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Discerning Risk of Disease Transition in Relatives of Systemic Lupus Erythematosus Patients Utilizing Soluble Mediators and Clinical Features

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Objective. **Systemic lupus erythematosus (SLE) and other autoimmune diseases cause significant morbidity. Identifying populations at risk of developing SLE is essential for curtailing irreversible inflammatory damage. The aim of this study was to identify factors associated with transition to classified disease that would inform our understanding of the risk of SLE.**

Methods. **Previously identified blood relatives of patients with SLE, who had <4 American College of Rheumatology (ACR) classification criteria for SLE at baseline, were enrolled in this follow-up study (n = 409 unaffected relatives). Participants provided detailed family, demographic, and clinical information, including the SLE-specific portion of the Connective Tissue Disease Screening Questionnaire (SLE-CSQ). Serum and plasma samples were tested for the presence of lupus-associated autoantibodies and 52 soluble mediators. Generalized estimating equations (GEEs) were applied to identify factors predictive of disease transition.**

Results. **Of the 409 unaffected relatives of SLE patients, 45 (11%) had transitioned to classified SLE at follow-up (mean time to follow-up 6.4 years). Relatives who transitioned to SLE displayed more lupus-associated autoantibody specificities and higher SLE-CSQ scores ($P < 0.0001$) at baseline than did relatives who did not transition. Importantly, those who had developed SLE during the follow-up period also had elevated baseline plasma levels of inflammatory mediators, including B lymphocyte stimulator, stem cell factor (SCF), and interferon-associated chemokines ($P \leq 0.02$), with concurrent decreases in the levels of regulatory mediators, transforming growth factor β (TGFβ), and interleukin-10 ($P \leq 0.03$). GEE analyses revealed that baseline SLE-CSQ scores or ACR scores (number of ACR criteria satisfied) and plasma levels of SCF and TGFβ, but not autoantibodies, were significant and independent predictors of SLE transition ($P \leq 0.03$).**

Conclusion. **Preclinical alterations in levels of soluble mediators may predict transition to classified disease in relatives of SLE patients. Thus, immune**

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perturbations precede SLE classification and can help identify high-risk relatives for rheumatology referral and potential enrollment in prevention trials.

Autoimmune diseases, including type 1 diabetes, rheumatoid arthritis, and systemic lupus erythematosus (SLE), are increasingly prevalent (1,2), with irreversible morbidity and early mortality due to immune dysfunction, chronic inflammation, and end-organ damage (3). Ongoing studies revealing the benefits of early intervention for patients at high risk of type 1 diabetes (2) and rheumatoid arthritis (4) suggest that early intervention could also be particularly beneficial in SLE, where irreversible organ damage is often present by the time patients are diagnosed and treated (5). Identifying preclinical factors that herald disease transition is essential. Although healthy relatives of SLE patients have an increased risk of developing SLE compared to the general population (6), the vast majority will never transition to classified disease (7,8).

Accumulation of lupus-associated autoantibodies prefaces SLE classification (9); however, autoantibody specificities alone appear to be insufficient to identify relatives at highest risk of developing lupus. Indeed, as many as 37% of unaffected relatives (10–12) and up to 14% of unrelated individuals (13) are antinuclear antibody (ANA) positive yet remain healthy, suggesting that other forms of immune dysregulation coincide with autoantibody production to precipitate transition to SLE. Interferon (IFN) pathways are associated with autoantibodies against DNA/RNA-binding proteins (6) and with the development of SLE (14). Indeed, IFN-induced levels of downstream mediators are also increased in the peripheral blood of SLE patients, including the chemokines monocyte chemotactic protein 1 (MCP-1), MCP-3, and macrophage inflammatory protein 1β (15), as well as B lymphocyte stimulator (BLyS), a tumor necrosis factor receptor (TNFR) superfamily member (16). BLyS contributes to altered B lymphocyte activation and autoantibody production (15) and is a current therapeutic target in SLE (16). The levels of stem cell factor (SCF), a molecule that is associated with hematopoiesis, T cell differentiation, and chemokine release (17,18), are also elevated in SLE patients before clinical flares (15). Other immunoregulatory mechanisms, including levels of circulating interleukin-10 (IL-10) and transforming growth factor β (TGFβ), may also be altered in SLE (15).

Although extensive studies have investigated the pathophysiologic mechanisms of SLE in patients with established disease, little is known about dysregulation of inflammatory pathways in the preclassification time period. Confounding immunomodulatory therapy and organ damage are often absent or limited during the preclassification period, and this would facilitate the identification of targets for pathway-directed therapy. We therefore assembled a unique cohort of previously unaffected blood relatives of SLE patients to investigate demographic, familial, clinical, and biologic factors that could distinguish relatives who transitioned to SLE in this follow-up cohort from relatives who did not transition to classified disease during the follow-up period.

PATIENTS AND METHODS

Study population and sample collection. Experiments were performed in accordance with the Declaration of Helsinki and approved by the Oklahoma Medical Research Foundation (OMRF) and Medical University of South Carolina Institutional Review Boards. All participants provided their written informed consent prior to study enrollment. Unaffected blood relatives (those meeting <4 cumulative American College of Rheumatology [ACR] criteria for SLE [19,20]) were previously enrolled in the Lupus Family Registry and Repository (21) or the Systemic Lupus Erythematosus in Gullah Health cohort (22) between 1992 and 2011. These previously unaffected relatives were recruited to participate in a follow-up study (between March 2010 and May 2012), in order to identify individuals who transitioned to classified SLE (meeting ≥ 4 cumulative ACR classification criteria for SLE [19,20], as ascertained by medical record review).

Upon enrollment in the initial cohort (baseline) and in the current study cohort (follow-up), participants provided serum and plasma samples, along with demographic and clinical information. Samples were stored at -20°C and assays were performed on freshly thawed samples. Participants completed the SLE-specific portion of the Connective Tissue Disease Screening Questionnaire (SLE-CSQ) at baseline and at the follow-up time point. All responses were scored using the SLE-CSQ algorithm (23). All relatives who had transitioned to classified SLE at follow-up (transitioned relatives) were compared to all relatives who had not transitioned to classified SLE (nontransitioned relatives). In addition, for case–control analyses within unique families, each transitioned relative was matched by race, sex, and age (± 5 years) to 1 ANA-positive nontransitioned relative and 1 ANA-negative nontransitioned relative (as determined using indirect immunofluorescence [IIF]), to identify factors elucidating the risk of transition to classified SLE (Table 1) (additional details are available in Supplementary Patients and Methods, available on the Arthritis & Rheumatology web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40004/abstract).

Detection of SLE-associated autoantibodies and soluble mediators. Serum samples were screened for ANAs and SLE-associated autoantibodies in the OMRF College of American Pathologists–certified clinical immunology laboratory, as previously described (12). Briefly, ANAs (detected using HEp-2 cells) and anti–double-stranded DNA (anti-dsDNA) antibodies (determined using *Crithidia luciliae* assays) were measured in the serum using IIF (Inova Diagnostics); seropositivity for ANAs was defined as a titer of $\geq$1:120, and seropositivity for anti-dsDNA was defined as a titer of $\geq$1:30. Anticardiolipin (aCL) antibodies were measured by enzyme-linked immunosorbent assay (ELISA); seropositivity for aCL antibodies was defined as a titer of >20 IgG

Table 1. Characteristics of the study participants

	SLE transition status at follow-up		Nontransitioned relatives (matched to transitioned relatives)*	
	Nontransitioned relatives (n = 364)	Transitioned relatives (n = 45)	ANA positive (n = 45)	ANA negative (n = 45)
Female, no. (%)†	304 (84)	40 (89)	40 (89)	40 (89)
Age, mean ± SD years				
Baseline	47.3 ± 15.9	47.2 ± 12.8	47.9 ± 13.7	48.0 ± 17.0
Follow-up	53.8 ± 15.5	53.4 ± 12.6	54.0 ± 13.2	55.3 ± 16.9
Time to follow-up, mean ± SD years	6.5 ± 3.9	6.4 ± 3.6	6.1 ± 3.5	7.3 ± 3.5
Race, no. (%)				
European American	270 (74.2)	36 (80.0)	36 (80.0)	36 (80.0)
African American	52 (14.3)	5 (11.1)	5 (11.1)	5 (11.1)
American Indian	15 (4.1)	4 (8.9)	4 (8.9)	4 (8.9)
Asian	14 (3.8)	–	–	–
Hispanic	11 (3.0)	–	–	–
Pacific Islander	2 (0.6)	–	–	–
Relationship status, no. (%)				
Parent of SLE patient	167 (45.9)†	10 (22.2)	24 (53.3)†	23 (51.1)†
Child of SLE patient	30 (8.2)†	10 (22.2)	3 (6.7)	4 (8.9)
Sibling of SLE patient	255 (70.0)‡	24 (53.3)	37 (82.2)†	27 (60.0)
Non-FDR of SLE patient	115 (31.6)‡	23 (51.1)	5 (11.1)§	14 (31.1)

* Each relative who transitioned to classified systemic lupus erythematosus (SLE) over the follow-up period was matched by race, sex, and age (±5 years) to 2 relatives who did not transition to SLE over the follow-up period (nontransitioned), including 1 antinuclear antibody (ANA)–positive nontransitioned relative and 1 ANA-negative nontransitioned relative (ANAs were determined at baseline by indirect immunofluorescence; positive titer defined as ≥1:120). Distributions of race and sex were not significantly different (by Fisher's exact test) between the groups. Moreover, age and time to follow-up were not significantly different (by unpaired *t*-test with Welch's correction) between the groups. A parent, child, or sibling of an SLE patient (from simplex or multiplex families) was considered to be a first-degree relative (FDR). Non-FDRs were an aunt, uncle, niece, nephew, first cousin, grandparent, grandchild, or other distant relative of an SLE patient.
† $P < 0.01$ versus transitioned relatives, by Fisher's exact test.
‡ $P < 0.05$ versus transitioned relatives, by Fisher's exact test.
§ $P < 0.0001$ versus transitioned relatives, by Fisher's exact test.

units or >20 IgM units. Plasma samples were assessed by xMAP BioPlex 2200 assay (Bio-Rad Technologies) for autoantibody specificities, including SLE-associated specificities toward dsDNA, chromatin, Ro/SSA, La/SSB, Sm, Sm/RNP complex, and RNP (12). In addition, specific ELISAs were used to assess the plasma levels of BLyS (R&D Systems) and APRIL (eBioscience/Affymetrix), in accordance with the manufacturers' protocols. An additional 50 analytes, including innate and adaptive cytokines, chemokines, and soluble TNF superfamily members, were assessed by xMAP multiplex assays (eBioscience/Affymetrix) on a BioPlex200 (Bio-Rad Technologies) (15) (additional details available in Supplementary Patients and Methods).

Statistical analysis. Relatives who underwent transition to classified SLE were compared to nontransitioned relatives at baseline (pretransition) and at follow-up (posttransition). Chi-square or Fisher's exact tests were used, as appropriate, to determine differences in sex, race, and familial relationship. Chi-square or Fisher's exact tests were used, as appropriate, with Bonferroni adjustment to determine differences in the presence of ACR criteria and lupus-associated autoantibody specificities. Age differences were assessed by unpaired *t*-test, with Welch's correction. The number of ACR criteria (ACR scores), SLE-CSQ scores, ANA titers, number of autoantibody specificities, and plasma soluble mediator levels were compared by Mann-Whitney test. Correlations between plasma soluble mediator levels and SLE-CSQ or ACR scores were determined by Spearman's rank correlation. Generalized estimating equations (GEEs), adjusted for correlation within families, were used to assess whether univariately associated demographic, familial, clinical, and serologic factors at baseline could forecast which relatives would transition to classified SLE at follow-up and which would remain unaffected (24). Unless noted otherwise, analyses were performed using GraphPad Prism software (version 6.02). GEE analyses were carried out in SAS, version 9.3 (SAS Institute) (additional details available in the Supplementary Patients and Methods).

RESULTS

Identification of relatives who transitioned to classified SLE during the follow-up period. We recruited previously identified, unaffected (meeting <4 cumulative ACR classification criteria for SLE) blood relatives of patients with medical record–confirmed SLE

(21,22) to participate in this follow-up study (n = 3,645; mean time to follow-up 8.0 years). Of the 409 previously unaffected relatives who agreed to participate in the current follow-up study (mean time to follow-up 6.4 years), the majority (364 relatives [89%]) had not transitioned to classified disease by the time of follow-up, while 45 relatives (11%) had transitioned to classified SLE (19,20). There were no differences in age at baseline, nor were there differences in time to follow-up, between relatives who did and those who did not have transition to classified SLE (Table 1). There was also no difference in time to follow-up between relatives who transitioned to classified SLE and ANA-positive relatives who did not transition (mean ± SD time to follow-up 6.4 ± 3.6 years versus 6.0 ± 3.7 years; $P = 0.5339$). Transitioned relatives were demographically similar to all of the enrolled participants; the majority of relatives who transitioned to SLE were of European American descent (36 European Americans, 5 African Americans, and 4 American Indians). Among European American relatives, 11.6% transitioned to classified SLE, and 11.8% of nonEuropean American relatives transitioned.

Although relatives of lupus patients are at increased risk of developing SLE (25), families with >1 SLE patient at baseline (multiplex families) were not enriched for relatives who subsequently transitioned to classified disease ($P = 0.7462$) (results available upon request from the corresponding author). Transition to classified SLE at follow-up was observed both in first-degree relatives (comprising parents, children, and siblings) and in non–first-degree blood relatives of SLE patients, regardless of whether they were from a simplex family or a multiplex family (Table 1).

Increased baseline SLE clinical features in relatives who transitioned to classified SLE during the follow-up period. Transitioned relatives, compared to nontransitioned relatives, displayed higher numbers of medical record–confirmed ACR criteria at baseline (mean ± SD ACR score 4.8 ± 0.8 in transitioned relatives versus 1.2 ± 0. 9 in nontransitioned relatives; $P < 0.0001$) (Figure 1) and also had higher self-reported SLE-CSQ scores (23) (mean ± SD 6.1 ± 3.0 in transitioned relatives versus 2.1 ± 2.2 in nontransitioned relatives; $P < 0.0001$) (results available upon request from the corresponding author). At baseline (pretransition), the majority of relatives (294 [72%]) met only 0 or 1 ACR criterion. Moreover, the mean ACR score at baseline was higher in relatives who transitioned to classified SLE than in nontransitioned relatives (mean ± SD 2.3 ± 0.7 versus 0.8 ± 0.8; $P < 0.0001$) (results available upon request from the corresponding author).

In addition to ACR criteria, baseline SLE-CSQ scores (23) were significantly higher in relatives who transitioned to SLE than in nontransitioned relatives (mean ± SD 5.9 ± 2.7 versus 2.2 ± 2.2; $P < 0.0001$) (results available upon request from the corresponding author). Compared to the ANA-positive subset (≥1:120 titer by IIF) of nontransitioned relatives, the relatives who transitioned to classified SLE still displayed higher ACR scores at baseline (mean ± SD 2.3 ± 0.7 in transitioned relatives versus 1.4 ± 0.6 in ANA-positive nontransitioned relatives; $P < 0.0001$) and had higher SLE-CSQ scores at baseline (mean ± SD 5.9 ± 2.7 in transitioned relatives versus 2.6 ± 2.4 in ANA-positive nontransitioned relatives; $P < 0.0001$). Thus, the mean ACR and SLE-CSQ scores were higher at baseline in relatives who transitioned to SLE during the follow-up period than in those who did not transition to classified disease.

ACR scores reflect a combination of currently observed and previously documented criteria, including clinical criteria, serum ANA positivity (≥1:120 titer by IIF), and immunologic criteria (antibody reactivity to dsDNA, Sm, or cardiolipin) (12). Thus, differences in ACR scores could be attributed to distinctions in clinical, ANA, and/or immunologic parameters between relatives who later transitioned to classified SLE and those who did not subsequently transition to classified disease over this follow-up period (Table 2). Relatives who transitioned to SLE, as well as ANA-positive and ANA-negative relatives who did not transition to classified disease met the clinical and immunologic ACR criteria for SLE both at baseline and at follow-up, including mucocutaneous criteria, arthritis, and aCL autoantibodies (Table 2). However, transitioned relatives were more likely than nontransitioned relatives to meet 1 clinical criterion at baseline, and had a higher prevalence of malar rash, photosensitivity, arthritis, and serositis, than did ANA-positive or ANA-negative nontransitioned relatives, at baseline (each $P < 0.0001$ versus nontransitioned relatives) (Table 2). At follow-up, only relatives who transitioned to classified SLE met ACR criteria for discoid rash (7 [16%]), serositis (20 [44%]), or renal disease (5 [11%]) (Table 2).

In all relatives, regardless of subsequent SLE classification status, ANA positivity (≥1:120 titer by IIF) was common at baseline (52% of the total cohort; 89% of transitioned relatives and 48% of nontransitioned relatives). Moreover, at follow-up, the frequency of ANA positivity was even higher (70% of the total cohort; 96% of transitioned relatives and 67% of nontransitioned relatives) (Table 2). However, relatives who transitioned to SLE had higher ANA titers ($P \leq 0.0007$) and more lupus-specific autoantibody specificities against DNA- and RNA-binding proteins, both at baseline and at follow-up ($P < 0.0001$ versus nontransitioned relatives), with the greatest number of autoantibody specificities observed in non–European

Table 2. ACR criteria in nontransitioned and transitioned relatives of SLE patients*

ACR criterion	Nontransitioned relatives, by ACR score				Transitioned relatives, by ACR score				Total with each ACR criterion		
	0	1	2	3	4	5	6	7	Nontransitioned relatives	Transitioned relatives	P†
Baseline											
Malar rash	–	–	2	1	2	3	1	–	3 (1)	6 (13)	<0.0001
Discoid rash	–	–	–						0	0	–
Photosensitivity	–	–	1	7	5	4	1	1	8 (2)	11 (24)	<0.0001
Oral ulcers	–	–	1			1	–	–	1 (0.3)	1 (2)	0.2082
Arthritis			3	6	7	6	2	–	9 (2.5)	15 (33)	<0.0001
Serositis	–	–	–	–	3	–	1	–	0	4 (9)	0.0001
Renal disease	–	–	–	–	–	–	1	–	0	1 (2)	0.1103
Neurologic	–	–	–	–	–	1	–	–	0	1 (2)	0.1100
Hematologic	–	–	1	5	1	2	1	–	6 (1.6)	4 (9)	0.0164
Immunologic	–	21	71	13	7	10	2	1	105 (29)	20 (44)	0.0394
ANA positivity	–	77	75	22	17	15	6	2	174 (48)	40 (89)	<0.0001
Follow-up											
Malar rash	–	–	3	3	11	7	6	2	6 (1.6)	26 (58)	<0.0001
Discoid rash	–	–	–	–	1	5	–	1	0	7 (16)	<0.0001
Photosensitivity	–	–	1	9	10	9	4	2	10 (2.7)	25 (56)	<0.0001
Oral ulcers	–	–	1	2	9	7	3	1	3 (1)	20 (44)	<0.0001
Arthritis	–	–	5	14	13	14	5	2	19 (5.2)	34 (76)	<0.0001
Serositis	–	–	–	–	6	7	5	2	0	20 (44)	<0.0001
Renal disease	–	–	–	–	1	2	2	–	0	5 (11)	<0.0001
Neurologic	–	–	2	–	–	3	1	1	2 (0.6)	5 (11)	0.0002
Hematologic	–	–	1	6	2	3	2	–	7 (1.9)	7 (16)	0.0002
Immunologic	–	30	101	17	8	12	2	1	148 (41)	23 (51)	0.2014
ANA positivity		115	106	24	19	16	6	2	245 (67)	43 (96)	<0.0001

* Values are the number of subjects with each American College of Rheumatology (ACR) criterion at baseline or follow-up, stratified either by the ACR score (total number of ACR criteria satisfied) at follow-up or by the total number (%) of relatives who did not transition to classified SLE over the follow-up period (nontransitioned) compared to relatives who did transition at follow-up (transitioned). ANA = antinuclear antibody.

† P values were determined by Fisher's exact test. The Bonferroni-adjusted P values for multiple comparisons were $P = 0.0050$ at baseline and $P = 0.0045$ at follow-up.

American relatives who transitioned to SLE (at baseline, mean ± SD 0.63 ± 0.90 in European Americans versus 1.67 ± 1.32 in non–European Americans [$P = 0.0194$]; at follow-up, mean ± SD 0.56 ± 0.88 in European Americans versus 1.67 ± 1.32 in non–European Americans [$P = 0.0077$]). Of the tested autoantibody specificities, titers of anti-Ro/SSA were significantly higher, both at baseline (preclassification) and at follow-up (postclassification) (each $P = 0.0004$, after Bonferroni correction), in relatives who transitioned to classified SLE compared to relatives who did not transition (at baseline, 27% versus 7.7%) (results available upon request from the corresponding author). Relatives who transitioned to classified SLE were also more likely to be positive for anti–nuclear RNP antibodies at baseline (13% of transitioned relatives versus 2.2% of nontransitioned relatives; $P = 0.0020$).

Altered plasma soluble mediator levels in relatives who transitioned to classified SLE. Altered levels of immune mediators are linked to SLE pathogenesis (15) and appear before disease classification (26). Utilizing a nested case–control approach, we assessed the plasma levels of 52 soluble mediators from multiple immune pathways (15) in the 45 relatives who transitioned to classified SLE compared to 90 nontransitioned relatives who were matched by race, sex, and age (±5 years) (comprising 45 ANA-positive nontransitioned relatives and 45 ANA-negative nontransitioned relatives) (Table 1). Similar to the findings in the whole cohort, in this subset analysis, transitioned relatives had significantly higher baseline ACR scores (mean ± SD 2.3 ± 0.7 in transitioned relatives versus 0.8 ± 0.8 in nontransitioned relatives; $P < 0.0001$) and significantly higher SLE-CSQ scores (mean ± SD 5.9 ± 2.7 in

Baseline ACR score	Follow-up ACR score							
	0	1	2	3	4	5	6	7
0	84	47	12	1				
1		98	42	5	5		1	1
2			56	8	8	9	1	
3				11	7	8	4	1

Figure 1. Values show the number of subjects with each American College of Rheumatology (ACR) score (number of ACR criteria) at follow-up according to baseline ACR scores in relatives of patients with systemic lupus erythematosus (SLE). Vertical line indicates the cutoff for defining relatives who transitioned to classified SLE over the follow-up period (those with ≥4 ACR criteria).

A

Biomarker	Spearman r	95% CI	p value
SLE-CSQ	0.613	0.492 to 0.711	***<0.0001***
SCF	0.393	0.236 to 0.531	***<0.0001***
MCP-3	0.304	0.138 to 0.454	***0.0003***
BLyS	0.256	0.086 to 0.3411	***0.0028***
MCP-1	0.230	0.059 to 0.389	***0.0072***
MIP-1β	0.219	0.047 tp 0.379	***0.0107***
TGF-β	-0.194	-0.356 to -0.021	***0.0241***
IL-10	-0.128	-0.295 to 0.047	*0.1385*
TNFRII	0.134	-0.041 to 0.300	*0.1225*

B

Biomarker	Spearman r	95% CI	p value
ACR Score	0.421	0.266 to 0.554	***< 0.0001***
SCF	0.356	0.194 to 0.499	***< 0.0001***
TNFRII	0.284	0.116 to 0.436	***0.0009***
MCP-3	0.277	0.108 to 0.430	***0.0011***
MIP-1β	0.230	0.059 to 0.389	***0.0072***
BLyS	0.209	0.036 to 0.369	***0.0151***
MCP-1	0.110	-0.065 to 0.279	*0.1102*
TGF-β	-0.091	-0.261 to 0.084	*0.2941*
IL-10	-0.064	-0.235 tp 0.111	*0.4601*

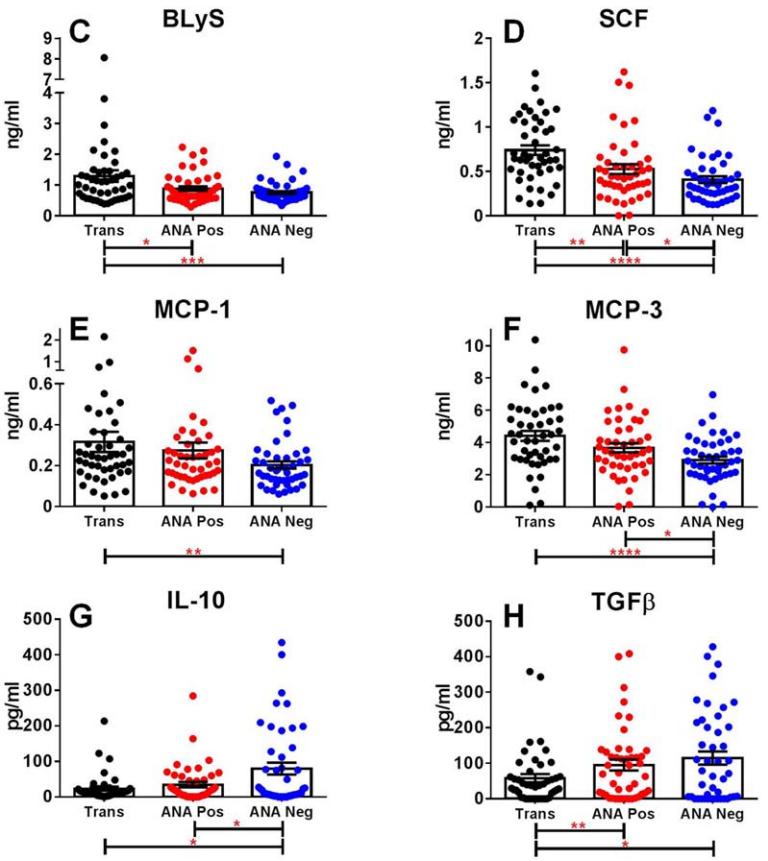

Figure 2. Altered baseline levels of soluble inflammatory mediators in relatives who transitioned to classified systemic lupus erythematosus (SLE) at follow-up. **A** and **B,** Spearman's correlation analyses were used to assess correlations of baseline SLE-CSQ scores (SLE-specific portion of the Connective Tissue Disease Screening Questionaire) and plasma soluble mediator levels with American College of Rheumatology (ACR) scores at follow-up **(A)** and correlations of baseline ACR scores and plasma soluble mediator levels with SLE-CSQ scores at follow-up **(B)**. Values are Spearman's rho with 95% confidence interval (95% CI). **C–H,** Plasma levels of B lymphocyte stimulator (BLyS) **(C)**, stem cell factor (SCF) **(D)**, monocyte chemotactic protein 1 (MCP-1) **(E)**, MCP-3 **(F)**, interleukin-10 (IL-10) **(G)**, and transforming growth factor β (TGFβ) **(H)** were measured at baseline in 45 relatives of SLE patients who transitioned to classified SLE at follow-up (Trans) compared to unaffected relatives who were antinuclear antibody (ANA) positive (Pos) or ANA negative (Neg) (as determined by indirect immunofluorescence) and who were matched by race, sex, age (±5 years) and time of sample procurement. Symbols represent individual subjects; bars show the mean ± SEM. $* = P < 0.05$; $** = P < 0.01$; $*** = P < 0.001$; $**** = P < 0.0001$, by Kruskal-Wallis test with Dunn's correction for multiple comparisons. MIP-1β = macrophage inflammatory protein 1β; TNFRII = tumor necrosis factor receptor type II.

transitioned relatives versus 2.0 ± 1.9 in nontransitioned relatives; $P < 0.0001$). However, no significant differences in SLE-CSQ scores were observed between ANA-positive and ANA-negative matched nontransitioned relatives (mean ± SD 2.3 ± 2.0 versus 1.6 ± 1.7; $P = 0.0669$).

Consistent with their putative contributions to SLE pathogenesis, baseline (pretransition) levels of a number of soluble mediators correlated with evidence of SLE at follow-up (Figure 2). Baseline plasma levels of BLyS ($P = 0.0028$), SCF ($P < 0.0001$), MCP-1 ($P = 0.0072$), and

MCP-3 ($P = 0.0003$) positively correlated with cumulative follow-up ACR scores (Figure 2A). In parallel, baseline plasma levels of BLyS ($P = 0.0151$), SCF ($P < 0.0001$), and MCP-3 ($P = 0.0011$) positively correlated with follow-up SLE-CSQ scores (Figure 2B).

Furthermore, baseline levels of BLyS (Spearman's rho = 0.208, $P = 0.0156$), SCF (Spearman's rho = 0.345, $P < 0.0001$), and MCP-3 (Spearman's rho = 0.300, $P = 0.0004$) significantly correlated with ACR scores at baseline. In addition, baseline levels of BLyS (Spearman's rho = 0.291, $P = 0.0006$), SCF (Spearman's rho = 0.306, $P = 0.0003$), and MCP-3 (Spearman's rho = 0.288, $P = 0.0007$) significantly correlated with the SLE-CSQ scores at baseline, prior to disease transition. Conversely, the levels of the regulatory mediator TGFβ at baseline ($P = 0.0241$) (Figure 2A) and at follow-up ($P = 0.0054$) negatively correlated with cumulative follow-up ACR scores (results available upon request from the corresponding author).

Baseline soluble mediator levels could be used to identify relatives who transitioned to classified SLE. Transitioned relatives had higher baseline plasma levels of BLyS and SCF compared to relatives who remained unaffected (Figures 2C and D), including ANA-positive nontransitioned relatives ($P = 0.0229$ for BLyS and $P = 0.0004$ for SCF) and ANA-negative nontransitioned relatives ($P = 0.0003$ for BLyS and $P < 0.0001$ for SCF). Relatives who transitioned to SLE and matched, ANA-positive nontransitioned relatives had similar baseline plasma levels of the IFN-driven chemokines MCP-1 and MCP-3 (both $P < 0.0001$) (Figures 2E and F). Transitioned relatives and ANA-positive nontransitioned relatives had significantly higher baseline plasma levels of these chemokines compared to matched, ANA-negative nontransitioned relatives. In addition, compared to relatives who did not transition, lupus relatives who transitioned to SLE had significantly reduced levels of the regulatory mediators IL-10 ($P = 0.0284$ versus ANA-negative nontransitioned relatives) and TGFβ ($P = 0.0082$ versus ANA-positive nontransitioned relatives and $P = 0.0121$ versus ANA-negative nontransitioned relatives) (Figures 2G and H).

After transition to classified disease, follow-up levels of multiple inflammatory mediators remained positively correlated with ACR scores and SLE-CSQ scores. Conversely, at follow-up, levels of the regulatory mediators IL-10 ($P = 0.0039$) and TGFβ ($P = 0.0054$) were negatively correlated with ACR scores (results available upon request from the corresponding author). Follow-up plasma levels of BLyS, SCF, MCP-1, MCP-3, IL-10, and TGFβ continued to be altered in relatives who transitioned to SLE compared to matched relatives who remained unaffected (results available upon request from the corresponding author). In addition, the levels of a number of mediators at follow-up were altered in relatives of SLE patients compared to matched, unrelated healthy controls with no medical or family history of SLE. Transitioned relatives had significantly higher levels of BLyS ($P < 0.0001$), MCP-1 ($P < 0.0001$), MCP-3 ($P = 0.05$), and IL-10 ($P = 0.0002$) compared to healthy controls. Furthermore, ANA-negative and ANA-positive nontransitioned relatives also had higher plasma levels of BLyS ($P \leq 0.01$), MCP-1 ($P \leq 0.003$), IL-10 ($P \leq 0.0002$), and TGFβ ($P \leq 0.01$) compared to healthy controls (results available upon request from the corresponding author).

Forecasting transition to SLE in relatives based on baseline levels of SCF and TGFβ, independent of clinical measures. We ascertained several factors that could serve as potential predictors of transition to classified disease in previously unaffected relatives of SLE patients. GEE analyses, adjusted for familial correlation, were performed to determine whether multivariable models that included univariate-associated demographic and familial relationship variables, SLE-CSQ scores, ACR classification criteria, autoantibody status, and/or levels of select soluble mediators at baseline could be used to forecast transition to SLE in unaffected relatives (Tables 3 and 4). All models were adjusted for age, sex, and race to verify effective demographic matching of transitioned and nontransitioned relatives. Levels of the soluble mediators MCP-1, MCP-3, and BLyS did not reach significance alone or in combination, and therefore these 3 variables were excluded from the final models.

The familial relationship to patients with confirmed SLE (blood relative, parent, child, or sibling) did not determine which relatives would transition to classified SLE (model 1 in Tables 3 and 4). However, increased baseline levels of SCF and decreased baseline levels of TGFβ were associated with transitioning to SLE (model 2 in Tables 3 and 4). Increased SLE-CSQ scores (model 3 in Table 3), as well as the number of baseline ACR criteria (model 3 in Table 4), were significantly associated with transitioning to SLE.

In addition, altered levels of SCF and TGFβ reached significance independent of SLE-CSQ scores (model 4 in Table 3) and ACR scores (model 4 in Table 4). These associations were attenuated only slightly by adjustment for SLE-CSQ scores (model 4 in Table 3) and ACR scores (model 4 in Table 4), indicating that immune dysregulation alone may help identify relatives at high risk of developing SLE. Although relatives who transitioned to classified SLE had more autoantibody specificities compared to nontransitioned relatives (results available upon request from the corresponding author), neither ANA positivity (model 5 in Tables 3 and 4) nor the number of

Table 3. Effects of biologic factors and SLE-CSQ scores on multivariable models forecasting the risk of transition to classified SLE in relatives of SLE patients*

	Model 1		Model 2		Model 3		Model 4		Model 5	
Baseline parameter	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Demographic										
Age	1.01 (0.97–1.04)	0.7099	1.01 (0.97–1.05)	0.6389	1.01 (0.97–1.06)	0.5230	1.01 (0.97–1.06)	0.5961	1.01 (0.96–1.06)	0.6360
Sex	0.61 (0.17–2.19)	0.4500	0.47 (0.11–2.10)	0.3241	0.42 (0.08–2.10)	0.2899	0.30 (0.05–1.72)	0.1764	0.27 (0.05–1.52)	0.1377
Race										
European American	1		1		1		1		1	
African American	0.60 (0.12–2.91)	0.5243	0.74 (0.13–4.08)	0.7255	0.73 (0.13–4.28)	0.7296	0.84 (0.13–5.45)	0.8528	0.73 (0.11–4.90)	0.7416
Other	1.68 (0.46–6.17)	0.4381	1.00 (0.21–4.76)	0.9985	4.17 (0.88–19.80)	0.0727	2.28 (0.39–13.33)	0.3612	2.39 (0.41–14.08)	0.3353
Relationship to SLE patient										
Blood relative	1		1		1		1		1	
Parent	0.90 (0.09–18.83)	0.9240	0.88 (0.07–10.53)	0.9195	4.19 (0.36–149.37)	0.2548	5.98 (0.39–91.44)	0.1989	7.30 (0.44–120.37)	0.1644
Child	2.92 (0.65–13.1)	0.1621	4.56 (0.79–26.47)	0.0910	1.79 (0.33–9.72)	0.4988	3.37 (0.45–25.30)	0.2383	3.73 (0.49–28.62)	0.2055
Sibling	1.19 (0.43–3.28)	0.5317	1.51 (0.46–4.91)	0.4956	1.44 (0.45–4.63)	0.5367	2.53 (0.60–10.75)	0.2078	2.66 (0.62–111.49)	0.1891
Clinical										
SLE-CSQ score	–	–	–	–	1.64 (1.35–1.98)	<0.0001	1.62 (1.29–2.02)	<0.0001	1.61 (1.28–2.02)	<0.0001
ANA positivity	–	–	–	–	–	–	–	–	1.78 (0.49–6.47)	0.3831
Biologic										
TGFβ levels	–	–	0.20 (0.08–0.52)	0.0010	–	–	0.27 (0.10–0.69)	0.0067	0.25 (0.10–0.67)	0.0058
SCF levels	–	–	3.96 (2.19–57.16)	<0.0001	–	–	3.78 (1.94–7.35)	<0.0001	3.62 (1.84–7.12)	0.0002
Test data set (n = 158)										
AUC (95% CI)	0.60 (0.47–0.72)		0.84 (0.76–0.92)		0.86 (0.80–0.92)		0.92 (0.88–0.97)		0.93 (0.89–0.97)	
Sensitivity	0.35		0.86		0.93		0.97		0.97	
Specificity	0.85		0.72		0.70		0.81		0.81	
LR+	2.33		3.07		3.10		5.11		5.11	
LR−	0.76		0.19		0.10		0.04		0.04	
PPV	0.22		0.28		0.28		0.39		0.39	
NPV	0.91		0.98		0.99		1.00		1.00	
Validation data set (n = 77)										
AUC (95% CI)	0.57 (0.39–0.75)		0.73 (0.56–0.88)		0.77 (0.64–0.91)		0.81 (0.66–0.95)		0.80 (0.65–0.95)	
Sensitivity	0.50		0.69		0.63		0.75		0.75	
Specificity	0.71		0.79		0.89		0.87		0.87	
LR+	1.72		3.29		5.73		5.77		5.77	
LR−	0.70		0.39		0.42		0.29		0.29	
PPV	0.18		0.29		0.41		0.42		0.42	
NPV	0.92		0.95		0.95		0.97		0.97	

* Relatives who transitioned to classified systemic lupus erythematosus (SLE) over the follow-up period were matched to relatives who did not transition by race, sex, and age (±5 years). Antinuclear antibody (ANA) status was determined by indirect immunofluorescence. *P* values were determined by Wald chi-square test. Odds ratios (ORs), with 95% confidence intervals (95% CIs), were determined per standard deviation (SD) increase in each variable (for stem cell factor [SCF], SD 329.2; for transforming growth factor β [TGFβ], SD 147.2). The positive likelihood ratio (LR+), negative likelihood ratio (LR−), positive predictive value (PPV), and negative predictive value (NPV) were each based on a cohort SLE transition prevalence/pretest probability of 0.11. SLE-CSQ = SLE-specific portion of the Connective Tissue Disease Screening Questionnaire; AUC = area under the receiver operating characteristics curve.

Table 4. Effects of biologic factors and ACR scores on multivariable models forecasting the risk of transition to classified SLE in relatives of SLE patients*

Baseline parameter	Model 1		Model 2		Model 3		Model 4		Model 5	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Demographic										
Age	1.01 (0.97–1.04)	0.7099	1.01 (0.97–1.05)	0.6389	1.00 (0.96–1.05)	0.8822	1.00 (0.95–1.05)	0.9988	1.00 (0.96–1.05)	0.9356
Sex	0.61 (0.17–2.19)	0.4500	0.47 (0.11–2.10)	0.3241	0.37 (0.07–1.97)	0.2450	0.39 (0.06–2.38)	0.3074	0.47 (0.07–3.07)	0.4268
Race										
European American	1		1		1		1		1	
African American	0.60 (0.12–2.91)	0.5243	0.74 (0.13–4.08)	0.7255	0.50 (0.08–3.29)	0.4679	0.47 (0.06–3.43)	0.4533	0.55 (0.07–4.09)	0.5552
Other	1.68 (0.46–6.17)	0.4381	1.00 (0.21–4.76)	0.9985	1.10 (0.18–6.65)	0.9218	0.57 (0.07–4.57)	0.5948	0.47 (0.05–4.50)	0.5149
Relationship to SLE patient										
Blood relative	1		1		1		1		1	
Parent	0.90 (0.09–18.83)	0.9240	0.88 (0.07–10.53)	0.9195	3.79 (0.24–60.23)	0.3452	4.44 (0.23–85.97)	0.3241	3.68 (0.21–63.35)	0.3693
Child	2.92 (0.65–13.1)	0.1621	4.56 (0.79–26.47)	0.0910	5.57 (0.83–37.16)	0.0763	6.94 (0.83–58.32)	0.0744	5.87 (0.70–49.52)	0.3693
Sibling	1.19 (0.43–3.28)	0.5317	1.51 (0.46–4.91)	0.4956	1.34 (0.38–4.72)	0.6467	1.61 (0.38–6.85)	0.5210	1.45 (0.33–6.41)	0.6256
Clinical										
ACR score	–	–	–	–	7.40 (3.54–15.45)	<0.0001	5.96 (2.69–13.19)	<0.0001	6.62 (2.98–14.72)	<0.0001
ANA positivity	–	–	–	–	–	–	–	–	0.40 (0.09–1.81)	0.2324
Biologic										
TGFβ levels	–	–	0.20 (0.08–0.52)	0.0010	–	–	0.29 (0.11–0.79)	0.0156	0.30 (0.11–0.83)	0.0203
SCF levels	–	–	3.96 (2.19–57.16)	<0.0001	–	–	2.69 (1.42–5.10)	0.0024	2.81 (1.46–5.38)	0.0019
Test data set (n = 158)										
AUC (95% CI)	0.60 (0.47–0.72)		0.64 (0.76–0.92)		0.90 (0.84–0.96)		0.93 (0.88–0.98)		0.93 (0.87–0.98)	
Sensitivity	0.35		0.86		0.93		0.86		0.90	
Specificity	0.85		0.72		0.70		0.90		0.87	
LR+	2.33		3.07		3.10		8.60		6.92	
LR−	0.76		0.19		0.10		0.16		0.11	
PPV	0.22		0.28		0.28		0.51		0.46	
NPV	0.91		0.98		0.99		0.98		0.99	
Validation data set (n = 77)										
AUC (95% CI)	0.57 (0.39–0.75)		0.73 (0.56–0.88)		0.87 (0.79–0.96)		0.89 (0.80–0.97)		0.89 (0.81–0.98)	
Sensitivity	0.50		0.69		0.63		0.81		0.81	
Specificity	0.71		0.79		0.89		0.89		0.92	
LR+	1.72		3.29		5.73		7.36		10.13	
LR−	0.70		0.39		0.42		0.21		0.21	
PPV	0.18		0.29		0.41		0.48		0.56	
NPV	0.92		0.95		0.95		0.97		0.97	

* Relatives who transitioned to classified systemic lupus erythematosus (SLE) over the follow-up period were matched to relatives who did not transition by race, sex, and age (±5 years). Antinuclear antibody (ANA) status was determined by indirect immunofluorescence. *P* values were determined by Wald chi-square test. Odds ratios (ORs), with 95% confidence intervals (95% CIs), were determined per standard deviation (SD) increase in each variable (for stem cell factor [SCF], SD 329.2; for transforming growth factor β [TGFβ], SD 147.2). The positive likelihood ratio (LR+), negative likelihood ratio (LR−), positive predictive value (PPV), and negative predictive value (NPV) were each based on a cohort SLE transition prevalence/pretest probability of 0.11. ACR = American College of Rheumatology; AUC = area under the receiver operating characteristics curve.

DNA- and RNA-binding autoantibody specificities (adjusted odds ratio 1.74, 95% confidence interval [95% CI] 0.79–3.85; $P = 0.1726$) informed the risk of SLE transition.

Overall, the best models for identifying relatives who would subsequently transition to SLE were those that combined soluble mediator information with clinical criteria derived from either SLE-CSQ scores (model 4 in Table 3) (area under the receiver operating characteristics [ROC] curve [AUC] 0.92, 95% CI 0.88–0.97 in the test data set [n = 158]; AUC 0.81, 95% CI 0.66–0.95 in the validation data set [n = 77]) or ACR scores calculated from the medical record (model 4 in Table 4) (AUC 0.93, 95% CI 0.88–0.98 in the test data set [n = 158]; AUC 0.89, 95% CI 0.80–0.97 in the validation data set [n = 77]). Significantly more relatives who transitioned to SLE at follow-up were positive for SCF at baseline (positive cutoff level of 486.1 pg/ml as determined by ROC curve/Youden index analysis) and negative for TGFβ at baseline (positive cutoff level of 62.77 pg/ml as determined by ROC curve/Youden index analysis) compared to matched ANA-positive and ANA-negative relatives who remained unaffected ($P < 0.0001$ for SCF and $P = 0.0028$ for TGFβ, by chi-square test). However, neither SCF positivity nor TGFβ negativity associated with any particular ACR criterion, either in the relatives who transitioned to SLE or in those who did not transition to SLE. Rather, baseline levels of these mediators were positively correlated (SCF) or negatively correlated (TGFβ) with overall ACR and SLE-CSQ scores at follow-up (Figures 2A and B).

Based on a pretest probability of transitioning to classified SLE of 0.11 (11% of the cohort transitioned to classified SLE at follow-up), combining self-reported SLE-CSQ data with soluble mediator data at baseline increased the posttest probability of transitioning to classified SLE to 0.41 (average of the test and validation sets; model 4 in Table 3). Moreover, combining physician-confirmed ACR criteria with soluble mediator data at baseline increased the posttest probability of transitioning to classified SLE to 0.50 (model 4 in Table 4).

In addition, among relatives who transitioned to SLE, we compared baseline differences in the levels of SCF and TGFβ between relatives and who had a baseline ACR score of 1 or 2 (ANA positivity and/or meeting immunologic criteria, n = 25) and those who had a baseline ACR score of 3 (also meeting clinical criteria, n = 20). Levels of SCF and TGFβ were not different between these groups (results available upon request from the corresponding author). Furthermore, no significant differences in either SCF or TGFβ levels were noted based on a history of prednisone or hydroxychloroquine use (results available upon request from the corresponding author). For those relatives remained unaffected (pretest probability of remaining unaffected 0.89), the posttest probability of remaining unaffected based on baseline SLE-CSQ scores and levels of soluble mediators was 0.99 (model 4 in Table 3), while the posttest probability of remaining unaffected was 0.98 when the model was based on baseline ACR scores and levels of soluble mediators (model 4 in Table 4).

DISCUSSION

Early intervention may ameliorate some autoimmune diseases, but this is currently not possible for lupus because those at highest risk of SLE development cannot be reliably identified. As a step toward developing both monitoring strategies and early intervention strategies to limit the accrual of SLE-induced organ damage (3), this study provides critical new information to help identify relatives of SLE patients at the highest risk of transition to SLE. Furthermore, it enables identification of those relatives who are less likely to develop SLE and may not require the same level of clinical monitoring. A strength of the current study is that we were able to re-enroll relatives of SLE patients positioned across the spectrum of SLE preclassification at baseline, ranging from meeting no criteria to exhibiting ANA positivity along with clinical features. This study identified blood relatives who transitioned to classified disease during the relatively short follow-up period of this study (mean $\pm$ SD 6.4 $\pm$ 3.9 years). Although some who transitioned to SLE were ANA positive with clinical features at baseline (pretransition), a number of the transitioned relatives exhibited no clinical features at baseline. Yet, the vast majority of relatives did not transition to classified SLE despite the fact that many of them exhibited ANA positivity and/or clinical features at baseline, with 68% exhibiting no change in ACR criteria between the baseline and follow-up evaluations (ACR scores of 0–3 at baseline and follow-up).

Although ANA positivity was more frequent and the number of autoantibody specificities greater in relatives who transitioned to SLE, neither factor independently identified future SLE classification in multivariable models. Thus, ANA positivity alone does not reliably denote future disease transition, as 85% of relatives who were ANA positive at baseline did not develop SLE during the period of observation. Rather, increased levels of SCF and decreased levels of TGFβ, independent of the ACR and SLE-CSQ scores, identified individuals who would transition to SLE (while those who would remain unaffected were identified by decreased levels of SCF and increased levels of TGFβ) in multivariable models. Measurement of these select soluble mediators could help

identify individuals in need of rheumatology referral, closer monitoring, or early intervention. Moreover, our findings support the emerging paradigm that SLE pathogenesis involves both enhanced proinflammatory pathways and insufficient compensatory regulatory pathways (27,28).

The levels of several inflammatory mediators were elevated at baseline in relatives who subsequently developed SLE. In particular, baseline plasma SCF levels were highest in relatives who transitioned to classified disease, and these levels were significantly predictive of SLE development. Taken together with our previous results showing that increased SCF levels immediately precede disease flare in patients with active SLE (15), these results suggest that SCF may promote the pathogenesis of SLE. Although typically known for its role in hematopoiesis, SCF has also been shown to drive IL-6 production and influence Th2 and Th17 pathways in several inflammatory conditions, by interacting with the receptor c-kit (18). Such mechanisms may drive SLE pathogenesis by inducing the secretion of MCP chemokines (17). Indeed, the chemokines MCP-1 and MCP-3 and their downstream mediator BLyS (29) showed similar patterns of significantly increased plasma levels at baseline and follow-up in relatives who underwent transition to SLE. Although it is considered a promising therapeutic target in SLE (16), BLyS did not contribute independently to the risk of transitioning to SLE in any of our models. Thus, upstream inflammatory factors, rather than downstream mediators such as BLyS, may be primary independent factors in early pathogenesis (15,17,18,29,30).

Along with enhanced inflammatory pathways, SLE patients showed signs of inadequate regulatory mechanisms as compared to healthy, ANA-positive individuals, suggesting that a failure of active regulation contributes to SLE pathogenesis in relatives of SLE patients (31–33). Indeed, TGFβ levels were lowest in relatives who transitioned to SLE, thereby differentiating them from unaffected relatives. Baseline IL-10 levels were also reduced in relatives who transitioned to SLE. TGFβ and IL-10 are required for the development and propagation of T regulatory cells (33), which may have altered numbers and/or functions in SLE (31). The effectiveness of regulatory pathways in SLE patients may be further reduced by resistance of T effector cells to T regulatory cells (32). Conversely, compensatory T regulator functions may help mitigate the risk of SLE in unaffected relatives (34), as the highest levels of TGFβ at baseline and follow-up were in those relatives who did not transition to classified SLE, irrespective of ANA status. Future studies could address these possibilities.

Among the previously unaffected relatives of SLE patients, 11% transitioned to classified SLE in this follow-up cohort (n = 409), highlighting the likelihood of identifying at-risk relatives for early intervention or clinical trial enrollment. Even in this primarily European American cohort with limited numbers of individuals meeting renal and neurologic classification criteria, utilizing the multivariable model incorporating both clinical features (self-reported SLE-CSQ scores or physician-confirmed ACR criteria) and serologic features increases the baseline risk of transitioning to SLE to 42% for those relatives who demonstrate clinical criteria, increased SCF levels, and decreased TGFβ levels. Such individuals may benefit from clinical trials to prevent or delay SLE classification.

Those relatives who are found to be autoantibody positive and yet exhibit elevated levels of regulatory mediators may be identified as having a decreased risk of transitioning to classified disease. Utilization of the multivariable model incorporating clinical and serologic features increases the negative predictive value to $\geq$98% for those relatives who demonstrate few clinical criteria, decreased SCF levels, and increased TGFβ levels. Analysis of such a population would have the potential to reveal novel mechanisms of incomplete breaks in tolerance that can be harnessed and applied to high-risk individuals to delay or prevent disease transition. This is particularly tantalizing given that we have observed differences in immune profiles between relatives of lupus patients who transitioned to SLE compared to matched, unrelated healthy controls with no family history of SLE, with increases in both inflammatory and regulatory mediators in the relatives of lupus patients. The increased inflammatory profile in relatives of lupus patients may be due to the presence of heritable risk factors (35), offset by enhanced regulatory mechanisms that have been detected in the current study and in other studies (34,36).

Although we were able to re-enroll a little more than 400 relatives of SLE patients and to confirm the presence of ACR classification criteria in the medical record, this study is limited in that only a single follow-up point was used, and the clinical data were collected prior to publication of the proposed Systemic Lupus International Collaborating Clinics classification criteria for SLE (37). Prospective, longitudinal monitoring of the relatives of lupus patients over a long period of time (>10 years), during which serial collection of clinical data and biologic specimens can occur, would improve our ability to pinpoint immune dysregulation associated with the natural progression to classified SLE (38,39). Such studies could potentially provide insight into the relationship between

genetic, epigenetic, and environmental risk factors (40) and immune dysregulation that leads to the accrual of autoantibody specificities and development of clinical disease.

Currently, ACR criteria and serology findings, particularly soluble mediator levels, may be used to evaluate unaffected relatives to help identify individuals at the highest risk of developing SLE. This evaluation requires a trained rheumatologist and may miss more subtle signs and symptoms that result in a clinician identifying a patient as having "potential SLE" (41). Screening families of lupus patients with the SLE-specific portion of the CSQ and serology may substantially facilitate the identification of relatives who are at increased risk of disease compared to relatives who do not require enhanced monitoring or treatment with potentially toxic medications.

Such information may be useful when counseling family members about future disease risk and may allow for the identification of relatives for inclusion in prospective prevention trials. Given the humanistic and economic burden of SLE (42,43), addressing immune dysregulation prior to disease classification may prove beneficial (44). Although SLE presents therapeutic challenges (45), this study reveals inflammatory and regulatory mechanisms that may be applied to the development of novel SLE therapies (46). In addition, early intervention with hydroxychloroquine has been shown to reduce organ damage (47), decrease the accumulation of lupus-associated autoantibodies, and delay the transition to classified SLE (48). Such an approach may allow for a decreased rate of damage and a reduced need for multiple and/or immunosuppressant treatments that perpetuate morbidity and increase healthcare costs (49) in relatives of SLE patients at high risk of transitioning to classified SLE.

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AUTHOR 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.

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Acquisition of data. Munroe, Young, Kamen, Guthridge, Niewold, Costenbader, Weisman, Ishimori, Wallace, Gilkeson, Karp, Harley, Norris, James.

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