11, specifically HLA-DRB1*1104 allele frequency, showed only a trend towards an increase in dcSSc, in contrast to previous reports, which found a statistically significant increase[1,6,7,12]. This may be due to the small number of patients with dcSSc in our series.

Autoantibody specificities. The HLA-DRB1*1104 was significantly increased in anti-Topo I-positive patients, in agreement with results from other series with Caucasians[8,9,12]. However, although some studies found a significant increase in the frequency of HLA-DQB1*03 subtype in anti-Topo I patients, in American Caucasians, Blacks, and Choctaw Indians, these findings were not confirmed in our population[9,11].

Previous studies have associated ACA response with increased frequencies of several alleles located on HLA-DR and HLA-DQ genes, for instance, HLA-DRB1*01 in Caucasians[4] and HLA-DQB1*05 in Caucasians and Japanese[13,14]. Interestingly, we also found an increased frequency of both alleles in ACA-positive patients.

Pulmonary involvement. The frequency of the HLA-DRB1*11 allele was increased in patients with PF. Although some studies have also shown a relationship between PF and HLA-DR3, our data showed that no other alleles were associated with either PF or PAH in SSc[8].

This is the first study to examine the role of HLA-DRB1 and HLA-DQB1 alleles in the genetic susceptibility to SSc and its clinical and serological expression in a Spanish Caucasian SSc population. Our data support the hypothesis that HLA-DRB1*11 confers susceptibility to SSc and that certain alleles are more frequently related to specific antibody responses and organ involvement. Our data are similar to those reported in other Caucasian populations. However, it is evident that SSc does not have a single dominant genetic pattern because other HLA alleles have also been associated with aspects of the disease in different ethnic groups.

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