

# Anti-Fibrillar Antibody in African American Patients with Systemic Sclerosis: Immunogenetics, Clinical Features, and Survival Analysis

ROOZBEH SHARIF, MARVIN J. FRITZLER, MAUREEN D. MAYES, EMILIO B. GONZALEZ, TERRY A. McNEARNEY, HILDA DRAEGER, MURRAY BARON, the Canadian Scleroderma Research Group, DANIEL E. FURST, DINESH K. KHANNA, DEBORAH J. DEL JUNCO, JERRY A. MOLITOR, ELENA SCHIOPU, KRISTINE PHILLIPS, JAMES R. SEIBOLD, RICHARD M. SILVER, ROBERT W. SIMMS, GENISOS Study Group, MARILYN PERRY, CARLOS ROJO, JULIO CHARLES, XIAODONG ZHOU, SANDEEP K. AGARWAL, JOHN D. REVEILLE, SHERVIN ASSASSI, and FRANK C. ARNETT

**ABSTRACT. Objective.** Anti-U3-RNP, or anti-fibrillar antibodies (AFA), are detected more frequently among African American (AA) patients with systemic sclerosis (SSc) compared to other ethnic groups and are associated with distinct clinical features. We examined the immunogenetic, clinical, and survival correlates of AFA in a large group of AA patients with SSc.

**Methods.** Overall, 278 AA patients with SSc and 328 unaffected AA controls were enrolled from 3 North American cohorts. Clinical features, autoantibody profile, and HLA class II genotyping were determined. To compare clinical manifestations, relevant clinical features were adjusted for disease duration. Cox proportional hazards regression was used to determine the effect of AFA on survival.

**Results.** Fifty (18.5%) AA patients had AFA. After Bonferroni correction, HLA-DRB1\*08:04 was associated with AFA, compared to unaffected AA controls (OR 11.5,  $p < 0.0001$ ) and AFA-negative SSc patients (OR 5.2,  $p = 0.0002$ ). AFA-positive AA patients had younger age of disease onset, higher frequency of digital ulcers, diarrhea, pericarditis, higher Medsger perivascular and lower Medsger lung severity indices ( $p = 0.004$ ,  $p = 0.014$ ,  $p = 0.019$ ,  $p = 0.092$ ,  $p = 0.006$ , and  $p = 0.016$ , respectively). After adjustment for age at enrollment, AFA-positive patients did not have different survival compared to patients without AFA ( $p = 0.493$ ).

**Conclusion.** Our findings demonstrate strong association between AFA and HLA-DRB1\*08:04 allele in AA patients with SSc. AA SSc patients with AFA had younger age of onset, higher frequency of digital ulcers, pericarditis and severe lower gastrointestinal involvement, but less severe lung involvement compared to AA patients without AFA. Presence of AFA did not change survival. (J Rheumatol First Release May 15 2011; doi:10.3899/jrheum.110071)

## Key Indexing Terms:

SCLERODERMA  
HLA-DRB1

GENISOS

ANTI-U3-RNP  
SCLERODERMA FAMILY REGISTRY

DIGITAL ULCER

From the Division of Rheumatology and Immunogenetics, University of Texas Health Science Center at Houston, Houston, Texas; Department of Medicine, University of Calgary, Calgary, Alberta, Canada; Division of Rheumatology, University of Texas Medical Branch at Galveston, Galveston, Texas; Division of Rheumatology, University of Texas Health Science Center at San Antonio, San Antonio, Texas; Jewish General Hospital and McGill University, Montreal, Quebec, Canada; Division of Rheumatology, Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, California; Division of Rheumatic and Autoimmune Diseases, University of Minnesota, Minneapolis, Minnesota, USA; University of Michigan Scleroderma Program, Ann Arbor, Michigan, USA; Division of Rheumatology and Immunology, Medical University of South Carolina, Charleston, South Carolina, USA; and Rheumatology Section, Department of Medicine, Boston University School of Medicine, Boston, Massachusetts, USA.

Supported by the National Institute of Health (NIH) Center of Research Translation P50AR054144 (F.C. Arnett, M.D. Mayes); NIH Training grant 5T32-AR052283-03 (J.D. Reveille); NIH Family Registry and DNA Repository N01-AR0-2251 (M.D. Mayes); NIH-KL2RR024149-04 (S. Assassi); NIH-U01-AI090909-01 Studies of HLA Region Genomics in Systemic Sclerosis and Ankylosing Spondylitis (X. Zhou); the United States Army Medical Research and Materiel Command PR064251 Candidate Gene Polymorphisms in Scleroderma: Defining Genetic

Susceptibility Factors (M.D. Mayes); University Clinic Research Center grants M01-RR00073 (UTMB) and M01-RR01346 (UT-HSC-SA); and NIH Clinical and Translational Sciences Award U11-RR024148 and T11-RR024147 from the National Center for Research Resources.

R. Sharif, MD, Postdoctoral Fellow, University of Texas Health Science Center at Houston; M.J. Fritzler, MD, PhD, Professor of Medicine, University of Calgary; M.D. Mayes, MD, MPH, Professor of Medicine, University of Texas Health Science Center at Houston; E.B. Gonzalez, MD, Professor of Medicine, University of Texas Medical Branch; T.A. McNearney, MD, Professor of Medicine, University of Texas Medical Branch (currently employed at Eli Lilly and Company, Indianapolis, IN); H. Draeger, MD, Assistant Professor of Medicine, University of Texas Health Science Center at San Antonio; M. Baron, MD, Professor of Medicine, Jewish General Hospital, Director, Canadian Scleroderma Research Group; D.E. Furst, MD, Professor of Medicine; D.K. Khanna, MD, MS, Associate Professor of Medicine, University of California at Los Angeles; D.J. del Junco, PhD, Director of Outcome Research Center for Translational Injury Research (CeTIR), University of Texas Health Science Center at Houston; J.A. Molitor, MD, PhD, Associate Professor of Medicine, University of Minnesota; E. Schiopu, MD, Assistant Professor of Medicine; K. Phillips, MD, PhD, Assistant Professor of Medicine, University of Michigan; J.R. Seibold, MD, Professor of Medicine, University of Michigan (currently at Scleroderma

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2011. All rights reserved.

Research Consultants, LLC, Avon, CT); R.M. Silver, MD, Professor of Medicine and Pediatrics, Medical University of South Carolina; R.W. Simms, MD, Professor of Medicine, Boston University; M. Perry, BSc, Senior Research Associate; C. Rojo, BSc; J. Charles, BSc, Senior Research Associate; X. Zhou, MD, Associate Professor of Medicine; S.K. Agarwal, MD, Associate Professor of Medicine; J.D. Reveille, MD, Professor of Medicine; S. Assassi, MD, MS, Assistant Professor of Medicine; F.C. Arnett, MD, Professor of Medicine, University of Texas Health Science Center at Houston.

Address correspondence to Dr. R. Sharif, University of Texas Health Science Center, 6431 Fannin Street, MSB 5.261, Houston, TX 77030, USA. E-mail: roozebeh.sharif@uth.tmc.edu

Accepted for publication March 11, 2011.

African American (AA) patients with systemic sclerosis (SSc; scleroderma) are reported to have a worse overall prognosis than Caucasians, which might be explained by a younger age of disease onset, higher frequency of diffuse cutaneous involvement, more severe lung involvement, and younger age at onset of pulmonary artery hypertension (PAH)<sup>1,2,3,4,5</sup>.

Anti-U3-RNP, or anti-fibrillarin antibody (AFA), is directed against a 35-kDa protein component of a nucleolar ribonucleoprotein called fibrillarin, which is an early marker for the formation site of nucleolus in dividing cells<sup>6</sup>. The frequency of AFA differs across ethnic groups, ranging from zero in a large cohort of Italian patients with SSc<sup>7</sup> to 50% in an African American SSc population<sup>8</sup>. The higher prevalence of AFA in the sera of AA patients with SSc has been noted in several studies<sup>9,10,11,12,13</sup>.

Studies have shown that HLA-DRB1\*08 and DQB1\*03:01 are associated with AFA in African Americans<sup>10,14</sup>. Clinically, SSc patients with AFA have been reported to have younger ages of disease onset, higher frequency of diffuse cutaneous involvement, PAH, SSc-associated musculoskeletal and cardiac involvement, and lower frequency of arthritis<sup>9,10,11,15,16,17</sup>. However, there is a lack of large robust studies on the immunogenetic associations, clinical manifestations, and survival effect of AFA in AA patients with SSc.

We compared the HLA class II alleles in AA SSc patients with AFA with unaffected controls matched for ethnicity and sex and with SSc patients without AFA. We investigated the clinical features and survival effect of AFA in AA patients with SSc.

## MATERIALS AND METHODS

**Study population.** Between 1985 and 2010, 3033 patients with SSc were enrolled in the following cohorts: (1) the Genetics versus ENvironment In Scleroderma Outcomes Study (GENISOS)<sup>3,5,18</sup>; (2) the NIH/NIAMS Scleroderma Family Registry and DNA Repository<sup>19</sup>; and (3) the Division of Rheumatology, University of Texas Health Science Center at Houston (UTHSC-H)<sup>10</sup>. Patients were included if they met the American College of Rheumatology (formerly American Rheumatism Association) classification criteria for SSc<sup>20</sup> or had at least 3 of the 5 CREST features (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasias)<sup>21</sup>. We included all AA patients from these cohorts (n = 278). Patients enrolled in more than one of the cohorts were identified and duplicate

entries were omitted. We enrolled 328 unaffected AA controls to determine any HLA class II allele associations with AFA. The unaffected AA individuals were volunteers with no personal or family history of SSc or other autoimmune disease by screening questionnaire. All study subjects enrolled (SSc patients and unaffected controls) provided written informed consent, and the institutional review board of all participating institutions approved the study.

**Autoantibody profile and HLA class II allele genotyping.** All autoantibody determinations and HLA class II allele typing were conducted in the Division of Rheumatology at UTHSC-H and the Mitogen Advanced Diagnostics Laboratory, University of Calgary, Calgary, Canada. Antinuclear antibodies (ANA) and anticentromere antibodies were determined using indirect immunofluorescence with HEp-2 cells as substrate (Antibodies Inc., Davis, CA, USA). Passive immunodiffusion gels against calf thymus extract were used to examine sera for antitopoisomerase-I (ATA; Scl-70), anti-Ro/SSA, anti-La/SSB, and anti-U1-RNP autoantibodies (Inova Diagnostics, San Diego, CA, USA). Anti-RNA polymerase III (RNAP III) was detected by ELISA kits (MBL Co. Ltd., Nagoya, Japan) and AFA were determined by a line immunoassay at a serum dilution of 1:1000 using purified recombinant fibrillarin protein (Euroline-WB; Euroimmun, Lubeck, Germany) in patients who had a positive ANA in anti-nucleolar pattern on the indirect immunofluorescence.

As described<sup>22</sup>, we genotyped HLA class II alleles (DRB1, DQA1, DQB1, and DPB1) on extracted and purified genomic DNA. Further, we examined the HLA class II allele-binding peptide using the ProPred MHC Class II Binding Peptide Prediction Server<sup>23</sup> in order to predict binding peptides of human fibrillarin protein. This prediction is based on quantitative matrices derived from the literature<sup>23,24</sup>.

**Clinical manifestation.** Age, sex, disease type (categorized as limited or diffuse cutaneous involvement at time of enrollment<sup>21</sup>), disease duration (calculated from the onset of the first non-Raynaud's phenomenon symptom attributable to SSc), and modified Rodnan skin score (MRSS)<sup>25</sup> were recorded.

To assess the severity of individual organ system involvement, the Medsger severity indices<sup>13,26</sup> of 8 organ systems were measured: peripheral vessels, skin, joints/tendons, skeletal muscle, gastrointestinal (GI) tract, lung, heart, and kidney. However, these data were available only for the patients enrolled in the GENISOS cohort (n = 78). The presence of digital ulcers was determined based on the participating rheumatologist's clinical assessment. Arthritis was defined as presence of joint swelling and tenderness on examination not attributable to osteoarthritis, crystalline arthropathy, or trauma. A decrease in range of motion > 25% in at least one joint axis was defined as joint contracture. Dysphagia, diarrhea attributable to SSc, and history of SSc renal crisis were recorded. Electrocardiography and 2-dimensional echocardiography findings and/or presence of an auscultatory friction rub determined the presence of pericarditis or clinically significant pericardial effusion.

As described<sup>18</sup>, pulmonary function tests were obtained at enrollment. Interstitial lung fibrosis, defined as chest radiograph showing fibrosis and/or forced vital capacity (FVC) < 75% of predicted value, was recorded.

For the purpose of our review, PAH was defined if the patient had (1) mean pulmonary artery pressure  $\geq$  25 mm Hg on right heart catheterization; (2) right ventricular systolic pressure  $\geq$  40 mm Hg on 2-dimensional echocardiography; or (3) if the ratio of FVC% predicted to diffusion capacity of carbon monoxide (DLCO)% predicted was  $\geq$  1.6. Serum creatine kinase (CK) levels were recorded and myositis was diagnosed if the patient had proximal muscle weakness with at least one of the following: elevated levels of CK, features of myositis on electromyography, and/or a characteristic muscle biopsy.

**Death search.** The vital status of patients was determined through the National Death Index (NDI) at the US Centers for Disease Control and Prevention, which provided data up until 2007. We then reviewed the US Social Security Death Index (SSDI) to update our results as of August 2010. SSDI is an online death search tool that provides fatality reports

based on death certificates and family confirmation. Patients not found on NDI or SSDI were assumed to be alive.

**Statistical analysis.** Homozygosity for alleles at each of the tested HLA loci was not suggestive of recessive inheritance, regardless of whether the referent comparison group comprised disease-free controls or AFA-negative cases. There were too few homozygous subjects to distinguish additive from dominant modes of inheritance, regardless of the referent. Therefore, a dominant mode of inheritance approach was used to compare the HLA association with AFA. Heterozygosity and homozygosity for a particular allele were both recoded as "1" in a binary (zero or 1) variable created for each specific HLA gene of interest. In other words, subjects negative for the gene on both their alleles for the particular HLA locus were coded "0" for the gene on the new binary variable. Bonferroni correction for multiple comparisons was performed for HLA allelic analyses.

Age, sex, disease type, and disease duration of AFA-positive and AFA-negative patients were evaluated utilizing chi-square and Student t test accordingly. SSc clinical manifestations might have changed over the disease course; therefore logistic regression was used to adjust for disease duration as a possible confounding factor in clinical features and to examine the independent effect of AFA.

We utilized Cox proportional hazards regression analysis to examine the association of AFA with survival. We investigated the potential association of relevant HLA class II with survival of the AA patients with SSc. Survival analysis was corrected for age at enrollment. Survival was calculated from the date of enrollment.

ATA and AFA are the 2 most common antinuclear antibodies among AA patients with SSc. We also compared the clinical features and survival of AA scleroderma patients with AFA (n = 50) to those with ATA (n = 61) for comparative analysis between more homogeneous groups.

All the statistical analyses were performed with SAS Version 9.2 (SAS Institute Inc., Cary, NC, USA) and Stata 11 (StataCorp., College Station, TX, USA). Hypothesis testing was 2-sided with a  $p \leq 0.05$  significance level.

## RESULTS

**Study population, disease, and autoantibody characteristics.** All 278 AA scleroderma patients from the 3 cohorts were included in the study. The mean age ( $\pm$  SD) of patients at enrollment was 46.9 (13.9) years, and 237 (85.3%) were female. At enrollment, 171 (61.5%) AA patients with SSc were diagnosed with diffuse cutaneous involvement. Average disease duration ( $\pm$  SD) was 6.0 (6.5) years.

ANA on HEp-2 substrate were detected in 93.1% of AA SSc patients. ATA, RNAP-III, and AFA were present in 21.8%, 15.4%, and 18.5% of patients, respectively (Table 1).

**HLA class II allelic frequencies.** As illustrated in Table 2, comparison of HLA class II allelic frequencies of AFA-positive patients with 329 ethnically matched unaffected controls revealed the HLA-DRB1\*08:04 allele more frequently in AFA-positive patients (47.6% vs 6.4%, respectively; OR 11.52, 95% CI 5.43, 24.40; corrected  $p < 0.0001$ ). Two other alleles located on the same haplotype, DQA1\*04:01 and DQB1\*03:01, had similar patterns. However, the increased frequency of DQA1\*04:01 was not statistically significant.

The frequency of HLA-DRB1\*08:04 in AFA-positive patients also was higher in comparison to AA patients without AFA, even after correction for multiple comparisons (47.6% vs 14.9%; OR 5.21, 95% CI 2.44, 11.09; corrected  $p = 0.0002$ ). Both HLA-DQA1\*04:01 and DQB1\*03:01

Table 1. Characteristics of the study population (n = 278).

Characteristics	
Age, mean (SD), yrs	46.9 (13.6)
Female, n (%)	237 (85.3)
Diffuse cutaneous involvement, n (%)	171 (61.5)
Disease duration, mean ( $\pm$ SD), yrs	6.0 (6.5)
Modified Rodnan skin score, mean ( $\pm$ SD)	17.3 (12.3)
Deceased patients, n (%)	83 (29.7)
Survival time (from time of enrollment), mean ( $\pm$ SD), yrs	5.8 (5.0)
Autoantibody profile, %	
Antinuclear	93.1
Anticentromere	6.2
Antitopoisomerase I	21.8
Antinuclear	44.5
Antifibrillar	18.5
RNA polymerase III	15.4
U1-ribonucleoprotein	12.2
Polymyositis/scleroderma	3.2
Ro/SSA60	9.3

showed similar trends. However, neither of them remained significant after correction for multiple comparisons.

HLA-DPB1\*01:01 was also seen more frequently among AFA-positive AA patients compared to unaffected controls and SSc patients without AFA, whereas HLA-DRB1\*11:01 seemed to be protective. HLA-DPB1\*01:01 and HLA-DRB1\*11:01 are not in linkage disequilibrium with HLA-DRB1\*08:04. However, the association of these 2 alleles with AFA did not withstand correction for multiple comparisons. The frequencies of all relevant HLA class II alleles in AA SSc patients and unaffected individuals are illustrated in Table 3.

**HLA-DRB1\*08:04 binding peptides.** Using virtual matrix for HLA-DRB1\*08:04, at a threshold of 1% (the percentage best scoring natural peptides), we identified 4 binding peptides (FRSKLAAAI, FRGRGRGGG, IHKPGAKV, and FVISIKANC) from the human fibrillar protein that could serve as potential binding sites within the antigen-binding groove.

**Clinical features.** AA SSc patients with AFA were younger at disease onset ( $p = 0.004$ ) but sex, disease type, and duration were not significantly different compared to AA SSc patients with AFA. Table 4 illustrates the comparison of clinical manifestations between AFA-positive and AFA-negative AA patients with SSc.

After adjustment for disease duration, AA SSc patients with AFA were 3.31-times more likely to have digital ulcers ( $p = 0.014$ ). Diarrhea and pericarditis occurred more frequently in AFA-positive AA SSc patients (OR 4.84,  $p = 0.019$ ; OR 2.45,  $p = 0.092$ , respectively) than AA patients without AFA. However, there were no differences between AFA-positive and AFA-negative AA SSc patients in MRSS, dysphagia, PAH, SSc-associated interstitial lung fibrosis, FVC and DLCO predicted values, SSc renal crisis, myositis

Table 2. Frequency of HLA class II alleles in antifibrillar (AFA)-positive African American (AA) patients with SSc compared to ethnically matched AFA-negative patients and unaffected controls.

HLA Class II Alleles	AFA-Positive, n = 50, %	AFA-negative, n = 221, %	Unaffected Controls, n = 329, %	AFA-positive AA Patients vs Unaffected AA Controls			AFA-positive vs negative AA Patients		
				OR (95% CI)	p	p*	OR (95% CI)	p	p*
DRB1*08:04	47.6	14.9	6.4	13.20 (6.24, 27.94)	< 0.001	< 0.001	5.21 (2.44, 11.09)	< 0.001	< 0.001
DQA1*04:01	33.3	17.5	20.6	1.95 (0.97, 3.90)	0.060	NS	2.37 (1.10, 5.09)	0.026	NS
DQB1*03:01	69.1	56.5	39.0	3.49 (1.75, 6.95)	< 0.001	0.005	1.72 (0.83, 3.56)	0.153	NS
DPB1*01:01	80	50.8	47.8	4.38 (1.08, 25.21)	0.019	NS	4.12 (1.07, 15.89)	0.041	NS
DRB1*11:01	0	16.9	11.8	NA	0.019	NS	NA	0.004	NS

\* Corrected p value. NS: not significant; NA: not applicable.

or muscle weakness, serum CK, joint contracture, or sicca symptoms.

AFA-positive patients had higher Medsger peripheral vascular severity index scores (regression coefficient [b] = 0.79, 95% CI 0.27, 1.30; p = 0.003), indicating more severe peripheral vascular involvement, and lower Medsger lung severity index (b = -0.82, 95% CI -1.50, -0.14; p = 0.019), indicating less severe lung involvement. The other Medsger severity indices were not significantly different (Table 4).

**Survival analysis.** At the time of analysis, 30% of AFA-positive AA SSc patients and 29.5% of AFA-negative patients were deceased (Table 4). After correction for age at enrollment, AFA-positive patients did not have different survival compared to AFA-negative patients (hazard ratio = 0.79, p = 0.493). In addition, none of the relevant HLA class II was a predictor of mortality in AA patients with SSc (Table 5).

**AFA and ATA among AA patients with SSc.** Although age at onset of the first non-Raynaud's symptom was not statistically different between these 2 groups, the AA scleroderma patients with AFA had higher frequency of digital ulcers, and lower GI tract involvement, pericarditis, and Medsger peripheral vascular severity index scores (Table 6). AFA-positive patients had lower Medsger lung severity index, higher FVC and DLCO predicted values, and fewer cases of PAH. Despite less severe lung disease, after adjusting for age of disease onset, AFA-positive patients did not have better or worse survival compared to ATA-positive (Table 5).

## DISCUSSION

At a frequency of 18.5%, AFA is the second most common antinuclear antibody among AA patients with SSc (second to ATA). Our report represents the first study of the genetic associations, clinical manifestations, and influence of AFA on survival in a large population of AA patients with SSc.

Distinct HLA class II allelic associations of SSc-specific

autoantibodies in different ethnic groups have been described in several studies<sup>5,10,14,27,28</sup>. In a large sample of Caucasian patients, we previously reported that HLA-DRB1\*13:02, DQB1\*06:04/06:05 haplotype correlated with AFA<sup>14</sup>. In the current study, we did not observe a similar pattern among AA patients with AFA. Our results indicated that HLA-DRB1\*08:04 is strongly associated with AFA in AA patients with SSc, compared to unaffected individuals or AFA-negative AA patients with SSc.

Previous studies investigated potential association of HLA-DRB1\*08:04 with other rheumatic conditions such as systemic lupus erythematosus (SLE)<sup>29</sup> and rheumatoid arthritis (RA)<sup>30</sup>. Reveille, *et al*<sup>29</sup> detected no difference in frequency of HLA-DRB1\*08:04 between 88 AA patients with SLE and 88 unaffected AA controls. Hughes, *et al*<sup>30</sup> reported no difference in frequency of HLA-DRB1\*08:04 between 321 AA patients with RA and 564 unaffected individuals. Previously, we showed that HLA-DRB1\*08:04 might be a susceptibility gene for SSc among AA<sup>14</sup>; whereas the results of the current study demonstrated that the higher frequency of HLA-DRB1\*08:04 with SSc in AA patients is mainly driven by its strong association with AFA in this ethnic group. Through the Binding Peptide Prediction Server<sup>23</sup> for HLA-DRB1\*0804, we identified 4 potential binding peptides from the human fibrillar protein that could serve as potential binding sites within the antigen-binding groove. The large effect sizes (Table 2) and predicted binding peptides should prompt more studies to investigate potential causal and/or environmental relationships of these autoantibodies.

An animal model for induction of AFA has been studied extensively and may provide clues to an environmental trigger in humans with AFA-positive SSc. Certain mouse strains possessing specific H2 (the murine counterpart for HLA) haplotypes develop a non-SSc autoimmune disease and high-titer AFA following administration of mercuric chloride or silver nitrate<sup>31,32,33,34</sup>. Of note, one study of urinary

Table 3. Frequency of HLA class II alleles in AFA-positive AA patients with SSc compared to ethnically matched AFA-negative patients and unaffected controls. Data are percentages. The results of the prevalence of all HLA class II alleles with frequency  $\geq 5\%$  in at least one group are included.

HLA Class II Alleles	AFA-positive, n = 50	AFA-negative, n = 221	Unaffected Controls, n = 329	AFA-positive AA and Control AA		AFA-positive and negative Patients	
				OR (95% CI)	p	OR (95% CI)	p
<b>DRB1</b>							
01:01	0	2.7	7.7	0.14 (0.01, 2.33)	0.06	0.38 (0.02, 7.16)	0.28
01:02	9.5	4.1	4.9	2.05 (0.64, 6.56)	0.22	2.49 (0.67, 9.28)	0.16
03:01	9.5	16.2	15.7	0.57 (0.19, 1.66)	0.30	0.54 (0.18, 1.66)	0.28
03:02	7.1	5.4	14.3	0.46 (0.14, 1.56)	0.20	1.35 (0.34, 5.32)	0.67
04:01	0	4.7	5.9	0.18 (0.01, 3.08)	0.11	0.22 (0.01, 3.97)	0.15
07:01	11.9	6.8	14.6	0.79 (0.29, 2.12)	0.64	1.86 (0.60, 5.79)	0.28
08:04	47.6	14.9	7.3	11.51 (5.43–24.4)	< 0.0001	5.21 (2.44–11.09)	< 0.0001
11:01	0	16.9	11.8	NA	0.02	NA	0.004
13:02	21.4	16.2	10.4	2.34 (1.02, 5.35)	0.04	1.41 (0.60, 3.32)	0.43
14:01	0	0.7	6.3	NA	0.10	NA	0.59
15:03	14.3	27.0	16.7	0.83 (0.33, 2.08)	0.69	0.45 (0.18, 1.15)	0.09
<b>DQA1</b>							
01:01	14.3	15.6	21.2	0.62 (0.25, 1.53)	0.30	0.90 (0.34, 2.38)	0.84
01:02	42.9	52.6	38.0	1.22 (0.64, 2.34)	0.55	0.68 (0.34, 1.35)	0.26
01:03	14.3	7.1	17.2	0.80 (0.32, 2.00)	0.64	2.17 (0.75, 6.25)	0.15
02:01	9.5	11.7	19.6	0.43 (0.15, 1.25)	0.11	0.80 (0.25, 2.49)	0.69
04:01	33.3	17.5	20.6	1.93 (0.96–3.87)	0.06	2.35 (1.09–5.05)	0.03
05:01	50.0	59.1	46.3	1.16 (0.61, 2.20)	0.65	0.69 (0.35, 1.37)	0.29
<b>DQB1</b>							
02:01	11.9	18.2	16.2	0.70 (0.26, 1.87)	0.48	0.61 (0.22, 1.69)	0.34
02:02	11.9	13.6	20.1	0.54 (0.20, 1.42)	0.20	0.86 (0.30, 2.42)	0.77
03:01	69.1	56.5	39.0	3.49 (1.75–6.95)	0.0002	1.72 (0.83–3.56)	0.14
03:02	2.4	6.5	9.8	0.22 (0.03, 1.70)	0.11	0.35 (0.04, 2.83)	0.31
04:02	7.1	11.0	16.2	0.40 (0.12, 1.34)	0.12	0.62 (0.17, 2.22)	0.46
05:01	16.7	18.8	22.3	0.70 (0.30, 1.64)	0.41	0.86 (0.35, 2.13)	0.75
06:02	26.2	35.1	28.7	0.88 (0.43, 1.83)	0.74	0.66 (0.31, 1.41)	0.28
06:04	16.7	9.7	8.5	2.14 (0.87, 5.27)	0.09	1.85 (0.70, 4.89)	0.21
06:05	0	7.1	1.8	NA	0.38	NA	0.07
<b>DPB1</b>							
01:01	80	50.8	47.8	4.38 (1.08, 25.21)	0.02	4.12 (1.07, 15.89)	0.04
02:01	26.7	23.1	18.9	1.56 (0.32, 5.94)	0.48	1.21 (0.34, 4.37)	0.77
03:01	6.7	16.9	14.4	0.42 (0.01, 3.20)	0.41	0.35 (0.04, 2.95)	0.32
04:01	20.0	27.7	16.2	1.29 (0.21, 5.49)	0.71	0.65 (0.16, 2.59)	0.54
04:02	20.0	15.4	26.1	0.71 (0.11, 2.89)	0.61	1.38 (0.33, 5.76)	0.66
17:01	6.7	20.0	18.0	0.33 (0.01, 2.40)	0.27	0.29 (0.03, 2.38)	0.22
18:01	0	6.2	2.4	NA	0.54	NA	0.96

NA: Not applicable.

mercury levels in SSc patients noted higher levels in those with AFA. However, this observation did not maintain statistical significance following corrections<sup>35</sup>. Interestingly, heavy metals have been noted to be highly concentrated in the nucleolus<sup>36</sup>. It was reported by Pollard, *et al*<sup>37</sup> that most if not all of the SSc-specific autoantigens were at some time during their life cycle localized to the nucleolus. Clearly, larger and more targeted studies of heavy metal and other environmental exposures are warranted in AFA-positive SSc patients, perhaps selected for the associated HLA class II alleles (DRB1\*13:02 in Caucasians and DRB1\*08:04 in AA) and AFA-negative SSc patients, as well as well matched healthy controls.

Confirming our previous findings<sup>10</sup>, we showed that HLA-DQB1\*03:01 had a higher frequency among

AFA-positive AA patients compared to unaffected AA individuals. However, there was no difference between AFA-positive and negative AA patients with SSc.

AA patients with SSc are younger at SSc onset compared to other ethnic groups<sup>1,2,5,28</sup>. Moreover, other studies show that SSc patients with AFA have younger age of disease onset<sup>2,12,13,16,17</sup>. In support of these findings, we demonstrated that AA SSc patients with AFA had younger age of onset in comparison to AA patients without AFA.

The higher Medsger peripheral vascular severity index and prevalence of digital ulcers in AA patients with AFA compared to those without AFA are novel findings. These findings were also present when we compared AFA-positive and AFA-negative AA patients with SSc. Previous studies have shown higher rates of digital ulcers among AA patients

Table 4. Clinical manifestations of African American patients with AFA compared to those without AFA (adjusted for disease duration).

Characteristic	AFA-positive, n = 50	AFA-negative, n = 221	OR (95% CI)	p
Age*, mean (SD), yrs	41.7 (13.31)	47.9 (13.25)	-6.29 (-10.59, -1.99)**	0.004
Female*, %	86.0	84.6	0.89 (0.31, 2.24)	0.806
Cutaneous involvement*, diffuse, %	36.0	39.5	0.86 (0.43, 1.69)	0.642
Disease duration at enrollment*	5.34 (5.14)	6.28 (6.85)	-0.93 (-3.51, 1.64)**	0.475
Deceased, %	30.0	29.5	0.99 (0.47, 2.03)	0.892
Modified Rodnan skin score, mean (± SD)	14.31 (7.62)	17.83 (13.50)	-2.55 (-9.66, 4.54)**	0.476
Digital ulcer, %	79.3	53.8	3.31 (1.27, 8.62)	0.014
Dysphagia, %	60.0	54.8	1.24 (0.49, 3.19)	0.619
Diarrhea, %	53.9	19.2	4.84 (1.29, 18.13)	0.019
Pericarditis, %	28.2	12.8	2.45 (0.86, 6.93)	0.092
Pulmonary artery hypertension, %	10.0	22.4	0.38 (0.09, 1.19)	0.081
SSc interstitial lung fibrosis, %	28.2	47.3	0.64 (0.28, 1.52)	0.322
FVC % predicted, mean (± SD)	77.8 (17.7)	72.9 (23.2)	6.08 (-4.54, 16.69)**	0.259
DLCO % predicted, mean (± SD)	67.7 (17.2)	57.9 (24.6)	9.45 (-2.24, 21.14)**	0.112
SSc renal crisis, %	10.5	8.4	1.28 (0.28, 4.54)	0.921
Myositis or muscle weakness, %	30.0	40.6	1.05 (0.21, 5.28)	0.953
Elevated serum creatine kinase	28.6	30.9	0.88 (0.31, 2.54)	0.817
Arthritis, %	21.9	31.0	0.53 (0.13, 2.13)	0.370
Joint contracture	22.9	21.4	1.44 (0.56, 3.72)	0.447
Sicca symptoms†	20.0	28.1	0.64 (0.11, 3.69)	0.621
Medsgger severity index, mean (± SD)				
General	0.6	0.5	0.06 (-0.53, 0.64)**	0.838
Peripheral vascular	2.2	1.4	0.79 (0.27, 1.30)**	0.003
Skin	1.5	1.7	-0.27 (-0.84, 0.31)**	0.361
Joint	0.8	0.9	-0.09 (-0.92, 0.73)**	0.813
Muscle	0.2	0.3	-0.10 (-0.41, 0.21)**	0.521
GI tract	0.6	0.6	0.02 (-0.43, 0.39)**	0.935
Lung	1.1	1.9	-0.82 (-1.50, -0.14)**	0.019
Heart	0.5	0.3	0.16 (-0.27, 0.58)**	0.457
Kidney	0.1	0.3	0.19 (-0.68, 0.29)**	0.441

\* These comparisons were not adjusted for disease duration. Student's t test and chi-square were utilized for comparisons, accordingly. \*\* Mean differences. † Two of 3 symptoms of dry mouth, dry eye, and/or enlarged parotid.

Table 5. Cox proportional hazards regression analysis of African American patients with systemic sclerosis (SSc).

	Hazard Ratio (95% CI)	p
AFA	0.80 (0.41, 1.53)	0.493
AFA vs ATA	0.84 (0.42, 1.69)	0.623
HLA-DRB1*08:04	1.00 (0.55, 1.83)	0.996
HLA-DQA1*04:01	1.50 (0.84, 2.68)	0.170
HLA-DQB1*03:01	0.85 (0.51, 1.41)	0.520
HLA-DQB1*01:01	1.13 (0.49, 2.60)	0.766
HLA-DRB1*11:01	1.17 (0.57, 2.39)	0.671

with SSc compared to Caucasians<sup>2,4</sup>. Higher frequencies of AFA in AA might contribute to this finding. Steen<sup>12</sup> reported higher frequency of digital ulcers in AFA-positive patients; however, these findings were not stratified for ethnic background.

In agreement with studies reporting more severe GI

involvement in AFA-positive patients (regardless of ethnicity)<sup>10,12</sup>, we observed a higher frequency of SSc-associated diarrhea in AFA-positive AA patients. The higher frequency of lower GI tract involvement was more significant when AFA-positive AA patients were compared to ATA-positive patients. It is possible that AFA-positive patients have more severe lower GI tract hypomotility and bacterial overgrowth that contribute to diarrhea.

Our results imply a less severe lung involvement among AFA-positive AA patients with SSc, as assessed by lower scores of the Medsgger lung severity index. The comparison of AFA-positive and ATA-positive AA scleroderma patients further demonstrated less severe lung involvement (higher FVC and DLCO predicted values and lower Medsgger lung severity index). In agreement with our findings, in an ethnically homogenous cohort of Japanese patients with SSc, AFA-positive patients had less severe lung involvement<sup>17</sup>. While data from several multiethnic cohorts suggested a

Table 6. Comparison of clinical manifestations of African American SSc patients with AFA to ATA-positive patients, adjusted for disease duration.

	AFA-positive, n = 50	ATA-positive, n = 61	OR (95% CI)	p
Age*, mean (SD), yr	41.7 (13.31)	45.9 (11.81)	-4.17 (-9.16, 0.81)**	0.099
Female*, %	86.0	74.6	2.09 (0.71, 6.66)	0.139
Cutaneous involvement*, diffuse, %	36.0	42.4	0.77 (0.33, 1.78)	0.498
Disease duration at enrollment*	5.34 (5.14)	5.77 (6.83)	-0.43 (-3.32, 2.47)**	0.769
Deceased, %	30.0	33.9	0.57 (0.19, 1.73)	0.325
MRSS, mean (± SD)	14.31 (7.62)	23.32 (13.19)	-7.38 (-15.35, 0.58)**	0.078
Digital ulcer, %	79.3	59.1	2.68 (0.90, 8.01)	0.078
Dysphagia, %	60.0	50.0	1.50 (0.43, 5.24)	0.314
Diarrhea, %	53.9	0	N/A	0.002
Pericarditis, %	28.2	10.6	3.30 (0.92, 13.29)	0.037
Pulmonary artery hypertension, %	10.0	34.0	0.28 (0.07, 1.13)	0.075
SSc interstitial lung fibrosis, %	28.2	55.1	0.48 (0.18, 1.29)	0.144
FVC % predicted, mean (± SD)	77.8 (17.7)	66.2 (18.4)	11.82 (1.37, 22.18)**	0.030
DLCO % predicted, mean (± SD)	67.7 (17.2)	47.5 (18.8)	20.14 (0.35, 30.93)**	0.004
SSc renal crisis, %	10.5	6.9	1.01 (0.16, 6.52)	0.995
Myositis or muscle weakness, %	30.0	22.2	2.46 (0.21, 28.69)	0.471
Elevated serum creatine kinase	28.6	30.4	0.94 (0.25, 3.47)	0.925
Arthritis, %	21.9	13.0	1.12 (0.14, 8.68)	0.914
Joint contracture	22.9	20.4	1.82 (0.57, 5.84)	0.314
Sicca symptoms†	20.0	19.7	1.01 (0.06, 17.08)	0.991
Medsgger severity index, mean (± SD)				
General	0.6	0.3	0.36 (-0.39, 1.12)**	0.329
Peripheral vascular	2.2	1.1	1.15 (0.47, 1.82)**	0.018
Skin	1.5	1.9	-0.46 (-1.23, 0.32)**	0.237
Joint	0.8	0.9	-0.14 (-1.23, 0.93)**	0.780
Muscle	0.2	0.3	-0.08 (-0.44, 0.27)**	0.633
GI tract	0.6	0.6	0.00 (-0.62, 0.62)**	1.000
Lung	1.1	2.4	-1.21 (-1.94, 0.48)**	0.014
Heart	0.5	0.5	0.01 (-0.62, 0.63)**	0.982
Kidney	0.1	0.4	-0.34 (-1.02, 0.35)**	0.317

\* Comparisons were not adjusted for disease duration. Student t test and chi-square were utilized accordingly.

\*\* Mean difference. † Two of 3 symptoms of dry mouth, dry eye, and/or enlarged parotid. MRSS: modified Rodnan skin score; GI: gastrointestinal.

higher frequency of isolated PAH and/or pulmonary fibrosis in the SSc patients with AFA<sup>9,10,12,13,38</sup>, these comparisons were not adjusted for ethnicity or for other antibodies, i.e., ATA, as potential confounders. Therefore, the higher frequency of lung fibrosis and PAH might be due to a sizeable AA population in the AFA-positive group and a large number of Caucasian SSc patients in the AFA-negative group. More severe SSc-associated lung involvement in AA patients with SSc compared to other ethnic groups has been reported in several studies<sup>2,18,28,39,40</sup>.

Based on our findings, AFA-positive AA patients with SSc have a higher prevalence of pericarditis, compared to AFA-negative as well as and ATA-positive patients. This is in agreement with studies indicating higher frequency of cardiac involvement in AFA-positive<sup>10,12</sup> and AA patients with SSc<sup>39</sup>.

Our study did not confirm reports of worse<sup>13</sup> or better<sup>11</sup> survival in AFA-positive patients with SSc. The poorer survival of AFA-positive patients in one report<sup>13</sup> might be

attributable to the confounding or modifying effects of ethnicity in studies that are not stratified by ethnicity, as AA ethnicity is associated with AFA positivity as well as poorer survival<sup>1,10,12,13</sup>.

This study has limitations. Although potentially important, data on heavy metal exposure were not collected. Medsgger severity indices were available only for patients from the longitudinal GENISOS cohort. High-resolution computed tomography scans and echocardiography were not performed on all patients, which might have led to underreporting of pulmonary involvement; and despite being the largest genetic study reported to date in AA patients with SSc, the findings might be underpowered to detect more subtle HLA associations with AFA in the AA population.

Anti-fibrillar antibody was the second most common antinuclear antibody in African Americans with SSc. Presence of AFA was strongly associated with the HLA-DRB1\*08:04 in the AA patients with SSc. In addition,

AA SSc patients with AFA had a younger age of disease onset, higher frequency of digital ulcers and pericarditis, more severe lower GI involvement, and less severe pulmonary involvement. Future studies should focus on environmental factors, such as heavy metal exposure, that may influence the B cell response and the immunopathology of the disease.

## APPENDIX

List of study collaborators: The Canadian Scleroderma Research Group: Janet E. Pope, Janet Markland, David Robinson, Niall Jones, Nader Khalidi, Peter Docherty, Maysan Abu-Hakima, Sharon LeClercq, Evelyn Sutton, Douglas Smith, Jean-Pierre Mathieu, Alejandra Masetto, Elzbieta Kaminska, Sophie Ligier.

## REFERENCES

- Mayes MD, Lacey JV Jr, Beebe-Dimmer J, Gillespie BW, Cooper B, Laing TJ, et al. Prevalence, incidence, survival, and disease characteristics of systemic sclerosis in a large US population. *Arthritis Rheum* 2003;48:2246-55.
- Nietert PJ, Mitchell HC, Bolster MB, Shaftman SR, Tilley BC, Silver RM. Racial variation in clinical and immunological manifestations of systemic sclerosis. *J Rheumatol* 2006;33:263-8.
- Assassi S, Del Junco D, Sutter K, McNearney TA, Reveille JD, Karnavas A, et al. Clinical and genetic factors predictive of mortality in early systemic sclerosis. *Arthritis Rheum* 2009;61:1403-11.
- Beall AD, Nietert PJ, Taylor MH, Mitchell HC, Shaftman SR, Silver RM, et al. Ethnic disparities among patients with pulmonary hypertension associated with systemic sclerosis. *J Rheumatol* 2007;34:1277-82.
- Reveille JD, Fischbach M, McNearney T, Friedman AW, Aguilar MB, Lisse J, et al. Systemic sclerosis in 3 US ethnic groups: a comparison of clinical, sociodemographic, serologic, and immunogenetic determinants. *Semin Arthritis Rheum* 2001; 30:332-46.
- Ochs RL, Lischwe MA, Spohn WH, Busch H. Fibrillarin: a new protein of the nucleolus identified by autoimmune sera. *Biol Cell* 1985;54:123-33.
- Ceribelli A, Cavazzana I, Franceschini F, Airo P, Tincani A, Cattaneo R, et al. Anti-Th/To are common antinucleolar autoantibodies in Italian patients with scleroderma. *J Rheumatol* 2010;37:2071-5.
- Kuwana M, Okano Y, Kaburaki J, Tojo T, Medsger TA Jr. Racial differences in the distribution of systemic sclerosis-related serum antinuclear antibodies. *Arthritis Rheum* 1994;37:902-6.
- Okano Y, Steen VD, Medsger TA Jr. Autoantibody to U3 nucleolar ribonucleoprotein (fibrillarin) in patients with systemic sclerosis. *Arthritis Rheum* 1992;35:95-100.
- Arnett FC, Reveille JD, Goldstein R, Pollard KM, Leaird K, Smith EA, et al. Autoantibodies to fibrillarin in systemic sclerosis (scleroderma). An immunogenetic, serologic, and clinical analysis. *Arthritis Rheum* 1996;39:1151-60.
- Tormey VJ, Bunn CC, Denton CP, Black CM. Anti-fibrillarin antibodies in systemic sclerosis. *Rheumatology* 2001;40:1157-62.
- Steen VD. Autoantibodies in systemic sclerosis. *Semin Arthritis Rheum* 2005;35:35-42.
- Aggarwal R, Lucas M, Fertig N, Oddis CV, Medsger TA Jr. Anti-U3 RNP autoantibodies in systemic sclerosis. *Arthritis Rheum* 2009;60:1112-8.
- Arnett FC, Gourh P, Shete S, Ahn CW, Honey RE, Agarwal SK, et al. Major histocompatibility complex (MHC) class II alleles, haplotypes and epitopes which confer susceptibility or protection in systemic sclerosis: analyses in 1300 Caucasian, African-American and Hispanic cases and 1000 controls. *Ann Rheum Dis* 2010;69:822-7.
- Satoh M, Akizuki M, Kuwana M, Mimori T, Yamagata H, Yoshida S, et al. Genetic and immunological differences between Japanese patients with diffuse scleroderma and limited scleroderma. *J Rheumatol* 1994;21:111-4.
- Reimer G, Steen VD, Penning CA, Medsger TA Jr, Tan EM. Correlates between autoantibodies to nucleolar antigens and clinical features in patients with systemic sclerosis (scleroderma). *Arthritis Rheum* 1988;31:525-32.
- Kuwana M, Kaburaki J, Okano Y, Tojo T, Homma M. Clinical and prognostic associations based on serum antinuclear antibodies in Japanese patients with systemic sclerosis. *Arthritis Rheum* 1994;37:75-83.
- Assassi S, Sharif R, Lasky RE, McNearney TA, Estrada YMR, Draeger H, et al. Predictors of interstitial lung disease in early systemic sclerosis: a prospective longitudinal study of the GENISOS cohort. *Arthritis Res Ther* 2010;12:R166.
- Mayes MD. The establishment and utility of a population-based registry to understand the epidemiology of systemic sclerosis. *Curr Rheumatol Rep* 2000;2:512-6.
- Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. *Arthritis Rheum* 1980;23:581-90.
- Leroy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988;15:202-5.
- Olsen ML, Arnett FC, Reveille JD. Contrasting molecular patterns of MHC class II alleles associated with the anti-Sm and anti-RNP precipitin autoantibodies in systemic lupus erythematosus. *Arthritis Rheum* 1993;36:94-104.
- Singh H, Raghava GP. ProPred: prediction of HLA-DR binding sites. *Bioinformatics* 2001;17:1236-7.
- Sturniolo T, Bono E, Ding J, Radrizzani L, Tuereci O, Sahin U, et al. Generation of tissue-specific and promiscuous HLA ligand databases using DNA microarrays and virtual HLA class II matrices. *Nat Biotechnol* 1999;17:555-61.
- Clements PJ, Lachenbruch PA, Seibold JR, Zee B, Steen VD, Brennan P, et al. Skin thickness score in systemic sclerosis: an assessment of interobserver variability in 3 independent studies. *J Rheumatol* 1993;20:1892-6.
- Steen VD, Medsger TA Jr, Rodnan GP. D-Penicillamine therapy in progressive systemic sclerosis (scleroderma): a retrospective analysis. *Ann Intern Med* 1982;97:652-9.
- Reveille JD, Durban E, Goldstein R, Moreda R, Arnett FC. Racial differences in the frequencies of scleroderma-related autoantibodies. *Arthritis Rheum* 1992;35:216-8.
- Reveille JD. Ethnicity and race and systemic sclerosis: how it affects susceptibility, severity, antibody genetics, and clinical manifestations. *Curr Rheumatol Rep* 2003;5:160-7.
- Reveille JD, Moulds JM, Ahn C, Friedman AW, Baethge B, Roseman J, et al. Systemic lupus erythematosus in three ethnic groups: I. The effects of HLA class II, C4, and CR1 alleles, socioeconomic factors, and ethnicity at disease onset. LUMINA Study Group. *Lupus in Minority Populations, Nature versus Nurture. Arthritis Rheum* 1998;41:1161-72.
- Hughes LB, Morrison D, Kelley JM, Padilla MA, Vaughan LK, Westfall AO, et al. The HLA-DRB1 shared epitope is associated with susceptibility to rheumatoid arthritis in African Americans through European genetic admixture. *Arthritis Rheum* 2008; 58:349-58.
- Hultman P, Enestrom S, Pollard KM, Tan EM. Anti-fibrillarin autoantibodies in mercury-treated mice. *Clin Exp Immunol*



- 1989;78:470-7.
32. Chen M, Rockel T, Steinweger G, Hemmerich P, Risch J, von Mikecz A. Subcellular recruitment of fibrillarin to nucleoplasmic proteasomes: implications for processing of a nucleolar autoantigen. *Mol Biol Cell* 2002;13:3576-87.
  33. Havarinasab S, Pollard KM, Hultman P. Gold- and silver-induced murine autoimmunity — requirement for cytokines and CD28 in murine heavy metal-induced autoimmunity. *Clin Exp Immunol* 2009;155:567-76.
  34. Pollard KM, Hultman P, Kono DH. Toxicology of autoimmune diseases. *Chem Res Toxicol* 2010;23:455-66.
  35. Arnett FC, Fritzler MJ, Ahn C, Holian A. Urinary mercury levels in patients with autoantibodies to U3-RNP (fibrillarin). *J Rheumatol* 2000;27:405-10.
  36. Rosen A, Casciola-Rosen L, Wigley F. Role of metal-catalyzed oxidation reactions in the early pathogenesis of scleroderma. *Curr Opin Rheumatol* 1997;9:538-43.
  37. Pollard KM, Reimer G, Tan EM. Autoantibodies in scleroderma. *Clin Exp Rheumatol* 1989;7 Suppl 3:S57-S62.
  38. Sacks DG, Okano Y, Steen VD, Curtiss E, Shapiro LS, Medsger TA Jr. Isolated pulmonary hypertension in systemic sclerosis with diffuse cutaneous involvement: association with serum anti-U3RNP antibody. *J Rheumatol* 1996;23:639-42.
  39. Laing TJ, Gillespie BW, Toth MB, Mayes MD, Gallavan RH Jr, Burns CJ, et al. Racial differences in scleroderma among women in Michigan. *Arthritis Rheum* 1997;40:734-42.
  40. McNearney TA, Reveille JD, Fischbach M, Friedman AW, Lisse JR, Goel N, et al. Pulmonary involvement in systemic sclerosis: associations with genetic, serologic, sociodemographic, and behavioral factors. *Arthritis Rheum* 2007;57:318-26.