Novel Rat Model of Diabetic Nephropathy Displaying Tubular Fibrosis and Glomerular Alterations

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INTRODUCTION AND AIM

Diabetic nephropathy (DN) is a long-term complication of diabetes characterized by proteinuria and loss of kidney function. Up to one third of patients with diabetes develop DN, and no new therapies targeting DN have been introduced for more than a decade. The lack of treatment options may in part be caused by the scarcity of translatable rodent models.

To address these challenges, we aimed to develop a novel surgery-induced rat model of DN by 90% pancreatectomy (Px) and uni-nephrectomy (UNx).

STUDY DESIGN AND GROUPS

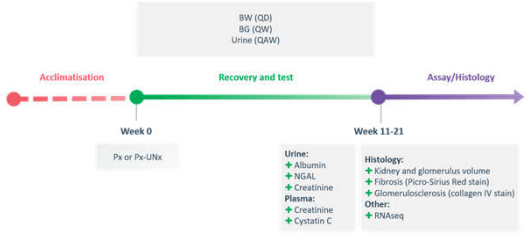


Figure 1 | Male rats underwent 90% pancreatectomy (Px) alone or combined with unilateral nephrectomy (UNx) of the left kidney. Body weight and blood glucose was recorded for 11-21 weeks, with spot urine collected every other week. At termination, plasma was collected for biochemical analyses, while the right kidney was sampled for histological analyses and next generation RNA sequencing.

Color	Group name	N	Surgery	Post-surgery duration
●	Ctrl	6	None	3 weeks
●	Px 11 wks	7	Pancreatectomy	11 weeks
●	Px 21 wks	8	Pancreatectomy	21 weeks
●	Px-UNx 11 wks	8	Pancreatectomy and uni-nephrectomy	11 weeks

RESULTS

Plasma markers indicated reduced GFR in Px-UNx rats

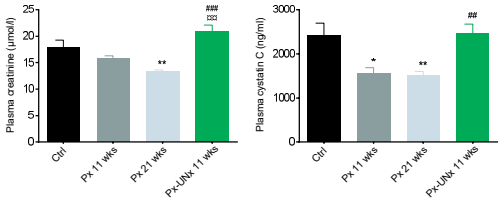


Figure 1 | Plasma creatinine and cystatin C at termination. Data are mean + SEM. One-way ANOVA with Tukey's post hoc test; *p<0.05, **p<0.01 vs Ctrl; ¤¤p<0.01 vs Px 11 wks; ##p<0.01, ###p<0.001 vs Px 21 wks.

Diabetes developed similarly in Px and Px-UNx rats

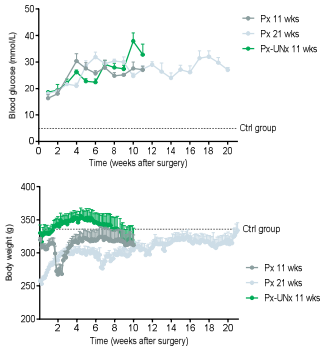


Figure 2 | Weekly blood glucose (non-fasted) and daily body weight throughout the study. Px and Px-UNx rats were considered diabetic and included in study if non-fasted blood glucose was >12 mmol/L within 14 days after surgery. Data are mean + SEM.

Px-UNx increased urinary albumin and NGAL excretion

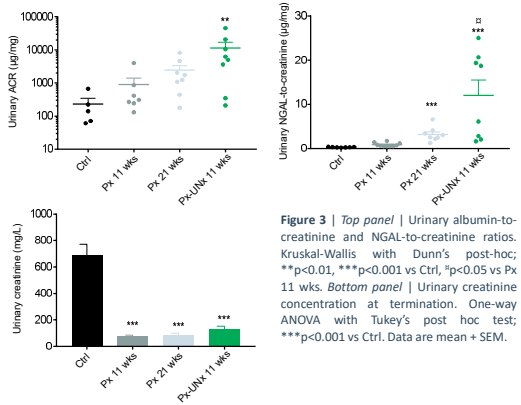


Figure 3 | *Top panel* | Urinary albumin-to-creatinine and NGAL-to-creatinine ratios. Kruskal-Wallis with Dunn's post-hoc; **p<0.01, ***p<0.001 vs Ctrl, ¤p<0.05 vs Px 11 wks. *Bottom panel* | Urinary creatinine concentration at termination. One-way ANOVA with Tukey's post hoc test; ***p<0.001 vs Ctrl. Data are mean + SEM.

Px-UNx caused renal and glomerular hypertrophy

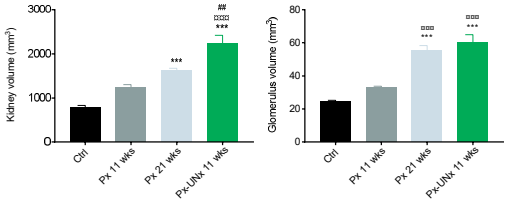


Figure 4 | Stereological volume estimates of the right kidney and glomeruli. Data are mean + SEM. One-way ANOVA with Tukey's post hoc test; ***p<0.001 vs Ctrl; ¤¤¤p<0.001 vs Px 11 wks; ##p<0.01 vs Px 21 wks.

Px-UNx caused renal fibrosis

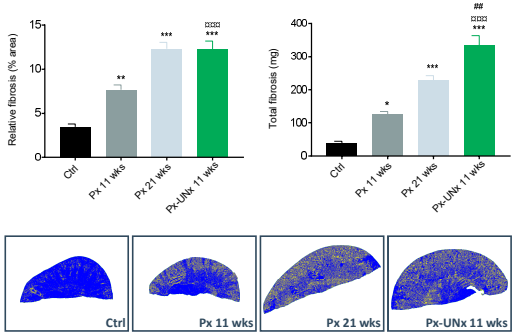


Figure 5 | Relative and total fibrosis of right kidney at termination with representative images of fibrosis quantification on Picro-Sirius red stained kidney sections. Data are mean + SEM. One-way ANOVA with Tukey's post hoc test; *p<0.05, **p<0.01, ***p<0.001 vs Ctrl; ¤¤¤p<0.001 vs Px 11 wks; ##p<0.01 vs Px 21 wks.

Px/Px-UNx surgery altered gene expression in renal cortex

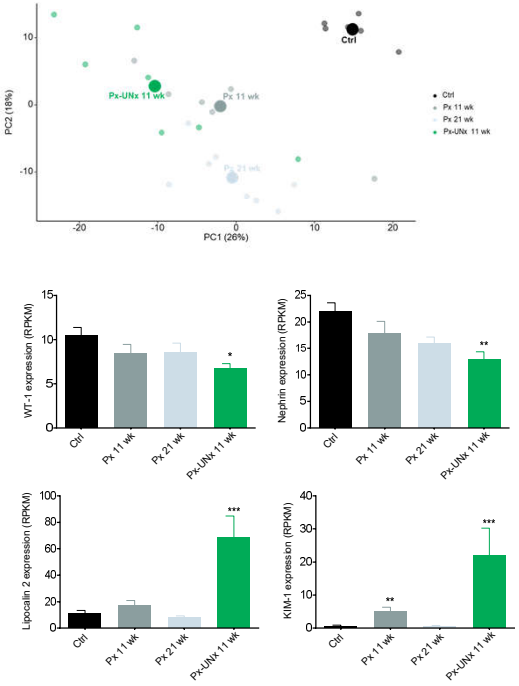
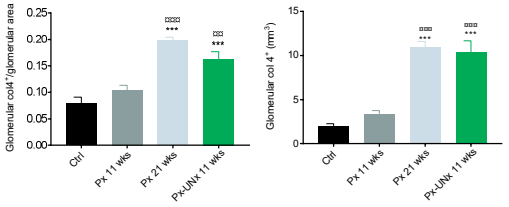


Figure 6 | *Top panel* | Principal component analysis (PCA) of the 500 most variable renal cortex genes as assessed by next generation sequencing. *Bottom panel* | Renal cortex expression of selected genes. Data are mean + SEM. *p<0.05, **p<0.01, ***p<0.001 vs Ctrl after correction for gene-wise multiple testing.

Px-UNx displayed increased glomerulosclerosis



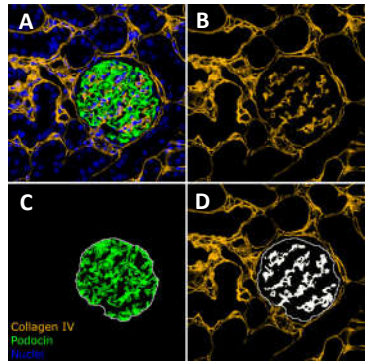


Figure 7 | Glomerulosclerosis quantification in podocin and collagen IV double-strained kidney sections. A | Podocin and collagen IV overlay. B | Collagen IV stain alone. C | Podocin stain alone and definition of region on interest (ROI). D | Quantification of glomerular collagen IV area inside ROI. Data are mean + SEM. One-way ANOVA with Tukey's post hoc test; ***p<0.001 vs Ctrl; ¤¤¤p<0.01, ¤¤¤¤p<0.001 vs Px 11 wks.

CONCLUSION

- ✓ **We present a novel combined Px-UNx rat model of DN characterized by:**
  - **Increased urinary albumin and NGAL excretion**
  - **Renal hypertrophy, increased renal fibrosis, and clear evidence of glomerulosclerosis**
  - **Altered gene expression in the renal cortex supporting a DN phenotype**
- ✓ **This advanced surgical approach represents a strong and short-duration alternative to streptozotocin-induced diabetes and genetic models (e.g. ZDF rats) for preclinical studies of DN drug candidates**