Glomerular Exostosin-Positivity is Associated With Disease Activity and Outcomes in Patients With Membranous Lupus Nephritis

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Introduction: The relationship of exostosin 1 and exostosin 2 (EXT1/EXT2) expression and outcomes in membranous lupus nephritis (MLN) was controversial.

Methods: EXT1/EXT2 was performed by immunohistochemistry (IHC) in 283 consecutive patients with MLN. Clinicopathological characteristics and outcomes of EXT1/EXT2-positive patients were compared with EXT1/EXT2-negative patients. The primary end points were adverse renal events, including death, dialysis, and renal transplantation.

Results: Of the patients with MLN, 29.3% were positive for EXT1/EXT2. The prevalence of EXT1/2-positive MLN was significantly higher in pure class V MLN than those for mixed class V MLN (44.2% vs. 19.4%, $P <$ 0.001). For EXT1/EXT2-positive patients, the median time between onset of lupus and renal biopsy, and lupus nephritis and renal biopsy is shorter (6 [interquartile range, IQR: 2–25] months vs. 12 [IQR: 3–49] months, $P = 0.008$ and 3 [IQR: 2–18] months vs. 6 [IQR: 2–23] months, $P = 0.039$) and they had significantly lower systemic lupus erythematosus Disease Activity Index (SLEDAI) scores ($P = 0.015$) and lower serum creatinine levels ($P < 0.001$), higher hemoglobin ($P = 0.006$) as well as lower blood pressure. The EXT1/EXT2-positive patients had significantly fewer chronicity features (glomerulosclerosis, $P < 0.001$; interstitial fibrosis, $P = 0.006$; and tubular atrophy, $P = 0.002$) and fewer activity indicators (endocapillary hypercellularity, $P = 0.012$; cellular crescents, $P = 0.007$; and fibrocellular crescents, $P < 0.001$) on renal biopsy. After a median follow-up of 65 (28–126) months, EXT1/EXT2-positive patients were less likely to experience adverse renal events (2.4% vs. 16.0%, $P = 0.001$).

Conclusion: Compared with EXT1/EXT2-negative patients, the EXT1/EXT2-positive patients presented with lower disease activity and were less likely to experience adverse renal events in relationship with the chronicity index.

Membranous nephropathy (MN) with no underlying etiology are defined as primary or idiopathic MN, shown as elevated pathogenetic antigens such as phospholipase A2 receptor and thrombospondin type 1 domain-containing protein 7A expression on podocytes and antibodies of these antigens in the serum.[1-3] Secondary MN, are diagnosed when MN developed secondary to infections, medications, malignancies, and various autoimmune diseases such as systemic lupus erythematosus, etc.[4] More recently, a fraction of potential antigens such as EXT1/EXT2, neural cell adhesion molecule 1, and transforming growth factor beta receptor 3, have been identified as candidates in secondary MN with autoimmune diseases.[5-9] Notably, EXT1/EXT2 are expressed in about one-third of patients with MLN.[6] Thus, relationships between EXT1/EXT2 and MLN are raising the public's attention.

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EXT1/EXT2 were first proposed by Sethi et al.[5] as 2 putative antigens in MLN (including class V and class V+III/IV lupus nephritis), and EXT1/EXT2-positive MLN had lower scores of chronicity index in renal biopsy and lower rate of histological transition in rebiopsy.[6,10] However, the relationship of EXT1/EXT2 expression and outcomes in MLN was controversial. In a cohort of 160 patients with MLN from the USA, the patients who were EXT1/EXT2-negative evolved to end-stage renal diseases faster and more frequently compared with EXT1/EXT2-positive patients (18.8% vs. 3.1%; $P = 0.003$).[6] However, a Chinese cohort that enrolled 165 patients with class V MLN showed comparable time to kidney end point between EXT1/EXT2-negative and EXT1/EXT2-positive patients.[10]

In our study based on the Chinese population, we aim to validate the distribution of EXT1/EXT2 as well as to further elucidate and provide new insight into the correlations between EXT1/EXT2 expression and clinicopathological characteristics, and prognosis in a large consecutive series of patients with MLN.

METHODS

Study Design
Patients who fulfilled the diagnostic criteria for systemic lupus erythematosus that were revised in 1997 by the American College of Rheumatology and had first renal biopsy with a diagnosis of MLN in The Department of Nephropathy, The First Affiliated Hospital of Sun Yat-sen University between January 1, 2006 and June 1, 2022 were screened for this study.[11] Participants had to be at least 14 years old and provide written consent, whereas exclusion criteria were as follows: (i) end-stage renal disease (depend on renal replacement therapy) on renal biopsy, (ii) insufficient renal tissue for EXT1/2 detection, and (iii) loss to follow-up. Clinicopathological specimens of the patients were collected, followed by dividing them into 2 groups (including EXT1/EXT2-positive and EXT1/EXT2-negative groups), for further analyses and interpretation. The study protocol adhered strictly to the Declaration of Helsinki and received approval from the Ethics Committee at the First Affiliated Hospital of Sun Yat-sen University. Prior to participation, all patients were adequately informed and gave their consent.

Demographic and Laboratory Data
Baseline demographic and laboratory data were collected from time of renal biopsy. If multiple sets of data were available, the value was selected for each individual participant at a time point closer to renal biopsy. Clinical disease activity was assessed by the SLEDAI.[12] Leukocytopenia was defined as a blood leukocyte concentration less than 4×10^9/L.

Thrombocytopenia was defined as a platelet concentration less than 100×10^9/L. C3 and C4 hypocomplementemia were defined as serum C3 concentration less than 0.79 g/l and serum C4 concentration less than 0.17 g/l, respectively. Nephrotic syndrome was defined as levels of serum albumin less than 30 g/l and 24-hour proteinuria more than 3.5 g.

### Histopathological Data and IHC Staining
Renal biopsy specimens were examined using light microscopy, immunofluorescence microscopy, and electron microscopy by 2 independent pathologists and reported according to the ISN/RPS 2018 classification,[13] The 4-μm thick paraffin-embedded tissue sections of the kidney were further processed through IHC technology, using antibodies against EXT1 (Abcam, ab126305) and EXT2 (Arigo, ARG58606). Briefly, after dewaxing and rehydration, a microwave pretreatment in citrate buffer (pH 6.0) was performed to unmask antigens present in the renal tissue. Tissue sections were then incubated with rabbit anti-EXT1 (1:200) or rabbit anti-EXT2 (1:200) antibodies overnight at 4 °C. After rinsing in phosphate-buffered saline, tissue slides were exposed for 1 hour to the secondary antibody (Dako, K5007). The sections were later washed with phosphate-buffered saline, followed by incubation with diaminobenzidine for 1 minute. The microscopic examination was carried out by using a ZEISS Axio Imager Z2 light microscope system (Zeiss, Göttingen, Germany). The stain was judged to be positive if there was granular staining along the glomerular basement membrane (GBM) in the glomeruli, and negative if there was no staining along the GBM in glomeruli.

Follow-up and Outcomes
The primary end points were adverse renal events, including death and renal replacement therapy that lasts for more than 3 months. The deadline for follow-up was February 10, 2023.

Statistical Analysis
Normally-distributed quantitative data were expressed as mean $\pm$ SD and were compared by independent-sample t-test; nonnormally distributed data were expressed as the median (quartile) and were compared by the Wilcoxon rank-sum test. The qualitative data were expressed as frequency (percentage) and were compared by chi-square test or Wilcoxon rank-sum test. Kaplan-Meier curves of survival analysis were performed for renal event-free survival rate assessment. Log-rank test and Cox regression model were performed for comparison of the difference between groups and detection of the hazard ratios with a 95% confidence interval, respectively. The multivariate Cox
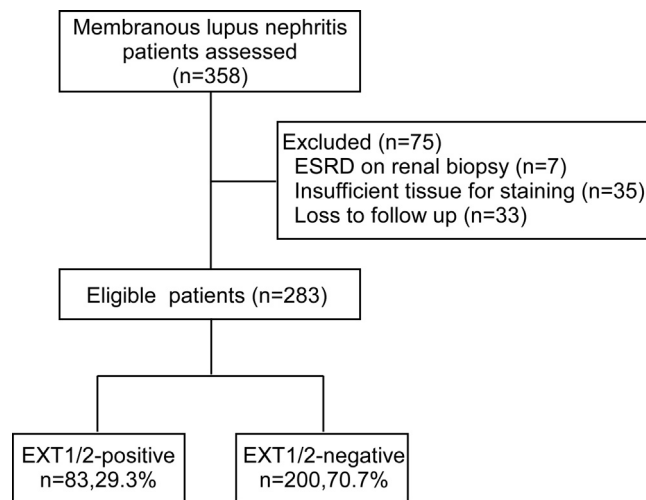
Figure 1. Flowchart of the study. ESRD, end-stage renal disease; EXT1/2, exostosin1/2.

regression model was constructed using eligible covariates that were important for clinical concern. IBM SPSS statistics version 20.0 software (SPSS Inc, Chicago, IL) was used for statistical analysis, and *P* values less than 0.05 in 2-sided tests were considered to be statistically significant.

RESULTS

Of the 358 patients with MLN, 283 were identified to fulfill the criteria and included in the study (Figure 1). Among the 283 patients with MLN, 83 (29.3%) patients had positive EXT1/2 staining along the GBM in the glomeruli and 200 (70.7%) had negative EXT1/2 staining. The prevalence of EXT1/2-positive MLN was significantly higher in pure class V MLN than those for mixed class V MLN (44.2% vs. 19.4%, *P* < 0.001). IHC showed granular staining for both EXT1 and EXT2 along the GBM in the positive cases and no granular staining for both EXT1 and EXT2 along the GBM in the negative cases; and a serum phospholipase A2 receptor antibody-positive primary MN patient as a negative control (Supplementary Figures S1–S3).

Comparison of Demographic, Clinical, and Laboratory Characteristics Between EXT1/EXT2-Positive and EXT1/EXT2-Negative MLN

Out of 283 patients with MLN, 85% of whom were female, the median age was 27 (IQR: 21–35) years in EXT1/EXT2-positive patients and 30 (IQR: 23–39) years in EXT1/EXT2-negative patients (*P* = 0.072) (Table 1). Compared with EXT1/EXT2-negative counterparts, the EXT1/EXT2-positive patients had a significantly shorter duration of systemic lupus erythematosus and lupus nephritis (*P* = 0.008 and *P* = 0.039), lower SLEDAI scores (12, IQR: 8–16 vs. 14, IQR: 10–18; *P* = 0.015), as well as lower systolic blood pressure (120,

Table 1. Baseline characteristics of exostosin-positive and exostosin-negative membranous lupus nephritis patients

Variables	Exostosin-positive (*n* = 83)	Exostosin-negative (*n* = 200)	*P* value
Age(y)	27 (21,35)	30 (23,39)	0.072
Female, *n* (%)	71 (85.5)	170 (85.0)	1.00
Lupus duration (months)	6 (2, 25)	12 (3, 49)	0.008
Lupus nephritis duration (mos)	3 (2, 18)	6 (2, 23)	0.039
SLEDAI score	12 (8, 16)	14 (10, 18)	0.015
Systolic blood pressure (mm Hg)	120 (109, 131)	127 (114, 142)	0.002
Diastolic blood pressure (mm Hg)	79 (70, 85)	81 (72, 90)	0.029
Leukocytopenia, *n* (%)	15 (18.1)	41 (20.5)	0.744
Thrombocytopenia, *n* (%)	3 (3.6)	8 (4.0)	1.000
Hemoglobin (g/l)	114 (100, 128)	106 (90, 122)	0.006
24-h proteinuria (g)	3.6 (1.2, 7.5)	2.8 (1.0, 5.5)	0.168
Proteinuria ≥3.5 g/24h, *n* (%)	42 (50.6)	76 (38.0)	0.064
Serum creatinine (μmol/l)	57 (50, 74)	73 (57,119)	<0.001
Serum albumin (g/l)	23 (18, 31)	25 (20, 30)	0.611
Nephrotic syndrome, *n* (%)	39 (47.0)	74 (37.0)	0.143
C3 (g/l)	0.57 (0.36, 0.82)	0.52 (0.37, 0.77)	0.297
Low C3, *n* (%)	59 (71.1)	157 (78.5)	0.219
C4 (g/l)	0.12 (0.07, 0.19)	0.11 (0.07, 0.18)	0.550
Low C4, *n* (%)	56 (67.5)	145 (72.5)	0.392
ANA, *n* (%)	81 (97.6)	194 (97.0)	1.000
Anti-dsDNA, *n* (%)	63 (75.9)	158 (79.0)	0.636
Anti-Sm, *n* (%)	25 (30.1)	42 (21.0)	0.124
Anti-SSA, *n* (%)	49 (59.0)	119 (59.5)	1.000
Anti-SSB, *n* (%)	19 (22.9)	36 (18.0)	0.409

ANA, antinuclear antibodies; anti-dsDNA, antidouble-stranded DNA; anti-Sm, anti-Smith; eGFR, estimated glomerular filtration rate; NA, not applicable; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; SSA, Sjogren's-syndrome-related antigen A; SSB, Sjogren's-syndrome-related antigen B.
Normal distribution data were presented as mean ± SD; and nonnormal distribution data as median (Q25, Q75).

IQR: 109–131 mm Hg vs. 127, IQR: 114–142 mm Hg; *P* = 0.002) and diastolic blood pressure (79, IQR: 70–85 mm Hg vs. 81, IQR: 72–90 mm Hg; *P* = 0.029). According to laboratory data at presentation, patients with positive EXT1/EXT2 had lower levels of serum creatinine (57 umol/l, IQR: 50–74 vs. 73 umol/l, IQR: 57–119; *P* < 0.001) and higher hemoglobin (114g/l, IQR: 100–128 vs. 106 umol/l, IQR: 90–122; *P* = 0.006) compared with those negative patients. Between the 2 groups, no significant difference was found in either 24-hour proteinuria or other immunologic measurements.

Comparison of Histopathological Features Between EXT1/EXT2-Positive and EXT1/EXT2-Negative MLN

Of the EXT1/EXT2-positive patients, 60.2% were categorized as pure class V MLN, whereas a majority of EXT1/2-negative patients (68.5%) were classified into class V+III/IV MLN. There was a significant statistical difference in the pathological pattern (*P* < 0.001) between these 2 groups (Table 2). The scores of activity

Table 2. Renal pathological features and outcomes of exostosin-positive and exostosin-negative membranous lupus nephritis patients
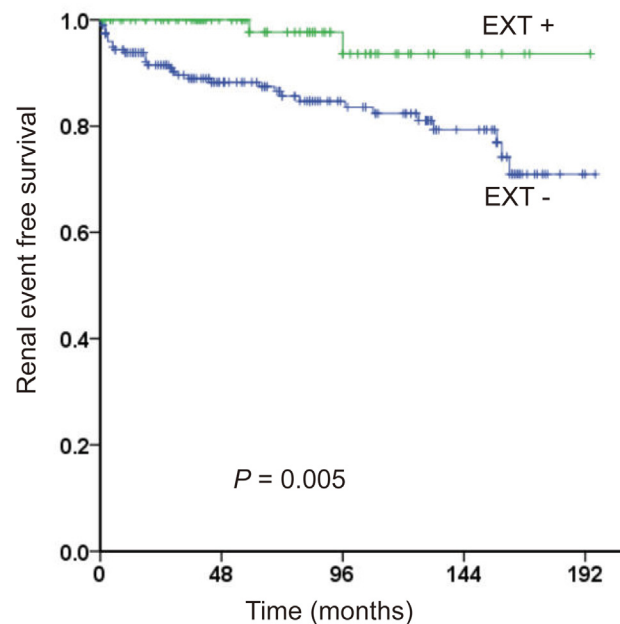
Variables	Exostosin-positive (n = 83)	Exostosin-negative (n = 200)	P value
Pathological pattern, n (%)			<0.001
Class V	50 (60.2)	63 (31.5)	
Class III+V	18 (21.7)	44 (22.0)	
Class IV+V	15 (18.1)	93 (46.5)	
Activity index	3 (2, 5)	7 (4, 9)	<0.001
Chronicity index	2 (1, 3)	3 (2, 4)	<0.001
Glomerulosclerosis, %	0 (0,5)	0 (0,9)	<0.001
Endocapillary hypercellularity, n (%)			0.012
0%	10 (12.0)	32 (16.0)	
<25%	49 (59.0)	78 (39.0)	
25%–50%	20 (24.1)	63 (31.5)	
>50%	4 (4.8)	27 (13.5)	
Fibrinoid necrosis, n (%)	2 (2.4)	10 (5.0)	0.519
Cellular crescents, %	0(0-0)	0(0-3)	0.007
Fibrocellular crescents, %	0(0-0)	0(0-6)	<0.001
Fibrous crescents, %	0(0-0)	0(0-0)	0.417
Interstitial Inflammation, n (%)			0.073
0%	41 (49.4)	84 (42.0)	
<25%	37 (44.6)	81 (40.5)	
25%–50%	5 (6.0)	29 (14.5)	
>50%	6 (3.0)	0 (0.0)	
Tubular atrophy, n (%)			0.002
0%	47 (56.6)	72 (36.0)	
<25%	33 (39.8)	93 (46.5)	
25%–50%	3 (3.6)	29 (14.5)	
>50%	0 (0)	6 (3.0)	
Interstitial fibrosis, n (%)			0.006
0%	48 (57.8)	82 (41.0)	
<25%	32 (38.6)	83 (41.5)	
25%–50%	3 (3.6)	29 (14.5)	
>50%	0 (0.0)	6 (3.0)	
Treatment, n (%)			0.105
Pred+IV-CYC	29 (34.9)	79 (39.5)	
Pred+MMF	20 (24.1)	46 (23.0)	
Pred+CNIs	13 (15.7)	26 (13.0)	
Pred	20 (24.1)	32 (16.0)	
Others[a]	1 (1.2)	17 (8.5)	
Outcomes			
Follow-up time (mos)	59.0 (37.0–102.0)	70.5 (26.3–131.8)	0.439
Adverse renal events, n (%)	2 (2.4)	32 (16.0)	0.001

CNIs; calcineurin inhibitors; ESRD, end-stage renal disease; IV-CYC, intravenous cyclophosphamide; MMF, mycophenolate mofetil; Pred, prednisone.
Normal distribution data were presented as mean ± SD; and nonnormal distribution data as median (Q25, Q75).
[a]Other treatments included prednisone + mycophenolate mofetil + belimumab, prednisone + calcineurin inhibitors + mycophenolate mofetil, prednisone + rituximab, prednisone + methotrexate, prednisone + azathioprine, prednisone + rituximab+ belimumab, prednisone + leflunomide, prednisone + oral cyclophosphamide.

Figure 2. Kaplan-Meier plots of renal event-free survival rate in membranous lupus nephritis patients with positive exostosin (EXT) and negative EXT.

cellular crescents, $P = 0.007$; and fibrocellular crescents, $P < 0.001$).

Outcomes of EXT1/EXT2-Positive and EXT1/EXT2-Negative MLN

Treatments were similar between EXT1/EXT2-positive and EXT1/EXT2-negative MLN (Table 2). The median follow-up time of EXT1/EXT2-positive and EXT1/EXT2-negative groups were 59.0 (IQR: 37.0–102.0) and 70.5 (IQR: 26.3–131.8) months, respectively ($P = 0.439$) (Table 2). Before the follow-up deadline, 2 (2.4%) EXT1/EXT2-positive patients and 32 (16.0%) EXT1/EXT2-negative patients had adverse renal events, with a significant statistical difference ($P = 0.001$). Among the EXT1/EXT2-positive patients, 1 patients died, and 1 patients had maintenance dialysis, whereas among the EXT1/EXT2-negative patients, 20 patients died, and 12 patients had maintenance dialysis. Kaplan-Meier curves of survival analysis presented that EXT1/EXT2-positive patients were less likely to reach adverse renal events compared to EXT1/EXT2-negative patients (Figure 2).

Cox regression analysis was used to predict the prognosis and outcomes of EXT1/EXT2-positive patients with MLN compared with EXT1/EXT2-negative patients (Table 3). In unadjusted model, the risk of adverse renal outcomes decreased in EXT1/EXT2-positive patients with MLN compared with EXT1/EXT2-negative patients (hazard ratio, 0.17; 95%

index and chronicity index were significantly lower in EXT1/EXT2-positive patients than in those of the EXT1/EXT2-negative group (both $P < 0.001$). EXT1/EXT2-positive patients showed less chronicity features (glomerulosclerosis, $P < 0.001$; interstitial fibrosis, $P = 0.006$; and tubular atrophy, $P = 0.002$) and less active features (endocapillary hypercellularity, $P = 0.012$;

Table 3. Association of exostosin positivity with outcomes in membranous lupus nephritis patients

Variables	Adverse renal Events	
	HR (95% CI)	P value
Unadjusted	0.17 (0.04–0.70)	0.014
Model 1[a]	0.17 (0.04–0.72)	0.016
Model 2[b]	0.17 (0.04–0.71)	0.015
Model 3[c]	0.25 (0.06–1.07)	0.062

CI, confidence interval; HR, hazard ratio; LN, lupus nephritis; MLN, membranous lupus nephritis.
[a]Model 1: adjusted for age and gender.
[b]Model 2: adjusted for Model 1 plus serum creatinine, and 24-hour proteinuria.
[c]Model 3: adjusted for Model 2 plus Chronicity index.

confidence interval, 0.04–0.70; $P = 0.014$). After adjusting for age, gender, serum creatinine, and 24-hour proteinuria, EXT1/EXT2-positive patients with MLN were less likely to develop adverse renal outcomes compared to EXT1/EXT2-negative patients (hazard ratio, 0.17; 95% confidence interval, 0.04–0.71; $P = 0.015$). However, the difference disappeared (hazard ratio, 0.25; 95% confidence interval, 0.06–1.70; $P = 0.062$) after the inclusion of the chronicity index variable, as shown in Model 3.

EXT Expression in Rebiopsy of MLN

Of 283 included patients with MLN, 7 cases underwent rebiopsy during the follow-up period (Table 4). It was found that all of their specimens showed EXT1/EXT2-negative IHC staining for the first time. One case, in particular, became EXT1/EXT2 positive after a 4-year interval. This case was initially diagnosed as MN without any clinical manifestation of lupus 4 years earlier but was later diagnosed as pure class V MLN accompanied with a normal serum creatinine level (35 μmol/l). The rate of interstitial fibrosis and tubular atrophy remained at 0%, whereas an ascendence was observed in the sclerosed glomeruli rate (from 0%–13%). On the other hand, the other 6 patients who were concordant EXT1/EXT2-negative in both renal biopsies had a more severe rate of interstitial fibrosis and tubular atrophy and worsening kidney function, as evidenced by serum creatinine elevation or the need for dialysis treatment, including a death report.

DISCUSSION

In this study of 283 patients with MLN, 29.3% were positive for EXT1/EXT2. Patients with EXT1/EXT2-positive MLN have lower serum creatinine and lower SLEDAI scores at presentation, less chronicity and activity features on kidney biopsy and on follow-up; and develop a markedly decreased incidence of adverse renal outcomes compared to patients with EXT1/EXT2-negative MLN.

The EXT-positive rate was 29.3% in our cohort, which was comparable to 32.6% reported by Sethi et al.[6] The prevalence of EXT1/2-positive MLN was significantly higher in pure class V MLN than in mixed class V MLN (44.2% vs. 19.4%, $P < 0.001$) in our study, which was consistent with the study by Hu et al. (46% vs. 9%).[10] The EXT-positive rate for pure class V MLN was 44.2% in our cohort, which was higher than 38.4% reported by a study of 86 patients with pure class V MLN from Europe.[14] However, Sethi et al.[6] and Xie et al.[15] found that the EXT-positive rate among the pure class V cases and mixed class V MLN were similar. Our cohort had the largest sample of 170 patients with mixed class V MLN whereas there were only 47 to 111 patients with mixed class V MLN in other studies. The difference in results of EXT-positive rate for the patients with mixed class may be due to differences in subject selection and sample sizes.

Compared with the EXT1/EXT2-negative patients, the EXT1/EXT2-positive patients with MLN appeared to represent a milder subgroup and had shorter onset of lupus nephritis, lower SLEDAI scores, lower serum creatinine levels, and lower renal activity index and chronicity index scores in renal biopsy. These results were consistent with the findings of 2 recent studies.[6,10] We suggested that EXT1/EXT2-positive patients were less likely to experience adverse renal events, which aligned with the findings of Sethi et al.,[6] who also found that the incidence of end-stage renal diseases remarkably decreased in EXT1/EXT2-positive patients over a 10-year follow-up period. However, the outcomes showed no significant difference between EXT1/EXT2-negative and EXT1/EXT2-positive patients

Table 4. The changes of exostosin expression and renal pathological findings in lupus nephritis patients on rebiopsy

Case	1st biopsy	EXT	Glomeruli/Sclerosed	IFTA, %	Time from 1st to 2nd biopsy, yr	2nd biopsy	EXT	Glomeruli/Sclerosed	IFTA, %	Outcome, Last Scr (umol/l)
1	MN, no SLE	neg	13/0 (0%)	0%	4	V	pos	23/3 (13.0%)	0%	7 yr, Scr 38
2	III + V	neg	27/0 (0%)	0%	7	III + V	neg	33/14 (42.4%)	50%	8 yr, Scr 95
3	IV + V	neg	18/0 (0%)	15%	7	III + V	neg	27/14 (51.9%)	50%	13 yr, dialysis 14 yr, death
4	IV + V	neg	32/9 (28.1%)	40%	3	IV	neg	20/16 (80%)	80%	5 yr, dialysis
5	III	neg	34/0 (0%)	5%	5	IV + V	neg	29/8 (51.9%)	35%	7yr, dialysis
6	IV	neg	16/0 (0%)	0%	10	IV+V	neg	21/7 (33.3%)	20%	13yr, Scr 149
7	IV	neg	19/1(5.3%)	0%	13	IV+V	neg	24/2 (8.3%)	10%	14yr, Scr 92

EXT, exostosin; IFTA; interstitial fibrosis and tubular atrophy; MN, membranous nephropathy; neg, negative; pos, positive; SLE, systemic lupus erythematosus; Scr, serum creatinine.

in 165 patients with pure class V MLN.[10] According to our multivariable Cox regression analysis, the significant difference of outcomes vanished after including the chronicity index. Thus, we conjectured that those patients with MLN with positive EXT1/EXT2 contributed to better prognoses and outcomes, which was possibly due to their lower chronicity kidney injure. Further multicenter and large investigations are required to validate the effect of EXT1/EXT2 on prognosis in patients with MLN.

Notably, both Sethi *et al.*[6] and Hu *et al.*[10] discovered significantly increasing proteinuria (more than 3.5 g/d) in EXT-positive MLN. Neither Xie *et al.*[15] nor our study found statistical differences; however, we both clarified that EXT1/EXT2-positive patients were prone to show greater proteinuria. Though remaining unexplained and elusive, one hypothesis was that the EXT1/EXT2 as glycosyltransferases being transported from podocytes to GBM might facilitate the synthesis of the heparan sulfates.[16,17] However, severe albuminuria did not develop in podocyte-specific EXT1 knockout mice despite podocyte abnormalities and loss of heparan sulfate glycosaminoglycans.[18] In addition, Wada *et al.* in Japan proposed that apart from proteinuria level or renal function, the EXT1/EXT2 positivity is more associated with the concentration of serum autoantibodies (anti-dsDNA) and circulating immune complexes.[19] Indeed, our study showed a similar positive rate of serum lupus autoantibodies in EXT1/EXT2-positive and EXT1/EXT2-negative patients.

The EXT1/EXT2-negative pure class V MLN had a higher rate of histology transition in rebiopsy.[10,14] In a repeat renal biopsies study, 10% (3/31) of patients with MLN had discordant EXT1 staining between their first and last biopsies, and 2 of the 3 patients with discordant EXT1 staining developed end-stage renal diseases during the follow-up period.[20] In particular, a case in our study transformed from EXT1/EXT2-negative to EXT1/EXT2-positive had relatively favorable renal function and prognosis whereas the other 6 patients who were concordant EXT1/EXT2-negative in both renal biopsies had a more severe rate of interstitial fibrosis and tubular atrophy and worsening kidney function. However, the sample size for rebiopsy patients was very limited, and larger studies exploring the temporal dynamics of EXT1/EXT2 are warranted.

EXT1 and EXT2 were coexisting along the thickened GBM and the staining of EXT1 was inclined to be slightly brighter than EXT2 on IHC, according to the previous study and our study.[5,6] There was a view that EXT1/EXT2 granularly expressed on the GBM functioned as biomarkers, antigens, or both.[6,10,21,22] Miller *et al.*[20] speculated that EXT1/EXT2 might be dynamic at the course of the disease, which may partially explain why serum EXT1/EXT2-antibodies have never been detected in patients with MLN. EXT1/EXT2-positive MLN was deemed to be a distinct subtype of MLN and denoted a favorable kidney prognosis. Patients with MLN are suggested to perform a regular EXT1/EXT2 staining followed by renal biopsy,[6] for clinically better understanding and predicting the prognosis. Herein, the specific mechanism of EXT1/EXT2 is urgent to be explored in the near future.

Some limitations are inevitable and have to be admitted in our study. First, it was a single-center retrospective analysis based on the Chinese population; and thus, the results may not be generalizable to other populations. Nonetheless, our study represents the largest cohort with regular follow-up and enrolled both patients with pure class V MLN and mixed class V MLN. Second, there was a limited sample size of repeat renal biopsy, so the change of EXT1/EXT2 throughout the disease course of MLN were largely unknown. Third, this study did not examine the potential mechanisms of EXT-positive and EXT-negative MLN; and so more studies in the future are needed to explore the underlying genotype or mechanisms that may result in distinctive phenotypic differences between these 2 groups.

To summarize, 29.3% (83/283) of patients with MLN were positive for EXT1/EXT2. Compared with EXT1/EXT2-negative patients, the EXT1/EXT2-positive patients appeared to represent a milder subgroup and less likely to progress to adverse renal events. EXT1/EXT2 may be used as a potential subtype and prognosis marker for patients with MLN.

DISCLOSURE

ACKNOWLEDGMENTS

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared upon reasonable request by the corresponding authors.

AUTHOR CONTRIBUTIONS

XX, SL, ZW, RT, and WC designed the study. ZW drafted the manuscript. XX, WC, and RT conducted statistical data analysis and critically reviewed the manuscript. XX, SL, SY, YF, and WP performed the experiments. XX, SL, ZW, SY, YF, WP, WenC, FH, RT, and WC participated in material preparation, data collection, results interpretation, and manuscript polish and final approval.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Immunohistochemistry of exostosin (EXT)1/2-positive membranous lupus nephritis (MLN).

Figure S2. Immunohistochemistry of exostosin (EXT)1/2-negative membranous lupus nephritis (MLN).

Figure S3. Immunohistochemistry of exostosin (EXT) 1 (A) and EXT 2 (B) in a serum phospholipase A2 receptor antibody positive primary membranous nephropathy patient as a negative control for the staining pattern.

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