Fabry meets Markov: evaluating biochemistry, disease course and costs in support of health care policy
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Summary, general discussion and future perspectives
Summary

After the introduction of enzyme replacement therapy, research on the nature and treatment of Fabry disease has markedly increased, resulting in many publications on these topics. Although the last few years further knowledge on the treatment of Fabry disease has expanded, also new questions have emerged. This thesis addressed some of these questions. Chapter 1 is an introduction on Fabry disease covering the clinical presentation, pathophysiology and treatment options. Briefly, Fabry disease presents as a cardiovascular disease including cardiac, renal and cerebrovascular complications but the underlying pathophysiology is still only partially understood. Traditionally, it has been suggested that prominent accumulation of globotriasylceramide (Gb3) in endothelial cells sets off the pathophysiology of the disease, and hence evaluation of therapeutic intervention has primarily focused on the clearance of this compartment from stored Gb3.

Chapter 2 describes the discovery of the presence of elevated levels of a new biomarker, deacylated Gb3, globotriaosylsphingosine or lysoGb3, in plasma of Fabry disease patients. Compared to Gb3, lysoGb3 is much more elevated compared to healthy controls and is also clearly elevated in symptomatic females. With regard to the pathophysiology of Fabry disease, it is of note that increased levels of plasma lysoGb3 induce smooth muscle cell proliferation in vitro, while Gb3 does not. In addition, lysoGb3 at high concentration can inhibit alfa-Galactosidase A activity, the enzyme that is deficient in Fabry disease. It can not be excluded that this metabolite plays a role in the vascular pathology of Fabry disease. This consideration led to our hypothesis that not only primary endothelial accumulation of Gb3 but in addition rather smooth muscle cell proliferation sets off a sequence of events, resulting in disease manifestations. This hypothesis is further explored in chapter 3 which contains a review of the literature on vascular complications and its pathophysiology in relation to Fabry disease. Even though some literature studies suggested storage in the vessel wall leads to accelerated atherosclerosis, this chapter provides evidence that, in contrast to some literature reports, end organ complications do develop without the manifestation of atherosclerotic lesions. The pathophysiology is different. In patients with residual enzyme activity, including females and patients with the cardiac variant, there is little to no endothelial Gb3 lipid storage suggesting that another process, such as smooth muscle cell proliferation, plays a role in the pathophysiology. Following this line of argument, it is hypothesized that smooth muscle cell proliferation is probably the earliest feature of vasculopathy leading to a process that resembles neo-intima formation in diabetes mellitus. This does not exclude that additional vascular risk factors could contribute to the variable expression of disease manifestations. In chapter 4 it is illustrated that plasma lysoGb3 can be used as diagnostic marker. The lyso-lipid is clearly elevated above normal in plasma of classically affected males and females, with the exception of pre-symptomatic pediatric females. Plasma lysoGb3 levels distinguish classically affected male patients from those with a non-classical (atypical) course of
disease. Plasma lysoGb3 also correlates with some disease symptoms; it correlates with left ventricular mass (LVmass) and disease severity in females and cerebral white matter lesions in males. Already very low levels of lysoGb3 seem to occur in association with vascular disease manifestations, as is described in chapter 5. It should be kept in mind that correlations between a biochemical analyte and symptoms do not prove a causal relationship. Several vascular measurements, including intima media thickness (IMT), flow mediated dilation (FMD), pulse wave velocity and advanced glycation end products were measured in males and females with Fabry disease. In atypical patients, with very low levels of plasma lysoGb3, there were no clear abnormalities in the studied vascular parameters. The somewhat higher levels of plasma lysoGb3 in females correlated with the increase in IMT and the decrease in FMD independent of age. Classic Fabry males all showed increased IMT and decreased FMD, but their elevated plasma lysoGb3 did not correlate with the extent of these abnormalities. This lack of correlation might be attributed to a ceiling effect, implicating an additional rise in lysoGb3 does not add to an increased effect or it might simply reflect that there is another causative factor of these vascular abnormalities.

During ERT, levels of plasma lysoGb3, Gb3 and urinary Gb3 decrease. In chapter 6 it is shown that lysoGb3 decrease occurs mainly within the first three months of ERT. Thereafter it remains stable or decreases further in the case of increase of enzyme dose (chapter 7). However in both treated males and females, plasma lysoGb3 as well as urinary Gb3 remain usually elevated compared to levels in healthy controls (chapter 7). In males who develop antibodies against the infused enzyme, plasma lysoGb3, Gb3 and urinary Gb3 remain higher compared to males without antibodies. The decrease in plasma lysoGb3, Gb3 and urinary Gb3 is correlated with the decrease in LVmass in females and males that show reduction of LVmass. In addition, it was observed that the lower the level of plasma lysoGb3, Gb3 and urinary Gb3, the lower the hazard ratio of developing cerebral white matter lesions.

Renal function is an important parameter to assess organ damage in patients with Fabry disease. To evaluate treatment effects, an accurate measurement is therefore of importance. In chapter 8 the glomerular filtration rate (GFR) was estimated in adult patients with Fabry disease using 11 different formulas based on plasma creatinine, cystatine C or β-trace protein and compared to the measured GFR with $^{125}$I -labelled iothalamate/ $^{131}$I-labelled hippuran. Of all formulas, the Stevens-formula, a creatinine and cystatin C-based formula, most closely approximated the measured GFR. Of the creatinine based formulas the abbreviated MDRD (Modification of Diet in Renal Disease) and CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula showed the best performance. Both formulas are regularly used as measure of effectiveness of ERT.

The first placebo-controlled trials reporting the effect of ERT on renal function and other clinical parameters, including acroparesthesia, had a follow-up of maximally 6 months. Thereafter several studies have been published on the long-term effect of ERT, but few
reports dealt with end-organ complications. The long-term effect of enzyme replacement therapy on renal function, LVmass, cerebral white matter lesions and end-organ complications in patients with Fabry disease is evaluated in chapter 9. During a median treatment duration of five years, renal function in males declined compared to the general population. Decline in renal function in females was comparable to healthy controls. Cardiac mass in males increased and remained stable in females during ERT. Occurrence of new cerebral white matter lesions occurred despite treatment. Even adolescents who started ERT relatively early developed cerebral white matter lesions despite ERT. In both the untreated as well as treated patients who showed end-organ symptoms (left ventricular hypertrophy, chronic kidney disease or one or more cerebral white matter lesions), the odds of developing a major complication (i.e. heart failure, end stage renal disease, stroke) increased with age. The odds for a major complication did decrease in treated patients with increasing treatment duration, independent of gender. Also, in patients with one complication, the odds of developing a second major complication declined with increasing treatment duration.

As the costs for enzyme replacement are extremely high, a cost-effectiveness analysis of enzyme replacement therapy was performed and presented in chapter 10. A life time Markov model was developed with use of the current knowledge of the disease course, with the use of prospective and retrospective data of the Dutch Fabry cohort. The natural (untreated) disease course was compared to the course of disease during ERT, including utilities as well as costs. The primary outcomes were the costs per year without end-organ damage and the costs per quality adjusted life year (QALY). In symptomatic patients, ERT has limited effect on quality of life and reduction of development of end-organ complications. As a consequence, the costs per year without end-organ damage and costs per QALY are high (€2.9 million - €3.8 million discounted and €5.5 - €7.5 million undiscounted).

Chapter 11 includes a meta-analysis and systematic review on the course of disease during ERT compared to the natural course of disease. Disease progression was seen both in patients with advanced disease as well as those with milder symptoms. Decline of renal function during ERT was comparable to the natural disease course in females and males with GFR>60 ml/min/1.73 m². LVmass in males increased during ERT, but this was less pronounced compared to the natural disease course. In females, LVmass decreased or remained stable. For white matter lesions and major complications the number of available data was too limited for a meta-analysis. In conclusion, ERT in combination with supportive care and co-medication including angiotensin-converting enzyme-inhibitors and angiotensin-II-receptor blockers is effective in reducing LVmass to a certain extent, but progression of renal decline cannot be prevented. In chapter 12 the topics of this thesis are discussed and put in perspective of the latest advances in research. Finally some suggestions are made for future studies.
General discussion

Glycosphingolipids and the pathophysiology of Fabry disease

Fabry disease is a vascular disease causing cardiovascular complications and stroke \(^1-^4\) Furthermore neurological symptoms may occur, including particular neuropathic pains, for which accumulation of Gb3 in dorsal rood ganglia is held responsible \(^5\). Although stored Gb3 is generally believed to be pivotal in the pathophysiology of Fabry disease, the mechanism by which this causes the several clinical manifestations characteristic for Fabry disease remains still elusive. There is no straightforward correlation between plasma Gb3 levels and disease manifestations and even though ERT results in clearance of stored Gb3 in endothelial cells and reduces plasma Gb3 levels, progression of disease has been observed. Furthermore, cellular Gb3 storage is already detected in fetal tissue of hemizygotes and it seems to clearly precede clinical manifestations \(^6-^8\). Barbey and coworkers reported on an unidentified factor in plasma of patients with Fabry disease capable of inducing proliferation of vascular smooth muscle cells and cardiomyocytes in vitro \(^3\). The same group suggested that elevated sphingosine-1-phosphate in patients with Fabry disease was responsible for vascular remodeling, though it was found to be only modestly increased in Fabry patients \(^9\). It was hypothesized by us that in analogy to Krabbe disease, another lysosomal storage disorder, a potentially toxic deacetylated Gb3 might exist. The existence of this compound was indeed demonstrated and it was found to be markedly elevated in plasma of patients with classic Fabry manifestations \(^10\). An in vitro study showed that lysoGb3, at concentrations as found in plasma of Fabry hemizygotes, can induce smooth muscle cell proliferation contrary to Gb3. This might support, but does not proof, the hypothesis that lysoGb3 plays a direct role in vascular changes in Fabry disease (chapter 3). A review of the literature led to the hypothesis that the first step in the pathophysiology is smooth muscle cell proliferation (chapter 3). Thickening of the intima media may lead to a hyper dynamic circulation as described by others \(^11-^13\). This may lead to a less compliant vascular wall, which can make the vessels more susceptible to vessel alterations, such as dolichoectasia \(^14-^16\). In chapter 3 we hypothesized that in analogy to diabetes mellitus, a process called neo-intima formation may occur. This process involves up-regulation of AT1 and AT2 receptors. Indeed, a recent study showed that ACE activity was up-regulated and plasma levels of angiotensin II increased after 14 days, just before ERT (agalsidase beta 1.0 mg/kg/ 2 weeks) was administered and angiotension II decreased four hours after ERT infusion \(^17\). Up-regulation of the renin angiotensin system may induce release of adhesion molecules and prothrombotic factors \(^18-^20\). This can impair release of nitric oxide \(^4\). Dysregulation of the NO-pathway was also observed in patients with Fabry disease \(^12\). A combination of reduced vascular compliance and activation of prothrombotic factors may then lead to vascular complications and ultimately result in end organ damage in Fabry disease patients (chapter 3). To further investigate whether lysoGb3 plasma levels in Fabry patients are associated with vascular changes, plasma
lysoGb3 levels in Fabry patients were measured as well as vascular parameters including carotid intima media thickness in arteries (IMT), flow mediated dilation (FMD), pulse wave velocity (PWV) and advanced glycation end products (chapter 5). Though in female Fabry patients, IMT was not significantly increased its values correlated positively with plasma lysoGb3 levels. In classically affected males, IMT was increased compared to age and gender matched controls, but there was no correlation between increase in IMT and that in plasma lysoGb3. As there was an association between IMT and lysoGb3 in females, and classically affected males showed higher IMT and higher levels of lysoGb3 compared to female patients, a ceiling effect might be considered as explanation. In the entire cohort, males and females with highest lysoGb3 (above the median of 7 nmol/L) showed the highest IMT on top of age and gender. LysoGb3 above 7 nmol/l was also associated with a 2.2% lower flow mediated dilation (FMD), independent of age and gender. Of interest, the atypical Fabry patients studied neither showed elevated plasma lysoGb3 levels nor abnormalities in vascular parameters. These observations suggest that plasma lysoGb3 is associated with, but not necessarily causing, vascular changes in Fabry disease. Studies on the correlation of biomarkers are hampered by lack of a clear definition on the disease severity or burden. There are commonly used disease severity scores, such as the Mainz severity scoring index, the DS3 or the Fabry International Prognostic Index. However, these scoring tools all are partially based on clinical parameters that are not specific for Fabry disease (eg LVH, proteinuria). A golden standard to assess the total amount of storage is also lacking. Thus, linking clinical symptoms and/or biomarkers to a phenotype of the disease is extremely difficult, especially if cohorts of patients consist of mixed populations of classical (severe) and non-classical (less severe, or atypical) patients. What is clear from these observations is that a high lysoGb3 is characteristic for a classical phenotype and these high levels may be related to vascular abnormalities already at an early stage of the disease, as suggested by preclinical data (chapter 2). It can be speculated whether chronic exposure to lower levels of lysoGb3 may also have a detrimental effect on the vascular wall as suggested in chapter 5, even though in preclinical studies this was not apparent. Most likely these lower lysoGb3 levels, as occurring in female heterozygotes, may result in less extensive smooth muscle cell proliferation, not to the extent seen with the lysoGb3 levels as found in classical male patients. Vascular remodelling might result in upregulation of AT1 and AT2 receptors and subsequent abnormal vascular responses and increases in IMT. This possible mechanism warrants further investigation. Increased IMT was found to be related to LVmass, renal function and cerebral white matter lesions, but lost significance when adjusted for age and gender (chapter 5). This is not surprising as age and gender are important predictors for complications in Fabry disease. In the general population IMT increases with age and an increase in IMT is associated with an increased risk of stroke 21. Comparable analysis in a larger Fabry cohort, with strict stratification for other cardiovascular risk factors, would clarify how IMT is related to clinical symptoms. Others have studied the vasculopathy in the alpha-
Galactosidase A deficient Fabry mouse, and reported that the reaction of the vascular wall to certain stimuli is mediated through the endothelium.\textsuperscript{22-24} The alpha-Galactosidase A deficient mouse model of Fabry disease warrants further discussion. The animals (male hemizygotes and female homozygotes) develop extensive storage of Gb3 in the endothelium, kidney and liver. Interestingly, no lipid deposition is noted in podocytes and no renal complications develop. The animals neither develop a marked LVmass increase.\textsuperscript{26} It could be that these mice do not develop the cardiac, renal and cerebral vascular complications due to their short life span of two years. The Fabry mouse model does not seem to be an ideal model to study the formation and correction of lipid deposits in podocytes and cardiomyocytes, phenomena associated with long term complications and atypical manifestations. The storage of lipid material in podocytes in Fabry patients remains intriguing: the lipid deposits are larger in podocytes compared to those in endothelial cells and located more perinuclear.\textsuperscript{27} Moreover, these are not strikingly reduced by enzyme replacement therapy, in contrast to the lipid material in various types of endothelial cells in the same patients.\textsuperscript{28} The same holds for the lipid deposits in cardiomyocytes.\textsuperscript{29} Puzzling is the fact that recombinant alfa-galactosidase A is reported to be taken up by podocytes, however not resulting in prominent clearance of lipid deposits.\textsuperscript{30} Although the accumulation of Gb-3 in podocytes in Fabry disease males and females, is dramatic, most females show little alteration in renal function.\textsuperscript{31-34} Apparently some threshold needs to be exceeded.

It remains of interest to study biopsies or tissues available from (untreated) patients at different stages of disease, and relate these findings to their clinical parameters. Interventions such as ACE-ARB treatment for prevention of renal complications in Fabry disease, should ideally be studied in placebo-controlled trials, with clinical outcome parameters as well repeated kidney biopsies.

**Fabry disease: the need for predictors of disease manifestation**

It has become clear that Fabry patients may present with a wide spectrum of disease manifestations from asymptomatic to severe end-organ involvement (chapter 4). Classical Fabry is defined by the occurrence of typical early manifestations of the disease, such as acroparesthesia, angiokeratoma, and hypo-or anhydrosis followed by late manifestations of which left ventricular hypertrophy and rhythm abnormalities, renal insufficiency and cerebral involvement are the most prominent. In some cases there is a clear link between the presence of this classical phenotype and the presence of a disruptive mutation of the GLA gene (e.g. a nonsense mutation or a large deletion). In these cases a very low to completely absent alpha Galactosidase A activity can be demonstrated as well. In contrast, patients with a non-classical phenotype (such as late-onset patients) often show significant residual enzyme activities and in these cases mutations (or variations) of the GLA gene usually cause amino acid substitutions with unclear consequences. In addition, due to many screening initiatives other mutations in the alpha-Galactosidase A gene are
encountered that are topic of debate whether they are disease-causing by themselves at all, only constitute a risk factor or are true pseudo-deficiencies. Among these non-classical two distinct (mono-organic) phenotypes are relatively frequently discerned: a cardiac and a renal phenotype. These patients are usually diagnosed through screening initiatives. It remains to be seen whether the reported patients actually have mono-organ involvement only. In the discussions on the non-classical patients, a few mutations are reported more often. These will be discussed here.

The mutation N215S is reported to be associated with the cardiac phenotype. However, it is not known whether N215S in every individual actually causes cardiac disease (or even involvement). This is of particular interest since the N215S is one of the most prevalent mutations found in both post-marketing disease registries (personal observation).

Another interesting mutation that raises a lot of discussion is the mutation that leads to the amino-acid substitution R112H. This mutation is reported in connection with very different disease manifestations, such as a single report on a male with the R112H mutation with a classical Fabry phenotype, but also other males with a mild cardiac phenotype only. The Dutch cohort includes 11 individuals with this mutation (4 males), of which one male presented with severe renal insufficiency necessitating dialysis (chapter 4). One other male family member recently developed proteinuria and decline of renal function. In both these males, kidney biopsy showed extensive lipid storage in podocytes but not in endothelial cells as is usually seen in classic Fabry disease (chapter 4). Whether storage in podocytes only results in moderate proteinuria and limited renal insufficiency in contrast to the additional storage in endothelial cells as seen in classical disease with its severe phenotype is unclear. In this respect it is of interest to note that myelin-like lipid storage in podocytes may also be seen in non-Fabry patients, for example Niemann-Pick patients and individuals with IgA nephropathy. In the aforementioned two R112H cases kidney-pathologists reported a definitive diagnosis of Fabry disease, and deemed other diagnoses unlikely apart from chloroquine or amiodarone use. The present lack of a definite diagnosis of Fabry disease based on kidney pathology in Fabry disease hampers clinical decision making and needs urgent evaluation. It is apparent that the presence of R112H in an individual as such does not obligatory predict disease or specific organ involvement. It may also be an example of a mild mutation that dependent on other (epigenetic or life style) factors may constitute a risk for disease development.

Other relatively common alterations in the alfa-galactosidase A gene result in the aminoacid changes D313Y and A143T, which encode enzymes with impaired activity. In these cases the predictive value for disease manifestation is even more obscure. The association between LVH and the presence of one of these mutations does not prove a causal relationship since both LVH and these mutations occur relatively frequent. The same holds for the co-occurrence of stroke and these mutations. These examples illustrate the complexity of predicting the clinical phenotype based on genotype. The term fringe
alleles has been coined to describe mutations that do not obligatory cause disease manifestation but are risk factors\textsuperscript{45}.

**LysoGb3 as diagnostic marker for classical Fabry disease**

A marked increase in plasma lysoGb3 has been identified as a new hallmark in both males and females with classical Fabry disease. Of interest, the data so far indicate that individuals with GLA mutations presenting with a non-classical course do not show marked plasma lysoGb3 abnormalities as classically affected patients do. For example, the two symptomatic R112H males described above show only very slightly increased plasma lysoGb3 as compared to healthy controls. Their values are in the middle of the range of those in Gaucher patients (unpublished observations), a disease with a totally different phenotype. Unfortunately, data on plasma lysoGb3 levels in patients with renal insufficiency but without GLA mutations are lacking. Thus, the sensitivity and specificity of lysoGb3 in patients with Fabry disease and without Fabry disease (but with similar clinical disease) is unknown. However, following the identification of lysoGb3 as a possible biomarker, recently several studies were published on lysoGb3 in different phenotypes of the disease. In males presenting with a cardiac phenotype modestly elevated lysoGb3 was detected\textsuperscript{46}. Another Japanese study reported no lysoGb3 elevations in a similar cohort\textsuperscript{47}. The interpretation of these discrepant findings is complicated by the fact that there might be concern about the validity of the clinical diagnosis in these non-classical patients: left ventricular hypertrophy is present in 15\% of the general population and related to hypertension, obesity, valvular heart disease, coronary disease and increases with age\textsuperscript{48}. Despite these uncertainties, compared to plasma Gb3, lysoGb3 appears to be much more sensitive and discriminative, especially between healthy controls and Fabry disease patients (chapter 2). In order to delineate whether lysoGb3 in urine may have additional diagnostic value, urinary lysoGb3 was measured in a Fabry disease cohort\textsuperscript{31}. It should be kept in mind that given the very low concentration of lysoGb3 in urine, plasma lysoGb3 offers a much better tool for analysing Fabry patients (article in press). Nevertheless, it is claimed that there is a clear correlation between urinary lysoGb3 elevation and severity of mutations in alfa-Galactosidase A\textsuperscript{49}.

In conclusion, based on the present data it can be concluded that demonstration of markedly elevated plasma lysoGb3 is extremely useful to assist in the biochemical confirmation of classical Fabry disease. On the other hand, absence of clearly elevated lysoGb3 does not a priori exclude a non-classical phenotype. Current knowledge only warrants exclusion of the classical phenotype in the case of a very low plasma lysoGb3 in males. It is presently not known whether a restricted accumulation of Gb3 in few cells in a single organ, e.g. podocytes, results in sufficient formation of lysoGb3 to be reflected in a significant elevation of plasma lysoGb3 levels. In young pre-symptomatic females, lysoGb3 can (still) be normal. In view of this, screening for Fabry disease based on plasma lysoGb3 is not ideal since it will not help to identify young pre-symptomatic
females and individuals with non-classical disease. This was very recently demonstrated in a study on the value of lysoGb3 levels in dried blood spots from newborns with the IVS4+919G>A alteration. In males of all ages with this alteration, lyso-Gb3 levels were elevated and were higher than in age-matched controls. The authors concluded that measurement of lyso-Gb3 levels could be useful in the diagnosis of Fabry disease in males, including the non-classical phenotype. However the lyso-Gb3 level was not elevated in the IVS4+919G>A heterozygotes. Whether the IVS4+919G>A substitution actually causes the non-classical form of Fabry disease remains to be established. It has been shown that a substantial proportion of older males with this gene alteration does not have any cardiac symptoms.

In conclusion, accurate prediction of nature and severity of disease manifestations is not possible based on genotype in the case of some abnormalities in the alpha-Galactosidase A gene that are not clearly known to be obligate-disease causing. This poses an enormous dilemma, since treatment with enzyme replacement therapy is available, and its limited effectiveness has pointed into the direction of early initiation of therapy. However, treatment of individuals with Fabry-like symptoms and a mutation of unknown clinical significance in the GLA gene with ERT should be avoided. Thus, early demonstration of true onset of disease should be focus of further research. Surrogate markers should be discovered that unequivocally demonstrate onset of disease, preferably at a very early stage. Their regular monitoring may then assist in decision making on initiation of therapeutic interventions, ranging from co-medication to invasive ERT. In the meantime, selection of patients for treatment and for further family screening should be performed with the utmost care and based upon extremely careful clinical, laboratory and imaging investigations. In case of persisting doubt on a definite diagnosis, patients should not receive the diagnosis of Fabry disease and subsequently would not be eligible for treatment (primum non nocere).

LysoGb3 and clinical manifestations of Fabry disease

Our first investigations already indicated a correlation between lysoGb3 in females and LVmass (chapter 2). Classical male Fabry patients, presenting already with high lysoGb3 levels during childhood, showed no correlation of plasma lysoGb3 levels with LVmass or other disease manifestations (chapter 2). The first investigations dealt with a subset of the Dutch cohort. Extension of the study cohort and adjusting for important co-variables as age, revealed some interesting relationships. The more detailed analysis showed that there was a clear correlation of lysoGb3 with LVmass in females (n=55) and white matter lesions in males (n=37) (chapter 4). Again these analyses suffered from a relatively small cohort size: when combining data of both males and females, and adjusting for age and gender, there was a correlation between plasma lysoGb3, white matter lesions and LVmass (chapter 4). Despite the interesting associations between plasma lysoGb3 levels and certain disease manifestations, these observations offer no proof for a causal relationship.
Further experimental studies will be needed to test the possibility that increased lysoGb3 itself is causing specific symptoms, either directly or as risk factor. There was absence of correlation of lysoGb3 with some other characteristic Fabry disease features, including acroparesthesia, angiokeratoma and anhidrosis (chapter 4). This could be interpreted in different ways. One might suggest that there is no causal relation between lysoGb3 and these disease characteristics because another (currently unknown) mechanism is responsible for development of these outings. On the other hand, all included males in that particular study were classically affected and all demonstrated acroparesthesias and elevated lysoGb3, making a clear correlation impossible.

It is possible that high levels of lysoGb3 are associated with acroparesthesia as some of the neurological features in classical phenotypes were associated with lifetime lysoGb3 exposure (thesis M.G. Biegstraaten). These results are in line with the aforementioned statement that high lysoGb3 predicts a severe phenotype. To further delineate the relation between lysoGb3 and clinical symptoms assessments of these in a larger and diverse cohort should be performed.

Intriguingly there was no correlation between lysoGb3 and renal function in males nor females, which may suggest that there is another causal factor for kidney damage; it either develops because of another toxic factor or it develops secondary to other processes. Interestingly, others showed urinary lysoGb3 was correlated with serum creatinine as well as microalbuminuria and proteinuria which would suggest, lysoGb3 might be involved in kidney pathology at least to some extent 49. Future pathology studies, analysing the outcome of exposure to lysoGb3, could be helpful in elucidating the role of the lyso-lipid in the pathophysiology.

**LysoGb3 and response to enzyme replacement therapy**

LysoGb3 levels decreased after start of enzyme replacement therapy (chapter 2). The effect of treatment regimen (agalsidase alfa 0.2 mg/kg/2 weeks, agalsidase beta 0.2 mg/kg/2 weeks, agalsidase beta 1.0 mg/kg/2 weeks) and dose was investigated (chapter 6). Plasma lysoGb3 levels decreased dose-dependently within three months reaching almost stable, still elevated, levels within the first year of treatment (chapter 6). After the first year, plasma lysoGb3 levels remain reduced or may slowly decrease further (chapter 7). LysoGb3 level correlated with LVmass in females during ERT and in males that showed decline of LVmass (chapter 7). Furthermore, a lower lysoGb3 level correlated with a lower hazard ratio for developing cerebral white matter lesions (chapter 7). The fact that the amount of lysoGb3 reduction correlates with the amount of LVmass reduction and a lower lysoGb3 level is associated with a decreased hazard ratio of developing white matter lesions, suggests that a more robust decline of lysoGb3 is associated with a more favourable outcome on these clinical parameters. As lysoGb3 decreases in the first year after ERT and remains stable thereafter unless a change of dose is initiated, the most dramatic response of ERT is expected the first year after start of ERT. Indeed, for example
in males without severe kidney involvement, LVmass reduction was seen the first year of ERT, while after a few years of ERT LVmass increased in most male patients (chapter 9). Antibodies can be induced by enzyme replacement therapy in individuals without residual enzyme protein and activity (e.g. males). Such antibodies have a negative impact on reduction of Gb3 levels. The variable nature and slowly progressive course of disease as well as the limited efficacy of ERT, make it difficult to assess the exact impact of antibodies.

In a further study we noted that males with antibodies treated with a lower enzyme dose (agalsidase alfa 0.2 mg/kg/2 weeks or agalsidase beta 0.2 mg/kg/2 week), tended to show less decline of lysoGb3 compared to males without antibodies (chapter 6). Decline of lysoGb3 was less pronounced in males treated with agalsidase alfa or beta 0.2 mg/kg/2 weeks compared to antibody positive males treated with agalsidase 1.0 mg/kg/2 weeks and males without antibodies. In six males with antibodies the dose was increased during follow-up (agalsidase beta 1.0 mg/kg/2 weeks), showing an additional decrease of lysoGb3, though these levels remained higher compared to average levels in antibody negative patients. This suggests that a higher dose partly overcomes the negative antibody effects in lysoGb3 correction (chapter 7). Further evidence for a tight relationship between plasma lysoGb3 levels and enzyme dose was provided by dose reduction as result of temporary enzyme shortage demonstrating that a reduction of the dose from 1.0 mg to 0.2 mg/kg/2 weeks was followed by increases in plasma lysoGb3 levels. These findings concerning lysoGb3 reductions with different dosing regimens suggest that at a dose of 0.2 mg/kg/2 weeks the biochemical response is suboptimal. This points to the need for a higher dosage of enzyme for a maximal biochemical response. It once more highlights the question on the present use of a lower dose of agalasidase alfa being 5-fold more expensive per mg of enzyme as compared to agalsidase beta.

**Effectiveness of enzyme replacement therapy**

The first reports on clinical outcome of ERT were promising, showing decrease of acroparesthesia, decrease of LVmass and stabilisation of renal function. Since then many long term follow-up studies have been performed up to 5 years of ERT. In these studies, it is reported that LVmass remains stable or decreases slightly. Furthermore it is reported that renal function remains stable in patients without kidney involvement at baseline. In other patients, renal function declines despite therapy but ERT is suggested to slow the rate of decline. It has to be kept in mind that the effect of co-medication was not described or accounted for in a number of the reported studies, hampering the interpretation of the findings. In addition it is of importance that accurate methods for follow-up are used. As shown in chapter 8 there is considerable variation in accuracy and precision of the formulas used for estimation of glomerular filtration rate (GFR) as compared to the measured GFR.
Studies on development of cerebral white matter lesions in patients treated with ERT are limited. Furthermore, there are few studies on development of end-organ complications. There is only one placebo-controlled study with a mean follow-up of 18 months that studied the time to stroke, cardiac event, renal event or death. Presence of proteinuria was a major determinant for development of complications. If corrected for baseline proteinuria, the hazard ratio for developing a composite endpoint appeared lower in the ERT cohort compared to the placebo group (HR 0.47 (95% CI: 0.21-1.03), p=0.06). One important inclusion criterion in this study was decreased renal function defined as a creatinine of 106 umol/l on two consecutive measurements or a GFR< 80 ml/min. To evaluate the long-term outcome in patients with all degrees of organ involvement, as opposed to patients with kidney involvement only, we evaluated the course of disease during ERT treatment in the Dutch cohort, as described in chapter 9. In 30 adult males with Fabry disease, renal function declined independent of CKD stage, also in less severely affected patients. Furthermore LVmass decreased after the first year, followed by a gradual increase. In 27 females with Fabry disease, decline of renal function was comparable to the general population and LVmass remained stable. Cerebral white matter lesions developed despite treatment in both males and females. The time to the first end-organ complication was compared for ERT treated patients and symptomatic patients that did not start ERT because ERT was not available or that remained untreated after ERT became available because of various reasons. The odds to develop a first complication increased with age (OR 1.05 (95% CI: 1.0-1.1) per year, p= 0.012). For development of the first and the second complication in another end-organ the odds declined with longer treatment duration (OR 0.82 (95% CI: 0.68-0.96) per year of ERT, p=0.015 and OR 0.52 (0.31-0.88), p=0.014 respectively). Disease progression occurred also in patients with less severe organ involvement at start of ERT. These data were also analyzed in the meta-analysis performed in chapter 11. In this meta-analysis the course of disease during treatment was compared to the natural course, available from the literature. There was no difference in the course of renal function compared to the natural history in males nor females, with the exception of males with a estimated GFR < 60 ml/min/1.73 m². In this subgroup the decline of renal function was less severe in males with proteinuria also treated with ACE-ARB treatment. Therefore, whether this less severe decline of renal function was caused by ERT, by ACE or ARB or both could not be assessed. On the contrary, change of LVmass in males with Fabry disease compared to the natural course showed that despite increase of LVmass during ERT, this increase was less severe compared to natural history data. In females, LVmass remained stable or decreased as opposed to increase during the natural course. New cerebral white matter lesions are noted during ERT, however, no comparison with natural history data could be performed as different effect estimates were used (chapter 11). These data show that ERT has limited effect on most disease manifestations, in particular males. Probably, in some patients the disease progression is too advanced and reversal of tissue damage is not feasible anymore. However prolonged...
treatment duration reduces the odds of developing disease complications (Chapter 9). This is felt by some to offer an argument to initiate ERT as early as possible. In 6 adolescents (2 males, 4 females) that started ERT during childhood, renal function declined which could be interpreted as normalisation of renal function during ERT, LVmass remained stable but new cerebral white matter lesions appeared (Chapter 9). In view of this ERT might even be needed to be initiated before symptoms have developed. The outcome of treatment in minimally affected boys with Fabry disease is still awaited. Unfortunately the present study lacks a placebo-arm and moreover it seems likely that a large proportion of boys from families with classic Fabry disease will develop persistent neutralizing antibodies during ERT. The appearance of persistent antibodies against the infused enzyme might interfere with the effectiveness of ERT (Chapter 7).

An explanation of the limited effectiveness of ERT could be that current dosing regimens are not high enough to reach all relevant cells and tissues. Previous pharmacology data demonstrated that after 20 minutes after cessation of the infusion, the maximum plasma concentration of agalsidase beta has diminished by 50% when given at 1.0 mg/kg/2 weeks. Plasma levels of agalsidase are undetectable after 24 hours, indicating rapid uptake (or degradation). Recombinant alpha-galactosidase A is taken up by mannose 6-phosphate (M6P) receptors and variation in expression of these receptors on target cells may lead to insufficient delivery of enzyme to cells, cells that do not need clearance of storage material. In addition, the means by which recombinant alpha-Galactosidase A is taken up by endothelial cells have also recently been questioned, as uptake of the recombinant enzyme does not seem to be facilitated by the M6P receptors.

Health technology assessment, costs and societal perspectives

The increased awareness of efficacy of medical treatments and raise of medical costs has led to development of health technology assessments worldwide. The Dutch Health Insurance Board has requested investigation of the cost-effectiveness of new therapies, in particular orphan drugs because of their extreme costs. Agalsidase alfa and agalsidase beta, both orphan drugs, are previously approved under exceptional circumstances, as little information from clinical trials was available to determine the effectiveness of treatment. Both drugs are extremely expensive: around 200 000 euro for an average 70 kg adult per year.

To assess the cost-effectiveness of ERT for Fabry disease, a health technology assessment (HTA) study with data from the Dutch Fabry cohort was conducted (Chapter 10). Previous studies on HTA in Fabry disease were hampered by the limited data available leading to assumptions as complete recovery of disease that were rather optimistic. The costs per QALY were estimated £ 252 000,- in the UK and $ 300 000 per QALY in the US. Recently a health technology study in England showed that at least 4 discounted QALYs for an adult patient with Fabry disease are needed for ERT to be cost-effective, considering a willingness to pay of £ 30 000. Analysis of the data from the
Dutch cohort, revealed the costs per QALY are much higher than previously estimated (chapter 10). Over a 70 year lifetime, the costs of ERT starting in the symptomatic stage are €2,947,380 per QALY (€5,451,797 undiscounted) for a male and €3,742,702 (€6,955,612 undiscounted) for a female during a patient’s lifetime. Taking in consideration, an untreated Fabry patient will on average experience 55.0 years (53.5 years in males and 56.9 years in females) free of end-organ damage (i.e. complications) and starting ERT increases the number of years without end-organ complications by 1.5 years (1.6 in males and 1.3 in females), the effectiveness of therapy is disappointing. The present study highlights the enormous costs associated with ERT and shows costs per QALY are much higher than previously estimated 74, 75. However, further scenario analyses showed that ERT is more effective in certain subgroups. ERT is most cost-effective in males with a classic phenotype, not taking in account the beneficial effect of ACE-ARB. As can be expected, ERT is less cost-effective in milder affected patients, such as women en atypical phenotypes. These observations may lead to reconsider whether ERT should be initiated in all patients, especially patients with a mild disease course. Ideally more data from other countries would be combined to further identify subgroups that benefit most from ERT. Last but not least, new research should focus on increasing effectiveness of ERT treatment by optimizing dosing and timing of treatment. The effectiveness of ERT and its tremendous costs will lead to discussion between government, health insurance companies, health professionals, patient organizations and industries, on indication, price and future therapies for Fabry disease.

Clinical implications and future perspectives

It is of importance to gain better understanding of the pathophysiology of Fabry disease to optimize treatment. From this thesis it can be concluded, that lysoGb3 response after start of ERT is indicative for amelioration of the course of disease, at least for some clinical parameters (chapter 7). In addition, the development of antibodies interferes with the lysoGb3 response, indirectly indicating that appearance of antibodies hampers the effectiveness of ERT. Indeed, increase of dose, showed a further decrease of lysoGb3, although levels of lysoGb3 remained higher in the antibody positive patients as compared to the antibody negative patients. This would implicate that dose as well as antibodies do matter. Based on the results described in chapter 2, 4, 6 and 7, absence of response to lysoGb3 suggests treatment failure. Studies in Gaucher disease, clinically much easier to monitor, since hepatosplenomgaly and cytopenia are specific features that response quickly to therapeutic intervention, point to the same direction. In patients without a response in chitotriosidase, a very reliable marker of Gaucher cell burden, clinical response is absent 77.
Collection of more data through international collaborations will increase the knowledge of Fabry disease, and render more insight in the implications of lysoGb3 response, development of antibodies and dosing. In addition questions about which patient characteristics determine the course of disease and treatment outcome could be answered when data from larger patient cohorts are available. Currently ERT is the only registered treatment for Fabry disease. Though data are derived from small study cohorts, it is clear that ERT is not as effective as initially hoped and expected by some. Considering the limited clinical effectiveness and its extreme costs, a more rigorous assessment of its pharmacological effectiveness should be performed. For example, insight on the therapeutic enzyme’s actual tissue and cell type distribution could in principle be obtained after administration of labelled recombinant alfa-Galactosidase A at different time points. Gaining more knowledge on the activity of recombinant alfa-Galactosidase A in vivo could lead to optimization of this treatment modality. The limited clinical effectiveness of ERT could partially be explained by anti-bodies against the infused enzyme (chapter 7). There remains an urgent need for alternative therapies for Fabry disease. A modified alpha-N-acetylgalactosaminidase (NAGA) with alpha-Galactosidase A like substrate specificity was designed and found to cleave Gb3 in cultured fibroblasts from a patient with Fabry disease. Studies demonstrated that there was no immunological cross-reactivity between the modified NAGA and alpha-Galactosidase A. Thus, this approach could result in a promising new enzyme therapy in Fabry disease males lacking aGalA protein, but such an approach is not likely to demonstrate more effectiveness than current ERT in patients who do not develop antibodies. Alternative therapies for Fabry disease, not based on enzyme supplementation, are being developed or still investigated in clinical trials.

Chaperone therapy, a molecule that binds to aGal non-covalently and which stabilizes it, is currently investigated (phase III trial). As such an approach requires the synthesis of a (though mutated) enzyme, it is only applicable for patients producing an enzyme with a particular (missense) mutation. Often these specific missense mutations also harbour limited but significant residual enzyme activity and thus may express less severe disease. Therefore it is of great importance to compare the outcome of such a therapeutic approach to a group of non-treated patients with similar mutations. In addition, the natural course of Fabry disease in such a cohort needs to be studied carefully, to assess the impact of this treatment.

Another modality of chaperone treatment is the combination of ERT with the chaperone, in order to promote (recombinant) enzyme stability and possibly prolong plasma half life. Pharmacokinetic studies with chaperone and ERT are currently ongoing. If this results in a prolonged plasma clearance, additional studies are needed to demonstrate whether a prolonged plasma half life is beneficial for therapeutic effectiveness.
Finally, selective inhibition of Gb3 synthase may also offer an attractive target. A natural deficiency of Gb3 synthase in humans is known without any complications. An additional positive outcome of this approach is that no antibody formation is likely. While gene therapy has been promoted as the ultimate cure, the formation of antibodies towards (endogenously) produced enzyme can be expected.

The last decade, after the introduction of enzyme replacement therapy, it has become clear, that ERT, combined with other supportive treatment, can slow disease progression, though disease progression is not halted. Further understanding of the pathophysiology of the disease, the pharmacokinetics of ERT and use of biomarkers to predict prognosis and response to therapeutic intervention are required to improve the treatment of patients with Fabry disease.
Reference List


