Genetic Ancestry, Serum Interferon-α Activity, and Autoantibodies in Systemic Lupus Erythematosus

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Abstract

Objective—To investigate and refine the relationships among systemic lupus erythematosus (SLE) and related autoantibodies, interferon-α (IFN-α), and various ancestral backgrounds.

Methods—We investigated quantitatively defined genetic ancestry through principal component analysis in place of self-reported ancestry.

Results—African ancestry was found to be associated with presence of anti-RNP antibody (p = 0.0026), and anti-RNP was correlated with high levels of IFN-α (p = 2.8×10^{-5}).

Conclusion—Our data support a model in which African ancestry increases the likelihood of SLE-associated autoantibody formation, which subsequently results in higher levels of serum IFN-α.

Key Indexing Terms

SYSTEMIC LUPUS ERYTHEMATOSUS; AUTOANTIBODIES; INTERFERONS; GENETICS

Systemic lupus erythematosus (SLE) is a heterogeneous disease characterized by different clinical and serological manifestations, which vary in prevalence across self-reported ancestral backgrounds[1,2,3,4]. Anti-Sm and anti-RNP antibodies are more prevalent in African American (AA) patients with SLE than in European (EA) and Hispanic American (HA) cohorts[2,4]. Moreover, AA and HA patients with SLE have a higher incidence of SLE-related renal disease than EA patients[3]. EA and HA patients tend to have more photosensitivity and oral ulcers compared to AA patients[2,4]. High levels of interferon-α (IFN-α), which plays an important pathogenic role in SLE, have been associated with positive serology and self-reported non-European ancestry[5].

Using self-reported ancestry to categorize subjects can be problematic, as many systems for self-report are designed to indicate 1 ancestral background per subject, and proportional admixture is not taken into account. Assessment of genetic ancestry using ancestry-informative markers (AIM) provides a means to address incomplete data provided by self-report systems[6,7,8]. Principal component analysis using AIM can provide covariates designating genetic ancestry that can be used to control for admixture in diverse study

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populations[6,7,8,9]. We investigated the relationships among ancestry, serum IFN-α levels, and autoantibodies using quantitatively defined genetic ancestry.

MATERIALS AND METHODS

Patients, samples, and data

Serum samples were obtained from 220 patients with SLE (140 AA, 71 EA, and 9 HA by self-reports) from the Translational Research Initiative in the Department of Medicine at the University of Chicago Medical Center (UCMC). All the patients met the American College of Rheumatology criteria for SLE[1,10]. Autoantibodies to Ro, La, Sm, and RNP were measured by ELISA methods (Inova Diagnostics, San Diego, CA, USA) at UCMC, and standard clinical laboratory cutoff points were used to categorize them as positive or negative. Anti-dsDNA levels were measured using *Crithidia luciliae* immunofluorescence.

Serum IFN-α activity was measured through a reporter cell bioassay (WISH cells, ATCC no. CCL-25) that assesses the ability of patient sera to cause IFN-induced gene expression[11]. IFN-α activity was categorized into high versus low based on a cutoff point of 2 SD above the mean of healthy donors. A total of 374 AIM were genotyped in the context of a larger experiment[12], and principal components derived from these AIM were used to designate genetic ancestry.

Statistical analysis

As a first-pass analysis, we performed multivariate logistic regression analysis on IFN-α and each autoantibody using principal components 1, 2, and 3 (PC1, PC2, PC3) as predictor variables. Sex and age were used as covariates in all regressions, and IFN-α was also included as a covariate in the autoantibody regressions because of the reported associations between IFN-α and autoantibodies[5,13]. We then carried forward variables with a p value < 0.20 for further analysis. Next, we performed backward stepwise regression modeling using each autoantibody and IFN-α as outcome variables with all the other variables as predictors for network analysis. Results with p < 0.05 in this overall model were considered significant, with OR to indicate the magnitude of the relationship.

RESULTS

The average age of subjects was 45 ± 14.1 years and 85.5% were women. Figure 1 shows plots of the first 3 principal components derived from the AIM, with each subject color-coded by self-reported ancestry. PC1 separated African from non-African ancestry, and the second and third components corresponded to some degree with self-reported Hispanic ancestry. First, we detected associations between genetic ancestry and each antibody and IFN-α separately (Table 1). Lower values of PC1 were correlated with high IFN-α activity and positive anti-Ro, anti-Sm, and anti-RNP (p = 0.0025, 0.0194, 0.0112, and 0.0002, respectively), thus linking them to African ancestry. Lower values of PC2 were associated with the presence of anti-La (p = 0.0397), suggesting a positive connection between Hispanic ancestry and anti-La.

We next considered the significant variables in an overall logistic model to account for between-variable relationships. Significant results from this overall regression (p < 0.05) are shown in Figure 2. Interestingly, African ancestry was associated only with anti-RNP (p = 0.0026), suggesting that other serologic relationships with African genetic ancestry may be secondary to anti-RNP. Additionally, IFN-α was not directly associated with African ancestry, but instead was correlated with anti-RNP ($p = 2.8 \times 10^{-5}$).

DISCUSSION

A study by Weckerle, *et al*[5] showed that self-reported non-European ancestry was associated with high serum IFN-α activity and positive autoantibodies in SLE, and after control for autoantibodies, a residual association remained between non-European ancestry and high IFN-α. We used genetic assessment of ancestry to investigate this question in detail, and this allowed us to resolve these overlapping associations to some degree. Our study supports a model in which African ancestry increases the likelihood of SLE-associated autoantibody formation, which subsequently results in higher levels of serum IFN-α. This finding is supported by previous *in vitro* work showing that SLE autoantibody immune complexes were able to induce IFN-α production, likely through Toll-like receptors[14]. We also replicated the relationship between higher IFN-α and younger age in SLE, and this finding continues to be robust across multiple analyses and cohorts[5,15].

Previous studies have shown other autoantibodies such as anti-Sm, anti-Ro, and anti-DNA also were associated with AA patients[2,3,5]. That anti-RNP was the only independently linked antibody in our study could be related to the high prevalence of this antibody in AA patients with SLE and reduced statistical power to detect associations with other less prevalent autoantibodies. It is also possible that the careful designation of genetic ancestry increased our ability to distinguish associations particular to African ancestry, eliminating potential residual effects from genetic admixture. Future larger-scale efforts will be able to answer this question more definitively.

IFN-α is an important pathogenic mediator in SLE[5,13], and improved understanding of the factors related to high IFN-α levels in humans is critical to our understanding of SLE. Our data support a model in which African genetic ancestry is linked to autoantibody production, leading to higher IFN-α activity.

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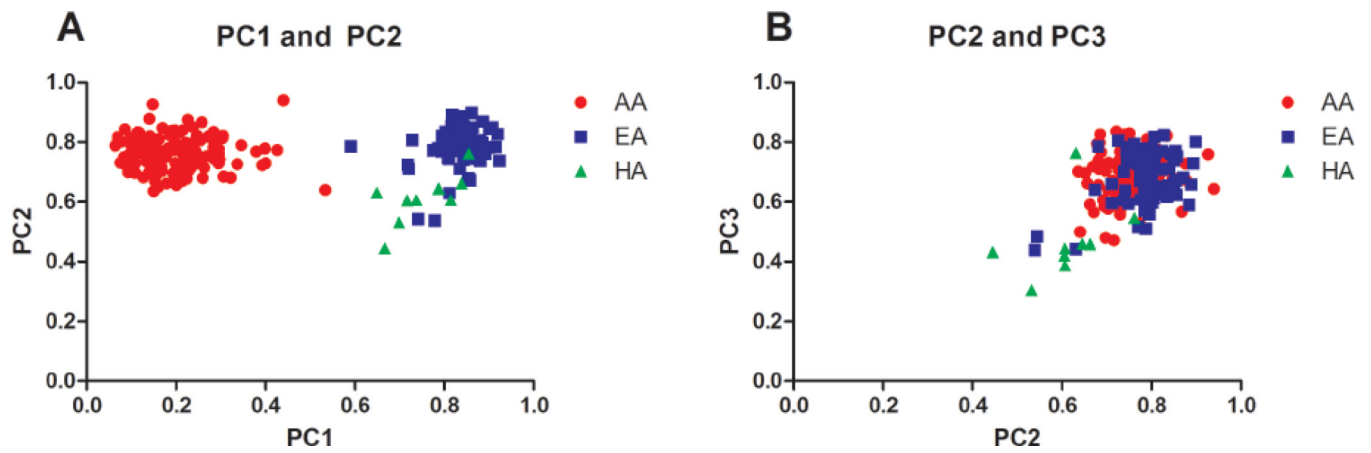
Figure 1.
Principal component (PC) analysis of ancestry-informative genetic markers in patients with systemic lupus erythematosus of various self-reported ancestral backgrounds. PC1, PC2, and PC3 correspond to the first, second, and third principal components from this analysis, and each dot represents 1 subject who is color-coded by self-reported ancestral background. A. PC1 vs PC2; B. PC2 vs PC3. AA: African-American; EA: European-American; HA: Hispanic American.
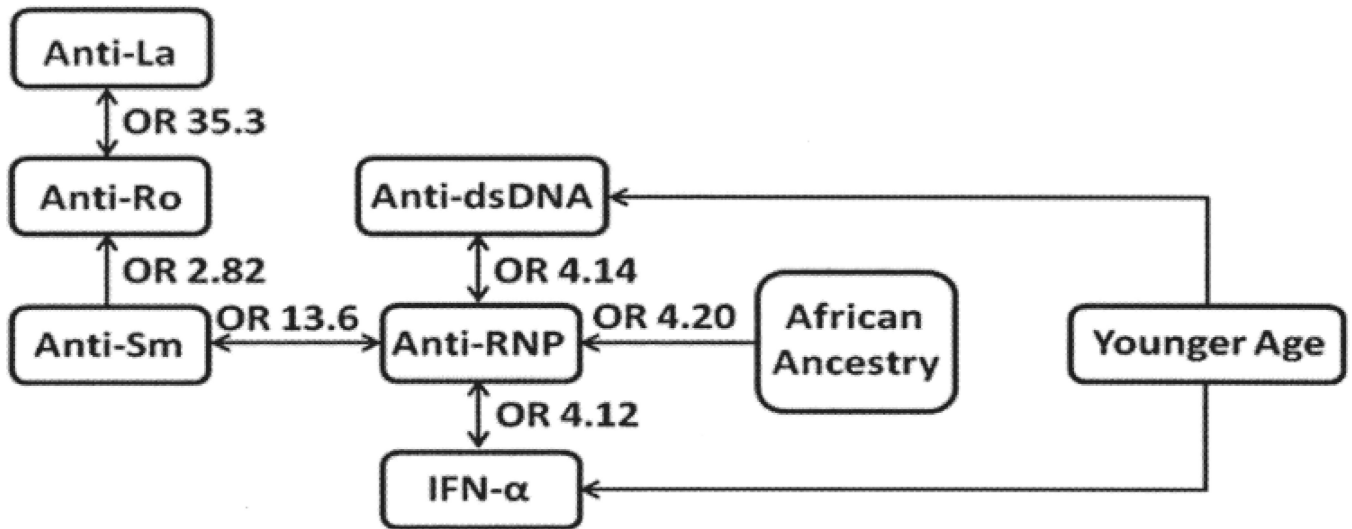
Figure 2.
Network diagram shows relationships among genetic ancestry, age, autoantibodies, and interferon-α (IFN-α), with OR indicating the magnitude of the relationship.

Table 1

Associations between genetic ancestry and autoantibodies with age, sex, and interferon-α (IFN-α) as covariates.

	IFN-α			Anti-RNP		
	β	SE	p	β	SE	p
Sex	-0.6465	0.431	0.1336	-0.0362	0.4689	0.9385
Age	-0.0408	0.0111	0.0002	-0.0162	0.0122	0.1848
IFN-α [*]	NA	NA	NA	1.4942	0.3303	6.1×10^{-6}
PC1	-1.4764	0.4889	0.0025	-2.1128	0.5576	0.0002
PC2 [**]	-3.6297	2.3468	0.1219	—	—	—
PC3 [**]	—	—	—	—	—	—

	Anti-RO			Anti-SM		
	β	SE	p	β	SE	p
Sex	-0.1825	0.4459	0.6823	0.0313	0.5299	0.9529
Age	0.0072	0.5235	0.5235	-0.0263	0.0132	0.0466
IFN-α [*]	0.7732	0.3179	0.0150	0.7354	0.3605	0.0413
PC1	-1.2298	0.5261	0.0194	-1.6780	0.6612	0.0112
PC2 [**]	—	—	—	-4.3808	2.4314	0.0716
PC3 [**]	1.9206	1.7241	0.2653	—	—	—

	Anti-La			Anti-dsDNA		
	β	SE	p	β	SE	p
Sex	0.1040	0.686	0.8795	0.2188	0.4638	0.6372
Age	0.0129	0.0155	0.4038	-0.0273	0.012	0.023
IFN-α [*]	1.0511	0.494	0.0334	0.8224	0.3168	0.0094
PC1 [**]	-1.5001	0.8949	0.0937	—	—	—
PC2 [**]	-6.1517	2.991	0.0397	—	—	—
PC3 [**]	—	—	—	—	—	—

[*] IFN-α was the dependent variable, so it was not used as a covariate.

[**] Values were omitted if components did not meet the first-pass criterion (p < 0.20), indicated with a dash. PC: principal component; NA: not applicable.