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Enzyme replacement therapy in Fabry disease, towards individualized treatment

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Summary and general discussion

Summary

After more than 15 years of market authorization of enzyme replacement therapy (ERT) for Fabry disease (FD), it is still not fully elucidated which patients benefit most from treatment with ERT. Lack of high quality, long-term data on clinically relevant endpoints has hampered the identification of subgroups that benefit most from ERT. Moreover, phenotypic differences often have not been taken into account. 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. These studies have been supported by a grant from ZonMw (project number: 836011009) in the program 'Goed Gebruik Geneesmiddelen', a program initiated by the Dutch Ministry of Health in order to stimulate effective, safe and appropriate use of medicines.

Most of the studies included in this thesis originate from a collaboration between three treatment centers of excellence: the Academic Medical Center, Amsterdam, The Netherlands; the Royal Free London NHS Foundation Trust, London, United Kingdom and the University Clinic Wuerzburg, Wuerzburg, Germany. Data on all FD patients from these three centers were merged into one database and are used for the studies published in chapters 3 to 7.

Chapter 2 comprises a systematic review on the quality of life (QoL) in FD. Most studies used the 36 item Short Form Health Survey (SF36) or the Euroqol five dimensions questionnaire (EQ5D) to measure QoL in FD. Furthermore, the Brief Pain Inventory was used to assess (the impact of) pain. Both men and women with FD had lower QoL scores compared to the general population. The QoL of FD patients was comparable to patients with other chronic conditions, *i.e.* rheumatoid arthritis and multiple sclerosis. Renal disease, disease severity and age were related to QoL in FD. No clear effect of ERT on QoL could be found. A limitation of the included studies was that no difference between gender or phenotype was made. Hence, we analyzed the QoL in patients from two referral centers from the Netherlands and the United Kingdom **(chapter 3)**. We confirmed that age was an important predictor of QoL. Men with a classical FD phenotype had a lower QoL compared to non-classical men and women. In addition, the decline in QoL with increasing age was more pronounced in men with classical FD. This might be caused by a higher prevalence of FD related complications, which are associated with lower QoL. The BPI pain scores correlated with the QoL scores. Interestingly, high proportions of pain were reported irrespective of phenotype and the presence of acroparesthesia, suggesting that pain in FD comprises more than acroparesthesia alone.

Since natural history data, especially stratified for phenotype and gender, is scarce, we evaluated the event rate before first visit as well as the renal function and cardiac mass at first visit in almost 500 patients from the three centers of excellence **(chapter 4)**. As one might expect, men with classical FD had a higher risk to develop complications compared to men

with non-classical FD and women. In women, a classical phenotype was also associated with a history of more events compared to the non-classical phenotype. Increasing age was an important predictor of lower renal function and higher cardiac mass. In addition, renal function was worse in men with classical FD compared to the other groups. The cardiac mass in patients with classical FD was higher compared to those with non-classical FD. Furthermore, late gadolinium enhancement, as marker for cardiac fibrosis, was associated with left ventricular hypertrophy in men. Interestingly, in women there was no such relationship: women frequently developed cardiac fibrosis in the absence of cardiac hypertrophy.

In **chapter 5** we evaluated which prognostic factors predicted the response on ERT. We therefore investigated clinical parameters of 293 patients receiving ERT. As expected, age, sex and phenotype were important predictors of event rate. Clinical events before ERT, increased cardiac mass and especially lower renal function at baseline were associated with an increased event rate. In addition, patients with impaired renal function at baseline showed a stronger decline in renal function during treatment; this effect was most prominent in men with classical FD. Another important predictor of the decrease in renal function was the presence of proteinuria. Furthermore, there was a trend towards an association between cardiac mass at baseline and the clinical event rate. The presence of cardiac fibrosis was associated with a stronger increase in cardiac mass. Of other cardiovascular risk factors, hypertension significantly predicted the risk for clinical events. We concluded that besides increasing age, male sex and classical phenotype, faster disease progression while on enzyme replacement therapy is predicted by renal function, proteinuria and to a lesser extent cardiac fibrosis and hypertension.

In **chapter 6**, we compared the effect of ERT with agalsidase alfa and agalsidase beta at their registered dose (0.2 mg/kg every other week and 1.0 mg/kg every other week, respectively) on clinical and biochemical outcomes. Data from more than 100 Canadian FD patients were added to the existing data of the three European centers. In total, almost 400 patients were included. There were considerable differences in both treatment groups at baseline. After adjusting for renal function, cardiac mass, events before baseline and age, no difference in event rate was found which was confirmed by a propensity score matched analysis. However, we observed a slightly better decline in cardiac mass with agalsidase beta. In addition, plasma globotriaosylsphingosine (lysoGb3), a biomarker in FD, decreased significantly better in the agalsidase beta group. Despite the fact that antibodies were more frequent following treatment with agalsidase beta in men witch classical FD, the decrease in lysoGb3 was still more robust compared to the men with classical FD receiving agalsidase alfa. This could be partially explained by the impaired lysoGb3 response in patients receiving agalsidase alfa who were antibody positive, while antibodies did not lead to a reduced lysoGb3 response in patients treated with agalsidase beta.

Because it is frequently suggested that early treatment is important, particularly in classically affected men, we compared the response in lysoGb3 in men with classical disease treated before (early-treatment) and after (late-treatment) the age of 25 **(chapter 7)**. Patients from the early-treatment group had lower lysoGb3 levels during ERT compared to the late-treatment group. Twice as many patients in the early-treatment group reached a lysoGb3 level <20 nmol/l one year after treatment start. Interestingly, a higher proportion of antibodies was found in the late-treatment group. Adjusting the lysoGb3 analysis for the presence of antibodies did not change the results. These findings suggested that initiation of ERT at younger age results in a better biochemical response in men with classical FD.

In **chapter 8** we described our experiences with the discontinuation of ERT in FD. Seventy-five patients started with ERT treatment between 1999 and 2015 in the Netherlands; 21 of these patients discontinued ERT during that period. Patients who discontinued were generally more severely affected. In the first ten years after introduction of ERT, six patients died while receiving ERT or ERT was stopped because of a very limited life expectancy (<3 months). More recently, treatment failure became a more important reason for cessation of ERT. Furthermore, in eight patients, primarily women, ERT was stopped at their own request, most often because of a disbalance between experienced benefit and burden of ERT. We concluded that in patients with combined end stage renal disease and heart failure, despite ERT, discontinuation of ERT should be considered. However, decisions to stop ERT should always be made on an individual basis, taking into account the disease severity and personal circumstances of the patient.

General discussion

The heterogeneity of Fabry disease

The disease course in Fabry disease (FD) is highly heterogeneous. The differences in onset and severity of disease manifestations between men and women are evident.[1] As a result of the enhanced interest in the disease following the introduction of enzyme replacement therapy (ERT), differences in phenotypes have been increasingly recognized. Because it is likely that patients differ in disease course and response to therapy, criteria for phenotypical classification have been proposed.[2] By applying these criteria, it is clear that in many studies, phenotypes are mixed up, which hampers interpretation of data.[2] For instance, natural history data for the different phenotypes are scarce, which led us to characterize the course of disease in classical and non-classical FD patients. In **chapter 4** we show that major FD related events occur more frequently and at younger age in patients with a classical phenotype. This difference is observed in men as well as women. A classical phenotype was also associated with worse renal function and higher left ventricular mass compared to the non-classical phenotype. These differences are also reflected by lower quality of life in patients with classical FD, especially at older age **(chapter 3)**. Interestingly, men with non-classical FD

and women with classical FD are more or less comparable in terms of event rate, eGFR and quality of life. These results show that adjusting or stratification for phenotype is crucial in any clinical study on FD.

Heterogeneity in FD is not surprising: this is in almost all lysosomal storage disorders the rule rather than the exception. For example, patients with alfa-l-iduronisdase deficiency have mucopolysaccharidosis type I (MPS I), but can be phenotypically distinguished in the severe neuropathic Hurler phenotype and non-neuropathic Scheie syndrome as extremes of a wide phenotypic spectrum.[3] Similarly, Gaucher disease, caused by beta-D-glucosidase deficiency, can be generally classified into attenuated non-neuropathic and more severe neuropathic phenotypes. The most severe form has an infantile onset phenotype (Gaucher diseae type 2), which leads to early death.[4] In Pompe's disease, caused by alfa glucosidase deficiency, a rapidly progressive infantile phenotype and a more attenuated late-onset phenotype are distinguished.[5] In these lysosomal storage disorders, genotype-phenotype relationships are usually only clear for infantile forms: in general, infantile forms are related to absent or strongly reduced residual enzyme activity. However, the more attenuated forms have less clear relationship with genotype and residual enzyme, suggesting that the disease course is influenced by other (epi) genetic or environmental factors.[4] In FD, no infantile form exists. Here, the genotype/phenotype correlations are complicated due to the many private mutations. Some 'null' mutations, which evidently lead to no (functional) enzyme, are associated with the classical phenotype in men. In women the genotype-phenotype relation is even less clear due to the potential influence of skewed X-inactivation.[6] Consequently, even within families patients may have different phenotypes. In addition, there is considerable overlap of symptomatology with more common disorders such as hypertension and diabetes, which, like FD, can lead to left ventricular hypertrophy, albuminuria and impaired renal function. The heterogeneity of FD across and within phenotypes as well as the overlap of symptomatology with more common disorders hampers the study of the effects of medications, including ERT. In the search for underlying causes for the heterogeneity, the (epi) genome of discordant phenotypes with a similar genetic background should be studied to find additional genetic factors that contribute to the phenotype.[7-9]

Biochemistry

In line with previous publications from our group and others, we have shown that plasma lysoGb3 almost completely discriminates between the classical and non-classical phenotype in men **(chapter 3)**.[10,11] A value of 45 nmol/l was proposed as cut off value for classical FD in men by Smid et al.[10] In our study, there was some overlap in plasma lysoGb3 of untreated patients in the range between 32 and 47 nmol/l. This is probably caused by misclassification of some patients due to the retrospective character of the study. Nonetheless, lysoGb3 levels above this range are strongly suggestive for a classical phenotype in men. In women, lysoGb3 levels were significantly higher in those with a classical phenotype than in women with non-classical FD, but there was considerable more overlap than in men. In men

with non-classical FD and women higher plasma lysoGb3 levels were associated with more severe disease **(chapter 3)**. Aurey-Blais et al showed that not only plasma lysoGb3, but also urinary lysoGb3 and analogues correlated positively with disease severity.[12] LysoGb3 analogous in plasma as well as urine might provide valuable additional information on phenotypes and specific organ involvement, and therefore should be studied in more detail.[13,14] Such a study should aim to find specific patterns and/or ratio's of lysoGb3 analogous which could predict phenotype, renal or cardiac disease in a well-defined Fabry population. Such a metabolic fingerprint could then be used in the diagnosis and management of FD.

Antibodies against the administered enzymes might negatively influence the efficacy of ERT trough altered tissue distribution, metabolism, receptor binding, subcellular trafficking or catalytic activity.[15] The development of antibodies is uncommon in men with non-classical FD and women and seems to be limited to men with a classical phenotype **(chapter 6)**.[16,17] This is most likely caused by the (near) absence of endogenous alpha galactosidase in these patients, similar to the CRIM negative status in Pompe's disease.[18,19] In classical infantile Pompe's disease the presence of antibodies is predictive of clinical outcome and immunomodulation has successfully been applied.[20] In FD one could debate if the side-effects of immunomodulation therapy outweigh the potential benefits. Immune tolerance induction by higher doses have proved to be very successful in hemophilia, but seems unfeasible for lysosomal disorders due to the already extreme high costs of ERT.[21,22] Nevertheless, there are several opportunities to increase our understanding of the antibody development in FD, such as characterization of the IgG subclasses or characterization of the binding sites of the antibodies. These studies could aid in the development of better treatment strategies. Also, antibody development should be an important item in the development and studies of new therapies in any lysosomal storage disorder.

Effectiveness of enzyme replacement therapy in relation to sex and phenotype

So far ERT is the only specific treatment in FD, which is extended to real-life experience. Both agalsidase alfa (Replagal®) and agalsidase beta (Fabrazyme®) have been licensed for more than fifteen years. Long-term observational studies have shown that the effectiveness of ERT is modest: FD related complications may be postponed but cannot be prevented and there is a limited effect on renal function.[23-26] However, these studies looked at the overall ERT effect across the entire patient population. Since the disease course highly depends on sex and phenotype **(chapter 4)**, different effects of ERT might be expected.

The potential benefit of treatment is largest in the most severely affected patients (*i.e.* men with classical FD). This principle is illustrated by the relation between cardiac mass at baseline and the decrease in cardiac mass following ERT. However, with more advanced FD the risk of irreversible organ damage also increases. We have shown, for example, that the presence of cardiac fibrosis was associated with accelerated progression of cardiac hypertrophy, confirming previous findings of Weidemann et al.[27] In analogy to irreversible cardiac fibrosis,

renal involvement may eventually result in irreversible damage to the kidneys. Banikazemi et al showed that ERT was able to slow down disease progression in patients with mild renal impairment, but not in those with severe renal impairment.[28] In line with these results, we showed that the risk of developing events increased with lower renal function at baseline **(chapter 5)**. Consequently, initiation of ERT before the occurrence of irreversible disease manifestations (*i.e.* cardiac fibrosis, extensive glomerular sclerosis) is warranted.

In contrast to classically affected men, the disease is more limited in men with non-classical FD. The primary involved organ in this group is the heart. Some develop severe renal impairment, but at a much later stage compared to classically affected men. The impaired kidney function in these patients probably has a different cause and occurs most likely secondary to congestive heart failure in combination with involvement of the kidney by FD itself. Similar to the classical FD group, ERT seems relatively effective in reducing the cardiac mass, provided that cardiac fibrosis is absent.[27,29] Consequently, men with non-classical FD with cardiac hypertrophy may benefit from ERT. However, when comparing the course of treated patients with the natural history data presented in chapter 4 one could doubt if treatment with ERT is beneficial since the disease course does not seem to be significantly altered by ERT. Moreover, other cardiovascular risk factors might be more important determinants of the overall morbidity and mortality in these patients. We would propose to decide on initiation of ERT in this patient group on a case by case basis; if there is evidence of a considerable increase of cardiac mass and/or family members have shown severe cardiac disease at young age, treatment with ERT is recommended.

The differences between classical and non-classical disease in women are less prominent compared to the differences in men, but the disease course is more heterogeneous. Clearly, the disease is in general milder than in men, and severe renal involvement leading to end stage renal disease is only very rarely observed in women with either classical or non-classical FD **(chapter 4)**. On the other hand, women may develop severe cardiac disease, including left ventricular hypertrophy and arrhythmias.[1,30] Of importance is that, in contrast to men with FD, in women cardiac fibrosis was frequently observed in the absence of left ventricular hypertrophy **(chapter 4)**.[31] Consequently, if ERT is only started if left ventricular hypertrophy is present, treatment may be started too late in these women considering that fibrosis is irreversible and therefore unresponsive to ERT.[32] So far, NT-proBNP and strain rate by speckle-tracking echocardiography have been identified as early markers of cardiac disease. These parameters are both associated with diastolic dysfunction but there is insufficient information on the predictive value of these markers.[32-35] Moreover, diagnostic accuracy is probably limited. Alternatively, cardiac biopsy may be considered to confirm (the extent of) cardiac involvement in women with FD. However, such biopsies are not without risks. Finally, T1-mapping on MRI may provide a valuable alternative with high specificity. Accumulation of Gb3 and/or related changes results in a decreased signal on T1 mapping.[36,37] Certainly, this technique needs further study and it would be interesting to investigate if T1 mapping

values are predictive for the development of cardiac fibrosis in patients with but also without left ventricular hypertrophy.

Effectiveness of ERT in relation to co-medication and co-morbidities

It has been hypothesized that FD and more prevalent diseases such as diabetes and hypertension have a final common pathway. Hypertension eventually leads to hypertrophic cardiomyopathy, which is often difficult to distinguish from cardiomyopathy caused by FD.[38] If patients have both hypertension and FD, the relative contribution of each of these diseases is difficult to determine. Similarly, some of the pathways implicated in the pathophysiology of glomerulosclerosis in FD are also suggested to play a role in the development of diabetic nephropathy.[39] Finally, cerebrovascular disease in patients with FD as well as the general population is associated with an increased intima-media thickness. Unlike in the general population, however, its presence in FD has not been associated with atherosclerosis which points to different, probably FD-related alterations in the vessel walls.[40,41] On the other hand, FD mice with apolipoprotein E deficiency show accelerated atherosclerosis. This suggests that Fabry disease might pose a risk for the development of atherosclerosis in the context of other risk factors. With respect to potential risk factors for cerebrovascular events, we showed that triglyceride levels, but not cholesterol, were related to an increased event rate **(chapter 5)**. Although the pathophysiological relationship between triglyceride levels and the occurrence of events is not well understood, we argue that it is of importance to explore whether lipid-lowering therapy is useful. We also found an association between the presence of hypertension and an increased risk for clinical events. Elevated systolic blood pressure has previously been reported to be associated with progression of cardiac fibrosis in FD.[32] Strict control of blood pressure is therefore of utmost importance in order to reduce the risk for vascular events. This is supported by the observation of a marked reduction of renal and cardiovascular events with strict blood pressure control in patients with diabetes.[42] Indeed, adjunctive treatments in FD are widely implemented. Angiotensin-converting-enzyme inhibitors (ACEi) and angiotensin-receptor-blockers (ARBs) are the cornerstone of treatment of proteinuria, but are also frequently prescribed for cardiac failure and hypertension.[43] A previous study evaluating the effect of combined treatment with an higher ERT dose and ACEi/ARBs showed that in patients who reached a protein-creatinine ratio <50 mg/mmol, kidney function was perserved.[44] We were not able to confirm a relation between the use of ACEi/ARBs and clinical outcomes, which may have been due to the fact that we used real-world data and therefore no uniform criteria for that start of ACEi/ARBs were used, but still endorse the use of ACEi/ARBs in patients with FD and proteinuria. A recent study has suggested an additional renoprotective effect of add-on paricalcitol, a synthetic vitamin D analog with potential selective vitamin-D-receptor activing properties.[45] Vitamin-D-receptor polymorphisms and vitamin D deficiency have been associated with the severity of FD providing an additional argument for a potential role for vitamin D (analogs) in the treatment of FD.[9,46]

Impact of dose on biochemical and clinical outcomes

Since the introduction of ERT for the treatment of FD, there has been a lot of discussion about differences and similarities of agalsidase alfa and beta, both on a biochemical and clinical level. It is surprising that after 15 years, these issues have still not been resolved. We have studied both the biochemical impact and clinical outcomes of the two enzymes, adjusted for differences in sex, phenotype and disease stage. This has resulted in some important new insights: firstly, we have shown a clear dose effect on clearance of lysoGb3 from plasma following ERT **(chapter 6)**, which is most robust in men with classical FD **(chapter 4)**. This is in line with the observation that patients who switched or received a lower dose during the shortage of agalsidase beta showed a small increase in lysoGb3.[47] There also seems to be a better clearance of globotriaosylceramide (Gb3) from podocytes with a higher cumulative dose.[48,49] Moreover, we found that timing of treatment initiation was an important predictor of the decrease in lysoGb3 in men with classical FD: initiation of ERT before the age of 25 led to lower lysoGb3 values when compared to patients who started ERT above that age **(chapter 7)**. The reduction in plasma lysoGb3 concentrations following ERT could be either caused by intra-lysosomal degradation of lysoGb3 or result from the clearance of accumulated Gb3, limiting the source of production of lysoGb3.[50] Compared to Gb3, the degradation of lysoGb3 by recombinant aGAL was found to be at least 50-fold less efficient in vitro.[50] Furthermore, it has been shown that outside the acid environment of the lysosome, hydrolysis of lysoGb3 by recombinant aGAL is virtually absent due to the pH optimum of alpha galactosidase.[50] The decline of lysoGb3 concentrations following ERT is therefore most likely primarily the result of a reduction of the amount of accumulated Gb3. Previous studies in children with FD have shown that the amount of podocyte Gb3 inclusions increased progressively with age.[49,51] In analogy to Gaucher disease we hypothesize that more extensive glycosphingolipid storage inhibits the effectiveness of ERT in reducing lysoGb3. This, in relation to more extensive Gb3 inclusions in podocytes with older age, could explain why there is a better treatment response if ERT is started at younger age. In addition, considering the inhibitory effect of lysoGb3 on aGAL activity, high intracellular lysoGb3 levels could in fact reduce the effect of ERT.[50] Although useful in evaluating treatment response, lysoGb3 has its limitations. Cardiac fibrosis and glomerulosclerosis represent irreversible disease manifestations. Consequently, ERT may lower lysoGb3 values, but this will probably not affect outcome. For example, a man with classical FD who started ERT at the age of 45 may show a decrease in lysoGb3 from 100 nmol/l to 17 nmol/l. However, if this man had severely impaired renal function (eGFR 30 ml/min/1.73m^2) before the start of ERT, renal function will worsen despite ERT, eventually necessitating renal replacement therapy, despite his "favorable" biochemical response. Thus, lysoGb3 reflects the clearance of accumulated sphingolipids, however this has no influence on the already ongoing process of sclerosis and fibrosis. On the other hand, in the evaluation of the effect of antibodies, lysoGb3 has been proven useful. In line with others, we found that antibodies against the recombinant alpha galactosidase were more prevalent during treatment with agalsidase beta than with agalsidase alfa **(chapter 6)**.[52-54] However, despite a higher proportion of patients with antibodies, lysoGb3

values were lower in patients treated with agalsidase beta. In **chapter 6** we showed that the presence of antibodies in patients receiving agalsidase alfa leads to a less prominent decrease in lysoGb3, while the decrease in lysoGb3 is unaffected by antibody formation during treatment with agalsidase beta. Presumably, the fivefold higher dose overcomes the negative effects of antibody formation.

In other lysosomal storage disorders, such as Gaucher disease, biomarkers, including substrates such as glucosylceramide and glucosylsphingosine, correspond well with disease severity and can be used to evaluate the effect of therapeutic interventions.[55,56] This suggests that in FD the difference in lysoGb3 response to higher dose is reflected in a clinical effect as well. Although in our study no difference in event rate was observed, a slightly better effect on the cardiac mass was found with a higher dose, *i.e.* agalsidase beta **(chapter 6)**. In line with our results, no change in clinical event rate was observed in studies that evaluated the effect of switching or dose reduction during the worldwide shortage of agalsidase beta.[47,57-59] These studies were, however, often small and had a relatively short follow-up. To increase power, data from more than 100 patients who were followed in the context of the Canadian Fabry Disease Initiative (CFDI) were added to the existing data of the three European centers, but even with almost 400 patients our study was probably underpowered as well. The investigators of the CFDI calculated that 600 patients would be needed to detect a ten percent difference.[60] More generally, the potential beneficial effect of ERT has probably been underestimated since patients with different disease stages have been analyzed, thus including patients with irreversible organ damage as well whose organ involvement is unresponsive to treatment. Consequently, a high number of FD patients without signs of irreversible organ damage is needed if an effect on clinical events is to be shown. It is unlikely, however, that such a study will be feasible. Alternatively, randomized studies with surrogate endpoints (*i.e.* plasma lysoGb3 or renal biopsies) and clear definitions of classical and non-classical disease would be helpful in substantiating our findings. Such a study could have a relatively short follow up, as the greater part of the effect of ERT on biochemical endpoints is seen in the first years after initiation.[49] Up until then, we would recommend to switch patients with persistent high levels of plasma lysoGb3 combined with high antibody titers while treated with agalsidase alfa to a higher dose (*i.e.* agalsidase beta).

What is left untreated

It has become clear from our as well as previous studies that disease progression despite ERT is present. There are some Fabry disease manifestations, which seem particularly unresponsive to ERT, including cerebrovascular disease, neuropathic pain, gastro-intestinal symptoms and quality of life.[23-25] Besides strokes and transient ischemic attacks (TIAs), cerebral involvement in FD is characterized by the presence of white matter lesions (WMLs).[61] In the general population, WMLs are clearly associated with an increased stroke risk,[62] but in our study, the presence of WML was not predictive for the development cerebral events **(chapter 5)**. This could have been due to the relatively small sample size but also the exclu-

sion of patients who cannot undergo MRI scans (*e.g.* with ICDs or pacemakers) may have biased our results. Previous studies have shown that WMLs as well strokes and TIAs are unresponsive to ERT.[24,63-65] Since life expectancy of FD patients has increased with the availability of ERT and improvement of adjunctive treatments with amongst others ACEi/ARBs, cerebral manifestations are more prevalent. Even vascular dementia is regularly seen in classically affected men with advanced disease. In some cases this was one of the underlying reasons to discontinue ERT in advanced FD **(chapter 8)**. It is unknown if cerebral manifestations are caused by small vessel disease alone, or if accumulation of sphingolipids in neurons plays a part as well.[66] In the latter case, the blood-brain-barrier opposes a boundary for ERT to reach brain neurons, similar to almost all other lysosomal storage disorders.[67] Emerging therapies, including small molecules and perhaps nanotechnology might overcome this obstacle.[67,68]

Studies on the effect of ERT on neuropathic pain have shown conflicting results. Beneficial effects of agalsidase alfa on pain have been observed in the pivotal trial.[54] This double-blind placebo-controlled trial of 26 adult male patients used the Brief Pain Inventory neuropathic pain severity score as primary outcome measure. A significant decrease of 2 points in ERT treated patients versus no significant change in the placebo group was found, but interpretation of these results were hampered by an imbalance in pain scores at baseline. Moreover, these findings were not corroborated by the pivotal study on the efficacy of agalsidase beta in which statistically significant decreases in pain scores were observed in both the ERT and the placebo group.[53] Long term observational studies showed no reduction of pain severity with ERT,[69,70] while there is some evidence for a beneficial effect on neuropathic pain in children with FD.[71,72] In our studies we did not evaluate the effect of ERT on neuropathic pain, but clinical experience has taught us that there is in general no sustainable effect of ERT. Still, the presence of neuropathic pain may be a reason to start ERT in FD patients, mainly because classical acroparesthesia in young patients are in general associated with more progressive disease. In this light, careful assessment of pain is of utmost importance; only those patients who exhibit chronic, burning pain in hands and feet with pain crises provoked by heat or exercise should be diagnosed with Fabry neuropathic pain. If there is doubt about the character of pain, additional investigations are needed. Besides, symptomatic pain treatment may be indicated. A recent systematic review demonstrated that the currently available effectiveness data are mainly derived from case reports and small observational studies.[73] It was concluded that large, high quality, long duration studies with robust clinical endpoints on different pain management strategies, preferably in a uniform group (*i.e.* men with classical FD), are needed.[73] Pain relief is of particular importance since pain has a great impact on the quality of life of FD patients **(chapter 2 and 3)**. Thus, pain should be adequately assessed and treated in FD patients.

Clinical implications

In 2015, our hospital organized a consensus procedure among European experts to define recommendations on initiation and cessation of ERT in FD.[74] During this procedure many

decisions were made on the basis of expert opinion, and it became clear that studies addressing the effectiveness of ERT in patients with various degrees of kidney, heart or brain involvement were urgently needed.[74] With the studies presented in this thesis some of these issues have been addressed. Firstly, in the procedure no consensus was reached whether severely impaired renal function (<45 ml/min/1.73m^2) should be an indication to start ERT or not. We showed that renal function is the most important predictor for the effectiveness of ERT. Already with a renal function below 60 ml/min/1.73m^2 the risk to develop clinical events increases significantly. Also, the yearly decline in renal function is considerable in this group of patients **(chapter 5)**, and comparable to untreated patients.[24] Therefore, doctors and patients should be reticent to initiate ERT in patients with an impaired renal function (<60 ml/min/1.73m^2), while treatment with ERT should be discouraged in patients with a renal function below 45 ml/min/1.73m^2. However, it should be noted that renal replacement therapy (*i.e.* dialysis or kidney transplantation) is an effective treatment option in FD patients with end stage renal disease. Since end stage renal disease is very uncommon in patients with the non-classical phenotype, impaired renal function in these patients should be carefully evaluated in order to find other (treatable) causes of renal dysfunction **(chapter 4)**. If another underlying disease is found and no other FD manifestations are present, treatment with ERT should be discouraged. If no other cause for the renal dysfunction is found, ERT may be indicated, but only if the GFR is >45 ml/min/1.73m^2.

We have shown that women with non-classical FD generally have very limited disease. We therefore recommend restrictive use of ERT in these patients. Only in case of cardiac disease that can be unambiguously ascribed to FD, initiation of ERT may be considered. Subsequently, the effect of treatment should be carefully evaluated by predefined parameters determined by the treating physician and patient. If no treatment effect is observed, discontinuation of ERT should be considered. Our findings combined with the knowledge from pre-existing literature suggest that agalsidase beta has superior effects compared to agalsidase alfa. We therefore recommend to prescribe agalsidase beta in ERT naïve patients with an indication to start ERT **(chapter 6)**. Only if a patient strongly prefers a short initial infusion time, agalsidase alfa can be considered. The prevalence of antibodies and infusion reactions is higher in patients treated with agalsidase beta compared to alfa. Although not specifically studied, these findings suggest that switching to agalsidase alfa may be beneficial in patients with severe infusion related reactions. Finally, an open discussion between doctor and patients on discontinuation of ERT is needed, especially in patients with advanced FD **(chapter 8)**. In any case, decisions on the initiation or cessation of ERT should be made on an individual basis taking disease severity, comorbidities and personal circumstances into account.

Future perspectives

Clearly, new treatments should be developed in order to improve the prospect for patients with Fabry disease. Recently, migalastat (Galafold®) has received marketing authorization for the treatment of FD. This is an oral chaperone therapy, developed to enhance endog-

enous GLA enzyme activity. Inherent to this type of therapy, some degree of functional residual enzyme activity needs to be present. Consequently, patients with null mutations or large deletions (*i.e.* men with classical disease), and thus zero residual enzyme activity, cannot benefit from migalastat.[75] The pivotal study on the efficacy of migalastat compared to placebo has shown that this drug may reduce cardiac mass and lysoGb3 concentrations in previously untreated patients, although considerable variation was seen.[76] The future should learn if there is a subgroup of patients that may benefit from treatment with migalastat in a real-world setting. Substrate reduction therapy - a therapy by which the formation of the accumulated lipid is blocked - might be another potential treatment modality for FD. There are already several substrate reduction therapies available for the treatment of other lysosomal storage disorders. Last year, the FDA granted a 'Fast Track Designation' to a novel glucosylceramide synthase inhibitor. This is a small molecule that blocks the formation of glucosylceramide, an upstream metabolite in the formation of Gb3. By inhibiting one of the first steps in sphingolipid formation it might also be used in other lysosomal storage disorders, including Gaucher disease. Recent results in FD mice showed promising results on the clearance of sphingolipids from several tissues, including the brain.[77] Finally, a chemically modified version of the recombinant alpha galactosidase produced in plant cells has been developed by Protalix. The protein subunits are bound via chemical cross-linking, which results in a more active and stable molecule and potentially also less antibody development.[78,79] Future studies will show if these therapies have superior effects compared to the currently available ERTs. In addition to these studies, several other research questions have been identified in this thesis, amongst others: epigenetic factors influencing disease severity, optimization of supportive care, further characterization of antibodies to ERT and the development of an immune modulation protocol, and the prevalence and treatment of psychological symptoms and cognitive impairment in FD.

These questions require further independent collaboration. Real world data (*i.e.* post marketing data) can provide valuable information. Obligatory post-marketing drug registries set up and managed by manufactures have resulted in several important new insights but also have severe shortcomings such as limited quality and incompleteness of data.[80] In the most recent publications the percentage of patients with missing renal data was up to 65%, while for cardiac outcomes this percentage was even higher.[81,82] In addition, the fact that there is more than one product on the market for the treatment of FD, each with its own drug registry, has led to fragmentation of data, hampering comparative studies.[80] These issues are not limited to FD.[80,83] Connock et al, investigating the long term effectiveness and cost-effectiveness of ERT in Gaucher disease, stated that "Gaucher Registry data, which potentially represented the richest source of observational data for this purpose, were inadequate for the task in hand". Furthermore, they expressed their concern that the analysis of the data are in hands of the people who have an interest in the product.[83]

To overcome these problems, it has been proposed to set up disease-centered instead of drug-centered registries that are supervised by health-care professionals, patients and other relevant stakeholders, but not by the market authorization holder.[84] In addition, raw data should be accessible upon request and independent statisticians should perform analyses. Realization of such registries requires collaboration among all stakeholders, including doctors, patients, regulatory authorities (*i.e.* European Medicines Agency and the Food and Drug Administration) and industry. Alternatively, a bottom-up approach as depicted in this thesis has been shown to be useful and feasible. A collaboration between three centers of excellence across Europe, and for one of the chapters also Canada, has resulted in high quality and complete data which have proven to be useful to address relevant issues in FD. For this project we had to go back to the patient charts. This has been a time consuming process, but ultimately resulted in a dataset with uniform and reliable data. As said before, ideally, data should be obligatory entered into an independent, international disease-based registry in a consistent manner.[84] However, until that time, we make a plea for sufficient resources for the setup and maintenance of such databases in order to improve diagnosis, management and treatment of rare (inherited metabolic) disorders can be made.

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