THE GENETIC BASIS OF STEROID-RESISTANT NEPHROTIC SYNDROME

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Nephrotic syndrome (NS) represents disruption of the glomerular filtration barrier which is responsible for plasma ultrafiltration during urine formation. Steroid-resistant nephrotic syndrome (SRNS) progressing to advanced kidney failure, although representing approximately 15% of all cases of childhood NS, is the common phenotype of most of the hereditary forms of NS whether recessive or dominant. Most gene-products associated with SRNS are expressed predominantly in the podocytes or the slit diaphragm.

The most frequent histologic feature of SRNS is focal segmental glomerulosclerosis (FSGS), followed by minimal change disease (MCD) and diffuse mesangial proliferation (DMP).

NS represents disruption of the glomerular filtration barrier, which is responsible for plasma ultrafiltration during urine formation and is composed of three layers: the innermost fenestrated endothelial cells followed by the glomerular basement membrane (GBM) and the visceral epithelial cells, better known as podocytes. Since most identified causes point to the podocytes or the slit diaphragm as pathogenetically involved, the various forms of NS are referred to as podocytopathies.

A seminal work by Tryggvason's group detected mutations in NPHS1 encoding nephrin, a central component of the slit diaphragm, to be responsible for the autosomal recessive congenital nephrotic syndrome (CNS) of the Finnish type (CNSF) (2). The slit diaphragm joins the podocyte foot processes and assists in maintaining their proper structure and function. Two founder mutations, designated Fin-major and Fin-minor, are responsible for the vast majority of patients with CNS (1).

Since 1994, the first example of podcytopathy. It was followed by the identification of mutations in NPHS2, encoding podocin, causing SRNS in children (3). The common phenotype was of SRNS presenting in early childhood with gradual decline in kidney function until ESKD is reached usually during the first or second decade of life. None of the affected patients in the original report experienced recurrence of FSGS following kidney transplantations. Podocin is a stomatin protein family member with a predicted hairpin-like structure localizing to the insertion site of the slit diaphragm of podocytes. In a study by Benzing's group it was demonstrated that wild-type podocin is targeted to the plasma membrane, and forms homo-oligomers involving the carboxy- and amino terminal cytoplasmic domains (4). The association of podocin with specialized lipid raft microdomains of the plasma membrane was a prerequisite for recruitment of nephrin into rafts.

In contrast, disease-causing mutations of podocin failed to recruit nephrin into rafts either because these mutants were retained in the endoplasmic reticulum (R138Q), or because they failed to associate with rafts (R138X) despite their presence in the plasma membrane. As lipid raft targeting facilitates nephrin signaling, failure of mutant podocin to recruit nephrin into lipid rafts may be essential for the pathogenesis of NPHS2 mutations.

Over the ensuing 15 years, mutations in a growing number of genes have been identified in patients with SRNS. The common denominator of these genes is that they are involved in the development, structure or function of components of the glomerular barrier, most often the podocytes. The rare exception is LAMB2 whose gene product is laminin-β2 (LAMB2) (5).

It has previously been shown that 85% of patients with CNS, manifesting before the age of 3 months, and 66% of individuals with infantile NS, presenting before one year of age, are explained by causative mutations in one of the following genes: NPHS1, NPHS2, LAMB2 and WT1 (7). Since data derived from large cohorts were available for SRNS patients presenting over a shorter period of time, most recently, a large cohort of patients with SRNS were studied to detect the frequency of causative mutations. A strategy of high-throughput, bar-coded exon sequencing using the Fluidigm platform

A list of 20 recessive and 6 dominant genes identified in cases of NS with the corresponding phenotype are depicted in Tables 1 and 2, respectively.

Table 1: Etiology of autosomal recessive nephrotic syndrome

<table>
<thead>
<tr>
<th>Genes</th>
<th>Locus</th>
<th>Phenotype / comments</th>
</tr>
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<tbody>
<tr>
<td>NPHS1</td>
<td>19q13.1</td>
<td>CNF, occasionally FSGS</td>
</tr>
<tr>
<td>NPHS2</td>
<td>1q25–q31</td>
<td>SRNS: MCD, DMP, FSGS</td>
</tr>
<tr>
<td>NPHS3PLCE1</td>
<td>1q23–q24</td>
<td>DMS, occasionally FSGS</td>
</tr>
<tr>
<td>SMARCAL1</td>
<td>2q34–q36</td>
<td>Schindek immune-ossseous dysplasia</td>
</tr>
<tr>
<td>LAMB2</td>
<td>3p1</td>
<td>Pierson syndrome (DMS)</td>
</tr>
<tr>
<td>SCARB2</td>
<td>4q21</td>
<td>NS with Clq deposits; progressive myoclonic epilepsy</td>
</tr>
<tr>
<td>COQ6</td>
<td>1q42</td>
<td>Syndrome NS (sensorineural deafness, seizures, ataxia)</td>
</tr>
<tr>
<td>PTPRO-GLEP1</td>
<td>12p2</td>
<td>FSGS/MCD</td>
</tr>
<tr>
<td>MYOEO</td>
<td>15q21</td>
<td>FSGS</td>
</tr>
<tr>
<td>ITGA3</td>
<td>1q21</td>
<td>Syndrome NS: CNS; renal tubular acidosis, skin fragility</td>
</tr>
<tr>
<td>COQ2</td>
<td>4q21.23</td>
<td>SRNS, ESKD, epileptic encephalopathy</td>
</tr>
<tr>
<td>CUSB</td>
<td>19p13</td>
<td>Intermittent proteinuria (tubular proteinuria not excluded)</td>
</tr>
<tr>
<td>A2CK4</td>
<td>19q13.2</td>
<td>SRNS, occasionally infantile</td>
</tr>
<tr>
<td>ITGB4</td>
<td>17q25.1</td>
<td>Cong. FSGS, epidermolysis bullosa</td>
</tr>
<tr>
<td>PDHS2</td>
<td>6q21</td>
<td>Risk allele for FSGS; independently, CoQ10 def</td>
</tr>
<tr>
<td>ARKADA</td>
<td>15q23.3</td>
<td>DMS</td>
</tr>
<tr>
<td>CD2AP</td>
<td>6p21.3</td>
<td>FSGS, also dominant inheritance</td>
</tr>
<tr>
<td>NEU1</td>
<td>1q42.4</td>
<td>SRNS</td>
</tr>
<tr>
<td>MEFV</td>
<td>1p13.3</td>
<td>Familial Mediterranean Fever</td>
</tr>
</tbody>
</table>

Table 2: Etiology of autosomal dominant nephrotic syndrome

<table>
<thead>
<tr>
<th>Genes</th>
<th>Locus</th>
<th>Phenotype / comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD2AP</td>
<td>6p12.3</td>
<td>FSGS; occasionally recessive inheritance</td>
</tr>
<tr>
<td>WT1</td>
<td>11p13</td>
<td>Deny-Drash syndrome; Frasier syndrome; DMS; FSGS</td>
</tr>
<tr>
<td>α-actinin-4</td>
<td>19q3</td>
<td>FSGS</td>
</tr>
<tr>
<td>TRPC6</td>
<td>11p12.3</td>
<td>FSGS</td>
</tr>
<tr>
<td>LMX1B</td>
<td>9q34.1</td>
<td>Nail patella syndrome</td>
</tr>
<tr>
<td>INF2</td>
<td>14q2</td>
<td>FSGS; also Charcot- Marie-Tooth disease</td>
</tr>
<tr>
<td>ARKAP24</td>
<td>4q20</td>
<td>FSGS</td>
</tr>
</tbody>
</table>
with consecutive next-generation sequencing was implemented (6). A proof of concept platform covering 27 genes that have been associated with NS and/or chronic kidney disease (CKD) is justified in the following subgroups of patients with NS:

- All children with CNS (onset prior to 3 months of age).
- All children with infantile NS (onset 3-12 months of age).
- All patients with a family history of NS and/or chronic kidney disease (CKD).

Whether it should be recommended in every child with SRNS, is still question- able. If the treating physician has free access to the high throughput screening platform covering 27 genes that have been associated with SRNS, this should be implemented. Otherwise, a reasonable approach would be to screen those patients for the 4 genes that account for over half of the patients with congenital nephrotic syndrome (NPHS1, NPHS2, WT1 and PLECE1). The order of screening may be affected by the age of onset of proteinuria: NPSI1, WT1 and PLECE1 first, in congenital or infantile NS and NPHS2 first in older children.

Although it may change in the near future, the main challenge is the advancement in the field, at this point in time, next-generation sequencing should still be reserved for well selected cases performed in research laboratories. The advantage of this approach is that it can screen all of the coding regions or the entire genome in one run and may yield not only causative mutations in known genes but may also discover new genes that have not been associated with NS before. Identifying new genes may shed light on the pathophysiological mechanisms underlying various forms of NS and additionally, may pave the path for the development of specific therapeutic modalities. The amount of data generated by this technique requires a well trained team in the area of bioinformatics in order to wisely analyze all the genetic variants which may bear deleterious effect. This will carry significant ethical dilemmas which should be taken seriously into consideration.

The availability of rapid and cost- effective genetic tests, should not replace the meticulous clinical evaluation of patients with SRNS by their treating physicians. Furthermore, this would have a favorable impact in the likelihood of a genetic test to yield the correct diagnosis.

### Availability of genetic testing

One of the challenges faced by physicians taking care of children with SRNS is to determine what genetic tests are currently available. The following is the updated information on the disease, gene, protein and the laboratory performing the tests. These data are country-specific or even insurer-specific. The type of laboratory performing the test (research- versus approved clinical testing) is to determine what genetic tests should be taken more seriously in domin- ant forms which may involve poorly penetrant alleles resulting in asymptomatic affected individuals. Living-related potential donors would have to be screened for the relevant mutation prior to declara- ting them suitable for kidney donation. Obviously, living-related donations from family members of individuals with hereditary kidney disease but unknown gen- etic defects should be discouraged.

Is routine genetic testing for NS justified?

- Any genetic test ordered should address the following three goals (22):
  - Would the result of this test help estab- lishing the diagnosis?
  - Would the result likely to assist in planning the treatment?
  - Is the result from the proband likely to produce data on the risk of other family members?

Given the data presented here, it is reasonable to conclude that genetic test- ing is justified in the following subgro- ups of patients with NS:

- All children with CNS (onset prior to 3 months of age).
- All children with infantile NS (onset 3-12 months of age).
- All patients with a family history of NS and/or chronic kidney disease (CKD).

It occurs almost exclusively among individuals without a genetic diagnosis which implies that a circulating permea- bility defect may be more likely a conse- quence of treatment with immunosuppressants and plasmapheresis. Proteinuria post kidney transplant has been detected in child with bi-allelic mutations in NPHS2 encoding podocin, although antibodies to podocin could not be identified (20). This is in contrast to children with CNF due to mutations in NPHS1 encoding nephrin which may develop proteinuria after kidney transplantation secondary to acquired circulating anti-nephrin antib- oodies (21).

It is unlikely that living-related asymptomatic kidney donors who are carriers of recessive mutations have additional risk of developing CKD in the future. This has been exemplified by a report of 4 living-related kidney donors, heterozygous for mutations in NPHS2, who remain healthy. Also, none of the corresponding children with congenital nephrotic syndrome followed up post kidney transplant have developed proteinuria following kidney transplantati- on (20). The only reservation would be a yet unknown interaction with a modifier gene or an environmental factor. This should be taken more seriously in domin- ant forms which may involve poorly penetrant alleles resulting in asymptomatic affected individuals. Living-related potential donors would have to be screened for the relevant mutation prior to declara- ting them suitable for kidney donation. Obviously, living-related donations from family members of individuals with hereditary kidney disease but unknown gen- etic defects should be discouraged.

### Conclusion

The genetic basis of steroid-resistant nephrotic syndrome (SRNS) has been explored in a small cohort of 96 patients, previ- ously diagnosed by Sanger sequencing of the relevant gene (6). This technique was then implemented in a large internatio- nal cohort of 2,016 cases from 1,783 fa- milies. Disease-causing mutations were found in 526 of 1783 families, represen- ting 9.83% of the families with proven genet- ic NS had mutations in NPHS2, 7.34% - in NPSI1, 4.77% - in WT1 and 2.17% - in PLECE1 implicating that in 82% of the families who tested positive, pathogenic mutations were detected in 4 out of 27 genes examined. No gender difference in the likelihood of disease-causing mutation was noted. There was a negative correlation between the like- lihood of identifying a disease-causing mutation and the age of onset of prote- inuria: 61.3% of children with infantile NS compared to approximately 10% of children over 12 years were found to have a single- gene mutation in one of the 21 genes. As expected, the detection rate of disease-causing mutations was much higher in highly consanguinous fami- lies.

Mutations in recessive genes are more likely to cause SRNS in childhood whereas dominant mutations are more prevalent among young adults. The ma- jor genetic causes of dominant forms of NS include mutations in NPHS1, NPHS2 (INF2), transient receptor potential ca- tion channel protein, type C6 (TRPC6) and actinin-alpha 4 (ACTN4). It has been shown that mutations in INF2 account for approximately 16% of all cases of AD SRNS/FSGS mostly in the adult po- pulation (9). The consequences of domi- nant variants are less penetrant and as a result affected individuals may even be asymptomatic. The ultimate phenotype may possibly result from further gene- and gene-environment interactions.

### Genotype-phenotype correlation

The age of onset of NS was signifi- cantly earlier for children with splice site mutations than with exonic mutations which may be a consequence of different splicing patterns or regulation of gene expression. The correlation between mutation type and age of onset of proteinuria was much higher in highly consanguineous families whereas dominant mutations are more prevalent among young adults. The ma- jor genetic causes of dominant forms of NS include mutations in NPHS1, NPHS2 (INF2), transient receptor potential ca- tion channel protein, type C6 (TRPC6) and actinin-alpha 4 (ACTN4). It has been shown that mutations in INF2 account for approximately 16% of all cases of AD SRNS/FSGS mostly in the adult po- pulation (9). The consequences of domi- nant variants are less penetrant and as a result affected individuals may even be asymptomatic. The ultimate phenotype may possibly result from further gene- and gene-environment interactions.

### Renal pathology

It has been appreciated that the absence of therapeutic response to steroid- is a better prognostic factor in chil- dren with nephrotic syndrome than the underlying histology. In fact, patients with bi-allelic mutations in NPHS2 may have a full range of histopathologic find- ings including minimal change, diffuse mesangial proliferation and FSGS (3, 11).

Furthermore, we have occasionally encountered IgM and/or C3 mesangial deposits with otherwise DMP, in these children which may represent non-speci- fic inflammatory events (13). Identifying a single-gene cause of SRNS renders renal biopsy unnecessary as this will have no bearing on the treatment choice or the long-term prognosis. Furthermore, the histopathological fin- dings cannot distinguish genetic from non-genetic disease etiologies (12).

### Steroid-Sensitive Nephrotic Syndrome (SSNS)

Not surprisingly, none of the 185 children with SSNS, who composed a control group with the international cohort of children with SRNS, was fo- und to carry bi-allelic mutations in any of the 27 genes which were associated with SRNS (6). This supports the notion that patients with single-gene cause of SRNS are unlikely to respond to steroids but may rather suffer from side-effects of this therapeutic modality (11, 15).

Most recently, it was shown that hete-rogenous genetic variants in sporadic nephrotic syndrome associate with not only resistance to steroids but to other immunosuppressive agents (16). A re- cent report from Hildebrandt’s group described mutations in EMP2, encoding epithelial membrane protein 2, in 4 individuals from 3 unrelated fami- lies with NS (17). Interestingly, in two families from Turkey the disease respon- ded to steroids and in the fourth child of African-American ancestry it was ste- roid-resistant. The precise pathogenic mechanism is yet to be determined with special emphasis on its varying response to steroids remains unclear.

There are anecdotal reports of pa- tients with genetic forms of NS who re- sponded to immunosuppressive regimen.

Two patients with bi-allelic mutations in PLECE1 seemed to attain remission following cyclosporine A (CsA) (18). Whether this represents the non-immunological anti-proteinemic effect of CsA remain to be proven. It has been shown that CsA can stabilize the actin cytoskeleton by blocking the calcineurin-mediated dephosphorylation of synaptotogin (19). Alternatively, this may be an example of the phenotypic va- riability which can be mutation-specific even within the context of the same genotype.

### Non-immunological modalities

have been shown to slow the progression of proteinuric renal diseases, including angiotensin converting enzyme inhibitors (ACEi) or lipid lowering agents should be considered in every child with SRNS regardless of whether they have genetic forms of NS.

### Post-transplant recurrence of FSGS

Recurrence of FSGS was found in 15% of patients undergoing kidney trans- plantation in the large international cohort of children with SRNS, was noted in 4 individuals from 3 unrelated families. Living-related po- donucin, although antibodies to podocin could not be identified (20). This is in contrast to children with CNF due to mutations in NPHS1 encoding nephrin which may develop proteinuria after kidney transplantation secondary to acquired circulating anti-nephrin antib- oodies (21).

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### steroid-resistant nephrotic syndrome. Paediatr Croat. 2015; 59 (Supl 1): 44-49
The discovery that mutations in NPHS1 cause CNF had great impact not only on our understanding of the pathogenesis of this clinical entity but also provided the medical community with a tool to diagnose a significant portion of their patients with CNF. CNF is inherited in an autosomal recessive fashion and has a worldwide distribution. It was realized that distinct mutations than those detected in Finland are responsible for CNF in other ethnic groups. It was quite striking to detect three different mutations in NPHS1 in one village near Jerusalem, Israel (23). Most of the inhabitants of this community are descendants of one Muslim family and have maintained their isolation by preference of consanguineous marriages.

As more and more genes have been identified as associated in their mutated form with SRNS, it was clear that every "new" gene provides explanation to a very small portion of the patient population. Mutations in 4 genes are responsible for SRNS in 24.2% of the 1,783 families tested, whereas mutations in 17 different genes cause SRNS in only additional 5.3% of the families (6). The genetic basis of SRNS in the remaining 70.5% of the families remains elusive. Also, mutations in EMP2, leading to NS, were identified in a single family from Turkey and subsequent screening of 1,600 individuals from an international cohort of patients with NS yielded two additional cases, implying that this is a rare cause of NS (17). Taken together, there seems to be a shift from "private" mutations in common genes to mutations in "private" genes. This may dictate novel strategies to identify genetic causes of renal diseases which may include searching for genetic variation in noncoding regions or epigenetic alterations.

From private mutations to private genes


