Comparative tissue transcriptomics of human and mouse diabetic kidney disease

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BACKGROUND & AIM

Transcriptome profiling is important for defining molecular mechanisms involved in the progression of diabetic kidney disease (DKD). However, a major impediment in DKD research is the scarcity of frozen kidney tissue specimens from patients with DKD. To overcome this challenge, we applied RNA sequencing of formalin-fixed paraffin-embedded (FFPE) kidney samples from a prospective study in patients with DKD enabling assessment of the clinical translatability of a state-of-the-art mouse model of advanced DKD.

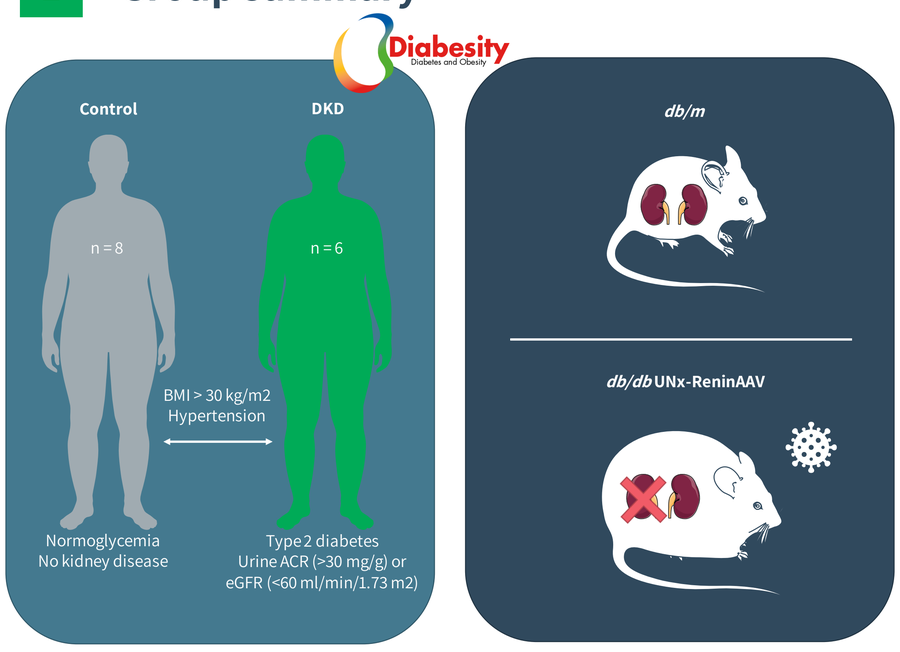
METHODS

RNA sequencing was performed on FFPE kidney samples from patients undergoing nephrectomy as part of the European Nephrectomy BioBank project. All patients included were diagnosed with obesity and hypertension, and were divided into two groups based on presence or absence of type 2 diabetes. Also, RNA sequencing was performed on snap-frozen kidney samples from female uninephrectomized (UNx) *db/db* mice that received adeno-associated virus (AAV)-mediated renin overexpression and non-diabetic *db/m* control mice.

CONCLUSION

- FFPE samples are fully applicable for RNA sequencing
- In DKD patients, renal gene regulations correlate with clinical diagnostic parameters (eGFR, plasma creatinine, HbA1c and urine ACR)
- Kidney transcriptome changes in DKD patients and *db/db* UNx-ReninAAV mice show significant overlap
- The *db/db* UNx-ReninAAV mouse model shows particularly good human translatability with regards to kidney transcriptome signatures of fibrosis
- Scan the QR code to see more

1 Group summary



2 Clinical and preclinical parameters

Human				Mouse			
Clinical features	Control (n = 8)	DKD (n = 6)	P value (DKD vs Control)	**Preclinical features**	*db/m* (n = 12)	*db/db* UNx-ReninAAV (n = 5)	P value (*db/db* UNx-ReninAAV vs *db/m*)
Age (years)	58.1±1.5	63.5±3.7	0.19	**Age (weeks)**	24	24	NA
Sex (male, %)	87.5	83.3	NA	**Sex (female, %)**	100	100	NA
BMI (kg/m²)	34.5±1.6	35.0±1.5	0.85	**BW (g)**	24.4 ± 0.4	43.6 ± 5.3	<0.001
HbA1c (%)	5.4±0.1	6.6±0.4	<0.01	**BG**	6.98 ± 0.2	23.91 ± 3.8	<0.001
ACR (µg/mg)	5.5±1.7	698.5±272.1	<0.05	**ACR (µg/mg)**	113 ± 36.1	25245 ± 7650	<0.001
eGFR (ml/min/1.73m²)	85.5±2.3	45.2±9.0	<0.001	**Glomerulo-sclerosis (total PAS mass, mg)**	0.69 ± 0.1	2.89 ± 0.3	<0.001

Table 1. Clinical characteristics of human DKD and Control, and preclinical characteristics of db/m and db/db UNx-ReninAAV mice. ACR, albumin-to-creatinine; BG, blood glucose; BMI, body mass index; BW, body weight; eGFR, estimated glomerular filtration rate; NA, not applicable; PAS, Periodic acid–Schiff.

3 Clear clustering of human DKD samples

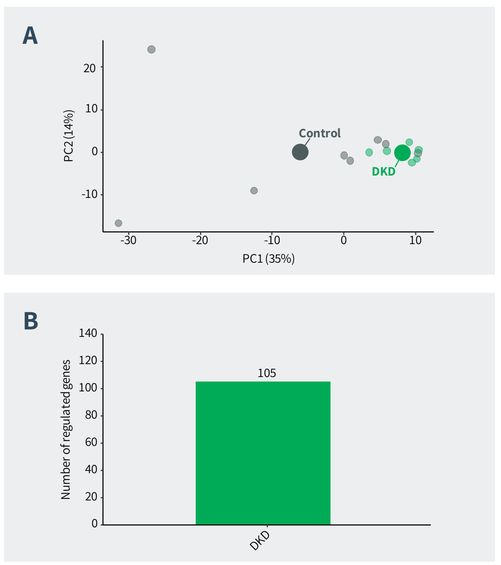


Figure 1. Transcriptome profile of human DKD using FFPE kidney samples. (A) Principal component analysis (PCA) of samples based on top 500 most variable gene expression levels for human DKD and Control. **(B)** Total number of regulated genes between human DKD and Control.

4 Gene expression changes correlate with clinical diagnostic parameters in DKD patients

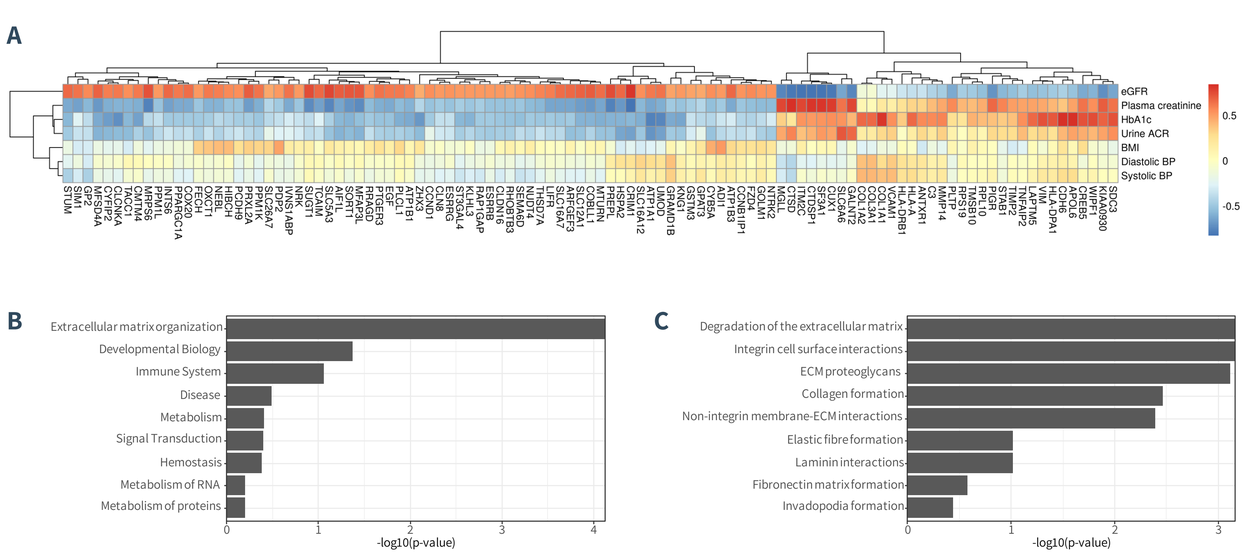


Figure 2. Correlation of gene expression changes with clinical parameters in DKD patients. (A) Heatmap showing the correlation of significantly regulated renal genes vs. clinical parameters (eGFR, plasma creatinine, HbA1c and urine ACR) in DKD patients as compared with healthy controls. **(B, C)** Reactome pathway gene enrichment analysis for human DKD compared to healthy controls. Degree of perturbation is indicated by the p-value after correction for multiple testing.

5 Transcriptomic profile of key regulated genes in DKD patients and *db/db* UNx-ReninAAV mice

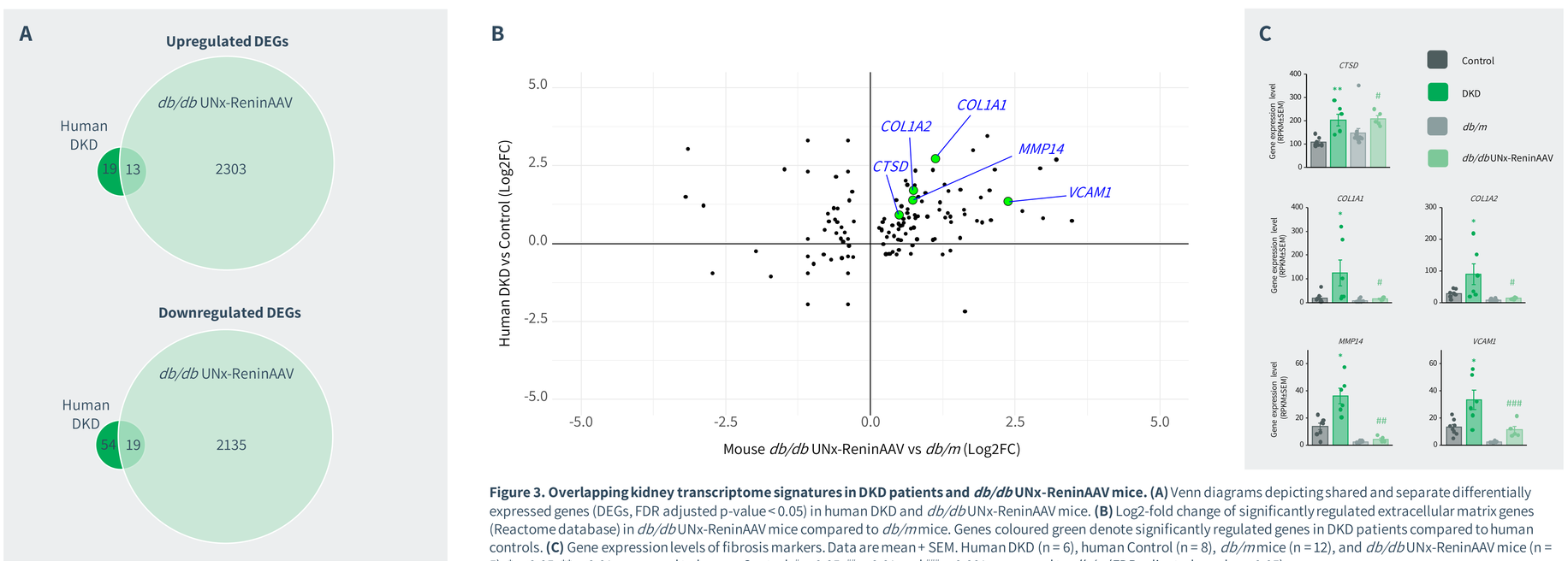


Figure 3. Overlapping kidney transcriptome signatures in DKD patients and *db/db* UNx-ReninAAV mice. (A) Venn diagrams depicting shared and separate differentially expressed genes (DEGs, FDR adjusted p-value < 0.05) in human DKD and *db/db* UNx-ReninAAV mice. **(B)** Log2-fold change of significantly regulated extracellular matrix genes (Reactome database) in *db/db* UNx-ReninAAV mice compared to *db/m* mice. Genes coloured green denote significantly regulated genes in DKD patients compared to human controls. **(C)** Gene expression levels of fibrosis markers. Data are mean + SEM. Human DKD (n = 6), human Control (n = 8), *db/m* mice (n = 12), and *db/db* UNx-ReninAAV mice (n = 5). *p<0.05, **p<0.01 compared to human Control; #p<0.05, ##p<0.01 and ###p<0.001 compared to *db/m* (FDR adjusted p-value < 0.05).