Clinical Study

HLA Markers for Poor Prognosis in Systemic Sclerosis Brazilian Patients

Ana Paula Toledo Del Rio, Zoraida Sachetto, Percival Degrava Sampaio-Barros, João Francisco Marques-Neto, Ana Carolina Santos Londe, and Manoel Barros Bertolo

1 Unit of Rheumatology, Department of Internal Medicine, Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas, SP, Brazil
2 Division of Rheumatology, University of São Paulo School of Medicine (USP), São Paulo, SP, Brazil

Correspondence should be addressed to Ana Paula Toledo Del Rio; anapauladelrio@hotmail.com

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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 \( \leq 0.05 \) 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 4:1, 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 [II], 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-DQAI*0501 and DQBI*0301 alleles in all ethnic groups. The DRBI*1104 allele was associated with SSc in Caucasian and Hispanic patients, whereas DRBI*0804 was correlated with the disease in African Americans [13]...
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 ≥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 hypodense 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, Bückinghamshire, 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 $\leq 0.05$ were considered significant after correction
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.

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 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.

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

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 DRBI*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 = 0.944), 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).

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 genotypic 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, DRBI*11, and DRBI*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,
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.

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 DQBI*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 DRBI*01 and DQBI*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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References
[1] H. Chifflet, B. Fautrel, C. Sordet, E. Chatelus, and J. Sibilia, “Incidence and prevalence of systemic sclerosis: a systematic literature review,” Seminars in Arthritis and Rheumatism, vol. 37, no. 4, pp. 223–235, 2008.
[2] S. Bernatsky, L. Joseph, C. A. Pineau, P. Belisle, M. Hudson, and A. E. Clarke, “Scleroderma prevalence: demographic variations in a population-based sample,” Arthritis Care and Research, vol. 61, no. 3, pp. 400–404, 2009.
[3] V. D. Steen and T. A. Medsger, “Changes in causes of death in systemic sclerosis, 1972–2002,” Annals of the Rheumatic Diseases, vol. 66, no. 7, pp. 940–944, 2007.
[4] D. D. Gladman, T. N. Kung, F. Siannis, F. Pellett, V. T. Farewell, and P. Lee, “HLA markers for susceptibility and expression in scleroderma,” Journal of Rheumatology, vol. 32, no. 8, pp. 1481–1487, 2005.
[5] M. Kuwana, J. Kaburaki, Y. Okano, H. Inoko, and K. Tsuji, “The HLA-DR and DQ genes control the autoimmune response to DNA topoisomerase I in systemic sclerosis (scleroderma),” Journal of Clinical Investigation, vol. 92, no. 3, pp. 1296–1301, 1993.
[6] F. K. Tan, D. N. Stivers, F. C. Arnett, R. Chakraborty, R. Howard, and J. D. Reveille, “HLA haplotypes and microsatellite polymorphisms in and around the major histocompatibility complex region in a Native American population with a high prevalence of scleroderma (systemic sclerosis),” Tissue Antigens, vol. 53, no. 1, pp. 74–80, 1999.
[7] J. D. Reveille, M. Fischbach, T. McNearney et al., “Systemic sclerosis in 3 US ethnic groups: a comparison of clinical, sociodemographic, serologic, and immunogenetic determinants,” Seminars in Arthritis and Rheumatism, vol. 30, no. 5, pp. 332–346, 2001.
[8] D. Frezza, V. Giambra, B. Tolusso et al., “Polymorphism of immunoglobulin enhancer element HSI:1A allele 2 associates with systemic sclerosis. Comparison with HLA-DR and DQ allele frequency,” Annals of the Rheumatic Diseases, vol. 66, no. 9, pp. 1210–1215, 2007.
[9] L. S. Loubière, N. C. Lambert, M. M. Madeleine et al., “HLA allelic variants encoding DRII in diffuse and limited systemic sclerosis in Caucasian women,” Rheumatology, vol. 44, no. 3, pp. 318–322, 2005.
[10] C. P. Simeón, V. Fonollosa, C. Tolosa et al., “Association of HLA class II genes with systemic sclerosis in Spanish patients,” Journal of Rheumatology, vol. 36, no. 12, pp. 2733–2736, 2009.
[11] D. R. Karp, N. Maranthan, S. G. E. Marsh et al., “Novel sequence feature variant type analysis of the HLA genetic association in systemic sclerosis,” Human Molecular Genetics, vol. 19, no. 4, pp. 707–719, 2009.
[12] L. Beretta, B. Rueda, M. Marchini et al., “Analysis of class II human leucocyte antigens in Italian and Spanish systemic sclerosis,” Rheumatology, vol. 51, no. 1, pp. 52–59, 2012.
[13] F. C. Arnett, P. Gourh, S. Shete et al., “Major histocompatibility complex (MHC) class II alleles, haplotypes and epitopes which confer susceptibility or protection in systemic sclerosis: analyses in 1300 Caucasian, African-American and Hispanic cases and 1000 controls,” Annals of the Rheumatic Diseases, vol. 69, no. 5, pp. 822–827, 2010.
[14] M. Kuwana, Y. Okano, J. Kaburaki, and H. Inoko, “HLA class II genes associated with anticientromere antibody in Japanese patients with systemic sclerosis (scleroderma),” Annals of the Rheumatic Diseases, vol. 54, no. 12, pp. 983–987, 1995.
[15] N. C. Lambert, O. Distler, U. Müller-Ladner, T. S. Tyley, D. E. Furst, and J. L. Nelson, “HLA-DQA1*0501 is associated with diffuse systemic sclerosis in Caucasian men,” Arthritis Rheum, vol. 43, pp. 2005–2010, 2000.
[16] T. R. D. J. Radstake, O. Gorlova, B. Rueda et al., “Genome-wide association study of systemic sclerosis identifies CD247 as a new susceptibility locus,” Nature Genetics, vol. 42, pp. 426–429, 2010.
[17] X. Zhou, J. E. Lee, F. C. Arnett et al., “HLA-DPB1 and DPB2 are genetic loci for systemic sclerosis: a genome-wide association study in Koreans with replication in North Americans,” Arthritis and Rheumatism, vol. 60, no. 12, pp. 3807–3814, 2009.
[18] J. D. Reveille, E. Durban, M. J. MacLeod-St. Clair et al., “Association of amino acid sequences in the HLA-DQB1 first domain with the antitopoisomerase I autoantibody response in scleroderma (progressive systemic sclerosis),” Journal of Clinical Investigation, vol. 90, no. 3, pp. 973–980, 1992.
[19] F. C. Arnett, J. D. Reveille, R. Goldstein et al., “Autoantibodies to fibrillin in systemic sclerosis (scleroderma): an immunogenetic, serologic, and clinical analysis,” Arthritis and Rheumatism, vol. 39, no. 7, pp. 1151–1160, 1996.
[20] J. D. Reveille, D. Overbach, R. Goldstein, R. Moreda, R. A. Isern, and F. C. Arnett, “Association of polar amino acids at position 26 of the HLA-DQB1 first domain with the anticientromere autoantibody response in systemic sclerosis (scleroderma),” Journal of Clinical Investigation, vol. 89, no. 4, pp. 1208–1213, 1992.
[21] C. Marguerie, C. C. Bunn, J. Copier et al., “The clinical and immunogenetic features of patients with autoantibodies to the nucleolar antigen PM-Scl,” Medicine, vol. 71, no. 6, pp. 327–336, 1992.
[22] P. A. Morel, H. J. Chang, J. W. Wilson et al., “Severe systemic sclerosis with anti-topoisomerase I antibodies is associated with an HLA-DRw11 allele,” Human Immunology, vol. 40, no. 2, pp. 101–110, 1994.
[23] D. Briggs, C. Stephens, R. Vaughan, K. Welsh, and C. Black, “A molecular and serologic analysis of the major histocompatibility complex and complement component C4 in systemic sclerosis,” Arthritis and Rheumatism, vol. 36, no. 7, pp. 943–954, 1993.
[24] G. C. Fanning, K. I. Welsh, C. Bunn, R. du Bois, and C. M. Black, “HLA associations in three mutually exclusive autoantibody subgroups in UK systemic sclerosis patients,” British Journal of Rheumatology, vol. 37, no. 2, pp. 201–207, 1998.
[25] J. D. Reveille, M. Fischbach, T. McNearney et al., “Systemic sclerosis in 3 US ethnic groups: a comparison of clinical, sociodemographic, serologic, and immunogenetic determinants,” Seminars in Arthritis and Rheumatism, vol. 30, no. 4, pp. 332–346, 2001.
[26] P. Langevitz, D. Buskila, D. D. Gladman, G. A. Darlington, V. T. Farewell, and P. Lee, “HLA alleles in systemic sclerosis: association with pulmonary hypertension and outcome,” British Journal of Rheumatology, vol. 31, no. 9, pp. 609–613, 1992.
[27] A. Santaniello, G. Salazar, S. Lenna et al., “HLA-B35 upregulates the production of endothelin-1 in HLA-transfected cells: a possible pathogenetic role in pulmonary hypertension,” Tissue Antigens, vol. 68, no. 3, pp. 239–244, 2006.
[28] S. Lenna, D. M. Townsend, F. K. Tan et al., “HLA-B35 upregulates endothelin-1 and downregulates endothelial nitric oxide synthase via endoplasmic reticulum stress response in endothelial cells,” *Journal of Immunology*, vol. 184, no. 9, pp. 4654–4661, 2010.

[29] F. C. Arnett, “HLA and autoimmunity in scleroderma (systemic sclerosis),” *International Reviews of Immunology*, vol. 12, no. 2–4, pp. 107–128, 1995.

[30] J. Whyte, C. Artlett, G. Harvey et al., “HLA-DQBI associations with anti-topoisomerase-1 antibodies in patients with systemic sclerosis and their first degree relatives,” *Journal of Autoimmunity*, vol. 7, no. 4, pp. 509–520, 1994.

[31] F. C. Gilchrist, C. Bunn, P. J. Foley et al., “Class II HLA associations with autoantibodies in scleroderma: a highly significant role for HLA-DP,” *Genes and Immunity*, vol. 2, no. 2, pp. 76–81, 2001.

[32] E. Carwile LeRoy, C. Black, R. Fleischmajer et al., “Scleroderma (systemic sclerosis): classification, subsets and pathogenesis,” *Journal of Rheumatology*, vol. 15, no. 2, pp. 202–205, 1988.

[33] H. Poormoghim, M. Lucas, N. Fertig, and T. A. Medsger Jr, “Systemic sclerosis sine scleroderma: demographic, clinical, and serologic factors and survival in forty-eight patients,” *Arthritis Rheum*, vol. 43, pp. 444–451, 2000.

[34] A. Ramirez and J. Varga, “Pulmonary arterial hypertension in systemic sclerosis: clinical manifestations, pathophysiology, evaluation, and management,” *Treatments in Respiratory Medicine*, vol. 3, no. 6, pp. 339–352, 2004.

[35] N. K. Harrison, A. R. Myers, B. Corrin et al., “Structural features of interstitial lung disease in systemic sclerosis,” *American Review of Respiratory Disease*, vol. 144, no. 3, pp. 706–713, 1991.