Mapping Progression of DKD in ReninAAV UNx db/db mice utilizing time-series RNA sequencing

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Background & Aim

The adeno-associated virus-mediated renin (ReninAAV) overexpressing and uninephrectomized (UNx) *db/db* mouse model of diabetic kidney disease (DKD) recapitulates hallmarks of DKD complicated by hypertension. Gaps persist in our understanding regarding temporal patterns of glomerular gene expression and pathophysiology during hypertensive DKD. The present longitudinal study therefore characterized glomerular transcriptome changes during disease progression in the ReninAAV-UNx *db/db* mouse.

Methods

Female *db/db* (BKS.Cg-Dock7m +/+ Leprdb/J) received a single i.v. dose of murine renin-encoding AAV. Untreated *db*/m mice (BKS.Cg-Dock7m +/+ Leprdb/J) served as healthy controls. One week after the AAV injection, ReninAAV mice underwent UNx and subsequently randomized and stratified into 4 groups according to fed blood glucose levels and body weight measured 3 weeks post-UNx. ReninAAV UNx mice were terminated at 4, 8, 12 or 16 weeks (week 1-week 12) after UNx. *db*/m mouse controls were terminated at 12 weeks. Endpoints included urine and plasma biochemistry, kidney histology (Col1a1 and glomerulosclerosis scoring) and RNA sequencing of glomeruli isolated by laser-capture microdissection (LCM).

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1 Study design

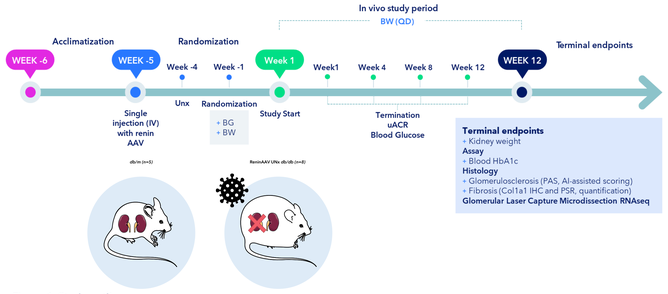

Figure 1. Study outline

2 Progression of diabetes

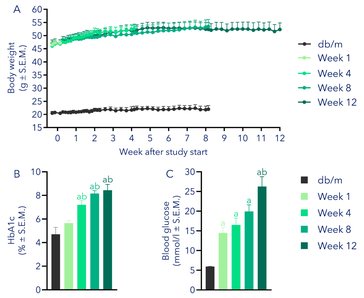

Figure 2. ReninAAV-UNx *db/db* mice develop a progressive, robust diabetic phenotype alongside obesity. A) Mean body weight (g ± S.E.M) **B)** HbA1c (% ± S.E.M.). **C)** Blood glucose (mmol/l ± S.E.M.). a, p<0.05 compared to *db*/m; b, p<0.05 compared to Week 1.

3 Histological hallmarks of progressive DKD

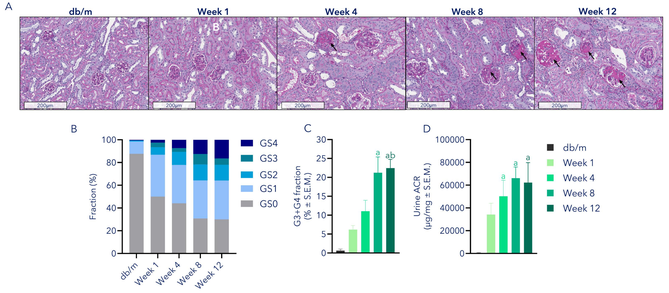

Figure 3. Progressive worsening of DKD hallmarks in ReninAAV-UNx db/db mice. (A) Representative images of kidney histological slides stained with PAS in ReninAAV-UNx db/db mice compared to healthy *db*/m control mice. Arrows indicate PAS-stained sclerosed glomeruli. **(B)** Group-wise distribution (fraction %) of glomerulosclerosis scores. **(C)** Fraction of glomeruli showing severe or global glomerulosclerosis (GS3+4). (D) Urine Albumin-to-Creatinine ratio (ACR). a, p<0.05 compared to *db*/m; b, p<0.05 compared to Week 1.

4 Progressive changes disease-associated signaling pathways

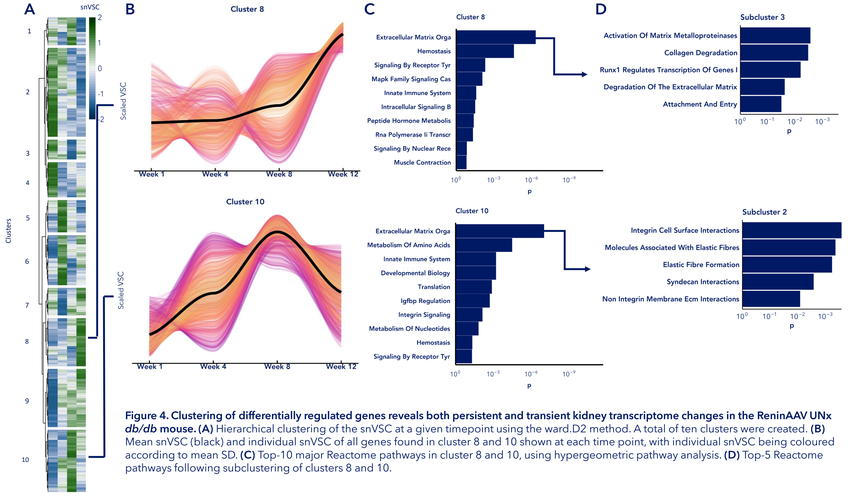

Figure 4. Clustering of differentially regulated genes reveals both persistent and transient kidney transcriptome changes in the ReninAAV UNx *db/db* mouse. (A) Hierarchical clustering of the snVSC at a given timepoint using the ward.D2 method. A total of ten clusters were created. **(B)** Mean snVSC (black) and individual snVSC of all genes found in cluster 8 and 10 shown at each time point, with individual snVSC being coloured according to mean SD. **(C)** Top-10 major Reactome pathways in cluster 8 and 10, using hypergeometric pathway analysis. **(D)** Top-5 Reactome pathways following subclustering of clusters 8 and 10.

5 Temporal variation in selected renal cell type markers

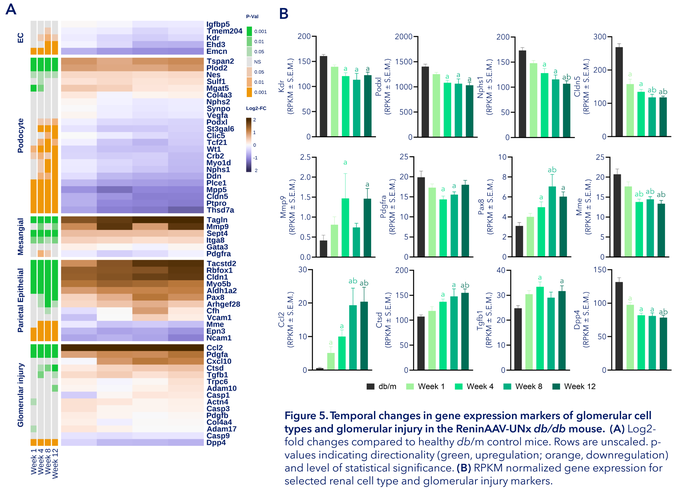

Figure 5. Temporal changes in gene expression markers of glomerular cell types and glomerular injury in the ReninAAV-UNx *db/db* mouse. (A) Log2-fold changes compared to healthy *db*/m control mice. Rows are unscaled. p-values indicating directionality (green, upregulation; orange, downregulation) and level of statistical significance. **(B)** RPKM normalized gene expression for selected renal cell type and glomerular injury markers.

Conclusion

- The ReninAAV-UNx *db/db* mouse model of hypertensive DKD demonstrates progressive glomerulosclerosis and severe albuminuria
- Temporal shifts in glomerular gene expression parallel the progressive glomerular disease phenotype
- The identification of temporal patterns of gene expression provides novel insights into the underlying molecular pathology of DKD
- Our data underscores the ReninAAV-UNx *db/db* mouse as an applicable, progressive model of human DKD in preclinical target and drug discovery

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