

# The Kidney in Fabry Disease: More Than Mere Sphingolipids Overload

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Hernán Trimarchi, MD, PhD<sup>1</sup>

## Abstract

Fabry disease is a rare cause of end-stage renal disease. Renal pathology is notable for diffuse deposition of glycosphingolipid in the renal glomeruli, tubules, and vasculature. Classical patients with mutations in the  $\alpha$ -galactosidase A gene accumulate globotriaosylceramide and become symptomatic in childhood with pain, gastrointestinal disturbances, angiokeratoma, and hypohidrosis. Classical patients experience progressive loss of renal function and hypertrophic cardiomyopathy, with severe clinical events including end-stage renal disease, stroke, arrhythmias, and premature death. The pathophysiological mechanisms by which endothelial cells, podocytes, smooth muscle cells, and tubular dysfunction occur in Fabry disease are poorly characterized and understood. This review evaluates the new evidence in pathophysiology of Fabry nephropathy, highlighting the necessity of early identification of individuals with Fabry disease.

## Keywords

Fabry disease, globotriaosylceramide, podocyte, nitric oxide, angiotensin II

## Introduction

Fabry disease is an X-linked genetic disorder of glycosphingolipid catabolism resulting from deficient activity of the lysosomal enzyme  $\alpha$ -galactosidase A ( $\alpha$ -gal A). As a consequence, the substrates of  $\alpha$ -gal A, which are neutral glycosphingolipids, mainly globotriaosylceramide (Gl3) and lyso-Gl3, accumulate in a variety of cells and tissues, leading to a wide clinical spectrum of clinical manifestations.<sup>1–3</sup> At the cellular level, the endothelium, podocytes, tubular cells, vascular smooth muscle cells, and mesangial cells are simultaneously affected. As a result, the glomerular basement membrane, vessels, and interstitium are gradually and progressively involved, resulting in proteinuria and progressive renal dysfunction. Chronic kidney disease is a prominent feature of Fabry disease that accounts for 0.01% of European and US dialysis registries.<sup>1,2–6</sup> However, enzymatic screening studies suggest that the true prevalence for male dialysis patients may be 10- to 100-fold higher.<sup>7,8</sup> Glomerular basement membrane damage can be observed either at the podocyte or at the endothelial side. However, the pathophysiological mechanisms by which endothelial cells, podocytes, smooth muscle cells, and tubular dysfunction occur in Fabry disease are poorly characterized and understood.<sup>3,9</sup>

## Vascular Compromise

The classic suggested vascular mechanisms of renal injury in Fabry disease include an initial compromise due to deposition

of Gl3 in the endothelium and within the arterial wall, mainly in smooth muscle cells. This Gl3 and lyso-Gl3 accumulation in the endothelium leads to a secondary decrease in nitric oxide (NO) synthesis and a trend to microthrombotic events that lead to local ischemic events. In this regard, 2 primary hypotheses have emerged to explain the pathogenesis of this vasculopathy. The first hypothesis states that circulating lyso-Gl3 deposits in the medial layer of the arterial vasculature stimulate smooth muscle cell proliferation and remodeling of the subendothelial compartment. The increase in shear stress results in an augmented expression of angiotensin receptors with the secondary formation of reactive oxygen species and nuclear factor kappa B activation. All these changes cause a decrease in NO synthesis and increase in  $\beta$ -integrin subunits expression.<sup>10</sup> An alternative but not contradictory hypothesis proposes that endothelial NO synthase (eNOS) dysregulation is the initial step for the development of Fabry-associated vasculopathy and that the

<sup>1</sup> Nephrology Service, Hospital Británico de Buenos Aires, Buenos Aires, Argentina

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## Corresponding Author:

Hernán Trimarchi, Nephrology Service Hospital Británico de Buenos Aires, Perdriel 74, Buenos Aires 1280, Argentina.

Email: htrimarchi@hotmail.com



Gl3 accumulation in the endothelium alone is sufficient to account for the dysregulation of eNOS. This phenomenon results in a lower NO bioavailability and eNOS uncoupling with the formation of reactive oxidants. These observations were consistent with a role for Gl3 in the assembly of the signalosome within the cytoplasmic caveolae, including eNOS. These experimental models of thrombosis, atherogenesis, and impaired relaxation underscore the role eNOS dysregulation plays as a fundamental basis for the inducible models of vasculopathy in Fabry disease. Clinical studies have been less consistent with the primary role NO may play in this scenario but support a critical role for eNOS dysregulation.<sup>11-15</sup> Shu et al have recently shown that in Fabry disease, levels of 3-nitrotyrosine, a specific marker for reactive nitrogen species, increased by 40- to 120-fold without corresponding changes in other oxidized amino acids, consistent with eNOS-derived reactive nitrogen species as the source of oxygen reactants. In conclusion, 3-nitrotyrosine may serve as a biomarker for the vascular involvement in Fabry disease.<sup>16</sup>

### Podocyte Mass

Parallel to the above-mentioned vascular compromise, a crucial event takes place. While endothelial and vascular smooth muscle cell damage leads to vasoconstriction and hypoxia, irreversible podocyte injury and detachment potentiate this progression to renal failure with the resultant glomerulosclerosis.<sup>17,18</sup>

The mechanisms of podocyte detachment are caused by the accumulation of Gl3, which interacts with podocyte cytoplasmic actin causing cell contraction, slit diaphragm widening, and the coupling with integrins. In this regard,  $\alpha_v\beta_3$  integrin and  $\alpha_3\beta_1$  are some of the main molecules that anchor podocytes to the basement membrane. In Fabry disease, when the  $\alpha_v\beta_3$  becomes activated, it triggers podocytic contraction and migration, finally contributing to podocyte detachment from the glomerulus and podocyturia.<sup>19,20</sup> Noteworthy, Utsumi et al have found elevated levels of  $\alpha_v\beta_3$  integrin in the urine of individuals with Fabry disease, probably due to the accumulation of Gl3. The augmented localization of the  $\beta_3$  component was seen mainly in podocytes and in epithelial cells of Bowman capsule.<sup>20</sup> Finally, the amount of vitronectin (a molecule involved in adhesion and fibrinolysis) was moderately increased in the kidney in patients with Fabry disease.<sup>20</sup> This finding is very interesting, as vitronectin couples with the urokinase-plasminogen activator receptor (uPAR). The uPAR actively participates in podocyte signal transduction via the  $\alpha_3\beta_1$  integrin, which in turn interacts with actin to cause podocyte contraction.<sup>21,22-26</sup> Therefore, in Fabry disease, the activation of certain integrins appears to be actively involved in podocyte detachment from the glomerular basement membrane.<sup>20,22-27</sup> With respect to the progression of kidney disease and proteinuria, *in vitro* studies of Fabry-cultured human podocytes have shown that the accumulation of Gl3 or lyso-Gl3 stimulated the secretion of transforming growth factor  $\beta_1$  and fibrosis.<sup>27,28</sup> Moreover, autophagy is dysregulated in Fabry podocytes due

to the inhibition of mammalian target of rapamycin (mTOR), a key enzyme that inhibits autophagy.<sup>29</sup> The accumulation of Gl3 and autophagosomes and a loss of mTOR kinase activity may indicate that autophagy may be an additional mechanism to podocyte depletion and proteinuria.<sup>29</sup> Finally, angiotensin II also plays a role in podocyte damage due to the fact that podocytes contain angiotensin II receptors. Angiotensin II leads to podocyte hypertrophy, stimulates transforming growth factor  $\beta_1$  synthesis, and depolymerizes podocyte actin, causing a deep disorganization of the cytoskeleton. The addition of converting enzyme inhibitors or angiotensin receptor blockers reduces podocyturia and proteinuria, as they are capable of decreasing podocyte contraction and also reducing the size pores of the glomerular basement membrane and the width of the slit diaphragms.<sup>30</sup>

In this respect, despite proteinuria is a useful marker of kidney disease and of glomerular injury, it is not specific of the stage of kidney damage, as it can be found at any stage of chronic kidney disease. Albeit a specific treatment for the disease exists, proteinuria frequently persists, particularly as renal disease worsens.<sup>17</sup> However, an excessive loss of podocytes in the urine could have been indicating an established structural glomerular abnormality and would herald the ulterior appearance of proteinuria. In addition, as podocytes do not replicate, once podocytes are detached from the glomerular basement membrane, the filtration barrier becomes denuded and proteinuria ensues when contiguous podocytes are unable to cover the function of the lost ones. It has been reported that when the population of podocytes per glomerulus is reduced to 20% to 40%, the process of glomerular obliteration is initiated.<sup>31,32</sup> Finally, it has been calculated that around 400 podocytes are lost in the urine everyday, which explains that the control patient also presents with podocyturia but of lower quantity.<sup>31,32</sup>

### Tubular Aspects

Tubular damage is mainly caused by the ischemic disturbances secondary to the vascular accumulation of Gl3 and lyso-Gl3 within the arterial wall, coupled with the podocyte loss and glomerulosclerosis, which determine the irreversible picture of tubular atrophy and interstitial fibrosis.<sup>17</sup> In addition, the tubular accumulation of sphingolipids within their cytoplasm translates into different electrolyte disturbances according to the involved segment of the nephron. For instance, Gl3 is mainly expressed in the proximal tubules with concomitant expression of angiotensin-converting enzyme. This finding may suggest that Gl3 and angiotensin II could be normally implicated in sodium and bicarbonate homeostasis.<sup>33</sup>

### The Inflammatory Milieu

The renal sphingolipid overload leads to a reactive local inflammatory response. The parenchymal renin-angiotensin system and steroid-dependent inflammatory pathways must

be involved in Fabry disease.<sup>9</sup> To our knowledge, many biomarkers are elevated in Fabry disease with glomerulosclerotic implications that explain a response to steroid therapy. The expression levels of renal thrombospondin 1, transforming growth factor  $\beta$ 1, vascular endothelial growth factor, and fibroblast growth factor 2 are higher in kidneys of Fabry mice compared to wild-type mice. Activities of caspases are also higher in kidneys of Fabry mice. These results may suggest that overexpression of transforming growth factor  $\beta$ 1 and vascular endothelial growth factor in the Fabry mouse kidney might contribute to Fabry glomerulosclerosis by inducing fibrosis and apoptosis.<sup>3</sup> Expression of thrombospondin 1 is increased in progressive renal disease and is associated with renal fibrosis and stimulates transforming growth factor  $\beta$ 1 in diabetes. Thrombospondin 1 is a possible activator of transforming growth factor  $\beta$ 1 in kidney injury and can induce apoptosis of endothelial cells. In addition, vascular endothelial growth factor increases vascular permeability, prevents apoptosis in endothelial cells, and induces apoptosis in cerebral endothelial cells after cell injury. It has been suggested that transforming growth factor  $\beta$ 1 activates expression of fibroblast growth factor 2 in endothelial cells, which then promotes vascular endothelial growth factor production, and vascular endothelial growth factor-induced fibroblast growth factor 2 expression in injured endothelial cells leads to migration and proliferation of smooth muscle cells. Vascular endothelial growth factor stimulation results in transforming growth factor  $\beta$ 1-induced fibrosis in proximal tubular cells. Upregulation of transforming growth factor  $\beta$ 1 and vascular endothelial growth factor may be associated with dysfunction of endothelial cells. Similarly, Sanchez-Niño et al reported that the expression of transforming growth factor  $\beta$ 1, CD74, and extracellular matrix protein were increased by adding Lyso-GL3 to human podocytes, showing that transforming growth factor  $\beta$ 1 and CD74 are mediators of podocyte injury.<sup>28</sup> CD74, the macrophage inhibitory factor receptor, is a potent receptor of kidney injury in diabetic nephropathy and sclerotic lesions. Increased expression of transforming growth factor  $\beta$ 1 and/or vascular endothelial growth factor in podocytes is associated with apoptosis or nephropathy.<sup>3</sup> Finally, GL3 correlates with oxidative stress and inflammation in Fabry disease. Patients presented decreased levels of antioxidant defenses, reduced glutathione, reduced glutathione peroxidase activity, increased superoxide dismutase–catalase ratio in erythrocytes, and interleukin 6 and tumor necrosis factor  $\alpha$  increments.<sup>34</sup> However, other studies show a decrease in tumor necrosis factor  $\alpha$ .<sup>35</sup> Decreased  $\alpha$ -2-antiplasmin was also associated with a parallel increase in circulating vascular endothelial growth factor and contributing to prothrombotic events in Fabry disease.<sup>10</sup> Sclerotic and thrombotic events can certainly contribute to ischemia and hypoperfusion, eventually leading to renal insufficiency. All these biomarkers and cytokines have been described to be elevated in focal and segmental glomerulosclerosis<sup>36,37</sup> and may explain the response to low-dose steroid therapy that exceptionally patients with Fabry may require as adjunctive therapy.

## ***Angiotensin II: A Link Between Inflammation and Hypoxia in Fabry Disease***

Interestingly, besides the well-known roles of angiotensin II in vasoconstriction, inflammation, and fibrosis, it is also involved in the pathogenesis of Fabry disease. Angiotensin-converting enzyme, a pivotal component of the renin–angiotensin system that converts angiotensin I to angiotensin II, is expressed in the plasma membrane of vascular endothelial cells, epithelial cells of renal proximal tubules, gastrointestinal tract, heart, and in various regions of the brain, the main tissues affected in Fabry disease.<sup>38</sup> It appears that treatment with recombinant  $\alpha$ -gal A decreases angiotensin-converting enzyme activity probably mediated by the release of the galactose residues from the angiotensin-converting enzyme molecule. The degree of angiotensin-converting enzyme glycosylation is important for the catalytic properties of the enzyme. In addition, glycosylation plays an important role in the release of angiotensin-converting enzyme from the membrane. Interestingly, 2 weeks later, angiotensin-converting enzyme activity was significantly upregulated, and the plasma levels of angiotensin II increased in the patients treated with  $\alpha$ -gal A following the elevations in activity of angiotensin-converting enzyme.<sup>38</sup> Proteinuria, which has emerged as an important risk factor for progression of kidney disease and considered the most important biomarker of disease progression in Fabry nephropathy, does not respond to enzyme replacement therapy alone using either recombinant agalsidase- $\alpha$  or recombinant agalsidase- $\beta$ .<sup>39</sup> In this regard, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers therapy has been shown to be effective in lowering proteinuria in Fabry disease as an adjunctive therapy.<sup>40,41</sup>

## ***The Implications of the Enzyme Mutation Involved***

Only to be mentioned, the inflammatory cytokine patterns of expression in Fabry disease may be subjected to the polymorphisms and mutations encountered and may be independent of the levels of  $\alpha$ -gal A.<sup>35</sup> An example to this phenomenon could be the reported different levels of tumor necrosis factor  $\alpha$  in patients with Fabry disease,<sup>34,35</sup> although it could also be due to the stage of the disease under consideration.<sup>9</sup>

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