Determinants of cerebral radiological progression in Fabry disease

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ABSTRACT

Background and aim It is unclear which patients with Fabry disease (FD) are at risk for progression of white matter lesions (WMLs) and brain infarctions and whether enzyme replacement therapy (ERT) changes this risk. The aim of this study was to determine the effect of ERT and clinical characteristics on progression of WMLs and infarctions on MRI in patients with FD.

Methods MRIs were assessed for WMLs (Fazekas scale), infarctions and basilar artery diameter (BAD). The effect of clinical characteristics (renal and cardiac involvement, cardiovascular risk factors, cardiac complications, BAD) and ERT on WM and infarction progression was evaluated using mixed models.

Results One hundred forty-nine patients were included (median age: 39 years, 38% men, 79% classical phenotype). Median follow-up time was 7 years (range: 0–13 years) with a median number of MRIs per patient of 5 (range: 1–14), resulting in a total of 852 scans. Variables independently associated with WML and infarction progression were age, male sex and a classical phenotype. Progression of WMLs and infarctions was not affected by adding ERT to the model, neither for the whole group, nor for early treated patients. Progression was highly variable among patients which could not be explained by other known variables such as hypertension, cholesterol, atrial fibrillation and changes in kidney function, left ventricular mass or BAD.

Conclusion Progression of WMLs and cerebral infarctions in FD is mainly related to age, sex and phenotype. Additional effects of established cardiovascular risk factors, organ involvement and treatment with ERT are probably small to negligible.

INTRODUCTION

Fabry disease (FD; OMIM 301500) is a rare X-inherited lysosomal storage disorder. A mutation in the GLA gene leads to a deficiency of α-galactosidase A activity (enzyme commission no. 3.2.1.22). This results in accumulation of globotriaosyleceramide (Gb3) and related compounds in various cell types throughout the body, leading to cellular damage and loss of function of especially the kidney, heart and brain. 1 Male or female sex is an important predictor of disease severity in FD, with a higher complication rate in men. 2 Phenotypically, patients can be classified as having classical or non-classical disease, with a more attenuated disease course in non-classical patients. 2

Recommended follow-up of patients with FD includes routine brain MRIs. 3 Commonly detected cerebral manifestations of FD on structural MRIs are white matter lesions (WMLs), 4 lacunar infarctions 5 and an increased basilar artery diameter (BAD). 6 WMLs and silent infarctions have been related to cognitive decline, clinical stroke risk and early death in the general population. 7 8 The consequences of WMLs and brain infarctions are less clear in FD, but there are indications that WMLs are related to cognitive impairment and clinical stroke. 4 9

Until recently, the only available specific treatment for FD was enzyme replacement therapy (ERT). The effect of ERT on cerebral manifestations, assessed by MRI, is unclear. Studies report contrasting results, 10–13 most likely due to small sample sizes, short follow-up duration and lack of stratification for phenotype in most studies. Moreover, while factors like hypertension, decreased renal function and atrial fibrillation (AF) have been related to WMLs and infarctions in the general population, 14–16 little is known about the effect of these factors on cerebral manifestations of FD. Determining which patients are at risk for progression of cerebral disease, establishing the importance of potentially modifiable risk factors and determining the effect of ERT could support patient management.

The aim of this study was twofold: 1) to describe WML, BAD and infarction progression in FD and 2) to investigate the effect of clinical characteristics, cardiovascular risk factors and ERT on the progression of WMLs and infarctions in a large retrospective cohort study conducted at a reference centre for FD.
scanner were extracted from the database for this retrospective longitudinal cohort study.

According to Dutch law, no approval of the study protocol was needed as this is a retrospective study and patients were not subjected to procedures or rules of behaviour additionally to regular clinical follow-up. Patient records were de-identified prior to analysis.1

In addition to the methods described below, please see online supplementary methods for more detailed information on phenotypic classification, data collection, demographics, cardiac complications and statistical methods.

**Diagnosis, phenotype, benign variants and genetic variants of unknown significance**

A diagnosis FD was made if: 1) a pathogenic mutation in the GLA gene was present (men and women) and 2) α-galactosidase A activity was decreased in leucocytes (men). Pathogenicity of the mutation was supported by: typical FD symptoms (Fabry-specific neuropathic pain, angiokeratoma and/or cornea verticillata in the patient or a family member), increased lysoGb3 levels, biopsy of an affected organ with typical zebra body inclusions, the mutation being described as pathogenic in literature and/or (more recently) presence of decreased T1 values on cardiac MRI. All patients were classified as having classical or non-classical disease based on strictly defined criteria.2 18 19

The following genetic variants were regarded as benign and subjects carrying these variants were not included in this study: p.A143T, p.D313Y, p.R118C.2 Subjects with the variants p.L106F (n=1) and p.P60L (n=7) were excluded since we were unsure about the pathogenicity of these variants.20 21

**Imaging protocol and assessment procedure**

All MRI data were obtained using 3T scanners. Scans before October 2012 were made on the Intera system (Philips Intera, Philips Medical Systems, Best, The Netherlands) and scans after October 2012 on the Ingenia system (Philips Ingenia, Philips Medical Systems, Best, The Netherlands). A change in scan acquisition parameters occurred simultaneously with the switch in MRI systems (online supplementary table 1). Scans were assessed by two neuroradiologists (MRL and MGFL). MRL assessed the BAD and MGFL assessed WMLs and infarctions. All identifying data (eg, age, sex, scan date) were removed from the MRI scans and both neuroradiologists were also blinded for scan order (baseline or follow-up).

**MRI brain assessment**

**White matter lesions**

WMLs were defined as hyperintensities on axial T2-weighted and fluid-attenuated inversion recovery-weighted (FLAIR-weighted) imaging without cavitation.22 23 WMLs were visually assessed using both the Fazekas scale24 and the Scheltens scale25 (online supplementary table 2). The Fazekas scale rates WMLs in two locations: periventricular and deep. Severity is rated per location from 0 (no WML) to 3 (severe confluent WMLs), resulting in a total score between 0 and 6.

The Scheltens scale is semi-quantitative and provides regional information for both periventricular and deep WMLs. Periventricular WMLs are rated in three regions resulting in a score from 0 (no WMLs) to 6 (severe periventricular lesions) and deep WMLs are rated in four regions resulting in a score from 0 (no WMLs) to 24 (severe deep lesions), with the total score ranging from 0 to 30.26

**Infarctions**

Infarctions were defined as focal lesions ≥3 mm, with an irregular hyperintense rim and central cavitation on axial T2-weighted and FLAIR-weighted imaging22 and were scored as present or absent.

**Basilar artery**

The BAD was assessed on both axial T2 images and Multiple Overlapping Thin Slab Acquisition (MOTSA) images (high-resolution cross-sectional MRA image of vessels) and was calculated as the average of three measures (caudal, intermediate and rostral) in mm (online supplementary methods: basilar artery diameter).6 12 26

**Disease characteristics and treatment data**

To assess the effect of patient characteristics and ERT on MRI brain parameters, we combined the scans with clinical data obtained at a nearby time point (with a maximum of 1 year time difference). If a patient experienced a clinical event (eg, AF, kidney transplantation), the time up to the event was classified as ‘event-free time’ and time after the event was classified as ‘postevent time’. Cardiovascular risk factors and cholesterol levels were assessed once, either before or during follow-up.

Renal function was evaluated by calculating the estimated glomerular filtration rate (eGFR).27 Left ventricular mass index (LVMI) was measured on cardiac MRI.28

Years treated with ERT were calculated. Inhibitory antidrug antibodies to ERT (from here on referred to as antibodies) were rated as positive (inhibitory titre ≥6) or negative (inhibitory titre <6).2 20 If the antibody response was transient it was classified as negative. LysoGb3 levels at diagnosis were measured in plasma using tandem mass spectrometry.2

Hypertension was defined as two outpatient blood pressure measurements with a systolic pressure of >140 mm Hg and/or diastolic pressure of >90 mm Hg or use of antihypertensive medication. Clinical cerebrovascular complications were defined as a stroke or TIA diagnosed by a neurologist.

Data on cardiac complications were gathered by one of the authors (M.E.S.) by reviewing all patient charts, clinical letters, echocardiography and/or cardiac MRIs from birth until January 2019, extracting predefined cardiac complications (online supplementary table 3). For this study, we extracted data on AF, ischaemic heart disease, valve dysfunction, systolic dysfunction and left ventricular outflow tract obstruction (LVOTO).

**Statistical methods**

Data are presented as median and range or mean±SD where appropriate. R (V3.5.1) was used for statistical analyses.30

The intrarater reliability of the Fazekas scale, Scheltens scale and presence, absence of infarctions and BAD measurements were assessed using Kendalls coefficient of concordance (W) and the intraclass correlation coefficient (ICC)31 in a randomly selected subsample of 30 reassessed scans.

Cumulative logistic mixed effect models (which preserve the ordinal nature of the data, package: ordinal; clmm232) were used to evaluate the importance of variables on the progression risk of WMLs (Fazekas score) and the progression risk of infarctions on MRI (absence or presence). A random patient effect was introduced into all mixed models to account for interpatient differences. The following variables were regarded as potentially important in relation to the risk of progression of both the WMLs and infarctions: age, sex, phenotype, years on ERT, eGFR, LVMI, hypertension, BAD, low-density lipoprotein (LDL)
cholesterol, AF, ischaemic heart disease and the MRI system used. The following variables were assumed to influence the progression risk of infarctions only: valve dysfunction, systolic dysfunction, LVOTO and Fazekas score.

Variables included were identified through a combination of potential importance in the literature (FD or general population), availability in our local database and aetiological plausibility. We created a baseline model for progression of both the WMLs and infarctions that included age, sex and phenotype as fixed effects, since we expected these to be most important for progression. The other variables of interest (eg, hypertension, eGFR) were tested for relevance by adding these to the baseline model (online supplementary table 4). Due to the exploratory nature of this study we tested many hypotheses. To reduce the false positive rate (type I errors), we regarded p values<0.01 as significant.

In this study, 149 patients with FD were included (79.2% with a classical phenotype; 37.6% men) with a median age of 38.8 years (range: 9.1–72.3 years) (table 1). Eighty-eight patients (59.1%) were treated with ERT at any time during follow-up.

In addition to the results below, please see online supplementary results for more detailed information on: intrarater reliability, relation between basilar artery diameter on MOTSA and T2-weighted imaging and adjustment of variables for mixed models.

### Intrarater reliability

Intrarater reliability was excellent for the Fazekas scale (W: 0.95), Scheltens scale (W: 0.97), infarctions (W: 1.00) and BAD (ICC: 0.96).

### Brain MRIs and involvement

During a median follow-up of 7.0 years (range: 0.0–13.1 years), patients were scanned a median of 5 times (range: 1–14) resulting

### Table 1 Patient characteristics

|                                | All                  | Men                  | Women                |
|--------------------------------|----------------------|----------------------|----------------------|
| **Events before first brain MRI** |                      |                      |                      |
| Cerebrovascular event, n (%)   | 11 (7.4%)            | 5 (11.1%)            | 6 (9.1%)             |
| Stroke, n (%)                  | 6 (4.0%)             | 3 (6.7%)             | 3 (4.1%)             |
| TIA, n (%)                     | 6 (4.0%)             | 2 (4.4%)             | 4 (4.1%)             |
| Ischaemic heart disease, n (%) | 2 (1.3%)             | 0 (0.0%)             | 1 (1.4%)             |
| Atrial fibrillation, n (%)     | 6 (4.0%)             | 3 (6.7%)             | 3 (4.1%)             |
| Systolic dysfunction or LVOTO, n (%) | 5 (3.4%)       | 1 (2.2%)             | 1 (1.4%)             |
| Moderate/Severe valve dysfunction, n (%) | 4 (2.7%)        | 3 (6.7%)             | 0 (0.0%)             |
| Renal event, n (%)             | 1 (0.7%)             | 0 (0.0%)             | 1 (1.4%)             |
| **Kidney function at first MRI** |                      |                      |                      |
| eGFR in ml/min/1.73 m², median (range) | 103.9 (11.4–147.3) | 109.5 (19.4–147.3) | 89.7 (11.4–122.7) |
| Albuminuria >A1, n (%)         | 63/143 (44.1%)       | 23/45 (51.1%)        | 9/11 (81.8%)         |
| **Cardiovascular risk factors** |                      |                      |                      |
| Hypertension, n (%)            | 26 (18.7%)           | 5 (11.4%)            | 21 (16.9%)           |
| Type 2 diabetes, n (%)         | 2 (1.4%)             | 0 (0.0%)             | 1 (1.4%)             |
| LDL-cholesterol in mmol/L, median (range) | 2.32 (0.95–4.77) | 2.13 (1.36–4.77)     | 2.93 (1.14–4.02)     |
| **Medication**                 |                      |                      |                      |
| Statin, n (%)                  | 20 (13.4%)           | 8 (17.8%)            | 12 (16.9%)           |
| ACE/ARB, n (%)                 | 73 (49.0%)           | 23 (51.1%)           | 39 (53.4%)           |
| Antplatelet, n (%)             | 68 (45.6%)           | 25 (55.6%)           | 41 (56.2%)           |

Continuous variables are presented as median (range) and discrete variables as number (percentages).

*history of antibodies in one man with classical disease (before stopping ERT) was counted as positive, transients antibodies in two men with classical disease were counted as negative.

**One patient with a history of renal transplantation, no patients on dialysis.

ACE, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blockers; eGFR, estimated glomerular filtration rate; ERT, enzyme replacement therapy; LDL, low-density lipoproteins; LVOTO, left ventricular outflow tract obstruction; NA, not assessed; TIA, transient ischaemic attack.

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**RESULTS**

**Patient characteristics**

A total of 149 patients with FD was included (79.2% with a classical phenotype; 37.6% men) with a median age of 38.8 years (range: 9.1–72.3 years) (table 1). Eighty-eight patients (59.1%) were treated with ERT at any time during follow-up.

Fourteen patients developed AF during follow-up, resulting in a total of 20 patients with a history of AF (13.4%) (online supplementary table 5).

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**Brain MRIs and involvement**

During a median follow-up of 7.0 years (range: 0.0–13.1 years), patients were scanned a median of 5 times (range: 1–14) resulting in a total of 20 patients with a history of AF (13.4%) (online supplementary table 5).
in a total of 852 scans (table 2). Infarctions on MRI were present in 23 patients (15.6%) at baseline and in 42 patients (28.2%) at the end of follow-up. The median BAD was 3.33 mm (range: 1.85–5.83 mm) at baseline and increased to 3.67 mm (range: 1.85–7.25 mm) at follow-up.

Both WML severity and BAD progressed with age, with differences in rate of progression between the sex and phenotype divided subgroups (figures 1 and 2). Infarction rate was highest in men with a classical phenotype, with a median infarction-free survival of 46.5 years (figure 3).

Variables related to white matter lesion and infarction progression risk

Adjusted variables

We adjusted four variables for the mixed models. First, in 40 scans (4.7%) the BAD was measured in two instead of three slices, mostly because of severe caudal tortuosity. There was no significant effect of the number of slices on BAD ($\beta$: −0.09; 95% CI: −0.23 to 0.04, p=0.18), so we included BAD measurements irrespective of the number of slices. Second, the only variable with data missing for ≥5% was LVMi measured with cardiac MRI (online supplementary table 6) and multiple imputation was used to impute these missing data (online supplementary figure 1). Third, we combined Fazekas score 5 and 6 to improve power, since these scores were relatively rare. Lastly, years treated with ERT correlated strongly with the variable age ($r=0.88$; 95% CI 0.87 to 0.91; $p\leq0.0001$), increasing the risk of invalid results due to collinearity. Therefore, we analysed the effect of treatment with ERT both as a continuous variable (years treated) and as a binary variable. In this analysis, some patients were started on ERT just before their MRI, while it is known that biomarkers decrease gradually over the 6 months to 1 year after the start of ERT. Therefore, we classified ‘untreated’ as no ERT or a treatment duration <6 months and ‘treated’ as ≥6 months of treatment.

Continuous variables are presented as median (range) or mean (±SD) and discrete variables as number (percentages).

BAD, basilar artery diameter.

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**Table 2** Number of scans and brain involvement at first MRI and last MRI

|                    | All          |            |          |            |          |
|--------------------|--------------|------------|----------|------------|----------|
| Number of scans, n (%) | 852          | 321 (37.7%)| 46 (5.4%) | 446 (52.3%)| 39 (4.6%) |
| Scans per patient, median (range) | 5 (1–14)   | 7 (1–13)   | 2 (1–12)  | 6 (1–14)   | 1 (1–5)  |
| Patients with one scan, n (%) | 32 (21.5%)  | 4 (8.9%)   | 5 (45.5%) | 10 (13.7%) | 13 (65.0%)|
| Follow-up time in years, median (range) | 7.0 (0.0–13.1) | 7.9 (0.0–13.0) | 1.0 (0–13.0) | 9.2 (0–13.1) | 0.0 (0.0–11.9) |
| Numbers of scans on Intera system, n (%) | 512 (60.1%) | 187 (58.3%)| 29 (63.0%)| 275 (61.7%)| 21 (53.8%)|
| Fazekas first MRI, median (range) | 0 (0–6)     | 0 (0–6)    | 0 (0–3)  | 0 (0–6)    | 0 (0–4)  |
| Fazekas last MRI, median (range) | 1 (0–6)     | 1 (0–6)    | 1 (0–3)  | 1 (0–6)    | 0 (0–4)  |
| Fazekas first MRI, mean (±SD) | 1.17 (±1.62) | 1.42 (±1.97) | 1.00 (±1.10) | 1.20 (±1.56) | 0.60 (±1.05) |
| Fazekas last MRI, mean (±SD) | 1.46 (±1.84) | 2.07 (±2.37) | 1.09 (±1.04) | 1.37 (±1.63) | 0.65 (±1.04) |
| Scheltens first MRI, mean (±SD) | 4.7 (±7.3)  | 6.0 (±9.3) | 3.6 (±4.8) | 4.7 (±6.4) | 2.7 (±6.1) |
| Scheltens last MRI, mean (±SD) | 7.4 (±8.8)  | 10.1 (±11.2) | 5.0 (±5.4) | 7.3 (±7.6) | 2.7 (±6.0) |
| Infarctions first MRI, n (%) | 23 (15.6%)  | 12 (26.7%) | 2 (18.2%) | 8 (11.3%)  | 1 (5.0%)  |
| Infarctions last MRI, n (%) | 42 (28.2%)  | 21 (46.7%) | 3 (27.3%) | 17 (23.3%) | 1 (5.0%)  |
| BAD first MRI in mm, median (range) | 3.33 (1.85–5.83) | 3.88 (2.45–5.83) | 3.29 (2.77–3.80) | 3.17 (1.99–5.55) | 3.18 (1.85–4.61) |
| BAD last MRI in mm, median (range) | 3.67 (1.85–7.25) | 4.35 (2.87–7.25) | 3.55 (3.18–4.27) | 3.54 (2.46–5.84) | 3.13 (1.85–4.61) |

Continuous variables are presented as median (range) or mean (±SD) and discrete variables as number (percentages).
Cerebrovascular disease

Figure 3 Infarction-free survival stratified for sex and phenotype. Grey lines and black lines represent patients with a classical phenotype and non-classical phenotype, respectively. Continuous lines represent men and dotted lines represent women. Shaded areas represent the 95% CIs; | indicates censoring. Age was defined as age at first infarction present on MRI if no infarction was present, patients were censored at the end of follow-up time (at the last MRI).

Relation of variables to progression risk

Age, sex and phenotype

Variables independently related to the Fazekas score progression risk were a classical FD phenotype (OR: 52.9; 95% CI 11.0 to 254.8; p<0.0001), male sex (OR: 7.4; 95% CI 2.4 to 23.2; p<0.0006) and age (OR per 1 year increase: 1.3; 95% CI 1.2 to 1.3; p<0.0001) (table 3). Variables independently related to infarction progression risk were male sex (OR: 169; 95% CI 17 to 1697; p<0.0001) and age (OR 1-year increase: 1.3; 95% CI 1.2 to 1.4; p<0.0001) (table 3). Risk of progression of both infarctions and the Fazekas score was increased in men with classical disease compared with the other sex and phenotype divided subgroups. Men and women with non-classical disease had a decreased risk of progression of the Fazekas score compared with men and women with classical disease. Although age, sex and phenotype explained differences in progression risk of both the Fazekas score and infarctions, highly variable ‘random effects’ per patient remained present (online supplementary figure 2-3).

Enzyme replacement therapy

The random effects per patient were not explained by adding the treatment with ERT (<6 months vs ≥6 months) to the model (table 3). When including ‘treatment in years’ the model did not converge for both WMLs and infarction progression, probably due to collinearity of age and years treated. Removing the factor age resulted in a strong positive relation between years treated and progression, but the effect of age could not be separated of the ERT effect in this analysis.

We also found no significant interaction between an ‘early’ treatment start (<30 years) and treatment with ERT (online supplementary table 7), meaning that we did not find a difference in treatment effect in ‘early’ treated patients compared with patients treated at older age.

Of note, in contrast to recent findings,12 we found no relation between treatment with ERT and BAD progression.

Disease characteristics

Fazekas score and infarction progression risks were not related to changes in eGFR, changes in BAD, changes in LVMI, presence of hypertension, level of LDL-cholesterol, ischaemic heart disease or a history of AF (table 3). Additionally, the risk of infarction progression was not related to valve dysfunction, systolic dysfunction or LVOTO. Increasing Fazekas scores were related to a higher risk of infarction progression (OR per point increase: 1.94; 95% CI 1.32 to 2.85, p<0.0008).

The change in MRI system and simultaneous change in scan acquisition parameters influenced Fazekas score progression risk (Intera system: OR: 2.36; 95% CI 1.56 to 3.57). Age, sex and phenotype remained independently associated with Fazekas score progression risk when including the system type to the model.

In explorative analyses trying to explain the progression in men with classical disease, we found that higher Fazekas score progression risks were related to higher baseline lysoGb3 levels and to the presence of a nonsense/frameshift mutation but not to changes in BAD (online supplementary table 7).

Suggested follow-up frequency

Since age, sex and phenotype are the main variables related to progression, scan frequency should be adjusted for each patient group (suggested follow-up in online supplementary table 8).

DISCUSSION

In this study, a large cohort of patients with FD with a known disease phenotype was followed for a median of 7 years, providing a unique dataset of >850 brain MRIs. Our analyses showed major differences in risk of progression of WMLs and infarctions in different patient groups, with a high progression rate from an early age in men with classical disease, while women with non-classical disease had very limited cerebral disease manifestations. Also, despite treatment with ERT, both WMLs and infarctions progressed. Progression was not related to differences in vascular risk factors (hypertension, LDL-cholesterol) or FD organ involvement (changes in eGFR, LVMI, BAD, AF and other cardiac complications).

Evaluating the effect of ERT in non-randomised and uncontrolled studies warrants further discussion. Because no similar untreated cohort is studied, it is still possible that the progression rate is changed by ERT. While most FD studies evaluating the effect of ERT on WMLs have found no benefits in complete group analyses,4 10 11 it has been suggested that ERT might stabilise WMLs and prevent stroke in ‘early’ treated patients.10 35

In the current study, after correction for age, sex and phenotype, we found no relation between ERT, WML or infarction progression, even in our ‘early’ treated patients. We were not able to assess the effect of very early treatment initiation (eg, before the age of 16) as these patients were underrepresented in the current study.

Previous studies in FD have shown a strong progression of the WML burden starting from the fourth decade of life,3 4 36 which was also seen in our cohort (figure 1). While WMLs are also prevalent in the general population, progression in FD starts at an earlier age and the burden is higher: in our cohort, ~15% of patients had a periventricular and deep Fazekas score ≥2 at a median age of 39 years, a WML incidence and severity observed in the general population three decades later.3 37 Similarly, radiological infarctions were present in 15% of our cohort at baseline increasing to 28% during 7 years of follow-up, while the prevalence of (silent) brain infarctions in the general population at 40 years would be <5%, increasing to 15% two to three decades later.3 38

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The risk of WML and infarction progression was not equally distributed: subgroups divided by sex and phenotype showed large differences in risk of progression in our study, this differentiation was not addressed in previous studies. The limited WML acquisition parameters, which probably affected assessment of severity within the phenotypes, reflected by the relation between WML progression and lysoGb3 levels and mutation type in men with classical disease in this study.

This study has several strengths and limitations. The combination of the large dataset, long follow-up and clinical data-matching to all separate scans, in combination with the use of mixed models improved power and maximised data use.

The study was limited by the use of MRIs with changes in acquisition parameters, which probably affected assessment of WML progression. Nevertheless, the relation to age, sex and phenotype did not change with the switch in acquisition parameters, indicating decent reliability of the use of WML rating scales in these circumstances. Second, the sensitivity of the Fazekas and enormous sample sizes would be required to demonstrate any additional effects after taking the effects of age, sex and phenotype into account. Another possible explanation for differences in progression could be additional subtle differences in disease severity within the phenotypes, reflected by the relation between WML progression and lysoGb3 levels and mutation type in men with classical disease in this study.

### Table 3 Mixed models assessing the relation between variables and progression risk of the Fazekas score and infarctions

| Fixed effects | Fazekas score | Infarctions |
|---------------|---------------|-------------|
| Age           | 1.28 (1.24 to 1.33) | 1.26 (1.17 to 1.35) |
| Sex           | --            | --          |
| Men           | 7.4 (2.4 to 23.2) | 169 (17 to 1697) |
| Non-classical | 1             | 1           |
| Classical     | 52.9 (11.0 to 254.8) | 64 (2.5 to 1625) |
| Age           | 1.28 (1.24 to 1.33) | 1.26 (1.17 to 1.35) |
| Sex, phenotype | --           | --          |
| Men, classical | 11.0 (3.2 to 38.4) | 224 (19 to 2618) |
| Men, non-classical | 0.06 (0.01 to 0.49) | 1.42 (0.04 to 52.6) |
| Women, non-classical | 0.05 (0.01 to 0.39) | 0.07 (0.00 to 7.61) |
| Model 2 + <6 months of ERT | 1 | -- |
| ≥6 months of ERT | 134 (0.96 to 1.88) | 1.43 (0.73 to 2.81) |
| Model 2 + years treated with ERT | -- | -- |
| Model 2 + changes in eGFR | 0.99 (0.98 to 1.01) | 0.96 (0.93 to 0.99) |
| Model 2 + changes in LVMi on MRI | 1.03 (1.00 to 1.05) | 1.03 (0.99 to 1.07) |
| Model 2 + no hypertension | 1 | -- |
| Hypertension | 1.12 (0.26 to 4.82) | 0.61 (0.05 to 6.88) |
| Model 2 + changes in BAD | 0.90 (0.57 to 1.43) | 1.62 (0.65 to 4.05) |
| Model 2 + LDL-cholesterol | 1.21 (0.58 to 2.64) | 1.26 (0.31 to 5.13) |
| Model 1 + AF | -- | -- |
| AF | 0.77 (0.32 to 1.85) | 0.45 (0.08 to 2.49) |
| Model 2 + no ischaemic heart disease | 1 | -- |
| Ischaemic heart disease | 1.92 (0.57 to 6.49) | 0.75 (0.09 to 6.47) |
| Model 2 + no valve dysfunction | -- | -- |
| Valve dysfunction | -- | -- |
| Model 2 + no systolic dysfunction or LVOTO | -- | -- |
| Systolic dysfunction or LVOTO | -- | -- |
| Model 2 + MRI scanner Ingenia | 1 | -- |
| Intera | 2.36 (1.56 to 3.57) | 1.74 (0.75 to 4.03) |
| Model 2 + Fazekas scale | -- | -- |

In all models adding additional variables to model 2, the effect sizes of age and sex and phenotype divided subgroups remained similar and are therefore not presented. To reduce the false positive rate (type I errors), we regarded p values <0.01 as significant.

*Models did not converge, probably due to collinearity.
†The model was unable to run with non-classical patients included, probably due to the low number of non-classical patients with infarctions. Thus, for this analysis only classical patients were included.

AF, atrial fibrillation; BAD, basilar artery diameter; eGFR, estimated glomerular filtration rate; ERT, enzyme replacement therapy; LDL, low-density lipoprotein; LVMi, left ventricular mass index; LVOTO, left ventricular outflow tract obstruction.
Scheltens scale for progression of WMLs is variable and volumetric measurement of the white matter lesion load is seen as the golden standard. We choose assessment with visual semi-quantitative measurement of the white matter lesion load is seen as the Scheltens scale for progression of WMLs is variable and quantitative scales since these are fast, easy to use, allowed full end points require unrealistic large sample sizes and follow-up MRI or WMLs measured using the Fazekas scale as clinical treatment strategies for FD, longitudinal data collection infestations is clearly insufficient. With multiple newly emerging MRIs, since the effect of current treatment on cerebral manifestations is clearly insufficient. With multiple newly emerging treatment strategies for FD, longitudinal data collection in large, international, industry-independent registries are needed to facilitate comparison of effectiveness. However, infarctions on MRI or WMLs measured using the Fazekas scale as clinical end points require unrealistic large sample sizes and follow-up duration (see online supplementary figure 4-5 for trial sample size calculations). Diffusion-weighted and quantitative imaging of the brain should be longitudinally explored, as there is a clear need for validated surrogate markers for the occurrence of cerebral infarctions in FD.

Correction notice This article has been corrected since it appeared Online First. The license type has been corrected to CC BY.

Contributors SK: study design, acquisition, analysis and interpretation of data, first draft of manuscript. MGF: acquisition and interpretation of data, critical revision of manuscript. MJH: acquisition and interpretation of data, critical revision of manuscript. CEMH: study concept, study design, interpretation of data, study supervision, critical revision of manuscript. MES: acquisition and interpretation of data, critical revision of manuscript. KV: acquisition and interpretation of data, study supervision, critical revision of manuscript. LV: critical revision of manuscript. MGD: statistical support, critical revision of manuscript. ML: interpretation of data, study supervision, critical revision of manuscript.

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Patient consent for publication Not required.

Ethics approval Patient records were de-identified prior to analysis. The Medical Ethics Review Committee of the AMC confirmed that the Medical Research Involving Human Subjects Act (WMO) does not apply to this study (W19-417 # 19.484). This study was conducted in accordance with the Declaration of Helsinki of 2013.

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Data availability statement Data are available on reasonable request. The datasets generated and analysed during the current study are not publicly available. Because of the rarity of the disease, even anonymised data can be linked to a

Table 4 Main findings, recommendations and future research directions

| Topic | Main findings | Future research directions and recommendations |
|-------|---------------|-----------------------------------------------|
| Age, sex and phenotype | Are strongly related to progression of WMLs and infarctions on MRI | Should be corrected for in any analysis of variables relating to WMLs or infarctions and evaluating treatment effects in FD. |
| Treatment | Patients and doctors should expect progression of WMLs over time, independent of treatment status | The effect of very early ERT initiation, before any visual cerebral involvement is present, should be evaluated in high-risk patients (men with classical disease). |
| WML assessment | Semi-quantitative scales are able to detect WML progression in patients with FD in a long-term follow-up setting | Presence of some punctate WMLs in patients aged >50 years is not necessarily FD related and treatment initiation should not be solely based on this finding. |
| Biomarkers, variables of interest and pathology | Changes in BAD, eGFR and LMVi are not related to WML and infarction progression after correction for age, sex and phenotype | Randomised controlled trials for new treatment modalities in FD should include MRIs of the brain. |

BAD, basilar artery diameter; eGFR, estimated glomerular filtration index; ERT, enzyme replacement therapy; FD, Fabry disease; LMVi, left ventricular mass index; WML, white matter lesion.
specific individual. In case of a specific scientific question, requests to make part of the dataset available will be reviewed and seriously considered.

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