SRNS: when and how to diagnose?

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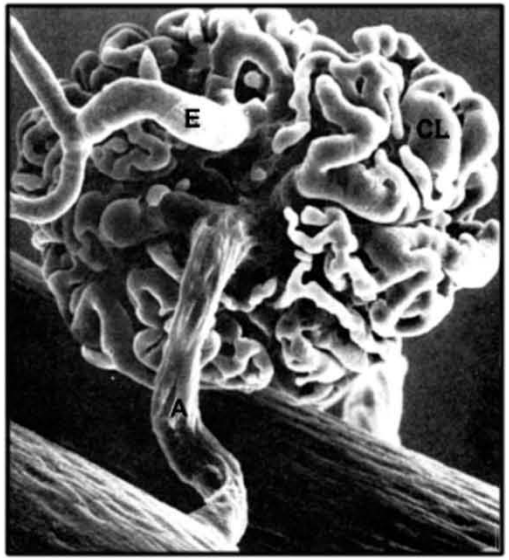
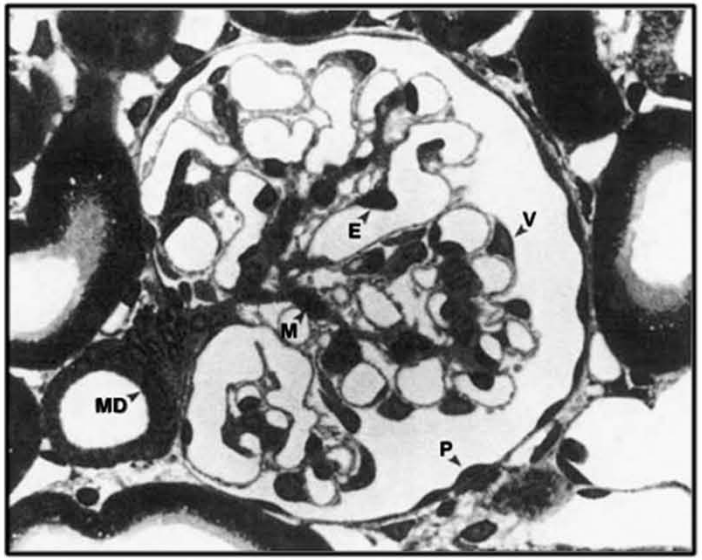
Bambino Gesù
OSPEDALE PEDIATRICO


E
CL
A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V
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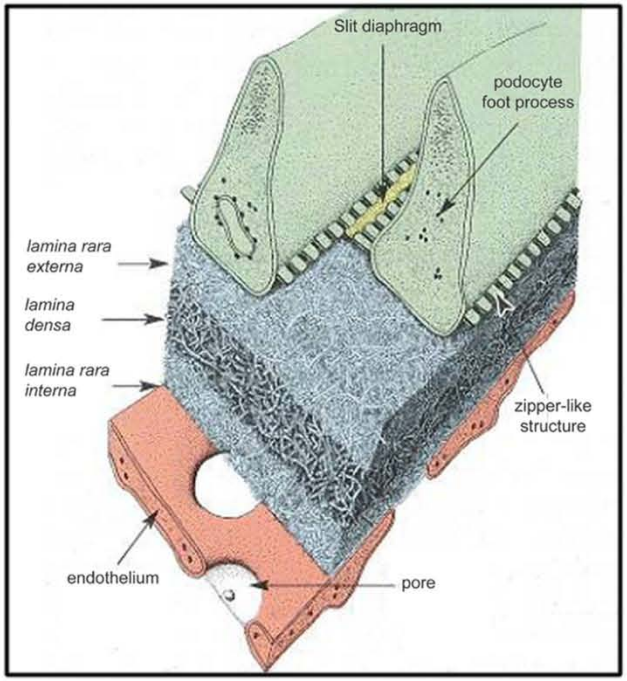
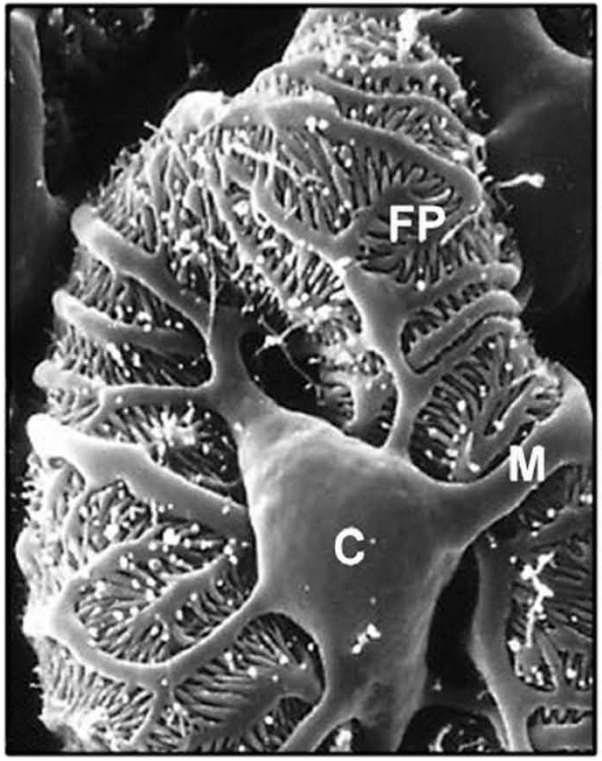
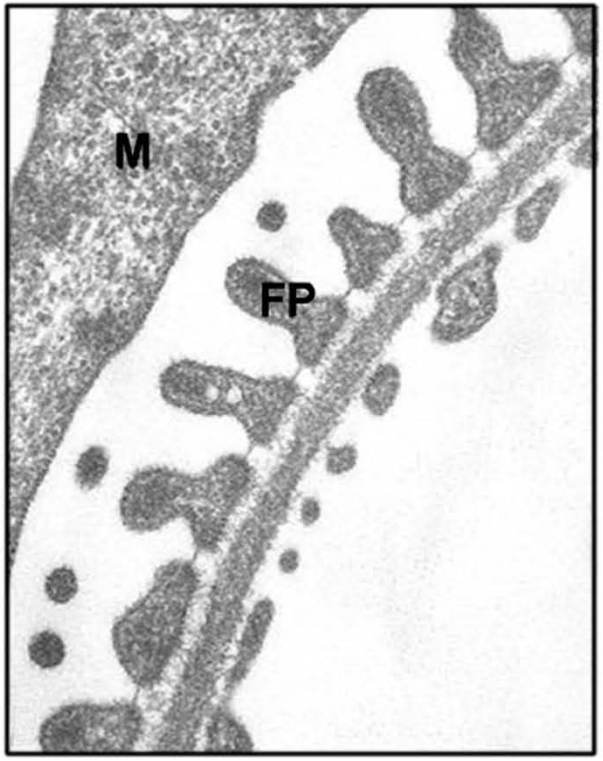
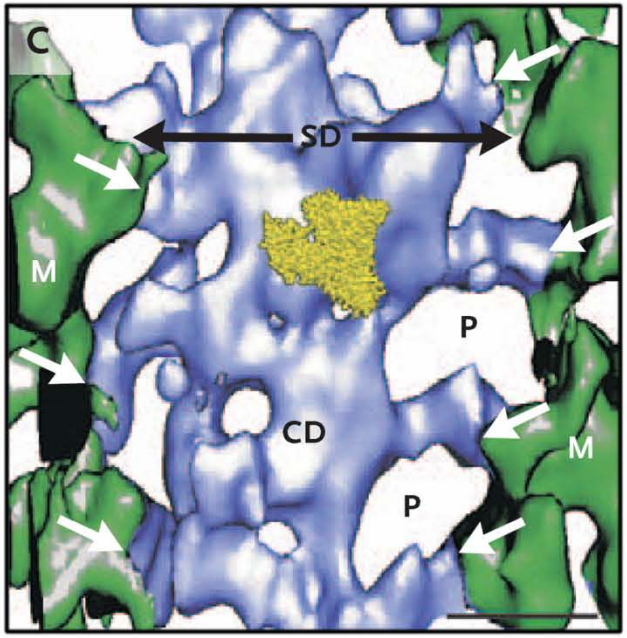
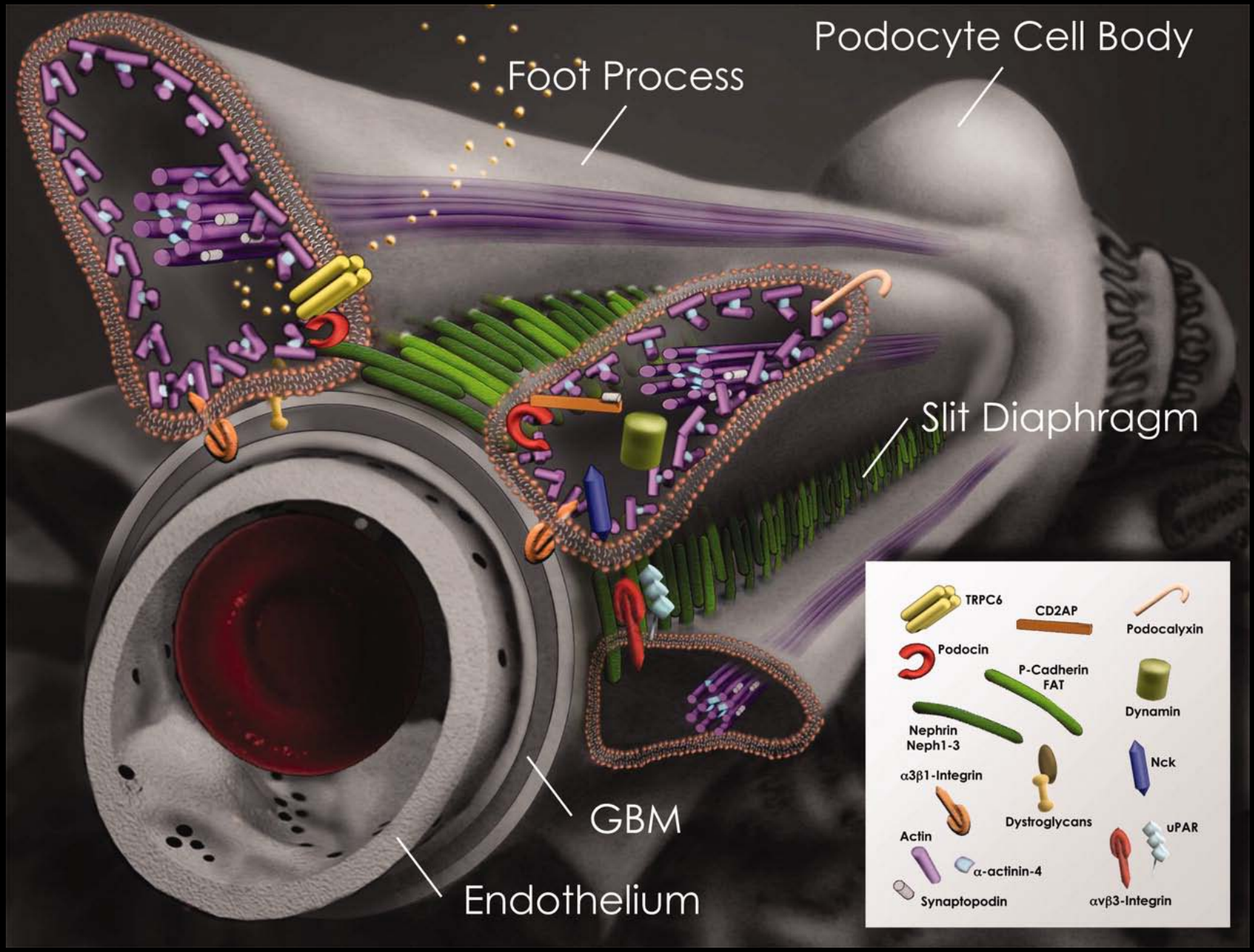

Slit diaphragm
podocyte
foot process
lamina rara
externa
lamina
densa
lamina rara
interna
zipper-like
structure
endothelium
pore

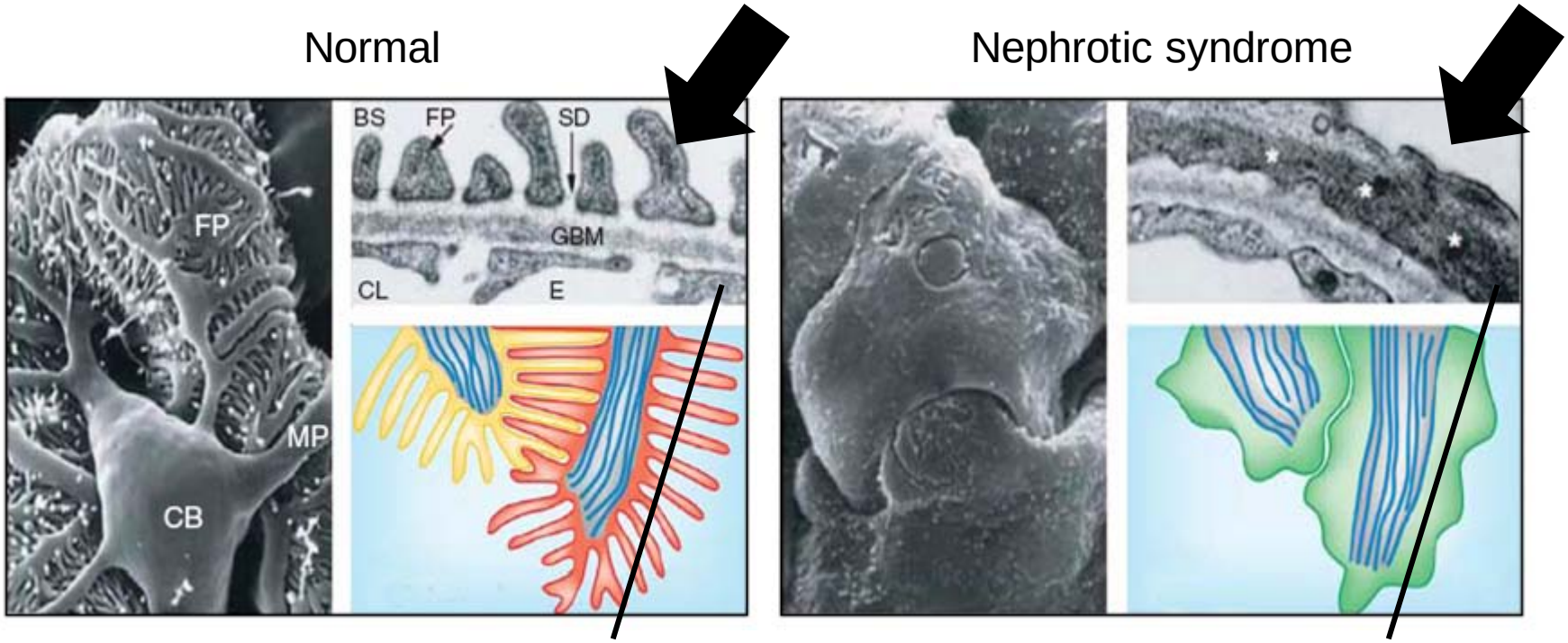

FP
M
C


M
FP

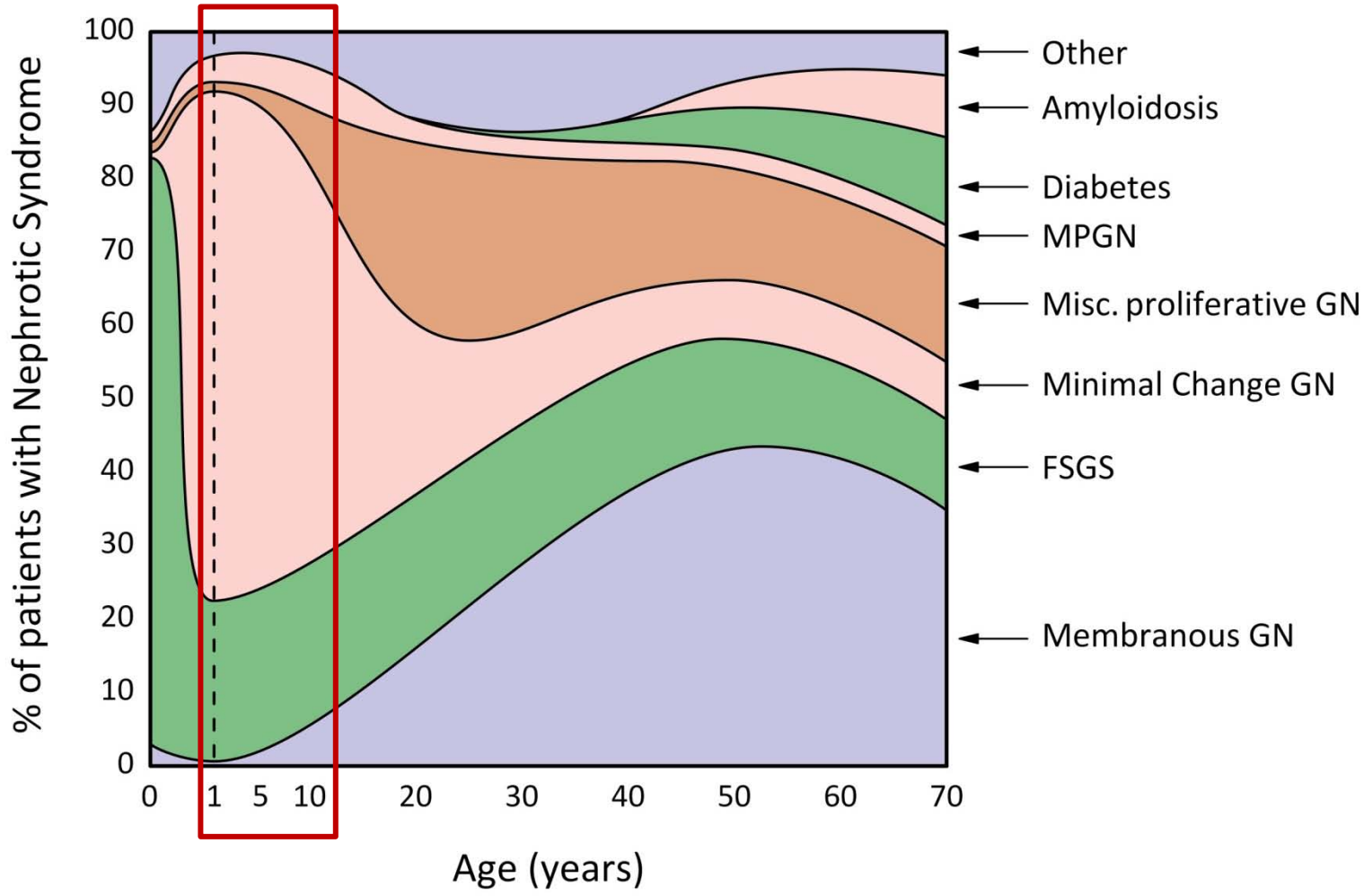

C
SD
M
P
CD
M
P


Podocyte Cell Body
Foot Process
Slit Diaphragm
GBM
Endothelium
TRPC6
CD2AP
Podocalyxin
Podocin
P-Cadherin
FAT
Dynamin
Nephrin
Neph1-3
Nck
α3β1-Integrin
Dystroglycans
uPAR
Actin
α-actinin-4
Synaptopodin
αvβ3-Integrin

Foot processes effacement

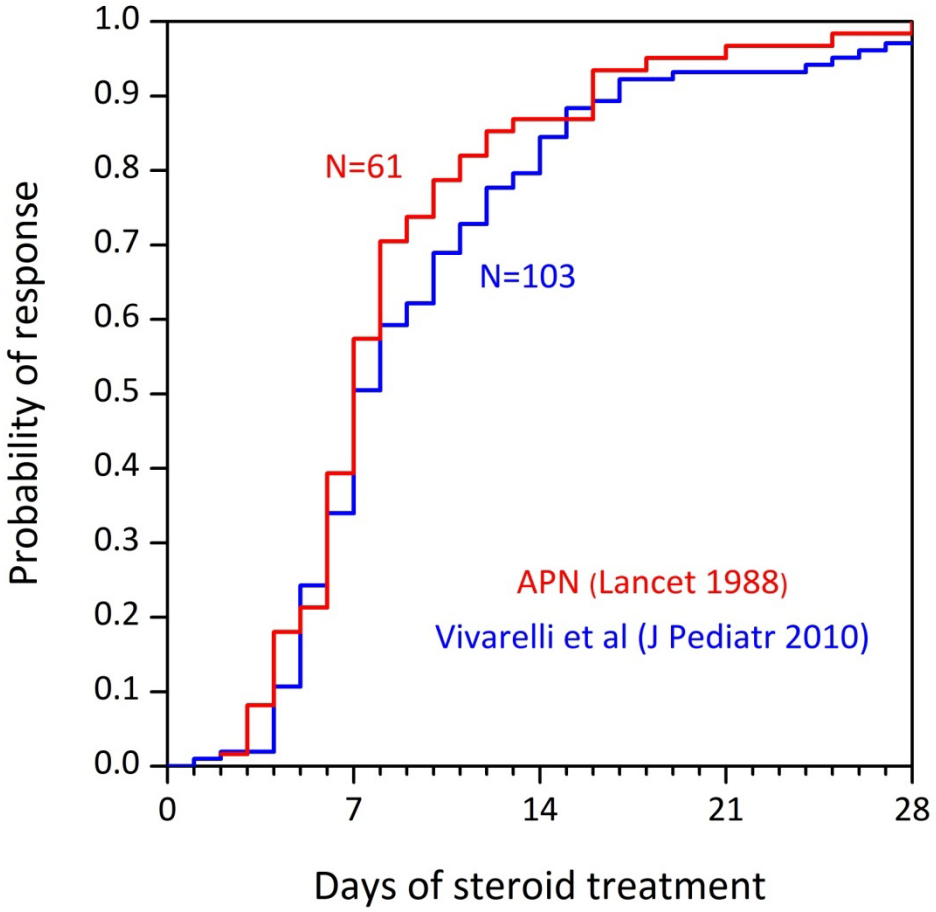


Causes of nephrotic syndrome



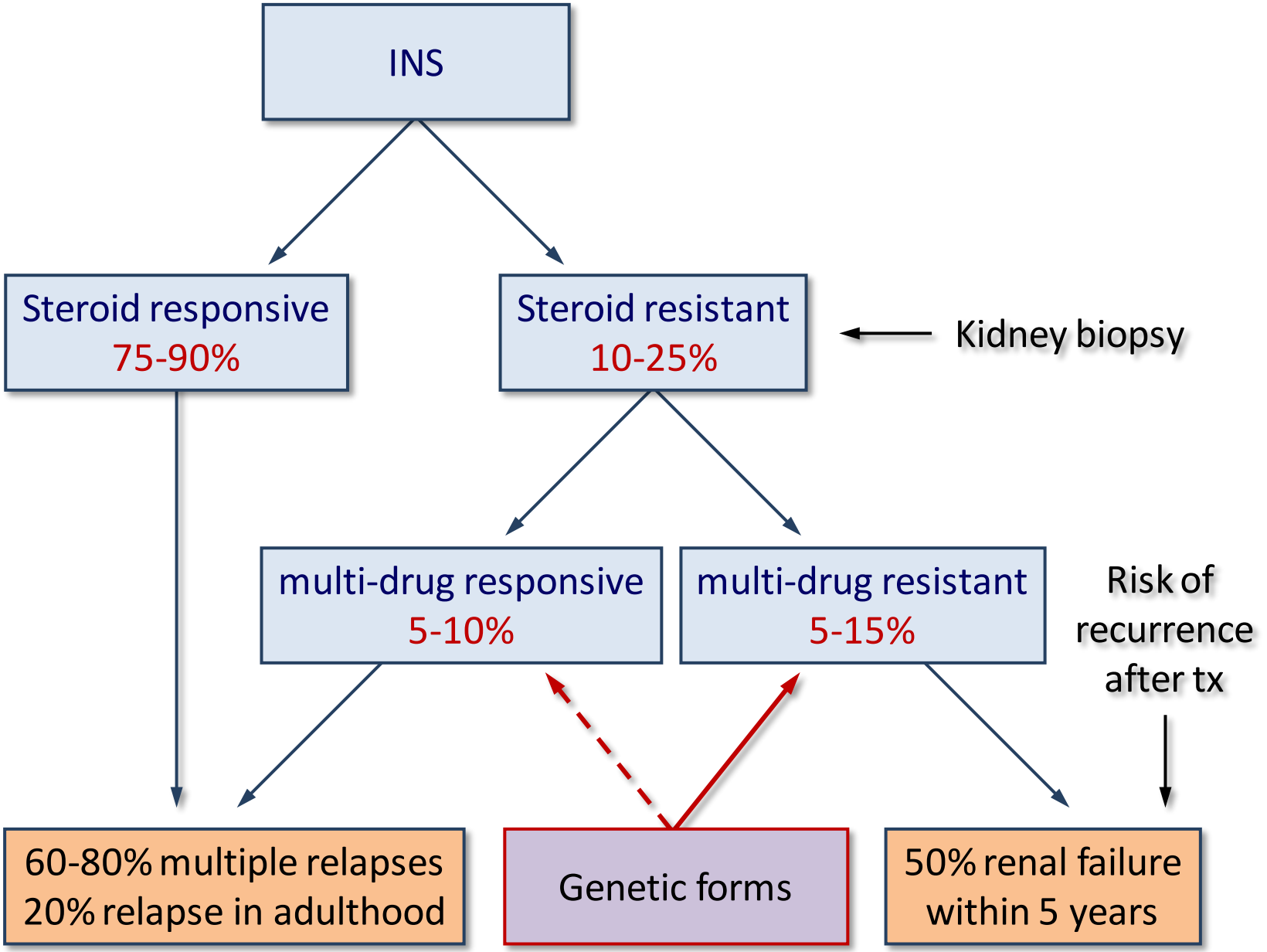
Adapted from Nachman, Jenette and Falk, Brenner & Rector, The kidney, 2008

Definition of steroid resistance in children

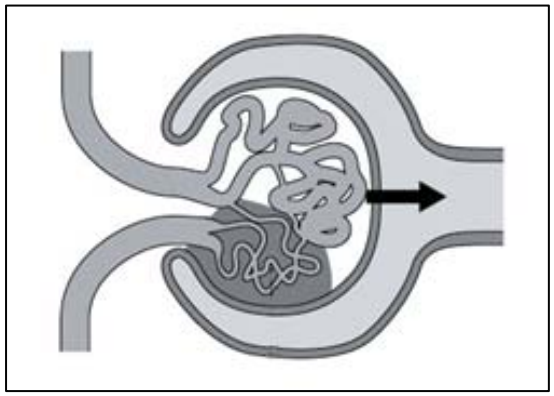


<u>Steroid resistance</u> = non response after 4 wks of PDN 60 mg/m^2 ± IV MP

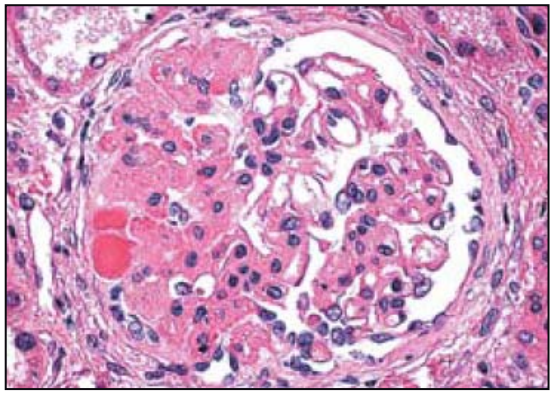
Idiopathic nephrotic syndrome in children



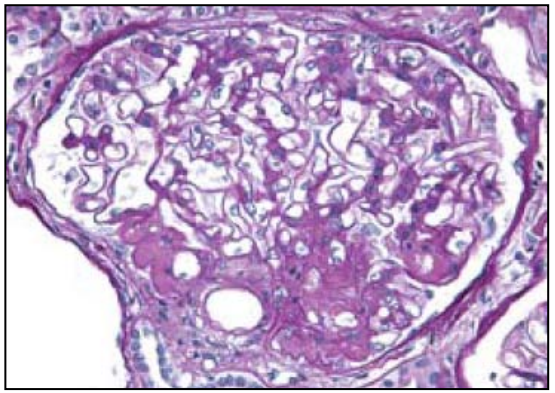
Pathology of FSGS



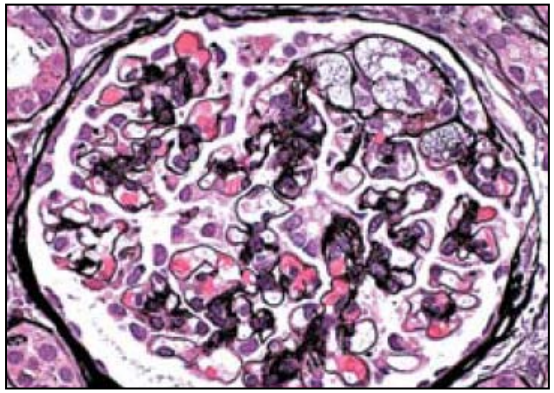
NOS



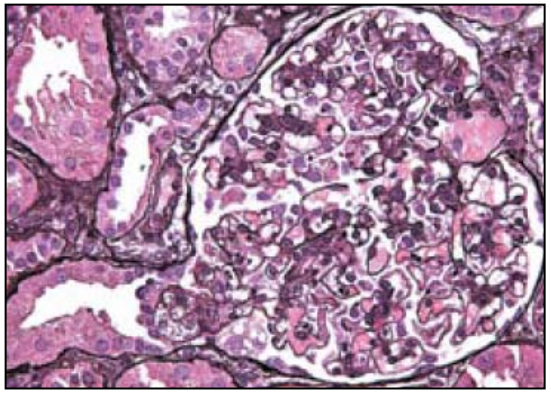
Perihilar



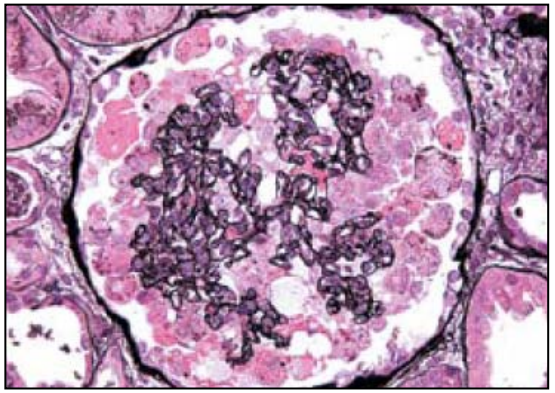
Cellular



Tip lesion



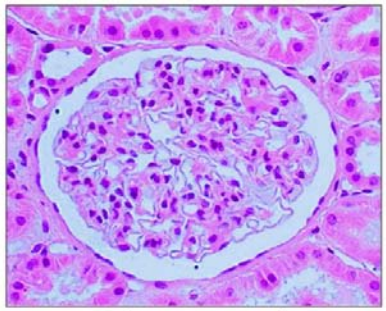
Collapsing



D'Agati et al, NEJM 2011

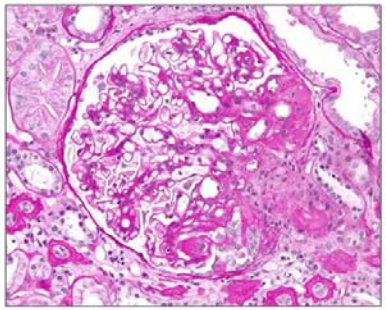
Some rules…

Minimal change disease
MCD



5 days post-transplant

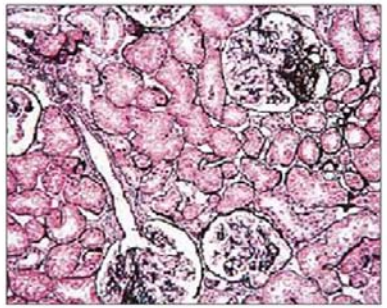
Focal segmental glomerulosclerosis
FSGS



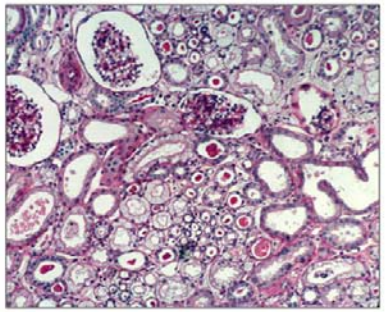
37 days post-transplant

Acute → *Chronic*

FSGS with low grade proteinuria

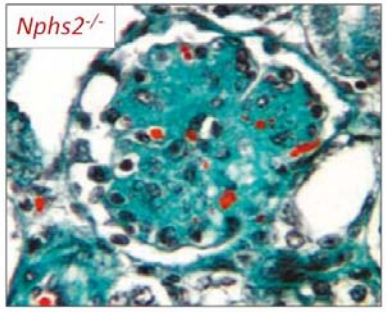


FSGS with massive proteinuria

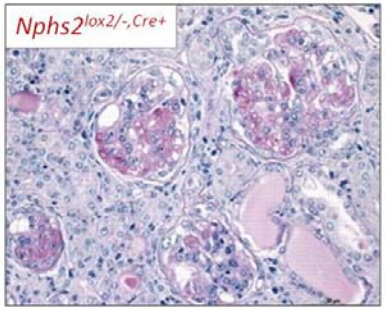


No tubular cysts → *Tubular cysts*

Diffuse mesangial sclerosis
DMS

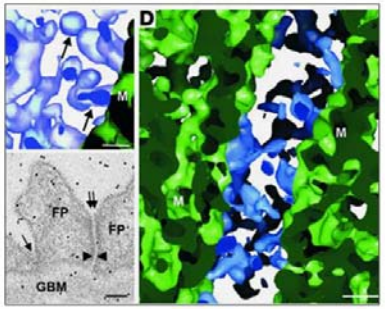


Focal segmental glomerulosclerosis
FSGS

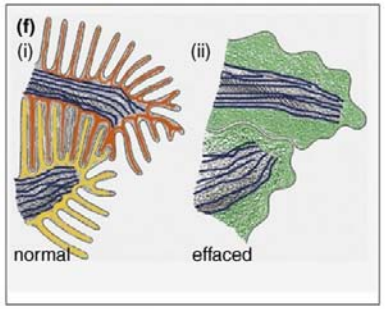


Early onset → *Late onset*

Slit diaphragm, GBM and developmental gene mutations



"Functional" and cytoskeleton gene mutations



RESPONSE TO TREATMENT

No response → *Partial (complete)*

Roselli et al, Mol Cell Biol 2004 - Mollet et al, JASN 2009

Direct effect of calcineurin inhibitors on podocyte cytoskeleton

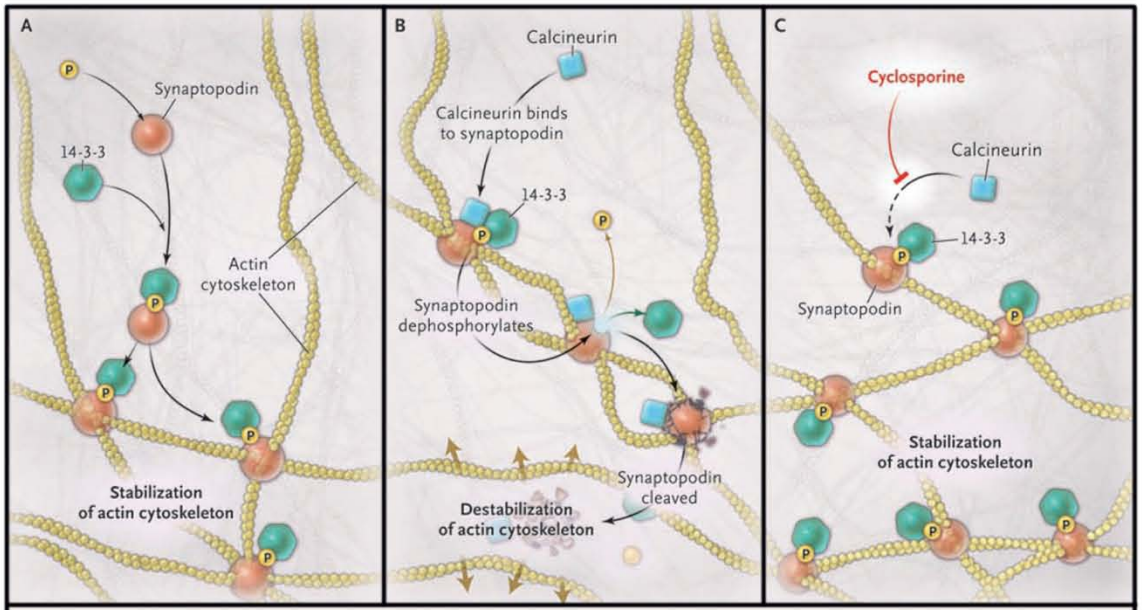
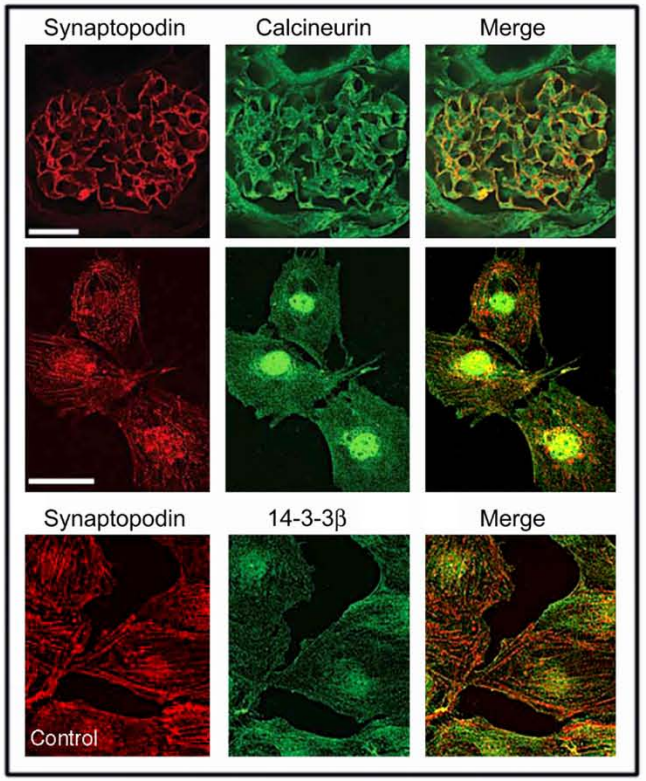


Figure 2. The Effect of Calcineurin on Synaptopodin.

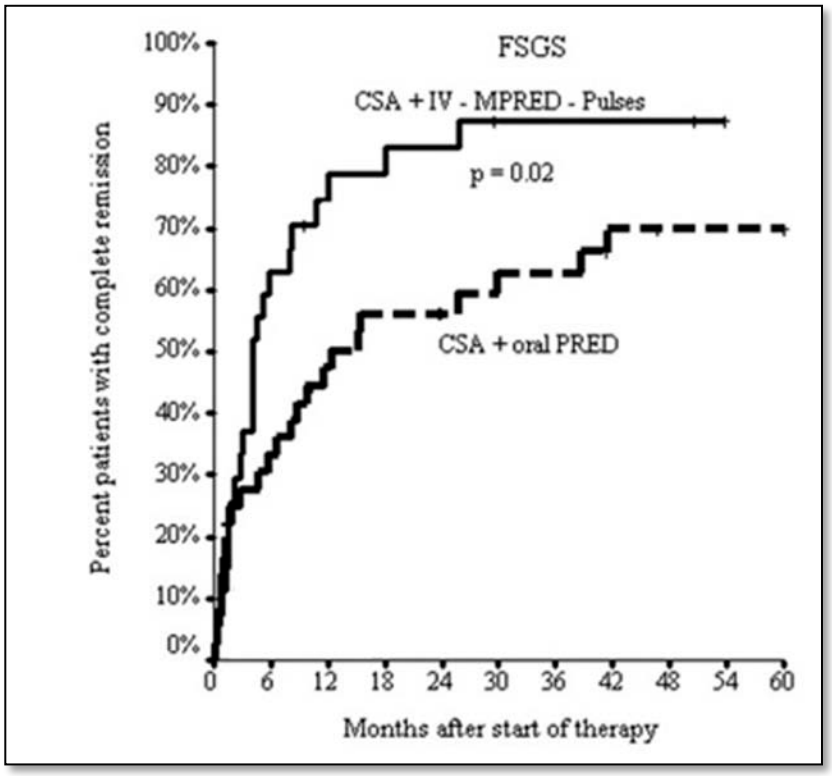
Synaptopodin, when phosphorylated, binds to the 14-3-3 protein and is protected from degradation. Synaptopodin stabilizes the actin cytoskeleton, allowing the podocyte to maintain its shape (Panel A). Calcineurin dephosphorylates synaptopodin, which then separates from 14-3-3 and can be degraded by cathepsin L. Its stabilizing effect on the actin cytoskeleton is lost, and the cell loses its shape (Panel B). Cyclosporin inhibits the action of calcineurin, preventing dephosphorylation of synaptopodin and allowing its actin-stabilizing effect to continue (Panel C). P denotes phosphorylation.

Mathieson, N Engl J Med, 2008



Faul et al. Nature Med, 2008

Response to treatment in childhood SRNS



Ehrich et al, Nephrol Dial Transpl 2007

Rome: 48 patients with SRNS since 2004

- ~ 30-40% CR with MP+CsA
- 6 *NPHS2*
  3 *NPHS1*
  3 *WT1*
  2 *COQ2*
  2 *SMARCAL1*
  1 *LAMB2*
  1 *tRNA^{LEU}*

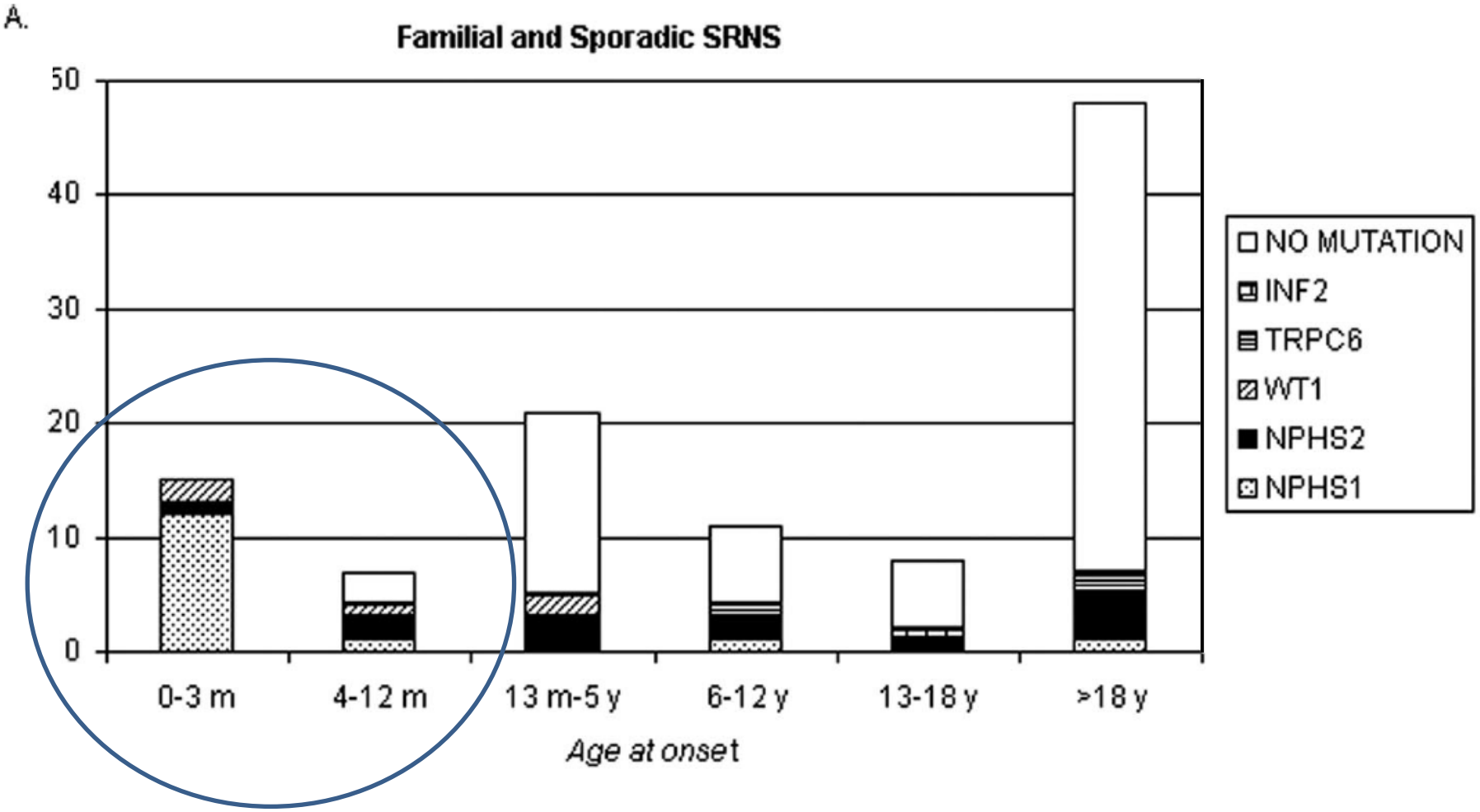
  none achieved CR

✓ *Children with genetic forms of SRNS often present with less overt proteinuria*

Genetics of SRNS

Gene	Chromosome	Protein name	FSGS	Collapsing GN	DMS	MCD	Kidney / Syndromic	Inheritance
Slit diaphragm complex								
NPHS1	19q13.1	Nephrin	+		+		Kidney	AR
NPHS2	1q25.2	Podocin	+		+	+	Kidney	AR
CD2AP	6p12	CD2 associated protein	+				Kidney	AR
Actinomyosin complex								
ACTN4	19q13	Alpha-actinin 4	+				Kidney	AD
MYH9	22q13.1	Non-muscle myosin IIA heavy chain	+	+			Kidney	ND
Cell signaling								
TRPC6	11q21.22	Transient receptor potential cation channel C6	+				Kidney	AD
PLCE1	10q23	Phospholipase epsilon 1			+		Kidney	AR
Membrane repair and turnover and vesicle function								
DYSF	2p13.2–13.1	Dysferlin				+	Syndromic	AR
GLA	Xq22	Alpha galactosidase	+				Syndromic	X linked
SCARB2	4q21.1	Scavenger receptor class B, member 2		+			Syndromic	AR
Transcription factors								
WT1	11p13	Wilms tumor 1	+		+		Both	Sporadic
PAX2	10q24	Paired homeobox 2	+				Syndromic	AR
LMX1B	9q34	LIM homeobox domain transcription factor 1	+				Syndromic	AR
Extracellular matrix and receptors								
LAMB2	3p21	Laminin Beta 2			+		Syndromic	AR
ITGB4	17q25	Integrin beta 4	+				Syndromic	AR
COL4A3	2q36–37	Collagen 4A3					Both	AR
COL4A4	2q36–37	Collagen 4A4					Both	AR
COL4A5	Xq22.3	Collagen4A5					Both	X-linked
Mitochondrial function								
mtDNA tRNA ^{leu}	mtDNA	Not applicable	+				Both	Maternal
COQ2	4q21.23	Co-enzyme Q enzyme 2	+	+			Both	AR
COQ6	14q24.3	Co-enzyme Q enzyme 6	+				Both	AR

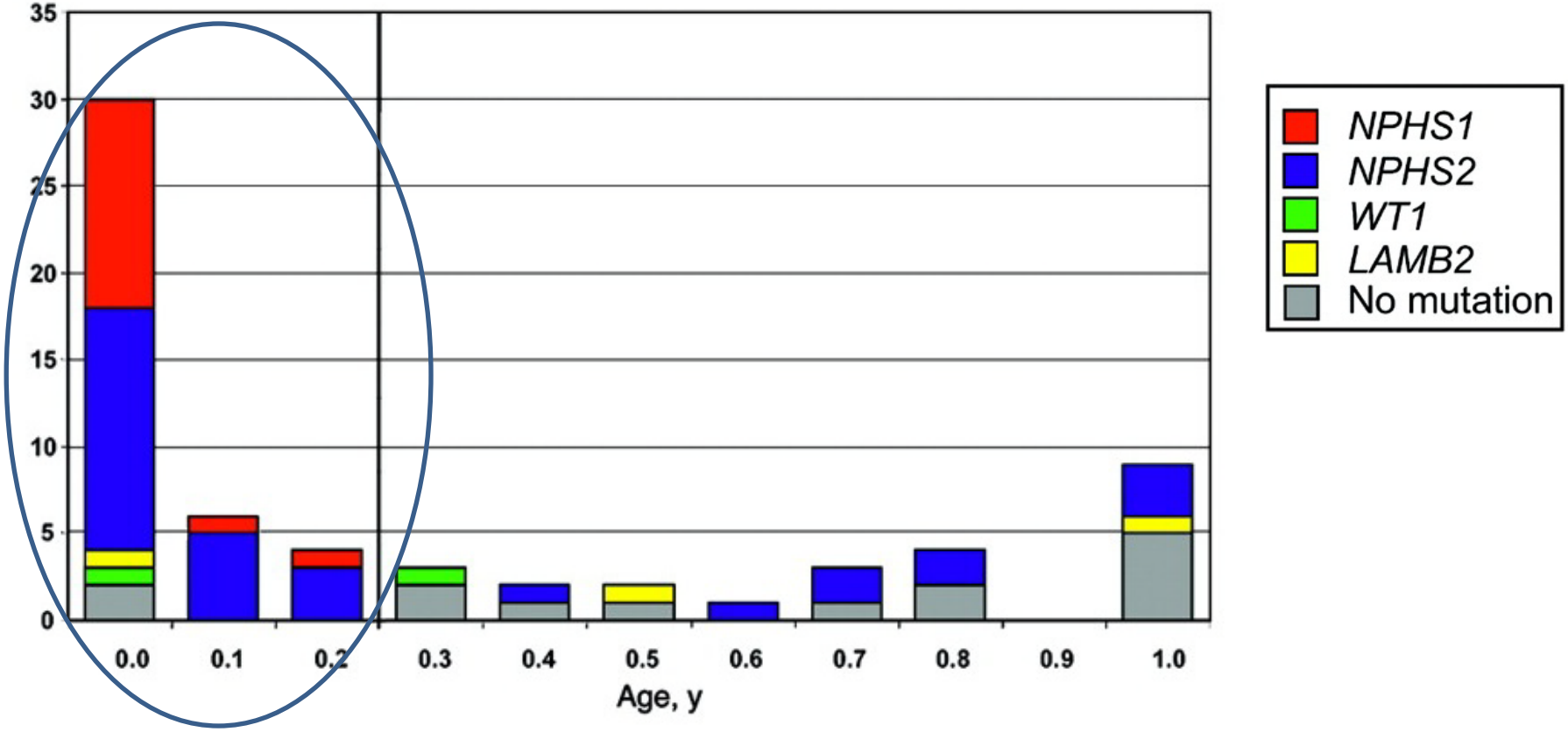
Gene mutations in SRNS



Most SRNS that begin before 1 year have an identifiable genetic cause …

Santin et al, Clin J Am Soc Nephrol, 2011

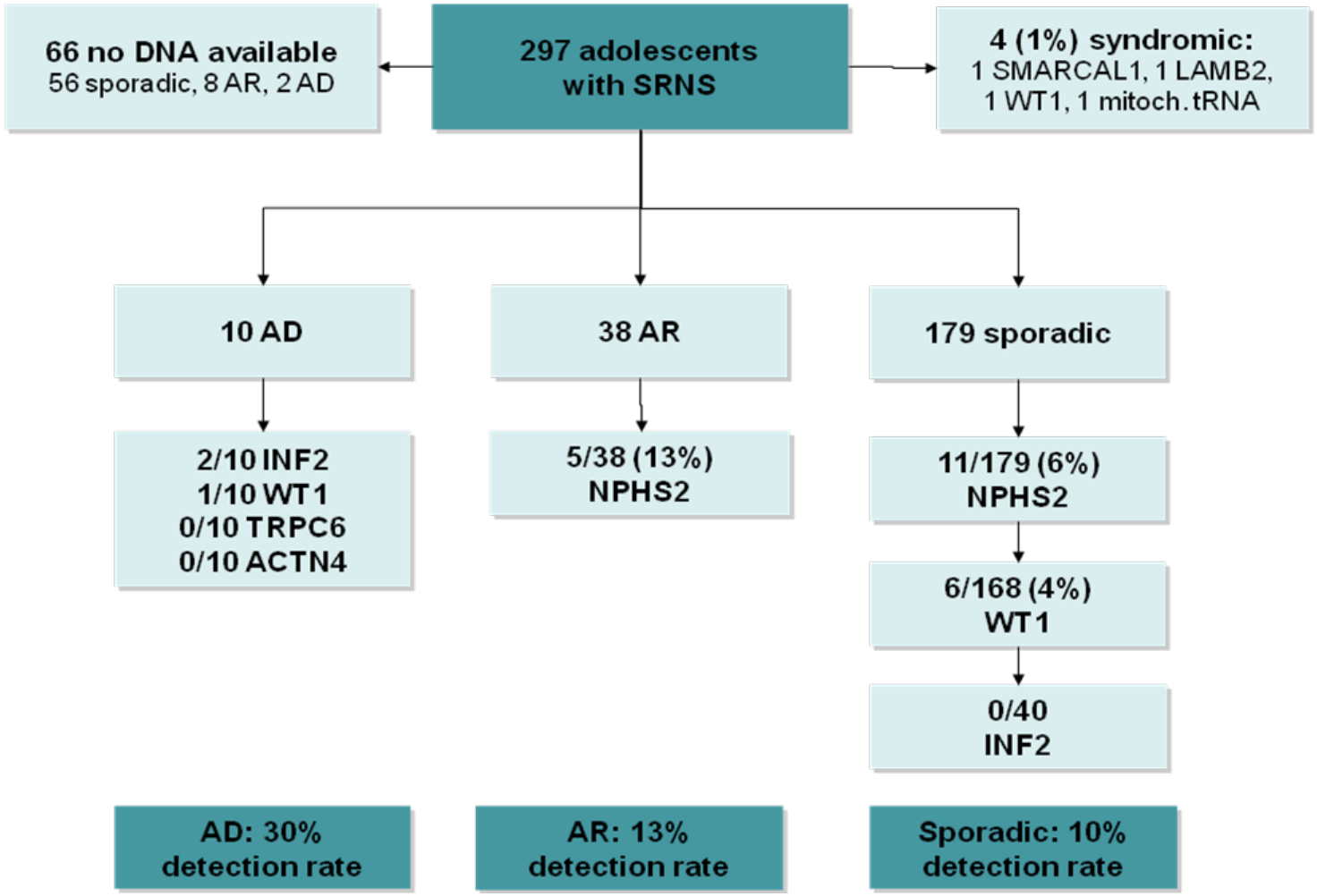
Gene mutations in NS during the 1st year of life



… especially if "congenital" (i.e. < 3 months)

Hinkes BG et al, Pediatrics 2007

Genetic Screening in Adolescents with SRNS



Lipska et al, Podonet consortium, KI 2013

Gene mutations in NS during the 1st year of life

Pediatr Nephrol (2011) 26:1897–1901
DOI 10.1007/s00467-011-1911-0

BRIEF REPORT

Nephrotic syndrome in infancy can spontaneously resolve

Jon Jin Kim · Joanna Clothier · Neil J. Sebire · David V. Milford · Nadeem Moghal · Richard S. Trompeter

4 patients with onset of NS at 0.5, 2.5, 4 and 7 months

SRNS in ubiquinone biosynthesis defects

Gene	Number of patients	Renal phenotype	Other features	Response to therapy
COQ1-PDSS1	2	No	Multisystem disorder	±
COQ1-PDSS2	1	SRNS	Progressive encephalomyopathy	+++
COQ2	6	SRNS	Progressive encephalomyopathy with liver failure	+++
COQ6	10	SRNS	Deafness seizures	+++
COQ7	-	?	(Mouse knock-out is lethal)	?
COQ8-ADCK3	>10	No	Cerebellar ataxia	±
COQ9	1	Tubulopathy	Lactic acidosis, encephalomyopathy	±

Emma et al, Pediatr Nephrol, 2011

Possible undiagnosed cases that could benefit from early treatment

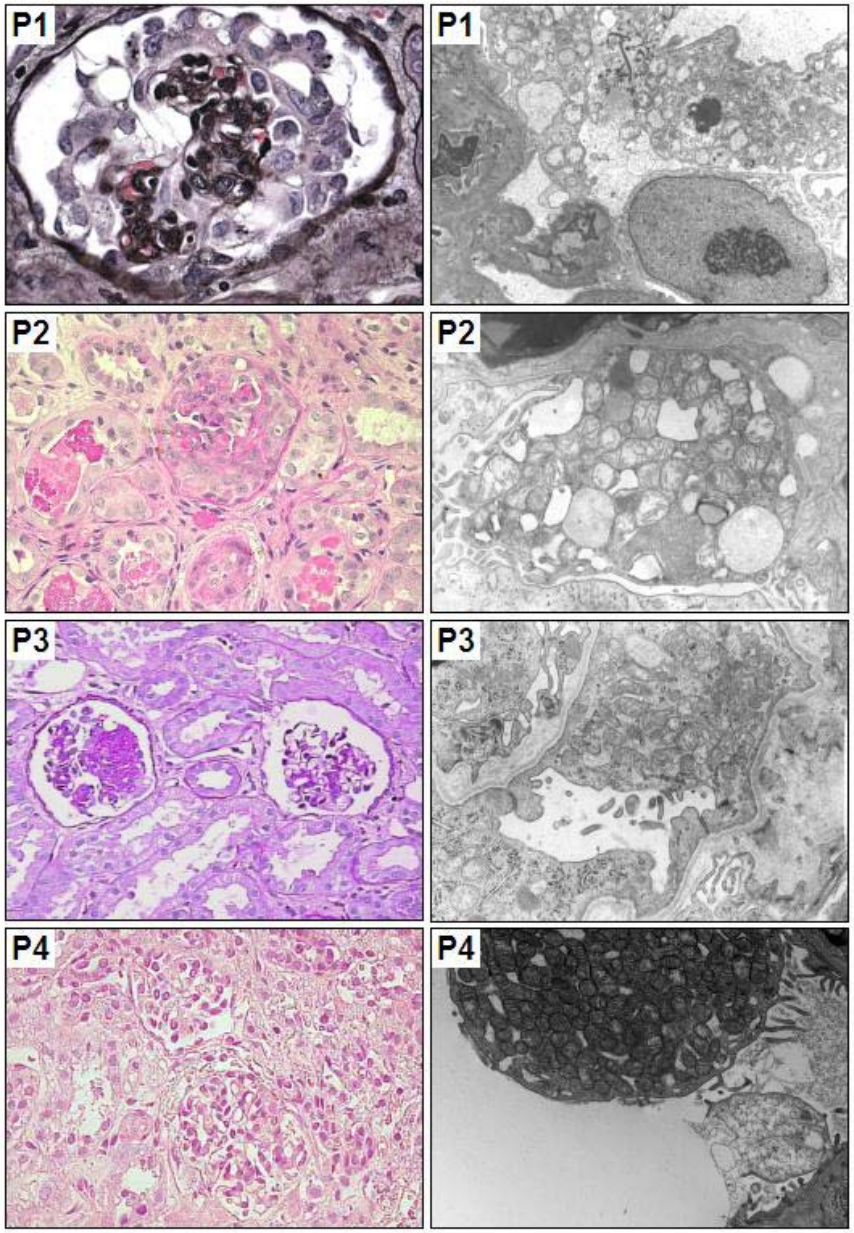
Simultaneous Sequencing of 24 Genes Associated with Steroid-Resistant Nephrotic Syndrome

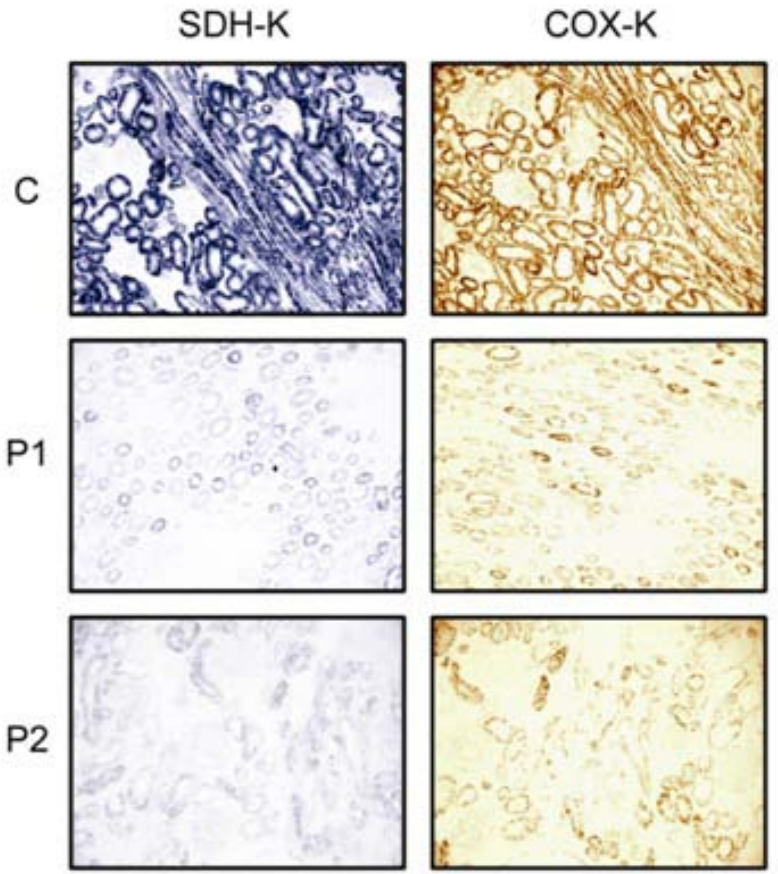
Hugh J. McCarthy, Agnieszka Bierzynska, Matt Wherlock, Milos Ognjanovic, Larissa Kerecuk, Shivaram Hegde, Sally Feather, Rodney D. Gilbert, Leah Krischock, Caroline Jones, Manish D. Sinha, Nicholas J.A. Webb, Martin Christian, Margaret M. Williams, Stephen Marks, Ania Koziell, Gavin I. Welsh, and Moin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

Clin J Am Soc Nephrol 8: 637–648, 2013.

- NGS in 36 patients with SRNS.
- Compound heterozygous mutation in *COQ2* in 1 patient with isolated SRNS at 2 years of age, rapidly evolving into ESRD (c.683 A>G, c.701delT).

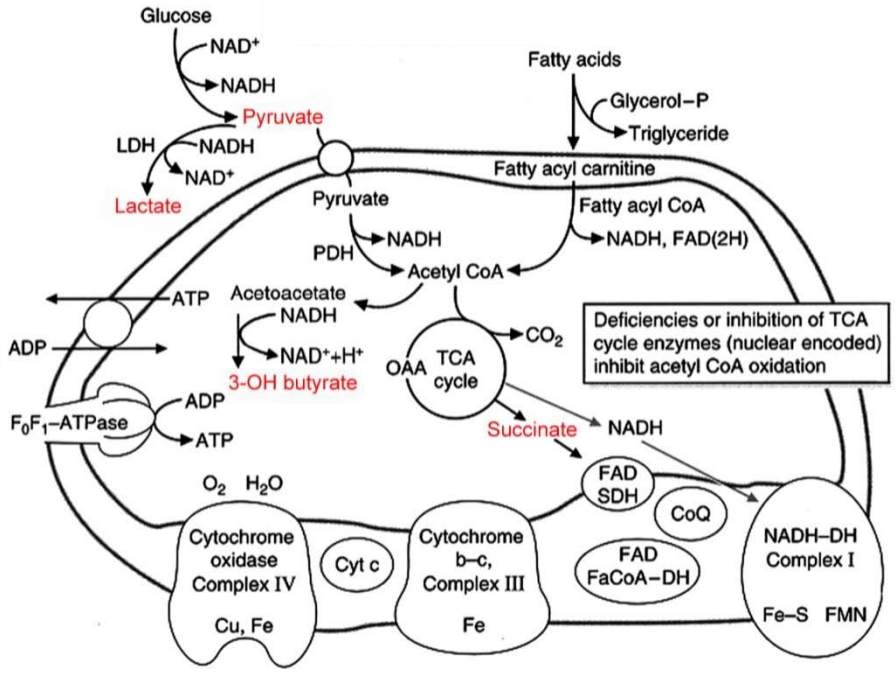
CoenzymeQ10 synthesis defects in early onset SRNS

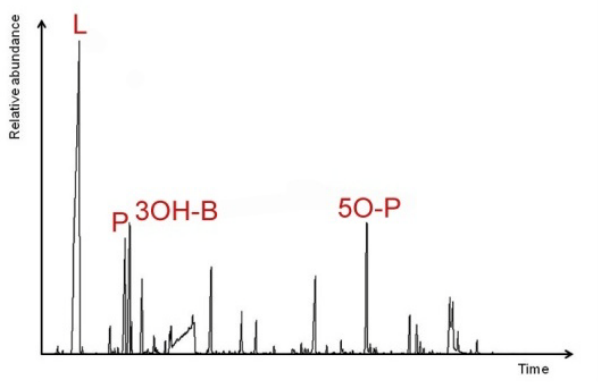


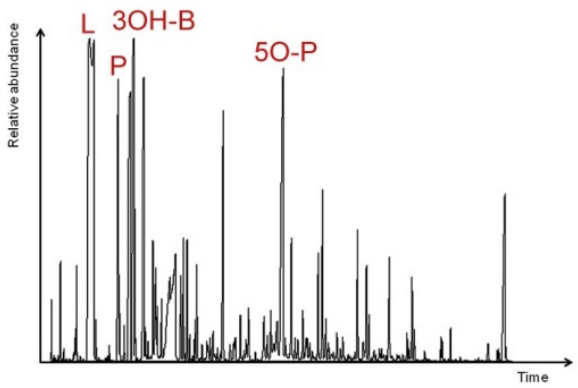


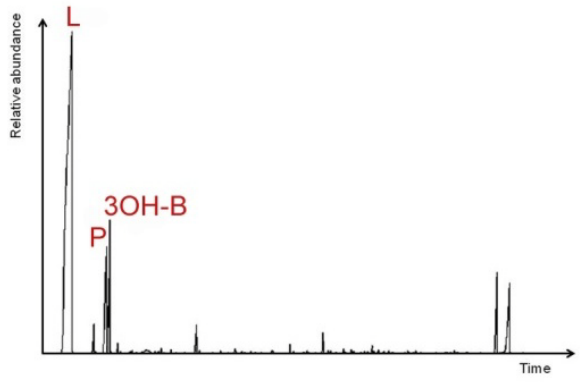
Diomedi-Camassei et al. JASN, 2007

Urinary organic acids in mitochondrial SRNS

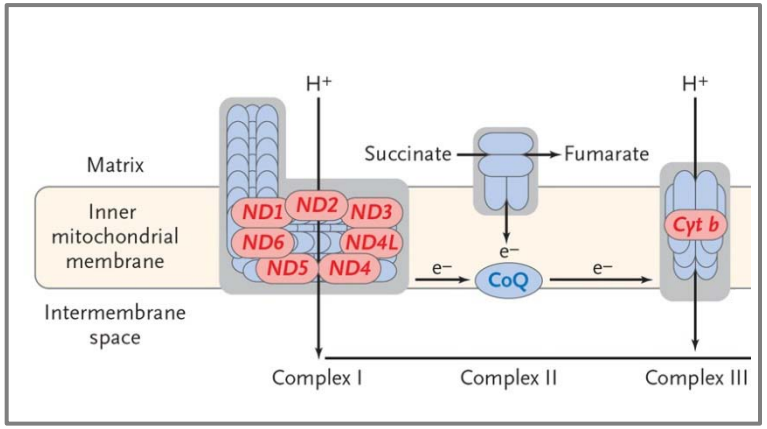


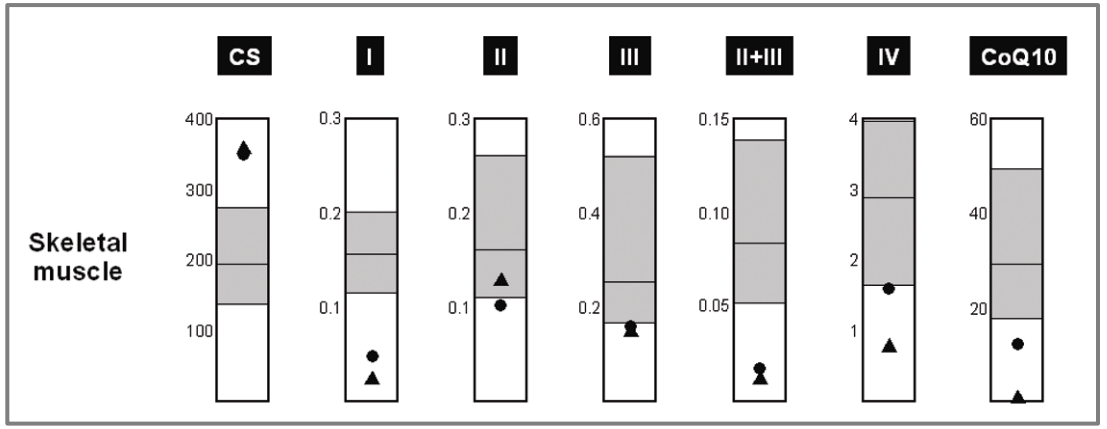






CoenzymeQ10 treatment of SRNS





Diomedi-Camassei et al. JASN, 2007

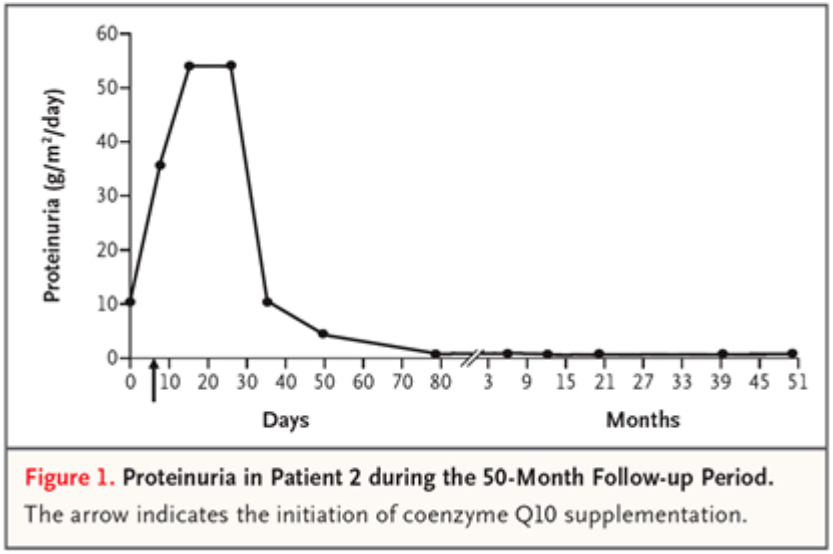
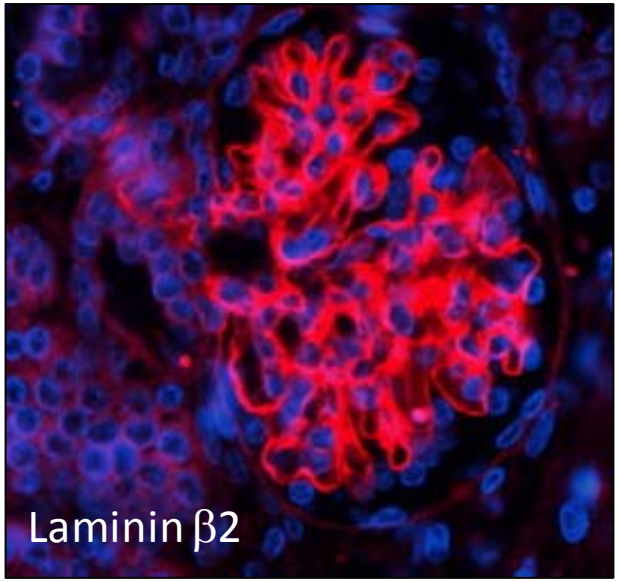
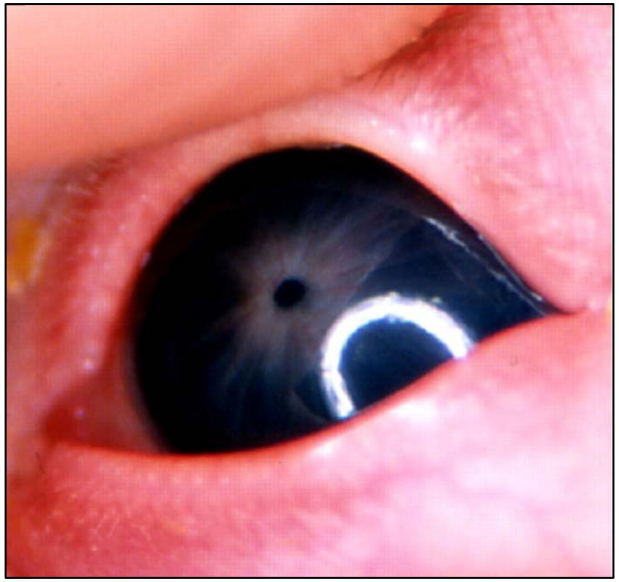


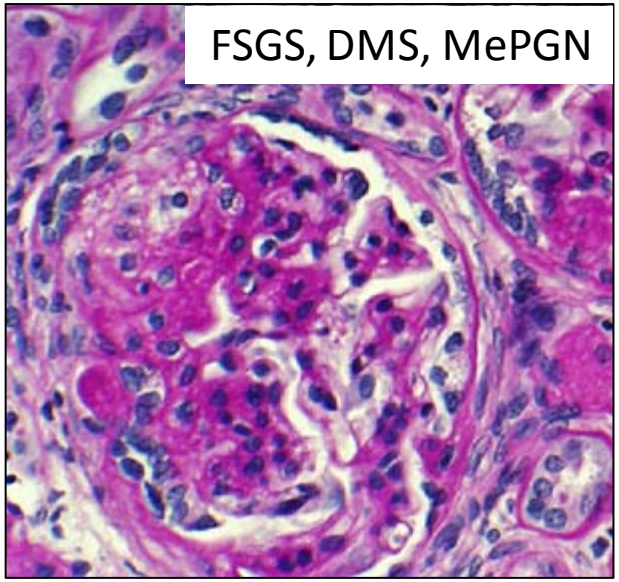
Figure 1. Proteinuria in Patient 2 during the 50-Month Follow-up Period.
The arrow indicates the initiation of coenzyme Q10 supplementation.

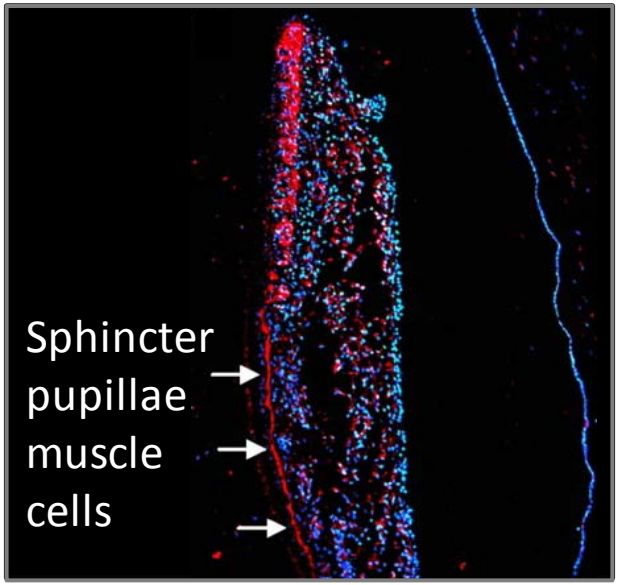
Montini et al. NEJM , 2008

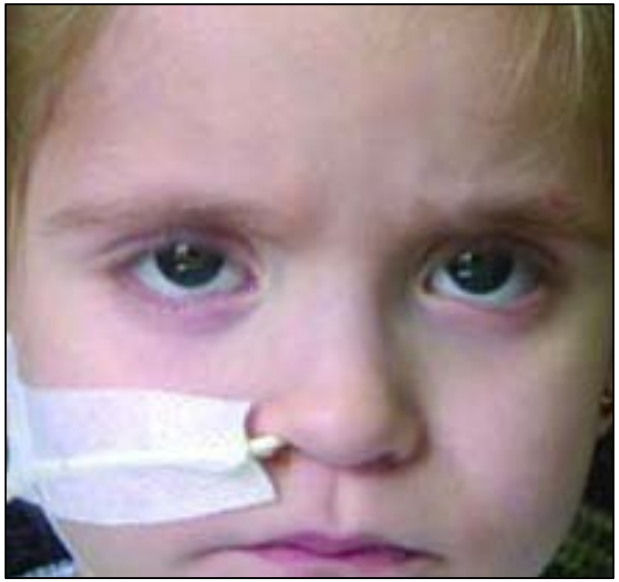
Pierson syndrome (microcoria-congenital nephrosis) syndrome (LAMB2)

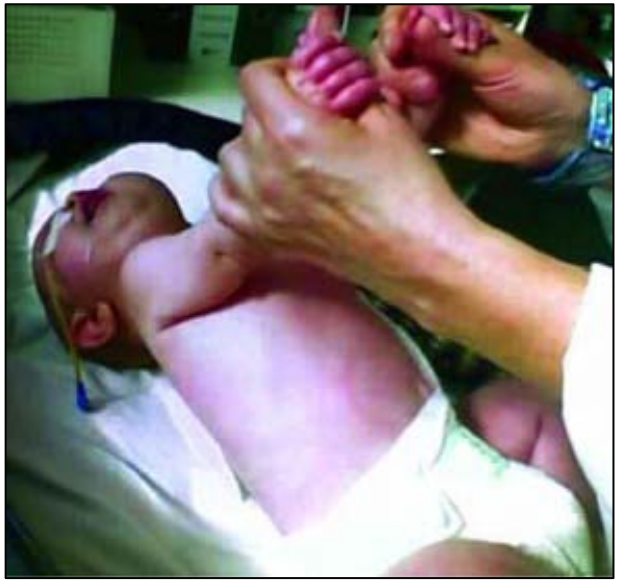




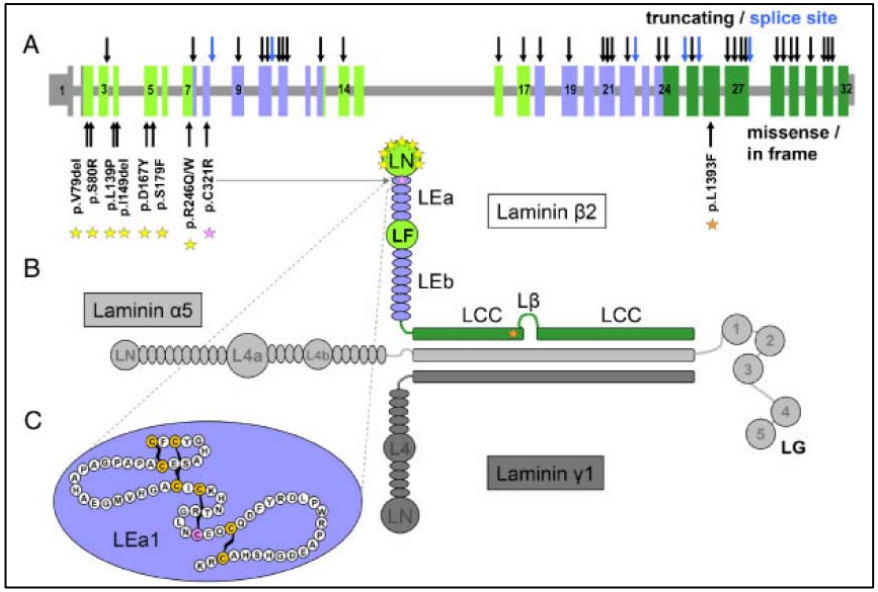




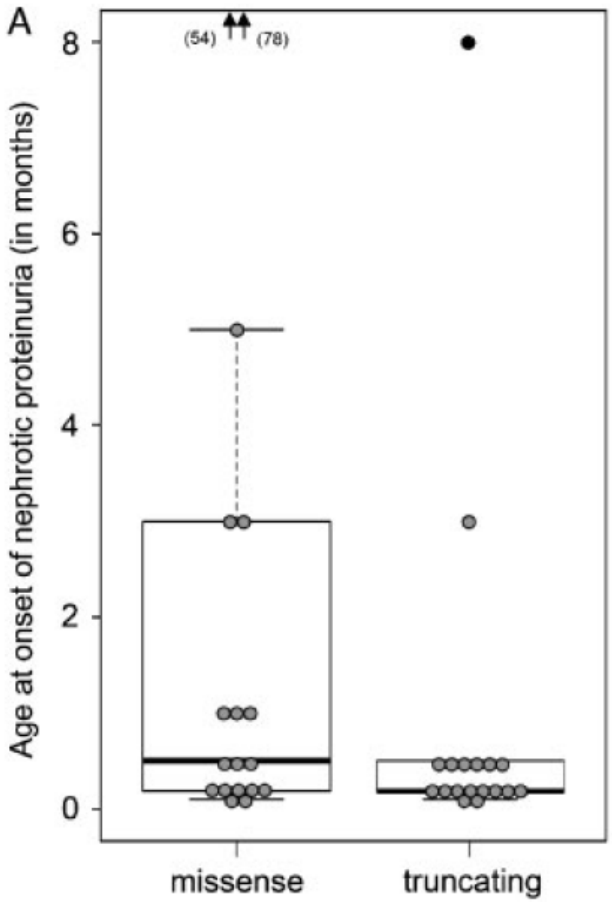




Age of onset of NS in LAMB2 mutations

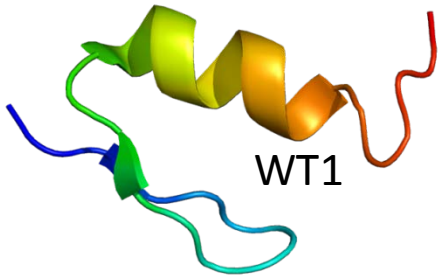


34/49	microcoria
25/47	visual imapirment
19/47	cataract or lenticonus
13/47	retinal detachment
13/47	nystagmus
9/47	microphtalmia
7/47	severe myopia



Matejas et al, Hum Mut, 2010

Denys-Drash and Frasier syndromes

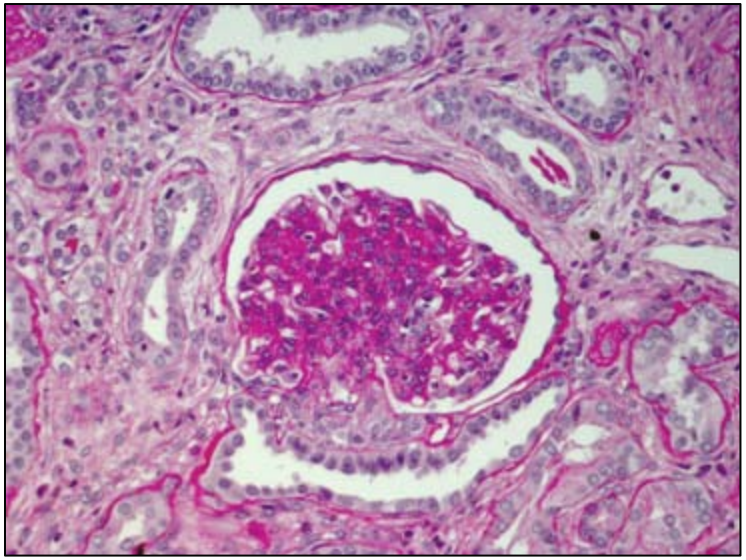


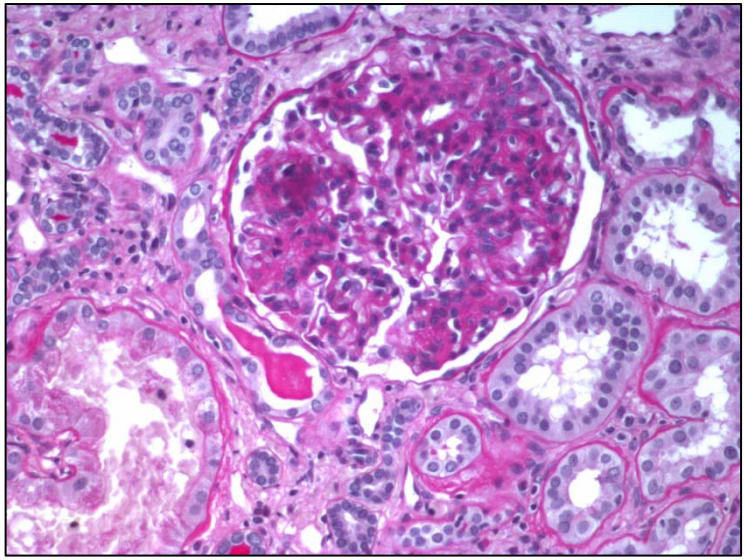
→Always check the karyotype of young girls with SRNS

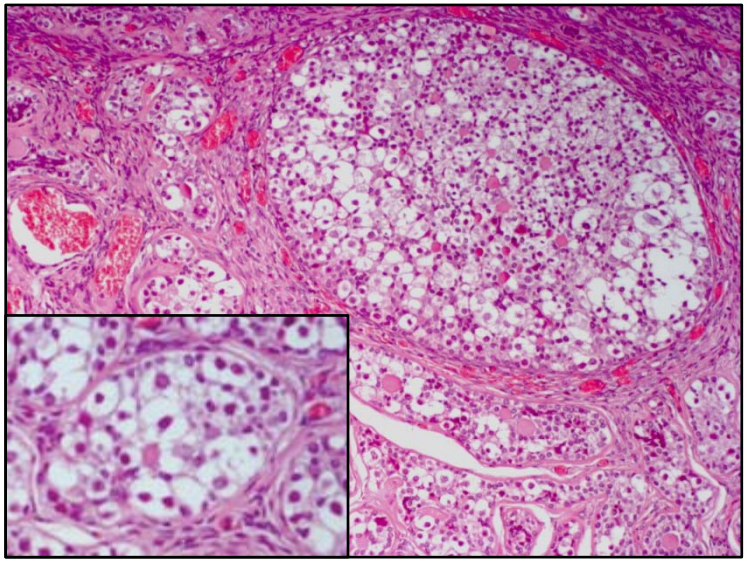
	Denys-Drash	Frasier
Onset of NS	< 2 years	2-6 years
Progression to ESRD	rapid	slow
Histology	DMS	FSGS>DMS
Gender	male pseudo-hermaphroditism	
Other	Wilms tumor	primary amenorrhea gonadal dysgenesis risk of gonadoblastoma
WT1 mutation	nearly all patients: - germline mutations - mutations in exon 8 and 9	donor splice site in intron 9 resulting in the loss of the +KTS isoform

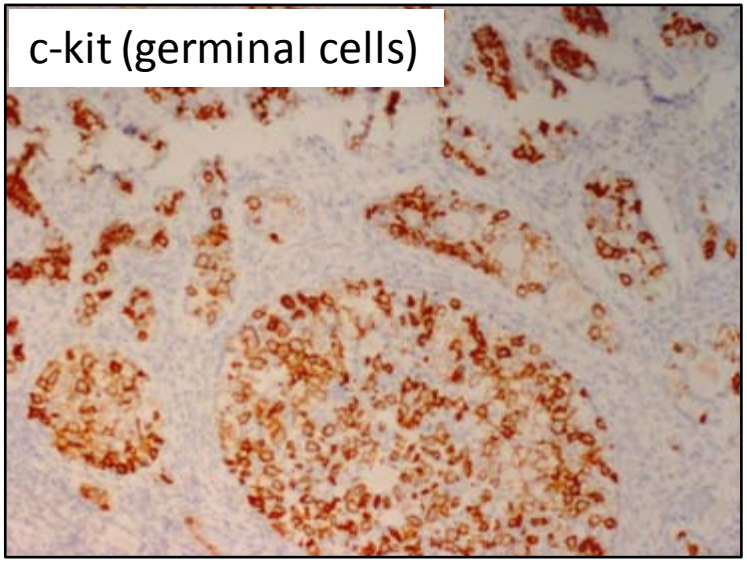
Frasier syndrome

2.2 y/o girl with SRNS – karyotype: 46XY









Denys-Drash and Frasier syndromes

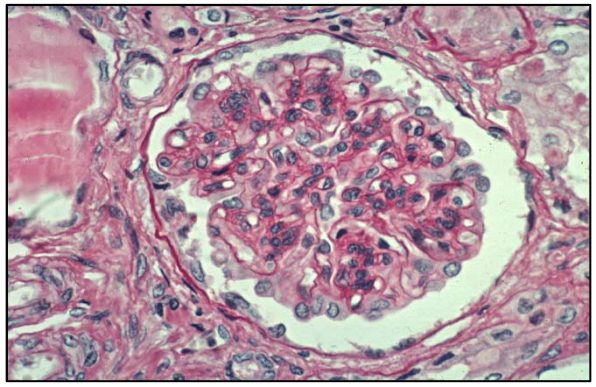
Screening for WT1 mutations in 114 children with FSGS and SRNS (neg for NPHS2)

- 3/32 girls classical WT1 splice mutation (Frasier syndrome)
  (2 patients 46 XY, 1 patient 46 XX)
- 1/32 girl D396N exon 9 (Denys-Drash syndrome)

Aucella F et al. Pediatr Nephrol. 2006

PLCE1 (NPHS3) mutations in early-onset NS

- Phospholipase C ε1
- Hydrolysis of membrane phospholipids → inositol triphosphate (IP3)
  → diacylglycerol
- Localization in the podocyte
  Interaction between PLCε1 and IQGAP1 (which interacts with nephrin)
- Mutations found in:
  ~30% DMS
  ~8% early onset SRNS with FSGS
  (only in familial cases)
- High phenotypic variability (protective effect from other phopholipases?)



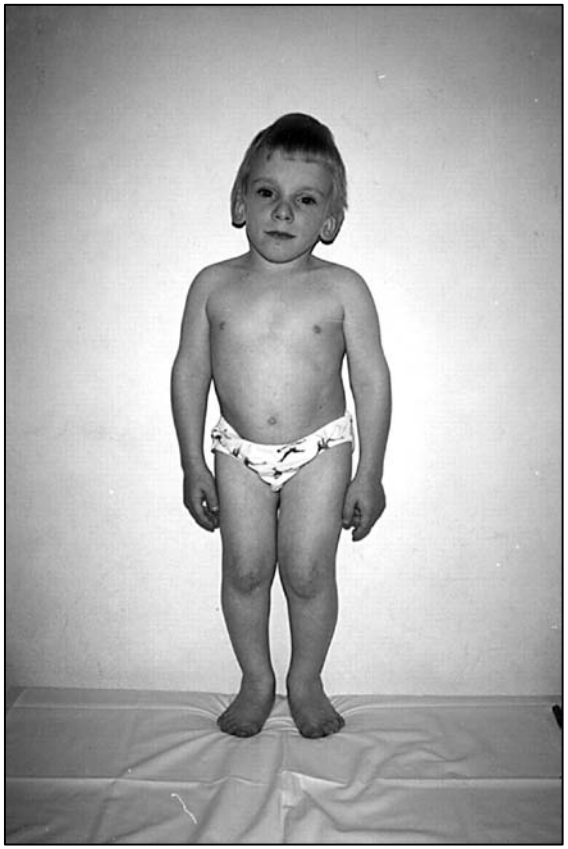
Diffuse Mesangial Sclerosis (DMS)

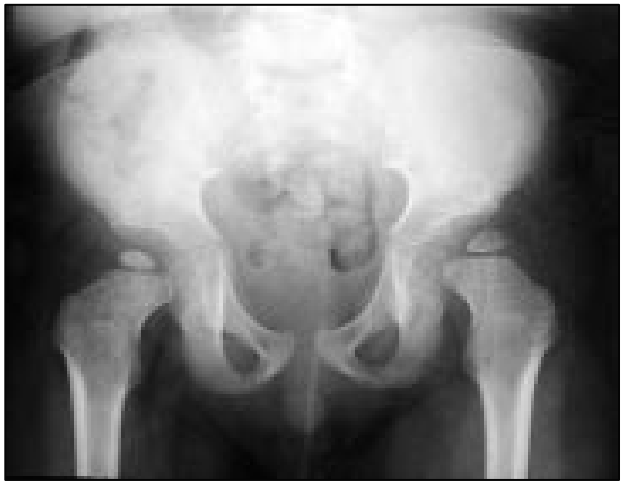
Courtesy C. Antignac

Schimke-Immuno-Osseous Dysplasia [*SMARCAL1* - MIM 242900]

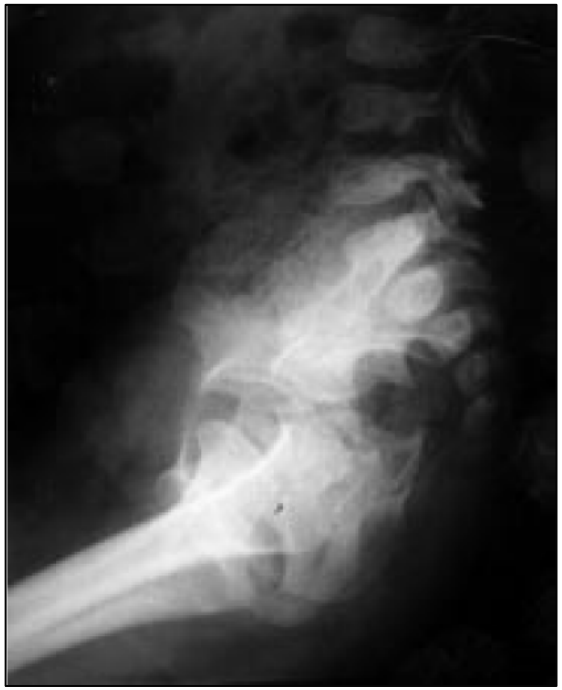
- AR mutations of the chromatin remodeling protein SMARCAL1
- Nephrotic syndrome with FSGS and progressive renal failure
- Recurrent lymphopenia , defective cellular immunity, chronic diarrhea, autoimmune thrombocytopenia, polyneuropathy, severe hypertension…
- Severe infantile form: dystrophia at birth, early renal insufficiency, neurologic complications (transient ischemic attacks , cerebral infarctions)
- Milder form: FSGS with short stature

Schimke-Immuno-Osseous Dysplasia (SIOD) [MIM 242900]





Small iliac wings and small ossification centres of the capital femoral epiphyses, laterally placed, with mild hip subluxation.

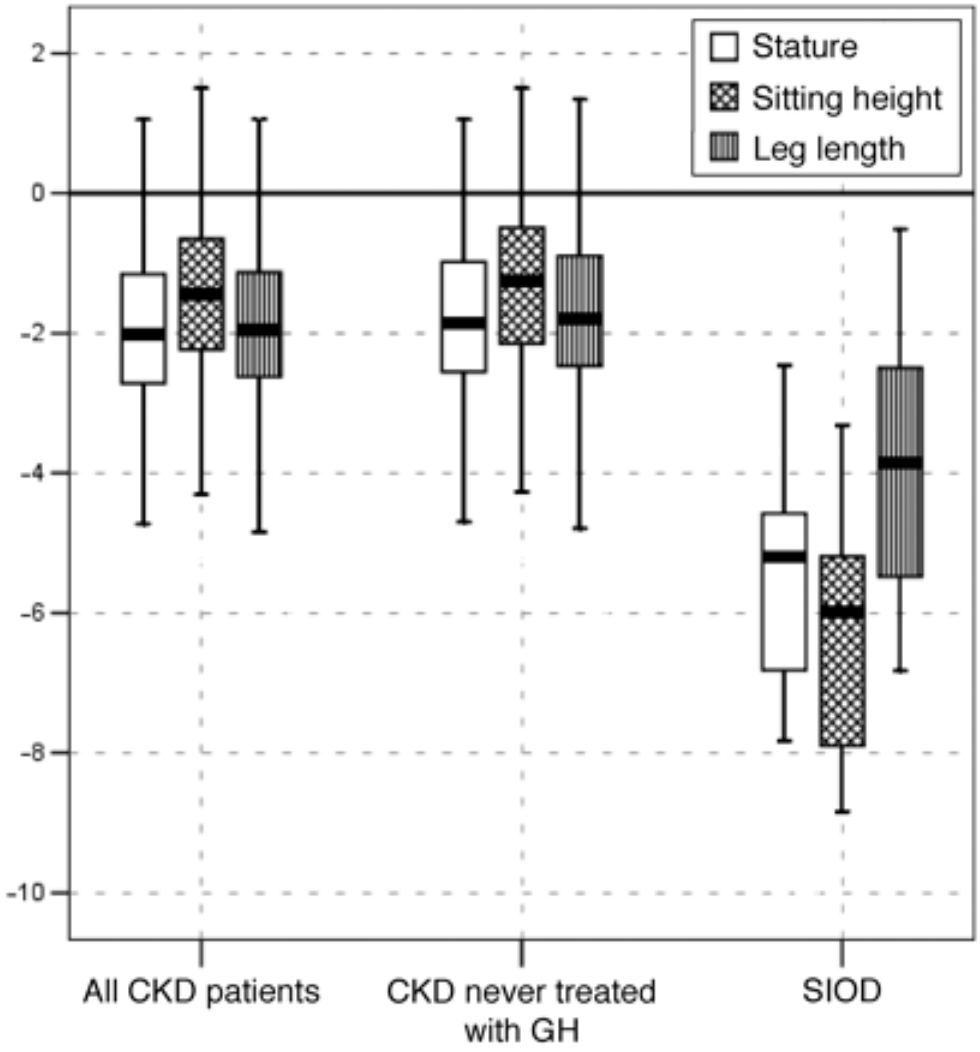


Platyspondyly with markedly short and rounded vertebral bodies.

- Spondyloepiphyseal dysplasia with disproportionate growth failure (ovoid and dorsally flat vertebrae, hypoplastic pelvis, dental abnormalities)
- Triangular face, short neck and trunk, lumbar lordosis, and protruding abdomen

Saraiva et al, J Med Genet

Schimke-Immuno-Osseous Dysplasia (SIOD) [MIM 242900]



Lücke el al, Pediatrics 2006

Inherited forms of SRNS: when should we suspect them?

A: in all congenital NS and SRNS

A: positive family history

A: extrarenal symptoms

(genitalia, ocular, neurological, skeletal malformation, deafness)

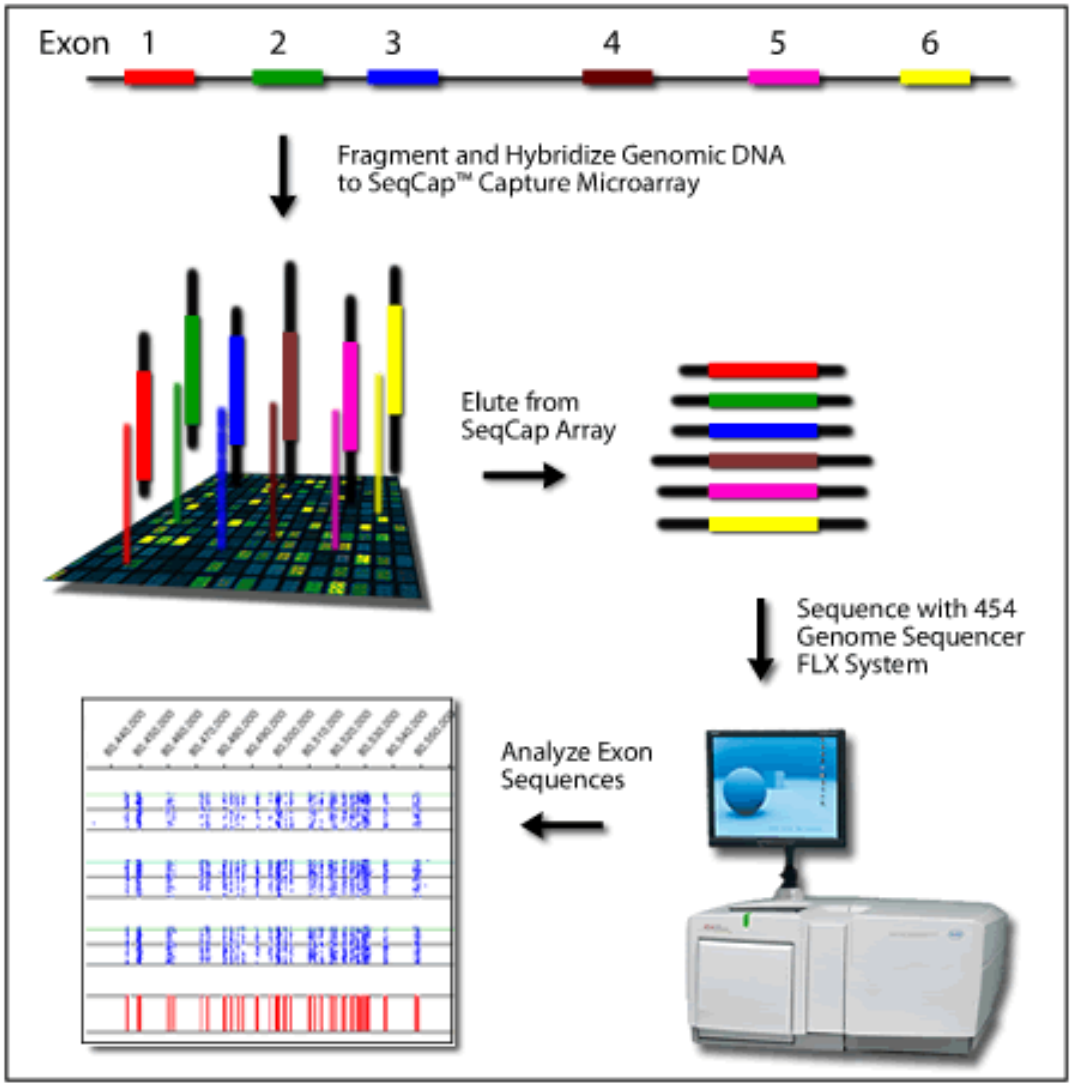
A: think of possible mitochondrial disorder in children with onset of SRNS < 2 years of age

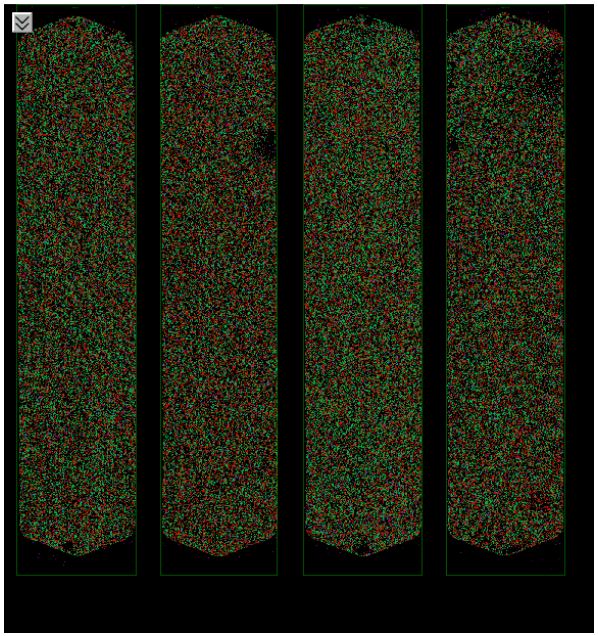
Targeted gene testing for SRNS

Congenital NS	FSGS	DMS
NPHS1	*NPHS2*	*WT1*
NPHS2	*WT1* in females	*PLCE1*
LAMB2	*NPHS1*	
PLCE1	*PLCE1*	

Courtesy C. Antignac

Next Generation Sequencing





Late Steroid resistance

- Rare, but exist
  (<2-3% of steroid responders; depends on your population)
- Generally develops 2-10 years after disease onset
- Difficult cases of SDNS from onset
- FSGS > MCD-MesP
- No genetic mutation
- Frequent relapses after transplantation

Akchurin and Kakel 2003, personal observations

Late Steroid resistance

- Treatment regimens:
  - mycophenolate mofetil
  - cyclosporine
  - tacrolimus
  - alkylating agents
  - rituximab
  - plasma exchange
- Remission in ≥50% of cases

Straatmann et al, Pediatr Nephrol 2013