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### Enzyme replacement therapy in Fabry disease, towards individualized treatment

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**Publication date**

2017

**Document Version**

Other version

**License**

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**Citation for published version (APA):**

Arends, M. (2017). *Enzyme replacement therapy in Fabry disease, towards individualized treatment*. [Thesis, fully internal, Universiteit van Amsterdam].

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# 1

## **General Introduction**

# General introduction

## Background

Fabry disease (FD) is a rare lysosomal storage disorder with an estimated prevalence of 1:40.000 – 170.000 males.<sup>1-4</sup> The disease is caused by a deficiency of the lysosomal enzyme alpha galactosidase A (aGAL, enzyme commission number: 3.2.1.22)<sup>5,6</sup> due to mutations in the *GLA* gene, which is located on the long arm of the X-chromosome (Xq22.1). Currently, more than 600 mutations are known, resulting in either a decreased or absent enzyme activity.<sup>7</sup> aGAL is involved in the degradation of glycosphingolipids with terminal  $\alpha$ -galactosyl residues. An important substrate of aGAL is globotriaosylceramide (Gb3). Gb3 is found on the plasma and intracellular membranes of many different cell types, and is also known as the P<sup>k</sup> blood group antigen on red blood cells, as the Shiga toxin receptor on endothelial cells, and as CD77 antigen on leucocytes.<sup>8,9</sup> Decreased or absent aGAL activity leads to the accumulation of Gb3 and related sphingolipids in the lysosomes of various cell types, in particular endothelial and vascular smooth muscle cells, cardiomyocytes, kidney cells and ganglia of the peripheral nervous system. Lysosomal inclusions in these cells can be seen on electron microscopy and are also referred to as “zebra bodies” because the concentric lamellar appearance resembles the stripes of zebras. The storage of glycosphingolipids subsequently results in disease manifestations in several organs, including the heart, kidneys and brain which eventually leads to a significantly reduced life expectancy in untreated FD patients.<sup>10,11</sup>

## Pathophysiology

Vasculopathy, fibrosis and systemic inflammation have all been implicated in the pathophysiology of Fabry disease, although the precise mechanism is still only partially understood. Extensive deposits of Gb3 and related compounds are usually found in endothelial and vascular smooth muscle cells, leading to functional and structural changes of the peripheral vascular system, including endothelial dysfunction, aortic stiffness and an increased intima-media thickness.<sup>12,13</sup> Several hypotheses have been proposed which link the stored sphingolipids to the observed pathophysiological changes at the cellular level in FD. First, Gb3 is suggested to induce oxidative stress and to up-regulate cell adhesion molecule expression in FD endothelial cells.<sup>14-16</sup> The observation that plasma of FD stimulates the proliferation of vascular smooth muscle cells<sup>17</sup> has led to further investigations showing that not Gb3, but exposure to globotriaosylsphingosine (lysoGb3), formed by deacetylation of Gb3, induced proliferation.<sup>18,19</sup> Secondly, fibroblast growth, cell differentiation and collagen expression is inhibited by lysoGb3 in aortic fibroblasts through the downregulation of a specific calcium channel (KCa3.1).<sup>20</sup> This could explain the higher proportion of aortic dilation/aneurysm in FD patients. Moreover, increasing accumulation and release of lysoGb3 may also play an important part in the development of the fibrotic and inflammatory changes observed in Fabry patients. In vitro studies have shown that lysoGb3 promotes the release of secondary mediators of glomerular injury similar to those found in diabetic nephropathy.<sup>21-23</sup>

In addition, lysoGb3 promotes Notch1, a mediator of podocyte injury leading to fibrotic and inflammatory changes.<sup>22</sup> These fibrotic changes are predominantly found in the kidneys and the heart. Preceding to glomerular sclerosis, tubular atrophy and interstitial fibrosis, renal involvement is characterized by foot process effacement and podocyte injury leading to proteinuria.<sup>24-26</sup> Besides fibrosis, cardiac manifestations in FD include concentric left ventricular hypertrophy and arrhythmias.<sup>27,28</sup> Interestingly, Gb3 accumulation accounts for only 1-3% of the mass of the hypertrophied hearts in FD,<sup>29,30</sup> and thus the majority of the increased mass is due to an increased extra cellular matrix and hypertrophy of the cardiac muscle cells. This illustrates that secondary processes, whether or not mediated through lysoGb3, may be even more important in the development of disease manifestations than the accumulation of sphingolipids itself.

### Phenotypes

Different phenotypes can be distinguished in FD. Patients may have the severe, classical phenotype or the more attenuated, non-classical phenotype.<sup>4</sup> The following symptoms are associated with the classical phenotype: Angiokeratoma: dark purple skin lesions clustered and located in the bathing trunk area; acroparesthesia: childhood-onset of tingling and burning pains in hands and feet with exacerbations during heat, fever and exercise; and/or cornea verticillata: a whorl-like pattern of corneal opacities.<sup>31,32</sup> Later in life, cardiac, renal and cerebral complications may develop. Men with this phenotype generally have very low to absent aGAL activity and high concentrations of lysoGb3.<sup>31</sup> Women do have residual enzyme activity since they harbor a “healthy” X-chromosome. Nevertheless, they may develop FD manifestations, with a disease course ranging from severe and progressive similar to men with classical FD to a more attenuated course.<sup>11</sup> This can be partly ascribed to skewed X-inactivation.<sup>33-35</sup> This phenomenon has also been described in other lysosomal storage disorders such as mucopolysaccharidosis type II (MPS II, Hunter syndrome).<sup>36</sup> A role for X-chromosome inactivation is further supported by a study on renal biopsies in which a relationship between podocyte mosaicism and podocyte injury in women with FD was found. Interestingly, no cross correction between “wild type” and “FD” podocytes was observed in this study,<sup>37</sup> which explains why women with FD may develop symptoms despite residual enzyme activity.

Patients with non-classical FD do not have the typical FD symptoms, such as angiokeratoma, neuropathic pain and cornea verticillata.<sup>31</sup> They usually present in adulthood, and disease manifestations may be limited to a single organ. Detailed studies on the natural course of non-classical disease - defined according to strict criteria - are, however, lacking. In the United Kingdom, a large cohort of non-classical FD patients is followed up who all harbor the N215S mutation and who predominantly suffer from cardiac disease. The N215S mutation is one of the few non-private FD mutations, and shows a relatively strong genotype-phenotype correlation.<sup>38</sup> For other mutations, a prediction of phenotype cannot always be made since the majority of FD mutations are unique to one family. Exceptions include large deletions,

frameshift mutations and nonsense mutations leading to a “null allele” which are consistently associated with a classical phenotype.<sup>31</sup> In the last decade, more common genetic variants (e.g. D313Y, R118C) of the gene have been discovered, and, after thorough evaluation, these variants are now considered to be non-pathogenic.<sup>39-42</sup> For other newly discovered genetic variants it may be unclear if they are causing FD or not. These variants are called “genetic variants of unknown significance” (GVUS). Due to screening (studies) in high risk populations and the widely available genetic screening tools (e.g. in cardiomyopathy patients), non-pathogenic mutations and GVUS are increasingly found.<sup>4</sup> Van der Tol and Smid have developed organ specific algorithms to support a definite diagnosis of FD in these patients.<sup>4,32,43,44</sup>

### **Clinical manifestations**

Disease severity and clinical manifestations largely depend on gender and phenotype. However, within phenotypes and even within families considerable heterogeneity of the disease course may exist.<sup>45</sup> As previously mentioned, patients with classical FD often have angiokeratoma, cornea verticillata and acroparesthesia. Other commonly reported symptoms are absent or reduced sweating (anhidrosis or hypohidrosis), hearing difficulties and gastro-intestinal complaints such as diarrhea, constipation and abdominal pain.<sup>43,46-48</sup>

#### *Cardiac manifestations*

Left ventricular hypertrophy is one of the hallmarks of FD.<sup>1,49</sup> Systolic function is generally preserved while diastolic function is often compromised resulting in exercise intolerance, dyspnea and fatigue.<sup>50</sup> In addition, rhythm disturbances are frequently found in patients with FD,<sup>51-53</sup> with sinus bradycardia and short PR-interval commonly being the first manifestations. Later in life, atrial fibrillation, ventricular tachycardia and episodes of asystole may develop which may necessitate the implantation of a cardiac defibrillator or pacemaker.<sup>53-55</sup> In more advanced disease, cardiac fibrosis may be observed,<sup>28</sup> which increases the risk of malignant ventricular arrhythmias and death.<sup>51</sup> Recent data suggests a shift in cause of death from renal complications towards cardiac deaths.<sup>56,57</sup>

Mild structural and morphologic abnormalities of the heart valves are relatively common in FD.<sup>58,59</sup> Although it has long been thought that FD patients have a higher risk of myocardial infarctions (MI) and related complications, more recent evidence suggests that the MI rate is not increased.<sup>55,60</sup> However, symptoms of angina are frequently reported by patients and most likely are related to sub-endocardial ischemia in hypertrophic hearts.<sup>60</sup>

#### *Renal manifestations*

Progressive decline in function and proteinuria are the most important renal manifestations of FD. Loss of renal function may ultimately result in the need of either dialysis or renal transplantation.<sup>55,61</sup> Severe proteinuria is associated with a steeper decline of renal function.<sup>55,62</sup> Notably, women are only rarely affected by severe proteinuria or end stage renal disease.<sup>10,11,55,61</sup>

### *Cerebral and psychological manifestations*

White matter lesions (WML) are frequently found on brain MRI which are thought to be related to the observed increased risk of transient ischemic attacks and strokes.<sup>10,11,55,63,64</sup> Furthermore, previous studies have suggested that cognitive impairment and psychiatric disorders occur more often in Fabry patients compared to the general population.<sup>65</sup> Since Fabry disease is a chronic, debilitating disorder, which impacts on the social and emotional wellbeing of patients, depression and other mood disorders have been reported.<sup>65</sup> Both physical and psychological consequences of the disorder result in reduced quality of life.<sup>66,67</sup>

### **Supportive care**

Supportive care for FD includes symptomatic and preventive treatments. Several drugs are prescribed to relieve neuropathic pain, anti-platelet drugs and statins are used to prevent thrombotic complications, and angiotensin converting enzyme inhibitors (ACEi) and angiotensin-II receptor blockers (ARBs) have been shown to be very effective in the treatment of proteinuria.<sup>68</sup> The use of ACEi and ARBs is associated with a better outcome in patients with chronic kidney disease due to type 2 diabetes which can probably be extrapolated to the FD population.<sup>69,70</sup> Furthermore, FD patients benefit from improvements in renal transplantation and dialysis techniques. In addition, cardiac devices, especially ICDs, have a place in the therapeutic arsenal to prevent sudden cardiac death.<sup>53</sup>

### **Fabry-specific treatments**

In general, lysosomal storage disorders can be treated by reducing the production of substrates of the deficient enzyme (*i.e.* substrate reduction therapy) or by increasing the degradation of storage products. The latter can be achieved through intravenous administration of the deficient enzyme (*i.e.* enzyme replacement therapy [ERT]), or enhancement of the activity of the endogenous produced enzyme (*i.e.* chaperone therapy). Recently, migalastat (Galafold®) produced by Amicus Therapeutics (Cranbury, NJ, United States) has been introduced for the treatment of FD.<sup>71</sup> Migalastat is an oral small molecule designed to bind and stabilize the endogenous aGAL (*i.e.* chaperone therapy). Only patients with endogenous aGAL production potentially benefit from treatment with migalastat. Consequently, many classical patients with null mutations (*i.e.* no endogenous enzyme production) are not eligible for treatment with migalastat.<sup>72</sup> To date migalastat is not (yet) reimbursed in the Netherlands. Advances in therapy may provide additional treatment options by endogenous *in vivo* production of enzyme (*i.e.* bone marrow transplantation, stem cell therapy or gene therapy) or correcting the underlying genetic defect (*i.e.* gene therapy, CRISPR-Cas9 editing).<sup>73</sup>

### *Enzyme replacement therapy*

In 2001, two ERT formulations have been approved by the European Medicines Agency for the treatment of FD: agalsidase alfa (Replagal®) produced in a human cell line by Shire (Dublin, Ireland), and agalsidase beta (Fabrazyme®) produced in Chinese hamster ovarian cells by Sanofi Genzyme (Cambridge, MA, United States). In the United States only agalsidase beta

has been authorized. Although both preparations are biochemically and structurally very similar,<sup>74-76</sup> there is a fivefold difference in registered dose with agalsidase alfa registered at a dose of 0.2 mg/kg/every other week (EOW) and agalsidase beta at 1.0 mg/kg/EOW. The price per patient per year is comparable.<sup>77,78</sup> Marketing approval was obtained based on relatively short and small trials.<sup>77,78</sup> Both pivotal studies used different (surrogate) endpoints; agalsidase alfa was approved based upon a reduction in pain compared to placebo whereas the pivotal trial of agalsidase beta used Gb3 clearance from renal biopsies as primary outcome. Consequently, these studies are hard to compare. Interestingly, neither of the pivotal studies was able to show a significant difference on the primary outcome of the other study. In a phase IV trial with a longer follow up period on the efficacy of agalsidase beta, ERT reduced the rate of important clinical events compared to placebo. However, cardiac, renal and cerebral complications were observed despite ERT.<sup>79</sup> The further occurrence of disease related major events despite treatment is supported by observational cohort studies with longer follow up, especially in patients with pre-existing advanced kidney involvement and/or cardiac fibrosis.<sup>26,27,64,80,81</sup> A meta-analysis on the effectiveness of ERT pointed to a favorable effect on cardiac mass while the effect on renal function was only limited.<sup>61</sup> Contradictory results have been published concerning the effect of ERT on WMLs: some reported progression during treatment while others suggested stabilization of the WML load.<sup>63,64,82-84</sup>

#### *Comparative studies*

Only two clinical trials directly compared the effectiveness of agalsidase alfa and beta. The first was a trial in 34 patients who were randomized to receive either 0.2 mg/kg/EOW agalsidase alfa or 0.2 mg/kg/EOW agalsidase beta. After 24 months of follow-up, no differences in clinical and biochemical outcomes between both groups were found. Comparison with patients on the regular 1.0 mg/kg/EOW dose of agalsidase beta suggested that the decline of lysoGb3 was dose dependent.<sup>85</sup> The second concerned a RCT comparing both agents at their registered dose which showed no difference in clinical event rate after a median follow up of approximately 50 months in 92 patients.<sup>86</sup> In addition, several switch studies were performed after a period of shortage of agalsidase beta, which arose after a viral infection at a Genzyme manufacturing facility.

As a consequence of the shortage, the majority of patients either received a lower dose of agalsidase beta or were switched to agalsidase alfa. None of these studies were able to show a difference in clinical event rate.<sup>87-90</sup> One study suggested a steeper decline in renal function and higher Mainz Severity Score Index (MSSI) scores in patients who had been switched to agalsidase alfa or had received a lower dose.<sup>90</sup> However, this was not confirmed by others. In a study from our center, a decrease in dose, switch to the lower dose of agalsidase alfa or interruption resulted in increases in plasma lysoGb3, suggesting, again, a dose effect.<sup>87</sup> It should be noted that most of these studies were small and had a relatively short follow-up. Also, interpretation of this data is hampered by bias by indication and selection bias. The

question as to whether the one product is superior over the other is therefore still to be answered.

### *Antibodies*

Another yet unresolved issue concerns the potentially negative influence of antibodies that may develop upon treatment with ERT. In other LSDs,<sup>91,92</sup> but also in hemophilia,<sup>93</sup> the formation of antibodies against the recombinant enzyme/protein is associated with a reduced treatment effect. In FD, antibodies frequently develop in those patients with absent enzyme activity (*i.e.* men with classical FD), while they are uncommon in women with FD.<sup>94,95</sup> There is some evidence that a higher proportion of patients treated with agalsidase beta develop neutralizing antibodies compared to those treated with agalsidase alfa,<sup>94</sup> while others did not find any difference in antibody development between the two compounds.<sup>95</sup> In the pivotal trials the number of patients who developed antibodies was higher in patients treated with agalsidase beta than in those treated with agalsidase alfa (88% vs 21%), but different assays were employed.<sup>77,78</sup> Clinical studies have suggested that antibody formation has no significant effect on the safety or efficacy of ERT.<sup>77,78,96</sup> On the other hand, there is robust evidence that urinary Gb3 clearance as well as the improvement in plasma lysoGb3 concentrations is reduced in patients with antibodies.<sup>95,97-99</sup> A more recent cross-sectional study showed an association between the presence of antibodies, cardiac mass and renal function. However, definite conclusions cannot be drawn since no distinction was made between men with classical FD (having a high risk of developing antibodies, and have more severe disease) and those with non-classical FD (having a low risk of developing antibodies, and have less severe disease).

### *Costs, reimbursement and appropriate use of expensive medication*

In 2012, the high costs, limited effectiveness in the whole group and consequently unfavorable cost-benefit ratio of ERT in FD (price per QALY €5.5-7.5 million) resulted in a fierce, partially public, discussion on the acceptability of reimbursement of ERT for FD.<sup>100</sup> It was eventually decided to continue reimbursement under strict conditions including pricing negotiations by the ministry of health, welfare and sports with the manufactures, development of start and stop criteria, and the establishment of an independent committee advising on the initiation and discontinuation of ERT.<sup>101</sup> In order to provide a basis for discussions, clearly more data were needed regarding the effectiveness of treatment. In addition, since the disorder is so heterogeneous, ideally data should be generated that would allow the committee to make evidence based decisions for starting or stopping treatment, also called appropriate use of treatment. Although appropriate use should be common practice for all medication used, it is even more pressing for extremely expensive and burdensome treatment such as ERT. Thus, a protocol to investigate the treatment effects in subgroups of FD patients was proposed, including identification of patient characteristics determining the response on ERT and comparing the effects of agalsidase alfa and beta. These challenges formed the basis of the studies presented in this thesis.



## Aims and outline

The purpose of this thesis is to support the appropriate use of agalsidase alfa or beta, by studying all clinical and biochemical aspects of FD and its untreated and treated disease course. Because of the low prevalence of FD, it was necessary to set up an international collaboration. A database was established that included patient data from the Academic Medical Center (The Netherlands), the University Clinic Wuerzburg (Germany) and the Royal Free London NHS Foundation Trust (United Kingdom). For the analysis of agalsidase alfa versus beta, a cohort from the Canadian Fabry Disease Initiative was added as well.

In **Chapter 2** we provide a systematic review of the available literature on the quality of life in FD. In **Chapter 3** we present the results of a detailed study on the quality of life in patients from Amsterdam and London, with a special focus on different phenotypes and disease states. **Chapter 4** reports on the natural course of classical and non-classical FD, describes the event free survival of untreated patients, and provides a cross-sectional analysis of cardiac involvement, renal function and the presence of WMLs. In **Chapter 5** we evaluated the long term effects of ERT in FD and provide patient characteristics associated with a more favorable or unfavorable disease course. In **Chapter 6** the outcome of patients treated with either agalsidase alfa or agalsidase beta is compared, including the effect of antibodies. In **Chapter 7** the biochemical response of patients who started ERT at young age is compared to those who started at older age. **Chapter 8** describes our experiences with discontinuation of ERT. Finally, **Chapter 9** includes a summary and general discussion of this thesis.

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