Protocol

PROTOCOL NUMBER:
EC22-008

STUDY TITLE:
A Real World Evidence Study of Danish Fabry patients: a > 20-year Longitudinal Retrospective Analysis of Prospectively Collected Data.

VERSION: 1.0 (Original)
DATE: 30 June 2023

SPONSOR:
Amicus Therapeutics Ltd.
One Globeside, Fieldhouse Ln, Marlow SL7 1HZ, UK

Study information

Title	Real World Evidence study of Danish Fabry patients: a >20-year longitudinal retrospective analysis of prospectively collected data.
Protocol version identifier	V1
Date of last version of protocol	
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Product reference	*NA*
Procedure number	
Marketing authorisation holder(s)	
Joint PASS	
MAH(s) contact	
Research question and objectives	**Research Question** Is the incidence and prevalence of Fabry associated clinical events (FACEs) (cardiac, renal and cerebrovascular) associated with sex, genotype, phenotype at time of diagnosis, biomarkers, and Fabry specific therapy? ***Objectives*** To investigate time to first Fabry associated clinical events (FACEs) (cardiac, renal, and cerebrovascular) with particular focus on Migalastat clinical outcomes and treatment outcomes preceding Migalastat therapy. To investigate the incidence and prevalence of FACEs with respect to Fabry specific treatment, Migalastat, ERT or no treatment. To describe FACEs in accordance with different geno- and phenotypic groups. To investigate the incidence and time to a first fatal or non-fatal cardiac, renal and cerebrovascular clinical event, separated by each category.

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Country(-ies) of study	Denmark

STATEMENT OF COMPLIANCE

The study will be conducted in accordance with the Declaration of Helsinki principles, International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines, and applicable Danish regulations and laws. All personnel involved in the conduct of this study have completed human subjects' protection training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable regulations and guidelines.

Principal Investigator or Clinical Site Investigator:

Signed: _____ Date: _____

Name:

Title:

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List of abbreviations

α-Gal A	Alpha Galactosidase A
ACE	Angiotensin-Converting Enzyme
ARB	Angiotensin-Receptor Blockers
BPI	Brief Pain Inventory
CRA	Clinical Research Assistant
CRO	Contract Research Organization
eCRF	electronic Case Report Form
EDC	Electronic Data Capture
ERT	Enzyme Replacement therapy
ESRD	End-Stage Renal Disease
FACE	Fabry Associated Clinical Event
FPI	First Patient In
GL-3/Gb3	Globotriaosylceramide
Lyso Gb3	Globotriaosylsphingosine
GCP	Good Clinical Practices
GLA	Galactosidase A
IgG	Immunoglobulin G
LPO	Last Patient Out
LV	Left Ventricle
LVH	Left Ventricular Hypertrophy
LVMI	Left Ventricular Mass Index
MedDRA	Medical Dictionary for Regulatory Activities
MSSI	Mainz Severity Score Index
PBI	Patient Benefit Index
PBQ	Patient Benefit Questionnaire
PCA	Principal Component Analysis
PI	Principal Investigator
PNQ	Patient Needs Questionnaire
PT	Preferred Term
Q1	First Quartile
Q3	Third Quartile
SF-36v2	The Short Form (36) Health Survey version 2
SIV	Site Initiation Visit
SmPC	Summary of Product Characteristics
SOC	System Organ Class
TSQM-9	Treatment Satisfaction Questionnaire for Medication v.9
QoL	Quality of Life
SAE	Serious Adverse Event

Responsible parties

SPONSOR CONTACT DETAILS

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CONTRACT RESEARCH ORGANIZATIONS INFORMATION

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Phone/Fax
Email

Participating clinical scientists and research staff will be described in Annex 1

Abstract

Title	Real World Evidence study of Danish Fabry patients: a > 20-year longitudinal retrospective analysis of prospectively collected data.
Rationale and background	Fabry is a rare X-linked metabolic lysosomal disorder caused by deficiency in the enzyme α-galactosidase A (α-Gal A) by mutations in the *GLA* gene, encoding the α-Gal A enzyme, which catalyses glycosphingolipids, namely globotriaosylceramide (Gb3). Reduced or absent α-Gal A activity leads to accumulation of Gb3 in various organs as well as cellular dysfunction and inflammation causing phsyical symptoms and eventual organ failure. Migalastat, an oral chaperone therapy was approved for Fabry patients in 2016 providing an alternative to infusions with enzyme replacement every 2 weeks. Although the initial trials of Migalastat had some both short and extended outcome treatment comparisons, the overall evidence of clinical efficacy is based on too small numbers considering the heterogeneity of the Fabry patient population as well as the very slow progression of the disease. Though the body of real-world evidence is growing, there is a need for more publications of real-world long-term data on clinical outcomes with a focus on treatment with Migalastat.
Research question and objectives	**Research Question** Is the incidence and prevalence of Fabry associated clinical events (FACEs) (cardiac, renal, and cerebrovascular) associated with sex, genotype, phenotype at time of diagnosis, biomarkers, and Fabry specific therapy? **Objectives** To investigate time to first Fabry associated clinical events (FACE) (cardiac, renal, and cerebrovascular) with particular focus on Migalastat clinical outcomes and treatment outcomes preceding Migalastat therapy. To investigate the incidence and prevalence of FACEs with respect to Fabry specific treatment, Migalastat, ERT or no treatment. To describe FACEs in accordance with different geno- and phenotypic groups. To investigate the incidence and time to a first fatal or non-fatal cardiac, renal, and cerebrovascular clinical event, separated by each category. **Primary outcomes** Time to first FACE (cardiac, renal, and cerebrovascular) with particular focus on Migalastat on clinical outcomes and treatment outcomes preceding Migalastat therapy.

	Secondary outcomes To investigate the incidence and prevalence of FACEs with respect to Fabry specific treatment, Migalastat, ERT or no treatment. To describe FACEs in accordance with different geno- and phenotypic groups To investigate the incidence and time to a first fatal or non-fatal cardiac, renal and cerebrovascular clinical event, separated by each category.
Study design	Retrospective observational, single-center study from the Danish National Fabry Center. Describing treatment related outcomes in Fabry Disease over time.
Population	• Male and female patients with a diagnosis of Fabry Disease. • Registred at the Danish National Fabry center, Rigshospitalet, Denmark from 01.01.2001.
Variables	The primary endpoint is time to first FACE (cardiac, renal, or cerebrovascular events) since each baseline, with particular focus on the added value of the recent addition of Migalastat as a treatment option. Secondary endpoints are prevalence and incidence of FACEs and time to first FACE. Exploratory endpoints: Organ-specific biomarkers across modalities (auditory, lung, heart, kidney, skin, and neurological), and patient-reported outcomes evaluated by questionnaires.
Data sources	Electronic medical records and paper records.
Study size	Estimated 115 patients, which constitute all patients with a genetically verified diagnosis of Fabry disease in Denmark.
Data analysis	Primary endpoint will be addressed using event-driven statistics from a baseline based on the date of diagnosis and treatment initiation. Secondary analyses will address the individual parameters of the FACEs. Exploratory endpoints will rely on repeated measures statistical models to investigate the change in organ-specific function during the 20-year follow-up. Detailed description is found in statistical analysis plan (SAP).
Milestones	
Ethics Committee Approval	Q3 2023
Start of data collection	Q3 2023
End of data collection	Q1 2024
Study progress report	Q1 2024
Interim report (baseline data)	Q2 2024

Registration in the EU PAS register	Q3-4 2023
Final report of study results	Q3 2024

Amendments and updates

None

Milestones

Milestone	
Ethics Committee Approval	Q3 2023
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Rationale and background

Fabry disease is a rare X-linked lysosomal disorder in lysosomes, resulting in a multisystemic disorder caused by deficiency in the enzyme α-galactosidase A (α-Gal A) due to mutations in the GLA gene, encoding the hydrolase α-Gal A enzyme, which catalyses glycosphingolipids namely globotriaosylceramide (Gb3). Reduced or absent α-Gal A activity leads to accumulation of Gb3 in the lysosomes of various organs, as well as cellular dysfunction and inflammation causing physical symptoms and eventual organ failure (1,2). Fabry disease as an X-linked disorder with a high penetrance, though not 100% in female heterozygotes, although the disease affects both males and females. With the X-linked nature, Fabry disease is suitable for an efficient, high diagnostic yield cascade screening over at least three generations surrounding an index-patient. The Danish National Fabry Centre has now more than 20 years of experience of cascade screening, which has provided a patient population of 115 male and female patients. The prevalence of Fabry disease in the Danish population is estimated to be 1:58000 (males 1:85000, females 1:44000) (3).

Primarily male patients, with early onset classical phenotypes have none or very low α-Gal A activity, and they inevitably develop multiorgan failure without treatment (4). Patients with late onset of Fabry disease represent both males and females with higher α-Gal A activity and variable degrees of organ involvement (5). Fabry disease can cause irreversible damage to multiple organs resulting in Fabry associated clinical events (FACEs) such as renal, cardiac, cerebrovascular events, and death (5).

The primary Fabry specific therapy has historically been enzyme replacement therapy (ERT), and as of 2016 an oral chaperone therapy, Migalastat, has been available, providing an alternative to ERT for patients with amenable mutations by increasing their endogenous α-Gal A enzyme activity (6–14). However, real-world long-term data from well-characterised cohorts with available detailed clinical consecutive observations with focus on treatment with Migalastat and Fabry associated events has not been fully investigated (12–14).

Research question and objectives

Research question

Is the incidence and prevalence of Fabry associated clinical events (FACEs) (cardiac, renal, and cerebrovascular) associated with sex, genotype, phenotype at time of diagnosis, biomarkers, and Fabry specific therapy?

Objectives

To investigate time to first Fabry associated clinical events (FACE) (cardiac, renal, and cerebrovascular) with particular focus on Migalastat clinical outcomes and treatment outcomes preceding Migalastat therapy.

To investigate the incidence and prevalence of FACEs with respect to Fabry specific treatment, Migalastat, ERT or no treatment.

To describe FACEs in accordance with different geno- and phenotypic groups.

To investigate the incidence and time to a first fatal or non-fatal cardiac, renal and cerebrovascular clinical event, separated by each category.

Primary objective

To investigate time to first Fabry associated clinical events (FACEs) (cardiac, renal, and cerebrovascular) with particular focus on Migalastat clinical outcomes and treatment outcomes preceding Migalastat therapy.

Secondary objectives

To investigate the incidence and prevalence of FACEs with respect to Fabry specific treatment, Migalastat, ERT or no treatment.

To describe FACEs in accordance with different geno- and phenotypic groups.

To investigate the incidence and time to a first fatal or non-fatal cardiac, renal and cerebrovascular clinical event, separated by each category.

Exploratory objectives

To describe disease progression with focus on organ involvement.

Research methods

Study design

Observational, retrospective, single-center study originating from the Danish National Fabry Centre, Centre of Cancer and Organ Disease, Department of Medical Endocrinology, Copenhagen University Hospital, Rigshospitalet. Patients are enrolled at the time of sampling leading to a genetically verified diagnosis of Fabry disease. Patients are followed from the day of enrolment until 31st of December 2022, having received yearly or bi-yearly evaluation during follow-up.

Method

Retrospective study of prospectively collected patient data with use of secondary patient data from both paper and electronic patient files. All patients are treatment naïve at time of diagnosis, which is defined as time point for a verified Fabry associated genetic mutation in the GLA gene. A baseline time point is further defined by switch of Fabry specific treatment. Both Migalastat and ERT will be studied retrospectively. Also untreated patients will be studied by assessment of relevant clinical and laboratory variables. The patients have been followed from 01.01.2001 until

31.12.2022. All patients with Fabry disease both receiving specific Fabry medication and untreated patients have been offered assessment yearly or every other year by thorough workup, including targeted examinations of organ function and symptoms known to be associated with Fabry Disease.

Endpoints

Primary endpoints

The primary endpoint is time to first FACE (cardiac, renal, or cerebrovascular events) since each baseline, with particular focus on the added value of the recent addition of Migalastat as a treatment option.

FACEs are defined as the following:

- Cardiac clinical events:
  - Myocardial infarction
  - New symptomatic arrhythmia requiring medication, direct current cardioversion, or interventional procedure (e.g., ablation, pacemaker, or defibrillator implantation)
  - Unstable angina defined by national practice guidelines and accompanied by electrocardiographic changes and non-ST segment elevation myocardial infarction [UA/NSTEMI]) and accompanied by electrocardiographic changes
  - Congestive heart failure requiring hospitalization
  - Any major cardiac medical procedure (e.g., valve replacement, stent-implantation, transplant or persistent atrial fibrillation)
- Cerebrovascular clinical events
  - Stroke (documented by a physician)
  - Transient ischemic attack (documented by a physician)
- Renal clinical events
  - Doubling of serum creatinine level from the start of analysis (2 consecutive values)
  - End-stage renal disease (ESRD) requiring long-term dialysis or transplantation
- Death due to FACEs

All FACEs will be analyzed together as a composite endpoint and separately in each category (cardiac, cerebrovascular, and renal).

Secondary endpoints

The secondary endpoints

- The prevalence of FACEs from each baseline (diagnosis, and start of treatment) to compare between Migalastat-treated, ERT-treated patients and non-treated patients
- The incidence of FACEs from each baseline (diagnosis, and start of treatment) to compare between Migalastat-treated, ERT-treated patients and non-treated patients

- Time to the first cardiac, cerebrovascular, and renal clinical event separately by each specific category (including death in these categories) from start of treatment or baseline
- Incidence of cardiac, cerebrovascular, and renal clinical events separately by each specific category (including death in these categories)
- Occurrence of cardiac, cerebrovascular, and renal clinical events separately by each specific category (including death in these categories)
- Annualized rate of change in eGFR based on the Chronic Kidney Disease Epidemiology Collaboration equation ($eGFR_{CKD\text{-}EPI}$) over time to compare between Migalastat-treated, ERT, and untreated patients
- The prevalence and incidence of a clinically significant change in mGFR and/or eGFR (according to KDIGO guidelines rapid progression is defined as a sustained decline in eGFR of more than 5 ml/min/1.73 m2 /yr) from each baseline (diagnosis, and start of treatment) to compare between Migalastat-treated, ERT-treated patients and non-treated pts
- The prevalence and incidence of micro (> 30 mg/g creatinine) or macroalbuminuria (> 300) by urinary albumin/creatinine ratio (ACR) or urinary 24 h protein excretion from each baseline (diagnosis, and start of treatment) to compare between Migalastat-treated, ERT-treated patients and non-treated pts

Exploratory organ specific Endpoints

Reflecting the programme of the annual clinical examinations and questionnaires of the Danish Fabry patients**.**

Lung capacity: clinically significant changes in spirometry measures of obstructive and restrictive lung function evaluate by body plethysmography

- Forced expiratory volume after 1 second (FEV1)

  A clinically relevant change is defined as 25% decrease from baseline or as incident volume of 20% below predicted value according to an age- and sex-adjusted reference range.

- Forced vital capacity (FVC)

  A clinically relevant change is defined as 25% decrease from baseline or as incident volume of 20% below predicted value according to an age- and sex-adjusted reference range.

Neurological, audiological assessments

- Standardized sweat test (hypo, normo, hyperhidrosis), standard unit of measure: mg pr. 30 minutes

Standardized tilt-test (Presence of orthostatism – yes/no)

- Heart rate variability (Age-dependent – impaired vs not)
- Audiogram (Impaired vs. not)
- Autonomic nerve function (small fibre assessment))
- Presence of Fabry associated abnormalities by brain MRI

Ophthalmological and dermatological assessments

- Presence of cornea versiculata
- Presence of angiokeratoma

Composite phenotypic score

- Mainz Severity Score Index (Composite score)

Cardiac function (evaluated by echocardiography)

- Left ventricular mass index (LVMi)
- Left ventricular ejection fraction (LVEF)
- Fractional shortening
- Left ventricular end diastolic and systolic volumes
- Mid-wall fractional shortening (MWFS)
- Left ventricular wall thickness
- NT-proBNP and high sensitive Troponin T – will be analysed from the Danish Fabry biobank.

Renal function

Laboratory parameters:

- serum creatinine
- urine creatinine
- urine protein
- urine albumin
- urine ACR
- measured glomerular filtration rate by 99Tc-DTPA or 51Cr-EDTA

Patient reported outcomes (PRO)

- SF-36 Health Questionnaire (Composite score)

- NHP-Health Profile (Daily living questionnaire – composite score)
- Major Depression Inventory (Becks Depression Index II - Composite score)

Pharmacodynamic Endpoints

Pharmacodynamic endpoints will be assessed by evaluations comparing Migalastat-treated and untreated patients and/or Migalastat-treated and ERT-treated patients:

- Plasma lyso-Gb_3
- WBC α-Gal A enzyme activity
- Urinary and plasma Gb3, lyso-Gb3, α-GAL A enzyme activity
- Neutralizing antibodies with ERT

Evaluation tools

Data extraction from electronic and paper medical records. No specific evaluation test or tool required.

Setting

Study population

All patients from the Danish National Fabry Centre will be included, estimating the inclusion of 115 individuals (sex ratio [female:male] – 2:1). Pre-specified subgroups includes: non-amenable/ amenable to Migalastat, males/females.

Inclusion criteria

- Established diagnosis of Fabry Disease based on genetic testing and alpha Gal-A activity in males and females.

- Data from baseline visit and at least one follow-up visit.

Exclusion criteria

Absence of data from baseline visit.

Treatments

Study treatment

No treatment will be evaluated prospectively. However, patients will be grouped according to treatment received during the treatment period (Migalastat, enzyme-replacement therapy or no treatment).

Patients who are switched from one treatment to another will be grouped according to current treatment at the assessment time point (if possible, according to a new baseline before switching).

Details of this procedure will depend on available information and numbers and cannot be described until all patients data have been collected.

Symptomatic therapies

Data on nonspecific Fabry therapy such as ace enzyme inhibitors (ACE-I), angiotension-II blockers (AT-II), anticoagulants, statin, antiarrhythmic therapy, asthma/bronchodilator therapy, antidepressive therapy, as well as use of analgesics and other important concomitant medication will be recorded.

Other measures

If possible, information on comorbidities at each treatment start and switch will be recorded.

Study workflow

Fabry patients entered the study at their baseline visit (first visit in the center) from 2001 with last follow-up December 2022. Patient data will be grouped for the whole duration of the defined study period, with individual datapoints registered at the specific date of event/measurement. Ideally the datapoints will be used according to their specific dates but may be aggregated according to availability. Details is further described in the statistical analysis plan.

Variables

Primary outcome variables (Form to be filled at untreated baseline and every change in treatment status: From untreated to treated or vice versa, from one treatment to another.

Registration of treatment

All patients included in the trial are considered treatment naïve at inclusion in the observational period (01.01.2001). Registration of initiation, change or cessation of treatment will follow the following structure:

Date (Date)	Drug (Name)	Action* (Categorical)	Dose (Integer)	Dose interval (Comment)	Comment (Comment)

*Action is restricted to four levels: initiation, increase, decrease, cessation.

Cardiac events		
Date of event	**Cardiac event**	**Yes/No**
	Myocardial infarction	Yes No
	Unstable angina pectoris	Yes

		No
	PCI / CABG	Yes No
	Heart Failure	Yes No
	Arrhythmia (New onset of symptomatic arrhythmia requiring anti-arrhythmic Medication)	Yes No
	Heart procedure (pacemaker/ICD/valve surgery)	Yes No

Renal events		
Date of event	**Renal event**	**Yes/No**
	Doubling of s-creatinine	Yes No
	ESKD	Yes No

Cerebrovascular events		
Date of event	**Cerebrovascular event**	**Yes/No**
	Stroke	Yes No
	TIA	Yes No

Variables of interest at inclusion

Variables of interest at inclusion consists primarily of time invariant variables.

<u>Demographics</u>

Date of Birth	• DDMMYYYY
Sex	• Male • Female
Ethnicity	White Black Asian

	Others
City	Residence City

Fabry-specific disease background

Age of first symptoms	Years
Age of diagnosis	Years
Fabry family history	Yes No Unknown
Index case	Yes No
ALPHA GAL ACTIVITY (LEUCO ASSAY) at inclusion	% of normal activity
MOLECULAR TEST	Amenable mutation found: Other mutations

Exploratory outcome variables

Variables concerning clinical presentation

ANGIOKERATOMA	Yes No
CORNEA VERTICILLATA	Yes No
ABDOMINAL PAIN	Yes No
REFLUX	Yes No
INDIGESTION	Yes No
DIARRHEA	Yes No
CONSTIPATION	Yes No
DYSPNOEA	Yes No

NEUROPATHIC PAIN	• Yes • No
ACROPARAESTHAESIA	• Yes • No
DEPRESSION SYMPTOMS	• Yes • No

Variables concerning organ dysfunction

Lung capacity	
Clinically significant Changes in spirometry of obstructive function	Yes No
Clinically significant changes in spirometry of restrictive function	Yes No
Neurological / Audiological	
Pathological heart rate variability	Yes No

Impaired hearing audiogram	Yes No
Vertigo	Yes No

BLOOD PRESSURE	mmHg
Heart Rate	BPM

BUN	mmol/l
Plasma CREATININE	mmol/l
URINARY CREATININE	mg/dl
ALBUMIN/CREATININE RATIO	mg/g
PROTEINURIA	mg
GFR CKD-EPI	ml/min/1,73m^2
mGFR	ml/min
Plasma Gb3	microgram/ml
Plasma lysoGb3	ng/ml
Urine GB3	microgram/mmol creatinine
Alpha GAL-A activity (DBS or Leuco assay)	% normal activity and quantitative concentration (Method reference)

Variables concerning concomitant medication

The included concomitant medication to be recorded is restricted to medications believed to be of clinical interest. Medication will be recorded as to their use at inclusion and regarding their change throughout the study if possible.

Concomitant medication at inclusion	
ACE-I/ ARB	Yes No
Beta-blocker	Yes No
Ca-blockers	Yes No

Diuretics	Yes No
Statins	Yes No

Date (Date)	Drug (Name)	Action* (Categorical)	Dose (Integer)	Dose interval (Comment)	Comment (Comment)

*Action is restricted to four levels: Initiation, decrease, increase, cessation.

Other variables and exploratory outcome variables

Safety variables

Safety Endpoints

Although the study collects retrospective secondary data, adverse event reporting will be conducted, if necessary, in line with the requirements of each study supporter.

- SAEs
- Vital signs:
- resting systolic blood pressure
  - resting diastolic blood pressure
- Body weight
- Overall survival among all patients enrolled, as assessed by recorded patient deaths from any cause
- Laboratory parameters:
- serum creatinine
  - urine creatinine
  - urine protein
  - urine albumin
  - urine ACR
- ECGs
- ECHOs, including left ventricular mass index (LVMi), if medically indicated and requested by a physician
- Occurrence of male infertility.

- Infusion related reactions and other key safety events

Data sources

Data sources for primary endpoints

Electronic medical records, paper records and national medical records

Other data sources

Not applicable

Study size

The study is estimated to comprise of approximately 115 individuals with genetically verified Fabry Disease (Ratio of sex [female:male] 2:1).

Data management

Data collection

Investigators or other designated site staff are implemented in the project group will be trained in the specific procedural steps of the protocol, data extraction.

Study variables will be recorded in an electronic database/ CRF using validated and secure database in RedCap©. Patient data will be handled while preserving confidentiality. Investigators, or other designated site staff, will analyze all Fabry patients irrespective of disease-causing mutation and will extract clinical and laboratory parameters from original medical records (primary data sources).

Validation procedures

A 10% random sample of records will be reviewed by a second independent observer to assure quality. The investigator team will discuss and resolve any noted discrepancies.

Case report form

All CRFs will be completed by the Investigators or other designated site staff who have been trained to record relevant information.

Data will be acquired retrospectively from medical documentation of enrolled patients.

Study variables will be recorded in a CRF designed for the specific purpose of this study. CRF design will be reviewed. At the end of data acquisition, the extent of missing values will be evaluated, and handled according to the description in the statistical analysis plan.

All patient data will be handled while preserving confidentiality.

Data management

All CRFs will be completed by the Investigators or other designated site staff who have been trained to record relevant information. Designated staff responsible for data acquisition should have higher medical education.

Data will be captured retrospectively from medical record documentation.

Study variables will be recorded in an electronic CRF by RedCap. CRFs will be reviewed for completeness at the end of document completion for data check (of missing data and/or specific questions requiring the investigators patient knowledge). All patient data will be handled preserving confidentiality.

Medical coding

Not applicable.

Serious Adverse Events/Adverse Events Data Reconciliation

Although the study collects retrospective secondary data, reporting of adverse events will be conducted, if necessary, in line with the requirements of each study supporter. In the event of not previously reported serious adverse events or suspected, unexpected serious adverse reactions to a subscriber's product, these events will be reported to Competent Authorities within 24 hours.

Database lock

At the completion of data acquisition, all interested parties will be informed of impending data lock. Data lock will transpire if all parties agree, but before the independent validation by an external party and before statistical analysis.

Data analysis

Descriptive statistics

Analyses will be performed by descriptive statistical methods. Continuous variables will be described with number of patients with valid/missing observations, mean, standard deviation, median, minimum and maximum. Non-normally distributed data will be presented as median and interquartile range as appropriate. Categorical variables will be described by frequencies and related percentages. In addition to descriptive analysis, many of the endpoints will undergo statistical testing (i.e., p-values, confidence limits) to provide inferential summaries of subgroup comparisons (such as gender, age, ethnicity).

Details are going to be described in the Statistical Analysis Plan (SAP).

Analysis will be stratified by sex, age (> or <40 years), Fabry specific therapy (ERT treated (agalsidase alfa and beta separately), Migalastat treated, treatment naïve), baseline organ involvement (cardiac, renal, cerebrovascular, peripheral nervous system, and gastrointestinal system). Additional analysis may be required and carried out based on the results of the analyses mentioned above.

Handling of missing data

Missing data will not be imputed.

Primary endpoints analysis

Kaplan-Meier plots will be used to assess time to first Fabry associated clinical event (FACEs). Cox proportional hazard models will be used to assess the association between baseline characteristics and incident Fabry associated clinical event. The following baseline variables will be used in the model as covariables: age, time since diagnosis of Fabry disease, previous FACE (renal, cardiac, or cerebrovascular).

Patient benefit index

Not applicable

Secondary endpoints analysis

Incidence rate of Fabry associated clinical events will be evaluated as events per 1000 patient – years

If data allows incidence and prevalence will be analyzed in subgroups (i.e., classic males, females, late-onset males, and females)

Renal, cerebrovascular, and cardiac function analyses will be performed in the same subgroups, as well as safety analyses per subgroup.

Quality control

Monitoring

Investigator training

Documents to perform the study will be mailed to physicians who agreed to participate, including the investigator agreements (fees agreement and confidentiality agreement).

Investigators and other designated site staff who have agreed to participate will be trained about the protocol, study procedures and CRF completion by teleconference or on-site visits.

Study variables will be recorded in a CRF, and patient data will be handled preserving confidentiality. Investigators, or other designated site staff will analyze all Fabry patients with amenable mutations and will extract clinical and laboratory features from original medical records (primary source).

Audits

Not applicable

Inspections

Not applicable

Limitations of the research methodology

The participating center has been selected as the Danish national Fabry center, with more than 20 years of experience managing patients with Fabry disease.

As the clinical data will be collected retrospectively, it is expected that some clinical data will be missing.

Protection of human subjects

Ethics and Good Clinical Practice

The study will be conducted in accordance with ICH GCP. All applicable subject privacy requirements, and the ethical principles that are outlined in the Declaration of Helsinki 2013, including, but not limited to: Danish legal and health authorities and Ethical committee's review and favourable approval of study protocol and any subsequent amendments. The study will be reviewed and approved by the Danish Data Protection Agency, and the Danish Health Data Authority. With special regards to enrolment of patients who is dead, the study will be reviewed and approved by the Scientific Ethics Committee, The Capital Region, Denmark according to the specific legal requirements. Data will be anonymized (coded) when entered in the database.

Regulatory framework of the study

No drug will be evaluated prospectively, therefore, regulatory submission to the Danish Medical Association is not required in Denmark. Regarding data-collection, approval will be acquired from the legal regulatory authorities of the Capital Region of Denmark (see above).

Independent Ethics Committee / Institutional Review Board

No other Institute Review Board.

Subject information and consent

All patients will have provided written informed consent. The Study Team must maintain the original, signed Informed Consent/Assent Form. A copy of the signed Informed Consent/Assent Form must be given to the patient and/or their legally authorized representative.

A dated entry will be available in the patient's chart to confirm that informed consent/assent was obtained prior to any extraction of data and that the patient or their legally authorized representative received a copy of the signed informed consent/assent.

Subjects and data confidentiality

Subject Confidentiality

Information on patients' identity shall be considered as confidential for all effects and purposes. The patients' identity should not be revealed nor published under any circumstances. Patient data recorded in the CRF will be documented anonymously, coded with a patient number in such a way that only the investigator and site staff may associate data with an identified or identifiable

individual or his/her medical record. All other parties involved in data management, analysis and storage will receive, and subsequently analyze, non-identifiable patient data.

Data Confidentiality

By signing the investigator's confidentiality agreement, the investigator affirms to Amicus that information furnished by Amicus to the investigator will be kept in confidence and such information will be divulged to any expert committee, affiliated institution, and employees only under an appropriate understanding of confidentiality with such committee, affiliated institution, and employees.

Data handling and record keeping

By signing the investigator's confidentiality agreement, the investigator agrees that, within local regulatory restrictions and ethical considerations, Amicus or any Regulatory Agency may consult and/or copy study documents to verify the included data.

Record keeping and archiving

According to Danish law and to Amicus policies.

The investigator will keep a separate log of subjects' unique identification numbers in a tracker.

Only authorized persons will have access to identifiable personal details, if required for data verification. Data protection and privacy regulations will be observed in capturing, forwarding, processing, and storing subject data. If applicable, subjects will be informed accordingly and will be requested to give their consent on data handling procedures in accordance with national regulations.

In any presentations or in publications of the results of the study, the patients' identities will remain anonymous and confidential. Investigators, or investigator partners defined in annex 1 if applicable and various government health agencies may inspect the records of the study. Every effort will be made to keep the patients' personal medical data confidential.

During the development and analysis of the study, statistical program codes will be locally stored as documents according to best practices. Data protection laws in Denmark will be adhered to at all times. Further, the log file produced during the final run of each program will be stored locally at the investigator site as accompanying documents along with the results.

Transfer of Essential Documents

Not applicable

Financing and insurance

Financing

Senior researchers 2x50% data management 700,000 DKK

Specialist nurse to extract patient data 390,000 DKK

2 Medical students to extract data and manage data files 240,000 DKK

Meetings, IT access, literature review, publication costs 70,000 DKK

Total 1,400,000 DKK

Amicus sponsored

Insurance

Not applicable

Management and reporting of adverse events/adverse reactions

Adverse event (AE)

Collection of adverse events as Individual Case Safety Reports (ICSRs) is not required from such retrospective analyses of patient medical records as it is secondary data.

Plans for disseminating and communicating study results

The study, and study results, will be disclosed on clinicaltrials.gov and other relevant clinical study disclosure websites in accordance with relevant AMICUS policies. The primary publication is expected to be in an internationally recognised journal, with the Scientific Committee forming the majority of the authorship. Thereafter, any secondary publications will reference the original publication and will be authored in accordance with International Committee of Medical Journal Editors (ICMJE) requirements.

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

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Annex 1. List of stand-alone documents

Documents listed in Annex 1 can be maintained separately from the study protocol. They should be clearly identifiable and provided on request. Write "None" if there is no document or list documents in a table as indicated below.

Number	Date	Section of study protocol	Amendment or update	Reason
1	*xx.xx.2023*	*Text*	*Update*	*Description of participating clinical scientists and research staff*

Annex 2. ENCePP checklist for study protocols

A copy of the ENCePP Checklist for Study protocols available at http://www.encepp.eu/standards_and_guidances/index.html completed and signed by the main author of the study protocol should be included in Annex 2.

The checklist will facilitate the review of the protocol and evaluation of whether investigators have considered important methodological aspects.

In question 9.5 of the Checklist, Revision 1:

"Study start" means "Start of data collection"

"Study progress" means "Progress report(s)"

"Study completion" means "End of data collection"

"Reporting" means "Final report of the study results"

Annex 3. Additional information

Additional annexes may be included if necessary.

List annex 1.1 Description of participating clinical scientists and research staff

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