Minireview

Vasculopathy in patients with Fabry disease: Current controversies and research directions

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\textbf{A B S T R A C T}

Fabry disease is an X-linked lysosomal storage disorder due to deficiency of the enzyme $\alpha$-galactosidase A. The principal clinical manifestations of Fabry disease consist of cardiovascular complications including cerebrovascular, renal and cardiac disease but the pathophysiology of this specific vasculopathy is unclear. With the development of targeted treatment for Fabry disease, i.e. enzyme replacement therapy, it has become apparent that the removal of stored glycosphingolipid from the endothelial cells does not prevent progression of vascular disease in many patients. The aim of this study is to review the current available literature on vascular function tests, imaging and pathology studies and propose a hypothesis on the evolution of arterial complications in Fabry disease. Clearly, although premature atherosclerosis is suggested to occur, most studies describe absence of characteristic plaque formation. Smooth muscle cell hypertrophy, is probably the earliest feature of a complex vasculopathy, as in females and atypical cardiac variants, who have residual enzyme activity, no endothelial storage of significance is found. Subsequently, processes occur as observed in neo intima formation however with formation of more fibrotic structures. In the presence of a hyperdynamic circulation in combination with a less compliant vascular wall, it is hypothesized that upregulation of local renin angiotensine systems may occur. Angiotensin II is known to increase adhesion molecules, cytokines and chemokines and exerts a pro-inflammatory effect on leucocytes, endothelial cells and vascular smooth muscle cells. This enhances release of pro-thrombotic factors and opposes actions mediated through angiotensin 2 (AT2) receptor, including the release of nitric oxide (NO). A combination of reduced vascular compliance and activation of pro-thrombotic factors can lead to vascular complications in Fabry disease.

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Introduction

Fabry disease is an X-linked disorder with a deficient expression of the key enzyme $\alpha$-galactosidase A \cite{1,2}. Patients with Fabry disease have a marked limited life expectancy which is due to extensive damage to cardiovascular tissues, such as the heart, brain and kidney \cite{3}. Although clinical features of the disease are mostly displayed in affected males, female carriers may suffer from Fabry related complications as well. The principal clinical manifestations in Fabry disease consist of artery associated complications (such as cerebral disease and nephropathy), but the pathophysiology of this specific vasculopathy is unclear. Several studies indicate that the specific vascular lesions that are present in Fabry disease occur as a result of vascular dysfunction with major components being endothelial dysfunction, alterations in cerebral perfusion and a pro-thrombotic phenotype \cite{4,5}. Possibly, other cardiovascular risk factors may contribute to enhanced athero-thrombogenesis and a worsening of arterial performance. Although some patients with Fabry disease may suffer from stroke by involvement of larger arteries, small-vessel disease causes cerebral complications and probably contributes to complications of the kidney and the heart \cite{6–8}. Controversy exists whether the storage in the endothelial cells and the pro-thrombotic state is the origin of arterial damage or whether smooth muscle cell proliferation in the arterial media layer is the initiating step in the cascade that leads to Fabry vasculopathy \cite{9,10}. In addition, small fiber neuropathy could influence vascular reactivity as well, by abnormal innervation or stimuli located in the adventitial layer of the arteries. Abnormal intima media thickness and vascular reactivity as well as smooth muscle cell proliferation and atherosclerosis have all been described in patients with different ages and degrees of organ damage. For
instance, advanced renal disease could markedly enhance atherosclerosis, which makes it difficult to delineate these different processes in arterial walls.

Treatment with enzyme replacement therapy (ERT, agalsidase alfa, Replagal, Shire or agalsidase beta, Fabrazyme, Genzyme) is available in developed countries for almost a decade \[11,12\]. Intra-venous supplementation of deficient cells with α-galactosidase A indeed results in removal of stored Gb3 in endothelial cells \[12\].

Currently, several hundreds of patients are being treated worldwide and it has gradually become clear that not all patients benefit from ERT. Indeed, Fabry patients with more advanced disease, specifically when renal function is impaired, had progression of disease despite ERT \[13–15\]. In particular the appearance of new white matter lesions has been reported to emerge despite treatment with enzyme replacement therapy \[16\]. Although ERT has shown to be capable of clearing the endothelium from stored glycosphingolipids, the more complex alterations in vascular function may not be influenced or may even progress despite therapeutic intervention. Improving our understanding of Fabry vasculopathy is needed to develop improved strategies for therapeutic intervention. Such knowledge could also support a decision whether additional treatment of cardiovascular risk factors, such as anti-platelet therapy and/or lipid lowering treatment (e.g. Statins) and/or inhibition of the renin angiotensin system (RAS), as anti-platelet therapy and/or lipid lowering treatment (e.g.

Methods

A literature search was conducted using the Medline and Embase database. The search was aimed to cover five main topics concerning Fabry disease and vasculopathy: Fabry disease and histopathology of the cardiovascular system, vascular dysfunction, thrombotic events, development of atherosclerosis and angiographic abnormalities. Search terms used included: Fabry disease, vasculopathy, vascular pathology, vascular function/dysfunction, autonomic function/dysfunction, vasomotor function/dysfunction, pro-thrombotic state, thrombosis, endothelial activation, atherosclerosis and angiography. The title and abstract were screened to select reports in which vascular aspects of Fabry disease were the primary focus of research. Reviews were only included when new data were presented. Of the initial 304 references, including 61 reviews, 94 focussed on vascular aspects. For 12 studies only an abstract was available or the report did not provide detailed data. A final number of 82 studies was included in the current review and the summaries of 55 articles are given in Tables 1–4.

Results

Histopathology of arteries in Fabry disease

Twenty four studies were identified in which the histology of arterial disease and/or the presence or absence of atherosclerosis in Fabry patients were described. Table 1 summarizes the findings in these studies. Nine studies on kidney biopsies, usually as part of a clinical study, with consistent findings of extensive storage of Gb3 in glomerular, tubular, vascular and interstitial cells and one

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Tissue</th>
<th>Remarks</th>
<th>Authors</th>
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<tbody>
<tr>
<td>Male, 50 years</td>
<td>Atheromatous stenosis of the superior mesenteric artery</td>
<td>PA post-mortem</td>
<td>Jardine et al. [26]</td>
</tr>
<tr>
<td>Male, 47 years</td>
<td>GB3 accumulation in vascular and nonvascular cells and organ systems, except for endothelial cells; atherosclerotic lesions in right and left coronary systems, aorta and basilar artery</td>
<td>After 2 years of ERT (PA post-mortem)</td>
<td>Schiffmann et al. [27]</td>
</tr>
<tr>
<td>Male, 65 years</td>
<td>Cholesterol emboli, atherosclerotic lesions in aorta and coronary arteries. GB3 in cardiomyocytes and kidney</td>
<td>Atypical Fabry disease, hypertension and diabetes (PA-post-mortem)</td>
<td>Shirai et al. [28]</td>
</tr>
<tr>
<td>4 Females, aged 32–50 years</td>
<td>GB3 storage in kidney, hypertrophy of smooth muscle cell layer</td>
<td>Atherosclerotic lesion in one patient with hypertension and hypercholesterolemia</td>
<td>Valbuena et al. [29]</td>
</tr>
<tr>
<td>Male, 54 years, cardiac variant, coronary stenosis</td>
<td>Arterial graft: GB3 accumulation in smooth muscle cells, patchily replaced by fibrous tissue, no endothelial storage</td>
<td>Occlusion of artery graft 1 year after surgery</td>
<td>Chimenti et al. [30]</td>
</tr>
<tr>
<td>Male, 38 years</td>
<td>Biopsy of aorta: no atherosclerosis, GB3 storage in all layers</td>
<td>Cardiac ischaemia and coronary artery stenosis</td>
<td>Fisher et al. [31]</td>
</tr>
<tr>
<td>Male, 47 years, renal insufficiency</td>
<td>Subarchnoidial arteries: medial thickening, adventitial fibrosis with lymphocytic infiltration, replacement of SMC by fibrosis in medium-sized vessels</td>
<td>PA post-mortem</td>
<td>Okseda et al. [32]</td>
</tr>
<tr>
<td>Male, 44 years</td>
<td>GB3 accumulation in small blood vessels, kidney, heart and nervous system</td>
<td>No atherosclerosis (PA post-mortem)</td>
<td>Takao et al. [33]</td>
</tr>
<tr>
<td>2 Males, one female, 44–75 years</td>
<td>Megadolichobasilar artery, dilatation of basilar artery</td>
<td>Thrombosis in 2 cases (PA post-mortem)</td>
<td>Garzuly et al. [34]</td>
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<tr>
<td>3 Males, 1 female, 44–64 years</td>
<td>GB3 accumulation in the endothelium and smooth muscle cells, thickened fibrous intima, thickened extracellular matrix layer and mediocalcinosis, no signs of atherosclerosis</td>
<td>First case: Fabry disease and diabetes (PA-post-mortem)</td>
<td>Desnick et al. [36]</td>
</tr>
<tr>
<td>2 Males, 47–50 years</td>
<td>GB3 accumulation in the endothelium and smooth muscle cells</td>
<td>Thrombotic events in all (PA post-mortem)</td>
<td>Schatzki et al. [37]</td>
</tr>
<tr>
<td>2 Males, 28–50 years</td>
<td>Vascularization and thickening of the smooth muscle</td>
<td>Mitral valve insufficiency (PA post-mortem)</td>
<td>Elleeder et al. [35]</td>
</tr>
<tr>
<td>7 Males, with cardiac variant, aged 63–83 years</td>
<td>Heart: GB3 accumulation in cardiomyocytes, no storage in endothelial cells, or other tissues</td>
<td>Normal angiography (PA post-mortem)</td>
<td>Takenaka et al. [38]</td>
</tr>
<tr>
<td>Female, 63 years</td>
<td>GB3 accumulation in cardiomyocytes, smooth muscle cells, renal cells; in endothelial cells at the borderline of detectibility</td>
<td>Hypertension, ischemia (PA post-mortem)</td>
<td>Hulikova et al. [39]</td>
</tr>
</tbody>
</table>

PA, pathology.
### Table 2
Vascular imaging and reactivity.

<table>
<thead>
<tr>
<th>Design study</th>
<th>Number of included subjects (case:control)</th>
<th>Gender (M/F)</th>
<th>Age (years) mean ± SD or range</th>
<th>Vessel type</th>
<th>Matched for</th>
<th>Outcome</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravascular ultrasound</td>
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<tr>
<td>Case control</td>
<td>9:10</td>
<td>4M/5F:5M/5F</td>
<td>50.4 ± 5.7; 60.0 ± 8.7</td>
<td>Coronary arteries</td>
<td>Type of lesion</td>
<td>More diffuse plaques than in controls</td>
<td>Kovarnik et al. [45]</td>
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<tr>
<td>IMT</td>
<td></td>
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<tr>
<td>Case control</td>
<td>53:120</td>
<td>24/29F vs 83M + 37F</td>
<td>45.0 ± 1.7; 55.0 ± 2.2; 52.7 ± 1.7; 49.5 ± 2.7</td>
<td>Common carotid artery</td>
<td>Age</td>
<td>Common carotid IMT increased, no atherosclerotic plaques</td>
<td>Barbey et al. [44]</td>
</tr>
<tr>
<td>Case control</td>
<td>68:324</td>
<td>30M + 38F vs 208M + 116F</td>
<td>40.7 ± 10.9; 44.7 ± 2.8; 50.7 ± 7.2; 49.8 ± 8.3</td>
<td>Common carotid artery</td>
<td>Age, cardiovascular risk factors</td>
<td>Common carotid IMT increased, correlation with LVmass</td>
<td>Barbey et al. [10]</td>
</tr>
<tr>
<td>Blood flow measurements: FMD</td>
<td></td>
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<tr>
<td>Case control</td>
<td>17:34</td>
<td>7M/10F vs 16M/18F</td>
<td>38 ± 14.42 ± 8</td>
<td>Brachial, carotid and aortic artery</td>
<td>Age, sex, smoking</td>
<td>Brachial, carotid and aortic IMT increased</td>
<td>Kalliokoski et al. [52]</td>
</tr>
<tr>
<td>Case-control</td>
<td>21:21</td>
<td>M</td>
<td>31 ± 13:(not reported)</td>
<td>Radial artery</td>
<td>Age, sex</td>
<td>Radial artery IMT increased</td>
<td>Boutouyrie et al. [53]</td>
</tr>
<tr>
<td>Case control</td>
<td>7:8</td>
<td>M</td>
<td>22–42:23–46</td>
<td>Radial artery</td>
<td>Age</td>
<td>No difference in internal or external arterial wall thickness</td>
<td>Moore et al. [56]</td>
</tr>
<tr>
<td>Blood flow measurements</td>
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<tr>
<td>Case controls</td>
<td>15:30</td>
<td>9M/6F:30M/0F</td>
<td>35 ± 12.35 ± 3</td>
<td>Brachial artery</td>
<td>Age, BMI</td>
<td>FMD brachial artery impaired/lower</td>
<td>Kalliokoski et al. [57]</td>
</tr>
<tr>
<td>Blood flow measurements</td>
<td></td>
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<tr>
<td>Case control</td>
<td>6:12</td>
<td>4M/2F:8M/4F</td>
<td>37:37</td>
<td>FMD after reactive hyperemia</td>
<td>Age, sex</td>
<td>Brachial artery FMD in rest and after hyperaemia: no difference Higher in controls compared to patients</td>
<td>Puccio et al. [58]</td>
</tr>
<tr>
<td>Case control</td>
<td>14:15</td>
<td>14M/15M + F</td>
<td>28.8 ± 9.1; 30.2 ± 8.4</td>
<td>Myocardial blood flow by PET scan and H2(15)O Plethysmography and laser Doppler flowmetry</td>
<td>Not reported (age)</td>
<td>Myocardial perfusion reserve decreased Forearm perfusion lower After ischaemia less hyper-perfusion, skin perfusion increased</td>
<td>Kalliokoski et al. [57]</td>
</tr>
<tr>
<td>Case control</td>
<td>8:10</td>
<td>2M/6F:10M/F</td>
<td>35.8 (9–46):36.2 (24–54)</td>
<td>Pneumoplethysmography and thermal probes</td>
<td>Not reported (age)</td>
<td>Forearm vascular resistance increased forearm venous capacitance lower/less finger and toe blood flow, finger and toe pulse volume</td>
<td>Seino et al. [60]</td>
</tr>
<tr>
<td>Case</td>
<td>1</td>
<td>F</td>
<td>48</td>
<td>Digital subtraction angiography</td>
<td>Matched for</td>
<td>FBF increased after substitution of Ach and L-NMMA</td>
<td>Martin et al. [61]</td>
</tr>
<tr>
<td>Case report</td>
<td>1 Case</td>
<td>M</td>
<td></td>
<td>Coronary flow reserve</td>
<td>Reduced coronary flow reserve</td>
<td>Reduced coronary flow reserve</td>
<td>Dimitrow et al. [62]</td>
</tr>
<tr>
<td>Case control</td>
<td>17:13</td>
<td>M</td>
<td>31 (21–49):34 (21–48)</td>
<td>Venous plethysmography, Forearm blood flow</td>
<td>Age, sex</td>
<td>FBF increased after substitution of Ach and L-NMMA</td>
<td>Altarescu et al. [63]</td>
</tr>
<tr>
<td>Case control</td>
<td>22:24</td>
<td>M</td>
<td>28.6 ± 8.3:28.6 ± 5</td>
<td>Transcranial Doppler ultrasound (TCD), middle cerebral artery</td>
<td>Age, sex</td>
<td>CBF decreased</td>
<td>Hilz et al. [66]</td>
</tr>
<tr>
<td>Case control</td>
<td>63:31</td>
<td>M</td>
<td>19–56:26–49</td>
<td>Transcranial Doppler ultrasound PET-scan</td>
<td>Age</td>
<td>rCBF increased</td>
<td>Moore et al. [67]</td>
</tr>
<tr>
<td>Case control</td>
<td>26:10</td>
<td>M:M/F</td>
<td>33.7 ± 8.1:33.4 ± 9.7</td>
<td>Transcranial Doppler ultrasound</td>
<td>Age</td>
<td>rCBF increased</td>
<td>Moore et al. [69]</td>
</tr>
</tbody>
</table>

**Abbreviations:** IMT, intima media thickness; CCA IMT: IMT of carotid artery; FMD, flow mediated dilatation; TCD, transcranial Doppler sonography; CBF, cerebral blood flow; FBF, forearm blood flow; BP, blood pressure; HT, hypertension; BMI, body mass index.
Factors of fibrinolysis: no increase of D-dimer, Acute phase response: increased IL-6 (Vedder et al.). Factors for leucocyte activation: increased CD11b, CD18 (De Graba et al.).

* Plasma markers of endothelial activation.

<table>
<thead>
<tr>
<th>Design study</th>
<th>Number of included subjects (case: control)</th>
<th>Gender (M/F)</th>
<th>Age (years)</th>
<th>Assessment</th>
<th>Outcome</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI and angiography studies</td>
<td>25/20</td>
<td>10M/15F:9M/11F</td>
<td>36.5 ± 11:36.8 ± 10</td>
<td>Cerebral magnetic resonance angiography</td>
<td>Basilar artery diameter enlarged in Fabry disease</td>
<td>Fellgiebel et al. [41]</td>
</tr>
<tr>
<td>Cohort study</td>
<td>57</td>
<td>F</td>
<td>43.3</td>
<td>Cerebral magnetic resonance imaging</td>
<td>10 out of 50 cases with megadolichoectasia or stenosis/occlusion</td>
<td>Gupta et al. [43]</td>
</tr>
<tr>
<td>Cohort study</td>
<td>8</td>
<td>7M/1F</td>
<td>32</td>
<td>Cerebral magnetic resonance angiography</td>
<td>Normal angiography, 1 case with vascular ectasia</td>
<td>Jardim et al. [46]</td>
</tr>
<tr>
<td>Cohort study</td>
<td>26</td>
<td>18M/8F:3M/1F</td>
<td>43 ± 14/48 ± 12</td>
<td>Coronary angiography</td>
<td>Normal angiography in all (n = 8)</td>
<td>Moon et al. [47], Funabashi et al. [48], Eckart et al. [49], Ogawa et al. [50], Erdmann et al. [51]</td>
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<tr>
<td>Case reports (4)</td>
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</table>

Imaging in clinical studies on thrombosis

| Case report | 9 | 8M/1F | 35–75 | MRI-angiography | 6 Cases with megadolichoarterial anomaly of which 2 cases with thrombosis | No microembolic sources | Garzuly et al. [34] |
| Case reports (3) | 4 | 2M/2F | 36–66 | Extracranial and transcranial Doppler of right middle cerebral artery | Retinal artery occlusion | No microembolic sources | Ritter et al. [73] |
| Case reports (5) | 3 | 2M/F | 16–31 | Ophthalmoscopy/scanner laser ophthalmoscopy | Retinal microvascular or choroidal changes/ischemia | No microembolic sources | Utsumi et al. [74], Andersen et al. [75], Sher et al. [76] |
| Case reports (5) | 5 | 3M/2F | 24–66 | Fundoscopy/indocyanine green/fluorescein angiography | Retinal microvascular or choroidal changes/ischemia | No microembolic sources | Kumagai et al. [79], Guenoun et al. [80], Dantas et al. [81], Abe et al. [82], Ohkubo et al. [83] |

Study on a muscle biopsy were not listed in the table, because specific alterations in the arterial layers, apart from storage in endothelium and smooth muscle cells, were not described [12,17–25]. Atherosclerosis is not a consistent finding in most of the studies. Only three studies indicated atherosclerosis as a major finding [26–28], which could be related to other risk factors such as diabetes and hypertension as well [28,29]. In two studies, arterial stenosis was present [30,31], which was not due to atheromatous plaque formation but explained by more diffuse involvement of endothelial or smooth muscle cell storage material. Furthermore, in six case-report studies cerebral, coronary, renal and/or intrarenal arteries from 11 hemizygotes and 2 carriers were studied post-mortem without evidence of atherosclerotic lesions, or without further description [32–37]. In atypical Fabry hemizygotes and in a heterozygote case, smooth muscle cells invariably showed storage, whereas the endothelial cells were clear [30,38,39]. Since residual enzyme activity is present both in atypical cardiac variants as well as in females, this indicates that the smooth muscle cells are probably more prone to Gb3 storage than endothelial cells. Using a more sensitive immunohistological technique to detect

Table 4

<table>
<thead>
<tr>
<th>First author</th>
<th>Endothelial activation</th>
<th>Coagulation factors</th>
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<tr>
<td></td>
<td>sVCAM-1</td>
<td>sICAM-1</td>
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<tr>
<td>DeGraba [84]</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td>Demuth [85]</td>
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<td>Shen [86]</td>
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<td>Geldermann [87]</td>
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<td>Vedder [88]</td>
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<td>Fedi [89]</td>
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</table>

Factors of platelet aggregation: increased thrombin-anti thrombin complexes (TAT) in females, beta-thrombo-globulin (beta-TG) in males and females, platelet factor 4 (PF-4) in females and CD63+ in females (Vedder et al.).

Factors of leucocyte activation: increased CD11b, CD18 (De Graba et al.).

Acute phase response: increased IL-6 (Vedder et al.).

Factors of fibrinolysis: no increase of D-dimer, α2-antiplasmin or Tplasminogen activator (De Graba et al., Vedder et al.).

Increased homocysteine levels (Demuth et al., Fedi et al.).

sVCAM-1: soluble vascular cell adhesion molecule-1; sICAM-1: soluble intercellular adhesion molecule-1; TFPI: tissue factor pathway inhibitor; EMP: endothelial micro-particles; stF: Soluble tissue factor.

PAI: plasminogen activator inhibitor.

vWF: von Willebrand factor.

↑ = increase; ↓ = decrease; — = normal.

M = male patients.

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↑ = increase; ↓ = decrease; — = normal.

M = male patients.
Gb3 storage in two classically affected patients, Gb3 deposits could be established even in the absence of visible inclusions in the cell membrane, in various cytoplasmic structures, and in the nucleus [40]. Although this phenomenon has not been studied in atypical cases and females, it leaves the possibility for subtle changes in endothelial cells that are not detected by conventional histology.

One study also describes the histochemistry of aneurysms and elongation of the larger arteries, mainly the basilar artery [34], a finding that has been described and reviewed previously [41–43]. Fibrous tissue and mediocalcinosis have been described to occur in the vessel wall [30,35], which may induce abnormal remodelling of the arterial wall, ultimately leading to aneurysm formation.

Imaging and vascular reactivity

Our search revealed 36 clinical studies and case reports on vascular imaging and reactivity, including 6 studies in which intima media thickness (IMT) is measured, 3 studies showing results for flow mediated dilation (FMD). Furthermore, 9 clinical studies described reactivity of blood flow by other techniques or in different organs (Table 2). Eighteen clinical studies were published on various forms of vascular imaging (Table 3). The IMT in specific arterial beds is an established surrogate endpoint for atherosclerosis and is therefore used as part of risk assessment for future ischemic events in clinical trials. However, increase in IMT can be caused by storage in the media and intima as well as by plaque formation. One study evaluated atherosclerotic plaque by IMT measurement, defined as a significant increase in common carotid IMT with a focal non-homogeneous intima media thickening more than 1.2 mm, in 53 Fabry patients and no atherosclerotic plaques were detected [44]. This is in line with the recent study by Kovarnik et al., showing diffuse hypechogenic plaques by intravascular ultrasound of the coronary arteries in patients with Fabry disease in contrast to 10 controls with stable angina, who exhibited more focal lesions [45]. In addition, in several studies angiography in patients with Fabry disease was normal [46–51] (Table 3).

In four other studies the intima media thickness of either the carotid artery, aorta or brachial artery was increased as well [10.52–54]. This was also observed in α-galactosidase A deficient mouse model [55]. However, Moore et al. did not find a significant increase in the radial artery IMT in a group of 7 patients [56].

A non-invasive evaluation to evaluate the endothelial function is flow mediated dilation (FMD). The brachial artery diameter is measured before and after an increase in shear stress following reactive hyperaemia. When a sphygmmomanometer cuff placed on the forearm distal to the brachial artery is inflated to 200 mm Hg and subsequently released 4–5 min later, FMD occurs predominantly as a result of local endothelial release of NO.

Although Kallikioski et al. initially reported no difference in patients with Fabry disease [57] compared to healthy control subjects, additional evaluation one year later showed an impaired FMD in patients with Fabry disease [52]. This last observation was in line with a previous observation [58]. Another method to evaluate forearm arterial blood flow was used in three other studies [59,60], showing that the blood flow is decreased [59,61] and vascular resistance is increased in patients with Fabry disease [60]. Others showed decreased coronary blood flow reserve [57,62]. Altarescu et al. showed an exaggerated increase in forearm arterial blood flow after intra-arterial infusion of acetycholine in Fabry patients which was still present after administering L-NMMA, an NO antagonist [63]. Acetycholine releases NO from vessels with an intact endothelium, which leads to vasodilatation. Infusion of sodium nitroprusside, which induces endothelium independent vasodilatation, was normal. The authors concluded that in Fabry disease the abnormal vascular responses were endothelium dependent, in a non-NO dependent way. They postulated that the NO-pathway is downregulated since the inhibitor l-NMMA showed less vasoconstriction in patients compared to control subjects. In contrast, in the α-galactosidase A deficient mouse model, there was a reduced reactivity to acetycholine [64]. Furthermore, they showed increased activity of COX-1 and COX-2 in the same model [65].

Two clinical studies reported alterations in cerebral blood flow by transcranial ultrasonography [66,67]. In a study by Hilz et al., a reduced cerebral blood flow velocity (CBFV) was reported by transcranial ultrasonography before start of enzyme substitution [66]. In the α-galactosidase A deficient mouse model, cerebral blood flow appeared diminished as well, although this finding was statistically not significant [68]. Other studies indicate enhanced blood flow: CBFV was increased in treated and untreated Fabry patients in a study by Moore et al. [67]. Studies that used PET scanning confirmed this [69,70]. Most affected regions in Fabry disease on MRI, mainly the posterior and periventricular white matter, were relatively hyperperfused. These observations could be explained by dysregulation of the nitric oxide pathway, leading to excessive protein nitration [69].

Pro-thrombotic phenotype and endothelial inflammation in Fabry disease

Cerebral white matter lesions are frequently found in Fabry disease patients, possibly related to microvascular ischemic lesions or thrombo-embolic complications [42,71,72]. These lesions may not always be symptomatic: in the Dutch cohort of 115 Fabry patients including children, eight had experienced a symptomatic cerebrovascular ischemic event while many more have white matters lesions (Rombach, personal observation). A pro-coagulant state might contribute to these ischemic lesions. The literature search revealed five clinical studies or case reports on arterial thrombosis (Table 3) [34,73–76], six laboratory studies (Table 4) and two experimental studies [77,78]. Occlusion of the central retinal artery was reported in three cases [74–76] and retinal microvascular or choroidal changes or ischemia in five cases (Table 3) [79–83]. As already described in the pathology studies (Table 1), autopsy of two hemizygotes revealed a megadolichobasilar artery with dilatation and thrombosis. Dilatation of arteries and cardiac abnormalities can contribute to the formation of microemboli, but Ritter et al. did not find evidence for this by using transcranial Doppler sonography to detect microembolic signals [73]. They suggest that a local process in the vessel wall is primarily responsible for the ischemic lesions. This is supported by four studies reporting increased markers for endothelial activation such as sVCAM [84–86], sICAM [84,86], P-selectin [84], E-selectin [86] and PAI [84] and endothelium derived micro-particles: CD144+ and CD105+ [87]. However, in other studies no increase in VCAM [88], ICAM and P-selectin [85] was detected. Table 4 summarizes these findings. One important factor that may contribute to the differences is the degree of renal impairment, since renal disease may induce endothelial activation by itself. In a recent study, we found that soluble tissue factor (sTF), a marker for activation of coagulation was increased [89] but was related to the severity of renal insufficiency. In the same cohort, pro-inflammatory markers were analyzed and slight elevations in some patients of interleukin-6, a pro-inflammatory cytokine, but grossly normal C-reactive protein levels were observed. Increased levels of homocysteine have been reported as a vascular risk factor as well [85,89]. Among the plasma abnormalities related to the vasculopathy, determinants of the NO-pathway are important. Moore showed that NO synthesis is probably downregulated in Fabry disease [69] which is likely induced by reactive oxygen species (ROS) [90]. Indeed, further evidence for this is provided by the presence of increased myeloperoxidase levels in the circulation, a local remodelling factor in atherosclerotic plaques [91]. The
susceptibility to alterations in NO regulation is further illustrated by the increased presence of eNOS polymorphism, eNOSG894T, which decreases NO synthesis [92]. This polymorphism was specifically frequently found in a subgroup of Fabry patients with renal failure [93].

Discussion

Patients with Fabry disease have a shortened life expectancy due to the development of a specific vasculopathy. Male patients typically develop renal impairment in their third or fourth decade of life, as well as cardiac hypertrophy and conduction abnormalities. Life expectancy is reduced with a median life expectancy between 50 and 57 years for the male population [94–96]. In females, the disease is more variable, with less involvement of the kidneys but life span is shortened as well [94,95,97]. In female patients, cardiac disease and cerebral white matter lesions dominate and contribute to survival. The understanding of the pathophysiology of the vasculopathy in Fabry disease is limited. Removal of glycosphingolipid from various cell types has been reported in studies investigating the efficacy of enzyme replacement therapy [21,98–100]. However, it has become apparent that the removal of stored glycosphingolipid from the endothelial cells as identified by conventional histological examination, does not prevent progression of vascular disease in many patients [13,15]. Thus, the traditional concept that prominent storage in endothelial cells is primarily responsible for the vascular dysfunction cannot be held. Several investigators have attempted to unravel the components that contribute to the vascular damage. Most studies were performed in limited patient populations, usually focusing on one aspect of the vascular dysfunction rather than on the complex interplay of the different involved factors. Furthermore, comparing the same techniques, such as FMD and cerebral autoregulation methods, still gave inconsistent results. The differences in disease stage, i.e. the degree of advanced organ damage such as renal dysfunction, may play a role in variability of the findings. In the current study we have reviewed existing literature on vascular dysfunction in Fabry patients in an attempt to provide a hypothesis for the vascular changes that may help to identify new areas for study. In summary, the histopathology of Fabry arteries shows that smooth muscle cell involvement with stored glycolipid is the most prominent and probably the earliest feature, as in females and atypical cardiac variants no endothelial storage of significance is found. The smooth muscle cells are hypertrophied as well [10,44,52–54]. Barbey et al. showed that Fabry plasma was capable of stimulating proliferation of vascular smooth muscle cells and cardiomyocytes [10] and Brack et al. recently reported sphingosine-1-phosphate as a growth-promoting factor in Fabry disease [101], while Aerts et al. identified a new substance, the lyso-compound of globotriaosylceramide (Gb3), lyso Gb3 or lyso-CTH, which indeed induces smooth muscle cell proliferation in vitro [102]. In contrast, no stimulating effect on smooth muscle proliferation was seen after exposure to Gb3. The precise trigger for the production of lyso-Gb3 is unclear, but the extent of increase in lyso-Gb3 in Fabry patients compared to controls is much more prominent than Gb3 itself and is already visible in very young patients, especially males. Storage in the arterial media and subsequent proliferation of smooth muscle cells are therefore thought to be the first manifestations of vascular involvement. This is very likely the explanation for the increased IMT as has been reported in several studies [10,44,52–54]. This increase in IMT is probably a more specific Fabry alteration in vessel wall than the more traditional IMT changes seen in premature atherosclerotic disease. Histologically

Fig. 1. In Fabry disease, excess of lyso-CTH in the circulation gives local depositions in the media layer resulting in smooth muscle cell proliferation and remodeling of both subendothelial layer and extracellular matrix. Shear stress increases expression of both angiotensin 1 receptor (AT1) and angiotensin 2 receptor (AT2). Stimulation of AT1 increases intracellular transcription of reactive oxygen species (ROS) and NF-κB with a subsequent increase in β-integrin (a key modulator for extracellular matrix) and a decrease in nitric oxide (NO) synthesis. RBC, red blood cell; WBC, white blood cell; ANG-II, angiotensin-II; PAI, plasminogen activator inhibitor.
as well as by imaging techniques, atheromatous plaque formation is rare and stenotic lesions have a different aspect with a more diffuse location. Barbee identified no marked atherosclerotic lesions by evaluation with carotid IMT measurements in a group of 53 Fabry patients. Fabry disease may pose a risk factor for the development of widespread atherosclerosis in the context of other risk factors, such as renal insufficiency, diabetes, and hypertension [27,28,39]. This is in line with the observation that α-galactosidase A deficiency accelerates atherosclerosis in mice in the context of another genetic defect, i.e. apolipoprotein E deficiency [103]. In addition, Vedder et al. were not able to identify one Fabry patient among 440 male patients with premature atherosclerosis [104]. We suggest therefore that Fabry disease vasculopathy is basically different from premature atherosclerosis, which has been suggested by others as well [45,105]. Fig. 1 shows the hypothesized order of events: the smooth muscle cell and not the endothelial cell is primarily involved in the accumulation of Gb3 and subsequently its proliferation. Smooth muscle cells will then respond to this local stimulus with processes that are similar as observed in neo intima formation: hypertrophy of smooth muscle cells with increased volume of the media layer (IMT) and influx of inflammatory cells [106]. In this specific form of vasculopathy, however, there is formation of more fibrotic structures and not an in volume growing atheroma. In the presence of a hyperdynamic circulation in combination with a less compliant vascular wall, upregulation of local renin angiotensin systems may occur. Angiotensin II increases adhesion molecules, cytokines and chemokines and exerts a pro-inflammatory effect on leucocytes, endothelial cells and vascular smooth muscle cells [107]. Acting via the angiotensin 1 (AT1) receptor, angiotensin II initiates an inflammatory cascade of reduced nicotinamide-adenine dinucleotide phosphate oxidase, formation of reactive oxygen species (ROS) and expression of nuclear factor-kB. These factors mediate transcription and gene expression leading to increases in adhesion molecules and chemokines. This can lead to subsequent increase in β-integrin, a key modulator for extracellular matrix [108]. The actions of angiotensin II oppose those mediated through the angiotensin 2 (AT2) receptor, which include the release of nitric oxide (NO). Whether upregulation of the AT1 receptor or the AT2 receptors in Fabry disease occurs has so far not been studied, but early vascular damage in diabetes suggest a role for this mechanism: in insulin resistant subjects without overt diabetes or vascular damage, Brillante et al. provide evidence of both increased AT1 and AT2 receptors in the small to medium-sized arteries [109]. They suggest that these changes may be compensatory mechanisms related to early vascular damage. Also, the observed increase in extracellular matrix as described by Elleder can be found in association with angiotensin II activity [35]. After binding to its AT1 receptor, angiotensin II activates integrin-mediated signaling, thereby inducing alterations of both extracellular matrix quality and quantity, and cytoskeletal protein composition and filament organization [108]. Another interesting role of angiotensin II could be the enhancement of neuropathic pain, an important and early feature of Fabry disease. Hyperalgesia, a typical symptom of small fibre neuropathy, can be enhanced after the iontophoresis of angiotensin II on capsaicin sensitized skin [110]. The angiotensin II induced production of ROS decreases nitric oxide bioavailability and ultimately causes endothelial dysfunction. These observations are in line with the findings by Moore et al. showing elevated levels of reactive oxygen species in Fabry disease resulting in positive staining for 3-nitrotyrosine present in dermal and cerebral blood vessels and increased plasma levels of nitrotyrosine and myeloperoxidase [69,90,91]. This phenomenon is further supported by the recent finding of reduction in endothelial NO activity and higher concentrations of ortho-tyrosine and nitrotyrosine in endothelial cells of alpha-galactosidase knockout mice [111]. It is plausible that secondary to this, endothelial dysfunction occurs, as evidenced by the abnormal FMD in Fabry patients. As in the study by Brillante et al., there may be a delicate balance between dysregulation of NO production and the formation of ROS, which may explain the different findings for several of the endothelial activation pro-coagulant markers in Fabry plasma [84–87]. Although the involvement of angiotensin and its receptors may provide a plausible explanation for the phenomena observed in Fabry vasculopathy, this needs to be proven. Other factors may contribute to the vasculopathy of Fabry disease. Among these, thrombomodulin dependent Activated Protein C (APC) is of interest as its formation is decreased in patients with diabetic nephropathy. APC can prevent glucose-induced apoptosis in endothelial cells and podocytes and resistance may pose a risk on development of renal injury. In Fabry disease, APC resistance has been reported in renal transplant candidates [112] and thrombomodulin was reported to be relatively low although this was not confirmed in another study [85]. The role of cyclooxygenase (COX) activity has recently been investigated by Park and co-workers. Vascular tone is dependent on COX activity and they established increased COX-1 and COX-2 activity in an α-galactosidase A deficient mouse model. Identical to the increased expression of AT1 and AT2 receptors, it is suggested that increased COX activity and the release of COX-derived products have a compensatory role in an attempt to maintain normal vascular smooth muscle contractility. During further development of organ damage, a vicious cycle will be induced: vascular damage leads to renal failure or hypertension which will further enhance endothelial cell activation. This accelerates the development of vascular damage, ultimately resulting in a pro-thrombotic state and atherosclerosis in some patients. Apart from endothelial dysfunction, ROS have also been implicated in the formation of aneurysms, since the inflammatory process leads to protease-mediated degradation of the extracellular matrix and apoptosis of smooth muscle cells. This process results in weakening of the aortic wall [113]. It is likely that this mechanism plays a role in the aneurysm formation of arteries in Fabry disease.

Treatment with enzyme therapy does not prevent the occurrence of new complications, although it is possible that earlier intervention may be more beneficial in this respect. ACE inhibition and angiotensin receptor blockers are widely used in Fabry disease, usually for renal protection. In early stages of the disease, when acroparesthesias and early vascular damage is already present, blockade of the RAS may help to slow down the progression of the vascular dysfunction. If the above outlined hypothesis is true, more selective blockade of the AT1 receptor could be of extra benefit.

In conclusion, we hypothesize that the smooth muscle cell is primarily involved in the vasculopathy of Fabry disease and that in an early stage of Fabry vasculopathy, angiotensin II production is upregulated. The proliferation of smooth muscle cells and Gb3 storage results in higher IMT. Increased ROS production as well as enhanced NO production may result in different findings with respect to endothelial activation markers, which can be severely enhanced in the context of other vascular risk factors. Selective AT1 receptor blockade may be an interesting option for optimal NO mediated vasodilatation and should be explored as adjunctive therapy.

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