RHEUMATOLOGY

Original article

Analysis of Class II human leucocyte antigens in Italian and Spanish systemic sclerosis

Lorenzo Beretta[1],*, Blanca Rueda[2],*, Maurizio Marchini[1],
Alessandro Santaniello[1], Carmen P. Simeón[3], Vicente Fonollosa[3],
Monica Caronni[1], Raquel Rios-Fernandez[4], Patricia Carreira[5],
Luis Rodriguez-Rodriguez[6], Spanish Systemic Sclerosis Group[†],
Antonia Moreno[7], Miguel A. López-Nevot[7], Ana Escalera[8],
Maria F. González-Escribano[8], Javier Martin[2],* and Raffaella Scorza[1],*

Abstract

Objective. To determine the role of Class II HLAs in SSc patients from Italy and Spain and in SSc patients of Caucasian ancestry.

Methods. Nine hundred and forty-four SSc patients (Italy 392 patients; Spain 452 patients) and 1320 ethnically matched healthy controls (Italy 398 patients; Spain 922 patients) were genotyped up to the fourth digit by PCR with sequence-specific oligonucleotides for HLA-DRB1, DQA1 and DQB1 loci. Patients included 390 ACA-positive and 254 anti-topo I-positive subjects. Associations between SSc or SSc-specific antibodies and HLA alleles or HLA haplotypes were sought via the chi-square test after 10 000-fold permutation testing. A meta-analysis including this study cohort and other Caucasoids samples was also conducted.

Results. In both the cohorts, the strongest association was observed between the HLA-DRB1*1104 allele and SSc or anti-topo I antibodies. The HLA-DRB1*1104 -DQA1*0501 -DQB1*0301 haplotype was over-represented in Italian [odds ratio (OR) = 2.069, 95% asymptotic CIs (CI_{95}) 1.486, 2.881; $P < 0.001$] and in Spanish patients (OR = 6.707, CI_{95} 3.974, 11.319; $P < 0.001$) as well as in anti-topo-positive patients: Italy (OR = 2.642, CI_{95} 1.78, 3.924; $P < 0.001$) and Spain (OR = 20.625, CI_{95} 11.536, 36.876; $P < 0.001$). In both the populations we also identified an additional risk allele (HLA-DQB1*03) and a protective allele (HLA-DQB1*0501) in anti-topo-positive patients. The meta-analysis showed different statistically significant associations, the most interesting being the differential association between HLA-DRB1*01 alleles and ACAs (OR = 1.724, CI_{95} 1.482, 2.005; $P < 0.001$) or topo I antibodies (OR = 0.5, CI_{95} 0.384, 0.651; $P < 0.001$).

Conclusions. We describe multiple robust associations between SSc and HLA Class II antigens in Caucasoids that may help to understand the genetic architecture of SSc.

Key words: systemic sclerosis, HLA, genetics, epidemiology

BASIC SCIENCE

[1]Referral Center for Systemic Autoimmune Diseases, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and University of Milan, Milan, Italy, [2]Instituto de Parasitología y Biomedicina López-Neyra, CSIC, Granada, [3]Servicio de Inmunología, Hospital Universitario Virgen del Rocío/Instituto de Biomedicina de Sevilla (IBIS), Sevilla, [4]Department of Internal Medicine, Hospital Vall d'Hebrón, Barcelona, [5]Department of Internal Medicine, Hospital Clínico San Cecilio, Granada, [6]Department of Rheumatology, Hospital Sant Pau, Barcelona, [7]Department of Rheumatology, Hospital Clínico, Madrid and [8]Department of Immunology, Hospital Virgen de las Nieves, Granada, Spain.

Introduction

SSc is a complex CTD characterized by fibrosis of the skin and internal organs, widespread vasculopathy and abnormalities of the immune system [1, 2]. Several lines of evidence underline the importance of the individual's genetic background in SSc susceptibility and/or phenotypic expression and associations with a number of single nucleotide polymorphisms (SNPs) and SSc have consistently been replicated in different populations [3]. Similarly, a number of discovery and replication studies describing associations with SSc and the HLA Class II alleles in different ethnic groups have been published so far [3, 4]. The major contribution in this field comes from reports conducted in North Americans, and most notably from the large case–control study recently published by Arnett *et al.* [5]. Conversely, the association between major histocompatibility Class II (MHC) antigens and SSc in other geographical regions and/or ethnicities, such as European Caucasoids, has been limited to reports with a small to moderate number of cases and/or controls, seldom reaching the hundreds [5–12]. The limited number of cases/controls employed in the vast majority of HLA case–control studies in Caucasoids may help to explain the variability of results observed in these samples, either in terms of pure associations with SSc susceptibility or in terms of associations with specific SSc autoantibodies. Indeed, while differences among ethnicities may account for the inconsistent results across the different reports, the lack of power should also be carefully considered as a potential source of variance. Owing to the existence of extended linkage disequilibrium (LD) within the HLA region, it may be difficult to ascertain the primary disease-associated HLA allele(s) in underpowered case–control studies.

In the present study, we aimed to elucidate the role of HLA Class II alleles in two vast case–control sets of European Caucasoid individuals recruited from tertiary referral centres in Spain and Italy. To our knowledge, this is the largest case–control MHC Class II-association study conducted in SSc patients of European ancestry.

Method

Patients selection

Four hundred and two consecutive SSc patients referring to one single centre in northern Italy and 398 unrelated ethnically and sex-matched healthy controls were considered (case–control Series 1). The majority of these patients (90.8%) fulfilled the preliminary criteria for the classification of SSc proposed by the ACR [13], yet a proportion of patients with definite SSc who did not fulfil these criteria was also considered [14]. Additionally, 452 patients, of whom 438 (96.9%) with SSc according to the ACR criteria, who were referred to 15 tertiary institutions in Spain and 922 unrelated ethnically and sex-matched controls were also considered (case–control Series 2). In both series, all the patients and the controls gave written consent for the research. The local ethic committees

approved the study (Comité de Bioética del Consejo Superior de Investigaciones Científicas and U.O. Comitato di Etica e Sperimentazione Farmaci Fondazione IRCCS Ca' Granda–Ospedale Maggiore Policlinico di Milano).

All the patients were categorized as having the lcSSc or dcSSc subset of the disease, according to LeRoy *et al.* [15]; patients with the CREST syndrome features were grouped into the lcSSc subset [16]. The patients' autoantibody profile was obtained by reviewing the patients' medical records.

HLA Class II genotyping

Case–control Series 1 was genotyped in one single laboratory; similarly, Series 2 genotyping was performed at a single institution [Instituto de Parasitologia y Biomedicina 'Lopez-Neyra' (CSIC), Granada]. In both institutions, high-resolution HLA Class II typing (up to the fourth digit) was performed by means of PCR with sequence-specific primers (PCR-SSP) as previously described [17].

Statistical analysis

Associations between HLA alleles and SSc or between HLA alleles and SSc-specific autoantibodies, such as ACA or anti-topo I (topo I, Scl70), were sought via the χ^2-test with 1 degree of freedom. Only alleles with a frequency >3% in cases/controls were considered for the analysis. To correct for multiple testing, 10 000 permutations were used; the advantage of permutation resampling, in this context, is the capability to derive the null distribution of high-dimensional test statistics while preserving the dependence structure (e.g. LD) among HLA alleles. Moreover, irregularities of the observed data are maintained in the permuted data sets and are included in the estimation of the permutation probability [18, 19]. Results were considered significant at the 0.05 level for the controls *vs* SSc comparison or at the 0.025 level for the auto-antibody *vs* controls or for the SSc subsets *vs* controls comparison with further account for multiple testings. For significant results, odds ratios (ORs) and their relative 95% asymptotic confidence intervals (CI_{95}) were calculated.

HLA haplotypes were derived in both cohorts via the PHASE software [20] (available at http://stephenslab.uchicago.edu/software.html). Associations between HLA Class II haplotypes and SSc or SSc-specific autoantibodies were then conducted as described above. Underrepresented HLA Class II haplotypes were excluded from the analysis.

Finally, to gauge the overall contribution of HLA alleles in Caucasoid populations, a meta-analysis that included the present and previously published data was conducted. To be considered for the meta-analysis, literature data had to report both high-resolution HLA typing results and HLA Class II allele frequencies. Meta-analysis was thus restricted to the present study, to the works by Vlachoyiannopoulos *et al.* [10] and by Arnett *et al.* [5]; in the latter case African-Americans and Hispanics were not considered for meta-analysis owing to the different genetic background from Caucasoids. Indeed, as observed by Bryc *et al.* [21], the Hispanic population is a genetic

amalgamation of native Americans, Europeans and West Africans. To perform the meta-analysis, the Microsoft Excel add-in Mix software was used [22] (available at http://www.meta-analysis-made-easy.com/) and the Mantel–Haenszel procedure with fixed effects was applied. When the percentage of total variation across studies that is due to heterogeneity (I^2) [23] was >50%, the meta-analysis was not performed to avoid any bias in the determination of the generalizability of the findings. Meta-analysis results were declared significant at the 0.001 threshold.

Result

Series 1 included 271 (67.4%) lcSSc and 94 (23.4%) dcSSc patients; 168 (41.8%) patients tested positive for ACAs, while 155 (38.6%) tested positive for Scl70 antibodies. Series 2 included 323 (71.5%) lcSSc and 115 (25.4%) dcSSc patients; 222 (49.1%) patients tested positive for ACAs, while 99 (21.9%) tested positive for Scl70 antibodies.

In both the cohorts, ACAs and Scl70 antibodies were strongly correlated with the lcSSc and the dcSSc subset, respectively ($P < 1 \times 10^{-20}$). For the sake of brevity, hereafter we will report only the most significant results, while an exhaustive description of the single-allele analysis in both series as well as of the meta-analysis is reported in the supplementary data, available at *Rheumatology* Online.

The HLA-DRB1*1104 allele and the DR11 haplotype are associated with SSc and anti-topo I antibodies

In both cohorts, the HLA-DRB1*1104 allele was strongly associated with SSc (Table 1) and to a somewhat stronger extent with Scl70 antibodies (Table 2). Due to heterogeneity issues an exhaustive meta-analysis for the HLA-DRB1*1104 was not possible, yet the HLA-DRB1*11 allele was found to be strongly associated with SSc in Caucasoids (cases: 999/3826, 26.1% and controls: 692/3980, 17.4%; $P < 0.001$; OR = 1.573, CI$_{95}$ 1.403, 1.764).

The prominent role for the HLA-DRB1*1104 allele in SSc susceptibility also emerged from haplotype analysis.

The DR11 haplotype (HLA-DRB1*1104 -DQA1*0501 -DQB1*0301) was overrepresented both in Italians (cases: 114/804, 14.2% and controls: 59/798, 7.2% haplotypes; $P < 0.001$; OR = 2.069, CI$_{95}$ 1.486, 2.881) and to a greater extent in Spaniards (cases: 59/904, 6.52% and controls 19/1844, 1% haplotypes; $P < 0.001$; OR = 6.707, CI$_{95}$ 3.974, 11.319). The DR11 haplotype was also significantly associated with anti-topo I antibodies (ATAs) both in the Italian (cases: 54/310, 17.4% and controls: 59/798, 7.4% haplotypes; $P < 0.001$; OR = 2.642, CI$_{95}$ 1.779, 3.924) and in the Spanish series (cases: 34/198, 17.7% and controls: 19/1844, 1% haplotypes; $P < 0.001$; OR = 20.625, CI$_{95}$ 11.536, 36.875).

The associations between the DR11 haplotype and SSc or between the DR11 haplotype and ATAs was previously described by Agarwal *et al.* [4]; nonetheless, these authors did not report the number and the proportion of cases/controls carrying the DR11 haplotype and thus it was not possible to conduct a proper meta-analysis to quantify the risk in Caucasoids.

The HLA-DRB1*01 allele is differently associated with ACAs and ATAs

The HLA-DRB1*01 allele was found to be underrepresented in Italian and Spanish patients with ATAs (Table 2) and overrepresented in Spanish subjects who tested positive for ACAs (Table 3). The opposite effects on risk susceptibility in patients with different anti-corpal specificities is also confirmed by the meta-analysis: Scl70 (cases: 73/818, 8.9% and controls: 588/3720, 15.8%; $P < 0.001$; OR 0.5001, CI$_{95}$ 0.384, 0.651) and ACAs (cases: 345/1342, 25.7% and controls: 588/372, 15.8%; $P < 0.001$; OR = 1.365, CI$_{95}$ 1.238, 1.511).

To isolate the effect of the HLA-DRB1*01 allele from the individuals' genetic background, we also performed a comparison between either ATA- or ACA-positive patients and SSc patients who tested positive for ANAs, but not to these specific SSc antibodies. Via a meta-analysis procedure, taking into account the Italian and the Spanish series, it was confirmed that the HLA-DRB1*01 allele is differently associated with ATAs or ACAs: Scl70

TABLE 1 HLA Class II associations with SSc

HLA	Ctrl, *n* (%)	SSc, *n* (%)	Uncorrected *P*	Corrected *P*	OR (CI$_{95}$)
Italy					
DRB1*1104	83 (10.40)	151 (18.78)	<0.001	<0.001	1.992 (1.493, 2.657)
DRB1*11	227 (28.45)	284 (35.32)	0.003	0.04	1.374 (1.124, 1.697)
DQA1*0501	250 (31.33)	337 (41.92)	<0.001	<0.001	1.582 (1.289, 1.942)
DQA1*05	326 (40.85)	396 (49.25)	0.001	0.01	1.405 (1.153, 1.712)
Spain					
DRB1*0701	283 (15.35)	91 (10.07)	<0.001	0.006	0.617 (0.48, 0.793)
DRB1*1104	20 (1.08)	64 (7.08)	<0.001	<0.001	6.499 (4.178, 11.556)
DRB1*11	230 (12.47)	153 (16.92)	0.002	0.03	1.43 (1.145, 1.785)

HLA Class II significant associations with SSc (after 10 000-fold permutation testing) in Italy and Spain. The table reports the number of positive alleles in cases and controls. Total number of alleles per series—Italy: SSc = 802; Ctrl = 798; Spain: SSc = 902; Ctrl = 1844. Ctrl: controls.

TABLE 2 HLA Class II associations with ATAs

HLA	Ctrl, n (%)	SSc, n (%)	Uncorrected P	Corrected P	OR (CI₉₅)
Italy					
DRB1*1104	83 (10.4)	64 (20.6)	<0.001	<0.001	3.75 (2.588, 5.436)
DQA1*01	316 (39.6)	84 (27.1)	<0.001	<0.001	0.567 (0.425, 0.756)
DQA1*0501	250 (31.3)	140 (45.2)	<0.001	<0.000	1.784 (1.369, 2.334)
DQA1*05	326 (40.9)	159 (51.3)	0.002	0.03	1.525 (1.171, 1.985)
DQB1*03	301 (37.7)	151 (48.7)	0.001	0.02	1.569 (1.189, 2.017)
DQB1*05	192 (24.1)	42 (13.5)	<0.001	<0.001	0.491 (0.341, 0.706)
Spain					
DRB1*1104	20 (1.10)	35 (17.70)	<0.001	<0.001	19.58 (11.049, 34.707)
DRB1*11	230 (12.50)	52 (26.30)	<0.001	<0.001	2.493 (1.796, 3.53)
DRB1*1501	142 (7.70)	38 (19.20)	<0.001	<0.001	2.847 (1.922, 4.217)
DRB1*15	165 (8.90)	43 (21.70)	<0.001	<0.001	2.823 (1.943, 4.102)
DQB1*0301	359 (19.50)	61 (30.80)	<0.001	0.02	1.842 (1.333, 2.544)
DQB1*0501	291 (15.80)	12 (6.10)	<0.001	0.02	0.344 (0.189, 0.625)
DQB1*05	358 (19.50)	17 (8.60)	<0.001	0.004	0.39 (0.234, 0.65)
DQB1*0602	121 (6.60)	34 (17.20)	<0.001	<0.001	2.952 (1.954, 4.461)
DQB1*06	316 (17.20)	57 (28.80)	<0.001	<0.001	1.955 (1.404, 2.721)

HLA Class II significant associations with ATAs (after 10 000-fold permutation testing) in Italy and Spain. Total number of alleles per series—Italy: SSc = 310; cases (Ctrl) = 798; Spain: SSc = 198; Ctrl = 1844. Ctrl: controls.

TABLE 3 HLA Class II associations with ACAs

HLA	Ctrl, n (%)	SSc, n (%)	Uncorrected P	Corrected P	OR (CI₉₅)
DRB1*0101	139 (7.54)	62 (13.96)	<0.001	<0.001	1.991 (1.447, 2.739)
DRB1*0701	283 (15.35)	28 (6.31)	<0.001	<0.001	0.373 (0.248, 0.556)
DRB1*0801	46 (2.49)	30 (6.76)	<0.001	<0.001	2.832 (1.766, 4.542)
DRB1*1104	20 (1.08)	23 (5.18)	<0.001	<0.001	4.982 (2.711, 9.156)
DQA1*0201	282 (15.29)	28 (6.31)	<0.001	<0.001	0.373 (0.249, 0.558)
DQA1*0401	58 (3.15)	36 (8.11)	<0.001	<0.001	2.717 (1.768, 4.175)
DQB1*0202	232 (12.58)	29 (6.53)	<0.001	0.007	0.485 (0.325, 0.725)
DQB1*02	468 (25.38)	68 (15.32)	<0.001	<0.001	0.532 (0.402, 0.703)
DQB1*0402	61 (3.31)	37 (8.33)	<0.001	<0.001	2.657 (1.742, 4.054)
DQB1*0501	291 (15.78)	113 (25.45)	<0.001	<0.001	1.822 (1.422, 2.336)

HLA Class II significant associations with ACAs (after 10 000-fold permutation testing) in Spain. No significant associations were found in Italy. Number of positive alleles in cases = 444 and controls = 1844. Ctrl: controls.

(cases: 29/508, 5.7% and SSc-ANA-positive subjects: 30/252, 11.9%; $P = 0.002$; OR = 0.417, CI₉₅ 0.235, 0.74); ACAs (cases: 130/776, 16.7% and SSc-ANA-positive subjects: 30/252, 11.9%; $P = 0.005$; OR = 1.844, CI₉₅ 1.2071, 2.816).

Summary of the associations between HLA Class II antigens and ACAs or ATAs

Table 2 lists all the significant associations we observed performing the ATAs vs controls comparisons, while Table 3 depicts the significant associations for the ACAs vs controls comparison; in this latter case, no significant results were observed in the Italian series. An exhaustive description of the comparison between SSc-specific antibodies and healthy controls is reported in the supplementary data, available at Rheumatology Online.

As described above, we also performed the ATAs vs ANAs and the ACAs vs ANAs comparisons in both series, yet a significant loss of power was observed due to the reduced sample size. When the two populations were analysed together via the meta-analysis procedure, we found an exclusive association between ATAs and the DR11 haplotype or the HLA-DRB1*1104 allele, which was not observed when the ACAs vs ANAs comparison was taken into account. Table 4 summarizes the significant results after the above-mentioned meta-analysis procedure. It should be observed that as far as the ACAs vs ANAs analysis is concerned, many comparisons were not possible due to heterogeneity issues, yet in the sole Spanish series, the associations with HLA-DRB1*01, DRB1*0701, DQA1*0201, DQB1*02 or DQB1*0501, previously found when healthy subjects were used as the control group, were confirmed (detailed results not shown).

Associations with SSc subsets

In both the case series, the association between the HLA-DRB1*1104 allele and SSc was not restricted to any of the two cutaneous subsets (Tables 5 and 6) despite its strong association with ATAs, and the tight link between these antibodies and the dcSSc subset. Similarly, the DR11 haplotype was found to be significantly increased in dcSSc and lcSSc patients, both in Italian subjects (dcSSc cases: 32/188, 17% and controls: 59/798, 7.4% haplotypes; $P < 0.001$; OR = 2.569, CI$_{95}$ 1.616, 4.085; lcSSc cases: 77/542, 14.2% and controls: 59/798, 7.4% haplotypes; $P < 0.001$; OR = 20.625, CI$_{95}$ 11.536, 36.876) and in Spanish patients (dcSSc cases: 29/230, 12.6% and controls: 63/1844, 3.41% haplotypes; $P < 0.001$; OR = 3.69, CI$_{95}$ 2.328, 5.85; lcSSc cases: 57/646, 8.8% and controls: 63/1844, 3.4% haplotypes; $P = 0.008$; OR = 2.583, CI$_{95}$ 1.785, 3.736).

Meta-analysis for the HLA-DRB1*1104 yielded inconsistent results due to heterogeneity issues. The DQA1*0501 and the DQB1*0301 alleles, which are in tight LD with HLA-DRB1*1104 in the DR11 haplotype, were associated with the dcSSc and the lcSSc subset, respectively. These results suggest and confirm that indeed, in Caucasoids, the HLA-DRB1*1104 allele or the DR11 haplotype do not associate with a specific cutaneous subset.

As far as other associations with the dcSSc or lcSSc subsets were concerned (Tables 5 and 6), they mostly mirrored associations with ATAs or ACAs, respectively. A larger number of associations were observed in the Spanish series, most likely owing to the larger number of subjects or to the tighter link between autoantibodies and SSc subsets (Italy: $P = 1 \times 10^{-22}$; Spain: $P = 1 \times 10^{-38}$ for the ATA/ACA *vs* dcSSc/lcSSc comparison).

Discussion

The present study represents the largest ever performed in European Caucasoids and confirms and extends previous observations from North American Caucasoids [5].

TABLE 4 HLA Class II associations with ATA or ACA using anti-nuclear-positive patients as controls

HLA	ANA, *n* (%)	Specific antibodies, *n* (%)	*P*-value	OR (CI$_{95}$)
ATAs				
HLA-DRB1*01	30 (11.9)	29 (5.7)	0.002	0.468 (0.235, 0.739)
DRB1*1104	21 (8.3)	99 (19.4)	<0.001	2.392 (1.460, 3.919)
DQB*0501	30 (11.9)	32 (6.3)	0.01	0.506 (0.297, 0.861)
DQB*05	30 (11.9)	59 (11.6)	0.002	0.513 (0.336, 0.782)
DR11 haplotype	15 (5.9)	86 (16.9)	<0.001	3.073 (1.738, 5.432)
ACAs				
HLA-DRB1*01	30 (11.9)	130 (16.7)	0.005	1.744 (1.207, 2.816)

HLA Class II significant associations with ATAs or ACAs after meta-analysis in the Italian and the Spanish series using ANA-positive patients as controls. Total number of alleles: ATAs = 508; ACAs = 776; ANAs = 252.

TABLE 5 HLA Class II associations with dcSSc

HLA	Ctrl, *n* (%)	dcSSc, *n* (%)	Uncorrected *P*	Corrected *P*	OR (CI$_{95}$)
Italy					
DRB1*1104	83 (10.4)	38 (20.2)	<0.001	0.001	2.182 (1.43, 3.329)
DQA*0501	250 (31.3)	87 (46.3)	<0.001	<0.001	1.888 (1.366, 2.609)
DQB*03	301 (37.7)	95 (50.5)	0.001	0.02	1.687 (1.225, 2.322)
Spain					
DRB1*01	255 (13.8)	12 (5.2)	<0.001	0.004	0.343 (0.189, 0.622)
DRB1*0402	27 (1.5)	11 (4.8)	<0.001	0.02	3.38 (1.654, 6.909)
DRB1*1104	20 (1.1)	26 (11.3)	<0.001	<0.001	11.623 (6.375, 21.193)
DRB1*11	230 (12.5)	49 (21.3)	<0.001	0.004	1.9 (1.346, 2.681)
DRB1*15	165 (8.9)	36 (15.7)	0.001	0.02	1.883 (1.278, 2.789)
DQB*0501	291 (15.8)	14 (6.1)	<0.001	0.008	0.346 (0.199, 0.602)
DQB*05	358 (19.4)	23 (10)	<0.001	0.007	0.461 (0.295, 0.72)

HLA Class II significant associations with the diffuse cutaneous subset (dcSSc) (after 10 000-fold permutation testing) in Italy and Spain. Total number of alleles per series: Italy, dcSSc = 188; controls = 798; Spain, dcSSc = 230; Ctrl = 1844. Ctrl: control.

TABLE 6 HLA Class II associations with lcSSc

HLA	Ctrl, n (%)	lcSSc, n (%)	Uncorrected P	Corrected P	OR (CI₉₅)
Italy					
DRB1*1104	83 (10.4)	100 (18.5)	<0.001	<0.001	1.949 (1.423, 2.669)
DQA*0501	250 (31.3)	231 (42.6)	<0.001	<0.001	1.628 (1.298, 2.042)
DQA*05	326 (40.8)	274 (50.5)	<0.001	0.01	1.48 (1.188, 1.844)
Spain					
DRB1*0701	283 (15.3)	59 (9.1)	<0.001	0.003	0.055 (0.412, 0.745)
DRB1*0801	46 (2.5)	36 (5.6)	<0.001	0.004	2.307 (1.477, 3.602)
DRB1*1104	20 (1.1)	38 (5.9)	<0.001	<0.001	5.7 (3.291, 9.871)
DRB1*1301	135 (7.3)	22 (3.4)	<0.001	0.01	0.446 (0.282, 0.707)
DQA*0103	158 (8.6)	26 (4.0)	<0.001	0.004	0.447 (0.293, 0.684)
DQA*0401	58 (3.1)	43 (6.7)	<0.001	0.003	2.196 (1.464, 3.293)
DQB*0402	61 (3.3)	44 (6.8)	<0.001	0.004	2.136 (1.434, 3.182)
DQB*05	358 (19.4)	163 (25.3)	0.002	0.02	1.401 (1.133, 1.731)

HLA Class II significant associations with the limited cutaneous subset (lcSSc) (after 10 000-fold permutation testing) in Italy and Spain. Total number of alleles per series—Italy: lcSSc = 542; Ctrl = 798; Spain: lcSSc = 646; Ctrl = 1844. Ctrl: controls.

We were able to replicate the strong association between the HLA-DRB1*11(04) allele(s) and SSc and, more notably, the association between the DR11 haplotype and scleroderma. To our knowledge, ours and Arnett *et al.*'s [5] studies are the first to describe a full HLA Class II haplotype analysis in SSc. The study of haplotypes provides several advantages over an analysis based on individual polymorphisms in the presence of multiple susceptibility alleles, including an increased power to detect an association in case–control studies, even when markers with multiple alleles, such as HLA, are taken into account [23].

Our analysis confirms that the well-documented association between the HLA-DRB1*11(04) allele and SSc in Caucasians is not restricted to any of the cutaneous subsets and that this allele is overrepresented in Scl70-positive patients. The latter finding may be related to the unique presence of a valine at position 86 of the antigen-binding region, which may affect peptide binding [24] and autoantigen presentation, promoting autoimmunity [25]. A different, yet similar, mechanism may favour the presentation of self or foreign peptides to autoreactive T lymphocytes via the HLA-DRB1*01 allele to promote the production of ACAs. The peculiar conformation of the antigen-presenting cleft of the HLA-DRB1*01 or HLA-DRB1*11(04) alleles would thus determine the targeting of particular autoantigens, which eventually results in specific associations with disease-specific autoantibodies. The finding that in SSc patients ACAs and ATAs are substantially mutually exclusive [26] suggests that their production is the result of a different activation of the immune system. These autoimmune processes may eventually lead to different clinical entities where, for instance, ATAs are associated with interstitial pulmonary fibrosis and cardiac involvement, whereas ACAs are associated with pulmonary arterial hypertension [27–29]. Preliminary studies suggest that it could be possible to

dampen MHC-driven autoimmune processes by directly and selectively targeting the antigen-presenting region on MHC molecules [30]. Whether this strategy could provide an effective means of immunointervention in SSc remains an intriguing possibility to be verified in the future.

HLA Class II may also contribute to the pathogenesis and progression of SSc by other mechanisms that do not involve antigen presentation. It has been observed that HLA Class II may transduce the signals into fibroblasts promoting the secretion of IL-6 and monocyte chemo-attractant protein 1 (MCP-1) [31], whose levels are increased in SSc patients, particularly in those clinical forms that are associated with ATAs [32].

The robust identification of SSc-associated HLA alleles in large cohorts may have important practical implications for an improved understanding of SSc development, progression and treatment. Despite high ORs and very low P-values, the discriminative and prognostic value of HLA Class II alleles in SSc remains weak, with a balanced predictive accuracy (e.g. the mathematical mean of sensitivity and specificity) no greater than 55% in the meta-analysis that we conducted (results not shown). Thus, we believe our findings, especially those from the meta-analysis, may primarily serve as a tool to focus future research on the characterization of the mechanisms that promote the development of SSc, may they be related or not to antigen presentation.

Rheumatology key message

- HLA Class II alleles are strongly associated with Caucasian SSc patients.
- SSc-specific autoantibodies are strongly and differently associated with HLA Class II alleles.
- HLA Class II alleles may drive immunological processes in the pathogenesis of SSc.

Acknowledgements

Supplementary data

Supplementary data are available at *Rheumatology* Online.

References

1 Silver RM. Clinical aspect of systemic sclerosis (scleroderma). Ann Rheum Dis 1991;50:854–61.

2 Varga J, Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. J Clin Invest 2007;117:557–67.

3 Agarwal SK, Reveille JD. The genetics of scleroderma (systemic sclerosis). Curr Opin Rheumatol 2010;22:133–8.

4 Agarwal SK, Tan FK, Arnett FC. Genetics and genomic studies in scleroderma (systemic sclerosis). Rheum Dis Clin North Am 2008;34:17–40.

5 Arnett FC, Gourh P, Shete S *et al*. Major histocompatibility complex (MHC) class II alleles, haplotypes, and epitopes which confer susceptibility or protection in the fibrosing autoimmune disease systemic sclerosis: analyses in 1300 Caucasian, African-American and Hispanic cases and 1000 controls. Ann Rheum Dis 2010;69:822–7.

6 Briggs D, Welsh KI. Major histocompatibility complex class II genes and systemic sclerosis. Ann Rheum Dis 1991;50:862–5.

7 Simeón CP, Fonollosa V, Tolosa C *et al*. Association of HLA class II genes with systemic sclerosis in Spanish patients. J Rheumatol 2009;36:2733–6.

8 Frezza D, Giambra V, Tolusso B *et al*. Polymorphism of immunoglobulin enhancer element HS1,2A: allele *2 associates with systemic sclerosis. Comparison with HLA-DR and DQ allele frequency. Ann Rheum Dis 2007;66:1210–5.

9 Favalli E, Ingegnoli F, Zeni S *et al*. [HLA typing in sistemi sclerosis]. Reumatismo 2001;53:210–4.

10 Vlachoyiannopoulos PG, Dafni UG, Pakas I *et al*. Systemic scleroderma in Greece: low mortality and strong linkage with HLA-DRB1*1104 allele. Ann Rheum Dis 2000;59:359–67.

11 Fanning GC, Welsh KI, Bunn C *et al*. HLA associations in three mutually exclusive autoantibody subgroups in UK systemic sclerosis patients. Br J Rheumatol 1998;37:201–7.

12 Hietarinta M, Ilonen J, Lassila O, Hietaharju A. Association of HLA antigens with anti-Scl-70-antibodies and clinical manifestations of systemic sclerosis (scleroderma). Br J Rheumatol 1994;33:323–6.

13 Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutics Criteria Committee. Arthritis Rheum 1980;23:581–90.

14 Hudson M, Taillefer S, Steele R *et al*. Improving the sensitivity of the American College of Rheumatology classification criteria for systemic sclerosis. Clin Exp Rheumatol 2007;25:754–7.

15 LeRoy EC, Black C, Fleischmajer R *et al*. Scleroderma (systemic sclerosis): classification, subset and pathogenesis. J Rheumatol 1988;15:202–5.

16 Le Roy EC, Medsger TA Jr. Criteria for the classification of early systemic sclerosis. J Rheumatol 2001;28:1573–6.

17 Dalva K, Beksac M. Sequence-specific primed PCR (PCR-SSP) typing of HLA Class I and Class II alleles. Methods Mol Med 2007;134:51–60.

18 Good PI. Permutation tests. A practical guide to resampling methods for testing hypotheses. New York: Springer, 2000.

19 Cheverud JM. A simple correction for multiple comparisons in interval mapping genome scans. Heredity 2001;87:52–8.

20 Stephens M, Smith N, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–89.

21 Bryc K, Velez C, Karafet T *et al*. Colloquium paper: genome-wide patterns of population structure and admixture among Hispanic/Latino populations. Proc Natl Acad Sci USA 2010;107:8954–61.

22 Bax L, Yu LM, Ikeda N *et al*. Development and validation of MIX: comprehensive free software for meta-analysis of causal research data. BMC Med Res Methodol 2006;6:50.

23 Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539–58.

24 Ong B, Willcox N, Wordsworth P *et al*. Critical role for the Val/Gly86 HLA-DR beta dimorphism in autoantigen presentation to human T cells. Proc Natl Acad Sci USA 1991;88:7343–7.

25 Busch R, Hill CM, Hayball JD *et al*. Effect of natural polymorphism at residue 86 of the HLA-DR beta chain on peptide binding. J Immunol 1991;147:1292–8.

26 Dick T, Mierau R, Bartz-Bazzanella P *et al*. Coexistence of antitopoisomerase I and anticentromere antibodies in patients with systemic sclerosis. Ann Rheum Dis 2002;1:121–7.

27 Steen VD, Powell DL, Medsger TA Jr. Clinical correlations and prognosis based on serum autoantibodies in patients with systemic sclerosis. Arthritis Rheum 1988;31:196–203.

28 Ferri C, Valentini G, Cozzi F *et al*. Systemic sclerosis: demographic, clinical, and serologic features and survival in 1,012 Italian patients. Medicine 2002;81:139–53.

29 Hamaguchi Y. Autoantibody profiles in systemic sclerosis: predictive value for clinical evaluation and prognosis. J Dermatol 2010;37:42–53.

30 Oshima M, Deitiker P, Atassi MZ. Targeting the antigen-binding site of HLA-restricting alleles in treatment of autoimmune disease. Crit Rev Immunol 2007;27: 271–88.

31 Ohyama H, Nishimura F, Meguro M *et al*. Counter-antigen presentation: fibroblasts produce cytokines by signalling through HLA class II molecules without inducing T-cell proliferation. Cytokine 2002;17:175–81.

32 Scala E, Pallotta S, Frezzolini A *et al*. Cytokine and che-mokine levels in systemic sclerosis: relationship with cutaneous and internal organ involvement. Clin Exp Immunol 2004;138:540–6.