Cerebrovascular Involvement in Fabry Disease
Current Status of Knowledge

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Fabry disease (FD) is a rare and highly debilitating lysosomal storage disorder that results from a total lack of, or deficiency in, the enzyme α-galactosidase A (α-Gal A) because of mutations in the GLA gene.1 FD is inherited as an X-linked trait; many of the male patients develop a classic severe phenotype with early onset of symptoms, whereas heterozygous females exhibit phenotypes ranging from asymptomatic to major involvement of vital organs.2 Most families inherit private mutations; to date, >600 mutations have been identified and are listed in the online FD database (Fabry-database.org).3 The deficiency in α-Gal A causes the accumulation of globotriaosylceramide (GL-3; also abbreviated Gb3) in various cellular compartments, particularly lysosomes, causing structural damage and cellular dysfunction, as well as triggering secondary, tissue-level responses, such as inflammation, ischemia, hypertrophy, and the development of fibrosis resulting in progressive organ dysfunction.4 Deacetylated globotriaosylceramide (lyso-globotriaosylceramide [lyso-GL-3]) has also been shown to be present in increased concentrations in the plasma of patients with FD. It has been suggested that lyso-GL-3 promotes GL-3 accumulation, induces proliferation of smooth muscle cells in vitro, and may have deleterious effects on the intima and media of small arterioles.5 Many cell types are involved in FD pathology, including vascular cells (endothelial and smooth muscle cells), cardiac cells (cardiomyocytes and valvular cells), a variety of renal cells (tubular and glomerular cells, and podocytes), and nerve cells.2

The underlying pathophysiological mechanisms of Fabry disease (FD) are complex and incompletely understood.6 Early pathophysiological changes are thought to predominantly involve the microvasculature.7 As age increases, arterial remodeling and intima-media thickening in medium-to-large caliber vessels occur.2 The first clinical symptoms of FD occur in childhood (eg, neuropathic pain, hypohidrosis, and gastrointestinal problems)8 and are primarily because of autonomic neuropathy.9 As the disease pathology progresses, organ damage occurs and patients are at risk of developing life-threatening complications mainly because of damage to the kidneys, heart, and the brain. Cerebrovascular complications caused by cerebral vasculopathy are a major cause of morbidity and early mortality in both male and female patients with FD.10

This review is based on a roundtable discussion that was held at an International Expert Panel on Cerebrovascular Involvement in FD, in Boston, MA, in January 2013. The panel comprised 9 experts in cerebrovascular complications in FD and clinical neuroimaging from Europe, South America, Australia, and the United States. Relevant literature published during the development of this review was also considered. This review provides a synopsis of the state of knowledge on the clinical and neuroradiological aspects, as well as the neurophysiology of cerebrovascular involvement in Fabry patients. It aims to identify developments that could lead to improved prediction and monitoring of cerebrovascular events in patients with FD. Of note, the expert panel also discussed therapeutic aspects (enzyme replacement therapy: agalsidase β [Fabrazyme; Genzyme, a Sanofi company] and agalsidase α [Replagal; Shire Human Genetic Therapies], conventional treatment) and intends to describe these separately.

Cerebral Manifestations of FD

Although ischemic strokes and transient ischemic attacks are the most prevalent types of overt cerebrovascular events in FD,10 cases of intracerebral hemorrhages,11 subarachnoid hemorrhage,12 microbleeds,13 cerebral venous thrombosis,14 and cervical carotid dissection15 have also been reported. To our knowledge, no cases of vertebral dissection or spinal cord infarction have been documented in the literature to date. Although silent infarcts are common events, also

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among young patients with stroke, there are no reports on the frequency of silent brain infarcts in FD. Aseptic meningitis can occur concomitantly in Fabry patients who have had cerebrovascular complications. One case of prolonged transient global amnesia has been reported in a Fabry patient. Dementia, cognitive impairment, and depression occur in patients with FD although additional studies are needed to establish a direct link to FD.

**Stroke**

**Prevalence, Age at Onset, and Influence of Sex**

Retrospective studies in small cohorts of Fabry patients have reported a wide range of stroke incidence (24%–48%). In patients with Fabry-related stroke, the neurological symptoms experienced are normally consistent with the vascular territory affected and the extent of the stroke. Although many patients have clear neuroradiological findings on MRI, the causes of clinically asymptomatic brain lesions and chronic white matter hyperintensities (CWMH) in Fabry patients remain controversial.

An analysis of a large cohort of 2446 patients in the Fabry Registry (Fabryregistry.com) reported that stroke occurs in 6.9% of men and 4.3% of women. Of these, 87% of first strokes were found to be ischemic, with hemorrhagic stroke reported in 13% of cases. Although the incidence of stroke increases with age in patients with FD, as in the general population, analysis of the Fabry Registry has indicated that the incidence of stroke among patients with FD is markedly higher than that observed in the general US population across all age categories. For example, in men aged 35 to 45 years, the relative risk of stroke is 12.2× higher in Fabry patients compared with healthy subjects. The relative risk of stroke is lower in women when compared with that in men, but still higher in Fabry patients when compared with that in healthy subjects: risk is highest at 4.2 in the 35 to 45 year age category. In the Fabry Registry cohort, a majority of Fabry patients experienced a first stroke between the age of 20 and 50 years, with 22% of patients having a first stroke at <30 years. For the majority of patients (>70%), stroke was the first serious FD complication and a high proportion (50% of men and 38% of women) had, therefore, not yet been diagnosed with FD. Another large-scale analysis of the Fabry Outcome Survey reported that 13.2% (15.1% men and 11.5% women) of a cohort of 688 Fabry patients had either a stroke or transient ischemic attack. In the Fabry Outcome Survey, the mean age at first stroke was <30 years (men) and <45 years (women). Small studies reported that the median age at first stroke ranged from 28 to 54 years.

Data from the Fabry Registry reported that women with FD were more likely to experience a stroke than men with FD although female patients were likely to be older at first stroke. However, hemorrhagic stroke has also been reported to be more common among men than women with FD (16.9% versus 6.9%, respectively). The observed differences in cerebrovascular involvement among male and female Fabry patients are difficult to explain. It seems that, physiologically, hemizygous male Fabry patients are unable to compensate for the physiological effect of the GL-3 accumulation, whereas heterozygous female Fabry patients may be able to compensate for the deleterious effects of the GL-3 accumulation, at least to some extent, resulting in a later onset of cerebrovascular complications.

Data from a recent in vivo study did not support the hypothesis that the number of X chromosomes could affect the degree of cerebral ischemia. Differences in X-chromosome gene expression between men and women with ischemic stroke have been reported previously. Moreover, α-Gal A showed sex-specific gene upregulation at 5 hours.
after an ischemic stroke for women and at 24 hours for men, in comparison with control subjects.29

The severity of FD manifestations in heterozygous female patients is complex because of the process of X chromosome inactivation (XCI). In female mammals, this process leads to a selective, irreversible inactivation of the maternal or paternal X chromosome in somatic cells during early embryogenesis. The pattern of XCI is clonally inherited; therefore, human females are mosaics of cell populations with different patterns of XCI and consequently, in female heterozygous Fabry patients, a mosaic of cells with different GLA expression and α-Gal A activity levels.30 Both random and skewed patterns of XCI have been reported in female heterozygous Fabry patients.31 Patients with random XCI patterns exhibited age-dependent worsening of the clinical severity of their condition.31 In contrast, heterozygous females with skewed XCI leading to the predominant expression of the wild-type GLA gene experienced an attenuated clinical phenotype, and disease progression was no longer correlated with age. These data suggest that even if the incidence of acute events, such as ischemic stroke, may not be directly linked to sex, XCI could be a general prognostic factor for FD in heterozygous female patients.31

Prevalence of FD in Patients With Ischemic Stroke

The cause of ischemic stroke remains undetermined in a significant proportion of younger patients. Previous hypotheses about FD prevalence in the cryptogenic (undetermined) young stroke cohort32 could not be supported by several subsequent studies (Table 1).14,15,32–38 Therefore, a regular FD screening tool remains out of reach. Three screening studies did not identify any patients with FD,33,35,36 but others have reported FD in 0.5% to 3.9% of the stroke population.14,15,32–38 This variation in reported prevalence may be because of differences in the studied stroke populations (age and sex ratios), the stroke types studied, and the diagnosis of FD. Some studies investigated ischemic stroke, whereas others included hemorrhagic stroke, which may have underestimated the prevalence of FD.30,40 In addition, the screening methods used to test for the presence of FD were inconsistent across studies.

Although analyses of dried blood spots and whole blood samples are practical for screening for enzyme activity in male patients with FD,41,42 all suspected cases of FD must be confirmed using the gold standard mutation analysis.1 Assessment of other biomarkers, such as GL-3 and lyso-GL-3 levels in blood, and GL-3 levels in urine, can be useful in suggesting a diagnosis of FD, particularly in female patients with FD who show partial enzyme activity.38 Despite this, screening for FD using urinary GL-3 levels is not recommended because these markers have been shown to be increased in patients who do not have FD but have common forms of heart disease, such as coronary artery disease or valvular dysfunction.35 An FD screening study of patients with stroke reported biologically significant Fabry mutations in 21 male (4.9%) and 7 (2.4%) female patients with stroke,32 but did not publish details on the type of mutations identified. This study could, therefore, have included intronic mutations and other mutations of undetermined clinical significance.

In FD screening studies, the screening population must be well defined, and a thorough family history remains crucial. It is clear that the prevalence of FD in young patients with stroke is low, notwithstanding the variations in FD incidence among young patients with stroke. Nevertheless, in case of cryptogenic strokes in younger patients, a clinical and biochemical workup for FD is appropriate.

Genotype–Phenotype Correlations

More than 600 different mutations are known in the GLA gene coding for α-Gal A.3 Mutations are not limited to active site residues, but include those predicting changes related to stability, and indirectly, to catalytic activity.44,45 Most mutations are family specific, but few occur with sufficient frequency to permit genotype–phenotype correlation.46 Even within families, phenotypic heterogeneity is often present, suggesting the possibility of gene–environment interaction. In a study of 210 male and female Fabry patients, Froissart et al37 identified 55 different mutations in 65 families, with only 8 mutations occurring in >1 family and 2 mutations in >2 families. The study also found an occurrence of cerebrovascular disease in a patient with R112H, a mutation that has been found to be

### Table 1. Incidence of Fabry Disease Among Younger Patients With Stroke in the General Population

<table>
<thead>
<tr>
<th>Trial</th>
<th>Patients Screened, n</th>
<th>Patients With Fabry Disease, n (%)</th>
<th>Age Range, Y</th>
<th>Mean Age at First Stroke, Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rolfs et al32</td>
<td>721</td>
<td>Men 21 (4.9)  Women 7 (2.4)</td>
<td>18–55</td>
<td>38.4 (men); 40.3 (women)</td>
</tr>
<tr>
<td>Brouns et al33</td>
<td>103</td>
<td>0 0</td>
<td>16–60</td>
<td>51.3</td>
</tr>
<tr>
<td>Wozniak et al34</td>
<td>154</td>
<td>1* (0.6)</td>
<td>15–49</td>
<td>Not stated</td>
</tr>
<tr>
<td>Brouns et al33</td>
<td>1000</td>
<td>2 (0.4)  3 (0.7)</td>
<td>18–60</td>
<td>48.2</td>
</tr>
<tr>
<td>Rolfs et al34</td>
<td>5023</td>
<td>11 (0.4)  16 (0.8)</td>
<td>18–55</td>
<td>Not stated</td>
</tr>
<tr>
<td>Baptista et al34</td>
<td>493</td>
<td>7 (2.3)  5 (2.6)</td>
<td>18–55</td>
<td>45.4</td>
</tr>
<tr>
<td>Marquardt et al35</td>
<td>1046</td>
<td>0 0</td>
<td>24–103</td>
<td>...</td>
</tr>
<tr>
<td>Sankaya et al35</td>
<td>150</td>
<td>0 0</td>
<td>18–55</td>
<td>43</td>
</tr>
<tr>
<td>Dubuc et al37</td>
<td>100</td>
<td>1 (1.0)</td>
<td>16–55</td>
<td>40.5</td>
</tr>
</tbody>
</table>

*All patients in this study were men.
associated with myocardial hypertrophy. Therefore, a clear genotype–phenotype correlation in FD has not yet been established, underlining the need for additional studies.

Several surveys have revealed a small percentage of young patients with stroke and a D313Y alteration in the GLA gene (Gly937Ala alteration at cDNA level)\(^ {14,15,48}\). Although these individuals were presumed to have FD, there was no evidence of other FD manifestations. It has been suggested that the D313Y variant, which results in normal α-Gal A activity in leukocytes and severely reduced α-Gal A activity in plasma, is a pseudodeficiency polymorphism. The identification of D313Y in 0.45% of normal X chromosomes further supports this theory.\(^ {49}\) Nevertheless, an association between the D313Y variant and CWMH has been shown in the absence of other FD-specific symptoms, suggesting that D313Y carriers may be more susceptible to neural tissue damage.\(^ {44}\) Nevertheless, the pathogenic role of the D313Y GLA variant continues to be debated.\(^ {50,51}\) Recently, Rolfs et al\(^ {48}\) also hypothesized that the mutations S126G and A143T, previously reported by Brouns et al,\(^ {15}\) are associated with a stroke-only phenotype in FD.

The question of whether there is a cerebrovascular variant of FD can be approached by classifying mutations by their effect on residual enzyme activity.\(^ {52}\) Lukas et al\(^ {52}\) divided GLA mutations into 4 classes according to in vitro enzyme activity measurement. In those with severe mutation and classic disease, mutation-specific enzyme activity correlated well with elevation in lyso-GL-3, albeit less so in heterozygous female patients. Patients with the most stable estimated glomerular filtration rate had fewer strokes than those Fabry patients with rapidly progressing renal disease.\(^ {64}\) An analysis of 2500 patients in the Stroke In Young Fabry Patients (SIFAP) study\(^ {65}\) demonstrated that lower estimated glomerular filtration rates within the normal range were associated with the presence of a moderate to severe CWMH load. In this study, increasing severity of CWMH was significantly associated with a lower estimated glomerular filtration rate,\(^ {65}\) raising the possibility that reduced estimated glomerular filtration rates could indicate an increased risk of CWMH and early stroke in patients with FD. Evidence is also emerging of an age-independent association between CWMH load and cardiomypathy in patients with FD,\(^ {66}\) which may relate to the risk of ischemic stroke.

Other possible influences on stroke severity in FD include polymorphisms in interleukin-6, endothelial nitric oxide synthase, and protein Z\(^ {57}\); fibrinolysis and angiogenesis factors\(^ {60,60}\); elevations in serum myeloperoxidase\(^ {56}\) and C-reactive protein\(^ {57}\); paraoxonase gene polymorphism\(^ {58}\); and angiotensinogen promoter and angiotensinogen receptor type I.\(^ {59}\) The risk of stroke in FD seems, therefore, to be related to residual enzyme activity as determined by GLA mutations, as well as other genetic and epigenetic factors not yet characterized fully. Careful follow-up studies for clinical manifestations of FD in young patients with cryptogenic stroke and sequence alterations in the GLA gene are needed to elucidate these factors further.

**Risk Factors of Ischemic Stroke in FD**

Several modifiable, lifestyle-related factors are likely to increase the risk of stroke in Fabry patients, as in the general population. These factors include smoking, obesity, lack of physical exercise, dyslipidemia, and arterial hypertension.\(^ {53}\) However, genetic modifiers, such as paraoxonase gene polymorphisms,\(^ {54}\) angiotensin promoter, and angiotensin II receptor type I gene polymorphism, interleukin-6, protein Z,\(^ {55}\) inflammatory factors (myeloperoxidase, C-reactive protein),\(^ {56,57}\) and hyperhomocysteinemia,\(^ {58,59}\) could also predispose a young Fabry patient to stroke. It has been suggested that the presence of factor V Leiden may be a link between FD and stroke.\(^ {60,61}\) However, caution must be exercised in drawing strong conclusions from case reports because the factor V Leiden mutation is also common in the general community.\(^ {52}\) Further evidence is needed to clarify the role of factor V Leiden, the above-mentioned genetic modifiers, and serum molecules in the genesis of stroke in patients with FD.

Fabry-related cardiac and renal disease may also affect the risk of cerebrovascular complications. The vasoregulation of the microvasculature of the brain shares similar hemodynamic properties with the kidney, and the 2 organs have common vascular risk factors, such as hypertension and diabetes mellitus. Accordingly, researchers have looked for associations between cerebrovascular involvement (determined as severity of CWMH load) and kidney function, as well as possible links to stroke risk.\(^ {62}\) A recent study reported that Fabry patients with the most stable estimated glomerular filtration rate were less likely to have a stroke compared to those with a rapidly progressing renal disease.\(^ {44}\) The studies for clinical manifestations of FD in young patients with cryptogenic stroke and sequence alterations in the GLA gene are needed to elucidate these factors further.

**Cerebrovascular Involvement in Fabry Disease**

Cerebrovascular manifestations in patients with FD can be classified as either large- or small-vessel disease. Large-vessel stroke occurs because of occlusion of the large intracranial vessels or because of cardiac embolism (from the heart or large-vessel disease).\(^ {68}\) Small-vessel disease is more commonly seen in patients with FD, manifesting as either subcortical stroke or the frequently asymptomatic CWMH and subcortical infarcts seen in neuroimaging studies.\(^ {60}\)

In patients with FD, stroke occurs in both the anterior and the posterior circulatory systems, as well as in cortical and subcortical locations. However, the mechanism and topography of stroke in FD have not been systematically studied because of the fact that the evaluation of FD has been focused on patients with cryptogenic stroke rather than all types of stroke. Because patients with FD can have large artery disease and arrhythmia as a result of cardiomyopathy, the observed pattern of infarcts in descriptive studies may not reflect the true topography of infarcts in FD.\(^ {60,60}\)

A significantly enlarged basilar artery diameter has been reported in patients with FD (compared with the general population),\(^ {70}\) the cause of which is postulated to be insufficient autoregulation leading to aberrant vascular remodeling. Importantly, the basilar artery diameter seems to be a promising radiological parameter for separating patients with FD from controls and may be a useful predictive tool for Fabry-related stroke. Recently, the basilar artery diameter was confirmed to be significantly increased in male patients with FD when compared with healthy controls.\(^ {71}\) Furthermore, a recent study of 70 Fabry patients suggested that vertebrobasilar doli-chocectasia could serve as an early marker of neurovascular involvement, as it was present in 56% of men and 35% of
women and was apparent at a younger age when compared with signs of ischemia. Tortuous, dilated cerebral vessels are also found in FD, and similar anatomic findings are also present in ocular vessels of Fabry patients. However, largely overlapping diameter ranges strongly reduce the diagnostic value of this measure in routine clinical practice. Moreover, no difference was found between male patients with FD and patients with general stroke, or among female subgroups.

**Chronic White Matter Hyperintensities**

Single, multiple, or confluent hyperintensities on T2-weighted images of white matter were considered characteristic neuroradiological signs of FD (Figure 2). Microvascular degeneration because of GL-3-related endothelial damage can lead to injury and appears as abnormal hyperintensities in the white matter on T2-weighted or fluid attenuated inversion recovery images. It should be noted that the CWMH seen in MRI studies of patients with FD may be distinct from, and not related to, white matter stroke. CWMH, especially when there is a low burden of lesions, are not usually associated with any neurological abnormalities and may be distinguished from stroke lesions according to the sequences of the MRI (T1 and T2).

**Figure 2.** A brain MRI (A–C) and MR angiography (D) of a 53-year-old male Fabry patient. Axial fluid attenuated inversion recovery images showing a left acute temporo-parietal-occipital ischemic stroke and several lacunar strokes (A); white matter hyperintensities are present bilaterally (B). An axial T1 image (C) disclosing a bilateral pulvinar hyperintensity (pulvinar sign), MR angiography (D): although the vessel diameter is relatively increased (the basilar artery is usually smaller than the carotid arteries), this patient does not present an increased tortuosity of the basilar artery. Adapted from Burlina et al with permission of the publisher. Copyright ©2015, Oxford University Press. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

CWMH occur in the subcortical, deep, and periventricular white matter, usually in a symmetrical manner, and have been shown to increase in number with age progressively. In a longitudinal MRI study of 50 patients with FD (mean age, 33 years), only 32% of patients did not demonstrate CWMH; strikingly, by the age of 54 years, all patients had CWMH. In children with FD, lesions, if present, are mostly minimal. CWMH load has been found to be similar in male and female Fabry patients, and the regional distribution of CWMH in patients with FD is comparable with that seen in normal aging and in hypertensive patients, particularly in the periventricular area. Furthermore, the load of white matter lesions may mimic the appearance of multiple sclerosis; therefore, a careful differential diagnosis should be performed.

Highly sensitive quantitative imaging methods, such as diffusion tensor imaging (DTI)-MRI, may be useful for monitoring the progression of CWMH load during the course of FD (see Neuroimaging Techniques section of this article).

A higher CWMH load may be an indicator of progressive cerebrovascular disease in Fabry patients. Although the majority of ischemic strokes involve both white and gray matter, 25% of strokes affect the white matter. The functional impairments seen in patients with ischemic stroke are most often because of the degree and extent of white matter involvement. Other risk factors for CWMH in the general population include hypertension, small-vessel disease, periventricular leukomalacia, neurometabolic disorders (phenylketonuria, leukodystrophies, mitochondrial disorders), demyelinating disorders, and aging. It is likely that similar risk factors are associated with CWMH in patients with FD. Arteriolar tortuosity, which appears at the age of 50 years in the general population, causes white matter perfusion deficits. Other factors that contribute to the chronic hypoperfusion of white matter include impaired interstitial fluid circulation and vasogenic periventricular edema.

**Pulvinar Sign**

Little has been published on the role of the pulvinar in humans. Interestingly, the pulvinar neurons of the primates have been identified as a unique substrate for rapid detection of threatening visual stimuli. Bilateral hyperintensity on T1-weighted images of the thalamic posterior area (pulvinar sign) was first observed by 2 separate groups in 2003. Unilateral involvement of the pulvinar has also been described. The pulvinar sign may be caused by subtle dystrophic calcifications and end-organ damage associated with regional hyperperfusion although the pathophysiology is not well understood. Although the pulvinar sign is characteristic of FD, it is not pathognomonic because it has also been detected in patients with central nervous system (CNS) infections, as a sequel of chemotherapy and radiotherapy, and in phakomatoses. The pulvinar sign is observed in ≤20% of male patients with FD. No reports had been documented in female patients before the study, in 2012, of 2 female Fabry patients with weak pulvinar signs. This suggested that the pulvinar sign is more typical for male patients with FD than for female patients with FD, possibly because of the effect of residual enzyme activity. No correlation between stroke and pulvinar sign has been reported although the pulvinar sign was
associated with cardiac signs (eg, hypertrophic cardiomyopathy) and kidney failure.88

Pathophysiology of Cerebral Involvement in FD
Cerebrovascular events in FD can be attributed to vasculopathy characterized by abnormalities in blood vessel walls, altered blood components, and altered blood flow.68 Alterations in the diameter of cerebral blood vessels develop because of endothelial proliferation, stenosis, or obstruction leading to impaired cerebral glucose metabolism and ischemia. As a result, increased platelet reactivity,90,91 altered cerebrovascular reactivity because of autonomic dysfunction, and cardiogenic embolism may occur.8 Although there is evidence supporting a pathogenic role of GL-3 accumulation in vascular endothelial and smooth muscle cells,92 studies in Fabry patients are few and the underlying pathophysiological mechanisms are complex and incompletely understood.

Lysosomal accumulations of GL-3 have been shown to induce oxidative stress and promote the formation of reactive oxygen species,93 causing sustained dilation of the cerebral vessels, which increases the vulnerability of cerebral vasculature, and promoting the development of endothelial dysfunction.94 However, oxidative stress is also increased by plasma from Fabry patients, in the absence of GL-3, suggesting a role for other circulating plasma factors (possibly inflammatory cytokines).93 The increased generation of reactive oxygen species also reduces the bioavailability of nitric oxide and causes endothelial dysfunction (through vascular autonomic dysfunction and altered vascular tone and reactivity), promoting atherosclerosis.68,94

Intracellular GL-3 deposition has also been shown to upregulate the expression of adhesion molecules in vascular endothelial cells.95 Other identified effects of GL-3 accumulation include dynamic changes in the composition of plasma membrane lipid microdomains, mediating endothelial dysfunction,95 and reduced K_{_Ca}3.1 channel expression, resulting in K_{_Ca}3.1 channel dysfunction in vascular endothelium.96

In FD, GL-3 accumulation also occurs in vascular smooth muscle cells and is thought to play a prominent role in development of early Fabry pathology.6 In vitro experiments have shown that vascular smooth muscle cell proliferation can be induced using plasma from Fabry patients97 and lyso-GL-3.5 Proliferation of these cells may also be triggered by circulating growth-promoting factors present in plasma from Fabry patients.97,98 Increased intima-media thickness occurs in patients with FD because of the combination of smooth muscle cell proliferation and vascular endothelial GL-3 storage, resulting in disturbances in the mechanical properties of vessel walls and decreased flow-mediated dilatation in the peripheral vasculature.99 An increased intima-media thickness has been demonstrated in large- and medium-sized arteries (eg, carotid artery, abdominal aorta, brachial artery, and radial artery) of patients with FD.99-101

In vivo models indicate that acute augmentation of blood flow increases wall shear stress in blood vessels and dilates affected arteries. This stimulates endothelial cells to translate the mechanical stimulus into biochemical signals via tyrosine kinase and integrin signaling.102 Dilation is associated with large increases in early growth response factor 1 levels in endothelial cells, and the expression of this transcription factor is closely linked to the expression of matrix metalloproteinases (MMPs),103 especially MMP-9.104 Additional evidence indicates that MMPs break down the elastic lamina, further aiding the migration of smooth muscle cells from the tunica intima to the stressed medial layer.105 MMP-9 levels have been shown to be significantly higher in Fabry patients than in controls, and a positive correlation has been shown between MMP-9 levels and the Mainz Severity Score Index.106 Negative correlations have also been found between MMP-9 and fractional shortening.106

Alterations in regional cerebral blood flow have been observed in Fabry patients. Hilz et al107 using transcranial Doppler sonography reported a reduction in cerebral blood flow velocity (CBFV) and found signs of impaired cerebral autoregulation and of increased resistance in small intracerebral vessels. A knockout mouse model of FD also demonstrated reduced cerebral blood flow with significant alterations in local glucose metabolism.108 However, other studies reported normal and increased CBFV, as measured by Doppler sonography,109,110 or cerebral regional hyperperfusion at rest with a posterior predominance, as measured using 15O-labeled water and positron emission tomography.111 The discrepancy in findings (normal CBFV, increased CBFV, and reduced CBFV) may be because of the heterogeneity of cerebrovascular pathology from different brain regions and at different stages of the disease.104 Hyper- and hypoperfusion can both lead to regional metabolic vulnerability (decreased cerebral glucose metabolism), whereas hyperperfusion is associated with an increase in interstitial pressure, fluid shifts, demyelination (gliosis, leukoaraiosis), and subsequently, CWMH.6,68

Other factors, which affect constant blood flow and organ perfusion in patients with FD, include abnormal autonomic cardiac and vascular modulation responses to instantaneously occurring cardiovascular challenges, such as orthostasis. Furthermore, small fiber neuropathy in Fabry patients adversely affects sympathetic modulation of skin blood flow,112 also leading to altered perfusion rates in limbs and peripheral circulation. Small fiber neuropathy might also influence vascular reactivity because of impaired sympathetic vasomotor function by abnormal innervation.112 In untreated Fabry patients, the dysfunction of afferent and efferent small nerve fibers also contributes to a subtle impairment of the baroreflex, as shown with both orthostatic, sympathetic,113 and metronomic deep-breathing induced parasympathetic challenge.114 The finding is particularly relevant because baroreflex dysfunction generally correlates with progression and poor prognosis in various diseases.112

Functional Parameters and Potential Biomarkers of Vascular Disease in FD
Potential Biomarkers
Stroke and transient ischemic attacks occur as highly invalidating and potentially life-threatening events in FD. Biomarkers are needed to facilitate early detection of patients at risk for CNS events and to monitor disease
progression and results of treatment. Potential biomarkers of cardiac and renal involvement have been described previously, but few have shown any correlation with cerebrovascular injury or dysfunction. For example, serum cystatin C seems to be an effective prognostic indicator of early renal dysfunction and heart failure, but its correlation correlates only weakly with CNS involvement, and only in men, in a comparison using the neurological component of the Mainz Severity Scoring Index.113 Biomarkers have also been discovered in urine using quantitative proteomics, but these have yet to be correlated with cerebrovascular manifestations. Interestingly, these include the lysosomal proteins prosaposin and GM2 activator protein.116 Other potential lysosomal protein markers, such lysosome-associated protein 1 and lysosome-associated protein 2, are found in phagocytes.117 Proteomic profiles of peripheral blood mononuclear cells from Fabry patients have shown upregulation of γ-enolase and galectin-1. γ-enolase increases significantly in cerebrovascular accidents and in brain trauma, and galectins are involved in the regulation of inflammation.118

GL-3 concentrations, although elevated in plasma and urine of male Fabry patients, may be normal in heterozygous female Fabry patients and do not correlate with clinical symptoms119 or serve as a predictor of disease progression or outcome after enzyme replacement therapy.120 With the discovery of high levels of lyso-GL-3 in plasma from patients with FD,5 it was anticipated that this deacetylated form of GL-3 might serve as a reliable biomarker of FD.119 A correlation between plasma lyso-GL-3 levels and the development of cerebrovascular white matter lesions in male patients has been shown, but no correlations with disease severity score have been found. In females, however, plasma lyso-GL-3 does correlate with total disease severity. It has been suggested that intima-media vessel wall thickness, which is known to correlate with risk for stroke, may be increased in FD because of the exposure to lyso-GL-3. However, plasma concentrations in female patients are only about a tenth of those seen in hemizygotes and may be normal in some women.119 Lyso-GL-3 levels are also much lower in asymptomatic and mildly affected patients, those with late-onset variants, patients with nonclassical mutations, and heterozygous female patients.46,121 Furthermore, although it seems to be a reliable marker of treatment efficacy in the early months of enzyme replacement therapy, lyso-GL-3 may not prove as sensitive in later stages.122 Six known analogs of lyso-GL-3 account for less than half of the plasma lyso-GL-3, but for >90% of the lyso-GL-3 in urine.123 Because of their potential pathological effects on the cerebrovascular system, additional study of lyso-GL-3 and its analogs is needed.

Measurements of 3-nitrotyrosine in biobanked plasma samples from male Fabry patients have shown a 6-fold increase over controls, suggesting lower nitric oxide (NO) availability because of significant decreases in endothelial NO synthase (eNOS) activity. Shu et al124 have reported decreased eNOS activity in primary cultures of aortic endothelial cells exposed to GL-3, proposing this as a mechanism for the vasculopathy seen in FD. Other candidates serving as potential markers of endothelial dysfunction include soluble vascular cell adhesion molecule-1, high-sensitivity C-reactive protein, tumor necrosis factor-α, and P-selectin.125 Segura et al125 also hypothesize that, because an increased endothelial inflammatory profile is found in FD, serum biomarkers would be better indicators than brain hemodynamic parameters to estimate stroke risk in patients with FD. In addition, it is important to consider the presence of specific polymorphisms in genes, such as angiotensin and angiotensinogen II receptor type 1, which serve as prognostic indicators of stroke in FD.55

Functional Parameters

Abnormalities in several measures of brain function and circulation have been shown to exist early in the course of FD while patients are asymptomatic. Of note, basilar artery diameter seems to be increased in Fabry patients,126 and basilar dolichoectasia in younger patients should be intensively evaluated by a neuroimaging expert as part of risk assessment for Fabry stroke. In addition, it has recently been shown that hippocampal atrophy is a surrogate marker correlating with early neuronal involvement in FD.127 Longitudinal studies are now required to determine whether this hippocampal degeneration will progress with advancing FD and be predictive of CNS involvement.

Hyperexcitability in the primary motor cortex has also been demonstrated in Fabry patients with no cerebrovascular symptoms,128 and functional transcranial Doppler has revealed vascular dysfunction and abnormalities in the posterior cortical circulation in asymptomatic Fabry patients.129 In addition, abnormal cerebral autoregulation may be predictive of future cerebrovascular events in patients with FD. Presymptomatic screening for these subtle indicators of cerebrovascular dysfunction may allow early identification of patients at risk of stroke.

Neuroimaging Techniques in Patients With FD

There are no specific techniques for detecting acute and chronic cerebrovascular lesions in patients with FD. Conventional neuroimaging methods used in the diagnosis of cerebrovascular disorders (ie, stroke and CWMH as risk factors for stroke, depression, and cognitive decline) are also used in the neurological investigation and imaging surveillance of patients with FD. Standard CNS imaging modalities include computed tomography (CT) and MRI (Table 2).13,88,126,130–132 Contrast media are usually not required. The MRI protocol should include: T1-weighted; fluid attenuated inversion recovery/T2-weighted; T2* susceptibility; and diffusion-weighted imaging sequences. T2*/susceptibility is sensitive for hemorrhage, and fluid attenuated inversion recovery/T2-weighted images are sensitive for the detection of CWMH burden and for identifying both lacunar and territorial stroke; T1-weighted images are sensitive to pulvinar signal changes (Figure 2).73 MR angiography and CT angiography are of value for vessel imaging, such as in cases of carotid artery dissection, intracranial vessel stenosis, or dolichoectasia. Unlike CT angiography and contrast-enhanced MR angiography, the use of time-of-flight
MR angiography avoids the risks associated with the administration of contrast media and is the preferred method for routine evaluation, especially in patients with end-stage renal disease.

In the authors’ experience, MRI is required only approximately every 3 years for patients with stable FD, but it is indicated in the event of clinical signs of a stroke. Only CT scan has an immediate indication. Currently, many asymptomatic Fabry patients are not being monitored regularly, but increasing awareness of the importance of silent infarcts and other stroke risk factors from the general stroke population should encourage neurologists to monitor Fabry patients for signs of early CNS involvement periodically, at least on a clinical basis, using appropriate imaging studies when indicated (Table 2).

Table 2. Neuroradiological Techniques for the Assessment of Central Nervous System Involvement in Patients With Fabry Disease

<table>
<thead>
<tr>
<th>Imaging Technique</th>
<th>Purpose</th>
<th>Advantages/Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recommended routine assessments</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2-weighted MRI/FLAIR sequences</td>
<td>To quantify chronic white matter hyperintensity load to assess stroke risk</td>
<td>...</td>
</tr>
<tr>
<td>To identify lacunar and territorial stroke</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2* MRI (gradient echo, susceptibility-weighted imaging)</td>
<td>To detect hemorrhage (macro-/microbleeds)</td>
<td>Can assess parenchymal lesion load, as an indication of stroke risk and disease progression</td>
</tr>
<tr>
<td>T1-weighted MRI</td>
<td>To detect pulvinar sign</td>
<td>Low sensitivity and relatively high specificity for FD</td>
</tr>
<tr>
<td>DWI</td>
<td>To assess stroke</td>
<td>No correlation with risk of stroke</td>
</tr>
<tr>
<td>CT/contrast-enhanced MR angiography</td>
<td>To assess vessel stenosis and vessel diameter</td>
<td>Can assess acute ischemic lesions, minor stroke, and TIAs</td>
</tr>
<tr>
<td>TOF MR angiography</td>
<td>To assess vessel diameter, particularly basilar artery diameter</td>
<td>No contrast agents used, preferred method for routine assessment</td>
</tr>
<tr>
<td><strong>Additional, nonroutine techniques</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proton MR spectroscopy</td>
<td>To assess metabolic state of tissues, such as ischemia (lactate peak) or presence of certain metabolites</td>
<td>Single voxel spectroscopy requires prior knowledge of where to place the region of interest Multivoxel spectroscopy possibly better but requires significant investment to extract quantitative data</td>
</tr>
<tr>
<td>DTI</td>
<td>Can be combined with a voxel-based approach to evaluate regional changes in white matter integrity</td>
<td>Able to detect early white matter injury</td>
</tr>
<tr>
<td>Volumetric analysis of white matter hyperintensity</td>
<td>To monitor progression of white matter hyperintensity load</td>
<td>Automated and semiautomated methods are available</td>
</tr>
<tr>
<td>3D T1 images</td>
<td>For hippocampal atrophy and voxel-based assessment (voxel-based morphometry, cortical thickness measurement)</td>
<td>...</td>
</tr>
<tr>
<td>Perfusion imaging (dynamic contrast perfusion-weighted MRI; ASL perfusion MRI; perfusion CT)</td>
<td>To measure and monitor regional blood flow</td>
<td>Provides indicators of the pathogenesis of signal abnormalities (white matter lesions, pulvinar sign) Exogenous agents required (except for ASL) Perfusion CT involves use of ionizing radiation Contrast media required for dynamic contrast perfusion-weighted MRI and perfusion CT</td>
</tr>
</tbody>
</table>

3D indicates 3-dimensional; ASL, arterial spin labeling; CT, computed tomography; DTI, diffusion tensor imaging; DWI, diffusion-weighted imaging; FD, Fabry disease; FLAIR, fluid attenuated inversion recovery; TIA, transient ischemic attack; and TOF, time-of-flight.

These techniques may have value in longitudinal studies of disease progression or treatment effects. Although perfusion imaging does not yet play a role in the routine evaluation of FD, it has some useful research benefits. Compared with the contrast-based MR and CT methods, arterial spin labeling perfusion has the advantage of using magnetically labeled tagged spins and does not require the use of an exogenous contrast agent. As such, arterial spin labeling can be used in the assessment of regional perfusion abnormality, which has been observed in the posterior circulation in the presymptomatic state. Examination of resting state functional MRI is being used to explore the network effect on blood oxygen level-dependent activity in patients with white matter disease, which may be of use in exploring early changes in FD.
Conclusions
Cerebrovascular complications are a major cause of early morbidity and mortality in patients with FD. Ischemic stroke and transient ischemic attacks are the most prevalent cerebrovascular events and occur at an earlier age than is usual in the general population. For many patients with FD, stroke is the first serious clinical manifestation of the disease and may be the event that leads to a diagnosis of FD. Although routine screening is not yet warranted in young patients with stroke, greater awareness of FD as a cause of early stroke is needed. Unfortunately, a cerebrovascular event may occur at any point, often without clinical warning signs, with potentially fatal consequences. Additional studies should, therefore, focus on the identification of biomarkers for FD or functional neurological parameters, measures that can be used to monitor disease progression and signs of neurological involvement in patients with FD. Furthermore, the evaluation of neurological imaging techniques should be more sensitive than the current MRI methods in clinical practice. A better understanding of the risk factors for stroke in patients with FD will lead to improved strategies for the prevention of cerebrovascular complications in FD and is an important area for further clinical study.

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References


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