



Latent autoimmunity across disease-specific boundaries in at-risk first-degree relatives of SLE and RA patients

Judith A. James^{a,b,*}, Hua Chen^a, Kendra A. Young^c, Elizabeth A. Bemis^c, Jennifer Seifert^c, Rebecka L. Bourn^a, Kevin D. Deane^c, M. Kristen Demoruelle^c, Marie Feser^c, James R. O'Dell^d, Michael H. Weisman^e, Richard M. Keating^f, Patrick M. Gaffney^a, Jennifer A. Kelly^a, Carl D. Langefeld^g, John B. Harley^h, William Robinsonⁱ, David A. Hafler^j, Kevin C. O'Connor^j, Jane Buckner^k, Joel M. Guthridge^a, Jill M. Norris^c, V. Michael Holers^c

^a Oklahoma Medical Research Foundation, Oklahoma City, OK, USA

^b University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

^c University of Colorado, Aurora, CO, USA

^d University of Nebraska Medical Center, Omaha, NE, USA

^e Cedars-Sinai Medical Center, Los Angeles, California, USA

^f The University of Chicago, Chicago, IL, USA

^g Wake Forest School of Medicine, Winston-Salem, NC, USA

^h Cincinnati Children's Hospital Medical Center, The University of Cincinnati College of Medicine, and Cincinnati US Department of Veterans Affairs VA Medical Center, Cincinnati, OH, USA

ⁱ Stanford University, Palo Alto, CA, USA

^j Yale School of Medicine, New Haven, CT, USA

^k Benaroya Research Institute at Virginia Mason, Seattle, Washington, USA

ARTICLE INFO

Article history:

Received 11 February 2019

Received in revised form 15 March 2019

Accepted 21 March 2019

Available online 3 April 2019

Keywords:

Autoantibodies

Lupus

Rheumatoid arthritis

Type I diabetes

Relatives

Genetic risk

ABSTRACT

Background: Autoimmune disease prevention requires tools to assess an individual's risk of developing a specific disease. One tool is disease-associated autoantibodies, which accumulate in an asymptomatic preclinical period. However, patients sometimes exhibit autoantibodies associated with a different disease classification. When and how these alternative autoantibodies first appear remain unknown. This cross-sectional study characterizes alternative autoimmunity, and associated genetic and environmental factors, in unaffected first-degree relatives (FDRs) of patients, who exhibit increased future risk for the same disease.

Methods: Samples ($n = 1321$) from disease-specific autoantibody-positive (aAb+) systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and type 1 diabetes (T1D) patients; and unaffected aAb+ and autoantibody-negative (aAb-) SLE and RA FDRs were tested for SLE, RA, and T1D aAbs, as well as anti-tissue transglutaminase, anti-cardiolipin and anti-thyroperoxidase. FDR SLE and RA genetic risk scores (GRS) were calculated.

Findings: Alternative autoimmunity occurred in SLE patients (56%) and FDRs (57.4%), RA patients (32.6%) and FDRs (34.8%), and T1D patients (43%). Expanded autoimmunity, defined as autoantibodies spanning at least two other diseases, occurred in 18.5% of SLE patients, 16.4% of SLE FDRs, 7.8% of RA patients, 5.3% of RA FDRs, and 10.8% of T1D patients. SLE FDRs were more likely to have alternative (odds ratio [OR] 2.44) and expanded (OR 3.27) autoimmunity than RA FDRs. Alternative and expanded autoimmunity were associated with several environmental exposures. Alternative autoimmunity was associated with a higher RA GRS in RA FDRs (OR 1.41), and a higher SLE GRS in aAb+ RA FDRs (OR 1.87), but not in SLE FDRs.

Interpretation: Autoimmunity commonly crosses disease-specific boundaries in systemic (RA, SLE) and organ-specific (T1D) autoimmune diseases. Alternative autoimmunity is more common in SLE FDRs than RA FDRs, and is influenced by genetic and environmental factors. These findings have substantial implications for preclinical disease pathogenesis and autoimmune disease prevention studies.

Fund: NIH U01AI101981, R01AR051394, U19AI082714, P30AR053483, P30GM103510, U54GM104938, U01AI101934, R01AI024717, U01AI130830, I01BX001834, & U01HG008666.

© 2019 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author at: Arthritis & Clinical Immunology, Oklahoma Medical Research Foundation, 825 NE 13th Street, Oklahoma City, OK 73104, USA.
E-mail address: judith-james@omrf.org (J.A. James).

Research in context

Evidence before this study

The source material for the questions addressed in this included: (1) Prior work of the authors and roles as content experts, (2) References identified within the manuscript, and (3) Ovid Medline search terms “autoimmune disease”, “autoantibodies”, and “familial relationships” or “first-degree relatives” (last search 11 December 2018, all languages, no beginning or end dates). Articles were reviewed for relevance and included in the manuscript if the primary disease or autoantibodies studied were related to rheumatoid arthritis, systemic lupus erythematosus or type 1 diabetes.

Added value of this study

The results of this study point to the importance of determining how and why both disease-specific autoantibodies and those that are associated with other related autoimmune diseases are initiated and evolve over time prior to the development of a clinically classified disease. It also identifies genetic and environmental factors which influence this process.

Implications of all the available evidence

Autoimmune disease prevention and preclinical therapeutic intervention will require tools to assess an individual's risk of developing a specific autoimmune disease. The current study provides new fundamental information across organ-specific and systemic autoimmune diseases regarding autoantibody profiles, genetic risk profiles, and environmental associations to assess an individual's risk of developing a specific autoimmune disease, as well as the precision of these assessments. This information will have substantial importance in the development and choice of therapeutic strategies for prevention or early intervention across select autoimmune diseases.

1. Introduction

Autoimmune diseases are a major cause of morbidity and mortality and have substantial direct and indirect economic costs [1,2]. Therefore, understanding the underlying mechanisms of disease initiation, developing novel therapeutics, and identifying prevention strategies are paramount goals. As a unifying characteristic of most autoimmune diseases, autoantibodies precede clinical disease onset, often exhibit pathogenic potential, and associate with specific autoimmune diseases. The precise autoantibody repertoire varies among patients with the same disease, is consistent with the significant overlap of genetic variants underlying autoimmune disease [3], and may cross boundaries of clinical disease specificity in patients with active classified disease.

A large majority of patients who will develop systemic lupus erythematosus (SLE) accumulate autoantibodies as they approach clinical disease onset and formal classification [4]. By the time of SLE classification, nearly all SLE patients exhibit anti-nuclear autoantibodies (ANA) against a variety of nuclear antigens, such as Sm, ribonucleoprotein (RNP), Sm/RNP, dsDNA, chromatin, ribosomal P, Ro/SSA, and La/SSB [4]. Highlighting their importance in SLE pathogenesis, various ANA specificities have been shown to cause direct tissue damage and stimulate lupus-like disease in animal models [5,6]. Similarly, rheumatoid factor (RF) and/or anti-citrullinated peptide autoantibodies (ACPAs)

appear before diagnosis in future seropositive rheumatoid arthritis (RA) patients [7,8], and ACPA levels are correlated with disease activity in new-onset RA [9]. In both SLE and RA, disease-associated autoantibodies undergo epitope spreading as patients approach disease classification [4,10]. In type 1 diabetes (T1D), hyperglycemia develops after the appearance of autoantibodies against islet antigens, such as glutamic acid decarboxylase, insulin, and islet antigen 2, and islet autoreactivity contributes to diabetes in murine models [11]. In addition, the number of diabetes-specific autoantibodies increases prior to diagnosis [12], and relatives of T1D patients with multiple diabetes-specific autoantibodies have significantly increased risk of developing future disease [13].

In addition to disease-specific autoantibodies, patients with one clinically defined and classified autoimmune disease can demonstrate autoantibodies associated with another disease. This phenomenon has been designated latent polyautoimmunity [14,15]. For example, a subset of SLE patients exhibits RF and/or anti-cyclic citrullinated peptide (CCP) autoantibodies typically associated with RA [16], and organ-specific latent autoantibodies have been observed in juvenile SLE patients [17]. RA patients and pediatric T1D patients [18] may also exhibit antinuclear autoantibodies typically associated with SLE. Latent autoantibodies could plausibly affect the disease course, but whether they develop in the preclinical period and/or at a later stage, and how genetic risk and environmental exposures might influence their development, is unknown. These questions are central to understanding immune mechanisms responsible for the initial loss of self-tolerance and accurately predicting the future development of a specific autoimmune disease.

While most relatives of autoimmune disease patients will remain healthy, they are more likely than the general population to exhibit disease-specific autoantibodies and to develop the same autoimmune disease as an affected relative [19,20]. Up to 32% of unaffected relatives of SLE patients are ANA-positive [21], and 16–24% of unaffected first-degree relatives of RA patients exhibit ACPAs, anti-CCP autoantibodies, and/or RF [22,23]. T1D autoantibodies, particularly anti-glutamic acid decarboxylase autoantibodies, occur in unaffected relatives of T1D patients [12]. Relatives of autoimmune disease patients are also more likely to develop autoimmune diseases besides the proband's diagnosis [24], and genome-wide association studies (GWAS) demonstrate overlap of risk loci between autoimmune conditions [3].

We hypothesized that alternative autoantibodies not associated with the patient/proband diagnosis occur in relatives of autoimmune disease patients, and that the presence of alternative autoantibodies is influenced by factors associated with autoimmune disease. This study examines the prevalence, genetic relationships and environmental determinants of disease-specific and alternative autoantibodies in several informative populations: SLE, RA, and T1D patients, and first-degree relatives (FDRs) of SLE and RA patients.

2. Methods

2.1. Study participants

This study was approved by the Institutional Review Boards of the participating institutions. All participants provided written informed consent prior to study-specific procedures. Serum samples were previously collected from T1D, SLE, and RA patients and FDRs. In this study, no patients and FDRs were related to each other. In addition, only one FDR was chosen from each family so that no FDRs were related to other FDRs.

The SLE cohort was derived from the Lupus Family Registry and Repository (LFRR) [19,25]. SLE patients met ≥ 4 American College of Rheumatology (ACR) SLE classification criteria. The SLE cohort consisted of SLE patients with SLE autoantibodies; unaffected, SLE autoantibody-positive FDRs of confirmed SLE patients (aAb+ SLE FDRs); and unaffected, SLE autoantibody-negative FDRs of confirmed SLE patients (aAb- SLE FDRs). All subjects were Caucasian non-Hispanic. Subjects

completed an LFRR questionnaire at the time of blood collection, assessing demographics and environmental exposures [25].

The RA cohort was derived from the Studies of the Etiology of RA (SERA) [26]. RA patients met ≥ 4 1987 ACR RA classification criteria. The RA cohort consisted of RA patients with RA autoantibodies; unaffected, RA autoantibody-positive FDRs of confirmed RA patients (aAb+ RA FDRs); and unaffected, RA autoantibody-negative FDRs of confirmed RA patients (aAb- RA FDRs). All subjects were Caucasian non-Hispanic. Subjects completed a SERA questionnaire at the time of the blood collection, assessing demographics and environmental exposures [26].

The T1D cohort, established at Benaroya Research Institute, included individuals with established T1D. All subjects were participants in the BRI Immune-Mediated Disease Registry and Repository. Healthy subjects had no history of autoimmune disease. T1D subjects were T1D-autoantibody positive and insulin dependent.

2.2. Autoantibodies

Serum and plasma were procured at enrollment and stored at -20°C (LFRR, Benaroya) and -80°C (SERA); assays were performed on freshly thawed serum samples in blinded batches.

The CAP (College of American Pathologists)-certified OMRF Clinical Immunology Laboratory tested SLE-associated autoantibodies and anti-cardiolipin. SLE-associated autoimmunity was defined as positivity for any of the following by an FDA-approved, multiplexed, bead-based assay (BioPlex 2200, Bio-Rad, Hercules, CA): Sm, Sm/RNP, RNP (nRNP 70 kD and/or nRNP A), dsDNA, chromatin, ribosomal P, Ro/SSA, and/or La/SSB [19]. Anti-dsDNA was reported in IU/mL and others as an antibody index (AI) value (range 0–8) with manufacturer-recommended positive cutoffs (10 IU/mL and AI = 1, respectively) [19]. Additionally, ANA titer and pattern were determined by indirect immunofluorescence, with $\geq 1:120$ considered positive. Anti-cardiolipin IgA, IgG, and IgM were assessed by enzyme-linked immunosorbent assay (ELISA) with ≥ 20 units considered positive [27].

RA autoantibody positivity, tested in the CAP-certified Exsera BioLabs at the University of Colorado, was defined as positivity for any of the following: RF (IgA, IgG, IgM), RF by nephelometry, CCP2, and/or CCP3.1. RF IgM, IgG, and IgA were assessed by commercial ELISA (QUANTA Lite, Inova Diagnostics, San Diego, CA) and quantified in units/mL. RF was also assessed by nephelometry (Dade-Behring, Newark, DE). RF positive cutoffs were previously established at the 95th percentile of 491 randomly selected healthy blood donors from the Denver area [7]. Anti-CCP was tested using commercial ELISAs (CCP2: Diastat, Axis-Shield Diagnostics, Dundee, Scotland; CCP3.1: IgA/IgG Inova Diagnostics) and quantified in units, with manufacturer-recommended positive cutoffs (CCP2 > 5 units; CCP3.1 ≥ 20 units).

T1D autoantibody positivity was defined as positivity for any of the following: micro-insulin autoantibodies (mIAA), glutamic acid decarboxylase (GAD65), and/or islet antigen 2 (IA-2). These were measured in the Clinical Immunology Laboratory at the Barbara Davis Center using radioimmunoassays with positive cutoffs at the 99th percentile of healthy controls, as described [28]. Anti-thyroperoxidase (TPO) autoantibodies were assessed by commercial ELISA (QUANTA Lite® TPO, Inova Diagnostics) per manufacturer protocol at OMRF. Anti-tissue transglutaminase (tTG) autoantibodies were assessed by radioimmunoassay using ^{35}S -labeled tTG (20,000 cpm) as described [29]. This assay has an interassay coefficient of variance (COV) of 18% and intraassay COV of 4.8% [29].

2.3. Outcomes

The primary study outcome was the presence of at least one autoantibody associated with one disease other than the patient/proband classification, designated *alternative autoimmunity*. A secondary outcome of *expanded autoimmunity* was defined as the presence of autoantibodies

associated with two or more diseases other than the patient/proband classification.

2.4. Data harmonization and environmental exposure assessment for RA and SLE cohorts

Environmental exposures were only examined in the SLE and RA FDRs. Exposure variables were analyzed only if they were collected in a similar manner across cohorts or could be derived from other questions, and had $\leq 10\%$ missing values.

2.5. Genotyping and calculation of genetic risk scores

SLE FDR DNA samples were genotyped using the ImmunoChip-1™ and read on the Illumina iScan in the OMRF Clinical Genomics Center [30]. Genotypes were called via Optical [31] using the default options plus the -nointcutoff option to manually remove intensity outliers. RA FDR DNA samples were genotyped using the Illumina MEGA^{EX} BeadChip per Illumina protocols. Quality control (QC) for both arrays included removing single nucleotide polymorphisms (SNPs) and samples with missing call rates >10%, minor allele frequency < 0.00001, and Hardy Weinberg Equilibrium $p < 0.001$. SNPs that indicated known QC errors (e.g. poor clustering) were also removed.

Genetic risk scores (GRS) were utilized to explore how the number of established risk polymorphisms (47 SLE, 40 RA; Supplementary Table 1) affected alternative and expanded autoimmunity [32,33]. If SNPs within a gene showed high linkage disequilibrium ($r^2 > 0.80$), only one SNP was included. This analysis excluded genetic variants in the HLA region. SLE- and RA-specific GRS for each individual were calculated as the sum of the beta coefficient for the risk allele association multiplied by the number of risk alleles [30]. The GRS included 87 risk polymorphisms; eleven (2 SLE, 9 RA) were not common across the two genotyping platforms and were imputed with IMPUTE2 using two phased reference panels (ImmunoChip and 1000 Genomes Phase 3) [34]. Imputed variants with an $R^2 < 0.3$ or a minor allele frequency < 1% were excluded.

2.6. Statistics

All statistical analyses were performed in SAS version 9.4 (Cary, NC). Means were compared by Mann-Whitney test, and proportions by chi-square, or Fisher's exact test when <10 events were expected for any category. Crude odds ratios (OR) and 95% confidence intervals (CI) were calculated for differences in autoimmunity by RA, SLE and T1D patients, and by RA and SLE FDRs.

Multivariable modeling among SLE and RA FDRs was performed by logistic regression with alternative autoimmunity (+/–) or expanded autoimmunity (+/–) as the dependent variable. A $P < 0.05$ was considered significant for model inclusion, and if an interaction was significant ($P < 0.05$), stratified analyses were reported. For general models, predictors included proband disease (SLE/RA), disease-specific autoimmunity (aAb+/aAb-), sex (female/male), and age (years). Given the exploratory nature of this study, effect modification was assessed to determine whether the association between alternative/expanded autoimmunity and disease differed by disease-specific autoimmunity, sex, and age. Three-way interactions were included in the initial models to determine whether alternative or expanded autoimmunity within each disease differed by disease-specific autoimmunity, age, and sex. For environmental exposures, each model was adjusted for proband disease, disease-specific autoimmunity, age, and sex. Effect modification was also assessed between each exposure, proband disease, and disease-specific autoimmunity. For associations with GRS (as a continuous variable) models were adjusted for proband disease, disease-specific autoimmunity, sex and age. Effect modification was assessed between GRS and proband disease and disease-specific autoimmunity. Results are presented for a 1-unit increase in GRS.

2.7. Role of the funding source

The funding source had no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; nor in the decision to submit the paper for publication. The corresponding author (JAJ) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

3.1. Demographics of SLE, RA, and T1D cohorts

To elucidate the development of alternative autoimmunity in autoimmune disease patients and at-risk preclinical populations, we evaluated autoantibodies in sera previously collected from SLE, RA, and T1D patients; and SLE and RA FDRs (Table 1). By design, patients and aAb + FDRs exhibited autoantibodies corresponding to the patient/proband diagnosis, while aAb- FDRs lacked such autoantibodies. The SLE and RA cohorts were predominantly female and were older than the T1D cohort (Table 1; Supplementary Fig. 1). The T1D cohort was evenly divided between males and females. All cohorts were predominantly non-Hispanic European-American.

3.2. Autoantibodies cross disease-specific boundaries in SLE, RA, and T1D patients and FDRs

Alternative autoimmunity occurred in SLE patients (56.0%), RA patients (32.6%), and T1D patients (43.0%) (Fig. 1A). SLE patients were the most likely to have alternative autoimmunity (vs. RA patients: OR 2.63, 95% CI 1.74–3.96; $p < 0.0001$, vs. T1D patients: OR 1.69, 95% CI 1.13–2.52; $p = 0.0144$), and RA patients the least likely (vs. T1D patients: OR 0.64, 95% CI 0.42–0.98; $p = 0.0482$). Additionally, 18.5% of SLE patients, 7.8% of RA patients, and 10.8% of T1D patients demonstrated expanded autoimmunity (Fig. 1B). FDRs of SLE patients and RA patients also exhibited alternative autoimmunity, and less commonly, expanded autoimmunity (Fig. 1).

3.3. Patterns of alternative autoimmunity associated with systemic and organ-specific disease

We next compared patterns of systemic autoimmunity (SLE, RA, and anti-phospholipid syndrome autoantibodies) and organ-specific autoimmunity (T1D autoantibodies, anti-TPO, and anti-tTG) in each cohort (Fig. 2, Supplementary Fig. 2). Alternative autoimmunity in the SLE cohort was predominantly systemic (patients 42.5%, FDRs 41.0%) rather than organ-specific (patients 27.0%, FDRs 27.6%). The most common alternative autoantibodies in the SLE cohort were RA-associated, followed by anti-phospholipid and anti-TPO (Fig. 2B, C, E). The RA cohort exhibited both systemic (patients 15.5%, FDRs 14.9%) and organ-specific (patients 21.2%, FDRs 23.4%) alternative autoimmunity, most

Table 1
Subject demographics.

	Total n	% Female	Age, mean (SD), years
SLE			
SLE Patients	200	85.5	39.2 (12.4)
aAb+ SLE FDRs	168	72.6	59.0 (15.0)
aAb- SLE FDRs	198	82.8	55.4 (14.5)
RA			
RA Patients	193	83.4	58.8 (13.3)
aAb+ RA FDRs	192	75.0	51.8 (16.0)
aAb- RA FDRs	184	86.4	47.3 (15.2)
T1D			
T1D Patients	186	47.8	25.3 (11.2)

All subjects in the SLE and RA cohorts, and 97.4% of the T1D cohort, were Caucasian Non-Hispanic. aAb+: positive for disease-specific autoantibodies. aAb-: negative for disease-specific autoantibodies. FDRs: First-degree relatives of confirmed patients.

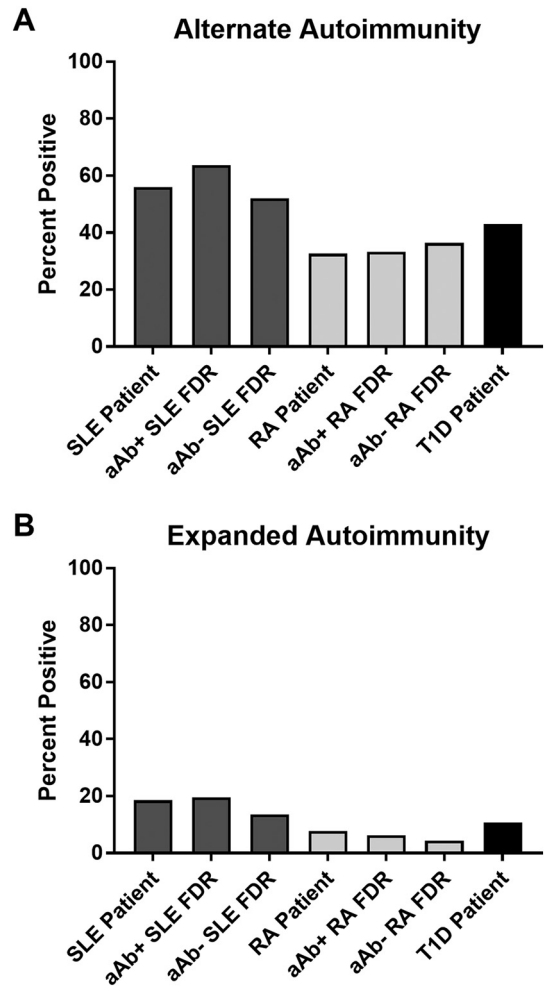


Fig. 1. Autoimmunity crosses disease-specific boundaries in SLE patients and FDRs, RA patients and FDRs, and type 1 diabetes patients. Autoantibodies associated with SLE, RA, anti-phospholipid syndrome, T1D, autoimmune thyroid disease, and celiac disease were assessed in three cohorts. The SLE cohort included SLE patients and FDRs with or without SLE-associated autoantibodies (aAb+ SLE FDR and aAb- FDR, respectively). The RA cohort included RA patients and FDRs with or without RA-associated autoantibodies (aAb+ RA FDR and aAb- RA FDR, respectively). The T1D cohort included T1D patients. (A) Alternative autoimmunity was defined as the presence of at least one autoantibody not associated with disease of interest. For example, SLE-associated autoantibodies represent alternative autoimmunity in the RA cohort, but not in the SLE cohort. (B) Expanded autoimmunity was defined as the presence of autoantibodies associated with at least two alternative diseases.

commonly anti-TPO (15.5%–23.4%) (Fig. 2E) and SLE-related specificities (13.0–13.6%) (Fig. 2A). In T1D patients, alternative autoimmunity was primarily organ-specific (36.6%) rather than systemic (15.0%), and anti-TPO was the most common alternative autoantibody (Fig. 2E). As expected, of all groups, T1D patients had the highest prevalence of the celiac disease autoantibody anti-tTG (8.1% vs. 0.5%–3.5% in other groups; Fig. 2F).

3.4. SLE autoantibodies in RA patients, RA FDRs, and T1D patients

Similar rates of SLE-associated autoantibodies were observed in RA patients (13.5%), aAb+ RA FDRs (13.0%), and aAb- RA FDRs (13.6%) (Fig. 2A). RA patients and FDRs with SLE autoantibodies typically had one SLE specificity ($n = 20/26$ patients, $n = 47/50$ FDRs). Some RA patients had two ($n = 4$) or four ($n = 2$) SLE specificities, and three aAb + RA FDRs had two SLE specificities (Fig. 3A).

The most common SLE specificities in RA patients were Ro/SSA (5.7%) and La/SSB (4.7%), followed by RNP (2.6%) (Fig. 3B–I). Each linked autoantibody system appeared in 3.3–5.4% of RA FDRs (aAb+ :

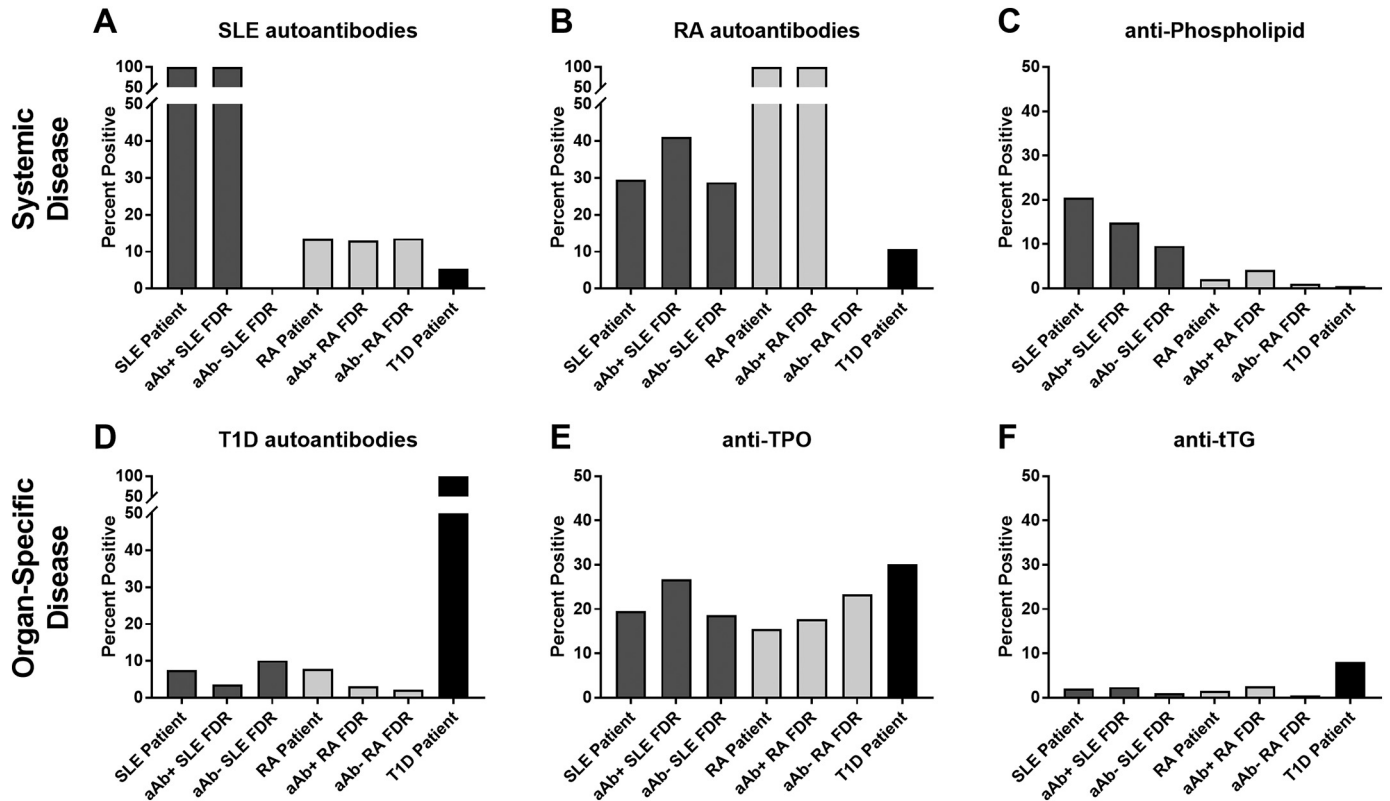


Fig. 2. Systemic and organ-specific autoimmunity in SLE patients and FDRs, RA patients and FDRs, and T1D patients. Patients, FDRs, and controls were assessed for autoantibodies associated with systemic disease, including (A) SLE (dsDNA, chromatin, Sm, SmRNP, RNP, Ribosomal P, Ro/SSA, La/SSB), (B) RA (CCP2, CCP3.1, RF IgA, RF IgM, RF IgG, RF-neph), and (C) anti-phospholipid, as well as organ-specific antibodies, including (D) T1D autoantibodies (anti-GAD, IA2, and IMAA), (E) anti-thyroperoxidase, and (F) anti-tissue transglutaminase. Note that the axis is broken in panels A, B, and D.

5.2% Ro/La, 4.0% Sm/SmRNP/RNP, 3.6% dsDNA/chromatin. aAb-: 4.9% Ro/La, 5.4% Sm/SmRNP/RNP, 3.3% dsDNA/chromatin).

Levels of each SLE autoantibody were lower in RA patients and FDRs compared to SLE patients (Fig. 3B–I; each $p < 0.0001$). High levels of anti-dsDNA (≥ 100 IU/mL), which associate with renal disease, occurred only in the SLE cohort (Fig. 3B). High levels of other SLE autoantibodies (antibody index ≥ 8) occurred in three RA patients, one aAb + RA FDR, and one aAb- RA FDR (Fig. 3B–H). Similarly, by indirect immunofluorescence, ANA titers were lower in ANA+ RA patients (49.2% prevalence, 1:120 median titer), aAb + RA FDRs (35.9%, 1:40), and aAb- RA FDRs (34.2%, 1:120) than in SLE patients (97.9%, 1:1080) (Fig. 3J). No T1D patients had high levels of an SLE-associated autoantibody nor more than one SLE-associated specificity.

3.5. RA autoantibodies in SLE patients, SLE FDRs, and T1D patients

RA-associated reactivity was prevalent among SLE patients (29.5%) and FDRs (34.4%). CCP reactivity was slightly more common in SLE FDRs than SLE patients (Fig. 4A–B), and SLE FDRs occasionally showed high CCP3.1 levels (40 U), which correlate with RA onset within 2 years [7] (SLE patients 0% vs. aAb + 7.3%, aAb- 3.1%; $p < 0.001$, $p = 0.014$, respectively). RF reactivity occurred in 18% of SLE patients, 34.1% of aAb + SLE FDRs, and 22.0% of aAb- SLE FDRs (Fig. 4C–F). Among T1D patients, 10.8% had RA-associated reactivity, and two (1.1%) had a high CCP3.1 titer.

3.6. Multivariable environmental analysis of alternative and expanded autoimmunity in RA and SLE FDRs

To identify factors that influence rates of alternative and expanded autoimmunity in the preclinical period, we performed multivariable analyses in RA and SLE FDRs. Compared to RA FDRs, SLE FDRs were

more likely to have alternative autoimmunity after adjusting for disease-specific aAb status, sex, and age (OR 2.44, 95% CI 1.80–3.31; $p < 0.0001$), and were more likely to exhibit expanded autoimmunity (OR 3.27, 95% CI 1.90–5.61; $p < 0.0001$). These associations showed no evidence of significant effect modification by disease-specific aAb status, sex, or age.

We next examined environmental factors for association with alternative and expanded autoimmunity in RA and SLE FDRs (Table 2). Smoking status was not associated with alternative or expanded autoimmunity. Some factors showed effects that lost their significance after multivariable adjustment. These include decreased odds of alternative and expanded autoimmunity in participants with an education beyond high school, and increased odds of alternative and expanded autoimmunity in women with a hysterectomy (alternative), increased number of pregnancies (expanded), and non-use of hormonal contraceptives (alternative and expanded). The effect of disease specific autoimmunity on alternative autoimmunity was significantly modified by both season of blood draw and season of birth. Among aAb- RA FDRs, samples drawn in fall and winter were more likely to show alternative autoimmunity than samples drawn in spring (fall v. spring: OR 3.00, 95% CI 1.04–8.60, $p = 0.04$; winter v. spring OR 5.05, 95% CI 1.68–15.13, $p = 0.004$). aAb + RA FDRs born in fall and summer were more likely to have alternative autoimmunity than those born in spring, after adjusting for age and sex (fall v. spring OR 3.59, 95% CI 1.36–9.50, $p = 0.01$; summer v. spring OR 6.24, 95% CI 2.31–18.87, $p = 0.0003$).

3.7. Genetic risk score analyses with alternative or expanded autoimmunity in SLE and RA FDRs

Composite genetic risk scores (GRS) for SLE and for RA were calculated for FDRs and examined for associations with alternative and expanded autoimmunity (Table 3). Neither GRS significantly associated

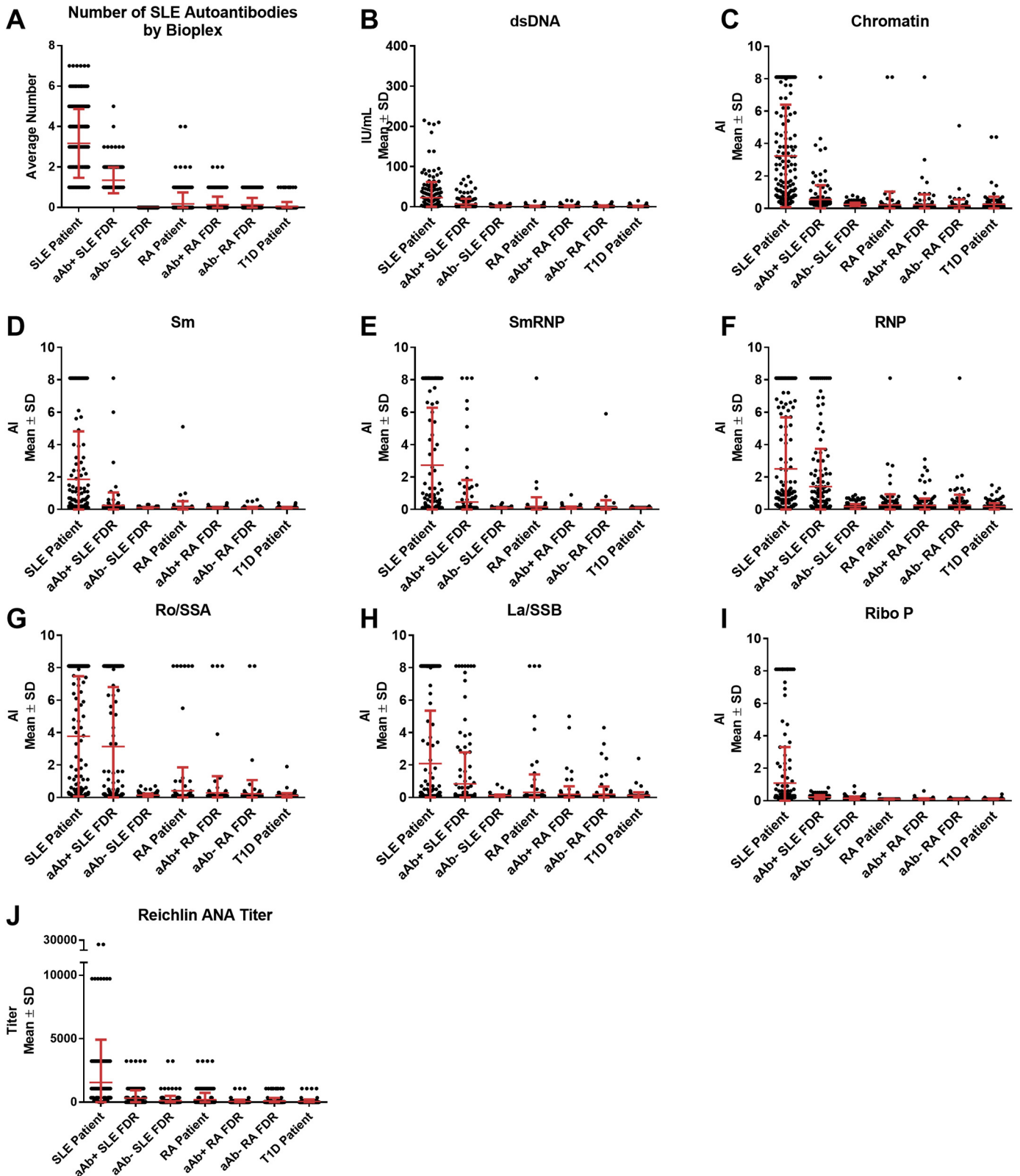


Fig. 3. Antinuclear autoantibodies are present in RA patients at low titer. (A) The number of positive SLE-associated specificities assessed by Bioplex is shown for SLE patients and autoantibody-positive (aAb+) and autoantibody-negative (aAb-) SLE FDRs; RA patients, aAb+ and aAb- RA FDRs; and type 1 diabetes (T1D) patients. (B–I) Individual SLE-associated specificities indicated were assessed by multiplex assay. Levels of anti-dsDNA are reported in IU/mL, with a positive cutoff of 10 IU/mL, and others are reported as antibody index values (range 0–8), with a positive cutoff of AI = 1. (J) ANA titer was determined by indirect immunofluorescence.

with expanded autoimmunity, and the effects of genetic risk on alternative autoimmunity varied by proband disease and disease-specific autoimmunity. A significant effect modification occurred between proband

disease and RA GRS on alternative autoimmunity (interaction $p = 0.048$); a higher RA GRS significantly associated with alternative autoimmunity in RA FDRs, but not in SLE FDRs (Table 3).

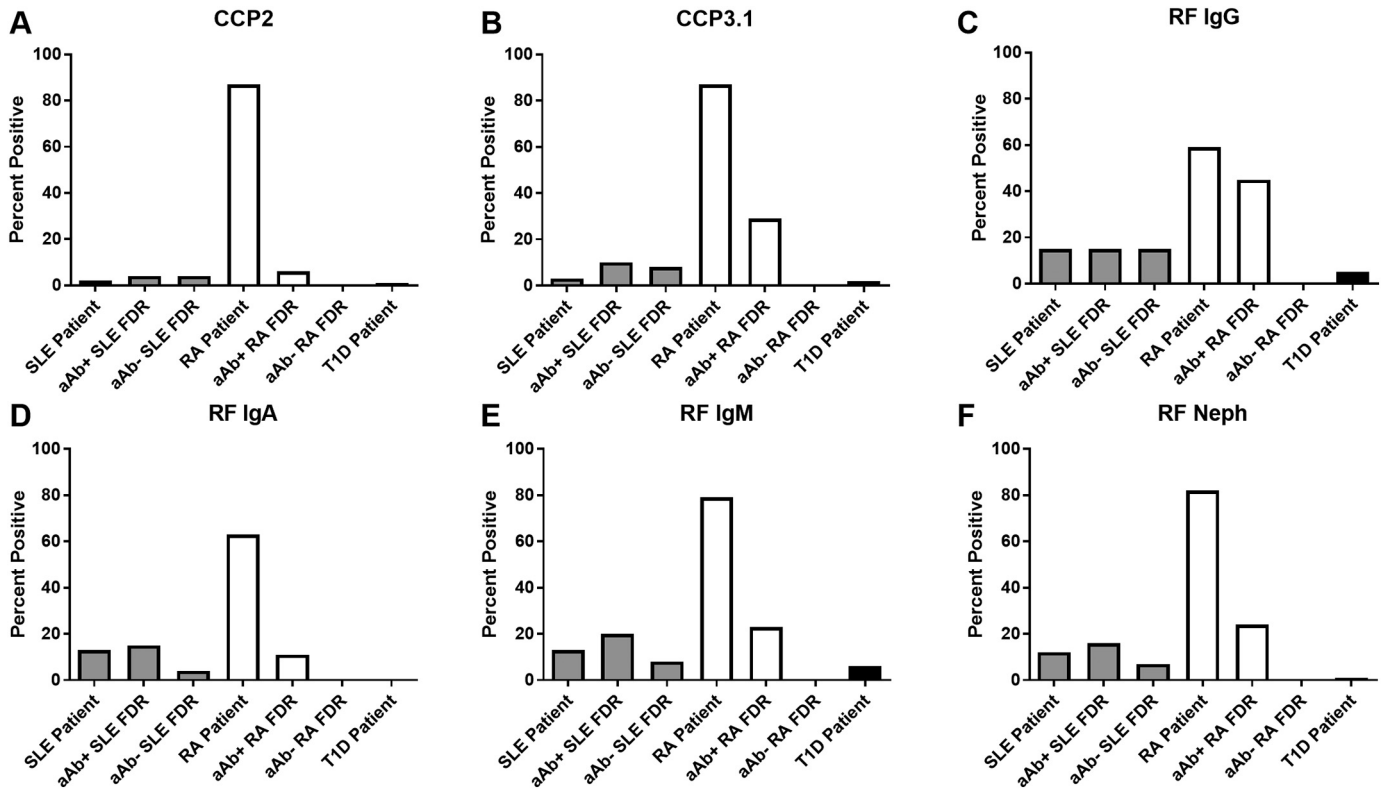


Fig. 4. Anti-CCP and RF are prevalent in SLE patients and FDRs of SLE patients. The prevalence of autoantibodies considered for RA classification was evaluated in SLE patients and SLE autoantibody-positive (aAb+) and autoantibody-negative (aAb-) FDRs; RA patients, aAb+ FDRs and aAb- FDRs; and type 1 diabetes (T1D) patients. (A) Anti-cyclic citrullinated protein (CCP)-2, (B) CCP3.1, (C) rheumatoid factor (RF) IgG, (D) RF IgA, and (E) RF IgM were determined by ELISA. (F) RF was also determined by nephelometry.

When assessing the impact of SLE GRS on alternative autoimmunity, disease-specific autoimmunity significantly interacted with SLE GRS ($p = 0.04$) and proband disease ($p = 0.03$). Although the 3-way

interaction between SLE GRS, proband disease, and disease-specific autoimmunity was not significant ($p = 0.10$), the effect modification between autoantibody positivity and SLE GRS was significant only in

Table 2

Presence of major epidemiologic variables in RA and SLE FDRs by alternative and expanded autoimmunity status.

Epidemiologic variables	Alternative autoimmunity status			Expanded autoimmunity status		
	Positive	Negative	p-value	Positive	Negative	p-value
General exposures						
N	341	401		80	662	
Cohort: % SLE FDR	61.6	38.9	<0.01	75.0	46.2	<0.01
Disease-specific aAb Status: % Positive	50.2	47.1	0.41	56.3	47.6	0.14
BMI: mean \pm sd ^a	28.1 \pm 5.8	27.3 \pm 5.8	0.09	28.4 \pm 6.8	27.6 \pm 5.7	0.33
Education: %beyond High School ^a	60.8	72.4	<0.01	54.0	68.6	0.01
Ever Smoker: %Yes ^a	48.5	48.1	0.91	50.0	48.1	0.75
Current Smoker: %Yes ^a	12.0	14.3	0.30	12.8	13.2	0.87
Mononucleosis Ever: %Yes ^a	13.5	12.3	0.60	7.5	13.5	0.13
Season of Blood Draw: % Yes			0.54			0.34
Fall	24.3	24.7		20.0	25.1	
Spring	23.8	25.9		28.8	24.5	
Summer	25.2	27.2		21.3	26.9	
Winter	26.7	22.2		30.0	23.6	
Season of Birth: %Yes ^a			0.08			0.27
Fall	30.3	24.9		32.5	26.8	
Spring	19.1	26.2		20.0	23.3	
Summer	25.3	22.4		28.8	23.2	
Winter	25.3	26.4		18.8	26.8	
Women-specific exposures						
N	273	316		64	525	
Age of first menstrual period: mean \pm sd ^a	12.7 \pm 1.5	12.7 \pm 1.5	0.84	12.7 \pm 1.6	12.7 \pm 1.5	0.93
Hysterectomy: %Yes ^a	38.3	28.4	0.01	39.3	32.2	0.26
Menopause: %Yes ^a	65.7	58.1	0.06	72.6	60.3	0.06
Number of pregnancies: mean \pm sd ^a	2.7 \pm 2.2	2.5 \pm 2.1	0.30	3.3 \pm 2.4	2.5 \pm 2.1	<0.01
Number of live births: mean \pm sd ^a	2.2 \pm 1.8	2.0 \pm 1.8	0.21	2.6 \pm 1.9	2.1 \pm 1.8	0.02
Hormone contraceptive use ever: %Yes ^a	73.9	82.0	0.02	67.3	79.6	0.04

p-values in bold indicate statistical significance.

^a Subjects with missing data.

Table 3
Genetic Risk Scores associate with alternative autoimmunity in RA FDRs.

Group	RA GRS		SLE GRS	
	Median (IQR)	Alternative autoimmunity OR (95% CI) p-value	Median (IQR)	Alternative autoimmunity OR (95% CI) p-value
RA FDRs	1.04 (1·10)	1·40 (1·04–1·89) p = 0·02		
RA aAb+			1.41 (0·67)	1·87 (1·04–3·36) p = 0·04
RA aAb-			1.37 (0·76)	0·82 (0·48–1·41) p = 0·47
SLE FDRs	1.38 (0·77)	0·88 (0·66–1·16) p = 0·36		
SLE aAb+			1.71 (1·46)	0·97 (0·79–1·18) p = 0·73
SLE aAb-			1.50 (1·57)	1·01 (0·74–1·39) p = 0·64

Bold indicates $p < 0.05$.

RA FDRs (interaction $p = 0.04$), not SLE FDRs (0.80). Therefore, analyses were stratified by FDR autoantibody status. A higher SLE GRS increased the odds of alternative autoimmunity in aAb + RA FDRs, but not in aAb- RA FDRs (Table 3). To determine if shared SNPs between SLE and RA drove the association between alternative autoimmunity and the SLE GRS, we removed 6 SNPs common to both the RA and SLE GRS (Supplementary Table 1). This revised SLE GRS maintained a similar association with alternative autoimmunity in both aAb + (OR 1·86, 95% CI 0·93–3·71) and aAb- (0·66, 0·34–2·17) RA FDRs, indicating the risk is not due to the shared SNPs alone.

4. Discussion

Although extensive research has focused on the evolution of disease-specific autoantibodies, little is known about the development of alternative autoantibodies, especially during the preclinical period and as clinical disease evolves. This study provides new insights into the development of alternative autoantibodies in SLE, RA, and T1D patients and at-risk FDRs.

The high rates of alternative and expanded autoimmunity among SLE patients and FDRs in our study are consistent with the recent observation that SLE patients have higher rates of latent polyautoimmunity than patients with RA, Sjogren's syndrome, or systemic sclerosis [15]. Because FDRs have heightened risk of developing autoimmune disease [20], the presence of alternative autoantibodies in FDRs suggests that alternative autoimmunity appears before clinical disease onset, likely very early in the disease course. Indeed, in both SLE and RA cohorts, the frequency and patterns of alternative autoimmunity in FDRs closely resembled those of patients, implying that the fundamental pattern of autoimmunity may be established in early pathogenesis. However, within the SLE cohort FDRs were more likely than patients to exhibit high levels of anti-CCP3.1 [7]. Because we do not know the disease risk for RA of these FDRs, it is uncertain whether these antibodies would impact the clinical trajectory in these FDRs, or whether this preclinical pattern of broad autoimmunity would narrow to focus on disease-specific autoantibodies as the clinically apparent disease approaches. In addition, similar to known clinical disease associations [35,36] and recent descriptions of latent and overt polyautoimmunity in SLE and RA patients [15], we observed a predominance of RA autoantibodies and anti-phospholipid in SLE patients, anti-TPO and SLE autoantibodies in RA patients, and anti-TPO in T1D patients. To formally delineate the development of alternative and expanded autoantibodies and define their clinical significance, longitudinal studies are needed that link temporal changes in preclinical autoantibodies with post-classification clinical characteristics and genetic/epidemiologic factors.

Since inflammation caused by target organ damage would be unexpected in unaffected FDRs, these results suggest that genetic or environmental factors may influence development of alternative autoantibodies in this at-risk population. GWAS have identified several hundred polymorphisms associated with various autoimmune diseases [37]. In an analysis integrating 39 GWAS, each immune-mediated disease shared on average 69% of its risk loci with other immune-mediated diseases; any two diseases shared up to 38% of their loci [3]. SLE, primary biliary cirrhosis, systemic sclerosis and RA correlated more strongly with each other than with T1D and other autoimmune diseases [3], consistent with a previously demonstrated cross-phenotypic effect of SNPs shared between RA and SLE [38]. Similarly, our data suggest that autoantibodies cross specificity boundaries between RA and SLE, but rarely cross boundaries between either of these diseases and T1D. Therefore, alternative autoantibodies may reflect genetic associations shared between diseases.

Our environmental analyses showing increased rates of alternative autoimmunity in aAb- FDRs in the fall and winter compared to the spring are consistent with other studies showing seasonal variation in the incidence and prevalence of autoimmune diseases and disease activity [39]. Specific environmental exposures associated with SLE and RA are known to vary with season, including vitamin D levels and infectious disease. Additional studies designed to analyze these exposures are needed to more clearly define the relationship between season and alternative autoimmunity. In addition to genetic factors, differences in age, environmental exposures, and systemic immune dysregulation may also contribute to differences in alternative autoimmunity in T1D patients compared to SLE and RA patients.

Although these results highlight the importance of understanding both shared and differentiating factors in the development of different autoimmune diseases, there are limitations. For example, this study does not indicate the true prevalence of alternative and expanded autoimmunity, in part because the study selected subjects who were not related to each other and who had a predetermined autoantibody status for their patient/proband diagnosis. To confirm and extend our findings, a larger study with a prevalence design is needed, with appropriate statistics to control for familial relationships between participants. In addition, while our findings are consistent with recent descriptions of polyautoimmunity and latent polyautoimmunity in patients with autoimmune diseases, the concepts of latent polyautoimmunity and alternative/expanded polyautoimmunity are not perfectly interchangeable, especially as they are currently presented in the context of distinct study designs. Studies that systematically ascertain a wide range of autoantibodies and clinical disease in large familial collections would elucidate the relationship between autoantibodies, overt clinical disease, and shared disease risk. Moreover, longitudinal studies and broader discussions within the field of autoimmunity are needed to review and further define the relationships between these concepts, and to confirm their relationship to clinical outcomes.

Collectively, these findings demonstrate the presence of both a disease-specific and an alternative/expanded disease autoantibody repertoire during the preclinical period, which may influence the subsequent clinical presentation and progression. In addition, the results suggest that preclinical autoantibody screening focused on a single disease may hinder accurate classification for natural history studies and disease-specific prevention trials. Perhaps most notably, FDRs of patients with SLE and RA commonly develop alternative autoantibodies, which vary substantially in these unaffected family members of the two disorders.

Acknowledgements

We thank Dr. Carla Greenbaum and the Diabetes Clinical Research Program at the Benaroya Research Institute for providing sera from individuals with established T1D.

Funding sources

Research reported in this publication was made possible by collaborations and funding through the Cooperative Study Group for Autoimmune Disease Prevention, supported by the NIH Division of Allergy, Immunology, and Transplantation (U01AI101934, U01AI101981, U01AI130830) (JA, VMH, JBH). This study was also supported by the National Institute of Allergy and Infectious Disease (U19AI082714 [JA]), R01AI024717 [JBH]), the National Institute of Arthritis, Musculoskeletal and Skin Diseases (P30AR053483 [JA]), R01AR051394 [VMH]), National Human Genome Research Institute (U01HG008666 [JBH]) and the National Institute of General Medical Sciences (P30GM103510, U54GM104938 [JA]) of the National Institutes of Health and I01BX001834 (JBH) of the US Department of Veterans Affairs.

Role of the funding source

The study sponsors had no role in study design, data collection, data analysis, interpretation, writing of the report, or the decision to submit the paper for publication. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the United States government.

Declaration of interests

Dr. James, Dr. Chen, Dr. Bourn, Dr. Gaffney, Ms. Kelly, and Dr. Hafler report grants from NIH, during the conduct of the study. Dr. Deane reports non-financial support from Inova Diagnostics, Inc., personal fees from Inova Diagnostics, Inc., outside the submitted work. Dr. Harley reports grants from NIH, grants from US Department of Veterans Affairs, during the conduct of the study. Dr. O'Connor reports grants from Ra Pharmaceuticals, personal fees from Genentech, personal fees from Momenta Pharmaceuticals, and personal fees from Editas Medicine, outside the submitted work. Dr. Guthridge reports grants from NIH, during the conduct of the study; other from Dxterity, Inc. outside the submitted work. Dr. Holers reports grants from National Institutes of Health – NIAID, during the conduct of the study; personal fees from Janssen, outside the submitted work; in addition, Dr. Holers has a patent with Stanford University. Dr. Young, Ms. Bemis, Ms. Seifert, Dr. Demoruelle, Dr. Feser, Dr. O'Dell, Dr. Weisman, Dr. Keating, Dr. Langefeld, Dr. Robinson, Dr. Buckner, and Dr. Norris have nothing to disclose.

Author contributions

J.A. James, D.A. Hafler, J. Buckner, J.M. Norris, and V.M. Holers contributed to the conception and/or design of the study. J.A. James, J. Seifert, K.D. Deane, M.K. Demoruelle, M. Feser, J.R. O'Dell, M.H. Weisman, R.M. Keating, P.M. Gaffney, J.A. Kelly, C.D. Langefeld, J.B. Harley, W. Robinson, D.A. Hafler, K.C. O'Connor, J. Buckner, J.M. Guthridge, and V.M. Holers contributed to data acquisition. J.A. James, H. Chen, K.A. Young, E.A. Bemis, J. Seifert, R.L. Bourn, K.D. Deane, M.K. Demoruelle, M. Feser, J.A. Kelly, C.D. Langefeld, J.B. Harley, J. Buckner, J.M. Guthridge, J.M. Norris, and V.M. Holers contributed to data analysis and/or interpretation. J.A. James, R.L. Bourn, J.M. Guthridge, and V.M. Holers drafted the manuscript. All authors revised the manuscript critically for important intellectual content.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ebiom.2019.03.063>.

References

- Malinowski KP, Kawalec PP, Mocko P. Indirect costs of absenteeism due to rheumatoid arthritis, psoriasis, multiple sclerosis, insulin-dependent diabetes mellitus, and ulcerative colitis in 2012: a study based on real-life data from the Social Insurance Institution in Poland. *Expert Rev Pharmacoecon Outcomes Res* 2016;16(2):295–303.
- Barber MRW, Clarke AE. Socioeconomic consequences of systemic lupus erythematosus. *Curr Opin Rheumatol* 2017;29(5):480–5.
- Farh KK, Marson A, Zhu J, et al. Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature* 2015;518(7539):337–43.
- Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med* 2003;349(16):1526–33.
- Putterman C, Diamond B. Immunization with a peptide surrogate for double-stranded DNA (dsDNA) induces autoantibody production and renal immunoglobulin deposition. *J Exp Med* 1998;188(1):29–38.
- Raz E, Brezis M, Rosenmann E, Eilat D. Anti-DNA antibodies bind directly to renal antigens and induce kidney dysfunction in the isolated perfused rat kidney. *J Immunol* 1989;142(9):3076–82.
- Demoruelle MK, Parish MC, Derber LA, et al. Performance of anti-cyclic citrullinated peptide assays differs in subjects at increased risk of rheumatoid arthritis and subjects with established disease. *Arthritis Rheum* 2013;65(9):2243–52.
- Verheul MK, Bohringer S, van Delft MAM, et al. Triple positivity for anti-citrullinated protein autoantibodies, rheumatoid factor, and anti-carbamylated protein antibodies conferring high specificity for rheumatoid arthritis: implications for very early identification of at-risk individuals. *Arthritis Rheum* 2018;70(11):1721–31.
- Kastbom A, Roos Ljungberg K, Ziegelsch M, Wettero J, Skogh T, Martinsson K. Changes in anti-citrullinated protein antibody isotype levels in relation to disease activity and response to treatment in early rheumatoid arthritis. *Clin Exp Immunol* 2018;194(3):391–9.
- Sokolove J, Bromberg R, Deane KD, et al. Autoantibody epitope spreading in the pre-clinical phase predicts progression to rheumatoid arthritis. *PLoS One* 2012;7(5):e35296.
- Silva DG, Daley SR, Hogan J, et al. Anti-islet autoantibodies trigger autoimmune diabetes in the presence of an increased frequency of islet-reactive CD4 T cells. *Diabetes* 2011;60(8):2102–11.
- Bingley PJ, Boulware DC, Krischer JP, Type 1 Diabetes TrialNet Study G. The implications of autoantibodies to a single islet antigen in relatives with normal glucose tolerance: development of other autoantibodies and progression to type 1 diabetes. *Diabetologia* 2016;59(3):542–9.
- Xu P, Krischer JP, Type 1 Diabetes TrialNet Study G. Prognostic classification factors associated with development of multiple autoantibodies, dysglycemia, and type 1 diabetes—A recursive partitioning analysis. *Diabetes Care* 2016;39(6):1036–44.
- Anaya JM, Restrepo-Jimenez P, Rodriguez Y, et al. Sjogren's syndrome and autoimmune thyroid disease: two sides of the same coin. *Clin Rev Allergy Immunol* 2018. <https://doi.org/10.1007/s12016-018-8709-9> [Epub ahead of print].
- Molano-Gonzalez N, Rojas M, Monsalve DM, et al. Cluster analysis of autoimmune rheumatic diseases based on autoantibodies. New insights for polyautoimmunity. *J Autoimmun* 2019;98:24–32.
- Skare TL, Nishihara R, Barbosa BB, da Luz A, Utiyama S, Picceli V. Anti-CCP in systemic lupus erythematosus patients: a cross sectional study in Brazilian patients. *Clin Rheumatol* 2013;32(7):1065–70.
- Aikawa NE, Jesus AA, Liphaus BL, et al. Organ-specific autoantibodies and autoimmune diseases in juvenile systemic lupus erythematosus and juvenile dermatomyositis patients. *Clin Exp Rheumatol* 2012;30(1):126–31.
- Notsu K, Note S, Nabeya N, Kuno S, Sakurami T. Antinuclear antibodies in childhood diabetes. *Endocrinol Jpn* 1983;30(4):469–73.
- Bruner BF, Guthridge JM, Lu R, et al. Comparison of autoantibody specificities between traditional and bead-based assays in a large, diverse collection of patients with systemic lupus erythematosus and family members. *Arthritis Rheum* 2012;64(11):3677–86.
- Sparks JA, Chen CY, Hiraki LT, Malspeis S, Costenbader KH, Karlson EW. Contributions of familial rheumatoid arthritis or lupus and environmental factors to risk of rheumatoid arthritis in women: a prospective cohort study. *Arthritis Care Res (Hoboken)* 2014;66(10):1438–46.
- van der Linden MW, Westendorp RG, Zidane M, Meheus L, Huizinga TW. Autoantibodies within families of patients with systemic lupus erythematosus are not directed against the same nuclear antigens. *J Rheumatol* 2001;28(2):284–7.
- Young KA, Deane KD, Derber LA, et al. Relatives without rheumatoid arthritis show reactivity to anti-citrullinated protein/peptide antibodies that are associated with arthritis-related traits: studies of the etiology of rheumatoid arthritis. *Arthritis Rheum* 2013;65(8):1995–2004.
- Koppejan H, Trouw LA, Sokolove J, et al. Role of anti-carbamylated protein antibodies compared to anti-citrullinated protein antibodies in indigenous north Americans with rheumatoid arthritis, their first-degree relatives, and healthy controls. *Arthritis Rheum* 2016;68(9):2090–8.
- Kuo CF, Grainge MJ, Valdes AM, et al. Familial aggregation of systemic lupus erythematosus and coaggregation of autoimmune diseases in affected families. *JAMA Intern Med* 2015;175(9):1518–26.
- Rasmussen A, Sevier S, Kelly JA, et al. The lupus family registry and repository. *Rheumatology (Oxford)* 2011;50(1):47–59.
- Kolfenbach JR, Deane KD, Derber LA, et al. A prospective approach to investigating the natural history of preclinical rheumatoid arthritis (RA) using first-degree relatives of probands with RA. *Arthritis Rheum* 2009;61(12):1735–42.
- McClain MT, Arbuckle MR, Heinlen LD, et al. The prevalence, onset, and clinical significance of antiphospholipid antibodies prior to diagnosis of systemic lupus erythematosus. *Arthritis Rheum* 2004;50(4):1226–32.
- Bonifacio E, Yu L, Williams AK, et al. Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for national institute of diabetes and digestive and kidney diseases consortia. *J Clin Endocrinol Metab* 2010;95(7):3360–7.

- [29] Bao F, Yu L, Babu S, et al. One third of HLA DQ2 homozygous patients with type 1 diabetes express celiac disease-associated transglutaminase autoantibodies. *J Autoimmun* 1999;13(1):143–8.
- [30] Langefeld CD, Ainsworth HC, Cunninghame Graham DS, et al. Transancestral mapping and genetic load in systemic lupus erythematosus. *Nat Commun* 2017;8:16021.
- [31] Shah TS, Liu JZ, Floyd JA, et al. optiCall: a robust genotype-calling algorithm for rare, low-frequency and common variants. *Bioinformatics (Oxford, England)* 2012;28(12):1598–603.
- [32] Chen L, Morris DL, Vyse TJ. Genetic advances in systemic lupus erythematosus: an update. *Curr Opin Rheumatol* 2017;29(5):423–33.
- [33] Knevel R, Huizinga TWJ, Kurreeman F. Genomic influences on susceptibility and severity of rheumatoid arthritis. *Rheum Dis Clin N Am* 2017;43(3):347–61.
- [34] Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* 2009;5(6):e1000529.
- [35] Gergianaki I, Bertias G. Systemic lupus erythematosus in primary care: an update and practical messages for the general practitioner. *Front Med* 2018;5:161.
- [36] Bliddal S, Nielsen CH, Feldt-Rasmussen U. Recent advances in understanding autoimmune thyroid disease: the tallest tree in the forest of polyautoimmunity. *F1000Research* 2017;6:1776.
- [37] Parkes M, Cortes A, van Heel DA, Brown MA. Genetic insights into common pathways and complex relationships among immune-mediated diseases. *Nat Rev Genet* 2013;14(9):661–73.
- [38] Marquez A, Vidal-Bralo L, Rodriguez-Rodriguez L, et al. A combined large-scale meta-analysis identifies COG6 as a novel shared risk locus for rheumatoid arthritis and systemic lupus erythematosus. *Ann Rheum Dis* 2017;76(1):286–94.
- [39] Watad A, Azrielant S, Bragazzi NL, et al. Seasonality and autoimmune diseases: the contribution of the four seasons to the mosaic of autoimmunity. *J Autoimmun* 2017;82:13–30.