Clinical Rheumatology (2019) 38:3099–3107
https://doi.org/10.1007/s10067-019-04624-z

ORIGINAL ARTICLE

The expression and clinical significance of different forms of LILRA3 in systemic lupus erythematosus

Yan Du[1] · Fengyin Sun[2] · Meiju Zhou[1] · Xinyu Wu[1] · Wenjia Sun[1] · Yujie Jiang[1] · Qi Cheng[1] · Xiaochan Chen[1] · Huaxiang Wu[1] · Jing Xue[1]

Received: 18 January 2019 / Revised: 22 April 2019 / Accepted: 27 May 2019 / Published online: 17 June 2019
© International League of Associations for Rheumatology (ILAR) 2019

Abstract

Objective Our previous study has shown that functional leukocyte immunoglobulin-like receptors A3 (*LILRA3*) contributes to susceptibility and subphenotypes of systemic lupus erythematosus (SLE). However, the mechanism remains unclear. We aimed to evaluate the role of LILRA3 in SLE.

Methods One hundred twenty-six SLE patients and 48 healthy controls were recruited in this study. Functional studies were performed using intracellular flow cytometry and ELISA.

Results Both LILRA3 levels in serum and $CD14^+$ monocytes were significantly elevated in SLE patients compared with healthy controls. Elevated LILRA3 level was found positively correlated with SLEDAI. Furthermore, more elevated LILRA3 levels were found in patients with higher SLEDAI, presence of lupus nephritis, and thrombocytopenia.

Conclusions Both LILRA3 levels in serum and $CD14^+$ monocytes significantly increased in SLE and positively correlated with disease activity and severity. The upregulation of LILRA3 expression may serve as a biomarker of disease activity and severity of SLE.

Key Points

- *LILRA3 contributes to susceptibility and subphenotypes of SLE; LILRA3 is elevated in SLE patients.*
- *Increased LILRA3 correlated with disease activity and severity.*
- *LILRA3 may serve as a biomarker of disease activity and severity of SLE.*

Keywords CD14+ monocytes · LILRA3 · Systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is a classic autoimmune disease, characterized by multiple systems and organs damage and a large number of autoantibodies. Both genetic and environment factors contribute to SLE pathogenesis, but the etiology of SLE is not fully understood. Impaired clearance of the apoptotic cells may play a central pathogenic role in human lupus. The accumulated apoptotic cells release auto-antigens, which are presented by antigen presenting cells, further breaking down the immune tolerance of T and B cells and triggering SLE.

Leukocyte immunoglobulin-like receptors (LILRs), also named immunoglobulin-like transcripts (ILTs), are a family of immune-modulatory proteins localized on human

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10067-019-04624-z) contains supplementary material, which is available to authorized users.

✉ Huaxiang Wu
wuhx8855@zju.edu.cn

✉ Jing Xue
jingxue@zju.edu.cn

[1] Department of Rheumatology, The Second Affiliated Hospital of Zhejiang University School of Medicine, 88 Jiefang Road, Hangzhou 310009, China

[2] Department of Pediatric Surgery, Qilu Hospital, Shandong University, 107 Wenhua Xi Road, Jinan 250000, China

chromosome 19 in the region 19q13.4 [1, 2]. A total of 13 different LILRs have been identified to date, mainly expressed on myeloid cells, natural killer (NK) cells, and T cells [1, 3].

LILRA3 is a member of the LILR family produced as a soluble molecule by monocytes and macrophages. A 6.7-kb deletion in the gene of LILRA3 results in a null allele and an absence of function [2, 4]. The frequencies of the 6.7-kb deletion vary greatly in different populations, considerably higher in Northeastern Asians (0.56–0.84) compared with Africans (0.10) or Europeans (0.17) [5]. The homozygous LILRA3 deletion (nonfunctional *LILRA3*) has been demonstrated to be associated with Sjogren's syndrome (SS) and multiple sclerosis (MS) in Caucasians [6–8]. However, in our previous studies, we found that in Chinese Han population, the LILRA3 non-deletion (namely functional *LILRA3*) contributes to susceptibility and subphenotypes of SLE and SS [9]. With respect to the significant role of LILRA3 in immune-modulatory functions, we studied the role of LILRA3 in SLE.

Materials and methods

Patients and controls

One hundred twenty-six patients with SLE and 48 healthy controls were enrolled at the Department of Rheumatology, the Second Affiliated Hospital Zhejiang University School of Medicine, and blood samples were obtained from these subjects. All of the enrolled patients fulfilled at least four of the 1997 update of American College of Rheumatology (ACR) revised criteria for SLE [10]. All participants signed the informed consent to donate their blood samples and de-identified clinical information for research. The study was approved by the ethics committee of the Second Affiliated Hospital Zhejiang University School of Medicine. The intracellular LILRA3 expression was detected for 61 of the 126 patients and 20 of the 48 healthy controls.

Clinical data analysis

Demographic, clinical and laboratory data, obtained from the medical records of the patients, included age, sex, disease duration, clinical symptoms, anti-double-stranded DNA antibody (anti-dsDNA Ab), anticardiolipin antibody (aCL), anti-nucleosome antibody (AnuA), anti-Sm antibody (Sm), anti-SSA antibody (SSA), blood cell counts, urinalysis, 24-h proteinuria excretion, IgG, IgM, IgA, complement component 3 (C3), complement component 4 (C4), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR). Anti-dsDNA, 24-h proteinuria excretion, C3, and C4 were used to predict SLE disease activity. Increased SLE severity was defined by the presence of SLEDAI ≥ 8, higher SLE-associated autoantibody levels (anti-dsDNA Ab, aCL, AnuA, anti-Sm Ab, and anti-SSA Ab), decrease of C3 or C4 levels or one of the following features: nephritis, thrombocytopenia, leukocytopenia, and lupus encephalopathy. Clinical features defined by the SLEDAI scoring system were seizure, psychiatric symptoms, encephalosis, visual injury, cranial neuropathy, lupus headache, cerebrovascular insult, vasculitis arthritis, myositis, cylinderuria (hemoglobin/red blood cell cylinder), granular cast, hemoglobinuria (> 5 red blood cells/high-power field (HP)), proteinuria (> 0.5 g/24 h), pyuria (> 5 white blood cells/HP), alopecia, rash, oral ulcers, pleuritis, fever (temperature (T) ≥ 38 °C), leukopenia ($< 3 \times 10^9$/L), and thrombocytopenia ($< 100 \times 10^9$/L).

Lymphocyte separation and intracellular flow cytometry

Peripheral blood was obtained from patients and healthy controls; lymphocytes were separated using Biocoll density centrifugation. Lymphocytes were adjusted to a concentration of 1×10^6 cells per ml, fixed with 2% paraformaldehyde and washed three times in PBS with BSA 0.1%. Cells were first stained with allophycocyanin (APC) anti-human CD14 (Biolegend, San Diego, CA, USA), and then washed three times in PBS/BSA after 25-min incubation. Secondly, cells were permeabilized with saponin; the Alexa Fluor®488 anti-human LILRA3 (R&D Corporation, Minneapolis, MN, USA) antibody was added and incubated for 25 min. The stained cells were processed in flow cytometry (BD FACS Aria™ II). Forward scatter and side scatter were used to gate lymphocytes, monocytes and granulocytes (Fig. 1a). Monocytes initially were gated using the monocyte marker anti-human CD14 APC conjugate IgG1 (Fig. 1a). The expression of LILRA3 in CD14+ monocytes was quantified by means of fluorescence intensity (MFI) (Fig. 1a). The results were analyzed using Flow Jo 7.6.5 (USA).

Enzyme-linked immunosorbent assay

All serum samples were split into aliquots and stored at − 80 °C until use. ELISA kits were used for measuring Sema5A levels in serum (Jiyinmei, Wuhan, P.R. China), according to the manufacturer instructions. The difference between intra-assay and inter-assay are less than 6% and 10%, respectively.

Statistical analysis

Data analyses were performed using SPSS software for Windows 16.0. For normally distributed data expressed as mean values ± SD (standard deviation), the differences between groups were analyzed by Student's *t* test. Comparisons of categorical variables were conducted using Pearson chi-square tests. For nonparametric data, results were

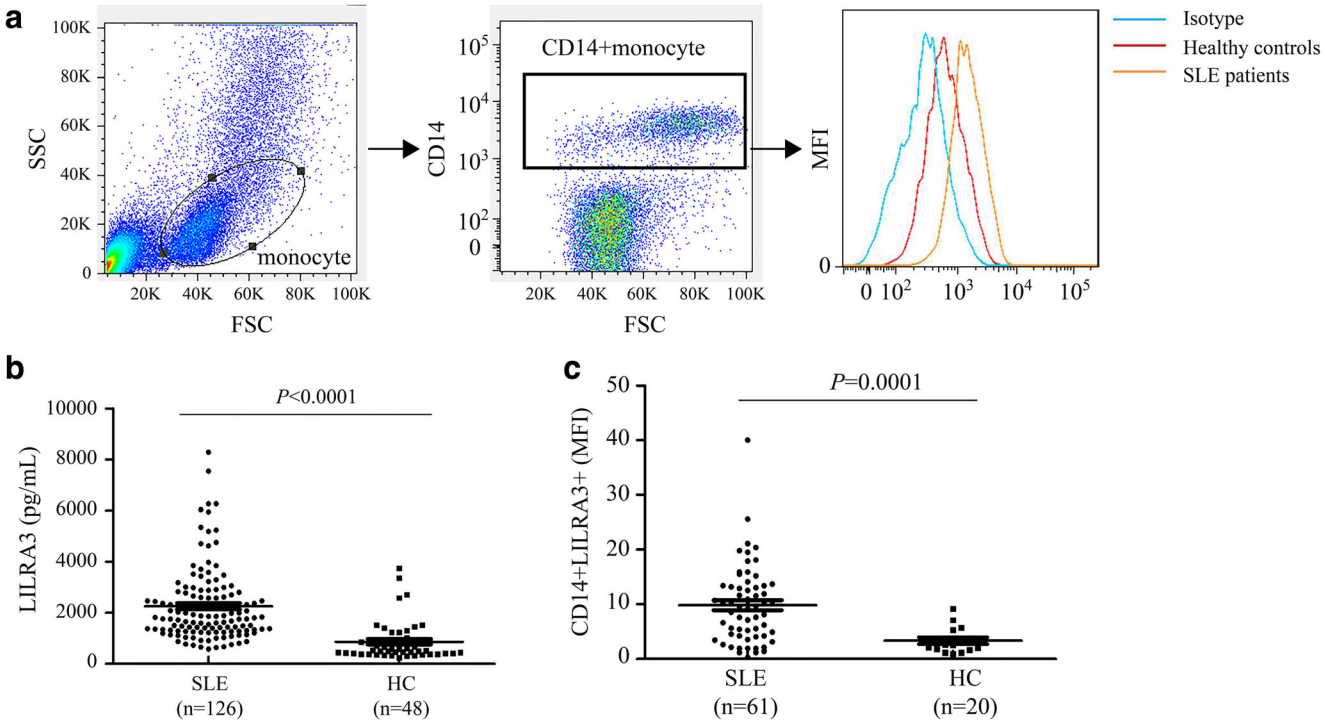

Fig. 1 Example of quantification of blood CD14+ monocytes subset. Intracellular LILRA3 expression was measured by flow cytometry as mean fluorescence intensity (MFI). **a** Cell distribution based on forward-scatter and side-scatter; the monocyte population is identified and gated accordingly. The fraction of monocyte positive for CD14 is identified and gated. Histogram shows the MFI of CD14+ monocytes positive for LILRA3 in IgG1 isotype (blue line), healthy controls (red line) and SLE patients (orange line). LILRA3 expression in CD14+ monocytes subset was significantly higher in patients with SLE compared with healthy controls. **b** Serum LILRA3 levels were significantly higher in patients with SLE than healthy controls. **c** The LILRA3 levels in the CD14+ monocytes were significantly higher in SLE patients compared with healthy controls

expressed as median (range) values, and the differences between groups were analyzed by the Mann–Whitney *U* test. Spearman's correlation coefficient was applied to detect the correlation between two groups. *P* values less than 0.05 were considered significant.

Results

Characteristics of SLE patients

One hundred twenty-six SLE patients with a mean age of 36.85 ± 14.3 years, and 48 healthy controls with a mean age of 37.05 ± 14.51 years were enrolled to detect the serum LILRA3 expression by ELISA. Among them, 61 SLE patients and 20 matched controls were enrolled in the flow cytometry study to detect LILRA3 expression in CD14+ monocytes. The SLE patients consisted of 111 female and 15 male subjects, and the healthy controls consisted of 43 females and 5 males. The median disease duration was 48 months with a range of 1 to 480 months. No significant differences were observed in age ($P = 0.2$) and gender ($P = 0.73$) between SLE patients and healthy controls. The SLEDAI score of the SLE patients was 9.31 ± 5.98. The clinical and laboratory characteristics of the studied SLE patients and healthy controls are shown in Table 1.

LILRA3 levels in serum and CD14+ monocytes in patients with SLE

The results of ELISA revealed that the mean values of LILRA3 were (2246.1 ± 123.7) pg/mL in serum from SLE patients and (858.2 ± 112.5) pg/mL in those from healthy controls, and the difference was statistically significant ($P < 0.0001$, Fig. 1b). Besides the elevated LILRA3 expression level in serum, the levels in monocytes examined by FACS were also significantly higher among 61 SLE patients (MFI 9.81 ± 0.91) than those from the 20 healthy controls (MFI 3.31 ± 0.63) ($P = 0.0001$, Fig. 1c).

Elevated LILRA3 levels were correlated with disease activity and severe clinical manifestations in SLE

In patients with SLE, the serum LILRA3 concentrations were positively correlated to disease activity quantified by

Table 1 Clinical and laboratory characteristics in patients with SLE and healthy controls

Clinical characteristics	SLE, N (%)[a]	Healthy controls	*P* value[b]
Age	36.85 ± 14.3	37.05 ± 14.51	0.200
Sex (female:male)	115:11	43:5	0.730
Disease duration (median, months)	(1–480, 48)	NA	
ANA (%)	119/126 (94.4)	NA	
Anti-dsDNA Ab (%)	85/126 (67.5)	NA	
ACL (%)	20/126 (15.9)	NA	
AnuA (%)	50/126 (39.7)	NA	
Sm (%)	37/126 (29.4)	NA	
SSA (%)	99/126 (78.6)	NA	
24 h proteinuria (%)	61/126 (48.4)	NA	
Lupus nephritis (%)	56/126 (44.4)	NA	
Decreased C3 (%)	104/126 (82.5)	NA	
Decreased C4 (%)	74/126 (58.7)	NA	
SLEDAI	9.31 ± 5.98	NA	

SLEDAI systemic lupus erythematosus disease activity index, *ANA* antinuclear antibody, *Anti-dsDNA Ab* anti-double strand DNA antibody, *ACL* anticardiolipin antibody, *AnuA* antinucleosome antibody, *Sm* anti-Smith antibody, *SSA* anti-SSA antibody, *C3* Complement component 3, *C4* Complement component 4; *NA* not applicable

[a] Values are represented as either mean or number *N* (%). Numerical data were presented as mean ± SD and analyzed using the Student *t* test or Pearson's chi-squared test.

[b] $P < 0.05$

SLEDAI score ($r = 0.257$, $P = 0.0036$) (Table 2, Fig. 2a). The LILRA3 expression in CD14+ monocyte was also positively associated with SLEDAI score ($r = 0.398$, $P = 0.0018$) (Table 2, Fig. 2b), and significant correlation was detected between LILRA3 in monocytes and 24-h proteinuria excretion ($r = 0.328$, $P = 0.024$) (Table 2, Fig. 2c).

Table 2 Correlations of LILRA3 levels with the studied parameters in SLE patients

Clinical manifestations	LILRA3 in monocytes (MFI)		LILRA3 in serum (pg/mL)	
	Spearman's *r*	*P* value	Spearman's *r*	*P* value
SLEDAI	0.398	0.0018**	0.257	0.0036**
24 h proteinuria excretion	0.328	0.024*	0.159	0.09
Leucocytes	− 0.033	0.680	− 0.253	0.0037**
Thrombocytes	− 0.176	0.213	− 0.144	0.11
Anti-dsDNA Ab	0.066	0.627	− 0.061	0.498
C3	− 0.138	0.290	− 0.076	0.392
C4	− 0.140	0.282	− 0.025	0.777
IgA	0.068	0.604	0.017	0.852
IgM	0.107	0.417	0.053	0.557
IgG	0.143	0.218	0.228	0.01*
CRP	− 0.164	0.237	0.088	0.339

Spearman's correlation coefficient (*r*) was applied to detect correlation between two types of numerical data

SLE systemic lupus erythematosus, *SLEDAI* systemic lupus erythematosus disease activity index, *LILRA3* leukocyte immunoglobulin-like receptor A3, *ANA* antinuclear antibody, *Anti-dsDNA Ab* anti-double strand DNA antibody, *ACL* anticardiolipin antibody, *AnuA* antinucleosome antibody, *Sm* anti-Smith antibody, *SS* anti-SSA antibody, *C3* Complement component 3, *C4* Complement component 4, *CRP* C-reactive protein.

*$P < 0.05$

**$P < 0.01$

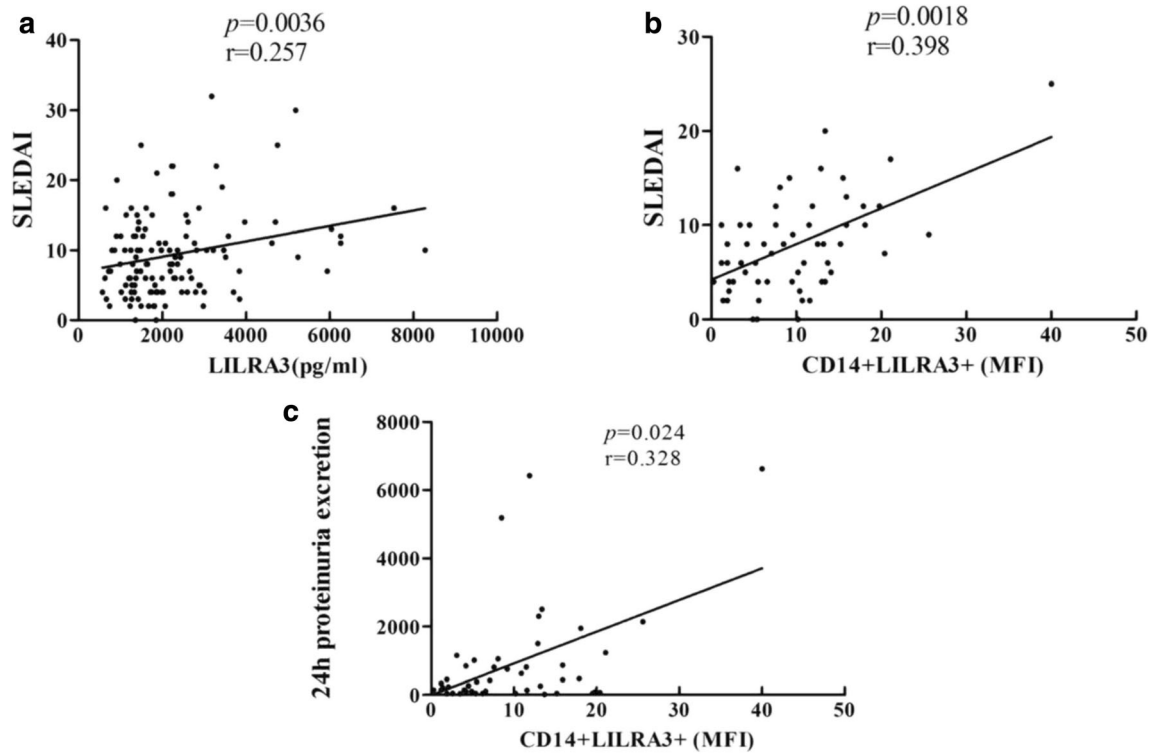

Fig. 2 Correlation of LILRA3 levels with different clinical parameters such as SLEDAI score and 24 h proteinuria excretion in patients with SLE. **a** Serum LILRA3 was positively correlated to **b** SLEDAI score, **c** LILRA3 in CD14+ monocytes had positive correlation with SLEDAI score and 24 h proteinuria excretion. 24 h, 24 h proteinuria excretion; SLEDAI, SLE disease activity index. Spearman's rank correlation test was used to assess correlations

Besides the correlation between LILRA3 expression and disease activity, both LILRA3 levels in CD14+ monocytes and circulating LILRA3 levels in serum were associated with more severe clinical and laboratory manifestations in SLE patients (Fig. 3). We grouped patients by SLEDAI or presence of clinical and laboratory features. The patients with SLEDAI more than 8 showed higher LILRA3 levels in serum (2646.5 ± 215.8 pg/mL vs. 1873.6 ± 115.2 pg/mL, $P = 0.011$) and in CD14+ monocytes (MFI 13.35 ± 1.85 vs. 7.44 ± 0.81, $P = 0.0049$). Serum LILRA3 levels were significantly higher ($P = 0.04$) in patients with leukocytopenia (2455.4 ± 202.8 pg/mL) than those without leukocytopenia (2119.5 ± 155.2 pg/mL). LILRA3 levels in CD14+ monocytes were significantly higher in patients with proteinuria (MFI 12.34 ± 1.62) than those without protein excretion (MFI 8.06 ± 0.97). Patients with lupus nephritis or thrombocytopenia showed both higher LILRA3 levels in serum and CD14+ monocytes than those without lupus nephritis or thrombocytopenia (serum LILRA3 lupus nephritis+ 2510.3 ± 195.0 pg/mL vs. lupus nephritis—1849.3 ± 113.0 pg/mL, $P = 0.013$; thrombocytopenia+2872.7 ± 319.7 pg/mL vs. thrombocytopenia—2061.8 ± 124.8 pg/mL, $P = 0.012$; LILRA3 in CD14 + monocytes: lupus nephritis+ MFI 13.66 ± 1.89 vs. lupus nephritis—7.94 ± 0.87, $P = 0.0056$; thrombocytopenia+14.83 ± 2.39 vs. thrombocytopenia—8.92 ± 0.77, $P = 0.016$) (Table 3, Fig. 3).

Comparison of clinical features between the LILRA3-positive group and LILRA3-negative SLE patients

The cutoff value for positivity was established 2SD above the mean value of healthy controls. Based on this cutoff value, the sensitivity and specificity of serum LILRA3 levels for identification of SLE patients in this cohort were 33.3% and 91.6%, respectively.

Among the 126 patients with SLE, there were no significant differences between the LILRA3-positive and LILRA3-negative subgroups with respect to sex, autoantibodies, and most other features (Supplementary Table 1). However, LILRA3-positive group showed shorter disease duration (serum LILRA3 36 (1–480) vs. 60 (6–360), $P = 0.045$; LILRA3 in CD14 + monocytes 36 (1–240) versus 120 (1–360), $P = 0.032$) and higher incidence of nephritis (serum LILRA3: 27/42 vs. 29/84, $P = 0.002$; LILRA3 in CD14 + monocytes 16/34 vs. 4/27, $P = 0.007$) compared

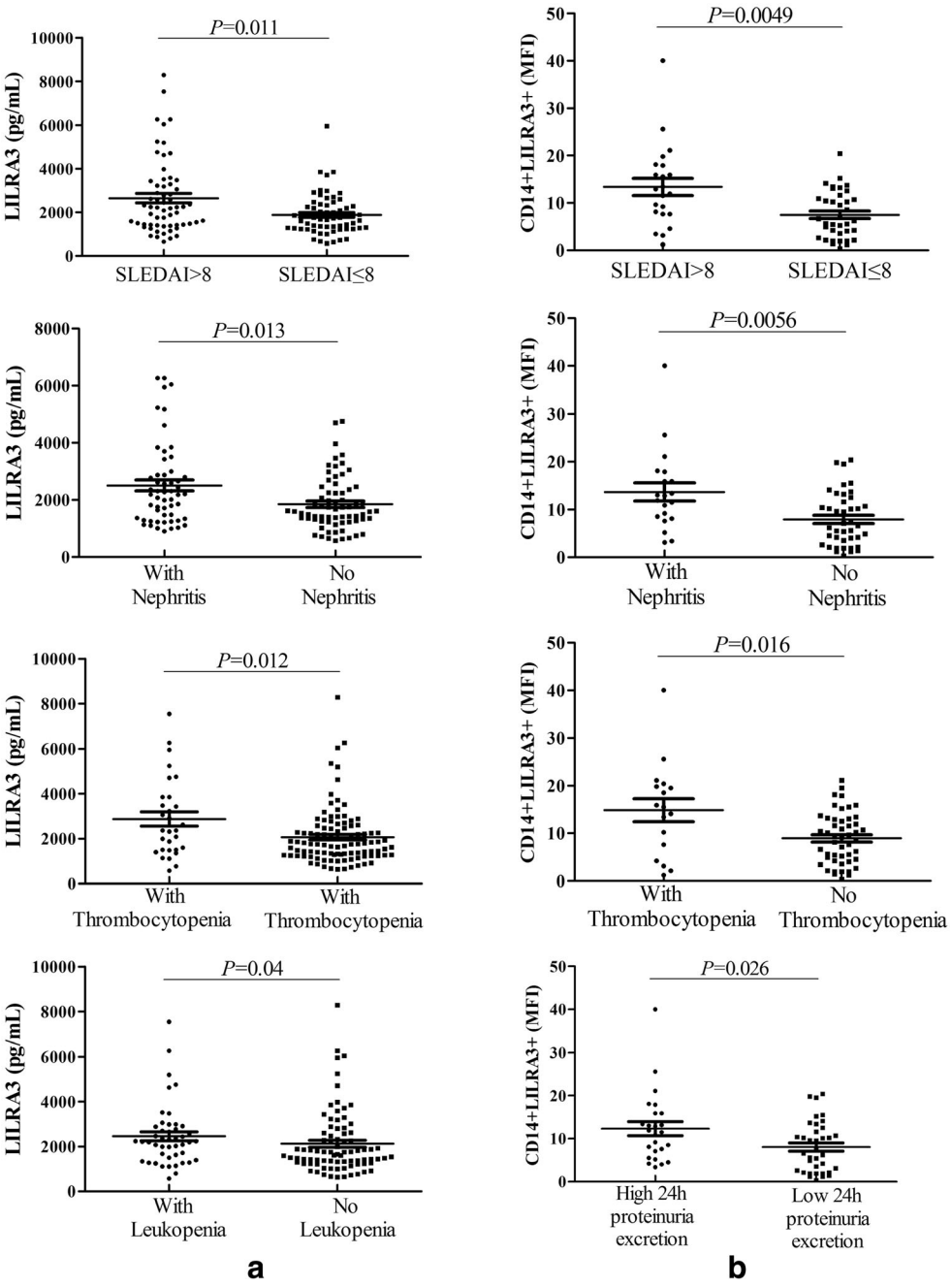

Fig. 3 Serum LILRA3 levels and LILRA3 in CD14+ monocytes according to the clinical manifestations in SLE. a Serum LILRA3 concentrations in patients with a SLEDAI more than or equal to 8, WBC less than 4×10^9/L, PLT less than 100×10^9/L, and with lupus nephritis. **b** LILRA3 in CD14+ monocytes in patients with a SLEDAI more than or equal to 8, PLT less than 100×10^9/L, 24 h proteinuria excretion more than or equal to 0.1 g/d, and with lupus nephritis. SLEDAI, SLE disease activity index; WBC, leukocyte; PLT, thrombocyte

with LILRA3-negative group (Supplementary Table 1). Moreover, the value of SLEDAI was significant higher in LILRA3-positive group compared with LILRA3-negative group (serum LILRA3 11.85 ± 7.39 vs. 7.59 ± 4.01; P = 0.0018; LILRA3 in CD14 + monocytes 9.34 ± 5.61 vs. 6.11 ± 3.76, P = 0.023), but the values of WBC (serum LILRA3: 4.0 (1.9–8.1) vs. 5.0 (1.5–13.1), P = 0.01) and PLT count (serum LILRA3 132 (5–259) vs. 170 (3–496), P = 0.021; LILRA3 in CD14 + monocytes: 118 (16–460) vs. 164 (7–363), P = 0.03) were lower in LILRA3-positive group compared with LILRA3-negative group (Supplementary Table 1).

Discussion

The LILR family can be divided into two classes: the activating LILR subfamily A (LILRA1-6) and the inhibitory LILR

Table 3 Comparison of LILRA3 levels in the presence or absence of SLE clinical and laboratory manifestations

Manifestations	LILRA3 in CD14+ monocytes (MFI)		*P*	LILRA3 in serum (pg/mL)		*P*
	Presence (*n*)	Absence (*n*)		Presence (*n*)	Absence (*n*)	
SLEDAI	13.4 ± 1.9 (SLEDAI > 8, *n* = 22)	7.4 ± 0.8 (SLEDAI ≤ 8, *n* = 39)	0.0049**	2646. 5 ± 215.9 (SLEDAI > 8, *n* = 62)	1873.6 ± 115.2 (SLEDAI ≤ 8, *n* = 64)	0.011*
Lupus nephritis	13.7 ± 1.9 (*n* = 20)	7.9 ± 0.9 (*n* = 41)	0.0056**	2613.3 ± 217.5 (*n* = 56)	1930.5 ± 137.8 (*n* = 70)	0.013*
proteinuria	12.3 ± 1.6 (*n* = 25)	8.1 ± 0.9 (*n* = 36)	0.026*	2261.4 ± 279.4 (*n* = 61)	2243.6 ± 142.3 (*n* = 65)	0.434
Leukocytopenia	9.9 ± 1.7 (*n* = 14)	9.4 ± 1.1 (n = 41)	0.917	2455.4 ± 202.8 (*n* = 46)	2119.5 ± 155.2 (*n* = 80)	0.04*
Thrombocytopenia	14.8 ± 2.4 (*n* = 17)	8.9 ± 0.7 (*n* = 44)	0.016*	2872.7 ± 319.7 (*n* = 30)	2061.8 ± 124.8 (*n* = 96)	0.012*
Anti-dsDNA Ab	10.4 ± 1.5 (*n* = 38)	8.8 ± 1.1 (*n* = 23)	0.665	2483.3 ± 383.1 (*n* = 85)	2480 ± 257.3 (*n* = 41)	0.898
ACL	13.6 ± 4.2 (*n* = 8)	9.0 ± 0.8 (*n* = 53)	0.298	2442.3 ± 352.4 (*n* = 20)	2548.9 ± 346.0 (*n* = 106)	0.776
SSA	9.6 ± 1.2 (*n* = 49)	9.7 ± 1.5 (*n* = 12)	0.879	2556.5 ± 340.6 (*n* = 99)	2523.6 ± 380.7 (*n* = 27)	0.583
Sm	10.3 ± 3.0 (*n* = 13)	8.4 ± 1.4 (*n* = 48)	0.298	2207.8 ± 233.1 (*n* = 37)	2253.0 ± 151.8 (*n* = 89)	0.832
Decreased C3	10.1 ± 1.2 (*n* = 43)	9.2 ± 1.4 (*n* = 18)	0.831	2261.7 ± 141.7 (*n* = 105)	2045.3 ± 173.9 (*n* = 21)	0.856
Decreased C4	10.7 ± 1.4 (*n* = 34)	8.7 ± 1.1 (*n* = 27)	0.376	2373.9 ± 185.3 (*n* = 74)	2084.9 ± 162.5 (*n* = 52)	0.674

An independent student's *t* test was used for statistical comparison of LILRA3 levels between the presence and absence of manifestations group in SLE

SLE systemic lupus erythematosus, *SLEDAI* systemic lupus erythematosus disease activity index, *LILRA3* leukocyte immunoglobulin-like receptor A3, *Anti-dsDNA Ab* anti-double strand DNA antibody, *ACL* anticardiolipin antibody, *Sm* anti-Smith antibody, *SSA* anti-SSA antibody, *C3* Complement component 3, *C4* Complement component 4

*$P < 0.05$

**$P < 0.01$

subfamily B (LILRB1-5). LILRs are in close linkage with the human killer cell inhibitory receptor (KIR) family, and both LILRs and KIR share similar Ig-like structure and cytoplasmic signaling domains. LILRA receptors have a transmembrane domain containing a charged arginine or lysine residue that associated with the (YxxI/$Lx_{6\text{-}12}$YxxI/L) ITAM-containing FcRγ [11]. ITAM activation recruits Syk/ZAP70 family kinases to drive downstream activation pathways important for immunity [12]. Conversely, LILRB receptors contain cytoplasmic (S/I/V/LxYxxI/V/L) ITIM domains to recruit Src homology 2 domain-containing phosphatases, SHP1/SHP2/SHIP, leading to inhibited immune signaling cascades [13]. The LILR family comprises primate-specific receptors in terms of sequence homology, and PIR-A and PIR-B have been proposed as the murine orthologs of LILRs.

Several LILR members were found in association with autoimmune diseases. Abnormal expressions of LILRBs have been demonstrated to be associated with SLE, RA and MS [14–16]. Compared with healthy donors, SLE patients showed significantly less inhibitory activity by LILRB1s on T cells and reduced expression of LILRB1 on B cells [14]. Phenotypic analysis of IL-10–treated, monocyte-derived DCs in patients with SLE suggested that enhanced LILRB1 expression might have a role in tolerogenic DC functions [17]. In RA patients, elevated serum LILRA3 levels were correlated with disease activity [18]. Upregulated LILRA2, LILRB2, and B3 expressions were found in RA synovial tissue, and the number of LILRB-expressing inflammatory cells significantly decreased in those patients who responded to anti-rheumatic treatment, because of partial inhibition of LILRA2-mediated TNF-α production [19].

In the previous study, for the first time, we showed that the functional rather than nonfunctional *LILRA3* was associated with SLE in Han population. The significant elevation of disease activity in SLE patients homozygous for functional *LILRA3*, and the notable higher level of *LILRA3* transcripts

in *LILRA3* carriers, supported a functional role of *LILRA3* in regulation of SLE susceptibility and disease activity [9]. The mechanism(s) underlying this genetic association of functional *LILRA3* remains unknown.

Deregulation of innate immunity and clearance of apoptotic cells have been implicated in the pathogenesis of SLE [20, 21]. In SLE, cell debris produced by impaired apoptosis may serve as danger signals to break immune tolerance and result in autoimmune inflammation and autoantibody production [22]. In this study, we revealed for the first time that LILRA3 levels were significantly higher in SLE patients compared with healthy controls. LILRA3 expression in both SLE serum and CD14+ monocytes was positively correlated with SLEDAI.

In terms of clinical and laboratory variables, both LILRA3 levels in CD14+ monocytes and circulating LILRA3 levels in serum were associated with more severe clinical and laboratory manifestations in SLE patients. LILRA3 positive group showed higher incidence of nephritis compared with LILRA3 negative group. Moreover, the value of SLEDAI was significant higher in LILRA3-positive group than in LILRA3-negative group, but the values of WBC and PLT count were lower in LILRA3-positive group than in LILRA3-negative group. Taken together, these results demonstrate that LILRA3 might play an important role in the development of SLE.

Several limitations should be considered in this study. First, a large sample size is required to validate the results, and because of the relatively small number of patients, the research lacks multivariate analysis. Second, a longitudinal study would be beneficial to better understand the LILRA3 level changes that are associated with disease activity and severity of SLE.

In conclusion, the findings of the present study demonstrate that LILRA3 is elevated in SLE patients and correlated with lupus nephritis and blood system damage, although our results stem from a single-center study with a relatively small sample size that could bias the association between LILRA3 and SLE. The upregulation of LILRA3 may serve as a biomarker of the disease activity and severity of SLE. Further investigations on the exact role of LILRA3 signaling will provide novel insights into the pathogenesis of SLE.

Author contributions Study conception and design: Du, Xue, Huaxiang Wu.

Acquisition of data: Du, Fengyin Sun, Xinyu Wu, Zhou, Jiang, Cheng, Xue, Wenjia Sun, Chen, Huaxiang Wu.

Analysis and interpretation of data: Du, Fengyin Sun, Xue, Huaxiang Wu.

Manuscript preparation: Du, Xue, Huaxiang Wu.

Statistical analysis**:** Du, Fengyin Sun, Huaxiang Wu.

Funding Supported by the National Natural Science Foundation of China (No. 81501388), Zhejiang Provincial Public Technology Applied Research Project (No. 2015C33177, No. 2017C33032, No. LGF19H100003) and Zhejiang Provincial Medical Science and Technology Plan Project (No. 2017KY381).

Compliance with ethical standards

Disclosures None.

References

1. Brown D, Trowsdale J, Allen R (2004) The LILR family: modulators of innate and adaptive immune pathways in health and disease. Tissue Antigens 64(3):215–225
2. Norman PJ, Carey BS, Stephens HAF, Vaughan RW (2003) DNA sequence variation and molecular genotyping of natural killer leukocyte immunoglobulin-like receptor, LILRA3. Immunogenetics 55(3):165–171
3. Colonna M, Nakajima H, Navarro F, Lopez-Botet M (1999) A novel family of Ig-like receptors for HLA class I molecules that modulate function of lymphoid and myeloid cells. J Leukoc Biol 66(3):375–381
4. Torkar M, Haude A, Milne S, Beck S, Trowsdale J, Wilson MJ (2000) Arrangement of the ILT gene cluster: a common null allele of the ILT6 gene results from a 6.7-kbp deletion. Eur J Immunol 30(12):3655–3662
5. Hirayasu K, Ohashi J, Tanaka H, Kashiwase K, Ogawa A, Takanashi M, Satake M, Jia GJ, Chimge NO, Sideltseva EW, Tokunaga K, Yabe T (2008) Evidence for natural selection on leukocyte immunoglobulin-like receptors for HLA class I in northeast Asians. Am J Hum Genet 82(5):1075–1083
6. Koch S, Goedde R, Nigmatova V, Epplen JT, Muller N, de Seze J, Vermersch P, Momot T, Schmidt RE, Witte T (2005) Association of multiple sclerosis with ILT6 deficiency. Genes Immun 6(5):445–447
7. Kabalak G, Dobberstein SB, Matthias T, Reuter S, The YH, Dorner T, Schmidt RE, Witte T (2009) Association of immunoglobulin-like transcript 6 deficiency with Sjogren's syndrome. Arthritis Rheum 60(10):2923–2925
8. Ordonez D, Sanchez AJ, Martinez-Rodriguez JE, Cisneros E, Ramil E, Romo N, Moraru M, Munteis E, Lopez-Botet M, Roquer J, Garcia-Merino A, Vilches C (2009) Multiple sclerosis associates with LILRA3 deletion in Spanish patients. Genes Immun 10(6):579–585
9. Du Y, Su Y, He J, Yang Y, Shi Y, Cui Y, Luo C, Wu X, Liu X, Hu F, Ma X, Zheng L, Zhang J, Zuo X, Sheng Y, Wu L, Zhang X, Guo J, Li Z (2015) Impact of the leucocyte immunoglobulin-like receptor A3 (LILRA3) on susceptibility and subphenotypes of systemic lupus erythematosus and Sjogren's syndrome. Ann Rheum Dis 74(11):2070–2075
10. Hochberg MC (1997) Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 40(9):1725
11. Nakajima H, Samaridis J, Angman L, Colonna M (1999) Human myeloid cells express an activating ILT receptor (ILT1) that associates with fc receptor gamma-chain. J Immunol 162(1):5–8
12. Mocsai A, Ruland J, Tybulewicz VL (2010) The SYK tyrosine kinase: a crucial player in diverse biological functions. Nat Rev Immunol 10(6):387–402
13. Ravetch JV, Lanier LL (2000) Immune inhibitory receptors. Science 290(5489):84–89
14. Monsivais-Urenda A, Nino-Moreno P, Abud-Mendoza C, Baranda L, Layseca-Espinosa E, Lopez-Botet M, Gonzalez-Amaro R (2007) Analysis of expression and function of the inhibitory receptor ILT2 (CD85j/LILRB1/LIR-1) in peripheral blood mononuclear cells

from patients with systemic lupus erythematosus (SLE). J Autoimmun 29(2–3):97–105
15. Wiendl H, Feger U, Mittelbronn M, Jack C, Schreiner B, Stadelmann C, Antel J, Brueck W, Meyermann R, Bar-Or A, Kieseier BC, Weller M (2005) Expression of the immune-tolerogenic major histocompatibility molecule HLA-G in multiple sclerosis: implications for CNS immunity. Brain 128(Pt 11:2689–2704
16. Kuroki K, Tsuchiya N, Shiroishi M, Rasubala L, Yamashita Y, Matsuta K, Fukazawa T, Kusaoi M, Murakami Y, Takiguchi M, Juji T, Hashimoto H, Kohda D, Maenaka K, Tokunaga K (2005) Extensive polymorphisms of LILRB1 (ILT2, LIR1) and their association with HLA-DRB1 shared epitope negative rheumatoid arthritis. Hum Mol Genet 14(16):2469–2480
17. Figueroa-Vega N, Galindo-Rodriguez G, Bajana S, Portales-Perez D, Abud-Mendoza C, Sanchez-Torres C, Gonzalez-Amaro R (2006) Phenotypic analysis of IL-10-treated, monocyte-derived dendritic cells in patients with systemic lupus erythematosus. Scand J Immunol 64(6):668–676
18. An H, Chandra V, Piraino B, Borges L, Geczy C, McNeil HP, Bryant K, Tedla N (2010) Soluble LILRA3, a potential natural antiinflammatory protein, is increased in patients with rheumatoid arthritis and is tightly regulated by interleukin 10, tumor necrosis factor-alpha, and interferon-gamma. J Rheumatol 37(8):1596–1606
19. Huynh OA, Hampartzoumian T, Arm JP, Hunt J, Borges L, Ahern M, Smith M, Geczy CL, McNeil HP, Tedla N (2007) Down-regulation of leucocyte immunoglobulin-like receptor expression in the synovium of rheumatoid arthritis patients after treatment with disease-modifying anti-rheumatic drugs. Rheumatology (Oxford) 46(5):742–751
20. Choi J, Kim ST, Craft J (2012) The pathogenesis of systemic lupus erythematosus-an update. Curr Opin Immunol 24(6):651–657
21. Gaipl US, Munoz LE, Grossmayer G, Lauber K, Franz S, Sarter K, Voll RE, Winkler T, Kuhn A, Kalden J, Kern P, Herrmann M (2007) Clearance deficiency and systemic lupus erythematosus (SLE). J Autoimmun 28(2–3):114–121
22. Munoz LE, Lauber K, Schiller M, Manfredi AA, Herrmann M (2010) The role of defective clearance of apoptotic cells in systemic autoimmunity. Nat Rev Rheumatol 6(5):280–289

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.