

Systemic lupus erythematosus

Novel IgG and IgA autoantibodies validated in two independent cohorts are associated with disease activity and determine organ manifestations in systemic lupus erythematosus: implications for anti-LIN28A, anti-HMGN5, anti-IRF5, and anti-TGIF1

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ABSTRACT

Objectives: This study aimed to identify and validate novel autoantibodies that reflect global and organ-specific disease activity in systemic lupus erythematosus (SLE).

Methods: Plasma samples were screened for IgG and IgA seroreactivity against 1609 protein autoantigens using a microarray (i-Ome Discovery; Sengenics). We determined differentially abundant autoantibodies (daAABs) in patients with SLE vs healthy controls within a discovery (n = 196 vs n = 110; NCT02890121) and an independent validation cohort (n = 30 vs n = 83; NCT02890134) from the European PRECISESADS project. Validated daAABs were analysed in relation to global and organ-specific disease activity using linear and logistic regression, along with daAAB target pathway enrichment analysis.

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Results: We validated 89 IgG and 66 IgA daAAbs. IgG anti-LIN28A, IgG anti-HMGN5, and both isotypes for anti-IRF5 and anti-TGIF1 were associated with a SLE disease activity index 2000 score of ≥ 10 , negatively associated with lupus low disease activity state, and highly prevalent in subgroups with active disease across organ manifestations. IgG anti-LIN28A levels exceeded the cutoff for positivity in 53% of patients with central nervous system (CNS) involvement, a prevalence higher than that observed for anti-double-stranded DNA (20%) and 47% of patients with renal activity. A cluster of IgG and IgA daAAbs against RNA-binding proteins, including anti-LIN28A, was linked to CNS involvement. IgA anti-FOSL2 was elevated uniquely in patients with musculoskeletal activity. Enriched pathways involving DNA binding and repair showed considerable overlap across manifestations.

Conclusions: Novel IgG and IgA autoantibodies, including IgG anti-LIN28A, IgG anti-HMGN5, IgG and IgA anti-IRF5, and IgG and IgA anti-TGIF1 were associated with SLE disease activity and highly abundant across organ manifestations.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Autoantibodies are a hallmark of systemic lupus erythematosus (SLE), but among a multitude of autoantigen specificities, few are mapped and used in routine clinical practice.
- Autoantibodies currently used for surveillance, such as anti-double-stranded DNA (anti-dsDNA), show only modest associations with SLE disease activity.

WHAT THIS STUDY ADDS

- IgG anti-LIN28A, IgG anti-HMGN5, IgG and IgA anti-IRF5, and IgG and IgA anti-TGIF1 were associated with high disease activity and negatively associated with lupus low disease activity state and were highly prevalent in subgroups of patients across organ manifestations.
- Extensive profiling of IgA autoantibodies in SLE is novel and suggests a potential role for mucosal immunity in SLE pathogenesis.
- IgG anti-LIN28A levels exceeded the cutoff for positivity in more than half of patients with central nervous system (CNS) involvement, whereas only a fifth of patients with CNS activity were anti-dsDNA positive.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- This study demonstrated associations between novel IgG and IgA autoantibodies, identified in a large discovery SLE cohort and validated in an independent inception cohort, and SLE disease activity, as well as high abundance of several of these autoantibodies across organ manifestations; autoantibody profiling using these novel autoantibodies may better characterise patients with SLE, predict impending flares, and guide personalised therapies.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototypical autoimmune disease, characterised by the presence of multiple autoantibodies that have been associated with its pathogenesis and clinical manifestations. These autoantibodies target self-antigens exposed during cell death or tissue damage, forming immune complexes that either get deposited from circulation or form locally in tissues and trigger inflammatory responses, ultimately resulting in organ damage [1,2]. Additionally, autoantibodies contribute to SLE pathogenesis by interacting with cell surface receptors and penetrating cells, thereby altering cellular processes and contributing to the phenotypical heterogeneity of SLE [3,4].

Antinuclear antibodies (ANAs) are the most frequently tested in clinical practice, with specific antibodies such as anti-double-stranded DNA (dsDNA) being both diagnostic and correlating, although modestly, with disease activity [5]. However, many other autoantibodies have been identified, expanding our understanding of the complexity of SLE [6]. Notably, autoantibody testing in clinical practice and research has predominantly focused on the immunoglobulin (Ig)G isotype. However, other isotypes, such as IgA and IgM, may also have relevance in SLE. For example, while non-IgG antiphospholipid antibodies are not universally accepted for their relevance in the classification of antiphospholipid syndrome, studies suggest they may contribute to disease mechanisms [7]. This underscores the potential value of expanding autoantibody profiling to obtain a more comprehensive understanding of the immunologic landscape of SLE.

Understanding the role of autoantibodies in SLE is essential for improving diagnostics, monitoring, prognostication, and the development of novel therapies, particularly in the era of personalised medicine. The chronic nature of SLE, characterised by alternating episodes of flares and remission, necessitates regular monitoring [8]. Identifying an increase in SLE disease activity can be challenging in routine clinical practice due to the heterogeneity of the disease and the subtlety of early signs of flares or unspecific symptoms. Early identification of increasing disease activity and evolution towards a flare is crucial for rapid treatment intervention, as high disease activity is associated with poor outcomes, including accrual of organ damage, which is linked to increased morbidity and mortality [9]. The 2023 European Alliance of Associations for Rheumatology recommendations state that the aim of SLE treatment is remission or low disease activity when remission is not achievable [10]. These goals are of clinical [11–13] and biological [14] relevance in SLE, and optimising surveillance and treatment evaluation is of great importance towards their induction and maintenance, especially in the light of new targeted therapies for SLE [15] and the growing awareness of the long-term adverse effects of glucocorticoids (GCs) [1]. Hence, to maximise the benefits of advanced treatments, identifying novel and reliable biomarkers of disease activity and impending flares remains an urgent unmet need.

To address this need, we aimed to uncover novel biomarkers that could enhance our ability to monitor SLE disease activity more accurately and tailor treatments more effectively using the i-Ome Discovery protein microarray (Sengenics Corporation) that encompasses 1609 protein autoantigens. We specifically screened for IgG and IgA specificities against these autoantigens. This high-throughput immunoassay platform uses KoRectly EXpressed (KREX) technology, which preserves the structure of

correctly folded full-length proteins, ensuring specific and biologically meaningful binding. Our primary goal was to identify novel autoantibodies that reflect global and organ-specific SLE disease activity.

METHODS

Study population

We used plasma samples from 2 independent multicentre cohorts of patients with SLE and healthy controls (HCs) collected within the frame of the 5-year European PRECISESADS (Molecular reclassification to find clinically useful biomarkers for systemic autoimmune diseases) project [16]. Specifically, 199 patients with SLE from a cross-sectional cohort and 111 HCs formed a discovery cohort (NTC02890121), and 30 patients with SLE from an inception cohort, enrolled within 1 year from diagnosis, along with 84 HCs formed a validation cohort (NCT02890134). Patients with SLE in the validation cohort underwent longitudinal sampling at baseline and at 6 and 14 months. All patients with SLE met the revised 1997 American College of Rheumatology criteria for SLE [17]. Patients were excluded if they had received high doses of prednisone equivalents (>15.0 mg/d), intravenous GCs, or high doses of immunosuppressants within 3 months preceding recruitment. Additionally, treatment with cyclophosphamide or belimumab in the past 6 months and rituximab in the past year was not permitted. The complete set of inclusion and exclusion criteria is available online in [Supplementary Table S1](#).

Before recruitment in PRECISESADS, all patients and HCs provided written informed consent. The PRECISESADS protocol was approved by local ethics review boards at all participating centres ([Supplementary Material, page 3](#), for a list of local investigators). This study was approved by the Swedish Ethical Review Authority (registration number: 2022-03907-01).

Clinical definitions

The Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) [18] and physician's global assessment (PGA; on a scale 0–100) were used to evaluate global SLE disease activity, with a SLEDAI-2K score ≥ 10 indicating high disease activity, as previously suggested [9].

Low disease activity was defined according to the Lupus Low Disease Activity State (LLDAS) criteria and required a SLEDAI-2K score of ≤ 4 , excluding major organ activity and fever, no new activity since the previous assessment, and a Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA)-SLEDAI PGA score of ≤ 1 (on a scale 0–3), allowing for a prednisone or equivalent dose ≤ 7.5 mg/d and immunosuppressive or approved biological agents at standard and tolerable doses [19].

Remission was defined according to the Definition Of Remission In Systemic lupus erythematosus (DORIS) criteria and required a clinical SLEDAI-2K score of 0 and a SELENA-SLEDAI PGA score of <0.5 (on a scale 0–3), allowing for serological activity and use of low-dose GCs (prednisone or equivalent ≤ 5.0 mg daily) and immunosuppressive or biological agents at standard and tolerable doses [20].

Active disease within an organ system was defined as a score above zero in at least 1 descriptor within the respective SLEDAI-2K organ system. Based on the central nervous system (CNS) organ system of SLEDAI-2K, we subgrouped the patients with active CNS lupus into patients with focal and patients with

diffuse syndromes [21]. In cases where both focal and diffuse syndromes were present, major involvement was given priority over minor, as previously defined by Ainiola et al. [22]. We defined current or past involvement of a particular organ system using information from SLEDAI-2K organ system scores, indicating active disease, and report of previous involvement as per the case report form of the PRECISESADS study protocol ([Supplementary Material, pages 4–5](#)).

Autoantibody measurement and gene expression analysis

Plasma samples were screened for IgG and IgA autoantibody specificities against a panel of 1609 human proteins using the i-Ome Discovery protein microarray (Sengenics Corporation; [Supplementary Material, sheet 1](#)) [23]. This high-throughput immunoassay platform uses KREX technology, which preserves the native 3-dimensional conformation of full-length proteins, thereby preserving *in vivo*-relevant epitopes and ensuring specific and biologically meaningful binding of autoantibodies to their targets. A detailed description is provided in [Supplementary Material, page 6](#).

Comparisons with conventional autoantibody specificities were enabled by measurements of IgG anti-dsDNA, IgG anti-Smith (anti-Sm), and IgG anti-U1RNP levels using an automated chemiluminescent immunoassay (IDS-iSYS; Immunodiagnostic Systems Holdings). IgG antichromatin levels were measured using an anti-dsDNA–NcX ELISA assay (EUROIMMUN Medizinische Labordiagnostika AG). Anti-dsDNA and antichromatin positivity were defined as levels ≥ 40.0 and ≥ 100.0 units/mL, respectively, as per assay protocols [24]. Genome-wide RNA sequencing of peripheral whole-blood samples was performed using Illumina assays (Illumina), as previously detailed [16].

Bioinformatics and statistical analysis

We conducted an analysis of differentially abundant autoantibodies (daAABs) using the limma R package [25], adjusting for polyspecific antibody (PSA) status, recruiting centre, age, and batch, all of which significantly impacted data composition. PSA status was determined based on antigens known to be targeted by the PSA phenotype ([Supplementary Material, sheet 2](#)). We first analysed daAABs in patients with SLE vs HC from the discovery and validation cohorts separately. daAABs in the discovery cohort with a false discovery rate–corrected *P* value of <0.05 that were also found among daAABs in the validation cohort with a *P* value of <0.05 were considered validated. Validated daAABs qualified for subsequent analysis in relation to SLEDAI-2K using multivariable linear regression and in relation to a SLEDAI-2K score of ≥ 10 , LLDAS, and DORIS remission using multivariable logistic regression in the discovery cohort, adjusting for age and sex. Participant sex was self-reported. Validated daAABs that were both positively associated with a SLEDAI-2K score of ≥ 10 and negatively associated with LLDAS qualified for analysis of autoantibody positivity, defined as levels higher than the median plus 2 IQRs of the distribution of levels of the autoantibody in the HC population of the discovery cohort. A comparison of autoantibody positivity between subgroups of patients with focal and diffuse CNS lupus was performed using the Fisher exact test. These daAABs also qualified for longitudinal assessments in relation to PGA in the validation cohort and for correlation analysis between levels of daAABs and conventional autoantibody specificities currently used in clinical practice in the discovery cohort. In addition, gene expression of the genes encoding the daAAB protein targets was analysed in relation to

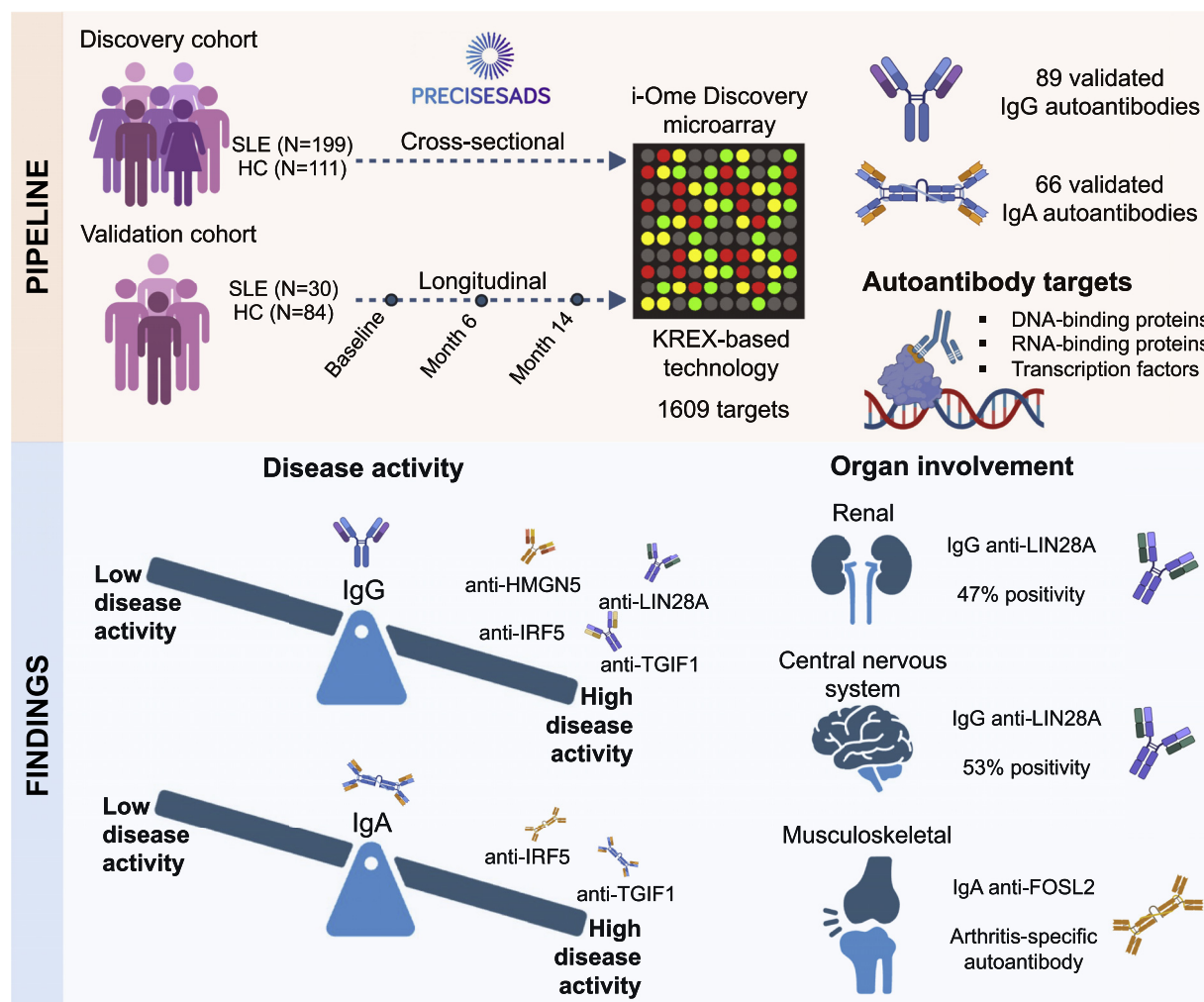


Figure 1. Schematic overview of the analytical pipeline as well as validated autoantibodies and their association with global disease activity and organ involvement. Plasma from patients with SLE in 2 independent cohorts was screened for IgG and IgA seroreactivity against 1609 protein autoantigens using the i-Ome Discovery microarray (Sengenics), with 3 longitudinal assessments conducted in the smaller validation cohort. The analysed autoantibody targets included DNA-binding and RNA-binding proteins as well as transcription factors. Among autoantibodies associated with high disease activity, IgG anti-LIN28A positivity was observed in about half of the patients with active renal and central nervous system involvement. HC, healthy control; KREX, KoRectly EXpressed; SLE, systemic lupus erythematosus.

SLEDAI-2K using multivariable linear regression and in relation to SLEDAI-2K of ≥ 10 , LLDAS, and DORIS remission using multivariable logistic regression in the discovery cohort, adjusting for age and sex.

Validated daAAbs also qualified for subsequent analysis of daAAbs across SLE manifestations. A detailed description of the analytical pipeline is found in [Supplementary Material, pages 6–8](#). The analytical pipeline as well as key findings are summarised in [Figure 1](#).

Patient and public involvement

The patient research partners Yvonne Enman and Karin Blomkvist Sporre were involved in the design and dissemination plans of this research. The public was not involved in the design, conduct, reporting, or dissemination plans of this research.

RESULTS

Characteristics of the 196 patients with SLE and 110 HCs in the discovery cohort as well as the 30 patients with SLE and 83 HCs in the validation cohort are presented in [Table](#).

Differentially abundant autoantibodies associated with SLE

We identified 109 IgG daAAbs in patients with SLE ($n = 195$) vs HC ($n = 108$) in the discovery cohort, of which 89 were validated in the independent inception cohort ([Fig 2A,B](#); [Supplementary Material, sheets 2–3](#)). We identified 115 IgA daAAbs in patients with SLE ($n = 196$) vs HC ($n = 110$) in the discovery cohort, of which 66 were validated in the independent inception cohort ([Fig 2C,D](#); [Supplementary Material, sheets 3–6](#)).

Differentially abundant autoantibodies associated with SLE disease activity

To explore potential associations between validated daAAbs and SLE disease activity, we assessed IgG and IgA daAAb levels in relation to several key clinical measures: SLEDAI-2K, high disease activity (SLEDAI-2K ≥ 10), LLDAS, and DORIS remission ([Supplementary Material, sheets 7–14](#)). Among 89 validated IgG daAAbs, 17 IgG daAAbs were associated with SLEDAI-2K ([Fig 3A](#)), with anti-ACPP and anti-PABPC1 exhibiting the lowest and highest coefficients, respectively. Additionally, 21 IgG daAAbs were associated with SLEDAI-2K of ≥ 10 ([Fig 3B](#)), including anti-ACPP and anti-PABPC1; 56 IgG daAAbs were

Table
Baseline characteristics of patients with SLE and healthy controls in the discovery and validation cohorts

	Discovery cohort		Validation cohort	
	SLE (n = 199)	HC (n = 111)	SLE (n = 30)	HC (n = 84)
Demographics				
Age (y)	46.9 ± 14.2	48.8 ± 15.0	35.2 ± 11.9	48.6 ± 13.2
Female sex	188 (94.5)	105 (94.6)	24 (80.0)	79 (94.0)
Ancestry				
Black/African American	0 (0.0)	0 (0.0)	3 (10.0)	0 (0.0)
Caucasian/White	199 (100.0)	111 (100.0)	25 (83.3)	84 (100.0)
Other	0 (0.0)	0 (0.0)	2 (6.7)	0 (0.0)
Clinical data				
Disease duration (y)	14.7 ± 10.2	NA	NA	NA
SLEDAI-2K score	6.9 ± 6.2; n = 193	NA	NA	NA
CNS (vs active non-CNS SLE)	18 (39.1); n = 46	NA	NA	NA
Vascular (vs active nonvascular SLE)	12 (25.5); n = 47	NA	NA	NA
Musculoskeletal (vs active nonmusculoskeletal SLE)	31 (67.4); n = 46	NA	NA	NA
Renal (vs active nonrenal SLE)	45 (52.9); n = 85	NA	NA	NA
Dermal (vs active nondermal SLE)	103 (90.4); n = 114	NA	NA	NA
Serosal (vs active nonserosal SLE)	5 (4.4); n = 113	NA	NA	NA
Immunologic (vs active nonimmunologic SLE)	128 (84.8); n = 151	NA	NA	NA
Constitutional (vs active nonconstitutional SLE)	3 (2.2); n = 139	NA	NA	NA
Haematologic (vs active nonhaematologic SLE)	39 (57.4); n = 68	NA	NA	NA
PGA score (0-100)	NA	NA	28.8 (22.6)	NA
LLDAS	73 (38.6); n = 189	NA	NA	NA
DORIS remission	29 (15.2); n = 191	NA	NA	NA
SLE manifestations (ever)	n = 193			
Neuropsychiatric	32 (16.6)	NA	NA	NA
Vascular	16 (8.3)	NA	NA	NA
Musculoskeletal	164 (85.0)	NA	NA	NA
Renal	71 (36.8)	NA	NA	NA
Mucocutaneous	172 (89.1)	NA	NA	NA
Serosal	43 (22.3)	NA	NA	NA
Immunologic	163 (84.5)	NA	NA	NA
Constitutional	12 (6.2)	NA	NA	NA
Haematologic	144 (74.6)	NA	NA	NA
Medications (current use)				
Glucocorticoids	102 (51.3)	NA	6 (20.0)	NA
Prednisone equivalent dose during follow-up (mg/d); median (IQR)	1.3 (0.0-5.0)	NA	NA	NA
Antimalarial agents	156 (78.4)	NA	16 (53.3)	NA
Immunosuppressants				
Azathioprine	60 (30.3); n = 198	NA	0 (0.0)	NA
Calcineurin inhibitors	2 (1.0)	NA	0 (0.0)	NA
Leflunomide	0 (0.0); n = 198	NA	0 (0.0)	NA
Methotrexate	16 (8.1); n = 198	NA	0 (0.0)	NA
Mycophenolic acid	25 (12.6)	NA	0 (0.0)	NA

ANA, antinuclear antibody; CNS, central nervous system; DORIS, definition of remission in SLE; HC, healthy control; LLDAS, lupus low disease activity state; NA, not applicable; PGA, physician's global assessment; SLE, systemic lupus erythematosus; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000.

Data are presented as numbers (%) or means ± standard deviation. In case of nonnormal distributions, the medians (IQR) are indicated. In case of missing values, the total number of patients with available data is indicated.

associated with LLDAS (Fig 3C), including anti-NHLH1 and anti-PRSS50; and 28 IgG daAABs were associated with DORIS remission (Fig 3D), including anti-RPL5 and anti-PRKACB.

Among 115 validated IgA daAABs, we found anti-RNF7 and anti-FEN1 to be associated with SLEDAI-2K (Fig 3E), and 6 with a SLEDAI-2K score of ≥10 (Fig 3F), including anti-RNF7 and anti-TSC22D4. We also identified 34 IgA daAABs that were associated with LLDAS (Fig 3G), including anti-ATF2 and anti-RNF7. IgA anti-E2F6, IgA anti-APOBEC3G, and IgA anti-MAPK12 were associated with DORIS remission (Fig 3H).

Autoantibody positivity across SLE manifestations

To investigate potential associations between validated daAABs and SLE manifestations, we calculated the proportions of autoantibody-positive patients stratified by organ system and compared them with the proportion of autoantibody-positive

patients in DORIS remission as well as HCs. To qualify for this investigation, validated daAABs were required to be both positively associated with high disease activity (a SLEDAI-2K score of ≥10) and negatively associated with LLDAS. Nine IgG daAABs fulfilled this requirement; ranked in decreasing order of log₂ fold change (FC) in patients with SLE vs HCs in the discovery cohort, those were anti-LIN28A, anti-HMG5, anti-IRF5, anti-TGIF1, anti-NHLH1, anti-ZNF276, anti-PABPC1, anti-IGF2BP3, and anti-RARA (Fig 4A-I; Supplementary Material, sheet 15). Notably, 53% (9/17) of patients with CNS activity and 47% (20/43) of patients with renal activity exhibited positive levels of IgG anti-LIN28A, in comparison with 21% (6/29) of patients in DORIS remission. In a subgroup analysis of patients with active CNS lupus, no difference was observed in the proportion of patients with positive levels of IgG anti-LIN28A between those with diffuse (3/4; 75%) and those with focal syndromes (3/6; 50%; *P* = .571). Additionally, 29% (9/31) of patients with

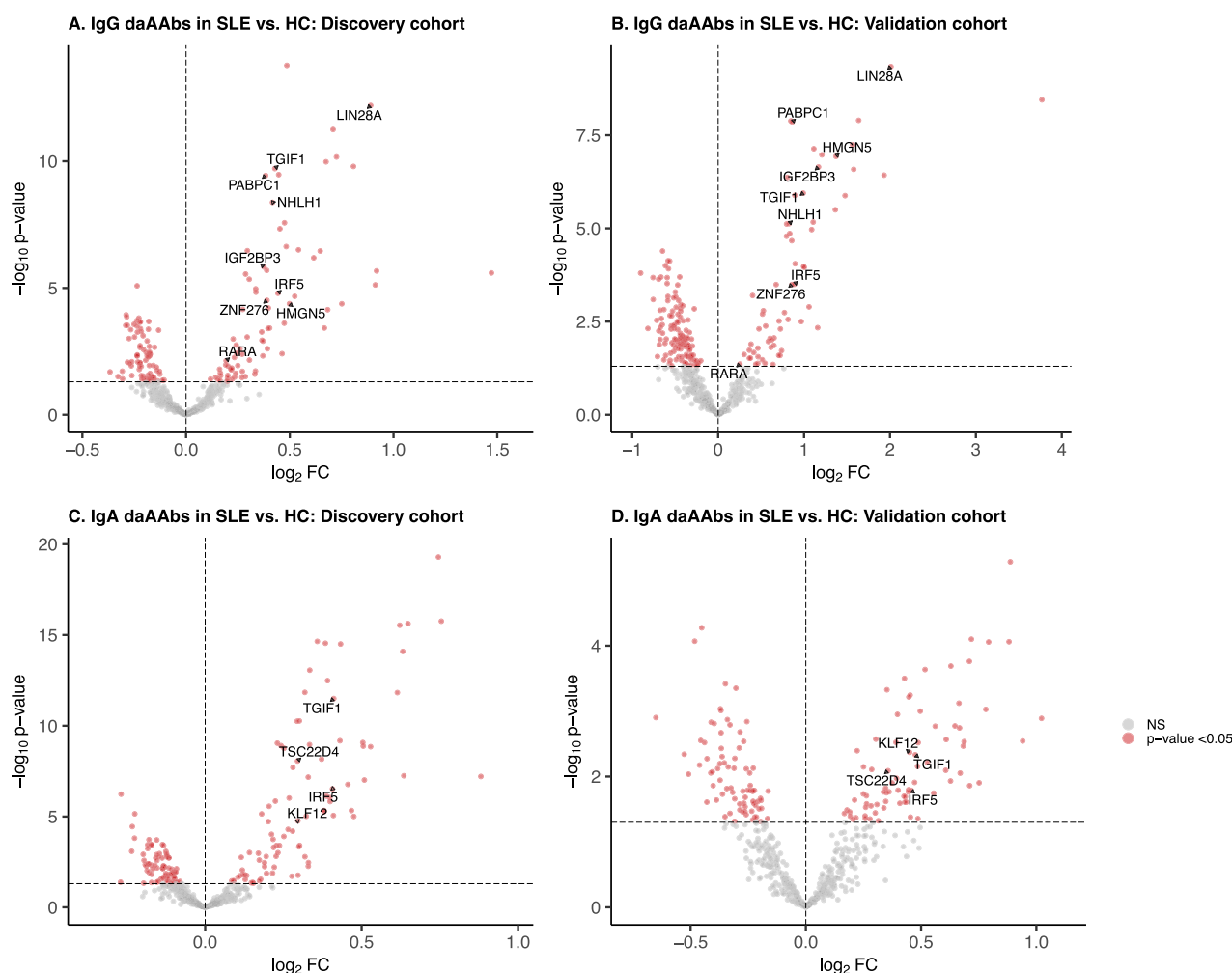


Figure 2. IgG and IgA daAABs in patients with SLE vs HC. The volcano plots display IgG daAABs in the (A) discovery and (B) validation cohorts, as well as IgA daAABs in the (C) discovery and (D) validation cohorts. daAABs with a $P < .05$ are highlighted in red, while nonsignificant daAABs are in grey. Selected daAABs are labelled, if associated with SLEDAI-2K of ≥ 10 and negatively associated with LLDAS, with the full list of daAABs provided in [Supplemental Material](#), sheets 3-6. daAAB, differentially abundant autoantibody; FC, fold change; HC, healthy control; LLDAS, lupus low disease activity state; NS, nonsignificant; SLE, systemic lupus erythematosus; SLEDAI-2K, systemic lupus erythematosus disease activity index 2000.

musculoskeletal activity were IgG anti-HMGN5 positive, and the proportions were even higher for the other SLE manifestations (30%–67%) except for vascular activity (1/11; 9%), in comparison with only 3% (1/29) in patients with DORIS remission.

Similarly, 4 validated IgA daAABs fulfilled the requirement of associations with high disease activity and LLDAS; ranked in decreasing order of $\log_2$ FC in patients with SLE vs HC in the discovery cohort, those were anti-TGIF1, anti-IRF5, anti-KLF12, and anti-TSC22D4 (Fig 4J–M; [Supplementary Material](#), sheet 16). The proportion of patients with positive levels of IgA anti-TGIF1 was 33% (6/18) in patients with CNS activity and ranged between 41% and 80% across the other organ systems except for vascular disease (2/11; 18%), in comparison with 28% (8/29) in patients with DORIS remission. A similar pattern was observed for IgG anti-TGIF1. The proportion of patients with positive levels of IgA anti-IRF5 were 28% (5/18) in patients with CNS activity and ranged between 33% and 60% across the other organ systems except for vascular disease (2/11; 18%), in comparison with 17% (5/29) in patients with DORIS remission.

The proportion of patients with positive levels of anti-dsDNA ranged between 20% and 100% across SLE manifestations. Among patients with CNS activity, 20% (3/15) were anti-dsDNA positive. The proportion of patients with positive levels of

antichromatin ranged between 57% and 100% across SLE manifestations ([Supplementary Table S2](#)).

Longitudinal variation in disease activity and levels of differentially abundant autoantibodies in SLE

PGA scores and levels of all validated IgG and IgA daAABs that were associated with high disease activity and negatively associated with LLDAS generally decreased over the 14-month follow-up in the validation cohort ([Supplementary Fig S1](#); [Supplementary Material](#), sheet 17). Autoantibody levels showed mild reductions, with the possible exception for IgG RARA.

Correlations between levels of differentially abundant autoantibodies and conventional autoantibody specificities

Levels of validated IgG daAABs that met the criteria for associations with high disease activity and LLDAS correlated most strongly with anti-dsDNA and antichromatin, with modest correlations observed for IgG Sjögren's-syndrome-related antigen B (SSB) (Fig 5A; [Supplementary Table S3](#)). Levels of validated IgA daAABs that met the same criteria correlated most strongly with

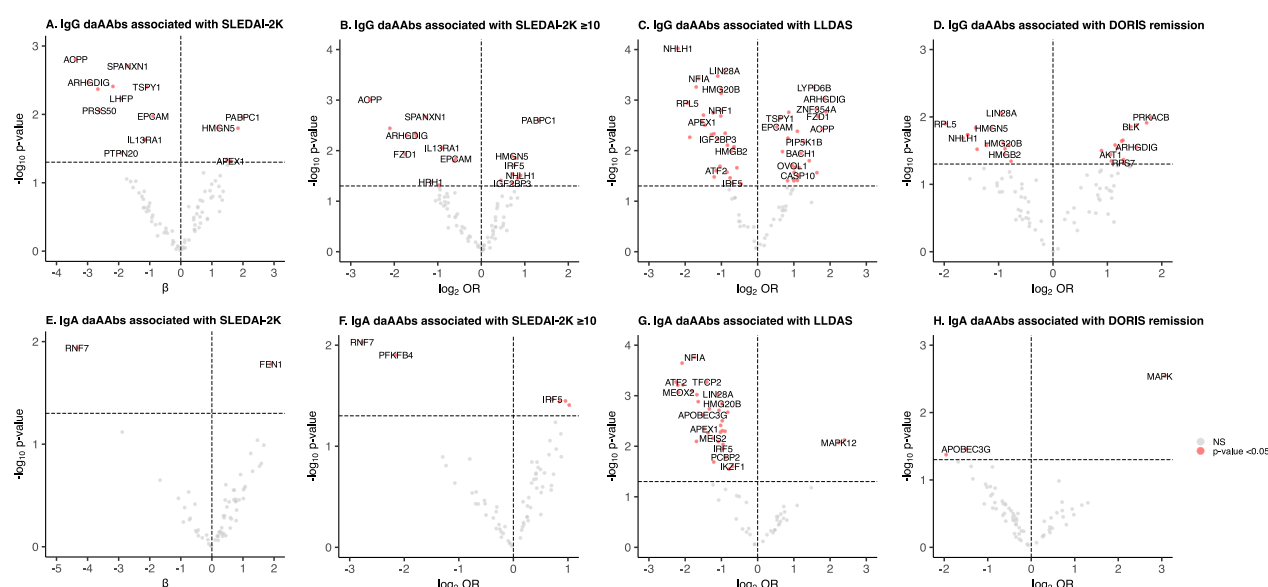


Figure 3. IgG and IgA daAABs associated with SLE disease activity. The volcano plots illustrate IgG daAABs in patients with SLE vs HC in the discovery cohort, associated with (A) SLEDAI-2K using multivariable linear regression, and (B) SLEDAI-2K ≥ 10 , (C) LLDAS, and (D) DORIS remission using multivariable logistic regression. The volcano plots show IgA daAABs in patients with SLE vs HC in the discovery cohort, associated with (E) SLEDAI-2K using multivariable linear regression and (F) SLEDAI-2K ≥ 10 , (G) LLDAS, and (H) DORIS remission using multivariable logistic regression. daAABs with a P value < 0.05 are highlighted in red, while nonsignificant daAABs are in grey. The labelling of daAABs is stochastic, and a full list of daAABs are provided in [Supplemental Material, sheets 7–14](#). daAAB, differentially abundant autoantibody; DORIS, definition of remission in SLE; HC, healthy control; LLDAS, lupus low disease activity state; NS, nonsignificant; SLE, systemic lupus erythematosus; SLEDAI-2K, systemic lupus erythematosus disease activity index 2000.

IgA anti-SSB, while modest correlations were seen with anti-dsDNA and antichromatin ([Fig 5B](#); [Supplementary Table S3](#)).

Gene expression of differentially abundant autoantibody targets associated with SLE disease activity

To investigate potential associations between the blood transcripts of daAAb targets linked to high disease activity and LLDAS, we assessed gene expression in relation to clinical measures, following a similar approach to that used for the validated daAABs ([Supplementary Material, sheets 18–21](#)). Among the 11 genes corresponding to the IgG and IgA daAAb targets, 6 were associated with SLEDAI-2K ([Supplementary Fig S2A](#)), with *PABPC1* and *RARA* showing the lowest and highest coefficients, respectively. Additionally, 4 genes were associated with SLEDAI-2K of ≥ 10 ([Supplementary Fig S2B](#)), including *PABPC1* and *NHLH1*, 7 genes were associated with LLDAS ([Supplementary Fig S2C](#)), including *RARA* and *PABPC*, and 4 genes were associated with DORIS remission ([Supplementary Fig S2D](#)), including *NHLH1* and *TGIF1*.

Differentially abundant autoantibodies associated with SLE manifestations

To also examine organ-specificity for validated daAABs in an exploratory manner, we performed subgroup daAAb analysis stratified by organ system vs HC in the discovery cohort ([Fig 6A, B](#); [Supplementary Material, sheets 22–23](#)). IgG anti-*RARA* and IgG anti-*SOX15* were solely elevated in patients with renal and immunologic activity, IgG anti-*ELF1* and IgG anti-*ERG* were exclusively elevated in patients with dermal and immunologic activity, and IgG anti-*USF2* was uniquely elevated in patients with serosal and immunologic activity. IgA anti-*E2F6* and IgA anti-*SRPK1* were solely elevated in patients with renal and

immunological activity, IgA anti-*FOSL2* was exclusively elevated in patients with musculoskeletal activity, and IgA anti-*FEN1* was uniquely elevated in patients with dermal and immunologic activity.

Among IgG and IgA daAABs in patients with current or past activity in one or more organ systems compared with HCs in the discovery cohort ([Supplementary Fig S3A,B](#); [Supplementary Material, sheets 24–25](#)), IgG anti-*SOX15* was solely elevated in patients with current or past musculoskeletal and immunologic activity. IgA anti-*FOSL2* was uniquely elevated in patients with current or past serosal activity, and IgA anti-*UBE2I* was exclusively elevated in patients with current or past musculoskeletal and immunologic activity.

Clusters of differentially abundant autoantibodies across SLE manifestations

We further performed cluster analysis for elevated IgG and IgA daAABs in patients with active disease and identified 3 autoantibody clusters for each isotype ([Fig 6C,D](#)). For IgG daAABs, cluster 1 comprised RNA-binding proteins such as SSB and *LIN28A* and was predominantly represented in patients with CNS, vascular, and musculoskeletal activity. Among the other 2 clusters, which were represented in patients with varying activity patterns, IgG cluster 2 included transcription factors like *IRF5* and *ATF2*, while IgG cluster 3 encompassed DNA repair proteins such as *APEX1* and *FEN1*. For IgA daAABs, clusters 1 and 2 were represented in patients with varying activity patterns, whereas cluster 3 was predominantly represented in patients with CNS and vascular activity. IgA cluster 1 comprised DNA-binding proteins such as *HMGN5* and *ZNF276*, cluster 2 included the DNA repair proteins *APOBEC3G* and *FEN1*, while cluster 3 comprised RNA-binding proteins such as SSB and *LIN28A* as well as transcription factors such as *IRF5* and *ATF2*.

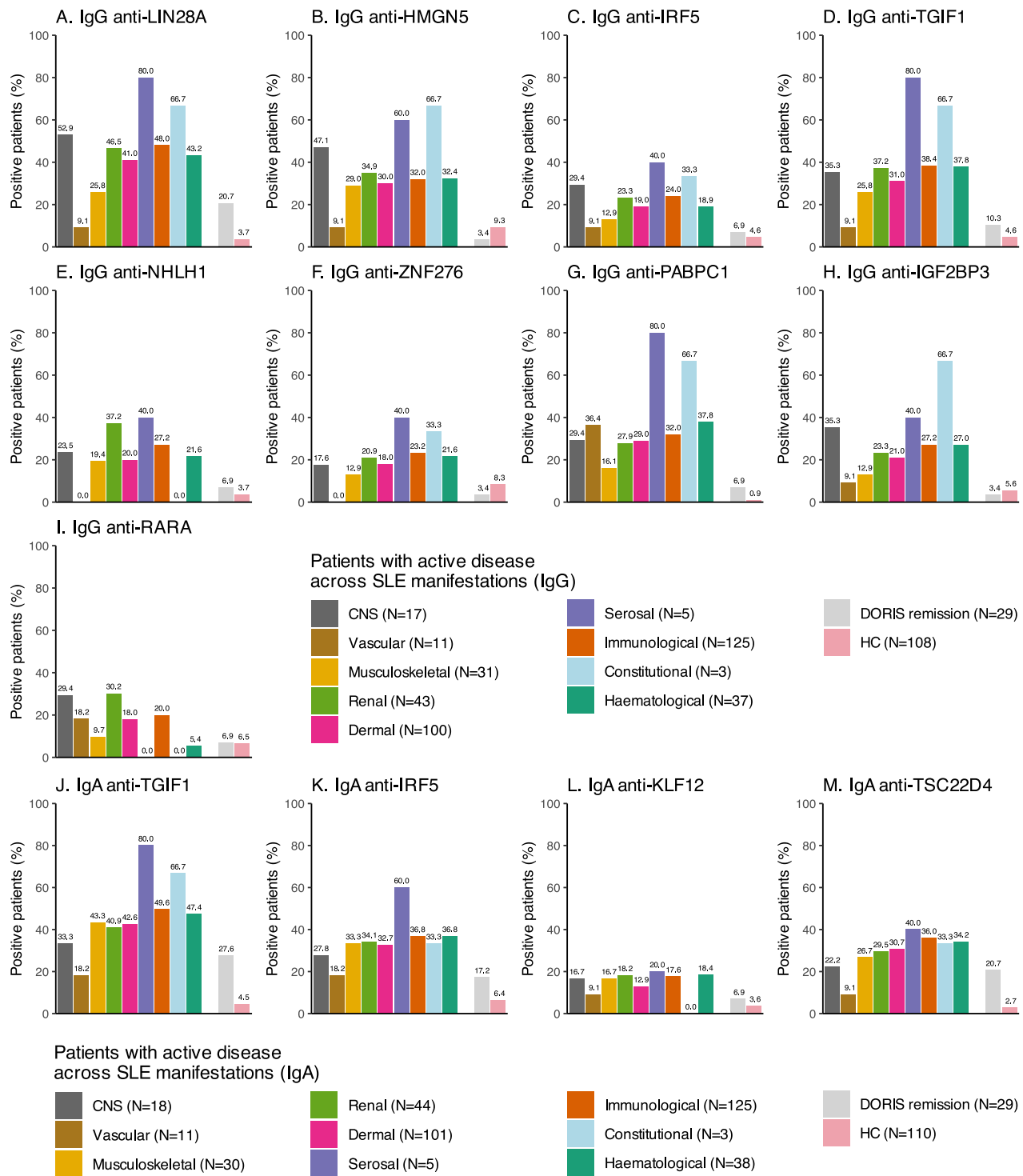
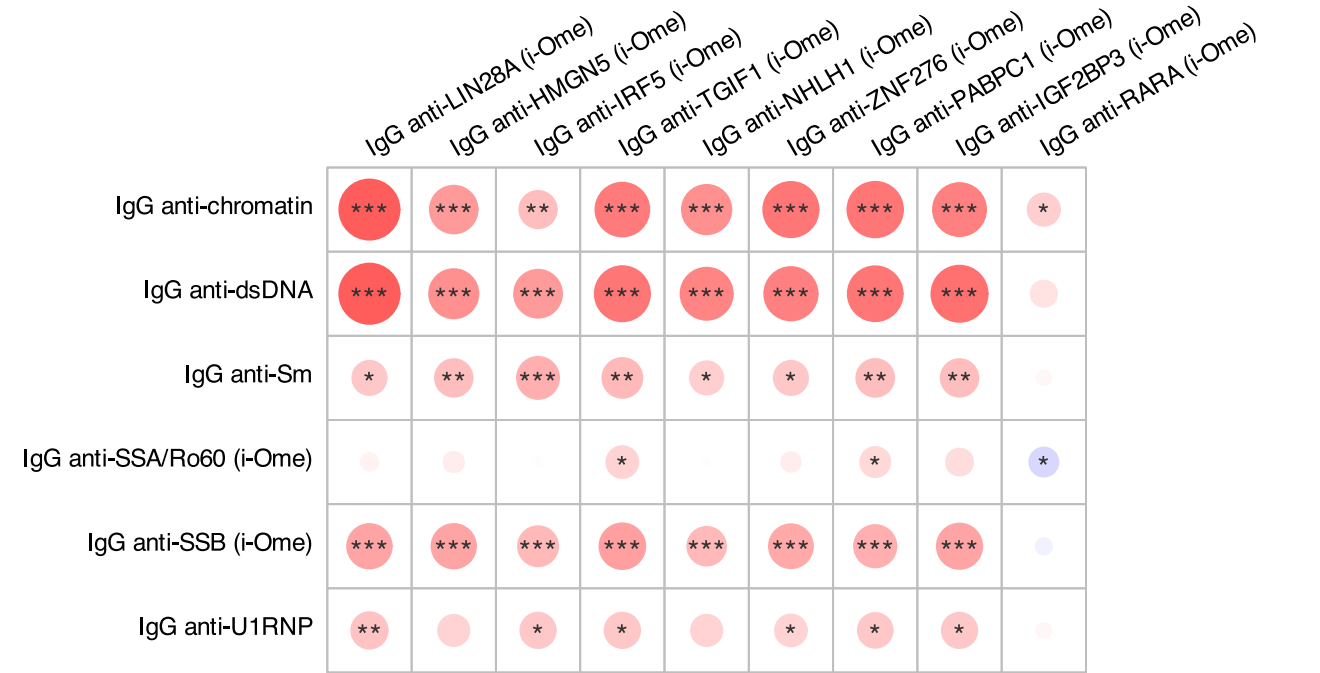


Figure 4. Proportions of patients with IgG and IgA autoantibody-positive SLE stratified by organ system and in comparison with the proportion of patients in DORIS remission as well as HC. The bar plots display the proportions of patients with autoantibody-positive SLE in the discovery cohort stratified by organ system and in comparison with the proportion of patients in DORIS remission for (A) IgG anti-LIN28A, (B) IgG anti-HMG5, (C) IgG anti-IRF5, (D) IgG anti-TGIF1, (E) IgG anti-NHLH1, (F) IgG anti-ZNF276, (G) IgG anti-PABPC1, (H) IgG anti-IGF2BP3, (I) IgG anti-RARA, (J) IgA anti-TGIF1, (K) IgA anti-IRF5, (L) IgA anti-KLF12, and (M) IgA anti-TSC22D4. The IgG and IgA daAabs are ranked in decreasing order of log₂ fold change in patients with SLE vs healthy controls. CNS, central nervous system; daAab, differentially abundant autoantibody; DORIS, definition of remission in SLE; HC, healthy control; SLE, systemic lupus erythematosus.

Similarly, we identified 3 autoantibody clusters of elevated IgG and IgA daAabs associated with current or past involvement of the different organ systems (Supplementary Fig S3C,D). We identified a cluster with RNA-binding proteins such as SSB and LIN28A,

which was predominantly represented in patients with current or past neuropsychiatric, vascular, and constitutional activity in the analysis of both isotypes, as well as in patients with current or past musculoskeletal activity in the analysis of IgA daAabs.

A. Correlations between levels of IgG daAAbs and conventional autoantibody specificities



B. Correlations between levels of IgA daAAbs and conventional autoantibody specificities

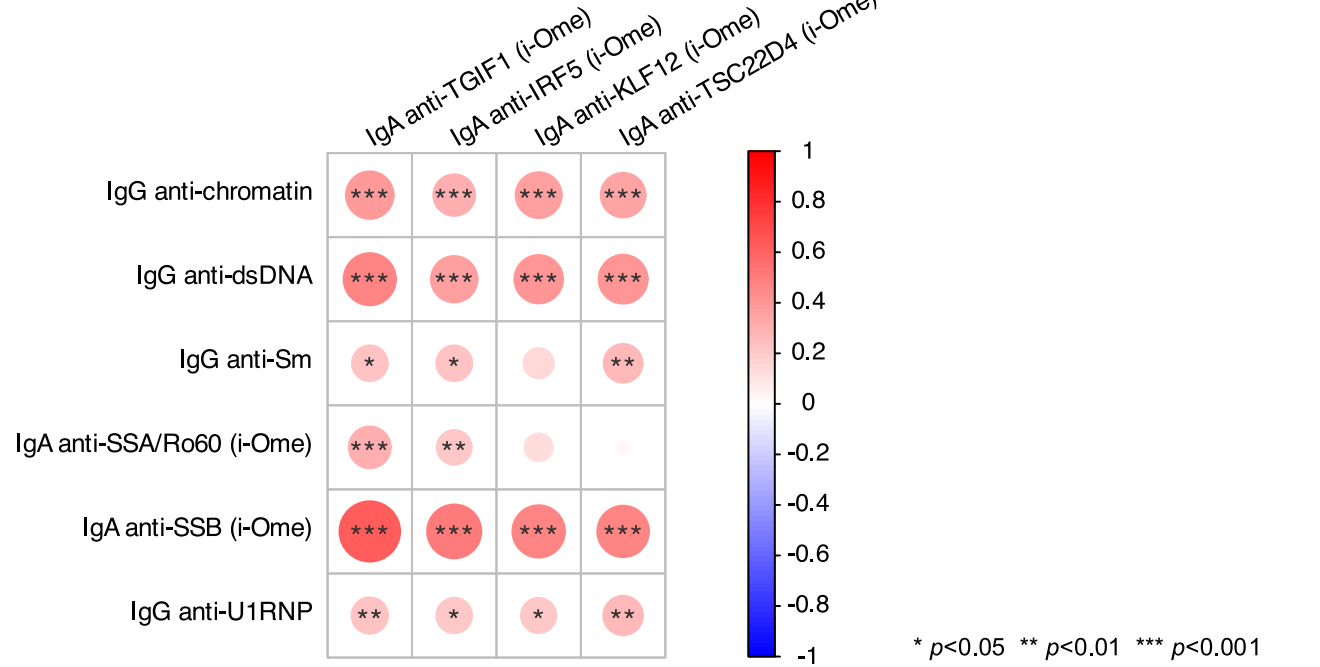


Figure 5. Correlations between levels of daAAbs and conventional autoantibody specificities. The correlation heatmaps shows Spearman rank correlation coefficients for correlations between levels of (A) IgG and (B) IgA daAAbs (measured using the i-Ome Discovery protein microarray) and levels of

Enriched pathways associated with differentially abundant autoantibody targets across SLE manifestations

Figure 7A illustrates the enriched gene ontology (GO) terms associated with IgG daAAb targets across SLE manifestations (Supplementary Material, sheet 26). Several SLE manifestations exhibited common enriched GO terms, which formed 3 pathway

clusters. Cluster 1 was linked to DNA repair, cluster 2 was related to cartilage development, RNA regulation, and serine/threonine kinase activity, and cluster 3 was linked to DNA binding, neuronal differentiation, and ubiquitin-protein ligase activity. Notably, LIN28A was included among the autoantigens within the enriched ‘positive regulation of neuron differentiation’ GO term and FOSL2 was included among the

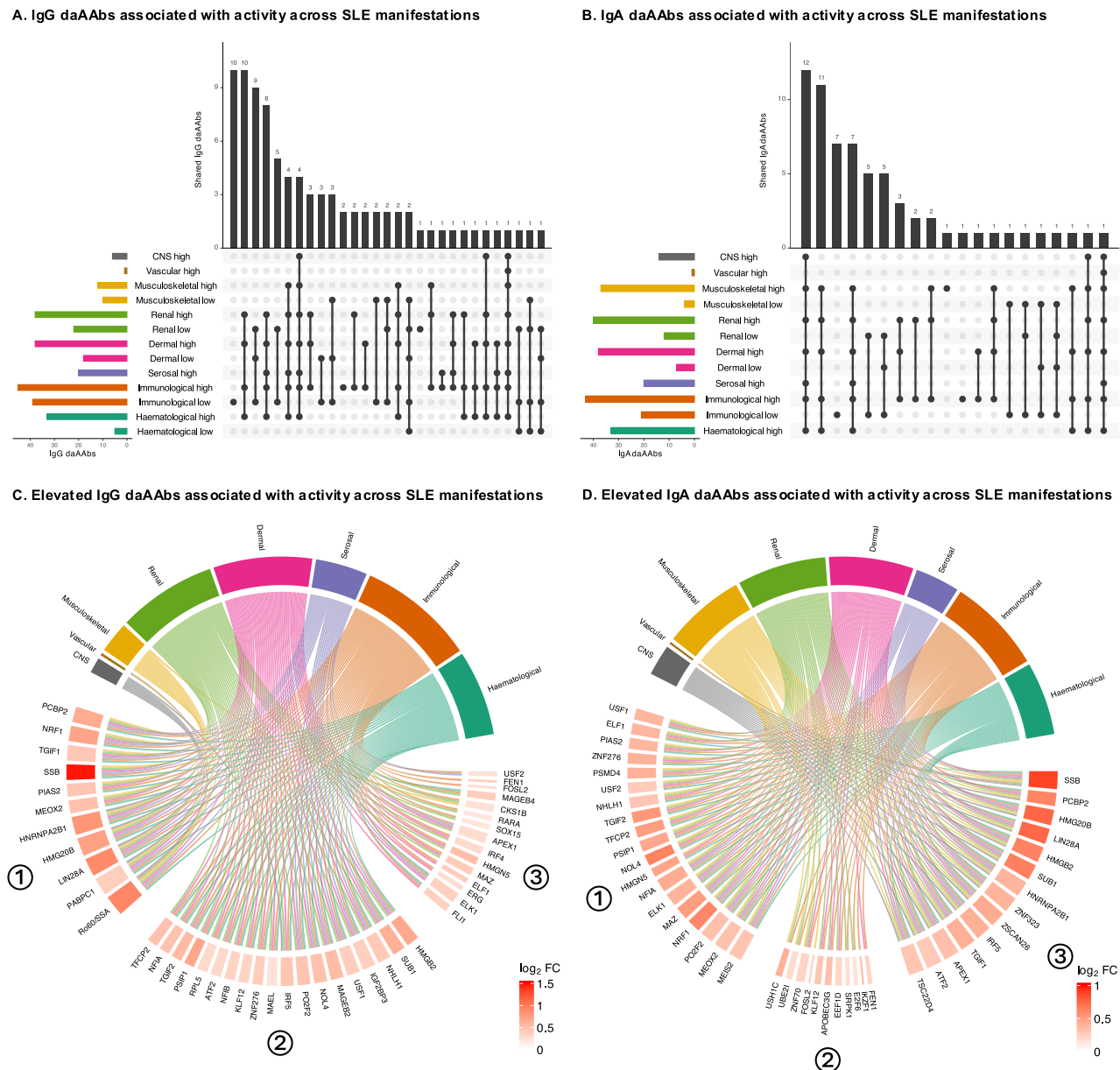


Figure 6. IgG and IgA daAAb in patients with active disease across SLE manifestations. The UpSet plots display (A) IgG and (B) IgA daAAb from subgroup daAAb analysis in patients with active SLE stratified by organ system with current activity vs healthy controls in the discovery cohort. The chord diagrams illustrate the log₂ FC of elevated (C) IgG and (D) IgA daAAb from the subgroup daAAb analysis. CNS, central nervous system; daAAb, differentially abundant autoantibody; FC, fold change; SLE, systemic lupus erythematosus.

autoantigens within the enriched ‘growth plate cartilage development’ GO term. No enriched KEGG or Reactome pathways were identified.

Similarly, SLE manifestations shared enriched GO terms associated with IgA daAAb targets, forming 3 pathway clusters (Fig 7B; Supplementary Material, sheet 27). Cluster 1 was related to DNA binding, cluster 2 was linked to RNA–protein interactions, and cluster 3 was related to nuclease activity. No enriched KEGG or Reactome pathways were identified.

DISCUSSION

Autoantibodies are a hallmark of autoimmunity, SLE in particular. In this study, we screened plasma samples from 2 independent cohorts of patients with SLE and HC for IgG and IgA seroreactivity against 1609 protein autoantigens. We identified and validated several novel IgG and IgA autoantibodies in

patients with SLE. Of particular interest were anti-LIN28A, anti-HMG5, anti-IRF5, and anti-TGIF1 antibodies, ranked in decreasing order of elevation in comparison with HC, whose IgG levels were associated with high disease activity and negatively associated with LLDAS. More than half of patients with CNS involvement had IgG LIN28A levels that exceeded the cutoff for positivity, whereas only a fifth of patients in DORIS remission were positive. Additionally, IgG and IgA LIN28A formed autoantibody clusters predominantly represented in patients with CNS activity. Upon further validation, these novel autoantibodies offer promise for early identification of active SLE or evolving flares, thus timely intervention and prevention of organ damage. Importantly, the IgA isotype of autoantibodies has not been sufficiently explored in previous literature of SLE and represents a significant novelty of this study, highlighting a potential role for mucosal immunity in SLE pathogenesis.

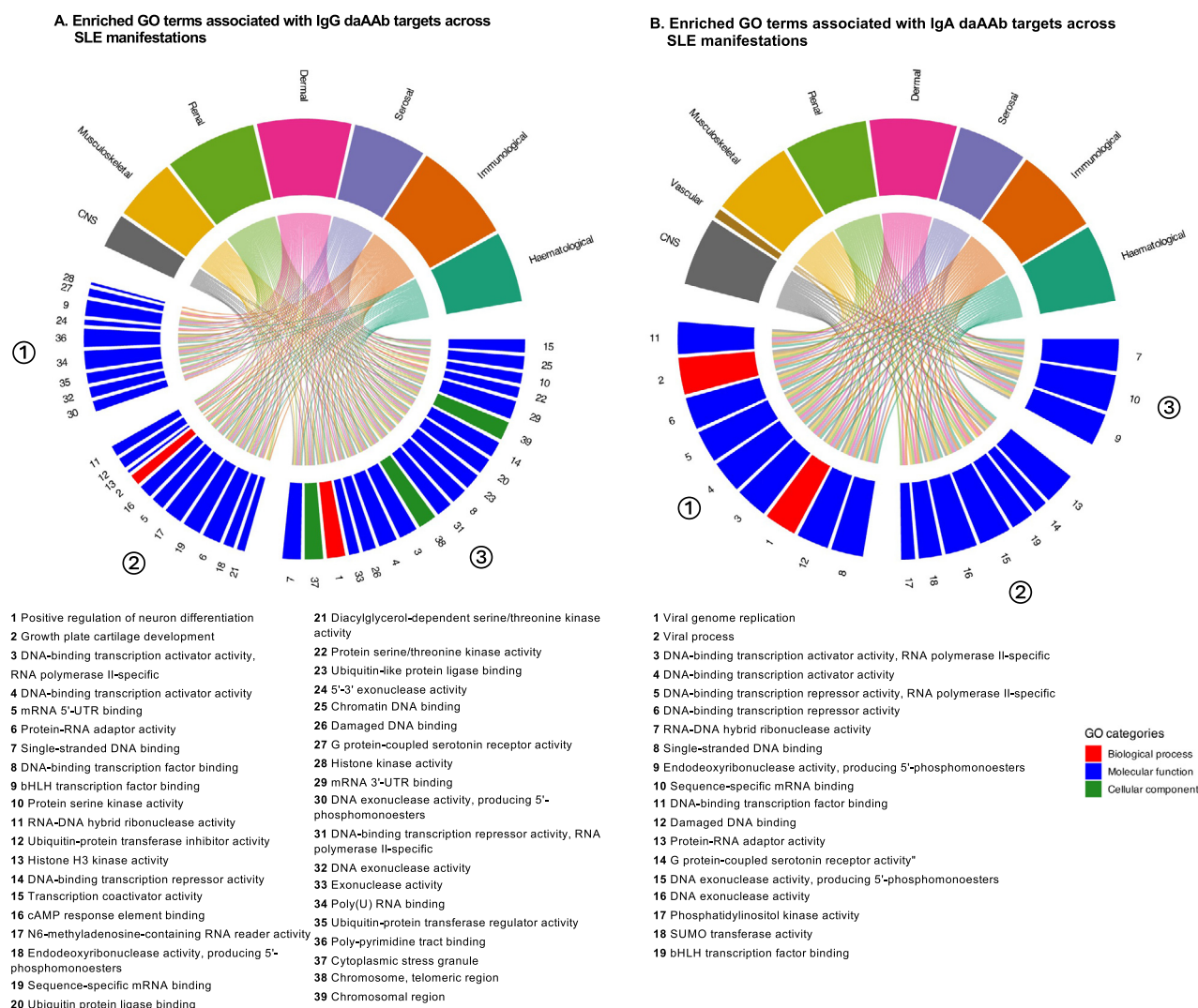


Figure 7. Enriched GO terms associated with IgG and IgA daAAb targets across SLE manifestations. The chord diagrams illustrate enriched GO terms from the enrichment analysis of (A) IgG and (B) IgA daAAb targets from subgroup daAAb analysis in patients with active SLE stratified by organ system with current activity vs healthy controls in the discovery cohort. CNS, central nervous system; daAAb, differentially abundant autoantibodies; GO, gene ontology; SLE, systemic lupus erythematosus.

Anti-LIN28A was associated with high disease activity (IgG) and negatively associated with LLDAS (IgG and IgA) and DORIS remission (IgG). Furthermore, in subgroup analysis across organ manifestations, IgG anti-LIN28A levels exceeded the cutoff for positivity in approximately half of the patients with CNS and renal activity, whereas only 21% of patients in DORIS remission were positive. Additionally, the IgG and IgA clusters that included anti-LIN28A were predominantly represented in patients with CNS activity, and LIN28A was found in an enriched pathway involving neuronal differentiation. In a recent analysis of the same cohorts, we found high specificity for SLE for IgG and IgA LIN28A compared with primary Sjögren's disease, systemic sclerosis, and HCs combined [26]. Interestingly, IgG anti-LIN28A was identified as one of the most prevalent novel autoantibodies in a previous study by Lewis et al. [27] using a protein microarray by Sengenics to screen for 1543 IgG autoantibodies in 2 independent cohorts of patients with SLE [27]. Similar to our findings, Lewis et al. [27] also identified IgG anti-IRF5, IgG anti-TGIF1, IgG anti-PABPC1, IgG anti-IGF2BP3, and IgG anti-RARA as autoantigens in SLE, whereas IgG anti-NHLH1 did not reach statistical significance, and IgG anti-HMG5 and anti-ZNF276 were not included in their panel

[27]. That study reported IgG LIN28A to be present in 22% of patients with SLE, defining positivity as levels higher than the mean distribution plus 2 SDs in the HC population. Despite using a more stringent cutoff to define autoantibody positivity in our study, requiring levels higher than the median plus 2 IQRs in the HC population, we found a higher prevalence of IgG anti-LIN28A than that reported by Lewis et al. [27] across all SLE manifestations but not vascular disease. It is worth noting that anti-dsDNA has been reported in up to 50% of patients with active neuropsychiatric SLE [28] and 20% of patients in our study population, which anti-LIN28A exceeded. This is particularly interesting in light of LIN28A being an RNA-binding protein involved in embryonic development and the promotion of CNS axon regeneration [29]. LIN28A has also been associated with numerous cancers, including brain tumours [30]. Targeting the LIN28A and tumour suppressor lethal-7 (*let-7*) pathway has been implicated as a potential therapy in certain paediatric CNS tumours [30,31]. Interestingly, high expression of LIN28A in mouse nephrons was recently shown to lead to inflammatory kidney injury rather than oncogenesis [32]. In addition to anti-LIN28A, we also identified autoantibodies against the RNA-binding protein IGF2BP3 among IgG autoantibodies associated

with high disease activity and negatively associated with LLDAS. This is of particular interest in light of that LIN28A forms an RNA-binding complex with IGF2BP3 to promote DNA repair in response to oxidative stress [33]. Whether these mechanisms hold true for SLE specifically and targeting the LIN28A protein or pathway has merit for drug development for SLE warrants further study.

Similarly, IgG anti-HMG5 was associated with high disease activity, and a considerable proportion of patients exhibited positive levels across organ manifestations, notably 47% in patients with CNS activity. HMG5 is a nucleosome-binding protein that interacts with histone H1 to unfold chromatin, thereby influencing transcription and DNA repair [34]. Besides HMG5, targets of validated IgG and IgA daAABs included the chromatin proteins HMGB2 and HMG20B. Reassuringly, a high proportion of patients had positive levels of antichromatin in subgroups of patients stratified by organ-specific disease activity. HMG5 has been implicated in development and oncogenesis [35], but its role in SLE remains unclear. To the authors' knowledge, this is the first report of IgG anti-HMG5 in SLE.

IgG and IgA anti-IRF5 were associated with high disease activity, and at least 28% of patients had positive IgA levels across all but vascular SLE manifestations, whereas 17% of patients in DORIS remission were positive. In addition, IgA anti-IRF5 was included in an autoantibody cluster that was predominantly represented in patients with CNS and vascular activity. IRF5 is a transcription factor that regulates type I IFN production as well as IgG switch in B cells, and genetic variants of IRF5 constitute a known risk factor for SLE [36,37]. In addition to the implicated role of IRF5 in SLE pathogenesis, Lewis et al. [27] identified IgG IRF5 as a novel autoantigen in 2 independent cohorts of patients with SLE [27]. Besides corroborating the presence of IgG anti-IRF5 in SLE, to our knowledge, we, in this study, for the first time revealed the presence of IgA anti-IRF5 in patients with SLE with active disease. IgA anti-IRF5 levels exhibited a strong correlation with IgA autoantibodies against the RNA-binding protein SSB, which is typically present in interferonopathies such as SLE and Sjögren's disease [16]. However, as expected, we did not observe an association between IgG SSB or IgG SSA and global disease activity, as these autoantibodies are primarily considered diagnostic markers and have been linked to specific disease manifestations [38]. This reinforces the significance of our findings and the autoantibodies identified herein, which demonstrated associations with disease activity. Notably, IRF5 has been shown to distinguish patients with SLE from population-based non-SLE controls, and IRF5-positive microparticles have been associated with higher disease activity in patients with SLE [39]. Epstein–Barr virus triggers antibody responses towards IRF5 epitopes, and the levels of those antibodies have been linked to systemic inflammation in patients with rheumatoid arthritis [40]. Moreover, a weak immune response to an IRF5 peptide was reported among patients with SLE [41]. It is therefore unclear whether anti-IRF5 antibodies are driving inflammatory processes in SLE in a disease-specific manner or reflect a consequence of a generic autoimmune response. In light of interferon pathways being important in SLE pathogenesis and precipitation [42,43], linking anti-IRF5 to activity positions this autoantibody as an emerging biomarker of disease activity and IRF5 as a potential target for therapeutic interventions.

IgG and IgA anti-TGIF1 were associated with high disease activity and a substantial proportion of patients had positive levels across all but vascular manifestations. In contrast, *TGIF1* gene expression was negatively associated with increasing disease

activity and was associated with LLDAS and DORIS remission. The opposing trends observed between anti-TGIF1 levels and *TGIF1* gene expression may be explained by a counterregulatory mechanism, potentially involving a negative feedback loop that downregulates *TGIF1* expression during active SLE. Furthermore, in daAAB cluster analysis, IgG and IgA anti-TGIF1 were predominantly represented in patients with CNS activity. Transforming growth factor (TGF)- β 1 possesses a wide repertoire of anti-inflammatory and immunosuppressive effects and has been associated with disease activity in SLE [44]. TGIF1 acts as a transcriptional repressor capable of inhibiting TGF- β 1 signalling by repressing SMAD transcriptional activity [45]. *TGIF1* has been linked to a common gene expression signature in patients with SLE and patients with multiple sclerosis [46], and our finding of IgG and novel IgA anti-TGIF1 complements the previously reported IgG autoantibodies targeting SMAD pathways in SLE [27].

We found IgA anti-MAPK12 to be associated with LLDAS and anti-IgA MAPK associated with DORIS remission. Notably, similar associations were not found for the corresponding IgG autoantibodies, which could potentially reflect the phase of the autoimmune response at the time of sampling. The p21ras/MAPK pathway is crucial for T cell signalling [47], and its upregulation has been linked to increased disease activity in SLE [48]. The c-Jun N-terminal kinase (JNK) pathway-associated phosphatase (JKAP) activates the MAPK JNK, and downregulation of JKAP in T cells has been correlated with SLEDAI scores and in patients with active lupus nephritis (LN), it has been associated with poor renal outcome [49]. In light of these reports, the observed associations between IgA anti-MAPK12 and anti-MAPK with low activity states in our study suggest that these autoantibodies may signify protective or restorative states in the immune dysregulation of SLE.

Among the few antibodies displaying organ specificity, we found IgA anti-FOSL2 to be elevated exclusively in patients with SLE with musculoskeletal activity. Additionally, FOSL2 was identified in an enriched pathway involving growth plate cartilage development. FOSL2 is an oncogenic transcription factor implicated in T cell malignancies [50]. It has been reported to be increased in the kidney tissue of patients with LN, and it was specifically localised in the glomerular podocytes in a murine model of lupus [51]. Interferon gamma (IFN- γ) significantly increases FOSL2 expression, and knockdown of FOSL2 was shown to attenuate podocyte damage induced by IFN- γ treatment *in vitro* in the same study. Interestingly, reduced binding of FOSL2 to the single variant rs7034653, using an anti-FOSL2 antibody, was shown to reduce TRAF1 expression and increase tumour necrosis factor production in patients with juvenile idiopathic arthritis [52]. Whether a similar mechanism applies to SLE requires further investigation, yet the organ-specific association of IgA anti-FOSL2 with musculoskeletal activity supports this notion.

Several SLE manifestations exhibited common enriched pathways associated with IgG and IgA daAAB targets. These pathways included those linked to DNA-binding, RNA regulation, serine/threonine kinase activity, and ubiquitin-protein ligase activity, all of which are known to be relevant to SLE pathogenesis [53,54]. The binding of ANAs to DNA and RNA to form immune complexes leading to tissue injury and, ultimately, damage is central to SLE pathogenesis [55], and study of immune complex formation engaging the IgG and IgA daAABs discovered in this study would be intriguing. The serine/threonine kinase ρ -associated, coiled-coil-containing protein kinase 2 (ROCK2) has been shown to activate IRF4, an essential transcription factor for the production of interleukins 17 and 21,

and inhibition of ROCK dampened inflammatory and autoantibody responses in mouse models of autoimmunity [56]. Notably, IgG anti-IRF4 was implicated in 1 of the 3 autoantibody clusters in our study, with representation across various SLE manifestations. Furthermore, we recently identified a dysregulated ubiquitination gene module in the peripheral blood of patients with SLE, which, again, was not manifestation specific [57]. In the same study, druggability suggested bortezomib, an inhibitor of the ubiquitin-proteasome system, as a potential treatment for SLE, which has also been supported by results from a phase II clinical trial and real-world experience [57–60].

The exclusion criteria set by the PRECISESADS study protocol may have introduced selection bias, as patients with severe SLE manifestations requiring high doses of GCs or other potent immunomodulatory treatments were excluded. However, this approach minimised the potential confounding effects of such treatments on the molecular aberrations investigated within the PRECISESADS project, including autoantibody levels and gene expression in this study. The small sample size of the validation cohort may have limited us from uncovering autoantibodies of relevance in SLE pathogenesis yet with a smaller magnitude of differentiation. Furthermore, it restricted our ability to assess correlations between longitudinal autoantibody levels and disease activity. Nevertheless, both autoantibody levels and disease activity exhibited a downwards trend, with the possible exception of IgG RARA, which may suggest differential cellular origin for these autoantibodies, warranting further investigation. While the associations between daAAs and disease activity need further validation, the use of a validation cohort to determine which autoantibodies would qualify for downstream analysis enhanced the robustness of our pipeline and the reliability of our findings. While validation using a solid-phase quantitative assay remains a future objective due to several factors, including logistical and financial constraints, the validation performed in independent cohorts in this study, along with the corroboration of findings consistent with previous investigations [27,61], strengthens our results. Moreover, methodological crossvalidation presents inherent challenges, including the lack of commercial kits for the novel autoantibodies identified. Additionally, such crossvalidation would overlook the advantage of the KREX platform used in this study, which offers high sensitivity and preserves protein conformation. Future translational studies dedicated to assay development and validation in larger, more ethnically diverse SLE populations will be essential to further advance our findings. Importantly, the comprehensive exploration of IgA autoantibodies in SLE is novel. This adds a new dimension to our understanding of the immunologic landscape in SLE, revealing potential roles of IgA autoantibodies in disease activity and organ involvement, and a potential role of mucosal immunity in SLE pathogenesis. Investigation of autoantibody isotypes beyond IgG and IgA, as well as subclass profiling might provide further insight.

In conclusion, this study identified and validated novel IgG and IgA autoantibodies. Among these, IgG anti-LIN28A, IgG anti-HMGN5, and both isotypes for anti-IRF5 and anti-TGIF1 were associated with high disease activity and were highly prevalent in subgroups of patients with active disease across organ manifestations. IgG anti-LIN28A levels exceeded the cutoff for positivity in more than half of the patients with CNS involvement, a prevalence higher than that observed for the routine clinical marker anti-dsDNA, and almost half of the patients with renal activity. Additionally, IgG and IgA LIN28A formed autoantibody clusters predominantly represented in patients with CNS activity. These findings indicate a potential role of mucosal

immunity in SLE pathogenesis and have direct implications for the early identification of patients with active or evolving disease and for timely and informed therapeutic intervention.

Competing interests

IP has received research funding and/or honoraria from Amgen, AstraZeneca, Aurinia, BMS, Elli Lilly, Gilead, GSK, Janssen, Novartis, Otsuka, and Roche. The other authors declare no conflicts of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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Contributors

JL, DN, HI, P-JJ, MEA-R, and IP were involved in the conceptualisation of the study. JL, DN, DL, LB, MOB, GB, MEA-R, and IP were involved in data curation. JL, DN, DL, and IP were involved in the visualisation of the findings. JL, DN, DL, HI, LB, GB, P-JJ, MEA-R, NS, and IP were involved in the interpretation of results. JL and IP were involved in the manuscript drafting. All authors were involved in data analysis, critical review of manuscript drafts, and approval of the final draft before submission. IP acts as the guarantor of the study.

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Patient consent for publication

All patients and healthy controls provided written informed consent before recruitment.

Ethics approval

This study involved human participants. It was reviewed and approved by local ethics committees at all recruiting centres: Comitato Etico Area 2, Fondazione IRCCS Ca Granda Ospedale Maggiore Policlinico di Milano and University of Milan (approval no. 425 bis 19 November 2014 and no. 671_2018 19 September 2018); Klinikum der Universität zu Köln, Cologne, Germany; Comité d'Éthique Hospitalo-Facultaire, Pôle de pathologies rhumatismales systémiques et inflammatoires, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Brussels, Belgium; Csongrad Megyei Kormányhivatal, University of Szeged, Szeged, Hungary; Comité

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Provenance and peer review

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Data availability statement

Data are available upon reasonable request. Raw data remain the property of the PRECISESADS consortium and are protected under the European General Data Protection Regulation (GDPR). Metadata and aggregated processed data are available upon reasonable request from the corresponding author and from the European Genome-Phenome Archive (EGA).

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.04.008.

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