



UNIVERSITÀ  
DEGLI STUDI  
DI FOGGIA

Dipartimento di Scienze Biomediche  
Centro di Medicina Molecolare  
Ospedali Riuniti - Foggia



# LA GENETICA NELLA SINDROME NEFROSICA

*2° CONGRESSO NAZIONALE*

*medici-famiglie sulla*

*SINDROME NEFROSICA IDIOPATICA*

*Pavia 14 Novembre 2009*

**M. Gigante, PhD**



# SINDROME NEFROSICA IDIOPATICA

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## ▪SN CORTICO-SENSIBILE

**85%**

## ▪SN CORTICO-DIPENDENTE O CON FREQUENTI RECIDIVE

## ▪SN CORTICO-RESISTENTE

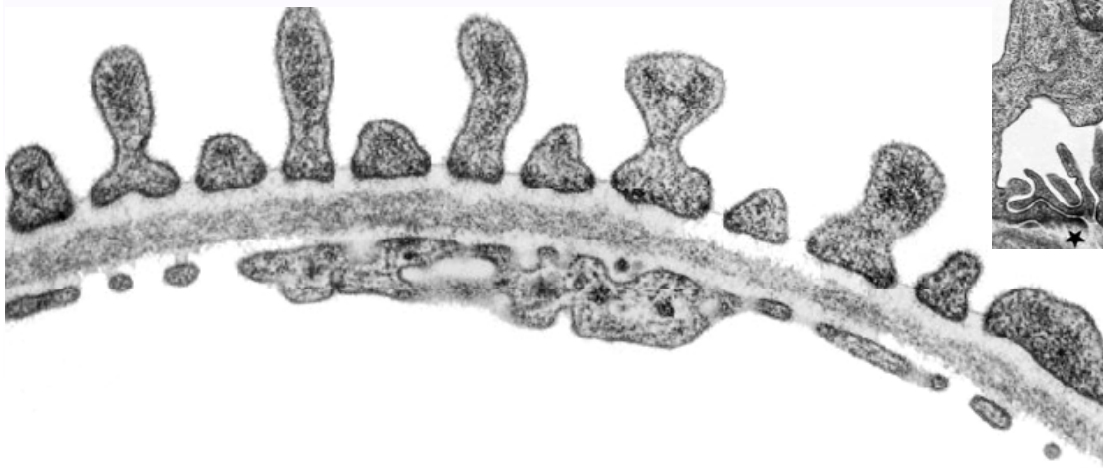
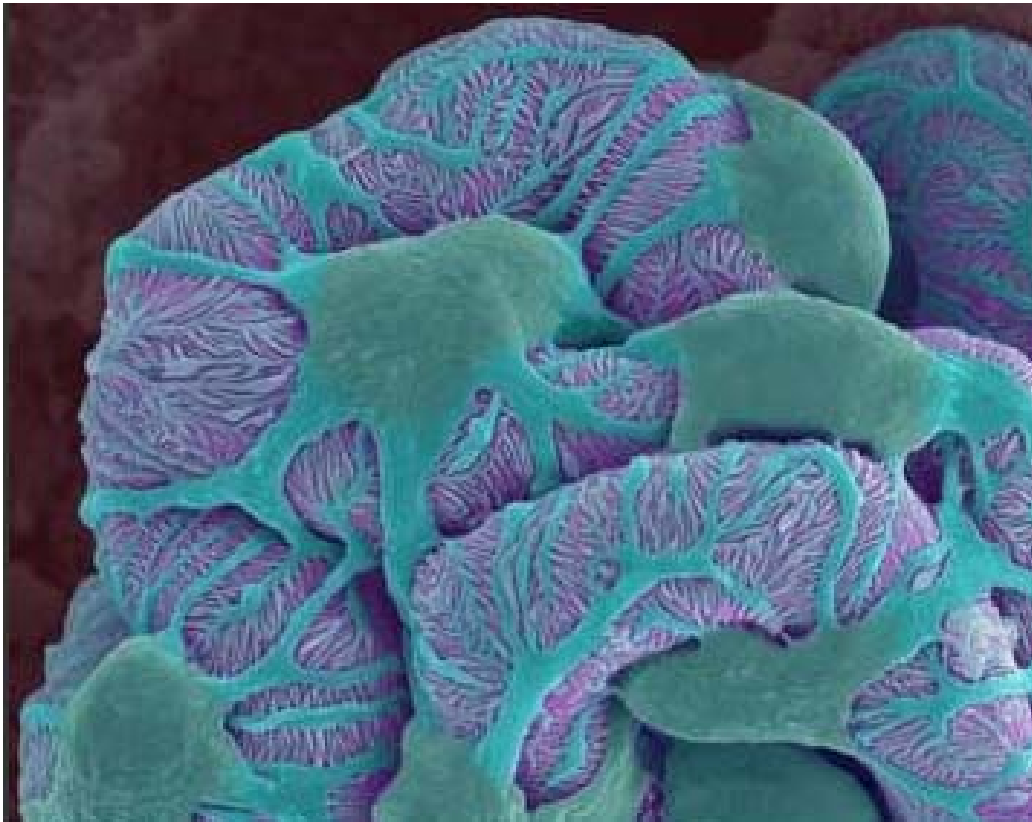
**15%**

**Forme  
familiari**

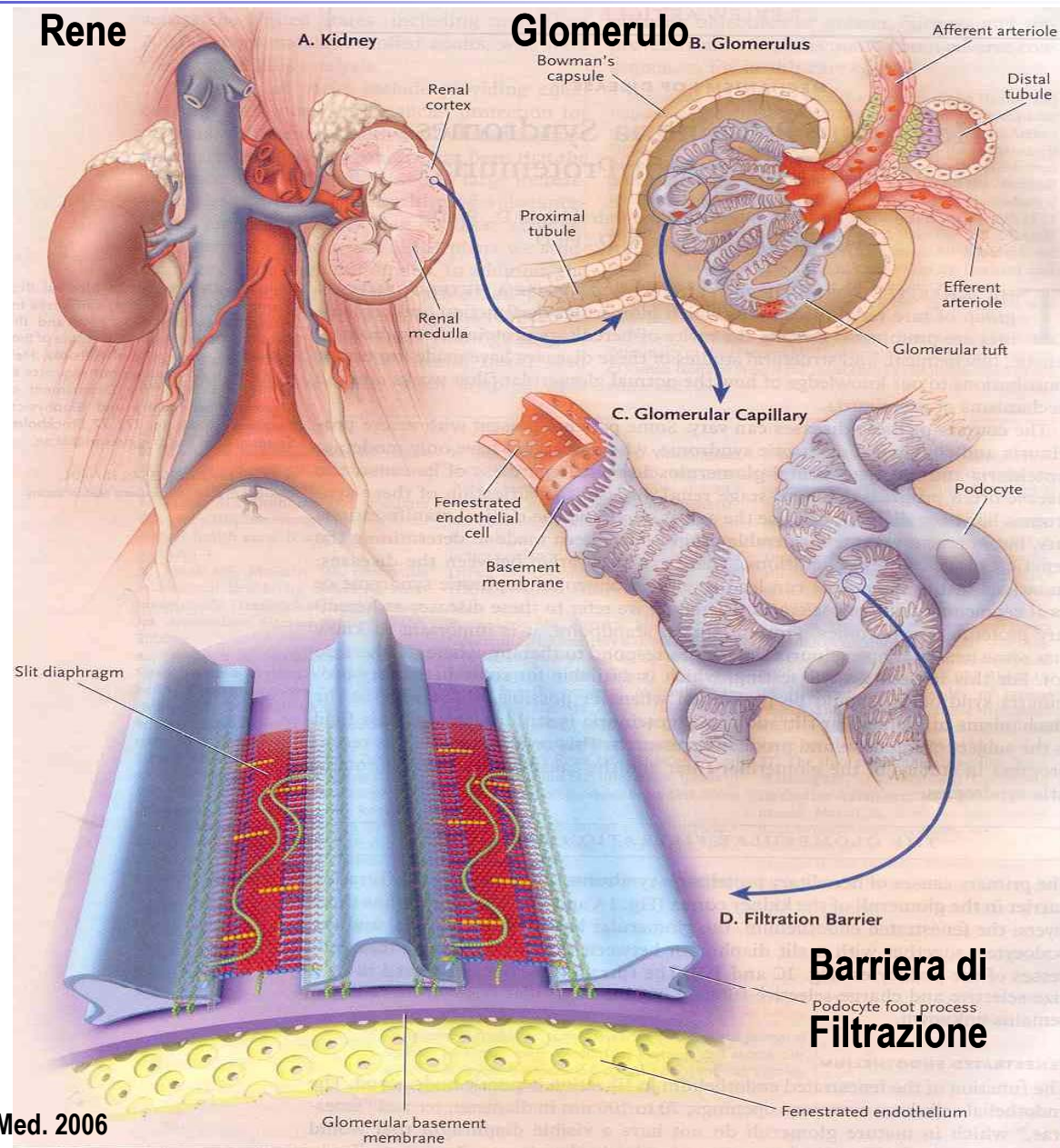
**Alterazioni dei geni  
podocitari**

**Forme  
sporadiche**

# PODOCYTES AND GLOMERULAR DISEASES

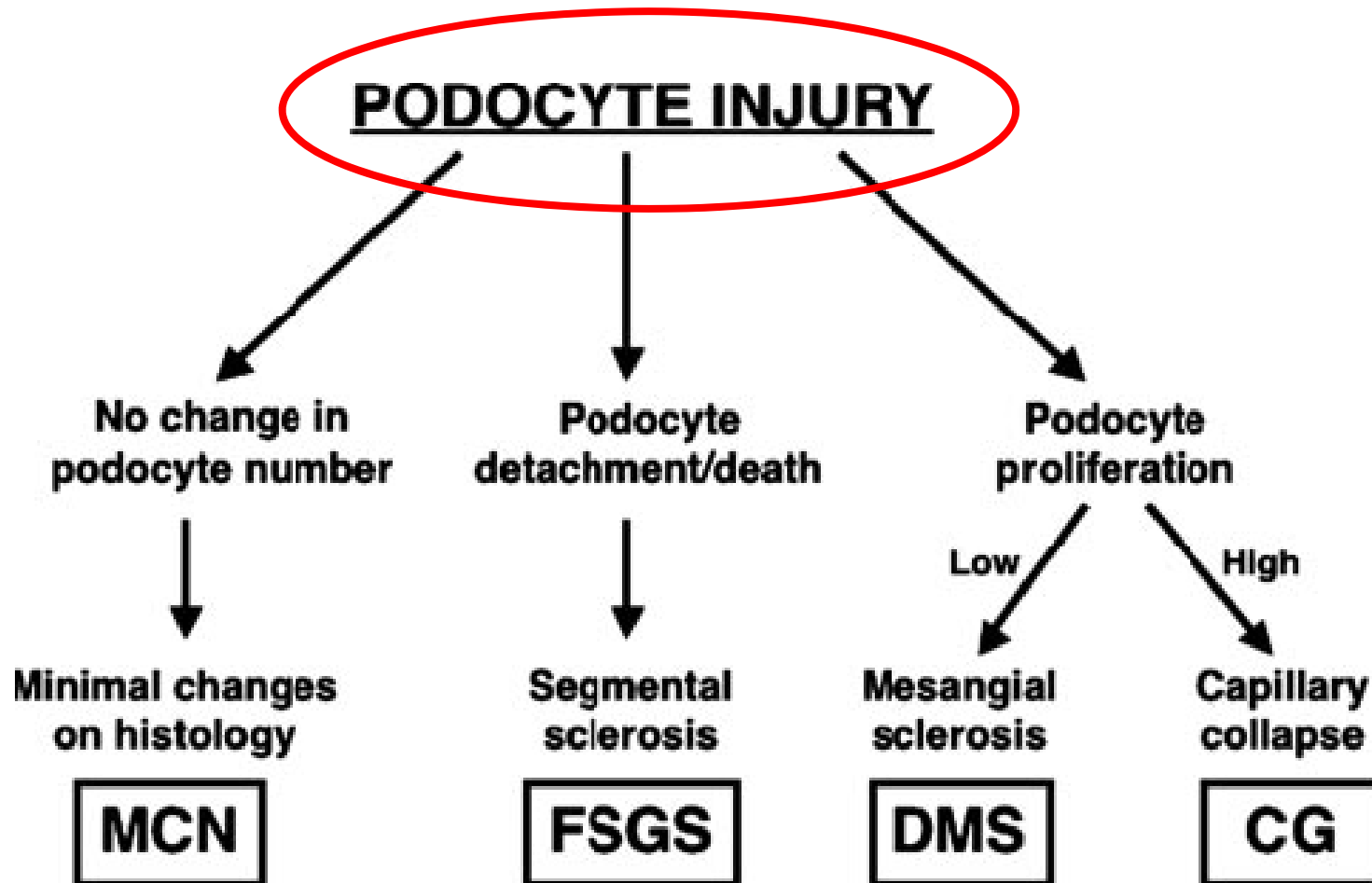


# La barriera di Filtrazione



# PODOCYTOPATHIES

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# Taxonomy of podocytopathies

	Idiopathic Forms	Genetic Forms	Reactive Forms
<b>MCN</b>	<b>Idiopathic MCN</b> Steroid sensitive Steroid resistant	<b>Nonsyndromic</b> NPHS2 <b>Syndromic</b> DYSF (limb-girdle muscular dys-trophy 2B)	<b>Reactive MCN</b> Hodgkin disease Immunogenic stimuli Medication associated
<b>FSGS</b>	<b>Idiopathic FSGS</b>	<b>Nonsyndromic</b> NPHS1 + NPHS2 NPHS2 ACTN4 CD2AP TRPC6 WT1 mtDNA tRNA <sup>Leu</sup> mtDNA tRNA <sup>Tyr</sup> PLCE1 <b>Syndromic</b> WT1 (Frasier) mtDNA tRNA <sup>Leu</sup> (MELAS) PAX2 (renal-coloboma syndrome with oligomeganephronia) LMX1B (nail-patella) COQ2 COQ8± PDSS2 (Leigh) ITGB4 COL4A3, A4, A5 (Alport) GLA (Fabry)	<b>Postadaptive FSGS</b> Reduced nephron mass: renal dysplasia, surgical renal mass reduction, reflux nephropathy, chronic interstitial nephritis Initially normal nephron mass: obesity, increased muscle mass, sickle cell anemia, hypertension Medication associated Cyclosporine, tacrolimus, interferon $\gamma$ , lithium, bisphosphonates

# Taxonomy of podocytopathies

	Idiopathic Forms	Genetic Forms	Reactive Forms
<b>DMS</b>	<b>Idiopathic DMS</b>	<b>Nonsyndromic</b> NPHS1 (CNF†) WT1 NPHS2 PLCE1 LAMB2 <b>Syndromic</b> LAMB2 (Pierson) WT1 (Denys-Drash) COQ6‡	Infection Viruses (HIV-1, parvovirus B19, CMV) Others (Campylobacter enteritis, Mycobacterium tuberculosis) Disease associations Autoimmune diseases, Guillan-Barre syndrome, thrombotic microangiopathy, hematologic malignancy Medications Interferon $\gamma$ , biophosphonates, calcineurin inhibitors Others Permeability factor Severe hyaline arteriopathy
<b>CG</b>	<b>Idiopathic CG</b>	<b>Nonsyndromic</b> COQ2 <b>Syndromic</b> SCARB2/Limp2 (action myoclonus-renal failure) ZMPSTE24 (mandibuloacral dysplasia)	

# I Geni e le Sindromi Nefrosiche Ereditarie

Malattia	Trasmissione	Locus	Gene	Proteina	Referenza
Sindrome nefrosica congenita di tipo Finlandese (CNF)	Autosomica recessiva	19q13.1	<i>NPHS1</i>	Nefrina	<i>Molec Cell</i> , 1998
Sindrome Nefrosica Steroido-Resistente (SRNS)	Autosomica recessiva	1q25-q31	<i>NPHS2</i>	Podocina	<i>Nature Genet</i> , 2000
Sclerosi Mesangiale Diffusa	Autosomica recessiva	10q23	<i>PLCE1</i>	fosfolipasi C epsilon 1 (PLCε1)	<i>Nature Genet</i> , 2006
•Sindrome di Denys-Drash •Sindrome di Frasier •Wilms Tumor	Autosomica dominante	11p13	<i>WT-1</i>	Fattore di trascrizione WT1 (Wilms tumor 1)	<i>Cell</i> , 1990
Sindrome di Pierson	Autosomica recessiva	3p21	<i>LAMB2</i>	Catena b2 della laminina	<i>Hum Mol Genet</i> , 2004
Glomerulosclerosi segmentale focale (FSGS)	Autosomica dominante	6p12	<i>CD2AP</i>	CD2-associated protein	<i>Science</i> , 2003
Glomerulosclerosi segmentale focale (FSGS)	Autosomica dominante	19q13	<i>ACTN4</i>	α-actinina-4	<i>Nature Genet</i> , 2000
Glomerulosclerosi segmentale focale (FSGS)	Autosomica dominante	11q21-q22	<i>TRPC6</i>	Transient receptor potential channel 6	<i>Science</i> , 2005 <i>Nat Genet</i> , 2005
Sindrome nail-patella	Autosomica dominante	9q34.1	<i>LMX-1B</i>	Fattore di trascrizione LMX-1b	<i>Nature Genet</i> , 1998
Sindrome di Alport (AS)	X-linked	Xq22.3	<i>COL4A5</i>	Catena α 5 del collagene di tipo IV	<i>Science</i> , 1990
•Sindrome di Fechtner (FTNS) •Sindrome di Epstein (EPTS)	Autosomica dominante	22q11.2	<i>MYH9</i>	Catena pesante della miosina non muscolare IIA (NMMHC-IIA)	<i>Am J Hum Genet</i> , 2001

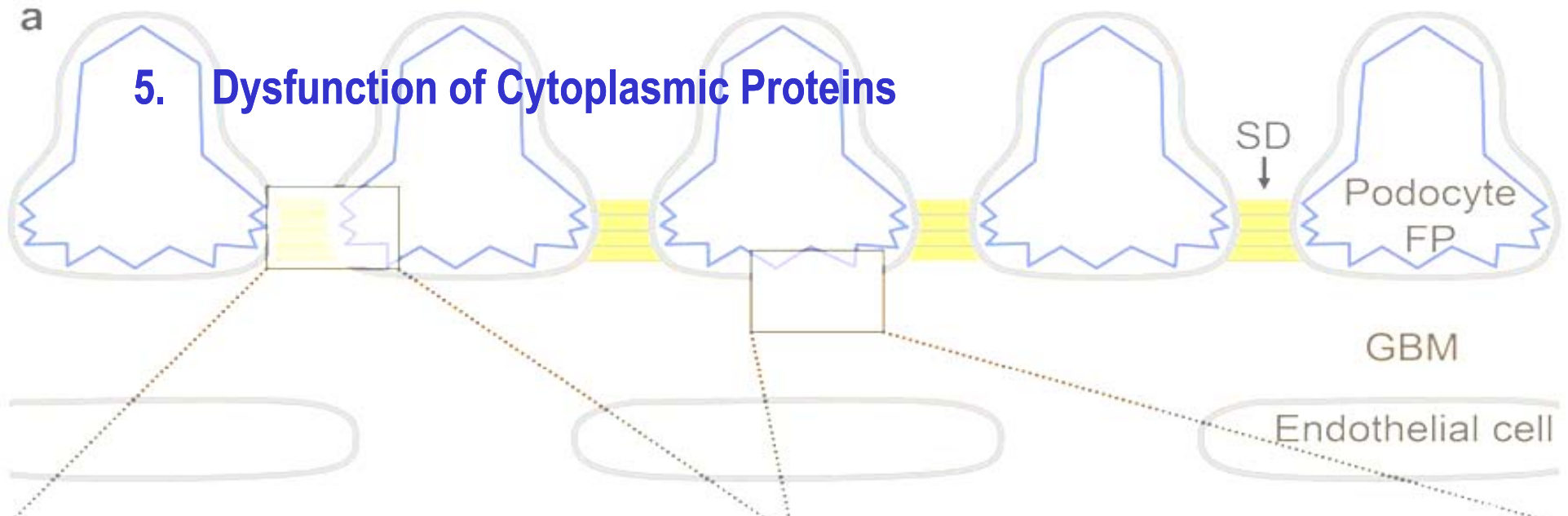


# MECHANISMS OF INJURY IN THE PODOCYTOPATHIES

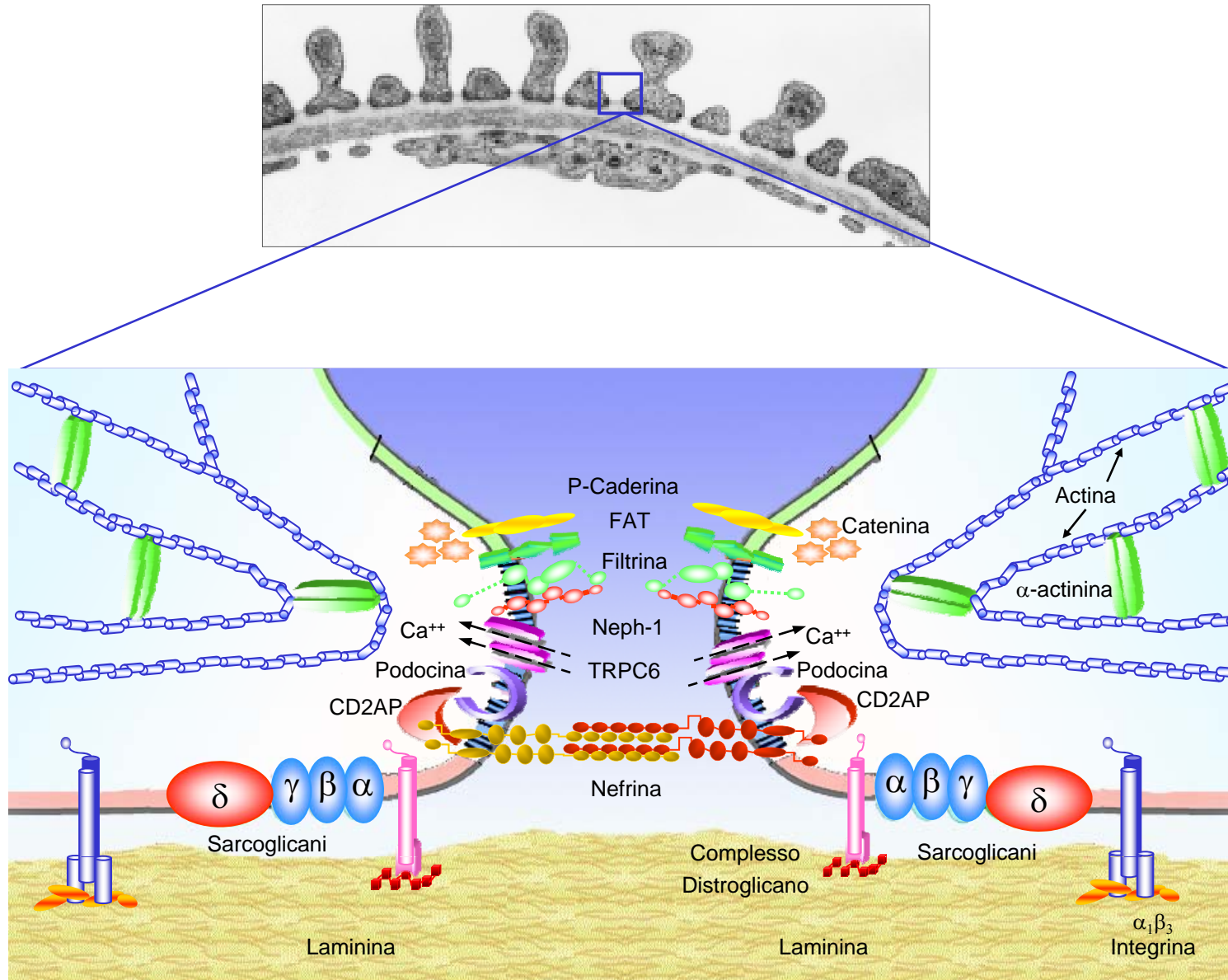
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1. Altered Components of the SD Complex
2. Abnormal Assembly or Function of the Actin-Based Cytoskeleton
3. Expression and Localization of Membrane Proteins
4. Transcriptional Regulators in Podocyte Injury

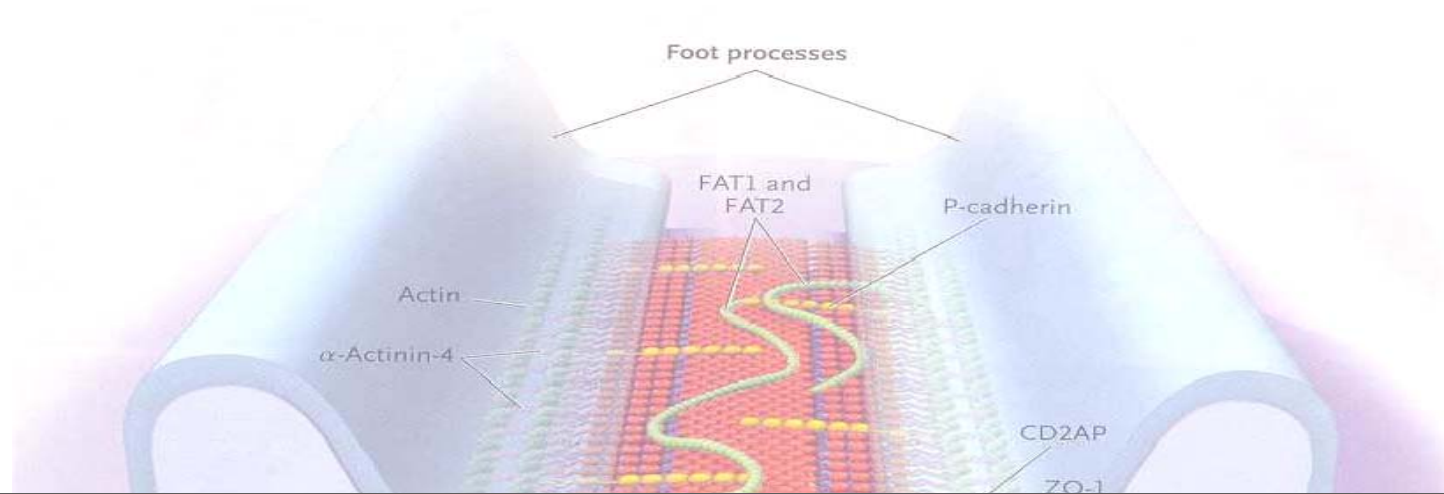
## 5. Dysfunction of Cytoplasmic Proteins



# 1. Altered Components of the SD Complex



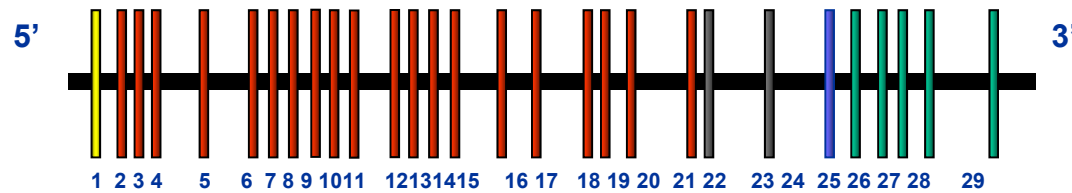
# 1. Altered Components of the SD Complex



List of diseases	Mode of inheritance	Protein	Protein function	Gene	Chrom.	Protein expression slit membrane
Congenital nephrotic syndrome of the Finnish type (CNF)	Autosomal recessive	Nephrin	Key component of the podocyte slit diaphragm	<i>NPHS1</i>	19q13.1	Podocyte slit membrane
Steroid-resistant nephrotic syndrome (SRN1)	Autosomal recessive	Podocin	Establishment of the podocyte slit diaphragm	<i>NPHS2</i>	1q25–q31	Podocyte slit membrane
Familial FSGS	Autosomal recessive	CD2-associated protein	Cytoskeletal remodeling, cell motility, endocytosis	<i>CD2AP</i>	6	Podocyte slit membrane

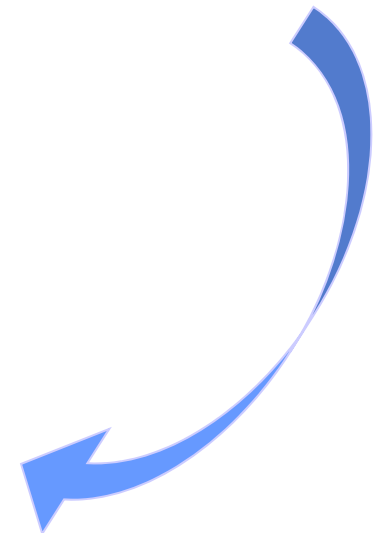
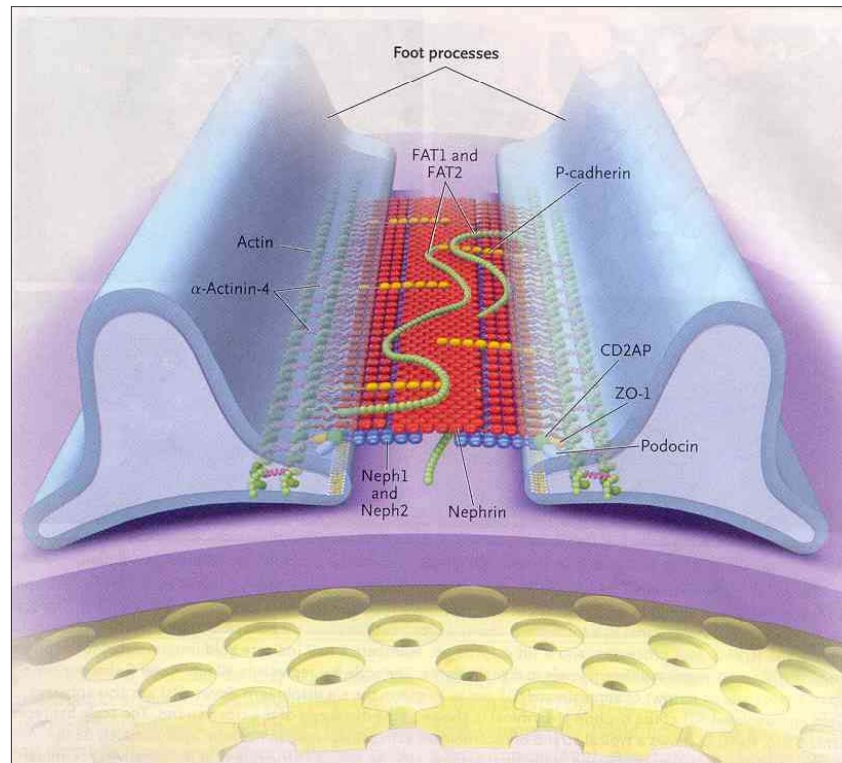
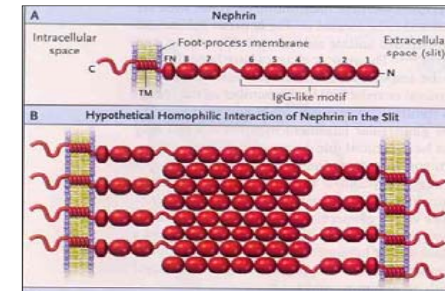
# NEPHRIN

## ❖ Gene NPHS1



Kestilä M *et al*, *Mol Cell* 1998

## NEFRINA



# SN Congenita di tipo Finnico

- Malattia Autosomica Recessiva

- Frequenza: 1:10.000 in Finlandia meno frequente in altri paesi (MALATTIA RARA)

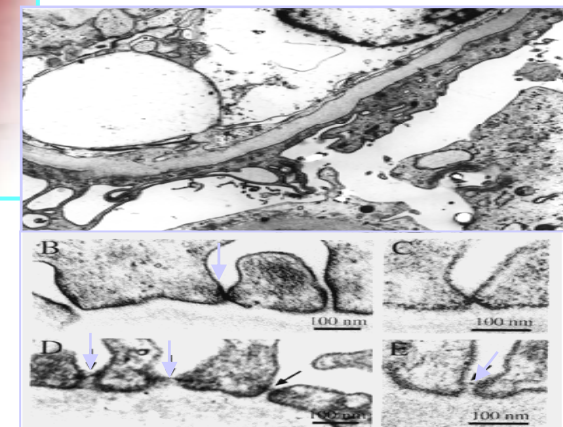
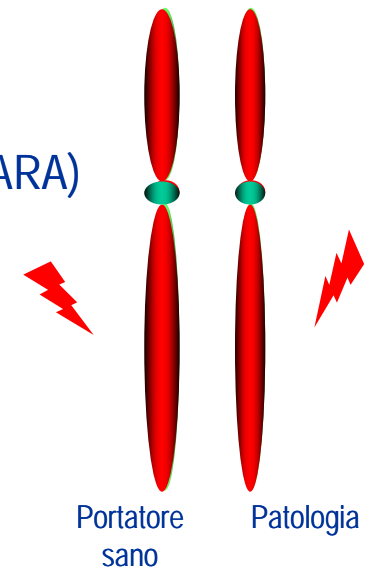
## CLINICA

### Prenatale:

- sviluppo anomalo della placenta
- proteinuria (*in utero*)
- elevati livelli di  $\alpha$ -feto proteina

### ➤ Postnatale

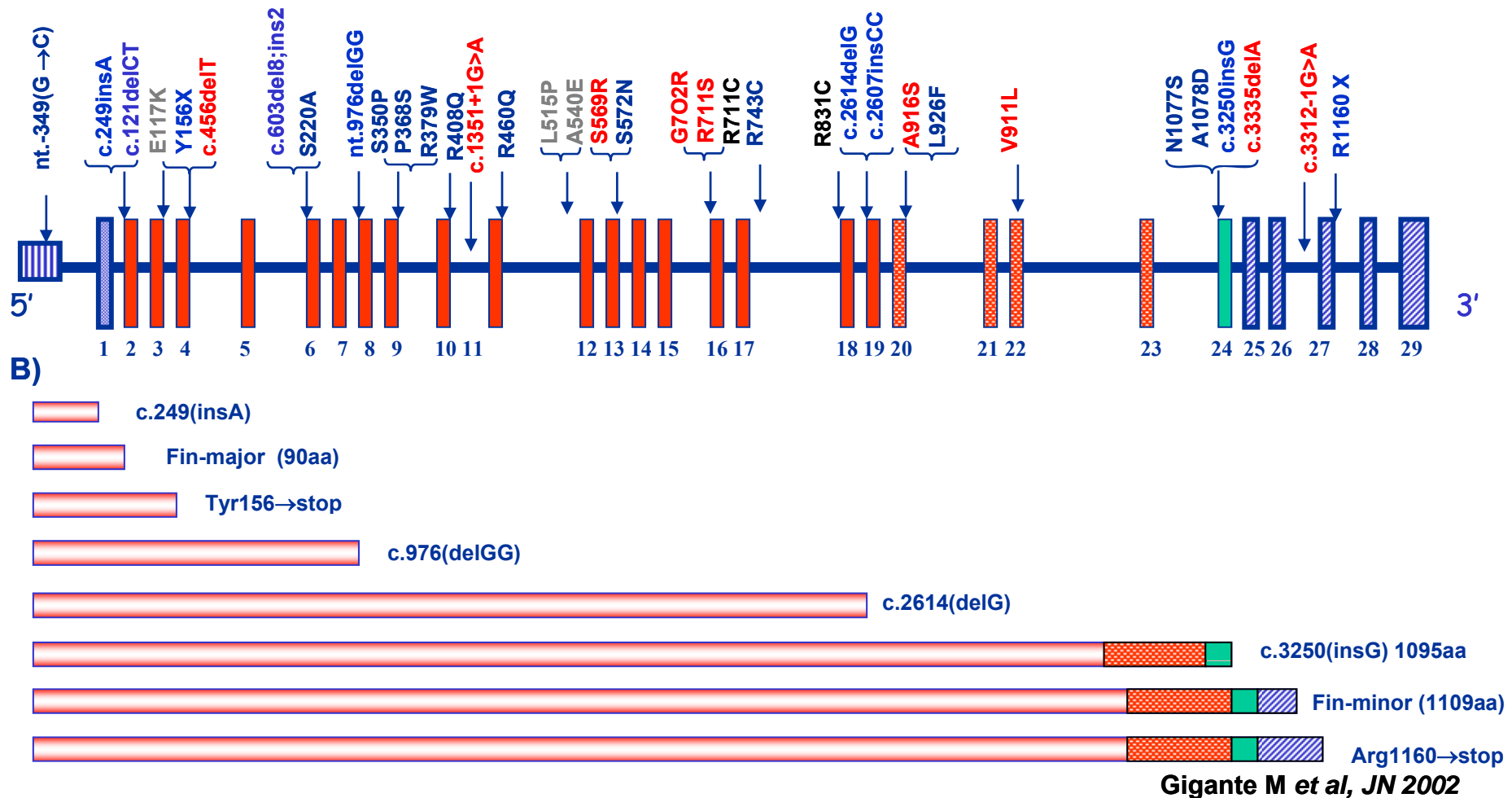
- età di insorgenza: congenita
- prematurità, ipoproteinemia, edema
- biopsia: fusione dei processi pedicellari





# SN Congenita di tipo Finnico

- ❖ In Finlandia: Fin-major e Fin-minor, mutazioni trovate nel 90% dei pazienti
- ❖ In Italia..





# SNC di tipo Finnico in altri paesi

EU Ptz	NPHS1 MUTATIONS	PROTEIN	EXON	HOMO/HETERO	REFERENCE
24s	c.1048T>C	p.S350P	9	Hetero	Lenkkeri, 1999; Gigante, 2002
	<b>1351+1 G&gt;A</b>	<b>Aberrant Splicing</b>	<b>IVS10</b>	<b>Hetero</b>	<b>novel</b>
25s	c.1223G>A	p.R408Q	10	Hetero	Lenkkeri, 1999; Gigante, 2002
	c.1544 T>G	p.L515P	12	Hetero	novel
	c.3335delA; 1112delA	t. p.1142	26	Hetero	novel
26s	c.2104G>C	p.G702R	16	Homo	novel
27s	c.121del CT	p.t. di 90 aa	2	Hetero	Kestila, 1998
	c.1135 C>T	p.R379W	9	Hetero	Beltcheva, 2001
	c.1619 C>A	p.A540E (polim?)	12	Hetero	novel
28s	c.2746 G>A	p.A916S	20	Hetero	novel
29s	c.2746 G>A	p.A916S	20	Hetero	novel



A/NZ Ptz	NPHS1 MUTATIONS	PROTEIN	EXON	HOMO/HETERO	REFERENCE
30a	nt.3478(C>T)	R1160X	27	Hetero	Lenkkeri, 1999; Gigante, 2002
	c.603(delCACCCCGG; insTT)	p.T205;	6	Hetero	Lenkkeri, 1999
	c.603del8;ins2	del P206 e R207			
31a	c.3312 - 1 G>A	Aberrant Splicing	IVS26	Homo	novel
32a	c.2104G->A	p.G702R	16	Hetero	novel
★ 33a	c.456delT	t. p. 174aa	4	Hetero	novel
	c.2131 C>A	p.R711S	16	Hetero	novel
★ 34a	c.2973 G>C	V911L (polim?)	22	Hetero	novel
	c.2131(C>A)	p.R711S	16	Homo	novel
★ 35a	c.2131 C>A	p.R711S	16	Hetero	novel
	c.2607insCC (codon869)	t. p. 904aa	19	Hetero	Lenkkeri, 1999
★ 36a	c.2131 C>A	p.R711S	16	Hetero	novel
	c.2607insCC (codon869)	t. p. 904aa	19	Hetero	Lenkkeri, 1999
★ 37a	c.2131 C>A	p.R711S	16	Homo	novel

# NPHS1

**HGMD® professional release 2009.3 (2009-09-25)**

Mutation type	Total number of mutations	View mutation data sorted by location
Missense/nonsense	91	<a href="#">Get missense/nonsense</a>
Splicing	14	<a href="#">Get splicing</a>
Regulatory	0	No mutations
Small deletions	21	<a href="#">Get small deletions</a>
Small insertions	10	<a href="#">Get small insertions</a>
Small indels	2	<a href="#">Get small indels</a>
Gross deletions	0	No mutations
Gross insertions	0	No mutations
Complex rearrangements	0	No mutations
Repeat variations	0	No mutations
<b>TOTAL</b>	<b>138</b>	<a href="#">Get all mutations</a>

Disease/phenotype	Number of mutations	Mutation data by disease/phenotype
Congenital nephrotic syndrome, Finnish type	96	<a href="#">Get mutations</a>
Nephrotic syndrome	33	<a href="#">Get mutations</a>
Minimal change nephrotic syndrome ?	6	<a href="#">Get mutations</a>
Focal segmental glomerulosclerosis	2	<a href="#">Get mutations</a>

# NEPHRIN

BRIEF COMMUNICATION

www.jasn.org

JASN 2008

## Nephrin Mutations Can Cause Childhood-Onset Steroid-Resistant Nephrotic Syndrome

Aurélie Philippe,<sup>\*†</sup> Fabien Nevo,<sup>\*†</sup> Ernie L. Esquivel,<sup>\*†</sup> Dalia Reklaityte,<sup>\*†</sup>  
Olivier Gribouval,<sup>\*†</sup> Marie-Josèphe Tête,<sup>\*‡</sup> Chantal Loirat,<sup>§</sup> Jacques Dantal,<sup>||</sup>  
Michel Fischbach,<sup>¶</sup> Claire Pouteil-Noble,<sup>\*\*</sup> Stéphane Decramer,<sup>††</sup> Martin Hoehne,<sup>‡‡</sup>  
Thomas Benzing,<sup>‡‡</sup> Marina Charbit,<sup>‡</sup> Patrick Niaudet,<sup>\*†‡</sup> and Corinne Antignac<sup>\*†§§</sup>

- 160 patients (142 unrelated families) with nephrotic syndrome at least **3 mo** after birth were screened for *NPHS1* variants once mutations in *NPHS2* had been excluded.
- Compound heterozygous *NPHS1* mutations were identified in **one** familial case and **nine** sporadic cases. Mutations included protein-truncating nonsense and frameshift mutations, as well as splice-site and missense variants.

## Nephrin Mutations Can Cause Childhood-Onset Steroid-Resistant Nephrotic Syndrome

JASN 2008

Table 1. Clinical data of patients who had SRNS and in whom *NPHS1* mutations were identified<sup>a</sup>

Patient	Gender	Age of Onset Pu (NS) (yr)	Biopsy	Therapy	Evolution	Mutation 1 Severe	Mutation 2 Mild
1420	F	0.25 (3.00)	MCNS	CS, CP	Normal Cr at 14 yr	c.609-2A→C (M)	c.319G→A p.A107T (P)
446	F	0.80 (0.80)	MCNS	CS	ESRF at 9 yr	c.3720_3735del16 p.L1240fs1286X <sup>b</sup> (P)	c.1724C→A p.P575Q (M)
1075	F	0.50 (0.50)	FSGS	Unknown	ESRF at 13 yr	c.1379G→A p.R460Q <sup>b</sup> (?)	c.2928G→T p.R976S (?)
841	F	3.80 (3.80)	FSGS	CS, CsA	Normal Cr at 6 yr	c.468C→G p.Y156X (P)	c.2928G→T p.R976S (M)
466	F	0.25 (0.75)	MCNS	CS, CP	Normal Cr at 10 yr	c.2479C→T p.R827X (M)	c.2928G→T p.R976S (P)
1167	F	3.10 (3.10)	FSGS	CS, CP	ESRF at 15 yr	c.516delC p.T712fs175X (M)	c.2928G→T p.R976S (P)
693	M	8.00 (8.00)	MCNS	CS	Normal Cr at 16 yr	c.1134-1135delGC p.R379fs417X (M)	c.286C→G p.L96V (P)
1407	F	5.00 (5.00)	MCNS	CS, CP	ESRF at 25 yr	c.516delC p.T712fs175X (M)	c.2928G→T p.R976S (P)
634	M	3.00 (3.00)	MP	Unknown	ESRF at 6 yr	c.1491delC p.S494fs547X (M)	c.2072-6C→G (P)
771 <sup>c</sup>	F	2.20 (2.80)	MCNS	CS, CsA	Normal Cr at 9 yr	c.2495T→C	c.2928G→T
1462 <sup>c</sup>	M	2.50 (2.50)	Not performed	No treatment	Normal Cr at 6 yr	p.L832P (P)	p.R976S (M)

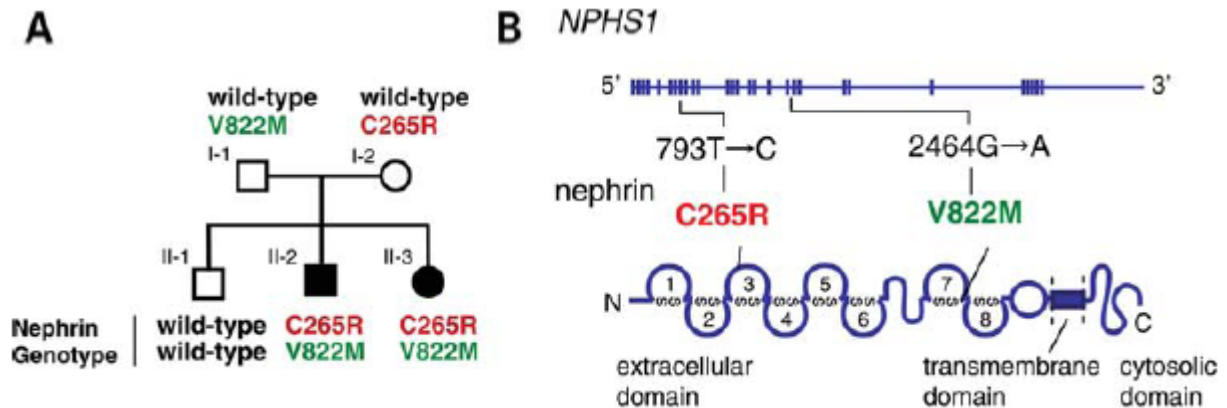
➤ Mutations were classified as “severe” or “mild” using prediction algorithms and functional assays. Most missense variants trafficked normally to the plasma membrane and maintained the ability to form nephrin homodimers suggesting retained function.

➤ The presence of at least one “mild” mutation in these patients likely explains the later onset and milder course of disease.

# Predisposition to relapsing nephrotic syndrome by a nephrin mutation that interferes with assembly of functioning microdomains

Japan

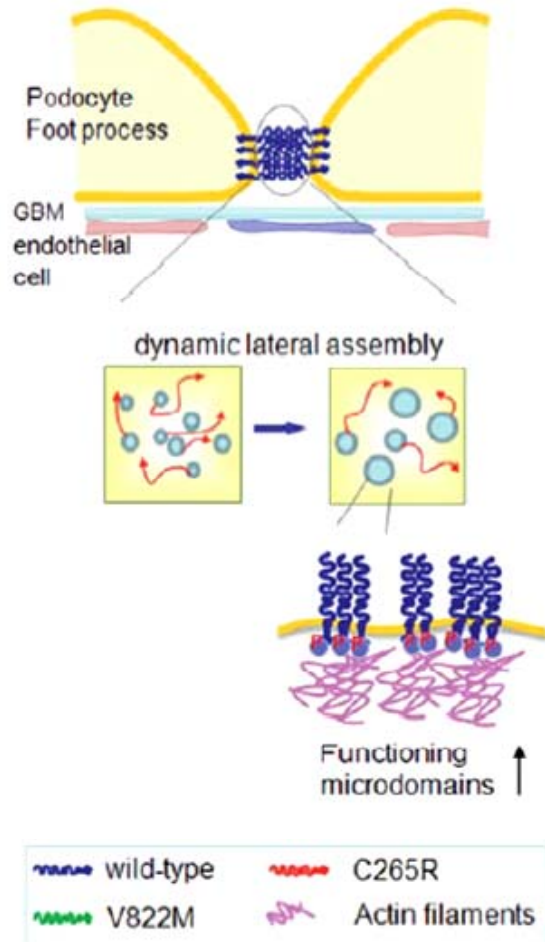
Akemi Shono<sup>1</sup>, Hiroyasu Tsukaguchi<sup>1,3,\*</sup>, Akiko Kitamura<sup>2</sup>, Ryugo Hiramoto<sup>4</sup>, Xiao-Song Qin<sup>1,†</sup>, Toshio Doi<sup>1</sup> and Kazumoto Iijima<sup>5</sup>



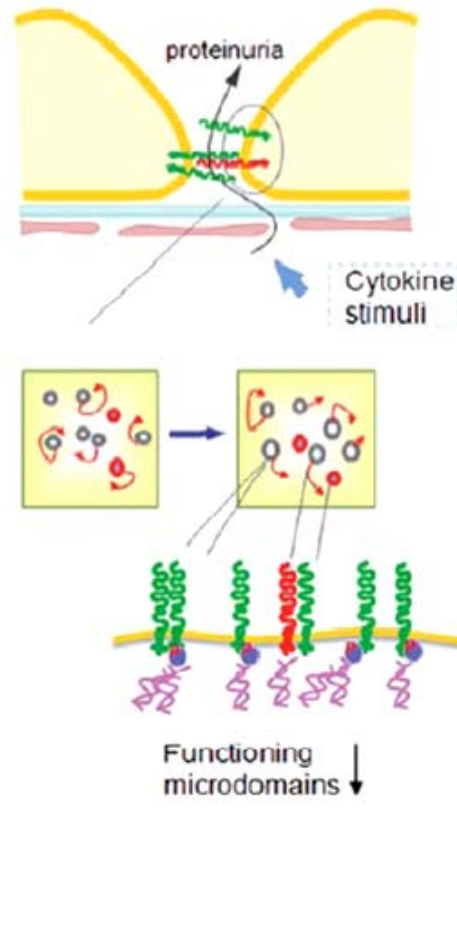
A rare familial NS with minimal-change histology. Both patients exhibited a similar mild and early onset NS with the following characteristic features: (i) NS manifested in the first year of life, (ii) spontaneous though partial remissions with no requirement for steroid therapy, (iii) recurrent NS two to three times per annum that were associated with respiratory infections. The patients exhibited mild but persistent proteinuria.

# NEPHRIN

**A stable SD (wild-type nephrin)**



**B labile SD (C265R+V822M)**



- Surface expression and trafficking of nephrin variants (C265R is largely retained within the cytoplasm)
- Aberrant assembly of membrane rafts upon clustering of V822M
- V822M fails to activate phosphorylation signaling
- V822M is unable to reorganize actin filaments

## DEFECTIVE MICRODOMAIN ASSEMBLY

(B) Defective SD composed of C265R and V822M leading to mild, relapsing NS. The unstable SD complex comprising C265R and V822M is the molecular basis for mild and recurrent NS with minimal-change histology. C265R is a class II mutation that reduces the surface availability at the SD. V822M is mainly class III, which fails to efficiently assemble into functional nephrin complexes at the plasma membrane. C265R and V822M build up the partially defective, labile SD that permits transient massive protein leakage upon cytokine stimulation (i.e. infection). The dysfunctional SD also appears to be leaky to some extent even during the remission period between nephrotic episodes, as evident by the sustained mild proteinuria. Nephrotic episodes presumably arise from the inability of nephrin variants to fully reassemble into the complete SD complex after cytokine injury.



# PHENOTYPIC CLASSES OF NEPHRIN VARIANTS

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Nephrin mutations may lead to dysfunction of the SD via at least three distinct mechanisms:

- **Class I** mutations (nonsense or frameshift mutations), that disrupt the transcription or translation process. Total absence of functioning nephrin leads to a severe, congenital phenotype.
- **Class II** mutations, as observed in most early-onset steroid resistant NS, or C265R in this case, result in the miss-trafficking of proteins that are trapped in the ER.
- **Class III** mutations yield mutant proteins that traffic to the cell membrane but are dysfunctional at the plasma membrane. V822M, for example, does not properly assemble into functional SD complexes.

# Clinical Features and Long-Term Outcome of Nephrotic Syndrome Associated with Heterozygous *NPHS1* and *NPHS2* Mutations

Gianluca Caridi,\* Maddalena Gigante,<sup>†</sup> Pietro Ravani,<sup>‡</sup> Antonella Trivelli,\*  
 Giancarlo Barbano,\* Francesco Scolari,<sup>§</sup> Monica Dagnino,\* Luisa Murer,<sup>||</sup> Corrado Murtas,<sup>¶</sup>  
 Alberto Edefonti,\*\* Landino Allegri,<sup>||</sup> Alessandro Amore,<sup>††</sup> Rosanna Coppo,<sup>††</sup>  
 Francesco Emma,<sup>‡‡</sup> Tommaso De Palo,<sup>§§</sup> Rosa Penza,<sup>|||</sup> Loreto Gesualdo,<sup>†</sup> and  
 Gian Marco Ghiggeri\*

cJASN 2009

NPHS1	Mutations and Variants Nt change; Aa Change (n)	N	Gender	Age at Onset (yr)	Histology	Response to Steroids	Response to CsA	ESRD (n)	Age at ESRD (yr)
Homozygous or compound heterozygous	c.3233; p.A1078D Homo (1) c.1707 C→A; p.S596R Homo (1) c.3312-1G→A; frameshift Homo (1) [c.658T→G; p.S220A + c.3230A→G; p.N1077S] (1) [c.468C→G; p.Y156X – c.3230A→G; p.N1077S] (1) [c.2491C→T; p.R831C + c.3250insG; p.G1083fsX] (2) [c.2491C→T; p.R831C + c.2131C→T; p.R711C] (1) [c.2614delG; p.N870fsX + c.2776C→T; p.L926F] (1) [c.456delT; p.G153fsX; + c.2131C→A; p.R711S] (1) [c.121delCT; p.N40fsX; + c.1135C→T; p.R379W] (1)	11	M: 6 F: 5	0.9 (0 to 3)	CNF: 11	NA: 11	NA: 11	9 <sup>b</sup>	4 (0 to 14)
Heterozygous	c.59-5C→G; frameshift (1) c.563A→T; p.N188I (2) c.1379G→A; p.R460Q (1) c.2491C→T; p.R831C (2) c.2746G→T; p.A916S (1)	7	M: 4 F: 3	6.5 (1 to 16)	None: 1 FSGS: 2 MCN: 3	Good: 5 Poor: 2	NA: 2 Good: 4 Poor: 1	0	
No <i>NPHS1</i> / <i>NPHS2</i> mutations		221	M: 138 F: 83	17 (0 to 68)	None: 51 FSGS: 129 IgM: 22 MCN: 19	NA: 5 Good: 33 Poor: 133	NA: 128 Good: 46 Poor: 47	92	21 (3 to 64)

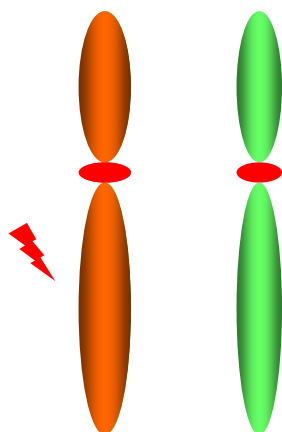
\*Continuous variables are mean (range). Aa, amino acid; CNF, congenital nephrosis of the Finnish type; Homo, homozygous; IgM, mesangial proliferation with IgM deposition; MCN, minimal-change nephropathy; NA, not administered; NS, nephrotic syndrome; Nt, nucleotide.

<sup>b</sup>Two patients died before ESRD.

# NPHS1:

## mutazioni in eterozigosi

1 allele NPHS1 affetto



- Diagnosi precoce
- Patologia meno severa
- Risposta alla terapia



**Background and objectives:** Mutations in nephrin (*NPHS1*) and podocin (*NPHS2*) genes represent a major cause of idiopathic nephrotic syndrome (NS) in children. It is not yet clear whether the presence of a single mutation acts as a modifier of the clinical course of NS.

**Design, setting, participants, & measurements:** We reviewed the clinical features of 40 patients with NS associated with heterozygous mutations or variants in *NPHS1* ( $n = 7$ ) or *NPHS2* ( $n = 33$ ). Long-term renal survival probabilities were compared with those of a concurrent cohort with idiopathic NS.

**Results:** Patients with a single mutation in *NPHS1* received a diagnosis before those with potentially nongenetic NS and had a good response to therapies. Renal function was normal in all cases. For *NPHS2*, six patients had single heterozygous mutations, six had a p.P20L variant, and 21 had a p.R229Q variant. Age at diagnosis and the response to drugs were comparable in all NS subgroups. Overall, they had similar renal survival probabilities as non-*NPHS1*/*NPHS2* cases (log-rank  $\chi^2$  0.84,  $P = 0.656$ ) that decreased in presence of resistance to therapy ( $P < 0.001$ ) and in cases with renal lesions of glomerulosclerosis and IgM deposition ( $P < 0.001$ ). Cox regression confirmed that the only significant predictor of dialysis was resistance to therapy.

**Conclusions:** Our data indicate that single mutation or variant in *NPHS1* and *NPHS2* does not modify the outcome of primary NS. These patients should be treated following consolidated schemes and have good chances for a good long-term outcome.

# Marco e Giulia: Fratelli

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**Marco**  
**Età**  
**Clinica**



**3 anni**  
**addome globoso**  
**algie addominali**  
**edemi palpebrali e aa. inferiori**  
**edema scrotale importante**



**Giulia**  
**Età**  
**Clinica**



**13 anni**  
**modesti edemi palpebrali da 3 gg.**  
**urine ipercromiche da 12 ore**

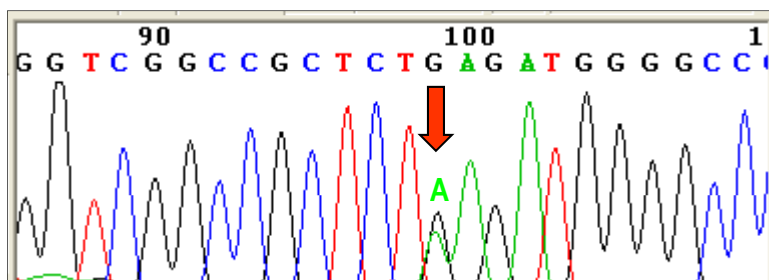


**Screening dei geni NPHS1, NPHS2 e hot spot WT1**

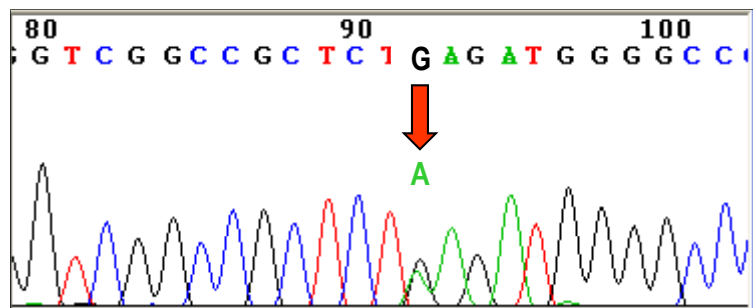


**Nessuna mutazione nei geni NPHS2 e WT1**

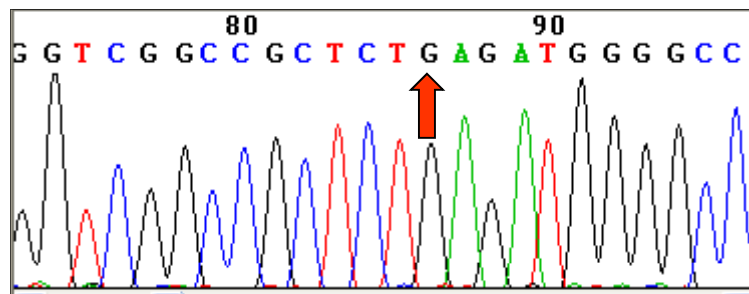
# NPHS1: esone 3 : c.349 G>A; p.Glu117Lys (p.E117K) POLIMORFISMO



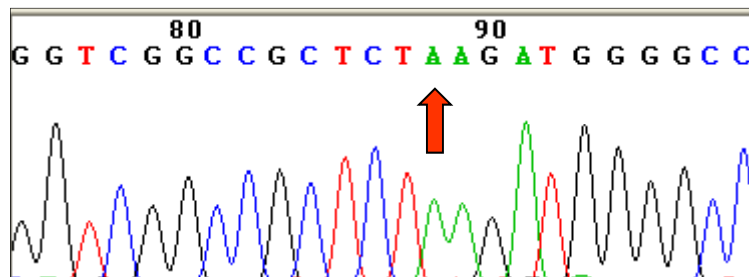
eterozigote



eterozigote



wilde type

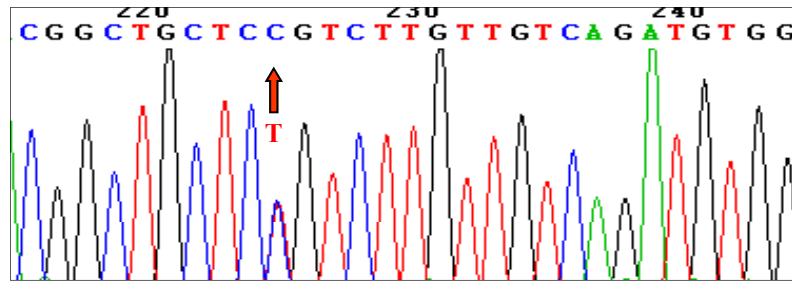


omozigote

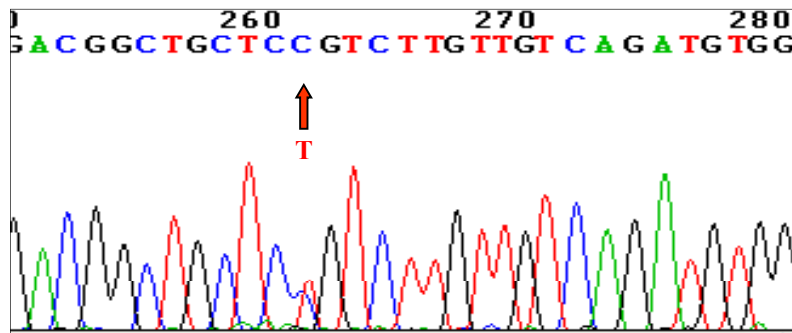




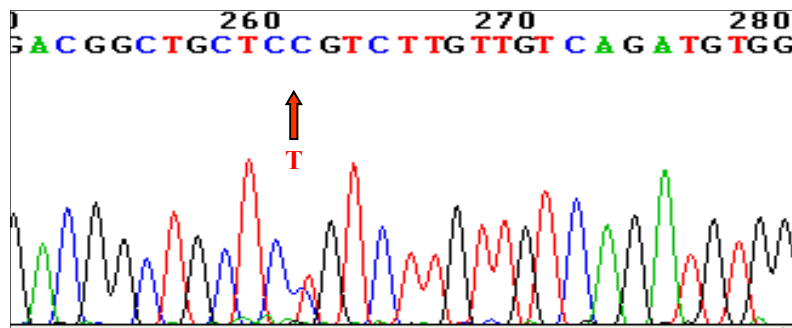
**NPHS1: esone 18: c.2491 C>T; p.Arg831Cys (p. R831C)**  
**MUTAZIONE**



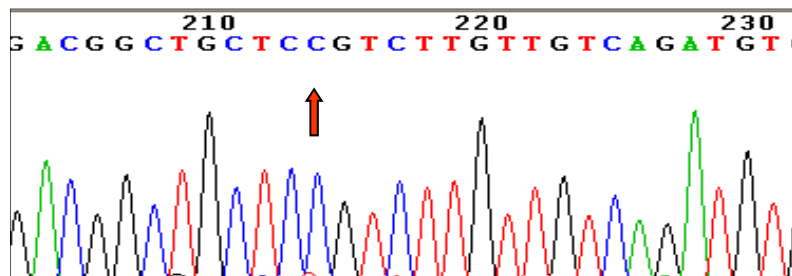
**eterozigote**



**eterozigote**



**eterozigote**



**wilde type**



**MARCO**

**BR**

ottobre '02

gennaio '03

maggio -settembre '03

gennaio '07

aprile '08



**glomerulopatia a lesioni minime**

**inizia CsA (4 mg/kg)**

**morbillo → sospende CsA**

**RECIDIVE → riprende CsA (4 → 2 mg/kg)**

**REMISSIONE COMPLETA**

**RECIDIVE**

**riprende CsA (3 → 2 mg/kg)**

**REMISSIONE COMPLETA**



**GIULIA**

**BR**

giugno '07

luglio '07

settembre '07

aprile '08



**Nefropatia a depositi mesangiali di IgA**

**inizia CsA (4 mg/kg) + prednisone**

**intossicazione da steroide → riduce lo steroide**

**IRA da CsA → dimezza la dose**

**creatinina ↑ → dimezza la dose**

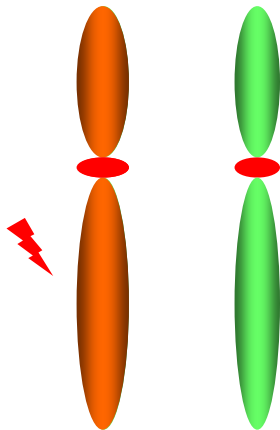
**REMISSIONE COMPLETA**



# NPHS1:

## mutazioni in eterozigosi

1 allele NPHS1 affetto



- Diagnosi precoce
- Patologia meno severa
- Risposta alla terapia

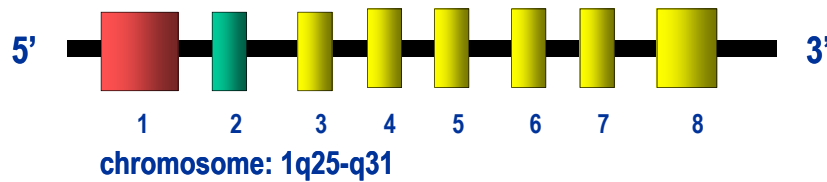


➤ **Singole mutazioni nel gene NPHS1 non modificano l'outcome: tali pazienti devono essere trattati secondo i consolidati schemi terapeutici ed hanno delle ottime prospettive di un outcome a lungo termine.**

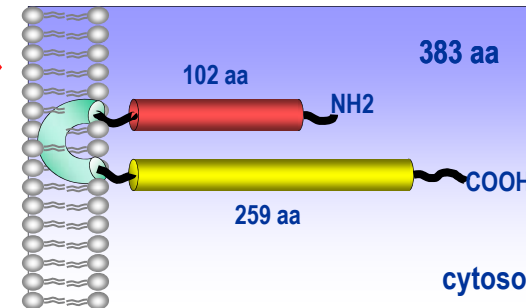
# PODOCINA

Boute et al, *Nat Genet* 2000

## ❖ Gene NPHS2



## PODOCINA



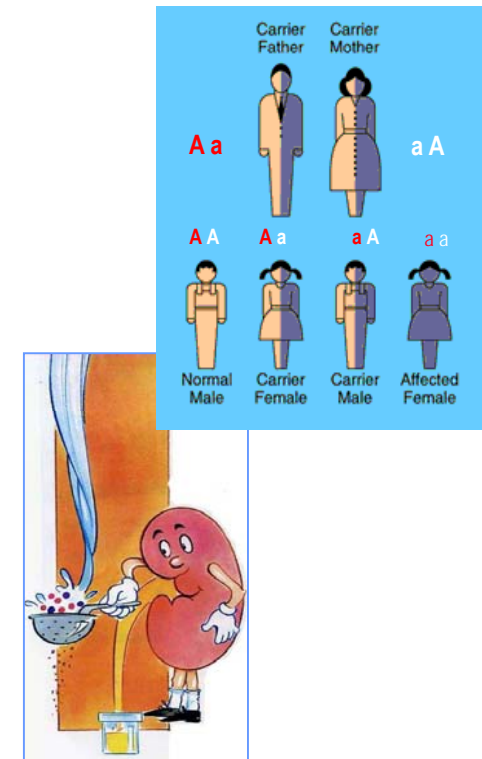
## ❖ Sindrome Nefrosica Steroido-Resistente (SRNS)

Forma familiare con ereditarietà autosomica recessiva

## ❖ Precoce età di esordio (3 mesi/5 anni)

## ❖ Resistenza al trattamento immuno-soppressivo

## ❖ Rapida progressione verso l'insufficienza renale terminale



# NPHS2

**HGMD® professional release 2009.3 (2009-09-25)**

<b>TOTAL</b>	<b>110</b>
<b>Disease/phenotype</b>	<b>Number of mutations</b>
Nephrotic syndrome, steroid resistant	51
Nephrotic syndrome	34
Focal segmental glomerulosclerosis	16
Nephrotic syndrome, steroid resistant ?	3
Reduced expression, association with	3
Focal segmental glomerulosclerosis, assoc. with	1
Nondiabetic end-stage renal disease, assoc. with	1
Thin basement membrane disease ?	1

## Specific Podocin Mutations Correlate with Age of Onset in Steroid-Resistant Nephrotic Syndrome

Bernward Hinkes,<sup>\*†</sup> Christopher Vlangos,<sup>\*</sup> Saskia Heeringa,<sup>\*</sup> Bettina Mucha,<sup>\*</sup> Rasheed Gbadegesin,<sup>\*</sup> Jinhong Liu,<sup>\*</sup> Katrin Hasselbacher,<sup>\*</sup> Fatih Ozaltin,<sup>‡</sup> Friedhelm Hildebrandt,<sup>\*§</sup> and Members of the APN Study Group

Departments of <sup>\*</sup>Pediatrics and <sup>§</sup>Human Genetics, University of Michigan, Ann Arbor, Michigan; <sup>†</sup>Kinder- und Jugendklinik, Universität Erlangen-Nürnberg, Erlangen, Germany; and <sup>‡</sup>Department of Pediatric Nephrology, Hacettepe University, Ankara, Turkey

JASN 2008

**Table 1.** A total of 430 patients from 404 families with SRNS analyzed for mutations and sequence changes in *NPHS2*<sup>a</sup>

Group	<i>NPHS2</i> Mutation	Affected Individuals		Affected Families		Mean Onset (yr)	Onset Range (yr)
		Total	Onset known	Total	Onset known		
A	Truncating × any <sup>b</sup>	32	31	29	28	1.75 <sup>c</sup>	0.0 to 9.1
B	R138Q × R138Q	27	26	22	21	1.77 <sup>c</sup>	0.0 to 5.4
C	R138Q × missense	10	9	9	8	5.95	0.0 to 14.3
D	Missense × missense	13	12	13	12	4.17	0.0 to 16.6
		82	78	73	69	2.61	0.0 to 16.6
E	Single × R229Q	14	13	13	12	6.74	0.8 to 16.3
F	Single × ?	9	9	8	8	8.12	1.6 to 14.7
G	R229Q × R229Q	2	2	2	2	6.48	0.0 to 13.0
	R229Q × ?	24	23	24	23		
		49	47	47	45	6.87	0.0 to 16.3
H	No mutation	299	267	284	253	6.4	0.0 to 21.0
Total		430	392	404	367	5.71	0.0 to 21.0

All patients affected by truncating or homozygous R138Q mutations developed SRNS before 6 yr of age. Patient groups with other recessive podocin mutations, with single heterozygous podocin mutations, with sequence variants, and with no podocin changes could not be distinguished from each other on the basis of age of onset.



# PODOCIN

---

## Novel mutations in NPHS2 detected in both familial and sporadic steroid-resistant nephrotic syndrome.

Karle SM, Uetz B, Ronner V, Glaeser L, Hildebrandt F, Fuchshuber A.

J Am Soc Nephrol. 2002 Feb;13(2):388-93.

PMID: 11805166 [PubMed - indexed for MEDLINE]

[Related Articles](#) [Free article at journal site](#)

•Sporadic

## Not all in the family: mutations of podocin in sporadic steroid-resistant nephrotic syndrome.

Winn MP.

J Am Soc Nephrol. 2002 Feb;13(2):577-9. Review. No abstract available.

PMID: 11805190 [PubMed - indexed for MEDLINE]

[Related Articles](#) [Free article at journal site](#)

•Late-onset

## Genotype/phenotype correlations of NPHS1 and NPHS2 mutations in nephrotic syndrome advocate a functional inter-relationship in glomerular filtration.

Koziell A, Grech V, Hussain S, Lee G, Lenkkeri U, Tryggvason K, Scambler P.

Hum Mol Genet. 2002 Feb 15;11(4):379-88.

PMID: 11854170 [PubMed - indexed for MEDLINE]

[Related Articles](#) [Free article at journal site](#)

## NPHS2 mutations in late-onset focal segmental glomerulosclerosis: R229Q is a common disease-associated allele.

Tsukaguchi H, Sudhakar A, Le TC, Nguyen T, Yao J, Schwimmer JA, Schachter AD, Poch E, Abreu PF, Appel GB, Pereira AB, Kalluri R, Pollak MR.

J Clin Invest. 2002 Dec;110(11):1659-66.

PMID: 12464671 [PubMed - indexed for MEDLINE]

[Related Articles](#) [Free article in PMC | at journal site](#)

## Broadening the spectrum of diseases related to podocin mutations.

Caridi G, Bertelli R, Di Duca M, Dagnino M, Emma F, Onetti Muda A, Scolari F, Miglietti N, Mazzucco G, Murer L, Carrea A, Massella L, Rizzoni G, Perfumo F, Ghiggeri GM.

J Am Soc Nephrol. 2003 May;14(5):1278-86.

PMID: 12707396 [PubMed - indexed for MEDLINE]

[Related Articles](#) [Free article at journal site](#)

## Podocin mutations in sporadic focal-segmental glomerulosclerosis occurring in adulthood.

Caridi G, Bertelli R, Scolari F, Sanna-Cherchi S, Di Duca M, Ghiggeri GM.

Kidney Int. 2003 Jul;64(1):365. No abstract available.

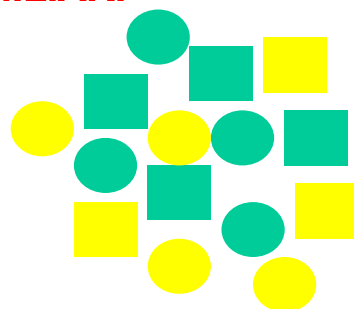
---

# Mutazioni NPHS2 e forme sporadiche

Identificazione di mutazioni NPHS2

in casi sporadici **NON FAMILIARI**

(10-30%)



Importante per l'approccio

terapeutico



Pazienti con SNSR e mutazioni NPHS2 non presentano **mai completa remissione** dopo trattamento con ciclosporina o ciclofosfamide; una **terapia prolungata** con ciclosporina può essere nefrotossica.



Di fronte a casi sporadici di SNSR, prima di intraprendere la terapia immunosoppressiva, è sempre consigliabile un **rapido screening del gene NPHS2**

PEDIATRICS, 2007

## Nephrotic Syndrome in the First Year of Life: Two Thirds of Cases Are Caused by Mutations in 4 Genes (*NPHS1*, *NPHS2*, *WT1*, and *LAMB2*)

Bernward G. Hinkes, MD<sup>a</sup>, Bettina Mucha, MD<sup>a</sup>, Christopher N. Vlangos, PhD<sup>a</sup>, Rasheed Gbadegesin, MD, FAAP<sup>a</sup>, Jinhong Liu, MD<sup>a</sup>, Katrin Hasselbacher<sup>a</sup>, Daniela Hangan, MD<sup>a</sup>, Fatih Ozaltin, MD<sup>b</sup>, Martin Zenker, MD<sup>c</sup>, Friedhelm Hildebrandt, MD<sup>a</sup>, and members of the Arbeitsgemeinschaft für Paediatrische Nephrologie Study Group

- 
- (1) NSFL is a monogenic disease and two thirds of NSFL can be explained by mutations in these 4 genes: *NPHS1*, *NPHS2*, *WT1*, or *LAMB2* only;
  - (2) children with *NPHS1* mutations manifest as CNS only;
  - (3) **mutations in *NPHS2* are an additional frequent cause of CNS among central European children;**
  - (4) Infants with causative mutations in any of the 4 genes do not respond to steroid treatment, and, therefore, unnecessary treatment attempts can be avoided.

# Clinical Features and Long-Term Outcome of Nephrotic Syndrome Associated with Heterozygous *NPHS1* and *NPHS2* Mutations

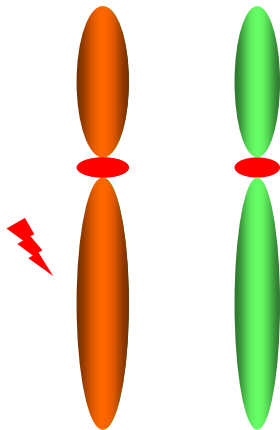
cJASN 2009

NPHS2	Mutations and Variants Nt change; Aa Change (n)	N	Gender	Age at Onset (yr)	Histology	Response to Steroids	Response to CsA	ESRD (n)	Age at ESRD (yr)
Homozygous or compound heterozygous	c.419delG; p.G140fsX Homo (5) c.413G→A; p.R138Q Homo (3) c.506T→C; p.L169P Homo (2) [c.412C→T; p.R138X + c.413G→A; p.R138Q] (2) [c.419delG; p.G140fsX + c.506T→C; p.L169P] (1) [c.413G→A; p.R138Q + c.538G→A; p.V180M] (1) [c.413G→A; p.R138Q + c.855_56delAA; p.Q285fsX] (1) [c.413G→A; p.R138Q + c.973C→T; p.H325Y] (1) [c.467_468insT; p.L156fsX + c.538G→A; p.V180M] (1)	17	M: 11 F: 6	4 (0 to 18)	None:4 FSGS:11 IgM:1 MCN: 1	Poor: 17	NA:9 Good:1 Poor: 8	13	8 (3 to 20)
p.P20L heterozygous	c.59C→T; p.P20L (6)	6	M: 5 F: 1	15 (2 to 64)	None:2 FSGS:3 IgM: 1	Good:2 Poor: 4	NA:4 Good:1 Poor: 1	2	8 (7 to 9)
p.R229Q heterozygous	c.686G→A; p.R229Q (21)	21	M: 13 F: 8	12 (1 to 42)	None:7 FSGS:8 IgM:3 MCN: 3	Good:6 Poor: 15	NA:12 Good:8 Poor: 1	6	22 (1 to 50)
Others	c.419delG; p.L139fsX (1) c.451 + 3insA; frameshift (1) c.555delT; p.M184fsX (1) c.631T→A; p.S211T (1) c.872G→A; p.R291Q (2)	6	M: 4 F: 2	10 (1 to 34)	None:2 FSGS:2 MCN: 2	Good:3 Poor: 3	NA:4 Good:1 Poor: 1	2	22 (6 to 38)
No <i>NPHS1</i> / <i>NPHS2</i> mutations		221	M: 138 F: 83	17 (0 to 68)	None:51 FSGS:129 IgM:22 MCN:19	NA:5 Good:33 Poor:183	NA:128 Good:46 Poor:47	92	21 (3 to 64)

# NPHS2:

## mutazioni in eterozigosi

1 allele NPHS2 affetto

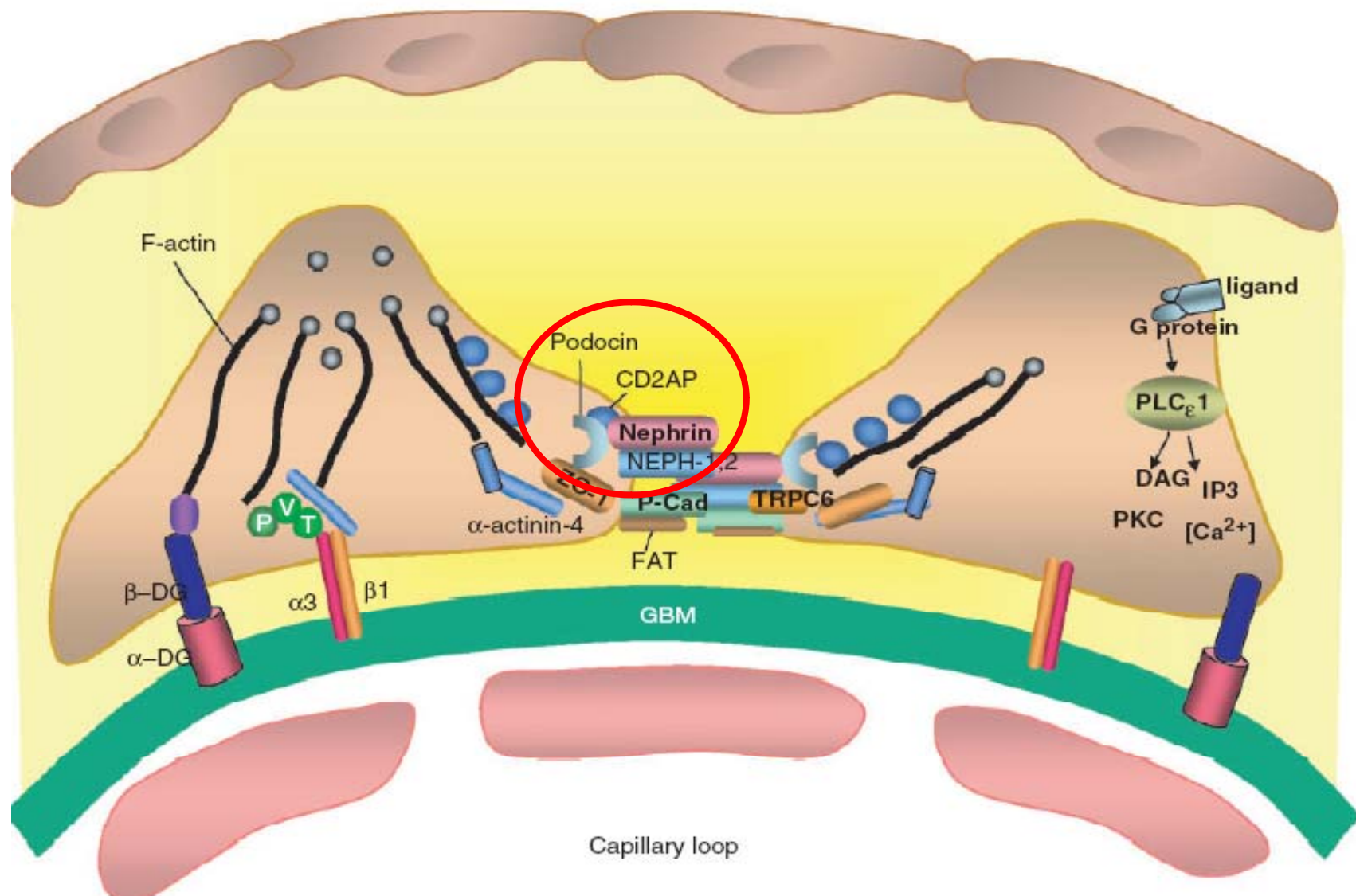


- Nessuna differenza nell'outcome
- Risposta alla terapia



➤ **Singole mutazioni nel gene NPHS2 non modificano l'outcome: tali pazienti devono essere trattati secondo i consolidati schemi terapeutici ed hanno delle ottime prospettive di un outcome a lungo termine.**

# CD2AP





# CD2AP

**HGMD<sup>®</sup> professional release 2009.3 (2009-09-25)**

**Missense/nonsense : 4 mutations** [\[back to top\]](#)

Codon change	Amino acid change	Codon number	Phenotype	Reference
AAG-ATG	Lys-Met	301	Nephrotic syndrome	<a href="#">Gigante (2009) Nephrol Dial Transplant epub, epub</a>
aACA-GCA	Thr-Ala	374	Nephrotic syndrome	<a href="#">Gigante (2009) Nephrol Dial Transplant epub, epub</a>
ATGc-ATA	Met-Ile	496	Glomerulosclerosis, focal segmental	<a href="#">Löwik (2008) Nephrol Dial Transplant 23, 3146</a>
gCGA-TGA	Arg-Term	612	Glomerulosclerosis, focal segmental	<a href="#">Lowik (2007) Kidney Int 72, 1198</a>

**Small deletions : 1 mutation** [\[back to top\]](#)

Deletion	Codon (^)	Phenotype	Reference
TACCA^AAAGaagaAGACAGTGCC	523	Nephrotic syndrome	<a href="#">Gigante (2009) Nephrol Dial Transplant epub, epub</a>

**Small indels : 1 mutation** [\[back to top\]](#)

Deletion	Insertion	Codon(^)	Phenotype	Reference
TTCTATTCTAg_I6E7_cCC^TTAATCCT	ct	245	Glomerulosclerosis, focal segmental	<a href="#">Kim (2003) Science 30, 1298</a>



# CD2AP

## CD2-Associated Protein Haploinsufficiency Is Linked to Glomerular Disease Susceptibility

Jeong M. Kim,<sup>1\*</sup> Hui Wu,<sup>1\*</sup> Gopa Green,<sup>1</sup> Cheryl A. Winkler,<sup>3</sup>  
Jeffrey B. Kopp,<sup>4</sup> Jeffrey H. Miner,<sup>2</sup> Emil R. Unanue,<sup>1</sup>  
Andrey S. Shaw<sup>1†</sup>

*Science* 2003

In casi sporadici di FSGS ad esordio  
tardivo, è stata trovata una variazione  
CD2AP in eterozigosi

Anche in omozigosi...

## Focal segmental glomerulosclerosis in a patient homozygous for a CD2AP mutation

MM Löwik<sup>1</sup>, PJTA Groenen<sup>2</sup>, I Pronk<sup>1</sup>, MR Lilien<sup>3</sup>, R Goldschmeding<sup>4</sup>, HB Dijkman<sup>2</sup>, EN Levtchenko<sup>1</sup>,  
LA Monnens<sup>1</sup> and LP van den Heuvel<sup>1</sup>

KI 2007

Nephrol Dial Transplant (2009) 1 of 7  
doi: 10.1093/ndt/gfn712

*Original Article*

**NDT**  
Nephrology Dialysis Transplantation

## CD2AP mutations are associated with sporadic nephrotic syndrome and focal segmental glomerulosclerosis (FSGS)

Maddalena Gigante<sup>1</sup>, Paola Pontrelli<sup>1,\*</sup>, Eustacchio Montemurno<sup>1,\*</sup>, Leonarda Roca<sup>1</sup>, Filippo Aucella<sup>1</sup>,  
Rosa Penza<sup>1</sup>, Gianluca Caridi<sup>2</sup>, Elena Ranieri<sup>1</sup>, Gian Marco Ghiggeri<sup>2</sup> and Loreto Gesualdo<sup>1</sup>

# CD2AP

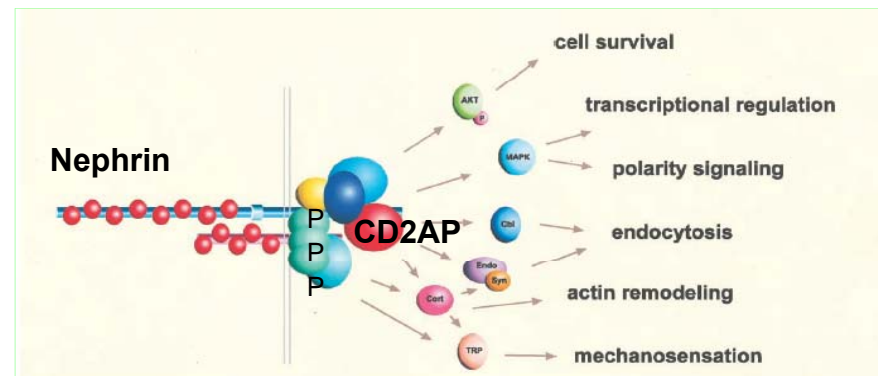
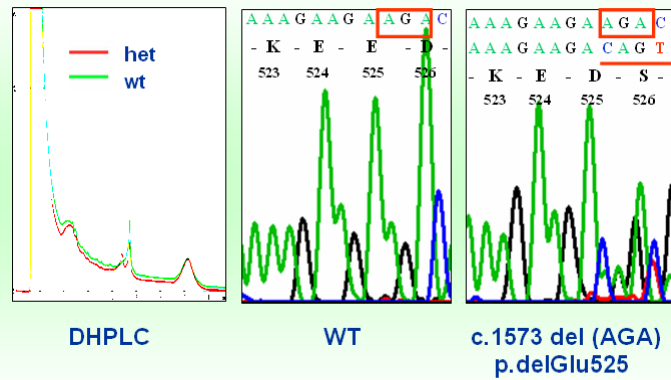
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Patient ID	Sex	Nucleotide Change	Amino Acid Change	Age at onset (years)	Therapy sensitivity
1	F	c.904A>T	p.K301M	23	Resistant
2	F	c.1120A>G	p.T374A	2	Resistant
3	M	c.1573delAGA	p.delGlu525	2	Resistant

# CD2AP

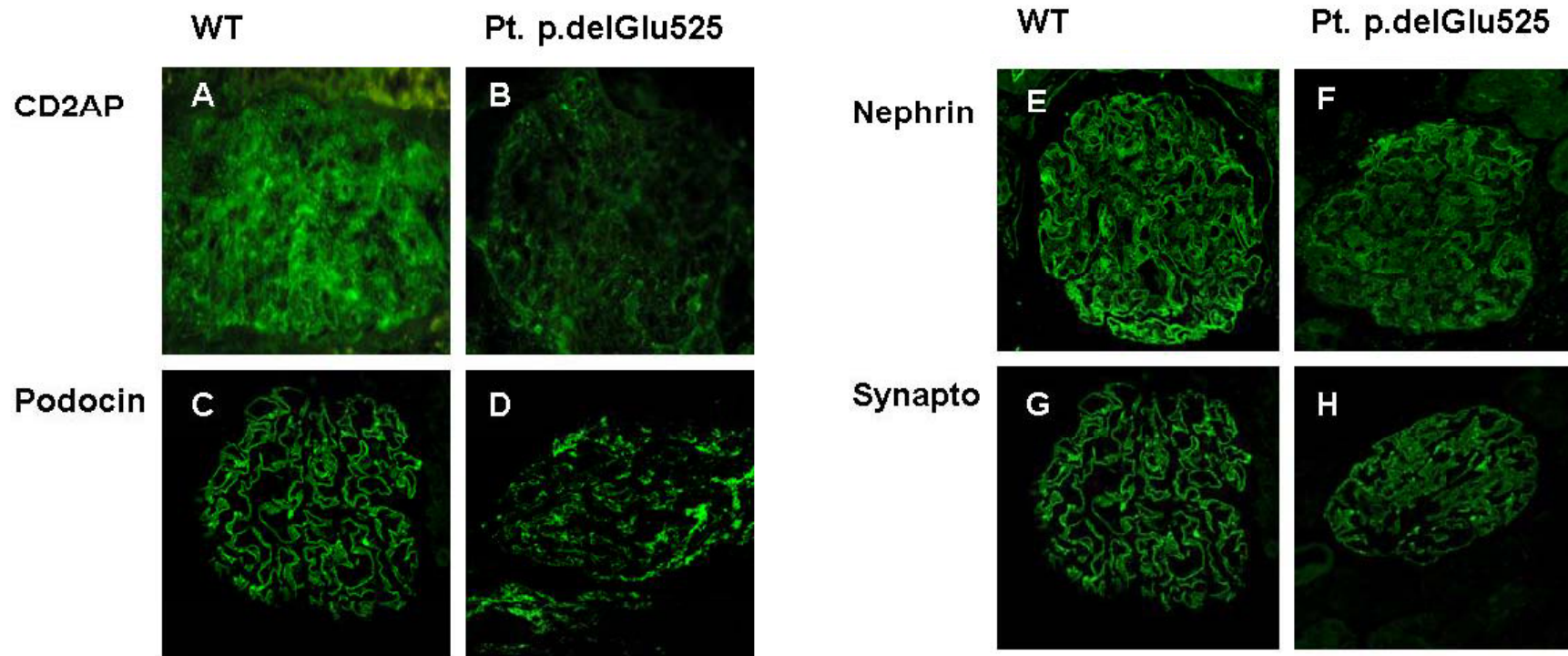


Exon 15



# CD2AP

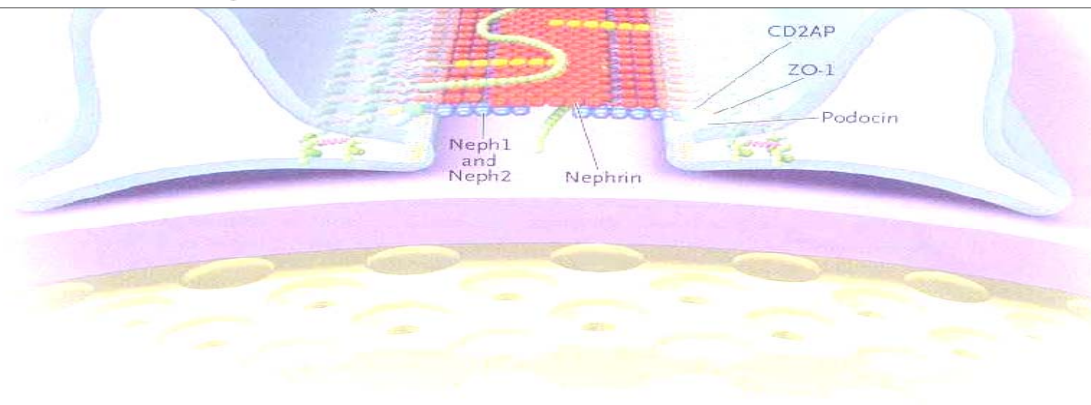
## Immunofluorescence on renal biopsy



## 2. Abnormal Assembly or Function of the Actin-Based Cytoskeleton

**Table 1** Characteristics of hereditary diseases involved in nephrotic syndrome

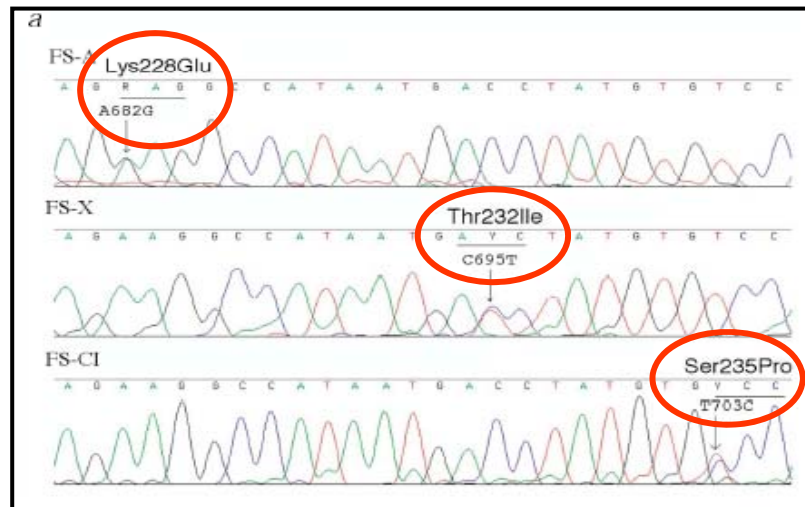
List of diseases	Mode of inheritance	Protein	Protein function	Gene	Chrom.	Protein expression slit membrane
Familial focal segmental glomerulosclerosis (FSGS1)	Autosomal dominant	Alpha-actinin-4	Anchoring protein	<i>ACTN4</i>	19q13	Predominantly podocyte cell body
Fechtner syndrome	Autosomal dominant	Nonmuscle myosinIIA heavy chain	Actin-based motility	<i>MYH9</i>	22q12.3	Tubular epithelia, mesangial cells and podocyte



# ACTN4 (19q13)

Mutations in *ACTN4*, encoding  $\alpha$ -actinin-4, cause familial focal segmental glomerulosclerosis

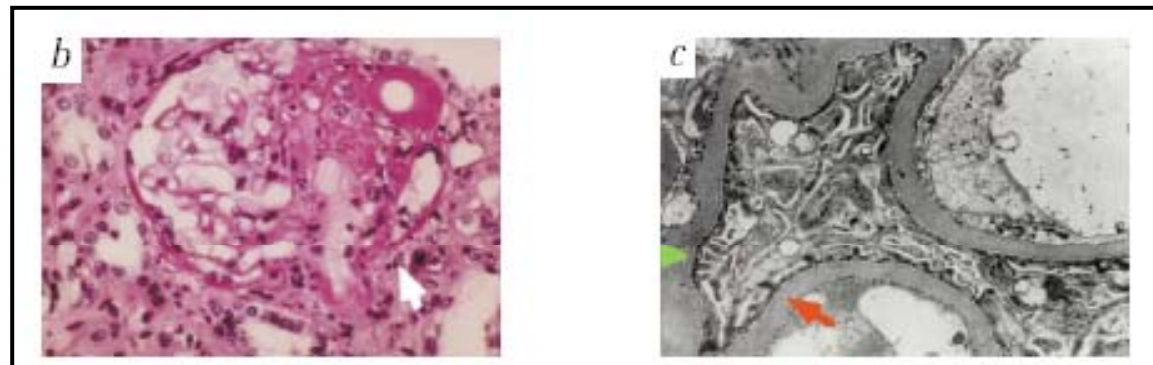
Nat Genet, Kaplan et al., 2000



Three missense mutations in *ACTN4* co-segregate with disease in families with autosomal dominant inheritance.



These mutations increase the binding of *ACTN4* to actin filaments



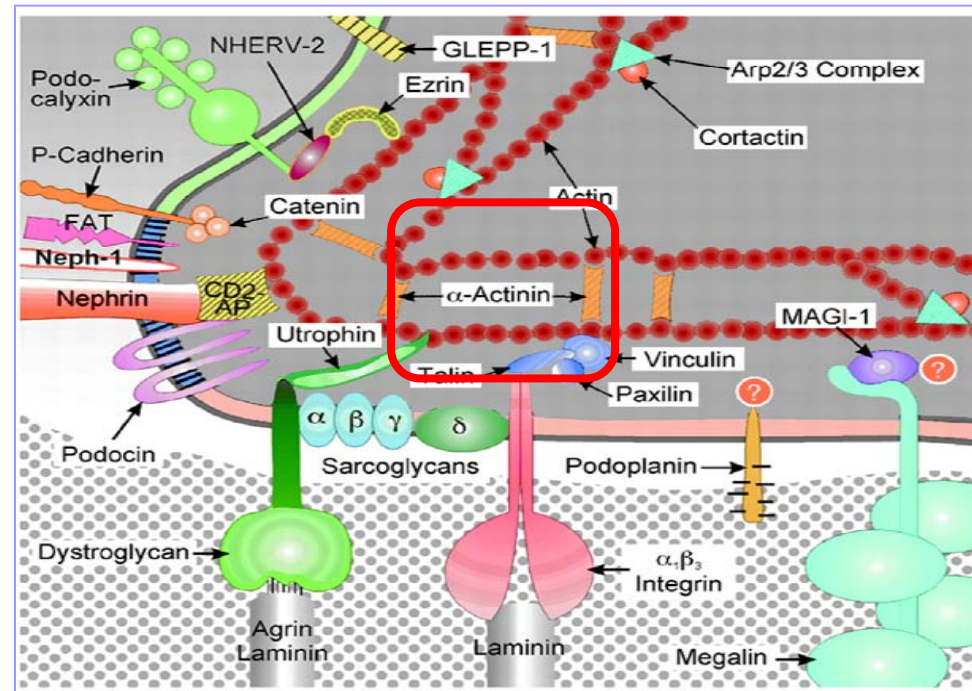


# ACTN4 (19q13)

Weins et al, *JASN* 2005

Analizzati 141 pedigrees con forme familiari di FSGS con esordio tardivo

Mutazioni ACTN4 rappresentano circa il 4% delle forme familiari di FSGS



**La proteina, actinina-4 è importante per il mantenimento della struttura dei podociti**



# “Myosin related disease”

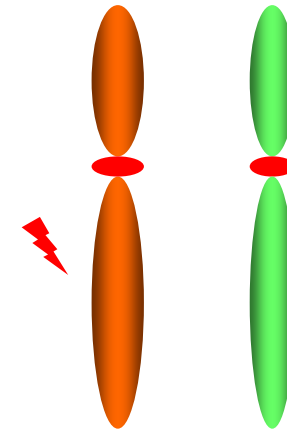
## ➤ Nefriti ereditarie (autosomiche dominanti):

Sindrome di Fechtner (FTNS; OMIM 153640)

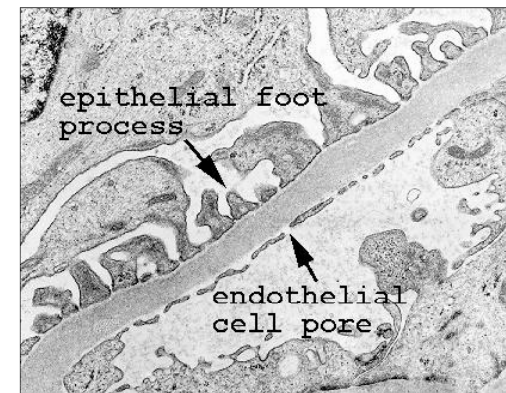
Sindrome di Epstein (EPTS; OMIM 153650)

Sindrome di Sebastian (SBS; OMIM 605249)

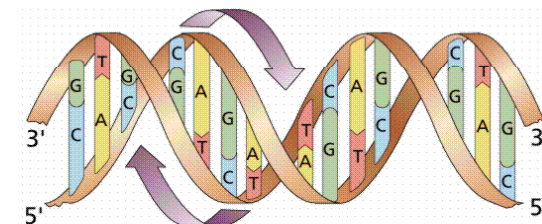
Anomalia di May-Hegglin (MHA; OMIM 155100)



➤ Caratterizzate da alterazioni della membrana basale, inclusioni “Dohle-like” a livello delle cellule polimorfonucleate e trombocitopenia



➤ Mutazioni nel gene MYH9, codificante per la catena pesante della miosina non muscolare IIA (NMMHC-IIA)



# “Myosin related disease”

## Prediction of susceptibility for FSGS

***MYH9* is a major-effect risk gene for focal segmental glomerulosclerosis. Kopp JB et al, Nature Genetics, 2008**

- Increase incidence of FSGS: **3% of ESKD cases**
- **African Americans** have a fourfold increased risk for sporadic FSGS and an 18- to 50-fold increased risk for HIV-1–associated FSGS

Admixture-mapping linkage-disequilibrium genome (MALD) study on **190 African American individuals with FSGS and 222 controls** was done.

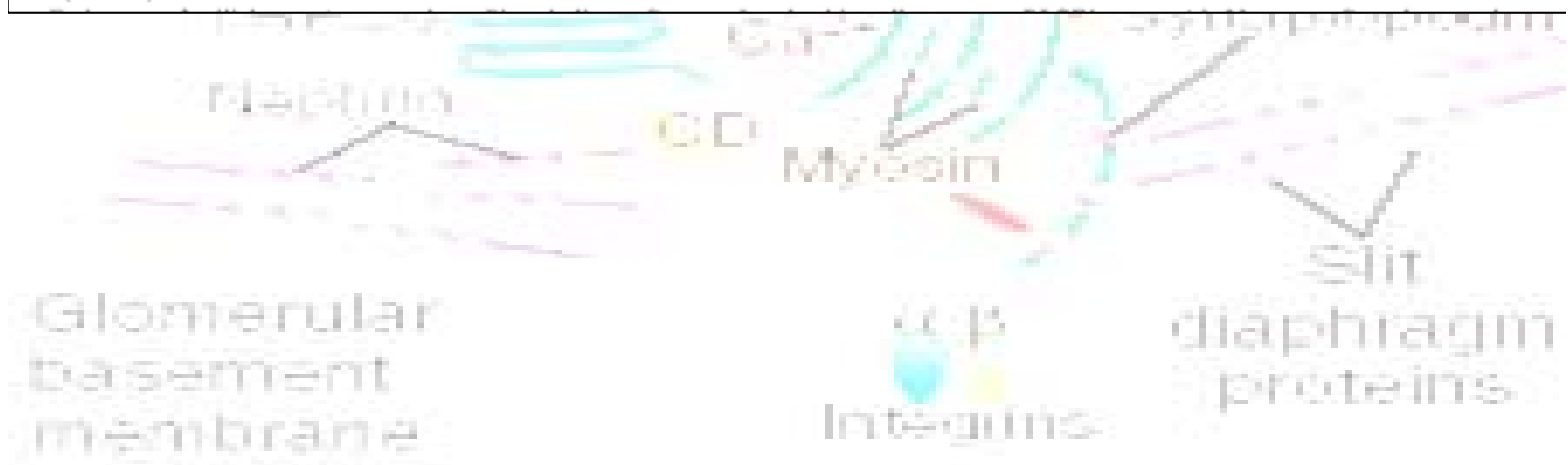
## Catena pesante della miosina non muscolare IIA

**A chromosome 22 region and multiple *MYH9* SNPs and haplotypes were recessively associated with FSGS, most strongly a haplotype spanning exons 14 through 23. This association extended to hypertensive ESKD but not type 2 diabetic ESKD ( $n = 476$ ). Genetic variation at the *MYH9* locus substantially explains the increased burden of FSGS and hypertensive ESKD among African Americans.**

### 3. Expression and Localization of Membrane Proteins

**Table 1** Characteristics of hereditary diseases involved in nephrotic syndrome

List of diseases	Mode of inheritance	Protein	Protein function	Gene	Chrom.	Protein expression slit membrane
Familial focal segmental glomerulosclerosis (FSGS2)	Autosomal dominant	Transient receptor potential cation channel 6	Ca <sup>2+</sup> entry during cell proliferation	<i>TRPC6</i>	11q21–q22	Tubules, podocytes, mesangial and endothelial cells



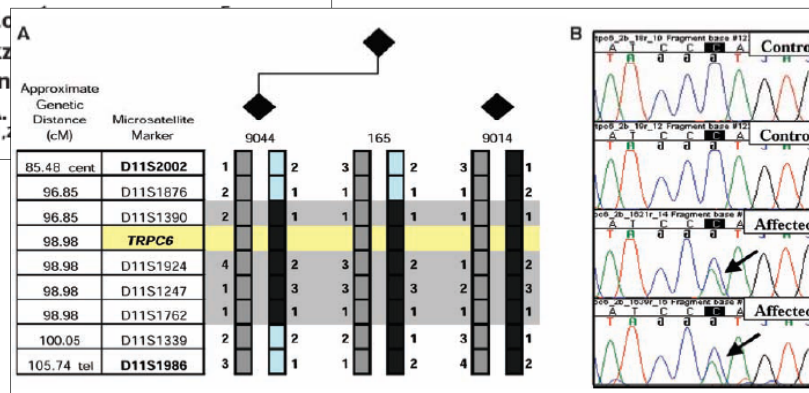
# TRPC6 (11q)

Winn et al, *Science* 2005

## A Mutation in the *TRPC6* Cation Channel Causes Familial Focal Segmental Glomerulosclerosis

Michelle P. Winn,<sup>1,2\*</sup> Peter J. Conlon,<sup>1,2</sup> Merry Kay Farrington,<sup>1,2</sup> Tony Creazzo,<sup>1,2</sup> Nikki Daskalakis,<sup>1,2</sup> Shu Ying Kwan,<sup>1,2</sup> James L. Burchette,<sup>5</sup> Margaret A. David N. Howell,<sup>5</sup> Jeffery M. Vance,<sup>1,2</sup>

**Mutation  
P112Q**



TRPC6 is a glomerular slit diaphragm-associated channel required for normal renal function

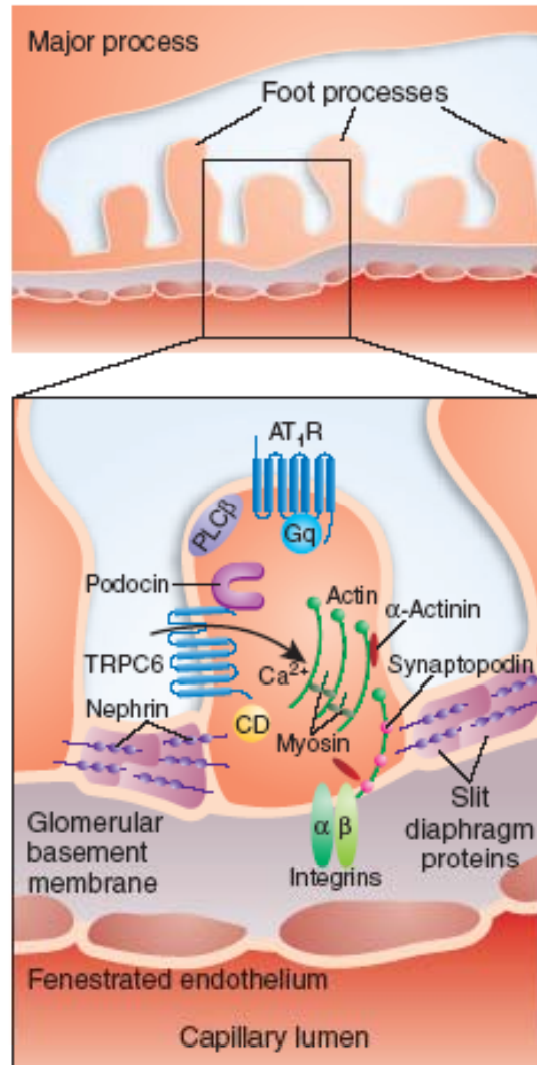
Jochen Reiser<sup>1</sup>, Krishna R Polu<sup>2</sup>, Clemens C Möller<sup>1</sup>, Pe Christian Faul<sup>3</sup>, Stephanie Herbert<sup>2</sup>, Ivan Villegas<sup>4</sup>, Car Dennis Brown<sup>6</sup>, Raghu Kalluri<sup>7</sup>, Peter Mundel<sup>3</sup>, Paula L

**NATURE GENETICS | VOLUME 37 | NUMBER 10 | OCTOBER 2005**

Family	Ethnicity	Mutation	Exon	Age at disease presentation	Number of family members with ESRD	Change in current amplitude
FG-EA	African American	N143S	2	27-39	5 of 36	No
FG-BN	Colombian	S270T	2	17-52	3 of 12	No
FS-Z	Polish	K874X	12	27-57	9 of 53	No
FG-FQ	Mexican	R895C	13	18-46	6 of 25	Yes
FS-XR	Irish and German	E897K	13	24-35	2 of 12	Yes

Summary of families and TRPC6 variants identified, including range of ages at disease presentation (in y), number of family members known to have end-stage renal disease (ESRD) and indication of whether the variant protein produced an altered current amplitude when expressed in HEK cells. Additional clinical details are given in **Supplementary Note online**.

# TRPC6

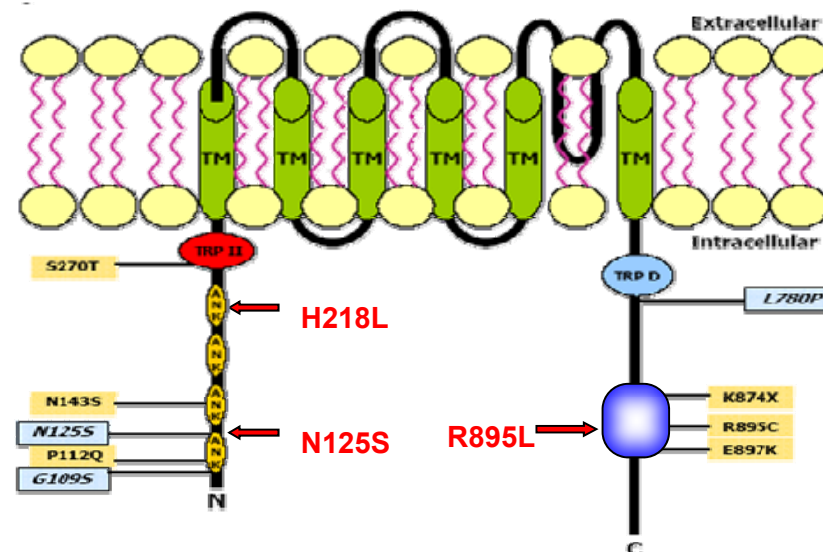


- **Forma familiare di FSGS**
- **Età adulta**
- **Lenta progressione della malattia**

# TRPC6

Missense/nonsense : 7 mutations [\[back to top\]](#)

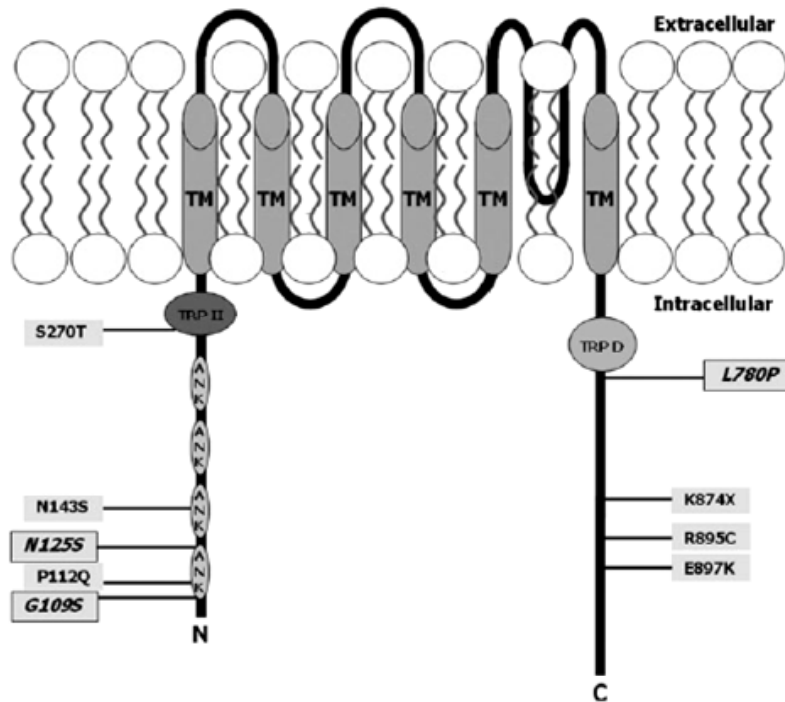
Codon change	Amino acid change	Codon number	Phenotype	Reference
CCA-CAA	Pro-Gln	112	Focal segmental glomerulosclerosis	<a href="#">Winn (2005) Science 308, 1801</a> <a href="#">Schlönörf (2009) Am J Physiol Cell Physiol 296: C558</a> [Functional characterisation]
AAT-AGT	Asn-Ser	143	Focal segmental glomerulosclerosis	<a href="#">Reiser (2005) Nat Genet 37, 739</a> <a href="#">Schlönörf (2009) Am J Physiol Cell Physiol 296: C558</a> [Functional characterisation]
cTCC-ACC	Ser-Thr	270	Focal segmental glomerulosclerosis	<a href="#">Reiser (2005) Nat Genet 37, 739</a> <a href="#">Schlönörf (2009) Am J Physiol Cell Physiol 296: C558</a> [Functional characterisation]
tAAG-TAG	Lys-Term	874	Focal segmental glomerulosclerosis	<a href="#">Reiser (2005) Nat Genet 37, 739</a> <a href="#">Schlönörf (2009) Am J Physiol Cell Physiol 296: C558</a> [Functional characterisation]
gCAG-AAG	Gln-Lys	889	Focal segmental glomerulosclerosis	<a href="#">Zhu (2009) Mutat Res 664, 84</a>
cCGC-TGC	Arg-Cys	895	Focal segmental glomerulosclerosis	<a href="#">Reiser (2005) Nat Genet 37, 739</a> <a href="#">Schlönörf (2009) Am J Physiol Cell Physiol 296: C558</a> [Functional characterisation]
tGAA-AAA	Glu-Lys	897	Focal segmental glomerulosclerosis	<a href="#">Reiser (2005) Nat Genet 37, 739</a> <a href="#">Schlönörf (2009) Am J Physiol Cell Physiol 296: C558</a> [Functional characterisation]



Original Article

## TRPC6 mutational analysis in a large cohort of patients with focal segmental glomerulosclerosis

Sheila Santín<sup>1</sup>, Elisabet Ars<sup>1</sup>, Sandro Rossetti<sup>2</sup>, Eduardo Salido<sup>3</sup>, Irene Silva<sup>1</sup>, Rafael García-Maset<sup>4</sup>,



- Descritte per la prima volta mutazioni TRPC6 sia in adulti che **bambini** con **FSGS non familiare**
- Gene con una penetranza estremamente variabile
- Fattore predisponente allo sviluppo di FSGS



## 4. Transcriptional Regulators in Podocyte Injury

Table 1 Characteristics of hereditary diseases involved in nephrotic syndrome

List of diseases	Mode of inheritance	Protein	Protein function	Gene	Chrom.	Protein expression slit membrane
Denys-Drash syndrome (DDS)/ Frasier syndrome (FS)	<i>de novo</i> (dominant)	Wilms tumor 1	Transcription factor	<i>WT1</i>	9q34.1	Nucleus and cytoplasm of podocyte cell body
Nail-patella syndrome	<i>de novo</i> (dominant)	LIM homeobox transcription factor 1 beta	Transcription factor	<i>LMX1B</i>	17q11	Nucleus and cytoplasm of podocyte cell body

# GENE WT1

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**Mutazioni WT1**  
**(Fattore di trascrizione WT1)**

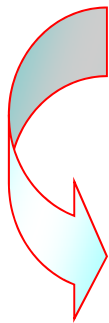


**Sindrome di Denys-Drash (DDS)**  
**Sindrome di Frasier**

- **severa glomerulopatia (DMS e SN)**
- **pseudoermafroditismo maschile (46,XY )**
- **tumore di Wilms**



- **esordio: neonatale/infanzia**
- **frequenza: rara**
- **terapia: resistente**



# WT1

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<b>TOTAL</b>	<b>102</b>
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<b>Disease/phenotype</b>	<b>Number of mutations</b>
Wilms tumour	48
Denys-Drash syndrome	33
Frasier syndrome	7
Nephrotic syndrome	5
Diffuse mesangial sclerosis	2
Focal segmental glomerulosclerosis	2
Hypospadias	1
Nephrotic syndrome?	1
Renal dysfunction & renal blastema	1
Ureteropelvic junction obstruction	1
Wilms tumour, adult	1

## ***WT1* mutations in nephrotic syndrome revisited. High prevalence in young girls, associations and renal phenotypes**

Filippo Aucella • Luigi Bisceglia • Patrizia De Bonis •  
Maddalena Gigante • Gianluca Caridi •  
Giancarlo Barbano • Gerolamo Mattioli •  
Francesco Perfumo • Loreto Gesualdo •  
Gian Marco Ghiggeri

Am J Transplant, 2006

### **Posttransplant Recurrence of Proteinuria in a Case of Focal Segmental Glomerulosclerosis Associated with *WT1* Mutation**

G. M. Ghiggeri<sup>a,b,\*</sup>, F. Aucella<sup>c</sup>, G. Caridi<sup>a</sup>,  
L. Bisceglia<sup>d</sup>, L. Ghio<sup>e</sup>, M. Gigante<sup>f</sup>, F. Perfumo<sup>b</sup>,  
M. Carraro<sup>g</sup> and L. Gesualdo<sup>f</sup>

**Mutazioni *WT1* non sono rare in ragazze con SRNS di età < 18 anni (10-12%)  
anche in assenza di un chiaro quadro renale istologico e delle anomalie genitali  
tipiche delle sindromi di DDS e Frasier**



**Rapida analisi molecolare degli esoni 8 e 9 del  
gene *WT1* al fine di evitare inutili terapie**

## 5. Dysfunction of Cytoplasmic Proteins

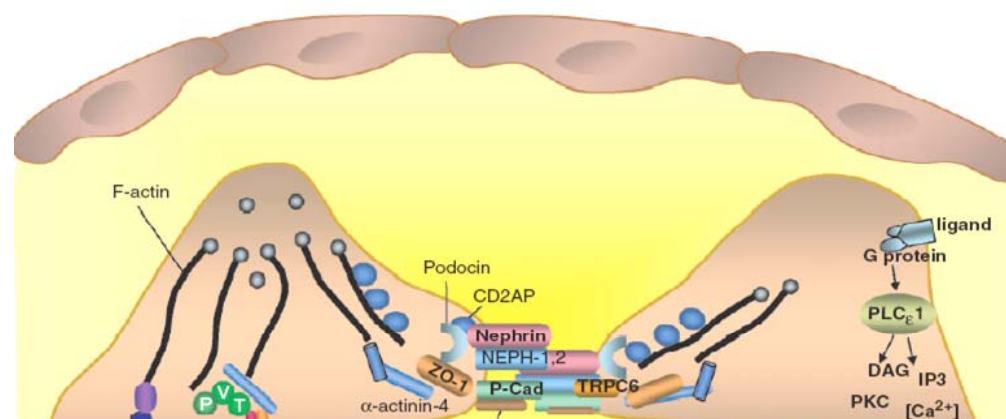


Table 1 Characteristics of hereditary diseases involved in nephrotic syndrome

List of diseases	Mode of inheritance	Protein	Protein function	Gene	Chrom.	Protein expression slit membrane
Early-onset familial nephrotic syndrome	Autosomal recessive	Phospholipase C epsilon	Involved in cell growth and differentiation, gene expression	<i>PLCE1</i>	10q23	Cytoplasm podocyte cell body

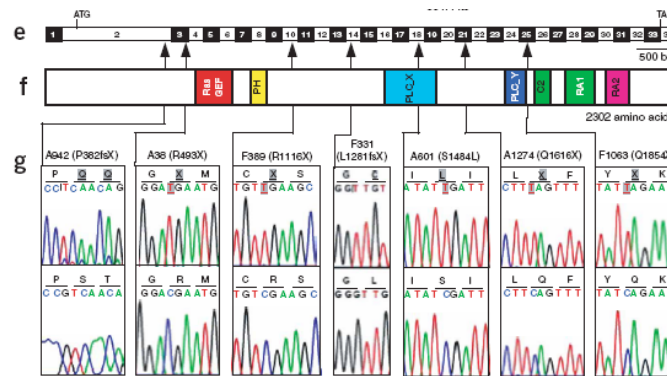
# GENE *PLCE1* (10q23) *NPHS3*

Hinkes B et al, *Nat Genet* 2006

Mutazioni nel gene *PLCE1*  
(fosfolipasi C epsilon)



Variante di Sindrome Nefrosica  
(autosomica recessiva)



- causa più frequente di DMS (28,6%)
- frequente progressione verso IRT
- esordio: infanzia
- frequenza: rara
- terapia: casi di risposta a terapia con CsA e ACEI

# PLCE1

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Mutation type	Total number of mutations
Missense/nonsense	7
Splicing	2
Regulatory	0
Small deletions	6
Small insertions	1
Small indels	0
Gross deletions	0
Gross insertions	0
Complex rearrangements	0
Repeat variations	0
<b>TOTAL</b>	<b>16</b>

Disease/phenotype	Number of mutations
Diffuse mesangial sclerosis	7
Nephrotic syndrome 3, early onset	7
Glomerulosclerosis, focal segmental	1
Glomerulosclerosis, focal segmental ?	1



# Bigenic heterozygosity and the development of steroid-resistant focal segmental glomerulosclerosis

Nephrol Dial Transplant (2008) 1 of 6  
doi: 10.1093/ndt/gfn208

Marije Löwik<sup>1</sup>, Elena Levtchenko<sup>1</sup>, Dineke Westra<sup>1</sup>, Patricia Groenen<sup>2</sup>, Eric Steenbergen<sup>2</sup>, Jan Weening<sup>2</sup>, Marc Lilien<sup>3</sup>, Leo Monnens<sup>1</sup> and Lambert van den Heuvel<sup>1</sup>

**Table 2.** Mutations detected in non-familial steroid-resistant FSGS patients

P	<i>WT-1</i>			<i>NPHS1</i>			<i>NPHS2</i>			<i>CD2AP</i>			<i>PLCE1</i>		
	nt	eff.	st.	nt	eff.	st.	nt	eff.	st.	Nt	eff.	st.	nt	eff.	st.
	sub.	mut	mut	sub.	mut	mut	sub.	mut	mut	sub.	mut	mut	sub.	mut	mut
1							622 G>A	A208T	het	1488 G>A <sup>a</sup>	M496I	het			
2				791 C>G	P264R	het	413 G>A	R138Q	het						
							948 DelT <sup>a</sup>	frame shift	het						
3	1228+5 G>A	splice	het	1126 C>G	L376V	het									
4										1834 C>T	R612X	hom			
5													1807 G>T <sup>a</sup>	E603X	het
6													3491 C>T <sup>a</sup>	T1164M	het
													3518 C>T <sup>a</sup>	S1177F	het

These data demonstrate that combined genetic defects in podocyte genes may play a role in the development of FSGS: altered interactions between several podocyte proteins can make podocytes vulnerable for the 'second hit' factors and result in genetic susceptibility

# CONCLUSIONI

**Nei casi di SN cortico-resistente è utile effettuare sempre uno screening genetico (1° step: geni NPHS1, NPHS2 e WT1 hot spot):**

1. **Bambini omozigoti per mutazioni nei geni NPHS1/NPHS2 sono notoriamente resistenti alla terapia steroidea e con ciclosporina: terapia inutile**
2. **WT1 mutations are not rare in females under 18 years with SRNS. WT1 hot spot mutational analysis could avoid potentially harmful therapeutic approaches**
3. **In nephrotic syndrome secondary to mutations in PLCE1, the majority of children had a poor prognosis; however, two patients are responsive to therapy**



**UNIVERSITÀ  
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DI FOGGIA**

**CENTRO DI MEDICINA MOLECOLARE  
Ospedali Riuniti - Foggia**

# **DIAGNOSI MOLECOLARE: COME?**

C T G T C  
T T C C C  
T T C G A  
T A C T G  
G A A C A  
C T G T C  
G G A C A  
C T G T C  
T A C T C  
A C C T A  
G A T A C  
C A G T A  
C T C G A  
T T C C C  
T T C G A  
T A C T G  
G A A C A  
C T G T C



# TEST GENETICI

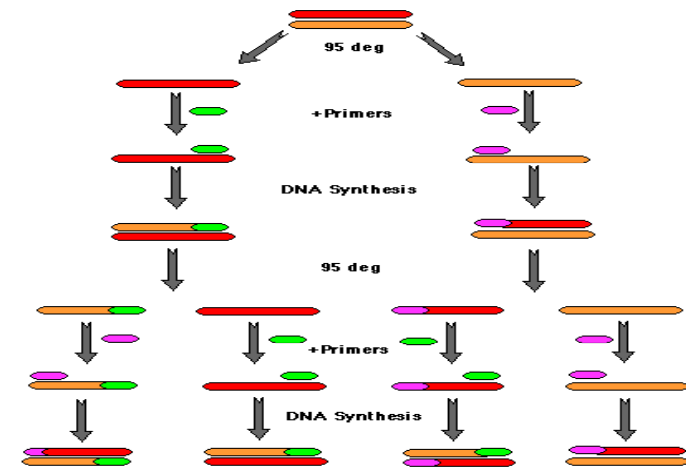
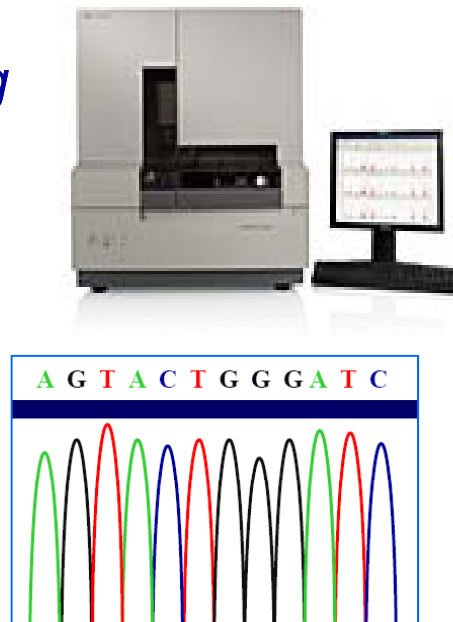


•PRELIEVO DI SANGUE

•ESTRAZIONE DI DNA

- *Analisi dei geni di interesse mediante tecniche di biologia molecolare*

•DNA sequencing



•PCR

# TEST GENETICI

Perchè?

- TERAPIA

- PROGNOSI

- DIAGNOSI PRENATALE  
nelle SN congenite con ereditarietà AR

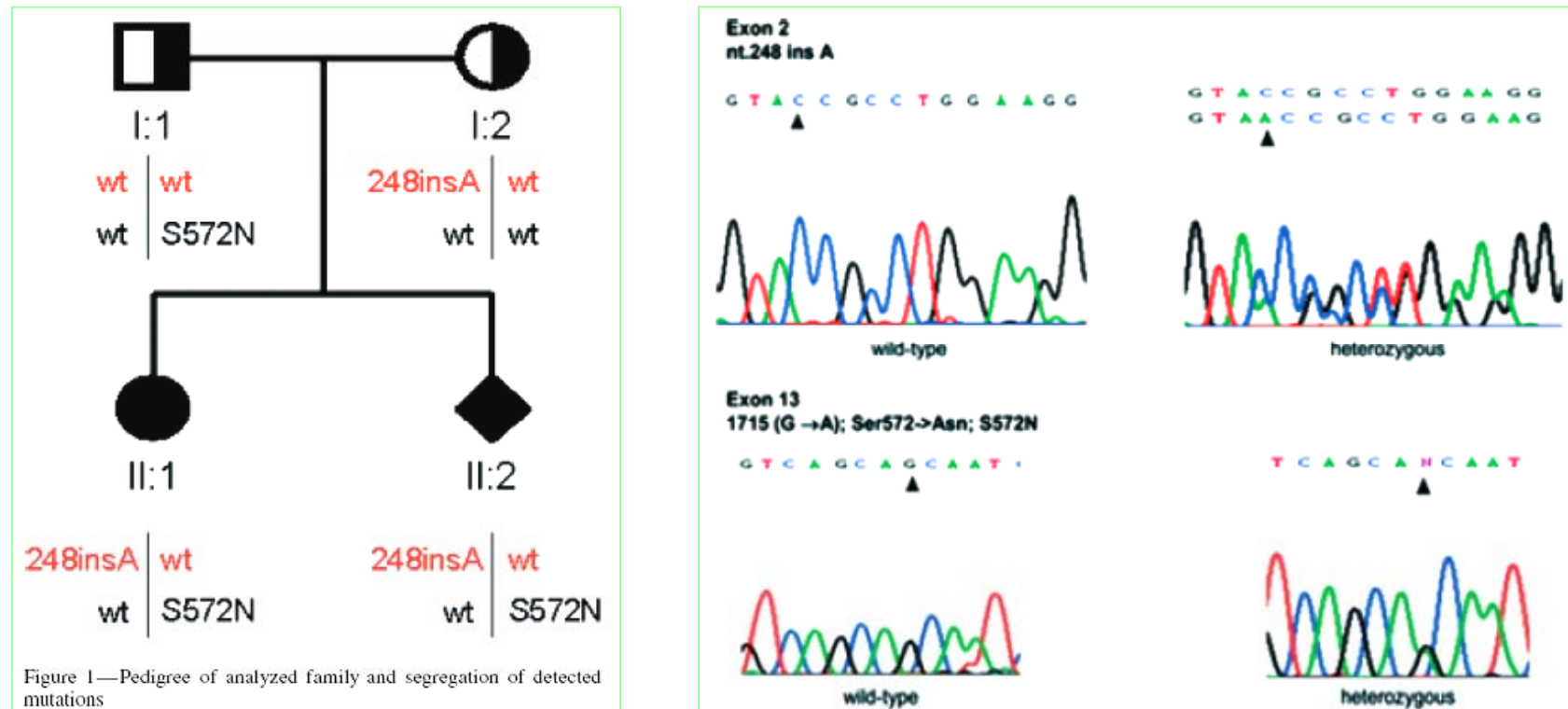


René Magritte 1898 – 1967  
The lovers 1928



## Congenital nephrotic syndrome of Finnish type: detection of new nephrin mutations and prenatal diagnosis in an Italian family

Maddalena Gigante<sup>1\*</sup>, Pantaleo Greco<sup>2</sup>, Vincenza Defazio<sup>1</sup>, Marco Lucci<sup>3</sup>, Maurizio Margaglione<sup>4</sup>, Loreto Gesualdo<sup>1</sup> and Achille Iolascon<sup>5\*</sup>



# TEST GENETICI

**Dove?**

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**Centro di Medicina Molecolare - Bioagromed**

**Università di Foggia-c/o Ospedali Riuniti Foggia**

**Istituto Giannina Gaslini**

**Ospedale Pediatrico IRCCS - GENOVA**

**Ospedale Pediatrico Bambino Gesù**

**ROMA**

**U.O. Pediatria Nefrologica - Azienda Ospedaliera - Universitaria**

**Padova**



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  - Leonarda Varraso
  - Leonarda Roca

- Gian Marco Ghiggeri (Genova)
- Gianluca Caridi (Genova)
- Achille Iolascon (Napoli)
- Filippo Aucella (S.G. Rotondo)
- Penza Rosa (Bari)
- Maurizio Margaglione (Foggia)



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