Fabry disease: studies on diagnosis, screening and patients' perspectives
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GENERAL INTRODUCTION
This chapter provides a concise overview of the current knowledge and emerging issues concerning Fabry disease, the subject of this thesis. It includes a short summary of historical aspects of the disease, clinical characteristics, pathophysiology, diagnosis and treatment.

1. HISTORY OF FABRY DISEASE

In 1898 two dermatologists, William Anderson from the UK and Johannes Fabry from Germany, independently described for the first time the dermatological characteristics of what is now known as Fabry disease or Anderson-Fabry disease. The disease was named after the typical dermatological findings as ‘angiokeratoma corporis diffusum’ and ‘purpura haemorrhagica nodularis’. More than 50 years later, Ruiter and co-workers reported the post-mortem examinations in two boys with Fabry disease and suggested that the disorder was not merely a dermatologic disease, but a systemic vascular disease.

In 1955, the Belgian scientist Christian de Duve discovered distinct intracellular particles which appeared to have digestive function within cells and named these particles lysosomes, work for which he was awarded the Nobel prize for Physiology or Medicine in 1974. One of his co-workers, Henri-Gery Hers, involved in research on glycogen metabolism, discovered the enzyme deficiency responsible for Pompe disease. The fact that this enzyme worked best at an acid pH, made him consider that the enzyme could be localized in the lysosome. In 1965 Hers established the concept of lysosomal storage disorders, based on his research on Pompe disease. Until now, more than 50 diseases are recognized as caused by an inherited defect in the lysosomal machinery.

In 1967, Brady et al demonstrated that Fabry patients were deficient in the activity of the lysosomal enzyme ceramide-trihexosidase. Kint et al subsequently demonstrated that this enzyme was actually an alpha-galactosidase. Enzyme analysis could now be performed, allowing reliable diagnosis of male Fabry patients. The X-linked nature of the disease was first recognized in 1965 and the location of the alpha-galactosidase A gene at Xq22 was established by Hamers et al. In 1972, Desnick performed the first study aimed at correction of the enzyme deficiency in a Fabry patient by means of kidney transplantation. This was the first step to a more recent hallmark in the history of Fabry disease: the development of enzyme supplementation therapy as a targeted treatment. The introduction of this potentially disease modifying treatment generated a tremendous impulse for research on this disease (figure 1), which resulted in an enormous increased knowledge, but also raised many new questions.
2. GENERAL CLINICAL DESCRIPTION

Fabry disease is a multisystemic disorder, with a remarkable phenotypic heterogeneity, predominantly affecting skin, heart, kidney, peripheral nerves and brain. Fabry disease is caused by a genetic defect in the alpha-galactosidase-A gene (GLA-gene), which is localized on the long arm of the X-chromosome (Xq22.1). Thus far more than 500 different mutations have been described, predominantly consisting of missense and nonsense mutations, but small deletions, insertions and splicing defects have also been reported (www.hgmd.cf.ac.uk/ac/lost.php). Biochemically, Fabry disease is characterized by a deficiency of the lysosomal enzyme alpha-galactosidase A. This hydrolase is responsible for the breakdown in lysosomes of glycosphingolipids, predominantly globotriaosylceramide or ceramide trihexoside (Gb3, Gl3 or CTH) by catalyzing the removal of a terminal α-galactose. Glycosphingolipids are important components of the cell membrane, and are presumed to play an important role in signal transduction and immunology. In Fabry disease, Gb3 has been found to accumulate in many cells, including renal epithelial cells, endothelial cells, vascular smooth muscle cells, cardiomyocytes, and neuronal cells.

Classically, early clinical manifestations of the disease include skin abnormalities (angiokeratoma), hypo- or anhydrosis, painful acroparesthesia, gastro-intestinal complaints and typical corneal deposits on slit-lamp examination (cornea verticillata) (see Table 1). Complications of Fabry disease as the result
of progressive organ involvement consist of renal failure, stroke and cardiac complications and may develop as early as the second decade of life. However, signs and symptoms of organ disease generally start only in the 3rd-5th decade, which results in a significant decreased life expectancy especially in males. Males are generally more severely affected, while females show a more variable and protracted disease course.

Fabry disease exhibits a broad phenotypic spectrum and also includes patients who develop only late onset complications, such as renal failure or cardiac complications, in the absence of a history of acroparesthesia and anhydrosis; these phenotypes are reported as the cardiac and renal variant (see figure 2). Relatively high residual enzyme activity is often noted in patients with a late disease onset, often referred to as an atypical variant of Fabry disease.

### 3. MANIFESTATIONS IN HETEROZYGOTES

Until only 10 years ago, Fabry disease was considered to affect almost exclusively adult males, which was in accordance with, and largely based on, the X-linked inheritance pattern of Fabry disease. Females with Fabry disease were considered to be asymptomatic heterozygotes (hence the term ‘carriers’) with only a few rare exceptions. However, during the last decade it has become evident that there is considerable variability in disease expression in females, ranging from asymptomatic to as severe as seen in male patients. Compared to male Fabry patients, symptoms usually develop later in life and are generally less severe. The

<table>
<thead>
<tr>
<th>Early signs and symptoms</th>
<th>Description</th>
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| Acroparesthesia          | Burning, tingling pains of hands and feet  
                          | Chronic and acute  
                          | Provoked by exercise, heat and stress  
                          | Caused by small fiber neuropathy |
| An/hypohidrosis          | Absence of sweating or decreased ability to sweat |
| Angiokeratoma            | Typical skin lesions  
                          | Localization: trunk, swimsuit area |
| Cornea verticillata      | Corneal clouding  
                          | Detectable on split lamp examination |
| Hearing loss             | High frequency hearing loss  
                          | Tinnitus |
| Gastro-intestinal symptoms | Abdominal pain  
                          | Diarrhea |
| Renal disease            | Micro-albuminuria  
                          | Proteinuria  
                          | Hyperfiltration |
residual enzyme activity in women varies considerably, ranging from normal to nearly completely absent \(^{24}\), but this is not an unequivocal predictor of clinical severity \(^{15}\). The presence of moderate and even severe disease expression in female Fabry heterozygotes is remarkably higher when compared to heterozygotes in e.g. the other X-linked lysosomal storage disorder (mucopolysaccharidosis type II, MPS-II or Hunter Syndrome). It has been hypothesized that skewed inactivation of the X-chromosome may explain disease expression in Fabry females, but the results of studies on this are conflicting \(^{12;25;26}\) and do not explain the preponderance of clinically affected females in Fabry disease compared to other X-linked disorders.

4. FABRY DISEASE IN PEDIATRICS

In 2003 a multinational cohort study reported for the first time extensively on the phenotype of Fabry disease in children, with data on 35 European children with this disorder \(^{27}\). This study revealed that Fabry disease may become clinically manifest during childhood. All males (age 1-21 years) and 85% of the females (age 1.5-20 years) had signs and symptoms of the disease. Following this study, three other important studies on pediatric patients have been published, one presenting the results from another cohort study and two presenting the data on pediatric patients from international registries on Fabry disease (the Fabry Registry and the Fabry Outcome Survey) \(^{28-30}\). It should be taken in consideration that these studies may be biased by inclusion of more severely affected children, as they will be more willing to participate, resulting in an overestimation of the prevalence of symptoms in children. The most recent and largest study on 352 pediatric patients demonstrated that the presenting and most prominent symptom is neuropathic pain in hands and feet, the so-called acroparesthesia \(^{28}\). Boys reported the onset of acroparesthesia at a median age of 7 years, compared to the age of 9 in girls. Acroparesthesia, caused by peripheral small fiber neuropathy \(^{31}\),

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**Figure 2. Phenotypic spectrum of Fabry disease.**

- Classical Fabry disease
  - Renal failure
  - Stroke
  - LVH
  - Proteinuria
  - Acroparesthesia
  - Angiokeratoma
  - Hypohidrosis
- Atypical Fabry disease
  - Isolated LVH
  - Isolated renal insufficiency
generally aggravate during exercise, temperature change and stress. The pain can be chronic or intermittent and is usually resistant to normal analgesics such as paracetamol and NSAID’s. These pains can be severe and disabling. Misdiagnoses are common, and include mainly rheumatic fever and growing pains. In the absence of a family member known with Fabry disease, diagnosis is often delayed and as a consequence these specific pains are not treated adequately.

Other disease manifestations during childhood may include angiokeratoma, which is reported in up to 53% of the boys and 38% of the girls. The lesions are typically localized on the trunk, scrotum and the umbilical area (see figure 3), but may also be found on hands, feet and in the face. Patients may have a (partial) inability to sweat, resulting in heat intolerance and episodes of fever of unknown origin. Ophthalmic manifestations of Fabry disease are also common, even in young children. The ocular findings typically do not impair vision, but are almost exclusively seen in Fabry disease and include cornea verticillata and conjunctival and retinal vessel tortuosity. Cornea verticillata is found in up to 73% of the boys and 70% of the girls. Furthermore, gastro-intestinal symptoms, including abdominal pain, diarrhea and nausea, have been frequently reported.

Involvement of kidneys, heart and central nervous system in pediatric patients is generally rare, however several patients with early involvement of these organs have been reported in the literature. Cardiac involvement has been described in a high number of children with Fabry disease in one cohort study. In this study on 20 children (8 boys), 35% already had mild left ventricular hypertrophy, and all patients had a left ventricular mass of >75th percentile of that in healthy controls. However, the mild increase in left ventricular mass did not result in either systolic or diastolic dysfunction. Overt renal disease with glomerular filtration rates (GFR) below normal usually does not develop until the third and fourth decade, but proteinuria may occur in adolescents and decreased GFR has been occasionally detected. Micro-albuminuria is usually the first clinical sign of renal involvement. A renal biopsy study in pediatric Fabry patients has demonstrated that significant

Figure 3. Angiokeratoma in a 12 year old Fabry patient localized in the umbilicus.
morphologic renal involvement may already occur before the onset of proteinuria and decreased glomerular filtration rate 36. Cerebral white matter lesions seen on MRI in Fabry patients and strokes usually do not occur during childhood, but have been reported in young males with Fabry disease 37-39.

Quality of life in pediatric patients has been addressed in a few studies and it has been shown that boys may already have significant lower scores in the domains of bodily pain and mental health before the age of 10 years 28,30; however, these results should be taken with caution, since in one of these studies 28 normative data from an adult population was used.

5. PATHOPHYSIOLOGY AND GENOTYPE-PHENOTYPE CORRELATION

The pathophysiology of Fabry is still largely unknown. Accumulation of Gb3 in endothelial cells and other cell types has long been considered as the cause of the progressive clinical manifestations of the disease. However, several findings suggest that other factors play an important role. First of all, it was demonstrated that in males accumulation of Gb3 already starts in utero 40,41, while clinical symptoms develop much later in life. Small amounts of storage material were already seen in kidney and myenteric plexuses cells of an aborted fetus at a gestational age of 19 weeks 40, while renal disease usually does not become manifest until after the second decade of life. In addition, there is no good correlation between Gb3 levels and clinical symptoms of Fabry disease 15. Finally, reduction in Gb3 levels with treatment of enzyme replacement therapy does not necessarily prevent disease progression 42,43.

Recently a new potential biomarker, globotriaosylsphingosine (lysoGb3), the deacylated form of Gb3, was found to be highly elevated in plasma of all male patients with classic Fabry disease 44. LysoGb3 was also increased in plasma of symptomatic female patients, although to a lesser extent. It was demonstrated that lysoGb3 inhibits alpha-galactosidase A and stimulates proliferation of smooth muscle cells and thus might be a critical factor in the pathophysiological cascade in Fabry disease. In addition, another factor, sphingosine-1-phosphate, was recently shown to be a growth-promoting factor involved in cardiovascular remodeling in Fabry disease 45.

Genotype-phenotype correlation is hampered by the large number of ‘private’ mutations, being confined to individual families. In addition, the disease manifestations may vary within families carrying the same mutation 46, suggesting that modifier genes or other genetic factors may be important. In general, however, mutations that have been described as associated with more attenuated late-onset disease are frequently missense mutations, e.g. the N215S genotype. One missense variant is considered to be a polymorphism, the D313Y genotype 47.
6. TREATMENT

Before enzyme replacement therapy was available, treatment of Fabry disease was only supportive and symptomatic. Enzyme replacement therapy is the first specific and disease modifying therapy for Fabry disease. In 2001, following the results of two randomized controlled trials (RCTs), two different recombinant enzyme preparations were approved in the Netherlands agalsidase alfa (Replagal®, registered dose 0.2 mg/kg, produced by human skin fibroblasts overexpressing the human alpha-galactosidase A gene), and agalsidase beta (Fabrazyme®, registered dose 1 mg/kg, produced by CHO cells; both administered intravenously every other week). Agalsidase alfa was found to have a favorable effect on the severity of acroparesthesia in 26 adult male patients compared with placebo, during a treatment period of six months \(^{11,12,43,48-52}\). Treatment with agalsidase beta of 56 men and two women during 20 weeks reduced storage of Gb3 in plasma and renal, endomyocardial and skin microvascular endothelium \(^{11,12}\). The only phase IV long term study (35 months) that included a placebo controlled-arm was performed with agalsidase beta \(^{48}\). This study established that treatment with agalsidase beta delayed the time to clinical events (renal, cardiac, or cerebrovascular event or death) and identified proteinuria as a predictor of clinical outcome, suggesting that a certain degree of organ damage unfavorably influences outcome \(^{48}\). Other studies reported improvements in cardiac function \(^{53-55}\), gastrointestinal symptoms \(^{56}\), as well as stabilization of kidney function \(^{42,43,52}\) and reduction of pain \(^{12}\).

To date, the optimal timing of the start of enzyme replacement therapy has not been elucidated. Although it is generally hypothesized that early initiation of treatment may prevent irreversible damage and disease progression, the efficacy of presymptomatic treatment has currently not been established. Only a few studies have focused on treatment efficacy of enzyme replacement therapy in children \(^{57}\). In the first clinical trial published in 2007, 13 patients (9 boys) were studied for 23 weeks \(^{57}\). Treatment was in general well tolerated and was found to reduce storage of Gb3 in plasma. In 2008 the results of another study on efficacy of enzyme replacement therapy in 16 pediatric patients (14 boys) were published. After 48 weeks, reduced Gb3 accumulation in dermal capillary endothelial cells and plasma was observed \(^{58}\). All studies in pediatric patients are limited by their open label design, the short term follow-up and the small number of patients studied. Furthermore, in the absence of an untreated placebo group, these studies are complicated by subjective clinical outcome parameters, e.g. pain and gastro-intestinal symptoms.

Other therapeutic approaches are under investigation, including substrate-reduction therapy, chaperones and the combination therapy of enzyme replacement therapy and chaperones.
CHAPTER 1

7. DIAGNOSIS AND PREVALENCE

Currently, most patients with Fabry disease are diagnosed through family screening, i.e. after a diagnosis of Fabry disease in a family member. In the absence of a known family member with Fabry disease, time between the onset of symptoms and diagnosis may be as long as 16 years and the diagnosis is often not established until adulthood. Due to the variability in clinical expression, the nonspecific signs and symptoms and the rarity of the disease, early recognition by physicians is difficult.

To detect patients that may otherwise remain undiagnosed and who may benefit from early recognition and initiation of disease modifying treatment, several screening studies have been performed in populations at high risk for Fabry disease, e.g. patients with unexplained renal failure or left ventricular hypertrophy. In addition to these selected screening studies, a number of studies have focused on the feasibility of newborn screening for Fabry disease, including studies analyzing enzyme activity in newborn dried blood spots. This resulted in three pilot studies on newborn screening (see Table 2). The first study was done in Italy. Enzyme analysis was performed in blood spots of total 37,104 male neonates. In twelve neonates with deficient alpha-galactosidase A activity, mutations were found in the GLA-gene, including three mutations previously described in late-onset patients, one classical mutation and four novel missense mutations. This results in a calculated birth prevalence of Fabry disease in 1 in 3,100 newborns, with 92% of the patients having mutations associated with late-onset disease. The second study was performed in Taiwan and included 90,288 male neonates. An incidence of 1 in 1,250 neonates was found, 86% of which had the intronic mutation IVS4+919G->A associated with late onset disease. Finally, Lin et al screened 57,451 male neonates in Taiwan and found an incidence of 1 in 1,400, 83% carrying the same intronic mutation as reported in the other Taiwan study. According to these studies the prevalence of Fabry disease is much higher than previously assessed on the basis of the number of patients

Table 2. Overview of pilot newborn screening studies for Fabry disease.

<table>
<thead>
<tr>
<th>Study</th>
<th>Newborns screened n</th>
<th>aGal A deficiency n, dried blot spots</th>
<th>GLA mutation n</th>
<th>Late onset</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Spada et al Italy</td>
<td>37.104</td>
<td>-</td>
<td>41*</td>
<td>-</td>
</tr>
<tr>
<td>Hwu et al Taiwan</td>
<td>90,288</td>
<td>81,689</td>
<td>638b</td>
<td>325</td>
</tr>
<tr>
<td>Lin et al Taiwan</td>
<td>57,451</td>
<td>52,576</td>
<td>58c</td>
<td>9</td>
</tr>
</tbody>
</table>

* aGalA activity <20% of normal mean, b aGalA activity <30% of normal mean, c aGalA activity <40% of normal mean (2 x)
diagnosed as a result of clinical signs and symptoms (1:40.000-117.000 live male births). This finding has several implications: perhaps there is underdiagnosis of true Fabry disease patients, who may benefit from early therapeutic intervention. On the other hand, a high number of “patients” may be identified in whom only minor or even no abnormalities will ever occur and thus should not be labeled as Fabry disease patients.

8. AIMS AND OUTLINE OF THIS THESIS

This thesis comprises a number of studies, aimed to gain more insight into the pathophysiology of Fabry disease, to improve delineation of phenotypic expression of the disease in heterozygotes and to enhance knowledge on quality of life and psychosocial development of Fabry patients. Finally our aim was to summarize current screening strategies and to identify issues to be considered in relation to the discussion on population (newborn) screening for Fabry disease.

In chapter 2 the histopathological findings in two placentas of pregnancies from mothers with Fabry disease are studied, to evaluate the spectrum of placental storage of Gb3. The diagnostic value of plasma lysoGb3 is studied in the Dutch cohort of Fabry patients and is described in chapter 3. Furthermore the relation between plasma lysoGb3 and clinical manifestations of Fabry disease is evaluated. In chapter 4 and 5 the clinical characteristics of heterozygotes with Fabry disease are studied. In chapter 6 the quality of life and psychosocial development of young adults grown up with Fabry disease is studied. Chapter 7 illustrates the delay in diagnosis that is common in Fabry disease. Chapter 8 studies the long term efficacy of enzyme replacement therapy in the Dutch cohort of Fabry patients. Chapter 9 provides a summary of different studies on screening for Fabry disease in high risk populations. Finally, we performed qualitative research exploring experiences and opinions of Fabry patients, Fabry experts and ethicists to identify issues that need to be considered before the introduction of newborn screening. These studies are described in chapter 10 and 11. In a general discussion, chapter 12, an overview of the results is given and directions for future research are outlined.
REFERENCES


