HLA Markers for Susceptibility and Expression in Scleroderma

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ABSTRACT. ***Objective.*** Reported associations between HLA alleles and both susceptibility to and features of scleroderma have been conflicting. Our objective was (1) to determine the role of HLA alleles in the susceptibility to scleroderma; and (2) to determine the role of HLA alleles in various aspects of disease expression.

Methods. Consecutive patients were followed in the scleroderma clinic between 1996 and 1998. Clinical data were obtained through chart review. Healthy volunteers as well as cadaveric donors served as controls. Molecular HLA typing was performed (polymerase chain reaction/sequence-specific oligonucleotides). Statistical analysis included Fisher's exact test and multivariate analyses, using logistic and linear regression models.

Results. Ninety-five Caucasian patients (75 women, 20 men, age 43.9 yrs, disease duration 11.9 yrs) with scleroderma and 416 controls were studied. HLA-DRB1*01 and HLA-DRB1*11 were associated with susceptibility to scleroderma, whereas HLA-DRB1*07 was protective. HLA-A*30 and HLA-A*32 were also associated with susceptibility to scleroderma, while HLA-B*57 and HLA-Cw*14 were protective. HLA-B*62 and HLA-DRB1*07 had a significant correlation with the presence of diffuse skin involvement in both univariate and multivariate analyses. HLA-DRB1*11 was associated with high skin score values, while lower values were related to the presence of HLA-Cw*14 and HLA-DQB1*06. Both alleles retained significance in a linear regression model. High skin score values were related to the absence of anticentromere antibodies. Pulmonary fibrosis was associated with HLA-B*62 and HLA-Cw*0602, whereas pulmonary hypertension was associated with HLA-B*13 and HLA-B*65.

Conclusion. HLA alleles play a role in susceptibility to scleroderma and its disease expression. (J Rheumatol 2005;32:1481–7)

Key Indexing Terms:
SCLERODERMA SUSCEPTIBILITY HLA ALLELE

Scleroderma is an autoimmune disease of unknown etiology. Women are more frequently affected than men with a ratio of 4:1[1] and the peak incidence is in the fourth and fifth decades of life. The main disease manifestations are skin thickening and a small-vessel vasculopathy, with a high prevalence of Raynaud's phenomenon. The diffuse form of the disease with more extensive skin involvement is more likely to affect internal organs, particularly the lungs, heart, and kidneys. Although the pathogenesis of scleroderma remains unclear, genetic, immunologic, and environmental factors are thought to contribute to disease mechanisms. In particular, HLA genes on the short arm of chromosome 6 have been implicated in the susceptibility to scleroderma.

Several studies have investigated the relationship between HLA and scleroderma. Serologic studies found a significant increase in the Class II HLA-DR5 antigen in patients with scleroderma compared to healthy controls[2-4]. An increased frequency of the Class II HLA-DRw52 (HLA-DRB3 found in HLA-DR3/DR5/DR6)[4,5] and decreases in the Class II HLA-DRw53 antigen (HLA-DRB4 found in HLA-DR4/DR7/DR9) and HLA-DRw6[4] have also been observed.

Molecular technology has facilitated precise definition of HLA alleles and allowed detection of 2 alleles (HLA-DRB1*11 and HLA-DRB1*12) within the serologically defined single antigen HLA-DR5. Subsequent molecular studies found the HLA-DR11 allele was elevated in patients with scleroderma compared to healthy controls[6,7]. Results suggesting increases in frequency of HLA-DRB3 support findings of increased HLA-DRw52[1,6].

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Two conflicting studies in the early 1990s highlighted the importance of ethnicity in HLA and scleroderma studies. Briggs, *et al*[6] reported a decreased frequency of HLA-DR2 in their Caucasian patient population compared to healthy controls, whereas Sasaki, *et al*[8] reported an increased frequency of HLA-DR2 in their Japanese patients with scleroderma. These contrasting findings pointed to the importance of ethnically stratifying patient populations. Subsequent studies illustrated further ethnic differences, with Choctaw Native Americans having increased levels of HLA-DRB1*1602 (HLA-DR2) compared to controls[9], and African American patients having increased levels of HLA-DRB1*08[10].

Molecular studies examining the relationship between the HLA-DQ and scleroderma have found variable results. An early study of Caucasian patients found an increased frequency of HLA-DQA2[6]. Further investigation suggested an increased incidence of HLA-DQA1*0501 spanning both Choctaw Native American patients[9] and Caucasian men[1]. The latter study found an elevated HLA-DQB1*0301 in Caucasian, African American, and Hispanic patients compared to race-matched controls[10].

Moreover, investigations into associations between disease severity and serologic HLA status again suggested the importance of HLA-DR5. Gladman, *et al*[2] found that Class II HLA-DR5 was associated with higher disease and skin scores within scleroderma patients. Black, *et al* [3] indicated that Class II HLA-DR1 was associated with those with non-diffuse scleroderma compared to diffuse disease. Langevitz, *et al*[4] found an increased frequency of the HLA-DRw52 group (HLA-DRB3 found in HLA-DR3/DR5/DR6) as well as HLA-DRw6 in their scleroderma patients with pulmonary hypertension compared to scleroderma patients without pulmonary hypertension. It was further noted that the mortality rate for patients with pulmonary hypertension and HLA-DRw6 was significantly greater than those patients without the HLA-DRw6 antigen. Subsequent studies looking at pulmonary fibrosis and HLA have found an increased frequency of HLA-DR3 (HLA-DR52a) in patients with pulmonary fibrosis compared to patients without fibrosis[6,7].

Several studies have looked into the association between autoantibodies detected among patients with scleroderma and HLA. Two autoantibodies of particular interest are the antitopoisomerase antibodies (anti-Scl-70) and the anticentromere antibodies (ACA). Anti-Scl-70 have been found more frequently in scleroderma patients with pulmonary fibrosis and diffuse disease, whereas ACA are more frequently found in scleroderma patients with more limited forms of disease[11]; however, these relationships are not mutually exclusive. Molecular studies indicate that Caucasian patients with antitopoisomerase antibodies have a higher frequency of HLA-DR5 than Caucasian patients lacking the antibody[6,7,10]. A similar molecular study of Japanese patients with antitopoisomerase antibodies found elevated HLA-DR2 compared to race-matched patients without the antibody[12]. Molecular studies of Caucasian patients with the ACA found an increased frequency of HLA-DRB1*01[6,10] and HLA-DRB1*04[10] compared to patients lacking the antibody. Reveille, *et al*[10] also found elevated HLA-DRB1*01 and HLA-DRB1*04 in Hispanic patients with ACA compared to race-matched patients without the antibody.

Thus the associations between HLA alleles and both susceptibility to and characteristic features of scleroderma have been conflicting. Our objective was 2-fold: to determine the role of HLA alleles in the susceptibility to scleroderma; and to determine the role of HLA alleles in various aspects of disease expression.

MATERIALS AND METHODS

Setting. The University of Toronto Scleroderma Clinic, where patients have been followed prospectively according to a standard protocol since 1978.

Patient selection. Consecutive patients followed in the Scleroderma Clinic between 1996 and 1998 who agreed to provide a DNA sample were studied. The study was approved by the Research Ethics Board of the University Health Network. Patients and controls provided informed consent.

Clinical data collection. Patients had been followed prospectively in the Scleroderma Clinic, with clinical and laboratory data recorded according to a standard protocol. Clinical data on participating patients were obtained through a review of the clinic charts. For each clinic visit, skin thickening was assessed according to a validated, quantitative scoring system, similar to the Modified Rodnan Skin Score. This scoring method has a maximum value of 54, and has been shown to have a high correlation with the Rodnan scoring system[13]. For this study, the highest recorded skin score for each patient was noted. Occurrences of Raynaud's phenomenon and digital necrosis (ulceration or gangrene) were recorded. Limited disease was defined as definite skin thickening confined to the distal extremities, whereas in diffuse disease there was in addition involvement of skin proximal to the knees and elbows. Lung involvement was defined by the presence of pulmonary hypertension (right ventricular pressure > 35 mm Hg by Doppler echocardiogram), radiological evidence of interstitial lung disease, and pleuritis or abnormalities on pulmonary function testing [DLCO, forced vital capacity, or total lung capacity (TLC) < 70% of predicted]. Cardiac involvement was defined by the presence of cardiomegaly, congestive heart failure (with elevated jugular venous pressure), or pericarditis (friction rub or fluid detected by an echocardiogram). Hypertension was defined as systolic blood pressure > 140 mm Hg and/or diastolic blood pressure > 90 mm Hg. The presence of renal disease was defined by an increased serum creatinine concentration (> 110 mmol/l). The presence of trigeminal neuralgia and entrapment neuropathy (confirmed by nerve conduction studies) was also recorded. The presence of the following autoantibodies was also noted where available: antinuclear (ANA), anti-Scl-70, ACA, and anti-double stranded DNA (dsDNA).

Controls. DNA samples were obtained from healthy volunteers as well as cadaveric donors from the regional HLA laboratory of the University Health Network.

Laboratory methods. DNA was extracted from whole blood using a salting-out technique (Puregene DNA isolation kit; Gentra Systems, Minneapolis, MN, USA). Genomic DNA was amplified in a generic polymerase chain reaction amplification using biotinylated primers specific for each of the HLA-A, -B, -Cw, -DRB, and –DQB loci. The resultant amplicons were hybridized to an array of immobilized sequence-specific probes. The bound amplicons were detected using a colorimetric reaction, and the binding pat-

tern was interpreted using a computer matching program that assigns HLA type (Dynal Reli-SSO, Oslo, Norway).

Statistical analysis. Fisher's exact test for 2 × 2 tables was used to assess the significance of the relationship between each one of the HLA alleles and the binary outcomes of interest. The usual 5% significance level was used as a rough guide to relationships of possible interest. We performed a global likelihood ratio test of significance for each locus, including only alleles that were present in at least 10% in at least one of the populations tested (scleroderma patients or controls), followed by more specific tests. This conservative strategy was used to allow for multiple testing considerations.

Multivariate analyses were performed, using logistic regression, to examine the role of alleles jointly in disease incidence and expression. In the single situation where the significance of a continuous variable is tested, the nonparametric Wilcoxon rank-sum test was used to explore the association with binary variables. In this case, multivariate analysis was performed using a linear regression model.

Based on the previous studies we were particularly interested in alleles at the HLA-DR and DQB. Our hypothesis was that HLA-DRB1*11 would be associated with scleroderma and worse skin scores. However, we performed complete HLA typing on our subjects since the number of previous studies including Class 1 alleles is limited. The analysis of Class 1 alleles is exploratory.

RESULTS

There were 103 patients with scleroderma in the clinic at the time of the study. From those, only the 95 Caucasians were enlisted (Table 1), together with 416 Caucasian controls who were HLA typed. The patients included 75 women and 20 men, with a mean age at diagnosis of 43.9 years (range 23–72) and mean disease duration at the time of the study of 11.9 years (range 0–30). The organ manifestations identified in the scleroderma cohort are listed in Table 1.

HLA alleles and susceptibility to scleroderma. Initially, we investigated the role of each one of the alleles in disease incidence. Since our interest was in the HLA-DR and DQB regions, we concentrated on those first. There were 7 HLA-DR alleles that occurred in at least 10% of the subjects. The global test of significance provided a p value of 0.0023, showing there is a relationship with scleroderma in the HLA-DR region. A closer look at individual alleles revealed an association of HLA-DRB1*01 and HLA-DRB1*11 with scleroderma, but a "protective" effect of HLA-DRB1*07. A similar analysis in the HLA-DQB region revealed a marginal association with alleles at this region (p = 0.051), with no individual allele having a strongly observed association.

For the Class 1 region, the global test for HLA-A suggested some relationships (p = 0.08). The most significant are HLA-A*32 (nominal p value of 0.02) and HLA-A*30 (nominal p value of 0.05). A global test for HLA-B locus revealed no association (p = 0.45). However, HLA-B57 did come up significantly associated with scleroderma in the univariate analysis, with only 1% of the 94 patients and 9% of the controls having this allele (nominal p value of 0.01). This allele likely deserves further study. A global test for HLA-C generated a p value = 0.22. No patient was observed to have HLA-C*14, compared to 4% of the controls. This observation requires future assessment.

Table 2 gives the results of univariate analyses — the frequencies and proportions as well as the estimated odds ratio and corresponding p value are given. We present the complete results for all alleles so that this information may be included in future metaanalyses.

Based only on significant variables from univariate analyses, the best multivariate model includes HLA-A*30, HLA-A*32, HLA-B*57, HLA-Cw*14, HLA-DRB1*01, and HLA-DRB1*11 (Table 3). HLA-DRB1*07 was omitted from the model because of its linkage disequilibrium with HLA-B*57[14].

Table 1. Characteristics of study population.

	N (%)	Total Tested
Sex: F:M	75:20	
Age at diagnosis, mean (SD) yrs	43.8 (11.5) (range 23–72)	94
Disease duration, mean (SD) yrs	12.0 (6.4) (range 0–30)	93
No. of patients deceased	16 (16.8)	95
Diffuse disease	53 (56.4)	94
Limited disease	41 (43.6)	94
Raynaud's phenomenon	94 (98.9)	95
Digital necrosis	35 (37.2)	94
Pulmonary fibrosis	20 (22.7)	88
Pulmonary hypertension	20 (21.7)	92
Pleuritis	5 (5.3)	94
Heart failure	12 (12.6)	95
Pericarditis	15 (16.0)	94
Hypertension	56 (59.6)	94
Entrapment neuropathy	10 (10.6)	94
Trigeminal neuralgia	3 (3.2)	94
ANA	85 (96.6)	88
ACA	22 (25)	88
Anti-Scl-70	11 (22)	50
Anti-dsDNA	4 (8.9)	45

HLA and disease manifestations in scleroderma

Diffuse/limited disease. Through logistic regression, we explored the influence of the HLA alleles on various aspects of disease expression. Table 4A shows the significant relationships, based on univariate analyses, between HLA alleles and the extent of skin involvement in scleroderma. Both HLA-B*62 (p = 0.02) and HLA-DRB1*07 (p = 0.05) have a significant correlation with the presence of diffuse skin involvement. Note that 10 out of the 11 patients who have the HLA-B*62 allele have diffuse skin disease. Finally, both variables retained significance in a multivariate model (Table 5A). Further, we explored the possible connection between the extent of skin involvement (diffuse/limited disease) and other clinical and laboratory variables. Only the presence of ACA appears to be significantly associated with limited scleroderma (p = 0.01), with pulmonary hypertension (associated with limited scleroderma; p = 0.08) and serum creatinine (associated with diffuse scleroderma; p = 0.07) being just above the 0.05 threshold (Table 4A). As

Table 2. HLA allele distribution in scleroderma patients and controls.

HLA-A	Controls N=416 # (%)	Patients n=94 # (%)	p*	OR**
A*01	111 (26.7)	28 (29.8)	0.52	1.17
A*02	207 (49.8)	43 (45.7)	0.50	0.85
A*03	119 (28.6)	21 (22.3)	0.25	0.72
A*11	49 (11.8)	9 (9.6)	0.72	0.79
A*23	15 (3.6)	5 (5.3)	0.39	1.50
A*24	59 (14.2)	11 (11.7)	0.62	0.80
A*25	14 (3.4)	3 (3.2)	1.00	0.95
A*26	25 (6.0)	7 (7.5)	0.64	1.26
A*29	39 (9.4)	6 (6.4)	0.43	0.66
A*30	20 (4.8)	10 (10.6)	0.05	2.36
A*31	15 (3.6)	4 (4.3)	0.76	1.19
A*32	25 (6.0)	13 (13.8)	0.02	2.51
A*33	16 (3.9)	6 (6.4)	0.27	1.70
A*34	2 (0.5)	0 (0)	1.00	-
A*36	1 (0.2)	0 (0)	1.00	-
A*66	6 (1.4)	1 (1.1)	1.00	0.73
A*68	33 (7.9)	8 (8.5)	0.83	1.08
A*69	2 (0.5)	0 (0)	1.00	-

HLA-C	Controls N=408 #(%)	Patients n=90 #(%)	p*	OR**
C*01	29 (7.1)	6 (6.7)	1.00	0.93
C*02	24 (5.9)	8 (8.9)	0.34	1.56
C*03	93 (22.8)	23 (25.6)	0.58	1.16
C*04	67 (16.4)	19 (21.1)	0.28	1.36
C*05	60 (14.7)	13 (14.4)	1.00	0.98
C*06	85 (20.8)	12 (13.3)	0.11	0.58
C*07	220 (53.9)	50 (55.7)	0.82	1.07
C*08	28 (6.9)	7 (7.8)	0.82	1.14
C*12	46 (11.3)	14 (15.6)	0.28	1.45
C*14	18 (4.4)	0 (0)	0.05	-
C*15	26 (6.4)	7 (7.8)	0.64	1.24
C*16	44 (10.8)	4 (4.4)	0.08	0.38
C*17	9 (2.2)	0 (0)	0.37	-
C*18	3 (0.7)	0 (0)	1.00	-

HLA-B	Controls n=415 #(%)	Patients n=94 #(%)	p*	OR**
B*07	106 (25.5)	22 (23.4)	0.79	0.89
B*08	76 (18.3)	21 (22.3)	0.38	1.28
B*13	24 (5.8)	5 (5.3)	1.00	0.91
B*14	27 (6.5)	9 (9.6)	0.27	1.52
B*15	51 (12.3)	13 (13.8)	0.73	1.15
B*18	40 (9.6)	13 (13.8)	0.26	1.50
B*27	27 (6.5)	10 (10.6)	0.18	1.71
B*35	63 (15.2)	17 (18.1)	0.53	1.23
B*37	16 (3.9)	3 (3.2)	1.00	0.82
B*38	12 (2.9)	4 (4.3)	0.51	1.49
B*39	10 (2.4)	3 (3.2)	0.72	1.33
B*41	7 (1.7)	0 (0)	0.36	-
B*42	3 (0.7)	0 (0)	1.00	-
B*44	103 (24.8)	23 (24.5)	1.00	0.98
B*45	7 (1.7)	0 (0)	0.36	-
B*47	4 (1.0)	0 (0)	1.00	-
B*48	2 (0)	0 (0)	1.00	-
B*49	17 (4.1)	3 (3.2)	1.00	0.77
B*50	4 (1.0)	1 (1.1)	1.00	1.10
B*51	51 (12.3)	10 (10.6)	0.73	0.85
B*52	8 (1.9)	1 (1.1)	1.00	0.55
B*53	5 (1.2)	1 (1.1)	1.00	0.88
B*55	10 (2.4)	0 (0)	0.22	-
B*56	3 (0.7)	0 (0)	1.00	-
B*57	36 (8.7)	1 (1.1)	0.01	0.11
B*58	15 (3.6)	3 (3.2)	1.00	0.88
B*60	42 (10.1)	9 (9.6)	1.00	0.94
B*61	12 (2.9)	5 (5.3)	0.22	1.89
B*62	30 (7.2)	11 (11.7)	0.15	1.70
B*63	9 (2.2)	1 (1.1)	0.70	0.48
B*64	8 (1.9)	2 (2.1)	1.00	1.11
B*65	19 (4.6)	7 (7.5)	0.30	1.68
B*70	10 (2.4)	1 (1.1)	0.70	0.44
B*73	2 (0.5)	1 (1.1)	0.46	2.22
B*78	1 (0.2)	0 (0)	1.00	-

HLA-DR	Controls n=415 #(%)	Patients n=95 #(%)	p*	OR**
DR*01	73 (17.6)	28 (29.5)	0.01	1.96
DR*03	95 (22.9)	21 (22.1)	1.00	0.96
DR*04	135 (32.5)	27 (28.4)	0.47	0.82
DR*07	116 (27.9)	15 (15.8)	0.01	0.48
DR*08	24 (5.8)	8 (8.4)	0.35	1.50
DR*09	10 (2.4)	2 (2.1)	1.00	0.87
DR*10	7 (1.7)	0 (0)	0.36	-
DR*11	65 (15.7)	27 (28.4)	0.005	2.14
DR*12	20 (4.8)	3 (3.2)	0.59	0.64
DR*13	89 (21.5)	15 (15.8)	0.26	0.69
DR*14	23 (5.5)	3 (3.2)	0.44	0.55
DR*15	113 (27.2)	19 (20)	0.16	0.67

HLA-DQ	Controls n=415 #(%)	Patients n=94 #(%)	p*	OR**
DQ2	167 (40.2)	29 (30.5)	0.08	0.65
DQ4	27 (6.5)	5 (5.3)	0.82	0.80
DQ5	119 (28.7)	36 (37.9)	0.08	1.52
DQ6	169 (40.7)	32 (33.7)	0.24	0.74
DQ7	144 (34.7)	37 (39.0)	0.48	1.20
DQ8	85 (20.5)	20 (21.1)	0.89	1.04
DQ9	45 (10.8)	5 (5.3)	0.13	0.46
DQ0501	80 (19.3)	27 (28.7)	0.05	1.69
DQ0502	24 (5.8)	7 (7.5)	0.48	1.31
DQ0503	21 (5.1)	2 (2.1)	0.28	0.41
DQ0601	11 (2.7)	0 (0)	0.23	-
DQ0602	94 (22.7)	14 (14.9)	0.12	0.60
DQ0603	44 (10.6)	11 (11.7)	0.72	1.12
DQ0604	25 (6.0)	5 (5.3)	1.00	0.88
DQ0605	11 (2.7)	0 (0)	0.23	-
DQ0609	0 (0)	1 (1.1)	0.18	-
DQ0301	143 (34.5)	37 (39.4)	0.40	1.23
DQ0302	85 (20.5)	20 (21.3)	0.89	1.05
DQ0303	45 (10.8)	5 (5.3)	0.13	0.46
DQ0304	1 (0.2)	0 (0)	1.00	-
DQ0305	0 (0)	1 (1.1)	0.18	-
DQ0401	2 (0.5)	0 (0)	1.00	-
DQ0402	25 (6.0)	5 (5.3)	1.00	0.88

*** Fisher's Exact Test**
**** Exact Odds Ratio**

expected, only ACA is included in the best multivariate model (OR 3.91, 95% CI 1.40–10.97; p = 0.01), while its significance remained unchanged when HLA-B*62 and HLA-DRB1*07 were added in the model.

We further explored which HLA alleles and other variables were related to quantitative expression of the severity of skin disease based on the skin score. Since all the explanatory variables used are binary, we used the Wilcoxon rank-sum test to analyze skin score levels in each of the groups. From the HLA alleles, only the presence of HLA-DRB1*11 is associated with high skin score values (p = 0.02), while lower values are related to the presence of HLA-DQB1*0604 (p = 0.04). Both alleles retain significance in a linear regression model, with respective significance levels of p = 0.01 and p = 0.05. Furthermore, we observed that high skin score values were related to the absence of ACA (p = 0.003) and the presence of ANA (p = 0.05), although only 2 patients were ANA-negative. However, these 2 patients had 2 of the lowest skin score values — 4 and 6 (skin scores ranged from 4 to 54). Thus, although the 2 groups (ANA-positive and negative) are so unevenly split, the difference in the mean skin score values, 5 and 22.4, is so great that it is significant. If these variables are added to the regression model together with the HLA

Table 3. Multivariate analysis for disease incidence.

Effect	OR	95% CI	p
HLA-A*30	2.45	1.09–5.55	0.03
HLA-A*32	2.64	1.25–5.55	0.01
HLA-B*57	0.12	0.02–0.92	0.04
HLA-Cw*14	∞	—	0.01†
HLA-DRB1*01	1.98	1.16–3.37	0.01
HLA-DRB1*11	2.22	1.29–3.82	0.004

† Based on likelihood ratio test.

alleles, we observe that both ACA (p = 0.0006) and ANA (p = 0.01) retain their significance. Finally, we note that the HLA alleles and the other factors that are related to the binary classification of skin disease (diffuse/limited) and the skin scores are not the same. This is likely due to the different aspects of skin disease that are reflected in these variables, although they are correlated.

Pulmonary fibrosis. Of the HLA alleles, only HLA-Cw*0602 showed a significant relationship with pulmonary fibrosis (OR 4.92, p = 0.02), as shown in Table 4B. From the clinical and laboratory variables, the TLC value (p < 0.0001) and ACA (p = 0.02) are significant variables, with pericarditis (p = 0.08) being marginally important (Table 4B). In a multivariate model, only TLC is included (Table 5B), while the association between TLC and ACA is probably the reason the latter is not part of it. TLC retains its significance if HLA-Cw*0602 is also included in the model. No association was observed between pulmonary fibrosis and HLA-DRB3 alleles (Table 6A).

Pulmonary hypertension. Analysis including only the HLA alleles showed that only HLA-A*30 (p = 0.02), HLA-B*13 (p = 0.03), HLA-B*65 (p = 0.04), and HLA-DRB1*03 (p = 0.03) had significant association with pulmonary hypertension (Table 4C). The best multivariate model (Table 5C) includes only HLA-B*13 and HLA-B*65. Additionally, from the remaining clinical and laboratory variables, DLCO value (p = 0.006), heart failure (p = 0.01), and pericarditis

Table 4. Univariate analyses for disease expression.

A. Effect	Diffuse n/N (%)	Limited n/N (%)	OR	p
HLA-B*62	10/52 (19.2)	1/41 (2.4)	9.52	0.02
HLA-DRB1*07	12/52 (22.6)	3/41 (7.3)	3.71	0.05
PHT	8/53 (15.1)	12/39 (30.8)	2.5	0.08
Serum creatinine	8/52 (15.4)	1/39 (2.6)	0.14	0.07
ACA	7/49 (14.3)	15/38 (39.5)	3.91	0.01
B. Effect	**No PF n/N (%)**	**PF n/N (%)**	**OR**	**p**
HLA-Cw*0602	6/65 (9.2)	6/18 (33.3)	4.92	0.02
TLC	9/58 (15.5)	16/20 (80)	21.78	< 0.0001
Pericarditis	8/67 (11.9)	6/20 (30)	3.16	0.08
ACA	19/61 (31.2)	1/20 (5)	0.12	0.02
C. Effect	**No PHT n/N (%)**	**PHT n/N (%)**	**OR**	**p**
HLA-A*30	4/71 (5.6)	5/20 (25.0)	5.58	0.02
HLA-B*13	1/71 (1.4)	3/20 (15.0)	12.35	0.03
HLA-B*65	3/71 (4.2)	4/20 (20.0)	5.67	0.04
HLA-DRB1*03	12/71 (16.7)	8/20 (40.0)	3.33	0.03
Pleuritis	2/72 (2.8)	3/20 (15.0)	6.18	0.07
DLCO	33/65 (50.8)	16/18 (88.9)	7.76	0.006
Heart failure	5/72 (6.9)	6/20 (30.0)	574	0.01
Pericarditis	7/71 (9.9)	7/20 (35.0)	4.92	0.01
Anti-DNA	1/30 (3.3)	3/12 (25.0)	9.67	0.06
Skin involvement	27/72 (37.5)	12/20 (60.0)	2.5	0.08
D. Effect	**No Mortality n/N (%)**	**Mortality n/N (%)**	**OR**	**p**
HLA-B*27	6/78 (7.7)	4/16 (25.0)	4	0.06
PHT	12/76 (15.8)	8/16 (50.0)	5.33	0.006
DLCO	36/70 (51.4)	14/15 (93.3)	13.22	0.03

PHT: pulmonary hypertension, ACA: anticentromere antibody, TLC: total lung capacity, PF: pulmonary fibrosis.

Table 5. Multivariate analyses for disease expression.

A. Effect	Diffuse/Limited OR	95% CI	p
HLA alleles			
HLA-B*62	9.91	1.20–82.20	0.03
HLA-DRB1*07	3.97	1.01–15.52	0.05
Other variables			
ACA	3.91	1.40–10.97	0.01
B. Effect	**Pulmonary Fibrosis OR**	**95% CI**	**p**
Other variables			
TLC	0.05	0.02–0.17	< 0.0001
C. Effect	**Pulmonary Hypertension OR**	**95% CI**	**p**
HLA alleles			
HLA-B*13	10.90	1.00–118.47	0.05
HLA-B*65	5.08	0.97–26.76	0.05
Other variables			
DLCO	0.15	0.03–0.73	0.02
Heart failure	4.70	1.14–19.31	0.03
D. Effect	**Mortality OR**	**95% CI**	**p**
Other variables			
PHT	3.78	1.03–13.80	0.05
DLCO	0.12	0.01–0.98	0.05

(p = 0.01) are significant variables, with anti-dsDNA antibody (p = 0.06) and pleuritis (p = 0.07) being marginally important (Table 5C). In a separate multivariate model (Table 5C), DLCO and heart failure are included, while the strong correlation between heart failure and pericarditis is probably the reason the latter is not part of it. Both DLCO and heart failure retain significance in a multivariate model that also includes HLA variables. Other variables do not add anything to the model. No association was observed between pulmonary hypertension and HLA-DRB3 alleles (Table 6B).

An interesting question is whether hypertension is related to renal disease. Nevertheless, univariate analysis showed no indication of a connection between pulmonary hypertension, secondary pulmonary hypertension, or systemic hypertension and serum creatinine.

Mortality. Mortality was defined as a binary outcome indicating death during the study period or survival to the end of the study period. Sixteen patients (16.8%) died during the study period. In univariate analysis of HLA alleles, only HLA-B*27 (p = 0.06) was weakly associated with mortality (OR 4, 95% CI 0.71–19.60), while from all the other factors pulmonary hypertension (OR 5.33, p = 0.006) and DLCO (OR 13.22, p = 0.003) appear to have a strong association with mortality (Table 4D). In the multivariate analysis (Table 5D), the final model, which is adjusted by the age of patients at time of entry to the trial, includes pulmonary hypertension and DLCO. None of the remaining variables adds significantly to the multivariate model.

DISCUSSION

Our study shows that HLA alleles do play a role in susceptibility to scleroderma. As expected, HLA-DRB1*01 and HLA-DRB1*11 were associated with susceptibility to scleroderma. HLA-DRB1*07 was found to be protective. There was no association with HLA-DQB1 alleles. In addition, we found that Class 1 alleles HLA-A*30 and HLA-A*32 were associated with an increased risk of scleroderma. HLA-B*57 and HLA-Cw*14 appear to be protective. The significance level of this latter association is marginal, with the global test for both the B and C loci not being significant. However, these results suggest that these areas deserve further consideration. Our study thus supports previous HLA studies in scleroderma that show that HLA confers susceptibility to this disease[2-8]. Moreover, our study confirms our previous observation of the association of scleroderma with HLA-DR5[2]. It should be noted that the patient group in the current study is different from that of our previous study.

Table 6. DRB3 alleles.

A. Effect	No PF n/N (%)	PF n/N (%)	OR	p
HLA-DRB3*01	15/41 (36.6)	6/15 (40.0)	1.16	1.00
HLA-DRB3*02	26/41 (63.4)	11/15 (73.3)	1.59	0.54
HLA-DRB3*03	4/41 (9.8)	0/15 (0)	—	0.56
B. Effect	**No PHT n/N (%)**	**PHT n/N (%)**	**OR**	**p**
HLA-DRB3*01	15/46 (32.6)	7/14 (50.0)	2.07	0.34
HLA-DRB3*02	30/46 (65.2)	8/14 (57.1)	0.71	0.75
HLA-DRB3*03	6/46 (13.0)	0/14 (0)	—	0.32

PF: pulmonary fibrosis, PHT: pulmonary hypertension.

More importantly, our study documents the role of HLA alleles in disease expression in scleroderma. HLA-B*62 and HLA-DRB1*07 are protective for diffuse skin involvement in scleroderma. We previously reported that HLA-DR5 was associated with higher skin scores in scleroderma[2]. Our current study documents the association between diffuse skin involvement and HLA-DRB1*11, an allele belonging to the HLA-DR5 serologic specificity. We further identified several HLA alleles that were protective for severe skin involvement, including HLA-Cw*14 and HLA-DQB1*06. With regard to internal organ involvement in scleroderma, pulmonary manifestations were also found to be associated with specific HLA alleles. Pulmonary fibrosis was associated with HLA-B*62 and HLA-Cw*0602, whereas pulmonary hypertension was associated with HLA-B*13 and HLA-B*65. Of note, in this study, unlike our previous report, we did not identify HLA-DRB3 as a risk factor for pulmonary hypertension. This may be because our previous study used serologic typing, and in the current study we used molecular techniques to identify these alleles.

Although our study was not designed to predict mortality, we were able to investigate whether the presence of HLA alleles was associated with mortality in our patients. As previously reported[4,15], pulmonary hypertension was associated with increased mortality. However, in our current study, the presence of HLA-B27 was also found to have an adverse effect on mortality, although its frequency among patients with scleroderma is similar to that of the general population.

From this and previous studies, it is evident that genetic factors play an important role in susceptibility to scleroderma as well as disease expression with respect to disease type, organ involvement, and the development of specific autoantibodies. However, it is also evident that in scleroderma there is no single dominant genetic pattern, and various HLA alleles have been found to be associated with different aspects of the disease. The development of scleroderma and its many disease patterns is multifactorial and the result of interaction between multiple genetic factors, the immune system, and exposure to environmental agents.

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