Genetic Testing of Children with Steroid Resistant Nephrotic Syndrome

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Peking University First Hospital
Nephrotic Syndrome (NS)

- Clinical manifestations
  massive proteinuria, hypoalbuminemia, edema and hyperlipidemia
- The most common glomerular disease of childhood
- An incidence in an unselected cohort of children approximately 2/100,000
- Initial treatment: steroid

J Paediatr Child Health, 2007
Steroid resistant Nephrotic Syndrome (SRNS)

- A clinical entity
- Represents approximately 22% of cases of idiopathic childhood nephrotic syndrome
  - Progression to end-stage renal disease
  - 70-75% will have biopsy-proven focal segmental glomerulosclerosis (FSGS)

Advances in Chronic Kidney Disease, 2011
Steroid resistant Nephrotic Syndrome (SRNS)

- Represents structural changes in the glomerulus or, more specifically, the podocyte
- the slit diaphragm
Morphological structure was described for several decades

“Zipper-like”

Molecular constitute?

Until 1998

**NP**HS1

Encoding nephrin

Causative gene for Finish type congenital NS

The first molecule on slit diaphragm
Milestones for Podocyte Molecular Study

- **NPHS1**: Molec. Cell, 1998
- **CD2AP**: Science, 2000
- **PLCE1**: Nature Genet., 2003
- **LAMB2**: Kidney Int., 2005
- **INF2**: Nature Genet., 2006
- **ACTN4**: Nature Genet., 2008
- **TRPC6**: Science, 2010
- **APOL1**: Nature Genet., 2011
- **DKGE**: Nature Genet., 2013
Podocyte genes associated with SRNS
Accurate diagnosis of SRNS

- Many single genes, more than 30
- Similar clinical features
  - Heavy proteinuria
  - Steroid resistance
  - Renal pathology: FSGS, DMS, MCD
  - Progression to ESRD
- Problems
  - Diversity of clinical and genetic findings
  - Application of steroid?
  - Recurrent after renal transplantation?
Accurate diagnosis of SRNS

- **Genetic testing**: a revolution on diagnosis for pediatric nephrologists
  - Accurate diagnosis
  - Precise genetic counseling
  - Appropriate therapy
  - Predict risk of post transplant recurrence
  - Better understanding of pathophysiology
    - Identify crucial molecular target, therapy
    - Clarify specific pathway, blockage
Suggested approach for genetic testing in SRNS

**GENETICS OF STEROID-RESISTANT NEPHROTIC SYNDROME: A REVIEW OF MUTATION SPECTRUM AND SUGGESTED APPROACH FOR GENETIC TESTING**

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**Keywords**
Gene mutations, Genetic testing, Nephrotic syndrome, Podocyte, Steroid resistance

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**ABSTRACT**
Identification of genes, associated mutations and genotype-phenotype correlations in steroid-resistant nephrotic syndrome (SRNS) is being translated to clinical practice through genetic testing. This review provides an update on the genes and mutations associated with SRNS along with a suggested approach for genetic testing in patients with SRNS.

**Conclusion:** The number of identified genes associated with SRNS is increasing along with our understanding of their impact on treatment response and risk of recurrence. A systematic approach to genetic testing in patients with SRNS might aid the physician in selecting appropriate treatment.
Suggested approach for genetic testing in SRNS

- **Congenital onset**
  - Microcystic dialation of tubules and PMS
    - Yes: Genetic diagnosis, Stop
    - No: MCNS/FSGS
      - Yes: NPHS1
      - No: NPHS2
        - Yes: Genetic diagnosis, Stop
        - No: NPHS1
          - Yes: Genetic diagnosis, Stop
          - No: PLCE1*
            - Yes: Genetic diagnosis, Stop
            - No: Genetic diagnosis

- **Childhood onset**
  - DMS
    - Yes: Karyotype
    - No: MCNS/FSGS
      - Yes: NPHS2
      - No: WT1
        - Yes: Genetic diagnosis, PS
        - No: Genetic diagnosis
          - Yes: WT1*
          - No: Genetic diagnosis
            - Yes: PLCE1*
            - No: Genetic diagnosis
              - Yes: CD2AP*, INF2*, ACTN4*, TRPC6*
              - No: Genetic diagnosis
Targeted next generation sequencing is a cost-effective strategy

Simultaneously investigate all relevant genes
Friedhelm Hildebrandt, et al:
Multicenter Study

A Single-Gene Cause in 29.5% of Cases of Steroid-Resistant Nephrotic Syndrome


*Division of Nephrology, Department of Medicine, Boston Children’s Hospital, Harvard Medical School, Boston, Massachusetts; †Department of Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, Michigan; ‡Department of Genetics and Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut; §Institute of Child Health, University College London, London, United Kingdom; ¶Pediatric Nephrology Unit, King Abdulaziz University Hospital, Jeddah, Kingdom of Saudi Arabia; ‖Department of Human Genetics, Otto von Guericke University, Magdeburg, Germany; **Department of Pediatrics, University Hospital Hamburg-Eppendorf, Hamburg, Germany; ††Department of Pediatric Nephrology, Medical Faculty of the Charité, Berlin, Germany; ‡‡The Pediatric Nephrology Unit, Alexandria University, Alexandria, Egypt; §§Department of Pediatrics, Center of Pediatric Nephrology & Transplantation, Kasr Al Ainy School of Medicine, Cairo University, Cairo, Egypt; ‖‖Egyptian Group for Orphan Renal Diseases, Cairo, Egypt; and ¶¶Howard Hughes Medical Institute, Chevy Chase, Maryland
an international cohort
- 1783 different families
- 2016 individuals
- SRNS
- 253 (14.1%) presented with congenital NS
all known 27 monogenes causing SRNS
- microfluidic multiplex PCR
- next-generation sequencing
Friedhelm Hildebrandt, et al: Multicenter Study
*J Am Soc Nephrol, 2015*

- Disease-causing mutations
  - 526 of 1783 families
  - 29.5% with monogenic mutation
- Mutations in 21 of the 27 known SRNS genes
  - 129 novel mutations (48 truncating alleles)
- Previously 1115 reported mutations
  - Human Gene Mutation Database ([http://www.hgmd.org](http://www.hgmd.org))
- An additional 11.6%
Large cohort study

- Most SRNS families from Europe and North America
- No patients from Russia, China, sub-Saharan Africa, or Pacific Rim countries
- 127/1783 (7%) families from India

- Is there a geographic or ethnic difference?
  - *NPHS2* mutation in Chinese children with SRNS, 4% vs 20%-30% in Europe

*Nephrol Dial Transplant, 2005*
A Multicenter Study for Chinese Children with SRNS

- Patients

- Inclusion criteria (at least one of two criteria):
  - SRNS
  - Isolated proteinuria + highly suspected genetic causes

- Exclusion criteria:
  - Age of onset of disease was more than 18 years
  - Diagnosed as Alport syndrome
A Multicenter Study for Chinese Children with SRNS

- Targeting next generation sequencing
- NGS detection included 28 genes
- 21 genes with autosomal recessive mode of inheritance
- 7 genes with autosomal dominant mode of inheritance
- To predict the functional significance of all variants
  - SIFT
  - PolyPhen
  - Condel
  - Mutation Taster
  - Sanger validation
A Multicenter Study for Chinese Children with SRNS

- 120 patients were included
  - SRNS: 110 cases
  - Isolated proteinuria: 10 cases
- From 5 centers
  - Beijing
  - Hangzhou
  - Fuzhou
  - Changsha
  - Guangzhou
## Age and Causative Gene Distribution

<table>
<thead>
<tr>
<th>Age of onset</th>
<th>≤3 mo</th>
<th>4-12 mo</th>
<th>13 mo-5 yr</th>
<th>6-12 yr</th>
<th>13-17 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mutation detection rate</strong></td>
<td>9/12 (75%)</td>
<td>6/18 (33.3%)</td>
<td>14/54 (25.9%)</td>
<td>5/34 (14.7%)</td>
<td>1/2 (50%)</td>
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<td><strong>Causative genes</strong></td>
<td>WT1 NPHS1 ADCK4 LAMB2</td>
<td>TRPC6 WT1 NPHS1 PLCE1 COQ2</td>
<td>WT1 LMX1B NPHS2 CUBN ADCK4</td>
<td>ADCK4 SMARCAL1</td>
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Causative Gene Distribution among Different Ages

Age of onset

Percent patients with causative mutations detected

- **0-3 mo**
  - SMARCAL1
  - NPHS1
  - NPHS2
  - LAMB2
  - PLCE1
  - ADCK4
  - CUBN
  - COQ2
  - WT1
  - TRPC6
  - LMX1B

- **4-12 mo**
  - SMARCAL1
  - NPHS1
  - NPHS2
  - LAMB2
  - PLCE1
  - ADCK4
  - CUBN
  - COQ2
  - WT1
  - TRPC6
  - LMX1B

- **13 mo-5 yrs**
  - SMARCAL1
  - NPHS1
  - NPHS2
  - LAMB2
  - PLCE1
  - ADCK4
  - CUBN
  - COQ2
  - WT1
  - TRPC6
  - LMX1B

- **6-12 yrs**
  - SMARCAL1
  - NPHS1
  - NPHS2
  - LAMB2
  - PLCE1
  - ADCK4
  - CUBN
  - COQ2
  - WT1
  - TRPC6
  - LMX1B

- **13-17 yrs**
  - SMARCAL1
  - NPHS1
  - NPHS2
  - LAMB2
  - PLCE1
  - ADCK4
  - CUBN
  - COQ2
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Comparison of causative genes: China vs others

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*J Am Soc Nephrol, 2015*
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Percent of patients with molecular diagnosis

- NPHS2: Hildebrandt's study (9.00%), Our study (9.00%)
- NPHS1: Hildebrandt's study (9.00%), Our study (9.00%)
- WT1: Hildebrandt's study (9.00%), Our study (9.00%)
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- LMX1B: Hildebrandt's study (9.00%), Our study (9.00%)
- ADCK4: Hildebrandt's study (9.00%), Our study (9.00%)
- SMARCAL1: Hildebrandt's study (9.00%), Our study (9.00%)
- Others: Hildebrandt's study (9.00%), Our study (9.00%)

Hildebrandt's study vs Our study

Korkmaz study

*ADCK4* mutations were found in 10 patients among 534 SRNS cases (1.9%)

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<tr>
<td><strong>n</strong></td>
<td>26</td>
</tr>
<tr>
<td>Age at first reported manifestation, years</td>
<td>14.1 (10.8–17.0)</td>
</tr>
<tr>
<td>Histopathological diagnosis</td>
<td></td>
</tr>
<tr>
<td>FSGS/global glomerulosclerosis</td>
<td>61.5%</td>
</tr>
<tr>
<td>Diffuse mesangial sclerosis</td>
<td>0</td>
</tr>
<tr>
<td>Mesangioproliferative GN</td>
<td>0</td>
</tr>
<tr>
<td>Minimal change GN</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
</tr>
<tr>
<td>No data/ not performed</td>
<td>38.5%</td>
</tr>
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Conclusion

Children with Steroid Resistant Nephrotic Syndrome

- Genetic testing is important
  - Making therapeutic strategy
  - Predict the recurrent risk for renal transplantation

- Genetic testing strategy for Chinese children with SRNS
  - *ADCK4* was the most frequent causative gene
    - Experimental treatment with coenzyme Q10 may be tried
  - *NPHS2* mutations was lower than reported previously
Acknowledgement

Grants
- Ministry of Science and Technology
- National Nature Science Foundation of China
- National Nature Science Foundation of Beijing

Colleagues and Collaborators
- Jianhua Mao, Hangzhou
- Zihua Yu, Fuzhou
- Zhuwen Yi, Changsha
- Li Yu, Guangzhou
- Jie Ding, Yanqin Zhang, Fangrui Ding, Hongwen Zhang, Xiaoyu Liu, Huijie Xiao, Yong Yao, Xuhui Zhong, Jingcheng Liu, Lixia Yu

Patients and their families
THANK YOU