Clinical prodromes of neurodegeneration in Anderson-Fabry disease

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ABSTRACT

Objective: To estimate the prevalence of prodromal clinical features of neurodegeneration in patients with Anderson-Fabry disease (AFD) in comparison to age-matched controls.

Methods: This is a single-center, prospective, cross-sectional study in 167 participants (60 heterozygous females and 50 hemizygous males with genetically confirmed AFD, 57 age-matched controls) using a clinical screening program consisting of structured interview, quantitative tests of motor function, and assessments of cognition, depression, olfaction, orthostatic intolerance, pain, REM sleep behavior disorder, and daytime sleepiness.

Results: In comparison to age-matched controls (mean age 48.3 years), patients with AFD (mean age 49.0 years) showed slower gait and transfer speed, poorer fine manual dexterity, and lower hand speed, which was independent of focal symptoms due to cerebrovascular disease. Patients with AFD were more severely affected by depression, pain, and daytime sleepiness and had a lower quality of life. These motor and nonmotor manifestations significantly correlated with clinical disease severity. However, patients with AFD did not reveal extrapyramidal motor features or signs of significant cognitive impairment, hyposmia, orthostatic intolerance, or REM sleep behavior disorder, which commonly precede later neurodegenerative disease. In our cohort, there were no differences in neurologic manifestations of AFD between heterozygous females and hemizygous males.

Conclusions: Aside from cerebrovascular manifestations and small fiber neuropathy, AFD results in a distinct neurologic phenotype comprising poorer motor performance and specific nonmotor features. In contrast to functional loss of glucocerebrosidase in Gaucher disease, α-galactosidase deficiency in AFD is not associated with a typical cluster of clinical features prodromal for neurodegenerative diseases, such as Parkinson disease. *Neurology*® 2015;84:1454–1464

GLOSSARY

α-Gal = α-galactosidase A; **AFD** = Anderson-Fabry disease; **ALP** = autophagy-lysosomal pathway; **BDI** = Beck Depression Inventory; **BPI** = Brief Pain Inventory; **ERT** = enzyme replacement therapy; **ESS** = Epworth Sleepiness Scale; **GD** = Gaucher disease; **MDS-UPDRS** = Movement Disorders Society–revised version of the Unified Parkinson's Disease Rating Scale; **MMSE** = Mini-Mental State Examination; **MoCA** = Montreal Cognitive Assessment; **MSSI** = Mainz Severity Score Index; **NIHSS** = NIH Stroke Scale; **PD** = Parkinson disease; **RBD** = REM sleep behavior disorder; **RBDSQ** = REM Sleep Behavior Disorder Screening Questionnaire; **SF-36** = 36-item Short-Form health survey; **SS-16** = Sniffin' Sticks 16-item smell identification test.

Anderson-Fabry disease (AFD) is a rare X-linked lysosomal storage disorder resulting in deficiency of the lysosomal enzyme α-galactosidase A (α-Gal)[1] and progressive accumulation of undegraded glycosphingolipids in various tissues. Neurologic manifestations of AFD include small fiber neuropathy associated with pain and reduced temperature sensation[2] and premature cerebrovascular events,[3] which are attributed to complex vasculopathy secondary to progressive glycosphingolipid accumulation in vessels and to cardiac involvement.

Neuropathologic studies have shown that glycosphingolipid storage in AFD is not restricted to the vasculature but also present in neurons of brain regions known to be affected in

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neurodegenerative diseases, such as the dorsal motor nucleus of the vagus, substantia nigra, and neocortex.[4,5] Moreover, disruption of the autophagy-lysosomal pathway (ALP) and focused presence of phosphorylated α-synuclein-containing lesions in the pons have been recently demonstrated in α-Gal-deficient mice.[6] Although Gaucher disease (GD), another lysosomal storage disorder, has been shown to be associated with at least a fivefold risk for Parkinson disease (PD),[7] and case reports have described L-dopa-responsive parkinsonism in patients with AFD,[8,9] the prevalence of prodromes of neurodegeneration has not been examined in this rare disease.

We report the results of a prospective, cross-sectional study investigating the clinical significance of neuronal glycosphingolipid storage in AFD by examining prodromal features of neurodegeneration in a large cohort of patients and age-matched controls. We hypothesized that neuronal glycosphingolipid storage and ALP disruption in AFD would result in clinical manifestations similar to those seen in GD patients and carriers.[10]

METHODS

Standard protocol approvals, registrations, and patient consents. This prospective, cross-sectional study had ethical approval from the North West London Research Ethics Committee (REC number 10/H0720/21). Written informed consent was obtained from all participants.

Participants. A total of 110 patients with AFD (60 heterozygous females, 50 hemizygous males) were recruited between April 2011 and February 2012 from the Lysosomal Storage Disorders Unit at the Royal Free London Hospital. Diagnosis was confirmed by molecular genetic analysis in all patients except one female (genetic data available on request). Fifty-seven controls, matched to patients for age and ethnicity, were identified from volunteers and nonmedical staff at the hospital. Controls were required to have a negative family history for lysosomal storage disorders and had no clinical signs of AFD. Neither patients nor controls had been diagnosed with neurodegenerative diseases in the past.

Procedures. Test procedures were performed identically in patients and controls. All participants were assessed by the same movement disorders specialist (M.L.) and underwent a structured interview and detailed clinical screening for motor and nonmotor prodromes of neurodegenerative diseases as explained below. Disease severity in patients with AFD was rated with the Mainz Severity Score Index (MSSI).[11] The presence of white matter abnormalities, lacuna, and territorial infarctions on latest cerebral MRI scans was used to document cerebrovascular sequelae of AFD. Renal involvement was assessed by serum creatinine, serum urea, glomerular filtration rate estimated by ^{51}Cr-EDTA clearance, and presence of proteinuria. Cardiac manifestations were assessed by the interventricular septal diameter measured by the latest transthoracic echocardiography.

Evaluation of motor function. Three tests were performed for quantitative assessment of motor function: (1) the Timed Up and Go, which is a basic measure of gait and transfer speed[12]; (2) the Purdue Pegboard, a test of fine manual dexterity, motor speed, and finger-eye coordination[13]; and (3) a shortened, 30-second version of the alternate tap test, used for the assessment of motor speed in the hands with a moderate requirement of coordination and accuracy.[14] Extrapyramidal motor symptoms were evaluated with the activities of daily living and motor subscales of the Movement Disorders Society–revised version of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS parts II and III).[15] The NIH Stroke Scale (NIHSS), a graded neurologic examination rating speech and language, cognition, visual field deficits, motor and sensory impairments, and ataxia, was used to identify potential focal neurologic deficits due to cerebrovascular disease.[16]

Evaluation of nonmotor function and quality of life. Cognitive function was assessed with the Mini-Mental State Examination (MMSE)[17] and the Montreal Cognitive Assessment (MoCA).[18] The MoCA has been shown to be more sensitive for the detection of mild cognitive impairment or dementia in PD than other scales, with optimal cut-off scores of <26 indicating mild cognitive impairment and <21 dementia.[19] Depressive symptoms were evaluated with the Beck Depression Inventory (BDI) II.[20] Olfactory function was assessed with the 16-item smell identification test from Sniffin' Sticks (SS-16; Burghart Messtechnik, Wedel, Germany). Individuals with anatomical upper airway abnormalities, respiratory tract infections, or a history of traumatic head injury were excluded from the analysis. Hyposmia was diagnosed at SS-16 scores <11, which in the UK population have shown to provide a PD probability of $\geq 50\%$.[21] Resting blood pressure and heart rate were taken after participants had been resting for 5 minutes in supine position and 2 minutes after standing up to check for signs of orthostatic intolerance. Drops of ≥ 20 mm Hg in systolic blood pressure were considered to be clinically significant. Pain was quantified with the Brief Pain Inventory (BPI), which measures intensity of pain and the interference of pain with the participant's life.[22] Features of REM sleep behavior disorder (RBD) were identified with the REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ), which was considered positive at scores of ≥ 5.[23] Daytime sleepiness was screened for with the Epworth Sleepiness Scale (ESS), with ESS values >10 points indicating significant sleepiness.[24] Quality of life was assessed with the EQ-5D-5L[25] and the 36-item Short-Form health survey (SF-36).[26]

Statistical analyses. Statistical analyses were performed with SPSS software, version 21.0 (SPSS, Chicago, IL). Since we expected sex differences due to X-linked inheritance in AFD, statistical comparisons were initially carried out separately for female and male participants with the unpaired t test or Mann-Whitney U test (continuous variables) and the χ^2 or Fisher exact test (discrete variables), as appropriate. For comparison of continuous variables between all participants, we used a general linear model with disease and sex as fixed factors and age as covariate, which was valid in all outcome parameters. The Spearman correlation was used to assess the association of clinical outcomes and the MSSI. Pairwise deletion was used for missing data. Unless stated otherwise, values are displayed as unadjusted means ± SD. Two-sided p values <0.05 were deemed statistically significant.

RESULTS

Study participants. A total of 110 patients with AFD (60 heterozygous females, 50 hemizygous males) and 57 controls (29 female, 28 male) were

included in our cross-sectional study. Mean age, age range, and ethnic background were similar between patients and controls (table 1). Patients with AFD were less likely to have university education and to be employed and more likely to be medically retired than controls. Hyperlipidemia and hypertension had been diagnosed more often in patients, whereas other cardiovascular risk factors were similar between groups. Cerebrovascular events were more often reported by patients with AFD, of whom 13.6% reported TIAs or strokes in the past. Antidepressants were more frequently found in the concomitant medication of patients with AFD, whereas other CNS medications were not different.

Disease characteristics in patients with AFD. Female and male patients with AFD examined in this study had been diagnosed a mean of 9.6 and 11.8 years ago, respectively (table e-1 on the *Neurology*® Web site at Neurology.org). Male patients were less likely to have a positive family history for AFD at the time of diagnosis than their female counterparts, indicating that diagnosis in male patients had been made more frequently following prior organ involvement.

Clinical disease severity rated with the MSSI was higher in male than in female patients. In keeping with higher disease severity, male patients with AFD were treated more often with enzyme replacement therapy (ERT), which had been initiated in 88% of male and 60% of female patients. The difference in total MSSI scores between sexes was due to higher renal and cardiac subscores in male participants, whereas general and neurologic MSSI subscores as well as the point distribution in individual components of the neurologic subscore were not different. Accordingly, male patients showed more pronounced renal and cardiac involvement on laboratory markers of renal function and transthoracic echocardiography, respectively, whereas cerebral MRI reports did not indicate significant differences in cerebrovascular manifestations between female and male patients.

Structured interview. All participants were interviewed for motor and nonmotor symptoms frequently observed in neurodegenerative diseases (table e-2). In brief, patients with AFD did not report extrapyramidal motor features more often than controls. When asked for nonmotor symptoms, patients more frequently noted orthostatic problems, urinary dysfunction, constipation, depression, neuropathic pain, and impaired hearing, whereas hyposmia, visual disturbances, and sleep problems were not reported differently in patients and controls.

Motor function. Quantitative assessments of motor function in patients with AFD demonstrated lower gait and transfer speed on the Timed Up and Go test and poorer fine manual dexterity and hand speed on the Purdue Pegboard and the alternate tap test, respectively (table 2 and figure 1). Interestingly, these impairments were still evident when we used the NIHSS as covariate instead of age in order to correct for potential focal neurologic deficits due to cerebrovascular disease (results not shown). Evaluation of extrapyramidal motor function with the MDS-UPDRS parts II and III revealed higher scores in patients with AFD compared to controls, indicating more impairment in motor experiences of daily living and extrapyramidal motor function. Evaluation of extrapyramidal symptoms demonstrated more asymmetric motor slowing and a trend for more postural instability in patients with AFD, whereas frequencies of tremor, rigidity, and reduced arm swing during gait were similar between groups (figure 2). Assessment for focal neurologic deficits with the NIHSS demonstrated very low scores in all groups, although mean NIHSS scores were slightly higher in patients with AFD, as expected in a disease with known potential cerebrovascular manifestations.

Motor function worsened with increasing age, which we used as covariate in our statistical model. Female participants performed better on the Purdue Pegboard test, whereas male participants performed better on the alternate tap test; otherwise, there were no significant differences between sexes. Importantly, we did not observe a combined effect of disease and sex on motor function, arguing for similar disease effects in female and male patients with AFD.

Nonmotor function and quality of life. Evaluation with MMSE and MoCA did not reveal significant cognitive deficits in patients with AFD, although mean MoCA scores were slightly lower than in controls (figure 1) due to reduced performance in abstraction and delayed recall (table 3). Depressive symptoms on the BDI were more frequent and severe in patients with AFD, who had significant and severe depression in 26.8% and 8.2% of cases, respectively. Testing with the SS-16 did not show differences in olfactory function between patients and controls, which was also illustrated by similarly low frequencies of hyposmia in all groups. During orthostatic challenge, patients with AFD did not show more evidence for orthostatic hypotension than age-matched controls. BPI assessments revealed higher severity and functional interference of pain in patients with AFD than in age-matched controls, which upon visual inspection of the questionnaires was frequently due to a combination of joint problems and neuropathic pain. Evaluation with the ESS revealed significantly higher scores for daytime sleepiness in patients, whereas screening with the RBDSQ provided no evidence for a higher frequency of RBD in AFD. Although most patients were treated with ERT, both female and male patients with AFD had

Table 1 Demographic data of patients with Anderson-Fabry disease and age-matched controls

	Female controls	Female patients	Male controls	Male patients	All controls	All patients	*p* Values[a]
No. of participants	29	60	28	50	57	110	
Age, y							
Mean (SD)	47.5 (17.9)	47.8 (16.1)	49.1 (17.1)	50.5 (15.9)	48.3 (17.4)	49.0 (16.0)	
Min-max	21.6-88.2	17.3-84.4	23.7-86.3	19.0-81.2	21.6-88.2	17.3-84.4	
Ethnic groups, n (%)							
White	26 (89.7)	59 (98.3)	27 (96.4)	50 (100.0)	53 (93.0)	109 (99.1)	
Asian	2 (6.9)	1 (1.7)	1 (3.6)	0 (0.0)	3 (5.3)	1 (0.9)	
Black	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Mixed	1 (3.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	
School education, y, n (%)							
>12	21 (72.4)	22 (36.7)[b]	23 (82.1)	24 (48.0)[b]	44 (77.2)	46 (41.8)	<0.05[b]
12 (A-Levels)	4 (13.8)	10 (16.7)	2 (7.1)	3 (6.0)	6 (10.5)	13 (11.8)	
11 (GCSE)	3 (10.3)	15 (25.0)	0 (0.0)	13 (26.0)[b]	3 (5.3)	28 (25.5)	<0.05[b]
<11	1 (3.4)	13 (21.7)[b]	3 (10.7)	10 (20.0)	4 (7.0)	23 (20.9)	<0.05[b]
Profession, n (%)							
Studying	5 (17.2)	4 (6.7)	1 (3.6)	2 (4.0)	6 (10.5)	6 (5.5)	
Employed	20 (69.0)	35 (58.3)	22 (78.6)	29 (58.0)	42 (73.7)	64 (58.2)	<0.05[b]
Housewife	0 (0.0)	5 (8.3)	NA	NA	0 (0.0)	5 (4.5)	
Pensioned	4 (13.8)	10 (16.7)	5 (17.9)	9 (18.0)	9 (15.8)	19 (17.3)	
Medically retired	0 (0.0)	4 (6.7)	0 (0.0)	5 (10.0)	0 (0.0)	9 (8.2)	<0.05[b]
Unemployed	0 (0.0)	2 (3.3)	0 (0.0)	5 (10.0)	0 (0.0)	7 (6.4)	
Cardiovascular risk factors							
Adipositas, n (%)	4 (13.8)	12 (20.0)	5 (17.9)	9 (18.0)	9 (15.8)	21 (19.1)	
Body mass index, kg/m^2, mean (SD)	25.3 (4.2)	25.9 (4.7)	26.9 (4.1)	25.7 (5.1)	26.1 (4.2)	25.8 (4.9)	
Diabetes mellitus type 2, n (%)	1 (3.4)	2 (3.3)	1 (3.6)	4 (8.0)	2 (3.5)	6 (5.5)	
Hyperlipidemia, n (%)	3 (10.3)	22 (36.7)[c]	4 (14.3)	19 (38.0)[c]	7 (12.3)	41 (37.3)	0.001[c]
Hypertension, n (%)	3 (10.3)	17 (28.3)	4 (14.3)	16 (32.0)	7 (12.3)	33 (30.0)	0.011[c]
Smoking, n (%)	4 (13.8)	9 (15.0)	4 (14.3)	10 (20.0)	8 (14.0)	19 (17.3)	
Pack-years in smokers, mean (SD)	18.9 (16.6)	12.6 (16.1)	10.5 (11.7)	16.4 (11.8)	14.7 (14.1)	14.6 (12.4)	
Cerebrovascular events, n (%)							
None	28 (96.6)	50 (83.3)	28 (100.0)	45 (90.0)	56 (98.2)	95 (86.4)	0.013[c]
TIA	0 (0.0)	5 (8.3)	0 (0.0)	2 (4.0)	0 (0.0)	7 (6.4)	
Stroke	1 (3.4)	5 (8.3)	0 (0.0)	3 (6.0)	1 (1.8)	8 (7.3)	
Concomitant CNS medications, n (%)							
Antidepressants	0 (0.0)	9 (15.0)[d]	0 (0.0)	4 (8.0)	0 (0.0)	13 (11.8)	0.005[d]
Antiparkinsonian agents	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Dopamine antagonists	1 (3.4)	0 (0.0)	0 (0.0)	1 (2.0)	1 (1.8)	1 (0.9)	
Neuroleptics	0 (0.0)	0 (0.0)	0 (0.0)	2 (4.0)	0 (0.0)	2 (1.8)	

Abbreviations: GCSE = General Certificate of Secondary Education; NA = not applicable.
Nonsignificant *p* values were omitted for clarity. Significant differences within female and male participants with $p < 0.05$ additionally indicated by [c]Pearson χ^2 test, [d]Fisher exact test, or [b]*Z* test. Means are provided as unadjusted means.
[a] *p* Values for comparisons between patients with Anderson-Fabry disease and age-matched controls.
[b] χ^2 test for equality of 2 proportions (*Z* test).
[c] Pearson χ^2 test.
[d] Fisher exact test.

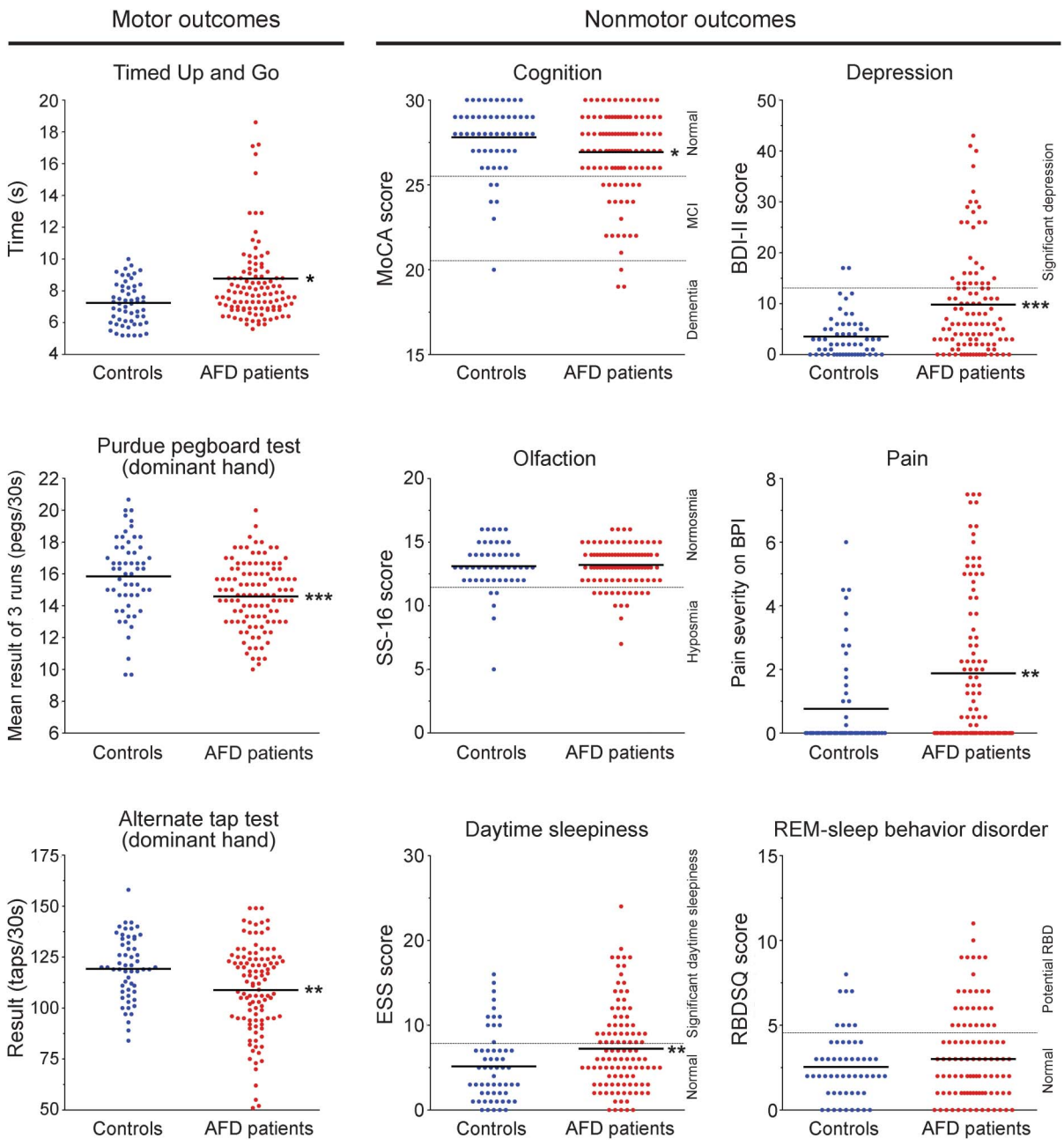
Table 2 Motor function and focal neurologic symptoms in patients with Anderson-Fabry disease and age-matched controls

							F and *p* values after GLM analysis	
	Female controls	Female patients	Male controls	Male patients	All controls	All patients	Disease	Other factors
No. of participants	29	60	28	50	57	110		
Right-handedness, n (%)	24 (82.8)	48 (80.0)	26 (92.9)	44 (88.0)	50 (87.7)	92 (83.6)		
Quantitative tests of motor function								
Timed Up and Go, s, mean (SD)	7.2 (1.6)	8.7 (2.9)[a]	7.2 (1.1)	8.8 (6.1)[a]	7.2 (1.4)	8.8 (4.6)	$F = 5.8$, $p = 0.018$	Age: $F = 8.4$, $p = 0.004$
Purdue Pegboard test, pegs/30 s, mean (SD)								
Dominant hand	16.4 (2.6)	15.0 (2.3)[a]	15.3 (2.3)	14.1 (2.5)[a]	15.9 (2.5)	14.6 (2.4)	$F = 13.2$, $p < 0.001$	Age: $F = 57.1$, $p < 0.001$; sex: $F = 5.4$, $p = 0.021$
Nondominant hand	15.0 (2.4)	14.0 (2.2)	14.6 (2.3)	13.7 (2.1)	14.8 (2.4)	13.9 (2.2)	$F = 7.4$, $p = 0.007$	Age: $F = 65.1$, $p < 0.001$
Alternate tap test, taps/30 s, mean (SD)								
Dominant hand	114.9 (17.1)	105.2 (22.7)	123.6 (13.9)	113.2 (22.0)[a]	119.1 (16.1)	108.9 (22.6)	$F = 9.2$, $p = 0.003$	Age: $F = 14.2$, $p < 0.001$; sex: $F = 8.0$, $p = 0.005$
Nondominant hand	105.1 (17.1)	96.4 (17.8)	111.0 (16.1)	101.8 (16.9)[a]	108.0 (16.7)	98.9 (17.5)	$F = 10.4$, $p = 0.002$	Age: $F = 15.1$, $p < 0.001$; sex: $F = 5.4$, $p = 0.021$
Extrapyramidal function, mean (SD)								
MDS-UPDRS part II (M-EDL)	0.9 (1.8)	2.8 (4.5)	0.9 (1.6)	2.4 (6.2)	0.9 (1.7)	2.6 (5.3)	$F = 5.5$, $p = 0.021$	Age: $F = 5.6$, $p = 0.019$
MDS-UPDRS part III (motor examination)	3.3 (5.2)	5.6 (6.1)	4.2 (3.6)	6.0 (6.0)	3.7 (3.9)	5.8 (6.0)	$F = 5.6$, $p = 0.019$	Age: $F = 33.0$, $p < 0.001$
Focal neurologic deficits, mean (SD)								
NIHSS score, mean (SD)	0 (0)	0.28 (0.85)[a]	0.04 (0.19)	0.20 (0.78)	0.02 (0.13)	0.25 (0.82)	$F = 4.1$, $p = 0.046$	
Neurologic deficit, NIHSS ≥1, n (%)	0 (0)	9 (15.0)[b]	1 (3.6)	5 (10.0)	1 (1.8)	14 (12.7)	(0.019)	

Abbreviations: GLM = general linear model; M-EDL = motor experiences of daily living; MDS-UPDRS = Movement Disorder Society–sponsored new version of the Unified Parkinson's Disease Rating Scale; NIHSS = NIH Stroke Scale.

Statistical comparison between controls and patients was performed with a GLM using disease and sex as fixed factors and age as covariate. Additional statistical comparison within female and male participants was performed with Fisher exact test or Mann-Whitney test, as appropriate: [a]$p < 0.05$ within same sex with Mann-Whitney *U* test; [b]$p < 0.05$ within same sex with Fisher exact test. Values in parentheses represent group analysis of NIHSS ordinal data performed with χ^2 test instead of GLM. Means are provided as unadjusted means. Nonsignificant *p* values have been omitted for clarity.

Figure 1 Motor and nonmotor outcomes in patients with Anderson-Fabry disease (red circles) and age-matched controls (blue circles)

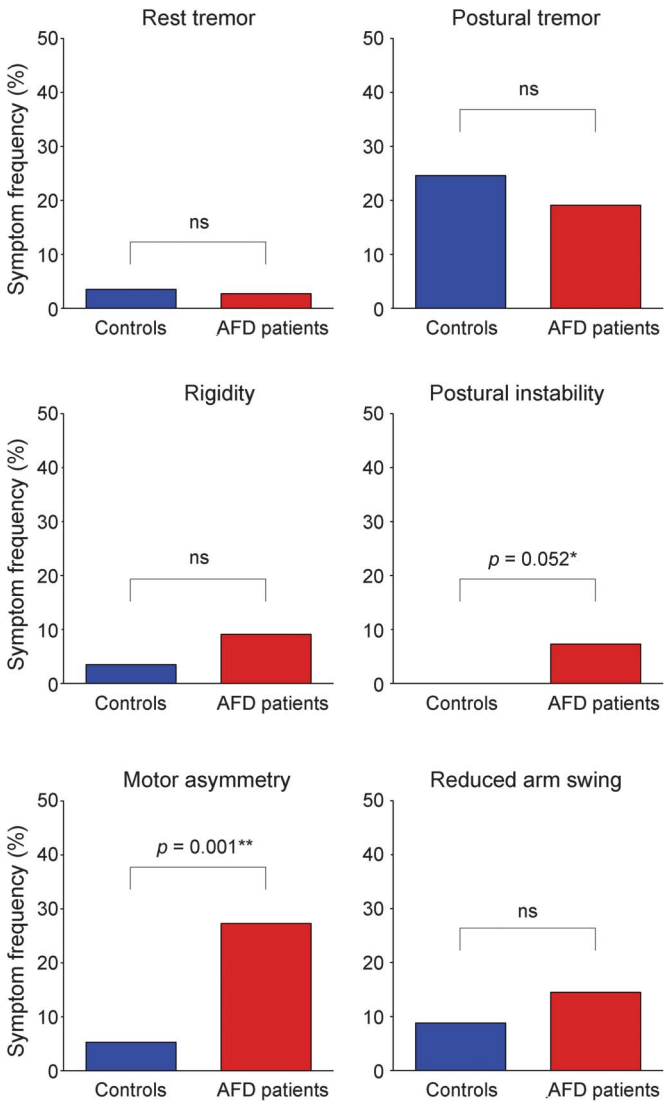


Black bars indicate unadjusted mean values. N = 110 and n = 57 for patients with Anderson-Fabry disease (AFD) and age-matched controls, respectively. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in comparison to age-matched controls with a general linear model using disease and sex as fixed factors and age as covariate. Only disease effects are shown. BDI = Beck Depression Inventory; BPI = Brief Pain Inventory; ESS = Epworth Sleepiness Scale; MCI = mild cognitive impairment; MoCA = Montreal Cognitive Assessment; RBD = REM sleep behavior disorder; RBDSQ = REM Sleep Behavior Disorder Screening Questionnaire; SS-16 = 16-item smell identification test from Sniffin' Sticks.

a markedly reduced quality of life, as documented by the EQ-5D-VAS and the SF-36 total scores (table 3).

Similar to motor function, age also affected several nonmotor outcomes in patients and controls (table 3). Higher age was associated with cognitive decline on the MMSE and MoCA, a reduction of olfactory function on the SS-16, a lower drop of blood pressure and reduced compensatory increase of heart rate upon standing up, higher pain severity and interference indices on the BPI, more daytime sleepiness on the ESS, and a lower quality of life on both EQ-5D-VAS and SF-36 (not shown). Sex effects were only identified during olfactory testing, where female participants performed significantly better than male participants (table 3). In keeping with our observations on motor function, we did not identify any differences in nonmotor outcomes between female and male patients with AFD, again indicating that the disease similarly affected both sexes.

Figure 2 Extrapyramidal motor symptoms in patients with Anderson-Fabry disease (red bars) and age-matched controls (blue bars)



N = 110 and n = 57 for patients with Anderson-Fabry disease (AFD) and age-matched controls, respectively. Symptoms were assessed during clinical evaluation with the Movement Disorders Society–revised version of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS). Postural instability was evaluated with the pull test. Motor asymmetry was defined as right-minus-left difference score ≥2 on side-specific MDS-UPDRS items. *Fisher exact test. **Pearson χ^2 test.

Correlation of study outcomes with disease severity. In order to assess whether neurologic symptoms would also correlate with disease severity in AFD, we performed bivariate correlations of all outcome parameters with the MMSI (table e-3). Significant and moderate correlations were found for all motor scales, depression (BDI-II), pain (BPI), and quality of life (EuroQoL Visual Analogue Scale, SF-36 score).

DISCUSSION We report a prospective, cross-sectional study on prodromal symptoms of neurodegeneration in a large cohort of patients with AFD aiming to evaluate the clinical relevance of neuronal glycosphingolipid accumulation in this rare disease. We found that AFD is associated with impaired motor function and various nonmotor symptoms, but unlike GD does not lead to a pattern of extrapyramidal symptoms, significant cognitive problems, hyposmia, or RBD commonly preceding neurodegenerative diseases, in particular PD[27] and dementia with Lewy bodies.[28]

Aside from cardinal neurologic manifestations, such as stroke[29] and small fiber neuropathy,[2] previous studies have described depression,[30] pain,[31] and daytime sleepiness[32] in 46%, 53%, and 68% of patients with AFD, respectively. Our study was able to reproduce these findings, but prevalence of these nonmotor symptoms was lower in our cohort, possibly due to ongoing symptomatic treatment or ERT in the majority of cases. Nevertheless, it is noteworthy that 4 out of 9 severely depressed patients did not receive treatment with antidepressants, reemphasizing the need for recognition of depressive symptoms in this disease. AFD was not associated with cognitive impairment or autonomic dysfunction, which is in keeping with previous, smaller studies.[33–35] Moreover, we were able to show that AFD does not result in hyposmia or RBD, which are prodromal for later neurodegenerative disease.

In addition, our study suggests that AFD does not lead to extrapyramidal symptoms or parkinsonian motor features but instead is associated with motor impairments during gait and transfer and poorer fine manual dexterity and hand speed. These deficits in motor function correlated to clinical disease severity similar to depression and pain, which emphasizes that motor impairments are an integral part of the disease. The pathophysiologic reasons for poorer motor performance in AFD cannot be answered by our clinical study and remain to be elucidated. Although cerebrovascular manifestations of AFD may lead to motor impairment, it must be noted that frequency and burden of cerebrovascular symptoms in our AFD cohort was very low and that disease effects on motor function were still evident when results were corrected for the presence of focal neurologic deficits. None of our patients complained about sensory loss, which is in agreement with previous neurophysiologic studies demonstrating that small fiber dysfunction predominates in the disease[2] and argues against peripheral neuropathy as the main reason for impaired motor function. Interestingly, former neuropathologic studies have shown that glycosphingolipid accumulation in AFD is not limited to the vasculature but can be found in neurons of various brain regions, in particular the brainstem.[4,5] Recently, a study has demonstrated ALP disruption and focused presence of phosphorylated α-synuclein-containing lesions in the pons of α-Gal-deficient mice, which were colocalized with large axonal spheroids indicating axonal degeneration.[6] We therefore speculate that

Table 3 Nonmotor function and quality of life in patients with Anderson-Fabry disease and age-matched controls

	Female controls	Female patients	Male controls	Male patients	All controls	All patients	*F* and *p* values after GLM analysis for	
							Disease	Other factors
No. of participants	29	60	28	50	57	110		
Autonomic function (orthostatic challenge)								
BP change at 2 minutes, mm Hg, mean (SD)	−5.6 (11.1)	−4.4 (10.6)	−3.9 (14.1)	0.4 (11.9)	−4.8 (12.6)	−2.2 (11.4)		Age: $F = 5.0$, $p = 0.026$
HR change at 2 minutes (1/min), mean (SD)	9.2 (9.1)	11.1 (9.2)	8.6 (7.0)	10.0 (6.5)	8.9 (8.0)	10.6 (8.0)		Age: $F = 26.8$, $p < 0.001$
Significant BP drop (>20 mm Hg), n (%)	2 (6.9)	4 (7.4)	4 (14.3)	2 (4.3)	6 (10.5)	6 (6.0)		
Cognition								
MMSE								
Total score (0–30), mean (SD)	28.7 (1.5)	28.3 (2.0)	29.0 (1.3)	28.5 (1.5)	28.8 (1.4)	28.4 (1.8)		Age: $F = 17.1$, $p < 0.001$
Dementia (<24 points), n (%)	0 (0)	2 (3.3)	0 (0)	0 (0)	0 (0)	2 (1.2)		
MoCA								
Visuospatial and executive, mean (SD)	4.5 (0.7)	4.2 (1.0)	4.6 (0.7)	4.3 (0.8)	4.5 (0.7)	4.3 (0.9)		Age: $F = 17.3$, $p < 0.001$
Naming, mean (SD)	3.0 (0.2)	3.0 (0.2)	3.0 (0)	3.0 (0.1)	3.0 (0.2)	3.0 (0.2)		
Attention, mean (SD)	5.4 (0.7)	5.4 (0.9)	5.8 (0.6)	5.8 (0.5)	5.6 (0.7)	5.6 (0.8)		Age: $F = 7.7$, $p = 0.006$; sex: $F = 10.0$, $p = 0.002$
Language, mean (SD)	2.4 (0.7)	2.1 (0.9)	2.4 (0.7)	2.3 (0.8)	2.4 (0.7)	2.2 (0.9)		
Abstraction, mean (SD)	1.8 (0.4)	1.8 (0.4)	2.0 (0.0)	1.8 (0.4)[a]	1.9 (0.3)	1.8 (0.4)	$F = 6.1$, $p = 0.014$	
Delayed recall, mean (SD)	4.4 (1.0)	3.8 (1.5)[b]	4.2 (0.8)	3.8 (1.2)	4.3 (0.9)	3.8 (1.4)	$F = 6.3$, $p = 0.013$	Age: $F = 25.3$, $p < 0.001$
Orientation, mean (SD)	5.9 (0.3)	5.9 (0.3)	5.9 (0.3)	5.9 (0.3)	5.9 (0.3)	5.9 (0.3)		
Total score (0–30), mean (SD)	27.6 (2.3)	26.6 (2.8)	28.0 (1.6)	27.3 (2.0)	27.8 (2.0)	26.9 (2.5)	$F = 5.0$, $p = 0.027$	Age: $F = 26.1$, $p < 0.001$
MCI (<26 points), n (%)	4 (13.8)	15 (25.0)	2 (7.1)	8 (16.0)	6 (10.5)	23 (20.9)	($p = 0.093$[c])	
Dementia (<21 points), n (%)	1 (3.4)	3 (5.0)	0 (0)	0 (0)	1 (1.8)	3 (2.7)		
Depression								
BDI-II								
Total score, mean (SD)	3.8 (4.7)	11.3 (11.1)[a]	3.2 (3.5)	8.0 (8.6)[a]	3.5 (4.1)	9.8 (10.2)	$F = 18.7$, $p < 0.001$	
Depression (>13 points), n (%)	2 (6.9)	19 (31.7)[c]	0 (0)	10 (20.0)[d]	2 (3.5)	29 (26.8)	($p < 0.001$[c])	
Severe depression (≥30 points), n (%)	0 (0)	5 (8.3)	0 (0)	4 (8.2)	0 (0)	9 (8.2)	($p = 0.028$[c])	
Olfaction								
SS-16								
Total score (0–16), mean (SD)	13.5 (1.5)	13.5 (1.7)	12.7 (2.1)	12.9 (1.5)	13.1 (1.8)	13.2 (1.6)		Age: $F = 4.5$, $p = 0.035$; sex: $F = 5.6$, $p = 0.02$
Hyposmia (<11 points), n (%)	1 (3.4)	3 (5.3)	2 (7.4)	2 (4.2)	3 (5.4)	5 (4.8)		

Continued

Table 3 Continued

	Female controls	Female patients	Male controls	Male patients	All controls	All patients	*F* and *p* values after GLM analysis for	
							Disease	Other factors
Pain								
BPI								
Pain severity index (0–10), mean (SD)	0.9 (1.6)	2.2 (2.4)[b]	0.7 (1.4)	1.5 (2.2)[a]	0.8 (1.5)	1.9 (2.3)	$F = 9.8$, $p = 0.002$	Age: $F = 9.6$, $p = 0.002$
Function interference index (0–10), mean (SD)	0.6 (1.2)	1.9 (2.5)[b]	0.6 (1.4)	1.5 (2.6)[a]	0.6 (1.3)	1.7 (2.5)	$F = 9.1$, $p = 0.003$	Age: $F = 4.8$, $p = 0.03$
Sleep								
RBDSQ								
Total score, mean (SD)	2.5 (2.0)	3.2 (2.8)	2.6 (1.9)	2.8 (2.7)	2.5 (1.9)	3.0 (2.8)		Age: $F = 6.7$, $p = 0.01$
RBD (≥5 points), n (%)	5 (17.2)	16 (26.7)	3 (10.7)	13 (26.5)	8 (14.0)	29 (26.6)		
ESS								
Total score (0–24), mean (SD)	5.6 (3.9)	7.4 (5.1)	4.7 (4.4)	7.1 (4.8)[b]	5.1 (4.2)	7.2 (5.0)	$F = 7.0$, $p = 0.009$	Age: $F = 5.7$, $p = 0.018$
Significant sleepiness (≥10 points), n (%)	5 (17.2)	16 (26.7)	6 (21.4)	12 (24.5)	11 (19.3)	28 (25.7)		
Quality of life								
EuroQol Visual Analogue Scale (0–100), mean (SD)	85.6 (19.8)	76.8 (19.0)[a]	83.9 (11.6)	72.6 (20.1)[b]	84.8 (16.1)	74.9 (19.5)	$F = 10.9$, $p = 0.001$	Age: $F = 8.9$, $p = 0.003$
SF-36 total score (0–100), mean (SD)	84.6 (12.5)	62.8 (24.4)[a]	86.3 (12.0)	68.2 (23.9)[a]	85.4 (12.2)	65.2 (24.2)	$F = 34.4$, $p < 0.001$	Age: $F = 11.3$, $p = 0.001$

Abbreviations: BDI = Beck Depression Inventory; BP = blood pressure; BPI = Brief Pain Inventory; ESS = Epworth Sleepiness Scale; GLM = general linear model; HR = heart rate; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; MoCA = Montreal Cognitive Assessment; RBD = REM sleep behavior disorder; RBDSQ = REM Sleep Behavior Disorder Screening Questionnaire; SF-36 = Short Form Health Survey; SS-16 = Sniffin' Sticks 16-item smell identification test.

Statistical comparison between controls and patients was performed with a GLM using disease and sex as fixed factors and age as covariate. Additional statistical comparison within female and male participants was performed with the χ^2 test, Fisher exact test, or Mann-Whitney *U* test: [a]$p < 0.01$, [b]$p < 0.05$, within same sex with Mann-Whitney *U* test. [c]$p < 0.05$ within same sex with χ^2 test. [d]$p < 0.05$ within same sex with Fisher exact test. Values in parentheses represent *p* values derived from group analysis of MCI and depression rates performed with χ^2 test instead of GLM. Means are provided as unadjusted means. Nonsignificant *p* values have been omitted for clarity.

neuronal glycosphingolipid storage and ALP disruption in AFD lead to focused brainstem pathology, which results in a distinct clinical phenotype with mild motor impairment and nonmotor symptoms such as depression, pain, daytime sleepiness, and hearing loss, but is not associated with cardinal clinical prodromes of neurodegenerative diseases, such as parkinsonian motor signs and impairments of cognition and olfaction that are found in GD.[10] However, we would not completely rule out a contribution of α-Gal deficiency to the development of neurodegenerative disease based on the lack of clinical prodromes, especially as neuropathologic brainstem involvement has also been demonstrated in presymptomatic stages of PD.[36]

Another interesting observation of our study is the similarity of neurologic manifestations in female and male patients with AFD, which is in keeping with other studies showing significant organ involvement in female heterozygotes despite X-chromosomal inheritance.[37] Skewed inactivation of the X-chromosome was suspected to explain disease manifestations in female patients, which is supported by recent research demonstrating correlations between X-inactivation and clinical disease severity in female patients.[38] Molecular interference by the mutant enzyme protein exerting a dominant negative effect on the normal gene product has also been suggested,[39] but this explanatory theory remains to be proven. While the reasons for sex parity of disease manifestations in AFD remain to be elucidated, our study should raise physicians' awareness for symptoms in female patients with AFD, who are often considered as carriers of but not patients with the disease.

Strengths of our study in this rare disease include its size, range, and complexity of clinical testing, and the use of age-matched controls to enhance external validity of the results. Due to its observational and cross-sectional design, our study has some limitations, which may have influenced its outcome. First, the majority of patients in our AFD cohort were treated with ERT, which may have partly altered the natural phenotype of the disease and explain lower severity of peripheral nonmotor symptoms, such as pain. However, ERT is unlikely to influence neurologic symptoms caused by central manifestations of AFD, such as motor features, hyposmia, or RBD, since it cannot cross the blood–brain barrier. Secondly, it must be acknowledged that blinded examination was not possible due to facial stigmata and skin manifestations in AFD, which may have subconsciously influenced our assessments, although we applied well-established and highly standardized clinical tests to minimize observer bias. Third, sex comparison in our study may not entirely reflect natural conditions in AFD carriers, since our cohort was recruited from a university-based specialty center, in which more severely affected female patients may be overrepresented. Moreover, patients' perception of having AFD may have contributed to worse outcomes on questionnaires in comparison to controls.

Taken together, our study argues for a distinct neurologic phenotype in AFD that lacks classical prodromal features of neurodegeneration that have been demonstrated in GD. Unlike functional loss of glucocerebrosidase in GD, which has been shown to be involved in accumulation of α-synuclein and results in neurotoxicity,[40] AFD-linked deficiency of α-Gal is apparently not associated with neurodegeneration on the clinical level.

AUTHOR CONTRIBUTIONS

M. Löhle: design and conceptualization of the study, analysis and interpretation of the data, drafting and revising the manuscript for intellectual content. D. Hughes: revising the manuscript for intellectual content. A. Milligan: revising the manuscript for intellectual content. L. Richfield: revising the manuscript for intellectual content. H. Reichmann: revising the manuscript for intellectual content. A. Mehta: revising the manuscript for intellectual content. A. Schapira: design and conceptualization of the study, interpretation of the data, revising the manuscript for intellectual content.

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