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Expanded Autoantibody Profiles for Subsetting of Native American, African American, and European American Patients With Systemic Lupus Erythematosus

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Objective. Many Native American (NA) patients with systemic lupus erythematosus (SLE) do not exhibit the classical SLE autoantibody profiles of European American (EA) and African American (AA) patients with SLE. The poorer SLE disease outcomes noted in NA patients highlights a need for more equitable diagnostic and prognostic tools for NA patients with SLE. The objective was to identify informative autoantibody profiles for NA, AA, and EA patients with SLE using an expanded set of autoantigens.

Methods. Sera from 49 NA, 49 AA, and 49 EA age-, sex-, and antinuclear autoantibody titer–matched patients with SLE who met the American College of Rheumatology classification criteria and 10 ethnicity-, sex-, and age-matched controls were tested for autoantibody reactivity by autoantigen microarrays. Autoantibodies that were significantly elevated in patients with SLE compared with ethnicity-specific controls were selected for hierarchical clustering. Differences in clinical criteria between patient clusters were determined by Fisher's exact test and corrected for multiple comparisons.

Results. NA, AA, and EA patients with SLE each had a cluster distinguished by higher levels of anti-Ro52 and another cluster distinguished by nucleic acid–specific autoantibodies. Additional clusters were distinguished in NA patients by elevated extracellular matrix autoantibodies and were distinguished in AA patients by elevated Sm/RNP autoantibody and elevated nucleolin/histone autoantibody. Two EA patient clusters with similar nucleic acid– and Ro52-specific autoantibodies were distinguished by either high or low histone 2A reactivity. Renal manifestations trended higher in the NA Ro52 cluster and were significantly enriched in the AA nucleolin/histone cluster. The AA nucleolin/histone cluster and EA H2A cluster had higher disease activity.

Conclusion. Expanded autoantibody profiles can identify informative subsets of patients with SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by the development of autoantibodies toward a variety of cellular autoantigens (1–3). Several autoantibodies develop in patients long before disease classification (1), and autoantibodies play direct pathogenic roles (4–12). Anti-Ro/SSA autoantibodies are associated with cutaneous and hematologic manifestations as well as with neonatal lupus and congenital heart block in babies of anti-Ro–positive mothers

(8,9,13–15). Anti–double-stranded DNA (dsDNA) and anti-Sm are associated with lupus nephritis (11,12), and anti-dsDNA contributes to lupus nephritis pathogenesis (16). Combinations of anti-Ro, anti-Sm, and anti-RNP are associated with more severe forms of lupus (2). Therefore, autoantibodies may provide information on biomarkers associated with the development of specific disease manifestations.

Various North American indigenous populations have higher incidence and prevalence rates of SLE (17) and higher SLE mortality rates than other racial/ethnic groups (18). Clinical SLE diagno-

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sis may be more difficult in Native American (NA) patients because they are more likely to have concurrent rheumatic diseases or symptoms, including Raynaud phenomenon, interstitial lung disease, Sjögren syndrome, and systemic sclerosis, compared with patients of other races/ethnicities (19). NA patients with SLE may also be more likely than European American (EA) patients to have severe manifestations of disease such as lupus nephritis (19,20) and hemolytic anemia (19). Given the importance of autoantibodies in diagnosing and classifying disease manifestations, there is a need to identify autoantibody profiles that will be more useful in characterizing disease in NA patients with SLE.

The frequencies and titers of classical SLE autoantibodies vary among racial/ethnic groups. NA patients with SLE exhibited higher rates of anti-dsDNA, anti-Sm, anti-RNP, anti-Ro, and anti-cardiolipin compared with EA patients but had lower rates of anti-RNP compared with African American (AA) patients. Unknown specificities detected by precipitin are more common among NA patients with SLE than among AA, EA, or Hispanic patients with SLE (19). Given the high frequency of precipitating levels of unknown specificities in NA patients with SLE, standard assays for detecting autoantibodies do not adequately define autoantibody profiles of the NA population with SLE. The number of different autoantibodies that have been identified in patients with SLE is much greater than the number of autoantibodies typically used for SLE clinical evaluation, diagnosis, and classification, suggesting that unique combinations of autoantibodies potentially exist that may allow for more thorough disease assessment across a broader range of racial populations (3,21).

Protein autoantigen arrays have been a useful tool for identifying additional autoantibodies in patients with SLE that are associated with disease manifestations such as lupus nephritis (22–24). The goal of this discovery-based study was to use protein autoantigen arrays to identify autoantibody specificities that may be informative for characterizing SLE in NA patients and to identify a set of autoantibodies that may be useful for identifying SLE patients with more severe disease.

PATIENTS AND METHODS

Cohorts and samples. This study was approved by the institutional review boards of the Oklahoma Medical Research Foundation (OMRF) and the University of Oklahoma Health Sciences Center and was conducted in accordance with the Helsinki Declaration. All participants provided written informed consent prior to study-specific procedures. Serum samples from 49 NA, 49 AA, and 49 EA age-, sex-, and antinuclear autoantibody (ANA) titer–matched patients with SLE and age- and sex- matched ANA-negative control subjects from each ethnicity were selected from the Lupus Family Registry and Repository (LFRR) (25). Individuals interested in participating in the LFRR directly contacted the OMRF for enrollment, and no recruitment occurred at tribal facilities. Demographics, including self-reported race, were collected on questionnaires. Patients with SLE met both the American College of Rheumatology (ACR) and Systemic Lupus International Collaborating Clinics classification criteria (26–28). ACR criteria were extracted from patient medical records and used to calculate lupus severity index (LSI) scores as previously described (29).

Autoantibody assays. Standardized serological tests were performed in the College of American Pathologists and Clinical Laboratory Improvement Amendments–certified OMRF Morris Reichlin Clinical Immunology Laboratory at the time of enrollment in the LFRR. ANAs were determined by indirect immunofluorescence with human epithelial type 2 cells and anti-dsDNA autoantibodies by immunofluorescence against Crithidia (Inova Diagnostics). Precipitating levels of autoantibodies directed against Ro/SSA, La/SSB, Sm, RNP, and ribosomal P were detected by immunodiffusion (30). Samples were also tested for 11 autoantibody specificities using an xMAP multiplex assay (BioPlex 2200; Bio-Rad Technologies). The BioPlex 2200 ANA kit uses fluorescently labeled magnetic beads for the simultaneous detection of 11 autoantibody specificities, including dsDNA, ribosomal P, chromatin, Ro/SSA, La/SSB, Sm, the Sm/RNP complex, RNP, Jo-1, Scl-70, and centromere B. Serum verification beads specific for factor XIII are included in each reaction as a positive control (Bio-Rad Technologies). Levels of Ro52 autoantibodies were confirmed by enzyme-linked immunosorbent assay (ELISA) (Inova Diagnostics).

Autoantigen arrays. Serum samples were used to probe microarrays containing 123 autoantigens along with three immunoglobulin (Ig) and two anti-Ig controls (University of Texas Southwestern, Microarray Core Facility). Autoantigens and control proteins were spotted in duplicate in a randomized manner onto nitrocellulose-coated 16 pad Fast slides using a MicroGrid II microanalyzer as previously described (23). Both IgM and IgG autoantibodies were detected using the appropriate fluorescent conjugated anti-human IgG or IgM. Because only IgG reactivities

were found to be informative for diagnosis and staging of disease, only IgG was analyzed for this study.

All arrays were scanned using the GenePix 4000B Microarray Scanner (Molecular Devices), and spot intensities were extracted using GenePix software (Molecular Devices). Background fluorescence was subtracted from the median fluorescence intensity for each reactivity. The resulting values were divided by the median fluorescence intensity of the Ig control spots; then the ratio was multiplied by 1000 to generate normalized fluorescence intensities (NFIs) for each autoantigen reactivity (23,24). To account for variability in total IgG levels, a relative NFI was calculated as the ratio of the NFI for each autoantigen to the total serum IgG spot NFI.

Data availability. Data generated during and/or analyzed during the current study are available from the corresponding author on reasonable request after approval by our institutions and tribal partners.

Hierarchical clustering and statistical analysis. Relative NFIs were transformed into z scores relative to all individuals in the same racial/ethnic cohort. For each reactivity, the mean NFI for all samples in the population (μ) was subtracted from the individual NFI (x), and the difference was divided by the population SD (σ):

$$z = \frac{x - \mu}{\sigma}$$

To select autoantigens for use in clustering, we conservatively identified an autoantibody profile associated with SLE in each racial cohort. Autoantigen reactivities that differed between patients with SLE and race-matched controls were determined by Kruskal-Wallis analysis of the z scores (TIBCO Spotfire Analyst version 7.11.0.68; TIBCO Software); autoantigen reactivities with a P value of less than 0.06 were considered relevant for clustering. The z scores from the selected autoantibodies were used for hierarchical clustering of patients with SLE using TIBCO Spotfire Analyst version 7.11.0.68. Complete linkage was used as the

hierarchical clustering method, and correlation was used as the distance measure. Clinical and serologic differences between patient populations and the enrichment of SLE clinical criteria within each cluster was determined by Fisher's exact test. To account for multiple comparisons, Kruskal-Wallis analysis was followed by Dunn's post hoc test, and the false discovery method of Benjamini, Krieger, and Yekutieli was used for other analyses, with q values of less than 0.05 considered significant (GraphPad Prism version 8.3.1; GraphPad Software).

RESULTS

Demographics of study participants. All groups were 90% women, with a median age of 45 years. There were no significant differences in age for patients with SLE or controls within a cohort or between cohorts (Table 1). ANA titers were similar among patients with SLE from all three ethnicities, and all controls were ANA negative by design (Table 1). The three cohorts showed similar autoantibody specificities by multiplex assay, except that AA patients had higher rates of anti-SmRNP (71% in AA patients vs. 31% in EA patients; $q = 0.0001$) than EA patients and had higher rates of anti-Sm (49% in AA patients vs. 18% in NA patients; $q = 0.0053$), anti-SmRNP (71% in AA patients vs. 33% in NA patients; $q = 0.0001$) and anti-RNP (61% in AA patients vs. 31% in NA patients; $q = 0.0090$) than NA patients (Supplemental Table 1). The ACR criteria were similar among cohorts except for higher rates of malar rash in EA patients (59% in EA patients vs. 31% in NA patients; $q = 0.0041$; 29% in AA patients; $q = 0.0041$) and discoid rash in AA patients vs. NA patients (22% in AA patients vs. 4% in NA patients; $q = 0.0305$) (Supplemental Table 1).

Identification of expanded autoantibody profiles associated with lupus in NA, AA, and EA cohorts. Because the classical autoantibodies used for diagnosing and classifying lupus are not represented the same in NA patients with SLE as in patients with SLE from other ethnicities, we used expanded autoantigen arrays to identify lupus-associated autoantibody profiles in NA, AA, and EA cohorts. Out of 123 autoantigens

Table 1. Demographics and ANA positivity of the study populations[a]

| | Native American | | African American | | European American | |
Characteristics	Control (n = 10)	SLE (n = 49)	Control (n = 10)	SLE (n = 49)	Control (n = 10)	SLE (n = 49)
Age,[b] median (range), y	45 (27-61)	45 (18-73)	45 (27-61)	45 (21-72)	45 (27-61)	45 (13-72)
Female sex, n (%)	9 (90)	44 (90)	9 (90)	44 (90)	9 (90)	44 (90)
Anti-ANA positive, n (%)	0 (0)	...	0 (0)	...	0 (0)	...
1:360[c]	...	15 (31)	...	15 (31)	...	15 (31)
1:1080[c]	...	15 (31)	...	16 (33)	...	16 (31)
1:3240[c]	...	12 (24)	...	12 (24)	...	12 (26)
1:9720[c]	...	7 (14)	...	6 (12)	...	6 (12)

Abbreviation: ANA, antinuclear antibody; SLE, systemic lupus erythematosus.
[a] Patients with SLE and controls were matched based on age, sex, and ANA titer.
[b] The median ages of controls and patients with SLE within and across ethnicities were compared using the Kruskal-Wallis test with Dunn's post hoc test for multiple comparisons; all P values > 0.9999.
[c] ANA titers of patients with SLE were compared across ethnicities using the Kruskal-Wallis test with Dunn's post hoc test for multiple comparisons; all P values > 0.9999.
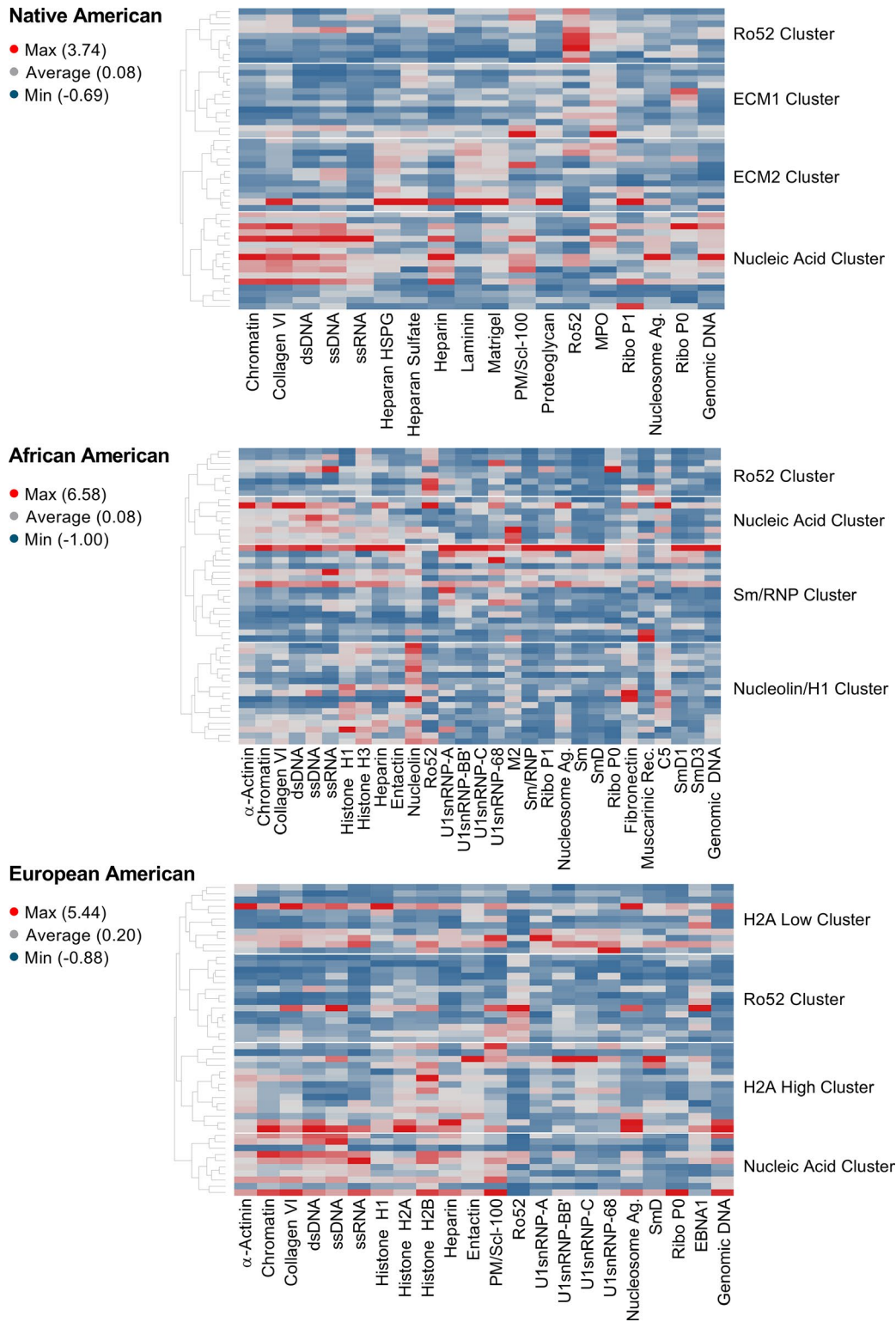
Figure 1. Hierarchical clustering of Native American (NA), African American (AA), and European American (EA) patients with systemic lupus erythematosus (SLE) using disease-associated reactivity on a directed autoantigen array. Autoantigens with different reactivity (*P* < 0.06) between patients and ethnicity-matched controls were selected for hierarchical clustering of NA, AA, and EA patients with SLE. Four clusters in each cohort were identified based on *z* scores representing autoantigen reactivity (see Methods). The *z* scores are shown on a gradient scale with maximum autoantigen reactivity in red, average reactivity in gray, and minimum reactivity in blue. Clusters were named after their most distinct autoantibodies (NA clusters: Ro52 [n = 9], ECM1 [n = 12], ECM2 [n = 12], and nucleic acid [n = 16]; AA clusters: Ro52 [n = 8], nucleic acid [n = 8], Sm/RNP [n = 16], and nucleolin/H1 [n =1 7]; EA clusters: histone 2A low [n = 11], Ro52 [n = 14], histone 2A high [n = 14], and nucleic acid [n = 10]). Ag, antigen; dsDNA, double-stranded DNA; ECM, extracellular matrix; ssDNA, single-stranded DNA; ssRNA, single-stranded RNA.

representing nuclear, extracellular matrix, cytoplasmic/membrane, circulating, microfilament, and pathogen-associated proteins (Supplemental Table 2 and Supplemental Table 3), the lupus-associated autoantibody profiles included 18 autoantigens in the NA cohort, 29 autoantigens in the AA cohort, and 22 autoantigens in the EA cohort (bolded P values in Supplemental Table 2).

All of the ethnicity-specific autoantibody profiles included nuclear autoantibodies commonly associated with SLE (chromatin, dsDNA, single-stranded DNA [ssDNA], genomic DNA, nucleosome antigen, Ribo P0, Ro52/SSA, and single-stranded RNA [ssRNA]) as well as the extracellular matrix autoantigens collagen VI and heparin (Supplemental Table 2). In addition, the NA-specific SLE autoantibody profile included increased reactivity to the nuclear autoantigens PM/Scl-100 and Ribo P1 (Supplemental Table 2) and the neutrophil granule-associated protein myeloperoxidase as well as decreased reactivity to the extracellular matrix autoantigens heparan HSPG, heparan sulfate, laminin, matrigel, and proteoglycan compared with NA controls (Supplemental Table 2). The AA-specific SLE autoantibody profile included increased reactivity to histone proteins (H1 and H3), nucleolin, members of the Sm/RNP complex (Sm, SmD, SmD1, SmD3, Sm/RNP, U1-sn-RNP-A, U1-snRNP-BB', U1-snRNP-C, and U1-snRNP-68), extracellular matrix molecules (entactin and fibronectin), M2 protein, muscarinic receptor, complement C5, and α-actinin compared with AA controls (Supplemental Table 2). The EA-specific SLE autoantibody profile also included increased reactivity to PM/Scl-100, histone proteins (H1, H2A, and H2B), members of the Sm/RNP complex (SmD, U1-snRNP-A, U1-snRNP-BB', U1-sn-RNP-C, and U1-snRNP-68) (Supplemental Table 2), entactin, α-actinin, and the Epstein-Barr virus EBNA1 protein (Supplemental Table 2) compared with EA controls. Autoantigen reactivities that did not vary significantly between NA, AA, or EA patients with SLE and their controls are listed in Supplemental Table 3.

Hierarchical clustering of NA, AA, and EA patients with SLE using NA-, AA-, and EA-specific lupus-associated autoantibody profiles. To identify subsets of patients with SLE with similar autoantigen reactivity, the ethnicity-specific autoantibody profiles were used to perform hierarchical clustering of NA, AA, and EA patients with SLE. Using this approach, four distinct clusters of patients with SLE were identified for each ethnicity (Figure 1). The clusters showed no significant differences in ANA titers (Supplemental Figure 2) but had different patterns of autoantigen reactivity (Figures 1 and 2).

One cluster of patients with SLE from each ethnicity was characterized by higher reactivity to Ro52 (Figures 1 and 2). The Ro52 clusters in the NA and EA cohorts had significantly higher reactivity to Ro52 compared with the other three clusters in the same cohorts (Figures 1 and 2). In the AA cohort, the Ro52 cluster had significantly higher reactivity to Ro52 than the SmRNP cluster and had a nonsignificant increase in Ro52 reactivity compared with the nucleolin/H1 and nucleic acid clusters (Figures 1 and 2). Results of a Ro52-spe-

cific ELISA (Supplemental Figure 1) were consistent with increased reactivity to Ro52, particularly in the NA and EA Ro52 clusters.

A second cluster of patients with SLE from each ethnicity was characterized by higher reactivity to nucleic acids, with higher reactivity to chromatin, dsDNA, ssDNA, and ssRNA compared with the other clusters (Figures 1 and 2). The nucleic acid clusters in the NA and EA cohorts also had significantly higher reactivity to genomic DNA (Figures 1 and 2). In addition, the nucleic acid clusters in the AA and EA cohorts, but not in the NA cohort, had significantly higher reactivity to heparin, α-actinin, and collagen VI compared with the other clusters in the same cohort (Figures 1 and 2).

The third and fourth clusters of NA patients with SLE, designated ECM1 and ECM2, had significantly higher reactivity to different sets of extracellular matrix autoantigens compared with the other clusters (Figures 1 and 2). The ECM1 NA patient cluster had higher reactivity to proteoglycan ($P = 0.0435$ vs. the Ro52 NA patient cluster and $P = 0.0156$ vs. nucleic acid NA patient clusters). The ECM2 NA patient cluster had higher reactivity to heparan HSPG ($P < 0.0001$ vs. the Ro52 cluster, $P = 0.0005$ vs. the ECM1 cluster, and $P = 0.0078$ vs. the nucleic acid cluster), laminin ($P = 0.0004$ vs. the Ro52 cluster, $P = 0.0105$ vs. the ECM1 cluster, and $P < 0.0001$ vs. the nucleic acid cluster), and matrigel ($P = 0.0023$ vs. the Ro52 cluster and $P = 0.0026$ vs. the ECM1 cluster). Both the ECM1 and ECM2 NA patient clusters had higher reactivity to heparan sulfate compared with the nucleic acid NA patient cluster (Figures 1 and 2). Reactivity to myeloperoxidase trended higher in the ECM1 cluster ($P = 0.0865$ vs. the Ro52 cluster and $P = 0.0547$ vs. the nucleic acid cluster).

The third cluster of AA patients with SLE, designated the SmRNP cluster, had high reactivity to Sm ($P = 0.0053$ vs. the Ro52 cluster), SmRNP ($P = 0.0066$ vs. the Ro52 cluster and $P = 0.0277$ vs. the nucleolin/histone H1 cluster), and SmD ($P = 0.0085$ vs. the Ro52 cluster). Additionally, the SmRNP cluster had higher reactivity to complement C5 ($P = 0.0137$ vs. the nucleolin/histone H1 cluster) (Figures 1 and 2). The fourth cluster of AA patients with SLE, designated the nucleolin/H1 cluster, had high reactivity to histone H1 ($P = 0.0069$ vs. the Ro52 cluster), nucleolin ($P = 0.0002$ vs. the Ro52 cluster; $P = 0.0012$ vs. the NA cluster, and $P = 0.0086$ vs. the SmRNP cluster) (Figures 1 and 2).

The additional EA SLE clusters, designated H2A high and H2A low, had either significantly higher or significantly lower reactivity to histone H2A, respectively ($P = 0.0120$) (Figures 1 and 2). The EA SLE H2A high patients also had significantly higher levels of reactivity to histone H2A ($P = 0.0113$ vs. the Ro52 cluster), histone H2B ($P = 0.0079$ vs. the Ro52 cluster), U1-snRNP68 ($P = 0.0025$ vs. the Ro52 cluster), U1-snRNPC ($P = 0.0082$ vs. the Ro52 cluster), heparin ($P = 0.0356$ vs. the Ro52 cluster), entactin ($P = 0.0018$ vs. the Ro52 cluster), and α-actinin ($P = 0.0035$ vs. the Ro52 cluster) (Figures 1 and 2).

Renal manifestations of SLE are significantly higher in the nucleolin/histone H1 cluster for AA patients with SLE and trend
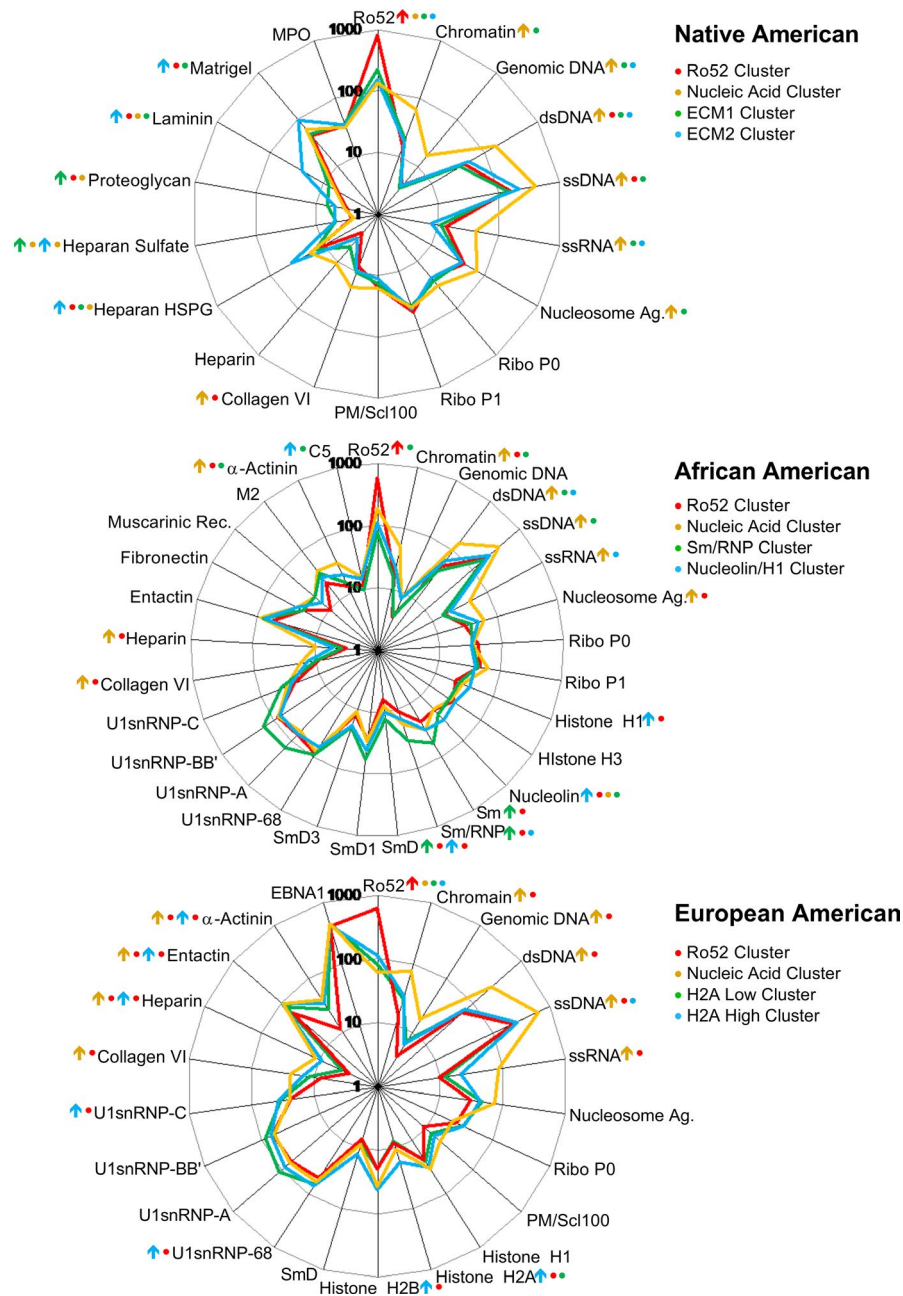
Figure 2. Autoantibody enrichment observed in Native American, African American, and European American systemic lupus erythematosus patient clusters. Autoantibody levels in each sample were calculated as a ratio of the normalized fluorescence intensity divided by the total serum immunoglobulin G for that sample. The median autoantibody levels in each cluster are shown using a log_{10} scale, using the color schemes as shown. Autoantibody levels that differed significantly between clusters were determined by Kruskal-Wallis analysis with Dunn's post hoc test. Colored arrows indicate clusters with significantly higher (up arrows) autoantibody levels compared with the clusters indicated by colored dots. *P* values are provided in the text. Ag, antigen; dsDNA, double-stranded DNA; HSPG, heparan sulfate proteoglycans; Rec, receptor; ssDNA, single-stranded DNA; ssRNA, single-stranded RNA.

higher in the Ro52 cluster for NA patients with SLE and the H2A cluster of EA patients with SLE.

Disease manifestations in NA, AA, and EA patients with SLE clustered by expanded autoantibody profiles.
To determine whether autoantibody profiles were associated with differences in disease manifestations within SLE cohorts, we com-

pared the ACR criteria and disease severity between patient clusters within each ethnicity. Among NA patients with SLE, the Ro52 cluster had a high rate of renal disease, but this did not reach significance when correcting for multiple comparisons (55.6% in the Ro52 cluster vs. 16.7% in the ECM1 cluster [*P* = 0.0873] and 8.3% in the ECM2 cluster [*P* = 0.0464; *q* = 0.2750]) (Figure 3A and Supplemental Table 4). Although dsDNA has been associated

with lupus nephritis, the Ro52 cluster of NA patients did not exhibit increased reactivity to dsDNA in the autoantigen array (Figure 2) or high rates of anti-dsDNA by multiplex (11.1% vs. 8.3%-25.0%) or indirect immunofluorescence (22.2% vs. 8.3%-75%) (Supplemental Table 4). Rates of other ACR criteria did not differ between

clusters of NA patients, and no clusters showed differences in length of disease (Supplemental Figure 3). The LSI did not differ between clusters (Figure 3B).

Among AA patients with SLE, the nucleolin/H1 cluster had significantly higher rates of renal involvement and significantly
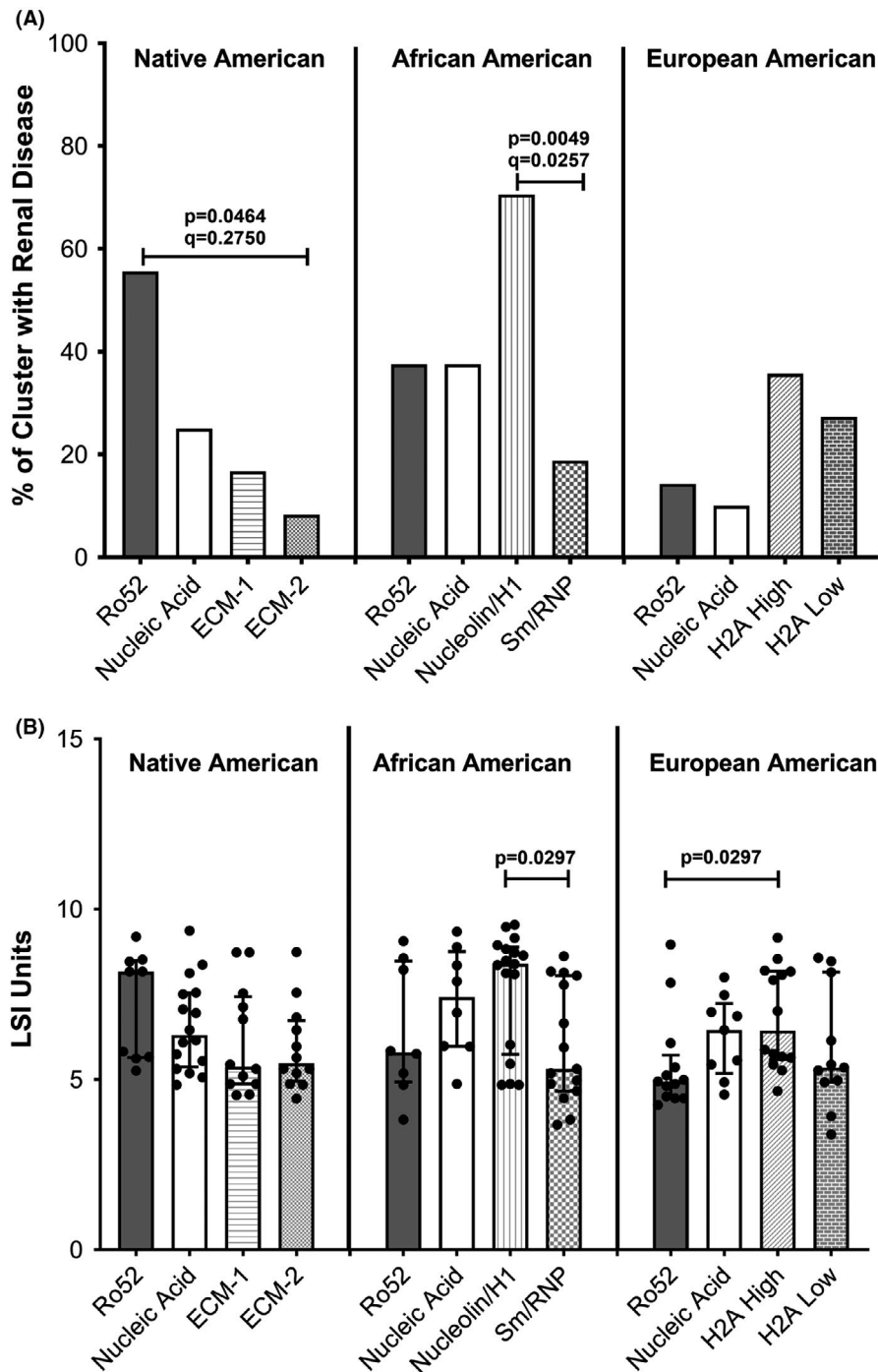

Figure 3. Renal manifestations in the Native American Ro52 and the African American nucleolin/H1 patient clusters. **A**, The percentage of patients with systemic lupus erythematosus in each patient cluster that met the American College of Rheumatology (ACR) criteria for renal disease was determined from data extracted from medical records. Enrichment for renal activity in clusters was determined using Fisher's exact test and q values calculated by the false discovery method. **B**, The lupus severity index (LSI) score for each patient was calculated using ACR data extracted from medical records. The median LSI score for each cluster was compared using Kruskal-Wallis analysis with Dunn's post hoc test. P values of less than 0.05 are indicated.

higher LSI scores than the SmRNP cluster (Figure 3 and Supplemental Table 5). Other differences between AA clusters did not reach significance (Figure 3 and Supplemental Table 5). Among EA patients with SLE, individual ACR criteria showed no significant differences between clusters, but the H2A cluster had significantly higher disease severity compared with the Ro52 cluster using the LSI (Figure 3 and Supplemental Table 6).

DISCUSSION

Current tools used to detect autoantibodies for SLE diagnosis and classification were largely developed with study cohorts in which NA populations were underrepresented (31). As a result, these tests are not optimal for identifying informative autoantibody specificities for NA patients with rheumatic disease, and there is a need to identify autoantibody profiles that will facilitate the development of diagnostic and disease-monitoring tools that are more appropriate for NA patients with SLE.

This study builds on previous work that used autoantigen arrays to identify autoantibodies associated with lupus nephritis and to differentiate patients with SLE from those with incomplete SLE (22–24). We utilized autoantigen arrays to define a novel autoantigen reactivity profile that associates with SLE in a NA cohort and reveals clusters of patients with SLE defined by distinct patterns of autoantigen reactivity. The cluster of NA patients characterized by increased Ro52 reactivity had a trend toward higher rates of renal disease, which was not significant after correcting for multiple comparisons. The time since diagnosis also trended higher in the Ro52 cluster of NA patients with SLE but did not differ significantly compared with the other NA SLE patient clusters. Renal disease in the Ro52 cluster of NA patients was not explained by anti-dsDNA, which is typically associated with lupus nephritis, because the Ro52 cluster had significantly lower levels of anti-dsDNA (4,7,11,12,32,33). Interestingly, recent publications demonstrated that anti-Ro autoantibodies, in combination with anti–early apoptotic cell autoantibodies, were linked to poor outcomes in patients with SLE and nephritis (34) and that Ro52-positive patients with SLE and higher levels of serum calreticulin had higher disease activity (35). Therefore, further exploration with a larger population of NA patients with SLE is warranted to clarify the potential relevance of Ro52 antibodies to renal disease in NA patients.

In the AA cohort, renal disease was most common in the cluster characterized by higher reactivity to the nucleosome components nucleolin and histone H1, with no increases in anti-dsDNA. This result might not be surprising, considering that autoantibodies to nucleosomes have been associated with renal disease (32). This cluster of AA patients with SLE also had significantly higher LSI scores than the other AA SLE patient clusters.

Unlike the Ro52 cluster of NA patients, the Ro52 cluster of EA patients had low rates of renal disease and significantly lower LSI scores compared with the H2A high cluster of EA patients with SLE. The H2A high cluster exhibited the highest LSI scores and a trend toward increased renal disease compared with the other EA patient clusters. The H2A cluster showed equal levels of reactivity to dsDNA.

These results identify interesting differences between the lupus-associated autoantibody profiles in NA, AA, and EA patients with SLE, and additional analysis of expanded autoantigens may support more equitable tools for characterizing SLE in these different populations. Indeed, others have suggested that autoantibodies traditionally associated with SLE are not the only autoantibodies that should be considered, nor should they be considered alone when identifying the best biomarkers of SLE with renal manifestations (36,37). Because of the discovery-based study design with a large number of comparisons and a relatively small sample size, our findings require confirmation in larger studies designed for hypothesis testing. Additionally, because some of these findings may be influenced by genetic admixture, ideally our observations need to be confirmed in a larger cohort of NA, AA, and EA patients with SLE whose races/ethnicities have been established by pedigree analysis rather than self-report. Furthermore, analysis of expanded autoantibody profiles in a longitudinal cohort with detailed disease activity assessments, such as Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) or SLEDAI-2K scores, would provide more robust data for understanding the value of autoantibody profiles for prognostic assessments.

In summary, the results of our study demonstrate that an expanded autoantibody profile can be used to identify informative autoantibodies for SLE patient populations that have been traditionally underrepresented in biomarker development.

AUTHORSHIP CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. James had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study conception and design. Carla J. Guthridge, Gross, Joel M. Guthridge, James.
Acquisition of data. Carla J. Guthridge, Gross, Quintero, Kheir.
Analysis and interpretation of data. Carla J. Guthridge, Gross, Levin, Bourn, Khan, Peercy, Saunkeah, Joel M. Guthridge, James.

REFERENCES

1. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, et al. Development of autoantibodies before

the clinical onset of systemic lupus erythematosus. N Engl J Med 2003;349:1526–33.

2. Reichlin M, Harley JB. Antibodies to Ro/SSA and La/SSB. In: Wallace DJ, Hahn BH, editors. Dubois' lupus erythematosus. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2002. p. 467–80.

3. Sherer Y, Gorstein A, Fritzler MJ, Shoenfeld Y. Autoantibody explosion in systemic lupus erythematosus: more than 100 different antibodies found in SLE patients. Semin Arthritis Rheum 2004;34:501–37.

4. Gaynor B, Putterman C, Valadon P, Spatz L, Scharff MD, Diamond B. Peptide inhibition of glomerular deposition of an anti-DNA antibody. Proc Natl Acad Sci U S A 1997;94:1955–60.

5. Poole BD, Gross T, Maier S, Harley JB, James JA. Lupus-like autoantibody development in rabbits and mice after immunization with EBNA-1 fragments. J Autoimmun 2008;31:362–71.

6. Qiao B, Wu J, Chu YW, Wang Y, Wang DP, Wu HS, et al. Induction of systemic lupus erythematosus-like syndrome in syngeneic mice by immunization with activated lymphocyte-derived DNA. Rheumatology (Oxford) 2005;44:1108–14.

7. Raz E, Brezis M, Rosenmann E, Eilat D. Anti-DNA antibodies bind directly to renal antigens and induce kidney dysfunction in the isolated perfused rat kidney. J Immunol 1989;142:3076–82.

8. LeFeber WP, Norris DA, Ryan SR, Huff JC, Lee LA, Kubo M, et al. Ultraviolet light induces binding of antibodies to selected nuclear antigens on cultured human keratinocytes. J Clin Invest 1984;74:1545–51.

9. Casciola-Rosen L, Rosen A. Ultraviolet light-induced keratinocyte apoptosis: a potential mechanism for the induction of skin lesions and autoantibody production in LE. Lupus 1997;6:175–80.

10. Kurien BT, Newland J, Paczkowski C, Moore KL, Scofield RH. Association of neutropenia in systemic lupus erythematosus (SLE) with anti-Ro and binding of an immunologically cross-reactive neutrophil membrane antigen. Clin Exp Immunol 2000;120:209–17.

11. Alba P, Bento L, Cuadrado MJ, Karim Y, Tungekar MF, Abbs I, et al. Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis. Ann Rheum Dis 2003;62:556–60.

12. McCarty GA, Harley JB, Reichlin M. A distinctive autoantibody profile in black female patients with lupus nephritis. Arthritis Rheum 1993;36:1560–5.

13. Oke V, Wahren-Herlenius M. The immunobiology of Ro52 (TRIM21) in autoimmunity: a critical review. J Autoimmun 2012;39:77–82.

14. Yoshimi R, Ueda A, Ozato K, Ishigatsubo Y. Clinical and pathological roles of Ro/SSA autoantibody system. Clin Dev Immunol 2012;2012:606195.

15. Ottosson L, Salomonsson S, Hennig J, Sonesson SE, Dörner T, Raats J, et al. Structurally derived mutations define congenital heart block-related epitopes within the 200-239 amino acid stretch of the Ro52 protein. Scand J Immunol 2005;61:109–18.

16. Yung S, Chan TM. Mechanisms of kidney injury in lupus nephritis: the role of anti-dsDNA antibodies. Front Immunol 2015;6:475.

17. Ferucci ED, Johnston JM, Gaddy JR, Sumner L, Posever JO, Choromanski TL, et al. Prevalence and incidence of systemic lupus erythematosus in a population-based registry of American Indian and Alaska Native people, 2007-2009. Arthritis Rheumatol 2014;66:2494–502.

18. Hurd K, Barnabe C. Mortality causes and outcomes in Indigenous populations of Canada, the United States, and Australia with rheumatic disease: a systematic review. Semin Arthritis Rheum 2018;47:586–92.

19. Kheir JM, Guthridge CJ, Johnston JR, Adams LJ, Rasmussen A, Gross TF, et al. Unique clinical characteristics, autoantibodies and medication use in Native American patients with systemic lupus erythematosus. Lupus Sci Med 2018;5:e000247.

20. Feldman CH, Hiraki LT, Liu J, Fischer MA, Solomon DH, Alarcón GS, et al. Epidemiology and sociodemographics of systemic lupus erythematosus and lupus nephritis among US adults with Medicaid coverage, 2000-2004. Arthritis Rheum 2013;65:753–63.

21. Yaniv G, Twig G, Shor DB, Furer A, Sherer Y, Mozes O, et al. A volcanic explosion of autoantibodies in systemic lupus erythematosus: a diversity of 180 different antibodies found in SLE patients. Autoimmun Rev 2015;14:75–9.

22. Haddon DJ, Diep VK, Price JV, Limb C, Utz PJ, Balboni I. Autoantigen microarrays reveal autoantibodies associated with proliferative nephritis and active disease in pediatric systemic lupus erythematosus. Arthritis Res Ther 2015;17:162.

23. Li QZ, Zhou J, Wandstrat AE, Carr-Johnson F, Branch V, Karp DR, et al. Protein array autoantibody profiles for insights into systemic lupus erythematosus and incomplete lupus syndromes. Clin Exp Immunol 2007;147:60–70.

24. Zhu H, Luo H, Yan M, Zuo X, Li QZ. Autoantigen microarray for high-throughput autoantibody profiling in systemic lupus erythematosus. Genomics Proteomics Bioinformatics 2015;13:210–8.

25. Rasmussen A, Sevier S, Kelly JA, Glenn SB, Aberle T, Cooney CM, et al. The lupus family registry and repository. Rheumatology (Oxford) 2011;50:47–59.

26. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725.

27. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.

28. Petri M, Orbai AM, Alarcón GS, Gordon C, Merrill JT, Fortin PR, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum 2012;64:2677–86.

29. Bello GA, Brown MA, Kelly JA, Thanou A, James JA, Montgomery CG. Development and validation of a simple lupus severity index using ACR criteria for classification of SLE. Lupus Sci Med 2016;3:e000136.

30. Swaak AJ, Huysen V, Nossent JC, Smeenk RJ. Antinuclear antibody profiles in relation to specific disease manifestations of systemic lupus erythematosus. Clin Rheumatol 1990;9 Suppl 1:82–95.

31. Bruner BF, Guthridge JM, Lu R, Vidal G, Kelly JA, Robertson JM, et al. Comparison of autoantibody specificities between traditional and bead-based assays in a large, diverse collection of patients with systemic lupus erythematosus and family members. Arthritis Rheum 2012;64:3677–86.

32. Sui M, Lin Q, Xu Z, Han X, Xie R, Jia X, et al. Simultaneous positivity for anti-DNA, anti-nucleosome and anti-histone antibodies is a marker for more severe lupus nephritis. J Clin Immunol 2013;33:378–87.

33. Yung S, Chan TM. Anti-dsDNA antibodies and resident renal cells: their putative roles in pathogenesis of renal lesions in lupus nephritis. Clin Immunol 2017;185:40–50.

34. Aktar K, Liu X, Yang X. Combination of anti-early apoptotic cell autoantibodies and anti-SSA autoantibodies in lupus nephritis. Cell Mol Biol (Noisy-le-grand) 2018;64:48–54.

35. Wang Y, Xie J, Liu Z, Fu H, Huo Q, Gu Y, et al. Association of calreticulin expression with disease activity and organ damage in systemic lupus erythematosus patients. Exp Ther Med 2017;13:2577–83.

36. Fu SM, Dai C, Zhao Z, Gaskin F. Anti-dsDNA Antibodies are one of the many autoantibodies in systemic lupus erythematosus. F1000Res 2015;4:939.

37. Mok CC. Biomarkers for lupus nephritis: a critical appraisal. J Biomed Biotechnol 2010;2010:638413.