Brigham and Women's Hospital
Founding Member, Mass General Brigham

RENAL PATHOLOGY: NEPHROTIC SYNDROME

(Part II; 2024)

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Assistant Professor of Pathology, Harvard Medical School

Renal pathologist, attending on the BWH diagnostic renal pathology service since 2011

I direct an active translational research program studying proteinuric kidney diseases

My research focuses on understanding the pathobiology of acute nephrotic syndrome and podocyte injury by integrating morphologic observations with imaging and tissue interrogation techniques

Disclosures (last 12 months)

- Patent

  “Methods for identifying and treating patients with antibody-mediated acquired primary or recurrent idiopathic nephrotic syndrome”

- Speaker fees

  Sysmex

  Novartis

- Advisory Board

  Vera Therapeutics

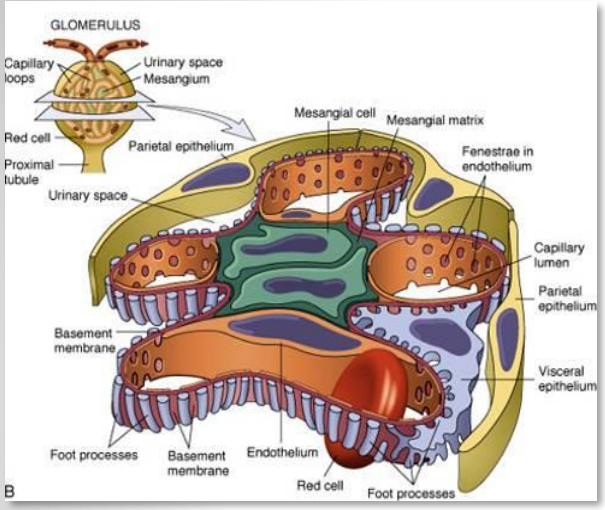
- Funding

  NIDDK, DoD

Objectives

- Brief review of glomerular structure
- Use a case-based discussion to highlight histopathologic findings in diffuse podocyte injury
- Correlate histopathologic findings with known etiologies in Minimal Change Disease and Membranous Nephropathy

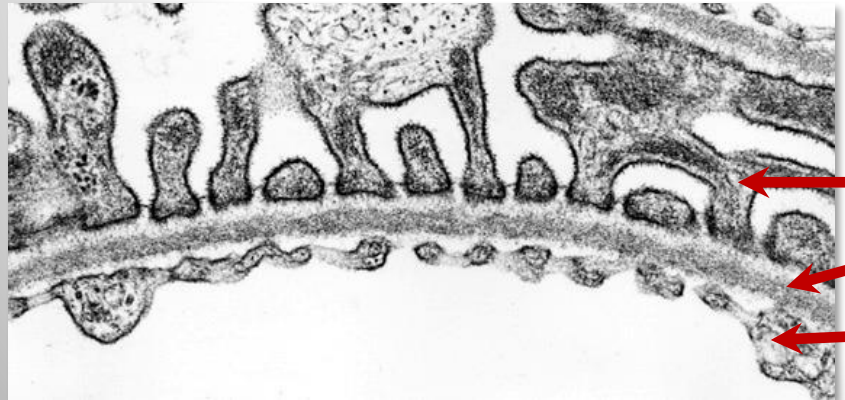
Glomerular Structure - Recap



Cells: Endothelium
Epithelium (visceral and parietal)
Mesangium

Space: Capillary lumen
Urinary space

Matrix: Glomerular Basement Membrane
Mesangial Matrix



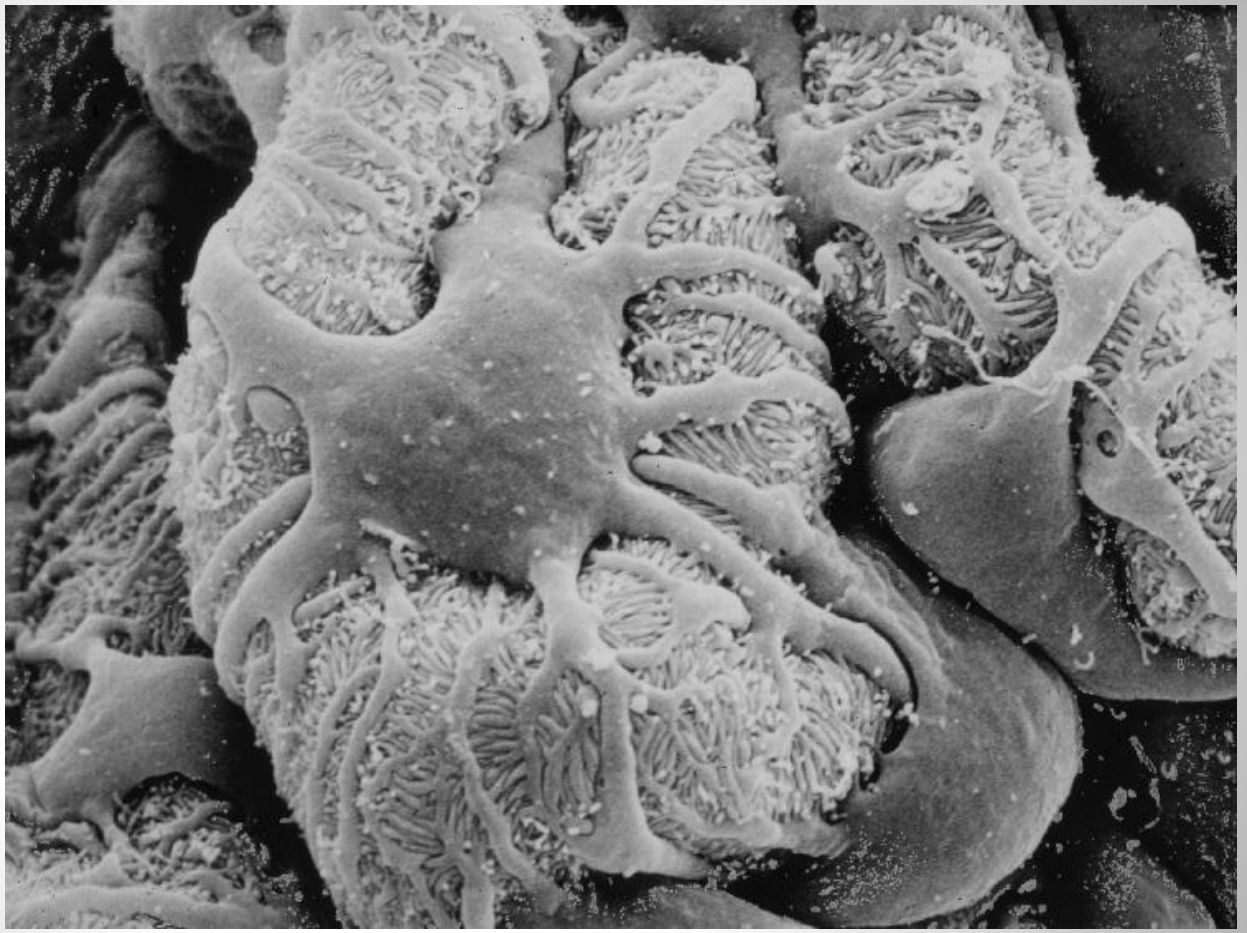
Podocyte

Basement Membrane

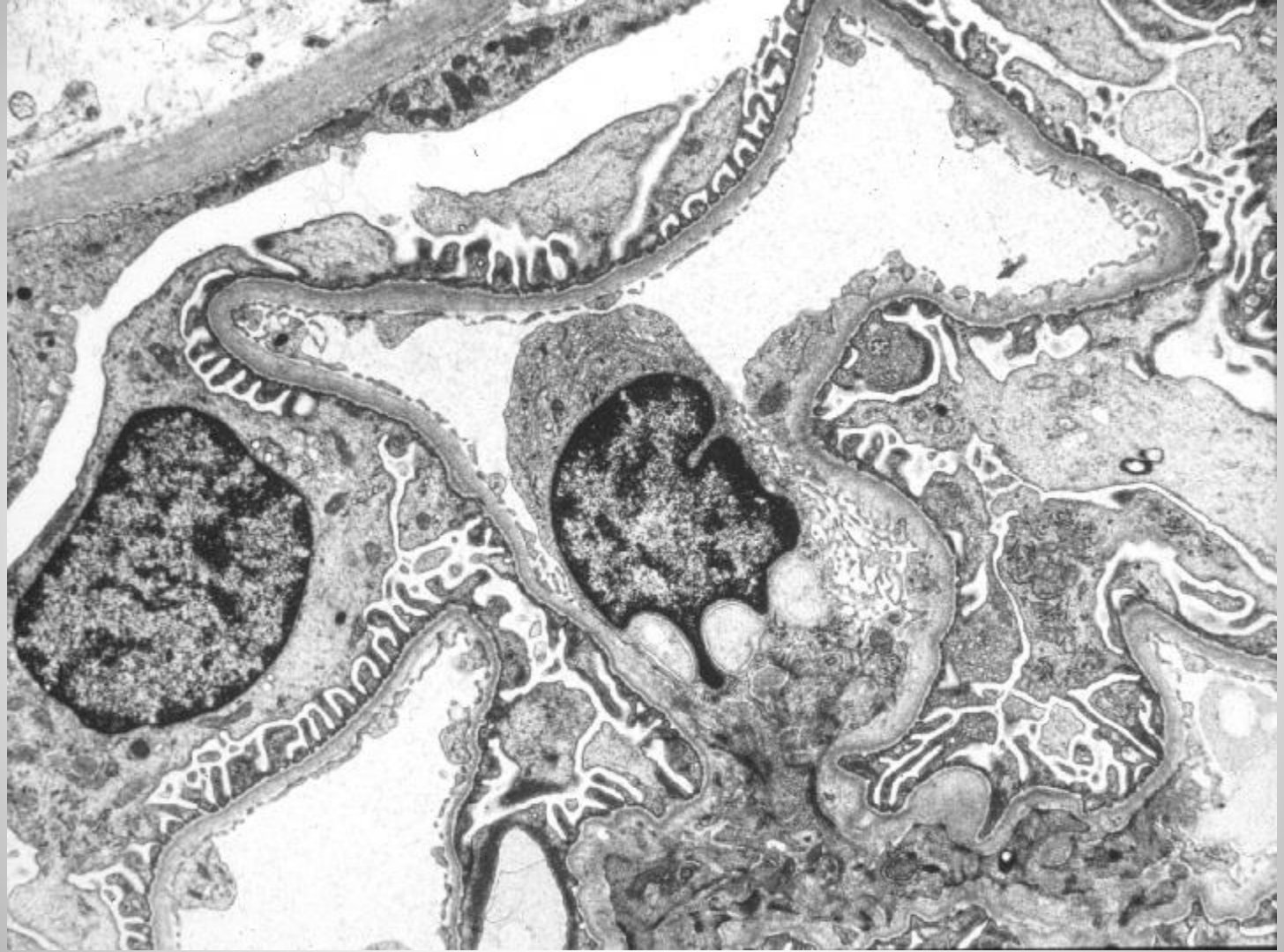
Endothelial cell

The podocyte –

a terminally differentiated, postmitotic epithelial cell enveloping the glomerular capillaries



SEM

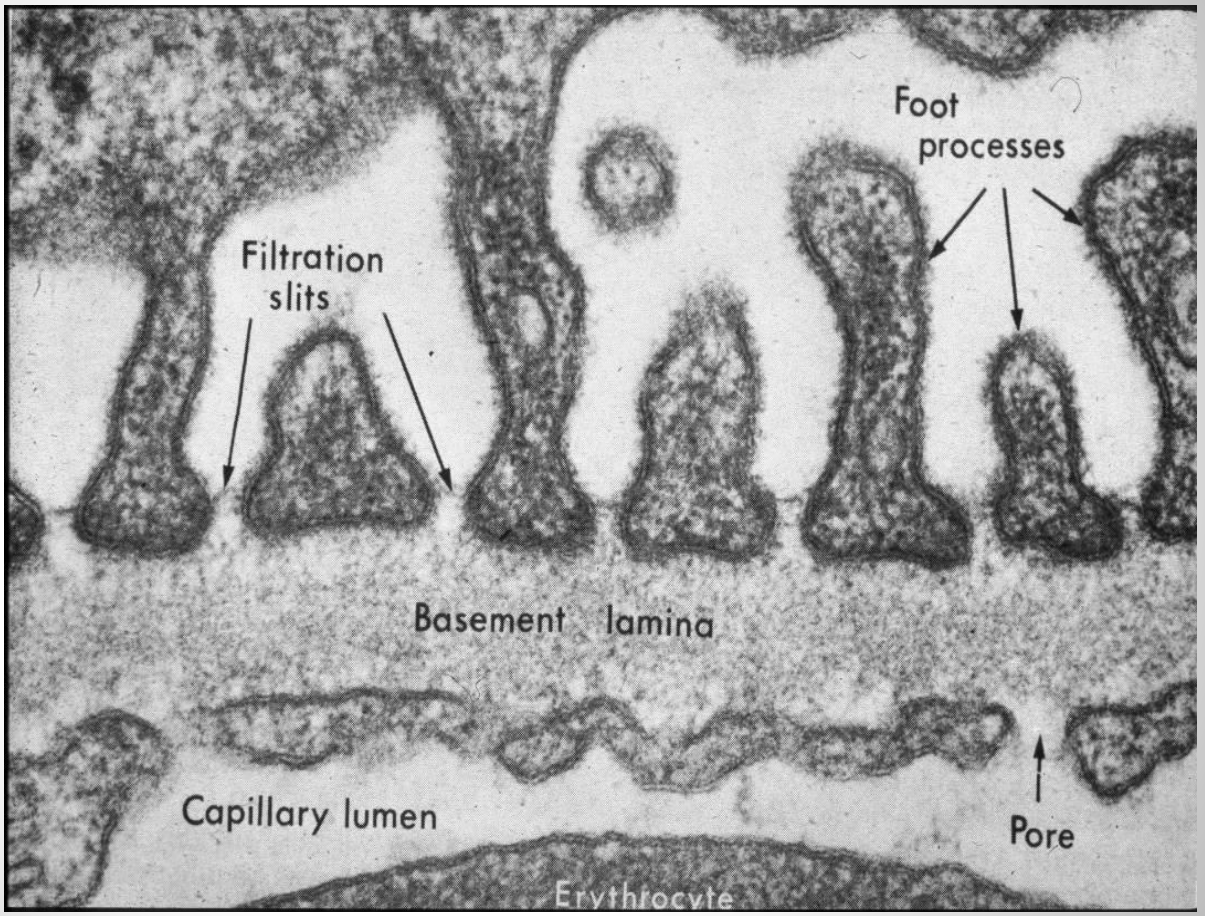


TEM

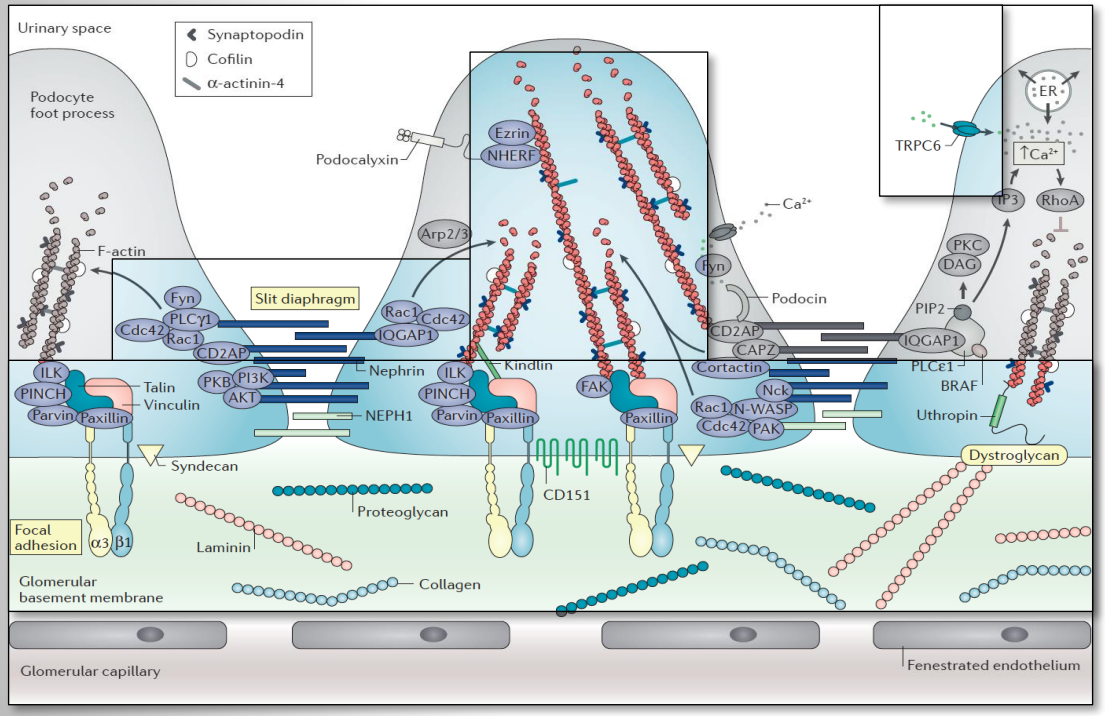
High power cross section of the glomerular filtration barrier

Elements:

1. Fenestrated and charged endothelium
2. Dense GBM
3. Podocyte foot processes with slit diaphragms



Molecular architecture of podocyte foot processes



Slit diaphragm complex

Nephrin
Podocin
Neph1-3
CD2AP

Actin cytoskeleton

ACTN4
Synaptopodin
Formins
Rac1

Cell-Matrix Interaction

Integrins/Focal Adhesions
Components of the GBM

Channels

TRPC6, TRPC5

Perico et al, Nat Rev Nephrol. 2016 Nov;12(11):692-710

Diseases with direct and diffuse podocyte injury and/or loss:

“PRIMARY” PODOCYTOPATHIES

1. Minimal Change Disease
2. Primary (Idiopathic) Focal and Segmental Glomerulosclerosis
3. Collapsing Glomerulopathy

(4. Membranous Nephropathy)

Case 1

This 41-year-old man from Brazil presents to the ED with chief complaint of a sore throat. A diagnosis of a URI is made.

Two weeks later, he notices swelling in his legs. This quickly progresses to total body swelling.

He is referred to your clinic by his PCP with 4+ proteinuria on dipstick. An UA shows a protein-to-creatinine ratio of 15 g/gCr.

He denies NSAID use, use of any other medication or use of illicit drugs.

There is no significant past medical history.

Physical examination:

Unremarkable, except for a body weight of 237 lbs (up from 220lbs 3 months ago), 2+ edema of LE

U/A :

1+ blood, 3+ protein; the sediment shows 0-5 RBC/hpf, 0 WBC/hpf, no cellular casts, few hyaline casts

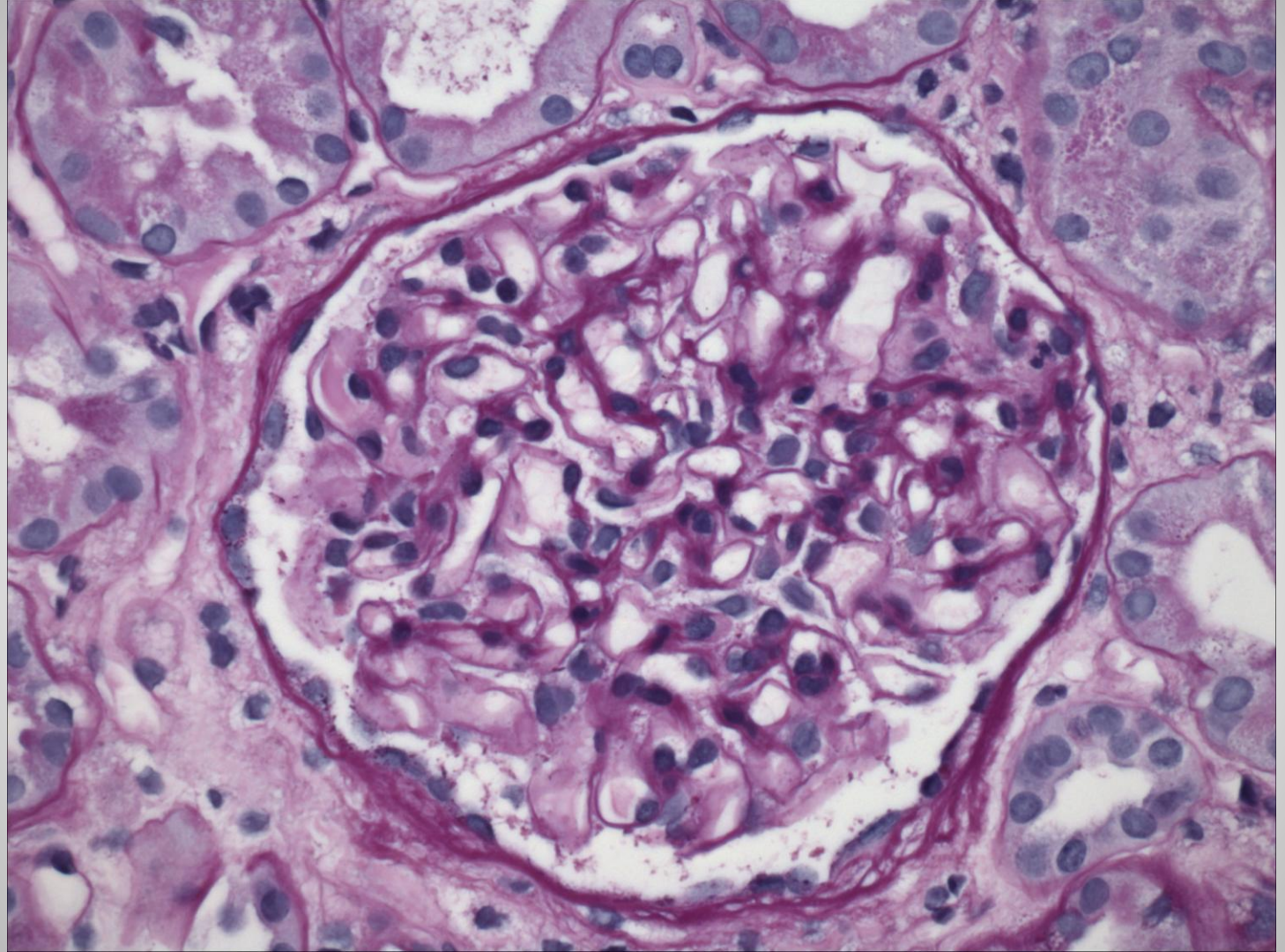
Laboratory testing:

Hgb 17.7, WBC 7.1K, Plt 373; Na 139, K 4.6, Cl 107; Creatinine 1.5, BUN 14, glucose 90; Ca 7.6, TSH 6.93.

ANA negative, ASO negative, ESR 121

An SPEP and UPEP are negative for paraproteins.

A **kidney biopsy** is performed.



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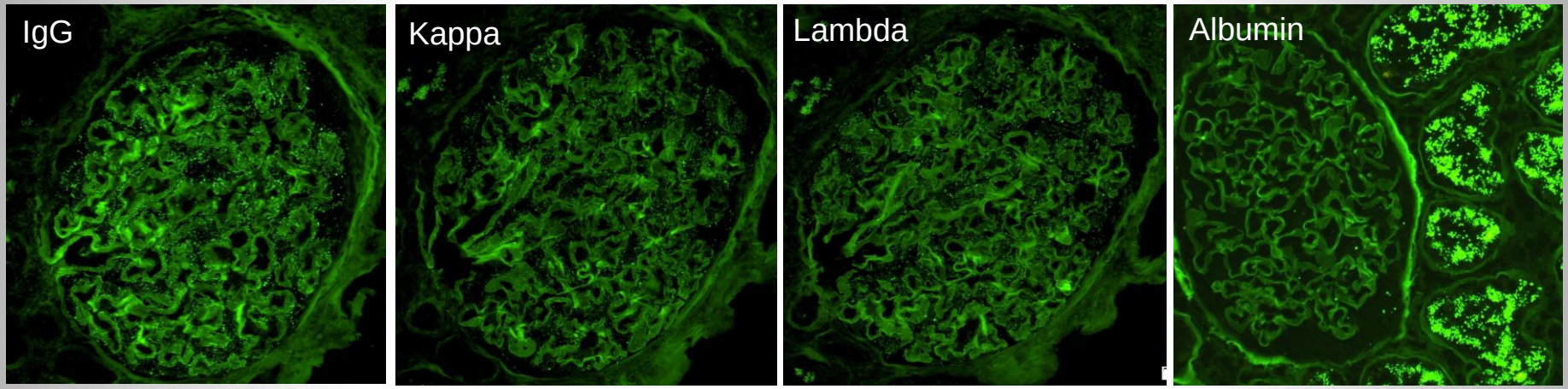

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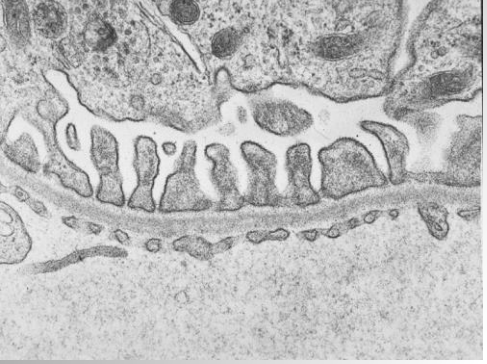

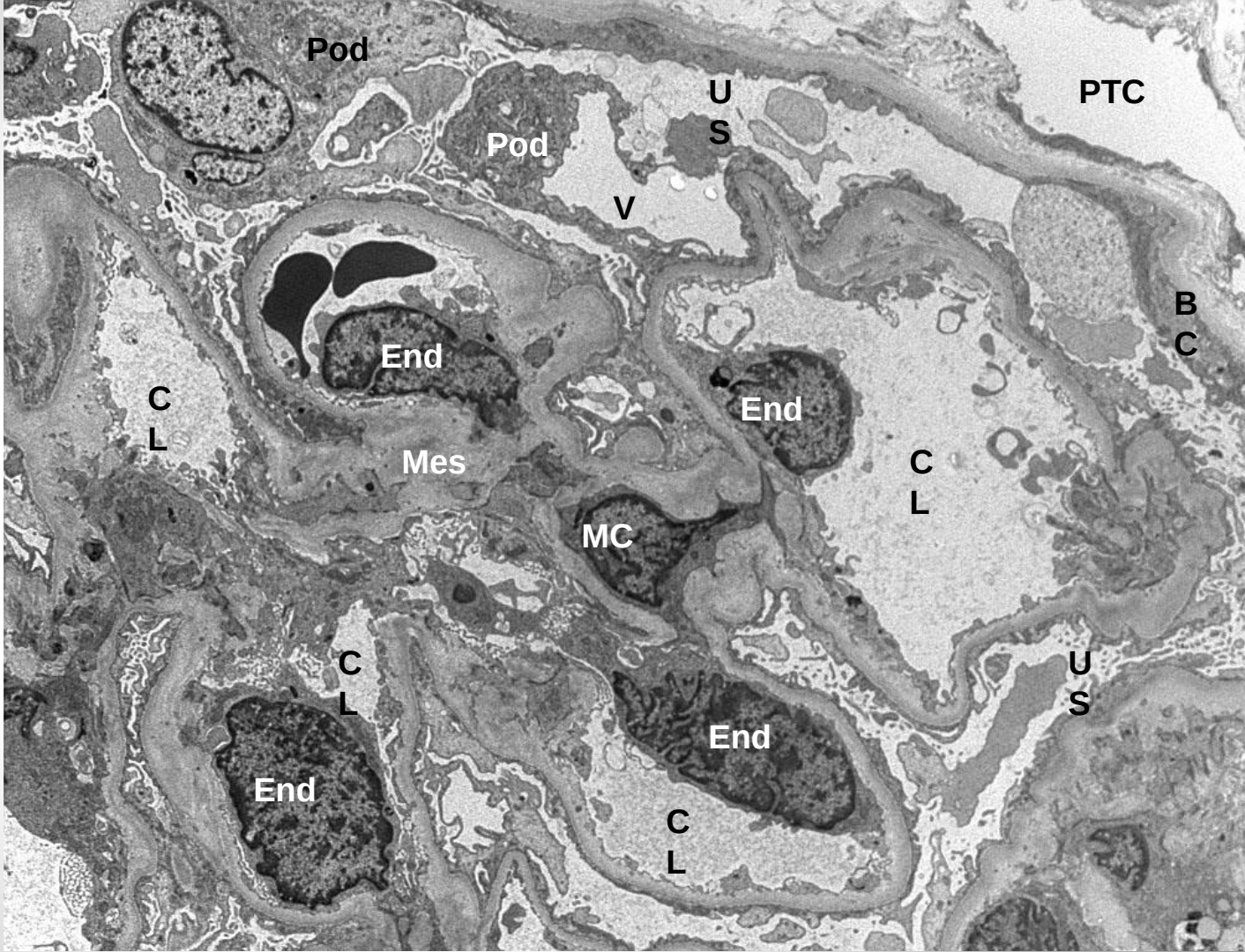

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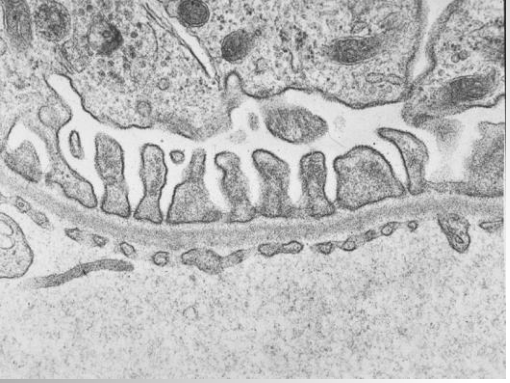

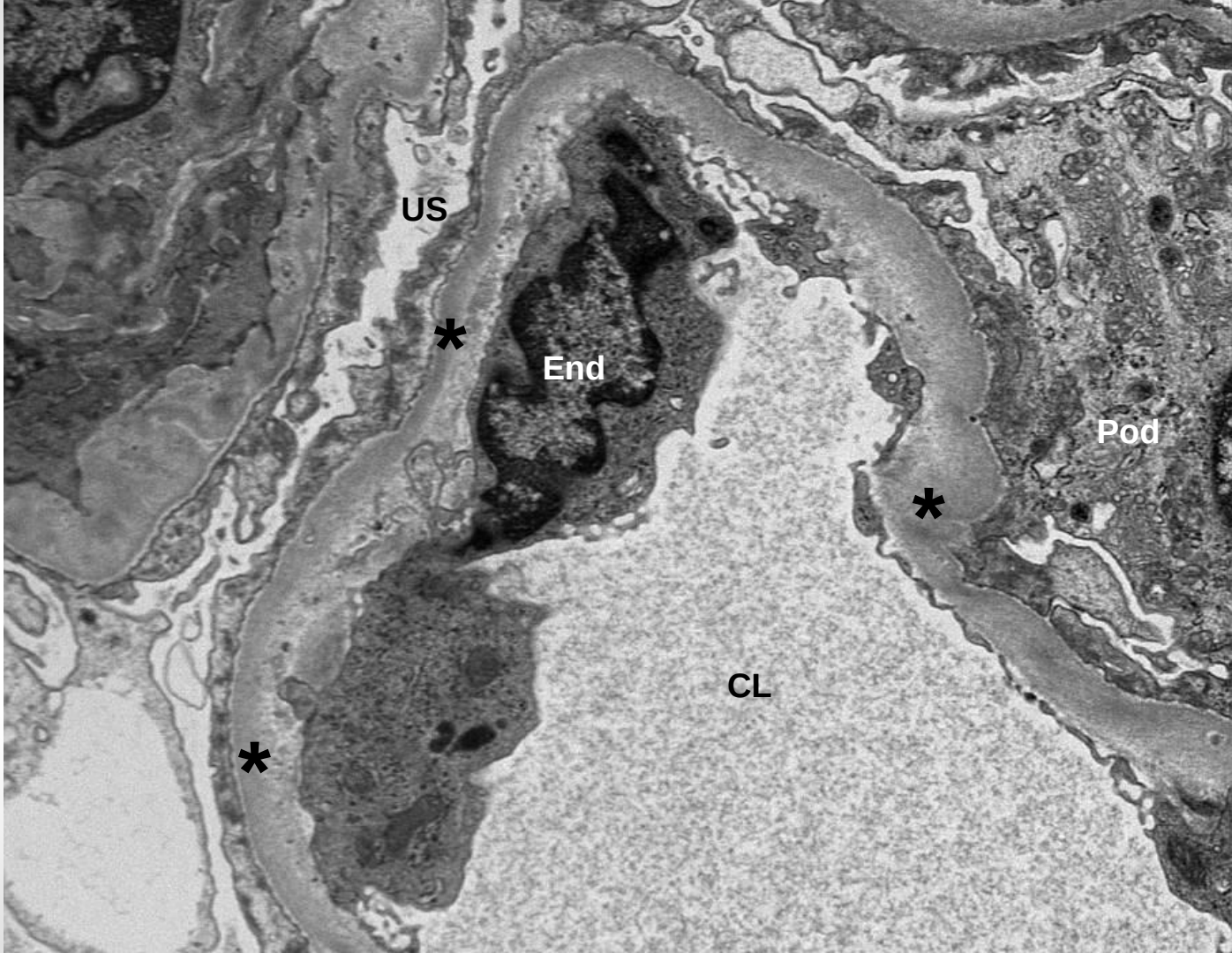

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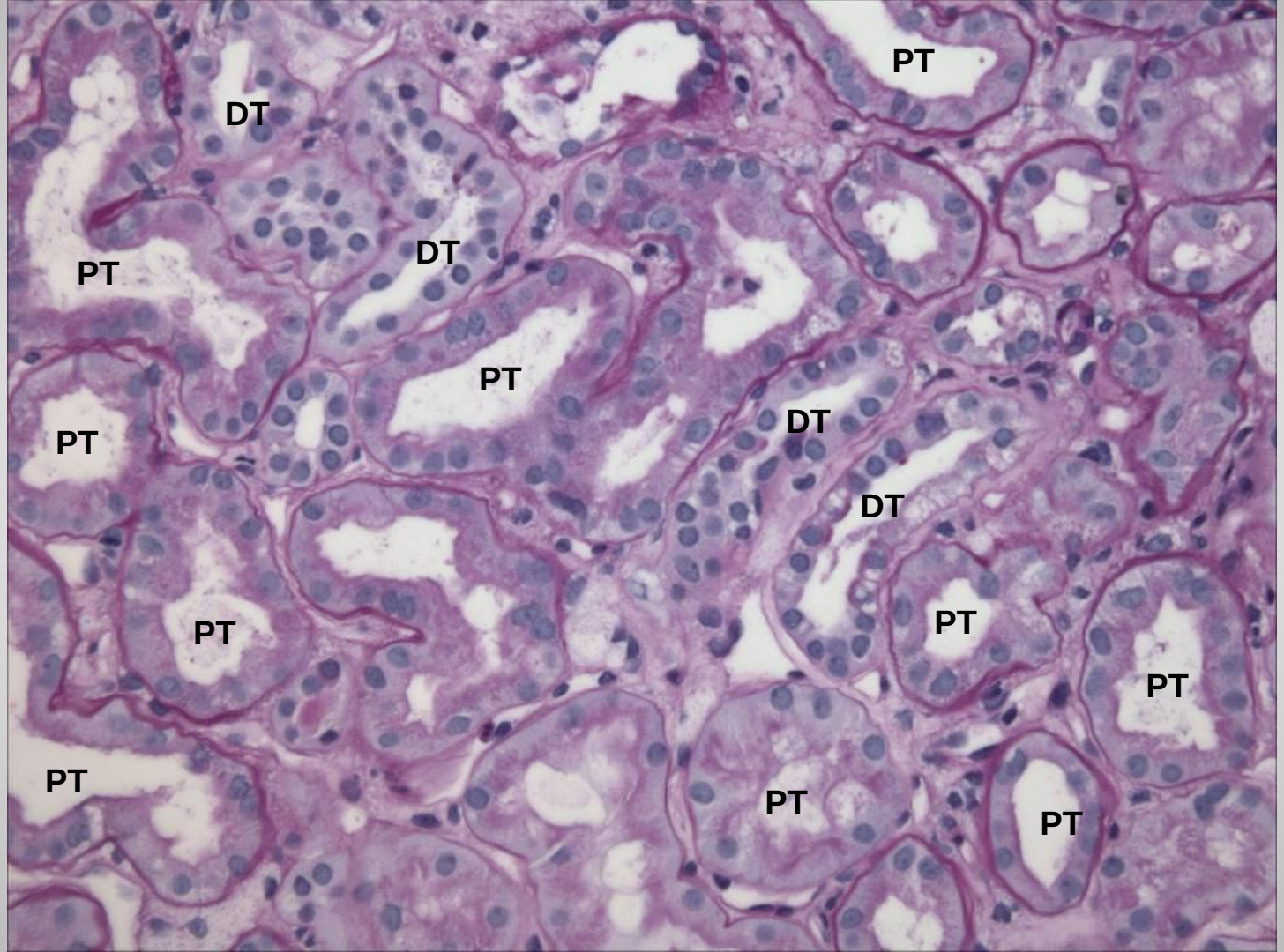

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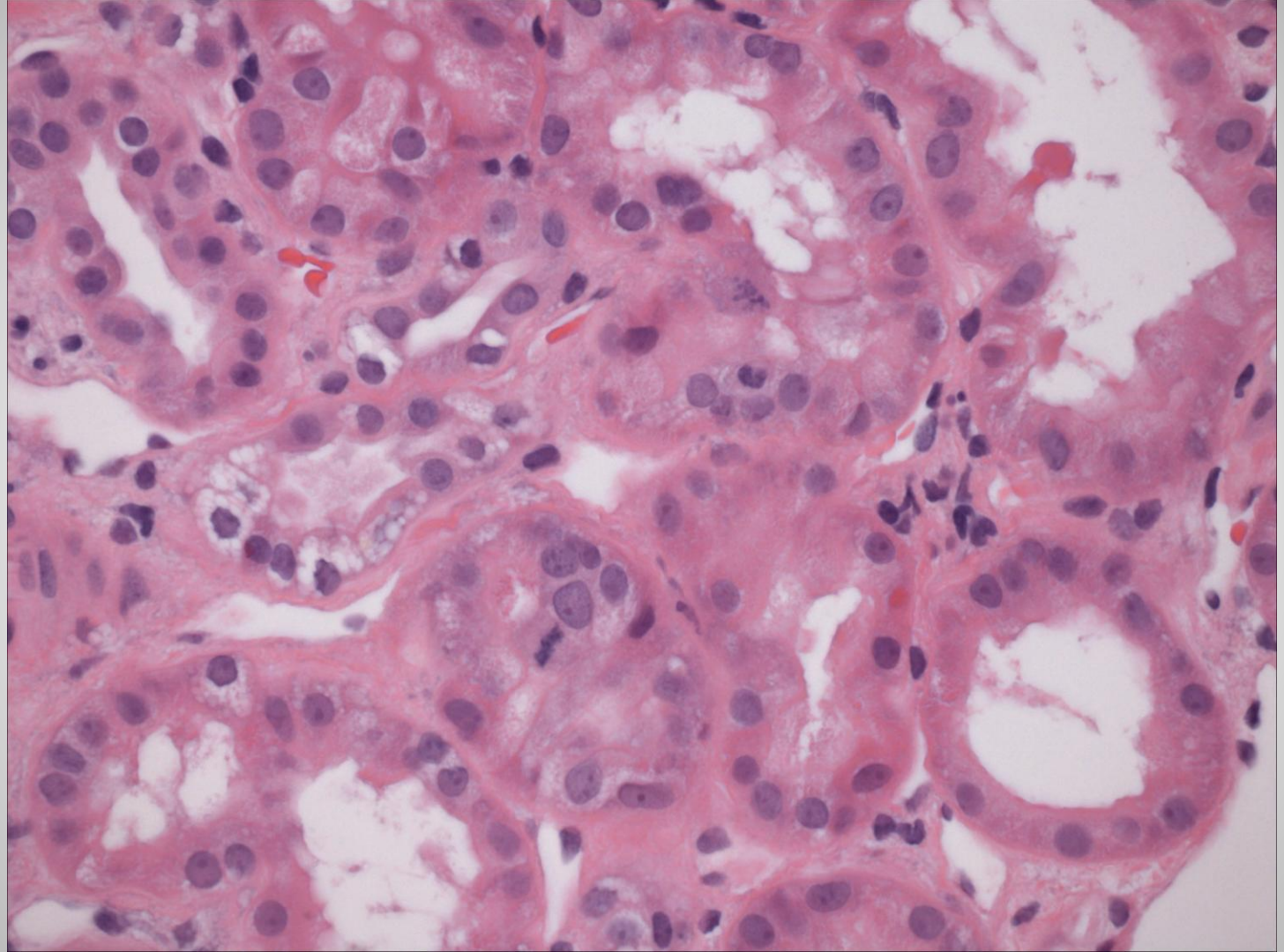


H&E

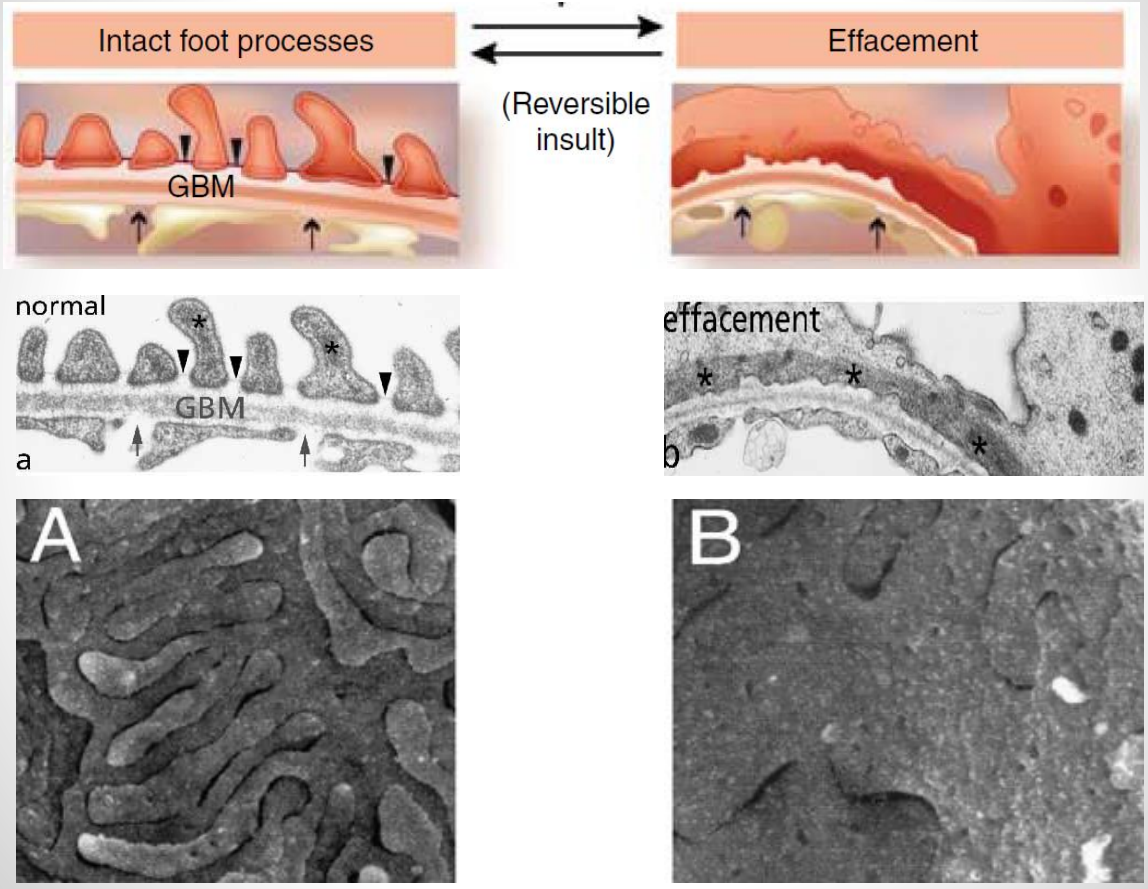
Diagnosis

Diffuse podocytopathy with minimal glomerular changes

(Minimal Change Disease)

Acute Tubular Injury

Podocyte injury leads to generally reversible structural changes



(adapted from d'Agati et al., 2003)

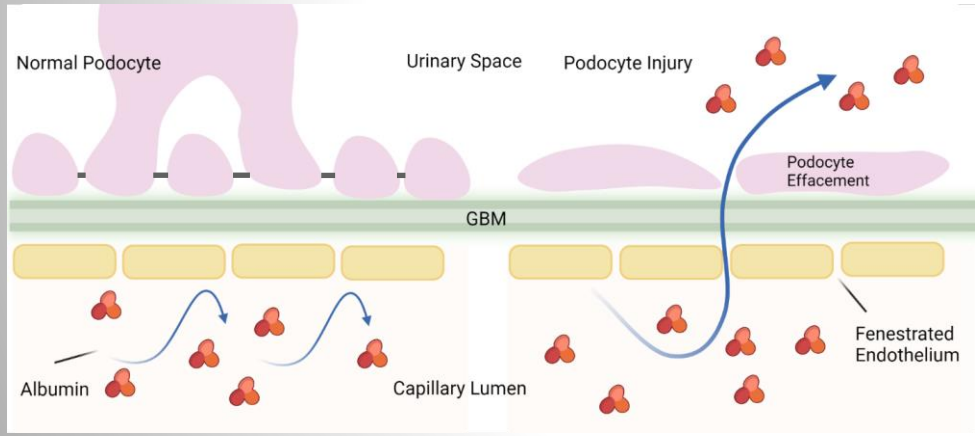
The consequence of these alterations is severe albuminuria

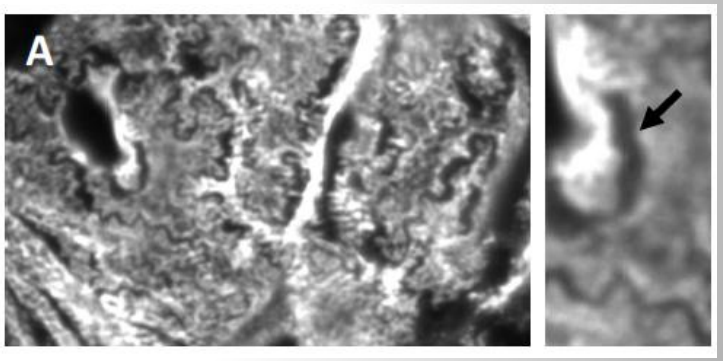
Foot process effacement and loss of filtration surface

Focal defects in podocyte coverage

Increased hydraulic conductivity and ineffective reflection

→ **albuminuria**





Based on: Rennke HG and Denker BM. Renal Pathophysiology. The Essentials. Wolters Kluwer 2020.

Evidence for Autoimmunity

- **Corticosteroids** are effective at inducing remission, especially in children, yet relapse and steroid dependence are common, and in some patients, additional immunosuppressive agents are successfully used.

- **B-cell targeted therapies** (Rituximab) are effective in both adults and children with relapsing or steroid-dependent NS**,** suggesting a **potential autoantibody mediated etiology**.

- **Memory B cells** are selectively increased in patients with SSNS

- Further evidence for **B-cell involvement:** MCD was cured by measles, a disease that leads to B cell depletion and immune amnesia

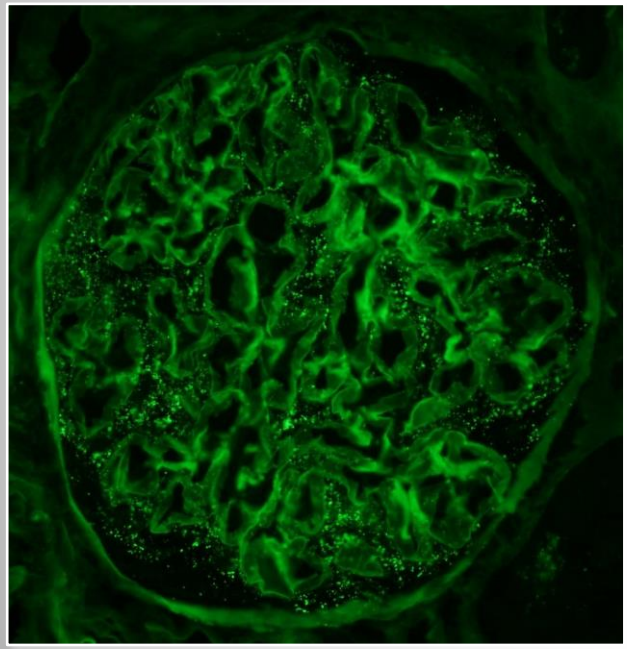
Elie et al, Ped Nephrol 2012. Coward et al, JASN 2005. Ravani et al, NTA 2017. Basu et al, JAMA 2018. Fenoglio, Oncotarget 2018.

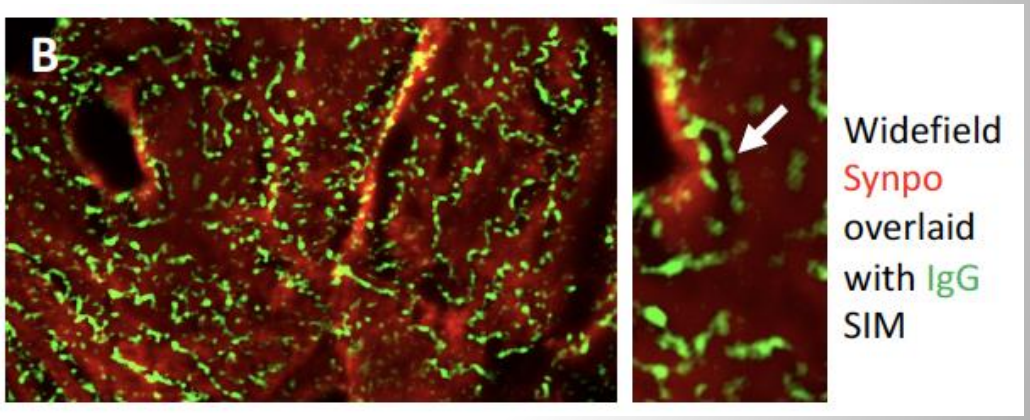
Proposed antigenic podocyte targets in MCD

Antigen	Localization	Method of discovery	References
Ubiquitin Carboxyl Terminal Hydrolase L1 (UCHL1)	Cytoplasmic and nuclear (not expressed in normal glomeruli)	Proteomic Analysis	*Jamin et al, J Autoimmun 2018;* *Chebotareva et al, Front Med 2023*
Annexin A2 (ANXA2)	Cytoplasmic and membrane bound, phospholipid-binding	2D electrophoresis and Mass Spec	*Ye et al, Ann Transl Med, 2021*
Crumbs cell polarity complex component 2 (CRB2)	Junctional transmembrane/slit diaphragm	Mouse model only	*Hada et al, JASN 2022*
CD40	Cell membrane/ligand	Protein array (rFSGS)	*Delville et al, Sci Transl Med 2014;* *Chebotareva et al, 2023*
Nephrin	Transmembrane/slit diaphragm	Immunostaining and ELISA/IP	*Watts, Keller et al, JASN 2022* *Hengel et al, NEJM, 2024; KI 2025*
Podocin Neph1/Kirrel1	Transmembrane/slit diaphragm	STED, ELISA	*Raglianti et al, KI 2024, JASN 2025*

Evidence for IgG in our MCD biopsies

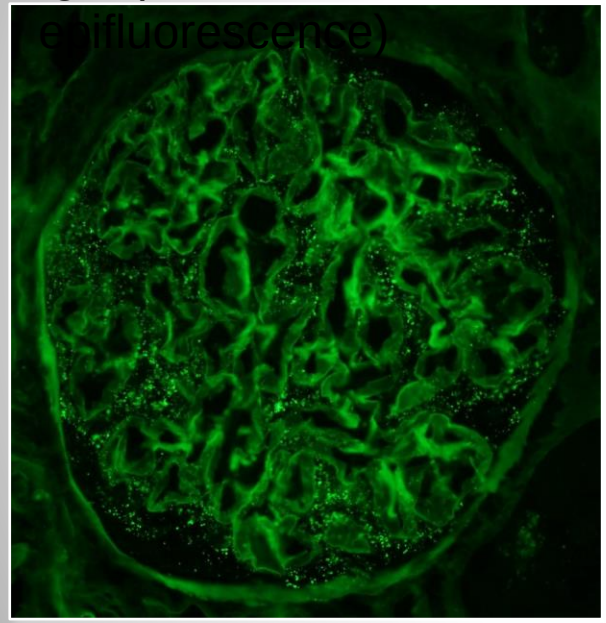
- Over many years, we have observed a **delicate extracapillary, punctate and diffuse glomerular staining for IgG** in ~64% of our MCD biopsies.



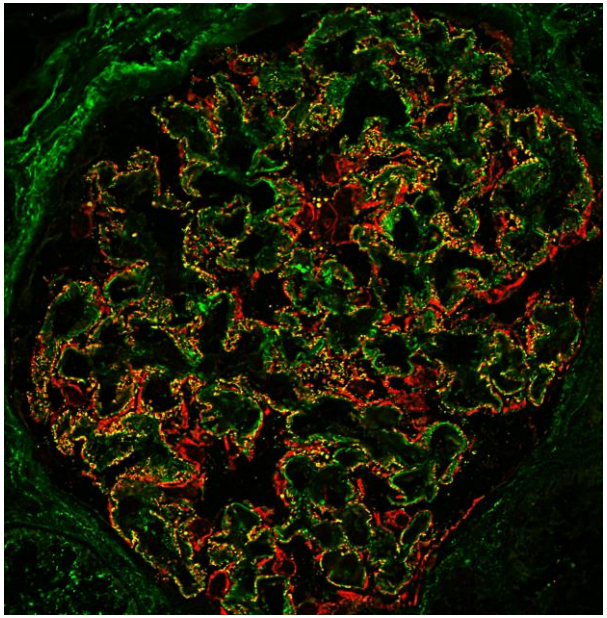


Punctate IgG staining in MCD biopsies colocalizes with nephrin

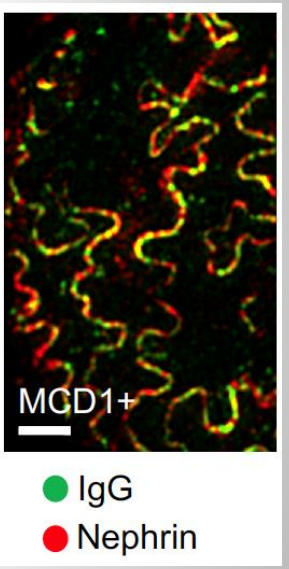
IgG (routine epifluorescence)



IgG/nephrin (confocal)



SIM



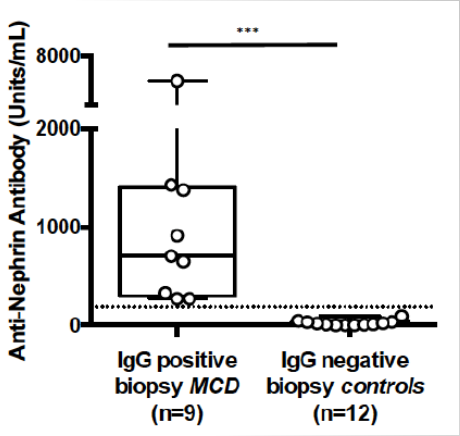
Watts, Keller et al, JASN 2022

Anti-nephrin antibodies are in circulation

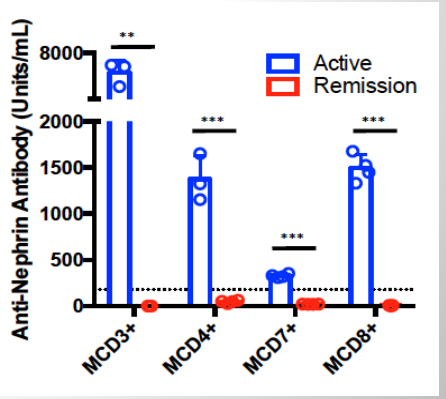
We obtained **serum during active disease and tested for anti-nephrin by indirect ELISA** on some of our in-house patients.

All IgG+ MCD patients (MCD+) were **positive for anti-nephrin** antibodies.

In contrast, all non-nephrotic or nephrotic control patients, including 3 IgG- MCD patients, were serologically negative.



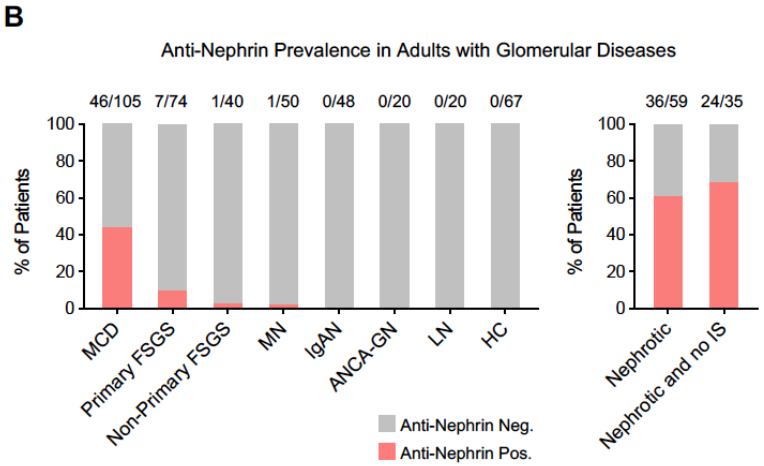
In all patients in whom we were also able to obtain a **remission** sample, anti-nephrin antibody levels were significantly **reduced**.

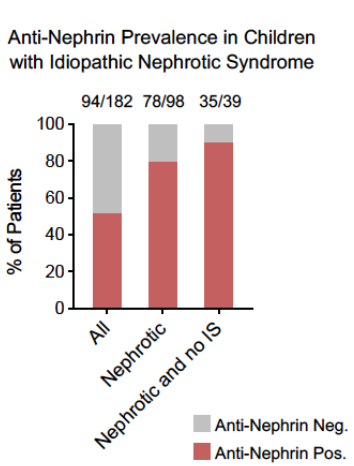


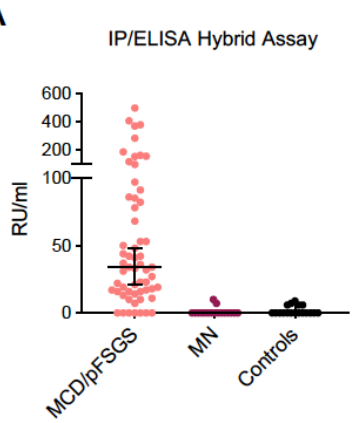
Watts, Keller et al, JASN 2022

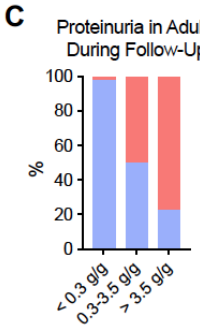
High % of anti-nephrin positivity in adults and kids

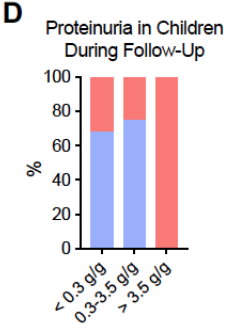
Hengel et al, NEJM 2024

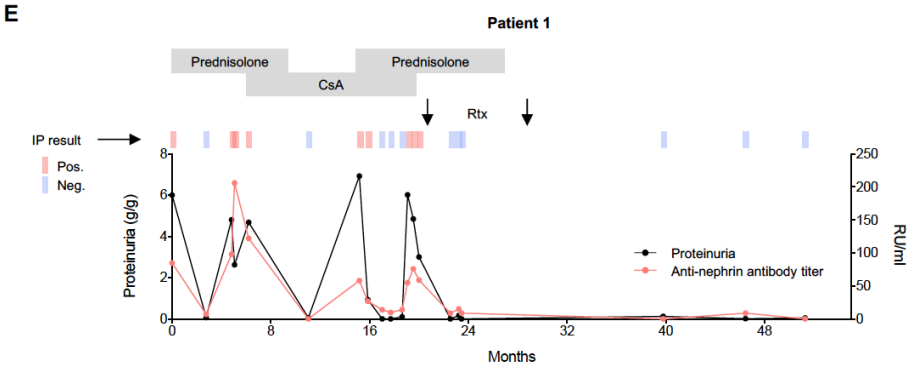




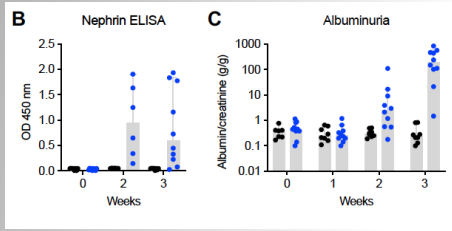


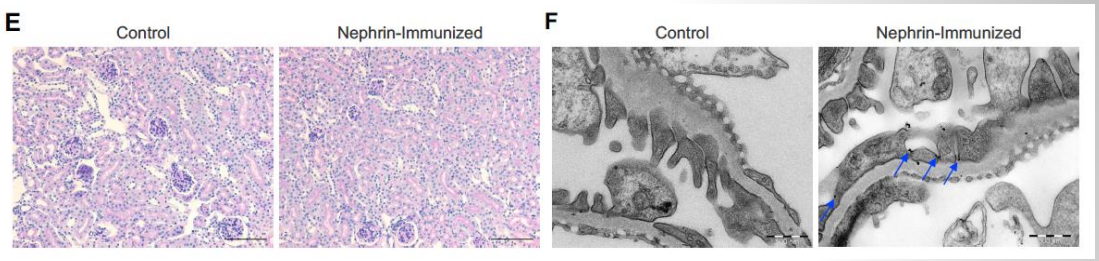


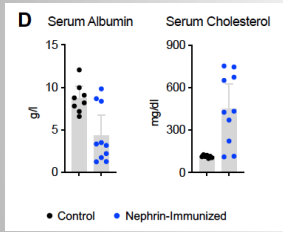


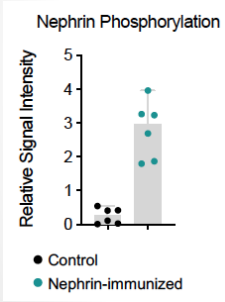


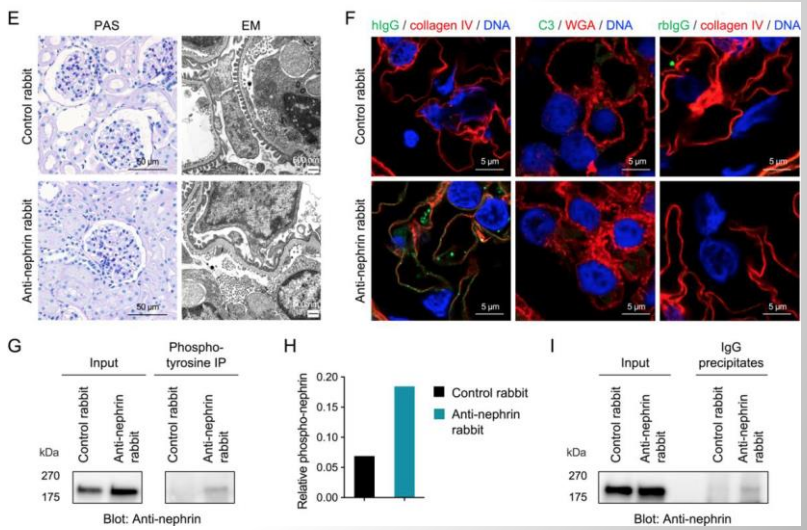
Proof of causality in immunization and passive transfer mod











Hengel et al, NEJM 2024; Hengel et al, KI 2025

Patient follow up

Several months later…

Chief complaints:

- Persistent lower extremity edema, otherwise normal physical exam
- Hyperlipidemia

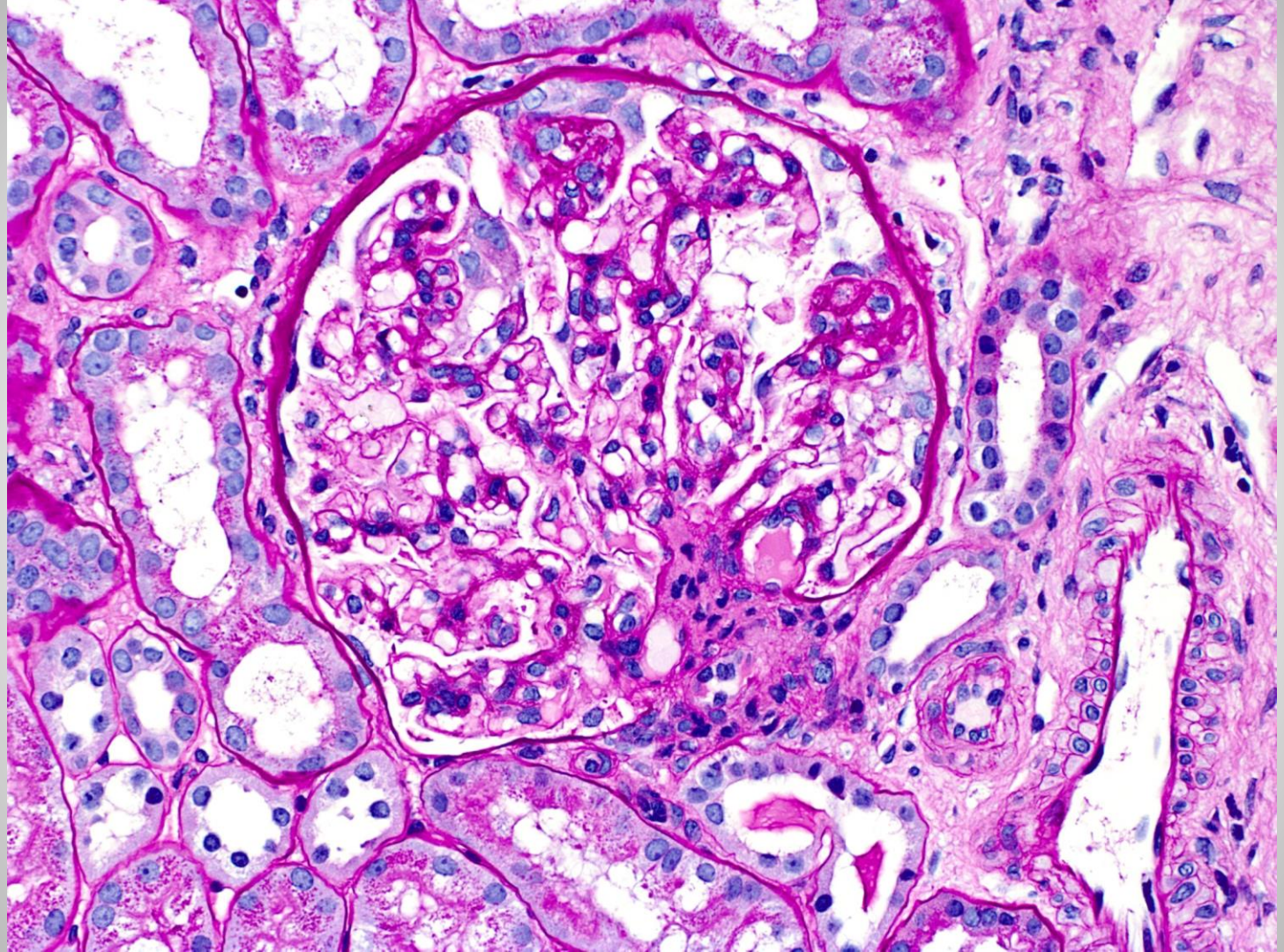
Blood:

- Creatinine: 0.8 mg/dl
- BUN: 15 mg/dl
- Glucose: 85 mg/dl
- Serum Albumin: 3.1 mg/dl

Urine:

- Urine protein 3.6 g/24h
- No hematuria

- All other tests and serologies were still negative



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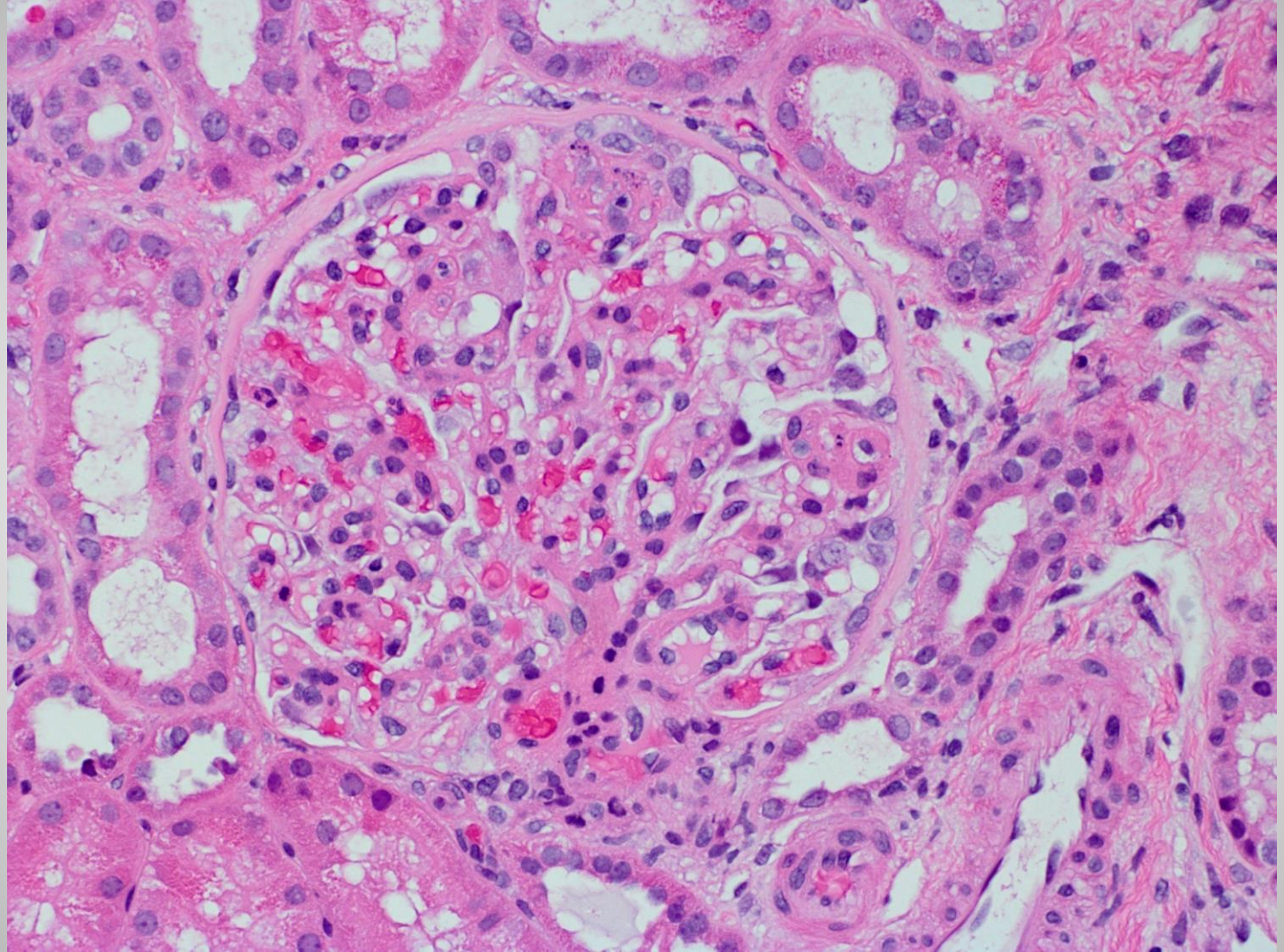
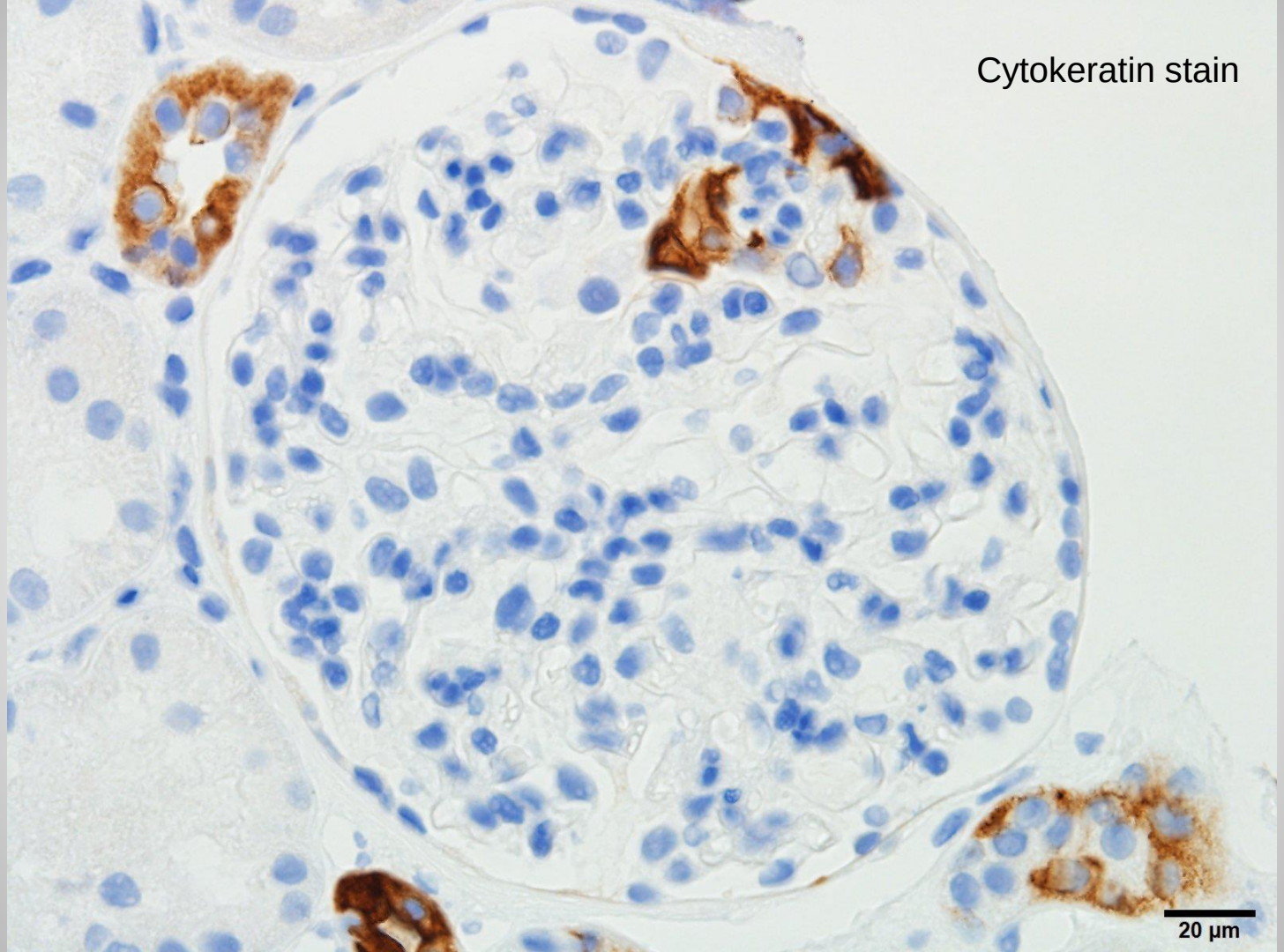
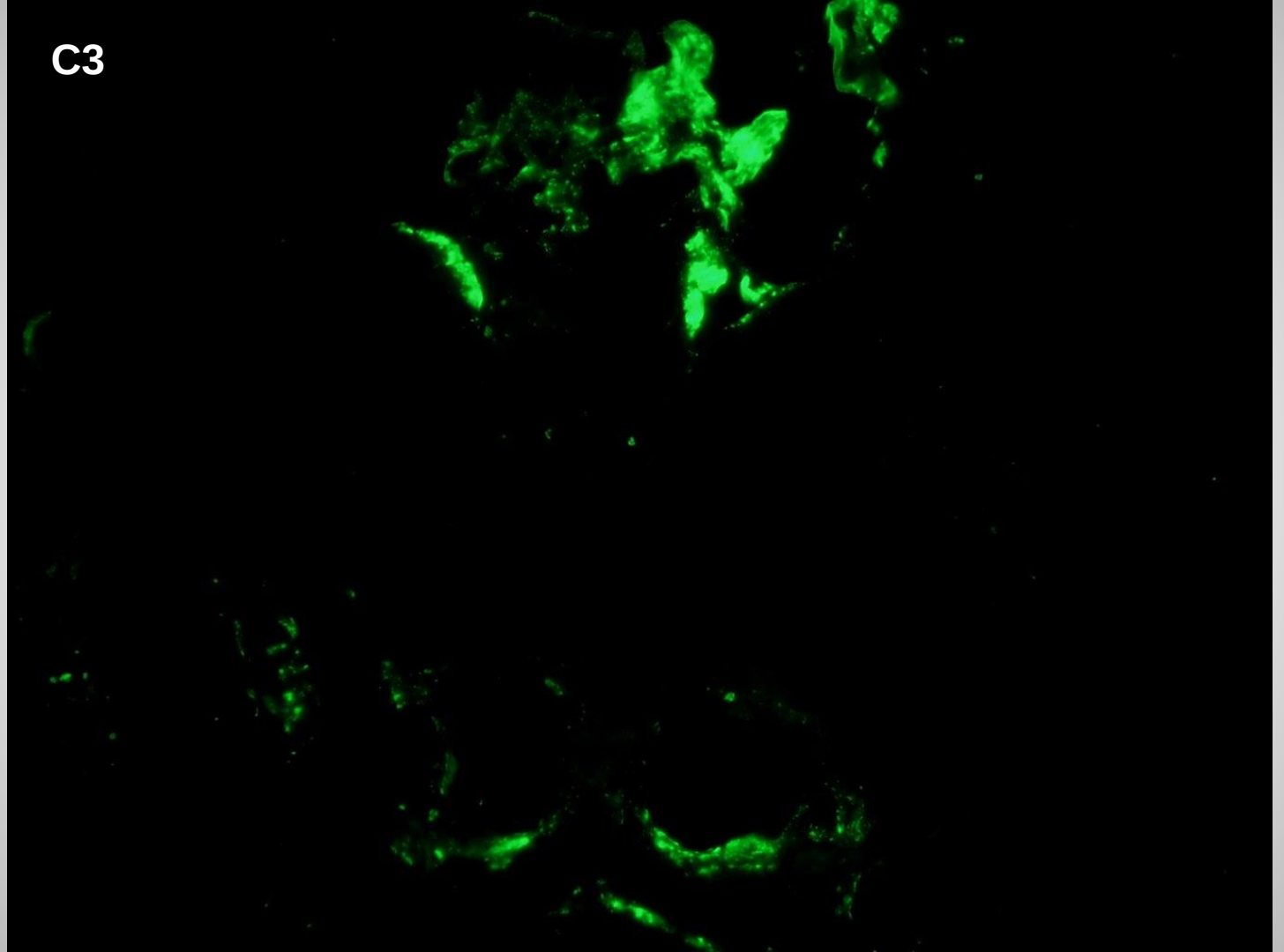


H&E



IHC



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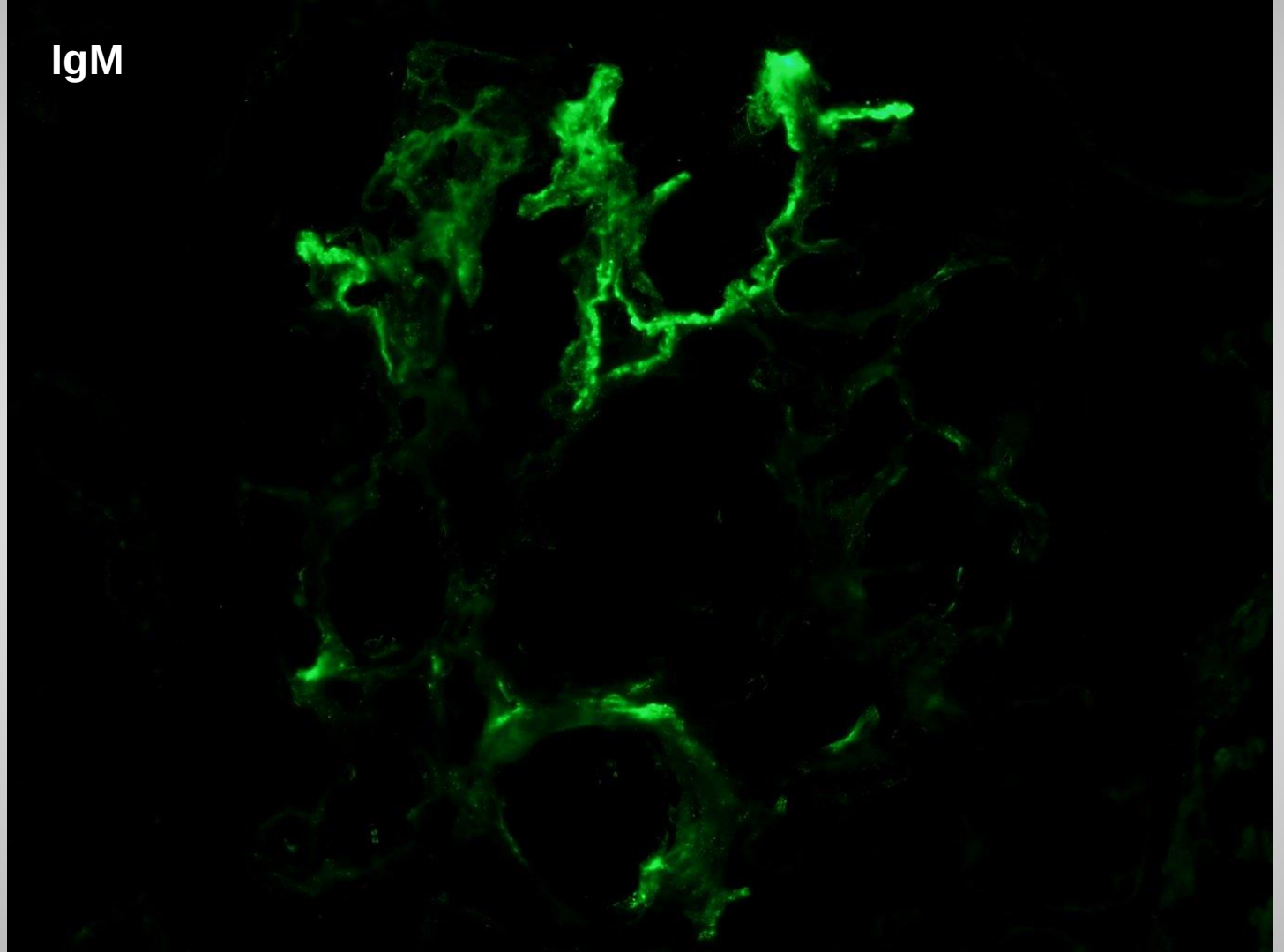

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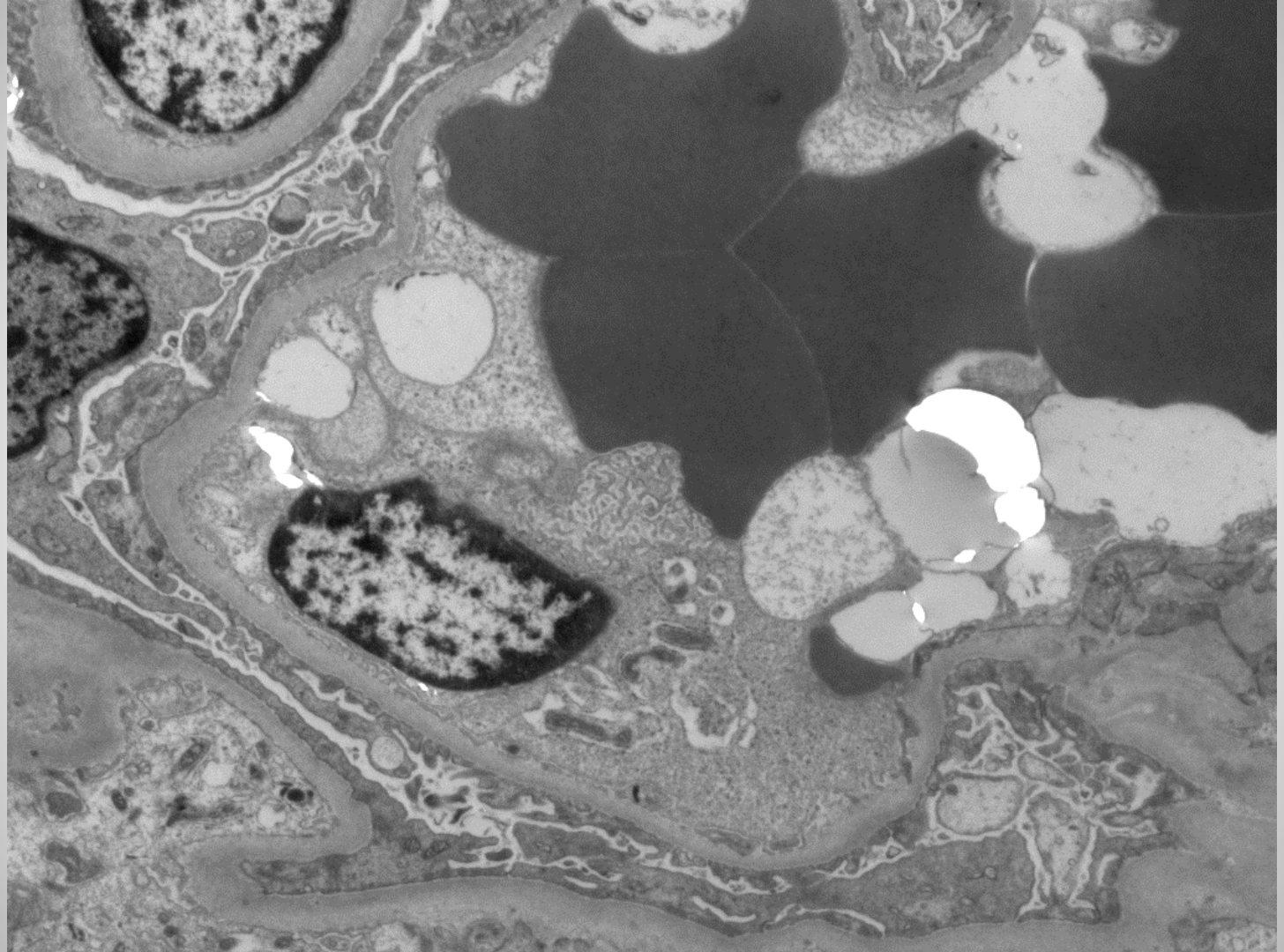

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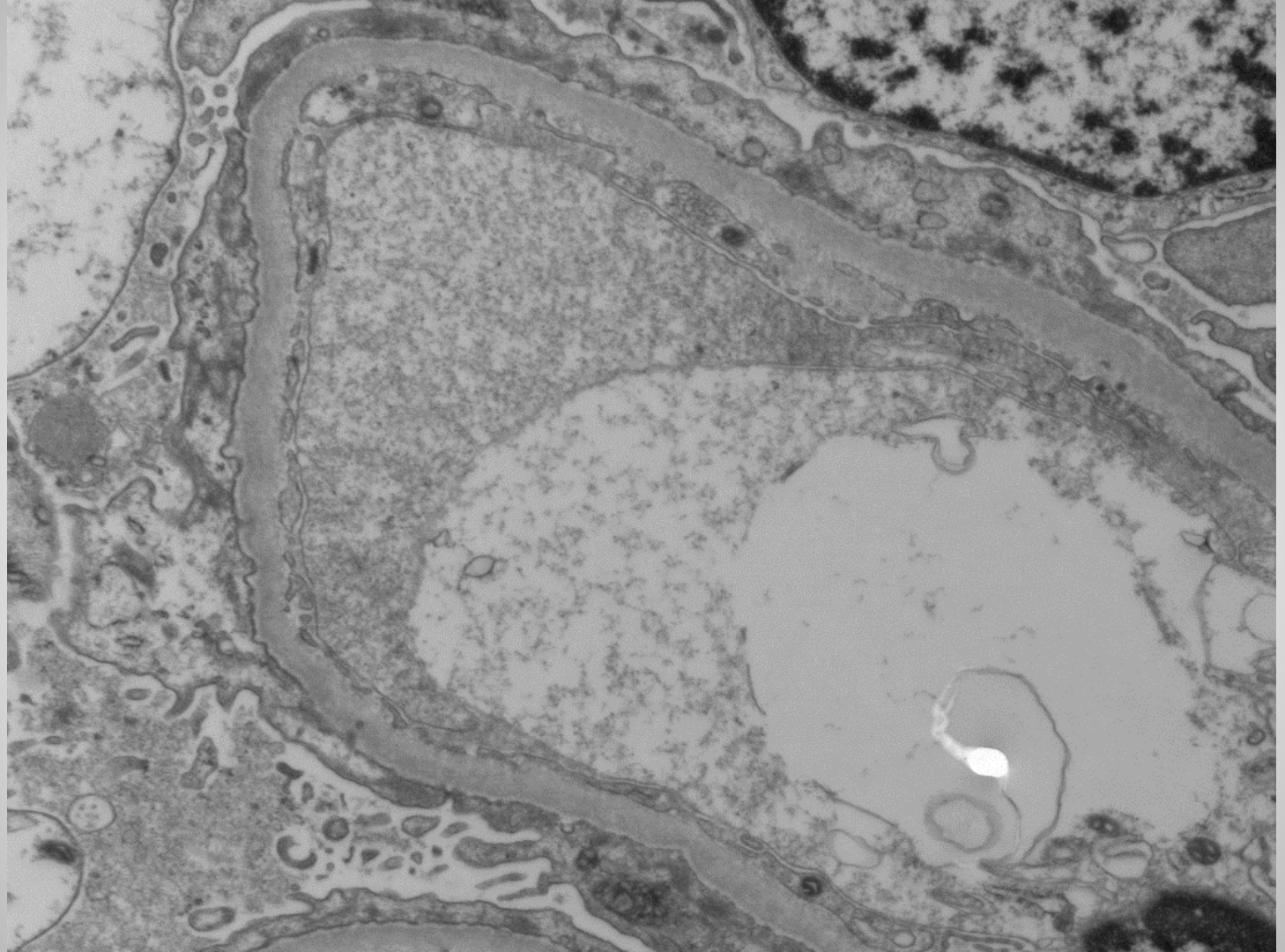


EM

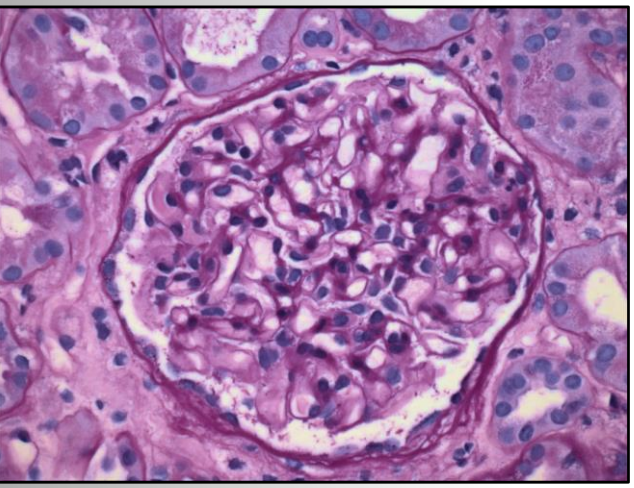
Diagnosis

Progressive diffuse podocytopathy with early focal and segmental glomerulosclerosis

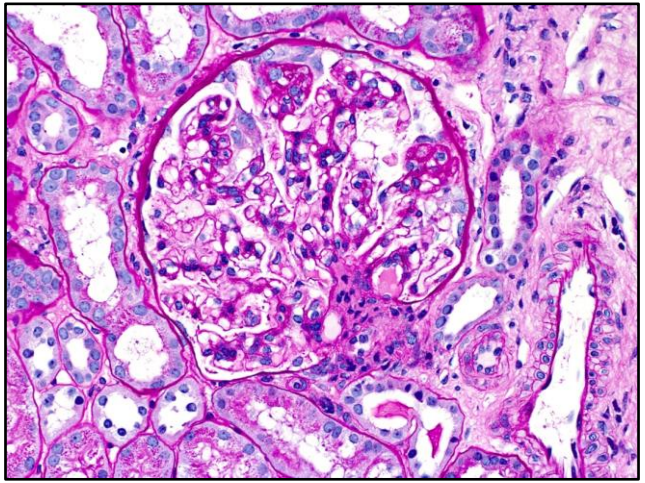
?Collapsing features

All 3 morphological patterns can be seen in the same patient, at different time points!

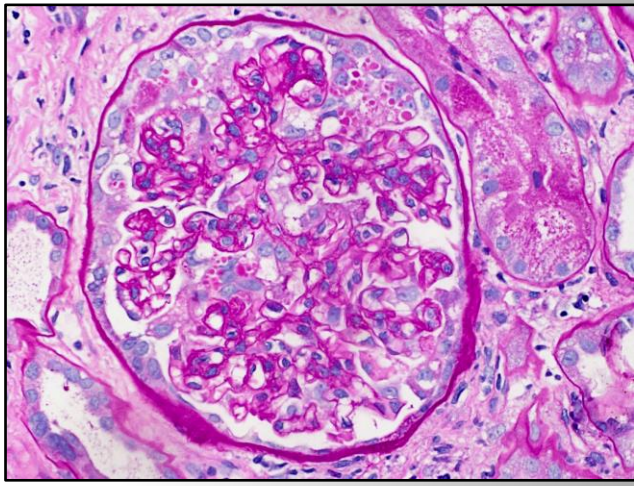
MCD



FSGS



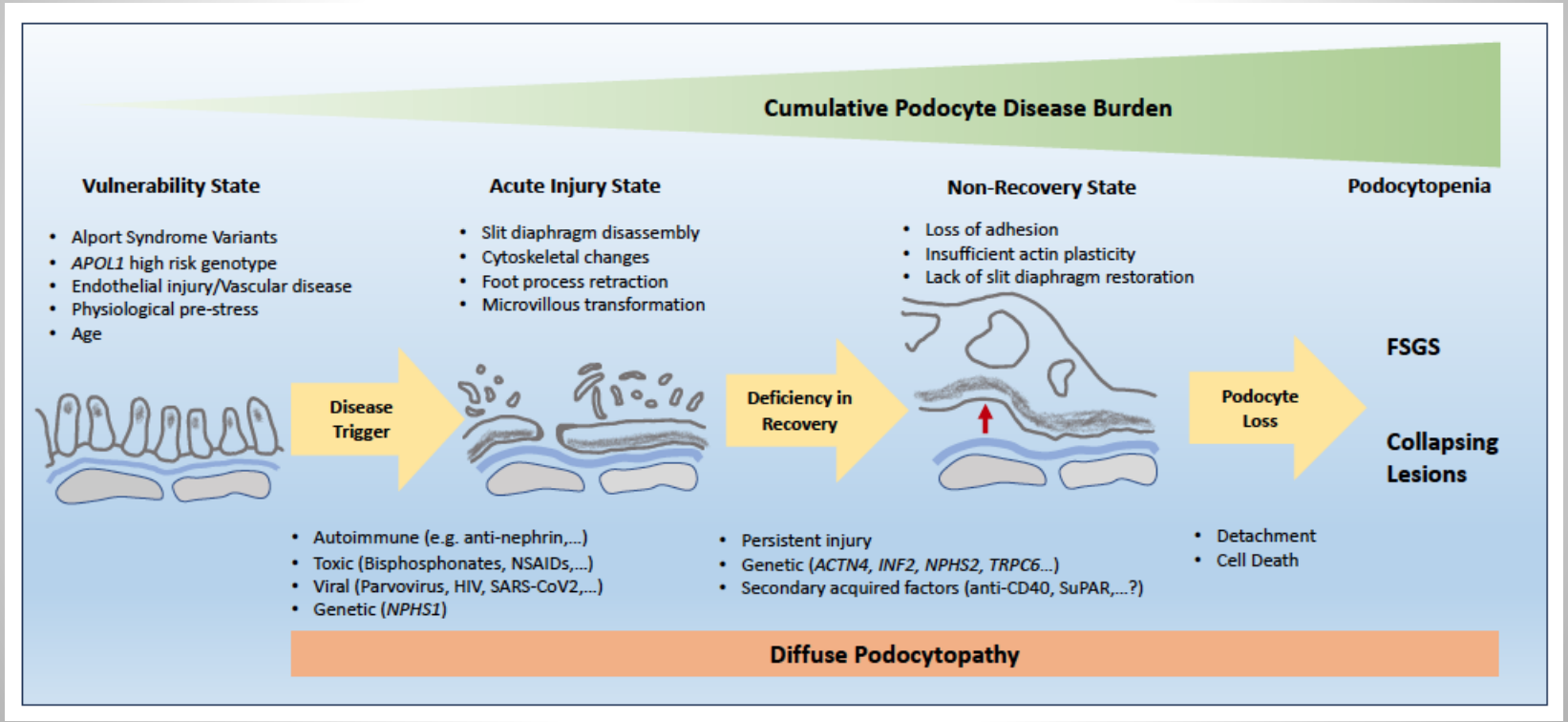
Collapsing glomerulopathy



It is important to establish:

1. Disease-initiating mechanisms (incl anti-nephrin)
2. Disease-perpetuating/progressing factors (environmental, genetic, other)
3. Predisposing factors (genetic background)

My concept of diffuse podocytopathies



Common diagnostic patterns observed in patients with Nephrotic Syndrome always affect the podocyte

"Podocytopathies":

- Minimal Change Disease
- Focal Segmental Glomerulosclerosis
- Collapsing Glomerulopathy

Membranous Nephropathy (idiopathic MN, Lupus)

Renal Amyloidosis

Nodular Glomerulosclerosis (Diabetic Nephropathy)

Case 3

A 63 yo man presents with “ankle swelling” which improves with a diuretic.

Heart: normal systolic function, LVEF 60%.

Hypercholesterolemia – started on simvastatin

No arthralgias, no hematuria.

Serology negative for HIV and Hepatitis.

Labs: BUN: 35 mg/dL; Cr: 0.92 mg/dL Alb: 2.5 g/dL Chol: 347 mg/dL

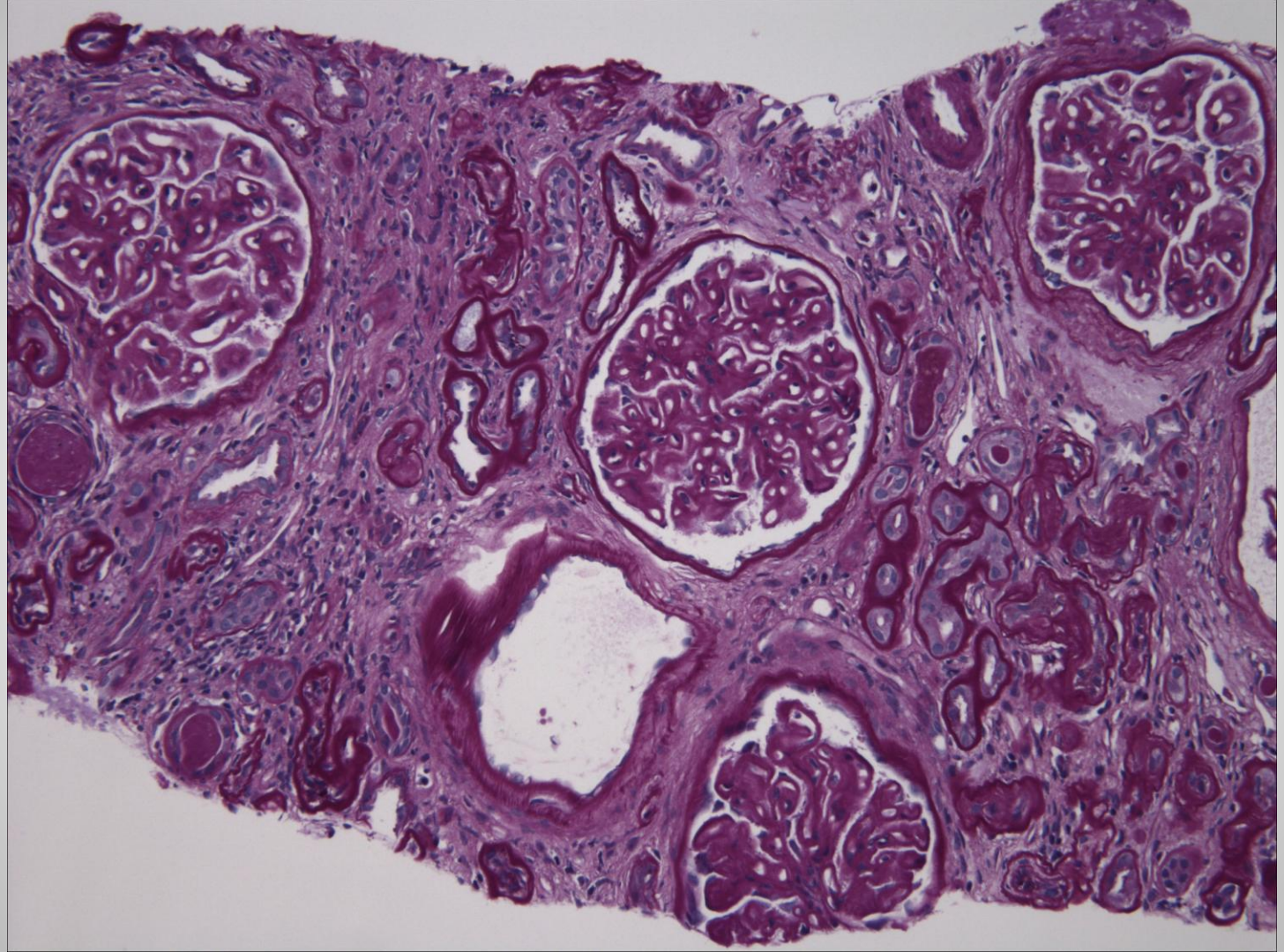
Urinalysis: 4+ protein/ +blood

24h urine: 9.7g protein/24hr

Urine sediment: Hyaline casts, oval fat bodies

Renal U/S: normal, 11cm left kidney; 11.8cm right kidney

A renal biopsy is performed.



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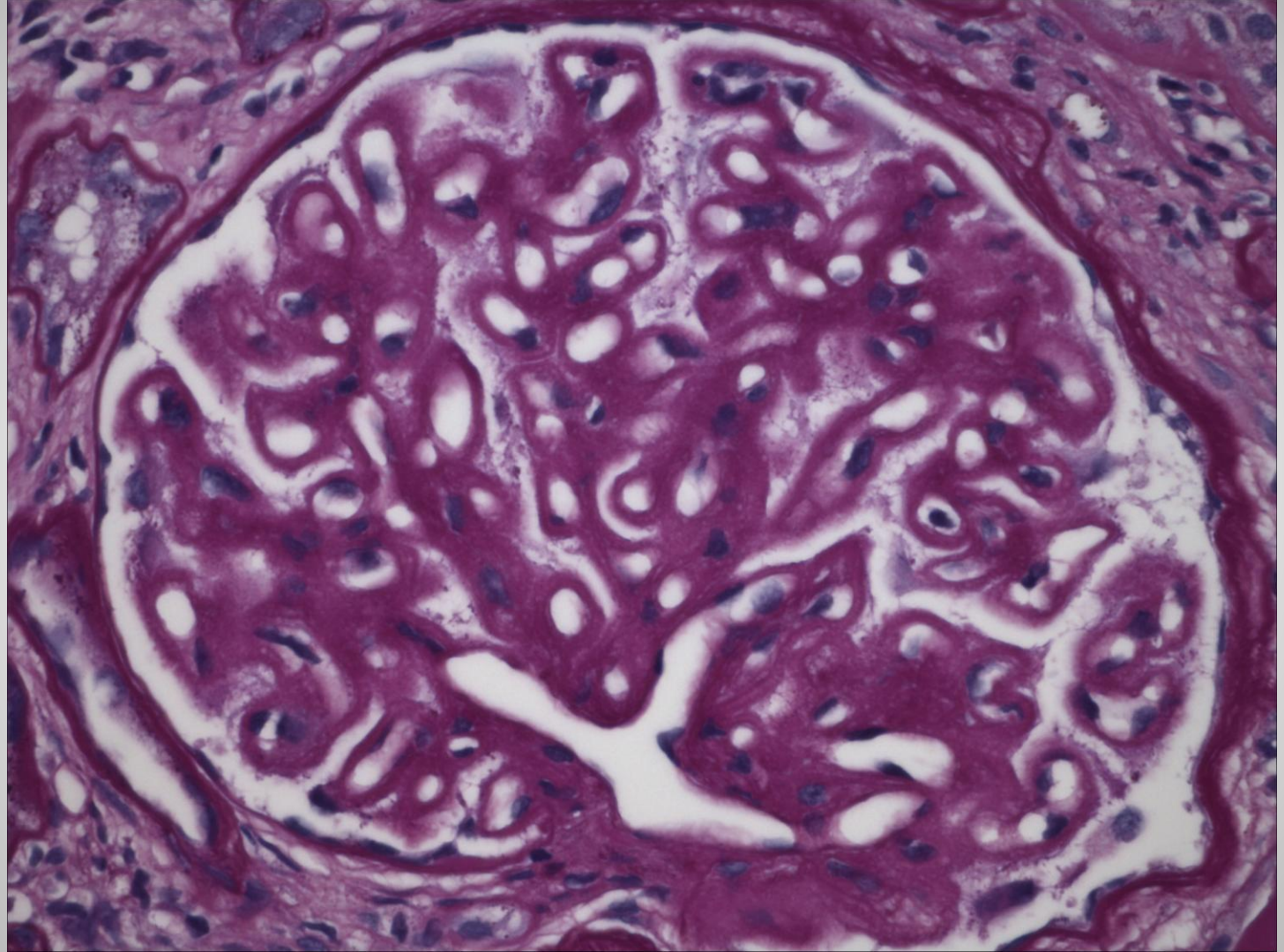

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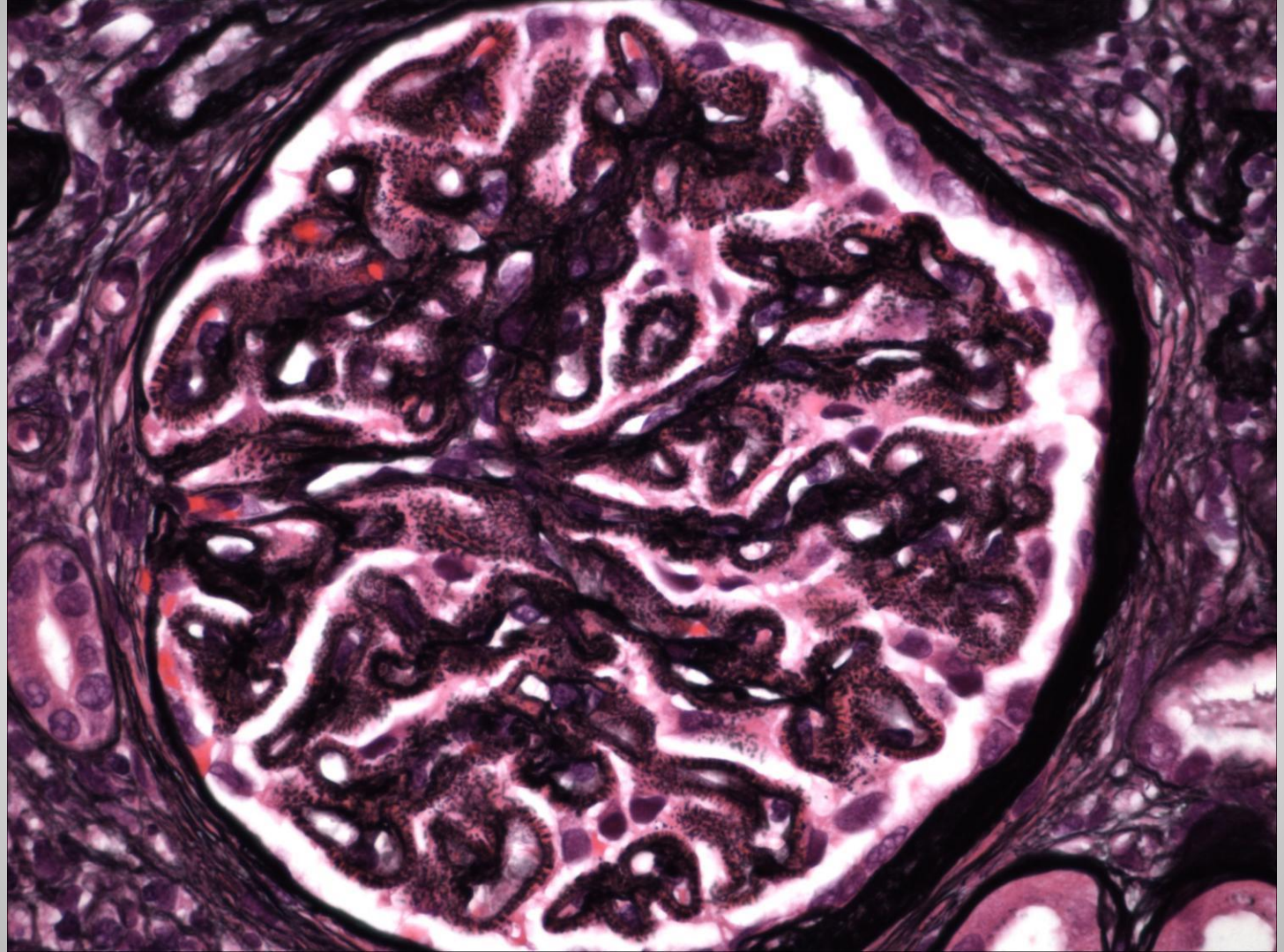

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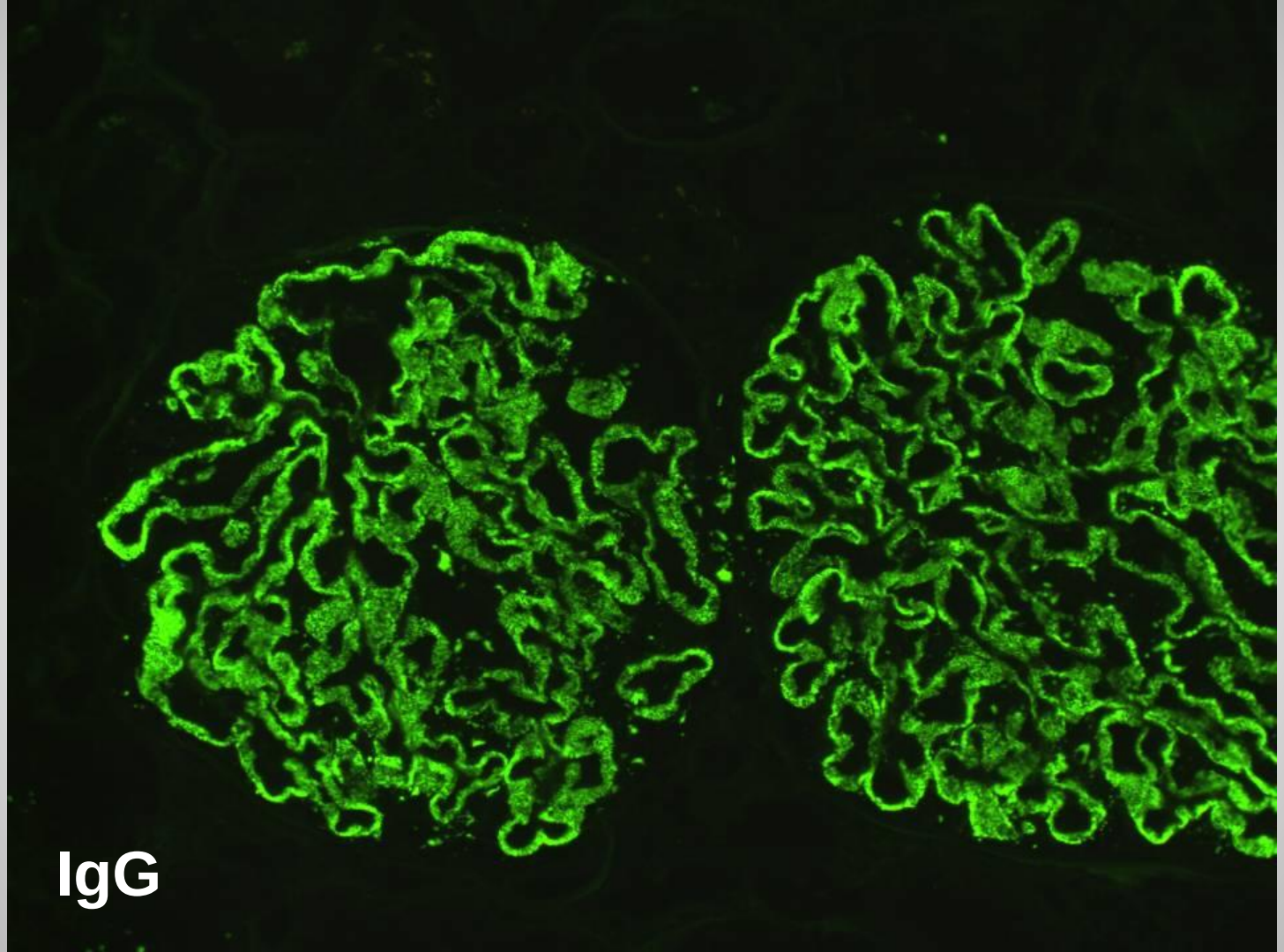

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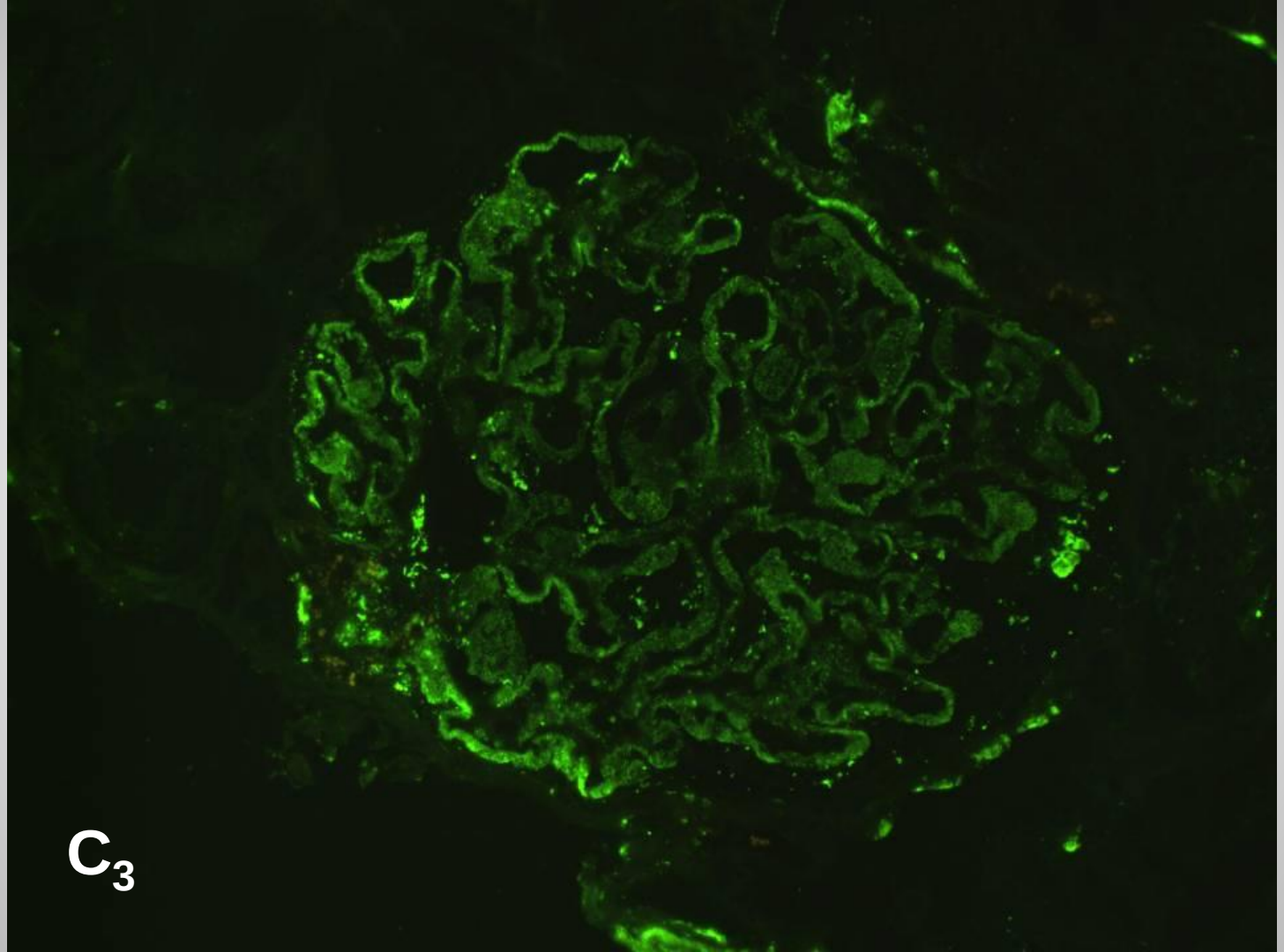

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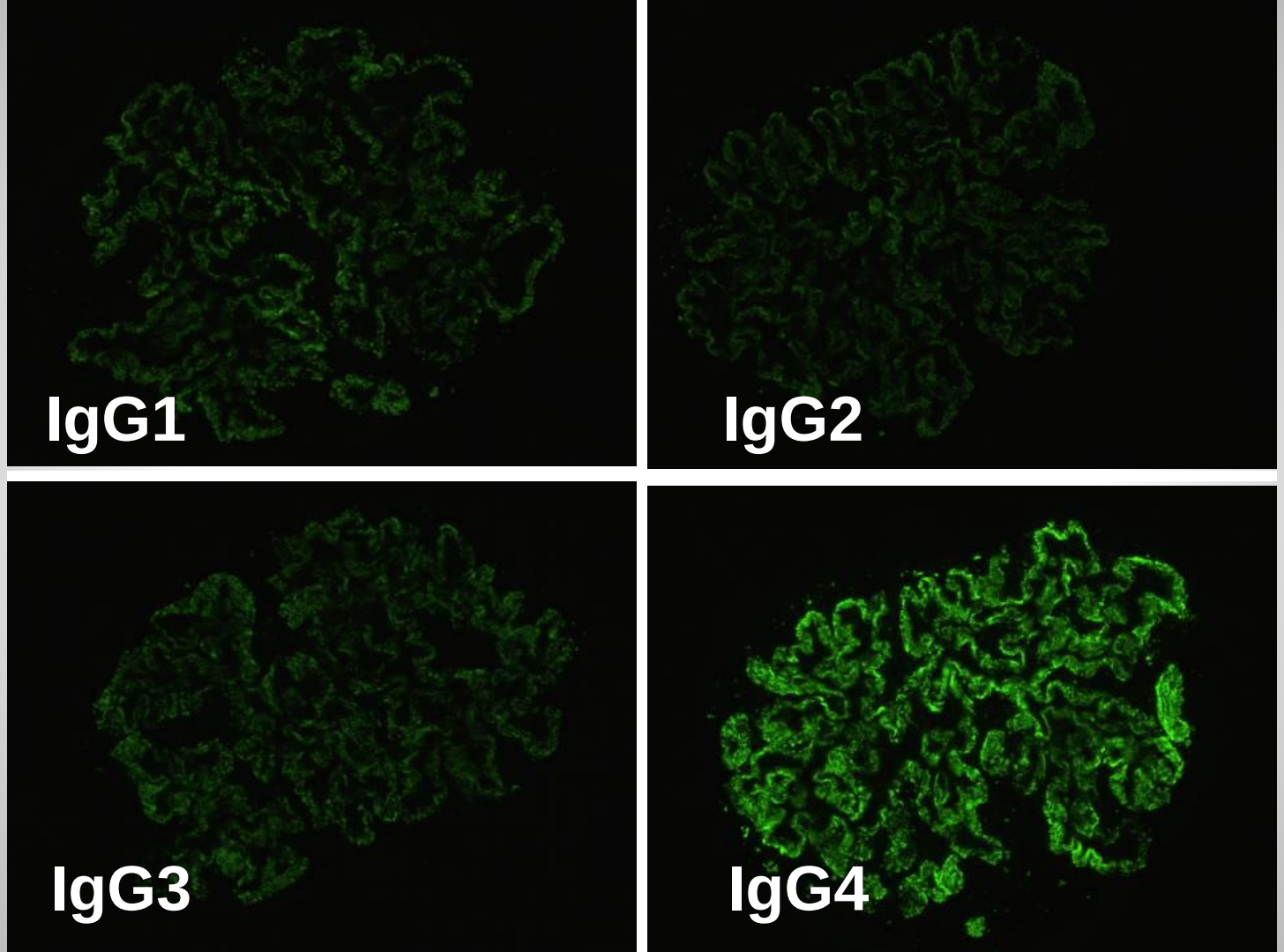

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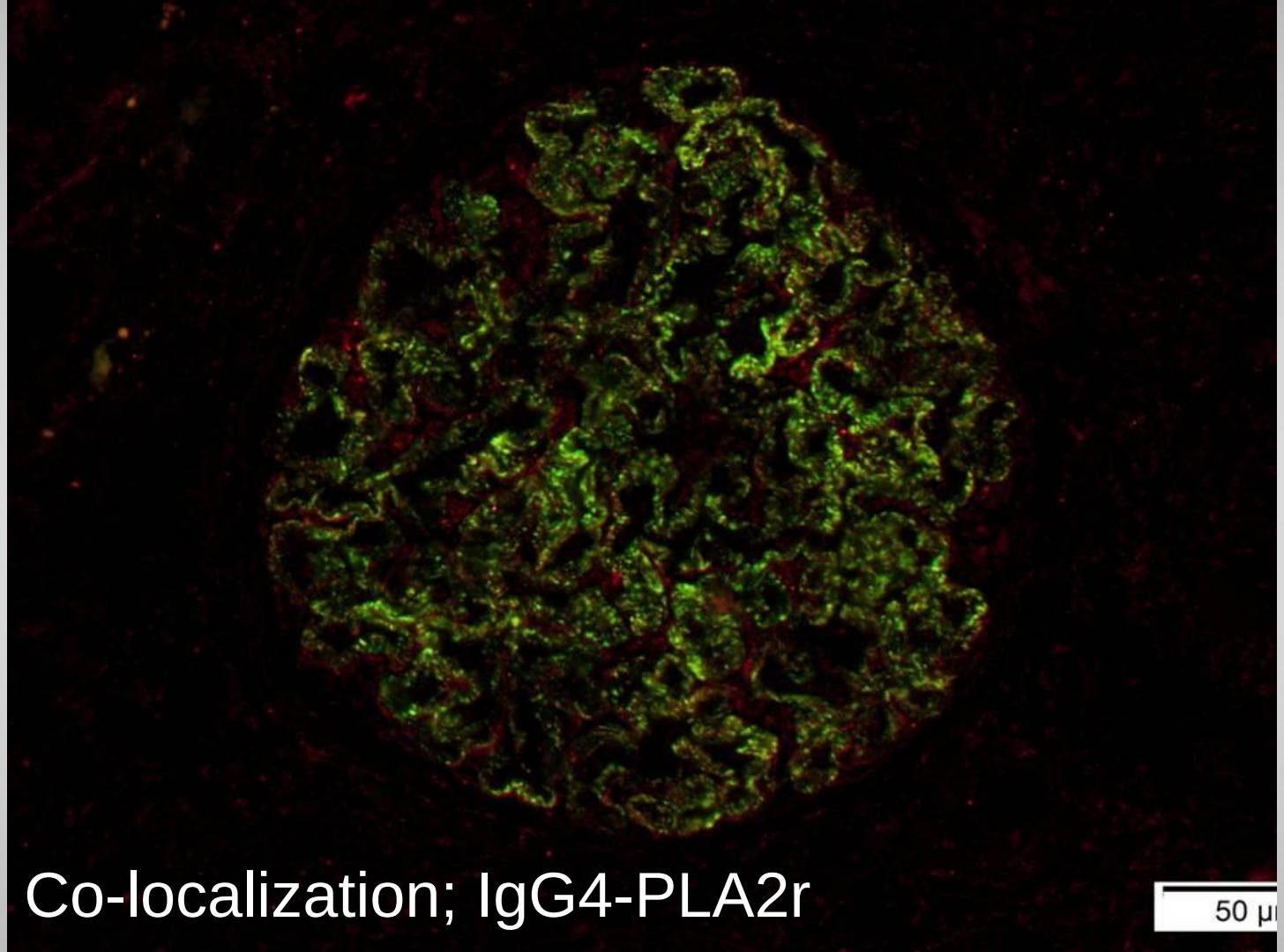

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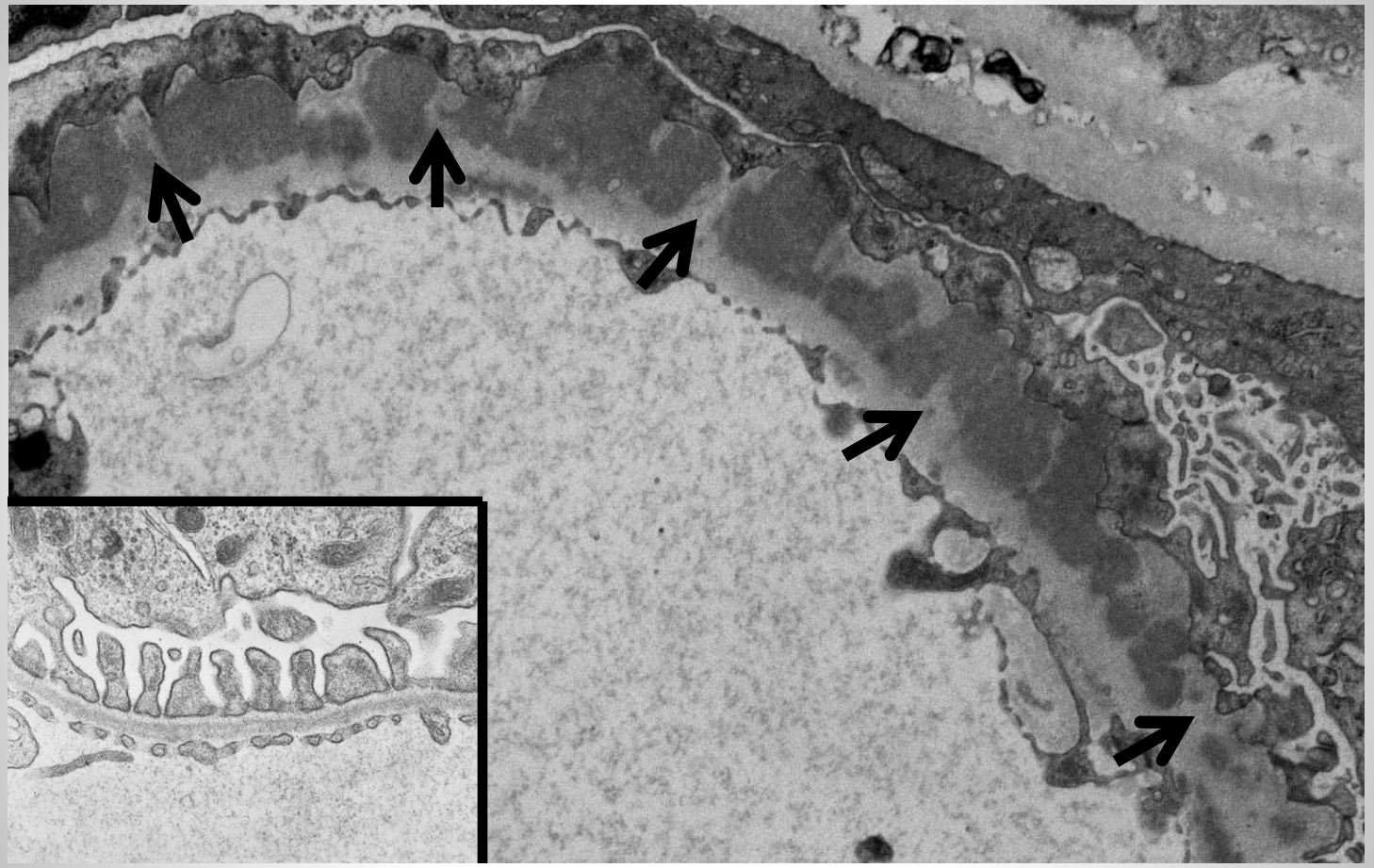
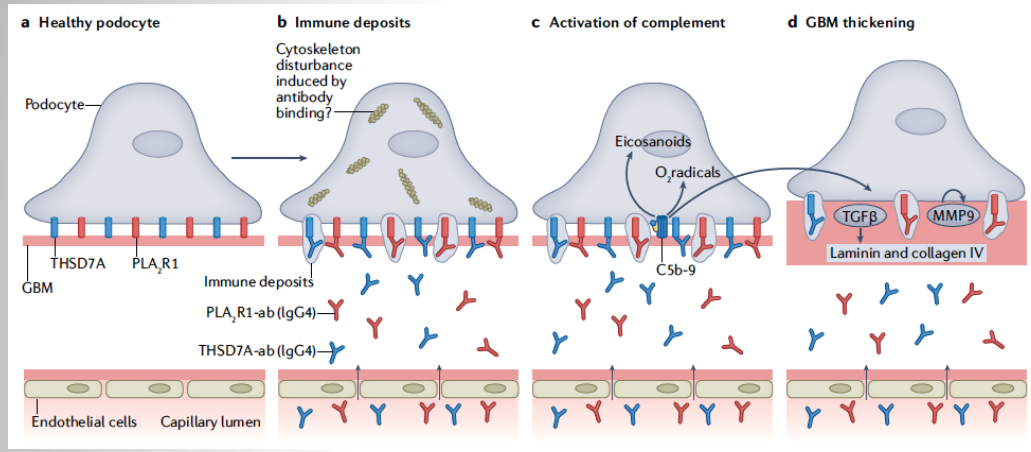


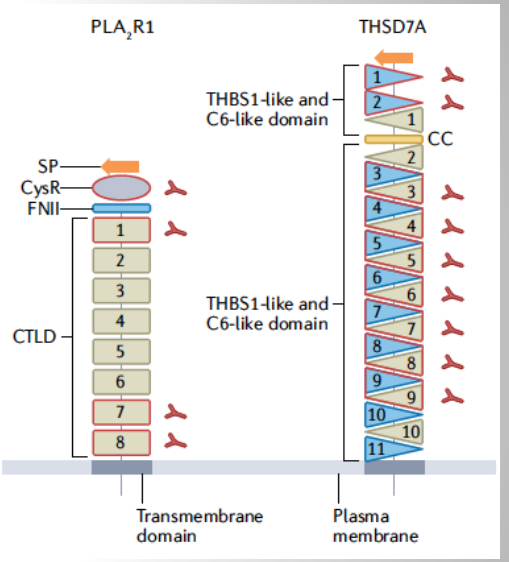
EM

Diagnosis

Membranous Nephropathy Stage II, PLA2r-positive

Membranous Nephropathy – explained!



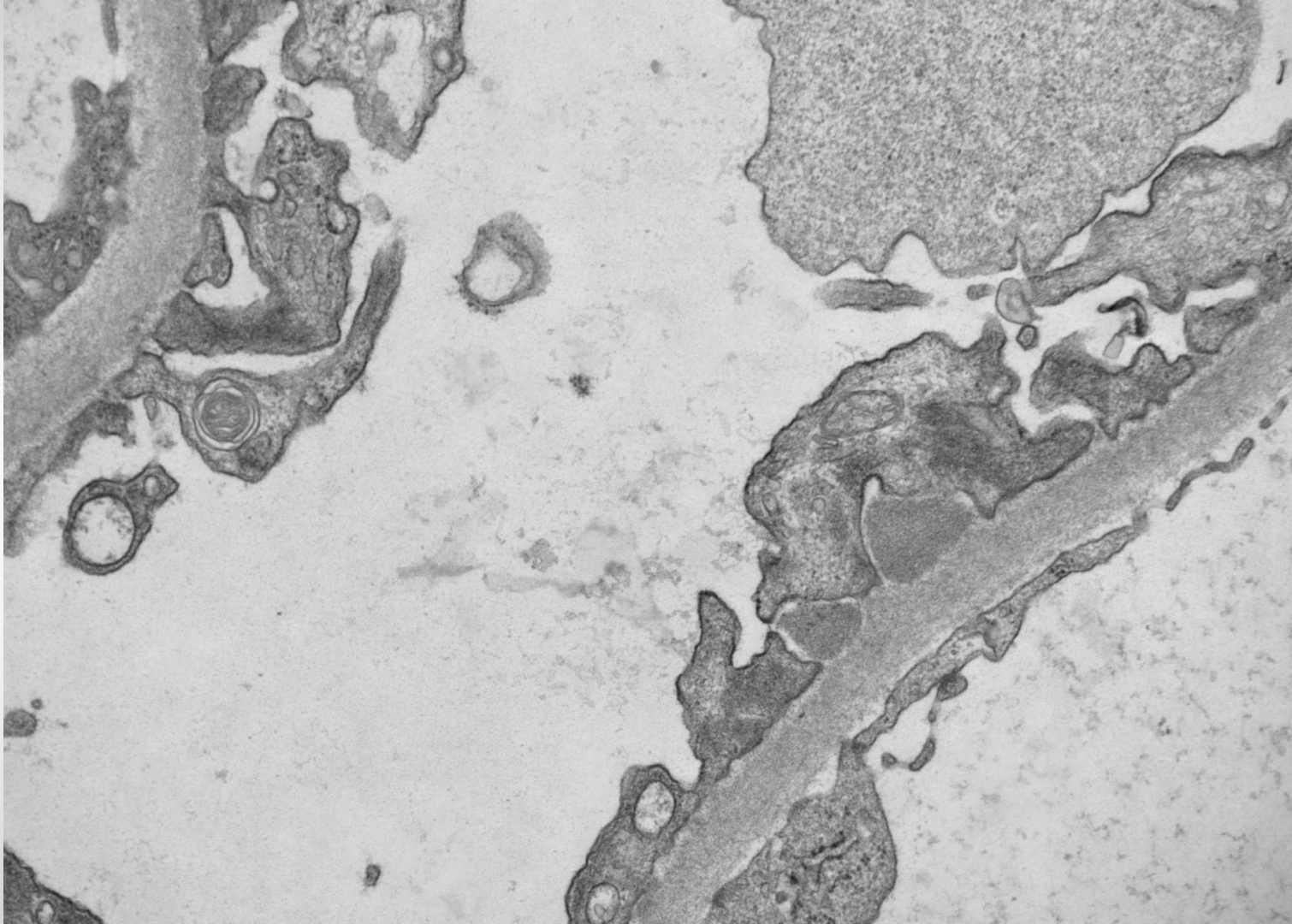


- Process of immune complex deposition is understood and accepted.
- Specific epitopes are identified for the majority of antigens/cases.
- Immunofluorescence and serological tests are available.

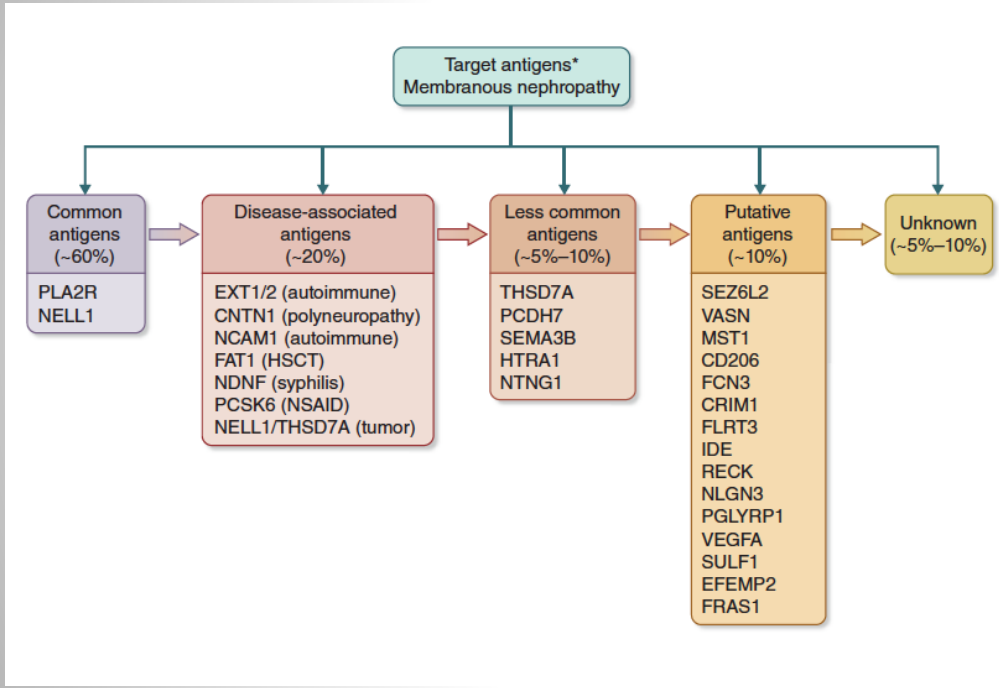
Hoxha E, Reinhard L, Stahl RAK. Nat Rev Nephrol. 2022 Apr 28.

“Lifting” of a podocyte by a growing deposit in early membranous nephropathy

Interference with podocyte attachment to the GBM



Membranous Nephropathy - Idiopathic no more!



Major **discoveries** of endogenous and exogenous antigens in last 2 decades, associated with various disorders

Presence of specific autoantibodies in the serum is **diagnostic for MN**, and predicts treatment response, disease activity and outcome

Slow disease **progression** and long **persistence** of immune complexes in the glomerulus leave a **long window** for both serological and histological detection

Sethi and Madden, Kidney Int 2023

TARGETS TO REMEMBER IN MEMBRANOUS NEPHROPATHY

1. “Primary” Membranous Nephropathy
   - M type phospholipase A2 receptor (PLA_2r)
   - Exostosin 1/2 (EXT) (SLE)
   - Neural epidermal growth factor-like1 prot (NELL1)
   - Thrombospondin Type-1 Domain-Containing 7A (THSD7A)
   - Semaphorin 3B
   - alpha-enolase

PLA_2R>EXT> NELL1>THSD7A and Semaphorin 3B

Congenital Membranous Nephropathy

- neutral endopeptidase (NEP)

Question 1

1) The target protein of the newly discovered and validated autoantibodies in minimal change disease is a component of the

a) Glomerular basement membrane

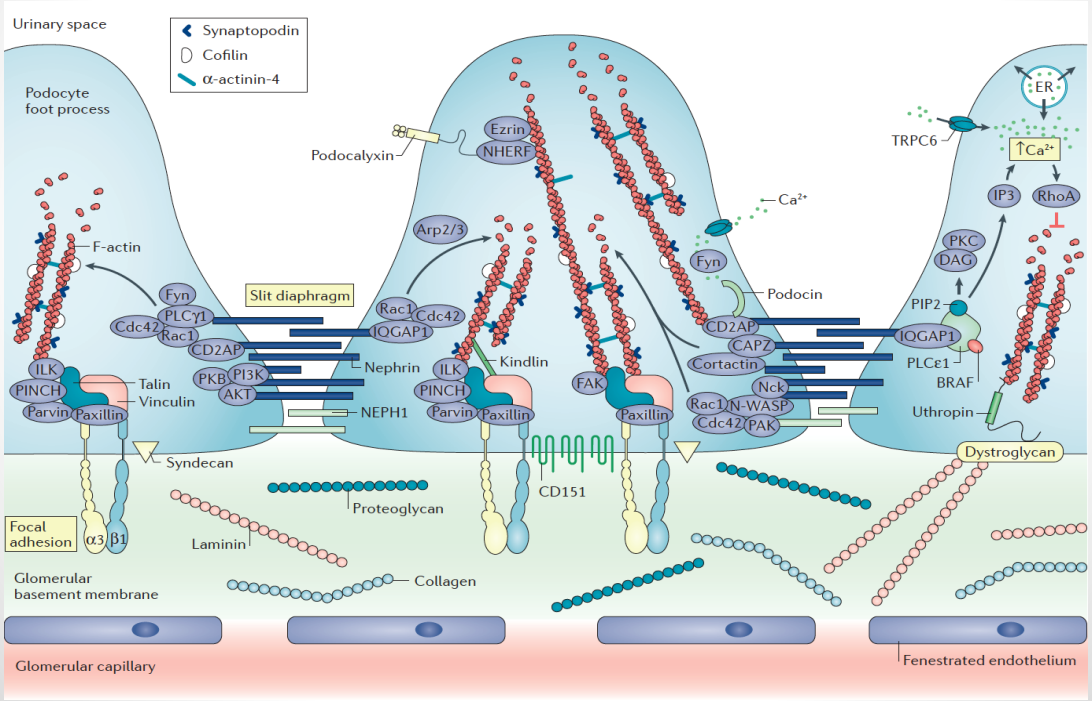
b) Podocyte actin cytoskeleton

c) Slit diaphragm

d) Focal adhesion complex

Answer 1

c) Nephrin is an essential component of the podocyte slit diaphragm.



Perico et al, Nat Rev Nephrol. 2016 Nov;12(11):692-710

Question 2

2) The most common autoantibody target in membranous nephropathy is

a) NELL1

b) Phospholipase A2 receptor 1

c) dsDNA

d) nephrin

Answer 2

b) Autoantibodies against the Phospholipase A2 receptor 1 account for 70-80% of cases of Membranous Nephropathy.

PLA2r > EXT > NELL1 > THSD7A and Semaphorin 3B

Beck et al, N Engl J Med, 2009 Jul 2;361(1):11-21

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