Genetics of Steroid Resistant Nephrotic syndrome

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Nephrotic syndrome - definition

- Oedema
- Massive proteinuria (> 50mg/kg/d or > 40mg/m²/h)
- Hypoalbuminemia
- Hyperlipidemia
Glomerular barrier

- Composed of a basement membrane covered by fenestrated endothelium on the inner surface and podocytes on the outer surface
Classification

• It is separated to steroid-sensitive or steroid-resistant (SRNS) forms in respect to response to intensive steroid therapy.

• SRNS usually progress to end-stage renal failure.

• According to the NorthAmerican Pediatric RenalTrials and Collaborative Studies SRNS constitutes the second most frequent cause of ESRD in the first two decades of life.

• Unfortunately, there is no curative treatment for majority of patients.
Histology

- Minimal change disease
- Diffuse mesangial proliferation
- Focal segmental glomerulosclerosis

- Often resistant to other immunosuppressive agents and tend to progress to ESRD
Genetic forms of steroid resistant nephrotic syndrome

- The genetic basis of several conditions leading to SRNS have been identified
- They all result in a structural and/or functional defect in the glomerular barrier
- This explains their unresponsiveness to immunosuppressive agents
- There is no recurrence of the disease in the kidney graft
Congenital nephrotic syndrome (Finnish type)

- Autosomal recessive disease
- Prenatal presentation (αFP; large placenta)
- Massive proteinuria > 20g/L
- Preserved renal function
- Infections and thromboembolic events maybe fatal within the first year of life
CNF-Improved outcome

- Daily albumin infusions
- Indomethacine
- Captopril
- Unilateral nephrectomy
- Bilateral nephrectomy, PD and Tx
Genetics of CNF

• The gene – \textit{NPHS1} (nephrin) localized on 19.13.1 chromosome

• The protein product of \textit{NPHS1}, named Nephrin, is specifically expressed in podocytes

• The precise location of nephrin is in the slit diaphragm

Podocin (NPHS2)

• Using positional cloning the NPHS2 gene was identified consisting of 8 coding exons
• NPHS2 encodes a 383-amino acid protein named podocin
• An integral membrane protein with one transmembrane domain

SRNS Genes

• The etiology and pathogenesis of SRSN has remained enigma for decades.

• The discovery of 27 dominant or recessive SRNS genes enabled better understanding of the function of the glomerular podocytes and slit membrane.

• Hildebrandt’s group has shown that 85% of SRNS cases with onset by 3 months of age and 66% with onset by 1 year of age can be explained by recessive mutations in one of four genes only (NPHS1, NPHS2, LAMB2, or WT1).

SRNS Genes

• The same group used modern diagnostic techniques such as next generation sequencing and tested a large international cohort of SRNS patients (n=1783 families).

• The diagnostic panel included 21 genes with a recessive mode of inheritance (NPHS2, NPHS1, PLCE1, LAMB2, SMARCAL1, COQ6, ITGA3, MYO1E, COQ2, CUBN, ADCK4, DGKE, PDSS2, ARHGDIA, CD2AP, CFH, ITGB4, NEIL1, PTPRO/GLEPP1, SCARB2, and MEFV) and

• 6 genes with a dominant mode of inheritance (WT1, INF2, TRPC6, ARHGAP24, ACTN4 and LMX1B).

Podocyte genes associated with steroid resistant nephrosis
Table 1. International cohort of 526 of the 1783 families, in whom a single-gene cause of SRNS was detected in 1 of 21 monogenic causes of SRNS (27 genes examined)

<table>
<thead>
<tr>
<th>Gene Causing SRNS</th>
<th>Mode of Inheritance</th>
<th>SRNS Families Molecularly Diagnosed by Sanger Sequencing (Published Previously), n&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SRNS Families Molecularly Diagnosed by Multiplex PCR (n)</th>
<th>Total SRNS Families with Molecular Diagnosis (% of Families)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPHS2</td>
<td>AR</td>
<td>170 (42)</td>
<td>7</td>
<td>177 (9.93)</td>
</tr>
<tr>
<td>NPHS1</td>
<td>AR</td>
<td>93 (61)</td>
<td>38</td>
<td>131 (7.34)</td>
</tr>
<tr>
<td>WT1</td>
<td>AD</td>
<td>78 (50)</td>
<td>7</td>
<td>85 (4.77)</td>
</tr>
<tr>
<td>PLCE1</td>
<td>AR</td>
<td>23 (16)</td>
<td>14</td>
<td>37 (2.17)</td>
</tr>
<tr>
<td>LAMB2</td>
<td>AR</td>
<td>10 (6)</td>
<td>10</td>
<td>20 (1.12)</td>
</tr>
<tr>
<td>SMARCA1</td>
<td>AR</td>
<td>1 (0)</td>
<td>15</td>
<td>16 (0.89)</td>
</tr>
<tr>
<td>INF2</td>
<td>AD</td>
<td>2 (0)</td>
<td>7</td>
<td>9 (0.5)</td>
</tr>
<tr>
<td>TRPC6</td>
<td>AD</td>
<td>1 (1)</td>
<td>8</td>
<td>9 (0.53)</td>
</tr>
<tr>
<td>COQ6</td>
<td>AR</td>
<td>6 (5)</td>
<td>2</td>
<td>8 (0.45)</td>
</tr>
<tr>
<td>ITGA3</td>
<td>AR</td>
<td>3 (3)</td>
<td>2</td>
<td>5 (0.28)</td>
</tr>
<tr>
<td>MYO1E</td>
<td>AR</td>
<td>0 (0)</td>
<td>5</td>
<td>5 (0.28)</td>
</tr>
<tr>
<td>CUBN</td>
<td>AR</td>
<td>1 (1)</td>
<td>4</td>
<td>5 (0.28)</td>
</tr>
<tr>
<td>COQ2</td>
<td>AR</td>
<td>0 (0)</td>
<td>4</td>
<td>4 (0.22)</td>
</tr>
<tr>
<td>LMX1B</td>
<td>AD</td>
<td>0 (0)</td>
<td>4</td>
<td>4 (0.22)</td>
</tr>
<tr>
<td>ADCK4</td>
<td>AR</td>
<td>3 (3)</td>
<td>0</td>
<td>3 (0.17)</td>
</tr>
<tr>
<td>DGKE1</td>
<td>AR</td>
<td>0 (0)</td>
<td>2</td>
<td>2 (0.11)</td>
</tr>
<tr>
<td>PDS2</td>
<td>AR</td>
<td>0 (0)</td>
<td>2</td>
<td>2 (0.11)</td>
</tr>
<tr>
<td>ARHGAP24</td>
<td>AD</td>
<td>0 (0)</td>
<td>1</td>
<td>1 (0.06)</td>
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<tr>
<td>ARHDIA</td>
<td>AR</td>
<td>1 (1)</td>
<td>0</td>
<td>1 (0.06)</td>
</tr>
<tr>
<td>CFH</td>
<td>AR</td>
<td>0 (0)</td>
<td>1</td>
<td>1 (0.06)</td>
</tr>
<tr>
<td>ITGB4</td>
<td>AR</td>
<td>0 (0)</td>
<td>1</td>
<td>1 (0.06)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>392 (189)</td>
<td>134</td>
<td>526 (29.5)</td>
</tr>
</tbody>
</table>

<sup>a</sup> AR = autosomal recessive, AD = autosomal dominant
Causative mutations in 502 families

- 1st year applied: 61.3% (n=244/398)
- Total: 69.4% (n=163/235)
- 49.7% (n=81/163)
- NPHS1
- LAMB2
- PLCE1
- NPHS2
- WT1
- SMARCAL1
- COQ6/COQ2/ADCK4/PDSS2
- INF2
- TRPC6
- Other

Sadowski et al, JASN 2015; 26:1279-89
Mutations are more often detected in the young
Extra-renal manifestations

- The most common organ system involved is the CNS (structural anomalies, mental retardation)
- Sexual differentiation defects and tumors due to mutations in WT1 (Denys-Drash or Frasier)
- Cardiac anomalies in homozygotes for the R138X mutation in NPHS2

<table>
<thead>
<tr>
<th>Type of Extrarenal Abnormality</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients without reported</td>
<td>1368</td>
<td>82.7</td>
</tr>
<tr>
<td>extrarenal abnormalities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mental retardation</td>
<td>65</td>
<td>3.9</td>
</tr>
<tr>
<td>Anomalies of central nervous system</td>
<td>42</td>
<td>2.5</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>17</td>
<td>1.0</td>
</tr>
<tr>
<td>Visual impairment</td>
<td>32</td>
<td>1.9</td>
</tr>
<tr>
<td>Hearing disorder</td>
<td>25</td>
<td>1.5</td>
</tr>
<tr>
<td>Anomalies of peripheral nervous system</td>
<td>7</td>
<td>0.4</td>
</tr>
<tr>
<td>Myopathy</td>
<td>13</td>
<td>0.8</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>6</td>
<td>0.4</td>
</tr>
<tr>
<td>Urogenital abnormalities</td>
<td>33</td>
<td>2.0</td>
</tr>
<tr>
<td>Impaired sex differentiation</td>
<td>16</td>
<td>1.0</td>
</tr>
<tr>
<td>Short stature</td>
<td>84</td>
<td>5.1</td>
</tr>
<tr>
<td>Facial dysmorphism</td>
<td>37</td>
<td>2.2</td>
</tr>
<tr>
<td>Spondyloepiphyseal dysplasia</td>
<td>8</td>
<td>0.5</td>
</tr>
<tr>
<td>Polydactyly</td>
<td>5</td>
<td>0.3</td>
</tr>
<tr>
<td>Nail patella syndrome</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>Cardiac structural disorder</td>
<td>36</td>
<td>2.2</td>
</tr>
<tr>
<td>Malignant disorder</td>
<td>21</td>
<td>1.3</td>
</tr>
<tr>
<td>Hematologic disorders</td>
<td>7</td>
<td>0.4</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>7</td>
<td>0.4</td>
</tr>
<tr>
<td>Other endocrine abnormalities</td>
<td>23</td>
<td>1.4</td>
</tr>
<tr>
<td>Skin abnormalities</td>
<td>13</td>
<td>0.8</td>
</tr>
<tr>
<td>Abnormalities of the gastrointestinal tract</td>
<td>5</td>
<td>0.3</td>
</tr>
<tr>
<td>Autoimmune disorder</td>
<td>9</td>
<td>0.5</td>
</tr>
<tr>
<td>Connalal cytomegaly virus</td>
<td>9</td>
<td>0.5</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>6</td>
<td>0.4</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>10</td>
<td>0.6</td>
</tr>
</tbody>
</table>
Patient with Denys Drash Syndrome

- Male pseudohermaphroditism
- Nephromegaly
- Proteinuria progressing to full nephrotic syndrome
- Septic complications
- Death
Patient with Denys Drash Syndrome

Mutational analysis of WT1
(mutation 1267T>C in exon 8)
Pediatric versus Adult SRNS

- Mutations in recessive genes are more likely to cause SRNS in childhood
- Dominant genes are less penetrant and cause SRNS in adults with phenotypic variability
- The most prevalent dominant genes are *INF2*, *TRPC6* and *ACTN4*
Why should pediatric and adult nephrologists learn about the genetics of SRNS?

• Identification of causative single-gene mutations may have important therapeutic consequences in some cases.
• This is very important for patients who carry mutations in a gene of coenzyme Q10 biosynthesis (COQ2, COQ6, ADCK4, or PDSS2).
• In these patients treatment with coenzyme Q10 may be indicated.
• Also patients with recessive mutations in PLCE1 may respond fully to treatment with steroids or cyclosporine A.
• Patients with CUBN may benefit the treatment with vitamin B12.
• Individuals with ARHGDIA may theoretically be responsive to the eplerenone treatment.
Response of SRNS to oral coenzyme Q10 (CoQ10) in monogenic SRNS due to a mutation in an enzyme of the coenzyme Q10 biosynthesis pathway

Lovric et al. Nephrol. Dial. Transplant. 2015; ndt.gfv355
Detection of causative mutations

- No benefit from immunosuppressants
- No recurrence after Tx
- No need for renal biopsy
- Living Related Tx donors acceptable
- Familial genetic counseling
- Prenatal diagnosis
- Carriers of WT1 mutations should be monitored for Wilm’s TU and gonadoblastoma
Proteins involved in single-gene causes and pathogenic pathways of SRNS. Identification of single-gene (monogenic) causes of SRNS has revealed the renal glomerular epithelial cell, the podocyte, as the center of action in the pathogenesis of SRNS, because all of the related genes are highly expressed in podocytes.

Lovric et al. Nephrol. Dial. Transplant. 2015;ndt.gfv355
Is routine genetic testing for SRNS justified?

• There is no consensus!
• Justified in the following groups of patients:
  – All children with CNS (onset < 3 mts)
  – All children with infantile NS
  – If multi-organ involvement
  – Positive family history of NS or CKD
But Hildebrandt’s group proposes when to start mutational analysis!

• all individuals with FSGS or with persistent proteinuria that manifests before age of 25 years
• panel of \(~39\) monogenic genes that are known to cause SRNS if mutated can now be examined
• Mutations in recessive disease genes are found more frequently in early-onset disease, whereas mutations in dominant genes more frequently cause adult-onset disease
• the likelihood of finding a causative (recessive) mutation is very high in individuals with SRNS from consanguineous marriages

Lovric et al. Nephrol. Dial. Transplant. 2015;ndt.gfv355
Research Laboratories

- [www.renalgenes.org](http://www.renalgenes.org)
  
  Contact. Prof. F. Hildebrandt, Boston Children’s Hospital, Harvard Medical School

- [www.podonet.org](http://www.podonet.org)
  
  Contact Prof. F. Schaefer, Division of Pediatric Nephrology, Center for Pediatric and Adolescent Medicine Heidelberg University Hospital