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the Role of cArdiac inflammation, endoThelial dysfunction, and fibrosis in FabrY disease: the RATIFY study

Project group

- Caroline Kistorp[1],
- Niels H. Brandt-Jacobsen[1, 2],
- Philip Hasbak[2]
- Niels Vejlstrup[3]
- Redi Pecini[3]

Institution

[1]Department of Nephrology and Endocrinology, Center of Cancer and Organ Diseases, University Hospital of Copenhagen - Rigshospitalet, Copenhagen, Denmark

[2]Department of Clinical Physiology and Nuclear Medicine, Center of Diagnostics, University Hospital of Copenhagen – Rigshospitalet, Copenhagen, Denmark

[3]Department of Cardiovascular Imaging, the Heart Center, University Hospital of Copenhagen – Rigshospitalet, Copenhagen, Denmark

Sponsor, initiator, and principal investigator

Caroline M. Kistorp, MD, PhD, Professor,
Department of Neprhology and Endocrinology,
Centre of Cancer and Organ Disease,
University Hospital of Copenhagen – Rigshospitalet
Blegdamsvej 9
DK-2100 Copenhagen, Denmark
Telephone: +45 22 47 56 99 / +45 35 45 96 42
Email: caroline.micheala.kistorp@regionh.dk

Specialist registrar

Niels Høeg Brandt-Jacobsen, MD, Ph.D, Post.doc

Overreaching aim

The overall objective of this study is to investigate Fabry-related cardiomyopathy, evaluating native T1-mapping, coronary microvascular function, cardiac inflammation, and cardiac injury in an effort to improve the ability to detect disease and ease diagnostics. The study aims to achieve this by:

1) Investigating the association between cardiac inflammation, fibrosis, and injury against the distribution and degree of microvascular disease in patients with Fabry disease with and without left ventricular hypertrophy (LVH) using cardiac magnetic resonance (CMR) imaging and ^{82}Rubidium Positron emission tomography and computer tomography (^{82}Rb-PET/CT).
2) Using an extensive, in-depth biomarker blood panel to investigate the pathological pathways associated with Fabry disease and Fabry-related cardiomyopathy.

Introduction

Fabry disease is a rare X-linked lysosomal disorder affecting 1:58,000 in the Danish population (males 1:85,000; females 1:44,000) [1]. A mutation in the gene encoding the enzyme α-galA, an essential enzyme in normal lysosomal function, causes progressive cellular accumulation of the glycosphingolipids, especially globotriaosylceramide (Gb3), leading to a severe disruption of cellular function. Men with a classic phenotype present at an early age with no or very low α-galA-activity and develop early multi-organ involvement, especially renal and cardiac disease, resulting in a severely impaired prognosis [2]. However, both men and women can be affected in the presence of a disease-bearing mutation [2,3]. Although often less severe, females and men with a non-classic phenotype also present with early organ involvement, but in a much more heterogeneous degree. While the classic male phenotype evidently need early initiation of therapy, the need of initiating treatment is less clear in females and in males with a non-classic phenotype [3]. Furthermore, incidence of new genetic variants of uncertain clinical significance, which could indicate a Fabry diagnosis, has increased due to the general implementation of genetic screening programs, especially in hypertrophic cardiomyopathy [4–6].

At present, approximately 100 patients in Denmark are diagnosed with a disease-bearing mutation and followed at the Danish National Fabry Centre at Copenhagen University Hospital - Rigshospitalet. The continuing clinical challenge of who will need and when to initiate treatment necessitates close observation of patients at risk and, thus, a continued search for precise, reliable methods able to detect early cardiac involvement. Early initiation of therapy prior to the full manifestation of Fabry disease has shown to impede progression while evidence suggests a late

initiation of treatment has reduced effects [7–10], further stressing the importance of early detection of Fabry cardiomyopathy and thus, early initiation of treatment.

Cardiomyopathy in Fabry disease

In Fabry disease, the complication of greatest prognostic impact is the cardiac manifestations, leading to arrhythmias, heart failure and cardiac death [2,3,11]. Although, the progressive deposition of Gb3 accounts for a maximum of 5% of total cardiac volume [12–14], a disproportionate cardiomyocyte hypertrophy, coronary wall thickening and endothelial dysfunction have been the general findings [12–14]. Indeed, left ventricular hypertrophy (LVH) has long been a hallmark of Fabry cardiomyopathy [15], however, the disproportionate relationship between a relatively small accumulation of Gb3 and the clinical cardiac manifestation of pronounced LVH has led to the proposal of the accumulation of Gb3 *per se* causes an early disruption of cellular function by pathways involving oxidative stress and inflammation [14–18]. The stress induced by Gb3 is believed to exacerbate left ventricular mass increase, cellular apoptosis, and causes the irreversible substitution of functioning tissue with reparative fibrosis.

A key site and mechanism of stress and perhaps an early indicator of disease may, therefore, be found investigating changes in exchange across the vascular wall. Not only does Gb3 accumulation cause structural changes [12–14,19], Gb3 have been shown to induce the production of reactive oxygen species (ROS) through important inflammatory pathways such as transforming growth factor (TGF) β-dependent signaling, a key step in the Fabry-related vasculopathy preceding fibrosis [18]. The early structural changes in endothelial structure ties directly to early endothelial dysfunction and detrimental changes in microvascular function [18,19].

Fabry-related cardiomyopathy and imaging

As one of the most distinguishing factors of Fabry cardiomyopathy, the ability to accurately detect LVH is paramount. Recognizing the improved spatial resolution of CMR imaging, a shift from echocardiography to CMR has recently caused CMR to be recommended as part of routine clinical practice in supplement to echocardiography to improve detection of changes in left ventricular mass [15,20,21].

However, the addition of CMR-based approach has revealed several image-derived parameters of interest, which may provide insight into key aspects of the underlying mechanisms of Fabry disease, such as ongoing Gb3 accumulation, changes in fibrotic burden, and ongoing inflammation in the early stages of disease [15]. In general, Fabry cardiomyopathy often presents with low native T1 values irrespective of the presence of LVH, which have been suggested as an indirect measure

of Gb3 burden [15,16,22]. In comparison, reparative fibrosis increases T1-values [15,16,22]. Furthermore, increased T2-values could be an indirect measure of ongoing inflammation [15–17], and interestingly, T2-values have been shown to decrease in concert with decreases in left ventricular mass following enzyme replacement therapy (ERT) [16,21]. Despite its promise, the overall use of T1 and T2 mapping has, however, not yet been implemented in clinical practice to guide clinical decision making in Fabry cardiomyopathy.

In comparison, although PET/CT-based imaging has shown promise by detecting early Fabry-related changes such as coronary microvascular disease (CMD), which by itself provides important prognostic information [23], use is limited due to radiation. However, the detection of CMD can elucidate on the progression of vascular endothelial dysfunction and may even be a key step in detecting early disease. Not only is the degree of CMD associated with the degree of LVH [24–26], of note, CMD seem to precede changes in left ventricular mass, as signs of CMD have been found irrespective of sex or the presence of LVH [24–26], suggesting its use is instrumental in detecting the early steps of Fabry-related cardiomyopathy.

Heterogeneity in presentation and regional disease progression?

In Fabry, the cardiac involvement is believed to progress diffusely throughout the myocardium, with symmetric LVH as a key finding. However, of note, previous reports show great regional heterogeneity in the measured T1- and T2-values as well as regional differences using strain analysis to detect functional decline [15–17,22]. Furthermore, low T1-values, believed to be a pathognomonic feature of Fabry-associated cardiomyopathy, has been proposed to increase and pseudo-normalize in tandem with disease progression and development of fibrosis, making measuring change over time especially important [16].

CMR and PET/CT separately provide global measures of fibrosis, inflammation, and microvascular function, therefore, the combination of modalities may explain the regional differences specific to the individual patient, which cannot be detecting using one approach alone. A combined approach may therefore provide key insights into the pathology of Fabry-associated cardiomyopathy – especially important in distinguishing early and late-stage disease.

Hypothesis

In the current study, we aim to investigate the association between inflammatory and fibrotic pathways and microvascular dysfunction with the presentation of Fabry cardiomyopathy to elucidate on the underlying mechanisms of Fabry-related cardiomyopathy, thereby further improving the

diagnostic approach in early disease detection, and the role of the chosen modalities in monitoring disease progression.

We hypothesize patients with Fabry disease exhibit

- Increased T2 values as a sign of active inflammation – a sign which will exhibit regional differences
- Reduced native T1-values as a sign of Gb3-burden and fibrosis – a value which changes dependent on the presence of fibrosis
- Reduced myocardial flow reserve (MFR) - a sign of microvascular dysfunction and CMD, which further decreases with disease progression.

Furthermore, we hypothesize the values mentioned above are highly reliant on the presence of reparative fibrosis, with areas exhibiting the most pronounced impairment correlating across modalities.

Participants and methods

Study design

A longitudinal, non-interventional cohort study and will consist of a group of patients from the Danish National Fabry Cohort followed at the Danish National Fabry Centre, Department of Neprhology and Endocrinology, Copenhagen University Hospital, Rigshospitalet. Furthermore, a control group of healthy age- and sex-matched individuals will be included to comprise a contemporary control cohort and will undergo the same program.

Participants

We will include 36 participants with Fabry disease previously verified by genetic testing. The Fabry patients will be stratified by echocardiograph-verified left ventricular hypertrophy (LVH evaluated as LVMi, Men/female: ≥115 / ≥95 g/m2) [27]. A control group will consist of 18 healthy age- and sex-matched controls without LVH.

Inclusion and exclusion criteria

Fabry cohort

Inclusion criteria

- Male and female individuals with a genetically-verified diagnosis of Fabry disease
- ≥ 18 years of age

- Able to give informed consent

Exclusion criteria*

- Any contraindication against a pharmacologically induced rest-stress PET/CT protocol according to local safety procedures such as acute coronary syndrome, severe bronchospasm, severe chronic obstructive pulmonary disease, cardiac arrhythmia.
- Any contraindication for MRI according to standard checklist used in clinical routine, including claustrophobia or metallic foreign bodies, metallic implants, internal electrical devices, or permanent makeup/tattoos that cannot be declared MR compatible.
- Pregnancy

* Individuals with a contraindication for one of the two imaging modalities will be able to participate in the study, but cannot undergo the imaging protocol in question. However, the proportion of individuals who cannot undergo both imaging protocol are not allowed to contribute more than 20% of participants in total.

Control cohort

Inclusion criteria

- Male and female individuals ≥ 18 years of age.
- Able to give informed consent

Exclusion criteria

- A genetically-verified diagnosis of Fabry disease.
- Family member to a patient with a genetically-verified diagnosis of Fabry disease
- Cancer expected to influence life expectancy.
- Known heart failure, previous apoplexy or previously established kidney disease.
- Initiation or change of antihypertensive therapy within 3 months of enrollment.
- Known LVH as evaluated on echocardiography
- Any contraindication for a medicine-induced stress PET/CT protocol according to local safety procedures such as acute coronary syndrome, severe bronchospasm, severe chronic obstructive pulmonary disease, cardiac arrhythmia.
- Any contraindication for MRI according to standard checklist used in clinical routine, including claustrophobia or metallic foreign bodies, metallic implants, internal electrical devices, or permanent makeup/tattoos that cannot be declared MR compatible.
- Pregnancy

Endpoints

Primary endpoint on ^{82}Rb-PET/CT

- A between-group difference in change in global myocardial flow reserve (MFR) evaluated by ^{82}Rb-PET/CT, comparing Fabry patients with controls irrespective of the presence of LVH.

Primary endpoint on CMR

- A between-group difference in change in global native T1 evaluated by MRI, comparing Fabry patients with controls irrespective of the presence of LVH.

Secondary endpoints

- A between-group difference in change in global myocardial flow reserve (MFR) evaluated by ^{82}Rb-PET/CT, comparing Fabry patients with controls accounting for the presence of LVH.
- A between-group difference in change in global native T1 evaluated by MRI, comparing Fabry patients with controls accounting for the presence of LVH.
- A between-group difference in change in global T2 values evaluated by MRI, comparing Fabry patients with controls accounting for the presence of LVH.
- A between-group difference in change in global T2 values evaluated by MRI, comparing Fabry patients with controls irrespective of the presence of LVH.

Exploratory endpoints

Analysis on exploratory endpoints consist of both the comparison of across the observed parameters at baseline and the observed change over time. Furthermore, analysis are performed both irrespective of and according to LVH and predefined Fabry-specific subgroups.

On ^{82}Rb-PET/CT

- A between-group difference in regional MFR evaluated by ^{82}Rb-PET/CT according to the 17-segment model.

On cardiac MRI

- A between-group difference in regional native T1 evaluated by MRI according to the 17-segment model.
- A between-group difference in regional native T2 values evaluated by MRI according to the 17-segment model.

Cross-modality comparison

- An association between regional impairment in global T1, T2 and MFR and according to the 17-segment model.

- An association between regional impairment in T1, T2, MFR, and the placement of irreversible reparative fibrosis detected using late-gadolinium enhancement.
- An association between extent and size of irreversible reparative fibrosis detected using late-gadolinium enhancement and total perfusion defect by Rb-PET/CT.

Additional pre-defined endpoints of interest (Rb-PET/CT):

Agatson score, myocardial perfusion during rest and pharmacologically induced stress, cardiac volumes measured on both modalities (Absolute and indexed; Left ventricular mass [LVM], end-diastolic volume [EDV], end-systolic volume [ESV], left ventricular ejection fraction [LVEF]).

Additional pre-defined endpoints of interest (CMR):

Cardiac volumes (Absolute and indexed; Left ventricular mass [LVM], end-diastolic volume [EDV], end-systolic volume [ESV], left ventricular ejection fraction [LVEF]), Left and right ventricular strain analysis (longitudinal, circumferential, and radial strain), indices of diastolic function.

Cross-modality endpoints of interest:

Cross-modality bias and variability in the assessment of cardiac volumetry and cardiac mass with CMR as the reference standard.

Predefined subgroups

Presence of left ventricular hypertrophy, biological sex, disease severity by Mainz Severity Score Index (MSSI) and Fabry International Index (FPI).

Procedures

Imaging procedures

^{82}Rb-PET/CT protocol

^{82}Rb-PET/CT provides regional information on myocardial flow, with CMD evaluated by MFR – the difference between myocardial flow at rest and at pharmacologically induced stress. Regionality is evaluated according to the 17-segment model. A routine clinical ^{82}Rb-PET/CT protocol will be performed according to local standard. Patients are to meet after a 4 hour fast, with the restriction of not having consumed substances containing caffeine at least 12 hours prior to scanning.

Table 1: ^{82}Rb-PET/CT protocol

Procedures	Sequences	Time (app.)
PET/CT (rest)	- Scan	10 min

PET/CT (stress)	- Repeat scan during adenosine infusion (mL/kg)	10 min

Cardiac magnetic resonance protocol

CMR imaging-derived values elucidating on fibrosis and inflammation provides insights on regional differences. Regionality is evaluated according to the 17-segment model. A routine CMR protocol will be performed as is standard in the current Fabry observation program followed by patients at the Danish National Fabry Center. Patients are to meet subsequent to a 4-hour fast, with the additional restriction of not having consumed substances containing caffeine 12 hours prior to scanning.

Table 2: Overview of cardiac MRI protocol

Procedures	Sequences	Time (app.)
MRI session	- Scan	30 min
	- Scan post-contrast	20 min

Clinical procedures

In addition to the imaging protocol, the participants will receive a full clinical work-up, fill out a questionnaire and undergo blood- and urine-sampling (Table 2).

Table 2: Clinical procedures

Procedures	**Outcome**
Semi-structured interview	- Medical history, including concomitant chronic disease, current medication, and dietary supplements. - Alcohol, tobacco, and substance abuse - Socioeconomic status and educational background

Clinical examination	- Anthropomorphic measures (Age, height, weight, waist and hip circumference, body mass index [BMI, kg/m^2])
	- Office blood pressure and pulse
	- Electrocardiogram
	- Examination as specified in the
	  - Mainz Severity Score Index
	  - Fabry international prognostic index

Patient reported outcome	- ”Dit helbred og velbefindende” (SF-36®; *Quality of life*)
Blood and urine samples	Research Biobank focusing on

	- Heart function, inflammation, and fibrosis and cellular signaling in the kidney - Metabolic dysfunction - Risk markers of disease - Markers related to Fabry disease risk and progression <u>Establishing a biobank of future research</u> - Surplus biological material acquired will be kept in a biobank for future research. *See table 3 for overview of biomarkers*

Medical interview and clinical examination.

Participants will undergo a semi-structured interview to acquire relevant patient information. The consists of their previous medical history and their current medication, established concomitant disease, their use of dietary supplements, their use of alcohol, tobacco and substance abuse, their current employment status and choice of field, and educational background (i.e. length of education).

They will undergo a focused clinical examination acquiring basic anthropomorphic measures age, height, weight, hip- and waist circumference. Furthermore, the clinical examination will consist of general health measures, consisting of a measurement of their office blood pressure, pulse, an electrocardiogram, and a physical examination specified to fulfill the elements of a modified Mainz Severity Score Index and the Fabry International Prognostic Index.

Patient reported outcome

Participants will be asked to answer a health-related questionnaire. The questionnaire is used and validated in Fabry disease:

- The SF36 Quality of Life-questionnaire

Blood and urine sampling

Participants will be required to undergo standard antecubital venipuncture to sample blood. The amount drawn will be 300 mL at the study visit. Regarding urine sampling, participants are to provide a first-voided, morning urine sample.

All samples are acquired and immediately frozen as aliquots and subsequently stored at -80 degrees (Celsius) until the end of the trial, comprising a research biobank. The research biobank is to cease 6 months after last patient last visit; scheduled at 01.12.2029. At the day the research biobank ceases, the remaining material will be considered surplus and will be transferred to a

biobank of future research. Regarding the research biobank, when performing the planned analysis, samples are to be thawed and analyzed in immediate succession, with repeat intra- and inter-assay analyses to determine and validate analysis-specific variation according to local laboratory standards. Postponing analysis until end of study is done to minimize time-dependent variation and is deemed necessary as part of trial conduct. Surplus material – both blood and urine – is stored and kept, comprising a biobank of future research. The biobank of future research is established and kept in accordance with the Danish laws on Data Protection and Data Security.

Biomarkers of interest to be analyzed on samples of blood or urine are specifically defined in protocol (table 5). Although all biomarkers are chosen due their prospective or established clinical value, further biomarkers may be necessary to be defined at a later date (e.g. omcis-based blood or urinary biomarker panel). If one or more biomarkers of interest are thought of post-hoc, a new informed consent is required and an amendment to the original protocol will be made to be accepted by the relevant ethical committee prior to performing the analyses. In this instance, the relevant ethical committee can provide dispensation regarding the need for renewed consent. Separate consent forms will be signed in regard to the research biobank and the biobank of future research, respectively (See Consent forms).

Table 5: Overview of pre-specified biomarkers in research biobank

Sample	Biomarker	Volume
Blood sample	*Circulating biomarkers specific to Fabry's Disease* - α-gal A activity in leucocytes, plasma Lyso-Gb3 and Gb3, and urine Gb3 *Cardiac function, fibrosis and inflammation, and cellular signaling in the heart* - GDF-15, TGF-β, FGF-21, FGF-23, VEGF, collagens, TnI, hsTNT, proANP, proCNP, NT-proBNP *Clinical biomarkers of metabolic dysfunction* - fasting plasma glucose, insulin, 3-OH-hydroxybutyrate, HbA1c, and lipid profile. *General biomarkers of risk deemed of clinical value* - Creatinine	300 mL
Urine sample	*Urinary excretion* - urinary podocyte excretion, albumin and creatinine excretion	200 mL

Study visits

Participants eligible for inclusion in the study will be required to attend 2 study visits to participate in the study program at baseline and again after 3 years.

Statistical considerations

Justification of sample size

^{82}Rb-PET/CT

From our own experience regarding normal values on MFR on healthy individuals, a baseline mean MFR can be expected to be 3.7 (SD 0.8) (Unpublished). In another cohort, baseline mean MFR in Fabry disease has previously been reported as 1.5 (SD 0.5) [26].

Conversely, not much is known as to the expected time-dependent change, however, a 3-year mean decrease of 0.5 units is deemed to be a clinically significant difference. In a prospective cohort, a between-group mean change of 0.5, an SD of the difference of 0.5, 80% power, a type 1 error of 5%, the final design necessitates the inclusion of 51 participants 1:1:1 to detect a 3-year-change – corresponding to 17 participants in each group.

CMR

Previously collected data has provided scanner-specific normal values for baseline mean T1 values, furthermore, a 40/60 ratio of men vs women included is expected. Therefore, a healthy, age- and sex-matched to cohort would be expected to present with a baseline mean T1-value of approximately 1013 ms (SD 23). Consequently, patients with Fabry disease have previously been reported to have a mean value in the range of 917-981 ms (SD 49-61) [16].

The expected time-dependent change in T1-values as a sign of progression is currently unknown, however, a 3-year decline of 50 ms when compared to an expected stable healthy control cohort is deemed to be clinically significant. Thus, in a prospective cohort, a between-group mean change of 50 ms, an SD of the difference of 50, 80% power, a type 1 error of 5%, the final design necessitates the inclusion of 51 participants 1:1:1 to detect a 3-year-change – corresponding to 17 participants in each group.

Additional considerations

The primary analysis is a direct comparison between patients with Fabry and healthy controls. To account for incidental claustrophobia and the inability to scan using all three modalities causing an unexpected loss of data, we aim to include 54 participants, preferably grouped 18:18:18 participants (Fabry w/ LVH; Fabry w/o LVH; age- and sex-matched controls).

The current Danish National Fabry cohort consists of a little more than 100 individuals, with approximately 33% believed to have a history of LVH. Therefore, a Fabry-specific study size consisting of 36 patients with genetically verified Fabry disease - of which 18 are expected to have

LVH - is deemed feasible using the proposed patient population. However, if the goal distribution according to the presence of LVH is determined to be impossible during the conduct of the study, the study will allow a skewered distribution in the Fabry cohort.

A control cohort of 18 healthy age- and sex-matched controls included as part of the study will constitute a basis for an in-depth comparison on the clinical implication of the proposed biomarkers and the prognostic implication of change in an ultra-rare genetic disease (prevalence below 1:50.000). This information is currently unknown; therefore, the inclusion of this group is considered an essential part of study conduct.

Risks and discomforts

There can be unexpected risks and discomforts attributable to participation in the study, which cannot be predicted prior to trial conduct. The following will elaborate on known risks and discomforts.

Risks related to clinical procedures

Blood sampling

Venipuncture to acquire blood samples can be uncomfortable but is considered safe and performed as a routine procedure in standard medical practice. Although a small hematoma may occur, and a minimal risk of infection is present, proper technique will be applied to ensure a minimal level of risks. In comparison to blood donation, where 500 mL is drawn, the current study will require a sample of 300 mL and may induce slight, transient lightheadedness, but is otherwise not associated with any known risk.

Risks related to scanning procedures

Radiation in general

In general, Danish citizens' lifetime risk of cancer is approximately 25.00%. Any exposure of radiation is believed to cause a stochastic risk of inducing spontaneous mutation and an increase in incident cancer. The ^{82}Rb-PET/CT scan delivers radiation to a much lesser degree than a single photon emission tomography (SPECT) scan, which is the current noninvasive clinical standard of diagnosing microvascular coronary artery disease. Radiation doses of ^{82}Rb-PET/CT scans performed as clinical standard are approximately 4 mSv per investigation, including both rest and stress. Thus, the individual dose experienced by each participant would amount to 8 mSv during the study period of 3 years. Assuming a linear relationship between radiation exposure and risk, participating in this study

increases the risk of cancer to approximately 25,04% (risk category: IIb) [28]. Due to the stochastic risk of mutation, pregnancy is a contraindication to trial participation.

^{82}Rb-PET/CT

When disregarding the specific risks attributed to radiation discussed in the above, PET/CT is considered safe and is not related to short- or long-term risk. The infusion of adenosine can induce discomfort, nausea, and a transient shortness of breath. However, due to the short biological half-life of the drug, symptoms will disappear within seconds of terminating transfusion. If a participant becomes claustrophobic and wants to terminate an ongoing exam, the physician is obliged to do so.

Cardiac magnetic resonance imaging

If an implanted device is deemed MRI-compatible or MRI-safe and no contraindication is found, CMR is considered safe and is not related to short- or long-term risk. If a prospective patient has an implanted device which is at least MRI compatible (e.g. pacemaker), participation in the study will require additional safety precautions prior to and after scans are performed according to local standards. If a participant becomes claustrophobic and wants to terminate an ongoing exam, the physician is obliged to do so. The contrast-agent, Gadobutrol (Gadovist), is a paramagnetic CMR contrast agent and is not associated with any radiation risk. Similar contrast agents have previously been associated with post-contrast cutaneous manifestations in patients with a severely impaired kidney function receiving increased doses (eGFR < 30 mL/min/1.73m^2), however, the use is safe among individuals with normal kidney function when receiving standard doses (0.2-0.4 mmol/kg) or receiving reduced doses in patients with low kidney function [29]. Injection with contrast may be associated a short temporary dizziness or local sense of local warming at the site of injection as a transient phenomenon. Patients deemed eligible to participate will receive an appropriate dose of contrast agent according to local safety guidelines.

Data protection of Personal Information in the Study

All legislations, regulations, and laws on Data Protection and Data Security and the directives of the Danish Protection Agency will be complied with during the conduct of the trial. Permission to handle personal data will be sought and approved by the Danish Data Protection Agency. All data are stored pseudo-anonymized and analyzed electronically and no unauthorized access to data is allowed. Original data is filed according to a unique participant number. REDCap, hosted by OPEN (Open Patient data Explorative Network) will be used for registration of clinical data. REDCap meets the safety requirements set by the Danish Data Protection Agency for storage of person-sensitive data;

prescribed medication, medical history, height, weight are examples of such data. Data will be encrypted and stored for 15 years in accordance with recommendations on data storage from the Danish Data Protection Agency and thereafter transferred to the Danish Data Archives.

The study is reported to the Danish Data Protection Agency in Region H and will be handled according to the regulations of the General Data Protection Regulation: GDPR: REGULATION (EU) 2016/679 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27th of April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC and the Data Potection Act. Data is stored for 15 years.

Procedures for recruiting participants and informed consent

Initial contact

Fabry patients are recruited from the Department of Hormone and Metabolism, Copenhagen University Hospital, Rigshospitalet. The department is assigned as the national center concerning the treatment and monitoring of patients with Fabry disease. Eligibility to participate is evaluated by the doctor or other personnel of the patient's usual care at a regularly scheduled outpatient visit. Eligibility is evaluated using a confirmed diagnosis of Fabry disease using the current clinical standard and the presence of left ventricular hypertrophy, and these two parameters (i.e. the inclusion criteria) will be transferred to the project doctor – the presence of left ventricular hypertrophy is the key grouping parameter used in the project. Patients who fulfill the inclusion criteria and none of the exclusion criteria will be informed of the study by the personnel at the department involved in their usual care. By acceptance, they will receive a written patient information. If the patient favors contact initiated by an investigator from the study and provides oral consent, the patients contact information (name, email, phone number) will be forwarded to an investigator. The oral information for the study is then presented by a medical doctor, who is a member of the study group at the department, given in quiet surroundings. Individuals acting as controls in the study will be recruited from varies homepages (sundhed.dk, facebook, and forsoegspersonen.dk), where contact information on investigators can be found. The recruitment material will be reviewed and approved by the Ethics Committee prior to use. Potential participants can contact the Departments of Nephrology and Endocrinology, Copenhagen University Hospital, Rigshospitalet by email or telephone. Regarding advertisements on Facebook, any advertisement/post will have its ability to be shared or write a comment to the advertisement/post disabled. In addition to the written information pertaining to the study, it will be accompanied by the brochure: "Forsøgspersoners rettigheder i et

sundhedsvidenskabeligt forskningsprojekt" (Your rights as a participant in medical research) and "Dit væv, din ret" (Your tissue, your right). If the individual accepts further information on the study, a meeting in a quiet, undisturbed room will be scheduled with one of the investigators, and the person will be informed of the right to have a bystander present at that moment. The patient will be offered at least 24 hours reflection period before signing the consent form. The informed consent to participate in the study gives the research responsible doctors, sponsor and their representatives, and potential control authorities access to the information on kidney function, and relevant medical information for the study from the patient's medical record.

Scheduled meeting

At the scheduled meeting, the individual will receive additional oral information, from an investigator, and it will be clarified whether the patient fulfill the general participation criteria. The patient will be informed about the terms and restrictions of the form of 'informed written consent'. The investigator will ensure that the patient is adequately informed about the study background and design both orally and in writing. It will be made clear that the patient can withdraw from the study at any time without repercussions.

Acquisition of informed consent

If the patient is interested in participating in the study after the initial information meeting and after the allotted deciding time, a meeting will be scheduled where oral and written informed consent will be obtained. After consent to participate in the study has been obtained, the first study visit will be scheduled. No study-related examinations will be conducted prior the acquisition of informed consent. Investigators will only access electronic hospital records to transfer or record information after acquisition of written informed consent.

Informed consent

The participant will be required to sign the individual forms of informed consent applicable.

<u>Consent regarding study participation</u>

The participant's consent includes the right to read and transfer information from electronical hospital records to RED-Cap by investigators. The data transferred include information such as medical history, blood and urine test results, prescribed and over-the-counter medication. Further, the informed consent includes permission to establish a research biobank regarding the analyses of biomarkers specified in the protocol. In addition, consent gives direct access to electronic hospital records for the primary investigator, the sponsor or their representatives, as well as authorized

auditors in order to retrieve the health-related information necessary to perform the study and to perform quality control and monitoring. The access to electronic hospital records gives access to information from previous clinical evaluations performed by doctors or other hospital staff in relation to hospitalizations or outpatient visits. The information accessed will be used to assist in attaining a contemporary assessment of the individual's organ involvement and concomitant disease (i.e. stage of disease according to current clinical standard). This includes access to previously performed diagnostic analyses (e.g. biochemical analysis of blood, urine or other tissue samples), image-based evaluations (e.g. by ultrasound, computer-tomography or magnetic resonance imaging), and current use of prescribed medication. These sources of information are vital in order to acquire a sufficient image of the patient's current medical health status.

The informed consent will be valid for the duration of the study.

Consent regarding establishment of a biobank of future research

A separate form of informed consent gives permission to establish a biobank of future research consisting of surplus material, if any, acquired at the initial visit of the core program. The aim of the biobank for future research is to ensure the ability to answer contemporary research questions in a rapidly evolving field. Future analyses may therefore include, but not be limited to, analyses of circulating levels of hormones, proteins, DNA-excerpts, not specified in the current protocol. Future analyses will be required to be approved by a relevant regional ethics committee prior to analysis.

The informed consent will be valid for the duration of the study.

Time schedule

- Ethics committee approval Q2-3, 2024
- First patient first visit Q4, 2024
- Last patient last visit Q2, 2029
- Cessation of Research Biobank Q4.2029
- End of data analysis and manuscript submission Q3, 2030

Funding

The primary investigator, sponsor and initiator of the study is professor, Caroline Kistorp from the department of Nefrology and Endocrinology at Copenhagen University Hospital, Denmark. The study will be funded by external independent private and public foundations. Any funding is deposited in a research account administered at Rigshospitalet, Copenhagen University Hospital - Rigshospitalet, Denmark. None of the investigators or departments will have any financial gain from

conducting the study. As funding is secured, the ethical committee (VEK) and the included patients will be informed, and the patient information will be accordingly updated to include the information on funding sources.

Current funding sources

- Unrestricted grant from Sanofi A/S: 250.000 DKK

Patient insurance and reimbursement

The study is conducted at the Department of Nephrology and Endocrinology, Rigshospitalet and covered by the departments insurance. Participants in the trial are covered by the existing patient insurance (”Patienterstatningen”). The participants will not receive payment. If the study requires extra visits to the department, transportation cost will be covered according to the guidelines for voluntary research subject’s appendix 1 of the National Scientific Committee (NVK) . Furthermore, expenses as to their nutritional need during visits will be provided for.

Ethical considerations and clinical implications

The study will be conducted in accordance with the Helsinki II declaration, the regulations of the General Data Protection Regulation, and will follow the directives of the laws on Data Security (‘Datasikkerhed’) and on Data Protection (‘Databeskyttelsesloven’). The study will be approved by the Danish Data Protection Agency and the Regional Ethics Committee of Copenhagen Denmark. Furthermore, the trial will be conducted following the current clinical research standard.

Heart disease is the complication with the greatest clinical impact in Fabry disease. All scheduled procedures – except for ^{82}Rb-PECT/CT – are part of the current observational program followed by patients at the Danish National Fabry Center. Although the addition of ^{82}Rb-PECT/CT to current diagnostic standard - using echocardiography and CMR - involves exposure to radiation, information on endothelial dysfunction derived from ^{82}Rb-PET/CT, we believe, is of incremental value in the detection of early-stage disease and would be the clinical alternative to CMR in the presence of an implantable pacemaker/cardioverter-defibrillator or any implantable device not compatible with CMR.

When evaluated by CMR- and PET-imaging, current evidence suggests distinct differences in Fabry disease when compared with other conditions causing hypertrophic cardiomyopathy, therefore, we believe the current study will improve the diagnostic approach and add to the ability to detect patients in early stages of disease. Furthermor, we find that the potential therapeutic gain

for the participants and future patients outweigh the risks and discomforts of participating in the study, and therefore, we believe conducting the study is justified.

Patient participation will depend on receiving informed oral and written consent, with participants reserving the right to withdraw consent at any time during trial conduct without further consideration.

Dissemination of results

The investigators oblige themselves to publish all clinically relevant findings in peer-reviewed journals irrespective of their ability to achieve statistical significance – i.e. publishing all results irrespective of being positive, neutral, or negative. Results will be published following the International Committee of Medical Journal Editors (ICMJE) recommendations.

The investigators will present findings at national and international conferences with an aim to strengthen the current understanding of, to substantiate or challenge the current clinical approach to Fabry disease. Furthermore, the authors will disseminate the results of the overreaching aims among patients with Fabry disease through patient organizations on the national level.

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Overview of Appendix

- Informed consent form
    - Participation in study (Patient with Fabry)
    - Participation in study (Healthy control)
    - Biobank of future research
- Documentation of Primary
- Curriculum Vitae of Primary
- Participant information
- Information Pamphlets
    - “Forsøgspersonens rettigheder i et sundhedsvidenskabeligt forskningsprojekt”
    - “Dit væv, din ret”
- Questionnaires
    - SF-36 Quality of Life questionnaire