BMJ Open

Rationale and design of a prospective, clinical study of kidney biopsies in people with type 2 diabetes and severely increased albuminuria (the PRIMETIME 2 study)

Marie Møller ,[1] Rikke Borg,[2,3] Iain Bressendorff,[1] Lisbeth N Fink,[4] Eva Gravesen,[5] Karina Haar Jensen,[2,6] Torben Hansen,[7] Dorrit Krustrup,[5] Frederik Persson ,[6] Peter Rossing,[3,6] Frederikke E Sembach,[4] Anne C B Thuesen,[7] Ditte Hansen[1,3]

To cite: Møller M, Borg R, Bressendorff I, *et al*. Rationale and design of a prospective, clinical study of kidney biopsies in people with type 2 diabetes and severely increased albuminuria (the PRIMETIME 2 study). *BMJ Open* 2023;**13**:e072216. doi:10.1136/bmjopen-2023-072216

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2023-072216).

Received 31 January 2023
Accepted 14 May 2023

For numbered affiliations see end of article.

Correspondence to
Dr Marie Møller;
marie.moeller@regionh.dk

ABSTRACT

Introduction Diabetic kidney disease is a severe complication of diabetes. The diagnosis is based on clinical characteristics such as persistently elevated albuminuria, hypertension and decline in kidney function, although this definition is not specific to kidney disease caused by diabetes. The only way to establish an accurate diagnosis—diabetic nephropathy—is by performing a kidney biopsy. The histological presentation of diabetic nephropathy can be associated with a heterogeneous range of histological features with many pathophysiological factors involved demonstrating the complexity of the condition. Current treatment strategies aim to slow disease progression and are not specific to the underlying pathological processes. This study will investigate the prevalence of diabetic nephropathy in individuals with type 2 diabetes (T2D) and severely elevated albuminuria. The deep molecular characterisation of the kidney biopsy and biological specimens may pave the way for improved diagnostic accuracy and a better understanding of the pathological processes involved and may also reveal new targets for individualised treatment.

Methods and analysis In the PReclsion MEdicine based on kidney TIssue Molecular interrogation in diabetic nEphropathy 2 study, research kidney biopsies will be performed in 300 participants with T2D, urine albumin/creatinine ratio ≥700 mg/g and estimated glomerular filtration ratio >30 mL/min/1.73 m^2. Cutting-edge molecular technologies will be applied to the kidney, blood, urine, faeces and saliva samples for comprehensive multi-omics profiling. The associated disease course and clinical outcomes will be assessed by annual follow-up for 20 years.

Ethics and dissemination The Danish Regional Committee on Health Research Ethics and the Knowledge Center on Data Protection (in the Capital Region of Denmark) have granted approval for the study. The results will be published in peer-reviewed journals.

Trial registration number NCT04916132.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ A deep phenotypic profile of this patient group will be created using cutting-edge molecular technologies applied to the kidney, blood, urine, faeces and saliva samples for comprehensive multi-omics profiling.
⇒ With a post-biopsy follow-up period of 20 years, all findings will be associated with disease course and clinical outcomes, creating an opportunity to identify biomarkers that will identify rapid progressors.
⇒ The study is a broad national project with endorsement from multiple specialties and sites, building a scientific bridge between diabetology, nephrology, clinical biochemistry and pathology.
⇒ The study will only include people from Denmark with type 2 diabetes (T2D) and severely elevated albuminuria, and the findings may not be generalisable to individuals with lesser degrees of albuminuria or different demographics.
⇒ All people with T2D and severe albuminuria from the participating sites will be invited for a research kidney biopsy, which creates a unique and unbiased cohort.

INTRODUCTION

Approximately 10% of the world's population has diabetes, and type 2 diabetes (T2D) accounts for more than 90%. Of these, approximately 40% will develop diabetic kidney disease (DKD) during their lifetime.[1–3] DKD is the leading cause of kidney failure, it is associated with cardiovascular diseases, and accounts for a large part of the excess mortality associated with diabetes.[2]

The clinical diagnosis of DKD is based on clinical characteristics such as persistently severely elevated albuminuria, hypertension, diabetic retinopathy, a decline in kidney function, and absence of clinical or laboratory evidence of other kidney or urinary tract disease.[4 5] These characteristics overlap with features of many non-diabetic kidney diseases (NDKD), and

clinical characteristics alone cannot reliably differentiate between the two. At present, only a kidney biopsy can give a definitive diagnosis.[6] However, there are no standardised criteria for when to perform a kidney biopsy in individuals with T2D. Previous studies are heterogeneous with different criteria or indications for the procedure, much like everyday clinical decision-making. Persistent proteinuria despite optimal treatment, absence of retinopathy, haematuria, active urinary sediment and rapid decrease of kidney function are some of the indications for a kidney biopsy.[6] However, most people with T2D, impaired kidney function or albuminuria will never have a kidney biopsy performed and will instead have the clinical diagnosis of DKD. As a consequence of this pragmatic approach, cases with NDKD may be missed.

When a biopsy is performed and the histological examination reveals pathological changes caused by diabetes, the patient is diagnosed with diabetic nephropathy. The diabetic nephropathy diagnosis has several clinical phenotypes and can be associated with a heterogeneous range of histological features, including nodular or diffuse glomerulosclerosis, tubulointerstitial fibrosis, tubular atrophy and kidney arteriolar hyalinosis, alone or in combination.[7] Despite this knowledge, current treatments aimed at slowing disease progression focus little on underlying and individual pathological processes. An understanding of these processes for each individual provides an opportunity for precision medicine in DKD to be introduced and further developed.

Rationale and aims for this project

The PRIMETIME (PRecIsion MEdicine based on kidney TIssue Molecular interrogation in diabetic nEphropathy) 2 study is a Danish national prospective cohort study that will investigate individuals with T2D and severely elevated albuminuria. All participants will undergo research kidney biopsies to apply the full force of modern molecular biological characterisation of kidney tissue in DKD. This setup will provide a unique opportunity to achieve the aims of the PRIMETIME 2 study: to describe the true prevalence of diabetic nephropathy in people with T2D and severely elevated albuminuria, identify disease mechanisms, improve diagnostication, prognostication, tailor medicine and potentially identify new treatment targets (figure 1).

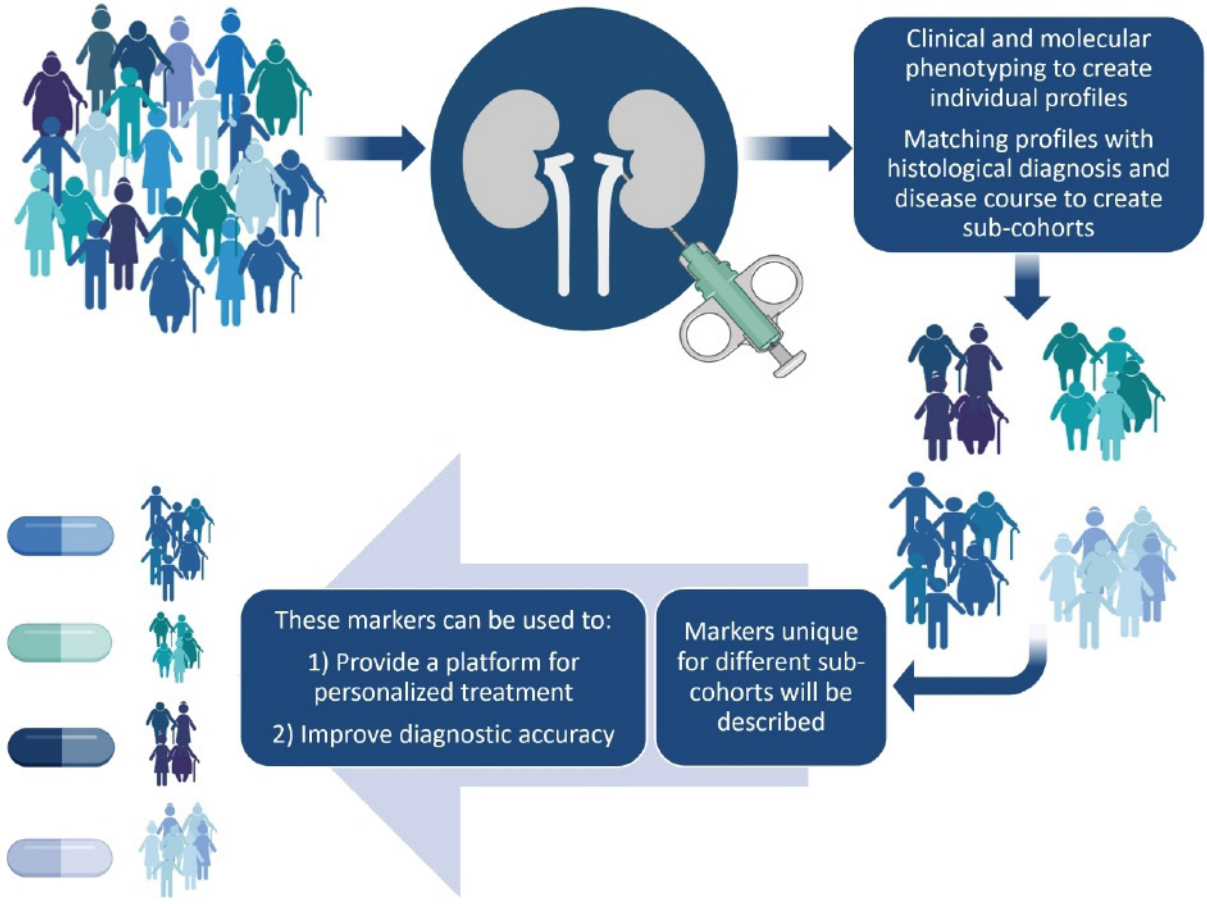


Figure 1 Aims of the PRecIsion MEdicine based on kidney TIssue Molecular interrogation in diabetic nEphropathy 2 study: to describe the true prevalence of diabetic nephropathy in people with type 2 diabetes and severe albuminuria, identify disease mechanisms, improve diagnostication, prognostication, tailor medicine and potentially identify new treatment targets.

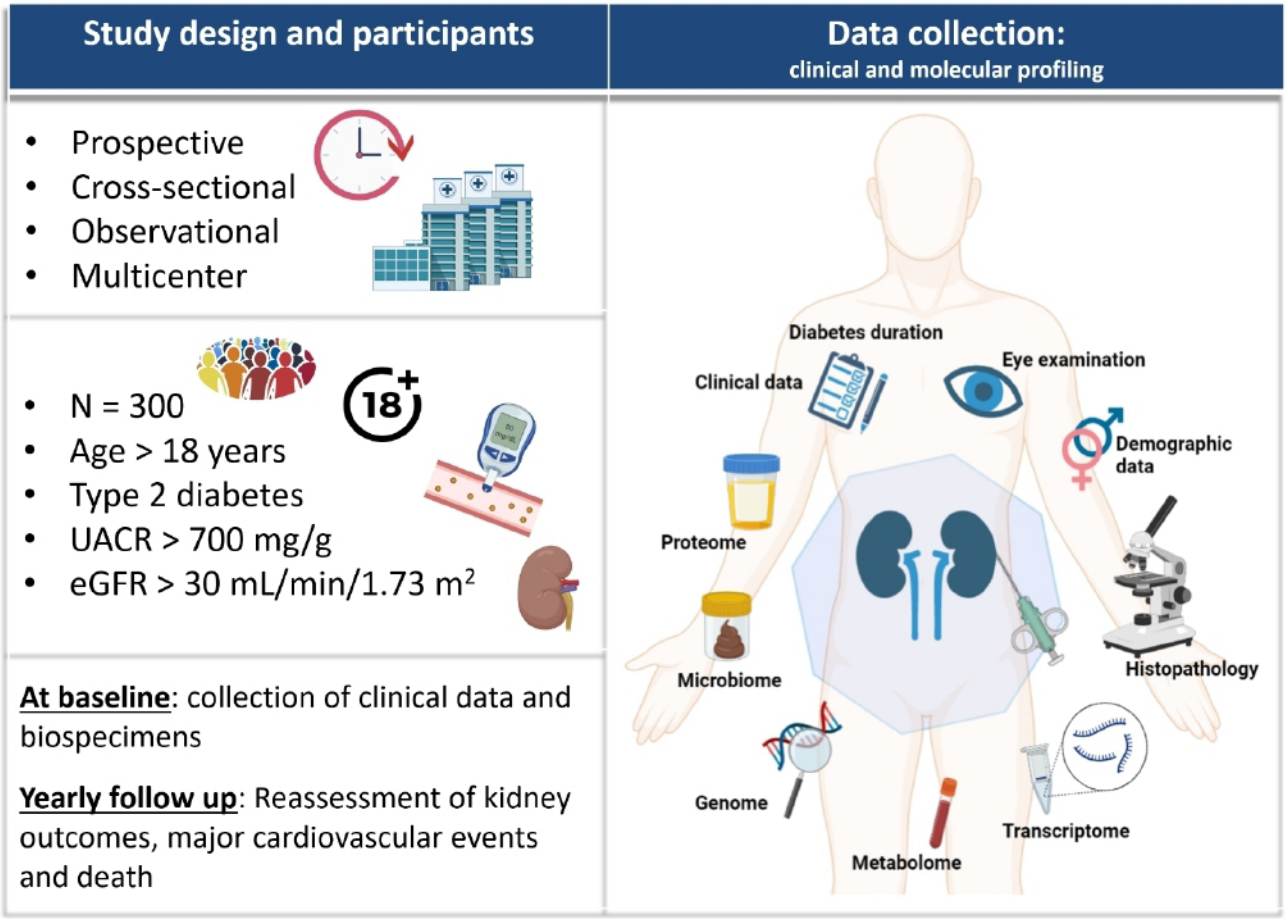


Figure 2 Study design and set-up. eGFR, estimated glomerular filtration ratio; UACR, urine albumin/creatinine ratio.

METHODS AND STUDY DESIGN

Overall study design

The research collaboration PRIMETIME was established in 2019, and the PRIMETIME 2 study is conducted under this collaboration. Where PRIMETIME 1 is a retrospective analysis of historic samples,[8] PRIMETIME 2 is a prospective, cross-sectional, observational, Danish multicentre study. Research kidney biopsies will prospectively be collected from a cohort of 300 participants with T2D and severely elevated albuminuria. The kidney tissue, blood, urine, faeces and saliva samples will be thoroughly investigated with cutting-edge molecular technologies for comprehensive profiling and later associated with the disease course. For an overview of the study setup, see figure 2.

The executive PRIMETIME committee designed this study in collaboration with the PRIMETIME steering committee, Gubra ApS and Novo Nordisk Center for Basic Metabolic Research, University of Copenhagen (for a full list of committee members see online supplemental file 1).

Before any study activity, the study was approved by the Danish Regional Committee on Health Research Ethics (Ethical Committee number: H-20080050) and the Knowledge Center on Data Protection (in the Capital Region of Denmark). The results will be published in peer-reviewed journals.

Objectives

The primary objective is to investigate the prevalence of biopsy-proven diabetic nephropathy in individuals with T2D and a history of severely elevated albuminuria.

The secondary objectives are divided among the cross-sectional and prospective observations as listed in Box 1. A range of study outcomes to reflect the prognostic values of these objectives will be evaluated annually for 20 years after the biopsy (Box 1).

Study population

The participants must be adults, diagnosed with T2D, have an estimated glomerular filtration rate (eGFR) (CKD-EPI)[9] >30 mL/min/1.73 m^2 and a history of severely elevated albuminuria with urine albumin/creatinine ratio (UACR) ≥700 mg/g. For a detailed list of inclusion criteria and main exclusion criteria, see Box 2 (for a full list of exclusion criteria see online supplemental table S1).

Recruitment and enrolment

Participants will be recruited from Steno Diabetes Centers and departments of endocrinology and nephrology in Denmark. Eligible participants will be identified during routine outpatient visits by the local clinician or

Box 1 Study objectives and outcomes

Primary objective:
⇒ To investigate the prevalence of biopsy-proven diabetic nephropathy in individuals with T2D and a history of severe albuminuria
Primary outcome
⇒ The prevalence of biopsy-proven diabetic nephropathy in individuals with T2D and a history of severe albuminuria
Secondary objectives:
Cross-sectional objectives:
⇒ To investigate whether clinical variables, transcriptomic, proteomic, and metabolomic profiles, and genetic variation can be associated with the presence of diabetic nephropathy in a kidney biopsy
⇒ To describe the sensitivity and specificity of diabetic retinopathy in predicting biopsy-proven diabetic nephropathy
Prospective objectives are to describe the prognostic value of:
⇒ Different histological and molecular findings on kidney biopsy in individuals with biopsy-proven diabetic nephropathy
⇒ Different histological and molecular findings on the kidney biopsy in individuals with NDKD compared to biopsy-proven diabetic nephropathy
⇒ The proteomic and metabolomic profiles in biopsy-proven diabetic nephropathy; different forms of genetic variation in biopsy-proven diabetic nephropathy
⇒ Different microbiome compositions and their relation to biopsy and clinical findings
Secondary (prospective) study outcomes
⇒ Changes in kidney status (defined by the binary outcomes: initiation of dialysis, kidney transplantation, death from kidney disease or decrease in eGFR > 40 % compared to baseline)
⇒ Annual decline in eGFR
⇒ Annual changes in UACR
⇒ Events of cardiovascular disease (fatal cardiovascular events, non-fatal stroke, non-fatal myocardial infarction, hospitalization for heart failure, percutaneous coronary intervention, or bypass surgery (coronary or lower extremities), limb amputations due to ischaemia and unstable angina)
⇒ Death (any cause)

eGFR, estimated glomerular filtration rate; NDKD, non-diabetic kidney disease; T2D, type 2 diabetes; UCAR, urine albumin/creatinine ratio.

Box 2 List of inclusion and exclusion criteria.

Inclusion criteria
⇒ Age ≥18 years
⇒ Diagnosis with T2D according to the American Diabetes Association[42]
⇒ eGFR>30 mL/min/1.73 m^2
⇒ Urine albumin/creatinine ratio (UACR) >700 mg/g or 24 hours urine albumin >700 mg on more than one historical measurement
⇒ Written informed consent
Exclusion criteria
⇒ Signs of acute kidney failure according to the KDIGO classification[43] at the time for kidney biopsy or the last 6 months before kidney biopsy
⇒ Kidney transplant recipient
⇒ Previous medical kidney biopsy
⇒ Factors that increase the risk of complications due to kidney biopsy:
⇒
1. Haemoglobin < 96.7 g/L
2. International Normalised Ratio (INR) >1.4 at the time of biopsy
3. Platelet count <100×10^9 /L
4. Uncontrolled high blood pressure
5. Only one functioning kidney
6. Evidence of urinary tract obstruction or hydronephrosis at the time of biopsy
7. Multiple bilateral kidney cysts
8. Kidney infection, peri-renal infection or cutaneous infection that overlies the kidney the time of biopsy
9. Unwilling to receive a blood transfusion
10. Unable to lie flat in bed 6 hours after the biopsy
11. Inability to withdraw anticoagulation, antiplatelet therapy or NSAID before the biopsy

KDIGO, Kidney Disease Improving Global Outcomes; NSAID, nonsteroidal anti-inflammatory drug; T2D, type 2 diabetes; UACR,urine albumin/creatinine ratio.

by retrieving patient lists based on International Classification of Diseases (ICD-10) codes for T2D, as well as biochemistry values of eGFR and UACR. After written informed consent is obtained, the collection of data and biospecimens for comprehensive profiling will take place at the nephrology departments.

Collection of biospecimens and data for comprehensive profiling

The comprehensive collection of biospecimens and data and the subsequent phenotyping will only occur at baseline concomitantly with performing the kidney biopsy. The clinical information to describe disease course and prospective observations will be obtained by review of the participants' medical records.

Kidney tissue sample collection and processing

The central part of the extensive profiling of this cohort is the kidney biopsy and the subsequent analysis of the kidney tissue. All participants will be admitted to a nephrology ward and the kidney biopsy will be performed according to local clinical guidelines. A minimum of three biopsy cores will be harvested to ensure sufficient tissue for all subsequent analyses. The tissue will be divided and placed in formalin, Histocon (Histolab ApS, Copenhagen, Denmark), glutaraldehyde and RNAlater (Thermo Fisher Scientific, Roskilde, Denmark) for subsequent light microscopy, immunofluorescence microscopy, electron microscopy and RNA sequencing, respectively. Tissue RNA sequencing will be substituted with single nuclei RNA sequencing in selected participants.

A detailed histopathological examination of the kidney tissue will be carried out by the same two trained kidney pathologists at the Department of Pathology, Herlev Hospital, Copenhagen, to ensure an accurate diagnosis of all participants. Electron microscopy will be performed at the Department of Pathology, Odense University Hospital, if it is clinically indicated to determine the diagnosis. The two kidney pathologists will also divide the biopsies into diabetic nephropathy, NDKD and mixed (both diabetic nephropathy and NDKD) kidney disease and score the

severity of the findings according to the Renal Pathology Society Classification of Diabetic Nephropathy.[10]

In collaboration with Gubra ApS, the kidney tissue will be explored with RNA sequencing and, in some cases, single nuclei RNA-sequencing, which will allow for global differential gene expression analysis, gene expression analysis in a specific cell type and provide an understanding of the molecular features of DKD. For a more detailed description of kidney tissue handling, see online supplemental file 2 and figure S1.

Clinical information collection

At baseline, a full medical and pharmacological history will be obtained. Data on physical examination, blood pressure, heart rate, body mass index, gender, race (Northern European/non-Northern European), duration of diabetes diagnosis, smoking history and retinopathy (as registered in DiaBase, a register of The Danish Clinical Quality Programme, National Clinical Registries) will be registered.[11] Data will be collected from participant interviews and medical records.

Blood and urine sample collection and processing

Blood and urine (24-hour and spot) samples will be collected for routine clinical biomarker evaluation from each participant. Blood haemoglobin, leucocytes, thrombocytes, and glycated haemoglobin (HbA1c) and plasma albumin, creatinine, carbamide, sodium, potassium, bicarbonate, ionised calcium (free), phosphate, magnesium, uric acid, glucose, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, parathyroid hormone and C reactive protein will be measured. Twenty-four hour-urine is analysed for albumin, protein, creatinine, sodium, albumin/creatinine ratio, and protein/creatinine ratio, and a rapid urine dipstick test is performed to look for haematuria. All samples will be collected and analysed at the local laboratories on each study site.

EDTA-plasma and a spot urine sample of 20 mL will be collected and stored for later proteomics and metabolomics analysis.

Stool and saliva sample collection and processing for microbiome and additional analyses

Two faeces samples will be collected in the participants' home with a home collection tray. The samples will be stored at −20°C in the participants' home freezer and transferred to the hospital in a cooling bag within 48 hours after collection and then stored at −80°C (if collected less than 24 hours before depositing at the hospital, the samples can be handed in non-frozen). The Novo Nordisk Foundation Center for Basic Metabolic at the University of Copenhagen will perform the laboratory and data analysis. Microbial DNA will be extracted from the samples and subjected to sequencing, microbial gene analyses, taxonomy analyses, including enterotypes of known species and unknown meta-species, and functional annotation.

A saliva sample will be obtained from the oral cavity from each participant by use of gum-base (Fertin Pharma, Vejle, Denmark). Immediately after collection, the saliva will be transferred to dry-ice or to RNAlater (saliva preserved in RNAlater will be stored at 4°C for 24 hours before being transferred to −80°C) and then transferred to −80°C. The saliva will be used for microbial DNA extraction, metabolomics, proteomics and studies of saliva microbial flora and saliva biochemistry. Participants can partake in the study without contributing with a faecal and saliva sample.

Whole genome sequencing

Buffy coat containing peripheral blood mononuclear cells from an EDTA blood sample will be isolated and transferred to a separate vial and frozen at −80°C until transfer to the Novo Nordisk Foundation Center for Basic Metabolic, University of Copenhagen, Denmark, who will perform the laboratory and data analysis. A standard whole genome sequencing approach will be applied with standard depth, coverage and bioinformatics pipelines to assemble the data. The generated data will investigate the genetic variation underlying kidney disease. Analyses will include the entire genome (both exons and non-coding areas), common variants, rare variants suspected to be involved in the development of kidney disease and structural variants.

Follow-up

After the kidney biopsy, the participants' kidney function and cardiovascular status will be assessed annually for 20 years by review of the participants' medical records. In Denmark, data describing the above factors are routinely obtained and registered in the participants' medical records during their standard outpatient follow-ups for diabetes and chronic kidney disease, when they are admitted to the hospital for other reasons, or in case of death. Therefore, the follow-up data will be obtained by review of the participants' medical records, laboratory analyses and national registers for hospital admissions (LPR) and not from a study visit. We will register measurements for kidney function (eGFR and UACR), HbA1c, blood pressure, pharmacological changes, dialysis initiation, and events of kidney transplantation, cardiovascular disease, or death.

Biobank for future research

Blood, urine, kidney tissue, faeces and saliva will be stored in a research biobank for future research. All samples will be marked with a unique study identification number and kept at −80°C for optimal storage. The kidney tissue for future research will be placed in a cryomold with optimal cutting temperature compound (Tissue-Tek, Sakura Finetek, Denmark) immediately after the biopsy and transferred to dry ice within 1–2 min. When completely frozen, the tissue is transferred to a −80°C freezer for long-term storage.

Current status

Recruitment for the PRIMETIME 2 study started on December 2021. At the time of submission, four sites

are actively including subjects, and we have collected 39 kidney research biopsies. End of inclusion was initially scheduled for December 2023, but the study initiation was delayed due to the COVID-19 pandemic, and the inclusion period will therefore be extended.

Statistical analysis

Number of participants (power calculation)

With an expected test sensitivity of 90% and a 95% probability that the estimated 95% lower confidence limit is above the minimum acceptable limit of 80%, 235 cases are required.[12] In accordance with the findings by Basu *et al*, we expect a prevalence of 20% of NDKD in the included population.[13] Therefore, 59 extra cases are required yielding a minimum sample size of 294. The study will therefore include 300 participants with T2DM. We expect a research core to apply to 90% or 270 participants.[14]

Statistical considerations

Baseline variables will be described and compared between participants with biopsy-proven diabetic nephropathy and NDKD. Categorical data will be compared by χ^2 or Fisher's exact test and continuous data by parametric and non-parametric statistics, as appropriate.

For diabetic nephropathy, the specificity, sensitivity and positive predictive value of clinical and biochemical variables, including retinopathy, transcriptomic, proteomic and metabolomic profiles will be examined by multivariable logistic regression analysis.

Kaplan-Meier curves and Cox-regression adjusted for baseline variables will be used to evaluate the association between baseline histopathological findings and molecular profiles and progression to end-stage kidney disease (dialysis >3 months, kidney transplantation, death from kidney disease), cardiovascular events and mortality, and all-cause mortality. A two-sided p value <0.05 will be considered statistically significant. All available data from test subjects will be part of the statistical analysis. All data will be described, including data incompleteness and reasons for data incompleteness.

Patient and public involvement

None. The results of this study will be shared with enrolled participants by letter after the end of the study.

Complications/safety assessment

All complications, both minor (such as pain and microscopic haematuria) and major (gross haematuria, blood transfusion, prolonged admission at the hospital, embolisation, nephrectomy or death), and time of the complications after biopsy will continually be registered in the electronic case report file and reported to the principal investigator. The principal investigator and the Executive Committee will investigate all major complications and act immediately when necessary. In addition, severe complications (such as need for embolisation) and unexpected complications will immediately be reported to the Danish Ethical Health Committee, which will investigate the event and demand changes when necessary. Furthermore, a yearly safety report for the Danish Ethical Health Committee will be conducted. An unexpected number of complications from one of the participating centres will then be revealed, so that necessary changes can be made.

QUALITY CONTROL

Standard operating procedures for all procedures and data collection have been developed. All sites will receive training in these procedures prior to the start of recruitment to train study investigators. Site-specific feedback will be provided and when necessary, plans for improvement will be made. The biopsy material will continuously be evaluated to ensure sufficient tissue for each analysis, and protocol changes will be made if necessary to achieve this. The study will be conducted with continuous monitoring by the Executive Committee.

DISCUSSION

Diabetes and diabetes-related kidney disease are global problems. The multiple clinical phenotypes and numerous disease courses illustrate its complexity and emphasises the need for a better understanding of the disease, improved diagnostics and individualisation of treatment. To achieve this, we must gain a deeper understanding of the condition and its pathophysiology.

Rationale for objectives

Several retrospective studies and meta-analyses have investigated the prevalence of biopsy-proven diabetic nephropathy in people with diabetes.[6 15–19] They all suggest a high prevalence of NDKD (~37%). However, all these studies fail to identify the true prevalence of diabetic nephropathy and NDKD in people with diabetes and impaired kidney function since all the examined biopsies were performed in a selected cohort of patients and for clinical indications. Basu *et al* investigated the prevalence unbiasedly by inviting 818 people with T2D and an eGFR of 30–60 mL/min/1.73 m^2 and/or UACR>300 mg/g for a kidney biopsy, 110 accepted and had a kidney biopsy performed. Among those 110 subjects, 66.4% had diabetic nephropathy, 18.2% had NDKD and 15.4% had mixed kidney disease.[13]

Thus, it remains unknown how many people with the clinical diagnosis of DKD and severely elevated albuminuria have biopsy-proven diabetic nephropathy. Therefore, the primary objective of the PRIMETIME 2 study is to describe the prevalence of diabetic nephropathy in people with T2D and severely elevated albuminuria in an unbiased manner. This will help us to understand the predictive value of known biomarkers and the need for new biomarkers in this patient cohort.

RNA sequencing applied to kidney tissue allows for the identification of upregulated and downregulated cellular pathways in glomerular and tubular cells with the potential to identify diagnostic, prognostic and therapeutic

targets to advance patient care.[20 21] Proteomics and metabolomics can characterise different disease processes in a non-invasive manner,[22] and mass spectrometry has identified a large number of autoantigens involved in kidney disease and revolutionised the care of patients with various antibody-mediated kidney diseases (in particular membranous nephropathy and monoclonal gammopathies of renal significance).[23] Inspired by these approaches, the PRIMETIME 2 study will look for new biomarkers and investigate known biomarkers for DKD and disease progression such as KIM1, TNFR1 and TNFR2.[24 25] These investigations may help clarify which patients with T2D and severely elevated albuminuria should be further examined with a kidney biopsy to diagnose an NDKD, thus potentially sparing some patients from a kidney biopsy, while also identifying patients in need of a kidney biopsy, who would otherwise be diagnosed with DKD. We hope to develop a non-invasive multimarker score based on clinical and molecular information that can be validated and introduced in the clinic to replace the invasive kidney biopsy, thereby widening correct diagnoses and improving therapy and outcome.

Additionally, we wish to identify groups with a high risk of rapidly progressive diabetic nephropathy, who would likely benefit from closer follow-up and additional treatment.[26 27] Suppose a biochemical, genetic, transcriptomic, proteomic or metabolomic profile or a composition of the microbiome with a high risk of progression is identified in the group with rapid progressors. In that case, this may indicate a group of individuals with diabetes who needs more aggressive treatment and further investigation.

Precision medicine is the idea of custom-made healthcare decisions or interventions based on individual characteristics instead of the 'one drug fits all'-model.[28] The ultimate goal with precision medicine in DKD is to introduce individualised and impactful treatments to improve survival rates and prevent end stage kidney disease. To do so, it is essential to identify pathways that are causal to disease development as targets for therapeutics. Second, these targets must be paired with clinical profiles or biomarkers, to permit prescription of specific and individualised treatment to each patient.

Several novel kidney-protective therapies have been introduced the last couple of decades and more are to come.[29] With this study, we hope to pave the way for the tentative beginning and implementation of precision medicine in DKD so a combination of these medicaments can be tailored to each patient. Furthermore, the comprehensive multi-omics profiling have the potential to reveal new targets for new therapeutics.

Rationale for inclusion and exclusion criteria

The study was designed to include an unselected cohort of people with T2D and severe albuminuria as a sign of kidney disease. The lower limit of eGFR on 30 mL/min/1.73 m^2 was chosen since the risk of bleeding complications due to a kidney biopsy increases significantly with declining eGFR,[30] biopsies in patients with severe chronic kidney disease yield little useful information since the microscopic appearance of the tissue is similar regardless of the cause,[31] and because we wish to investigate the active mechanisms that result in the pathological changes in diabetic nephropathy representing possible targets for future interventions.[32] The chosen range of albuminuria was defined semi-arbitrarily as >700 mg/g since this reflect limits for referral to departments of nephrology in Denmark at the time the study was designed, thus defining a group of patients who are followed in outpatient nephrology clinics, but who in many cases would not undergo a kidney biopsy due to presumed diagnosis of diabetic nephropathy.

Most exclusion criteria are defined to protect the participants from bleeding complications after a kidney biopsy.

Considerations regarding the kidney biopsy procedure and the risk of bleeding complications

Percutaneous needle kidney biopsy from living individuals has been performed since the early 1960s and is considered a safe procedure. When performing clinical kidney biopsies, the overall risk of complications is small but still present. Incidences of serious complications after a kidney biopsy has been described as 1.2%–1.9%, for macroscopic haematuria, 0.9%–1.1% for blood transfusion, 0.2%–0.6% for invasive procedures and 0.02% for death.[33 34] Guidelines for kidney biopsy already take into consideration known risk factors for these complications (eg, acute kidney injury, severely increased serum creatinine, hypertension, thrombocytopenia, and platelet dysfunction, antithrombotic and anticoagulation medication, coagulopathy, and size of biopsy needle (14–18 gauge)).[30 35] In the PRIMETIME 2 study, many of these risks will be eliminated by excluding individuals with contra-indications for percutaneous kidney biopsy according to local clinical guidelines combined with the extra safety measures described in the exclusion criteria.

Recently, several studies have investigated the risk of complications when performing kidney biopsies in people with diabetes. The TRIDENT research group investigated the feasibility and safety of obtaining kidney biopsy cores in patients with T2D, and as in this study, Hogan *et al* harvested an extra core biopsy for research on 160 participants. They found a risk of gross haematuria on 2%, the need for blood transfusion 2%, surgery/arterial embolisation 0%.[14] Hence, the rate of complications when obtaining an extra core for research is similar to the risk described in large biopsy cohorts. Two retrospective national studies from Japan and France with 76 320 and 52 138 patients, respectively, examined the risks of major bleeding complications after kidney biopsies performed between 2012 and 2018.[36 37] Both studies identified patients with diabetes and investigated the rate of complications in this subcohort. The Japanese study found that diabetes was significantly associated with major bleeding complications (RR=2.41 (95% CI 2.00 to 2.90)) and multiagent or insulin treatment (probably reflecting more advanced disease) was significantly associated with major

bleeding complications compared with single-agent treatment. The French study found the opposite, that diabetes was a protective factor (adjusted OR for major bleeding after a biopsy with a known history of diabetes was 0.91 (95% CI 0.81 to 1.02)). Our study will allow us to prospectively registerer all complications (both major and minor in accordance with Tøndel *et al*)[34] in an unbiased manner in order to assess the true risk of complications in patients similar to our study cohort.

Study limitations

The study will only include people with T2D, and severely elevated albuminuria and the findings may not be generalisable to individuals with lesser degrees of albuminuria. There are clinical phenotypes of diabetic nephropathy without albuminuria as described in autopsy studies showing lesions consistent with diabetic nephropathy even though there were no clinical signs of DKD.[38 39] Thus, the incidence of diabetic nephropathy in people with lesser degrees of albuminuria cannot be assessed based on the results of our study.

All participants will be included in Denmark, which has a population consisting of 89.6% people with Danish origins and 7.9% are immigrants of whom 41.7% are from non-Western countries.[40] Whether the results of our study can be generalised to describe other demographics is unknown.

Finally, our study will only include people recruited from departments of nephrology and endocrinology, and thus not the large majority of people with T2D who are treated by their general practitioners. Therefore, the study may only reflect the prevalence of diabetic nephropathy among people with a presentation of T2D, which leads to referral to a specialised treatment of T2D. However, the included population should represent all which according to guidelines are to be referred for specialist evaluation.

Study strength

One of this study's main strengths is the cohort's composition. There is no clinical indication for performing the biopsy, which creates an unselected cohort that enables us to describe the true prevalence of diabetic nephropathy.

Besides the kidney biopsy and the subsequent translational analyses of the kidney tissue, the participants' proteomic, metabolomic, and genomic profile and the microbiome composition will be characterised, creating a very profound profiling of this patient group. Furthermore, the participants' associated disease course and clinical outcomes will be assessed by an exceptionally long follow-up (20 years), creating an opportunity to identify biomarkers that will identify rapid progressors.

The study was designed in close collaboration with other research groups within this research field, enabling us to compare our results with other cohorts and to use data beyond this study.[41]

Finally, this study is a broad national project with great endorsement from multiple specialties and sites, building a scientific bridge between diabetology, nephrology, clinical biochemistry and pathology. The project will bring together molecular, translational and clinical scientists, ultimately bringing forward an improved understanding of the most frequent cause of end-stage kidney disease: DKD.

Author affiliations
[1]Department of Nephrology, Copenhagen University Hospital – Herlev and Gentofte, Herlev, Denmark
[2]Department of Medicine, Zealand University Hospital, Roskilde, Denmark
[3]Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark
[4]Gubra ApS, Hørsholm, Denmark
[5]Department of Pathology, Copenhagen University Hospital – Herlev and Gentofte, Herlev, Denmark
[6]Steno Diabetes Center Copenhagen, Herlev, Denmark
[7]Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Twitter Marie Møller @MarieMoeller_DK and Karina Haar Jensen @KarinaHaar

Acknowledgements The study setup was designed in dialogue with the Kidney Precision Medicine Project.[43] All figures are created with BioRender.com.

Collaborators Collaborators are Departments of Nephrology at Copenhagen University Hospital—Herlev and Gentofte, Copenhagen University Hospital—North Zealand Hilleroed, Center of Cancer and Organ Diseases, Copenhagen University Hospital—Rigshospitalet Copenhagen, Zealand University Hospital, Roskilde, Holbæk Hospital, Aalborg University Hospital, The Region Hospital of Gødstrup, Odense University Hospital and Aarhus University Hospital, Departments of Endocrinology at Center of Cancer and Organ Diseases, Copenhagen University Hospital—Rigshospitalet Copenhagen and Copenhagen University Hospital—North Zealand Hilleroed, Steno Diabetes Center in Copenhagen, Aarhus, Odense, The Zealand Region, and the North Denmark Region, Departments of Pathology at Copenhagen University Hospital—Herlev and Gentofte and Odense University Hospital, The Novo Nordisk Foundation Center for Basic Metabolic Research at Copenhagen University and Gubra ApS.

Contributors The research collaboration PRIMETIME was established in 2019 by PR, FP, RB, IB and DH. In collaboration with MM and KHJ, they designed the study, and MM finished the method protocol, the biospecimens protocols, and the pathology protocol in close cooperation with DK and EG from the Department of Pathology, Herlev Hospital, LNF and FES from Gubra, and TH and ACBT from Center for Basic Metabolic Research. All authors contributed to designing the protocol. MM wrote the draft of the manuscript. All authors revised the manuscript and have read and agreed to the submitted version.

Funding The work presented in this article was supported by the Novo Nordisk Foundation with a Steno Collaborative Grant (NNF19OC0058900) and Herlev and Gentofte Hospital with a PhD Scholarship. The funding source has no role in the design, conduct, analysis or reporting of the study.

Competing interests FP reports receiving honoraria for lectures and consultancy from AstraZeneca, Bayer, Boehringer Ingelheim, Novo Nordisk and Sanofi. PR reports honoraria to Steno Diabetes Centre Copenhagen for education and consultancy from Astellas, Abbott, AstraZeneca, Bayer, Boehringer Ingelheim, Novo Nordisk, Merck and Sanofi. RB reports honoraria for education and consultancy from Astra Zeneca, Bayer, Mundipharma, Vifor and Boehringer Ingelheim. DH reports honoraria for lectures from Gedeon Richter and advisory board attendance from Pharmacosmos. IB reports honoraria for lectures from Bayer and Amgen. FSE and LNF are employee and former employee, respectively, of Gubra Aps. MM, IB and KJ receive funding from the Novo Nordisk Foundation. The other authors report no conflicts of interest in this work.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iDs
Marie Møller http://orcid.org/0000-0002-1248-1740
Frederik Persson http://orcid.org/0000-0001-6242-6638

REFERENCES

1 Saeedi P, Petersohn I, Salpea P, *et al*. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International diabetes Federation diabetes Atlas. *Diabetes Res Clin Pract* 2019;157:107843.
2 Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: challenges, progress, and possibilities. *Clin J Am Soc Nephrol* 2017;12:2032–45.
3 Sun H, Saeedi P, Karuranga S, *et al*. IDF diabetes Atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract* 2022;183:109119.
4 Chertow G, Luyckx V, Marsden P, *et al*. Brenner and Rector's the kidney. In: *Diabetic Nephropathy*. Elsevier, 2019: 39.
5 Persson F, Rossing P. Diagnosis of diabetic kidney disease: state of the art and future perspective. *Kidney Int Suppl (2011)* 2018;8:2–7.
6 Gonzalez Suarez ML, Thomas DB, Barisoni L, *et al*. Diabetic nephropathy: is it time yet for routine kidney biopsy. *World J Diabetes* 2013;4:245–55. 10.4239/wjd.v4.i6.245 Available: https://pubmed.ncbi.nlm.nih.gov/24379914/
7 Thomas MC, Brownlee M, Susztak K, *et al*. Diabetic kidney disease. *Nat Rev Dis Primers* 2015;1:15018. 10.1038/nrdp.2015.18 Available: https://pubmed.ncbi.nlm.nih.gov/27188921/
8 Haar K, Rossing P, Persson F, *et al*. *Design and methodology of The PRIMETIME 1 cohort study, PRecIsion MEdicine based on kidney TIssue Molecular interrogation in diabetic nEphropathy*. To be Submitt,
9 Levey AS, Stevens LA, Schmid CH, *et al*. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;150:604–12.
10 Tervaert TWC, Mooyaart AL, Amann K, *et al*. Pathologic classification of diabetic nephropathy [Internet]. *J Am Soc Nephrol* 2010;21:556–63. 10.1681/ASN.2010010010 Available: https://pubmed.ncbi.nlm.nih.gov/20167701/
11 Andersen N, Hjortdal JØ, Schielke KC, *et al*. The Danishregistry of diabetic retinopathy. *Clin Epidemiol* 2016;8:613–9.
12 Flahault A, Cadilhac M, Thomas G. Sample size calculation should be performed for design accuracy in diagnostic test studies. *Journal of Clinical Epidemiology* 2005;58:859–62. 10.1016/j.jclinepi.2004.12.009 Available: https://pubmed.ncbi.nlm.nih.gov/16018921/
13 Basu M, Pulai S, Neogi S, *et al*. Prevalence of non-diabetic kidney disease and inability of clinical predictors to differentiate it from diabetic kidney disease: results from a prospectively performed renal biopsy study. *BMJ Open Diab Res Care* 2022;10:e003058.
14 Hogan JJ, Owen JG, Blady SJ, *et al*. The feasibility and safety of obtaining research kidney biopsy cores in patients with diabetes an interim analysis of the Trident study [Internet]. *Clin J Am Soc Nephrol* 2020;15:1024–6. 10.2215/CJN.13061019 Available: https://pubmed.ncbi.nlm.nih.gov/32341009/
15 Sharma SG, Bomback AS, Radhakrishnan J, *et al*. The modern spectrum of renal biopsy findings in patients with diabetes. *Clin J Am Soc Nephrol* 2013;8:1718–24.
16 Fiorentino M, Bolignano D, Tesar V, *et al*. Renal biopsy in patients with diabetes: A pooled meta-analysis of 48 studies. *Nephrol Dial Transplant* 2017;32:97–110. 10.1093/ndt/gfw070 Available: https://pubmed.ncbi.nlm.nih.gov/27190327/
17 Tong X, Yu Q, Ankawi G, *et al*. Insights into the role of renal biopsy in patients with T2Dm: A literature review of global renal biopsy results. *Diabetes Ther* 2020;11:1983–99.
18 Tan J, Zwi LJ, Collins JF, *et al*. Presentation, pathology and prognosis of renal disease in type 2 diabetes. *BMJ Open Diabetes Res Care* 2017;5:e000412.
19 Liang S, Zhang X-G, Cai G-Y, *et al*. Identifying parameters to distinguish non-diabetic renal diseases from diabetic nephropathy in patients with type 2 diabetes mellitus: A meta-analysis. *PLoS One* 2013;8:e64184.
20 Ellison DH. Me or your own eyes: RNA-seq and the kidney. *J Am Soc Nephrol* 2021;32:768–71. 10.1681/ASN.2021010017 Available: https://jasn.asnjournals.org/content/32/4/768
21 Fan Y, Yi Z, D'Agati VD, *et al*. Comparison of kidney transcriptomic profiles of early and advanced diabetic nephropathy reveals potential new mechanisms for disease progression. *Diabetes* 2019;68:2301–14. 10.2337/db19-0204 Available: https://doi.org/10.2337/db19-0204
22 Dubin RF, Rhee EP. Proteomics and Metabolomics in kidney disease, including insights into etiology, treatment, and prevention. *Clin J Am Soc Nephrol* 2020;15:404–11. 10.2215/CJN.07420619 Available: https://doi.org/10.2215/CJN.07420619
23 Sethi S. New "Antigens" in membranous nephropathy [Internet]. *J Am Soc Nephrol* 2021;32:268–78. 10.1681/ASN.2020071082 Available: https://doi.org/10.1681/ASN.2020071082
24 Niewczas MA, Pavkov ME, Skupien J, *et al*. A signature of circulating inflammatory proteins and development of end stage renal disease in diabetes. *Nat Med* 2019;25:805–13.
25 Coca SG, Nadkarni GN, Huang Y, *et al*. Plasma biomarkers and kidney function decline in early and established diabetic kidney disease. *J Am Soc Nephrol* 2017;28:2786–93. 10.1681/ASN.2016101101 Available: https://pubmed.ncbi.nlm.nih.gov/28476763/
26 Parsa A, Kao WHL, Xie D, *et al*. APOL1 risk variants, race, and progression of chronic kidney disease. *N Engl J Med* 2013;369:2183–96. 10.1056/NEJMoa1310345 Available: https://www.nejm.org/doi/10.1056/NEJMoa1310345
27 Macisaac RJ, Ekinci EI, Jerums G. Markers of and risk factors for the development and progression of diabetic kidney disease. *Am J Kidney Dis* 2014;63(2 Suppl 2):S0272-6386(13)01431-5):S39–62.:. 10.1053/j.ajkd.2013.10.048 Available: https://pubmed.ncbi.nlm.nih.gov/24461729/
28 Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease. Towar Precis MED build a Knowl NETW Biomed Res a new Taxon DIS. 2011: 1–128. Available: https://pubmed.ncbi.nlm.nih.gov/22536618/
29 Yamazaki T, Mimura I, Tanaka T, *et al*. Treatment of diabetic kidney disease: current and future. *Diabetes Metab J* 2021;45:11–26.
30 Corapi KM, Chen JLT, Balk EM, *et al*. Bleeding complications of native kidney biopsy: a systematic review and meta-analysis. *Am J Kidney Dis* 2012;60:62–73. 10.1053/j.ajkd.2012.02.330 Available: https://pubmed.ncbi.nlm.nih.gov/22537423/
31 Examination of the end-stage kidney. *Diagnostic Pathol Kidney Dis* 2016:1040–5.
32 Gluck C, Ko YA, Susztak K. Precision medicine approaches to diabetic kidney disease. *Curr Diab Rep* 2017;17.
33 Andrulli S, Rossini M, Gigliotti G, *et al*. The risks associated with percutaneous native kidney biopsies: a prospective study. *Nephrol Dial Transplant* May 19, 2022. Available: https://academic-oup-com.ep.fjernadgang.kb.dk/ndt/advance-article/doi/10.1093/ndt/gfac177/6589424
34 Tøndel C, Vikse BE, Bostad L, *et al*. Safety and complications of percutaneous kidney biopsies in 715 children and 8573 adults in Norway 1988-2010. *Clin J* 2012;7:1591–7. 10.2215/CJN.02150212 Available: https://pubmed.ncbi.nlm.nih.gov/22837269/
35 Bakdash K, Schramm KM, Annam A, *et al*. Complications of percutaneous renal biopsy. *Semin Intervent Radiol* 2019;36:97–103. 10.1055/s-0039-1688422 Available: https://pubmed.ncbi.nlm.nih.gov/31123379/
36 Hasegawa S, Okada A, Aso S, *et al*. Association between diabetes and major bleeding complications of renal biopsy. *Kidney International Reports* 2022;7:232–40.
37 Halimi J-M, Gatault P, Longuet H, *et al*. Major bleeding and risk of death after percutaneous native kidney biopsies a French nationwide cohort study. *CJASN* 2020;15:1587–94.
38 Oshima M, Shimizu M, Yamanouchi M, *et al*. Trajectories of kidney function in diabetes: a Clinicopathological update. *Nat Rev Nephrol* 2021;17:740–50. 10.1038/s41581-021-00462-y Available: https://pubmed.ncbi.nlm.nih.gov/34363037/
39 Klessens CQF, Woutman TD, Veraar KAM, *et al*. An autopsy study suggests that diabetic nephropathy is Underdiagnosed. *Kidney Int* 2016;90:S0085-2538(16)00313-6:149–56.:. 10.1016/j.kint.2016.01.023 Available: https://pubmed.ncbi.nlm.nih.gov/27165826/
40 Indvandrere og efterkommere. Danmarks Statistik. n.d. Available: https://www.dst.dk/da/Statistik/emner/borgere/befolkning/indvandrere-og-efterkommere
41 Welcome to the kidney precision medicine project. 2022. Available: https://www.kpmp.org/
42 American Diabetes Association, 2020. Classification and diagnosis of diabetes: standards of medical care in Diabetes-2020. *Diabetes Care*

2020;43(Supplement_1):S14–31. Available: https://pubmed.ncbi.nlm.nih.gov/31862745/

43 Kellum JA, AspelinP, KarolinskaUH, *et al*. *KDIGO Clinical Practice Guideline for Acute Kidney Injury*. 2012.

Supplementary Study organization
Executive and Steering committee members.

Executive Committee

Chair of the committee
Frederik Persson.
MD DMSc
Steno Diabetes Center Copenhagen
Niels Steensens Vej 2
2820 Gentofte

Voting members
Ditte Hansen
MD PhD
Dept of Nephrology
Herlev Hospital
Borgmester Ib Juuls Vej 1
2730 Herlev

Frederik Persson.
MD DMSc
Steno Diabetes Center Copenhagen
Niels Steensens Vej 2
2820 Gentofte

Iain Bressendorff
MD PhD
Dept of Nephrology
Herlev Hospital
Borgmester Ib Juuls Vej 1
2730 Herlev

Peter Rossing
Professor, MD DMSc
Steno Diabetes Center Copenhagen
Niels Steensens Vej 2
2820 Gentofte

Rikke Borg
MD PhD
Dept of Nephrology
Roskilde University Hospital
Sygehusvej 10
4000 Roskilde

Non-voting members
Karina Haar Jensen
MD PhD-student
Dept of Nephrology
Roskilde University Hospital
Sygehusvej 10
4000 Roskilde

Marie Møller
MD PhD-student
Dept of Nephrology
Herlev Hospital
Borgmester Ib Juuls Vej 1
2730 Herlev

Steering Committee

Chair of the committee
Ditte Hansen
MD PhD
Dept of Nephrology
Herlev Hospital
Borgmester Ib Juuls Vej 1
2730 Herlev

Pathologist
Dorrit Krustrup
MD, PhD
Dept of Pathology, Herlev Hospital
Herlev University Hospital
Borgmester Ib Juuls Vej 1
2730 Herlev

Eva Gravesen
MD, PhD
Dept of Pathology, Herlev Hospital
Herlev University Hospital
Borgmester Ib Juuls Vej 1
2730 Herlev

Nephrologists
Bjarne Ørskov
MD, PhD
Dept of Nephrology
Roskilde University Hospital
Sygehusvej 10
4000 Roskilde

Bo Broberg
MD
Dept of Nephrology
Herlev University Hospital
Borgmester Ib Juuls Vej 1
2730 Herlev

Johan Povlsen
MD, DMSc
Dept of Nephrology
Århus University Hospital
Palle Juul-Jensens Boulevard 99
8200 Århus

Kristine Dyhr Schandorff
MD
Dept of Nephrology
Nordsjællands Hospital
Dyrehavevej 29
3400 Hillerød

Mads Hornum
Professor, MD, PhD
Dept of Nephrology P
Rigshospitalet
Blegdamsvej 9
2100 Kbh Ø

Morten Lindhardt
MD, PhD
Dept of Nephrology
Holbæk Hospital
Smedelundsgade 60
4300 Holbæk

Charlotte Strandhave
MD, PhD
Dept of Nephrology
Aalborg University hospital
Mølleparkvej 4
9000 Aalborg

Frank Holden Mose
MD, PhD
Dept of Nephrology
Regionshospitalet Gødstrup
Hospitalsparken 15
7400 Herning

Hans Dieperink
MD DMSc
Dept of Nephrology
Odense University Hospital
J. B. Winsløws Vej 4
5000 Odense C

Endocrinologists

Allan Kofoed-Enevoldsen
MD
Dept of Endocrinology
Nykøbing F. Sygehus
Hospitalsvej, 4800
Nykøbing Falster

Karoline Schousboe
MD, PhD
Steno Diabetes Center Odense
Kløvervænget 10, 3
5000 Odense C

Signe Sabine Sætre Rasmussen
MD, PhD
Dept of Endocrinology
Nordsjællands Hospital
Dyrehavevej 29
3400 Hillerød

Thomas Almdal
MD, DMSc
Dept of Endocrinology
Rigshospitalet
Blegdamsvej 9
2100 Kbh Ø

Peter Vestergaard
MD, DMSc
Steno Diabetes Center North Denmark
Aalborg University Hospital
Søndre Skovvej 3E
9000 Aalborg

Peter Haulund Gæde
MD, DMSc
Departments of Internal Medicine,
Endocrinology,
Slagelse Hospital
Ingemannsvej 30
4200 Slagelse

Peter Rossing
MD, DMSc
Steno Diabetes Center Copenhagen
Borgmester Ib Juuls Vej 83
2730 Herlev

Søren Tang Knudsen
DMSc, PhD
SDCA – Steno Diabetes Center Aarhus
Århus University Hospital
Palle Juul-Jensens Boulevard 99
8200 Århus

Urd Lynge Kielgast
MD, PhD
Dept of Endocrinology
Sjællands Universitetshospital, Køge
Lykkebækvej 1
4600 Køge

Supplementary Table S1

Full list of exclusion criteria

Exclusion criteria

- Signs of acute kidney failure according to the KDIGO classification (16) at the time for kidney biopsy or the last six months before kidney biopsy
- Kidney transplant recipient
- Previous medical kidney biopsy
- Women who are pregnant or planning to become pregnant before the kidney biopsy is performed
- Hemoglobin < 96.7 g/L
- International Normalised Ratio (INR) >1.4 at the time of biopsy
- Platelet count < 100 x 10^9/l
- Uncontrolled high blood pressure (defined as systolic blood pressure > 160 mmHg and/or diastolic blood pressure > 100 mmHg)
- Only one functioning kidney
- Evidence of urinary tract obstruction or hydronephrosis at the time of biopsy
- Multiple bilateral kidney cysts
- Kidney infection, peri-renal infection, or cutaneous infection that overlies the kidney the time of biopsy
- Unwilling to receive a blood transfusion
- Unable to lie flat in bed six hours after the biopsy
- Any other contra-indications for percutaneous kidney biopsy according to local clinical guidelines
- Inability to withdraw nonsteroidal anti-inflammatory drugs (NSAID) 7 days before the biopsy
- Treatment with Marcoumar (all other anticoagulants are accepted when paused appropriately)
- High thromboembolic risk combined with held in anticoagulation or antiplatelet therapy according to the report "Perioperative regulation of antithrombotic treatment", a Danish guideline* (17)
- Unable to understand written and oral information

*** Mechanical heart valve, atrial fibrillation,** AND CHA2DS2-VASc> 5 and/or stroke within the last three months, recurrent **venous thromboembolism** OR venous thromboembolism within the last three months, less than six weeks after uncomplicated **Acute Coronary Syndrome (ACS)** with or without revascularization (Percutaneous Coronary Intervention (PCI)) with Bare Metal Stents (BMS) or Coronary Artery Bypass Grafting (CABG)), less than three months after uncomplicated **ACS** with revascularization (PCI with Drug Eluting Stent (DES)), less than 9-12 months after complicated **ACS** (e.g., reinfarction or stent thrombosis), less than one month after revascularization in individuals with **stable Coronary Artery Disease (CAD)** (PCI with BMS or CABG), less than three months after revascularization in individuals with **stable CAD** (PCI with DES), less than three months after **stroke**, or **Transient Ischemic Attack (TIA)**

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 flourescence microspopy (LSFM)

Furthermore, Light-sheet flourescence microspopy (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 Scientific, Roskilde, Denmark) and stored at 4 ^{o}C for 24 hours to allow the RNAlater to infiltrate the tissue. After 24 hours, the tube is transferred to -80 ^{o}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

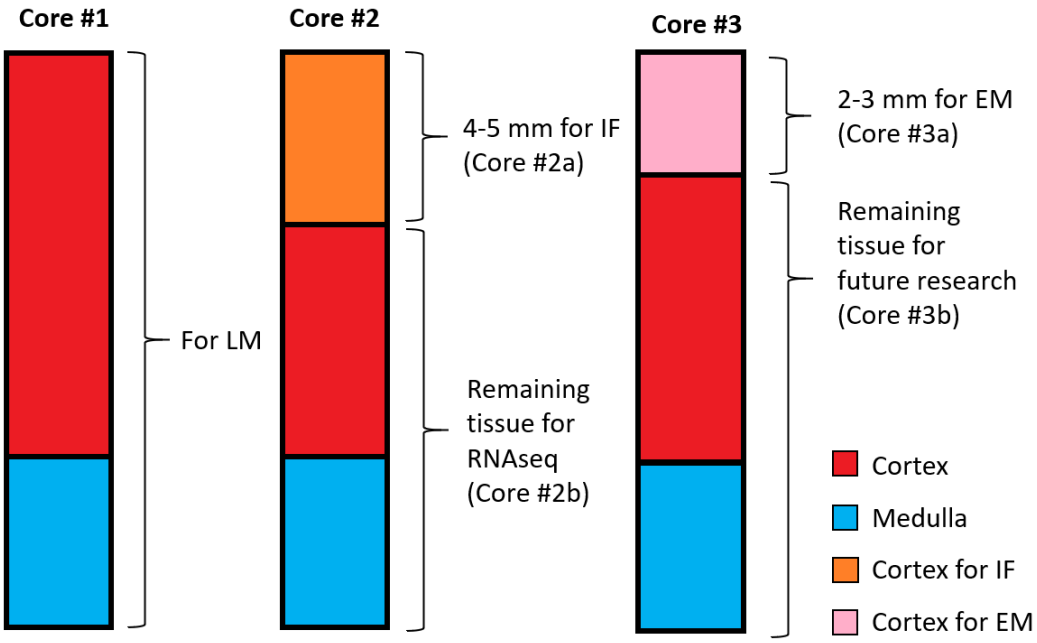
Tissue RNA sequencing will be substituted with single nuclei RNA sequencing in selected participants The kidney tissue will be snap frozen in liquid nitrogen 1-2 minutes after the biopsy procedure. Nuclei are isolated from frozen tissue, and mRNA transcripts derived from every single nucleus will be 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 the protein/metabolite in question.

1. Tervaert TWC, Mooyaart AL, Amann K, Cohen AH, TerenceCook H, Drachenberg CB, et al. Pathologic classification of diabetic nephropathy [Internet]. Vol. 21, Journal of the American Society of Nephrology. J Am Soc Nephrol; 2010 [cited 2020 Sep 1]. p. 556–63. Available from: https://pubmed.ncbi.nlm.nih.gov/20167701/


Core #1
Core #2
Core #3
For LM
4-5 mm for IF
(Core #2a)
Remaining
tissue for
RNAseq
(Core #2b)
2-3 mm for EM
(Core #3a)
Remaining
tissue for
future research
(Core #3b)
Cortex
Medulla
Cortex for IF
Cortex for EM