

# Linc8986 and linc0597 in plasma are novel biomarkers for systemic lupus erythematosus

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Received November 27, 2020; Accepted June 22, 2021

DOI: 10.3892/etm.2021.10644

**Abstract.** Despite increasing evidence that large intergenic non-coding RNAs (lincRNAs) are widely involved in human disease, the role of lincRNAs in the development of systemic lupus erythematosus (SLE) has remained largely elusive. The purpose of the present study was to investigate the expression of three lincRNAs (linc0597, linc8986 and linc7190) in the plasma of patients with SLE and their potential use as biomarkers for the diagnosis and treatment of SLE. Plasma samples were obtained from 54 patients with SLE, 24 patients with rheumatoid arthritis (RA), 24 patients with Sjögren's syndrome (SS) and 22 healthy controls. LincRNA expression levels were measured by reverse transcription-quantitative PCR. Compared with those in the healthy controls, the plasma levels of linc0597 and linc8986 were significantly increased in the patients with SLE ( $P<0.001$ ), while the difference in the level of linc7190 was not significant ( $P=0.052$ ). In addition, there was no significant difference in the levels of linc0597 and linc8986 among patients with RA, patients with SS and the healthy controls ( $P>0.05$ ). Compared with patients with SLE without lupus nephritis (LN), the levels of linc0597 were significantly higher in patients with LN ( $P=0.044$ ). For

linc7190 and linc8986, there was no significant difference between patients with and without LN ( $P>0.05$ ). Furthermore, complement component 3 (C3) levels were used to evaluate whether the expression of linc8986 and linc0597 is related to the activity of SLE. The results indicated that the levels of linc8986 and linc0597 were negatively correlated with the level of C3 ( $P<0.001$  and  $P=0.004$ , respectively). Further analysis suggested that linc0597 and linc8986 were able to specifically identify patients with SLE and that a combination of linc0597 and linc8986 may improve the diagnostic accuracy. Therefore, the plasma levels of linc0597 and linc8986 may be suitable biomarkers for diagnosing SLE.

## Introduction

Systemic lupus erythematosus (SLE) is a serious chronic autoimmune disease characterized by loss of tolerance to autoantigens, a variety of immune abnormalities and high titer of autoantibodies against nuclear components (1). It is a highly heterogeneous disease and different patients may have distinct symptoms and clinical characteristics (2). The pathogenesis of SLE is complex and it is thought that genetic susceptibilities and environmental factors have a key role in the development of SLE (3). Lupus nephritis (LN), one of the most serious manifestations of SLE, is the major cause of a large number of SLE-related deaths (4,5). In the internationally recognized SLE classification standard, complement component 3 (C3) and C4 are among the immunological diagnostic items for SLE (6,7). Although plasma levels of C3 and C4 are of certain diagnostic value for SLE, they are not specific diagnostic indicators (8). Therefore, it is critical to identify more diagnostic indicators and therapeutic targets for SLE.

The importance of non-coding RNAs (ncRNAs), such as microRNAs (miRNAs), has been emphasized in numerous biological and pathological processes (9). Several studies have demonstrated the feasibility of using miRNAs in bodily fluids as a biomarker for the diagnosis of SLE (10,11). Although miRNAs have an important role in SLE, they account for only a small fraction of the non-coding region of the mammalian genome. Unlike miRNAs, long non-coding RNAs (lncRNAs) are highly expressed, including large intergenic ncRNAs (lincRNAs) (12). LincRNAs have been indicated to have an important role in the course of autoimmune diseases and

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**Abbreviations:** lincRNA, large intergenic noncoding RNA; SLE, systemic lupus erythematosus; LN, lupus nephritis; RA, rheumatoid arthritis; SS, Sjögren's syndrome; ACR, American College of Rheumatology; C3, complement component 3; ESR, erythrocyte sedimentation rate; dsDNA, double-stranded DNA; RT-qPCR, reverse transcription-quantitative PCR

**Key words:** systemic lupus erythematosus, large intergenic non-coding RNA, linc0597, linc8986, linc7190

studies on rheumatoid arthritis (RA) (13) and autoimmune thyroid diseases (14) have provided evidence to confirm this hypothesis. The relationship between SLE and lincRNAs has been a research hotspot and has been preliminarily explored in several reports. Wu *et al* (15) reported that the expression levels of linc0597 and linc0949 in peripheral blood mononuclear cells of patients with SLE were significantly downregulated compared with those of healthy controls, suggesting that linc0597 and linc0949 may be biomarkers for the diagnosis of SLE. Zheng *et al* (16) determined that soluble TNF receptor 1 (sTNF-R1 and linc0597 may serve as biomarkers for the diagnosis of LN and were associated with disease activity in SLE. In another study of 24 patients with SLE, the lincRNA growth arrest-specific 5 was identified as a potential biomarker for SLE (17). Li *et al* (18) measured the expression of lincRNAs by reverse transcription-quantitative PCR (RT-qPCR) after 8 h of treatment of THP-1 macrophages with Pam3-Cys-Ser-Lys4 (Pam3CSK4). Among them, there was a clear decrease in the expression levels of linc7190 and linc0597, while the expression of linc8986 increased significantly. In addition, linc7190 was indicated to have a regulatory effect on the secretion of interleukin-6 (IL-6) and TNF- $\alpha$ , both of which have been indicated to be involved in the pathogenesis of SLE (19).

There is evidence that lincRNAs are stable in human plasma. In the present study, the expression levels of three lincRNAs, namely linc7190, linc0597 and linc8986, we measured in the plasma of patients with SLE and their relationship with the clinical characteristics and organ damage status of the patients was analyzed. The present study provided a basis for the use of new potential biomarkers for the diagnosis and treatment of SLE.

## Materials and methods

**Patients and healthy controls.** A total of 54 patients with SLE, 24 patients with RA and 24 patients with Sjögren's syndrome (SS) were enrolled at the Department of Rheumatology and Immunology of the First Affiliated Hospital of Harbin Medical University (Harbin, China), as well as 22 healthy individuals matched to the patients with SLE for sex and age from the Physical Examination Center at the First Affiliated Hospital of Harbin Medical University (Harbin, China) between April 2018 and October 2018. Demographic characteristics, disease activity and laboratory parameters were collected from the patients' medical records. All patients with SLE were diagnosed according to the American College of Rheumatology (ACR) 1997 revised criteria (20). Renal damage in SLE was defined by the ACR standard and includes any one of the following: i) Persistent proteinuria  $\geq 0.5$  g/day; ii) presence of active tubular cells; iii) biopsy evidence of LN. RA was diagnosed according to the ACR/European League Against Rheumatism 2010 classification criteria (21). SS was diagnosed in accordance with the revised 2002 American-European criteria (22). The exclusion criteria for all patients were as follows: i) Patients with malignant tumors; ii) patients with acute infection within one month prior to admission; and iii) patients with other autoimmune diseases.

The present study was approved by the Research Ethics Committee of the First Affiliated Hospital of Harbin Medical University (Harbin, China). All participants included in the study provided written informed consent.

Table I. Clinical features of patients with systemic lupus erythematosus.

Characteristic	HC (n=22)	Patients (n=54)	P-value
Age, years	35.9 $\pm$ 5.5	39.4 $\pm$ 6.3	0.245
Sex (male/female)	3/19	4/50	0.406
Anti-dsDNA (P/N) <sup>a</sup>	-	27/22	
Disease duration, years	-	2.5 $\pm$ 1.5	
LN (P/N)	-	24/30	
ESR (P/N) <sup>a</sup>	-	37/14	
C3 level, mg/dl <sup>a</sup>			
<80	-	38	
$\geq$ 80	-	13	
Methylprednisolone dose <sup>a</sup> , mg/day			
$\geq$ 30	-	33	
<30	-	19	

<sup>a</sup>Since certain patients were not examined, the number of patients listed in this feature is less than the total number of patients. The normally distributed data are presented as the mean  $\pm$  standard deviation. The abstract independent-samples t-test was used for age and Fisher's exact test was used for sex to calculate P-values for SLE patients vs. healthy controls. HC, healthy controls; anti-dsDNA, anti-double-stranded DNA; LN, lupus nephritis; P/N, positive/normal; SLE, systemic lupus erythematosus; ESR, erythrocyte sedimentation rate.

**Obtainment of peripheral blood samples and RNA processing.** Blood samples from each donor were collected in EDTA-anticoagulant tubes. The plasma was separated by centrifugation at 1,500  $\times$  g for 10 min at room temperature, followed by high-speed centrifugation at 12,000  $\times$  g for 10 min at room temperature to completely remove cell debris. The supernatant plasma was recovered and stored at -80°C until further analysis. Total RNA was extracted from 400  $\mu$ l plasma by TRIzol<sup>®</sup> reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The total RNA concentration was measured using a spectrophotometer (Thermo Fisher Scientific, Inc.) and 200-600 ng of total RNA was obtained from 400  $\mu$ l plasma.

**RT-qPCR.** The PrimeScript<sup>™</sup> RT Kit (Takara Bio Inc.) was used to eliminate genomic DNA from the RNA samples and to reverse-transcribe RNA into cDNA. To quantify the expression of the three lincRNAs (linc0597, linc8986 and linc7190), cDNA was amplified by PCR using a SYBR Green kit (SYBR<sup>®</sup> PremixEx Taq<sup>™</sup> II; Takara Bio, Inc.) in 20- $\mu$ l reactions containing 10  $\mu$ l SYBR Green, 0.4  $\mu$ l ROX Reference Dye II, 0.2  $\mu$ M forward primer, 0.2  $\mu$ M reverse primer, 6  $\mu$ l sterile deionized water and 2  $\mu$ l cDNA. The relative expression level of each lincRNA was normalized to GAPDH (23). The following thermocycling conditions were used for PCR: 95°C for 1 min, followed by 40 cycles of 95°C for 10 sec, 60°C for 30 sec and 72°C for 1 min. After the reaction, the quantification cycle (Cq) value was determined using a fixed threshold

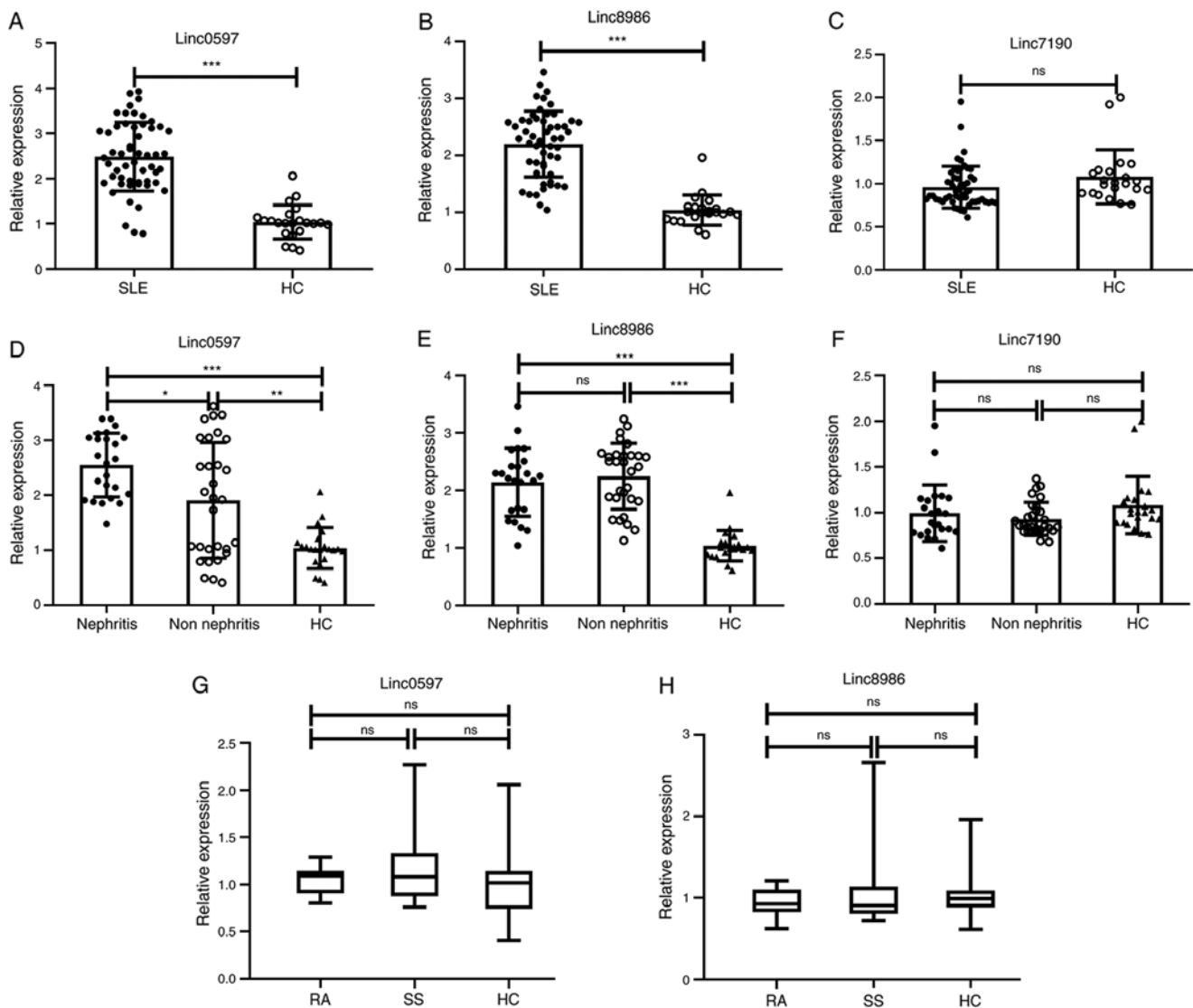


Figure 1. Validation of candidate lncRNAs (linc0597, linc8986 and linc7190) identified in plasma of patients with SLE and HCs. The expression levels of the three candidate lncRNAs in 54 patients with SLE and 24 HCs were analyzed by reverse transcription-quantitative PCR and normalized to GAPDH. (A) Increased expression of linc0597 in total patients with SLE vs. HCs. (B) Increased expression of linc8986 in total patients with SLE vs. HCs. (C) Expression of linc7190 in total patients with SLE vs. HCs. (D) Increased expression of linc0597 in patients with SLE with and without nephritis vs. HCs. (E) Increased expression of linc8986 in patients with SLE with and without nephritis vs. HCs. (F) Expression of linc7190 in patients with SLE with and without nephritis vs. HCs. Each symbol represents an individual subject; horizontal lines indicate median values. (G) Expression of linc0597 in patients with RA vs. patients with SS vs. HCs. (H) Expression of linc8986 in patients with RA vs. patients with SS vs. HCs. The three horizontal lines in the figure indicate the upper quartile, the median and the lower quartile values, respectively. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ . ns, no significance; HC, healthy control; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; SS, Sjögren's syndrome; lncRNA, long non-coding RNA; linc, large intergenic non-coding RNA.

setting. The relative expression of the lincRNAs was calculated using the  $2^{-\Delta\Delta C_q}$  method (24). The PCR primer sequences were as follows: Linc0597 forward, TTGGATTCATCCCGTTCACCTCCA and reverse, AAAGAAGCAGGACTACCCACT; linc8986 forward, ATCTTGGCCACAGAGGAGGAAT and reverse, ATTCCAGTGACTGCACTGAAGGT; linc7190 forward, CTGCTTTGGAGCAGTTGGGAAGTT and reverse, AGACAGGTTTGCTGACGAAGGTCT; and GAPDH forward, CCAACATGCTGACTCACCTTCC and reverse, ATGGAGTCTCGCTCTGTCAACCA.

**Statistical analysis.** All statistical analyses were performed using SPSS statistical software version 25.0 (IBM Corporation). Values are expressed as the mean  $\pm$  standard deviation. The t-test was used to compare age and the Fisher's exact test was

used to compare sex between patients with SLE and healthy controls. The nonparametric Mann-Whitney U-test was used to compare differences in the expression of each lincRNA within two groups. The nonparametric Kruskal-Wallis test was used to compare data among three groups. Spearman's rank correlation analysis was used to analyze the correlation between lincRNAs and clinical characteristics and to calculate the correlation coefficient. Receiver operating characteristic (ROC) curve analysis was performed by MedCalc version 18.9 (MedCalc Software bvba).  $P<0.05$  was considered to indicate statistical significance.

## Results

**Identification of candidate lincRNAs in SLE.** The expression levels of three unique lincRNAs, namely linc0597, linc8986

Table II. Correlation of the expression of plasma linc0597 and linc8986 with clinical parameters of patients with systemic lupus erythematosus.

Clinical feature	N	Linc0597		Linc8986	
		$r_s$	P-value	$r_s$	P-value
C3	51	-0.380	0.004	-0.565	<0.001
Anti-dsDNA	51	0.127	0.116	0.114	0.545
Prednisone	52	0.076	0.339	0.174	0.194
ESR	51	0.291	0.039	0.143	0.051
Age	54	0.02	0.783	0.077	0.294
Duration of disease	54	0.128	0.081	0.075	0.308

Spearman's correlation analysis was used. ESR, erythrocyte sedimentation rate; Anti-dsDNA, anti-double-stranded DNA; linc, large intergenic non-coding RNA; C3, complement component 3.

and linc7190, in human plasma were analyzed in 54 patients with SLE (50 females and 4 males; mean age,  $39.4 \pm 6.3$  years) and 22 healthy donors (19 females and 3 males; mean age,  $35.9 \pm 5.5$  years) using RT-qPCR. The mean age and sex distribution did not differ significantly between the patients with SLE and the healthy donors (Table I). As presented in Fig. 1A and B, patients with SLE had significantly higher levels of linc0597 and linc8986 than the healthy controls (both  $P < 0.001$ ). However, the difference in linc7190 levels between patients with SLE and the healthy donors was not significant ( $P = 0.052$ ; Fig. 1C). In addition, in order to determine whether there is an association between lincRNA levels and LN, the patients with SLE were divided into an LN group ( $n = 24$ ) and a non-LN group ( $n = 30$ ). The results indicated that the expression level of linc0597 in the LN and the non-LN groups were significantly higher compared with the healthy controls ( $P < 0.001$  and  $P = 0.006$ ) and the linc0597 in the LN group was significantly higher than that in the non-LN group ( $P = 0.044$ ; Fig. 1D). In addition, the levels of linc8986 in the LN and the non-LN groups were significantly higher compared with those in the healthy controls ( $P < 0.001$ ), but there was not significantly different between the LN and non-LN groups ( $P = 0.523$ ; Fig. 1E). The levels of linc7190 in the LN and the non-LN groups were not significantly different compared with the healthy controls ( $P > 0.05$ ), and there was also no significant difference between the LN and non-LN groups ( $P = 0.122$ ; Fig. 1F). To further evaluate the diagnostic capacity of linc0597 and linc8986, 24 patients with RA and 24 patients with SS were selected for analysis and comparison with the healthy control group. As presented in Fig. 1G and H, no significant difference in the plasma levels of linc0597 and linc8986 was observed between the patients with RA, the patients with SS and the healthy controls ( $P = 0.468$  and  $P = 0.674$ , respectively). Overall, the results suggested that increased levels of linc0597 and linc8986 in plasma may be associated with SLE.

**Correlation between the candidate lincRNAs and clinical features of SLE.** To determine whether the expression levels of lincRNA was related to clinical features, autoantibody profiles and hormone therapy, we analyzed the correlation of

the expression of plasma linc0597 and linc8986 with clinical parameters of patients with SLE (Table II). The expression levels of linc0597 and linc8986 were both negatively correlated with C3 levels ( $P = 0.004$  and  $P < 0.001$ , respectively; Table II) and the expression levels of linc0597 was positively correlated with erythrocyte sedimentation rate (ESR) ( $P = 0.039$ ; Table II).

**Association between the expression levels of linc0597 and clinical characteristics in patients with SLE.** According to the expression levels of C3 and ESR in patients with SLE, the group was divided into a low C3 group and normal C3 group, as well as a high ESR group and a normal ESR group. It was revealed that the level of linc0597 was significantly higher in the low C3 group than in the normal C3 group ( $P = 0.03$ ; Fig. 2A) and correlation analysis between C3 and linc0597 indicated that the level of linc0597 in patients with SLE was negatively correlated with C3 levels ( $r = 0.380$ ,  $P = 0.004$ ; Fig. 2B). In addition, the level of linc0597 in the high ESR group was significantly higher than that in the normal ESR group ( $P = 0.032$ ; Fig. 2C) and there was a weak positive correlation between linc0597 levels and ESR ( $r = 0.291$ ,  $P = 0.039$ ; Fig. 2D).

The association between lincRNA levels and autoantibody profiles and medical treatments were also analyzed and no significant difference in the level of linc0597 between the high anti-double-stranded DNA (anti-dsDNA) antibodies group and the normal anti-dsDNA antibody level group was obtained ( $P = 0.609$ ; Fig. 2E). Furthermore, patients with SLE receiving drug therapy were divided into a middle-to-high dose of prednisone group ( $\geq 30$  mg/day) and a low dose of prednisone group ( $< 30$  mg/day). There was no significant difference in the expression level of linc0597 between the two groups ( $P = 0.247$ ; Fig. 2F).

**Relationship between the expression level of linc8986 and clinical characteristics of patients with SLE.** Compared to patients with normal C3 levels, the expression level of linc8986 was significantly higher in patients with SLE with low C3 levels ( $P < 0.001$ ; Fig. 2G). Further analysis revealed a negative correlation between the level of linc8986 and C3 levels ( $r = 0.565$ ,  $P < 0.001$ ; Fig. 2H). In contrast to linc0597, there was no significant difference in the expression level of linc8986

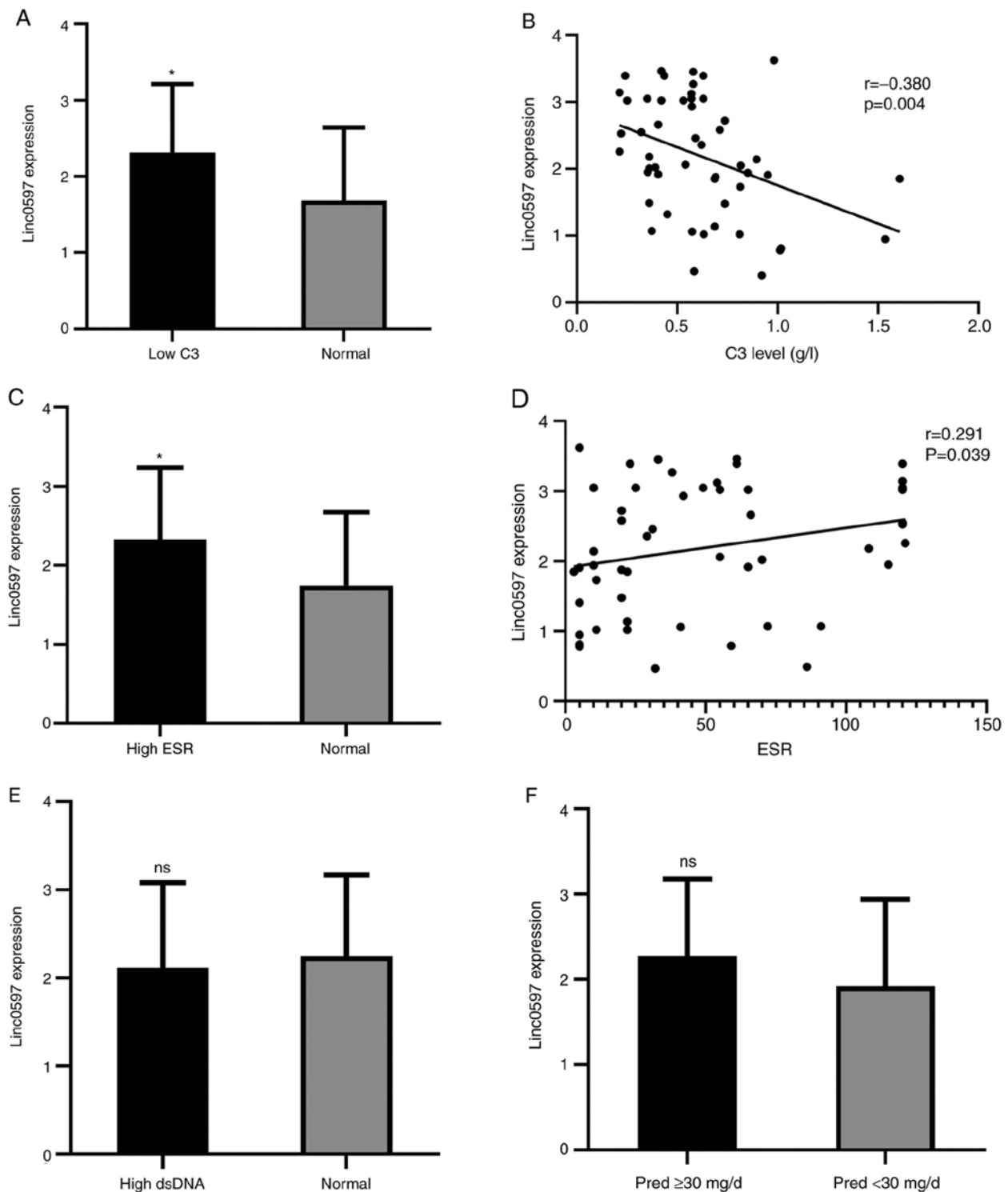


Figure 2. Continued.

between the high ESR group and the normal ESR group ( $P=0.135$ ; Fig. 2I). However, the expression level of linc8986 in the high anti-dsDNA group was significantly higher than that in the normal anti-dsDNA group ( $P=0.009$ ; Fig. 2J), but further analysis indicated no correlation between linc8986 and anti-dsDNA ( $r=0.114$ ,  $P=0.545$ ; Fig. 2K). In addition, there was no significant difference in the expression level of linc8986 ( $P=0.055$ ; Fig. 2L) between the middle-to-high dose prednisone group and the low-dose prednisone group.

**Identification of linc0597 and linc8986 as potential biomarkers of SLE.** In order to determine whether linc0597 and linc8986 may be used as potential biomarkers of SLE, ROC curve analysis was used to assess linc0597 and linc8986 separately and in combination, as presented in Fig. 3.

The area under the ROC curve (AUC) of linc0597 was 0.768 (95%CI: 0.652-0.883;  $P=0.0015$ ), the cut-off value was 0.515, the sensitivity and the specificity was 64.8 and 78.7%. The AUC of linc8986 was 0.827 (95%CI: 0.728-0.925;

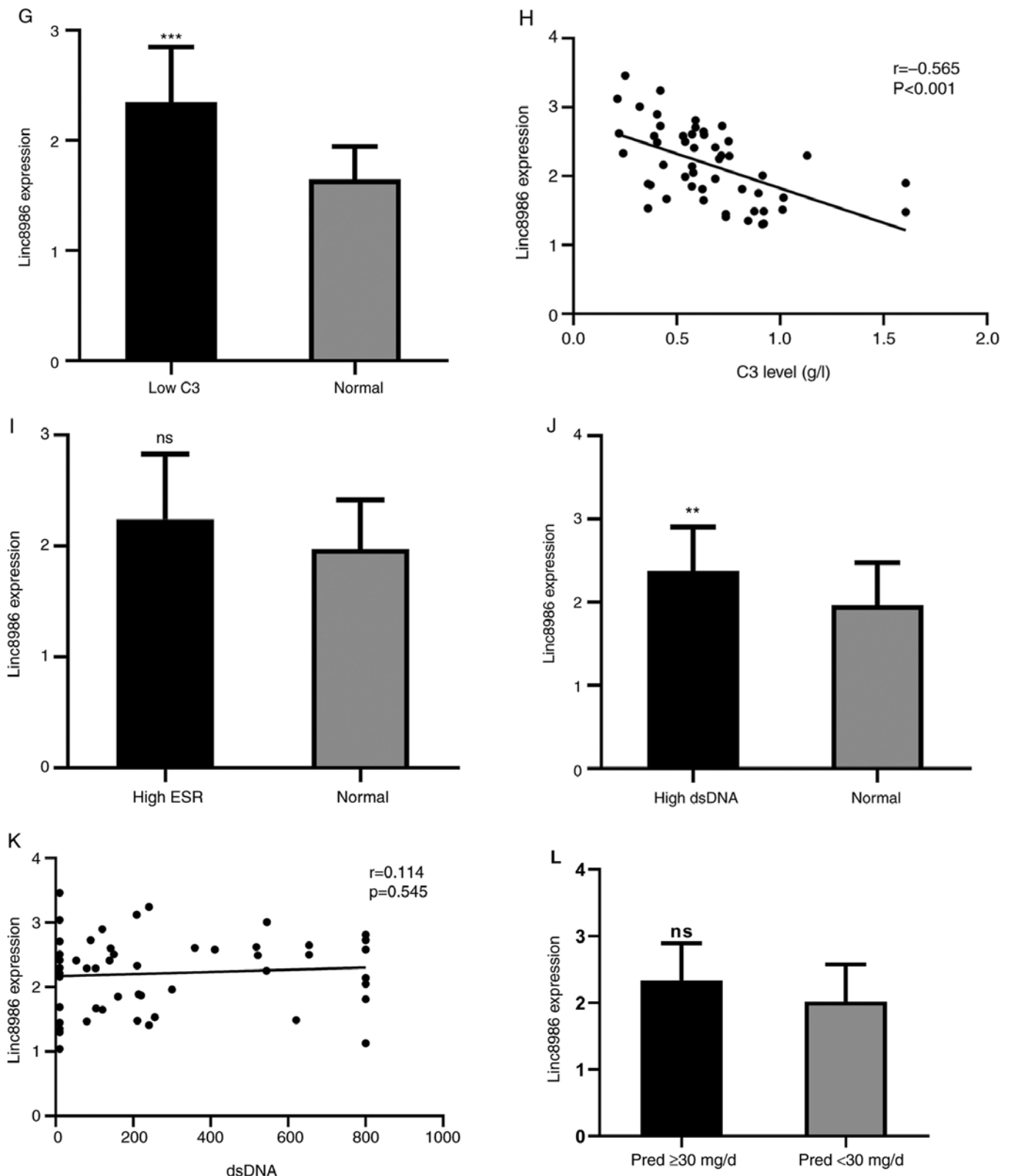


Figure 2. Relationship between the expression levels of linc0597 and linc8986 and clinical characteristics of patients with SLE. (A) Increased expression of linc0597 in the low level of C3 group vs. normal C3 levels group. (B) A significant negative correlation was observed between linc0597 expression and the level of C3 in patients with SLE. (C) Increased expression of linc0597 in the high level of ESR group vs. normal level of ESR group. (D) Linc0597 expression was positively correlated with ESR levels in patients with SLE. (E) Expression of linc0597 in the high level of anti-dsDNA group vs. normal level of anti-dsDNA group. (F) Expression of linc0597 in the middle-to-high level of predgroup vs. low level of pred group. (G) Increased expression of linc8986 in the low level of C3 group vs. normal level of C3 group. (H) A significant negative correlation was observed between linc8986 expression and the level of C3 in patients with SLE. (I) Expression of linc8986 in the high level of ESR group vs. normal level of ESR group. (J) Increased expression of linc8986 in the high level of anti-dsDNA group vs. normal level of anti-dsDNA group. (K) Correlation analysis between linc8986 and dsDNA levels in patients with SLE. (L) Expression of linc8986 in the middle-to-high level of pred group vs. low level of pred group. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . ns, no significance; C3, complement component 3; ESR, erythrocyte sedimentation rate; anti-dsDNA, anti-double-stranded DNA antibody; linc, large intergenic non-coding RNA; SLE, systemic lupus erythematosus; pred, prednisone.

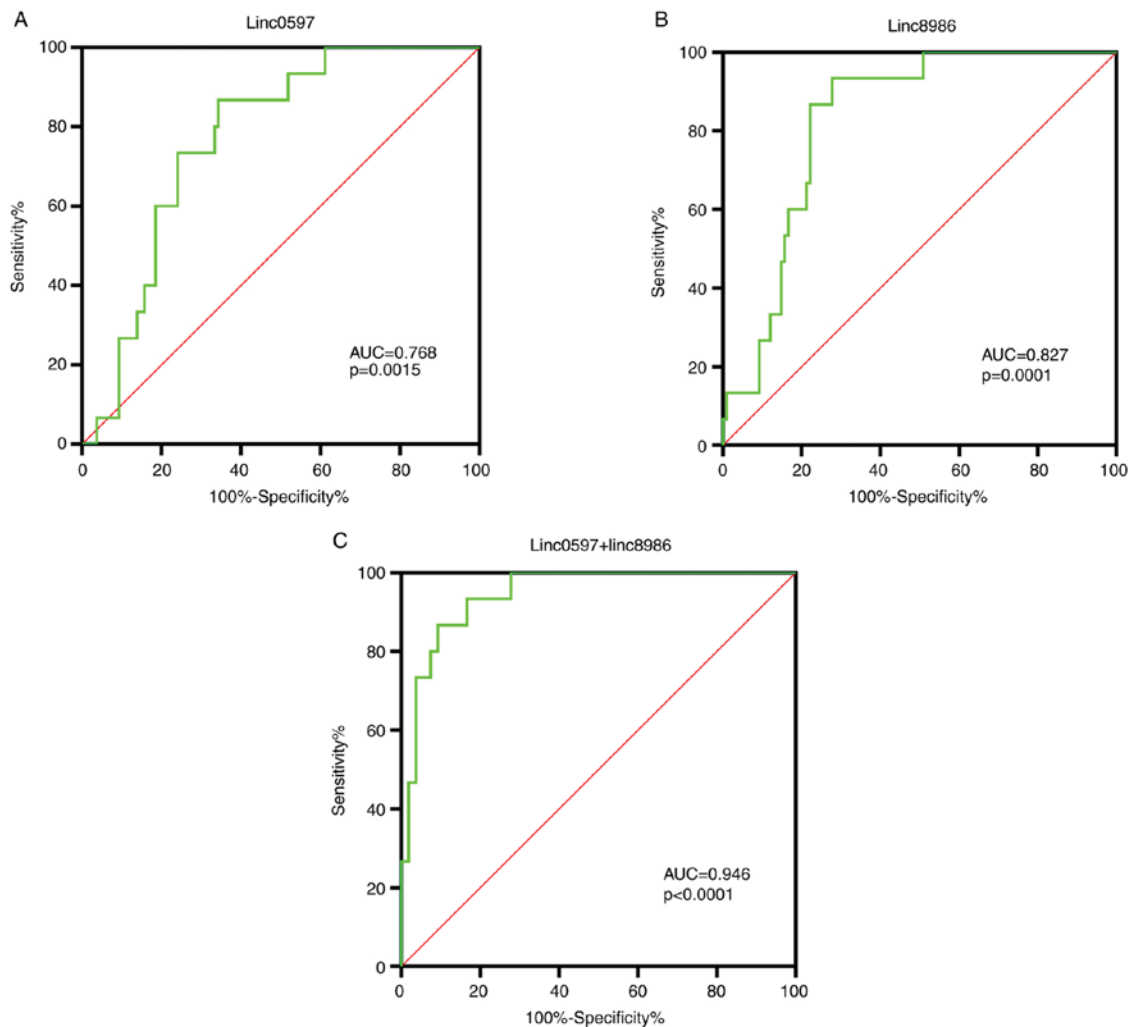


Figure 3. ROC curve analysis for the ability of (A) linc0597 alone, (B) linc8986 alone and (C) linc0597 combined with linc8986 to distinguish systemic lupus erythematosus from healthy controls. ROC, receiver operating characteristic; AUC, area under the curve; linc, large intergenic non-coding RNA.

$P=0.0001$ ), the cut-off value was 0.655, the sensitivity and the specificity was 72.2 and 84.6%. (Fig. 3A and B). The results revealed that both linc8986 and linc0597 may be associated with the pathogenesis of SLE, but that linc8986 has higher sensitivity and may be a potential biomarker for the diagnosis of SLE. Furthermore, the results suggested that the combined application of linc8986 and linc0597 had an AUC of 0.946 (95% CI: 0.893-0.998,  $P<0.0001$ ; Fig. 3C) and may thus provide higher diagnostic accuracy. The cut-off value was 0.774, the sensitivity and specificity of the combined application of linc8986 and linc0597 were 90.7 and 86.7%, respectively.

## Discussion

Increasing evidence has indicated that lincRNAs have important roles in vast biological processes, including stem cell biology, cell differentiation, embryonic development and tissue-specific expression (25). LincRNAs, which were initially studied in the context of genomic imprinting and cell differentiation, have been determined to function as key regulators of various processes, particularly in the molecular mechanisms of immune cells and autoimmunity (26).

In the present study, the expression levels of three different lincRNAs (linc0597, linc8986, and linc7190) were analyzed in plasma samples and investigated the association between their expression and specific clinical features of SLE was determined. The results indicated that linc0597 and linc8986 may be suitable biomarkers for the diagnosis of SLE, while linc7190 is not. In addition, the plasma level of linc0597 was correlated with the levels of C3 and the ESR, while the plasma level of linc8986 was correlated with the levels of C3 and anti-dsDNA antibodies. This further raised the question of how complements are affected by these lincRNAs. Song *et al* (27) indicated that elevated levels of the lincRNA RPA1N regulate invasion and apoptosis of trophoblast cells through complement C1q. Furthermore, it was indicated that overexpression of RPA1N inhibits the expression of C1q and that C1q was the functional target of RPA1N (27). It was also previously reported that C3 and C4 have diagnostic value for SLE (6,7). In the present study, elevated levels of linc8986 and linc0597 in the plasma of patients with SLE were indicated to be negatively correlated with C3, suggesting that high expression of linc8986 and linc0597 may inhibit the expression of C3. Whether C3 is also the target of these two lincRNAs requires further investigation.



Compared with the previously reported expression levels of linc0597 in monocytes of patients with SLE, the plasma level of linc0597 in patients with SLE is relatively high (15,28). This discrepancy may be due to a number of reasons. One explanation for the increased levels of linc0597 in the plasma of patients with SLE may be the increase of cellular exocytosis resulting in a decrease in intracellular content (29). In addition, the sequence length of lincRNAs is high and it is unlikely to exist in its complete form in bodily fluids, i.e., the lincRNAs in plasma may exist in a fragmented form (30). Another reason is that the expression of lincRNAs may be tissue-specific (31).

To the best of our knowledge, the present study was the first to identify a relationship between the plasma levels of linc8986 and SLE. The results suggested that the expression level of linc8986 in patients with SLE was higher than that in the healthy controls. Further analysis revealed that the plasma levels of linc8986 were not significantly different between patients with RA, patients with SS and healthy controls, indicating that the level of linc8986 in plasma was specific for the diagnosis of SLE. Li *et al* (18) used Pam3CSK4 to stimulate THP-1 macrophages and determined that the expression level of linc8986 increased significantly. However, only a small number of studies have examined linc8986 and the next step is to identify the molecular mechanisms in detail. Regarding linc7190, Li *et al* (18) indicated that the level of linc7190 increased significantly after 8 h of treatment of THP-1 macrophages with Pam3CSK4 and linc7190 regulates the secretion of IL-6 and TNF- $\alpha$ . Of note, both IL-6 and TNF- $\alpha$  are involved in the pathogenesis of SLE. Previous studies suggested that Toll-like receptors may trigger the regulatory factors NF- $\kappa$ B and IFN, which regulate the expression of hundreds of genes involved in the immune response, including the pro-inflammatory cytokines TNF- $\alpha$ , IL-1 and IL-6 (32,33). Therefore, Li *et al* (18) revealed that under the stimulation of Pam3CSK4, the regulation of linc7190 may have a priming process and linc7190 further regulates the secretion of IL-6 and TNF- $\alpha$  and participates in the pathogenesis of SLE.

LN is one of the most common complications of SLE. Depending on the population, the incidence of LN may be as high as 50% and the incidence of end-stage nephropathy is 20% (5). Although significant progress has been made in the diagnosis and treatment of LN in recent years, the therapeutic effect remains unsatisfactory and the incidence of the disease is on the rise (34). While renal pathological biopsy is still the gold standard for diagnosing patients suspected to have LN, it has a number of disadvantages, such as increased trauma and pain associated with the procedure (35). Wu *et al* (17) reported that plasma lincRNA dendritic cells may be used as a biomarker for the diagnosis of SLE and to distinguish between patients with and without LN in SLE. Zheng *et al* (16) reported that serum sTNF-R1 and linc0597 were upregulated in patients with SLE and LN, suggesting that serum sTNF-R1 and linc0597 may be biomarkers for SLE and LN. Therefore, in the present study, the relationship between linc8986, linc0597 and linc7190 was also analyzed in patients with LN. The present results indicated that the expression level of linc0597 in patients with SLE with LN was higher than that in patients with SLE without LN, but the expression levels of linc8986

and linc7190 were not significantly different between the two groups. This suggests that linc0597 and linc8986 may be utilized for the diagnosis of SLE and that high levels of linc0597 may be helpful for the differential diagnosis of LN in patients with SLE.

Over the past few decades, much effort has been invested into the study of SLE biomarkers and numerous researchers have proposed potential biomarkers for SLE. For instance, IFN-induced genes and chemokines may be used to determine the activity and severity of SLE in patients (36,37). However, the limitations of these biomarkers have gradually emerged. Previous studies have reported that overexpression of the IFN I type pathway has been confirmed in patients with RA, SS, myositis and scleroderma. Thus, IFN is not sufficiently specific for the diagnosis of SLE (38,39). This highlights the urgent requirement for a specific biomarker to diagnose SLE. As biomarkers, lincRNAs have a number of characteristics that make them well-suited for use as biomarkers, including being stable in plasma and their ease and low cost of detection (40), making them ideal biomarkers to evaluate disease activity and judge the severity of SLE in patients.

In the present study, it was demonstrated that the expression levels of linc0597 are specific to patients with SLE and that the levels of linc0597 may be helpful for evaluating disease activity and distinguishing between patients with LN and without LN. However, further validation of linc0597 in large-scale multicenter trials is necessary. In addition, the present study also suggested that linc8986 in plasma may be used as a promising diagnostic marker for SLE. To our knowledge, the present study was the first to report the association of linc8986 with SLE. Furthermore, in the ROC curve analysis, the AUC value of linc0597 and linc8986 was 0.768 and 0.827, respectively and combination of linc8986 and linc0597 improved the diagnostic accuracy. Therefore, the use of lincRNAs as novel biomarkers may become a valuable tool for the clinical diagnosis of SLE.

However, there are still certain shortcomings to the present study. The functions of the lincRNAs and their potential mechanisms in SLE were not investigated. In addition, the level of lincRNAs released from local target tissues was not evaluated. In future studies, it will be investigated how these lincRNAs affect IFN, IL-6, TNF and TGF signals and their relationship with LN within a larger cohort and the influence of these molecules on the STAT signaling pathway and the regulation of lincRNAs on the post-transcriptional process will be further explored.

In conclusion, linc8986 and linc0597 may be used to specifically identify patients with SLE and linc0597 has an important role in the detection of nephritis in patients with SLE. Combined application of linc0597 and linc8986 may improve the diagnostic accuracy of SLE.

## Acknowledgements

Not applicable.

## Funding

This study was supported by the National Natural Science Foundation of China (grant no. 81772261).



## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Authors' contributions

CR proposed the research, performed the data analysis and wrote the manuscript. HX helped with the formal analysis and data collection. CY and FW helped with the data acquisition. HZ was involved in funding acquisition, and analysis and interpretation of data for the study. XG was involved in supervision and conception and design of the study. XG and HZ confirmed the authenticity of the raw data. All authors read and approved the final version of the manuscript.

## Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the First Affiliated Hospital of Harbin Medical University (Harbin, China; approval no. IRB-AF/SC-04/01.0). All participants provided written informed consent.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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