The 5th Global Congress For Consensus in Pediatrics & Child Health

Advances in hereditary Renal diseases

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Hereditary renal diseases

- Are hereditary renal diseases rare?
  - 10% of adults and almost all children with renal-replacement therapy
  - The fifth most common cause of end-stage renal disease after diabetes, hypertension, glomerulonephritis, and pyelonephritis
  - About 30% of childhood renal diseases
A retrospective study from China **Chin J Pediatr, 2004**
- 1268 hospitalized children with chronic renal failure
- 24.2% caused by congenital/inherited renal diseases
Hereditary renal diseases

- Overall prevalence
  - about 60–80 cases per 100,000 in Europe and the USA

*Lancet, 2014; Nephron Clin Pract 2012*

- constitute at least **160** different disorders
Classification

According to inherited mode/pattern

The transmission usually follows Mendel

- **Autosomal dominant**
  - *e.g.* ADPKD

- **Autosomal recessive**
  - *e.g.* ARPKD

- **X linked**
  - *e.g.* Alport syndrome
Classification

- Renal function
  - Glomerular diseases
  - Renal tubular diseases and metabolic diseases
  - Nephrolithiasis
- Renal growth and structure
  - Congenital abnormalities of the kidney and urinary tract (CAKUT)
  - Ciliopathies

Inherited renal diseases

Genetics were first used in nephrology in the 1980s


Disease-causing genes coding for a wide range of proteins: receptors, channels and transporters, enzymes, transcription factors, and structural components

- Several hundreds genes

- Constitute at least 160 different disorders
Inherited renal diseases

- The advances of relevant studies on genes as well as encoding proteins
  - Accurate diagnosis
  - Appropriate therapy
  - Genetic counseling
  - Predict risk of post transplant recurrence
  - Shed light on pathogenesis of renal diseases
    - Identify crucial molecular target, therapy
    - Clarify specific pathway, blockage
Inherited renal diseases

- Proteinuric disorders/nephrotic syndrome and podocyte molecules
- Alport syndrome and progression mechanism
- Atypical hemolytic uremic syndrome and complement genes defects
Inherited renal diseases

- Proteinuric disorders/nephrotic syndrome and podocyte molecules
- Alport syndrome and progression mechanism
- Atypical hemolytic uremic syndrome and complement genes defects
Glomerular Filtration Barrier

- endothelial cells
- glomerular basement membrane
- the specialized epithelial cells - podocytes
Slit Diaphragm

- Morphological structure was describe for several decades
  - “Zipper-like”
  - Molecular constitute?
  - Untill 1998
    - NPHS1
    - Encoding nephrin
    - Causative gene for Finish type congenital NS
    - The first molecule on slit diaphragm
# Partial Hereditary Nephrotic Syndrome

<table>
<thead>
<tr>
<th>Gene</th>
<th>Histology</th>
<th>Inheritance</th>
<th>Age at Present</th>
<th>Age at ESRD</th>
<th>Transplant Recurrence</th>
<th>Comments Nonrenal Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPHS1</td>
<td>Large kidneys, progressive fibrosis</td>
<td>AR</td>
<td>Infant</td>
<td>Infant</td>
<td>No</td>
<td>Rare types may present later in life</td>
</tr>
<tr>
<td>NPHS2</td>
<td>FSGS, DMS, MCD</td>
<td>AR</td>
<td>Infant-adult</td>
<td>Infant-adult</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>INF2</td>
<td>FSGS</td>
<td>AD</td>
<td>Child-adult</td>
<td>Child-adult</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>ACTN4</td>
<td>FSGS</td>
<td>AD</td>
<td>Child-adult</td>
<td>Adult</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>CD2AP</td>
<td>FSGS</td>
<td>AD</td>
<td>Child-adult</td>
<td>Child-adult</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>TRCP6</td>
<td>FSGS</td>
<td>AD</td>
<td>Adult</td>
<td>Adult</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>PLCE1</td>
<td>DMS, FSGS</td>
<td>AR</td>
<td>Infant-child</td>
<td>Child-adult</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>WT1</td>
<td>FSGS</td>
<td>AD</td>
<td>Child-adult</td>
<td>Child-adult*</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>LMX1B</td>
<td>FSGS, abnormal GBM</td>
<td>AD</td>
<td>Child-adult</td>
<td>Adult*</td>
<td>No</td>
<td>Isolated NS; Wilms tumor, Denys-Drash, WAGR, or Frasier syndrome</td>
</tr>
<tr>
<td>LAMB2</td>
<td>Mesangial sclerosis, abnormal GBM</td>
<td>AR</td>
<td>Infant-child</td>
<td>Child-adult</td>
<td>No</td>
<td>Nail-patella syndrome</td>
</tr>
<tr>
<td>MYH9</td>
<td>FSGS, abnormal GBM</td>
<td>AD</td>
<td>Infant-adult</td>
<td>Child-adult*</td>
<td>No</td>
<td>Isolated NS; Epstein, Frechtned, or Sebastian syndrome</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Fibrillar deposits</td>
<td>AD</td>
<td>Child-adult</td>
<td>Adult</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>
Accurate diagnosis

- 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

- A “revolution” on genetic testing
  - Next generation sequencing
  - Simultaneous investigate all relevant genes
A Single-Gene Cause in 29.5% of Cases of Steroid-Resistant Nephrotic Syndrome


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Friedhelm Hildebrandt, et al: Multicenter Study
_J Am Soc Nephrol, 2015_

- 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
  and next-generation sequencing
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)

an additional 11.6%
A negative correlation between the likelihood of identifying the causative gene and the age of onset of proteinuria. 


**Graph:**
- **Y-axis:** Percentage of families with molecular diagnosis [%]
- **X-axis:** Age of onset [years]
- **Line:** % of families with causative mutation identified (for each year of age of onset)
- **Trendline:** \( y = 35.84e^{-0.094x} \)
Renal Pathology Findings

- 337/544 (61.9%) performed renal biopsies
- Infants
  - 26.9% DMS
- >7 yrs
  - >90% FSGS, no DMS
- DMS
  - WT1, 23.1%
  - PLCE1, 17.8%
  - LAMB2, 13.6%
  - NPHS1, 4.9%

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

- 120 patients
- NGS detection included 28 genes

### 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>Mutation detection rate</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>
</tr>
<tr>
<td>Causative genes</td>
<td>WT1, NPHS1, ADCK4, LAMB2</td>
<td>TRPC6, WT1, NPHS1, PLCE1, COQ2</td>
<td>WT1, LMX1B, NPHS2, CUBN, ADCK4</td>
<td>ADCK4</td>
<td>ADCK4</td>
</tr>
</tbody>
</table>
The detection of causative genes

- Avoid unnecessary initiation or extension of steroid treatment
- Refine management
  - mutation in coenzyme Q10 biosynthesis pathway (COQ2, COQ6, ADCK4, or PDSS2)
  - can be treated with coenzyme Q10
- Lower recurrent rate post renal transplantation
  - <5% vs 30%-50%  
  
  *Pediatr Nephrol 2009*
- Prenatal or preimplantation diagnosis
- Promote studies on specific molecular target or pathway
Milestones for Podocyte Molecular Study

- **NPHS1** Molec. Cell 1998
- **CD2AP** Science 2000
- **PLCE1** Nature Genet. 2003
- **LAMB2** Kidney Int. 2005
- **INF2** Nature Genet. 2006
- **DKGE** Nature Genet. 2008
- **ARHGAD2A** J. Clin. Invest. 2010
- **NPHS2** Nature Genet. 2011
- **TRPC6** Science 2013
- **APOL1** Nature Genet. 2010
- **MYO1E** New Eng. J. Med. 2011
- **ACTN4** Nature Genet. 2013
- **PTPRO** Am. J. Hum. Genet. 2013
Milestones for Podocyte Molecular Study

1998: NPHS1, Molec. Cell
2000: CD2AP, Science
2003: PLCE1, Nature Genet.
2005: LAMB2, Kidney Int.
2011: TRPC6, Science
2013: APOL1, Nature Genet.
TRPC6

- Transient Receptor Potential cation Channel 6
- A member of nonselective cation channels
- Mutations were detected in autosomal dominant FSGS families *Science, 2005*
- Increased expressions in inherited and acquired renal diseases *JASN, 2007*
  - gain of function

Questions

- Can we get the expression down?
- What is the pathway to regulate TRPC6 expression?
**Hypothesis: mTOR pathway**

- mTORC1? Or mTORC2?

**Graphs and Images:**

- Bar graphs comparing TRPC6/β-actin fold increase under different conditions.
- Western blots showing protein expression levels of rictor, raptor, TRPC6, p70s6k (pan), p-p70s6k, akt (pan), p-akt, and β-actin.

**Images:**

- Fluorescence images of cells treated with control, rapamycin, and ku0063794 at 0s, 12s, and 60s.

**Significance:**

- NS indicates non-significant differences.
- * indicates significant differences.
TRPC6

- The mTORC2 signaling pathway regulates TRPC6 in podocytes
- a novel regulator

The results of this study may provide a new therapeutic target for proteinuric renal diseases

Fangrui Ding, et al. PLOS ONE, 2014
Inherited renal diseases

- Proteinuric disorders/nephrotic syndrome and podocyte molecules
- Alport syndrome and progression mechanism
- Atypical hemolytic uremic syndrome and complement genes defects
Alport Syndrome

- Clinical Manifestations
  - Hematuria and proteinuria
  - Progressive renal failure
- Need renal replacement therapy
- Typical renal pathology findings
  - Diffused thickening and splitting of GBM
  - Glomerulosclerosis and kidney fibrosis
- Type IV collagen alpha3-5 chains mutation
Thin basement membrane nephropathy

<table>
<thead>
<tr>
<th>Characteristic Feature</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent glomerular hematuria, minimal proteinuria, normal BP, and normal renal function</td>
<td>High (80%)</td>
<td>Moderate</td>
<td>TBMN is the most common cause; occurs in IgA disease too but often with higher urinary red blood cell counts and (less commonly) proteinuria</td>
</tr>
<tr>
<td>Family history of hematuria</td>
<td>Moderate (70%)</td>
<td>High</td>
<td>Family history of hematuria is also common in X-linked Alport syndrome</td>
</tr>
<tr>
<td>Generally thinned GBM without focal lamellation</td>
<td>95%</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Collagen IV α3α4α5 network present in GBM</td>
<td>100%</td>
<td>Moderate</td>
<td>Supports but does not prove the diagnosis of TBMN</td>
</tr>
<tr>
<td>α5(IV) collagen chain present in skin</td>
<td>40%</td>
<td>High</td>
<td>Supports but does not prove the diagnosis of TBMN</td>
</tr>
<tr>
<td>Hematuria segregates with COL4A3/COL4A4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematuria does not segregate with the COL4A5 locus</td>
<td>High</td>
<td>High</td>
<td>Linkage studies require careful characterization of other family members but are possible with very few members</td>
</tr>
<tr>
<td>Single mutation in COL4A3 or COL4A4</td>
<td>80%</td>
<td>Very high</td>
<td></td>
</tr>
</tbody>
</table>

JASN, 2013
Pathogeneses of Alport syndrome

What is the progression mechanism?
Correlation between proteinuria in Alport and podocytes?

- The degree of footprocess effacement correlated to the level of proteinuria

中华肾脏病杂志，26(10):748-52, 2010
Are podocyte molecules involved in Alport?

nephrin

podocin

control

Alport with heavy proteinuria
Pathogeneses of Alport syndrome

- Conclusion
  - Podocytes involve in the progression of Alport syndrome

- Question:
  - Alport is caused by monogenic mutation encoding type IV collagen alpha chains (extracellular matrix)
  - Alport is a GBM disease
  - What is the interactions between podocytes and the extracellular matrix?
Potential mechanisms underlying chronic renal disease occurring in Alport syndrome

Potential mechanisms underlying chronic renal disease occurring in Alport syndrome

Potential mechanisms underlying chronic renal disease occurring in Alport syndrome

Potential mechanisms underlying chronic renal disease occurring in Alport syndrome

Management of Alport Syndrome

- Early angiotensin-converting enzyme inhibition in Alport syndrome delays renal failure and improves life expectancy

Kidney Int, 2012
Management of Alport Syndrome

- Early and longterm ACEi and ARB treatments in children is efficient and well tolerated
- The antiproteinuric effect of ACEi and ARB is of equal value in children with severe and less severe mutations in the COL4An gene

<table>
<thead>
<tr>
<th>Length of treatment (years)</th>
<th>Number of children</th>
<th>Proteinuria (mg/kg/day)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>70</td>
<td>37.4±31.8</td>
<td>25.6±23.1</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>39.3±31.8</td>
<td>34.8±31.7</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>34.2±25.9</td>
<td>35.8±35.0</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>38.8±31.0</td>
<td>41.7±35.4</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>34.7±27.8</td>
<td>42.2±45.5</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± standard deviation (SD)
Inherited renal diseases

- Proteinuric disorders/nephrotic syndrome and podocyte molecules
- Alport syndrome and progression mechanism
- Atypical hemolytic uremic syndrome and complement genes defects
Atypical Hemolytic Uremic Syndrome

- Not caused by microbial toxins, but may be triggered by infections, etc
- Life-threatening, chronic disease of complement-mediated thrombotic microangiopathy
- Mutations in the gene encoding complement factor H (CFH) was identified in 1998
- Advance of the pathogenesis of aHUS
  - 50% to 60% identified with a genetic defect
  - All related to the alternative complement pathway activation

*Kidney Int, 1998*
Complement activation and regulations

Classical
Antibody/antigen
\[ \rightarrow \]
C1

Lectin
MBL/carbohydrate
\[ \rightarrow \]
MASPs

Alternative/properdin
C3b-microbe GAG, microbe
\[ \rightarrow \]
C3b/properdin

Amplification loop

Factors B, D, P

C3 Convertases
C4, C2

C3b
C4b

Opsonization
CD59, S protein clusterin

MAC
Lysis

Factor I + CD35, CD55, fH, or C4bp (CR1g ?)

MAC
Inflammation

Factor I + CD35, CD46, fH, or C4bp

C3a, C5a

C1 inhibitor

Clinical and Experimental Immunology, 2011
Complement activation and regulations

Clinical and Experimental Immunology, 2011
Hereditary complement-mediated TMA (aHUS)

- Complement mutations
  - either a loss-of-function mutation in a regulatory gene \((CFH, CFI, \text{ or } CD46)\)
  - or a gain-of-function mutation in an effector gene \((CFB \text{ or } C3)\)
Eculizumab used in hereditary complement-mediated TMA, aHUS

- Eculizumab is a monoclonal antibody
- Inhibits the cleavage of C5
- blocking complement activation
- effect limited among patients C5 mutations

Summary

- More causative genes identified
  - Several hundreds genes
- More inherited renal disease diagnosis
  - 160 different disorders

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**Glomerular diseases**
- Congenital steroid-resistant nephrotic syndrome
- Denys-Drash syndrome, Frasier's syndrome
- Wilms' tumour, aniridia, genitourinary abnormalities, and mental retardation (WAGR) syndrome
- Pierson's syndrome
- Nail-patella syndrome
- Schimmel immuno-osseous dystrophy
- Mitochondrial disorders with steroid-resistant nephrotic syndrome
- Fabry's disease
- Alport's syndrome
- Benign familial haematuria (thin basement membrane)
- Fechtner syndrome (Alport's syndrome with macrothrombocytopenia)
- Alport's syndrome with leiomyomatosis
- Familial amyloidosis

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**Proximal tubule**
- Renal glucosuria
- Dicarboxylic aminoaciduria
- Lysinuric protein intolerance
- Proximal renal tubular acidosis
- Hypophosphataemic rickets
- Nephropathic cystinosis
- Primary renal Fanconi’s syndrome
- Fanconi-Bickel syndrome (hepatorenal glycogenosis)
- Lowe's syndrome
- Dent's disease, types 1 and 2
- Hereditary renal hypouricaemia
- Cystinuria, types 1-3

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**Thick ascending limb and distal convoluted tubule**
- Bartter’s syndrome, types 1-4
- Familial hypocalciuric hypercalcaemia
- Neonatal severe hyperparathyroidism
- Autosomal dominant hypocalcaemia
- Gitelman’s syndrome
- Pseudohypoaldosteronism type 2 (Gordon’s syndrome)
- SeSAME syndrome (EAST syndrome)
- Hypomagnesaemia, types 1-6
- Familial juvenile hyperuricaemic nephropathy

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**Collecting duct**
- Liddle’s syndrome
- Distal renal tubular acidosis
- Pseudohypoaldosteronism type 1
- Nephrogenic diabetes insipidus, types 1 and 2
- Nephrogenic syndrome of inappropriate antidiuresis

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*Lancet, 2014*
Summary
Opportunities and challenges

- Diagnosis accurately and efficiently
  - New technology
  - Correlations of phenotype and genotype
- Understanding pathogenesis
  - Identify crucial molecular targets for intervention
  - Develop novel drugs
- Clinical trials in monogenic disorders
  - Biomarkers for specific evaluation
  - Specific cohort with a monogenic disorder
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