



Novel systemic lupus erythematosus autoantigens identified by human protein microarray technology

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ABSTRACT

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease affecting many organs. Many autoantibodies have been associated with the disease, but either in low specificity or low sensitivity of detection. In an aim to screen for better autoantibodies, we profiled the autoantibody repertoire in sera from 30 SLE patients versus 30 healthy controls using a protein microarray containing 5011 non-redundant human proteins, and identified four candidates. We then selected CLIC2 for further verification by ELISA in an extended cohort including 110 SLE, 121 non-AD, 118 RA, 117 SSs, and 105 pSS patients. The positive rate of anti-CLIC2 was 28.18% in SLE patients, significantly higher than those in non-AD, RA, and SSs patients. The presence of anti-CLIC2 in SLE had positive correlation with disease activity in terms of SLEDAI score and several indexes ($p < 0.05$).

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1. Introduction

Systemic lupus erythematosus (SLE) is a common autoimmune disease that can affect any part of body [1], severely harming the health of humans, especially women at bearing age. As with other autoimmune diseases, the impairment of the immune system results in the production and accumulation of abnormally high amounts of autoantibodies and immune complexes [2], which attack the cells and tissues of the own body and lead to inflammation and tissue damage [3,4]. The course of the disease is unpredictable, with periods of illness alternating with remissions. SLE is currently incurable, and the main causes of death are infection [1], end-stage renal failure [5], and neuropsychiatric disorders [6]. But with early

detection and treatment, the survival rate has increased dramatically in the past decades [7].

At present, the diagnosis of SLE mainly depends on disease history, clinical manifestation, symptoms, and laboratory test results. Tests of autoantibodies are important for diagnosis, differential diagnosis and evaluation of severity of the disease [1,4,8]. For the past century more than 100 autoantibodies have been identified to be associated with SLE [9] using various techniques including several high throughput methods used recently, serological analysis of expression cDNA libraries [10,11] and serological proteomics analysis [12–14]. The diversity of autoantibodies in SLE is most complex, the targets of these autoantibodies include nuclear antigens, cytoplasmic antigens, cell membrane antigens, phospholipid-associated antigens, blood cells, endothelial cells, and nervous system antigens, plasma proteins, matrix proteins, and miscellaneous antigens [9]. The most important autoantibodies include anti-nuclear antibodies, anti-dsDNA, anti-Sm and anti-phospholipid [15], which have been used in the clinic. However, autoantibodies with high specificity and high sensitivity have yet to be found.

Protein microarray technology has emerged as a new high throughput method, which can detect low abundant proteins and consume minimal volume of samples. Protein microarray has already been successfully used in identification of new autoantibodies in many diseases, including autoimmune hepatitis [16], rheumatoid

Abbreviations: Non-AD, non-autoimmune diseases; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSs, systemic sclerosis; VEEV, venezuelan equine encephalomyelitis virus.

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arthritis [17], breast cancer [18], ovarian cancer [19], and muscle invasive diseases [20].

In an aim to screen for better autoantibodies with protein microarray technology, we profiled the autoantibody repertoires of sera from 30 SLE patients versus 30 healthy controls using a protein microarray containing 5011 non-redundant human proteins, and identified four candidates. We then selected CLIC2 for further verification by ELISA in an extended cohort and analysis of association with disease activity and the potential usefulness in diagnosis.

2. Materials and methods

2.1. Serum samples

The 30 SLE and 30 healthy control sera used for autoantibody profiling by microarray were from the serum bank of Beijing BGI–GBI Biotech Co. Ltd. The 571 serum samples used in ELISA were collected at the Peking Union Medical College Hospital, including 121 non-AD, 110 SLE, 118 RA, 105 pSS, and 117 SSc samples. The study was approved by the Ethic Committee of Peking Union Medical College Hospital. Healthy samples were from regular physical exams, and sera of non-AD and various autoimmune diseases were from both outpatients and inpatients. The diagnosis of SLE, RA, SSc, and pSS was based on the respective classification criteria set by American College of Rheumatology. Non-AD samples were patients without any autoimmune diseases.

2.2. Preparation of protein microarrays

Expression strains of 5090 recombinant human proteins with GST tags, representing 5011 non-redundant ORFs, were previously constructed in our laboratory [16]. Protein expression and purification were conducted as described previously [21]. Purified human proteins, proteins for CV measurement (series dilution of VEEV-E2 protein), and other controls were spotted in duplicate onto OPEpoxyslide™ (BioCapital Corp., Beijing, China) using a SpotArray-72 printer (Perkin Elmer, MA, USA). The printed human protein microarrays were vacuum sealed and stored at 4 °C.

2.3. Serum assay on protein microarrays

Serum assays were carried out according to Song et al. [16] with minor modification. Protein microarrays were blocked in blocking buffer containing 3% (w/v) BSA at 37 °C for 1 h before incubation with 200 µL of serum (1:500 in blocking buffer) and anti-VEEV-E2 mouse mAb (1:1000, Beijing Protein Innovation Co. Ltd., Beijing, China) under a glass coverslip (LifterSlip, Erie Scientific Company, Portsmouth, NH, USA) for 1 h at 37 °C. Cy5 conjugated anti-human IgG (1:2000, Santa Cruz Company, CA, USA) and Cy5 conjugated anti-mouse IgG (1:2000, Beyotime Institute of Biotechnology, Shanghai, China) were added for detection of bound human IgG and anti-VEEV-E2 mAb, respectively. After repeated washes with PBST (PBS with 0.05% (v/v) Tween 20) and double-deionized water, the microarray was spun at 2000 rpm for 3 min to dryness and scanned with a LuxScan-10K/A scanner (BioCapital Corp., Beijing, China). The binding signals were acquired and analyzed using the GenePix Pro 5.1 software (Molecular Devices, Sunnyvale, CA, USA).

2.4. Analysis of microarray data

Two methods were used to analyze the microarray data. In the first method, the signal intensity of each protein feature was determined by first subtracting the background median value from the foreground mean value and then adding 10. If the result is less than

10, a minimal value of 10 was assigned. The geometric average of duplicates was used as the final signal intensity for a given protein. To perform inter-microarray normalization, individual signal intensity was further divided by the total signals of its microarray. The mean value of a given protein for all control serum samples was then calculated, and a protein in the individual serum with >2-fold signal intensity increase was considered as positive.

In the second method, the signal intensity of each protein feature was normalized according to Hu et al. [22]. Shortly, the signal intensity of each protein feature was defined as the ratio of foreground median value to background median value. Then the median value of all spots in each block was set as 1 for intra-microarray normalization. All the signals were plotted, and the signals with intensities less than 1 were used to create a mirror image using 1 as the axis of symmetry, thus generated a symmetric pseudo-data for estimation of the standard deviation (SD). The cutoff was set at 6 SD away from the mean signal intensity of the whole microarray. Only when both duplicates were above the cutoff, the protein feature would be considered as positive.

For both methods, the number of positives for each protein in patient and control sera was counted, and the SLE specific autoantigen candidates were identified according to the following criteria: positive rate $\geq 30\%$ in patient sera and $\leq 5\%$ in control sera.

2.5. Preparation of CLIC2 protein without GST tag

GST-tagged CLIC2 proteins were expressed in yeast and bound to GST beads for purification as described previously [21]. Instead of using elution buffer to elute the bound GST–CLIC2 protein, 4 U/mL Thrombin (Sigma–Aldrich, St. Louis, MO, USA) in cleavage buffer (150 mM NaCl, 2.5 mM CaCl₂, 1 mM DTT in 50 mM Tris–Cl (pH8.0)) was added to the beads and incubated overnight to cleave the protein at the Thrombin site between CLIC2 and GST sequences, thus releasing GST-free CLIC2 proteins. The beads were treated with Thrombin a second time to assure a maximum recovery of CLIC2 proteins. The quality of the protein was checked by 12% SDS–PAGE, and the identity of the protein was confirmed by Mass spectrometry on UltraFlex MALDI–TOF/TOF (Bruker Daltonics, MA, USA).

2.6. Enzyme linked immune absorption assay (ELISA)

ELISA was performed according to Song et al. [16] with minor modification. Purified recombinant protein (100 µL at 1 µg/mL) was coated in a carbonate buffer (50 mM sodium carbonate (pH 9.6)) overnight at 4 °C. Subsequent blocking (1% BSA), sera incubation (1:100 dilution), and incubation with horseradish peroxidase (HRP)-conjugated mouse anti-human IgG antibody (diluted 1:25,000) (Beijing Protein Innovation Co. Ltd., Beijing, China) were all carried out for 1 h at 37 °C. After stopping a 4 min of HRP substrate reaction, the absorption at 450 nm (OD₄₅₀) was measured using an automatic microplate reader Model 680 (Bio-Rad Laboratories Inc., Hercules, CA, USA). The relative absorption value for a given sample was calculated by dividing the average absorption value of duplicate by means of duplicate reference samples in the same ELISA plate. The cutoff was set at the 95th percentile of the relative absorption value of non-AD samples.

2.7. Western blotting

Western blotting was carried out according to Altrichter et al. [23] with minor modification. Purified GST-free CLIC2 (200 ng/lane) was used in 12% SDS–PAGE. After transferring protein bands to 0.45 µm polyvinylidene fluoride membrane (Millipore, Billerica, MA, USA) by wet electroblotting, blocking (5% non-fat milk), sera incubation (1:200 dilutions), and incubation with HRP-conjugated

mouse anti-human IgG monoclonal antibody (diluted 1:25,000) were all carried out for 1 h at 37 °C. The immunoreactive bands were detected by addition of enhanced chemiluminescence reagents (GE Healthcare, Waukesha, WI, USA) and were visualized by ImageQuant RT ECL (GE Healthcare, Waukesha, WI, USA).

2.8. Statistical analysis

Open source statistical software R was used for statistical analysis. *P* values were calculated with Pearson's Chi-square, Fisher's exact, or Wilcoxon rank sum tests. *P* value less than 0.05 was considered to be statistically significant.

3. Results

3.1. Identification of SLE-associated autoantigens using the 5011 human protein microarray

Previously, we constructed a 5011 human protein microarray and used it to have successfully identified three novel autoantigens for autoimmune hepatitis [16]. To identify novel autoantigens for SLE, we employed the same human protein microarray to perform serum profiling assays using samples collected from SLE patients and healthy individuals. Because the human proteins on the microarray were GST-tagged, quality of the microarray was evaluated by anti-GST antibody. Approximately 84.3% of the proteins had signal to noise ratio over 2 and the correlation coefficient between duplicate spots was 0.9859 (Supplementary Fig. 1). In addition, we included in the microarray a series dilution of a recombinant venezuelan equine encephalomyelitis virus antigen VEEV-E2 since our serum samples are unlikely to have its antibodies. When performing serum assay, we also added the antibody against anti-VEEV-E2 to monitor the CV of VEEV-E2 between microarrays, which was 6.17–8.36% among all tested samples, indicating the high quality of the microarray and the hybridizing system.

Thirty SLE patient and 30 healthy control samples were individually probed to the human protein microarray, followed by detection of bound human autoantibodies using a Cy5-conjugated anti-human IgG antibody (Fig. 1). To identify potential SLE-associated autoantigens, we used Genepix Pro 5.1 to acquire signal intensity for all protein spots in each assay, and identified the positives using two methods detailed in the Material and Methods. When at most one positive in 30 controls were allowed, the number of positive hits in SLE samples at or above 30% reached 60 and 11, respectively (listed in Supplementary Table 1). Both lists contain several known SLE-related autoantigens, such as HIST2H2BF, HIST1H2AI, HIST1H1T, RPS20, PRTN3, FBL, HMGB1 in the first list and HIST1H3A, HIST1H2AA in the second, indicating the reliability of both methods. Between the two lists, four (MORF4L1, CLIC2, GSTP1, and PBOV1) are in common (Table 1).

3.2. ELISA for anti-CLIC2 in extended samples

Among the four candidates, CLIC2 (chloride intracellular channel protein 2) is of particular interest. It is a new member of the chloride intracellular channel protein family [24], expressed in many tissues including cardiac muscle and skeletal muscle [25], and its receptors RyR1 [26] and RyR2 [27] are known to function in excitation-contraction coupling [25,28]. Because myocarditis, cardiac insufficiency, cardiac conduction abnormalities [29,30], and extensive muscle pain and tenderness are common symptoms in SLE, CLIC2 and/or its autoantibodies may potentially play roles in the disease. Thus, we selected CLIC2 for further verification by ELISA.

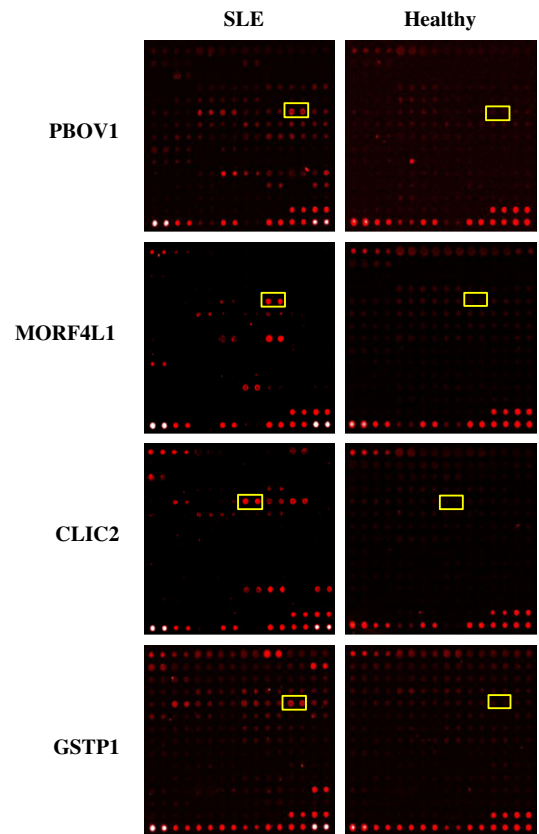


Fig. 1. Probing of the 5011 human protein microarray with SLE and control sera. Thirty SLE and 30 healthy serum samples were diluted 1:500 and individually incubated with the 5011 human microarray, followed by the addition of Cy5-conjugated anti-human IgG antibody. Representative areas of the immunoreaction images in SLE and healthy samples are shown for anti-PBOV1, anti-MORF4L1, anti-CLIC2, and anti-GSTP1.

We prepared GST-free CLIC2 protein, and then performed ELISA to determine the positive rate of anti-CLIC2 in sera from 110 SLE and 121 non-AD patients (Table 2). Using the 95th percentile of signals in non-AD samples as a cutoff, 28.18% SLE and 4.96% non-AD sera were positive, which was similar to the microarray data.

It is well known that many autoantibodies are common in multiple autoimmune diseases. To see the occurrence of anti-CLIC2 in other autoimmune diseases, we also included in the ELISA test the sera from 118 RA, 117 SSC, and 105 pSS patients (Table 2), and their positive rates for anti-CLIC2 were 14.41%, 13.68%, and 20.00%, respectively. Chi-squared test showed that the positive rate of anti-CLIC2 in SLE was significantly higher than those in non-AD, RA, and SSC ($p < 0.05$).

To verify the reliability of ELISA data, we tested several positive and negative sera by Western blotting (Fig. 2). The results clearly showed that the judgment by ELISA was the same as those by Western blotting.

3.3. Association between anti-CLIC2 autoantibodies and SLE manifestations

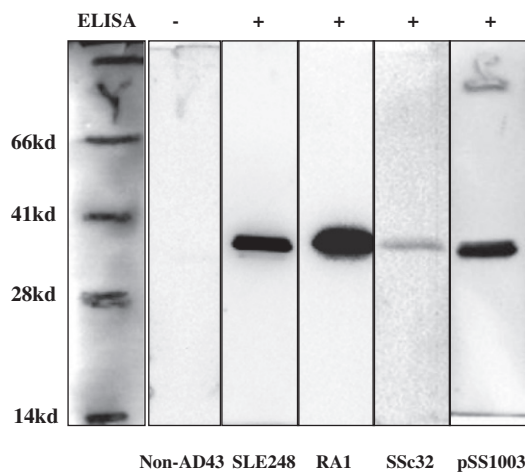
SLE disease activity index 2000 (SLEDAI-2K) is a scoring system indicating SLE disease activity using 24 indexes covering 9 organs. To analyze the potential association between anti-CLIC2 autoantibodies and SLE disease activity, we reviewed the records of all 110 SLE patients and calculated their SLEDAI-2K scores at the time of sample collection. Based on their score, we divided the SLE patients into four groups, none (0–4 points), mild (5–9 points), moderate (10–14 points), and severe activity group (≥ 15 points). As shown

Table 1
SLE-related autoantigen candidates.

Official symbol	Name	GeneBank gene ID	Positives in SLE (Method 1)	Positives in SLE (Method 2)
MORF4L1	Mortality factor 4 like 1	10933	9/30	9/30
CLIC2	Chloride intracellular channel 2	1193	10/30	9/30
GSTP1	Glutathione S-transferase pi 1	2950	10/30	9/30
PBOV1	Prostate and breast cancer overexpressed 1	59351	13/30	9/30

Table 2
Anti-CLIC2 positive rates in different groups.

Group	Cases	Female (%)	Age mean $\pm$ SD (year)	Anti-CLIC2 positive rate (cases)	P value (Chi-squared test)
SLE	110	92.72	33.1 $\pm$ 12.4	28.18% (31)	–
Non-AD	121	47.13	40.6 $\pm$ 14.0	4.96% (6)	1.537e-06
RA	118	83.90	46.6 $\pm$ 13.2	14.41% (17)	0.01079
SSc	117	91.50	45.9 $\pm$ 11.8	13.68% (16)	0.007023
pSS	105	97.98	46.0 $\pm$ 12.0	20.00% (21)	0.1614

**Fig. 2.** Validation of anti-CLIC2 autoantibodies by Western blotting. Blotted recombinant CLIC2 (~30 kDa) was probed with ELISA-tested anti-CLIC2 positive sera from various autoimmune disease groups and negative serum from non-AD group. Representative Western blotting results are shown.

in Table 3, the prevalence of anti-CLIC2 increased with aggravated disease activity. The positive rates of anti-CLIC2 in none and mild activity groups were 0%, while those in moderate and severe activity groups went up to 28.85% and 34.04%, respectively. Fisher's exact test showed that the difference in anti-CLIC2 positive rate between none activity and severe activity group was statistically significant ($p < 0.05$) (Table 3).

To see which index might be associated with anti-CLIC2, we first analyzed the relationship of the positive rate of anti-CLIC2 in individual SLEDAI-2K index using Fisher's test and found no significant association (data not shown). We then compared the level of anti-CLIC2 in SLE patients with or without each index using Wilcoxon rank sum test, and found significant positive association between anti-CLIC2 and seizure, visual disturbance, arthritis, and thrombocytopenia, but negative correlation with alopecia (Table 4).

Table 3
Association between disease activity and anti-CLIC2 positive rate.

Disease stage	Cases	Female (%)	Age mean $\pm$ SD (year)	SLEDAI-2K score (mean $\pm$ SD)	Anti-CLIC2 positive rate (cases)	P value (Fisher's test)
None	9	89	41.0 $\pm$ 12.9	2.7 $\pm$ 0.8	0.00% (0)	–
Mild	2	50	27.5 $\pm$ 6.5	6.5 $\pm$ 1.5	0.00% (0)	1
Moderate	52	92	32.6 $\pm$ 11.6	11.7 $\pm$ 1.4	28.85% (15)	0.09657
Severe	47	94	32.2 $\pm$ 12.6	26.6 $\pm$ 9.6	34.04% (16)	0.04778

4. Discussion

Systemic lupus erythematosus is a prototype of autoimmune disease resulting from abnormality of cellular and humoral immunity. More than 100 autoantibodies have been found in SLE patients [9], but autoantibodies with high specificity and sensitivity in SLE have not been discovered. In recent years, several high throughput technologies were developed to search for distinctive autoantibodies. Protein microarray has its unique advantage, detecting low abundant proteins and consuming minimal volume of samples. In this study, we have profiled the sera autoantibodies on a protein microarray containing 5011 unique human proteins, identified and confirmed CLIC2 as a new autoantigen for SLE, with a sensitivity of 28.18%.

CLIC2 is a new member of chloride intracellular channel proteins [24]. The CLIC2 gene locates in Xq28, encoding 243 amino acids [24]. It is widely expressed in multiple tissues, including cardiac and skeletal muscles [26]. CLIC2 possesses the remarkable property of being able to convert from a water-soluble state to a membrane channel state at low pH levels [31,32]. As an ion channel, CLIC2 may be involved in regulating secretion, cell division and apoptosis; as a soluble globular protein, CLIC2 can interact with the cardiac ryanodine receptor (RyR2) or skeletal ryanodine receptor (RyR1) and modulate its calcium release channel activity, implying that CLIC2 may play a role in excitation–contraction coupling or cardiac conduction. A higher than normal level of anti-CLIC2 may impair its normal function.

CLIC2 has never been reported as an autoantigen before. Here we found anti-CLIC2 present in SLE patients' sera and mainly in patients with higher SLEDAI-2K score, indicating anti-CLIC2 is associated with the severity of the disease. Lupus may be mild or life-threatening, and closely monitoring the disease activity and customizing treatment will effectively deal with lupus flare-ups as well as prevent infection due to overdose of steroid. Among 116 reported autoantibodies related to SLE, 19 have association with SLE disease activity, including anti-dsDNA, anti-nucleosomes, and anti-ribosomal P proteins [9] which are routinely tested in some clinic for SLE patients. We analyzed their association with anti-CLIC2 in SLE

Table 4

Association analysis between anti-CLIC2 relative O.D. value and individual SLEDAI-2K index.

Group	Number of Samples	ELISA relative O.D. median (quartile range)	P value (Wlixon rank sum test)
Seizure	8	0.92 (1.02)	0.03375*
Non-seizure	102	0.50 (0.86)	
Visual disturbance	5	1.25 (1.42)	
Non-visual disturbance	105	0.52 (0.78)	0.03609*
Arthritis	41	0.62 (0.88)	
Non-arthritis	69	0.44 (0.62)	
Alopecia	34	0.31 (0.66)	0.01164**
Non-alpecia	76	0.59 (0.83)	
Thrombocytopenia	27	0.76 (0.75)	0.04714*
Non-thrombocytopenia	83	0.43 (0.70)	

* Denotes the titer of anti-CLIC2 was greater in patients with the indicated symptom than patients without.

** Denotes the titer of anti-CLIC2 was less in patients with the indicated symptom than patients without.

patients by Fisher's exact test and found no association ($p > 0.05$) (Supplementary Table 2). Considering the positive rate of anti-CLIC2 increases with the aggravation of patient's condition, anti-CLIC2 tests may serve as a useful supplement in monitoring the disease activity of SLE.

Further looking into the association between anti-CLIC2 and individual disease index, seizure, visual disturbance, arthritis, and thrombocytopenia showed positive correlation with anti-CLIC2 level ($p < 0.05$). However, we did not see the correlation among these symptoms.

Similar to many other autoantibodies reported for SLE, anti-CLIC2 is not unique to SLE, it also presents in other autoimmune diseases. We found anti-CLIC2 in RA, SSc and pSS, though at a lower rate. The difference in the presence of anti-CLIC2 between SLE and RA, and between SLE and SSc patients were statistically significant. However, the presence of anti-CLIC2 in pSS patients was not significantly less than that in SLE. Whether anti-CLIC2 also indicates a severity in pSS is worthy investigation.

Protein microarray is a robust technology for profiling autoantibodies, but the analysis of huge data is a big challenge. Here, we combined two data analysis methods to narrow down to several candidate autoantigens, which would save money and time for further validation. We have successfully validated one candidate, and the usefulness of this strategy will require the validation of the other candidates in the future.

In conclusion, we have identified and verified CLIC2 as a novel autoantigen in SLE. The presence of anti-CLIC2 was associated with the severity of the disease, and may help in diagnosis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2012.01.001.

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Supplementary Material

Supplemental figure legends

Supplemental Figure 1 Detection of recombinant proteins on 5,011 human protein microarray. (A) Image of the microarray hybridized with anti-GST antibody followed by Cy5 labeled anti-mouse IgG antibody. The proteins positively detected by the anti-GST antibody are represented in red or white (saturated intensity). (B) Scatterplot of logarithmic signal intensity of duplicate spots. The correlation coefficient of duplicate spots was 0.9859, indicating high reproducibility of the protein spotting.

Supplemental table legends

Supplemental Table 1 List of SLE autoantigen candidates identified by 5,011 protein microarray

Supplemental Table 2 Association between anti-CLIC2 and three disease activity-related autoantibodies

Supplemental tables

Supplemental Table 1

Locations on microarray (B-C-R)	Gene Name	Accession Number	Description	Method for candidate Identification
14-11-5	MORF4L1	10933	mortality factor 4 like 1 [Homo sapiens]	1, 2
19-9-5	CLIC2	1193	chloride intracellular channel 2 [Homo sapiens]	1, 2
25-13-5	GSTP1	2950	glutathione S-transferase pi 1 [Homo sapiens]	1, 2
38-13-6	PBOV1	59351	prostate and breast cancer overexpressed 1 [Homo sapiens]	1, 2
27-15-12	TP53TG3	24150	TP53 target 3 [Homo sapiens]	1
19-15-12	RNASET2	8635	ribonuclease T2 [Homo sapiens]	1
30-7-8	unknown	ENST00000333859	unknown	1
35-15-12	MYNN	55892	myoneurin [Homo sapiens]	1
38-3-10	TICAM2	353376	toll-like receptor adaptor molecule 2 [Homo sapiens]	1
46-15-11	unknown	ENSG00000187654	unknown	1
38-15-12	unknown	gi 51468651 ref XM_370665.3	PREDICTED: similar to DnaJ (Hsp40) homolog, subfamily B, member 6 isoform a; heat shock protein J2	1
4-11-11	RND1	27289	Rho family GTPase 1 [Homo sapiens]	1
25-15-12	TLN1	7094	talin 1 [Homo sapiens]	1
19-11-12	unknown	ENST00000355352	unknown	1
42-15-5	HIST2H2BF	440689	histone cluster 2, H2bf [Homo sapiens]	1
46-3-10	THAP5	168451	THAP domain containing 5 [Homo sapiens]	1
40-13-11	METTL6	131965	methyltransferase like 6 [Homo sapiens]	1
4-15-11	FAM204A	63877	family with sequence similarity 204, member A [Homo sapiens]	1
38-1-9	unknown	gi 66472899 ref NM_001023565.1	hypothetical protein LOC401286 [Homo sapiens].	1
38-13-8	unknown	gi 51464308 ref XM_498986.1	PREDICTED: hypothetical protein XP_498986 [Homo sapiens].	1
48-11-8	unknown	ENST00000258728	unknown	1
33-15-6	SLC22A7	10864	solute carrier family 22 (organic anion transporter), member 7 [Homo sapiens]	1
22-15-11	unknown	ENSG00000181495	unknown	1
48-7-11	unknown	ENSG00000198866	unknown	1
29-13-2	RPE	6120	ribulose-5-phosphate-3-epimerase [Homo sapiens]	1
36-7-3	EEF1G	1937	eukaryotic translation elongation factor 1 gamma [Homo sapiens]	1
46-13-6	HIST1H2AI	8329	histone cluster 1, H2ai [Homo sapiens]	1
22-15-9	CDCA4	55038	cell division cycle associated 4 [Homo sapiens]	1
32-3-6	HIST1H1T	3010	histone cluster 1, H1t [Homo sapiens]	1
46-3-8	RPL21	6144	ribosomal protein L21 [Homo sapiens]	1

Supplemental Table 1 (Continued)

32-13-9	CXorf56	63932	chromosome X open reading frame 56 [Homo sapiens]	1
16-11-8	unknown	ENST00000338928	unknown	1
5-7-7	unknown	ENSG00000175789	unknown	1
38-7-11	unknown	ENSG00000178385	unknown	1
6-7-8	unknown	ENSG00000184767	unknown	1
46-7-11	unknown	ENSG00000196370	unknown	1
5-15-4	APOM	55937	apolipoprotein M [Homo sapiens]	1
16-11-2	MGST2	4258	microsomal glutathione S-transferase 2 [Homo sapiens]	1
9-9-6	TFB1M	51106	transcription factor B1, mitochondrial [Homo sapiens]	1
40-11-11	RPS20	6224	ribosomal protein S20 [Homo sapiens]	1
1-15-7	unknown	ENSG00000198757	unknown	1
2-11-11	PRTN3	5657	proteinase 3 [Homo sapiens]	1
25-3-5	GLRX	2745	glutaredoxin (thioltransferase) [Homo sapiens]	1
48-11-11	unknown	ENSG00000179175	unknown	1
22-15-7	unknown	ENSG00000198152	unknown	1
3-3-3	MRPL16	54948	mitochondrial ribosomal protein L16 [Homo sapiens]	1
46-15-7	unknown	ENSG00000165740	unknown	1
47-7-2	FBL	2091	fibrillarin [Homo sapiens]	1
28-5-3	GNB2L1	10399	guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1 [Homo sapiens]	1
21-9-2	CNBP	7555	CCHC-type zinc finger, nucleic acid binding protein [Homo sapiens]	1
20-3-12	HMGB1	3146	high mobility group box 1 [Homo sapiens]	1
24-5-11	unknown	ENSG00000174092	unknown	
17-11-5	SHMT1	6470	serine hydroxymethyltransferase 1 (soluble) [Homo sapiens]	1
27-7-12	MAGEA5	4104	melanoma antigen family A, 5 [Homo sapiens]	1
48-9-8	KRT8	3856	keratin 8 [Homo sapiens]	1
46-15-8	unknown	ENST00000339359	unknown	1
33-3-5	ZDHHC17	23390	zinc finger, DHHC-type containing 17 [Homo sapiens]	1
46-11-8	unknown	ENST00000306292	unknown	1
46-15-12	unknown	ENST00000305704	unknown	1
46-3-9	unknown	ENST00000310106	unknown	1
30-9-6	HIST1H3A	8350	histone cluster 1, H3a [Homo sapiens]	2
6-11-6	PURB	5814	purine-rich element binding protein B [Homo sapiens]	2

Supplemental Table 1 (Continued)

38-5-6	HIST1H2AA	221613	histone cluster 1, H2aa [Homo sapiens]	2
22-11-8	unknown	ENST00000355037	unknown	2
30-11-8	unknown	ENST00000361502	unknown	2
8-5-6	FERD3L	222894	Fer3-like (Drosophila) [Homo sapiens]	2
45-13-7	unknown	HIX0021857	Hypothetical protein.	2

Note: Accession numbers are shown as GeneBank Gene ID, Gi number, or Ensembl transcript ID. Gene name bolded were known autoantigens associated with SLE.

Supplemental Table 2

Test	Anti-CLIC2(+) n=20	Anti-CLIC2(-) n=62	P value (Fisher test)
Anti-ribosomal P proteins (+)	9	21	0.43
Anti-nucleosomes (+)	7	15	0.39
Anti-dsDNA (+)	6	22	0.79

Note: Sera sample from 82 SLE patients were tested for multiple autoantibodies including AMA-M2, ribosomal P-proteins, histones, nucleosome, dsDNA, PCNA, CENP-B, Jo-1, PM-Scl, Scl-70, SS-B, Ro-52, SS-A, Sm, nRNP/Sm, using the commercial kit (EUROLINE Test Systems: ANA Profile 3) (EUROIMMUN Medizinische Labordiagnostika AG, Germany) according to the operating manual, three of them relate to disease activity in SLE and are listed here.