# Stratified Medicine in Nephrology

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# Nephrotic Syndrome

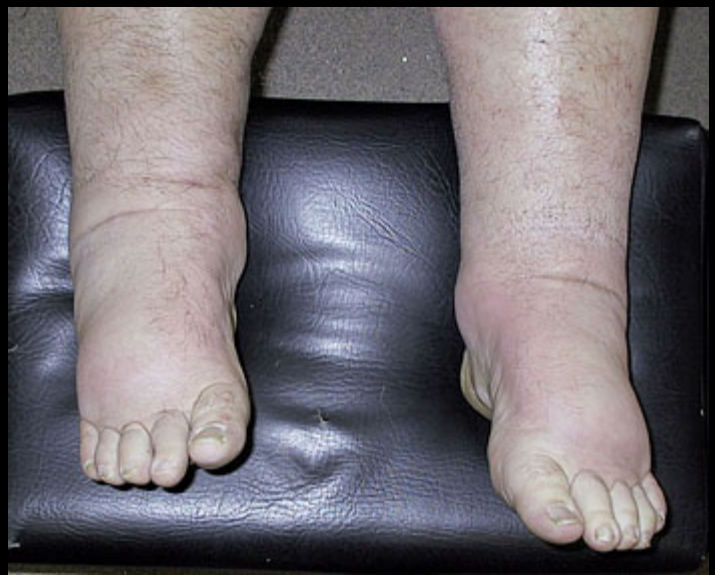
Heavy proteinuria

↓

Hypoalbuminaemia

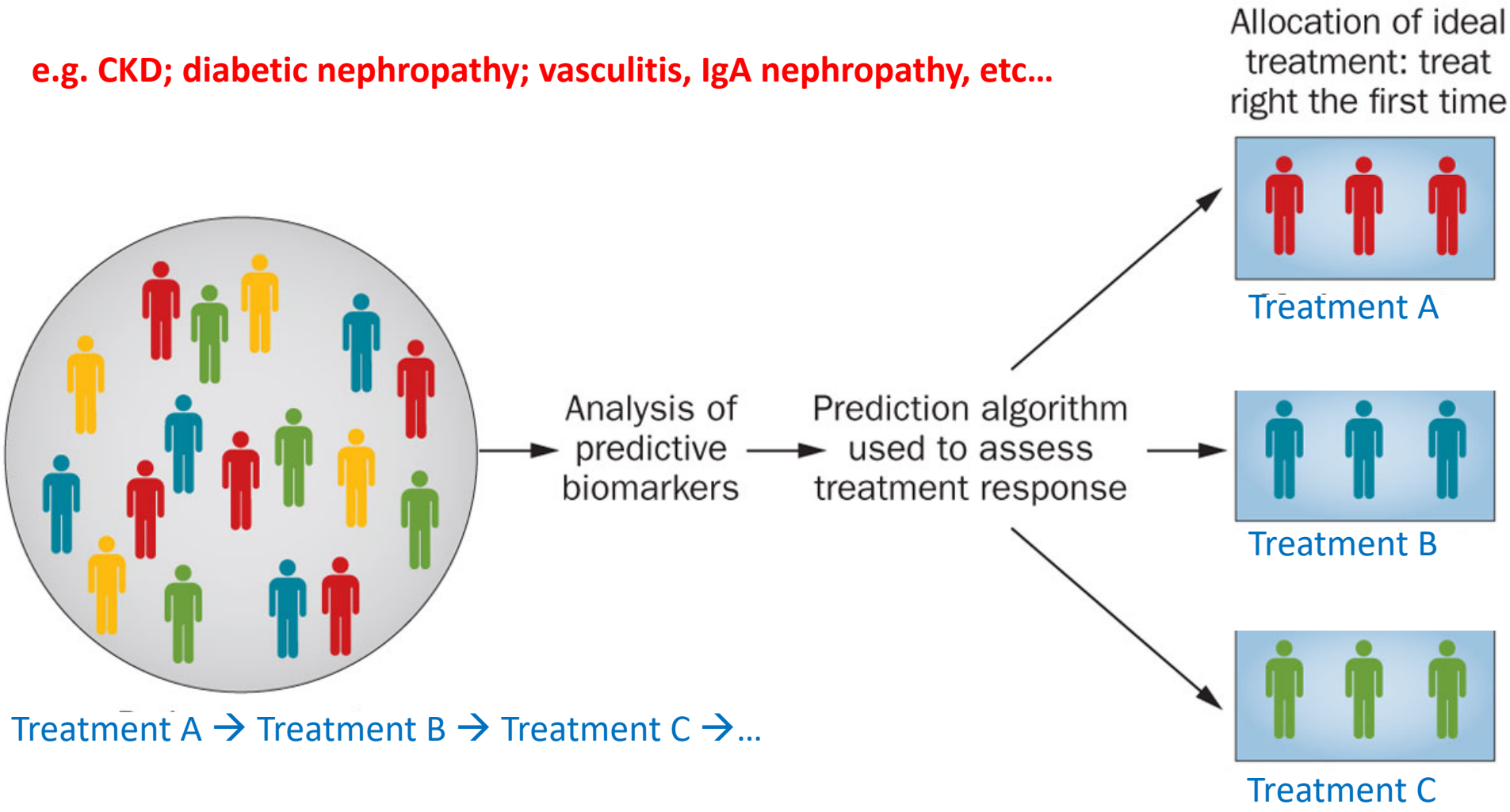
↓

Oedema



# The era of molecular medicine is changing the way we investigate, classify and treat our patients

**e.g. CKD; diabetic nephropathy; vasculitis, IgA nephropathy, etc…**




Treatment A → Treatment B → Treatment C →…

RaDaR

RareRenal
Information on rare kidney diseases

- UK Rare Disease Registry - Original target 500 patients
- Over 20,000 patients, 34 patient groups, 93 renal units
- Nephrotic Syndrome was a pilot group, now >2600 patients recruited

Industry Collaborators

North Bristol NHS
NHS Trust

BioResource
NHS
National Institute for Health Research

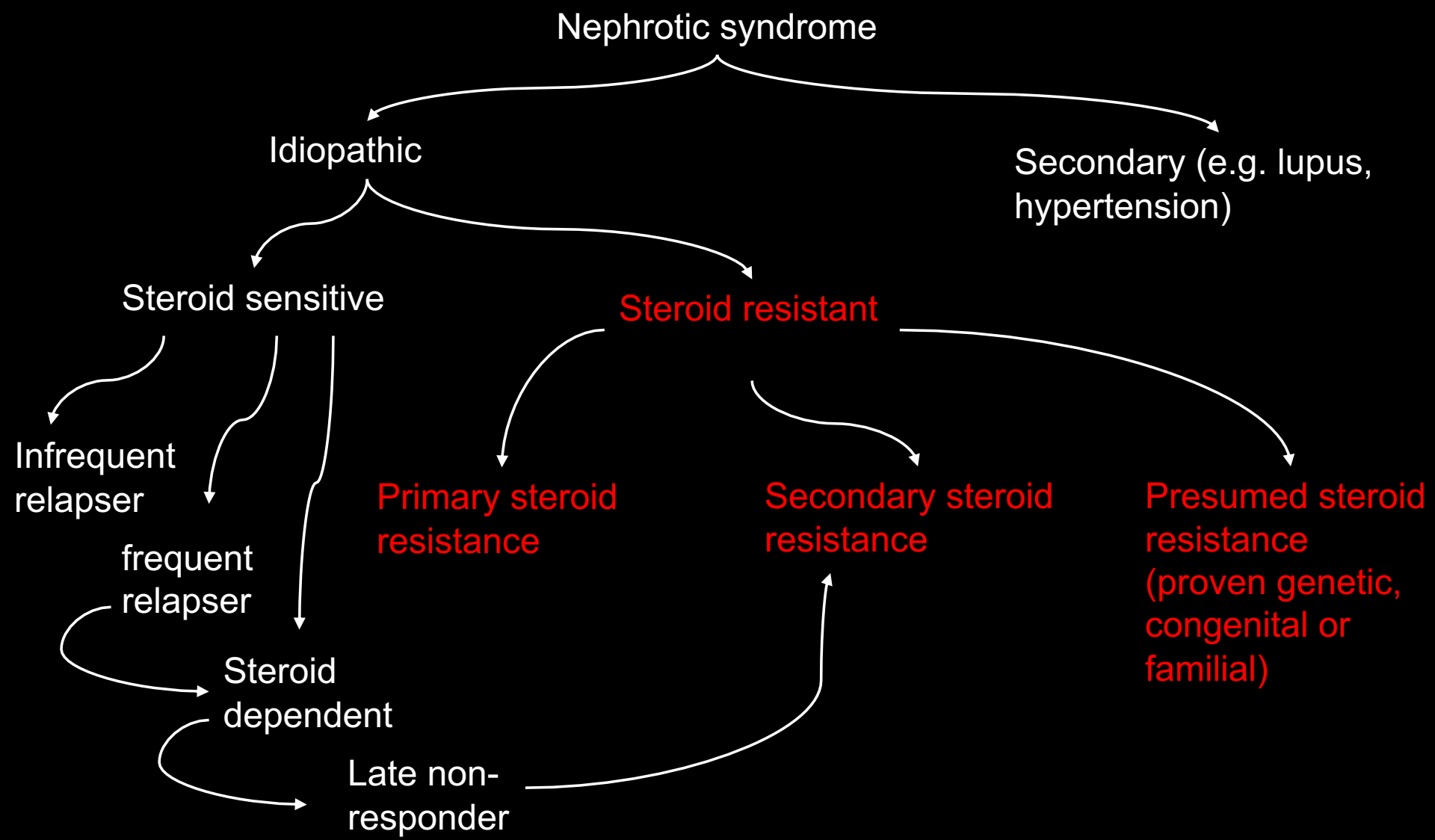
RaDaR

NephroS – the UK study of Nephrotic Syndrome

NURTURE

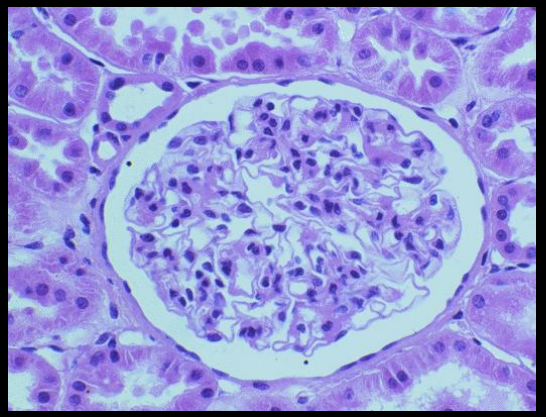
National Unified Renal Translational Research Enterprise

# Nephrotic syndrome – Imprecision medicine
# Classification based on steroid response

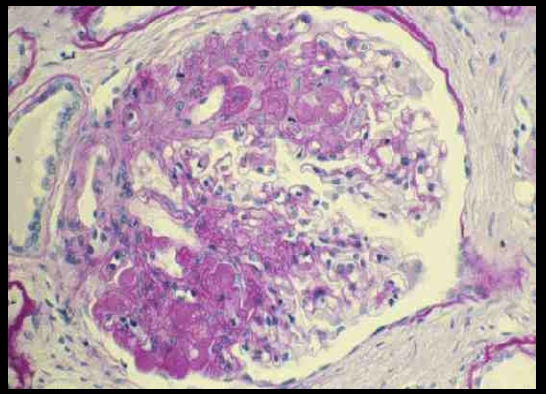


# Nephrotic Syndrome -Histological Classification

1. Minimal change nephrotic syndrome

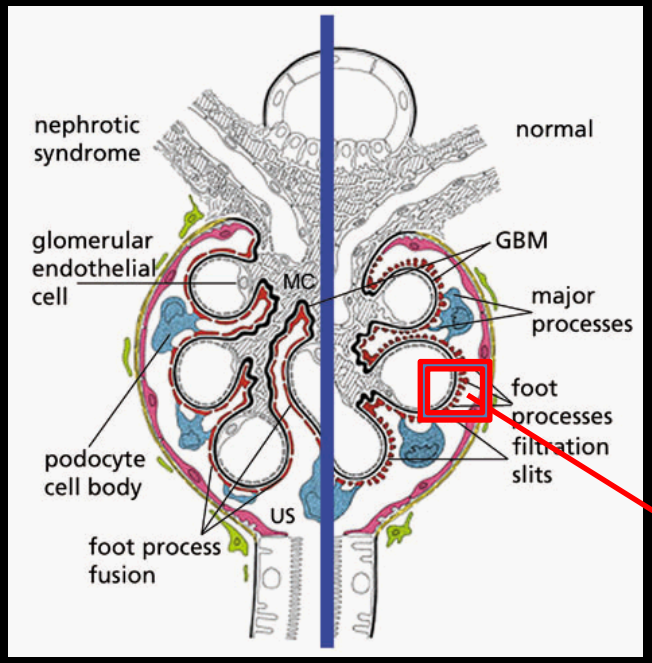


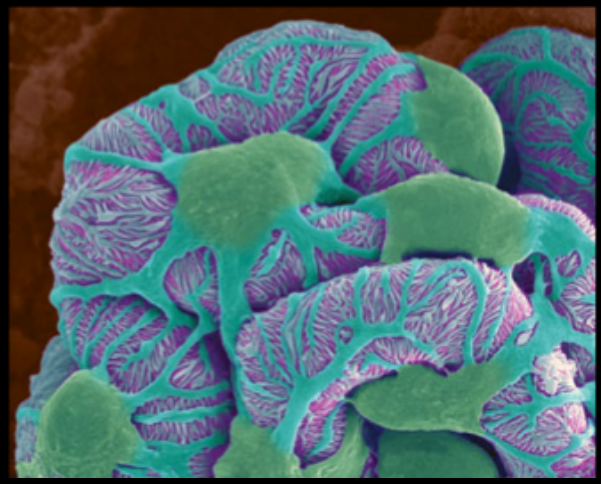
1. Focal segmental glomerulosclerosis (FSGS)

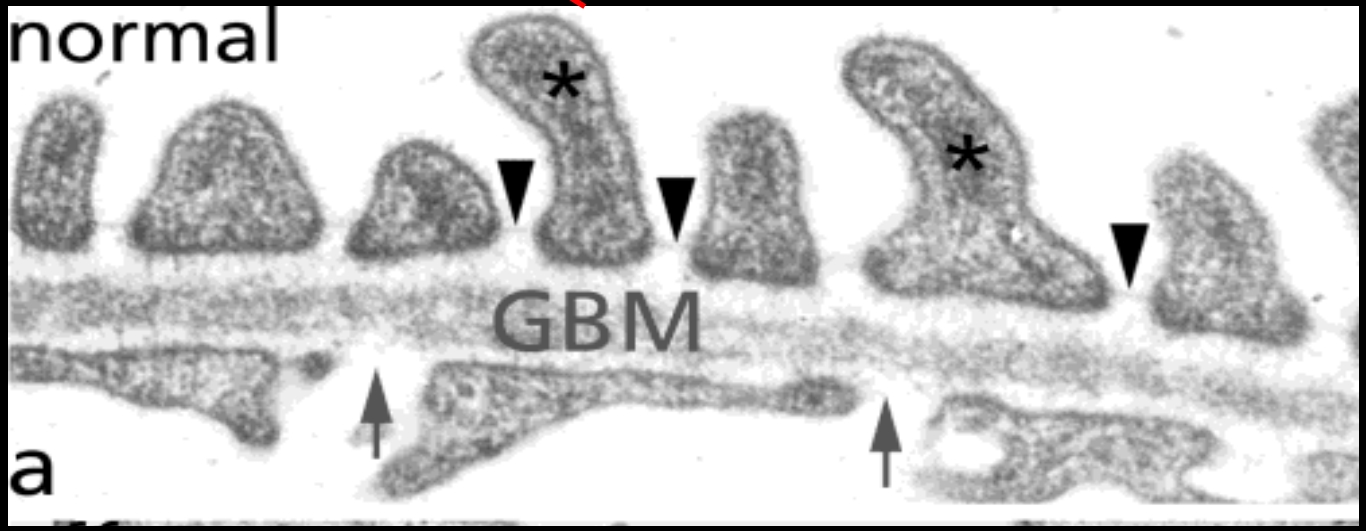


3. Others – e.g. membranous nephropathy, MPGN, IgA nephropathy

# The podocyte - the central cell of the glomerular filtration barrier



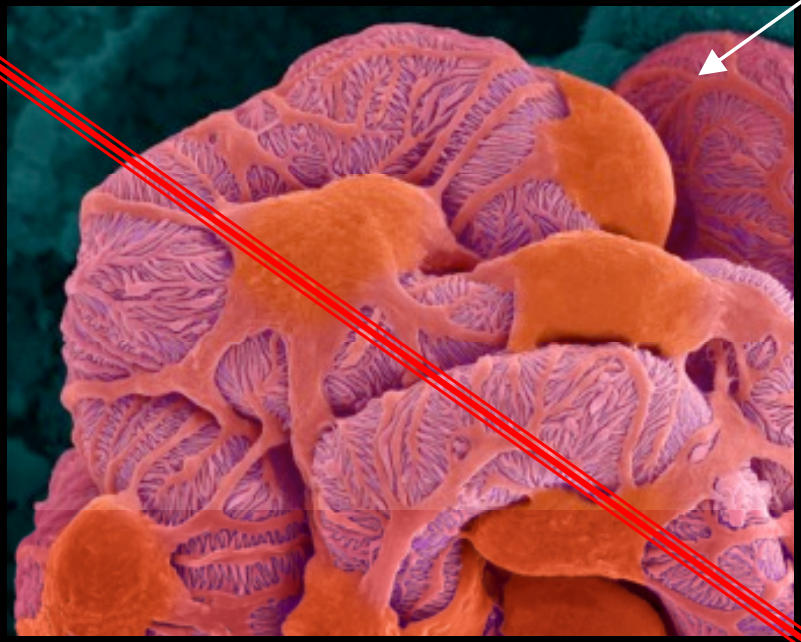


# Signals to control podocyte health

Genes
(nephrin, WT1 etc)

**MOLECULAR SIGNALS**

GBM signals
- Integrins



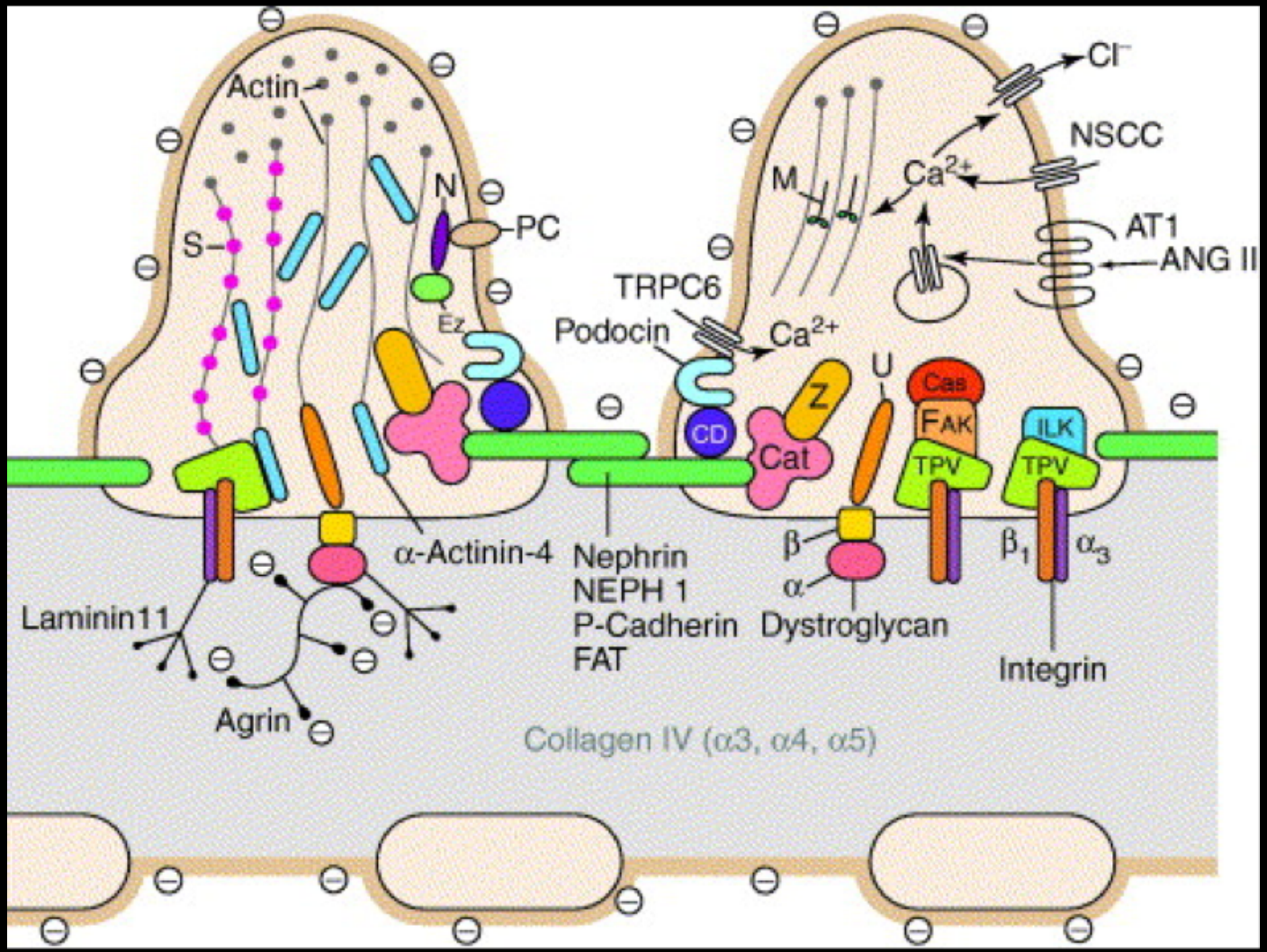
*VEGF*
*Endothelin*
*Etc.*

**SOLUBLE FACTORS**

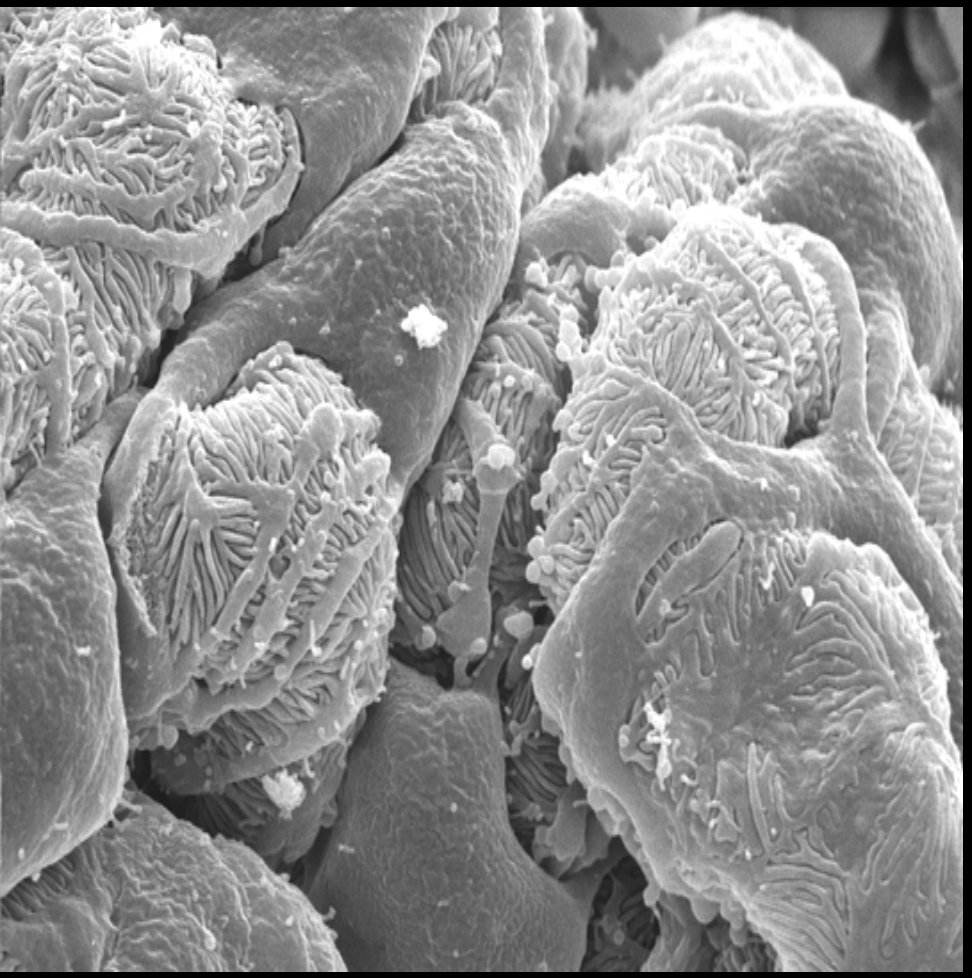
Endothelial cells
Mesangial Cells

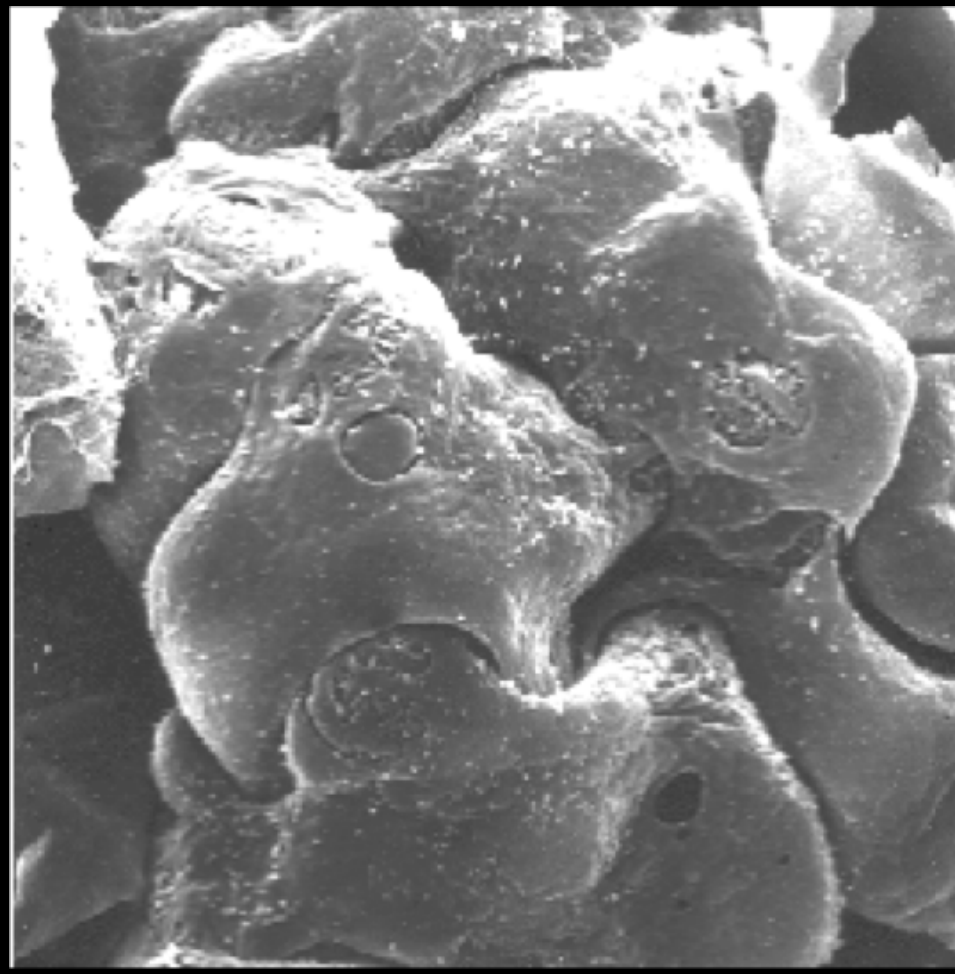
Circulating mediators
- NS (immune derived)
-Diabetic Nephropathy
- insulin
- toxins, e.g shigatoxin

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



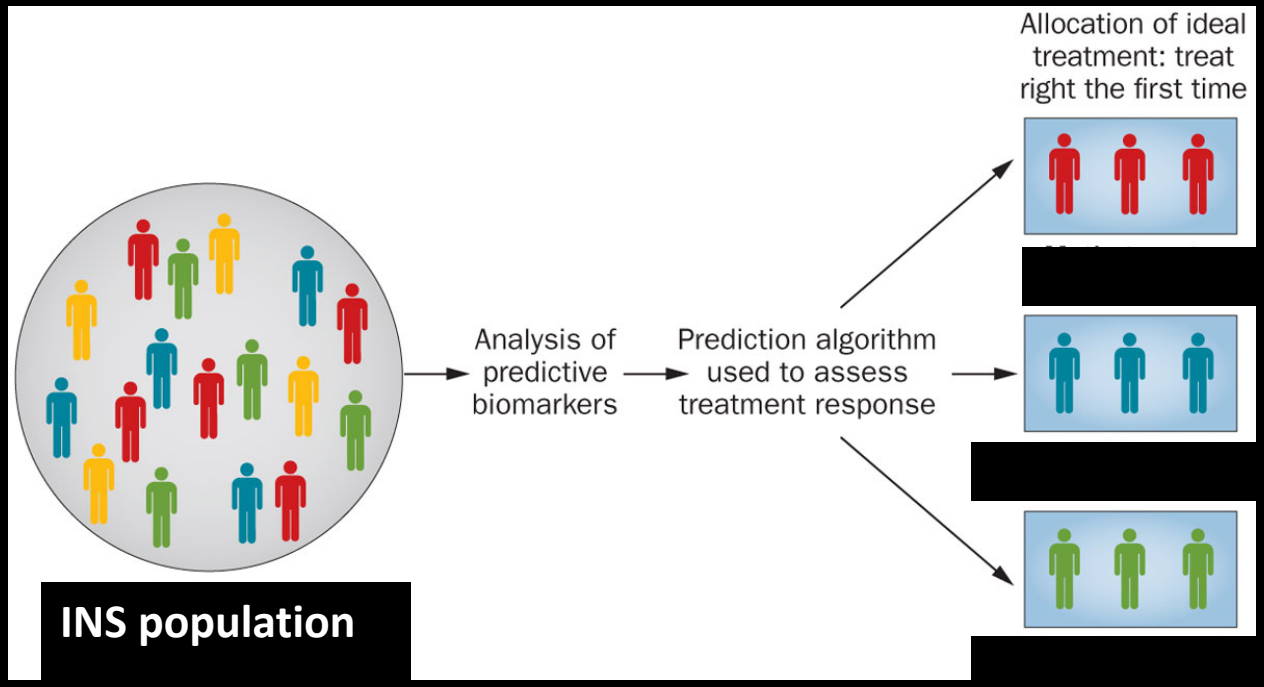
# Podocyte morphology in health and disease





*If the signals are disrupted, the structure of the cell is destroyed*

# Reclassification of idiopathic nephrotic syndrome – signal disruption




Single Gene Disorder

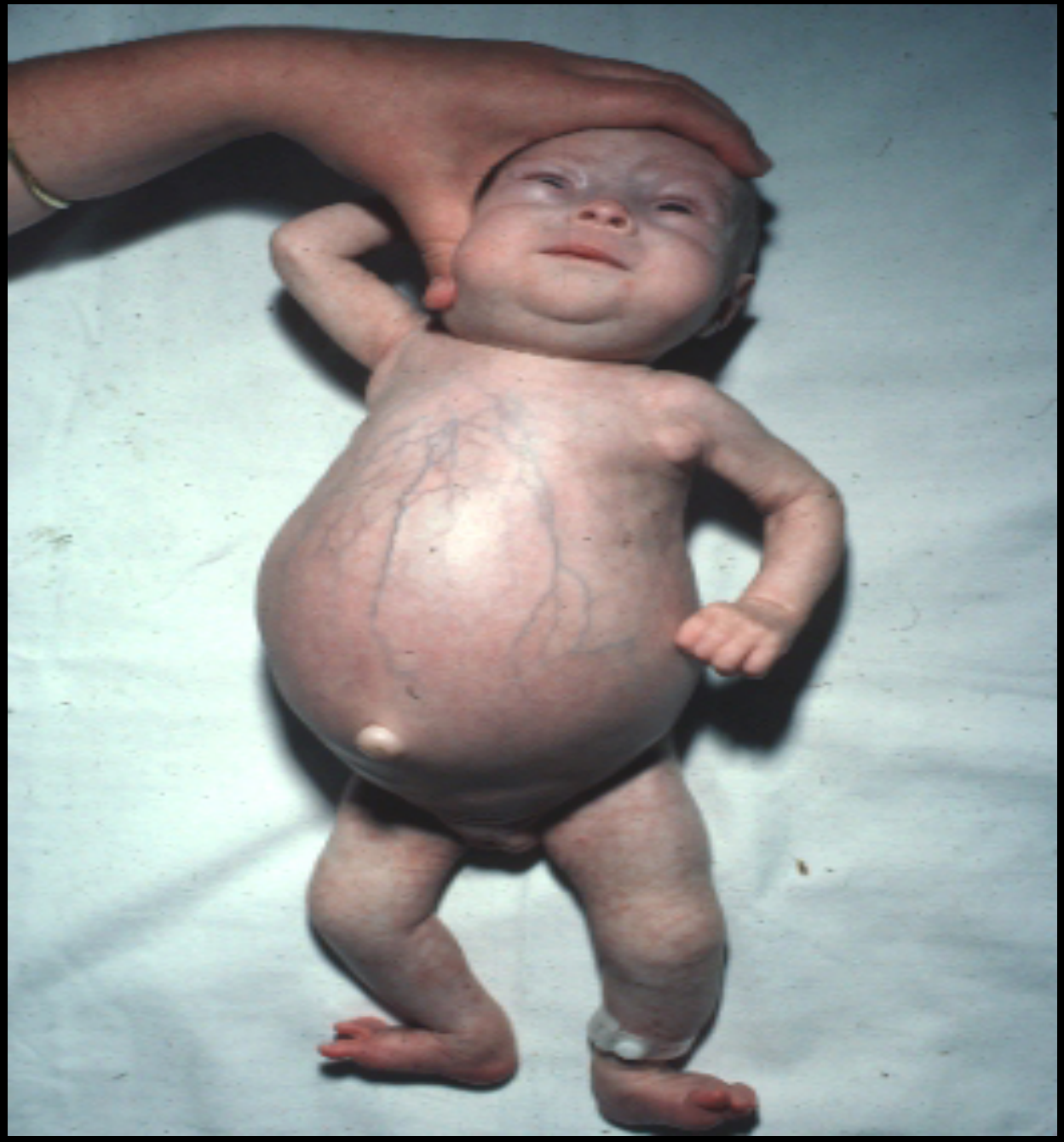
Circulating Factor Disease

?Other mechanism

# Reclassification - Two main mechanistic categories of idiopathic nephrotic syndrome

- Circulating factor disease – probably immune derived

- Monogenic disease (currently 55 different genes discovered)
  - Who are the most relevant patients to test
  - Which test should we use
  - Are we missing patients with our current knowledge



# Exome Sequencing results – 196 children

| Gene | Inheritance | Disease |
|---|---|---|
| ACTN4 | AD | Familial and sporadic SRNS (usualy adult) |
| ADCK4 | AR | SRNS |
| ALG1 | AR | Congenital disorder of glycosylation |
| ANLN | AD | FSGS (mainly adult) |
| ARHGAP24 | AD | FSGS |
| ARHGDIA | AR | CNS |
| CD151 | AR | NS,pretibial bullous skinlesions, neurosensory deafness, bilateral lacrimal duct stenosis, nail dystrophy, and -thalassemia minor |
| CD2AP | ADAR | 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; DMS |
| CRB2 | AR | SRNS |
| CUBN | AR | Intermittent nephrotic range proteinuria +/- with epilepsia |
| DGKE | AR | Atypical hemolytic uremic syndrome-7 +/- ,NS, Nephrotic syndrome, type 7 |
| E2F3 | AD | FSGS+mental 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(A) |
| KANK1 | AR | SSNS |
| KANK2 | AR | SSNS/SDNS +/- hematuria |
| KANK4 | 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 |
| PLCe1 | AR | CNS/SRNS |
| PMM2 | AR | Congenital disorder of glycosylation |
| PODXL | AD | FSGS |
| PTPRO | AR | NS |
| SCARB2 | AR | Action myoclonus renal failure syndrome +/-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 |

*Bierzynska et al ,Kidney International – 2017*

# What proportion of patients with SRNS have a monogenic disease?

- Podonet Study (Schaefer)
  - 1655 patients, 21 countries, 26% familial, between 1 and 31 genes screened per patient
  - Genetic cause in 23.6%

- US study (Hildebrandt)
  - 1783 families (2016 individuals), 27 genes tested
  - Genetic cause in 29.5%

- UK study (RaDaR)
  - 196 patients, 55 SRNS genes screened (whole exome)
  - Unselected cohort of all UK children (0-18), 12% familial
  - 33% incidence of monogenic disease

*Bierzynska et al ,Kidney International – 2017*

# Clinical Genetic Testing

Bristol Genetics Laboratory

- Clinical genetics test developed and approved for SRNS
- 70 genes on the panel
- Over 700 patients worldwide tested to date

- Rapid turnaround - Result aimed for in 4-12 weeks
- Cost £600

- Who should be tested?

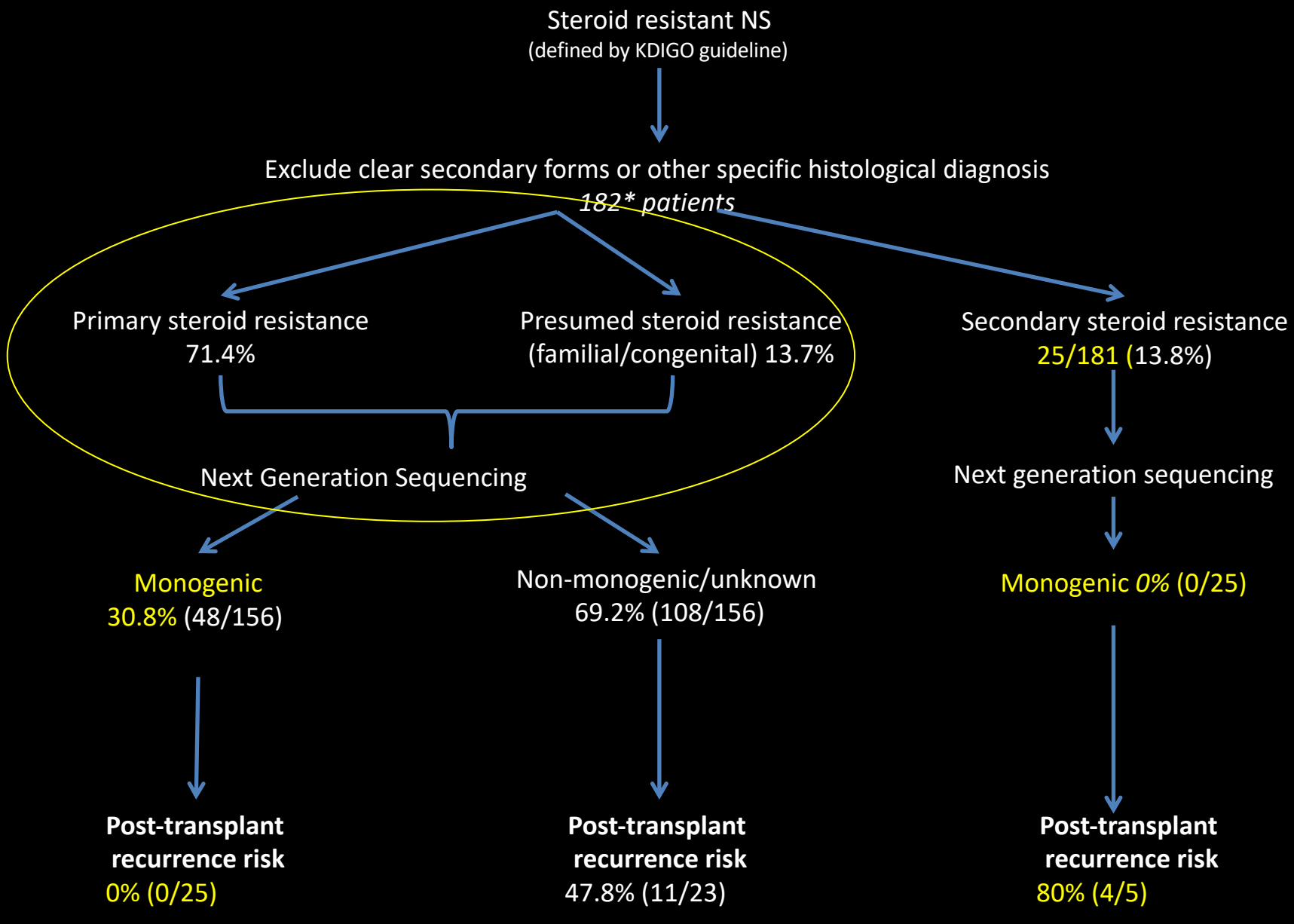
  All children with SRNS

  All adults with primary SRNS? – 11.1% of sporadic non-familial SRNS adults (n=135) have a mutation (Antignac et al, ASN 2017).

# New Genes discovered

- ADCK4 – mitochondrial function - *J Clin Invest 2013*
- CRB2 - polarity protein– *Am J Hum Genet 2015*
- *FAT1* – glomerulotubular feedback - *Nature Commun – 2016, Feb*
- MAGI2 – Causes CNS - *J Am Soc Nephrol – 2017*
- *SGPL1* – adrenal insufficiency and FSGS - *J Clin Invest – 2017*
- *2 other current candidates (collaboration with Corinne Antignac, Guillaume Dorval)*

# A precision medicine based approach to a national cohort of paediatric SRNS
# (187 patients, exome sequenced and with deep phenotyping)



# How can we distinguish genetic NS from circulating factor NS at an early stage?

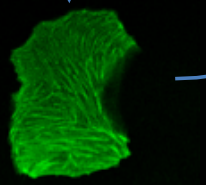
1. Clinical Biomarkers

2. Biological Biomarkers

# Post transplant recurrence in SRNS, the archetypal Circulating Factor Disease

- **The risk of recurrence post-transplant is up to 50% in those without a known genetic mutation**
- **Recurrence occurs within minutes or hours**
- **Pre-transplant plasmapheresis has been very effective in delaying or even preventing post-transplant recurrence of disease**

Serum



mRNA. Protein expression, localisation and phosphorylation, etc.

Human podocytes in culture


Saleem, M.A., *The phenomenon of focal segmental glomerulosclerosis post-transplantation--a one-hit wonder?* Pediatr Nephrol, 2012. **27**(12): p. 2163-6.
Gallon, L., et al., *Resolution of recurrent focal segmental glomerulosclerosis after retransplantation.* N Engl J Med, 2012. **366**(17): p. 1648-9.
Gohh, R.Y., et al., *Preemptive plasmapheresis and recurrence of FSGS in high-risk renal transplant recipients.* Am J Transplant, 2005. **5**(12): p. 2907-12.

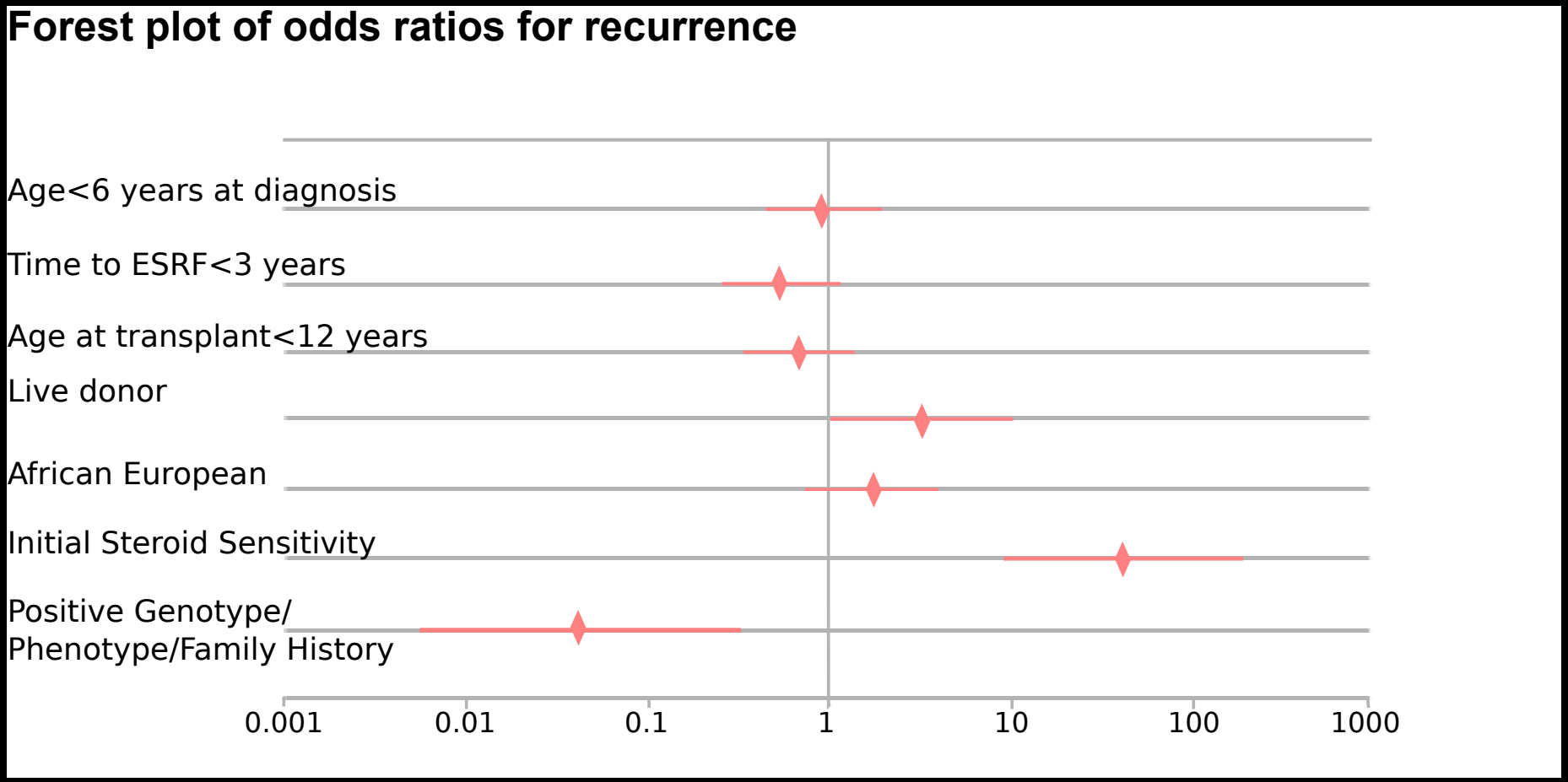
# How can we distinguish genetic NS from circulating factor NS at an early stage? A clinical clue

- *Post-transplant recurrence is the clearest manifestation of circulating factor disease*
  - *Can this be predicted by an initial response to steroids?*

- 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 – a highly sensitive and specific predictor of circulating factor disease




*90% of patients with initial steroid sensitivity developed post-transplant recurrence (total cohort of 150 patients)*

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

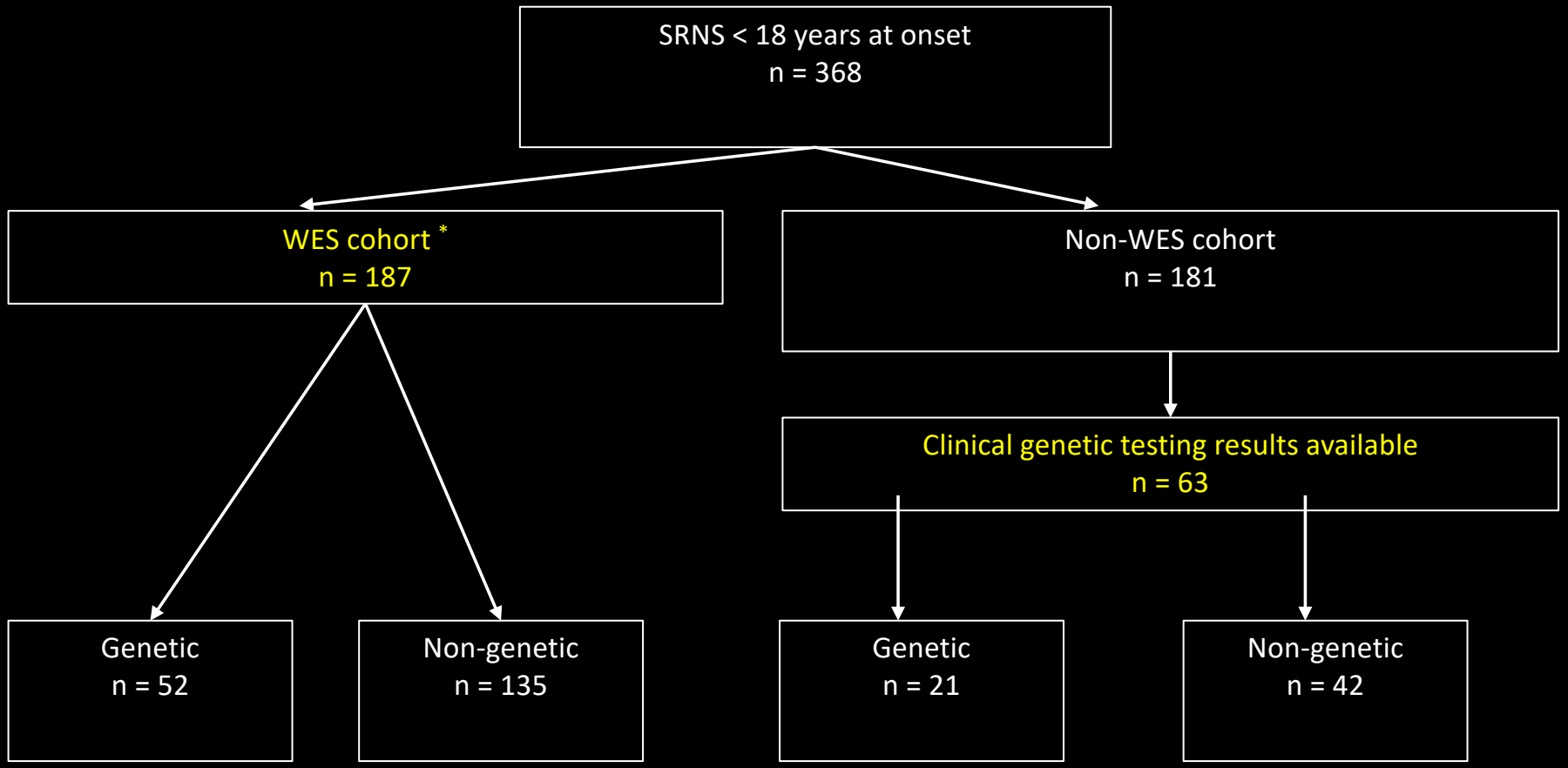
Does Response to **intensified immunosuppression** and subsequent long-term outcome further stratify patients

- Steroid-resistant nephrotic syndrome
  - What proportion of patients have complete response to intensified immunosuppression (IIS)?
  - Is there a difference in outcome between those who respond to their first IIS, and those who don't?

# Methods – Medication response

- All IS medications
- Complete response (CR)
  - Urine protein:creatinine ratio (PCR) < 20 mg/mmol or negative/trace dipstick proteinuria within 6 months of starting therapy
- Partial response (PR)
  - PCR > 20 mg/mmol or dipstick ≥ 1+ but plasma albumin > 25 g/L within 6 months of starting therapy

# Patient selection – all SRNS patients with exome or gene panel test results




* Bierzynska et al. Genomic and clinical profiling of a national nephrotic syndrome cohort advocates a precision medicine approach to disease management. Kidney international. 2017.

# Re-classification of idiopathic NS

## Monogenic disease

*55 single gene causes*
*Currently known*

Test using Next Generation Sequencing

Clinical prediction using
Family History
Extrarenal features

## Circulating Factor disease

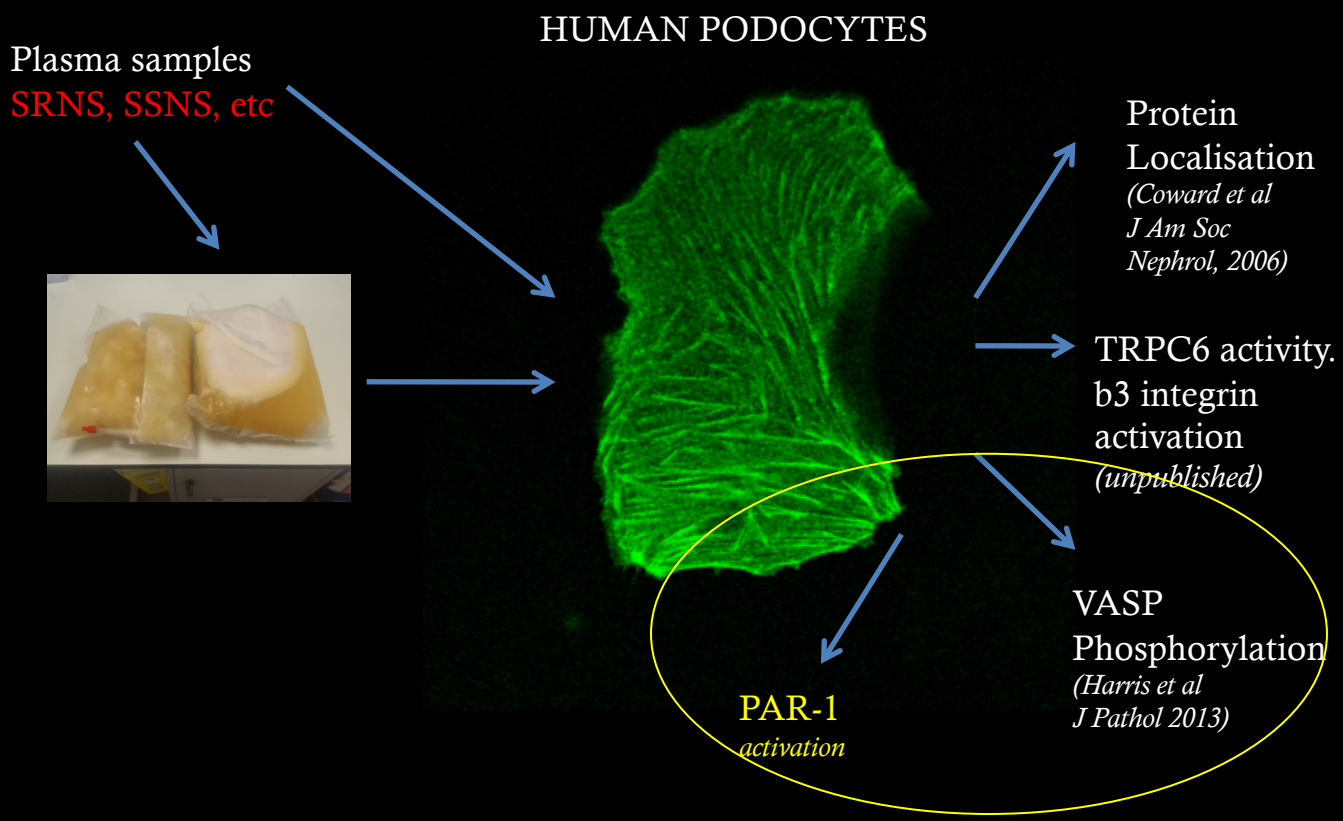
*?2 (or more) types*

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

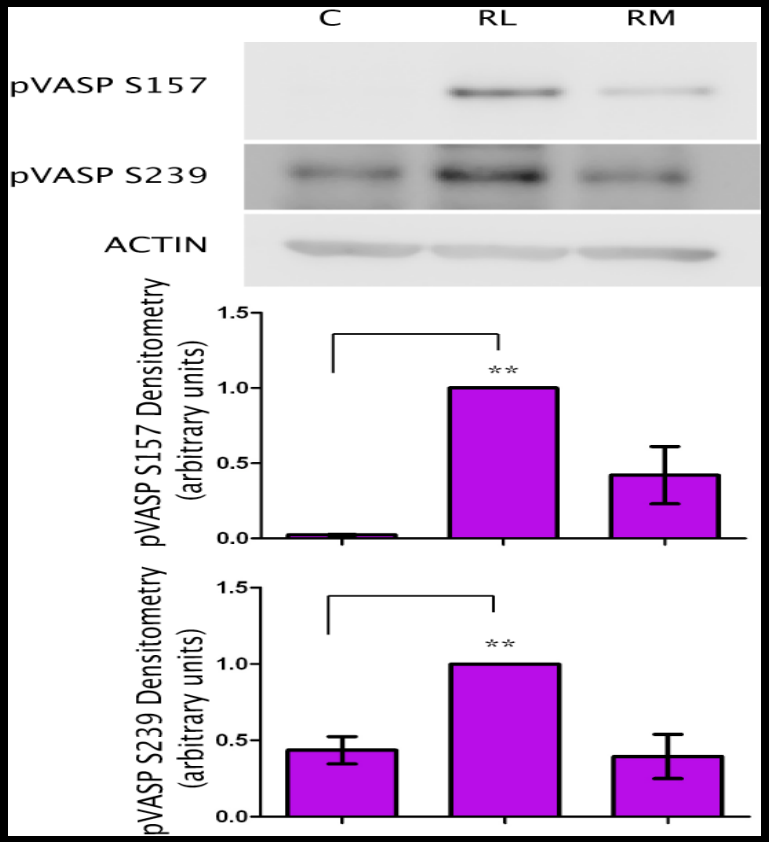
Clinical prediction using
Initial Steroid Sensitivity

## Other?

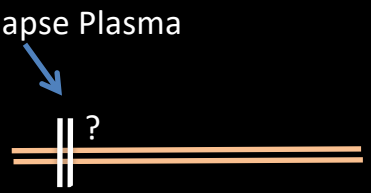
# How do we discover robust *biological biomarkers* that identify patients with 'Circulating Factor Disease'?



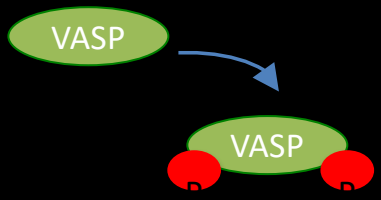
# Actin associated protein VASP is phosphorylated in response to nephrotic plasma




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




*Cytoskeleton reorganisation*



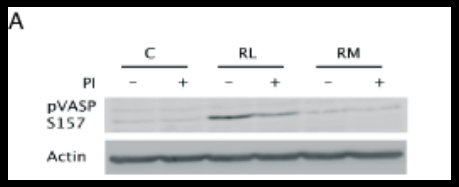
What ‘factor’ is circulating in plasma that could be causing this effect?

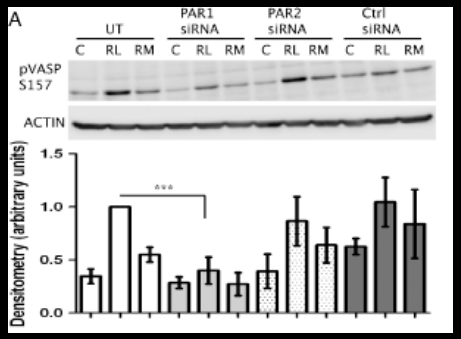
*Hypothesis – an imbalance of circulating plasma proteases*

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

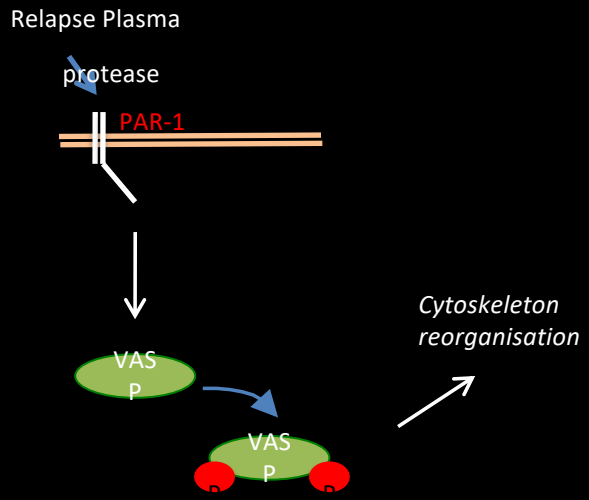
*Hypothesis – Protease Activated Receptors (PARs)?*

# Hypothesis - The Effector is a Protease that Signals Through PAR-1



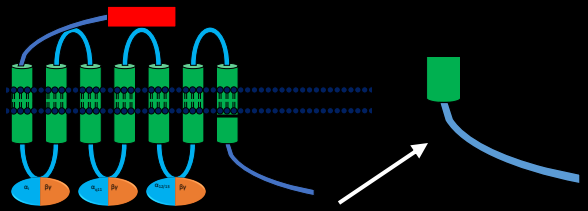



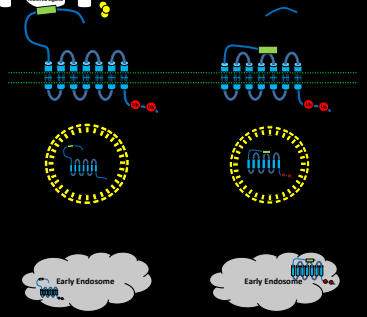

- Protease inhibition significantly reduces VASP phosphorylation in response to relapse plasma
- Knock down of PAR-1 using siRNA also significantly reduces VASP phosphorylation in response to relapse plasma




Harris, J.J., et al., *Active proteases in nephrotic plasma lead to a podocin-dependent phosphorylation of VASP in podocytes via protease activated receptor-1.* J Pathol, 2013. **229**(5): p. 660-71.

# Transgenic Par-1 Active and Mouse Model

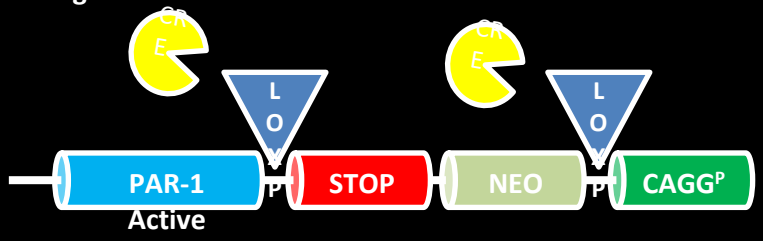


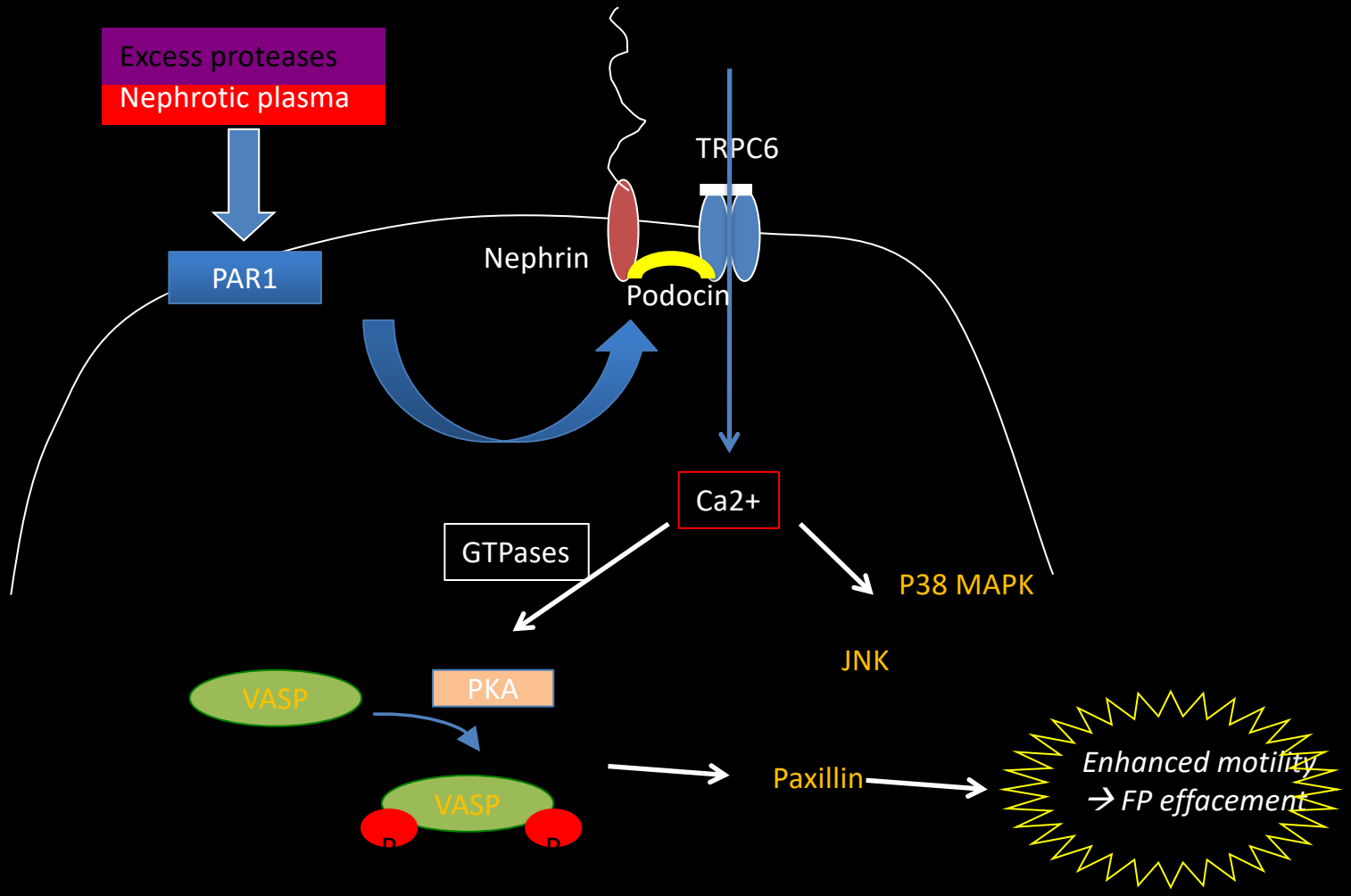



Developmental Driver


Inducible Driver

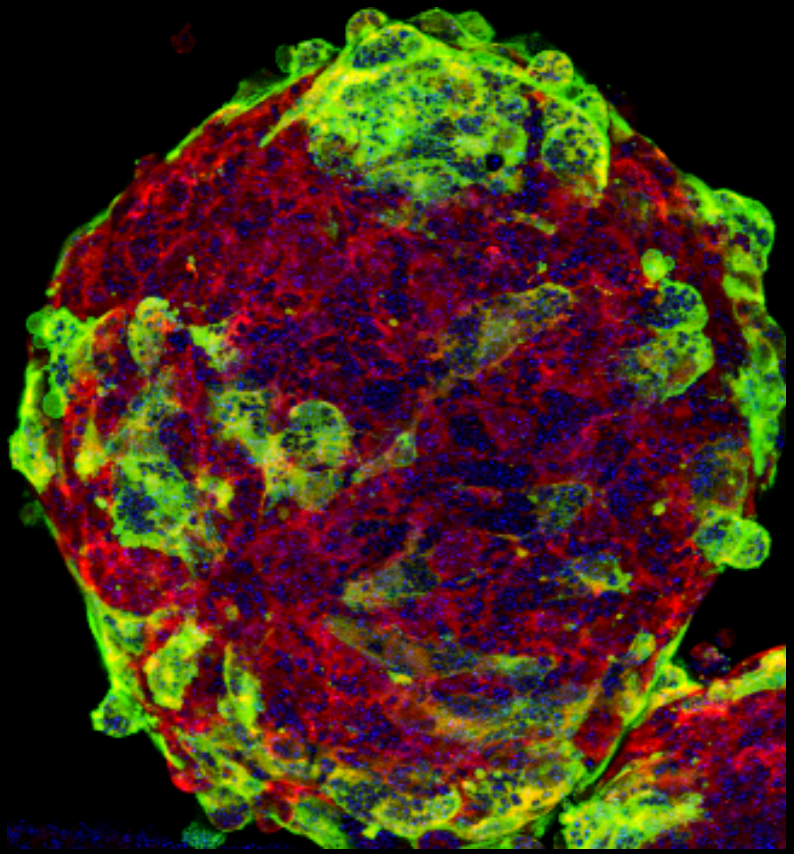

Transgenic Cassette




Excess proteases
Nephrotic plasma
PAR1
TRPC6
Nephrin
Podocin
Ca2+
GTPases
P38 MAPK
JNK
VASP
PKA
VASP
P
P
Paxillin
Enhanced motility
→ FP effacement

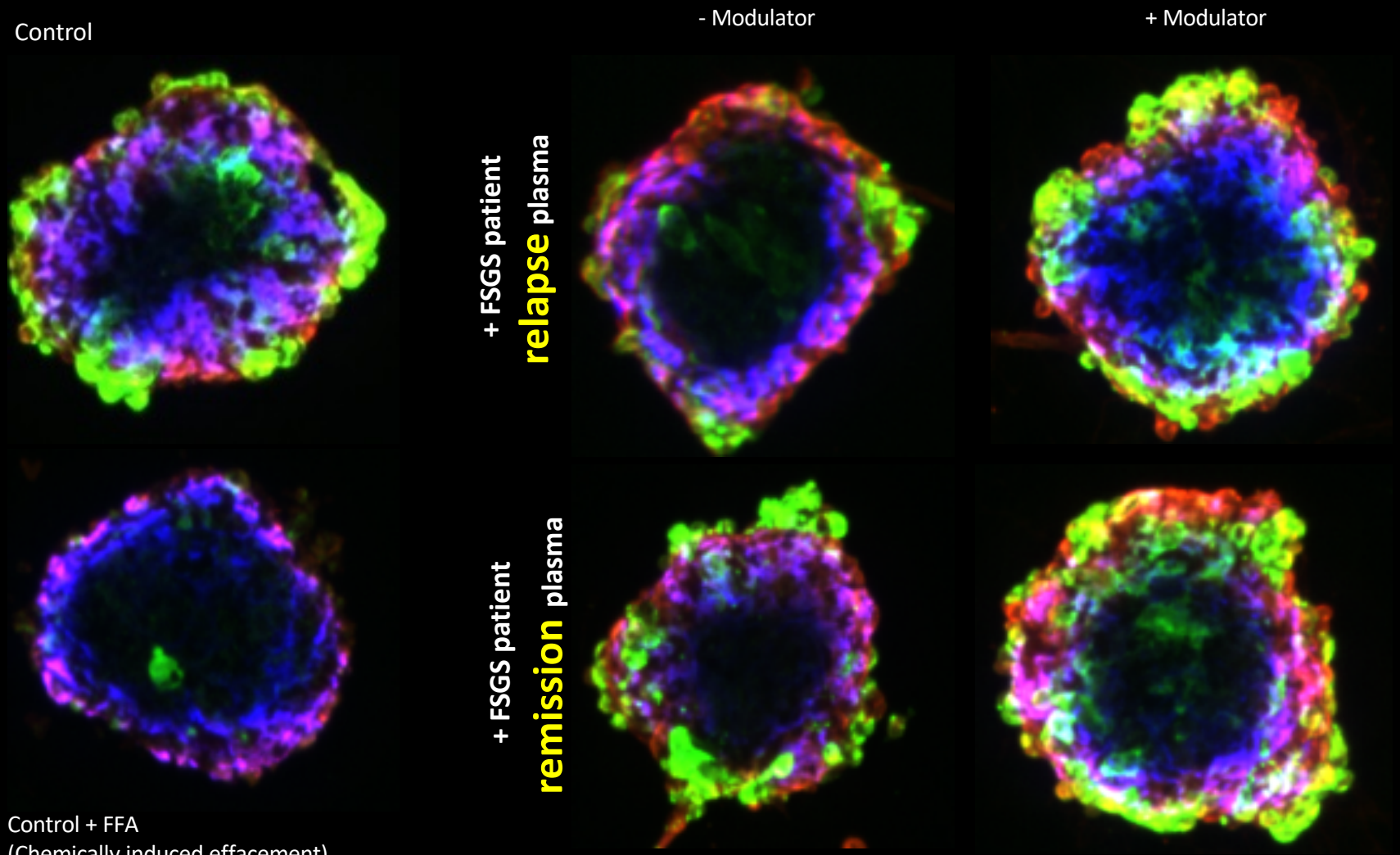
# Development of novel biomarkers for INS – enhancing the model system

**3D spheroids created by magnetic levitation**
podocytes and glomerular endothelial cells,
with collagen staining at the interface



*Jack Tuffin*
*Simon Satchell*

# Modulator prevents podocyte loss caused by SRNS patient relapse plasma in a Glomerulosclerosis spheroid model



# Re-classification of idiopathic NS

## Monogenic disease

*55 single gene causes*
*Currently known*

Test using Next Generation Sequencing

Clinical prediction using
Family History
Extrarenal features

Withdraw Immunosuppression
OR – specific therapies for genetic Mutations…

## Circulating Factor disease

Test by induced podocyte expression of b1, b3 integrins, pVASP
T cell transcriptomics, - SYSTEMS BIOLOGY

Clinical prediction using
Initial Steroid Sensitivity

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

## Other?

The NEW ENGLAND JOURNAL of MEDICINE

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ORIGINAL ARTICLE

## Adenovirus-Associated Virus Vector–Mediated Gene Transfer in Hemophilia B

Amit C. Nathwani, M.B., Ch.B., Ph.D., Edward G.D. Tuddenham, M.B., B.S., M.D., Savita Rangarajan, M.B., B.S., Cecilia Rosales, Ph.D., Jenny McIntosh, Ph.D., David C. Linch, M.B., B.Chir., Pratima Chowdary, M.B., B.S., Anne Riddell, B.Sc., Arnulfo Jaquilmac Pie, B.S.N., Chris Harrington, B.S.N., James O'Beirne, M.B., B.S., M.D., Keith Smith, M.Sc., John Pasi, M.D., Bertil Glader, M.D., Ph.D., Pradip Rustagi, M.D., Catherine Y.C. Ng, M.S., Mark A. Kay, M.D., Ph.D., Junfang Zhou, M.D., Yunyu Spence, Ph.D., Christopher L. Morton, B.S., James Allay, Ph.D., John Coleman, M.S., Susan Sleep, Ph.D., John M. Cunningham, M.D., Deokumar Srivastava, Ph.D., Etiena Basner-Tschakarjan, M.D., Federico Mingozzi, Ph.D., Katherine A. High, M.D., John T. Gray, Ph.D., Ulrike M. Reiss, M.D., Arthur W. Nienhuis, M.D., and Andrew M. Davidoff, M.D.

N Engl J Med 2011; 365:2357-2365 | December 22, 2011 | DOI: 10.1056/NEJMoa1108046

# Hypothesis

AAV is an efficient specific vector for gene therapy in podocytes

Wen Ding – KRUK Clinical Training Fellow

# Nephrotic Syndrome – A Precision Medicine approach

**Deep Phenotyping**
NURTuRE

**Genotyping**
World's first NGS Gene panel

**Biomarkers**
Biological, and from deeper understanding of genetic architecture

Clinical Trials

Treatment A

Treatment B

Treatment C

A mechanistic reclassification of INS

# Thank you ☺

- **Liz Colby**
- **Agnieszka Bierzynska**
- **Gavin Welsh**
- **Richard Coward**
- **Simon Satchell**
- Ethan Sen
- Jack Tuffin
- Wen Ding
- **Valerya Kuzmuk**

- UK Renal Registry
  - Fiona Braddon

Kidney Research UK

Michael Nation

Elaine Davies

- Caroline Jones
- Carol Inward
- Rodney Gilbert
- Martin Christian
- Larissa Kerecuk

- Ania Koziell
- Paul Hartley
- Tim Johnson

- Olivia Boyer
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