# White matter integrity correlates with cognition and disease severity in Fabry Disease

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**Word count**: 4843

**Abstract word count**: 398

**Tables**: 4

**Figures**: 4

**References**: 58

## Abstract

Cerebral white matter pathology is a common central nervous system manifestation of Fabry disease, visualised as white matter hyperintensities on MRI in 42-81% of patients. Diffusion tensor imaging MRI is a sensitive technique to quantify microstructural damage within the white matter with potential value as a disease biomarker. We evaluated the pattern of diffusion tensor imaging abnormalities in Fabry disease, and their correlations with cognitive impairment, mood, anxiety, disease severity and plasma lyso-Gb3 levels in 31 patients with genetically proven Fabry disease and 19 age-matched healthy controls. We obtained average values of fractional anisotropy and mean diffusivity within the white matter and performed voxelwise analysis with Tract-Based Spatial Statistics. Using a standardised neuropsychological test battery, we assessed processing speed, executive function, anxiety, depression and disease severity. The mean age (% male) was 44.1 (45%) for patients with Fabry disease and 37.4 (53%) for the healthy control group. In patients with Fabry disease, compared to healthy controls the mean average white matter fractional anisotropy was lower in (0.423(SD 0.023) vs. 0.446(SD 0.016), p=0.002) while mean average white matter mean diffusivity was higher ($749x10^{-6}$ $mm^2/s$ (SD $32x10^{-6}$) vs $720x10^{-6}$ $mm^2/s$ (SD $21x10^{-6}$), p=0.004). Voxelwise statistics showed that the diffusion abnormalities for both fractional anisotropy and mean diffusivity were anatomically widespread. A lesion probability map showed that white matter hyperintensities also had a wide anatomical distribution with a predilection for the posterior centrum semiovale. However, diffusion abnormalities in Fabry disease were not restricted to lesional tissue; compared to healthy controls, the normal appearing white matter in patients with Fabry disease had reduced fractional anisotropy (0.422(SD 0.022) vs 0.443(SD 0.017) p=0.003) and increased mean diffusivity ($747x10^{-6}$ $mm^2/s$ (SD $26x10^{-6}$) vs $723x10^{-6}$ $mm^2/s$ (SD $22x10^{-6}$), p=0.008). Within patients, average white matter fractional anisotropy and white matter lesion volume showed statistically significant correlations with Digit Symbol Coding Test score (r=0.558, p=0.001; and r=-0.633, p=<0.001, respectively). Average white matter fractional anisotropy correlated with the overall Mainz Severity Score Index (r=-0.661, p=<0.001), while average white matter mean diffusivity showed a strong correlation with plasma lyso-Gb3 levels (r=0.559, p=0.001). Our findings using diffusion tensor imaging confirm widespread areas of microstructural white matter disruption in Fabry disease, extending beyond white matter hyperintensities seen on conventional MRI. Moreover, diffusion measures show strong correlations with cognition (processing speed), clinical disease severity and a putative plasma biomarker of disease

activity, making them promising quantitative biomarkers for monitoring Fabry disease severity and progression.

**Keywords:** Fabry Disease; Diffusion Tensor Imaging; Central Nervous System; White Matter Pathology; Cognition.

**Glossary:**

**CADASIL** = Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy; **CNS** = Central Nervous System; **cSVD** = cerebral small vessel disease; **DSC** = Digit Symbol Coding; **DTI** = Diffusion Tensor Imaging; **DW** = Diffusion weighted; **ERT** = Enzyme Replacement Therapy; **FA** = Fractional Anisotropy; **HADS-A** = Hospital Anxiety and Depression Scale for Anxiety; **HADS-D** = Hospital Anxiety and Depression Scale for Depression; **Lyso-Gb3** = globotriaosylsphingosine**; MD** = Mean Diffusivity; **MNI**=Montreal Neurological Institute; **MSSI** = Mainz Severity Score Index; **NA WM =** Normal Appearing White Matter; **PDT2** = Proton Density T2-weighted; **TIA** = Transient Ischaemic Attack; **TMT** = Trail Making Test; **WM** = White Matter; **WMLs**= White Matter Lesions

# Introduction

Fabry disease is an X-linked lysosomal storage disorder caused by deficiency of the enzyme α-galactosidase A (Zarate and Hopkin, 2008) which leads to a widespread cellular accumulation of glycosphingolipids. The incidence is estimated between 1:40,000 to 1: 117,000 male live births. Although females are heterozygote carriers of the disease, many present with clinically significant symptoms (MacDermot *et al.*, 2001). The main clinical manifestations are cutaneous lesions (angiokeratomata), peripheral neuropathic pain (acroparaesthesia), renal failure, cardiomyopathy and cerebrovascular disease. The accumulation of globotriaosylceramide (Gb3)(Zarate and Hopkin, 2008) leads to endothelial dysfunction, which is likely to be the cause of some of the cerebrovascular manifestations of Fabry disease (Satoh, 2014); plasmatic levels of globotriaosylsphingosine (lyso-Gb3) have been proposed as a biomarker of disease activity. Ischaemic stroke is estimated to occur in up to 20% of Fabry disease patients (Grewal, 1994; Mehta *et al.*, 2005), often due to small vessel occlusion (Burlina, 2010). Patients with Fabry disease characteristically have cognitive impairments affecting executive functioning, information processing speed and attention (Bolsover *et al.*, 2014; Loeb *et al.*, 2018). Psychiatric manifestations such as anxiety and depression are also common, with an estimated prevalence of around 46%(Cole *et al.*, 2007).

Brain MRI in Fabry disease typically shows features of cerebral small vessel disease (cSVD) such as white matter hyperintensities (Korver *et al.*, 2018), lacunes (Fazekas *et al.*, 2013) and cerebral microbleeds (Reisin *et al.*, 2011; Politei *et al.*, 2014; Kono *et al.*, 2016). White matter hyprintensities are common, affecting 42-81% of patients, with no gender difference or reported specific anatomical localization (Korver *et al.*, 2018; Stefaniak *et al.*, 2018).

Although cSVD (and in particular, white matter pathology) is likely to contribute to neurological manifestations in Fabry disease patients, previously reported associations between cSVD and cognitive dysfunction have been weak (Lelieveld *et al.*, 2015) or absent (Schermuly *et al.*, 2011) possibly due to small sample sizes including patients with mild white matter hyperintensities load and cognitive impairment.

Diffusion tensor imaging (DTI) is a sensitive technique that allows the quantification of microstructural tissue alterations, which are not visible on conventional MRI (Nucifora *et al.*, 2007); the typical findings in pathological white matter are an increase in mean diffusivity (MD) and a reduction in fractional anisotropy (FA). Previous studies suggest that these DTI

metrics are superior to conventional imaging markers in assessing disease burden in cSVD (Nitkunan *et al.*, 2008; van Norden *et al.*, 2012; Tuladhar *et al.*, 2015; Stefaniak *et al.*, 2018). There are few studies of DTI findings in Fabry disease: three studies showed an increase in global MD in the white matter of patients with Fabry disease (Fellgiebel *et al.*, 2006a; Albrecht *et al.*, 2007; Fellgiebel *et al.*, 2009), while reduced FA was reported in a small cohort study (n=12) (Paavilainen *et al.*, 2013). DTI measures are thus promising as a potential quantitative biomarker of disease severity and progression, but whether they correlate with cognitive function or measures of Fabry disease severity remains unclear.

We used DTI MR analysis to test the following hypotheses: (1) FA and MD are altered in Fabry disease white matter compared to a group of healthy controls; (2) the alterations are diffuse, with no specific anatomical distribution; (3) in the Fabry disease group, white matter integrity, measured with FA and MD, is correlated with processing speed, executive dysfunction, disease severity (measured with Mainz severity score index (MSSI) (Whybra *et al.*, 2004) and levels of plasma lyso-Gb3 (a putative marker of disease activity).

# Methods

## Patient selection

We enrolled 31 Fabry disease patients (14 men, mean age 39.6 years; 17 females, mean age 47.7 years) and 19 healthy controls (10 men, mean age 38.1 years, 9 females, mean age 36.5 years), matched closely by group for age and sex. 25 patients and 18 healthy controls were included in a previously published study on perfusion MRI (Phyu *et al.*, 2018). Inclusion criteria were genetically confirmed Fabry disease referred to the National Hospital for Neurology and Neurosurgery, above the age of 18 years, and with no contraindication to MRI scanning. Patients with other known CNS diseases were excluded. Eligible participants were recruited consecutively from April 2012 to July 2013; each participant underwent detailed clinical assessment, MRI brain scanning and blood testing during a single hospital visit.

## Standard protocol approval and patient's consent

The Joint Research Ethics Committee of the UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery, London, UK, approved the study. We obtained written informed consent from each participant.

## MRI data acquisition and post-processing

We used a 3.0T MRI scanner with a 32-channel receive coil (Phillips Healthcare Systems, Best, the Netherlands). The central axial section was orientated along the subcallosal line.

The standardised structural MRI protocol and sequence parameters were as follows:

1. 3D Sagittal T1-weighted Gradient Turbo Field Echo (TFE) sequence, TFE factor: 230, shot interval: 3000 ms, voxel size: 1 $mm^3$ isotropic with no slice gaps, 256 mm x 180 mm x 256 mm (AP x RL x FH) field-of-view.

2. Axial T2-weighted Dual Echo Fast Field Echo (FFE) sequence, first echo proton-density (PD) weighted TE: 20.7 ms, second echo T2 weighted TE: 85 ms, TR: 6000 ms, no slice gaps, 240 mm x 240 mm x 144 mm (AP x RL x FH) field of view; matrix size 240 x 240 x 72 voxels.

3. Axial Phase Sensitive Inversion Recovery (PSIR) Turbo Spin Echo (TSE) sequence, TE: 13 ms, IR delay: 400 ms, no slice gaps, 240 mm x 240 mm x 144 mm (AP x RL x FH) field-of-view; matrix size 480 x 480 x 72 voxels.

4. High Angular Resolution Diffusion Imaging (HARDI) scan consisted of a spin-echo (SE) sequence with echo planar imaging (EPI) readout: TR = 4000 ms; TE = 68 ms; 72 axial slices with an isotropic resolution of 2x2x2 $mm^3$; 61 volumes: one volume without directional weighting (b-value of 0) and 60 volumes with non-collinear diffusion gradients (b-value of 1200 s $mm^{-2}$). Afterwards, we acquired 6 extra volumes without directional weighting (b-value of 0).

4. High Angular Resolution Diffusion Imaging (HARDI) scan consisted of a spin-echo (SE) sequence with echo planar imaging (EPI) readout: TR = 4000 ms; TE = 68 ms; 72 axial slices with an isotropic resolution of 2x2x2 mm3; 61 volumes containing a volume without directional weighting (b-value of 0) and 60 volumes with non-collinear diffusion gradients (b-value of 1200 s mm-2). Afterwards, we acquired 6 extra volumes without directional weighting (b-value of $b_0$).

## Whole brain white matter lesion volume quantification

We assessed the white matter lesion volume on the PDT2 scans as hyperintense >2 mm lesions, using the phase sensitive inversion recovery sequences for anatomical referencing. We excluded bright lesions >2 mm in the anterior commissure given that enlarged perivascular

spaces are often seen in this area. We then segmented WM lesions in each participant MRI using JIM version 5.0 (Xinapse Systems, Northants). An experienced neuroradiology consultant delineated lesions semi-automatically using a local thresholding technique and manually adjusted by consensus between the neuroradiology consultant and a trained observer (a neurology research fellow). The total ROI volume was then automatically calculated. We segmented tissue into cortical grey matter (CGM), WM, deep grey matter, brainstem and cerebrospinal fluid using GIF framework (Cardoso *et al.*, 2015) over T1w images in native space. GIF is free available as a webservice at http://niftyweb.cs.ucl.ac.uk/. Afterwards, using GIF tissue segmentation we computed brain parenchyma fraction (BPF), grey matter fraction (GMF) and white matter fraction (WMF); we then obtained the normalised tissue volumes and vScaling factor using SIENAX (Smith *et al.*, 2002).

## White matter lesion segmentation and probability maps

We first affine and then non-rigidly registered T1-weighted images to the MNI atlas using the NiftyReg package. We resampled each lesion mask to MNI atlas space. Finally, to obtain the white matter lesion probability map, we summed all the lesion masks and divided by the number of patients to give a lesion probability at each voxel.

## DTI analysis

Diffusion weighted (DW) images were corrected for miss-alignment between the extra $B_0$s and the DW images, as well as eddy current and susceptibility distortions. First, we rigidly registered without any symmetric optimization the 6 extra $b_0$ images to the $b_0$ image acquired as the first of the 61 DWI volumes. Then, using FSL (Smith *et al.*, 2004) over the previously corrected DWI data we performed eddy current and head motion correction using affine registration to the first $b_0$ (Andersson and Sotiropoulos, 2016). Afterwards, using Brainsuite, we corrected for susceptibility induced distortions caused by the EPI sequences (Bhushan *et al.*, 2012). Finally, using the NiftyFit software package, we computed the following classical DTI metrics fractional anisotropy (FA) and mean diffusivity (MD).

In order to perform regional analyses, using Brainsuite, we rigidly registered the subject structural 3D T1-weighted image (3DT1) to the corresponding DW image (DWI) (Bhushan *et al.*, 2012), resulting in a structural image of resolution 2x2x2 $mm^3$ aligned to the DWI. Then,

we segmented the 3DT1w image in DWI space into cortical grey matter, white matter, deep grey matter and CSF according to Desikan–Killiany–Tourville atlas protocol (Klein and Tourville, 2012) using GIF. Finally, for each region, we obtained FA and MD from the DTI maps.

## TBSS analysis and voxelwise statistics

We performed voxelwise statistical analysis of the FA data using TBSS (Tract-Based Spatial Statistics (Smith *et al.*, 2006)), part of FSL (Smith *et al.*, 2004). First, we performed a skull-stripping of the FA maps using the GIF segmentation of the 3DT1 volume in DWI space. We then aligned all subjects' FA data into a common space using the nonlinear registration tool FNIRT (Andersson *et al.*, 2007a; Andersson *et al.*, 2007b), which uses a b-spline representation of the registration warp field (Rueckert *et al.*, 1999). Next, we created and thinned the mean FA image to create a mean FA skeleton representing the centres of all white matter tracts common to each group. We projected each subject's aligned FA data onto this skeleton and fed the resulting data into voxelwise cross-subject statistics.

## Clinical measures

We collected demographic and clinical data on the same day as the MRI using a standardized case report form. Participants underwent a comprehensive neuropsychological assessment including the Digit Symbol Coding (DSC) sub-test from the Wechsler Adult Intelligence scale 3$^{rd}$ edition (Wechsler, 1997) and the trail making test part B (TMT-B) (Reitan, 1958) as a measure of processing speed and executive function respectively. To measure the burden of anxiety and depression, we used the Hospital Anxiety and Depression scale (HADS) (Zigmond and Snaith, 1983). Fabry disease severity was measured according to the Mainz Severity Score Index (MSSI) (Whybra *et al.*, 2004). Finally, we used a mass spectroscopy based rapid multiplexed assay developed at UCL Institute of Child Health (Dr. Kevin Mills) to measure plasma globotriaosylsphingosine (lyso-Gb3) with a reference range 0–1.8 ng/mL.

## Statistical analysis

We performed statistical analysis using SPSS version 24.0 (IBM Corp, Armonk, NY). Normal distribution of continuous variables was assessed with the Shapiro-Wilk test. Where possible, we used logarithm transformation to normalize the data. We then used parametric tests for normally distributed variables and non-parametric test for non-normal ones. We compared white matter average FA and MD and Digit symbol coding scores between patients and controls using a general linear model analysis, after regressing for the effect of age. White matter lesion volume, TMT – B and HADS -A and – D scores were compared between the two groups with a Mann-Whitney U test. Within the patient group, we compared neuroimaging biomarkers between males and females using a general linear model analysis, correcting for the effects of age (FA and MD, for both whole and normal appearing white matter) or a Mann Whitney U test (white matter lesion volume). The correlations between imaging biomarkers and processing speed, executive function, anxiety, depression and MSSI were investigated in a linear regression analysis. In order to perform the regression analysis, we applied the logarithm transformation to the white matter lesion volume, the TMT - B and the HADS-A and HADS-D scores; we then performed the analysis on both logarithm transformed and non-logarithm transformed variables. We used the scaling factor as a covariate when we ran correlations with white matter lesion volume, adjusting for the possible effects of different head sizes. Assuming that white matter integrity loss leads to worse cognitive function, we used single tailed tests; we set the threshold below p values $\leq 0.01$ and above r values $\geq 0.5$ (or $r \leq -0.5$ for negative correlation) because this is generally considered to be evidence of at least a moderately strong relationship between variables (Mukaka, 2012). Correlation between neuroimaging markers and plasma lyso-Gb3 levels was investigated (in the 29 out of 31 patients who consented for blood collection) with a non-parametric two tailed Spearman rank correlation coefficient, since its values showed a non-normal distribution even after logarithm transformation; as above, we considered significant the correlations with an $r_s \geq 0.5$ (or $r_s \leq -0.5$ for negative correlation) a with a p value $\leq 0.01$. In order to reduce type 1 errors, we used the Benjamini–Hochberg procedure to perform the False Discovery Rate (FDR) multiple comparison correction analysis on all correlation analyses. We used the Mann Whitney U test to assess difference in neuroimaging findings in patients receiving versus those not receiving ERT; we then used a two-tailed Spearman rank correlation coefficient to assess the correlation between the duration of ERT and neuroimaging biomarkers with a significance threshold of $r_s \geq 0.5$ or $\leq -0.5$. All correlation analyses were performed in the patient group only.

## Data availability

The study data are available from the corresponding author, upon reasonable request.

# Results

### Study population

Thirty-one patients and nineteen healthy controls were included in the study. The demographic characteristics are shown in Table 1. The patient group showed reduced executive function and processing speed scores compared with controls, but no significant differences in anxiety or depression scores (Table 2).

### Diffusion tensor imaging: quantitative findings

Table 3 shows the comparison of neuroimaging findings between patients and controls. The mean average white matter FA was 0.423 (SD 0.23) for patients and 0.446 (SD 0.16) for healthy controls (p=0.002). The mean average white matter MD was 749 x 10-6 $mm^2/s$ (SD 32 x 10-6) in patients and 720 x 10-6 $mm^2/s$ (SD 21 x 10-6) in healthy controls (p=0.004). With regards to normal appearing white matter, the mean white matter FA was 0.422 (SD 0.022) for patients and 0.443 (SD 0.017) for healthy controls (p=0.003); the mean average white matter MD was 747 x 10-6 $mm^2/s$ (SD 26 x 10-6) in patients and 723 x 10-6 $mm^2/s$ (SD 22 x 10-6) in healthy controls (p=0.008). The white matter lesion volume was 3815 $mm^3$ (95% CI 569-7060) in the patient group and 221 $mm^3$ (95% CI 0-427) in healthy controls (p=0.005). The difference in FA and MD remained significant even after excluding patients with severe white matter lesion volume load (p=0.009 for FA; p=0.017 for MD). Within the patient group, we found no difference between males and females in FA, MD or white matter lesion volume.

### Voxelwise analysis of diffusion tensor imaging and white matter lesion probability maps

In voxelwise analyses, FA values were globally reduced in the patient group compared to healthy controls; this reduction of FA was significant in bilateral regions of the internal capsule, corona radiata, centrum semiovale, parietal, frontal, temporal and occipital white matter, and,

less markedly, in the brainstem (Fig 1). MD elevation was seen in the patient group in similar regions (Fig. 1). There were no areas with a higher FA or a reduced MD in patients compared to controls.

An example of an individual patient white matter lesion segmentation map is shown in Fig 2. Within the patient group, white matter lesion probability maps showed a widespread distribution (3.2-12.9%) with a higher presence in the posterior periventricular white matter (32.5%) (Fig 3).

## Clinical and radiological correlation analyses

The results of correlation analyses are shown in Table 4 and Figure 4. In a linear regression model white matter FA and white matter lesion volume showed a correlation with the Digit Symbol Coding score ($r=0.558$, $R^2=0.312$, $p=0.001$ and $r=-0.633$, $R^2=0.400$, $p<0.001$, respectively). White matter FA and white matter MD showed a significant correlation with the overall Mainz Severity Score Index (MSSI) ($r=-0.661$, $R^2=0.380$, $p<0.001$ and $r=0.532$, $R^2=0.283$, $p=0.002$) No statistically significant correlation was seen between any imaging biomarker and TMT–B scores, HADS-A or HADS-D. When repeated using non-logarithm transformed variables, we found similar significant results. White matter MD was significantly correlated with plasma lyso-Gb3 levels ($r_s= 0.559$, $p=0.001$). All the above-mentioned significant correlations survived the FDR analysis for multiple comparison.

Finally, regarding enzyme replacement therapy (ERT) within the patients group, 22 patients with Fabry disease (71%, mean age 46.3±14, 13(59%) males) were receiving treatment whereas 9 (mean age 40±18, 1 (11%) male) were not under ERT. The mean duration of therapy was 7(±4) years (Table 1). We found no difference between patients receiving enzyme replacement therapy (ERT) and those who were untreated in FA (0.42 vs 0.43, $p = 0.38$) and MD ($754x10^{-6}$ vs $735x10^{-6}$, $p = 0.24$) values, nor any correlation between FA and MD values and the number of years of ERT treatment ($r = -0.223$, $p = 0.23$ and $r = 0.332$, $p = 0.07$, respectively).

## Discussion

Our findings using diffusion tensor imaging confirm a widespread loss of white matter integrity in Fabry disease, which extends beyond white matter hyperintensities seen on conventional MRI. Moreover, diffusion tensor imaging measures show strong correlations with cognition (processing speed), clinical disease severity and a putative plasma biomarker of disease activity (lyso-Gb3), making them promising quantitative biomarkers for monitoring Fabry disease severity and progression.

The pattern of reduced FA and increased MD in white matter that we observed is not pathologically specific, but is consistent with increased water content, loss of myelin, axons, or both, resulting increased diffusivity and decreased anisotropic water diffusion. A similar pattern has been found in other diseases affecting CNS white matter, such as multiple sclerosis (Werring *et al.*, 1999). There are few data on the neuropathology of Fabry disease, although vasculopathy has been considered the main mechanism of neurological injury. An autopsy case report concerning a patient with dementia associated with Fabry Disease showed diffuse axonal damage and leukoencephalopathy with multisegmental hydropic irregular swelling of axons in the bilateral cerebral deep white matter, particularly around small arteries and arterioles (Okeda and Nisihara, 2008), suggesting a contribution from axonal injury. In keeping with this, an animal mode of alpha galactosidase deficiency showed large, swollen axonal spheroids indicating axonal degeneration (Nelson *et al.*, 2014). Further studies could make use of other advanced neuroimaging methods to extract biophysically meaningful features that are more specific to axonal or myelin loss.

Group maps of the distribution of DTI abnormalities showed that microstructural injury extends beyond the extent of MRI-visible white matter lesion shown using a probability map. Our finding of reduced FA and increased MD in Fabry disease NAWM confirms that white matter integrity is impaired regardless of the presence of MRI-visible white matter lesion, and that DTI provides additional information regarding tissue injury. These findings suggest that DTI might detect pathological changes in NAWM before such abnormalities are visible on conventional MRI; mechanisms could include Wallerian degeneration associated with neuronal injury. Our findings are in keeping with previous studies which showed structural and metabolic brain abnormalities in patients with Fabry disease without a significant white matter lesion load using DTI (Fellgebiel et al, 2006; Albrecht et al 2007), magnetic resonance spectroscopy (Tedeschi et al 1999) and 18-fluoro-deoxyglucose PET (Moore et al, 2003). If

diffusion changes do precede MRI-visible white matter lesion, DTI could be an early biomarker of Fabry disease; testing this hypothesis will require longitudinal studies.

Our finding of a diffuse MD elevation within white matter tracts in Fabry disease is consistent with that described in previous studies (Fellgiebel *et al.*, 2006a; Albrecht *et al.*, 2007; Paavilainen *et al.*, 2013). However, only one of these studies reported reduced FA (Paavilainen *et al.*, 2013). There are several explanations for this difference: first, we used an advanced DTI protocol with isotropic resolution and 60 diffusion gradient directions, which is more sensitive in detecting FA changes compared to the one used by the previous studies, which used 6 diffusion gradient directions and non-isotropic resolution (Fellgiebel *et al.*, 2006a; Albrecht *et al.*, 2007). Furthermore, our study and the most recent previous study (Paavilainen *et al.*, 2013) used advanced TBSS analyses rather than region of interest (Fellgiebel *et al.*, 2006a) or voxel-based (Albrecht *et al.*, 2007) analyses. TBSS analysis has higher sensitivity in detecting white matter microstructural damage compared to other techniques (Smith *et al.*, 2006). Finally, differences between the cohorts involved could also be contributory: in one of the previous studies (Albrecht *et al.*, 2007) about half of the participants had no white matter lesion suggesting mild CNS involvement.

The topographical distribution of the DTI abnormalities showed a widespread, non-specific localization of damage within periventricular and deep white matter. These white matter findings are consistent with a process affecting small vessels (Crutchfield *et al.*, 1998). Abnormalities in small perforating arteries related to Gb-3-related endothelial damage could lead to white matter injury due to altered blood flow or ischaemia (Crutchfield *et al.*, 1998; Moore *et al.*, 2003) in Fabry disease. Our findings are also consistent with previous reports of the an anatomical distribution of DTI white matter changes (Fellgiebel *et al.*, 2006a; Albrecht *et al.*, 2007; Paavilainen *et al.*, 2013) and white matter lesion (Assareh *et al.*, 2011; Korver *et al.*, 2018) in Fabry disease.

The white matter lesion probability map also showed a diffuse distribution of white matter hyperintensities, but with a higher lesion probability in the posterior periventricular regions. These findings are consistent with previous reports of preferential involvement of posterior brain regions in Fabry disease, including hyperperfusion or metabolic disturbance (Moore *et al.*, 2003, Phyu *et al.*, 2018). The mismatch between the distribution of DTI abnormalities and MRI-visible white matter lesion probability maps suggests that microstructural damage might occur independently of haemodynamic or metabolic factors. An

immunohistochemistry study showed glycolipid accumulation within neurons (deVeber *et al.*, 1992), which could lead to axonal damage in connected white matter regions through Wallerian degeneration.

Our findings of impaired processing speed and executive function in Fabry disease are consistent with previous reports (Bolsover *et al.*, 2014). Regarding the correlations of diffusion tensor imaging measures with cognition, average white matter FA and total white matter lesion volume showed an association with the Digit Symbol Coding (DSC). DTI measures have been shown to be associated with processing speed in sporadic cSVD (O'Sullivan *et al.*, 2004; Nitkunan *et al.*, 2008; van Norden *et al.*, 2012; Baykara *et al.*, 2016) and in Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) (Chabriat *et al.*, 1999; Baykara *et al.*, 2016). Our observations suggest that slower processing speed is related to the integrity of normal-appearing white matter in Fabry disease. Processing speed is considered to be a distributed cognitive ability spanning different domains and dependent on the integrity of many anatomically widespread white matter tracts. The widespread diffusion changes we have observed suggest that widespread disorganization of white matter fibres underlies slower processing speed in Fabry disease.

Anxiety and depression scores did not differ between patients and controls and were not correlated with any neuroimaging biomarker; depression is reported to affect almost one-half of Fabry disease patients (Cole *et al.*, 2007). Our findings suggest that this might be due either to the difficulty of coping with the severity of the disease (particularly with neuropathic pain) (Bolsover *et al.*, 2014) rather than to white matter microstructural alterations, although the majority of our patients had mild Fabry disease. In addition, structural brain changes other than white matter integrity such as hippocampal and amygdala atrophy could affect anxiety and depression levels and might usefully be investigated in further studies (Hamilton *et al.*, 2008; Santos *et al.*, 2018); mild progressive hippocampal atrophy has been demonstrated in Fabry disease patients in over 8 years of follow up (Lelieveld *et al.*, 2015).

We have shown that brain DTI measures correlate with the overall severity of Fabry disease, assessed by the MSSI composite score including cerebrovascular, cardiac and renal impairment. All of these multisystem features could contribute directly or indirectly to white matter damage. The lack of association between white matter lesion volume and disease severity suggests that DTI provides new quantitative information that is more relevant to Fabry disease pathophysiology than conventional MRI. Systemic nitric oxide pathway dysregulation

(Altarescu *et al.*, 2005; Fellgiebel *et al.*, 2006b) and inflammatory processes are important factors in developing small vessel injury and white matter disease. Multi-system involvement including cardiac and renal impairment are also likely to be related to systemic inflammation and the degree of nitric oxide pathway dysregulation (Modlinger *et al.*, 2004; Van Linthout and Tschöpe, 2017), which could cause explain the correlation of disease severity with microstructural damage in cerebral white matter. Consistent with our findings, previous studies did not find a strong correlation between MSSI and white matter lesion volume (Reisin *et al.*, 2011; Schermuly *et al.*, 2011). The lack of association between disease severity and white matter lesion load, lacunes, or microbleeds suggests that and DTI measures of small vessel injury are a more sensitive marker of white matter microstructural damage. The correlation we found between plasma lyso-Gb-3 levels and MD also supports the hypothesis that DTI measures are relevant to Fabry disease severity; moreover, lyso-Gb3 levels have been shown to correlate with MSSI score and to be associated with a higher risk of developing white matter lesion (Satoh, 2014).

As well as showing strong correlations with cognition and disease severity, DTI also has potential practical advantages as a biomarker since it can be obtained rapidly and automatically; moreover, as long as the same methods are used, DTI indices are rotationally invariant and totally replicable. Furthermore, DTI based biomarkers are more sensitive in detecting changes over time than white matter hyperintensities volume in sporadic small vessel disease (Nitkunan *et al.*, 2008).

We did not find any difference in white matter lesion or microstructure between patients receiving ERT and those who were untreated, nor any correlation between white matter measures and the duration of the treatment. Although ERT might be effective in stabilizing the progression of white matter hyperintensities (Rombach *et al.*, 2013), the evidence remains controversial since its efficacy has been proven only in a single study (Fellgiebel *et al.*, 2014) but was not confirmed by a larger meta-analysis (Rombach *et al.*, 2014) or two more recent studies (Stefaniak et al., 2018; Körver et al., 2020). However, due to methodological limitations, our data are inconclusive: first, our study was cross-sectional, so could not assess any change in white matter disease over time; and second, the treated and non-treated patients showed differences in gender, age and disease severity, which we could not adjust for due to the limited sample size. Nevertheless, given its sensitivity in detecting microstructural damage beyond that seen on conventional MRI, and its face validity in its correlations with important disease severity measures, DTI could be useful for investigating the potential benefit of ERT

on white matter health in Fabry disease. Future longitudinal studies using DTI will therefore be of interest. Large randomised controlled trials of ERT using DTI as a biomarker would be an ideal study design but are not likely to be considered ethical in Fabry disease.

Our study has strengths. All the subjects and controls were prospectively recruited and studied using a standardized protocol. Our cohort size is quite large for a rare disease such as Fabry disease, involving both males and female patients. All the MRIs were evaluated by a single trained observer and the ROI for white matter lesion volume quantification were placed by a single expert neuroradiologist.

Some limitations should be noted. First, patients with contraindication to MRI were excluded, for example those with pacemakers or implantable cardiac devices, thus influencing the severity of cardiac disease in those included, potentially reducing the generalisability of our findings to the whole Fabry disease population. In addition, even though quite large for a rare disease, our sample size did not allow us to run more complex multivariable analyses. Moreover, the non-specific nature of DTI findings along with the lack of reliable pathological studies did not allow us to definitively clarify the pathology of white matter abnormalities in Fabry Disease. Finally, the cross-sectional nature of this study did not allow to evaluate the progression of white matter damage over time.

Nevertheless, our data show that DTI measures are related to cognition and disease severity in Fabry disease, so could be a helpful tool in the monitoring of disease severity and progression. Further longitudinal studies are needed to assess the potential role of DTI in the follow-up of patients with Fabry disease, including the impact of ERT.

## Funding

Shire Pharmaceuticals provided a project grant to RH Lachmann and DJ Werring. This work was undertaken at UCLH/UCL who received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme. FP is a Guarantors of Brain Postdoctoral fellow.

## Competing Interests

The authors declare no other competing interests.

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# Tables

**Table 1. Demographics characteristics of patients with Fabry Disease and healthy controls**

| | **Patients** (n= 31) | **Healthy Controls** (n= 19) | ***p*** |
|---|---|---|---|
| Age | 44.4 (24-80) | 37.4 (27-65) | 0.20 |
| Male | 14 (45.2%) | 10 (52.6%) | 0.43 |
| Yrs of education | 15.4 (± 2.7) | 16.4 (± 3.5) | 0.45 |
| Right handedness | 29 (93.5%) | 18 (94.7%) | 1 |
| Hypertension | 2 (10.5%) | 7 (22.6%) | 0.45 |
| Diabetes | 0 | 0 | 1 |
| Hyperlipidaemia | 3 (9.6%) | 5 (26.3%) | 0.23 |
| Smoking | 13 (41.9%) | 5 (26.3%) | 0.36 |
| Renal Disease | 5 (16.1%) | 0 | 0.14 |
| Ischemic Stroke | 2 (6.9%) | 0 | 0.52 |
| TIA | 1 (3.5%) | 0 | 1 |
| Migraine | 1 (3.5%) | 1 (5.2%) | 1 |
| Receiving ERT | 22 (71%) | - | - |
| ERT yrs | 7 (±4) | - | - |
| MSSI Overall Score | 17.5 (± 8.6) | - | - |
| Mild FD | 20 (64.5%) | - | - |
| Moderate FD | 11 (35.5%) | - | - |
| Severe FD | 0 | - | - |

Characteristic of patients and healthy controls shown as mean (±SD) for continuous variables and as number (%) for proportions. Age is shown as mean (range). **TIA**: Transient Ischaemic Attack; **ERT**: Enzyme Replacement Therapy; **MSSI**: Mainz Severity Score Index; **FD:** Fabry disease.

**Table 2. Neuropsychological findings**

| Neuropsychological test | Patients (n=31) | HC (n=19) | *p* |
|---|---|---|---|
| **DSC** | 76.3 (± 18.7) | 85.6 (± 12.3) | **0.03** |
| **TMT-B** | 68 (50.5-84) | 51 (39.5-65) | **0.037** |
| **HADS A** | 6 (4.5-11.5) | 6 (2.5-10.5) | 0.26 |
| **HADS D** | 3 (1-8) | 2 (0.5-5) | 0.51 |

Findings are shown as mean (±SD) or median (IQR). Statistically significant differences are in bold. **DSC**: Digit Symbol Coding; **TMT-B**: Trail Making Test B; **HADS A** and **D**: Hospital Anxiety and Depression Scale for Anxiety and Depression respectively.

**Table 3. Neuroimaging findings in patients and healthy controls.**

| Imaging Parameter | Patients (n=31) | HC (n=19) | p |
|---|---|---|---|
| **NAWM FA** | 0.422 (± 0.022) | 0.443 (± 0.017) | **0.003** |
| **NAWM MD** | 747 x $10^{-6}$ (± 26 x $10^{-6}$) | 723 x $10^{-6}$ (± 22 x $10^{-6}$) | **0.008** |
| **WMLs Vol*** | 225 $mm^3$ (0-834) | 92 $mm^3$ (0-476) | **0.005** |
| **WMLs No*** | 2 (0-5) | 0 (0-2) | **0.035** |
| **No WMLs** | 9 (29%) | 11 (58%) | 0.073 |
| **Mild WMLs** | 15 (48%) | 8 (42%) | 0.77 |
| **Severe WMLs** | 7 (23%) | 0 | **0.03** |

Findings are shown as mean (± SD) for continuous variable and as number (%) for proportions; significant difference are in bold. All the results were corrected for age. NA**WM FA**: The average value of Fractional Anisotropy value in the normal appearing white matter; NA**WM MD**: The average value of mean diffusivity in the normal appearing white matter volume; **WMLs:** White matter lesions. **WMLs Vol:** White matter lesions volume; **WMLs No:** White matter lesions number. *Median (IQR).

| | | DSC | TMT - B | HADS A | HADS D | Fabry disease severity (MSSI) | Lyso-Gb3* |
|---|---|---|---|---|---|---|---|
| **WM FA** | Pearson r | **-0.558** | -0.394 | -0.128 | -0.110 | **-0.661** | -0.413 |
| | $R^2$ | **0.312** | 0.155 | 0.016 | 0.012 | **0.380** | |
| | *p* | **0.001** | 0.034 | 0.493 | 0.570 | **<0.001** | 0.26 |
| **WM MD** | Pearson r | -0.471 | 0.199 | 0.219 | 0.167 | **0.532** | **0.559** |
| | $R^2$ | 0.221 | 0.040 | 0.013 | 0.028 | **0.283** | |
| | *p* | 0.008 | 0.301 | 0.550 | 0.370 | **0.002** | **0.002** |
| **WMLs Volume** | Pearson r | **-0.633** | 0.470 | 0.128 | 0.333 | 0.394 | 0.220 |
| | $R^2$ | **0.400** | 0.221 | 0.048 | 0.111 | 0.155 | |
| | *p* | **<0.001** | 0.011 | 0.240 | 0.070 | 0.030 | 0.25 |

**Table 4. Correlation between neuroimaging biomarkers and clinical features**

All the correlations are from a linear regression model. Statistically significant results (with r or $r_s \geq 0.5$, $p \leq 0.01$) are in **bold**. **WM FA**: The average value of Fractional Anisotropy value in the white matter; **WM MD**: The average value of mean diffusivity in the white matter; **WMLs volume**: Total volume of white matter lesions. **DSC**: Digit Symbol Coding; **TMT – B**: Trail making test (part B); **HADS A** and **D**: Hospital Anxiety and Depression Scale for Anxiety and Depression respectively; **MSSI**: Mainz Severity Score Index.

*Correlations with lyso-Gb3 were investigated with a 2-tailed Spearman rank correlation coefficient.

# Figure legends

Figure 1. Figure 1. Fractional anisotropy (FA) and mean diffusivity (MD) voxelwise analysis, comparing Fabry disease patients and healthy controls. Areas where FA values in the patient group are significantly lower than in healthy controls within white matter skeleton (displayed in green) are reported in a red-yellow scale (p values ranging from 0.05 to p. 0.005) on the upper row. Areas where MD values in the patient group are significantly higher than in healthy controls within white matter skeleton (displayed in green) are reported in a blue-lightblue scale (p values ranging from 0.05 to 0.005) on the bottom row. All p values have been family-wise error-corrected for multiple comparisons after regressing for the effect of age. Results have been overlaid on a skull-stripped MNI152 atlas and smoothed with a mean kernel of 3x3x3 voxels to aid visual interpretation.

**Figure 2.** Two individual examples of Fabry Disease patients with white matter lesions (A, C) are displayed on the left column. On the right column the correspondent white matter lesion segmentation maps are shown (B, D, respectively).

**Figure 3.** Lesion probability map TODO caption. Several MNI ATLAS axial slices with the lesion probability map overlaid are displayed in this picture. Within the patient group, white matter lesion probability maps showed a widespread distribution (3.2-12.9%) with a higher presence in the posterior periventricular white matter (32.5%).

**Figure 4.** Correlations between neuroimaging markers, neuropsychological assessment, disease severity and biomarkers. Both WM FA (A) and WMLs Volume (B) have a strong correlation with DSC score, positive ($r=0.558$, $p=0.001$) and negative ($r=-0.633$, $p<0.001$) respectively. DTI measures are correlated either with disease severity measured with Mainz Severity Score Index ($r=-0.661$, $p<0.001$) (C) and circulating lyso-Gb3 levels ($r_s=0.559$, $p=0.002$) (D).