A short-term adenine diet–induced mouse model of progressive renal dysfunction and chronic kidney disease

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Background & Aim

Translational models are essential to identify improved treatment options for chronic kidney disease (CKD) patients. However, most preclinical CKD models do not demonstrate reduced glomerular filtration rate (GFR).

Here, we characterized the temporal development of functional, histopathological, and molecular changes during a short-term 3-week ADI protocol to define the progression of disease and its suitability for preclinical drug evaluation.

Methods

See the study outline. Male C57BL/6JRj mice (11 weeks old) were randomised into study groups based on body weight. From day 1, control mice received control diet (S9352-E064, Ssniff, Germany), and adenine mice received 0.2% adenine (S9352-E060, Ssniff, Germany). Adenine diet-induced (ADI) mice were either treated with vehicle (PO, QD). At week 1, 2, and 3, urine albumin-to-creatinine ratio (uACR) was measured and at week 2 and 3 the glomerular filtration rate (GFR) was measured and calculated based on T1/2 of FITC-sinistrin clearance. Terminal plasma was collected for evaluation of urea and creatinine levels. The left kidney was weighed and processed for histomorphometric assessment of fibrosis (α-SMA, Col1a1), tubular injury (KIM-1) and macrophage infiltration (F4/80). Bulk RNA sequencing was performed on kidney samples for differential gene expression and pathway enrichment analysis.

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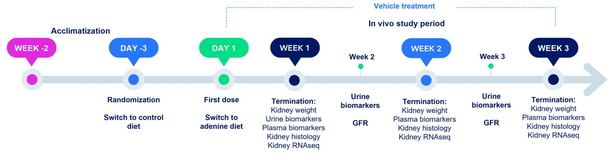
1 Study design and group overview



Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing volume
1	Control diet (Ctrl) week 3	Male	8	Vehicle	PO	Once daily	5 ml/kg
2	Adenine diet (ADI) week 1	Male	9	Vehicle	PO	Once daily	5 ml/kg
3	Adenine diet (ADI) week 2	Male	9	Vehicle	PO	Once daily	5 ml/kg
4	Adenine diet (ADI) week 3	Male	9	Vehicle	PO	Once daily	5 ml/kg

Figure 1. Study outline and group table

2 Kidney functional parameters

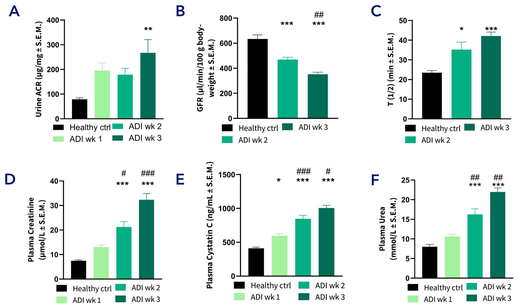


Figure 2. Kidney functional parameters. (A) Urine albumin-to-creatinine ratio (uACR). **(B)** Glomerular filtration rate (GFR). **(C)** Elimination half-life (T ½) for GFR. **(D, E, F)** Plasma creatinine, cystatin C and urea. Values expressed as mean of n = 8-9 + SEM. Dunnett's test one-factor linear model. *p<0.05, **p<0.01, ***p<0.001 compared to healthy controls. #p<0.05, ##p<0.01, ###p<0.001 vs ADI week 1 or 2.

3 Progressive kidney inflammation and tubular injury

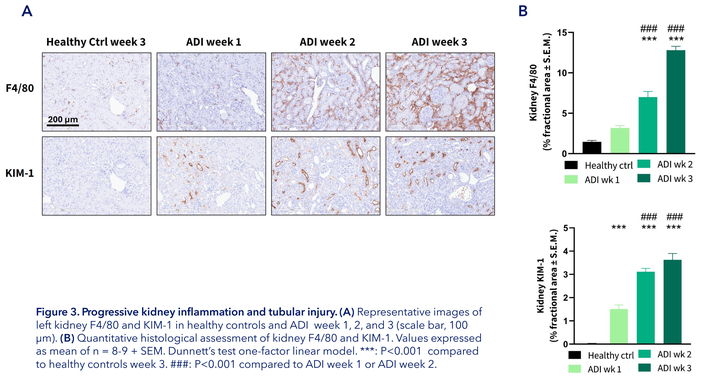


Figure 3. Progressive kidney inflammation and tubular injury. (A) Representative images of left kidney F4/80 and KIM-1 in healthy controls and ADI week 1, 2, and 3 (scale bar, 100 µm). **(B)** Quantitative histological assessment of kidney F4/80 and KIM-1. Values expressed as mean of n = 8-9 + SEM. Dunnett's test one-factor linear model. ***: P<0.001 compared to healthy controls week 3. ###: P<0.001 compared to ADI week 1 or ADI week 2.

4 Progressive fibrosis development

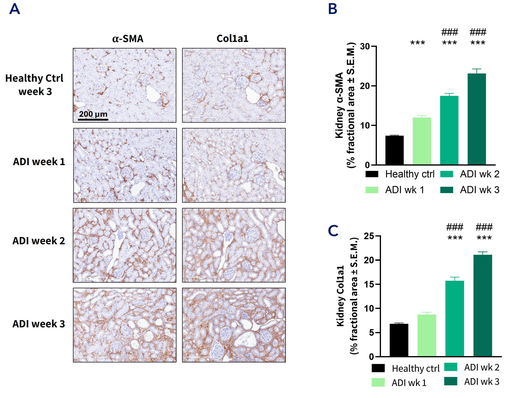


Figure 4. Title. (A) Representative images of left kidney α-SMA and Col1a1 in healthy controls and ADI week 1, 2, and 3 (scale bar, 100 µm). **(B)** Quantitative histological assessment of kidney α-SMA and Col1a1. Values expressed as mean of n = 8-9 + SEM. Dunnett's test one-factor linear model. ***: P<0.001 compared to healthy controls week 3. ###: P<0.001 compared to ADI week 1 or ADI week 2.

5 Progressive dysregulation of genes associated with CKD

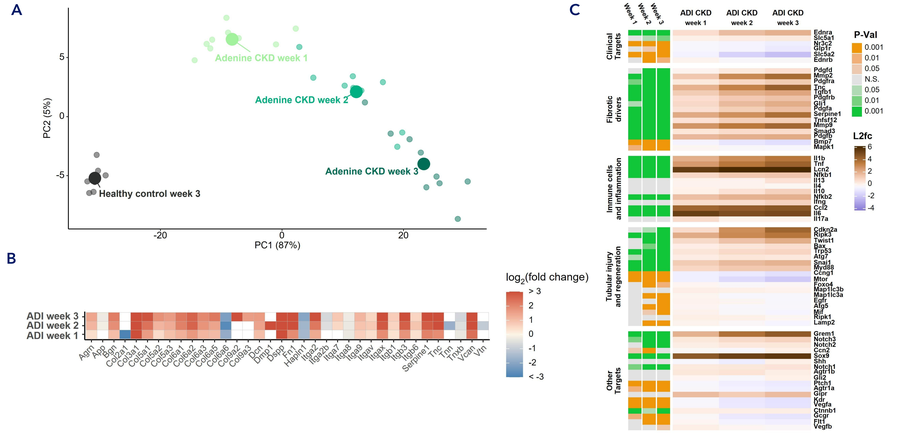


Figure 5. Progressive dysregulation of genes associated with CKD. (A) Principal component analysis based on the top 500 most varying genes. The axes describe the percentage of variability explained by the principal component 1 (PC1) and component 2 (PC2). Each small point represents a sample, and each big point represents the group average. **(B)** Heatmap of ECM proteoglycans pathway (Reactome). **(C)** Heatmap of gene expression of CKD drug targets. Log2-fold change in gene expression of CKD drug targets from controls to ADI mice over time. Rows are unscaled. Drug targets are separated into groups, based on putative function.

Conclusion

- The short-term ADI mouse model develops progressive CKD within 3 weeks
- Declining GFR was accompanied by increasing tubular injury, inflammation, and fibrosis
- RNAseq confirmed progressive dysregulation of CKD-associated signaling pathways
- The model provides a rapid platform for evaluating candidate drugs for CKD

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