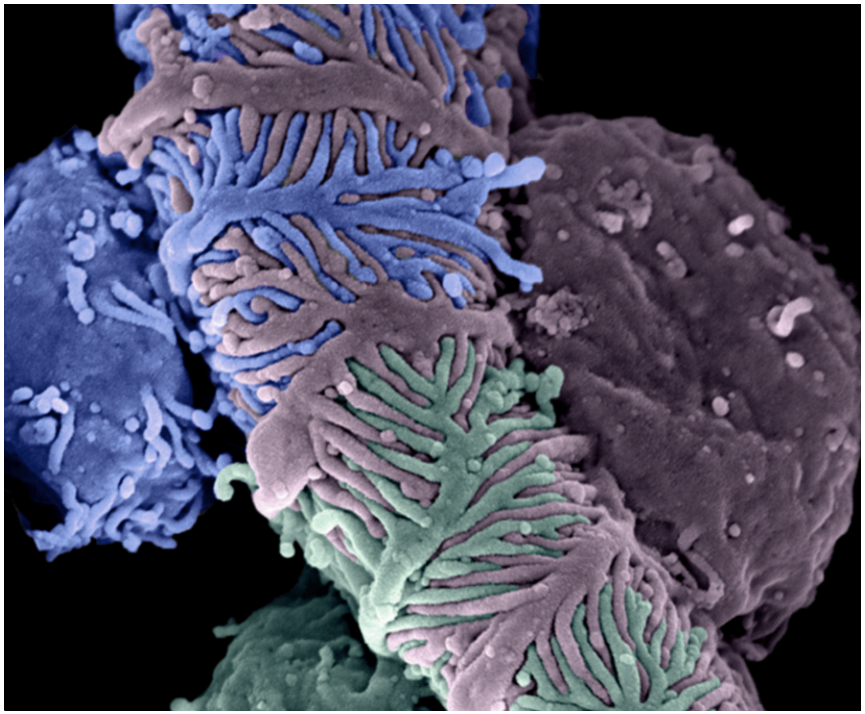
ERKNet Webinar

ERKNet

Monogenic podocytopathies in adults



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ORKiD FILIÈRE ORPHAN KIDNEY DISEASES

imagine INSTITUT DES MALADIES GÉNÉTIQUES

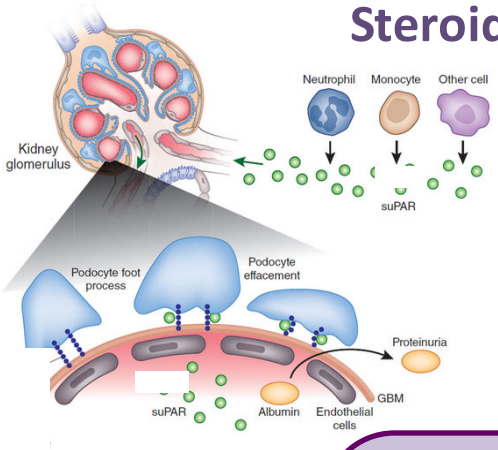
UNIVERSITÉ PARIS DESCARTES

Different etiologies of nephrotic syndrome (NS)

Idiopathic NS

Steroid sensitive

Steroid resistant



Shankland et al.
Nature Med, 2011

Complete remission with intensified immunosuppression

Recurrence after RTx

Dysimmune NS

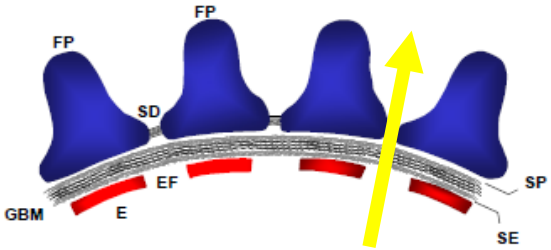
↓

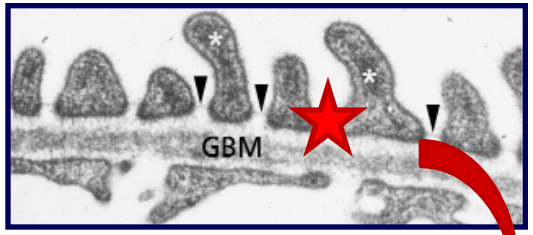
Circulating factor of glomerular permeability

Monogenic NS

↓

Structural anomalies of the glomerular filtration barrier



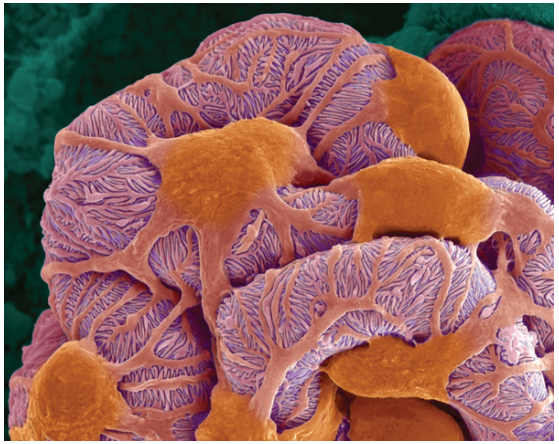


Glomerular filtration barrier

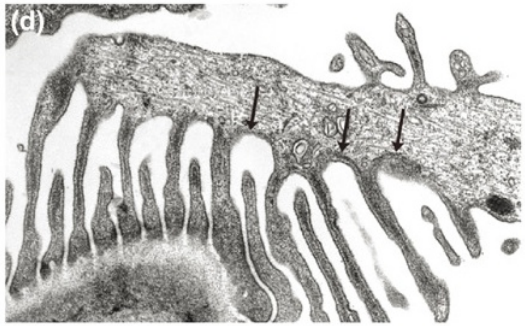
- Congenital NS
- Steroid resistant NS
- AD FSGS

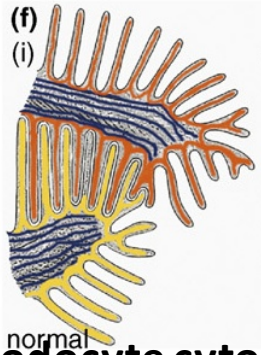
Partial remission may be observed with CNIs

Podocyte is a fascinating cell

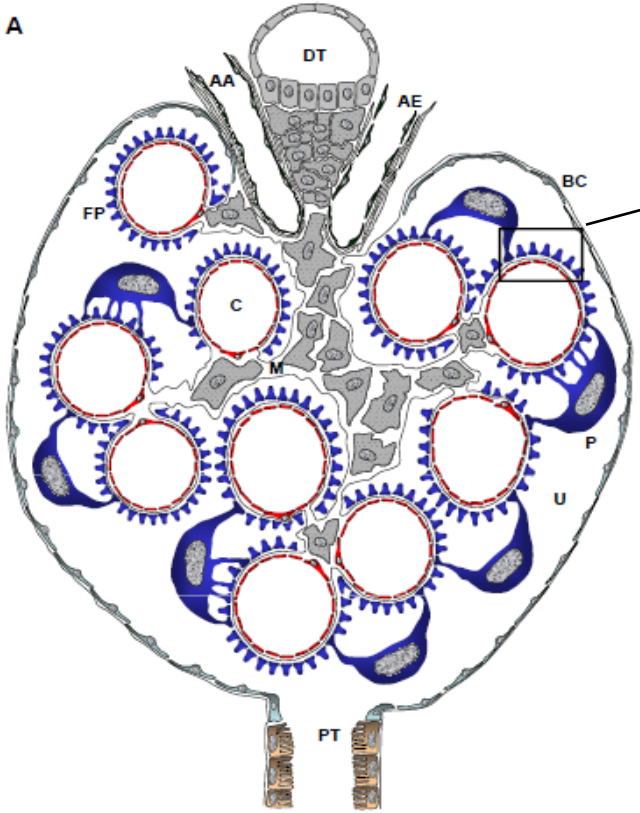


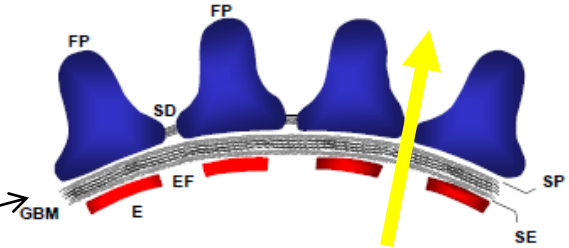
Octopus-like shape

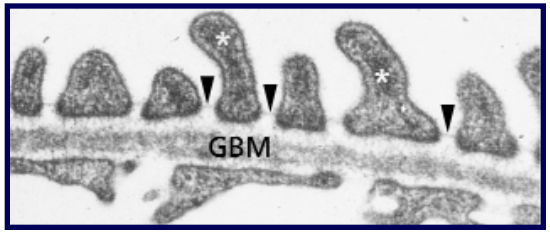




Podocyte cytoskeleton







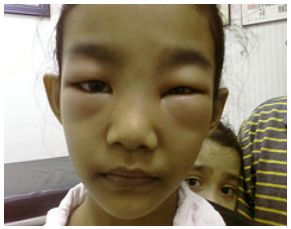
Glomerular Filtration Barrier

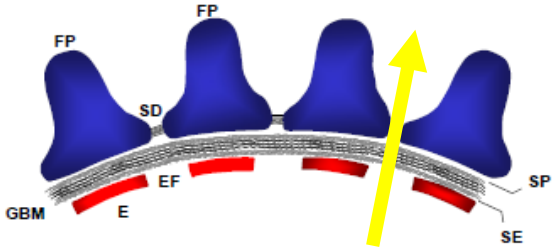
Hereditary podocytopathies

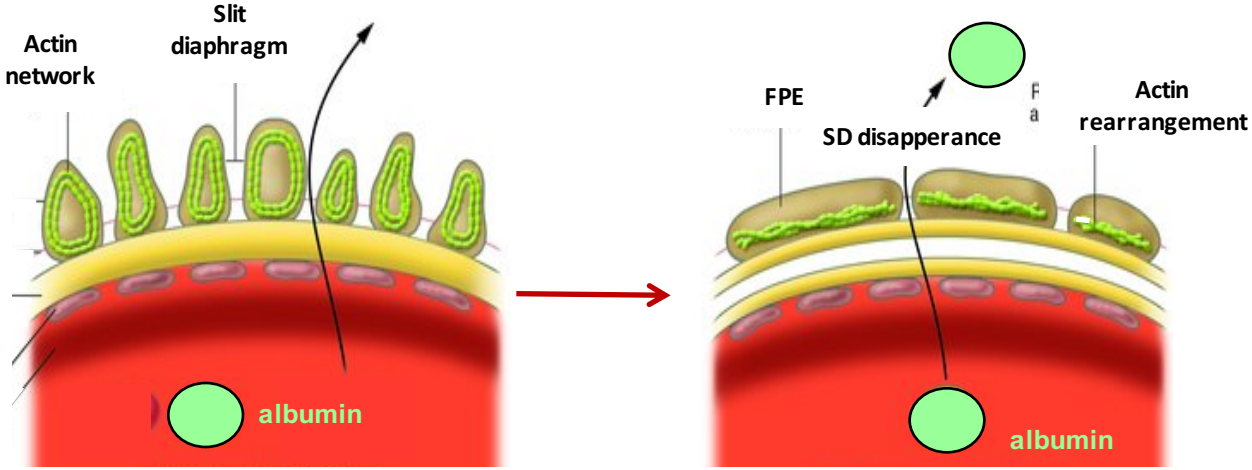
- Congenital NS
- Steroid-resistant NS
- Idiopathic FSGS

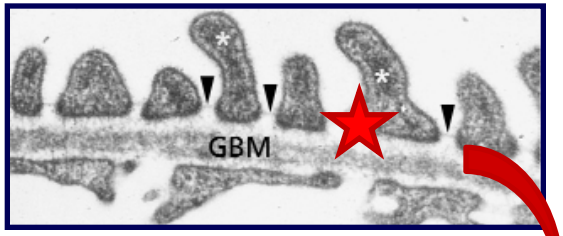
Machucca et al. HMG 2009
Faul, Trends Cell Biol 2007
Welsh, Nat Rev Nephrol 2012

Podocyte gene mutations result in actin rearrangement and foot process effacement

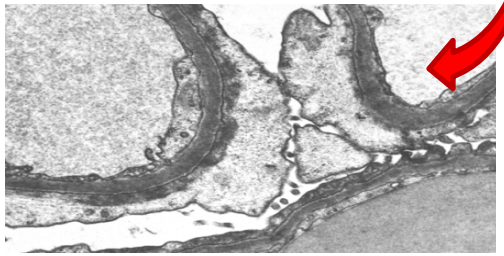




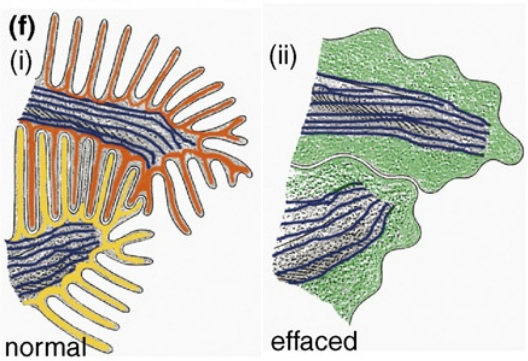


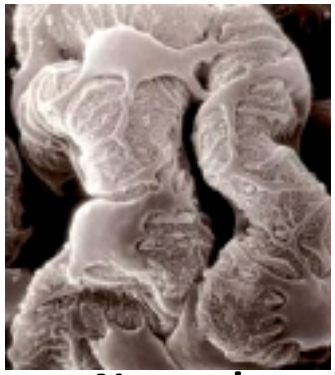


Glomerular Filtration Barrier

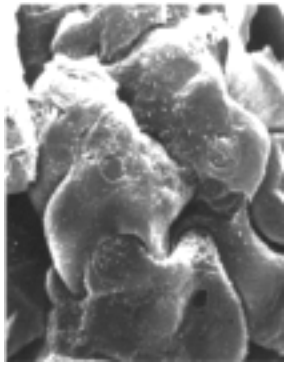


Foot Process Effacement





Normal



Effaced

Machucca et al. HMG 2009
Faul, Trends Cell Biol 2007
Welsh, Nat Rev Nephrol 2012

Clinical forms of hereditary podocytopathies

Congenital & infantile NS

Antenatal onset – 1 yr
Enlarged hyperechoic kidneys
Increased α-FP
Enlarged placenta
Massive Pu, anasarca
➔ ESKD

Tubular dilation (Finnish NS) or DMS or FSGS

Steroid-resistant NS

Childhood onset
Pu > 50 mg/kg/d
Albuminemia < 25-30g/d

No remission with steroids and other IS (ciclosporine, MMF, …)
➔ 50% ESKD at 10 years

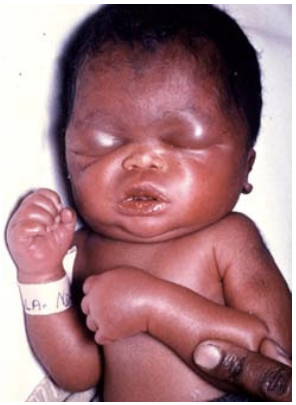
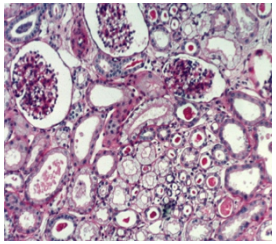
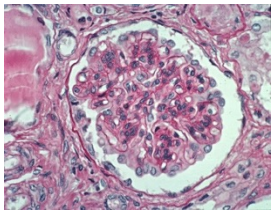
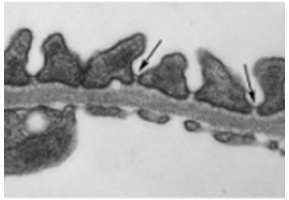
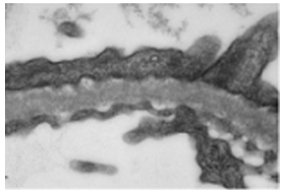
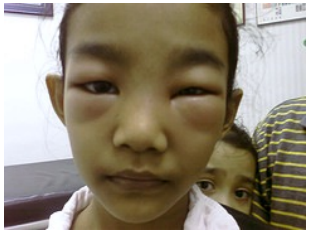
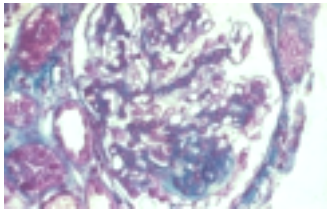
MCD or FSGS

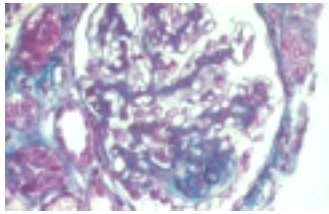
Glomerular Pu ± NS with FSGS

Late-onset (adolescent, adult)

Fortuitous Dx ++
MCD or FSGS

IS rarely used
Traitement with ACEi/ARBs



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Antenatal onset – 1 yr
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➔ ESKD
Tubular dilation (Finnish NS) or DMS

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No remission with steroids and other IS (ciclosporine, MMF, …)
➔ 50% ESKD at 10 years

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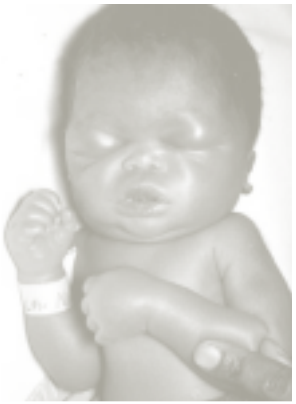
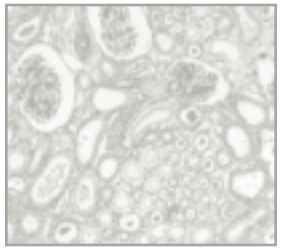
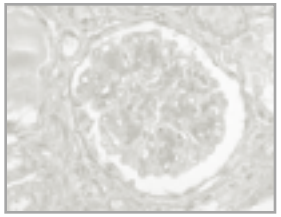
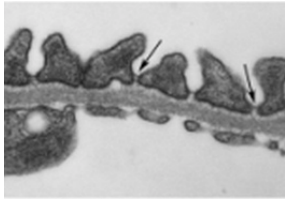
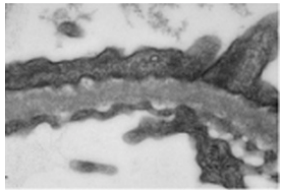
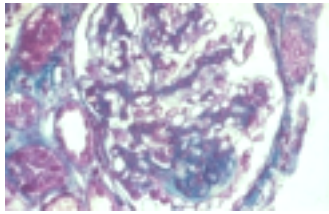
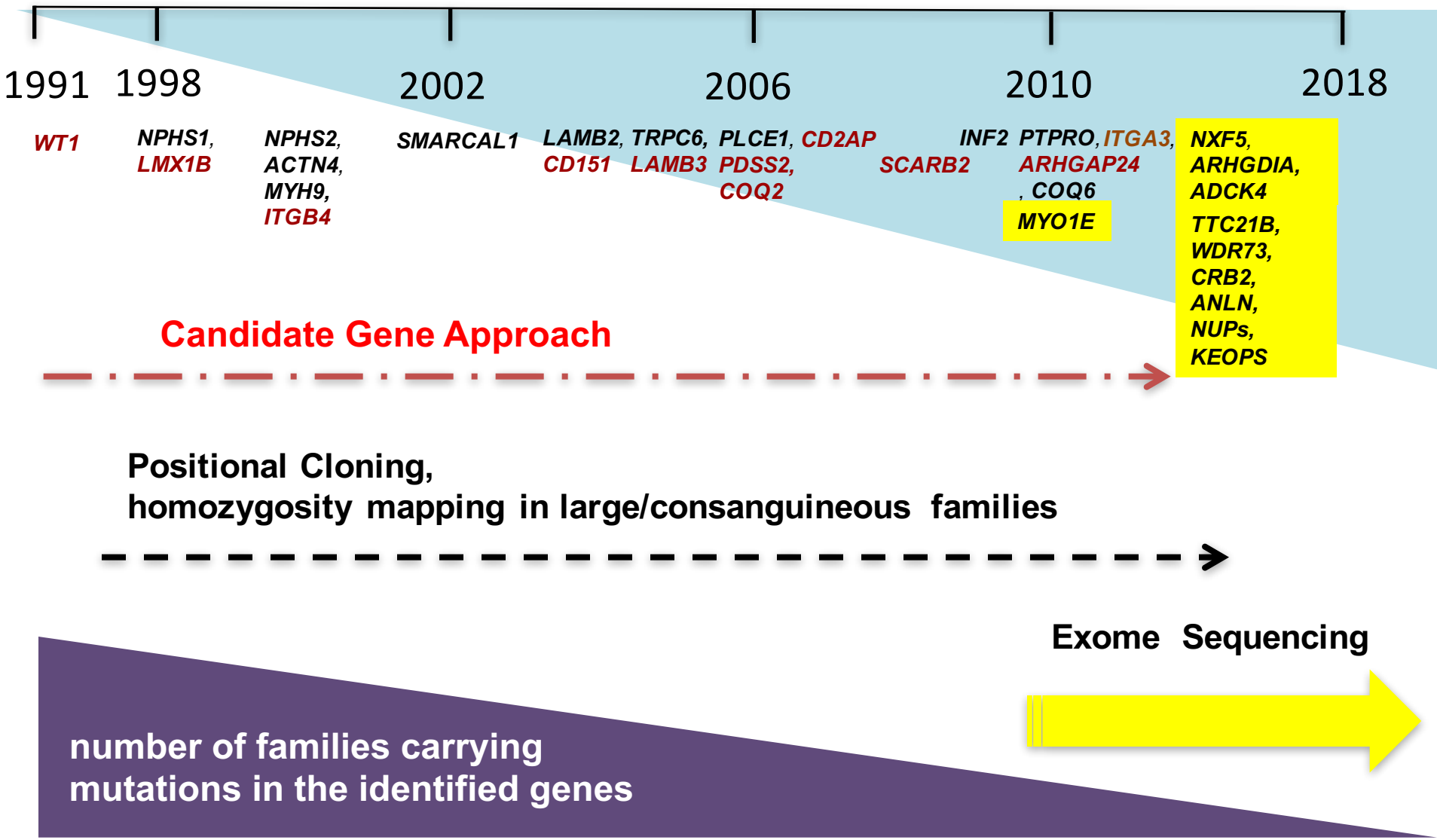
Fortuitous Dx ++
K Bx: MCD or FSGS

IS rarely used
Traitement with ACEi/ARBs

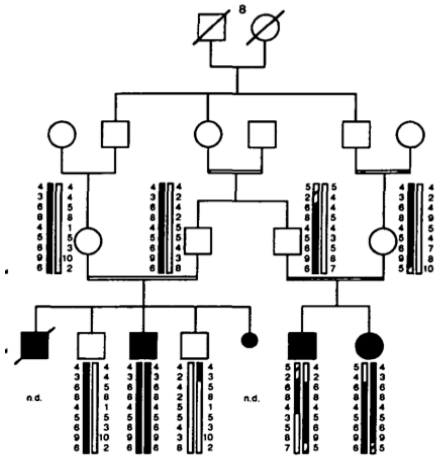
Growing number of genes involved in podocytopathies



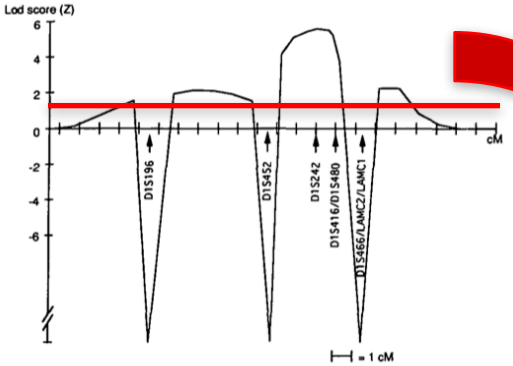
C. Antignac

Input from positonal cloning

Analyses the cotransmission of a morbide trait (NS/Pu) with microsatellite markers spred along the whole genome (linkage analyses, homozygosity mapping) ➔ location in the genome



Sequencing of candidate genes at the locus ➔ mutation



Direct identification of genes involved in hereditary diseases, which pathophysiology is unknown

Requires several informative families or large consanguineous familles
Limited by incomplete penetrance

NPHS2 mutations (podocin) are the 1st cause of autosomal recessive steroid-resistant nephrotic syndrome

AR-SRNS

NS ≅ 4 years, ESKD ≅ 10 years
> 110 mutations
- 42% familial cases
-10-30% sporadic cases

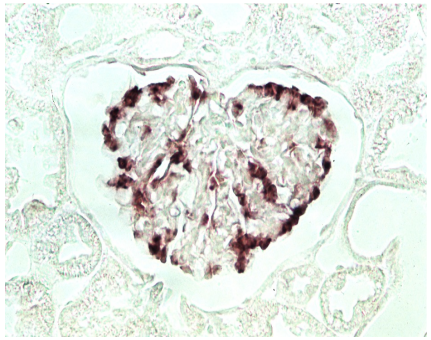
R138Q (32%): retained in the endoplasmic reticulum
Age at Dx 2 years < 10 years for mutants located at the plasma membrane

Congenital NS

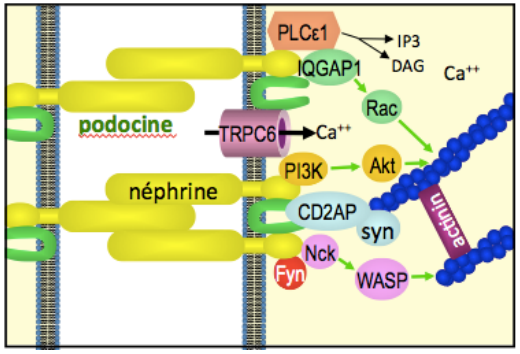
15-40 % of cases (ESKD ≅ 6 years)

Late onset NS/Pu

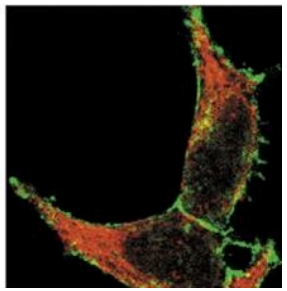
R229Q + 1 pathogenic mutation
19% of adult NS (Pu ≅ 25 years, ESKD ≅ 32 years)



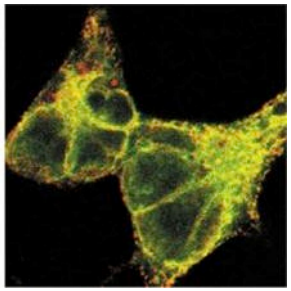
Expression in podocytes



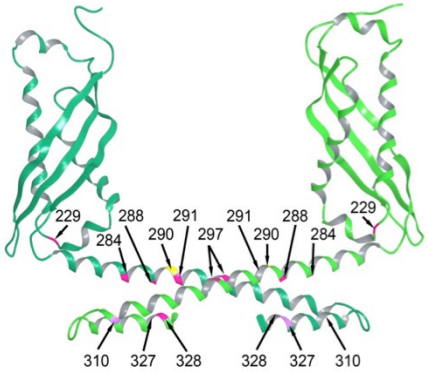
Slit diaphragm protein



WT



R138Q



Homodimer

Podocine **Calnexin (RE)**

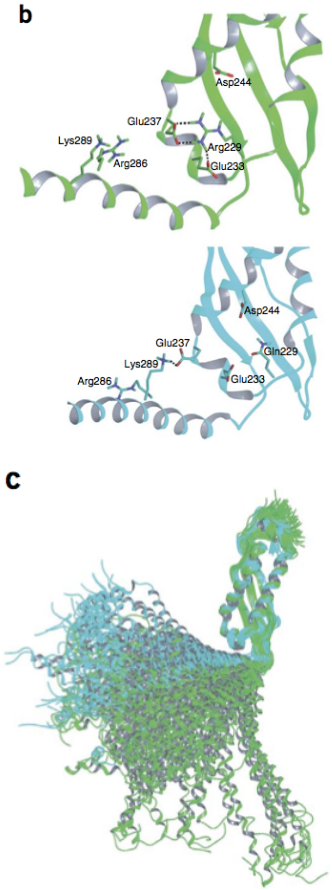
Boute et al., Nat Genet 2000
Roselli et al., Traffic 2004

Tsukagushi, JCI 2002
Bouchireb K, Boyer O … Antignac C., Hum Mut 2014

Adult patients carry the *NPHS2* polymorphism p.R229Q + 1 pathogenic mutation

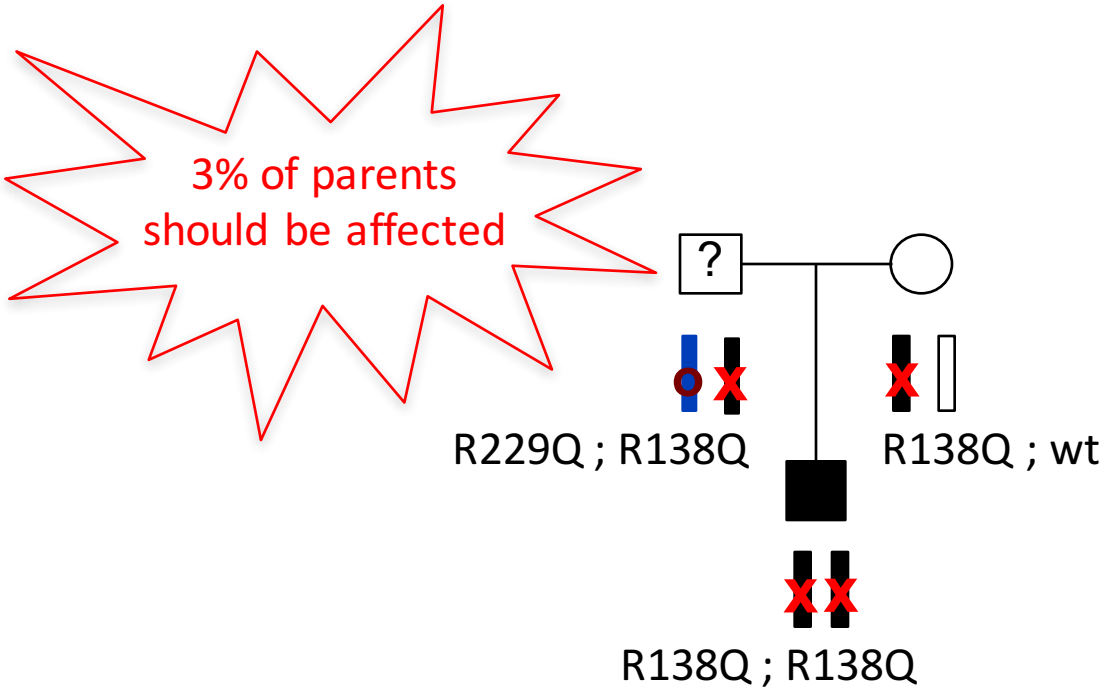
Series of 105 cases / 96 families

- **No adult patient with 2 pathogenic mutations**
- **18 patients with 1 pathogenic mutation + p.R229Q**
- P.R229Q: non neutral polymorphism (~ 3%) ➔ reduced interaction with nephrin
- Age at Dx: 24.9 years (18.5 – 39 years)
- Age at ESKD: 32 years (27.7 - 38.5 years)



Tsukagushi, JCI 2002
Machuca, … Antignac C. KI 2009

NPHS2 mutations and incomplete penetrance

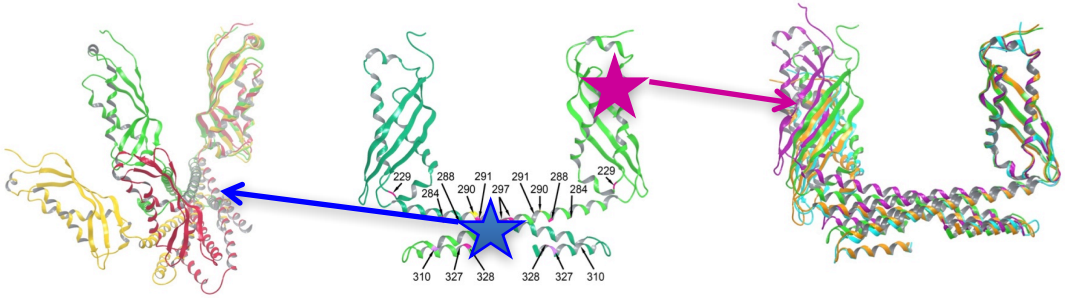


➔ 6/129 parents of affected children are asymptomatic carriers of the association [**p.R229Q**];[mutation]

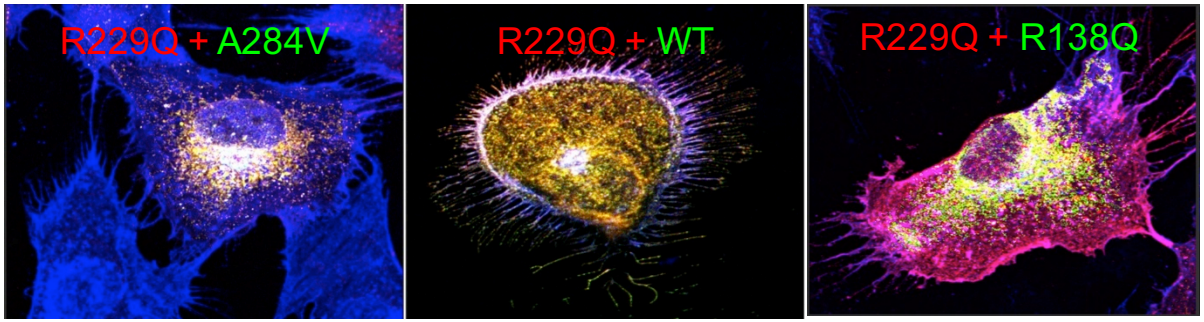
Tory K… Antignac C. Nat Genet 14

The effect of the p.R229Q *NPHS2* mutation depends on the 2nd mutation

Pathogenic dimers
[**p.R229Q**];[**C-terminal** mutation]

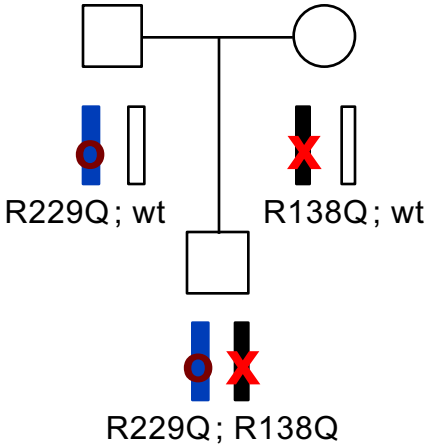


Non pathogenic dimers
[**p.R229Q**];[**N-terminal** mutation]



Podocin retained in the cell
FSGS

Podocin at the plasma membrane
unaffected



➔ [**p.R229Q**];[**N-terminal mutation**] DO NOT EXPLAIN NS => **immune NS?**

➔ **Genetic counselling**: A couple carrying an *NPHS2* mutation in exons 1–6 in one member and R229Q in the other is not at risk of having an affected child

Tory K... Antignac C. Nat Genet 14

TRPC6 and *ACTN4* mutations are responsible for ≈ 5 and 4% of AD FSGS

TRPC6

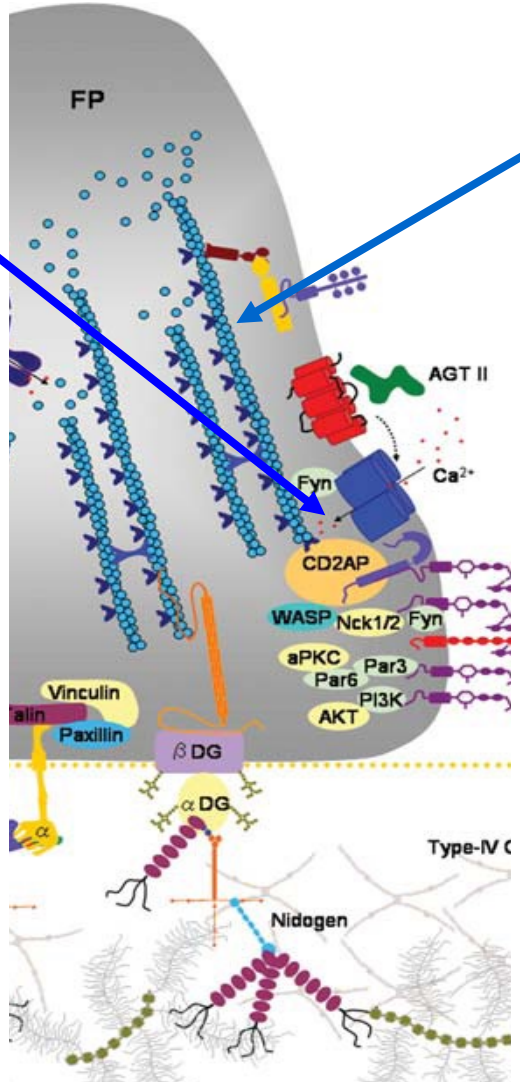
Calcium channel

Identified in a large family from New Zealand with late onset Pu and ESKD in 60% of patients

Mutations in **9/179 families (5%)**

↑ Intracellular Ca influx

Incomplete penetrance



ACTN4 (α-actinin 4)

Actin-bundling protein

Missense mutations in 3 large pedigrees with **AD FSGS**

5/141 familial FSGS (4%)

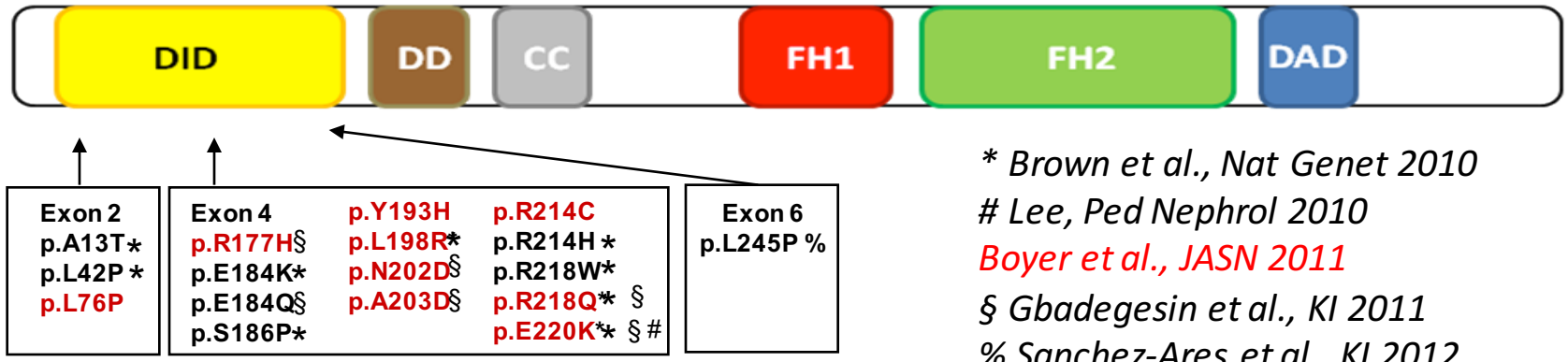
Proteinuria with FSGS (2nd decade), progressive CKD **late-onset ESKD in some patients (~50 yrs)**

Incomplete penetrance

Winn et al., Kidney Int 1999
Winn et al., Science 2005
Reiser et al., 2005

Kaplan et al., Nat Genet 2000
Weins et al., JASN 2005
Weins et al., PNAS 2007

Mutations in *INF2* are the main causes of AD FSGS



** Brown et al., Nat Genet 2010*
 # Lee, Ped Nephrol 2010
Boyer et al., JASN 2011
§ Gbadegesin et al., KI 2011
% Sanchez-Ares et al., KI 2012

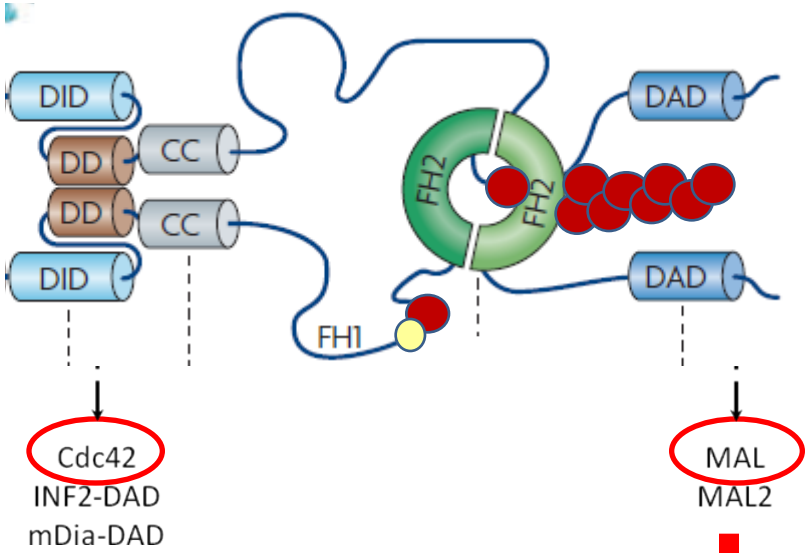
Reference	# tested families	# mutated families	% mutations	Age at onset Pu	Age at ESKD
Brown 2010 Barua 2012	215	20	9%	11-72 yrs	13-67 yrs
Lee 2010	9	1	11%	7-30 yrs	14 yrs
Boyer 2011	54	9	17%	5-44 yrs	20-70 yrs
Gbadegesin 2011	49	8	16%	14-46 yrs	33-45.5 yrs
TOTAL	**327**	**38**	**12%**	**5-72 yrs**	**13-70 yrs**

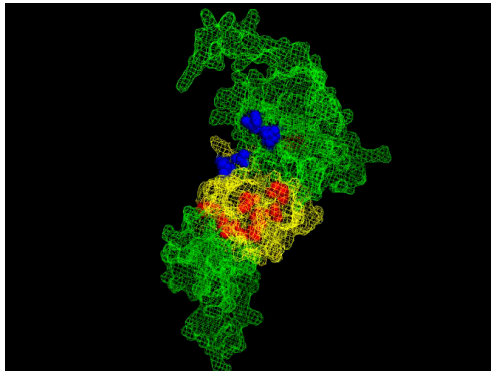
Interindividual variability & incomplete penetrance

3/405 = 0.8% of sporadic cases

Bown E … Pollak M. Nat Genet 10
Boyer O, … Antignac C. JASN 11

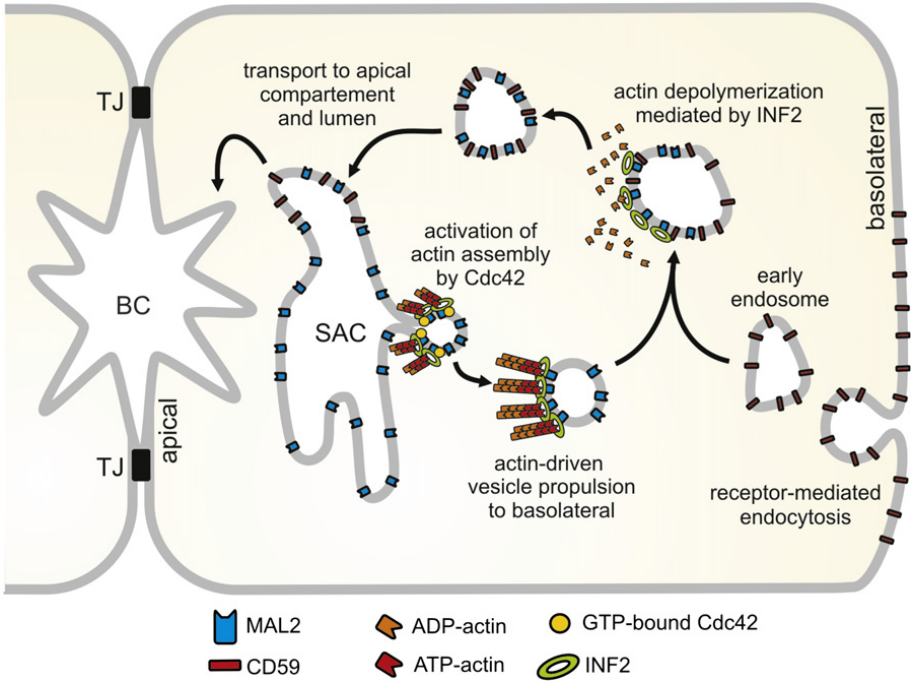
Mutations in the INF2 DID alter the localisation of the protein and actin polymerization





MYELIN And Lymphocyte protein

- ➔ Enhances actin polymerization
- ➔ Promotes actin depolymerization and filament sievering
- ➔ **Role in intracellular protein transport in association with Cdc42 and MAL**

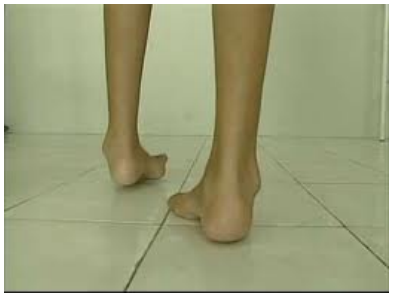


Madrid, Dev Cell 2010

Andres-Delgado, Blood 2010

Bown E … Pollak M. Nat Genet 10

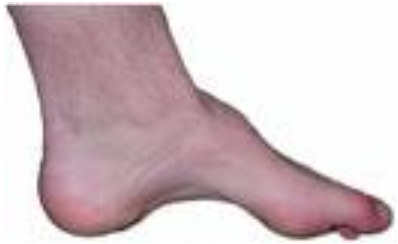
Input from candidate gene approaches



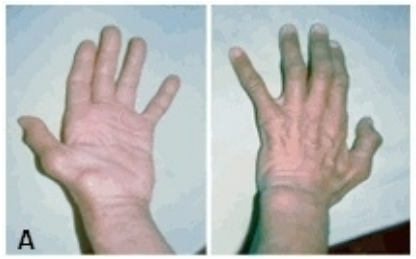
Amyotrophy

Steppage gait

Prevalence: 1/2500



Pes cavus



Reduced deep tendon reflexes

CMT + FSGS

13 reported cases

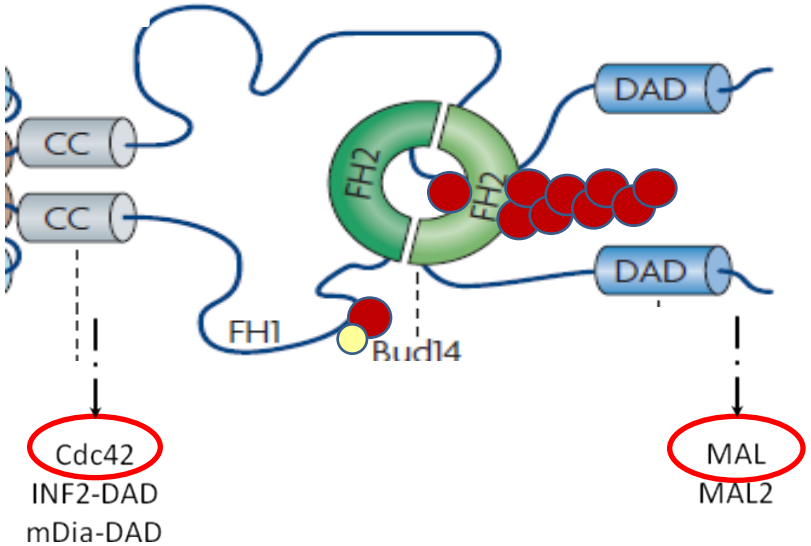
Age Pu 1-28 yrs

Nephrotic syndrome 3/11

ESKD after 0.5-15 yrs

Common genetics?

INF2 partners Cdc42 and MAL are crucial for myelination



INF2 = good candidate?

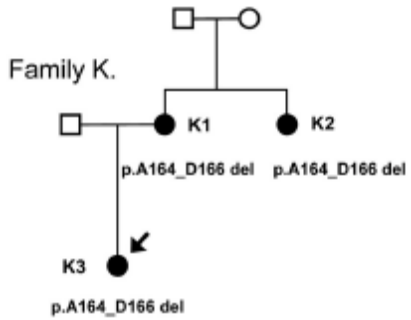
Lemieux et al., Can Med Assoc J 1967

Mutations in *INF2* are the main causes of FSGS associated with Charcot Marie Tooth disease

Cohort of 16 unrelated families with FSGS-CMT :

➔ **mutations in 75% cases**

No mutation in **150 patients with CMT** **and no renal involvement**

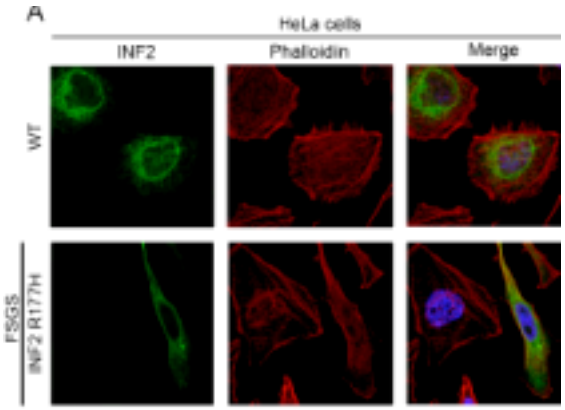


Dominant inheritance

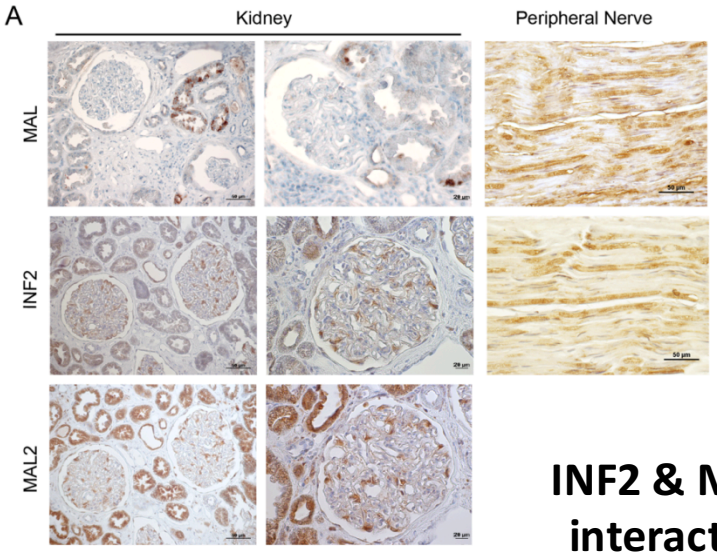
CMT : 13 years (5-28 years), 4 with deafness

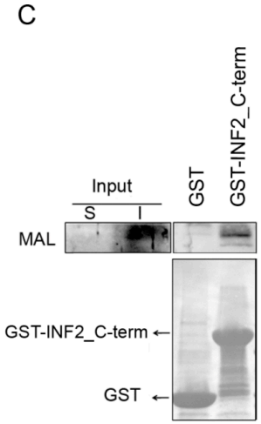
Proteinuria : 18 years (10-21 years)

ESKD: 21 years (12-47 years)



Mutants alter the INF2-mediated polymerization & depolymerization

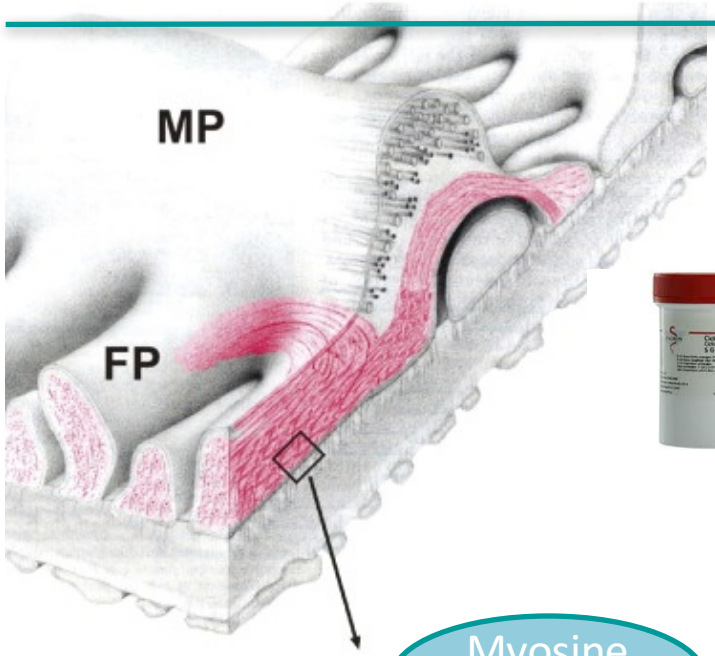


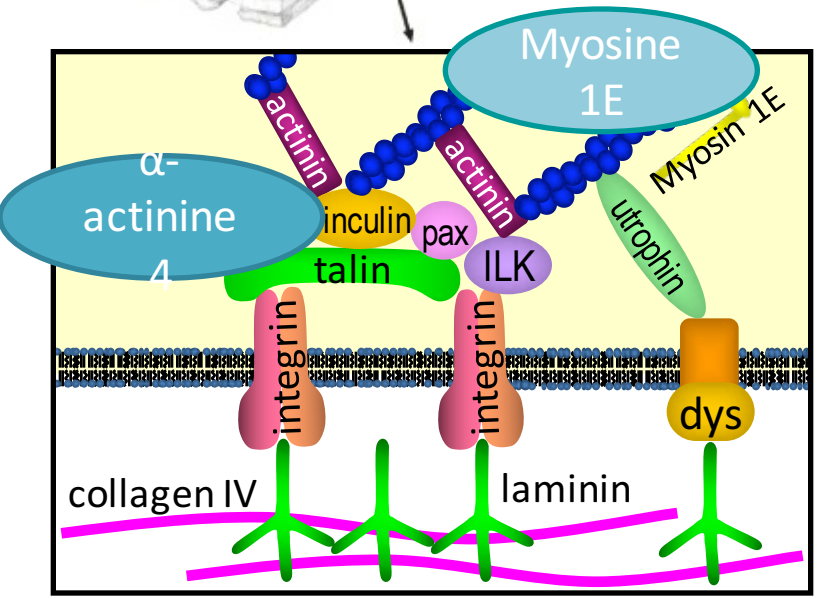


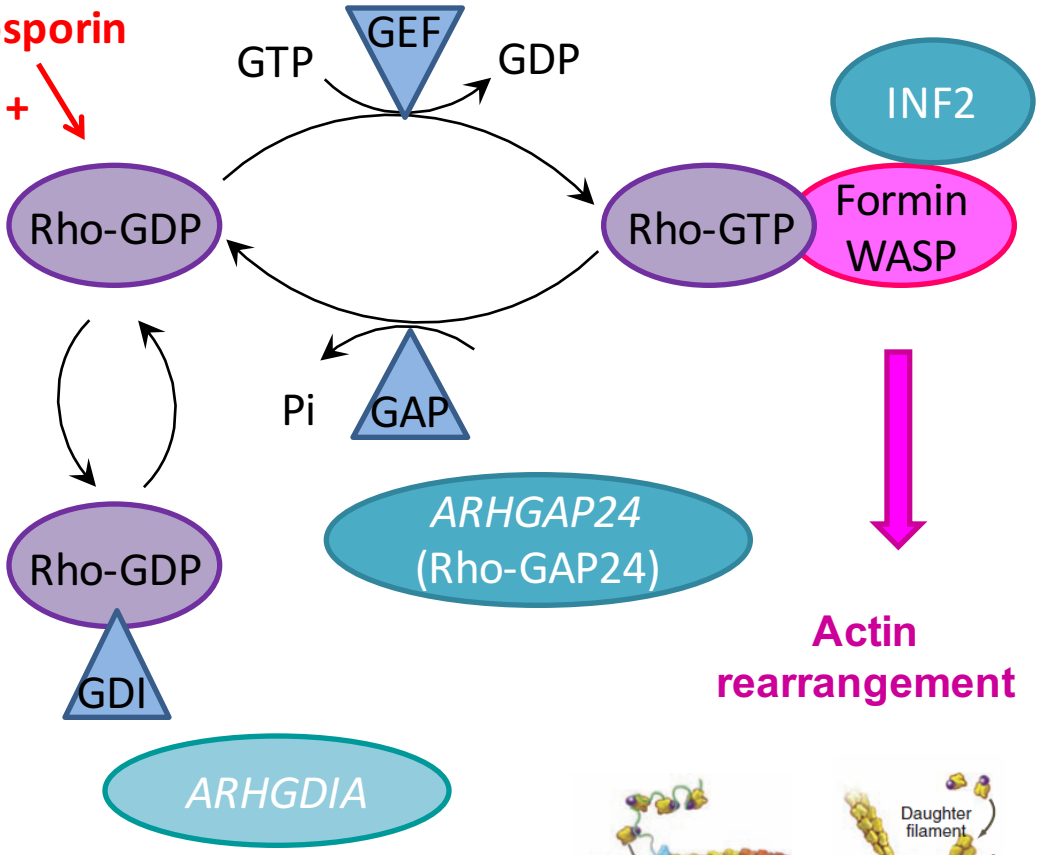
INF2 & MAL colocalize and interact in Schwann cells

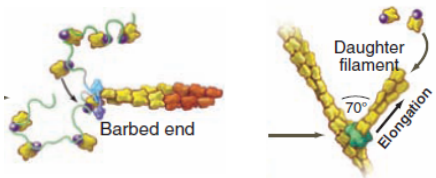
Boyer O, … Antignac C. N Engl J Med

Input from candidate gene approaches
Other podocyte cytoskeleton genes



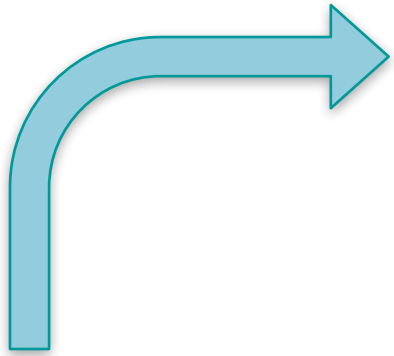






Faul, Trends Cell Biol 2007
Akilesh S, et al., JCI 2011

Input from high throughput sequencing



XXXXX variants

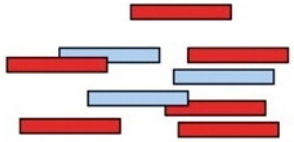
Filters

Candidate genes
Segregation analysis
Functional studies

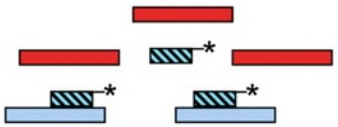
Disease-causing variant

Exome sequencing

Genomic DNA

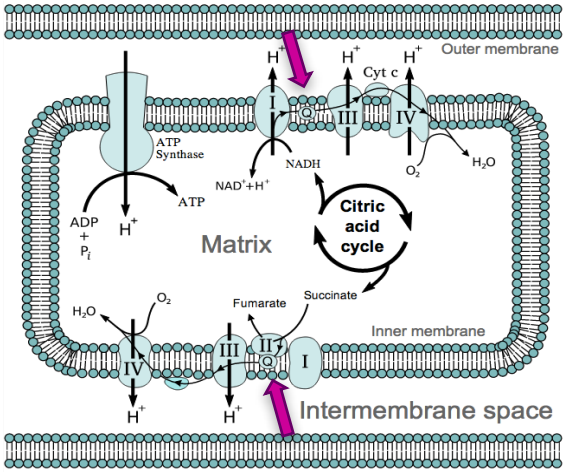


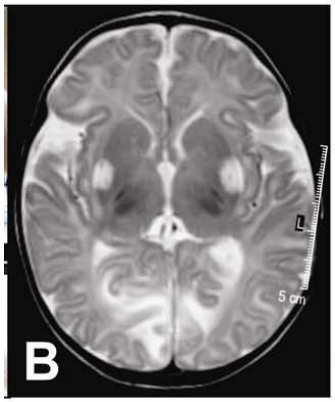
Exome capture

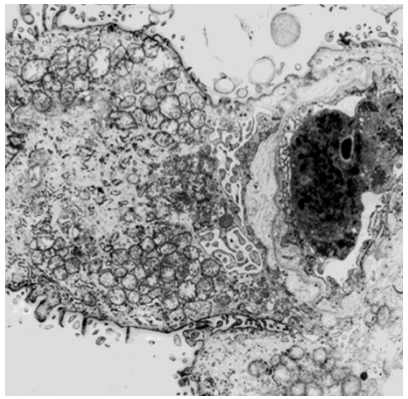


Sequencing

1) Identification of new genes: *ADCK4* involved in coenzyme Q_{10} (CoQ_{10}) biosynthesis



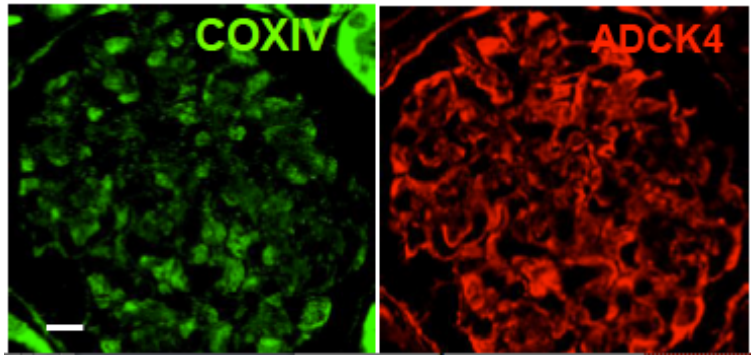




Gene	Kidney phenotype	Extra-renal features	COQ10 effects
COQ2	CNS, SRNS (FSGS)	Encephalopathy, MOF	?
PDSS2	Congenital/infantile NS	Deafness, MR, Leigh	?
COQ6	CNS, SRNS (FSGS/DMS)	± Deafness, seizures, ataxia	↓ Pu ± deafness
ADCK4	SN infantile, SNCR (HSF)	1 heart diasease, 1 MR/15	↓ Pu

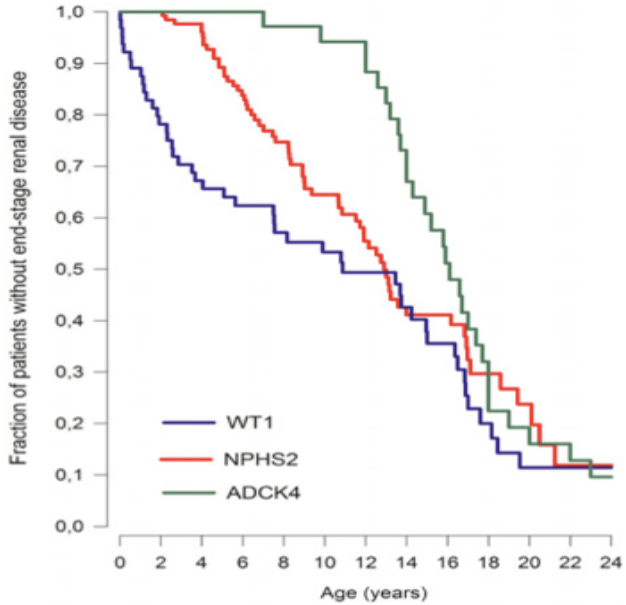
Heeringa et al., JCI 2011
Ashraf et al., JCI 2013

1) Identification of new genes: *ADCK4* (COQ8B) involved in coenzyme Q_{10} (CoQ_{10}) biosynthesis



- Identification of *ADCK4* mutations in 15 patients (8 families) with SRNS by linkage analyses and exome sequencing
- Expression in podocytes (mitochondria and foot processes)
- ↓ level of CoQ_{10} in lymphocytes or fibroblasts
- ↓ cell migration by ADCK4 knockdown, reversed by CoQ_{10}
- ↓ proteinuria in 1 treated patient

Ashraf et al., JCI, 2013



- 26 patients from 12 families
- median age, 14.1 years (11-17)

- nephrotic-range proteinuria in 44%
- advanced CKD in 46% of patients at time of diagnosis
- Age at start RRT 14-18 years
- Mostly isolated FSGS

Kormaz, JASN 2015

2) Mutations in "syndromic NS genes" in patients with isolated glomerular diseases

Family F.

36 yrs: Pu
58 yrs: ESKD
Cause?
RTx

40 yrs: ESKD
Cause?

I:1 I:2

II:1 II:2 II:3 het II:4 II:5 II:6 II:7 No mutation II:8 II:9

III:1 het III:2 III:3 het III:4 No mutation III:5 III:6 het

IV:1 het

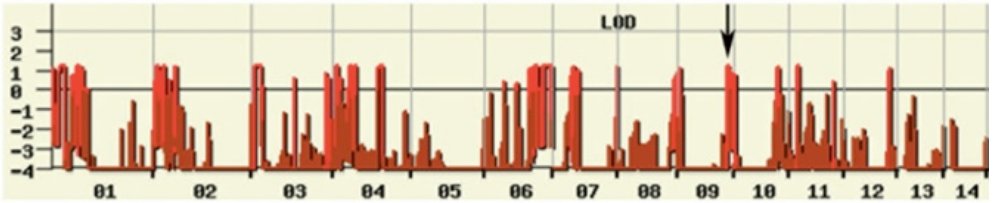
26 yrs: gravidic NS
K Bx: FSGS
GFR 120 ml/min/1.73m2

22 yrs: Pu
K Bx: FSGS
59 yrs: GFR 61 ml/min/1.73m2

6 yrs: SRNS
K Bx: MC
Variable Pu
Lost to F/U
37 yrs: NS, FSGS, CKD
54 yrs: ESKD

7 yrs: microalbuminuria
GFR 115 ml/min/1.73m2

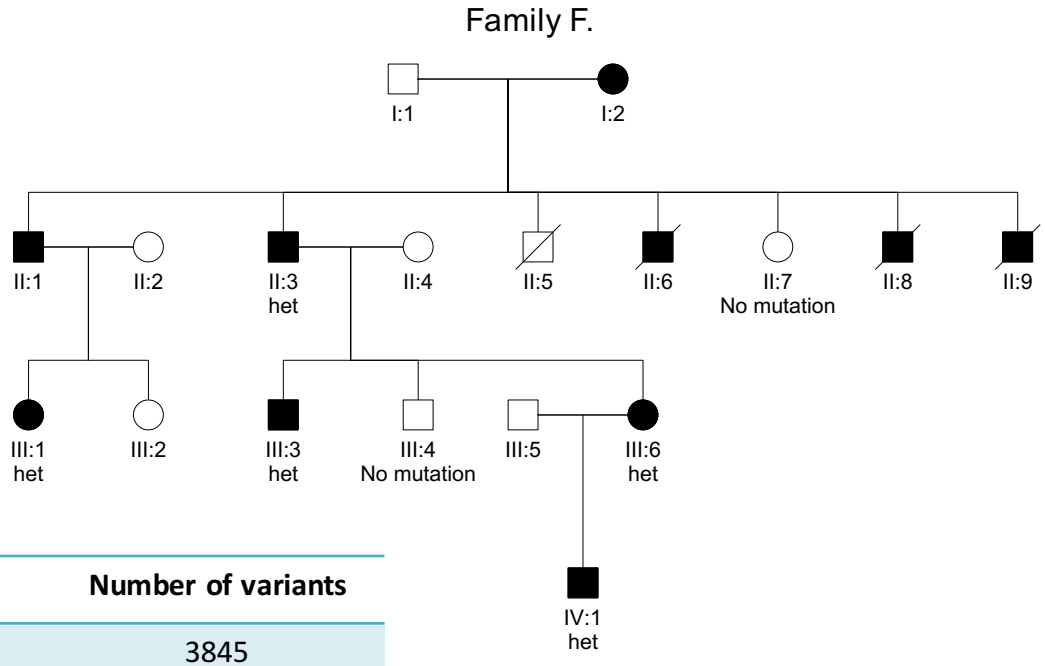
10 affected
4 generations
5 with DNA
No extra-renal involvement



Linkage analyses:
Large region of interest
118 Mb on chromosomes
1,2,3,4,6,9,11,15,17,19
1000 genes

2) Mutations in "syndromic NS genes" in patients with isolated glomerular diseases

3845 variants



Needle in a haystack

Filters	Number of variants
Common to the 5 pts	3845
Within the regions of interest	315
- non exonic regions	140
- synonymous variants	20
- polymorphisms dbSNP	13
- polymorphisms 1000 genomes	11
- polymorphisms EVS	8
- Polymorphisms Necker	2

LMX1B Mutation
p.R246Q segregating in the family
p.R246Q and p.R246P in 2 others

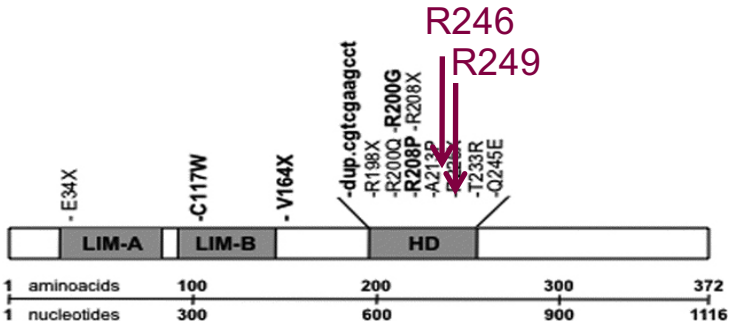
Boyer O et al., *JASN* 2013

2) Mutations in "syndromic NS genes" in patients with isolated glomerular diseases: *LMX1B* mutations without Nail Patella

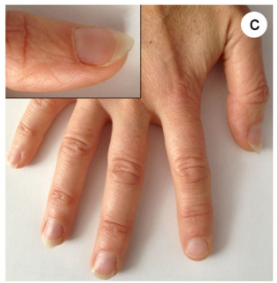
Nail-Patella syndrome

Developmental defects of dorsal limb structures, kidney and eye, with **nail dysplasia, patellar abnormalities**, elbow dysplasia, **iliac horns**, nephropathy **(2-65%)** and glaucoma

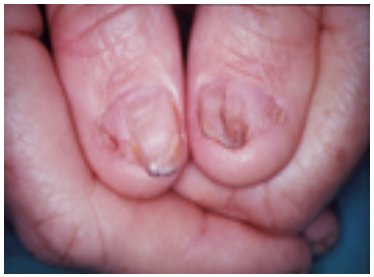
- **No extra-renal disorder** recorded in the patients
- No typical EM lesions of the glomerular basement membrane in the biopsy sample



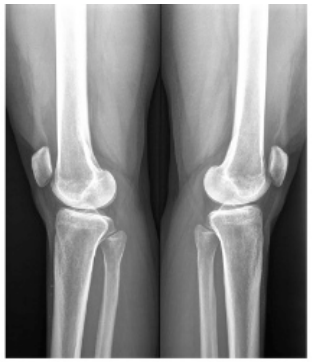
Edwards et al., *Clin Kidney J*, 2015



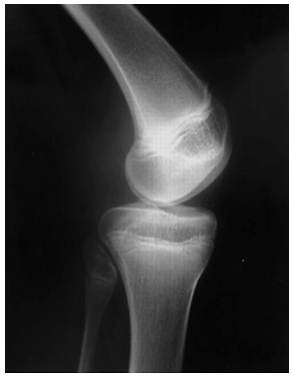
Patients

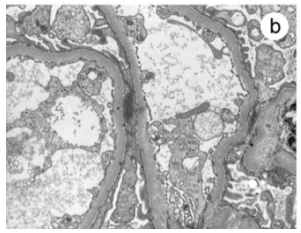


Nail Patella

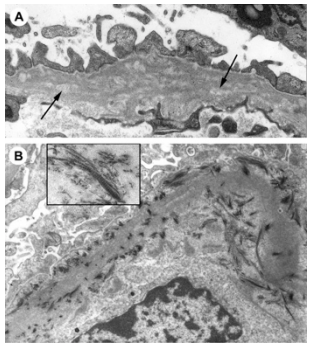


Patients





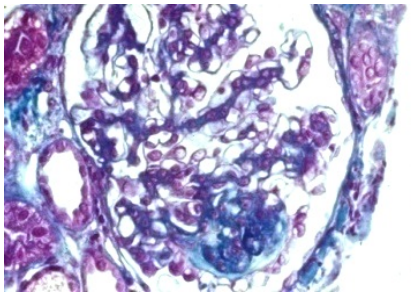
Patients



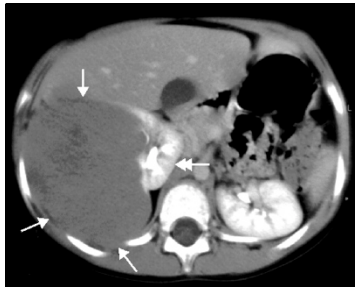
Nail Patella

2) Mutations in "syndromic NS genes" in patients with isolated FSGS: *WT1* mutations without Denys Drash or Frasier Sd

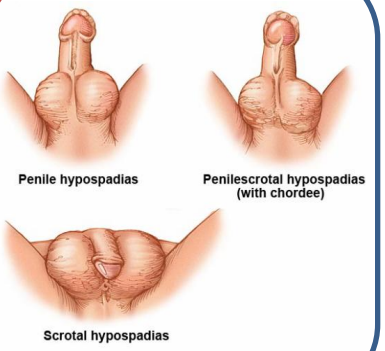
Denys Drash



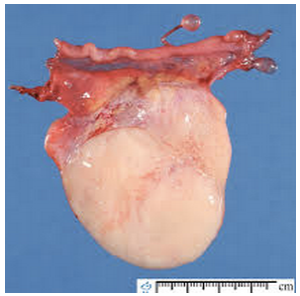
FSGS



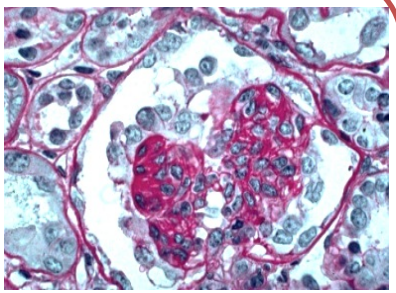
Wilm's



Frasier

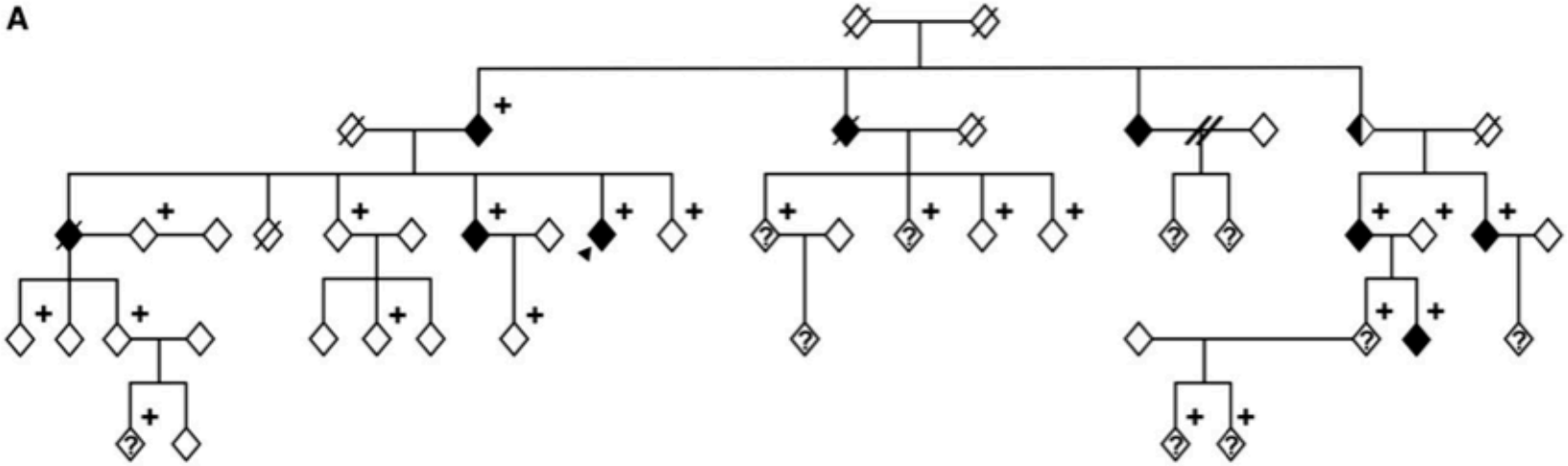


gonadoblastoma



DMS

Identification of a *WT1* mutation segregating with the disease in 2 large families (with at least 5 males) with FSGS (diagnosed between 16-30 yrs)



Hall G et al., *JASN 2015*

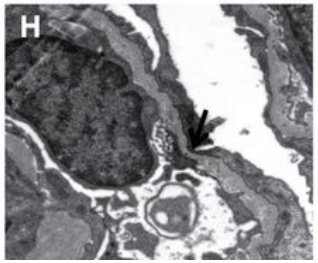
3) Mutations in genes involved in other kidney diseases: *COL4A3-5* and *PAX2* gene

COL4A3-5 mutations

⇒ *COL4A3* and *COL4A4* mutations: AD and AR Alport Sd and Familial Benign hematuria

⇒ *COL4A3* heterozygous mutations in **~10% families with AD FSGS** – **no deafness before molecular testing**

- Some missing clinical data (Hu? EM?)
- Some patients clearly have isolated proteinuria without hematuria or any GBM changes

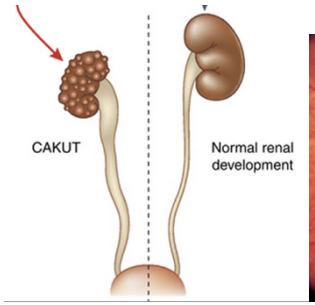


Malone et al., *KI 2014*
Xie et al., *J Mol Cell Biol 2014*

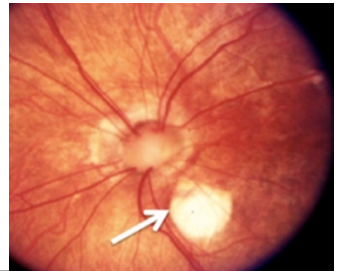
PAX2 mutations

⇒ CAKUT and papillo-renal syndrome

⇒ Identified in 4% of adult-onset familial FSGS and no extra-renal features



CAKUT



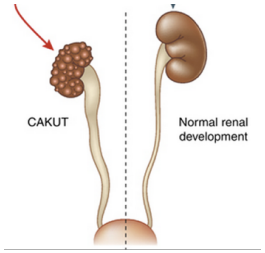
coloboma

Barua et al, *JASN* 2014

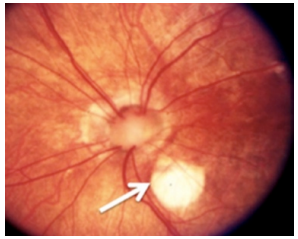
Include *COL4A3-5* & *PAX2* genes in FSGS genetic testing

PAX2 (CAKUT)

⇒ CAKUT and papillo-renal syndrome
⇒ in 4% of adult-onset familial FSGS and no extra-renal features



CAKUT

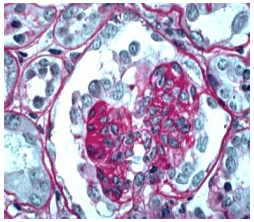


coloboma

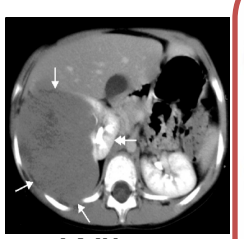
Barua et al, *JASN* 2014

WT1 (syndromic NS)

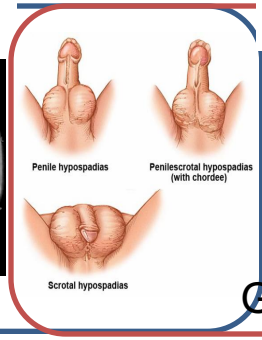
Denys Drash



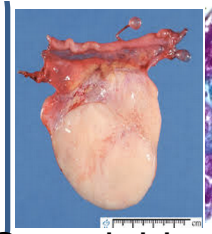
DMS



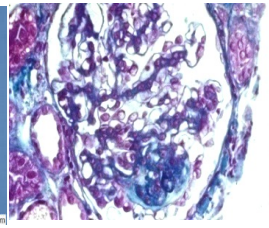
Wilms



Frasier

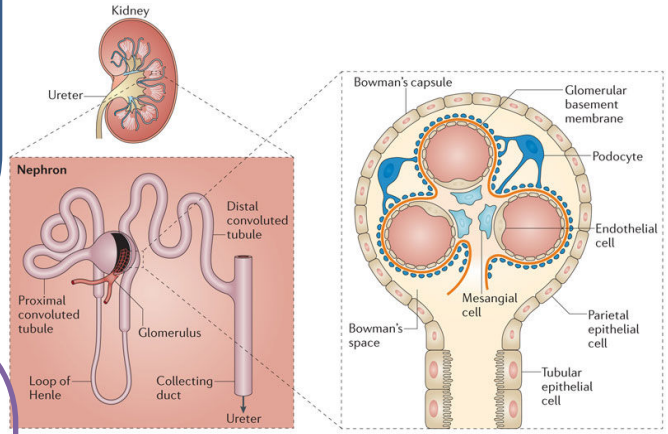


Gonadoblastoma



FSGS

Hall G et al., *JASN* 2015



Tubulopathies

- *CBN* (cubilin) (Megaloblastic anemia)
- *OCRL* et *CLCN5* (Dent)

=> Chronic Pu and FSGS
=> ↓ albumin reabsorption in the DCT
⇒ Secondary FSGS? Intrisic role ?

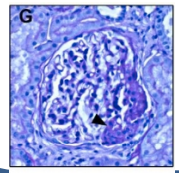
Ovuncet al, *JASN* 2011

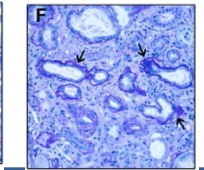
TTC21B (ciliopathy)

- Pu (1-7 g/l) et HBP ++
- ESKD 22 yrs

⇒ FSGS and nephronophtisis

- Magrheb and Portugal

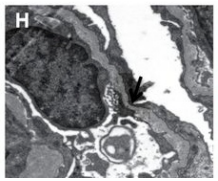




Huynh Cong E et al., *JASN* 2014

COL4A3-5 (Alport)

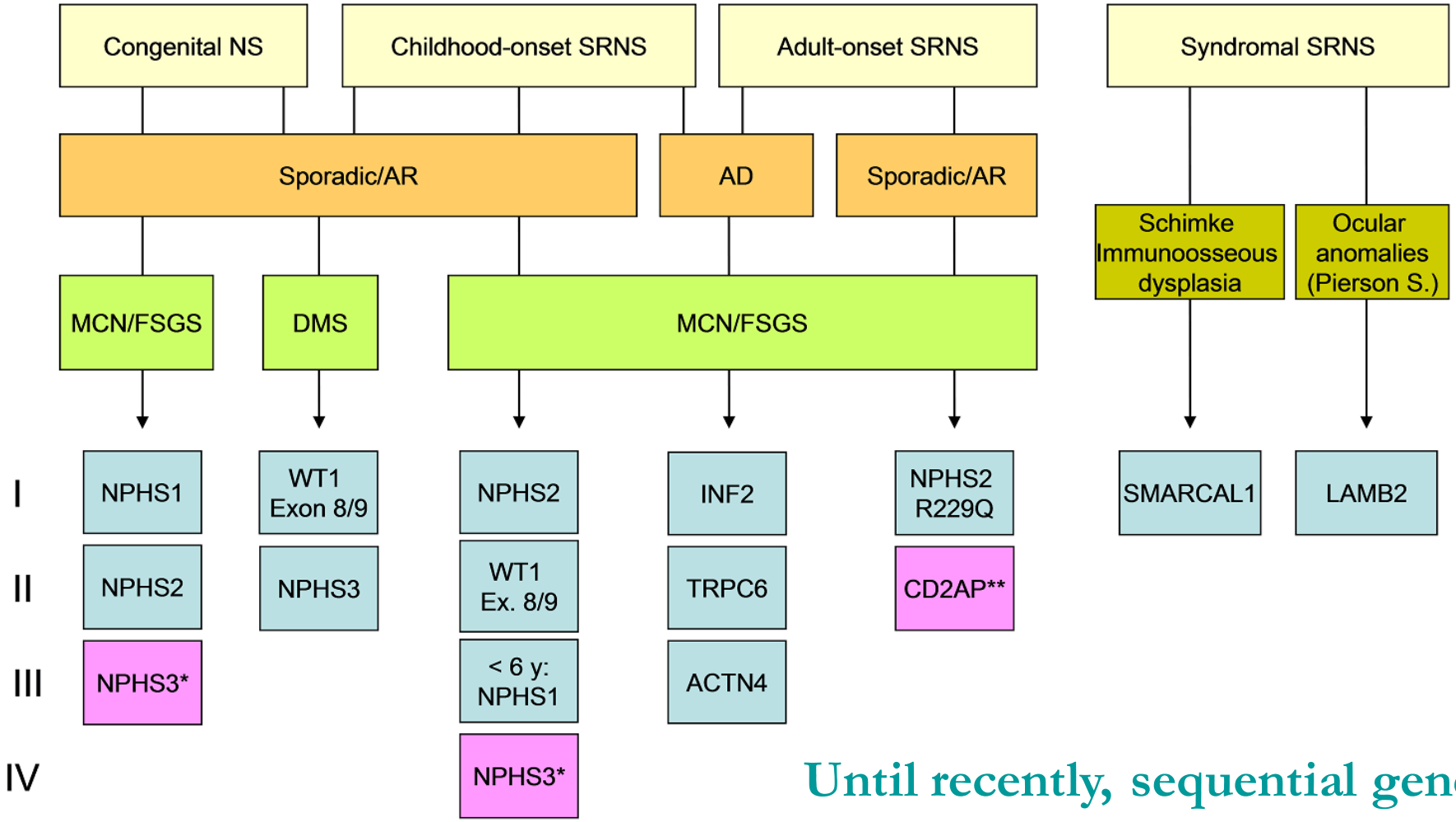
⇒ Heterozygous *COL4A3* mutation in ~10% AD-FSGS families
No deafness before genetic testing



Malone et al., *KI* 2014

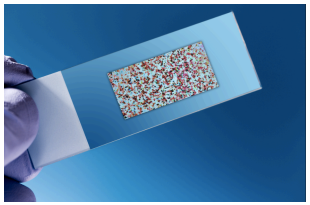
4) Strategies for molecular diagnosis of SRNS/FSGS ?

Suggested Strategy of Genetic Mutation Screening in SRNS



Until recently, sequential gene screening by Sanger sequencing

4) Strategies for molecular diagnosis in FSGS/SRNS



Exome sequencing

- Cost: still expensive to get good accuracy
- Long turnaround time
- Very large data sets

⇒ interpretation challenge

- Incidental findings

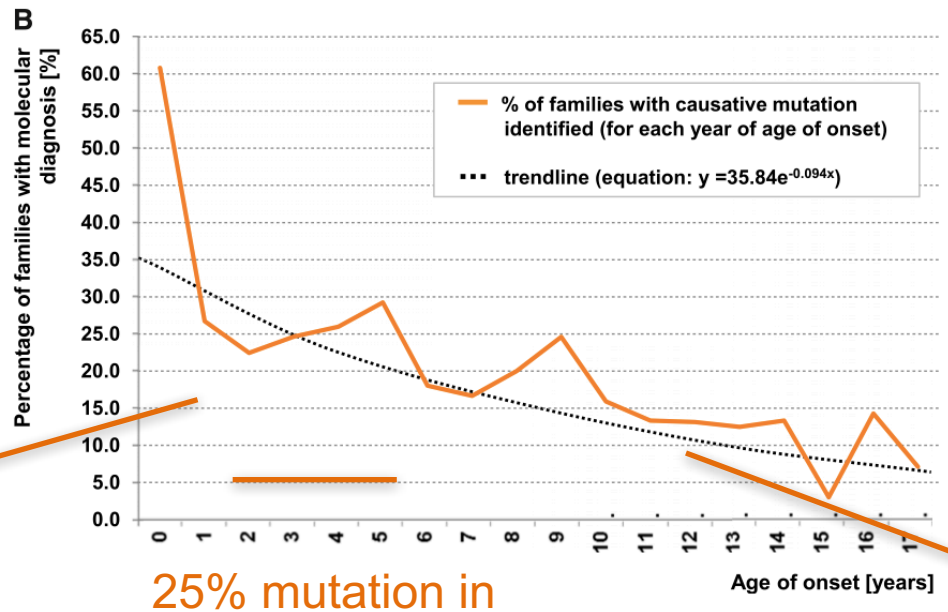
Targeted panels (selected genes)

(FSGS panel HSF ~30 gènes)

- More cost-effective
- Faster
- Less data to interpret in few selected genes
- Less ethical issues

Targeted sequencing – Next Generation Sequencing (NGS) panel sequencing in SRNS-FSGS

- 1783 families with SRNS < 25 yrs : Sanger and targeted NGS of 27 genes
- 29.5% disease causing mutations in a single gene
- 49.5% in consanguineous families and only 25% in non consanguineous families



61% mutation in CNS and infantile NS

25% mutation in children 2-5 yrs at NS onset

10% mutation in children > 12 yrs at NS onset

Sadowski et al. *JASN, 2014*

Adult forms (Necker's cohort)

- 135 adult patients
- Sporadic cases
- Mean age at Pu : 30 (18-84) years
- 58% edema at diagnosis
- 50% ESKD at a median age of 37 (20-72) years
- **16/135 (12%) patients with identified mutation**
  - Mean age at Pu 25 yrs (18-46)
  - All diagnosed < 25 yrs except 2 *COL4* gene mutations

A. Servais

C. Antignac
Necker, Paris

imagine
INSTITUT DES MALADIES GÉNÉTIQUES

Autosomal Dominant (31%)		
INF2	**2**	
WT1	**1**	**(Frasier)**
PAX2	1	
LMX1B	1	
TRPC6	0	
ACTN4	0	

Collagen genes (44%)	
COL4A3	**4**
COL4A4	**1**
COL4A5	**2**

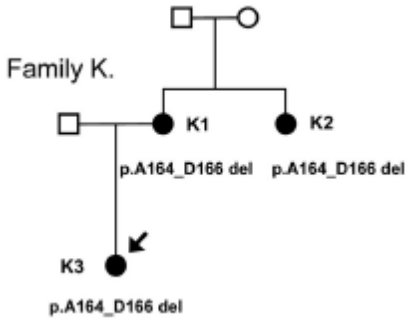
Autosomal Recessive (31%)	
NPHS2	**2**
MYO1E	1
CD2AP	1

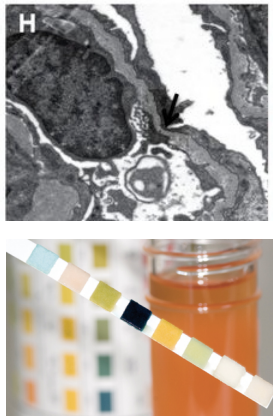
Servais A, Boyer O, … Antignac C. *submitted*

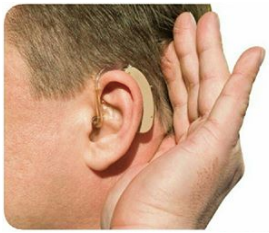
Genetic testing in nephrotic syndrome: who, when, how?

Search for hereditary forms

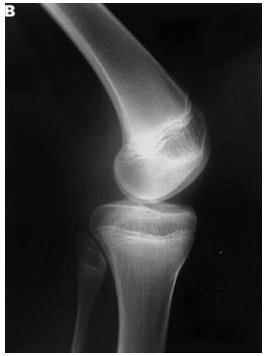
* Tests the parents: Pu, Hu
* search extraR features

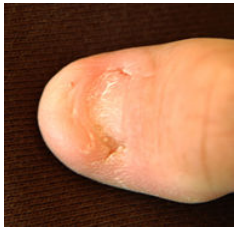






Alport Sd

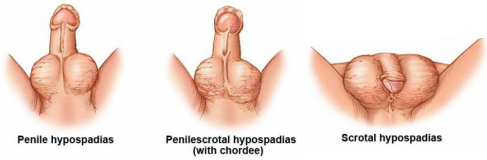




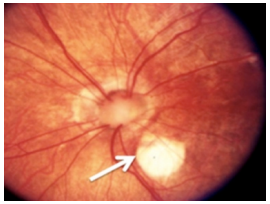
Nail Patella

Mitochondrial disease

CMT



DDS and Frasier Sd



Papillo-renal

Genetic testing in nephrotic syndrome: who, when, how?

Who?

➔ **All childhood cases with SRNS: no or partial remission** under immunosuppressive therapy

➻ **Not in sporadic immune forms (negative Pu or post-Tx recurrence)**

➔ Familial adult cases : Yes

➔ Sporadic adult cases ? < 25 years ?

When?

At the end of the first failed corticosteroid course.

How?

Gene panels …. Until whole exome/genome sequencing for all…

Acknowlegdments

EURenOmics ERKNet

Imagine Institute - Necker

Olivier Gribouval
Aude Servau
Géraldine Mollet
Kalman Tory
Sophie Saunier
Marie-Claire Gubler
Corinne Antignac

Pediatric Nephrology - Necker

Marie-Josèphe Tête
Patrick Niaudet
Rémi Salomon

Imagine Genomic Core Facility

Christine Bole-Feysot

Bioinformatic Core Facility

Patrick Nitschké

All the clinicians who referred the patients

Collaborations

Franz Schaefer (Heidelberg)
Beata Lipska (Gdansk)

ORKiD FILIÈRE ORPHAN KIDNEY DISEASES

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