Time course of hypertensive diabetic kidney disease in uninephrectomized *db/db* mice: Progressive albuminuria, glomerulosclerosis, and gene regulation

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Background & Aim

Preclinical models that combine diabetes with hypertension and impaired kidney function is a good tool to recapitulate progressive diabetic kidney disease (DKD). Here, we profiled disease progression in a translational mouse model of DKD, induced by renin overexpression combined with uninephrectomy in *db/db* mice.

Methods

Female *db/db* (BKS.Cg-Dock7m +/+ Leprdb/J) mice, 11–12 weeks of age, were administered a single i.v. dose of renin-encoding AAV in week -5. Age-matched untreated *db/+* (BKS.Cg-Dock7m +/+ Leprdb/J) mice and untreated *db/db* mice served as healthy controls. In week -4, *db/db* ReninAAV mice underwent uninephrectomy (UNx). *db/db* ReninAAV-UNx (DKD) mice were randomized into four groups based on urine albumin-to-creatine ratio (uACR), fed blood glucose levels and body weight measured in week -1. The study was initiated 4 weeks after UNx and the *db/db* ReninAAV-UNx mice were terminated at day 1, week 4, week 8, and week 12. Untreated *db/db* mice and healthy db/+ mice were terminated at week 12, serving as controls. Study endpoints included urine biochemistry, and renal histopathology (glomerulosclerosis, fibrosis, tubular injury and inflammation) and kidney bulk RNA sequencing.

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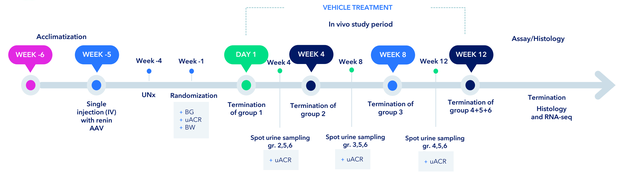
1 Model study design and protocol



Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing volume
1	*db/db* ReninAAV-UNx day 1	Female	9	**Vehicle**	SC	Once daily	5 ml/kg
2	*db/db* ReninAAV-UNx week 4	Female	8	**Vehicle**	SC	Once daily	5 ml/kg
3	*db/db* ReninAAV-UNx week 8	Female	10	**Vehicle**	SC	Once daily	5 ml/kg
4	*db/db* ReninAAV-UNx week 12	Female	10	**Vehicle**	SC	Once daily	5 ml/kg
5	*db/db* control week 12	Female	11	**Vehicle**	SC	Once daily	
6	*db/+* control week 12	Female	9	**Vehicle**	SC	Once daily	

Figure 1. Study outline and protocol.

2 Metabolic phenotype and albuminuria

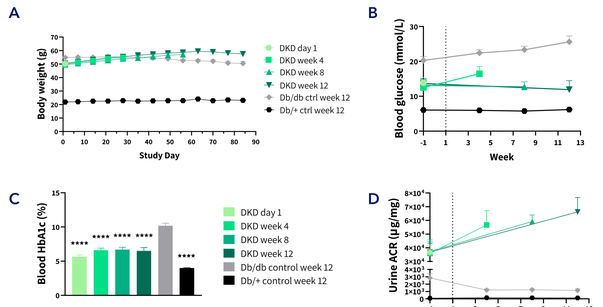


Figure 2. *db/db* ReninAAV-UNx mice display progressive hyperglycemia and albuminuria. (A) Mean body weight profile. **(B)** Fed blood glucose profile. **(C)** Quantification of blood HbA1c at termination. **(D)** Urine albumin-to-creatinine ratio (uACR) profile. Values expressed as mean of n = 7-11 + SEM. Dunnett's test one-factor linear model. ********p<0.0001 compared to db/db control week 12 mice.

3 Kidney dysfunction and structural remodelling

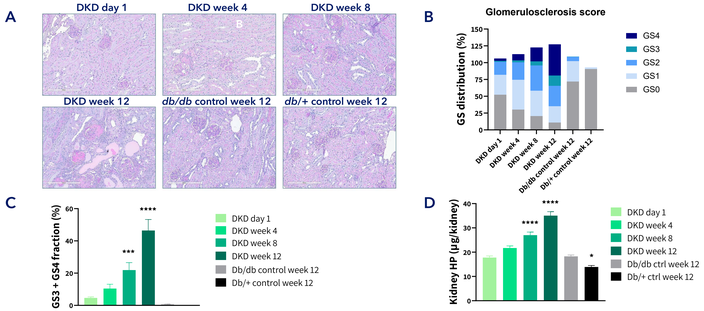


Figure 3. *db/db* ReninAAV-UNx mice display progressive, advanced glomerulosclerosis. (A) Representative images of kidney sections stained with Periodic acid-Schiff (PAS). **(B)** Distribution of glomerulosclerosis score (GS). The percentage of animals with score 0 (normal), score 1 (up to 25% involvement), score 2 (up to 50% involvement), score 3 (up to 75% involvement), and score 4 (global, more than 75% involvement). **(C)** Fraction (%) of score 3 (GS3) and 4 (GS4). **(D)** Quantification of total kidney hydroxyproline (HP). Values expressed as mean of n = 8-11 + SEM. Dunnett's test one-factor linear model. *****: P < 0.05 compared to Db/db control week 12 mice.

4 Progressive kidney fibrosis, inflammation and tubular injury

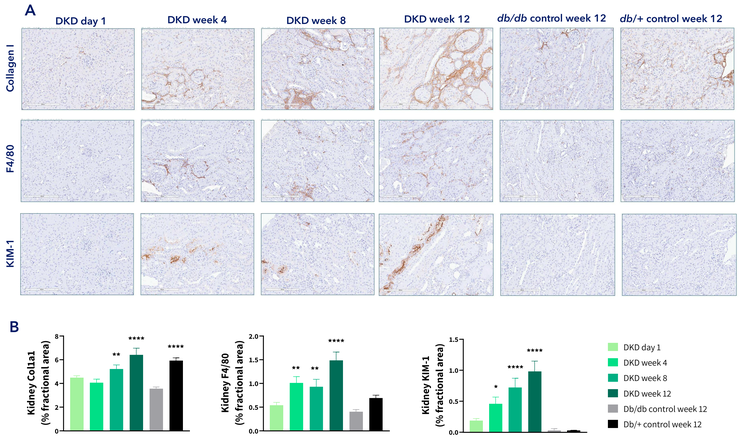


Figure 4. *db/db* ReninAAV-UNx mice display progressive kidney fibrosis, inflammation and tubular injury. (A) Representative images of right kidney Col1a1, F4/80 and KIM-1 expression during disease progression (scale bar, 100 µm). **(B)** Quantitative histological assessment of right kidney Col1a1, F4/80 and KIM-1. Values expressed as mean of n = 8-11 + SEM. Dunnett's test one-factor linear model. *****: P < 0.05 compared to *db/db* control mice.

5 Time-dependent transcriptomic changes during DKD progression

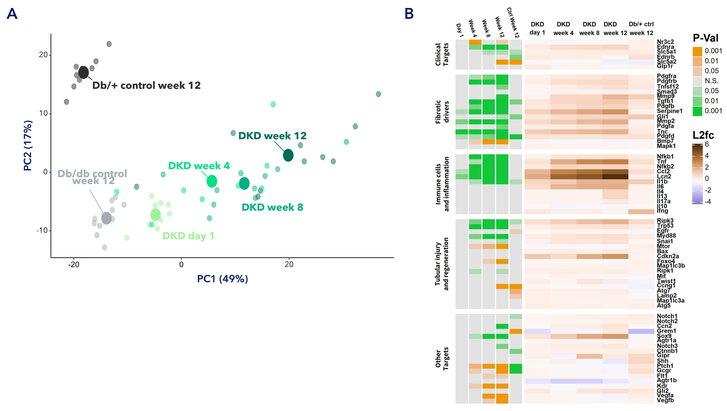


Figure 5. Bioinformatics analysis on kidney samples for differential gene expression and pathway enrichment analysis. (A) Principal component analysis based on the top 500 most varying genes across the dataset. The axes describe the percentage of variability explained by the principal component 1 (PC1) and principal component 2 (PC2). Each small point represents a sample, and each big point represents the study group average. **(B)** Heatmap of gene expression of CKD drug targets. Log2-fold change in gene expression of CKD drug targets from Db/+ control 12 weeks mice to DKD animals over time. Rows are unscaled. Drug targets are separated into several groups, based on putative function.

Conclusion

The *db/db* ReninAAV-UNx mouse model of hypertension-accelerated DKD exhibits:

+ Severe development of albuminuria
+ Progressive kidney histopathology showing glomerulosclerosis, macrophage infiltration, tubular injury and fibrosis
+ Time-dependent dynamic changes in renal gene expression

In conclusion, the *db/db* ReninAAV-UNx mouse model resembles human DKD and is a valuable tool in preclinical target discovery and drug development

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