

File ID 352578  
Filename Chapter 12: General discussion and future perspectives

---

SOURCE (OR PART OF THE FOLLOWING SOURCE):

Type Dissertation  
Title Fabry disease: studies on diagnosis, screening and patients' perspectives  
Author M.G. Bouwman  
Faculty Faculty of Medicine  
Year 2012  
Pages 207  
ISBN 978-94-6182-058-7

FULL BIBLIOGRAPHIC DETAILS:

<http://dare.uva.nl/record/408102>

---

*Copyright*

*It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use.*

---

12

---

---

GENERAL DISCUSSION  
AND FUTURE PERSPECTIVES



## 1. INTRODUCTION

Following the availability of enzyme replacement therapy (ERT) for Fabry disease a decade ago, the number of studies on Fabry has increased tremendously (see **chapter 1**, figure 1). This has resulted in an increase in observations and some improvement in the understanding of this rare disease. Indeed, many questions remain to be answered. The discovery and availability of a potentially disease modifying treatment for Fabry disease in the form of ERT also raised new concerns, such as how to determine treatment efficacy and how to assess the optimal timing of initiation of treatment. Finally, the notion that early start of treatment may improve treatment efficacy and thus the prognosis of patients with Fabry disease, in combination with the fact that first signs and symptoms of Fabry disease are often not recognized resulting in a long diagnostic delay, raised questions on the feasibility of selected and/or population screening.

This thesis comprises several studies on different aspects of Fabry disease with a focus on psychosocial development of adolescents, the clinical phenotype in heterozygotes, long term treatment efficacy and aspects of screening for Fabry disease. In the next section several issues will be highlighted and possible research strategies will be discussed.

## 2. WHAT IS THE IMPACT OF A LONG DIAGNOSTIC DELAY AND ACROPARESTHESIA?

Fabry disease is a rare, heterogeneous, lysosomal storage disorder which involves primarily the skin, heart, kidney and nervous system. The rarity of the disease, in combination with the nonspecific signs and symptoms and the heterogeneity of the course of the disease, complicates recognition of the disorder by physicians. In the absence of affected and previously diagnosed family members, the first clinical manifestations during childhood – generally being acroparesthesia, angiokeratoma and anhydrosis - are often not recognized as signs and symptoms of Fabry disease. Therefore, the time from onset of symptoms to diagnosis may be more than a decade (**chapter 7**). Although this delay in diagnosis is not unique for Fabry disease and is an issue complicating many rare disorders, this diagnostic delay may have significant impact on patients with the severe symptoms of Fabry disease as it may prevent timely start of both symptomatic as well as disease modifying treatment. Although it still remains to be proven that early start of ERT can prevent the occurrence of complications, it has been shown that in advanced cases ERT has little effects<sup>1-3</sup>. Of less dispute is adequate and early symptomatic treatment, including adequate pain medication and the use of ACE-inhibitor and/or AT-2 antagonists. We explored experiences of Fabry patients with regard to the timing of their diagnosis, and described the results in **chapter 9**. Patients with Fabry disease that experienced a delay in diagnosis reported that their symptoms, mainly

consisting of acroparesthesia, fatigue and heat intolerance, were misunderstood and not taken seriously by parents, physicians and teachers at school and that misdiagnosis was common. Patients were frequently told that they “were being overdramatic”. They often had to go through a prolonged diagnostic trajectory before the correct diagnosis was finally established and symptoms were often attributed to psychological problems instead of a somatic disease. Therefore, a timely diagnosis is highly relevant for these patients, providing early and accurate acknowledgement for their symptoms and to prevent the impact of growing up with severe and unrecognized clinical symptoms.

Secondly, growing up with a chronic disease might have significant impact on the psychosocial developmental trajectory resulting in achievement of fewer psychosocial milestones, or at an older age, compared to peers <sup>4-8</sup>. Achievement of milestones (e.g. having friends, participating in sports, having chores, a first job, the first boy-/girlfriend) during childhood is important for transition from childhood into adult life <sup>9</sup>. In **chapter 6** we demonstrated that Fabry disease has an impact on several aspects of the social development in males, with less participation in sports and less going out to bars and discos compared to peers. As this is likely to be related to the presence of acroparesthesia, optimizing the treatment of pain by analgesics may help to achieve optimal social development. However, providing adequate pain treatment in Fabry disease can be very difficult. Only few studies report on the efficacy of different drugs for acroparesthesia. No prospective trials on treatment of acroparesthesia have been published. Case series suggest that carbamazepine, intravenous morphine and lidocain may provide optimal pain relief <sup>10-12</sup>, however it is difficult to determine whether reported improvements are the result of the treatment or the natural, often fluctuating, course of the symptoms. In our experience, carbamazepine is highly effective in treating chronic acroparesthesia, however the excruciating pains during the so called ‘Fabry crises’, may occur despite carbamazepine. Other options such as antidepressive agents and opiates should be tried in addition to carbamazepine in that case. Our experience is that even high dosed polypharmacy may fail to ameliorate the pain crises (unpublished observations). Other causes of small fiber neuropathy should be excluded. A stepwise approach how to manage of acroparesthesias in Fabry is lacking, with most observations or reviews documenting expert opinion. A prospective study on the management of acroparesthesias is warranted. Nevertheless, it is clear that optimal treatment of pain in Fabry disease can only be achieved when the diagnosis of Fabry disease is established as normal pain treatment regimes in children will fail. An early diagnosis is therefore relevant to optimize psychosocial development, especially in boys.

### 3. DOES EARLY TREATMENT WITH ERT PREVENT DISEASE PROGRESSION?

ERT is the first specific and potentially disease modifying therapy available for Fabry disease. Two different recombinant alpha-galactosidase A preparations are currently available in the Netherlands agalsidase alfa (Replagal®) and agalsidase beta (Fabrazyme®). The first short term studies on efficacy of these novel therapies reported mainly a beneficial effect on clinical outcome (pain, renal function)<sup>13</sup>, and reductions in accumulation of the substrate globotriaosylceramide (Gb3) levels<sup>14</sup>. Additional follow-up demonstrated stabilization of renal function in some, but not all patients<sup>15-19</sup>. Cardiac mass stabilized (or was slightly reduced) in some cases, but again others demonstrated progression.<sup>2;18;20;21</sup>. Of interest, several studies indicated that disease progression occurred more rapidly in patients started on ERT while having severe disease at baseline, especially kidney disease (proteinuria and chronic kidney disease (CKD) stage > 3)<sup>1;2;15;19;22;23</sup> and cardiac fibrosis<sup>21</sup>. As a result, it is generally hypothesized that treatment with ERT in younger, less severely affected patients is more effective.

In our study on efficacy of long term treatment, we demonstrated that ERT combined with optimal supportive care does not prevent disease progression, especially in males (loss of GFR, increase in left ventricular mass and occurrence of white matter lesions), but may stabilize disease in some patients (**chapter 8**). No differences were found in progression of the disease between patients with and without signs of organ damage at initiation of ERT (CKD stage, presence of left ventricular hypertrophy and white matter lesions). However, although no differences were found between disease progression in treated versus untreated patients, increased treatment duration did reduce the odds of developing a first and second complication. These findings emphasize the need to delineate more precisely who will benefit from ERT and who will not. The suggestion of a positive outcome of longer treatment (eg earlier initiation) strengthens the necessity to study the efficacy of early treatment. In the small group of adolescents (n=6) that started treatment early, during childhood, left ventricular mass remained stable, nevertheless GFR declined (-7.3±1.0 ml/min/1.73m<sup>2</sup>) during a median follow-up of 4.7 years. Most adolescents however already had hyperfiltration at start of therapy, thus the decrease in GFR may reflect normalization rather than progression. Development of white matter lesions occurred despite treatment.

Further studies are needed to investigate the true benefit of early intervention on disease progression in Fabry disease. Currently, a multinational, open-label study with a duration of five years is ongoing, evaluating treatment with ERT (agalsidase beta, Fabrazyme®) in treatment-naïve male pediatric patients with Fabry disease without severe symptoms (NCT00701415; clinicaltrials.gov). Two dosages are being compared: 1 mg/kg every month and 0.5 mg/kg every two weeks. Unfortunately, no untreated control group is included in this study. Results therefore will have to be compared to natural history data from untreated pediatric

patients, usually having less severe disease, with less rigorous follow-up. This, in combination with the slow rate of progression of disease, might make it difficult to draw definite conclusions from this study and patients will need to be included in further long term follow-up studies.

Another factor which might influence treatment efficacy is the emergence of antibodies against exogenous administered alpha-galactosidase A in some patients. As males with Fabry disease generally do not express residual enzyme activity, exogenous administration of the enzyme alpha-galactosidase A may lead to an immune response as the enzyme is seen as a foreign protein by the immune system. Antibody formation towards alpha-galactosidase A has been reported to occur in over 40% of males treated with agalsidase alfa and over 80% of males treated with agalsidase beta, although the use of different assays hampers comparison<sup>19;22;24</sup>. Almost all of the antibody responses involve immunoglobulin G (IgG) subclass antibodies; however immunoglobulin E (IgE) antibody reactions have been described<sup>25</sup>. Antibodies have been demonstrated to exhibit neutralizing capacities, inhibiting the enzyme activity *in vitro*<sup>26</sup>. In addition, a major proportion of circulating enzyme during infusion is bound to IgG and as a result altered enzyme targeting and trafficking has been shown<sup>26</sup>. Several studies demonstrated that Gb3 clearance in urine, and to a lesser extent in plasma, decreases in the presence of antibodies<sup>24;26-28</sup> and it was shown that this causes re-accumulation of Gb3 in skin capillaries<sup>29</sup>. Thus far, no correlation between antibody titers and onset of clinical events has been established, however it seems very likely that antibodies have a negative effect on treatment efficacy and future research should focus on strategies that overcome these effects.

Several other therapeutic approaches are currently under investigation, including substrate-reduction therapy, chaperone therapy and the combination therapy of ERT and chaperones. Substrate-reduction therapy reduces the biosynthesis of glycosphingolipids and has been shown to be effective for Gaucher disease type 1, another lysosomal storage disorder. *In vivo* and *in vitro* studies of substrate deprivation therapy for Fabry disease have demonstrated a reduction of Gb3<sup>30;31</sup> and an additive reduction when combined with ERT<sup>32</sup>. Treatment with pharmacological chaperones is based on the selective binding of small molecules to proteins in cells, leading to improved protein folding and trafficking, and increased activity<sup>33</sup>. This approach might be an alternative treatment option for a subset of patients with missense mutations leading to protein misfolding and subsequent rapid degradation while the mutated enzyme may still have significant residual activity if it had reached the lysosomal compartment. A phase III, double blind, randomized, placebo controlled study currently investigates the efficacy of a pharmacological chaperone (migalastat hydrochloride) in Fabry disease (NCT 00925301, [clinicaltrials.gov](http://clinicaltrials.gov)). In addition, studies with the combination of chaperones with ERT in patients are initiated (NCT 01196871, [clinicaltrials.gov](http://clinicaltrials.gov)).

## 4. WHAT DEFINES FABRY DISEASE?

During the last years an increasing number of individuals have been identified with a decreased activity of alpha-galactosidase A and mutations in the coding *GLA* gene of which the clinical relevance is unknown. These individuals are generally identified because of selective screening of patients with relatively few or isolated symptoms of Fabry disease or within the scope of pilot studies on the feasibility of newborn screening programs for Fabry disease. This raises the essential question on the definition of Fabry disease. The following four approaches are, often, but not always, used in combination to establish a diagnosis of Fabry disease.

Firstly, diagnosis can be highly suspected on the basis of clinical signs and symptoms. The combination of typical acroparesthesia, angiokeratoma and anhydrosis in a male, is a strong indicator for Fabry disease. However, classical symptoms may be less prominent or even absent in milder or later-onset forms of the disease. In addition, several Fabry related signs and symptoms are rather aspecific. For instance, fatigue, palpitations, chest pain, abdominal pain and diarrhea are frequently reported in relation to Fabry disease, but are also common symptoms in the general population. This is illustrated by our study on the prevalence of symptoms in heterozygotes compared to controls, in which we detected that, although all reported gastrointestinal symptoms combined are significantly more often reported by Fabry heterozygotes (82%), the prevalence of these symptoms in controls is also very high (51%) (**chapter 5**). In addition, several signs and symptoms related to Fabry disease are found in normal elderly people, such as mild left ventricular hypertrophy and cerebral white matter lesions. A large study on the prevalence of white matter lesions revealed that of 1077 randomly selected Dutch elderly people, aged 60-90 years, only 8% was free of cerebral white matter lesions <sup>34</sup>. Thus, care should be taken to attribute the presence of white matter lesions directly to Fabry disease, and this emphasizes the need to further specify these signs of Fabry disease. So, only in the case of a highly classical presentation in males, diagnosis of Fabry disease can be established with a high degree of certainty on the basis of signs and symptoms; however, this should still be confirmed by enzyme analysis.

Secondly, in male Fabry patients with a classical form of the disease, the activity of alpha-galactosidase A in leukocytes or fibroblasts is generally very low to absent, which confirms a definite diagnosis. However, significant residual enzyme activity has been reported in males and appears to be related to late onset disease <sup>35,36</sup>. Furthermore, in heterozygotes (females), the residual enzyme activity varies considerably, ranging from normal to nearly completely absent and studies have shown that one third of females will be missed when diagnosis is established based on enzyme activity alone <sup>37</sup>. There is no clear correlation between the disease severity and the residual enzyme activity in females <sup>38</sup>. So, diagnosing heterozygotes based on enzyme activity is not reliable and complementary diagnostic approaches are needed.

Sequencing the *GLA* gene may also provide essential information on defining Fabry disease. However, in the absence of classical symptoms, or a family member carrying the same mutation with classical Fabry disease, predicting whether a mutation is pathogenic can be very difficult, especially in heterozygotes. To determine whether known gene variants are disease-causing or not, genotype-phenotype relations can be studied in large databases, allowing pooling of data for Fabry disease. Two registries have been set up, the Fabry Outcome Survey (FOS, Shire) and the Fabry Registry (Genzyme) to study post-marketing safety and efficacy of ERT. One study using data from the FOS on 114 different mutations, studied genotype-phenotype correlation, defining the phenotype based on the number of affected organs and a composite score using the FOS-Mainz Severity Score Index (FOS-MSSI). These registries are unfortunately limited by incompleteness of datasets and variable quality of data, due to lack of standardization of assessments<sup>39</sup>, thus hampering phenotypic characterization of patients. The currently used registries are not primarily designed to study genotype-phenotype correlations as the main objectives of these registries are related to post-marketing surveillance. Genotype - phenotype correlation of unknown mutations may be studied by mapping *GLA* mutations onto a three-dimensional structural model of the enzyme. Structural modeling provides information on the structural location (e.g. localization in the functionally important region or further away) and the number of atoms influenced by the amino acid substitution<sup>40-42</sup>. Another approach of studying the impact of a mutation is to express a mutation in a cell system, and measure in vitro enzyme activity. However no studies have shown the correlation between enzyme activity as determined in vitro and clinical disease. The high number of private mutations, finally, hampers this approach and more in general defining clear genotype-phenotype correlations in Fabry disease. Variable disease severity within families carrying identical mutations<sup>43</sup> suggest that other factors also influence phenotype in Fabry disease.

Finally, Fabry disease might also be defined on the basis of the finding of accumulation of the substrate of alpha-galactosidase A, mainly being globotriaosylceramide (Gb3). This can be found by performing histology, demonstrating Gb3 inclusions with light or electron microscopy in different cells, or by analyzing the metabolites in a quantitative way in plasma or urine. The majority of Fabry males show increased plasma Gb3, however in females this is usually normal<sup>38</sup>. In contrast urinary Gb3, has been found to be increased in the majority of both Fabry males and females<sup>38</sup>. A clear relation between plasma and urinary Gb3 levels and the occurrence of clinical manifestations has not been established<sup>38</sup>. Globotriaosylsphingosine (lyso-Gb3), which is the deacylated form of Gb3, was more recently found to be increased in males as well as most females with Fabry disease<sup>44</sup>. Plasma lysoGb3 exposure was shown to correlate with disease severity and was found to be an independent risk factor for development of cerebrovascular signs in male patients and left ventricular mass in females (**chapter 3**). Also, lyso-Gb3 allowed differentiating between patients with classical

Fabry disease and patients with mutations presumed to be associated with a milder phenotype or perhaps even no Fabry disease (the R112H and P60L genotype; **chapter 3**). These observations suggest that plasma lysoGb3 measurement may prove to be an useful additional assessment for confirmation of Fabry disease in individuals with an alpha-galactosidase A mutation with unknown consequence.

In conclusion, diagnosing Fabry disease should be the result of a combination of clinical, biochemical and molecular characteristics, because the isolated approach will result in either overdiagnosis (patients with non-pathogenic mutations) or underdiagnosis (heterozygotes with normal enzyme activity). Measuring urinary Gb3 and plasma lysoGb3 should be further explored as a marker to confirm a biochemical diagnosis in heterozygotes and atypical Fabry patients.

## 5. FABRY DISEASE IN HETEROZYGOTES

It is currently well established that, despite its X-linked inheritance, severe disease manifestations may also occur in females with Fabry disease. Females generally have a milder disease course and complications generally occur later in life. In **chapter 2** we describe that storage may already be present at birth in females, as we found inclusion bodies in smooth muscle cells of the umbilical cord. However, there was no storage in placental tissue in contrast to previously found in males. In **chapter 5** we explored the prevalence of symptoms in Fabry heterozygotes compared to age-matched controls, using a retrospective survey. Fatigue, palpitations, pains in hands and feet, joint pain, dizziness, loss of libido and proteinuria during pregnancy were significantly more common in Fabry females. Even though these symptoms are present in a significant proportion of normal controls, they deserve further attention by treating physicians to better understand their significance in Fabry disease and to study whether these symptoms are amenable by ERT or by symptomatic treatment. Development of symptoms in females has been attributed to skewed inactivation of the X chromosome. There is some evidence supporting this hypothesis. One case report describes uneven X-inactivation in a female monozygotic twin with discordant phenotypic expression (15). Secondly, Drobovolny et al studied the relation between X-inactivation and clinical manifestations and found a more rapid disease progression in females with predominantly inactivated wild type X chromosomes<sup>13;45;46</sup>. However, another study failed to confirm the relation between X inactivation and disease severity<sup>47</sup>. This study was limited by the inability to determine which allele was inactivated in a substantial portion of the patients included. An interesting finding though was that the frequency of occurrence of skewed X-inactivation in Fabry heterozygotes did not differ from that in controls. In **chapter 4** we report on a young female Fabry patient with already severe manifestation of the disease at an early age and 100% skewed X-inactivation of the wild type X-chromosome in leukocytes. Although the pattern of X-inactivation was determined only in leukocytes and it

is uncertain whether the same 100% skewed inactivation pattern is present in all cell types and tissues, this case-study supports the hypothesis that X-inactivation pattern plays a role in the phenotype in heterozygotes. Another possible mechanism may be a lack of metabolic cross-correction. Lysosomal enzymes excreted by cells can be taken up by deficient cells and may subsequently correct the enzyme deficiency to some extent. Although intercellular transfer of alpha-galactosidase A and metabolic cross-correction have been reported *in vitro*<sup>48</sup>, apparently this mechanism is insufficient to prevent storage and clinical symptoms in a considerable proportion of Fabry heterozygotes. An explanation may be that formation of a specific metabolite in alpha-galactosidase A deficient cells could potentially affect alpha-galactosidase A competent cells. Indeed it has been shown that lysoGb3, formed in alpha-galactosidase A deficient cells, inhibits residual alpha-galactosidase A activity<sup>44</sup>.

## 6. IS FABRY DISEASE A CANDIDATE FOR POPULATION SCREENING?

An approach to early diagnosis of Fabry disease is newborn screening. The general aim of newborn screening is to detect disease in apparently healthy newborns, that if left untreated cause severe disability or death. In general, newborn screening programs have expanded during the last decades as a result of both technological developments, especially the development of tandem mass spectrometry, and advances in disease-modifying therapy. Also in the Netherlands, the newborn screening program was expanded from three to 17 diseases in 2007, following the advice of the Dutch Health Council<sup>49</sup>. Inclusion of a number of lysosomal storage disorders in newborn screening programs has gained considerable interest owing to the availability of ERT<sup>50-53</sup>. Studies have focused on measuring enzyme activity in dried blood spots<sup>54-58</sup>. For Fabry disease, three pilot newborn screening programs have been performed<sup>59-61</sup>. These studies, one performed in Italy, the other two in Taiwan, revealed a much higher prevalence of Fabry disease than previously estimated on the basis of the number of patients diagnosed as a result of clinical signs and symptoms and family studies. However, many patients had significant residual enzyme activity and the majority of the detected mutations were associated with late onset disease. In Taiwan, one specific mutation appeared to be highly prevalent. Further studies are needed to delineate the natural history of late onset disease associated with these mutations.

In **chapters 9 and 10**, relevant issues to be considered when discussing newborn screening for Fabry disease are identified. Our interview study highlights important patient perceptions about the timing of the diagnosis of Fabry disease, reflecting the heterogeneity of the disease. The impact of a delayed diagnosis when having severe symptoms is illustrated (see above), as well as the drawback

of a presymptomatic diagnosis, i.e. the risk of medicalization and labeling. Furthermore, a frequently mentioned disadvantage of an early diagnosis was the loss of a carefree life and increased anxiety about the future (**chapter 9**). During explorative focus group discussions with Fabry experts, ethicists and Fabry patients, it was generally agreed that the current difficulties with predicting the severity and course of Fabry disease in asymptomatic patients, in combination with the lack of knowledge on optimal timing of treatment and the efficacy of treatment, argue strongly against inclusion of Fabry disease in newborn screening programs (**chapter 10**).

It can be argued that detection of mutations causing mild or unknown phenotypes during newborn screening is a common problem. Indeed, following the expansion of the program in the Netherlands, the total number of individuals detected with an abnormal screening result and subsequent diagnosed with an inborn error was considerably higher than the number of patients diagnosed following clinical signs and symptoms, especially for biotinidase deficiency and MCAD deficiency (19-fold and 3-fold respectively) <sup>62</sup>. Detection of patients with mild phenotypes is not the goal of newborn screening and should be carefully balanced to the benefits of newborn screening for a particular disease, i.e. early, presymptomatic diagnosis of more severe phenotypes.

## 7. WHAT ARE THE ALTERNATIVE APPROACHES TO ALLOW EARLY DIAGNOSIS OF FABRY DISEASE?

One alternative approach to allow timely diagnosis of Fabry patients would be to increase awareness amongst physicians who might be confronted with Fabry patients. The feasibility to achieve this for a general practitioner is probably low, since general practitioners are confronted with a large group of patients with unexplained symptoms. In addition, there is a great number of rare diseases and it is unrealistic to expect general practitioners to be aware of the initial presenting signs and symptoms of all of them. It is more feasible to try to increase awareness of the presenting signs and symptoms of Fabry disease amongst pediatricians, thus allowing early diagnosis in those patients before the occurrence of irreversible organ damage, when treatment might be most effective. In general, educating and stimulating physicians to perform internet search strategies for patients with persistent signs and symptoms of unknown origin might be a feasible approach for identification of rare disorders (**chapter 7**). Another approach to achieve early diagnosis could be by optimizing family screening, allowing early diagnosis in relatives of index patients. Applied strategies for the identification of affected family members will be different according to local protocols for genetic counseling and ethical regulations, and the diagnosis of Fabry disease can be missed or delayed if family screening is not actively pursued. A recent study on the results of family screening for Fabry disease revealed that, on average, five

additional patients with Fabry disease may be diagnosed in a family pedigree following the identification of one proband or index case <sup>63</sup>. At present, index patients in the Netherlands are provided with an oral and written explanation on the hereditary nature of the disorder. They are given general advice to inform potentially affected family members and to suggest that they seek genetic counseling. Approximately two patients per proband are currently identified (unpublished data). A more active approach, using a system of cascade screening to intensify the search for family members at risk, and offering counseling and diagnostic studies, might result in a much higher yield. Such cascade family screening approaches are currently applied in the Netherlands to patients with familial hypercholesterolaemia <sup>64</sup>. However, as early treatment of Fabry disease is currently not proven to be as effective as in familial hypercholesterolaemia, an active approach might not be appropriate yet. Finally, high risk populations, i.e. patient populations that express a specific symptom that could be caused by Fabry disease, such as renal failure or left ventricular hypertrophy, can be screened for Fabry disease, the so-called selected screening. The need for early diagnosis has prompted several screening studies on the prevalence of Fabry disease in high risk populations. In **chapter 9** we summarized these studies and calculated the combined prevalence. These combined studies demonstrated that the prevalence of Fabry disease in the dialysis population was 0.33% for men and 0.10% for women. The prevalence found in cohorts of patients with LVH varies from 0.9% to 3.9% in men. As this approach will only lead to the identification of patients in whom organ damage is already present, who might not benefit from treatment with ERT, selected screening will only be beneficial for the relatives of these patients.

## 8. QUALITATIVE RESEARCH AND PATIENTS' PERSPECTIVE

This thesis includes several chapters in which the patients' perspective has been taken into account. In **chapter 5** we evaluated the prevalence of symptoms in Fabry heterozygotes, with the use of a questionnaire, which was largely developed by Fabry heterozygotes themselves. The aim was to identify possible unrecognized symptoms in females and to better understand aspecific complaints brought forward by Fabry heterozygotes. In this study none of the symptoms that were specifically suggested by the Fabry females as probably related to Fabry disease were found to be significantly more prevalent amongst Fabry females than in controls. However, this study revealed that fatigue, palpitations, pains in hands and feet, joint pain, dizziness, loss of libido and proteinuria during pregnancy were more common in Fabry females. Although these symptoms were present in a significant proportion of normal controls, they deserve further attention

by treating physicians to better understand their significance, treatment and relationship with Fabry disease.

Furthermore, we used a qualitative research approach to explore the pros and cons of newborn screening for Fabry disease (**chapters 10 and 11**). In general, qualitative research is becoming more prominent in medicine and health care. During the last decades increasing number of qualitative studies appeared in medical journals. Several medical journals with high impact factors have set criteria specifically for qualitative studies (e.g. BMJ, <http://resources.bmj.com/bmj/authors/checklists-forms/editors-checklists> and PLOS medicine<sup>65</sup>). Qualitative research addresses different kinds of research questions compared to the more well-known quantitative research, because it investigates patients' needs, beliefs and experiences. We performed an interview study, exploring patients' experiences with the timing of their diagnosis, with the primary aim of identifying relevant themes to fuel the discussion about expanding newborn screening programs to include Fabry disease. To date, the debate about the potential benefits and drawbacks of screening for Fabry disease had focused on technical aspects and expert opinions, rather than the opinions or experiences of Fabry patients. We feel that it is highly relevant to take the experiences of patients into consideration when discussing complex issues such as these.

In our experience, performing qualitative research on patients may also prove to be of value for the involved physicians. Firstly, although physicians are familiar with the competence to openly explore issues when taking the clinical history of their patients, these communication skills are more consequently used during in-depth interviews. In our study we have experienced how this intense communication led to a better understanding of the patients' perspective in relation to health and disease, and provided a more complete, holistic, insight in the patient and his/her disease, including social and psychological aspects. Although it is clear that this open explorative approach is very time-consuming, the use of this method of communication may prove to be of value to physicians, and consequently to patients, also during their normal practice. In addition, we noticed that the detailed process of data analysis, exploring and analyzing patients' experiences, brings considerable nuances to the surface and, without doubt, prevents the tendency to generalize.

## 9. FUTURE PERSPECTIVES

Further studies are needed to investigate the true benefit of early treatment with ERT. The results of the current multinational, open-label, study evaluating treatment with ERT in young male pediatric patients with no or only mild signs and symptoms of Fabry disease, have to be awaited and efforts should be made to compare these results with reliable natural history data. In addition, a long term follow study of treatment efficacy in patients included in this study will need to

be done. Moreover, efforts should be made to collect multi-center, international, data on patients treated early with ERT.

In the absence of clear objective clinical endpoints in pediatric patients with Fabry disease, the psychosocial development and quality of life are important outcome measures that need to be carefully considered for inclusion in follow-up studies and treatment efficacy-studies. As all adolescents included in our current study on course of life grew up without ERT, it would be of interest to repeat the study on the course of life in a cohort of children that grew up treated with ERT, to evaluate the impact of treatment on psychosocial development.

For all future studies on Fabry disease, it will prove to be essential to have full international agreement on the definition of Fabry disease based on clinical, biochemical and molecular characteristics. International consensus meetings may be instrumental to achieve consensus on this definition.

Finally, it is of utmost importance to study the natural course of patients with late onset signs and symptoms and of patients with mutations with unknown clinical relevance. This will prove to be essential for decisions on treatment initiation and follow-up of these individuals.

Based on the results of our studies, it is my personal opinion that, at this moment, there are insufficient grounds to include Fabry disease in a newborn screening program. Indeed, several important issues need to be resolved before newborn screening for Fabry disease should be reconsidered. The focus of these studies should be on unraveling how to identify those patients who will develop severe symptoms at an early age and will benefit from introduction of disease modifying treatment.

**Table 1:** Direction for future studies.

---

Long term efficacy of early initiation of enzyme replacement therapy
Prospective study on the management of acroparesthesias
Studies on predictors of phenotype
Consensus on definition of Fabry disease
Natural course in patients with mild and late onset Fabry disease

---

## REFERENCES

1. Banikazemi M, Bultas J, Waldek S et al. Agalsidase-beta therapy for advanced Fabry disease: a randomized trial. *Ann. Intern. Med.* 2007;146:77-86.
2. Breunig F, Weidemann F, Strotmann J, Knoll A, Wanner C. Clinical benefit of enzyme replacement therapy in Fabry disease. *Kidney Int.* 2006;69:1216-1221.
3. Vedder AC, Linthorst GE, Houge G et al. Treatment of Fabry disease: outcome of a comparative trial with agalsidase alfa or beta at a dose of 0.2 mg/kg. *PLoS. ONE.* 2007;2:e598.
4. Bosch AM, Maurice-Stam H, Wijburg FA, Grootenhuus MA. Remarkable differences: the course of life of young adults with galactosaemia and PKU. *J. Inher. Metab. Dis.* 2009;32:706-712.
5. Grootenhuus MA, Stam H, Last BF, Groothoff JW. The impact of delayed development on the quality of life of adults with end-stage renal disease since childhood. *Pediatr. Nephrol.* 2006;21:538-544.
6. Linthorst Homan MW, de KJ, Grootenhuus MA et al. Impact of childhood vitiligo on adult life. *Br. J. Dermatol.* 2008;159:915-920.
7. Stam H, Grootenhuus MA, Last BF. The course of life of survivors of childhood cancer. *Psychooncology.* 2005;14:227-238.
8. Stam H, Hartman EE, Deurloo JA, Groothoff J, Grootenhuus MA. Young adult patients with a history of pediatric disease: impact on course of life and transition into adulthood. *J. Adolesc. Health* 2006;39:4-13.
9. Garber J. Classification of childhood psychopathology: a developmental perspective. *Child Dev.* 1984;55:30-48.
10. Gordon KE, Ludman MD, Finley GA. Successful treatment of painful crises of Fabry disease with low dose morphine. *Pediatr. Neurol.* 1995;12:250-251.
11. Politei JM. [Intravenous lidocaine as treatment for the painful episodes in Fabry's disease]. *Rev. Neurol.* 2009;49:166-167.
12. Filling-Katz MR, Merrick HF, Fink JK et al. Carbamazepine in Fabry's disease: effective analgesia with dose-dependent exacerbation of autonomic dysfunction. *Neurology* 1989;39:598-600.
13. Schiffmann R, Kopp JB, Austin HA, III et al. Enzyme replacement therapy in Fabry disease: a randomized controlled trial. *JAMA* 2001;285:2743-2749.
14. Eng CM, Guffon N, Wilcox WR et al. Safety and efficacy of recombinant human alpha-galactosidase A--replacement therapy in Fabry's disease. *N. Engl. J. Med.* 2001;345:9-16.
15. Germain DP, Waldek S, Banikazemi M et al. Sustained, long-term renal stabilization after 54 months of agalsidase beta therapy in patients with Fabry disease. *J. Am. Soc. Nephrol.* 2007;18:1547-1557.
16. Lubanda JC, Anijalg E, Bzduch V et al. Evaluation of a low dose, after a standard therapeutic dose, of agalsidase beta during enzyme replacement therapy in patients with Fabry disease. *Genet. Med.* 2009;11:256-264.
17. West M, Nicholls K, Mehta A et al. Agalsidase alfa and kidney dysfunction in Fabry disease. *J. Am. Soc. Nephrol.* 2009;20:1132-1139.
18. Whybra C, Miebach E, Mengel E et al. A 4-year study of the efficacy and tolerability of enzyme replacement therapy with agalsidase alfa in 36 women with Fabry disease. *Genet. Med.* 2009;11:441-449.
19. Wilcox WR, Banikazemi M, Guffon N et al. Long-term safety and efficacy of enzyme replacement therapy for Fabry disease. *Am. J. Hum. Genet.* 2004;75:65-74.
20. Hughes DA, Elliott PM, Shah J et al. Effects of enzyme replacement therapy on the cardiomyopathy of Anderson-Fabry disease: a randomised, double-blind, placebo-controlled clinical trial of agalsidase alfa. *Heart* 2008;94:153-158.
21. Weidemann F, Niemann M, Breunig F et al. Long-term effects of enzyme replacement therapy on fabry cardiomyopathy: evidence for a better outcome with early treatment. *Circulation* 2009;119:524-529.
22. Schiffmann R, Ries M, Timmons M, Flaherty JT, Brady RO. Long-term therapy with agalsidase alfa for Fabry disease: safety and effects on renal function in a home infusion setting. *Nephrol. Dial. Transplant.* 2006;21:345-354.
23. Warnock DG, Ortiz A, Mauer M et al. Renal outcomes of agalsidase beta treatment for Fabry disease: role of proteinuria and timing of treatment initiation. *Nephrol. Dial. Transplant.* 2011
24. Vedder AC, Breunig F, Donker-Koopman WE et al. Treatment of Fabry disease with different dosing regimens of agalsidase: effects on antibody formation and GL-3. *Mol. Genet. Metab* 2008;94:319-325.
25. Bodensteiner D, Scott CR, Sims KB et al. Successful reinstitution of agalsidase beta

- therapy in Fabry disease patients with previous IgE-antibody or skin-test reactivity to the recombinant enzyme. *Genet. Med.* 2008;10:353-358.
26. Linthorst GE, Hollak CE, Donker-Koopman WE, Strijland A, Aerts JM. Enzyme therapy for Fabry disease: neutralizing antibodies toward agalsidase alpha and beta. *Kidney Int.* 2004;66:1589-1595.
  27. Ohashi T, Sakuma M, Kitagawa T et al. Influence of antibody formation on reduction of globotriaosylceramide (GL-3) in urine from Fabry patients during agalsidase beta therapy. *Mol.Genet.Metab* 2007;92:271-273.
  28. Whitfield PD, Calvin J, Hogg S et al. Monitoring enzyme replacement therapy in Fabry disease--role of urine globotriaosylceramide. *J.Inherit.Metab Dis.* 2005;28:21-33.
  29. Benichou B, Goyal S, Sung C, Norfleet AM, O'Brien F. A retrospective analysis of the potential impact of IgG antibodies to agalsidase beta on efficacy during enzyme replacement therapy for Fabry disease. *Mol.Genet.Metab* 2009;96:4-12.
  30. Abe A, Gregory S, Lee L et al. Reduction of globotriaosylceramide in Fabry disease mice by substrate deprivation. *J.Clin. Invest* 2000;105:1563-1571.
  31. Abe A, Arend LJ, Lee L et al. Glycosphingolipid depletion in fabry disease lymphoblasts with potent inhibitors of glucosylceramide synthase. *Kidney Int.* 2000;57:446-454.
  32. Marshall J, Ashe KM, Bangari D et al. Substrate reduction augments the efficacy of enzyme therapy in a mouse model of Fabry disease. *PLoS.ONE.* 2010;5:e15033.
  33. Smid BE, Aerts JM, Boot RG, Linthorst GE, Hollak CE. Pharmacological small molecules for the treatment of lysosomal storage disorders. *Expert.Opin.Investig. Drugs* 2010;19:1367-1379.
  34. De Leeuw FE, De Groot JC, Achten E et al. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. *The Rotterdam Scan Study.* *J.Neurol. Neurosurg.Psychiatry* 2001;70:9-14.
  35. Nakao S, Takenaka T, Maeda M et al. An atypical variant of Fabry's disease in men with left ventricular hypertrophy. *N.Engl.J.Med.* 1995;333:288-293.
  36. Nakao S, Kodama C, Takenaka T et al. Fabry disease: detection of undiagnosed hemodialysis patients and identification of a "renal variant" phenotype. *Kidney Int.* 2003;64:801-807.
  37. Linthorst GE, Vedder AC, Aerts JM, Hollak CE. Screening for Fabry disease using whole blood spots fails to identify one-third of female carriers. *Clin.Chim.Acta* 2005;353:201-203.
  38. Vedder AC, Linthorst GE, van Breemen MJ et al. The Dutch Fabry cohort: diversity of clinical manifestations and Gb3 levels. *J.Inherit.Metab Dis.* 2007;30:68-78.
  39. Hollak CE, Aerts JM, Ayme S, Manuel J. Limitations of drug registries to evaluate orphan medicinal products for the treatment of lysosomal storage disorders. *Orphanet.J.Rare.Dis.* 2011;6:16.
  40. Garman SC, Garboczi DN. Structural basis of Fabry disease. *Mol.Genet.Metab* 2002;77:3-11.
  41. Garman SC, Garboczi DN. The molecular defect leading to Fabry disease: structure of human alpha-galactosidase. *J.Mol.Biol.* 2004;337:319-335.
  42. Garman SC. Structure-function relationships in alpha-galactosidase A. *Acta Paediatr.Suppl* 2007;96:6-16.
  43. Verovnik F, Benko D, Vujkovic B, Linthorst GE. Remarkable variability in renal disease in a large Slovenian family with Fabry disease. *Eur.J.Hum.Genet.* 2004;12:678-681.
  44. Aerts JM, Groener JE, Kuiper S et al. Elevated globotriaosylsphingosine is a hallmark of Fabry disease. *Proc.Natl.Acad. Sci.U.S.A* 2008;105:2812-2817.
  45. Dobrovolny R, Dvorakova L, Ledvinova J et al. Relationship between X-inactivation and clinical involvement in Fabry heterozygotes. Eleven novel mutations in the alpha-galactosidase A gene in the Czech and Slovak population. *J.Mol.Med.* 2005;83:647-654.
  46. Redonnet-Vernhet I, Ploos van Amstel JK, Jansen RP et al. Uneven X inactivation in a female monozygotic twin pair with Fabry disease and discordant expression of a novel mutation in the alpha-galactosidase A gene. *J.Med.Genet.* 1996;33:682-688.
  47. Maier EM, Osterrieder S, Whybra C et al. Disease manifestations and X inactivation in heterozygous females with Fabry disease. *Acta Paediatr.Suppl* 2006;95:30-38.
  48. Medin JA, Tudor M, Simovitch R et al. Correction in trans for Fabry disease: expression, secretion and uptake of alpha-galactosidase A in patient-derived cells driven by a high-titer recombinant

- retroviral vector. *Proc.Natl.Acad.Sci.U.S.A* 1996;93:7917-7922.
49. Health Council of the Netherlands. Neonatal Screening. The Hague: Health Council of the Netherlands, 2005; publication no. 2005/11.
  50. Chien YH, Chiang SC, Zhang XK et al. Early detection of Pompe disease by newborn screening is feasible: results from the Taiwan screening program. *Pediatrics* 2008;122:e39-e45.
  51. Kemper AR, Hwu WL, Lloyd-Puryear M, Kishnani PS. Newborn screening for Pompe disease: synthesis of the evidence and development of screening recommendations. *Pediatrics* 2007;120:e1327-e1334.
  52. Marsden D, Levy H. Newborn screening of lysosomal storage disorders. *Clin.Chem.* 2010;56:1071-1079.
  53. Nakamura K, Hattori K, Endo F. Newborn screening for lysosomal storage disorders. *Am.J.Med.Genet.C.Semin.Med.Genet.* 2011;157:63-71.
  54. Duffey TA, Bellamy G, Elliott S et al. A tandem mass spectrometry triplex assay for the detection of Fabry, Pompe, and mucopolysaccharidosis-I (Hurler). *Clin. Chem.* 2010;56:1854-1861.
  55. Gelb MH, Turecek F, Scott CR, Chamoles NA. Direct multiplex assay of enzymes in dried blood spots by tandem mass spectrometry for the newborn screening of lysosomal storage disorders. *J.Inherit. Metab Dis.* 2006;29:397-404.
  56. Zhang XK, Elbin CS, Chuang WL et al. Multiplex enzyme assay screening of dried blood spots for lysosomal storage disorders by using tandem mass spectrometry. *Clin.Chem.* 2008;54:1725-1728.
  57. Zhang XK, Elbin CS, Turecek F et al. Multiplex lysosomal enzyme activity assay on dried blood spots using tandem mass spectrometry. *Methods Mol.Biol.* 2010;603:339-350.
  58. Zhou H, Fernhoff P, Vogt RF. Newborn Bloodspot Screening for Lysosomal Storage Disorders. *J.Pediatr.* 2011
  59. Hwu WL, Chien YH, Lee NC et al. Newborn screening for Fabry disease in Taiwan reveals a high incidence of the later-onset GLA mutation c.936+919G>A (IVS4+919G>A). *Hum.Mutat.* 2009;30:1397-1405.
  60. Lin HY, Chong KW, Hsu JH et al. High incidence of the cardiac variant of Fabry disease revealed by newborn screening in the Taiwan Chinese population. *Circ. Cardiovasc.Genet.* 2009;2:450-456.
  61. Spada M, Pagliardini S, Yasuda M et al. High incidence of later-onset fabry disease revealed by newborn screening. *Am.J.Hum.Genet.* 2006;79:31-40.
  62. Visser G, van Spronsen FJ, de Sain-van der Velden MG, Blom HJ, Wijburg FA. Uitgebreide neonatale hieprikscreening op stofwisselingsziekten in Nederland. *Ned Tijdschr Geneesk* 2009;153:
  63. Laney DA, Fernhoff PM. Diagnosis of Fabry disease via analysis of family history. *J.Genet.Couns.* 2008;17:79-83.
  64. Ned RM, Sijbrands EJ. Cascade Screening for Familial Hypercholesterolemia (FH). *PLoS.Curr.* 2011;3:RRN1238.
  65. The PLOS Medicine Editors. Qualitative research: Understanding patients' needs and experiences. *PLoS Med* 2007; 4 (8):e258