Supplemental methods

Contents

Phenotypic classification

In men a classical FD phenotype was defined as: leucocyte α-Gal A activity ≤5% of the median of the reference range and one or more typical FD symptoms (Fabry specific neuropathic pain, angiokeratoma, and/or cornea verticillata).[1] A lysoGb3 >40 nmol/L before start of ERT supported the diagnosis of a classical phenotype. In women a classical phenotype was defined as: presence of one or more typical FD symptoms (in the patient or a male family member with the same mutation). Patients not fulfilling these criteria were classified as non-classical.

Data collection – visit frequency

In patients treated with enzyme replacement therapy (ERT) biannual evaluation at the outpatient clinic includes routine urine- and blood tests and yearly cardiac and brain MRIs. For untreated patients with classical disease, frequency of these evaluations are yearly (urine- and blood tests) and biyearly (MRIs). For untreated non-classical patients this frequency is biyearly and once every four years.

Basilar artery diameter

MRA is the reference standard when measuring the basilar artery diameter.[2] In our center, Multiple Overlapping Thin Slab Acquisition (MOTSA) imaging (high-resolution cross-sectional MRA image of vessels) [3] was added in to the scan protocol in the year 2012. As included scans range back to 2004, using MOTSA for the assessment of basilar artery diameter would have resulted in a major loss of data. When reviewing the literature, axial T2 seemed to be an acceptable alternative if MRA is not available in non-FD [4-7] and FD populations.[8] In a previous study, there was a strong correlation between MRA and T2-weigthed measurement of the basilar artery diameter.[9] To confirm this relation in our own population we assessed the BAD in T2-weighted imaging and if available in the MOTSA images as well.

Demographics, treatment, evaluation of complications

Renal function was evaluated by calculating the estimated glomerular filtration rate using the CKD-EPI formula for patients ≥18 years old[10] and the creatinine-based bedside Schwartz formula for patients <18 years.[11] Renal events were defined as a history of renal transplantation or dialysis. Albuminuria at baseline was graded using the KDIGO categories as A1 (normal to mildly increased) to A3 (severely increased).[10]

The left ventricular mass index (LVMi) was measured on MRI without the papillary muscles,[12] adjusted for body surface area (Dubois formula).

Mutations were classified as missense, nonsense or "other". Because of similar effects on enzyme activity we included frameshift mutations in the nonsense mutations category.

Two ERTs are approved in Europe: agalsidase alpha (Replagal, Shire, 0.2 mg/kg/every other week (EOW)) and agalsidase beta (Fabrazyme, Sanofi Genzyme, 1.0 mg/kg/EOW). Years treated were calculated as the time difference between the start of ERT and every MRI. Zero years treated was assigned for scans made in untreated patients.

LysoGb3 levels before start ERT were measured in plasma using tandem mass spectrometry with isotope labeled or glycine labeled lysoGb3 as internal standard. If both were available at the same time point we preferred the measurement using glycine labeled internal standard. Both internal standards show excellent intra-class correlation.[13]

Presence of hypertension, type 2 diabetes or the use of antidiabetic medication was extracted from

medical history. LDL-cholesterol levels were extracted from our local database. Medication use was defined as the use of a specific drug at any time prior to or during the follow-up time.

Cardiac complications

Data on cardiac complications were gathered in an observational retrospective longitudinal cohort study on the progression of cardiac involvement in FD. Using all patient charts, cardiac MRIs, echocardiography and clinical letters from birth to last follow-up date, data on predefined cardiac events were extracted including date of occurrence (for event definition see **Supplemental table 3**).

Statistical methods

To evaluate the intra-rater reliability, a random subsample of 30 scans was selected and reassessed 17 months after initial scan assessment. Both neuroradiologists were blinded for their initial assessment. The intra-rater reliability of the basilar artery diameter was assessed using the intra-class correlation coefficient (2-way mixed effects model, absolute agreement, single ratings).[14] The intra-rater reliability of the Fazekas scale, Scheltens scale and presence or absence of infarctions were assessed using Kendall's concordance coefficient, corrected for ties.

In the cumulative logistic mixed effect models, if patient received renal replacement therapy or a kidney transplantation, the subsequent scans after this date were not used in the evaluation of the effect of eGFR change on progression of the WMLs or infarctions on MRI.

Supplemental table 1 Acquisition parameters for brain MRI

Parameter	**FLAIR**	**T2**	**T1**
Plane	Axial	Axial	Axial
Voxel volume (mm^3), median (range)	1.0 (0.7-3.3)	1.0 (0.5-1.2)	1.0 (0.7-3.3)
Slice thickness (mm), median (range)	5.0 (1.1-5.0)	5.0 (3.0-5.0)	5.0 (3.0-5.0)
Interslice gap (mm), median (range)	5.5 (0.6-6.5)	5.5 (3.3-6.5)	5.5 (3.0-6.5)
TR (msec), median (range)	10113 (4000-11000)	4206 (2489-5938)	530 (7-600)
TE (msec), median (range)	100 (100-365)	80 (80-80)	9.8 (3.1-9.8)
TI (msec), median (range)	2600 (1650-2600)	0 (0-0)	0 (0-0)
Flip angle (degree), median (range)	90 (90-90)	90 (90-90)	90 (8-90)
Matrix, median (range) * median (range)	256 (208-312) * 166 (145-312)	400 (328-408) * 307 (240-377)	256 (232-288) * 256 (205-288)

FLAIR = Fluid-Attenuated Inversion Recovery, FOV = Field of View, TE = Echo Time, TI = Inversion Time, TR = Repetition Time

Supplemental table 2 MRI brain assessment

Description	Scale	Response options
Periventricular WMLs	Fazekas[15]	0: Absence 1: "caps" or pencil-thin lining 2: Smooth "halo" 3: Irregular periventricular lesions extending into deep white matter
Deep WMLs	Fazekas[15]	0: Absence or a single punctate lesion 1: Multiple punctate lesions 2: Beginning confluency of lesions (bridging) 3: Large confluent lesions Total score: 0 to 6
Periventricular WMLs: - Occipital - Lateral - Frontal	Scheltens[16]	0: Absence 1: ≤5 mm 2: >5 mm and <10 mm Periventricular WMLs exceeding 10 mm were per definition scored as deep Total score: 0 to 6
Deep WMLs: - Frontal - Parietal - Temporal - Occipital	Scheltens[16]	0: Absence 1: ≤ 3mm and n≤5 2: ≤ 3mm and n≥6 3: 4-10mm and n≤5 4: 4-10mm and n≥6 5: ≥11 mm and n≥1 6: Confluent WMLs Total score: 0 to 24
Infarctions	-	Presence or absence of infarctions
Basilar artery diameter (mm)	-	1: Caudal (shortly after the confluence of the vertebral arteries) 2: Intermediate (in the middle of the basilar artery) 3: Rostral (just before the bifurcation) Total score: average of 1, 2 and 3

WMLs = White matter lesions

Supplemental table 3 Definitions cardiac events

Events	Definition
Ischemic heart disease	
Coronary artery bypass graft	Open heart surgery where a bypass is placed around one or more (stenotic) coronary arteries
Percutaneous coronary intervention	Non-surgical intervention in which coronary stenosis is resolved with coronary angioplasty with or without the placement of a coronary stent
Coronary atherosclerosis*	>50% stenosis of luminal diameter of left main coronary artery or >70% stenosis of luminal diameter of at least one of the major epicardial coronary arteries one CAG[17] Patients were also classified as having coronary atherosclerosis if there were indications of myocardial ischemia on: - Stress ECG - First pass perfusion cardiac MRI - Regional wall movement abnormalities seen on echocardiography with ischemic changes on ECG - Dobutamine stress MRI
Atrial fibrillation	Irregular heart rhythm without identifiable p-waves recorded on ECG
Moderate to severe valve dysfunction	First ultrasound report mentioning moderate to severe stenosis of insufficiency of the mitral, tricuspid or aortic valve. Or heart valve dysfunction that required surgery where no previous ultrasound reports were available[18 19]
Systolic dysfunction	Left ventricular ejection fraction <50% on MRI.[20] If no MRI is available: left ventricular ejection fraction <55% on echocardiography[21]
Left ventricular outflow tract obstruction	Dynamic gradient of ≥30 mmHg on echocardiogram in the left ventricular outflow tract measured during rest, Valsalva procedure or exercise[22]

** Events discussed with the expert panel*

AV = atrioventricular, bpm = beats per minute, CAG = coronary angiogram, ECG = electrocardiogram, ICD = implantable cardioverter-defibrillator, CRT-D = cardiac resynchronization therapy with defibrillator

Supplemental table 4 Categorization of variables included in mixed models

Variable	Type	Options	Details	Time dependent[†]
Dependent variables				
Fazekas scale	Ordinal	Score range: 0-6	-	Yes
Infarctions	Ordinal/categorical	Absent (0)* / present (1)	Included as ordinal to improve comparability of results to Fazekas scale	Yes
Independent variables				
Age	Continuous	-	-	Yes
Sex	Categorical	Women*/Men	-	No
Phenotype	Categorical	Non-classical*/classical	-	No
Years treated ERT	Categorical	<6 months/≥6 months	-	Yes
Years treated ERT	Continuous	-	-	Yes
Changes in eGFR	Continuous	-	Scans after renal replacement therapy or renal transplantation were removed	Yes
Changes in LVMi	Continuous	-	Missing values were imputed	Yes
Hypertension	Categorical	No hypertension*/ Hypertension	-	No
Changes in BAD	Continuous	-	-	Yes
LDL-cholesterol	Continuous	-	-	No
AF	Categorical	No AF*/AF	-	Yes

Ischemic heart disease	Categorical	No IHD*/IHD	Composite of coronary artery bypass graft, percutaneous coronary intervention, coronary atherosclerosis	Yes
Valvular dysfunction	Categorical	No valvular dysfunction*/Valvular dysfunction	-	Yes
Systolic dysfunction and LVOTO	Categorical	No SD or LVOTO*/SD or LVOTO	-	Yes
MRI scanner	Categorical	Ingenia*/Intera	All scans before October 2012 were made on the Intera system, all scans afterwards on the Ingenia system. The changes in acquisition parameters were simultaneous with the system switch	Yes
Fazekas scale	Continuous	Score range: 0-6	The Fazekas scale was only used in relation to presence or absence of infarctions	Yes

† Some variables were time-dependent. This included continuous variables (e.g. different eGFR measurements of a patient were used during follow-up) and categorical variables (e.g. a patient developed AF during follow-up. Scans beforehand were coded as "No AF", scans afterwards as "AF present"). Other variables were time-independent. This included continuous variables (e.g. the LDL-cholesterol measurements were extracted once per patient before or during follow-up and this value was matched to all scans) and categorical variables (e.g. hypertension present before or during follow-up. All scans of this patient were coded as "Hypertension present").

** Reference category*

AF = atrial fibrillation, BAD = basilar artery diameter, eGFR = estimated glomerular filtration rate, ERT = enzyme replacement therapy, IHD = ischemic heart disease, LDL = low density lipoprotein, LVMi = left ventricular mass index, LVOTO = left ventricular outflow tract obstruction

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