

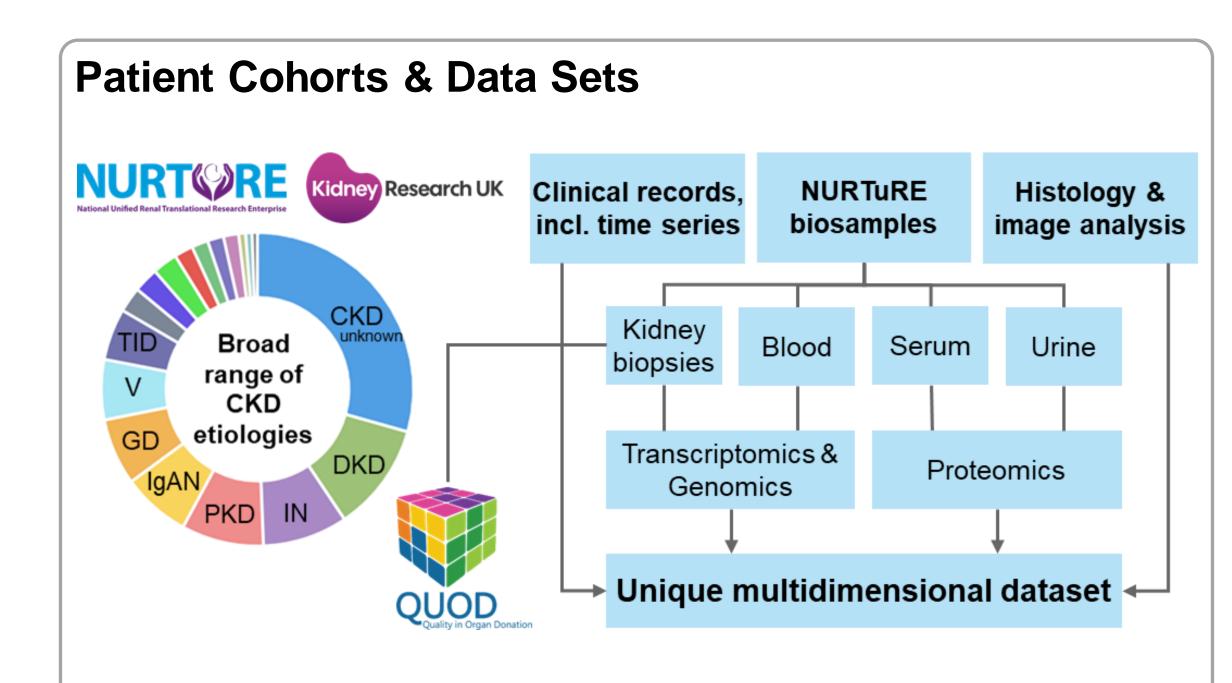
Unsupervised Characterization of the NURTuRE Cohort Reveals Gene Expression and Tissue Remodeling Dynamics along a Synthetic CKD Progression Axis

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Background

- Conventional stratification by clinical and histopathological phenotypes is insufficient to describe the heterogeneity of chronic kidney diseases (CKD). Recent advances in CKD classification¹ integrate real-world molecular, morphological and clinical data from large patient cohorts to improve mechanistic disease understanding.
- Here, we combined molecular groups identified by unsupervised characterization of the NURTuRE² and QUOD³ patient cohorts into a synthetic disease progression axis (sDPA) ranging from healthy to severe CKD, with the aim to explore gene expression and tissue remodeling dynamics along this pseudotime trajectory.

We will use this framework for a human data-driven, patient-centric and omics-enabled target identification focused on common cellular and molecular mechanisms of disease



- NURTuRE² is a unique prospective cohort study involving > 3500 CKD patients that is linked to a biobank of matched patient samples covering a broad range of diagnoses and kidney functional states.
- A rich multidimensional dataset was generated by combining clinical and histopathological records with multiomics analyses of kidney and liquid biopsies.
- A data-driven (clinical reports, serum chemistry) selection of biopsies from the QUOD³ initiative, representing kidney-healthy donors after brain death, was processed simultaneously to provide reference kidney transcriptomes.

Methods

- Unsupervised classification of NURTuRE kidney transcriptomes via self-organizing maps⁴ and characterization via PHATE⁵ dimensionality reduction inferred 5 groups with distinct molecular profiles that aligned with clinical and histopathological disease progression.
- A data-driven selection of QUOD (healthy, n = 36) and NURTuRE (CKD, n = 139) kidney biopsy transcriptomes (FFPE, RNA-seq) representative of molecular groups was combined into a synthetic disease progression axis (sDPA) via PHATE⁵ embedding.
- Groups of genes with similar expression dynamics were derived via local regression and hierarchical clustering enabling a pseudotemporal interpretation of gene expression dynamics along the sDPA.
- Gene set overrepresentation analysis based on > 600 manually curated kidney-focused scRNA-seq signatures and additional public sources (evoGO⁶, Reactome, Hallmark) was employed to support a mechanistic interpretation of molecular disease progression.

A human data-driven, patient-centric and omics-enabled target identification framework focused on common mechanisms of CKD Molecular disease progression Molecular group OUOD F E C Unsupervised molecular stratification and Construction of a molecular disease Functional enrichment analysis and Characterization of molecular mechanisms of multimodal characterization of kidney biopsy mechanistic interpretation of molecular disease progression axis and pseudotemporal disease in normal and maladaptive cell states transcriptomes progression stratification of gene expression Pseudotime Overrepresentation Kidney function analysis along the sDPA KRAS SIGNALING UP enables a mechanistic ALLOGRAFT REJECTION INFLAMMATORY RESPONSE **interpretation** of Adaptive / Maladaptive / Repairing Thick Ascending Limb Cell IL6 JAK STAT3 SIGNALING **Disease progression** pseudotemporal disease Vascular Smooth Muscle Cell / Pericyte ANGIOGENESIS Fibroblast aligns with molecular APICAL JUNCTION progression. Adaptive / Maladaptive / Repairing Fibroblast INTERFERON GAMMA RESPONSE stratification largely independent of clinical Molecular group ● QUOD ● F1 ● F2 ● C ● A ● AB diagnosis. Combined **similarity** Proximal Tubules Distal Tubules Regulatory T Cell L2 STAT5 SIGNALING network analysis of A data-driven selection (e.g. exclusion of biopsies affected by NTERFERON ALPHA RESPONS Interstitium Natural Killer Cell enriched kidney scRNAsampling bias) of molecular subgroups was combined into a XENOBIOTIC METABOLISM FATTY ACID METABOLISM reference degenerative adaptive seq signatures and other synthetic disease progression axis (sDPA) ranging from healthy OXIDATIVE PHOSPHORYLATION M2 Macrophage mechanistic genes sets to severe CKD. ADIPOGENESIS Classical Dendritic Cell Immune reponse interferon-secreting BILE ACID METABOLISM unravels complex MTORC1 SIGNALING cellular and molecular mechanisms of CKD. HEME METABOLISM Cell type-specific pseudotemporal expression profiles reflect Immune reponse PEROXISOME tubulointerstitial tissue remodeling dynamics characterized by signature expression a gradual loss of healthy tubular gene expression and a characterizes the complementary increase of maladaptive tubular, fibrotic and polarized global data 830 genes immune gene expression. structure. 614 genes Chemotaxis and cell projections (singscore of 200 genes, 227 genes Hallmark M5932) 141 genes Collagen deposition and fibroblasts 398 genes **Tubular function and** 502 genes 369 genes 433 genes Metabolism 260 genes 198 genes Fatty acid metabolism FA and AA metabolism -2 -1 0 1 2 Molecular group signature expression Myeloid cell activation characterizes the polarized global data Genes highly correlated with the sDPA (~ 5000) were clustered structure. hierarchically by their expression dynamics resulting in 12 clusters with different pseudotemporal change. Hallmark M5935) scRNAseq signatures evoGO:BP Reactome Our framework captures accumulating maladaptive Integration of selected molecular subgroups into a Tubular injury and atrophy, interstitial fibrosis and Molecular groups represent distinct transcriptomic disease progression axis reveals pseudotemporal gene immune response are the main functional concepts tubules and associated molecular programs as disease states aligned with disease progression common drivers of CKD progression associated with molecular disease progression expression dynamics

Conclusions and Outlook

- Unsupervised cohort characterization and multimodal data exploration enabled the careful selection and integration of disease-relevant biopsy transcriptomes into a molecular CKD progression axis. Pseudotemporal stratification of gene expression along this axis revealed groups of genes with shared expression dynamics corresponding to CKD tissue remodeling.
- Our framework captures major concepts of CKD progression, including but not limited to tubular injury and atrophy, interstitial fibrosis, inflammation and immune infiltration as reflected by the enrichment of curated scRNA-seq-derived cell type-specific signatures and other mechanistic gene sets.
- Importantly, our human data-driven and omics-enabled analysis provides translational evidence for an early accumulation of profibrotic and proinflammatory maladaptive cell tubular states reflecting failed repair as common drivers of CKD progression in this large patient cohort.

Chinook Therapeutics and Evotec joined forces for a patient-centric target identification supported by strong translational evidence to initiate drug discovery programs with a focus on tubular failed repair and cross-talk in the tubulointerstitial niche

References and Acknowledgement

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