DETAILED STATISTICAL ANALYSIS PLAN (SAP)

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

Contents

1. Administrative information

1.1. Title, registration, version and revisions

Full study title	the Role of cArdiac inflammation, endoThelial dysfunction, and fibrosis in FabrY disease: the RATIFY study
Acronym	RATIFY
Local project number	112403 / H-24052180
Clinicaltrials.gov number	Provided when registered
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1.2. Revision history

SAP revision history	None
SAP revision justification	-
SAP revision timing	-

1.3. Roles and responsibility

Author	Niels Høeg Brandt-Jacobsen[1,2]
Statistician	NA
Principle investigator	Caroline M. Kistorp[2]
Contributors and roles	-
Affiliations	[1]Department of Clinical Physiology and Nuclear Medicine, Centre for Diagnostics, Copenhagen University Hospital - Rigshospitalet [2]Department of Nephrology and Endocrinology, Centre for Cancer and Organ Disease, Copenhagen University Hospital - Rigshospitalet

1.4. Signatures

We the undersigned, certify that we read this SAP and approve it as adequate in scope of the main-analyses of the RATIFY study.

1.4.1. Author

Name: Niels H. Brandt-Jacobsen

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Date:

1.4.2. Principle investigator

Name: Caroline M. Kistorp

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Date:

1.5. Abbreviations

SAP	Statistical analysis plan
LVH	Left ventricular hypertrophy
Gb3	Globotriaocylceramide
CMR	Cardiac magnetic resonance
CMD	Coronary microvascular disease
PET/CT	Positron emission tomography/computer tomography
MFR	Myocardial flow reserve
SD	Standard deviation

2. Introduction

This document outlines the statistical methods to be implemented during the analyses of data collected within the scope of the Role of cArdiac inflammation, endoThelial dysfunction, and fibrosis in FabrY disease (RATIFY study). The purpose of this statistical analysis plan (SAP) is to provide a framework in which answers to the protocol objectives may be achieved in a statistically rigorous fashion, without bias or analytical deficiencies. Specifically, this plan has the following purposes:

- To outline the specific types of analyses and presentations of data prospectively that will form the basis for conclusions.
- To explain in detail how the data will be handled and analyzed, adhering to commonly accepted standards and practices of biostatistical analysis. Any deviations from these guidelines must be substantiated by sound statistical reasoning and documented in writing in the final study report.

2.1. Background and rationale

Fabry disease is a rare X-linked lysosomal disorder. An enzyme deficiency causes progressive accumulation of glycosphingolipids, mainly globotriaocylceramide (Gb3), in virtually all organs, leading to dysfunction and eventually organ failure. Due to disease heterogeneity all patients are at increased risk of multisystem organ involvement and must attend extensive screening, continuous monitoring in order to decide who are in need of treatment, when to initiate, and to document the treatment effects [1,2].

Fabry cardiomyopathy is characterized left ventricular hypertrophy (LVH) [3], 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 mechanisms of inflammation, energy dysmetabolism, and oxidative stress. A key site and mechanism of stress and perhaps an early indicator of disease may, therefore, be found investigating changes across the vascular wall.

Recognizing the improved spatial resolution of cardiac magnetic resonance (CMR) imaging, CMR is recommended as supplement to echocardiography as part of routine clinical practice to improve detection of changes in left ventricular mass [3–5]. 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 [3]. In addition, the detection of coronary microvascular disease (CMD) by positron emission tomography/computer tomography (PET/CT) has been proposed as a marker of early disease. Not only is the degree of CMD

associated with the degree of LVH [6–8], 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 [6–8]. While the recent advances in imaging present a unique possibility for early detection, validation is needed to establish that a high-precision diagnostic approach adds clinical value in supplement clinically established biomarkers of risk [9–14].

CMR and PET/CT separately provide global and regional 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 detected 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.

2.2. Objectives

2.2.1. Objectives and research questions

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. This is divided in two major objectives:

- 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 /computer tomography (Rb-PET/CT).
- Using an extensive, in-depth biomarker blood panel to investigate the pathological pathways associated with Fabry disease and Fabry-related cardiomyopathy.

2.2.2. Hypotheses

The hypothesis of research question 1, *hereafter the cardiac imaging study*, is:

- Null hypothesis: there is no true correlation between any Fabry-related cardiomyopathy and MR- and PET/CT-based imaging – mainly focusing on change in T1-mapping and microvascular function, but also including inflammation, fibrosis, and cardiac injury.
- Alternative hypothesis: MR- and PET/CT-based cardiac impairmentis associated with the degree of Fabry-related cardiomyopathy – mainly focusing on change in T1-mapping and microvascular function, but also including inflammation, fibrosis, and cardiac injury –.

As research question 2, hereafter *the biomarker study*, is exploratory in nature no formal hypothesis test is pre-specified.

2.2.3. Scope

This SAP will be the guiding document, creating the outline for the analyses that will be conducted throughout the study. Any additional aims will be included in an appendix of this SAP as an addendum in the future and be added in the revision history of the document (section 1.2).

3. Study methods

3.1. General study design and plan

The study is initiated by Caroline Kistorp, professor at the Danish National Fabry Centre, at the Department of Nephrology and Endocrinology, Copenhagen University Hospital - Rigshospitalet. The RATIFTY study is a non-interventional, longitudinal cohort study and therefore purely observational in nature. No interventions are used as part of study design.

The study will consist of a group of patients with genetically verified Fabry disease, dichotomized by the presence of LVH. 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.

The protocol of this study was reviewed and approved by the regional scientific ethics committee (Project number: 112403 / H-24052180) and registered at clinicaltrials.gov prior to the inclusion of the first patient in the study. The original SAP was written prior to the enrollment of the first patient, with revisions and timing of revisions evident in the revision history (section 1.2).

3.2. Sample size, power, and detectable difference

Use of similar CMR- and PET/CT-based cardiac imaging parameters have previously been reported in patients with Fabry disease prior to the initiation of the current study. However, disease heterogeneity makes it difficult to extrapolate results and to calculate sample size based on previous literature.

3.2.1. The cardiac imaging study

Rb-PET/CT

From our own experience regarding normal values on myocardial flow reserve (MFR) on healthy individuals, a baseline mean MFR can be expected to be 3.7 (SD 0.8) (Unpublished). In a Fabry cohort, baseline mean MFR has previously been reported as 1.5 (SD 0.5) [8].

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 is expected among participants. 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) [15].

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.

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 both modalities causing an unexpected loss of data, we aim to include 54 participants, preferably grouped 18:18:18 participants (Fabry patients with LVH; Fabry patients without LVH; age- and sex-matched controls).

3.2.2. Biomarker study

Recognizing the exploratory nature of a biomarker study, including multiple biomarkers, no formal hypothesis is pre-specified as part of the SAP, thus a sample size calculation is not pre-specified. Results based on these analyses are therefore perceived as hypothesis-generating.

3.2.3. Additional considerations

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 skewed distribution in the Fabry cohort.

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

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.

3.3. Timing of final analysis

3.3.1. Image acquisition and quality assurance

The evaluation of images procured from MR and PET/CT scans as to whether they achieve the necessary quality to support image analysis will be performed as an integral part of the imaging procedure by experienced personnel. Similarly, reasons regarding incomplete scanning procedures and missingness will be documented concurrently. Images procured will be extracted, pseudo-anonymized, and stored until the day of analysis. The key used to identify patients will be stored separately.

3.3.2. Image analysis

As it has already been established certain image-derived biomarkers, such as left ventricular mass, have clinical value. A clinical image analyses will be performed at an *ad hoc* basis by experienced personnel. These values will be recorded and stored in the database as 'clinical values'. If the results of this analysis is cause to any change in risk assessment and/or treatment, this change will be recorded and stored in the database.

The final image analysis of baseline values will be performed upon inclusion of the last patient in the study. The final image analysis of follow up-values will be performed upon completion of the last visit in the study. To minimize bias in the study, all images analyzed in a blinded form, where the investigator responsible will be blinded from clinical information. These values will be recorded and stored as 'study values'.

Discrepancies between 'study values' and 'clinical values' will be subject to further review to uncover any bias.

3.3.3. Biomarkers analysis

The analysis of blood and urine of baseline or follow-up will be grouped to minimize possible bias.

4. Statistical principles

4.1. Testing and multiplicity

We have two primary endpoints: 1) change in global native T1-value irrespective of LVH, and 2) change in global MFR irrespective of the presence LVH. Both analyses investigate patients with disease against the healthy controls. The primary endpoints will be reported in an unadjusted form. If either analysis achieve nominal statistical significance a supplementary analysis will be performed using p-value adjustment and subsequently be reported [16].

We will encounter further multiplicity issues due to the multiple secondary and exploratory outcomes that are tested for significance on the same cohort (table 1, section 7). The study measures four pre-defined as secondary outcomes (table 2, section 7). These outcomes will be subject to adjustment for multiplicity as part of standard reporting, reported as a supplementary analysis to assess the robustness of the conclusions drawn from these results. The remaining exploratory outcomes of the cardiac study and outcomes of the biomarker study will be reported as exploratory and will not be subject to subject to adjustment for multiplicity.

4.1.1. Adjustment procedure

We will apply an adjustment for multiplicity using the False Discovery Rate-adjustment proposed by Benjamini-Hochberg [16], adjustning the p-values. Adjustment of the primary outcomes will be performed separately from all other outcomes. The adjustment procedure will be performed as a supplementary analysis, reporting unadjusted and adjusted p-values in order to create full transparency of the statistical procedure and the strength of the analyses performed.

4.2. Statistical significance and confidence interval

An unadjusted p-value below 0.050 will be reported as achieving nominal statistical significance for both our primary outcome and secondary outcomes. Regarding results of secondary outcomes, an adjusted p-value below 0.050 will be concluded as robust. To account for discrepancies due to multiplicity adjustment, results which achieve nominal statistical significance (unadjusted p-value below 0.050) but fail to be shown as robust (adjusted p-values above 0.050) will be highlighted to emphasize the increased chance of a type I error.

Baseline characteristics will be reported according to their data structure and their conformity to normality (mean/SD, median/interquartile range etc.). Results will be presented by their values (e.g. regression coefficients, mean difference etc.) with 95% confidence intervals.

4.3. Adherence and protocol deviations

4.3.1. Definitions of protocol deviations

Protocol deviations are defined as the activities which diverge from the protocol approved by the local institutional review board.

5. Study population

5.1. Screening data

Eligible patients who were not included will be summarized as to the reason of exclusion.

5.2. Eligibility

All eligible patients have a genetically-verified diagnosis of Fabry disease and are currently followed at the Danish National Fabry Center, Copenhagen University Hospital - Rigshospitalet. Review of eligibility and inclusion in the study consists of a protocolized clinical examination and subsequent blood and urine analysis. The examination is performed by study personnel.

5.2.1. Inclusion criteria – Fabry cohort

- Male and female individuals with a genetically-verified diagnosis of Fabry disease
- $\geq$ 18 years of age.
- Able to give informed consent

5.2.2. Exclusion criteria – Fabry cohort

- 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 CMR 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

5.2.3. Inclusion criteria – Control cohort

- Male and female individuals ($\geq$18 years of age)
- Able to give informed consent

5.2.4. 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 CMR 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

5.3. Recruitment

A flow diagram will be used to visualize the flow of patients throughout the study. In this flow diagram, we will report the population from which the eligible patients were selected, reasons for exclusion and procedures performed at both baseline and follow-up visit.

5.4. Patient characteristics

5.4.1. Collected patient characteristics

The cohort study was designed to register a set of clinical, biochemical and MR and PET/CT imaging variables in each included patient at baseline and at follow up. We register baseline demographic data by a semi-structured clinical interview and examination. We obtain the biochemical values from analyses on venous blood and urine samples acquired at the day of inclusion in the study and at the follow up visit. Finally, cardiac imaging variables are obtained by CMR and PET/CT (**Table 1** provides an overview of all collected variables and indicates for each variable whether it is categorized as a clinical, biochemical or imaging variable).

5.4.2. Patient disease history

As Fabry disease is an ultra-rare disease, all patients with Fabry disease in Denmark are subject to monitoring as part of their clinical follow-up at the Danish National Fabry Centre at the Department of Nephrology and Endocrinology - Rigshospitalet, Copenhagen University Hospital. The yearly monitoring program is part of the patients' clinical routine, and not a part of the study design. However, the individual patient will be evaluated prior to inclusion according to their individual risk-profile and whether their disease is stable, in regression or is progressing based on the monitoring parameters used in the program.

During the observation period, all patients will be monitored yearly according to the routine clinical and evaluated as part of their clinical routine.

5.4.3. Repeated measures

Change between repeated measurements on the same individual is defined as the change from baseline to follow up.

5.5. Assumed confounding covariates

5.5.1. Time-invariant variables

Given the size of the study, time-invariant factors will inevitably give rise to variation, however, a completely adjusted model is deemed impossible. Factors believed to have the greatest possibility of skewing the results are 1) age, 2) sex, 3) known pathogenic vs late onset-variant 4) disease stability 5) disease stage 6) Recipient of Fabry-specific therapy. These variables are predefined confounding variables to be used in a supplementary adjusted model as covariates.

While further measured and unmeasured variables, such as environmental, genetic, or psychological factors, may further influence the analyses, further confounding results is not be pre-specified. Any parameters added post hoc will be reported as hypothesis-generating.

5.5.2. Time-variant variables

As the study is a non-interventional, longitudinal cohort study, time-variant factors will undoubtedly have influence on the results. Among the time-variant factors believed to have the greatest influence is 1) disease stability and 2) stage of disease 3) changes in ongoing Fabry-specific and non-specific treatment. Regarding each of the abovementioned factors, patients followed at the Danish National Fabry Centre are evaluated as part of their routine clinical monitoring program and will therefore be evaluated prior to inclusion, yearly during the observation period, and prior to change in Fabry-specific treatment.

While these variables are predefined covariates of interest, an analysis cannot be pre-specified due to the inherent complexity of such a model and the constraint created by the overall size of the trial. Any analysis using the pre-specified time-variant variables will therefore be reported as hypothesis-generating.

6. Analysis

6.1. Outcome definitions

The focus of the study is the cardiac imaging study.

6.1.1. Primary outcomes

Primary endpoint on Rb-PET/CT

- A between-group difference in change in global myocardial flow reserve (MFR) evaluated by 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 CMR, comparing Fabry patients with controls irrespective of the presence of LVH.

6.1.2. Secondary outcomes

- A between-group difference in change in global myocardial flow reserve (MFR) evaluated by 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 CMR, comparing Fabry patients with controls accounting for the presence of LVH.
- A between-group difference in change in global T2 values evaluated by CMR, comparing Fabry patients with controls accounting for the presence of LVH.
- A between-group difference in change in global T2 values evaluated by CMR, comparing Fabry patients with controls irrespective of the presence of LVH.

6.1.3. Exploratory endpoints

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

On Rb-PET/CT

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

On CMR

- A between-group difference in regional native T1 evaluated by CMR according to the 17-segment model.
- A between-group difference in regional native T2 values evaluated by CMR 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 during 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, known pathogenic vs late-onset variant, disease stability, disease stage/severity by Mainz Severity Score Index (MSSI) and Fabry International Index (FPI), receiving Fabry-specific therapy.

6.2. Analysis methods

6.2.1. Primary analysis

The primary analysis on will be performed using a simple linear mixed model with the primary outcome as stated above. The grouping variable (Fabry disease vs healthy control), visit and their interaction will be used as the explanatory variables. To account for the correlation between repeated measures on the same individual an unstructured covariance pattern will be used. Reporting will include coefficients, 95% confidence intervals, and un-adjusted p-values. The statistical analysis will use all available information.

6.2.2. Secondary analyses

Secondary will be analyzed using the same basic framework as the primary analysis. When accounting for the presence of LVH, the Fabry group will be divided by the presence of LVH, creating three groups (Fabry patients with LVH, Fabry patients without LVH, healthy controls). Reporting will include coefficients, 95% confidence intervals, and unadjusted p-values. The statistical analysis will use all available information.

6.2.3. Multiple linear regression, and correlation between variables

Multiple linear regression models will include the pre-specified time-invariant confounding variables defined previously. Correlation between variables will be tested using a linear model. Reporting will include coefficients, 95% confidence intervals, and unadjusted p-values. The statistical analysis will use all available information.

6.2.4. Descriptive summarization of baseline patient characteristics

General patient characteristics will be listed in a baseline table. Data will be presented as mean with standard deviation (SD) when normally distributed or as median with interquartile range in case of skewed data as deemed appropriate. Dichotomous and categorical data will be presented in proportions. Normality of the data will be assessed using P-P plots, Q-Q plots, and/or histograms. Linearity will be assessed using scatter plots. Differences between continuous variables will be assessed using appropriate parametric or nonparametric test (e.g. Student's t-tests or Mann-Whitney-U test) depending on normality as deemed appropriate. Fischer's exact test will be used for categorical values.

6.3. Subgroup and sensitivity analyses

Although the overall statistical power of the study is low, the value of the even hypothesis-generating results is deemed of inherent value due to the scarcity of evidence specific to Fabry disease despite the potential lack of statistical strength to provide robust statistical inference. Therefore, subgroup analyses will be performed, however, these results will be reported as exploratory and hypothesis-generating.

Pre-defined subgroups include presence of left ventricular hypertrophy (yes vs no), biological sex (male vs female), known pathogenic variant (yes vs no), disease stability, disease stage according to the Mainz Severity Score and the Fabry International Prognostic Index, and receiving of Fabry-specific treatment (yes vs. no).

Sensitivity analyses will include a direct comparison of the previously stated 'clinical values' and the 'study values' (*see 3.3.2*). Any other sensitivity analysis will be defined at an *ad hoc* basis.

6.4. Missing data

6.4.1. Reasons for missing data

We will strive to achieve a complete dataset, containing an valid observation for all variables. Where easy-accessible clinical variables such as blood pressure, heart rate, blood, and urine sampling may be easier to acquire, in case of patient claustrophobia or discomfort during CMR and PET/CT scans, the imaging protocol can be terminated per patient request, causing an inevitable loss of data. Furthermore, the overall length of the observation may cause drop-out due to attrition among patients and controls. In the event of missingness, reasons as to why will be sought answered to assess the impact on the conclusions drawn from the results.

As the overall ability to undergo imaging is not dependent on cardiac impairment, the reasons for missingness are viewed *a priori* as being missing at random (MAR) or missing completely at random (MCAR), thereby having little influence the overall conclusions of the study.

6.4.2. Imputation method

Our primary model analysis will rely on model-based imputation using expectation maximization within a linear mixed model. If need be, a supplementary sensitivity analysis may be performed to investigate the influence of changes in time-variant variables and patients who do not undergo a full imaging protocol. Multiple imputation using the MI impute command in SAS or similar will be used. Included in the imputation procedure will be all available imaging variables, weight, height, age, sex, and all available blood- and urine-variables. The random seed used will be (45361) with imputation repeated 50 times or more until the final estimates are deemed stable.

6.5. Statistical software

Statistical analyses will be performed using SAS version 9.4 (SAS institute, Cary, NC, USA) or R.

7. Tables and figures

Table 1. Overview of all pre-specified outcomes measured in the renal study and biomarker study.

	Examinations
Clinical examination	
	Sex
	Age
	Genetic variant
	Height
	Weight
	Waist circumference
	Hip circumference
	Electrocardiogram
	Office blood pressures
	Pulse (Office blood pressure)
	Mainz Severity Score Index
	Fabry International Prognostic Index
	SF36 Quality of Life-questionnaire
Cardiac imaging	
CMR	T1
	T2
	Left ventricular mass
	Extent of fibrosis defined using late gadolinium enhancement
	Left ventricular mass
	Left ventricular end-diastolic volume
	Left ventricular end-systolic volume
	Left ventricular ejection fraction
	Right ventricular strain indices (longitudinal, circumferential, radial)
	Left ventricular strain indices (longitudinal, circumferential, radial)
	Indices of diastolic function
PET/CT	Myocardial flow reserve
	Perfusion at rest
	Perfusion during stress
	Agatson score
	Left ventricular mass
	End-diastolic volume
	End-systolic volume
	Left ventricular ejection fraction
	Total perfusion defect
Biomarker analysis	
	α-gal A activity in leucocytes
	Lyso-Gb3
	Gb3
	urine Gb3
	Creatinine
	GDF-15
	TGF-β
	FGF-21
	FGF-23
	VEGF

	Collagens
	fasting plasma glucose
	Insulin
	3-OH-hydroxybutyrate
	HbA1c
	lipid profile
	NT-proBNP
	TnI
	hsTnT
	proANP
	proCNP
	urinary podocyte excretion,
	Urinary albumin/creatinine ratio

Table 2. Overview of all pre-specified primary and key secondary outcomes measured in the cardiac study.

Examination	**Primary outcome (P) or key secondary outomes (S)**
Cardiac imaging	
CMR	
Global T1; Irrespective of LVH	P
Global T2; Irrespective of LVH	S
Global T1; Accounting for LVH	S
Global T2; Accounting for LVH	S
Rb-PET/CT	
MFR, Irrespective of LVH	P
MFR, Accounting for LVH	S

The study includes two primary outcome and 4 secondary outcomes. P-value adjustment will performed separately for primary and secondary outcomes, and will be performed to assess the robustness of the conclusions based on the results, reporting both unadjusted and adjusted p-values. Any other pre-specified or post hoc analyses performed will not be subject to p-value adjustment.

8. References

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