Transcriptomic Atlas Reveals Molecular Dynamics of Congenital Bladder Outflow Obstruction

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Background

Congenital urinary **bladder outflow obstruction (BOO) or megacystis** is characterized by an anatomical or functional blockage of the urethra, during fetal development preventing the emptying of the bladder[1]. We hypothesize that BOO arises from an incomplete developmental process that leads to altered cellular and molecular heterogeneity compared with the normal bladder.

Aims

- Characterize the cellular heterogeneity in the fetal bladder
- Map the developmental processes of the human urinary bladder
- Identify molecular processes that define congenital BOO

Experimental design

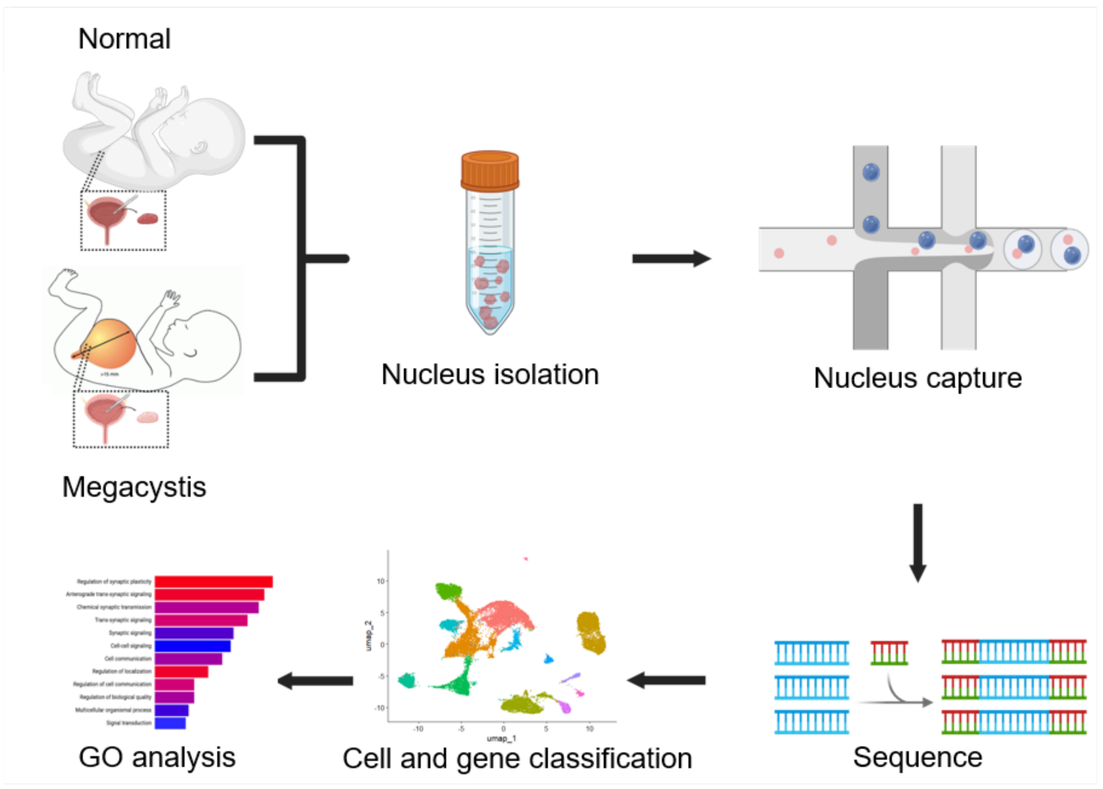


Figure 1: Experimental overview. Fetal bladder dome was collected[2] from normal (n=2) and megacystis (n=2) bladder at gestational weeks 16 - 20. The nuclei were isolated and processed for snRNA-sequencing. The captured nuclei were sequenced and later analyzed in R using the Seurat pipeline. Results showed cell and gene classification as well as gene ontology (GO) analysis.

Results: New cell clusters in the developing urinary bladder

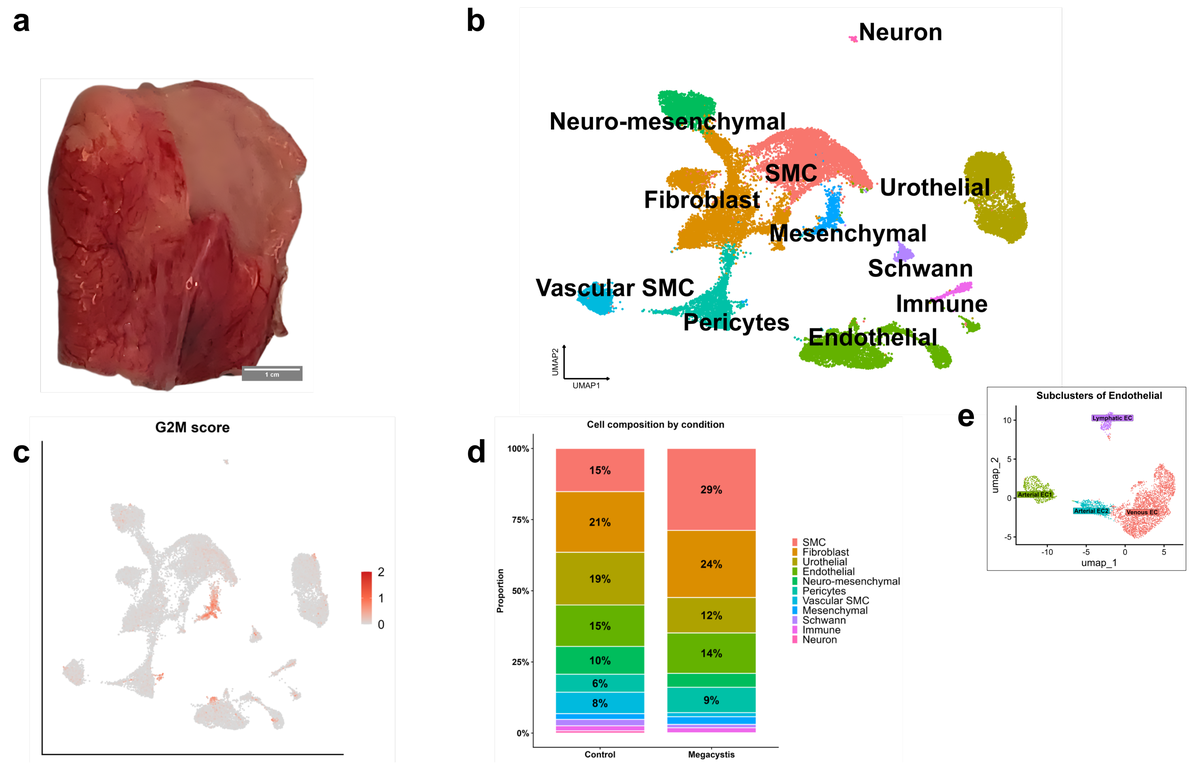


Figure 2: Gross morphology and cell classification. a. Fetal megacystis resected from anatomical site measured approx. 4.5 cm at gestational week 20. Scale bar: 1 cm **b**. Unsupervised clustering analysis of the fetal bladder revealed 11 distinct cell clusters. Of the 11 clusters, we identified two newly unexplored clusters: **mesenchymal and neuro-mesenchymal**. **c**. Cell cycle scoring computed by G2M score confirmed that mesenchymal population had the highest score of dividing cells. **d.** The fibroblast cluster accounted for the largest proportion of cells (21%) in control samples, while megacystis samples had a higher percentage of SMC (29%). **e.** An example of subclusters of endothelial cells (EC): Arterial EC1 (green), Arterial EC2 (cyan), Lymphatic EC (purple) and Venous EC (salmon).

References

1. Roberts N, Chan M, Woolf A, The biology of congenital urinary bladder outflow obstruction, Trends in Molecular Medicine, 2025;0
2. Ethical Approval by the Danish Ethics Committee (VEK: H-20084445)
3. Mitsioni, A., et al. ROBO2 gene variants in children with primary nonsyndromic vesicoureteral reflux with or without renal hypoplasia/dysplasia.

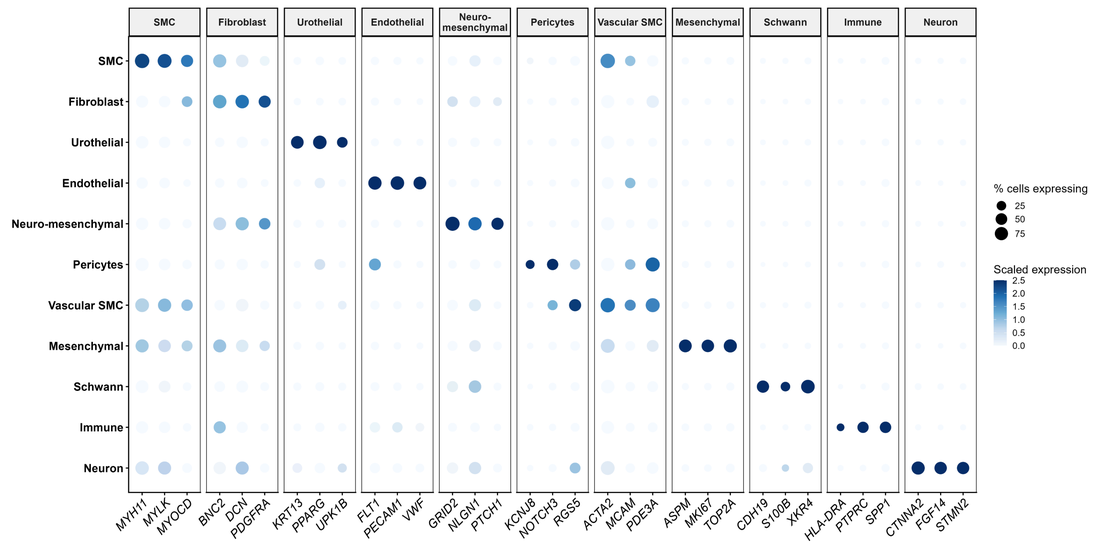


Figure 3: Dot plot representation of selected marker genes. Major cell types in the developing bladder were annotated based on canonical markers. Notably, pericytes and vascular SMC (vSMC) shared similar marker genes; however, vSMC additionally expressed myogenic genes. Neuro-mesenchymal cells expressed both neural and fibroblast-like genes representing a cluster largely unexplored in the developing bladder.

Results: Pseudobulk analysis compared congenital BOO to control

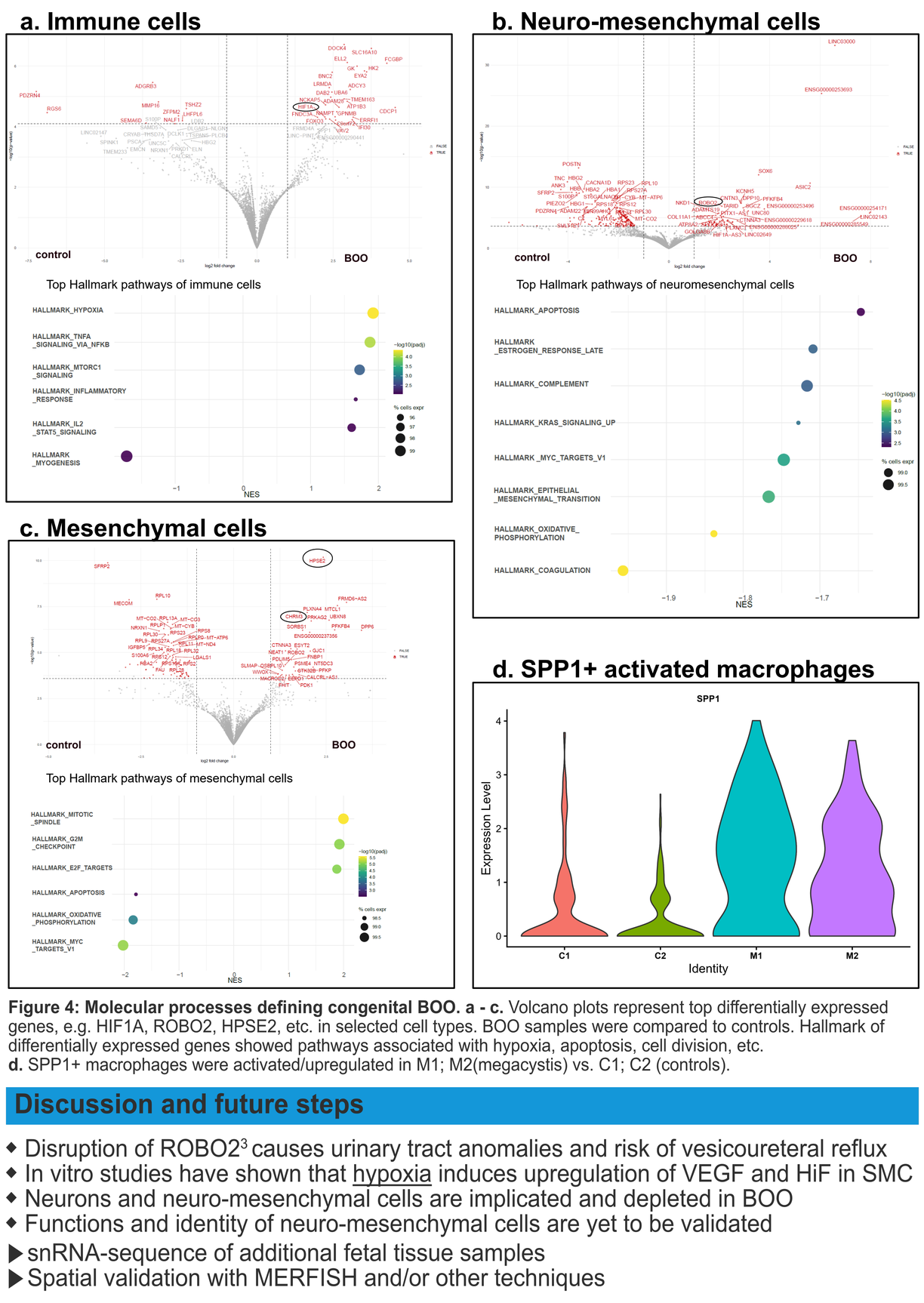


Figure 4: Molecular processes defining congenital BOO. a - c. Volcano plots represent top differentially expressed genes, e.g. HIF1A, ROBO2, HPSE2, etc. in selected cell types. BOO samples were compared to controls. Hallmark of differentially expressed genes showed pathways associated with hypoxia, apoptosis, cell division, etc.
d. SPP1+ macrophages were activated/upregulated in M1; M2(megacystis) vs. C1; C2 (controls).

Discussion and future steps

- Disruption of ROBO2[3] causes urinary tract anomalies and risk of vesicoureteral reflux
- In vitro studies have shown that hypoxia induces upregulation of VEGF and HiF in SMC
- Neurons and neuro-mesenchymal cells are implicated and depleted in BOO
- Functions and identity of neuro-mesenchymal cells are yet to be validated

▶ snRNA-sequence of additional fetal tissue samples
▶ Spatial validation with MERFISH and/or other techniques

Key takeaways

- Identification of mesenchymal and neuro-mesenchymal cells in the developing bladder
- Verification of known genes from mouse studies of BOO
- Illustration of unprecedented insights on the developmental processes of BOO
- Impaired SMC, immune upregulation, and upregulation of key genes: HPSE2[1] & ROBO2

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