Never in the Urinary Tract –
Causing Urinary Tract Malformations: the case of $Tbx6$

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Congenital anomalies of the kidney and urinary tract (CAKUT): Conditions and Epidemiology

Genetics of CAKUT

- **Point mutations**: 6–20% of CAKUT caused by single-gene defects with over 50 genes identified thus far (most commonly *HNF1B, PAX2, EYA1, SALL1* and others)

- **Structural variants / Copy number variations** (CNVs; i.e. deletions or duplications of germline DNA often affecting multiple genes): additional 2-10% of CAKUT caused by large CNVs associated to genomic disorders (ex. 22q11.2, 17q12, and others)

Verbitsky & Westland, *Nat Genet.* 2019
Sanna-Cherchi & Westland, *J Clin Invest.* 2018
Standard paradigm for gene identification in humans

- **Step 1**: unbiased, hypothesis-free genetic study to localize a gene or region of the genome associated with the phenotype (linkage studies in families, GWAS, exome or genome sequencing…)

- **Step 2**: candidate gene selection / prioritization. Classically it has been recognized that, if a gene causes a phenotype when mutated, it should be expressed in the tissue where the phenotype occurs

- **Step 3**: generation of a vertebrate model that recapitulates the human phenotype
CNV study in 2,824 CAKUT cases and 21,498 controls identifies the chromosome 16p11.2 microdeletion syndrome as a cause of CAKUT

Common phenotypes with 16p11.2 microdeletion:
- Congenital scoliosis
- Spondylocostal dysostosis
- Autism spectrum disorder

Classic association between congenital scoliosis and CAKUT

Verbitsky & Westland, Nat Genet. 2019

Hanson et al., Biol Psychiatry. 2015
Vitko, Cass & Winter, J Urol. 1972
Defining a driver for CAKUT at the 16p11.2 locus: deletion mapping

Verbitsky & Westland, Nat Genet. 2019
Plausibility for *TBX6* as a CAKUT gene

- Involved in early mouse development
- Human truncating mutations associated with congenital scoliosis
- Heterozygous mutations are very rare in humans

### Mouse *Tbx6* allelic series

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
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<tbody>
<tr>
<td><em>Tbx6</em>&lt;sup&gt;+&lt;/sup&gt;/+</td>
<td>wild type</td>
</tr>
<tr>
<td><em>Tbx6</em>&lt;sup&gt;rv&lt;/sup&gt;/+</td>
<td>wild type</td>
</tr>
<tr>
<td><em>Tbx6</em>&lt;sup&gt;+&lt;/sup&gt;/–</td>
<td>minor vertebral abnormalities</td>
</tr>
<tr>
<td><em>Tbx6</em>&lt;sup&gt;rv&lt;/sup&gt;/–</td>
<td>fused ribs and vertebrae</td>
</tr>
<tr>
<td><em>Tbx6</em>&lt;sup&gt;rv&lt;/sup&gt;/&lt;sup&gt;rv&lt;/sup&gt;</td>
<td>severe fusion of ribs and vertebrae</td>
</tr>
<tr>
<td><em>Tbx6</em>&lt;sup&gt;–&lt;/sup&gt;/–</td>
<td>lethal E9.5</td>
</tr>
</tbody>
</table>
Severe reduction in $Tbx6$ gene dosage causes CAKUT with complete penetrance $\rightarrow$ supporting causality

Verbitsky & Westland, *Nat Genet.* 2019
Milder mutations recapitulate CAKUT phenotypes in 16p11.2 deletion including obstructive uropathy and duplicated ureters→ supporting pleiotropic effect

Verbitsky & Westland, Nat Genet. 2019
Analysis of the lower urinary tract for CAKUT phenotypes identifies anorectal malformations → further pleiotropy

<table>
<thead>
<tr>
<th>Wild Type</th>
<th>Tbx6^{rv/ rv}</th>
<th>Tbx6^{rv/-}</th>
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<tbody>
<tr>
<td>E15.5</td>
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- Bladder
- Hindgut
- Urethra
- Anus
Additional lower urinary tract defects that correlate with urethra malformations and PUV
Initial conclusions and questions

• *TBX6* reduced gene dosage is the cause of CAKUT in patients with the chromosome 16p11.2 microdeletion

• *Tbx6* reduced gene dosage has a profound pleiotropic effect similar to the 16p11.2 microdeletion causing upper and lower urinary tract defects

• **Open question**: How do *TBX6* mutations cause CAKUT and what are the mechanisms for such pleiotropic effect?
*Tbx6* is expressed in the intermediate mesoderm surrounding the cloaca at E9.5 but **NEVER** in the developing urinary tract.

*How is it possible?*
Some hint: Does the nephric duct insert into the cloaca?
Tbx6 gene dose reduction affects the position & angle of ureteric bud formation and disrupts the normal interactions between the ureteric bud and nephric progenitors. Mendelsohn, *Organogenesis*. 2009
Rationale for urinary tract defects in *Tbx6* mutants

- *Tbx6* appears to be critical for insertion of CND into the cloaca
  - Expressed at the right place at the right time and gene dose reduction results in failure of CND insertion
  - CND insertion is critical for normal UB induction and insertion of ureter into bladder → mechanism for VUR, OU, DCS, KA (Mackie Stephens hypothesis)

- *Tbx6* dose reduction results in ectopic neural tubes in place of posterior somites
  - Grobstein et al in the 1950’s demonstrated that neural tube is capable of attracting and inducing mesenchyme *in vitro*
  - Abnormal position of kidney mesenchyme → mechanism of DCS, KA
Conclusions and Future Directions

- *Tbx6* gene-dose reduction is sufficient to cause all categories of human CAKUT observed in the chromosome 16p11.2 microdeletion syndrome

- *Tbx6* is expressed in peri-cloacal mesenchyme at E9.5, but, surprisingly, never in the developing ureter or kidney

- We propose different mechanisms and explanations for the *TBX6* mutations causal role and the observed pleiotropy:
  - Failure of nephric duct insertion
  - Ectopic neural tubes
  - Failed or incomplete cloacal septation

- Future directions: studies aimed at understanding the transcriptional dysregulation resulting from TBX6 mutations and identification of potential intervention targets
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