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)

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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 PReCIson 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 m². 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.

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.1–3 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. 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. 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. 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.
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 m² 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 1.

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; 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 RNA later (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

Box 2  List of inclusion and exclusion criteria.

Inclusion criteria
⇒ Age ≥18 years
⇒ Diagnosis with T2D according to the American Diabetes Association
⇒ eGFR>30 mL/min/1.73 m²
⇒ 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 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 <100×10⁹ /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, antplatelet 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.
severity of the findings according to the Renal Pathology Society Classification of Diabetic Nephropathy. 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. 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 20mL 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. In accordance with the findings by Basu et al., we expect a prevalence of 20% of NDKD in the included population. 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.

**Statistical considerations**

Baseline variables will be described and compared between participants with biopsy-proven diabetic nephropathy and NDKD. Categorical data will be compared by \( \chi^2 \) 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. 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.

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.\textsuperscript{20, 21} Proteomics and metabolomics can characterise different disease processes in a non-invasive manner,\textsuperscript{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).\textsuperscript{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.\textsuperscript{24–26} 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.\textsuperscript{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.\textsuperscript{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 in the last couple of decades and more are to come.\textsuperscript{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 medications 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,\textsuperscript{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,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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)).\textsuperscript{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%\textsuperscript{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 76320 and 52138 patients, respectively, examined the risks of major bleeding complications after kidney biopsies performed between 2012 and 2018.\textsuperscript{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 multi-agent 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 register 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.

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**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.

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