



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

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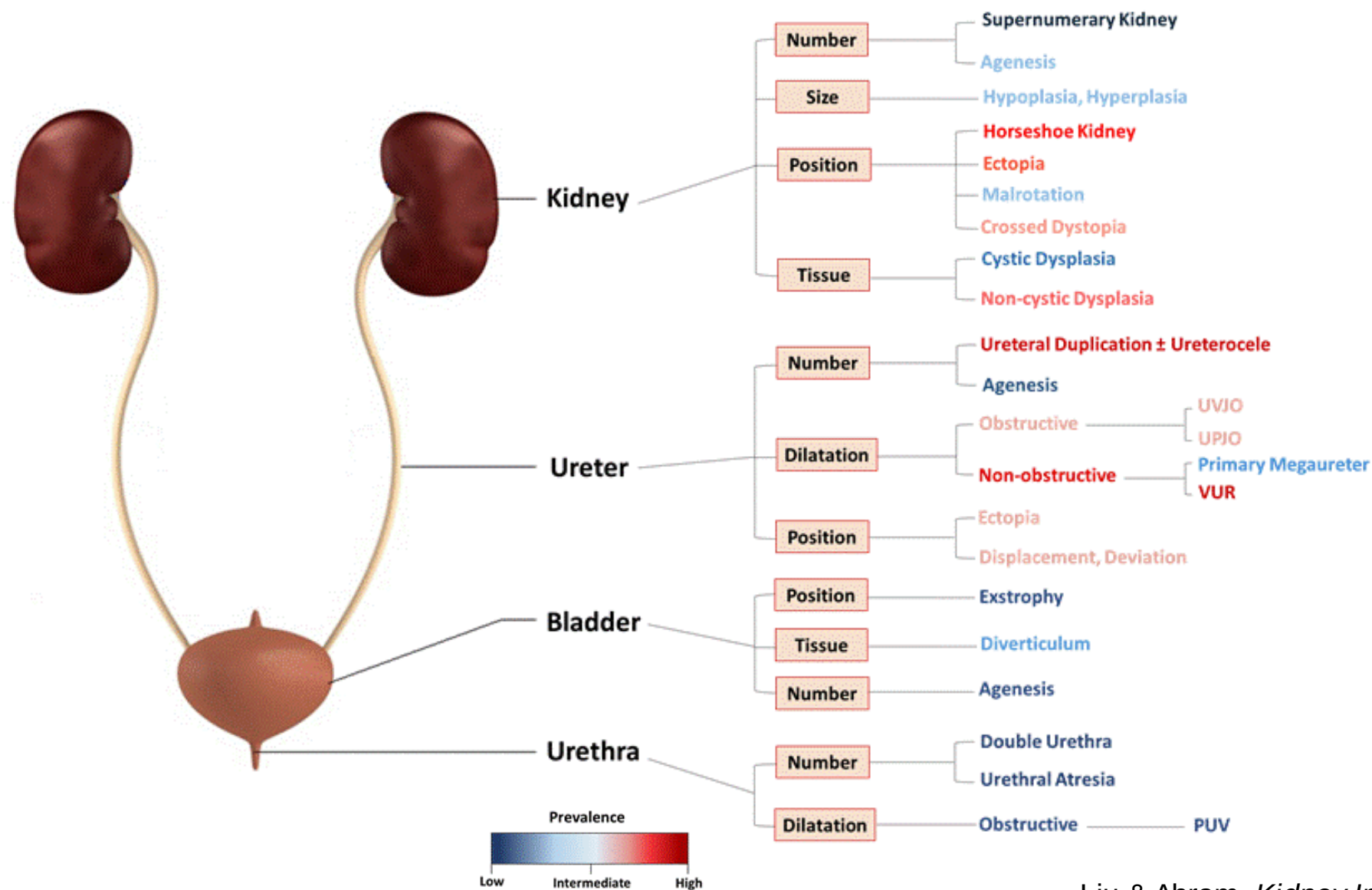
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Congenital anomalies of the kidney and urinary tract (CAKUT) : Conditions and Epidemiology



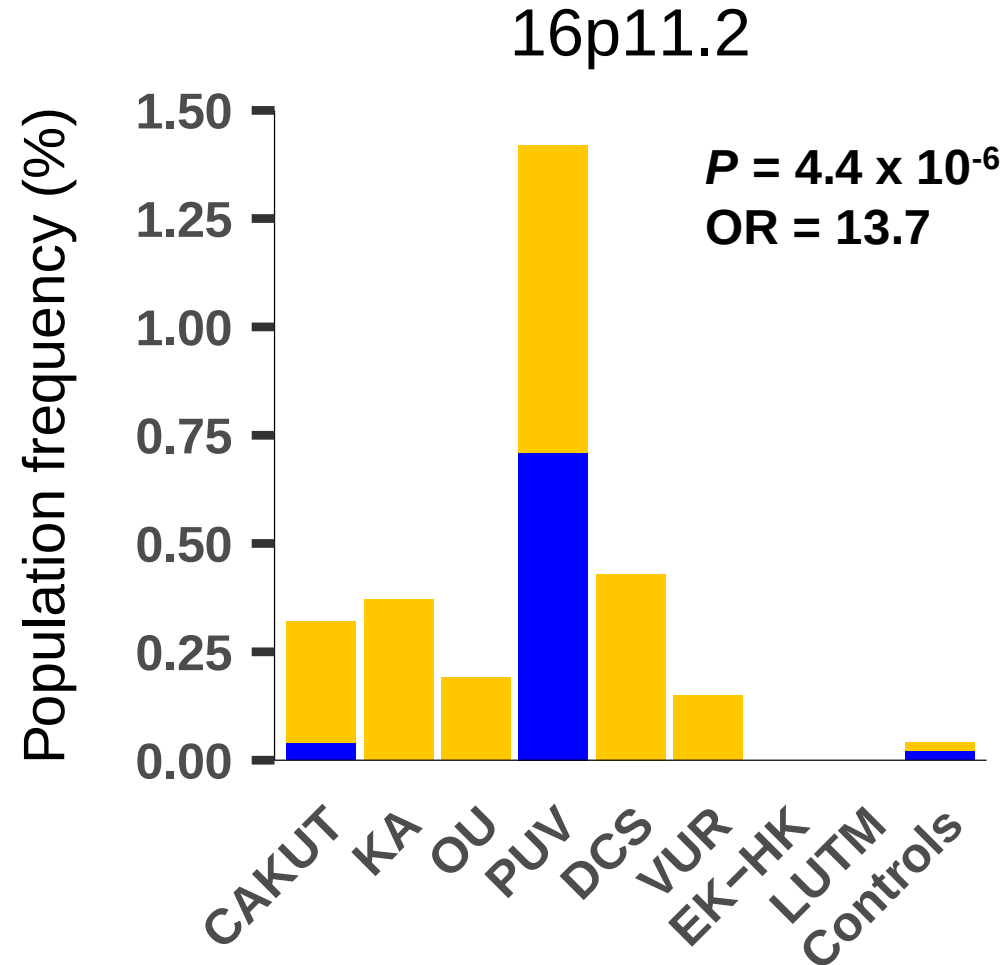
Genetics of CAKUT

- 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)

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



Verbitsky & Westland, *Nat Genet.* 2019

Common phenotypes with 16p11.2 microdeletion:

- Congenital scoliosis
- Spondylocostal dysostosis
- Autism spectrum disorder

Classic association between congenital scoliosis and CAKUT

El-Kateb et al, *Am J Med Genet A.* 2014

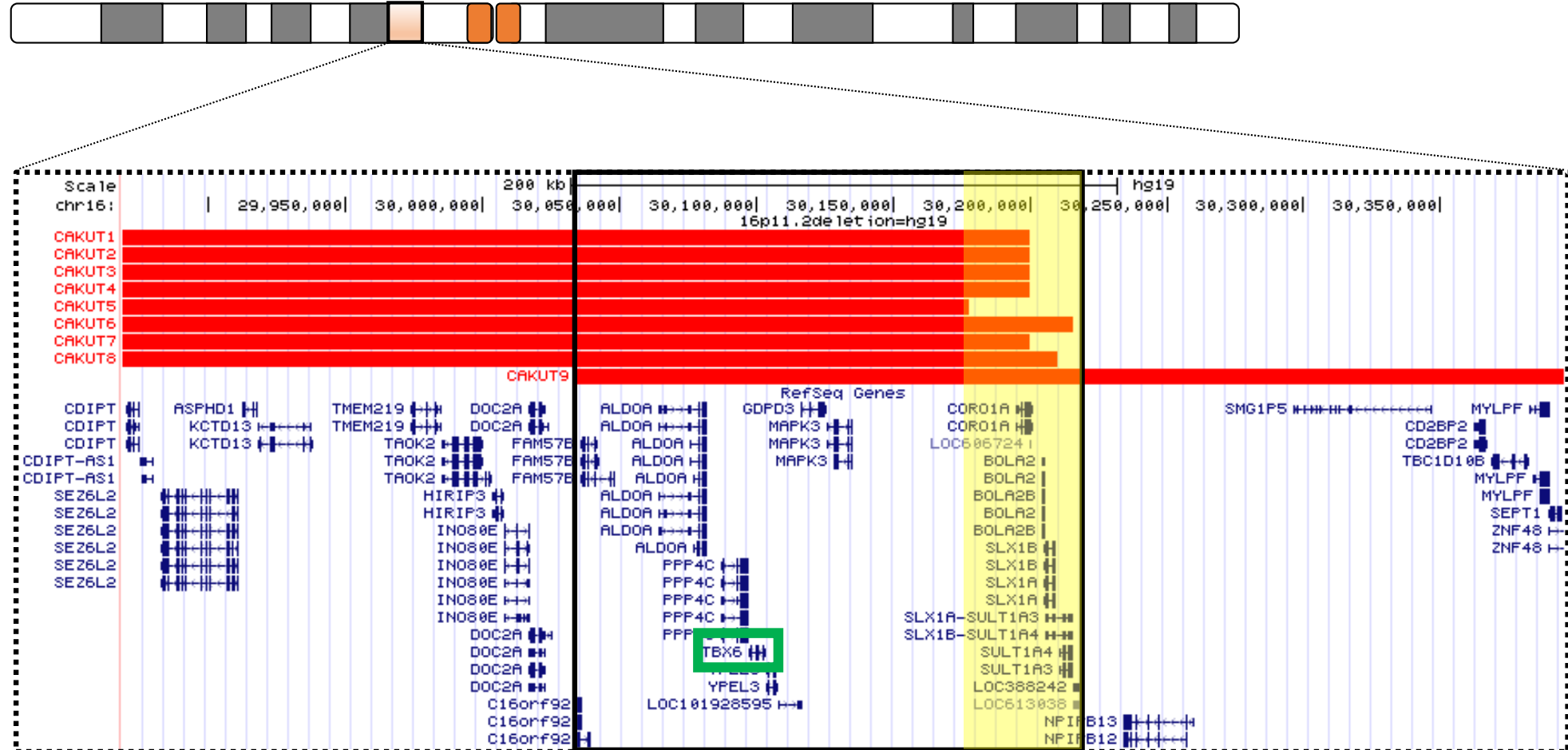
Hanson et al., *Biol Psychiatry.* 2015

Vitko, Cass & Winter, *J Urol.* 1972

MacEwen, Winter & Hardy, *J Bone Joint Surg.* 1972

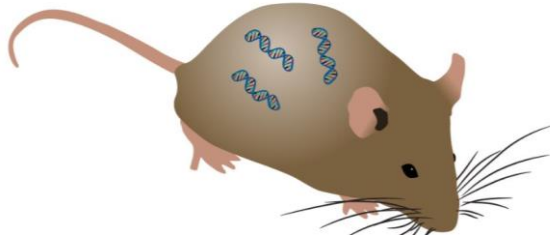
Defining a driver for CAKUT at the 16p11.2 locus: deletion mapping

Chromosome 16 p11.2



Plausibility for *TBX6* as a CAKUT gene

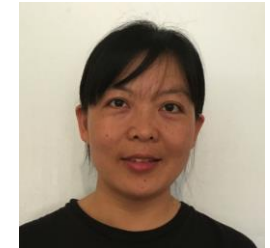
- Involved in early mouse development
- Human truncating mutations associated with congenital scoliosis
- Heterozygous mutations are very rare in humans



Mouse *Tbx6* allelic series



Alejandra Perez



Qingxue Liu

Tbx6⁻
Tbx6^{rv}

null allele
spontaneous hypomorphic allele

Genotype

Tbx6^{+/+}

Tbx6^{rv/+}

Tbx6^{+/-}

Tbx6^{rv/rv}

Tbx6^{rv/-}

Tbx6^{-/-}

Phenotype

wild type

wild type

minor vertebral abnormalities

fused ribs and vertebrae

severe fusion of ribs and vertebrae

lethal E9.5

In collaboration with Mendelsohn and Papaioannou labs

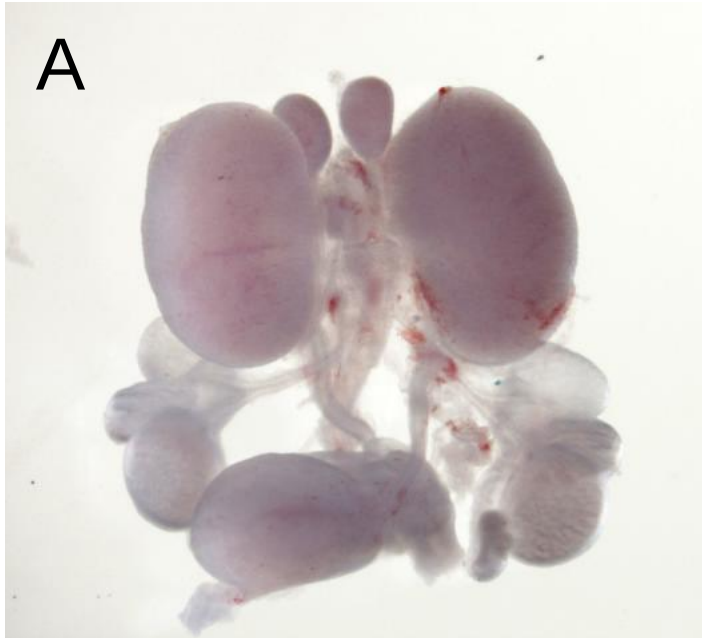
Severe reduction in *Tbx6* gene dosage causes CAKUT with complete penetrance → supporting causality

Wild type

Tbx6^{rv/-}

E18.5

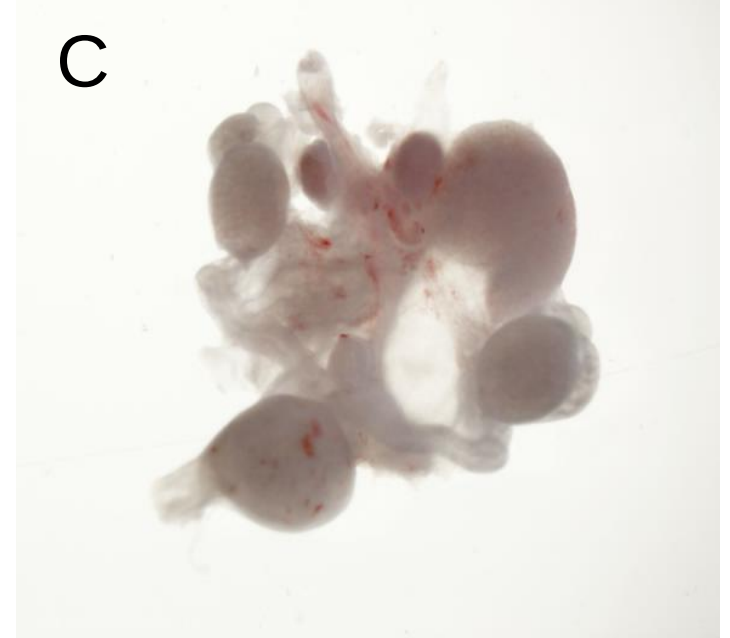
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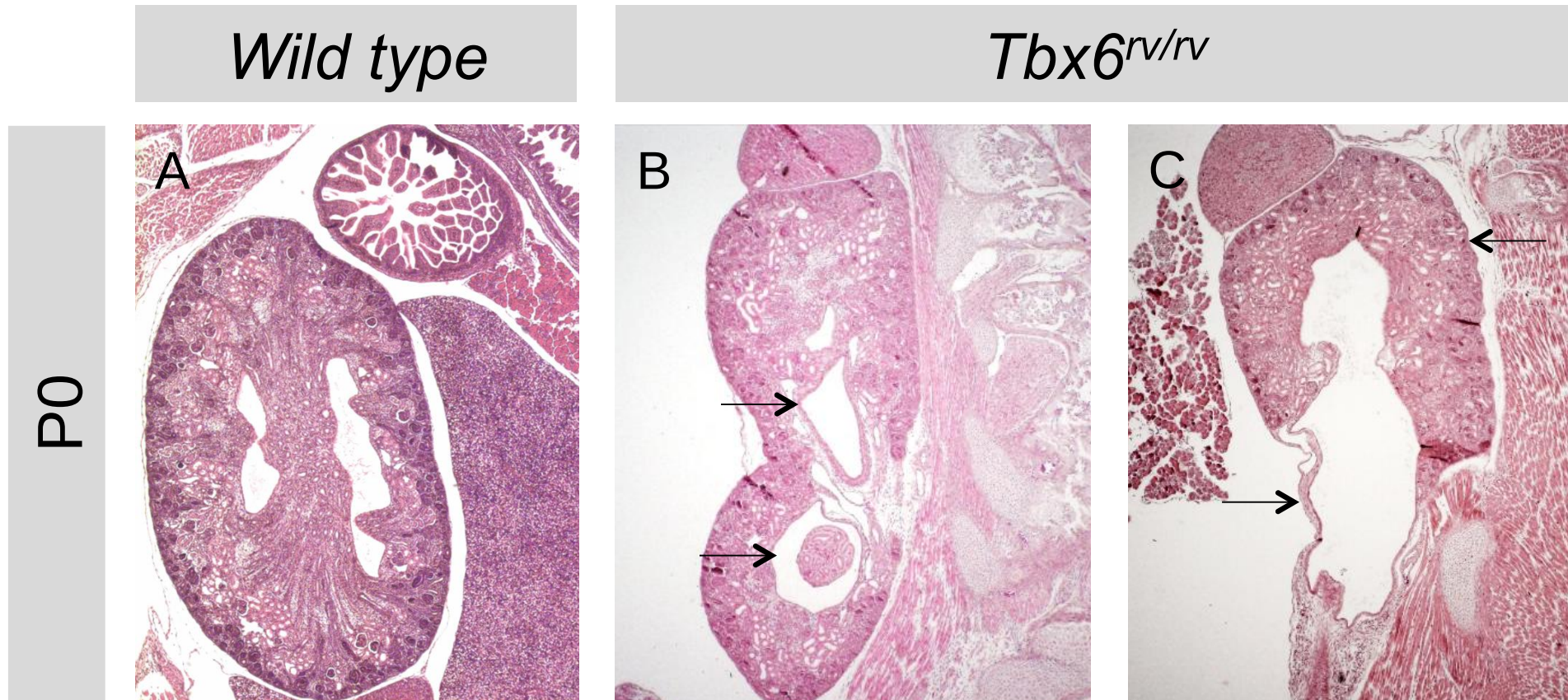
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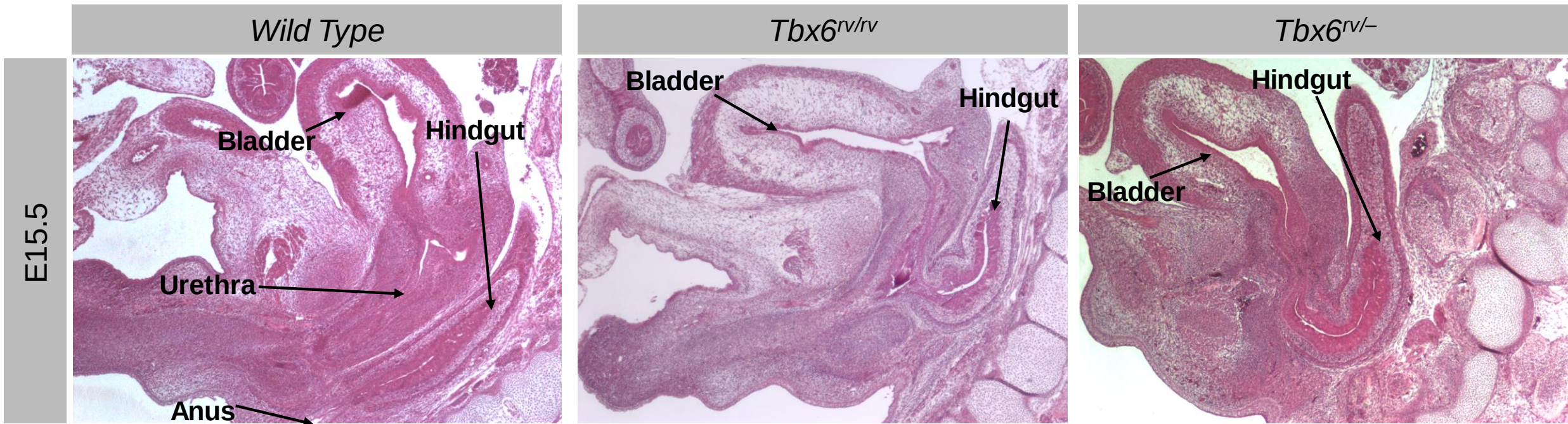
C



Milder mutations recapitulate CAKUT phenotypes in 16p11.2 deletion including obstructive uropathy and duplicated ureters → supporting pleiotropic effect



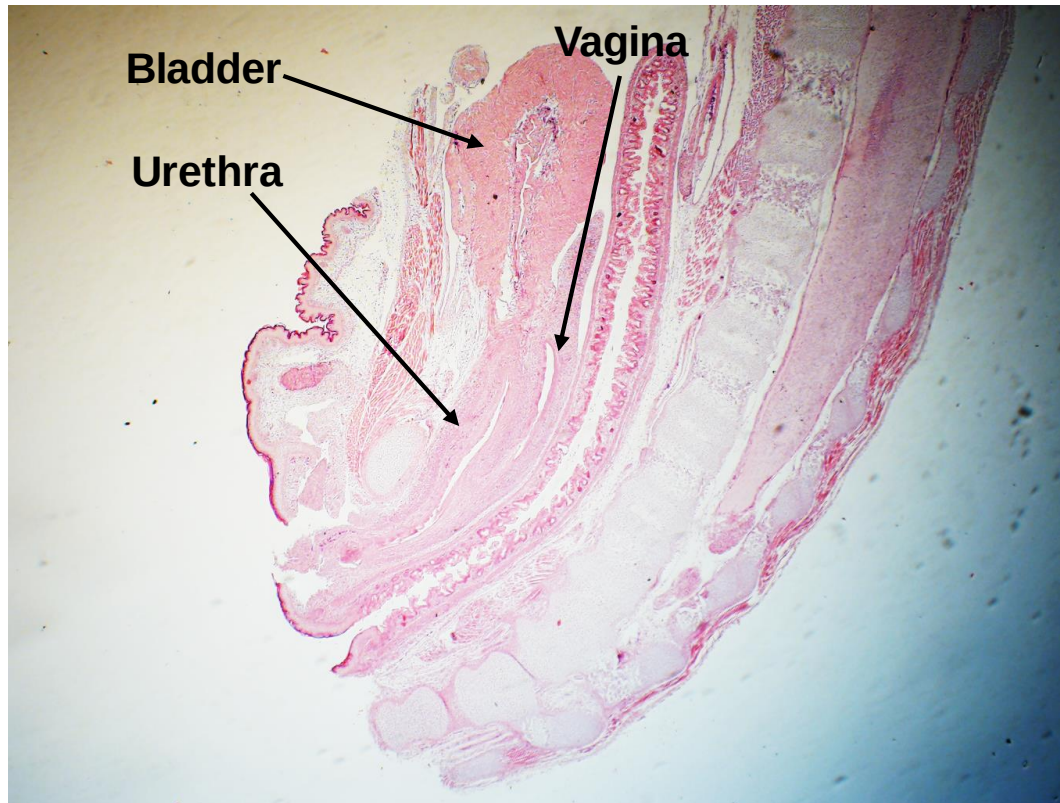
Analysis of the lower urinary tract for CAKUT phenotypes identifies anorectal malformations → further pleiotropy



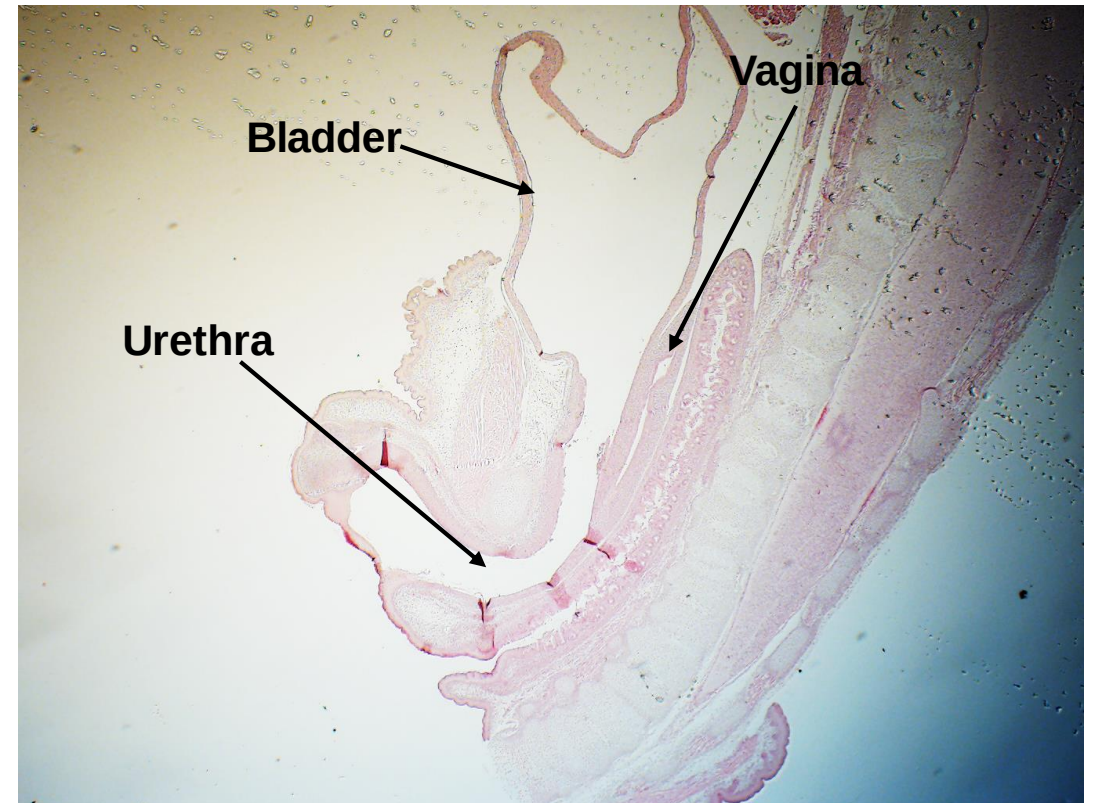
Additional lower urinary tract defects that correlate with urethra malformations and PUV

E18.5

Wild Type



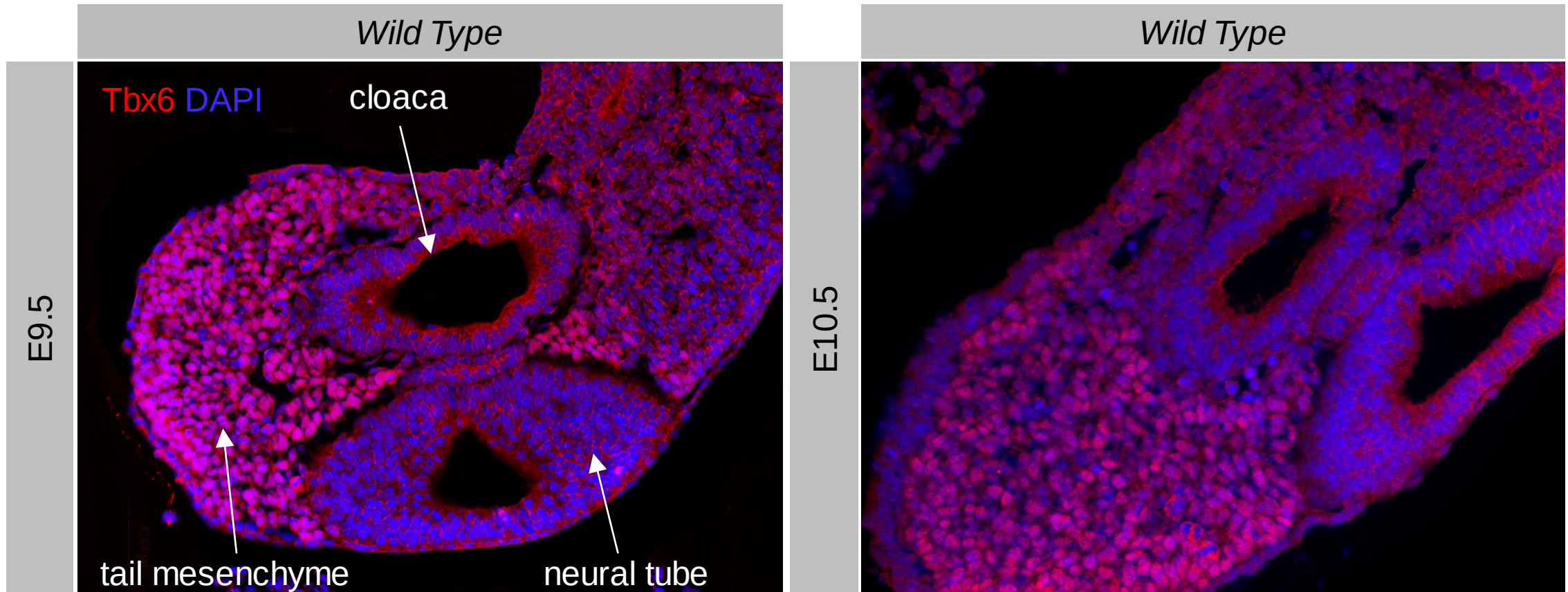
Tbx6^{rv/rv}



Initial conclusions and questions

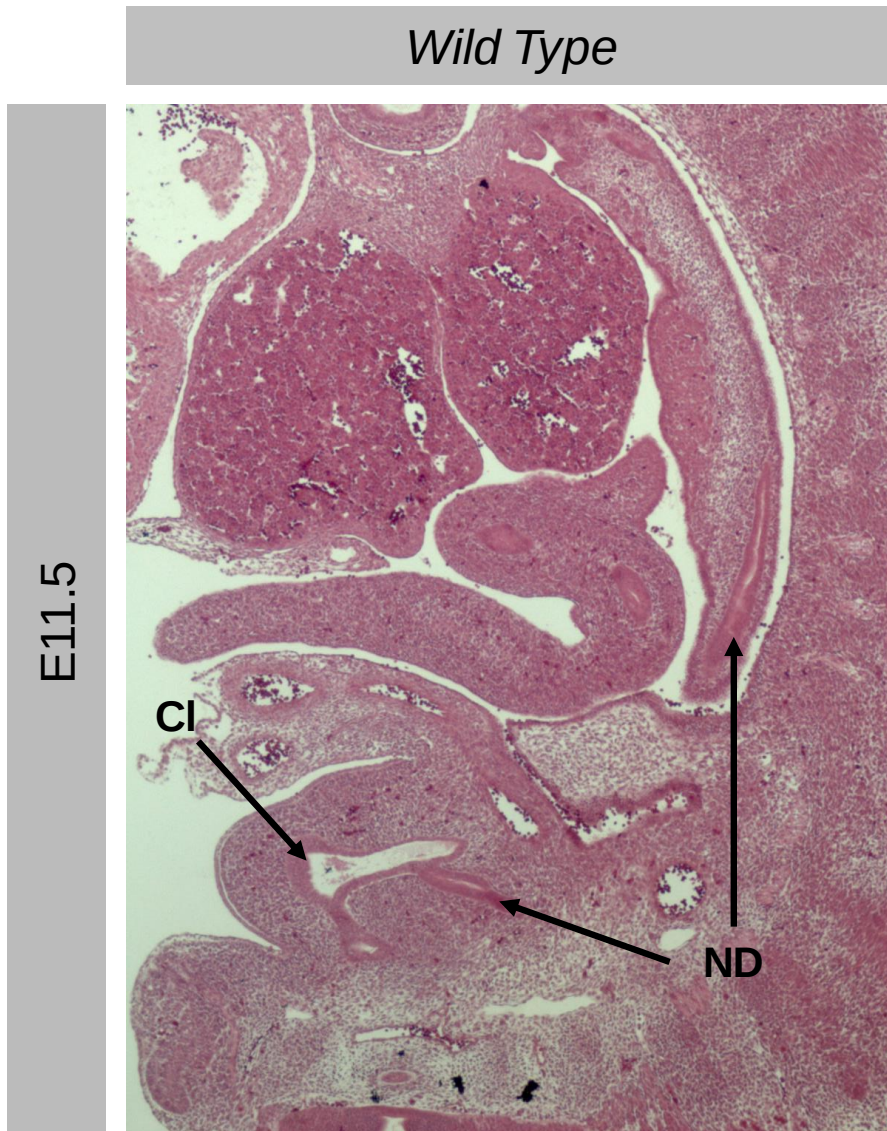
- *TBX6* reduced gene dosage is the cause of CAKUT in patients with the chromosome 16p11.2 microdeletion
- *Tbx6* reduced gene dosage has a profound pleiotropic effect similar to the 16p11.2 microdeletion causing upper and lower urinary tract defects
- Open question: How do *TBX6* mutations cause CAKUT 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

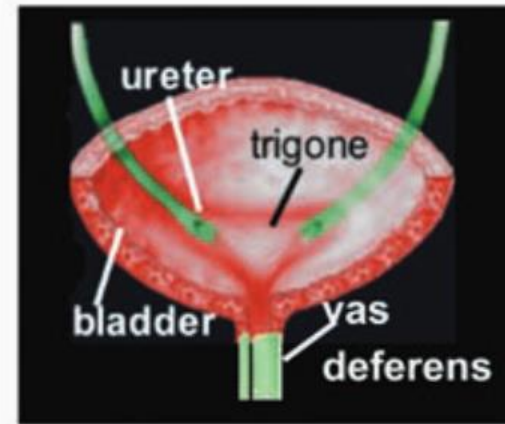


How is it possible?

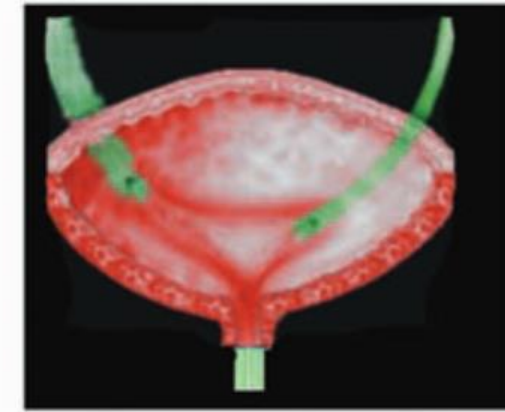
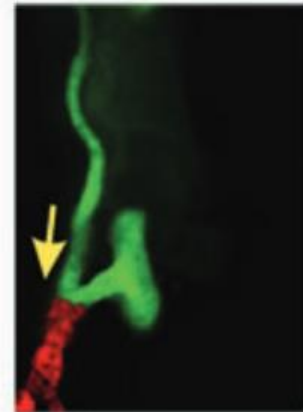
Some hint: Does the nephric duct insert into the cloaca?



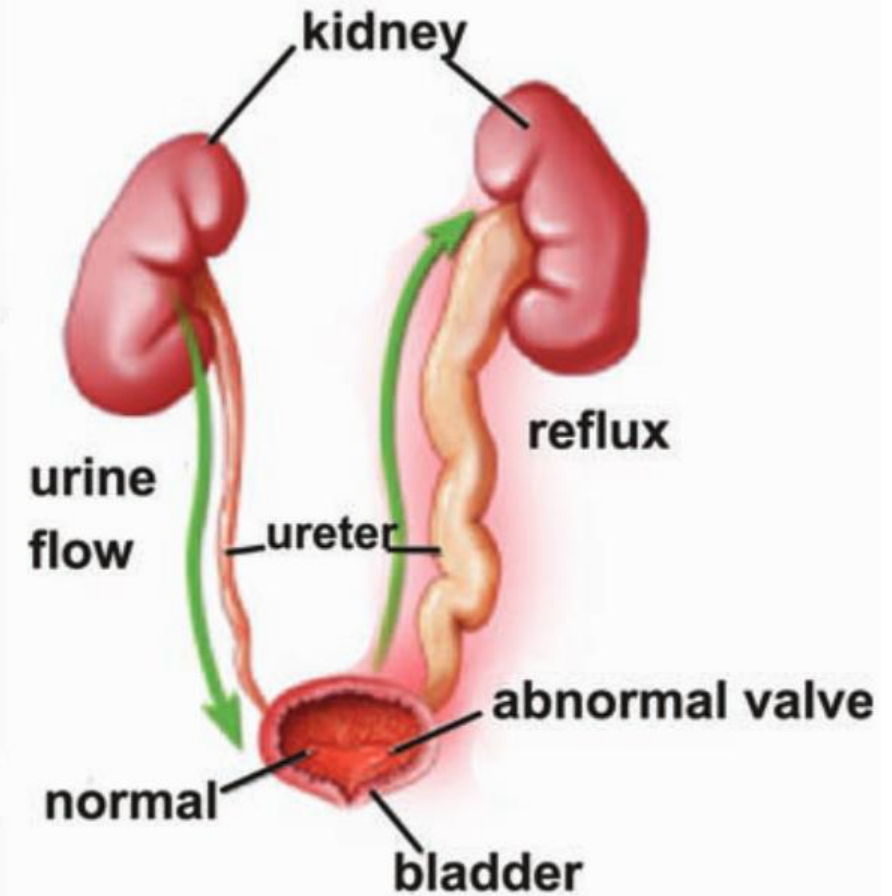
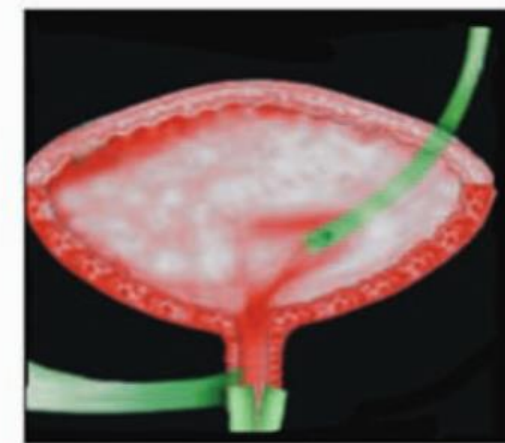
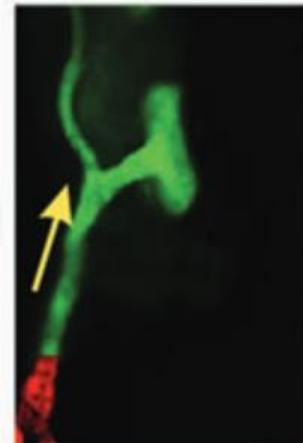
normal ureteric bud



low ureteric bud



high ureteric bud



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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