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Clinical Study

HLA Markers for Poor Prognosis in Systemic Sclerosis Brazilian Patients

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Objectives. The aim of this study was to evaluate human leukocyte antigen (HLA) involvement in the disease expression and poor prognostic clinical features (pulmonary fibrosis and pulmonary arterial hypertension) in patients diagnosed with systemic sclerosis (SSc) in a multiethnic population. *Methods*. SSc patients followed up between 2008 and 2011 were included, and clinical data were obtained through records review. Molecular HLA typing was performed (polymerase chain reaction amplification technique using specific primer sequences). The statistical analysis involved Fisher's exact test and Pearson's corrected chi-square test. P values ≤ 0.05 were considered significant. The delta method was used to estimate the variance of the prevalence ratio (PR). *Results*. A total of 141 patients (120 women and 21 men) with SSc were studied, including 33.3% with diffuse cutaneous SSc (dcSSc), 62.4% with limited cutaneous SSc (lcSSc), and 4.3% with sine scleroderma. Pulmonary fibrosis was present in 61 patients (43.3%), and the HLA-A*30 and DQB1*04 alleles were related to susceptibility. In contrast, the HLA-DRB1*01 and DQB1*05 alleles were protective. Pulmonary arterial hypertension was diagnosed in 19 patients (13.5%) and was associated with HLA-B*35 and C*04; in contrast, C*03 seemed to be protective. *Conclusions*. Our current study documents the association of some classes I and II HLA alleles with the most severe clinical manifestations in a multiethnic case series. Our findings differed slightly from the previous data in other populations.

1. Introduction

Systemic sclerosis (SSc) is an autoimmune disease of unknown etiology that is characterized by vascular dysfunction and cutaneous and visceral fibrosis. Women are more frequently affected than men at a ratio of 4 : 11, and the peak incidence occurs in the fourth and fifth decades of life. The incidence varies from 2.3 to 22.8/1 million/year [1]. The prevalence appears to be increasing due to improved survival in recent decades [2]. The symptoms with greater prognostic impact that are the main causes of death in SSc patients are pulmonary fibrosis (PF) and pulmonary arterial hypertension (PAH) [3].

Although the pathogenesis of SSc remains unclear, genetic factors are thought to contribute to the disease. The HLA (*human leukocyte antigen*) genes have been implicated in the susceptibility to SSc and in its clinical and serological manifestations [4–10].

HLA widely participates in immunological processes. Class I (HLA A, B, and C) and class II (HLA DR, DQ, and DP) molecules are expressed on the cell surface and participate in antigen presentation and T lymphocyte activation. Class I molecules also activate natural killer (NK) cell receptors [11], and class II molecules appear to stimulate the secretion of IL-6 and monocyte chemotactic protein (MCP-1) by fibroblasts, especially in antibody antitopoisomerase (ATA) associated clinical forms [12].

Susceptibility to the disease was found to be associated with the HLA-DQA1*0501 and DQB1*0301 alleles in all ethnic groups. The DRB1*1104 allele was associated with SSc in Caucasian and Hispanic patients, whereas DRB1*0804 was correlated with the disease in African Americans [13]

and DRB1*1502 with Japanese and Koreans [14]. HLA-DRB1*01 and DRB1*11 (mainly DRB1*1101 and DRB1*1104) were also associated with SSc [15]. A multicenter study (GWAS, Genome Wide Association Study) established HLA-DQB1 as the main allele related to susceptibility [16]. Zhou et al. found an association of HLA-DPB1 and DPB2 with increased susceptibility to the disease, primarily associated with ATA [17]. HLA-A*30, A*32, B*57, Cw14, DRB1*0701, DQA1*0201, DQB1*0202, and DRB1*1501 were identified as protectors [4, 13].

However, much stronger correlations have been demonstrated between certain HLA alleles and each of the SSc-specific autoantibodies, which may be clinically relevant because each autoantibody subset of scleroderma is associated with certain disease features and has different prognostic implications [14, 18–22]. ATA antibodies have a higher frequency of the HLA-DRB1*1104, DQA1*0501, DQB1*0301, and DPB1*1301 alleles [13]. Caucasian patients were associated with HLA-DR5 [23–25], corresponding to DRB1*05, and Japanese patients were associated with HLA-DR2 [5], DRB1*15, or DRB1*16 in the current nomenclature. The anticentromere antibody (ACA) was related to the HLA-DRB1*01, DRB1*04, DQA1*0101, and DQB1*0501 alleles [7, 12, 13, 23].

Considering the clinical features, the HLA-DRB1 allele has been associated with the limited cutaneous form (lcSSc) [4] and B*62, DRB1*11 (DRB1*1104), and DRB1*07 with the diffuse cutaneous form (dcSSc) [4, 9]. An association between HLA alleles and visceral involvement in SSc is not well established. Some previous studies reported a correlation of the internal organ involvement of SSc and autoimmune patterns with different specific serological HLA statuses, and these associations appeared to differ according to ethnicity [7, 13].

A high frequency of the DRw6 and DRB3 alleles related to PAH has been described by Langevitz et al., with DRw6 associated with a higher mortality [26]. Some class I alleles were also related, such as A*30, B*13, B*65 [4], and B*35, which appear to play a role in the production of endothelin-1 (ET-1) in SSc [27, 28]. HLA-DRB1*01, DRB1*03, and DQB1*0501 were also related to PAH and ACA [29, 30].

Similarly, the DR52a and Cw*0602 alleles were found more frequently in SSc with PF [4, 26]. Other alleles described for PF in the presence of ATA are DRB1*11 (DRB1*1104) [10, 31], DPB1*1301 [29], and DQB1*0301 [29, 30].

In Brazil, a South American country with a multiethnic population, there has been no study concerning HLA and SSc. The aims of this study were to evaluate HLA involvement in the disease expression and to correlate the HLA alleles with the poor prognostic clinical features in patients diagnosed with SSc at a university referral hospital in the city of Campinas, state of Sao Paulo, Brazil.

2. Subjects and Methods

2.1. Patient Selection. We evaluated patients diagnosed with SSc who were followed for a period of 3 years (2008–2011) in the Rheumatology Department at the University of Campinas Teaching Hospital, a tertiary referral hospital located in the state of São Paulo, Brazil. The clinical data on the patients, who were all unrelated ethnically, were obtained through a records review.

This study was approved by the Ethics Committee of Campinas State University. The patients provided informed consent.

The SSc diagnosis was based on the American College of Rheumatology (ACR) criteria for SSc [10]. Patients under 18 years old and with overlap syndrome were excluded. All patients were evaluated for gender, visceral involvement, and laboratory test results and underwent a routine rheumatology examination. They were classified according to cutaneous involvement as having the diffuse, limited, or sine scleroderma forms. Gastrointestinal (GI) involvement, PF, and PAH were the visceral involvements that were considered. Limited disease was defined as definite skin thickening confined to the distal extremities, whereas diffuse disease showed the additional involvement of the skin proximal to the knees and elbows. The sine scleroderma form was defined according to established criteria [32, 33].

The presence and pattern of the antinuclear antibody (ANA) were also evaluated.

GI tract involvement was confirmed by imaging studies (contrast radiography, esophageal emptying scintillography, and intestinal transit) and upper gastrointestinal endoscopy. PAH was defined when the right ventricular pressure was higher than 40 mmHg by Doppler echocardiogram. The alteration in the systolic pulmonary artery, as it is an examiner-dependent result, was confirmed with another echocardiogram after a minimum interval of two months. When possible, these patients underwent confirmatory cardiac catheterism (medium pulmonary arterial pressure $\geq$ 25 mmHg) [34]. PF was investigated by pulmonary function testing and high resolution computed tomography (HRCT) and was diagnosed when the forced vital capacity or total lung capacity (TLC) was less than 70% of the predicted value. The main CT findings were hyperdense pulmonary nodules, ground glass, reticular opacities, and traction bronchiectasis [35].

2.2. Laboratory Methods. DNA was extracted from whole blood using a *commercial kit* (Illustra Blood genomic Prep Mini Spin Kit/GE Healthcare Bio-Sciences AB, Buckinghamshire, UK).

2.3. HLA Genotyping. HLA classes I and II genotyping was performed by the polymerase chain reaction amplification (PCR) technique using specific primer sequences (One Lambda INC/Generic Class I and Generic Class II, CA, USA).

3. Statistical Analysis

The patients' data were analyzed by software SPSS for Windows release 13. The association among the HLA alleles, different clinical features, and gender was investigated using Fisher's exact test and Pearson's corrected chi-square test. P values ≤ 0.05 were considered significant after correction

Table 1: Characteristics of the study case series.

	N	%
Gender F : M	120 : 21	
Diffuse disease	47	(33.3)
Limited disease	88	(62.4)
Sine scleroderma	6	(4.3)
Pulmonary fibrosis	61	(43.3)
Pulmonary arterial hypertension	19	(13.5)
Gastrointestinal involvement	112	(79.4)
ANA	109	(77.3)

for the number of alleles with a frequency higher than 5% (nonrare allele). The delta method was used to calculate the confidence interval (variance) of the prevalence ratio (PR).

4. Results

This series included 141 patients, with 120 (85.1%) women and 21 (14.9%) men. Regarding the clinical form, 47 (33.3%) had dcSSc, 88 (62.4%) had lcSSc, and 6 (4.3%) had sine scleroderma SSc.

We also classified patients based on the main antinuclear antibody (ANA) patterns. The centromeric pattern was present in 25 patients (17.7%), nucleolar in 23 (16.3%), and other patterns in 61 (43.3%). In addition, 32 patients (22.7%) had a negative ANA result.

A confirmatory cardiac catheterism was performed in 14 of 19 patients diagnosed with PAH. The clinical features identified in this case series are listed in Table 1.

There was no evidence of an association between gender and any of the features considered (clinical forms, visceral involvement, or ANA and HLA alleles).

We found no association of the HLA alleles with the clinical forms of the disease (diffuse, limited, and sine scleroderma) or with the main patterns of ANA that were considered (centromeric and nucleolar).

Concerning the clinical manifestations, evidence of an association was found between PF and PAH ($P = 0.017$). GI involvement was not associated with any HLA alleles. With respect to pulmonary fibrosis, there was an association with class I HLA-A*30 ($P = 0.020$) and class II DRB1*01 ($P = 0.004$), DQB1*05 ($P < 0.001$), and DQB1*04 ($P = 0.019$). The A*30 and DQB1*04 alleles were more frequent in patients with PF than in patients without PF. The presence of these two alleles was related to a higher risk for interstitial lung disease (PR = 1.86 and PR = 1.70, resp.). However, the HLA-DRB1*01 and DQB1*05 alleles appeared to be protective (PR = 0.38 and PR = 0.41, resp.) (Tables 2, 3, and 4). Pulmonary arterial hypertension was related only to class I B*35 ($P = 0.035$), C*03 ($P = 0.044$), and C*04 ($P = 0.015$) alleles. The HLA-B*35 and C*04 alleles were more frequent, resulting in a greater predisposition to this feature (PR = 2.58 and PR = 2.71, resp.). The C*03 allele was less frequent in these patients and was negatively correlated with this feature (PR = 0.13) (Tables 2, 3, 4 and 5).

Table 2: Frequency of class I HLA alleles in pulmonary fibrosis (PF) and pulmonary arterial hypertension (PAH).

HLA	PF			PAH		
	+ *N* (%)	− *N* (%)	*P*	+ *N* (%)	− *N* (%)	*P*
	61	80		19	122	
A∗01	13 (21.3)	18 (22.5)	0.866	4 (2.0)	27 (22.1)	1
A∗02	24 (39.3)	40 (50.0)	0.208	7 (36.8)	57 (46.7)	0.421
A∗03	12 (19.6)	24 (30.0)	0.163	5 (26.3)	31 (25.4)	1
A∗33	5 (8.2)	5 (6.2)	0.746	1 (5.3)	9 (7.4)	1
A∗23	6 (9.8)	6 (7.5)	0.622	2 (10.6)	10 (8.2)	0.665
A∗11	7 (11.5)	5 (6.2)	0.271	3 (15.8)	9 (7.4)	0.207
A∗29	6 (9.8)	8 (10.0)	0.974	2 (10.6)	12 (9.8)	1
A∗30	9 (14.7)	3 (3.7)	0.020	3 (15.8)	9 (7.4)	0.207
A∗24	9 (14.7)	12 (15.0)	0.968	1 (5.3)	20 (16.4)	0.307
A∗68	7 (11.5)	9 (11.2)	0.967	0 (0)	16 (13.1)	0.129
B∗15	9 (14.7)	8 8 (10.0)	0.390	1 (5.3)	16 (13.1)	0.469
B∗07	6 (9.8)	13 (16.2)	0.269	3 (15.8)	16 (13.1)	0.722
B∗44	9 (14.7)	15 (18.7)	0.532	2 (10.6)	22 (18.0)	0.530
B∗18	12 (19.6)	13 (16.2)	0.598	5 (26.3)	20 (16.4)	0.333
B∗35	13 (21.3)	18 (22.5)	0.866	8 (42.1)	23 (18.8)	0.035
B∗39	5 (8.2)	9 (11.2)	0.548	2 (10.6)	12 (9.8)	1
B∗51	7 (11.5)	7 (8.7)	0.592	4 (21.0)	10 (8.2)	0.098
B∗08	8 (13.1)	6 (7.5)	0.269	2 (10.6)	12 (9.8)	1
B∗14	6 (9.8)	10 (12.5)	0.621	3 (15.8)	13 (10.6)	0.454
C∗03	8 (13.1)	14 (17.5)	0.477	0 (0)	22 (18.0)	0.044
C∗06	14 (22.9)	18 (22.5)	0.950	4 (21.0)	28 (22.9)	1
C∗07	28 (46.0)	33 (41.3)	0.581	10 (52.6)	51 (41.8)	0.376
C∗04	19 (31.1)	22 (27.5)	0.637	10 (52.6)	31 (25.4)	0.015
C∗05	4 (6.6)	11 (13.7)	0.170	3 (15.8)	12 (9.8)	0.428
C∗12	7 (11.5)	12 (15.0)	0.544	1 (5.3)	18 (14.7)	0.470
C∗08	5 (8.2)	11 (13.7)	0.303	3 (15.8)	13 (10.6)	0.454
C∗02	4 (6.6)	10 (12.5)	0.242	1 (5.3)	13 (10.6)	0.693

5. Discussion

Our study investigated the frequencies of HLA class I and II alleles in ethnically unrelated Brazilian patients with SSc and the relationships of these alleles with the main clinical manifestations. This work represents the first HLA genotyping study performed in our multiethnic population. Studies involving HLA and SSc are important from an etiopathogenic point of view, especially the clinical perspective, as an attempt to identify patients with more severe disease.

In our series, there was no evidence of an association between the clinical forms of the disease and the HLA alleles. Our results did not support the previous reports of a higher skin score in the presence of the HLA-B*62, DRB1*11, and DRB1*07 alleles [4, 9].

Concerning the clinical manifestations, there are few reports about the relationship between GI tract involvement and the HLA alleles. We did not find any predisposing allele, which may be due to the high frequency of this feature,

Table 3: Frequency of class II HLA alleles in pulmonary fibrosis (PF) and pulmonary arterial hypertension (PAH).

HLA	PF			PAH		
	+	–		+	–	
	N (%)	*N* (%)	*P*	*N* (%)	*N* (%)	*P*
	61	80		19	122	
DRB1∗01	5 (8.2)	22 (27.5)	0.004	4 (21.0)	23 (18.9)	0.761
DRB1∗04	14 (22.9)	18 (22.5)	0.950	5 (26.3)	27 (22.1)	0.769
DRB1∗16	3 (4.9)	8 (10.0)	0.350	0 (0)	11 (9.0)	0.360
DRB1∗11	24 (39.3)	23 (28.7)	0.186	7 (36.8)	40 (32.8)	0.727
DRB1∗10	1 (1.6)	6 (7.5)	NC	0 (0)	7 (5.7)	0.594
DRB1∗13	10 (16.4)	13 (16.2)	0.086	5 (26.3)	28 (22.9)	0.773
DRB1∗08	10 (16.4)	6 (7.5)	0.099	3 (15.8)	13 (10.7)	0.454
DRB1∗15	14 (22.9)	16 (20.0)	0.671	3 (15.8)	27 (22.1)	0.764
DRB1∗03	14 (22.9)	17 (21.2)	0.809	4 (21.0)	27 (22.1)	1
DRB1∗07	9 (14.7)	12 (15.0)	0.968	1 (5.3)	20 (16.4)	0.307
DRB1∗09	3 (4.9)	1 (1.2)	NC	1 (5.3)	3 (2.6)	NC
DRB1∗12	3 (4.9)	1 (1.2)	NC	1 (5.3)	3 (2.6)	NC
DRB1∗14	2 (3.3)	1 (1.2)	NC	0 (0)	3 (2.6)	NC
DRB5	13 (21.3)	18 (22.5)	0.866	2 (10.6)	29 (23.8)	0.246
DRB5∗01	4 (6.6)	3 (3.7)	NC	1 (5.3)	6 (4.9)	1
DRB3	11 (18.0)	10 (12.5)	0.361	2 (10.6)	19 (15.6)	0.739
DRB3∗01	2 (3.3)	7 (8.7)	0.299	0 (0)	9 (7.4)	0.690
DRB3∗02	28 (46.0)	36 (45.0)	0.915	10 (52.6)	54 (44.3)	0.496
DRB4	3 (4.9)	0	NC	1 (5.3)	2 (1.6)	NC
DRB4∗01	22 (36.0)	31 (38.7)	0.744	5 (26.3)	48 (39.3)	0.275
DQB1∗03	34 (55.8)	44 (55.0)	0.930	11 (57.9)	67 (55.0)	0.808
DQB1∗05	11 (18.0)	38 (47.5)	<0.001	4 (21.0)	45 (36.9)	0.178
DQB1∗06	23 (37.7)	32 (40.0)	0.782	7 (36.8)	48 (39.3)	0.835
DQB1∗02	19 (31.1)	24 (30.0)	0883	4 (21.0)	39 (31.9)	0.336
DQB1∗04	14 (22.9)	7 (8.7)	0.019	5 (26.3)	16 (13.1)	0.163

NC: non calculable.

Table 4: Risk and protective alleles for pulmonary fibrosis.

PF-related alleles	PR	(95% CI)	P^*
HLA-A∗30	1.86	(1.26–2.74)	0.020
HLA-DQB1∗04	1.70	(1.17–2.48)	0.019
HLA-DQB1∗05	0.41	(0.24–0.72)	<0.001
HLA-DRB1∗01	0.38	(0.17–0.85)	0.004

95% CI: 95% confidence intervals; PR: prevalence ratio; * univariate *P* values calculated with Chi-square and Fisher's exact tests where appropriate.
The PR and *P* values were obtained by comparing patients with and without PF and PAH.

found in almost 80% of the patients. We found evidence of an association between PF and PAH, which are both determinants of poor prognosis.

Regarding PAH, we found a higher risk in the presence of HLA-B*35 and C*04. The association with HLA-B*35 had already been described, and this allele appears to be involved in the upregulation of ET-1 and the pathogenesis of PAH [27, 28]. Gladman et al. found a significant association between PAH and the HLA-A*30, B*13, B*65, and DRB1*03 alleles [4]. Our results did not confirm these findings. The frequency of these alleles in our sample was 15.8% for A*30, 5.3% for B*13, 21% for DRB1*03, and 0% for B*65. In this analysis, C*03 was noted as a protective allele, being absent in all patients with PAH. We emphasize that C*04 and C*03 were not previously described to be related to PAH. Moreover, the alleles associated with an increased risk for PAH, B*35 and C*04, are often interrelated.

Table 5: Risk and protective alleles for pulmonary arterial hypertension.

PAH-related alleles	PR	(95% CI)	P^*
HLA-B∗35	2.58	(1.14–5.85)	0.035
HLA-C∗04	2.71	(1.19–6.18)	0.015
HLA-C∗03	0.13	(0.01–2.14)	0.044

95% CI: 95% confidence intervals; PR: prevalence ratio; * univariate *P* values calculated with Chi-square and Fisher's exact tests where appropriate.
The PR and *P* values were obtained by comparing patients with and without PF and PAH.

The analysis of patients with PF revealed intriguing results. The previously described associations between PF and HLA alleles were usually linked to ATA [10, 29–31], but we did not consider the autoantibody profiles. The Cw*0602 and DR52a alleles were associated with PF [4, 26], but we did not identify these alleles as risk factors in our study. None of our patients presented these alleles. In our sample, both HLA-A*30 and DQB1*04 played a role in the susceptibility to this manifestation. An interesting finding was A*30 as a risk factor in PF but not in PAH as described by Gladman et al. [4]. In addition, the presence of the DRB1*01 and DQB1*05 alleles was negatively related to PF and, therefore, considered protective.

The delta method was chosen due to the study design, a cross-sectional in contrast to case-controlled study, where the odds ratio should be more suitable. A limitation of this study was the genotyping resolution. The technique used for HLA typing (PCR-based sequence analysis using specific primers) does not always provide the gene subtype.

Our current study documents the association of some class I and II HLA alleles with the most severe clinical manifestations in a multiethnic case series. Our findings slightly differed from the previous data in other populations. Based on these and previous results, there are clearly multiple genetic patterns in SSc, and various HLA alleles were associated with different clinical and serological aspects of this disease. In conclusion, new descriptive and multicentric genetic studies are necessary for a better comprehension and definition of the disease expression in Brazil.

Conflict of Interests

The authors declare no conflict of interests.

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