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Clinical significance of exostosin 1 in confirmed and suspected lupus membranous nephropathy

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ABSTRACT

Objective This study aimed to investigate the clinical significance of exostosin 1 (EXT1) in confirmed and suspected lupus membranous nephropathy (LMN).

Methods EXT1 was detected in 67 renal tissues of M-type phospholipase A2 receptor (PLA2R)-negative and ANA-positive membranous nephropathy by immunohistochemistry, and cases were divided into confirmed LMN and suspected LMN. The clinicopathological data were compared among the above groups, as well as EXT1-positive group and EXT1-negative group.

Results Twenty-two cases (73.3%) of confirmed LMN and six cases (16.2%) of suspected LMN exhibited EXT1 expression on the glomerular basement membrane and/or mesangium area, showing a significant difference ($p<0.001$). Concurrently, lupus nephritis (LN) of pure class V demonstrated a lower frequency of EXT1 positivity compared with mixed class V LN in the confirmed LMN group (31.8% vs 68.2%, $p=0.007$). EXT1-positive patients in the confirmed and suspected LMN group showed significant differences in some clinicopathological data comparing with EXT1-negative patients ($p<0.05$). Follow-up data revealed that a greater proportion of patients in the EXT1-positive group achieved complete remission post-treatment ($p<0.05$). Cox regression analysis showed that EXT1 positivity was significantly correlated with complete remission across the entire study cohort (HR 5.647; 95% CI, 1.323 to 12.048; $p=0.019$). Kaplan-Meier analysis indicated that the EXT1-positive group had a higher rate of accumulated nephrotic remission compared with the EXT1-negative group in the whole study cohort ($p=0.028$).

Conclusions The EXT1-positive group exhibited a higher active index and a more favourable renal outcome than the EXT1-negative group. It would be better to recognise suspected LMN with EXT1 positivity as a potential autoimmune disease and maintain close follow-up due to its similarities with confirmed LMN.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Lupus membranous nephropathy (LMN) exhibited positive exostosin (EXT) 1/2 expression in renal tissue, and patients testing positive for EXT1/2 appeared to have a better prognosis.

WHAT THIS STUDY ADDS

⇒ EXT1 is positive in both pure and mixed classes of LMN, and it deposits in multiple locations of glomeruli.

⇒ Suspected LMN with EXT1 positivity would be better recognised as a potential autoimmune disease due to its similarities with confirmed LMN.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Highlighting the importance of EXT1 staining in confirmed and suspected LMN would help elucidate the pathogenesis of EXT1 and maintain close follow-up to suspected LMN with EXT1 positivity.

INTRODUCTION

Membrane nephropathy is one of the most common kidney diseases of adult nephrotic syndrome.[1] About 10–20% of patients with lupus nephropathy (LN) exhibit pure class V lupus, also known as lupus membranous nephropathy (LMN), which usually manifests a large amount of proteinuria.[2] Compared with other classes of LN, LMN is more insidious with a slow onset; furthermore, its renal function is relatively stable and the prognosis is relatively good. The 10-year renal survival rate is 72–98%; however, it would be significantly lower when accompanied by proliferative lesions (classified as III/IV LN).[3 4] In clinical practice, suspected LMN from atypical MN often presents with mild haematuria and/or proteinuria, and atypical abnormalities in serum autoantibodies, such as being ANA positive but lacking specific antibody markers like Smith (SM) antibody and double-stranded DNA (dsDNA) antibody.[5] A recent report showed multiple pathological findings strongly suggest the diagnosis of LN, including: (1) 'full-house' immunofluorescence (IF) staining for IgG, IgM, IgA, C3 and C1q; (2) high intensity of C1q; (3) extraglomerular immune deposits; (4) combined subendothelial

and subepithelial deposits; and (5) the presence of endothelial tubuloreticular inclusions.[6] Renal prognosis for patients exhibiting pathological features of lupus membranous, but without a clinical diagnosis of systemic lupus (termed lupus-like membranous glomerulonephritis), is reported to fall between that of LMN and idiopathic membranous glomerulonephritis or may even be worse than both.[7 8] Therefore, new biological markers are sought to indicate the antigen of LMN and further predict the prognosis of LMN.

Since exostosin 1 (EXT1) and EXT2 have shown similar expression in LMN,[9–11] our study aimed at detecting EXT1 expression and exploring its clinical significance through comparing EXT1-positive and negative groups in both confirmed and suspected LMN.

MATERIALS AND METHODS

Patients

Six hundred and thirty-five patients from February 2015 to February 2021 with kidney biopsies-confirmed phospholipase A2 receptor (PLA2R)-negative MN were selected at Hangzhou TCM Hospital Affiliated to Zhejiang Chinese Medical University. Thirty patients of confirmed LMN (including 14 pure class V, 8 class V+III, and 8 class V+IV LN) and 37 patients of suspected LMN were screened. Criteria for confirmed LMN were as follows: (1) 2019 American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) criteria for SLE[12]; (2) 2018 International Society of Nephrology (ISN)/Renal Pathology Society (RPS) criteria for LN.[13] Criteria for suspected LMN were as follows: (1) ANA was positive but specific lupus antibodies (SM and dsDNA antibodies) were negative; (2) atypical MN with at least two of the specific pathological features mentioned above and negative PLA2R[6 14]; (3) not meeting the 2019 ACR/EULAR criteria for SLE. Patients who had received immunosuppressive therapy were excluded. Primary MN with PLA2R positivity was selected as the negative control for EXT1 detection.

Immunohistochemical, IF double-staining and immunoelectron microscopy detection of EXT1

Paraffin-embedded renal tissues were dewaxed and dehydrated, followed by antigen repair using both the citric acid and gastric enzyme methods. Rabbit anti-human EXT1 antibody (ThermoFisher Scientific, PA5-106907, 1:100 dilution) and rabbit anti-human C4d antibody (Sigma, 404A-1, 1:100) were incubated overnight at 4°C. Subsequently, the samples were incubated with horseradish peroxidase-conjugated mouse anti-rabbit antibody (Sigma, MAB201P, 1:500) at 37°C for 30 min. All cases were observed and scored by two renal pathologists. The intensity of EXT1 was scored from 0 to 3 (negative: score 0; mild and diffuse positive: score 1; moderate positive: score 2; strong positive: score 3). The positive samples were repeated by IF double-staining of EXT1 (red) and Collagen IVα5 (green) with Alexa Fluor 594-conjugated donkey anti-rat IgG antibody (1:100 dilution; Life Technologies, Carlsbad, California, USA) and fluorescein isothiocyanate (FITC)-conjugated polyclonal rabbit anti-human IgA antibody (1:50 dilution; Dako) as second antibodies, and detected under IF microscope (OPLENIC, SCOPE 53). Some positive samples were detected by immunoelectron microscopy (IEM) of EXT1 with immunogold labelling antibody (1:40 dilution; Aurion, Wageningen, the Netherlands). Negative control of IF and IEM was set by PLA2R antibody as the primary antibody. C4d detection by immunohistochemistry was performed in the same manner as EXT1.

IF detection of other markers

FITC-conjugated IgG subclasses: IgG_1, IgG_2, IgG_3 and IgG_4 (all from Southern Biotech, 1:100), along with IgA, IgG, IgM, C3 and C1q (Dako, 1:50), were incubated at 4°C overnight and detected by a BX53 fluorescence microscope (Olympus Corporation, Tokyo, Japan).

Clinical and pathological characteristics of LMN

The clinical data were collected, including age, sex and mean arterial pressure (MAP). Clinical manifestations included erythema, joint pain, multiple serous cavity effusions, etc. Laboratory parameters encompassed serum C3/C4, haemoglobin, and blood counts of white cell count, red cell count and platelets. Serum autoantibodies included ANA, dsDNA, anti-SM, anti-Sjögren syndrome A and anti-Sjögren syndrome B antibodies. Other parameters were haematuria, 24-hour proteinuria, serum albumin level, serum creatinine (SCR) level and estimated glomerular filtration rate (eGFR, calculated by the Chronic Kidney Disease Epidemiology Collaboration formula) level. All cases with renal biopsy underwent light microscopy, IF and electron microscopy detection. Histological parameters included glomerulosclerosis, segmental glomerular sclerosis, crescents, endothelial cell proliferation, activity index (AI), chronicity index (CI) and interstitial fibrosis and tubular atrophy (IFTA), as well as the location of electron dense deposits. 'Full house' was defined as positive expression of all immunoglobulins and complements.[15] The histological classification of lupus nephritis, as well as AI and CI, was based on the ISN/RPS 2018 Classification Standard. The AI and CI scores were evaluated in both confirmed and suspected LN groups to present active and chronic features with quantitative data. IFTA were scored as follows: <25% of IFTA in the entire cortical area (score of 1); 25–50% (score of 2); >50% (score of 3).[13 16]

Treatments and outcomes

Treatments were categorised into: (1) immunosuppressive therapy, which included 25 cases of prednisone+cyclophosphamide, 8 cases of prednisone+mycophenolate

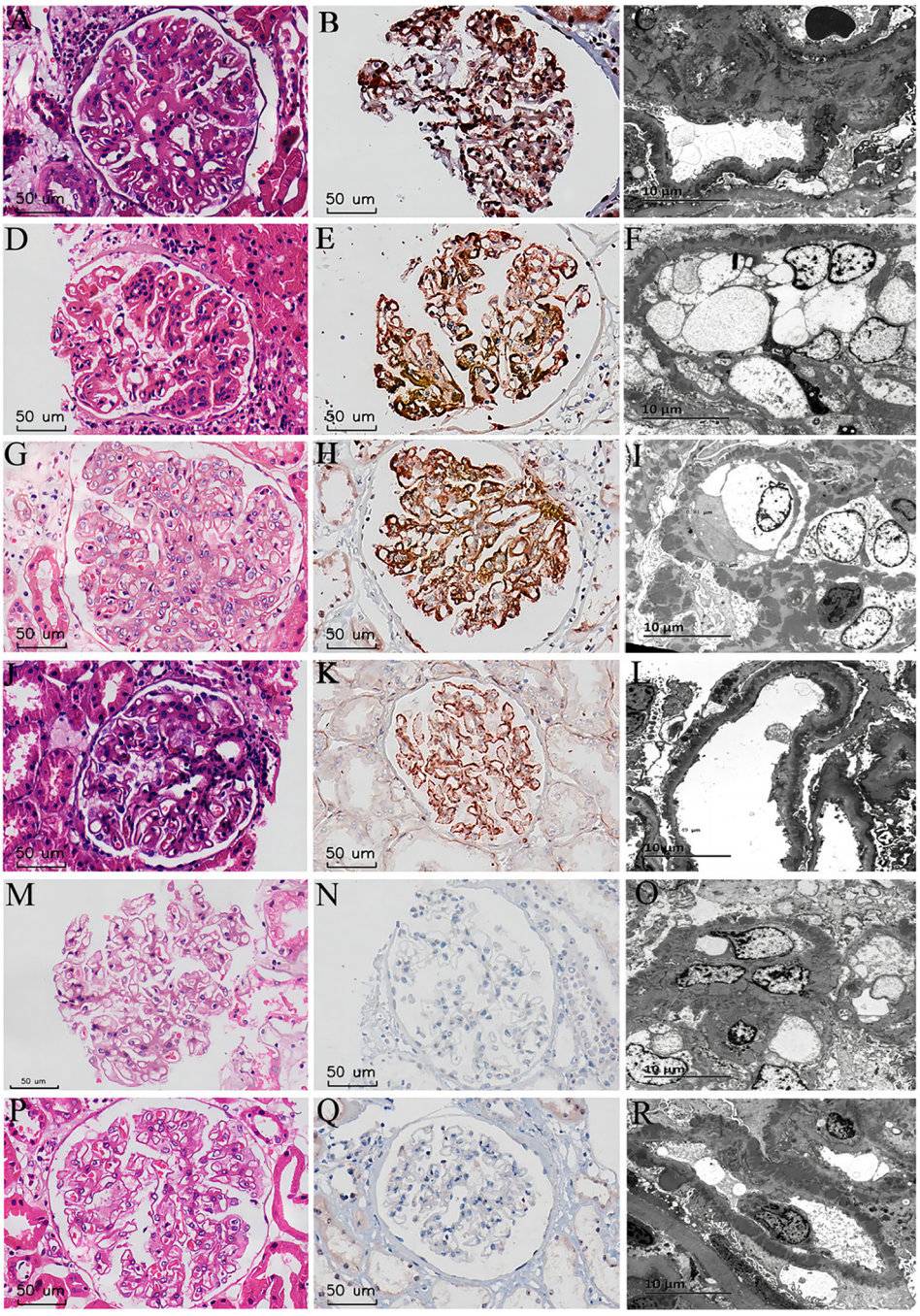

Figure 1 Light microscopy, immunohistochemistry (IHC) and electron microscopy (EM) of EXT1-positive, EXT1-negative LMN and PLA2R-positive MN. (A–C) Pure class V LN; (D–F) class V+III LN; (G–I) class V+IV LN; (J–L) suspected LMN with positive EXT1; (M–O) suspected LMN with negative EXT1; (P–R) PLA2R-positive MN. (D and G) Endothelial cell proliferation (H&E, 400×). (B, E, H and K) Positive EXT1 expression along the GBM and mesangial area (IHC, 400×). (N and Q) Negative EXT1 expression (IHC, 400×). (C, F, I and O) Multiloci deposits and (L and R) merely subepithelial deposits (EM, 2500×). EXT1, exostosin 1; GBM, glomerular basement membrane; LMN, lupus membranous nephropathy; LN, lupus nephropathy; MN, membranous nephropathy; PLA2R, phospholipase A2 receptor.

mofetil and 10 cases of prednisone+hydroxychloroquine; (2) non-immunosuppressive therapy, which included 12 cases treated with ACE inhibitors+angiotensin receptor blocker and 4 cases treated with traditional Chinese medicine; (3) dialysis with 4 cases.

The categories for treatment response were: (1) complete remission (CR) characterised by urinary protein levels of <0.3 g/day, accompanied by normal serum albumin and creatinine; (2) partial remission marked by urinary protein levels of <3.5 g/day (which is reduced by ≥50%), serum albumin levels of >30 g/L and stable SCR; (3) no remission denoted by urinary protein levels of >3.5 g/day, no reduction or a reduction of <50%, and an eGFR decrease of <30%[17]; (4) end-stage kidney disease (ESKD) indicated by SCR levels of >442 μmol/L or eGFR levels of <15 mL/min/1.73 m^2.[18]

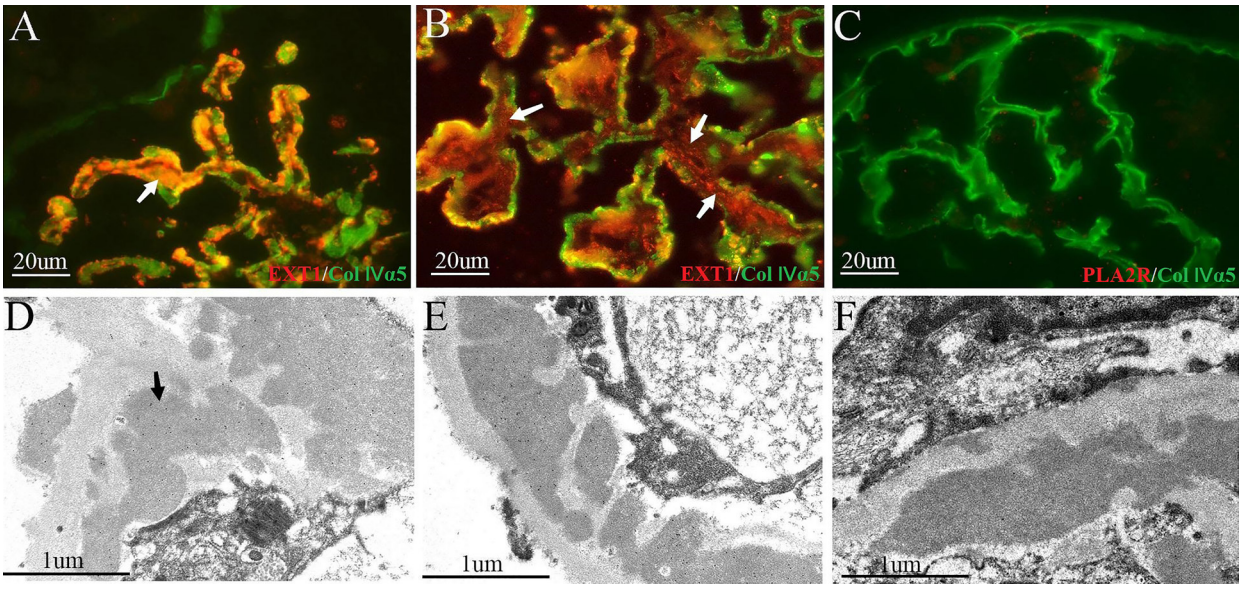

Figure 2 The EXT1-positive sites detected by immunofluorescence (IF) double-stained with Collagen IVα5 and immunoelectron microscopy (IEM) in both pure and mixed V LN. (A, B) IF revealed EXT1-positive staining (red) on multilocations in pure V LN (A) and mixed V LN (B), including mesangial area (arrow) (IF, 1000×). (C) Negative control of IF with PLA2R as primary antibody (IF, 1000×). No obvious positive staining (red) of PLA2R was shown on multilocations. (D,E) IEM showed EXT1-positive sites by immunogold labelling on multilocations, including mesangial area (arrow) (IEM, 30 000×). (F) Negative control of IEM with PLA2R as primary antibody (IEM, 30 000×). No obvious immunogold labelling was shown in deposits on multilocations. EXT1, exostosin 1; LN, lupus nephropathy; PLA2R, phospholipase A2 receptor.

Statistical analysis

Statistical analysis was conducted using SPSS V.20.0 (IBM). Data with a normal distribution were expressed as mean±SD. For non-normal distribution variables, data were expressed as median and interquartile intervals. Clinical and pathological characteristics among groups were assessed using t-tests or analysis of variance for continuous variables and non-parametric tests for discontinuous variables. Categorical variables were expressed as percentages, and comparisons between groups were evaluated using the X^2 test or Fisher's exact test. A p value less than 0.05 (two sided) was considered statistically significant. Kaplan-Meier (KM) analysis was used to plot curves of cumulative CR rate. Log-rank tests were used to calculate differences between EXT1-positive and EXT1-negative groups. Cox proportional hazards regression models determined predictive factors for CR to treatment after adjustment for other factors such as eGFR and numbers of LN flare.[19]

RESULTS

EXT1 expression in confirmed LMN and suspected LMN

Immunohistochemical staining of EXT1 showed diffuse granular expression along the capillary wall of glomerular basement membrane (GBM) in both confirmed and suspected LMN cases, while PLA2R-positive MN was negative. Moreover, granular EXT1 expression was observed in the mesangium area aside from the GBM. The EXT1 expression and histological features of confirmed and suspected LMN were shown in figure 1. The further detection of EXT1 deposit sites revealed EXT1-positive staining on multilocation by both double-staining method and IEM (figure 2).

Sixty-seven cases of PLA2R-negative membranous nephropathy with serum ANA abnormality were screened, including 30 confirmed LMN (14 cases of pure class V LN and 16 cases of mixed class V LN) and 37 suspected LMN. In the confirmed group, 22 (73.3%) were EXT1 positive, while in the suspected group, only 6 (16.2%) were EXT1 positive, showing a significant difference (p<0.001). Furthermore, the EXT1 positive rate was significantly higher in the mixed class V LN than in the pure class V LN (68.2% vs 31.8%, p=0.007) (figure 3).

The statistical analysis of EXT1-positive sites and intensity was shown in figure 4. Pure V LN displayed the highest positive rate of EXT1 in the mesangial area compared with the other groups, and it was statistically higher than suspected LMN (p=0.02). Suspected LMN exhibited a higher EXT1 positive rate along GBM than both pure V LN (p=0.007) and mixed V LN (p=0.001). Meanwhile, mixed V LN had a higher positive rate involving areas of both GBM and mesangium than pure V LN (p=0.006) and suspected LMN (p=0.002). The intensity of EXT1 expression was the highest along the GBM in mixed V LN, though not significantly (p=0.286). In pure V LN, the intensity was the highest in the mesangial area and was significantly higher than in suspected LMN (p=0.013) (figure 4). As shown above, higher frequency of EXT1 expression was detected in confirmed LMN than suspected LMN, and the EXT1 expression pattern was not limited on GBM but also in mesangial area.

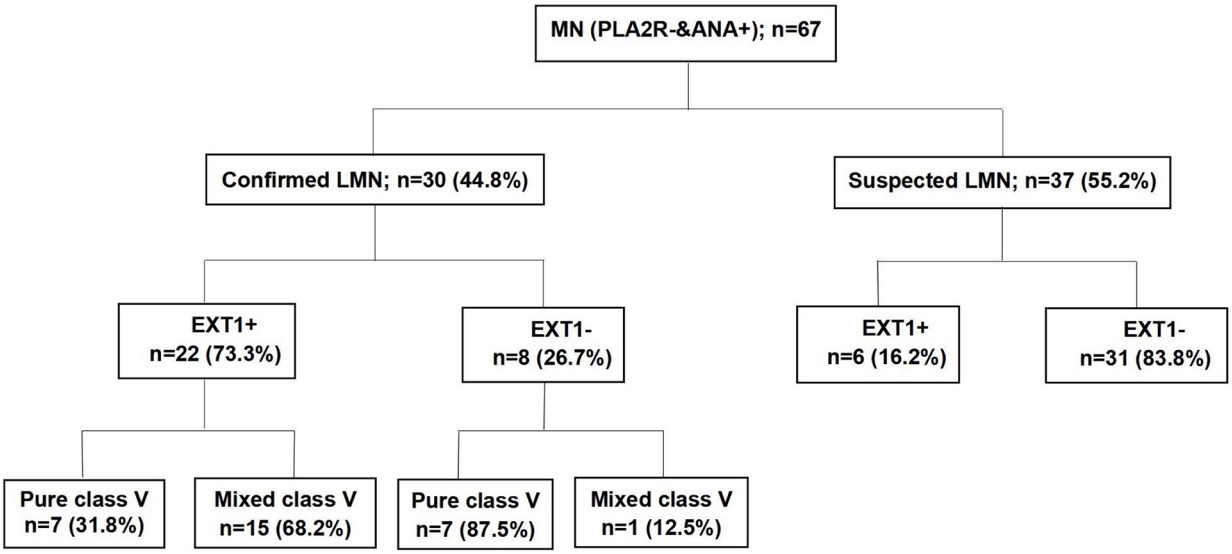

Figure 3 Study cohort of membrane nephropathy with PLA2R-negative and ANA-positive membranous nephropathy (MN). MN was categorised into confirmed LMN and suspected LMN and they were further subcategorised according to EXT1 expression. EXT1, exostosin 1; LMN, lupus membranous nephropathy; PLA2R, phospholipase A2 receptor.

Comparison of clinicopathological characteristics of EXT1-positive and EXT1-negative patients in confirmed LMN and suspected LMN groups

Cases of confirmed LMN and suspected LMN were subdivided into the EXT1-positive and EXT1-negative groups. Clinical characteristics were compared, as shown in table 1 and figure 3. In the confirmed LMN group, EXT1-positive patients had a higher level of MAP (p=0.040), serum C3/C4 (p=0.024), more urinary red cell counts (p=0.031), higher frequency of proteinuria ≥3.5 g/day (p=0.032) and shorter duration of lupus (p=0.004) and nephropathy (p=0.027). They also had a lower platelet count (p=0.020) and eGFR (p=0.029) than EXT1-negative patients. In the suspected LMN group, EXT1-positive patients exhibited some similar differences compared with EXT1-negative patients as observed in the confirmed LMN, though not statistically significant. However, they were younger (p=0.028) and had fewer platelets (p=0.028) and white cell counts (p=0.023) than EXT1-negative patients. Furthermore, when comparing EXT1-positive patients in the two different groups, those in the confirmed LMN group had a higher MAP (p=0.012), SCR level (p=0.024), a lower eGFR (p=0.020) and higher frequency of proteinuria ≥3.5 g/day (p=0.007) than the suspected LMN group.

The pathological characteristics of the two groups were shown in table 2. Within the confirmed LMN group, there were more crescents (p=0.024) and endothelial cell proliferation (p=0.010), and a higher AI score (p=0.013) in EXT1-positive patients compared with EXT1-negative patients. In the suspected LMN group, EXT1-positive patients exhibited more C4d (p=0.027) and IgG_2 (p=0.020) positivity. Additionally, when comparing

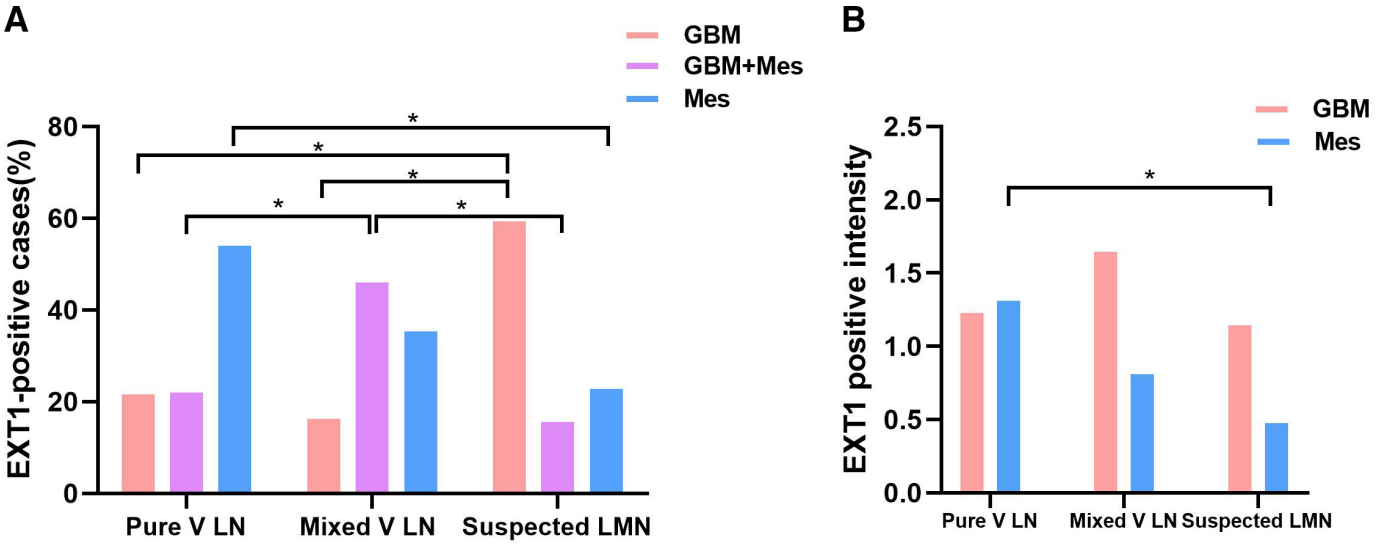

Figure 4 Comparison of deposition sites (A) and intensity of EXT1 (B) in different groups. *p<0.05. EXT1, exostosin 1; GBM, glomerular basement membrane; LMN, lupus membranous nephropathy; LN, lupus nephropathy; Mes, mesangium.

Table 1 Comparison of clinical data between EXT1-positive and EXT1-negative cases in confirmed and suspected LMN

	Confirmed LMN		Suspected LMN	
	EXT1 positive (n=22)	**EXT1 negative (n=8)**	**EXT1 positive (n=6)**	**EXT1 negative (n=31)**
Male, n (%)	3.0 (13.6)	0.0 (0.0)	0.0 (0.0)	9.0 (29.0)
Age (year)	33.6±14.5	33.0±12.0	**40.8±18.1***	**49.8±12.3**
Duration of lupus (month)	**1.0 (0.5–3.3)***	**60.0 (7.5–96.0)**	—	—
MAP (mm Hg)	**103.5 (94.5–112.8)*†**	**88.5 (81.8–104.3)**	**94.0 (78.0–101.0)**	92.5 (84.3–107.0)
Erythema, n (%)	15 (68.2)	5 (62.5)	0 (0.0)	0 (0.0)
Joint pain, n (%)	13 (59.0)	5 (62.5)	0 (0.0)	5 (16.1)
Multiple serous cavity effusions, n (%)	6 (27.2)	4 (50.0)	0 (0.0)	6 (19.3)
Serum C3/C4 (mg/dL)	**6.7 (4.6–9.0)***	**4.7 (4.0–5.2)**	5.8 (4.8–7.4)	4.7 (3.9–6.1)
Hb (g/L)	111.0 (85.0–126.5)	126.0 (120.0–126.0)	123.0±12.2	118.5±15.8
RBC ($\times10^{12}$/L)	3.7±0.7	4.0±0.1	4.1±0.4	4.0±0.6
WBC ($\times10^{9}$/L)	6.1±2.7	6.1±2.2	**3.7±1.0***	**5.7±1.8**
PLT ($\times10^{9}$/L)	**188.2±66.5***	**213.4±41.3**	**171.6±89.6***	**217.8±62.8**
ANA	1:160 (1:200–1:80)	1:160 (1:200–1:64)	1:80 (1:80–1:40)	1:40 (1:80–1:20)
dsDNA, n (%)	10 (45.5)	1 (12.5)	0 (0.0)	2 (6.5)
Anti-SM, n (%)	9 (40.9)	3 (37.5)	0 (0.0)	0 (0.0)
Anti-SSA, n (%)	13 (59.1)	3 (37.5)	5 (83.3)	16 (51.6)
Anti-SSB, n (%)	8 (36.4)	1 (12.5)	1 (16.7)	2 (6.5)
Duration of nephropathy (month)	**12.0 (1.8–42.0)***	**65.0 (12.0–115.0)**	2.5 (0.8–7.5)	3.0 (1.0–24)
Haematuria (/HP)	**15.0 (6.5–41.5)***	**6.0 (2.0–19.7)**	7.0 (7.0–19.0)	6.0 (3.0–11.5)
Proteinuria (g/24 hours)	2.5 (1.2–5.2)	1.2 (0.7–4.3)	1.4 (0.7–4.0)	2.2 (1.2–3.6)
Proteinuria ≥3.5/day, n (%)	**18 (81.8)*†**	**3 (37.5)**	**1 (16.7)**	8 (25.8)
Albumin (g/L)	24.6±8.1	26.1±8.0	29.9±7.2	25.6±6.7
SCR (μmol/L)	**68.1 (49.5–100.7)†**	55.0 (43.7–68.3)	**54.0 (42.5–61.3)**	54.7 (49.4–70.5)
eGFR (mL/min/1.73 m^2)	**83.1±35.8*†**	**108.8±31.4**	**104.3±12.7**	91.5±37.0

Data were presented as n (%) or medians (IQR). Data with significant differences are highlighted in bold.
*EXT1-positive cases compared with EXT1-negative cases; p<0.05.
†EXT1-positive cases in confirmed LMN compared with EXT1-positive cases in suspected LMN; p<0.05.
anti-SSA, anti-Sjögren syndrome A antibody; anti-SSB, anti-Sjögren syndrome B antibody; dsDNA, double-stranded DNA; eGFR, estimated glomerular filtration rate; EXT1, exostosin 1; Hb, haemoglobin; LMN, lupus membranous nephropathy; MAP, mean arterial pressure; PLT, platelets; RBC, red blood cell; SCR, serum creatinine; SM, Smith antibody; WBC, white blood cell.

EXT1-positive patients in confirmed and suspected LMN groups, those in the confirmed LMN group had more crescents (p=0.018) and endothelial cell proliferation (p=0.021), and a higher AI score (p=0.006) than EXT1-positive patients in the suspected LMN group (table 2). In brief, EXT1-positive LMN had significantly more active features (crescents, endothelial cell proliferation and AI score) than EXT1-negative LMN.

Clinical follow-up of EXT1-positive and EXT1-negative patients in confirmed LMN and suspected LMN groups

The treatment parameter indicated that all but one of the EXT1-positive cases in both confirmed LMN and suspected LMN received immunosuppressive treatment, showing no statistical difference. However, more patients in the EXT1-negative group underwent dialysis and developed ESKD compared with the EXT1-positive group in confirmed LMN (p=0.026). When comparing treatment responses, both confirmed and suspected LMN had a higher CR rate in the EXT1-positive group than in the EXT1-negative group (77.3% vs 50.0% in confirmed LMN and 50.0% vs 9.6% in suspected LMN) (p<0.05). The suspected LMN had a lower non-remission rate in the EXT1-positive group compared with the EXT1-negative group (p=0.016). None of the patients with EXT1 positivity developed ESKD, while 3 of 8 (37.5%) and 1 of 31 (3.2%) of EXT1-negative cases in both confirmed and suspected LMN developed ESKD, respectively (table 3). Furthermore, when comparing treatment outcomes of EXT1-positive cases in confirmed LMN with those in suspected LMN, no significant differences were found

Table 2 Comparison of pathological data between EXT1-positive and EXT1-negative cases in confirmed and suspected LMN

	Confirmed LMN		Suspected LMN	
	EXT1 positive (n=22)	**EXT1 negative (n=8)**	**EXT1 positive (n=6)**	**EXT1 negative (n=31)**
IgG_1, n (%)	22 (100.0)	8 (100.0)	6 (100.0)	27 (87.1)
IgG_2, n (%)	19 (86.4)	5 (62.5)	**6 (100.0)***	**13 (41.9)**
IgG_3, n (%)	15 (68.2)	4 (50.0)	1 (16.7)	10 (32.3)
IgG_4, n (%)	15 (68.2)	3 (37.5)	6 (100.0)	18 (58.1)
C3 positivity, n (%)	22 (100.0)	8 (100.0)	6 (100.0)	27 (87.1)
C4d positivity, n (%)	18 (81.8)	4 (50.0)	**6 (100.0)***	**15 (48.4)**
C1q positivity, n (%)	22 (100.0)	7 (87.5)	6 (100.0)	17 (54.8)
'Full-house', n (%)	6 (27.3)	1 (12.5)	4 (66.7)	20 (64.5)
GS/SS, n (%)	11 (50.0)	5 (62.5)	2 (33.3)	21 (67.7)
Crescents, n (%)	**13 (59.1)*†**	**1 (12.5)**	1 (16.7)	2 (6.5)
Endothelial cell proliferation, n (%)	**19 (86.4)*†**	**2 (25.0)**	2 (33.3)	9 (29.0)
AI	**2.5 (2.0–4.0)*†**	**1.0 (1.0–1.0)**	0.5 (0.0–1.0)	0.0 (0.0–1.0)
CI	2.0 (2.0–4.0)	2.0 (0.3–2.0)	0.5 (0.0–2.0)	2.0 (1.0–2.0)
IFTA	1.0 (1.0–1.0)	1.0 (0.3–1.0)	0.0 (0.0–1.0)	1.0 (0.0–1.0)
Multiloci deposits, n (%)	11 (50.0)	1 (12.5)	3 (50.0)	16 (51.6)

Data were presented as n (%) or medians (IQR). Data with significant differences are highlighted in bold.
*EXT1-positive cases compared with EXT1-negative cases; p<0.05.
†EXT1-positive cases in confirmed LMN compared with EXT1-positive cases in suspected LMN; p<0.05.
AI, activity index; CI, chronicity index; EXT1, exostosin 1; GS, glomerulosclerosis; IFTA, interstitial fibrosis and tubular atrophy; LMN, lupus membranous nephropathy; SS, segmental glomerular sclerosis.

(p>0.05). KM survival analysis revealed a significantly higher renal CR accumulation rate in the EXT1-positive group compared with the EXT1-negative group for the entire study cohort (p=0.028) (figure 5). Moreover, when subdividing into confirmed and suspected LMN groups, there was no significant difference in renal CR between EXT1-positive and EXT1-negative cases (p>0.05). In general, the follow-up data revealed that EXT1-positive patients had better outcomes.

The Cox regression analysis of predictive factors for CR to treatment

The Cox regression analysis of predictive factors for CR to treatment was summarised in table 4 (only data

Table 3 Treatment response and outcomes in the EXT1-positive and EXT1-negative cases in confirmed and suspected LMN

	Confirmed LMN		Suspected LMN	
	EXT1 positive (n=22)	**EXT1 negative (n=8)**	**EXT1 positive (n=6)**	**EXT1 negative (n=31)**
Immunosuppression, n (%)	21 (95.5)	5 (62.5)	5 (83.3)	12 (38.7)
Non-immunosuppression, n (%)	1 (4.5)	0 (0.0)	1 (16.7)	14 (45.2)
Dialysis, n (%)	**0 (0.0)***	**3 (37.5)**	0 (0.0)	1 (3.2)
Follow-up time, month	48 (24–120)	97.5 (17.3–174)	12 (10–15)	24 (8–60)
Complete remission, n (%)	**17 (77.3)***	**4 (50.0)**	**3 (50.0)***	**3 (9.6)**
Partial remission, n (%)	3 (13.6)	2 (25.0)	2 (33.3)	9 (29.0)
No remission, n (%)	2 (9.1)	2 (25.0)	**1 (16.6)***	**19 (61.3)**
ESKD, n (%)	**0 (0.0)***	**3 (37.5)**	0 (0.0)	1 (3.2)

Data were presented as n (%) or medians (IQR). Data with significant differences are highlighted in bold.
Immunosuppression: prednisone+cyclophosphamide/mycophenolate mofetil/hydroxychloroquine; non-immunosuppression: ACE inhibitors+angiotensin receptor blocker or traditional Chinese medicine.
*EXT1-positive cases compared with EXT1-negative cases; p<0.05.
ESKD, end-stage kidney disease; EXT1, exostosin 1; LMN, lupus membranous nephropathy.

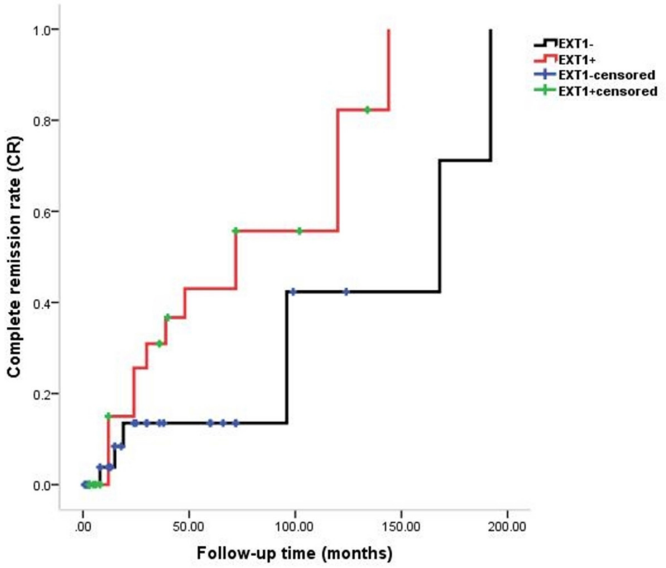

Figure 5 Comparison of accumulation rates of complete remission (CR) between EXT1-positive and EXT1-negative group in the whole study cohort. EXT, exostosin 1.

with p≤0.2 in the univariate analysis were listed). Using univariate Cox regression, EXT1 positivity was found to be associated with CR (HR 2.895; 95% CI 1.035 to 8.101; p=0.043) after adjustment for other factors such as eGFR and numbers of LN flare; while using multivariable Cox regression, EXT1 positivity was found to be the only independent factor of CR (HR 5.647; 95% CI 1.323 to 12.048; p=0.019) (table 4).

DISCUSSION

In 2019, Sethi *et al* reported that only LMN exhibited positive EXT1/2 expression in renal tissue, and patients testing positive for EXT1/2 appeared to have a better prognosis.[9 10] However, studies on the expression of EXT1/2 and their clinical significance in both confirmed and suspected LMN are limited. Recent research suggests that EXT1/EXT2-positive MN may help identify LMN, and no individual case demonstrated positivity for just a single EXT marker.[20] Furthermore, EXT1 exhibited a stronger positive intensity in glomeruli compared with EXT2.[21] Therefore, we chose EXT1 as the representative marker for detection.

According to the study reported in 2021, EXT1/2 staining was observed in autoimmune diseases, such as membranous lupus nephritis and mixed connective tissue disease. In positive cases, EXT1/2 displayed granular deposition along the GBM.[21] However, we observed that mixed class V LN exhibited a higher rate of EXT1-positive cases compared with pure class V LN. Moreover, EXT1 showed a higher deposition rate in the mesangium area than in the GBM for pure V LMN. We believe the results were not falsely positive, as none of the PLA2R-positive MN exhibited mesangium EXT1 deposition, and EXT1-positive area was verified by IF double-staining and IEM. EXTs, similar to other glycosyltransferases, are secreted in a truncated form into the extracellular medium, including the GBM.[22 23] The mesangial area is rich in extracellular matrix components, like collagen and fibronectin, which might retain chemicals such as EXTs.[24 25] EXT1/EXT2, in conjunction with heparan sulfates, may coat immune complexes, preventing these deposits from initiating an inflammatory response.[26 27] It remains unclear whether the mesangial deposition of EXT1 plays a pathogenetic role, acts as a standby mechanism or accelerates the healing process. Overall, the impact of the deposition region of EXT1 on LN biological behaviour warrants further investigation.

Choung *et al* reported that some cases with lupus-like features were positive for EXT1.[28] They speculated that lupus-like MN might be significantly associated with an underlying autoimmune disease. In our study, we observed similar clinicopathological differences, particularly in renal outcomes, between EXT1-positive and EXT1-negative cases in both confirmed and suspected LMN. During follow-up, one patient with positive EXT1 in suspected LMN developed SLE, whereas no patient with negative EXT1 met the diagnostic criteria for SLE. We speculate that EXT1-positive cases might have

Table 4 The Cox regression analysis of predictive factors for complete remission to treatment

	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
EXT1	**2.895**	1.035 to 8.101	**0.043**	**5.647**	1.323 to 12.048	**0.019**
C3	0.990	0.975 to 1.005	0.191	0.994	0.970 to 1.019	0.640
MAP	1.034	0.995 to 1.073	0.087	1.037	0.988 to 1.088	0.143
Anti-SSA	3.074	1.003 to 9.417	0.051	2.525	0.623 to 10.227	0.149
Anti-SSB	3.344	1.502 to 7.447	0.087	1.577	0.517 to 4.810	0.423
WBC	0.817	0.655 to 1.019	0.073	0.853	0.703 to 1.035	0.108
AI	1.288	0.890 to 1.865	0.179	0.500	0.252 to 0.992	0.055

P<0.05 is considered significant. Data with significant differences are highlighted in bold.
AI, activity index; anti-SSA, anti-Sjögren syndrome A antibody; anti-SSB, anti-Sjögren syndrome B antibody; EXT1, exostosin 1; MAP, mean arterial pressure; WBC, white blood cell.

a pathogenesis similar to LN, which is consistent with Choung *et al*, even if they initially presented atypically. However, EXT1-positive patients in confirmed LMN demonstrated more severe renal damage, higher SCR, lower eGFR levels and more active lesions like increased crescent frequency, endothelial cell proliferation and higher AI scores compared with suspected LMN. The mechanism remains unclear, but we hypothesise it might relate to the intensity and deposition area of EXT1 to a certain extent, since confirmed LMN exhibited less EXT1 expression in the GBM and a lower frequency of multisite positivity compared with suspected LMN.

Ravindran A *et al* reported that the EXT1/2-positive patients presented more frequently with proteinuria ≥3.5 g/day.[21] Our study showed similar result in EXT1-positive LMN, although the total 24-hour proteinuria had no significant difference. At the same time, our study showed that EXT1-positive patients in the confirmed LMN group had shorter duration of lupus and nephropathy than EXT1-negative patients. This result suggests that LN with EXT1 deposits probably lead to an earlier onset of proteinuria and diagnosis of LN, and EXT1 deposits may act as a cofactor for renal disease manifestations detected during screening patients with SLE for LN. Furthermore, compared with the EXT1-negative group, the EXT1-positive group was reported to have slower chronic kidney progression and a better prognosis.[20 21] Saidi *et al* found that EXT-negative patients had significantly more repeat biopsies with proliferative class 3 or 4 lupus nephritis since the diagnosis of SLE-MN. Multivariable analysis suggested that the EXT status independently predicted clinical remission at the end of follow-up, which was similar to our results.[29] It is worth noting that, despite EXT1-positive patients in the confirmed LMN group displaying more active and severe characteristics, the renal outcome was notably better after immunosuppressive therapy compared with the EXT1-negative patients. We speculate that active lesions might be more responsive to immunosuppressive therapy, leading to improved outcomes. EXTs are present in the podocyte Golgi apparatus, where they are responsible for the glycosylation of heparan sulfates that are eventually transported to the GBM. Heparan sulfates can act as clearance receptors for aberrant extracellular proteins, thus facilitating the removal of immune complexes and proteins and associating with a favourable prognosis clinically.[30] In our study, we found EXT1-positive patients had a higher CR rate in the whole study cohort than EXT1-negative patients, consistent with a previous study.[20] However, there was no significant difference when subdividing cases into confirmed and suspected LMN groups, possibly due to the limited number of cases. The suspected LMN group was clinically heterogeneous, as we observed 2 cases combined with colon adenoma, 1 with liver cirrhosis and 11 that developed other systemic autoimmune diseases like antiphospholipid antibody syndrome, mixed connective tissue disease, rheumatoid arthritis, Sjögren syndrome and autoimmune hepatitis during follow-up. This diversity might explain the low EXT1 positivity rate and unclear aetiology. Yet, EXT1-positive cases might have some innate similarities, especially in renal outcomes across both groups. Therefore, it is noteworthy that suspected LMN with positive EXT1 should be considered as a potential autoimmune disease, as it might later develop into LN.

The limitation of this study is that it was a single-centre, retrospective study with a limited number of renal biopsy samples, which causes bias in comparing clinicopathological data between subgroups and adds some difficulty in the interpretation of some data. Further research would benefit from multicentre collaboration and a larger database.

In conclusion, this study highlighted the importance of EXT1 staining in LMN, particularly emphasising the significance of EXT1 positivity in suspected LMN. Future studies should explore the pathogenic role and potential mechanisms of deposition area of EXT in LMN.

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Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

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