Ethnic Differences in Autoantibody Diversity and Hierarchy: More Clues from a US Cohort of Patients with Systemic Sclerosis

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ABSTRACT. ***Objective.*** To determine the autoantibody repertoire and clinical associations in a multiethnic cohort of American patients with systemic sclerosis (SSc).

Methods. There were 1000 patients with SSc (196 Hispanic, 228 African American, 555 white, and 21 other) who were screened for antinuclear antibodies (ANA), including anticentromere antibodies (ACA) by indirect immunofluorescence assay, antitopoisomerase-1 (topo-1/Scl-70) by immunodiffusion, and anti-RNA polymerase III (RNAP III) by ELISA. Sera from 160 patients with mainly nucleolar and/or speckled ANA pattern, but negative for ACA, Scl-70, and RNAP III, were further characterized by immunoprecipitation for SSc-specific antibodies.

Results. The prevalence of antibodies against RNAP III, Th/To, and PM/Scl did not differ significantly among the ethnic groups. The frequency of anti-Scl-70 was lowest in whites (18.0%) compared with 24.0% and 26.8% in Hispanics and African Americans ($p = 0.01$), respectively. Compared with African American patients, Hispanic and white subjects had a higher frequency of ACA ($p < 0.0001$) and lower frequency of U3-RNP ($p < 0.0001$). U3-RNP antibodies were uniquely higher in African American patients, independent of clinical subset, while Th/To autoantibodies were associated with limited cutaneous SSc in white subjects. Overall, Hispanic and African American patients had an earlier age of onset and a predominance of diffuse cutaneous SSc compared with their white counterparts.

Conclusion. SSc-specific antibodies may predict disease subset; however, the hierarchy of their prevalence differs across ethnic groups. This study provides the most extensive analysis to date on the relevance of autoantibodies in the diagnosis and clinical manifestations of SSc in Hispanic American patients. (J Rheumatol First Release August 1 2016; doi:10.3899/jrheum.160106)

Key Indexing Terms:
SYSTEMIC SCLEROSIS AUTOANTIBODIES ANTINUCLEAR ANTIBODIES

Systemic sclerosis (SSc) is characterized by fibrosis of skin and diverse internal organs, vascular damage, and altered immune functions. It is a chronic and potentially life-threatening disease. Based on the extent of skin involvement, the disease can be classified into diffuse cutaneous SSc (dcSSc) and limited cutaneous SSc (lcSSc)[1]. lcSSc is characterized by fibrosis of the skin confined to the distal extremities, a lower incidence of renal involvement, and restrictive pulmonary disease with a better prognosis[1]. In contrast, dcSSc is characterized by skin fibrosis involving the proximal extremities and/or the trunk, in addition to acral skin thickening, and has a poorer prognosis[1,2,3].

The immunopathology associated with SSc involves multiple compartments of the immune system, including T

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cells, B cells, and cytokines/chemokines, with fibroblasts and endothelial cells as both effectors and targets[4]. Abnormal activation of the immune system is reflected by the presence of circulating, essentially mutually exclusive SSc-specific antinuclear antibodies (ANA) with striking associations between autoantibody specificities and clinical phenotypes[4,5,6,7,8]. SSc autoantibodies target both nuclear and nucleolar proteins, and certain specificities have diagnostic and predictive relevance[7,8,9,10]. Of the ANA, centromere (ACA), topoisomerase-I (topo-I/Scl-70), RNA polymerase III (RNAP III), U3-RNP (fibrillarin), PM/Scl, and Th/To constitute ~80% of SSc-specific autoantibodies[5,6,7]. Autoantibodies less specific for SSc, targeting the nucleolar organizing region 90/human upstream-binding factor (NOR90/hUBF), U1RNP, Ro52, and Su/Argonaute2 (Ago2) antigens, have been described as well[5,7,9]. Currently, only ACA, anti-Scl-70, and anti-RNAP III are included in the 2013 classification guidelines for SSc[10].

The presence of specific antibodies has been associated with lcSSc and dcSSc, as well as certain SSc clinical manifestations. ACA are mainly associated with lcSSc with more favorable prognosis[11,12,13,14]. Antinucleolar antibodies (ANoA) such as anti-Th/To and anti-PM/Scl are also associated with lcSSc[15,16], whereas anti-Scl-70 and RNAP III and ANoA that react with U3-RNP are associated with dcSSc and poorer prognosis[7,11,12,16,17,18]. Patients with anti-RNAP III usually have dcSSc, with an increased risk of renal crisis and a shorter survival time[16,17,18]. At disease onset, dcSSc is more prevalent with poorer outcomes in African Americans than white Americans[19,20,21]. These associations correlate with higher incidences of anti-Scl-70, anti-U3-RNP, and anti-RNAP III compared with ACA in individuals of African American descent relative to their white and Hispanic counterparts[16,20,21,22]. Thus, specific ANA have diagnostic, prognostic, and predictive relevance in the evaluation and management of SSc and tend to cluster within particular ethnic and/or racial groups[16,23,24].

Our study examined the presence of SSc-associated autoantibodies in a large US multiethnic cohort of patients with SSc and associations with clinical subsets and/or certain manifestations in Hispanic subjects compared with their white and African American counterparts.

MATERIALS AND METHODS

Patient population. Patients enrolled in the Scleroderma Family Registry and DNA Repository were characterized clinically based on the extent of skin involvement, age at disease onset, disease duration at blood draw, sex, and ethnicity. All patients with SSc either fulfilled the 1980 American College of Rheumatology classification criteria for SSc[25] or had at least 3 of the 5 features of CREST syndrome (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, telangiectasias)[26]. Patients with SSc were further characterized as having lcSSc or dcSSc based on their skin involvement[1]. Disease duration was measured from the onset of the first non-Raynaud phenomenon symptom attributed to SSc. Clinical information regarding renal crisis was available for 183 of the patients. Ethnic categories were determined by self-report and defined as follows: African American (non-Hispanic African American), white (non-Hispanic white), Hispanic, and other (Hispanic African American, American Indian/Alaskan Native, more than 1 race, and other). The institutional review boards at the University of Utah School of Medicine, Salt Lake City, and the University of Texas Health Science Center at Houston approved our study. Written informed consent was obtained from all subjects at the University of Texas Health Science Center at Houston according to the Declaration of Helsinki.

Detection of autoantibodies. Sera from a multiracial cohort of 1000 patients with SSc (Scleroderma Family Registry and DNA Repository) were screened for immunoglobulin ANA by indirect fluorescent assay (IFA; Inova Diagnostics Inc.) with specific patterns identified if present [centromere (ACA), nuclear speckled, and nucleolar]. All ANA-positive and a subset of ANA-negative samples were screened for anti-Scl-70 by immunodiffusion (Inova Diagnostics Inc.) and anti-RNAP III by ELISA (MBL Co. Ltd.; Figure 1). From 346 ANA-positive patients negative for ACA, anti-Scl-70, and anti-RNAP III, 160 sera [ANoA alone (n = 142) or ANoA and nuclear speckled patterns (n = 18)] were tested for autoantibody characterization by radioimmunoprecipitation (IP) assay. Autoantibodies detected by IP included anti-Scl-70, anti-RNAP I/III, anti-U3-RNP, anti-Th/To, anti-PM/Scl, anti-NOR90, and anti-Su/Argonaute2. Unlike the anti-RNAP III ELISA, the IP assay allows classification of RNAP antibody types (I, II, III) based on the presence of characteristic bands allowing for differentiation. Antibodies to RNAP I and III coexist in most cases and are specific for SSc; specimens with these autoantibodies are reported as RNAP III.

Statistical analysis. Significance of associations between sets of categorical data was determined using a chi-square test or a Fisher's exact test when expected values were low. The ANOVA test was used to determine the difference between continuous variables (mean ages or mean disease duration in yrs) in the different subset classifications for the markers with pairwise comparisons performed posthoc on groups that showed significance with a Tukey test. To determine the predictive power of specific markers, a logistic regression analysis was performed in the model using the presence or absence of specific autoantibodies. All statistical analyses were performed using SAS 9.4 (SAS Institute) or Prism 5.0 (GraphPad Software). Differences were considered statistically significant for p values < 0.05.

RESULTS

Demographics and characteristics of the study cohort. Table 1 summarizes the demographic and clinical information for the study cohort. The mean age at disease onset differed significantly among ethnicities: Hispanic and African American patients had a similar age of onset (40.2 ± 14.0 yrs vs 40.6 ± 13.0 yrs) that was significantly earlier than that observed in the white subjects (44.4 ± 14.1 yrs, $p < 0.01$). There was no difference in disease duration at the time of blood draw between the Hispanic and African American patient groups (6.7 ± 6.9 yrs vs 6.6 ± 6.2 yrs). Disease duration at the time of blood draw was significantly longer in whites (9.2 ± 9.0 yrs) compared with Hispanic ($p < 0.01$) and African American patients ($p < 0.05$; Table 1). Frequencies of female and male patients were comparable in all ethnic groups with more women than men, irrespective of ethnicity.

Hispanic (57.2%) or African American patients (66.2%) had a significantly higher prevalence of dcSSc ($p < 0.0001$) compared with whites (38.0%). Conversely, the prevalence of lcSSc was significantly higher in the white group (61.4%) compared with the Hispanic (41.3%) or African American cohort (32.0%, $p < 0.0001$; Table 1). Of the documented patients with renal crisis (n = 180), African Americans had the highest frequency (13/53, 24.5%), followed by Hispanics (9/43, 20.9%) and whites (13/84, 15.5%).

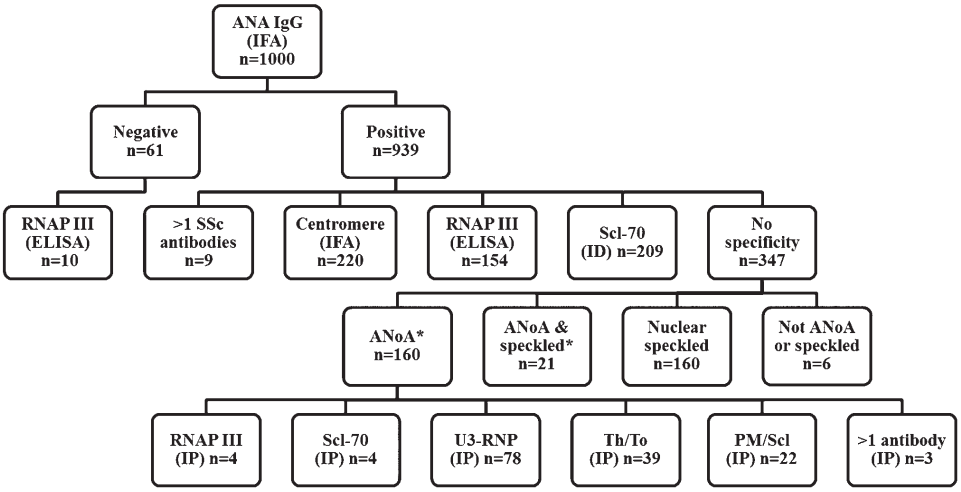

Figure 1. Algorithm for identifying autoantibodies in patients with SSc. Sera from 1000 patients with SSc were screened for ANA by IFA with specific patterns identified if present (centromere, nuclear speckled, and nucleolar). All ANA+ and a subset of ANA– samples were screened for anti-Scl-70 by ID and RNAP by ELISA. From 346 ANA+ patients negative for ACA, anti-Scl-70, and anti-RNAP III, 160 sera were selected for autoantibody characterization by IP assay. * The 142 patients with an ANoA IFA pattern and 18 patients with an ANoA and nuclear speckled IFA pattern were tested by IP. ANA: antinuclear antibodies; IgG: immunoglobulin G; RNAP III: RNA polymerase III antibodies; SSc: systemic sclerosis; IFA: indirect fluorescent assay; ID: immunodiffusion; ANoA: antinucleolar antibodies; IP: radioimmunoprecipitation.

Table 1. Demographic characteristics of study cohort (n = 1000). Values are % (n) unless otherwise specified.

Characteristics	African American, n = 228	White, n = 555	Hispanic, n = 196	Other, n = 21	p
Age at disease onset, yrs, mean ± SD	40.6 ± 13.0[1]	44.4 ± 14.1[1,4]	40.2 ± 14.0[4]	40.9 ± 13.6	0.0002
Disease duration, yrs, mean ± SD	6.6 ± 6.2[2]	9.2 ± 9.0[2,4]	6.7 ± 6.9[4]	6.6 ± 5.6	< 0.0001
Female, n = 828	84.7 (193)	83.1 (461)	85.7 (168)	76.2 (16)	NS
Male, n = 162	15.3 (35)	16.9 (94)	14.3 (28)	23.8 (5)	NS
dcSSc, n = 483	66.2 (151)[3]	38.0 (211)[3,5]	57.2 (112)[5]	42.9 (9)	< 0.0001
lcSSc, n = 507	32.0 (73)[3]	61.4 (341)[3,5]	41.3 (81)[5]	57.1 (12)	< 0.0001
Unspecified, n = 10	1.8 (4)	0.5 (3)	1.5 (3)	0.0 (0)	NS

African American versus white: [1] $p < 0.01$ (age at disease onset), [2] $p < 0.05$ (disease duration), and [3] $p < 0.0001$ (diffuse, limited). African American versus Hispanic: NS for all categories. White versus Hispanic: [4] $p < 0.01$ (age at disease onset, disease duration) and [5] $p < 0.0001$ (diffuse, limited). SSc: systemic sclerosis; dcSSc: diffuse cutaneous SSc; lcSSc: limited cutaneous SSc; NS: not significant.

Autoantibody profiling in patients with SSc: an extended approach. The majority of the patients in our study were ANA-positive (93.9%); 60.3% (603/1000) of the patients were identified as ACA-, Scl-70–, or RNAP III antibody-positive (Figure 1). There were 593 patients who had a single SSc-specific antibody, 10 of which had more than 1 autoantibody specificity (1 with ACA and anti-Scl-70, 4 with ACA and anti-RNAP III, and 5 with anti-Scl-70 and anti-RNAP III). Interestingly, 10 samples that were ANA-negative by IFA were found to be anti-RNAP III-positive by ELISA. Of the remaining 346 ANA-positive samples with no defined autoantibody specificity (346/939, 37.1%), 160 had an ANoA pattern, 158 had a nuclear speckled pattern, 22 were positive for both patterns, and 6 were negative for both patterns. Of the patients with no defined autoantibody specificities, 142 cases with ANoA only and 18 with ANoA and speckled patterns were further characterized by IP.

IP identified autoantibodies in 152 of 160 patients with SSc with an ANoA IFA pattern (Figure 1); 149 had SSc-specific antibodies and 3 had non-SSc–specific autoantibodies. SSc-specific antibodies identified by IP included U3-RNP (78/160, 48.8%), Th/To (39/160, 24.4%), PM/Scl (22/160, 13.8%), Scl-70 (4/160, 2.5%), and RNAP III (4/160, 2.5%; Figure 1). Two patients with dcSSc had dual-positive SSc-specific antibodies [an African American patient (U3-RNP and Th/To) and a Hispanic patient (U3-RNP and PM/Scl)]. Of the 3 patients with autoantibodies not specific for SSc, 1 had anti-NOR90, another anti-NOR90 and anti-Su, and the third only had anti-Su (data not shown). Interestingly, in the patients with both an ANoA and a speckled pattern, there was a higher frequency of antibodies against Th/To

(6/18, 33.3%), PM/Scl (4/18, 22.2%), Scl-70 (3/18, 16.7%), and NOR90 (1/18, 5.6%) detected by IP compared with patients with a purely nucleolar pattern. Eight of the ANoA-positive patients had no identifiable autoantibodies (4 had only an ANoA pattern and 4 had an ANoA and speckled pattern).

For all of the autoantibodies detected, women represented the majority of the positive patients, which may be skewed owing to the composition of the cohort (data not shown). The mean age at disease onset differed significantly among autoantibodies, with anti-U3-RNP associated with the earliest age at onset. Anti-U3-RNP (35.6 ± 14.6 yrs) and anti-Scl-70 (40.3 ± 14.4 yrs) were associated with a significantly earlier age of onset compared with ACA (44.7 ± 14.9 yrs; $p < 0.0001$ and $p = 0.0009$, respectively) and RNAP III (45.8 ± 12.6 yrs; $p < 0.0001$ and $p = 0.0001$, respectively). Patients with ACA (12.5 ± 10.7 yrs) had a significantly longer duration of disease compared with patients with anti-Scl-70 (7.6 ± 8.7 yrs, $p < 0.0001$), anti-RNAP III (6.3 ± 7.5 yrs, $p < 0.0001$), and anti-U3-RNP (5.9 ± 6.4 yrs, $p < 0.0001$). ACA (88.1%) and anti-Th/To (84.6%) were found at higher frequencies in patients with lcSSc compared with those with dcSSc. In contrast, anti-Scl-70 (63.4%), anti-RNAP III (78.6%), and anti-U3-RNP (78.2%) were more prevalent in patients with dcSSc than those with lcSSc, which was consistent with published findings[7,11,12,13,14,15,16,17,18]. Information about kidney involvement was only available for 18.3% of the cohort. However, anti-RNAP III (37.5%) was detected at a significantly higher frequency than ACA (3.2%, $p = 0.0003$) and anti-Scl-70 (8.8%, $p = 0.0042$) in patients with renal crisis. Anti-U3-RNP (33.3%) was also present at a higher frequency compared with anti-Scl-70 (8.8%, $p = 0.0466$) in patients with renal crisis.

Autoantibody hierarchy is dependent on ethnicity. Of the SSc-associated antibodies, ACA, Scl-70, and RNAP III have been shown to be the most common, are routinely available for testing, and are included in the 2013 classification criteria for SSc[9,10]. In our cohort, ACA, Scl-70, and RNAP III made up 79.9% of the antibodies detected (Table 2). These 3 antibodies represented significantly less of the autoantibodies detected in African American (63.6%) compared with both white (86.0%, $p = 0.0003$) and Hispanic patients (82.8%, $p = 0.0023$). ACA was significantly more prevalent in individuals of white and Hispanic descent, while anti-Scl-70 had the highest frequency in African American patients (Table 2). There was no significant difference in the prevalence of anti-RNAP III among ethnic groups. Although these 3 specificities represented the majority of SSc-specific autoantibodies in all ethnic groups, the hierarchies of these antibodies differ among racial groups (Table 2, Figure 2).

In the Hispanic group, anti-Scl-70 (24.0%), anti-RNAP III (20.9%), and ACA (19.4%) were the most prevalent autoantibodies and were detected at similar frequencies (Table 2). Anti-U3-RNP, anti-Th/To, and anti-PM/Scl were uncommon in Hispanic patients. However, the prevalence of anti-U3-RNP was significantly higher in Hispanic patients compared with white patients (6.1% vs 1.8%, $p < 0.0001$). In African American patients, anti-Scl-70 (26.8%) was the most prevalent autoantibody specificity included in the current SSc diagnostic criteria, followed by anti-RNAP III (14.0%) and ACA (6.6%), with each of these comparisons achieving statistical significance (ACA vs Scl-70, $p < 0.0001$; ACA vs RNAP III, $p = 0.0112$; Scl-70 vs RNAP III, $p = 0.0006$; Table 2). Interestingly, anti-U3-RNP (22.8%) was the second most prevalent autoantibody overall in African American patients. Anti-Scl-70, anti-U3-RNP, and anti-RNAP III made up 88% of the identified SSc-specific autoantibody repertoire in African American individuals. Compared with white and Hispanic patients with SSc, ACA, anti-Th/To, and anti-PM/Scl were uncommon in African American patients.

Table 2. Frequency of SSc-specific autoantibodies in the different ethnic groups. Values are % (n) unless otherwise specified.

Autoantibody	Entire Cohort, n = 1000	African American, n = 228	White, n = 555	Hispanic, n = 196	Other*, n = 21	p
ACA	22.0 (220)	6.6 (15)[1,3]	29.2 (162)[1,6]	19.4 (38)[3,6]	23.8 (5)	< 0.0001
Scl-70**	21.3 (213)	26.8 (61)[2]	18.0 (100)[2]	24.0 (47)	23.8 (5)	0.04
RNAP III**	16.8 (168)	14.0 (32)	16.6 (92)	20.9 (41)	14.3 (3)	NS
U3-RNP	7.8 (78)	22.8 (52)[1,4]	1.8 (10)[1,5]	6.1 (12) [4,5]	19.1 (4)	< 0.0001
Th/To	3.9 (39)	1.8 (4)	4.5 (25)	4.0 (8)	9.5 (2)	NS
PM/Scl	2.2 (22)	0.9 (2)	2.9 (16)	2.0 (4)	0 (0)	NS
More than 1	1.2 (12)	0.9 (2)	1.1 (6)	2.0 (4)	0 (0)	NS
Undefined	19.7 (197)	21.9 (50)	20.0 (111)	17.9 (35)	4.8 (1)	NS
ANA–	6.1 (61)***	5.7 (13)***	6.7 (37)***	5.1 (10)***	4.8 (1)	NS

* Excluded in statistical analyses. ** Includes 4 samples that were negative by immunodiffusion or ELISA, but positive by immunoprecipitation. *** There are 10 overlapping RNAP III and ANA– samples (3 African American, 4 white, and 3 Hispanic). African American versus white: [1] $p < 0.0001$ (ACA and U3-RNP), [2] $p = 0.008$ (Scl-70). African American versus Hispanic: [3] $p < 0.0001$ (ACA), [4] $p = 0.033$ (U3-RNP). White versus Hispanic: [5] $p < 0.0001$ (U3-RNP), [6] $p = 0.007$ (ACA). No other significant differences in antibody prevalence were detected between any 2 ethnic groups. ANA+ samples negative for ACA, Scl-70, RNAP III, U3-RNP, Th/To, and PM/Scl were classified as undefined. SSc: systemic sclerosis; ACA: anticentromere antibodies; RNAP III: RNA polymerase III antibodies; ANA: antinuclear antibodies; NS: not significant.

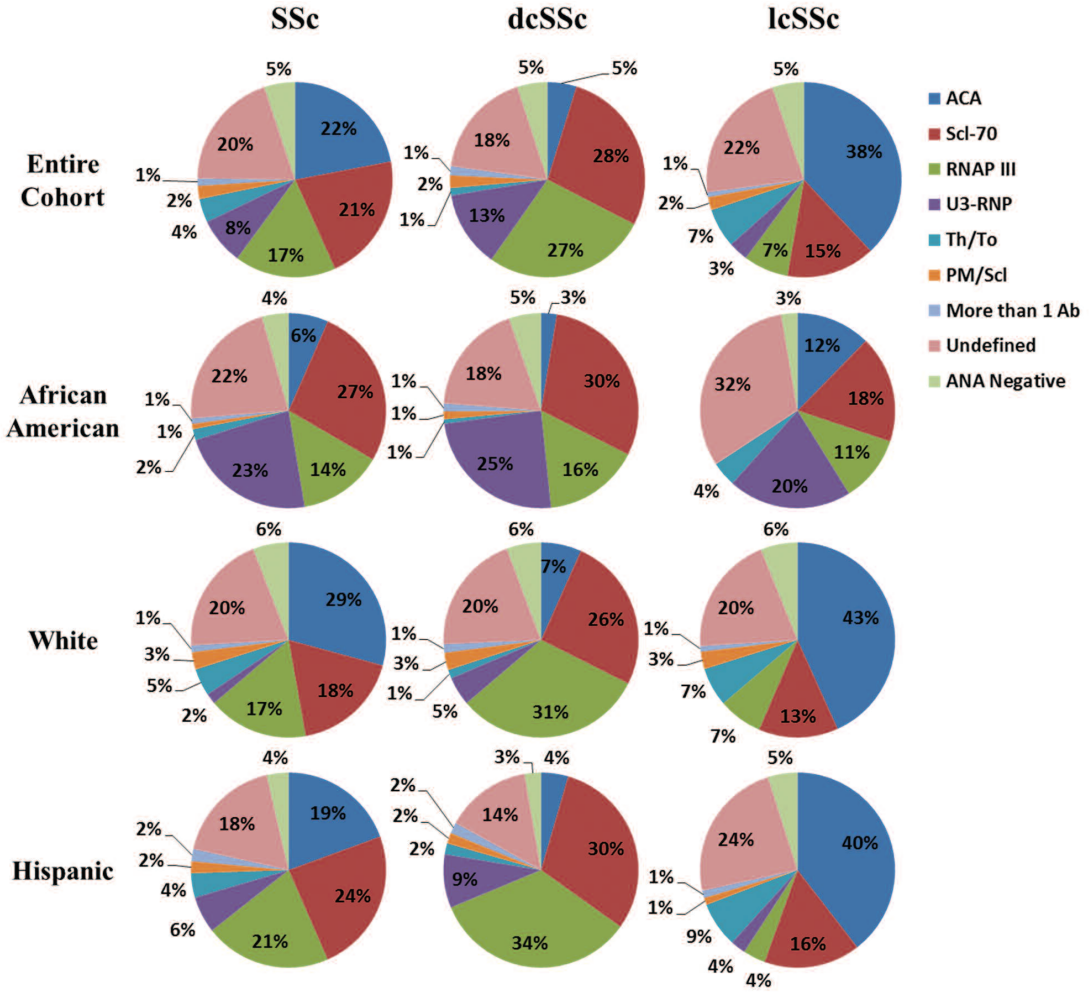

Figure 2. Autoantibody hierarchy in SSc is dependent on ethnicity. Pie charts show positivity rates for defined autoantibodies, undefined ANA specificities, or ANA– results in the entire SSc cohort, patients with dcSSc, or patients with lcSSc based on ethnicity. SSc: systemic sclerosis; dcSSc: diffuse cutaneous SSc; lcSSc: limited cutaneous SSc; ACA: anticentromere antibodies; RNAP III: RNA polymerase III antibodies; Ab: antibody; ANA: antinuclear antibodies.

In white patients, ACA (29.2%) was present at a significantly higher frequency than anti-Scl-70 (18.0%) and anti-RNAP III (16.6%, both $p < 0.0001$; Table 2), whereas anti-Scl-70 and anti-RNAP III did not differ in prevalence. Relative to all other ethnic groups, anti-U3-RNP was uncommon in white patients. Although significantly less common than the SSc antibodies included in the criteria, anti-Th/To and anti-PM/Scl were detected in white (4.5% and 2.9%, respectively), Hispanic (4.0% and 2.0%, respectively), and African American patients with SSc (1.8% and 0.9%, respectively). The prevalence of ANA-negative or -positive cases with no identifiable SSc-specific autoantibodies using standard assays as well as the IP method was similar (~20%) across the 3 ethnic groups.

Associations between autoantibodies, clinical subset, and ethnicity in patients with SSc. To determine the involvement of antibodies in SSc stratification by ethnicity, the prevalence of all identified autoantibodies in the 2 main clinical subsets were analyzed. Comparison of antibody frequencies between ethnic groups for patients with dcSSc demonstrated that anti-RNAP III and anti-U3-RNP had significantly different prevalences based on ethnicity (Table 3). Anti-RNAP III occurred in higher frequencies in Hispanic (33.9%) and white patients with dcSSc (31.4%) compared with individuals of African American descent (15.9%, $p = 0.0002$). While the Hispanic and white cohorts had comparable prevalence of anti-RNAP III, there were significant differences between the African American and Hispanic groups, as well as African American and white ($p = 0.0011$ and $p = 0.0008$, respectively). Anti-U3-RNP was unique in that it was present in a higher frequency in African American (24.5%) relative to Hispanic (8.9%, $p = 0.0011$) and white patients with dcSSc (4.8%, $p < 0.0001$). No significant difference in prevalence was noted for the other autoantibodies based on ethnicity in our investigation.

In patients with lcSSc, only ACA and anti-U3-RNP had

Table 3. Correlation between specific SSc autoantibodies and clinical subsets within race. Results for patients with a defined subset of SSc are shown. Of the 1000 patients with SSc, 10 were unspecified for dsSSc or lcSSc (4 African American, 3 white, and 3 Hispanic patients). ANA+ samples negative for ACA, Scl-70, RNAP III, U3-RNP, Th/To, and PM/Scl were classified as undefined. Values are % (n) unless otherwise specified.

Race	Autoantibody	Disease Subsets		p
African American, n = 224		dcSSc, n = 151	lcSSc, n = 73	
	ACA, n = 13	2.6 (4)	12.3 (9)	0.011
	Scl-70, n = 58	29.8 (45)	17.8 (13)	NS
	RNAP III, n = 32	15.9 (24)	11.0 (8)	NS
	U3-RNP, n = 52	24.5 (37)	20.5 (15)	NS
	Th/To, n = 4	0.7 (1)	4.1 (3)	NS
	PM/Scl, n = 2	1.3 (2)	0.0 (0)	NS
	More than 1, n = 2	1.3 (2)	0.0 (0)	NS
	Undefined, n = 51	18.5 (28)	31.5 (23)	0.041
	ANA–, n = 13*	7.3 (11)*	2.7 (2)	NS
White, n = 552		dcSSc, n = 210	lcSSc, n = 342	
	ACA, n = 162	6.7 (14)	43.2 (148)	< 0.0001
	Scl-70, n = 99	25.7 (54)	13.2 (45)	0.0002
	RNAP III, n = 91	31.4 (66)	7.3 (25)	< 0.0001
	U3-RNP, n = 10	4.8 (10)	0.0 (0)	< 0.0001
	Th/To, n = 25	1.4 (3)	6.4 (22)	0.005
	PM/Scl, n = 16	2.9 (6)	2.9 (10)	NS
	More than 1, n = 6	1.4 (3)	0.9 (3)	NS
	Undefined, n = 110	20.0 (42)	19.9 (68)	NS
	ANA–, n = 37*	6.7 (14)*	6.7 (23)*	NS
Hispanic, n = 193		dcSSc, n = 112	lcSSc, n = 81	
	ACA, n = 37	4.5 (5)	39.5 (32)	< 0.0001
	Scl-70, n = 47	30.4 (34)	16.0 (13)	0.0271
	RNAP III, n = 41	33.9 (38)	3.7 (3)	< 0.0001
	U3-RNP, n = 12	8.9 (10)	2.5 (2)	NS
	Th/To, n = 8	1.8 (2)	7.5 (6)	NS
	PM/Scl, n = 3	1.8 (2)	1.2 (1)	NS
	More than 1, n = 3	1.8 (2)	1.2 (1)	NS
	Undefined, n = 35	14.3 (16)	23.5 (19)	NS
	ANA–, n = 10*	5.4 (6)*	4.9 (4)	NS
Other, n = 21		dcSSc, n = 9	lcSSc, n = 12	
	ACA, n = 5	0.0 (0)	41.7 (5)	0.045
	Scl-70, n = 5	11.1 (1)	33.3 (4)	NS
	RNAP III, n = 3	33.3 (3)	0 (0)	NS
	U3-RNP, n = 4	44.4 (4)	0 (0)	0.021
	Th/To, n = 2	0.0 (0)	16.7 (2)	NS
	PM/Scl, n = 0	0.0 (0)	0 (0)	NA
	More than 1, n = 0	0.0 (0)	0 (0)	NA
	Undefined, n = 1	0.0 (0)	8.33 (1)	NS
	ANA–, n = 1	11.1 (1)	0 (0)	NS

* There are 10 overlapping RNAP III and ANA– samples (3 African Americans with dcSSc, 2 whites with dcSSc, 2 whites with lcSSc, and 3 Hispanics with dcSSc). SSc: systemic sclerosis; dcSSc: diffuse cutaneous SSc; lcSSc: limited cutaneous SSc; ANA: antinuclear antibodies; ACA: anticentromere antibodies; RNAP III: RNA polymerase III antibodies; NS: not significant; NA: not applicable.

significant differences in frequency based on ethnicity. African American patients (12.3%) had a significantly lower prevalence of ACA compared with their Hispanic (39.5%, p = 0.0002) or white counterparts (43.2%, p = 0.0002). Similar to what was observed for dcSSc, the prevalence of anti-U3-RNP was higher in African American (20.5%) compared with Hispanic (2.5%, p = 0.0004) and white patients with lcSSc (0%, p < 0.0001). Although limited by sample size, more Hispanic patients with lcSSc were positive for anti-U3-RNP relative to white patients (p = 0.0363). In addition to the prevalence of specific autoantibodies based on ethnicity, the associations of these antibodies between clinical subsets in each ethnic cohort were also evaluated and compared (Table 3). Anti-Scl-70 and anti-RNAP III were more prevalent in patients with dcSSc for all ethnic groups, but this association only reached statistical significance for Hispanic (p = 0.0271 and p < 0.0001, respectively) and white patients (p = 0.0002 and p < 0.0001, respectively). In the white group, anti-U3-RNP was detected exclusively in patients with dcSSc (p < 0.0001), while anti-Th/To was

significantly more frequent in the lcSSc subset (p = 0.005). Similar trends were observed in the Hispanic group, but were not statistically significant. Anti-U3-RNP was more prevalent in African American than in Hispanic or white patients, and was associated with patients with dcSSc in general. In African American patients, the frequency of anti-U3-RNP was similar in dcSSc and lcSSc. No significant difference in prevalence of anti-PM/Scl was found between disease subsets for any ethnic group. Thus, in addition to the antibody hierarchies differing between the 3 ethnic groups, they also differed based on disease type within each ethnic group (Figure 2).

Regression analysis of the data showed that the presence of ACA, anti-Scl-70, anti-RNAP III, and anti-U3-RNP were significant for predicting disease type ($p < 0.0001$ for each autoantibody) and age of onset ($p = 0.0033$, $p = 0.0394$, $p = 0.0003$, and $p < 0.0001$, respectively) in patients with SSc, independent of ethnicity (Table 4). Anti-Th/To was significant for predicting disease type ($p = 0.0002$), but not age of onset. In addition, anti-RNAP III was found to be predictive of renal crisis ($p = 0.0012$). When the interaction between antibody and race was factored into the analysis, only ACA ($p < 0.0001$) and anti-Th/To ($p = 0.0068$) retained their significance for predicting disease type (data not shown).

DISCUSSION

The presence of specific autoantibodies associated with ANA is of diagnostic relevance in SSc, and may help predict the likelihood of certain clinical manifestations, allowing for patient risk stratification and optimal disease management. Further, certain SSc autoantibodies tend to be associated with ethnicity, and correlate with manifestations involving frequency and severity of lung and renal involvement, as well as dcSSc or lcSSc. Most of these observations are based on studies composed primarily of white, African American, or Japanese patients, with limited information on Hispanic American SSc populations. In our study, we report unique differences in the frequencies, hierarchies, and associations of specific autoantibodies in the 3 US ethnic groups, including a significant number of Hispanic American patients with SSc.

Table 4. Predictive power of the antibodies to determine disease status, renal crisis, or age at disease onset in cohort by regression analysis.

Autoantibody	Disease Subset	Renal Crisis	Age at Disease Onset, Yrs
ACA	< 0.0001	NS	0.0033
Scl-70	< 0.0001	NS	0.0394
RNAP III	< 0.0001	0.0012	0.0003
U3-RNP	< 0.0001	NS	< 0.0001
Th/To	0.0002	NS	NS
PM/Scl	NS	NS	NS
More than 1	NS	NS	NS
Undefined	NS	NS	NS
ANA–	NS	NS	NS

ACA: anticentromere antibodies; RNAP III: RNA polymerase III antibodies; ANA: antinuclear antibodies; NS: not significant.

Studies that have included a sufficient number of Hispanic American patients with SSc have not presented an analysis of autoantibody profiles regarding race or ethnicity[27,28,29]. The Genes versus Environment in Scleroderma Outcomes Study (GENISOS) included 77 Hispanic patients (29% of cohort), but evaluated only 3 antibody markers (ACA, Scl-70, and U3-RNP)[19]. In a study of Mexican Mestizo patients with SSc, a higher frequency of ACA, anti-PM/Scl, anti-Ku, and anti-Scl-70 were observed compared with African American and/or white subjects with a lower prevalence of anti-RNAP III relative to the other ethnicities investigated[30]. Differences between the results from the Mexican Mestizo SSc cohort and data presented in our study can likely be explained by the heterogeneity within the Hispanic populations, as well as environmental factors[31]. Arnett, *et al* reported that the HLA class II alleles and haplotypes that showed the strongest association with SSc in Hispanic patients were also associated with SSc in white patients[27], which could explain some of the similarity in autoantibody profiles between these groups.

The relevance of autoantibody testing in the evaluation of SSc cannot be overemphasized. Indeed, the majority of the patients in our cohort were ANA-positive, with a large proportion identified as ACA, Scl-70, or RNAP III antibody-positive. Although significant differences in the prevalence of ACA were observed among ethnic groups, the high frequency in white subjects and overall association with lcSSc within the different categories in our study is consistent with previous reports[16,19,20]. No ethnic differences were observed in the prevalence of anti-Scl-70 in the lcSSc or dcSSc clinical subset. However, the frequency of anti-Scl-70 appears to be highest in African American patients with SSc, irrespective of clinical subset relative to other ethnic groups investigated. Previous studies have shown that a significant proportion of anti-Scl-70–positive African American patients had dcSSc (71% and 87%)[19,23]. In our cohort, there was a trend toward a higher prevalence of anti-Scl-70 in African American patients with dcSSc, but these associations did not reach statistical significance. This may be due to the sample size of antibody-positive patients (n = 61) or to other characteristics of our cohort. In the case of anti-RNAP III, white and Hispanic patients with dcSSc had significantly higher frequencies compared with their African American counterparts. A very limited number of African American patients were positive for anti-RNAP III, likely contributing to the lack of association with dcSSc compared with lcSSc. Increased occurrence of anti-U3-RNP in African American relative to white or Hispanic patients was previously reported in our cohort and is similar to findings by other investigators in the United States[16,19,20,23,32]. In both white and Hispanic patients with SSc, the prevalence of anti-U3-RNP was

relatively higher in dcSSc than in lcSSc. However, unlike the white cohort where anti-U3-RNP was associated with dcSSc, the presence of anti-U3-RNP was not associated with dcSSc or lcSSc in African American patients. The lack of association between anti-RNAP III or anti-U3-RNP (and possibly anti-Scl-70) and dcSSc in the African American patients differs from published reports[7,11,12,16,17,18,19,23], which were mainly based on data on white and Japanese patients. Last, the frequency of anti-Th/To did not differ significantly among ethnic groups, and was only associated with lcSSc in white patients. One European and 2 US studies reported a high prevalence of anti-Th/To and/or its correlation with lcSSc in whites[15,16,33]. However, correlation between anti-Th/To and lcSSc has not always been consistent, which may be attributable to the study size and number of anti-Th/To–positive cases[11,16,19,34]. It also remains to be clarified whether these variations are related to the diagnostic methods used for detecting the antibody marker. For example, to detect Th/To antibodies, Kuwana, *et al* used both RNA and protein immunoprecipitation assays[34] while other investigators have predominantly used the protein immunoprecipitation method[15,16,19,33] or radioactively labeled antisense riboprobes[11]. More studies are needed to further define the association between the presence of Th/To antibodies with clinical subsets and/or ethnicity in SSc.

Currently, only ACA, anti-Scl-70, and anti-RNAP III are included in the 2013 classification guidelines for SSc[8] and are widely available for testing in clinical laboratories. The analysis of autoantibody prevalence by ethnicity and/or disease subset in patients with SSc presented in our study provides compelling evidence for testing antibodies to U3-RNP, PM/Scl, and Th/To in patient care. In the absence of specific autoantibody tests, the data also suggest that ANA IFA pattern(s) may be useful in predicting clinical subset. Alternatively, the ANA IFA pattern, clinical presentation, and/or ethnic background may inform the request for specific autoantibody tests. Further, for every subset of SSc in patients, the results indicate that there exist a substantial number (14%–32%) of cases that are ANA-positive but negative for known SSc-specific autoantibodies. Thus, there exists a significant gap in the autoantibody repertoire in SSc and this highlights opportunities for identifying novel diagnostic markers for this disease.

Our study has limitations. Similar to most studies of this design, ours is cross-sectional and limited by the paucity of clinical data for internal organ involvement and survival. Further, IP was not performed on all samples, but limited to those with mainly nucleolar staining. Thus it is likely that the frequency of some autoantibodies may be underestimated or skewed toward specificities associated with ANoA patterns. While the ANoA pattern is strongly associated with a number of antibody specificities in SSc, its association is not always consistent for some autoantibodies, including RNAP III as observed in our investigation and previously reported by others[35]. Another limitation is the likely heterogeneity within each ethnic group, which could affect the interpretation of our results. Despite these limitations, our study further highlights the relevance of specific autoantibody detection in the evaluation and management of SSc, providing the most extensive characterization of the autoantibody profile in Hispanic American patients thus far, to our knowledge. Overall, serologic testing represents a cost-effective and personalized approach for this rare and heterogeneous disease with its diverse clinical outcomes.

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