Molecular Genetics of Renal Failure
(all genes that cause kidney disease?)

When I was your age, there was polycystic kidney disease, nephronophthisis, Alport syndrome, and an obscure type of congenital nephrotic syndrome in Finland. The idea that we would ever understand how any of these worked, was like landing a man on the moon.

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Focal-segmental glomerulosclerosis (FSGS)

1. Involves 20% of children and 40% of adults with NS
2. Is the most common cause of ESRD in the US
3. Is largely due to “secondary” avoidable causes
4. Is a manifestation of sickle-cell anemia
5. Could be elucidated by the urokinase receptor pathway

So what about genetics and FSGS???
An entire host of these syndromes are genetic; we will discuss three
Nephrin extends into the center of the slit from adjacent podocyte foot processes and form homophilic and heterophilic interactions with NEPH. The slit diaphragm complex includes podocin. Through interaction with CD2-associated protein (CD2AP), the slit diaphragm molecules are linked to the actin cytoskeleton, which is regulated by $\alpha$-actinin-4, inverted formin 2 (INF2), and myosin 1E (Myo1E). Calcium generated by phospholipase C epsilon 1 (PLCε1) through diacylglycerol (DAG) and inositol triphosphate (IP3) enters the cell through transient receptor potential cation channel 6 (TRPC6) to regulate actin polymerization. At the basal surface, adhesion molecules $\alpha3\beta1$ integrin and $\alpha$-dystroglycan are linked to laminin. Integrin is coupled to the actin cytoskeleton through talin, vinculin, and paxillin, whereas adhesion molecule $\alpha$-dystroglycan links to actin through utrophin. Negatively charged podocalyxin and glomerular epithelial protein 1 (GLEPP-1) are arrayed on the apical-cell membrane.
<table>
<thead>
<tr>
<th>Human gene product</th>
<th>Gene</th>
<th>Inheritance</th>
<th>Chromosome</th>
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</thead>
<tbody>
<tr>
<td>Slit Diaphragm Proteins</td>
<td>NPHS1</td>
<td>AR</td>
<td>19q13.1</td>
</tr>
<tr>
<td>Podocin</td>
<td>NPHS2</td>
<td>AR</td>
<td>1q21-31</td>
</tr>
<tr>
<td>CD2-associated protein</td>
<td>CD2AP</td>
<td>AD; rarely AR</td>
<td>6p12</td>
</tr>
</tbody>
</table>

**Cell Membrane-Associated Proteins**
- TRPC6          | AD       | 11q11-12    |
- PTPRO          | AR       | 12p22       |
- LAMIN-B2       | AR       | 3p21        |
- ITG4           | AR       | 17q11       |
- CD151          | AR       | 11p15       |

**Cytosolic or Cytoskeletal Proteins**
- ACTN4          | AR       | 19q13       |
- PLCE1          | AR       | 10q23-24    |
- MYH9           | AD       | 22q12.3     |
- INF2           | AD       | 14q32       |
- MYO1E          | AR       | 13q21-26    |

**Nuclear Proteins**
- WT1            | AD       | 11p13       |
- SMARCA-like protein | SMARCA1 | AR       | 2q34-36    |

**Mitochondrial Components**
- mtDNA-A3243G   | Maternal | mtDNA      |
- COQ2           | AR       | 4q11-12     |
- COQ6           | AR       | 14q24.3     |

**Lysosomal Protein**
- SCARB2         | AR       | 4q13-21     |

**Unknown Cellular Location**
- APOL1          | AR       | 22q12       |

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**Histologic Subtype**

1. **NOS**
2. **Perihilar**
3. **Cellular**
4. **Tip**
5. **Collapse**

**Generic FSGS**

**Genetic forms**

**Obesity, hypertension**

**Primary or secondary**

**Best prognosis**

**HIV, parvovirus SV40, EBV, CMV, pamidronate, interferon, CNI, CAN**
We performed whole-genome linkage analysis followed by high-throughput sequencing of the positive-linkage area in a family with autosomal recessive focal segmental glomerulosclerosis (index family) and sequenced a newly discovered gene in 52 unrelated patients with focal segmental glomerulosclerosis. Immunohistochemical studies were performed on human kidney-biopsy specimens and cultured podocytes. Expression studies in vitro were performed to characterize the functional consequences of the mutations identified.

A non-muscle myosin

Whole-genome linkage analysis was performed in the index family with the use of an array of 1 million single-nucleotide polymorphisms (SNPs).
The family tree shows

1. Incest
2. Autosomal dominant
3. Autosomal recessive
4. Spontaneous mutations
5. Cannot be determined
### 900 K SNP chip for linkage

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age at Diagnosis (yr)</th>
<th>Age at Onset of ESRD (yr)</th>
<th>Treatment</th>
<th>Finding on Renal Biopsy</th>
<th>First Observation</th>
<th>Last Observation</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Age (yr)</td>
<td>Urinary Protein g/24 hr</td>
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<tr>
<td><strong>Index family</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>9</td>
<td>13</td>
<td>Glucocorticoids (NR), cyclosporine (NR), ACE inhibitor (NR)</td>
<td>Advanced FSGS</td>
<td>9</td>
<td>3.00</td>
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<td>Patient 2</td>
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<td>Cyclosporine (PR), ACE inhibitor (PR)</td>
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<td>4</td>
<td>1.56</td>
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<tr>
<td>Patient 3</td>
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<td>—</td>
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<td>3.40</td>
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<tr>
<td><strong>Family 2</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 4</td>
<td>1</td>
<td>—</td>
<td>Glucocorticoids (NR), cyclosporine (PR), ACE inhibitor (PR)</td>
<td>FSGS</td>
<td>1</td>
<td>+++‡</td>
</tr>
</tbody>
</table>

#### A Whole-Genome Multipoint Linkage Analysis in Index Family

![Linkage Analysis Graph](image)

#### B Direct Sequencing

**Index Family**

- c.475G>C (Ala159Pro)
- c.2085T>G (Tyr695*)

**Family 2**

- c.475G>C (Ala159Pro)
- c.2085T>G (Tyr695*)
Whole-genome linkage analysis was performed in the index family with the use of an array of 1 million single-nucleotide polymorphisms (SNPs).
Immunoperoxidase staining of Myo1E, synaptopodin and podocin in control human and patient 4 biopsies.

Scanning electron microscopy images of wild-type and myo1e-ko mouse glomeruli showing podocyte microvillous transformation.
A, B, C are normal kidney. Myo1E (red) is mainly expressed in podocytes (synpo green), as is the case in mice.

D, E, F, are cultured human podocytes. Myo1E (red) localized close to the cytoplasmic membrane, with enrichment at the lamellipodia tips.

H, I, are immuno-gold labeling for Myo1E in control human glomeruli revealed gold particles almost exclusively on the cytoplasmic side of the podocyte plasma membrane.
Human podocytes transfected with green fluorescent protein (GFP)–tagged wild-type Myo1E or E753K, I531M, D465N, and F307L (control SNPs) showed predominant localization at the plasma membrane, whereas podocytes transfected with GFP-tagged mutant A159P Myo1E showed diffuse cytoplasmic localization, which at a higher plasmid dose acquired a punctate pattern.

Wild-type Myo1E overexpression increased human podocyte motility (Figure 4K). In contrast, A159P Myo1E had no effect on podocyte motility. Conversely, Myo1E knockdown in human podocytes (Panel K) and Myo1E knockout in mouse podocytes impaired migration (unpublished data).
Panel A shows views of the Myo1E protein showing the ATP pocket with the Switch-1 (green) and the P-loop (blue) domains, the actin binding site (orange), the IQ (light blue), the TH1 (pink), the TH2 (violet) and the SH3 (dark violet) domains. A detail of the ATP pocket with the position of the Ala159 is shown in panel B (red).

Nonmuscle myosin activity generates tension, and the interaction among actin, myosins, and alpha-actinin-4 probably allows the foot processes to generate the contractile forces that help the glomerular capillaries to resist the high intraluminal hydrostatic pressure and to change their morphologic structure actively, modifying the permeability of the glomerular filtration barrier. MYO1E mutations have a role in focal segmental glomerulosclerosis and suggest the importance of Myo1E in podocyte homeostasis and the consequent integrity of the glomerular filtration barrier.
Beneficial effect of CsA on proteinuria is not dependent on NFAT inhibition in T cells, but rather results from the stabilization of the actin cytoskeleton in kidney podocytes.
This patient has

1. Lead poisoning
2. Peroneal palsy
3. Flat feet
4. Proteinuria

Charcot-Marie-Tooth disease is the most common inherited disorder of the peripheral nervous system. The disease is characterized by a progressive muscle weakness and atrophy, sensory loss, foot (and hand) deformities and steppage gait. While many of the genes associated with axonal CMT have been identified, to date it is unknown which mechanism(s) causes the disease. However, genetic findings indicate that the underlying mechanisms mainly converge to the axonal cytoskeleton.
Mutations in inverted-formin 2 (INF2) were recently identified in patients with autosomal dominant FSGS. INF2 encodes a formin protein that interacts with the Rho-GTPase CDC42 and myelin and lymphocyte protein (MAL) that are implicated in essential steps of myelination and myelin maintenance. We therefore hypothesized that INF2 may be responsible for cases of Charcot-Marie-Tooth neuropathy associated with FSGS. We performed direct genotyping of INF2 in 16 index patients with Charcot-Marie-Tooth neuropathy and FSGS who did not have a mutation in PMP22 or MPZ, encoding peripheral myelin protein 22 and myelin protein zero, respectively. Histologic and functional studies were also conducted.
Mode of inheritance

1. X-linked recessive
2. X-linked dominant
3. Autosomal recessive
4. Autosomal dominant
5. Damned if I know!

Mutations in PMP22 or MPZ were ruled out in all patients.
INF2 mutations account for 12 to 17% of autosomal dominant cases of FSGS. The gene encodes a member of the diaphanous-related formin family, which is involved in remodeling the actin and microtubule cytoskeletons. INF2 possesses functional domains characteristic of other diaphanous-related formins: an N-terminal diaphanous-inhibitory domain (DID), the formin homology domains FH1 and FH2, and a C-terminal diaphanous-autoregulatory domain (DAD). However, INF2 has a unique ability to promote not only actin polymerization but also filament severing and depolymerization.

All were new mutations located in exons 2 and 3, which encode the DID domain (Panel A). All caused nonconservative changes in highly conserved amino acids. We mapped mutants associated with FSGS alone and those associated with FSGS and Charcot–Marie–Tooth neuropathy onto a human INF2 DID in silico model (Panel B).

All involved DID residues, mutations in the two groups of patients were distinctly localized, the latter being located mostly in the second and third DID armadillo repeats and the former mostly in the fourth armadillo repeat
All patients had FSGS, but full-blown nephrotic syndrome was noted in only 5. Magnetic resonance imaging of the brain showed central nervous system anomalies characterized by white-matter hyperintensity and ventricular dilation, which were more severe in the older patient. Sural-nerve biopsy specimens all showed a pattern of lesions with a combination of axonal and demyelinating changes, characterized by a marked decrease in myelinated fibers, as compared with that in age-matched controls, and numerous multilayered “onion bulbs”. These data suggest an intermediate Charcot–Marie–Tooth phenotype in patients with INF2 mutations.
We investigated whether the mutations in INF2 proteins affect their binding to CDC42, an actin-regulating Rho-GTPase known to interact with the INF2 DID. An enhanced interaction was observed between the INF2 mutants and a constitutively active form of CDC42 (CDC42-Q61L) as compared with the wild-type INF2 protein (panel A and B). INF2 mutants affected the subcellular localization of CDC42-Q61L, with the fraction of active CDC42 at the plasma membrane being lost in a large proportion of mutant cells as compared with cells expressing wild-type INF2 (panel C).

The formin INF2 as a crucial molecular entity in the occurrence of FSGS and Charcot–Marie–Tooth neuropathy provides additional insight into the role of similar cellular machinery in podocytes and Schwann cells, even though these two highly specialized cell types have distinct functions.
Stressed-out podocytes seeking to avoid proteinuria

1. Rearrange their actin cytoskeleton
2. Retract or efface their foot processes
3. Abandon their RhoA-dependent stationary state
4. Assume a CDC42- and Rac1-dependent migratory state
5. Require a functioning Rac1 GTPase-activating (GAP) protein
The Ras superfamily is a protein superfamily of small GTPases, which are all related, to a degree, to the Ras protein subfamily (the key human members of which are KRAS, NRAS, and HRAS). There are more than a hundred proteins in the Ras superfamily. Based on structure, sequence and function, the Ras superfamily is divided into eight main families, each of which is further divided into subfamilies: Ras, Rad, Rab, Rap, Ran, Rho, Rheb, Rit, and Arf. Miro is a recent contributor to the superfamily. Each subfamily shares the common core G domain, which provides essential GTPase and nucleotide exchange activity. The surrounding sequence helps determine the functional specificity of the small GTPase, for example the 'Insert Loop', common to the Rho subfamily, specifically contributes to binding to effector proteins such as IQGAP and WASP. The Ras family is generally responsible for cell proliferation, Rho for cell morphology, Ran for nuclear transport and Rab and Arf for vesicle transport.

Like for instance Arhgap24
Ras homolog gene family, member A (RhoA) is a small GTPase protein known to regulate the actin cytoskeleton in the formation of stress fibers. In humans, it is encoded by the gene RHOA. It acts upon two known effector proteins: ROCK1 (Rho-associated, coiled-coil containing protein kinase 1) and DIAPH1 (diaphanous homolog 1 (Drosophila)). RhoA is part of a larger family of related proteins known as the Ras superfamily; proteins involved in the regulation and timing of cell division.
We found that decreased membrane ruffling in differentiated podocytes was dependent on the presence of the GTPase-activating protein (GAP), Rho-GAP 24 (Arhgap24). Previous work from Stossel and colleagues has shown that Arhgap24 (also known as Filamin A–binding RhoGAP [FilGAP]) is a GAP for Rac1 and that it suppresses lamellipodia formation and cell spreading downstream of RhoA signaling. Their work showed that the highest level of Arhgap24 transcript was present in the kidney. Here we show that Arhgap24 was highly expressed in podocytes of the kidney and was upregulated as these cells differentiate in vivo. The ARHGAP24 gene is highly conserved, implying an important role for the gene product. When we sequenced the DNA from patients with FSGS, we identified a loss-of-function mutation in the ARHGAP24 gene in a kindred with familial kidney disease. Taken together, these results suggest that Arhgap24 controls the RhoA-Rac1 signaling balance in podocytes that appear to be dysregulated in proteinuric kidney diseases, such as FSGS.
When undifferentiated podocytes were cultured at the permissive temperature, they exhibited highly ruffled plasma membranes. In contrast, the plasma membranes of the differentiated podocytes had a very smooth, flat appearance.

Cells were outfitted with a temperature-sensitive SV40 large T antigen. Heat them up and off they go.

Podocytes upregulate Arhgap24 when they differentiate.

Arhgap24 colocalizes with the focal adhesion marker vinculin at the tips of actin stress fibers.
Arhgap24 is expressed in kidney podocytes in vivo.

Arhgap24 knockdown in differentiated podocytes increases membrane ruffling.

Arhgap24 knockdown in differentiated podocytes increases active Rac1 and Cdc42 levels and accelerates epithelial monolayer wound closure. (Cells migrate and close the gap)

Recall that CDC42 was also up in INF2 mut
The effect of variations in the Arhgap24 gene on Rac1-GAP activity and dimerization with the wild-type protein is shown. The table below lists the incidence of nonsynonymous sequence variations in patients with biopsy-proven FSGS ($n = 310$) and controls ($n = 180$). Arhgap24 Q158R has defective Rac1-GAP activity and dimerizes with the wild-type protein.

### Sequence alignment of the Arhgap24 protein across various species

<table>
<thead>
<tr>
<th>Species</th>
<th>aa</th>
<th>Sequence</th>
<th>aa</th>
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</thead>
<tbody>
<tr>
<td>Homo sapiens</td>
<td>141</td>
<td>vryekrygnr lamlveQcv dfirqrglkie</td>
<td>170</td>
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<tr>
<td>Pan troglodytes</td>
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<td>vryekrygnr lamlveQcv dfirqrglkie</td>
<td>359</td>
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<tr>
<td>Mus musculus</td>
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<td>vryekrygnr lamlveQcv dfirqrglkie</td>
<td>168</td>
</tr>
<tr>
<td>Rattus norvegicus</td>
<td>140</td>
<td>vryekrygnr lamlveQcv dfirqrglkie</td>
<td>169</td>
</tr>
<tr>
<td>Callictrix jacobus</td>
<td>141</td>
<td>vryekrygnr lamlveQcv dfirqrglkie</td>
<td>170</td>
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<tr>
<td>Equus caballus</td>
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<td>vryekrygnr lamlveQcv dfirqrglkie</td>
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<td>vryeryrnk mapmlveQcv dfirwglre</td>
<td>173</td>
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</table>

The figure shows the linkage disequilibrium of the Arhgap24 gene, with specific markers and allele frequencies. Arhgap24 Q158R has defective Rac1-GAP activity and dimerizes with the wild-type protein.
This genetic syndrome was solved by

1. Linkage analysis
2. Genetic association
3. Positional cloning strategy
4. Haplotype analysis
5. Basic research