Whole-kidney 3D imaging and transcriptome assessment in the UNx db/db (renin-AAV) mouse model of diabetic nephropathy

Brandon B. Boland, Frederikke E. Sembach, Lisbeth N. Fink, Urmas Roostalu, Jacob Lerche Skytte, Thomas Secher, Keld Fosgerau, Jacob Hecksher-Sørensen, Jacob Jelsing, Niels Vrang, and Mette V. Østergaard
Gubra, Hørsholm, Denmark
Corresponding author: mvo@gubra.dk

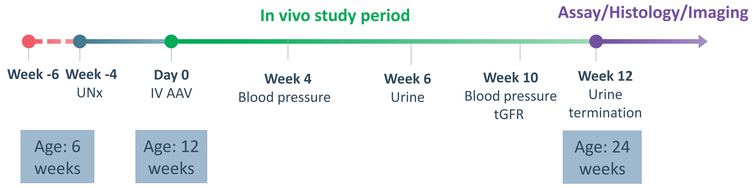
BACKGROUND AND AIMS

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease and is associated with increased cardiovascular risk and shortened survival. With the exception of recent data supporting the use of SGLT-2 inhibitors and GLP-1R agonists in DN, the therapeutic landscape has remained almost unchanged for decades, largely due to the lack of translatable preclinical models.

In this study, we assessed renal changes in uninephrectomized (UNx) C57BLKS/J db/db mice overexpressing renin using light sheet microscopy, histology and RNA sequencing to better define the structural and functional changes associated with progression of advanced DN.

STUDY DESIGN

UNx was performed on 8 wk old female C57BLKS/J db/db mice, while db/m mice served as controls. ReninAAV or LacZAAV were IV injected via tail vein 4 weeks following UNx (study day 0). ReninAAV was injected at 1X, 2X, or 4X10^{10} GC. Blood pressure was assessed week 4 and 10 via tail cuff measurement. Transdermal GFR was assessed via FITC-Sinistrin and transdermal recording during week 10, and albuminuria assessed week 6 and 12. Animals were terminated week 12 and injected with lectin-594 5 minutes prior to termination to visualize glomerular morphology from the entire kidney using light sheet fluorescent microscopy. Terminal plasma biochemistry, kidney histology, AI-assisted glomerular identification and quantification of glomerulosclerosis, and kidney cortex RNAseq were performed.



RESULTS

ReninAAV-induced hypertension does not influence body weight

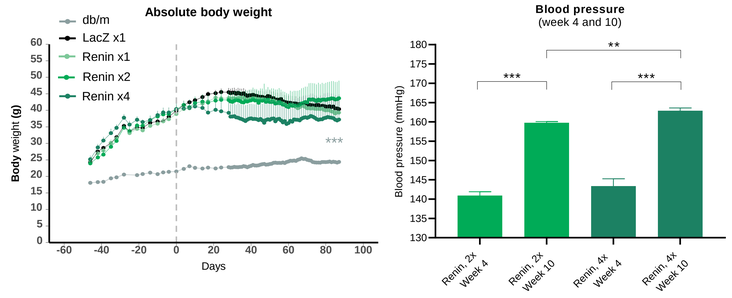


Figure 1| Absolute body weight during the study and tail-cuff blood pressure measurements at weeks 4 and 10 in the ReninAAV 2X and 4X groups.
Absolute Body weight: *** p ≤ 0.001 vs LacZ, N=3-13.
Hypertension: ** p ≤ 0.01 between groups, *** p ≤ 0.001 within groups, N=5-6.

ReninAAV increases UACR and kidney weight, but does not affect GFR

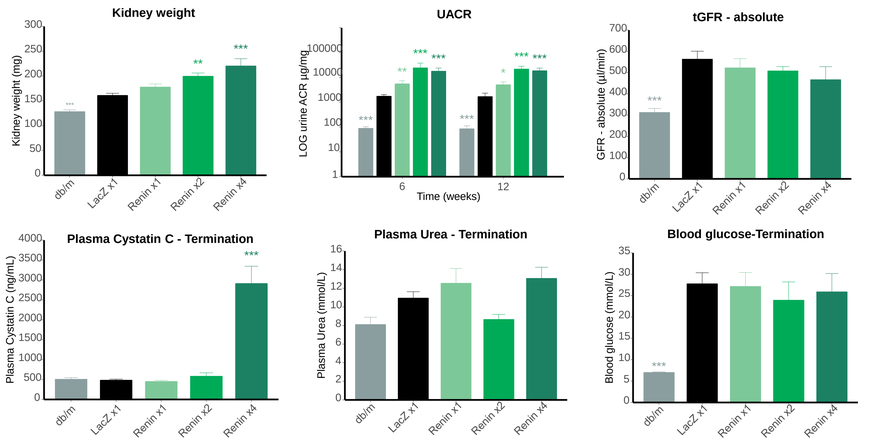


Figure 2 |Urine ACR, blood glucose, plasma cystatin C, plasma urea, tGFR and terminal liver and heart weights. * p ≤ 0.05 vs LacZ ** p ≤ 0.01 vs LacZ *** p ≤ 0.001 vs LacZ , N=4-11.

Light sheet microscopic quantification of UNx kidneys reveals glomerular hypertrophy

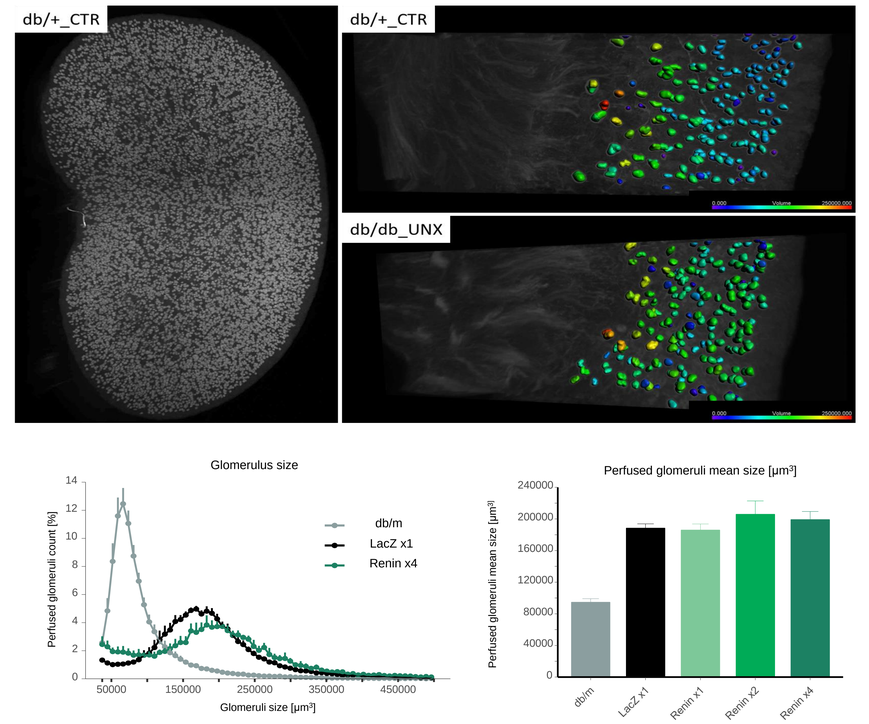


Figure 3 | Detection of total glomeruli number from lectin_594 injected db/+ mouse kidney scanned using light sheet microscopy. Representative color-coded volume rendering of individual glomeruli shows a shift in size (blue-green-red) of glomeruli in db/db_UNX kidneys as compared to the db/+ controls. Calculation of the size of all glomeruli in each kidney revealed a clear shift in glomerulus volume in hypertensive db/db UNx animals. N=3-13.

AI-assisted quantification reveals dose-dependently increased glomerulosclerosis in ReninAAV mice

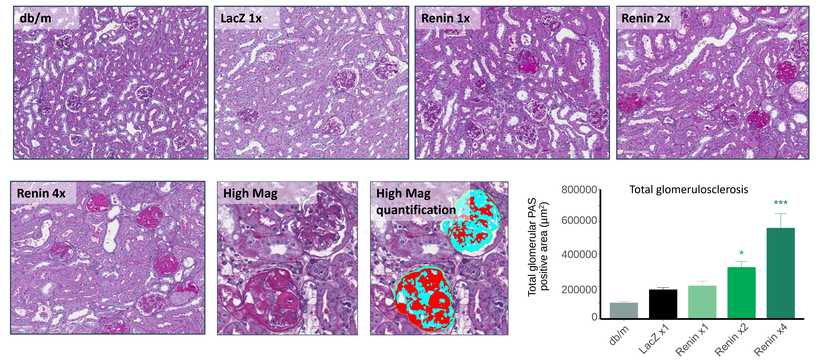


Figure 4 | AI-assisted identification of PAS-stained glomeruli and subsequent quantification of PAS positive area within total glomerular area. Red = PAS positive area; Blue = Remaining glomerular tissue. * p ≤ 0.05 vs LacZ ,*** p ≤ 0.001 vs LacZ N=3-13.

Kidney cortex RNAseq reveals altered expression of pathological genes in ReninAAV mice

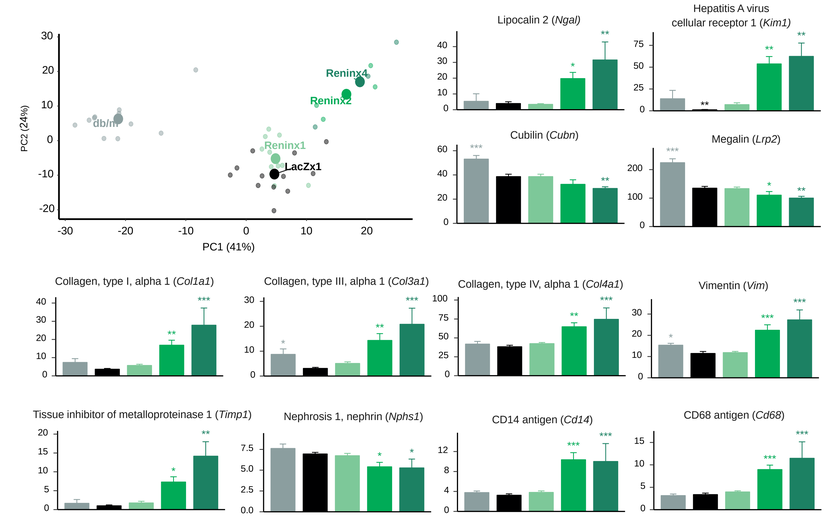


Figure 5 | PCA plot of the 500 most variable genes. RPKM values for genes related to kidney injury (*Ngal*, *Kim1*), tubular reuptake (*Cubn*, *Lrp2*), fibrotic fiber formation (*Col1a1, Col3a1, Col4a1*, *Vim*), ECM remodeling (*Timp1*), podocyte markers (*Nphs1*), and inflammation (*Cd14, Cd68*) are shown.
* p ≤ 0.05 vs Renin1X ** p ≤ 0.01 vs Renin1X *** p ≤ 0.001 vs Renin 1X after correction for gene-wise multiple testing. N=4-13.

CONCLUSION:

ReninAAV administration in uninephrectomized C57BLKS/J *db/db* mice dose-dependently induces glomerulosclerosis, increases urine ACR, and alters kidney cortex gene expression of pathological genes, indicating a viable translatable model of advanced human diabetic nephropathy.