# Transcriptomic profiling of renal cortical and glomerular changes in a uninephrectomized, hypertension-accelerated mouse model of advanced diabetic kidney disease

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## BACKGROUND AND AIM

Our current understanding of molecular mechanisms involved in diabetic kidney disease (DKD) progression is challenged by the complex structure of the kidney and the specialized functions of the nephron.

To define molecular mechanisms involved in DKD progression and identify novel therapeutic targets, we determined glomerular and renal cortical gene expression profiles using RNA sequencing and single-nucleus RNAseq in a uninephrectomized, hypertension-accelerated *db/db* mouse model of advanced DKD.

## STUDY OUTLINE

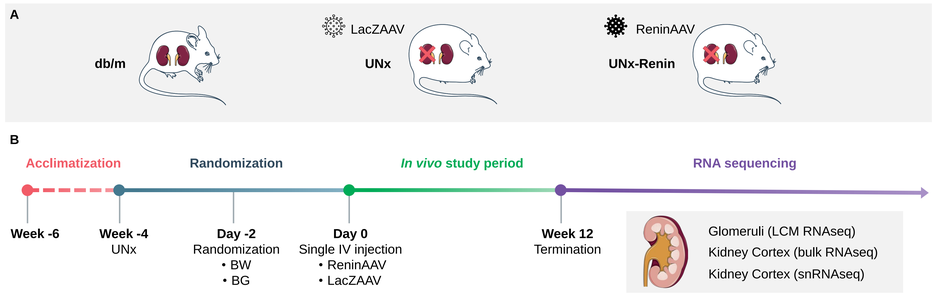


**Figure 1. Graphic illustration of the study outline. (A)** Study groups. **(B)** Study outline. The top and bottom parts of the kidney cortex were sequestered for bulk and single-nucleus RNA sequencing. The remaining part of the kidney cortex was cryosectioned and glomeruli were isolated using laser-capture microdissection. AAV, adeno-associated virus; BG, blood glucose; BW, body weight; LCM, laser-capture microdissection; RNAseq, RNA sequencing; snRNAseq, single-nucleus RNA sequencing; UNx, uninephrectomy.

## Worsening of metabolic and biochemical parameters in UNx-Renin mice

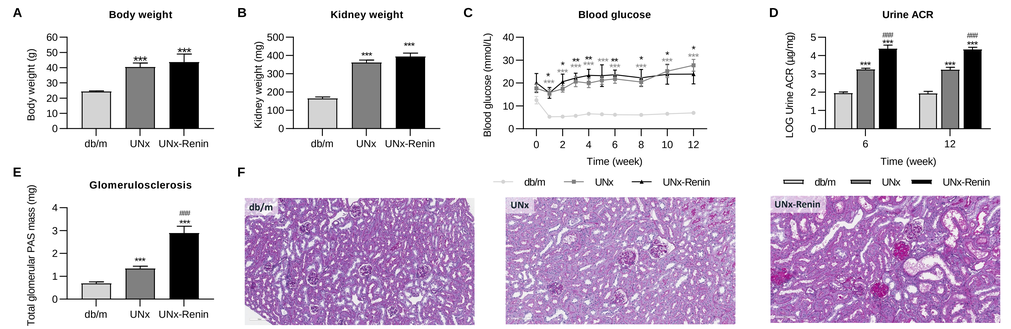


**Figure 2. Measurements 12 weeks after injection with ReninAAV or LacZAAV in diabetic UNx mice and age-matched db/m controls. (A)** Body weight. **(B)** Kidney weight. **(C)** Blood glucose measured biweekly throughout the study. **(D)** LOG10-transformed urine albumin-to-creatinine ratio (ACR) at week 6 and 12 in the study. **(E)** Quantification of glomerulosclerosis. **(F)** Representative images of PAS-stained kidney sections. Data is presented as mean ± SEM (n = 5-13). One-way ANOVA with Tukey's post hoc test (Fig. 1A, B and E) or two-way ANOVA with Bonferroni's post hoc test (Fig. 1C and D). *:P < 0.05, **:P < 0.01, ***: P < 0.001 compared to db/m. ###: P < 0.001 compared to UNx.

## Highly specific gene expression profiles in glomeruli versus kidney cortex

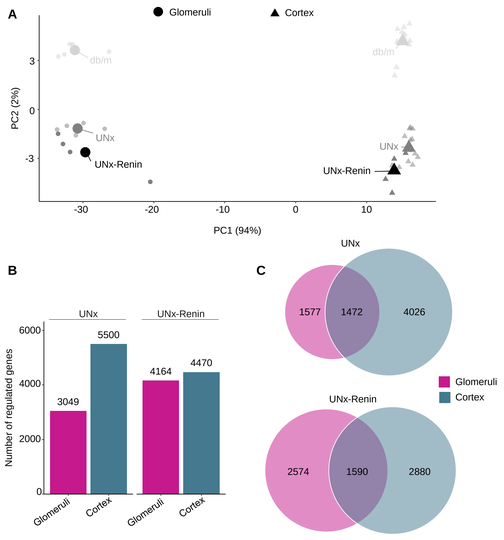


**Figure 3. Highly specific gene expression profiles in glomeruli versus kidney cortex for UNx and UNx-Renin mice. (A)** Principal component (PC) analysis of the 500 most variable genes. Small points indicate a sample and large points the group centre. **(B)** Total number of differentially expressed genes (DEGs) in glomeruli and kidney cortex from UNx and UNx-Renin mice compared with db/m controls, respectively. **(C)** Venn diagrams depicting shared and separate DEGs in glomeruli and kidney cortex from UNx or UNx-Renin mice (n=5-13).

## Gene enrichment analysis in glomeruli and kidney cortex from UNx or UNx-Renin mice

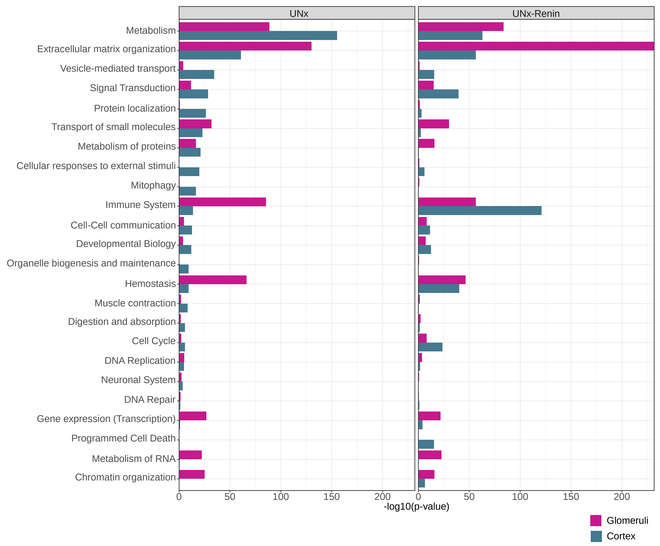


**Figure 4. Gene enrichment analysis in glomeruli and kidney cortex from UNx or UNx-Renin mice**. Reactome pathway gene enrichment analysis in glomeruli and kidney cortex from UNx or UNx-Renin mice. Degree of perturbation is presented as the −log10(p-value) after correction for gene-wise multiple testing (n=5-13).

## Single-nucleus RNA sequencing reveal cell-specific regulations in DKD pathogenesis

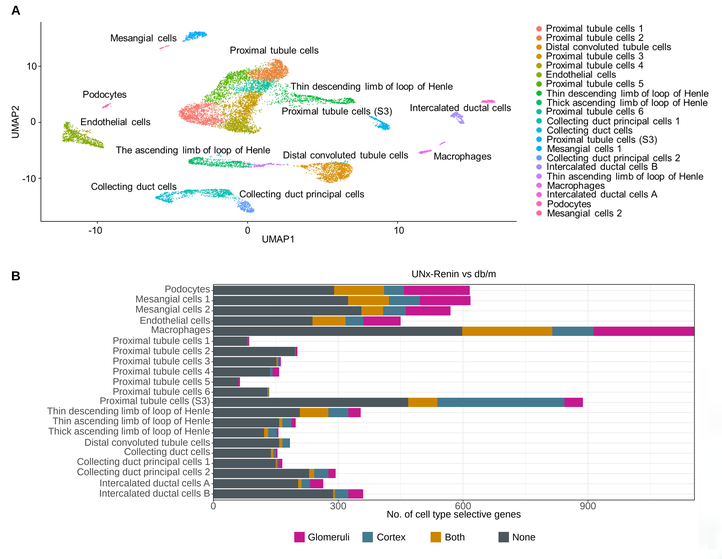


**Figure 5. Number of differentially expressed genes in cortex and glomeruli of UNx-Renin mice mapped to a specific cell type using single-nucleus RNAseq. (A)** UMAP projection of 12.840 nuclei from kidney cortex of db/m and UNx-Renin mice (n = 1-2). Each dot represents a nucleus coming from a single cell. **(B)** Number of cell type specific genes significantly regulated between UNx-Renin and db/m mice in glomeruli, cortex, both or none of the two tissue areas. Genes were defined as specific to a cell population, if the expression was increased by 2-fold as compared to the cell population with the second highest expression level.

## Regulation of podocyte-specific genes in glomeruli and kidney cortex

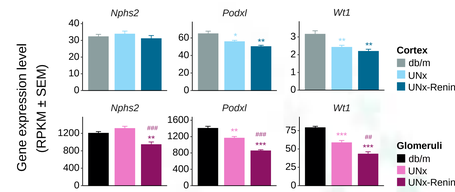


**Figure 6. Regulation of podocyte specific genes in kidney cortex and glomeruli of UNx-Renin mice.** Expression levels presented as mean ± SEM RPKM values. *:P < 0.05, **:P < 0.01, ***: P < 0.001 compared to db/m. ##:P < 0.01, ###: P < 0.001 compared to UNx (false discovery rate adjusted p-values).

## CONCLUSIONS

+ ReninAAV administration in UNx *db/db* mice increases glomerulosclerosis and urine ACR.
+ RNAseq of isolated glomeruli identifies transcriptome changes not found in kidney cortex.
+ Combining single-nucleus RNAseq with bulk gene expression profiling can link gene expression changes to specific cell types.
+ This approach provides a strong tool to identify novel disease-related genes and pathways in specific cell types.