Supplementary Methods

A detailed description of kidney tissue handling

A minimum of three biopsy cores will be harvested to ensure sufficient tissue for all subsequent analyses. The tissue will be divided under a magnifier or dissecting microscope and apportioned as showed in Figure S1. If it is not possible for the investigator to distinguish medulla from cortex, the superficial part of the biopsy will be assigned to immunofluorescence microscopy (IFM)/electron microscopy (EM), and the more profound part (pointing towards medulla) will be assigned to research.

Light microscopy

One core will be subjected to light microscopy (LM) histopathology. Tissue for LM is placed in formalin and processed into formalin-fixed paraffin-embedded (FFPE) blocks. Serial 3-µm-thick sections are cut from FFPE blocks onto glass slides and stained with Hematoxylin & Eosin (H&E), Periodic Acid Schiff (PAS), Masson’s Trichrome (MT), Jones Methenamine Silver (Silver), and Congo Red (amyloid).

Immunofluorescence microscopy

Approximately 1/3 of a core will be used for IFM. Tissue for IFM will be placed in histocon (Histolab ApS, Copenhagen, Denmark), for transportation and subsequently embedded in optimal cutting temperature (OCT) compound (Tissue-Tek, Sakura Finetek, Denmark)) and frozen in isopentane. The OCT frozen section tissue block is cryosectioned in serial 2-µm-thick sections for staining with fluorescein-conjugated anti-IgG, IgA, IgM, κ- and λ-light chains, C3 and C1q. Digital photographs of representative glomeruli and tubulointerstitium will be obtained when showing positive staining.
Electron microscopy

Approximately 1/4 of a core will be used for EM. Tissue for EM is placed in 2.5% glutaraldehyde and processed into plastic embedded blocks. Thick plastic sections are cut and stained with Toluidine blue and reviewed by light microscopy. Electron microscopic examination is performed on thin sections, and digital photographs of representative glomeruli and tubulointerstitium are obtained. EM will only be performed when it is required for the diagnostic assessment in accordance with standard procedure.

Classification of kidney disease and class of diabetic nephropathy

All biopsies will be categorized as “representative” (ten or more glomeruli for LM) or “not representative” (nine or fewer glomeruli for LM). If the biopsy for LM contains less than ten glomeruli, the biopsy can be categorized as “inconclusive” or “conclusive despite sparing material”. If there are no signs of other medical kidney diseases than diabetic nephropathy in the tissue for LM, we will accept the biopsy despite missing glomeruli in the core for IFM. If necessary for the final diagnosis, the tissue in OCT for future research can be used for IFM. A sensitivity analysis will be conducted to verify whether this approach applies.

All LM slides will be scored according to the Renal Pathology Society Classification of Diabetic Nephropathy (1). In brief, the classification scheme categorizes the biopsy into four hierarchical classes of glomerular lesions based on the extent of glomerular basal membrane thickening, mesangial expansion, and glomerulosclerosis. Separate evaluation of interstitial and vascular involvement, which includes the degree of interstitial fibrosis and tubular atrophy and the presence of arteriolar hyalinosis and large vessel arteriosclerosis, will be made. Furthermore, we will register the presence or absence of tip lesions, crescents, mesangiolysis (dissolution of...
mesangial matrix with or without microaneurysms), glomerular insudative lesions (capsular drops or fibrin caps) or thrombotic microangiopathy.

**Light-sheet fluorescence microscopy (LSFM)**

Furthermore, Light-sheet fluorescence microscopy (LSFM) may be performed by Gubra ApS in a subset of biopsies. The method adds a deeper understanding of the developement and progression of the disease by analysis and visualization of e.g. fibrosis in 3D imaging.

**RNA sequencing**

Approximately 2/3 of one core will be used for RNA sequencing. The tissue for RNA sequencing will be placed in a tube with RNAlater (Thermo Fisher Scientifc, Roskilde, Denmark) and stored at 4 °C for 24 hours to allow the RNAlater to infiltrate the tissue. After 24 hours, the tube is transferred to -80 °C until shipped to Gubra ApS on wet ice or ice packs. Excess RNAlater is then aspirated, and the tissue will be mechanically separated in glomerular and tubulointerstitial fractions. RNA is isolated from these fractions and stored at -80°C until analyzed by next-generation sequencing technology. RNA sequencing quantifies messenger RNA and thereby gene expression. Data analyses will show whether specific genes show changes in their expression.

**Single nuclei RNA sequencing**

Tissue RNA sequencing will be substituted with single nuclei RNA sequencing in selected participants The kidney tissue will be snap frozen in liquid nitrogen 1-2 minutes after the biopsy procedure. Nuclei are isolated from frozen tissue, and mRNA transcripts derived from every single nucleus will be sequenced. This methodology permits the analysis of gene expression in the single nuclei derived from frozen tissue, each representing a single cell. Each cell is classified according to its gene expression of key cellular markers (e.g., PECAM-1 and VEGFR2, etc., for
endothelial cells). Subsequently, the expression level of additionally expressed genes in the specific cell types can be studied.

*Biomarker assays*

For genes encoding circulating proteins or relevant metabolites (potential biomarkers), regulation at the gene level may be validated in matched plasma samples from the research biobank employing enzyme-linked immunosorbent assays (ELISA) or mass spectrometry methods tailored to the protein/metabolite in question.