Pleiotropy of Congenital Anomalies of the Kidney and Urinary Tract in the Chromosome 16p11.2 Microdeletion Syndrome Is Recapitulated in Mouse Models of *Tbx6* Deletion

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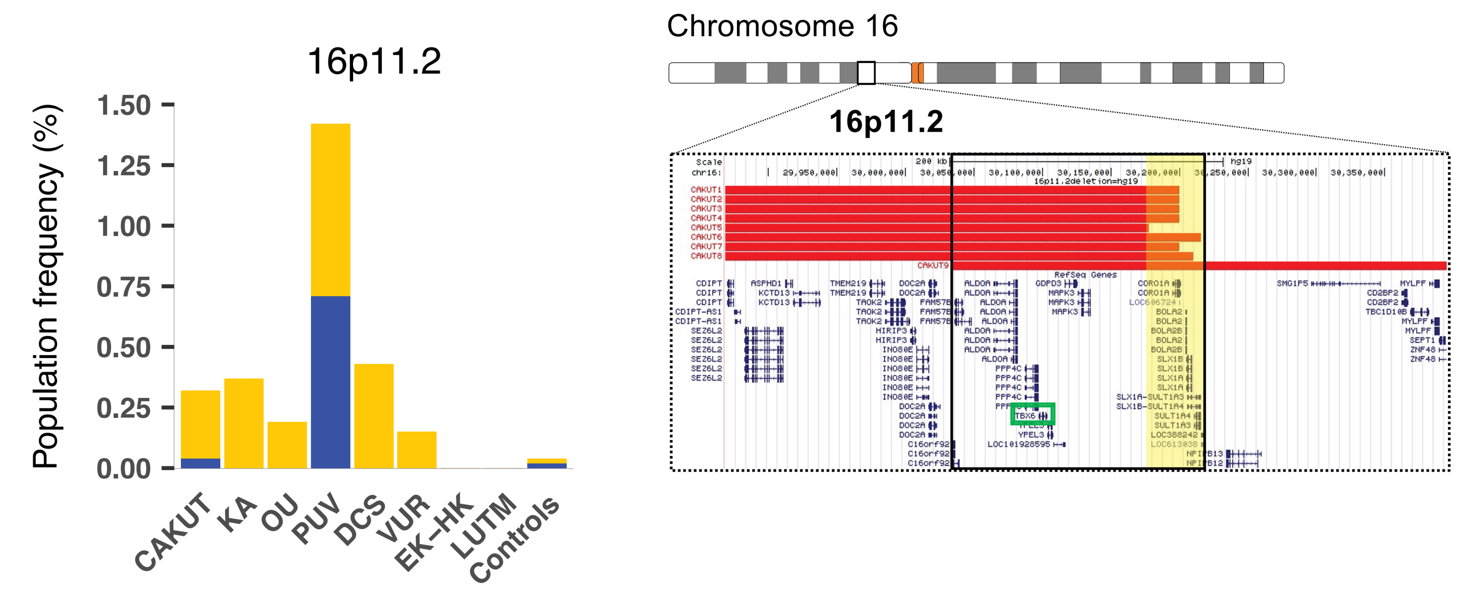
Introduction

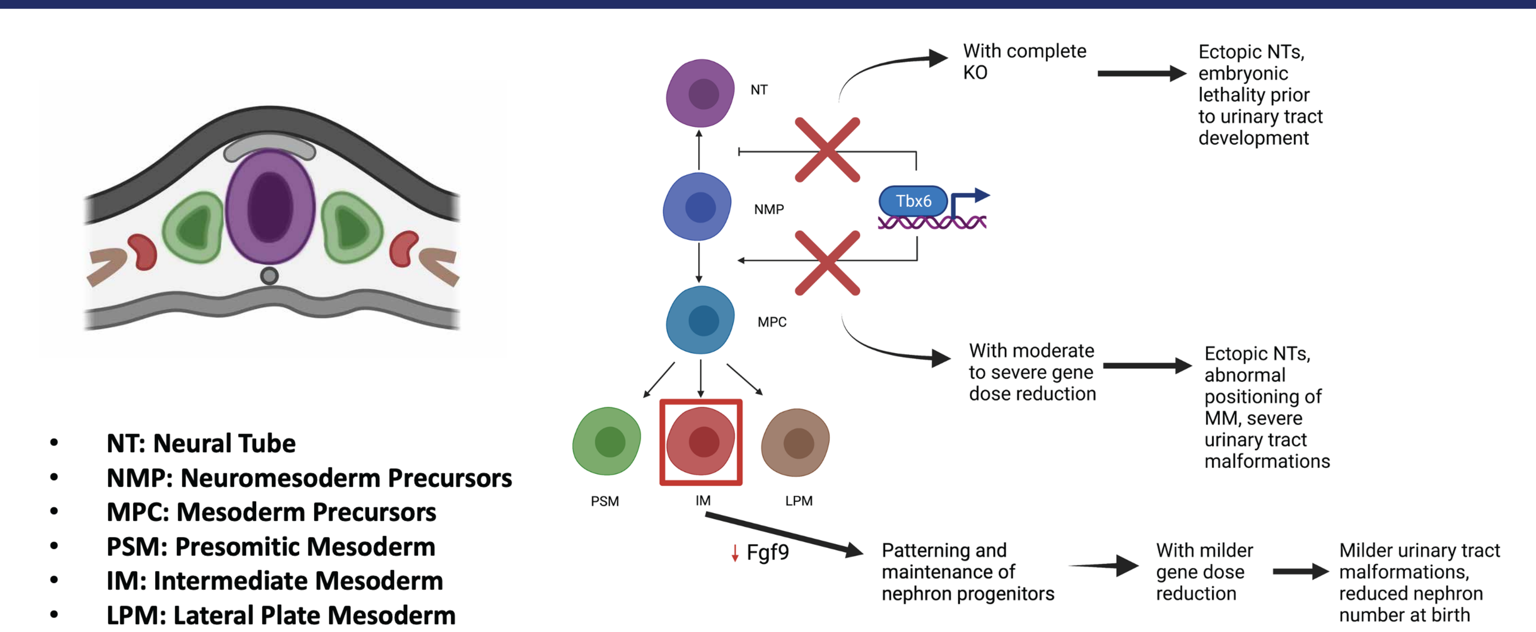


We previously showed 16p11.2 microdeletion to be a major cause of congenital anomalies of the kidney and urinary tract (CAKUT), and identified *TBX6* as the most likely driver.[1] Whether *TBX6* dose reduction causes the full spectrum of CAKUT seen in 16p11.2 microdeletion has yet to be demonstrated, and consequent mechanisms remain elusive. Here we show the full phenotypic spectrum of CAKUT in *Tbx6* mouse models and identify, in silico, downstream signaling pathways and targets that impact development of the genitourinary (GU) tract.

Methods

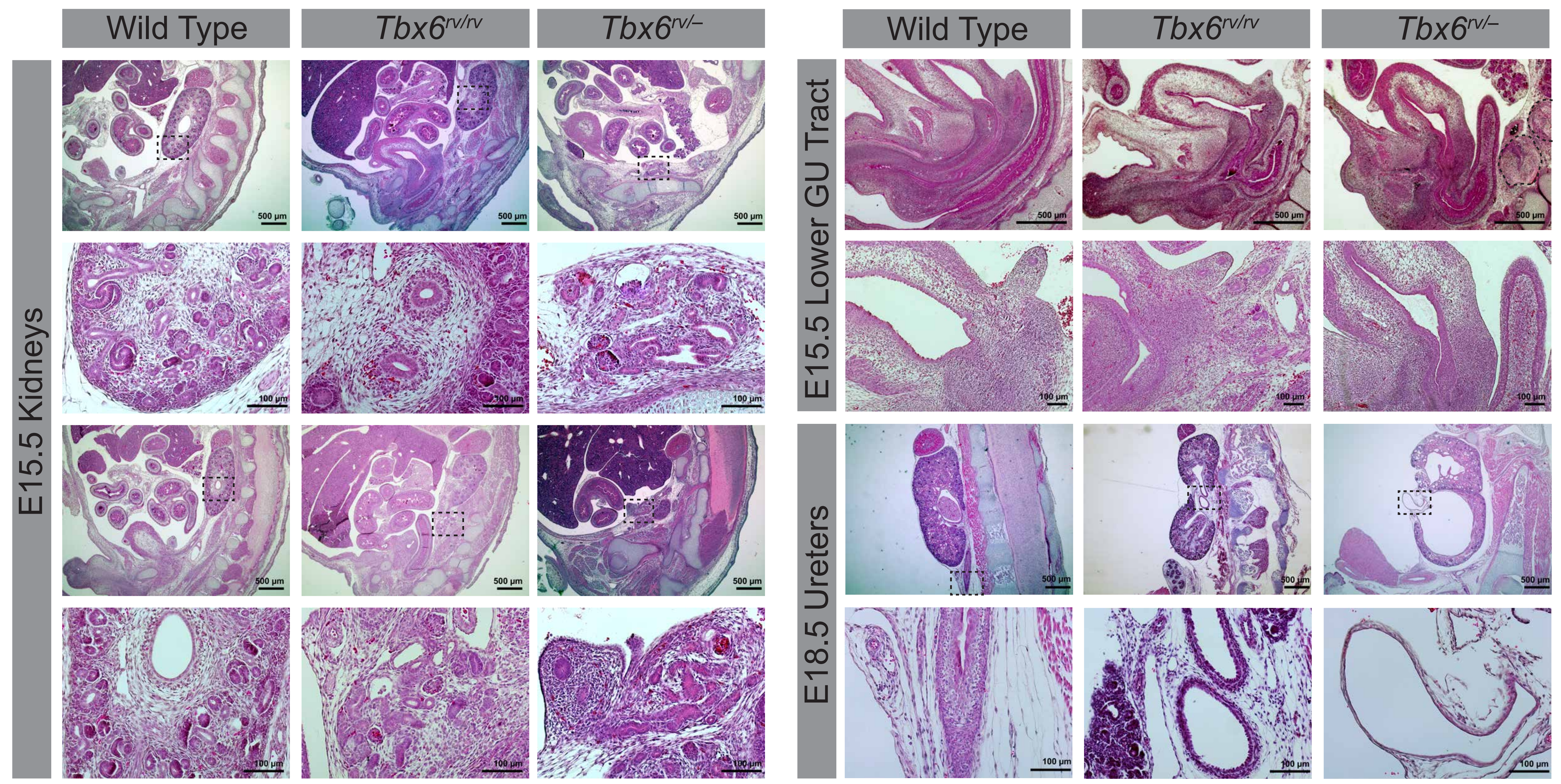
We studied a Tbx6 allelic series using two independent alleles: a null allele (*Tbx6*^{tm2pa}, hereafter *Tbx6*$^{-}$) and a hypomorphic allele (*Tbx6*^{rv}). We conducted detailed phenotypic analysis on histological sections of these models across developmental stages. Gene expression analysis was performed on dissected mesenchyme from E9.5 tails with neural tubes and ectoderm removed, keeping the two most recently segmented somites. Gene-set enrichment analysis (GSEA) and deconvolution with publicly available single-cell RNA-seq (scRNA-seq) were perfomed to identify disrupted biological processes, pathways, and cell types.[2] Transcription factor binding site (TFBS) analysis was conducted to identify potential targets of *Tbx6*. TFBS predictions were subsequently prioritized by intersection with ATAC-seq of E9.5 tail and intersection with our expression data.[3] Coexpression with *Tbx6* was then determined using the scRNA-seq data used for deconvolution.

Graphical Abstract



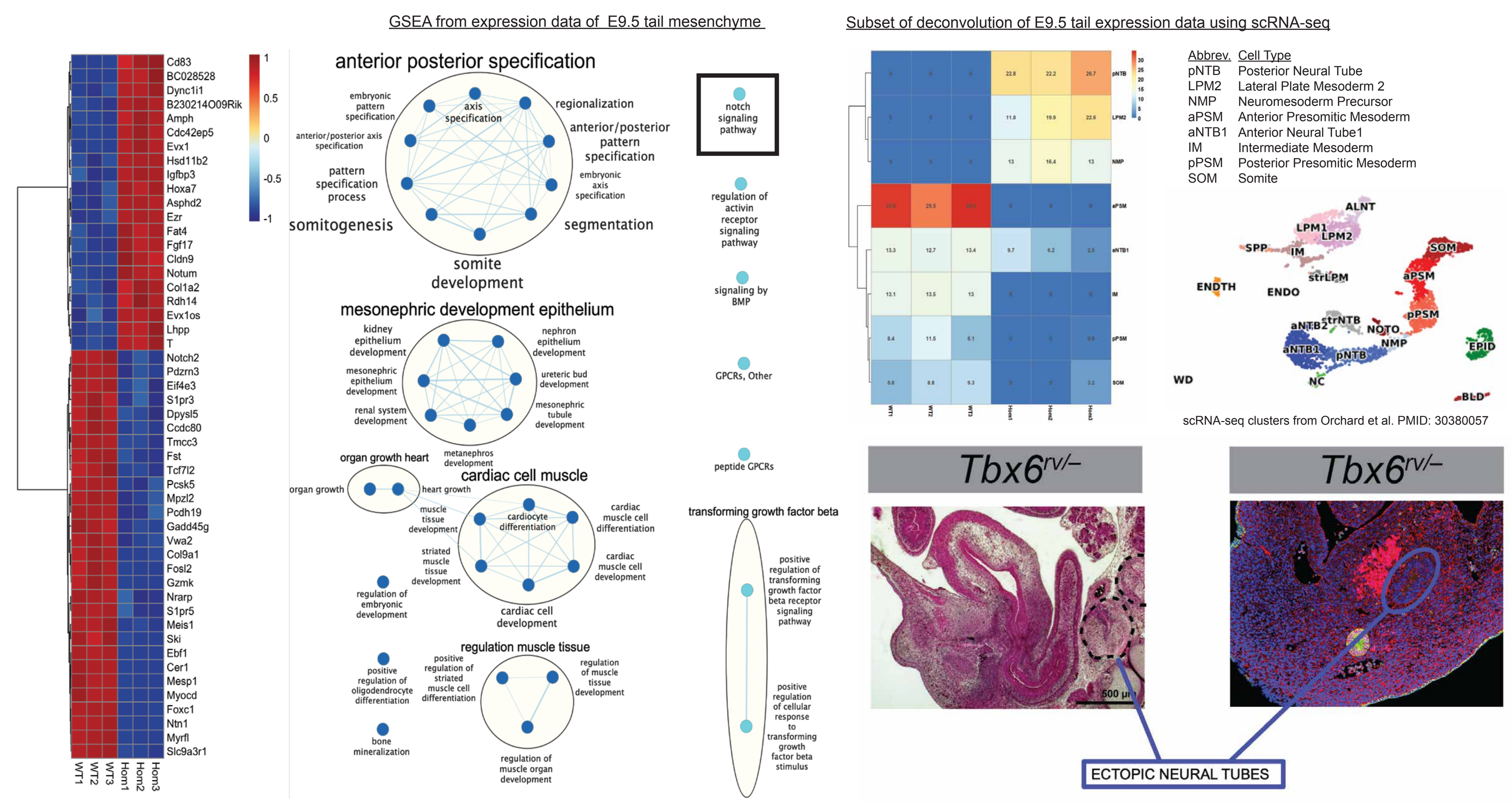
Results

Pleiotropy of CAKUT in mice with reduced Tbx6 gene dosage



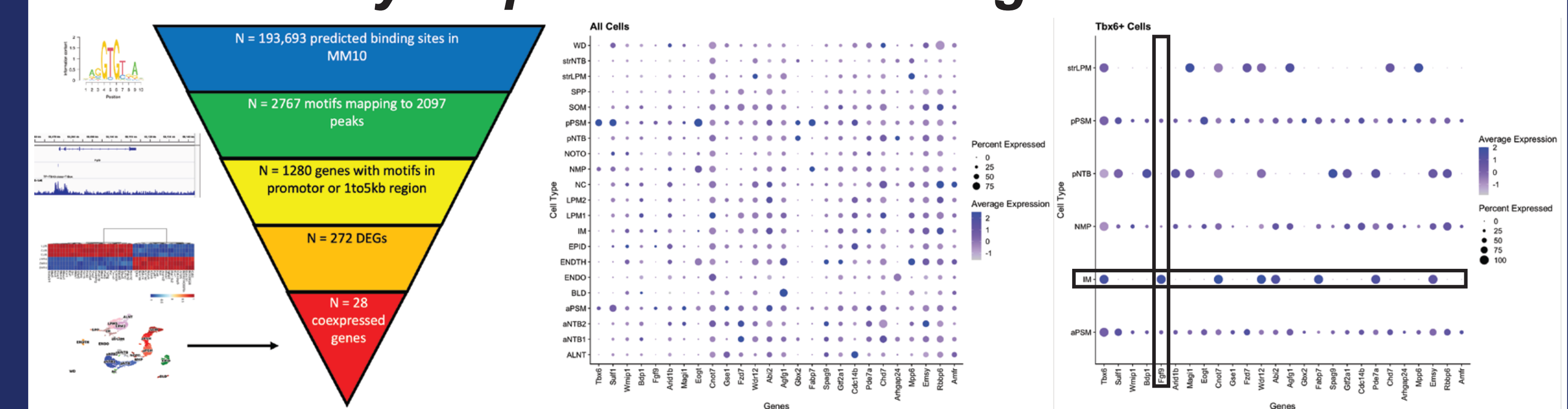
Phenotype analysis recapitulated the full spectrum of CAKUT observed in 16p11.2 patients, with profound upper and lower urinary tract defects (renal hypodysplasia, hydronephrosis, ureter duplication, cloacal septation defects, failed ureter and reproductive duct insertions, urethral and genital tubercle malformations). This indicates an early role of Tbx6 in GU development.

Gene set enrichment analysis and deconvolution of E9.5 tail gene expression identify disrupted cell types and pathways in Tbx6 mutants



GSEA of differentially expressed genes (DEGs) suggested a critical role of *Tbx6* in somitogenesis, Notch signaling, and Bmp signaling, which in turn play a role in GU development. Deconvolution identifies disruption in the normal patterning of all three populations of the mesoderm (presomitic, intermediate, and lateral plate) and an over abundance of neuromesoderm precurors and neural tube cells, despite dissecting them out. The over representation of the posterior neural tube cells seen in deconvolution correlated with the presence of ectopic neural tubes and neural tube cells in the *Tbx6* mutants as shown by histology and immunohistochemistry.

ATAC-seq DEG and scRNA-seq prioritized TFBS analysis predicts novel targets of Tbx6



Prioritization of predicted *Tbx6* binding motifs by open chromatin regions, differential expression, and coexpression from scRNA-seq data identifies 28 potential targets of *Tbx6*. Of these genes, 4 have been previously identified in the literature as causes of CAKUT, including *Sulf1*, *Chd7*, *Arid1b*, and *Kmt2d*.[4,5] This analysis also identifies *Fgf9* as a promising new cause of CAKUT and target of *Tbx6*, as that the two are significantly coexpressed in the intermediate mesoderm. *Fgf9* is important for patterning of the intermediate mesoderm from embryonic stem cells *in vitro*,[6,7,8] and has been shown *in vivo* to maintain stemness of nephron progenitors along with *Fgf20*.[9]

Conclusions

This study strongly supports a role of *TBX6* in pleiotropic CAKUT, uncovers causal mechanisms, and identifies genes and pathways regulated by *Tbx6*. Involvement of Notch signaling is of particular relevance as mutations of NOTCH2 and related genes cause Alagille syndrome and spondylocostal dysostosis, characterized by CAKUT and spine defects similar to those observed both in our *Tbx6* mouse models and in patients with 16p11.2 microdeletion. These data implicate loss of TBX6-mediated regulation of Notch signaling as critical to the development of CAKUT and spine defects. The integration of large scale TFBS analysis with single-cell transcriptomics and epigenomics allowed for the identification of downstream mechanisms, pathways, and target genes. Of these targets, *Fgf9* is attractive as a novel candidate CAKUT gene, given its importance in specification and maintenance of nephron progenitors.

Future Directions

We are conducting confirmatory studies to validate the differential expression of genes from our E9.5 tail dataset. Protein-DNA interactions will be investigated to confirm newly predicted targets of *Tbx6*. We will also examine the utility of *TBX6* dose reduction as a model of low nephron number at birth and subsequent risk for kidney diseases across the lifespan.

References

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