Why do children get Nephrotic Syndrome?

Moin Saleem
Professor of Paediatric Renal Medicine
University of Bristol, UK

Nephrotic Syndrome

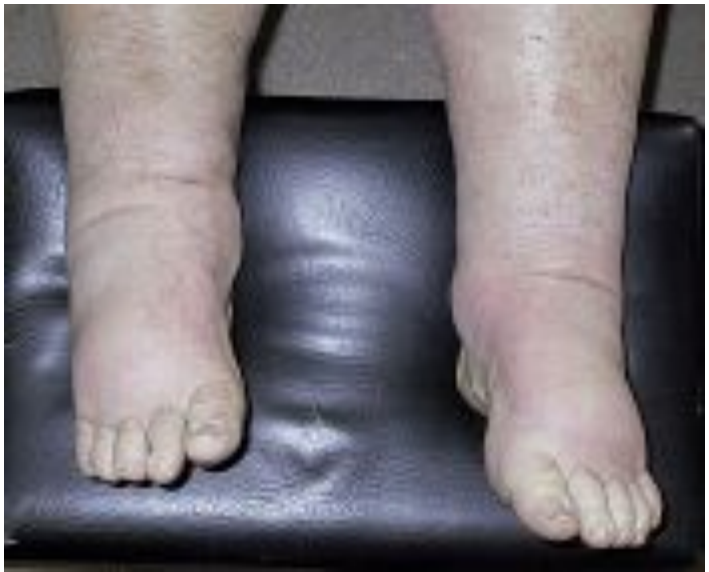
Heavy proteinuria

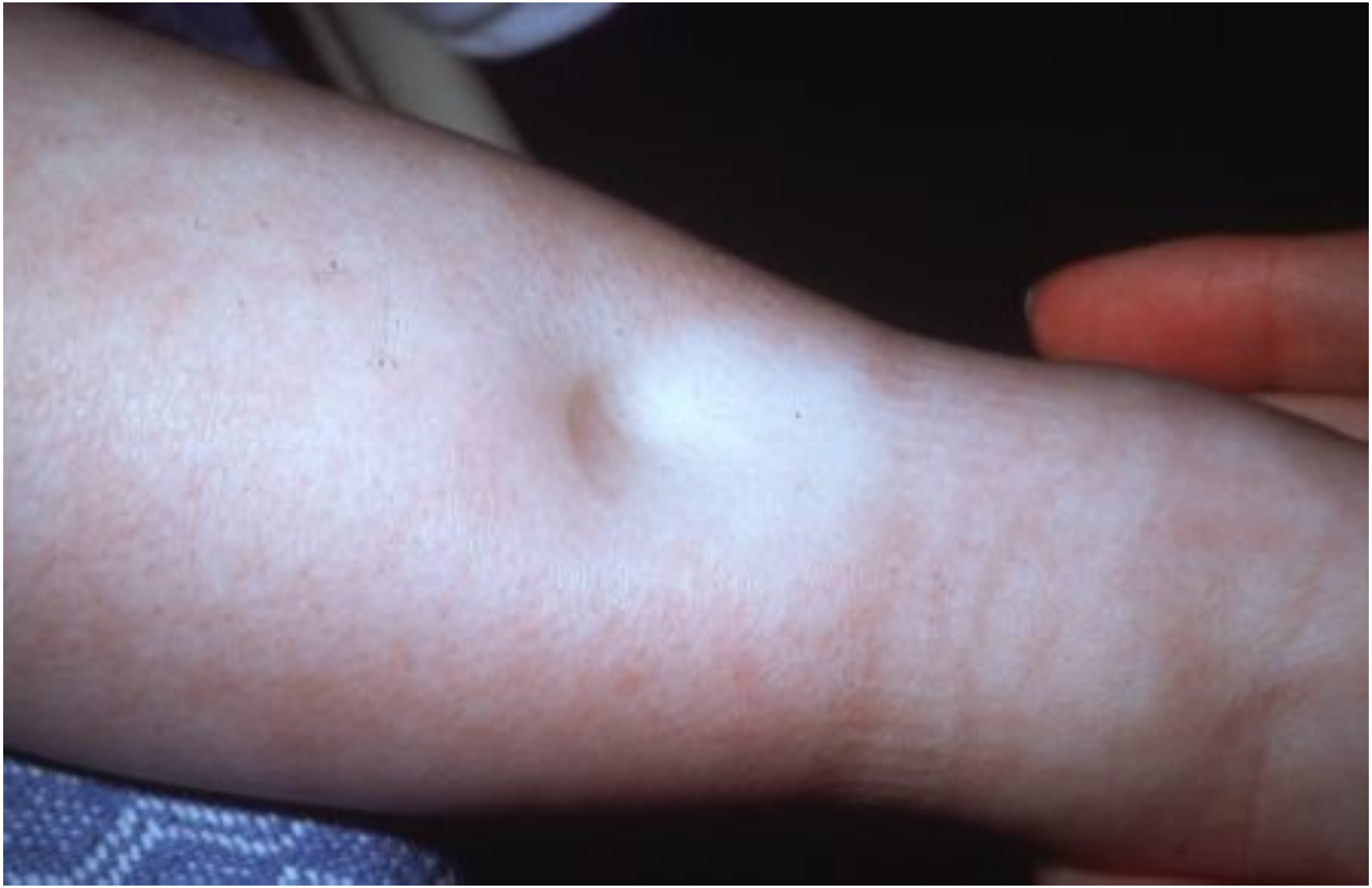
↓

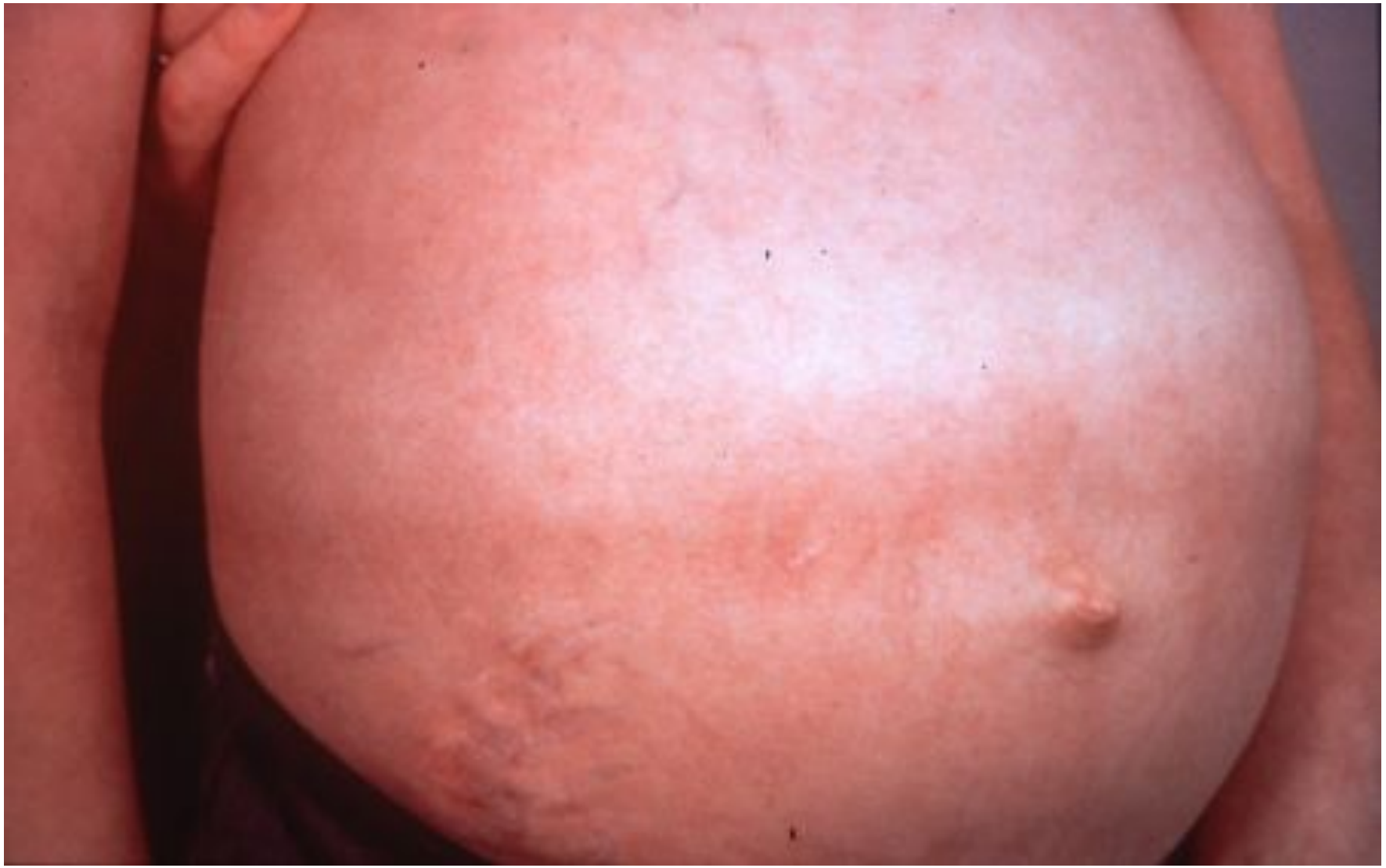
Hypoalbuminaemia

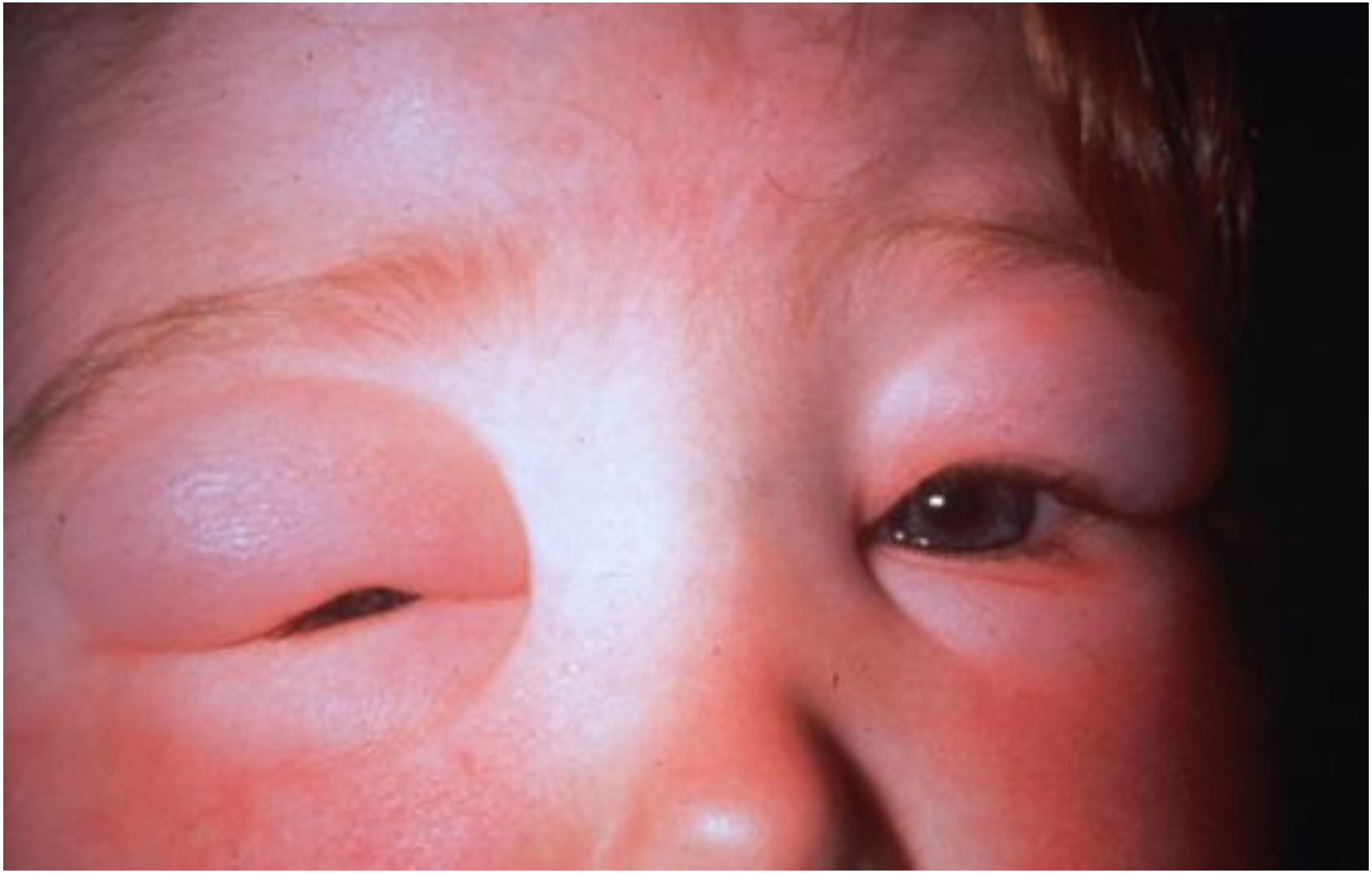
↓

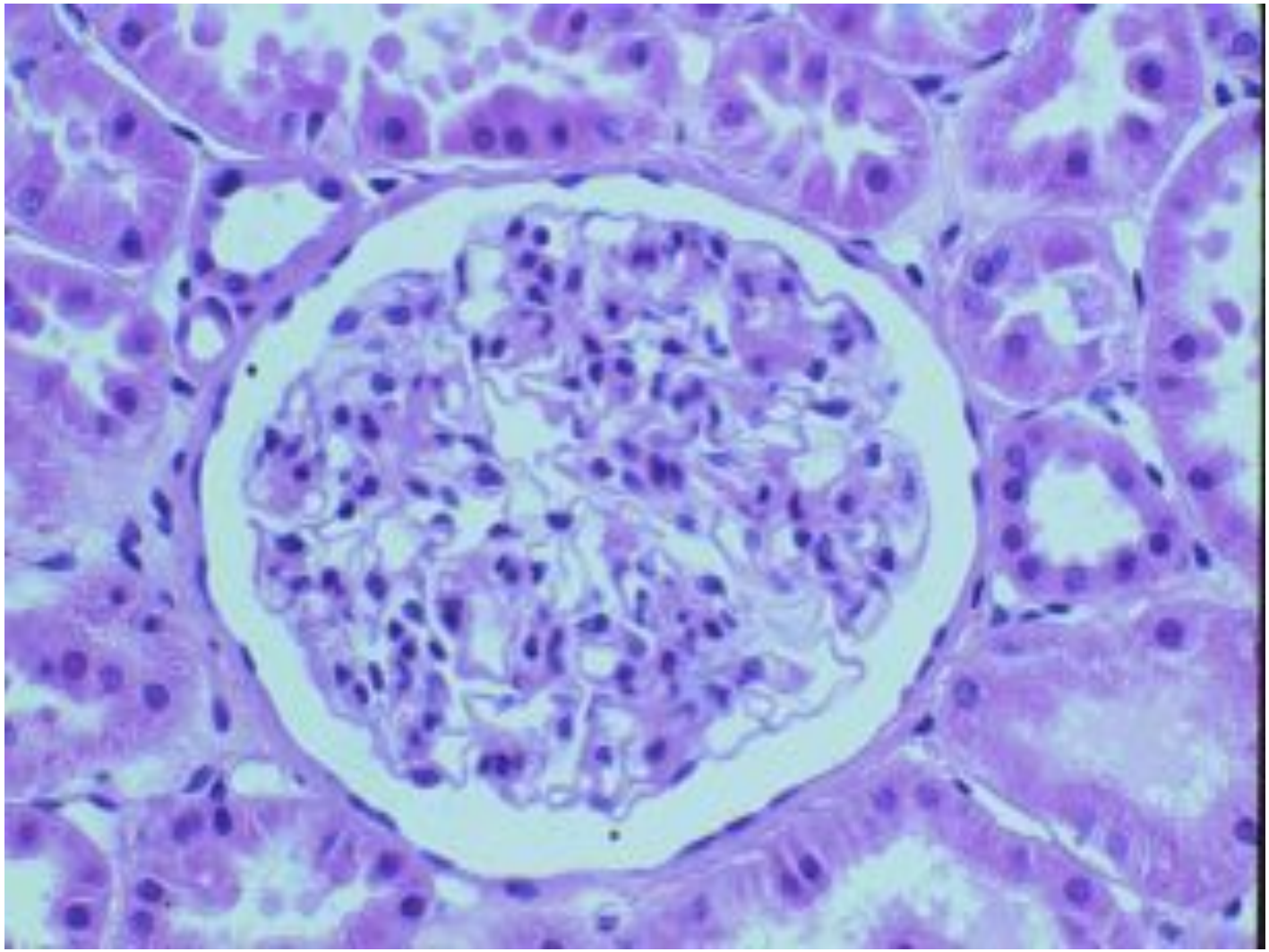
Oedema

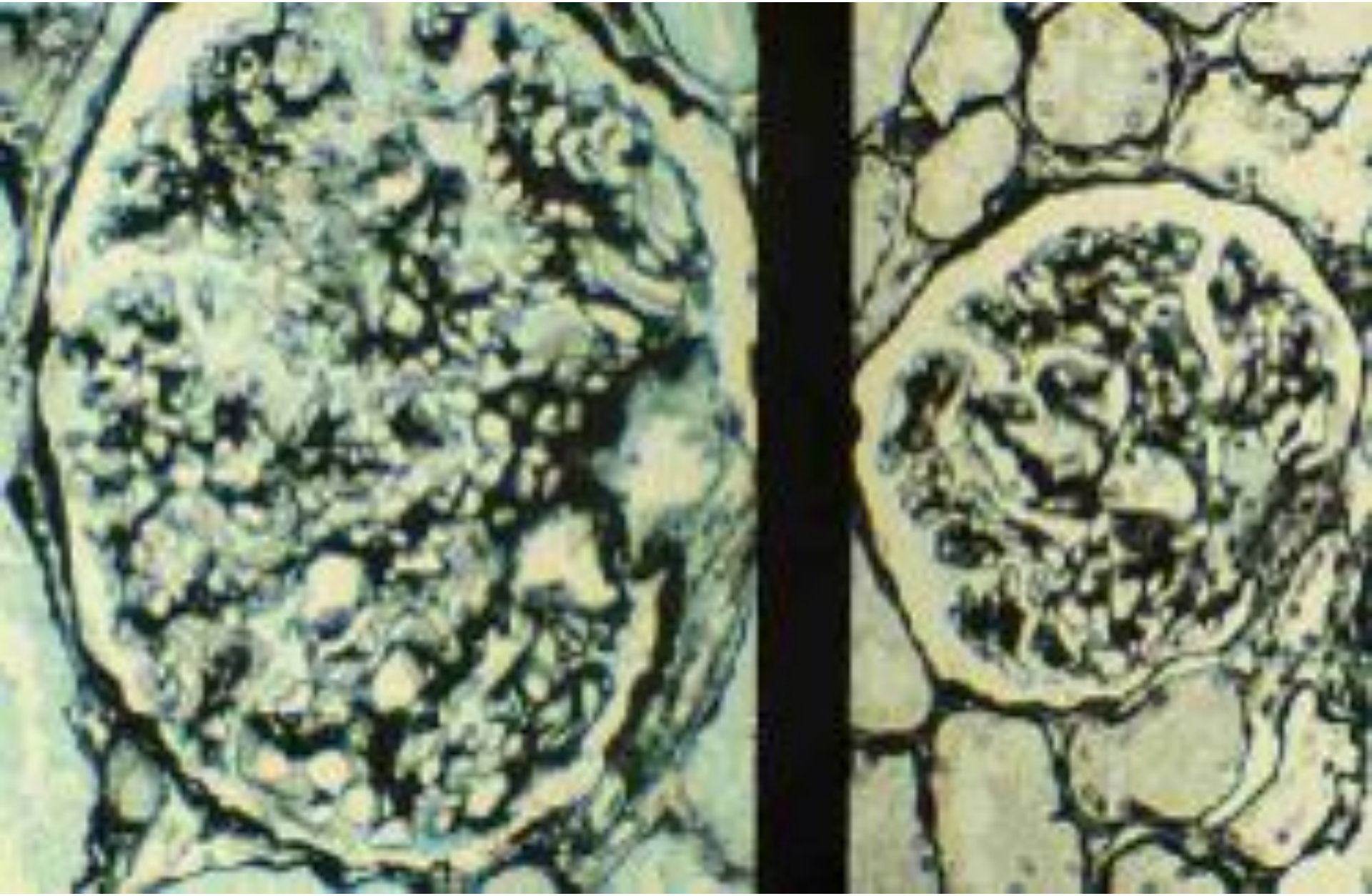




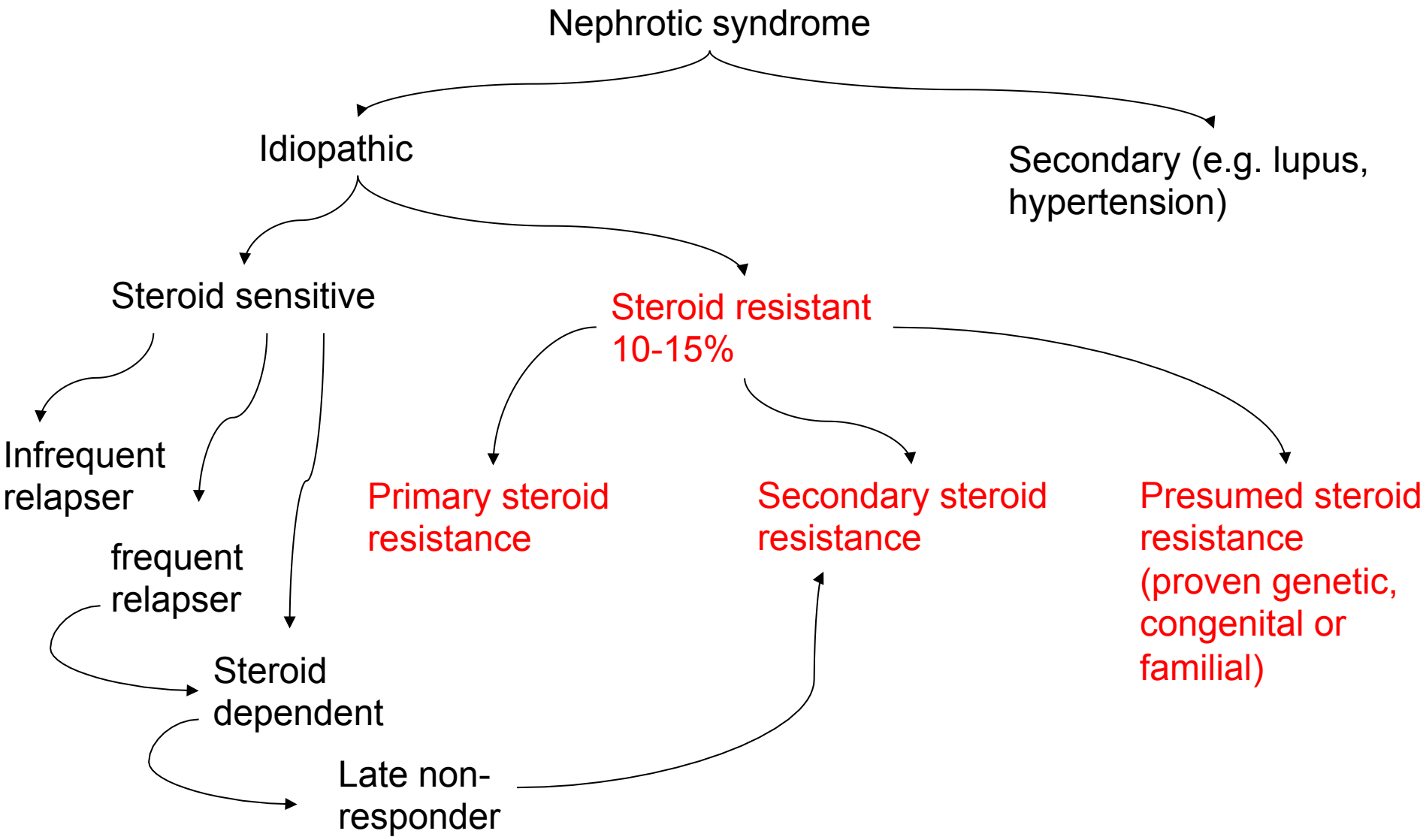








Nephrotic syndrome – response based classification



Nephrotic Syndrome – a disease of the filtration barrier

- Is this an immune disease?
  - Viral triggers; response to steroids/immunosuppression
  - BUT – no immune infiltrates
  - 'Circulating' factor is likely
- Is this non-immune?
  - Some patients never respond to immunosuppression
  - Can occur in families
  - Can occur with extra-renal features

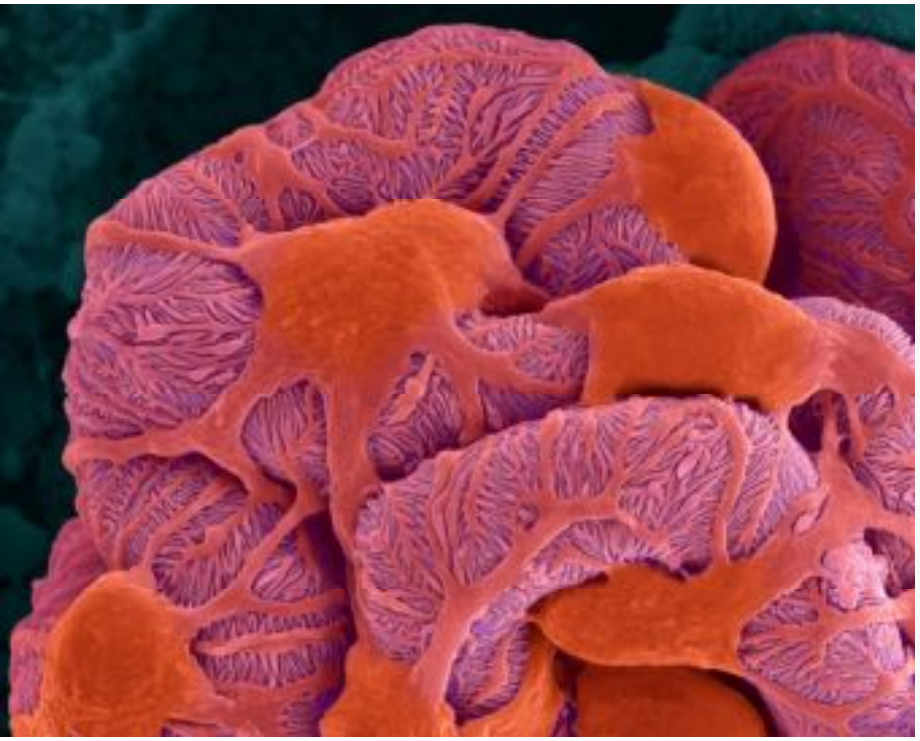
- How can we understand the disease mechanism underlying the clinical (and molecular) observations we are making

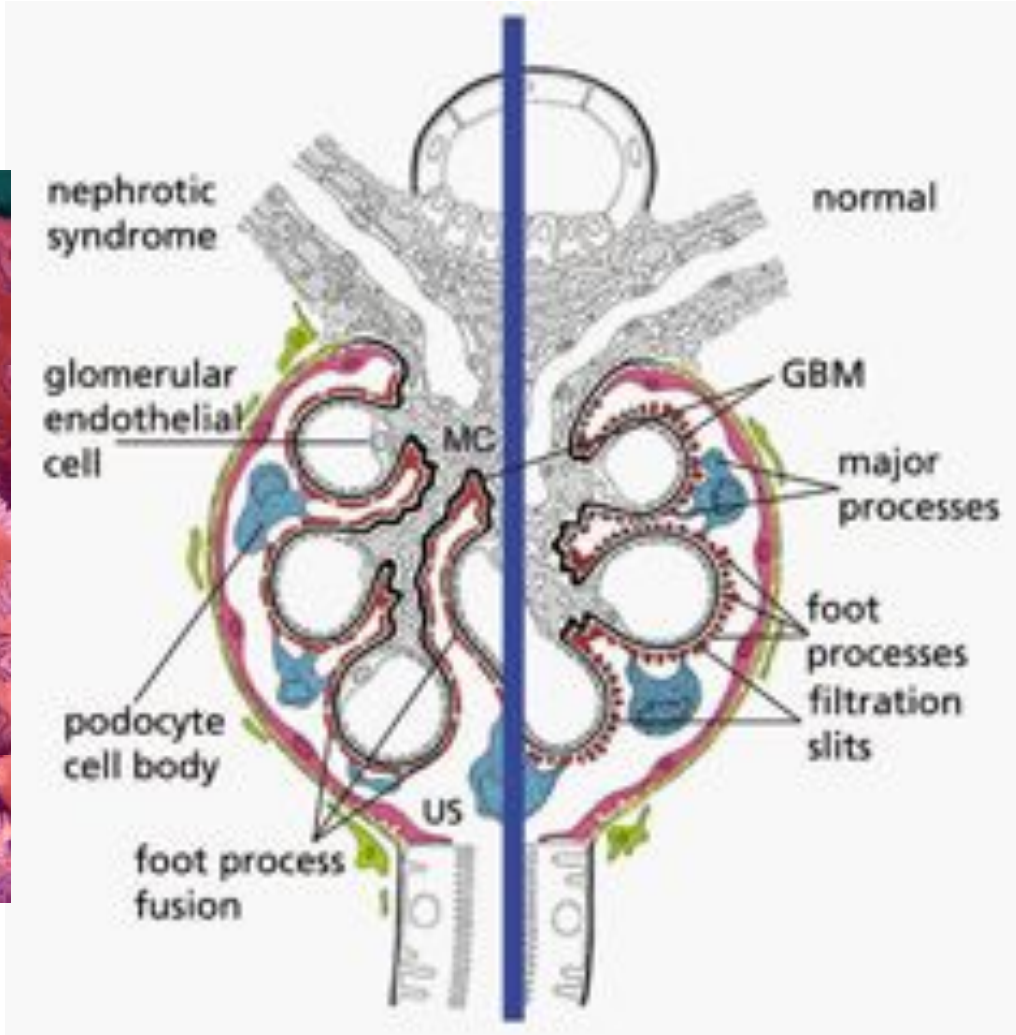
- How can we stratify our patients better?

Idiopathic Nephrotic Syndrome - Current clinical classification

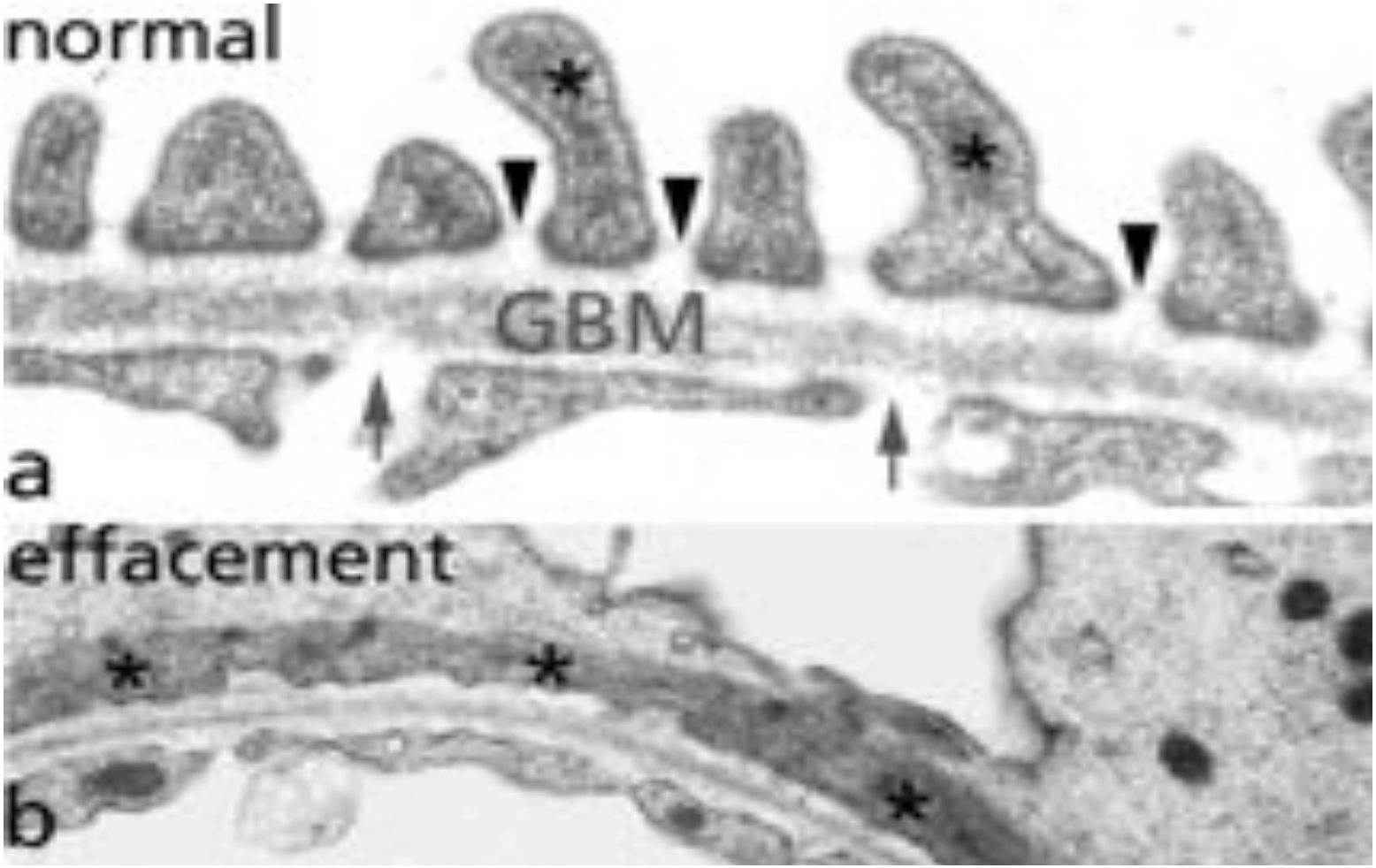
- Unsatisfactory
- Mechanistically - Are these the same or different diseases?
- The final common target is the podocyte

Structure of the glomerulus

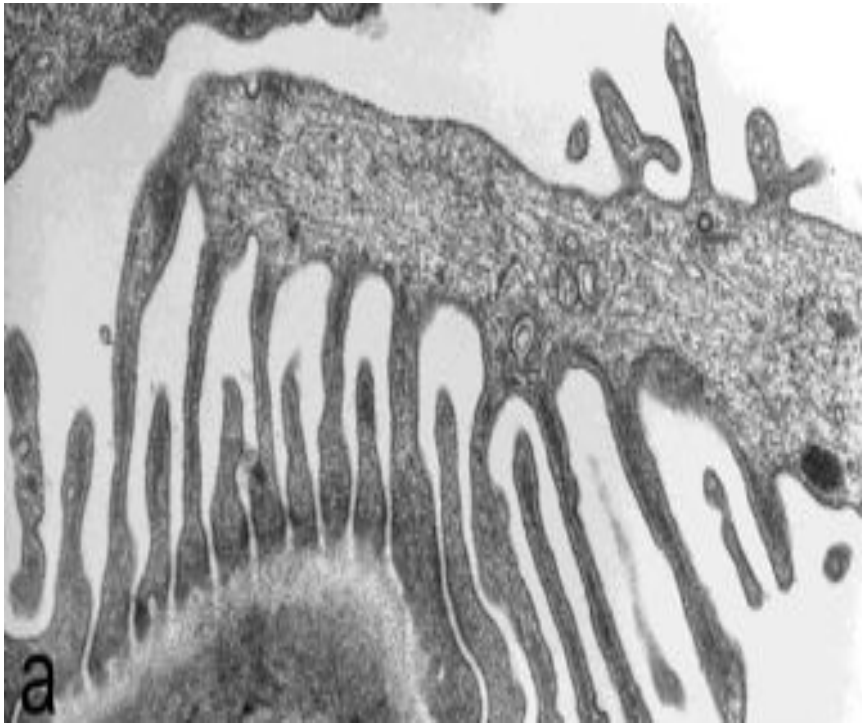


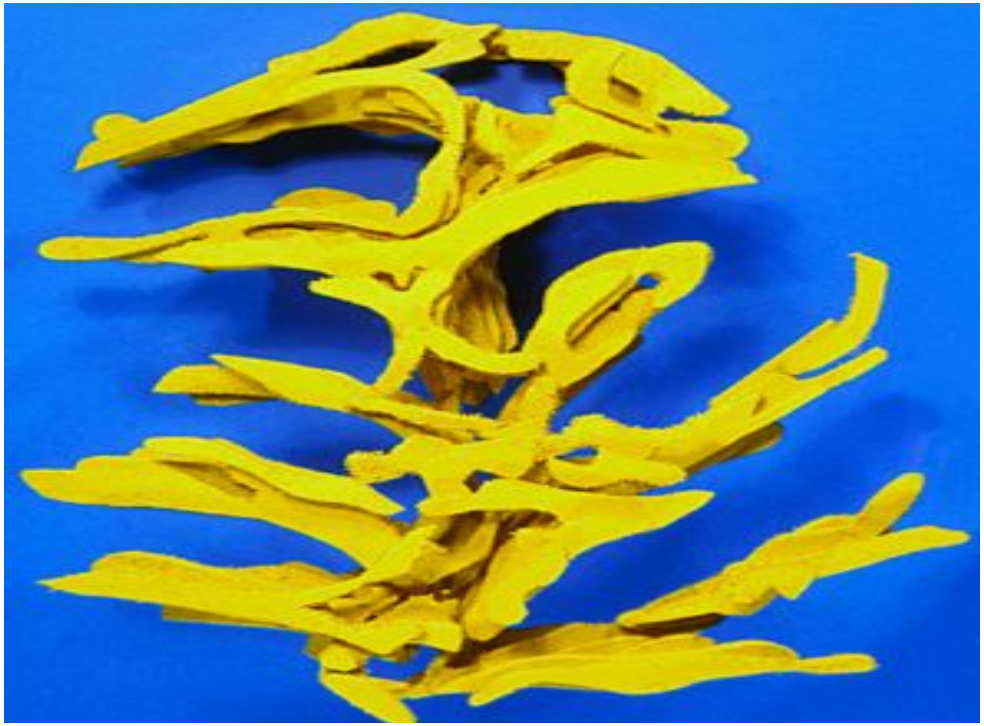


The glomerular filtration barrier



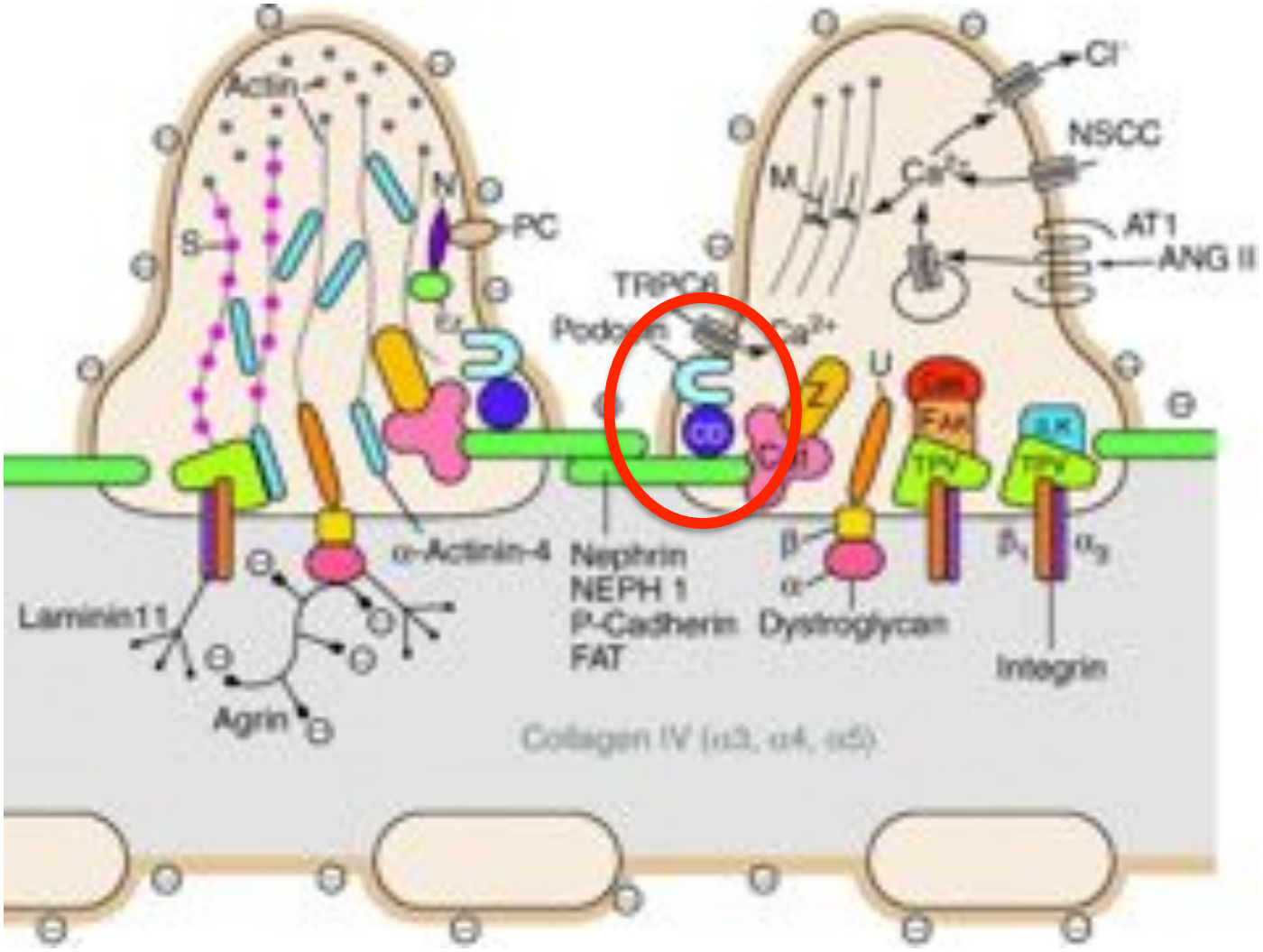
Actin filaments connect neighbouring foot processes



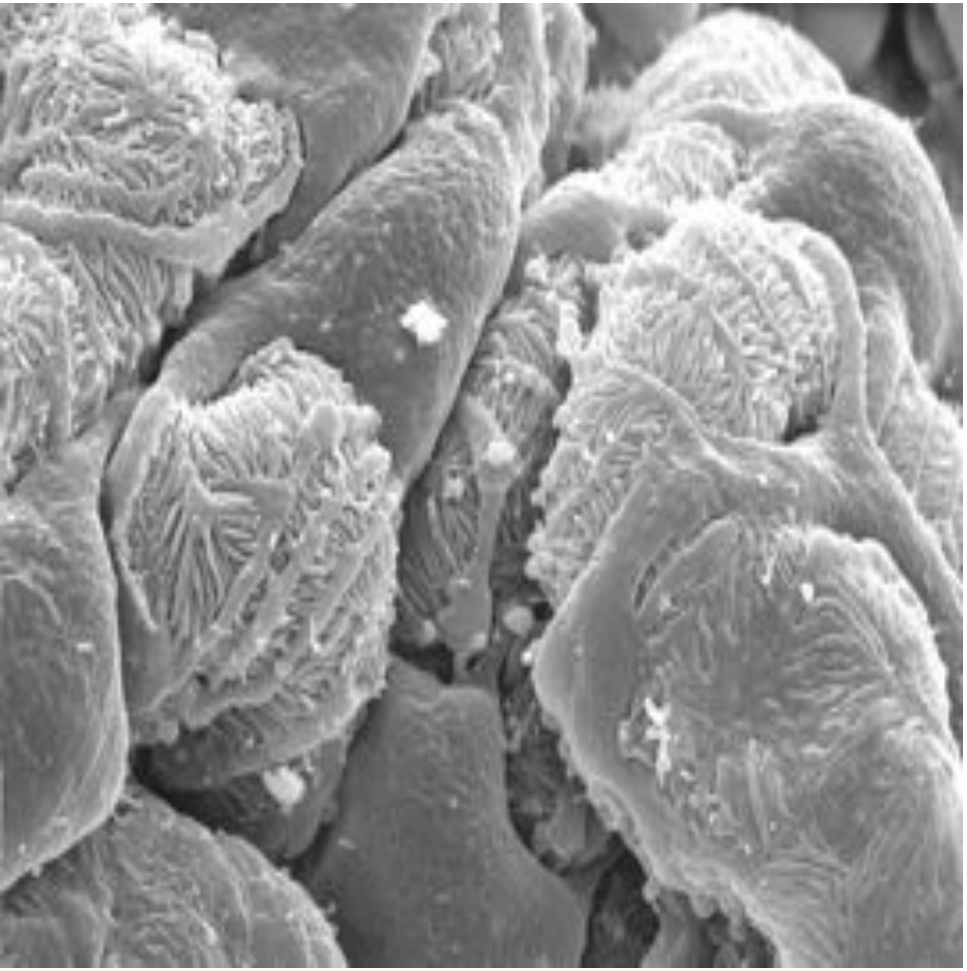


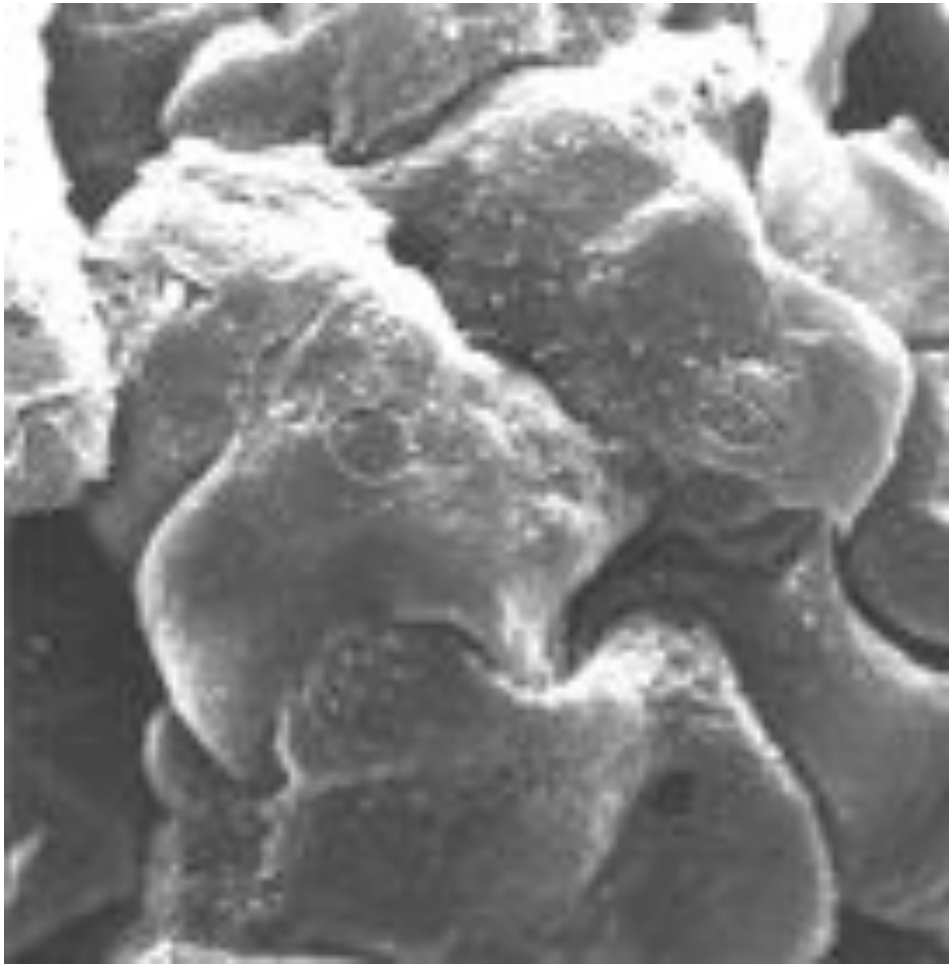
- Actin filaments form an integrated and highly organised structure, supporting foot processes

The podocyte slit diaphragm is the ‘nerve centre’ of the podocyte



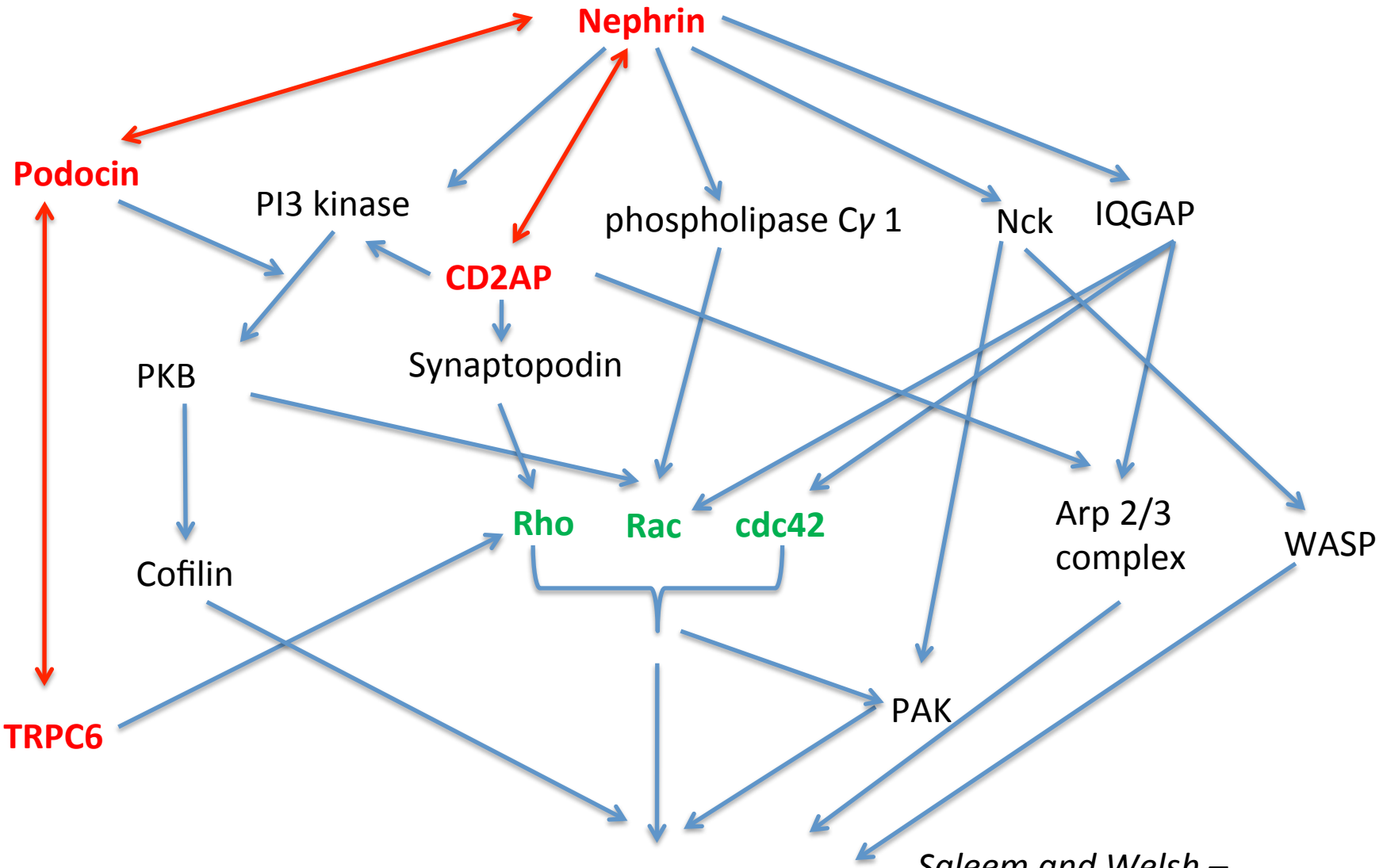
Podocyte morphology in health and disease





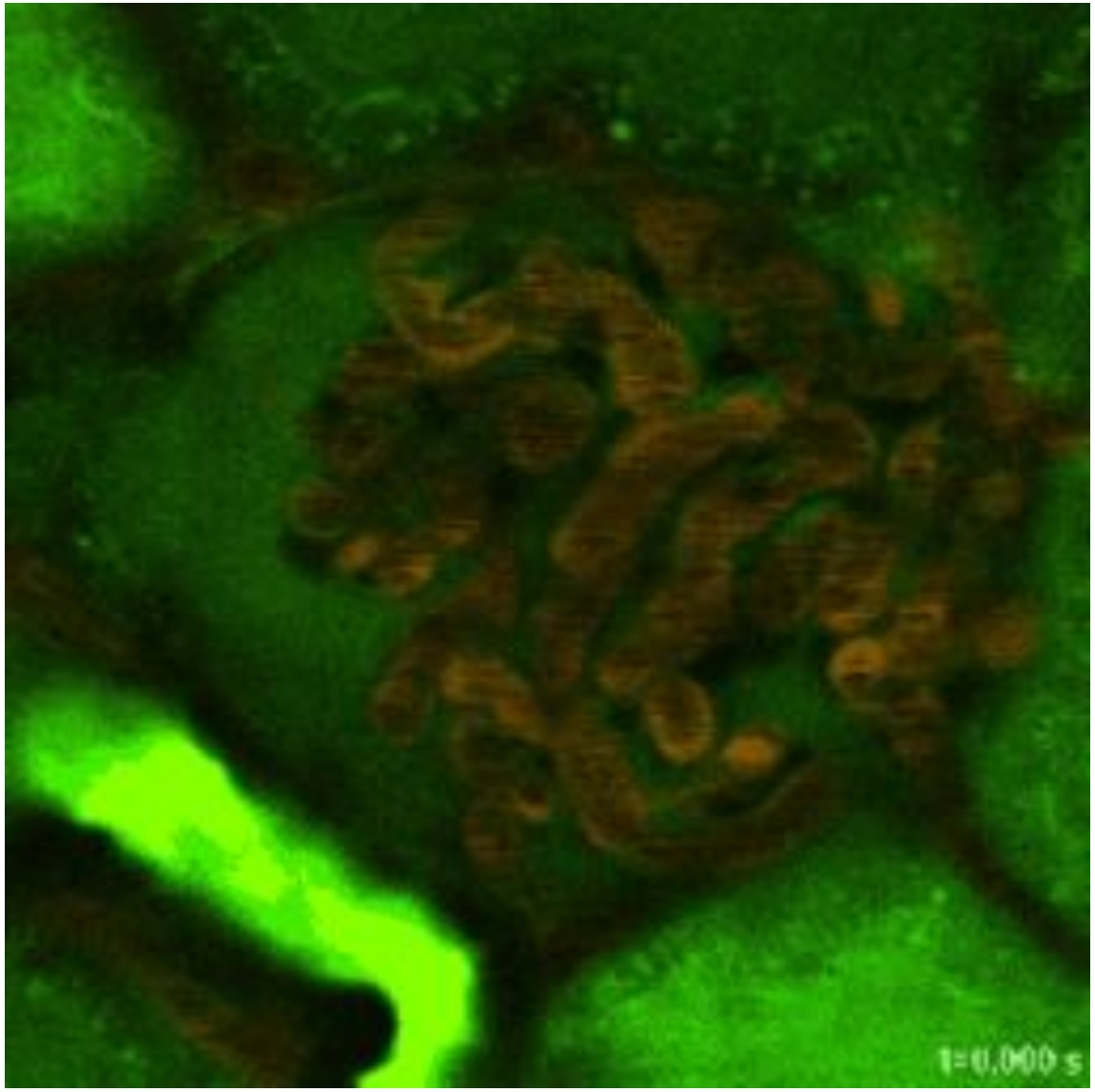
What are the molecular signals leading to foot process effacement?

Slit diaphragm based Cytoskeletal arrangement is based upon small GTPase regulation



Saleem and Welsh – Nature Reviews Nephrology, 2012

The podocyte is a dynamic cell



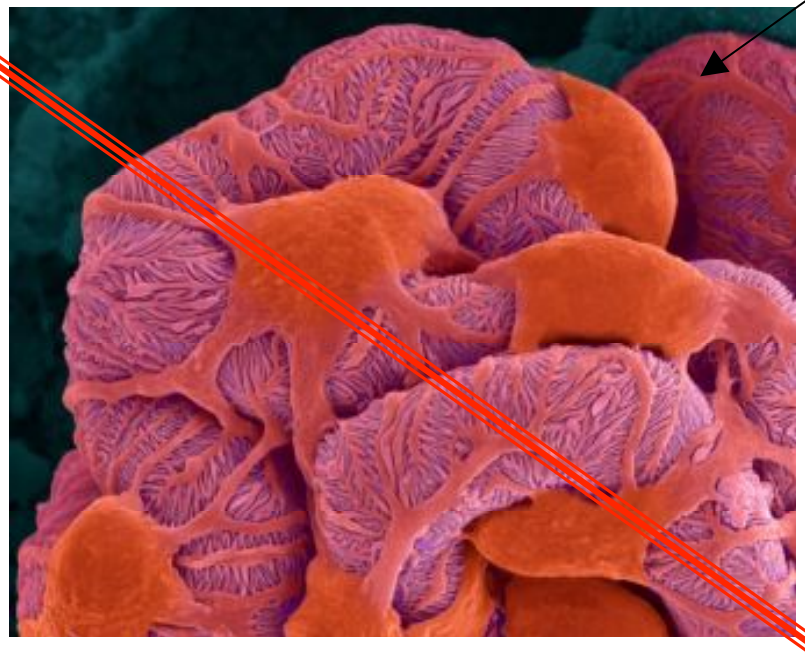
Janos Peti-Peterdi
USC

Signals to control podocyte behaviour

Genes
(nephrin, WT1 etc)

MOLECULAR SIGNALS

GBM signals
- Integrins



VEGF
Endothelin
Etc.

SOLUBLE FACTORS

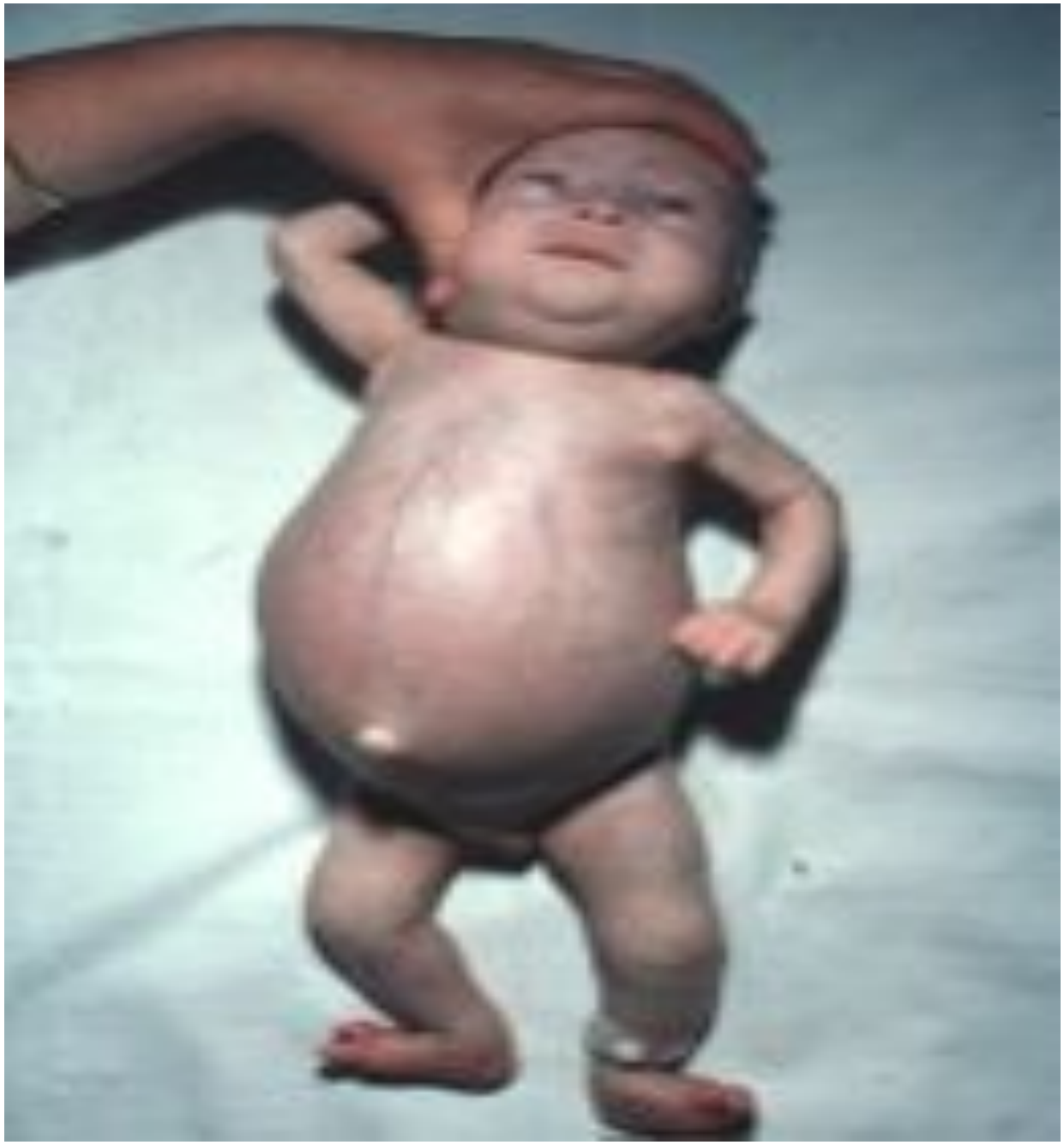
Endothelial cells
Mesangial Cells

Circulating mediators
- NS
-Diabetic Nephropathy

Steroid Resistant Nephrotic Syndrome

- A proportion will have a podocyte specific genetic defect causing their disease

- The rest will have another ‘insult’ to the podocyte, probably externally derived



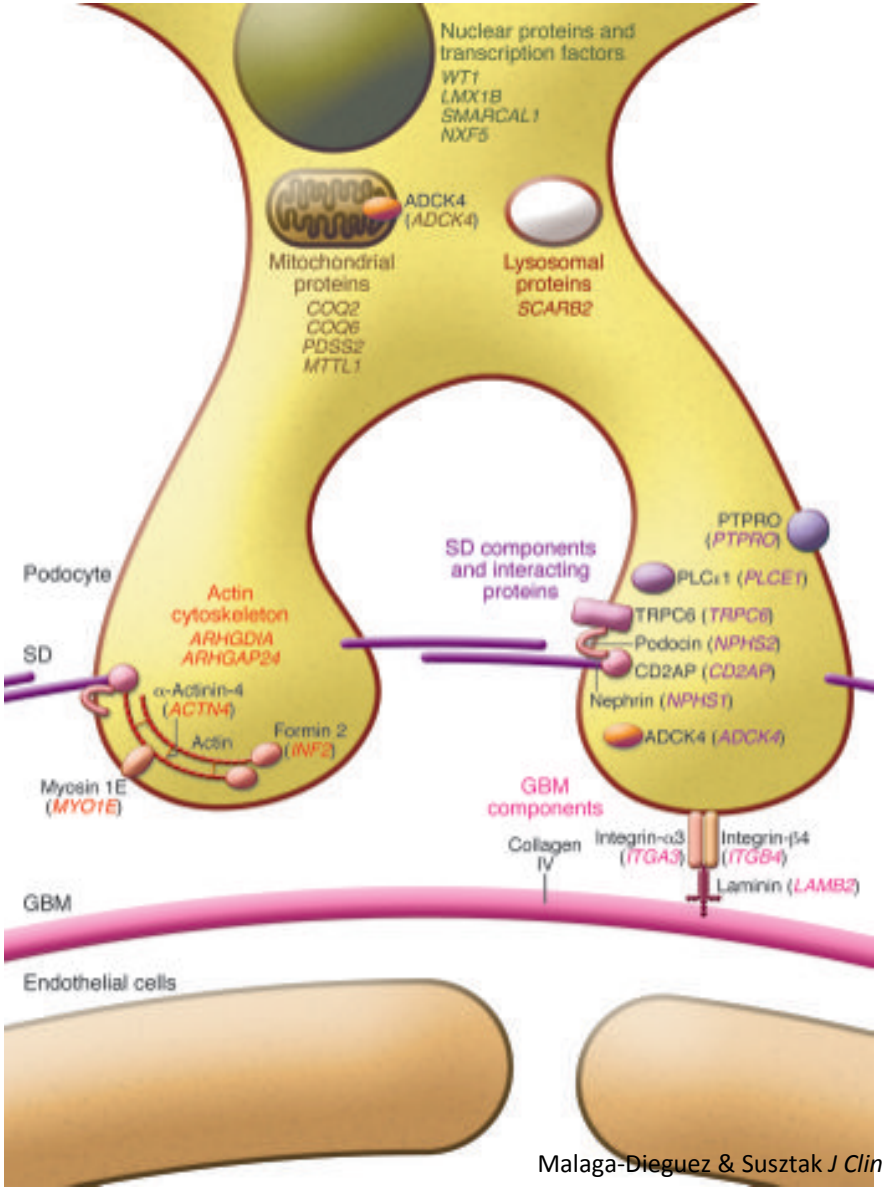
Genes associated with isolated renal disease

Gene	Disease association
ACTN4	SRNS (most commonly adult autosomal dom)
APOL1	Increased susceptibility to FSGS
ARHGAP24	FSGS/SRNS
ARHGAP4	FSGS/SRNS
ADCK4	FSGS/SRNS
CD2AP	FSGS/SRNS
INF2	Familial SRNS
MYO1E	Familial SRNS
NPHS1	Congenital Nephrotic Syndrome/SRNS
NPHS2	Congenital Nephrotic Syndrome/SRNS
PLCe1	Congenital Nephrotic Syndrome/SRNS
PTPRO	SRNS
TRPC6	SRNS (most commonly adult onset)
WT1	Childhood SRNS (may be associated with abnormal genitalia)

Genes associated with syndromes involving SRNS

Gene	Disease association
ALG1	Congenital disorder of glycosylation
COL4A3	Alport's disease
COL4A4	Alport's disease
COL4A5	Alport's disease
COQ2	Mitochondrial disease/isolated nephropathy
COQ6	Nephropathy with deafness
ITGA3	FSGS +/- respiratory & skin involvement
LAMB2	Pierson Syndrome
LMX1B	Nail Patella Syndrome
MHY9	MYH9 related disease
PDSS2	Leigh Syndrome
PMM2	Congenital Disorder of Glycosylation
SCARB2	Action Myoclonus Renal Failure Syndrome
SMARCAL1	Schimke Immuno-Osseous Dysplasia
ZMPSTE24	Mandibuloacral Dysplasia

Nephrotic genes expressed in the podocyte



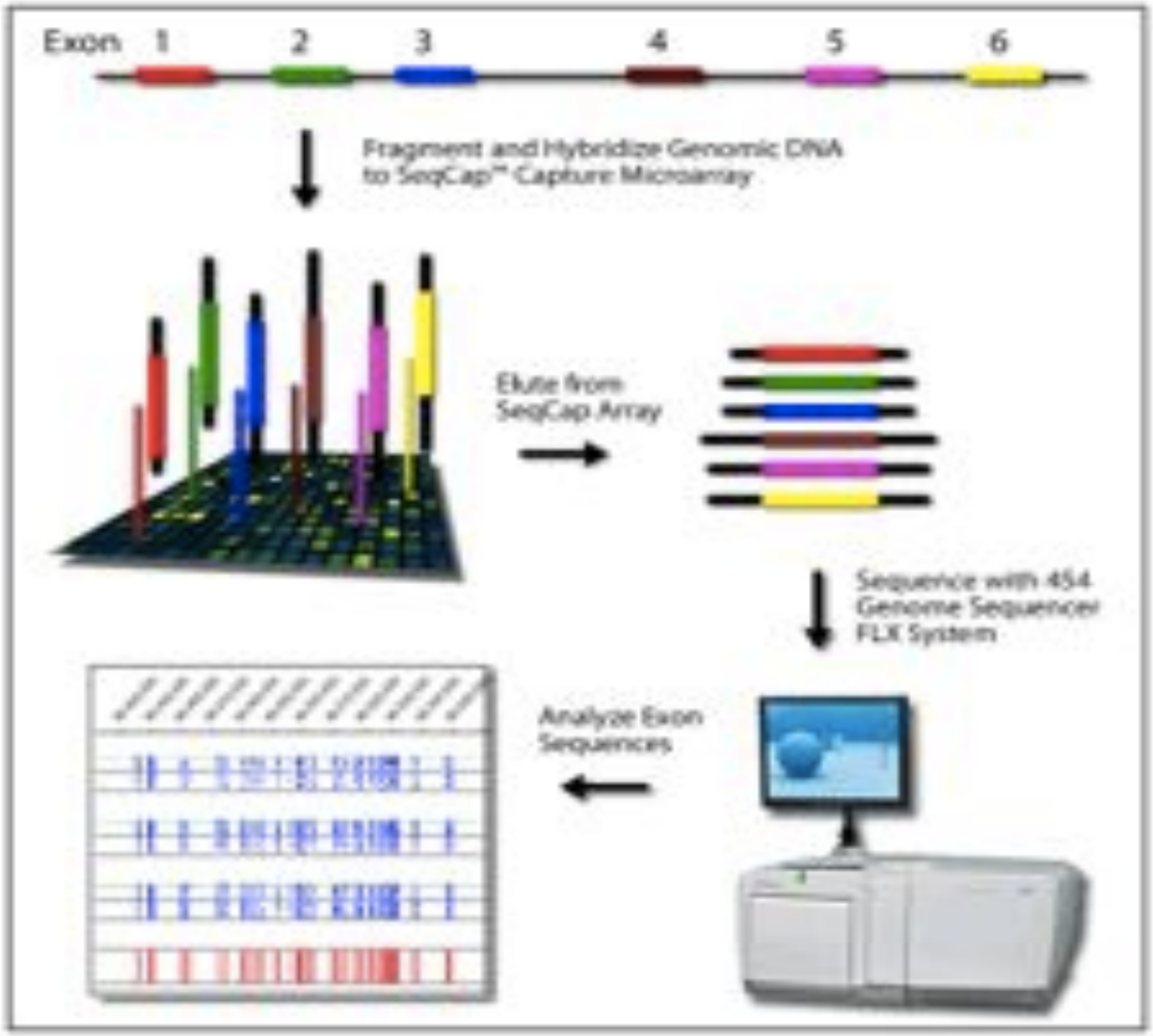
Malaga-Dieguez & Susztak *J Clin Invest.* 2013 Dec 2;123(12):4996-9.

The UK Study of Steroid Resistant Nephrotic Syndrome in Childhood

- A national web based registry for all children with SRNS
- Currently collected ~ 350 patients
- Used Next Generation Sequencing on 200 patients

RenalRadar.org

Next Generation (massively parallel) sequencing



The UK Study of Steroid Resistant Nephrotic Syndrome in Childhood

Simultaneous sequencing of 24 genes associated with steroid resistant nephrotic syndrome, harnessing **Next Generation Sequencing**

Hugh J McCarthy, Agnieszka Bierzynska, Matt Wherlock , Milos Ognjanovic, Larissa Kerecuk, Shivaram Hegde, Sally Feather, Rodney D Gilbert, Leah Krischock, Caroline Jones, Manish Sinha, Nicholas Webb, Martin Christian, Stephen Marks, Ania Koziell, Gavin I Welsh, Moin A Saleem on behalf of RADAR and the UK SRNS study group

Clinical Journal of The Am Soc Nephrol – *2013 Jan*

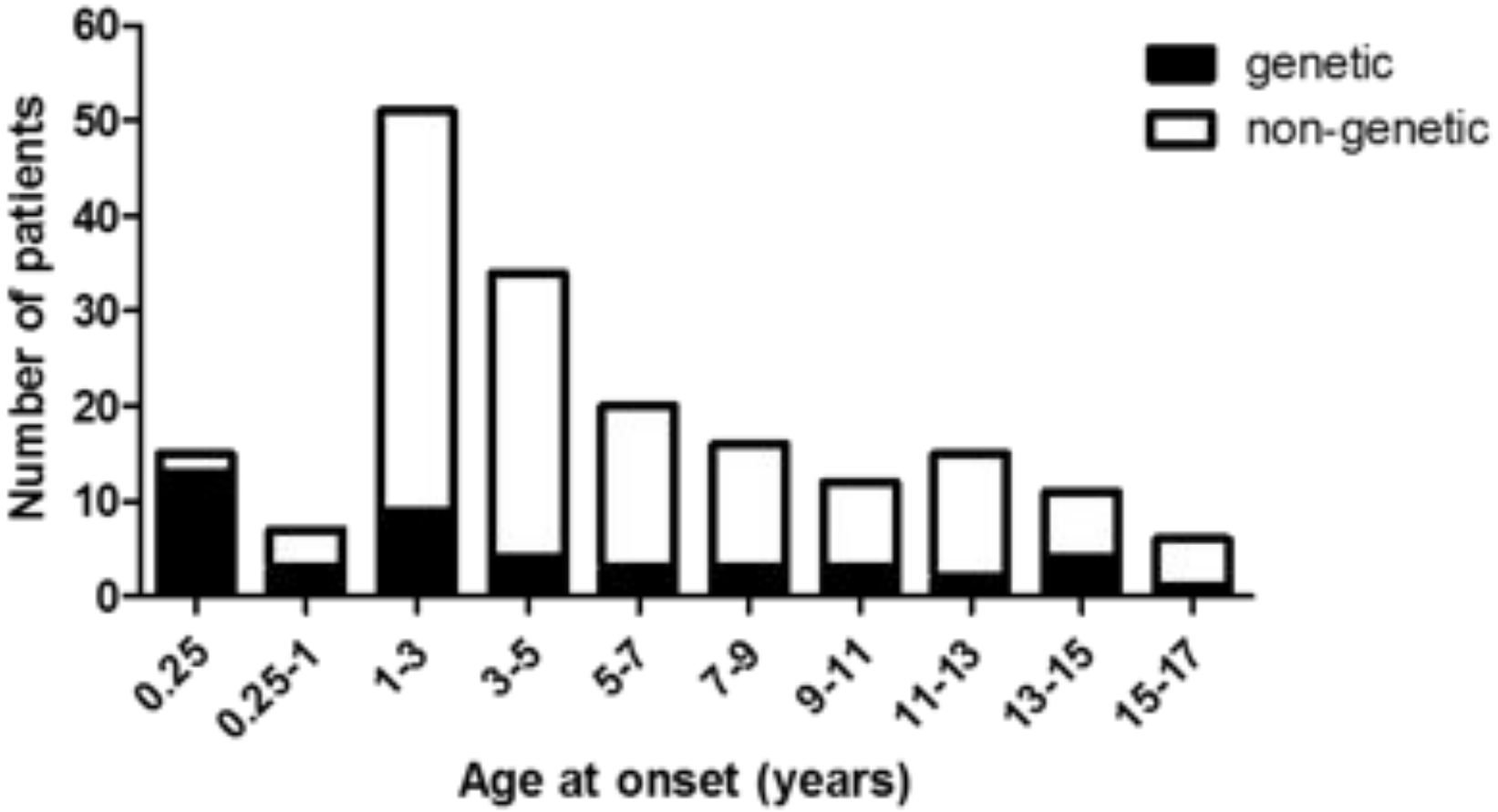
RenalRadar.org

Whole Exome Sequencing – an unselected cohort of 200 SRNS patients

- ✓ 38 (19%) patients had either a previously described mutation or a variant that is probably/possibly disease causing.
- ✓ 80 % of those were in the 4 commonest genes (Nephrin, Podocin, *WT1* and *LAMB2*)
- ✓ 10/200 (5%) patients had likely pathogenic variants identified in genes that would not routinely be screened under recent testing practice

What proportion of SRNS patients has a monogenic cause?

Unselected cohort of SRNS patients in the UK – whole exome screening
45/187 (24%) identified as having a mutation (same as PODONET cohort – cJASN 2015)



Nephrotic genes and associated syndromes

Gene	Inheritance	Disease
ACTN4	AD	Familial and sporadic SRNS (usually adult)
ADCK4	AR	SRNS
ALG1	AR	Congenital disorder of glycosylation
ANLN	AD	FSGS (mainly adult)
AP4B1/AP4E1	AD	FSGS
AP4S1/ZFYVE26	AR	CNS
CD151	AR	NS,perioral bullous skin lesions, neurosensory deafness, bilateral lacrimal duct stenosis, nail dystrophy, and α-thalassemia minor
CD2AP	AD/AR	FSGS/SRNS
COL4A3	AR	Alport's disease
COL4A4	AR	Alport's disease
COL4A5	X-linked AR	Alport's disease
COQ2	AR	Mitochondrial disease/Isolated nephropathy
COQ6	AR	NS +/- sensorineural deafness; CAMS
CRB2	AR	SRNS
CUBN	AR	Intermittent nephrotic range proteinuria +/- with epilepsia
DGKE	AR	Atypical hemolytic uremic syndrome-3 +/- NS, Nephrotic syndrome, type 7
EZH2	AD	FSGS/maternal retardation (whole gene deletion)
EMP2	AR	Childhood-onset SRNS and SSNS
INF2	AD	Familial and sporadic SRNS, FSGS- associated Charcot-Marie-Tooth neuropathy
ITGA3	AR	Congenital interstitial lung disease, nephrotic syndrome, and mild epidermolysis bullosa
ITGB4	AR	epidermolysis bullosa and pyloric atresia + FSGS(LA)
KANK1	AR	SSNS
KANK2	AR	SSNS/SDNS +/- hematuria
KIRREL1	AR	SRNS + hematuria
LAMB2	AR	Pierson syndrome
LMNA	AD	Familial partial lipodystrophy + FSGS
LMX1B	AD	Nail-patella syndrome; also FSGS without extrarenal involvement
MYO1E	AR	Familial SRNS
NPHS1	AR	CNS/SRNS
NPHS2	AR	CNS, SRNS
NXF5	X-linked recessive	FSGS + heart block disorder
OCRL	X-linked recessive	Dent disease2, Lowe syndrome, +/- FSGS, +/- nephrotic range proteinuria
PAX2	AD	adult onset FSGS without extrarenal manifestations
PDSS2	AR	Leigh syndrome
PLCε1	AR	CNS/SRNS
PMM2	AR	Congenital disorder of glycosylation
PODXL	AD	FSGS
PTPRO	AR	NS
SCARB2	AR	Action myoclonus renal failure syndrome of hearing loss
SMARCAL1	AR	Schimke immuno-osseous dysplasia
SYNPO	AD	sporadic FSGS (promoter mutations)
TRPC6	AD	Familial and sporadic SRNS (mainly adult)
TTC21B	AR	FSGS with tubulointerstitial involvment
WT1	AD	Sporadic SRNS (children—may be associated with abnormal genitalia); Denys-Drash and Frasier syndrome
ZMPSTE24	AR	Mandibuloacral dysplasia with FSGS
MYH9	AD/assoc.	MYH9-related disease; Epstein and Fechtner syndromes
APOL1	COMPLEX?	Increased susceptibility to FSGS and ESRD in African Americans, Hispanic Americans and in individuals of African descent

UK SRNS cohort - expanding the range of genes causing SRNS

- COL4a5, COL4a3 – Alports or Thin Basement Membrane disease
- LAMB2 – Piersons syndrome
- OCRL - Dent's disease
- DGKE – atypical haemolytic uraemic syndrome

- Testing of SRNS patients using Next Generation Sequencing will yield a high number of positive results

(clinical service currently offered by Bristol Genetics Laboratory)

New genes discovered

- *CRB2* - crumbs homolog 2 (Drosophila) – *Am J Hum Genet – 2015*
- ARHGAP4 - Rho GTPase activating protein 4 – *J Clin Invest – 2014*

- *2 others currently being validated*

Potential disease modifiers?

Variants with higher frequency than in a general population – controls must be from the same population

Gene	Variant	dbSNP#	Allele freq	Bris (mix) vs KCL (mix)	Bris (white) vs CEU	Bris (white) vs GBR
CYP11B2	p.V386A	RS61757294	0.044	0.001	0.002	0.0008
CYP11B2	p.A297A	RS4543	0.096	0.0053	0.0164	0.0179

CEU - Utah residents with Northern and Western European ancestry
GBR - British in England and Scotland

Fisher's exact test-> p value: extremely- very- significant

Bristol Genetics Laboratory

North Bristol NHS Trust

Steroid Resistant Nephrotic Syndrome (SRNS)

Contact details:
Bristol Genetics Laboratory
Pathology Sciences
Southmead Hospital
Bristol, BS10 5NB
Enquiries: 0117 323 6271
FAX: 0117 323 5572

Head of department:
Eileen Roberts FRCPath

Consultant Lead for Molecular Genetics:
Maggie Williams FRCPath
Email: Maggie.Williams@nbt.nhs.uk

Service Lead:
Laura Yarram
Email: Laura.Yarram@nbt.nhs.uk

Sample Required:

Adult: 5mls blood in EDTA
Paediatric: at least 1ml EDTA (preferably >2ml)

Samples should be accompanied by a FULLY completed request form (available as download at www.nbt.nhs.uk/genetics or from the laboratory).

Please include details of test, family history, address and POSTCODE, NHS number, referring clinician and unit/hospital.

Consent and DNA Storage:

All genetic testing requires consent. It is the responsibility of the referring clinician to ensure that appropriate consent has been obtained.

DNA is stored from **all** patients unless consent for this is specifically denied.

Stored samples may be used for quality assurance purposes and may be used anonymously for the development of new tests for the disorder in question.

Clinical Background and Genetics

- Alternative name: Focal Segmental Glomerulosclerosis (FSGS)
- SRNS is a disease of kidney filtration, resulting in massive and unremitting protein loss into the urine. It is managed with heavy immunosuppression, but despite this the majority of patients eventually suffer irreversible kidney failure.
- Diagnosis is made clinically, and it is now clear the disease segregates into genetic and non-genetic forms.
- SRNS is defined as:
  Presence of nephrotic syndrome (Serum albumin < 25g/l and urine albumin > 4 mg/m2/h or urine albumin/creatinine ratio >100 mg/mmol), that is either:
  1) resistant to treatment with steroids, or
  2) present in the first 3 months of life, or
  3) has a histological picture of FSGS on biopsy.

Service offered

- 16* genes are targeted using a custom designed HaloPlex Target Enrichment System kit and sequenced using a MiSeq (Illumina) analyser. Analysis is performed using an open source in-house pipeline (alignment: BWA; alignment modification and variant calling: GATK; variant annotation: Annovar).

  *Genes include: *ACTN4, APOL1, ARHGAP24, CD2AP, COQ2, COQ6, INF2, LAMB2, LMX1B, MYO1E, NPHS1, NPHS2, PLCe1, PTPRO, TRPC6* and *WT1*.
  An extended assay to include >30 SRNS associated genes is currently under service development.
- Familial tests are available for known mutations using Sanger sequencing.

Quality

- BGL participates in the EMQN scheme for DNA sequencing.

Referrals

Referrals are accepted nationally from Consultant Nephrologists and Consultant Clinical Geneticists only.

Target reporting Time and Cost (where appropriate)

Diagnostic screen of 16 genes:	*20-30 days*
Known Mutation:	*10 days (by Sanger sequencing)*
Urgent:	*3 days*

Please contact the laboratory for up to date prices

Clinical Advice

If clinical discussion is required we would recommend contact with:
Prof. Moin A Saleem FRCP, PhD, Professor of Paediatric Renal Medicine, University of Bristol. Email: m.saleem@bristol.ac.uk

References

- Simultaneous Sequencing of 24 Genes Associated with Steroid-Resistant Nephrotic Syndrome, McCarthy *et al.* 2013, Clin J Am Soc Nephrol **8**.

Information document No.75 Version 1
Active date of this version: 05/07/2013
DETAILS CORRECT AT DATE OF PRINTING ONLY.
Approved by: Maggie Williams

Exceptional healthcare, personally delivered

CPA
Accredited Medical Laboratory
Reference No: 2907

www.nbt.nhs.uk/genetics

All known genes for SRNS tested (currently 42 genes)

Rapid turnaround (4 weeks)

£600 per patient

Re-classification of idiopathic NS

Monogenic disease

50 single gene causes
Currently known

Circulating Factor disease

Other?

Circulating factors in NS – the debate rages on…

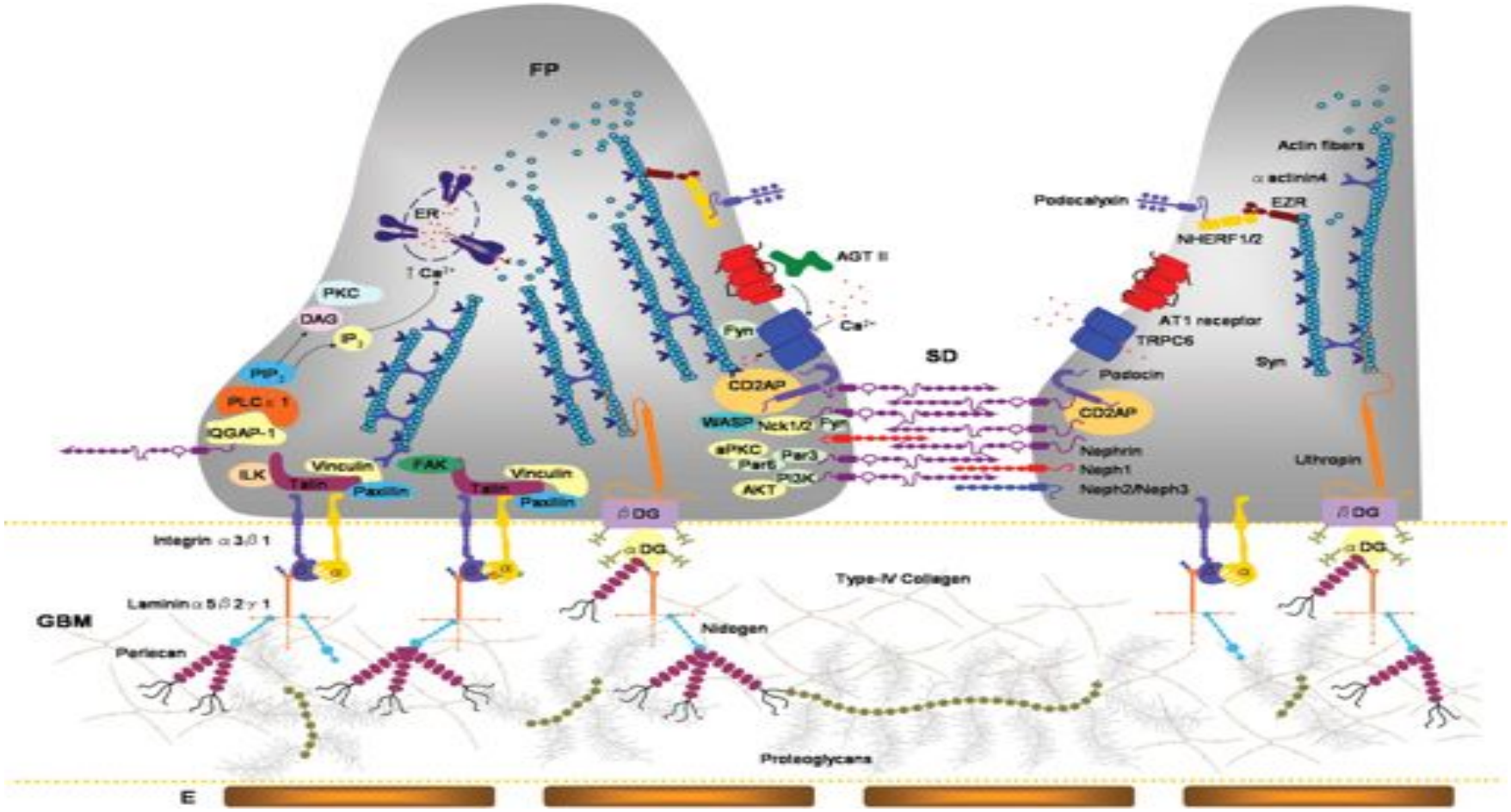
- Hemopexin *(Bakker et al J Am Soc Nephrol 1999)*
- TNF-α *(Torban et al, Ped Neph 2012)*
- suPAR *(Wei et al, Nat Med 2011)*
  - Elevated in patients with primary and recurrent FSGS
  - Causes downstream effects on podocyte motility
  - Clinical data not yet replicated consistently in other cohorts (*e.g. Sinha, Bagga et al KI 2014*)

How does one search for a circulating factor?

- Look for evidence of a high level of a factor in patient plasma

- Study podocyte changes at the molecular level in NS, and infer the factor that may cause those effects

Integrin activation is a key downstream feature of podocyte motility changes



Integrins are important effectors in podocyte motility

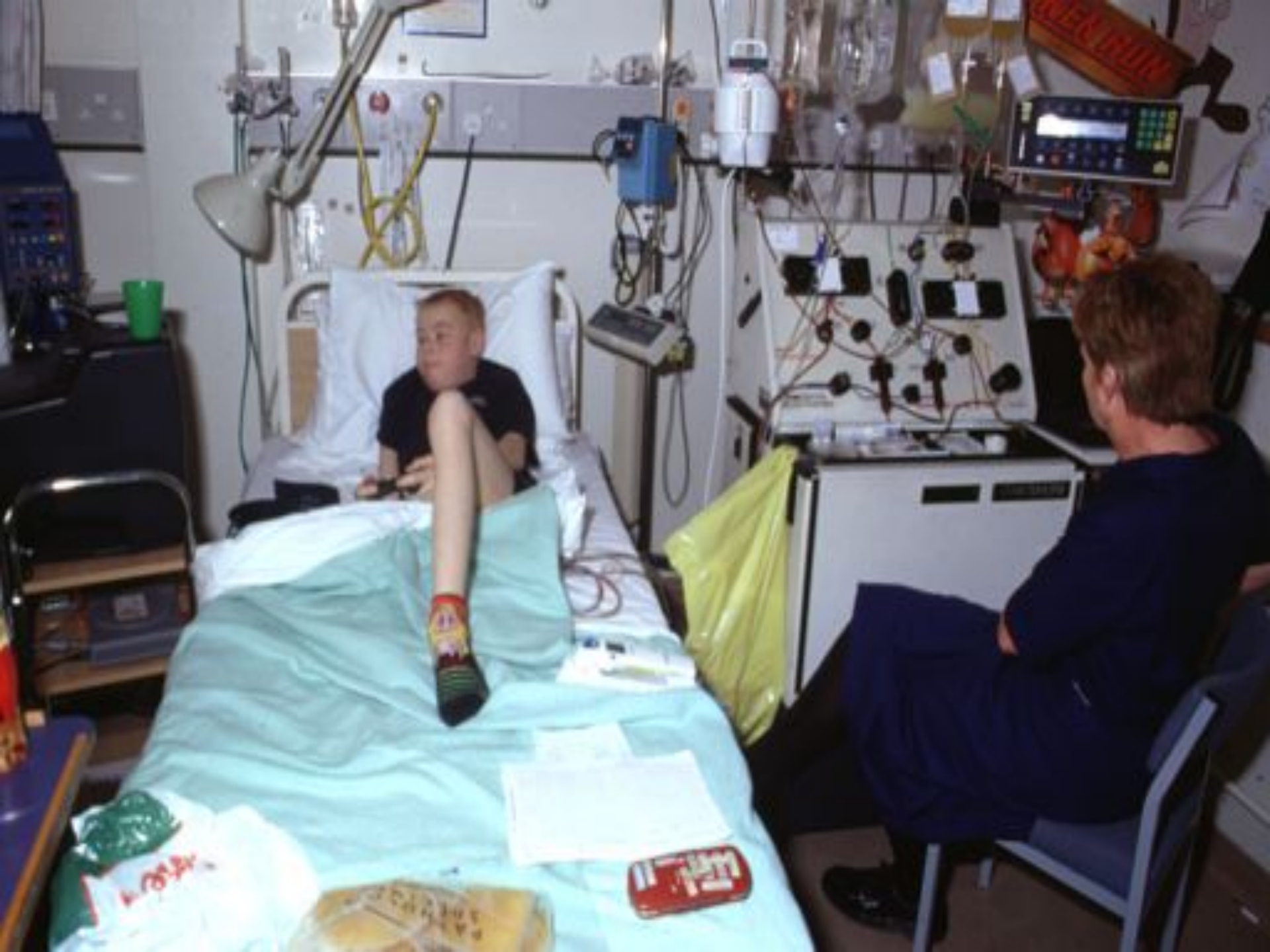
- β3 integrin activation noted in PAN model of NS, and in FSGS biopsies
- α5β3 integrin activation – increases podocyte motility in response to puromycin, and suPAR

- B7-1 positivity has been proposed as a marker of active glomerular disease (*Yu et al NEJM 2013*)
- β1 integrin is suppressed by B7-1 expression in podocytes, which results in decreased cell motility
  - Abatacept postulated as a treatment for B7-1 positive SRNS

A direct test of circulating factor on human podocytes *in vitro*

- Circulating Factor disease
  - Patient derived plasma during active disease, is having a direct pathogenic effect on the target cell – the podocyte
- If we apply patient plasma on human podocytes *in vitro*, specific markers should be seen in podocytes

- The archetypal circulating factor disease is Post Transplant Recurrence of SRNS



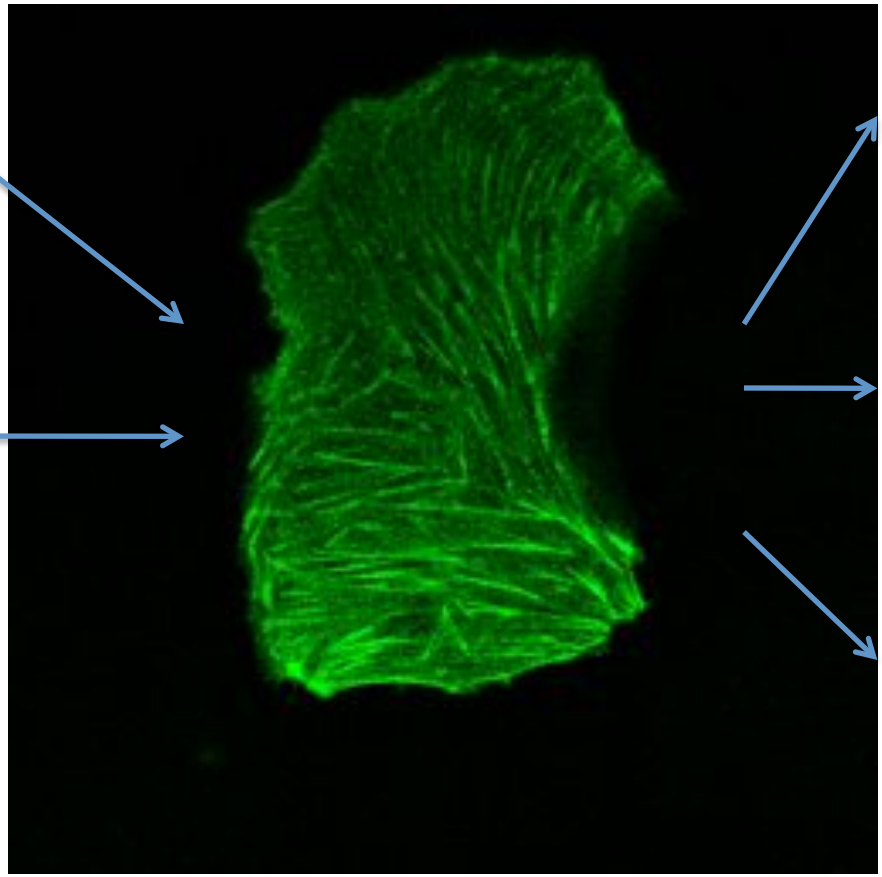
A toolbox to define NS pathogenesis

HUMAN PODOCYTES

Plasma samples
SRNS, SSNS, etc

Fractionated

Proteomics
Biological NMR
etc



Protein
localisation

TRPC6 activity

VASP
phosphorylation

Is there a specific actin remodelling pathway that is triggered by disease plasma?

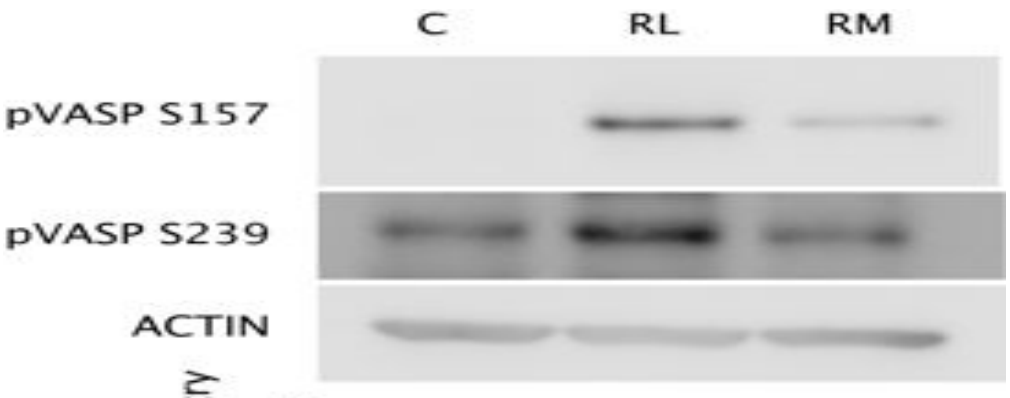
Actin is a polymer – extending into long filaments, and constantly dynamically rearranging in a cell

We screened several molecules involved in actin dynamic regulation using disease plasma on podocytes

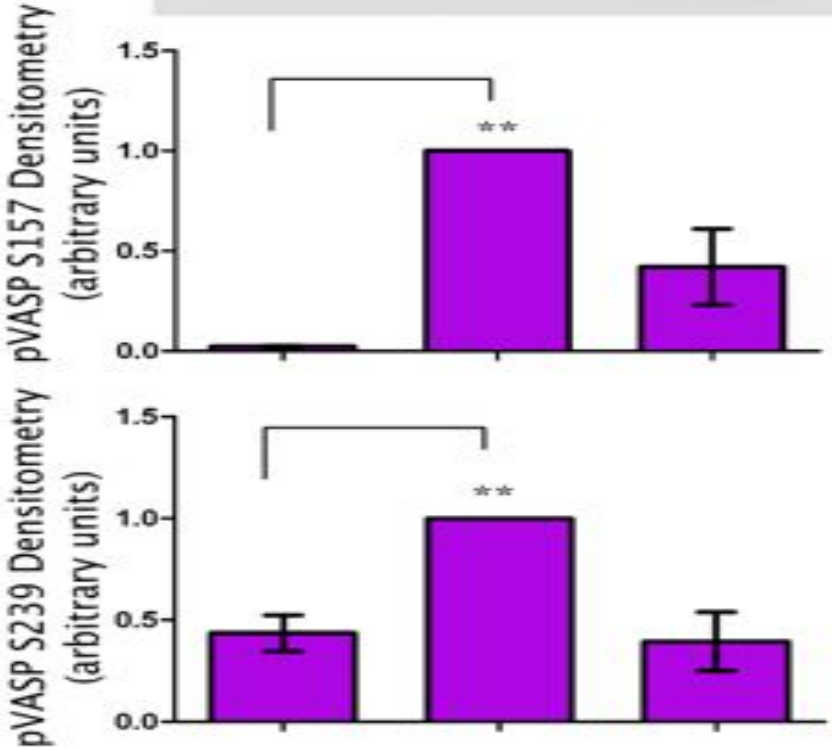
VASP – an important regulator of actin polymerisation, involved in cell morphogenesis –

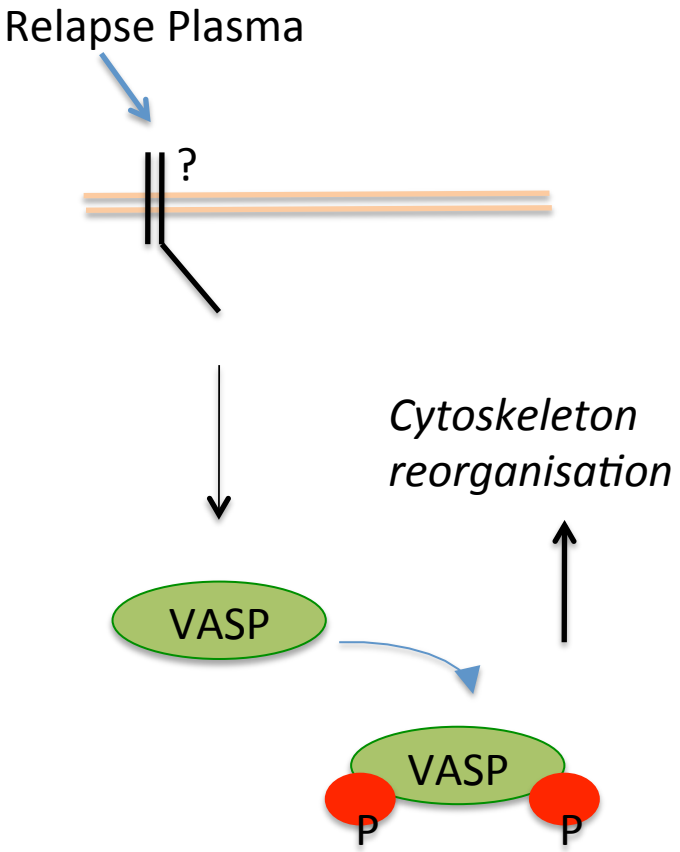
- Aberrant activation of VASP could therefore cause key changes in podocyte dynamics

Actin associated protein VASP is phosphorylated in response to nephrotic plasma

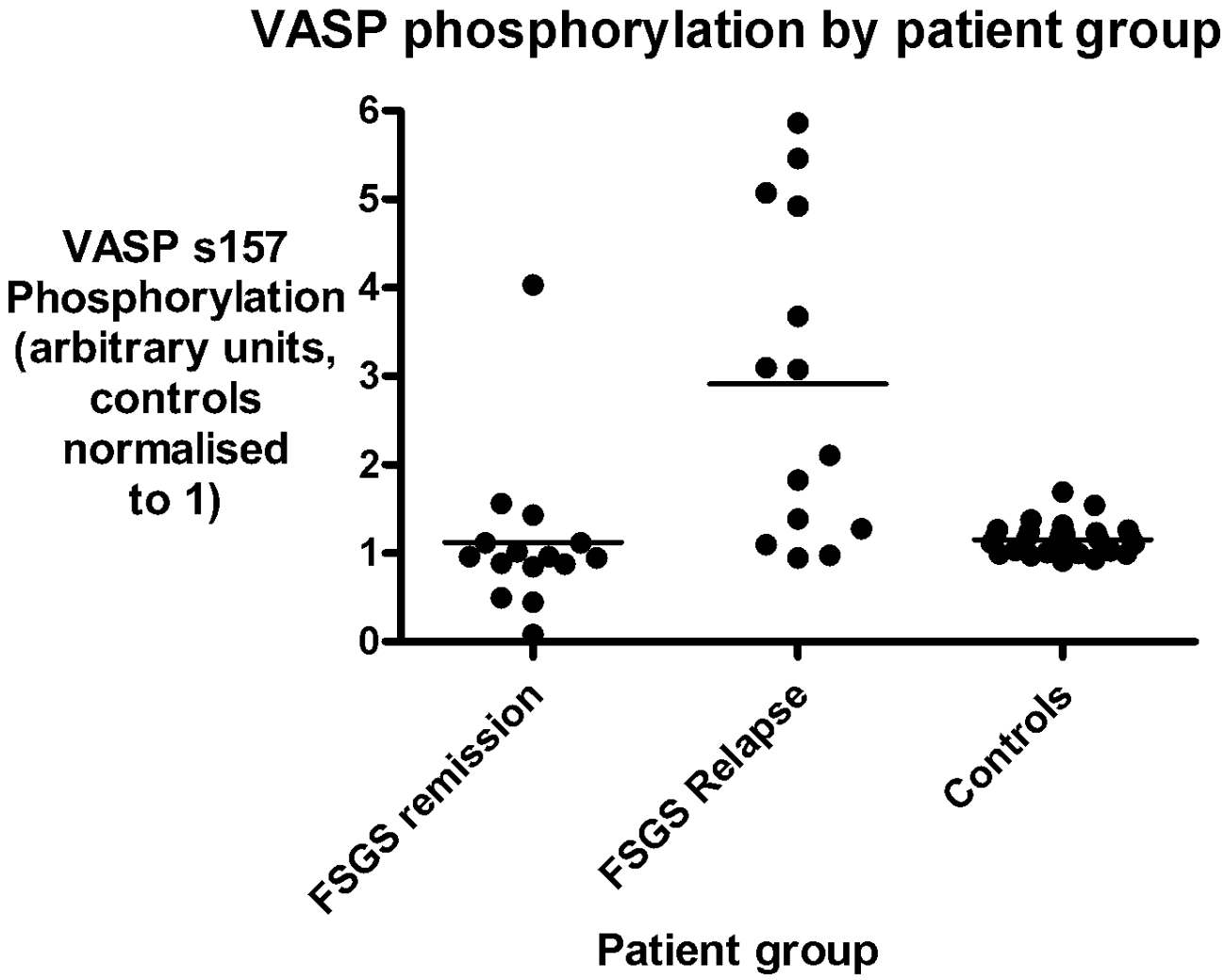


C = control plasma
RL = Relapse plasma
RM = remission plasma



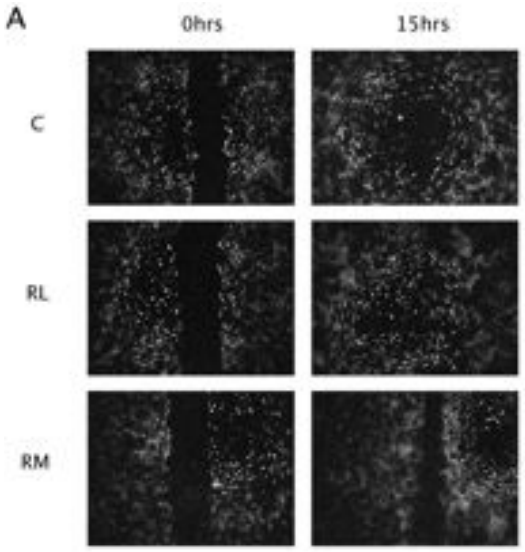


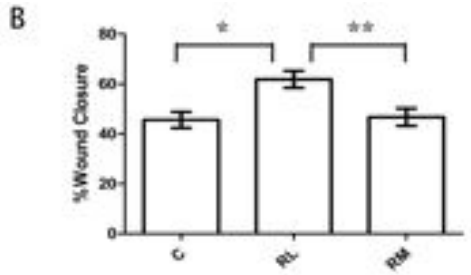
VASP phosphorylation in podocytes is specific to SRNS plasma



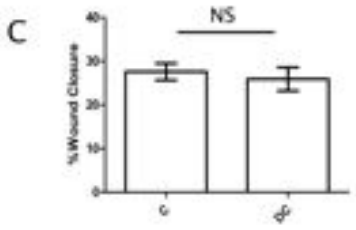
Disease plasma causes a VASP dependent increase in podocyte motility

Podocyte Migration Assay



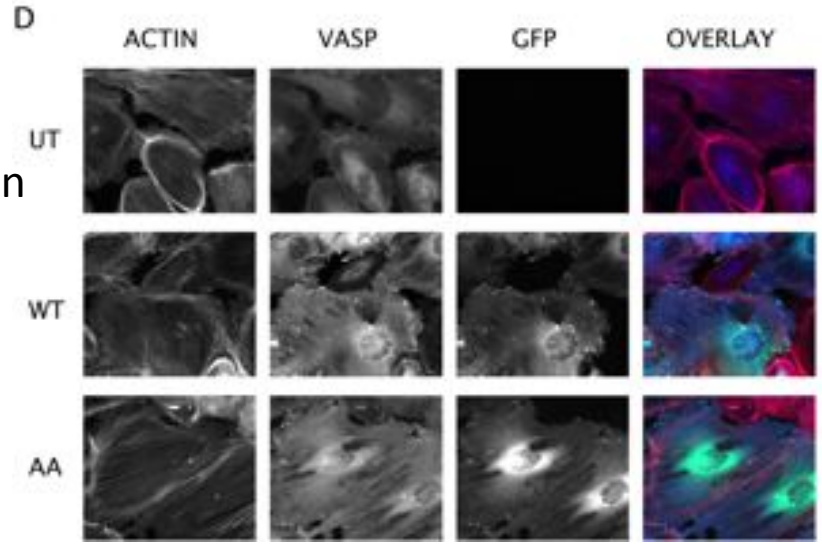


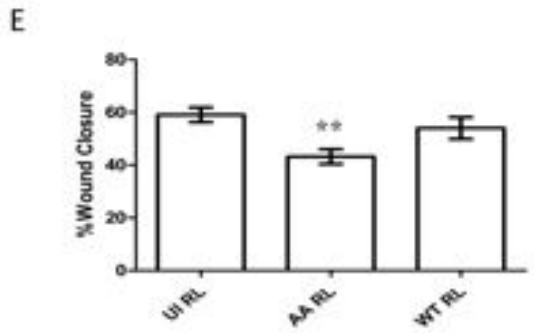
FSGS plasma



Non-FSGS plasma

VASP Phosphorylation mutant





FSGS Plasma On VASP Inhibited podocytes

Harris et al, J Pathol 2013

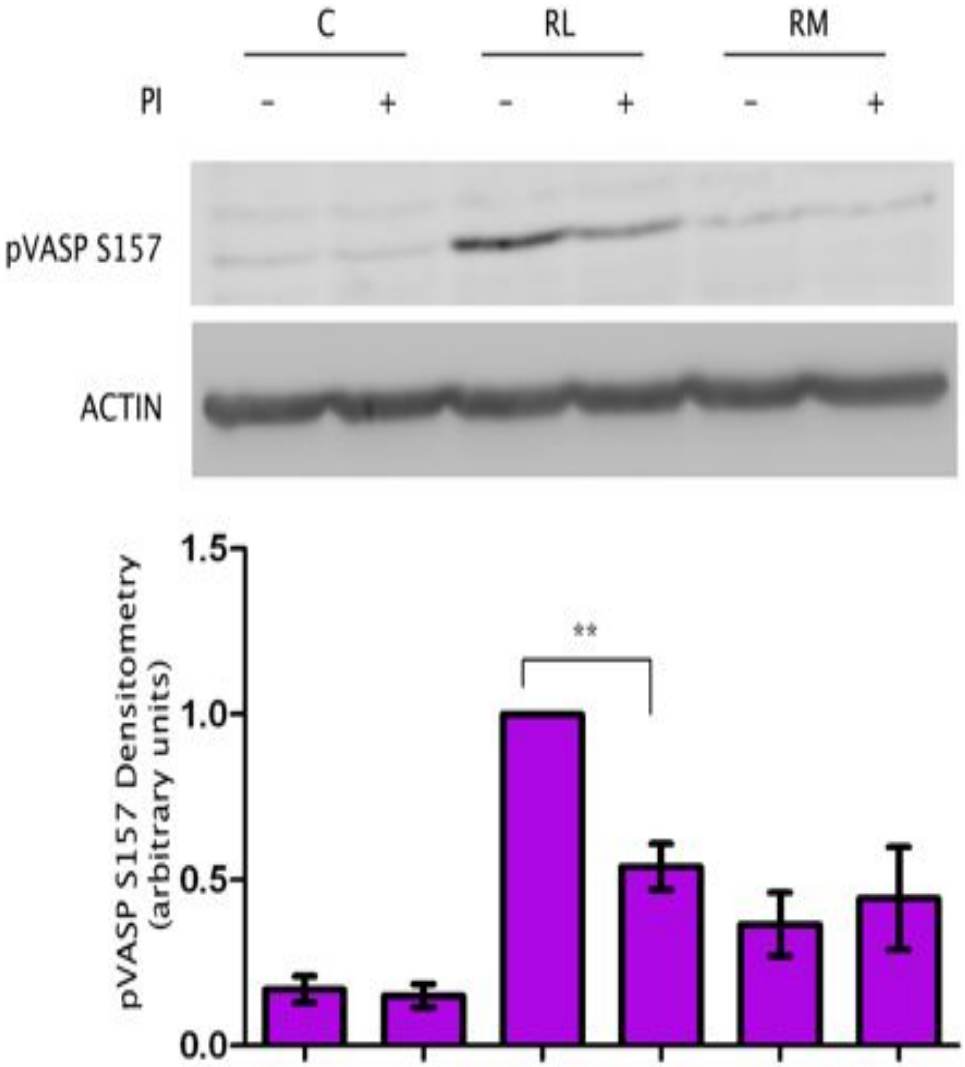
What ‘factor’ is circulating in plasma that could be causing this effect?

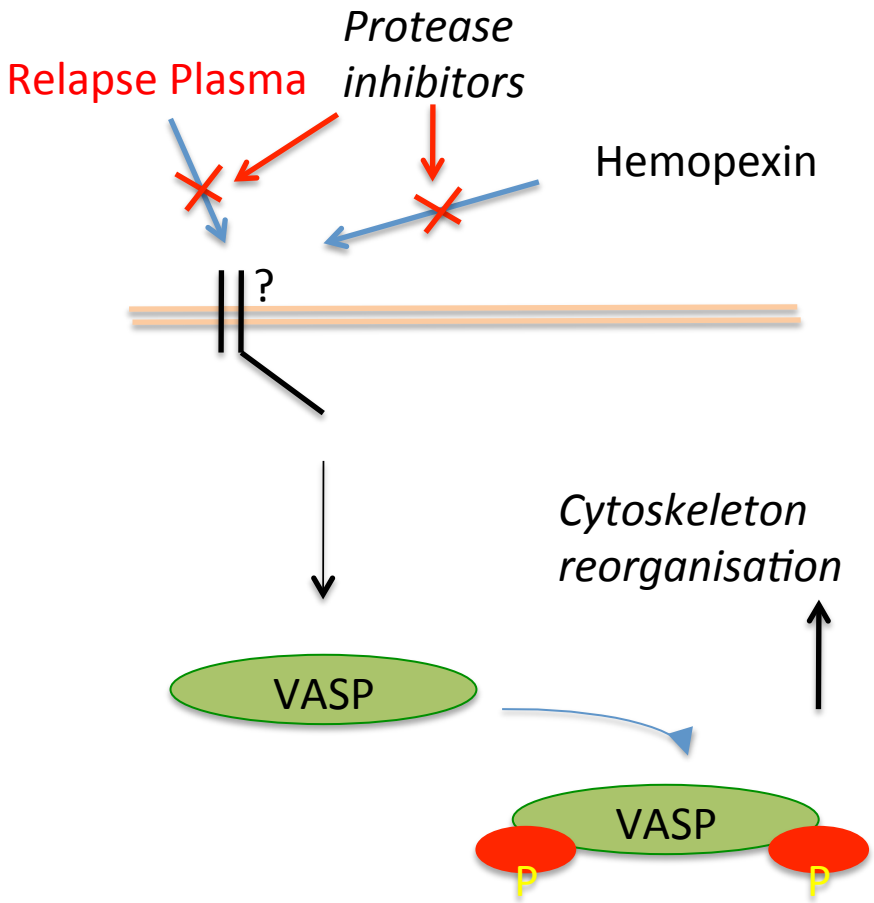
Hypothesis – an imbalance of circulating plasma proteases

What is the surface receptor through which nephrotic plasma is working?

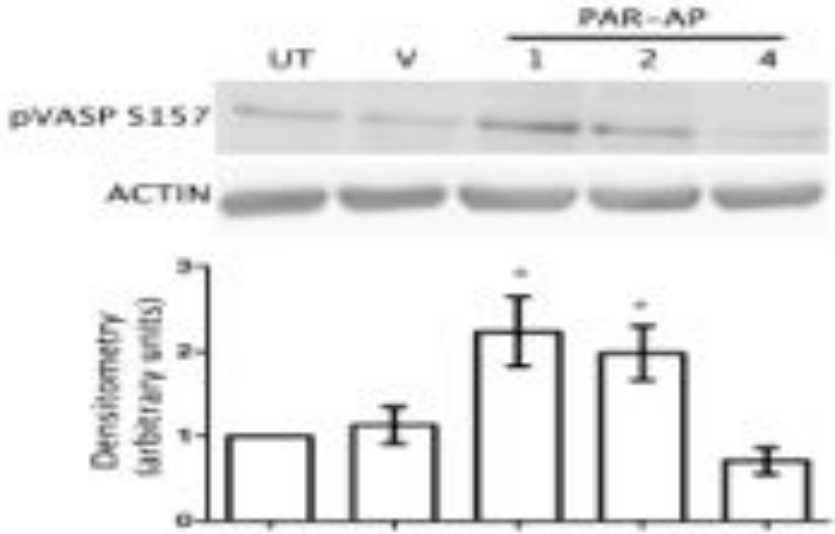
Hypothesis – Protease Activated Receptors (PARs)?

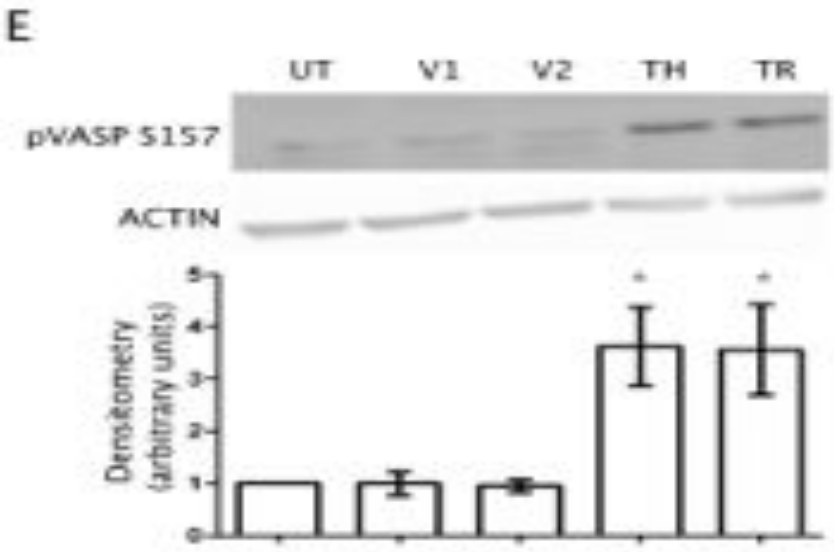
Does FSGS relapse plasma contain excess protease activity? – the use of Protease inhibitors

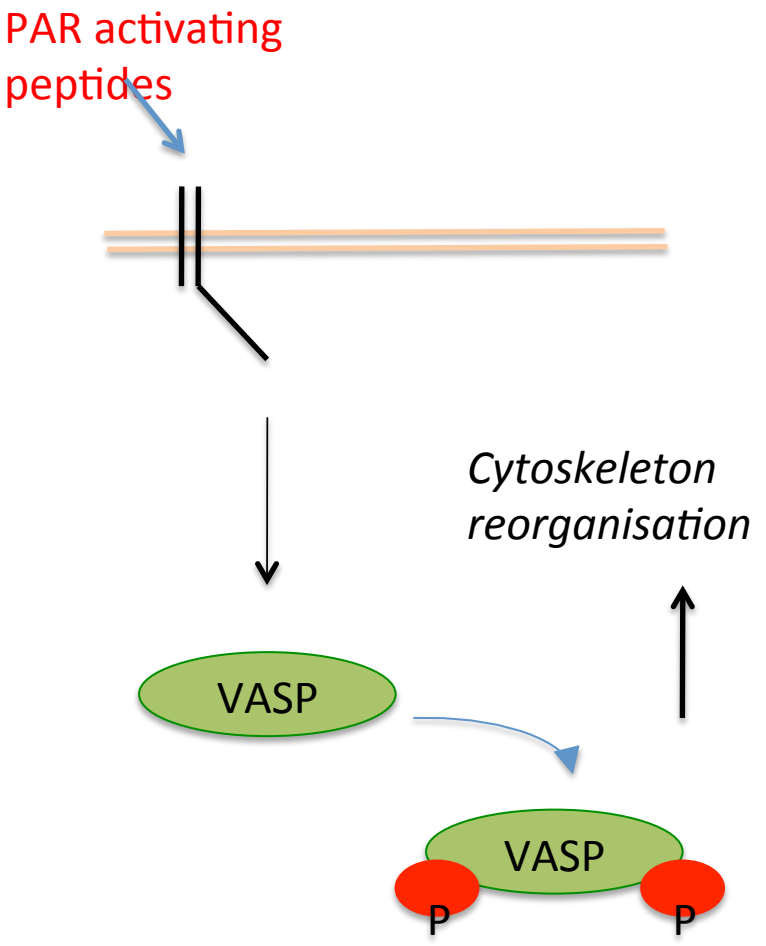




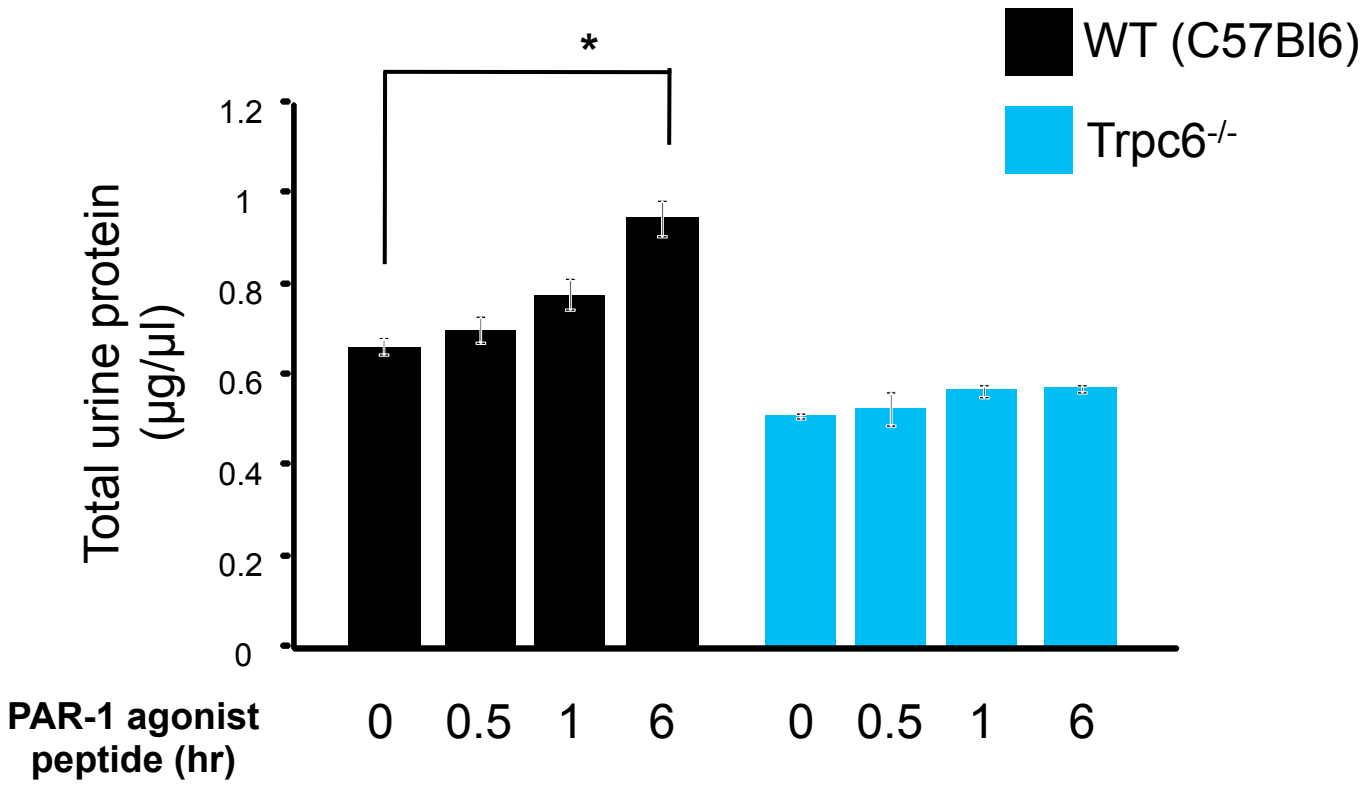
Protease Activated Receptors (PAR) activated in Podocytes via PAR-Activating Peptides

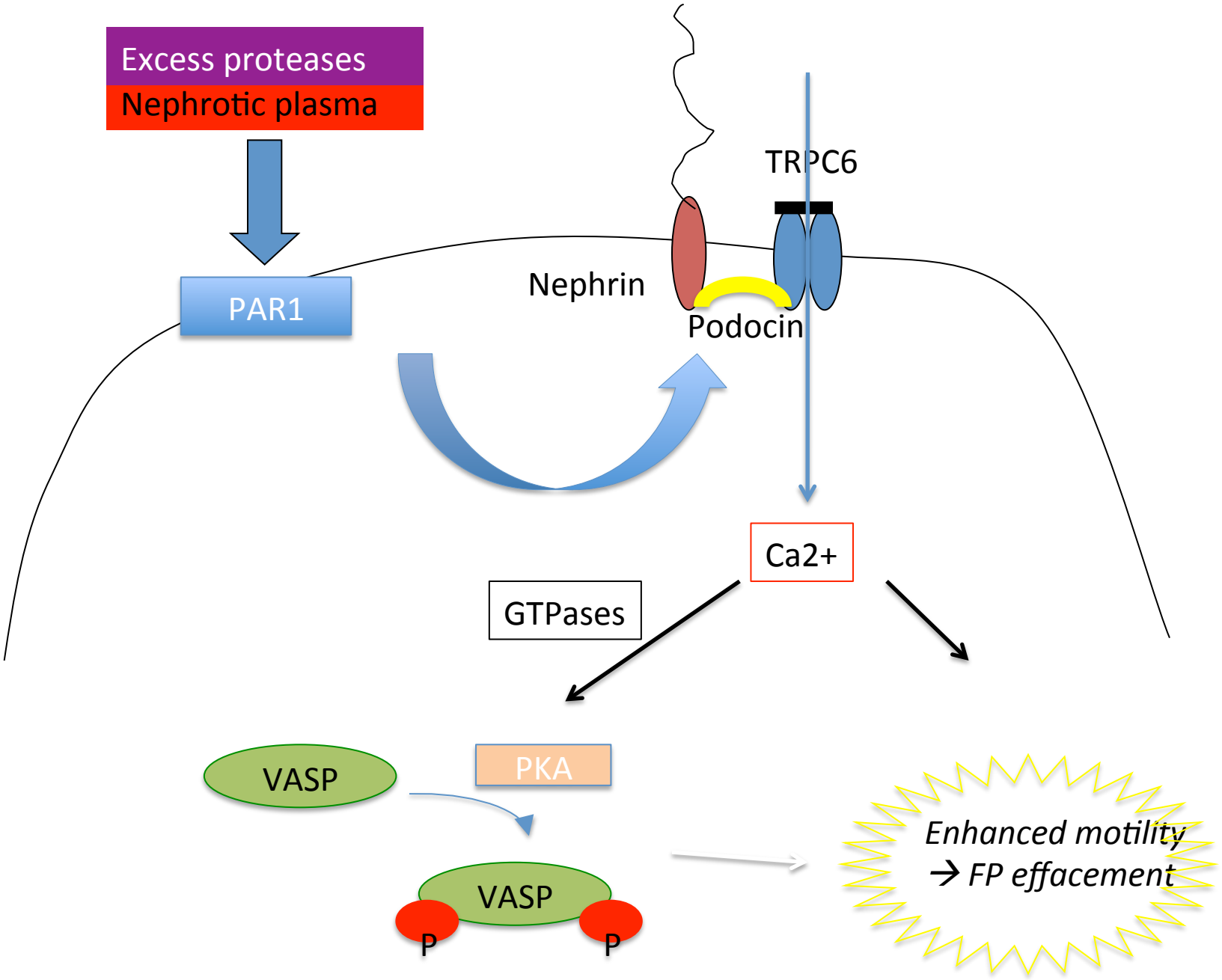


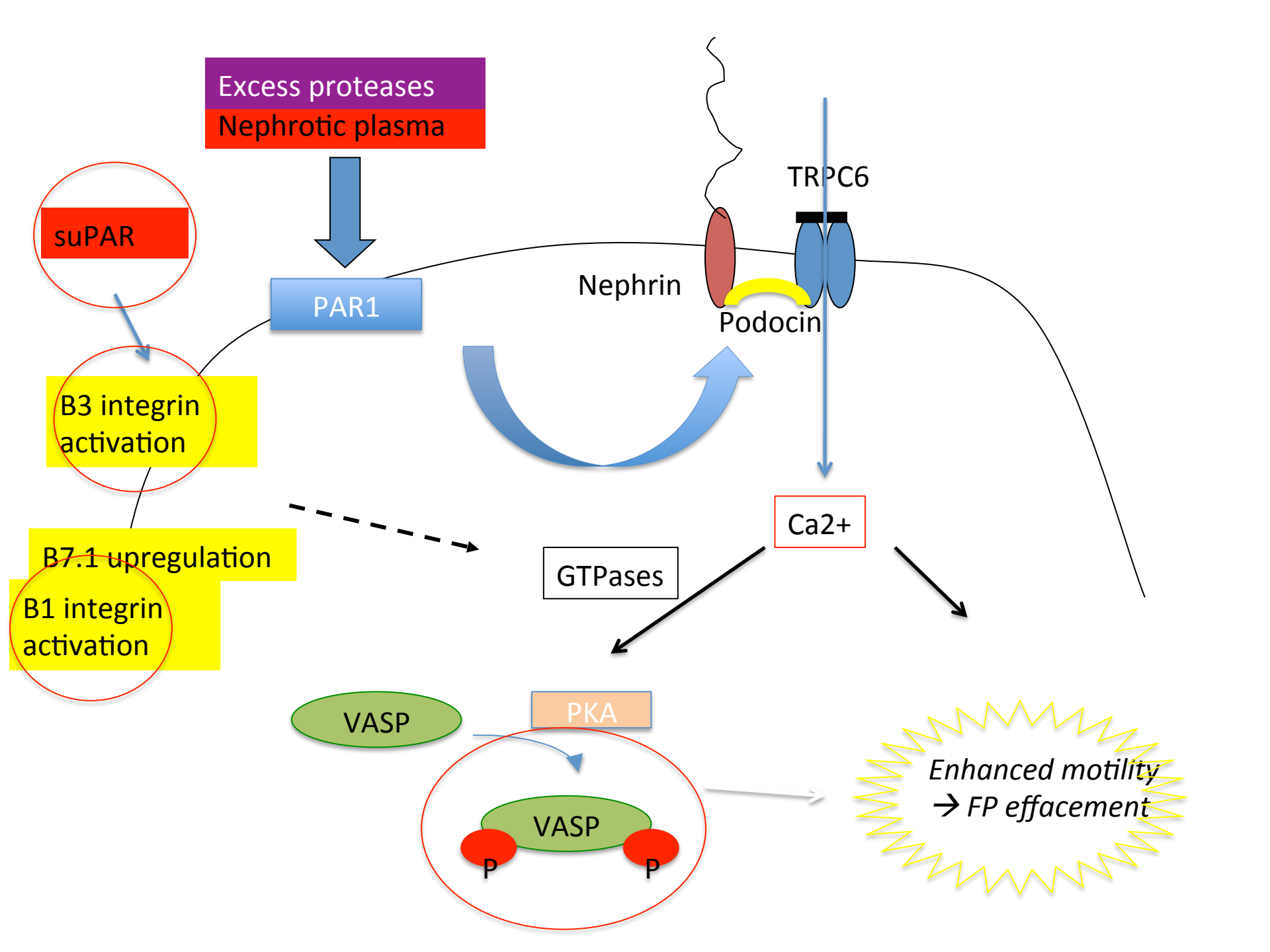




PAR1 agonist causes proteinuria in mice - which is TRPC6 dependent




Excess proteases
Nephrotic plasma
PAR1
TRPC6
Nephrin
Podocin
Ca2+
GTPases
VASP
PKA
VASP
P
P
Enhanced motility
→ FP effacement


Excess proteases
Nephrotic plasma
suPAR
PAR1
TRPC6
Nephrin
Podocin
B3 integrin activation
B7.1 upregulation
B1 integrin activation
Ca2+
GTPases
VASP
PKA
VASP
P
P
Enhanced motility
→ FP effacement

Re-classification of idiopathic NS

Monogenic disease

42 single gene causes
Currently known

Test using Next Generation Sequencing

Circulating Factor disease

Test by plasma induced podocyte expression of b1, b3 integrins, pVASP
Etc?

Other?

Are there any *clinical* clues to identify circulating factor disease?

Early steroid sensitivity is highly likely to indicate circulating factor disease

- Outcomes of 150 children with SRNS who were transplanted (Bristol, Evelina–London, and Necker-Paris)

- 57/150 (38%) patients developed post-transplant recurrence

Initial steroid sensitivity and risk of recurrence

- 29/150 (19%) showed initial steroid sensitivity defined as complete remission of proteinuria on at least one episode after steroid therapy

- 26/29 (90%) patients with initial steroid sensitivity developed recurrence
  (p<0·0001; OR=42, 95%CI 9·33-189.05)

- 26/110 (23·6%) of those without documented steroid sensitivity recurred

Ding et al, J Am Soc Nephrol – *Feb 2014*

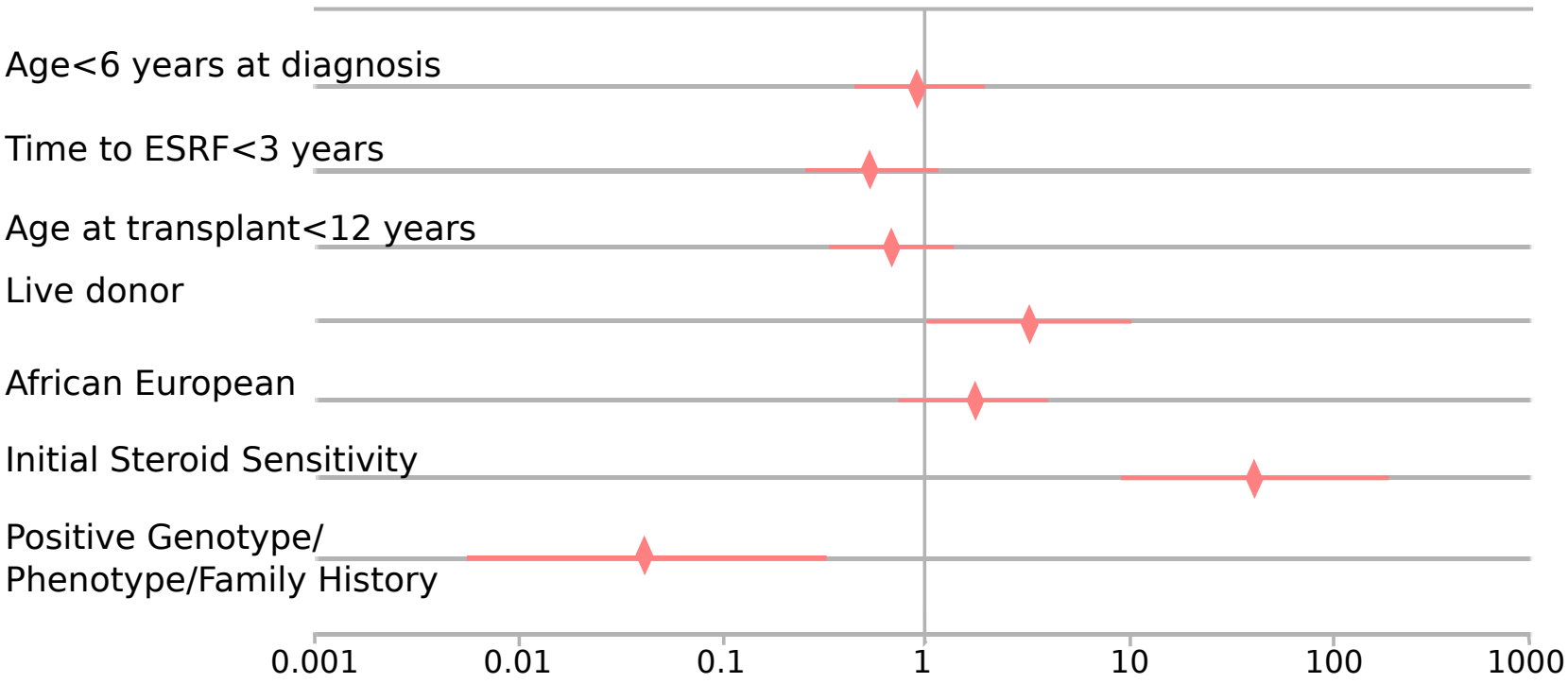
Genetic abnormalities and risk of recurrence

- 18/62 tested patients had a pathogenic single gene mutation
- A further 11/150 patients had extrarenal features suggestive of a syndrome, or a positive Family History of SRNS
- Only 1 of those patients developed recurrence

(n=1/29; p<0·0001; OR=0·042, 95% CI 0·0056-0·32).

Ding et al, J Am Soc Nephrol – *Feb 2014*

Forest plot of odds ratios for recurrence



Ding et al, J Am Soc Nephrol – *Feb 2014*

Re-classification of idiopathic NS

Monogenic disease

42 single gene causes
Currently known

Test using Next Generation Sequencing

Clinical prediction using
Family History
Extrarenal features

Targeted Treatment
Withdraw Immunosuppression

Circulating Factor disease

Test by induced podocyte expression of b1, b3 integrins, pVASP
Etc?

Clinical prediction using
Initial Steroid Sensitivity

Targeted Treatment
e.g. Rituximab, Abatacept, specific b1 or b3 inhibitors

Other?

Conclusions

- Our understanding of podocyte biology by genetic and molecular markers is changing the way we view the disease
- The current classification of NS by steroid response is unsatisfactory and mechanistically unsafe
- Systematic examination of patient samples, biopsy and plasma derived, will allow us to directly identify circulating factor disease

Take home messages

The podocyte is the central cell of the glomerular filtration barrier

Its function is achieved by fine regulation of its cytoskeleton

Cytoskeletal dynamics can be pathologically altered by:

(a) Intrinsic defects in the molecular control of the cytoskeleton

- Genetic defects

(b) External signals dysregulating homeostatic state of the cytoskeleton

- Circulating factors

These pathways can be targeted by novel drugs/compounds to restore the normal balance

This will lead to stratified, individualised therapy, with better outcomes

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