Genetic aspects of CAKUT

Stefanie Weber
Laboratory for Molecular Genetics
Pediatric Nephrology
University-Children’s Hospital Essen

31.10.2014 Antalya, Turkey
Congenital anomalies of the kidney and urinary tract

- Rising detection rate of anomalies of the kidney and urinary tract due to routinely performed pre- and postnatal ultrasound examinations

- Abnormal ultrasound results in 0.2-0.8% of all pregnancies

- Incidence in the general population approx. 1%
CAKUT: congenital anomalies of kidney and urinary tract

A congenital nephropathies

1.1 kidney agenesis/hypoplasia
1.2 hypoplasia with dysplasia
1.3 oligomeganephronia

2.1 dysplasia without cysts
2.2 dysplasia with cysts
2.3 multicystic dysplasia
2.4 obstructive forms

B congenital uropathies

1.1 ureteropelvic junction stenosis

2.1 primary non-refluxive megaureter (distal ureter obstruction)
2.2 primary refluxive megaureter
2.3 secondary megaureter with infravesical obstruction (urethral valve, meatus stenosis)

3.1 ureter ectopia with/without doubling malformations of the upper tract
Kidney survival in CAKUT phenotypes

Sanna-Cherchi et al., *Kidney Int* 2009
CAKUT with renal insufficiency

Diagnosis of CAKUT in 70% of pediatric CNI patients (n=466)
Early kidney development

Rat/Mouse:
- 11 d: ureteral budding
- 21 d: disappearance of 'septa'

Human:
- 35 d: growth of the ureter & vessels
- 40 wk: Birth
A Ectopic Budding of the Ureter

Kidney dysplasia
Molecular mechanism of the regulation of budding

Miyazaki et al., JCI 2000
Why genetics in CAKUT patients?

A  Familial clustering observed in ~ 10% of patients
• 218 index patients and their were enrolled in the study

• All asymptomatic first-degree relatives were screened for CAKUT by ultrasound.

• New anomalies were diagnosed in 116 asymptomatic first-degree relatives (23%)

• When family histories and ultrasound findings of 180 index patients were evaluated together, CAKUT was diagnosed in 129 first-degree relatives in 92 families (51.1%)
Why genetics in CAKUT patients?

A  Familial clustering observed in ~ 10% of patients

B  Monogenic gene *knock-out* of developmental genes in mice results in a phenotype highly reminiscent of human CAKUT

- kidney agenesis
- kidney hypoplasia/dysplasia
- double kidneys
- ureteral anomalies/hydronephrosis

CAKUT
### Knock-out mice with CAKUT phenotype

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Renal phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt1</td>
<td>transcription factor</td>
<td>bilateral agenesis</td>
</tr>
<tr>
<td>Eya-1</td>
<td>transcription factor</td>
<td>-/-: bilateral aplasia, +/-: hypoplasia</td>
</tr>
<tr>
<td>Lim1</td>
<td>transcription factor</td>
<td>bilateral agenesis</td>
</tr>
<tr>
<td>Foxc1/2</td>
<td>transcription factor</td>
<td>double kidneys, hydroureter, hypopl.</td>
</tr>
<tr>
<td>Pax2</td>
<td>transcription factor</td>
<td>-/-: bilateral agenesis, +/-: hypoplasia</td>
</tr>
<tr>
<td>Bmp-4</td>
<td>secreted signal molecule</td>
<td>+/-: hypo/dysplasia, hydroureter</td>
</tr>
<tr>
<td>Bmp-5</td>
<td>secreted signal molecule</td>
<td>hydroureter</td>
</tr>
<tr>
<td>Bmp-7</td>
<td>secreted signal molecule</td>
<td>dysplasia</td>
</tr>
<tr>
<td>Fgf-7</td>
<td>secreted signal molecule</td>
<td>+/-: hypoplasia</td>
</tr>
<tr>
<td>Wnt4</td>
<td>secreted signal molecule</td>
<td>dysplasia</td>
</tr>
<tr>
<td>Gdnf</td>
<td>secreted signal molecule</td>
<td>+/-: unilat. agenesis, bilat. dysplasia</td>
</tr>
<tr>
<td>Ret</td>
<td>receptor tyrosine kinase</td>
<td>uni/bilat. aplasia/dysplasia</td>
</tr>
<tr>
<td>Agtr2</td>
<td>angiotensin receptor</td>
<td>hypo/dysplasia, hydroureter</td>
</tr>
<tr>
<td>Adams-1</td>
<td>metalloproteinase/disintegrin</td>
<td>-/-: proximal ureter stenosis</td>
</tr>
</tbody>
</table>
CAKUT phenotype in 53% of $Bmp4$ +/- pups

- 60% kidney hypoplasia/dysplasia
- 32% UVJ/hydronephrosis
- 8% bifid ureter/double kidneys

Miyazaki et al., *JCI* 2000
Results of mutational analysis in **BMP4** and **SIX2**

<table>
<thead>
<tr>
<th>Index Patient</th>
<th>Herkunft</th>
<th>SIX2 Mutation (Nukleotide)</th>
<th>SIX2 Mutation (Aminosäuren)</th>
<th>Ultraschallbefundb</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Polen</td>
<td>402 C-&gt;T</td>
<td>Leu43Phe (het)</td>
<td>DYS(l)/VUR(r)</td>
</tr>
<tr>
<td>P2</td>
<td>Polen</td>
<td>997 C-&gt;T</td>
<td>Pro241Leu (het)</td>
<td>CYS-DYS(r,l)/VUR(r,l)</td>
</tr>
<tr>
<td>P3</td>
<td>Deutschland</td>
<td>997 C-&gt;T</td>
<td>Pro241Leu (het)</td>
<td>CYS-DYS(r,l)</td>
</tr>
<tr>
<td>P4</td>
<td>Italien</td>
<td>997 C-&gt;T</td>
<td>Pro241Leu (het)</td>
<td>HYPO(r)/VUR(r)</td>
</tr>
<tr>
<td>P5</td>
<td>Portugal</td>
<td>1100-1101 GG-&gt;AA</td>
<td>Asp276Asn (het)</td>
<td>CYS-DYS(r,l)/HYPO(r)</td>
</tr>
</tbody>
</table>

**BMP4 m Mutation (Nukleotide)**

<table>
<thead>
<tr>
<th>Index Patient</th>
<th>Herkunft</th>
<th>BMP4 Mutation (Nukleotide)</th>
<th>Aminosäuren</th>
</tr>
</thead>
<tbody>
<tr>
<td>P6</td>
<td>Polen</td>
<td>272 C-&gt;G</td>
<td>Ser91Cys (het)</td>
</tr>
<tr>
<td>P7</td>
<td>Deutschland</td>
<td>272 C-&gt;G</td>
<td>Ser91Cys (het)</td>
</tr>
<tr>
<td>P8</td>
<td>Turkei</td>
<td>347 C-&gt;G</td>
<td>de novo Thr116Ser (het)</td>
</tr>
<tr>
<td>P9</td>
<td>Turkei</td>
<td>450 C-&gt;G</td>
<td>Asn150Lys (het)</td>
</tr>
<tr>
<td>P10</td>
<td>Turkei</td>
<td>450 C-&gt;G</td>
<td>Asn150Lys (homo)</td>
</tr>
</tbody>
</table>

---

**Notes:**

- Patients n= 250 ; controls n= 150
- DYS=dysplasia, VUR = vesico-ureteral reflux, CYS-DYS=cystic dysplasia, HYPO=hypoplasia, AGEN=agenesis, l=left, r=right

Weber/Taylor et al., JASN 2006; Tabatabaei Far et al., Ped Nephrol 2009
Many novel CAKUT associated genes have been identified

Ruf et al., *PNAS* 2004
Jenkins et al., *JASN* 2005
Jenkins et al., *NDT* 2006
Wu et al., *AJHG* 2007
Weber/Taylor et al., *JASN* 2008
Weber et al., *AJHG* 2011
Saisawat et al., *Kidney Int* 2013
Schild et al., *NDT* 2013
Vivante et al., *JASN* 2013
Sanna-Cherchi et al., *NEJM* 2013
Shukrun et al., *PLoS One* 2014
Hwang et al., *Ped Nephrol* 2014
Results of the ESCAPE study

Mutational analysis in 99 patients CAKUT:

*PAX2:*

*EYA1:*

*SIX1:*

*SALL1:*

*HNF1B:*

Severe prenatal renal anomalies associated with mutations in *HNF1B* or *PAX2*:

- Analysis of *HNF1B* and *PAX2* in 103 fetuses (91 families) with termination of pregnancy due to severe congenital anomalies of the kidney

Madariaga et al., CJASN March 2013
HNF-1β

- Tissue specific transcription factor
- Expressed in pancreas, liver and kidney
- In the kidney, expression was observed in the developing ureter and tubular system
- First mutations in the human \( HNF1B \) gene were identified in patients with MODY5, then in Renal cysts and diabetes (RCAD) syndrome
Renal Phenotypes Related to Hepatocyte Nuclear Factor-1β (TCF2) Mutations in a Pediatric Cohort

Tim Ulinski,* Sandra Lescure,† Sandrine Beaufils,‡ Vincent Guigonis,† Stéphane Decramer,§ Denis Morin,‖ Séverine Clauin,‡ Georges Deschênes,* François Bouissou,§ Albert Bensman,* and Christine Bellanne-Chantelot¶


HNF1B mutations were identified in 25 / 80 patients with predominant cystic RHD (30%)

Figure 1. Prevalence of TCF2 anomalies in patients with multicytic dysplasia, isolated renal cysts, hypo/dysplastic kidneys, and single kidneys. □, patients with TCF2 anomalies; □, patients without TCF2 anomalies.
• 6 / 24 patients of the total cohort with cystic lesions have a mutation in \textit{HNF1B} (25%)

• in contrast: only 2 / 76 patienten without cystic lesions (3%)
HNF1B analysis in 104 CAKUT patients

- 81 patients with renal hypodysplasia
- 39 patients with kidney dysplasia with cysts
- 9 patients with HNF1B mutation: all dysplasia with cysts (8 bilateral)
Anomalies of the *TCF2* Gene Are the Main Cause of Fetal Bilateral Hyperechogenic Kidneys

Stéphane Decramer,*† Olivier Parant,† Sandrine Beaufils,‡ Séverine Clauin,‡ Cécile Guillou,* Sylvie Kessler,† Jacqueline Aziza,§ Flavio Bandin,*† Joost P. Schanstra,* and Christine Bellanné-Chantelot‖


62 fetuses with bilateral hyperechogenic kidneys

- 18/62 (29%) → positive *HNF1B* (*TCF2*) mutation analysis
  → 15/18 whole gene deletions (83%)

- 34/62 → ARPKD/ ADPKD/ tubulopathy
Phenotypic variability in *HNF1B* mutation carriers

Renal cysts, CRI
uterus bicornis

Vester et al., *Ped Nephrol* 2010
Extrarenal malformations/symptoms in 28%
Genetic testing in kidney dysplasia

Sequential testing

- HNF1B
- PAX2
- others

Diagnostic panel

- HNF1B, PAX2, EYA1, SIX1, SIX2, BMP4, RET, GDNF, DSTYK, SOX17, SALL1, FRAS1, UPIIIA, UMOD, ...

WES

in selected cases

-> dependent on national resources, clinical course, and extrarenal symptoms
The phenotypic spectrum of CAKUT is broad. The renal prognosis is largely dependent on the phenotype of anomaly.

Family history is positive in about 10-15%. Mouse model studies support a genetic contribution.

Mutations in PAX2 and HNF1B seem to be a relevant cause of CAKUT in a subset of patients.

High detection rate of HNF1B mutations in bilateral cystic dysplasia (23-25%) and hyperechogenic large kidneys (29%) in antenatal ultrasound.

Renal function and progression of renal insufficiency can be very variable, also within the same family.

Family analysis and genetic counseling can be important in CAKUT patients (frequently autosomal dominant disease, extrarenal symptoms).
Genome-wide linkage analysis and NGS in a family with bladder outflow obstruction (BOO)

kindly provided by Sevgi Mir, Betül Sozeri, University of Izmir
Causative mutation in M3 acetylcholine receptor \textit{CHRM3}

Weber et al., \textit{AJHG} 2011
Chrm3 knock-out mouse

- megacystis
- hydrenephrosis
- impaired eye reaction to light

Matsui et al, *PNAS* 2000
CHRM3 encodes the muscarinic acetylcholine receptor
Causative mutation in M3 acetylcholine receptor – a major receptor for bladder contraction

kindly provided by Sevgi Mir, Betül Sozeri, University of Izmir

Weber et al., AJHG 2011
DD: Ochoa syndrome

Ochoa syndrome = uro-facial syndrome (UFS)

Mutations in Heparanase 2 (HPSE2) or LRIG2

Pang, AJHG 2010 & Daly, AJHG 2010; Stuart et al., AJHG 2013

reviewed in Stuart et al., Ped Nephrol 2013

Ochoa, Ped Nephrol 2004: 19:6
Ochoa syndrome, related to $HPSE2$ mutations

duly provided by Aysun Karabay Bayazit, University of Adana
Urinary Tract Effects of HPSE2 Mutations


JASN, Aug 2014
Establishment of ESPN Working Groups

- Transplantation
- Dialysis
- CKD-MBD
- CAKUT/UTI/bladder dysfunction
- Inherited renal disorders
- Nephrotic syndrome
- Immune-mediated disorders
ESPN Working Group CAKUT/UTI/Bladder dysfunction

- Chair: S. Weber
- Fatos Yalcinkaya
- Giovanni Montini
- Amira Peco-Antic
- Ann Raes
- Khalid Ismaili

Current projects: Establishment of a web-based European Registry for Familial Cases of CAKUT, establishment of an HNF1B-observational study

http://www.esp-nephrology.org
Thank you very much for your attention