


Machine Learning for the Identification of a Common Signature for Anti-SSA/Ro 60 Antibody Expression Across Autoimmune Diseases

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Objective. Anti-Ro autoantibodies are among the most frequently detected extractable nuclear antigen autoantibodies, mainly associated with primary Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), and undifferentiated connective tissue disease (UCTD). This study was undertaken to determine if there is a common signature for all patients expressing anti-Ro 60 autoantibodies regardless of their disease phenotype.

Methods. Using high-throughput multiomics data collected from the cross-sectional cohort in the PRECISE Systemic Autoimmune Diseases (PRECISESADS) study Innovative Medicines Initiative (IMI) project (genetic, epigenomic, and transcriptomic data, combined with flow cytometry data, multiplexed cytokines, classic serology, and clinical data), we used machine learning to assess the integrated molecular profiling of 520 anti-Ro 60+ patients compared to 511 anti-Ro 60– patients with primary SS, patients with SLE, and patients with UCTD, and 279 healthy controls.

Results. The selected clinical features for RNA-Seq, DNA methylation, and genome-wide association study data allowed for a clear distinction between anti-Ro 60+ and anti-Ro 60– patients. The different features selected using machine learning from the anti-Ro 60+ patients constituted specific signatures when compared to anti-Ro 60– patients and healthy controls. Remarkably, the transcript Z score of 3 genes (*ATP10A*, *MX1*, and *PARP14*), presenting with over-expression associated with hypomethylation and genetic variation and independently identified using the Boruta algorithm, was clearly higher in anti-Ro 60+ patients compared to anti-Ro 60– patients regardless of disease type. Our findings demonstrated that these signatures, enriched in interferon-stimulated genes, were also found in anti-Ro 60+ patients with rheumatoid arthritis and those with systemic sclerosis and remained stable over time and were not affected by treatment.

Conclusion. Anti-Ro 60+ patients present with a specific inflammatory signature regardless of their disease type, suggesting that a dual therapeutic approach targeting both Ro-associated RNAs and anti-Ro 60 autoantibodies should be considered.

INTRODUCTION

Anti-Ro autoantibodies are among the most frequently detected extractable nuclear antigen (ENA) autoantibodies and are mainly associated with primary Sjögren's syndrome (SS). These autoantibodies are also frequently observed in systemic lupus erythematosus (SLE)

and undifferentiated connective tissue disease (UCTD) (1,2). Additionally, anti-Ro autoantibodies have been reported in other autoimmune diseases such as systemic sclerosis (SSc), mixed connective tissue disease (MCTD), rheumatoid arthritis (RA), and myositis (3).

Anti-Ro autoantibodies include reactivity against 2 autoantigens (Ro 52 and Ro 60) encoded by separate genes and are

Supported by the Innovative Medicines Initiative Joint Undertaking (grant 115565; PRECISE Systemic Autoimmune Diseases [PRECISESADS] study). The PRECISESADS study is supported by the European Union Seventh Framework Programme (FP7/2007–2013) and in-kind contribution from companies in the European Federation of Pharmaceutical Industries and Associations. The B Lymphocytes, Autoimmunity and Immunotherapies Laboratory is supported by the Agence Nationale de la Recherche Investissement d'Avenir program (reference no. ANR-11-LABX-0016-001) (Laboratoire d'Excellence IGO).

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Author disclosures are available at <https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fart.42243&file=art42243-sup-0001-Disclosureform.pdf>.

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Submitted for publication December 24, 2021; accepted in revised form May 17, 2022.

found in distinct cellular compartments (4). Ro 52 is a type I interferon (IFN)- and type II IFN-inducible protein (5,6) and is a negative regulator of proinflammatory cytokine production (7). Ro 60 antigen binds to ~100 nucleotide noncoding RNAs called human Y RNA (8) and acts as a quality checkpoint for RNA misfolding with molecular chaperones for defective RNA (9).

Findings from previous studies have shown variation in clinical manifestations or outcomes according to the presence or absence of anti-Ro autoantibodies. Thus, SLE subjects with anti-Ro 60 antibodies have an increased prevalence of skin disease, photosensitivity, and nephritis, along with elevated expression of IFN-inducible genes in immune cells and tissue samples (10). In primary SS, patients with both anti-Ro 60 and Ro 52 antibodies were distinguished by a higher prevalence of markers of B cell hyperactivity and glandular inflammation (11). Those patients also had earlier disease onset and presented with more systemic extraglandular manifestations, such as leukopenia, hypergammaglobulinemia, and major salivary gland swelling (12). Recently, 2 subgroups of patients with primary SS were defined according to HLA association, Ro 60/SSB antibodies, and clinical manifestations. The Ro 60/SSB antibody-positive subgroup was younger at disease onset and diagnosis and more frequently presented with anemia, leukopenia, hypergammaglobulinemia, purpura, major salivary gland swelling, lymphadenopathy, and lymphoma. These results confirmed an overall more severe disease phenotype in patients who were Ro 60/SSB antibody-positive compared to patients negative for both anti-Ro 60 and anti-SSB antibodies (13). Anti-Ro 60 reactivity alone strongly correlated with oral ulcers, a characteristic manifestation of SLE, while the combination of anti-Ro 60 and anti-Ro 52 was significantly more prevalent in patients with interstitial kidney disease and sicca syndrome symptoms (14).

Due to the presence of anti-Ro 60 antibodies in different autoimmune diseases and the reported clinical manifestations that characterize this expression, the question remains of if there is a common signature for all patients expressing anti-Ro 60 autoantibodies that would allow physicians to consider a suitable therapy regardless of disease phenotype.

Using algorithms derived from machine learning, the present study was undertaken to determine the precise signature of anti-Ro 60+ patients in diseases where this autoantibody is the most frequently observed (primary SS, SLE, and UCTD) using high-throughput multiomics data collected in the PRECISE Systemic Autoimmune Diseases (PRECISESADS) study Innovative Medicines Initiative Joint Undertaking project (genetic, epigenomic, and transcriptomic data, combined with flow cytometry data, multiplexed cytokines, classic serology, and clinical data). In this study, we performed integrated molecular profiling of 520 anti-Ro 60+ patients compared to 511 anti-Ro 60- patients and 279 healthy controls. We then observed whether this signature was also present in the 41 of 725 anti-Ro 60+ patients with other autoimmune diseases such as MCTD, RA, and SSc, all from the

same PRECISESADS cohort. See Appendix A for a list of members of the PRECISESADS Clinical Consortium and members of the PRECISESADS Flow Cytometry Consortium.

PATIENTS AND METHODS

Patient population. The present study was conducted in 1,755 patients (367 with primary SS, 508 with SLE, 156 with UCTD, 307 with RA, 327 with SSc, and 90 with MCTD) and 279 healthy controls included in the European multicenter cross-sectional study of the PRECISESADS IMI consortium (15). Classification criteria were the 2010 American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) RA classification criteria (16), the 1997 update of 1982 ACR SLE criteria (17), 2013 ACR/EULAR SSc classification criteria (18), or the American-European Consensus Group primary SS classification criteria (19) with at least the presence of anti-Ro and/or a positive focus score on minor salivary gland biopsy, MCTD using the Alarcon-Segovia criteria (20), and UCTD in patients with clinical features of systemic autoimmune diseases that did not fulfill any of the above criteria or any other systemic autoimmune disease criteria for at least 2 years (with the presence of nonspecific antibodies, antinuclear antibodies [ANAs] $\geq 1:160$). Patients fulfilling 3 of 4 SLE classification criteria and patients with early SSc (21) were not classified as having UCTD. Recruitment occurred between December 2014 and October 2017 involving 19 institutions in 9 countries (Austria, Belgium, France, Germany, Hungary, Italy, Portugal, Spain, and Switzerland).

The PRECISESADS study adhered to the standards set by International Council for Harmonisation Guidelines for Good Clinical Practice and the ethics principles that have their origin in the 2013 Declaration of Helsinki. Informed consent was obtained from each patient prior to inclusion in the study. This study protocol was approved the Ethics Review Boards of the 19 participating institutions. Protection of the confidentiality of records that could identify included subjects was ensured as defined according to the European Union Directive 2001/20/EC and the applicable national and international requirements relating to data protection in each participating country. The cross-sectional cohort study and inception cohort study are registered on [ClinicalTrials.gov](https://clinicaltrials.gov) ([clinicalTrials.gov](https://clinicaltrials.gov) identifiers: NCT02890121 and NCT02890134, respectively). The anti-Ro 60+ signature identified using machine learning was validated using the transcriptome of 106 patients in the PRECISESADS inception study ([ClinicalTrials.gov](https://clinicaltrials.gov) identifier: NCT02890134), who were followed up and had samples collected at the time of recruitment and at 6 and/or 14 months. Of note, patients in the inception cohort were diagnosed within less than a year and had not received high doses of immunosuppressants, cyclophosphamide, or belimumab at least 3 months prior to recruitment. For time points 6 and 14 months, patients could receive any standard of care therapy prescribed by their physician. Healthy controls were individuals not receiving

Table 1. Demographic and clinical characteristics of the healthy controls, patients with primary SS, those with SLE, and those with UCTD according to anti-Ro 60 expression (anti-Ro 60+ patients compared to anti-Ro 60- patients)*

Characteristic	Value	Characteristic	Value
Healthy controls		Steroid usage (<i>cont'd</i>)	
No. of patients	279	Yes	
Age, mean ± SD years	52 ± 9	Anti-Ro 60-	16 (26)
Sex, female	262 (94)	Anti-Ro 60+	62 (20)
Sex, male	17 (6.1)	<i>P</i>	0.3
Ethnicity		Antimalarials	
American Indian/Alaska Native	0 (0)	No	
Asian	2 (0.7)	Anti-Ro 60-	37 (61)
Black/African American	0 (0)	Anti-Ro 60+	194 (63)
Caucasian/White	277 (99)	Yes	
Native Hawaiian/Other Pacific Islander	0 (0)	Anti-Ro 60-	24 (39)
Other	0 (0)	Anti-Ro 60+	112 (37)
Obesity†	16 (5.8)	<i>P</i>	0.7
Smoking‡	38 (14)	Immunosuppressants	
Primary SS		No	
No. of patients	367	Anti-Ro 60-	47 (77)
Anti-Ro 60-	61	Anti-Ro 60+	264 (86)
Anti-Ro 60+	306	Yes	
Age, mean ± SD years		Anti-Ro 60-	14 (23)
Anti-Ro 60-	59 ± 13	Anti-Ro 60+	42 (14)
Anti-Ro 60+	57 ± 13	<i>P</i>	0.067
<i>P</i> §	0.3	Biologics**	
Sex, female		No	
Anti-Ro 60-	57 (93)	Anti-Ro 60-	10 (100)
Anti-Ro 60+	293 (96)	Anti-Ro 60+	29 (100)
Sex, male		Yes	
Anti-Ro 60-	4 (6.6)	Anti-Ro 60-	0 (0)
Anti-Ro 60+	13 (4.2)	Anti-Ro 60+	0 (0)
<i>P</i>	0.5	<i>P</i>	>0.9
Ethnicity		PhGA, mean ± SD score††	
American Indian/Alaska Native		Anti-Ro 60-	30 ± 18
Anti-Ro 60-	0 (0)	Anti-Ro 60+	24 ± 19
Anti-Ro 60+	0 (0)	<i>P</i>	0.007
Asian		ESSDAI, mean ± SD score‡‡	
Anti-Ro 60-	0 (0)	Anti-Ro 60-	4 ± 6
Anti-Ro 60+	2 (0.7)	Anti-Ro 60+	5 ± 5
Black/African American		<i>P</i>	0.11
Anti-Ro 60-	0 (0)	ESSPRI, mean ± SD score§§	
Anti-Ro 60+	1 (0.3)	Anti-Ro 60-	5.59 ± 2.29
Caucasian/White		Anti-Ro 60+	4.71 ± 2.33
Anti-Ro 60-	61 (100)	<i>P</i>	0.029
Anti-Ro 60+	299 (98)	SLE	
Native Hawaiian/Other Pacific Islander		No. of patients	508
Anti-Ro 60-	0 (0)	Anti-Ro 60-	333
Anti-Ro 60+	0 (0)	Anti-Ro 60+	175
Other		Age, mean ± SD years	
Anti-Ro 60-	0 (0)	Anti-Ro 60-	46 ± 14
Anti-Ro 60+	4 (1.3)	Anti-Ro 60+	45 ± 13
<i>P</i>	>0.9	<i>P</i>	0.6
Obesity§		Sex, female	
Anti-Ro 60-	7 (12)	Anti-Ro 60-	302 (91)
Anti-Ro 60+	37 (12)	Anti-Ro 60+	163 (93)
<i>P</i>	>0.9	Sex, male	
Smoking¶		Anti-Ro 60-	31 (9.3)
Anti-Ro 60-	7 (12)	Anti-Ro 60+	12 (6.9)
Anti-Ro 60+	29 (10.0)	<i>P</i>	0.3
<i>P</i>	0.6	Ethnicity	
Disease duration, mean ± SD years#		American Indian/Alaska Native	
Anti-Ro 60-	9 ± 8	Anti-Ro 60-	0 (0)
Anti-Ro 60+	10 ± 8	Anti-Ro 60+	0 (0)
<i>P</i>	0.2	Asian	
Steroid usage		Anti-Ro 60-	3 (0.9)
No		Anti-Ro 60+	1 (0.6)
Anti-Ro 60-	45 (74)		
Anti-Ro 60+	244 (80)		

(Continued)

Table 1. (Cont'd)

Characteristic	Value	Characteristic	Value
Ethnicity (cont'd).		UCTD	
Black/African American		No. of patients	156
Anti-Ro 60-	3/333 (0.9)	Anti-Ro 60-	117
Anti-Ro 60+	9/175 (5.1)	Anti-Ro 60+	39
Caucasian/White		Age, mean ± SD years	
Anti-Ro 60-	318 (95)	Anti-Ro 60-	47 ± 12
Anti-Ro 60+	162 (93)	Anti-Ro 60+	46 ± 12
Native Hawaiian/Other Pacific Islander		<i>P</i>	0.6
Anti-Ro 60-	0 (0)	Sex, female	
Anti-Ro 60+	1 (0.6)	Anti-Ro 60-	108 (92)
Other		Anti-Ro 60+	36 (92)
Anti-Ro 60-	9 (2.7)	Sex, male	
Anti-Ro 60+	2 (1.1)	Anti-Ro 60-	9 (7.7)
<i>P</i>	0.010	Anti-Ro 60+	3 (7.7)
Obesity¶¶		<i>P</i>	>0.9
Anti-Ro 60-	23 (7.2)	Ethnicity	
Anti-Ro 60+	15 (8.8)	American Indian/Alaska Native	
<i>P</i>	0.5	Anti-Ro 60-	1 (0.9)
Smoking##		Anti-Ro 60+	0 (0)
Anti-Ro 60-	60 (19)	Asian	
Anti-Ro 60+	30 (18)	Anti-Ro 60-	0 (0)
<i>P</i>	0.8	Anti-Ro 60+	1 (2.6)
Disease duration, mean ± SD years		Black/African American	
Anti-Ro 60-	14 ± 10	Anti-Ro 60-	1 (0.9)
Anti-Ro 60+	12 ± 9	Anti-Ro 60+	0 (0)
<i>P</i>	0.079	Caucasian/White	
Steroid usage		Anti-Ro 60-	113 (97)
No		Anti-Ro 60+	38 (97)
Anti-Ro 60-	179 (54)	Native Hawaiian/Other Pacific Islander	
Anti-Ro 60+	77 (44)	Anti-Ro 60-	0 (0)
Yes		Anti-Ro 60+	0 (0)
Anti-Ro 60-	154 (46)	Other	
Anti-Ro 60+	98 (56)	Anti-Ro 60-	2 (1.7)
<i>P</i>	0.037	Anti-Ro 60+	0 (0)
Antimalarials		<i>P</i>	0.6
No		Obesity#	
Anti-Ro 60-	112 (34)	Anti-Ro 60-	17 (15)
Anti-Ro 60+	45 (26)	Anti-Ro 60+	6 (15)
Yes		<i>P</i>	>0.9
Anti-Ro 60-	221 (66)	Smoking§§§	
Anti-Ro 60+	130 (74)	Anti-Ro 60-	17 (15)
<i>P</i>	0.066	Anti-Ro 60+	6 (15)
Immunosuppressants		<i>P</i>	>0.9
No		Disease duration, mean ± SD years¶¶¶	
Anti-Ro 60-	234 (70)	Anti-Ro 60-	6 ± 6
Anti-Ro 60+	111 (63)	Anti-Ro 60+	7 ± 8
Yes		<i>P</i>	>0.9
Anti-Ro 60-	99 (30)	Steroid usage	
Anti-Ro 60+	64 (37)	No	
<i>P</i>	0.12	Anti-Ro 60-	78 (67)
Biologics***		Anti-Ro 60+	38 (97)
No		Yes	
Anti-Ro 60-	27 (100)	Anti-Ro 60-	39 (33)
Anti-Ro 60+	17 (100)	Anti-Ro 60+	1 (2.6)
Yes		<i>P</i>	<0.001
Anti-Ro 60-	0 (0)	Antimalarials	
Anti-Ro 60+	0 (0)	No	
<i>P</i>	>0.9	Anti-Ro 60-	62 (53)
PhGA, mean ± SD score+++		Anti-Ro 60+	23 (59)
Anti-Ro 60-	19 ± 18	Yes	
Anti-Ro 60+	21 ± 17	Anti-Ro 60-	55 (47)
<i>P</i>	0.067	Anti-Ro 60+	16 (41)
SLEDAI, mean ± SD score+++		<i>P</i>	0.5
Anti-Ro 60-	4 ± 6		
Anti-Ro 60+	5 ± 5		
<i>P</i>	0.2		

(Continued)

Table 1. (Cont'd)

Characteristic	Value	Characteristic	Value
Immunosuppressants		Biologics (cont'd)	7 (100)
No		Anti-Ro 60+	
Anti-Ro 60-	100 (85)	Yes	
Anti-Ro 60+	38 (97)	Anti-Ro 60-	4 (15)
Yes		Anti-Ro 60+	0 (0)
Anti-Ro 60-	17 (15)	<i>P</i>	0.6
Anti-Ro 60+	1 (2.6)	PhGA, mean ± SD score****	
<i>P</i>	0.045	Anti-Ro 60-	26 ± 21
Biologics###		Anti-Ro 60+	18 ± 15
No		<i>P</i>	0.12
Anti-Ro 60-	23 (85)		

* Except where indicated otherwise, values are the number (%) of patients. Significance was determined by Wilcoxon's rank sum test, Fisher's exact test, or Pearson's chi-square test. SS = Sjögren's syndrome; SLE = systemic lupus erythematosus; UCTD = undifferentiated connective tissue disease; PhGA = physician global assessment of disease activity; ESSDAI = European Alliance of Associations for Rheumatology (EULAR) Sjögren's Syndrome Disease Activity Index; ESSPRI = EULAR Sjögren's Syndrome Patient Reported Index.

† Data were missing from 1 patient.

‡ Data were missing from 10 patients.

§ Data were missing from 3 patients and 10 patients who were anti-Ro 60- and anti-Ro 60+, respectively.

¶ Data were missing from 4 patients and 15 patients who were anti-Ro 60- and anti-Ro 60+, respectively.

Data were missing from 1 patient each who was anti-Ro 60- and anti-Ro 60+.

** Data were missing from 51 patients and 277 patients who were anti-Ro 60- and anti-Ro 60+, respectively.

†† Data were missing from 2 patients and 26 patients who were anti-Ro 60- and anti-Ro 60+, respectively.

‡‡ Data were missing from 15 patients and 112 patients who were anti-Ro 60- and anti-Ro 60+, respectively.

§§ Data were missing from 17 patients and 167 patients who were anti-Ro 60- and anti-Ro 60+, respectively.

¶¶ Data were missing from 12 patients and 15 patients who were anti-Ro 60- and anti-Ro 60+, respectively.

Data were missing from 21 patients and 11 patients who were anti-Ro 60- and anti-Ro 60+, respectively.

*** Data were missing from 306 patients and 158 patients who were anti-Ro 60- and anti-Ro 60+, respectively.

††† Data were missing from 16 patients and 15 patients who were anti-Ro 60- and anti-Ro 60+, respectively.

‡‡‡ Data were missing from 159 patients and 96 patients who were anti-Ro 60- and anti-Ro 60+, respectively.

§§§ Data were missing from 2 patients who were anti-Ro 60-.

¶¶¶ Data were missing from 1 patient who was anti-Ro 60-.

Data were missing from 90 patients and 32 patients who were anti-Ro 60- and anti-Ro 60+, respectively.

**** Data were missing from 5 patients and 2 patients who were anti-Ro 60- and anti-Ro 60+, respectively.

long-term medication, without any inflammatory autoimmune, allergic, or infectious condition, and without a history of autoimmune disease, particularly thyroid disease or other diseases that may modify cellular profiles in blood.

Determination of autoantibodies, ANAs, free light chains, and complement fractions. For all samples collected between March 2016 and June 2019, all autoantibodies were determined at a single center (University Hospital of Brest). Anti-ENA (comprising Sm, U1 RNP, Scl-70, Ro 52, Ro 60, and SSB) and specific autoantibodies anti-Ro 52 and anti-Ro 60, anti-cyclic citrullinated peptide 2 (anti-CCP2), IgG and IgM anti-β₂GPI, IgG, and IgM anticardiolipin [aCL], anti-double-stranded DNA (anti-dsDNA), and antacentromere autoantibody levels were determined using a chemiluminescent IDS-iSYS immunoanalyser (Immunodiagnostic Systems). Rheumatoid factor (RF) was determined regardless of the isotypes measured by turbidimetry using SPA_{PLUS} (Binding Site), as well as C3 and C4 complement fractions and kappa and lambda free light chains. Samples from all patients and healthy controls were tested. See Supplementary Methods for more details regarding sample and data collection (available on the *Arthritis & Rheumatology* website at <http://onlinelibrary.wiley.com/doi/10.1002/art.42243>). Autoantibodies and RF distribution were described according to

concentration level (negative, low, medium, or elevated/high), and the proportion and the concentration across anti-Ro 60+ patients and anti-Ro 60- patients in each disease were compared using Fisher's exact test. Complements C3 and C4 and circulating free light chains have been described in continued concentrations expressed in gm/liter and mg/liter respectively, and Kruskal-Wallis testing was used to compare the concentration level across the anti-Ro 60+ patients and anti-Ro 60- patients in each disease. Anti-Ro 60 autoantibody-positive samples were also classified according to their degree of positivity. Patients with positive samples with concentrations between 10 and 640 arbitrary units (AU)/ml were considered anti-Ro 60^{low} patients, whereas those with samples with a concentration > 640 AU/ml were considered anti-Ro 60^{high} patients.

ANA detection was performed using an in-house technique on HEp-2 cells (ATCC strain CCL23). Each sample was systematically tested at 5 successive dilutions (1:80, 1:160, 1:320, 1:640, 1:1,280), and the threshold of positivity was set at 1:160, according to international recommendations (22). Information regarding current or past presence of hypergammaglobulinemia was collected in each center at the time of inclusion and defined as, within 12 weeks, a serum IgG value greater than the upper limit of normal and/or gammaglobulin values >20%.

Clinical data. Clinical data obtained from 520 anti-Ro 60+ patients (306 with primary SS, 175 with SLE, and 39 with UCTD), 511 anti-Ro 60- patients (61 with primary SS, 333 with SLE, and 117 with UCTD), and 279 healthy controls were collected using an electronic case report form. Clinical data included patient's age, sex, ethnicity, disease duration, the physician global assessment of disease activity (PhGA), fulfillment of Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (23) for SLE, fulfillment of EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) (24) for primary SS, and current use of treatments.

Other available data. High-dimensional omics genotypes, RNA-Seq data, DNA methylation data, and the proportions of relevant cell types using custom flow cytometry marker panels were analyzed in whole blood samples. Additional information, such as cytokine levels, chemokine levels, and inflammatory mediator expression levels were obtained from serum samples. All of these parameters are described in greater detail in the Supplementary Methods (<http://onlinelibrary.wiley.com/doi/10.1002/art.42243>), and repartition of patients with a full data set per omic type and across diseases is shown in Supplementary Table 1.

Dimensionality reduction. Our strategy for dimensionality reduction was driven by artificial intelligence approaches involving machine learning. Patients were first grouped according to their disease (primary SS, SLE, or UCTD). We then separately considered each of the data sets describing these patients (RNA-Seq, DNA methylation, GWAS, and flow cytometry associated with cytokine expression). For each of these data sets, we performed a Boruta analysis (25) to discriminate between anti-Ro 60+ patients and anti-Ro 60- patients in order to extract features that significantly distinguish the 2 groups within each data set. The Boruta algorithm was used to create an extended data set by adding copies of each feature in the original data set. Values of the duplicated features were then shuffled, and the resulting features were called "shadow features." The random permutation of modality within these features lead to the removal of any preexisting correlation with the target variable, in our case, anti-Ro 60 positivity. Once shadow features were generated, a random forest classifier was run on the whole data set and Z scores were computed for all clinical features (real and shadow). Shadow features were then sorted according to their Z score, and the maximum score was stored in memory as a threshold. The algorithm marked each non-shadow feature with a Z score above this threshold. Finally, Boruta findings indicated the clinical features that had Z scores that were significantly lower than the shadow features with maximum Z scores. These features were considered unimportant and were removed from the data set before removing all shadow features and returning a clean data set.

We ran the Boruta algorithm for 300 iterations with a maximum depth set to 5. Extracted features were run on a linear discriminant analysis (LDA), which was only used to visually assess the distinction between anti-Ro 60+ patients, anti-Ro 60- patients, and healthy controls. No classification metrics were computed using LDA.

RESULTS

Specific biologic and clinical features of anti-Ro 60+ patients. We compared the characteristics of the 279 anti-Ro 60- healthy controls and 520 anti-Ro 60+ patients (306 with primary SS, 175 with SLE, and 39 with UCTD) to the 511 anti-Ro 60- patients (61 with primary SS, 333 with SLE, and 117 with UCTD) (Table 1). Regarding the antibody profile, compared to anti-Ro 60- patients, anti-Ro 60+ patients from the 3 diseases had significantly increased levels of ANAs, kappa and lambda free light chains, RF, anti-Ro 52, and anti-SSB antibodies (Figure 1 and Supplementary Table 2). Both anti-Ro 52 autoantibody levels and anti-SSB autoantibody levels were also significantly increased in anti-Ro 60^{high} patients compared to anti-Ro 60^{low} patients. Past and/or present hypergammaglobulinemia was more common in anti-Ro 60+ patients regardless of the disease (Supplementary Figure 1A, <http://onlinelibrary.wiley.com/doi/10.1002/art.42243>). No difference in terms of the disease activity score (ESSDAI, SLEDAI, PhGA) was observed between anti-Ro 60- patients and anti-Ro 60+ patients (Supplementary Figure 1B). However, in those with primary SS, anti-Ro 60+ patients had lower EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) (26) scores, and the higher the anti-Ro 60 scale, the lower the ESSPRI score and its components (dryness, fatigue, and pain) (Supplementary Figure 1C, <http://onlinelibrary.wiley.com/doi/10.1002/art.42243>).

Identification of a specific signature common to anti-Ro 60+ patients in the different omics data sets using machine learning. We used the Boruta algorithm (25) with all data sets to extract features that significantly contributed to the prediction of which patients were anti-Ro 60+ according to the different omics (RNA-Seq, DNA methylation, GWAS, and cytokine expression associated with cell subset distribution). A total of 923 features were selected from RNA-Seq variables, 64 features were selected from DNA methylation variables, 5,749 features were selected from GWAS variables (Supplementary Tables 3–5 respectively, <http://onlinelibrary.wiley.com/doi/10.1002/art.42243>), and 8 features were selected from the association of cytokine expression levels and cell subset distribution. An LDA for each omics is shown in Figure 2. We considered the combined analysis of patients with primary SS, patients with SLE, and patients with UCTD within the framework of the Boruta results. Features were selected from disease data sets to capture the maximum amount of discriminating information. We then considered the combination

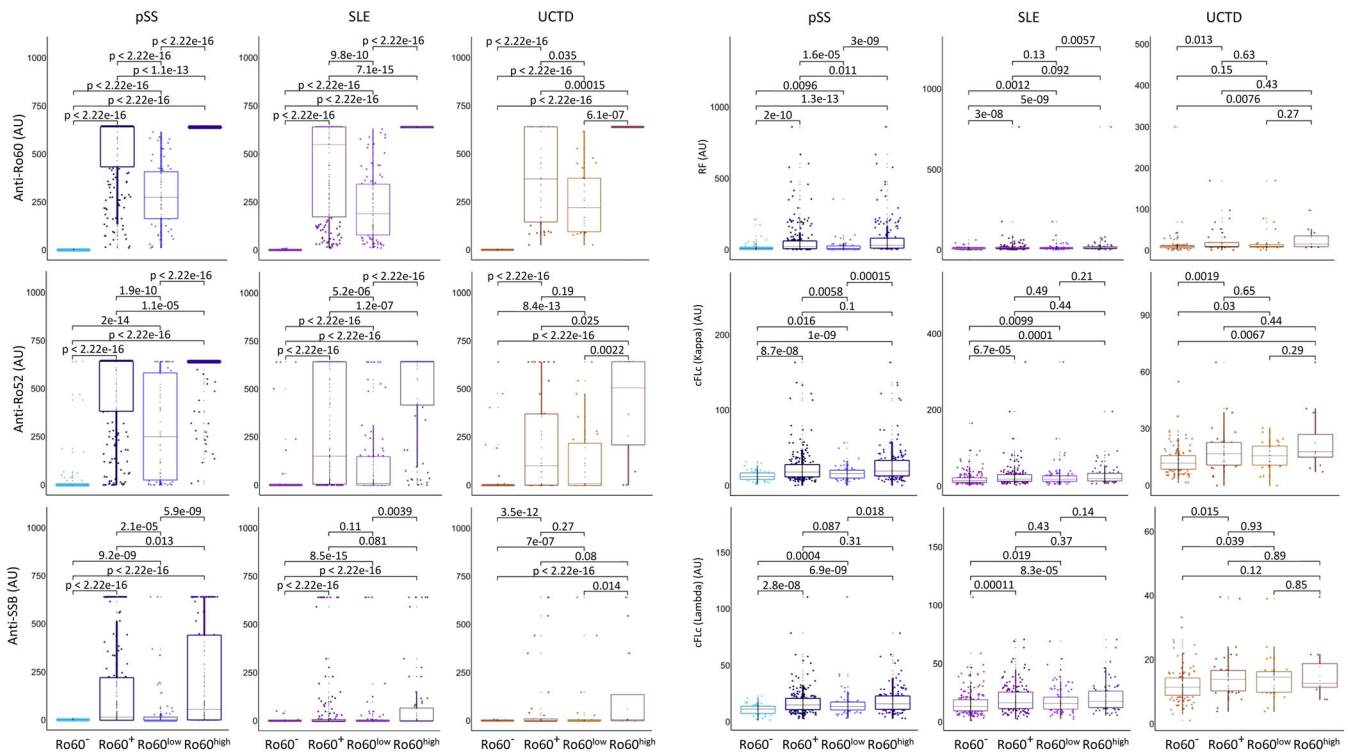


Figure 1. Serologic distributions in patients with primary Sjögren's syndrome (pSS), those with systemic lupus erythematosus (SLE), and those with undifferentiated connective tissue disease (UCTD). The presence of anti-Ro 52, anti-Ro 60, anti-SSB antibodies, rheumatoid factor (RF), and circulating free light chains (cFLc) were measured in serum samples from 520 anti-Ro 60+ patients (306 with primary SS, 175 with SLE, and 39 with UCTD) and 511 anti-Ro 60- patients (61 with primary SS, with 333 SLE, and 117 with UCTD) from the same center using an automated chemiluminescent IDS-iSYS immunoanalyser. Turbidimetry was used for the detection of RF and circulating free light chains (kappa and lambda). Anti-Ro 60+ patients were divided in 2 groups: anti-Ro 60^{low} patients (samples with concentrations between 10 and 640 arbitrary units [AU]/ml) and anti-Ro 60^{high} patients (samples with concentrations >640 AU/ml). Statistical significance was determined by 2-tailed pairwise Wilcoxon's rank sum test. Results are shown as box plots, in which each box represents the interquartile range, lines inside the box represent the median, and lines outside the box represent the 10th and 90th percentiles; symbols represent individual samples. Color figure can be viewed in the online issue, which is available at <http://onlinelibrary.wiley.com/doi/10.1002/art.42243/abstract>.

of Boruta results for primary SS, SLE, and UCTD to generate the final signature. Of note, selected features for RNA-Seq and GWAS data clearly distinguished between anti-Ro 60+ patients and anti-Ro 60- patients. Remarkably, even if data from healthy controls were not used for feature selection, their integration into the different LDA based on the Boruta algorithm-identified features that discriminate between anti-Ro 60+ patients and anti-Ro 60- patients, resulted in a separation from patients. These results demonstrate that the different features selected using machine learning from the anti-Ro 60+ patients constitute specific signatures when compared to anti-Ro 60- patients and healthy controls.

Characterization and pathway analysis of the transcriptomic signature found in anti-Ro 60+ patients. The 923 transcripts identified using machine learning to discriminate anti-Ro 60+ patients (Figure 2A) were analyzed using Reactome (27). The 25 most relevant pathways are shown in Supplementary Table 6 (<http://onlinelibrary.wiley.com/doi/10.1002/art.42243>). Anti-Ro 60+ patients were enriched in genes involved in IFN signaling (type

I and II), cytokine signaling, activation of C3 and C5, antiviral response by IFN-stimulated genes, and interleukin-10 (IL-10) signaling.

To further understand the IFN signature, we analyzed IFN-annotated modules previously described as strongly up-regulated in SLE (28,29). The different type I and type II IFN Z scores were increased in anti-Ro 60+ patients regardless of the disease (Figure 3).

Characterization and pathway analysis of the DNA methylation signature found in anti-Ro 60+ patients.

The 37 genes associated with the 64 CpGs identified using machine learning to discriminate anti-Ro 60+ patients (Figure 2B) were analyzed using Reactome. Interestingly, the most relevant pathways were the same as those previously found in the transcriptome analysis, such as type I and type II IFN signaling, cytokine signaling in the immune system, and antiviral response by IFN-stimulated genes (Supplementary Table 7, <http://onlinelibrary.wiley.com/doi/10.1002/art.42243>).

Among these 37 differentially methylated genes, 33 methylated genes were also found using the Boruta algorithm in the RNA-Seq analysis. The interaction networks of these 33 common genes,

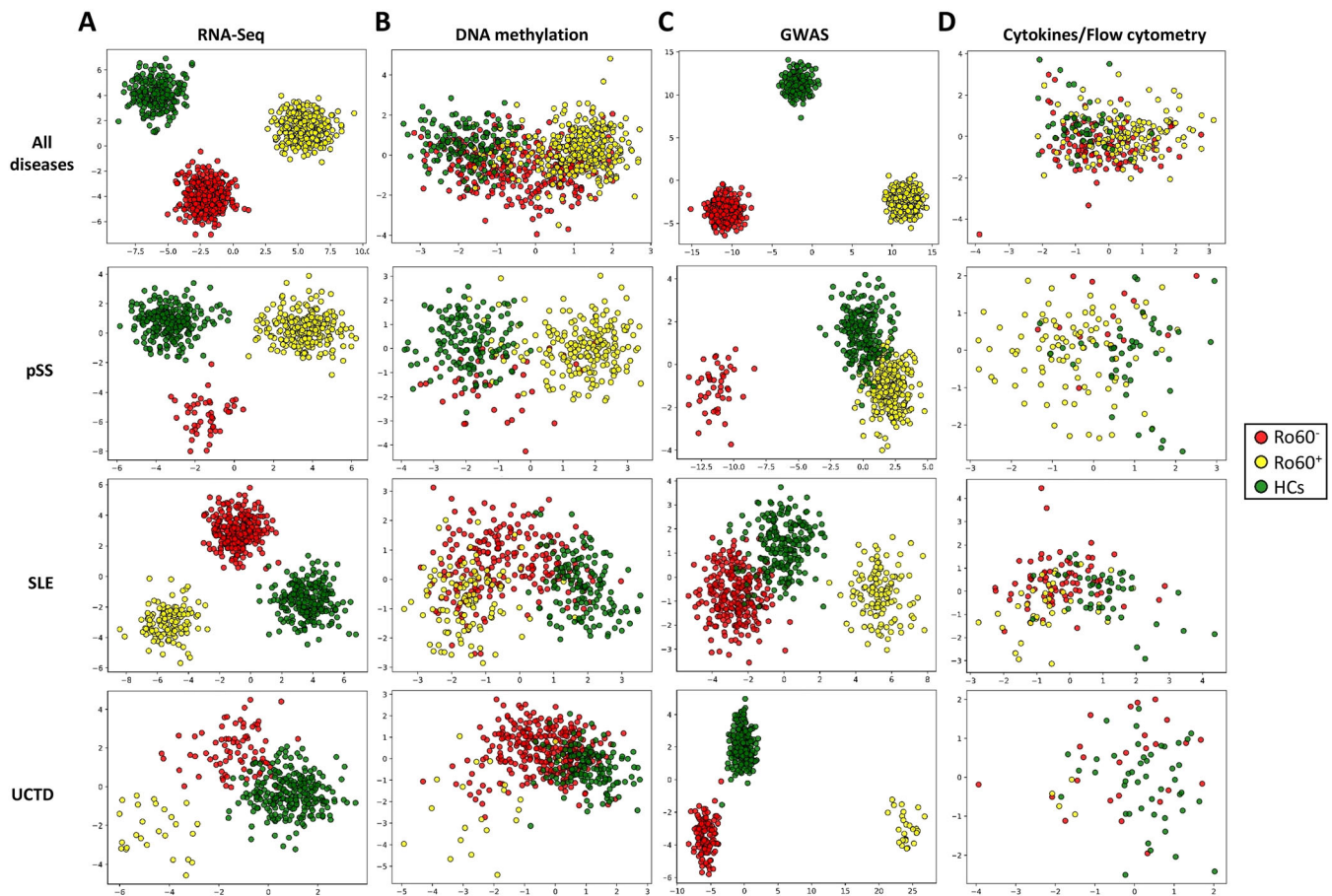


Figure 2. Identification of specific signatures common to anti-Ro 60+ patients in different omics data sets using machine learning. Linear discriminant analysis of features selected with the Boruta algorithm, assessed among all 3 diseases combined or separately among patients with SLE, those with UCTD, and those with primary SS. A total of 923 features were selected from RNA-Seq data (A), 64 features were selected from methylation data (B), 5,749 features were selected from genome-wide association study (GWAS) data (C), and 8 features were selected from flow cytometry distribution and cytokine expression data (D). Symbols represent individual samples from anti-Ro 60- patients, anti-Ro 60+ patients, and healthy controls (HCs). See Figure 1 for other definitions.

determined using STRING with a confidence cutoff of 0.4, revealed and confirmed the common IFN signature (Supplementary Figure 2, <http://onlinelibrary.wiley.com/doi/10.1002/art.42243>) (30). Of note, all transcripts were overexpressed in anti-Ro 60+ patients regardless of the disease, and global hypomethylation of CpGs was observed for all but 1 gene (*ISG15*). For 1 gene (*IFITM1*), up to 8 hypomethylated CpGs were assessed (Supplementary Figure 3, <http://onlinelibrary.wiley.com/doi/10.1002/art.42243>).

GWAS analysis of anti-Ro 60+ patients. Our machine learning approach identified 5,749 single-nucleotide polymorphisms (SNPs) that were able to discriminate anti-Ro 60+ patients from anti-Ro 60- patients (Figure 2C). Interestingly, 3 of these SNPs were located on genes previously indicated by the algorithm from the previous RNA-Seq and DNA methylation analyses (Figure 4A). The 3 corresponding genes were *ATP10A*, *MX1*, and *PARP14*. Remarkably, the transcript Z score of these 3 genes was clearly higher in anti-Ro 60+ patients compared to

anti-Ro 60- patients but was also higher in anti-Ro 60^{high} compared to anti-Ro 60^{low} patients when all diseases were merged (Figure 4B). The same was true in all the diseases and constituted a clear signature (Figure 4C). Given the strong association between anti-Ro 60 and anti-Ro 52/tripartite motif-containing protein 21 (TRIM21) antibodies, we considered that positivity for anti-Ro 52/TRIM21 may define the signature. We then divided patients with primary SS, patients with SLE, and patients with UCTD into 3 groups (anti-Ro 60-/anti-Ro 52-, anti-Ro 60+/anti-Ro 52-, and anti-Ro 60+/anti-Ro 52+) and assessed the anti-Ro 60 signature identified using the transcript Z score of the 3 genes (*ATP10A*, *MX1*, and *PARP14*). Z scores were higher in anti-Ro 60+/anti-Ro 52- patients compared to anti-Ro 60-/anti-Ro 52- patients with primary SS and those with SLE. In contrast, the Z scores were only higher in anti-Ro 60+/anti-Ro 52+ primary SS patients compared to anti-Ro 60+/anti-Ro 52- patients with primary SS, and no significant difference was observed in SLE patients and UCTD patients (Supplementary Figure 4A, <http://onlinelibrary.wiley.com/doi/10.1002/art.42243>).

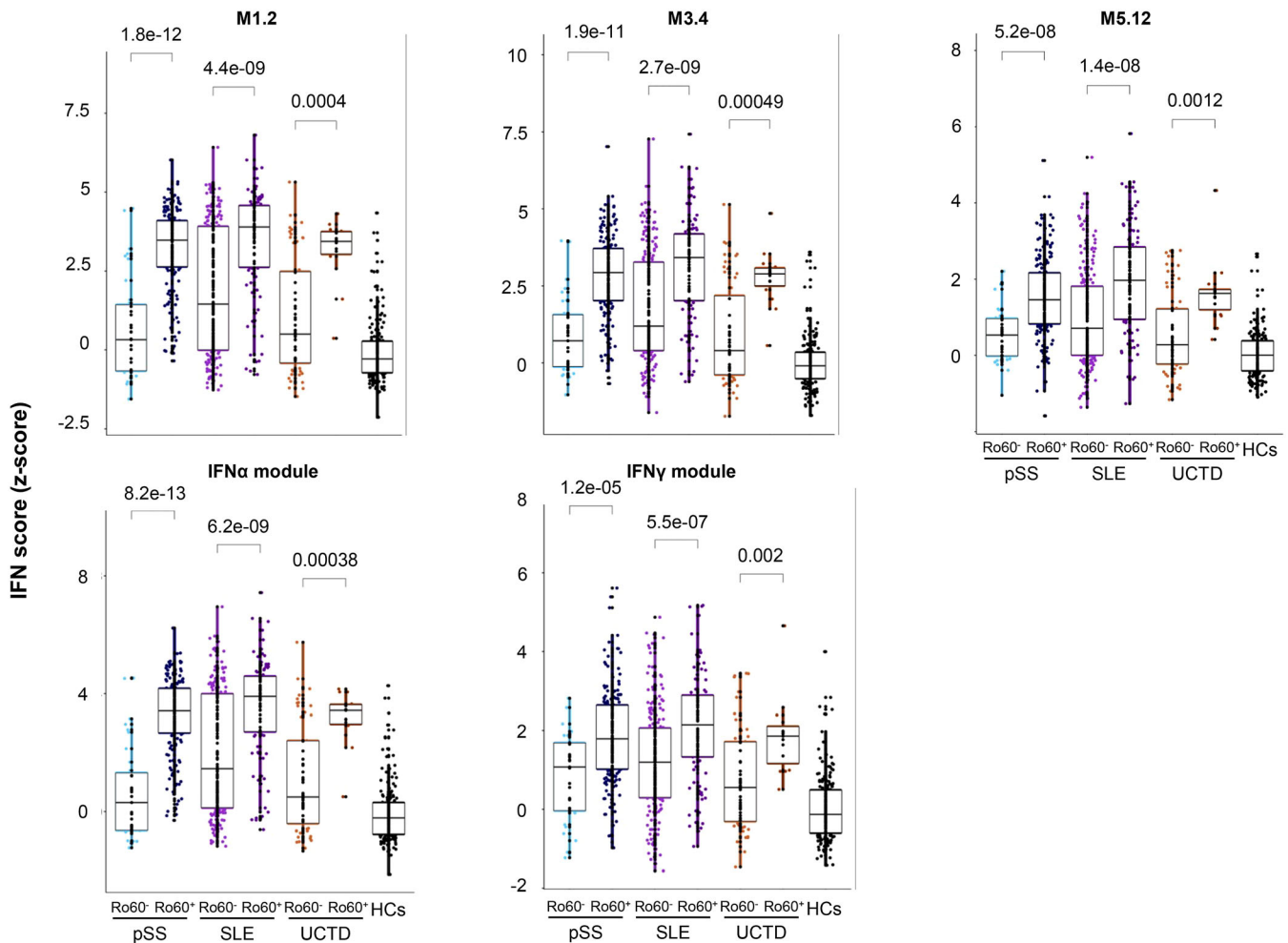


Figure 3. Anti-Ro 60+ patients have a higher interferon (IFN) signature regardless of the disease. IFN Z score analyses were performed with 411 anti-Ro 60+ patients (249 with primary SS, 136 with SLE, and 26 with UCTD) compared to 392 anti-Ro 60- patients (46 with primary SS, 267 with SLE, and 79 with UCTD) and 254 healthy controls. The genes from the M1.2 module (*IFI44*, *IFI44L*, *IFIT1*, and *MX1*) are induced by IFN α , while genes from both M1.2 and M3.4 (*ZBP1*, *IFIH1*, *EIF2AK2*, *PARP9*, and *GBP4*) are up-regulated by IFN β . The genes from the M5.12 module (*PSMB9*, *NCOA7*, *TAP1*, *ISG20*, and *SP140*) are poorly induced by IFN α and IFN β alone, while they are up-regulated by IFN γ . Moreover, transcripts belonging to M3.4 and M5.12 are only fully induced by a combination of type I and type II IFNs. Other modules identified genes preferentially induced by IFN α (*IFIT1*, *IFI44*, and *EIF2AK2*) or IFN γ (*IRF1*, *GBP1*, and *SERPINC1*). Statistical significance was determined by 2-tailed pairwise Wilcoxon's rank sum test. Results are shown as box plots, in which each box represents the interquartile range, lines inside the box represent the median, and lines outside the box represent the 10th and 90th percentiles; symbols represent individual samples. HCs = healthy controls (see Figure 1 for other definitions). Color figure can be viewed in the online issue, which is available at <http://onlinelibrary.wiley.com/doi/10.1002/art.42243/abstract>.

We also considered that anti-Ro 60 positivity may just be a marker of B cell reactivity, given that the majority of anti-Ro 60- patients were ENA negative. We then assessed the transcript Z score of the 3 genes (*ATP10A*, *MX1*, and *PARP14*) in 5 groups of patients regardless of disease: patients without any autoantibodies, anti-Ro 60-/anti-Ro 52+ patients negative for any of the autoantibodies analyzed (anti-RNP, anti-Sm, anti-SSB, anti-Scl-70, anti-CCP, anti-dsDNA, antimyeloperoxidase, anti-proteinase 3, anti-CENP-B), anti-Ro 60-/anti-Ro 52+ patients who were positive for any other autoantibodies, anti-Ro 60-/anti-Ro 52- patients who were positive for any of the autoantibodies, and anti-Ro 60+ patients. Z scores were clearly higher in anti-Ro 60+ patients compared to all the other groups (Supplementary Figure 4B,

<http://onlinelibrary.wiley.com/doi/10.1002/art.42243>). All these data confirm that the determined signature is specific to anti-Ro 60+ patients and is not just a marker of B cell activation or due to the presence of any other autoantibody.

Characterization of the flow cytometry signature and cytokine expression in anti-Ro 60+ patients.

Machine learning was used to identify 6 parameters among flow cytometry data and 2 parameters among cytokine expression data (assessed using Luminex-based quantitative assay) to discriminate anti-Ro 60+ patients from anti-Ro 60- patients (Figure 2D). The robustness of the 6 flow cytometry features was poor and was only associated with 1 disease (Supplementary Figure 5,

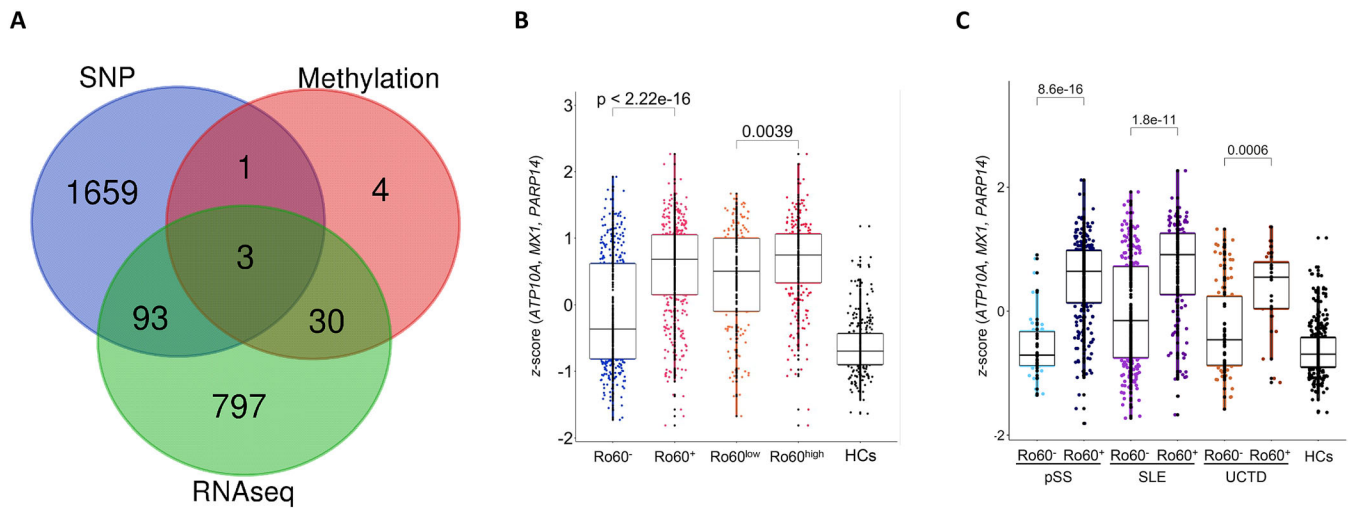


Figure 4. Three genes common to RNA-Seq data, DNA methylation data, and GWAS findings used to characterize anti-Ro 60+ patients. **A**, Venn diagram showing the number of overlapping genes according to the different omics data analyses conducted using machine learning (RNA-Seq, DNA methylation, and single-nucleotide polymorphisms [SNPs]) to discriminate anti-Ro 60+ patients from anti-Ro 60- patients. **B**, *ATP10/MX1/PARP14* Z score analyses in 803 patients and 254 healthy controls (HCs) according to anti-Ro 60 expression. **C**, *ATP10/MX1/PARP14* Z score analyses in 295 patients with primary SS, 403 patients with SLE, and 105 patients with UCTD and 254 healthy controls. Statistical significance was determined by 2-tailed pairwise Wilcoxon's rank sum test. Results are shown as box plots, in which each box represents the interquartile range, lines inside the box represent the median, and lines outside the box represent the 10th and 90th percentiles; symbols represent individual samples. See Figure 1 for other definitions. Color figure can be viewed in the online issue, which is available at <http://onlinelibrary.wiley.com/doi/10.1002/art.42243/abstract>.

<http://onlinelibrary.wiley.com/doi/10.1002/art.42243>). Interestingly however, cytokine expression in serum samples showed an increase of IFN γ -induced protein (CXCL10/IFN γ -inducible 10-kd protein) and down-regulation of IL-1 R2, the decoy receptor for cytokines belonging to the IL-1 family, in anti-Ro 60+ patients regardless of disease (Supplementary Figure 5).

Established common signature between anti-Ro 60+ patients with primary SS, those with SLE, or those with UCTD and patients with RA, those with SSc, or those with MCTD expressing anti-Ro 60 antibodies and showing stable antibody expression over time. To confirm the robustness of the identified signature, we observed whether this signature was also present in an independent cohort of 106 newly diagnosed patients with primary SS, those with SLE, or those with UCTD from the inception cohort of the PRECISESADS study, which provided an additional validation data set to test the generalization of our signature in patients whose samples were not used for the feature selection process. At inclusion (first time point), our study included 46 anti-Ro 60+ patients and 60 anti-Ro 60- patients. Again, using the 923 transcripts of the RNA-Seq signature, the LDA findings showed a clear separation between anti-Ro 60+ and anti-Ro 60- patients regardless of the disease (Supplementary Figure 6A). Furthermore, the Z scores for the 33 genes identified using the Boruta algorithm that were common to RNA-Seq and DNA methylation data were significantly increased in anti-Ro 60+ patients (Supplementary Figure 6B). This was also true with the Z scores for

the 3 genes (*ATP10A*, *MX1*, and *PARP14*) previously selected using the algorithm from the RNA-Seq, GWAS, and DNA methylation analyses (Supplementary Figure 6C).

Finally, the signature's robustness was also assessed in 724 patients with other autoimmune diseases, such as MCTD, RA, and SSc, all from the PRECISESADS cross-sectional cohort. A clear separation between anti-Ro 60+ patients ($n = 40$) and anti-Ro 60- patients ($n = 684$) using the representation space generated by the LDA is shown in Figure 5A. In all diseases except MCTD, anti-Ro 60+ patients had significantly increased Z scores for the 33 common genes (Figure 5B) and for the 3 genes constituting the signature (Figure 5C). We can therefore conclude that anti-Ro 60+ patients have a specific signature regardless of disease.

Additionally, we assessed the transcript Z scores of the 3 genes (*ATP10A*, *MX1*, and *PARP14*) in the inception cohort restricted to 86 patients (primary SS, SLE, UCTD, RA, SSc, and MCTD) who were followed up and had samples collected at 3 time points (recruitment, at 6 and/or 14 months). At the 6- and 14-month time points, patients could receive any standard of care therapy prescribed by their physician (Supplementary Table 8, <http://onlinelibrary.wiley.com/doi/10.1002/art.42243>). Anti-Ro 60+ patients remained positive and anti-Ro 60- patients remained negative over time (data not shown). We confirmed that the Z score remained stable in anti-Ro 60+ patients and anti-Ro 60- patients over time (Figure 5D). Overall, the signature identified for anti-Ro 60+ patients does not depend on treatment and is stable over time.

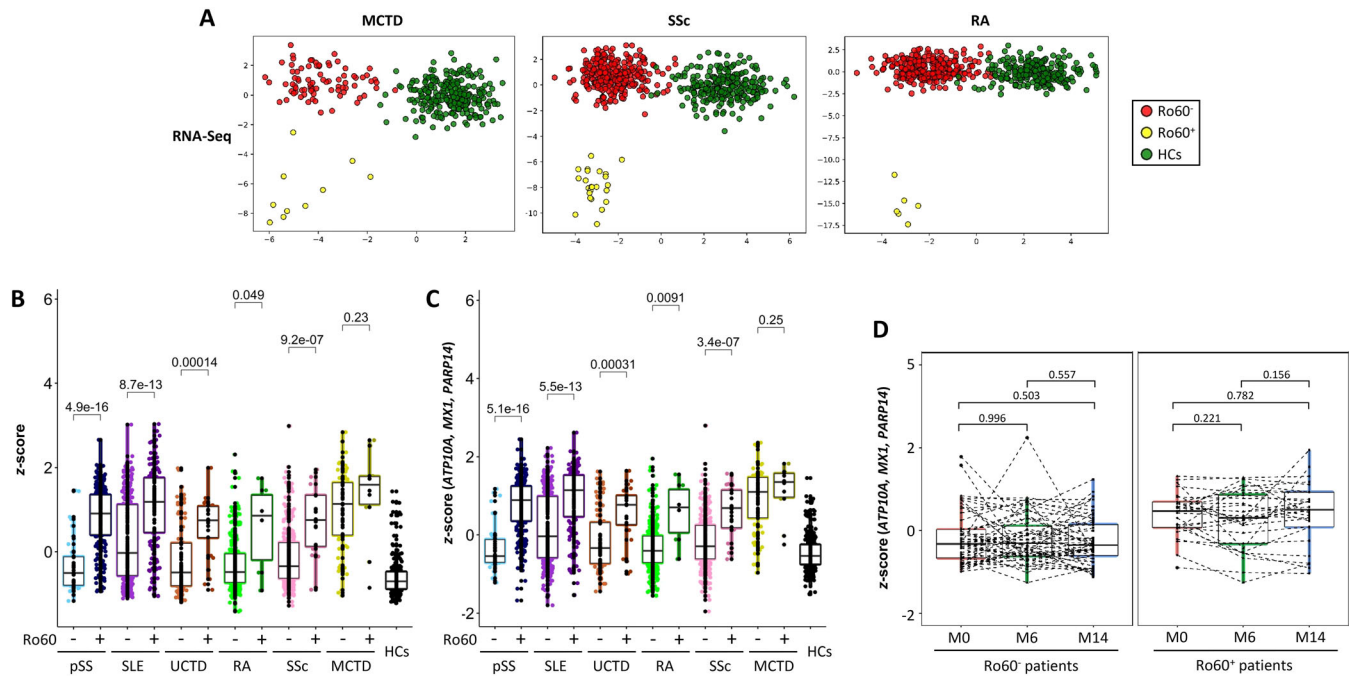


Figure 5. Confirmation of the established signature of anti-Ro 60+ patients common to patients with rheumatoid arthritis (RA), those with systemic sclerosis (SSc), or those with mixed connective tissue disease (MCTD) expressing anti-Ro 60 antibodies. **A**, Linear discriminant analysis of 923 features selected from RNA-Seq data (obtained using the Boruta algorithm in patients with SLE, UCTD, or primary SS), to discriminate anti-Ro 60+ patients from anti-Ro 60- patients with mixed connective tissue disease (MCTD), those with systemic sclerosis (SSc), and those with rheumatoid arthritis (RA). **B**, Z score analyses of the 33 genes, identified using the Boruta algorithm and common to RNA-Seq and methylome data, in 295 patients with primary SS, 403 patients with SLE, 105 patients with UCTD, 307 patients with RA, 327 patients with SSc, and 90 patients with MCTD, and 254 healthy controls (HCs). **C**, *ATP10A/MX1/PARP14* Z score analyses in 295 patients with primary SS, 403 patients with SLE, 105 patients with UCTD, 307 patients with RA, 327 patients with SSc, and 90 patients with MCTD and 254 healthy controls. Statistical significance was determined by 2-tailed pairwise Wilcoxon's rank sum test. **D**, *ATP10A/MX1/PARP14* Z score analyses in 86 patients from the inception cohort who were followed up and had samples collected at the time of recruitment (M0) and at month 6 (M6) and/or month 14 (M14). Patients were grouped as anti-Ro 60+ ($n = 29$) and anti-Ro 60- ($n = 57$) regardless of the disease (primary SS, SLE, UCTD, RA, SSc, or MCTD). Statistical significance was determined by pairwise *t*-test. Results are shown as box plots, in which each box represents the interquartile range, lines inside the box represent the median, and lines outside the box represent the 10th and 90th percentiles; symbols represent individual samples. Color figure can be viewed in the online issue, which is available at <http://onlinelibrary.wiley.com/doi/10.1002/art.42243/abstract>.

DISCUSSION

Our study demonstrates that anti-Ro 60+ patients have a specific signature regardless of disease. Anti-Ro 60+ patients compared to anti-Ro 60- patients presented with the same clinical and biologic characteristics as those previously described in the literature, such as hypergammaglobulinemia (12,13) and an association with other autoantibodies (anti-Ro 52, anti-SSB, and RF) (14). Anti-Ro 60 positivity was reported to be higher in the low symptom burden subgroups of patients with primary SS (31) in accordance with our observation that anti-Ro 60+ patients had a lower ESSPRI score.

Our study has some limitations. First, it could be argued that this was a cross-sectional study and it is assumed that single samples (cells and sera) were collected at an arbitrary time point during the disease course of different autoimmune diseases. However, in our inception cohort with a 14-month follow-up period, we demonstrated that the identified signature in anti-Ro

60+ patients remained stable over time and was not influenced by treatment. Second, virtually all subjects were Caucasian, and although common variants were expected to be old in evolution and shared across ethnicities, some risk loci show considerable ethnic differences in frequency and/or effect size.

The novelty of our study was our use of machine learning to identify a robust signature specific to anti-Ro 60+ patients through dimensionality reduction approaches, using high-throughput multi-omics data. Assessment of the signature's robustness occurred in 3 steps. First, we used discriminant features extracted from the different omics data sets to perform LDA. The new data representation spaces generated using the selected features through this analysis allowed for sufficient separation of anti-Ro 60+ patients and anti-Ro 60- patients in each of the 3 diseases studied (SLE, primary SS, and UCTD). Second, we considered the overlap of the selected features in RNA-Seq data and DNA methylation data and the overlap of the selected features in RNA-Seq data, DNA

methylation data, and GWAS data, narrowing down the original selection to 2 signatures composed of 33 genes and 3 genes, respectively. Both Z scores, generated by either the 33 genes or the 3 genes, were significantly different between anti-Ro 60+ patients and anti-Ro 60- patients in SLE, primary SS, and UCTD. Third, because we used a machine learning approach to identify the features, we assessed the possibility of overfitting by testing the validity of these signatures in patients whose samples were not used in the training process of the algorithm. Generalizability of the signature was evaluated by determining RNA-Seq features from another cohort of patients with primary SS, patients with SLE, and patients with UCTD and from RA patients, SSc patients, and MCTD patients. LDA findings consistently showed a clear distinction between anti-Ro 60+ patients and anti-Ro 60- patients.

Again, Z scores were significantly different between anti-Ro 60+ patients and anti-Ro 60- patients with RA and those with SSc but not for those with MCTD. Consequently, the discriminating properties of the representation space obtained through the computation of LDA, the statistical tests of distributions, and the generalizability to other diseases constituted strong indicators of the signature's robustness.

Reactome pathway analysis of the 33 differentially expressed and methylated genes showed a link between anti-Ro 60 antibodies and IFN signature, cytokine secretions, and IFN regulatory factor 7, which were associated with Toll-like receptor (TLR) signaling. The notable association between anti-Ro 60 autoantibodies and inflammation in autoimmune diseases led to the hypothesis that the RNA-binding properties of Ro 60 produce aberrant TLR signaling (32). Alu retroelements activate TLR-7 and TLR-8 as oligoribonucleotides and associate with Ro 60 in cell lines (33); consequently, inflammatory and IFN signatures associated with anti-Ro 60 autoantibodies may be due to the RNA-binding properties of Ro 60.

Remarkably, the transcript Z scores of 3 genes (*ATP10A*, *MX1*, and *PARP14*) were clearly higher in anti-Ro 60+ patients compared to anti-Ro 60- patients in all the diseases and constituted a clear signature. The first gene, *ATP10A*, encoded 1 of the 5 P4 ATPase that requires interaction with transmembrane protein 30A to exit from the endoplasmic reticulum to the plasma membrane. *ATP10A* was recently linked to autoimmunity, as one study demonstrated that methylation qualitative trait loci regulated the methylation of the *ATP10A* gene in blood samples from patients with primary SS (34). Since this enzyme mainly transports 2 aminophospholipids: phosphatidylserine and phosphatidylethanolamine, which may be the target of minor autoantibodies in antiphospholipid syndrome (APS) (35), it is reasonable to speculate that there is a link between the presence of antiphospholipid antibodies and the increase in *ATP10A* transcript. We re-ran the analysis excluding patients positive for the major autoantibodies found in APS (i.e., anti- β_2 GPI and IgG and IgM aCLs) to exclude potential patients with secondary APS, and the signature that was identified persisted ($P = 4.2 \times 10^{-10}$) (data not shown).

Moreover, to our knowledge, no association with APS has been described in the literature to date. Thus, the signature carried by *ATP10A* appears to be specific to anti-Ro 60+ patients. Another GWAS study on cytokine responses found that genetic variants of *ATP10A* were associated with IFN α production (36). The second gene, *PARP14*, encoded for a member of poly(ADP-ribose) polymerase (PARP) family proteins which contain macrodomain binding proteins influencing many biologic processes (37). PARP-14 suppressed proinflammatory IFN/STAT1 signaling and activated the antiinflammatory IL-4/STAT6 pathway in primary human macrophages (38). PARP-14 also enhanced histone activation to promote transcription of type I IFN genes such as *IFNB1* after lipopolysaccharide stimulation in RAW264.7 cells (39). Interestingly, *PARP14* was identified as 1 of the 5 genes that can distinguish patients with primary SS from controls (40). The third gene, *MX1*, encoded the Mx dynamic, MX dynamin-like GTPase 1 or MxA, which participates in the cellular antiviral response by antagonizing the replication processes of several different RNA or DNA viruses. *MX1* gene expression is induced by IFN via JAK1A/Tyk-2 followed by the activation of the STAT1/STAT2 pathway (41). Furthermore, MX1 protein levels were recently reported as a surrogate for the type I IFN gene scores in SLE (42). Consequently, these 3 overexpressed, hypomethylated, and mutated genes in anti-Ro 60+ patients were significantly associated with the IFN signature regardless of the autoimmune disease.

To control the IFN signature in anti-Ro 60+ patients with autoimmune diseases, a key challenge would be to break the continual turnover of Ro 60-specific clones that seems to drive lifelong Ro 60 humoral autoimmunity (43). This may entail a dual approach targeting both Ro 60-associated RNAs (including Alu transcripts and Y RNAs) and Ro 60-specific autoantibody clonotypes as suggested by Reed and Gordon (32).

ACKNOWLEDGMENTS

We would like to particularly express gratitude to the patients, nurses, technicians, and many others who directly or indirectly helped in this study. We are also grateful to the Institut Français de Bioinformatique, the Roscoff Bioinformatics platform Analysis and Bioinformatics for Marine Science (URL: <http://abims.sb-roscoff.fr>), for providing computing and storage resources, and the HYPERION platform at Lymphocytes B et Autoimmunité, for providing flow cytometry facilities.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Pers had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Pers.

Acquisition of data. Bettacchioli, Jamin, Alarcón-Riquelme.

Analysis and interpretation of data. Foulquier, Le Dantec.

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