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

Frank C Arnett,1 Pravitt Gourh,1 Sanjay Shete,2 Chul W Ahn,1 Robert E Honey,1 Sandeep K Agarwal,1 Filemon K Tan,1 Terry McNearney,3 Michael Fischbach,4 Marvin J Fritzler,5 Maureen D Mayes,1 John D Reveille1

ABSTRACT
Objective To determine human leucocyte antigen-class II (HLA-class II) (DRB1, DQB1, DQA1 and DPB1) alleles, haplotypes and shared epitopes associated with scleroderma (systemic sclerosis (SSc)) and its subphenotypes in a large multi-ethnic US cohort by a case–control association study.

Patients and methods 1300 SSc cases (961 white, 178 black and 161 Hispanic subjects) characterised for clinical skin forms (limited vs diffuse), SSc-specific autoantibodies (anticientromere (ACA), anti-topoisomerase I (ATA), anti-RNA polymerase III (ARA), anti-U3 ribonucleoprotein (fibrellarin) and others) and autoantibodies were performed using exact logistic regression modelling for dominant, additive and recessive effects from HLA.

Results The strongest positive class II associations with SSc in white and Hispanic subjects were the DRB1*1104, DQA1*0501, DQB1*0301 haplotype and DQB1*03 alleles encoding a non-leucine residue at position 57. ATA and ACA was best explained by DRB1*0804, DQA1*0501, DQB1*0301 alleles. DRB1*1301 showed the highest odds ratio for ATA (OR = 14). Moreover, it showed no linkage disequilibrium or gene interaction with DR/DQ. ACA was best explained by DQB1*0501 and DQB1*26 alleles and ARA by DRB1*0404, DRB1*11 and DQB1*03 alleles in white and Hispanic subjects but DRB1*08 in black subjects.

Conclusion These data indicate unique and multiple HLA-class II effects in SSc, especially on autoantibody markers of different subphenotypes.

INTRODUCTION
Scleroderma (systemic sclerosis, SSc) is a chronic complex autoimmune disease characterised by (a) organ fibrosis involving the skin, lungs, gastrointestinal tract and/or heart; (b) a proliferative vasculopathy primarily affecting small blood vessels and capillaries; (c) immune activation with production of disease-specific autoantibodies.1–4 The disease is further classified into limited and diffuse forms based on extent and distribution of cutaneous thickening.

The most common SSc-specific autoantibodies are directed against centromeric proteins anticientromere (ACA) (CENP B and A), anti-topoisomerase I (ATA) (also termed Scl-70) and anti-RNA polymerase III (ARA); however, a variety of less common specificities, typically antinucleolar, can be found, which include anti-U3 ribonucleoprotein (fibrellarin) AFA, anti-Th/To, anti-Pm-Scl, anti-RNA polymerase I and anti-U1-ribonucleoprotein (RNP).5 Importantly, each patient with SSc typically produces only one of these autoantibodies and each one currently serves as a biomarker for different patterns of skin and visceral involvement, as well as prognosis. In addition, certain SSc-specific antibodies occur in different frequencies among different ethnic groups.

Scleroderma is thought to be a complex genetic disease, influenced by multiple genes, with a substantial environmental or non-germline component based on twin studies.6 African-American and Hispanic patients with scleroderma tend to have more severe disease than their Caucasian counterparts and disease in African-American subjects begins at an earlier age.7 The Choctaw Indians of southeastern Oklahoma have a nearly 10-fold prevalence of the disease compared with other ethnicities.8 It appears likely that there are different combinations of genes, the interacting effects of which influence disease susceptibility and severity. Recently, we have found that the same polymorphism in the PTPN22 gene associated with rheumatoid arthritis, systemic lupus erythematosus and other autoimmune diseases is also associated with SSc, especially in those patients having ATA or ACA antibodies.9–11 Additional ‘autoimmunity’ genes now reported to be associated with SSc include allograft inflammatory factor, IL1A, IRF5, STAT4 and FAS.12–16

Major histocompatibility complex (MHC) or human leucocyte antigen-class II (HLA-class II) allelic associations with SSc have been reported in European and North American Caucasian subjects (DRB1*0301, DRB1*11, DRB1*07) and Japanese and Koreans (DRB1*1502) over the past two decades but have been relatively weak.5 17–22

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Much stronger correlations, however, have been demonstrated between certain HLA-class II alleles and each of the SSC-specific autoantibodies. Different HLA-DRB1, DQB1, DQA1 and/or DPB1 alleles, or combinations thereof, are associated with expression of ACA (DQβ1*0501 and other DQB1 alleles encoding non-leucine residues at position 26 in the peptide binding groove), ATA (DRB1*11, especially the DRB1*1104, DQβ1*0301 haplotype) in Caucasian subjects and DRB1*1502, DQβ1*0601 in Japanese and Korean subjects. AFA antibody (the DRB1*1302, DQβ1*0604 haplotype) and anti-PM-Scl (DRB1*0301). No consistent HLA correlations heretofore have been made with ARA. Only a few studies have examined HLA-DPB1 alleles in SSC; however, one such allele (DPB1*1301) has been associated with ATA. It has been unclear whether this allele is an independent disease correlate or the result of linkage disequilibrium (LD) with HLA-DRB1 and DQB1 haplotypes. Although genetic influences are thought to have an important role in susceptibility to SSC, genetic studies of scleroderma have included relatively small numbers of patients, especially black and Hispanic subjects. HLA associations with specific autoantibodies may be clinically relevant because each of the autoantibody subsets of scleroderma is associated with certain disease features and different prognostic implications. Thus, the overall aims of this study were to determine specific HLA-class II alleles, haplotypes and epitopes influencing susceptibility to, and/or expression of, SSC itself, or its limited or diffuse forms, or its various autoantibody subsets across ethnic lines in the largest cohort yet of American patients.

METHODS
Selection of SSC cases and normal controls
A case–control association study was performed (total SSC cases 1300; total controls 1000) along with subanalyses by ethnicity, gender, clinical subsets and specific autoantibody profiles. Patients were included from the NIH/NIAMS Scleroderma Family Registry and DNA Repository, the Genetics versus Environment in Scleroderma Outcomes Study (GENISOS) cohort followed up in the NIH/NIAMS Center of Research Translation in Scleroderma, and the UT-Houston Rheumatology Division. Patients with SSC included 961 Caucasian, 178 African-American and 161 Hispanic subjects; normal local control totals were 539 Caucasian, 263 African-American and 198 Hispanic subjects. Only Hispanic patients with SSC and controls of Mexican or Central American ancestry were included.

HLA-DPB1 alleles were determined in 705 Caucasian patients with SSC and 287 Caucasian controls in the Scleroderma Registry. Only 82 African-American and Hispanic cases were studied for HLA-DPB1 alleles but were added to the Caucasian subjects for an overall comparison. All patients fulfilled the preliminary American College of Rheumatology criteria for the diagnosis of SSC or had three of the five clinical features of the CREST syndrome (calcinosis, Raynaud’s phenomenon, esophageal dysfunction, sclerodactyly or telangiectasia). Patients having overlapping Sjögren’s syndrome, myositis or features of rheumatoid arthritis or systemic lupus were not excluded. Results of HLA allele frequencies in some of these patients have been reported previously and are included here. Because of the cross-sectional nature of two of these three cohorts, reliable clinical information on specific organ involvement, such as pulmonary fibrosis, pulmonary hypertension or renal crisis, was not available to assess HLA associations. HLA registry controls were primarily spouse or friend controls, and GENISOS/Division controls were healthy medical centre personnel or blood bank donors from the local Houston area. All controls were screened for a history of any autoimmune diseases and excluded if positive. All study subjects provided written informed consent and the study was approved by the UTH Committee for the Protection of Human Subjects.

Autoantibody identification
Antinuclear antibodies and ACA were determined in all patients with SSC by indirect immunofluorescence on HEp-2 cells (Antibodies, Davis, California, USA). Immunodiffusion against calf thymus extract was used to determine the presence of ATA (Scl-70), anti-Ro/SS-A, anti-La/SS-B, anti-Smith (Sm) and anti-RNP autoantibodies (Inova Diagnostics, San Diego, California, USA). ARA were determined using a commercially available enzyme-linked immunoassay (EIA) kit (MBL, Nagoya, Japan). AFA were determined only in those Division and GENISOS patients with SSC with a nucleolar antinuclear antibody pattern using immunoprecipitation.

HLA-class II genotyping
HLA-DQA1, -DQB1 and -DRB1 alleles were oligotyped and DRB1 alleles directly sequenced from extracted genomic DNA as previously described.

Statistical analyses
We used statistical analysis software SAS 9.1.3 and the SAS Genetic Package (SAS Institute, Cary, North Carolina, USA). Tests or Fisher’s exact tests were used to compare HLA-class II allele frequencies between the normal control and SSC groups. Mantel–Haenszel tests were performed for the analysis of HLA-class II allele frequencies to control for the confounding effects of ethnicity. In the online supplementary tables, we only reported the odds ratio (OR) and the corresponding 95% CI for p values <0.017, which accounts for the Bonferroni corrections by the number of comparisons for each HLA genotype (0.017 = 0.05/3). LD coefficients and the corresponding p values were computed to examine the allelic associations that occur between alleles at different loci using SAS Genetics software. These χ2 data are presented in the supplementary tables. We then performed exact logistic regression for each of the HLA variants using dominant (D), additive (A) and recessive (R) modeling. To account for the multiple comparisons, we used a false discovery rate approach. We used an experimental threshold of α = 0.05 and accounted for 1000 potential multiple comparisons that we performed. Using these values for α and number of tests performed, a point significance of 0.004 is considered statistically significant. Nonetheless, p values of <0.05 also are selectively shown for alleles of interest and those which have been associated with SSC or its subgroups in other reports. Use of the 0.004 as the threshold to declare significance leads to a rough false discovery rate of 0.025. For LD analyses we used the procedure haplotype in the SAS Genetic Package. In addition, we examined frequencies of the presence of non-leucine residues at position 26 in the DQβ chain based on our previous studies implicating this ‘epitope’ (DQB1*26 epitope) in susceptibility to the ACA in SSC.

RESULTS
HLA-class II alleles in SSC
HLA-class II (DRB1, DQA1 and DQB1) genotyping was completed in 1300 SSC cases and 1000 normal controls and HLA-DPB1 in 705 white SSC cases and 287 white controls (tables 1–3 and supplementary tables 1 and 2).
Table 1  HLA-class II allelic frequencies significantly associated with Caucasian patients with systemic sclerosis (SSc) compared with Caucasian normal controls using logistic regression

|                | SSc (%) | Controls (%) | Model (D/R/A) | p Value | OR    | 95% CI   |
|----------------|---------|--------------|---------------|---------|-------|----------|
| *0404          | 4.3     | 1.9          | D             | 0.003   | 2.33  | 1.3 to 4.1 |
| *0701          | 8.0     | 14.2         | A             | <0.0001 | 0.39  | 0.1 to 1.1 |
| *1            | 13.5    | 8.2          | D             | 0.0002  | 1.81  | 1.3 to 2.4 |
| *1104          | 7.1     | 3.0          | D             | <0.0001 | 2.48  | 1.6 to 3.9 |
| *1501          | 11.0    | 14.3         | R             | 0.0003  | 0.22  | 0.1 to 0.5 |
| DQA1†          |         |              |               |         |       |          |
| *0102          | 18.1    | 21.3         | R             | 0.0002  | 0.36  | 0.2 to 0.6 |
| *0201          | 7.6     | 14.4         | A             | <0.0001 | 0.20  | 0.1 to 0.6 |
| *0501          | 31.6    | 24.0         | A             | <0.0001 | 2.29  | 1.4 to 3.7 |
| *3101          | 5.4     | 10.3         | D             | <0.0001 | 0.46  | 0.3 to 0.7 |
| *0301          | 23.0    | 16.8         | D             | 0.002   | 1.50  | 1.2 to 1.9 |
| *0602          | 11.5    | 14.6         | R             | 0.003   | 0.29  | 0.1 to 0.7 |
| *0801          | 64.4    | 53.8         | –              | 0.0003  | 1.59  | 1.2 to 2.0 |
| *0804          | 3.7     | 1.4          | D             | 0.004   | 3.18  | 1.4 to 7.3 |

Table 2  HLA-class II allelic frequencies significantly associated with African-American patients with systemic sclerosis (SSc) compared with normal African-American controls using logistic regression

|                | SSc (%) | Controls (%) | Model (D/R/A) | p Value | OR    | 95% CI   |
|----------------|---------|--------------|---------------|---------|-------|----------|
| *0801          | 10.2    | 3.6          | D             | 0.0003  | 3.31  | 1.7 to 6.5 |
| *0501          | 34.2    | 24.1         | A             | 0.003   | 3.55  | 1.6 to 8.1 |
| *0301          | 33.0    | 21.9         | D             | <0.0001 | 2.44  | 1.6 to 3.7 |

Table 3  HLA-class II allelic frequencies significantly associated with Hispanic patients with systemic sclerosis (SSc) compared with normal Hispanic controls using logistic regression

|                | SSc (%) | Controls (%) | Model (D/R/A) | p Value | OR    | 95% CI   |
|----------------|---------|--------------|---------------|---------|-------|----------|
| *0104          | 8.8     | 2.0          | D             | 0.0003  | 4.99  | 2.0 to 12.4 |
| *0701          | 3.8     | 7.6          | D             | 0.01    | 0.39  | 0.2 to 0.8 |
| *0501          | 29.9    | 21.7         | R             | 0.002   | 4.09  | 1.6 to 10.4 |
| *0201          | 3.8     | 7.8          | D             | 0.01    | 0.39  | 0.2 to 0.8 |
| *0301          | 24.2    | 14.9         | D             | 0.003   | 2.13  | 1.3 to 3.5 |
| *0801          | 72.7    | 61.7         | –             | 0.019   | 1.81  | 1.1 to 3.0 |

1SSc cases (n = 961) versus normal controls (n = 539); 2SSc cases (n = 705) versus normal controls (n = 287) typed for HLA-DPB1.

For all white patients with SSc versus ethnically matched controls, a strong association was found with HLA-DRB1*11, but specifically with DRB1*1104 and not with DRB1*1101, DQA1*0501 and DQB1*0301 also were significantly increased. These three alleles constitute a haplotype with most DRB1 alleles including both DRB1*1101 and DRB1*1104. Given the highest odds ratio (OR = 2.48) being conferred by DRB1*1104 rather than DQA1*0501 (OR = 2.29) or DQB1*0301 (OR = 1.50), the primary associated allele appeared likely to be DRB1*1104 in a dominant model. Also, the shared DQB1*26 epitope (absence of leucine in position 26) was increased (OR = 1.59). HLA-DRB1*1104 also was significantly increased (OR = 2.33); however, the strongest HLA-class II association with SSc was with HLA-DRB1*1104 (OR = 3.18). Testing for LD between HLA-DRB1*1104 and DRB1*1101 was not associated with SSc (table 2).

The HLA-DRB1*0701, DQA1*0201, DQB1*0202 haplotype was negatively associated with SSc in an additive model (HLA-DRB1*0701, DQA1*0201) and in dominant model (HLA-DRB1*0701, DQB1*0202) suggesting that it conferred a ‘protective’ effect (table 1). Similarly, the HLA-DRB1*1501, DQA1*0102, DQB1*0602 haplotypes were significantly decreased but in a recessive model.

In African-Americans, the HLA-DRB1*0804/DQA1*0501/DQB1*0301 haplotype was significantly increased, while DRB1*1104 was not associated with SSc (table 2).

Significant associations with SSc in Hispanic subjects were found for DRB1*1104 (OR = 4.99), DQA1*0501 (OR = 4.09) and DQB1*0301 (OR = 2.13), along with the DQB1*26 epitope (OR = 1.81) (table 3). Again, DRB1*0701 and DQA1*0201 were decreased in frequency.

HOLA-DRB1*0501 was significantly more frequent in male (101/165 or 61%) than in female (583/1135 or 51%) patients with SSc (p = 0.02, OR = 1.49, 95% CI 1.06 to 2.12), thus confirming one earlier report.

**HLA alleles in limited versus diffuse SSc**

HLA associations with both limited and diffuse forms of SSc largely mirrored the findings in the disease group as a whole, except in black patients with limited disease whose numbers were small (n = 54) (supplementary tables 3a and 4a by logistic regression and supplementary tables 3, 4 and 5 by χ²).

**HLA allele frequencies in autoantibody subsets of SSc**

Frequencies and ethnic differences in SSc autoantibodies are shown in online supplementary table 5. ACA were found to be associated with several class II haplotypes in white subjects, including HLA-DRB1*0101, DQA1*0101 and DQB1*0301, DRB1*04, especially DRB1*0401 and weakly DRB1*0402 which carried DQB1*0301 alleles and weakly with DRB1*08 (8501) and DQA1*0501 (table 4, supplementary table 6). The strongest associated alleles were DRB1*0401 (OR = 8.95) in a recessive model, DRB1*0404 (OR = 4.18) in a dominant model, DQB1*0301 in a recessive model (OR = 3.41), DQB1*0501 (dominant OR = 2.56) and the DQB1 26 epitope (OR = 2.95). No HLA-DPB1 alleles were associated with ACA.

No HLA alleles were associated with ACA in black subjects and only HLA-DRB1*0407 in Hispanic subjects.

ATA in white subjects were strongly associated with the HLA-DRB1*1104, DQA1*0501, DQB1*0301 haplotype (OR = 6.95) and even more so with HLA-DRB1*1301 (OR = 14.02), both showing dominant models (table 5) (supplementary table 7).

The DRB1*1104 association also was present in the black subjects and Hispanic subjects, as was the DQB1 26 epitope (table 5, supplementary table 7). In addition, HLA-DRB1*08 alleles, DRB1*0804 in black subjects (OR = 3.42) and DRB1*0802 in Hispanic subjects (OR = 1.91) also were increased in these groups showing both additive and dominant effects. There appeared to be no negative or ‘protective’ effect from the DRB1*0701 haplotype. HLA-DRB1*1301 was highly significantly associated with 1SSc cases (n = 161) versus normal controls (n = 198).
**Table 4** HLA-class II allelic frequencies significantly associated with anticientromere antibody positive Caucasian and Hispanic patients with systemic sclerosis (SSc) compared with ethnically matched normal controls using logistic regression

| Allele | Caucasians | Hispanics | OR | 95% CI |
|--------|------------|-----------|----|--------|
|        | SSc        | Controls  | Model | p Value |
| DRB1   |            | D/R/A     |      |         |
| *01    | 21.0       | 12.5      | D    | <0.0001 | 2.19 | 1.5 to 3.2 |
| *04    | 24.9       | 11.7      | D    | <0.0001 | 2.72 | 1.8 to 4.1 |
| *0401  | 11.0       | 8.3       | R    | <0.03    | 0.89 | 1.4 to 5.72 |
| *0404  | 5.5        | 1.9       | D    | <0.0001 | 4.18 | 2.0 to 8.6 |
| *0701  | 2.9        | 14.2      | A    | <0.0001 | 0.10 | 0.01 to 0.8 |
| *08    | 6.1        | 3.4       | D    | <0.04    | 1.90 | 1.1 to 3.4 |
| *0801  | 4.8        | 2.0       | D    | <0.03    | 3.17 | 1.5 to 6.6 |
| *11    | 11.9       | 8.2       | D    | 0.02     | 1.67 | 1.1 to 2.6 |
| *1104  | 5.0        | 3.0       | NS   |         |      |              |
| *1501  | 8.9        | 14.3      | R    | 0.006    | 0.67 | 0.4 to 1.02 |
| DQA1   |            | D/R/A     |      |         |
| *0101  | 23.1       | 13.4      | D    | <0.0001 | 2.57 | 1.8 to 3.7 |
| *0102  | 14.4       | 21.3      | R    | <0.001   | 0.25 | 0.1 to 0.8 |
| *0201  | 2.0        | 14.4      | A    | <0.0001 | 0.07 | 0.01 to 0.8 |
| DQB1   |            | D/R/A     |      |         |
| *0202  | 5.3        | 2.8       | D    | <0.0001 | 0.14 | 0.07 to 0.3 |
| *0301  | 23.5       | 16.8      | R    | 0.004    | 3.41 | 1.4 to 8.0 |
| *0302  | 14.6       | 9.4       | D    | 0.007    | 2.04 | 1.2 to 3.4 |
| *0501  | 22.8       | 12.6      | D    | <0.0001 | 2.56 | 2.0 to 3.7 |
| *DQB1 26| 75.1      | 53.8      | A    | <0.0001 | 2.93 | 2.0 to 4.2 |

**Table 5** HLA-class II allelic frequencies significantly associated with antitopoisomerase I antibody positive Caucasian, African-American and Hispanic patients with systemic sclerosis (SSc) compared with ethnically matched normal controls using logistic regression

| Allele | Caucasians | African-Americans | Hispanics | OR | 95% CI |
|--------|------------|-------------------|-----------|----|--------|
|        | SSc        | DRB1              | DRB1      | Model | p Value |
|        | Controls   |                  |           | (D/R/A) |       |
| *0401  | 3.6        | 8.3               | D         | <0.0001 | 0.03 | 0.35 to 0.7 |
| *1101  | 6.2        | 3.8               | D         | 0.03    | 2.13 | 1.1 to 4.1 |
| *1104  | 16.6       | 3.0               | D         | <0.0001 | 6.93 | 3.9 to 12.2 |
| DQA1   |            |                  |           |       |       |
| *0501  | 34.7       | 24.0              | A         | <0.0001 | 2.01 | 0.9 to 4.7 |
| DQB1   |            |                  |           |       |       |
| *0202  | 5.2        | 10.3              | D         | 0.006   | 0.44 | 0.2 to 0.8 |
| *03    | 45.1       | 30.1              | A         | 0.0004  | 3.30 | 1.8 to 6.2 |
|        |            |                  |           |       |       |

**DISCUSSION**

new to this study was the finding that the African HLA-DRB1*0804 allele, along with DQA1*0501 and DQB1*0301, showed the most significant associations in black subjects and recurred in those with diffuse disease, ATA, ARA and AFA.

Perhaps equally important was the susceptibility effect of other DQB1 alleles (besides *0301 and *0501) encoding polar amino acids at position 26 of the DQ β chain (DQB1*26 epi). Previously, we reported this shared epitope in the antigen binding cleft to be most important in the ACA autoimmune response25; however, this current larger study suggests that it occurs in the majority of patients with SSc regardless of ethnicity or autoantibody.

A second unreported and potentially ‘protective’ haplotype in white subjects, HLA-DRB1*1501, DQA1*0501 and DQB1*0602, was significantly decreased in a recessive model in Caucasian alleles (OR = 3.92), primarily DQB1*0301 allele, along with DQA1*0501 (OR = 2.88) for two copies, in an additive model and DQB1*0301 (OR = 3.60) showing a dominant effect. Among Hispanic subjects, DRB1*11, DQA1*0501 and the DQB1 26 epitope showed positive weak positive correlations, but the strongest association was with DQB1*0301 (OR = 4.07).

**Other autoantibodies and HLA**

The HLA-DRB1*1302, DQB1*0604 haplotype was most strongly associated with AFA in white subjects (p=0.0003, OR=6.67), along with the DQB1 26 epitope (p=0.0009, OR=1.60), while in black patients DRB1*08 alleles (p=0.0003, OR=5.76), especially DQB1*0804 (p=0.002, OR=5.70), showed the strongest associations (supplementary tables 9 and 9a).

ATA when all ethnic groups were combined (p<0.0001, OR = 9.96) (supplementary table 7). Using logistic regression, there was no evidence of gene–gene interaction between DBB1*1301 and DRB1*1104 (p = 0.9999) or between DBB1*1301 and DQB1*0301 (p = 0.9999) in the ATA positive group or in the total SSc group.

ARA were found to be most strongly associated in white subjects with HLA-DRB1*0404 (OR = 5.13) and DRB1*11 alleles (OR = 1.55 for one copy, 6.78 for two copies), especially DRB1*1104 in additive and dominant models, respectively (table 6) (supplementary table 8). In addition, a positive recessive effect was seen from DQB1*03 alleles (OR = 2.58), especially DQB1*0301 (OR = 1.50 for one copy, 3.77 for two copies). In black patients, the strongest associations were with DRB1*08 alleles (OR = 3.92), primarily DRB1*0804 (OR = 2.98), along with DQA1*0501 (OR = 3.10 for one copy, 6.05 for two copies) in an additive model and DQB1*0301 (OR = 3.60) showing a dominant effect. Among Hispanic subjects, DRB1*11, DQA1*0501 and the DQB1 26 epitope showed possible weak positive correlations, but the strongest association was with DQB1*0301 (OR = 4.07).
Table 6  HLA-class II allelic frequencies significantly associated with anti-RNA polymerase III positive antibody positive Caucasian, African-American and Hispanic patients with systemic sclerosis (SSc) compared with ethnically matched normal controls using logistic regression.

| Allele  | Caucasian (%) | Controls (%) | Model (D/R/A) | p Value | OR | 95% CI |
|---------|---------------|--------------|---------------|---------|----|-------|
| DRB1    |               |              |               |         |    |       |
| *0404   | 7.5           | 1.9          | D             | <0.0001 | 5.13 | 2.4 to 11.0 |
| *11     | 12.4          | 8.2          | A             | 0.02    | 6.78 | 1.2 to 37.7 |
| *1104   | 6.1           | 3.0          | D             | 0.02    | 2.34 | 1.2 to 4.6  |
| DQB1    |               |              |               |         |    |       |
| *03     | 40.6          | 30.1         | R             | 0.004   | 2.38 | 1.3 to 4.2  |
| *0301   | 22.9          | 16.8         | A             | 0.01    | 3.77 | 1.3 to 11.0 |
|        |               |              |               |         |    |       |
| African-American‡ | |              |               |         |    |       |
| DRB1    |               |              |               |         |    |       |
| *08     | 13.6          | 5.5          | D             | 0.003   | 3.92 | 1.7 to 4.2  |
| *0804   | 9.1           | 3.6          | D             | 0.05    | 2.98 | 1.04 to 8.5 |
| *1104   | 3.0           | 1.9          | NS            |         |    |       |
| DQA1    |               |              |               |         |    |       |
| *0501   | 42.2          | 24.1         | A             | 0.003   | 6.03 | 1.5 to 23.8 |
| DQB1    |               |              |               |         |    |       |
| *0301   | 37.5          | 21.9         | D             | 0.002   | 3.60 | 1.6 to 8.0  |
|        |               |              |               |         |    |       |
| Hispanic§ |            |              |               |         |    |       |
| DRB1    |               |              |               |         |    |       |
| *11     | 12.5          | 5.6          | D             | 0.10    | 2.17 | 1.2 to 11.6 |
| *1104   | 3.8           | 2.0          | D             | 0.005   | 10.50| 2.3 to 46.8 |
| DQA1    |               |              |               |         |    |       |
| *0501   | 28.8          | 21.7         | R             | 0.047   | 3.65 | 1.5 to 12.6 |
| DQB1    |               |              |               |         |    |       |
| *03     | 50.0          | 34.1         | D             | 0.04    | 2.33 | 1.1 to 5.1  |
| *0301   | 32.5          | 14.9         | D             | 0.0002  | 4.07 | 2.0 to 8.5  |
|        |               |              |               |         |    |       |

Extended report

subjects with SSc overall and in both limited and diffuse subgroups, as well as in those patients with ACA.

An HLA-DBP*1301 allele, DBP1*1301 in a dominant model, showed the highest odds ratios in SSc (OR = 3.18) and in both the limited (OR = 4.20) and diffuse (OR = 4.38) forms in Caucasian subjects; however, these associations were completely explained by its strong correlation with ATA (OR = 14.02). Testing for LD of this DBP1 allele with the DB1*1104 and DQB1*0301 alleles showed that these were independently associated class II effects. Interestingly, the ATA response is strongly associated with pulmonary fibrosis in SSc and certain HLA-DP alleles have been clearly shown to promote susceptibility to occupationally acquired berylliosis, another fibrosing lung disease. 30

Finally, ARA characteristically are markers of rapidly progressive skin thickening and renal crises and this study is the first to demonstrate that they occur in similar prevalences in each of these ethnic groups and are associated with certain class II MHC alleles.

In previous studies of HLA and ARA, there have been contradictory findings, perhaps because small samples have been studied. 28 38 39

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