Supplemental Data

Supplemental Materials and Methods

Detection of Glomerular PLA2R Expression and Circulating Anti-PLA2R Antibodies

Paraffin-embedded kidney tissues were cut into 4 μm-thick sections, and antigen retrieval involved autoclave heat repair (in citrate solution, pH 6.0) plus trypsin repair-induced epitope retrieval. The rabbit anti-human PLA2R polyclonal antibody (Sigma, 1:800 dilution) was added as the primary antibody and incubated at 4°C overnight. The ALP-labeled goat anti-rabbit IgG antibody (Beijing Zhongshan) was used as the secondary antibody and incubated for 60 minutes at room temperature. ALP-Red was used as a substrate of ALP.

The serum level of anti-PLA2R antibodies was detected by means of ELISA (EUROIMMUN, Lübeck, Germany). The microplate was coated with the PLA2R isoform. The serum samples were diluted 1:100 in sample buffer. A positive reaction was defined as a concentration of anti-PLA2R antibody in serum > 20 RU/ml.

Detection of Glomerular THSD7A Expression and Circulating Anti-THSD7A Antibodies

Paraffin-embedded kidney tissues were cut into 4 μm-thick sections and then processed by autoclave heating-induced epitope retrieval (in citrate solution, pH 9.0). The rabbit anti-human THSD7A polyclonal antibody (Sigma, 1:1500 dilution) was used as the primary antibody, and the ALP-labeled goat anti-rabbit IgG antibody (Beijing Zhongshan) was used as the secondary antibody. ALP-Red was used as a substrate of ALP.

The circulating anti-THSD7A antibody was detected by an indirect immunofluorescence assay kit

(EUROIMMUN AG, Lübeck, Germany) following standard instructions. Patient serum was diluted 1:10 and incubated on the reaction fields of slides at room temperature for 30 minutes. FITC-conjugated anti-human IgG antibodies were used for the detection of bound total IgG antibodies. Cytoplasmic fluorescence of the transfected cells only at this dilution was considered positive.

The Selection Criteria of PLA2R Positive Patients Who were Stained with NELL-1

we selected 31 patients with PLA2R-positive MN for NELL-1 staining. The selection criteria were as follows: ① patients who were positive for renal PLA2R staining and serum PLA2R antibody; ② patients who received formal immunosuppressive therapy; and ③ in the course of immunosuppressive therapy, the titer of serum PLA2R antibody was detected at least once every 6 months, and a titer > 300 was maintained for at least one year.

Supplemental Table 1. Clinical and pathological characteristics of dual PLA2R and THSD7A positive patients

	Sex	Age (year)	Urinary Protein (g/24h)	Albumin (g/L)	SCr (μmol/L)	eGFR (ml/min/1.73m^2)	anti-PLA2R antibody (RU/ml)	PLA2R antigen	Anti-THSD7A antibody	THSD7A antigen	Anti-NELL-1 antibody	NELL-1 antigen	Malignancy
Patient 1	M	44	2	25	71	109	226	+	-	+	-	-	No
Patient 2	M	77	5	16	97	48	42	-	+	+	-	-	No

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Renal Pathology						Treatment	Prognosis			
Stages	IgG1	IgG2	IgG3	IgG4	Electron microscopy		Complete remission/Partial remission	Relapse	Kidney Dysfunction	Follow-up Time (month)
II	-	+	+	+++	Subepithelial electron dense deposits	Pred and Cyclophosphamid	CR	No-	No	24
I	-	+	-	++	Subepithelial electron dense deposits	Cyclosporin A	No	No	No	5

SCr, serum creatinine; eGFR, estimated glomerular filtration rate; IgG, immunoglobin G; NA, not available; Pred, prednisone

Estimated glomerular filtration rate (eGFR) of patients was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Immunofluorescence 2 + and above were positive.

The definitions of PR and CR are the same as described above.

Supplemental Table 2. Clinical and pathological data of the 16 primary Membranous Nephropathy patients with glomerular staining for NELL-1

	Sex	Age (year)	Urinary Protein (g/24h)	Albumin (g/L)	SCr (μmol/L)	eGFR (ml/min/1.73m^2)	Cholesterol (mmol/L)	Renal Pathology				
								Stages	IgG1	IgG2	IgG3	IgG4
1	F	44	4	29	60	107	6	I	++	-	-	++
2	F	49	2	26	61	102	10	I	++	-	-	-
3[#]	F	44	7	28	61	106	8	I	+~++	+	-	++
4[#]	F	50	14	21	106	53	13	I	++ ~ +++	+ ~ ++	-	++
5	F	48	5	20	39	119	10	I	++	-	-	+++
6	F	50	3	33	76	79	7	II	-	-	-	++ ~ +++
7	F	48	5	29	61	103	11	I	++	-	-	-
8	F	63	5	30	89	59	6	I	-	++	-	++
9	F	51	2	43	70	86	7	II	-	-	-	++
10	F	50	0.6	44	42	115	5	I	++	+	-	++
11	F	43	5	26	50	114	7	I	++ ~ +++	-	-	++
12[##]	M	36	18	16	72	114	11	I	++	++	-	++
13	M	49	5	16	63	110	9	II	++	+	-	++~+++
14[##]	M	38	6	29	76	110	10	II	++~+++	++	+	++~+++
15[##]	M	60	5	18	117	58	10	I	++	+	-	-
16*	F	68	5	29	59	99	6	II	+++	-	-	+++

F, female; M, male; SCr, serum creatinine; eGFR, estimated glomerular filtration rate; IgG, immunoglobin G; IgA, immunoglobin A; NA, not available; Pred, prednisone; RASI, renin-angiotensin system inhibitors

Estimated glomerular filtration rate (eGFR) of patients was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Immunofluorescence 2 + and above were positive.

Complete remission was defined as urinary protein excretion<0.3 g/d, confirmed by 2 values at least 1 week apart, accompanied by normal serum albumin and normal serum creatinine levels.

Partial remission was defined as urinary protein excretion <3.5 g/d and a 50% or greater reduction from peak values, accompanied by an improvement or normalization of serum albumin and stable serum creatinine levels.

Kidney dysfunction was defined as a decrease of 30% of the initial eGFR and below 60 ml/min/1.73 m^2.

[#] Patients with expression of NELL-1 antigen in glomeruli and serum anti-NELL-1 antibody positivity.

*Patient with glomerular dual positive for NELL-1 and PLA2R.

[##] Patients who have received immunosuppressive therapy before kidney biopsy.

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	Treatment	Pregnosis			
		Complete remission/Partial remission	Relapse	Kidney Dysfunction	Follow-up Time (month)
1	Pred and cyclophosphamide	Partial remission	No	No	2
2	Cyclosporin A	Partial remission	No	No	77
3	Cyclosporin A	NA	NA	NA	NA
4	Pred and Cyclosporin A	Partial remission	No	No	20
5[#]	Pred and cyclophosphamide	Partial remission	No	No	6
6[#]	RASI	Partial remission	No	No	5
7	NA	NA	NA	NA	NA
8	Cyclosporin A	Complete remission	No	No	39
9	Pred and Cyclosporin A	Complete remission	No	No	6
10	NA	NA	NA	NA	NA
11	Cyclosporin A	Complete remission	No	No	28
12	Cyclosporin A	Partial remission	No	No	32
13	Herbs	Partial remission	No	No	33
14	Pred and Cyclosporin A	No	No	No	31
15	Pred and Cyclosporin A	Complete remission	No	No	24
16[*]	Cyclosporin A	Complete remission	Yes	No	42

Supplemental Table 3. Comparison of clinical and pathological data between female and male patients with NELL-1-positive membranous nephropathy

	Male (n=4)	Female (n=11)	*P* value
Age, year	49(37-64)	49(44-50)	0.39
Urinary Protein, g/d	5.2(4.6-22.3)	4.5(2.3-5.1)	0.13
Albumin, g/dl	1.7(1.6-2.7)	2.9(2.6-3.3)	0.04
Nephrotic syndrome, n (%)	4(100)	8(73)	0.24
eGFR, ml/min per 1.73m^2	98±27	95±23	0.81
Cholesterol, mg/dl	386(386-425)	270(232-386)	0.24
Hypertension, n (%)	3(75)	5(46)	0.31
Diabetes mellitus, n (%)	1(25)	0	0.09
Interstitial fibrosis, n (%)			0.51
<25%	0	0	
25-50%	4(100)	8(73)	
50-75%	0	2(18)	
>75%	0	1(9)	
IgG1 positive, n (%)	4(100)	7(64)	0.16
IgG2 positive, n (%)	2(50)	1(9)	0.08
IgG3 positive, n (%)	0	0	
IgG4 positive, n (%)	3(75)	9(82)	0.77
IgG4 predominant positive, n (%)	3(75)	7(64)	0.68
IgA positive, n (%)	0	1(9)	0.53
Segmental sclerosis, n (%)	0	0	

eGFR, estimated glomerular filtration rate; BMI, body mass index; IgG, immunoglobin G; IgA, immunoglobin A; NA, not available; Pred, prednisone

Immunofluorescence 2 + and above were positive.

Estimated glomerular filtration rate (eGFR) of patients was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Supplemental Table 4. Comparison of treatment and prognosis data between female and male patients with NELL-1-positive membranous nephropathy

	Male (n=4)	Female (n=8)	*P* value
Immunosuppressive therapy (%)	3(75)	6(75)	1.0
Pred and Cyclosporin A (%)	2(50)	2(25)	0.39
Cyclosporin A (%)	1(25)	2(25)	1
Pred and Cyclophosphamide (%)	0	2(25)	0.27
Remission (%)	3(75)	8(100)	0.14
Complete remission (%)	1(25)	3(38)	0.67
Partial remission (%)	2(50)	5(63)	0.68

Pred, prednisone
The definitions of partial remission and complete remission are the same as described above.