Fabry and the brain

Incorporating patients' illness perceptions into the physicians' practice

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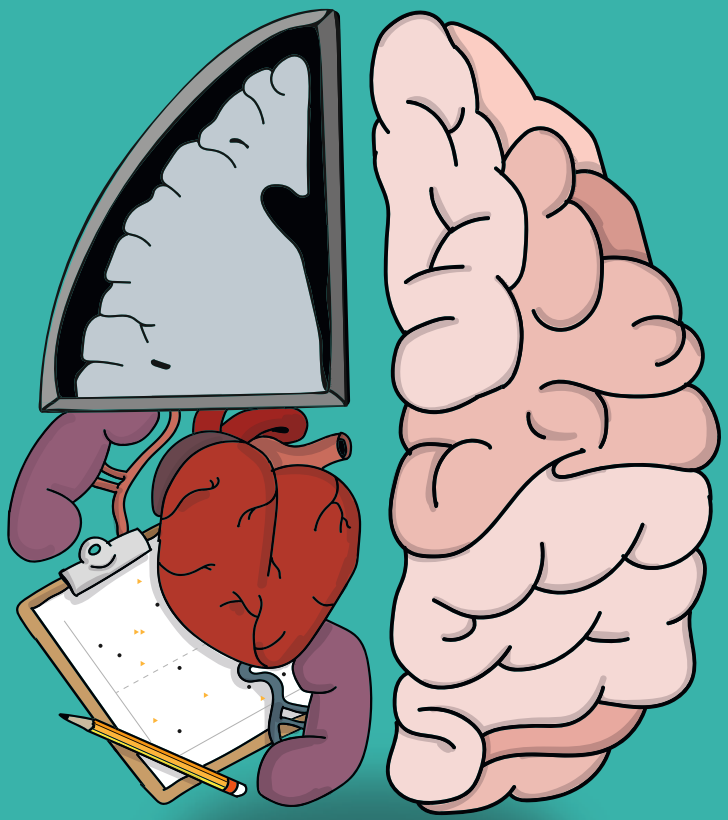

Incorporating patients' illness perceptions into the physicians' practice

Simon Körver

FABRY AND THE BRAIN

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Fabry and the Brain

Incorporating patients' illness perceptions into the physicians' practice

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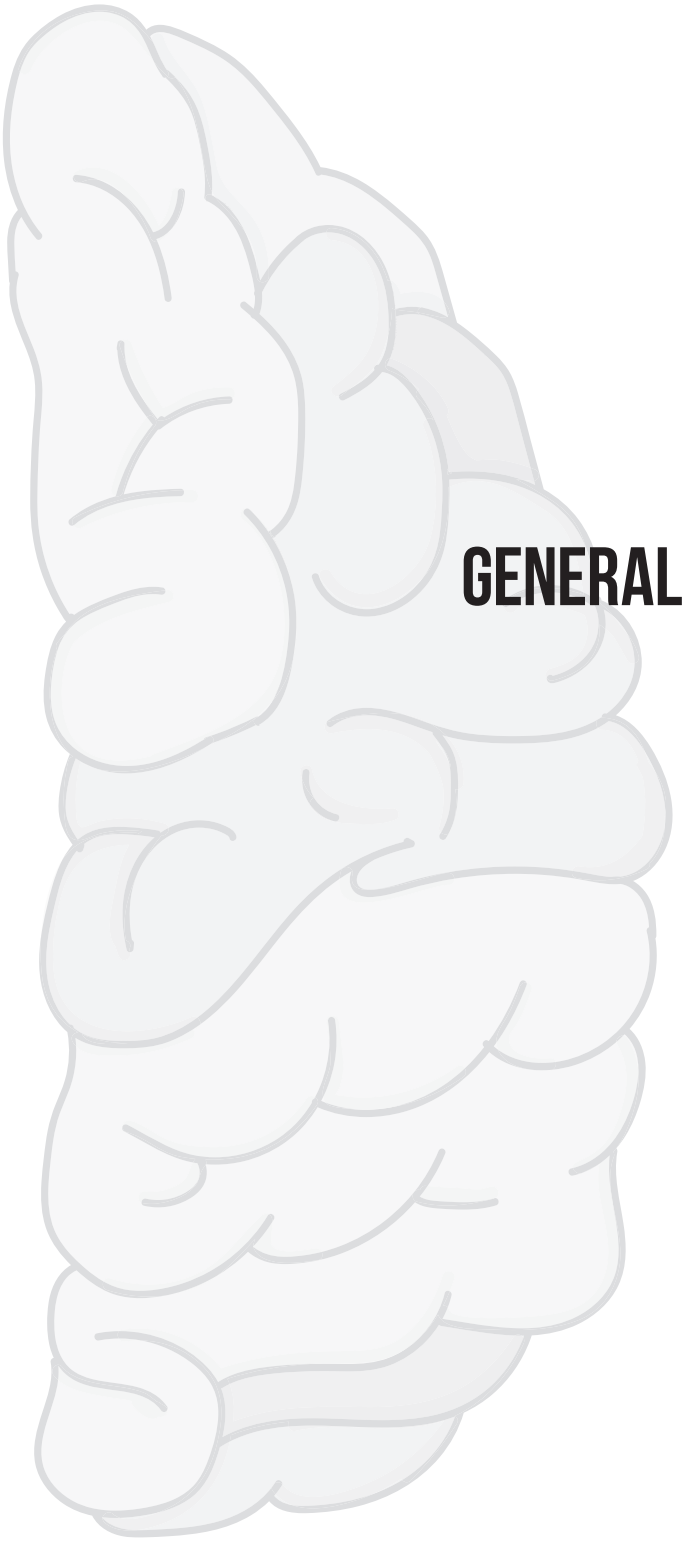
Contents



1

GENERAL INTRODUCTION

General introduction

Background

Fabry disease (FD) is a rare, heritable, X-linked disorder and can be classified as a lysosomal storage disease (OMIM 301500). Lysosomes are acidic organelles with multiple functions including breakdown and recycling of macromolecules, monitoring of nutrient status and calcium signaling [1, 2]. Deficiencies of lysosomal hydrolases are the most common cause of lysosomal storage diseases [2]. Patients with FD lack activity of the enzyme α-galactosidase A (enzyme commission no. 3.2.1.22) [3, 4] due to mutations in the GLA-gene [5]. In the presence of normal enzyme activity, α-Galactosidase A hydrolyzes the terminal α-galactose moiety from glycosphingolipids [5]. Lack of enzyme activity results in cellular storage of glycosphingolipids, such as globotriaosylceramide (Gb3), galactosylceramide [6] and increased plasma levels of globotriaosylsphingosine (lysoGb3; a deacylated form of Gb3) [7]. The exact function of Gb3 is unknown but it has been studied mostly for its role in binding the Shiga toxin [8] and it might be involved in lipid rafts, small membrane domains related to cellular signaling [9]. In FD, this abnormal storage occurs in many cell types in organs throughout the body such as the kidney, heart, intestines and brain [5, 10, 11].

Cerebral involvement in FD is common, particularly white matter lesions (WMLs), transient ischemic attacks and stroke [12, 13]. Also, cognitive impairment and a high prevalence of depressive symptoms have been described in patients with FD [14]. This thesis is mainly focused on the effects of FD on the brain and the relation between disease manifestations captured by brain MRI and clinical consequences such as cognitive functioning, depressive symptoms and clinical stroke.

Pathophysiology

While Gb3 and related glycosphingolipids accumulate in many cell types in FD, an important part of the pathology has been historically ascribed to the Fabry vasculopathy [15]. Accumulation of storage material in the vascular endothelial and vascular smooth muscle cells has been a recurrent finding in biopsies of FD patients [15]. As a consequence, smooth muscle cell hypertrophy occurs, possibly resulting in shear stress due to a less compliant vascular wall and a less dynamic reaction to changes in blood pressure [15-17]. Dysfunction of the vascular endothelium has also been shown [18, 19], as well as imbalance in reactive oxygen species [20], pro-thrombotic factors [21] and renin-angiotensin system [15, 22] in FD patients' blood samples. Although direct toxic effects of Gb3 [23] and lysoGb3 [24] are probable, secondary disruption of cellular and systemic processes in response to the storage material is likely to have major effects [25]. These disruptions continue even after the storage material has been removed [15]. This hypothesis has been instigated

by the finding that many patients still have complications while being on enzyme replacement therapy (ERT), despite clearance of Gb3 from the vascular endothelium [26, 27]. Consequentially, it is thought that starting treatment early (before major secondary storage effects occur) might prevent or slow the secondary consequences and result in more positive treatment effects [27, 28].

Recently, there has been increasing attention for these secondary consequences of storage. Normal lysosomal function is needed for regular function of the immune system and FD related lysosomal dysfunction might therefore be related to the low grade chronic inflammatory state found in FD [25]. Cellular substrate accumulation and subsequent lysosomal dysfunction might also dysregulate processes such as autophagy [29], cholesterol trafficking [30] and mitochondrial function [29]. The combination of pathological cascades will probably ultimately result in a final common pathway, shared with other lysosomal storage disorders and characterized by fibrosis and cell death [31].

FD pathophysiology of the brain

Brain autopsy studies reveal Gb3 storage in both cerebral vascular smooth muscle and endothelial cells [32-34]. In an autopsy case of a patient treated with ERT, the cerebral vascular endothelium was almost completely cleared from Gb3 deposits, but vascular smooth muscle cell deposits were still present [32]. Interestingly, Gb3 deposits have also been found widespread throughout the brain in deceased patients, often in regions that are generally not affected by WMLs or infarctions on MRI [32, 33]. Nevertheless, some of these areas with Gb3 deposits, such as the hippocampus and substantia nigra seem to be affected by atrophy in FD patients, without clear clinical consequences [35, 36].

The regions that are affected by WMLs in FD, the periventricular and deep white matter, correspond with the regions in which WMLs occur in the general population [16]. Both WMLs and lacunar infarctions, often found on FD patients brain MRIs, have been classified as small cerebral vessel disease in the general population [37]. The periventricular and deep white matter areas susceptible to WML development are watershed areas, which are vascularized by long penetrating arterioles and therefore seem sensitive to chronic ischemia [38]. However, the idea that chronic ischemia is the sole driving factor of these changes has been abandoned in WMLs research, with additional contributing factors being blood brain barrier and endothelial dysfunction, involvement of immune and glial cells and genetic modifiers [39, 40]. In the general population, small vessel disease has been marked as a whole brain disease, also affecting white matter that appears normal on structural imaging (*normal appearing white matter;* NAWM) [39].

Similarly, involvement of NAWM in FD patients was shown in diffusion weighted imaging studies [16, 41]. As in the general population, glial involvement [33], genetic modifiers of WMLs [42] and immune dysfunction [25] have all been described to play a role in WML development in FD. Cerebral blood flow in FD is probably affected, although studies have shown inconsistent results [43-45]. There might also be regionally decreased cerebral glucose metabolism [46, 47].

Next to small vessel involvement, the large cerebral vessels are affected in some FD patients with an increased basilar artery diameter (BAD) as its most prominent feature [48]. Again, this pathology might be related to Gb3 depositions in the vascular smooth muscle cells [49].

Lastly, large meta-analyses in the general population have shown a clear relation between chronic kidney disease and stroke [50], hypertrophic cardiomyopathy and atrial fibrillation and stroke [51] and cardiovascular risk factors and stroke [52]. Consequently, FD related cardiac and renal dysfunction and cardiovascular risk factors might also affect the pathology and prevalence of cerebral involvement in FD [53].

Overall, although poorly understood, the cerebral involvement of FD seems to be a combination of primary storage effects in the cerebral vasculature and secondary cellular and systemic effects, resulting in alterations in blood components, endothelial function and blood flow and disruption of cellular and systemic processes. Risk factors such as hypertension and FD related renal or cardiac dysfunction might also contribute the cerebral involvement.

Diagnosis

The conclusive diagnosis FD can be difficult to establish in some patients, because of the many different variants and mutations found in the GLA-gene and, mainly in women and non-classical patients, the lack of FD specific symptoms. Not all mutations in the GLA-gene result in disease and some DNA alterations are considered benign variants [54].

A diagnosis of FD is made if: 1) a pathogenic mutation in the GLA-gene is present (men and women) and 2) α-galactosidase A activity is decreased in leukocytes (men). Furthermore, typical FD related symptoms (Fabry specific neuropathic pain, angiokeratoma, and/or cornea verticillata) in the patient or a family member with the same mutation are strongly supportive of the diagnosis. If someone presents with a new GLA variant, without typical FD symptoms in the patient or a family member, pathogenicity can be supported by the following findings: increased lysoGb3 levels (although positive predictive value decreases in the lower regions, especially in women [7, 55]), biopsy of an affected organ with typical

zebra body inclusions, the mutation being described as pathogenic in literature and/or (more recently) presence of decreased T1-values on cardiac MRI [56, 57].

Phenotype and genotype

FD presents itself with a wide range of disease severity. Phenotypically, patients with FD can be classified as classical and non-classical (also: late-onset) [54, 58]. The classical phenotype is defined by a triad of typical symptoms: 1) neuropathic pains in hand and feet, often exacerbated by fever, exercise or heat, 2) cornea verticillata, a whorl-like pattern of corneal opacities and 3) angiokeratomas, red/purple skin lesions that cluster in the bathing trunk area, fingertips and on mucous membranes. Men with at least one of these symptoms combined with an enzyme activity below 5 percent and/or a plasma lysoGb3 over 40 nmol/L are classified as having classical FD [54, 58]. Male FD patients not fulfilling these criteria are classified as having non-classical disease.

The classification in women is more difficult since enzyme activity can be within the normal range and there is considerable overlap in lysoGb3 levels between classical and non-classical women [58]. Women with (a family history of) at least one of the typical FD related symptoms are classified as having classical FD. All other female FD patients not fulfilling these criteria are classified as having non-classical FD. Some GLA-mutations are more commonly detected and have been consequently reported as resulting in a classical or non-classical phenotype. In these specific cases, a phenotypical classification can be based mainly on the genotype.

The sex and phenotypical classification supports prognostication. That is, subgroups divided by sex and phenotype show major differences in their natural disease course and risk of progression of organ manifestations, which results in different recommendations for follow-up and treatment. For example, risks of complications are substantially higher in men with classical FD compared to all other patient groups. After age 30 there are few untreated male patients with classical FD that are free of organ involvement [58]. Treatment before signs of organ involvement [59] and strict monitoring is therefore recommended in this patient group [58].

Many different mutations and variants in the GLA-gene have been found. In combination with the low number of patients, this results in difficulties defining the relation between genotype and phenotype. Moreover, some genetic variants result in a modest reduction in enzyme activity and are therefore classified as non-pathogenic. Recently, efforts have been made to link specific mutations to phenotypes in genotype-phenotype databases [60, 61]. International collaborations have increased the number of known patients per mutation and therewith the accuracy of classification.

Cardiac and renal involvement

Cardiac involvement

Patients with FD can have a broad range of cardiac signs and symptoms, ranging from decreased exercise intolerance [62] and atrial fibrillation [63] to life threatening ventricular arrhythmias [64] and (diastolic) heart failure [65]. Follow-up of cardiac involvement consists of blood tests, ECGs, echocardiography and cardiac MRI (cMRI). Early features of cardiac involvement are shortening of the PR-interval on ECG [66] and a decreased native T1 value on cMRI [67]. With progression of disease, left ventricular hypertrophy and fibrosis formation may occur [58]. Eventually, the risk of life threatening arrhythmias also increases with age, left ventricular mass and area of fibrosis [64, 68].

Therefore, in patients with advanced disease, cardioverter/defibrillators are implanted regularly, but clear eligibility criteria and guidelines for this intervention in FD are lacking [64, 68]. Clear differences can be found in cardiac involvement between patient groups divided by sex and phenotype. Men with classical FD develop cardiac complications at an earlier age compared to the other subgroups [58]. However, a high prevalence of cardiac involvement is also found in men with non-classical FD and women with classical disease [58].

Renal involvement

In the early stages of FD, urinary and blood sample analyses for kidney involvement often do not show abnormalities [69], despite clear accumulation of Gb3 in renal biopsies [70]. Early biochemical signs of kidney involvement seen in patients with FD are hyperfiltration and (micro)albuminuria [71, 72]. During their lifetime, patients with FD are at risk for chronic kidney disease [73] and those with high levels of proteinuria show faster decline in kidney function [58, 74]. Differences in kidney function decline between sex and phenotype divided patient groups are even more striking compared to the differences in cardiac involvement. In men with classical FD kidney function may start to decline after adolescence [58]. In women, kidney function declines more slowly and end stage renal disease rarely occurs [58].

Cerebral involvement

Clinically, patients with FD are at risk for TIAs and strokes at an early age, in higher rates compared to the general population [12]. In clinical practice, assessment of cerebral involvement in FD consists mainly of MRIs of the brain with different sequences.

Conventional MRI findings

Using structural MRI, the most common abnormalities found in FD are WMLs [53]. The prevalence of WMLs is already increased in pediatric FD patients compared to age

matched control subjects [75] and WMLs are present in almost all men over 50 years with a classical FD phenotype [76]. Other important findings are both lacunar and large vessel infarctions on MRI [12, 77]. One large cohort study described an increased risk of hemorrhagic stroke in FD patients [12], a finding that was since then never investigated. Lastly, the prevalence of microbleeds might also be increased in patients with FD [78].

In contrast to the heart and the kidney, biopsy of the brain in suspected FD cases is considered to be unethical given the invasive nature of the procedure with a relative high complication risk. Since WMLs, TIAs and strokes are not FD specific, some studies tried to identify FD specific signs on MRIs of the brain to use as a screening parameter in high risk populations. The pulvinar sign, a hyperintensity of the lateral pulvinar on T1-weighted imaging, was initially considered a pathognomic sign of FD, but is neither sensitive nor specific for FD [79]. Basilar artery diameter (BAD) showed a more promising sensitivity and specificity compared to the general population, but was not able to accurately distinguish FD and stroke patients [49, 80]. Nevertheless, the increased BAD gained further attention in FD patients as a potential cause of stroke [81] or as a variable to evaluate treatment efficacy [82].

Phenotypical differences have been largely unexplored, but the risk of TIA and stroke is probably higher in men with classical disease [58]. Follow-up data on structural MRI findings in FD has been scarce but most studies suggest progression of WMLs despite treatment with ERT [83].

Experimental imaging findings

Researchers have used experimental imaging techniques to search for other prognostic markers of FD and to further unravel the FD brain pathophysiology. Most promising results have been found using diffusion weighted imaging. FD patients had both increased mean diffusivity and decreased fractional anisotropy compared to control groups [41, 84], which can be interpreted as markers of early loss of cellular structure and axon degeneration [85]. These changes were also found in the NAWM in patients with little to no WMLs and might therefore be a precursor of WMLs.

Cerebral blood flow and cerebral glucose uptake have also received considerable attention. An early study in FD patients reported an increased cerebral blood flow in men with FD [86]. Multiple studies have tried to replicate this finding using different methods, with conflicting results [43-45, 47]. Similarly, regional glucose metabolism might be decreased [47], but decreased glucose metabolism might also be a representation of regions with microbleeds or infarctions [46]. Possibly, cerebral blood flow and glucose metabolism change depending on disease severity [53], or simply due to differences in methodology used to measure these parameters.

Functional MRI studies in FD patients are sparse and have mainly focused on motor tasks and circuits [84, 87, 88]. All studies found changes in activation of different cerebral networks and motor regions in FD patients compared to control subjects, some of which resembled changes found in early stages of Parkinson's disease. In addition, FD patients scored poorer on motor performance tasks compared to a control group [89]. Extrapyramidal involvement has therefore been suggested in FD. However, clinically, these symptoms are not widely recognized and are probably subclinical [89].

Lastly, although volumetric studies found decreases in volume of the substantia nigra [36], hippocampus [35] and total intracranial brain [90], the importance of these findings remains unknown.

These experimental imaging methods have not been broadly applied in clinical follow-up. Most results are inconsistent and even contrasting or found only in a single small cohort study, without later replication in other studies. Moreover, longitudinal data are lacking and relations to clinical outcomes are often not tested or not present.

Cognitive functioning

Cognitive functioning can be divided in several functions such as memory, processing speed and executive functioning. Using a neuropsychological test battery, cognitive subdomains can be tested separately and test results can give a representation of everyday functioning [91].

In the general population, a relation between the presence of WMLs [92], stroke [93], major depressive disorder [94] and cognitive decline has been established. It is therefore not surprising that it was hypothesized that patients with FD might be at risk for cognitive impairment. An early case report linked FD to (vascular) dementia [95]. Subsequent studies systematically assessing cognitive functioning in FD patients showed mixed results [96, 97]. A systematic review concluded that there is some evidence for neuropsychological impairment, mainly affecting executive functioning, processing speed and attention in FD patients [14]. The link between WMLs and decline in processing speed and executive functioning in the general population [92] strengthens the suggestion between brain involvement and cognitive impairment in FD. Unfortunately, comparability between studies in FD patients was limited due to the use of different neuropsychological tests and approaches (e.g. computerized, paper based, through telephone). Also, simultaneous cerebral imaging and assessment of cognitive functioning and depressive symptoms lacked in most studies [14]. Therefore, the relation between cognitive impairment in FD and depressive symptoms, cerebral imaging parameters or other disease related parameters remains unknown.

Depressive symptoms and coping

It has been increasingly recognized that FD patients are at risk for depressive disorder [98] and depressive symptoms are found in up to 46% of FD patients [14]. This is not unique for FD: depressive symptoms are highly prevalent in common chronic diseases such as stroke or heart disease [99] and in rare diseases in general [100].

Previous studies hypothesized that FD related cerebral pathology might be a biological substrate for depressive disorder [101] and that cognitive impairment and depressive symptoms might be interrelated [14]. However, up until now no clear relation between depressive symptoms and WMLs [96] or cognitive impairment [102] has been found in FD patients.

In previous studies, no or only a minor relation between cardiac and renal organ involvement and depressive symptoms in FD patients was shown [96, 102]. Alternatively, FD patients' subjective perception of their own health status might be more important in relation to depressive symptoms [103, 104]. Subjective health perception is probably a combination of a patients' current disease status (organ involvement) combined with other elements such as expected future (disease) progression [105]. Having FD can cause uncertainty about the future and may require constant adaption as the disease progresses [106]. This process is hampered in patients with chronic diseases [107]. An important factor in the psychological adaptation to a chronic disease is coping, the cognitive and behavioral efforts to manage stressors that tax and exceed the resources of a person [108].

The (expected) progression and recurring symptoms and complications of a chronic disease such as FD are probably perceived as stressful events by patients. According to the most commonly adhered coping theory by Lazarus and Folkman, a new stressor is first appraised in terms of predictability, controllability and expectancies for the individual [108]. If this event or stressor is potentially threatening for this person (e.g. for their health, goals or values), this will result in the employment of coping styles [108]. Whether individuals have a preferred way of coping, or have a different set of coping skills for every situation has been extensively debated [109]. Taking both sides into account, coping can be conceptualized as a personality style, meaning that patients have a preferred way of coping with stressors but might adjust their style somewhat depending on the stressor [109]. It has been shown in other chronic diseases such as type 2 diabetes and rheumatoid arthritis that differences in coping styles are related to differences in both psychological outcomes [110] and physical outcomes [110, 111]. Coping styles and their relation to depressive symptoms have not yet been addressed in FD.

Treatment in Fabry disease

Enzyme replacement therapy

In 2001, enzyme replacement therapy (ERT), an intravenous administration of the recombinant α-galactosidase A, was approved for patients with FD. In the European Union, the European Medicines Agency approved two formulations: agalsidase alfa (Replagal, Shire, Dublin, Ireland) and agalsidase beta (Fabrazyme, Sanofi Genzyme, Cambridge, United States). Replagal is dosed at 0.2mg/kg every other week, Fabrazyme at 1.0 mg/kg every other week.

The approval was based upon relatively small randomized controlled trials (RCTs) using surrogate endpoints, almost exclusively in men suspected to have classical FD [112-115]. Over the years multiple studies have shown that in most patients disease progresses despite treatment [26, 116] and effectiveness is suspected to be limited in delaying complications [27]. Head to head comparisons of Replagal and Fabrazyme are scarce and no differences in organ complications were found when regarding all RCTs [112, 116]. Nevertheless, some studies have argued a favorable effect of Fabrazyme over Replagal [117, 118].

Cerebral outcomes

As the pivotal trials did not include cerebral outcomes, the effect of ERT on the brain in FD is largely unknown. A post-hoc analysis of a phase IV trial showed no differences between Fabrazyme and placebo regarding WML progression [83]. A meta-analysis of cohort studies showed less cerebrovascular events (stroke or TIA) in patients treated with Fabrazyme compared to both Replagal and untreated patients [117]. However, as age, sex and phenotype were often not adequately described, it is unknown whether the cohorts in these studies were balanced, which might have had a major effect on the meta-analysis' outcome. In an international consensus meeting it was agreed that starting ERT *may* be considered in patients with WMLs and *should* be considered in patients with a history of TIA and stroke [59].

Other therapies

In 2016, the European Medicine Agency approved migalastat (Galafold, Amicus Therapeutics, Cranbury, United States), an oral small molecule chaperone therapy with the potential to stabilize endogenous α-Galactosidase A, improving enzyme function. In the Netherlands migalastat was not approved after reconsideration by the National Healthcare Institute (ZIN) [119]. It was concluded that, given the current level of evidence from the pivotal trials, no conclusions on its effectiveness in comparison to ERT were possible. While migalastat crosses the blood brain barrier in mice [120], pivotal trials did not include MRIs of the brain and follow-up time and power were insufficient to detect differences in TIA and stroke incidence [121, 122].

Currently, several other new treatments are being tested in phase II and phase III RCTs or at an earlier stage of development, such as second generation ERTs, substrate reduction therapy, gene therapy and mRNA therapy. Unfortunately, none of the ongoing trails include cerebral outcomes as part of their primary or secondary outcomes [123].

Supportive treatment

Supportive treatment mainly focusses on decreasing FD related neuropathic pain and preventive strategies focus on reduction of cardiovascular complication risk and renal function decline [124]. FD specific neuropathic pain can be treated with anti-epileptics such as carbamazepine and gabapentin [125] or prevented by limiting high intensity exercise and exposure to heat [124]. Proteinuria is managed using angiotensin converting enzyme inhibitors or angiotensin receptor blockers [126].

Considering cerebral involvement, it is recommended to follow primary and secondary stroke prevention guidelines for the general population [124, 127, 128]. Both primary and secondary preventive management consists of aggressive control of risk factors such as smoking, hypertension and dyslipidemia, but also increasing physical activity, decreasing excessive alcohol intake and dietary intake and composition [127-129]. In addition, strict control of glucose in patients with diabetes mellitus and anticoagulation in patients with AF are recommended [127, 128]. In secondary prevention screening for diabetes mellitus, sleep disordered breathing, heart valve disease, carotid disease and atrial fibrillation are recommended [128]. In primary prevention, it is recommended to start treatment with aspirin in patients with a 10-year risk ≥10% of coronary heart disease or stroke. There is no evidence that antiplatelet medications reduce the risk of stroke in low(er) risk populations [127].

FD patients presenting with acute stroke should be evaluated in the emergency department. There have been some documented cases of intravenous thrombolysis in FD patients, with variable results [130, 131]. There are no published cases of mechanical thrombectomy in patients with FD. The combination of aspirin and clopidogrel might be considered for the first 21 days after a TIA or stroke [132] and continuation of clopidogrel monotherapy afterwards [128].

Caveats in knowledge on Fabry disease and the brain

Consultation and treatment of patients with FD can be difficult, partly due to caveats in the knowledge of the relation between FD and the brain. For example, it is unknown which patients are at risk for brain involvement, cognitive impairment and depressive symptoms, what effects can be expected from ERT and which patients will progress over time. In addition, small sample sizes, lack in methodological rigor and lack of follow-up

complicate interpretation of published research. Taking this into account, communicating risks, progression and potential consequences of brain involvement in FD to patients and family is currently inaccurate at best.

Aims and outline

The purpose of this thesis is to establish which patients are at risk for brain involvement, what the progression is in these patients and whether an effect of ERT is seen. Secondly, this thesis focuses on the potential consequences of brain involvement, depressive symptoms and cognitive impairment and their interrelation. Using this knowledge, we aim to improve prognostication, find potential targets to treat or prevent cerebral involvement and its consequences and to improve the appropriate use of ERT. For this thesis two cohorts were created: 1) a cohort of Dutch FD patients for prospective assessment of cognitive functioning, depressive symptoms and coping and 2) a cohort including all Dutch FD patients with ≥1 MRI of the brain for the longitudinal retrospective analysis of brain involvement in FD. One chapter uses the data from a combined cohort with patients from the Netherlands and the United Kingdom. Together, this has resulted in the following work:

In **Chapter 2** we provide a systematic review of all available literature on the prevalence, severity, location, progression, effect of ERT, related patient characteristics and potential consequences of WMLs in FD. In **Chapter 3** we use the retrospective MRI data to explore progression of WMLs and infarctions and to evaluate the importance of treatment and different patient characteristics such as phenotype and cardiac or renal involvement. **Chapter 4** reports on the effect of clinical characteristics and events such as stroke on quality of life in FD patients, using data from Dutch patients as well as patients from the United Kingdom. In **Chapter 5** the profile of cognitive impairment in FD and its relation to disease manifestations and patient characteristics in the Dutch FD cohort is evaluated. Since the assessment of cognitive functioning is time and labor intensive, we evaluated the accuracy of the mini mental state examination to screen for cognitive impairment in FD in **Chapter 6**. In **Chapter 7** the prevalence of depressive symptoms and the employed coping styles of FD patients are presented. Relationships between depressive symptoms and cognitive functioning, disease manifestations and patient characteristics are studied. In **Chapter 8** the follow-up data of both cognitive functioning and depressive symptoms are compared to the baseline data. Finally, **Chapter 9** includes a summary and general discussion of this thesis.

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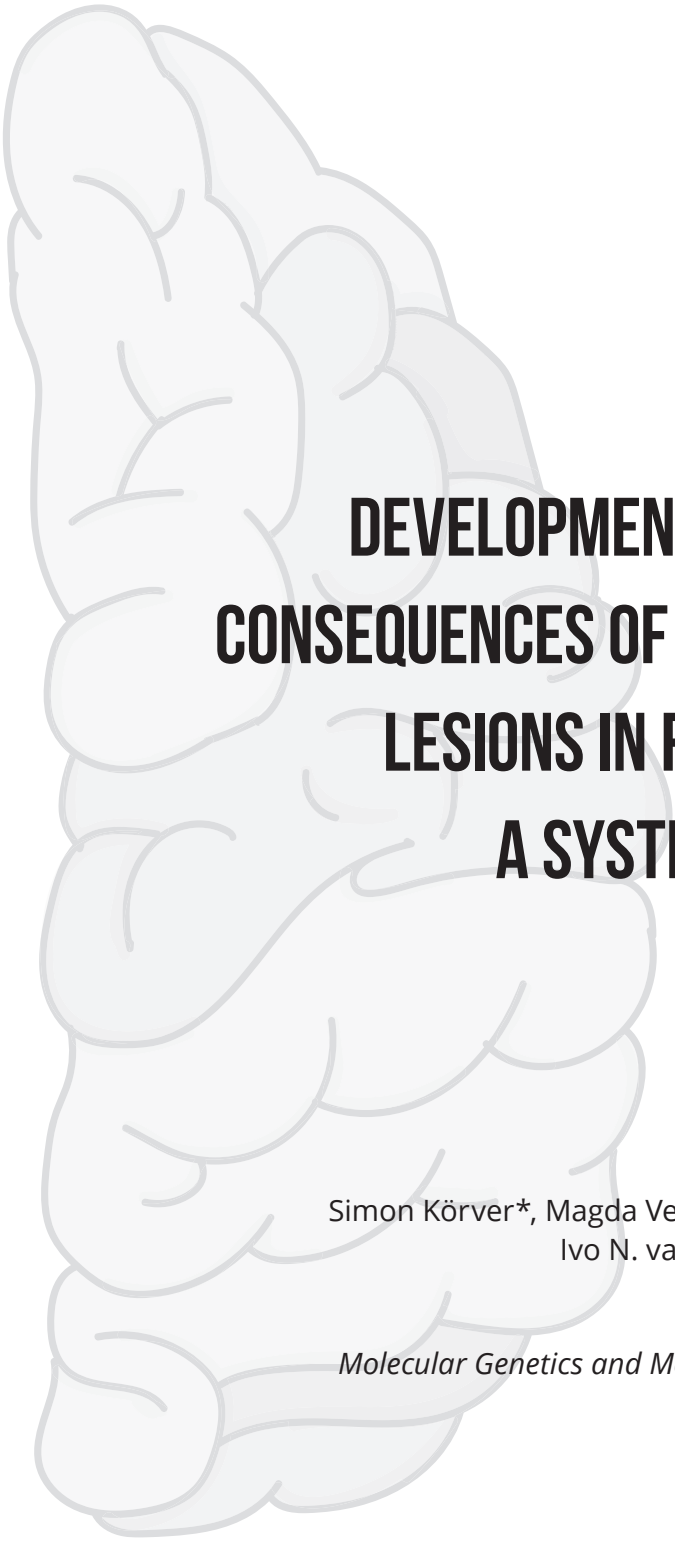
PART 1

CEREBRAL INVOLVEMENT IN FABRY DISEASE



2

DEVELOPMENT AND CLINICAL CONSEQUENCES OF WHITE MATTER LESIONS IN FABRY DISEASE: A SYSTEMATIC REVIEW

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Abstract

Background:
Fabry disease (FD) is a rare lysosomal storage disorder that might result in, amongst other complications, early stroke and white matter lesions (WMLs). More insight in WMLs in FD could clarify the role of WMLs in the disease presentation and prognosis in FD. In this systematic review we assessed the prevalence, severity, location and course of WMLs in FD. We also systematically reviewed the evidence on the relation between WMLs, disease characteristics and clinical parameters.

Methods:
We searched Pubmed, EMBASE and CINAHL (inception to Feb 2018) and identified articles reporting on FD and WMLs assessed with MRI. Prevalence and severity were assessed for all patients combined and divided by sex.

Results:
Out of 904 studies a total of 46 studies were included in the analyses. WMLs were present in 46% of patients with FD (581 out of 1276 patients, corrected mean age: 38.8 years, range 11.8-79.3) and increased with age. A total of 16.4% of patients (31 out of 189 patients, corrected mean age: 41.1 years, range 35.8-43.3 years) showed substantial confluent WMLs. Men and women showed comparable prevalence and severity of WMLs. However, men were significantly younger at time of WML assessment. Patients with classical FD had a higher chance on WMLs compared to non-classical patients.

Progression of WMLs was seen in 24.6% of patients (49 out of 199 patients) during 38.1 months follow-up. Progression was seen in both men and women, with and without enzyme replacement therapy, but at an earlier age in men. Stroke seemed to be related to WMLs, but cerebrovascular risk factors, cardiac and renal (dys)function did not. Pathology in the brain in FD seemed to extend beyond the WMLs into the normal appearing white matter.

Conclusions:
A significant group of FD patients has substantial WMLs and male patients develop WMLs earlier compared to female patients. WMLs could be used in clinical trials to evaluate possible treatment effects on the brain. Future studies should focus on longitudinal follow-up using modern imaging techniques, focusing on the clinical consequences of WMLs. In addition, ischemic and non-ischemic pathways resulting in WML development should be studied.

Introduction

Fabry disease (FD; OMIM 301500) is an X-inherited lysosomal storage disease. A mutation in the GLA-gene causes a deficiency of the enzyme α-galactosidase A (enzyme commission no. 3.2.1.22), resulting in accumulation of globotriaosylceramide (Gb3) and its derivatives in various cell types. FD is a multi-organ disease and disease manifestations occur predominantly in kidney, heart and brain [1, 2]. To detect and monitor organ involvement systematic follow-up of patients is strongly recommended. This should include MRI brain on a regular basis, since early cerebral manifestations of FD can be asymptomatic but might warrant treatment [2]. These cerebral manifestations are early (transient ischemic attack) TIA, stroke, lacunar infarctions and white matter lesions (WMLs) [1, 3]. Despite many reports on WMLs, information on the development and consequences are scarce. As FD is an X-linked disorder, men are generally more severely affected and develop disease complications earlier in life then women. In addition, disease severity is variable between patients who can traditionally be classified as having classical or non-classical FD. Men with a classical phenotype typically have no residual enzyme activity and affected family members generally have earlier and more widespread disease manifestations and complications compared to non-classical patients [1]. Interestingly, differences in the development of WMLs between men and women have so far not been found [4, 5]. This may be the result of lack of statistical power, or because no distinction is made between patients with classical and non-classical disease or stratified by age. The latter is supported by the recent observation of a higher prevalence of WMLs in men with classical disease versus those with non-classical FD [1]. This emphasizes the need to classify patients by sex, age and and phenotype, when studying cerebral involvement in FD.

Detection of WMLs can also have diagnostic implications: in some diseases other than FD, the specific location and distribution of WMLs suggests a specific underlying disease, such as corpus callosum involvement in multiple sclerosis [6]. This has so far not been established for FD: WML distribution in FD has been described as aspecific and multifocal [7]. Moreover, despite the status of WMLs as an early marker of cerebral involvement in FD [8], their clinical consequences and response to enzyme replacement therapy (ERT) have been rarely addressed in follow-up studies.

In view of the paucity of analyses on this topic, the following points of interest were raised. Firstly, more insight in the prevalence, severity and course of WMLs may help to identify patients who are likely to develop WMLs. Secondly, a detailed exploration of the location of WMLs by magnetic resonance imaging (MRI) may possibly assist in the diagnosis of FD. Lastly, the relationship between WMLs and occurrence and severity of

FD complications can help to identify whether WMLs can be used as a prognostic factor and/or parameter to evaluate treatment efficacy. Hence, in this systematic review we assessed the prevalence, severity, location and course of WMLs in FD as well as the relation between WMLs, disease characteristics and clinical parameters.

Methods

For this systematic review we adhered to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement [9].

Data sources and search

We searched Pubmed, EMBASE and CINAHL from inception to the 15th of February 2018. No restrictions were applied on language or publication date. The search included synonyms of "Fabry disease" and "White matter lesions". To include studies that did not use a synonym of WMLs in the title or abstract but might report on WMLs in full text we used terms related to MRI brain and other cerebral manifestations of FD, see **Supplemental File A** for the search terms used. The search strategy was adapted for each database to increase sensitivity. Reference lists of reviews and included studies were checked and did not identify missing studies.

Study selection

Randomized controlled trials (RCTs) and cohort studies (≥5 included patients) were included, using MRI brain to report on WMLs in adults and children with FD. Excluded were: 1) Case-series since these are prone for publication bias [10], 2) Studies screening for FD in high risk populations (e.g. in a young stroke cohort), 3) Studies focusing on a group of patients with one specific mutation. Studies reported as congress abstracts only were included for the calculation of the prevalence of WMLs.

The most relevant report of consecutive studies in the same cohort was included. If studies reported on the same or an overlapping cohort, but provided information on different research questions, both studies were included.

Two reviewers (SK and MV) independently screened all titles and abstracts using Covidence, an online article screening tool [11]. Reviewers resolved disagreement by discussion, if necessary a third reviewer (ML) was consulted.

Data extraction

Two reviewers (SK and MV) individually extracted data using a standardized report form. The following data were extracted: number of patients in the study, sex, phenotype,

age, treatment status, previous TIA or Stroke, MRI sequence used to identify WMLs, MRI field strength (Tesla (T)), definition that was used for WMLs and data reporting on WML frequency, severity, location and course and the relation of WMLs to clinical parameters and patients characteristics. Data were extracted for the whole study cohort and by disease phenotype and sex if specified. If data were reported for adults and children both groups were included separately. Primary outcome was the prevalence of WMLs. Secondary outcomes were WML severity, location and course. In addition, data on the relation of WMLs to clinical parameters and patient characteristics were extracted.

Definitions

We used the definitions for WMLs as provided by the authors of the individual articles. Prevalence was defined as the number of patients with WMLs on MRI divided by the number of patients who underwent a brain MRI. Severity was defined as the size or number of WMLs, WML volume or by using a WML rating scale (e.g. Fazekas). Location was defined as the region in which the WMLs were observed on MRI and was classified as periventricular, deep or subcortical or per anatomical or circulatory area. Course was defined as whether WMLs were progressive or stable over time as reported by a radiologist or quantitatively assessed on at least one follow-up MRI.

Statistical analysis

Data are presented as median and range or mean ± SD where appropriate. R (version 3.3.1) was used for statistical analysis. Prevalence of WMLs was calculated with and without information obtained from abstracts only (studies for which no full article is available). When combining variables (e.g. age or Fazekas score), correction for cohort size was performed by using the number of patients per study as a weight factor. These variables are referred to as "corrected mean age" and "corrected mean Fazekas score". Prevalence of WMLs (present versus absent) and Fazekas scores (Fazekas ≥2 versus Fazekas <2) in men and women were compared using the chi^2-test. Ages in studies reporting on the prevalence and/or severity of WMLs were considered non-normally distributed after visual inspection using histograms and Q-Q plots and the Shapiro-Wilk test and were compared using the Mann-Whitney U test.

Results

A total of 904 studies was identified through our search of which 256 were duplicates, see **Supplemental File B** for the PRISMA flow diagram. After screening for title and abstract 170 articles and 17 abstracts were assessed for eligibility. A total of 46 articles [1, 3-5, 12-53] and eight abstracts were included in the qualitative synthesis [54-61], see **Supplemental Table A** for an overview table of all articles.

MRI field strength and sequences

Different field strengths and MRI sequences were used in the assessment of WMLs. Of the 46 studies, 25 (54.3%) reported both field strength and sequence(s) used to assess WMLs and eight (17.4%) reported neither. MRI field strengths of 0.5-3T were reported for 27 studies and sequences used to assess WMLs were reported for 36 studies. Most used field strength was 1.5T and most used sequence was the fluid attenuation inversion recovery (FLAIR) sequence. For details see **Supplemental Table A**.

Prevalence of WMLs

Thirty-one articles [1, 3, 4, 12-39] and eight abstracts [54-61] assessed the prevalence of WMLs in FD in a total of 1577 patients, of which 1276 patients were described in the articles (**Table 1**). Since abstracts often did not provide information on patient characteristics these were only extracted from the articles. Corrected mean age of patients was 38.8 years (range 11.8-79.3 years). A total of 372 patients (33.6%) were treated with ERT and 76 patients (12.7%) had a history of TIA and/or stroke (TIA/stroke data missing in 46.8% of patients).

WMLs were present in 45.5% of all patients (581 out of 1276 patients). When including abstracts WML prevalence was 44.8% (707 out of 1577 patients). The prevalence of WMLs visually increased with age (**Figure 1**). One cohort with six geriatric patients (mean age: 79.3 years (range: 75-87 years), prevalence WMLs: 50%) was removed for visual purposes as their average age extended the x-axis with 20 years.

In approximately half of the 1276 patients, sex was reported (309 men and 317 women). None of the pediatric cohorts provided the prevalence of WMLs divided by sex (**Figure 2**). One cohort with five geriatric women (mean age: 80.2 years (range 75-87 years), prevalence WMLs: 40%) was removed for visual purposes as their average age extended the x-axis with 20 years.

WML prevalence was 46.9% in men at a corrected mean age of 36.4 years and 41.0% in women at a corrected mean age of 43.1 years (**Table 2**). No differences were found when comparing prevalence of WMLs between men and women ($\chi^2(1)=2.0$, $p=0.15$). However comparing uncorrected age showed that men were younger at time of WML assessment compared to women ($U=10$, $p<0.0001$).

Table 1 Characteristics of patients with reported prevalence of WMLs

	All patients*	Sex known	
		Men†	Women‡
Patients (including abstracts), n	1577	368	346
Patients (articles only), n	1276	309	317
Patient characteristics (articles only)#			
Patients per article, median (range)	26 (6-283)	15 (4-52)	15 (4-57)
Number of patients with age reported, n	930	261	302
Corrected age (years), mean (range)	38.8 (11.8-79.3)	36.4 (27.4-41.9)	43.1 (35.0-80.2)
Number of patients with ERT use reported$, n	1106	188	147
Number of patients on ERT, n (%)	372 (33.6%)	96 (51.1%)	60 (40.8%)
History of TIA and/or stroke reported, n	597	172	193
History of TIA and/or stroke, n (%)	76 (12.7%)	29 (16.9%)	35 (18.1%)

**Includes mixed cohorts, pediatric cohorts, men only cohorts and women only cohorts, †Includes all articles from the "All patients" group that presented data on prevalence of WMLs divided by sex, #Abstracts often did not provide information on ERT use, TIA or stroke and age and were subsequently not included in the patient characteristics, $Two articles were published before the availability of ERT. These patients were classified as not using ERT.*
ERT = Enzyme Replacement Therapy, WMLs = White matter lesions

Table 2 Prevalence of WMLs

	All patients*	Sex known	
		Men†	Women†
Number of patients with WMLs (articles only), n	581	145	130
WML prevalence (articles only), %	45.5%	46.9%	41.0%
Number of patients with WMLs (including abstracts), n	707	168	139
WML prevalence (including abstracts), %	44.8%	45.7%	40.2%

**Includes mixed cohorts, pediatric cohorts, men only cohorts and women only cohorts, †Includes all articles from the "All patients" group that presented data on prevalence of WMLs divided by sex*
WML(s) = White matter lesion(s)

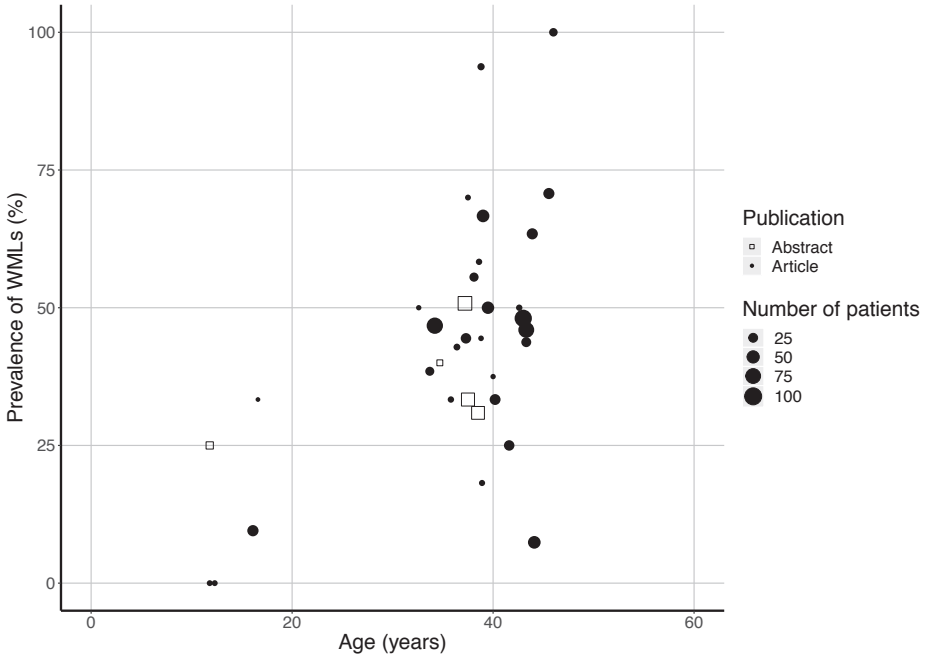

Figure 1 Prevalence of white matter lesions (WMLs) per study in relation to age

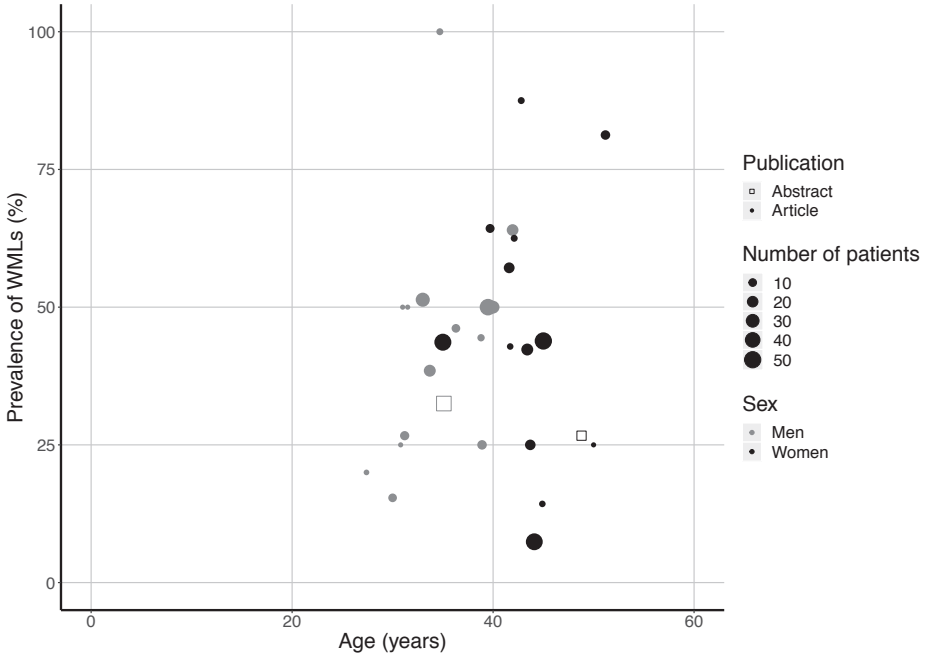

Figure 2 Prevalence of white matter lesions (WMLs) per study, divided by sex in relation to age

Severity

Twenty-six studies assessed WML severity [3-5, 13, 14, 17-21, 23, 26-28, 31, 32, 34-37, 39-44]. Methods used to assess WML severity were the Fazekas scale, subjective assessment, white matter lesion number and/or size and white matter lesion volume.

Fazekas scale

The original Fazekas scale describes WML severity in deep and periventricular white matter [62]. It ranges from 0 (no WMLs) to 3 (confluent WMLs) for both locations, resulting in a total score from 0 to 6. A modified version of the Fazekas scale is often used, primarily focusing on deep white matter lesions, resulting in a score from 0 (no deep WMLs) to 3 (confluent deep WMLs), with a score of ≥2 considered as the presence of significant WMLs (**Figure 3**). The Fazekas scale was used in eleven studies, in a total of 405 patients with FD of which seven studies used the modified scale (n= 212; **Table 3**).

The corrected mean Fazekas score was 0.76 (n= 185, range: 0.53-1.90; **Figure 4**) at a corrected mean age of 42.1 years (range: 35.8-46.0). A score of ≥2 was found in 16.4% of the patients (31 out of 189 patients). Equal percentages were found in men and women, respectively 16.9% (11 out of 65 men) and 16.1% (18 out of 112 women), ($\chi^2(1)=0.0$, p=1.0) at a corrected mean age of 35.8 years for men and 43.6 years for women. Importantly, when comparing uncorrected mean age men were younger at the time of assessment compared to women (U=1, p=0.016).

Size and number of white matter lesions

Seven studies reported lesions number and/or size [3, 14, 17, 19, 21, 39, 44]. The three studies measuring WML size used different methods, complicating comparability (**Supplemental Table B**). Methods varied from measuring total length of all lesions to WML diameter per lesion normalized for head size. Five studies reported the number of lesions, with lesion counts ranging from a single lesion to >10 lesions (**Supplemental Table B**).

White matter lesion volume

Six studies (on four different cohorts) reported total WML volume [5, 20, 27, 40-42], in 279 patients with a corrected mean age of 39.5 years (range 36.5-46.0 years) (**Table 4**). In the largest cohort, a mixed group of 223 patients, men and women showed comparable mean WML volumes (4.7 ml in men, 4.9 ml in women) [5]. Again, in this study men were significantly younger compared to women.

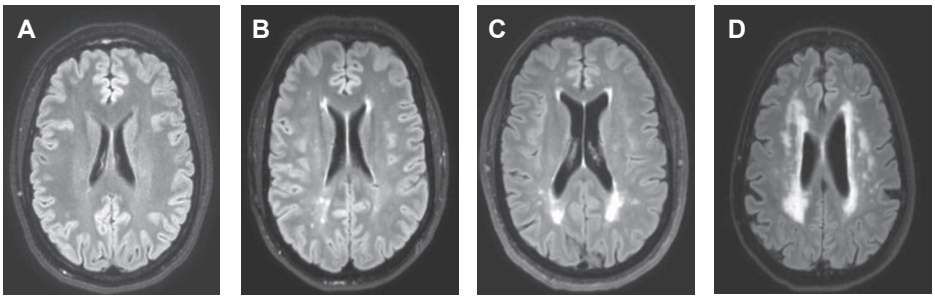

Figure 3 Modified Fazekas scale in Dutch patients with Fabry disease
A Fazekas score 0, B Fazekas score 1, C Fazekas score 2, D Fazekas score 3

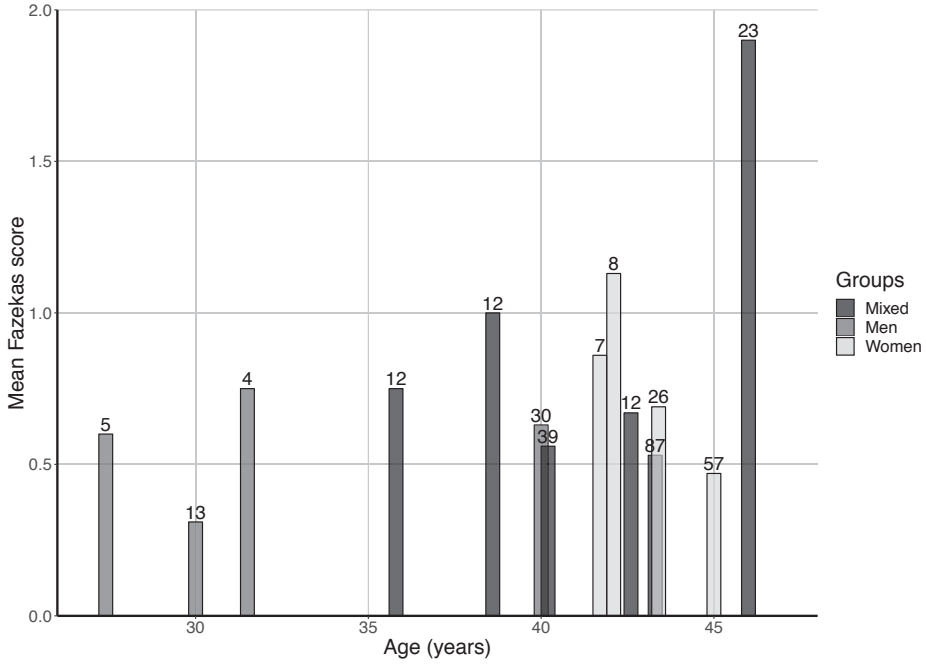

Figure 4 Mean Fazekas score per study in relation to age. Whole study cohorts are labeled as "Mixed" and if available as sex divided subgroups. Number of patients is displayed at the top of each bar

Table 3 Fazekas score per study

First author, groups (sex)	Patients per study, n (men)	Age (years), median or mean ±SD (range)	Faz score, mean	Faz 0	Faz 1	Faz 2	Faz 3	Faz 4	Faz 5	Faz 6
Fazekas (0-3), modified										
Paavilianen et al [28], All	12 (4)	38.6±17.8 (16-68)	1.00	5 (41.7%)	3 (25.0%)	3 (25.0%)	1 (8.3%)	-	-	-
Men	4	31.6±16.1 (17-54)	0.75	2 (50.0%)	1 (25.0%)	1 (25.0%)	0 (0.0%)	-	-	-
Women	8	42.1±18.6 (16-68)	1.13	3 (37.5%)	2 (25.0%)	2 (25.0%)	1 (12.5%)	-	-	-
Üçeyler et al [31], All	87 (30)	43.3 (16-73)	0.53	47 (54.0%)	35 (40.2%)	4 (4.6%)	1 (1.1%)	-	-	-
Men	30	40.0 (16-40[$])	0.63	15 (50.0%)	12 (40.0%)	2 (6.7%)	1 (3.3%)	-	-	-
Women	57	45.0 (16-73)	0.47	32 (56.1%)	23 (40.4%)	2 (3.5%)	0 (0.0%)	-	-	-
Korsholm et al [32], All	39 (13)	40.2±14.7 (10-66)	0.56	27 (69.2%)	5 (12.8%)	7 (17.9%)	1 (2.6%)	-	-	-
Men	13	30.0±10.6 (10-47)	0.31	11 (84.6%)	0 (0.0%)	2 (15.4%)	0 (0.0%)	-	-	-
Women	26	43.4±13.9 (15-66)	0.69	15 (57.7%)	5 (19.2%)	5 (19.2%)	1 (3.8%)	-	-	-
Azevedo et al [37], All	12 (5)	35.8±12.8	0.75	8 (66.7%)	1 (8.3%)	1 (8.3%)	2 (16.7%)	-	-	-
Men	5	27.4±11.5	0.60	4 (80.0%)	0 (0.0%)	0 (0.0%)	1 (20.0%)	-	-	-
Women	7	41.7±10.6	0.86	4 (57.1%)	1 (14.3%)	1 (14.3%)	1 (14.3%)	-	-	-
Fellgiebel et al [4], All	27 (13)	38.1 (12-69)	-	12 (44.4%)	6 (22.2%)	9 (33.3%)	*	-	-	-
Men	13	36.3±9.9 (12-51)	-	7 (53.8%)	2 (15.4%)	4 (30.8%)	*	-	-	-
Women	14	39.7±13.5 (19-69)	-	5 (35.7%)	4 (28.6%)	5 (35.7%)	*	-	-	-
Lee et al [34], All (Mixed)	12 (4)	42.6±14.3 (18-61)	0.67	6 (50.0%)	4 (33.3%)	2 (16.7%)	0 (0.0%)	-	-	-
Duning et al [27], All (Mixed)	23 (12)	46.0 (29-61)	1.9	-	-	-	-	-	-	-

Table 3 Fazekas score per study (continued)

First author, groups (sex)	Patients per study, n (men)	Age (years), median or mean ±SD (range)	Faz score, mean	Faz 0	Faz 1	Faz 2	Faz 3	Faz 4	Faz 5	Faz 6
Fazekas (0-6), original										
Cocozza et al [36], All (Mixed)	32 (12)	43.3±12.2 (20-68)	0.66	18 (56.3%)	11 (34.4%)	2 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (3.1%)
Gavazzi et al [13], All	16 (8)	38.8±13.9 (17-58)	2.06	1 (6.3%)	8 (50.0%)	3 (18.8%)	0 (0.0%)	2 (12.5%)	1 (6.3%)	1 (6.3%)
Men	8	34.7±10.0 (22-58)	2.13	0 (0.0%)	4 (50.0%)	2 (25.0%)	0 (0.0%)	1 (12.5%)	1 (12.5%)	0 (0.0%)
Women	8	42.8±16.6 (17-58)	2.00	1 (12.5%)	4 (50.0%)	1 (12.5%)	0 (0.0%)	1 (12.5%)	0 (0.0%)	1 (12.5%)
Buechner et al [23], All	41 (25)	45.6 (19-74)	-	12 (29.3%)	6 (14.6%)	23 (56.1%)	#	#	#	#
Men	25	41.9±10.8 (21-62)	-	9 (36.0%)	3 (12.0%)	13 (52.0%)	#	#	#	#
Women	16	51.2±18.0 (19-74)	-	3 (18.8%)	3 (18.8%)	10 (62.5%)	#	#	#	#
Cocozza et al [35], All (Mixed)	104 (40)	43.0±13.4 (13-72)	2.3	-	-	-	-	-	-	-

** Fazekas score 2 and 3 were grouped, # Fazekas 2-6 were grouped, $Adopted from the original article. However, it was considered unlikely that median and maximum age were both 40 years. Faz = Fazekas, - = Not available*

Table 4 White matter lesion volume per study

First author, group (Sex)	Patients per study, n (men)	Age (years), median/mean	Volume WMLs (ml), mean	Volume WMLs (ml), range
Marino et al [20], All	8 (4)	40.0	0.0	0.9-18.2
Men	4	31.0	0.5	0.9-3.5
Women	4	50.0	0.0	18.2
Rost et al [5], All	223 (91)	39.2	4.7	0.3-61.2
Men	91	34.7	4.7	-
Women	132	42.3	4.9	-
Duning et al [27], All (Mixed)	23 (12)	46.0	3.0	-
Fellgiebel et al [40, 42], All (Mixed)	25 (10)	36.5	2.0	0-24.1
Lelieveld et al [41]*, All (Mixed)	14 (4)	46.1	1.0	0-2.8

** Provided eight-year follow-up data on 14 patients from the earlier studies by Fellgiebel et al [40, 42]. ml = milliliter, - = Not available*

Location

Twelve studies reported the location of WMLs [3, 14, 18, 19, 22, 26, 27, 35-37, 39, 44]. WML locations were classified as periventricular, deep and subcortical or by specific anatomical or circulatory area.

Periventricular, deep and subcortical WMLs

In eight publications the location of WMLs was described as periventricular, deep and/or subcortical [3, 14, 18, 19, 22, 36, 37, 44], two of which report on the same cohort during one and two year follow-up [19, 44]. Periventricular WMLs were found in all eight articles [3, 14, 18, 19, 22, 36, 37, 44], with periventricular involvement ranging from 21% [36] to 100% [22] of included patients. Deep WMLs were found in seven studies [3, 14, 18, 19, 36, 37, 44], with deep white matter involvement ranging from 25% [19] to 100% [36] of included patients. Subcortical lesions were described in two cohorts [18, 44].

Specific anatomical and circulatory areas

Seven studies located WMLs according to anatomical areas and two studies according to circulatory areas (**Supplemental Table C**). White matter involvement occurred in the anterior, middle as well as the posterior circulation, and WMLs were mostly found in the frontal, parietal and temporal lobes. The brainstem, cerebellum and grey matter were less frequently affected.

Since some patients with FD are initially misdiagnosed as having multiple sclerosis one study used corpus callosum involvement to differentiate between 117 multiple sclerosis patients and a mixed cohort of 104 FD patients [35]. Only 3% of FD patients had WMLs in

the corpus callosum compared to 90% of the multiple sclerosis patients. This finding may assist in the differential diagnosis of these disorders.

Course

Eleven studies assessed the course of WMLs over time [3, 12, 14, 16, 23, 26, 29, 30, 32, 41, 44] either by assessment of a radiologist or by quantitative measurement using volume or diameter.

Assessment by radiologist

In ten studies WMLs were reported as progressive or stable, in a total of 241 patients (corrected mean baseline age: 37.4 years, range: 11.8-46.1) (**Table 5**).

A total of 24.6% of patients showed progression of WMLs (49 out of 199 patients) and 75.4% of patients had stable WMLs (150 out of 199 patients) over a corrected mean follow-up time of 38.1 months (range: 6-96 months). A higher percentage of patients on ERT showed progression versus untreated patients (21.6%, 30 out of 139 patients versus 13.9%, 5 out of 36 patients respectively), most likely because untreated patients had milder disease. Men and women showed comparable rates of progression (20.7% of men over a corrected mean follow-up time of 24.9 months (17 out of 82 men, corrected mean baseline age: 34.5 years) versus 23.1% of women over a corrected mean follow-up time of 46.1 months (15 out of 65 women, corrected mean baseline age: 42.1 years)). Again men were significantly younger compared to women when comparing uncorrected baseline age (U=0, p=0.004). Uncorrected mean follow-up time was not different between men and women (U=10, p=0.41).

Quantitatively measured course

Two studies assessed WMLs quantitatively over time [14, 41]. In one study, a mixed cohort of 14 patients (4 men, 10 on ERT), significant progression of the median white matter lesion load (WMLL) from 0.12 ml to 1.03 ml was found [41]. The second study, a post-hoc analysis of a RCT comparing agalsidase-beta (Fabrazyme; Genzyme Corp., Cambridge, Massachusetts, USA) showed significant progression of WML diameter in both treated and untreated patients (n=41, 38 men, 25 treated, mean follow-up time 27 months) [14].

Table 5 Radiological assessment of white matter lesion course per study

First author, groups (Sex)	Patients in study, n (men)	Patients in follow-up, n	Age at first MRI (years), median/mean	Patients on ERT, n	Follow-up time (months), mean	Progression, n (n no ERT, n ERT)		Stable, n (n no ERT, n ERT)	
Buechner et al [23], All	41 (25)	24 (13)	41.7	15	30 m	9	(4 no ERT, 5 ERT)	15	(5 no ERT, 10 ERT)
Men	25	13	37.2	10	17 m	4	(1 no ERT, 3 ERT)	9	(2 no ERT, 7 ERT)
Women	16	11	47.1	5	44 m	5	(3 no ERT, 2 ERT)	6	(3 no ERT, 3 ERT)
Ortu et al [12], All	11 (4)	9 (2)	40.8	9	12 m	0	(0 no ERT, 0 ERT)	9	(0 no ERT, 9 ERT)
Men	4	2	26.5	2	12 m	0	(0 no ERT, 0 ERT)	2	(0 no ERT, 2 ERT)
Women	7	7	44.9	7	12 m	0	(0 no ERT, 0 ERT)	7	(0 no ERT, 7 ERT)
Korsholm et al [32], All	40 (14)	34 (12)	39.2	29	47 m	3	(0 no ERT, 3 ERT)	31	(5 no ERT, 26 ERT)
Men	14	12	29.5	11	52 m	0	(0 no ERT, 0 ERT)	12	(1 no ERT, 11 ERT)
Women	26	22	44.5	18	45 m	3	(0 no ERT, 3 ERT)	19	(4 no ERT, 15 ERT)
Rombach et al [29], All	63 (32)	56 (27)	39.4	56	-	21	(0 no ERT, 21 ERT)	35	(0 no ERT, 35 ERT)
Men	30	25	37.3	25	37 m	12	(0 no ERT, 12 ERT)	13	(0 no ERT, 13 ERT)
Women	27	25	46.6	25	48 m	7	(0 no ERT, 7 ERT)	18	(0 no ERT, 18 ERT)
Pediatric (Mixed)	6 (2)	6 (2)	16.6	6	-	2	(0 no ERT, 2 ERT)	4	(0 no ERT, 4 ERT)
Jardim et al [44], All	8 (7)	6 (5)	35.0	6	24 m	1	(0 no ERT, 1 ERT)	3	(0 no ERT, 3 ERT)[$]
Men	7	5	32.8	5	24 m	1	(0 no ERT, 1 ERT)	3	(0 no ERT, 3 ERT)[$]
Woman	1	1	46.0	1	24 m	0	(0 no ERT, 0 ERT)	0	(0 no ERT, 0 ERT)[$]
Reisin et al [26], All (Mixed)	46 (18)	22 (? + 3)	-	-	-	5	(1 no ERT, 4 ERT)	17	(17 unknown)
Pediatric (Mixed)	10 (3)	10 (3)	11.8	1	-	1	(0 no ERT, 1 ERT)	9	(9 no ERT, 0 ERT)
Borgwardt et al [30], Pediatric (Mixed)	10 (6)	10 (6)	12.3	10	-	0	(0 no ERT, 0 ERT)	10	(0 no ERT, 10 ERT)
Lelieveld et al [41], All (Mixed)	25 (10)	14 (4)	46.1	10	96 m	10	(10 unknown)	4	(4 unknown)
Crutchfield et al [3], Men	52 (52)	40 (40)	-	0	-	4 + ?*	(4 + ? no ERT, 0 ERT)	9 + ?*	(9 + ? no ERT, 0 ERT)
Moore et al [16], Men	26 (26)	26 (26)	33.7	14	6 m	0	(0 no ERT, 0 ERT)	26	(12 no ERT, 14 ERT)[#]

*$ Description in study: "MRI was stable in 3 (normal in 2 and showing the same lesions in the other). WML worsened in patient 1, fluctuated in patient 2 and, surprisingly, disappeared in patient 6." The patients with fluctuating and disappearing WMLs were removed from the analysis. * Description in study: "Patients who had lesions and who were studied multiple times usually had increased lesion load on subsequent testing." This study was excluded from further analyses, # Description in study: "There was no significant progression of the lesion burden over the 6-month trial period." Patients were classified as being stable.*

- = Not available, m = months, unknown = ERT status unknown

Relation of white matter lesions to cerebral parameters

To gain more insight in the pathophysiology of WML development in FD, 18 studies assessed the relation between WMLs and other cerebral parameters (**Supplemental Table D**).

Brain metabolism, cerebral blood flow and diffusion of water molecules

Twelve studies used imaging techniques to quantify water molecule diffusivity, brain metabolism and cerebral blood flow (CBF) (**Supplemental Table D**).

Hypometabolic areas were found in correspondence with infarctions and hemorrhages, but also in deep and periventricular white matter of patients with and without WMLs, compared to controls. N-acetylaspartate, a nervous system-specific metabolite of which decreases have been linked to neuronal damage [63], was found to be decreased in areas with WMLs. In some patients, these areas extended into the normal appearing white matter (NAWM). Moreover, regions of increased CBF showed a similar pattern, extending from areas with WMLs into the NAWM. It was therefore hypothesized that microstructural alterations happen in areas with WMLs but also in adjacent areas with NAWM.

Diffusion weighted imaging (DWI), an imaging technique using random motion of water molecules, was performed in six studies in five different cohorts, in a total of 111 patients (corrected mean age: 41.9 years, range: 38.1-46.0, 58 men) (**Supplemental Table D**). Motion of water molecules can be influenced by changes in structural organization, permeability and cellularity of brain tissue [64]. Derivative variables are mean diffusivity (MD), the average random molecular diffusion rate and fractional anisotropy (FA), as well as the preferred direction of diffusion [65]. Increased MD and decreased FA can be the result of cell damage (increasing random diffusion, and thereby MD) and decreased fiber integrity (decreasing anisotropy and thereby FA) [66]. Increased MD was found in men and women with FD compared to controls, especially in the temporal, frontal and parietal white matter. Three studies showed reduced FA in men and women with FD compared to controls, with no clear regional preference. In contrast, two studies assessing the same cohort, did not find reduction of FA compared to controls.

In five out of six studies increased MD and/or decreased FA were found both in areas with WMLs and in NAWM [27, 28, 45, 46, 48]. One of these reported a positive relation between MD and the WMLL, and a negative relation between FA and the WMLL [48].

Other cerebral parameters

In FD patients with microbleeds WMLs were more often present, compared to patients without microbleeds (**Supplemental Table D**). No strong relation was found between

the diameter of large intracranial arteries and WMLs. Moreover, studies reported no relation between WMLL and hippocampal volume and atrophy, white and grey matter volume, functional connectivity of the motor cortex and between presence of WMLs and increased motor cortex excitability. In one study an abnormal pattern of brain activation was found during a simple motor task (finger tapping) in 16 FD patients compared to healthy control subjects [13]. While no relation was established between the WMLL and motor functions, the sensorimotor cortex activation contralateral of the tapping fingers correlated with the WMLL.

Relation of white matter lesions to patient characteristics and clinical parameters

Twenty-four studies assessed the relation of WMLs to patient characteristics and clinical parameters [1, 3-5, 14, 17, 19, 21, 22, 25-27, 29, 31, 32, 40, 41, 44, 48-53].

White matter lesions, TIA and/or stroke

Two follow-up studies reported that WML severity at baseline was related to progression of WML severity during follow-up, both in treated and untreated patients [14, 41].

The majority of seven studies [3, 5, 14, 31, 32, 41, 48] supported a positive relation between WMLs and TIA and/or stroke: a higher prevalence of TIA and/or stroke in patients with WMLs was found [3, 32], while a follow-up study reported that patients with a history of stroke had more WMLs at baseline and developed more WMLs [14]. A large mixed cohort study on 223 patients (91 men) showed that stroke was related to the WMLL in a multivariate model but TIA was not [5]. No relation between TIA and/or stroke and WMLs was reported in three articles [31, 41, 48], but the largest (n=87) did not formally test the relation [31] and the two other studies were relatively small.

Age, sex and phenotype

Six studies reported that patients with WMLs are significantly older compared to patients without WMLs [4, 17, 22, 26, 29, 50] and twelve studies showed a positive relation between age and presence of WMLs or WMLL [1, 3, 5, 14, 21, 25, 40, 41, 44, 48, 52, 53].

The four studies that assessed the relation between sex and WMLs did not find differences between men and women in the presence of WMLs or WMLL [4, 5, 21, 53]. However, in the largest study men were significantly younger compared to women, while WMLL was comparable [5].

Only one study, a mixed cohort including 283 patients with MRI of the brain, assessed subgroups divided by both sex and phenotype [1]. Men as well as women with classical

disease were more likely to have WMLs compared to men with non-classical disease. MRIs of 42 pediatric patients were also assessed and all four pediatric patients with WMLs (3 boys, 1 girl) had classical FD.

Cerebrovascular risk factors

Five studies assessed the relation between cerebrovascular risk factors and WMLs [3-5, 26, 52]. No relation was found between the presence of WMLs and cholesterol-level [52], hypertension [3, 52] or smoking [3] or between WMLL and smoking [5]. In a fourth study, a mixed cohort with 27 patients (13 men), there were no differences in WMLL between men and women and no differences in history of hypertension, serum LDL-cholesterol, smoking or APOE-4 frequency [4]. In the last study, a mixed cohort with 10 pediatric and 36 adult patients showed that patients with WMLs had more vascular risk factors (defined as a history of hypertension, dyslipidemia, diabetes and/or smoking), compared to patients without WMLs [26].

Renal and cardiac involvement and the Mainz Severity Score Index

Seven studies assessed the relation between renal involvement and WMLs [3, 5, 14, 26, 31, 41, 48]. No relation was found between WMLL and renal function [14, 41], WMLL or presence of WMLs and renal dysfunction/complications [3, 5, 26, 31, 48] or WMLL and decreasing estimated glomerular filtration rate during follow-up [14].

Five studies assessed the relation between cardiac involvement and WMLs [5, 14, 26, 41, 48]. No relation was found between the WMLL and cardiovascular dysfunction [48], WMLL or presence of WMLs and hypertrophic cardiomyopathy [5, 26, 41] or WMLL and arrhythmias [5, 41]. One follow-up study reported a relation between the left ventricular posterior wall thickness and WMLL at baseline and during follow-up [14].

Of two studies reporting on the Mainz Severity Score Index (MSSI) in relation to WMLs, one article found no significant differences between patients with WMLs and patients without WMLs on MSSI-scores [26]. The second study found that WMLL and MSSI-scores were moderately related [50].

Relations to other clinical parameters and patient characteristics

Ten studies assessed the relation between other clinical parameters, patient characteristics and WMLs (**Supplemental Table E**). No relation between WMLL and neuropsychological test scores or depression frequency and severity was found. However, during follow-up a relation was found between increased WMLL and decreased performance on an executive task.

The presence of WMLs was not related to residual enzyme activity, nor to the presence of antibodies against recombinant agalsidase A. On the other hand, high lysoGb3 level, a biomarker in FD, was related to an increased risk of developing WMLs in men. Subsequently, reduction in lysoGb3 and Gb3 predicted a decreased risk of WML development in the first year of treatment in both men and women with FD. Finally, some genetic polymorphisms related to cerebral ischemia in the general population were related to presence of WMLs in FD while others were not.

Discussion

WMLs are present in almost half of the patients with FD and their prevalence increases with age. In the majority of FD patients a mild WMLL is found (Fazekas 0-1), but 16% of FD patients have a substantial WMLL with (beginning) confluent WMLs (Fazekas ≥2). In the general population WMLs are commonly found, especially in the elderly [67]. However, the prevalence and severity of WMLs reported in FD corresponds to that found in individuals in the general population that are at least one to three decades older [67, 68].

When combining all studies, prevalence, severity and progression of WMLs are comparable in men and women. However, men were significantly younger compared to women at time of the WML assessment (approximately 6-7 years), supporting the concept that men are more severely affected than women, since WMLs tend to progress over time. Perhaps an even larger difference exist as most studies in the general population point to an increased risk of WMLs in women [68]. It is likely that phenotype plays a role as well: classically affected males have faster disease progression than classical females or non-classical patients. Most studies did not take phenotype into consideration, but a single report dividing patients by sex and phenotype suggests that indeed phenotype does play a role in the risk of WML development in FD [1].

To establish their role as a biomarker of cerebral involvement in FD, WMLs should be linked to clinically relevant endpoints. In the general population WMLs are related to stroke, cognitive dysfunction and mortality [69]. We found that, despite not uniformly shown in every study, WMLs and WMLL are most probably related to the occurrence of stroke in FD. Of importance is whether there is a link between high WMLL and cognitive dysfunction. This is currently being investigated at our center. In contrast, renal and cardiac disease were not found to be related to the presence and severity of WMLs. Conversely, decreased renal function is related to increased WMLL in the general population [70]. It is possible that men with classical FD (who are prone to develop kidney failure) have a higher WMLL, since studies did not incorporate phenotype in

their analyses and studies lacked power to detect this relation. Of clinical importance is the fact that two studies found no relation between arrhythmias and WMLs, because supraventricular rhythm disturbances, in particular atrial fibrillation, are a risk factor for ischemic stroke.

No specific location and distribution of WMLs can be established in FD: the frontoparietal and temporal white matter are most often affected and both the periventricular and deep white matter are involved. Despite early reports that the posterior circulatory areas are most severely affected in FD, this was not confirmed by analysis of the location and distribution. A major feature of posterior circulation alterations in FD, an increased basilar artery diameter, was also not related to WML development in FD. It is expected in FD that WMLs originate from pathological changes of the small cerebral vessels, and that large vessel abnormalities might play little to no role in the origin of WMLs. In the general population both periventricular and deep WMLs have been attributed to small vessels pathology, but with regional differences in pathology [71, 72].

Follow-up of WML development was performed in a minority of studies, despite the possible role of WMLs as biomarker for cerebral involvement in FD. We found that a quarter of patients show progression of WMLs during three years follow-up irrespective of sex and treatment status. Evaluating the effect of ERT on WMLs is unreliable due to four points: 1) most follow-up cohorts are subgroups of bigger cohort studies and no background characteristics are provided for these subgroups, 2) there is a treatment bias, with more severely affected patients being treated earlier, 3) there are very little follow-up data of untreated patients, 4) most RCTs comparing ERT to a placebo did not incorporate WML development. In a post-hoc analysis of a RCT comparing agalsidase-beta to a placebo, significant progression of WML diameter was seen, comparable in the treated versus untreated group [14]. Most of these patients were male, with advanced FD. In patients under 50 years old less progression of WML diameter and a more stable WMLL was found in the agalsidase-beta group compared to the placebo group. However, the subgroup included only 38% of the original patient cohort and the analysis of this subgroup was not predefined. A consensus document on treatment in FD stated that ERT "may be considered" for the treatment of WMLs (evidence class IIB) [73], a conclusion that cannot be changed after this systematic review. Unfortunately, none of the future, ongoing or recently published trials mention cerebral MRIs or WMLs as their primary or secondary outcome on ClinicalTrial.gov, except for a German observational prospective cohort study of patients treated with chaperone therapy [74].

In the general population, hypertension is considered the biggest risk factor for WML development, next to age [75]. Surprisingly, only one of five articles reported a

relation between cerebrovascular risk factors and WMLs in FD. Since the frequency of hypertension is similar to the general population (Korver et al, unpublished analysis based upon Arends [76]), the WMLs we see in the majority of younger patients with FD probably have a different pathological origin. Naturally, vascular risk factors are still very important to address in patients with FD, especially as with treatment and supportive care patients become older.

The pathophysiology of WML in FD is probably complex. Glycosphingolipid accumulation in the smooth muscle cell of the vessel wall may lead to a less compliant vascular wall due to fibrosis and impairment of autoregulation of cerebral perfusion [66, 77]. The shear stress due to a hyperdynamic circulation and an incompliant vessel wall might then lead to endothelial dysfunction. Combined with storage in the endothelium, especially prevalent in classical male patients, increases in reactive oxygen species and pro-thrombotic/pro-inflammatory cytokines as well as upregulation of the renin–angiotensin system are the result [77, 78]. Changes in regional metabolism, cerebral blood flow, MD and FA were found beyond the borders of the WMLs. As was previously hypothesized [66], this might indicate a pathological continuum extending into the NAWM, compatible with findings in the general population [75, 79]. In addition to the described vascular/perfusion pathology, non-ischemic contributors to WML development that have been found in the general population are glial dysfunction, neuro-inflammation, blood-brain barrier disruption and genetic predisposition and these factors may also contribute to WML development in FD [79]. Post mortem studies in FD have shown widespread glycosphingolipid accumulation in the brain itself [80-82]. Swelling of neurons, axons and glial cells was also noted [81, 82]. One post mortem study in a man with progressive cognitive complaints and severe WMLs on MRI showed astrocytic swelling and increased astrocytic processes, indicating glial dysfunction in FD [82]. Moreover, increased protein levels have been found in the cerebral spinal fluid of FD patients that were described as having aseptic meningitis and in patients misdiagnosed as having multiple sclerosis [83, 84]. This could indicate blood-brain barrier dysfunction. In the Fabry mouse a disruption of the autophagy-lysosome pathway has been described in the brain, possibly adding to axonal pathology [85]. A study by Altarescu et al. [52] showed that a number of genetic polymorphisms contributed to the risk of WML development in FD patients. Pathology studies in cell or mouse models and brain tissue samples obtained post mortem could further explore potential pathways resulting in WML development in FD.

Some included studies extensively described the definition and criteria to determine WMLs, while most did not. It might therefore be possible that some lacunar infarctions were classified as WMLs. We did not exclude studies with limited description of WML methods since: 1) we would be left with a small group of studies, 2) we expect that WMLs

and lacunar infarctions might represent a spectrum of cerebral disease and have similar pathology in FD and 3) the differentiation between WMLs and lacunar infarctions is difficult, even with extensive definitions [86]. However, we do encourage future studies to provide well defined criteria for WMLs.

In this study we systematically reviewed WMLs in FD. It is clear that many unresolved questions remain, which have been summarized in **Table 6** including proposals for future research directions.

Conclusions

A significant group of FD patients has confluent WMLs and a substantial WMLL, which progresses over time. As expected, men with FD start developing WMLs earlier compared to women and patients with classical disease are more severely affected compared to non-classical patients. WMLs seem to be related to stroke, represent cerebral small vessel dysfunction but systematic studies fail to address influence of treatment as well as important clinical outcomes such as cognitive function. Traditional cerebrovascular risk factors probably have a minor effect on development of WMLs in patients. Future studies should focus on longitudinal follow-up using modern imaging techniques, with emphasis on the clinical consequences of WMLs and use of WMLs in treatment trials. Last but not least, ischemic and non-ischemic pathways resulting in WML development should be studied.

Table 6 Main findings and future research directions on WMLs in FD

Topic	Main findings	Future research directions
Prevalence	· WMLs were present in 46% of patients with FD at 39 years of age and their number increased with age · Men with FD develop WMLs at a younger age compared to women with FD · Patients with classical FD appear to have a higher risk of WML development compared to patients with non-classical FD	· Strengthen the findings on the relations between phenotype, sex of FD patients and prevalence of WMLs · Correct for age when comparing subgroups of FD patients with WMLs · Avoid using only “presence” or “absence” of WMLs, since this provides minimal information and decreases power to detect risk factors

Table 6 Main findings and future research directions on WMLs in FD (continued)

Topic	Main findings	Future research directions
Severity	· 16% of patients with FD had a substantial amount of WMLs · Men with FD develop a higher WMLL at a younger age compared to women with FD	· Further explore the relation between phenotype and WML severity · Use well established scales or volumetric measurements of WMLL to assess WML severity
Location	· No clear pathognomonic location or distribution of WMLs in FD was found · WMLs were present in both the periventricular and deep white matter	· Include WML location and distribution since local differences in underlying pathology and consequences of WMLs might be present
Course	· WMLs progressed in 1/4th of patients with FD over three years follow-up · WMLs progressed in both men and women, with and without ERT · Men with FD show progression of WMLs at a younger age compared to women	· Report patient characteristics of (sub)groups in longitudinal studies · Report on the course of WMLs in untreated patients · Include WMLs as outcome parameters in trials evaluating (new) treatments · Use quantifiable methods of WML assessment
Clinical relations and consequences: brain parameters	· There might be changes in metabolism and CBF in areas with WMLs extending into the NAWM · Loss of cellular integrity and/or increased interstitial water content seem to be present, even in FD patients without WMLs · WMLs seemed to be related to stroke	· Explore whether changes in NAWM precede WML formation using longitudinal follow-up combining structural MRI modern imaging techniques · Confirm the relation between stroke (subtypes) and WML (severity) in longitudinal studies · Study the relationship between the presence of WMLs and clinical consequences (e.g. cognitive functioning) · Study model organisms to explore pathways resulting in WML development in FD
Clinical relations and consequences: other	· Renal and cardiac (dys)function did not seem to be (strongly) related to the amount of WMLs in FD · Cerebrovascular risk factors did not seem to be (strongly) related to the amount of WMLs in FD	· Identify factors contributing to risk of WML development in FD

WMLs = white matter lesions, WMLL = white matter lesion load, ERT = enzyme replacement therapy, CBF = cerebral blood flow, NAWM = normal appearing white matter

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Supplemental file A

Search Pubmed:

Cerebral/brain involvement:

((((((((((involvement[tiab]) OR structural alteration*[tiab]) OR microstructur*[tiab]) OR structural chang*[tiab]) OR micro-structur*[tiab])) AND ((brain[tiab]) OR Cerebral[tiab])))

OR

Cerebral pathology/diagnostics:

((“White Matter”[Mesh]) OR “Leukoencephalopathies”[Mesh:noexp]) OR “Ultrasonography, Doppler, Transcranial”[Mesh]) OR “Basilar Artery”[Mesh]) OR “pulvinar”[Mesh]) OR White Matter[tiab]) OR WML[tiab]) OR WMH[tiab]) OR Leukoencephalopath*[tiab]) OR Leuko-encephalopath*[tiab]) OR leukoaraios*[tiab]) OR leuko-araios*[tiab]) OR pulvinar sign*[tiab]) OR cerebral MRI*[tiab]) OR brain MRI*[tiab]) OR brain pathol*[tiab]) OR cerebral chan*[tiab]) OR brain chan*[tiab]) OR brain alter*[tiab]) OR cerebral alter*[tiab]) OR brain manifestat*[tiab]) OR cerebral manifestat*[tiab]) OR basilar artery[tiab]) OR pulvinar[tiab]) OR cerebral vasculopathy[tiab]) OR microbleed*[tiab]) OR fazekas[tiab]) OR cerebral blood flow[tiab]) OR “Cerebrovascular Circulation”[Mesh]) OR Cerebrovascular Circulation[tiab]) OR cerebrovascular complication*[tiab]) OR brain volume*[tiab]) OR cerebral volume*[tiab]) OR CNS involvement[tiab]) OR MRI lesion*[tiab]) OR cerebrovascular manifestation*[tiab]) OR intracranial abnormalit*[tiab]) OR transcranial doppler[tiab]) OR brain angiography[tiab])))

AND

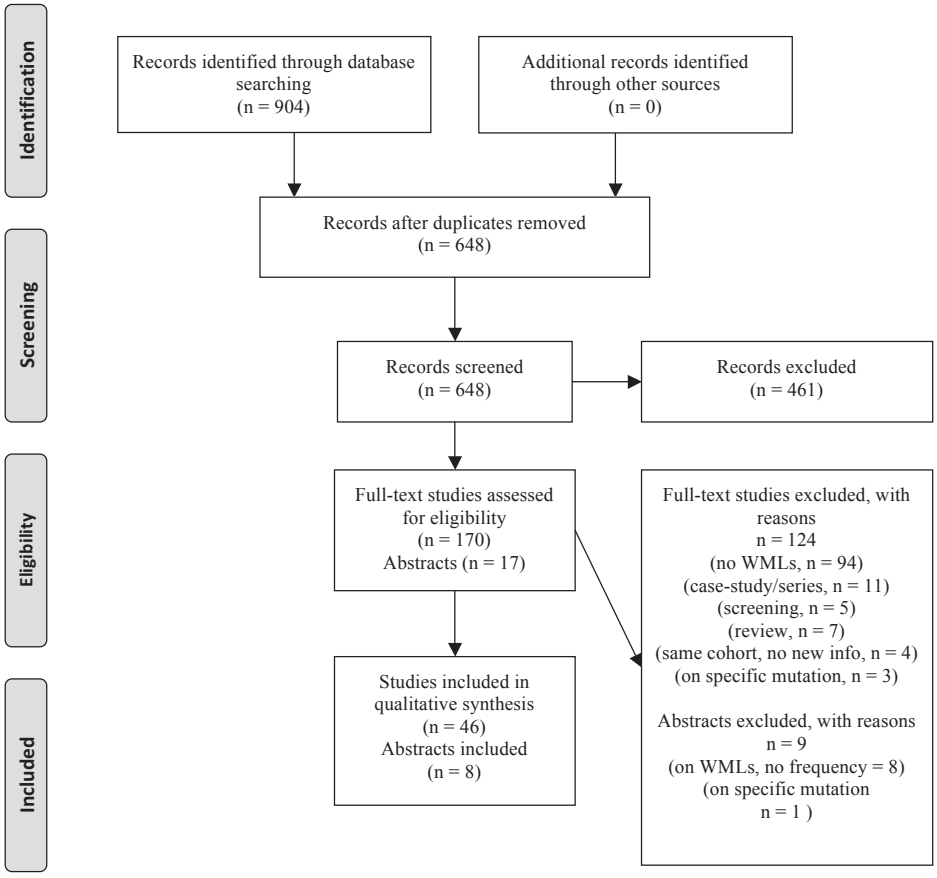
Fabry disease

((((((“Fabry Disease”[Mesh]) OR Fabry*[tiab]) OR alpha galactosidase a deficien*[tiab]) OR ((“alfa galactosidase a”[tiab] AND deficien*[tiab]))) OR GLA-deficien*[tiab]) OR angiokeratoma corporis diffusum[tiab])

CINAHL and EMBASE:

Abovementioned search terms were also used for the CINAHL and EMBASE databases. Searches were tweaked for specific search characteristics per database.

Supplemental file B



PRISMA 2009 Flow Diagram

Supplemental Table A

Supplemental Table 1 Study information in alphabetic order

Author, group (Sex)	Year	Patients, n (men)	Age (years), median or mean ±SD (range)	ERT, n	TIA and stroke, n	Tesla	Sequence	WML method	Study design
Albrecht et al [1], All	2007	25 (10)	36.4±11.5 (19-55)	20	5	1.5T	FLAIR	WMLL (-/±/+)	Pros, Cross
Men	2007	10	36.1±12.0	-	-	1.5T	FLAIR	WMLL (-/±/+)	Pros, Cross
Women	2007	15	36.7±11.5	-	-	1.5T	FLAIR	WMLL (-/±/+)	Pros, Cross
Altarescu et al [2], All (Men)	2005	57 (57)	36.0±12.0 (12-64)	-	8	-	FLAIR	WMLs (presence)	Pros, Cross
Arends et al [3], All (Mixed)	2016	283 (?)	-	-	-	-	-	WMLs (presence)	Retro, Long
Pediatric (Mixed)	2016	42 (20)	16.1 (5-18)	0	-	-	-	WMLs (presence)	Retro, Long
Azevedo et al [4], All	2012	12 (5)	35.8±12.8	8	0	1.5T	T2/PD	WMLL (Fazekas)	Pros, Cross
Men	2012	5	27.4±11.5	5	0	1.5T	T2/PD	WMLL (Fazekas)	Pros, Cross
Women	2012	7	41.7±10.6	3	0	1.5T	T2/PD	WMLL (Fazekas)	Pros, Cross
Barbey et al [5] , All	2015	6 (1)	79.3±4.8 (75-87)	3	2	-	-	WMLs (presence)	Retro, Cross
Man	2015	1	75.0	1	0	-	-	WMLs (presence)	Retro, Cross
Women	2015	5	80.2±4.8 (75-87)	2	2	-	-	WMLs (presence)	Retro, Cross
Borgwardt et al [6], Pediatric (Mixed)	2013	10 (6)	12.3±2.5 (9-16)	10	-	-	-	WMLs (presence)	Retro, Long
Buechner et al [7], All	2008	41 (25)	45.6 (19-74)	24	19	-	T2/FLAIR	WMLL (Fazekas)	Retro, Long
Men	2008	25	41.9±10.8 (21-62)	17	11	-	T2/FLAIR	WMLL (Fazekas)	Retro, Long
Women	2008	16	51.2±18.0 (19-74)	7	8	-	T2/FLAIR	WMLL (Fazekas)	Retro, Long
Burlina et al [8], All	2008	36 (16)	41.6±14.4 (18-72)	26	7	1T/1.5T	T2/FLAIR	WMLs (presence)	Pros, Long
Men	2008	16	38.9±9.3 (24-62)	16	5	1T/1.5T	T2/FLAIR	WMLs (presence)	Pros, Long
Women	2008	20	43.7±17.4 (18-72)	10	2	1T/1.5T	T2/FLAIR	WMLs (presence)	Pros, Long
Cocozza et al [9], All (Mixed)	2017	104 (40)	43.0±13.4 (13-72)	72	-	0.5T/1.5T/3T	FLAIR	WMLL (Fazekas)	Retro, Cross
Cocozza et al [10], All (Mixed)	2017	32 (12)	43.3±12.2 (20-68)	29	0	3T	FLAIR	WMLL (Fazekas)	Pros, Cross
Cocozza et al [11], All	2018	32 (12)	43.3±12.2 (20-68)	29	0	3T	FLAIR	WMLL (Fazekas)	Pros, Cross
Men	2018	12	-	-	0	3T	FLAIR	WMLL (Fazekas)	Pros, Cross
Women	2018	20	-	-	0	3T	FLAIR	WMLL (Fazekas)	Pros, Cross

Supplemental Table 1 Study information in alphabetic order (continued)

Author, group (Sex)	Year	Patients, n (men)	Age (years), median or mean ±SD (range)	ERT, n	TIA and stroke, n	Tesla	Sequence	WML method	Study design
Crutchfield et al [12], All (Men)	1998	52 (52)	39.5 (6-63)	0*	-	-	T2	WMLL (Length)	Retro, Long
Duning et al [13], All (Mixed)	2013	23 (12)	46.0 (29-61)	23	0	3T	FLAIR	WMLL (Fazekas) WMLL (Volume)	Pros, Cross
Fellgiebel et al [14], All	2005	27 (13)	38.1 (12-69)	22	5	1.5T	FLAIR	WMLL (-/±/+)	Pros, Cross
Men	2005	13	36.3±9.9 (12-51)	-	2	1.5T	FLAIR	WMLL (-/±/+)	Pros, Cross
Women	2005	14	39.7±13.5 (19-69)	-	3	1.5T	FLAIR	WMLL (-/±/+)	Pros, Cross
Fellgiebel et al [15], All	2006	27 (13)	38.1 (12-69)	22	5	1.5T	FLAIR	WMLs (Presence)	Pros, Cross
Men	2006	13	36.3±9.9 (12-51)	-	2	1.5T	FLAIR	WMLs (Presence)	Pros, Cross
Women	2006	14	39.7±13.5 (19-69)	-	3	1.5T	FLAIR	WMLs (Presence)	Pros, Cross
Fellgiebel et al [16], All (Mixed)	2009	25 (10)	36.5±11.0	21	5	1.5T	FLAIR	WMLL (Volume)	Pros, Cross
Fellgiebel et al [17], All (Mixed)	2012	25 (10)	36.5±11.0	-	-	1.5T	FLAIR	WMLL (Volume)	Pros, Cross
Fellgiebel et al [18], All (Mixed)	2014	41 (38)	43.9±9.7 (20-68)	25	2	1T	T2/FLAIR	WMLL (Diameter)	RCT
Gavazzi et al [19], All	2006	16 (8)	38.8±13.9 (17-58)	7	6	1.5T	FLAIR	WMLL (Fazekas)	Pros, Cross
Men	2006	8	34.7±10.0 (22-58)	6	2	1.5T	FLAIR	WMLL (Fazekas)	Pros, Cross
Women	2006	8	42.8±16.6 (17-58)	1	4	1.5T	FLAIR	WMLL (Fazekas)	Pros, Cross
Ginsberg et al [20], All	2006	47 (32)	-	-	2	-	-	WMLs (number)	Retro, Cross
Men	2006	32	-	-	-	-	-	WMLs (number)	Retro, Cross
Women	2006	15	-	-	-	-	-	WMLs (number)	Retro, Cross
Ginsberg [21], All (Mixed)	2006	84 (?)	-	-	6	-	-	WMLs (presence)	Retro, Cross
Gupta et al [22], All (Women)	2005	54 (0)	44.1±11.5 (21-72)	-	-	-	-	WMLs (number)	Pros, Cross
Jardim et al [23], All	2004	8 (7)	32.6±9.4 (24-47)	8	1	1.5T	T2/FLAIR	WMLs (number)	Pros, Long
Men	2004	7	30.7±8.4 (24-47)	7	1	1.5T	T2/FLAIR	WMLs (number)	Pros, Long
Women	2004	1	46	1	0	1.5T	T2/FLAIR	WMLs (number)	Pros, Long
Jardim et al [24], All	2006	8 (7)	32.6±9.4 (24-47)	8	1	1.5T	T2/FLAIR	WMLs (number)	Pros, Long
Men	2006	7	30.7±8.4 (24-47)	7	1	1.5T	T2/FLAIR	WMLs (number)	Pros, Long
Women	2006	1	46	1	0	1.5T	T2/FLAIR	WMLs (number)	Pros, Long

Supplemental Table 1 Study information in alphabetic order (continued)

Author, group (Sex)	Year	Patients, n (men)	Age (years), median or mean ±SD (range)	ERT, n	TIA and stroke, n	Tesla	Sequence	WML method	Study design
Kono et al [25], All (Mixed)	2016	54 (24)	39 (25-51)	-	-	1.5T	FLAIR	WMLs (presence)	Retro, Cross
Korsholm et al [26], All	2015	39 (13)	40.2±14.7 (10-66)	32	9	1.5T	FLAIR	WMLL (Fazekas)	Pros, Long
Men	2015	13	30.0±10.6 (10-47)	12	1	1.5T	FLAIR	WMLL (Fazekas)	Pros, Long
Women	2015	26	43.4±13.9 (15-66)	20	8	1.5T	FLAIR	WMLL (Fazekas)	Pros, Long
Lee et al [27], All (Mixed)	2017	12 (4)	42.6±14.3 (18-61)	12	4	1.5T/3T	T2/FLAIR	WMLL (Fazekas)	Retro, Cross
Lelieveld et al [28], All (Mixed)	2015	14 (4)	46.1±10.8 (27-64)	10	3	1.5T	FLAIR	WMLL (Volume)	Pros, Long
Low et al [29], All (Mixed)	2007	14 (13)	36.4±10.6	-	-	1.5T	T2/PD	WMLs (presence)	Pros, Cros
Marino et al [30], All	2006	8 (4)	40.0±12.4 (19-54)	-	3	1.5T	T2/T1/PD	WMLL (Volume)	Pros, Long
Men	2006	4	31.0±9.8 (19-43)	-	2	1.5T	T2/T1/PD	WMLL (Volume)	Pros, Long
Women	2006	4	50±4.7 (45-54)	-	1	1.5T	T2/T1/PD	WMLL (Volume)	Pros, Long
Moore et al [31], All (Men)	2001	26 (26)	(19-47)	14	1	-	FLAIR	WMLs (number)	RCT
Moore et al [32], All (Men)	2002	26 (26)	33.7±8.1 (19-47)	14	1	-	FLAIR	WMLs (number)	RCT
Moore et al [33], All (Men)	2002	17 (17)	(19-49)	-	-	1.5T	FLAIR	WMLL (length)	Pros, Cross
Moore et al [34], All (Men)	2003	16 (16)	(21-49)	-	-	-	FLAIR	WMLs (presence)	Pros, Cross
NIH cohort (Men)[#]	2003	79 (79)	35.0±12.0 (11-69)	-	-	-	FLAIR	WMLs (number) WMLL (length)	Retro, Long
Ortu et al [35], All	2013	11 (4)	38.9±16.8 (18-65)	-	3	-	T1/T2/ FLAIR	WMLs (presence)	Pros, Long
Men	2013	4	30.8±7.5 (23-41)	-	1	-	T1/T2/ FLAIR	WMLs (presence)	Pros, Long
Women	2013	7	44.9±18.4 (18-65)	-	2	-	T1/T2/ FLAIR	WMLs (presence)	Pros, Long
Paavilainen et al [36], All	2013	12 (4)	38.6±17.8 (16-68)	7	1	1.5T	-	WMLL (Fazekas)	Pros, Cross
Men	2013	4	31.6±16.1 (17-54)	3	0	1.5T	-	WMLL (Fazekas)	Pros, Cross
Women	2013	8	42.1±18.6 (16-68)	4	1	1.5T	-	WMLL (Fazekas)	Pros, Cross
Reisin et al [37], All	2011	36 (15)	37.3 (20-73)	18	0	-	FLAIR	WMLs (presence)	Pros, Long
Men	2011	15	31.2 (20-47)	-	0	-	FLAIR	WMLs (presence)	Pros, Long
Women	2011	21	41.6 (22-73)	-	0	-	FLAIR	WMLs (presence)	Pros, Long
Pediatric (Mixed)	2011	10 (3)	11.8 (6-16)	-	0	-	FLAIR	WMLs (presence)	Pros, Cross

Supplemental Table 1 Study information in alphabetic order (continued)

Author, group (Sex)	Year	Patients, n (men)	Age (years), median or mean ±SD (range)	ERT, n	TIA and stroke, n	Tesla	Sequence	WML method	Study design
Ries et al [38], All	2007	109 (85)	33.1±13.5 (6-72)	36	-	-	FLAIR	WMLL (-)	Retro, Cross
Men	2007	85	31.0±13.0 (6-58)	36	-	-	FLAIR	WMLL (-)	Retro, Cross
Women	2007	24	42.0±12.0 (22-72)	-	-	-	FLAIR	WMLL (-)	Retro, Cross
Rombach et al [39], All	2010	83 (33)	34.2	-	-	-	-	WMLs (presence)	Retro, Cross
Men	2010	33	33.0	-	-	-	-	WMLs (presence)	Retro, Cross
Women	2010	50	35.0	-	-	-	-	WMLs (presence)	Retro, Cross
Rombach et al [40], All	2012	59 (29)	39.7 (15-71)	59	-	-	-	WMLs (presence)	Retro, Long
Men	2012	29	33.6 (17-65)	29	-	-	-	WMLs (presence)	Retro, Long
Women	2012	30	45.6 (15-71)	30	-	-	-	WMLs (presence)	Retro, Long
Rombach et al [41], All	2013	50 (25)	42.0	50	-	-	T2/FLAIR	WMLs (presence)	Retro, Long
Men	2013	25	37.3±12.9	25	-	-	T2/FLAIR	WMLs (presence)	Retro, Long
Women	2013	25	46.6±12.8	25	-	-	T2/FLAIR	WMLs (presence)	Retro, Long
Pediatric	2013	6 (2)	16.6±0.7 (15-17)	6	0	-	T2/FLAIR	WMLs (presence)	Retro, Long
Rost et al [42], All	2016	223 (91)	39.2±14.9 (9-72)	136	35	-	T2/FLAIR	WMLL (Volume)	Retro, Long
Men	2016	91	34.7	-	-	-	T2/FLAIR	WMLL (Volume)	Retro, Long
Women	2016	132	42.3	-	-	-	T2/FLAIR	WMLL (Volume)	Retro, Long
Schermuly et al [43], All (Mixed)	2011	25 (10)	36.5±11.0 (21-56)	20	5	1.5T	FLAIR	WMLL (Volume)	Pros, Cross
Takanashi et al [44], All (Mixed)	2003	10 (9)	37.5±11.8 (19-59)	-	3	1.5T	T2	WMLs (presence)	Pros, Cross
Tedeschi et al [45], All (Men)	1999	9 (9)	38.8±8.8 (20-50)	0*	0	1.5T	T1/T2	WMLs (presence)	Pros, Cross
Üçeyler et al [46], All	2014	87 (30)	43.3 (16-73)	36	9	3T	FLAIR	WMLL (Fazekas)	Retro, Cross
Men	2014	30	40.0 (16-40[$])	23	4	3T	FLAIR	WMLL (Fazekas)	Retro, Cross
Women	2014	57	45.0 (16-73)	13	5	3T	FLAIR	WMLL (Fazekas)	Retro, Cross

** Study was published before the availability of ERT. Included patients were classified as not using ERT. [#]Study briefly mentions a Fabry "NIH cohort" and uses this cohort for the calculation of a hazard risk for white matter lesions. [$]Adopted from the original article. However, it was considered unlikely that median and maximum age were both 40 years. - = Not available, (-/±/+) = No WMLs, mild WMLs, significant WMLs, ERT = enzyme replacement therapy, TIA = transient ischemic attack, T = Tesla, WMLs = white matter lesions, WMLL = white matter lesion load, FLAIR = Fluid Attenuated Inversion Recovery, Pros = prospective, Retro = retrospective, Cross = Cross-sectional, Long = Longitudinal, PD = Proton density, RCT = randomized controlled trial, NIH = National Institutes of Health Fabry cohort*

Supplemental table B

Supplemental Table B Size and number of white matter lesions per study

First author, group	Patients, n (men)	Age (years), median or mean ±SD (range)	Method	Size (mm), mean±SD
Size				
Crutchfield et al [12], WM and GM lesions (men)	13 (13)	47.0±6.1	Longitudinal length of all lesions combined	485±525
Crutchfield et al [12], periventricular WM lesions (men)	13 (31)	42.6±9.2	Longitudinal length of all lesions combined	102±111
Fellgiebel et al [18], All (mixed)	41 (38)	43.9±9.7 (20-68)	Diameter per lesion normalized for head size	32±45
Moore et al [34], WM lesions (men)	10 (10)	42.6±5.6	Longitudinal length per lesion	21
First author, group	**Patients, n**	**Age (years), median or mean ±SD (range)**	**Method**	**Number of WMLs**
Number				
Moore et al [34], WM lesions (men)	10 (10)	42.6±5.6	Mean number of WMLs per patient	3
Jardim et al [23], All (mixed)*	8 (7)	32.6±9.4 (24-47)	Range number of lesions	0-6
Jardim [24], All (mixed)*	6 (5)	35.2±9.7 (24-47)	Range number of lesions	0-10
Gupta et al [22], All (women)	54 (0)	44.1±11.5 (21-72)	Range number of lesions	0-5
Ginsberg et al [20], All (mixed)	47 (32)	-	Number of lesions	0: 21 patients 1-5: 10 patients 6-10: 1 patients >10: 15 patients
Men	32	-	Number of lesions	0: 14 patients 1-5: 7 patients 6-10: 0 patients >10: 11 patients
Women	15	-	Number of lesions	0: 7 patients 1-5: 3 patients 6-10: 1 patients >10: 4 patients

** Describe the same cohort over a period of one and two years. Mm= millimeter, WM = White matter, GM = Grey matter*

Supplemental table C

Supplemental Table C White matter location per study

First author, group (sex)	Patients per study, n (men)	Age (years), median or mean ±SD (range)	Location: patients with involvement
Anatomical			
Gupta et al [22], All (Women)	54 (0)	44.1±11.5 (21-72)	Tempoparietal WM: 2 Deep GM: 3 Cerebellum: 1
Crutchfield et al [12], All (Men)	52 (52)	39.5 (6-63)	Deep GM: 24 Brainstem/cerebellum: 18 Cortical GM: 6
Duning et al [13], All (Mixed)	23 (12)	46.0 (29-61)	Parietal WM: * Frontal WM: * Brainstem: 0
Takanashi et al [44], All (Mixed)	10 (9)	37.5±11.8 (19-59)	Parietal WM: 5 Cerebellum: 0
Jardim et al [23], All (Mixed)	8 (7)	32.6±9.4 (24-47)	Parietal WM: 4 Frontal WM: 4 Temporal WM: 0 GM: 0 Posterior fossa: 0
Azevedo et al [4], All (Mixed)	12 (5)	35.8±12.8	Frontotempoparietal WM: #
Cocozza et al [9], All (Mixed)	104 (40)	43.0±13.4 (13-72)	Corpus callosum: 3
Circulatory			
Reisin et al [37], All (Mixed)	36 (15)	37.3 (20-73)	Posterior: 3 Anterior: 7 Equally divided: 6
Fellgiebel et al [18], All (Mixed)	41 (38)	43.9±9.7 (20-68)	Anterior: 12 Middle: 9 Posterior: 5

** Description in study: "The symmetrical WM changes were most prominent in the parietal and frontal regions.", # Description in study: "mainly located in periventricular and frontotemporoparietal WM regions." GM = gray matter, WM = white matter*

Supplemental table D

Supplemental Table D Relation of white matter lesions to cerebral parameters

First author, design	Number of pt:ct	Men pt:ct	Matched	Age (years), median or mean ±SD (range)	Imaging methods	Outcome
Brain metabolism and cerebral blood flow						
Korsholm et al [26], Pros, Long	39:-	13:-	Age	Pt: 40.2±14.7 (10-66) Ct: (9–34), (30–60), (55–90)*	FLAIR: WMLL (Fazekas) F-18 FDG-PET: CGM, voxelwise	**Regional CGM:** reduced compared to ct, corresponding with infarcts/ hemorrhage not with WMLs. No correction
Moore et al [34], Pros, Cross	16:7	16:7	Sex	Pt: (21-49) Ct: (26-49)	FLAIR: WMLs (mean length) F-18 FDG-PET: CGM, ROI, voxelwise	**Mean CGM:** no differences **Regional CGM:** reduced compared to ct, (deep/periventruclar WM) in pt with and without WMLs. No correction
Moore et al [34], Pros, Cross	26:10	26:10	Sex	Pt: (19-47) Ct: (21-48)	FLAIR: WMLs (mean length) H_2O PET: CBF, voxelwise	**Regional CBF:** increased compared to ct in pt with WMLs and pt without WMLs Correction for age
Tedeschi et al [45], Pros, Cross	9:20	9:20	Age Sex	Pt: 38.8±8.8 (20-50) Ct: -	T1/T2: WMLs (presence) $_1$H-MRSI: chemical composition brain, ROI (fr/par/tem/occ/ins cor, tha, cs, ln, cd, cere)	**NAA/Cre and NAA/Cho:** reduced compared to ct (fr/par/tem/occ/ins cor, cs, tha), exceed areas of WMLs on MRI **Cho and Cre:** No differences **Lactate:** not detectable No correction
Marino et al [30], Pros, Long	8:8	4:4	Age Sex	Pt: 40.0±12.4 (19-54) Ct: 40.0 (22-55)	PD/T2/T1: WMLL (Volume) MTr: interaction water protons and macromolecules, voxelwise, ROI (WMLs or NAWM (cr, cs, fr, occ, g and s cc) $_1$H-MRSI: chemical composition brain, voxelwise	**MTr:** reduced compared to ct in WMLs, exceed areas of WMLs on MRI **NAA/Cre:** reduced compared to ct No correction

Supplemental Table D Relation of white matter lesions to cerebral parameters (continued)

First author, design	Number of pt:ct	Men pt:ct	Matched	Age (years), median or mean ±SD (range)	Imaging methods	Outcome
Jardim et al [23], Pros, Long	8:-	7:-	-	Pt: 32.6±9.4 (24-47)	T2/FLAIR: WMLs (number of) $_{1}$H-MRSI: chemical composition brain, ROI (fr WM)	**NAA/Cre:** reduced in 1 pt compared to literature **Cho/Cre:** increased in 3 pt compared to literature Differences found in patients with and without WMLs. No correction
Gavazzi et al [19], Pros, Cross	16:16	8:8	Sex Handedness	Pt: 38.8±13.9 (17-58) Ct: 42.7±15.3	FLAIR: WMLL (Fazekas) $_{1}$H-MRSI: chemical composition brain, single voxel (fr subcortical)	**NAA, Cre, Cho:** no differences compared to ct **Lactate:** not present Correction for age and sex
Diffusion weighted imaging						
Duning et al [13], Pros, Cross	23:44	12:23	Age Sex	Pt: 46.0 (29-61) Ct: 46.0 (29-59)	FLAIR: WMLL (Fazekas, Volume) DTI: voxelwise	**FA:** reduced compared to ct (fr, midbrain, brainstem), exceed areas of WMLs on MRI Correction for age
Paavilianen et al [36], Pros, Cross	12:13	4:2	-	Pt: 38.6±17.8 (16-68) Ct: 46.2±10.1 (32-66)	-: WMLL (Fazekas) DTI: voxelwise, ROI	**MD:** increased compared to ct (fr/par WM, ic, cere, tha, g and s cc, cr), in pt with and without WMLs **FA:** reduced compared to ct (fr/par WM, cere, tha, cc, pons, cst, ic, ec, ilf, dc), in pt with and without WMLs Correction for sex and age
Moore et al [33], Pros, Cross	17:8	17:8	Sex	Pt: (19-49) Ct: (21-47)	FLAIR: WMLs (mean length) DWI: DAv, voxelwise	**DAv:** increased compared to ct, no difference in pt with WMLs and pt without WMLs No correction
Fellgiebel et al [15], Pros, Cross[#]	27:21	13:12	Age Sex	Pt: 38.1 (12-69) Ct: 35.2	FLAIR: WMLs (-) DTI: ROI (fr/par/tem/occ WM tha, pulv, hipp)	**MD:** increased compared to ct (fr/par/tem WM), in pt with and without WMLs **FA:** no differences compared to ct Correction for sex and age

Supplemental Table D Relation of white matter lesions to cerebral parameters (continued)

First author, design	Number of pt:ct	Men pt:ct	Matched	Age (years), median or mean ±SD (range)	Imaging methods	Outcome
Cocozza et al [11], Pros, Cross	32:35	12:14	Age Sex	Pt: 43.4±12.2 (20-68) Ct: 42.2±14.5 (19–70)	FLAIR: WMLL (Fazekas) DTI: voxelwise	**FA:** reduced compared to ct (major commissural tracts, sparing of tem/occ WM, g and s cc, cere) Correction for sex and age
Albrecht et al [1], Pros, Cross[#]	25:20	10:12	Age	Pt: 36.4±11.5 (19-55) Ct: 35.1±9.7 (22–55)	FLAIR: WMLL (absent, mild, severe) DTI: voxelwise	**MD:** increased compared to ct (fr/tem/central/par WM, tha), in pt with and without WMLs (similar location, smaller areas). Related to WMLL **FA:** no differences to ct, related to WMLL Correction for age
Other cerebral parameters						
Kono et al [25], Pros, Cross	54:-	24:-	-	Pt: 39 (25-51)	FLAIR: WMLL (Fazekas) SWI: microbleeds	**Microbleeds:** more WMLs in pt with microbleeds compared to pt without No correction
Üçeyler et al [46], Pros, Cross	87:36	30:14	-	Pt: 43.3 (16-73) Ct: 39.1 (16-84)	FLAIR: WMLL (Fazekas) MRA: cerebral artery diameter (CCA, MCA, ACA, PCA, BA)	**Artery diameter:** high BA diameter not related to a high WMLL Correction for sex
Lelieveld et al [28], Pros, Long	14:-	4:-	-	Pt: 46.1±10.8 (27-64)	FLAIR: WMLL (Volume) MRA: cerebral artery diameter (ICA, MCA, ACA, PCA, BA) 3DT1 MP-RAGE: HV	**Artery diameter:** difference baseline and follow-up left ACA related to WMLL, no relation to other arteries **HV atrophy:** not related to difference baseline and follow-up WMLL Correction for sex
Fellgiebel et al [17], Pros, Cross	25:20	10:9	Age Education	Pt: 36.5±11.0 Ct: 36.8±10.0	FLAIR: WMLL (Volume) T1: HV, WM and GM volume	**HV:** not related to WMLL **WM and GM volume:** not related to WMLL Correction for age

Supplemental Table D Relation of white matter lesions to cerebral parameters (continued)

First author, design	Number of pt:ct	Men pt:ct	Matched	Age (years), median or mean ±SD (range)	Imaging methods	Outcome
Gavazzi et al [19], Pros, Cross	16:16	8:8	Sex Handedness	Pt: 38.8±13.9 (17-58) Ct: 42.7±15.3	FLAIR: WMLL (Fazekas) fMRI: during finger tapping	**Finger tapping:** no relation to WMLL **fMRI:** increased activation of sc, is, cma, sma compared to ct. Activation of sc related to WMLL No correction
Cocozza et al [10], Pros, Cross	32:35	12:14	Age Sex	Pt: 43.3±12.2 (20-68) Ct: 42.1±14.5 (19–70)	FLAIR: WMLL (Fazekas) fMRI: resting state	**rPCG:** decreased functional connectivity bilaterally (cd, ln) compared to ct **lPCG:** decreased functional connectivity bilaterally (cd, ln), right cere, cluster with dn, vermis, cere compared to ct No relation to WMLL Correction for age and sex
Ortu et al [35], Pros, Long	11:11	4:4	Age Sex	Pt: 38.9±16.8 (18-65) Ct: 37.4±17.2 (21-65)	T1/T2/FLAIR: WMLs (Presence) TMS: motor evoked potentials	**Motor cortex excitability:** increased compared to ct. No relation to WMLs. No correction

** F-18 FDG-PET results were matched to one of three control scans depending on age, # Cohorts overlap almost entirely, differ in DTI analysis: ROI versus voxelwise*
- = not available, Pt = patients, Ct = controls, FLAIR = fluid attenuated inversion recovery, WMLs = white matter lesions, WMLL = white matter lesion load, pros = prospective, cross = cross-sectional, long = longitudinal, F-18 FDG-PET = 18-fluoro-deoxyglucose positron emission tomography, CGM = Cerebral Glucose Metabolism, WM = white matter, ROI = regions of interest, H_2O PET = PET with ^{15}O-labelled water, CBF = cerebral blood flow, $_1$H-MRSI = proton magnetic resonance spectroscopy imaging, fr = frontal, par = parietal, tem = temporal, occ = occipital, ins = insular, cor = cortex, tha = thalamus, cs = centrum semiovale, ln = lentiform nucleus, cd = caudate, cere = cerebellum, NAA = N-acetylaspartate, Cho = choline, Cre = creatine phosphocreatine, MTr = magnetization transfer ratios, NAWM = normal appearing white matter, cr = corona radiate, g and s = genu and splenium, cc = corpus callosum, DTI = diffusion tensor imaging, MD = mean diffusivity, FA = fractional anisotropy, ic = internal capules, cst = corticospinal tract, ec = external capsules, ilf = inferior longitudinal fasciculus, dc = dorsal cingulum, DWI = diffusion weighted imaging, DAv = average brain diffusion constant, pulv= pulvinar, hipp = hippocampus, SWI = susceptibility-weighted imaging, MRA = 3D-reconstructions of the time-of-flight-MR-angiography, CCA = common carotid artery, MCA = middle cerebral artery, ACA = anterior cerebral artery, PCA = posterior cerebral artery, BA = basilar artery, ICA = internal carotid artery, MP-RAGE = Magnetization Prepared Rapid Gradient Echo, HV = Hippocampal volume, GM = grey matter, fMRI = functional MRI, sc = sensorimotor cortex, is = intraprietal sulcus, cma = cingulated motor area, sma = secondary motor area, rPCG = right precentral girus, lPCG = left PCG, dn = dentate nuclei, TMS = transcranial magnetic stimulation

Supplemental table E

Supplemental Table E Relations of white matter lesions to other clinical parameters and patient characteristics

First author, design	Number of patients (men)	Age (years), median or mean ±SD (range)	WML method	Outcome
Schermuly et al [43], Pros, Cross	25 (10)	36.5±11.0 (21-56)	WMLL (Volume)	WMLL not related to neuropsychological test scores (after correction age) or to a history of depression and depressive symptoms High WMLL (median split or +2SD) not related to neuropsychological test scores (after correction age) or to a history of depression and depressive symptoms
Lelieveld et al [28], Pros, Long	14 (4)	46.1±10.8 (27-64)	WMLL (Volume)	Difference in WMLL related to change in performance on neuropsychological test (executive functioning) WMLL not related to depression severity, frequency or pain
Altarescu et al [2], Pros, Cross	57 (57)	36.0±12.0 (12-64)	WMLs (presence)	Presence of WMLs not related to residual enzyme activity Presence of WMLs related to polymorphisms G174C of IL6, G894T of eNOS, A-13G and G79A of protein Z and the factor V G1691A mutation Presence of WMLs not related to polymorphisms T786C of eNOS, G20210A of prothrombin or C677T of MTHFR
Rombach et al [39], Retro, Cross	83 (33)	34.2	WMLs (presence)	Presence of WMLs related to higher plasma lysoGb3 in untreated men
Rombach et al [40], Retro, Long	59 (29)	39.7 (15-71)	WMLs (presence)	Occurrence of WMLs not related to presence of antibodies Risk of developing WMLs related to decrease in lysoGb3, plasma Gb3 or urinary Gb3 within first year after start ERT
Duning et al [13], Pros, Cross	23 (12)	46.0 (29-61)	WMLL (Fazekas) WMLL (Volume)	WMLL (Fazekas or volume) not related to polysomnographic parameters (AHI, cAHI, oAHI, length of CSR episodes, SpO2, sleep efficiency)
Albrecht et al [1], Pros, Cross	25 (10)	36.4±11.5 (19-55)	WMLL (-/±/+)	WMLL not related to presence of angiokeratoma or neuropathic pain
Crutchfield et al [12], Retro, Long	52 (52)	39.5 (6-63)	WMLL (Length)	WMLL not related to pulmonary function abnormalities
Ries et al [38], Retro, Cross	109 (85)	33.1±13.5 (6-72)	WMLL (-)	WMLL related to hearing loss
Jardim et al [24], Pros, Long	8 (7)	32.6±9.4 (24-47)	WMLs (number)	Presence of WMLs showed trend relation to presence of hearing loss

(-/±/+) = No WMLs, mild WMLs, significant WMLs
WMLs = white matter lesions, Pros = prospective, Cross = Cross-sectional, Long = Longitudinal, WMLL = white matter lesion load, SD = standard deviation, eNOS = endothelial nitric oxide synthase, IL = interleukin, MTHFR = methylenetetrahydrofolate reductase, ERT = enzyme replacement therapy, AHI = apnea-hypopnea index, cAHI = central AHI, oAHI = obstructive AHI, CSR = Cheyne-Stokes respiration, SpO2 = Saturation of peripheral Oxygen

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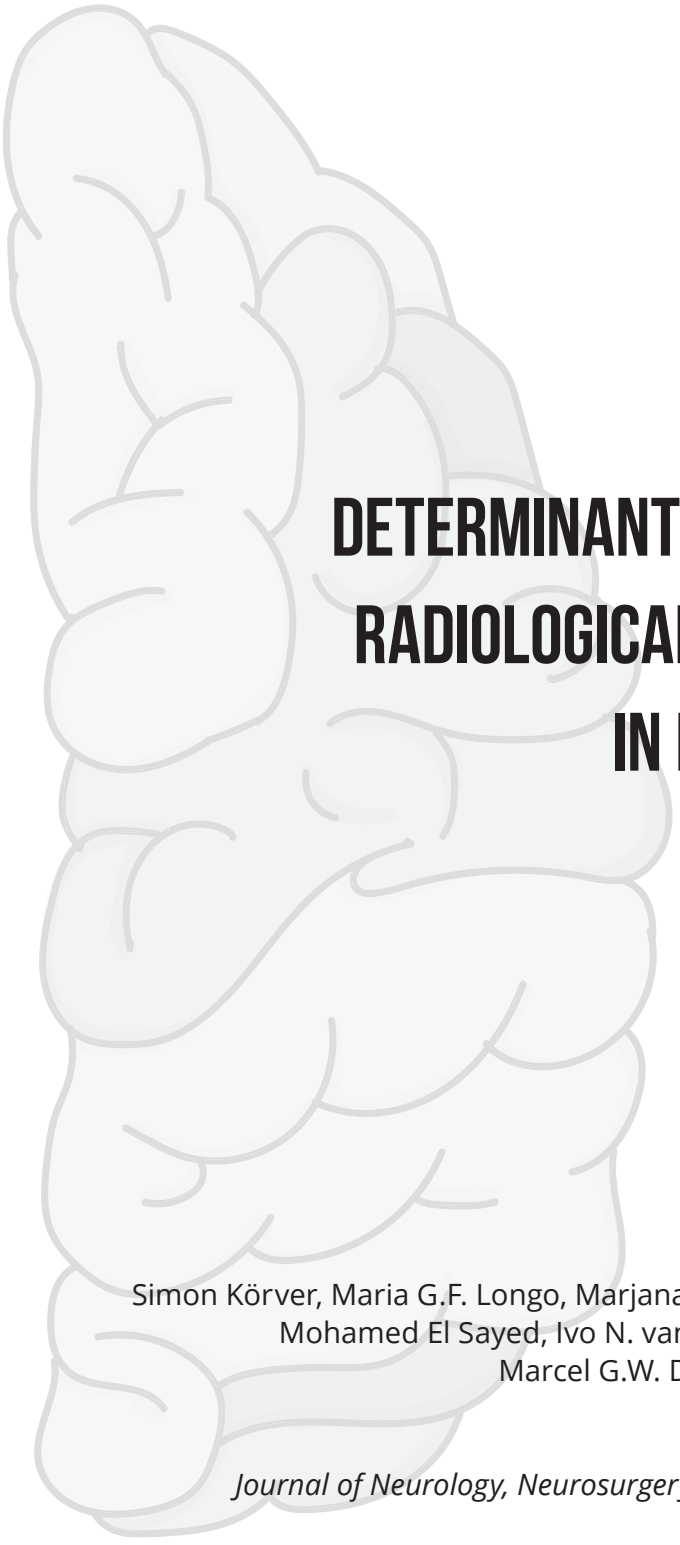

3

DETERMINANTS OF CEREBRAL RADIOLOGICAL PROGRESSION IN FABRY DISEASE

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Abstract

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

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

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

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

Introduction

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

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

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

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

Methods

Study design and data collection

The Amsterdam University Medical Centers (location Academic Medical Center (AMC)) is the national referral center for FD patients in the Netherlands. Follow-up at the outpatient clinic depends on disease phenotype and treatment status and ranges from half yearly to once every two years. It includes blood tests (kidney function and plasma globotriaosylsphingosine (lysoGb3)) and MRIs (cardiac and brain). Clinical follow-up data are collected in a local database after patients provide written informed consent. Data on patients with a definite FD diagnosis and ≥1 MRI scan of the brain on a 3T scanner were extracted from the database for this retrospective longitudinal cohort study.

According to Dutch law no approval of the study protocol was needed as this is a retrospective study and patients were not subjected to procedures or rules of behavior additionally to regular clinical follow-up. Patient records were de-identified prior to analysis. The Medical Ethics Review Committee of the AMC confirmed that the Medical Research Involving Human Subjects Act (WMO) does not apply to this study (W19-417 # 19.484). This study was conducted in accordance with the Declaration of Helsinki of 2013 [17].

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

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

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

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

Imaging protocol and assessment procedure

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

MRI brain assessment

White matter lesions

WMLs were defined as hyperintensities on axial T2 and FLAIR weighted imaging without cavitation [22]. WMLs were visually assessed using both the Fazekas scale [23] and the Scheltens scale [24] (**Supplemental methods: Supplemental table 2**). The Fazekas scale rates WMLs in two locations: periventricular and deep. Severity is rated per location from 0 (no WMLs) to 3 (severe confluent WMLs), resulting in a total score between 0 and 6.

The Scheltens scale is semi-quantitative and provides regional information for both periventricular and deep WMLs. Periventricular WMLs are rated in three regions resulting in a score from 0 (no WMLs) to 6 (severe periventricular lesions) and deep WMLs are rated in four regions resulting in a score from 0 (no WMLs) to 24 (severe deep lesions), with the total score ranging from 0 to 30 [25].

Infarctions

Infarctions were defined as focal lesions ≥3 mm, with an irregular hyperintense rim and central cavitation on axial T2 and FLAIR weighted imaging [22] and were scored as present or absent.

Basilar artery

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

Disease characteristics and treatment data

To assess the effect of patient characteristics and ERT on MRI brain parameters, we combined the scans with clinical data obtained at a nearby time point (with a maximum

of one year time difference). If a patient experienced a clinical event (e.g. atrial fibrillation (AF), kidney transplantation), the time up to the event was classified as "event free time" and time after the event was classified as "post-event time". Cardiovascular risk factors and cholesterol levels were assessed once, either before or during follow-up.

Renal function was evaluated by calculating the eGFR [27]. Left ventricular mass index (LVMi) was measured on cardiac MRI [28].

Years treated with ERT were calculated. Inhibitory anti-drug antibodies to ERT (from here on referred to as antibodies) were rated as positive (inhibitory titer ≥6) or negative (inhibitory titer <6) [2, 29]. If the antibody response was transient it was classified as negative. LysoGb3 levels at diagnosis were measured in plasma using tandem mass spectrometry [2].

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

Data on cardiac complications were gathered by one of the authors (*MES*) by reviewing all patient charts, clinical letters, echocardiography and/or cardiac MRIs from birth until January 2019, extracting predefined cardiac complications (**Supplemental methods: Supplemental table 3**). For this study we extracted data on atrial fibrillation (AF), ischemic heart disease, valve dysfunction, systolic dysfunction and left ventricular outflow tract obstruction (LVOTO).

Statistical methods

Data are presented as median and range or mean ± standard deviation (SD) where appropriate. R (version 3.5.1) was used for statistical analyses [30].

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

Cumulative logistic mixed effect models (which preserve the ordinal nature of the data, package: ordinal; clmm2 [32]) were used to evaluate the importance of variables on the progression risk of WMLs (Fazekas score) and the progression risk of infarctions on MRI (absence or presence). A random patient effect was introduced into all mixed

models to account for inter-patient differences. The following variables were regarded of potential importance for the progression risk of both the WMLs and infarctions: age, sex, phenotype, years on ERT, eGFR, LVMi, hypertension, BAD, low density lipoprotein (LDL) cholesterol, AF, ischemic heart disease and the MRI system used. The following variables were assumed to influence the progression risk of infarctions only: valve dysfunction, systolic dysfunction, LVOTO and Fazekas score.

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

Missing data of independent variables were assessed after data were matched to the cerebral MRI scans. If data were missing for <5% of the matched cerebral MRIs, this was assumed to have little influence on the analysis outcome. In case more data were missing, multiple imputation by chained equations was used to impute the missing data (package: mice [33]).

Results

Patient characteristics

A total of 149 FD patients was included (79.2% with a classical phenotype, 37.6% men) with a median age of 38.8 years (range: 9.1-72.3 years) (**Table 1**). Eighty-eight patients (59.1%) were treated with ERT at any time during follow-up. Fourteen patients developed AF during follow-up, resulting in a total of 20 patients with a history of AF (13.4%) (**Supplemental results: Supplemental table 5**).

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

Intra-rater reliability

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

Brain MRIs and involvement

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

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

Table 1 Patient characteristics

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
Patients, n (%)	149	45 (30.2%)	11 (7.4%)	73 (49.0%)	20 (13.4%)
Age at first MRI in years, median (range)	38.8 (9.1-72.3)	25.1 (11.0-60.5)	49.5 (24.0-63.9)	42.0 (11.2-71.3)	39.2 (9.1-72.3)
Patients <18 years, n (%)	24 (16.1%)	10 (22.2%)	0 (0.0%)	12 (16.4%)	2 (10.0%)
Missense mutation, n (%)	97 (65.1%)	22 (48.9%)	11 (100.0%)	45 (61.6%)	19 (95.0%)
Ever ERT, n (%)	88 (59.1%)	41 (91.1%)	4 (36.4%)	42 (57.5%)	1 (5.0%)
Years treated at last MRI, median (range)	7.9 (0.1-15.8)	7.9 (0.1-15.8)	7.5 (2.0-13.0)	8.1 (1.0-14.1)	2.2
Antibody positive[$], n (%)	NA	21 (51.2%)	NA	NA	NA
LysoGb3 before ERT in nmol/L, median (range)	9.1 (0.4-148.6)	99.0 (52.7-148.6)	7.5 (0.9-26.0)	7.4 (1.3-39.6)	2.1 (0.4-6.0)
Events before first brain MRI					
Cerebrovascular event, n (%)	11 (7.4%)	5 (11.1%)	1 (9.1%)	5 (6.8%)	0 (0.0%)
Stroke, n (%)	6 (4.0%)	3 (6.7%)	0 (0.0%)	3 (4.1%)	0 (0.0%)
TIA, n (%)	6 (4.0%)	2 (4.4%)	1 (9.1%)	3 (4.1%)	0 (0.0%)
Ischemic heart disease, n (%)	2 (1.3%)	0 (0.0%)	1 (9.1%)	1 (1.4%)	0 (0.0%)
Atrial fibrillation, n (%)	6 (4.0%)	3 (6.7%)	0 (0.0%)	3 (4.1%)	0 (0.0%)
Systolic dysfunction or LVOTO, n (%)	5 (3.4%)	1 (2.2%)	1 (9.1%)	1 (1.4%)	2 (10.0%)
Moderate/severe valve dysfunction, n (%)	4 (2.7%)	3 (6.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Renal event[¥], n (%)	1 (0.7%)	0 (0.0%)	0 (0.0%)	1 (1.4%)	0 (0.0%)
Kidney function at first MRI					
eGFR in ml/min/1.73m^2, median (range)	103.9 (11.4-147.3)	109.5 (19.4-147.3)	89.7 (11.4-122.7)	101.0 (46.4-140.1)	107.1 (53.6-134.4)
eGFR <60 ml/min/1.73m^2, n (%)	15/145 (10.3%)	6/44 (13.6%)	4/11 (36.4%)	4/71 (5.6%)	1/19 (5.3%)
Albuminuria > A1, n (%)	63/143 (44.1%)	23/45 (51.1%)	9/11 (81.8%)	28/69 (40.6%)	3/18 (16.7%)

Table 1 Patient characteristics (continued)

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
Cardiovascular risk factors					
Hypertension, n (%)	26 (18.7%)	5 (11.4%)	5 (50%)	12 (16.9%)	4 (28.6%)
Type 2 diabetes, n (%)	2 (1.4%)	0 (0.0%)	1 (11.1%)	1 (1.4%)	0 (0.0%)
LDL-cholesterol in mmol/L, median (range)	2.32 (0.95-4.77)	2.13 (1.36-4.77)	2.93 (1.14-4.02)	2.45 (0.95-4.48)	2.38 (1.35-4.56)
Medication					
Statin, n (%)	20 (13.4%)	8 (17.8%)	3 (27.3%)	9 (12.3%)	0 (0.0%)
ACE/ARB, n (%)	73 (49.0%)	23 (51.1%)	9 (81.8%)	39 (53.4%)	2 (10.0%)
Antiplatelet, n (%)	68 (45.6%)	25 (55.6%)	2 (18.2%)	41 (56.2%)	0 (0.0%)

Continuous variables are presented as median (range) and discrete variables as number (percentages).
$ A history of antibodies in one man with classical disease (before stopping ERT) was counted as positive, transients antibodies in two men with classical disease were counted as negative. ¥ One patient with a history of renal transplantation, no patients on dialysis.
ACE = angiotensin-converting enzyme inhibitor, ARB = Angiotensin receptor blockers, eGFR = estimated glomerular filtration rate, ERT = enzyme replacement therapy, LDL = low density lipoproteins, LVOTO = left ventricular outflow tract obstruction, NA = not assessed, TIA = transient ischemic attack

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

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

Continuous variables are presented as median (range) or mean (±SD) and discrete variables as number (percentages).
BAD = Basilar artery diameter

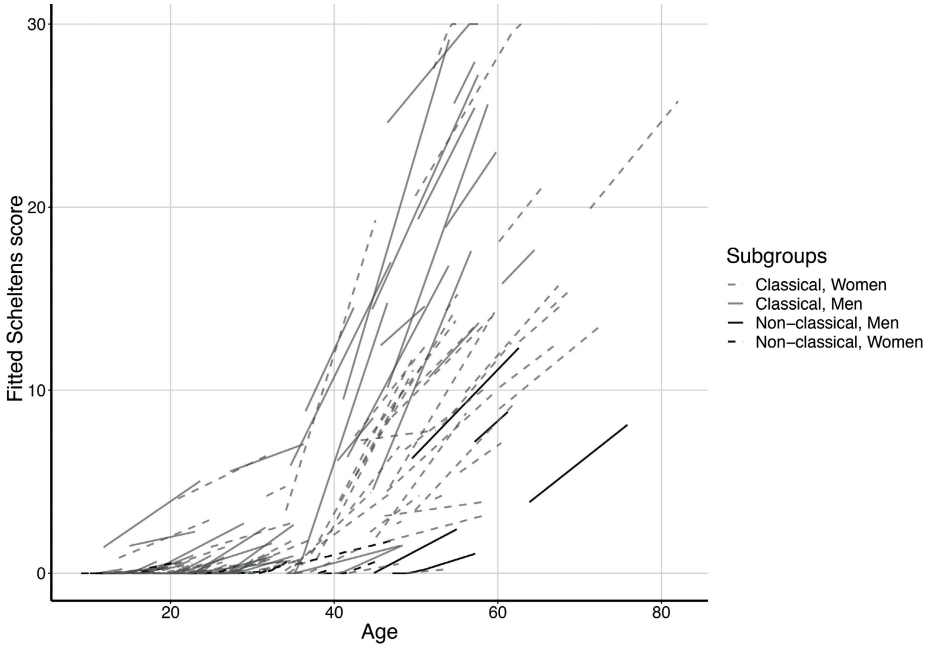

Figure 1 Relation between the Scheltens score (white matter lesion severity) and age. Grey lines and black lines represent patients with a classical phenotype and non-classical phenotype, respectively. Continuous lines represent men and dotted lines represent women. Individual patients' Scheltens scores were fitted using a linear mixed model.

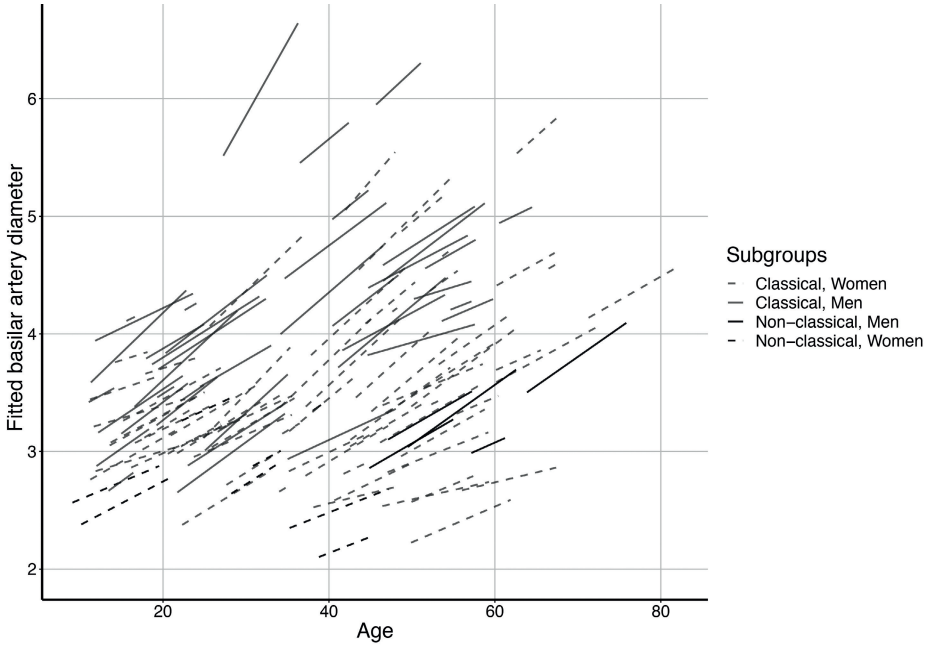

Figure 2 Relation between the basilar artery diameter and age. Grey lines and black lines represent patients with a classical phenotype and non-classical phenotype, respectively. Continuous lines represent men and dotted lines represent women. Individual patients' basilar artery diameters were fitted using a linear mixed model.

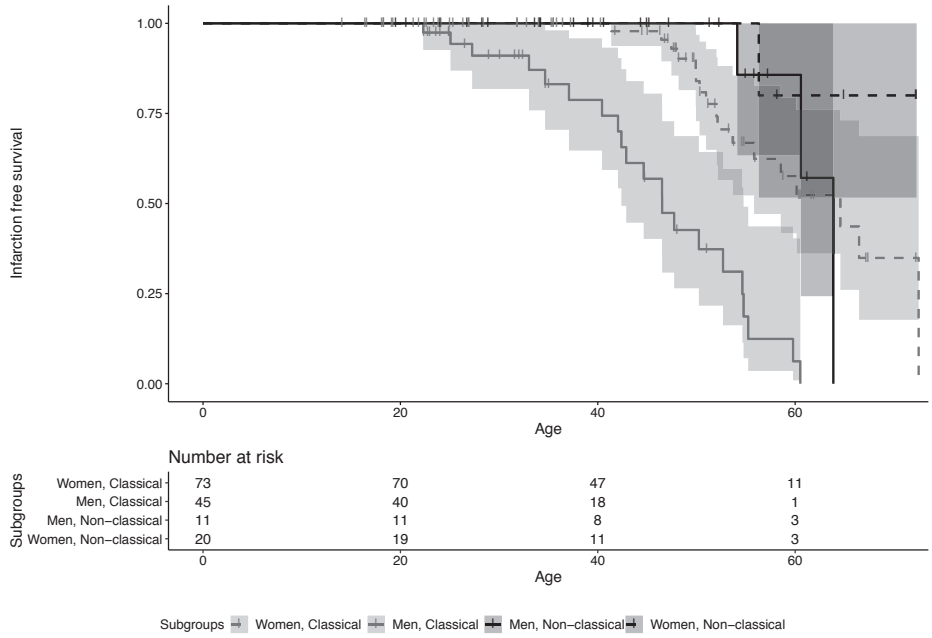

Figure 3 Infarction free survival stratified for sex and phenotype. Grey lines and black lines represent patients with a classical phenotype and non-classical phenotype, respectively. Continuous lines represent men and dotted lines represent women. Shaded areas represent the 95% CIs; | indicates censoring.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

Variables related to white matter lesion and infarction progression risk

Adjusted variables

We adjusted four variables for the mixed models. Firstly, in 40 scans (4.7%) the BAD was measured in two instead of three slices, mostly because of severe caudal tortuosity. There was no significant effect of the number of slices on BAD (β: -0.09; 95%CI: -0.23-0.04, p=0.18), so we included BAD measurements irrespective of the number of slices. Secondly, the only variable with data missing for ≥5% was LVMi measured with cardiac MRI (**Supplemental results: Supplemental table 6**) and multiple imputation was used to impute these missing data (**Supplemental results: Supplemental figure 1**). Thirdly, we combined Fazekas score 5 and 6 to improve power, since these scores were relatively rare. Lastly, years treated with ERT correlated strongly with the variable age (r = 0.88; 95%CI 0.87 – 0.91; p = <0.0001), increasing the risk of invalid results due to collinearity. Therefore, we analyzed the effect of treatment with ERT both as a continuous variable (years treated) and as a binary variable. In this study, some patients were started on ERT just before their MRI, while it is known that biomarkers decrease gradually over the six months to one year after the start of ERT.[34] Therefore we classified "untreated" as no ERT or a treatment duration <6 months and "treated" as ≥6 months of treatment.

Relation of variables to progression risk

Age, sex and phenotype

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

Enzyme replacement therapy

The random effects per patient were not explained by adding the treatment with ERT (<6 months versus ≥6 months) to the model (**Table 3**). When including "treatment in years" the model did not converge for both WMLs and infarction progression, probably due to collinearity of age and years treated. Removing the factor age resulted in a strong positive relation between years treated and progression, but the effect of age could not be separated of the ERT effect in this analysis. We also found no significant interaction between an "early" treatment start (<30 years old) and treatment with ERT (**Supplemental results: Supplemental table 7**), meaning that we did not find a difference in treatment effect in "early" treated patients compared to patients treated at older age.

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

Disease characteristics

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

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

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

Suggested follow-up frequency

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

Discussion

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

Evaluating the effect of ERT in non-randomized and uncontrolled studies warrants further discussion. Because no similar untreated cohort is studied, it is still possible that the progression rate is changed by ERT. While most FD studies evaluating the effect of ERT on WMLs have found no benefits in complete group analyses [4, 10, 11], it has been suggested that ERT might stabilize WMLs and prevent stroke in "early" treated patients [10, 35]. In the current study, after correction for age, sex and phenotype, we found no relation between ERT, and WML or infarction progression, even in our "early" treated patients. We were not able to assess the effect of very early treatment initiation (e.g. before the age of 16) as these patients were underrepresented in the current study.

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

			Fazekas score		Infarctions	
Fixed effects			**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Model 1 (sex and phenotype separate)						
Age			1.28 (1.24-1.32)	<0.0001	1.26 (1.17-1.35)	<0.0001
Sex						
	Women		1.0	-	1.0	-
	Men		7.4 (2.4-23.2)	0.0006	169 (17-1697)	<0.0001
Phenotype						
	Non-classical		1.0	-	1.0	-
	Classical		52.9 (11.0-254.8)	<0.0001	64 (2.5-1625)	0.0119
Model 2 (sex and phenotype combined groups)						
Age			1.28 (1.24-1.33)	<0.0001	1.26 (1.17-1.35)	<0.0001
Sex, phenotype						
	Women, classical		1.0	-	1.0	-
	Men, classical		11.0 (3.2-38.4)	0.0002	224 (19-2618)	<0.0001
	Men, non-classical		0.06 (0.01-0.49)	0.0090	1.42 (0.04-52.6)	0.8483
	Women, non-classical		0.05 (0.01-0.39)	0.0037	0.07 (0.00-7.61)	0.2619
Model 2 +	<6 months of ERT		1.0	-	1.0	-
	≥6 months of ERT		1.34 (0.96-1.88)	0.0821	1.43 (0.73-2.81)	0.2951
Model 2 +	years treated with ERT		#	#	#	#
Model 2 +	changes in eGFR		0.99 (0.98-1.01)	0.4997	0.96 (0.93-0.99)	0.0180
Model 2 +	changes in LVMi on MRI		1.03 (1.00-1.05)	0.0368	1.03 (0.99-1.07)	0.1726
Model 2 +	No hypertension		1.0	-	1.0	-
	Hypertension		1.12 (0.26-4.82)	0.8821	0.61 (0.05-6.88)*	0.6859
Model 2 +	Changes in BAD		0.90 (0.57-1.43)	0.6492	1.62 (0.65-4.05)	0.2983
Model 2 +	LDL-cholesterol		1.21 (0.58-2.64)	0.6264	1.26 (0.31-5.13)	0.7514
Model 2 +	No AF		1.0	-	1.0	-
	AF		0.77 (0.32-1.85)	0.5617	0.45 (0.08-2.49)	0.3584
Model 2 +	No ischemic heart disease		1.0	-	1.0	-
	Ischemic heart disease		1.92 (0.57-6.49)	0.2917	0.75 (0.09-6.47)	0.7960
Model 2 +	No valve dysfunction		-	-	1.0	-
	Valve dysfunction		-	-	0.43 (0.10-1.86)	0.2575
Model 2 +	No systolic dysfunction or LVOTO		-	-	1.0	-
	Systolic dysfunction or LVOTO		-	-	0.28 (0.04-2.09)	0.2132
Model 2 +	MRI-scanner	Ingenia	1.0	-	1.0	-
		Intera	2.36 (1.56-3.57)	<0.0001	1.74 (0.75-4.03)	0.1946
Model 2 +	Fazekas scale		-	-	1.94 (1.32-2.85)	0.0008

In all models adding additional variables to model 2, the effect sizes of age and sex and phenotype divided subgroups remained similar and are therefore not presented. To reduce the false positive rate (type-I errors) we regarded P-values <0.01 as significant.
*# Models did not converge, probably due to collinearity. * The model was unable to run with non-classical patients included, probably due to the low number of non-classical patients with infarctions. Thus, for this analysis only classical patients were included.*
AF = atrial fibrillation, BAD = basilar artery diameter, CI = confidence interval, eGFR = estimated glomerular filtration rate, ERT = enzyme replacement therapy, LDL = low density lipoprotein, LVMi = left ventricular mass index, LVOTO = left ventricular outflow tract obstruction, OR = odds ratio

Previous studies in FD have shown a strong progression of the WML burden starting from the fourth decade of life [4, 36], which was also seen in our cohort (**Figure 1**). While WMLs are also prevalent in the general population, progression in FD starts at an earlier age and the burden is higher: in our cohort, ~15% of patients had a periventricular and deep Fazekas score ≥2 at a median age of 39 years old, a WML incidence and severity observed in the general population three decades later [37]. Similarly, radiological infarctions were present in 15% of our cohort at baseline increasing to 28% during seven years of follow-up, while the prevalence of (silent) brain infarctions in the general population at forty years old would be <5%, increasing to 15% two to three decades later [8].

The risk of WML and infarction progression was not equally distributed: subgroups divided by sex and phenotype showed large differences in risk of progression in our study, this differentiation was not addressed in previous studies. The limited WML severity and the low number of infarctions in women with non-classical disease suggests that there may not be an increased risk of these complications in this patient group compared to the general population [8, 37]. These findings may have implications for future FD drug studies. If sex and phenotype are not equally distributed between the treated and control groups, this can lead to erroneous conclusions regarding treatment effectiveness.

No modifiable risk factors for progression of WMLs and infarctions were found in this study. Neither vascular risk factors, nor cardiac or renal disease were related to progression of WMLs or infarctions. This is in line with previous findings on WMLs [4, 11, 36] and infarctions on MRI [5]. Since the effects of most of these factors on progression are probably negligible to small, enormous sample sizes would be required to demonstrate any additional effects after taking the effects of age, sex and phenotype into account. Another possible explanation for differences in progression could be additional subtle differences in disease severity within the phenotypes, reflected by the relation between WML progression and lysoGb3 levels and mutation type in men with classical disease in this study.

This study has several strengths and limitations. The combination of the large dataset, long follow-up and clinical data-matching to all separate scans, in combination with the use of mixed models improved power and maximized data use. The study was limited by the use of MRIs with changes in acquisition parameters, which probably affected assessment of WML progression. Nevertheless, the relation to age, sex and phenotype did not change with the switch in acquisition parameters, indicating decent reliability of the use of WML rating scales in these circumstances. Secondly, the sensitivity of the Fazekas and Scheltens scale for progression of WMLs is variable and volumetric

measurement of the white matter lesion load is seen as the golden standard [38, 39]. We choose assessment with visual semi-quantitative scales since these are fast, easy to use, allowed full anonymization and have been broadly applied in earlier studies. This study shows that the use of these scales is feasible in studies with long term follow-up with changing scan parameters. Thirdly, one neuroradiologist assessed the BAD while the other neuroradiologist assessed all other pathology. While intra-rater reliability was excellent, we were not able to assess the inter-rater reliability. Lastly, evaluating the effect of ERT in cohort studies is difficult, and might result in erroneous conclusions. There is a strong indication bias: severely affected patients are more likely to be treated. Moreover, the use of the number of years on treatment is complicated due to collinearity with age. The latter cannot be excluded from analyses since it is the one of the most important factors in relation to progression.

To conclude, progression of cerebrovascular involvement in FD, regardless of ERT status, is to be expected with increasing age, especially in men with classical disease. This should be clearly communicated to patients. Surprisingly, cardiovascular risk factors were not related to the progression of WMLs and brain infarctions. While these factors should be managed rigorously, the effects of this management should not be overestimated and future studies should evaluate the effect of genetic modifiers and accurate measures of residual enzyme activity on progression of infarctions and WMLs (**Table 4**). Trials evaluating new treatment modalities for FD should incorporate brain MRIs, since the effect of current treatment on cerebral manifestations is clearly insufficient. With multiple newly emerging treatment strategies for FD, longitudinal data collection in large, international, industry-independent registries is needed to facilitate comparison of effectiveness. However, infarctions on MRI or WMLs measured using the Fazekas scale as clinical endpoints require unrealistic large sample sizes and follow-up duration (see **Supplemental discussion: Supplemental figure 4 and 5** for trial sample size calculations). Diffusion weighted and quantitative imaging of the brain [40] should be longitudinally explored, as there is a clear need for validated surrogate markers for the occurrence of cerebral infarctions in FD.

Table 4 Main findings, recommendations and future research directions

Topic	Main findings	Future research directions and recommendations
Age, sex and phenotype	· Are strongly related to progression of WMLs and infarctions on MRI	· Should be corrected for in any analysis of variables relating to WMLs or infarctions and evaluating treatment effects in FD · Women with non-classical FD should be compared to the general population to confirm the low rate of FD related cerebral involvement in this patient group
Treatment	· Patients and doctors should expect progression of WMLs over time, independent of treatment status · Older men with classical FD have a high risk of infarction progression, independent of treatment status	· The effect of very early ERT initiation, before any visual cerebral involvement is present, should be evaluated in high risk patients (men with classical disease) · Presence of some punctate WMLs in patients >50 years old is not necessarily FD related and treatment initiation should not be solely based on this finding · Randomized controlled trials for new treatment modalities in FD should include MRIs of the brain
WML assessment	· Semi-quantitative scales are able to detect WML progression in FD patients in a long term follow-up setting	· White matter lesion volume is preferable to semi-quantitative scales as they provide more detailed information on a continuous scale
Biomarkers, variables of interest and pathology	· Changes in BAD, eGFR and LMVi are not related to WML and infarction progression after correction for age, sex and phenotype	· WMLs and infarctions on MRI are probably end stage pathologic processes. Earlier biomarkers should be explored using sophisticated imaging techniques · The effect of differences in enzyme activity levels and genetic modifiers on progression of WMLs and infarctions should be explored within men with classical disease since interpatient variability is high · Potentially important variables should be assessed prospectively and with advanced methodology (e.g. volumetric atrial measurements instead of presence/absence atrial fibrillation)

BAD = basilar artery diameter, eGFR = estimated glomerular filtration index, ERT = enzyme replacement therapy, FD = Fabry disease, LVMi = left ventricular mass index, WML = white matter lesion, WMLs = white matter lesions

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Supplemental methods

Contents

Phenotypic classification

In men a classical FD phenotype was defined as: leucocyte α-Gal A activity ≤5% of the median of the reference range and one or more typical FD symptoms (Fabry specific neuropathic pain, angiokeratoma, and/or cornea verticillata) [1]. A lysoGb3 >40 nmol/L before start of ERT supported the diagnosis of a classical phenotype. In women a classical phenotype was defined as: presence of one or more typical FD symptoms (in the patient or a male family member with the same mutation). Patients not fulfilling these criteria were classified as non-classical.

Data collection – visit frequency

In patients treated with enzyme replacement therapy (ERT) biannual evaluation at the outpatient clinic includes routine urine- and blood tests and yearly cardiac and brain MRIs. For untreated patients with classical disease, frequency of these evaluations are yearly (urine- and blood tests) and biyearly (MRIs). For untreated non-classical patients this frequency is biyearly and once every four years.

Basilar artery diameter

MRA is the reference standard when measuring the basilar artery diameter [2]. In our center, Multiple Overlapping Thin Slab Acquisition (MOTSA) imaging (high-resolution cross-sectional MRA image of vessels) [3] was added in to the scan protocol in the year 2012. As included scans range back to 2004, using MOTSA for the assessment of basilar artery diameter would have resulted in a major loss of data. When reviewing the literature, axial T2 seemed to be an acceptable alternative if MRA is not available in non-FD [4-7] and FD populations [8]. In a previous study, there was a strong correlation between MRA and T2-weigthed measurement of the basilar artery diameter [9]. To confirm this relation in our own population we assessed the BAD in T2-weighted imaging and if available in the MOTSA images as well.

Demographics, treatment, evaluation of complications

Renal function was evaluated by calculating the estimated glomerular filtration rate using the CKD-EPI formula for patients ≥18 years old [10] and the creatinine-based bedside Schwartz formula for patients <18 years [11]. Renal events were defined as a history of renal transplantation or dialysis. Albuminuria at baseline was graded using the KDIGO categories as A1 (normal to mildly increased) to A3 (severely increased) [10].

The left ventricular mass index (LVMi) was measured on MRI without the papillary muscles [12], adjusted for body surface area (Dubois formula).

Mutations were classified as missense, nonsense or "other". Because of similar effects on enzyme activity we included frameshift mutations in the nonsense mutations category.

Two ERTs are approved in Europe: agalsidase alpha (Replagal, Shire, 0.2 mg/kg/every other week (EOW)) and agalsidase beta (Fabrazyme, Sanofi Genzyme, 1.0 mg/kg/EOW). Years treated were calculated as the time difference between the start of ERT and every MRI. Zero years treated was assigned for scans made in untreated patients.

LysoGb3 levels before start ERT were measured in plasma using tandem mass spectrometry with isotope labeled or glycine labeled lysoGb3 as internal standard. If both were available at the same time point we preferred the measurement using glycine labeled internal standard. Both internal standards show excellent intra-class correlation [13].

Presence of hypertension, type 2 diabetes or the use of antidiabetic medication was extracted from medical history. LDL-cholesterol levels were extracted from our local database. Medication use was defined as the use of a specific drug at any time prior to or during the follow-up time.

Cardiac complications

Data on cardiac complications were gathered in an observational retrospective longitudinal cohort study on the progression of cardiac involvement in FD. Using all patient charts, cardiac MRIs, echocardiography and clinical letters from birth to last follow-up date, data on predefined cardiac events were extracted including date of occurrence (for event definition see **Supplemental table 3**).

Statistical methods

To evaluate the intra-rater reliability, a random subsample of 30 scans was selected and reassessed 17 months after initial scan assessment. Both neuroradiologists were blinded for their initial assessment. The intra-rater reliability of the basilar artery diameter was assessed using the intra-class correlation coefficient (2-way mixed effects model, absolute agreement, single ratings) [14]. The intra-rater reliability of the Fazekas scale, Scheltens scale and presence or absence of infarctions were assessed using Kendall's concordance coefficient, corrected for ties.

In the cumulative logistic mixed effect models, if patient received renal replacement therapy or a kidney transplantation, the subsequent scans after this date were not used in the evaluation of the effect of eGFR change on progression of the WMLs or infarctions on MRI.

Supplemental table 1 Acquisition parameters for brain MRI

Parameter	FLAIR	T2	T1
Plane	Axial	Axial	Axial
Voxel volume (mm3), median (range)	1.0 (0.7-3.3)	1.0 (0.5-1.2)	1.0 (0.7-3.3)
Slice thickness (mm), median (range)	5.0 (1.1-5.0)	5.0 (3.0-5.0)	5.0 (3.0-5.0)
Interslice gap (mm), median (range)	5.5 (0.6-6.5)	5.5 (3.3-6.5)	5.5 (3.0-6.5)
TR (msec), median (range)	10113 (4000-11000)	4206 (2489-5938)	530 (7-600)
TE (msec), median (range)	100 (100-365)	80 (80-80)	9.8 (3.1-9.8)
TI (msec), median (range)	2600 (1650-2600)	0 (0-0)	0 (0-0)
Flip angle (degree), median (range)	90 (90-90)	90 (90-90)	90 (8-90)
Matrix, median (range) * median (range)	256 (208-312) * 166 (145-312)	400 (328-408) * 307 (240-377)	256 (232-288) * 256 (205-288)

FLAIR = Fluid-Attenuated Inversion Recovery, FOV = Field of View, TE = Echo Time, TI = Inversion Time, TR = Repetition Time

Supplemental table 2 MRI brain assessment

Description	Scale	Response options
Periventricular WMLs	Fazekas [15]	0: Absence 1: "caps" or pencil-thin lining 2: Smooth "halo" 3: Irregular periventricular lesions extending into deep white matter
Deep WMLs	Fazekas [15]	0: Absence or a single punctate lesion 1: Multiple punctate lesions 2: Beginning confluency of lesions (bridging) 3: Large confluent lesions Total score: 0 to 6
Periventricular WMLs: - Occipital - Lateral - Frontal	Scheltens [16]	0: Absence 1: ≤5 mm 2: >5 mm and <10 mm Periventricular WMLs exceeding 10 mm were per definition scored as deep Total score: 0 to 6
Deep WMLs: - Frontal - Parietal - Temporal - Occipital	Scheltens [16]	0: Absence 1: ≤ 3mm and n≤5 2: ≤ 3mm and n≥6 3: 4-10mm and n≤5 4: 4-10mm and n≥6 5: ≥11 mm and n≥1 6: Confluent WMLs Total score: 0 to 24
Infarctions	-	Presence or absence of infarctions
Basilar artery diameter (mm)	-	1: Caudal (shortly after the confluence of the vertebral arteries) 2: Intermediate (in the middle of the basilar artery) 3: Rostral (just before the bifurcation) Total score: average of 1, 2 and 3

WMLs = White matter lesions

Supplemental table 3 Definitions cardiac events

Events	Definition
Ischemic heart disease	
Coronary artery bypass graft	Open heart surgery where a bypass is placed around one or more (stenotic) coronary arteries
Percutaneous coronary intervention	Non-surgical intervention in which coronary stenosis is resolved with coronary angioplasty with or without the placement of a coronary stent
Coronary atherosclerosis*	>50% stenosis of luminal diameter of left main coronary artery or >70% stenosis of luminal diameter of at least one of the major epicardial coronary arteries one CAG [17] Patients were also classified as having coronary atherosclerosis if there were indications of myocardial ischemia on: - Stress ECG - First pass perfusion cardiac MRI - Regional wall movement abnormalities seen on echocardiography with ischemic changes on ECG - Dobutamine stress MRI
Atrial fibrillation	Irregular heart rhythm without identifiable p-waves recorded on ECG
Moderate to severe valve dysfunction	First ultrasound report mentioning moderate to severe stenosis of insufficiency of the mitral, tricuspid or aortic valve. Or heart valve dysfunction that required surgery where no previous ultrasound reports were available [18, 19]
Systolic dysfunction	Left ventricular ejection fraction <50% on MRI [20]. If no MRI is available: left ventricular ejection fraction <55% on echocardiography [21]
Left ventricular outflow tract obstruction	Dynamic gradient of ≥30 mmHg on echocardiogram in the left ventricular outflow tract measured during rest, Valsalva procedure or exercise [22]

** Events discussed with the expert panel AV = atrioventricular, bpm = beats per minute, CAG = coronary angiogram, ECG = electrocardiogram, ICD = implantable cardioverter-defibrillator, CRT-D = cardiac resynchronization therapy with defibrillator*

Supplemental table 4 Categorization of variables included in mixed models

Variable	Type	Options	Details	Time dependent†
Dependent variables				
Fazekas scale	Ordinal	Score range: 0-6	-	Yes
Infarctions	Ordinal/categorical	Absent (0)* / present (1)	Included as ordinal to improve comparability of results to Fazekas scale	Yes
Independent variables				
Age	Continuous	-	-	Yes
Sex	Categorical	Women*/Men	-	No
Phenotype	Categorical	Non-classical*/classical	-	No
Years treated ERT	Categorical	<6 months/≥6 months	-	Yes
Years treated ERT	Continuous	-	-	Yes
Changes in eGFR	Continuous	-	Scans after renal replacement therapy or renal transplantation were removed	Yes
Changes in LVMi	Continuous	-	Missing values were imputed	Yes
Hypertension	Categorical	No hypertension*/ Hypertension	-	No
Changes in BAD	Continuous	-	-	Yes
LDL-cholesterol	Continuous	-	-	No
AF	Categorical	No AF*/AF	-	Yes
Ischemic heart disease	Categorical	No IHD*/IHD	Composite of coronary artery bypass graft, percutaneous coronary intervention, coronary atherosclerosis	Yes
Valvular dysfunction	Categorical	No valvular dysfunction*/ Valvular dysfunction	-	Yes
Systolic dysfunction and LVOTO	Categorical	No SD or LVOTO*/SD or LVOTO	-	Yes
MRI scanner	Categorical	Ingenia*/Intera	All scans before October 2012 were made on the Intera system, all scans afterwards on the Ingenia system. The changes in acquisition parameters were simultaneous with the system switch	Yes
Fazekas scale	Continuous	Score range: 0-6	The Fazekas scale was only used in relation to presence or absence of infarctions	Yes

† Some variables were time-dependent. This included continuous variables (e.g. different eGFR measurements of a patient were used during follow-up) and categorical variables (e.g. a patient developed AF during follow-up. Scans beforehand were coded as "No AF", scans afterwards as "AF present"). Other variables were time-independent. This included continuous variables (e.g. the LDL-cholesterol measurements were extracted once per patient before or during follow-up and this value was matched to all scans) and categorical variables (e.g. hypertension present before or during follow-up. All scans of this patient were coded as "Hypertension present").
** Reference category AF = atrial fibrillation, BAD = basilar artery diameter, eGFR = estimated glomerular filtration rate, ERT = enzyme replacement therapy, IHD = ischemic heart disease, LDL = low density lipoprotein, LVMi = left ventricular mass index, LVOTO = left ventricular outflow tract obstruction*

Supplemental results

Contents

Intra-rater reliability

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

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

In total, 128 patients (82.6%) had at least one MOTSA scan and T2-weighted scan at the same time point. The spearman correlation of both basilar artery diameter (BAD) assessments was 0.80 (95%CI: 0.71-0.87, p = <0.0001). Considering the strong correlation between MOTSA and T2-weighted assessment of the BAD and increase of power using T2-weighted imaging, further analyses are performed using the T2-weighted BAD measurements.

Adjustment of variables for mixed models

Basilar artery diameter

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

Multiple imputation of left ventricular mass index

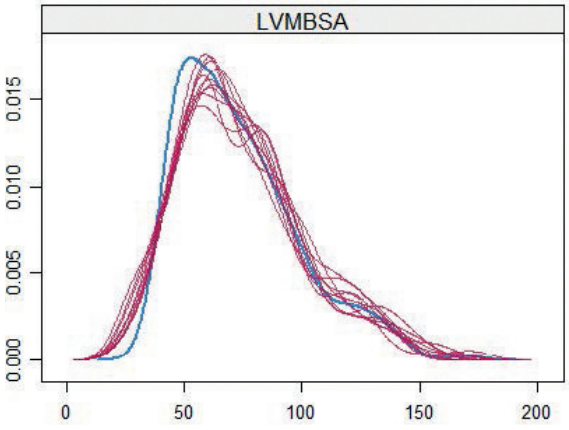
The only variable with >5% missing was the left ventricular mass index (LVMi) measured on cardiac MRI (**Supplemental table 6**). LVMi was mostly missing for scans before 2008 (the start of serial cardiac MRI imaging in our center). We assumed that data were missing at random and used multiple imputation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

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

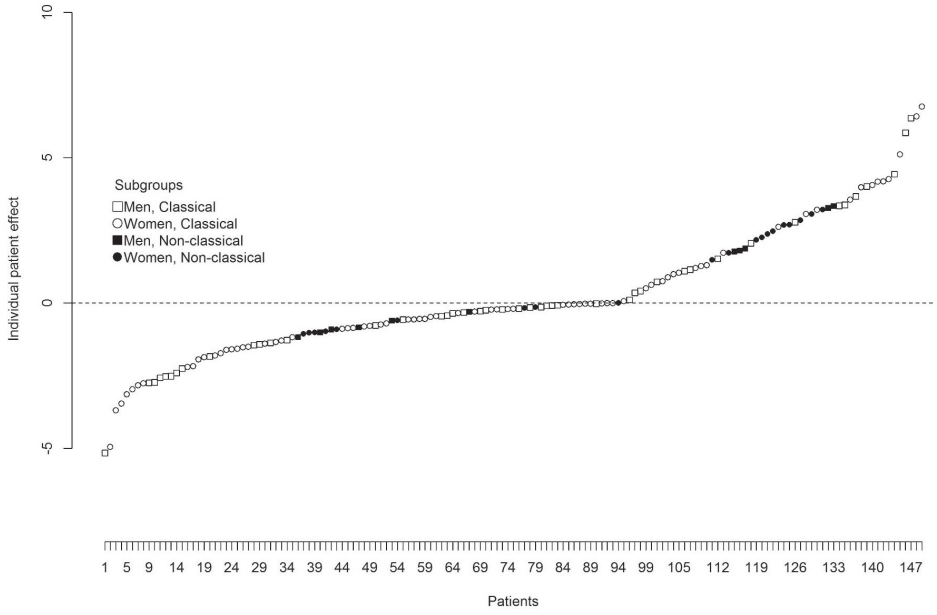
We used the following variables for imputation of cardiac LVMi:

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

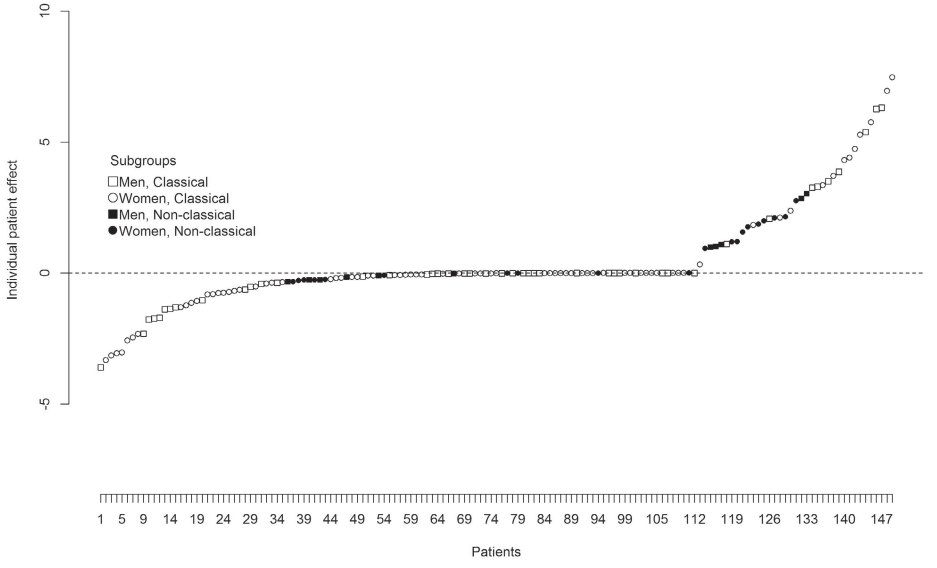
Patient id was classified as grouping variable, adapting the imputation to the multilevel structure. Cardiac LVMi was imputed with a method adapted to the multilevel structure of the data and continuous nature of the variable ("2l.pan"). The number of datasets created was set at 10 and the number of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



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



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



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

Basilar artery diameter and enzyme replacement therapy

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

Supplemental table 5 Patient characteristics at last brain MRI

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

Continuous variables are presented as median (range) and discrete variables as number (percentages).
** All five patients have had a renal transplantation, two patients are post-dialysis.*
eGFR = estimated glomerular filtration rate, ERT = enzyme replacement therapy, LVOTO = left ventricular outflow tract obstruction, TIA = transient ischemic attack

Supplemental table 6 Missing data of cerebral MRIs and other variables

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

Discrete variables are presented as number (percentages). Variables without missing data are not present (such as age, atrial fibrillation) BAD = Basilar artery diameter, eGFR = estimated glomerular filtration rate, LDL = low density lipoproteins, LVMi = left ventricular mass index, WMLs = white matter lesions

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

	Fazekas score		Infarctions	
Fixed effects	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Model 2 + Age ≥ 30 years	1.0	-	1.0	-
Age < 30 years	0.23 (0.10-0.58)	0.0016	0.26 (0.03-2.10)*	0.2068
<6 months of ERT	1.0	-	1.0	-
≥6 months of ERT	1.02 (0.67-1.56)	0.9292	1.86 (0.67-5.19)*	0.2349
Interaction treatment ERT and age < 30 years				
(≥6 months of ERT * Age ≥ 30 years)	1.0	-	1.0	-
(<6 months of ERT * Age < 30 years)	0.70 (0.47-1.05)	0.0820	1.33 (0.49-3.59)*	0.5739
Men with a classical disease phenotype				
Age	1.54 (1.41-1.69)	<0.0001	NA	-
LysoGb3 before start ERT	1.08 (1.02-1.14)	0.0073	NA	-
Age	1.58 (1.43-1.73)	<0.0001	1.28 (1.15-1.43)	<0.0001
Antibodies				
No antibodies present#	1.0	-	1.0	-
Antibodies present#	0.42 (0.11-1.65)	0.2130	4.57 (0.31-67.4)	0.2684
Age	1.55 (1.41-1.69)	<0.0001	1.30 (1.16-1.46)	<0.0001
Mutation				
Missense	1.0	-	1.0	-
Nonsene/frameshift	12.8 (1.04-158.9)	0.0469	23.5 (0.84-653.5)	0.0630
Other	190.5 (2.1-17241)	0.0224	64.6 (0.21-19527)	0.1525
Age	1.52 (1.38-1.68)	<0.0001	1.27 (1.12-1.44)	0.0002
Changes in BAD	1.38 (0.59-3.23)	0.4540	1.69 (0.38-7.61)	0.4912

** The model was unable to run with non-classical patients included, probably due to the low number of non-classical patients with infarctions. Thus, for this analysis only classical patients were included. # No antibodies present includes two men with transient antibodies. Antibodies present includes one man with a history of antibodies who stopped treatment with ERT on his own request.*
BAD = basilar artery diameter, CI = confidence interval, ERT = enzyme replacement therapy, NA = not available, does not converge, OR = odds ratio

Supplemental table 8 Scan frequency algorithm

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

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

Supplemental discussion

Power calculation

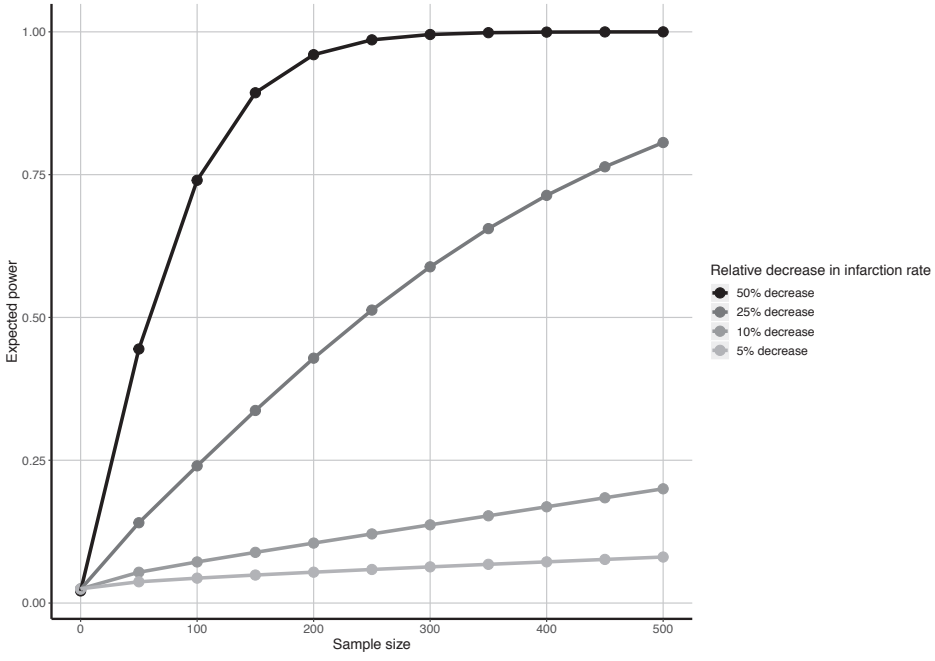
We used the Fazekas and infarction data within men with classical FD since progression risk was highest in this group. Effect of enzyme replacement therapy was regarded as negligible so we included all patients (treated and untreated). We calculated sample sizes needed for trials looking to decrease the infarction rate or to stabilize the Fazekas score, with the outcomes categorized as binary [23] and ordinal [23, 24], respectively.

Infarctions on MRI were present in 0% of men with classical disease at 20 years old and in 50% at ~45 years old (**Figure 1**). Hypothetically, a new treatment might relatively reduce the infarction rate with 50%, 25%, 10% or 5%, resulting in an absolute infarction rate reduction at 45 years of age of 25% (50% of 50%), 12.5% (25% of 50%), 5.0% (10% of 50%), and 2.5% (5% of 50%), respectively.

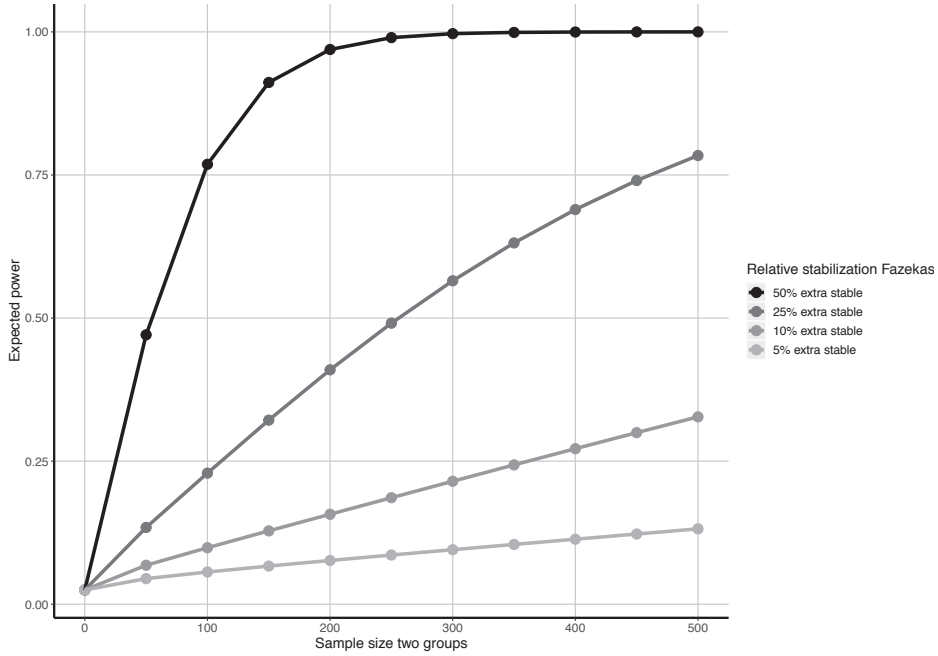
If a new treatment would result in a relative infarction reduction of 50%, a group of 116 men with classical disease (58 per treatment arm) should be followed for ~25 years (start treatment at 20 years and stop trial at 45 years), for a power of 0.8 and significance level of 0.05 (**Supplemental figure 4**). If a new treatment would result in a relative infarction reduction of only 5%, a group of 12548 men with classical disease (6274 per treatment arm) should be followed for ~25 years for a power of 0.8 and significance level of 0.05.

The Fazekas score started to increase from 20 years old (coded as Fazekas score 0: n=45) and increased until the end of follow-up at a median of 45 years old (data from study Fazekas score 0: n=19, 1: n=7, 2: n=4, 3: n=2, 4: n=4, 5 and 6: n=9). A total of 26 patients had a Fazekas score >0. Hypothetically, a new treatment might stabilize the Fazekas score in 50%, 25%, 10% or 5% of patients. Using a random number generator we randomly selected an additional 50% (n=13), 25% (n=5), 10% (n=3), or 5% (n=1) of patients that had a Fazekas score >0 and recoded them as 0. The new Fazekas score was then compared to the old score.

If a new treatment would result in a stabilization of 50% of men with classical disease at a Fazekas score of 0, a group of 106 men with classical disease (53 per treatment arm) should be followed for ~25 years (start treatment at 20 years and stop trial at 45 years), for a power of 0.8 and significance level of 0.05 (**Supplemental figure 5**). If a new treatment would result in a relative infarction reduction of only 5%, a group of 5518 men with classical disease (2759 per treatment arm) should be followed for ~25 years for a power of 0.8 and significance level of 0.05.



Supplemental figure 4 Power calculation for a relative reduction of the infarction rate of 50%, 25%, 10% and 5% in 0 to 500 men with classical disease.



Supplemental figure 5 Power calculation for a relative reduction of the infarction rate of 50%, 25%, 10% and 5% in zero to 500 men with classical disease.

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PART 2

QUALITY OF LIFE, DEPRESSIVE SYMPTOMS AND COGNITIVE FUNCTIONING IN FABRY DISEASE

4

PHENOTYPE, DISEASE SEVERITY AND PAIN ARE MAJOR DETERMINANTS OF QUALITY OF LIFE IN FABRY DISEASE: RESULTS FROM A LARGE MULTICENTER COHORT STUDY

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Abstract

Quality of life (QoL) is decreased in patients with Fabry disease (FD). To improve QoL, it is important to understand the influence of FD related characteristics, symptoms and complications. In this retrospective cohort study we explored the effect of pain (measured by the Brief Pain Inventory), phenotype, treatment and FD-related complications on QoL. QoL data of Fabry patients as assessed by the EuroQol five dimension questionnaire (EQ-5D) from two international centers of excellence were collected. The aim of this study was to evaluate the effect of sex, phenotype, age, different states of disease severity, pain and ERT on EQ-5D utilities.

For 286 adult FD patients (mean age 42.5 years, 40% men, 60% classical phenotype) 2240 EQ-5Ds were available. QoL is decreased in men as well as women with FD, especially in older men with a classical phenotype. At age 50, utility was lower in men with classical FD compared to those with non-classical disease (β =-0.12, 95% CI: -0.23 – 0.01, p=0.037) with further difference in the years thereafter. Cardiovascular complications, stroke or transient ischemic attacks, multiple FD-related complications and pain were also associated with decreased utilities. Overall, no change in utility was seen in patients on ERT over a mean follow-up of 6.1 years.

FD leads to a decreased QoL compared to the general population. Disease complications and pain both negatively influence QoL. Adequate assessment and treatment of pain as well as improved strategies to prevent disease complications are needed to improve QoL in the FD population.

Introduction

Fabry disease (FD; OMIM 301500) is a rare X-linked lysosomal storage disorder with a heterogeneous disease course. The disease is caused by a deficiency of the enzyme α-galactosidase A (enzyme commission no. 3.2.1.22) due to mutations in the GLA-gene. This results in accumulation of globotriaosylceramide (Gb3) and related sphingolipids in cells throughout the body and may cause clinical complications, especially in kidney, heart and brain. Despite the X-linked inheritance pattern, women are affected as well, and may develop similar symptoms and complications as men [1, 2]. Both men and women with FD experience a decreased QoL [3-6].

Phenotypically, FD can be divided into classical or non-classical disease. Men with classical FD generally have no residual enzyme activity and often exhibit Fabry-specific symptoms including neuropathic pain, cornea verticillata and angiokeratoma. Men with non-classical FD and women with either classical or non-classical FD have residual enzyme activity, usually resulting in a milder disease course. Older studies showed severely decreased QoL, predominantly in men who nowadays most likely would be considered to have classical FD [7, 8]. Also in more recent studies a distinction in phenotypes has not been made. In other words, the effect of phenotype on QoL has yet to be elucidated.

Part of the decreased QoL in patients with FD seems to be associated with the neuropathic pain often seen in men with classical FD [7]. Episodes of severe, debilitating burning pains can be alternated with chronic pain, mostly in hands and feet. Moreover, the presence of gastro-intestinal (GI) symptoms, including GI-pain, is also associated with lower QoL [9]. Several studies reported a positive effect of enzyme replacement therapy (ERT) on pain [10-12]. In contrast, no clear effect of ERT on QoL was established in a recent systematic review from our group [6]. Besides pain, complications linked to FD such as end stage renal disease (ESRD), cardiomyopathy and stroke have been associated with decreased QoL [7, 8, 13]. For the purpose of a cost-effectiveness analysis, Rombach et al. created mutually exclusive disease states to simulate the disease course of FD [14]. Lower QoL was found in patients in a more severe disease state. However, the sample size necessitated grouping of different complications in one single group, so the effect of individual cerebral, renal or cardiac complications on QoL remained unknown. Better understanding of QoL in different disease states and improved understanding of the influence of specific symptoms and complications on QoL may facilitate targeted treatment, and thereby improve well-being of Fabry patients. With this study we aim to gain insight into the influence of sex, phenotype, age, disease severity and ERT on QoL.

Methods

Study design

Using local databases containing prospectively collected data as well as medical records, demographic, clinical and laboratory data of all FD patients from two centers of excellence (Academic Medical Center (AMC), The Netherlands; and Royal Free London NHS Foundation Trust (RFH), United Kingdom) were merged into one database. This cohort represents the part of a larger study [15] with available EuroQol five dimension questionnaire (EQ-5D) data. Baseline was defined as the date of the first EQ-5D measurement, except for the evaluation of the influence of ERT on the QoL where the start date of ERT was used as baseline.

According to Dutch law, and after review of the AMC ethics committee, no approval of the study protocol was needed because of the observational nature of the study. All data were obtained from medical records. Patient records were anonymized and de-identified prior to analysis. All patients have provided consent for the use of their medical data and samples in accordance with local ethics requirements.

Study participants

Adult patients (≥18 years) with a definite FD diagnosis according to previously developed criteria [16] of whom sufficient data for phenotypical classification and one or more EQ-5D measurements were available, were included. They were categorized as classical or non-classical on the basis of enzyme activity and the presence or absence of characteristic FD symptoms (Fabry neuropathic pain, clustered angiokeratoma and/or cornea verticillata [17]). A detailed description of the classification method has been published earlier (**Supplement A** [15]).

EQ-5D

The EQ-5D is a QoL questionnaire that covers five different QoL domains: Mobility, Self-care, Anxiety/Depression, Usual activities and Pain/Discomfort [18]. Respondents are asked to choose per domain which one of the following three options describes their situation best: No problems, Some/Moderate problems or Extreme problems [19]. EQ-5D data can be presented as a health profile which shows the frequency of reported problems for each level for each dimension [19]. Also, a utility for the health status can be calculated by combining the responses on all five domains. A utility of 1 means perfect health and a score of 0 represents death. Negative scores can also be obtained representing health states that are considered worse than death. Utilities differ per country. For our study we used the Dutch and UK weighing for Dutch and English patients, respectively [20, 21].

Pain assessment

The AMC and the RHF both used the Brief Pain Inventory (BPI) to assess the presence and severity of pain and its influence on daily life. All BPI scores closest to the utility with a maximum window of ±3 months were used. The BPI assesses pain at its worst, average pain and pain interference with life. The interference score measures the influence of pain on general activity, walking, work, mood, enjoyment of life, relations and sleep. It is the mean of at least four of these items. Worst pain, average pain and the interference score are graded from 0 (pain is absent) to 10 (worst possible pain) [22].

Disease severity

To evaluate the effect of symptoms, organ involvement and complications in FD, patients were classified in ten mutually exclusive disease states with increasing severity [14] (**table 1**). According to strict criteria, patients can transition from one state to another in case of disease progression.

Clinical and laboratory measurements

Renal function was evaluated by the estimated glomerular filtration rate (eGFR) using the CKD-EPI formula [23] and proteinuria. Cardiac involvement was assessed by echocardiography. Left ventricular mass (LVM) was calculated using the Devereux formula and was corrected for height ($m^{2.7}$) [24]. Left ventricular hypertrophy was defined as LVM ≥49 and ≥45 gram/$m^{2.7}$ in men and women, respectively [24]. The presence of white matter lesions (WMLs)/ischemic lesions was investigated by cerebral MRI. Plasma lysoGb3 levels were measured with tandem mass spectrometry with glycine labeled (RFH and AMC after August 2015) or isotope labeled lysoGb3 (AMC before August 2015) as an internal standard [15, 25].

Statistical methods

R (version 3.1.5) and SPSS for Windows, version 22.0 (SPSS Inc. Chicago, Illinois, USA) were used. First, utilities per sex and phenotype were calculated, followed by a second order polynomial regression mixed effect model with a random intercept and slope to evaluate the effect of age on utilities, stratified for sex and phenotype. To evaluate the relation between BPI score and utility, the polynomial mixed effect model was extended by including BPI scores as covariate.

Second, the effect of ERT on utilities was investigated with a linear mixed model including time on ERT and age at ERT initiation as time-dependent covariates and a mixed model of the difference between the utility at baseline and follow up measurements. Since QoL is known to fluctuate over time, patients were only included in this analysis if they completed an EQ-5D within three months before the start of ERT. Finally, utilities per

disease state for the combined cohort of men and women with classical and non-classical disease were modeled.

To account for the fact that one patient may have filled in more than one EQ-5D per disease state, a linear mixed effect model with the disease state as covariate and a random intercept was used to evaluate the utility per disease state. Patient numbers were too small to include sex and phenotype. In order to analyze the effect of eGFR, LVM and WML on QoL within the "organ involvement" disease state, univariate and multivariate analyses were performed within this group. Data are presented as mean ± standard deviation (SD) or median and range dependent on the distribution of data. Where appropriate, 95% confidence intervals (95% CI) are given. P-values <0.05 were considered statistically significant.

Table 1 Description of disease states

Disease state*	**Description**
Asymptomatic	
No organ involvement	No left ventricular hypertrophy, kidney disease, white matter lesions or complications
Symptoms	
Neuropathic pain	A history of Fabry neuropathic pain in the extremities provoked by heat, fever or exercise (also referred to as acroparesthesia)
Organ involvement	Left ventricular hypertrophy, chronic kidney disease stages 2-4, albuminuria/proteinuria or white matter lesions
Single complication	
End stage renal disease	Chronic kidney disease stage 5 (eGFR <15ml/min/1.73m2), dialysis or kidney transplant
Cardiac complication(s)	Atrial fibrillation, any other rhythm disturbance needing hospitalization, pacemaker or implantable cardiac defibrillator (ICD) implantation, cardiac congestion for which hospital admittance was needed, myocardial infarction, percutaneous coronary intervention or coronary artery bypass graft
Cerebrovascular accident	Transient ischemic attack (TIA) or stroke, as diagnosed by a neurologist
Multiple complications#	
End stage renal disease and cardiac complication(s)	
End stage renal disease and cerebrovascular accident	
Cardiac complication(s) and cerebrovascular accident	
End stage renal disease and cardiac complication(s) and cerebrovascular accident	

** Typically, patients progress from the asymptomatic state or neuropathic pain state to the symptoms state; from the symptoms state to a single complication state; from a single complication state to a double complication state, and from a double complication state to the triple complication state. # Since the number of patients in the disease states representing more than one complication was low, one combined 'multiple complications' disease state was made.*

Results

The merged database contained data on 439 patients from the AMC and the RFH, of whom 27 patients did not fulfill the criteria for a definite diagnosis, 12 had insufficient baseline data for assessment of disease severity and no follow-up data, and 114 patients did not complete one or more EQ-5D measurements. Two-hundred-eighty-six FD patients (117 patients from the AMC and 169 patients from the RFH) with a mean age: 42.5 ± 12.5 years completed 2240 EQ-5Ds. Each patient completed on average 7.8 ± 4.5 EQ-5Ds during a mean follow-up period of 5.4 ± 3.2 years. Classically affected patients completed more EQ-5Ds compared to non-classically affected patients. **Table 2** shows the baseline characteristics at the time of completion of the first EQ-5D.

Health profile at baseline per phenotype and per disease state

The health profile of the first completed EQ-5D (**table 3**) shows that 35.5% of all men with classical FD reported some/moderate problems of mobility with one patient reporting extreme problems. Lower percentages (21.9% to 28.9%) of women and men with non-classical disease reported some/moderate mobility problems. Self-care was relatively preserved in all subgroups of patients, while 35.3% experienced some/moderate problems with their usual activities with percentages ranging from 25.0% in women with non-classical FD to 42.1% in men with classical disease. Twenty-one patients (7.3%) experienced extreme pain at baseline and almost two-thirds of the men with classical FD experienced at least some/moderate pain. Some/moderate anxiety or depressive symptoms were noted in about one third of men with classical FD and women with classical and non-classical FD.

The health profiles of the first EQ-5D per disease state (**supplemental table B**) indicated that a large proportion of patients in the 'neuropathic pain' disease state reported problems with their usual activities and anxiety/depression domain, and some/moderate to extreme problems in the pain/discomfort domain. Since the number of patients in the disease states representing more than one complication was low, one combined 'multiple complications' disease state was made, representing patients with complications in at least two organs (kidney, heart and/or brain). Patients in the 'single' and 'multiple complication' disease states reported problems across all domains.

Table 2 Characteristics of all patients at first EQ-5D measurement

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
Patients, n (%)	286	76 (26.6)	38 (13.3)	96 (33.6)	76 (26.6)
Age in years, mean (±SD)	42.5 (±15.5)	37.4 (±12.5)	54.2 (±15.4)	44.0 (±15.5)	40.7 (±15.2)
Age first visit, mean (±SD)	40 (±16.0)	34.2 (±12.5)	52.8 (±15.8)	42.6 (±15.6)	37.8 (±15.5)
History of ERT, n (%)	125 (43.7)	57 (75.0)	13 (34.2)	41 (42.7)	14 (18.4)
Currently on ERT, n (%)	117 (40.9)	52 (68.4)	13 (34.2)	39 (40.6)	13 (17.1)
Time on ERT in years, mean (±SD)	2.97 (±2.38)	3.69 (±2.61)	1.94 (±2.03)	2.73 (±2.05)	1.87 (±1.89)
Events before first EQ-5D					
Any event, n (%)	50 (17.5)	16 (21.1)	14 (36.8)	16 (16.7)	4 (5.3)
Cardiac event, n (%)	33 (11.5)	8 (10.5)	12 (31.6)	9 (9.4)	4 (5.3)
Renal event, n (%)	8 (2.8)	5 (6.6)	2 (5.3)	1 (1.0)	0 (0)
Cerebral event, n (%)	20 (7.0)	8 (10.5)	3 (7.9)	9 (9.4)	0 (0)
WML, n (%)	112 (39.2)	34 (44.7)	11 (28.9)	41 (42.7)	26 (34.2)
eGFR in ml/min/1.73m^2, mean (±SD)	93.8 (±25.9)	99.0 (±30.3)	80.6 (±26.3)	96.7 (±21.6)	91.5 (±23.7)
eGFR <60 ml/min/1.73m^2, n (%)	29 (10.3)	10/76 (13.2)	6/38 (15.8)	5/94 (5.3)	8/73 (11.0)
LVM in gr/m$^{2.7}$, median (range)	42.0 (16.2-139.9)	48.3 (23.6-110.4)	54.2 (16.2-99.9)	40.8 (18.2-139.9)	34.5 (17.1-96.2)
LVM >upper ref limit, n (%)	101 (37.4)	33/74 (44.6)	20/35 (57.1)	32/92 (34.8)	16/69 (23.2)
LysoGb3* in nmol/L, median (range)	7.5 (0.4-150.3)	105.5 (31.8-150.3)	6.0 (1.2-22.4)	7.6 (0.7-27.2)	2.0 (0.4-15.4)
BPI average pain*, median (range)	2 (0-8)	2 (0-8)	0 (0-7)	3 (0-8)	3 (0-8)
BPI worst pain*, median (range)	3 (0-10)	3 (0-10)	0 (0-9)	3 (0-9)	3 (0-10)
BPI average interference*, median (range)	0.6 (0-9.9)	0.5 (0.0-8.4)	0.1 (0.0-9.9)	0.5 (0.0-9.3)	1.1 (0.0-9.7)
EQ-5Ds#, n	2240	668	286	771	515
EQ-5Ds per patient#, mean (±SD)	7.8 (±4.5)	8.8 (±4.8)	7.5 (±4.1)	8.2 (±4.5)	6.8 (±4.4)
Follow-up time#, mean (±SD)	5.38 (±3.15)	5.73 (±3.46)	4.71 (±2.86)	5.56 (±2.98)	5.13 (±3.15)

Events represent the number of patients with one or more events before first EQ-5D. Events were defined as described at end stage renal disease, cardiac complications and cerebrovascular accident similar to the definition of the disease states (table 1).
*ERT = enzyme replacement therapy, WML = white matter lesions on MRI, eGFR = estimated glomerular filtration rate, LVM = left ventricular mass index on echocardiography, BPI = Brief Pain Inventory. Upper reference limit LVM: ♂ = 51 / ♀ = 48. Normal range lysoGb3 = 0.3-0.6 nmol/L. LysoGb3 represents values before start of ERT. * Values missing: LysoGb3 32%, BPI Average pain 16%, BPI Worst pain 15%, BPI Average Interference 12%. # Values acquired during follow-up*

Table 3 Health profile and utility of the first EQ-5D measurement stratified for sex and phenotype

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
Mobility	number of patients (%)				
1*	207 (72.4)	48 (63.2)	27 (71.1)	75 (78.1)	57 (75.0)
2	78 (27.3)	27 (35.5)	11 (28.9)	21 (21.9)	19 (25.0)
3	1 (0.3)	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
Self-care	number of patients (%)				
1	261 (91.3)	64 (84.2)	34 (89.5)	93 (96.9)	70 (92.1)
2	22 (7.7)	10 (13.2)	4 (10.5)	3 (3.1)	5 (6.6)
3	3 (1.0)	2 (2.6)	0 (0.0)	0 (0.0)	1 (1.3)
Usual activities	number of patients (%)				
1	173 (60.5)	40 (52.6)	25 (65.8)	57 (59.4)	51 (67.1)
2	101 (35.3)	32 (42.1)	12 (31.6)	38 (39.4)	19 (25.0)
3	12 (4.2)	4 (5.3)	1 (2.6)	1 (1.0)	6 (7.9)
Pain/discomfort	number of patients (%)				
1	138 (48.3)	29 (38.2)	22 (57.9)	47 (49.0)	40 (52.6)
2	127 (44.4)	43 (56.6)	15 (39.5)	40 (41.7)	29 (38.2)
3	21 (7.3)	4 (5.3)	1 (2.6)	9 (9.4)	7 (9.2)
Anxiety/depression	number of patients (%)				
1	191 (66.8)	51 (67.1)	27 (71.1)	65 (67.7)	48 (63.2)
2	90 (31.5)	25 (32.9)	9 (23.7)	31 (32.3)	25 (32.9)
3	5 (1.7)	0 (0.0)	2 (5.3)	0 (0.0)	3 (3.9)
Utility#, mean (±SD)	0.77 (±0.26)	0.75 (±0.25)	0.81 (±0.27)	0.79 (±0.23)	0.76 (±0.30)

** 1 = No problems, 2 = Some/Moderate problems, 3 = Extreme problems*
 # Please note that a baseline utility is presented in contrast to Figure 1 which presents longitudinal utilities

Relation between phenotype, age and utilities

A decrease in utilities with age was seen in all subgroups (**figure 1**), although the extent of this relation differed between the subgroups. The mixed model revealed that the utility of a 50-year-old man with classical FD is on average 0.12 points lower (95% CI: -0.23 – 0.01, p=0.037) compared to a man with non-classical FD of the same age. At age 60 this mean difference has increased to 0.21 points (95% CI: -0.36 – -0.07, p=0.004). This illustrates the progressive worsening of utilities in men with classical FD with increasing age. In women and men with non-classical FD the decline in utilities with age was less strong (**figure 1**).

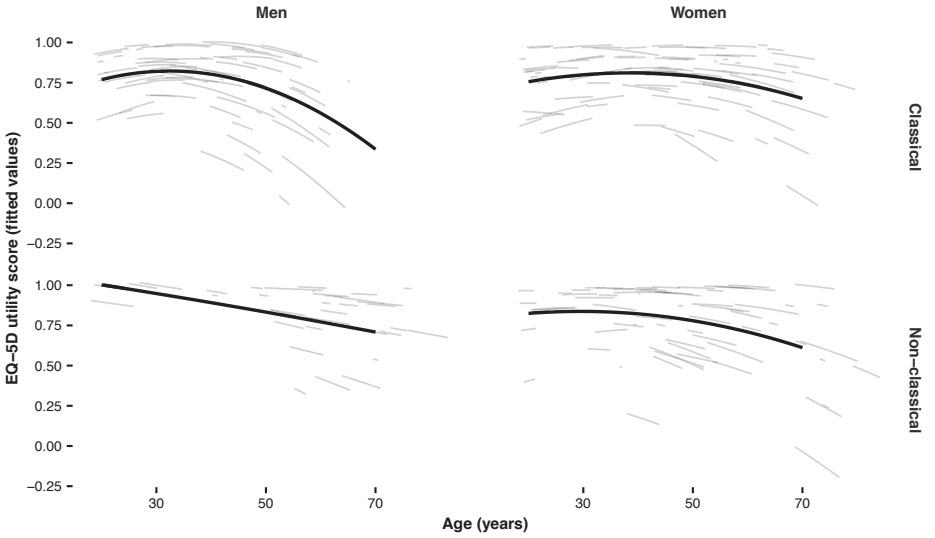

Figure 1 Relation between EQ-5D and age. Polynomial mixed effect model of EQ-5D in relation to age, stratified for sex and phenotype. Large lines represent fitted values at group level, the smaller lines represent the fitted values at individual patient level.

Relation between BPI scores and utilities

In total 1559 BPIs of 276 patients were matched to an EQ-5D. Of the patients that completed BPIs 45.3% were men, 61.2% had the classical phenotype and the mean age was 44.8 ± 14.9 years. Utilities decreased with higher BPI scores: with every one point higher BPI average pain score, the utility decreased on average with 0.045 points (β = -0.045, 95% CI: -0.049 – -0.040, p<0.001). Similarly, an increase in BPI worst pain score (β = -0.035, 95% CI: -0.039 – -0.031, p<0.001) or BPI interference score (β = -0.058, 95% CI: -0.063 – -0.053, p<0.001) resulted in lower utilities. A sensitivity analysis without the pain/discomfort domain in the calculation of the utilities did not change the results.

The effect of ERT on utilities

For the evaluation of the effect of ERT on utilities, 61 patients were analyzed who completed at least one EQ-5D before the initiation of ERT. The mean age of patients at ERT initiation was 44.2 ± 15.5 years, and the mean follow-up time after initiation of ERT was 6.1 ± 2.5 years. The median utility score before ERT was 0.796 (-0.166 – 1.000). Utility remained unchanged after start of treatment (β = -0.004, 95% CI: -0.066 – -0.058, p=0.89). Furthermore, there was no relation between change in utility, time on ERT (β = -0.005, 95% CI: -0.016 – -0.006, p=0.40) and age at ERT initiation (β=-0.002, 95% CI: -0.006 – 0.001). In a subgroup analysis, we found that utilities increased after initiation of ERT in the 13 men with classical FD (β = 0.17, 95% CI: 0.06 – 0.28, p=0.003). This was primarily

attributable to 3 patients with a very low utility before the start of ERT due to extreme pain/discomfort which improved substantially after the start of treatment. One of them started taking carbamazepine for his neuropathic pains during the same period, leading to a substantial decrease in neuropathic pain in the months thereafter. Without these three patients no change in utility was observed (β = 0.04, 95% CI: -0.07 – 0.14, p=0.50). Additional subgroup analyses revealed that no annualized change in utility was observed in women with classical or non-classical disease, while in men with non-classical FD a 0.027 point decline per year on ERT (95% CI: -0.053 – 0.001, p=0.04) was found.

Relation between disease severity and utilities

Table 4 shows the mean utility per disease state for men and women with classical and non-classical FD combined. Within the "organ involvement" disease state we found no relation between eGFR, LVMI and WML on the one hand and utilities on the other hand. Patients included in the advanced disease states, and thus with more severe disease, were older and more often men with classical FD. Compared to the 'no organ involvement' disease state, the utilities were significantly lower in the 'cardiac complication(s)', 'cerebrovascular accident' and the 'multiple complications' disease states but not the 'end stage renal disease' disease state. The utility of the latter was based on a low number of patients (n=7) who showed divergent utility scores. The lowest utility was found in the 'multiple complications' disease state (β = 0.530, 95% CI: 0.42-0.63). The 'neuropathic pain' disease state also showed a trend towards lower utility compared to the 'no organ involvement' disease state (p=0.054).

Discussion

This study shows that QoL in patients with FD is related to phenotype, age, pain and disease severity. Obviously, these features are related to each other; classically affected patients of older age will have more severe disease with a higher chance of developing FD-related complications and thus a decreased QoL. Additional analyses to study the independent effects of these features on QoL were not feasible due to limited patient numbers and the expectation of high multicollinearity.

The mean utility of FD patients in the present study ranged from 0.75 in men with classical FD to 0.81 in men with non-classical disease, and QoL decreased with advancing age. Comparison of the health profile of these patients to the health profile of the general population in the UK and the Netherlands supports a higher prevalence of impaired QoL in FD (**supplemental table C**) [26, 27]. In line with the current results, a recent study in a mixed cohort of treated and untreated men and women with FD showed a mean utility of 0.79 [28].

Table 4 Utility per disease state

Disease state	No organ involvement	Neuropathic pain	Organ involvement	End stage renal disease	Cerebrovascular accident	Cardiac complication(s)	Multiple complications
Patients*, n	31	21	221	7	16	45	18
EQ-5Ds, n	103	71	1521	56	100	290	99
Health utility, mean (95% CI)	0.851 (0.77 - 0.93)	0.725 (0.63-0.82)	0.783 (0.75-0.81)	0.828 (0.67-0.99)	0.705 (0.60-0.81)	0.732 (0.67-0.80)	0.530 (0.42-0.64)
P-value#	-	0.053	0.123	0.796	**0.037**	**0.026**	**<0.001**
Woman, n (%)	26 (83.9)	15 (71.4)	138 (62.4)	1 (14.3)	8 (50.0)	20 (44.4)	6 (33.3)
Classical phenotype, n (%)	5 (16.1)	19 (90.5)	136 (61.5)	6 (85.7)	15 (93.8)	21 (46.7)	14 (77.8)
Age in years, mean (±SD)	32.0 (±10.1)	26.5 (±8.6)	41.0 (±14.1)	45.8 (±12.9)	49.3 (±10.1)	59.2 (±11.0)	60.5 (±8.4)
History of ERT, n (%)	3 (9.7)	2 (9.5)	107 (48.4)	5 (71.4)	13 (81.2)	32 (71.1)	14 (77.8)
Now ERT, n (%)	3 (9.7)	2 (9.5)	97 (43.9)	5 (71.4)	13 (81.2)	31 (68.9)	13 (72.2)
Time on ERT in years, mean (±SD)	1.40 (±0.49)	0.98 (±1.31)	2.72 (±2.14)	3.06 (±1.72)	4.67 (±4.08)	4.91 (±4.04)	6.14 (±2.81)

ERT = Enzyme Replacement Therapy
** Patients may have more than one EQ-5D per disease state and may contribute to more than one disease state. # P-values were calculated with 'No organ involvement' as reference group.*

In contrast, a cohort study from the pre-ERT era in which 38 men of similar age and with presumed classical disease were included, showed a substantially lower utility of 0.56 [8]. These patients had more often suffered from one or more complications [8]. Therefore, a possible explanation for the difference in utilities is that in our cohort ERT delayed the occurrence of complications and therefore delayed the decrease in QoL caused by complications [29]. Indeed, in a recent study from our group on the natural course of FD stratified by sex and phenotype, men with classical disease were shown to have the highest risk of developing complications [15]. The median age at first complication was approximately 50 years, which corresponds with the age after which the decrease in QoL accelerates in these men in the present study. The fact that the utility in the pre-ERT cohort resembles the utility in our 'multiple complications' disease state further supports this. It is unlikely that QoL scores in the investigated FD populations are influenced by regional differences, since the QoL scores in the general population in the UK and the Netherlands are comparable (**supplemental table C**). However, other factors may have contributed to the higher QoL in the present study compared to the pre-ERT cohort, such as pain management. Indeed, a detailed look at the health profiles of the different subgroups of patients indicates that the prevalence of extreme pain seems to have decreased when compared to older studies (**supplemental table C**) [8, 11]. However, pain is still present in around half of the patients and associated with lower QoL in FD, as also established in a Fabry Outcome Survey study [11]. Moreover, chronic pain is related to decreased QoL in all domains in the general population [30]. Therefore, QoL is expected to increase if pain control is improved. An often mentioned cause of pain in patients with FD is neuropathic pain in the extremities (also called acroparesthesia), which is associated with decreased QoL [7]. However, in this study, men with non-classical FD and women, who are known to have a low prevalence of Fabry-related neuropathic pain, also frequently reported pain. Indeed, a previous study has shown that other types of pain (e.g. musculoskeletal pain or GI-pain) may play an important role in the life of FD patients [31]. As a consequence, it is recommended to assess individual causes of pain and manage it accordingly [31-33].

The reported percentage of extreme anxiety/depression in the health profile has also decreased compared to older studies [8, 11]. The availability of treatment in itself can provide hope or relief of complaints and might reduce anxiety/depressive complaints [34]. On the other hand, in other metabolic diseases it has been speculated that biweekly infusions are burdensome, especially in the hospital setting, thereby potentially affecting QoL [35, 36]. In our study, no effect of ERT was seen on the percentage of patients with anxiety/depression.

Previous studies have not been able to unequivocally determine the effect of ERT on QoL [6]. The current study showed that QoL did not change over six years of follow-up in patients receiving ERT. However, there are individual differences in the course of QoL with some patients deteriorating while others improve. Of interest to this end is the observation that three out of thirteen men with classical FD had higher utilities after start of ERT, especially in the Pain and Activity domain. However, it is difficult to attribute the improvement in QoL to the start of ERT, because pain may subside spontaneously [37, 38]. Moreover, we could not correct for concomitant analgesic or antidepressant treatment that might improve or stabilize QoL.

In patients with deteriorating QoL, ERT may have been started too late. Indeed, it has been previously shown that ERT is of limited benefit in patients with advanced organ involvement and complications [29]. In the present study complications are clearly associated with a decreased QoL: cerebrovascular accidents, cardiovascular complications and multiple complications resulted in a decrease in utilities of 0.15, 0.12 and 0.32 respectively, all exceeding the minimally clinically important difference of 0.074 [39].

Our study has several limitations. Firstly, the EQ-5D offers three answer options per domain, limiting the detection of small changes in health [40]. Secondly, questionnaire data were gathered during clinical visits, in some patients for up to 15 years in a row. Habituation to repeatedly filling out a questionnaire as well as coping might have influenced the questionnaire accuracy. Thirdly, the lack of a control group hampered the interpretation of the effect of ERT on QoL. Finally, since the QoL data were gathered in an uncontrolled, real-life environment, they were more prone to be influenced by known and unknown confounders, such as presence of concomitant diseases and the use of pain medication. On the other hand, real-life data provides an opportunity to assess QoL in actual practice conditions. Despite these shortcomings an insight has been gained into QoL in FD and its determinants, which can be used to improve the care for these patient

Conclusion

In conclusion, QoL is decreased in men as well as women with FD compared to the general population, especially in older men with a classical phenotype. QoL is lower in patients with FD-related complications and ERT does not seem to have a major impact on QoL. This necessitates the improvement of treatment and preventive strategies. Pain also has a severe impact on QoL. It is prevalent in both sexes and phenotypes and comprises more than neuropathic pain alone. Pain assessment should be an important part of routine follow-up and treatment should be standardized and evaluated accordingly.

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Supplemental A

Phenotypic classification (Adapted from Arends et al (2016)[1] with permission)
Patients were classified as classical or non-classical FD on the basis of their enzyme activity (men only) and the presence or absence of characteristic symptoms [2]. Men were considered to have a classical phenotype when they met the following criteria: 1) a GLA mutation, 2) enzyme activity ≤5% of the mean reference range, 3) ≥1 characteristic FD symptoms (i.e. Fabry neuropathic pain, angiokeratoma and/or cornea verticillata, for definitions see [3]). Men not fulfilling these criteria were categorized as non-classical FD.

Women with a GLA mutation and ≥1 characteristic FD symptoms (i.e. Fabry neuropathic pain, angiokeratoma and/or cornea verticillata [3]) were classified as having a classical phenotype. Women without these characteristic FD symptoms were classified as non-classical FD.

Classification on the basis of phenotypic features and residual enzyme activity was challenging in two groups of patients. It was decided that in these cases a final judgement was made by the treating physician. These groups were:

1) Patients with the N215S mutation: this group is especially prevalent in the UK. According to literature and physician experience, patients exhibit a non-classical (mostly cardiac) phenotype, but exceptions may occur. In this group of 90 patients, 12 had a characteristic symptoms, but without confirmatory deficiency of GLA activity in leucocytes in men (*n = 5*). Notably, one of the N215S patients presented with severe renal disease at young age and had a renal transplantation at age 29. According to the judgement of the treating physician this patient was classified as classical FD while the other N215S patients were all classified as non-classical FD. Similarly, three patients with characteristic symptoms and the P389A mutation (1 man, 1 woman) or R112H (1 woman) mutation were discussed with the treating physician. These patients all had a late onset presentation, only minimal cornea verticillata (no other characteristic FD symptoms) and a family history of non-classical FD. Consequently they were classified as non-classical FD.

2) Men with slightly higher than 5% enzyme activity in the presence of 1 or more characteristic symptoms (*n = 13*). Residual enzyme activity ranged from 6% to 10% in leucocytes (*n = 10)*, and from 6% to 20% in plasma (*n = 3*). All had at least one characteristic FD symptom and the majority had a relative with classical FD and consequently were considered having classical FD. In four men the enzyme activity and/or the data on characteristic FD symptoms were missing. These patients were classified as classical FD according to the opinion of the treating physician, which was mainly based on their family history.

Furthermore, we included three patients (one man, two women, all from the same family) with the A143T mutation. They were classified as having classical FD based on the combination of characteristic deposits on renal biopsy or post mortem biopsy, the presence of one or more characteristic FD symptoms, low enzyme activity (3.9%, 21% and 38% respectively) and high plasma lysoGb3 concentrations (men: 35-50 nmol/l while receiving ERT; woman 1: 16 nmol/l while receiving ERT; woman 2: 8 nmol/l while not receiving ERT). In these cases, a combination of the A143T mutation and an unknown mutation and/or other (genetic) disease modifiers may have caused the classical FD presentation.

Supplemental table A Criteria for phenotypic classification

Classical FD	
Men	**Women**
• A mutation in the GLA gene*	• A mutation in the GLA gene
• ≥ 1 of the following characteristic Fabry disease symptoms: Fabry neuropathic pain, angiokeratoma and/or cornea verticillata	• ≥ 1 of the following characteristic Fabry disease symptoms: Fabry neuropathic pain, angiokeratoma and/or cornea verticillata
• Severely decreased or absent leukocyte AGAL activity (<5% of the normal mean)	
Non-classical FD	
• A mutation in the GLA gene, and not fulfilling the criteria for classical FD	

**The following genetic variants were not considered FD (neutral variants): A143T, P60L, D313Y, R118C, T385A, IVS0-10 C>T, the complex haplotype: IVS0-10 C>T/IVS4-16A>G/IVS6-22C>T. In patients in whom classification on the basis of these criteria was not feasible, the final judgement was made by the treating physician.*

Supplemental table B

Supplemental table B Health profile of first EQ-5D measurement per disease state

Disease state	No organ involvement	Neuropathic pain	Organ involvement	End stage renal disease	Cerebrovascular accident	Cardiac complication(s)	Multiple complications
Patients#, n	31	21	221	7	16	45	18
Mobility (Number of patients (%))							
1*	28 (90.3)	17 (81)	169 (76.5)	4 (57.1)	9 (56.2)	24 (53.3)	5 (27.8)
2	3 (9.7)	4 (19)	52 (23.5)	3 (42.9)	7 (43.8)	21 (46.7)	12 (66.7)
3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)
Self-care (Number of patients (%))							
1	30 (96.8)	20 (95.2)	209 (94.6)	5 (71.4)	13 (81.2)	40 (88.9)	12 (66.7)
2	1 (3.2)	1 (4.8)	10 (4.5)	2 (28.6)	3 (18.8)	5 (11.1)	5 (27.8)
3	0 (0.0)	0 (0.0)	2 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)
Usual activities (Number of patients (%))							
1	25 (80.6)	11 (52.4)	143 (64.7)	4 (57.1)	7 (43.8)	20 (44.4)	5 (27.8)
2	5 (16.1)	10 (47.6)	69 (31.2)	3 (42.9)	9 (56.2)	24 (53.3)	11 (61.1)
3	1 (3.2)	0 (0.0)	9 (4.1)	0 (0.0)	0 (0.0)	1 (2.2)	2 (11.1)
Pain/discomfort (Number of patients (%))							
1	20 (64.5)	8 (38.1)	108 (48.9)	5 (71.4)	5 (31.2)	19 (42.2)	2 (11.1)
2	9 (29.0)	8 (38.1)	96 (43.3)	2 (28.6)	10 (62.5)	22 (48.9)	15 (83.3)
3	2 (6.5)	5 (23.8)	17 (7.7)	0 (0.0)	1 (6.2)	4 (8.9)	1 (5.6)
Anxiety/Depression (Number of patients (%))							
1	20 (64.5)	13 (61.9)	149 (67.4)	5 (71.4)	12 (75.0)	27 (60.0)	11 (61.1)
2	10 (32.2)	8 (38.1)	69 (31.2)	2 (28.6)	4 (25.0)	15 (33.3)	6 (33.3)
3	1 (3.2)	0 (0.0)	3 (1.4)	0 (0.0)	0 (0.0)	3 (6.7)	1 (5.60)

 # Patients may have more than one EQ-5D per disease state and may contribute to more than one disease state
** 1 = No problems, 2 = Some/Moderate problems, 3 = Extreme problems*

Supplemental table C

Supplemental table C Comparison of health profile first EQ-5D of present study with literature

	Present study	**Present study**	**Miners et al (2002)** [4]	**Kind et al (1998)** [5]	**Lamers et al (2006)** [6]
	FD (all patients)	**FD (men with classical disease)**	**FD (pre-ERT cohort)**	**Sample general population**	**Sample general population**
N	286	76	38	3395	298
Age, years (±SD)	42.5 (±15.5)	37.4 (±12.5)	37.2 (±9.2)	Unknown	43.4 (±15.0)
Men, n (%)	114 (39.8)	76 (100)	38 (100)	1562 (46)	152 (51.0)
Classical phenotype, n (%)	172 (60.1)	76 (100)	Unknown	-	-
Country	UK/NL	UK/NL	UK	UK	NL
Mobility	(Number of patients (%))				
1*	207 (72.4)	48 (63.2)	19 (50.0)	2424 (71.6)	258 (86.5)
2	78 (27.3)	27 (35.5)	18 (47.4)	620 (18.3)	40 (13.5)#
3	1 (0.3)	1 (1.3)	1 (2.6)	3 (0.1)	
Self-care	(Number of patients (%))				
1	261 (91.3)	64 (84.2)	28 (73.7)	3285 (95.8)	292 (98.0)
2	22 (7.7)	10 (13.2)	9 (23.7)	139 (4.1)	6 (2.0)#
3	3 (1.0)	2 (2.6)	1 (2.6)	5 (0.1)	
Usual activities	(Number of patients (%))				
1	173 (60.5)	40 (52.6)	17 (44.7)	2829 (83.7)	257 (86.2)
2	101 (35.3)	32 (42.1)	20 (52.7)	481 (14.2)	41 (13.8)#
3	12 (4.2)	4 (5.3)	1 (2.6)	70 (2.1)	
Pain/discomfort	(Number of patients (%))				
1	138 (48.3)	29 (38.2)	10 (26.3)	2268 (67.0)	193 (64.8)
2	127 (44.4)	43 (56.6)	21 (55.3)	988 (29.2)	105 (35.2)#
3	21 (7.3)	4 (5.3)	7 (18.4)	129 (3.8)	
Anxiety/depression	(Number of patients (%))				
1	191 (66.8)	51 (67.1)	19 (50.0)	2687 (79.1)	255 (85.6)
2	90 (31.5)	25 (32.9)	14 (36.8)	648 (19.1)	43 (14.4)#
3	5 (1.7)	0 (0.0)	5 (13.2)	62 (1.8)	

** 1 = No problems, 2 = Some/Moderate problems, 3 = Extreme problems*
N = Number of respondents, UK = United Kingdom, NL = Netherlands.
 # Dutch sample of general population only provided combination of some/moderate and extreme problems as "any problems".

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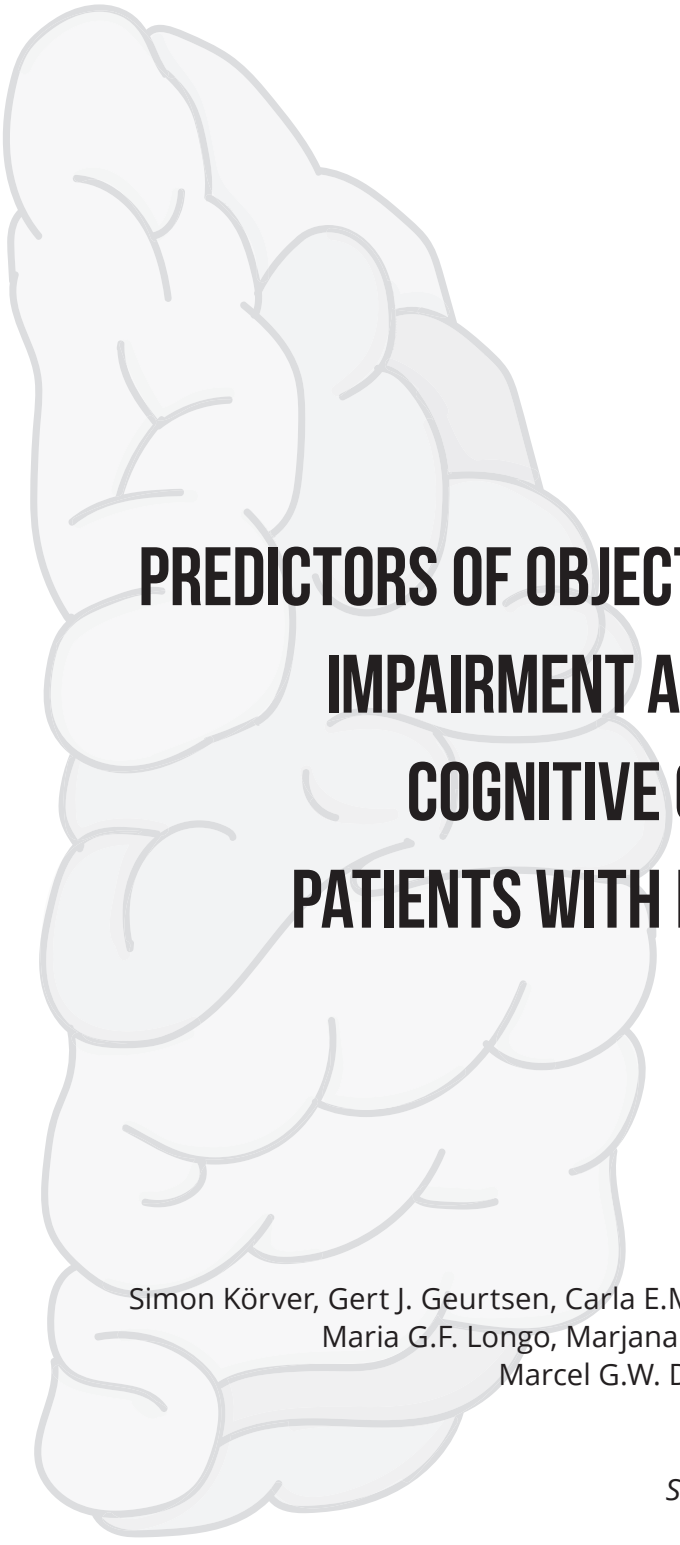

5

PREDICTORS OF OBJECTIVE COGNITIVE IMPAIRMENT AND SUBJECTIVE COGNITIVE COMPLAINTS IN PATIENTS WITH FABRY DISEASE

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Abstract

This study investigates the relationship between objective cognitive impairment (OCI), subjective cognitive complaints and depressive symptoms in men and women with classical and non-classical Fabry disease (FD). Cognitive functioning was assessed using a neuropsychological test battery, subjective cognitive complaints using a structured interview and depressive symptoms using a depression scale (CESD).

Eighty-one patients were included (mean age 44.5±14.3, 35% men, 74% classical). Subjective cognitive complaints were reported by 64% of all patients. OCI was present in thirteen patients (16%), predominantly in men with classical FD. Thirty-one patients (38%) had a high score (≥16) on the CESD scale. Male sex (OR, 6.8; 95%CI, 1.6-39.8; p=1.6*10-2) and stroke (OR, 6.4; 95%CI, 1.1-41.0; p=3.7*10-2) were independently positively associated with OCI, and premorbid IQ (one IQ point increase: OR, 0.91; 95%CI, 0.82-0.98; p=3.8*10-2) was independently negatively associated with OCI. The CESD-score (one point increase: OR, 1.07; 95%CI, 1.02-1.13; p=3.3*10-3) and a history of depression (OR, 2.7; 95%CI, 1.1-7.3; p=3.9*10-2) were independently positively associated with subjective cognitive complaints.

OCI is present in 16% of FD patients, warranting referral for neuropsychological assessment. Nevertheless, subjective cognitive complaints are related to depressive symptoms, emphasizing the importance of recognition and treatment of the latter.

Introduction

Fabry disease (FD; OMIM 301500) is a rare, X-linked, lysosomal storage disorder. A mutation in the GLA-gene causes a lack or absence of enzymatic activity of α-galactosidase A resulting in the accumulation of glycosphingolipids, mainly affecting the cardiovascular and nervous system. Sex and phenotype have been determined as important predictors of the disease course in FD [1]. Women generally have a more attenuated disease course compared to men [1]. Phenotypically, FD can be split in a milder, non-classical and a classical, more severe phenotype, with multi-organ involvement [2].

Frequent cerebral manifestations of FD are the occurrence of white matter lesions (WMLs), TIA and stroke. Furthermore, depressive symptoms are highly prevalent [3]. WMLs, stroke and depression in itself are known to result in objective cognitive impairment (OCI) in the general population [4]. Previous studies on OCI in FD were limited by small sample size, often did not incorporate neuroimaging, combined WMLs and stroke as a single entity and did not differentiate between patient groups with different FD phenotypes and sex [3, 5-7]. Small study populations also restricted the identification of variables related to OCI in FD.

Subjective cognitive complaints are frequently mentioned by FD patients during their routine clinic visits and were related to depressive symptoms but not to OCI in a recent study in a FD population [7]. Extending knowledge on the relationship between subjective cognitive complaints and depressive symptoms and/or OCI can have significant implications for the therapeutic measures indicated to address these complaints.

The objective of this study was to investigate the prevalence of OCI, subjective cognitive complaints, depressive symptoms and to explore their risk factors and interrelation in patients with FD in general as well as in subgroups defined by sex and FD phenotype.

Methods

Study design and phenotype

The baseline data of an ongoing prospective cohort study on neuropsychological functioning in adult patients (≥18 years) with a definite diagnosis of FD [8] are presented. All known adult Fabry patients (n=154) at the Academic Medical Centre (AMC), the national referral centre for patients with FD in the Netherlands, were screened for eligibility.

All patients were phenotypically characterized as having classical or non-classical FD [2], see **Supplemental file 1** for criteria. Demographic parameters, clinical and disease characteristics of all patients were gathered from the local Fabry database containing prospectively collected data as well as from medical records.

This study was approved by the Human Research Ethics Committee of the AMC and conducted in accordance with the Declaration of Helsinki in 2013 [9]. All participants provided informed consent prior to inclusion and all experiments were performed in accordance with relevant guidelines and regulations. The datasets generated and analyzed during the current study are not publicly available. Because of the rarity of the disease, even anonymized can be linked to a specific individual. In case of a specific scientific question, requests to make part of the dataset available will be reviewed.

Neuropsychological assessment

All included patients completed a neuropsychological test battery assessing language skills, memory, visuospatial perception, processing speed and executive functioning. Language skills were assessed using the Boston Naming Test (BNT) [10] and the Wechsler Adult Intelligence Scale IV: Similarities (WAIS-IV: S) [11]. Memory was assessed using the Dutch version of The Rey Auditory Verbal Learning Test (RAVLT) [12] and the Rivermead Behavioural Memory Test (RBMT): Storytelling [13], both assessing immediate recall (ir) and delayed recall (dr). Visuospatial perception was assessed using the WAIS IV: Block Design (BD) and the Judgement of Line Orientation (JLO) [11, 14]. Processing speed was assessed using the Trail Making Test part A (TMTA) [15], Stroop Word (W) and Stroop Colour (C) [16]. Executive functioning was assessed using the TMT part B (TMTB) [15], Stroop Colour-Word (CW) [16], semantic fluencies, referred to as Animal Fluency and Occupational Fluency [17] and phonetic fluency, referred to as Letter Fluency [18].

T-scores (mean of 50, standard deviation (SD) of 10) were calculated per test using extensive Dutch normative data, except for the JLO, for which we used normative data from the United States. The median normative data sample size was 471 healthy participants (range: 121-1000). All normative data were corrected for age and most subtests also for sex and educational level. See **Supplemental Table E-1** for additional information on the neuropsychological test battery.

General cognitive functioning was screened using the Mini Mental State Exam (MMSE) [19]. Motivation and underachievement were assessed using the Test of Memory Malingering (TOMM) [20]. An estimation of intelligence was done using the Dutch Adult Reading Test (DART), the Dutch version of the National Adult Reading which correlates strongly with intellectual ability and is relatively resistant to change by neurological impairment [21].

The neuropsychological test battery was preceded by a structured interview assessing subjective cognitive complaints, addressing the following domains: memory, attention and executive complaints. This resulted in a score ranging from 0 to 3: no subjective cognitive complaints (0), subjective cognitive complaints in one domain (1), in two domains (2) or in all three domains (3), see **Supplemental file 2**.

Objective cognitive impairment

OCI was defined as a T-score ≤33 (<5th percentile, -1.67 SD) on at least two neuropsychological tests, resembling statistical significance of two one-tailed tests with p<0.05. Severe OCI was defined as a T-score ≤30 (<2.3rd percentile, -2 SD) on at least two neuropsychological tests, resembling statistical significance of two two-tailed tests with p<0.05. We choose the cutoff of -1.67 SD to prevent high rates of false positives, a strategy which has been recommended for other diseases as well [22]. To decrease the family-wise error rate, one or more T-scores ≤33 or ≤30 in the following combination of tests assessing a similar cognitive domain, were regarded as a single abnormal T-score: Animal Fluency and/or Occupational Fluency and/or Letter Fluency, RAVLT ir and/or RBMT ir, RAVLT dr and/or RBMT dr, TMTA and/or Stroop W and/or Stroop C, TMTB and/or Stroop CW, WAIS-IV: BD and/or JLO.

Questionnaires

Depressive symptoms were measured using The Centre for Epidemiological Studies – Depression scale (CESD) [23]. Twenty items on depressive symptoms experienced in the last week are scored on a four point Likert scale (range 0 to 3). Total scores range from 0 to 60 points and patients with scores ≥16 were classified as having depressive symptoms [24].

Pain was measured using the Brief Pain Inventory (BPI) which is divided in three separate sub scores: 1) worst pain, 2) average pain and 3) pain interference with life. The latter is an average score of the influence of pain on general activity, mood, walking, work, enjoyment of life, relations and sleep. Pain scores are graded from 0 (absence of pain) to 10 (worst possible pain) [25].

Quality of life (QoL) was obtained using the Dutch version of the Short Form-36 Health Survey (SF-36). The SF-36 assesses eight domains of QoL which are calculated using Dutch normative data [26]. The eight domains can be grouped into two summary scores: the Physical Component Summary (PCS) and the Mental Component Summary (MCS) that are standardized using normative data from the US population and are presented as T-scores [27].

Sleep quality was measured using The Pittsburgh Sleep Quality Index (PSQI) [28]. The PSQI assesses seven domains which are graded from 0 to 3, resulting in a score ranging from 0 to 21. PSQI scores >5 are indicative for poor sleep quality.

Fabry Disease severity was assessed using the Mainz Severity Score index (MSSI, range: 0-76) [29]. The MSSI is composed of four subscales that cover general (range: 0-18), neurological (range: 0-20), renal (range: 0-18) and cardiac (range: 0-20) signs and symptoms of the disease.

Brain MRI

MRI of the brain was performed yearly or biannually as part of routine follow-up on a 3-T system (Philips Ingenia, Philips Medical Systems, Best, The Netherlands). All MRIs of the brain were made using the same standardized protocol, see **Supplemental table E-2** more information on MRI settings per sequence. Eight patients had no MRI of the brain, six because of the presence of an MRI non-compatible pacemaker or ICD and one because of claustrophobia. In one patient the brain MRI was made in a different hospital. MRIs were re-evaluated by two neuroradiologists, (MRL evaluated basilar artery pathology, MGL evaluated infarctions, WMLs and atrophy), blinded for all patient characteristics. White matter lesions (WMLs) were rated on axial FLAIR using the Fazekas scale, ranging from 0 (no WMLs) to 6 (confluent periventricular and deep WMLs) [30]. Presence and number of (lacunar) infarctions was rated on DWI, axial T2 and FLAIR images. Basilar artery diameter (BAD) was rated on axial T2 images [31]. Atrophy of medial and temporal lobe were rated on T1 3D GRE images using the Medial Temporal lobe Atrophy rating scale (MTA) [32]. See **Supplemental Table E-3** for additional information on the scales.

Statistical methods

Data are presented as median and range or mean ± SD where appropriate. R (version 3.3.1) and SPSS version 24.0 (SPSS Inc. Chicago, Illinois, USA) were used for statistical analysis. Between subgroup differences were compared using the Kruskall-Wallis test, one way ANOVA and Fisher's exact test where appropriate. Post hoc analyses were done with the Dunn test, Tukeys test and 2x2 Fisher's exact tests with Bonferroni-Holm correction for abovementioned tests, respectively. Included and excluded patients were compared as a whole group and per subgroup divided for sex and phenotype. To check if neuropsychological test results differed from the average from the reference cohort, T-scores were compared to a T-score of 50 using a one-sided sign test.

T-scores per cognitive domain were obtained calculating mean T-scores of all tests addressing this domain. Variables were included in the univariate analyses if they were

deemed as potentially related to OCI trough literature search in the general population or previous studies on OCI and FD. Next, the univariate models were used to identify variables for multivariate models. Linear regression was used to analyze the univariate relation between variables and T-scores per cognitive domain. Kendall's tau-b (r_τ) was used to analyze the univariate relation between variables and subjective cognitive complaints (range 0-3) and between variables and OCI (binary, yes/no) (**table 6**). Bootstrapping was used to calculate bias corrected accelerated 95% confidence intervals (CI) of Kendall's tau-b, stratified for sex. To minimize the effect of multiple testing, the relation between a variable and OCI was first tested. If the relation between a variable and OCI was (very) weak ($\approx r_\tau < 0.1$, $\approx p > 0.25$) a relation between the variable and T-scores per cognitive domain was not tested. To correct for false discovery rate we used the Benjamini-Hochberg procedure in **Table 6** [33].

A multiple logistic regression model was used to check which variables are independent risk factors of OCI and a proportional odds model was used to check which variables are independent risk factors of subjective cognitive complaints. Both models were iteratively built selecting variables from univariate models (inclusion if $p<0.10$). The Akaike Information Criterion (AIC) was used to optimize the models. We chose the simplest models and compared them to our theoretical concepts. Variance inflation factor was used to explore potential multicollinearity in the logistic regression model. A likelihood ratio test was used to test proportionality of odds, comparing the goodness of fit of the proportional odds model to its multinomial counterpart.

P-values <0.05 were considered statistically significant. If multiple tests were carried out regarding a single hypothesis, the results were corrected using the Bonferroni-Holm correction, to control for the family wiser error rate [34]. Results were reported in accordance with the STROBE guidelines [35].

Results

Baseline characteristics

Of the 154 known FD patients in the AMC, ten patients were not considered eligible because of comorbidity known to influence the neuropsychological test results (autism (n=2), blindness (n=1), intellectual and developmental disabilities (n = 3), severe aphasia (n=1)) or because of insufficient knowledge of the Dutch language (n=3).

Of the 144 contacted patients, 63 patients were not willing to participate (not interested (n=29), time constraints (n=8) and participation being too strenuous (n=26)). There were

no significant differences between participants and non-participants in sex, phenotype, age, TIA, stroke or median Fazekas score (see **Supplemental Table E-4** for data on non-participants).

A total of 81 patients were included with a mean age of 44.5±14.3 years (range: 19-76 years) (**Table 1**). Fifty-three patients were women (65%) and 60 patients had classical FD phenotype (74%). Twenty-two patients (27%) reported a history of or a current depression, as diagnosed by their general practitioner, psychologist or psychiatrist, without statistically significant differences between subgroups split by sex and phenotype ($p=6.6*10^{-1}$).

Subjective cognitive complaints

Fifty-two patients (64%) experienced subjective cognitive complaints in at least one domain, without statistically significant differences between subgroups split by sex and phenotype ($p=3.0*10^{-1}$) (**Table 2**).

Objective cognitive impairment

There were no signs of underachievement or lack of motivation in any of the patients based on the TOMM score.

A total of 13 (16%) patients had any OCI, of which four (5%) had severe OCI (**Table 2**). Seven men with classical FD (41%) had any OCI of which two (12%) had severe OCI. For men with non-classical FD this was three (27%) and one (9%), respectively, and in women with classical FD this was three (7%) and one (2%), respectively. OCI did not occur in women with non-classical FD.

Most abnormal T-scores (T-scores ≤33) were found in the attention and executive functioning domain (**Table 2**). Decreased T-scores were found in the attention and executive functioning domain in men with classical FD (T-score, 45.6; $p=1.4*10^{-2}$) and men with non-classical FD (T-score, 46.6; $p=1.2*10^{-2}$) (**Table 3**). None of the patients scored <24 on the MMSE, suggesting lack of sensitivity for the detection of OCI in FD using this cut-off.

After post-hoc correction there were no differences in premorbid IQ between the subgroups divided by sex and phenotype (**Table 3**). However, there was a difference looking at sex only: men had a lower premorbid IQ compared to women (W=468.5, $p=6.6*10^{-3}$).

Questionnaires

Thirty-one patients (38.3%) scored ≥16 on the CESD, indicating the presence of depressive symptoms (**Table 4**), with comparable scores in all subgroups. MSSI scores were higher in men and women with classical FD and men with non-classical disease compared to women with non-classical disease, indicating less severe disease in women with non-classical disease. BPI, MCS and PCS scores were comparable in all subgroups, indicating no differences in pain, mental QoL and physical QoL in all subgroups, respectively. Almost half of all patients (n=39) experienced poor sleep quality.

Cerebral involvement

Ten patients had a history of stroke as diagnosed by a neurologist, none of them were women with non-classical FD (**Table 5**). Seventy-three patients (90%) had an MRI brain (median: 0.7 years) before the neuropsychological assessment. WMLs were present in 43 patients (58.9%) and were most often mild (total Fazekas score of 1 or 2) (n=27).

Table 1 Patient characteristics

	All	Men		Women		Intergroup comparison	
		Classical (a)	Non-classical (b)	Classical (c)	Non-classical (d)	P	Post hoc
Patients, n (%)	81	17 (21.0%)	11 (13.6%)	43 (53.1%)	10 (12.3%)	-	-
Age in years, mean (±SD)	44.5 (±14.3)	38.6 (±13.5)	58.0 (±11.2)	43.5 (±13.9)	43.9 (±13.0)	**$3.5*10^{-3}$**	a,c<b
History of ERT, n (%)	48 (59.3%)	17 (100.0%)	3 (27.3%)	27 (62.8%)	1 (10.0%)	**$5.3*10^{-7}$**	b,c,d<a, d<c
Currently on ERT, n (%)	43 (53.1%)	15 (88.2%)	2 (18.2%)	25 (58.1%)	1 (10.0%)	**$3.2*10^{-5}$**	d<a,c, b<a
Replagal/Fabrazyme, n/n	11/32	5/10	0/2	6/19	0/1	-	-
Time on ERT in years, median (range)	8.6 (0.1-16.0)	12.4 (1.6-16.0)	9.5 (6.4-12.5)	7.6 (0.1-13.6)	0.2	$6.4*10^{-2}$	-
Current psychiatric medication, n (%)	15 (18.5%)	2 (11.8%)	3 (27.3%)	9 (20.9%)	1 (10.0%)	$6.5*10^{-1}$	-
Antidepressants†, n (%)	7 (8.6%)	1 (5.9%)	2 (18.2%)	3 (7.0%)	1 (10.0%)	$6.4*10^{-1}$	-
Benzodiazepines, n (%)	9 (11.1%)	1 (5.9%)	1 (9.1%)	7 (16.3%)	0 (0.0%)	$5.9*10^{-1}$	-
Unemployed%, n (%)	32 (39.5%)	9 (52.9%)	5 (45.5%)	15 (34.9%)	3 (30.0%)	$5.4*10^{-1}$	-
Unfit for work$, n (%)	20 (24.7%)	7 (41.2%)	2 (18.1%)	10 (23.3%)	1 (10.0%)	$3.1*10^{-1}$	-
Single#, n (%)	30 (37.0%)	9 (52.9%)	4 (36.4%)	14 (32.6%)	3 (30.0%)	$5.1*10^{-1}$	-
Years of education, mean (±SD)	13.8±3.0	14.4±2.8	13.9±4.9	13.3±2.7	14.9±1.8	$3.5*10^{-1}$	-
Depression*, n (%)	22 (27.2%)	3 (17.6%)	3 (27.3%)	12 (27.9%)	4 (40.0%)	$6.6*10^{-1}$	-
Burnout*, n (%)	12 (14.8%)	1 (5.9%)	0 (0.0%)	7 (16.3%)	4 (40.0%)	$5.8*10^{-2}$	-
Smoking, n (%)	36 (44.4%)	6 (35.3%)	6 (54.5%)	21 (48.8%)	3 (30.0%)	$5.6*10^{-1}$	-
Hypertension, n (%)	24 (29.6%)	2 (11.8%)	7 (63.6%)	14 (32.6%)	1 (10.0%)	**$1.5*10^{-2}$**	‡
Diabetes mellitus type 2, n (%)	3 (3.7%)	0 (0.0%)	1 (9.1%)	2 (4.7%)	0 (0.0%)	$1.0*10^{0}$	-
Dyslipidemia, n (%)	11 (13.6%)	1 (5.9%)	4 (36.4%)	6 (14.0%)	0 (0.0%)	$8.1*10^{-2}$	-
eGFR in ml/min/1.73m^2, median (range)	94.6 (11.4-141.0)	105.6 (25.4-141.0)	77.3 (11.4-109.9)	94.0 (45.6-131.1)	95.4 (73.6-118.3)	**$4.1*10^{-3}$**	b<a,c,d
eGFR <60 ml/min/1.73m^2, n (%)	11 (13.6%)	2 (11.8%)	4 (36.4%)	5 (11.6%)	0 (0.0%)	$1.2*10^{-1}$	-
Albuminuria in mg/day, median (range)	32 (3-2761)	60 (5-921)	100 (7-2761)	21 (3-1426)	13.5 (4-1422)	$7.4*10^{-2}$	-
Albuminuria >A1	41 (50.6%)	12 (70.6%)	7 (63.6%)	21 (48.8%)	1 (10.0%)	**$1.5*10^{-2}$**	d<a
LVMI in gr/m^2, median (range)	62.7 (33.4-139.6)	78.3 (45.9-139.5)	64.7 (50.1-136.9)	55.9 (36.6-119.1)	44.7 (33.4-77.6)	**$1.5*10^{-4}$**	c<a, d<a,b
LVMI > upper ref limit, n (%)	24/74 (32.4%)	9/17 (52.9%)	3/8 (37.5%)	11/39 (28.2%)	1/10 (10.0%)	$1.2*10^{-1}$	-
Cardiac fibrosis	23/72 (31.9%)	6/17 (35.3%)	2/6 (33.3%)	14/39 (35.9%)	1/10 (10.0%)	$4.4*10^{-1}$	-
LysoGb3 before ERT in nmol/L, median (range)	8.2 (0.6-150.3)	99.0 (36.8-150.3)	5.0 (1.2-16.5)	7.8 (1.3-22.6)	1.9 (0.6-5.0)	**$5.5*10^{-11}$**	b,c,d<a, d<c

Continuous variables are presented as median (range) or mean (±SD) and discrete variables as number (percentages). Intergroup differences were tested, results <0.05 are in bold. If <0.05 then post-hoc tests were performed. For representation of the results of the post-hoc analyses we allocated a letter (a,b,c or d) to each subgroup.
ERT = enzyme replacement therapy, eGFR = estimated glomerular filtration rate, LVMI = left ventricular mass index
*†Antidepressants taken for neuropathic pain not included, % Includes three retirees, $ Includes three patients regarded partially unfit for work, # Unmarried, divorced or widowed, * History of or current, as diagnosed by a general practitioner, psychologist or psychiatrist, ‡ Post-hoc Fisher's exact test was not significant after Bonferroni-Holm correction*
Upper reference limit LVM: ♂ = 79 / ♀ = 75. Normal range lysoGb3 = 0.3-0.6 nmol/L. Albuminuria >A1 = >30mg/day

Table 2 Number of patients with subjective cognitive complaints, objective cognitive impairment and T-scores ≤33

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
Cognitive dysfunction					
Subjective cognitive complaints[&]	52 (64.2%)	11 (64.7%)	5 (45.5%)	31 (71.1%)	5 (50.0%)
Any OCI	13 (16.0%)	7 (41.0%)	3 (27.3%)	3 (7.0%)	0 (0%)
Severe OCI	4 (4.9%)	2 (11.8%)	1 (9.1%)	1 (2.3%)	0 (0%)
	T≤33, n (%)	**T≤33, n (%)**	**T≤33, n (%)**	**T≤33, n (%)**	**T≤33, n (%)**
Intelligence estimation					
DART*	3 (3.8%)	0 (0%)	3 (27.3%)	0 (0%)	0 (0%)
Language					
BNT	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
WAIS-IV: S*	5 (6.3%)	0 (0%)	1 (9.1%)	4 (9.5%)	0 (0%)
Memory					
RAVLT ir	6 (7.4%)	4 (23.5%)	1 (9.1%)	1 (2.3%)	0 (0%)
RAVLT dr	4 (4.9%)	3 (17.6%)	0 (0%)	1 (2.3%)	0 (0%)
RBMT ir	2 (2.5%)	2 (11.7%)	0 (0%)	0 (0%)	0 (0%)
RBMT dr	3 (3.7%)	2 (11.7%)	0 (0%)	1 (2.3%)	0 (0%)
Visuospatial perception					
WAIS-IV: BD*	6 (7.5%)	2 (11.7%)	0 (0%)	3 (7.1%)	1 (10%)
JLO	3 (3.7%)	0 (0%)	1 (9.1%)	2 (4.7%)	0 (0%)
Processing speed					
TMT A	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Stroop W	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Stroop C	4 (4.9%)	2 (11.7%)	1 (9.1%)	1 (2.3%)	0 (0%)
Attention and executive functioning					
TMT B	3 (3.7%)	1 (5.9%)	0 (0%)	2 (4.7%)	0 (0%)
Stroop CW	2 (2.5%)	0 (0%)	1 (9.1%)	1 (2.3%)	0 (0%)
Fluency A	10 (12.3%)	3 (17.6%)	1 (9.1%)	5 (11.6%)	1 (10%)
Fluency O	11 (13.6%)	6 (35.3%)	0 (0%)	5 (11.6%)	0 (0%)
Fluency L	7 (8.6%)	2 (11.7%)	4 (36.3%)	1 (2.3%)	0 (0%)

Discrete variables as number (percentages).
OCI = objective cognitive impairment, DART = Dutch Adult Reading Test, BNT = Boston Naming Test, WAIS-IV: S = Wechsler Adult Intelligence Scale IV: Similarities, RAVLT = Rey Auditory Verbal Learning Test, ir = immediate recall, dr = delayed recall, RBMT = Rivermead Behavioural Memory Test, BD = Block Design, JLO = Judgement of Line Orientation, TMT = Trail Making Test, W = Words, C = Color, CW = Color-Word, A = Animal, O = Occupation, L = Letter.
*&Presence of subjective cognitive complaints on memory, attention and/or executive functioning in at least one domain, *One woman with classical Fabry disease used the DART, WAIS-IV: S and WAIS-IV: BD in her job setting so did not perform these cognitive tests.*

Table 3 Results cognitive subtests/domains and subgroup comparison

	All	Men		Women		Intergroup comparison	
		Classical (a)	Non-classical (b)	Classical (c)	Non-classical (d)	P	Post-hoc
General cognitve functioning							
MMSE†	29 (25-30)	29 (27-30)	29 (27-30)	29 (25-30)	29 (28-30)	-	-
Intelligence estimation							
DART#*	94.0 (68-133)	89.0 (83-114)	85.0 (68-133)	94.5 (82-121)	100.0 (84-121)	**$4.4*10^{-2}$**	‡
Language*	49.5 (32.0-63.0)	51.5 (39.5-62.0)	45.5 (36.0-61.5)	48.8 (32.0-59.5)	55.0 (42.0-63.0)	$7.1*10^{-2}$	-
BNT	50.0 (37-63)	54.0 (39-63)	46.0 (39-63)	50.0 (37-59)	47.5 (37-63)	$4.9*10^{-1}$	-
WAIS-IV: S*	50.0 (27-72)	50.0 (40-72)	44.0 (33-60)	50.0 (27-63)	59.0 (40-70)	**$2.3*10^{-2}$**	b<d
Memory	55.0 (22.8-71.5)	54.5 (22.8-69.5)	55.3 (38.5-64.3)	54.8 (24.8-71.5)	57.8 (42.8-71.0)	$2.2*10^{-1}$	-
RAVLT ir	52.0 (16-72)	49.0 (18-65)	57.0 (32-66)	52.0 (16-68)	57.5 (47-72)	$3.9*10^{-2}$	-
RAVLT dr	53.0 (21-71)	48.0 (21-69)	54.0 (34-64)	53.0 (27-71)	56.0 (44-64)	$3.7*10^{-1}$	-
RBMT ir	57.0 (27-81)	59.0 (27-73)	57.0 (34-68)	57.0 (34-81)	58.0 (41-75)	$3.2*10^{-1}$	-
RBMT dr	55.0 (22-76)	54.0 (25-76)	59.0 (41-69)	54.0 (22-74)	56.5 (39-75)	$8.0*10^{-1}$	-
Visuospatial perception*	54.0 (28.0-65.5)	54.5 (44.5-64.0)	48.0 (36.5-54.0)	55.8 (28.0-65.5)	58.5 (47.0-65.5)	**$6.1*10^{-3}$**	b<a,c,d
WAIS-IV BD*	50.0 (27-72)	50.0 (33-67)	*43.0 (34-50)[1]*	52.0 (27-72)	60.0 (33-70)	$2.8*10^{-2}$	-
JLO	61.0 (29-61)	61.0 (52-61)	52.0 (33-61)	61.0 (29-61)	61.0 (48-61)	$7.9*10^{-2}$	-
Processing speed	53.7 (32.3-74.7)	49.7 (42.0-60.0)	55.7 (40.3-74.7)	52.7 (32.3-63.3)	54.3 (45.7-70.3)	$3.4*10^{-2}$	-
TMT A	56.0 (34-77)	51.0 (38-61)	55.0 (34-63)	56.0 (34-77)	59.5 (43-71)	$9.0*10^{-2}$	-
Stroop W	56.0 (34-84)	56.0 (41-69)	51.0 (41-61)	60.0 (37-84)	54.0 (41-77)	$1.9*10^{-2}$	-
Stroop C	52.0 (29-88)	47.0 (29-59)	53.0 (29-88)	53.0 (33-71)	52.0 (39-71)	$1.0*10^{-1}$	-
Attention and executive functioning	48.8 (25.6-66.8)	*45.6 (35.2-58.8)[2]*	*46.6 (37.2-55.4)[3]*	50.2 (25.6-66.0)	52.6 (40.2-66.8)	$8.3*10^{-2}$	-
TMT B	51.0 (-1-74)	47.0 (33-58)	49.0 (35-54)	51.0 (-1-74)	51.0 (42-59)	$2.8*10^{-1}$	-
Stroop CW	50.0 (32-84)	48.0 (39-60)	43.0 (33-61)	51.0 (32-84)	53.5 (45-71)	$1.5*10^{-2}$	-
Fluency A	50.0 (29-75)	48.0 (29-75)	54.0 (29-61)	50.0 (29-69)	57.0 (29-69)	$4.9*10^{-1}$	-
Fluency O	50.0 (17-69)	43.0 (24-64)	50.0 (38-57)	48.0 (17-69)	56.0 (36-67)	$1.3*10^{-1}$	-
Fluency L	*45.0 (25-71)[4]*	*42.0 (25-64)[5]*	*43.0 (27-60)[6]*	50.0 (33-71)	43.5 (36-68)	**$1.7*10^{-3}$**	a,b<c

All variables are presented as median (range). The MMSE is presented as a raw score with range (<24 is considered a diagnostic clue for the presence of dementia), the DART is presented as IQ-score, all other results are presented as T-scores in comparison to the reference population, where the mean is 50 and one SD is 10. T-scores <50 were compared to a T-score of 50 and presented in italics if they were statistically significant after Bonferroni-Holm correction. Intergroup differences were compared. Significant results (after Bonferroni-Holm correction) are in bold and were followed by post-hoc testing. For representation of the results of the post-hoc analyses we allocated a letter (a,b,c or d) to each subgroup. MMSE = Mini Mental State Exam, DART = Dutch Adult Reading Test, BNT = Boston Naming Test, WAIS-IV: S = Wechsler Adult Intelligence Scale IV: Similarities, RAVLT = Rey Auditory Verbal Learning Test, ir = immediate recall, dr = delayed recall, RBMT = Rivermead Behavioural Memory Test, BD = Block Design, JLO = Judgement of Line Orientation, TMT = Trail Making Test, W = Words, C = Color, CW = Color-Word, A = Animal, O = Occupation, L = Letter

*† Raw MMSE score, # IQ score, * One classical women used the DART, WAIS-IV: S and WAIS-IV: BD in her job setting so did not perform these cognitive tests, ‡ Post-hoc Tukeys test was not significant* [1]$p=9.8*10^{-4}$, [2]$p=1.4*10^{-2}$, [3]$p=1.2*10^{-2}$, [4]$p=8.4*10^{-3}$, [5]$p=6.4*10^{-3}$, [6]$p=5.9*10^{-3}$

Variables affecting cognitive domains

Higher premorbid IQ was related to increased T-scores in all five domains (**Table 6**). Men generally scored lower on processing speed (β=-5.37; 95%CI -8.92 to -1.81; $p=3.6*10^{-3}$) compared to women. Other factors related to a lower T-score on processing speed were the pain score (one point increase BPI interference: β=-1.31; 95%CI -2.12 to -0.51; $p=1.8*10^{-3}$), being single (β=-5.19; 95%CI -8.69 to -1.68; $p=4.3*10^{-3}$), the MSSI score (one point increase: β=-0.20; 95%CI -0.33 to -0.06; $p=4.9*10^{-3}$) and BAD (one mm increase: β=-3.68; 95%CI -5.92 to -1.17; $p=4.1*10^{-3}$). Being employed was positively related to processing speed (β=4.94; 95%CI 1.46 to 8.42; $p=5.9*10^{-3}$). Being single was also negatively related to executive functioning (β=-5.15; 95%CI -8.33 to -1.97; $p=1.8*10^{-3}$). BAD and Fazekas score were related to a lower T-score on the memory domain (BAD: increase one mm β=-4.05; 95%CI -6.64 to -0.85; $p=1.2*10^{-2}$, Fazekas: one point increase β=-1.80; 95%CI -3.10 to -0.51; $p=7.1*10^{-3}$) as was being single (β=-5.15; 95%CI -10.32 to -2.12; $p=3.4*10^{-3}$).

Variables affecting objective cognitive impairment

Male sex was positively related to the presence of OCI (r_τ=0.39; 95%CI, 0.15 to 0.58; $p=5.1*10^{-4}$) (**Table 6**). This relation was still present when comparing men with classical FD to all other patients (r_τ=0.35; 95%CI, 0.09 to 0.60; $p=1.7*10^{-3}$). Higher premorbid IQ was negatively related to the presence of OCI (r_τ=-0.29; 95%CI, -0.45 to -0.079; $p=2.2*10^{-3}$). There was a positive relation between brain parameters and the presence of OCI (Fazekas score, r_τ=0.22; 95%CI, -0.02 to 0.41; $p=4.7*10^{-2}$, BAD, r_τ=0.22; 95%CI, -0.04 to 0.37; $p=2.1*10^{-2}$). This relation was more robust when only the relationship of severe OCI to the Fazekas score was considered (r_τ=0.31; 95%CI, 0.18 to 0.41; $p=5.2*10^{-3}$). There was a positive relation between a history of stroke and the presence of OCI (r_τ=0.25; 95%CI, -0.04 to 0.52; $p=2.9*10^{-2}$). There was no relation between OCI and the CESD score or between OCI and subjective cognitive complaints (**Table 6**).

Two logistic regression models were comparable in AIC and both were in agreement with our theoretical concepts. The first model showed that male sex (OR, 6.8; 95%CI, 1.6 to 39.8; $p=1.6*10^{-2}$) and a history of stroke (OR, 6.4; 95%CI, 1.1 to 41.0; $p=3.7*10^{-2}$) were independently positively associated with OCI and that premorbid IQ (one IQ point increase: OR, 0.91; 95%CI, 0.82 to 0.98; $p=3.8*10^{-2}$) was independently negatively related to OCI. The second model showed that male sex (OR, 5.9; 95%CI, 1.4 to 31.7; $p=2.3*10^{-2}$) and being single (OR, 4.8; 95%CI, 1.2 to 25.2; $p=3.9*10^{-2}$) were both independently positively associated with OCI and that premorbid IQ (one IQ point increase: OR, 0.91; 95%CI, 0.81 to 0.99; $p=4.6*10^{-2}$) was independently negatively associated with OCI. Including male sex, a history of stroke, being single and premorbid IQ in one model did not improve the model.

Variables affecting subjective cognitive complaints

The CESD score was most strongly positively related to subjective cognitive complaints (r_τ=0.36; 95%CI, 0.18 to 0.51; p=2.7*10^{-5}) (**Table 6**). The PSQI score (r_τ=0.30; 95%CI, 0.13 to 0.45; p=7.8*10^{-4}) and the MSSI score also showed a positive relation to subjective cognitive complaints, the latter relation was mostly driven by the MSSI general and MSSI neurological subscores (MSSI general, r_τ=0.32; 95%CI, 0.15 to 0.48; p=2.4*10^{-4}; MSSI neurological, r_τ=0.32; 95%CI, 0.15 to 0.46; p=2.7*10^{-4}). Being employed was negatively related to subjective cognitive complaints (r_τ=-0.29; 95%CI, -0.46 to -0.11 ; p=4.6*10^{-3}). MCS and PCS scores were also negatively related to subjective cognitive complaints (MCS, r_τ=-0.29; 95%CI, -0.45 to -0.10; p=6.9*10^{-4}; PCS, r_τ=-0.32; 95%CI, -0.46 to -0.18; p=1.9*10^{-4}).

In a proportional odds model, the CESD score (one point increase: OR, 1.07; 95%CI, 1.02 to 1.13; p=3.3*10^{-3}), a history of depression (OR, 2.7; 95%CI, 1.1 to 7.3; p=3.9*10^{-2}) and the MSSI general score (one point increase: OR, 1.3; 95%CI, 1.1 to 1.5; p=5.5*10^{-3}) were independently positively associated with subjective cognitive complaints.

Table 4 Questionnaires and indexes

	All	Men		Women		Intergroup comparison	
		Classical (a)	Non-classical (b)	Classical (c)	Non-classical (d)	P	Post hoc
CESD, median (range)	11 (0-44)	11 (0-40)	12 (0-37)	12 (0-44)	7.5 (0-20)	$6.3*10^{-1}$	-
CESD≥16, n (%)	31 (38.3%)	7 (41.2%)	4 (36.4%)	17 (39.5%)	3 (30.0%)	$9.7*10^{-1}$	-
MSSI, median (range)	24 (2-68)	32 (15-68)	24 (2-41)	23 (4-42)	6.5 (2-20)	**$3.3*10^{-5}$**	d<a,b,c, c<a
MSSI 20-40, n (%)	48 (59.3%)	13 (76.5%)	6 (54.5%)	28 (65.1%)	1 (10.0%)	-	-
MSSI>40, n (%)	7 (8.6%)	2 (11.8%)	2 (18.2%)	1 (2.3%)	0 (0.0%)	-	-
BPI worst, median (range)	2 (0-8)	2 (0-7)	4 (0-7)	3 (0-8)	0 (0-8)	$8.4*10^{-1}$	-
BPI average, median (range)	1 (0-7)	1 (0-7)	4 (0-7)	2 (0-7)	0 (0-6)	$6.6*10^{-1}$	-
BPI interference, median (range)	0.9 (0.0-6.9)	0.4 (0.0-5.0)	3.3 (0.6-6.4)	0.9 (0.0-6.9)	0.1 (0.0-6.3)	$5.0*10^{-1}$	-
PCS, median (range)	43.5 (18.4-62.9)	39.8 (20.6-62.9)	39.9 (18.4-60.2)	42.4 (22.5-59.6)	52.3 (24.1-59.4)	$3.9*10^{-1}$	-
MCS, median (range)	49.8 (13.2-65.1)	49.0 (13.2-65.1)	46.3 (23.3-60.8)	49.0 (21.4-62.2)	54.5 (34.6-61.6)	$3.3*10^{-1}$	-
PSQI, median (range)	5.0 (0.0-20.0)	4.0 (0.0-14.0)	6.0 (1.0-13.0)	6.0 (1.0-20.0)	5.5 (2.0-10.0)	**$4.7*10^{-2}$**	a<c
PSQI>5, n (%)	39 (48.1%)	4 (23.5%)	7 (63.6%)	23 (53.5%)	5 (50.0%)	-	-

Continuous variables are presented as median (range), discrete variables as number (percentages). Intergroup differences were compared. Significant results (after Bonferroni-Holm correction) are in bold and were followed by post-hoc testing. For representation of the results of the post-hoc analyses we allocated a letter (a,b,c or d) to each subgroup. For additional information on the interpretation of questionnaires and indexes please see: methods, questionnaires.
CESD = Centre for Epidemiological Studies – Depression scale, MSSI = Mainz Severity Score Index, BPI = Brief Pain Inventory, PCS = SF-36 physical component scale, MCS = SF-36 mental component scale, PSQI = Pittsburgh Sleep Quality Index

Table 5 Cerebral involvement and MRI brain assessment

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
History of cerebral event[#], n (%)	15 (18.5%)	4 (23.5%)	2 (18.2%)	9 (20.9%)	0 (0%)
History of TIA[#], n (%)	10 (12.3%)	3 (17.6%)	2 (18.2%)	5 (11.6%)	0 (0%)
History of stroke[#], n (%)	10 (12.3%)	2 (11.8%)	2 (18.2%)	6 (14.0%)	0 (0%)
MRI present, n (%)	73 (90.1%)	17 (100%)	7 (63.6%)	39 (90.7%)	10 (100.0%)
Time since MRI in years, median (range)	0.7 (0.0-2.9)	0.5 (0.0-2.8)	0.6 (0.0-1.5)	0.9 (0.2-2.9)	0.9 (0.1-2.6)
WMLs present, n (%)	43 (58.9%)	9 (52.9%)	5 (71.4%)	23 (59.0%)	6 (60.0%)
Fazekas					
Total score (0-6), median (range)	1 (0-6)	0 (0-6)	1 (0-3)	1 (0-6)	0.5 (0-2)
Deep WMLs					
None (0), n (%)	38 (52.1%)	9 (52.9%)	4 (57.1%)	20 (51.3%)	5 (50.0%)
Punctate (1), n (%)	22 (30.1%)	2 (11.8%)	2 (28.6%)	13 (33.3%)	5 (50.0%)
Early confluent (2), n (%)	9 (12.3%)	4 (23.5%)	1 (14.3%)	4 (10.3%)	0 (0%)
Confluent (3), n (%)	4 (5.5%)	2 (11.8%)	0 (0%)	2 (5.1%)	0 (0%)
Periventricular WMLs					
None (0), n (%)	46 (63.0%)	11 (64.7%)	2 (28.6%)	24 (61.5%)	9 (90.0%)
Caps/lines (1), n (%)	18 (24.7%)	1 (5.9%)	5 (71.4%)	11 (28.2%)	1 (10.0%)
Bands (2), n (%)	5 (6.8%)	3 (17.6%)	0 (0%)	2 (5.1%)	0 (0%)
Irregular extending into WM (3), n (%)	4 (5.5%)	2 (11.8%)	0 (0%)	2 (5.1%)	0 (0%)
(Lacunar) stroke on MRI, n (%)	13 (17.8%)	5 (29.4%)	3 (42.9%)	4 (10.3%)	1 (10.0%)
Number of (Lacunar) stroke(s) on MRI, median (range)	0 (0-8)	0 (0-5)	0 (0-8)	0 (0-3)	0 (0-1)
BAD in mm, median (range)	3.6 (2.5-5.9)	4.2 (3.1-5.6)	3.6 (3.3-4.3)	3.6 (2.5-5.9)	3.2 (2.5-3.6)
MTA, median (range)	1 (0-3)	1 (0-3)	1 (0-2)	1 (0-3)	1 (0-2)

Continuous variables are presented as median (range), discrete variables as number (percentages). TIA = Transient Ischemic Attack, WMLs = White matter lesions, WM = White matter, BAD = Basilar artery diameter, MTA = Medial temporal lobe atrophy scale [#]As diagnosed by a neurologist

Table 6 Univariate relations to subjective cognitive complaints, objective cognitive impairment and cognitive domains

	Men	Classical	Classical men	Age (years)	Education (years)	DART (premorbid IQ)	Employment	Single	Alcohol/drug abuse	CESD	BPI interference
Cognitive dysfunction											
SCC	-0.097	0.255*	0.017	-0.028	-0.069	-0.008	**-0.293****	0.106	0.056	**0.362*****	0.221*
OCI	**0.389*****	0.028	**0.353****	0.062	-0.115	**-0.288****	-0.128	**0.291****	0.225*	-0.108	0.131
Cognitive domains											
Language	0.49	-	2.99	-	**0.80****	**0.32*****	0.71	1.18	-1.20	-0.12	-0.725
Memory	*-2.79*	-	-4.87	-	-	***0.63****	2.91	**-6.22****	*-7.17**	*-0.06*	***-1.14****
Visuospatial perception	*-2.94*	-	1.47	-	**0.71***	**0.25****	-0.02	-0.18	2.66	-0.10	**-1.06****
Processing speed	**-5.37****	-	-5.09*	-	-	**0.23****	**4.94****	**-5.19****	2.38	-0.19*	**-1.31****
Executive functioning	-4.14*	-	-3.29	-	-	**0.33*****	3.78*	**-5.15****	2.28	-0.12	-0.93*

	PSQI	PCS	MCS	LVMI	eGFR	ERT use	Presence of fatigue	History of depression	MSSI	MSSI general	MSSI cardiac
Cognitive dysfunction											
SCC	**0.297*****	**-0.317*****	**-0.288*****	-0.005	0.020	0.252*	**0.287****	0.258*	**0.246****	**0.324*****	0.103
OCI	-0.055	-0.125	0.028	**0.281****	-0.102	0.074	-0.022	0.191	**0.256****	0.081	0.239*
Cognitive domains											
Language	-	0.06	-	0.02	-	-	-	-2.07	-0.02	-	-0.12
Memory	-	*0.17*	-	*-0.08**	-	-	-	*-1.60*	*-0.16**	-	*-0.37**
Visuospatial perception	-	*0.15**	-	-0.03	-	-	-	-0.93	-0.12	-	*-0.29**
Processing speed	-	0.12	-	-0.08*	-	-	-	0.60	**-0.20****	-	-0.25
Executive functioning	-	0.09	-	-0.01	-	-	-	-0.88	-0.11	-	-0.08

Table 6 Univariate relations to subjective cognitive complaints, objective cognitive impairment and cognitive domains (continued)

	MSSI Renal	MSSI neuro	Fazekas#	Number of Infarctions on MRI#	History of stroke	MTA#	BAD#
Cognitive dysfunction							
SCC	0.129	**0.319*** **	0.168	0.115	0.178	0.224*	0.160
OCI	0.201	0.150	0.218*	0.137	0.245*	0.076	0.224*
Severe OCI	-	-	**0.307****	0.217	0.261*	0.215	0.210*
Cognitive domains							
Language	-0.02	0.04	-1.01*	-0.88	-1.94	-	-0.98
Memory	*-0.13*	*-0.40*	**-1.80****	-1.99*	*-2.40*	-	**-4.05****
Visuospatial perception	-0.44*	-0.07	-1.38*	-1.26	-3.87	-	-1.34
Processing speed	-0.34	**-0.56****	-1.16*	-1.71*	-4.31	-	**-3.68****
Executive functioning	-0.13	-0.45*	-1.06	-1.49*	-2.94	-	-1.97

Univariate relations to subjective cognitive complaints (SCC) and objective cognitive impairment (OCI) were tested using kendalls tau-b. Univariate relations to the combined T-scores of the cognitive domains were tested using generalized linear models and are presented as beta's. For binary variables kendalls tau-b and beta's were calculated with the presented value coded as 1 (men, classical, classical men, employment, single, alcohol/drug abuse, ERT use, presence of fatigue, history of depression and history of stroke) and the other value coded as 0 (e.g. women, non-classical, unemployment). For continuous variables beta's were calculated for a 1 year increase (age, education), 1 point increase on IQ, index, questionnaire or scale (DART, CESD, BPI interference, PSQI, PCS, MCS, MSSI, Fazekas, MTA), 1 gr/m^2 increase (LVMI), 1 ml/min/1.73m^2 increase (eGFR), 1 extra infarction (number of infarctions) and 1 mm increase (BAD).

<0.05, **<0.01, *<0.001. Bold printed numbers are significant after Benjamini-Hochberg correction. In several cases the T-scores of cognitive domains Memory and Visuospatial perception violated the assumptions of the generalized linear model. In these cases the T-scores were squared to satisfy these assumptions. Consequently, instead of beta's, the difference between the square root of the predicted values is presented in italics. MRI brain variables were also related to severe CI.*

SCC = Subjective cognitive complaints, OCI = Objective cognitive impairment, DART = Dutch adult reading test, CESD = Centre for Epidemiological Studies – Depression scale, BPI = Brief Pain Inventory, PSQI = Pittsburgh Sleep Quality Index, PCS = SF-36 physical component scale, MCS = SF-36 mental component scale, LVMI = Left ventricular mass index, eGFR = Estimated glomerular filtration rate, ERT = Enzyme replacement therapy, MSSI = Mainz Severity Score Index, MTA = Medial temporal lobe atrophy scale, BAD = Basilar artery diameter

#After removal of two classical men with a history of severe drug abuse

Discussion

In this large sample of Dutch patients with FD we have, for the first time, shown a relationship between sex, phenotype and risk for OCI. OCI was present in 41% of men with classical disease, affecting mostly the executive functioning domain. In addition, OCI was found in a significant number (27%) of men with non-classical FD. In women with classical FD, however, the prevalence of OCI was markedly lower (7%) and none of the women with non-classical FD had OCI. The risk of OCI in patients with FD was independently related to male sex, a history of stroke and to premorbid IQ.

In a healthy population of male veterans from the United States (n=4371), slightly younger compared to our cohort (38.4±2.5 years), OCI was found in ~6-7% of this cohort when using similar criteria [36]. In a second healthy mixed control group (n=138) of a study on the cognitive effects of type 1 diabetes, with comparable age (49±7 years) to our study population, the prevalence of OCI was 5% [37]. This indicates that OCI in our population of FD patients is much higher in male patients than would be expected in this age group. In women with FD the prevalence of OCI is comparable to that in the general population. Loeb et al. [7] found OCI in 30% of patients with FD, but used different criteria to define OCI and a smaller reference population (n=80), possibly explaining the differences. The impaired executive domain found in our study is in accordance with previous studies in FD [3]. Moreover, our study confirms the preliminary finding of a study by Sigmundsdottir et al. [5] that men with FD are more likely to get OCI, especially in those with classical disease.

In addition, a relationship between the extent of the WMLs (Fazekas score) and the presence of severe OCI was established. In a previous study a subgroup of patients with markedly increased volumes of white matter lesions showed more cognitive deficits compared to patients with lower lesion volumes [6]. In the general population, as was found in our study, the positive relationship between WMLs and OCI is not very strong [38]. It has been postulated that a threshold of WML severity needs to be surpassed before OCI develops [38]. The relation between stroke and OCI in the general population has been more firmly established [39]. Likewise, in our study the relation between a history of stroke and OCI in FD is clearly present. This was also observed in previous studies in FD, albeit using univariate analyses [5, 7].

The positive relationship between (premorbid) IQ and neuropsychological test scores has been firmly established in the general population [36, 40]. It has been theorized that a higher premorbid IQ reflects a greater "cognitive reserve", thus more decline has to take place before OCI occurs [41, 42]. The observation that higher T-scores in FD patients with higher premorbid IQ lower the chance of OCI fits this hypothesis. We also found a

lower median premorbid IQ in men compared to women. Despite this difference, male sex was related to a higher risk of OCI independently of premorbid IQ. Premorbid IQ therefore does not fully explain the differences in prevalence of OCI between men and women with FD.

A new finding is that almost two-thirds of our cohort of FD patients experienced subjective cognitive complaints, without significant differences in prevalence between all subgroups. Interestingly, in our study the subjective cognitive complaints were not related to OCI, but showed a clear relation with both depression in the past and current depressive symptoms. In the general population, the relation between OCI and subjective cognitive complaints is still controversial [43]. More thoroughly established is that patients with depression have a higher prevalence of subjective cognitive complaints [44], as was also previously shown by Loeb at al. [7] in a population of FD patients. The relation of depressive symptoms to subjective cognitive complaints further emphasizes the importance of recognizing these symptoms. Conversely, Loeb et al. [7] concluded that in patients with FD prevalence of subjective cognitive complaints is not increased. It seems that, in our cohort of FD patients, subjective cognitive complaints were highly prevalent. Of these, subjective memory complaints were present in 46% of our cohort (data not shown), while these are found in 22% of the general population [45], indicating that the prevalence in patients with FD could be more than twice as high. The difference to our study might be caused by the difference in assessment (structured interview versus questionnaire) as well as the use of a high cutoff (mean +2SD compared to a healthy population) for detecting subjective cognitive complaints in the Loeb et al. [7] study.

The high prevalence (38%) of depressive symptoms in our study is in line with the previously found prevalence of 46% from a mixed cohort of 186 patients with FD [46]. In populations with chronic diseases or chronic pain various treatments have been shown to improve depressive complaints [47, 48]. Unfortunately, treatment effects and risk factors for depression in FD are largely unknown. Only one small, uncontrolled study (n=15) looked at the effect of psychological counseling and found all FD patients improving [49]. Therefore, efficacy of treatment options for depressive symptoms should be topic of further research in FD patients.

This study has some strengths and limitations. Strengths are the precise phenotyping of the studied cohort and the use of a reliable cognitive test battery. Moreover, this study is the first to combine data on subjective cognitive complaints, depressive symptoms, cognitive functioning and MRI brain parameters in subgroups of patients divided by sex and FD phenotype. We included 81 patients, more than half of the known patients in the

Netherlands, a fairly large group for a rare disease like FD. However, we cannot rule out inclusion bias: patients with subjective cognitive complaints might be more interested in participation. Conversely, patients with severe cognitive impairment might not participate due to participation being to strenuous. Nevertheless, we found no significant differences in patient characteristics, nor in the presence of TIA, stroke or the height of the Fazekas score between participants and non-participants in this study. MRIs were made using the same standardized protocol, as part of routine follow up. This means that sometimes there was a time gap between the MRI and the neuropsychological assessment, but in most patients this was not more than a year and WMLs are known to increase slowly. Of some patients MRI of the brain was not available, mostly due to the presence of non-MRI compatible ICD/pacemakers. Excluding these patients, however, might also lead to a bias towards less affected patients. We did not assess a healthy control group ourselves. Instead, we used large normative datasets (median sample size: 471) compiled of healthy control groups from multiple studies. Furthermore, most neuropsychological test results were corrected for age, sex and education level, although not for (premorbid) IQ. Lastly, we used the Fazekas-scale to assess WMLs in this study. It has been shown that visual rating scales have a lower ceiling compared to volumetric measurements of WMLs [50]. Perhaps, the use of volumetric measurement of WMLs would have strengthened the relation to OCI.

In conclusion, OCI is present in one-sixth of FD patients, predominantly in men with classical disease. The relation between a history of stroke and OCI in this study re-emphasizes the importance of prevention of stroke in patients with FD. Moreover, the presence of stroke or other clinical indications of OCI warrants referral of FD patients for neuropsychological assessment. The high prevalence of subjective cognitive complaints, equally distributed over the phenotypes and sexes, was not explained by OCI, but showed a clear relation with current or historical depressive complaints. Evaluation of subjective cognitive complaints in patients with FD should therefore include a psychological evaluation and healthcare professionals should focus on recognition and treatment of depressive symptoms.

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Supplemental file 1

Criteria classical and non-classical Fabry disease

In men, classical disease was defined as: 1) a mutation in the GLA-gene, 2) enzyme activity ≤5% of the mean reference range and 3) ≥1 characteristic FD symptoms (i.e. angiokeratoma, Fabry neuropathic pain, and/or cornea verticillata, see [1] for definitions), or an affected family member with a definite diagnosis according to abovementioned criteria. In women, classical disease was defined as: 1) a mutation in the GLA-gene and 2) ≥1 characteristic FD symptoms, or an affected family member with a definite classical diagnosis according to abovementioned criteria. Men and women, with a mutation in the GLA-gene not regarded as a neutral variant [2], and not fulfilling the criteria for a classical phenotype were diagnosed as having non-classical disease.

Supplemental file 2

Structured interview subjective cognitive complaints

SK and GG developed the structured interview in accordance with the neuropsychological history taking, recommended before all neuropsychological test assessments[3]. All structured interviews were conducted by SK or by a neuropsychologist that assisted with data collection.

Methodology

The interview focused on education, work, specific complaints concerning Fabry disease (FD), general medical history, medication, depressive complaints and subjective cognitive complaints.

First, to prevent framing of patients' perception of their own cognition, they were asked broadly about their perceived cognitive functioning. Thereafter all patients were asked the following question:

"Do you have any complaints in the process of thinking (e.g. memory, attention)."

It was verified if these complaints were considered severe to the patient compared to surrounding friends/family/coworkers without Fabry disease. If both questions were answered "Yes" then we considered subjective cognitive complaints as being present. Similar strategies were applied for the different cognitive domains and examples of complaints in specific situations were asked. When doubts were present whether these examples were in agreement with the domain at hand these were explored in more detail.

Supplemental Table E-1

Table E-1 Neuropsychological test battery

Cognitive domain	Test administered	Scoring
Intelligence estimation	Dutch Adult Reading Test[a]	Words correctly read out loud
Language	Boston Naming Test	Total correctly recognized drawings
	WAIS-IV: Similarities[b]	Total correct similarities
Memory	Rey Auditory Immediate recall[c]	Total immediately recalled words trials 1-5
	Rey Auditory Delayed recall[c]	Total words recalled after 20 minutes
	Rivermead Behavioural Memory Test: Story[c]	Parts of story immediately recalled
	Rivermead Behavioural Memory Test: Story[c]	Parts of story recalled after 15 minutes
Visuospatial perception	WAIS-IV: Block Design[b]	Correctly/timely matched patterns
	Judgement of Line Orientation[a]	Correctly matched line pairs
Processing speed	Trail Making Test-A[c]	Time to complete
	Stroop Words[c]	Time to complete
	Stroop Colour[c]	Time to complete
Attention and executive functioning	Trail Making Test-B[c]	Time to complete
	Stroop Colour-Word[c]	Time to complete
	Fluency Animals[c]	Total numbers of animals in 1 minute
	Fluency Occupation[c]	Total number of occupations in 1 minute
	Fluency Letters[c]	Total number of words with 3 letters 1 minute each

WAIS-IV = Wechsler Adult Intelligence Scale IV
[a] Corrected for age and sex, [b] Corrected for age, [c] Corrected for age, sex and level of education

Supplemental Table E-2

Table E-2 Acquisition parameters for brain MRI

Parameter	T1W 3D GRE	FLAIR 3D TSE	T2W 2D TSE	DWI EPI
Plane	Sagittal	Sagittal	Axial	Axial
FOV read (mm)	256	250	230	200
FOV phase (%)	100	100	80	100
Slice thickness (mm)	0.9	1.1	3.0	3.0
TR/TE/TI (msec)	9.0/4.1/-	4800/356/1650	4391/80/-	5770/80/-
Flip angle (degree)	8	40	90	90
Bandwidth (Hx/pz)	2.516/172.6	0.594/731.0	1.988/218.5	23.498/18.5

FLAIR = Fluid Attenuated Inversion Recovery; GRE = Gradient Echo; TSE = Turbo Spin Echo; SWI = Susceptibility Weighted Imaging; DWI = Diffusion-Weighted; EPI = Echo Planar Imaging; MOTSA = Multiple overlapping thin slab acquisition; FOV = Field of View; TR = Repetition Time; TE = Echo Time; TI = Inversion Time

Supplemental Table E-3

Table E-3 MRI brain assessment

Pathology	Description	Sequence	Response
WMLs	Presence of WMLs	FLAIR Axial	Yes/no
WMLs	Fazekas periventricular	FLAIR Axial	Fazekas 0: Absence Fazekas 1: "caps" or pencil-thin lining Fazekas 2: Smooth "halo" Fazekas 3: Irregular periventricular hyperintensities extending into deep white matter
WMLs	Fazekas deep white matter	FLAIR Axial	Fazekas 0: None or a single punctate WMH lesion Fazekas 1: Multiple punctate lesions Fazekas 2: Beginning confluency of lesions (bridging) Fazekas 3: Large confluent lesions
Infarctions	Presence of (lacunar) infarctions	T2/FLAIR	Yes/no
Infarctions	Number of (lacunar) infarctions	T2/FLAIR	Number of (lacunar) infarctions
Dilatation basilar artery	Basilar artery diameter (mm)	T2 Axial	Average of: 1. Caudal (shortly after the confluence of the vertebral arteries) 2. Intermediate (in the middle of the basilar artery) 3. Rostral (just before the bifurcation)
Hippocampal atrophy	Medial temporal lobe atrophy rating scale	T1 coronal	0: no CSF is visible around the hippocampus 1: choroid fissure is slightly widened 2: moderate widening of the choroid fissure, mild enlargement of the temporal horn and mild loss of hippocampal height 3: marked widening of the choroid fissure, moderate enlargement of the temporal horn, and moderate loss of hippocampal height 4: marked widening of the choroid fissure, marked enlargement of the temporal horn, and the hippocampus is markedly atrophied and internal structure is loss

WMLs = White matter lesions, FLAIR = Fluid Attenuated Inversion Recovery

Supplemental Table E-4

Table E-4 Characteristics non-participants

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
Patients, n (%)	73	18 (24.7%)	5 (6.8%)	34 (46.6%)	16 (21.9%)
Age in years, mean (±SD)	47.5 (±17.9)	38.3 (±14.4)	58.8 (±20.7)	50.0 (±17.8)	48.8 (±18.1)
History of cerebral event#, n (%)	9 (12.3%)	2 (11.1%)	3 (60.0%)	4 (11.8%)	0 (0.0%)
History of TIA#, n (%)	6 (8.2%)	2 (11.1%)	1 (20.0%)	3 (8.8%)	0 (0.0%)
History of stroke#, n (%)	6 (8.2%)	1 (5.6%)	2 (40.0%)	3 (8.8%)	0 (0.0%)
Fazekas					
Total score (0-6), median (range)	1 (0-6)	1 (0-6)	0.5 (0-1)	1 (0-6)	0 (0-1)

Continuous variables are presented as median (range) or mean (±SD), discrete variables as number (percentages).
TIA = Transient Ischemic Attack, #As diagnosed by a neurologist

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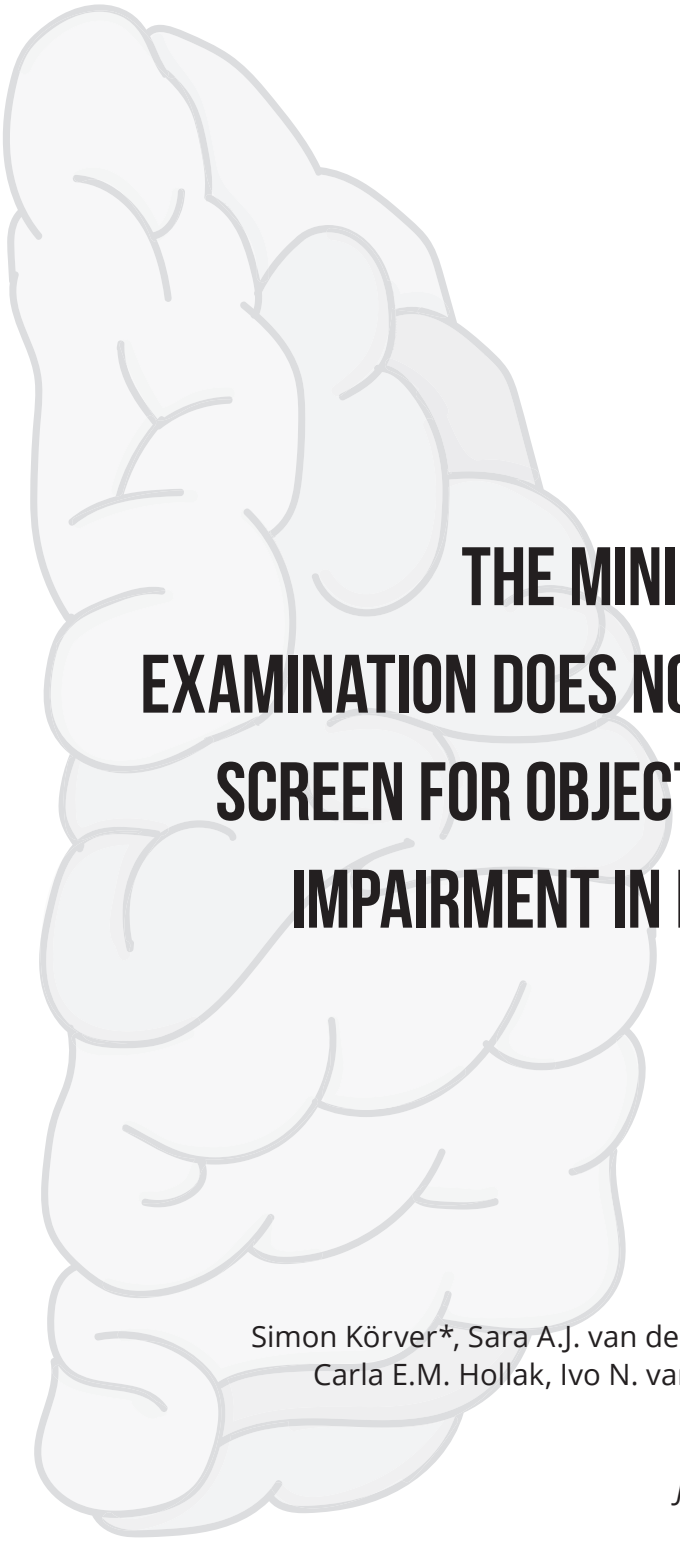

6

THE MINI MENTAL STATE EXAMINATION DOES NOT ACCURATELY SCREEN FOR OBJECTIVE COGNITIVE IMPAIRMENT IN FABRY DISEASE

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Abstract

Fabry disease (FD) patients may suffer from objective cognitive impairment (OCI). This study assessed the accuracy of the Mini Mental State Examination (MMSE) to screen for OCI in FD patients. Presence or absence of OCI was established using a neuropsychological test battery. For different MMSE cut-offs sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and clinical utility index (CUI) to identify OCI were calculated.

Eighty-one patients were included (mean age 44.5±14.3, 35% men, 74% classical phenotype) of which thirteen patients (16%) had OCI. The median MMSE score was 29 (range: 25-30). MMSE cut-offs ≤28 and ≤29 had the highest sensitivity and specificity, with higher specificity reached at cut-off ≤28 (sensitivity: .46, specificity: .73) and higher sensitivity at cut-off ≤29 (sensitivity: .92, specificity: .40). PPV was low for both cut-offs (PPV ≤28: .25, PPV ≤29: .23) resulting in a low positive CUI (case finding ability).

The results of our study indicate that the MMSE does not accurately screen for OCI in FD, with poor sensitivity-specificity trade-off at all cut-offs. The low PPV shows that the majority of FD patients that score below the cut-offs do not suffer from OCI. Administering the MMSE as a screening test will lead to unnecessary referrals for neuropsychological testing, which is time consuming and burdensome. Screening tools designed to accurately detect mild (executive) impairment might prove more appropriate to screen for OCI in FD.

Introduction

Fabry Disease (FD; OMIM 301500) is a rare lysosomal storage disorder caused by mutations in the GLA-gene, which codes for the enzyme α-galactosidase A (enzyme commission no. 3.2.1.22) [1]. Reduced or absent activity of this enzyme results in the accumulation of glycosphingolipids such as globotriaosylceramide (Gb3) in various cells types throughout the body. This leads to cardiac, renal and cerebral involvement and complications [2].

Common cerebrovascular manifestations of FD are white matter lesions, early transient ischemic attacks (TIA) and stroke [3]. In the general population, these cerebrovascular disorders cause cognitive deficits such as impaired executive functioning and vascular dementia [4]. Several studies have shown a relation between FD and objective cognitive impairment (OCI) [5-7]. In addition, we recently established that stroke is independently related to OCI in FD [7].

Interestingly, while subjective cognitive complaints are often mentioned by FD patients [7], these seem to be related to depressive symptoms rather than OCI [7, 8]. Subjective cognitive complaints therefore probably provide little information on the presence of OCI in FD, complicating the estimation of cognition by clinicians.

Neuropsychological examination, the golden standard in the assessment of cognitive function, is time consuming and burdensome [9]. The administration of cognitive screening instruments is a method to select patients that are likely to have OCI. The most widely used cognitive screening instrument is the Mini Mental State Examination (MMSE;[10] Folstein et al 1975). The MMSE was designed for clinicians to get a quick indication of cognitive performance [10]. It is most commonly used to screen for dementia for which it works reasonably well, with a sensitivity of 0.85 and specificity of 0.90 in elderly community samples [11]. Its accuracy for the detection of subtle cognitive deficits is less impressive, with sensitivity dropping to 0.60 [12]. Studies using the MMSE to assess cognitive functioning in FD [13, 14] reported that OCI was not present. Later studies, using a full neuropsychological test battery, have shown that the prevalence of OCI in FD is probably increased compared to the general population [7, 8], suggesting that the MMSE might not be sensitive enough to detect the cognitive deficits found in FD.

The purpose of the present study was to assess the usefulness of the MMSE to screen for OCI in FD.

Methods

Study design and participants

This study used the baseline data of a prospective cohort study assessing cognition in a cohort of FD patients at the Amsterdam University Medical Centre (Amsterdam UMC, location Academic Medical Centre (AMC)). The neuropsychological data were previously described in relation to predictors of OCI [7]. All adult FD patients (≥18 years) known at the AMC (n=154), the national referral centre for FD, were screened for eligibility. Ten patients were excluded according to preset criteria (**Supplemental figure 1**). Patients were phenotypically classified as classical or non-classical in accordance with previously published criteria [7, 15]. The study was approved by the Human Research Ethics Committee of the AMC. All participants signed informed consent prior to inclusion. This manuscript was written in accordance with criteria for appropriate reporting in diagnostic accuracy studies: the STARD [16] and STARDdem [17].

Data collection

Data collection for this study was performed at the AMC outpatient clinic or during a home visit (see **Supplemental methods** for additional information on data collection). The MMSE was administered on the same day as the neuropsychological test battery, always before the battery. Additional data, such as patient characteristics, were collected from the local Fabry database and cross-checked with digital medical records (see **Supplemental methods** for additional information on questionnaires and patient characteristics in **Table 1**).

The mini mental state examination

The MMSE screens general cognitive functioning with a score ranging from 0 to 30, with higher scores indicating better cognitive functioning [10]. The MMSE includes measures of memory, orientation in time and place, working memory, visuospatial skills, object naming, writing, reading and complex motor operation. The cut-off most often used for presence of dementia is ≤23/30 [18].

Neuropsychological test battery

Neuropsychological functioning was assessed across the following five domains: language, memory, visuospatial perception, processing speed and executive functioning. Raw test scores were converted to normative T-scores (mean = 50, standard deviation (SD) = 10, corrected for age, education and sex where possible) using extensive normative data (median sample size = 471, range 121-1000) [7]. Language skills were assessed using the 30-item short form of the Boston Naming Test (BNT) [19, 20] and the Wechsler Adult Intelligence Scale IV: Similarities (WAIS-IV: Sim) [21]. Memory was assessed with the Rey

Auditory Verbal Learning Test (RAVLT) [22] and the Rivermead Behavioural Memory Test (RBMT): Storytelling [23], both assessing immediate recall (IR) and delayed recall (DR). Visuospatial skills were assessed using the WAIS IV: Block Design (WAIS-IV: BD) [21]; and the Judgement of Line Orientation (JLO) [24]. Processing speed was assessed using the Trail Making Test Part A (TMTA) [25], Stroop Word (W) and Colour (C) [26]. Executive functioning was assessed using the TMT part B (TMTB) [25], Stroop Colour-Word (CW) [26], Category Fluency (categories: animals and occupations) [27] and Letter Fluency [28].

Objective cognitive impairment

OCI was defined as a T-score ≤33 on two or more distinct cognitive tests, resembling statistical significance of two one-tailed tests with $p<0.05$ (T-scores ≤33 imply scoring <5th percentile or 1.67 SD below the mean T-score of the normative population of 50). This cut off was chosen with the intention to identify milder cognitive impairment, while at the same time limiting the number of false-positives. Severe OCI was defined as a T-score ≤30 on at least two neuropsychological tests, resembling statistical significance of two two-tailed tests with $p<0.05$ (<2.3rd percentile, -2 SD). To decrease family-wise error rate two or more T-scores ≤33/≤30 on cognitive tests assessing a similar cognitive process were treated as a single deficient test score. This applied to the following cognitive processes: Verbal fluency/Executive functioning: category fluency animals, category fluency occupation and letter fluency. Memory, immediate recall: RAVLT IR and RBMT IR. Memory, delayed recall: RAVLT DR and RBMT DR. Processing speed: TMTA, Stroop W and Stroop C. Executive functioning: TMTB and Stroop CW. Visuospatial skills: WAIS-IV: BD and JLO.

Data analysis

Statistical analyses were performed using R (version 3.4.3). Patient characteristics and questionnaire scores for the different patient groups were compared using one-way ANOVAs, Kruskal Wallis tests and Fisher's exact tests where appropriate. For significant effects, post-hoc tests (Tukeys HSD, Dunn Test and 2x2 Fisher exact tests) were performed, corrected for multiple comparisons.

The diagnostic properties of the MMSE to screen for OCI at different cut-off scores of were assessed by calculating sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and the clinical utility index (CUI). The CUI takes both the discriminative ability of the test and the prevalence of the disease into account with a CUI ≥0.81 being excellent, ≥0.64 good, ≥0.49 satisfactory and <0.49 poor [29]. Positive CUI (CUI+: sensitivity*PPV) displays the case finding ability of the test. Negative CUI (CUI-: specificity*NPV) displays the ruling out ability of the test. An ROC-curve was plotted and the area under the curve (AUC) was calculated.

Results

Patient characteristics

Eighty-one FD patients were included in the study (flow chart in **Supplementary figure 1**). Participants and non-participants did not differ in age, sex, phenotype, median Fazekas score and the occurrence of TIA or stroke [7].

Participating patients' mean age was 44.5 years (SD: 14.3, range: 19-76), 53 were women (65.4%) and 60 (74.1%) were classified as having a classical phenotype (**Table 1**). Depressive symptoms were present in 31 patients (38.3%), with no significant differences between the subgroups divided by sex and phenotype. Disease severity as assessed by the Mainz severity score index ranged from mild in women with a non-classical phenotype (median: 6.5, range: 2-20) to moderate in men with a classical phenotype (median: 32, range: 15-68). Deep white matter lesions were present in 47.3% of all patients.

MMSE and OCI

The median MMSE score of the sample was 29 (range 25-30), with no differences across subgroups divided by sex and phenotype. In the neuropsychological test battery, reduced T-scores were predominantly found in male patients in the executive domain [7]. Thirteen patients were classified as having OCI of whom four had severe OCI. Men with a classical phenotype had the highest prevalence of OCI (n=7; 41.2%), whilst in women with a non-classical phenotype OCI was not present. In the other two subgroups (men with a non-classical and women with a classical disease phenotype), an intermediate prevalence of OCI was found (27.3% and 7.0% respectively).

Diagnostic properties of the MMSE

There were no properties calculated for cut-off scores below 25, as the range of scores was 25-30. The accuracy of the MMSE to screen for OCI was calculated at different cut-offs (**Table 2**). The best sensitivity-specificity trade-offs were reached at cut-off ≤28 and cut-off ≤29, with higher specificity reached at cut-off ≤28 (sensitivity: .46, specificity: .73, PPV: .25, NPV: .88) and higher sensitivity at cut-off ≤29 (sensitivity: .92, specificity: .40, PPV: .23, NPV: .96).

High NPV was found at all cut-offs (range: .85-.96), while the PPV was low at cut-offs ≤26/30 to ≤29/30 (range: .23-.50). The CUI+ (case finding ability) ranged from .08 to .21 and the CUI- (ruling out ability) ranged from .85 at cut-off ≤25/30 to .39 at cut-off ≤29/30. The ROC-curve is displayed in **Figure 1**; the AUC of the ROC-curve is 0.686 (95% confidence interval = 0.547-0.826).

Table 1 Patient characteristics, MMSE and objective cognitive impairment divided by disease phenotype and sex

	All	Classical men	Non-classical men	Classical women	Non-classical women	Intergroup Comparison†	
	(n=81)	(a: n=17)	(b: n=11)	(c: n=43)	(d: n=10)	p-value	post-hoc
Age in years, mean±SD	44.5±14.3	38.6±13.5	58.0±11.2	43.5±13.9	43.9±13.0	**.003**	a,c<b
MMSE score#, median (range)	29 (25-30)	29 (27-30)	29 (27-30)	29 (25-30)	29 (28-30)	.593	-
OCI, n (%)	13 (17.1%)	7 (41.2%)	3 (27.3%)	3 (7.0%)	0 (0.0%)	**.003**	c<a
Severe OCI, n (%)	4 (4.9%)	2 (11.8%)	1 (9.1%)	1 (2.3%)	0 (0.0%)	.268	-
DART IQ, median (range)	94.0 (68-133)	89.0 (83-114)	85.0 (68-133)	94.5 (82-121)	100.0 (84-121)	**.044**	n.s.
History of ERT, n (%)	48 (59.3%)	17 (100.0%)	3 (27.3%)	27 (62.8%)	1 (10.0%)	**<.001**	b,c,d<a; d<c
Currently on ERT, n (%)	43 (53.1%)	15 (88.2%)	2 (18.2%)	25 (58.1%)	1 (10.0%)	**<.001**	b,d<a; d<c
Education in years, mean±SD	13.8±3.0	14.4±2.8	13.9±4.9	13.3±2.7	14.9±1.8	.353	-
Unemployed, n (%)	32 (39.5%)	9 (52.9%)	5 (45.5%)	15 (34.9%)	3 (30.0%)	.543	-
Unfit for work*, n (%)	20 (24.7%)	7 (41.2%)	2 (18.2%)	10 (23.3%)	1 (10.0%)	.315	-
History of Depression, n (%)	22 (27.2%)	3 (17.6%)	3 (27.3%)	12 (27.9%)	4 (40.0%)	.656	-
CESD score, median (range)	11 (0-44)	11 (0-40)	12 (0-37)	12 (0-44)	7.5 (0-20)	.722	-
Above cut off ≥ 16, n (%)	31 (38.3%)	7 (41.2%)	4 (36.4%)	17 (39.5%)	3 (30.0%)	.969	-
MSSI score, median (range)	24 (2-68)	32 (15-68)	23 (4-42)	24 (2-41)	6.5 (2-20)	**<.001**	d<a,b,c; b<a
History of TIA or stroke, n (%)	15 (18.5%)	4 (23.5%)	2 (18.2%)	9 (20.9%)	0 (0.0%)	.482	-
Deep WMLs**, n (%)	35 (47.3%)	8 (47.1%)	3 (37.5%)	19 (48.7%)	5 (50.0%)	1	-
Fazekas score**, median (range)	1 (0-3)	0 (0-3)	1 (0-2)	0 (0-3)	0.5 (0-1)	.885	-
LVMI** in gr/m^2, median (range)	62.7 (33.4-139.6)	78.3 (45.9-139.5)	64.7 (50.1-136.9)	55.9 (36.6-119.1)	44.7 (33.4-77.6)	**<.001**	c<a, d<a.b
eGFR in ml/min/1.73m^2, median (range)	94.6 (11.4-141.0)	105.6 (25.4-141.0)	77.3 (11.4-109.9)	94.0 (45.6-131.1)	95.4 (73.6-118.3)	**.004**	b<a,c,d

Continuous variables were displayed as mean±SD or median (range) and discrete variables as n (%). CESD = Centre for Epidemiological Studies – Depression scale; DART = Dutch Adult Reading Test; eGFR = estimated Glomerular Filtration Rate; ERT = Enzyme Replacement Therapy; LVMI = Left Ventricular Mass Index; MMSE = Mini Mental State Examination; MSSI = Mainz Severity Score Index; OCI = Objective Cognitive Impairment; TIA = Transient Ischemic Attack; WMLs = White Matter Lesions

*† Intergroup comparisons were conducted with one-way ANOVAs, Kruskal Wallis tests and Fisher's exact tests where appropriate. Bold p-values are <.05. In case of p-values <0.05 post-hoc tests (Tukeys HSD, Dunn Test and 2x2 Fisher exact tests) were performed, corrected for multiple comparisons. The letters a,b,c,d denotes which groups differed from other groups. - = No post-hoc test performed; # In one 48-year old woman with a classical phenotype and without objective cognitive impairment, the MMSE was not administered due to logistical issues; n.s. = not significant after correcting for multiple comparisons. * Inability to work was defined as an official statement from the Dutch government that one is unfit for work; **Imaging data of seven patients (four classical women, three non-classical men) were not available (presence of non-MRI compatible ICD/pacemaker (n=6), claustrophobia (n=1)).*

Table 2 Accuracy of the Mini Mental State Examination to screen for objective cognitive impairment per cut-off for all Fabry patients

Cut-off score	TP	FP	TN	FN	Sensitivity	Specificity	PPV	NPV	CUI+	CUI-
≤ 25/30	1	0	67	12	0.08	1.00	1.00	0.85	0.08	0.85
≤ 26/30	1	1	66	12	0.08	0.99	0.50	0.87	0.04	0.83
≤ 27/30	3	7	60	10	0.23	0.90	0.30	0.86	0.07	0.77
≤ 28/30	6	18	49	7	0.46	0.73	0.25	0.88	0.12	0.64
≤ 29/30	12	40	27	1	0.92	0.40	0.23	0.96	0.21	0.39
AUC (95% C.I.)	0.686 (.547-.826)									

*AUC = Area under the curve, C.I. = Confidence interval, CUI+ = Positive Clinical Utility Index = Sensitivity*PPV, CUI- = Negative Clinical Utility Index = Specificity*NPV, FN = False Negative, FP = False positive, TN = True Negative, TP = True positive, NPV = Negative Predictive Value, PPV = Positive predictive value*

Post hoc analyses: MMSE and OCI in patient subgroups

We calculated the discriminant properties of the MMSE for different patient subgroups to evaluate whether the MMSE performed better between subgroups divided by sex or phenotype or when screening for severe OCI. The discriminant properties of the MMSE for women, men, classical and non-classical phenotype showed a similar pattern as for the patient group as a whole (**Supplementary table 1.1 – 1.4**).

The discriminant properties of the MMSE for *severe* OCI were better than for *any* OCI (**Supplementary Material 1.5**). The best sensitivity-specificity trade-off in severe OCI was reached at cut-off ≤27 (sensitivity: .75, specificity: .91, PPV: .30, NPV: .99). Again, the CUI+ (case finding ability) was low (≤27: .23).

Discussion

The results of our study indicate that the MMSE does not accurately screen for OCI in FD, with poor sensitivity-specificity trade-off at all cut-offs. Thirteen patients had OCI according to our preset criteria. The poor PPV, case finding ability (CUI+) and ruling out ability (CUI-) disqualify the MMSE as a cognitive screening instrument to determine which patients need comprehensive neuropsychological testing, as the majority of patients would still be referred for further testing, which is time consuming and burdensome.

Our results are in line with the consensus that the MMSE cannot accurately differentiate subtle cognitively impaired from cognitively unimpaired patients [12] and does not detect executive dysfunction [30]. Studies suggest that the MMSE is an adequate screening instrument in a setting with a high prevalence of disorders resulting in severe cognitive impairment. It loses predictive value when cognitive disturbances are milder, less prevalent and mainly occur in the executive domain [31-33], as seems to be the case in FD [5, 7].

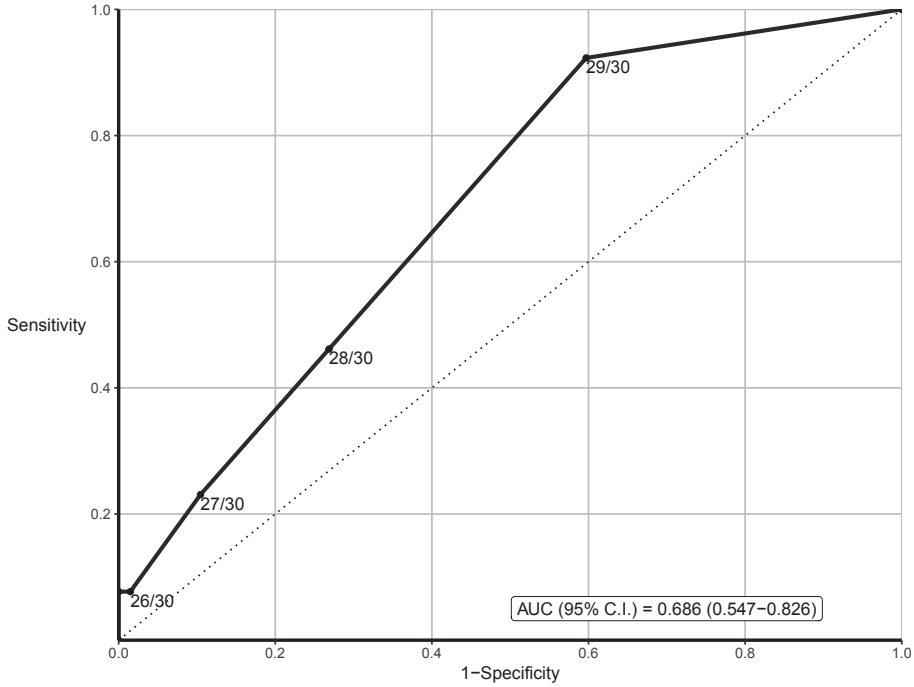

Figure 1 ROC curve portraying the accuracy of the Mini Mental State Examination at different cut-offs to identify objective cognitive impairment in Fabry patients

This is, to our knowledge, the first study on the accuracy and effectiveness of using a cognitive screening instrument in a FD population. Previous studies have used the MMSE to assess global cognitive functioning in FD patients [13, 14, 34] (**Supplementary table 2**). The conclusion reached in these studies, namely that cognition is unaffected when the MMSE scores are in the normal range (≥24/30) [13], is in disagreement with the results of the current study, in which we validated MMSE scores using individual neuropsychological test scores.

Although we assessed cognition using the gold standard, a neuropsychological test battery, the cut off for the presence of OCI is an arbitrary one. After reviewing FD literature we expected that most cognitive impairment found in this disorder would be mild [5]. As such, a cut-off T-score of ≤33 on two tests assessing different cognitive domains limited the number of false positives, while still including patients with milder cognitive impairment.

An alternative to using the MMSE could be to use alternative screening instruments such as the Montreal Cognitive Assessment (MoCA) [35]. This screening instrument includes

more cognitive domains that seem to be affected in FD [5], like executive functioning and sustained attention. Also, the MoCA is advised for use in populations with mild cognitive impairment or early stage dementia [12, 31]. Even though no cognitive impairment was found in FD patients at group level using the MoCA [13], the MoCA classified 21% of FD patients as possibly having mild cognitive impairment compared to 11% of controls. Nonetheless, it remains to be investigated whether the MoCA is able to accurately detect individual FD patients that show OCI in comparison to a neuropsychological test battery.

In conclusion, this study showed a poor ability of the MMSE to screen for OCI in patients with FD. Clinicians should be cautious in using the MMSE, as it is probably not time- or cost-effective as a screening tool and could burden patients with unnecessary assessments. Future research should find out whether alternatives show better accuracy to screen for OCI in FD.

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Supplemental methods

Data collection

Data collection for this study was performed from July 2016 to April 2017. All included participants were administered multiple questionnaires and a comprehensive neuropsychological test battery, either at the academic medical center (AMC) outpatient clinic, or during a home visit. The mini mental state examination (MMSE) was administered on the same day as the neuropsychological test battery, always as the first test in the battery. The test administration was performed by trained staff supervised by a clinical neuropsychologist. Patients completed a structured interview about their background characteristics and subjective (cognitive) complaints prior to the test battery.

Patient characteristics

Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI formula as recommended by the most recent KDIGO guideline [1]. Left ventricular mass (without papillary muscles) were assessed on MRI and adjusted for body surface area using the Dubois formula.

White matter lesions were rated on MRI according to the Fazekas score. The Fazekas score separately rates periventricular and deep white matter lesions from 0 (no white matter lesions) to 3 (confluent white matter lesions) [2]. A modified version only reporting the deep white matter lesions is reported in this study, which is common practice in Fabry disease [3].

Six MRIs of the heart and brain were missing due to presence of an MRI incompatible ICD or pacemaker (three women with a classical phenotype, three men with a non-classical phenotype). MRI of the heart and brain was missing in one woman with classical disease due to claustrophobia.

Mainz severity score index

Disease severity was rated using the Mainz severity score index, which is composed of four subscales (general, neurological, renal and cardiac) [4]. The four subscales were added to a total score ranging from 0-76 points. Patients can be classified as mildly (0-19), moderately (20-40) or severely affected (41-76).

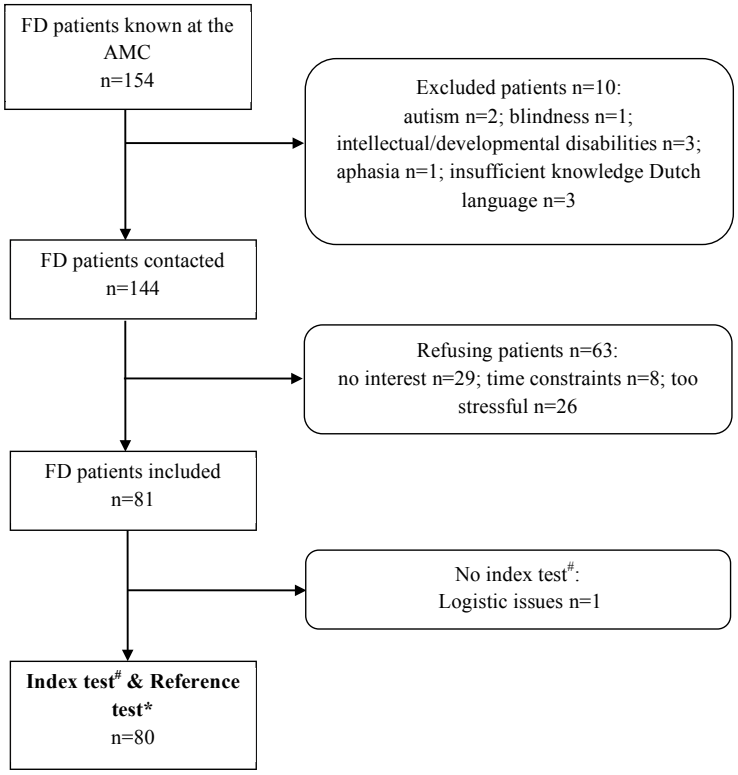
Centre for Epidemiological Studies – Depression scale

Depressive symptoms were quantified using the Centre for Epidemiological Studies – Depression scale with scores ≥16 indicating the presence of depressive symptoms (range score: 0-60) [5].

Dutch Adult Reading Test

We used the Dutch Adult Reading Test, the Dutch version of the National Adult Reading Test, as an estimate of intelligence (IQ) [6].

Supplemental figure 1



Supplementary Figure 1 Flow chart of participation**.** AMC = Academic medical center, FD = Fabry Disease, MMSE = Mini Mental State Examination, # Index test = MMSE, *Reference test = neuropsychological test battery

Supplemental table 1.1 – 1.5

Supplementary table 1.1 Accuracy of the Mini Mental State Examination to screen for OCI per cut-off for men with Fabry disease

Cut-off score	TP	FP	TN	FN	Sensitivity	Specificity	PPV	NPV	CUI+	CUI-
≤ 25/30	0	0	18	10	0.00	1.00	Na	0.64	Na	0.64
≤ 26/30	0	0	18	10	0.00	1.00	Na	0.64	Na	0.64
≤ 27/30	2	2	16	8	0.20	0.89	0.50	0.67	0.10	0.59
≤ 28/30	4	6	12	6	0.40	0.67	0.40	0.67	0.16	0.44
≤ 29/30	9	11	7	1	0.90	0.39	0.45	0.88	0.41	0.34

*CUI+ = Clinical Utility Index Positive = Sensitivity*PPV, CUI- = Clinical Utility Index Negative = Specificity*NPV, FN = False Negative, FP = False Positive, Na = Not applicable (cannot be calculated), NPV = Negative Predictive Value, OCI = objective cognitive impairment, PPV = Positive predictive value, TN = True Negative, TP = True Positive*

Supplementary table 1.2 Accuracy of the Mini Mental State Examination to screen for OCI per cut-off for women with Fabry disease

Cut-off score	TP	FP	TN	FN	Sensitivity	Specificity	PPV	NPV	CUI+	CUI-
≤ 25/30	1	0	49	2	0.33	1.00	1.00	0.96	0.33	0.96
≤ 26/30	1	1	48	2	0.33	0.98	0.50	0.96	0.17	0.94
≤ 27/30	1	5	44	2	0.33	0.90	0.17	0.96	0.05	0.86
≤ 28/30	2	12	37	1	0.66	0.76	0.14	0.97	0.10	0.74
≤ 29/30	3	29	20	0	1.00	0.41	0.09	1.00	0.09	0.41

*CUI+ = Clinical Utility Index Positive = Sensitivity*PPV, CUI- = Clinical Utility Index Negative = Specificity*NPV, FN = False Negative, FP = False Positive, NPV = Negative Predictive Value, OCI = objective cognitive impairment, PPV = Positive predictive value, TN = True Negative, TP = True Positive*

Supplementary table 1.3 Accuracy of the Mini Mental State Examination to screen for OCI per cut-off for Fabry patients with a classical phenotype

Cut-off score	TP	FP	TN	FN	Sensitivity	Specificity	PPV	NPV	CUI+	CUI-
≤ 25/30	1	0	49	9	0.10	1.00	1.00	0.84	0.10	0.84
≤ 26/30	1	1	48	9	0.10	0.98	0.50	0.84	0.05	0.82
≤ 27/30	2	6	43	8	0.20	0.88	0.25	0.84	0.05	0.74
≤ 28/30	4	14	35	6	0.40	0.71	0.22	0.85	0.09	0.61
≤ 29/30	9	26	23	1	0.90	0.47	0.26	0.96	0.23	0.45

*CUI+ = Clinical Utility Index Positive = Sensitivity*PPV, CUI- = Clinical Utility Index Negative = Specificity*NPV, FN = False Negative, FP = False Positive, NPV = Negative Predictive Value, OCI = objective cognitive impairment, PPV = Positive predictive value, TN = True Negative, TP = True Positive*

Supplementary table 1.4 Accuracy of the Mini Mental State Examination to screen for OCI per cut-off for Fabry patients with a non-classical phenotype

Cut-off score	TP	FP	TN	FN	Sensitivity	Specificity	PPV	NPV	CUI+	CUI-
≤ 25/30	0	0	18	3	0.00	1.00	Na	0.86	Na	0.86
≤ 26/30	0	0	18	3	0.00	1.00	Na	0.86	Na	0.86
≤ 27/30	1	1	17	2	0.33	0.94	0.50	0.89	0.17	0.85
≤ 28/30	2	4	14	1	0.67	0.78	0.33	0.93	0.22	0.73
≤ 29/30	3	14	4	0	1.00	0.22	0.18	1.00	0.18	0.22

*CUI+ = Clinical Utility Index Positive = Sensitivity*PPV, CUI- = Clinical Utility Index Negative = Specificity*NPV, FN = False Negative, FP = False Positive, NPV = Negative Predictive Value, OCI = objective cognitive impairment, PPV = Positive predictive value, TN = True Negative, TP = True Positive*

Supplementary table 1.5 Accuracy of the Mini Mental State Examination to screen for severe OCI per cut-off for all Fabry patients

Cut-off score	TP	FP	TN	FN	Sensitivity	Specificity	PPV	NPV	CUI+	CUI-
≤ 25/30	1	0	76	3	0.25	1.00	1.00	0.96	0.25	0.96
≤ 26/30	1	1	75	3	0.25	0.98	0.50	0.96	0.13	0.95
≤ 27/30	3	7	69	1	0.75	0.91	0.30	0.99	0.23	0.89
≤ 28/30	3	21	55	1	0.75	0.72	0.13	0.98	0.09	0.71
≤ 29/30	4	48	28	0	1.00	0.37	0.08	1.00	0.09	0.37

*CUI+ = Clinical Utility Index Positive = Sensitivity*PPV, CUI- = Clinical Utility Index Negative = Specificity*NPV, FN = False Negative, FP = False Positive, NPV = Negative Predictive Value, OCI = objective cognitive impairment, PPV = Positive predictive value, TN = True Negative, TP = True Positive*

Supplemental table 2

Supplementary table 2 Studies that administered the Mini Mental State Examination in patients with Fabry disease

Study	Patients, n (men)	Age (years), median or mean, ±SD or (range)	Study Design	MMSE scores, median or mean, ±SD or (range)	Other screening instruments, median or mean, ±SD or (range): domains impaired	Remarks by authors about MMSE outcomes
Low et al 2007 [7]	Total: 21 (19); with MMSE: 17 (15)	*Men*: 40.4±11.9 (20–62) *Women*: 20 and 56	prospective, cross-sectional	28.4	NuCOG, 90: language	"Fabry patients appear to have few higher-level deficits though formal detailed neuropsychological assessments would be needed to detect subtle deficits"
Lelieveld et al 2015 [8] Baseline	25 (10)	*All*: 39 (19-55)	prospective, longitudinal	30 (27-30)	-	-
Lelieveld et al 2015 [8] Follow-up	14 (4)	*All:* 47 (27-64)	prospective, longitudinal	29.5 (24-30)	-	-
Löhle et al 2015 [9]	110 (50)	*Men:* 50.5±15.9 (19-81) *Women:* 47.8±16.1 (17-84)	prospective, cross-sectional	*Men:* 28.5±1.5, no scores <24 *Women:* 28.4±1.8, two scores <24	MoCA, *Men:* 27.3±2.0, 8 scores <MCI cut-off *Women:* 26.6±2.8, 15 scores <MCI cut-off	"Evaluation with MMSE and MoCA did not reveal significant cognitive deficits in patients with FD, although mean MoCA scores were slightly lower than in controls [...] due to reduced performance in abstraction and delayed recall"

FD = Fabry disease, SD = standard deviation; MMSE = Mini Mental State Examination; NuCOG = Neuropsychiatry Unit Cognitive Assessment Tool; MoCA = Montreal Cognitive Assessment

Supplemental references

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7

DEPRESSIVE SYMPTOMS IN FABRY DISEASE: THE IMPORTANCE OF COPING, SUBJECTIVE HEALTH PERCEPTION AND PAIN

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Abstract

Background:
Despite the high prevalence of depressive symptoms in Fabry disease (FD), it is unclear which patient characteristics are important in relation to these symptoms. Additionally, the impact of coping styles in relation to depressive symptoms in FD has been unexplored. Determining the impact of different factors relating to depressive symptoms in FD can guide both prevention and treatment of these symptoms.

Methods:
Depressive symptoms (Center for Epidemiologic Studies Depression scale (CESD)) and coping styles (Utrecht Coping List) were assessed in a Dutch FD cohort. Other potentially important variables were identified from FD literature and assessed in this cohort. Relations were evaluated using multiple linear models.

Results:
Potentially important variables in FD literature were: pain, unemployment, health perception, being single, comorbidities and stroke. Employed coping styles were "avoidance and brooding", "positivity and problem solving" and "seeking social support". Thirty-one of the 81 FD patients (38%) had depressive symptoms. CESD-scores were lower in patients with better health perception and more "positivity and problem solving" and higher in patients with more pain and "avoidance and brooding". The best model explained 70% (95%CI: 54-76%) of observed variance of the CESD.

Conclusions:
Depressive symptoms in FD are related to pain, negative health perception and use of specific coping styles. Psychological interventions could be employed to alter coping behavior and alleviate depressive symptoms.

Introduction

Fabry disease (FD; OMIM 301500) is a rare X-inherited lysosomal storage disorder. Accumulation of globotriaosylceramide and related compounds occurs in various cell types due to deficiency of α-galactosidase A activity (enzyme commission no. 3.2.1.22). Accumulation of those substrates may result in damage of the kidneys, heart and brain [1]. Important predictors of symptoms and complications in FD are sex and phenotype [2]. Generally, men have more and earlier complications and are more severely affected compared to women. In addition, patients with a classical FD phenotype are often more severely affected compared to patients with a non-classical FD phenotype [2].

A high prevalence of depressive symptoms (46%) has been reported in patients with FD compared to the general population [3-6]. FD related factors, such as pain [3, 4] and non-FD related factors such as being single [4] or lack of social support [7] have been related to depressive symptoms in FD in earlier studies. It has been hypothesized that the cerebral pathology in FD might be a biological substrate for depressive symptoms [3, 6, 7].

Interestingly, while most studies failed to establish a relation between organ involvement and depressive symptoms [5, 8], FD patients' perception of their health was strongly related to depressive symptom severity [4, 9]. This relationship is not unique for FD and has been shown in other diseases as well [10]. While many patients living with a chronic disease show resilience and manage to adapt to new situations, such adjustment is hampered in a substantial subgroup [11]. Coping, a process of cognitive and behavioral effort to manage daily hassles as well as stressors that tax or exceed the resources of a person [12], might be an important factor in the psychological adjustment to a chronic disease like FD. In chronic diseases such as rheumatoid arthritis or type 2 diabetes, different coping styles have been related to both improvement and worsening of psychological [13] and physical outcomes [13, 14].

Determining the importance of different factors in relation to depressive symptoms in FD can support the identification of patients at risk as well as a starting point for FD specific (psychologic) interventions to prevent or treat depressive symptoms. Previous studies explored different variables in relation to depressive symptoms making it difficult to determine which factors should receive more attention and which can be ignored. Moreover, coping styles have not been previously assessed in relation to depressive symptoms in patients with FD. The purpose of this study was therefore: 1) To identify potentially important variables related to depressive symptoms in FD through a literature search and to evaluate the effect of these in our patient cohort; 2) To evaluate coping styles in relation to depressive symptoms in FD; 3) To explore further potential variables of interest in relation depressive symptoms in FD.

Methods

Study design and data collection

The Amsterdam University Medical Center (location Academic Medical Center (AMC)) is the national referral center for FD. Adult Fabry patients (n = 154) at the AMC were screened for eligibility (**Figure 1**). All included patients filled out questionnaires and completed a comprehensive neuropsychological assessment, between July 2016 and April 2017. The tests were performed at the AMC outpatient clinic or during a home visit. The neuropsychological data have been published elsewhere [15]. Demographic, clinical and disease characteristics were extracted from a local clinical database and cross-checked with medical records. Patients were phenotypically characterized as having classical or non-classical FD using established criteria [15, 16].

Identification of variables related to depressive symptoms in FD

Studies were identified using: 1) a systematic review giving an overview of studies on depressive symptoms in FD until November 2012 [3] and 2) a PubMed search until the 7th of January 2019. We used an extended version of the search from the systematic review [3] including synonyms of "Fabry disease", "depression" and "psychology" (see **Supplemental identified variables: Search for studies**). Variables were extracted and classified as "related to depressive symptoms in FD" or as "unrelated to depressive symptoms in FD".

Depressive symptoms

The Center for Epidemiologic Studies Depression scale (CESD) was used to quantify depressive symptoms [17]. Twenty items are scored on a four point Likert scale (range 0 to 3) resulting in a score between 0 and 60. Scores ≥16 indicate the presence of depressive symptoms [4, 17].

Coping

Coping was measured using the Utrecht Coping List (UCL, a Dutch version of the Coping Scale by Westbrook [18]), a questionnaire consisting of 47 items measuring seven coping styles (palliative, passive, active, avoiding, social support seeking, reassuring thoughts, expressing emotions) [19]. Responses are rated on a scale ranging from 1 (seldom or never) to 4 (very often) and can be added to a total score per coping style, with higher scores indicating stronger use of that coping style. Coping is regarded as a personality style, meaning that most people have a regular way of coping with stressors but might change this style somewhat depending on the situation [19].

Neuropsychological test battery and subjective cognitive complaints

All included patients completed 16 well-established neuropsychological tests assessing: language, memory, visuospatial perception, processing speed and executive functioning (for specific neuropsychological tests see [15]). Presence or absence of objective cognitive impairment was determined using preset criteria (see **Supplemental methodology: Objective cognitive impairment**). Subjective cognitive complaints were assessed in a structured interview and rated as present or absent.

Additional questionnaires

Pain was assessed using the Brief Pain Inventory (BPI) with scores graded from 0 (absence of pain) to 10 (worst possible pain) [20]. For this study we used the BPI severity score. This score is an average of four items: worst pain, least pain, average pain and pain right now [21].

The 36-item short form survey (SF-36) is a health related quality of life (QoL) questionnaire, consisting of 36 items. The SF-36 assesses eight domains of QoL on a scale from 0-100, with higher scores indicating better functioning [22]. In this study we focused on the following subscales: subjective health perception, fatigue and self-rated social functioning.

Sleep quality was measured using the Pittsburgh Sleep Quality Index (PSQI) [23]. Total scores range from 0 to 21 and a score >5 is indicative of poor sleep quality.

Clinical characteristics, complications and comorbidities

We calculated left ventricular mass, rated cardiac fibrosis and calculated estimated glomerular filtration rate (see **Supplemental methodology: Clinical characteristics and complications** for additional information). Stroke was diagnosed by a neurologist using a combination of clinical symptoms and MRI (if available). Comorbidity was defined as presence or absence of an additional (chronic) somatic disorder.

Brain MRI

Routine follow-up scans were performed on a yearly or biannual basis using a 3T system (Philips Ingenia, Philips Medical Systems, Best, The Netherlands), using a standardized protocol [15]. Two neuroradiologists rated the MRIs, (*MRL* evaluated basilar artery pathology, *MGL* evaluated infarctions and white matter lesions (WMLs)), blinded for all patient characteristics. WMLs were rated on axial FLAIR using the Fazekas scale, ranging from 0 (no WMLs) to 6 (confluent periventricular and deep WMLs) [24].

Statistical methods

R (version 3.5.1) was used for statistical analysis. P-values <0.05 were regarded as significant, unless stated otherwise.

Firstly, an exploratory factor analysis (EFA) was performed on the UCL. The rationale was that adding the original seven subscales of the UCL to a multiple regression analysis would complicate adding other variables due to limited power. The EFA reduced the number of UCL scales for the multiple regression analysis while providing a reflection of coping styles employed by FD patients. The EFA in short: We adjusted the EFA-methodology for the non-normality and ordinal nature of the data. Factors were named using the items with the strongest loading per factor and factor scores were calculated according to the Anderson-Rubin method [25]. This results in continuous scores with a mean of 0 and a change in factor score of 1 per SD increase or decrease. Most scores will range between -2 to 2, and higher scores indicate more extensive use of the coping style in question. UCL factor scores were split by sex and phenotype. A one-way ANOVA with Bonferroni correction was performed to compare factor scores.

Secondly, two multiple linear regression models were created with CESD score as the outcome variable. Model 1 was used to evaluate the effect of important variables identified in previously published FD research i.e. previously significantly related to depressive symptoms in FD and available in our cohort. In Model 2 we extended Model 1 with the coping styles identified using EFA. Assumptions of both models were assessed and we performed sensitivity analyses removing outliers/influential patients to test the robustness of the findings.

Lastly, we explored the effects of other potentially interesting variables in relation to depressive symptoms in FD, using an akaike information criterion based explorative automated model generating procedure. The explorative automated procedure specified all possible models with the given set of variables and presents model-averaged importance of variables (See **Supplemental methodology: statistical methods** for additional information on abovementioned analyses). In the explorative automated model generating procedure we added the variables of Model 2 as well as variables that are important in depression research in the general population but seemed less important or have never been explored in previous FD literature.

Results were reported in accordance with the Strengthening the Reporting of Observational studies in Epidemiology guidelines [26].

Results

Patients

There were no significant differences between participants and excluded patients/ non-participants in sex, phenotype, age, history of stroke or median Fazekas score [15]. A total of 81 patients were included, 52.6% of the Dutch Fabry cohort (**Figure 1**), with a mean age of 44.5±14.3 years (range: 19-76 years) (**Table 1**). Twenty-eight patients were men (34.6%), 60 patients (74.1%) had a classical phenotype and 43 patients (53.1%) were currently treated with enzyme replacement therapy. Twenty-two patients (27.2%) reported a history of, or current, depression. WML severity was generally mild, but in some patients with classical disease Fazekas scores ranged up to 6, indicating presence of severe confluent WMLs.

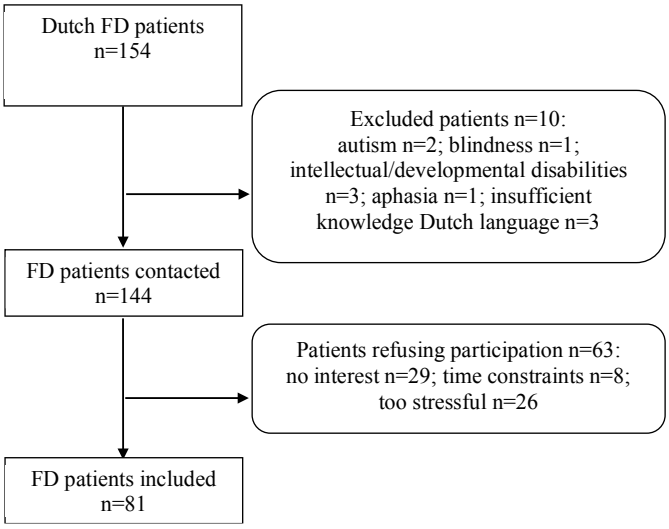

Figure 1 Flow chart of non-participants and in- and excluded patients
FD = Fabry disease

Depressive symptoms and neuropsychological functioning

A total of 31 patients (38.3%) experienced depressive symptoms (score of ≥16 on the CESD) and scores ranged from 0 to 44 (**Table 2**). The presence of depressive symptoms was evenly spread over subgroups defined by sex and phenotype.

Thirteen patients (16.0%) were classified as having objective cognitive impairment, of which seven were men with classical FD (41.0%) and none were women with non-classical FD.

Table 1 Patient characteristics

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
Patients, n (%)	81	17 (21.0%)	11 (13.6%)	43 (53.1%)	10 (12.3%)
Age in years, mean (±SD)	44.5 (±14.3)	38.6 (±13.5)	58.0 (±11.2)	43.5 (±13.9)	43.9 (±13.0)
Currently on ERT, n (%)	43 (53.1%)	15 (88.2%)	2 (18.2%)	25 (58.1%)	1 (10.0%)
Years treated with ERT, median (range)	1.6 (0.0-16.0)	12.4 (1.5-16.0)	0.0 (0.0-14.2)	1.6 (0.0-13.6)	0.0 (0.0-0.3)
Unemployed%, n (%)	32 (39.5%)	9 (52.9%)	5 (45.5%)	15 (34.9%)	3 (30.0%)
Unfit for work$, n (%)	20 (24.7%)	7 (41.2%)	2 (18.1%)	10 (23.3%)	1 (10.0%)
Single#, n (%)	30 (37.0%)	9 (52.9%)	4 (36.4%)	14 (32.6%)	3 (30.0%)
Years of education, mean (±SD)	13.8±3.0	14.4±2.8	13.9±4.9	13.3±2.7	14.9±1.8
Depression*, n (%)	22 (27.2%)	3 (17.6%)	3 (27.3%)	12 (27.9%)	4 (40.0%)
Burnout*, n (%)	12 (14.8%)	1 (5.9%)	0 (0.0%)	7 (16.3%)	4 (40.0%)
Current psychiatric medication, n (%)	15 (18.5%)	2 (11.8%)	3 (27.3%)	9 (20.9%)	1 (10.0%)
Antidepressants, n (%)	7 (8.6%)	1 (5.9%)	2 (18.2%)	3 (7.0%)	1 (10.0%)
Benzodiazepines, n (%)	9 (11.1%)	1 (5.9%)	1 (9.1%)	7 (16.3%)	0 (0.0%)
Loneliness, n (%)	11 (13.6%)	2 (11.8%)	2 (18.2%)	6 (14.0%)	1 (10.0%)
Comorbidity, n (%)	40 (49.4%)	8 (47.1%)	10 (90.9%)	19 (44.2%)	3 (30.0%)
Left ventricular hypertrophy‡†, n (%)	45 (55.6%)	13 (76.5%)	4 (36.4%)	24 (55.8%)	4 (40.0%)
Cardiac fibrosis, n (%)	23/72 (31.9%)	6/17 (35.3%)	2/6 (33.3%)	14/39 (35.9%)	1/10 (10.0%)
eGFR<60 ml/min, n (%)	11 (13.6%)	2 (11.8%)	4 (36.4%)	5 (11.6%)	0 (0.0%)
Fazekas score‡€, median (range)	1 (0-6)	0 (0-6)	1 (0-3)	1 (0-6)	0.5 (0-2)
Complications, n (%)	27 (33.3%)	7 (41.2%)	6 (54.5%)	14 (32.6%)	0 (0.0%)
Cardiac, n (%)	14 (17.3%)	4 (23.5%)	4 (36.4%)	6 (14.0%)	0 (0.0%)
Renal, n (%)	4 (4.9%)	1 (5.9%)	2 (18.2%)	1 (2.3%)	0 (0.0%)
Stroke, n (%)	10 (12.3%)	2 (11.8%)	2 (18.2%)	6 (14.0%)	0 (0.0%)

Continuous variables are presented as median (range) or mean (±SD) and discrete variables as number (percentages).
*% Includes three retirees, $ Includes three patients regarded partially unfit for work, # Unmarried, divorced or widowed, * History of or current, as diagnosed by a general practitioner, psychologist or psychiatrist, ‡ MRIs were unavailable in seven patients (three non-classical men, four classical women) due to presence of an MRI non-compatible pacemaker or ICD (n=6) and due to claustrophobia (n=1). † If MRI of the heart was not available then presence of left ventricular hypertrophy on echocardiography was used. € In one patient the brain MRI was performed in a different hospital.*
ERT = enzyme replacement therapy, eGFR = estimated glomerular filtration rate

Exploratory factor analysis of the Utrecht Coping List

EFA of the UCL data resulted in three coping styles. These styles will be referred to as *"avoidance and brooding"*, *"positivity and problem solving"* and *"seeking social support and comfort"*. See **Supplemental results: EFA** for additional information.

There were no significant differences in employment of coping styles between the FD subgroups divided by sex and phenotype (avoidance and brooding: $F(3,77) = 0.28$, $p = 0.84$; positivity and problem solving: $F(3,77) = 0.87$, $p = 0.46$; social support and comfort: $F(3,77) = 2.42$, $p = 0.07$).

Identified variables and multiple linear regression

A total of 16 studies assessed the relation between one or more variables and depressive symptoms in FD (see **Supplemental identified variables: Depressive symptoms in FD literature and variables of interest** for details). Six variables were found to be significantly related to depressive symptoms in earlier FD studies (i.e. BPI severity score, being unfit for work, SF-36 health perception score, being single, presence of comorbidities and history of stroke). These variables were added in Model 1. Model 1 explained 43.3% of CESD score variance ($F(6,74) = 9.43$, $p < 0.0001$, 95%CI 24.3 – 53.7%, adjusted R^2 39.3%) (**Table 3**). CESD scores were positively related to higher BPI severity scores and negatively related to higher SF-36 health perception and to presence of a comorbidity.

Model 2 investigated the coping styles identified with EFA in relation to the CESD scores (Avoidance and brooding, positivity and problem solving, seeking social support and comfort), in addition to the six variables from Model 1. Model 2 explained 70.3% of CESD score variance ($F(9,71) = 18.68$, $p < 0.0001$, 95%CI 53.9 – 75.9%, adjusted R^2 67.1%) (**Table 3**). CESD scores in this model were positively related to higher BPI severity scores and to higher avoidance and brooding. CESD scores were negatively related to higher SF-36 health perception and to more employment of positivity and problem-solving. The avoidance and brooding coping style had the greatest effect on CESD scores considering the standardized beta coefficients.

Overall, assumptions of both linear models were met. Sensitivity analyses, removing outliers and patients with most influence on the models fit, revealed no major differences in the model results (**Supplemental results: Assumption testing**).

Explorative automated model generation

Another seven variables of interest were identified and included in the automated explorative models with the CESD score as outcome variable (**Supplemental identified variables: Variables related to depressive symptoms in the general population)**.

Added to all variables from Model 2 were: presence of loneliness, cardiac and/or renal involvement, SF-36 fatigue scale, self-rated sleep quality (PSQI), history of depression, subjective cognitive complaints and SF-36 self-rated social functioning scale. Of all these variables the avoidance and brooding and the positivity and problem solving coping styles, the SF-36 social functioning scale, presence of loneliness, the BPI severity score and cardiac and/or renal involvement explained the most CESD variance (**Figure 2**).

Post hoc analyses

Model 1 and 2 showed that presence of a comorbidity was negatively related to the CESD score, mainly in Model 1. Hypertension and hypercholesterolemia were the most prevalent comorbidity noted in our cohort (~50% of patients with a comorbidity), which were well regulated and not leading to symptoms. The negative relation between comorbidities and the CESD score decreased when Model 1 was adjusted by excluding hypertension and hypercholesterolemia (*B* -3.58; p = 0.17; 95%CI -8.71 – 1.55).

Since the Fazekas score was not available for all patients we did not incorporate it in the explorative models. A linear model showed no relation between the Fazekas score and the CESD score (one-point increase: *B* 0.61; p = 0.43; 95%CI -0.92 – 2.13).

There was no relation between presence of objective cognitive impairment and the CESD score or between sex and phenotype and the CESD score [15]. Lastly, we found no relation between years treated with enzyme replacement therapy and the CESD score (one treatment year increase: *B* 0.06; p = 0.79; 95%CI -0.37 – 0.48).

Discussion

In this cross-sectional cohort study including more than half of the Dutch FD patients we found a high prevalence of depressive symptoms (38%), comparable to earlier work in FD patients [4]. We determined the importance of coping, in addition to variables identified from FD literature in relation to depressive symptoms in FD. An avoidant and brooding coping style was related to a higher depressive symptom score, while more a positive and problem-solving coping style was related to a lower score. Pain and a negative health perception, variables identified from FD literature, were also independently related to depressive symptoms. Of interest, while previous studies suggested a relation between unemployment and depressive symptoms, this was not confirmed in our model. Years treated with enzyme replacement therapy showed no relation to depressive symptoms. By using exploratory analyses we identified loneliness, experienced social functioning and cardiac/renal involvement as potentially important factors, which merit further research.

Table 2 Questionnaires, scales and cognition

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
CESD, median (range)	11 (0-44)	11 (0-40)	12 (0-37)	12 (0-44)	7.5 (0-20)
CESD≥16, n (%)	31 (38.3%)	7 (41.2%)	4 (36.4%)	17 (39.5%)	3 (30.0%)
Subjective cognitive complaints*, n (%)	52 (64.2%)	11 (64.7%)	5 (45.5%)	31 (72.1%)	5 (50.0%)
Objective cognitive impairment#, n (%)	13 (16.0%)	7 (41.0%)	3 (27.3%)	3 (7.0%)	0 (0%)
BPI severity, median (range)	1.0 (0.0-7.0)	0.8 (0.0-6.5)	4.0 (0.0-7.0)	2.0 (0.0-7.0)	0.0 (0.0-5.8)
PSQI, median (range)	5.0 (0.0-20.0)	4.0 (0.0-14.0)	6.0 (1.0-13.0)	6.0 (1.0-20.0)	5.5 (2.0-10.0)
PSQI>5, n (%)	39 (48.1%)	4 (23.5%)	7 (63.6%)	23 (53.5%)	5 (50.0%)
SF-36 Fatigue, mean (±SD)	50.5 (±23.0)	55.3 (±24.8)	54.5 (±22.2)	45.5 (±22.0)	59.5 (±22.4)
SF-36 Social functioning, mean (±SD)	71.5 (±26.8)	75.7 (±24.8)	69.3 (±28.2)	67.4 (±28.4)	83.8 (±18.7)
SF-36 Health perception, mean (±SD)	43.3 (±22.6)	41.2 (±25.0)	40.0 (±23.2)	40.5 (±19.1)	63.0 (±25.3)

Continuous variables are presented as median (range) or mean (±SD) and discrete variables as number (percentages).
** Presence or absence of subjective cognitive complaints, #presence or absence of objective cognitive impairment*
CESD = Center for Epidemiologic Studies Depression scale, BPI = Brief Pain Inventory, PSQI = Pittsburgh Sleep Quality Index, SF-36 = Short Form-36 Health Survey

While this study is the first to explore coping in relation to depressive symptoms in FD, similar relations between coping styles and depressive symptoms were found in more common chronic diseases such as type 2 diabetes and rheumatoid arthritis. Avoidance [13] and brooding [27] are generally considered maladaptive and have been related to a higher prevalence of depressive symptoms in these diseases [13, 14, 27]. A positive mentality has been consistently related to lower rates of depressive symptoms [28]. In addition, problem-solving interventions have been effectively employed to decrease depressive symptoms in the general population [29]. The underlying assumption for these interventions is that for rational problem solving, a positive problem orientation is indispensable [29]. While FD itself is not directly "solvable" for the patients, research has shown that a problem-solving approach of intermediate goals (e.g. lifestyle adjustments, scheduling hospital appointments) improves self-management in for example type 2 diabetes patients [30].

Table 3 Summary of multiple linear regression Model 1 and 2

Model 1					**Model 2**			
Independent variables	**B (95% CI)**	**SE B**	**β**	**p-value**	**B (95% CI)**	**SE B**	**β**	**p-value**
BPI severity	1.60 (0.63 – 2.58)	0.49	0.35	0.002	0.82 (0.04 – 1.59)	0.39	0.18	0.039
Unfit for work	0.23 (-5.22 – 5.69)	2.74		0.933	0.47 (-3.57 – 4.51)	2.03		0.817
SF-36 Health perception	-0.19 (-0.30 – -0.09)	0.05	-0.41	<0.001	-0.13 (-0.21 – -0.05)	0.04	-0.28	0.001
Single	-0.49 (-4.42 – 3.43)	1.97		0.804	-0.60 (-3.55 – 2.35)	1.48		0.687
Comorbidity	-6.15 (-10.20 – -2.10)	2.03		0.003	-2.92 (-6.07 – 0.23)	1.58		0.069
Stroke	3.18 (-3.02 – 9.39)	3.11		0.309	3.18 (-1.41 – 7.77)	2.30		0.171
Avoidance and brooding					5.39 (3.82 – 6.95)	0.79	0.50	<0.0001
Positivity and problem solving					-3.12 (-4.53 – -1.71)	0.71	-0.29	<0.0001
Seeking social support and comfort					-0.14 (-1.56 – 1.29)	0.72	-0.01	0.849
Intercept	20.74				18.14			
F-value	9.43			<0.0001	18.68			<0.0001
R2	43.3% (24.3 – 53.7)				70.3% (53.9 – 75.9)			
Adjusted R2	39.3%				67.1%			

B = beta coefficients, β = standardized beta coefficients for continuous variables, SE = standard Error, BPI = Brief Pain Inventory, SF 36 = Short Form-36 Health Survey

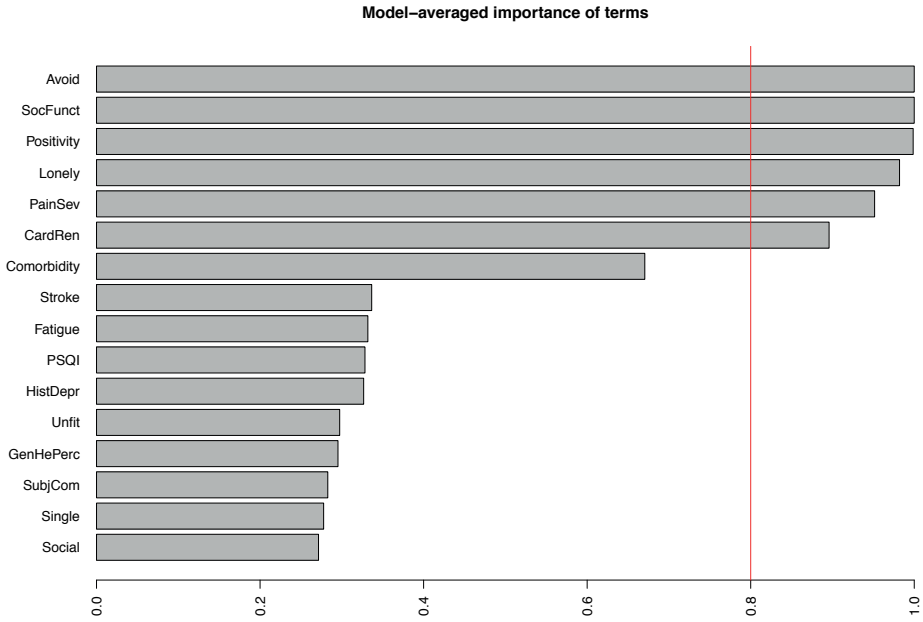

Figure 2 Results explorative models. Model averaged importance of the 6500 models explaining most variance of CESD-scores. Avoidance and brooding and SF-36 social functioning were included in all 6500 models and therefore set to 1.0. All variables with a model averaged importance >0.8 might be relevant variables in relation to the CESD score.
Avoid = Avoidance and brooding, SocFun = Short Form-36 Health Survey (SF-36) social functioning, Positivity = Positivity and problem solving, Lonely = Loneliness, PainSev = Brief pain inventory severity, CardRen = Cardiac and/or renal involvement, Fatigue = SF-36 fatigue, PSQI = Pittsburgh sleep quality index, HistDepr = History of depression, Unfit = unfit for work, GenHePerc = SF-36 general health perception, SubjCom = Subjective cognitive complaints, Social = Social support and comfort

The relation between social support and depressive symptoms has been less clear in chronic disease research. In early theoretical work, it was expected that seeking social support was related to better psychological outcomes [13]. However, we found no relation between seeking social support and depressive symptoms in the FD cohort. An explanation might be that chronic disease can complicate social support due to prolonged strain on the caregiver [31]. It has therefore been postulated that *seeking* social support is not similar to *receiving* social support and that social support might decrease during a prolonged disease course [13, 31]. In line with this, our explorative analyses showed that both subjective social impairment and loneliness may contribute to depressive symptoms in FD, meaning that expecting social support, but receiving less then desired, might increase depressive symptoms.

While we did not assess the relation between pain and coping in this study, the interrelation between pain, coping and depressive symptoms is likely complex [32]. Coping

styles probably influence pain experience and the effect of treatment on pain in FD [3]. Moreover, a study testing a psychological counseling intervention for depressive symptoms in FD patients showed that pain seems to improve when depressive symptoms decrease [33]. It is also likely that depressive symptoms will improve with adequate treatment of pain.

Interestingly, while subjective health perception has been repeatedly identified as an important factor in relation to depressive symptoms in FD, the observed relation between organ complications and depressive symptoms has been less straightforward. We propose that impact of FD on patients' perceived health extends beyond the physical symptoms and complications, to more subjective factors such as uncertainty about the future, difficulties surrounding heritability and stigmatization [34, 35]. In other words, complications and symptoms might have an effect on depressive symptoms, but the perception that patients have of their disease and the extent to which certain coping styles are employed will determine the individual outcome.

Of note, we could not confirm the previously observed relation [8] between a history of stroke and depressive symptoms, nor was there a relation between WMLs and depressive symptoms. This further strengthens the hypothesis that brain abnormalities are not the main cause of depressive symptoms in patients with FD [3, 5].

This study has several limitations. Although the sample size is large for a rare disease like FD, it limited our statistical analyses. Our multiple linear regression models are probably not adequate to detect small to medium effects, and results should be interpreted as such. Moreover, although background characteristics of included patients and non-participants were similar, there might be an inclusion bias: patients with more depressive symptoms might have had greater interest in participation. Conversely, severely depressed patients might have felt unable to participate due to depression related symptoms. Furthermore, we did not find a relation between years treated with enzyme replacement therapy and depressive symptoms. This analysis might be affected by indication bias: more severely affected patients are probably treated earlier and longer. This hampers strong conclusions on the effectiveness of enzyme replacement therapy on depressive symptoms. Lastly, we used an explorative automated model selection procedure. Since this automatically tested >65000 models this presents an extreme case of multiple testing, which warrants confirmation.

Future studies could further unravel the interrelation between pain, coping and depressive symptoms in FD patients by evaluating the mediating effect of coping between pain and depressive symptoms. Moreover, factors that influence patients'

health perceptions (e.g. illness perception, repeated medical testing) could be explored. Lastly, an extension to children and adolescents would be valuable, since coping strategies differ per life stage, as do FD related symptoms.

Finally, we recommend that pain, should be routinely assessed, monitored and treated according to published guidelines [36]. Considering the probable under-diagnosis and under-treatment of depressive symptoms in FD [4, 6] we further recommend to include a screening questionnaire (for example the CESD or the Beck Depression Inventory) in routine clinical care [37]. Patients with depressive symptoms should be referred, preferentially to psychologists with knowledge of chronic diseases [37].

Conclusions

Depressive symptoms are frequent in patients with FD and are related to pain, negative health perception and use of specific coping styles. Future psychological treatment can be tailored to coping styles, for example by focusing on improvement of problem solving or decreasing avoidant behavior, ideally in a research setting.

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Supplemental identified variables: Depressive symptoms in FD literature and variables of interest

Content

Search for studies on variables related to depressive symptoms in FD

In addition to the studies extracted from the systematic review [1], we searched Pubmed for studies on depressive symptoms in FD published until the 7th of January 2019 using the following criteria:

Depressive symptoms:
(((((“Neuropsychiatry”[Mesh]) OR Neuropsychiatr*)) OR ((depressive disorder) OR (((((depression) OR (“Depression”[Mesh] AND “Depressive Disorder”[Mesh])) OR depressive symptoms) OR psychology) OR psychiatry))))
Fabry disease:
AND
(fabry*[tiab]) OR (alpha galactosidase a deficien*[tiab]) OR (angiokeratoma corporis diffusum[tiab])

Identified studies on variables related to depressive symptoms in Fabry disease

Included studies

In total, we identified 16 studies assessing the relation between variables and depressive symptoms in Fabry disease. Seven studies by using the systematic review [2-8], eight studies using the Pubmed search [9-16] and one book chapter presenting new data by screening the reference lists of included studies [17].

Excluded studies

Five studies in the systematic review were excluded since no relations were tested between variables to depressive symptoms in FD [18-22].

Of the 104 studies from the Pubmed search, 92 were excluded after screening of title and abstract. Four more studies were excluded after full text evaluation (no relation tested between variables and depressive symptoms [23, 24], included only pediatric patients [25, 26]).

Supplemental table 3 Assessed variables related to depressive symptoms in Fabry disease literature per study (alphabetic order)

First author	Year	Patients, n (men)	Age (years), median or mean ±SD (range)	Study design	Measure of depressive symptoms	Variables significantly related to depressive symptoms in FD	Variables not significantly related to depressive symptoms in FD
Ali [12]	2017	10 (2)	42.1±12.0 (22-61)	Prospective, longitudinal	ASEBA ASR OASR	(Tele)counseling, change over time in SF-36 mental health, change over time in BPI severity	Change over time in adaptive functioning, change over time in SF-36 physical health, change over time in BPI interference with life
Cole [2]	2007	184 (74)	44±14 (18–76)	Prospective, cross-sectional	CESD	Other chronic illness present, interference of FD symptoms with life, acroparesthesia, anhidrosis, unemployement, abdominal symptoms, cardiac symptoms, ERT, No partner[&], Problems with income[&]	Age, sex, level of education, having child(ren) with FD, cerebrovascular symptoms, renal symptoms, time on ERT, response of symptoms to ERT
Crosbie [3]	2009	28 (16)	(18-60)	Prospective, cross-sectional	MMPI-2 depression subscale	Experienced symptom severity, pain severity	Time on ERT, time since diagnosis
Franzen [14]	2015	52 (17)	42.8±14.7	Prospective, cross-sectional	PHQ-9	Epworth sleepiness scale	-
Grewal [4]	1993	33 (6)	29.3 (23-37)[†]	Retrospective, longitudinal	Reported diagnosis of depression	Acroparesthesia and pain crises	-
Körver [16]	2018	81 (28)	44.5±14.3	Prospective, cross-sectional	CESD	Subjective cognitive complaints	Objective cognitive impairment, sex and phenotype
Laaksonen [5]	2008	12 (0)	45.5±15.1 (17-63)	Prospective, cross-sectional	GCPS depression questions	Age, experienced somatic symptoms, neuropathic pain, decreased IENFD	-
Laney [6]	2010	33 (15)	40 (18-59)	Prospective, cross-sectional	ASR and ABCL, DSM depression scale	Poor adaptive functioning	-
Lelieveld [11‡]	2015	14 (4)	46.1±10.8 (27-64)	Prospective, longitudinal	HAMD-17	-	Age, neuropsychological measures, brain structural parameters

Supplemental table 3 Assessed variables related to depressive symptoms in Fabry disease literature per study (alphabetic order) (continued)

First author	Year	Patients, n (men)	Age (years), median or mean ±SD (range)	Study design	Measure of depressive symptoms	Variables significantly related to depressive symptoms in FD	Variables not significantly related to depressive symptoms in FD
Loeb [13]	2018	41 (12)	47.2±14.7 (20-75)	Prospective, cross-sectional	HAMD-17	Subjective cognitive complaints	Sex, objective cognitive impairment
Löhle [10]	2015	110 (50)	49.0±16.0 (17-84)	Prospective, cross-sectional	BDI-II	MSSI total	-
Müller [17]	2006	36 (18)	36±10	Prospective, cross-sectional	HAMD, reported diagnosis of depression	Social support	-
Schermuly [7]	2011	25 (10)	36.5±11.0 (21-56)	Prospective, cross-sectional	HAMD-17	PANSS positive symptoms, PANSS negative symptoms, SF-36 mental health	Cognitive performance, SF-36 physical health, BPI severity, BPI interference with life, age, WMLL
Segal [8]	2010	16 (7)	29 (7-61)	Prospective, cross-sectional	DSM-IV criteria	Overall FD involvement, decreased cognitive function	-
Sigmundsdottir [9]	2014	17 (12)	46.6±11.8 (25-60)	Prospective, cross-sectional	DASS-21 depression subscale	MSSI neurologic, TIA/stroke, BPI severity, BPI intensity	Age, MSSI total, MSSI general, MSSI cardiac, MSSI renal, CKD
Talbot [15]	2016	20 (20)	43.9±10.7 (23-71)	Prospective, cross-sectional	Clinical diagnosis (symptoms of depression, treatment)	Periodic limb movement index	-

ABCL = Achenbach adult behavior checklist, ASEBA = Achenbach System of Empirically Based Assessment, ASR = Adult Self-Report, BDI-II = Beck Depression Inventory II, BPI = brief pain inventory, CESD = The Centre for Epidemiological Studies Depression scale, CKD = chronic kidney disease (stages according to KDIGO guidelines), Cross = cross-sectional, DASS-21 = Depression, Anxiety and Stress scale, DSM = Diagnostic and Statistical Manual of Mental Disorders, ERT = enzyme replacement therapy, FD = Fabry disease, GCPS = modified graded chronic pain status questionnaire, HAMD = Hamilton Rating Scale for Depression, HAMD-17 = HAMD 17-item version, IENFD = intraepidermal nerve fiber density, long = longitudinal, MMPI-2 = Minnesota Muliphasic Personality Inventory, MSSI = Mainz Severity Score Index, OASR = Older Adult Self Report, PANSS = The Positive and Negative Syndrome Scale, PHQ-9 = patient health questionnaire-9, pros = prospective, retro = retrospective, SF-36 = 36-Item Short Form Survey, WMLL = white matter lesion load

& Relation was claimed, however calculated odds ratios crossed 1. † Age at diagnosis. – Not available. ‡ This study is an eight year follow-up study of a subgroup of the study by Schermuly, Muller [7].

Supplemental table 4 Summary variables related to depressive symptoms in Fabry disease literature, use in models en description

Variables assessed in relation to depressive symptoms	Significant relation found between variable and depressive symptoms [references]	Included in model 1 and 2	Included in explorative models	Name variable assessed in relation to depressive symptoms current study
Age, sex and phenotype				
Age	No [2, 7, 9, 11], Yes [5]	No	No	NA
Sex	No [2, 13]	No	No	NA
Sex and phenotype	No [16]	No	No	NA
Subjective/reported symptoms				
Overall experienced symptoms	Yes [2, 3, 5]	Yes	Yes	SF-36 general health perception
Abdominal, renal, cerebrovascular, cardiac symptoms	Yes [2]	No	No	NA
Subjective cognitive complaints	Yes [13, 16]	No	Yes	Subjective cognitive complaints
Symptoms, organ involvement, disease severity				
Cerebral:				
TIA/stroke	Yes [9]	Yes	Yes	History of stroke
WMLs	No [7]	No	Yes	Fazekas scale
Brain structural parameters	No [11]	No	No	NA
Cognitive functioning	No [7, 11, 13, 16], Yes [8]	No	No	NA
MSSI neurologic	Yes [9]	No	No	NA
Clinical involvement:				
Overall	Yes [8]	No	No	NA
Anhidrosis	Yes [2]	No	No	NA
CKD	No [9]	No	Yes	Renal and/or cardiac involvement (see supplemental table 6 below)
MSSI total	No [9], Yes [10]	No	No	NA
MSSI general, cardiac, renal	No [9]	No	No	NA
Pain:				
Neuropathic pain (acroparesthesia)	Yes [2, 4, 5]	No	No	NA
Decreased IENFD	Yes [5]	No	No	NA
BPI subscales	No [7, 12], Yes [3, 9, 12]	Yes	Yes	BPI severity scale

Supplemental table 4 Summary variables related to depressive symptoms in Fabry disease literature, use in models en description (continued)

Variables assessed in relation to depressive symptoms	Significant relation found between variable and depressive symptoms [references]	Included in model 1 and 2	Included in explorative models	Name variable assessed in relation to depressive symptoms current study
Sleep:				
Periodic limb movement index	Yes [15]	No	No	NA
Excessive daytime sleepiness	Yes [14]	No	Yes	PSQI
Treatment				
(Tele)counseling	Yes [12]	No	No	NA
ERT use and subjective response	No [2]	No	No	NA
Time on ERT	No [2, 3]	No	No	NA
Background and patient characteristics				
Patient characteristics:				
Time since diagnosis	No [3]	No	No	NA
Other chronic illness	Yes [2]	Yes	Yes	Comorbidity
Adaptive functioning	No [12], Yes [6]	No	No	NA
Background:				
No partner	Unclear [2]	Yes	Yes	Relationship status (Partner/Single)
Problems with income	Unclear [2]	No	No	NA
Level of education	No [2]	No	No	NA
Unemployment	Yes [2]	Yes	Yes	Unfit for work
Child(ren) with Fabry disease	No [2]	No	No	NA
Social support	Yes [17]	No	Yes	SF-36 Social functioning
Other				
SF-36 mental health	Yes [7, 12]	No	No	NA
SF-36 physical health	No [7, 12]	No	No	NA
PANSS positive symptoms	Yes [7]	No	No	NA
PANSS negative symptoms	Yes [7]	No	No	NA

BPI = brief pain inventory, CKD = chronic kidney disease, ERT = enzyme replacement therapy, IENFD = intraepidermal nerve fiber density, MSSI = Mainz severity score index, NA = Not assessed, PANSS = positive and negative syndrome scale, SF-36 = 36-Item Short Form Survey, WMLs = white matter lesions

Supplemental table 5 Variables significantly related to depressive symptoms in the general population and unexplored in Fabry disease literature, use in models en description

Variables assessed in relation to depressive symptoms	Scientific foundation	Name variable assessed in relation to depressive symptoms current study (variable description and coding)	Included model 2	Included in explorative models
Coping	MA: Coping styles are positively and negatively related to depressive symptoms [27].	"Avoidance and brooding", "positivity and problem solving" and "seeking social support and comfort"	Yes	Yes
Renal and/or cardiac involvement	MA&R: chronic kidney disease, dialysis and kidney transplantation are associated with depression [28, 29]. MA & R: depression is a risk factor for cardiac disease [30] and occurs more often in patients with cardiac disease [31].	Renal and/or cardiac involvement (No renal or cardiac involvement (0), Renal involvement (eGFR <60ml/min/1.73m^2) and/or cardiac involvement (left ventricular hypertrophy or fibrosis) (1), Cardiac and/or renal complications (2))	No	Yes
History of depression	R: History of depression increases risk of recurrent depression [32, 33].	History of depression (Absent (0)/Present (1))	No	Yes
Loneliness	MA: Loneliness is associated with depression [34].	Loneliness (Absent (0)/Present (1))	No	Yes
Fatigue	SR: Fatigue associated with depression in cancer patients [35] and rheumatoid arthritis patients [36].	SF-36 Fatigue (scale, possible range 0-100)	No	Yes

CKD = chronic kidney disease, EFA = exploratory factor analysis, eGFR = estimated glomerular filtration rate, LVH = left ventricular hypertrophy, MA = Meta-analysis, PSQI = Pittsburgh sleep quality index, R = review, SF-36 = 36-Item Short Form Survey, SR = systematic review

Supplemental methodology

Contents

- Objective cognitive impairment
- Clinical characteristics
- Complications
- Statistical methods
  - Exploratory factor analysis (EFA) methodology
  - Assumption testing multiple linear regression models
  - Calculated statistics multiple linear models
  - Exploratory models

Objective cognitive impairment

Objective cognitive impairment (OCI) was defined as a T-score ≤33 on two or more distinct cognitive tests (T-scores ≤33 imply scoring <5th percentile or 1.67 SD below the mean T-score of the normative population of 50). To decrease family-wise error rate two or more T-scores ≤33 on cognitive tests assessing a similar cognitive process were treated as a single deficient test score.

Clinical characteristics

Kidney function was assessed using the estimated glomerular filtration rate (eGFR in ml/min/1.73m^2), calculated using the CKD-EPI formula [37]. Left ventricular mass on MRI was calculated without papillary muscles and adjusted for body surface area (Dubois formula). If no MRI was available left ventricular mass was calculated using echocardiography (Cube formula), adjusted for height$^{2.7}$ [38]. Presence of left ventricular hypertrophy was defined as >72 g/m^2 or >49 g/m$^{2.7}$ in men and >55 g/m^2 or >47 g/m$^{2.7}$ in women on MRI and echocardiography, respectively [39, 40]. Late gadolinium enhancement on cardiac MRI was regarded indicative of the presence of fibrosis.

For our explorative automated model selection procedure (see statistical methods) we created an ordinal scale rating severity of cardiac and renal involvement from 0-2: (0) No renal or cardiac involvement, (1) renal involvement (eGFR <60 ml/min) and/or cardiac involvement (left ventricular hypertrophy and/or fibrosis), (2) cardiac and/or renal complications.

Complications

An eGFR <15 ml/min/1.73m^2 [37], a history of renal transplantation and/or a history of dialysis were regarded as renal complications. A history of myocardial infarction, coronary artery bypass grafting, percutaneous transluminal coronary angiography, hospitalization due to heart failure, arrhythmias (including atrial fibrillation) and presence of a pacemaker or ICD were regarded as cardiac complications.

Statistical methods

Exploratory factor analysis (EFA) methodology

An exploratory factor analysis (EFA) was performed on the Utrecht Coping List (package: psych [41]). We used the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy to determine the underlying proportion explained by individual items [42]. Items with a KMO value <0.5 were iteratively removed, starting with the item with the lowest value until all individual remaining variables had a KMO value >0.5 [42]. Analyses were performed using the remaining items. Since data were not normally distributed we choose principal axis factoring as factor extraction method [43], and since data were ordinal we choose for

polychoric correlation [44]. We determined the number of factors in the EFA by performing parallel analysis (number of iterations: 1000). We first attempted oblique rotation ("Oblimin"). If factor intercorrelations were low (all <0.32), we used orthogonal rotation ("Equamax") [45]. Because of the low sample size: 1) only factors with at least four items loading >0.6 were viewed as reliable, 2) if this condition was satisfied all items loading >0.5 were used for factor naming [42].

Assumption testing multiple linear regression models

Standardized residuals (mean = 0, SD = 1) were used to identify possible outliers [46]. Influential points were identified using Cook's distance, with the cutoff set at 4/(n of patients – n of variables – 1). Scores >1 were regarded as potentially impactful [47]. If influential points or outliers were identified we performed sensitivity analyses (rerunning the model after removing the influential points). Multicollinearity was checked using variance inflation factor (VIF), with the cutoff set at (square root(VIF) ≤ 2) [47]. Homoscedasticity, linearity and multivariate normality were visually assessed using scale-location plots, residuals versus fitted value plots and a Q-Q plot of the studentized residuals. Lastly, independence of errors was tested using the Durbin-Watson test.

Calculated statistics multiple linear models

Next to regular beta coefficients, we calculated standardized beta coefficients for continuous variables to improve comparability. These can be interpreted as: how many SD will the dependent variable change per SD increase of the independent variable. Using bias corrected and accelerated ordinary non-parametric bootstrapping we calculated 95% confidence intervals of the R^2 of our multiple regression models (package: boot [48]). We calculated the adjusted R^2 (adjusting R^2 for number of independent variables) using the Pratt formula, which performs well if sample size to independent variables ratio is low [49].

Explorative models using glmulti [50]

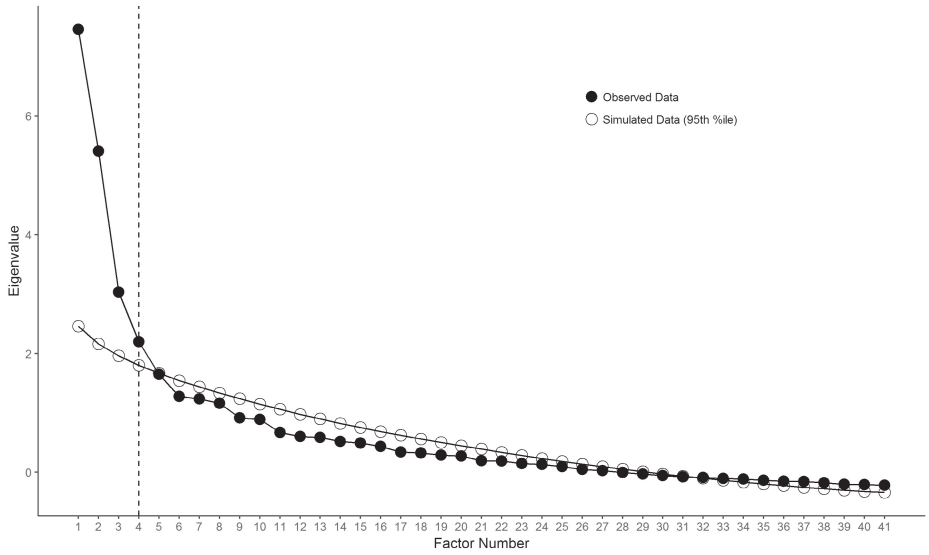
Models were specified as linear regressions. With 16 included possible variables a total of 2^{16}=65536 possible models were generated. Estimates were based on the ~10% (6500 models) with lowest Akaike information criterions. An average importance of 0.8 or higher was regarded as reliable concerning our sample size [50].

Supplemental results

Contents

Exploratory factor analysis of coping list

Two patients did not fully complete the Utrecht Coping List (both missing one item). These two items were assumed to be missing completely at random and were imputed using the median of the answers of all other patients. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was 0.58, showing mediocre proportion of variance explained by underlying factors [42]. We iteratively removed six items with an item KMO <0.5 (items 4, 9, 14, 27, 30, 40), which improved the overall KMO to 0.66. Following analyses were performed with the remaining 41 items. Using parallel analysis we determined that eigenvalues of four factors were higher compared to sampled data (**Supplemental figure 1**) [51].



Supplemental figure 1 Eigenvalues per factor of study dataset versus simulated dataset

After oblique rotation factor intercorrelations were low (all <0.32) [45]. Therefore we used orthogonal rotation ("Equamax") for the final model.

The four extracted factors explained 39.9% of variance. Since the fourth factor consisted of only one item loading >0.5, this factor was regarded as unreliable and discarded. Therefore, we also analyzed a three factor structure which explained 35.5% of variance (**Supplemental table 1**), showed comparable fit and more simplicity compared to the four factor structure and was therefore preferred. All three factors showed ≥4 items loading >0.6 and a total of 25 out of 41 variables loaded >0.5 (**Supplemental table 2**).

Factor naming

The first factor consisted of ten items loading >0.5. Four items were originally included in the "passive" coping style (items 3, 24, 31, 46), three items were originally included in the "avoidance" coping style (items 8, 19, 26) and two items were originally included in the "palliative" coping style (items 6, 34) and one item (items 28) was originally not included in any of the predefined coping styles [52]. The latter item was: in case of problems or unpleasant events how often do you "wait for better times". Recurring themes in the items in this factor were: "avoiding or evading problems", "seeking distraction to not think about the problem", "self-isolation" and "brooding on problems". We interpreted this as a combination of avoiding (thinking about) problems, partly by seeking (mental) distraction, but not being able to distract oneself completely resulting in brooding. We will refer to this factor as the "*avoiding and brooding*" coping style.

The second factor consisted of nine items loading >0.5. Six items were originally included the "active" coping style (items 13, 18, 21, 22, 23, 32). Two items were originally not included in any of the predefined coping styles. These two items were: in case of problems or unpleasant events how often do you "remain optimistic about the future" (item 20) and "see the humorous side of problems" (item 41). One item was originally included in the "Avoidance" coping scale. The item was: in case of problems or unpleasant events how often do you think "don't worry: everything will be fine" (item 45). Recurring themes in the items of this factor were: "remaining calm and positive", "using humor", "see problems as challenges" and "analyze problems and seek solutions". We interpreted this as having a positive attitude towards problems and seeing them as challenges to overcome. We will refer to this factor as the *"positivity and problem solving"* coping style.

The third factor consisted of six items loading >0.5. Five items were originally included in the "seeking social support" coping style (items 10, 38, 39, 42, 43). One item was originally included in the "reassuring thoughts" coping style (item 47). The item was: in case of problems or unpleasant events, how often do you "encourage yourself". Recurring themes in the items of this factor were: "sharing feelings and doubts", "discussing problems" and "seeking encouragement and understanding". We will refer to this factor as the *"seeking social support and comfort"* coping style.

Supplemental table 1 Variance explained by three factor structure

	Factor 1	Factor 2	Factor 3
Proportion Variance, %	13.8	11.5	10.3
Cumulative Variance, %	13.8	25.2	35.5

Supplemental table 2 Three factor structure matrix

Questions	Factor 1	Factor 2	Factor 3
UCL1	0,376	0,169	0,168
UCL2	0,070	0,305	0,210
UCL3	**0,696**	-0,062	-0,048
UCL5	0,479	-0,360	0,046
UCL6	**0,569**	-0,251	-0,036
UCL7	0,170	0,055	0,267
UCL8	**0,583**	-0,075	0,141
UCL10	-0,029	0,023	**0,723**
UCL11	-0,209	0,383	0,266
UCL12	0,271	0,373	0,045
UCL13	-0,105	**0,528**	-0,018
UCL15	0,359	-0,122	-0,072
UCL16	0,380	-0,099	0,102
UCL17	0,373	0,044	0,310
UCL18	-0,029	**0,649**	0,275
UCL19	**0,609**	-0,262	-0,042
UCL20	-0,366	**0,610**	-0,008
UCL21	-0,238	**0,540**	-0,097
UCL22	-0,290	**0,647**	0,212
UCL23	-0,241	**0,683**	0,162
UCL24	**0,531**	-0,252	0,035
UCL25	0,150	0,061	0,337
UCL26	**0,615**	-0,239	-0,129
UCL28	**0,690**	-0,046	0,031
UCL29	-0,048	0,261	0,452
UCL31	**0,638**	-0,068	0,031
UCL32	-0,082	**0,669**	0,119
UCL33	0,164	-0,080	0,214
UCL34	**0,537**	0,123	0,190
UCL35	0,363	0,209	0,370
UCL36	0,337	0,193	0,137
UCL37	0,194	0,484	0,137
UCL38	-0,082	0,198	**0,667**
UCL39	0,098	0,022	**0,798**
UCL41	-0,047	**0,574**	-0,041
UCL42	0,030	0,045	**0,717**
UCL43	0,010	0,091	**0,604**
UCL44	0,439	-0,040	-0,483
UCL45	-0,003	**0,504**	-0,307
UCL46	**0,630**	-0,107	0,053
UCL47	0,251	0,342	**0,534**

UCL = Utrecht Coping List

Assumption testing multiple linear regression models

Model 1: Sensitivity analyses removing the most influential points did not improve the R^2 and showed little effect on beta's of the included variables. Other assumptions were assessed as described in the extensive methodology and were met.

Model 2: One potential outlier was also marked as most influential using Cook's distance. Sensitivity analyses removing this point improved the R^2 with ~2.5%. This patient scored highest on the depression questionnaire and we regarded this as representative of the extreme end of depressive symptoms in Fabry disease. Therefore, we choose to present model 2 with this patient included. However, using model 2 a slight underestimation of depression scores might occur at the extreme end. Other assumptions were met.

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8

COGNITIVE FUNCTIONING AND DEPRESSIVE SYMPTOMS IN FABRY DISEASE: A FOLLOW-UP STUDY

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Abstract

Patients with Fabry disease (FD) have a high prevalence of depressive symptoms and can suffer from cognitive impairment, negatively affecting their life. The course of cognitive functioning and depressive symptoms in FD is unknown. The aim of this prospective cohort study was to describe changes in cognitive functioning and depressive symptoms and to identify related variables in patients with FD over one year. Assessments were conducted twice, using a neuropsychological test battery and the Centre of Epidemiological Studies Depression scale (CESD).

Eighty-one patients were included of which 76 patients (94%) completed both assessments (age: 44 years, 34% men, 75% classical phenotype). A significant decrease in cognitive functioning was found in four patients (5%), with patients regressing from excellent to average/good. Changes were not related to sex, phenotype, stroke, IQ or CESD scores. CESD scores ≥16 were present in 29 patients (38%) at baseline. Using the reliable change index a decrease in CESD scores was found in six patients (8%). Decreased CESD scores were independently related to employing a positive and problem solving coping style and increased CESD scores to an avoiding and brooding coping style and worsening health perception.

We found no major changes in cognitive functioning in patients with FD during one year follow-up making it an unsuitable outcome in FD treatment trials. Considering the high prevalence of persistent depressive symptoms, assessment of depressive symptoms should be part of routine follow-up. Altering coping styles and health perception may improve psychological wellbeing in FD.

Introduction

In Fabry disease (FD; OMIM 301500), a rare X-inherited lysosomal storage disorder, mutations in the GLA-gene result in a deficiency of α-galactosidase A activity (enzyme commission no. 3.2.1.22). Consequently, globotriaosylceramide and related compounds accumulate in various cell types, which often results in damage to the kidneys, heart and brain [1]. Strong predictors of disease progression in FD are age, sex and phenotype: older men with a classical disease phenotype have the highest complication risk while young women with a non-classical disease phenotype often do not display organ involvement [2].

Patients with FD are at risk for cognitive impairment [3-5] and depressive symptoms are present in a large proportion of patients [3, 6, 7]. People diagnosed with a major depressive disorder show more cognitive impairment compared to controls from the general population [8]. In FD, however, no relation between cognitive impairment and depressive symptoms could be established [4, 5, 9], but cognitive impairment is associated with male sex, a classical phenotype, a lower IQ [4] and stroke [4, 5].

Previous work on depressive symptoms in FD has shown a relation to pain and social factors such as economic status [3, 6]. Conversely, the relation of depressive symptoms to renal, cardiac or cerebral involvement is less prominent and patients' subjective health perception is probably more important [3, 6, 7]. Differences in coping, the process of cognitive and behavioral efforts to manage daily hassles and stressors [10], might influence the impact of subjective health perception on the psychological well-being of FD patients. In a recent study, we found that patients' use of an avoiding and brooding coping style was related to more depressive symptoms while positivity and problem solving was related to less depressive symptoms [7].

Since most studies on depressive symptoms and cognitive functioning in FD have been cross-sectional, little is known about their course over time. Follow-up data on depressive symptoms and cognitive functioning provide insight in the course of FD and offer an opportunity to explore variables that might be related to changes. This knowledge can guide decisions of treating physicians, might be of interest for future trials designs (especially patient reported outcomes) and may identify modifiable variables to decrease depressive symptoms or prevent cognitive decline.

The aim of this study was twofold: 1) to assess changes in both depressive symptoms and cognitive functioning after one year follow-up and 2) to explore disease related variables as well as coping styles in relation to changes in depressive symptoms and cognitive functioning.

Methods

Study design and rationale

Baseline data on cognitive functioning and depressive symptoms have been published elsewhere [4, 7]. The Amsterdam University Medical Center (location Academic Medical Center (AMC)) is the national referral center for FD. All Dutch adult (≥18 years old) FD patients were screened for eligibility [4]. Eighty-one patients (52.6%) were assessed at baseline and after one year all included patients were approached for a follow-up assessment (**Figure 1**). Both baseline and follow-up assessment included the same neuropsychological tests and questionnaires. The baseline assessments were completed between July 2016 and May 2017 and the follow-up assessments between May 2017 and May 2018. Between baseline and follow-up, patients received care as usual.

This study did not include an intervention. However, some patients were referred to their general practitioners or to local psychologists as not communicating potentially relevant depressive symptoms (Center of Epidemiological Studies Depression scale (CESD) score ≥16) was considered unethical and potentially harmful, see **Supplemental methods: data collection and referral**. Psychological interventions (pharmacological or non-pharmacological) between baseline and follow-up were registered.

A one year follow-up interval was chosen as (1) the course of depressive symptoms and cognitive functioning in FD is unknown as follow-up data are scarce [9, 11], and (2) this would be an achievable follow-up time for international trials, thus showing changes in cognitive functioning or depressive symptoms would provide evidence that these could potentially be used as a reliable outcome.

Phenotype

Patients were phenotypically characterized as having classical or non-classical FD using preset criteria [4, 12]. This study was conducted in accordance with the Declaration of Helsinki [13] and was approved by the local human ethics committee. All patients provided informed consent before inclusion.

Neuropsychological test battery

The neuropsychological test battery consisted of 16 subtests representing the following cognitive domains: language, memory, visuospatial perception, attention and executive functioning and processing speed (**Supplemental methods: Supplemental table 1**). If available, different test versions were used for baseline and follow-up to minimize training effects. The neuropsychological test battery was composited by a licensed clinical neuropsychologist (*GJG*). Included subtests are commonly used in neuropsychological

research in both the general population as well as in neurodegenerative diseases [14] and many have been used in earlier studies on cognitive functioning in patients with FD [3] (see [4] for a more elaborate description of the subtests). Raw test scores were converted to T-scores (mean of 50, standard deviation of 10) using normative data from Dutch healthy populations with a median sample size of 471 (range 121-1000). Most T-scores were adjusted for age, sex and education.

Additionally, the Dutch adult reading test (DART) provided an estimate of intelligence at baseline [15] and the test of memory malingering (TOMM) was used to assess malingering at baseline and follow-up [16].

Depressive symptoms

Depressive symptoms were measured using the CESD [17]. The CESD is a 20-item self-administered scale, has been validated in the Dutch population [18] and has previously been used in FD patients [6]. The total score ranges from 0 to 60 and scores ≥16 indicate the presence of depressive symptoms and that a depressive disorder may be present [17, 19].

Coping

Coping was assessed using the Utrecht Coping List (UCL), a questionnaire consisting of 47 items which can be combined to seven subscales [20]. Since power was limited due to the sample size, we used an exploratory factor analysis to reduce the number of subscales to three. The three coping styles mainly employed in our FD population were: "avoidance and brooding", "positivity and problem solving" and "seeking social support and comfort" (for more information on the exploratory factor analysis see: [7]). Scores per coping style were calculated for both baseline and follow-up using the Anderson-Rubin method [21]. This resulted in mean scores per coping style of 0 and a change in score of 1 per standard deviation increase or decrease. For both baseline and follow-up, most scores will range between -2 to 2 and higher scores indicate more employment of this coping style.

Pain

Pain was quantified using the Brief Pain Inventory (BPI) severity subscale [22]. Pain score was averaged from four items: pain right now, average, worst and least pain. Each item ranged from 0 (absence of pain) to 10 (worst pain imaginable).

Quality of life

Quality of life was assessed using the short-form 36 health survey (SF-36), which consists of 36 items [23]. It can be divided in eight different scales with scores ranging from 0-100 and higher scores indicating better functioning. For our analyses we focused on the "subjective health perception" scale and "self-rated social functioning" scale.

Clinical characteristics and complications

Kidney involvement was evaluated by calculating the estimated glomerular filtration rate (eGFR) [24]. Left ventricular hypertrophy (LVH) was rated as present or absent on MRI or echocardiography (if MRI was unavailable) [25-27]. Cardiac and renal complications were rated as present or absent. We created an ordinal scale rating cardiac and renal involvement (range 0-2): (0) No renal or cardiac involvement, (1) cardiac involvement (presence of LVH) and/or renal involvement (eGFR <60ml/min/1.73m^2) and (2) cardiac and/or renal complications (**Supplemental methods: Clinical characteristics and complications**).

Stroke was diagnosed by a neurologist using a combination of clinical symptoms and MRI. The diagnosis depressive disorder was made by a patient's general practitioner, psychologist or psychiatrist and was extracted from clinical letters and verified during the interview phase of the baseline and follow-up assessment.

Brain MRI

Brain involvement was rated on MRIs acquired during routine follow-up (Philips Ingenia, Philips Medical Systems, Best, The Netherlands), using a standardized protocol [4]. MRIs were rated by two neuroradiologists (*MRL* rated basilar artery diameter (BAD), *MGFL* rated white matter lesions (WMLs)). Deep and periventricular WMLs were rated using the Fazekas scale on FLAIR, resulting in a score ranging from 0 (no WMLs) to 6 (confluent deep and periventricular WMLs) [28]. The BAD was calculated as a mean of measurements in three slices (caudal, intermediate, rostral) on axial T2.

Statistical methods

R (version 3.5.1) was used for statistical analysis [29]. P-values <0.05 were considered statistically significant unless stated otherwise.

Cognitive domain scores were calculated by averaging T-scores on tests measuring a similar cognitive domain, for the domains language, memory, visuospatial perception, attention and executive functioning and processing speed (**Supplemental methods: Supplemental table 1**).

Whole group baseline and follow-up CESD and neuropsychological test scores were compared using paired t-tests or the Wilcoxon signed rank-test. Effect sizes of differences between baseline and follow-up were evaluated using cohen's d or a non-parametric equivalent [30]. In both, scores between 0.2 to 0.5, 0.5 to 0.8 and >0.8 indicate small, medium and large effects, respectively.

To evaluate if changes on individual patient level were reliable and clinically relevant we calculated a reliable change index (RCI) per patient for both the CESD score and the neuropsychological test results, with the latter adjusted for multiple testing. The RCI gives an indication whether the change within a patient is greater than what could be expected by measurement error alone [31] and is a reliable measure of change [32], see **Supplemental Methods: Statistical Methods**.

At baseline the following parameters independently correlated with cognitive impairment: male sex, a classical disease phenotype, a history of stroke and lower IQ as estimated with the DART [4]. We assessed whether changes in neuropsychological domain scores (T-scores follow-up minus T-scores baseline) were related to any of these variables using MANOVA's, Kruskal-Wallis tests and Kendall's Tau b. Considering the multiple relations tested we set the p-value at <0.01.

For depressive symptoms, the following parameters were associated with a higher CESD score at baseline: avoidant and brooding coping scale score, positivity and problem solving coping scale score, BPI pain severity score and SF-36 health perception score [7]. Two multiple linear models were created. In model 1 changes in CESD scores (CESD score follow-up minus CESD score baseline) were related to changes in these variables. In extended model 2, changes in variables that were identified in an explorative analysis as potentially relevant in relation to CESD scores at baseline were added [7]. These were: loneliness, SF-36 social functioning scores and cardiac and/or renal involvement.

To evaluate the potential effects of patients lost to follow-up we used multiple imputation by chained equations (package: mice [33]) to impute missing data and reran several analyses. The results presented in this study are the original unimputed data.

For additional information on the RCI, assumption testing multiple linear models and multiple imputation please see **Supplemental methods: Statistical methods**.

Results

Patient participation

No differences with respect to age, sex, phenotype, Fazekas score and stroke were found between the 81 included patients and the 73 non-participants (**Figure 1**) at baseline [4]. Seventy-six patients (93.8%) completed the follow-up assessment after a mean interval of 1.1 (±0.1) year (**Figure 1**). The five patients lost to follow-up assessment did not differ in age, sex, cognitive domain scores and CESD-score at baseline and were excluded from all analyses.

Patient characteristics

Of the 76 patients completing both assessments 26 were men (34.2%), 57 had a classical phenotype (75.0%) and mean age was 44.3 years (**Table 1**).

During follow-up three patients experienced a stroke, two of which had had one or more strokes in the past. Six patients developed a new cardiac complication. No new renal events occurred. Eight patients were started on enzyme replacement therapy between baseline and follow-up.

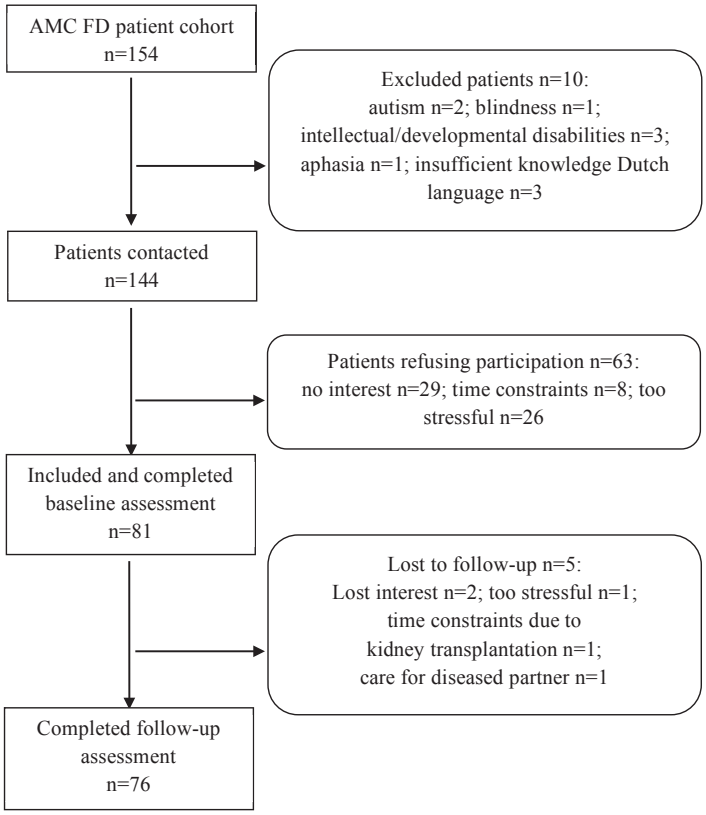

Figure 1 Flow chart of non-participants, in- and excluded patients and loss-to follow-up. AMC = Academic Medical Center, FD = Fabry disease

Follow-up cognitive functioning

There were no signs of underachievement in any of the patients based on the TOMM score. The Rey Auditory Verbal Learning Test immediate recall, delayed recall and the letter fluency T-scores increased between baseline and follow-up, while the Rivermead Behavioural Memory Test immediate recall and delayed recall decreased (**Table 2**). Effect sizes were small to medium.

For baseline and follow-up raw scores and T-scores please see **Supplemental results: Supplemental tables 4 to 7**.

Four patients (5.3%) showed reliable decrease in cognitive functioning, two women and one man with classical disease and one woman with non-classical disease (age range: 19-41 years). Changes were from excellent to good/average and from good to average. None had a history of stroke or extensive WMLs. Follow-up CESD scores were similar in two patients (+0 and +1) and increased in two others (+6, +11).

Variables related to cognitive changes

We found no significant relations between changes on neuropsychological domain scores and sex, phenotype, a history of stroke, estimated IQ, baseline Fazekas scores or changes in CESD scores (**Supplemental results: Supplemental table 2**).

Follow-up CESD scores

At baseline 29 patients (38.2%) scored ≥16 on the CESD and 22 patients (28.9%) had a history of depressive disorder (**Table 3**). Eighteen patients (23.7%) had psychological counselling between baseline and follow-up, mostly from the group scoring above the CESD cut-off at baseline (51.7%, n = 15). Between baseline and follow-up a new depressive disorder was diagnosed in six patients (7.9%) by their general practitioner or psychologist/psychiatrist. Five of these patients scored above the CESD cut-off at the baseline assessment and were subsequently referred to their general practitioner or psychologist/psychiatrist for further analyses. One patient had a CESD score of 15 at the baseline assessment and sought help with increasing depressive symptoms between baseline and follow-up. At follow-up 22 patients (29.3%) scored above the CESD cut-off.

Changes in CESD scores

Overall, no significant difference was found when comparing CESD scores between baseline and follow-up ($p = 0.096$, effect size: 0.14).

A change in CESD score of 13.6 points was calculated as reliable change. Six patients showed reliable decrease of the CESD score and one patient showed reliable increase (**Figure 2**). All six patients showing reliable decrease had a CESD score >16 at baseline and <16 at follow-up. Of the six patients with a new diagnosis of depressive disorder between baseline and follow-up, one had a reliable decrease in CESD score (-17 points) with CESD scores changes in the other five ranging from -9 to +8 points.

Table 1 Patient characteristics at baseline

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
Patients, n (%)	76	17 (22.4%)	9 (11.8%)	40 (52.6%)	10 (13.2%)
Age in years, mean (±SD)	44.3 (±14.3)	38.6 (±13.5)	60.5 (±10.2)	43.1 (±13.6)	43.9 (±13.0)
ERT at any time before baseline, n (%)	45 (59.2%)	17 (100.0%)	3 (33.3%)	24 (60.0%)	1 (5.0%)
Years treated with ERT, median(range)	8.8 (0.1-16.0)	12.4 (1.5-16.0)	12.5 (6.4-14.2)	8.1 (0.1-13.6)	0.3
Antidepressant use, n (%)	7 (9.2%)	1 (5.9%)	2 (22.2%)	3 (7.5%)	1 (10.0%)
Estimated IQ†, median (range)	94 (68-133)	89 (83-114)	84 (68-133)	95 (82-121)	100 (84-121)
Years of education, mean (±SD)	13.8 (±3.0)	14.4 (±2.8)	13.6 (±5.2)	13.4 (±2.6)	14.9 (±1.8)
Unemployed, n (%)	28 (36.8%)	9 (52.9%)	3 (33.3%)	13 (32.5%)	3 (30.0%)
Unfit for work$, n (%)	19 (25.0%)	7 (41.2%)	2 (22.2%)	9 (22.5%)	1 (10.0%)
Single#, n (%)	28 (36.8%)	9 (52.9%)	3 (33.3%)	13 (32.5%)	3 (30.0%)
Left ventricular hypertrophy, n (%)	42 (55.3%)	13 (76.5%)	3 (33.3%)	22 (55.0%)	4 (40.0%)
eGFR in ml/min/1.73m2, median (range)	95.4 (11.4-141.0)	105.6 (25.4-141.0)	77.3 (11.4-109.9)	93.4 (45.6-131.1)	95.4 (73.6-118.3)
eGFR<60 ml/min/1.73m2, n (%)	10 (13.2%)	2 (11.8%)	3 (33.3%)	5 (11.6%)	0 (0.0%)
Fazekas score‡, median (range)	1 (0-6)	0 (0-6)	1 (0-3)	1 (0-6)	0.5 (0-2)
BAD‡ in mm, median (range)	3.6 (2.5-5.6)	4.2 (3.1-5.6)	3.6 (3.3-4.3)	3.6 (2.5-5.6)	3.2 (2.5-3.6)
Complications, n (%)	27 (33.3%)	7 (41.2%)	6 (54.5%)	14 (32.6%)	0 (0.0%)
Cardiac, n (%)	14 (17.3%)	4 (23.5%)	4 (36.4%)	6 (14.0%)	0 (0.0%)
Renal, n (%)	4 (4.9%)	1 (5.9%)	2 (18.2%)	1 (2.3%)	0 (0.0%)
Stroke, n (%)	9 (11.8%)	2 (11.8%)	2 (22.2%)	5 (12.5%)	0 (0.0%)

Continuous variables are presented as median (range) or mean (±SD) and discrete variables as number (percentages).
† The IQ-score was estimated using the Dutch Adult Reading Test, $ Includes three patients regarded partially unfit for work, # Unmarried, divorced or widowed, ‡ MRIs were unavailable in seven patients (three non-classical men, four classical women) due to presence of an MRI non-compatible pacemaker or ICD (n=6) and due to claustrophobia (n=1)
BAD = basilar artery diameter, ERT = enzyme replacement therapy, eGFR = estimated glomerular filtration rate, IQ = intelligence quotient

Table 2 Comparison baseline and follow-up T-scores neuropsychological tests and domains

Neuropsychological tests and domains	Baseline T-score	Follow-up T-score	Median or mean change score (95% CI for mean scores)	p-value	Effect size*	Reliable decrease, n (%)
Language	49.5 (32-63)	51.5 (33.5-65.0)	2	0.093	0.14	
BNT	49 (37-63)	53 (37-63)	4	0.370	-0.07	2 (2.6%)
WAIS-IV: S	50 (27-72)	53 (27-72)	3	0.140	-0.12	3 (4.0%)
Memory	53.9 (±9.5)	53.4 (±9.4)	-0.5 (-1.9 to 0.8)	0.422	-0.09	
RAVLT ir	51.8 (±11.4)	55.9 (±11.5)	4.1 (2.2 to 6.0)	<0.001	0.48	0 (0.0%)
RAVLT dr	52.4 (±10.5)	54.9 (±10.5)	2.5 (0.6 to 4.3)	0.008	0.31	3 (3.9%)
RBMT ir	56.3 (±11.0)	51.5 (±11.2)	-4.8 (-6.7 to -2.8)	<0.001	-0.56	6 (7.9%)
RBMT dr	55.0 (±11.5)	51.1 (±12.1)	-3.9 (-6.2 to -1.7)	<0.001	-0.40	8 (10.5%)
Visuospatial perception	55 (32.5-67.0)	54 (28.0-65.5)	-1	0.406	0.07	
WAIS-IV: BD	50.1 (±10.9)	50.9 (±11.6)	0.8 (-1.0 to 2.5)	0.375	0.10	4 (5.3%)
JLO	61 (29-61)	61 (30-61)	0	0.266	-0.09	3 (3.9%)
Processing speed	54.1 (±8.1)	53.8 (±8.4)	-0.3 (-1.5 to 0.9)	0.599	-0.06	
TMT A	54.1 (±9.5)	53.8 (±9.9)	-0.3 (-2.7 to 2.1)	0.806	-0.03	5 (6.6%)
Stroop W	55.5 (34-79)	55.5 (30-93)	0	0.018	-0.19	7 (9.2%)
Stroop C	51.3 (±11.8)	52.0 (±10.5)	0.8 (-0.8 to 2.3)	0.322	0.11	3 (3.9%)
Attention and executive functioning	49.0 (±7.5)	50.0 (±8.6)	1.0 (-0.1 to 2.1)	0.07	0.21	
TMT B	51 (-1-74)	52 (-10-70)	1	0.389	0.07	4 (5.3%)
Stroop CW	50 (32-84)	51.5 (25-76)	1.5	0.022	0.19	3 (3.9%)
Fluency A	49.7 (±11.3)	50.1 (±11.4)	0.4 (-1.7 to 2.6)	0.706	0.04	2 (2.6%)
Fluency O	47.4 (±11.3)	47.6 (±12.9)	0.3 (-2.0 to 2.5)	0.828	0.03	4 (5.3%)
Fluency L	46.5 (±10.0)	49.4 (±11.4)	3.0 (1.3 to 4.7)	<0.001	0.40	1 (1.3%)

T-scores are presented as median (range) or mean (±SD). Reliable decrease is presented as n (%) with n = 75 at WAIS-IV: S and WAIS-IV: BD and n = 76 at the other tests. P-values <0.01 were considered statistically significant.

** For normally distributed data cohen's d was calculated and in case of non-normality a nonparametric equivalent BD = Block Design, BNT = Boston Naming Test, CI = confidence interval, dr = delayed recall, Fluency A = Animal, Fluency L = Letter, Fluency O = Occupation, ir = immediate recall, JLO = Judgement of Line Orientation, RAVLT = Rey Auditory Verbal Learning Test, RBMT = Rivermead Behavioural Memory Test, Stroop C = Color, Stroop CW = Color-Word, Stroop W = Words, TMT = Trail Making Test, WAIS-IV: S = Wechsler Adult Intelligence Scale IV: Similarities*

Table 3 Depressive symptoms and psychological follow-up

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
Baseline					
CESD, median (range)	11 (0-44)	11 (0-40)	12 (0-23)	12.5 (0-44)	7.5 (0-20)
CESD≥16, n (%)	29 (38.2%)	7 (41.2%)	3 (33.3%)	16 (40.0%)	3 (30.0%)
Depressive disorder*, n (%)	22 (28.9%)	3 (17.6%)	3 (33.3%)	12 (30.0%)	4 (40.0%)
Antidepressant use, n (%)	7 (9.2%)	1 (5.9%)	2 (22.2%)	3 (7.5%)	1 (10.0%)
Follow-up					
CESD, median (range)	8 (0-38)	6 (0-37)	11 (1-30)	9 (0-38)	5 (1-24)
CESD≥16, n (%)	22 (29.3%)	5 (29.4%)	2 (25.0%)	12 (30.0%)	3 (30.0%)
Newly diagnosed depressive disorder#, n (%)	6 (7.9%)	3 (17.6%)	0 (0.0%)	3 (7.5%)	0 (0.0%)
Psychological counseling after baseline, n (%)	18 (23.7%)	4 (23.5%)	1 (11.1%)	13 (32.5%)	0 (0.0%)
New antidepressant use, n (%)	1 (1.3%)	1 (5.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Continuous variables are presented as median (range) and discrete variables as number (percentages).
** (History of) depressive disorder as diagnosed by a psychologist, psychiatrist or general practitioner, # Newly diagnosed depressive disorder by a psychologist, psychiatrist or general practitioner.*
CESD = Center for Epidemiologic Studies Depression scale

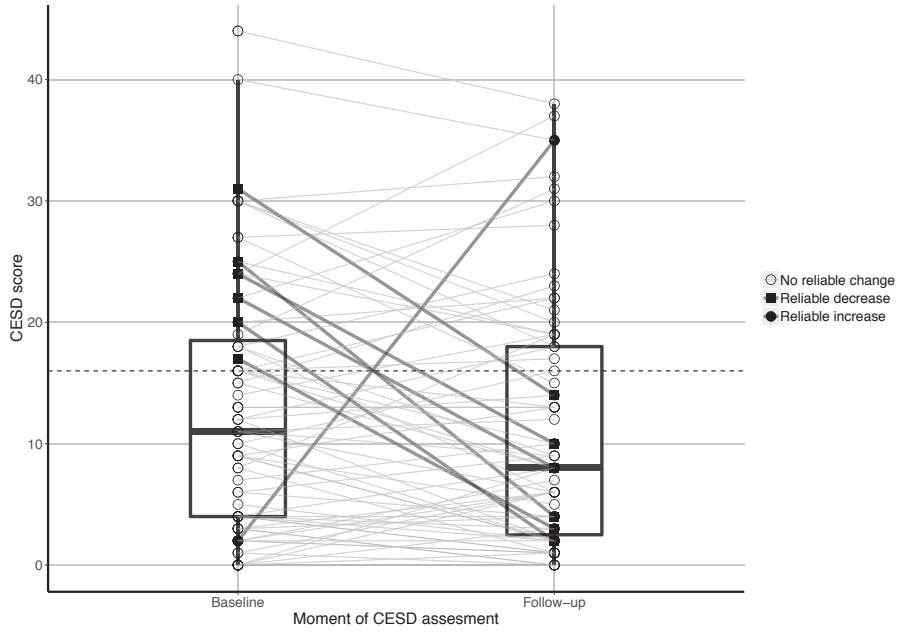

Figure 2 Changes in CESD scores between baseline and follow-up. The CESD scores at baseline and follow-up are visualized using two boxplots showing the median, interquartile range and total range. A scatterplot is projected over the boxplots with patients divided by the presence or absence of reliable change. Thick grey lines display the change in score in patients with reliable change, thin grey lines display change in scores in the remaining patients. There is considerable overlap in CESD scores, resulting in overlap within the scatters.

Variables related to the CESD change score

We created two multiple linear models evaluating potentially relevant variables in relation to the change in CESD scores. Model 1 significantly explained 27.4% of the variance in change scores (95%CI: 11.9 – 44.0%, F(4,69) = 6.52, p = 0.0002) (**Table 4**). CESD score changes were negatively related to changes in SF36 health perception scores and changes in positivity and problem solving scores, meaning that an increase in SF-36 health perception and more use of positivity and problem solving between baseline and follow-up were related to a decrease in CESD scores during follow-up. CESD scores changes were positively related to changes in avoidance and brooding scores, meaning that more use of avoidance and brooding during follow-up was related to an increase in CESD scores during follow-up.

None of the added variables in model 2 were significantly related to changes in CESD scores (**Table 4**). Model 1 was preferred over model 2 as it was simpler and explained an equal amount of the variance (after adjusting R^2 for number of variables).

In sensitivity analyses, removing two influential patients, the relation between the change in CESD score and the change in the SF-36 health perception score became less prominent (B: -0.09, 95%CI: -0.21 – 0.03, p = 0.14) (**Supplemental results: assumption checking and sensitivity analyses**).

We found no differences in the change in CESD scores between patients that did and did not receive psychological counselling between evaluations, regardless of whether their baseline score was above the cut off (≥16).

Missing data and multiple imputation

Using imputed data we compared baseline and follow-up cognitive domain scores and reran the multiple linear models relating variables to changes in CESD scores (**Supplemental results: multiple imputation**). Results were highly similar to the non-imputed analyses (**Table 2, Table 4**).

Table 4 Summary of multiple linear regression model relating change in CESD score to potentially relevant variables

	Model 1				*Model 2*			
Independent variables	*B* (95% CI)	SE *B*	β	p-value	*B* (95% CI)	SE *B*	β	p-value
Change in BPI severity	-0.11 (-1.30 to 0.88)	0.55	-0.04	0.7050	0.02 (-2.21 to 3.34)	0.57	0.00	0.9762
Change in SF-36 health perception	-0.13 (-0.26 to -0.00)	0.07	-0.22	0.0452	-0.09 (-0.22 to 0.08)	0.07	-0.12	0.3330
Change in avoidance and brooding	3.02 (0.51 to 5.53)	1.26	0.27	0.0192	2.84 (0.17 to 5.43)	1.32	0.25	0.0372
Change in positivity and problem solving	-4.37 (-6.94 to -1.79)	1.29	-0.40	0.0012	-4.14 (-6.95 to -1.61)	1.34	-0.40	0.0021
Loneliness at follow-up					0.76 (-4.78 to 6.05)	2.71		0.8145
Change in SF-36 social functioning					-0.05 (-0.14 to 0.04)	0.05	-0.14	0.2699
Cardiac and/or renal involvement								
eGFR<60ml/min and/or presence of LVH at baseline					-3.11 (-6.98 to 0.77)	1.94		0.1140
Cardiac or renal complications at baseline					-2.71 (-7.48 to 2.07)	2.39		0.2618
Intercept	-1.24				0.57			
F-value	6.52			0.0002	3.72			0.0012
R^2	27.4% (11.9 to 44.0)				31.4% (14.1 to 43.7)*			
Adjusted R^2	23.7%				23.4%			

B = beta coefficients, β = standardized beta coefficients for continuous variables, BPI = Brief Pain Inventory, CESD = Centre for Epidemiologic Studies Depression scale, eGFR = estimated glomerular filtration rate, LVH = left ventricular hypertrophy, SE = standard Error, SF-36 = Short Form-36 Health Survey

** Bootstrapping for 95%CI was performed without "Loneliness at follow-up" due to lack of variation in this variable*

Discussion

This is the largest longitudinal cohort study to date following the short term course of cognitive and psychological functioning in patients with FD. While cognitive impairment is present in FD patients [3-5], we found no major changes in cognitive functioning during one year of follow-up and did not identify factors related to changes in cognitive functioning. Changes in depressive symptoms were more variable and were related to changes in use of coping styles, and to patients' own health perception.

Four patients (5%) showed a reliable decrease in cognitive functioning according to our preset criteria. Most T-scores in these four patients decreased from excellent to good/average. In addition, the patient characteristics of these four patients did not correspond with our previously hypothesized risk groups (e.g. only one man with a classical phenotype, no patients with stroke or severe WMLs). We hypothesize that the decrease in these patients is not directly related to FD itself, however, the low number of patients with reliable decrease prevents strong conclusions. The only previously published follow-up study of cognitive functioning in FD also showed no cognitive decline after eight years, but was hampered by size (n=14) and loss to follow-up [9]. The methodology applied here is sensitive enough to detect short term changes as exemplified by a trial evaluating the effect of deep brain stimulation in patients with Parkinson's disease in which 34% of included patients showed a reliable decrease in cognitive functioning over one year using similar criteria [34].

Twenty-nine patients (38%) had depressive symptoms at baseline and six patients (8%) showed a reliable decrease in depressive symptoms between baseline and follow-up. Six patients were diagnosed with a new depressive disorder between baseline and follow-up by their general practitioner or psychologist/psychiatrist, five of which were referred after discussing their increased CESD scores. This could be explained by depressive disorder being underdiagnosed in FD, which has been suggested in FD [6]. Surprisingly, we found no differences in changes in CESD scores between patients that were counseled between baseline and follow-up for depressive symptoms and those that were not. Our findings might reflect general findings of depressive symptoms in a chronic disease population: remission may occur but depressive symptoms are generally more persistent in patients with a chronic disease when compared to those without [35]. Nevertheless, improvement may be achieved using a patient or disease adapted approach. In a small longitudinal study in FD patients a sustained decrease in depressive symptoms was achieved after employing individually tailored psychological interventions [11]. In contrast to the intervention study, we did not employ a standardized referral or intervention for all patients. Rather, as Dutch FD patients are spread throughout the country they were

referred to local healthcare practitioners. Since depressive symptoms may thus persist for prolonged periods of time, patients with FD might need specialized psychological interventions since rare inherited metabolic diseases present unique problems [36, 37] ideally offered by psychologists embedded in multidisciplinary care teams [38].

Potential factors of interest for psychological interventions in patients with FD are coping styles. A decrease in depressive symptoms was independently related to an increased use of positivity and problem solving while an increase in depressive symptoms was independently related to increased avoidance and brooding. While causality cannot be inferred from this study and these relations might be bi-directional, similar relations between coping styles and depressive symptoms have been published for other chronic disorders [39-41]. Moreover, coping intervention studies show potential to improve outcomes in chronic illnesses [42] and could therefore also be investigated in the FD population. In addition, adjusting perception of illness, referring to a patients' interpretation of a diseases' causes, symptoms, consequences, timeline and controllability (locus of control) [43] can be useful, as has been shown in patients with myocardial infarction [44].

Strengths of this study include the large sample size, the use of established neuropsychological tests, the low loss-to follow up and the evaluation of the effects of patients lost to follow-up using imputed data. This study has several limitations. Firstly, interpretation of the results is limited by the lack of a control group. Despite using large normative samples to evaluate neuropsychological test results, the effects of repeated testing (such as learning effects) could not be fully controlled for, although parallel test versions were used if available. Secondly, depressive symptoms were assessed using the CESD without simultaneous assessment of the DSM-V criteria for depressive disorder. Therefore, we were unable to analyze whether increased CESD scores reflected a current depressive disorder or were increased due to chronic pain or anxiety [45]. Thirdly, the effect of enzyme replacement therapy and other medications such as antidepressants on depressive symptoms or cognitive functioning in FD is unknown. Considering the indication bias in cohort studies (more severely affected patients will generally receive more and earlier treatment), we expected no verifiable effect of these treatments and regarded the analyses as unreliable and therefore did not include these in this study. Nevertheless, despite treatment with both enzyme replacement therapy and antidepressants, cognitive impairment was clearly present and depressive symptoms were widespread and persistent [3, 4, 7]. Fourthly, since time of day was not standardized and seasonal affective disorder was not controlled for, we cannot rule out their effects on the neuropsychological test results [46, 47]. As patients were assessed after one year, meaning that their baseline and follow-up assessment were both in the same season, the effect of seasonal affective disorder on the changes in neuropsychological test results

are expected to be small. Lastly, there is some evidence that both cognitive impairment and depressive symptoms might be already present in pediatric FD patients [48, 49]. Since we did not include patients <18 years old in our study population, we cannot exclude that early neuro- and psychologic development is affected in FD. Future studies should evaluate these early life effects of FD as this might also be important in relation to the timing of interventions to reduce or prevent depressive symptoms and cognitive impairment.

To conclude, no major changes in cognitive functioning were found over one year follow up and we did not identify patients at risk for cognitive decline. Hence, we do not recommend the use of cognitive functioning as a functional outcome for intervention trials in patients with FD. The fact that depressive symptoms may persist for longer periods of time, mandates assessment of depressive symptoms during routine follow-up. Future studies should explore whether individually tailored psychological interventions focused on combining adjustment of coping styles and illness perception in FD patients improve depressive symptoms.

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Supplemental methods

Content

- Data collection and referral
- Clinical characteristics and complications
- Statistical methods
- **Supplemental table 1** Neuropsychological test information

Data collection and referral

Follow-up of FD patients in the Academic Medical Center is adjusted for enzyme replacement therapy (ERT) treatment status and disease phenotype and ranges from half yearly to once every two years. Clinical data are collected in a local database and can be accessed for research. Follow-up includes cardiac imaging (echocardiography and MRI), cerebral imaging (MRI) and blood work (kidney function). ERT treatment status, renal and cardiac parameters and complications were extracted from our local clinical database and cross-checked with patients' electronic health records.

Patients' baseline results were discussed with a licensed clinical neuropsychologist (*GJG*). If results indicated significant depressive symptoms (Center of Epidemiological Studies Depression scale (CESD) score ≥16), we considered not intervening to be unethical as this could have potentially negative effects on patients' health. In these cases we discussed the results with the patient and, if preferred by the patient, we communicated the results with their general practitioner or psychologist/psychiatrist. We think that this would be the "natural course" of action if a depressive symptoms questionnaire is included in the standard follow-up of FD as in clinical practice referrals would be quite subjective, with the wants and needs of the individual patient in mind.

Clinical characteristics and complications

We calculated estimated glomerular filtration rate (eGFR) using the CKD-EPI formula [1]. Left ventricular mass index (LVMi) was measured on MRI [2], without papillary muscles and adjusted for body surface area (Dubois formula) and on echocardiography using the cube formula, adjusted for height$^{2.7}$ [3]. Cutoffs for LVH were >72 g/m^2 or >49 g/m$^{2.7}$ in men and >55 g/m^2 or >47 g/m$^{2.7}$ in women on MRI and echocardiography, respectively [4, 5].

Cardiac complications were present if any of the following events occurred: atrial fibrillation, any other rhythm disturbance or heart failure requiring hospitalization, pacemaker or ICD implantation, myocardial infarction, percutaneous coronary intervention or coronary artery bypass surgery. Renal complications were rated as present if a patient had a history of renal replacement therapy, kidney transplantation or an eGFR <15 ml/min.

The ordinal scale rating cardiac and renal involvement was created for the multiple regression in relation to depressive symptoms for three reasons. Firstly, FD specific severity scores often combine both measures of organ involvement and subjective health perception. We wanted to separate the effects of both as we found that subjective health perception is probably of more importance compared to organ involvement in relation to depressive symptoms in FD [6]. Secondly, FD is a rare disease and the multiple

regression had limited power. Including parameters related to organ involvement such as eGFR and LMVi separately would have required to leave out other variables which were also of interest (such as a history of stroke or coping styles). Thirdly, we regarded stepwise single linear regression to preselect variables for multiple regression inferior to theory driven multiple regression. The combined scale includes only organ involvement, used limited power and was theory driven.

Statistical methods

Baseline characteristics and outcomes of patients that were lost to follow-up were compared to patients that completed both assessments using Fishers exact test and the Mann-Whitney U test for binary (sex and phenotype) and continuous (age, total CESD-score, cognitive domain scores) variables, respectively.

Baseline and follow-up scores were compared using paired t-tests or the Wilcoxon signed rank-test. With 21 variables (neuropsychological test and domain scores), this increases the risk of type-I errors. Considering this, but also taking the explorative nature of this study into account we set the p-value at <0.01 for the neuropsychological comparisons.

The reliable change index is calculated as follows: (score follow-up minus score baseline)/ standard error of the difference between the scores [7,8]. Reliable change of the CESD score was defined as a change of >1.645 on the RCI (in a normal distribution, this represents the ~5% lowest and ~5% highest scores). To reduce the risk of type-I error reliable cognitive change was defined as a change of >1.645 on the RCI for at least three neuropsychological tests and RCI scores on neuropsychological tests assessing a similar cognitive process were treated as a single deficient test score. Considering the risk of family-wise error rate given the large number of neuropsychological tests, RCI scores changes >1.645 on neuropsychological tests assessing a similar cognitive process were treated as a single deficient test score. This applied to the fluencies (letter, animal, occupation), immediate recall tests (Rey auditory verbal learning test, Rivermead behavioral memory test), delayed recall (Rey auditory verbal learning test, Rivermead behavioral memory test), processing speed (trail making test A, Stroop word, Stroop color), executive functioning (trail making test B, Stroop color-word) and visuospatial skills (judgement of line orientation, block design).

The effect of sex and phenotype on changes in neuropsychological T-scores (T-scores follow-up minus T-scores baseline) was evaluated using MANOVAs. MANOVAs can combine multiple dependent variables: in this study all changes in scores of neuropsychological tests assessing a single cognitive domain. If multivariate normality and homogeneity of covariance matrices were violated, we used the Kruskal-Wallis test

to compare changes in domain scores divided by sex and phenotype. The effect of a history of stroke, IQ-scores, Fazekas scores and changes in CESD scores on changes in scores per cognitive domain was evaluated using Kendall's Tau.

Considering the loss of power and information in dichotomizing the CESD score [9] and the explorative nature of this study, we found the use of the continuous CESD score justifiable to explore potential relations of interest.

For the multiple linear models relating variables to changes in CESD scores we tested the following assumptions: possible outliers and influential points were assessed using standardized residuals and Cook's distance. If influential points and outliers were present we reran the analyses excluding these points and compared R^2 and beta's to the original model. Multicollinearity was assessed using variance inflation factor. Homoscedasticity, linearity and multivariate normality were visually inspected using scale-location plots, residuals versus fitted value plots and a Q-Q plot of the studentized residuals. Lastly, independence of errors was tested using the Durbin-Watson test.

We calculated 95% confidence intervals of R^2 (unadjusted) using ordinary non-parametric bootstrapping with bias corrected acceleration. An adjusted R^2 was also calculated, penalized for the number of included independent variables, using Pratt's formula [10]. Lastly, standardized beta-coefficients were calculated, improving comparability of continuous independent variables. Standardized beta-coefficients reflect how many standard deviations (SD) the dependent variable will change if the independent variables changes one SD.

Missing data were imputed using multiple imputation by chained equations (package: mice [11]). Missing values are filled in with plausible values in multiple copies of the same dataset. The copies are identical for the non-missing data entries, but differ in imputed values. These differences result from uncertainty in the imputation. During analyses the copies of the dataset are pooled [11]. The theoretical framework on which pooling after multiple imputation is based is designed for parametric tests. Testing the influence of missing data was therefore restricted to parametric tests.

Supplemental table 1 Neuropsychological test information

Cognitive domain	Neuropsychological tests	Scoring system	T-score corrected (age, sex, education)	Alternative versions
Intelligence estimation	Dutch adult reading test	Words correctly read out load	Age, sex	No
Language	Boston Naming Test	Drawings recognized	-	No
	WAIS-IV: Similarities	Similarities recognized	Age	No
Memory	RBMT: story immediate recall	Parts of story immediately recalled	Age, sex, education	Yes
	RBMT: story delayed recall	Parts of story recalled after 15 minutes	Age, sex, education	Yes
	RAVLT immediate recall	Immediately recalled words in 5 trials	Age, sex, education	Yes
	RAVLT delayed recall	Recalled words after 20 minutes in 1 trial	Age, sex, education	Yes
Visuospatial	WAIS-IV: Block design	Timely matched patterns	Age	No
perception	Judgement of line orientation	Correctly matched line pairs	Age, sex	Yes
Processing speed	Trail making test part A	Time to complete	Age, sex, education	Yes
	Stroop word	Time to complete	Age, sex, education	No
	Stroop color	Time to complete	Age, sex, education	No
Attention and	Trail making test part B	Time to complete	Age, sex, education	Yes
executive functioning	Stroop color-word	Time to complete	Age, sex, education	No
	Fluency animals	Number of animals in 1 minute	Age, sex, education	No
	Fluency occupation	Number of occupations in 1 minute	Age, sex, education	No
	Fluency letter	Number of words with 3 letters	Age, sex, education	Yes

RAVLT = Rey Auditory Verbal Learning Test, RBMT = Rivermead Behavioural Memory Test, WAIS-IV = Wechsler Adult Intelligence Scale

Supplementary results

Content

- Assumptions testing and sensitivity analyses multiple linear models
- Multiple imputation
  - Analytical choices
  - Results sensitivity analyses imputed data
  - **Supplemental table 2** Relation between changes in cognitive domain scores and relevant variables
  - **Supplemental table 3** Multiple linear regression model after imputation
  - **Supplemental table 4-7** Baseline and follow-up neuropsychological raw scores and T-scores

Assumption testing multiple linear regression models and sensitivity analyses

In both model 1 and model 2 two patients could be marked as influential outliers using standardized residuals and Cook's distance and potentially had disproportionate impact on the model. These two patients had the largest increase and decrease in CESD score between baseline and follow-up. Removing the two patients from model 1 resulted in a decrease of R^2 from 27.4% to 20.6%. Both avoidance and brooding and positivity and problem solving remained independently related to changes in the CESD score, however the relation to changes in the SF-36 health perception score was less prominent (B: -0.09, 95%CI: -0.21 – 0.03, p = 0.14).

Model 1 and 2 showed no multicollinearity or heteroscedasticity. Multivariate normality and independence of errors were acceptable.

Multiple imputation

Analytical choices

Beside the five patients lost to follow-up the following data was missing: one woman used three neuropsychological tests in her job setting (Dutch Adult Reading test (DART), two Wechsler Adult Intelligence Scale subtests). We therefore skipped these tests in her baseline and follow-up assessment. Follow-up questionnaires of one patient were lost in the mail.

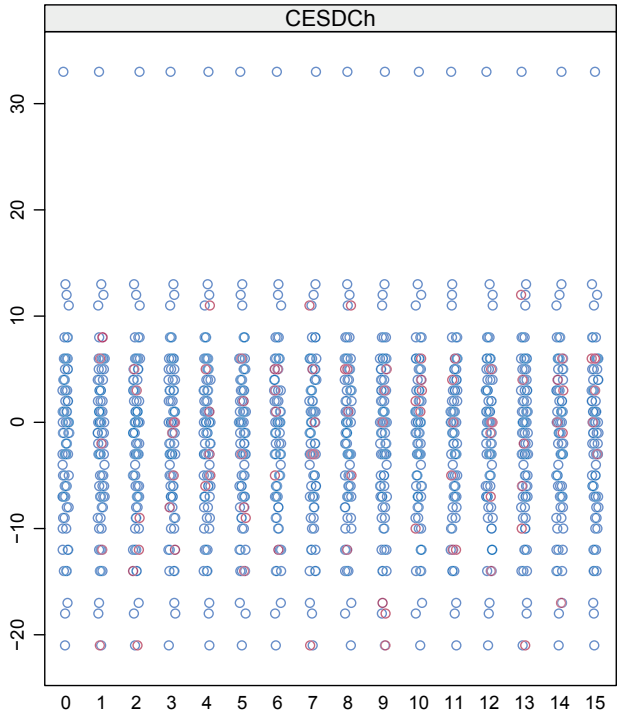
The variables used for the imputation of the cognitive domain scores were:

- Sex, phenotype, age at baseline, CESD-score at baseline, change in CESD score, history of depressive symptoms, DART IQ-score at baseline, history of stroke and all baseline and follow-up cognitive domain scores.

The variables used for the imputation of the changes in CESD scores, changes in Utrecht Coping List (UCL) coping style scores, changes in brief pain inventory (BPI) pain scores and changes in short form-36 (SF-36) subscale scores were:

- Sex, phenotype, age at baseline, history of depressive symptoms, CESD-score at baseline, changes in CESD scores, avoidance and brooding baseline score, changes in avoidance and brooding score, positivity and problem solving baseline score, changes in positivity and problem solving score, BPI pain severity score baseline, changes in BPI pain severity score, SF-36 health perception baseline score, changes in SF-36 health perception score, SF-36 social functioning baseline score, changes in SF-36 social functioning score, cardiac and/or renal involvement, loneliness at follow-up

All parameters were imputed using the predictive mean matching method. The number of dataset copies was set at 15 and the number of iterations at 10. For an example of imputed data please see **Supplemental figure 1**.



Supplemental figure 1 Multiple imputation of the changes in Centre for Epidemiologic Studies Depression scale score (CESDCh). The y-axis represents the change score (follow-up minus baseline). The x-axis represents the original data (0) and the 15 imputed datasets (1-15). Original measurements are shown with blue circles, imputed values in red.

Results sensitivity analyses imputed data

Using the multiple imputation paired t-test we compared cognitive domain scores that followed a normal distribution. We found no differences between baseline and follow-up in the imputed data for the attention and executive functioning domain (mean difference (95%CI): 1.0 (-0.1 to 2.2), p = 0.082), memory domain (mean difference (95%CI): -0.6 (-1.9 to 0.8), p = 0.407) and processing speed domain (mean difference (95%CI): -0.2 (-1.4 to 1.0), p = 0.730). Differences were highly similar to the non-imputed data (**Table 2**).

The pooled multiple linear regression model relating changes in CESD scores to potentially relevant variables showed highly similar results in beta's and explained variance (**Supplemental table 3**) when compared to the non-imputed data (**Table 4**).

Supplemental table 2 Relation between changes in cognitive domain scores and relevant variables

Cognitive domains	Sex and phenotype	Stroke	IQ	Changes in CESD scores	Fazekas score
Language	χ^2 = 7.0, p = 0.07	T = -0.15, p = 0.11	T = 0.09, p = 0.26	T = -0.01, p = 0.90	T = -0.03, p = 0.80
Memory	V = 0.2, p = 0.23	T = -0.03, p = 0.75	T = 0.03, p = 0.74	T = -0.09, p = 0.25	T = 0.05, p = 0.61
Visuospatial perception	χ^2 = 1.8, p = 0.61	T = -0.05, p = 0.60	T = -0.07, p = 0.39	T = 0.05, p = 0.56	T = -0.16, p = 0.10
Processing speed	V = 0.12, p = 0.42	T = 0.09, p = 0.35	T = -0.12, p = 0.13	T = -0.19, p = 0.02	T = -0.10, p = 0.27
Attention and executive functioning	χ^2 = 1.9, p = 0.59	T = -0.07, p = 0.44	T = 0.01, p = 0.86	T = -0.06, p = 0.44	T = 0.04, p = 0.69

The relation between sex and phenotype and changes in domain scores was tested using a MANOVA (presented as Pillai's trace (V) and p-value) or Kruskall-Wallis rank sum test (presented as chi-squared (χ^2) and p-value). Relations between IQ, stroke, changes in CESD scores, Fazekas scores and changes in cognitive domain scores were tested using Kendall's tau (presented as tau (T) and p-value).
CESD = Centre of Epidemiological Studies Depression scale, IQ = intelligence quotient

Supplemental table 3 Summary of multiple linear regression model after imputation relating changes in CESD scores to potentially relevant variables

	Model 1			Model 2		
Independent variables	**B (95% CI)**	**SE B**	**p-value**	**B (95% CI)**	**SE B**	**p-value**
Change in BPI severity	-0.16 (-1.29 to 0.97)	0.58	0.7797	0.09 (-1.06 to 1.24)	0.59	0.8803
Change in SF-36 health perception	-0.14 (-0.27 to -0.01)	0.07	0.0386	-0.07 (-0.22 to 0.08)	0.08	0.3409
Change in avoidance and brooding	2.99 (0.46 to 5.53)	1.29	0.0238	2.68 (0.12 to 5.23)	1.30	0.0438
Change in positivity and problem solving	-4.11 (-6.91 to -1.32)	1.42	0.0052	-4.01 (-6.76 to -1.25)	1.41	0.0058
Loneliness at follow-up				-0.70 (-6.29 to 4.89)	2.85	0.8056
Change in SF-36 social functioning				-0.06 (-0.15 to 0.03)	0.04	0.1735
Cardiac and/or renal involvement						
eGFR<60ml/min and/or presence of LVH at baseline				-3.14 (-6.86 to 0.59)	1.90	0.1035
Cardiac or renal complications at baseline				-2.64 (-7.31 to 2.02)	2.38	0.2701
Intercept	-1.30			1.26		
R^2	27.1% (10.7 to 45.2)			31.8% (14.6 to 50.0)		

B = beta coefficients, BPI = Brief Pain Inventory, CESD = Centre for Epidemiologic Studies Depression scale, eGFR = estimated glomerular filtration rate, LVH = left ventricular hypertrophy, SE = standard Error, SF-36 = Short Form-36 Health Survey

Supplemental table 4 Baseline raw neuropsychological test scores

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
Intelligence estimation					
DART, median (range)	82.2 (45.8-105)	77.2 (68.8-96.2)	71 (45.8-105)	82.9 (68.2-99.8)	86.1 (70-99.6)
Language					
BNT, median (range)	27.5 (19-30)	28.5 (21-30)	26.5 (21-30)	27.5 (19-29.5)	27.25 (19.5-30)
WAIS-IV: S, median (range)	24 (10-34)	24 (18-34)	20 (14-28)	24 (10-31)	29 (18-34)
Memory					
RAVLT ir, median (range)	53 (21-71)	48 (21-70)	53 (22-60)	53 (21-69)	56.5 (50-71)
RAVLT dr, median (range)	12 (2-15)	11 (2-15)	11 (3-13)	12 (4-15)	12.5 (10-15)
RBMT ir, median (range)	24 (8.5-35.5)	28 (9-34)	22.5 (8.5-31.5)	23.25 (11-34.5)	24.5 (15-35.5)
RBMT dr, median (range)	19.75 (0.5-32)	21 (4-32)	19.5 (6-28.5)	19.25 (0.5-31.5)	19.5 (12.5-31)
Visuospatial perception					
WAIS-IV BD, median (range)	41 (16-65)	46 (20-62)	28 (20-45)	43 (16-65)	49.5 (24-63)
JLO, median (range)	29 (14-33)	29 (25-30)	26 (18-30)	30 (14-33)	29.5 (24-33)
Processing speed					
TMT A, median (range)	23 (13-74)	26 (18-41)	30 (18-74)	23 (14-50)	20.5 (13-37)
Stroop W, median (range)	36 (26-74)	36 (30-46)	40 (34-74)	36 (26-50)	38 (28-48)
Stroop C, median (range)	50 (32-100)	56 (46-76)	54 (40-100)	50 (32-68)	50 (38-60)
Attention and executive functioning					
TMT B, median (range)	60 (27-370)	63 (41-97)	77 (62-200)	56 (27-370)	53.5 (39-99)
Stroop CW, median (range)	82 (46-214)	86 (66-122)	112 (68-214)	78 (46-132)	77 (56-92)
Fluency A, median (range)	24 (10-38)	25 (13-38)	24 (10-31)	24 (13-35)	27.5 (14-35)
Fluency O, median (range)	18 (4-29)	16 (9-26)	16 (9-22)	17 (4-29)	21 (12-26)
Fluency L, median (range)	34.5 (7-61)	31 (12-55)	30 (7-51)	36.5 (20-61)	31.5 (23-59)

All variables are presented as median (range).
Fluency A = Animal, BD = Block Design, BNT = Boston Naming Test, Stroop C = Color, CI = confidence interval, Stroop CW = Color-Word, DART = Dutch Adult Reading Test, dr = delayed recall, ir = immediate recall, JLO = Judgement of Line Orientation, Fluency L = Letter, Fluency O = Occupation, RAVLT = Rey Auditory Verbal Learning Test, RBMT = Rivermead Behavioural Memory Test, TMT = Trail Making Test, Stroop W = Words, WAIS-IV: S = Wechsler Adult Intelligence Scale IV: Similarities

Supplemental table 5 Follow-up raw neuropsychological test scores

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
Language					
BNT, median (range)	27.5 (19-30)	28.5 (21-30)	26.5 (21-30)	27.5 (19-29.5)	27.25 (19.5-30)
WAIS-IV: S, median (range)	24 (10-34)	24 (18-34)	20 (14-28)	24 (10-31)	29 (18-34)
Memory					
RAVLT ir, median (range)	53 (21-71)	48 (21-70)	53 (22-60)	53 (21-69)	56.5 (50-71)
RAVLT dr, median (range)	12 (2-15)	11 (2-15)	11 (3-13)	12 (4-15)	12.5 (10-15)
RBMT ir, median (range)	24 (8.5-35.5)	28 (9-34)	22.5 (8.5-31.5)	23.25 (11-34.5)	24.5 (15-35.5)
RBMT dr, median (range)	19.75 (0.5-32)	21 (4-32)	19.5 (6-28.5)	19.25 (0.5-31.5)	19.5 (12.5-31)
Visuospatial perception					
WAIS-IV BD, median (range)	41 (16-65)	46 (20-62)	28 (20-45)	43 (16-65)	49.5 (24-63)
JLO, median (range)	29 (14-33)	29 (25-30)	26 (18-30)	30 (14-33)	29.5 (24-33)
Processing speed					
TMT A, median (range)	23 (13-74)	26 (18-41)	30 (18-74)	23 (14-50)	20.5 (13-37)
Stroop W, median (range)	36 (26-74)	36 (30-46)	40 (34-74)	36 (26-50)	38 (28-48)
Stroop C, median (range)	50 (32-100)	56 (46-76)	54 (40-100)	50 (32-68)	50 (38-60)
Attention and executive functioning					
TMT B, median (range)	60 (27-370)	63 (41-97)	77 (62-200)	56 (27-370)	53.5 (39-99)
Stroop CW, median (range)	82 (46-214)	86 (66-122)	112 (68-214)	78 (46-132)	77 (56-92)
Fluency A, median (range)	24 (10-38)	25 (13-38)	24 (10-31)	24 (13-35)	27.5 (14-35)
Fluency O, median (range)	18 (4-29)	16 (9-26)	16 (9-22)	17 (4-29)	21 (12-26)
Fluency L, median (range)	34.5 (7-61)	31 (12-55)	30 (7-51)	36.5 (20-61)	31.5 (23-59)

All variables are presented as median (range).
Fluency A = Animal, BD = Block Design, BNT = Boston Naming Test, Stroop C = Color, CI = confidence interval, Stroop CW = Color-Word, dr = delayed recall, ir = immediate recall, JLO = Judgement of Line Orientation, Fluency L = Letter, Fluency O = Occupation, RAVLT = Rey Auditory Verbal Learning Test, RBMT = Rivermead Behavioural Memory Test, TMT = Trail Making Test, Stroop W = Words, WAIS-IV: S = Wechsler Adult Intelligence Scale IV: Similarities

Suppelemental table 6 Baseline T-scores neuropsychological tests

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
Language	49.5 (32.0-63.0)	51.5 (39.5-62.0)	45.5 (36.0-61.5)	48.8 (32.0-59.5)	55.0 (42.0-63.0)
BNT, median (range)	50.0 (37-63)	54.0 (39-63)	46.0 (39-63)	50.0 (37-59)	47.5 (37-63)
WAIS-IV: S, median (range)	50.0 (27-72)	50.0 (40-72)	44.0 (33-60)	50.0 (27-63)	59.0 (40-70)
Memory	55.0 (22.8-71.5)	54.5 (22.8-69.5)	55.3 (38.5-64.3)	54.8 (24.8-71.5)	57.8 (42.8-71.0)
RAVLT ir, median (range)	52.0 (16-72)	49.0 (18-65)	57.0 (32-66)	52.0 (16-68)	57.5 (47-72)
RAVLT dr, median (range)	53.0 (21-71)	48.0 (21-69)	54.0 (34-64)	53.0 (27-71)	56.0 (44-64)
RBMT ir, median (range)	57.0 (27-81)	59.0 (27-73)	57.0 (34-68)	57.0 (34-81)	58.0 (41-75)
RBMT dr, median (range)	55.0 (22-76)	54.0 (25-76)	59.0 (41-69)	54.0 (22-74)	56.5 (39-75)
Visuospatial perception	54.0 (28.0-65.5)	54.5 (44.5-64.0)	48.0 (36.5-54.0)	55.8 (28.0-65.5)	58.5 (47.0-65.5)
WAIS-IV BD, median (range)	50.0 (27-72)	50.0 (33-67)	43.0 (34-50)	52.0 (27-72)	60.0 (33-70)
JLO, median (range)	61.0 (29-61)	61.0 (52-61)	52.0 (33-61)	61.0 (29-61)	61.0 (48-61)
Processing speed	53.7 (32.3-74.7)	49.7 (42.0-60.0)	55.7 (40.3-74.7)	52.7 (32.3-63.3)	54.3 (45.7-70.3)
TMT A, median (range)	56.0 (34-77)	51.0 (38-61)	55.0 (34-63)	56.0 (34-77)	59.5 (43-71)
Stroop W, median (range)	56.0 (34-84)	56.0 (41-69)	51.0 (41-61)	60.0 (37-84)	54.0 (41-77)
Stroop C, median (range)	52.0 (29-88)	47.0 (29-59)	53.0 (29-88)	53.0 (33-71)	52.0 (39-71)
Attention and executive functioning	48.8 (25.6-66.8)	45.6 (35.2-58.8)	46.6 (37.2-55.4)	50.2 (25.6-66.0)	52.6 (40.2-66.8)
TMT B, median (range)	51.0 (-1-74)	47.0 (33-58)	49.0 (35-54)	51.0 (-1-74)	51.0 (42-59)
Stroop CW, median (range)	50.0 (32-84)	48.0 (39-60)	43.0 (33-61)	51.0 (32-84)	53.5 (45-71)
Fluency A, median (range)	50.0 (29-75)	48.0 (29-75)	54.0 (29-61)	50.0 (29-69)	57.0 (29-69)
Fluency O, median (range)	50.0 (17-69)	43.0 (24-64)	50.0 (38-57)	48.0 (17-69)	56.0 (36-67)
Fluency L, median (range)	45.0 (25-71)	42.0 (25-64)	43.0 (27-60)	50.0 (33-71)	43.5 (36-68)

All variables are presented as median (range).
Fluency A = Animal, BD = Block Design, BNT = Boston Naming Test, Stroop C = Color, CI = confidence interval, Stroop CW = Color-Word, dr = delayed recall, ir = immediate recall, JLO = Judgement of Line Orientation, Fluency L = Letter, Fluency O = Occupation, RAVLT = Rey Auditory Verbal Learning Test, RBMT = Rivermead Behavioural Memory Test, TMT = Trail Making Test, Stroop W = Words, WAIS-IV: S = Wechsler Adult Intelligence Scale IV: Similarities

Supplemental table 7 Follow-up T-scores neuropsychological tests

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
Language	51.5 (33.5-65)	53.5 (37.5-65)	44 (35-57.5)	51 (33.5-62.5)	55 (43-61.5)
BNT, median (range)	53 (37-63)	54 (38-63)	45 (37-63)	51.5 (37-63)	51.5 (39-63)
WAIS-IV: S, median (range)	53 (30-73)	53 (33-73)	43 (33-57)	53 (30-67)	60 (40-67)
Memory	54.25 (19.5-69)	55.5 (19.5-68.75)	54.25 (40-61.75)	53.1 (28.8-63.5)	61.75 (48.75-69)
RAVLT ir, median (range)	57 (18-75)	54 (18-68)	57 (41-69)	57 (19-72)	70 (54-75)
RAVLT dr, median (range)	57 (19-70)	50 (21-67)	57 (40-70)	57 (19-69)	63.5 (56-69)
RBMT ir, median (range)	51 (20-76)	54 (20-73)	50 (32-60)	50.5 (27-76)	55.5 (43-70)
RBMT dr, median (range)	52 (19-75)	54 (19-75)	54 (40-59)	51 (27-70)	56.5 (41-73)
Visuospatial perception	55 (32.5-67)	60.5 (42.5-67)	49.5 (40-55.5)	54 (32.5-65.5)	59.25 (47.5-64)
WAIS-IV BD, median (range)	50 (30-77)	60 (33-73)	43 (33-50)	49 (30-77)	59 (43-67)
JLO, median (range)	61 (30-61)	61 (48-61)	56 (43-61)	61 (30-61)	61 (52-61)
Processing speed	53.2 (36-78)	51.3 (40-61.7)	48.3 (36-63)	57.2 (36-78)	51 (39.3-67.3)
TMT A, median (range)	54 (31-79)	51 (42-62)	53 (31-62)	56.5 (31-79)	52.5 (31-70)
Stroop W, median (range)	55.5 (30-93)	53 (36-71)	45 (30-57)	58.5 (31-93)	52.5 (44-65)
Stroop C, median (range)	52 (27-76)	47 (31-66)	54 (27-71)	54 (33-76)	49.5 (33-67)
Attention and executive functioning	51 (17.6-66.4)	49.2 (33.2-63.4)	44.8 (37.8-64.4)	52.8 (17.6-64.4)	51.5 (39.2-66.4)
TMT B, median (range)	52 (-10-70)	50 (34-65)	45 (33-58)	54.5 (-10-70)	52 (39-59)
Stroop CW, median (range)	51.5 (25-76)	50 (37-69)	45 (32-63)	54.5 (25-76)	54.5 (48-72)
Fluency A, median (range)	50 (23-75)	54 (31-71)	46 (33-75)	50 (23-69)	54 (35-71)
Fluency O, median (range)	49 (19-79)	45 (19-74)	50 (33-79)	49 (19-67)	54.5 (26-62)
Fluency L, median (range)	49 (23-74)	44 (31-74)	42 (23-59)	54 (30-73)	51 (31-74)

All variables are presented as median (range).
Fluency A = Animal, BD = Block Design, BNT = Boston Naming Test, Stroop C = Color, CI = confidence interval, Stroop CW = Color-Word, dr = delayed recall, ir = immediate recall, JLO = Judgement of Line Orientation, Fluency L = Letter, Fluency O = Occupation, RAVLT = Rey Auditory Verbal Learning Test, RBMT = Rivermead Behavioural Memory Test, TMT = Trail Making Test, Stroop W = Words, WAIS-IV: S = Wechsler Adult Intelligence Scale IV: Similarities

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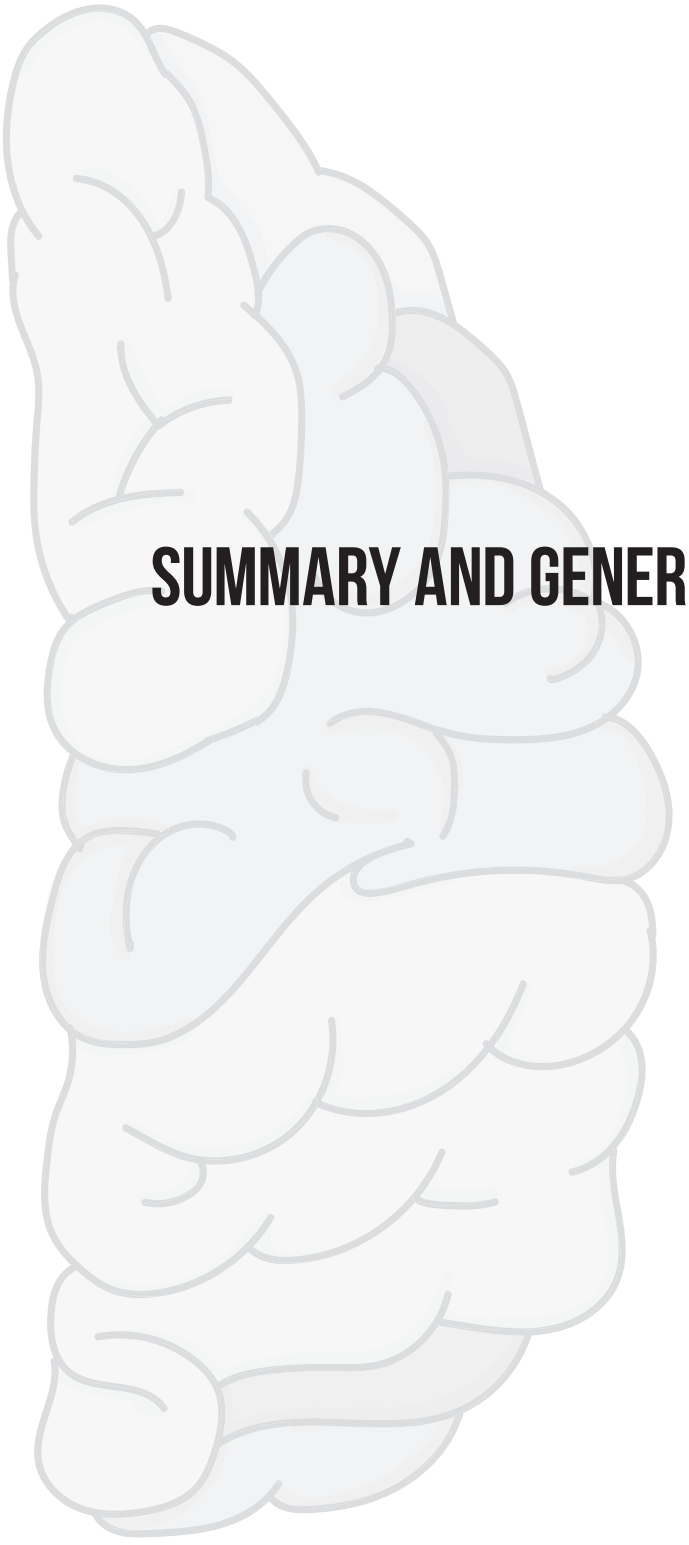

9

SUMMARY AND GENERAL DISCUSSION

Summary

It has long been known that cerebral involvement is common in Fabry disease (FD). Moreover, depressive symptoms are frequently present and mild cognitive impairment has been shown in FD patients. Nevertheless, decades later, many questions remain and published research in FD focusses mainly on heart and kidney related outcomes. The relation between symptoms experienced by patients, such as depressive symptoms, memory or attention deficits and brain involvement on MRI is largely unknown. Additionally, it is unclear which cognitive domains are affected, which patients are at risk and how cognitive functioning changes over time. Moreover, almost 20 years after the approval of enzyme replacement therapy (ERT), the effect on the cerebral manifestations of FD is still debated. Pivotal trials did not include cerebral imaging and were not powered to detect differences in cerebral events. Later published studies on the effect of ERT on cerebral FD manifestations often yielded limited information because of small sample sizes, lack of follow-up, poor methodological quality and did not incorporate sex and phenotype related differences. The caveats in the knowledge of the relation between FD and cerebral disease and the potential effect of ERT complicate counseling and treatment of FD patients.

In light of these questions, this thesis aimed to incorporate patients' illness perception (e.g. depressive symptoms, quality of life, coping) into the physicians' practice (e.g. MRIs of the brain, blood test results, treatment). To maximize the yield of the studies, we focused on large sample sizes and long follow-up duration, robust methodology and incorporation of sex and phenotype related differences. We aimed to identify important objective variables of cerebral disease, depressive symptoms and cognitive impairment and connect them to subjective symptoms of patients with Fabry disease. In addition, insight in the therapeutic effectiveness of ERT on the progression of cerebral involvement in FD was studied, which can support the appropriate use of this expensive treatment.

Cerebral involvement in Fabry disease

In **Chapter 2** we systematically reviewed all published studies on white matter lesions (WMLs) in patients with FD, with a focus on the prevalence, severity, location, progression, effect of ERT, related patient characteristics and potential clinical consequences of WMLs. Forty-six studies were included, with a total of 1276 patients with FD. In 46% of these patients WMLs were present and 16% had substantial confluent WMLs. WMLs in male patients with FD occurred at a younger age and were more severe compared to WMLs in female patients. Patients with classical FD had a higher risk of WMLs compared to non-classical patients. In patients treated with ERT, 25% showed WML progression during a follow-up time of three years. Patients with moderate to severe WMLs seemed to have

a higher stroke risk compared to patients with mild WMLs. Other relations between WMLs and clinical symptoms have been studied scarcely. Studies using modern imaging techniques, such as diffusion weighted imaging, showed involvement of the normal appearing white matter surrounding WMLs. This suggested that WMLs might be a tip of the iceberg of FD related changes in the brain. Unexpectedly, we found no relation between either cardiovascular risk factors, cardiac involvement or renal involvement and WMLs.

Consequently, the study described in **Chapter 3** aimed to find which patients are at risk for progression of WMLs and cerebral infarctions on MRI and what the effects were of changes in disease and treatment variables. A total of 852 MRI scans of 149 patients were assessed by two neuroradiologists on the presence of infarctions, presence and severity of WMLs and basilar artery diameter (BAD). Progression of WMLs and infarctions was mainly related to increasing age, male sex and having a classical FD phenotype. Both infarctions and WMLs progressed, regardless of ERT status. Additionally, progression could not be explained by other variables such as hypertension, dyslipidemia, atrial fibrillation or changes in kidney function, left ventricular mass or BAD.

Chapter 2 and 3 provide a sobering view of the treatability and manageability of WMLs and infarctions in FD. Patients should be clearly informed that WMLs will increase with age, despite treatment with ERT. Men with classical FD have a high risk of developing cerebral infarctions. Cardiovascular risk factors are probably of limited importance in progression of FD related WMLs and infarctions.

Quality of life, depressive symptoms and cognitive functioning in Fabry disease

In **Chapter 4**, we assessed the effect of different disease stages, complications, pain, age, sex and phenotype on quality of life (QoL) in FD patients. The data were gathered by Maarten Arends in the context of his PhD thesis work, in an international collaboration between three centers of excellence. QoL data were available from 286 Dutch and United Kingdom FD patients. QoL was worse in older men with a classical FD phenotype, compared to those with non-classical FD. Patients with higher pain scores, a history of cardiac complications or a history of stroke also reported lower QoL. Treatment with ERT did not appear to affect QoL. Considering this, adequate assessment and treatment of pain might improve QoL in FD patients.

For **Chapters 5-8** we used data of 81 Dutch FD patients who were assessed twice, with a year interval using a neuropsychological test battery and several questionnaires, which focused mainly on cognitive functioning, depressive symptoms and coping.

In **Chapter 5** the prevalence and profile of cognitive impairment in FD patients and their relation to disease manifestations and characteristics were evaluated, using data from the first assessment. Cognitive impairment was present in 16% of FD patients and was mostly mild. It mainly affected the executive functioning domain and processing speed. Subjective cognitive complaints of memory, executive functioning and attention were present in almost two-thirds of the FD patients. There was no relation between cognitive impairment and subjective cognitive complaints. Subjective cognitive complaints were related to both a history of depressive disorder and to higher scores on a depressive symptom questionnaire. Cognitive impairment, on the other hand, was related to male sex, classical FD phenotype, lower estimated IQ and a history of stroke. There was a univariate relation between severe WMLs and cognitive impairment, but this was not found in the multivariate analyses.

These results suggest that in our FD patient population cognitive impairment is subtle and is mainly seen in men with classical FD. In clinical practice, if subjective cognitive complaints negatively affect a patient's daily life, a neuropsychological test battery can be considered, especially in men with a classical disease phenotype and in patients with a history of stroke. Neuropsychological assessment should always be combined with the assessment of current depressive symptoms and a history of depressive symptoms.

Since the assessment of cognitive functioning is time and labor intensive, we evaluated the accuracy of the mini mental state examination (MMSE), a short screener for cognitive impairment, in FD in **Chapter 6**. We found that the MMSE did not accurately screen for cognitive impairment in FD, with poor sensitivity-specificity trade-off at all cutoffs. The majority of patients scoring below cutoffs did not have cognitive impairment. The application of the MMSE in clinical practice would therefore lead to unnecessary referrals for additional neuropsychological testing. Future studies could focus on screening tools designed to detect mild (executive) impairment in FD.

In **Chapter 7** we focused on the relation between depressive symptoms and coping styles in patients with FD. In addition, other variables that were potentially important in relation to depressive symptoms in FD were identified, using a systematic search of published literature. Coping styles employed by FD patients were "avoidance and brooding", "positivity and problem solving" and "seeking social support". Depressive symptoms were frequently present in our FD cohort (38%).

Higher depressive symptom scores were related to higher pain scores, worse health perception, more avoidance and brooding en less positivity and problem solving. Psychological counseling of FD patients can be adapted to improve coping styles and should be aimed at improving health perception.

In **Chapter 8** we investigated whether cognitive functioning and depressive symptoms changed during one year. At the follow-up assessment, 76 FD patients (94%) were available for re-evaluation. Changes in cognitive functioning and depressive symptoms were analyzed on an individual level and at group level. No major changes in cognitive functioning were found and we did not identify patients at risk for cognitive decline. Depressive symptoms were frequently present at both baseline (38%) and follow-up (29%). A major decrease in depressive symptoms was seen in six patients and a major increase in one patient. An increase in depressive symptoms was related to increased employment of an avoidant and brooding coping style and decreased use of positivity and problem solving. Considering the minimal changes in cognitive functioning over one year, we do not recommend using it as an outcome in clinical trials evaluating the effect of new treatment options. To evaluate the progression of cognitive impairment in FD patients, we recommend a longer follow-up time. Assessment of depressive symptoms using a questionnaire should be included in routine follow-up of FD patients.

Lastly, in **Chapter 9** we provided a summary of this thesis, followed by a discussion of the results in context of existing knowledge. Firstly, we speculate on the potential explanations of the lack of relation between WMLs in FD and cognitive functioning. Also, the importance of age, sex and phenotype in relation to progression in FD is stressed, while depressive symptoms have a different etiology. Evaluation of ERT effectiveness in cohort studies is discussed, with focus on major limitations and biases.

Secondly, recommendations for clinical practice are given. The follow-up frequency of cerebral manifestations in FD patients can be individually adjusted to risks for clinically relevant progression. Follow-up of relevant cerebral involvement on MRI is illustrated and the use of cerebral involvement in the decision on initiation and cessation of ERT is questioned. Lastly, current knowledge gaps are addressed and suggestions for future studies are given. Other variables (next to age, sex and phenotype) in relation to the progression of WMLs and stroke should be explored. Longitudinal cohort studies should focus on imaging biomarkers in relation to relevant clinical outcomes, which can be incorporated in future RCTs evaluating new FD specific treatments.

General discussion

Incorporating patients' illness perceptions into the physicians' practice

Patients with Fabry disease (FD) are at risk for white matter lesions (WMLs), transient ischemic attacks (TIAs) and strokes already from a young age [1, 2]. Psychological and cognitive impairment and an overall decreased quality of life (QoL) are common as well [3, 4]. In FD, clinical cohort studies and enzyme replacement therapy (ERT) trials have mainly focused on objective disease parameters, which provide support for the way physicians evaluate disease severity, progression and response to interventions (e.g. results from cerebral MRIs, blood tests, echocardiography). Outcomes as experienced by FD patients (e.g. depressive symptoms, health perception, quality of life) have been studied scarcely, while physicians and patients may have different perceptions of an illness. The differences in perception between a physicians' and patients' understanding of a disease can limit communication, treatment adherence and comprehension of the impact of the disease [5]. Incorporating patients' illness perceptions into the physicians' practice may reduce the difference in perspective between physicians' and patients' understanding of a disease, shifting focus in clinical practice and research to outcomes which are most relevant to patients. In this thesis, we therefore investigated the patients' perceptions (depressive symptoms, health perception) and the physicians' practice (cerebral MRIs, blood tests) and explored how these are related.

Cerebral involvement, cognitive functioning, quality of life and depressive symptoms: exploring the connections

In this thesis, it was shown that WMLs, cerebral infarctions, cognitive impairment and subjective cognitive complaints and depressive symptoms are all prevalent in patients with FD. As we investigated these outcomes simultaneously, we were able to explore their interrelation.

Cerebral involvement

In the general population, WMLs and infarctions on MRI are linked to a higher rate of clinical stroke [6, 7], cognitive decline [8, 9], depressive disorder [10] and death [8, 10]. Clinical stroke itself also gives a higher rate of cognitive decline [11], depressive disorder [12] and death [13].

In Fabry disease, as described in **chapters 2-5, chapter 7 and 8**, clinical stroke was related to the presence of cognitive impairment and decreased QoL, but not to depressive symptoms or changes in cognitive functioning. An increase in WMLs was associated with a higher risk of developing an infarction on MRI and a higher prevalence of TIA and clinical stroke. However, an increase in WMLs was not associated with (changes in) cognitive functioning, subjective cognitive complaints or depressive symptoms.

In this thesis, comparable to previous work in FD [3], the majority of the FD patients with cognitive impairment, were classified as having mild constraints. The mild executive functioning and attention deficits and mildly decreased information processing speed in FD [3] fits the profile of mild vascular cognitive impairment [14]. Four of the FD patients studied in this thesis were classified as having severe cognitive impairment, all with a history of stroke. In retrospect, three of these patients could have been classified as having a vascular like dementia considering the severity of cognitive impairment, its impact on their daily life and the history of stroke [15].

The lack of connection found between WMLs and other outcomes in this thesis might have several explanations. Firstly, although a relatively large cohort of patients was investigated, lack of statistical power is still a problem in a rare disease such as FD. Most relations between WMLs and other outcomes in the general population were shown in large meta-analyses [7, 8, 10]. Theoretically, this might be further complicated by individual differences in "brain reserve", the resilience of the brain to withstand pathology [16], and "cognitive reserve", the ability to efficiently use cognitive networks [16, 17]. Markers for brain reserve, such as brain size or neuronal structural integrity [18], were not assessed in this thesis. A previous study found a decreased intracranial volume (approximately 8%) in FD patients compared to controls [19]. Hypothetically, this would mean that, overall, brain reserve of FD patients could be somewhat decreased compared to the general population. It is unknown whether there are inter individual differences in brain volume within FD patients related to disease severity. IQ, a marker for cognitive reserve, was linked to cognitive impairment in this thesis, fitting the cognitive reserve hypothesis. It is probable that, comparable to the general population, differences in brain and cognitive reserves in FD patients influence the ability to withstand cerebral damage (such as WMLs). The "threshold" of WML severity after which cognitive decline becomes apparent will be different in individual FD patients. Therefore, relations between the severity of WMLs and cognitive functioning will probably be difficult to determine without taking markers for brain and cognitive reserve into account. Lastly, WMLs have been described as a "tip of the iceberg" of cerebral damage in the general population [20]. Beyond the regions with WMLs on structural imaging, signs of early cellular and axonic damage have been shown in "normal appearing white matter", using diffusion weighted imaging [21]. In multiple sclerosis and vascular dementia, DWI markers have been shown to explain cognitive impairment, independently of WMLs [22, 23]. In FD, changes in DWI markers are also present in normal appearing white matter (literature review **Chapter 2**). Whether DWI markers are of importance in relation to cognitive functioning in FD has not yet been studied.

During one year of follow up, we found no relevant changes in cognitive functioning. Neither in the whole patient group, nor in men with classical disease, nor in patients with a history of stroke. Probably, a one-year follow-up time period is too short to detect relevant differences in cognitive functioning in a slowly progressive disease such as FD. Since we did not include a control group, we cannot rule out a learning effect, the changes in test performance attributed to repeated test exposure, potentially masking small changes in cognitive functioning [24]. However, to reduce this risk we alternated test forms, if available. Undoubtedly, longer follow-up times are needed for more stringent conclusions.

Cognitive functioning and depressive symptoms

The presence of a major depressive disorder has a clear link to impairment in executive functioning and attention in the general population [25], domains of cognitive functioning that are affected in FD patients as well [3]. In the general population it has also been shown that cognitive impairment persists after depressive symptoms decrease [25, 26]. In contrast, we found no relation between (changes in) cognitive functioning and (changes in) depressive symptom severity in FD patients. FD patients with and without a history of depressive disorder had comparable cognitive functioning. The following factors could have contributed to these findings.

Firstly, in this thesis we assessed depressive symptoms using the Center for Epidemiological Studies Depression Scale (CESD). The CESD was developed to measure depressive symptomatology in the general population [27] and has been used mainly as a screening tool for major depressive disorder [28]. However, its validity might be reduced in patients with pain, chronic diseases and anxiety [29]. Thus, an increased score on the CESD does not necessarily reflect a diagnosis of major depressive disorder, possibly explaining the lack of relation found between cognitive impairment and depressive symptoms in FD patients. Secondly, a history of major depressive disorder in our FD cohort was defined as "diagnosed by a general practitioner, psychologist or psychiatrist". In the general population, persistent cognitive impairment has been shown more evidently in patients with recurrent or late onset major depressive disorder [26]. As we did not further specify the "type" of major depressive disorder, we cannot rule out that cognitive impairment between subtypes of major depressive disorder in FD might differ as well.

We also showed that subjective cognitive complaints were not related to cognitive impairment but were related to both depressive symptoms and a history of major depressive disorder. In the general population, the relation between subjective cognitive complaints and cognitive impairment is controversial [30], while the link between subjective cognitive impairment and both depressive symptoms or a history of major depressive

disorder has been shown more clearly [31]. Cognitive impairment in major depressive disorder can be split into (1) cognitive impairment as measured using neuropsychological tests and (2) cognitive biases including increased focus on negative stimuli [32]. The latter might be part of the explanation why FD patients with more depressive symptoms or a history of major depressive disorder reported more subjective cognitive complaints: "normal" forgetfulness or searching for words might be interpreted in a negative context. In other words: perceived cognitive functioning is not the same as actual cognitive functioning [31].

Cerebral involvement, cognitive functioning, quality of life and depressive symptoms: The importance of age, sex and phenotype

In this thesis, age, sex and disease phenotype were strongly related to progression of WMLs and infarctions on MRI, QoL and the presence of cognitive impairment. These findings fit into the growing body of evidence showing the importance of age, sex and phenotype in progression of all FD related organ involvement [33]. Generally, older men with a classical disease phenotype have a high risk of complications while (younger) women with a non-classical disease phenotype generally have a low risk.

It is not surprising that sex is an important variable in relation to progression of an X-linked disease such as FD. Similarly, the finding that age is strongly related to progression of cerebral involvement in a chronic disease such as FD is not groundbreaking. Also, structured phenotypical classification [33, 34] has gained more attention in recent guidelines as an important predictor of disease progression [35]. However, in the vast majority of, even recently published FD studies (e.g. [36-39]), only one or two of these factors are taken into account when assessing outcomes that have been previously shown to be related to age, sex as well as phenotype. It is probable that this results in erroneous conclusions, which is especially problematic in trials assessing new (and often expensive) treatments.

Organ involvement and cardiovascular risk factors

After taking age, sex and phenotype into account, we did not find any relation between either cardiac or renal organ involvement or cerebrovascular risk factors and cerebral involvement on MRI. This led us to speculate that most progression happens due to aging and severity of the disease itself (the latter shown by the importance of sex and phenotype) and that additional effects of cardiac or renal involvement or cardiovascular risk factors might be small to negligible in comparison. A potential explanation for the lack of relation between cerebrovascular risk factors and progression of WMLs and infarctions on MRI could be the observational nature of our studies. Follow-up of FD patients in the outpatient clinic also includes the evaluation of cerebrovascular risk factors [35]. If a FD patient is diagnosed with hypertension, antihypertensive treatment

is initiated and blood pressure is monitored strictly. This has a protective effect on the progression of WMLs in the general population [40, 41], potentially masking the true effects of hypertension on the brain in FD. Nevertheless, pathophysiology of small vessel disease in FD is in all probability different from the general population [42] and the effects of cerebrovascular risk factor management have not been systematically assessed in FD. In our view, the severity of WMLs in many FD patients without cerebrovascular risk factors or with strict cerebrovascular risk management suggests that in most patients these will not be the main contributor of cerebral disease progression.

It surprised us that we did not find any relation between renal and cardiac involvement, cerebrovascular risk factors and progression of WMLs and infarctions on MRI as major individual differences in progression risk remained after correction for age, sex and phenotype. This means that prognostication according to age, sex and phenotype is probably still inaccurate. In **chapter 3** we found that within the group of men with a classical disease phenotype, higher lysoGb3 levels and nonsense mutations were related to an increased risk of WML progression, after correction for age. Higher lysoGb3 levels might be a representation of minor differences in residual enzyme levels, potentially resulting in differences in disease severity. Nonsense mutations are associated with more severe FD compared to missense mutations [43]. It is therefore likely that a part of the unexplained risk of progression within subgroups after correction for age, sex and phenotype is due to additional differences in disease severity.

Depressive symptoms, the exception to the rule

In contrast to the other main outcomes in this thesis, severity of depressive symptoms in FD was not related to age, sex or disease phenotype, despite major differences in disease severity, in sex and phenotype defined subgroups. Depressive symptoms were observed in an equal proportion of women with non-classical disease, with no to minimal FD related complications, and severely affected men with classical FD. We did find that individual differences in coping styles were related to (changes in) depressive symptoms. We hypothesize that individual differences in coping influence the ability of FD patients to adapt to the likelihood of disease progression in a genetic disorder such as FD. As FD progresses it poses consecutive stressors on individuals, requiring constant adjustments to new situations. As described in **Chapter 1**, stressors are appraised by patients according to predictability, controllability and expectancies, which are all complicated in FD by the lack of precise prediction of the disease progression for an individual patient and the lack of adequate treatment options. Due to the many unknowns, health related changes might easily be considered as threatening for FD patients and uncertainties about the future probably affect mental health as well.

The individual differences in the adjustment to a chronic disease as FD might also be reflected in differences in FD patients' subjective health perception. Previous published work in FD patients, as reviewed in **Chapter 7**, and our current studies showed that subjective health perception appears to be more important in relation to depressive symptoms, compared to organ involvement or FD related complications. It is likely that the relation between depressive symptoms and health perception goes both ways: more depressive symptoms probably results in a worse health perception and a worse health perception may result in more depressive symptoms. While cardiac and renal involvement says something about current disease status, subjective health perception probably involves a broader depiction of patients' ideas about their current and future health, the perceived social implications and their experiences with the healthcare system [44, 45]. The finding that other factors than the disease itself explained a major part of depressive symptoms in FD patients, matches the pattern in other chronic disease patient populations [46].

We also assessed the effect of psychological counseling in our follow-up study. After the baseline assessment we referred several patients to local psychologists, but found no differences in depressive symptom severity between referred and non-referred patients after one year. As we did not randomize the referrals it is probable that these were biased. For example, to referral of patients with more obvious level of suffering, while patients who were likely to show improvement without counseling might not get referred. Altogether, while some patients do show clinically relevant improvement in depressive symptoms, and a previous study showed that personalized counseling improved depressive symptoms in FD [47], future studies should focus on which patients might benefit from counseling and whether psychological counseling aimed to adjust coping styles or health perception in FD can improve depressive symptoms.

Treatment with enzyme replacement therapy

The assessment of ERT efficacy and effectiveness in cohort studies is suboptimal. Unfortunately, none of the trials assessing the efficacy of ERT in FD patients included cerebral MRIs as a primary outcome [48-58] or were powered to detect differences in clinical TIA and/or stroke rate. The two RCTs that did include cerebral MRIs in their study protocol [53, 59] showed no difference in progression of MRI abnormalities between patients treated with agalsidase alfa and beta [53] and no difference in WML progression between patients treated with agalsidase beta and a placebo [59]. None of the recently published, currently active or planned trials for new FD treatments includes cerebral MRIs in their primary or secondary outcomes (**Chapter 1**). In this thesis, both WMLs and infarctions progressed despite treatment with ERT in the whole group as well as in "early treated" patients. We also found no effect of ERT on QoL, cognitive impairment or depressive symptoms.

Analyzing the effect of ERT in cohort studies is mainly limited by treatment bias. Patients with (suspected) severe FD will be treated earlier and follow-up will be more rigorous compared to patients with less severe FD. To overcome the treatment biases in analyses, many FD studies have resorted to the comparison of treated patients to historically untreated cohorts (mostly before the availability of ERT). Although this might be the best available option, it is probable that this comparison is also flawed. Compared to the pre-ERT era, current FD care is more standardized and protocolized. Standardized care generally results in improved outcomes [60-62]. Also, the availability of ERT increased the interest in FD screening studies and resulted in recognition of milder cases [63], further complicating the comparison of current cohorts to the mostly severely affected historic cases [64, 65]. In this thesis we were not able to compare cognitive functioning or depressive symptoms to untreated FD cohorts, as there are no cohort studies assessing depressive symptoms or cognitive functioning from the pre-ERT era. There are three cerebral MRI studies including only untreated patients [66-68]. As these three studies were >15 years ago, we expected the differences between our and the historic MRI assessments insurmountable and therefore did not pursue the comparison to these historical cohorts.

For **Chapter 3** we explored whether creating a variable "years treated" with ERT could provide more information on the effect of treatment. In an ideal world, receiving treatment for a longer period of time (and from an earlier age) would result in less disease progression. However, due to multicollinearity between age and years treated (and age being an important factor in relation to disease progression, thus non-removable) this was no reliable alternative.

Lastly, there is some evidence that agalsidase beta results in a better (biochemical) treatment response compared to algalsidase alfa [69, 70]. Again, comparing both types of ERT is limited by treatment bias. In the Amsterdam UMC, disease progression while being treated with agalsidase alfa is a reason to switch to the higher dosed agalsidase beta. Currently, agalsidase alfa prescription is restricted to specific cases, meaning that most patients will be treated with agalsidase beta. Therefore, we did not differentiate between treatment with agalsidase alfa and beta in this thesis.

Considering the abovementioned limitations, including the absence of an untreated cohort for comparison, it is impossible to conclude whether ERT altered the disease course. Nevertheless, we showed that despite being treated with ERT, progression of WMLs is seen in many patients, infarctions still occur, QoL is low and depressive symptoms are prevalent. The current treatment and management of FD patients, including ERT, therefore is insufficient. Discussions on the importance of FD specific treatments crossing the blood brain barrier can only be conducted in a meaningful way if relevant outcomes measures for cerebral involvement of FD are included in future trials.

Clinical implications

Follow-up frequency

In international FD treatment guidelines, recommended (cerebral) follow-up frequency is generally the same for all FD patients, independent of patients characteristics and risk of FD related complications [35, 71]. In **Chapter 3** we proposed an age, sex and phenotype dependent follow-up frequency for cerebral MRIs, with more stringent follow-up frequency in patients with higher risk of complications. Since cardiac and renal progression and complication risks are also strongly related to age, sex and phenotype [33], a similar adjustment in follow-up frequency could be considered for follow-up of kidney and heart involvement. Overall, this reduces the number of visits in patients with an expected low risk of clinically meaningful changes in organ involvement, reducing the burden of unnecessary testing on both patients and the medical system.

Cerebral imaging

Guidelines with recommendations for cerebral imaging in FD patients have been published [42, 72]. Markers that seem to be most relevant for clinical follow-up in FD are WMLs and (silent) infarctions on MRI. In this thesis we used the Fazekas and Scheltens scale for the follow-up of WMLs. As these were initially designed for cross-sectional assessment of WMLs severity [73, 74], we do not recommend these in a clinical follow-up setting. Alternatively, WMLs progression scales are also available and might provide a less crude assessment of progression [75, 76]. Potentially, automatically assessed white matter lesion load might be a feasible option for clinical practice as well [77].

Prevention of cerebrovascular events

There are no studies evaluating the effects of antiplatelet therapy in patients with FD. Increased markers of coagulation have been shown in FD patients, but it is unknown whether this contributes to the TIAs, strokes or development of WMLs [78, 79]. Nevertheless, as recommended for renal and cardiac primary and secondary prevention in FD, guidelines for the general population should be adhered to for primary and secondary prevention of WMLs, TIAs and strokes [6, 80], carefully considering the risk of side effects of antiplatelet drugs and the expected effects per individual patient. Generally, in patients with moderate to severe WMLs in whom the expected 10-year risk of (coronary heart disease or) stroke is ≥10% the start of acetylsalicylic acid should be considered [80]. Secondary prevention using antiplatelet therapy seems to be justifiable in FD patients after a TIA or stroke. Progression of WMLs on MRI might prompt more aggressive monitoring as this seems to be related to a higher risk of infarction. Patients with new infarctions on MRI should be interviewed for potentially missed (clinical) stroke symptoms. Patients with new infarctions on MRI, especially those in a subgroup with

low risk for infarctions (such as women with a non-classical phenotype and younger patients), should be referred to a neurologist for careful evaluation of other causes [81].

As discussed, the contribution of cerebrovascular risk factors in FD patients to cerebral disease severity is unknown, but overall expected to be limited. Nevertheless, primary and secondary preventive effects of the treatment of hypertension, treatment of dyslipidemia and glycemic control in type 2 diabetes have been firmly established in the general population [6, 80]. As side-effects of most common treatments for cerebrovascular risk factors are mild, the potential small benefit for the brain (but also the heart and kidney) should be aimed for in FD patients. Moreover, effect size of these interventions in subgroups with a lower risk of FD related cerebral progression might be greater. Nevertheless, expected effects and uncertainties should be explained to individual patients and not be overestimated, with a lenient view on cerebrovascular risk factor target values in individual cases.

Cognitive functioning and depressive symptoms

If patients mention subjective cognitive complaints, which negatively affect their daily lives, a referral for neuropsychological testing should be considered. Especially men with a classical disease phenotype and patients with a history of stroke seem to be at risk for cognitive impairment. As neuropsychological testing is labor intensive and exhaustive for patients, we also assessed the screening abilities of the Mini Mental State Examination (MMSE) for cognitive impairment in FD. However, the MMSE was unable to accurately screen for cognitive impairment in FD patients and is therefore not suitable to pre-select patients at risk for cognitive impairment. Neuropsychological testing should be combined with screening for depressive symptoms and assessment of psychiatric history, as these are likely to contribute to subjective cognitive complaints. In addition, we advise to include a depressive symptoms questionnaire (such as the CESD) in clinical follow-up as depressive symptoms are prevalent in all subgroups of FD patients and may persist over longer periods of time. In patients suffering from depressive symptoms, referral for psychological counseling should be discussed with the patient. It is to be expected that psychologists integrated in the multidisciplinary care teams might be able to identify the needs and challenges of FD patients more accurately, compared to psychologists without specific knowledge of FD [35, 44, 45].

Other patient reported outcomes

We recommend follow-up of both pain and QoL in the outpatient clinic. In this thesis, pain was related to decreased QoL and depressive symptoms. In FD, pain is often assessed using the brief pain inventory (BPI) [82] or FD specific pain questionnaires, the latter focusing on FD related neuropathic pain [83]. Since treatment of FD specific neuropathic

pain differs from "regular" pain treatment [84], a differentiation should be made (as much as possible). The diagnosis of FD related neuropathic pain is mainly based on the typical presentation and symptomology [82], but can be confirmed in atypical cases by somatosensory evaluation and a skin biopsy [85]. While antiepileptic drugs are the treatment of choice for neuropathic pain [84], "regular" pain should be treated according to published stepwise algorithms [86, 87].

QoL in FD is assessed with generic and widely used questionnaires, mainly the 36-Item Short Form Health Survey (SF-36) and the EuroQol 5D (EQ-5D), as FD specific QoL questionnaires are not available [4]. The EQ-5D can provide a quick overview of a patients QoL. The SF-36 is more comprehensive and can be divided in a physical and mental component. Decreased QoL should prompt further exploration of potentially contributing factors.

Generally, the use of questionnaires in longitudinal follow-up in clinical practice could be facilitated using digital tools. Information visualization systems can display individual patients' questionnaire results in comparison to reference populations, highlighting the most important changes and problems that require attention [88].

Enzyme replacement therapy

As discussed, the effectiveness of ERT on the brain is not known, but disease progression and complications occur despite treatment. Initiation and cessation criteria for ERT have been published, stating that the start of ERT may be considered in patients with WMLs and should be considered in patients with TIA or stroke, independent of sex and phenotype [89]. In clinical practice, if severe (irreversible) multi-organ damage is present at the time of FD diagnosis, ERT is generally not initiated. Considering the findings outlined in **chapter 2 and chapter 3** we evaluated the (non) initiation and cessation criteria for ERT.

There are some limitations regarding the current criteria for the presence of (FD related) cerebral involvement which might prompt ERT initiation. Firstly, "presence" or "absence" of WMLs is an insensitive description of FD related cerebral disease severity. Also, the presence of WMLs, TIA or stroke is not FD specific. Secondly, the effect of ERT on WMLs has not been established and progression of WMLs while on treatment is to be expected. The effect of ERT on TIA and stroke has also not been established either, although a reduction of complications (including TIA and stroke) was seen in patients receiving ERT compared to a placebo [51] and reduction of stroke risk was suggested in a meta-analysis [70].

Initiation of enzyme replacement therapy

Generally, cardiac and renal involvement should direct the decision to start ERT. In men with a classical disease phenotype, we recommend removing WMLs, TIAs and strokes from the decision to start ERT, since ERT initiation should be considered at an early age in this subgroup, independently of the presence of organ involvement. In women with a classical phenotype and men or women with a non-classical phenotype, (1) more severe WMLs than should be expected for their age (as assessed by a neuroradiologist) or TIA/stroke at a young age, (2) without other (potential) explanations for these WMLs, TIA or stroke could be indicative of (relatively) severe FD. This should prompt strict assessment of cardiac and renal involvement, plasma lysoGb3 concentration and disease expression in the family. If the additional assessments highlight a high risk of future organ involvement, ERT should be considered. It is unknown whether patients with severe cerebral disease, without renal or cardiac involvement, high lysoGb3 or severe disease in the family, have a higher risk of FD related complications in the future. Cerebral disease, without any additional arguments for relatively severe FD should not result in the start of ERT.

Non-initiation and cessation of enzyme replacement therapy

Irreversible cardiac or renal involvement should guide the decision of non-initiation and cessation of ERT. Presence of multiple infarctions on MRI, progression of severe confluent WMLs or having a history with multiple strokes should not be used as criteria for non-initiation or cessation of ERT, but severe cognitive or physical disability due to severe cerebral involvement should.

Important outcomes for future studies and future study directions

Age, sex and phenotype

We propose that all FD studies exploring new outcomes should initially evaluate the relation to age, sex and phenotype (simultaneously) before testing any other relation. All FD studies regarding renal, cardiac or cerebral outcomes should correct for age, sex and phenotype before exploring the effect of other variables.

Stroke

Clinical stroke is a relevant clinical outcome in FD, as it has been most thoroughly studied. Stroke prevention may have the potential to preserve QoL and cognitive functioning. However, it seems unfeasible to use stroke as an outcome in randomized controlled trials. For example, in **Chapter 3**, seven out of 149 patients developed a first time TIA or

stroke during a median follow-up time of seven years. In comparison, all published FD trials included less than 100 patients and (blinded) follow-up time generally lasted six months. Using power calculations, we showed it is unlikely that future trials will be able to detect differences in infarctions on MRI. For large multicenter, industry-independent databases including hundreds or potentially thousands of patients over a longer time period, clinical stroke might be a more feasible endpoint.

Previous randomized controlled trials have also used "cerebrovascular events" as an outcome, combining TIAs and strokes [58]. This increased the number of events, therewith decreasing the needed sample size and follow-up time. However, the diagnosis of a TIA is notoriously difficult as many other diseases can mimic the presentation [90]. While diagnosing a TIA requires cerebral imaging to exclude tissue ischemia [91], the diagnosis is often based on clinical interviewing in FD studies [56, 92, 93]. Therefore, when using TIA as an outcome, the international diagnostic criteria should be strictly adhered to.

Future studies should explore the presentation and clinical consequences of stroke in FD patients, using (adaptations of) well known scales and classifications used in general stroke research. It is unknown whether functional disability after stroke in FD is similar compared to that in stroke patients in the general population. Scales, such as the Modified Ranking Scale, are widely used in stroke research [94] might be valuable to assess post stroke functioning in FD patients as well.

FD seems to affect both the large and small cerebral arteries. It has also been speculated that atrial fibrillation might be an important cause of stroke in FD [42], although we did not find a relation between atrial fibrillation and progression of infarctions. Classifying subtypes of stroke in FD using established criteria might show differences in stroke etiology [95] and risk factors [96] per stroke subtype, which could influence individual diagnostic follow-up and secondary prevention. This would require large international collaborations as the number of FD patients with strokes in the Netherlands will be too low for subtype analyses. Lastly, prospective studies using more sophisticated assessment of risk factors (e.g. internal loop recorder for the assessment of atrial fibrillation) can improve the understanding of which FD patients are at risk for stroke and why.

Neuropsychological testing

Considering the lack of changes in cognitive functioning during one-year follow-up, we do not recommend using neuropsychological test battery outcomes as endpoints in short term FD trials. We are planning to reassess the FD patient cohort from **Chapter 8**, approximately five years after the baseline assessment, to analyze if FD patients are

at risk for cognitive decline and if so, at what rate cognitive decline occurs. Cognitive screeners sensitive to mild impairment in processing speed and executive functioning should be explored to see if FD patients needing neuropsychological evaluation can be pre-selected.

Patient reported outcomes

Although pain has been recognized as an important and disabling factor in FD, high quality research on the treatment of FD neuropathic pain is a lacking [84] and should be a focus of future trials testing not only FD specific treatments but also different anti-epileptic drugs. The effect of pain treatment on QoL and depressive symptoms would be of interest as well.

As we were the first to explore coping styles in FD, our findings should be confirmed in FD patients in other countries/cultures. Moreover, the interrelation between pain, coping and depressive symptoms is likely complex and unraveling this interrelation could provide more insight in the possibilities to decrease depressive symptoms. A single interventional pilot study explored personalized psychological counseling in FD patients, although it is unclear what kind of counseling was provided [47]. Considering the work of this thesis, future psychological counseling studies in FD might compare counseling with and without adjustment to coping styles and could be targeted at changing health perception.

Given the potential inflation of CESD scores in patients with chronic diseases and/or pain [29], it is uncertain whether FD patients with increased CESD scores should be regarded as having a major depressive disorder or if other factors contribute to these scores as well. It would be interesting to combine qualitative research with depressive symptom questionnaires, while simultaneous assessing the DSM-V criteria for major depressive disorder to discover important themes related to depressive symptoms and the prevalence of major depressive disorder in FD patients. This could provide opportunities to further tailor psychological counseling on an individual basis.

Cerebral imaging

In this thesis, we proposed that a large group of FD patients with a non-classical phenotype, especially women, have a similar WML and infarction burden compared to the general population. If this is supported by future studies, female patients with non-classical disease could be reassured that their risk of cerebral involvement is largely unchanged compared to the general population. This may also decrease unnecessary treatment with ERT in groups with low complication risks.

More sophisticated assessment of potential risk factors in prospective studies can improve the understanding of the remaining differences between WML and infarction severity after correcting for age, sex and phenotype. Whether small differences in residual enzyme activity might explain some of the remaining differences in disease severity within the group of men with a classical FD phenotype has to be shown in future research.

Lastly, the relation between WMLs and infarctions on MRI in FD and potential clinical consequences is largely unknown and should be further explored. However, this should not be restricted to WMLs or infarction only. We suggest that the search for relevant biomarkers for cerebral manifestations of FD on cerebral MRI should be intensified, including their relation with (future) clinical outcome parameters. Prospective cohort studies or trials with sophisticated imaging techniques simultaneously assessing patient reported outcomes could provide more insight in the relative importance of different imaging parameters such as cerebral blood flow or DWI markers [72]. Inclusion of these relevant biomarkers for cerebral outcomes in future trials needs to be encouraged by the European Medicines Agency and the Food and Drug Administration.

Currently, we are longitudinally exploring the relation between WMLs and DWI parameters. As discussed, it has been shown in the general population that WMLs are the "tip of the iceberg" [20] and that pathological changes can be found in regions appearing as normal white matter on structural MRIs. If DWI parameters change before the occurrence of WMLs, patients at risk for WMLs might be identified in an earlier stage.

Improving perceptions

In conclusion, we believe that this thesis may improve communication between doctors and patient, guides the appropriate use of ERT and gives clear directions for future research. Research in rare diseases is difficult due to low numbers of patients and the heterogeneity of disease phenotypes. International, large, industry-independent databases can increase the number of patients included in rare disease studies, resolving (some of) these problems. We also make a plea for longitudinal studies, with smart designs (with the potential to decrease the needed number of patients [97]) and robust methodology and reporting to improve comparability and interpretability of results.

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Chapter 9 | Summary and general discussion

A

APPENDIX

Nederlandse samenvatting

De ziekte van Fabry is een zeldzame X-gebonden, erfelijke lysosomale stapelingsziekte. Een genetische afwijking in het GLA-gen zorgt voor een verminderde activiteit van het enzym α-galactosidase A. Dit leidt tot stapeling van de stof globotriaosylceramide, wat uiteindelijk kan resulteren in complicaties van nieren, hart en hersenen. De gevolgen in de hersenen uiten zich vooral als herseninfarcten en TIA's; *transient ischemic attacks*. Op MRI-scans van de hersenen van Fabry patiënten worden regelmatige kleine infarcten en witte stofafwijkingen aangetroffen. Daarnaast benoemen patiënten in de praktijk een verminderde functie van geheugen, aandacht, concentratie en soms een verslechterde stemming.

Aangezien de ziekte X-gebonden is, verloopt deze anders bij mannen en vrouwen: mannen krijgen gemiddeld eerder én vaker complicaties. Fabry patiënten worden ingedeeld aan de hand van twee fenotypen: een klassiek fenotype en een niet-klassiek fenotype. Patiënten met een klassiek fenotype hebben een hogere kans op complicaties ten opzichte van niet-klassieke patiënten. Vanwege het verschil in natuurlijk beloop is de indeling van patiënten aan de hand van leeftijd, geslacht en fenotype noodzakelijk om zicht te krijgen op de prognose van een patiënt en het potentiële nut van behandeling met enzymtherapie. Oudere mannen met een klassiek fenotype hebben het hoogste risico op complicaties terwijl jongere vrouwen met een niet-klassiek fenotype het laagste risico op complicaties hebben. De behandeling met enzymtherapie lijkt vooral zinvol als er gestart wordt op een jongere leeftijd voordat er onomkeerbare schade ontstaan is, waarbij het profijt voor mannen met een klassiek fenotype het grootst lijkt. Enzymtherapie, een tweewekelijkse intraveneuze toediening van recombinant α-galactosidase A, was tot voor kort de enige ziekte-specifieke behandeling bij de ziekte van Fabry.

Ondanks het feit dat de gevolgen van de ziekte van Fabry al tientallen jaren bekend zijn, blijven er veel vragen bestaan. Veel van de gepubliceerde onderzoeken richten zich op hart en nieren en niet zozeer op de betrokkenheid van de hersenen in het ziekteproces. De relatie tussen symptomen die patiënten met Fabry ervaren, zoals depressieve klachten, geheugen- en aandachtsproblemen, en de met MRI aangetoonde afwijkingen in de hersenen, is grotendeels onbekend. Het is ook onduidelijk welke cognitieve domeinen betrokken zijn, welke patiënten een groter risico lopen om cognitieve stoornissen te krijgen en hoe het beloop van het cognitief functioneren is. Bovendien is het effect van enzymtherapie op de hersenen bij de ziekte van Fabry bijna 20 jaar na de goedkeuring nog steeds een punt van discussie. Tijdens de gerandomiseerde, placebo gecontroleerde studies die hebben geleid tot het toelaten van enzymtherapie werden geen MRIs van de hersenen uitgevoerd en was de steekproefgrootte (en daarmee het onderscheidend

vermogen) van de studies onvoldoende om verschillen te vinden in herseninfarcten en TIA's tussen behandelde en onbehandelde patiënten. Later gepubliceerde onderzoeken naar patiënten met de ziekte van Fabry zijn vaak van matige kwaliteit omdat ze (te) klein zijn, de methodiek onduidelijk beschreven is en er geen onderscheid wordt gemaakt aan de hand van geslacht en fenotypen. De vele opstaande vragen over de gevolgen van cerebrale schade en de effectiviteit van enzymtherapie bemoeilijken het bieden van een accurate prognose en een goed onderbouwde behandeling van Fabry patiënten.

Het doel van deze thesis was dan ook om een bijdrage te leveren aan het beantwoorden van deze vragen, waarbij de verbinding gezocht werd tussen ervaringen van patiënten (zoals depressieve klachten, kwaliteit van leven, coping strategieën) en de praktijk van de dokter (zoals MRIs van de hersenen, resultaten van bloedonderzoek). Om deze vragen zo accuraat mogelijk te beantwoorden lag de nadruk op grote patiëntgroepen, robuuste methodologie, longitudinaal onderzoek en het in acht nemen van verschillen tussen de fenotypes en geslacht. De uiteindelijke resultaten kunnen bijdragen aan het voorspellen van de prognose van individuele patiënten door identificatie van belangrijke patiëntkenmerken die gekoppeld zijn aan progressie. Het verhelderen van de rol die enzymtherapie heeft bij progressie van de cerebrale afwijkingen die gevonden worden bij de ziekte van Fabry kan de doelmatige inzet van deze dure therapie stimuleren.

Cerebrale betrokkenheid bij de ziekte van Fabry

Hoofdstuk 2 bevat een systematisch overzicht van alle studies over witte stofafwijkingen bij de ziekte van Fabry, gefocust op de prevalentie, ernst, locatie, progressie, effect van enzymtherapie, gerelateerde patiënt karakteristieken en potentiële gevolgen. In totaal werden er 46 studies met daarin 1276 patiënten met de ziekte van Fabry geanalyseerd. Zesenveertig procent had zichtbare witte stofafwijkingen op de MRI en 16% had uitgebreide samenvloeiende witte stofafwijkingen. Mannen hadden op jongere leeftijd meer toename en uitgebreidere witte stofafwijkingen dan vrouwen. Patiënten met een klassiek fenotype hadden een grotere kans op witte stofafwijkingen ten opzichte van patiënten met een niet klassiek fenotype.

Ondanks behandeling met enzymtherapie namen witte stofafwijkingen bij een kwart van de patiënten toe gedurende drie jaar vervolgonderzoek. Patiënten met matige tot ernstige witte stofafwijkingen hadden een hoger risico op herseninfarcten in vergelijking met patiënten met geringe witte stofafwijkingen. Andere potentiële gevolgen van witte stofafwijkingen zijn nauwelijks bestudeerd bij patiënten met de ziekte van Fabry. Studies waarin moderne scantechnieken gebruikt werden suggereerden dat er ook schade detecteerbaar is buiten de gebieden die nu als witte stofafwijkingen gezien worden op conventionele scans. Met diffusie gewogen opnames werden veranderingen

in de beweging van waterstofatomen vastgesteld in hersenweefsel zonder witte stofafwijkingen. Dit kan duiden op vroege schade aan hersencellen en zenuwbanen. Dit suggereerde dat witte stofafwijkingen nog maar het topje van de ijsberg zijn van de schade aan de hersenen bij de ziekte van Fabry. Onverwacht was dat we geen relatie vonden tussen andere patiënt karakteristieken zoals een verhoogde bloeddruk, schade aan de nieren of het harten witte stofafwijkingen.

Om dit beter te begrijpen was het doel van **hoofdstuk 3**: onderzoeken welke patiënten een hoger risico hebben op toename van witte stofafwijkingen en infarcten op MRI scans van de hersenen en evalueren welk effect hart en nieren, cerebrovasculaire risicofactoren en behandeling met enzymtherapie hebben.

Hiervoor zijn door twee neuroradiologen 852 MRI scans van 149 patiënten beoordeeld op de aanwezigheid van infarcten, witte stofafwijkingen en de diameter van de arteria basilaris. Veroudering, het mannelijk geslacht en een klassiek Fabry fenotype waren de belangrijkste factoren in relatie tot toename van zowel infarcten als witte stofafwijkingen. Ondanks behandeling met enzymtherapie namen zowel witte stofafwijkingen als infarcten toe. Toename van witte stofafwijkingen en infarcten was niet gerelateerd aan hypertensie of veranderingen in nierfunctie, linker ventrikel massa of de diameter van de arteria basilaris.

Hoofdstuk 2 en 3 geven een ontnuchterende kijk op de mate van beïnvloedbaarheid door risicofactoren buiten de ziekte van Fabry en de behandelbaarheid van witte stofafwijkingen en infarcten op MRI scans van de hersenen. Patiënten zouden dan ook duidelijk voorgelicht moeten worden over de toename van witte stofafwijkingen bij veroudering, onafhankelijk van behandeling met enzymtherapie. Mannen met een klassiek fenotype hebben een duidelijk verhoogd risico op infarcten. In tegenstelling tot studies in de algemene bevolking lijken cerebrovasculaire risicofactoren zoals hypertensie weinig tot geen invloed te hebben op de progressie van Fabry gerelateerde witte stofafwijkingen en infarcten. Dit past bij ons vermoeden dat de progressie hoofdzakelijk veroorzaakt wordt door de ernst van de ziekte van Fabry zelf en niet zo zeer door bijkomende factoren.

Kwaliteit van leven, depressieve klachten en cognitief functioneren bij de ziekte van Fabry

Hoofdstuk 4 beschrijft de kwaliteit van leven van 286 Fabry patiënten met gegevens uit Nederland en het Verenigd Koninkrijk met nadruk op de invloed van ziektestadia, complicaties, pijn, leeftijd, geslacht en fenotype. Mannen met een klassiek fenotype ervoeren een grotere achteruitgang van kwaliteit van leven ten opzichte mannelijke

leeftijdsgenoten met een niet-klassiek fenotype. Daarnaast werd er een verminderde kwaliteit van leven gevonden bij patiënten met meer pijn, na het doormaken van een herseninfarct of na cardiale complicaties. Enzymtherapie was niet gerelateerd aan een betere kwaliteit van leven. Het adequaat uitvragen en behandelen van pijn lijkt een mogelijke strategie om de kwaliteit van leven te verbeteren.

Voor **hoofdstukken 5-8** hebben wij de resultaten gebruikt van de gegevens van 81 Nederlandse Fabry patiënten die tweemaal onderzocht zijn met een neuropsychologische testbatterij en vragenlijsten naar onder andere depressieve klachten en coping.

De prevalentie van cognitieve stoornissen bij de ziekte van Fabry en de betrokken cognitieve domeinen werden geëvalueerd in **hoofdstuk 5** met data van de eerste meting. Cognitieve stoornissen waren aanwezig bij een subgroep van patiënten met de ziekte van Fabry (16%) en waren veelal gering. De executief functioneren en verwerkingssnelheid waren de meest betrokken cognitieve domeinen. Bijna twee derde van de patiënten ervoer klachten van het geheugen, executief functioneren en de aandacht, zogenoemde subjectieve cognitieve klachten. Patiënten met cognitieve stoornissen hadden geen hogere kans op subjectieve cognitieve klachten ten opzichte van patiënten zonder cognitieve stoornissen. Subjectieve cognitieve klachten waren wel sterk geassocieerd met de aanwezigheid van een depressieve stoornis in de voorgeschiedenis en met een hoge score op de depressieve klachten vragenlijst. Het risico op cognitieve stoornissen bleek hoger bij mannen met een klassiek Fabry fenotype, bij patiënten met een lager IQ en na een herseninfarct. Het hebben van ernstige witte stofafwijkingen vertoonde geen sterke relatie met de aanwezigheid van cognitieve stoornissen.

Cognitieve stoornissen komen voornamelijk voor bij mannen met een klassiek fenotype en blijken over het algemeen gering te zijn. In de praktijk zou er bij subjectieve cognitieve klachten en een normaal neuropsychologisch onderzoek verder gekeken moeten worden naar actuele (of een verleden van) depressieve klachten.

Het ondergaan van een neuropsychologisch onderzoek is mentaal belastend en vergt tijdsinvestering van zowel patiënt als neuropsycholoog. In **hoofdstuk 6** hebben we daarom gekeken of een korte screenende test, de M*ini Mental State Examination* (MMSE), Fabry patiënten met en zonder cognitieve stoornissen accuraat van elkaar kan onderscheiden. Wij vonden echter geen geschikte afkapwaarde waarbij de MMSE dit onderscheid accuraat kon maken. Het klinische gebruik van de MMSE zou tot inaccurate verwijzingen naar de neuropsycholoog kunnen leiden. Vervolgonderzoek zou ingezet kunnen worden naar andere screenende testen die gevoeliger zijn voor geringe afwijkingen in bijvoorbeeld het executief functioneren.

In **hoofdstuk 7** hebben we ingezoomd op de relatie tussen depressieve klachten en coping stijlen bij patiënten met de ziekte van Fabry. Coping, de cognitieve en gedragsmatige inspanningen die worden ingezet indien situaties als stressvol worden ingeschat, was nog nooit geëvalueerd bij patiënten met de ziekte van Fabry. Ook hebben we gezocht in eerder gepubliceerde onderzoeken welke andere factoren samengaan met depressieve klachten bij de ziekte van Fabry. Uit analyse van de vragenlijsten bleek dat de coping stijlen "vermijden en piekeren", "positiviteit en probleem oplossend gedrag" en "sociale steun zoeken" voornamelijk toegepast werden door de Fabry patiënten in dit cohort. Veel patiënten ervoeren depressieve klachten (38%).

Patiënten met meer pijnklachten, een negatievere ziekteperceptie, die meer vermeden en piekerden en minder positiviteit en probleem oplossend gedrag toonden, hadden meer depressieve klachten. De psychologische begeleiding van patiënten met de ziekte van Fabry met depressieve klachten zou in de toekomst kunnen worden aangepast aan de coping stijlen van individuele patiënten en kan gericht worden op het beïnvloeden van ziekteperceptie.

In **hoofdstuk 8** hebben wij gekeken of er veranderingen waren in het cognitief functioneren en depressieve klachten van Fabry patiënten na één jaar. Zesenzeventig patiënten (94%) waren beschikbaar voor het vervolgonderzoek. Voor zowel cognitief functioneren als depressieve klachten hebben we op groepsniveau maar ook op individueel niveau gekeken of er veranderingen waren ten opzichte van de eerste meting. We vonden geen opvallende veranderingen in cognitief functioneren na één jaar, niet op groepsniveau en niet op individueel niveau. Depressieve klachten waren veel aanwezig bij zowel de eerste meting (38%) als bij de tweede meting (29%). Depressieve klachten veranderden wel in enige mate, waarbij zes patiënten een relevante afname lieten zien en één patiënt een relevante toename. Op groepsniveau was toename van depressieve klachten gerelateerd aan meer gebruik van een "vermijdende en piekerende" coping stijl, een verslechterende ziekteperceptie en minder "positiviteit en probleem oplossend" gedrag. De afwezigheid van veranderingen in cognitief functioneren na één jaar in deze relatief grote groep Fabry patiënten maakt cognitief functioneren geen goede uitkomstmaat voor gerandomiseerd (medicijn)onderzoek. Om het effect van Fabry op cognitief functioneren beter te kunnen bestuderen is een langere volgduur nodig. Gezien de hoge aantallen Fabry patiënten met depressieve klachten raden wij aan deze klachten te meten met een vragenlijst bij een bezoek aan de polikliniek.

In **hoofdstuk 9** wordt de thesis samengevat en worden de resultaten in de context van huidige kennis bediscussieerd. Allereerst speculeren we over de mogelijke reden waarom bij patiënten met de ziekte van Fabry geen relatie gevonden wordt tussen witte

stofafwijkingen en cognitief functioneren. We benadrukken het belang van leeftijd, geslacht en fenotype in relatie tot progressie van de ziekte van Fabry, ook op het gebied van de betrokkenheid van de hersenen. Depressieve klachten volgen echter een ander patroon waarbij coping stijlen en ziekteperceptie van groter belang blijken te zijn. De effectiviteit van enzymtherapie op het ontstaan en de progressie van hersenafwijkingen, cognitief functioneren en depressieve klachten wordt bediscussieerd en de bias van effectiviteitsanalyses van enzymtherapie in cohortstudies wordt besproken.

Daarnaast geven we adviezen voor de praktijk, die voortvloeien uit deze thesis. De frequentie van MRI scans van de hersenen kan worden aangepast aan de hand van individuele risico's op klinisch relevante progressie. Het gebruik van cerebrale afwijkingen als criterium voor het starten of stoppen van enzymtherapie kan aan de hand van de huidige kennis worden aangescherpt. Afsluitend worden resterende kennislacunes uitgelicht en suggesties gedaan voor toekomstige studies. Het belang van andere variabelen (naast leeftijd, geslacht en fenotype) in relatie tot witte stofafwijkingen en infarcten zou moeten worden onderzocht. Longitudinale studies zouden ook moeten zoeken naar biomarkers op MRI scans van de hersenen die een sterke relatie hebben met klinisch relevante uitkomstmaten voor Fabry patiënten. Deze biomarkers kunnen in toekomstige gerandomiseerde studies worden opgenomen zodat het effect van nieuwe behandelingen op de hersenen van Fabry patiënten wel kan worden vastgesteld.

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Portfolio

Name PhD student: S. Körver
PhD period: Januari 2016 – September 2020
Name PhD supervisor: Prof. Dr. C.E.M. Hollak

PhD training

General courses	**Year**	**ECTS**
eBROK ('Basiscursus Regelgeving Klinisch Onderzoek')	2016	1.0
Practical Biostatistics	2016	1.4
Scientific Writing for publication	2016	1.5
The AMC World of science	2016	0.7
Advanced Topics in Biostatistics	2017	2.1
Computing in R	2017	0.4
Didactical Skills	2017	0.4
MRI: basic understanding for (bio)medical research	2017	1.0
Specific courses		
Openclinica Training	2016	0.6
Design week Endocrinology & Metabolism	2017	1.0
Seminars, workshops and master classes		
APROVE science night	2016	0.1
15th International Postgraduate Course on Lysosomal Storage Disorders, Nierstein, Germany	2016	1.1
ZonMW GGG-congres 2018, Amsterdam, The Netherlands	2018	0.2

Presentations	**Year**
Elevator pitch: The NeuroFab Study: Two year follow-up of Cognitive and Psychological functioning in patients with Fabry Disease, AG&M PhD-Student Retreat, Garderen, The Netherlands	2016
Oral presentation: Neuropsychologic complications of Fabry disease, 15th International Postgraduate Course on Lysosomal Storage Disorders, Nierstein, Germany	2016
Oral presentation: Quality of life in patients with Fabry disease, Symposium EFWG meeting, Amsterdam, The Netherlands	2017
Oral presentation: Subjective cognitive complaints and symptoms of depression are highly prevalent in Fabry disease and are not related to objective cognitive impairment, ESN Najaarssymposium 2017, Utrecht, The Netherlands	2017

Poster presentation: Cognitive functioning in Fabry disease: relation with stroke, depression and disease phenotype, 14th annual WORLD Symposium, San Diego, United States	2018
Oral presentation: Cognition in Fabry disease, relations to cerebral imaging and depression, FIN Fabry Expert Meeting, Vilnius, Lithuania	2018
Poster presentation: Cognitive functioning in Fabry disease: relation with stroke, depression and disease phenotype, SSIEM annual symposium, Athens, Greece	2018
Oral presentation: Fabry disease: brain, cognition and depressive complaints, Lysosomal Storage diseases: Meet the experts, Amsterdam, The Netherlands	2018
Elevator pitch: Development and clinical consequences of white matter lesions in Fabry disease: a systematic review, ESN Najaarssymposium 2018, Utrecht, The Netherlands	2018
Poster presentations: (1) Progression Of White Matter Lesions And Cerebral Infarctions In Fabry Disease: Effect Of Patient Characteristics And Enzyme Replacement Therapy and (2) Coping, Health Perception And Pain Are Related To Depressive Symptoms In Fabry Disease, 6th Update on Fabry disease, Prague, Czech Republic	2019
Oral presentation: Coping, Health Perception And Pain Are Related To Depressive Symptoms In Fabry Disease, SSIEM annual symposium, Rotterdam, The Netherlands	2019

(Inter)national conferences	**Year**	**ECTS**
AG&M PhD-Student Retreat, Garderen, The Netherlands	2016	0.5
ESN Voorjaarssymposium 2016, Groningen, The Netherlands	2016	0.5
ESN Voorjaarssymposium 2017, Leuven, Belgium	2017	0.5
21st 2017 ESGLD Workshop, Lyon, France	2017	0.8
ESN Najaarssymposium 2017, Utrecht, The Netherlands	2017	0.3
14th annual WORLD Symposium, San Diego, United States	2018	1.5
ESN Voorjaarssymposium 2018, Valkenburg a/d Geul, The Netherlands	2018	0.5
SSIEM annual symposium, Athens, Greece	2018	0.8
ESN Najaarssymposium 2018, Utrecht, The Netherlands	2018	0.1
6th Update on Fabry disease, Prague, Czech Republic	2019	1.3
SSIEM annual symposium, Rotterdam, The Netherlands	2019	0.2

Other

Derde lustrum patientenvereniging FSIGN, Almere, The Netherlands	2016	0.1
Fabry patiëntendag 2017, Almere, The Netherlands	2017	0.2
Symposium EFWG meeting, Amsterdam, The Netherlands	2017	0.3
FIN Fabry Expert Meeting, Vilnius, Lithuania	2018	0.3

Lysosomal Storage diseases: Meet the experts, Amsterdam, The Netherlands	2018	0.3
Fabry patiëntendag 2019, Almere, The Netherlands	2019	0.1

Teaching

Lecturing	**Year**
Oral presentation: Cognitie en stemming bij patiënten met de ziekte van Fabry, Fabry patiëntendag 2017, Almere, The Netherlands	2017
Oral presentation: De ziekte van Fabry, Onderwijsmiddag vereniging voor genetisch consulenten, Utrecht, The Netherlands	2018
Oral presentation: Fabry: het brein, de stemming en het denken, Fabry patiëntendag 2019, Almere, The Netherlands	2019

Tutoring, Mentoring	**Year**	**ECTS**
Bachelor thesis: mentoring 3rd year medical student in the writing process. Together we rewrote the end product into a systematic review on white matter lesions in patients with Fabry disease	2016-17	2.5

Supervising

Masterthesis and data-acquisition: supervision of a masters student psychology, 6 months	2016-17	4.0
Masterthesis and data-acquisition: supervision of a masters student psychology, 6 months. Resulted in a research article on the Mini Mental State Examination in patients with Fabry disease	2018	4.0

Other

Organized evening on intercultural communication together with Jong AMC, Jong VuMc and ICA	2018	1.0

List of publications

Journal articles in this thesis

Körver S, Vergouwe M, Hollak CEM, et al. Development and clinical consequences of white matter lesions in Fabry disease: a systematic review. *Molecular genetics and metabolism* 2018; 125: 205-216. DOI: 10.1016/j.ymgme.2018.08.014.

Körver S, Longo MGF, Lima MR, et al. Determinants of cerebral radiological progression in Fabry disease. *Journal of Neurology, Neurosurgery & Psychiatry* 2020: jnnp-2019-322268. DOI: 10.1136/jnnp-2019-322268.

Arends M, Körver S, Hughes DA, et al. Phenotype, disease severity and pain are major determinants of quality of life in Fabry disease: results from a large multicenter cohort study. *Journal of inherited metabolic disease* 2018; 41: 141-149. DOI: 10.1007/s10545-017-0095-6.

Körver S, Geurtsen GJ, Hollak CEM, et al. Predictors of objective cognitive impairment and subjective cognitive complaints in patients with Fabry disease. *Scientific reports* 2019; 9: 188. DOI: 10.1038/s41598-018-37320-0.

Körver S, van de Schraaf SAJ, Geurtsen GJ, et al. The Mini Mental State Examination does not accurately screen for objective cognitive impairment in Fabry Disease. *JIMD Reports* 2019; 0. DOI: 10.1002/jmd2.12036.

Körver S, Geurtsen GJ, Hollak CEM, et al. Depressive symptoms in Fabry disease: the importance of coping, subjective health perception and pain. *Orphanet Journal of Rare Diseases* 2020; 15: 28. DOI: 10.1186/s13023-020-1307-y.

Körver S, Geurtsen GJ, Hollak CEM, et al. Cognitive functioning and depressive symptoms in Fabry disease: a follow-up study. *Journal of inherited metabolic disease* 2020 In press. DOI: 10.1002/jimd.12271.

Other publications

Schuller Y, Arends M, Korver S, et al. Adaptive pathway development for Fabry disease: a clinical approach. *Drug discovery today* 2018; 23: 1251-1257. DOI: 10.1016/j.drudis.2018.02.004.

Körver S, Feldt-Rasmussen U, Svarstad E, et al. Oral Chaperone Therapy Migalastat for the Treatment of Fabry Disease: Potentials and Pitfalls of Real-World Data. *Clinical Pharmacology & Therapeutics* 2019; 0. DOI: 10.1002/cpt.1536.

De Bie AJR, Körver S, Kersten E, et al. A paraplegic patient with fever and leucocytosis: not always what it seems. *Oncology in Clinical Practice* 2019; 15. DOI: 10.5603/OCP.2019.0005.

Miscellaneous

Column FSIGNAAL

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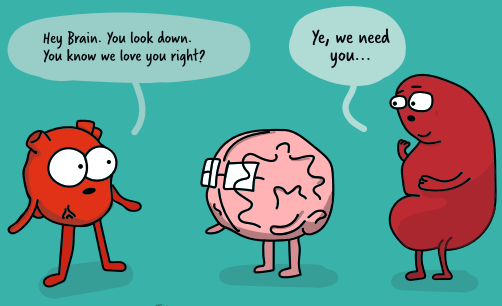
Maarten Dorr, het bovenstaande geldt natuurlijk ook voor jou. Maar een paranimf verdient wat extra aandacht. Afgelopen jaar hebben we elkaar vaak aan de lijn gehad op leuke momenten, maar ook als het minder ging. Er zijn weinig mensen die ik zo heb zien groeien in zowel hun visie op werk, als in persoonlijke sferen. Ik denk dat de bijnaam "kwakzalver" al lang niet meer aan de orde is. Ik verwacht nog veel van je te zien in de toekomst.

Lieve **papa en mama**, de appel valt niet ver van de boom: het vastbijten in een onderwerp en niet meer loslaten is jullie niet vreemd. Ik heb geluk dat ik in zo'n stimulerende omgeving ben opgegroeid en dat jullie me vanuit jullie beroepen verder

hebben laten kijken naar welzijn, thuissituatie, kwaliteit van leven en zingeving. Dit heeft mij gevormd tot de arts en onderzoeker die ik nu ben. Als ik iets meemaak staan jullie onder mijn sneltoets op mijn telefoon, jullie bieden altijd een luisterend oor als ik dat nodig heb. **Sarah**, (je)zus, bewust of onbewust heb ik altijd "jouw" pad gekozen (basisschool, middelbare school, atletiek, geneeskunde). Deels volg jij nu het mijne door ook te beginnen met onderzoek, al is dat niet volledig uit vrije wil. Je wordt hoe dan ook een uitstekende SEH-arts. **William**, halsoverkop naar Valkenswird verhuizen was vast niet altijd makkelijk. Desalniettemin is het mooi om je erbij te hebben tijdens familie-evenementen. Jouw hulp was onmisbaar bij de lay-out van het boekje!

Lieve **Christa**, tijdens mijn promotietraject zijn we van 5 jaar heen en weer reizen tussen verschillende steden uiteindelijk samen gaan wonen. Jij hebt dit hele traject van begin tot einde meegekregen en kreeg dag in dag uit werkverhalen te horen. Jij zorgt ervoor dat ik ontspanning zoek en me niet alleen mee laat slepen door de waan van de dag. We vullen elkaar denk ik steeds beter aan en dat maakt dat ik van je houd.

Als laatste wil ik alle patiënten bedanken die tijd vrij hebben gemaakt om mee te doen aan mijn onderzoek. Ook verdient (het bestuur van) de patiëntenvereniging, de FSIGN, hier een plek. Het belang van lotgenotencontact is groot en de stem van meerdere patiënten samen klinkt harder dan van één patiënt alleen.



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