

## CLINICAL VIGNETTE

# Exostosin 2 Associated Membranous Nephropathy

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### Introduction

Membranous nephropathy (MN) affects the glomerulus, leading to loss of protein into the urinary space. About 80% of cases are classified as primary with 20% associated with other secondary causes. Secondary causes include infections such as hepatitis B or human immunodeficiency virus, malignancies such as non-Hodgkin lymphoma or melanoma, autoimmune diseases including diabetes or Systemic lupus erythematosus (SLE), alloimmune processes such as graft versus host disease, or drugs including non-steroidal anti-inflammatory drugs.<sup>1</sup>

Primary MN is one of the most common causes of nephrotic syndrome in non-diabetic adults.<sup>1</sup> This autoimmune glomerular disease involves antibodies against antigens such as M-type phospholipase A2 receptor (anti-PLA2R)<sup>2</sup> and thrombospondin type 1 domain containing 7A (THSD7A)<sup>3</sup>. In a recent study, Sethi, et al, identified exostosin 1 (EXT1) and exostosin 2 (EXT2) as additional target antigens in MN<sup>4</sup>. While there are treatment recommendations for primary MN, including anti-PLA2R and anti-THSD7A associated MN, no treatment recommendations currently exist for EXT1 or EXT2 positive MN. We report a case of EXT2 positive MN and treatment with rituximab.

### Case Presentation

A male in his early 30s with hypertension for two years and obesity presented with bilateral lower extremity edema and proteinuria. The edema started in his calves and later spread to his feet, groin, and abdomen over four months. He acknowledged forgetting to take his blood pressure medicine, losartan with home blood pressures generally 140s/80-90s mmHg. The patient recalled being told of blood and protein in his urine as a teenager, without further evaluation. He denies any family history of renal disease.

He also reports prior history of heavy alcohol use of two beers per weekday and a 12 pack on weekends. He discontinued alcohol use one month prior to initial nephrology consultation. He smoked 1 pack of cigarettes per day since high school but recently quit about 2 months prior to consultation. He also reports cocaine use for 2 years, which he quit 7 months prior to presentation along with quitting marijuana.

On the initial nephrology he was well developed with BMI of 38.4kg/m<sup>2</sup> and BP of 146/92. Exam was remarkable for bilateral 3+ edema in lower extremities to his abdomen. Lungs were clear to auscultation and his heart sounds were regular with no murmurs. He had no skin rashes, oral ulcers, joint swelling, or tenderness.

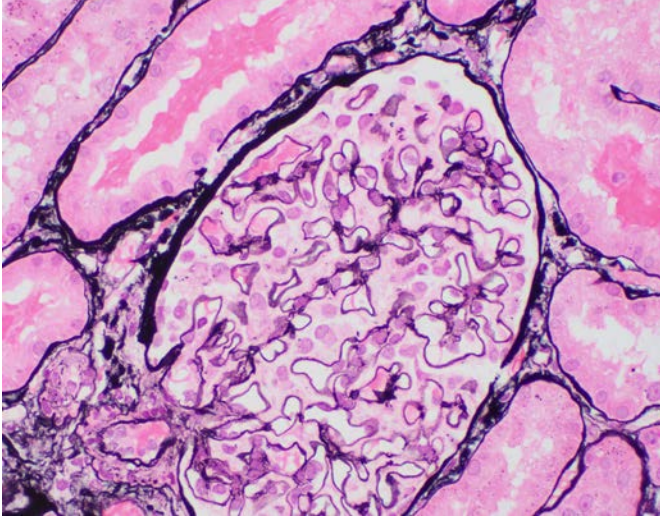
Laboratory testing included: Cr of 0.95 mg/dL, BUN 11mg/dL, with estimated glomerular filtration rate >89 mL/min/1.73m<sup>2</sup>. Serum calcium was 7.6 mg/dL, albumin 1.6 g/dL, and total protein 3.9 g/dL and cholesterol 512mg/dL. Urinalysis showed 13 red blood cells per high power field, with 4+ protein and albumin to creatinine ratio of 6862.4 and total protein to creatinine ratio of 10.1.

Antinuclear antibody was positive with titer of 1:320 in a speckled pattern. Anti-Smith and anti-ribonucleoprotein antibodies were positive. C-reactive protein was elevated at 1.8mg/dL and Sedimentation rate was elevated at 46mm/hour.

Negative labs included: for anti-PLA2R, anti-THSD7A, rheumatoid factor, anti-double stranded DNA, anti-Ro (SS-A), and anti-La (SS-B) antibodies. C3 and C4 were in the normal range. Immunofixation of serum and urine did not show monoclonal immunoglobulins. Immunoglobulin levels including IgG subclass 4 were not elevated. Hepatitis B surface antigen, hepatitis C antibody, rapid plasma regain test, and Mycobacterium tuberculosis Quantiferon Gold enzyme-linked immunosorbent assay test were also negative.

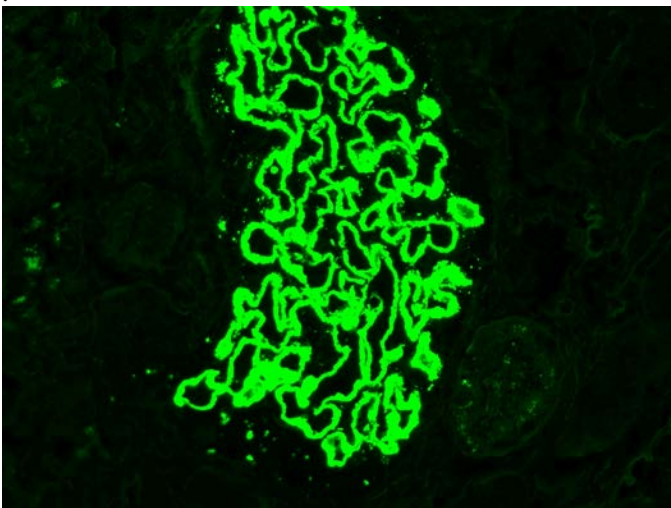
Renal biopsy was performed. Light microscopy revealed 23 glomeruli, one of which was globally sclerotic. The glomeruli were enlarged with focal mild mesangial hypercellularity.

Capillary loops showed numerous spikes and holes (Figure 1A).

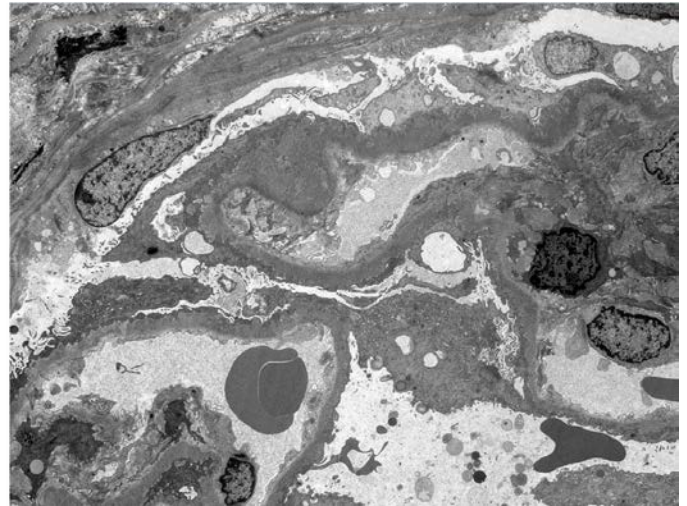


No segments of sclerosis or crescents were seen. Tubules showed patchy increased protein reabsorption droplets. There was no significant tubulointerstitial scar or inflammation. Arteries and arterioles were unremarkable.

On immunofluorescence microscopy glomeruli did not appear hypercellular. There was no significant tubulointerstitial inflammation or scar. Glomeruli showed global and granular, predominantly capillary loop staining for IgG (4+), IgA (trace), C3 (3-4+) and both light chains (2+ each). No significant staining was seen outside of the glomeruli. (Figure 1B)

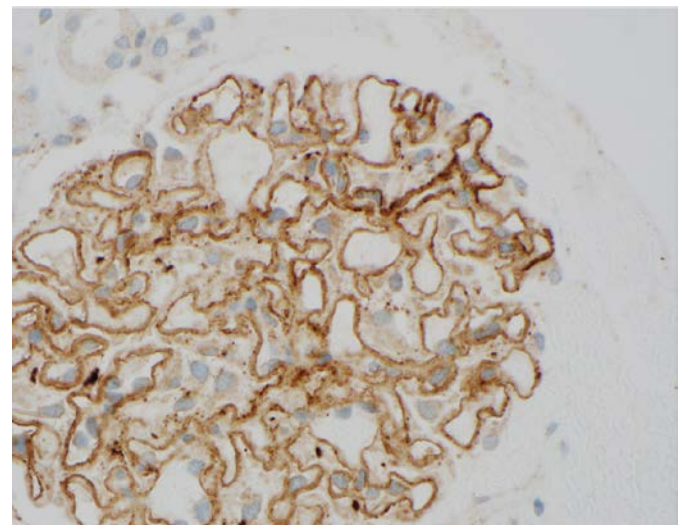


On electron microscopy, the glomeruli had normal cellularity with thickened capillary loops. There was no significant tubulointerstitial scar or inflammation. There was diffuse podocyte foot process effacement overlying numerous subepithelial deposits (Figure 1C).



Basement membranes showed extensive remodeling with spike formations interposition of basement membranes between deposits. The endothelium was attached and fenestrated. No tubuloreticular inclusions or subendothelial deposits were seen. Deposits extended along paramesangial basement membranes with limited mesangial involvement. Deposits were not seen outside of the glomerulus.

EXT2 staining demonstrated scattered positivity within subepithelial deposits. Figure 1 D.



Staining for PLA2R and THSD7A were negative. Overall, renal biopsy was suggestive of MN, stage 2 of 4, with PLA2R stain negative, THSD7A stain negative, and EXT 2 stain positive.

Computed tomography (CT) of the chest showed no evidence of intrathoracic malignancy but mild pulmonary edema and subcutaneous edema as well as small bilateral pleural effusions. CT of the abdomen and pelvis showed no masses or morphologically abnormal lymph nodes in the abdomen and pelvis. Imaging showed volume overload with small ascites, gallbladder wall and subcutaneous soft tissue edema.

**Treatment**

Patient was also seen by rheumatology and started on prednisone 20mg daily with a taper and hydroxychloroquine

200mg two times daily. Due to continued nephrotic range proteinuria, patient agreed to be treated with rituximab, two 1 gram doses, given fourteens days apart. Prior to each rituximab infusion patient was given methylprednisolone premedication of 100mg intravenously.

By five months after the second rituximab infusion, the patient’s urine protein creatinine ratio had decreased from 8.1, prior to rituximab treatment, to 1.7. His serum albumin level increased from 1.0 g/dL to 3.2 g/dL. (Figure 2). His lower extremity edema also clinically improved.

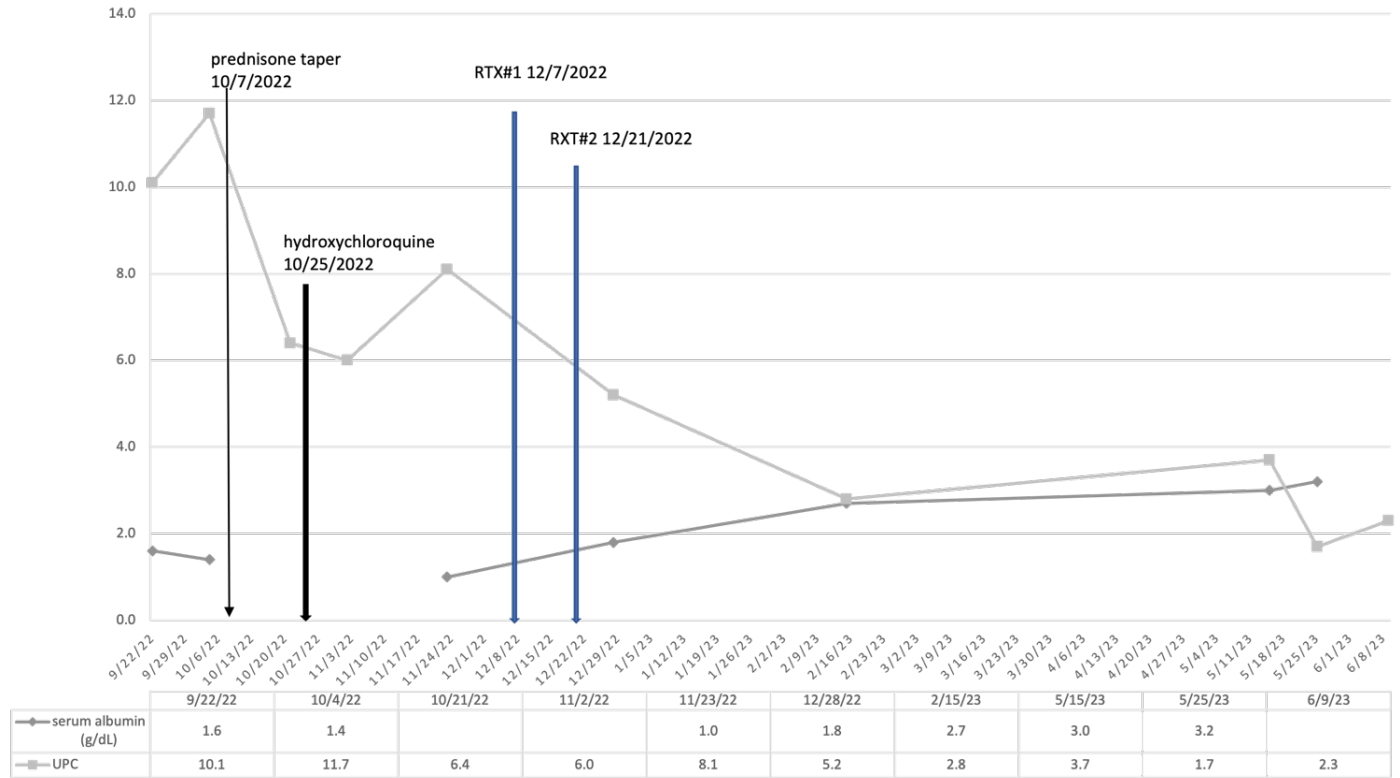


Figure 2

**Discussion**

Membranous nephropathy is the most common cause of idiopathic nephrotic syndrome in non-diabetic adults. While 80% of cases are primary, 20% are secondary to other causes. About 70-80% of primary cases show positivity to anti-PLA2R antibody, with about 2-7% of cases positive to anti-THS7DA antibody. 80% of patients with primary MN present with nephrotic syndrome. In untreated primary MN patients, about one third undergo spontaneous remission, one third progress to end stage renal disease, and the rest develop chronic kidney disease.<sup>1</sup>

Sethi, et al. 2019 identified a subset of MN, negative for PLA2R, with an accumulation of EXT1 and EXT2 in the glomerular basement membrane (GBM). Given the staining pattern of EXT1 and EXT2 are similar to the granular IgG

staining along the GBM, Sethi, et al. deduced that EXT1 and EXT2 are likely antigens in the immune complex. Clinical features and renal biopsy findings of EXT1 and EXT2 are present in patients with autoimmune disease such as lupus. Thus, EXT1 and EXT2 are suspected to be target antigens in secondary autoimmune MN.<sup>4</sup>

The patient had positive SLE-associated serologies including ANA, Smith and RNP antibodies, raising the initial possibility of membranous lupus nephropathy. Complicating the argument for SLE was the lack of both extrarenal SLE manifestations and typical lupus nephritis renal biopsy features including subendothelial deposits, tubuloreticular structures in glomerular endothelial cells and “full house” immunofluorescence staining i.e., positivity for IgA, IgM, IgG, C3 and C1q.

A key histological feature was the presence of EXT2 staining on renal biopsy. EXT2 positive staining in the context of positive SLE serologies raised the possibility of lupus membranous nephropathy, despite absence of other histological features of lupus nephritis.

Initial recommended treatment for MN is supportive care with blood pressure control using renin-angiotensin system inhibitors and evaluation for risk of progression of renal disease. Patients with continued elevated anti-PLA2R or anti-THSD7A levels, and proteinuria more than 3.5 grams per day, after 6 months of supportive care, or those with complications of nephrotic syndrome are recommended to be treated with immunosuppressive therapy. Immunosuppressive treatment options for primary MN include alternating courses of steroids and cyclophosphamide<sup>5</sup>, calcineurin inhibitor, or B cell depletion.<sup>1</sup> The Membranous Nephropathy Trial of Rituximab (MENTOR) trial showed that rituximab was noninferior to cyclosporine in inducing proteinuria remission at 12 months and superior in maintaining long-term proteinuria remission up to 24 months in patients with MN at high risk for progressive disease<sup>6</sup>.

Regarding treatment of lupus MN, expert opinion suggests that presence of nephrotic syndrome is an indication to initiate immunosuppressive treatment. First-line treatment in most cases consists of mycophenolate mofetil, cyclophosphamide or calcineurin inhibitor in combination with glucocorticoids<sup>7</sup>. Rituximab is considered an alternative treatment if first-line therapies are not effective or not tolerated<sup>8</sup>.

Our patient with EXT 2 positive membranous nephropathy, was treated with rituximab and demonstrated improved proteinuria and clinical parameters. As there is no established recommended immunosuppressant treatment for EXT2 associated MN, this case provides guidance in developing of treatment regimens for EXT2 positive MN.

### Disclosure

Verbal and written consent was obtained from the patient for this case presentation.

The authors have no conflicts of interest to disclose.

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