



Molecular Pathogenesis of Membranous Nephropathy

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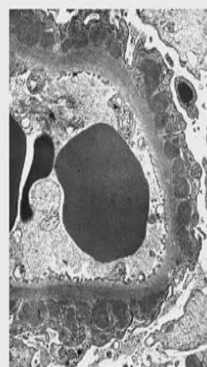
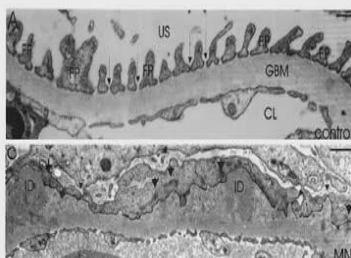
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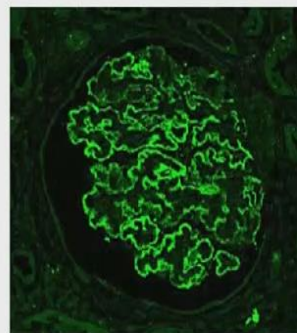
Brief summary of MN breakthroughs



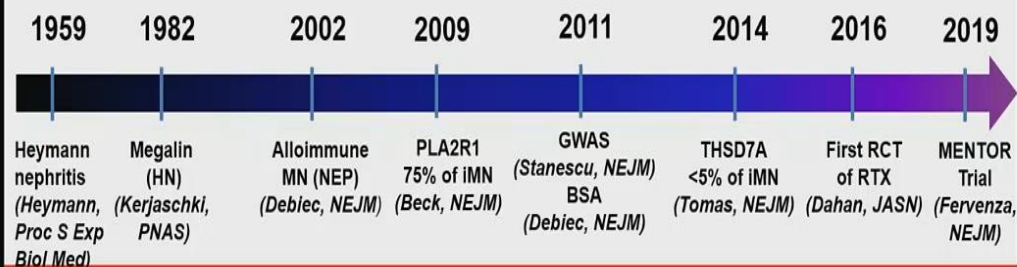
- Thickening of GBM
- Immune deposits
- Foot process retraction



Electron dense deposits



IF : immunoglobulin
(IgG4>IgG1)



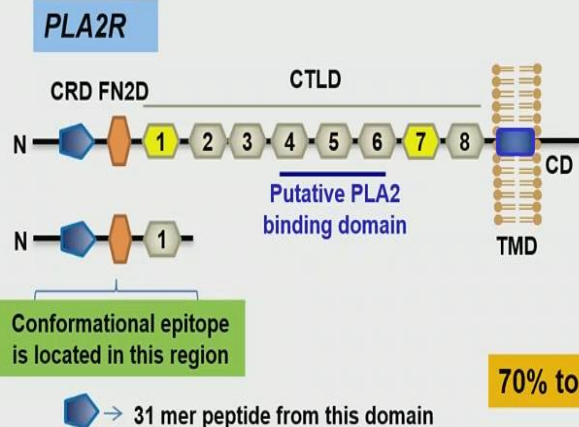
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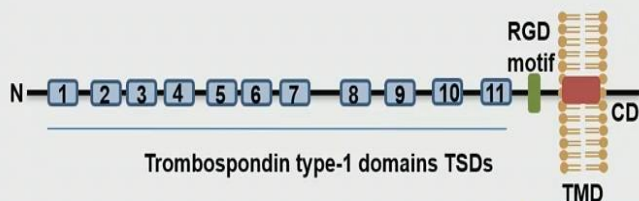
A paradigm shift in diagnostic, monitoring & classification of patients with MN



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 2017 Nov 7th (epitope spreading correlated with outcome)

70% to 85% of adult MN patients

Thrombospondin type-1 domain containing 7A (THSD7A)



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

10 % of PLA2R-negative patients

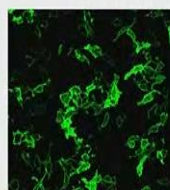
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Serological tests for diagnosis and monitoring

→ Indirect immunofluorescence
for PLA2R and THSD7A

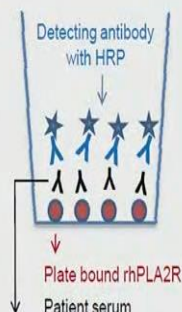


HEK293 cells
transfected with
cDNA for PLA2R



HEK293 cells non
transfected

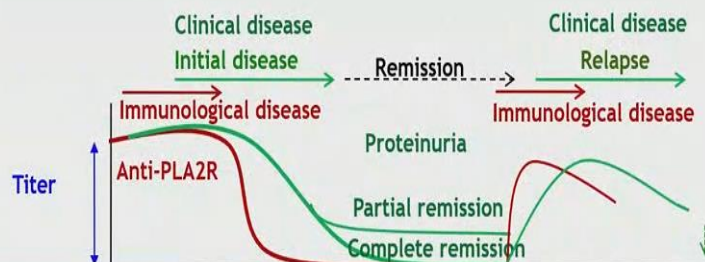
ELISA-PLA2R



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*



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



Sydney Brenner
Nobel Prize 2002

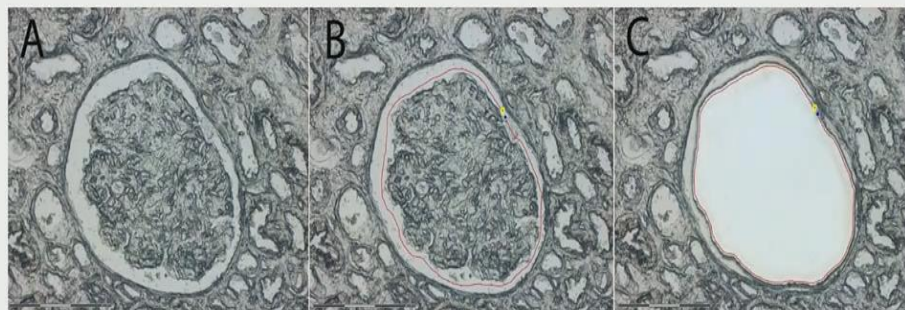
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Laser microdissection of glomeruli & MS of trypsin digested proteins



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

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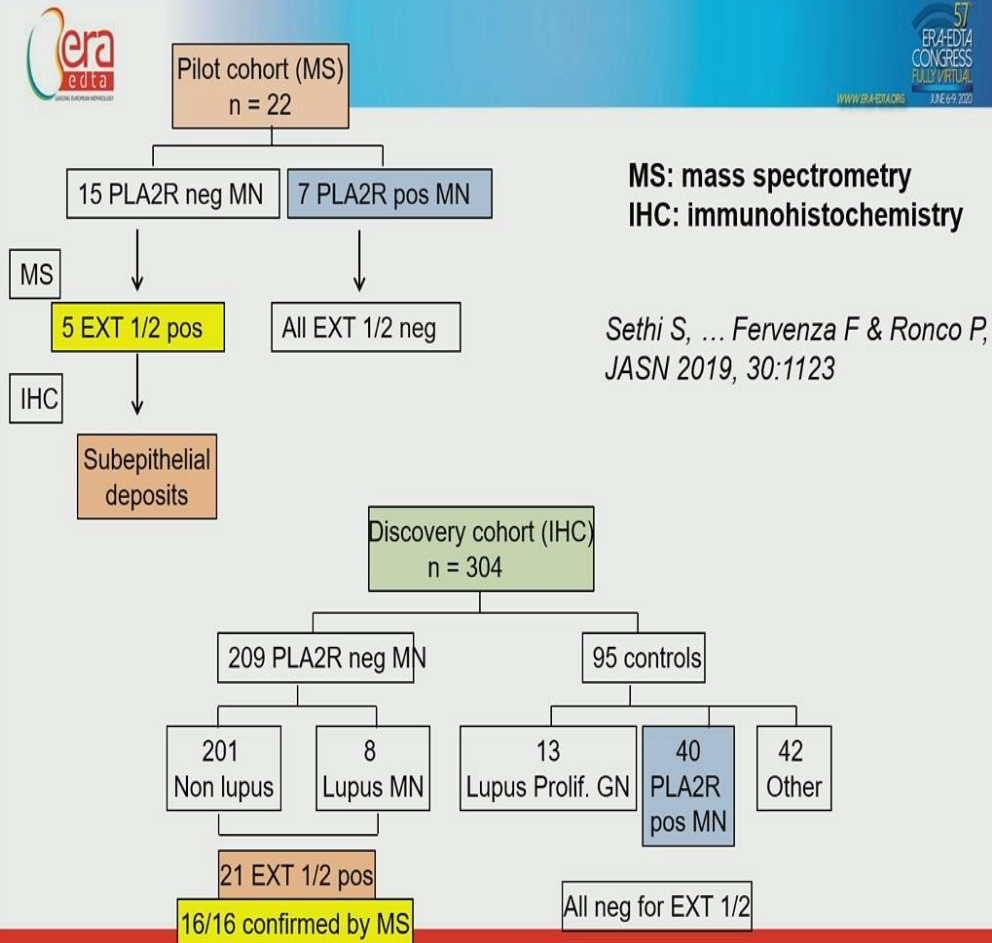
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Exostosins 1 and 2

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Coverage of exostosin-1 sequence by MS identified peptides



sp|Q16394|EXT1_HUMAN (100%), 86,257.4 Da

Exostosin-1 OS=Homo sapiens GN=EXT1 PE=1 SV=2

35 exclusive unique peptides, 54 exclusive unique spectra, 70 total spectra, 486/746 amino acids (65% coverage)

MQAKKRYFIL	LSAGSCLALL	FYFGGLQFRA	SRSHSRREEH	SGRNGLHHPS	PDHFWPRFPD
ALRPFVPWDQ	LENEDSSVHI	SPRQKRDNAS	SIYKGKKCRM	ESCFDFTLCK	KNGFKVYVYP
QQKGEKIAES	YQNILAAIEG	SRFYTSDPSPQ	ACLFVLSLDT	LDRDQLSPQY	VHNLRSKVQS
LHLWNNGRNH	LIFNLYSGTW	PDYTEDVGFD	IGQAMLAKAS	ISTENFRPNF	DVSIPLFSKD
HPRTGGGERGF	LKFNTIPPLR	KYMLVFKGKR	YLTGIGSDTR	NALYHVHNGE	DVVLLTTCKH
GKDWWQKHDS	RCDRDNTEYE	KYDYREMLHN	ATFCLVPRGR	RLGSFRFLEA	LQAACVPVML
SNGWELPFSE	VINWNQAAVI	GDERLLLQIP	STIRSIHQDK	ILALRQQTQF	LWEAYFSSVE
KIVLTTLEII	QDRIFKHISR	NSLIWNKHPC	GLFVLPQYSS	YLGDFPYYYA	NLGLKPPSKF
TAVIHAVTPL	VSQSQPVLKL	LVAAAKSQYC	AQIIVLWNCQ	KPLPAKHRWP	ATAVPVVIE
GESKVMSSRF	LPYDNIITDA	VLSLDEDTVL	STTEVDFAFT	VWQSFPERIV	GYPARSHFWD
NSKERWGYTS	KWTNDYSMVL	TGAAIYHKYY	HYLYSHYLP	SLKNMVDQLA	NCEDILMNFL
VSAVTKLPPI	KVTQKKQYKE	TMMGQTSRAS	RWADPDHFAQ	RQSCMNTFAS	WFGYMPLIHS
QMRLLDPVLFK	DQVSILRKKY	RDIERL			

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

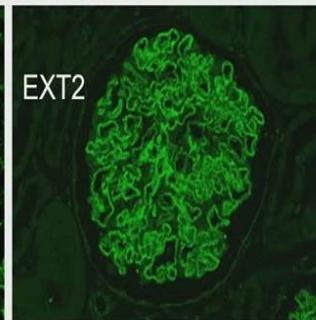
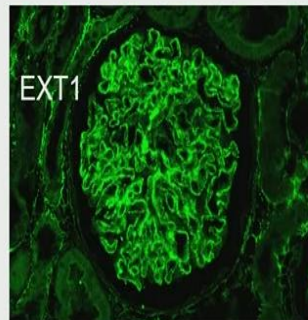
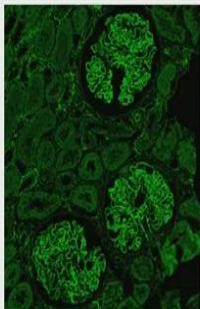
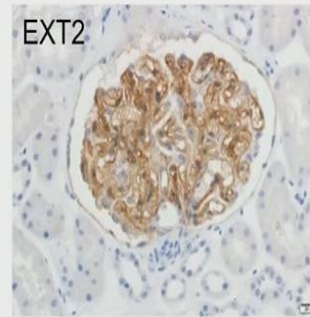
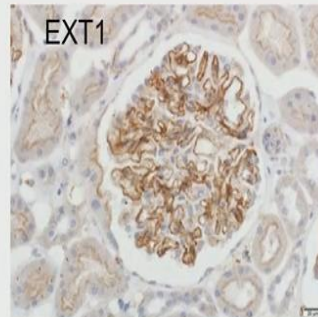
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IHC and IF labeling of the paraffin biopsy from the same patient



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

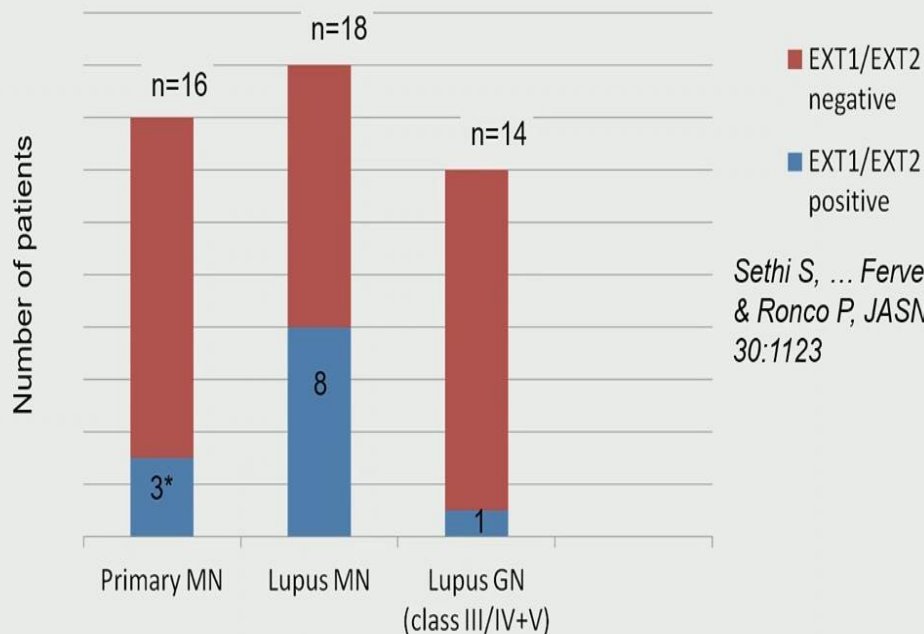
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Validation cohort (n=48)



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.

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Clinical features of EXT1/EXT2 patients



Mean age, (+/-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

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New data since the JASN paper



Mayo Clinic:

374 biopsies of MN including mixed class:

122 EXT1/EXT2 positive (32.6%)

30 with proliferative features (III/V, IV/V)

Tenon-Pitié (M. Saidi, Z. Amoura, P. Cacoub):

100 biopsies of pure class V:

32 EXT1/EXT2 positive (32%)

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NELL-1

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

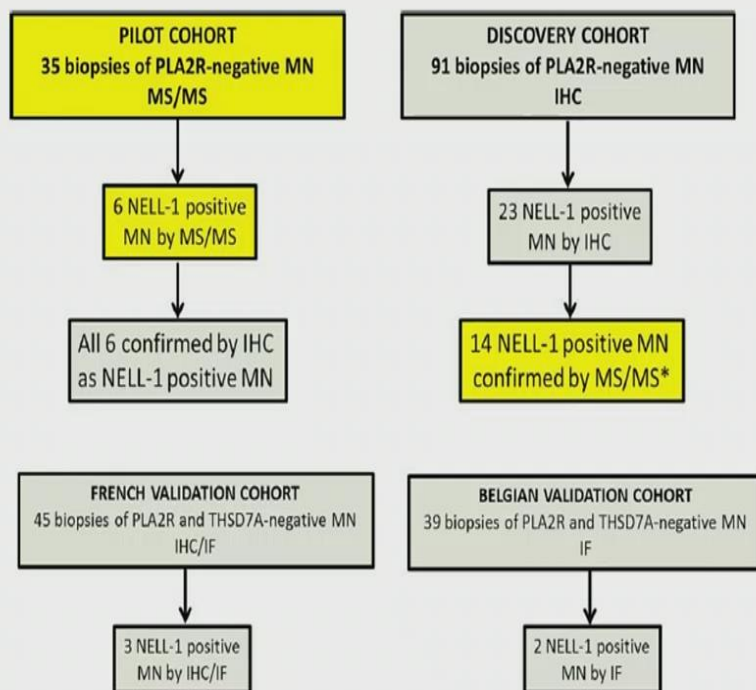
Sethi S, Debiec H, ... Fervenza F & Ronco P
([Kidney Int.](#) 2020 Jan;97(1):163-174)

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

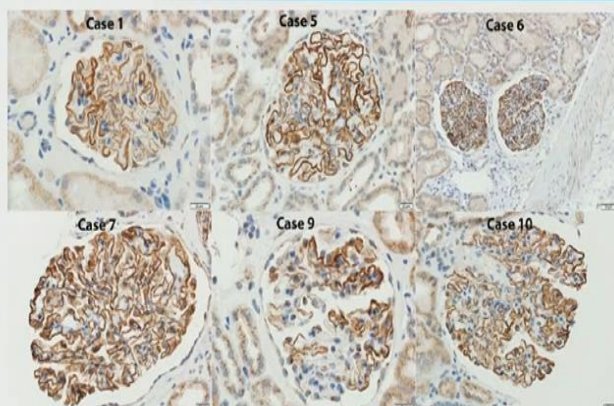


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Immunohistochemical stain for NELL-1

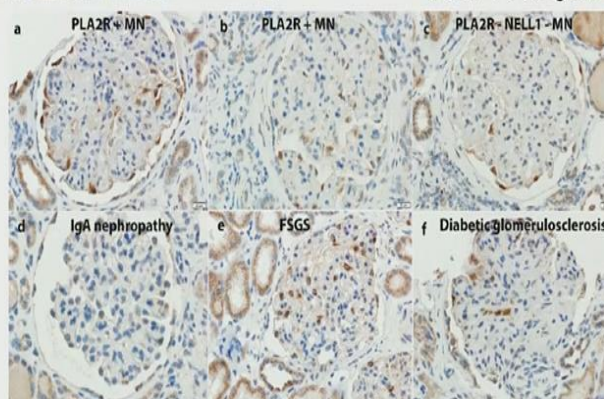


NELL-1 associated MN

PLA2R neg
NELL-1 neg MN

PLA2R pos MN (X2)

Other nephropathies

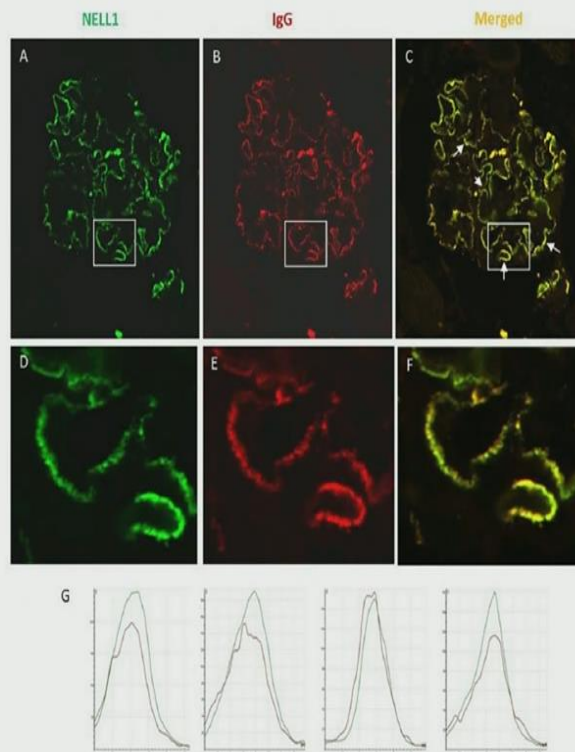


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Detection of NELL-1 and IgG by confocal IF



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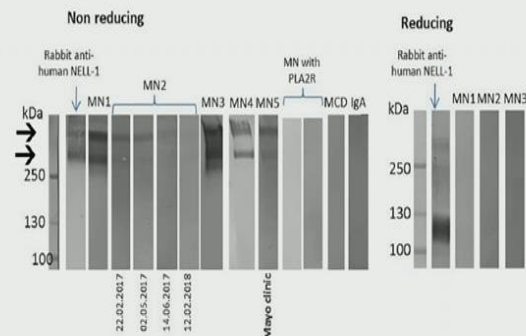


WB detection of anti-NELL-1 Ab in serum

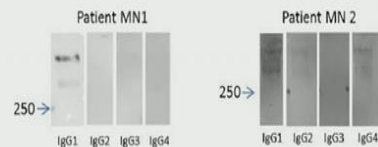


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A Western Blot with Recombinant NELL1



B IgG subclass



C Molecular architecture of NELL-1



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Epidemiology of NELL-1 associated MN



Same demographics and clinical characteristics as usual « primary MN »

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

4/5 cases associated with a K (pancreas, breast, lung, urinary bladder)

Association with K is most likely more frequent

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What is NELL-1?



- **NELL-1 is secreted (90kD) and osteoinductive: highly expressed in osteoblasts and promotes bone regeneration and osteoblastic adhesion via $\alpha 3\beta 2$**
- **NELL-1 is overexpressed in patients with craniosynostosis**
- **Kidney: very weak, may be deposited in ECM (culture), expressed in normal tubules**
- **Questions: ultrastructural localization, role of anti-NELL-1 antibodies, mechanism of immunization?**

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Toward a new serology/biopsy-based classification



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- PLA2R-related # 60 to 80%
- THSD7A-related <5%
- EXT1/EXT2, NELL-1, others #20% (identified by biopsy staining in double negative patients)
- Any of this antigen defined MN can be:
 - primary without known etiology.....as yet
 - secondary:
 - PLA2R-associated HepB, sarcoidosis
 - THSD7A, PLA2R, NELL-1- associated cancer
 - EXT1/EXT2- associated lupus-like auto-immunity

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What about childhood MN?



➤ Podocyte antigens as yet identified:

- Neutral endopeptidase (neonates only)
- THSD7A (rare)
- PLA2R (rare)

➤ Non-podocyte antigens

- Bovine serum albumin (BSA) (*Debiec et al, NEJM 2011, 364 : 2101*)
- Enzyme replacement therapy (*Debiec et al, JASN, 2014, 25:475*)

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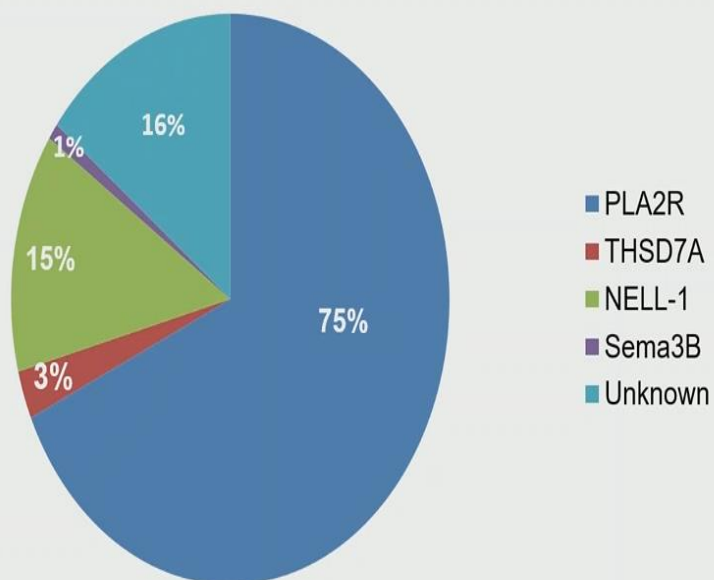
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Sethi, Debiec, Vivarelli, Fervenza, Ronco, Kidney Int, 2020, in press

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Distribution of podocyte antigens in "primary" MN



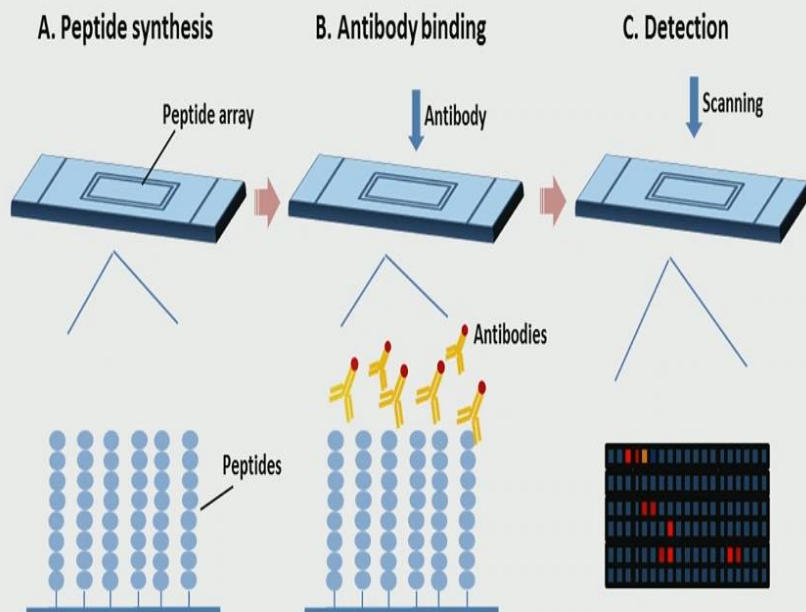
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Conclusion: Toward a MN-specific antigen/epitope chip array



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