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ORIGINAL ARTICLE

Familial aggregation and linkage analysis of autoantibody traits in pedigrees multiplex for systemic lupus erythematosus

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Autoantibodies are clinically relevant biomarkers for numerous autoimmune disorders. The genetic basis of autoantibody production in systemic lupus erythematosus (SLE) and other autoimmune diseases is poorly understood. In this study, we characterized autoantibody profiles in 1506 individuals from 229 multiplex SLE pedigrees. There was strong familial aggregation of antinuclear antibodies (ANAs), anti-double-stranded DNA (dsDNA), anti-La/SSB, anti-Ro/SSA, anti-Sm, anti-nRNP (nuclear ribonucleoprotein), IgM antiphospholipid (aPL) antibodies (Abs) and rheumatoid factor (RF) across these families enriched for lupus. We performed genome-wide linkage analyses in an effort to map genes that contribute to the production of the following autoantibodies: Ro/SSA, La/SSB, nRNP, Sm, dsDNA, RF, nuclear and phospholipids. Using an approach to minimize false positives and adjust for multiple comparisons, evidence for linkage was found to anti-La/SSB Abs on chromosome 3q21 (adjusted $P=1.9\times10^{-6}$), to anti-nRNP and/or anti-Sm Abs on chromosome 3q27 (adjusted $P=3.5\times10^{-6}$), to anti-Ro/SSA and/or anti-La/SSB Abs on chromosome 4q34–q35 (adjusted $P=3.4\times10^{-4}$) and to anti-IgM aPL Abs on chromosome 13q14 (adjusted $P=2.3\times10^{-4}$). These results support the hypothesis that autoantibody production is a genetically complex trait. Identification of the causative alleles will advance our understanding of critical molecular mechanisms that underlie SLE and perhaps other autoimmune diseases.
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Introduction

Autoimmune diseases are characterized by the activation of autoreactive lymphocytes and development of immune responses to many self-antigens. A low level of autoreactivity is physiologic.[1] The challenge, therefore, is to understand how normal lymphocyte activation, in the setting of complex interactions between environmental factors and polymorphic alleles of multiple genes, progresses to a pathologic process. Collectively, autoimmune diseases are estimated to affect at least 5–8% of the population;[2,3] thus, identifying genetic factors that contribute to these disorders is of high priority.

The clustering of multiple autoimmune disorders in families and the evidence for overlapping disease susceptibility loci between different autoimmune diseases[4–6] suggest that certain alleles contribute to multiple autoimmune disorders. In addition to well-known associations of certain human leukocyte antigen (HLA) loci with multiple human autoimmune diseases, recent studies have identified a growing number of non-HLA genes that may contribute across the spectrum of autoimmune disorders. Examples include associations of *CTLA4* with autoimmune thyroid disease (AITD), type I diabetes (T1D), celiac disease, Wegener's granulomatosis, systemic lupus erythematosus (SLE), vitiligo, Addison's disease and rheumatoid arthritis (RA),[7–15] *PD-1* with multiple sclerosis (MS), RA, T1D and SLE[16–18] and *PTPN22* with SLE, RA, T1D, Graves' disease, Wegener's granulomatosis, Addison's disease and Hashimoto thyroiditis.[19–29] Murine studies are consistent with models of multigenic inheritance, and many susceptibility loci that are shared across different autoimmune mouse models have been identified.[30]

SLE [MIM 152700] is often considered the prototypic systemic autoimmune disease, and is characterized by the production of high titers of autoantibodies directed

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against native DNA and other cellular constituents. SLE is estimated to affect about 1 in 2000 individuals, with greater than 90% of cases occurring in women.[31] SLE has a highly variable clinical presentation with potential involvement of multiple organs. The American College of Rheumatology (ACR) established criteria for the classification of SLE, which include 11 features related to dermatologic, renal, neurologic, hematologic and immunologic manifestations. Any four of the 11 items present at any time is sufficient for diagnosis.[32] Two of the 11 criteria focus on autoantibodies in SLE, and presence of antinuclear antibodies (ANAs) are far and above the most common classification criteria met (>95% of patients).[33]

Genetic susceptibility to SLE clearly involves multiple loci, but environmental factors such as infectious agents are likely to have a role in initiating and propagating the disease.[34] Multiple genome-wide screens for SLE susceptibility loci have been performed, producing nine chromosomal locations that reach the threshold for significant linkage:[35] 1q23, 1q31–32, 1q41–42, 2q37, 4p16, 6p21–p12, 12q24, 16q12 and 17q11.[36–43] Each of these loci has been confirmed in at least one independent collection.[39,40,43–51] Sixteen additional intervals with suggestive evidence for linkage to SLE have been identified in more than one data set and include 1p36, 1q24–25, 6q25–27, 7p22–21, 7q21, 7q36, 9p24, 13q32, 14q22–23, 15q26, 16p13, 17q21, 19q13, 20p12, 20q13 and 22q11–12.[36,38–43,45–50,52] Upon stratification of SLE pedigrees according to selected clinical manifestations, nine additional loci show significant evidence for linkage: 2q33 and 11p15 to nephritis, 5p15 to polyarthritis, 5q14–15 to AITD, 11p13 to thrombocytopenia and discoid rash, 11q14 to hemolytic anemia and antinucleolar autoantibodies, 17p13 to vitiligo and 18q21 and 19p13 to anti-double-stranded DNA (dsDNA).[53–63] In nearly all of these studies, linkage analyses were carried out using phenotypes defined by fulfillment of the ACR criteria for classification of SLE to determine affected individuals. As a result, substantial phenotypic heterogeneity among individuals considered as affected is inherent in these studies. The challenge is to identify the specific genes responsible, define their effects and to dissect their mechanism of pathogenesis.

Given the extraordinary complexity of SLE, analysis of intermediate phenotypes provides an additional strategy for meeting this challenge. SLE is characterized by numerous serologic abnormalities, including elevated levels of cytokines and various autoantibodies, often directed against nuclear components. Interestingly, autoantibodies are present and show an increase in titer and a broadening of epitope specificities many years before the development of clinically apparent disease.[64–66] Certain autoantibodies are highly specific for SLE, such as anti-dsDNA, anti-P and anti-Sm, whereas others, such as anti-Ro/SSA and anti-La/SSB, are common in SLE and also occur in patients with other rheumatic diseases.[67] Furthermore, many of these autoantibodies have shown association with specific clinical manifestations and are thought to contribute directly to pathogenesis. Examples include associations of anti-dsDNA with nephritis and antibodies (Abs) against the ribonucleoproteins Ro/SSA and La/SSB with subacute cutaneous lupus, photosensitive rash and congenital heart block.[33] Autoantibodies and other immunologic abnormalities are also enriched in apparently healthy family members of SLE probands.[68]

Given these observations, we hypothesized that the serological abnormalities found in healthy family members of SLE patients reflect an underlying genetic susceptibility to autoimmunity, and could be useful traits to map genes contributing to autoimmune disease. We characterized intermediate phenotypes based on lupus-related autoantibody (LRA) profiles and performed genome-wide linkage analyses to identify autoimmunity predisposing loci in families enriched for lupus. We also developed a novel strategy for addressing the multiple testing issues inherent in the analysis of such complex traits, based on the mean allele sharing at each locus and adjustment of the significance level. Our data illustrate the potential genetic complexity of autoantibody production.

Results

Autoantibody frequencies in SLE patients, their family members and controls

We first characterized autoantibody profiles in a collection of 1506 individuals from 229 multiplex SLE pedigrees and 877 unrelated controls. Tables 1 and 2 show the frequencies of the autoantibodies measured in this study. The frequencies observed in SLE patients are within the ranges generally reported, taking into consideration the methods used to identify these autoantibodies.[33,69,70] In general, the frequencies we observed in our controls were also within the ranges commonly reported in general populations.[67]

The median ANA titer observed in the SLE affecteds was 1:1080. Median titers for both SLE blood relatives and controls were less than 1:40. The maximum titers detected in any individual were 1:2 000 000, 1:9720 and 1:1080 for SLE patients, blood relatives and controls, respectively. For controls with an ANA titer of ⩾1:120, we observed frequencies of 20.7% in female subjects and 13.5% in male subjects (Table 1). At ANA titers of ⩾1:360, the frequencies for female and male controls were 6 and 2%, respectively (data not shown). The frequencies of ANAs observed in controls and family members appear elevated compared to previous studies[71] and are likely to reflect the fact that this assay has been optimized to obtain high-level sensitivity. Although we cannot rule out an increased rate of subclinical infections, autoimmune features or family history in the controls, we consider these possibilities unlikely. The lower incidence of specific autoantibodies measured by different methods (see below) in the relatives and control group suggests that our ANA assay is sensitively measuring IgG Abs to histones, chromatin and/or single-stranded DNA.

As expected, lupus probands have higher autoantibody frequencies when compared to their unaffected blood relatives, with the difference for most autoantibodies being highly statistically significant (Table 1, $P<0.0001$). As shown in Table 1, blood relatives who do not satisfy the ACR criteria for SLE had significantly higher frequencies of most autoantibodies compared to unrelated controls: $P<0.0001$ for ANA⩾1:40 and anti-Ro/SSA, $P=0.0002$ for anti-nRNP

Table 1 Frequency of autoantibodies (%) in individuals from 229 multiplex SLE pedigrees and unrelated controls

Trait	*SLE*			*Blood relatives*			*Controls*			P-*values for*				
										Major group differences		*Gender differences within group*		
	All (n=*519*)	*Females* (n=*474*)	*Males* (n=*46*)	*All* (n=*819*)	*Females* (n=*521*)	*Males* (n=*298*)	*All* (n=*877*)	*Females* (n=*589*)	*Males* (n=*288*)	*SLE versus blood relatives*	*Blood relatives versus controls*	*SLE*	*Blood Relatives*	*Controls*
ANA≥1:40	98.8	98.7	97.8	42.6	47.6	33.9	31.5	36.2	21.9	<0.0001	<0.0001	NS	NS	<0.0001
ANA≥1:120	89.2	89.0	89.1	16.0	19.2	10.4	18.4	20.7	13.5	<0.0001	NS	NS	0.0007	0.0154
α-dsDNA	54.9	54.4	58.7	0.9	1.3	0.0	0.1	0.2	0.0	<0.0001	0.0330	NS	NS	NS
α-Ro/SSA	30.3	30.8	23.9	2.9	3.6	1.7	0.3	0.5	0.0	<0.0001	<0.0001	NS	NS	NS
α-La/SSB	13.7	13.5	15.2	0.7	1.2	0.0	0.0	0.0	0.0	<0.0001	0.0126	NS	NS	NC
α-nRNP	28.1	27.0	39.1	1.5	1.7	1.0	0.0	0.0	0.0	<0.0001	0.0002	NS	NS	NC
α-Sm	13.7	13.1	19.6	0.2	0.4	0.0	0.0	0.0	0.0	0.0194	NS	NS	NS	NC
α-P	1.5	1.5	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0007	NC	NS	NS	NC
α-IgG aPL	16.6	15.8	23.9	3.5	3.3	4.0	2.6	2.7	2.4	<0.0001	NS	NS	NS	NS
α-IgM aPL	8.3	7.8	13.0	2.9	2.7	3.4	1.1	1.0	1.4	<0.0001	0.0092	NS	NS	NS
RF≥1:40	27.7	29.1	13.0	19.8	21.9	16.1	NM	NM	NM	0.0011	NM	0.0237	NS	NM
RF≥1:80	9.6	10.1	4.3	7.2	8.6	4.7	<5%[a]	NM	NM	NS	NM	NS	0.0353	NM

Abbreviations: ANA, antinuclear antibody; aPL, antiphospholipid; dsDNA, double-stranded DNA; nRNP, nuclear ribonucleoprotein; NS, not significant; NM, not measured; NC, *P*-value not calculated owing to all cell counts of 0; RF, rheumatoid factor; SLE, systemic lupus erythematosus.[2] Tests of association were performed by two-sided Fisher's exact tests.
[a]Data not available, value from population frequency is reported for comparison.[67]

Table 2 Frequency of autoantibodies (%) in individuals from multiplex SLE pedigrees by ethnic background

Trait	*SLE*						*Blood relatives*						P-*values for*					
	European-American			*African-American*			*European-American*			*African-American*			*Differences among SLE affecteds*			*Differences among blood relatives*		
	All (n=*306*)	*Females* (n=*279*)	*Males* (n=*27*)	*All* (n=*162*)	*Females* (n=*149*)	*Males* (n=*13*)	*All* (n=*524*)	*Females* (n=*311*)	*Males* (n=*213*)	*All* (n=*205*)	*Females* (n=*152*)	*Males* (n=*53*)	*EA*[a] *versus AA*[b]	*EA*[a] *gender*	*AA*[b] *gender*	*EA*[a] *versus AA*[b]	*EA*[a] *gender*	*AA*[b] *gender*
ANA≥1:40	98.4	98.2	100.0	100.0	100.0	100.0	40.8	46.3	32.9	44.4	48.0	34.0	NS	NS	NS	NS	NS	NS
ANA≥1:120	87.6	87.5	88.9	93.2	92.6	100.0	15.5	19.6	9.4	14.6	15.1	13.2	NS	NS	NS	NS	0.0013	NS
α-dsDNA	51.0	49.5	66.7	61.7	63.1	46.2	0.8	1.3	0.0	1.5	2.0	0.0	NS	NS	NS	NS	NS	NS
α-Ro/SSA	29.7	29.7	29.6	34.0	34.9	23.1	2.3	2.6	1.9	4.9	5.9	1.9	NS	NS	NS	NS	NS	NS
α-La/SSB	13.7	13.6	14.8	13.6	13.4	15.4	0.8	1.3	0.0	1.0	1.3	0.0	NS	NS	NS	NS	NS	NS
α-nRNP	17.0	16.1	25.9	52.5	51.0	69.2	0.6	1.0	0.0	4.4	3.9	5.7	<0.0001	NS	NS	0.0009	NS	NS
α-Sm	8.8	8.6	11.1	24.1	23.5	30.8	0.2	0.3	0.0	0.5	0.7	0.0	<0.0001	NS	NS	NS	NS	NS
α-P	0.3	0.4	0.0	4.3	4.0	7.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0031	NS	NS	NS	NC	NC
α-IgG aPL	18.6	17.2	33.3	13.0	13.4	7.7	3.6	3.2	4.2	3.9	3.9	3.8	NS	NS	NS	NS	NS	NS
α-IgM aPL	10.5	10.4	11.1	2.5	2.0	7.7	3.6	3.2	4.2	0.5	0.7	0.0	0.0016	NS	NS	0.0206	NS	NS
RF≥1:40	26.8	28.0	14.8	27.2	28.2	15.4	17.0	19.3	13.6	27.3	27.0	28.3	NS	NS	NS	0.0028	NS	NS
RF≥1:80	9.8	10.4	3.7	9.3	9.4	7.7	6.3	8.0	3.8	9.8	10.5	7.5	NS	NS	NS	NS	NS	NS

For abbreviations, see Table 1 footnote.
Tests of association were performed by two-sided Fisher's exact test.
[a]European-American.
[b]African-American.

Table 3 Familial aggregation of autoantibodies ($n = 1506$ individuals with serology)

Trait	*Only SLE*					*SLE+relatives*				
	Sibpair counts			*Odds ratio*[a]	P-*value*	*Sibpair counts*			*Odds ratio*[a]	P-*value*
	++[b]	+−[c]	−−[d]			++[b]	+−[c]	−−[d]		
ANA⩾1:40	161	0	0	NC	NC	393	128	373	1.5	**0.0052**
ANA⩾1:120	118	2	41	0.6	0.7609	193	292	409	1.4	**0.0132**
α-dsDNA	50	37	74	1.4	0.1611	50	569	275	1.5	**0.0152**
α-Ro/SSA	21	79	61	1.8	**0.0467**	28	665	201	1.9	**0.0049**
α-La/SSB	9	120	32	4.4	**0.0014**	10	783	101	3.1	**0.0012**
α-nRNP	14	99	48	2.5	**0.0134**	19	724	151	2.4	**0.0008**
α-Sm	4	131	26	3.2	**0.0339**	4	815	75	2.3	0.0608
α-P	0	157	4	NC	NC	0	833	11	NC	NC
α-IgG aPL	5	111	45	1.1	0.4169	7	756	131	1.2	0.3014
α-IgM aPL	1	138	22	1.2	0.4351	4	822	68	2.9	**0.0284**
RF⩾1:40	23	91	47	3.9	**0.0001**	49	573	272	1.5	**0.0141**
RF⩾1:80	2	137	22	2.4	0.1490	3	760	131	0.5	0.8493

For abbreviations, see Table 1 footnote.
Sibpair counts were used to estimate each trait's genetic component, as measured by its familial aggregation, according to Olson *et al.*[73] Bold results highlight significant *P*-values ($P<0.05$), indicating evidence for familial aggregation. NC = *P*-values and odds ratio could not be calculated for anti-P, nor for ANA⩾1:40 in the SLE affecteds group, owing to the absence of concordant sibling pairs for these traits.
[a]Given by the inverse of $(b^2-b)/(4ac)$.
[b]Number of sibling pairs that are concordant affected for each trait.
[c]Number of sibling pairs that are discordant for each trait.
[d]Number of sibling pairs that are concordant unaffected for each trait.

(nuclear ribonucleoprotein), $P = 0.0092$ for anti-IgM aPL (antiphospholipid), $P = 0.0126$ for anti-La/SSB and $P = 0.0330$ for anti-dsDNA. The increased frequencies of autoantibodies in relatives of SLE patients compared to unrelated controls support a predisposition for autoantibody production in these families.

As shown in Table 1, significant gender differences were seen for positive ANA titers among blood relatives ($P = 0.0007$ for ANA⩾1:120) and controls ($P<0.0001$ for ANA⩾1:40, $P = 0.0154$ for ANA⩾1:120), with frequencies higher in female subjects for both groups. However, the frequencies of ANAs among SLE female and male patients were similar. Rheumatoid factor (RF) was the only other autoantibody specificity in which we observed significant differences related to gender. The frequency of RF was significantly higher in female patients than male patients with SLE ($P = 0.0237$ for RF⩾1:40). Similarly, the frequency of RF was also higher in female blood relatives when compared to male blood relatives ($P<0.0353$ for RF⩾1:80).

Significant ethnic differences were observed for some autoantibodies (Table 2). Within the SLE patient group, anti-nRNP ($P<0.0001$) and anti-Sm ($P<0.0001$) were more frequent in African-American patients, as previously described.[33,72] We also found an increased frequency of anti-P Abs ($P = 0.0031$) in African-American SLE patients. Anti-IgM aPL Abs were more frequent in European-Americans ($P = 0.0016$). In the blood relatives, anti-nRNP ($P = 0.0009$) and RF⩾1:40 ($P = 0.0028$) frequencies were enriched in the African-American pedigrees, whereas anti-IgM aPL Abs ($P = 0.0206$) were significantly more common in European-Americans. When the sample was stratified by both gender and ethnic background, only ANA⩾1:120 was higher in European-American female blood relative ($P = 0.0013$).

Autoantibody-related phenotypes
In order to assess the potential for contribution of a genetic component to each autoantibody trait, we measured familial aggregation of a given trait among siblings as described by Olson *et al.*[73] Evidence for familial aggregation in the SLE affecteds was found for RF⩾1:40 ($P = 0.0001$), anti-La/SSB ($P = 0.0014$), anti-nRNP ($P = 0.0134$), anti-Sm ($P = 0.0339$) and anti-Ro/SSA ($P = 0.0467$) (Table 3). When we included the SLE blood relatives in the analysis, evidence for familial aggregation of anti-Ro/SSA, anti-La/SSB and anti-nRNP increased in statistical significance. Evidence for familial aggregation was also observed for ANAs, anti-dsDNA and anti-IgM aPL when both SLE affecteds and blood relatives were evaluated.

Given the number of traits that we had available for linkage analyses, we explored patterns of correlations among autoantibodies to determine if we could reduce the number of variables. We first assessed pairwise correlations between the various autoantibody specificities and generated a correlation matrix (Table 4), using our sample of 1506 individuals with available serology data. The ANA⩾1:120 titer was correlated with the presence of anti-dsDNA ($r = 0.6$, $P<0.0001$) and, to a lesser extent, RNA–protein autoantibodies (anti-Ro/SSA, anti-La/SSB, anti-nRNP and anti-Sm, $P<0.05$). This is expected, as the ANA test is known to detect a variety of autoantibodies, including the ones in which the correlations were significant. Of the remaining specific autoantibodies, the two most significant correlations were observed between anti-Ro/SSA and anti-La/SSB ($r = 0.6$, $P<0.0001$), and between anti-nRNP and anti-Sm ($r = 0.5$, $P<0.0001$), consistent with previously reported associations.[67]

A principal component (PC) analysis was performed to identify a subset of uncorrelated linear combinations

Table 4 Correlation matrix for pairwise comparisons of autoantibodies plus race ($n = 1506$ individuals)

Trait	*ANA⩾1:40*	*ANA⩾1:120*	*α-dsDNA*	*α-Ro/SSA*	*α-La/SSB*	*α-nRNP*	*α-Sm*	*α-P*	*α-IgG aPL*	*α-IgM aPL*	*RF⩾1:40*	*RF⩾1:80*	*Race*
ANA⩾1:40	1.00	**0.66***	**0.40***	**0.29***	**0.18***	**0.27***	**0.18***	**0.06**	**0.17***	**0.11***	**0.13***	0.02	0.037
ANA⩾1:120		1.00	**0.55***	**0.40***	**0.27***	**0.39***	**0.26***	**0.09**	**0.20***	**0.14***	**0.14***	0.05	**0.05**
α-dsDNA			1.00	**0.39***	**0.20***	**0.37***	**0.33***	**0.10***	**0.25***	**0.11***	**0.07***	0.01	**0.08***
α-Ro/SSA				1.00	**0.55***	**0.27***	**0.20***	0.00	**0.14***	**0.07***	**0.12***	**0.08***	**0.08***
α-La/SSB					1.00	**0.11***	**0.13***	0.02	**0.11***	0.05	**0.12***	**0.09**	0.02
α-nRNP						1.00	**0.53***	**0.15***	**0.09**	**0.06**	**0.08***	0.04	**0.22***
α-Sm							1.00	**0.15***	**0.06**	0.02	0.04	−0.01	**0.12***
α-P								1.00	0.01	0.03	0.03	0.05	**0.09**
α-IgG aPL									1.00	**0.27***	**0.08***	**0.05**	0.01
α-IgM aPL										1.00	**0.13***	**0.08***	**−0.09**
RF⩾1:40											1.00	**0.55***	**0.06**
RF⩾1:80												1.00	0.03
Race													1.00

For abbreviations, see Table 1 footnote.
Correlation coefficients with significant *P*-values ($P<0.05$) are shown in bold. *P*-values were obtained by a Fisher's r to z test.
*$P<0.01$, **$P<0.001$, ***$P<0.0001$.

Table 5 Coefficients derived from principal component (PC) analysis of autoantibody traits ($n = 1506$ individuals)

	PC												
Trait	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
ANA⩾1:40	0.67	−0.05	−0.20	−0.09	−0.50	0.02	0.21	0.12	−0.30	−0.05	−0.06	0.12	−0.29
ANA⩾1:120	0.79	−0.07	−0.16	−0.05	−0.36	0.04	0.02	0.07	−0.03	0.03	−0.06	−0.07	0.42
α-dsDNA	0.72	−0.16	−0.09	−0.11	−0.09	−0.03	0.01	−0.20	0.57	−0.16	−0.04	−0.14	−0.14
α-Ro/SSA	0.65	0.05	−0.21	0.49	0.23	0.025	−0.02	0.04	0.15	0.21	0.15	0.39	0.01
α-La/SSB	0.48	0.15	−0.25	0.59	0.37	0.17	0.03	0.05	−0.19	−0.14	−0.11	−0.32	−0.05
α-nRNP	0.63	−0.26	0.40	−0.12	0.06	−0.10	−0.29	0.05	−0.10	0.42	0.14	−0.23	−0.08
α-Sm	0.52	−0.31	0.40	−0.10	0.17	0.03	−0.48	−0.08	−0.17	−0.34	−0.13	0.19	0.05
α-P	0.19	−0.09	0.40	−0.25	0.23	0.69	0.45	−0.05	0.01	0.02	0.05	0.04	0.00
α-IgG aPL	0.35	0.17	−0.34	−0.43	0.37	−0.29	0.19	−0.50	−0.20	0.04	0.03	0.02	0.02
α-IgM aPL	0.23	0.30	−0.31	−0.56	0.35	0.01	−0.12	0.56	0.09	−0.02	−0.05	0.01	−0.01
RF⩾1:40	0.27	0.77	0.29	0.00	−0.15	−0.03	−0.06	−0.01	−0.03	−0.21	0.42	−0.05	0.02
RF⩾1:80	0.17	0.79	0.34	0.04	−0.09	0.03	−0.07	−0.12	0.06	0.18	−0.41	0.05	−0.02
Race	0.18	−0.12	0.52	0.13	0.19	−0.56	0.50	0.23	0.03	−0.10	−0.06	0.02	0.02
Proportion of variance	0.25	0.12	0.10	0.09	0.08	0.07	0.07	0.06	0.04	0.04	0.03	0.03	0.02
Cumulative proportion of variance	0.25	0.37	0.47	0.57	0.64	0.71	0.78	0.84	0.88	0.92	0.95	0.98	1.00

For abbreviations, see Table 1 footnote.

of the autoantibodies (Table 5). The first eight PCs accounted for 80% of the phenotypic variance, indicating that a reduction of the number of original variables was not well achieved. The first PC accounted for ~25% of the total variation and had high coefficients for Abs that target nuclear antigens. The second PC accounted for an additional 12% of the total variation and revealed high scores for individuals with the RF autoantibody traits. The third PC accounted for ~10% of the total variation. Individuals with highest scores for this PC were African-American with anti-nRNP and anti-Sm (consistent with the pairwise correlation and previous reports in the literature[67]), as well as anti-P Abs. These individuals tended not to have aPL, anti-Ro/SSA or anti-La/SSB Abs. In the fourth PC, individuals with anti-Ro/SSA and anti-La/SSB, consistent with the pairwise correlation analyses and previous findings reported in the literature,[67] but without aPL had the highest scores. The following PC was similar, with the exception of a positive correlation between anti-Ro/SSA, anti-La/SSB and aPL Abs. Individuals with anti-P scored the highest for the sixth and seventh PCs, and were European-Americans or African-Americans, respectively. The eighth PC was strongly weighted in individuals with anti-IgM aPL and without anti-IgG aPL. The remaining PCs each accounted for less than 5% of the total variation and reflected single traits of anti-dsDNA (PC9), anti-nRNP (PC10), RF⩾1:40 (PC11), anti-Ro/SSA (PC12) and ANA⩾1:120 (PC13). Overall, these results indicate that the production of most autoantibody specificities is independently controlled, with some weak clustering such as that observed with anti-Ro/SSA and anti-La/SSB, or anti-Sm and anti-nRNP. Thus, we did not perform linkage analysis on the PC traits.

Genome scans using autoantibody profiles

General approach. Based on our characterization of autoantibody traits in the 229 SLE pedigrees, we performed linkage analyses in 227 multiplex SLE pedigrees for which genome-wide microsatellite marker

data were available as previously described.[38,41] The autoimmunity traits used to classify individuals as affected for linkage analysis were defined by the presence of either (1) single autoantibody specificities ('specific autoantibody traits'), (2) combinations of autoantibodies with evidence for association ('associated autoantibody traits') or (3) general autoantibody production ('composite autoantibody traits').

Given our objective to identify loci involved in autoantibody production through an approach involving multiple genome scans, we chose a robust regression method with increased power to detect such loci, while recognizing a potential for increased Type I error. We used the revised Haseman–Elston regression algorithm for all linkage analyses.[74] In order to control for a potential increase in false positives, we carefully evaluated allele sharing patterns as illustrated in Figure 1 for each linkage peak reaching an asymptotic *P*-value of 1.7×10^{-3}.[35] A total of four loci were identified in which the mean proportion of allele sharing for the discordant sib pairs was above 0.5 and were immediately rejected as likely false positives. The remaining 49 loci reaching an asymptotic *P*-value of 1.7×10^{-3} are presented in Table 6. For each of these loci, we observed a statistically significant increase in mean allele sharing in the concordant pairs (>0.5) and decrease in the discordant pairs (<0.5). This pattern is consistent with true positive linkage and such loci were considered potentially important.

Next, we estimated empirical *P*-values and determined an adjustment factor to account for multiple testing inherent in our study. To estimate an adjustment factor, we performed permutation testing on our five most statistically significant linkage peaks. The average difference between asymptotic and empirical *P*-values was 2 orders of magnitude. Based on these results, we increased the initial asymptotic *P*-value by 2 orders of magnitude for each of the linkage peaks shown in Table 6. All *P*-values reported herein are asymptotic unless explicitly referred to as 'adjusted' *P*-values. Following adjustment, four loci retained statistically significant evidence for linkage at $P\leqslant1.7\times10^{-3}$ (Table 6). Detailed evaluations of allele sharing patterns for these four loci are presented in Table 7.

Specific autoantibody traits. We used each individual autoantibody trait (anti-dsDNA, anti-Ro/SSA, anti-La/SSB, anti-nRNP, anti-Sm, anti-IgG aPL, anti-IgM aPL, RF$\geqslant$1:40 and RF$\geqslant$1:80) as the dependent variable in the Haseman–Elston regression analyses, with the exception of anti-P owing to its extremely low frequency. Some of these autoantibody specificities were more prevalent (anti-dsDNA, anti-Ro/SSA and anti-nRNP), and others appeared to have a stronger genetic component as determined by the familial aggregation analyses (anti-La/SSB, anti-nRNP, anti-Ro/SSA and anti-Sm). Table 6 summarizes the results in which the evidence for linkage was at least suggestive before adjustment of the *P*-values.[35] Each autoantibody specificity showed at least suggestive levels of evidence for linkage to multiple chromosomal regions.

Significant evidence was observed for anti-IgM aPL on chromosome 13q14 with both the European-American subset ($n=131$ pedigrees, $P=2.3\times10^{-6}$) and all 227 pedigrees ($P=2.7\times10^{-5}$). Using the presence of anti-La/SSB Abs as the trait, we found significant evidence for

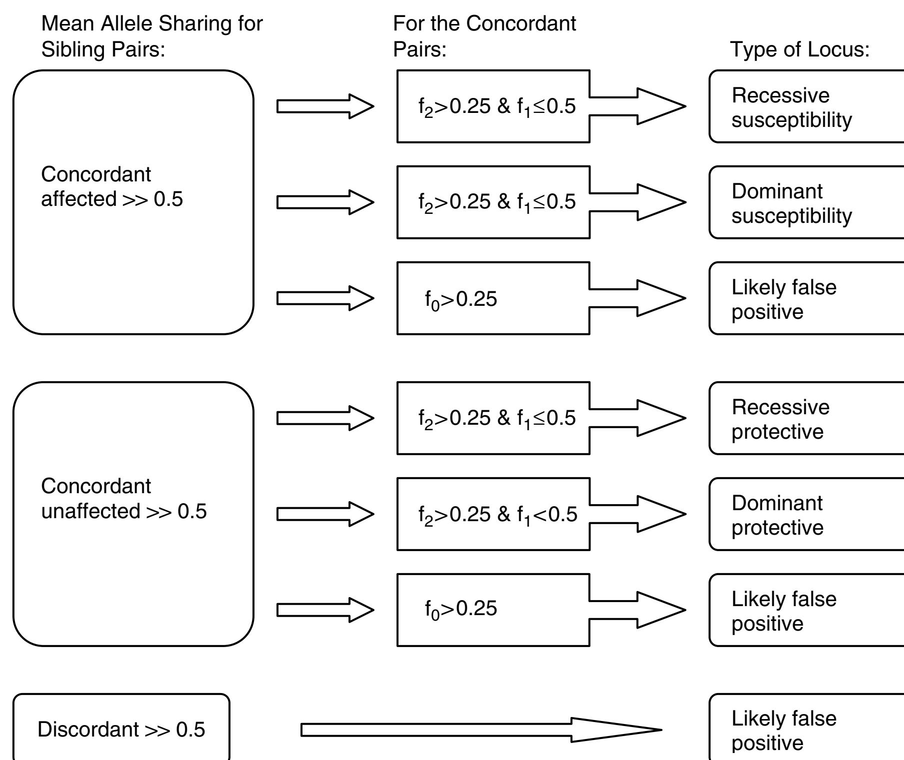

Figure 1 Flow chart for accepting a locus as a likely true positive based on the allele sharing at that locus. f_0 represents the proportion of sibpairs sharing 0 alleles identical-by-descent (IBD), f_1 the proportion of sibpairs sharing one allele IBD and f_2 the proportion of sibpairs sharing two alleles IBD. $\gg$ indicates 'significantly greater than'.

Table 6 Summary of genome scan results using autoantibody traits in 227 pedigrees multiplex for SLE

Chr[a]	*Markers*	*Band*	*Asymptotic P-value*													
			Specific autoantibody traits								*Associated autoantibody traits*		*Composite autoantibody traits*			
			dsDNA	*Ro/SSA*	*La/SSB*	*Sm*	*nRNP*	*IgG aPL*	*IgM aPL*	*RF≥1:40*	*nRNP/Sm*	*Ro/La*	*LRA*	*ANA≥ 1:120*	*ANA1–4*	*ANA rel*
1	D1S1622–D1S3721	p35.3–p34.2						0.00017								
	D1S1665–D1S551	p31.1–p31.1	*0.00108*													
	D1S1677–D1S2141	q23.3–q41	*0.00159*							**0.0016** 0.00039					*0.00023*	
	D1S549–D1S3462	q41–q42.2													*9.60×10^{-5}*	
2	D2S441	p14					*0.00047*									
	D2S1777	p12								**2.27×10^{-5}** 0.00013						
	D2S442	q21.3						*0.00077*								
	D2S1391–D2S1384	q32.1–q33.3				*1.91×10^{-5}*					*3.07×10^{-5}*			*0.00022*		
3	D3S2432	p24.1				**5.60×10^{-5}** *0.00089*										
	D3S3023–D3S1764	q13.33–q23			*1.99×10^{-8}* ◆ ◆ **0.00024**											
	D3S1744–D3S1763	q24–q26.1				**0.00045**										
	D3S3053–D3S2398	q26.31–q28				**0.00012**					*3.46×10^{-8}* **2.49×10^{-5}**					
4	D4S2366	p16.1			0.00087							0.00165				
	D4S2639–D4S2632	p15.32–p15.1										0.00052				
	D4S2367	q13.2			0.00035											
	D4S3243	q21.21				*0.00042*										
	D4S2394–D4S1644	q28.2–q31.21							**2.51×10^{-4}** 0.00010		*0.00029*					
	D4S1625	q31.21					*0.00094*									
	D4S2417–D4S408	q34.3–q35.1		0.00025								3.42×10^{-6} ◆ **0.00115**				
5	D5S2505–D5S807	p15.32–p15.31			**0.00143**											
	D5S817	p15.2													0.00063	
	D5S1470	p13.3					**0.00095**									
	D5S1453–D5S1505	q21.3–q23.1											**0.00104** 0.00031			
6	F13a1–D6S2434	p25.1–p23				**0.00027**					**0.00157** *0.00016*					
7	D7S513–D7S1802	p21.3–p21.1								**0.00060**						
	D7S1808	p15.1				*0.00112*										
	D7S817–D7S1818	p14.3–p12.3	**0.00035** 0.00158													
	D7S2204	q21.11											0.00046			
	D7S1799–D7S1842	q22.2–q31.32		0.00057								0.00013				
8	D8S264	p23.3			0.00144											
	D8S2324–D8S1119	q21.11–q21.3			0.00148						*0.00018*					
	D8S1132–D8S1179	q23.1–q24.13	0.00056													
	D8S1128	q24.21	0.00054													
9	D9S2169–D9S925	p24.1–p22.2			*3.73×10^{-5}*											
10	D10S1435	p15.3		0.00023												
	D10S189–D10S2325	p14–p13		**0.00087** 0.00091	*3.21×10^{-5}* **0.00033**											
11	D11S2002–D11S2000	q14.1–q22.3			*0.00097*			*0.00115*								
13	D13S325–D13S800	q14.11–q22.1					*6.37×10^{-5}*		2.27×10^{-6} ◆ **2.67×10^{-5}**							
14	D14S588	q24.1			0.00125											
	D14S1434	q32.13						**0.00019**								

Table 6 Continued

Chr[a]	Markers	Band	Asymptotic P-value													
			Specific autoantibody traits								*Associated autoantibody traits*		*Composite autoantibody traits*			
			dsDNA	*Ro/SSA*	*La/SSB*	*Sm*	*nRNP*	*IgG aPL*	*IgM aPL*	*RF ≥ 1:40*	*nRNP/Sm*	*Ro/La*	*LRA*	*ANA ≥ 1:120*	*ANA1–4*	*ANA rel*
15	actc	q14			0.00066							0.00052				
	D15S1507–D15S211	q22.31–q25.1					*0.00152*									**0.00049**
16	D16S753	p11.2			*0.00029*											
	D16S516	q23.1					0.00109				**0.00048** 0.00145					
17	D17S1294–D17S1299	q11.2–q21.2				**0.00068** *0.00044*					*0.00059*					
20	D20S482	p13			*0.00026*											
	D20S470–D20S478	p21.1–q11.23				*0.00014*										
	D20S481–D20S480	q13.12–q13.2					**8.20×10^{-5}**	*0.00040*								
21	D21S2052–D21S1440	q21.3–q22.13				*0.00035*			**0.000280** 0.000584	*0.00055*						

For abbreviations, see Table 1 footnote.
Pedigrees used for linkage: bold font = all; italic font = African-American; normal font = European-American. Diamonds represent the loci that meet evidence for linkage after *P*-value adjustment: suggestive (one diamond) or significant (two diamonds).
[a]Chromosome.

linkage ($P \leqslant 4.9 \times 10^{-5}$) to chromosomes 3q21 ($P = 1.9 \times 10^{-8}$), 9p23 ($P = 3.7 \times 10^{-5}$) and to 10p13 ($P = 3.2 \times 10^{-5}$), in the subset of 73 African-American pedigrees. The effects on chromosomes 13q14 and 3q21 are novel and have not been previously reported in linkage studies of SLE. The locus on 9p23 has been previously reported in linkage studies of SLE using this same data set[38] as well as an independent collection of SLE pedigrees.[40] Suggestive evidence for linkage of SLE to chromosome 10p13 was also previously reported in an independent data set.[49]

After *P*-value adjustment, the evidence for linkage between anti-La/SSB and chromosome 3q21 remained significant (adjusted $P = 1.9 \times 10^{-6}$). The significance level for linkage of anti-IgM aPL to 13q14 in European-American pedigrees was suggestive (adjusted $P = 2.3 \times 10^{-4}$). We found the effects at 3q21 and at 13q14 to most likely represent recessive susceptibility loci (Figure 1 and 2 and Table 7).

Associated autoantibody traits. We combined the presence of multiple autoantibodies into two single traits based on our data showing that certain specific autoantibodies were correlated (Tables 4 and 5) and based upon their physical association.[75,76] These two traits included (1) the presence of anti-nRNP and/or anti-Sm ('anti-nRNP/Sm'), as well as (2) the presence of anti-Ro/SSA and/or anti-La/SSB ('anti-Ro/La').

Significant evidence for linkage to anti-nRNP/Sm was observed on chromosome 3q27 in African-American pedigrees ($P = 3.5 \times 10^{-8}$) (Table 6). After *P*-value adjustment, this result remained above the threshold for significant linkage. Minimal evidence for linkage of SLE to chromosome 3q27 has previously been described in a small cohort of 12 Italian pedigrees (LOD = 2.00).[43]

Significant evidence was also found on chromosome 4q34.3–35.1 with the anti-Ro/La trait in the European-American pedigrees ($P = 3.4 \times 10^{-6}$). After *P*-value adjustment, this locus still met criteria for suggestive linkage. This linkage effect is novel and has not been previously reported in other SLE studies.

For the two effects on chromosomes 3q27 and 4q34–35, examination of allele sharing patterns led us to conclude that each appears to be recessive susceptibility loci (Figures 1 and Table 7). Other loci with suggestive evidence for linkage became insignificant after *P*-value adjustment (Table 6).

Composite autoantibody traits. The ANA serological test is commonly used clinically to screen for autoantibody production against extractable nuclear antigens (ENAs), and detects a variety of specificities. We used several approaches to classify this general autoimmunity trait for linkage analysis, including (1) as a dichotomous trait (individuals were classified as affected based on a threshold defined by ANA titer ('ANA ≥ 1:40', 'ANA ≥ 1:120')), (2) as an ordinal trait where affecteds were classified proportionately to their titer ('ANA1–4') and (3) limiting the analysis to consider only relatives of SLE affecteds with an ANA titer ≥ 1:120 ('ANArel'). We did not find significant evidence for linkage to any of the composite traits based on ANA data and as shown in Table 6, only five loci were identified where evidence for linkage was at least suggestive ($P \leqslant 1.7 \times 10^{-3}$). Interestingly, with the exception of the 15q22–25 locus, each of

Table 7 Estimates of mean allele sharing IBD for each sibpair type at the most significant linkage peaks

Trait	*Markers*	p_i[a]			f_0[b]			f_1[c]			F_2[d]			*Type of locus*
		0[e]	*1*[f]	*2*[g]	*0*[e]	*1*[f]	*2*[g]	*0*[e]	*1*[f]	*2*[g]	*0*[e]	*1*[f]	*2*[g]	
α-La/SSB	D3S3023-D3S1764	0.49	0.37	0.93	0.26	0.41	0.01	0.51	0.44	0.12	0.23	0.15	0.87	Recessive susceptibility
α-nRNP/Sm	D3S3053-D3S2398	0.52	0.42	0.55	0.23	0.34	0.17	0.50	0.47	0.56	0.27	0.19	0.27	Recessive susceptibility
α-Ro/La	D4S2417-D4S408	0.51	0.43	0.61	0.23	0.33	0.13	0.51	0.48	0.50	0.26	0.19	0.36	Recessive susceptibility
α-IgM aPL	D13S325-D13S800	0.49	0.46	0.64	0.26	0.31	0.14	0.51	0.46	0.45	0.23	0.23	0.41	Recessive susceptibility

For abbreviations, see Table 1 footnote.
[a]Estimate of the mean proportion of alleles shared identical-by-descent (IBD).
[b]Estimate of the proportion of sibpairs sharing 0 alleles IBD.
[c]Estimate of the proportion of sibpairs sharing one allele IBD.
[d]Estimates of the proportion of sibpairs sharing two alleles IBD.
[e]Concordantly unaffected sibpairs.
[f]Discordant sibpairs.
[g]Concordantly affected sibpairs.

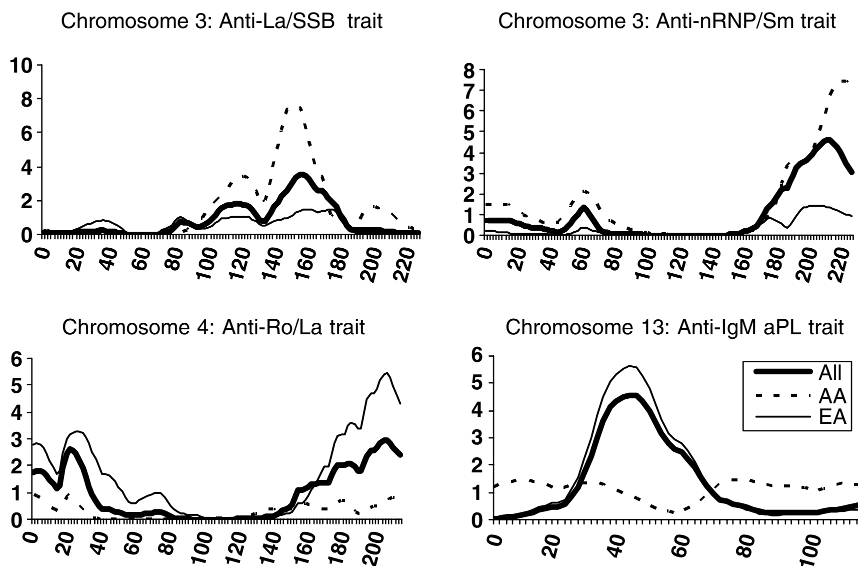

Figure 2 Results of the linkage analysis in the most significant peaks for all (All), African-American (AA) and European-American (EA) pedigrees. The Y axis indicates the $-\log(P$ value) and the X axis indicates the chromosomal length in cM of each chromosome.

these loci has been previously identified in genome scans of these same pedigrees and demonstrated stronger evidence for linkage when affecteds were classified based on criteria for SLE.[38,41] Thus, these loci may indeed be important for the overall phenotype of SLE, but appear to be less related to autoantibody production in general.

Based on the presence of autoantibodies that are common or specific for SLE, we defined a general lupus-related autoimmunity (LRA) trait. Individuals who were positive for at least one of the following were classified as affected for the LRA trait: ANA titer $\geqslant$1:120, anti-dsDNA, anti-Ro/SSA, anti-La/SSB, anti-nRNP, anti-Sm, anti-P, anti-IgG or anti-IgM aPL $>$20 units and RF titer$\geqslant$1:80. This reclassification of individuals in the SLE pedigrees with a new phenotype increased the number of affecteds from 511 with SLE to 850 with the LRA trait.

Similar to our findings for the ANA traits, we did not find significant evidence for linkage to any of the composite traits based on the LRA classification (Table 6). Only two loci with evidence for linkage based on asymptotic P-values reached a suggestive significance level (chromosome 5q21.3–q23.1, $P=3.2\times10^{-4}$ and 7q21.11, $P=4.6\times10^{-4}$). Evidence for linkage of SLE to 7q21 has been previously reported in an independent data set.[49]

Discussion

These studies represent the most extensive effort to date to map potential autoimmune disease predisposing loci using traits related to autoantibody production in SLE multiplex pedigrees. Bias and co-workers[77,78] were the first to develop a segregation model for autoimmunity. They hypothesized autosomal dominant inheritance of a major autoimmune locus based on a trait defined by an autoantibody profile, with additional genes conferring

specificity to the autoimmune disease. Whereas the classic study by Bias *et al.*[78] suggested the presence of a major dominant locus contributing to development of autoimmunity, our results support a more complex genetic architecture and the involvement of many genes in autoantibody production and the development of autoimmune disease.

Inflammatory processes in SLE are complex and involve lymphocyte activation, production of autoantibodies, formation of immune complexes and activation of complement pathways that culminate in causing organ damage.[79] The initiation and dysregulation of immune activation in SLE is thought to involve B-cell hyperactivity, aberrant T-cell signaling, defects in apoptosis and patterns of differential cytokine production and activity. Recent work has shown that the regulation of innate immune mechanisms are likely to play a major role in disease pathogenesis as well, involving the activity of toll-like receptors and genes known to be inducible by interferons (IFNs).[80] Thus, the potential for complex genetics underlying autoantibody production as demonstrated by our linkage studies is not unexpected.

Our observation that SLE patients and their blood relatives have higher frequencies of autoantibodies than controls supports a predisposition for autoantibody production in these families. Given that lupus-related autoantibodies progressively accumulate before the onset of SLE,[64] unaffected individuals in these families may carry genetic polymorphisms that confer a predisposition to autoimmunity that can be the first step toward pathogenesis in any of several autoimmune diseases. We assessed the potential genetic component of each autoantibody and found evidence of familial aggregation for anti-La/SSB, anti-Ro/SSA, anti-nRNP, anti-Sm and RF$\geqslant$1:40 among the individuals meeting criteria for SLE. We also found evidence of familial aggregation for ANAs, anti-dsDNA and anti-IgM aPL Abs when SLE patients plus their relatives were included in the analyses. Previous studies examining autoantibody profiles in family members have described variable results with respect to aggregation within families, but in general support a genetic contribution to autoantibody production.[45,81,82] Clearly, ethnic differences, stochastic variation in the relatively small samples reported to date and differences in sensitivity of autoantibody measurements may contribute to variability in familial aggregation measures of various autoantibody specificities. We cannot exclude potential contribution of shared environment; however, studies have shown that the profile of autoantibody specificities are virtually identical among monozygotic twins regardless of concordance for SLE[83] and suggest a major role for genetic influence on autoantibody production.

For our linkage analyses, we chose the revised Haseman–Elston regression method[74] as a powerful and robust but somewhat non-conservative approach to identify potentially important loci specific for autoantibody production in our SLE pedigrees. Our approach was to first use less stringent criteria for significance in order to maximize detection of possible autoantibody-predisposing loci. Out of 53 initial loci detected, four loci were removed based on allele sharing patterns that were inconsistent with true linkage. Of the remaining 49 loci, we identified four that withstood a filtering approach consisting of adjusting *P*-values and examining allele sharing patterns, leading us to consider these chromosomal regions as most likely to contain important loci involved in autoantibody production. Certainly, as with linkage analyses of any complex trait, replication in additional data sets will be important for confirming any loci identified and described herein. The most significant evidence for linkage was found at chromosomes 3q21 with the presence of anti-La/SSB (adjusted $P=1.99\times10^{-6}$) in African-American pedigrees, 3q27 with anti-nRNP/Sm (adjusted $P=3.46\times10^{-6}$) in African-American pedigrees, 4q34–q35 for the presence of anti-Ro/La autoantibodies (adjusted $P=3.42\times10^{-4}$) in European-American pedigrees and 13q14 with IgM aPL autoantibodies ($P=2.27\times10^{-3}$) in European-American pedigrees. By examining the allele sharing distribution, all four loci were consistent with a recessive mode of inheritance.

Clustering of multiple autoimmune diseases within SLE families suggests that certain susceptibility loci may predispose to more than one disease.[4–6] We hypothesized that the loci identified in this study may also contribute to susceptibility of other autoimmune diseases, particularly as autoantibody production is not specific to SLE. To explore the potential overlap, we compared our results to the results obtained in reported linkage analyses of other related human autoimmune diseases. We considered regions with evidence for linkage in other diseases that were mapped within 5 Mb of our linkage peaks as potentially overlapping loci. Diseases in which we have searched for overlapping loci include RA, T1D/insulin-dependent diabetes mellitus (IDDM), psoriasis, inflammatory bowel disease (IBD), MS and AITD. We found that nearly all of the 49 loci we have identified do overlap with linkages previously described for at least one of several autoimmune diseases.

The linkage observed with the presence of anti-La/SSB autoantibodies on chromosome 3q21 overlaps with linkages also observed for RA,[84] psoriasis,[85,86] MS[87] and T1D,[88,89] including the IDDM9 locus,[90] and thus may represent a locus that is important for multiple autoimmune diseases. Interestingly, the hematopoietic cell-specific Lyn substrate 1 (*HS1*) gene lies in this interval and has been associated with SLE.[91] This gene plays an important role in lymphocyte signaling through the B-cell receptor (BCR). Polymorphisms identified in SLE patients are thought to accelerate BCR-mediated signaling and contribute to increased rates of apoptosis.

Overlapping evidence for linkage to the anti-nRNP/Sm interval on chromosome 3q27 has been observed in IBD[92] and AITD.[93] No specific gene in this interval has yet been shown to be associated with an autoimmune disease through genetic studies. However, several interesting candidate genes are located in this region and include *TNFSF10/TRAIL* and *BCL6*. TRAIL is a tumor necrosis factor superfamily member that can trigger rapid apoptosis in multiple cell types. Differences in expression and activity of TRAIL have been observed in patients with SLE, Sjogren's syndrome and thyroid disease.[94–97] A polymorphism in exon 5 of *TRAIL* has been associated with MS,[98] but, to our knowledge, has not been evaluated for genetic association to other autoimmune diseases. *BCL6* is also an attractive candidate for SLE. This gene codes for a transcriptional

repressor that plays an important role in controlling lymphoid development and function.[99] *BCL6* is frequently involved in translocations that contribute to non-Hodgkin's B-cell lymphomas and involve juxtapositioning of immunoglobulin genes. Somatic hypermutation has also been observed and may contribute to increased expression of *BCL6*, which may in turn promote differentiation of germinal center B cells into Ab-secreting plasma cells.[99]

Modest evidence for linkage near the anti-IgM aPL interval on chromosome 13q14 has been previously reported for IDDM.[100] Of the potential candidate genes in this region, none has yet been shown to be associated with an autoimmune disorder. However, deletions at 13q14 are common in B-cell chronic lymphocytic leukemia and multiple myeloma and may involve loss of one or more tumor suppressor genes.[101] Whether or not the same genes could contribute to dysregulation of lymphocyte development in SLE will require further investigation.

The locus on chromosome 4q34–35 that provided evidence for linkage to the presence of anti-Ro/SSA or anti-La/SSB autoantibodies overlaps with the PSOR3 locus identified in psoriasis,[102] one of the autoimmunity clusters reported by Becker *et al*.[4] and linkage results from previous studies of SLE.[38,46] A whole-genome association study of MS has also found significant evidence for association of this same region.[103] In SLE, Rao *et al*.[46] used a PC approach to define traits based on combinations of clinical manifestations in SLE patients and reported significant evidence for linkage to this interval with a PC in which dermatologic, arthritic and cardiopulmonary traits were common among the affected individuals. Ongoing efforts to map the psoriasis gene, *PSOR3*, in this region have shown association between variants of the interferon-regulatory factor 2 (*IRF2*) gene and this disorder.[104] Evidence for association of *IRF2* has also been reported with atopic dermatitis.[105] IRF2 plays a repressive role in transcriptional regulation of multiple genes known to be induced by IFNs.[106,107] Mice lacking IRF2 are hyper-responsive to IFN-α/β, dramatically overexpress genes induced by type I IFN and develop inflammatory dermatological manifestations involving $CD8^+$ T cells.[108] Numerous lines of evidence suggest that pathways influenced by IFNs are dysregulated in multiple autoimmune diseases, including SLE, PS, AITD, Crohn's, RA and spondyloarthropathy,[109–113] supporting a potential role for genes such as *IRF2*.[17,114]

We did not observe significant linkage to the HLA region as one might expect, because HLA alleles have previously been associated with particular autoantibody specificities.[115] However, multiple risk haplotypes defined by *DR2*, *DR3* and *DR8* alleles are associated with SLE and most likely make it difficult to detect significant evidence for linkage using allele sharing methods among sibpairs as in our study.[116,117]

Autoantibody production that contributes to SLE and perhaps other related autoimmune diseases appears to be a genetically complex trait. Our data are consistent with the hypothesis that traits defined by individual autoantibody specificities are influenced by independent loci. Our study indicates that autoantibody production is not a simple monogenic trait, but rather is the result of an intricate interplay between multiple predisposing genes and potential environmental triggers. The complexity of autoantibody production may be similar to that observed for SLE, both in human and murine models, where multiple regions have modest linkage peaks, suggesting a small contribution for each gene(s) in each peak. Furthermore, because our data were largely reflective of high-titer autoantibody production, additional analyses of more standard quantitative Ab levels will be an interesting complementary approach that may uncover additional loci involved in autoantibody production. These analyses are currently underway. Identification and characterization of the genes responsible for these linkage effects will undoubtedly provide further insight into the pathophysiology of SLE, and perhaps underlying cause(s) of multiple other autoimmune diseases.

Subjects and methods

SLE families and controls

Ascertainment, genotyping and Mendelian inheritance testing of the family collection have been described previously.[38,41] Informed consent was obtained from all subjects enrolled in the study. Serological data were available from 229 families multiplex for SLE (1506 individuals) and 877 unrelated controls. Ethnic proportions were similar in both the family and the unrelated control groups and included 55% European-American and 24% African-American individuals. All controls denied a diagnosis or family history of SLE. Genotype data for 279 autosomal microsatellite markers were available for 1894 individuals from 227 families multiplex for SLE, including 511 individuals who met ACR classification criteria for SLE.[32] Subsets of families for analysis included 131 European-American and 73 African-American pedigrees.

Autoantibody measurements

Serum samples were evaluated for ANAs in a blinded fashion at the Oklahoma Medical Research Foundation Clinical Immunology Laboratory. Titers of IgG ANAs were determined by immunofluorescence on Hep2 cells (INOVA Diagnostics Inc., San Diego, CA, USA). Several different variables were used to analyze ANA measurements. First, two separate dichotomous traits were defined using two different thresholds of ANA titer. Relatively low titers were included in analyses by using a threshold of $\geqslant$1:40 to define presence or absence of the ANAs. Because low-titer ANAs can be transiently detected that are not thought to be indicative of autoimmune disease (for example, following certain infections), a more stringent threshold of $\geqslant$1:120 was also evaluated. Second, we created a dichotomous variable that identified only those individuals who had an ANA titer of $\geqslant$1:120 but did not meet ACR classification criteria for SLE (ANA-positive relatives, 'ANArel'). We reasoned that linkage analysis of individuals with ANAs, but not full expression of SLE, may facilitate identification of loci more specifically related to autoantibody production. Third, we included an ordinal trait with individuals classified proportionately to their titer as follows ('ANA1–4'): 'ANA1' = $\leqslant$1:120, 'ANA2' = >1:120$\leqslant$1:360, 'ANA3' = >1:360$\leqslant$1:640 and 'ANA4' = >1:640.

Precipitating (high-titer) levels of Abs reactive with ENAs included anti-Ro/SSA, anti-La/SSB, anti-Sm, anti-nRNP and anti-ribosomal P protein and were detected by Ouchterlony assays.[118] These autoantibodies were dichotomously classified as being 'present' or 'absent'. Abs reactive with anti-dsDNA were measured by *Crithidiae lucilliae* assays (Protrac Industries, Kerrville, TX, USA). All individuals with detectable anti-dsDNA Abs at a titer of $\geqslant$1:10 were considered positive.

Antiphospholipid IgG and IgM Abs (anti-IgG aPL and anti-IgM aPL), as well as RF levels, were measured by enzyme-linked immunosorbent assay.[119] For the anti-IgG aPL and anti-IgM aPL Abs, individuals with a titer >20 aPL units were classified as positive. For RF levels, separate analyses were carried out using two alternative thresholds defined as detectable Ab at serum dilutions of either $\geqslant$1:40 or $\geqslant$1:80.

Certain Ab measurements may fluctuate with disease activity or therapeutic interventions (e.g. anti-dsDNA Abs). In such cases, use of a single time point for autoantibody measurement (such as at the time of study enrollment) may underestimate the prevalence of certain autoantibodies. We compared autoantibody data obtained at the time of study enrollment with historical medical record data that were available for the 519 individuals with SLE. For the majority of Ab specificities, there was no change in classification status. For a small percentage of cases, we used medical record data to classify individuals with Abs against Ro/SSA (7%), La/SSB (6%), Sm (7%), nRNP (7%) and dsDNA (5%).

Characterization of autoantibody traits

The traits evaluated in this study were classified into one of three groups. The first group included nine traits defined by the presence of the particular autoantibody specificity (e.g. anti-Ro/SSA, anti-dsDNA, etc.). These were designated as specific autoantibody traits. The second group included two traits defined by combinations of highly associated autoantibodies determined through association analyses (see Results). These two combinations consisted of a trait defined by the presence of anti-nRNP and/or anti-Sm ('anti-nRNP/Sm'), and a trait defined by anti-Ro/SSA and/or anti-La/SSB ('anti-Ro/La'). The third group of traits included composite phenotypes chosen to represent generalized autoantibody production. Composite autoantibody traits included any of the variables described above for scoring the ANA immunofluorescence test results, plus an additional trait that we defined as 'lupus-related autoimmunity' or 'LRA', based on evidence for production of any autoantibody included in this study, regardless of antigenic specificity. Classification of individuals with the LRA trait was based on the presence of any of the following: ANA titer $\geqslant$1:120, any of the measured ENA autoantibodies, positive anti-dsDNA, IgG or IgM aPL Abs >20 units or RF $\geqslant$1:80.

Statistical analysis

Sample description. Fisher's exact test (GraphPad Software) was used to test for pairwise independence of each of the autoantibodies between each group of individuals (affecteds, relatives and unaffected, unrelated controls), including subgroups based on ethnicity and gender. Unadjusted *P*-values are reported here.

Trait association analyses. Familial aggregation of each autoantibody trait was evaluated using an approach that detects association in siblings to estimate the genetic component of a given trait.[73] In this analysis, we used dichotomous variables to classify individuals as affected or unaffected for the trait of interest. We then determined the number of concordant affected (n_1), discordant (n_2) and concordant unaffected (n_3) sibpairs and calculated odds ratios as $4n_1n_3/((n_2)^2-n_2)$. Odds ratios were calculated for sibpairs that included only individuals with SLE and for sibpairs that included individuals with SLE plus their siblings.

Standard pairwise correlations between autoantibody traits were calculated using all individuals (StatView Software). To assess potential higher-order relationships among autoantibodies, PC analysis (StatView Software) was conducted to create a set of variables composed of uncorrelated linear combinations of the original variables (autoantibodies) and explain the variance–covariance structure of the autoantibodies.[120] PC decomposition is achieved by an orthogonal transformation of original variables based on the standardized phenotypic variance–covariance structure of the data. The coefficients in each PC represent the weight that reflects the degree to which the original variable contributes to the new variable (PC).

Linkage analysis. SIBPAL v4.6, a part of the SAGE (Statistical Analysis for Genetic Epidemiology) package, was used as the primary analytical tool for linkage analysis. The revised Haseman–Elston regression algorithm[74] was used to screen each trait for genetic linkage on the basis of sibpair relationships. This algorithm is a weighted combination of the squared trait difference and squared mean corrected trait sum, adjusted for the non-independence of both the sibpairs and the squared trait differences and sums. Linkage analyses were performed using all pedigrees combined ($n=227$, 624 sibpairs), as well as subsets of European-American ($n=131$, 378 sibpairs) and African-American ($n=73$, 159 sibpairs) pedigrees alone.

Two approaches were used to address the issue of multiple testing and reduce potential Type I errors. First, the mean allele sharing identical-by-descent (IBD) was examined at each peak showing at least suggestive evidence for linkage (defined by a minimum asymptotic *P*-value of 1.7×10^{-3})[35] using detailed results generated by SIBPAL. This information includes the average allele sharing among sibling pair types (concordant affected, concordant unaffected and discordant), as well as proportions of siblings sharing 0, 1 and 2 alleles IBD for each estimate and the associated *P*-values. Loci with estimated proportions of mean allele sharing for discordant sibpairs >0.5 were considered to be likely false positive results and excluded from further consideration (Figure 1). Second, permutation tests were performed in the five most significant linkage peaks (representing approximately 10% of the total peaks identified) in order to calculate empirical *P*-values and estimate an adjustment factor that could be used to account for multiple testing. Empirical *P*-values of the five peaks obtained from permutation tests were compared to the asymptotic *P*-values obtained from linkage analyses. The average difference between empirical and asymptotic *P*-values was 2 orders of magnitude, so this value was used to

adjust the magnitude of asymptotic *P*-values for determining significance of linkage peaks. Four loci retained significance with a $P \leqslant 1.7 \times 10^{-3}$ after adjustment and were evaluated for detailed allele sharing patterns among various sibpair types (Figure 1).

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URLS

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim

Statistical Analysis for Genetic Epidemiology (SAGE) software, http://darwin.cwru.edu/sage/index.php

StatView software for Windows version 5.0, Cary, NC, http://www.statview.com

GraphPad Instat software version 3.0 for Windows XP, San Diego, CA, http://www.graphpad.com

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