Supplemental results

Contents

Intra-rater reliability

Kendall's concordance coefficients were: 0.95 (p=0.003), 0.97 (p=0.002) and 1.00 (p=0.001) for the Fazekas score, Scheltens score and presence or absence of infarctions, respectively. The intra-class correlation coefficient for the basilar artery diameter (BAD) was 0.96 (p<0.0001).

Relation assessment basilar artery diameter on MOTSA and T2-weighted imaging

In total, 128 patients (82.6%) had at least one MOTSA scan and T2-weighted scan at the same time point. The spearman correlation of both basilar artery diameter (BAD) assessments was 0.80 (95%CI: 0.71-0.87, p = <0.0001).

Considering the strong correlation between MOTSA and T2-weighted assessment of the BAD and increase of power using T2-weighted imaging, further analyses are performed using the T2-weighted BAD measurements.

Adjustment of variables for mixed models

Basilar artery diameter

In a total of 40 scans (4.7%) in 21 patients (range scans per patient: 1-5) the BAD was measured in two slices instead of three, since the BAD was too short for three measurements (n=3) or severe caudal tortuosity (n=37). We used a linear mixed effect model with BAD as dependent variable, number of slices as fixed effect (options: two or three slices) and a random patient effect to evaluate potential differences between two and three slice assessments. There was no significant effect of the number of slices on BAD, but it is possible that two slices might lead to slightly lower BAD values (β: -0.09; 95%CI: -0.23-0.04, p=0.18). Since the effect is probably small, we included all measured diameters.

Multiple imputation of left ventricular mass index

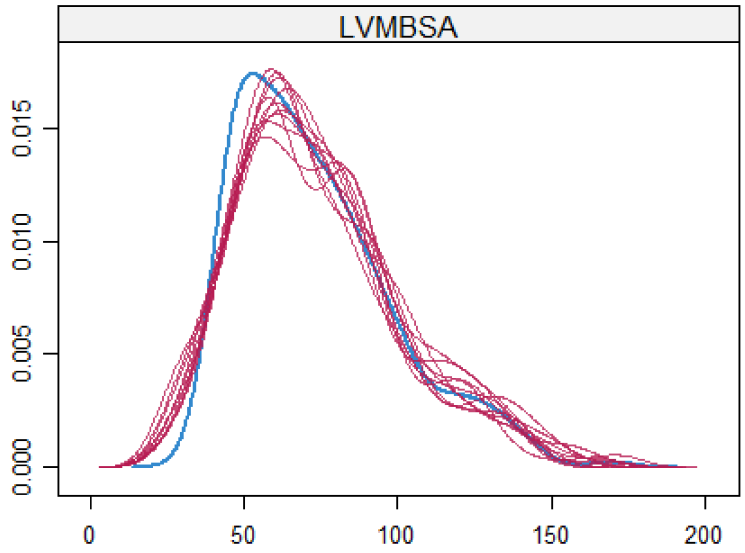
The only variable with >5% missing was the left ventricular mass index (LVMi) measured on cardiac MRI (**Supplemental table 6**). LVMi was mostly missing for scans before 2008 (the start of serial cardiac MRI imaging in our center). We assumed that data were missing at random and used multiple imputation by chained equations for the missing LVMi data (package: mice[1]). In short: mice replaces missing values with plausible values simultaneously in multiple copies of the same dataset. The copies of the same dataset are identical for non-missing data entries, but imputed values differ per dataset. Differences between datasets result from uncertainty in imputations. The results from analyses performed after imputation are pooled results from all imputed datasets.

Before multilevel imputation is performed the following specification should be made: which variables should be used for imputation, the method(s) used for imputation of the included variables and classification of the included variables (e.g. fixed effect, random effect).

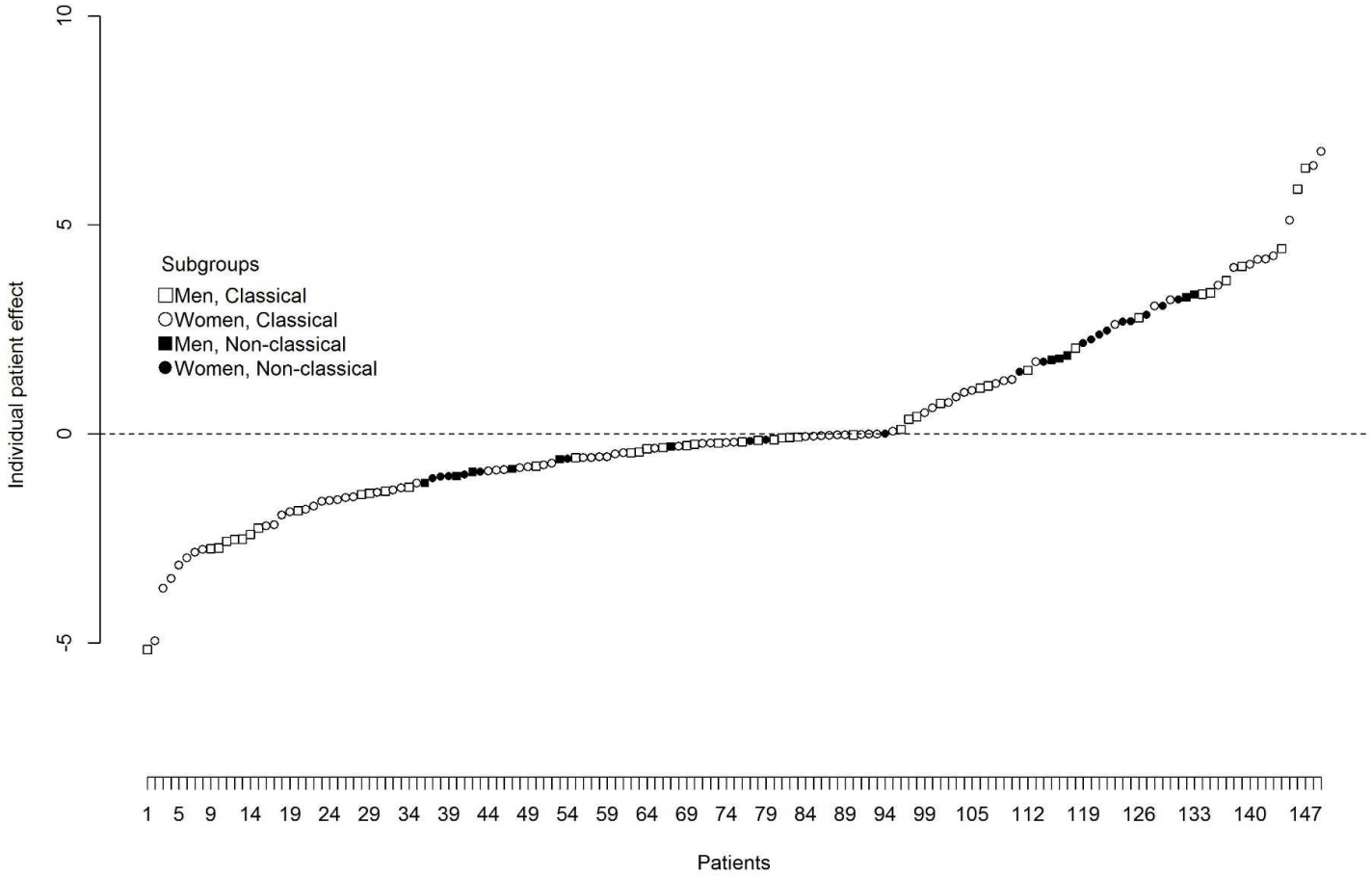
We used the following variables for imputation of cardiac LVMi:

- Continuous variables: body surface area (BSA), scan date, age, low density lipoprotein levels, estimated glomerular filtration rate, basilar artery diameter, years treated with enzyme replacement therapy, lysoGb3 levels before start treatment, Scheltens total score
- Categorical variables: sex, phenotype, cardiovascular events, atrial fibrillation, patient id
- Ordinal variables: Fazekas scale, presence or absence of infarctions

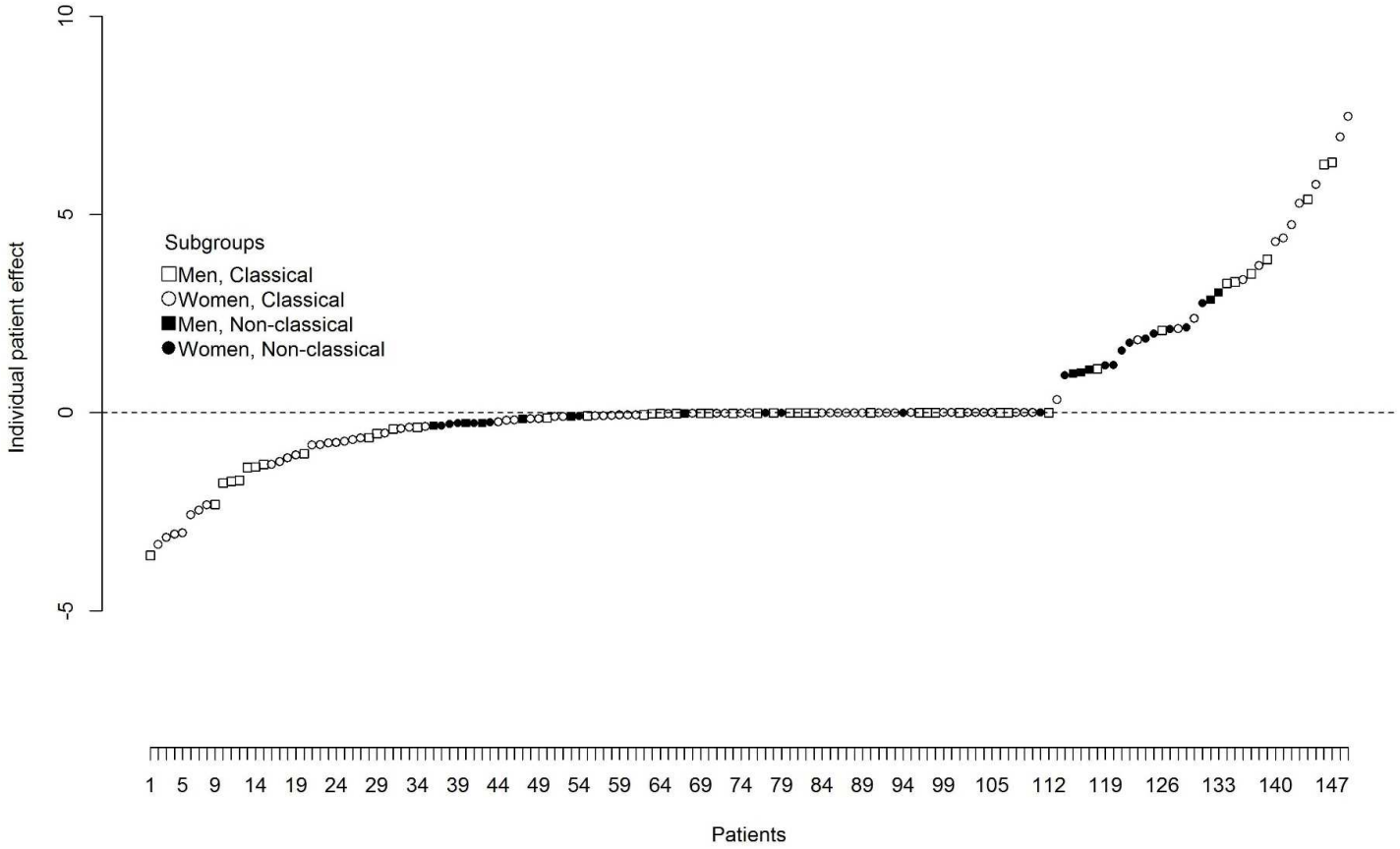
Patient id was classified as grouping variable, adapting the imputation to the multilevel structure. Cardiac LVMi was imputed with a method adapted to the multilevel structure of the data and continuous nature of the variable ("2l.pan"). The number of datasets created was set at 10 and the number of iterations at 5. Imputed LVMi values were allowed to range between 26-205 g/m^2, the minimum and maximum of the measured LVMi values ±20%. Imputed cardiac LVMi data were similarly distributed compared to the original data (**Supplemental figure 1**).



Supplemental figure 1 *Multiple imputation by chained equations of the left ventricular mass index (LVMi) assessed by MRI. The x-axis represents the LVMi corrected for body surface area in g/m^2. The y-axis represents the density, the probability of obtaining a range of values that the continuous variable can assume. The original data are shown with the blue line, the imputed values are represented by the red lines (n = 10, one for every created dataset).*



Supplemental figure 2 *Individual "random effects" per patient (n=149) for progression of WMLs assessed by using the Fazekas scale, divided by sex and phenotype. The dots represent individual patients' conditional modes (the difference between population averaged predictions and individual predictions). A positive score indicates an increased predicted progression for an individual patient compared to population averaged prediction, and a negative score indicates the opposite.*



Supplemental figure 3 *Individual "random effects" per patient (n=149) for progression of cerebral infarctions, divided by sex and phenotype. The dots represent individual patients' conditional modes (the difference between population averaged predictions and individual predictions). A positive score indicates an increased predicted progression for an individual patient compared to population averaged prediction, and a negative score indicates the opposite.*

Basilar artery diameter and enzyme replacement therapy

We tested the effect of treatment with ERT on the BAD in a linear mixed model with treatment with ERT, sex and phenotype as fixed effects and a random patient and age effect. BAD was significantly related to age (β age one year increase: 0.04, 95%CI: 0.04-0.05, P<0.0001), sex and phenotype but not to ERT (β ≥6 months of ERT: -0.03, 95%CI: -0.08-0.01, p<0.1198).

Supplemental table 5 Patient characteristics at last brain MRI

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
Patients, n (%)	149	45 (30.2%)	11 (7.4%)	73 (49.0%)	20 (13.4%)
Age at last MRI, median (range)	46.3 (14.1-82.0)	35.0 (14.1-64.5)	55.0 (24.0-75.8)	48.0 (18.1-82.0)	42.5 (19.5-72.3)
Events before last cerebral MRI					
Cerebrovascular event, n (%)	18 (12.1%)	9 (20.0%)	2 (18.2%)	7 (9.6%)	0 (0.0%)
Stroke, n (%)	12 (8.1%)	6 (13.3%)	1 (9.1%)	5 (6.8%)	0 (0.0%)
TIA, n (%)	9 (6.0%)	3 (6.7%)	2 (18.2%)	4 (5.5%)	0 (0.0%)
Cardiovascular events, n (%)	8 (5.4%)	5 (11.1%)	1 (9.1%)	2 (2.7%)	0 (0.0%)
Atrial fibrillation, n (%)	20 (13.4%)	10 (22.2%)	0 (0.0%)	10 (13.7%)	0 (0.0%)
Systolic dysfunction or LVOTO, n (%)	14 (9.4%)	7 (15.6%)	1 (9.1%)	4 (5.5%)	2 (10.0%)
Moderate/severe valve dysfunction, n (%)	21 (14.1%)	12 (26.7%)	0 (0.0%)	8 (11.0%)	1 (5.0%)
Renal event*, n (%)	5 (3.4%)	3 (6.7%)	1 (9.1%)	1 (1.4%)	0 (0.0%)
Kidney function at last MRI					
eGFR in ml/min/1.73m^2, median (range)	92.9 (11.4-144.0)	95.9 (15.3-144.0)	90.8 (11.4-122.7)	93.6 (28.1-131.6)	103.0 (53.6-120.7)
eGFR <60 ml/min/1.73m^2, n (%)	23/148 (15.5%)	10/45 (22.2%)	4/11 (36.4%)	8/73 (11.0%)	1/19 (5.3%)
Albuminuria > A1, n (%)	70/146 (47.9%)	27/45 (60.0%)	7/11 (63.6%)	33/71 (46.5%)	3/19 (15.8%)

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

** All five patients have had a renal transplantation, two patients are post-dialysis.*

eGFR = estimated glomerular filtration rate, ERT = enzyme replacement therapy, LVOTO = left ventricular outflow tract obstruction, TIA = transient ischemic attack

Supplemental table 6 Missing data of cerebral MRIs and other variables

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
Number of scans, n (%)	852	321 (37.7%)	46 (5.4%)	446 (52.3%)	39 (4.6%)
WMLs missing, n (%)	10 (1.2%)	3 (0.9%)	1 (2.2%)	6 (1.3%)	0 (0.0%)
Infarctions missing, n (%)	10 (1.2%)	3 (0.9%)	1 (2.2%)	6 (1.3%)	0 (0.0%)
BAD missing, n (%)	4 (0.5%)	1 (0.3%)	0 (0.0%)	3 (0.7%)	0 (0.0%)
eGFR, n (%)	6 (0.7%)	1 (0.3%)	0 (0.0%)	3 (0.7%)	2 (5.1%)
LVMi on MRI, n (%)	329 (38.6%)	117 (36.4%)	19 (41.3%)	177 (39.7%)	16 (41.0%)
Hypertension, n (%)	7 (0.8%)	2 (0.6%)	1 (2.2%)	1 (0.2%)	3 (7.7%)
LDL-cholesterol, n (%)	31 (3.6%)	18 (5.6%)	2 (4.3%)	7 (1.6%)	4 (10.3%)

Discrete variables are presented as number (percentages). Variables without missing data are not present (such as age, atrial fibrillation)

BAD = Basilar artery diameter, eGFR = estimated glomerular filtration rate, LDL = low density lipoproteins, LVMi = left ventricular mass index, WMLs = white matter lesions

Supplemental table 7 Mixed effect models to determine the importance of variables on the progression risk of the Fazekas scale and infarctions

	Fazekas score		Infarctions	
Fixed effects	OR (95% CI)	p-value	OR (95% CI)	p-value
Model 2 + Age ≥ 30 years	1.0	-	1.0	-
Age < 30 years	0.23 (0.10-0.58)	0.0016	0.26 (0.03-2.10)*	0.2068
<6 months of ERT	1.0	-	1.0	-
≥6 months of ERT	1.02 (0.67-1.56)	0.9292	1.86 (0.67-5.19)*	0.2349
Interaction treatment ERT and age < 30 years				
(≥6 months of ERT * Age ≥ 30 years)	1.0	-	1.0	-
(<6 months of ERT * Age < 30 years)	0.70 (0.47-1.05)	0.0820	1.33 (0.49-3.59)*	0.5739
Men with a classical disease phenotype				
Age	1.54 (1.41-1.69)	<0.0001	NA	-
LysoGb3 before start ERT	1.08 (1.02-1.14)	0.0073	NA	-
Age	1.58 (1.43-1.73)	<0.0001	1.28 (1.15-1.43)	<0.0001
Antibodies				
No antibodies present[#]	1.0	-	1.0	-

Antibodies present[#]	0.42 (0.11-1.65)	0.2130	4.57 (0.31-67.4)	0.2684
Age	1.55 (1.41-1.69)	<0.0001	1.30 (1.16-1.46)	<0.0001
Mutation				
Missense	1.0	-	1.0	-
Nonsene/frameshift	12.8 (1.04-158.9)	0.0469	23.5 (0.84-653.5)	0.0630
Other	190.5 (2.1-17241)	0.0224	64.6 (0.21-19527)	0.1525
Age	1.52 (1.38-1.68)	<0.0001	1.27 (1.12-1.44)	0.0002
Changes in BAD	1.38 (0.59-3.23)	0.4540	1.69 (0.38-7.61)	0.4912

** 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. # No antibodies present includes two men with transient antibodies. Antibodies present includes one man with a history of antibodies who stopped treatment with ERT on his own request.*
BAD = basilar artery diameter, CI = confidence interval, ERT = enzyme replacement therapy, NA = not available, does not converge, OR = odds ratio

Supplemental table 8 Scan frequency algorithm

Sex, phenotype	**Age in years**	**Scan frequency, every**
Men, classical	<10	No scans
	10-20	5 years
	20-30	3 years
	≥30	2 years
Men, non-classical	<30	No scans
	≥30	5 years
Women, classical	<15	No scans
	15-30	5 years
	30-40	3 years
	≥40	2 years
Women, non-classical	<40	No scans
	≥40	5 years
Specific indications		
History of stroke or confluent WMLs	-	2 years
At FD diagnosis	-	Once
Before start ERT	-	Once

ERT = enzyme replacement therapy, FD = Fabry disease, WMLs = white matter lesions

References

1. van Buuren S, Groothuis-Oudshoorn K. mice: Multivariate Imputation by Chained Equations in R. *Journal of Statistical Software; Vol 1, Issue 3 (2011)* 2011 doi: 10.18637/jss.v045.i03