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Cognitive dysfunction and white matter hyperintensities in Fabry disease

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Word counts
Abstract: 207
Text: 5333

Table count: 3
Figure count: 0

No colour picture provided for cover

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jimd.12472

Abstract

Fabry disease (FD) is an X-linked lysosomal storage disorder with multi-system involvement including cerebrovascular disease. Patients with FD also have a high risk of ischaemic stroke and TIA. White matter hyperintensities are common, but their clinical impact on cognition remains uncertain. Previous studies have examined the neuropsychological profile of FD, but have been inconclusive in part due to methodological limitations including small sample sizes. We sought to address these limitations in a case-control study of 26 patients with Fabry disease with mild to moderate disease symptoms matched with 18 healthy controls for age and premorbid intellectual level. We obtained detailed neuropsychological data and MRI neuroimaging data on the severity of white matter changes. Mood was accounted for as a possible confounder. Our results showed significant compromise of executive functions and information processing speed for the FD group. Error analyses suggested that the compromise of executive functions could not be entirely accounted for by slowed information processing speed. We demonstrated significant correlations between cognitive decline and the overall volume of white matter hyperintensities in the FD group. Our results point to significant compromise of cognition in FD even without stroke or mood difficulties. This suggests that neuropsychological assessment and rehabilitation should be routinely offered to patients with FD.

Synopsis

Compromise of executive function and information processing speed in patients with Fabry disease, unrelated to ischaemic stroke.

Acknowledgements and conflict of interest

Shire Pharmaceuticals provided unrestricted educational funding.

Patrick Murphy, Fay Williams, Indran Davagnanam, Edgar Chan, Graziella Quattrocchi, David Werring, Robin Lachman, Elaine Murphy Graziella Quattrocchi and Lisa Cipolotti are employed at UCLH/UCL, who received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme.

Robin Lachmann has received honoraria from Takeda and Genzyme.

David Werring receives research support from the Stroke Association and British Heart Foundation.

Elaine Murphy has received educational grants and honoraria from Genzyme.

Derralynn Hughes has received consultancy fees and honoraria for speaking from Amicus therapeutics, Sanofi Genzyme, Takeda through UCL consultants and used in part for Fabry-related research.

Author contributions

DJW, RHL, EM, DH and LC designed and supervised the study.

PM, FW, EC, and LC analysed and interpreted the neuropsychological data.

ID, GQ, DJW, RHL, FW and C.A.M. W-K analysed and interpreted the imaging data.

PM, LC, and EC wrote the paper.

DH and EM identified and screened suitable patients for inclusion in the study

All authors reviewed, commented and provided amendments for the submitted manuscript.

Ethics statement

The Joint Research Ethics Committee of the UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery, London, UK, approved the study.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

Proof that informed consent was obtained must be available upon request If doubt exists whether the research was conducted in accordance with the Helsinki Declaration, the authors must explain the rationale for their approach, and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Keywords

Fabry disease, cognition, neuropsychological testing, white matter hyperintensity

1. Introduction

Fabry disease (FD) is a progressive X-linked lysosomal storage disorder caused by a mutation in the α-galactosidase gene leading to deficient lysosomal α-galactosidase enzyme activity (Rolfs et al., 2010). This results in the accumulation of glycosphingolipids in the lysosomes of cells, including in the CNS (Desnick, Ioannou and Eng, 1995). FD is rare, with an estimated incidence of between 1 in 40,000 to 1 in 117,000 male live births (Meikle, Hopwood, Clague & Carey, 1999) and is a multisystem disease with variable clinical features. Classic Fabry disease is mostly seen in hemizygous males who present in childhood with acroparaesthesiae, angiokeratomata and sweating abnormalities. Women with FD have a heterogeneous expression but are on the whole less severely affected. Disease progression primarily affects the kidneys and heart but the CNS is also frequently involved (see Germain, 2010, for review).

Abnormal MRI scans have been noted in half of FD patients, with white matter hyperintensities (of presumed vascular origin, see Wardlaw et al., 2013) the most common change (Mehta and Ginsberg, 2005). These hyperintensities are thought to be associated with small vessel disease affecting periventricular deep white matter, brainstem, cerebellum and basal ganglia (Assareh et al., 2011). Ischaemic stroke and TIA can occur before the emergence of other symptoms (Sims, Politei, Banikazemi & Lee, 2009), although estimates of prevalence vary (e.g. 24%: Grewal, 1994; 13%: Mehta & Ginsberg, 2005).

Studies examining the neuropsychological profile of FD have commonly noted deficits in information processing speed (e.g. Sigmundsdottir et al., 2014) and executive functions (e.g Loeb et al., 2018). Difficulties with attention (Schermuly et al., 2011) language (Low et al., 2007) and current general intellectual level (Sigmundsdottir et al., 2014) have also been observed. In contrast, other studies have reported intact or normal cognition (Lohle et al., 2015; Loret et al., 2021) and another linked cognitive deficits with mood difficulties (Schermuly et al., 2011). Preserved memory has been noted despite significant hippocampal atrophy, suggesting that cognitive decline may be compensated by neural plasticity (Fellgiebel et al., 2012). However, in a review Bolsover et al. (2014) noted that previous studies have methodological limitations including small sample sizes and an absence of well-matched healthy controls. It was also noted that it was unclear in the reviewed studies whether cognition was compromised in the absence of stroke. Additionally, subsequent studies have tested a limited range of cognitive domains (e.g. Wadley et al. 2015),used cognitive screening tools that are less sensitive to subtle compromises in cognition (Löhle et al., 2015) or compared patients with FD with normative data, leaving it unclear whether cognition had declined from premorbid levels (Loret et al., 2021).

To our knowledge, only two studies have compared patients with FD with well-matched healthy controls on a comprehensive battery of neuropsychological tests. Sigmundsdottir et al. (2014) found that male (but not female) patients with FD performed significantly worse than healthy controls on tests of information processing speed, executive functions and general intellectual ability. No significant group differences were found on tests of attention, memory, language, visual perception and verbal attention. Unfortunately, the sample size in this study was small (N=17 in FD group) with a relatively high prevalence of stroke (33% of males). Weak general intellectual ability and elevated anxiety could also have accounted for the male participants' focal deficits (see Tsourtos, Thompson & Stough, 2002) and no imaging data was available to relate deficits to CNS changes. More recently, Loeb et al. (2018) found a high rate of impairment for patients with FD on tests of information processing speed, the Block Design subtest of the WAIS and on the Stroop Colour/Word test (a test of executive functions and information processing speed). Given the compromise of white matter in FD, these results appear to reflect the relationship between reduced information processing speed and compromised white matter integrity (Kusnetzova et al., 2016). However, the level of mood difficulties in the FD group relative to healthy controls was not entirely clear and is thus a potential confounder in this study. It was also unclear whether cognition was intact in the absence of stroke (which affected 17% of the FD group) and no imaging data was available to relate deficits to CNS changes.

The relationship between cognition and physiological markers of FD progression is also equivocal. White matter disease severity predicted performance on cognitive tests in a recent study (Körver et al., 2019), but not an earlier study (Schermuly et al. 2011). Cortical volume loss has been found in FD without a decline in cognition (Fellgiebel et al., 2012; Lelieveld et al., 2015). Neurological and renal dysfunction (but not cardiovascular dysfunction) predicted performance on some cognitive tests where performance was compromised in one study (Sigmundsdottir et al., 2014). One study (Loeb et al., 2018) found that greater FD severity, as measured by the Mainz Severity Score Index (MSSI, Whybra et al. 2004), was associated with poorer scores on only one of the five tests where the FD group demonstrated compromise.

In the current study we compared the performance of a group of patients with FD with a relatively low prevalence of stroke with a group of healthy controls matched for age, premorbid intellectual ability and mood difficulties on a comprehensive battery of neuropsychological tests. Where deficits were apparent within a given cognitive domain, we investigated whether these were accounted for by a more general cognitive process, such as slowed information processing speed or a decline in general intellectual ability. We also

examined the relationship between compromised test scores and white matter hyperintensity severity based on MRI scans and overall disease severity as measured by MSSI.

2. Materials and Methods

2.1 Participants

Patients with a diagnosis of Fabry disease aged 18 or over attending the Charles Dent Metabolic Unit at the National Hospital for Neurology & Neurosurgery or the Lysosomal Storage Disorders Unit at the Royal Free Hospital in London, UK, were invited to participate via letter and those who confirmed interest were screened for study eligibility. All participating patients had a genetically confirmed diagnosis of FD verified on the International Fabry Disease Genotype-Phenotype Database (Icahn School of Medicine at Mount Sinai, 2021). See Appendix B for details of mutations. Healthy volunteer control participants aged 18 or over were recruited by posters placed in public areas within University College London Hospital NHS Foundation Trust. Exclusion criteria were: i) a central nervous system (CNS) disease/injury or intellectual disability unrelated to Fabry disease, ii) ineligible to have a 3T MRI scan (exclusions mainly due to cardiac devices or renal devices/transplant). Additionally, one patient with a benign mutation who had never displayed symptoms of FD was excluded from the study and one patient with a benign mutation but with significant acroparesthesia and ECG abnormalities was included in the study. Overall, 26 patients with FD (N=18 classic variant, N=7 late onset, N=1 benign) and 18 healthy controls were included in the study.

One patient with FD had a previous ischaemic stroke. Thus, our FD patient group had a low burden of strokes (3.8%) compared with previous studies and with the estimated prevalence within FD.

Informed written consent was obtained from all participants and the protocol was reviewed and approved by the NHS London Bromley Research Ethics Committee and the NHS UCLH/UCL Joint Biomedical Research Unit.

2.2 Neuropsychological and mood assessment

Participants completed a battery of clinical neuropsychological tests with published standardised normative data from large control samples. All participants underwent neuropsychological assessment on the same day as their neurological assessment and MRI scan. All neuropsychological assessments were conducted by F.W. under the supervision of L.C., a qualified clinical neuropsychologist. Tests were administered according to test handbooks.

The cognitive domains assessed and tests used are shown in Appendix A. Raw scores were used when statistically comparing the FD group and healthy controls, with the exception of

NART IQ, Verbal IQ, Performance IQ and the Hayling Sentence Completion Test, where standardised scores obtained from test manuals were used.

2.3 Measures of disease severity

The main measures of FD severity were:

i. The Mainz Severity Score Index (MSSI, Whybra et al., 2004), consisting of four subscales covering the general, neurological, cardiovascular and renal signs of FD. Summed score of the subscales was taken as a measure of disease severity.

ii. The duration of disease measured as the time since first symptom, established via self-report, was also used as a measure of FD severity.

2.4 MRI acquisition and analysis

Patients with FD and healthy controls were scanned using a standardised brain MRI protocol performed on a 3 Tesla Achieva TX MRI System (Philips Healthcare, Best, The Netherlands) using a Sensitivity Encoding (SENSE) 32-channel coil. Two patients with FD were unable to attend their scheduled MRI scans on the date of testing and this unfortunately could not be rearranged due to travel and time constraints (neither of these patients had a stroke or TIA).

The standardised MRI protocol and sequence parameters for this component of the study are as follows:

i. 3D Sagittal T1-weighted Gradient Turbo Field Echo (TFE) sequence, TFE factor: 230, Shot interval: 3000ms, reconstructed slice thickness: 1 mm isotropic with no slice gaps, 256mmx180mmx256mm (APxRLxFH) field of view
ii. Axial T2-weighted Dual Echo Fast Field Echo (FFE) sequence, first echo proton-density (PDT2) TE: 20.7ms, second echo TE: 85ms, TR: 6000ms, slice thickness: 2mm with no slice gaps, 240mmx180mmx144mm (APxRLxFH) field of view
iii. Axial Phase Sensitive Inversion Recovery (PSIR) Turbo Spin Echo (TSE) sequence, TE: 13ms, IR delay: 400ms, slice thickness: 2mm with no slice gaps, 240mmx180mmx144mm (APxRLxFH) field of view.

MRI scans were systematically analysed by two observers (FW and ID), the latter being an experienced neuro-radiologist. Both observers were blinded to participant identity and all clinical and demographic data.

2.5 White matter hyperintensity rating

White matter changes were rated by consensus according to the Age Related White Matter Change scale (ARWMC, Wahlund et al., 2001) using PDT2 and PSIR sequences. White matter changes by this definition were hyperintensities ≥5mm. However, white matter changes > 2mm were also included if they appeared as lacunes, defined as appearing like cerebrospinal fluid. If these hyperintensities were ≤2mm they were considered to be perivascular spaces and were not included. Larger hyperintensities around the anterior commisure (perforating substance) were also excluded as larger perivascular spaces are commonly found in this area. The following regions were rated in each cerebral hemisphere: frontal lobe, parieto-occipital area, temporal lobe, infratentorial area (including brain stem and cerebellum) and basal ganglia (including the striatum, globus pallidus, thalamus, internal and external capsules and insula). The overall volume of white matter hyperintensities was also used as a measure of white matter disease severity.

2.6 Whole brain white matter hyperintensity quantification

White matter hyperintensities were assessed for volume on the PDT2 scans, according to Schermuly et al. (2011) as bright hyperintensities >2mm, using the PSIR images for anatomical referencing. Hyperintense hyperintensities (>2mm) around the anterior commisure (perforating substance) were again excluded as larger perivascular spaces are commonly found in this area.

By this definition, white matter hyperintensities on all participant MRI examinations were segmented using JIM version 5.0 (Xinapse Systems, Northants). A semi-automated method for region of interest (ROI) placement was undertaken by consensus by the observers whereby ROIs were automatically assigned over hyperintensities and refined manually. The mean total ROI volume was automatically calculated and tabulated.

To assess inter-rater reliability and intra-rater reliability of hyperintensity segmentation, this process of semi-automated segmentation and analysis was undertaken independently in 10 randomly selected scans (of both control participants and patients) by both observers, blinded to the others ROIs and analysis, at two time points at least two weeks apart.

2.7 Statistical analysis

All data were analysed using SPSS Version 24.0 (IBM Corp.). Where data were normally distributed (Kolmogorov–Smirnoff test: $p>0.05$) independent sample t-tests were used to compare performance of Patients with FD and healthy controls. A Mann–Whitney U test was used where data were non-parametric. A Pearson correlation coefficient was used to calculate correlations where data were normally distributed and a Spearman's rank correlation coefficient where data were non-parametric.

Effect size for significant results was estimated (Cohen, 1988). The strength of correlations was noted using the rule of thumb (Hinkle, Wiersma & Jurs, 2003).

To assess group matching, we compared the FD group and healthy controls in terms of background demographic data, premorbid intellectual level and current level of mood difficulties. Neuropsychological data was analysed using the following approach:

i. Comparison of the performance of FD group and healthy controls on the neuropsychological measures.

ii. An error analysis to establish whether compromised FD group performances reflected focal compromise or a more general process, such as slow information processing speed or a decline in general intellectual ability (see, e.g. Stuss et al., 1998; Robinson et al., 2015).

iii. Where the FD group performed significantly worse than healthy controls, we calculated the correlations between their scores on these tests and measures of overall FD severity and white matter disease severity.

iv. As sex is linked with disease severity, we compared the performance of male and female patients with FD on the tests where the FD group performed significantly worse than healthy controls.

The level of significance was set at p=0.05. A Bonferroni correction was used wherever more than one test or correlation was used within a single cognitive domain. For example, for the four executive functions tests, statistical significance was set at 0.05/4 = 0.0125.

3. Results

3.1 Demographic data

The mean age of the FD group (N=26, 12 female) was 41.69 years (SD = 12.28, range 24-61 years) and their mean years of education was 15.94 years (SD=2.20, range = 11-20). The mean age of the healthy controls (N=18, 10 female) was 38.44 years (SD=9.9, range=28-65) and their mean number of years of education was 16.36 years (SD=16.36, range=12-25). The groups did not differ in terms of age, $t(42)=0.931$, $p=0.357$, or number of years of education, $t(42)=0.471$, $p=0.640$. Three of the patients with FD were unemployed at the time of the assessment, with two of these patients receiving governmental support due to disability related to FD. One further patient was on illness leave due to FD symptoms at the time of the assessment, one was studying for a degree and one for a postgraduate professional qualification. The remaining 20 patients with FD were employed, with one employed in an elementary role and the remaining 19 either self-employed or employed in a professional, managerial, technical or other skilled role.

3.2 Clinical profile of FD group

Mean Lyso-Gb3 level for the FD group was 24.93 ng/ml (N=21, SD=34.57, range 0.07 – 132.14) with elevated levels observed for 16 patients with FD (≥2.0 ng/ml, Maruyama et al., 2018) and levels for five patients below this cut-off. None of the healthy controls had elevated Lyso-Gb3 levels.

In terms of treatment, 19 of the patients with FD were receiving enzyme replacement therapy (ERT) and none were receiving chaperone therapy. Mean duration of ERT was 4.96 years (SD=4.65 years, range:1.00-15.00 years). Three of the FD group were taking statins, noted as having an uncommon side-effect of cognitive difficulties in clinical pharmacy reference guidelines (Joint Formulary Committee, 2019), although systematic reviews have noted no associated cognitive difficulties at the group level (Swiger et al., 2013). No other medications being taking by the FD group were known to have an effect on cognition.

Data on GLA mutations is presented in Appendix B.

Using estimated glomerular filtration rate to characterise kidney disease (see Levin et al., 2013), we found that 14 of the FD group had normal kidney functioning, nine had mild loss of kidney functioning, one mild-to-moderate loss, one moderate to severe loss and one had severe loss. In terms of urinary protein/creatinine ratio the mean for the FD group was 31.19 mg/mmol (SD=18.55, minimum=5, maximum=247). Three of the patients with FD scored above the cut-off of 30mg/mmol indicating significant proteinuria (Cote et al., 2008).

The estimated premorbid / NART IQ for both the FD group and healthy controls was in the high average range, making our sample relatively high functioning (see Table 1). This might have reflected a selection bias in recruitment, as previous studies of cognitive functioning have often seen a bias in participation towards more higher-functioning individuals (e.g. Woolgar et al., 2010).

*3.*3 *Neuropsychological tests: comparison of FD group and healthy controls*

Table 1 details the performance of the FD group and healthy controls on the neuropsychological tests. The FD group performed significantly worse than healthy controls on all three information processing speed tests and on three of four executive function tests. The FD group also had a weaker Performance IQ. Effect sizes for the significant results were moderate (WAIS PIQ, d=0.65; Letter Fluency, d=0.77; Hayling Sentence Completion Test, d=0.68; Stroop CW, d=0.65; Symbol Search, d=0.70). and large (Digit Symbol Test, d=0.81) according to Cohen's rule of thumb.

INSERT TABLE 1 AROUND HERE

For the Performance IQ subtests, we found that the FD group performed significantly worse than healthy controls on the Block Design subtest, t(42)=2.291, p=0.027, but similarly on the Picture Completion subtest, t(42)=1.450, p=0.154 and Picture Arrangement subtest, t(42)=1.638, p=0.109. The healthy controls' Performance IQ was seven points above their predicted IQ, with seven scoring above the 98th percentile on Block Design. Furthermore, the Performance IQ of the FD group was closely in line with their predicted premorbid intellectual ability. Taken together, this suggests that the results for Performance IQ reflected an anomalous strength for the healthy controls on the Block Design subtest, rather than any weakness for the FD group in this area.

To confirm that our results were not influenced by the single patient with FD with a previous stroke, we re-ran the analyses while omitting this patient. We found a very similar pattern of results. The patients with FD again performed worse than healthy controls on Letter Fluency, Hayling Sentence Completion Test, Stroop Test, Digit Symbol Test and PIQ, with moderate effect sizes again observed. The difference in Symbol Search scores between the two groups was narrowly non-significant at the corrected level, t(41) = 2.152, p=0.019.

3.4 Error analysis

As three executive function tasks have a timed component, we conducted an error analysis to ascertain whether the FD group's performance on these tests reflected errors characteristic of executive dysfunction (see Shallice & Cipolotti, 2018, for review). The FD group made significantly more perseverative errors than healthy controls on the Letter

Fluency Test (U=153, p=0.003). In terms of gross connected errors on the Hayling Sentence Completion Test, the group differences approached significance (U=179, p=0.052), with the patients with FD again making more of these errors. Both groups made a similar number of "somewhat connected" errors on the Hayling Sentence Completion Test (U=191, p=0.146) and disinhibition errors on the Stroop Colour/Word test (U=214, p=0.070).

3.5 Mood status

Scores on the anxiety and depression components of the HADS were not significantly different between the groups (see Table 1), with mean for both groups within the normal range (<8, Crawford, Henry, Crombie & Taylor, 2001).

3.6 Overall disease severity, white matter disease severity and their association with cognitive compromise.

Overall FD severity and white matter disease severity data for the FD group are presented in Table 2a. Table 2b shows the results of the correlations between this data and scores on tests where the FD group's performance was compromised relative to healthy controls.

Insert Table 2a and 2b about here

In terms of overall FD severity, the MSSI score of 18 patients with FD fell within the mild/moderate range, eight within the moderate range and none within the severe range (Whybra et al., 2004).

As shown in Table 2b, correlations between overall disease severity/white matter disease severity and compromised test scores were generally higher for information processing speed tests than for executive function tests and overall in the low to moderate range.

In terms of white matter disease, hyperintensities were far more common in the frontal cortex (in 45% of patients with FD), parieto-occipital cortex (42%) and infratentorial areas (29%) than in the temporal lobes (13%) and basal ganglia (4%). The number of patients with FD at different points on the ARWMC scale in the regions of interest was low, which limited the statistical analysis of this data. However, we examined the performance of the seven patients with FD who scored 2 (beginning confluence of lesions) or 3 (diffuse involvement of entire region) on the ARWMC scale for any of the regions of interest. Six of these seven patients with FD (86%) scored at least one standard deviation worse than the FD group mean on at least one of the tests in Table 2b. By contrast, such weak scores were only noted for 42% of the patients with FD who had focal hyperintensities (score = 1) or no hyperintensities (score =0).

White matter hyperintensities were noted on scans for seven healthy controls, despite none reporting a history of neurological disease (mean overall volume: 206 mm^3, SD=408 mm^3). However, the mean overall volume of these hyperintensities, U=130, p=0.003, was far lower than the FD group.

3.7 Relationship between genotype and cognitive decline.

The mean age for the classic group was approximately 6 years younger than the late onset group, but this difference was not statistically significant, t(23)=1.200, p=0.242. As expected, age at first symptom was lower for the classic group, t(23)=2.137, p=0.023, but time since first reported symptom was unexpectedly similar for both groups, t(20)=0.450, p=0.327. MSSI score, t(23)=2.284, p=0.016, Lyso-Gb3 level, U=2.00, p<0.001 and urine protein/creatinine ratio, U=35.00, p=0.047 were all higher for the classic group as expected. There was no difference between the groups in terms of overall white matter hyperintensity volume, U=49.00, p=0.381.

No significant difference was found between the performance of patients with classic and late onset mutations on the tests where decline was noted. (Performance IQ: t(23)=0.432, p=0.335; Symbol Search: t(23)=1.287, p=0.106; Digit Symbol: t(23)=0.088, p=0.466; TMT Part A: U=53.00, p=0.287; Letter Fluency: t(23)=0.221, p=0.414; Stroop Colour/Word: U=62.50, p=0.488; Hayling Sentence Completion Test: U=56.00, p=0.351).

3.8 Other disease and treatment parameters and cognitive decline.

Chronic kidney disease has been shown to be associated with cognitive impairment (Drew, Weiner & Sarnak, 2019). Using protein/creatinine ratio, we examined the relationship between kidney functioning in the FD group and their scores on the neuropsychological tests where they performed significantly poorer than healthy controls. Worse kidney functioning was associated with weaker Performance IQ, $r_s(26)=-0.403$, p=0.021, with no other results significant. As already noted, the FD group Performance IQ score was in line with their premorbid baseline. Thus overall it seems that kidney functioning was not predictive of a decline in cognition.

As recent studies have examined the relationship between ERT and neurological progression in FD (Körver, Longo et al., 2020), we also calculated the correlations between duration of ERT and both volume of white matter hyperintensities and scores on tests where the performance of patients with FD was compromised. None of the correlations were significant.

3.9 Effect of pain on cognition

Pain has a known effect on cognition (Moriarty, McGuire, and Finn, 2011) and is a common issue in Fabry disease (Politei et al., 2016). 12 of the patients with Fabry disease were routinely taking pain medication (aspirin: N=9; paracetamol: N=1; amitriptyline: N=1; co-codamol: N=1; ibuprofen: N=1). Four of the patients with FD reported experiencing pain crises on the MMSI.

Unfortunately, a clinical measure of pain was not gathered at the time of assessment. Instead, to ascertain whether pain accounted for the results we compared the performance of the patients who were and were not taking pain medication on the tests where compromise was noted. None of the group differences were significant (Performance IQ $t(24)=0.584$, $p=0.282$; Symbol Search: $t(24)=0.540$, $p=0.291$; Digit Symbol: $t(24)=0.326$, $p=0.374$; TMT Part A: $U=75.50$, $p=0.334$; Letter Fluency: $t(24)=0.047$, $p=0.482$; Stroop Colour/Word: $U=80.00$, $p=0.430$; Hayling Sentence Completion Test: $U=84.00$, $p=0.500$). Due to the low number of patients reporting pain crises, a group comparison was not possible. However, aside from a slightly lower Performance IQ score, the group means for these four patients was strikingly similar to the FD group mean on the tests where compromise was already noted (Performance IQ, M=99.00, SD=11.58; Symbol Search: M=38.00, SD=7.53; Digit Symbol: M=69.25, SD=10.44; TMT Part A: M=29.50, SD=8.35; Letter Fluency M=19.50, SD=6.45; Stroop Colour/Word: M=100.00, SD=14.99; Hayling Sentence Completion Test: M=5.58, SD=1.06).

3.10 Relationship between sex and cognitive decline

For the FD group, males and females did not differ in terms of age, $t(24)=0.356$, $p=0.725$, years of education, $t(24)=0.946$, $p=0.353$ or premorbid IQ, $t(24) = 0.341$, $p = 0.736$. Age at first symptom was lower for males (mean=12.20 years, SD=10.67) than females (mean=23.45 years, SD=11.17), $t(24)=2.126$, $p=0.023$. The MSSI score for males (mean=20.66, SD=9.08) was higher than that of females (mean=15.29, SD=8.79), although this difference did not reach significance, $t(24)=1.533$, $p=0.069$. There was no sex difference in terms of overall volume of hyperintensities $U=65.00$, $p=0.367$.

We found no significant difference between the performance of male and female patients with FD on the neuropsychological tests where the FD group exhibited compromise (Performance IQ: $t(24)=0.577$, $p=0.285$;, $p=0.297$, Digit Symbol: $t(24)=1.070$, $p=0.146$, Symbol Search: $t(24)=0.554$, $p=0.293$, TMT Letter Fluency: $t(24)=0.182$, $p=0.429$, Stroop Colour/Word $U=72.00$, $p=0.280$, Hayling Sentence Completion Test: $U=62.50$, $p=0.138$).

4. Discussion

In this study we compared the performance of a group of patients with FD and healthy controls on an extensive battery of neuropsychological tests. The groups were matched for age, premorbid intellectual ability and reported mood difficulties. Our results showed that, compared with healthy controls, the FD group performance was weaker on all three information processing speed tests, on three of four executive function tests and on a test of Performance IQ, with moderate to large effect sizes noted. In contrast, there was no evidence of a compromise of memory, language, visual perceptual, arithmetic or attentional functions. An analysis of errors by the FD group on the executive function tests and of their performance on the Performance IQ subtests provided support for the conclusion that executive dysfunction could not be entirely accounted for by slowed information processing speed or a general decline in non-verbal intellectual ability. Our sample of FD patients was well-characterised in terms of disease severity and had a low prevalence of stroke. Pain and medication regimes appear unlikely to account for any weakness in cognition.

These results help clarify some issues left unresolved by previous studies. Our results reflect those of the two previous studies conducted with a similar methodology (Sigmundsdottir et al., 2014; Loeb et al., 2018), where prominent information processing speed and executive function deficits were observed for the FD group. However, in contrast to these two studies, the number of patients with cerebrovascular disease in our sample was very low, with only one patient with a history of stroke. The relative prevalence of mood difficulties in the healthy control and FD groups was also not entirely clear in these two previous studies. Thus, our results confirm that information processing speed and executive function deficits in FD cannot be solely accounted for by a high prevalence of stroke or the presence of mood difficulties (see Veiel, 1997). Furthermore, our matched case-control methodology provides insight into more subtle declines from baseline functioning that may not be evident when examining the frequency of gross impairment (e.g. Loeb et al., 2018).

Our findings indicate that the relationship between FD severity and progression, white matter damage and cognitive decline is complex. We observed low to moderate correlations (i.e. a relatively weak association) between white matter hyperintensity volume and cognitive deficits for the patients with FD, in line with previous studies (Körver et al., 2019; Schermuly et al., 2011; Ulivi et al., 2020) and reflecting the known complex relationship between white matter progressive disease and cognition (see van Norden et al. 2011, for review). Genotype did not predict cognitive decline nor was it associated with the level of white matter hyperintensity volume. The latter is at odds with the findings of Körver, Longo et al. (2020), which may reflect that the time since first reported symptom was unexpectedly similar for

both classic and late onset groups in our sample. The correlations between measures of disease severity (time since first symptom, MSSI) and cognitive decline were also low. A significantly higher MSSI score in males also did not predict greater compromise of cognition. This is at odds with the results reported by Sigmundsdottir et al.(2014), which may be explained by the far higher sex difference in MSSI score in that study. Furthermore, two further variables related to disease severity, kidney functioning and duration of ER, failed to predict cognitive decline. The overall picture that emerges is in line with conclusions of Loeb et al. (2018), who stated that “cognitive impairment in Fabry disease does not seem to occur solely by having symptoms for many years or by having high disease affection”. It seems likely that other variables mediate the impact of FD on neurological and cognitive symptoms, e.g. other vascular risk factors, demographics and lifestyle factors (see van Norden et al. 2011). Consideration of these may be an objective for future studies.

Information processing speed is known to be associated with global white matter integrity (Kuznetsova et al., 2016; Penke et al., 2010). Thus it is unsurprising that white matter hyperintensity volume was associated with slowed information processing speed. In terms of a basis for the observed executive function deficits, we note that the areas primarily affected by white matter hyperintensities in the FD group, namely frontal and parietal cortex and infratentorial regions, are associated with executive functions (Shallice & Cipolotti, 2018; Bettcher et al., 2016; Koziol, Budding, & Chidekel, 2012). Despite this, the strength of the correlations between white matter hyperintensity volume and executive function scores were relatively low. This may be explained by considering the imaging parameters we used. Conventional MRI parameters are known to be insensitive to additional white matter abnormalities in FD revealed by diffusion tensor imaging (DTI, Ulivi et al., 2020). Proton MRS imaging has also revealed broader cortical and subcortical abnormalities in FD, including abnormalities present within cell nuclei (Tedeschi et al.,1999). This reflects that, while white matter hyperintensity volume correlated with cognitive deficits in our sample, they did not explain them entirely. An analysis of the role of damage to specific white matter tracts in FD using DTI may also have been revealing, as this has previously revealed a basis for executive function deficits in small vessel disease (Taludhar et al., 2015), a condition also associated with white matter compromise and with a pattern of cognitive deficits similar to those seen in FD (Roman et al., 2002). Consideration of these techniques, along with regional CNS atrophy in FD (Cocozza et al., 2018) and grey matter changes (Buechner et al., 2008) may help shed further light on the neurological basis for executive function deficits in FD in future studies.

Our study has some limitations. Firstly, our sample was relatively high functioning, which as acknowledged above may have reflected a selection bias. Patients with cardiac devices and

renal devices/transplant were also excluded from the study, which likely precluded participation for many patients with more advanced disease. Deficits may have been more marked for patients with more severe FD and/or of lower premorbid intellectual ability (Stern, 2009), so caution is advised in generalising the findings. Related to this, the cognitive functioning of the FD group was within the normal range, with most employed in professional or other skilled roles. It is therefore unclear how activities of daily living were affected by weakened cognition, which could be assessed in future studies using an appropriate structured questionnaire (e.g. the Frontal Systems Behavior Scale, Grace & Malloy, 2001). Our sample also contained no patients with severe FD, limiting the generalisability of our findings to this group. We also lacked more finer-grained physiological measures such as residual enzyme activity, which could have shed further light on the relationship between disease severity and cognitive decline.

Our results suggest that cognitive difficulties may affect individuals with FD who have not been flagged as a concern to neurological or neuropsychiatric services. Given that most of the patients with FD were in employment, it seems likely that some of these deficits may be subtle or may not be creating significant difficulties with activities of daily living. Thus, patients to FD should be offered appropriate neuropsychological screening based on the known profile of deficits in FD, as is already standard practice in other conditions affecting white matter integrity (Salvadori et al., 2020).

In summary, our study found compromise of information processing speed and executive functions in a sample of patients with mild to moderately severe FD. The deficits observed were unrelated to problems with mood or the presence of stroke. Our results provided evidence for a focal compromise of executive functions, which indicates that this deficit along with slowed information processing speed is a central feature of FD.

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Table 1: Comparison of performance[a] of FD patients and healthy controls on neuropsychological tests

		FD patients[b] (N=30)		Healthy controls (N=18)		
Domain	**Test**	**Mean[a]**	**SD**	**Mean[a]**	**SD**	**p-value**
Premorbid intellectual functioning	NART IQ	111.19	8.29	113.72	8.46	0.160
Current intellectual functioning	Verbal IQ	108.00	15.64	109.94	13.39	0.335
	Performance IQ	**109.15**	**16.71**	**120.78**	**18.65**	**0.018***
Memory	Words recognition †	47.77	2.96	48.67	0.91	0.411
	Faces recognition	41.92	4.48	43.39	4.26	0.14
	Figure recall immediate†	80.96	17.21	90.03	12.16	0.035
	Figure recall delayed†	81.18	17.08	88.91	13.55	0.029
	Story recall immediate†	39.96	7.81	39.78	7.93	0.292
	Story recall delayed	38.69	8.39	39.78	8.28	0.337
Word finding	GNT†	21.42	4.32	21.83	5.93	0.283
	Animal fluency	23.27	6.62	25.17	5.06	0.184
Mental arithmetic	GDAT	14.69	5.34	17.22	4.47	0.053
Visual perception	Incomplete letters†	19.70	0.55	19.61	0.50	0.236
Executive function	**Letter Fluency**	**17.88**	**5.05**	**22.00**	**5.69**	**0.008***
	Stroop Colour/Word†	**101.31**	**12.85**	**109.78**	**4.39**	**0.01***
	Hayling Sentence Completion Test†	**5.57**	**1.06**	**6.28**	**0.89**	**0.008***
	MCST perseverations†	1.69	2.36	0.72	0.83	0.06
Attention	Elevator counting with distraction†	8.52	2.33	9.17	1.95	0.102
Information processing speed	**TMT Part A†**	**29.77**	**10.41**	**23.11**	**5.05**	**0.016***
	WAIS-III Digit Symbol	**74.27**	**16.31**	**86.00**	**12.34**	**0.007***
	WAIS-III Symbol Search	**35.38**	**6.74**	**40.00**	**6.47**	**0.014***
Mood	HADS Anxiety†	7.58	4.79	7.22	4.17	0.261
	HADS Depression†	3.81	4.12	4.33	4.10	0.500

Notes: FD=Fabry disease; SD=standard deviation; NART=National Adult Reading Test; GNT=Graded Naming Test; TMT= Trail Making Test; WAIS=Weschler Adult Intelligence Scale; MCST=Modified Card Sorting Test; HADS-Hospital Anxiety and Depression Scale. Statistically significant results shown in bold

[a]Raw scores are shown and were used in statistical comparisons, with the exception of IQ measures and the Hayling Sentence Completion Test, where standardised scores based on the test manual were used.

[b]Data from one FD patient missing for Hayling Sentence Completion Test

* $p<0.05$ (corrected)

† Data significantly deviates from normality at $p<0.05$, non-parametric statistics used

Table 2a: Clinical characteristics of FD patients

	Mean	SD	Range
Time since first symptom / years (N=23)	23.39	15.77	2-54
MSSI	17.76	9.16	2-38
White matter hyperintensity volume / mm^3 (N=24) †	2138	7133	0-35194

Notes: FD=Fabry disease; SD=standard deviation; MSSI=Mainz Severity Score Index
† Data significantly deviates from normality at $p<0.05$

Table 2b: Correlations between measures of disease severity and white matter hyperintensity volume and neuropsychological test scores[a] for FD patients

	Performance IQ	**TMT Part A**	**WAIS-III Digit Symbol**	**WAIS-III Symbol Search**	**Letter fluency**	**Stroop colour/word**	**Hayling Sentence Completion Test**
Time since first symptom	**-0.329**	**0.503***	**-0.339**	**-0.395**	-0.091	-0.175	-0.012
MSSI	**-0.455***	**0.363**	**-0.348**	-0.222	-0.075	**-0.406**	-0.202
Total white matter hyperintensity volume	**-0.368***	**0.384**	**-0.673****	**-0.514***	-0.133	**-0.428**	**-0.439**

Notes: FD=Fabry disease; WAIS=Weschler Adult Intelligence Scale; Low correlations (0.3 to 0.5) and moderate correlations (0.5 to 0.7) are shown in bold. Non-parametric correlations used where either variable deviated significantly from normality as detailed in Tables 1 and 2a.
[a]Correlations only calculated where FD patients performed significantly worse than healthy controls.
* $p<0.05$ (corrected). **$p<0.01$ (corrected).

Appendix A: List of neuropsychological tests and mood measures administered

Premorbid Intellectual functioning	
National Adult Reading Test (NART)	(Nelson, 1982)
General intellectual functioning	
Wechsler Adult Intelligence Scale – 3rd Edition (WAIS-III)	(Wechsler, 1997)
Memory	
Recognition Memory Tests (RMT), Words and Faces	(Warrington, 1984)
Adult Memory and Information Processing Battery (AMIPB), Story and Figure recall	(Coughlan & Hollows, 1985)
Word finding	
Graded Naming Test (GNT)	(McKenna & Warrington, 1980)
Verbal category fluency test (Animals)	(Tombaugh, Kazak & Rees 1999)
Mental Arithmetic	
Oral Graded Difficulty Arithmetic Test (GDAT)	(Jackson & Warrington, 1986)
Visual-perception	
Visual Object and Space Perception Battery (VOSP) Incomplete letters	(Warrington & James, 1991)
Executive functions	
Stroop Colour / Word Test	(Trenerry, Crosson, Deboe & Leber, 1989)
Hayling Sentence Completion Test	(Burgess & Shallice, 1997)
Modified Card Sorting Test (MCST)	(Nelson, 1976)
Letter fluency	(Tombaugh, Kozak, & Rees, 1999)
Attention	
Test of Everyday Attention Elevator Counting with Distraction	(Robertson, Ward, Ridgeway, & Nimmo-Smith, 1994)
Speed of Processing	
Trail-Making Test Part A (TMT Part A)	(Reitan, 1958)
WAIS-III Digit-Symbol and Symbol Search	(Wechsler, 1997)
Mood	
Hospital Anxiety and Depression Scale (HADS)	(Zigmond & Snaith, 1983)

Appendix B: Overview of GLA mutations in the FD group

Mutation	Number of patients with mutation
c.1033_1034del (p.Ser345Argfs*29)	2
c.1067G>A	1
c.1087C>T	1
c.1223delA	1
c.1229C>T (p.Thr410Ile)	1
c.334C>T (p. R112C)	1
c.335G>A	1
c.613C>A	2
c.644A>G	2
c.902G>A	1
D92H [mis]	2
g.IVS3+1G>C	3
P205T [mis]	1
Q107X	1
R118C	1
R227X	2
R301P	1
R301Q	1
W287G	1