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ERKNet
Advanced Webinars on Rare Kidney Disorders

Date: 15 December 2020

Topic: Membranous Nephropathy

Speaker: Pierre Ronco

Moderator: Jack Wetzels

New advances in membranous nephropathy: the antigenic revolution

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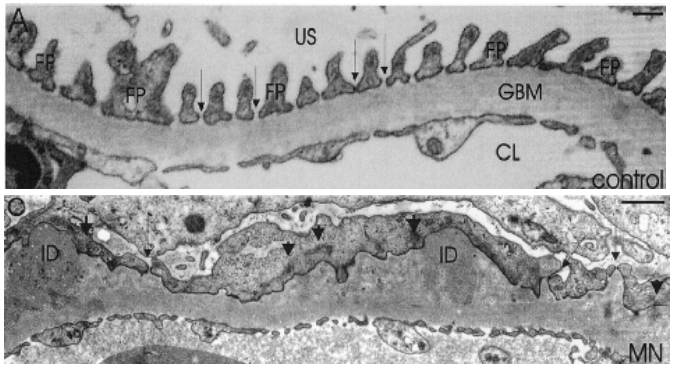
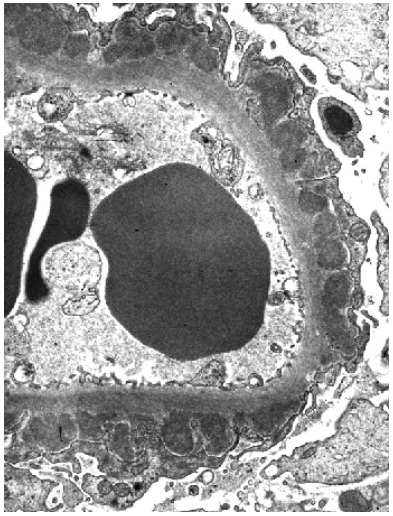
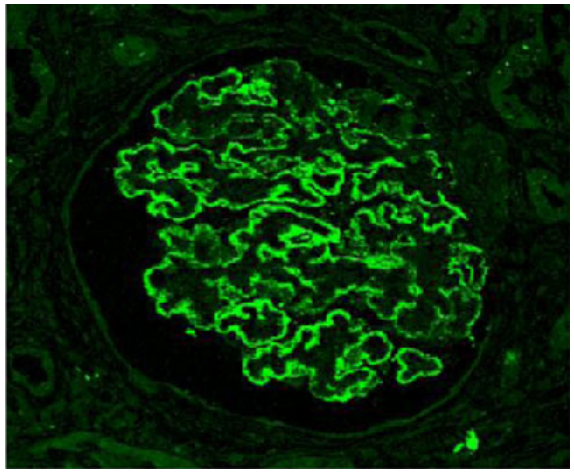
Brief summary of MN breakthroughs

- Thickening of GBM
- Immune deposits
- Foot process retraction

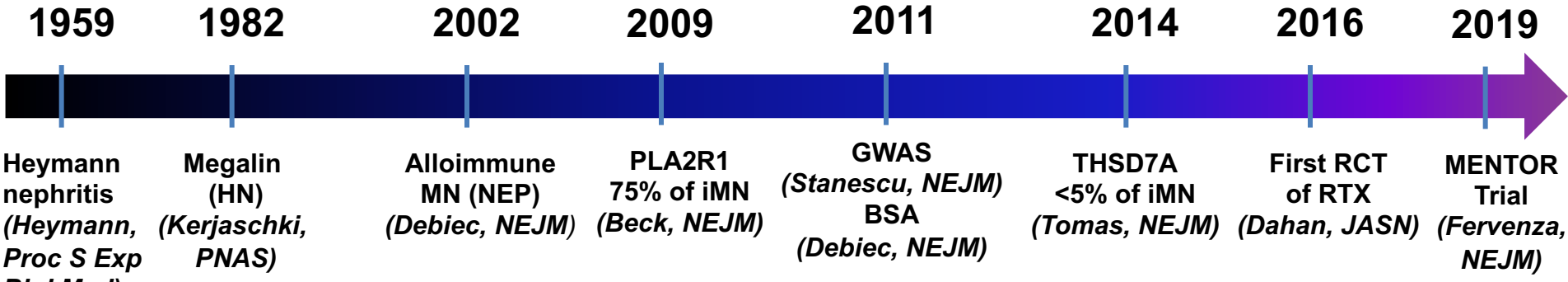




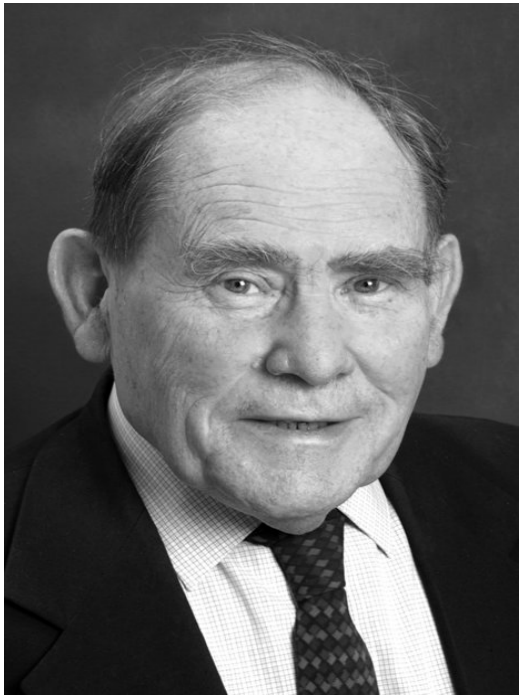
Electron dense deposits



IF : immunoglobulin (IgG4>IgG1)



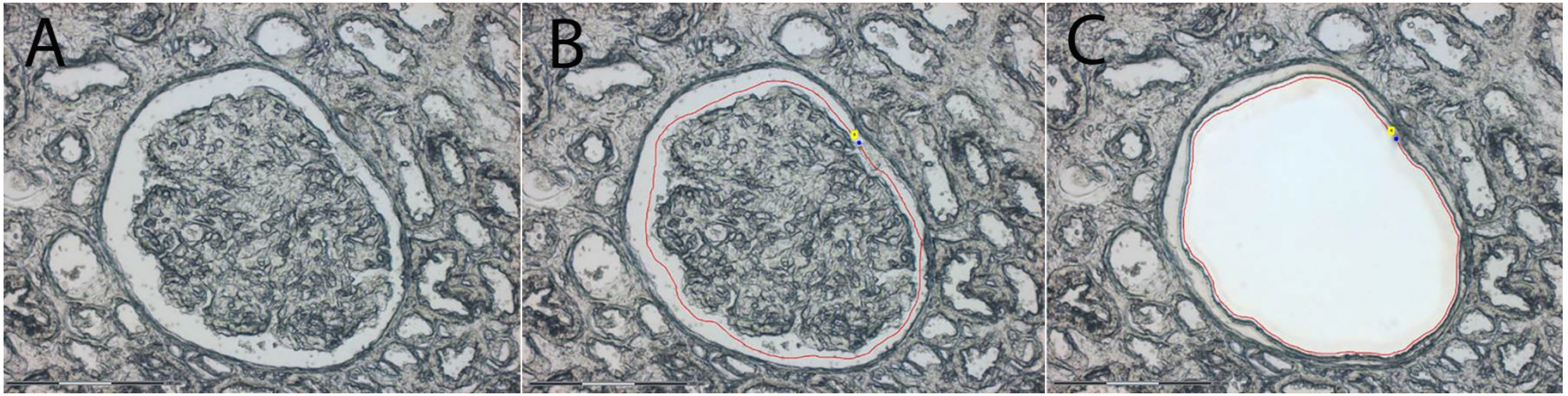
"Progress in science depends on new techniques, new discoveries and new ideas, ***probably in that order****"*



Sydney Brenner
Nobel Prize 2002

2019-2020
The third antigenic (r)evolution in membranous nephropathy

A major technological leap:
Laser microdissection of glomeruli followed by MS of trypsin digested proteins



Sethi S, Debiec H … Fervenza F & Ronco P, JASN 2019, 30:1123

New biomarkers/antigens: Will they change the concepts and the management?

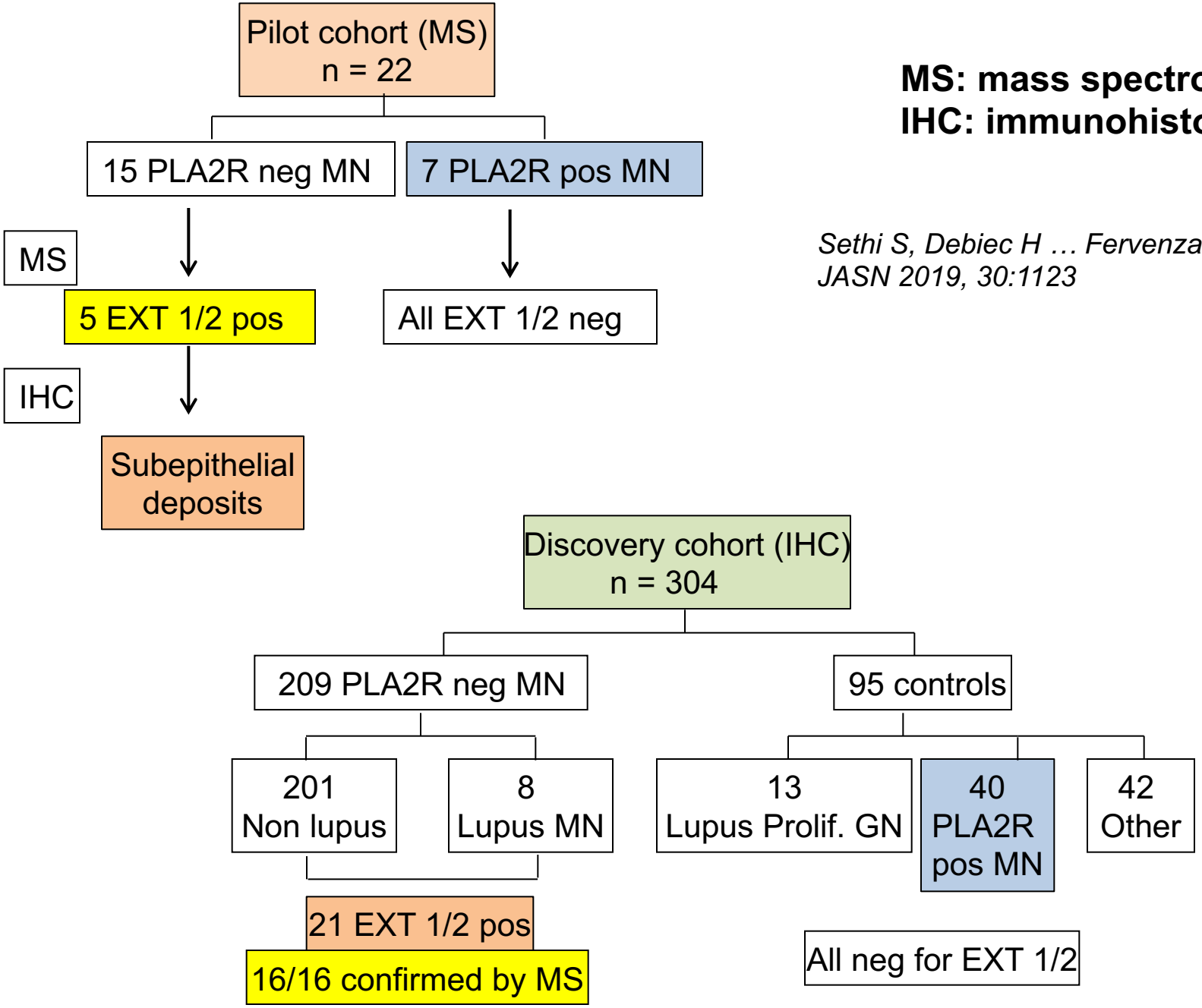
- **Which biopsy samples?**

  Double negative (PLA2R/THSD7A) MN >20% of all MN (up to 40% in Japan)

- **Initial step: Laser microdissection + mass spectrometry performed at Mayo (Sanjeev Sethi, Fernando Fervenza)**

- **All following steps: Identification of antibodies, co-localization by laser confocal microscopy, validation studies performed in Paris (Hanna Debiec, Pierre Ronco)**

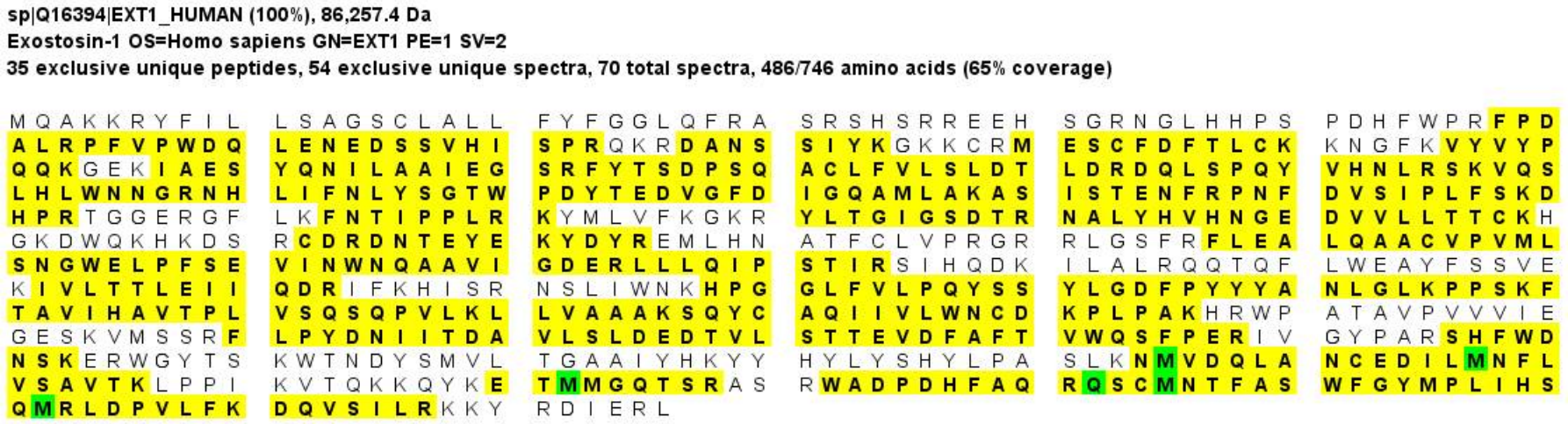
Exostosins 1 and 2



MS: mass spectrometry
IHC: immunohistochemistry

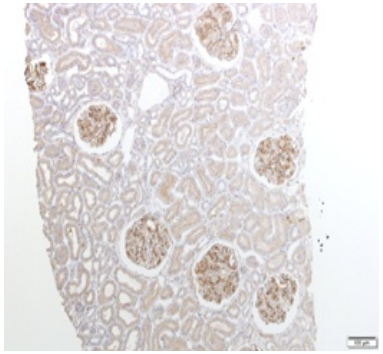
Sethi S, Debiec H … Fervenza F & Ronco P, JASN 2019, 30:1123

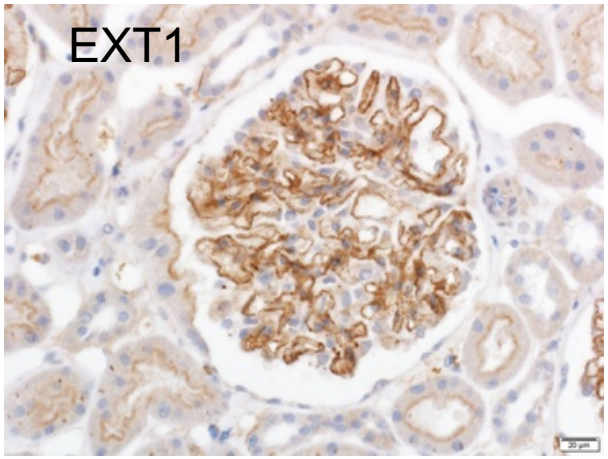
Coverage of exostosine-1 sequence by MS identified peptides

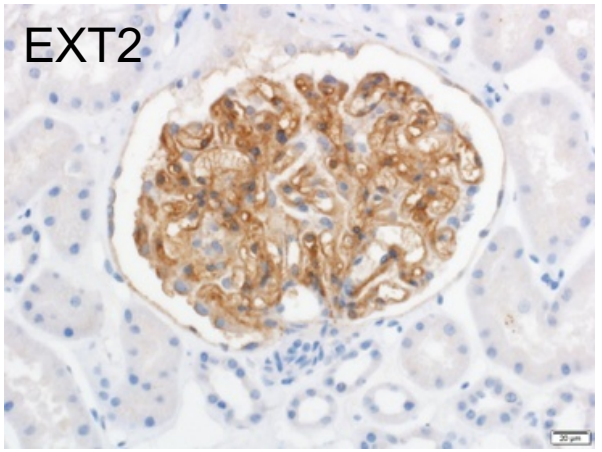


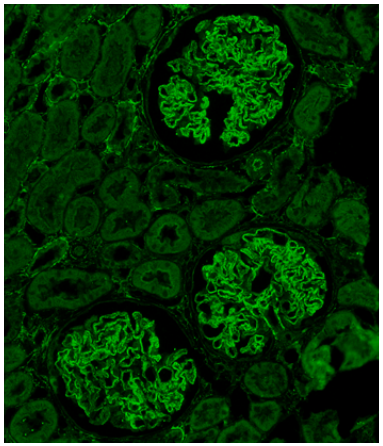
Sethi S, Debiec H … Fervenza F & Ronco P, JASN 2019, 30:1123

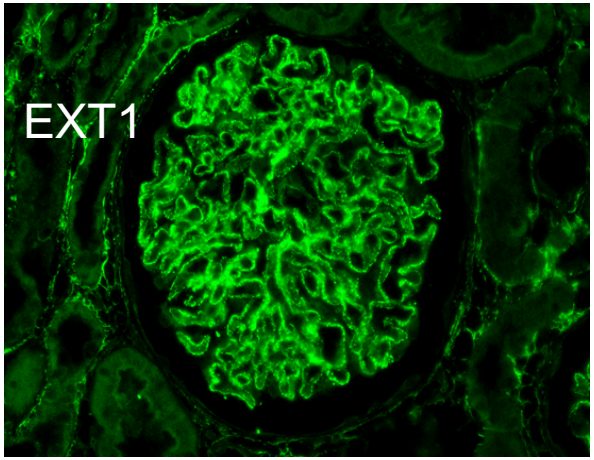
IHC and IF labeling of the paraffin biopsy from the same patient (paraffin sections)

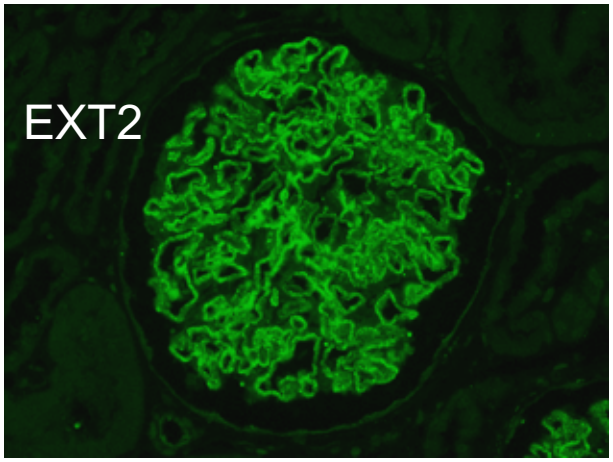










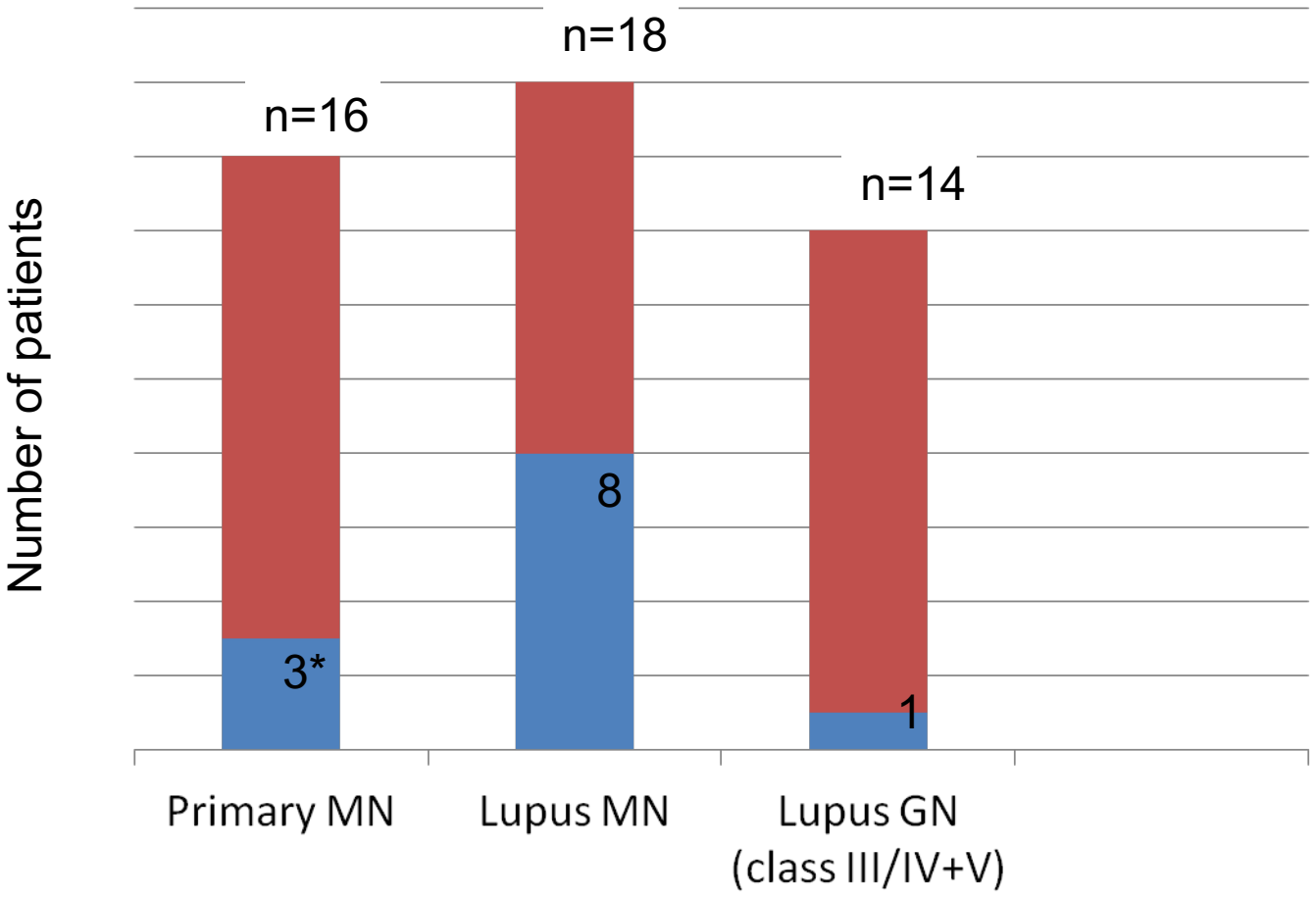


Sethi S, Debiec H … Fervenza F & Ronco P, JASN 2019, 30:1123

Clinical characteristics of EXT1/EXT2 patients

- Mean age, 35(+/-13.4) years; 81% females
- S creatinine, 10 (SD+/-0.9) mg/L
  proteinuria, 5.9 (SD +/-4.8) g/day
- 71% ANA, anti-DNA, -Smith, -SSA/B, -RNP
- 35% with a clinical diagnosis of lupus
- 85% , IgA or IgM (no « full-house »)
- 73%, C1q
- Ig1>>IgG4 (MS)
- PLA2R negative
- EM: 96% mesangial deposits ; 35% subendothelial deposits; 81% tubuloreticular inclusions

Validation cohort (n=48)



Sethi S, … Fervenza F & Ronco P, JASN 2019, 30:1123

*** The 3 cases of EXT1/EXT2 positive « primary » MN were associated with signs of auto-immunity (ANA without anti-DNA antibodies, no C1q or significant prolif.); Two patients later developed lupus disease.**

Exostosins ½: biomarkers of class V lupus MN and of MN with auto-immunity

- **EXT1/EXT2 (glycosyl transferase complex in Golgi) are novel biomarkers of lupus MN or MN with non-lupus auto-immunity**
- **Anti-EXT1/EXT2 antibodies are not detected in serum with native recombinant proteins (neo-epitope? low titer? epitopes specific for the glomerular podocyte)**

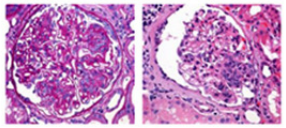
- **Detected in 30% of pure lupus MN (class V)**
- **EXT1/EXT2 are rare in mixed classes**
- **EXT1/EXT2 are not detected in the absence of subepithelial deposits**
- **In young female patients with a diagnosis of « primary » MN, EXT1/EXT2 staining may anticipate development of lupus disease**

Neural cell adhesion molecule 1 is a novel autoantigen in membranous lupus nephritis.

Mean age = 34 yr

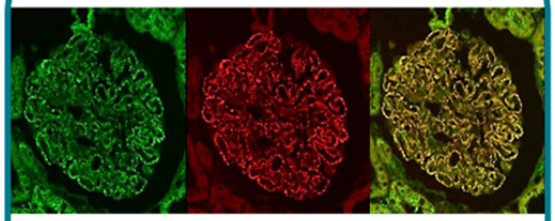
70% Female

ANA positive



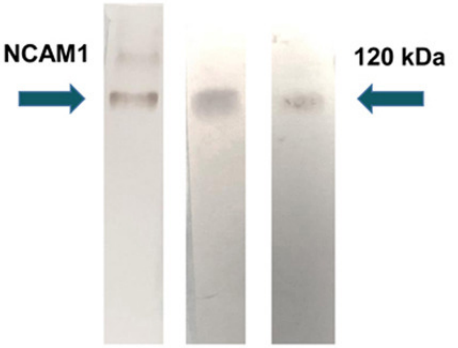
5/20 (25%) with proliferative component

NCAM1-associated membranous nephropathy (n=20 patients)



IgG NCAM1 Overlay

NCAM1 co-localizes with IgG within glomeruli in NCAM1-associated membranous nephropathy.



Antibodies against NCAM1 are identified in serum from NCAM1-associated MN patients.

CONCLUSION:

Nerve cell adhesion molecule-1 (NCAM1) is the target antigen of a subset of membranous lupus nephritis biopsies and anti-NCAM1 antibodies can be detected within patient sera.

ISN kidney INTERNATIONAL
OFFICIAL JOURNAL OF THE INTERNATIONAL SOCIETY OF NEPHROLOGY

Caza et al, 2020

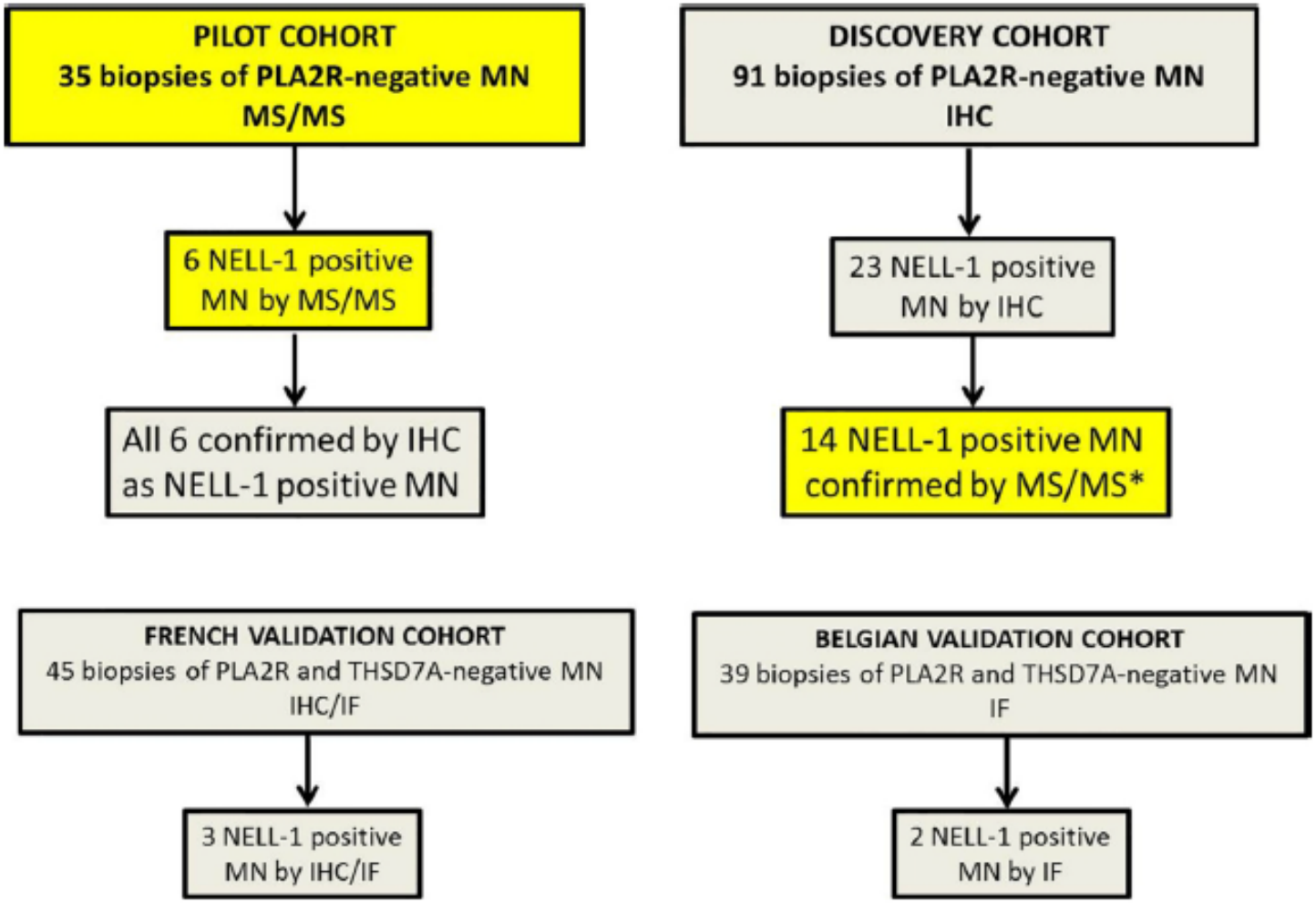
- **14/212 (6.6%)lupus MN with or without proliferative changes**
- **33/209 (15.8%) EXT2 positive**
- **2/101 (2.0%) « primary » MN**

Caza et al, Kidney Int, 2020 October 9

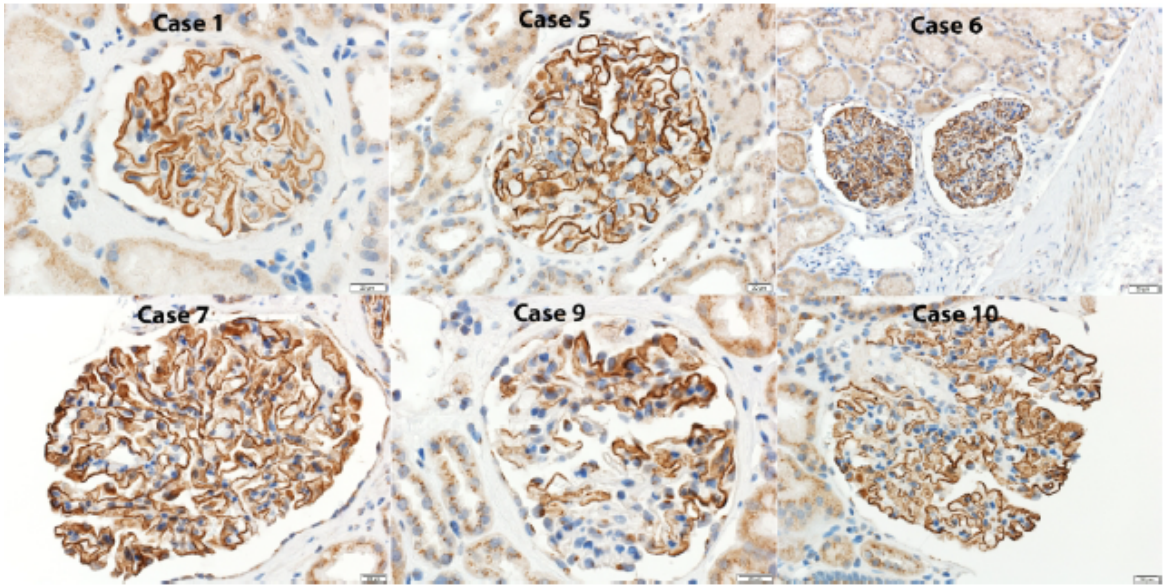
NELL-1
Neural EGF-like 1 protein
PK C-binding protein NELL-1

Sethi S, Debiec H, … Fervenza F & Ronco P
(Kidney Int. 2020 Jan;97(1):163-174)

NELL-1: Flowchart of the pilot, discovery, and validation cohorts



Immunohistochemical stain for NELL-1 in NELL-1-associated MN, PLA2R-associated MN, and control cases



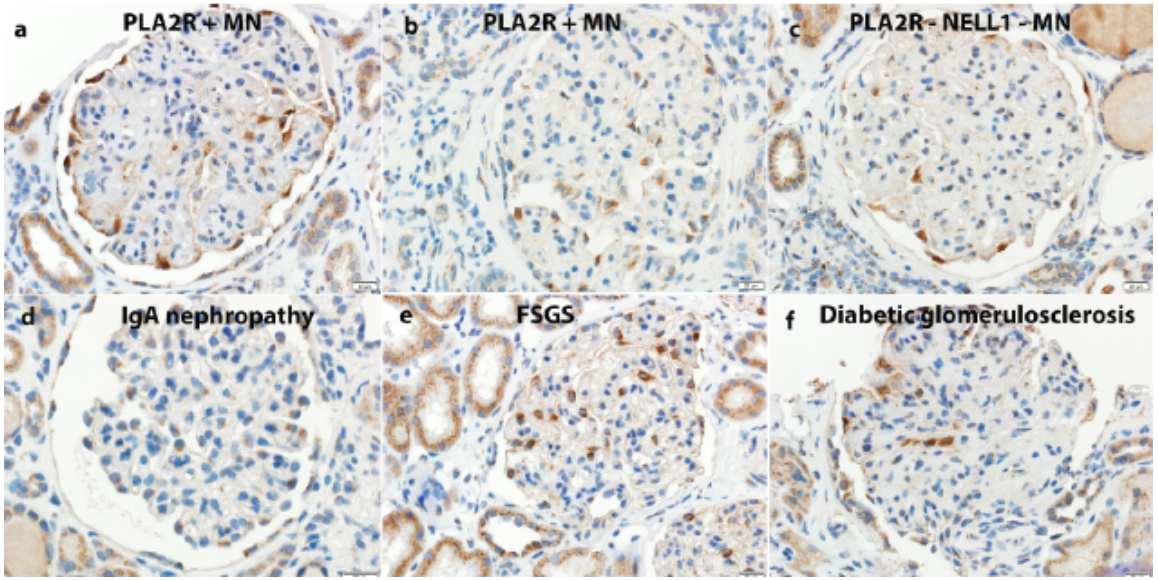
Six cases of NELL-1-associated MN

Segmental deposits in case#9

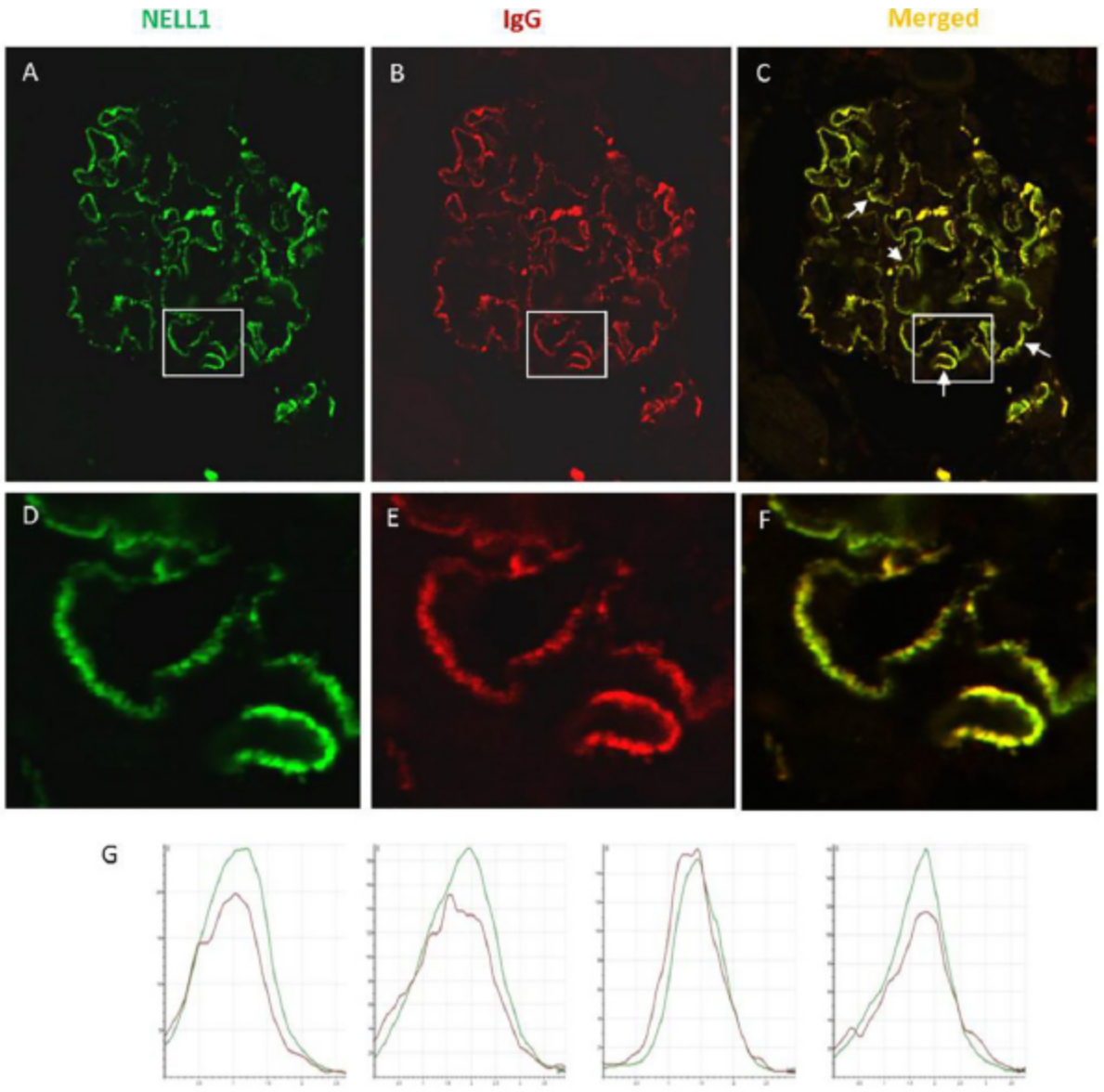
Controls

PLA2R-associated MN

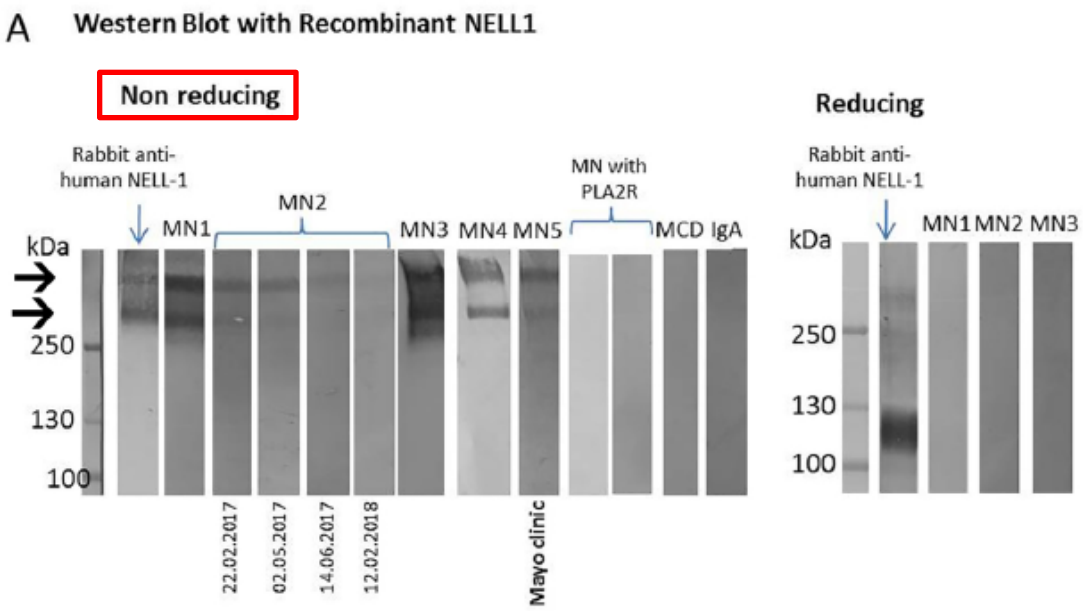
Other nephropathies

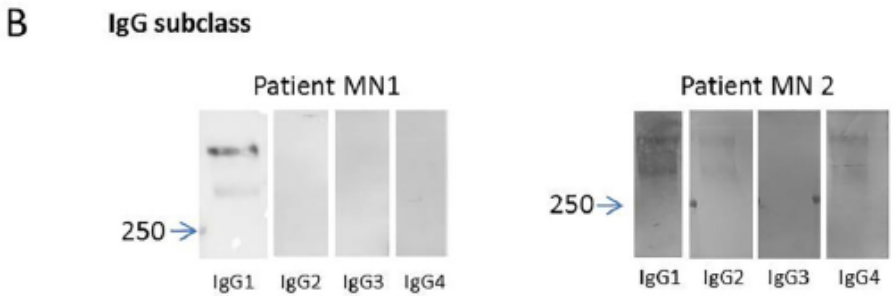


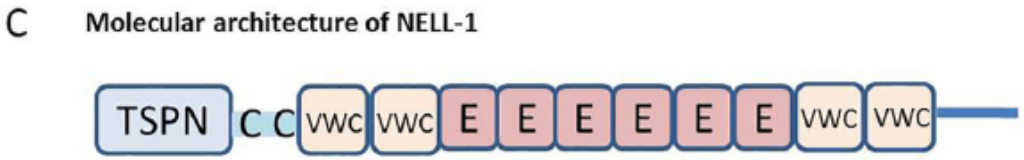
Detection of NELL-1 and IgG in glomerular immune deposits by confocal immunofluorescence microscopy



WB detection of anti-NELL-1 antibodies in the serum







What is NELL-1?

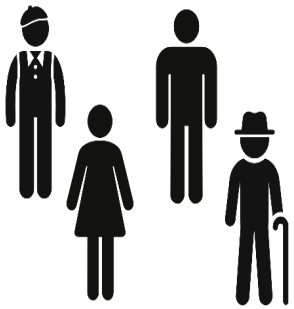
- **NELL-1 is secreted (90kD) and osteoinductive: highly expressed in osteoblasts and promotes bone regeneration and osteoblastic adhesion via integrin $\alpha 3\beta 2$**
- **NELL-1 is overexpressed in patients with craniosynostosis**
- **Kidney: very weak, may be deposited in ECM (culture), expressed in normal tubules but downregulated in areas of renal carcinoma**
- **Not reported in kidney disease as yet**
- **Questions: ultrastructural localization, role of anti-NELL-1 antibodies, mechanism of immunization?**

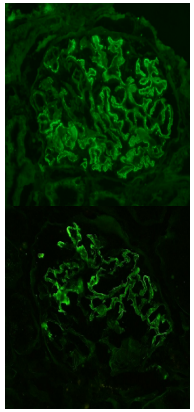
NELL-1: A new marker of K-associated MN?

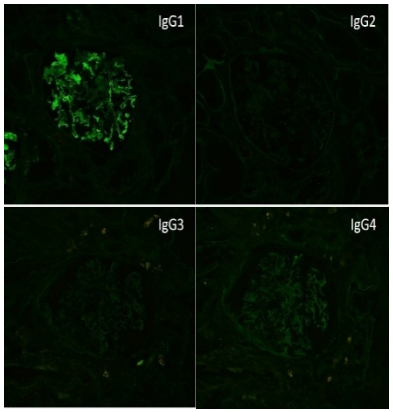
- At Mayo: 24% of double neg MN; no cancer detected in the 29 patients
- French and Belgium cohorts: 6% (5/84) of double neg MN

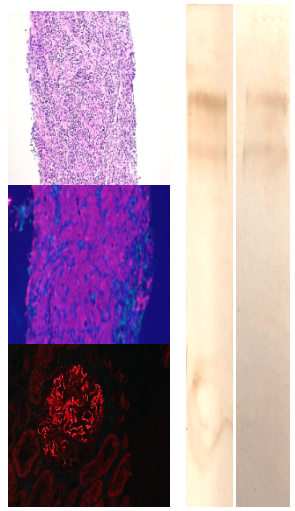
  4/5 cases associated with a K (pancreas, breast, lung, urinary bladder); parallel evolution of cancer remission, proteinuria and anti-NELL-1
- Association with K is most likely more frequent

NELL1: A Target Antigen in Malignancy-Associated Membranous Nephropathy.









NELL1-associated MN(n=91 patients)

Incomplete global or segmental IgG (93.4%)

IgG1 subclass staining in all cases (100%)

Malignancy 30/91 cases (33%)

NELL1 positivity within glomeruli, tumor, and sera

CONCLUSION:

Nerve epidermal growth factor-like 1 (NELL1), a recently identified antigen in membranous nephropathy, is enriched in patients with malignancy-associated membranous nephropathy and anti-NELL1 antibodies can be detected within serum.

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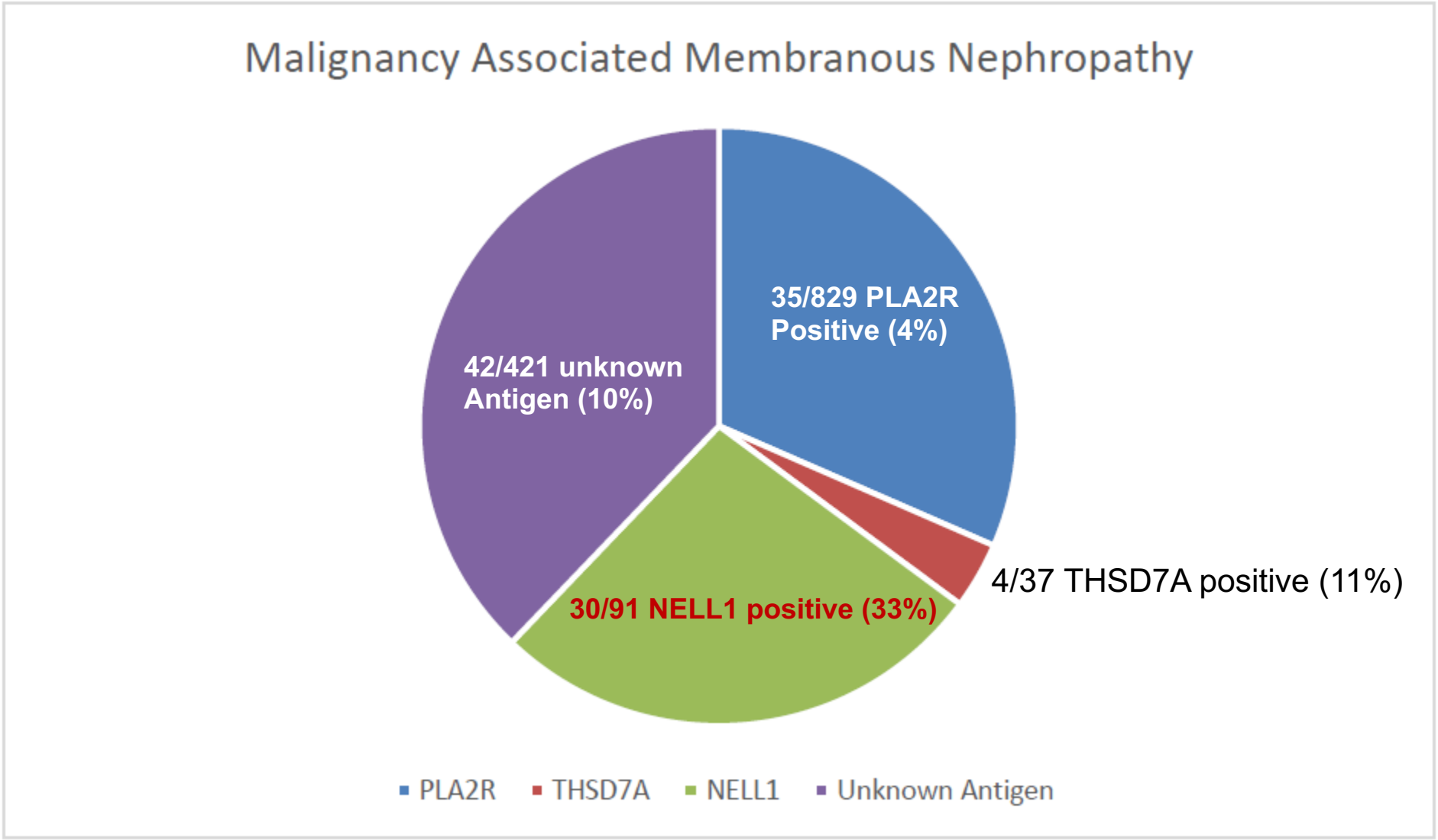
Caza et al, 2020

5 years of "primary" MN at Arkana L. (1378 biopsies)

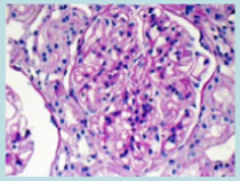


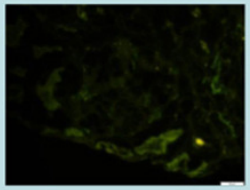
From Casa et al, Kidney Int, 2020, in press

Semaphorin 3B-associated membranous nephropathy is a distinct type of disease predominantly present in pediatric patients.

Patient Selection

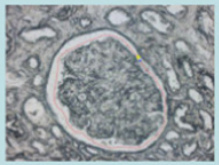
PLA2R-negative MN

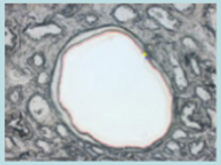


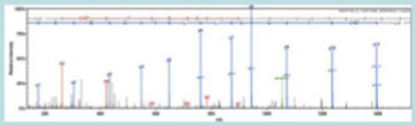


Laser Microdissection and Mass Spectrometry

Identification of novel protein Sema 3B

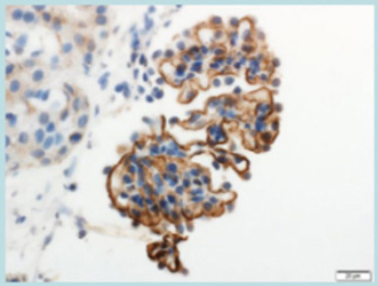






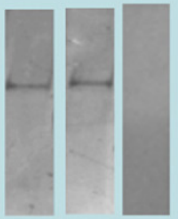
Immunohistochemistry /Immunofluorescence

Granular anti-Sema3B GBM staining



Western Blot Analysis

Serum antibodies to Sema3B



CONCLUSION:

Semaphorin 3B defines a distinct type of membranous nephropathy

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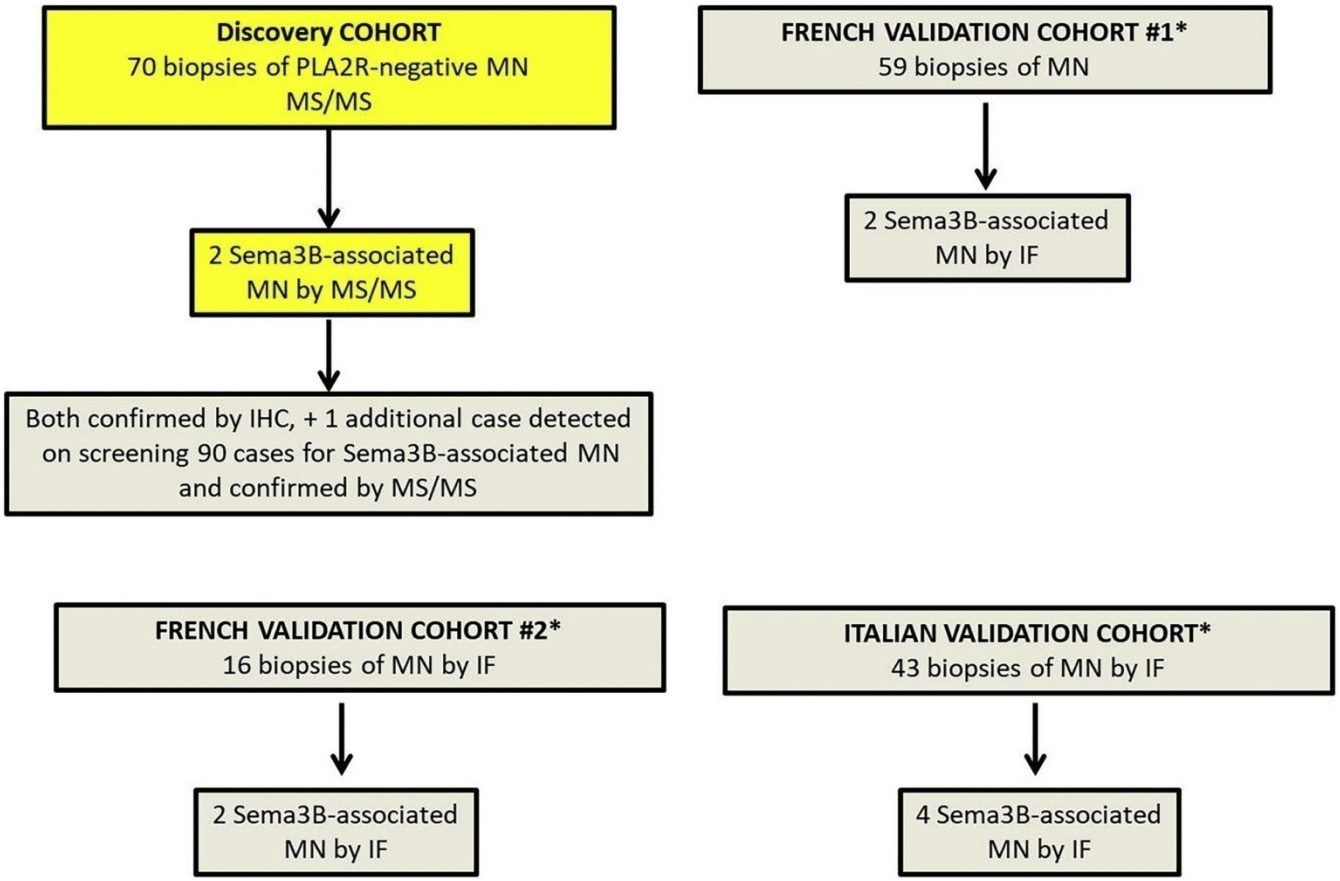
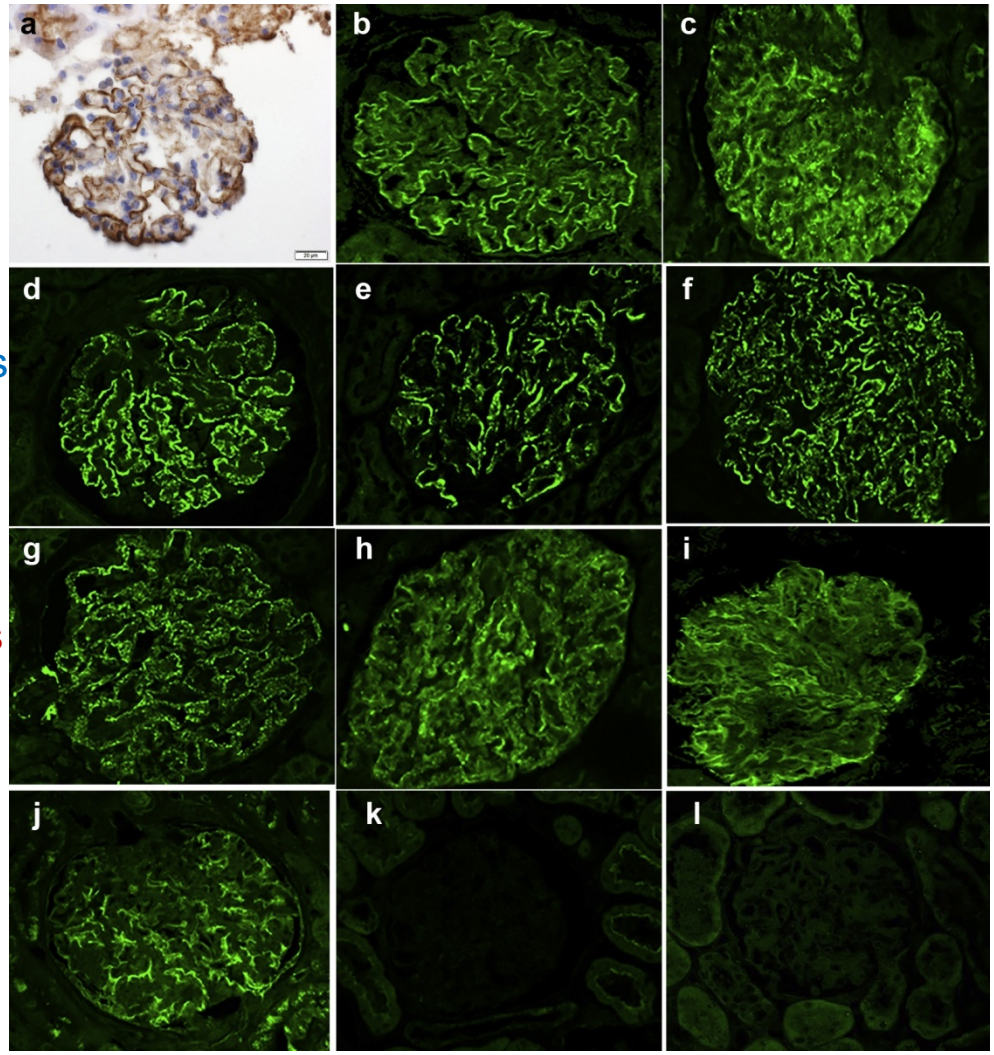
Sethi et al, 2020

Sema3B: Flowchart of the discovery and validation cohorts



Sethi S, Debiec H … Fervenza F & Ronco P: Kidney Int 2020, june 10

IHC and IF labeling of the paraffin biopsies from the European patients



c: Patient #5 at 1 year

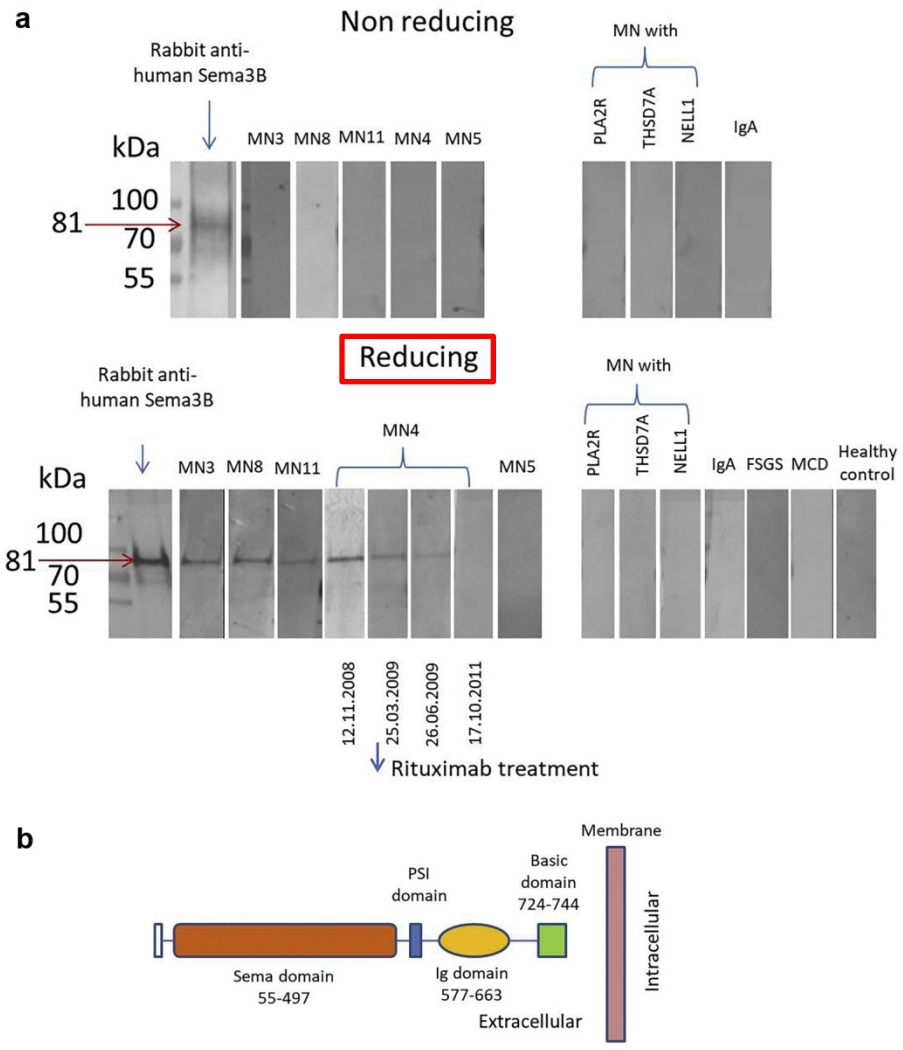
d: Patient #5 at 19 years

e-j: All pediatric patients

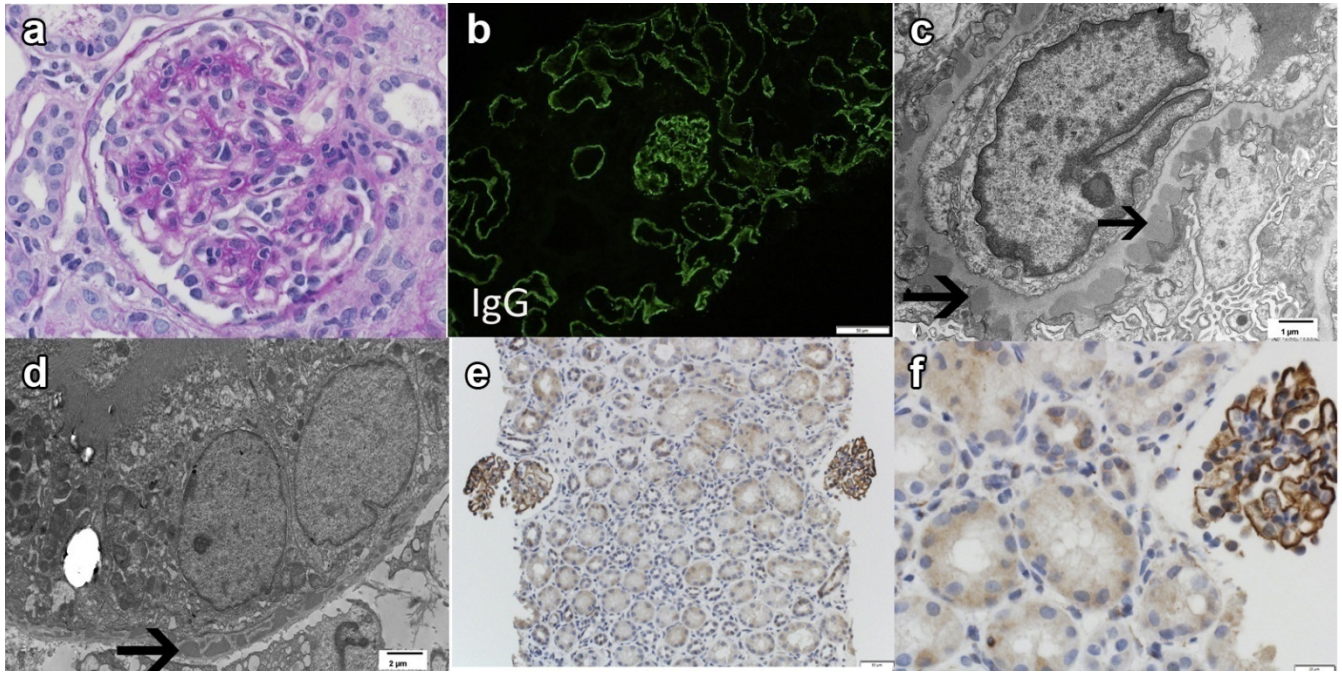
Control cases:

k: PLA2R positive MN
i: PLA2R negative, Sema3b negative child

WB detection of anti-Semaphorin3B antibodies in the serum



Representative kidney biopsy findings



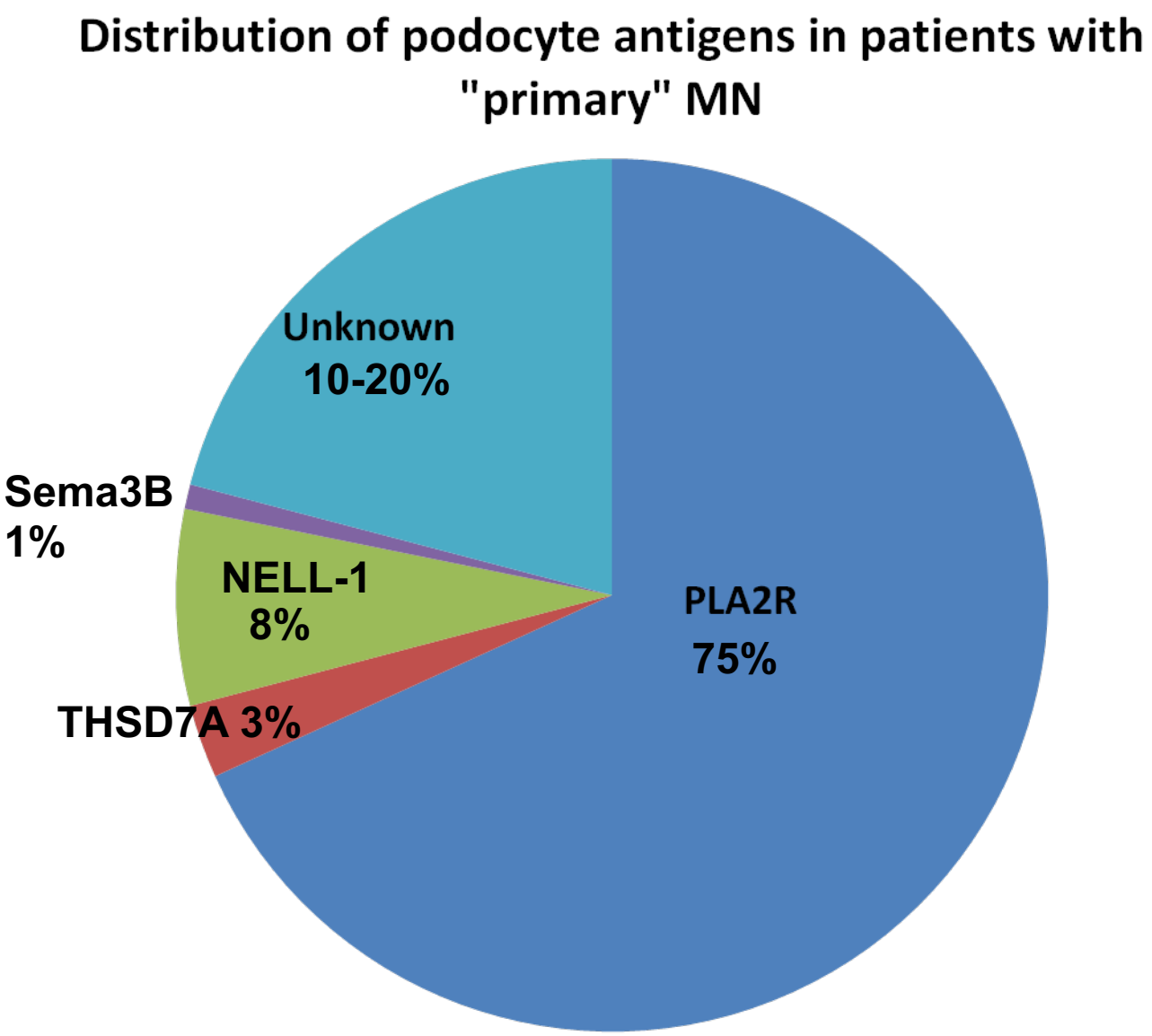
b, c, d: Patient with immune deposits in TBM (b & d)

e & f: Patient without TBM deposits

Sethi S, Debiec H … Fervenza F & Ronco P: Kidney Int 2020, june 10

What is Semaphorin 3B?

- Large family of proteins (>20, 8 subclasses), initially identified as proteins that guide neuronal axons to their targets
- Unlike Sema3A which controls Slit Diaphragm proteins and podocyte survival, Sema 3B seems to be weakly expressed in the podocyte
- All Sema 3s are secreted proteins
- Their receptors, plexins and neuropilins, have been detected in endothelial cells, podocytes and tubular epithelial cells
- The role and expression of sema 3B in the kidney is unkown


Distribution of podocyte antigens in patients with "primary" MN
Unknown 10-20%
Sema3B 1%
NELL-1 8%
THSD7A 3%
PLA2R 75%

A comparison of antigens/biomarkers in MN
From Famine to Plenty…..

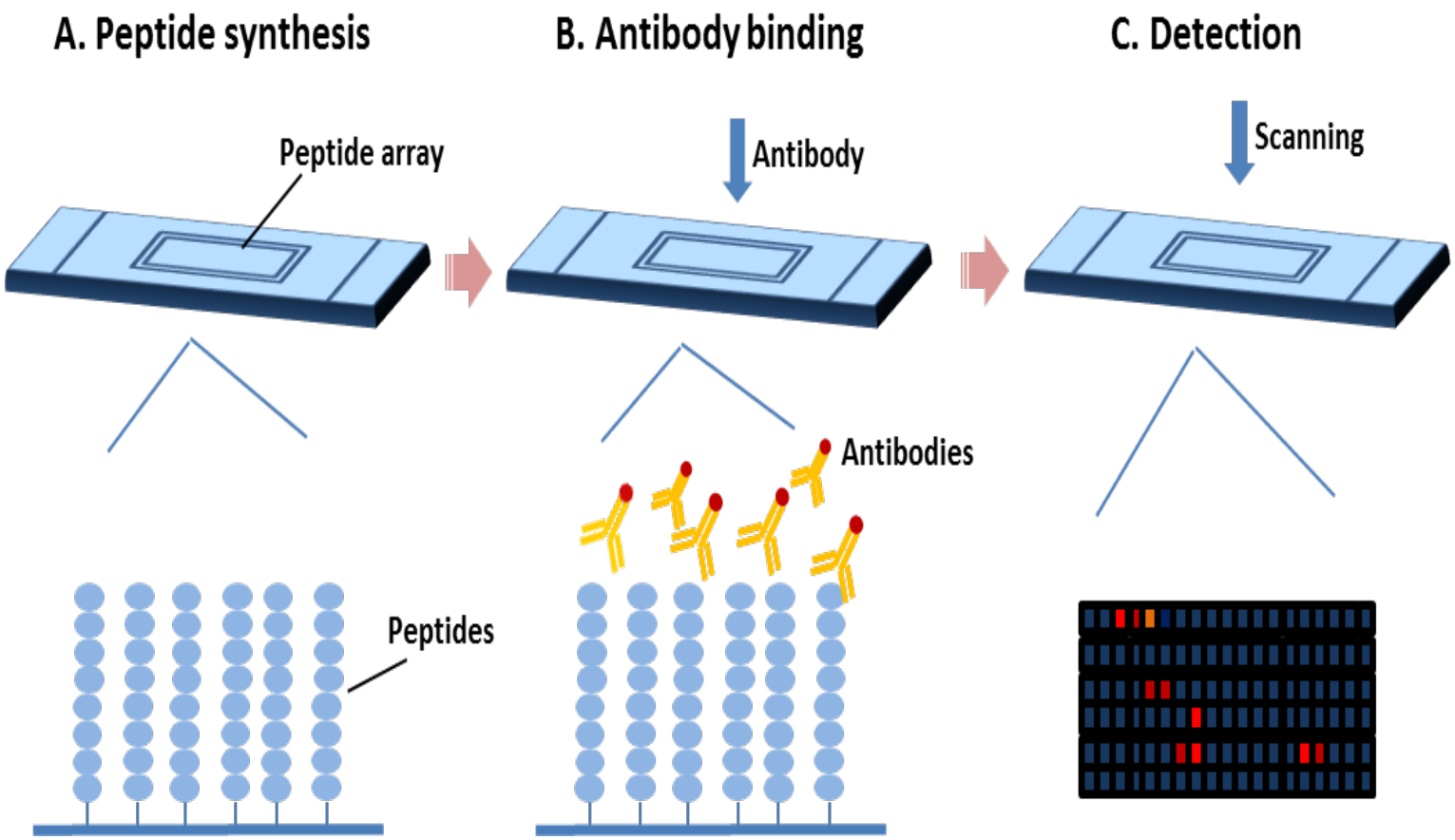
	PLA2R1	THSD7A	EXT1/EXT2	NCAM1	NELL-1	Sema3B
UniProt ID	Q13018	Q9UPZ6	Q16394, Q93063	P13591 (120 kDa isoform)	Q92832	Q13214
Size (in amino acids)	1463	1657	746, 718	858	810	749
Compartment	Transmembrane glycoprotein	Transmembrane glycoprotein	**Glycosyltransferase in Golgi and secreted**	**Transmembrane glycoprotein**	**Secreted**	**Secreted**
Evidence for expression by podocyte	Strong	Strong	**Moderate (EXT2 > EXT1)**	**Weak if any**	**Weak**	Strong Sema3A **??? Sema3B**
Presence in subepithelial deposits	Yes	Yes	Yes	Yes	Yes, **often segmental**	Yes
Circulating Ab	Yes	Yes	**No**	Yes	Yes	Yes, **reduced Ag**
Predominant subclass	IgG4	IgG4	**IgG1 in deposits**	**IgG1 +/- other subclasses**	**IgG1**	**IgG1 / not IgG4**
Distinctive associations	Prototype for primary MN	Malignancy in a minority of cases	**Lupus (#30%) or other systemic autoimmune disease**	**Lupus (#7%)**	**Possible association with malignancy**	**Pediatric MN; early onset**

From Beck et al. Kidney Int 2020, 98: 1081
(commentary accompanying our Sema3B paper)

The latest newcomers presented at ASN 2020!

- **Protocadherin 7 (Mayo, Tenon) #5% of PLA2R-negative cases (revision JASN)**
  - **8/150 PLA2R/THSD7A/NELL1/EXT/Sema3B neg biopsies in the discovery cohort**
  - **4/69 in the validation cohort (France & Belgium)**
  - **Circulating and deposited (eluates) antibodies to the non-reduced form**
  - **Minimal complement deposition (heavy glycosylation?), IgG1>G3>G2>G4**
  - **Association with Sjögren/lupus (x2), sarcoidosis (x1), prostate carcinoma (x1)**
  - **Transmembrane protein that mediates cell-cell recognition and adhesion**
- **High Temperature Recombinant protein A1 (Beck USA) #5% of PLA2R neg cases**
  - **Older patients**
  - **Mostly IgG4**
  - **Transmembrane protein (51kDa)**
  - **Few cases as yet, correlation with disease activity**

Toward a MN-specific antigen/epitope chip array in PLA2R-negative patients



Conclusion

- **"Technological advances have allowed for the demonstration of Moore's law (*a doubling every 2 years in the number of transistors that can be fit onto a computer chip*) in the field of MN" (N. Hayashi and L. H. Beck Jr, KI 2020,98:1081)**

- **For pathologists investigating PLA2R neg MN, staining of paraffin biopsies** can be performed with the antibodies that are all commercially available except for EXT1.
- **Choice of antibody for biopsy staining should be prioritized according to the clinical context: age, systemic manifestations, extent of the Ig deposits (segmental or global)**

- **For clinicians, an accurate histopathological diagnosis is pivotal for etiological investigation and treatment monitoring.**
- **Serological tests (chip test) will become available in a near future**

☞ **We should follow the road open by oncopathologists**

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Next Webinars

Working Group on Inherited Kidney Disorders

The European Rare Kidney Disease Reference Network

european society for paediatric nephrology

International Pediatric Nephrology Association
GREAT CARE FOR LITTLE KIDNEYS. EVERYWHERE

ERKNet/ERA-EDTA Advanced Webinars on Rare Kidney Disorders

Date: **12 Jan 2021**

Speaker: **Fadi Fakhouri**

Topic: **Pregnancy related TMA**

ERKNet/ERA-EDTA Advanced Webinars on Rare Kidney Disorders

Date: **19 Jan 2021**

Speaker: **Ben Walsh**

Topic**: dRTA**

ESPN/ERKNet Educational Webinars on Pediatric Nephrology & Rare Kidney Diseases

Date: **02 Feb 2021**

Speaker: **Christoph Licht**

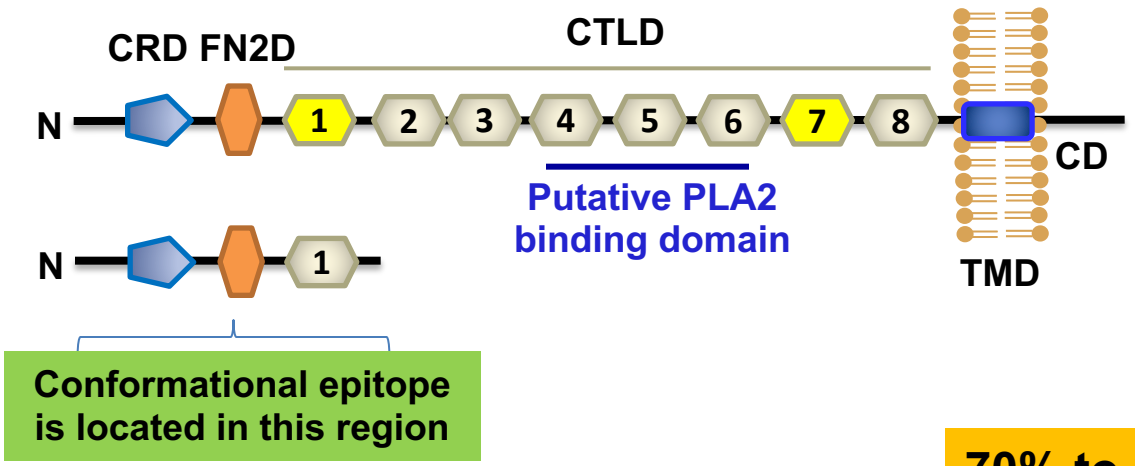
Topic**: C3 Glomerulopathy**

Subscribe the ERKNet and IPNA Newsletter and don‘t miss Webinars!

Thank you!

A paradigm shift in diagnosis, monitoring and classification of patients with MN

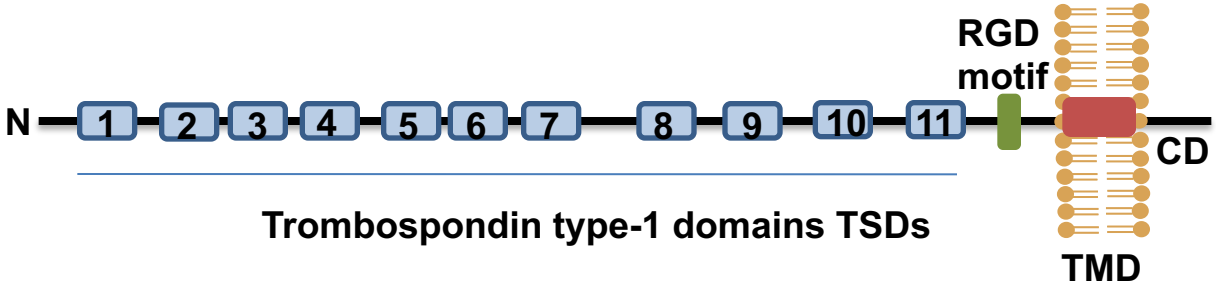
PLA2R



Beck et al, NEJM 2009,361:11
Kao et al, JASN 2015,26:291
Fresquet et al, JASN 2015,26:302
Seitz et al, JASN 2016, 27:1517; JASN 2018 29:401
(epitope speading correlated with outcome)

70% to 85% of adult MN patients

Thrombospondin type-1 domain containing7A(THSD7A)

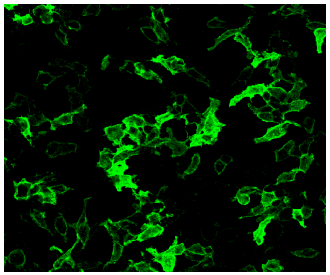


Tomas et al,
NEJM 2014, 371: 2277
Tomas et al,
J Clin Invest 2016, 126:2519

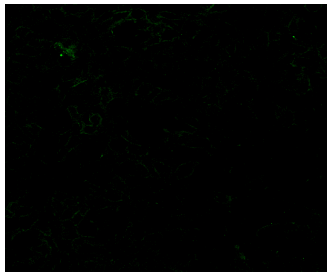
10 % of PLA2R-negative patients

Serological tests for the diagnosis and monitoring of patients with MN

→ **Indirect immunofluorescence for PLA2R and THSD7A** →

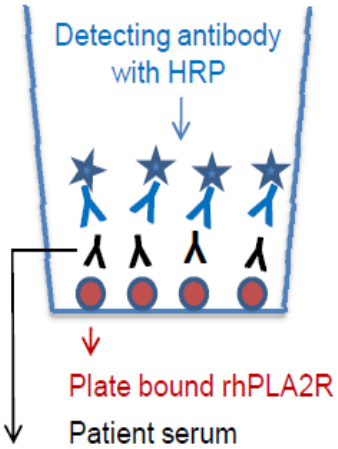


HEK293 cells transfected with cDNA for PLA2R



HEK293 cells non transfected

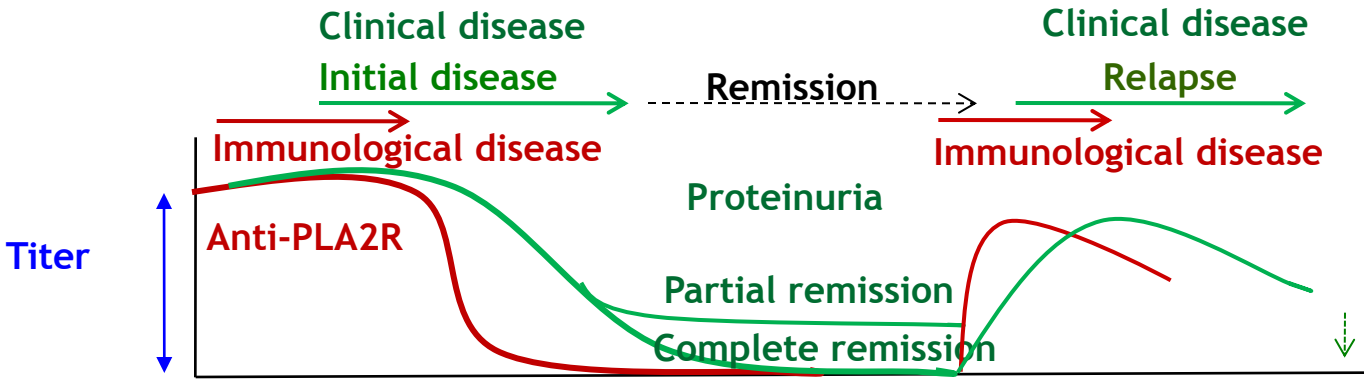
ELISA-PLA2R



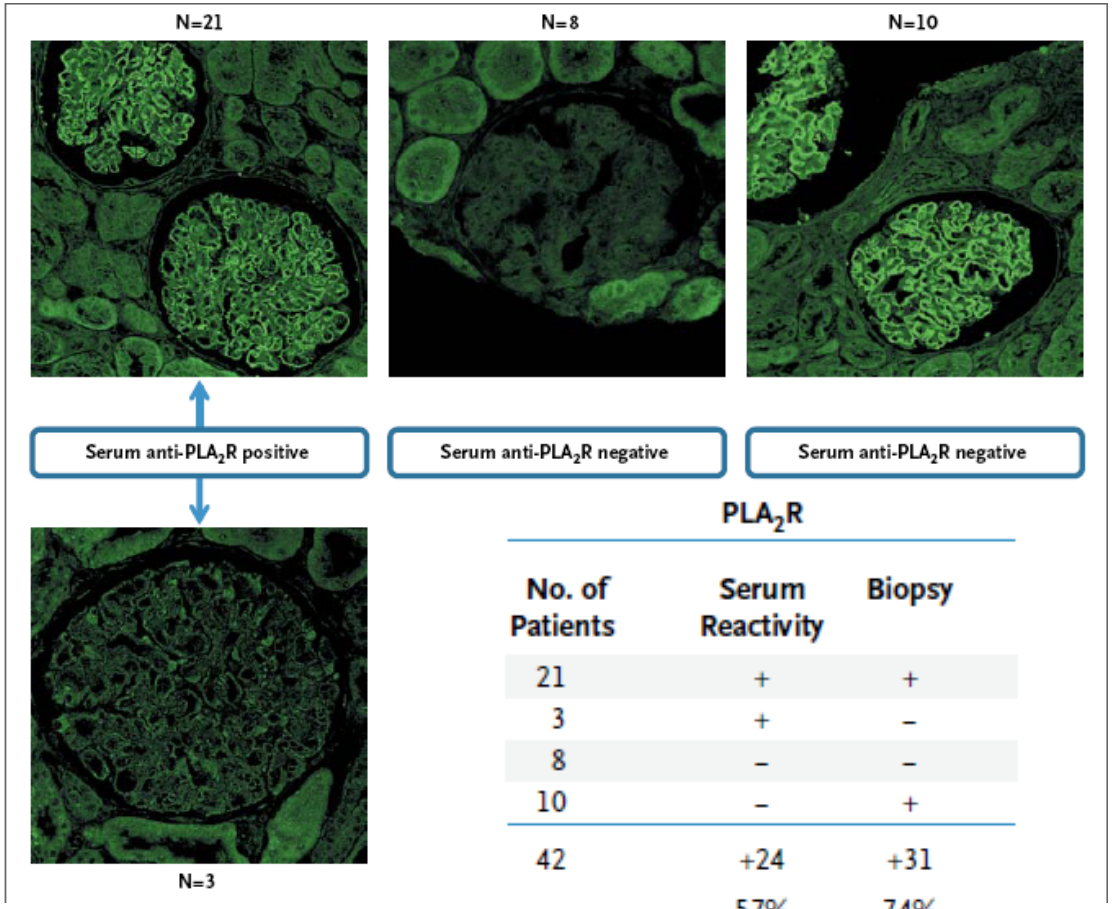
Meta-analysis (2014)

- 15 studies, 2212 patients
- **Specificity = 99%**
  (95% CI : 96-100%)
- **Sensitivity = 78%**
  (95% CI :66-87%)

Du et al, PLoSOne 2014, 9:e104936



Antigen detection in biopsy is more sensitive than serology



	PLA$_2$R	
No. of Patients	Serum Reactivity	Biopsy
21	+	+
3	+	–
8	–	–
10	–	+
42	+24	+31
	57%	74%

Tenon cohort 2000-2014

- n = 106 (84 iMN ; 22 sMN)

- sensitivity PLA2R - Ag : 86%

- ‘’ aPLA2R-Ab : 76%

Pourcine et al, PLoS One. 2017

Retrospective diagnosis

Debiec and Ronco, New Engl J Med, 2011, 364 :689