

Primary Membranous Nephropathy With Enhanced Staining of Exostosin 1/Exostosin 2 in the Glomeruli: A Report of 2 Cases



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Technological advances have allowed the discovery of 6 subtypes of membranous nephropathy based on target antigens: M-type phospholipase A₂ receptor (PLA₂R), thrombospondin type 1 domain-containing 7A (THSD7A), neural epidermal growth factor-like 1 protein, semaphorin 3B, exostosin 1 (EXT1), and EXT2. EXT1/EXT2 are thought to be associated with secondary (autoimmune) membranous nephropathy. Although it has been reported that PLA₂R- and THSD7A-associated membranous nephropathy have rarely been detected concomitantly, there have been no previous reports demonstrating PLA₂R- or THSD7A-associated membranous nephropathy with enhanced glomerular staining of EXT1/EXT2. We describe 2 cases of primary membranous nephropathy with enhanced glomerular staining of EXT1/EXT2. Patient 1 was diagnosed with PLA₂R-associated primary membranous nephropathy, and patient 2 was diagnosed with THSD7A-associated primary membranous nephropathy. Both patients achieved clinical remission in response to immunosuppressive therapy. Neither patient demonstrated signs of autoimmune diseases, and antinuclear antibodies were absent in their sera. Based on these 2 cases, enhanced staining of EXT1/EXT2 in glomeruli, although rare, can be detected in primary membranous nephropathy without autoimmune diseases.

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INTRODUCTION

Membranous nephropathy (MN) is the leading cause of nephrotic syndrome in adults.¹ Based on the underlying cause, MN is classified as either primary or secondary. Autoantibodies against M-type phospholipase A₂ receptor (PLA₂R), thrombospondin type 1 domain-containing protein 7A (THSD7A), neural epidermal growth factor-like 1 protein, and semaphorin 3B have been reported to be the primary cause of MN.²⁻⁵ Of these, PLA₂R and THSD7A are most frequently associated with the primary form. However, neural epidermal growth factor-like 1 protein has recently been identified as an antigen in many cases of secondary MN in which MN was associated with malignancies,⁶ and exostosin-1 and exostosin-2 proteins (EXT1/EXT2) have been identified as putative target antigens in autoimmune-type secondary MN.⁷ Although autoantibodies against PLA₂R and THSD7A are mutually exclusive in most cases of MN,^{2,8} some reports have demonstrated that these autoantibodies may be concomitantly produced through unknown pathophysiologic mechanisms.⁹⁻¹¹ EXT1/EXT2 were also thought to be exclusive to autoimmune-type secondary MN.⁷ In this report, we describe 2 patients with primary MN accompanied by enhanced granular expression of EXT1/EXT2 in glomeruli.

CASE REPORTS

Case 1

A man in his 40s was referred to our hospital due to sudden onset of edema. The patient's blood pressure on

admission measured 134/94 mm Hg. He displayed no signs of autoimmune disease. A history of the patient included only a duodenum ulcer, and infection caused by *Helicobacter pylori* was diagnosed after admission. Laboratory data are shown in Table 1. Serum creatinine and albumin levels on admission were 0.68 mg/dL and 3.0 g/dL, respectively. Measured proteinuria was protein excretion of 2.1 g/d. Antinuclear antibody (ANA) was absent, and serum complement levels (C3, C4, and CH50) were within the normal range.

A kidney biopsy was performed, and light microscopy demonstrated 43 glomeruli with spike formation and/or bubbling (Fig 1A), resulting in the diagnosis of MN. Focal and segmental mesangial proliferation was observed (Fig 1B). Immunofluorescence showed granular staining along the glomerular basement membrane (GBM) and was positive for immunoglobulin G (IgG; 3+) and C3 (1+) but negative for IgA, IgM, C4, and C1q. Electron microscopy was not performed.

A detailed screening process, including computed tomography from neck to pelvis and upper and lower gastrointestinal endoscopies, did not detect any underlying disease, a history of drug use, or other potential causes of secondary MN. The patient had idiopathic MN diagnosed and was treated with 40 mg (0.7 mg/kg) of prednisolone. The level of proteinuria decreased to protein excretion of 0.9 g/d over the following month, and prednisolone dosage was tapered. The patient achieved complete remission by 6 months and maintained the condition for approximately 20 years (Fig 2: clinical course is shown for only 2 years). Retrospectively, immunohistochemical

Table 1. Laboratory Data on Admission in Cases 1 and 2

	Case 1	Case 2
Complete blood cell count		
White blood cells, / μ L	6,000	7,150
Hemoglobin, g/dL	15.2	13.1
Platelets, $\times 10^4$ / μ L	25.2	32.9
Blood chemistry		
Aspartate aminotransferase, IU/L	14	24
Alanine aminotransferase, IU/L	12	18
Alkaline phosphatase, IU/L	175	276
Total protein, g/dL	6.1	4.5
Albumin, g/dL	3.0	1.5
Total cholesterol, mg/dL	320	375
Serum urea nitrogen, mg/dL	16.0	20.2
Creatinine, mg/dL	0.68	1.07
Uric acid, mg/dL	6.7	4.2
Sodium, mEq/L	143	145
Potassium, mEq/L	3.5	4.3
Chloride, mEq/L	105	108
Calcium, mg/dL	9.4	8.5
Inorganic phosphate, mg/dL	3.3	4.3
C-Reactive protein, mg/dL	0.1	0.15
Hemoglobin A _{1c} , %	6.6	6.6
Blood glucose, mg/dL	108	85
Serology		
C3, mg/dL	133	129
C4, mg/dL	22	38
CH50, IU/mL	58	>60
IgG, mg/dL	981	613
IgA, mg/dL	212	182
IgM, mg/dL	91	71
Antinuclear antibody	<40	<40
Anti-DNA antibody, IU/mL	<2.0	
HBS-Ag	Negative	Negative
HCV-Ab	Negative	Negative
Urinalysis		
pH	6.5	5.5
Protein, g/d	1	8.2
Red blood cells, /HPF	20-29	20-29
β_2 -microglobulin, μ g/L	84	14.4

Abbreviations: C3, complement component 3; CH50, complement activities; HBS-Ab, anti-hepatitis B surface antigen; HCV-Ab, anti-hepatitis C virus antigen; HPF, high-power field; IgG, immunoglobulin G.

analysis revealed enhanced granular staining of PLA₂R (Fig 1C) and IgG4 (Fig 1D), suggesting primary MN. In this case, enhanced staining of EXT1/EXT2 was also observed (Fig 1E and F). The patient did not display any sign indicative of an autoimmune disease for 20 years.

Case 2

A woman in her 60s was referred to our hospital due to sudden onset of edema. Blood pressure on presentation measured 159/99 mm Hg. The patient displayed no signs of any autoimmune disease. Laboratory data are summarized in Table 1. Serum creatinine and albumin levels on admission were 1.07 mg/dL and 1.5 g/dL, respectively. Measured proteinuria was protein excretion of 8.2 g/d.

ANA was absent, and serum complement levels (C3, C4, and CH50) were within the reference range.

A kidney biopsy was performed, and light microscopy demonstrated 35 glomeruli without apparent spike formation or bubbling (Fig 1G). Focal and segmental mesangial proliferation was found (Fig 1H). Enhanced granular staining of THSD7A (Fig 1I) and IgG4 (Fig 1J) in glomeruli suggested THSD7A-associated primary MN. Immunofluorescence showed granular staining along the GBM, which was positive for IgG (2+) and C3 (1+; Fig 1K and L) but negative for IgA, IgM, C4, and C1q.

Secondary MN was ruled out using a detailed screening process, including history of negative drug use, computed tomography from neck to pelvis, gastrointestinal

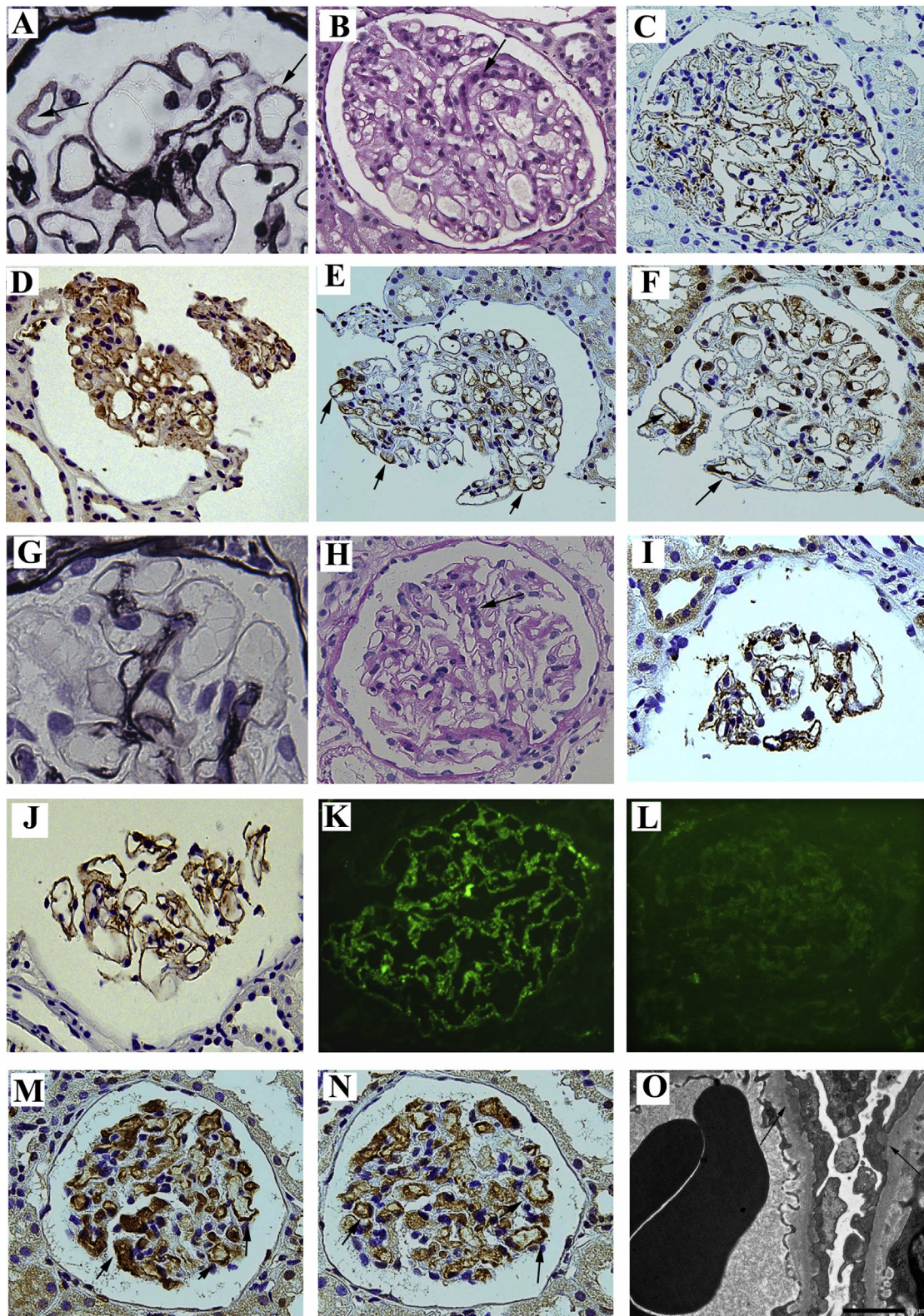
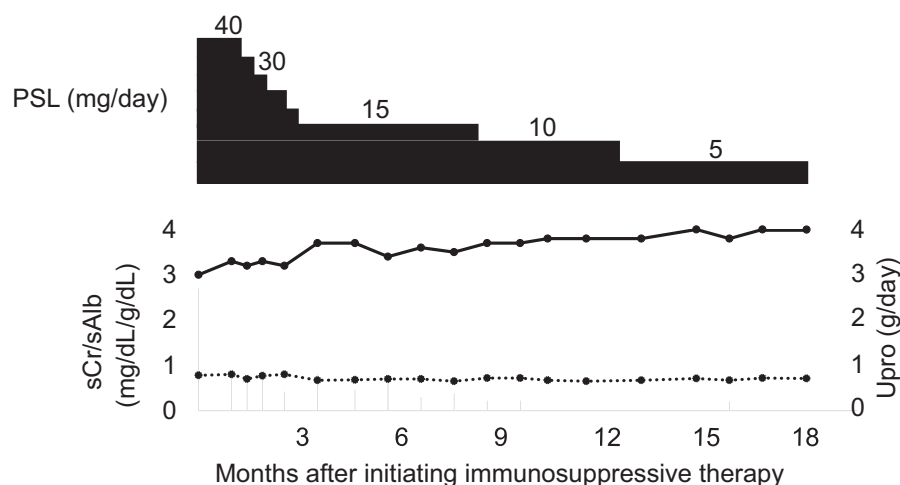


Figure 1. Photographs of glomeruli in the kidney. (A-F) Case 1, (G-O) case 2. (A, G) Periodic acid–methenamine silver staining shows spike formation in case 1 (arrows), but not in case 2 (original magnification, $\times 1,000$). (B, H) Periodic acid–Schiff staining of the kidney biopsy specimen shows slightly increased mesangial matrix (arrows) (original magnification, $\times 400$). Immunohistochemistry shows enhanced staining of (C) phospholipase A_2 receptor, (I) thrombospondin type 1 domain-containing 7A, (D, J) immunoglobulin G4 (IgG4), (E, M) exostosin 1 (EXT1), and (F, N) Ext2 on the glomerular basement membrane (GBM). Immunofluorescence shows granular staining along the GBM for (K) IgG and (L) C3 in case 2. (O) Electron microscopy reveals subepithelial deposits in the GBM (original magnification, $\times 8,000$).

Patient 1



Patient 2

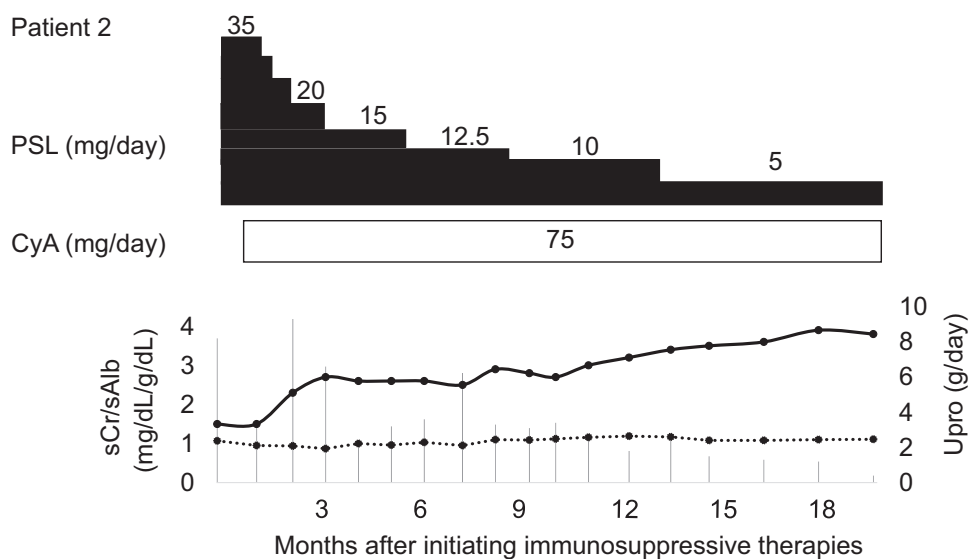


Figure 2. Clinical course after initiating immunosuppressive therapy. Levels of serum creatinine (sCr; dotted line), serum albumin (sAlb; solid line), and 24-hour urinary protein (Upro; bar graph) are shown. Abbreviations: CyA, cyclosporine A; PSL, prednisolone.

endoscopy, and fecal occult blood test. Angiotensin II receptor blocker and statin therapy were started; however, the nephrotic-range proteinuria continued and serum creatinine level increased over a period of 5 months. Thereafter, the patient was treated with 35 mg (0.7 mg/kg) of prednisolone as an induction therapy for 4 weeks and then with added 75 mg (1.5 mg/kg) of cyclosporine. The level of proteinuria decreased over time to protein excretion of 3.6, 1.8, and 0.4 g/d at 6, 12, and 18 months, respectively (Fig 2). Further analysis of immunohistochemistry revealed enhanced staining of EXT1/EXT2 in glomeruli (Fig 1M and N). Electron microscopic findings showed subepithelial electron-dense glomerular deposits but no tubuloreticular inclusion structure (Fig 1O). The patient had not displayed any sign indicative of

an autoimmune disease at the time of this report (2 years later).

DISCUSSION

We have reported 2 cases of primary MN accompanied by enhanced staining of EXT1/EXT2 in glomeruli: 1 case of PLA₂R-associated MN and 1 case of THSD7A-associated MN. EXT1/EXT2 are currently thought to be exclusive within MN.⁷ Because EXT1/EXT2 are newly discovered putative target antigens in MN, there have only been a few reports regarding EXT1/EXT2-associated MN.^{7,12} We conducted immunohistochemistry for EXT1/EXT2 in our cohort study (Iwakura et al, unpublished data, 2021). Of a total of 104 samples from patients with MN, enhanced

staining of EXT1/EXT2 was found in 17. PLA₂R-associated and THSD7A-associated MN were detected in 39 and 6 samples, respectively, and we found that 2 of these detected primary MN samples showed enhanced staining of EXT1/EXT2. It has been reported that EXT1/EXT2 represent potential biomarkers or target antigens in secondary MN with autoimmune disease.⁷ However, our cases demonstrated neither signs of autoimmune diseases nor the presence of ANA. In addition, our cohort study revealed that ANA was present in 6 cases (35.3%) and autoimmune features were found in 5 cases (29.4%) of patients with MN with enhanced staining of EXT1/EXT2, suggesting that EXT1/EXT2 might occur in settings other than autoimmune diseases. In the report from Sethi et al,⁷ 7.7% (2/26) of patients with EXT1/EXT2-associated MN had cancer, which raised a concern that EXT1/EXT2 could be associated with cancer. However, neither patient in the current report had cancer diagnosed during follow-up care.

The findings of dual positivity of PLA₂R and EXT1/EXT2, as well as THSD7A and EXT1/EXT2, suggest that EXT1/EXT2 are not primary antigens like PLA₂R and THSD7A; rather, they are secondary phenomena. The lack of antibodies against EXT1/EXT2 may support that these are not primary antigens but glomerular markers.⁷

Interestingly, EXT staining was segmental and weak in patient 1. Sethi et al⁷ reported that EXT proteins seem to shed from podocytes because of the uniformity of EXT1/EXT2 staining along the GBM and the presence of subepithelial deposits. However, our findings suggest that EXT proteins may derive from circulating antigens or immune complexes.^{13,14} If patient 1 had small amounts of circulating antigens or immune complexes, the trapped EXT protein along the GBM could be detected with segmental and weak staining. Because EXT1/EXT2 in MN is a new area of study, further accumulation of cases is necessary to examine whether similar cases exist, and if so, what characteristics these patients have in common.

This report highlights the possible concomitance of primary MN and glomerular overexpression of EXT1/EXT2. Because autoantibodies against EXT1/EXT2 have not been discovered in EXT1/EXT2-associated MN, overexpression of EXT1/EXT2 in glomeruli may play unique roles and have distinct characteristics as compared with autoantibody-associated MN. Further investigation is needed to clarify the roles and characteristics of EXT1/EXT2 in MN.

ARTICLE INFORMATION

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