Genetic Advances Towards Precision Medicine in Childhood CKD

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Steroid-Resistant Nephrotic Syndrome

Focal segmental glomerulosclerosis (FSGS)
Homozygous Point Mutation in the \textit{Podocin} Gene

A mutation in 1 bp of the 3,300,000,000 bp of the total genome is sufficient to cause FSGS.
Monogenic disease (Single-gene disorder)

Definition

• In 1 patient the disease is caused by mutation of 1 gene only of ~20,000 genes (recessive, e.g. NPHS2; dominant, e.g. INF2)

• In different patients different genes may cause a similar disease: ("genetic locus heterogeneity", e.g. podocin, nephrin)

• 47 recessive, 8 dominant genes for SRNS
Consequences from Disease Gene Identification

Gene mutation $\Rightarrow$ mRNA $\Rightarrow$ Protein $\Rightarrow$ Disease

“Pathogenesis”

Gene mutation $\Rightarrow$ Molecular Genetic Diagnosis $= Etiology$

“Pathogenesis” $\Rightarrow$ Novel Insights into Pathogenesis & Physiology

“Pathogenesis” $\Rightarrow$ Genotype/Phenotype Correlation „Precision Medicine“
<table>
<thead>
<tr>
<th>Chronic Kidney Disease</th>
<th>Cause</th>
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<tbody>
<tr>
<td>CAKUT (Congenital Anomalies of the Kidneys &amp; Urinary Tract)</td>
<td>50%</td>
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<td>6%</td>
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<tr>
<td>NEPHROLITHIASIS / NEPHROCALINOSIS</td>
<td>3%</td>
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<td>TOTAL (n=8,990) (NAPRTCS 2008)</td>
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Many monogenic genes cause CKD <25 yrs

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<td>30 (2)</td>
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Gene Identification Moved the Glomerular Podocyte to Center Stage of SRNS Pathogenesis

(Netter; www.georgetown.edu/.../GUE-scopeLibrary1.html)
Monogenic causes of SRNS/FSGS

CoQ_{10} biosynthesis

Mitochondria

Podocyte foot process

Nucleus

S1P metabolism

Lysosome

Integrin/Laminin

Actin-binding

Slit membrane

Podocyte

foot process

Integrin/

Laminin

Glomerular basement membrane

Endothelial Cell

CoQ_{10} biosynthesis

Mitochondria

Podocyte foot process

Nucleus

S1P metabolism

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Podocyte

foot process

Integrin/

Laminin

Glomerular basement membrane

Endothelial Cell
What percentage of Steroid-Resistant Nephrotic Syndrome (SRNS) is caused by single-gene mutations? (onset <25 yrs)
Causative mutation found (% cases)

- Germany: 25.6%
- Switzerland: 21.3%
- Turkey: 35.5%
- Egypt: 43.8%
- Saudi Arabia: 45.2%

Europe map with statistical data and pie charts indicating the percentage of cases with causative mutations.

Graph: Family with disease mutation detected vs. percentage of consanguineous families.

Equation: $y = 0.3681x + 15.184$, $R^2 = 0.9414$, p-value < 0.01
Q$_{10}$ treatment in SRNS & COQ6 mutation

Proteinuria mg/m$^2$/h

Weeks after start of Tx

(Ashraf JCI 123:5179, 2013)
Edema and treatment in a zebrafish model of nephrotic syndrome from *arhgdia* knockdown

Control MO

*arhgdia* MO

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**IC50 = 23.76 uM**

**RAC1 inhibitor**

**Log concentration**

**Periorbital edema (%)**

**Vehicle only**

**100 uM NSC23766**

**30 uM Rac1 inhibitor**

**50 uM Rac1 inhibitor II**

**2 uM Rho inhibitor I**

**10 uM Y-27632**

**GFP concentration (pg/mL)**

**p53 MO**

**arhgdia MO**

**Rac1 inhibitor**

**Y-27632**
Precision medicine in monogenic diseases: Jumping from a low yield dx measure to a specific diagnosis by whole exome sequencing
Increased renal echogenicity: Causative mutation detected in ~63% of cases

(Braun *Kidney Internat* 93:204, 2016)
### Indication-driven whole exome sequencing for single-gene causes of CKD <25 yrs

<table>
<thead>
<tr>
<th>Indication</th>
<th># of genes</th>
<th>Detect cause in</th>
</tr>
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<tbody>
<tr>
<td>Steroid-resistant nephrotic syndrome</td>
<td>55</td>
<td>~30% (12-45%)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystic kidney disease</td>
<td>100</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>US: cysts or echogenicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAKUT Imaging</td>
<td>45</td>
<td>&gt;22%</td>
</tr>
<tr>
<td>Renal stones</td>
<td>30</td>
<td>&gt;20%</td>
</tr>
<tr>
<td>Stone or nephrocalcinosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The “Nephrome” = All CKD &lt;25 yrs</td>
<td>~230</td>
<td>&gt;20%</td>
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www.renalgenes.org
Whole exome sequencing in 236 children with CAKUT: Causal mutation in 1 of 40 CAKUT genes in >13%

(A) Causative mutation in known CAKUT gene (32/236; 13%)

(B) Syndromic CAKUT gene in patient with isolated CAKUT (14/236; 6%)

(C) Phenocopy gene (4/236; 2%)

(D) Murine CAKUT gene (5/236; 2%)

(E) Novel, single candidate gene (26/236; 11%)

(F) Novel, multiple candidate genes per family (21/236; 9%)

(G) Disease-causing mutation in non-CAKUT gene (9/236; 4%)

(H) No mutation detected (125/236; 53%)

(van der Ven & Connaughton JASN 29:2348, 2018)
Clinical consequences from detection of monogenic causation in CAKUT patients

**METHOD:**
Recruitment of 104/263 patients with renal Tx at BCH in last 10 yrs

**RESULTS:**
Disease causing mutation detected in 33%
(NPHP 78%, SRNS 43%, CAKUT 18%)

(Mann JASN 30:201, 2019)
Clinical timeline for patients receiving Tx for Congenital Anomalies of the Kidneys and Urinary Tract (CAKUT)

= Important consequences for PKT management from WES Dx:
- HNF1B → cave! Tacro b/o diabetes
- EYA1 → hearing test/aid
In 20% of children with CKD you can now detect the cause of disease

Every patient with a kidney disease caused by a single-gene mutation should have a chance at having this mutation identified (if consenting), because:

... it is now feasible

... provides an etiologic diagnosis (= CAUSE of disease)

... may reveal a potential (personalized) treatment (CoQ_{10})

... allows etiologic classification for therapeutic trials

... provides the missing pieces for the puzzle of pathogenic pathways

... cellular animal models to screen for therapeutic molecules