The Role of Genetics in Congenital Anomalies of the Kidneys and Urinary Tract

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# Congenital Anomalies of Kidneys and Urinary Tract (CAKUT): Prevalence (per 1000 births)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vesicoureteral reflux</td>
<td>20-30 (100 during first days of life)</td>
</tr>
<tr>
<td>Duplex kidney/ureter</td>
<td>8.0</td>
</tr>
<tr>
<td>Obstruction of kidney or ureter</td>
<td>1.0</td>
</tr>
<tr>
<td>Kidney agenesis/hypoplasia/dysplasia</td>
<td>0.8</td>
</tr>
<tr>
<td>Obstruction of bladder/urethra</td>
<td>0.2</td>
</tr>
<tr>
<td>Horseshoe kidney</td>
<td>0.15</td>
</tr>
<tr>
<td>Bladder ekstrophy</td>
<td>0.06</td>
</tr>
<tr>
<td>Cystic kidney diseases</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Beetz et al. 1998
Causes of Chronic Kidney Disease in Children

4C Study, 700 European children with GFR 15-60 ml/min/1.73m²

- Congenital Anomalies of Kidneys and Urinary Tract: 63%
- Glomerulopathies: 8%
- Polycystic kidney disease: 6%
- Metabolic: 5%
- Nephropathia: 4%
- HUS: 3%
- Interstitial nephropathy: 2%
- Post-ischemic: 1%
- Vasculitis: 1%
- Unknown: 6%
- Other: 2%
- Unknown: 6%
# CAKUT Manifestations in CKD Cohort

<table>
<thead>
<tr>
<th>Condition</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilateral agenesis</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Unilateral hypoplasia</td>
<td>21</td>
<td>54%</td>
</tr>
<tr>
<td>Bilateral hypoplasia</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Unilateral dysplasia without cysts</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Bilateral dysplasia without cysts</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Unilateral cystic dysplasia</td>
<td>5</td>
<td>50%</td>
</tr>
<tr>
<td>Bilateral cystic dysplasia</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Multicystic-dysplastic kidney</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Associated VUR</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Associated distal ureteric obstruction</td>
<td>8</td>
<td>62%</td>
</tr>
<tr>
<td>Associated UPJ obstruction</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
CAKUT: Age at Start of Renal Replacement Therapy

ERA-EDTA Registry: % patients starting RRT per age group

Wühl et al. cJASN 2012
CAKUT: Familial Clustering

Unilateral renal agenesis in 9% of relatives of fetuses with bilateral renal agenesis


Offspring of CAKUT patients: CAKUT risk 15-20%

Carter et al. J Hum Genet 1984

Solitary, duplex kidneys, UPJ obstruction in 15% of 1st/2nd degree relatives of children with renal agenesis/adysplasia

Schwaderer Pediatr Nephrol 2007
Positive family history: 12%
Extrarenal anomalies: 28%
Ureteric bud

Pronephros

Wolffian Duct

Mesonephros

Metanephrone Mesenchyme

Budding

Branching

Epithelialization
## Knock-Out Mouse Models with Renal 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>Pax2</td>
<td>transcription factor</td>
<td>-/-: bilateral agenesis; +/-: hypoplasia</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, hypoplasia</td>
</tr>
<tr>
<td>BMP-4</td>
<td>secreted signaling molecule</td>
<td>+/-: hypo/dysplasia, hydronephrosis</td>
</tr>
<tr>
<td>BMP-5</td>
<td>secreted signaling molecule</td>
<td>hydronephrosis</td>
</tr>
<tr>
<td>BMP-7</td>
<td>secreted signaling molecule</td>
<td>dysplasia</td>
</tr>
<tr>
<td>FGF-7</td>
<td>secreted signaling molecule</td>
<td>-/-: hypoplasia</td>
</tr>
<tr>
<td>WNT4</td>
<td>secreted signaling molecule</td>
<td>dysplasia</td>
</tr>
<tr>
<td>GDNF</td>
<td>secreted signaling molecule</td>
<td>+/-: unilat.agenesis, bilat.dysplasia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-/-: bilat.agenesis</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, hydronephrosis</td>
</tr>
<tr>
<td>ADAMTS-1</td>
<td>metalloproteinase</td>
<td>-/-: stenosis of uretero-pelvic junction</td>
</tr>
</tbody>
</table>
Bardet-Biedl → **BBS1-12**
Beckwith-Wiedemann → **p57KIP2**
Cat-Eye
Renal cysts and diabetes → **HNF1β**
EEC
Fanconi
Fraser → **FRAS1**

Fryns
Jeune (thoracic dystrophy)
Johanson-Blizzard
Klippel-Feil (sequence)
Lateral (sequence)
Meckel-Grubel
Melnick-Fraser (BOR) → **EYA1, SIX1, SIX5**
MURCS (association)
Oral-Facial-Digital → **OFD1**
Pallister-Hall
Peter’s-Plus
Renal-coloboma → **PAX2**
Rubinstein-Taybi
Schinzel-Gideon
Tuberous sclerosis
Saldino-Noonan
Majewski
Townes-Brocks → **SALL1**
VATER (association)
Zellweger → **PAF1**

Aarskog
Acrodysostose
Apert → **FGFR2**
Baller-Gerold
Branchio-Oculo-Facial
Campomelic dysplasia → **SOX9**
CHARGE (association)
Chondroectodermal dysplasia
Elhers-Danlos

Kabuki
Kallmann → **KAL1, FGFR1, PROK2**
Langer-Gideon
Nail-patella → **LMX1B**
Opitz
Robinow
Russell-Silver
Saethre-Chotzen

Simpson-Golabi-Behmel → **GPC3**
Smith-Lemli-Opitz → **D(7)HCR**
Von Hippel Lindau
Williams → **elastine**
Deletions 3p, 4p, 5p, 9p, 13q, 18q
Duplications 4p, 9p
Trisomies 9 mosaïque, 13, 18
Identifying human CAKUT genes:
The *Syndrome Candidate Gene* Approach
100 CAKUT Patients from ESCAPE Study:
Search for Mutations in Genes Causing Syndromes with Mild Clinical Phenotype

**PAX2**: Renal-Coloboma Syndrome (RCS)
- RHD, MCDK, oligomeganephronia, VUR,
- optic nerve dysplasia / coloboma, rarely hypoacusis

**HNF1b (TCF2)**: Renal Cysts and Diabetes Syndrome
- RHD, glomerulocystic nephropathy,
  - MODY, hyperuricemia, hepatopathy

**EYA1 and SIX1**: Branchio-Oto-Renal Syndrome (BOR)
- RHD, lateral cervical cysts/fistulae, ear anomalies, hypoacusis

**SALL1**: Townes-Brocks Syndrome (TBS)
- RHD, VUR, PUV, skeletal, cardiac, hepatic and cerebral anomalies

Weber et al. JASN 2007
Heterozygous PAX2 mutations detected in 7 patients from 6 families

Multicystic-dysplastic kidney in 2 pts, UPJ obstruction in 1 patient

Ocular anomalies in 5 of 7 patients and 1 of 2 affected parents

Hypoacusis in 3 of 7 patients

Extrarenal anomalies minimal in 5 of 7 affected patients, diagnosed only after mutation findings
HNF1B

- *HNF1b* anomalies in 8/100 patients with renal hypo/dysplasia
- 4 heterozygous *HNF1b* deletions, 1x *de novo*
  - 3 heterozygous mutations in 4 patients
- **Cystic** RHD in 6 of 8 affected patients
- Extrarenal manifestations: 1 DM (13 y), 2 hyperuricemia
- Diabetic relatives in 3 of 8 families
HNF1B

Ulinski et al. JASN 2006:

- **HNF1b** mutations and gene deletions in 25/80 patients with mainly **cystic RHD**
- **de novo** in 9/17 families

-> Systematic **HNF1b** screening relevant
   - in children with **cystic RHD**
   - because of **metabolic risks** (diabetes, hyperuricemia, liver dysfunction) for patient and affected family members
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

- Antenatal cysts: 11 of 18 pts (unilateral in 8/11)
- Cysts appeared during 1st YoL in 17/18
- GFR decreased with time in all pts

Decramer et al. JASN 2007
Indications for HNF1B Screening

• 200 CAKUT patients screened in HNF1B
• Screening criteria: 1 major renal criterion, or 1 minor + extrarenal / family history
• 20 positive (10%) (10 mutations, 10 deletions)
ESCAPE CAKUT Cohort:
Gene Variations in 99 Patients with Renal Hypodysplasia

- **PAX2:** 7 patients with mutations
- **HNF1b:** 8 patients with deletions/mutations/variants
- **EYA1:** 1 patient with mutation
- **SALL1:** 1 patient with mutation

-> **17% prevalence** of mild syndromic forms in CAKUT with CKD
HNF1B and Pax2 Mutations in Fetuses with Severe CAKUT

103 fetuses from 91 families with termination of pregnancy due to severe CAKUT on ultrasound

HNF1B mutations in 12 fetuses from 11 families
- all bilateral hyperechogenic kidneys, cystic dysplasia
- 2 unilateral duplex ureter

PAX2 mutations in 5 unrelated fetuses
3 bilateral hypoplasia, 1 hypodysplasia, 1 single dysplastic kidney

50% isolated, 50% syndromic features

No HNF1B/PAX2 mutations in 27 cases of bilateral renal agenesis

-> 23% prevalence of HNF1B/PAX2 anomalies in non-BRA CAKUT

Madriaga et al. CJASN 2013
Identifying human CAKUT genes:
The **Family Gene Study** Approach
(genome-wide linkage, whole exome sequencing)
Bilateral Renal Agenesis

- **Recessive ITGA8** mutations identified by WES in 2/10 families
- Integrin-α8 expressed in metanephric mesenchyme
- Absent kidney development in KO mice
- Pathogenicity of mutations demonstrated

Humbert et al. Am J Hum Genet 2014
Bilateral Renal Agenesis

- Recessive FGF20 mutation identified in family with BRA

- FGF20 deficiency in mice induces renal hypoplasia due to premature differentiation of nephron progenitors (‘loss of stemness’)

Barak et al. Dev Cell 2012
**DSTYK: Novel Genetic Cause of CAKUT**

**Dual Serine-Threonine and tyrosine protein Kinase (DSTYK) gene identified by GWLA/WES in dominant CAKUT family**

Additional DSTYK mutations found in **7 of 311** CAKUT patients (2.3%)

**Phenotype:** **UPJO, UVJO, renal hypodysplasia**

DSTYK defects cause **loss of FGF signaling** in UB/MM

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*Sanna-Cherchi et al. NEJM 2013*
Identifying human CAKUT genes:
The Murine Candidate Gene Approach
CAKUT Candidate Gene: BMP4

- Bone morphogenetic proteins: secreted signaling proteins involved in **body patterning and morphogenesis**

- Bmp4 highly expressed in cells surrounding the Wolffian Duct, **inhibits abnormal ureteric budding**

- Bmp4 **promotes elongation of the branching ureter**

- Murine Bmp4 knock-out: Homozygous lethal, heterozygous **renal hypo/dysplasia, anomalies of urinary tract**
CAKUT Candidate Gene SIX2

- Six protein family has important functions as transcription factors for **early organogenesis**

- Human mutations in SIX1 and SIX5 are associated with BOR syndrome

- In the kidney, SIX1 and SIX2 have **similar expression patterns**

- Murine *Six2* knock-out is associated with **bilateral kidney dysplasia**

(Self et al., ASN 2004)
(Dominant?) Heterozygous Mutations in *SIX2* and *BMP4* in 250 Children with Renal Hypodysplasia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Origin</th>
<th><em>BMP4</em> mutation analysis</th>
<th><em>SIX2</em> mutation analysis</th>
<th>Kidney ultrasound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nucleotide exchange</td>
<td>Amino acid exchange</td>
<td></td>
</tr>
<tr>
<td>P6</td>
<td>Poland</td>
<td>272 C→G</td>
<td>Ser91Cys (het)</td>
<td>AGEN(r)</td>
</tr>
<tr>
<td>P7</td>
<td>Germany</td>
<td>272 C→G</td>
<td>Ser91Cys (het)</td>
<td>DYS(l)/VUR(r)</td>
</tr>
<tr>
<td>P8</td>
<td>Turkey</td>
<td>347 C→G</td>
<td><em>de novo</em> Thr116Ser (het)</td>
<td>HYPO(r)/VUR(l)</td>
</tr>
<tr>
<td>P9</td>
<td>Turkey</td>
<td>450 C→G</td>
<td>Asn150Lys (het)</td>
<td>HYPO(r)</td>
</tr>
<tr>
<td>P10</td>
<td>Turkey</td>
<td>450 C→G</td>
<td>Asn150Lys (homo)</td>
<td>CYS-DYS(r,l)</td>
</tr>
</tbody>
</table>

**BMP4**

<table>
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<th><em>BMP4</em> mutation analysis</th>
<th><em>SIX2</em> mutation analysis</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nucleotide exchange</td>
<td>Amino acid exchange</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>Poland</td>
<td>402 C→T</td>
<td>Leu43Phe (het)</td>
<td>DYS(l)/VUR(r)</td>
</tr>
<tr>
<td>P2</td>
<td>Poland</td>
<td>997 C→T</td>
<td>Pro241Leu (het)</td>
<td>CYS-DYS(r,l)/VUR(r,l)</td>
</tr>
<tr>
<td>P3</td>
<td>Germany</td>
<td>997 C→T</td>
<td>Pro241Leu (het)</td>
<td>CYS-DYS(r,l)</td>
</tr>
<tr>
<td>P4</td>
<td>Italy</td>
<td>997 C→T</td>
<td>Pro241Leu (het)</td>
<td>HYPO(r)/VUR(r)</td>
</tr>
<tr>
<td>P5</td>
<td>Poland</td>
<td>1100-1101 GG→AA</td>
<td>Asp276Asn (het)</td>
<td>CYS-DYS(r,l)/ HYPO(r)</td>
</tr>
</tbody>
</table>
Human mutations abolish *bmp4* function in zebrafish overexpression assay

Weber et al. JASN 2008
ESCAPE CAKUT Cohort:
Gene Variations in 99 Patients with Renal Hypodysplasia

**BMP4:** 3 patients with variants

**SIX2:** 3 patients with variants

**SIX1:** 2 siblings with variant

**RET:** 6 patients with mutations, 5 patients with variants

**GDNF:** 1 patient with variant

But: Some variants also found in healthy family members and patients with non-renal disease!
CAKUT: A Disease with Complex Genetics?

- **Rare alleles causing monogenic disease**: $CFTR$ in CF
- **Low-frequency variants with intermediate effect**
- **Common variants implicated in common disease**: $HHIP$ in COPD
Human Mutations in Genes Causing Recessive Unilateral Renal Agenesis in Mice

**NGS panel sequencing** of 12 recessive murine candidate genes

574 individuals with isolated CAKUT from 590 families

- **15 of 590 families**: recessive mutations in FRAS1 (n=7), FREM2, GRIP1, FREM1, ITGA8, and GREM1

- All genes involved in UB – MM interaction

- Missense FRAS mutations cause isolated CAKUT, truncating mutations multiorgan Fraser syndrome

- Mutations in 6 identified genes accounted for **2.5% of patients** in CAKUT cohort

Kohl et al. JASN 2014
Identifying human CAKUT genes:
The **Next Generation Panel Gene Sequencing** Approach
NGS Panel Sequencing of CAKUT Cohort

749 individuals from 650 families with CAKUT screened in
17 known dominant CAKUT-causing genes
39% VUR, 15% renal hypodysplasia, 12% unilateral renal agenesis

37 heterozygous mutations in 47 patients from 41 families (6.3%):


-> Cohort differences in CAKUT phenotype spectrum
-> Challenge to verify pathogenicity of mutations

Hwang et al. KI 2014
High-Throughput Research in Rare Kidney Diseases

WP2/3: Nephrotic glomerulopathies

WP5: Complement-mediated nephropathies

WP4: Tubulopathies

WP6: Kidney malformations
Targeted NGS Approach

Gene mutations identified in family studies

Human malformation syndromes

Animal models of CAKUT

CAKUTOME NGS Panel: 208 genes

453 CAKUT patients predominantly mild disease (VUR, PUJO...)

Massive parallel sequencing of pooled samples (196 samples per run)
Mean target coverage per sample of 135X
Variant Filtering

208 genes; 453 patients
11,588 variants

3,142 variants

remove synonymous

1,928 variants

Prioritization

Missense variants
n=1,642
Mean Pv ≤ 2.5
Mean Po ≤ 2.5
Novel, Deleterious
n=145

Truncating
n=78

HGMD variants
n=75
Kidney disease-related genes
n=44

Validated by Sanger sequencing
n=180

Classification
## Classification of the Validated Variants

<table>
<thead>
<tr>
<th>Group 1: Causal Mutations (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Criteria</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 2: candidate variants of pathogenicity (n=15)</th>
</tr>
</thead>
</table>
| **Criteria** | 1. Rare truncating variants in known kidney disease-related genes  
2. Variants previously reported in CAKUT patients with experimental evidence supporting the pathogenic effect on the protein function |

<table>
<thead>
<tr>
<th>Group 3: Probably deleterious (n=128)</th>
</tr>
</thead>
</table>
| **Criteria** | 1. Novel missense variants predicted to be deleterious (PolyPhen-2, SIFT) and conserved (GERP>4)  
2. Rare truncating variants in any of the 208 genes |

<table>
<thead>
<tr>
<th>Group 4: Variants of uncertain significance (VUS) (n=32)</th>
</tr>
</thead>
</table>
| **Criteria** | 1. HGMD variants with no functional evidence supporting their causality  
2. HGMD variants found in population databases and have no statistical evidence for an association with disease. |
Conclusions: CAKUT NGS Gene Panel Sequencing

- Successful high-throughput sequencing with high coverage

- Small patient subset carries known or novel pathogenic mutations in a known causal CAKUT gene

- 148 candidate variants in 82 genes in 151/453 (33%) CAKUT patients of which 143 variants need further investigation

- ~98% of these variants were predicted to be among 1% most deleterious of all possible substitutions in the human genome

- Testing of second CAKUT cohort with more severe kidney disease (4C/ESCAPE) underway
Can CAKUT Genetics Become Clinically Useful?

- Currently limited impact of genetic diagnosis on clinical management: Genetic counseling, syndromic cases

- NGS technologies making diagnostics quick and cheap

- Lack of detailed genotype-phenotype correlation studies

- **Crucial issue:** Does genetic defect predict risk and rate of renal failure progression?

  - Comprehensive NGS gene panel studies in large, well characterized CAKUT cohorts required
Role of Epigenetic Modifications in CAKUT?

Jin et al. AJKD 2014

• Monozygotic twins **discordant for unilateral renal agenesis**

• No discordant SNPs or DNA copy number variations

• 514 Differentially Methylated Regions, localized to 10 signaling pathways and 25 genes, including MAP kinase pathway and 6 genes involved in organ development (FGF18, FGF12, PDGFRA, MAPK11, AMH, CTBP1)

➢ Epigenetic mechanisms involved in renal agenesis?
Potential Causes for Impaired Nephrogenesis in Humans

- IUGR
  - Congenital renal anomalies
  - Preterm birth?

- ↓ Nephron number
  - Tubular changes

- Genetic/epigenetic factors
- Gender

- Drugs
  - Glucocorticoids
  - Chorioamnionitis

- Gestational diabetes
- Iron deficiency
- Vitamin A deficiency
- Maternal denutrition