Enzyme-Replacement Therapy in Classic Infantile Pompe Disease:

Long-term outcome, dosing, and the role of antibodies

Carin M. van Gelder

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Through enzyme-replacement therapy, infants with classic infantile Pompe disease grow older and achieve important motor milestones such as walking. However, the disease still leaves a trail. (background: muscle biopsy)
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Lange termijn uitkomst, dosering en rol van antilichamen

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CHAPTER 1

General introduction and scope of the thesis

The introduction of enzyme-replacement therapy (ERT) in 2006 has dramatically increased the interest in Pompe disease. In the three decades following the discovery of acid α-glucosidase deficiency as cause of the disease by H.G. Hers in 1963[1] only very few research laboratories world-wide were studying Pompe disease, investigating the genetic background, the molecular details of acid alpha-glucosidase deficiency, were cloning the gene,[2-4] making knockout mouse models,[5-8] and developing ERT.[9-13] After the start of the first clinical trial of ERT in 1999[14] more centers got gradually involved and at present over 1,800 patients world-wide are receiving ERT. The number of publications on Pompe disease has risen from 2 in 1963 to 140 in 2012.

While untreated patients with classic infantile Pompe disease usually die before the age of one year, the oldest treated patient with classic infantile Pompe disease at Erasmus MC University Medical Center is at present 15 years old. The aims of this thesis were to delineate the long-term outcome of infants treated with ERT, to identify and evaluate the influence of prognostic factors on the effect of ERT, and to explore the effects of a higher and more frequent dosing regimen and of neonatal screening.

This first introductory chapter provides information on the incidence and genetics, clinical spectrum, disease pathology, the natural course, and the diagnosis of Pompe disease. It also summarizes the reported effects of ERT and the current limitations that have led to the studies described in this thesis.

1.1 | POMPE DISEASE

Pompe disease is named after dr. J.C. Pompe, a Dutch pathologist who described in 1932 a 7-month-old baby girl who died with idiopathic hypertrophy of the heart associated with massive accumulation of glycogen within 'vacuoles'. The glycogen accumulation was difficult to explain, as no abnormality could be demonstrated in the classical scheme of glycogen degradation. The metabolic defect was elucidated more than 30 years later when H.G. Hers demonstrated a deficiency of acid α-glucosidase in five infantile patients with Pompe disease.[1] The lysosomal location of this enzyme was confirmed in the same year by Lejeune.[15]

Lysosomes are membrane-bound cytoplasmic organelles filled with acid hydrolases that are able to degrade a wide variety of macromolecular compounds. Acid α-glucosidase degrades glycogen that has entered the lysosomes via autophagy. When its activity is below a certain threshold glycogen starts to accumulate in almost all tissues but most so in skeletal

musle cells and in the heart.[16] The lysosomal glycogen storage ultimately results in dysfunction at a cellular and organ level.

Incidence

The reported incidence of Pompe disease varies among populations and ethnic groups. The current estimates put the overall disease incidence at approximately 1 in 40,000 live births.[17-20]

In a Dutch study, the incidence of classic infantile Pompe disease was estimated to be 1 in 138,000, and of non-classic Pompe disease in children and adults 1 in 57,000.[17] These estimates are based on calculated carrier frequencies of three common mutations in the Netherlands in an unselected sample of newborns. Pilot newborn screening programs reveal that Pompe disease is probably more prevalent: The combined incidence of Pompe disease in pilot studies in Taiwan was 1 in 17,000,[21] in Austria 1 in 8,684,[22] and in Hungary between 1 in 4,447 and 1 in 20,012.[23] Currently, 141 patients are known in the Netherlands: 14 of these patients have classic infantile Pompe disease.

Genetic heterogeneity

Pompe disease is inherited as an autosomal recessive trait. The gene for acid α-glucosidase (*GAA*) is located on chromosome 17q25.2–q25.3. The primary translation product, the acid α-glucosidase precursor, has a molecular mass of 110 kDa. The precursor is processed whereby molecular species of 95 kDa (intermediate) and 76 and 70 kDa (mature) arise.[24]

Patients with classic infantile Pompe disease carry two fully deleterious *GAA* mutations. They have virtually no residual acid α-glucosidase activity, but most of them produce some amount of acid α-glucosidase. Infants with a detectable amount of acid α-glucosidase (cross-reactive immunological material; CRIM) are said to be CRIM-positive (Figure 1). Those without acid α-glucosidase production are said to be CRIM-negative. Approximately 25% of the classic infantile patients are CRIM-negative.[25] They mostly carry two 'null' mutations, i.e. nonsense mutations or deletions/ insertions causing a frame shift resulting in a premature stop, or they have a multi-exon deletion.[25] Children and adults with Pompe disease carry a severe mutation in one allele and a milder mutation in the other, which results in residual enzyme activity of up to 30%.[26] All children and adults with Pompe disease are CRIM-positive (Figure 1).

At present, over 300 pathogenic mutations have been identified (www.pompecenter.nl). The majority of these mutations is private, but some mutations have been identified as more common within certain populations. For example, c.1935C>A (p.Asp645Glu) is common in Asians,[27,28] c.2560C>T (p.Arg854X) is common in Africans and African Americans,[29] while

c.2481+102_2646+31del (delexon18; p.Gly828_Asn882del), c.525delT (p.Glu176fsX45) and c.925G>A (p.Gly309Arg) are common in Caucasians.[30,31]

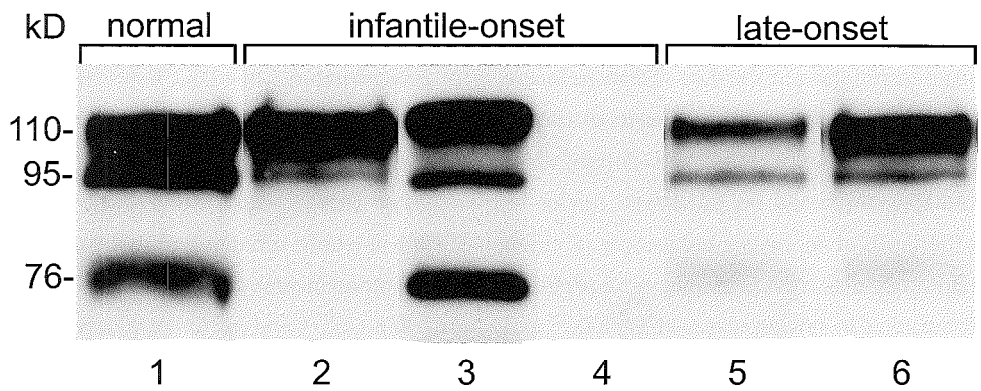

Figure 1 | Molecular forms of α-glucosidase in fibroblasts.

Examples of abnormal synthesis of α-Glucosidase in fibroblasts of patients with classic infantile Pompe disease and children and adults with Pompe disease. **1**: Normal synthesis of acid α-glucosidase in fibroblasts of a healthy person. **2**: Normal synthesis but no maturation. **3**: Normal synthesis, almost normal maturation, but no activity. **4**: No synthesis. **5**: Reduced synthesis, normal maturation. **6**: Normal synthesis, but little maturation. Lanes 1-5 represent CRIM-positive persons; lane 6 represents a CRIM-negative patient with classic infantile Pompe disease.

A single splice-site mutation, c.-32-13T>G (IVS1), is responsible for Pompe disease in the majority of Caucasian children and adults with Pompe disease.[31-33] This mutation leads to 10-20% residual activity of acid α-glucosidase and a broad clinical spectrum.[34]

1.2 | CLINICAL SPECTRUM, DISEASE PATHOLOGY, AND NATURAL COURSE

Clinical spectrum

As most lysosomal storage disorders, Pompe disease has a broad clinical spectrum with a continuum in age at onset, organ involvement, and rate of disease progression. Both for diagnostic and prognostic purposes and to study the effect of therapies, patients with Pompe disease have been classified in different subtypes. However, up until now even among experts no consensus is reached on the classification and nomenclature of the various subtypes of Pompe disease.[35]

In this thesis, a distinction is made between 1) classic infantile Pompe disease, characterized by onset of muscle weakness within 6 months of birth; hypertrophic cardiomyopathy; less than 1% acid α-glucosidase activity in fibroblasts; and severe mutations in both *GAA* alleles: and, 2) Pompe disease in children and adults (non-classic Pompe disease), characterized by disease onset between birth and adulthood, but without progressive cardiac hypertrophy and with residual enzyme activity. This thesis focuses on patients with classic infantile Pompe disease.

In general, the clinical phenotype correlates with the patients' level of residual acid α-glucosidase activity. However, molecular diagnosis and clinical correlation are also needed to define the subtype as some children and adults with Pompe disease can have nearly as low residual enzyme activity levels as patients with classic infantile Pompe disease.[36,37]

In children and adults with Pompe disease, the variation in age at onset and disease progression is not fully explained by the patients' *GAA* genotype and residual enzyme activity. For example, even within siblings with the same genotype, the course and severity of the disease can differ substantially.[38-40] This suggests that other factors, including environmental (e.g. exercise and diet) and genetic factors (e.g. somatic mosaicism and modifying genes), also play a role.

Disease pathology

In classic infantile Pompe disease, glycogen accumulation is found in almost all tissues. Storage is most marked in cardiac, skeletal, and smooth muscle, and in liver. Widespread accumulation of glycogen is also found in numerous other tissues, including endothelial cells, kidney, lymphocytes, the eye, and the skin.[16] In nervous tissue, glycogen accumulation is prominent in the brain stem and the anterior horn cells of the spinal cord and to a lesser extent in the cerebral cortex.[41-43]

In children and adults with Pompe disease, glycogen accumulation is virtually limited to skeletal and smooth muscle tissue, and is of lesser magnitude.[16,44]

The mechanism of lysosomal glycogen storage leading to skeletal muscle dysfunction is slowly being unraveled. As a result of progressive glycogen accumulation, the size and number of the lysosomes increases[45] and this by itself leads to loss of muscle contractility.[46] When the lysosomal compartment expands, other intracellular processes notably autophagy become impaired, and when the lysosomes reach a critical size they may ultimately burst and release their content in the cytoplasm.[47] All pathological processes together result in the deposition of centrally located inclusion bodies consisting of non-contractile cellular debris leading to loss of muscle structure and function.[48-50] Muscle biopsies have shown increased amounts

of lipofuscin, most likely reflecting enhanced muscle oxidative stress.[50,51] Altered localization and deposition of the cytoskeletal proteins desmin and titin could negatively affect muscle quality and, hence, contractile performance.[50,52]

Natural course

Classic infantile Pompe disease

The clinical picture of classic infantile Pompe disease is rather uniform and the course is predictable. Median age at onset of symptoms ranges from 1.6 to 2.8 months.[53-56] Common presenting symptoms are feeding difficulties and failure to thrive, muscle weakness, respiratory infections and difficulties, and cardiac problems like cardiac failure.[53-56] On clinical examination, patients show signs of generalized hypotonia, such as slipping through, a head lag, and a frog-like position of the legs. Despite the profound general muscle weakness, the muscles of the calves are generally firm and even hypertrophic in appearance.[16] Tendon reflexes are often decreased. Signs of respiratory distress, due to weakness of the diaphragm and surrounding muscles, or due to respiratory infections and/or cardiac enlargement, are often observed. Additional clinical features can be macroglossia, facial hypotonia, tongue weakness, and moderate enlargement of the liver. Swallowing dysfunction is common and often leads to airway invasion, i.e. penetration or aspiration of food.[57] A progressive hypertrophic cardiomyopathy is characteristic. Patients have an increased risk for tachyarrhythmia in conjunction with observed cardiac conduction abnormalities.[58] Motor development is severely delayed and major motor milestones such as rolling over, sitting, or walking are usually not achieved, or will be lost as the disease progresses.[53,54] Although untreated patients do not live long enough or are too ill to be reliably evaluated, mental development is apparently normal.[59-64] If patients with classic infantile Pompe disease are untreated, the rapid disease progression results in early death from cardiorespiratory failure, at a median age of 6.0 to 8.7 months.[53-55] Patients with classic infantile Pompe disease rarely survive beyond 1 year of age.

Pompe disease in children and adults

The course of the disease in children and adults with Pompe disease is very heterogeneous: onset of symptoms may range from the first year to the seventh decade of life.[65] The disease presents as a slowly progressive limb-girdle myopathy, resulting in both mobility and respiratory problems.[16,26,56,65-72] First symptoms are mostly related to impaired motor function, and comprise delayed motor development and problems in running and doing

sports, climbing stairs, rising from a chair, walking, and rising from a lying position. Fatigue, pain, and respiratory complaints are also frequently reported.[65,66]

On clinical examination, patients show signs of limb-girdle muscle weakness, including a Gowers sign and waddling gait. In general, weakness is more pronounced in the proximal muscles than in the distal muscles and more in the lower than in the upper extremities. Respiratory symptoms include breathing difficulties and symptoms of sleep-disordered breathing. Tendon reflexes may be low or absent.[65] Less familiar features such as ptosis, bulbar weakness, and scapular winging are increasingly being recognized.[56,68,73-75] Feeding and swallowing difficulties due to bulbar weakness can cause underweight.[65,67] Truncal weakness often results in scoliosis and/ or lordosis.[56,65,68] Osteoporosis is also frequently encountered.[76-78]

In contrast to patients with classic infantile Pompe disease, cardiac enlargement or conduction disturbances are rare,[16,79] and hearing loss is also less frequent, although contradictory findings have been reported.[80-82]

If untreated, mobility and respiratory problems progress at a group level, but at the individual level the rate and extent of progression varies highly between patients.[65,66,68,72,83-87] Furthermore, no clear pattern can be discerned in the sequence of involvement of respiratory and skeletal muscles.[68,69,85,86,88,89] Overall, disease severity is associated to disease duration and not to age, but in a subset of patients under the age of 15 years the course of the disease is more severe and rapid; they require more intensive follow-up and early intervention.[83] Though the disease course in children and adults with Pompe disease is less progressive compared to classic infantile Pompe disease, 'slow' clearly is a relative notion; 10 to 15 years after diagnosis approximately 50% of the patients has become wheelchair-bound or ventilator-dependent.[83] Adults with Pompe disease have a higher mortality rate than the general population, with a median survival after diagnosis of 27 years.[90] The most frequent cause of death in children and adults with Pompe disease is respiratory failure.[56,65] Vascular problems including cerebral aneurysms are increasingly recognized as life-threatening features.[44,91-96]

1.3 | DIAGNOSTIC PROCEDURES

Recognizing Pompe disease can be challenging, partly due to the rarity of the disease, and partly due to its atypical and heterogeneous presentation which often considerably overlaps with other diseases. As a result, Pompe disease may not be readily considered and significant diagnostic delays are common.

Several ancillary tests may help in the initial evaluation of a patient suspected of having Pompe disease. For example, a chest radiography and electrocardiography can reveal cardiomegaly and cardiomyopathy in classic infantile Pompe disease, and pulmonary function tests can reveal pulmonary impairment and hypoventilation in children and adults with Pompe disease. In all subtypes of Pompe disease, an electromyogram (EMG) may show myopathic patterns.[75,97]

Routine laboratory investigations may reveal elevated levels of biochemical markers indicative for muscle pathology. Levels of creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) are often increased in patients with Pompe disease, but they can also be within the normal range.[53,65,68,88,98,99] A definitive diagnosis of Pompe disease can be established by biochemical demonstration of acid α-glucosidase deficiency or by mutation analysis. Acid α-glucosidase is present in all tissues of the body, but for diagnostic purposes the enzyme activity is determined mostly in leukocytes or fibroblasts, less so in muscle tissue. Different substrates are being used, including the natural substrate glycogen and the artificial substrate 4-methylumbelliferyl-α-D-glucopyranoside (4-MU). Due to the presence of maltase-glucoamylase in mixed leukocytes, measurement of acid α-glucosidase in these cells was not reliable in the past. Inclusion of acarbose as inhibitor of maltase-glucoamylase has improved the quality of the assay tremendously.[100,101] Recently, assays in dried-blood spots were developed.[100,102-105] These assays are not only suitable for newborn screening, but also provide a quick and minimally invasive screening test to support the clinical suspicion of Pompe disease.

Another inexpensive and quick screening test to support the diagnosis of Pompe disease is the quantification of glycogen-filled vacuoles in peripheral blood lymphocytes using Periodic acid-Schiff (PAS) staining.[106] The finding of elevated levels of the urinary glucose tetrasaccharide Glc_4 is highly informative in classic infantile Pompe disease,[107] but this tetrasaccharide is also seen in other glycogen storage diseases and is not sensitive in children and adults with Pompe disease.[108,109] As a biomarker, Glc_4 has been shown to correlate well with the clinical response of patients with classic infantile Pompe disease to ERT.[110,111]

Muscle biopsies show the presence of vacuoles that stain positively for PAS, as well as for the lysosomal enzyme acid phosphatase.[16] The muscle glycogen content is increased in patients with classic infantile Pompe, but is normal in approximately 20% of the children and adults with Pompe disease.[65]

All patients with classic infantile Pompe disease show severely affected muscle fibers, but not all fibers in the same section are equally affected, and the pathological damage depends on the age and clinical condition of the patient (Figure 2).[112] In mildly affected classic infantile patients, glycogen accumulation is mainly lysosomal, and muscle architecture is relatively well preserved, whereas in severely affected patients almost all muscle fibers can be affected, vacuolization is more, larger fields of cytoplasmic glycogen are observed, and contractile elements are scarce.[45,47,112]

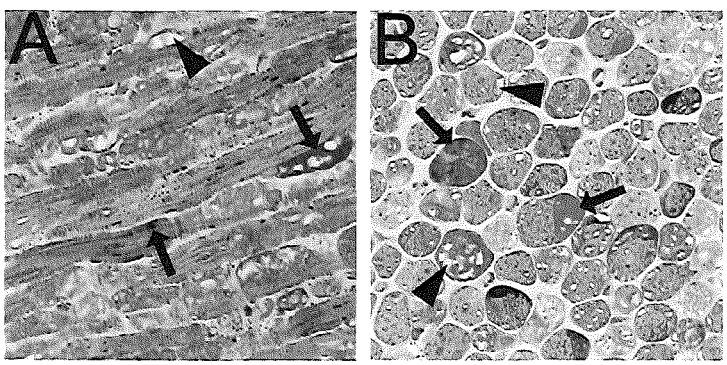

Figure 2 | Pretreatment pathology in skeletal muscle tissue.

Longitudinal (**A**) and transverse (**B**) sections of the quadriceps femoris in a moderately affected patient with classic infantile Pompe disease. All sections were stained with Periodic acid-Schiff (PAS) to demonstrate the glycogen storage (arrows). Open spheres represent lysosomes in which the glycogen was lost due to a fixation artifact (arrow heads).

In children and adults with Pompe disease, muscle biopsies reveal lysosomal pathology in most patients, but normal muscle morphology does not exclude Pompe disease.[65,69] Muscle tissue specimens are mostly heterogeneously affected. Vacuolization is observed in 10 – 50% of the muscle fibers, and the vacuoles stain positive for PAS when the tissue is properly fixed.[51] In advanced disease, muscle architecture is lost and muscle fibers are replaced by dense connective tissue and adipose tissue.[51]

Clearly, the diagnosis Pompe disease should always be confirmed by demonstrating α-glucosidase deficiency or deleterious mutations in both *GAA* alleles. DNA analysis has become an integral part of the diagnostic procedure for confirmation, for carrier detection, and for genetic counseling.[113]

1.4 | ENZYME-REPLACEMENT THERAPY

Until recently, Pompe disease was an untreatable disorder. The discovery of the metabolic and genetic defect together with the characterization of the biosynthesis, processing, and lysosomal targeting of acid α-glucosidase has resulted in the development of the first therapy for Pompe disease; enzyme-replacement therapy with recombinant human acid α-glucosidase. The rationale for this treatment is to correct the enzyme deficiency by intravenous administration of the enzyme (see **Chapter 2** for details).

Two different production systems were developed simultaneously: production of recombinant human acid α-glucosidase in the milk of transgenic rabbits and in Chinese hamster ovary cells (CHO-cells).[11-13] After establishing the effect of ERT in animal models of Pompe disease,[11,114] the first clinical trial was performed in 1999 at Erasmus MC University Medical Center.[14,59,115]

In 2006, treatment with recombinant human acid α-glucosidase derived from CHO-cells (alglucosidase alfa) obtained marketing approval by the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA). The availability of ERT has significantly improved the perspective for all patients with Pompe disease.

ERT in classic infantile Pompe disease

Table 1 summarizes the reported effects of ERT derived from the milk of transgenic rabbits and CHO-cells on the most important end-points in patients with classic infantile Pompe disease (i.e. survival, ventilator-free survival, cardiomyopathy, and motor function).[59-61,63,64,116-118] All these open-label studies evaluating the efficacy and safety of ERT show that, in the large majority of patients, ERT prolongs survival well beyond 1 year of age and improves hypertrophic cardiomyopathy. Many patients reach motor milestones such as standing and walking which are unmet in untreated patients.[53,54]

Table 1 | Summary of clinical studies with ERT in patients with classic infantile Pompe disease.

Author	Hout	Klinge	Amalfitano	Kishnani	Kishnani	Nicolino	Chakrapani	Cho
Year of publication	2000 2001 2004	2005 2005	2001	2006	2007 2009	2009	2010	2011
No. of patients	4	2	2[b]	8	18	21[d]	20[d]	4[d]
ERT duration in years, median (range)	> 3	1.8	1.4 (1.3-1.4)	> 1 (0.4-3.2)	2.3 (1.1-3.0)	2.5 (0-3.5)	2.6 (0.1-8.5)	1.9 (0.8-3.2)
Age at start of ERT > 6 mo **(age range in months)**	2/4 *(3-8)*	0/2 *(3-6)*	0/2 *(3-4)*	3/8 *(3-15)*	1/18 *(1-6)*	20/21 *(4-43)*	NA *(0.5-32)*	3/4 *(2-76)*
Type of ERT	R	R	CHO	CHO	CHO	CHO	CHO	CHO
Dose of ERT (mg/kg)	15-20/week	40/week	2x 5/week	10/week	20 or 40 eow[e]	20 eow	NA	20 eow[f]
Dose increased to (n)	40/week (4)	-	5 times 10/week (2)[c]	20/week or 20 eow (3)	-	40 eow (8)	-	-
Alive	3/4	2/2	2/2	2/8	13/18	15/21	13/20	4/4
Age study end in years, median (range)	>4	2.1 (2-2.2)	1.7 (1.5-1.8)	NA	2.9 (1.7-3.5)	NA (2.9-6.7)	3.8 (0.4-9)	2.6 (1.6-9.5)
Ventilator-free	1/3[a]	2/2	0/2	2/8	9/18	7/16[a]	7/20	3/3[a]
Walking	1/4	1/2	0/2	2/8	7/18	5/21	4/10 pts over 1 yr	2/4
Decrease in LVMI	4/4	2/2	2/2	8/8	17/18	17/21	NA	3/3[g]

Only studies with more than 2 patients are presented.
[a] Some patients already were ventilator dependent at the start of ERT; these patients are not listed.
[b] One patient did not have classic infantile Pompe disease; data from this patient are not listed.
[c] These patients have received immunomodulation in combination with a very high dose.
[d] These studies also describe patients with atypical infantile Pompe disease.
[e] Patients were randomized to a dose of 20 or 40 mg/kg eow.
[f] In one patient treatment was temporarily disrupted due to shortage/ supply difficulties.
[g] One patient did not have a hypertrophic cardiomyopathy; this patient is not listed.

Although significant treatment effects are observed, these studies also demonstrate that the outcome of patients with classic infantile Pompe disease treated with ERT is highly variable. The largest study comprised 18 patients, in whom treatment with ERT with either 20 or 40 mg/kg eow was initiated before 7 months of age: at 36 months of age, the mortality rate was 28% and the invasive ventilation rate was 51%.[116] Whereas the cardiac response is dramatic and improvement in cardiomyopathy is often noted even in advanced cases, only 22 of the 69 patients described learned to walk (Table 1).

ERT in children and adults with Pompe disease

The initial trials with ERT focused primarily on infants with Pompe disease. At the time of approval of alglucosidase alfa, the efficacy of ERT still needed to be established in children and adults with Pompe disease. At present, an increasing number of papers highlight the beneficial effects of ERT in these patients. A recent review of all twenty-one studies published before January 2012, containing clinical data from 368 children and adults with Pompe disease, shows that at least two-thirds of the patients showed stabilization or improvement in creatine kinase levels, motor performance, respiratory function, and the need for ambulatory and/ or ventilatory support following treatment with ERT.[99] One placebo-controlled trial was performed.[87] Sixty ambulant, non-ventilated patients of 8 years or older received ERT in the recommended dose of 20 mg/kg eow, and 30 patients received placebo. After 78 weeks, treatment with ERT was associated with improved walking distance on the 6-minute walk test and stabilization of pulmonary function. This treatment effect was maintained over an additional 26 weeks in an open-label extension study.[119] A recent international observational study in 283 adult Pompe patients demonstrated a positive effect of ERT on survival.[120]

As in classic infantile Pompe disease, not all children and adults benefit equally from ERT. A recent open-label study evaluating the effect of ERT and prognostic factors in 69 adult patients suggested that female gender is a favorable prognostic factor for the effect of ERT on muscle strength, and that younger age and better clinical status are favorable prognostic factors for improved pulmonary function.[121] This suggests that it is important to start treatment early in the course of disease. Further research is required to identify prognostic factors to enable better clinical and therapeutic management of these patients.

SCOPE OF THIS THESIS

The studies described in this thesis were started ten years after the first trial with ERT commenced. At that time knowledge on the effects of ERT on important major end-points including survival, cardiomyopathy, and motor status had grown substantially. Despite this, the oldest patients reported in clinical trials receiving ERT were only 4 years of age,[59] and data on the long-term outcome of patients treated with ERT was still missing. Little was known about residual muscle disease and on the long-term cognitive outcome. Although some prognostic factors had been identified, including the age at which ERT is initiated,[14] the disease severity at the start of ERT reflected by the degree of muscle pathology,[45,112] and the predominance of muscle fiber type,[122] the potential negative impact of the patients' CRIM status and antibodies to ERT was unclear. As not all patients benefit equally from ERT and as residual disease is common, ways to improve the treatment outcome needed to be explored. These gaps in knowledge gave rise to the studies described in this thesis.

The patients described in this thesis participate in a nationwide prospective observational cohort study evaluating the safety and efficacy of ERT in patients with Pompe disease. All patients are followed at the Center for Lysosomal and Metabolic Diseases at Erasmus MC University Medical Center on a regular basis, according to a standardized protocol (appendix I).

AIMS OF THIS THESIS

The studies described in this thesis had the following objectives:

1. To investigate and delineate the long-term outcome of patients with classic infantile Pompe disease treated with ERT,
2. To investigate the impact of CRIM-status and antibody formation on the effect of ERT in patients with classic infantile Pompe disease,
3. To evaluate the safety and efficacy of dose augmentation of ERT in patients with classic infantile Pompe disease, and to explore the potential benefits of earlier diagnosis by neonatal screening.

OUTLINE OF THIS THESIS

Chapter 2 reviews the applications and challenges of the current treatment options for lysosomal storage disorders, including ERT.

Chapters 3, 4, 5, and **6** delineate the emerging phenotype of patients with classic infantile Pompe disease treated with ERT, particularly the distribution, severity and consequences of residual muscle weakness, and the patients' cognitive outcome.

Chapter 7 discusses the role of the patients' CRIM status and antibody formation on the effect of ERT.

In **Chapter 8** and **9**, possibilities to improve the effect of ERT are being explored, by means of dose augmentation or earlier diagnosis by neonatal screening.

Finally, **Chapter 10** covers the general discussion and highlights future perspectives.

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Appendix I | Measurements performed in patients with classic infantile Pompe disease

Procedure	Baseline	t = 0 weeks	t = 2 weeks	t = 4 weeks	t = 8 weeks	t = 3 months	t = 6 months	t = 1 year	t = 2 years
Informed consent	X								
Inclusion/exclusion criteria	X								
General and neurological examination	X			X	X	X			
Weight	X					X			
Infusion with Myozyme		X	X[1]						
Vital signs	X	X	X[1]						
Adverse events assessment		X	X[1]						
Concomitant medication assessment		X	X[1]						
Muscle strength and function testing									
AIMS[2]	X					X			
BSID-II[3]	X					X			
M-ABC[4]	X					X			
Manual muscle testing (MRC score)[5]	X					X			
Hand-Held Dynamometry[5]	X					X			
Six-minute walk test[5]	X					X			
Timed tests[5]	X					X			
QMFT	X					X			
Griffith Mental Development Scales[6]	X					X			
Video monitoring of functional outcomes	X					X			
Pulmonary function testing									
Spirometry	X					X			
Sleep study	X							X	
Ventilator use assessment[7]	X		X						
Cardiac evaluation									
Electrocardiogram[8]	X			X	X	X			
Echocardiogram[8]	X			X	X	X			
Hearing testing									
Tone audiogram[8]	X						X		
Tympanography[8]	X						X		
BAEP [9]	X						X		

Procedure	Baseline	t = 0 weeks	t = 2 weeks	t = 4 weeks	t = 8 weeks	t = 3 months	t = 6 months	t = 1 year	t = 2 years
Blood sample collection									
Acid α-glucosidase acitiviy in leukocytes	X								
PAS-positive lymphocyte vacuoles	X								
DNA-mutation analysis[10]	X								
CK, AST, ALT, LDH	X			X	X	X			
Antibodies against α-glucosidase		X		X	X	X			
PK-analyse[11]		X							
Urine sample collection									
Oligosaccharides		X		X	X	X			
Skin biopsy[10]									
Acid α-glucosidase acitiviy in fibroblasts	X								
X-ray spine[12]	X								
DEXA scan[13]	X								X
CT/MRI-muscle[13]	X								X
Muscle biopsy[13]	X								X
Self-reported outcome measures[14]									
Fatigue Severity Scale	X						X		
Rotterdam handicap Scale	X						X		
SF-36/TAPQOL/TACQOL[15]	X						X		
HADS	X						X		
Health economic questionnaire	X						X		

MRC: Medical Research Council; QMFT: Quick Motor Function Test; AIMS: Alberta Infant Motor Scale; BSID-II: Bayley Scales of Infant Development II; M-ABC: Movement Assessment Battery for Children; BAEP: Brainstem Auditory Evoked Potentials; PAS: Periodic Acid Schiff; DNA: Deoxyribonucleic acid; CK: Creatine kinase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; LDH: Lactate dehydrogenase; PK: Pharmacokinetic; DEXA: Dual-energy X-ray absorptiometry; CT:Computed Tomography; MRI: Magnetic Resonance Imaging; SF-36: Medical Outcomes Study 36-item short-form health survey; TACQOL: TNO-AZL Child Quality of Life Questionnaire; HADS: Hospital Anxiety and Depression Scale.

1. Or every week.
2. In patients ≥18 years.
3. In patients <3.5 years
4. In patients ≥3.5 and <8 years
5. In patients >6 years
6. In patients <8 years.
7. If applicable.
8. Only when not performed prior to this study or when abnormalities were found at a prior investigation
9. Only in patients ≤5 years
10. Only when not performed prior to this study
11. Only in patients ≥18 years. PK analysis will be performed at week 0 and week 2.
12. Only when indicated
13. Not compulsory
14. Only in patients ≥16 years unless otherwise indicated
15. Depending on the patients' age.

CHAPTER 2

Treatment options for lysosomal storage disorders: developing insights

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ABSTRACT

Introduction

Lysosomal storage disorders (LSDs) are clinically heterogeneous disorders that result primarily from lysosomal accumulation of macromolecules in various tissues. LSDs are always progressive, and often lead to severe symptoms and premature death. The identification of the underlying genetic and enzymatic defects has prompted the development of various treatment options.

Areas covered

To describe the current treatment options for LSDs, we provide a focused overview of their pathophysiology. We discuss the current applications and challenges of enzyme-replacement therapy, stem-cell therapy, gene therapy, chaperone therapy, and substrate-reduction therapy, as well as future therapeutic prospects.

Expert opinion

Over recent decades, considerable progress has been made in the treatment of LSDs and in the outcome of patients. None of the current options are completely curative yet. They are complicated by the difficulty in efficiently targeting all affected tissues (particularly the central nervous system), in reaching sufficiently high enzyme levels in the target tissues, and by their high costs. The pathways leading from the genetic mutation to the clinical symptoms should be further elucidated, as they might prompt the development of new and ultimately curative therapies.

ARTICLE HIGHLIGHTS

- Lysosomal storage disorders (LSDs) are severe, progressive disorders, with a combined birth prevalence of 1:7000.
- Current insights in disease pathophysiology have enabled the development of treatment strategies; enzyme-replacement therapy (ERT) is currently the commonest.
- The field is developing rapidly; novel treatment options emerge and a growing number of agents is registered or in clinical trial.
- Existing therapies are complicated by the difficulty in efficiently reaching the target tissues and by their high costs.
- The complexity of LSDs and their treatment leaves ample space for the development of innovative and ultimately curative therapies.

1 | INTRODUCTION

1.1 | General introduction

Most lysosomal storage disorders (LSDs) are severe, progressive disorders that affect various tissues, often leading to severe symptoms and premature death. Over recent decades, major progress has been made and several therapies have been developed. Without being completely curative, these improve the clinical outcome of patients with several LSDs.

This review discusses the mechanism and current applications of enzyme-replacement therapy (ERT), hematopoietic stem-cell therapy (HSCT), gene therapy, chaperone therapy and substrate-reduction therapy (SRT), examining their pros and cons and the possible directions of future treatment options. First, we discuss lysosomal function and the pathophysiology of LSDs.

1.2 | Lysosomal function and lysosomal proteins

Lysosomes were first described in 1955 as membrane-bound cytoplasmic 'bodies' – i.e. organelles – filled with acid hydrolases that are able to degrade a wide variety of macro-molecular compounds, even including entire mitochondria.[1] They are currently known to play various critical roles in cellular function and tissue homeostasis. For instance, they are involved in pathogen defence (macrophages and neutrophilic granulocytes), bone remodeling (osteoclasts), cholesterol homeostasis, and tissue-wide cell renewal.[2]

The lysosomal membrane is particularly rich in two structurally related lysosomal-associated membrane proteins LAMP-1 and LAMP-2, proteins that are highly glycosylated on the luminal side.[3] Amongst others, these proteins are thought to protect the membrane against degradation by its highly aggressive content of an estimated 50 hydrolases, which together allow the degradation of the many biological substances that are brought to the lysosomes by three main processes: phagocytosis, endocytosis and autophagy.[4] The hydrolases include glycosidases, proteases, lipases and DNA/RNAases. Other lysosomal membrane proteins function as a proton pump to acidify the lysosomal interior, or as a transporter to export particular monosaccharides and aminoacids.[3] More functions of the lysosomal membrane proteins will certainly be clarified, such as those of the lysosomal integral membrane protein 2 (LIMP-2), which was fairly recently discovered to be involved in the lysosomal targeting of glucocerebrosidase.[5]

Another class of lysosomal proteins is the activator proteins, e.g. saposins, which assist sphingolipid-degrading enzymes by making the substrates accessible to the enzymes.[6] To fulfil their function, lysosomes constantly fuse with endosomes, autophagosomes, phagosomes, and other membranous structures including the cell membrane.[4]

The hydrolytic lysosomal proteins themselves are macromolecules that cannot pass the lysosomal membrane. They enter the endoplasmic reticulum (ER) enacted by a signal peptide at the extreme N-terminal end of the protein (Figure 1A). After co-translational entry into the ER lumen, lysosomal proteins become glycosylated, are folded, and undergo a series of post-translational modification events involving both the carbohydrate side chains and the protein core. In this process, most lysosomal enzymes acquire mannose 6-phosphate (M6P) as a recognition marker for mannose 6-phosphate receptor (M6PR) mediated vesicular transport from trans-Golgi to lysosomes.[7] Some lysosomal hydrolases reach the lysosomes in a different way, such as glucocerebrosidase which is directed to the lysosomes through binding to LIMP-2 in the ER.[5] In physiological conditions, about 5 to 20% of the newly synthesized lysosomal enzymes are secreted.[7]

The lysosomal membrane proteins bear cytosolic sorting signals that target them to the lysosomes through a direct intracellular route or indirectly through the plasma membrane.[7]

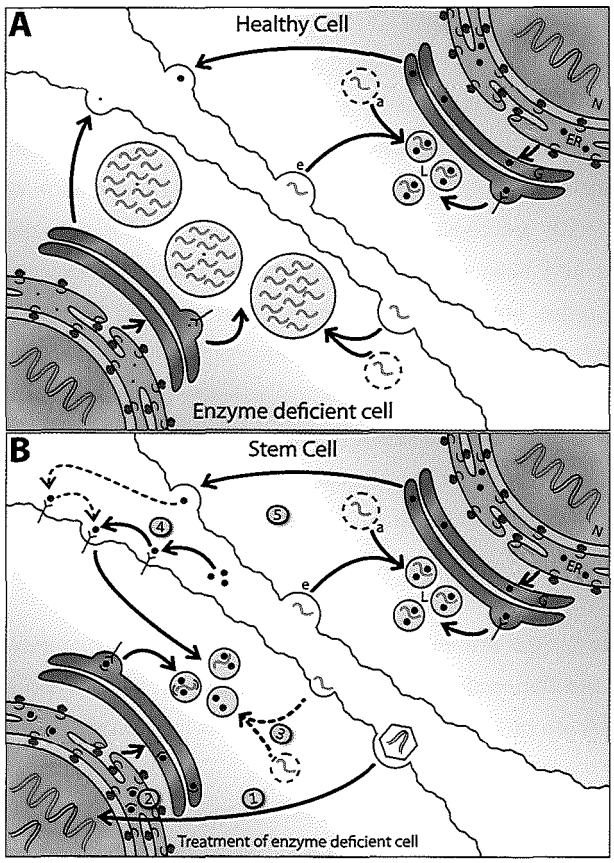

Figure 1 | Lysosomal function in health and disease, and options to treat lysosomal storage disorders.

A. Healthy cell: Newly synthesized lysosomal enzymes (large blue dots) are co-translationally imported into the lumen of the endoplasmic reticulum (ER). After folding and transport to the Golgi apparatus (G), they are glycosylated and most obtain a mannose-6 phospate marker for receptor-mediated transport to the lysosomes (L). Inside the lysosomes, they degrade various substances (orange ~) that enter the lysosomes via autophagy (a) or endocytosis (e). A small portion of the lysosomal enzymes is secreted from the Golgi apparatus. **Enzyme deficient cell:** Mutations (DNA region in red) either result in deficient synthesis or in the formation of structurally abnormal enzymes (small blue dots). Abnormally folded enzyme is often subject to ER-associated degradation. Shortage of enzyme in the lysosomes results in lysosomal storage and expansion of the lysosomal apparatus, affecting endocytosis, autophagy and ultimately cellular function.

B. Treatment options: Gene therapy (1): A viral vector is used to deliver DNA encoding for the missing enzyme (DNA region in green). The gene is expressed by the cellular machinery, and provides functional enzyme that can also be secreted and reach adjacent cells by receptor-mediated endocytosis. **Chaperone therapy (2):** Chaperones bind to misfolded enzymes in the ER and induce proper folding, thereby preventing ER-associated degradation and stimulating transport to the lysosomes. **Substrate-reduction therapy (3):** The synthesis of storage compounds is partially inhibited, thereby improving the balance between biosynthesis and impaired degradation. **Enzyme-replacement therapy (4):** Administered recombinant enzymes enter the cell by receptor-mediated endocytosis and are directed to the lysosomes. **Stem-cell therapy (5):** Healthy donor cells migrate to various tissues. They provide a permanent source of the missing enzyme to host cells via enzyme secretion and receptor-mediated uptake (dashed lines).

1.3 | Pathophysiology and clinical presentation

At present, up to fifty lysosomal storage disorders (LSDs) are known (see [8] for a detailed description). Most are caused by mutations in genes coding for one of the many hydrolases. These mutations lead to enzyme deficiency or dysfunction and subsequent accumulation of biological compounds. A minority of LSDs are caused by mutations in lysosomal membrane transporter proteins (e.g. cystinosis and Salla disease).[9] Interestingly, LAMP-2 deficiency hampers the fusion of autophagosomes and lysosomes, and is characterized by extra-lysosomal vesicular storage of glycogen in the heart (Danon disease).[10] LIMP-2 deficiency results in action myoclonus-renal failure syndrome, which is clinically similar to Gaucher type 3 disease,[11] and may serve as a modifier in Gaucher disease.[12] Mucolipidosis II (I-cell disease) and mucolipidosis III are exceptional in that malfunction of enzymes involved in the generation of the mannose 6-phosphate recognition marker leads to a plethora of lysosomal enzyme deficiencies.[13] Deficiencies of saposins, i.e. sphyngolipid activator proteins, clinically mimic the sphyngolipid storage disorders.[6]

All LSDs are monogenic disorders and are inherited as recessive traits. Mucopoly-saccharidosis type II, Fabry disease and Danon disease are X-linked disorders; all others are autosomal. Though the individual LSDs are rare, their combined prevalence is estimated to be 1:7,000 live births.[14,15]

Figure 1A shows the pathophysiological cascade. Deficiency in a lysosomal function leads primarily to the accumulation of one or more undegradable substances within the lysosomes. Lysosomal dysfunction occurs, with other cell systems becoming involved – e.g. reduced cell and tissue renewal through defective endocytosis and autophagy. Alterations in signal-transduction pathways and intracellular calcium homeostasis are tertiary events.[16,17] The initial cellular pathology causes tissue damage followed by organ dysfunction, and can lead to more distant effects, for instance through the formation and circulation of toxic substances or through immune irregularities.[18]

Due to the presence of lysosomes in all different cell types except erythrocytes, the pathologic events are rarely restricted to one cell type or tissue type, although there is usually one leading organ. Which organs are affected is largely determined by the cell- and organ-specific turnover of the storage compounds.

Most LSDs have a broad clinical spectrum, ranging from severe, rapidly progressing, infantile-onset forms to less progressive forms presenting in childhood or adulthood.[8] The differences in phenotype within a disease can be explained largely by the residual activity of

the deficient enzyme, which results from different mutations within the same gene.[19] However, this genotype-phenotype correlation is not strict, and disease severity and progression can differ substantially, even within siblings or twins, indicating the involvement of other genetic or non-genetic modifying factors.[20]

The pathophysiological mechanism of LSDs is complicated and still not fully understood. If it is further unravelled, new therapeutic targets may be identified.[17]

1.4 | Therapy

Several options for therapeutic intervention are provided by current insights not only into the role of lysosomes and their functioning through interaction with endocytic and autophagic systems, but also into the pathophysiology of lysosomal diseases. The objective of all these options is to relieve the burden of lysosomal storage and to restore cellular and organ functions by preventing storage or its recurrence. While certain therapeutic approaches apply overall, each disease offers unique challenges that are mainly related to the target tissues.

Figure 1B provides a schematic overview of the mechanisms of action of enzyme-replacement therapy, hematopoietic stem-cell therapy, gene therapy, chaperone therapy, and substrate-reduction therapy. These options are discussed in turn below. Table 1 provides an overview of the LSDs for which one or more of these treatments are presently available or evaluated in clinical trials.

2 | TREATMENT OPTIONS

2.1 | Enzyme-replacement therapy

2.1.1 | Principles

Enzyme-replacement therapy is currently the commonest treatment for LSDs. The discovery of the first lysosomal enzyme defect in 1963[21] led to the idea that lysosomal storage could be corrected by administering the missing enzyme. At that time, heterophagy (endocytosis) was already known to deliver exogenous materials to the lysosomes.[22]

ERT was first attempted in infants with Pompe disease through the intramuscular and intravenous administration of α-glucosidase from Aspergillus Niger and human placenta, but had little effect.[23] However, the clearance of glycogen from the liver might now be seen as a first hint that the strategy could work.[24] All subsequent attempts at ERT in a broad variety of

Table 1 | Summary of the treatments options available or in trial for lysosomal storage disorders*

Disease	Defective enzyme	Main clinical manifestations# [8]	Current treatment	Clinical trials (phase)[44]	OMIM
Mucopolysaccharidoses					
MPS type I	α-L-Iduronidase	CNS involvement, dysostosis multiplex, cardiac disease, respiratory and ENT problems, hearing loss, hepatosplenomegaly, corneal clouding, hernias, macroglossia	ERT, HSCT*	CSF-ERT (1), HSCT&ERT^ (2), HSCT&CSF-ERT (1)	#607014, #607015, #607016
MPS type II	Iduronate 2-sulfatase	CNS involvement, dysostosis multiplex, cardiac disease, respiratory and ENT problems, hearing loss, hepatosplenomegaly, hernias, macroglossia	ERT	CSF-ERT&ERT (1/2), HSCT (2)	#309900
MPS type IIIA	N-sulfoglucosamine sulfohydrolase	CNS involvement with relatively mild somatic disease, mild dysostosis multiplex, hepatosplenomegaly, hearing loss		CSF-ERT (1/2), GT (1/2), HSCT (2)	#252900
MPS type IIIB	N-alpha-acetylglucosaminidase	CNS involvement with relatively mild somatic features, mild dysostosis multiplex, hepatosplenomegaly, hearing loss		HSCT (2)	#252920
MPS type IIIC	Heparan acetyl-CoA:alpha-glucosaminide N-acetyltransferase	CNS involvement with relatively mild somatic disease, mild dysostosis multiplex, hepatosplenomegaly, hearing loss		HSCT (2)	#252930
MPS type IIID	*N*-Acetylglucosamine-6-sulfatase	CNS involvement with relatively mild somatic disease, mild dysostosis multiplex, hepatosplenomegaly, hearing loss		HSCT (2)	#252940
MPS type IVA	Galactosamine-6-sulfate sulfatase	Dysostosis multiplex, cardiac disease, respiratory and ENT problems, hearing loss, hepatomegaly, corneal clouding		ERT (3)	#253000
MPS type VI	*N*-Acetylgalactosamine 4-sulphatase	CNS involvement, dysostosis multiplex, cardiac disease, respiratory and ENT problems, hearing loss, hepatosplenomegaly, corneal clouding, hernias	ERT	HSCT (2)	#253200
MPS type VII	β-Glucuronidase	CNS involvement, dysostosis multiplex, cardiac disease, respiratory problems, hearing loss, hepatosplenomegaly, corneal clouding, hernias		HSCT (2)	#253220

Disease	Defective enzyme	Main clinical manifestations[#] [8]	Current treatment	Clinical trials (phase)[44]	OMIM
Oligosaccharidoses					
Fucosidosis	α-L-Fucosidase	CNS involvement, dysostosis multiplex, cardiac disease, respiratory and ENT problems, hearing loss, hepatosplenomegaly, angiokeratoma		HSCT (2/3)	#230000
α-Mannosidosis	α-Mannosidase	CNS involvement, dysostosis multiplex, ENT problems, hearing loss, hepatosplenomegaly, corneal clouding, hernias, immune deficiencies		ERT (2), HSCT (2)	#248500
Aspartylglucosaminuria	Aspartylglucosaminidase	CNS involvement, dysostosis multiplex, respiratory and ENT problems, hernias		HSCT (2)	#208400
Sphingolipidoses					
GM1 gangliosidosis/ Morquio B	β-Galactosidase	CNS involvement, dysostosis multiplex, hearing loss, hepatosplenomegaly,corneal clouding, loss of vision, macroglossia		HSCT (2/3)	#230500, #230600, #230650, # 253010
GM2 gangliosidosis (Tay-Sachs)	β -Hexosaminidase A	CNS involvement, ENT problems, hearing problems, loss of vision		CT (2), HSCT (2/3), SRT (3)	#272800
GM2 gangliosidosis (Sandhoff)	β -Hexosaminidase A and B	CNS involvement, ENT problems, hepatosplenomegaly, loss of vision		CT (2), HSCT (2/3), SRT (3)	#268800
Metachromatic leukodystrophy	Arylsulphatase A	CNS and PNS involvement, hearing problems, loss of vision	HSCT	HSCT&GT (1/2), ERT (1/2), CSF-ERT (1/2), ERT&HSCT (2)	#250100
Nieman-Pick A and B	Sphingomyelinase	CNS involvement, respiratory problems, gastrointestinal problems, hepatosplenomegaly		HSCT (2/3)	#257200
Gaucher	Glucocerebrosidase	CNS involvement, skeletal involvement, hepatosplenomegaly, hematologic manifestations	ERT§, SRT§	CT$ (2), SRT$ (3), HSCT$ (2/3)	#230800
Fabry	α-Galactosidase A	Renal and cardiac disease, cerebrovascular manifestations, pain/ paresthesia, angiokeratoma, hypohidrosis	ERT	CT (3), CT&ERT (2), SRT (3)	#301500

Disease	Defective enzyme	Main clinical manifestations# [8]	Current treatment	Clinical trials (phase)[44]	OMIM
Krabbe, Globoid cell leukodystrophy	Galactocerebrosidase	CNS and PNS involvement, hearing loss, gastrointestinal problems, loss of vision	HSCT		#245200
Mucolipidoses					
Mucolipidosis	lysosomal-enzyme *N*-acetylglucosaminyl-1-phosphotransferase	CNS involvement, dysostosis multiplex, cardiac disease, respiratory and ENT problems, hepatosplenomegaly, corneal clouding, hernias, macroglossia, gingival hyperplasia,		HSCT& (2)	#252500, #252600
Lipidoses					
Niemann-Pick C	NPC1	CNS involvement, liver failure, hearing loss, gastrointestinal problems, hepatosplenomegaly	SRT	HSCT (2/3)	#257220, #607625
Wolman	Acid lipase	Gastrointestinal problems, hepatosplenomegaly, hematologic manifestations, adrenal disease		HSCT (2/3), ERT (2/3)	# 278000
Neuronal ceroid lipofuscinosis 1 (NCL 1)	Palmitoyl-protein thioesterase-1	CNS involvement, loss of vision		Cysteamine (2)	# 256730
NCL 2	Tripeptidyl peptidase-1	CNS involvement, loss of vision		GT (1/2)	# 204500
Lysosomal transport defects					
Cystinosis	Cystinosin	CNS involvement, myopathy, hepatosplenomegaly, renal disease, endocrine involvement, short stature, corneal clouding, loss of vision	Cysteamine		#219800, #219900, #219750
Glycogen storage disorder type II					
Pompe disease	α-Glucosidase	Myopathy, cardiac disease, respiratory problems, hearing loss, hepatomegaly, macroglossia	ERT	GT (1/2), CT (2), CT&ERT (2), ERT (2)	# 232300

The LSDs are listed according to the chemical properties of the accumulating substances. * The LSDs for which no treatment is currently available or in clinical trial are not included; # As most LSDs present as a broad clinical spectrum, the main clinical manifestations per LSD are summarized, which may not be applicable for all patients individually. ^ Only for Hurler patients; § Only for Gaucher disease type I and III; $ Only for Gaucher disease type I; & Only for mucolipidosis type II.
CNS: Central nervous system; CSF-ERT: Intra-cerebrospinal fluid enzyme-replacement therapy; ERT: Enzyme-replacement therapy; HSCT: Hematopoietic stem-cell transplantation; GT: Gene therapy; CT: Chaperone therapy; SRT: Substrate-reduction therapy; PNS: peripheral nervous system.

LSDs over the following decades had limited effect. In retrospect, this lack of effect was due to low doses, short treatment duration, sub-optimal enzyme preparations, and the lack of knowledge about receptor-mediated endocytosis.[25]

The existence of carbohydrate specific receptors at the cell surface was first described by Ashwell and Morell,[26] who discovered the role of the asialoglycoprotein receptor in hepatocytes, which bind glycoproteins with exposed galactose residues. Within a few years, endocytosis and lysosomal delivery had been shown to occur, according to cell type, through various receptors. Many cell types turned out to expose M6PRs on their surface, allowing the endocytosis of mannose 6-phosphorylated lysosomal enzymes.[27,28] Dendritic cells, liver Kupffer cells, and macrophages were found to expose mannose receptors, binding mannose-residue-rich sugar chains.[29] However, it was not until 1978 that the development of receptor-mediated ERT was actively pursued.[30]

The first successful receptor-mediated ERT was achieved in Gaucher disease. In this case, human placental glucocerebrosidase was used as an enzyme source after partial deglycosylation with neuraminidase, galactosidase, and hexosaminidase to expose mannose residues, to enable uptake by the mannose receptor of macrophages.[31] This successful attempt led to the registration of alglucerase in 1991, and revived belief in ERT as a potential therapy for LSDs. Its broad application came after the introduction of recombinant DNA technology, which allowed the large-scale production of lysosomal enzymes.

To date, ERT has been registered for Gaucher disease (imiglucerase[32], velaglucerase alfa,[33] and taliglucerase alfa[34]), Fabry disease (agal-sidase alfa[35] and agalsidase beta[36]), Pompe disease (alglucosidase alfa[37-39]), and mucopoly-saccharidosis (MPS) types I (laronidase[40]), II (idursulfase[41]), and VI (galsulfase[42]). ERTs for MPS IVA (recombinant human galactosamine-6-sulfate sulfatase NCT01415427), α-mannosidosis (recombinant human alpha-mannosidase, NCT01285700), metachromatic leukodystrophy (MLD) (recombinant human arylsulfatase A, NCT00681811), Wolman disease (recombinant human lysosomal acid lipase, NCT01473875), and a second generation ERT for Pompe disease (GILT-tagged recombinant human acid α-glucosidase, NCT01230801) are in different phases of clinical evaluation.[43] For LSDs other than Gaucher disease, the mannose 6-phosphate receptor was chosen as the best target for efficient enzyme delivery.

2.1.2 | Disease-related challenges

Although pivotal trials and clinical follow-up studies have demonstrated the beneficial effects of ERT, the overall outcome varies from disease to disease (Table 2). The effect of ERT depends

first of all on the cell type primarily affected and on the accessibility of that cell type to ERT. Figure 2A shows that Kupffer cells, endothelial cells and hepatocytes in the liver have free access to the administered enzyme circulating in the blood. The accessibility of macrophages in the liver (Kupffer cells) and spleen contributes much to the success of ERT in Gaucher disease,[44] and the accessibility of the Kupffer cells and hepatocytes to the effect on liver in MPSs. Another example is Fabry disease, where the location of the podocytes outside the glomerular filtration barrier prevents their effective treatment, whereas the endothelial cells lining the glomerular capillaries are treated effectively (Figure 2B). The blood brain barrier (BBB) is a problem in its own right, as it prevents the access of the large therapeutic enzymes to the brain, which is clinically involved in many LSDs.

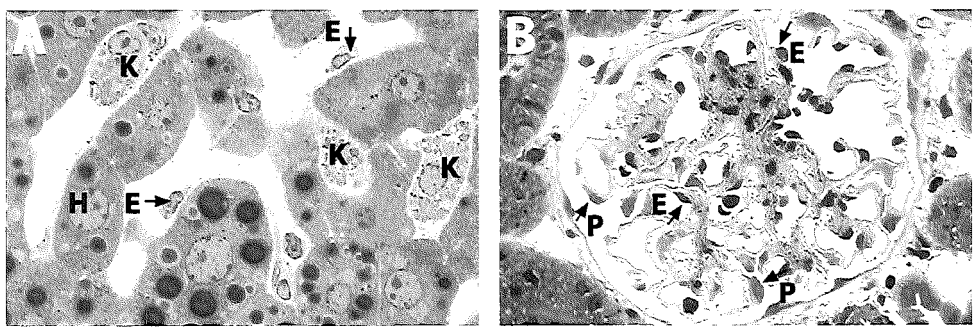

Figure 2 | Accessibility of cell types to circulating recombinant enzymes.

A. The Kupffer cells (K), endothelial cells (E) and hepatocytes (H) in the liver have free access to the administered enzyme-replacement therapy. **B.** In the kidney, the endothelial cells (E) have direct access to the administered enzyme, whereas the location of the podocytes (P) outside the glomerular filtration barrier prevents their effective treatment.

The effect of ERT is also determined by the molecular composition, the architecture, and the natural turnover of the target tissue. For instance, through the continuous generation of macrophages in the bone marrow through differentiation of hematopoietic stem-cells, the Gaucher cells are gradually replaced by enzyme-corrected macrophages. The bone problems in Gaucher disease are secondary to the storage of glucosylceramide in the Gaucher cells. By the time the Gaucher cells start to disappear, the bone infarctions appear to subside, but normalization of the bone structure – including bone density and Erlenmeyer flask shape – is delayed, most likely due to the slow turnover of bone.

Table 2 | Effects and challenges of approved enzyme-replacement therapies in lysosomal storage disorders.

Disease	Enzyme replacement	Recommended dose	Main target organs	Effects observed in clinical trials	Main challenges^
Fabry	Agalsidase alfa[35]	0.2 mg/kg/eow	Vascular endothelium Kidney Heart PNS	Stabilizes renal function and reduces cardiomyopathy in mildly affected patients, reduces pain, improves quality of life	Renal and cardiac disease
	Agalsidase beta[36]	1 mg/kg/eow			
Gaucher	Imiglucerase[32]*	1.5 mg/kg/eow	Liver Spleen Reticuloendothelial system Bone CNS**	Improves blood counts, reduces hepatosplenomegaly, improves bone pain, some improvements in bone mineral density	CNS disease** and skeletal pathology
	Velaglucerase alfa[33]*	1.5 mg/kg/eow			
	Taliglucerase alfa[34]*	60 U/kg/eow			
MPS I	Laronidase[40]	0.58 mg/kg/week	Liver Spleen Connective tissue Cartilage Bone CNS	Reduces hepatosplenomegaly, improves joint mobility, sleep apnea, and pulmonary function, some improvements in walking distance in mildly affected patients	CNS disease, bone manifestations, and corneal clouding,
MPS II	Idursulfase[41]	0.5 mg/kg/week	Liver Spleen Connective tissue Cartilage Bone CNS	Reduces hepatosplenomegaly, improves pulmonary function, walking distance and joint mobility	CNS disease and bone manifestations
MPS VI	Galsulfase[42]	1 mg/kg/week	Liver Spleen Connective tissue Cartilage Bone CNS	Reduces hepatosplenomegaly, improves pulmonary function and walking distance	Bone manifestations and corneal clouding
Pompe disease	Alglucosidase alfa[37-39]	20 mg/kg/eow	Skeletal muscle Smooth muscle Heart	Prolongs survival, improves motor function and walking distance, stabilizes pulmonary function, and reverses cardiac hypertrophy	Skeletal muscle weakness

eow: Every other week; * Indicated for patients with type I Gaucher disease; ** In Gaucher disease type II and III; ^ ERT can not pass the blood brain barrier PNS: Peripheral nervous system; CNS: Central nervous system

The skeletal abnormalities in several of the MPSs (such as dysostosis multiplex) are also very difficult to correct, as they arise in part from abnormal cartilage anlage during fetal development and early post-natal life, and from storage of glycosaminoglycans (GAGs) in osteoclasts and osteoblasts.[45] The correction of GAG storage in the chondrocytes of the avascular articular cartilage and in the growth plate is very challenging, as the therapeutic enzyme will only diffuse through the matrix very slowly. Similarly, the cardiac valves are only slightly accessible to ERT. While microvasculature can be present in the heart valves, the myofibroblasts composing the valves are supplied with oxygen mainly by diffusion from the valve surface, and are therefore not easy targets for the relatively large therapeutic enzymes.[46]

Animal studies have shown that some of the problems associated with cell and tissue accessibility may be overcome by increasing the dose of ERT.[47,48] In mice with Fabry disease, an effect on the heart was seen only with a dose of 10 mg/kg recombinant human alpha-galactosidase.[47] The therapeutic doses currently used in humans are 1 mg/kg for agalsidase alfa and 0.2 mg/kg for agalsidase beta. To optimize the therapeutic effect on cardiac and skeletal muscle, for instance, doses of up to 40 mg/kg of Myozyme are now administered to patients with Pompe disease.[37]

Another problem preventing the full effect of ERT is antibody formation against the therapeutic enzymes, which can counteract the effect of ERT.[49] This may partly explain the different response to ERT observed between patients with the same disease. Several strategies, including immune modulation, are now being explored to preclude or reduce the formation of antibodies.[50]

Irreversible cell damage is a general problem in the treatment of LSDs. More specifically, the effect of ERT is thought to be reduced by secondary effects of lysosomal storage; for example, autophagic build-up in severely affected muscle fibers in Pompe disease impairs intracellular trafficking, including the path from cell membrane to lysosomes that is essential in ERT.[51]

2.1.3 | How can the effect of ERT be improved?

Several strategies for enhancing the effect of ERT are now being explored. *In-vitro* studies and animal studies have shown that it is indisputably improved by higher doses. However, simply raising the dose is complicated by the fact that all ERTs are registered at a certain dose, and that higher dosing might prompt infusion-associated reactions or a more robust immunological response, though this has never been demonstrated.

A second consideration in this respect is that because the price of ERT is calculated per mg bodyweight, a higher dose automatically increases the cost of treatment. Although, to a certain

extent, this can be remedied by reducing the cost of treatment through the development of less expensive production systems such as genetically modified yeast or carrots or transgenic animals (milk), production accounts for only part of the total cost, which also includes development, registration and marketing. For rare disorders, these costs are extremely high.

Recently, human recombinant glucocerebrosidase produced in genetically modified plant cells (taliglucerase alfa) has been approved for the treatment of Gaucher disease.[34] Although the very first initiatives towards producing recombinant human acid α-glucosidase in the milk of transgenic rabbits were stopped for strategic reasons, 6 of the 7 patients with Pompe disease who participated 12 years ago in a phase II clinical trial involving recombinant human acid α-glucosidase from rabbit milk are still alive, and are currently treated with alglucosidase alfa.

Irrespective of production costs, another way of improving the effect of ERT beyond increasing the dose might be by ameliorating the uptake of ERT by the target cells. To this end, the enzyme can be conjugated with synthetic oligosaccharides bearing M6P. This is envisaged for Pompe disease (oxime-neo-recombinant human α-glucosidase). The artificial product was shown to clear the lysosomal glycogen storage in muscle of immunotolerized Pompe mice better than the unmodified enzyme.[52]

The appplication of GILT technology (glycosylation independent lysosomal targeting) may be another way of improving the effect of ERT in Pompe disease. GILT involves the replacement of the N-terminal propeptide of acid α-glucosidase by an N-terminal fragment of IGF-2 that includes the signal peptide.[53] The IGF-2 propeptide binds with high affinity to the M6PR. The fusion protein composed of IGF-2 and recombinant human acid α-glucosidase is currently being tested in a phase II clinical trial (NCT01230801).[43] In Pompe mice, the glycogen clearance effect of the fusion protein was generally equivalent to a corresponding 5-fold higher dose of alglucosidase alfa.[53]

To improve uptake in the central nervous system (CNS), recombinant proteins are being coupled to a monoclonal antibody against an endogenous BBB receptor, such as the human insulin receptor or the transferrin receptor.[54] This has increased enzyme uptake in the brain of MPS II animal models.[55]

The uptake of recombinant enzymes might also be improved through modulation of the M6PR expression on the target cell. Adjunctive treatment of Pompe mice with clenbuterol, a selective β2-agonist, enhanced the M6PR expression in skeletal tissue and increased efficacy of ERT as measured by motor function and glycogen clearance.[56]

Enzyme uptake in specific organs may be improved by local enzyme injection. Injection of enzyme into the intra-cerebrospinal fluid in animal models has reduced neuropathology and improved neurological function.[57] In humans, such a strategy poses several ethical and practical questions. Intrathecal administration of laronidase in a patient with MPS I seemed safe and appeared to alleviate some signs and symptoms of spinal cord compression.[58] The safety and efficacy of intrathecal application of ERT is currently evaluated in open-label phase I/II studies in MPS IIIA (recombinant human heparan N-sulfatase, NCT01299727), MLD (recombinant human arylsulfatase A, NCT01510028), and MPS I (laronidase, NCT00852358).[43] Other clinical studies currently investigate the safety of combined intrathecal and intravenous idursulfase administration in MPS II (NCT01506141), and the administration of intrathecal idursulfase around the time of HSCT in MPS I (NCT00638547).[43]

All forms of ERT are complicated by their burden on patients, who need to receive it life-long, at least twice a month. And, as stated above, ERT is also expensive: its annual cost can amount to USD 145,000-377,000 per patient.[59]

2.2 | Hematopoietic stem-cell transplantation

Although most LSDs do not result in hematopoietic defects, allogeneic hematopoietic stem-cell transplantation (HSCT) might offer an effective treatment. Through infiltration into various tissues, healthy donor cells can replace enzyme-deficient cells. In theory, they can serve as a permanent source of the missing enzyme, and correct neighbouring cells through enzyme secretion and uptake by enzyme-deficient host cells. Various graft sources can be used, including bone marrow (BM), peripheral blood (PB), and cord blood (CB).

In 1980, the first allogeneic HSCT was performed in a patient with MPS I, who received BM from a human leukocyte antigen (HLA) matched relative. He showed biochemical as well as clinical improvements for up to 13 months after transplantation; these included a reduction in hepatosplenomegaly and corneal clouding, and improvement in growth, development and cognition.[60]

Although HSCT has since been attempted in many of the LSDs,[61] its application is currently mainly limited to a subset of patients with MPS I (Hurler), Krabbe disease, and metachromic leukodystrophy (MLD).[62] The clinical effects in MPS I (Hurler) seem the most rewarding.

Hematopoietic stem-cells differentiate into white-blood-cell lineages and migrate to several organs in mice, such as bone marrow, liver (Kupffer cells), spleen, lung (alveolar macrophages), and the central nervous system (microglia).[63] However, they barely home in tissues such as skeletal muscle and cartilage, as shown in rats.[64] In patients with MPS I, HSCT is effective in reducing hepatosplenomegaly, improving airway obstruction and cardiac function, it can

improve corneal clouding, hearing, and psychomotor development, and improves survival. Although it can also improve joint mobility, it does not correct skeletal manifestations, and cardiac valvular deformities appear to resolve poorly.[65] Although one might have expected HSCT to have similar effects in other MPSs, a limited number of studies has shown less beneficial effects. This may be related to the fact that patients with other MPSs were usually transplanted at a later age, or to the use of other graft sources or conditioning regimens.[62] In MPSs with minimal neurological involvement, such as MPS VI, the possible benefits of HSCT have to be weighed against the risks.

Since it is biologically easier to prevent pathology than to correct it, the effect of HSCT is bound to depend on the disease status at the time of transplantation. Transplantation should therefore take place at a very young age, preferably before the clinical symptoms manifest themselves. This is particularly important for preventing irreversible damage in the CNS: as migroglia turnover is very slow, engraftment takes a considerable time.[63] An example of this was reported in Krabbe disease: although CB transplantation improved neurological and developmental outcome in asymptomatic newborns, minimal neurological improvement took place in symptomatic infants.[66] In 2006, these findings and considerations underlay the start of a screening programme for Krabbe disease in New York State.[67] However, the long-term effects of presymptomatic children transplanted for Krabbe disease are not optimal; progressive neurologic deterioration is still observed in many of the treated patients.[68]

The serious complications of HSCT are graft failure and procedure-related risks of mortality and long-term morbidity. To limit these risks, careful selection of a HLA-matched donor graft source and conditioning regimen used to eliminate the patient's own stem-cell population is required. In MPS I, the implementation of transplantation guidelines has significantly improved event-free survival (defined as alive and engrafted).[62] Similarly, the availability of banked cord-blood stem-cells has reduced the time to transplantation. CB has several other advantages, including reduced graft-versus-host disease despite a higher level of HLA incompatibility, the likelihood of sustained engraftment in MPS I, and the suggested capacity of multipotent stem-cells in CB to differentiate into osteoblasts, chondroblasts, and neurons,[69] as a result of which CB might be considered as the preferred cell source in HSCT for LSDs.[70]

As HSCT results in partial correction of the clinical manifestations, and as hematopoietic stem-cells do not engraft well in all tissues, the use of alternative donor cell sources is being explored. For example, to reduce residual disease in patients with MPS I and MLD who previously underwent successful HSCT, mesenchymal stem-cells, capable of differentiation into a large variety of tissues, were infused, but the clinical effect was limited.[71] The ability of

human neural progenitor cells to participate in brain repair has recently been demonstrated in several preclinical studies.[72] One phase I clinical trial has been completed using human central nervous system stem-cells directly implanted into the brains of children with infantile or late infantile neuronal ceroid lipofuscinosis; results are being awaited (NCT00337636).[43]

In practice, ERT is often used to optimize the patient's condition in anticipation of HSCT engraftment.[73] Stem-cell therapy can also be combined with gene therapy (see below).

2.3 | Gene therapy

Gene therapy is based on the idea of transferring DNA that encodes a functional enzyme into the patient's enzyme deficient cells so as to correct the enzyme deficiency and provide a potentially permanent source of therapeutic enzyme. The level of expression can be manipulated by using strong promoters. Since LSDs are monogenic disorders, and low enzyme activities are often sufficient to bring clinical improvement, LSDs are good candidates for gene therapy.

Gene transfer can be applied *in vivo* and *ex vivo*. For *in-vivo* gene therapy, a vector carrying the transgene is injected either into the circulation or directly into a target tissue (e.g. the brain). *Ex-vivo* gene therapy implies correction of the patient's cells by genetic modification *in vitro*, followed by re-implantation. The use of genetically modified hematopoietic stem-cells makes it possible to achieve migration of corrected cells into organs such as the brain, which are otherwise hard to access.[74] Since *ex-vivo* gene therapy entails autologous cells, transplant-related mortality and morbidity is lower than in allogeneic HSCT. *Ex-vivo* gene therapy can also involve the use of organoids (artificial organs).[75]

The feasibility of gene therapy has been demonstrated in various studies performed in a range of animal models. Both *in-vivo* and *ex-vivo* gene therapy can lead to biochemical and clinical improvements.[76,77] As for ERT, these studies have shown that transduction in certain tissues (such as brain) is more difficult than in others (such as liver). In addition, the effect of gene therapy depends on the level of transgene expression, which in turn determines how much therapeutic enzyme is secreted by the genetically corrected cells and potentially available for correction of more distant tissues and organs.[77] Although the outcome of gene-therapy studies in animal models has been fairly promising, many safety and efficacy issues need to be addressed for application in humans.

One major obstacle to the clinical application of gene therapy is the potential risk of oncogenesis due to undesirable integration of the transgene construct into the genome.

Attempts at gene therapy for patients with X-linked SCID using retroviral vectors led to successful treatment of 9 of 10 patients, but also led to lymphoproliferative disorders in four of these patients.[78,79] Viral vectors also risk eliciting inflammatory and immunological responses; the latter may increase with readministration.[80] The transgene product may also elicit a humoral immune response, which can reduce the effect of gene therapy. Restriction of enzyme expression to the liver can reduce this immune response, presumably by reducing expression of the transgene in antigen-presenting cells.[80]

The key issue in somatic gene therapy is finding the right vector for the delivery and long-term expression of the transgene in the target tissues. For gene transfer in LSDs, mainly viral vectors are used, particularly adeno-associated viruses (AAV) and lentiviruses.[81] These vectors can infect a wide range of tissues, infect dividing and non-dividing cells, and induce long-term transgene expression. Whereas lentiviruses insert into the genome, and can thus induce insertional mutagenesis and oncogenesis, present-day AAV-vectors are essentially episomal and are non-pathogenic.

Thus far, about 20 clinical trials have been performed with gene therapy in LSDs, but as yet none has reached phase III.[81] Recently, it was suggested that administration of an AAV serotype 2 vector expressing the human CLN2 cDNA into the brain of children with late infantile neuronal ceroid lipofuscinosis (Batten disease) slowed neurological progression in the treated children compared to untreated controls.[82] A follow-up study is currently ongoing (NCT01414985).[43] Phase I/II clinical trials are currently being performed for Pompe disease (NCT00976352) to evaluate the delivery of a recombinant AAV acid α-glucosidase gene vector to the diaphragm, and for MLD (NCT01560182) using gene-modified HSCT.[43] Direct intracranial injection of viral gene vectors has resulted in reduced lysosomal storage and functional improvement in some large animal models of LSDs.[76] A phase I/II trial evaluating the tolerance and safety of intracerebral administration of an AAV vector carrying the N-sulfoglucosamine sulfohydrolase and sulfatase-modifying factor 1 cDNAs for the treatment of MPS IIIA is currently ongoing (NCT01474343).[43]

As well as viral vectors, non-viral vectors are being developed as an alternative method of gene delivery and targeting. Methods of delivery include the encapsulation of DNA in polymers, liposomes or nanoparticles,[81] which may provide vehicles to cross the BBB.[83]

If the vectors and procedures become safer, more specific and more effective, gene therapy is likely to become applicable in clinical practice.

2.4 | Chaperone therapy

During co-translational import into the endoplasmic reticulum (ER), missense mutations in genes coding for lysosomal proteins often lead to improperly folded or unstable proteins. These proteins are either recognized by the ER quality-control system (ERAD), and then exported and degraded by proteasomes; or they continue their journey to the lysosomes, but fail to reach their final destination. The aim of chaperone therapy is to reduce the elimination of these proteins, which can be catalytically active. Chaperones are small molecules that selectively bind and stabilize target proteins, thereby mimicking normal folding, improving intracellular trafficking, and increasing lysosomal enzyme activity. While chaperone therapy is thus expected to restore small conformational changes, it is not expected to restore the effect of mutations that cause major structural changes (e.g. null-mutations or frameshifts mutations). The chaperones currently used or developed for the treatment of LSDs are reversible competitive inhibitors of their target enzyme.

Many *in vitro* studies have shown that a growing number of chaperones can restore enzyme transport and maturation, and increase residual enzyme activity in cells of patients with chaperone-sensitive mutations.[84]

The first clinical trial of chaperone therapy for Fabry disease using galactose claimed remarkably good results in a patient with the cardiac variant of Fabry disease.[85] Galactose was administered intravenously every other day, whereas the current chaperones have the advantage of oral administration.

Several phase I/II clinical trials have been conducted for Fabry (migalastat hydrochloride) and Gaucher disease (isofagomine), and GM2 gangliosidosis (pyrimethamine). Some patients were reported to respond positively to these chaperones, by increased enzyme activity levels in leukocytes.[86,87] Communications from the manufacturer reported that the renal function of responding Fabry patients remained stable during 2 – 3 years of treatment.[87] In Gaucher disease, clinically meaningful improvements in key measures of disease were reported in only one of the eighteen patients after six months of treatment.[87] In GM2 gangliosidosis, clinical effects were difficult to assess, mainly due to short treatment duration of up to 10 months and inter-individual variability.[86] Treatment was generally well tolerated in Fabry and Gaucher disease.[87] In GM2 gangliosidosis, significant side effects were experienced by most patients at or above 75 mg pyrimethamine per day.[88] A phase II clinical trial in Pompe disease with 1-deoxynojirimycin hydrochloride was suspended due to serious and probably treatment related adverse events (NCT00688597).[43,89]

At present, a randomized, placebo-controlled phase III clinical trial with chaperone therapy is ongoing for Fabry disease (migalastat hydrochloride, NCT00925301), and an uncontrolled open-label, phase II extension study is ongoing for Gaucher disease (isofagomine, NCT00813865).[43]

Whereas the first chaperones were identified by rational drug design, recently, high-throughput screening of libraries with chemicals and registered drugs has been successfully utilized to identify novel chaperones. This strategy led to the identification of the drug ambroxol as a potential chaperone for Gaucher disease.[90] Ambroxol was demonstrated to increase the activity of glucocerebrosidase in cultured fibroblasts from patients and in wild-type mice.[90,91] An open-label phase I/II clinical trial has been announced to assess its safety and proof-of-concept (NCT01463215).[43]

The continued interest in chaperone therapy is explained largely by their potential to target many organs and to cross the BBB. This has been illustrated in mouse models for Fabry,[92] Gaucher,[91,93] GM1 gangliosidosis,[94] and Pompe disease.[84] Administration of N-octyl 4-epi-β-valienamine in GM1 gangliosidosis mice starting at the early stage of disease resulted in remarkable arrest of neurological progression within a few months, and prolonged survival.[94] Other advantages of chaperones are that they can be administered orally, making therapy minimally invasive, and that these small molecules are relatively easy to produce, which reduces their cost. Neither is antibody formation likely to occur.

A real disadvantage of chaperone therapy is that their use is restricted to a small subset of mutations, as shown in Fabry,[95] Pompe,[96] and Gaucher disease,[97] and in GM1 gangliosidosis.[98] Secondly, as the current generation of chaperones competes with the endogenous substrates for binding to the active site of the lysosomal enzymes, dosing is very critical. The chaperone must correct protein folding as much as possible and inhibit the catalytic function as little as possible. To optimize the effect, dose optimization studies have been performed in animal models. For example, in Fabry mice that express a mutant form of human α-Galactosidase A (R301Q) on a knockout background, intermittent administration of chaperone therapy (4 days on/ 3 days off or every other day) resulted in greater substrate reduction than daily administration.[92] Another approach to circumvent the competition between chaperones and endogenous substrates is to search for chaperones that bind to alternative sites. For this purpose, novel high-throughput screening methodologies have been used to identify non-inhibitory chaperones for Gaucher disease[99] and to search for chaperones that act as enzyme activators in Pompe disease.[100]

It is being explored whether chaperones can have a synergistic effect when combined with ERT. Compared to ERT alone, co-administration of chaperones with ERT has been shown to

enhance substrate clearance and increase cellular enzyme activity in Gaucher,[101] Fabry,[102] and Pompe[103] disease *in vitro*, and in Fabry[104] and Pompe mice.[103] Drug-drug interaction studies of chaperone therapy and ERT in patients with Fabry (NCT01196871) and Pompe disease (NCT01380743) are currently ongoing, and the results of a study in patients with Gaucher are awaited (NCT00433147).[43]

2.5 | Substrate-reduction therapy

The objective of substrate-reduction therapy (SRT) is to minimize lysosomal storage by inhibiting the synthesis of the substrate and thereby improving the balance between lysosomal input and degradation. In this way, the low level of residual enzyme activity, usually found in patients with slowly progressive phenotypes, may become sufficient.

As glucosyltransferase is a key enzyme in the synthesis of glycosphingolipids, it was proposed that inhibition of this enzyme could reduce the production of glycosphingolipids.[105] The first iminosugar used for this purpose was *N*-butyldeoxynojirimycin (Miglustat), which reversibly inhibits ceramid-specific glucosyltransferase and shows structural similarities with active site-directed inhibitors such as chaperones.

The administration of *N*-butyldeoxynojirimycin to adults with Gaucher disease type I reduced hepatosplenomegaly and improved blood counts and disease biomarkers.[106] In 2003, these results led to the award of marketing approval for mild to moderately affected patients with Gaucher disease type I for whom ERT is not a therapeutic option, e.g. because of persistent side effects to ERT. Longer follow-up studies have shown significant improvements in all major efficacy endpoints, and have demonstrated effectiveness over time.[107] Although *N*-Butyldeoxynojirimycin has been shown to cross the BBB in several mouse models it showed no improvement in neurological symptoms over a two-year period in patients with Gaucher type III.[108] In a sibling study in two adults with Saposin C deficiency, *N*-butyldeoxynojirimycin had no significant effect after 2 years of treatment.[109]

In 2009, *N*-butyldeoxynojirimycin was also approved for the treatment of Niemann Pick disease type C (NPC), as it had been shown to stabilize or improve saccadic eye movements, a neurological symptom of NPC in a randomised controlled study.[110] The mechanistic action of *N*-butyldeoxynojirimycin in NPC, in which glycolipids and cholesterol accumulate, is not fully clear. It might act through a direct effect on lipid trafficking or indirectly by modulating intracellular calcium homeostasis or reduction of oxidative damage.[111]

N-butyldeoxynojirimycin causes side effects consisting of gastrointestinal problems such as flatulence, bowel cramps and diarrhea, which is probably caused by inhibition of the

intestinal carbohydrate-digesting enzyme sucrase-isomaltase.[112] Another inhibitor acting on glucosyltransferase is Eliglustat. Although the biochemical and clinical results obtained with Eliglustat were similar to those obtained with N-butyldeoxynojirimycin,[113] its higher specificity for glucosyltransferase and the absence of inhibition of intestinal glucosidases, meant that the treatment related gastrointestinal side effects were minimal.[114] Eliglustat is currently being evaluated in a phase III randomized, double-blind, placebo-controlled trial in patients with Gaucher disease type I (NCT00891202).[43]

Genistein is a potential SRT in the MPSs. Genistein is a soy-bean isoflavone that has non-specific glycosaminoglycan-reducing ability. In MPS II[115] and MPS IIIB mice[116], it has been shown to reduce GAG accumulation in non-CNS tissue. In an MPS IIIB mouse model, it had various effects on neuropathology; most notably, it reduced heparan sulfate storage in the brain, improved synaptic function and corrected behaviour abnormalities.[117]

Although the first clinical trial using genistein in patients with MPS III A and B for 12 months showed statistically significant improvements in urinary GAG concentration, hair morphology, and cognitive function,[118] a recent placebo-controlled cross-over study showed that genistein had little effect in MPS III patients.[119] Total GAGs in urine decreased significantly, but there were no measurable effects on overall behaviour or hair morphology. Furthermore, neither parents nor caregivers were able to distinguish the periods in which the patients received either treatment or placebo.

In summary, SRT seems a promising approach for treating some of the LSDs. Advantages include the potential for crossing the BBB, lower production costs than ERT, and oral administration. Conceivably, improvements will be achieved by searching for novel compounds with higher specificity and improved range of action.

2.6 | Other pharmacotherapeutic options

Cysteamine bitartrate

In 1994, cysteamine bitartrate was approved by the US Food and Drug Administration as a therapy for cystinosis, an LSD in which cystine accumulates due to deficiency of the cystine transporter. Cysteamine bitartrate enters the lysosome and reacts with cystine to form cysteine and cysteine-cysteamine complexes, which can leave the lysosome.[120]

In 2006 Lu et al. suggested that cysteamine might be useful for the treatment of infantile neuronal ceroid lipofuscinosis (NCL 1), since cysteamine acts on thioesther linkages and seems to cleave palmitoyl-CoA, which is the accumulating substrate in NCL 1. Cysteamine bitartrate resulted in a modest reduction of palmitoyl-CoA accumulation in isolated lymphoblasts of

NCL 1 patients.[121] Cysteamine bitartrate, alone and in combination with N-acetylcysteine, is currently being tested for the treatment of NCL 1 (NCT00028262).[43]

Nonsense mutation suppression

Some patients with LSDs have nonsense mutations that prematurely abort translation and give rise to a truncated protein, usually a non-functional one. In such cases, therapy can be targeted on suppressing the nonsense mutation with drugs[122] such as ataluren, which has been shown to increase enzyme activity in cultured fibroblasts of patients with infantile neuronal ceroid lipofuscinosis.[123] Ataluren is a non-toxic compound that promotes ribosomal read-through of premature stop codons caused by nonsense mutations. The compound is supposed to prevent translation termination and to stabilize nonsense-containing transcripts, thus enabling quasi-normal enzyme formation. Ataluren has had positive effects in patients with cystic fibrosis caused by nonsense mutations.[124] Though its clinical effects to date have been limited, this therapeutic approach is currently being evaluated in an uncontrolled open-label phase III clinical trial for the treatment of cystic fibrosis (NCT01140451).[43]

Targeted gene repair

Targeted gene repair can be seen as the ultimate art of gene therapy. It is intended to correct specific point mutations through the use of site-specific oligonucleotides.[125] Whatever its promise, it has not yet shown its potential value in clinical practice: targeted correction of a singe-base change using chimeric RNA/DNA oligonucleotides in a model system employing Gaucher fibroblasts produced no genomic correction.[126]

3 | EXPERT OPINION

Much progress has been made in the development of therapeutic strategies for LSDs. In the past decades, several therapies have been brought to market and have significantly improved the outcome of patients with LSDs. Unfortunately, none of the current therapies are fully curative yet. Interference with the pathologic process either has to start with cellular repair through any therapeutic means, or by replacing affected cells through stem-cell therapy. In all instances, the timing of the start of treatment is crucial, as tissue and organ damage become irreversible upon disease progression.

What prevents the current therapies from being fully curative is the multi-organ involvement in LSDs caused by the ubiquity of lysosomes in all cell types other than red blood cells; this requires the correction of many specialized cell and tissue types, which is difficult for ERT and gene therapy to target. While some tissue types seem to require a high dose of circulating enzyme, this automatically results in higher costs. A potentially useful feature of gene therapy is that it can be directed to a distant organ, for instance the liver to make it serve as permanent enzyme reservoir. The tightness of the BBB impedes correction of CNS pathology by ERT.

The BBB problem is partially circumvented by chaperone therapy and SRT. Their use is of limited applicability, as their effect depends on the patient's genetic mutation. These therapies are further complicated by their narrow therapeutically effective range: while too high a dose of chaperone therapy may suppress the enzyme's activity, as the chaperones currently available settle in the active site pocket, too low a dose is ineffective. Too high a dose of SRT risks harmfully inhibiting essential biosynthetic pathways.

New avenues to therapeutic intervention might be opened by greater insight into the pathophysiological processes leading to the complex phenotypes of LSDs. Currently, our knowledge is still scarce on how the lysosomal protein dysfunction caused by lysosomal gene defects eventually results in the clinical phenotype, and on the extent to which epigenetic, cell-type specific, and environmental factors play a role in this process. Knowledge of these processes might prompt the development of novel therapeutic targets.

With regard to the near future, we believe that the therapeutic effectiveness of ERT can be improved and costs be reduced by intelligent design of the therapeutic enzymes, for example by maximizing their M6P content or make them bind with higher affinity to the bifunctional M6P/IGF 2 receptor (GILT technology).[53] Costs might be reduced through alternative production methods such as the use of plant cells to produce recombinant human glucocerebrosidae for the treatment of Gaucher disease,[34] or yeast-cell systems to produce therapeutic enzymes for GM2-gangliosidosis.[127] In the past, production of acid α-glucosidase in rabbit milk and larger transgenic animals proved very efficient and therapeutically effective; its cost are potentially low.[128] In cases in which antibody formation clearly counteracts the effect of treatment, improvement can be achieved by eliminating the immune response.[50]

In the near future, once safe vectors with proper specificities have been developed, enzyme therapy will probably be replaced by the *in-vivo* or *ex-vivo* use of gene therapy. In the meantime, the search for modifying factors may lead to the identification of new therapeutic targets that can help to improve the response to existing methods. Modifying factors might then support the selection of the best therapy for individual patients.

In the more distant future, stem-cell therapy may provide a tool for local tissue replacement. Natural stem-cell sources and induced pluripotent stem-cells (iPS cells) are currently being considered for this purpose.[129] In theory, iPS cells have great potential, since they can be stimulated to differentiate into various lineages including neuronal stem-cells.[130,131] The cells can be derived from the patient and then genetically corrected before re-transplantation. This technology avoids the ethical dilemmas inherent to obtaining stem-cells, and also circumvents robust immunological responses.

It is crucial to the success of any type of therapy – existing or potential – that therapy starts before the disease process has caused irreversible tissue damage. Although precious time in this respect can be gained by neonatal screening programmes,[132] they raise ethical questions.[133] Most LSDs are characterized by a very broad clinical spectrum, and many patients will be diagnosed long before their first disease symptoms manifest. Patients are then condemned to live with the burden of a disease that may not develop until late adulthood. Clinical trials in LSDs are complicated by the rarity of the diseases, and the broad spectrum of phenotypes within each disease.[8] This demands that knowledge is preferably gathered and shared by Centres of Expertise. To investigate the effect of therapeutic interventions and to choose the best clinical endpoints for future trials, this process should start by delineating the natural course of the disease.

In conclusion, considerable progress has been made in establishing different therapies for patients with LSDs. As the number of studies is continuously expanding, the first fully curative treatment for LSDs is likely to become a reality within the next few decades.

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CHAPTER 3

Motor development, motor function, and muscle strength in patients with classic infantile Pompe disease treated with enzyme therapy; an open-label, single-center study

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ABSTRACT

Background

Enzyme-replacement therapy (ERT) has greatly improved motor development in patients with classic infantile Pompe disease. As only scarce information is available on long-term motor involvement, we aimed to establish a profile of the motor performance and complications in survivors who learned to stand or walk.

Methods

In 11 patients with classic infantile Pompe disease who were over 1.5 years of age and who were treated for up to 14 years, we prospectively assessed motor development (Alberta Infant Motor Scale, Bayley Scales of Infant Development II, and Movement Assessment Battery for Children), motor function (Quick Motor Function Test, 6-minute walk test, and timed tests), muscle strength (manual muscle testing and hand-held dynamometry), and the development of specific clinical features of muscle weakness in different age groups.

Results

Up to three years of age patients gradually gained motor skills, and motor development was close to normal. Three patients temporarily or completely lost motor milestones due to respiratory decline. At study end, six patients were over four years of age, of whom four could walk. In these four patients a characteristic phenotype emerged: All patients had difficulties in specific motor function items. A typical distribution of muscle weakness emerged: hip extensors and abductors, foot dorsal flexors, and neck flexors were affected most. One patient developed severe weakness of selective finger extensors. Specific clinical features of muscle weakness such as a weak facial expression, ptosis, and mild scoliosis were common. Strikingly, beyond three years of age certain motor functions such as squatting deteriorated, and gait became abnormal.

Discussion

Although initially motor development is close to normal, as patients grow older a typical phenotype emerges that differs from that of untreated children and adults with non-classic Pompe disease. Our findings emphasize the need to develop specific follow-up and multidisciplinary support programs for patients with classic infantile Pompe disease.

INTRODUCTION

Pompe disease (acid maltase deficiency or glycogen storage disease type II) is a rare neuromuscular disorder, in which deficiency of the lysosomal enzyme acid α-glucosidase leads to glycogen accumulation in many tissues; most notably in skeletal muscles.[1,2] The clinical spectrum is broad, and ranges from the most severe classic infantile form, characterized by general hypotonia, hypertrophic cardiomyopathy, and respiratory problems resulting in death before one year of age,[3,4] to less progressive forms in children and adults, in which weakness of the limb-girdle muscles and the diaphragm is the predominant feature.[5-7]

Enzyme-replacement therapy (ERT) with recombinant human acid α-glucosidase (alglucosidase alfa®) is the first available treatment for Pompe disease and was approved in 2006. Treatment with ERT improves ventilator-free survival and reverses cardiac hypertrophy in patients with classic infantile Pompe disease.[8-16] Many patients now reach previously unmet motor milestones such as standing or walking.

Slowly, it becomes clear that most patients treated with ERT exhibit residual muscle weakness,[17-22] and that the emerging gross motor and musculoskeletal phenotype of these infants differs from the phenotype of children and adults with non-classic Pompe disease.[21,22] Knowledge of motor development and distribution of muscle weakness in patients treated for many years with ERT is important to get insight in long-term outcome, to timely install appropriate supportive measures, and to provide adequate information to parents and health care practitioners. However, data on long-term motor outcome are scarce and longitudinal studies are hardly available.

Therefore, in this study we aimed to established a profile of the long-term motor performance and motor complications in infants with classic infantile Pompe disease treated with ERT. Data were collected as part of a longitudinal follow-up study since 1999. We examined motor development, motor function, and muscle strength in 11 patients who were more than 1.5 years old and who had learned to stand or walk. Patients were treated with ERT for up to 14 years.

METHODS

2.1 | Patients

Patients with classic infantile Pompe disease participated in a single-center, prospective, open-label cohort study on the effects of ERT (20 mg/kg every other week to 40 mg/kg weekly) conducted from 1999 to June 2013 at Erasmus MC University Medical Center, the designated center of expertise for Pompe disease in the Netherlands. All patients with classic infantile Pompe disease treated with ERT who had at least reached the age of 1.5 years and had learned to stand or walk were included. Classic infantile Pompe disease was defined as: 1) Symptoms of muscle weakness within six months of birth, 2) hypertrophic cardiomyopathy, and 3) severe *GAA* (the gene-encoding acid α-glucosidase) mutations on both alleles. Initially, four patients received recombinant human α-glucosidase from the milk of transgenic rabbits;[8] from 2004 onwards, all patients were treated with recombinant human α-glucosidase form CHO cells (alglucosidase alfa®). The Institutional Review Board approved all protocols, and all parents or guardians gave written informed consent.

2.2 | Motor development

Motor development was assessed every three months using the Alberta Infant Motor Scale (AIMS; 0 – 19 months),[23] the Bayley Scales of Infant Development II (BSID-II; 0 – 3.5 years),[24,25] and the Movement Assessment Battery for Children (M-ABC; 3 – 8 years)[26,27] depending on the patient's age. For the AIMS, the total score (range 0 – 58) obtained by patients was compared with the scores of age-related healthy peers,[23] for the BSID-II, age-equivalent scores were compared to the patients' chronological age,[25] and for the M-ABC, raw scores were converted into percentile scores for the total test score and for the three subtests manual dexterity, aiming and catching, and balance.[26,27] Whenever possible, a single therapist performed the subsequent assessments of a child. The age at the achievement of motor milestones was registered during regular examinations by a pediatrician.

2.3 | Motor function

From 2005 on, motor function was assessed using the Quick Motor Function Test (QMFT), the first motor function test designed and validated specifically for Pompe patients.[28] The performance of a patient on 16 motor skills was scored on a 5-point ordinal scale (with 0 representing "cannot perform task" and 4 "can perform task with no effort").

Functional activity assessments also included a six-minute walk test (6MWT) and two timed tests. The 6MWT was performed according to the guidelines of the American Thoracic Society.[29] The timed tests consisted of 10 meter running and rising from supine to standing position. Scores were compared to age-related healthy peers.[30-32]

2.3 | Muscle strength

Muscle strength was assessed by manual muscle testing using the Medical Research Council (MRC) grading scale.[33] Muscle strength was examined according to the patient's age: general strength of proximal and distal muscles was measured up to 1 year of age; between 1 and 3 years of age bilateral strength of proximal and distal muscles of the arms and legs was measured; and over four years of age, bilateral strength of 18 solitary muscles was measured and was averaged group wise to obtain scores for the proximal and distal muscles of the arms and legs. The muscle groups tested were: Shoulder abductors, elbow flexors and extensors (proximal muscles arms); wrist flexors and extensors, wrist pronators and supinators, grasp force, finger extensors (distal muscles arms); hip flexors and extensors, hip abductors and adductors, knee flexors and extensors (proximal muscles legs); foot dorsal flexors, and foot plantar flexors (distal muscles leg).

In patients older than six years, hand-held dynamometry (HHD) (Cytec dynamometer, Groningen, the Netherlands) was used as a second measure of muscle strength. The following muscle groups were examined: neck flexors, shoulder abductors, elbow flexors, wrist extensors, hip flexors, hip abductors, knee extensors, knee flexors, foot dorsal flexors. Individual scores for each muscle group were summed and compared to reference values of age related peers.[34]

2.4 | Other clinical features

During standardized examinations by a neurologist and pediatrician every three months information was gathered on the following: 1) the presence of specific features such as ptosis, lordosis, scoliosis, and muscle atrophy; 2) the use of a wheelchair; and 3) the use of splints.

RESULTS

3.1 | Patients

Between 1999 and June 2013, 24 patients with classic infantile Pompe disease were seen at Erasmus MC University Medical Center, the designated center of expertise for Pompe patients

in the Netherlands. Nineteen of them received ERT of whom eleven patients met the inclusion criteria (7 males, 4 females); four patients were younger than 1.5 years by study end, the other four patients never learned to stand or walk (Figure 1). All patients had severe *GAA* mutations.

The ages at start of treatment ranged from 0.1 to 4.8 months. At baseline, five required supplemental oxygen via nasal cannula; seven required nasogastric tube feeding. Ten patients were CRIM-positive and one was CRIM-negative. At last assessment the ages of these patients ranged from 1.5 to 14.9 years. Two patients became partially or completely ventilator dependent at the age of two years; one of these patients died at the age of four.

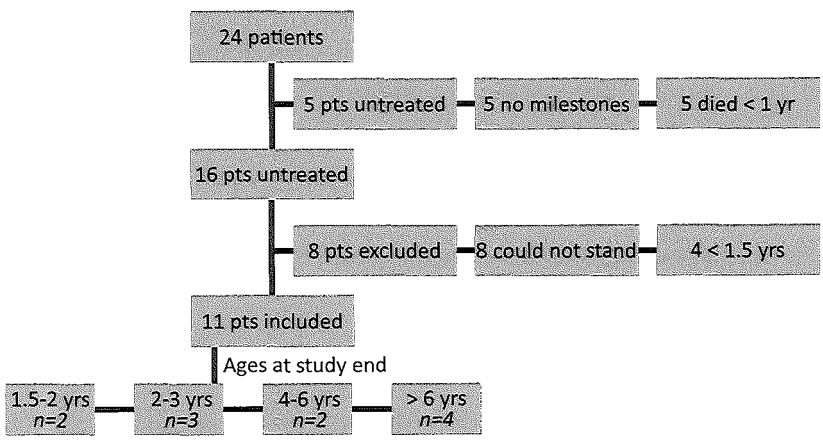

Figure 1 | Flowchart of patient selection.

3.2 | Motor development

All patients showed a gradual increase in AIMS score during the first 1.5 years (Table 1). Ten patients reached maximum scores in supine and sitting position, seven in standing position, and five in prone position. Five patients learned to crawl with reciprocal use of upper and lower extremities with a flat lumbar spine; the six others crawled with lumbar lordosis, indicative for weakness of the abdominal muscles. Seven patients learned to squat. Patients had particular difficulties with antigravity movements. By 18 months of age, five patients had reached the maximal AIMS score.

Table 1 | AIMS score and maximal motor milestone.

Patient	Raw AIMS score at baseline		Raw score at last AIMS^		Maximal motor milestone$
1	7	*(3)*	34#	*(19)*	Walking with support
2	5	*(5)*	44	*(17)*	Pulls to stand
3	6*	*(2)*	48	*(17)*	Walking&
4	6	*(5)*	50	*(17)*	Walking
5	3	*(1)*	52	*(16)*	Walking
6	1	*(2)*	56	*(17)*	Walking
7	10*	*(3)*	57	*(18)*	Walking
8	2	*(0)*	57*	*(19)*	Walking&
9	4*	*(0)*	57*	*(19)*	Walking
10	4	*(4)*	58*	*(18)*	Walking
11	2	*(0)*	58*	*(19)*	Walking
Total: median (range)	**4 (1-10)**	***2 (0-5)***	**56 (34-58)**	***18 (16-19)***	

Age in months is given between brackets.
* AIMS score >p5; ^ p5 and p50 at 18 months of age are 56.6 and 57.7 respectively; # Patients previously reached higher AIMS scores, but lost motor skills after respiratory infections; $ If maximal motor milestone is reached beyond 18 months of age, the age in months of maximal motor milestone is given between brackets; & Lost the ability to sit or walk respectively after they became ventilator dependent.

Nine patients learned to walk at ages ranging from 14-18 months (median 16 months), which is within normal limits. Two patients who were 1.5 and 1.8 years at study end are expected to walk alone soon; they now pull to stand or walk with support.

By three years of age, three patients had lost motor skills. One patient temporarily lost the ability to pull to stand at the age of one after an RSV infection; the AIMS score is now steadily increasing. Two ambulant patients lost the ability to walk at the age of two after they had become ventilator-dependent. These patients all showed a decrease in AIMS score at the time of clinical deterioration (Figure 2A).

One patient was 1.5 years at last assessment. The other seven patients continuously gained motor skills between the age of 1.5 and 3.5 years as assessed by the BSID-II (Figure 2B). None of the patients developed completely within the normal limits for age, but four patients showed normal age-equivalent motor development in at least one assessment. Subscores were available for six patients: all patients placed 10 pellets in a bottle in 60 seconds, 5/6 could squat briefly, and 5/6 walked on tiptoe for four steps. All could stand on one leg with help; 3/6 stood alone on one foot. Two patients swinged their leg to kick a ball, one patient

jumped distance of 10 cm, and none of the patients could run with coordination. Gross motor skills were more delayed than fine motor skills.

At study end, six patients were over 4 years of age, of whom four patients could walk. The M-ABC was performed in three of them at ages ranging from four to seven years. Manual dexterity was within normal limits in two patients. One patient could not post coins and thread beads within sufficient time. Borderline motor difficulties were present in two patients at testing aiming and catching; they had difficulties in catching a beanbag. Definite motor problems were present in one patient at testing aiming and catching, and in all patients at testing balance, i.e. standing on one leg, jumping and walking heels raised. The total tests scores of all patients were below the 5th percentile.

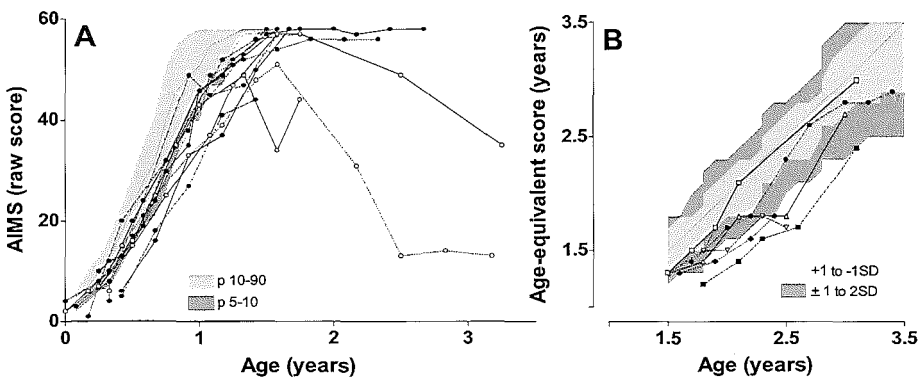

Figure 2 | Motor development as measured by AIMS and BSID-II.

Raw AIMS scores over time **A.** Three patients temporarily or completely lost motor skills (open circles). Age-equivalent scores of the BSID-II compared to the patient's chronological age **B.** The dashed areas provide reference data.

3.2 | Motor function

In the first 1.5 years of life, the QMFT was performed in seven patients. Motor function of all patients gradually increased. None of the patients had difficulties with crossing their hands across midline and extending the arms; one patient had difficulties with hip and knee flexion and two with raising the torso in prone position. Only one patient could flex the neck to 45 degrees while supine. All patients used their hands when transferring from supine to a sitting position.

Between the ages of two and three, the motor function of the two patients who lost the ability to walk deteriorated significantly. None of the other seven patients older than two years had difficulties with raising the torso, hand across midline, extending the arms, hip and knee flexion, and extending the legs. None of the patients used their hands while standing up from a chair or squatting; one patient used the hands to stand up from a squat and to pick up an object. Six patients walked 10 meter with a normal gait. One patient still could flex the neck to 45 degrees while supine. As appropriate for age, all patients had difficulties in standing on one leg, standing up from half knee, and jumping, and all used the railing to walk up steps.

Four of these seven patients, representative for this group (Figure 3A), were followed to the age of 4, 8, 9, and 14 years. Fiure 3B shows the scores per motor function item at last assessment. At last assessment, none of the patients had difficulties with hand across midline and extending the arms. The time standing on one leg and the distance jumped increased in two patients. Neck flexion remained difficult for all patients. Patients still used their hands to do a sit-up. Two patients now used their hands to stand up from a chair. Two patients could no longer squat, and one patient now used the hands to squat and stand up from a squat. All patients walked 10 meters, but all had developed an abnormal gait. None of the patients had a heelstrike, and three patients walked with circumduction of the legs. Hip extension was reduced in all patients; three had an anterior pelvic tilt and used compensatory trunk movements. All patients had endorotation of the legs, and one had a Trendelenburg gait. All patients had pes planus, genu valgum, and increased lumbar lordosis. One patient could no longer walk up steps; two others still used the railing.

Functional capacity with the 6MWT was impaired in three patients; one patient developed at or just below the borderline of normal (-2SD) (Figure 4A). The time to rise from supine to standing position and to walk 10 meters took longer for the patients compared to healthy peers (Figure 4B). Of note, none of the patients showed a Gowers sign, indicative for proximal muscle weakness, while rising from supine to stand. The flight phase of one patient was highly reduced; the three others did not show a flight phase at all while running. Over time, the absolute distance walked at the 6MWT deteriorated significantly in one patient; the time to rise from supine deteriorated in three patients; and the time to run 10 meter deteriorated in one patient and improved in another patient.

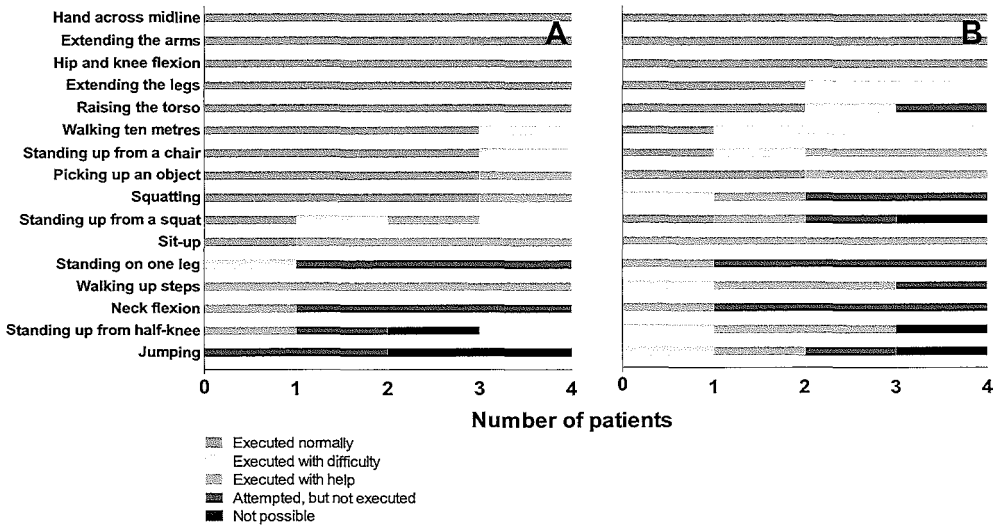

Figure 3 | Motor function as measured by QMFT.

Scores of the four ambulant patients over four years of age on the different motor function items of the QMFT between two and three years of age (A) and at the age of 4, 8, 9, and 14 (B).

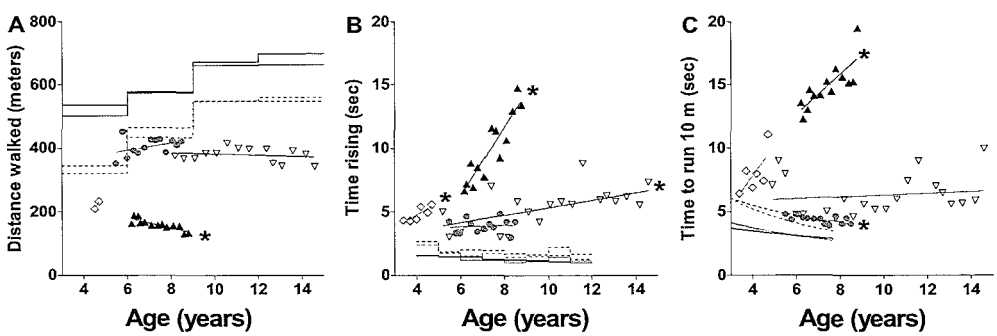

Figure 4 | Scores on the six-minute walk test and timed tests.

Scores on the six-minute walk test (A) and timed tests: Time to rise from supine to stand (B), and time to run 10 meter (C). The scores are compared to age-related healthy peers (solid lines represent 0 SD; dotted lines represent -2 SD (A) or + 2 SD (B and C); black represents boys; grey represents girls). The symbols represent different patients. The lines represent the linear regression line per patient.

3.3 | Muscle strength

At baseline, muscle weakness was more pronounced in the proximal muscles than distal muscles (Figure 5A). Proximal muscle strength tended to increase over time, whereas distal muscle strength tended to decrease. Comparisons of median MRC values for the distal and proximal muscles at baseline and last assessment in the four ambulant patients over four showed no significant difference when assessed using the Wilcoxon matched-pairs signed-rank test (p=0.20 and p=0.29 respectively). The legs were more affected than the arms (Figure 5B). At three years of age, none of the patients had an MRC score of 3 or lower in the distal legs; at last assessment two ambulant patients had an MRC of 0 and 1 in the distal legs.

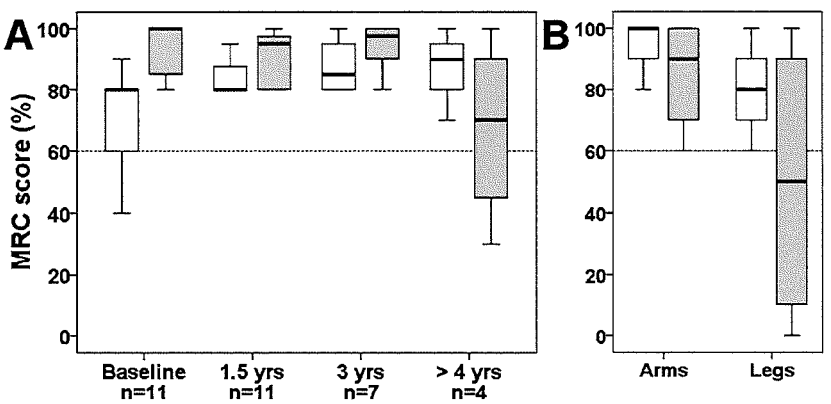

Figure 5 | Distribution of muscle weakness over time.

Box plots show the MRC scores of the proximal (black) and distal (grey) muscles at baseline and different age groups (**A**), and the severity of muscle weakness of the arms and legs in the four ambulant patients over four at last assessment (**B**) The dashed lines represent the median MRC score, boxes represent the p25 and p75. The dotted lines represent an MRC score of 3, indicative for severe muscle weakness.

Figure 6 shows the severity and distribution of muscle weakness of the four ambulant patients over four years at last assessment. The facial and bulbar muscles, neck flexors, abdominal muscles, hip flexors, hip extensors, hip abductors and adductors, and foot plantar and dorsal flexors were affected in at least three patients (Figure 6A). The strength of the quadriceps muscle and of the proximal muscles of the arms was reduced in only one or two patients. The facial and bulbar muscles, neck flexors, paraspinal muscles, hip extensors and abductors,

foot plantar and dorsal flexors were most severely affected (Figure 6B). One patient could not actively flex the wrist and could not actively straighten the middle and ring finger (Figure 7A and B). The pattern of muscle weakness was symmetrical in all patients.

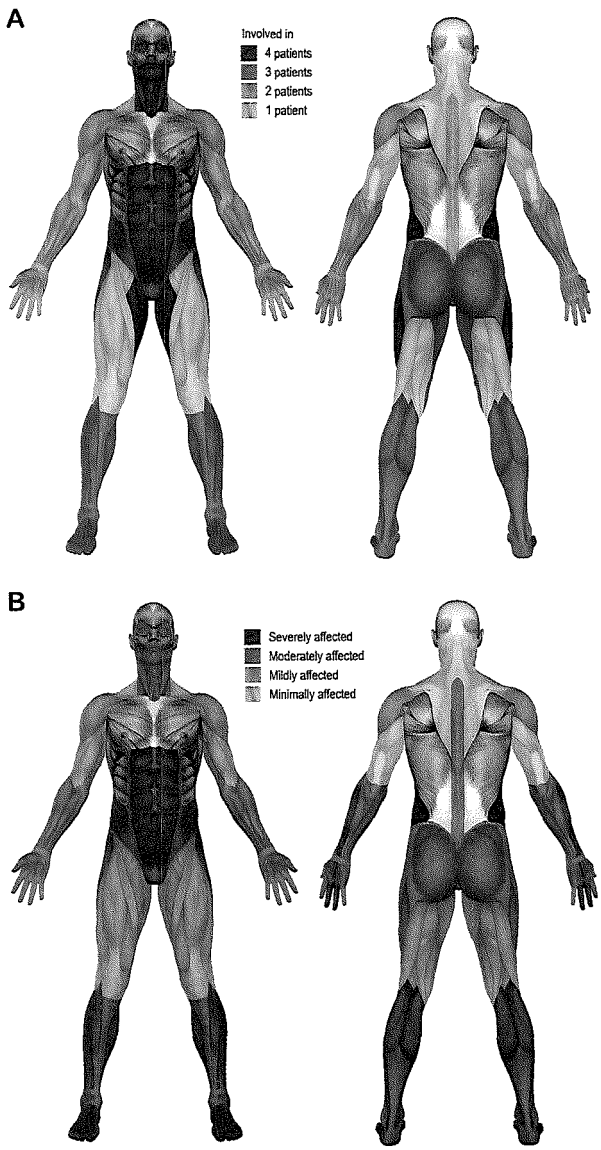

Figure 6 | Muscle weakness in ambulant patients over four years of age.

Distribution of skeletal muscle weakness (**A**) and severity of muscle weakness of the individual muscle groups (**B**) in four ambulant classic infantile Pompe patients over four years of age.

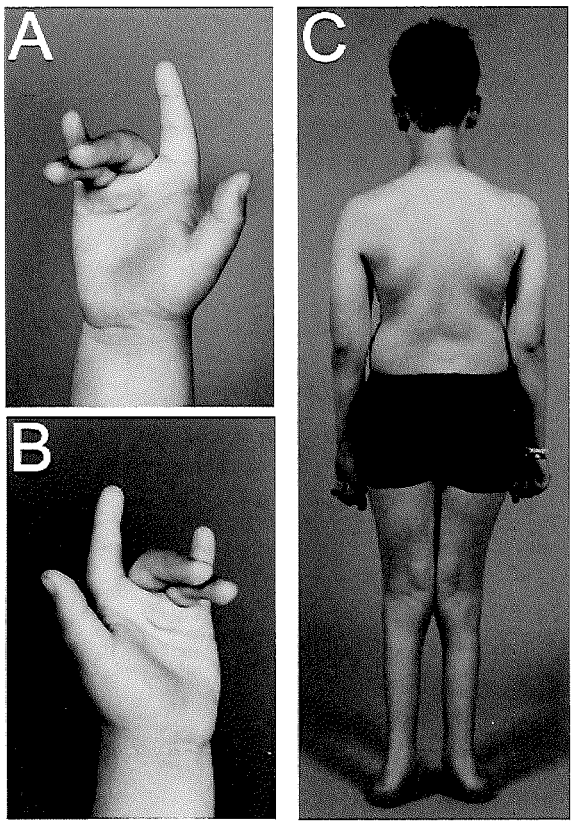

Figure 7 | Specific features of muscle weakness.

Selective weakness of finger extensors of the middle and ring finger in a 6-year old boy with classic infantile Pompe disease (**A** and **B**). Picture is taken at maximal extension. Muscle atrophy, predominantly in the lower legs, in an 11-year old boy with classic infantile Pompe disease (**C**). In addition, an abnormal posture with lordosis, mild scoliosis, flexion of the hip and knees and pes valgus can be seen.

Hand-held dynamometry was performed in the three ambulant patients older than six years. Total muscle strength increased significantly in two patients over time, but was lower than age related peers (Figure 8). Foot dorsal flexors were most affected; in two patients no active ankle dorsiflexion was possible in any measurement.

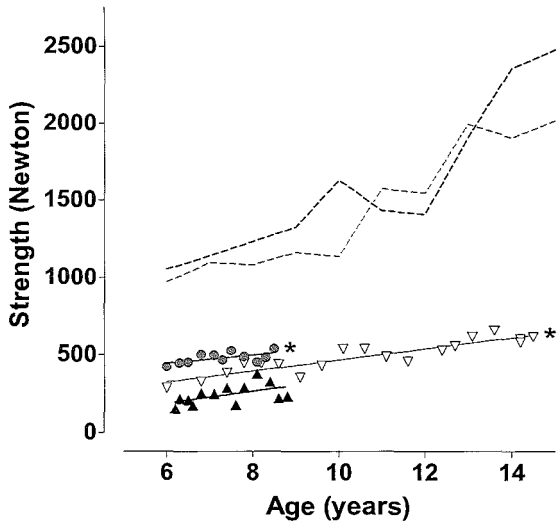

Figure 8 | Muscle strength as measured by Hand-Held Dynamometry.

Results of nine muscle groups are summed together to calculate a total sumscore. The dotted lines represent age related reference values (p50; boys in black, girls in grey).[34] The lines represent the linear regression line per patient.

3.4 | Other clinical features

At last assessment, all four ambulant patients over four years of age had other clinical features of muscle weakness. All patients had a weak facial expression and bulbar muscle weakness. Three patients had a ptosis; one of them also had an extraocular motility disorder.[20] All patients had a mild scoliosis and increased lumbar lordosis. All had contractures of the ankle dorsoflexors requiring intermittent use of orthoses; some patients had contractures of the wrist and hip. Three patients used a wheelchair for long distances. Muscle atrophy was not observed at baseline, but was observed in two patients at last assessment, particularly in the lower leg (Figure 7C).

DISCUSSION

The introduction of ERT has tremendously improved the prospect for patients with classic infantile Pompe disease. All patients in this study achieved previously unmet motor milestones.

As long-term motor outcome is largely unknown, we aimed to establish a profile of the motor performance and motor complications in long-term survivors with classic infantile Pompe disease. Whereas motor development appeared close to normal in the first years of life, a characteristic motor phenotype emerged beyond four years of age: patients developed severe distal muscle weakness and motor function plateaued or tended to deteriorate.

In the first 1.5 years of life, patients gradually gained motor skills and motor development was close to normal. Some postures indicated minor muscle weakness, and some patients needed more time to acquire antigravity movements. Eight patients continued to gain motor skills up to three years of age; their motor development was only slightly below age-appropriate level. Patients had more difficulties with gross motor skills than fine motor skills such as manual dexterity. Three patients temporarily or completely lost motor skills by one or two years of age due to respiratory decline. As respiratory decline is more often observed at the age of two,[8,14] patients seem particularly vulnerable to become ventilator dependent and lose motor skills at this age. Of note, the motor development beforehand did not differ from other patients who did not lose skills.

Beyond the age of four a characteristic phenotype emerged: 1) Specific motor function items appeared difficult for all patients, most important being neck flexion,[21,22] sit-up, and motor skills that require good body balance. 2) All patients developed a distinct pattern of muscle weakness. As increasingly recognized,[17-20] facial and bulbar muscle weakness and ptosis were common. In general agreement with the qualitative findings of Case and Prater et al.,[21,22] strength of the neck flexors, paraspinal muscles, hip extensors and abductors, and foot plantar and dorsal flexors was most severely reduced. In contrast, strength of the quadriceps was reduced only mildly, and none of the patients showed a Gowers sign at last assessment. Remarkably, one patient showed severe weakness of the finger extensors of the middle and ring finger; this unique pattern is not observed in any other neuromuscular disorder. While one study described mild scoliosis in 1 out of 11 long-term survivors only,[21] in our study all four ambulant patients over four had developed a mild scoliosis.

On pathophysiological grounds, the emerging phenotype of treated patients with classic infantile Pompe disease is expected to mimic the phenotype of patients with less progressive forms of the disease. While neck flexion and sit-up are also particularly difficult for untreated children and adults with Pompe disease,[6] the distribution of muscle weakness differs greatly. The latter patients typically develop limb-girdle muscle weakness.[7,35] Muscles of the hands and feet are affected in less than 10%,[7] and ptosis and bulbar muscle weakness is present in approximately 25% of patients. In contrast, survivors with classic infantile Pompe disease all

have profound facial and bulbar weakness and they develop profound weakness of the distal legs. Consequently, they developed a gait that differs from the waddling gait of children and adults with Pompe disease.[21]

Our long-term standardized follow-up study allowed us to establish a longitudinal profile of motor involvement, which has not been studied previously. Strikingly, whereas healthy children improve their performance with age, many motor functions plateaued or deteriorated beyond the age of four. Although in retrospect mild signs may have been present at younger ages, a clear deterioration in motor function became only apparent when patients grew and needed to learn more complex skills. Along with this deterioration, weakness of the foot dorsoflexors became more pronounced and patients started to develop contractures. Ultimately, three of the four oldest ambulant patients required a wheelchair for long distances.

Several factors might contribute to this deterioration in motor performance. Although muscle strength as measured by HHD remained stable or slightly increased, this was apparently not sufficient to compensate for the increase in body mass and height with age. Moreover, the disease process might continue despite ERT. This is suggested by the developing muscle atrophy in our patients, indicating a net loss of muscle tissue. Thirdly, it as hypothesized that the relative amount of ERT per gram of muscle-fiber decreases with age, due to two mechanisms: 1) Muscle mass increases with age, and 2) as the muscle-fiber size increases with age, the ratio between muscle-fiber area and muscle-fiber volume decreases. Consequently, the number of mannose 6-phosphate receptors – responsible for the uptake of enzymes – per muscle-fiber volume may decrease. Finally, as established muscle weakness causes compensatory movement patterns and postural habits, this will further impair the normal use of muscles, and likely magnifies muscle weakness on the long-term.

The exact cause for the in classic infantile Pompe patients treated with ERT is intriguing and requires further investigation. Several factors might play a role, including variation in response to ERT by different muscle-fiber types, and potential neurologic involvement due to glycogen storage in the nervous system. The effect of ERT seems to depend on the muscle-fiber types affected; especially type II muscle fibers appeared difficult to correct in mice,[36] although this is so far contradicted in humans.[37,38]

As the QMFT is originally designed and validated to rate motor function in children and adults with Pompe disease with ages between 5 and 76 years and as normative data are lacking,[28] we could only report absolute scores. Because the QMFT was constructed based on the Gross Motor Function Measure,[39] existing of 88 items that a child can perform by 5

years of age, the QMFT provides a good impression of the patients' motor function at last assessment.

Will this profile be the prospect for patients with classic infantile Pompe disease? At present, several options to improve the effect of ERT are being explored, including dose augmentation, immunomodulation in patients prone to develop high antibody titers to ERT,[40] second-generation products that may improve uptake in skeletal muscle tissue,[41-43] and co-administration of chaperones with ERT.[44] Although these interventions may improve motor outcome, it is likely that some motor features will remain difficult to correct and will remain characteristic for survivors with classic infantile Pompe disease.

To maintain and optimize motor function and to prevent secondary complications such as contractures, early intervention by physical, occupational, and speech therapists in all patients with classic infantile Pompe disease is of paramount importance. Patients might benefit from customized exercise and physical therapy programs.[45] Appropriate supportive measures such as splints are required. The patients' weight should be monitored carefully: whereas overweight might impair motor function, insufficient intake of food might cause breakdown of muscle tissue. Now the first cohort of treated infants reaches adolescence, close monitoring for possible complications in older patients such as scoliosis is important.

CONCLUSIONS

Our study provides a profile of the motor involvement in patients with classic infantile Pompe disease treated with ERT, which could serve as a reference base for the expected complications and management of patients at varying ages. We conclude that even though initially the motor development is close to normal, beyond four years of age a typical phenotype emerges consisting of profound facial, bulbar, and distal muscle weakness, and difficulties in specific motor function items such as neck flexion and sit-up. Remarkably, certain motor functions plateaued or deteriorated with age. These findings emphasize the need for the development of appropriate supportive measures and customized exercise or physical therapy programs. In order to improve motor outcome, further studies are needed to elucidate the cause of the emerging pattern of muscle weakness that is so distinct from children and adults with Pompe disease, and to clarify why certain motor functions deteriorate with age.

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CHAPTER 4

Facial-muscle weakness, speech disorders and dysphagia are common in patients with classic infantile Pompe disease treated with enzyme therapy

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ABSTRACT

Classic infantile Pompe disease is an inherited generalized glycogen storage disorder caused by deficiency of lysosomal acid α-glucosidase. If left untreated, patients die before one year of age. Although enzyme-replacement therapy (ERT) has significantly prolonged lifespan, it has also revealed new aspects of the disease. For up to 11 years, we investigated the frequency and consequences of facial-muscle weakness, speech disorders and dysphagia in long-term survivors. Sequential photographs were used to determine the timing and severity of facial-muscle weakness. Using standardized articulation tests and fibreoptic endoscopic evaluation of swallowing, we investigated speech and swallowing function in a subset of patients. This study included 11 patients with classic infantile Pompe disease. Median age at the start of ERT was 2.4 months (range 0.1 – 8.3 months), and median age at the end of the study was 4.3 years (range 7.7 months – 12.2 years). All patients developed facial-muscle weakness before the age of 15 months. Speech was studied in four patients. Articulation was disordered, with hypernasal resonance and reduced speech intelligibility in all four. Swallowing function was studied in six patients, the most important findings being ineffective swallowing with residues of food (5/6), penetration or aspiration (3/6), and reduced pharyngeal and/or laryngeal sensibility (2/6). We conclude that facial-muscle weakness, speech disorders and dysphagia are common in long-term survivors receiving ERT for classic infantile Pompe disease. To improve speech and reduce the risk for aspiration, early treatment by a speech therapist and regular swallowing assessments are recommended.

INTRODUCTION

Pompe disease (glycogen storage disease type II, acid maltase deficiency, OMIM # 232300) is a rare autosomal recessive lysosomal storage disorder caused by mutations in the gene-encoding acid α-glucosidase (EC3.2.1.20).[1,2] Severe mutations cause complete enzyme deficiency, resulting in the classic infantile form of Pompe disease, which was first described by Pompe in 1932.[3] Symptoms are caused by glycogen accumulation, mainly in skeletal, cardiac and smooth muscle, but also in other tissues, including the central and peripheral nervous system. In the first months of life, patients present with progressive muscle weakness, hypertrophic cardiomyopathy, respiratory problems and feeding difficulties. If untreated, this leads to death before the age of one year.[4,5]

Although the lifespan of classic infantile Pompe patients has been significantly prolonged, and although motor functioning is improved by enzyme-replacement therapy (ERT), various extents of muscle weakness remain.[6-16] This study focuses on weakness of the facial and bulbar muscles.

We simultaneously examined the prevalence and consequences of facial-muscle weakness, speech disorders and dysphagia in a cohort of patients with classic infantile Pompe disease who had been treated with ERT over a long period, in some cases up to 11 years.

PATIENTS AND METHODS

Patients

The study comprised 11 patients with classic infantile Pompe disease treated with ERT between 1999 and 2010 at Erasmus MC University Medical Center, Rotterdam, the Netherlands. Classic infantile Pompe disease was defined as 1.) symptoms of muscle weakness within six months of birth, 2.) hypertrophic cardiomyopathy, and 3.) severe GAA (the gene-encoding acid α-glucosidase) mutations on both alleles. The diagnosis was confirmed by an enzyme-activity assay in leukocytes or fibroblasts. Patients were enrolled in clinical trials that investigated the safety and efficacy of ERT with recombinant human α-glucosidase (20 mg/kg/two weeks to 40 mg/kg/week). The Institutional Review Board approved the studies, and written informed consent was obtained from all parents.

Facial-muscle weakness

To examine the onset of facial-muscle weakness, we collected photographs of the face taken over a period of 24 months from the start of ERT. For this we used standardized photographs and videos taken every three months. The photographs were ordered arbitrarily and evaluated independently by three neurologists. The evaluators stated whether facial-muscle weakness was present, and, whether it was mild or severe. Facial-muscle weakness was defined as an expressionless face with an open drooping or tent-shaped mouth.[17] To accept any judgement, the agreement of at least two evaluators was needed. If this was impossible, the evaluation was considered not to be applicable.

To further characterize facial-muscle weakness, the evaluators scored whether the following clinical features were present, absent or impossible to judge: ptosis, sunken cheeks, drooping of the lower lip, absence of the nasolabial folds, and absence of horizontal forehead lines. Ptosis was considered to be present when the upper eyelid was less than 2 mm from midpupil, or when asymmetry between the left and right upper eyelid was greater than 2 mm. A recent photograph of each patient was collected to analyse progression over time.

Speech and swallowing function

Between 2008 and 2010, speech was assessed in patients older than 24 months or in those who spoke more than ten words (n=4). Swallowing function was assessed in patients who were not fed by percutaneous endoscopic gastrostomy (n=6). Assessments were repeated after at least one year.

Speech

First, a speech therapist conducted a thorough orofacial observation to detect whether speech was impaired by weakness or reduced movements of the lip and tongue. To evaluate speech, a modified form of the Dutch Schisis Articulation Examination was used, which examines spontaneous language, and the repetition of phonemes and words. The following items were examined: 1.) articulatory disorders (i.e. mispronunciation of speech sounds), 2.) hypernasal resonance (i.e. increased resonance by the nasal cavity), and 3.) speech intelligibility.

Additionally, a neuropsychologist tested for dysarthria using the Mayo Clinic Lists,[18] which also investigates respiration, phonation (i.e. the characteristics of voice production by the larynx), and prosody (i.e. speed and rhythm of speech).

Swallowing function

The speech therapist obtained a feeding history from all parents.

Pharyngeal swallowing function was assessed by an experienced otolaryngologist using fibreoptic endoscopic evaluation of swallowing (FEES).[19] First, the masticatory pattern was investigated. Then, after the fibrescope had been introduced, the anatomy and function of the swallowing apparatus were examined: Velopharyngeal closure (i.e. sealing of the nasal cavity by the soft palate) was examined during speech, and the pharynx and larynx were screened for deviant anatomy, reduced pharyngeal squeeze, and impaired laryngeal function.

Next, pharyngeal swallowing function was examined while patients ingested food in a sitting position. Observation of swallowing function included premature spillage of food, delayed swallowing, nasal regurgitation, pharyngeal food residue, and penetration and aspiration of the food or pooling secretions. Penetration was defined as leakage of food into the laryngeal vestibule up to the level of the true vocal cords; aspiration was defined as leakage into the laryngeal vestibule below this level.[20] Finally, we observed the sensory reaction of the pharynx and larynx.

Associated clinical outcome measures

At the time of speech and swallowing assessments, relevant clinical data on feeding (orally or tube feeding), airway infections, motor development, and hearing loss[21] were collected.

RESULTS

Patients

Eleven patients participated in this study. Table 1 summarizes each patient's clinical features. At the start of ERT all patients had symptoms of Pompe disease. All were hypotonic, and eight were fed by nasogastric tube.

Facial-muscle weakness

In total, 96 photographs were collected of 11 patients. The median age at the first photo was 2.3 months (age range 0.1 – 8.8 months); the median age at the last was 49.2 months (age range 6.9 months – 11.6 years).

Between the ages of 1.0 and 15.0 months (Table 1, median 6.6 months), all patients developed evident signs of facial-muscle weakness, even when ERT was started very early.

When such weakness was first observed, its severity in most patients (9/11) was considered to be mild. The main characteristics were sunken cheeks (8/11) and a drooping lower lip (9/11). While the absence of the nasolabial fold and forehead lines were difficult to judge, four patients clearly had diminished nasolabial folds. Only one patient had ptosis.

The final photographs show that, despite ERT, facial-muscle weakness became severe in 7/11 patients (Figure 1). While the main features were still sunken cheeks (10/11) and drooping of the lower lip (9/11), facial expression was clearly reduced by diminished nasolabial folds (7/11) and forehead lines (5/11). The number of patients with ptosis rose to four.

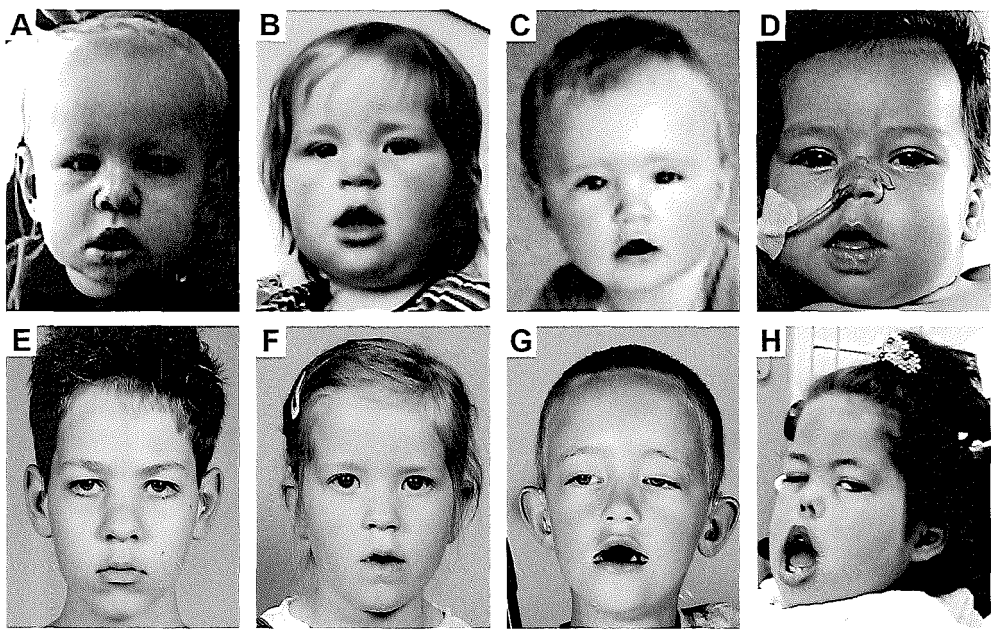

Figure 1 | Development of facial muscle weakness over time in four patients with classic infantile Pompe disease treated with ERT.

Per patient, comparison of the first photograph which showed the first signs of facial-muscle weakness (**A-D**) with the most recent photograph (**E-H**) showed that facial muscle weakness remained mild in two patients (**A** and **B** compared to **E** and **F**), but became severe in one patient (**C** compared to **G**). One patient presented with severe facial muscle weakness at the age of 1 month; this persisted over time (**D** compared to **H**).

Table 1 | Patient characteristics and development of facial muscle weakness in 11 patients with classic infantile Pompe disease treated with ERT

Patient	Gender	Age at diagnosis (months)	Age at the start of ERT (months)	NGT at the start of ERT	Age at study end (years)	Invasive ventilation (months)	Maximal motor milestone	Severity of first observed FMW (months)	Severity of FMW on most recent photo (years)
1	M	0.7	3.8	N	11	No	Walking	Mild (6.6)	Mild (11.6)
2	F	3.6	7.2	Y	12	7†	Tetraplegic	Severe (1.0‡)	Severe (11.4)
3	F	0.6	3.0	Y	*4**	26	Sitting	Mild (5.5)	Severe (3.4)
4	F	6.2	8.3	Y	12	11	Tetraplegic	Mild (9.0)	Severe (6.2§)
5	M	0.2	1.9	Y	*4**	24	Walking	Mild (13.8)	Severe (4.1)
6	M	0.7	1.2	Y	6	No	Walking	Mild (3.4)	Severe (6.0)
7	F	0.2	0.5	Y	5	No	Walking	Mild (12.4)	Mild (5.5)
8	F	3.2	3.6	Y	*0.8**	No	Minimal movements	Mild (6.9§)	Mild (0.6§)
9	M	0.1	0.1	Y	3	33	Walking	Mild (15.0)	Severe (3.0)
10	M	2.0	2.2	N	3	No	Sitting	Severe (2.0)	Severe (2.7)
11	F	2.3	2.4	N	2	No	Walking	Mild (2.3)	Mild (1.7§)

F: Female; M: Male; NGT: Nasogastric tube feeding at start ERT; Y: Yes; N: No; * Died, † Invasive ventilation before start of ERT; FMW: Facial muscle weakness; ‡: Photographs were available before start of ERT, §: last available picture due to referral to treatment abroad (4), early death (8), and short treatment duration at the end of this study (11).

Speech and swallowing function

Speech

Speech was assessed in four patients at a median age of 4.1 years (age range 2.0 – 9.9 years, supplementary Table 2). Orofacial observation showed that the speech of all four was impaired by reduced movement and/or weakness of the lip and/or tongue. Their articulation was disordered, featuring consonant substitutions, consonant omissions and cluster reductions, mild to moderate hypernasal resonance, and significantly impaired speech intelligibility. Together, this suggested velopharyngeal incompetence.

Three patients were reassessed at a median age of 5.5 years (age range 5.1 – 11.1 years). In the period between the first and second assessment, no major changes in orofacial hypotonia or speech were observed, although speech therapy had improved the active articulatory compensation. Additional investigation of dysarthria in these three patients showed disorders in respiration, phonation and prosody. They spoke in short sentences in a monotone, hoarse wet voice with monoloudness. These features are specific for flaccid dysarthria.

Swallowing function

Swallowing function was assessed in six patients at a median age of 3.0 years (age range 8.0 months to 9.9 years). Feeding difficulties were reported (5/6), and comprised all parameters (see supplementary Table 2). Patient 9 was fed completely by nasogastric tube, and ingested only water orally. Observation of mastication revealed impaired mastication in two patients.

Fibreoptic endoscopic examinations of swallowing showed that five of the six patients had varying extents of dysphagia; only the youngest had no swallowing abnormalities. Reduced velopharyngeal closure was found in four patients, and caused nasal regurgitation in two. Although the anatomy of the swallowing apparatus and function of the larynx were normal, pharyngeal muscle contraction was reduced (5/6). This resulted in pooling secretions in the pharynx (4/6), which, in two patients, contained remnants of a previous meal (Figure 2A).

Swallowing of various textures of food could be examined in four patients, whereas swallowing function of two patients who refused to eat during the examination (9 and 10) was evaluated on the basis of dry swallows. In all patients with insufficient muscle contraction of the pharynx (5/6), residues of food or saliva remained present at the valleculae, pharyngeal wall, pyriform sinuses and postcricoid. Because patients used no protective reflexes such as coughing or swallowing to try to clear the food, it was clear that the sensory reaction of the pharynx was impaired (2/6). In three patients, pharyngeal residues resulted in penetration of

food or saliva (Figure 2B). In two of the six patients, the sensory reaction of the larynx was impaired.

Swallowing function was reassessed in four patients, at a median age of 5.3 years (age range 2.0 – 11.1 years). In three patients it remained stable; in the other (Patient 7), it deteriorated, leading to aspiration, premature spillage of food, and delayed onset of swallowing.

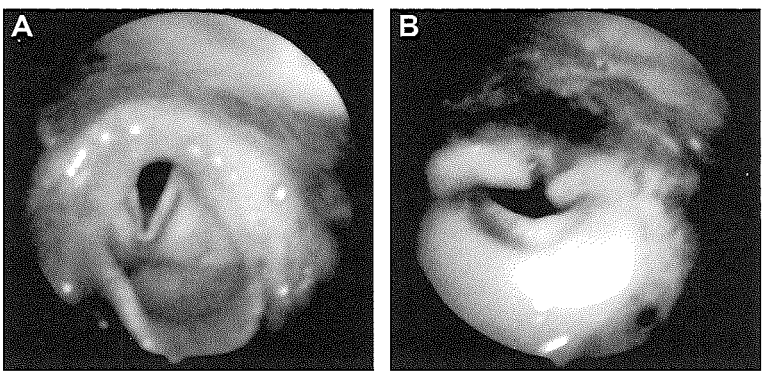

Figure 2 | FEES examination in a 6-year old boy with classic infantile Pompe disease treated with ERT.
Pooled secretions in the pharynx containing saliva and remnants of previously eaten food at FEES examination (**A**), and pharyngeal food residue with penetration and aspiration directly after food intake (**B**).

Associated clinical outcome measures

At the start of ERT, feeding through a nasogastric tube (NGT) was required by eight of the 11 patients. By the end of the study, five patients were completely orally fed. Their ages were 2, 3, 6, 6, and 11 years. Two patients have never required NGT feeding since ERT began.

Hearing was impaired in all patients except Patient 9, their hearing deficits ranging from 30 – 90 dB.[21] Three of the four patients whose speech was evaluated already had hearing aids at first evaluation. The other patient (7) needed hearing aids later; he had a mild hearing loss of 30 – 40 dB.

Five of the six patients whose swallowing function was assessed, learned to walk (see Table 1 and supplementary Table 2 for motor outcome), and three of the same six patients had recurrent respiratory infections.

Supplementary Table 2 | Initial assessment and reassessment of swallowing and speech function in patients with classic infantile Pompe disease treated with ERT.

	Initial assessment (reassessment)						
Patient	**1**	**6**	**7**	**9**	**10**	**11**	**Total**
Age (years)	9.9 (11.1)	4.3 (5.5)	3.9 (5.1)	2.1	1.6	0.7 (2.0)	
Nasogastric tube	- (-)	- (-)	- (-)	+	-	- (-)	1/6 (0/4)
Recurrent respiratory infections	- (-)	+ (+)	- (-)	+	+	- (-)	3/6 (1/4)
Gross motor development	Walking (Walking)	Walking (Walking)	Walking (Walking)	Walking	Sitting	Sitting (Walking)	
Speech				NA	NA		
Oral hypotonia	+ (+)	+ (+)	+ (NA)			+	4/4 (2/3)
Articulatory imprecision*	2 (1)	3 (3)	1 (1)			1	4/4 (3/3)
Passive compensation*	2 (1)	2 (1)	2 (2)			2	4/4 (3/3)
Active compensation*	2 (2)	2 (2)	1 (2)			1	4/4 (3/3)
Hypernasal resonance*	3 (3)	3 (1)	3 (3)			2	4/4 (3/3)
Reduced intelligibility*	2 (2)	3 (3)	2 (2)			3	4/4 (3/3)
Feeding difficulties							
Slow mastication	- (-)	- (-)	-(+)	NA	-	- (-)	0/6 (1/4)
Prolonged mealtimes	- (-)	+ (+)	+ (+)	NA	NA	- (-)	2/6 (2/4)
Modified food	- (-)	- (-)	+ (-)	+	+	- (-)	3/6 (0/4)
Choking	- (+)	- (+)	- (+)	+	+	- (-)	2/6 (3/4)
Clinical examination							
Slow mastication	- (-)	- (-)	- (-)	NA	-	- (-)	0/6 (0/4)
Impaired mastication	- (-)	+ (+)	- (-)	NA	+	- (-)	2/6 (1/4)
FEES							
Reduced VP closure	+ (+)	+ (+)	+ (+)	NA	+	- (-)	4/6 (3/4)
Deviant anatomy	- (-)	- (-)	- (-)	-	-	- (-)	0/6 (0/4)
Reduced pharyngeal squeeze	+ (+)	+ (+)	+ (+)	+	+	- (-)	5/6 (3/4)
Impaired larynx function	- (-)	- (-)	- (-)	-	-	- (-)	0/6 (0/4)
Pharyngeal pooling secretions	+ (+)	+ (+)	- (-)	+	+	- (-)	4/6 (2/4)
Premature spillage	- (-)	- (+)	- (-)	NA	NA	- (NA)	0/6 (1/4)
Delayed swallow	- (-)	- (+)	- (-)	NA	NA	- (NA)	0/6 (1/4)
Nasal regurgitation	- (-)	+ (+)	- (-)	NA	NA	- (NA)	1/6 (1/4)
Pharyngeal residue	+ (+)	+ (+)	+ (+)	+	+	- (-)	5/6 (3/4)
Penetration	- (-)	+ (+)	- (-)	+	+	- (-)	3/6 (1/4)
Aspiration	- (-)	- (+)	- (-)	-	-	- (-)	0/6 (1/4)
Impaired sensory reaction of pharynx	+ (+)	+ (+)	- (-)	-	-	- (NA)	2/6 (2/4)
Impaired sensory reaction of larynx	+ (+)	+ (+)	- (-)	-	-	- (NA)	2/6 (2/4)

Values between brackets are data obtained at reassessment; +: Present; -: Absent, * Speech items were tested: 0=absent; 1=mild; 2=moderate; 3=severe;NA: Not applicable; FEES: Fiberoptic endoscopic evaluation of swallowing; VP: Velopharyngeal.

DISCUSSION

The longest survivors receiving enzyme-replacement therapy for infantile Pompe disease are currently 12 years old. It is evident not only that ERT has significantly increased survival, but also that it greatly affects these children's motor performance. However, this longer survival has also highlighted previously unrecognized aspects of the disease. Noting that many children had developed facial-muscle weakness over time, we investigated the frequency and consequences of facial-muscle weakness, speech disorders and dysphagia in long-term survivors.

In all 11 patients, facial-muscle weakness had developed before the age of 15 months. When first observed, its main features were poor facial expression, sunken cheeks and drooping of the lip. Over the years, all facial-muscles seemed to become affected. FEES examinations showed weakness of the bulbar muscles, with velopharyngeal incompetence and reduced muscular contraction of the pharynx.

This muscle weakness affected daily functioning in four main ways. 1.) All patients had poor facial expression. 2.) Four children developed a bilateral ptosis over time, which was so severe in one child that surgical correction was required.[22] 3.) Speech was abnormal, characterized by disordered articulation, hypernasality, and lower intelligibility – all typical of velopharyngeal incompetence,[23] as suggested earlier by Muller et al.[10] 4.) Swallowing was generally weak and ineffective, leaving residues of food around the larynx, with penetration in three out of six patients. As some patients lack protective sensory reactions of the pharynx and larynx, aspirations and micro-aspirations may easily occur and go unnoticed. In retrospect, we suspect that this phenomenon explains the recurrent airway infections in three patients in our study. As respiratory muscle weakness can easily lead to respiratory insufficiency, such aspirations and aspiration pneumonias may be life threatening. As it has proved difficult to wean patients with classic infantile Pompe off the ventilator, this is particularly important. Earlier studies have shown that, despite treatment with ERT, 50% of classic infantile patients eventually become ventilator dependent, and that respiratory insufficiency is the main cause of death.[8]

We found no clear relationship between the age at start of ERT and the point at which facial-muscle weakness developed, although the severest facial muscle weakness was found in patients who started ERT late – at 7 and 8 months of age. ERT seemed to reduce feeding difficulties in some patients. During enzyme therapy, nasogastric tube feeding could be discontinued in three of eight patients who needed NGT at start. At the end of the study, five

patients in age ranges from 2 – 12 years were completely orally fed. Still, four of these patients showed some signs of dysphagia. It is noteworthy that the patients who were fed orally were the best performers. Four of these five patients learned to walk and were still walking at the end of the study. ERT could not prevent disordered speech, although the severest speech problems were observed in those with the poorest motor outcome.

Our study indicated that parents often underreport signs of choking and swallowing difficulties. Given the findings of our study, we attach paramount importance to assessments of swallowing function especially in young patients. To prevent aspiration and pneumonia, it may be advisable to modify dietary texture, or even to discontinue oral feeding in high-risk patients. A low maintenance dose of antibiotics may also be helpful. To improve speech, feeding and swallowing difficulties as much as possible, we recommend early examination and treatment by a speech therapist. In patients with severe hypernasal resonance, however, only slight gains on speech can be achieved by behavioural exercises. Other options to improve speech include a palatal lift prothesis, or surgical interventions such as pharyngoplasty or a pharyngeal flap.[24] But as these may also increase swallowing difficulties or cause obstructive sleep apnoea, they should be used with caution. Their overall effect may also be limited by the residual pharyngeal muscle weakness that remains in patients with classic infantile Pompe disease.

Hearing loss is common in classic infantile Pompe patients, and may also impact speech development. We earlier recommended regular auditory tests, and early implementation of hearing aids.[21]

The exact cause of bulbar muscle weakness is unknown. In infants with Pompe disease, it has been shown that glycogen accumulates in the tongue of an untreated infant,[25] but the effect of ERT on bulbar muscle pathology in these infants has not been studied. Only one case report addresses the effect of ERT on bulbar muscle pathology in an adult patient with Pompe disease and showed that, 21 months after treatment with ERT, residual storage of glycogen remained in the oesophagus.[26] This is in line with results obtained in Pompe knock-out mice, which showed that extensive glycogen storage present in bulbar muscles was not completely cleared by ERT.[27]

Together, these findings suggest that residual muscle pathology of the bulbar muscles almost certainly plays a major role in the speech and swallowing problems described in this study. It cannot be excluded that a role is also played by glycogen storage in the nervous system. Autopsies of untreated patients with classic infantile Pompe disease have shown glycogen accumulation in the glial cells of the cortex, thalamus, brainstem, and spinal anterior

motor horns.[28-30] Since ERT cannot cross the blood-brain barrier, ERT is unlikely to affect the glycogen storage in the central nervous system.[31]

Certain features of the speech of the children in our study may reflect flaccid dysarthria,[18,23] a condition caused by damage to the lower motor neurons emerging from the brainstem. The lower sensibility of the larynx and pharynx and the delayed swallowing seen in some patients might also indicate involvement of the nervous system. Further research is required.

All in all, we could not fully explain why obvious bulbar muscle weakness developed even in good responders to ERT with a good motor outcome. If muscle pathology indeed underlies the clinical problems, this may imply that bulbar muscles respond less to ERT than the muscles of the limbs and trunk.

Several studies have sought to explain the differential response of muscles to ERT. One potential explanation involved variation in response by different muscle-fibre types. It was shown in mice with Pompe disease that type 2 muscle fibres were largely resistant to ERT.[32] In humans, type 1 and type 2a muscle fibres both responded to enzyme therapy.[33] Comparison of skeletal muscles from the limb and trunk with bulbar muscles shows that bulbar muscles have a wider repertoire of contractile proteins, including developmental and specialized isoforms of myosin and hybrid fibers that express two or more isoforms.[34-36] This might contribute to a lower response to ERT in these muscles. While the results of our studies in knock-out mice with Pompe disease have not confirmed a smaller response of the bulbar muscles, the situation might be different in humans.[27]

In conclusion, we have shown that facial-muscle weakness, speech disorders and dysphagia are prominent in patients with classic infantile Pompe disease who survive due to enzyme therapy. Bulbar muscle weakness caused speech disorders, severely reducing speech intelligibility, thereby affecting communication and social interaction. Early treatment by a speech therapist might help to improve articulation and speech. Similarly, because ineffective swallowing puts patients at risk for the development of aspiration pneumonias and respiratory insufficiency, early and regular swallowing assessments and development of a safe feeding plan are recommended. Further research is necessary to elucidate the exact pathophysiology.

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CHAPTER 5

Ptosis, extraocular motility disorder, and myopia as features of Pompe disease

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ABSTRACT

Purpose

The assets of this report are a thorough description of new clinical findings, namely the combination of ptosis, extraocular motility disorder and myopia, in consequence of prolonged survival in classic infantile-onset Pompe disease through enzyme therapy.

Design

Single case description.

Results

This manuscript describes a combination of ptosis, extraocular motility disorder and myopia in a 4.5-year-old patient with classic infantile-onset Pompe disease, who survived through enzyme therapy. This patient was treated with a bilateral frontalis suspension (modified Crawford technique) using prolene 3-0 sutures.

Conclusions

The combination of ptosis, extraocular motility disorder and myopia, is a new clinical finding in children with classic infantile-onset Pompe disease.

INTRODUCTION

Pompe disease, also referred to as glycogen-storage disease type II or acid maltase deficiency (OMIM #232300), is an autosomal recessive disorder caused by mutations in the acid α-glucosidase (GAA) gene located on chromosome 17q25.2–q25.3. The disease affects people from all age groups. Patients with the classic infantile-onset form present with progressive generalized muscle weakness and hypertrophic cardiomyopathy. Without treatment, they die within the first year of life.

Children and adults exhibit a slowly progressive proximal myopathy. They ultimately become wheelchair and ventilator dependent.[1,2] Enzyme replacement therapy with recombinant human alpha-glucosidase is commercially available since 2006. It has prolonged survival in infantile patients and improved skeletal muscle and cardiac function.[3]

Myogenic ptosis has been described as a single feature in adults with Pompe disease.[4,5] Ptosis in combination with strabismus has been described once in a child with Pompe disease.[6] We present a patient with classic infantile-onset Pompe disease with ptosis, extraocular motility disorder and myopia. This combination has not been reported before.

Case Report

A boy with classic infantile-onset Pompe disease was referred to our ophthalmological clinic with complaints of blurred vision at the age of 2.5 years.

Enzyme replacement therapy was started immediately after diagnosis at the age of 4 weeks and had a positive effect on skeletal muscle function. At baseline ophthalmological examination, mild ptosis was noted combined with a myotonic face. Extraocular movements were within normal range.

Cycloplegic refraction revealed a high myopia, with spherical equivalent values of -7.75 D and -8.75 D, respectively, right and left eye. Fundus examination revealed no abnormalities. At the age of 4.5 years extraocular eye movements were consensual restricted compared with normal eye movement at that age (Figure 1A, B, C). The elevation was most restricted to 5° from primary position. The myopia progressed to -8.5 D and -10.0 D in the right and left eye, with an axial length of 24.81 mm and 25.14 mm, respectively. Palpebral fissure height was now 3 mm in the right eye and 2 mm in the left (Figure 2A). There was an obvious chin-up head tilt. Levator function measured 5 mm in both eyes. A bilateral frontalis suspension (modified Crawford technique) using prolene 3-0 sutures was performed.[7] Postoperatively, palpebral fissure height measured 8 mm in both eyes (Figure 2B).

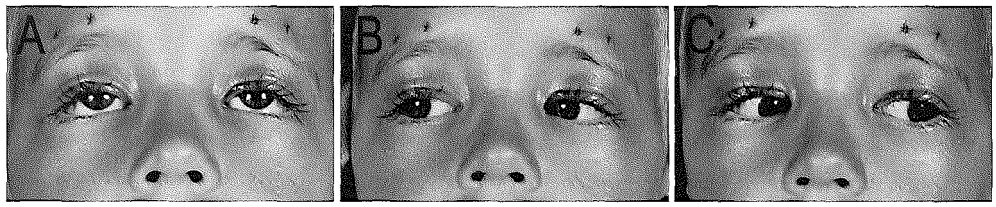

Figure 1 | Restricted elevation (A), restricted ab- en adduction of respectively the right and left eye on right gaze (B), and restricted ad- and abduction of the right and left eye respectively on left gaze (C).

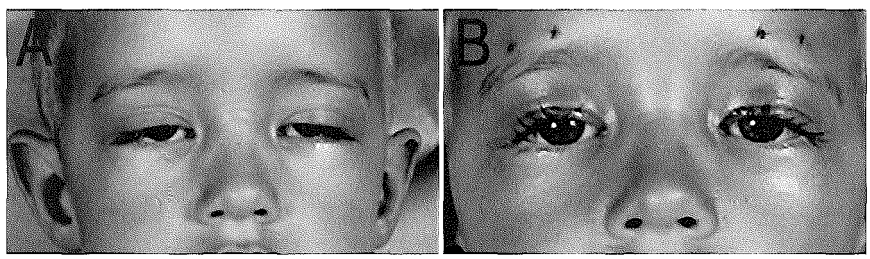

Figure 2 | Bilateral ptosis and myotonic face preoperatively (A), palpebral fissure height of 8 mm bilaterally at 1 week postoperatively (B).

COMMENT

To our knowledge, this is the first case report of a patient with classic infantile Pompe disease with the combination of ptosis, extraocular motility disorder and myopia. In adults with Pompe disease, ptosis as a single ophtalmological finding was reported before. Groen et al. found ptosis to occur in four out of twelve adult patients. Their average age was 55 years.[5] De Wilde et al. described an additional case.[4] Ptosis in combination with strabismus has been described once in a child with Pompe disease.[6]

In adults with Pompe disease the deposition of glycogen mainly occurs in skeletal muscle. In infants also other tissues may be affected. This difference in accumulation pattern and rate of disease progression is attributed to the virtual absence of alpha-glucosidase activity in infants and the presence of 10 – 30% residual enzyme activity in adults with Pompe disease. Enzyme

therapy aimed at increasing the level of alpha-glucosidase activity in tissues has changed the prognosis of infants significantly. The combination of ptosis and extraocular motility disorder in our 4.5-year-old survivor through enzyme therapy is a new finding and might be explained by glycogen storage in the levator palpebrae and the extrinsic eye muscles.

The myopia in our patient was progressive. The axial length corresponds with the refractive error. Most likely the myopia in our patient is inherited, as both parents are myopic.

Prall et al. found myopia in carriers of Danon disease, an X linked glycogen storage disease, possibly resulting from osmotic refractive changes in the lens due to glycogen depositions. No glycogen deposits in the lens have been described in Pompe's disease.[8] Cogan et al. described glycogen deposits in the retina in Pompe's disease, without any evident functional impairment of the retina.[9]

To treat the myogenic ptosis, a frontalis suspension with prolene 3-0 sutures was performed. Silicone rods secured through a silicone sleeve would be a suitable alternative. Adjusting the palpebral fissure height in case of progression can easily be done with these materials.

In conclusion, our case report shows that myogenic ptosis, extraocular motility disorder and myopia may occur in infants with Pompe disease that survive through enzyme therapy. We recommend including ophthalmic examination in the routine follow-up of patients with Pompe disease.

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CHAPTER 6

Cognitive outcome of patients with classic infantile Pompe disease receiving enzyme therapy

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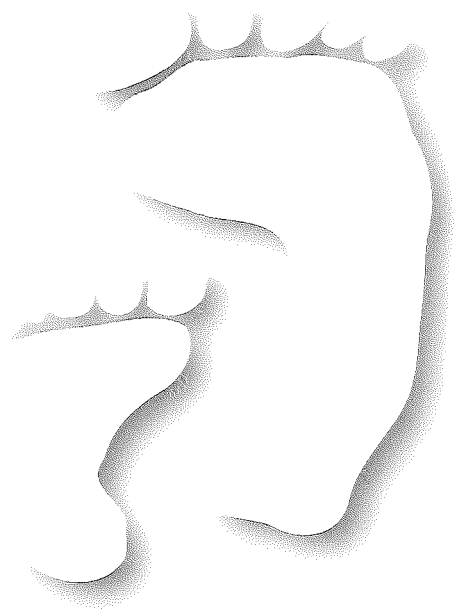

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ABSTRACT

Objective

Classic infantile Pompe disease affects many tissues, including the brain. Untreated infants die within their first year. Although enzyme-replacement therapy (ERT) significantly increases survival, its potential limitation is that the drug cannot cross the blood-brain barrier. We therefore investigated long-term cognitive development in patients treated with ERT.

Methods

We prospectively assessed cognitive functioning in 10 children with classic infantile Pompe disease who had been treated with ERT since 1999. Brain imaging was performed in 6 children.

Results

During the first 4 years of life, developmental scores in 10 children ranged from above average development to severe developmental delay; they were influenced by the type of intelligence test used, severity of motor problems, speech/language difficulties, and age at start of therapy. Five of the children were also tested from 5 years onward. Among them were 2 tetraplegic children whose earlier scores had indicated severe developmental delay. These scores now ranged between normal and mild developmental delay and indicated that at young age poor motor functioning may interfere with proper assessment of cognition. We found delayed processing speed in 2 children. Brain imaging revealed periventricular white matter abnormalities in 4 children.

Conclusions

Cognitive development at school age ranged between normal and mildly delayed in our long-term survivors with classic infantile Pompe disease treated with ERT. The oldest was 12 years. We found that cognition is easily underestimated in children younger than 5 years with poor motor functioning.

INTRODUCTION

Pompe disease is a progressive metabolic myopathy caused by lysosomal α-glucosidase deficiency. Patients with the classic infantile form have completely deleterious mutations in both acid α-glucosidase alleles, reducing residual enzyme activity to less than 1%. As a result, glycogen stores are excessive in skeletal, cardiac, and smooth muscle, and also in other tissues such as the brain. As a result of cardiorespiratory failure, patients rarely survive beyond 1 year.[1-4]

In 1999, we pioneered enzyme-replacement therapy (ERT) with recombinant human α-glucosidase in 4 children with classic infantile Pompe disease.[5] ERT demonstrably degrades glycogen in muscle tissue, improving motor development and increasing life expectancy,[6-9] but ERT cannot cross the blood-brain barrier.[10] Brain autopsies of untreated patients are limited and showed widespread glycogen storage in the CNS.[11-16] Mild white matter abnormalities have been reported in the brains of infants treated with ERT.[17,18]

So far it is not known whether glycogen storage in the CNS causes cognitive deficits. A precursor to this study found developmental delays at 3 years after the start of ERT.[19] To investigate the long-term cognitive outcome of children with classic infantile Pompe disease, we evaluated the results of a prospective follow-up study up to age 12.

METHODS

Patients

Patients with classic infantile Pompe disease participated in a long-term standardized follow-up study on the effects of ERT that started at Rotterdam's Center for Lysosomal and Metabolic Diseases at Erasmus MC University Medical Center in 1999. Diagnosis had been confirmed by enzyme activity assays and mutation analysis. The dose of recombinant human α-glucosidase ranged from 20 mg/kg every 2 weeks to 40 mg/kg weekly.

Standard protocol approvals, registrations, and patient consents

Study protocols were approved by the institutional review board, and written informed consent was obtained from the participating children's parents.

Assessments

Patients regularly underwent psychological assessments. Until 2004, infants and young children were assessed with the Bayley Scales of Infant Development, Second Edition (BSID-II) (0 – 42 months).[20] After 2004, we switched to the Griffiths Mental Developmental Scales (Griffiths) (0 – 2 months),[21,22] expecting it to differentiate better between various domains. Older children were assessed using the Wechsler Intelligence Scales for Children, Third Edition (WISC-III) (>72 months).[23] For children with tetraplegia, we used the Raven Coloured (4.06–11.06 years) or Standard Progressive Matrices (6 – 68+ years).[24,25] For those with impaired hearing, we used the Snijders Oomen Nonverbal Intelligence Test-Revised (2½ – 7 years).[26]

Children assessed at younger than age 5 were divided into 2 groups: group 1 consisted of children born between 1999 and 2003 (BSID-II; patients 1 – 5); group 2 consisted of those born after 2003 (Griffiths; patients 6 – 10). The children were assessed by 2 pediatric neuropsychologists (F.K.A. and B.J.E.) and a pediatrician specialized in psychological assessment (N.W.-K.). Parent's educational levels were assessed during interviews.

Statistics

Patients' test results were compared against the normative data of the Dutch population. The mean score for all tests is 100, with SD of 15 points. A score greater than 85 indicates normal development, a score between 84 and 70 indicates mild developmental delay, and a score less than 70 indicates severe developmental delay.[27] A disharmonic profile was defined as a discrepancy of more than 1 SD of the subscale from the personal mean score; its presence shows the impact of stronger or weaker domains on the total test score. We used the two-sided binomial test to determine whether the percentage of disharmonic profiles deviated from that in the normal Dutch population and the Mann-Whitney *U* test to determine differences between groups. All analyses were performed with SPSS for Windows (version 16; SPSS Inc., Chicago, IL). A p value of ≤ 0.05 was considered statistically significant.

RESULTS

Patient characteristics

Ten patients were included. Patient characteristics are summarized in Table 1.

Median age at diagnosis was 0.7 months (range 0.1 – 6.2 months). When ERT started (at a median age of 2.3 months [range 0.1 – 8.3 months]), all patients had signs of muscle

weakness. Two children aged 7.2 and 8.3 months were in the end stage of disease; they had lost virtually all muscle function and became ventilator-dependent before age 1. At last assessment, they were still tetraplegic at ages of 11 and 12 years. The other 8 children gained motor skills and learned to sit or walk unsupported as therapy proceeded. Three of these 8 patients also became ventilator-dependent at age 2 (patients 3, 5, and 8). Thereafter they lost all motor skills; patients 3 and 5 finally died at age 4, but patient 8 improved when the ERT dose was raised from 20 mg/kg every other week to 40 mg/kg weekly; at last assessment he was ventilator-dependent only during sleep and had regained his motor functions to the extent that he could sit up independently and also ride a tricycle. To date, however, he has not regained the ability to walk.

Nine of the 10 children had hearing deficits (ranging from 30 to 90 dB). Hearing aids were fitted in 7 patients at ages between 13 and 46 months. Seven of the 10 children had impaired vision, requiring glasses (ranging from +3.25 to -11 diopters).

Cognitive development during the first 4 years of life

Forty psychological assessments were performed in 10 children (age range 2 months – 4 years). Table 2 groups test results by 5 different time points during the first 3 years of therapy: before the start of ERT (median age 5 months), at 6 months of ERT (median age 8 months), at 1 year of ERT (median age 14 months), at 2 years of ERT (median age 27 months), and at 3 years of ERT (median age 37 months).

At baseline, the median developmental score was 79. It was then 88 at 6 months of ERT (73 in group 1 vs 98 in group 2), 92 at 1 year of ERT (65 vs 99), 67 at 2 years of ERT (56 vs 90), and 73 at 3 years of ERT (59 vs 81). Children tested in group 2 started therapy earlier (median 1.2 months) than those tested in group 1 (median 3.8 months) (z=-2.19, p=0.03). Relative to group 1 at 6 months after the start of therapy (z = -2.61, p = 0.01) and 2 years after the start of therapy (z=-2.25, p=0.04), group 2 scored better. In group 1, 4 of the 5 children were ventilator-dependent; they scored the lowest possible psychomotor development index (PDI) score of <50 and generally also had the lowest possible mental development index (MDI) score of <50 (highest MDI score was 67).

Table 1 | Patient characteristics.

Patient	Gender	Age diagnosis, mo	Age start ERT, mo	Age last assessment, y	Invasive ventilation, mo	Maximal motor milestone (Age, mo)	Hearing aids	Impaired vision	Radiologic imaging (Age, y)
1	M	0.7	3.8	11	No	Walking (16)	Yes	Yes	MRI (9)
2	F	3.6	7.2	11	7 [a]	MMF	Yes	Yes	MRI (0.5)
3	F	0.6	3.0	4 [b]	26 [a]	Sitting (19) [c]	Yes	No	CT, MRI (4.3)
4	F	6.2	8.3	12	11 [a]	MMF	Yes	Yes	MRI (8.7)
5	M	0.2	1.9	4 [b]	24 [a]	Walking (17) [c]	Yes	No	MRI (1.5)
6	M	0.7	1.2	6	No	Walking (18)	Yes	Yes	– [d]
7	F	0.2	0.5	5	No	Walking (17)	Yes	Yes	– [d]
8	M	0.1	0.1	3	33 [a]	Walking (14) [c]	No	Yes	– [d]
9	M	2.0	2.2	3	No	Sitting (12)	Yes	No	– [d]
10	F	2.3	2.4	1	No	Walking (15)	Yes	Yes	– [d]

Abbreviations: ERT: enzyme-replacement therapy; MMF: minimal motor function.

[a] Age in months at which invasive ventilation was started.

[b] Died at age 4 years.

[c] Lost virtually all motor milestones after becoming ventilator-dependent.

[d] —, not performed.

Table 2 | Total test scores per patient per assessment.

	BSID-II					SON-R	WISC-III				
Group 1, ERT duration	**0 y**	**0.5 y**	**1 y**	**2 y**	**3 y**	**5 y**	**6 y**	**7 y**	**8 y**	**10 y**	**11 y**
Chronologic age range. y	0.2-0.7	0.7-1.1	1.1-1.6	1.8-2.5	2.5-3.1	5.7-5.8		7.7	9.1	10.6-10.8	11.6-12.3
Patient 1	101 [a]	87 [a]	97 [a]	62 [a,b]	79 [a]	91		78 [a]	76 [a]	74	78 [a]
Patient 2	<50	62	<50	<50		80 [c]				76 [c]	
Patient 3 [d]	81	86 [a]	93 [a]	72 [a]	<50						
Patient 4	76 [a]	53	<50	56	67 [a]						92 [c]
Patient 5 [d]		73	65	<50	<50						
Median total test score	79	73	65	56	59						
	Griffiths						**WISC-III**				
Group 2, ERT duration	**0 y**	**0.5 y**	**1 y**	**2 y**	**3 y**	**5 y**	**6 y**	**7 y**	**8 y**	**10 y**	**11 y**
Chronologic age range. y		0.3-0.8	0.7-1.9	2.0-2.4	3.0-4.0	4.9-5.0	6.0				
Patient 6		112 [a]	99	90 [a]	77	75 [a]	75 [a]				
Patient 7		97 [a]	90 [a]	120 [a]	110 [a]	108					
Patient 8		89	102		69 [a,e]						
Patient 9		98 [a]	54 [a,e]	83 [a]	84 [a]						
Patient 10		105	110								
Median total test score		98	99	90	81						

Abbreviations: BSID II: Bayley Scales of Infant Development, Second Edition; Griffiths: Griffiths Mental Development Scales; SON-R: Snijders Oomen Nonverbal Intelligence Test-Revised; WISC-II: Wechsler Intelligence Scales for Children, Third Edition. <70, severe developmental delay; 70 – 84, mild developmental delay; 85 – 115, normal development; >115, advanced development.

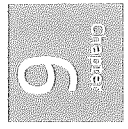
[a] Disharmonic profile.

[b] After this time point hearing aids were fitted.

[c] Raven (disharmonic profiles could not be calculated).

[d] Died at age 4.

[e] At this time point the patient had a serious respiratory infection.



Disharmonic profiles were found in 7 of 10 patients and in 21 of the 30 tests performed in these 7 patients. At each time point after the start of therapy, the percentage of patients with disharmonic profiles was higher than that of their healthy peers (p <0.05). The disharmonic profiles were due to lower gross motor functioning (15 of 21 Griffiths or BSID-II) and, to a lesser extent, also to higher Personal-Social scores (5 of 21), lower MDI vs PDI scores (2 of 21 BSID-II), higher Eye and Hand Coordination scores (1 of 21), higher Performance scores (2 of 21), lower Performance scores (1 of 21), and higher Hearing and Language scores (2 of 21). Some patients had an imbalance in their test profile for more than one subscale. Median subscale scores per measurement are presented in Table 3.

Table 3 | Median subscale scores on the Griffiths Mental Developmental Scales.

	Score after start of ERT			
Subscale	**0.5 y**	**1 y**	**2 y**	**3 y**
Locomotor	89	90	83	53
Personal-Social	103	96	108	93
Hearing&Language	114	100	81	78
Eye&Hand coordination	105	101	98	92
Performance	102	100	122	94

Cognitive development up to 12 years of age

Table 2 also presents the test results of 5 children who were followed regularly for a period of 5 – 11 years after the start of therapy (number of tests=11). Their ages at the latest assessment were 5.1, 6.0, 11.3, 11.6, and 12.2 years. Two of the 5 children had normal test scores. The scores of the other 3 children indicated mild developmental delays. The 5 children included 2 tetraplegic children with earlier scores indicating severe developmental delays. For more detail, see below.

Five years after the start of therapy, patient 1, who had hearing impairments and speech difficulties, scored in the normal range on a nonverbal intelligence test. Retesting using the WISC-III at 7 – 11 years after the start of therapy produced scores in the range of mild developmental delay. Figure 1 shows his factor scores on the WISC-III. The decline of almost 2 SDs on the factor processing speed is noteworthy. At last assessment (11 years of ERT), the score for the factor processing speed was lower than that for the other factors (p <0.05).

On the BSID-II, patients 2 and 4 (both tetraplegic and ventilator-dependent) scored severe developmental delays. From age 5 onward, they were tested with a motor-free nonverbal intelligence test, and their scores were markedly higher, indicating mild developmental delay or even normal development (Table 2).

Patient 6 had normal cognitive development at the start of therapy but declined toward mild developmental delay at age 5 as a result of problems in hearing/speech and motor functioning (Griffiths). At age 6, he was retested with the WISC-III and achieved the score he had last scored on the Griffiths scale at age 5. As in patient 1, we found a lower factor score on processing speed ($p<0.05$) (Figure 1).

Patient 7 had normal cognitive scores at the age of 5 years. She had the best motor performance of all patients.

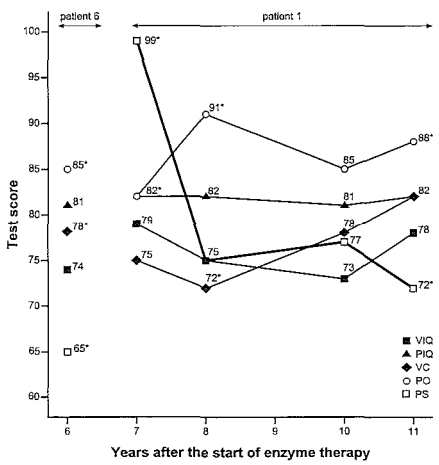


Figure 1 | Wechsler Intelligence Scales for Children, Third Edition, subscales and factor scores of patient 1 and 6.

PIQ: performal IQ; PO: perceptual organization factor; PS: processing speed factor; V: verbal comprehension factor; VIQ: verbal IQ. *Significant differences between the subscales conform to the Dutch norms at a given time point.[23]

Education

All 5 children aged 5 and older were attending school (patients 1, 2, 4, 6, and 7). One child attended a regular school (patient 7) and one child (patient 4) was educated at home, where she used an Internet link to attend classes at a special school for children with motor disabilities.

Both patients fulfilled the curricular requirements for their age group, in accordance with their normal intelligence test score. The other 3 (patients 1, 2, and 6) needed special education, mainly because of their motor disabilities, but also because of their learning disabilities. The overall educational levels of patients 1, 2 and 6 were below their intelligence score. Patients 1 and 2 had specific learning disabilities in mathematics and language comprehension (patient 2). At age 5, patient 2, who was completely paralyzed and was only able to communicate with eye movements and grunting, received a speech computer. Her learning disabilities may have been at least partly attributable to her limited exposure to the world outside.

Parents' highest educational level

The intelligence scores of patients 1, 6, 7, 9, and 10 were consistent with the highest educational level of their parents, but at the last assessment patients 1 and 6 scored below their parents' educational level. At all times, patients 2 and 4 had test scores below their parents' highest educational levels. The highest educational level of the parents of patient 3 was unknown. The parents of patients 5 and 8 had received their education in other countries; their highest educational level could not be calculated.

Adaptive skills

Except for the 2 tetraplegic patients, children had relatively normal adaptive skills. For example, they could eat independently (except for patients receiving tube feeding), have hobbies such as horse-back riding and swimming, go on errands to nearby shops, and make friends. Some children had difficulties performing tasks requiring motor skills, such as dressing and undressing.

Brain imaging

Ultrasound images of the brains were made of 6 patients at ages ranging from 9 days to 8 months (median age 2 months). Except for minor asymmetry of the lateral ventricles (patient 3) and a double contour in the right-sided choroid plexus (patient 2), there were no detectable abnormalities. One CT and 5 MRI images were made of the brains of 5 children between the ages of 6 months and 9 years (Table 1). No abnormalities were observed in the youngest patient at the age of 6 months (patient 2, MRI). Four patients showed periventricular white matter abnormalities (MRI, patients 1, 3, 4, and 5) (Fiure 2). Other observations included a thinner corpus callosum (patients 3 and 5, MRI), and white matter tract changes in the internal capsula (patient 3, CT), the cerebral peduncles (patient 1, MRI), and the mesencephalon/pons area (patient 3, MRI). Patient 3 had hyperthermia during scanning.

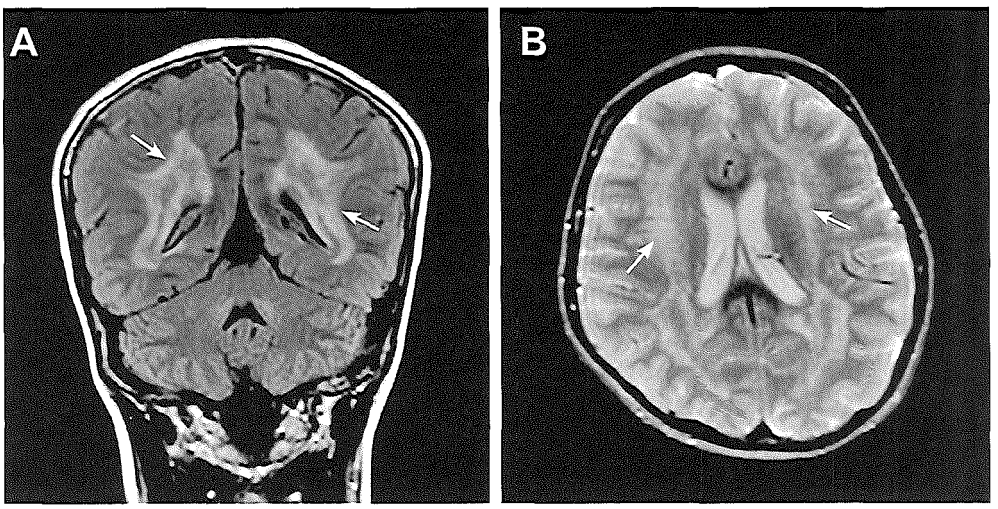


Figure 2 | Brain MRI scan of patient 1 at the age of 9 years.

(A) Coronal view of a fluid-attenuated inversion recovery image. (B) Transversal view of a T2-weighted image. Arrows show periventricular white matter abnormalities.

DISCUSSION

Eleven years ago we started ERT in children with classic infantile Pompe disease. Because glycogen storage is known to occur in the brains of untreated infants[11-15] and this therapy is unlikely to cross the blood-brain barrier,[10] we were concerned about the cognitive development of these children.

We found that cognition in children with infantile Pompe disease at school age was normal to mildly delayed. It should be noted that only 5 children in our study had reached school age, whereas one was aged 12 years. Although the story has not ended yet and mild delays may have developed over time, we can conclude that infantile Pompe disease differs substantially from other lysosomal storage diseases, such as Hurler disease, Hunter disease, Sanfilippo disease, and Niemann-Pick disease type C, in which progressive storage in the CNS and profound mental retardation occur at an early age.[28,29]

To date, the few reports on cognition in children with classic infantile Pompe disease treated with ERT[7,18,19,30,31] have presented data only on early development (until age 4), and their results have been ambiguous. Our study, too, found a wide range of early developmental scores.

Testing of mental capacities in young children younger than age 4 is driven largely by motor skills. Because severe muscle damage cannot be repaired by ERT,[32] children with end-stage Pompe disease at start of ERT will continue to perform poorly on the motor items of the developmental tests. This became evident when better nonmotor intelligence tests were used to test 2 children with complete paralysis after age 4: their test results were markedly higher.

During the first years in which we performed ERT, we used the BSID-II to test early development. Later, we decided to use the Griffiths instead. Although both tests contain similar items, we expected the Griffiths to differentiate better between various domains. It did indeed show that disharmonic profiles were mainly due to poor performance in the gross motor domain. Because these results reflect the difficulty of properly assessing the true mental capacities of young children with classic infantile Pompe disease, results of early developmental tests up to age 4 should be interpreted very carefully.

Does this mean that there are no concerns about the consequences of glycogen storage in the brain? The limited autopsy data available on storage of glycogen in the brains of untreated infants younger than age 1 show that glycogen is stored in the anterior horn cells of the spinal cord, the brain stem, the thalamus, the cerebellum, and, to a lesser extent, the cerebral cortex.[11-15] Previous radiologic studies in children treated with ERT until age 4 reported white matter changes,[17,18] as also demonstrated in our study. Although these changes were not observed in the youngest patient investigated at 6 months, it is difficult to assess white matter development in infants, and although we did not perform longitudinal MRI scans in any of our patients, white matter abnormalities seemed to become more evident on MRI scans over time. However, the extent of the abnormalities did not seem to increase.

It is conceivable that the white matter abnormalities noted in the periventricular areas and corpus callosum are related to the delays in processing speed found in some of the children.[33,34] Earlier a correlation was shown between white matter abnormalities in preterm children and mild cognitive deficits at follow-up.[35,36] Detailed research is needed on the potential relationship between subtle MRI changes, pathologic abnormalities, and the cognitive profiles of long-term survivors of classic infantile Pompe disease. This might be facilitated by regular brain imaging.

Problems with hearing, speech, and language should also be addressed when cognitive functioning is assessed in these children. Earlier we reported that many patients with classic infantile Pompe disease had hearing problems.[37] For optimal cognitive functioning, these patients' hearing should be tested regularly, and hearing aids should be fitted as early as possible. All these children had speech problems characterized by articulation disorders,

hypernasality, and poor phonation, all requiring the intervention of a speech therapist. These speech problems were probably caused by a combination of hearing problems and bulbar muscle weakness, although conceivably CNS pathology could also have a role.[38] Children tested with the Griffiths presented delays in the Hearing and Language domain; this result might be related to problems not only in hearing and speech production but also in language comprehension. Speech delays and language delays were reported earlier; it was suggested that delays in language development may be related to delays in myelination.[39] This suggestion requires further investigation.

The various limitations in our patient population raise the question of the most suitable education for school-age children with infantile Pompe disease. Because of severe motor impairments, speech/language problems, delays in processing speed, and/or mild developmental delays, 4 of our 5 children aged 5 – 12 years attended special schools. To determine the right education level and supportive measures, we recommend that all children have a regular neuropsychological examination. The most appropriate test should be chosen, in view of the possibility that the differences in psychometric properties between tests used during long-term follow-up may lead cognition to be mildly underestimated or overestimated, as may indeed have been the case in our study.

Before ERT became available in 1999, children with classic infantile Pompe disease would die before age 1. Since then we have had a unique opportunity to study cognition in the longest survivors of this disease. Despite the strong evidence of glycogen stores in the brain of these children, the impact on the function of the CNS seems to have been limited.

Here we show that cognitive development at school age ranged from normal to mildly delayed in our children with infantile Pompe disease, of whom the oldest was 12 years. Some children older than 5 had abnormalities in processing speed, which may be explained partly by mild white matter changes. Most developmental delays in young children were caused by muscle weakness and hearing and speech difficulties, making it easy to underestimate true levels of mental development. Because patients who started enzyme therapy early had the best motor outcome and the highest scores on early cognitive development, it is mandatory that enzyme therapy should start early.

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CHAPTER 7

Antibody titers in relation to CRIM status: Effects on enzyme therapy in classic infantile Pompe disease

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ABSTRACT

Background

Enzyme-replacement therapy (ERT) in Pompe disease – an inherited metabolic disorder caused by acid α-glucosidase deficiency and characterized in infants by generalized muscle weakness and cardiomyopathy – is complicated by immune responses. They occur particularly in infants that do not produce any endogenous enzyme; so called CRIM-negative patients. We examined the antibody response to ERT and the patients' clinical outcome in relation to their CRIM status.

Methods

Eleven patients were genotyped and their CRIM status (i.e. capacity to produce acid α-glucosidase) was determined. Antibody formation and clinical outcome were assessed for a minimum of 4 years and a maximum of 13.7 years (median 5.6 years).

Results

All patients developed antibodies to ERT with alglucosidase alfa. The titers varied substantially between patients and did not strictly correlate with the patients' CRIM status. The three CRIM-negative patients in our study succumbed by the age of 4 years seemingly unrelated to the height of their antibody titer. Patients starting relatively late with ERT tended to develop higher antibody titers than those who started very early. High antibody titers had a negative effect on the response to ERT and this seems effectuated by the stoichiometry of the antibody and the therapeutic enzyme in the immune reaction.

Conclusion

Antibody formation is a common response to ERT in classic infantile Pompe disease and counteracts the effect of treatment; its extent may be minimized by early start of ERT. The CRIM-negative status itself is possibly associated with poor outcome.

1 | INTRODUCTION

Immune responses are a common phenomenon in lysosomal storage disorders (LSDs) in which enzyme-replacement therapy (ERT) is applied.[1,2] The aim of ERT is to correct the enzyme deficiencies in LSDs by intravenous infusion of recombinant human enzymes. ERT is now available for Gaucher disease, Fabry disease, the mucopolysaccharidoses (MPS I, II and VI), and for Pompe disease. Immunological responses have been seen in all these diseases.[1,2]

Pompe disease (glycogen storage disease type II, acid maltase deficiency, OMIM # 232300) is a lysosomal storage disorder caused by mutations in the acid α-glucosidase gene (*GAA*, EC 3.2.1.20).[3,4] The disease is autosomal recessive and has a broad clinical spectrum.[3] At the severe end of the spectrum, patients with classic infantile Pompe disease present with muscle weakness, hypertrophic cardiomyopathy and respiratory insufficiency in the first few months of life. If untreated, they usually succumb to cardio-respiratory insufficiency before the end of their first year.[5,6] Their rapid demise is caused by a virtually total deficiency of acid α-glucosidase activity. Treatment with alglucosidase alfa reverses the cardiomyopathy, improves motor function, and extends the survival of patients with classic infantile Pompe disease.[7-15]

Over 95% of affected infants receiving ERT develop antibodies to alglucosidase alfa.[16] In classic infantile Pompe disease, a distinction is often made between CRIM-negative patients (cross reactive immunologic material) who lack any form of endogenous acid α-glucosidase and CRIM-positive patients who synthesize a certain amount of catalytically inactive acid α-glucosidase. High and sustained antibody titers occur particularly in CRIM-negative patients and are associated with a poor clinical outcome.[16,17] Here we report on the formation of antibodies to ERT in 11 patients with classic infantile Pompe disease who were treated for up to thirteen years. We examined 1.) the relationship between antibody formation and the patients' CRIM status and 2.) the impact of antibody formation on the patients' clinical outcome.

2 | MATERIALS AND METHODS

2.1 | Patients

We describe 11 patients with classic infantile Pompe disease who received ERT in our hospital between 1999 and 2012. Classic infantile Pompe disease was defined as symptoms of muscle

weakness within 6 months after birth; hypertrophic cardiomyopathy (left ventricular mass index (LVMI) >75 g/m^2 (>+2SD)[18]); less than 1% acid α-glucosidase activity in fibroblasts; and severe mutations in both *GAA* alleles. Enzyme-activity assays in fibroblasts and mutation analysis were performed as described previously.[19,20] All patients participated in consecutive trials investigating the safety and efficacy of ERT (20 mg/kg every other week to 40 mg/kg weekly). Initially, four patients received recombinant human α-glucosidase from the milk of transgenic rabbits.[7] From 2004 onwards, all patients were treated with alglucosidase alfa. The Institutional Review Board approved all studies and the parents of all patients gave written informed consent.

2.2 | CRIM status

Two methods were used to determine the patients' CRIM status. Cultured skin fibroblasts from the patients were used in the first method. They were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) and antibiotics, and were harvested with trypsin one week after reaching confluence. Frozen cell pellets were lysed in 10 mM phosphate-buffered saline (PBS) pH 7.0 containing 1% Triton-X100. A 10 µL aliquot containing 100 µg protein was mixed with 10 µL sample buffer (NuPAGE, LDS sample buffer, Life Technology, with 4% SDS instead of 0.84%) and applied to SDS-PAGE (Bio-Rad Criterion XT 4 – 12% MOPS gel system). After SDS-PAGE, the various molecular forms of acid α-glucosidase were visualized by immunoblotting using polyclonal rabbit anti recombinant-human acid α-glucosidase antiserum and goat anti-rabbit IRDye 680LT as secondary antibody on an Odyssey infrared imager (LI-COR Biosciences).

The second method for determining the CRIM status was based on transient expression of *GAA* cDNA constructs containing the patients' pathogenic mutations in HEK293T cells. The activity of acid α-glucosidase in these cells and in the culture medium was measured with the artificial substrate 4-methylumbelliferyl-α-D-glucopyranoside (MUGlc, Sigma) at 72 hours after transfection. The biosynthetic forms of acidα-glucosidase were separated by SDS-PAGE and visualized by immunoblotting.[21]

2.3 | Antibody detection

Blood samples for antibody titer measurements were drawn before the start of ERT and every three months thereafter, just before the start of enzyme infusion. The sera were stored at 20°C. An enzyme-linked immunosorbent assay (ELISA) was used to determine the titers. To this end a 96-well plate (Nunc, F96 Maxisorp, Denmark) was coated with 50 µL/well alglucosidase

alfa in a concentration of 5 μg/mL, diluted in PBS (pH 7.4), and incubated for 2 hours at room temperature under continuous shaking. Plates were blocked overnight at 4°C with a solution of bovine serum albumin (BSA; Sigma A7030) in PBS (1% weight/volume). The plates were rinsed six times at room temperature with 200 μL washing buffer (0.05% Tween-20 in PBS). Plates were then incubated with 50 μL of 5-fold serial dilutions of patients' sera for one hour during shaking. Samples were diluted from 50 to 3,906,250 fold in dilution buffer (BSA/PBS containing 0.05% Tween-20). Sera from healthy persons were used as negative controls, and rabbit antiserum prepared against alglucosidase alfa as a positive control. After washing of the plates, 50 μL conjugate was added to each well: polyclonal goat-anti-human-[IgG, IgA and IgM]-HRP (Acris) in a 20,000-fold dilution for human sera, and goat-anti-rabbit-IgG-HRP (Sigma) in a 10,000-fold dilution for rabbit serum. After 1 hour incubation at room temperature followed by thorough washing, 100 μL Tetramethylbenzidine Microwell Peroxidase substrate (Kirkegaard and Perry Laboratories, Maryland) was added, and the plates were incubated for 10 minutes. The colorimetric reaction was stopped by the addition of 100 μL 1M Phosphoric Acid (H_3PO_4). Absorbance was measured at 450 nm using a spectrophotometer (Thermo Electron Corporation, Vantaa, Finland). The maximal dilution at which absorbance was at least twice that of the negative control was taken as titer.

An antibody titer assay based on immunoprecipitation of alglucosidase alfa was used as second semi-quantitative method.[22]

2.3.1 | Inhibition of alglucosidase alfa uptake

Fibroblasts from a patient homozygous for the 525delT pathogenic *GAA* sequence variation and fully deficient in acid α-glucosidase production were seeded in 24-well tissue-culture plates and maintained at 37°C in Ham's F10 medium supplemented with 10% FCS and antibiotics. To measure the uptake of alglucosidase alfa, we first added Pipes as buffer to the medium in a final concentration of 3 mM, and then the enzyme in an amount equivalent to 200 nmol MUGlc/hr per 200 μL medium. Finally, 20 μL of the patients' sera were added. The acid α-glucosidase activity in the medium was measured directly after enzyme addition and just prior to cell harvest. Uptake of alglucosidase alfa was measured in cell homogenates with MUGlc as substrate.

2.4 | Clinical assessments

The clinical outcome was evaluated by analyzing survival, ventilator use, gross motor function, and cardiac dimensions at baseline and at regular intervals; the latter by 2D-guided M-mode

echocardiography. The left ventricular mass index (LVMI) was calculated as a measure for hypertrophic cardiomyopathy (LVMI >75 g/m^2 (>+2SD)[18]). Motor function was assessed using the Alberta Infant Motor Scale (AIMS).[23]

Safety assessments included the monitoring of infusion-associated reactions (IARs). All adverse events that were judged to be possibly, probably or definitely related to ERT were considered IARs.

2.5 | Statistical analysis

Due to limitations in patient numbers, the clinical data was summarized using descriptive statistics. All patients were followed-up for at least four years after the start of ERT, or until death. Data were analyzed using SPSS for Windows version 20, SPSS Inc., Chicago, IL.

3 | RESULTS

3.1 | Patients

This study describes 11 patients (5 boys and 6 girls) with classic infantile Pompe disease (Table 1). All had symptoms before the age of 3.5 months; nine started ERT before the age of 4 months. Two patients started ERT at the ages of 7 and 8 months when they were severely affected. All 11 patients had cardiac hypertrophy, less than 1% of average normal acid α-glucosidase activity in cultured fibroblasts, and a severe mutation in each of the two *GAA* alleles (Table 1). They were treated with ERT for 0.3 to 13.7 years (median duration 5.6 years).

3.2 | CRIM status

'CRIM status' in this study is defined by the ability to detect any form of acid α-glucosidase with immunological methods in either the patients' cultured fibroblasts or in HEK293 cells in which mutated *GAA* constructs carrying the patients' mutations were expressed. A CRIM-positive status indicates that forms of acid α-glucosidase were detected. A CRIM-negative status indicates that these were undetectable.

Figure 1 presents an example of the first type of analysis in fibroblasts. Cell homogenates were analyzed by SDS-PAGE followed by immunoblotting. Compared to normal synthesis of acid α-glucosidase in fibroblasts of a healthy person (Wt), represented by processing of the 110 kD acid α-glucosidase precursor to molecular species of 95, 76 and 70 kD, fully maturated enzyme of 76 kD was not detectable in cells from any of the patients. However, the 110 kD precursor was detected in 8 cases. In four of these cases (pts 1, 3, 10, and 11), the apparent

molecular mass of the precursor was near normal. In the other 4 cases (pts 2, 5, 6, and 9), the mass was abnormally low due to either homozygosity or heterozygosity for the rather common exon 18 deletion (c.2481+102_2646+31del). Proteolytic conversion of the 110 kD precursor to the 95 kD intermediate could be demonstrated only in cells of patient 9. Three patients did not have any trace of acid α-glucosidase due to either homozygosity for c.525delT (pt 7) or c.2741delinsCAG (pt 4), or to compound heterozygosity for c.525delT and c.378_379del (pt 8). Based on the outcome of this analysis, 8 of the 11 patients were designated CRIM-positive and 3 CRIM-negative.

Table 1 | Baseline characteristics.

			Parameters at start of ERT				
Pt	**Gender**	**Ethnic origin**	**Age (months)**	**Resp. function**	**AIMS score**	**LVMI in SD**	**Genotype**
1	M	Turkic	0.1	OS	N	+5	c.1460T>C c.1460T>C
2	F	Caucasian	0.5	OS	N	+22	c.2481+102_2646+31del c.2481+102_2646+31del
3	M	Caucasian	1.2	NS	A	+15	c.1933G>T c.525delT
4	M	Turkic	1.9	NS	N	+30	c.2741delinsCAG c.2741delinsCAG
5	M	Caucasian	2.2	NS	N	+10	c.2481+102_2646+31del c.525delT
6	F	Caucasian	2.4	NS	A	+23	c.2481+102_2646+31del c.2481+102_2646+31del
7[a]	F	Caucasian	3.0	NS	A	+31	c.525delT c.525delT
8	F	Caucasian	3.6	OS	A	+24	c.378_379del c.525delT
9[a]	M	Caucasian	3.8	NS	N	+14	c.2481+102_2646+31del c.1799G>A
10[a]	F	Caucasian	7.2	IV	A[b]	+18	c.1115A>T c.525delT
11[a]	F	Caucasian	8.3	OS	A[b]	+68	c.1913G>T c.1548G>A
	Male 45%	Caucasian 81%	Median 3.0	Ventilated 9%		Median +22	

Pt: patient; M: male; F: female; Resp: respiratory; OS: oxygen via nasal cannula; NS: no respiratory support; IV: invasive ventilation; N: within normal range of healthy peers; A: atypical motor development (AIMS score <-2SD); LVMI: left ventricular mass index; SD: standard deviation

[a] These patients initially received recombinant human acid α-glucosidase from transgenic rabbits

[b] Paresis arms and paralysis legs

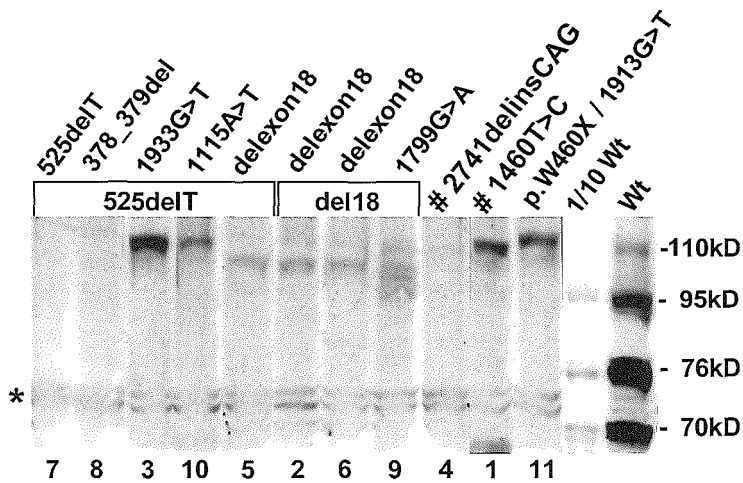

Figure 1 | Molecular forms of acid α-glucosidase in fibroblasts.

Cell homogenates were prepared as described in materials and methods. Equal amounts of protein were loaded per lane. SDS-PAGE followed by immunoblotting was used to reveal the biosynthetic forms of acid α-glucosidase. The numbers under the lanes refer to the patient numbers. The genotypes of the patients are presented above the lanes (delexon18 or del18 stands for c.2481+102_2646+31del; p.W460X stands for c.1548G>A). W: fibroblasts from a healthy individual; # Homozygous; * Nonspecific background staining.

As an alternative approach determining the patients' CRIM status, we introduced mutations in *GAA* cDNA constructs and studied their effect on acid α-glucosidase synthesis and maturation in HEK293T cells (Figure 2). Transient expression of the five missense mutations identified among the patients in this study revealed synthesis of the 110 kD acid α-glucosidase precursor with either normal (c.1933G>T, c.1913G>T, and c.1115A>T) or slightly higher than normal (c.1460T>C and c.1799G>A) molecular mass. Notably, the c.1460T>C precursor appeared in two subspecies (Figure 2; Cells).

The c.1799G>A and c.1460T>C encoded precursors were also detectable in the cell culture medium, but the c.1933G>T, c.1913G>T, and c.1115A>T encoded precursors were not (Figure 2; Medium). Figures 1 and 2 both illustrate that c.1799G>A does not block the conversion from 110 kD to 95 kD completely. Transient expression of c.2741delinsCAG did not reveal any acid α-glucosidase species (Figure 2; Cells) and also this finding is consistent with the lack of acid α-glucosidase expression in fibroblasts of the patient who is homozygous for this mutation (Figure 1). Expression of none of the mutagenized cDNA constructs led to acid α-glucosidase activity neither in the transfected cells nor in the medium.

On the basis of these two types of analyses, patients 4, 7 and 8 were designated CRIM-negative; the other 8 patients were designated CRIM-positive.

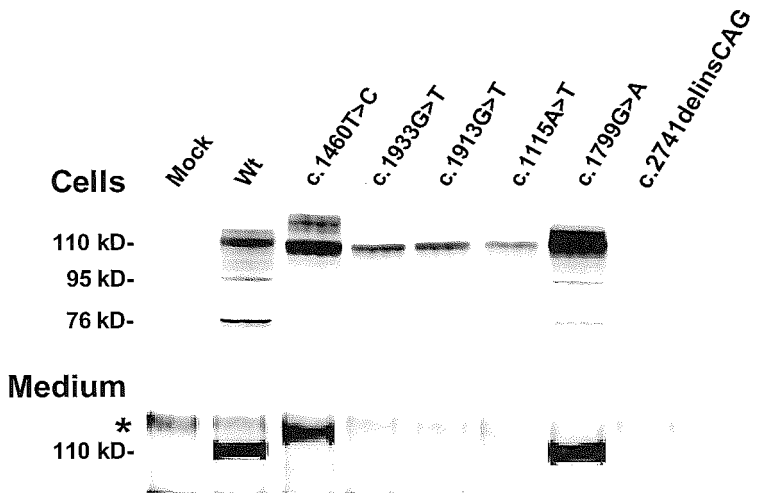

Figure 2 | Transient expression of wildtype and mutant acid α-glucosidase cDNA constructs.

Hek293T cells were transfected with wildtype (Wt) and mutant cDNA constructs to study abnormalities in the synthesis and post-translational modification of acid α-glucosidase. To visualize the molecular species, the same procedure was used as in Figure 1. * Nonspecific background staining.

3.3 | Antibody titers

All patients developed antibodies to alglucosidase alfa (Figure 3A, Table 2). Over four years of treatment the median peak antibody titer (ELISA) was 1:31,250 (range 1:1,250 – 1:156,250). These titers were reached within one year of ERT. Both low and high antibody titers were measured in CRIM-positive (median 1:18,750, range 1:1,250 – 1:156,250) and CRIM-negative patients (median 1:31,250, range 1:6,250 – 1:156,250). The peak antibody titers tended to be higher in patients who had started ERT relatively late (Table 2). Notably, none of the patients who received ERT before the age of 2 months developed titers above 1:6,250.

Six patients (pts 5-8, 10 and 11) developed titers ≥1:31,250 (Table 2); titers remained high in five cases and declined temporarily to 1:6,250 in one case (pt 10). The antibody titers of the other five patients remained ≤1:6,250 over the entire study period; one of these patients was CRIM-negative and his titer eventually declined to 1:50. There was no apparent relationship between the titer and the dose of ERT, nor between the titer and the use of recombinant human acid α-glucosidase from either rabbit milk or CHO cells (Supplemental Figure 1).

Table 2 | CRIM status, antibody titer, and clinical outcome.

	Age			Antibody titer				Clinical outcome at 4 years of ERT[b]		
Pt	At start of ERT (months)	Current (years)	CRIM status	Peak titer through 4 years of ERT	Titer at 4 years of ERT[b]	Last titer (years after start of ERT)		Age at respiratory insufficiency[c]	Major motor milestone	LVMI in SD
1	0.1	5.6	+	1:6,250	1:1,250	1:6,250	(5.0)	2.7 (1:250)	Sitting[e]	-0.6
2	0.5	8.0	+	1:1,250	1:250	1:250	(7.1)	-	Walking	-0.7
3	1.2	8.5	+	1:6,250	1:1,250	1:6,250	(8.0)	-	Walking	-1.5
4	1.9	*4.4*[a]	-	1:6,250	1:50	1:50	(3.4)	2.0 (1:6,250)	Sitting[e]	+0.9
5	2.2	5.2	+	1:31,250	1:31,250	1:31,250	(4.0)	-	Sitting	+1.1
6	2.4	4.1	+	1:31,250	1:31,250	1:31,250	(3.0)	-	Walking	+3.7
7	3.0	*4.3*[a]	-	1:156,250	1:156,250	1:156,250	(3.8)	2.2 (1:31,250)	Sitting	+4.4
8	3.6	*0.6*[a]	-	1:31,250	1:31,250	1:31,250	(0.3)	0.6 (1:31,250)	Minimal motor gain	+28.6
9	3.8	14.1	+	1:6,250	1:250	1:6,250	(12.8)	-	Walking	+0.4
10	7.2	14.3	+	1:31,250	1:6,250	1:156,250	(12.9)	0.6[d]	Tetraplegic	+4.9
11	8.3	14.3	+	1:156,250	1:156,250	1:156,250	(11.5)	0.9 (1:250)	Tetraplegic	+4.5

Pt: Patient number
[a] Age at death
[b] Last available data are presented if patient did not yet receive four years of ERT
c Age at respiratory insufficiency in years and (antibody titer around that time)
[d] Patient was ventilator dependent before start of ERT
[e] Patient lost ability to walk

An immunoprecipitation assay was used as alternative method determining the antibody titer, for which we selected three patients whose respective ELISA titers were 1:1,250, 1:6,250, and 1:156,250. The results are shown in Figure 4 and are consistent with the outcome of the ELISA assay in that both methods discriminate similarly between a low, intermediate and high titer.

3.3.1 | Inhibition of alglucosidasealfa uptake

To measure the effect of antibodies on the uptake of alglucosidase alfa, we used sera of all patients at the time that their antibody titer was highest (Table 3). Alglucosidase alfa and patients' sera were added together to the culture media of fibroblasts from a CRIM-negative infant. No effects on enzyme activity or uptake of alglucosidase alfa were observed when antibody titers were ≤1:6,250. Alglucosidase alfa activity in the medium was substantially inactivated (26 – 50% loss of enzymatic activity) in one of three cases with a titer of 1:31,250 and in all three cases with a titer of 1:156,250. Uptake of alglucosidase alfa by the cells was inhibited (24 – 42% inhibition) only in the three cases with a titer of 1:156,250.

3.4 | CLINICAL OUTCOME OVER FOUR YEARS OF ERT

3.4.1 | Survival and ventilator-free survival

One of the 11 patients was ventilator dependent before the start of ERT. Over four years of ERT, five more patients developed respiratory insufficiency (at ages 0.6, 0.9, 2.0, 2.2, and 2.7 years; Table 2) and three of them died (at ages 0.6, 4.3, and 4.4 years). The last antibody titers measured before the patients' deaths or respiratory insufficiency ranged from 1:50 to 1:31,250. The median peak antibody titer of these patients was 1:31,250 (range 1:6,250 – 1:156,250); the median peak antibody titer of the five patients who are still alive and ventilator-free at the time of reporting was 1:6,250 (range 1:1,250 – 1:31,250). All CRIM-negative patients died. All eight CRIM-positive patients are alive; two of them became ventilator dependent during treatment.

Table 3 | Uptake of alglucosidase alfa by cultured fibroblasts.

	Activity compared to control (%)		Antibody titer
	Medium	Cell[a]	
Pt 1	93	90	1:6,250
Pt 2	97	111	1:1,250
Pt 3	92	85	1:6,250
Pt 4	95	90	1:6,250
Pt 5	88	112	1:31,250
Pt 6	**74**	83	1:31,250
Pt 7	**58**	**24**	1:156,250
Pt 8	81	91	1:31,250
Pt 9	93	96	1:6,250
Pt 10	**50**	**42**	1:156,250
Pt 11	**60**	**41**	1:156,250
Rabbit antiserum	**49**	**1**	>1:156,250
Control	100	100	
Blanco		1	

Pt: Patient; Control, Serum from healthy individual

[a] Mean of two experiments

Figures in bold refer to substantial inhibition of alglucosidase alfa activity or uptake.

3.4.2 | Motor outcome

Over four years of ERT, eight patients achieved important motor milestones. Six learned to walk and two learned to sit, but three made minimal motor gains. Figure 3C depicts the patients' AIMS scores over time. The median peak titer of the six patients who learned to walk was 1:6.250 (range 1:1,250 – 1:31,250) against 1:31,250 (range 31,250 – 156,250) for the patients who did not learn to walk. One of the three CRIM-negative patients and five of the eight CRIM-positive patients learned to walk.

Two of the six walkers lost their ability to walk after developing respiratory insufficiency. Their antibody titers never rose above 1:6,250. One of them was CRIM-positive, the other CRIM-negative. One CRIM-positive patient with a peak titer of 1:31,250 temporarily lost the ability to attain a sitting position after a Respiratory Syncytial Virus infection at the age of 1.3 years (Figure 3C, pt 5, yellow line). None of the patients with a longer than four years follow-up lost or gained motor milestones beyond four years of ERT.

3.4.3 | Cardiac dimensions

At the start of ERT, all patients had hypertrophic cardiomyopathy. Over four years of ERT, the LVMI decreased significantly in all but one patient who died 4 months after ERT was initiated (Figure 3B). Six of the 11 patients had a normal LVMI at four years of ERT.

The median peak antibody titer of the patients with a normal LVMI after four years of ERT was 1:6,250 (range 1:1,250 – 31,250) against 1:31,250 (range 1:6,250 – 1:156,250) for those whose LVMI was still increased. The LVMI remained above the reference value in all three CRIM-negative patients, against two of the eight CRIM-positive patients. Beyond four years of ERT, no major changes in LVMI were observed.

3.4.4 | Infusion-associated reactions

Over four years of ERT, ten of the 11 patients (82%) experienced one or multiple IARs. The first IARs started 0.4 – 24.5 months after the start of ERT and comprised the following signs and symptoms: General malaise, hypoxia, bronchospasm, dyspnea, tachycardia, bradycardia, hypotension, cyanosis, sweating, fever, vomiting, flushing, and exanthema. All IARs could be controlled by slowing the infusion rate, with or without the administration of premedication.

The only patient who did not develop IARs was CRIM-positive and had the lowest peak antibody titer over the whole period of reporting.

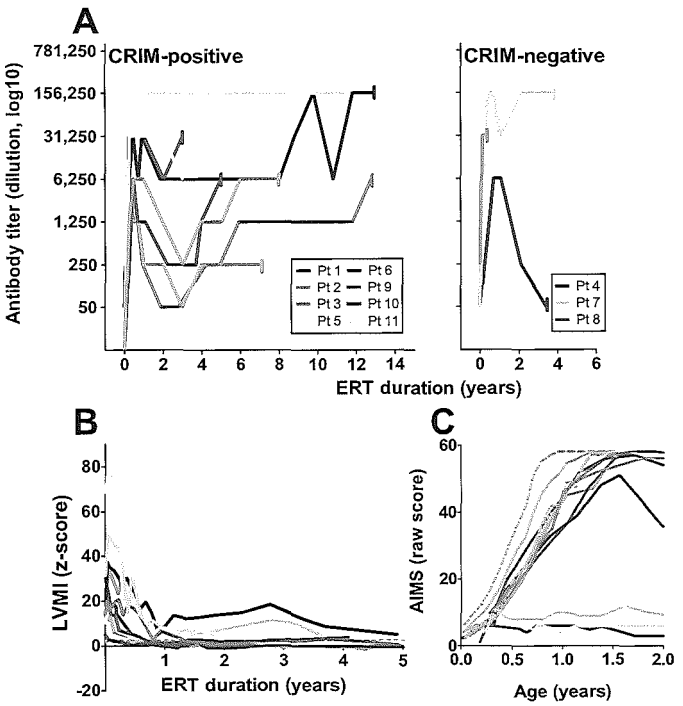

Figure 3 | Antibody titer and CRIM-status.

Antibody titers to alglucosidase alfa as measured by ELISA in CRIM-positive and CRIM-negative patients.

4 | DISCUSSION

Several studies have shown that antibody formation can occur in patients receiving recombinant human proteins for therapy.[2,16,17,24] Our patients with classic infantile Pompe disease who were treated with alglucosidase alfa showed various immune response patterns. Low and high titers were measured in CRIM-negative and CRIM-positive patients alike. While thorough statistical analysis was prevented by the small sample size and the heterogeneity of our patient population, we found that patients who did not learn to walk had a relatively high titer. We also found that patients who started ERT before the age of two months tended to have lower titers than those who started later. Notably, all CRIM-positive patients were still alive at the time of reporting, whereas all CRIM-negative patients had died.

4.1 | *GAA* genotypes and CRIM status

The quality and quantity of acid α-glucosidase in Pompe disease depends on the *GAA* genotype. Ten different mutations were identified, all causing complete loss of acid α-glucosidase activity.

The commonly used procedure to determine the CRIM status is immunoblotting and normally reveals four distinct acid α-glucosidase species that separately and collectively are called CRIM. In healthy people (Figure 1, Wt), the long-lived 76 and 70 kD species comprise approximately 80% of the total CRIM. In patients, however, the synthesis of acid α-glucosidase can be totally lost, which results in a CRIM-negative status. More often, the synthesis is derailed, and CRIM consists mainly of the 110 kD precursor (e.g. Figure 1, pts. 1 and 3) or a modified derivative thereof (Figure 1, pts. 2 and 6). As it stands, the CRIM-negative status is well defined, but the CRIM-positive status encompasses a collection of qualitatively and quantitatively abnormal immunoblot profiles.

By and large, a patient's CRIM status can be predicted on the basis of its genotype.[25] While most non-sense mutations and frame-shift mutations lead to mRNA decay and a CRIM-negative status (e.g. 525delT in pt. 3), truncated protein species are sometimes detectable by immunoblotting (e.g. c.2481+102_2646+31del in pts. 1, 7, 10 and 11), and thus lead to a CRIM-positive status. Although the effect of missense mutations is hard to predict, they usually lead to a CRIM-positive status.

Information on the effect of missense mutations can be obtained by transient expression (Figure 2), which is especially informative for the stage at which mutant forms of acid α-glucosidase are degraded. For instance, the c.1460T>C or c.1799G>A encoded acid a-glucosidase precursors must have traversed the Golgi complex since they are secreted into the medium, whereas the c.1833G>T, c1913G>T and c1115A>T encoded precursors do not appear in the medium (Figure 2) and are apparently degraded while passing through the ER/ Golgi complex. By the current definition of CRIM, these five missense mutations lead to a CRIM-positive status, but obviously do not represent one and the same CRIM-positive condition.

Our application of both methods to determine the CRIM status resulted in the same outcome: 8 patients are CRIM-positive and 3 are CRIM-negative. Notably, the patient homozygous for c.2741delinsCAG was previously designated CRIM-positive.[17,21]

4.2 | CRIM status and antibody titer

Antibody formation is a natural response to foreign invading proteins. Thus, ERT is prone to evoke an immunological response in CRIM-negative patients, but not necessarily in all CRIM-

positive patients. Patients with Pompe disease receiving ERT respond roughly according to these principles. Analysis of the immune response in 34 treated infants showed a strong tendency towards higher and sustained antibody titers in CRIM-negative compared to CRIM-positive infants.[16] However, CRIM-positive patients can also develop high titers,[17] and CRIM-negative patients low titers.[26,27] Our study in 11 infants has led to similar findings. The highest peak antibody titers were measured in four of the eight CRIM-positive patients and in two of the three CRIM-negative patients. The antibody titer of the third CRIM-negative patient did not exceed 1:6,250 and spontaneously regressed to 1:50 after 3 years of ERT. There were two CRIM-positive patients with the same *GAA* genotype; one developed a relatively high titer, and the other a relatively low titer.

Although the number of patients in our study is small, we can firmly conclude that the CRIM status alone predicts neither the level nor the duration of the immune response. This may relate to the imprecise definition of CRIM status, which does not describe the amount, the conformation or the location of the endogenous acid α-glucosidase. For instance, secretion of the 110 kD precursor as opposed to intracellular degradation might reduce the immune response and contribute to the relatively low antibody titers in patients 1 and 9. Notably, adult patients with a considerable amount of normally structured and catalytically active acid α-glucosidase can also develop high titers.[22,24,28,29] Altogether, we conclude that genotype and CRIM status are of limited value in predicting the height of the immunological response.

As previously suggested,[27,30] our study indicates that the age at start of ERT might play a role in the immune response since none of the patients who started ERT before 2 months of age developed titers >1:6,250. There are at least two plausible explanations for this: First, the neonatal immune system is immature, and very early administration of ERT might induce tolerance.[31,32] Second, the 'danger model' suggests that the immune system needs alarm signals from injured tissues to be activated. At a less advanced stage of disease these signals will be weaker.[33] Other models may equally apply.

4.3 | Consequences of antibody formation

Depending on their binding sites antibodies can inhibit catalytic function, block binding to the mannose 6-phosphate receptor and prevent uptake, or otherwise misdirect the protein to macrophages and neutrophils.[1,2] Antibodies and antigen can also form immune complexes and trigger a cascade of adverse events.[34]

In a previous case-report about an adult patient with Pompe disease we have calculated how the concentration of alglucosidase alfa specific antibodies relates to the concentration

of alglucosidase alfa during enzyme infusion.[22] In the present study we have applied the same arithmetic method to infants. For example, patient 11 had an ELISA titer of 1:156,250 at a certain point of treatment. At that time the corresponding titer as measured by immune-precipitation was 40 nmol MUGlc/h.μL (Figure 4) implying that 1 mL of the patient's serum contained enough antibodies to bind 0.13 mg alglucosidase alfa.[22] As a child receives 0.24 mg of alglucosidase alfa per mL blood (based on 20 mg/kg and a circulating blood volume of 80 mL/kg), antibodies may bind as much as 54% of the administered enzyme. By contrast, if the titer is only 4 nmol MUGlc/h.μL, corresponding with an ELISA titer of 1:6,250, the circulating antibodies can bind only 5% of the administered aglucosidase alfa.

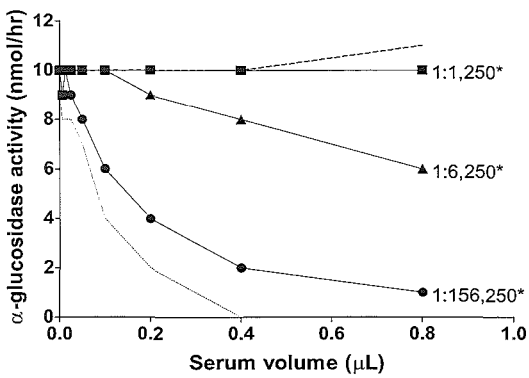

Figure 4 | Antibody titer by immunoprecipitation.

A fixed amount of alglucosidasealfa was incubated with different volumes of patients' sera: Patient 2 (■); patient 10 (▲); patient 11 (●); serum of a healthy individual (--); and rabbit serum raised against alglucosidasealfa (···). Antibody-bound enzyme was precipitated with Protein A sepharose beads, and the activity remaining in the supernatant was measured with MUGlc. *Corresponding ELISA titer.

Although these estimates are crude they indicate that ELISA titers of 1:6,250 and lower probably have no clinical significance, whereas titers of 1:31,250 and higher may counteract ERT at a dose of 20 mg/kg. Accordingly, titers above 1:60,000 are expected to counteract ERT at a dose of 40 mg/kg. Our arithmetic estimates of what should be considered a high titer and what should be considered a low titer remarkably correspond to the cut-off value of 1:51,200 chosen by Banaguria et al.[17]

Antibodies can impede the effect of ERT in several ways. In four cases we observed a decrease of catalytic activity suggestive for binding of antibodies to the enzyme's active site. Uptake was inhibited in three cases. In one case this was due to a combination of inactivation and uptake inhibition possibly by steric hindrance of the ligand-receptor binding. Of note, the effect of antibodies not only depends on the binding sites but also on the stoichiometry of antibodies and alglucosidase alfa in the experimental setting; changing the concentration of one of the two components can tip the balance. Whereas neutralizing antibodies were reported to be more common in CRIM-negative than in CRIM-positive patients,[16,17] in our study their presence was not related specifically to the patients' CRIM status but to the patients' antibody titer.

It has been shown that high antibody titers in CRIM-negative as well as CRIM-positive patients are associated with shorter ventilator-free survival.[16,17] In several adults with Pompe disease, a high titer was also accompanied by poor response to ERT.[22,24] Although our study group was too small and too varied to permit statistical analysis, patients with higher titers tended to attain fewer motor milestones. However, with respect to ventilator-free survival, only two of the six patients who developed respiratory insufficiency or died had a titer of $\geq$1:31,250 at the time that these events occurred. Two other patients had low titers, but nevertheless developed respiratory insufficiency at the age of 2. Two patients became ventilator dependent just before or soon after the start of ERT what we ascribe to their advanced disease at start of ERT.

4.4 | Conclusions and challenges

It is beyond question that high and sustained antibody titers need to be prevented in order to achieve a good response to ERT.[16,17,22] Our study indicates that the negative effect of antibodies starts at an antibody titer of approximately 1:31,250. It has been demonstrated that immune tolerance can be successfully induced by a combination of rituximab, methotrexate and intravenous immunoglobulins,[35-37] a regimen likely to be most effective when used prophylactically.[38,39] Very early start of ERT (<2 months), achievable by neonatal screening, can also help to keep the titers low as suggested by our study. The real challenge remains identifying patients who are prone to developing a strong immune response.

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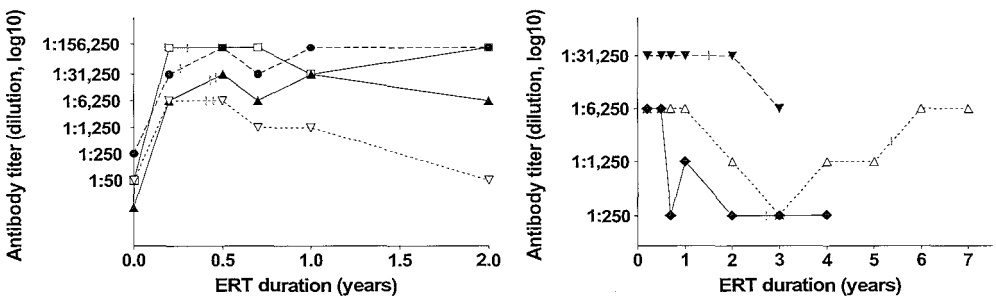

Supplemental Figure 1 | Dose augmentation in relation to antibody titer.

Antibody titers after dose augmentation (grey |) in patients receiving recombinant human acid α-glucosidase from the milk of transgenic rabbits (A; patient 7 (□); patient 9 (▽); patient 10 (▲); and patient 11 (●)) or from CHO cells (B; patient 1 (◆); patient 3 (△); patient 5 (▼)).

CHAPTER 8

A higher dose of alglucosidase alfa in classic infantile Pompe disease positively affects ventilator-free survival and motor outcome: an open-label single-center study

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Submitted

ABSTRACT

Background

Though enzyme-replacement therapy (ERT) with alglucosidase alfa has significantly improved the prospects for patients with classic infantile Pompe disease, some 50% of treated infants do not survive ventilator-free beyond the age of 3 years. We investigated whether higher and more frequent dosing of alglucosidase alfa improves outcome.

Methods

Ten patients were included in the study. All had fully deleterious mutations in both *GAA* alleles. Six received a dose of 20 mg/kg every other week (eow) and four received 40 mg/kg weekly. Survival, ventilator-free survival, left-ventricular mass index (LVMI), motor outcome, infusion-associated reactions (IARs), CRIM status and antibody formation were evaluated.

Results

Seven of the ten patients survived, six of them ventilator-free. The four patients who died and/or became ventilator dependent were treated with 20 mg/kg eow. Four of the six patients receiving 20 mg/kg eow learned to walk; two of them maintained this ability. All four patients receiving 40 mg/kg/week acquired and maintained the ability to walk until study end at ages of 2.7 – 5 years, even though their baseline motor functioning was poorer. There were no apparent differences between the two dose groups with respect to the effect of ERT on LVMI, the number of IARs, and antibody formation. Two patients receiving 20 mg/kg eow were CRIM-negative. All others were CRIM-positive.

Conclusions

Treatment with 40 mg/kg/week was well tolerated and resulted in better ventilator-free survival and motor outcome than treatment with 20 mg/kg eow.

BACKGROUND

Pompe disease (glycogen storage disease type II, OMIM #232300) is a rare, autosomal recessive lysosomal storage disorder caused by deficiency of acid α-glucosidase and characterized by lysosomal glycogen storage, mainly in muscle tissue.[1] Depending largely on how much enzyme activity is preserved, it can present at different ages, from soon after birth to late adulthood. The most severely affected patients – those with classic infantile Pompe disease – present in the first months of life with generalized muscle weakness, hypertrophic cardiomyopathy, respiratory problems, and feeding difficulties.[2,3] Their disease progresses rapidly. If untreated, they usually die before one year of age due to cardio-respiratory insufficiency.

Patients' prospects were significantly improved in 2006, when enzyme-replacement therapy (ERT) with recombinant human acid α-glucosidase (Myozyme®, alglucosidase alfa) was approved for the treatment of Pompe disease. ERT prolongs lifespan, improves cardiac hypertrophy, and enables patients to reach previously unmet motor milestones.[4-12] However, response to treatment is not yet optimal in all patients. When treated with either 20 or 40 mg/kg every other week (eow) , approximately half of patients with classic infantile Pompe disease do not survive ventilator-free beyond the age of three years.[10] Similarly, a substantial proportion of patients do not learn to walk, and nearly all retain residual muscle weakness.[13-15] Significant clearance of glycogen from skeletal muscle is reported in only a small number of patients.[5,9,10,16,17]

Though it remains unclear why skeletal muscle pathology is more difficult to correct fully than pathology in other tissues, several factors seem responsible. First, to reach the skeletal muscle fibers, the therapeutic enzyme has to cross the capillary endothelium and pass the interstitial tissue. This is the opposite of the situation with the macrophages (which are specifically affected in Gaucher disease), or with the endothelium (one of the primary target tissues in Fabry disease), which have direct access to the intravenously administered ERT.[16] Second, the number of cation-independent mannose 6-phosphate receptors (CI-MPR) on the cell surface of matured skeletal muscle cells and required for the cellular uptake of alglucosidase alfa is reportedly low.[18,19] Third, skeletal muscle – the most abundant tissue in the human body – comprises 15 – 40% of total body mass. Together, these three factors might explain the high recommended dose of alglucosidase alfa (20 mg/kg eow)[20] relative to that in other lysosomal storage disorders (for instance 0.2 – 1 mg/kg eow in Fabry disease and up to 1.5 mg/kg eow in Gaucher disease).

Preclinical[21,22] and clinical studies[4,5,9,10] have shown that the reduction in glycogen levels in skeletal muscle is dose-dependent. On the basis of these findings and of the published intracellular half-life of alpha-glucosidase,[23-28] we developed a theoretical model whereby we could calculate the increment in acid α-glucosidase activity in skeletal muscle by manipulating the dose and frequency of ERT. These calculations enabled us to conclude that patients might benefit from a higher and more frequent dose.

From the beginning of 2008 we treated affected infants with a dose of 40 mg/kg/week, i.e., the dose previously administered to four infants treated with recombinant human acid α-glucosidase from rabbit milk.[4,5] The safety and efficacy of this higher and more frequent dosing regimen was compared with that of the recommended dose of 20 mg/kg eow.

METHODS

1 | Patients

Classic infantile Pompe disease was defined as symptoms of muscle weakness within six months of birth, hypertrophic cardiomyopathy, and confirmation of total deficiency of acid α-glucosidase (GAA) activity by the finding of pathogenic mutations in both *GAA* alleles. Patients at our center who had started ERT with alglucosidase alfa before 2008 were treated with a dose of 20 mg/kg eow, whereas those who started ERT in 2008 or later received a dose of 40 mg/kg/week. All patients participated in clinical trials that investigated the safety and efficacy of ERT. The Institutional Review Board approved the protocols, and all parents or guardians gave written informed consent. The patients were followed at least to age 2.7 years or until death. The follow-up of one patient was limited to 1.7 years; this patient received 20 mg/kg eow (Table 1).

2 | Clinical efficacy

Clinical efficacy was measured by assessing survival, ventilator-free survival, number of hospitalizations for respiratory infections, cardiac dimensions, and motor function. Cardiac dimensions were measured by 2D-guided M-mode echocardiographic tracings at baseline and at regular intervals thereafter. Left-ventricular mass index (LVMI) was calculated as a measure for hypertrophic cardiomyopathy (LVMI >+2SD[29]). Motor function was examined using the Alberta Infant Motor Scale (AIMS)[30], and the achievement of motor milestones was examined during regular clinical assessments.

Table 1 | Baseline characteristics.

Patient	Gender (M/F)	Age at start of ERT in months	Age at study end in months (years)		CRIM status	Mutation I	Mutation II
20 mg/kg eow							
1	M	0.1	33	(2.7)#	+	c.1460T>C	c.1460T>C
2	F	0.5	106	(8.8)	+	c.2481+102_2646+31del	c.2481+102_2646+31del
3	M	1.2	66	(5.5)	+	c.1933G>T	c.525delT
4	M	1.9	53	(4.4) #*	-	c.2740dup;c.2742dup	c.2740dup;c.2742dup
5	M	2.2	20	(1.7)	+	c.2481+102_2646+31del	c.525delT
6	F	3.6	8	(0.6) #*	-	c.378_379del	c.525delT
40 mg/kg/week							
7	F	0.3	35	(2.9)	+	c.525delT	c.1933G>A
8	F	2.4	60	(5.0)	+	c.2481+102_2646+31del	c.2481+102_2646+31del
9	M	3.8	32	(2.7)	+	c.2481+102_2646+31del	c.525delT
10	F	4.6	34	(2.9)	+	c.378_379del	c.2104C>T

M: male; F: female; eow: every other week

Patients developed respiratory insufficiency; *Patient died

3 | Safety

Safety assessments included the monitoring of infusion-associated reactions (IARs). All adverse events that were judged to be possibly, probably or definitely related to ERT were considered to be IARs. The severity of each IAR was indexed on the basis of clinical judgment as mild, moderate or severe similar as described previously.[5]

Before enzyme infusions, blood samples were drawn at regular intervals so as to determine the development of antibodies to ERT on the basis of an enzyme-linked immunosorbent assay (ELISA) (van Gelder et al., unpublished results).

4 | Pharmacokinetic analysis

To determine the activity of acid α-glucosidase in the blood circulation and the rate of alglucosidase alfa clearance in relation to dosing, we measured the activity in plasma during an enzyme infusion with 20 mg/kg and one with 40 mg/kg of alglucosidase alfa. Blood samples were drawn before the start of the alglucosidase alfa infusion infusion, at 2 and 3 hours after this, at 15 min before the end of the infusion, at the end of infusion, and then 15, 30, 60, and 120 min thereafter.

To determine the percentage of the enzyme in the blood that was antibody-bound, patients' plasma samples were incubated in the presence of Protein-A Sepharose beads to bind antibody-bound alglucosidase alfa, and in parallel in the presence of Sepharose beads only (control). After removal of the beads by centrifugation, acid α-glucosidase activity was measured in the supernatant.[31] Pre-infusion serum samples were collected to determine the corresponding patients' antibody titer by ELISA (van Gelder et al., unpublished results).

RESULTS

1 | Patients

We included 10 patients with classic infantile Pompe disease, six of whom were treated with alglucosidase alfa in a dose of 20 mg/kg eow, while four were treated with 40 mg/kg/week. The patients' baseline characteristics are summarized in Table 1. Patients in the 20 mg/kg eow dose group started with ERT at a median age of 1.5 months (range 0.1 – 3.6 months) vs. a median age of 3.1 months (range 0.3 – 4.6 months) in the 40 mg/kg/week group. The median age at study end was 3.6 years (range 0.6 – 8.8 years) in the 20 mg/kg eow dose group, and 2.9 years (range 2.7 – 5.0 years) in the 40 mg/kg/week dose group.

All patients had severe mutations in the *GAA* gene. Two patients were CRIM-negative; both received 20 mg/kg eow.

2 | Clinical efficacy

2.1.1 | Survival and ventilator-free survival

At baseline, five of the ten patients required supplemental oxygen; 50% in both dose groups. During treatment, four patients were able to discontinue this. The one patient who continued to depend on oxygen supplementation received 20 mg/kg eow.

At study end, three of the six patients in the 20 mg/kg eow dose group had developed respiratory insufficiency and/or had become ventilator dependent (at ages of 0.6, 2.0, and 2.7 years, Table 1, Figure 1). Two of these three patients died (at ages 0.6 and 4.4 years). In the 40 mg/kg/week group, all four patients were alive at study end; none had developed respiratory insufficiency.

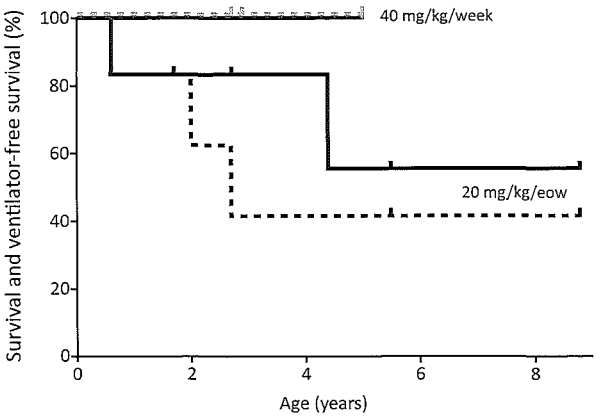

Figure 1 | Kaplan-Meier curves of survival and ventilator-free survival.

Survival (dashed lines) and ventilator-free survival (solid lines) of patients treated with 20 mg/kg eow (black) or 40 mg/kg/week (grey).

2.1.2 | Hospital admissions for respiratory infections

After the start of ERT, five of the six patients treated with 20 mg/kg eow were repeatedly hospitalized for respiratory infections or aspiration pneumonias. One of these patients (Patient 6) remained hospitalized until her death at the age of 6 months; for the other four, the number of admissions ranged from 3 – 6. In the 40 mg/kg/week group none of the patients was admitted for respiratory infections or aspiration pneumonias after the start of ERT, and all were discharged from hospital within 3 weeks of the start of ERT.

2.2 | Cardiac outcome

Median baseline LVMI was similar in the 20 mg/kg eow group (median +18.3 SD, range 4.9 – 30.0 SD) to that in the 40 mg/kg/week dose group (median +21.4 SD, range 6.4 – 25.8 SD). LVMI steadily decreased in 5/6 patients receiving 20 mg/kg eow, and in 4/4 patients receiving 40 mg/kg/week (Figure 2). At study end, LVMI was within normal limits in 4/6 patients in the 20 mg/kg eow dose group and in 3/4 patients in the 40 mg/kg/week dose group.

We note that one patient treated with 40 mg/kg/week had severe left-ventricular dilatation and severe mitral valve regurgitation at baseline, which was considered to be life threatening by the treating cardiologist. After 1.6 years of treatment, this patient's LVIDd had normalized and mitral regurgitation had become moderate.

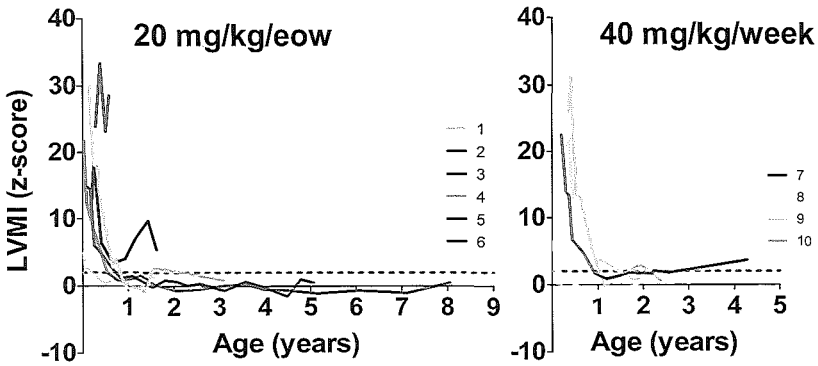

Figure 2 | Left-ventricular mass index (LVMI) z-scores over time.
The coloured lines represent different patients. The dashed line represents +2SD.

2.3 | Motor function

At baseline, all ten patients showed symptoms of muscle weakness, including head lag and axial hypotonia; seven had AIMS scores below the 5th percentile (3/6 in the 20 mg/kg eow, and 4/4 in the 40 mg/kg/week dose groups, Figure 3). During treatment, eight of the ten patients ultimately approached the maximal AIMS score and learned to walk – 4/6 in the 20 mg/kg eow dose group (median age at walking 17 months, range 14 – 18 months), and 4/4 in the 40 mg/kg/week dose group (median age at walking 15 months, range 14 – 17 months). The two patients who did not learn to walk were both receiving 20 mg/kg eow. One did not achieve any motor milestones and died 4 months after start of treatment; the other learned to sit independently.

Over time, some patients lost motor milestones (Figure 3). Two patients who had initially learned to walk lost this skill after becoming ventilator-dependent at the ages of 2.0 and 2.7 years. One other patient temporarily lost the ability to attain a sitting position after a Respiratory Syncytial Virus infection at the age of 1.3 years. Loss of motor milestones was observed only in the 20 mg/kg eow group and not in the 40 mg/kg/week dose group. At study end, two of the six patients in the 20 mg/kg eow group were able to walk, as were all four in the 40 mg/kg/week group.

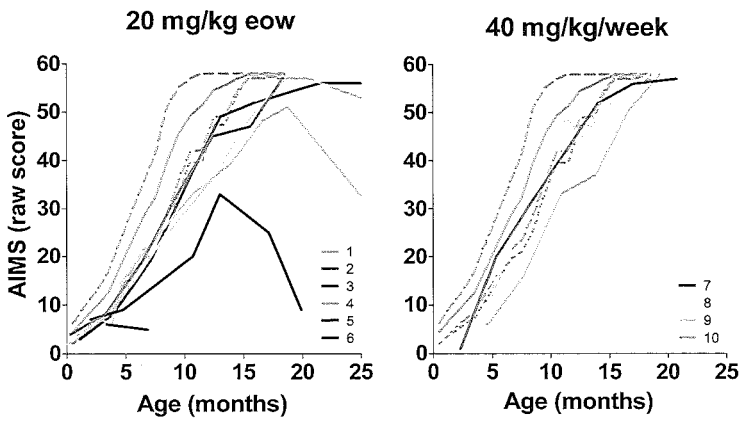

Figure 3 | Alberta Infant Motor Scale scores over time.
The coloured lines represent different patients. Solid line: p50; — line: p 10 or p 90; ··· line: p5.

3 | SAFETY

3.1 | Infusion-associated reactions

IARs were experienced by 5/6 patients treated with 20 mg/kg eow and by 4/4 patients treated with 40 mg/kg/week (Table 2). The number of IARs per patient varied substantially. One patient in the 40 mg/kg/week dose group had 70 IARs (50% of all IARs), six of them were severe. Three patients treated with 20 mg/kg eow had one severe IAR. The most common IARs were exanthema, fever, and decreased oxygen saturation. All IARs could be controlled by slowing the infusion rates and prolonging the duration of the infusion, with or without the administration of premedication (antihistamines and/or steroids). No patients discontinued treatment because of IARs, all recovered without sequelae, and premedication could be stopped in all patients. At the end of the study, all patients treated with 40 mg/kg/week had been IAR-free for at least nine months. All patients alive now have most of their infusions at home.

3.2 | Antibody formation

Figure 4A shows the antibody response to alglucosidase alfa. One patient – in the 40 mg/kg/week dose group – developed an exceptionally low antibody titer of 1:250 maximum, which was only 5 times (one grade) above her baseline level of 1:50. In the 20 mg/kg eow dose group

the median peak antibody titer was 1:6,250 (range 1:1,250 – 1:31,250); in the 40 mg/kg/week dose group, it was 1:31,250 (range 1:250 – 1:156,250). The titers of the two CRIM-negative patients were in the same order of magnitude as those of the CRIM-positive patients. Whereas the peak antibody titers of patients who started ERT before the age of 2 months ranged from 1:50-1:6,250, those of patients who started ERT later ranged from 1:31,250 – 1:156,250 (Figure 4B).

Table 2 | Number, severity, and onset of infusion-associated reactions.

Patient	No of IARs possibly related to ERT (per year)	No of severe IARs	ERT duration in months (years)			Home infusions
			At first IAR	At last IAR	After last IAR	
20 mg/kg eow						
1	18 (7)	1	2.9 (0.2)	29.5 (2.5)	3.0 (0.3)	Yes
2	-	-	-	-	-	Yes
3	27 (5)	0	3.2 (0.3)	19.9 (1.7)	44.3 (3.7)	Yes
4	2 (<1)	0	24.5 (2.0)	50.3 (4.2)	0.3 (0.0)	No
5	3 (2)	1	8.1 (0.7)	17.3 (1.4)	0.7 (0.1)	Yes
6	3 (3*)	1	1.8 (0.2)	2.7 (0.2)	1.3 (0.1)	No
Total	53	3	1.8-24.5	2.7-50.3		
40 mg/kg/week						
7	2 (1)	0	1.4 (0.1)	9.4 (0.8)	24.9 (2.1)	Yes
8	70 (15)	6	0.7 (0.1)	37.6 (3.1)	19.6 (1.6)	Yes[#]
9	10 (4)	0	0.9 (0.1)	10.3 (0.9)	18.3 (1.5)	Yes#
10	5 (2)	0	9.7 (0.8)	12.2 (1.0)	17.5 (1.5)	Yes[#]
Total	87	6	0.7-9.7	10.3-37.6		

IAR: infusion-associated reaction; ERT: enzyme-replacement therapy.

* This patient experienced a total of 3 IARs over a period of 0.3 years; # These patients received three out of four infusions at home.

4 | Pharmacokinetic profile

We studied differences in the pharmacokinetics of alglucosidase alfa administrations of 20 mg/kg and 40 mg/kg by giving both doses to the same patient at an interval of one week. A 40-mg/kg infusion led to approximately twice the enzyme activity in plasma as a 20-mg/kg infusion (Figure 5). The dose did not seem to influence the plasma half-life.

Around the time that these experiments were performed, the patient's antibody titer was 1:6,250. Using a Protein-A Sepharose based precipitation method, we could not detect substantial amounts of antibody-bound alglucosidase alfa during enzyme infusion (Figure 5). Neither could we detect antibody-bound alglucosidase alfa in the plasma of three other patients who received 40 mg/kg/week and had antibody titers ranging from 1:1,250 to 1:31,250 (pts. 8, 9, and 10) at the time of investigation.

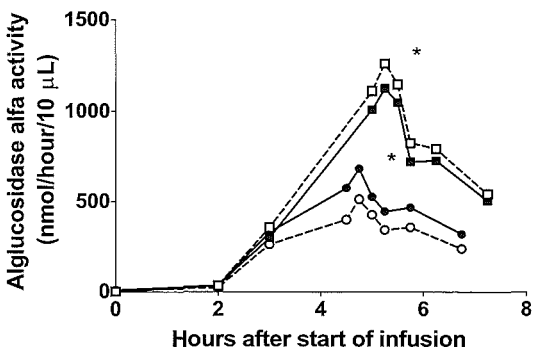

Figure 5 | Enzyme activity in plasma using doses of either 20 mg/kg or 40 mg/kg.

Blood samples were collected just before the start of infusion (0 h) and at regular time intervals thereafter. A dose of 20 mg/kg (circles) and 40 mg/kg (squares) were given to the same patient at one week's interval. Closed symbols represent total acid α-glucosidase activity in the plasma; open symbols represent the amount of activity that was not antibody-bound. The activity in the supernatant was measured with MUGlc and is expressed in nmol 4 MU liberated per 10 µl supernatant per hour. NB: Even though the enzyme-activity assay is a standardized and validated assay, there is always a slight variation in the figures obtained.

DISCUSSION

It is unquestionable that the introduction of enzyme replacement therapy has significantly improved the life expectancy of patients with infantile Pompe disease.[4-12] Nevertheless, nearly 50% of the infants treated do not survive ventilator-free.[9,10] In this study we evaluated the efficacy and safety of a higher and more frequent dosing regimen, which we hoped would improve the patients' clinical outcome. With regard to ventilator-free survival and motor outcome, we found that a dosing regimen of 40 mg/kg weekly was more effective than the

recommended dose of 20 mg/kg eow. In the 40 mg/kg/week dose group, the number of hospital admissions for respiratory infections was also notably lower.

Rationale for dose augmentation

Like other lysosomal storage disorders, Pompe disease is rare and clinically heterogeneous, a fact that limits the scope for extensive clinical trials and dose-finding studies. In the very first enzyme-therapy study for infantile Pompe disease, which used recombinant human α-glucosidase from rabbit milk, we used a weekly dosing regimen of 15 to 20 mg/kg, which was later increased to 40 mg/kg/week when one of the infants did not respond optimally.[4] The doses were based on studies in a mouse model of Pompe disease,[21] which had indicated that the uptake of enzyme by skeletal muscle is a dose-dependent process that is linear up to at least 80 mg/kg.[25] To partially correct enzyme activity in skeletal muscles, a minimum dose of 20 mg/kg was required; for full correction, 40 mg/kg or more were required.[21,25] This was supported by our findings in infants.[4,5] Similar results were obtained in studies with recombinant human α-glucosidase from CHO cells in mice; these showed an incremental uptake with doses ranging from 20 – 100 mg/kg.[22,32,33] However, it should be noted that even the highest dose did not prove sufficient to clear the lysosomal glycogen completely.[22,34,35]

Although different doses have been used in clinical trials with alglucosidase alfa (ranging from 10 mg/kg/week to 40 mg/kg/eow),[8-11] only one study has compared the safety and efficacy of two different doses.[9,10] In this study, 18 infants were enrolled, half of whom received 20 mg/kg eow, and half 40 mg/kg eow. Up to three years of age, the two dose groups did not differ clearly with regard to efficacy or safety.[10]

The theory behind dose augmentation

Since a weekly dosing regimen had not been tested in pivotal trials, we composed a theoretical model to demonstrate the consequences of weekly dosing versus dosing eow for both the 20 mg/kg and 40 mg/kg dose groups (Figure 6). For the sake of simplicity, the intra-lysosomal half-life of alglucosidase alfa was set at 7 days on the basis of the 2 to 9 day-intracellular half-life reported for acid α-glucosidase species in various tissues including cultured skeletal muscle cells of Pompe patients and skeletal muscle from Pompe mice.[23-28] We made two other assumptions: 1) To prevent symptoms, a level of at least 25% of average normal acid α-glucosidase activity is required (referred to below as the "critical threshold"). This assumption is based on the fact that symptoms do not present if the residual acid α-glucosidase activity is more than 25% of average normal[1,36]; 2) The uptake of alglucosidase alfa is dose-dependent in the range from 10 to 100 mg/kg.[4,22,25,32,33]

Figure 6 depicts the model, which applies four different dosing scenarios: 20 and 40 mg/kg eow (Panel A and B), and 20 and 40 mg/kg weekly (Panel C and D). In the model it is assumed that, in theory, a single dose of 20 mg/kg reaches the critical threshold of 25% of normal activity in muscle, and that a single dose of 40 mg/kg reaches 50% of normal. Panels A-D show that the level of the dose (20 or 40 mg/kg) and the frequency of dosing (once every week or once every two weeks) affect the maximal activity levels reached in the muscle. Surprisingly, the model reveals that the cumulative effect of repeated dosing subsides within 8 weeks. In the 20 mg/kg eow dosing scenario, while enzyme-activity level in the muscle is, at best, 33% of average normal (Panel A), it is below the critical threshold most of the time between infusions. In the weekly dosing scenario of 20 mg/kg, the situation is better: at best, enzyme activity in the muscle is 50% of average normal, falling back to the level of the critical threshold by the time the next infusion is given. While a dose of 40 mg/kg eow results in a slightly higher peak enzyme-activity level of 66%, activity in muscle now drops below the critical threshold for about 25% of the time, during which glycogen might start to re-accumulate. Only if the 40 mg/kg/week dosing scenario is applied do activity levels clearly remain consistently above the critical threshold, fluctuating between 50 and 100% of normal.

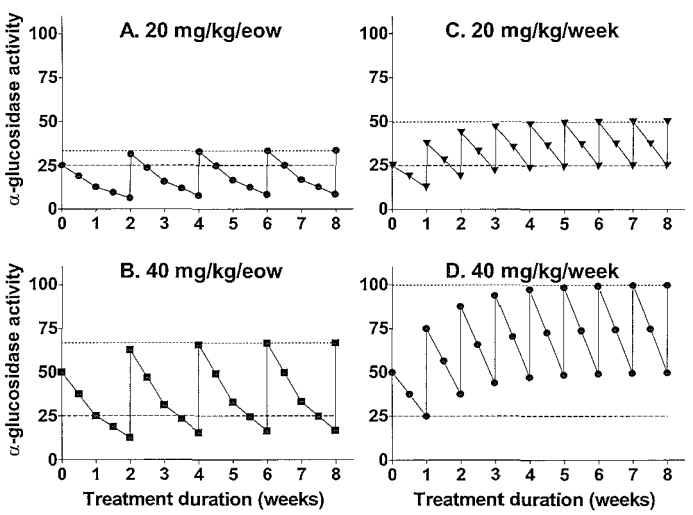

Figure 6 | Theoretical model showing the increment of acid α-glucosidase activity in skeletal muscle using four different dosing regimens

Panels A and B show the predicted intracellular activity in percentage of normal that is assumed to be achieved with doses of either 20 or 40 mg/kg eow based on the theoretical model; panels C and D show the same for doses of 20 and 40 mg/kg weekly. The model is based on a half-life of 7 days for acid α-glucosidase in skeletal muscle, and on the assumption that 25% of normal activity is achieved with a single doseof 20 mg/kg and that 50% is produced with a single dose of 40 mg/kg.

On the basis of this modelling, we conclude that weekly dosing leads to less fluctuation in intramuscular enzyme activities than dosing every other week. Theoretically, the 40 mg/kg/week regimen provides the far best scenario.

Our model is clearly limited in that some factors with great impact are uncertain, including the uptake efficiency of the recombinant enzyme and its half-life in skeletal muscle in patients *in vivo*. Studies in mice report varying enzyme activity levels in skeletal muscle after the administration of different doses of alglucosidase alfa; for example, a dosing regimen of 100 mg/kg achieved 33 – 44% of the normal activity level,[22] a regimen of 20 mg/kg twice a week achieved 28-100%,[34] another form of human GAA--transgenic enzyme constitutively produced in liver and secreted into the bloodstream of knockout mice (Gaa-/- a dose of 20 mg/kg/week achieved 10 to 20%,[22] while a study applying 20 mg/kg eow measured enzyme activities between 25 and 50% of normal.[33] If the enzyme activities reached with 20 and 40 mg/kg in human skeletal muscle are in fact higher or lower than assumed in our theoretical model, the effect of the dosing regimens will be different, changing either for the better or the worse.

Our decision to increase the dose from 20 mg/kg eow to 40 mg/kg/week was not based on this predictive model, but on the insufficient response of individual patients to ERT. The model provides us with a rationale.

40 mg/kg/week seems more effective than 20 mg/kg eow

Though the follow-up is still rather short and differs between patients, overall and ventilator-free survival in patients treated with 40 mg/kg/week appeared to be better than in those treated with 20 mg/kg eow. If three years of age is taken as a reference point, half of the patients in the 20 mg/kg eow dose group had become ventilator dependent or had died – a proportion similar to that in the pivotal trial in which patients were treated with 20 or 40 mg/kg eow.[9,10] In contrast, all four patients treated with 40 mg/kg/week in our study survived ventilator-free. The most notable contrast was the difference in overall clinical condition, which was reflected in the difference in hospital admissions for the two groups: while none of the patients treated with 40 mg/kg/week had ever had respiratory infections requiring hospitalization, 5/6 patients treated with 20 mg/kg eow required frequent readmissions or continuous hospitalization. Consequently, three of these patients developed respiratory insufficiency at the age of 2.7 years or younger.

Similarly, motor function appeared to be better in the 40 mg/kg/week dose group, all of whom learned to walk, and maintained the ability to so, unlike 4/6 patients treated with

20 mg/kg eow, only 2/6 of whom could still walk at the end of the study. In all cases, the loss of motor milestones in the 20 mg/kg eow dose group was preceded by infections requiring hospital admissions. Our study results suggest that the 40 mg/kg/week dosing regimen helps to stabilize or improve the respiratory condition of affected infants better than the 20 mg/kg eow dosing regimen.

With regard to cardiac hypertrophy, both dose regimens worked equally well, which is explained by the fact that a lower dose is required to correct or prevent cardiac hypertrophy.[4,8,21,22] For the same reason, adults with Pompe disease with residual α-glucosidase activities of up to 25% do not generally develop hypertrophic cardiomyopathy, while they do have skeletal muscle weakness.[1]

It should also be noted that response varies between patients treated with the same dose. This is illustrated by one of the six patients treated with the lower dose of 20 mg/kg eow, who performed extremely well until the end of the follow-up at the age of eight years.

Safety issues

Although we observed no clear differences in safety parameters between the two dose groups, the small numbers do not allow us to draw firm conclusions. While similar numbers of patients in each dose-group experienced IARs, the overall number of IARs was higher in the 40 mg/kg/week dose group, largely because a single patient had 50% of the total number of IARs. A similar pattern was observed in the pivotal trial.[9,10] The patient with most IARs in our study had recurrent episodes of exanthema, coughing and vomiting, occasionally accompanied by saturation drops. Remarkably, the IARs started within minutes of the start of the infusion, when the infusion rate was still slow. Total IgE, serum tryptase and complement levels were within the normal range. While this patient had a relatively high sustained antibody titer, the titer was similar to that of other patients who did not develop as many IARs. At the time of writing, the patient was receiving home-based enzyme therapy without problems.

It is well recognized that therapeutic proteins can induce an immunological response that neutralizes the effect of ERT. Three of the four patients treated with 40 mg/kg/week and two of the six treated with 20 mg/kg eow developed a peak antibody titer of 31,250, which was estimated to be the highest titer without significant consequences for ERT (van Gelder, et al. unpublished results). Using pharmacokinetic studies in the present study, we could not detect substantial amounts of antibody-bound alglucosidase alfa during enzyme infusion in patients whose antibody titers ranged from 1:6,250 to 1:31,250. One patient receiving 40 mg/kg/week had a peak antibody titer of 1:156,250, which later declined to 1:31,250. According to earlier

estimates, as much as 54% of the administered enzyme (about 10 mg/kg) is antibody-bound at a dose of 20 mg/kg and a titer of 1:156,250 (van Gelder, unpublished results). If a similar amount (10 mg/kg) were bound upon administration of 40 mg/kg, about 30 mg/kg would theoretically still be available for uptake in the target tissues.

Overall, we found no apparent correlation between the level of antibodies and the dose of ERT, although patients treated with 40 mg/kg/week tended to develop higher antibody titers than those receiving 20 mg/kg eow. This is consistent with a previous study that compared the level of antibody titers between patients treated with 20 or 40 mg/kg eow.[37] In line with previous observations ([38] and van Gelder, et al. unpublished results), the patient's peak antibody titer seemed to be related to the age at start of therapy.

CRIM status

Since the two CRIM-negative patients both received 20 mg/kg eow, and since CRIM-negative patients have been reported to have poorer outcome than CRIM-positive patients,[39] the CRIM-negative status per se might have contributed to the worse outcome of this dose group. However, exclusion of these patients from analysis shows that the number of patients who became ventilator dependent, required frequent hospital admissions, and did not learn to walk was still higher in the 20 mg/kg eow dose group than in the 40mg/kg/week dose group.

Residual disease

It should be noted that patients treated with 40 mg/kg/week also had minor motor problems such as facial-muscle weakness, and weakness of the neck flexors and ankle dorsiflexors. As glycogen also accumulates in neural tissues, including motor neurons of the spinal cord and peripheral nerves,[40,41] we cannot exclude the possibility that neurological damage plays a role as well.

CONCLUSIONS

Although this study is limited by its small sample size and limited follow-up duration, our preliminary data suggest that a dose of 40 mg/kg/week improves ventilator-free survival and motor outcome over that brought by the currently recommended dose of 20 mg/kg eow. More studies are recommended to evaluate the effects of weekly dosing regimens in larger cohorts of patients.

ACKNOWLEDGEMENTS

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CHAPTER 9

Severely impaired health status at diagnosis of Pompe disease: A cross-sectional analysis to explore the potential utility of neonatal screening

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ABSTRACT

Since the introduction of enzyme replacement therapy for Pompe disease, awareness and early diagnosis have gained importance. Because the therapy is most effective when started early and methods for dried bloodspot screening for Pompe disease are currently being explored, neonatal screening is getting increased attention. The objective of this study was to investigate the gains that might be achieved with earlier diagnosis by neonatal screening. For this purpose we analyzed the health and functional status of non-screened patients with Pompe disease at the time of diagnosis.

Previously collected clinical data and results of an international patient-reported questionnaire were used. Cross-sectional data of 53 patients with Pompe disease diagnosed between 1999 and 2009 (aged 0 – 64 years) were analyzed. According to the World Health Organization's International Classification of Functioning, Disability and Health the following domains are described: body function, activity, participation and contextual factors. In all patients with classic infantile Pompe disease cardiac function, hearing, muscle strength and motor development were considerably impaired at the time of clinical diagnosis. The use of oxygen and/or nasogastric tube-feeding was reported in more than 70% of these cases. Most children, adolescents and adults had advanced muscle weakness and impaired respiratory function at the time of their diagnosis, causing varying degrees of handicap. About 12% of them used a walking device and/or respiratory support at the time of diagnosis.

The severely impaired health status reported here provides a strong argument for earlier diagnosis and to further explore the potential of neonatal screening for Pompe disease.

1 | INTRODUCTION

Pompe disease, or glycogen storage disease type II [OMIM ID: 232300], is an autosomal recessive lysosomal storage disorder. It is caused by a deficiency of acid alpha-glucosidase (GAA) [EC 3.2.1.20] which leads to glycogen accumulation in the lysosomes, predominantly resulting in progressive weakening of the muscles. The frequency of Pompe disease in the Netherlands is about 1/40,000.[1] The genotype-phenotype correlation is largely understood in that the nature of the mutations in both *GAA* alleles determines the degree of enzyme deficiency, but secondary genetic factors or non-genetic factors also contribute to the broad clinical spectrum of phenotypes seen within Pompe disease.[2] Generalized hypotonia, respiratory difficulties and cardiomyopathy manifesting in the first months of life are characteristic features of the so-called classic infantile form of Pompe disease. Without treatment these patients die within the first year of life due to cardiorespiratory failure.[3] Other patients with Pompe disease have symptoms that can occur at any age, from early childhood until the sixth decade, and typically do not suffer from cardiomyopathy. In these patients the disease is characterized by a less progressive limb-girdle myopathy and decreased pulmonary function. When untreated these children, juveniles and adults with Pompe disease may become wheelchair-dependent or in need of respiratory support. Respiratory insufficiency is the major cause of death in these patients.[2,4]

Enzyme replacement therapy (ERT) with recombinant human acid alpha-glucosidase (alglucosidase alfa), the first available treatment for Pompe disease, received market approval in Europe and the United States in 2006. Although not all patients respond equally well, the outcomes of treatment are promising. Previously published studies among patients with classic infantile Pompe disease (n=18, aged 1 – 6 months) showed a prominent effect of ERT on cardiac hypertrophy and function and a substantial effect on survival. However, 4 of the 18 studied patients died before the age of 36 months and half of the studied patients required invasive ventilation at age 36 months, at the time of death or by the end of the study.[5] In children and adults with Pompe disease (n=60, aged 15 – 70 years) treatment with ERT is associated with improved walking distance and stabilization of pulmonary function over an 18-month period.[6,7] Clinical trials indicate a better clinical outcome if ERT is started early in the course of the disease.[5,8,9] Due to rarity of the disease and the variation in disease presentation, the diagnosis of Pompe disease is often considerably delayed.[2,4] In the Netherlands, the median age of symptom onset of classic infantile Pompe disease has been earlier described as 1.6 months, whereas the median age of diagnosis was around 5 months of age.[3] For the less

progressive phenotypes a median doctors' delay of 7 years has been described.[4,10] Diagnosing patients in an earlier stage of the disease, or even before onset of symptoms, enables early intervention which could prevent or postpone further health damage.

The most promising option to enable earlier diagnosis is neonatal screening. It is conceivable to integrate the screening for Pompe disease into current neonatal screening programs. Recently it has been shown in Taiwan that nationwide testing of GAA-activity in dried bloodspots substantially reduced the diagnostic delay for infants with classic infantile Pompe disease.[11] This screening program confirms that early diagnosis and subsequent early treatment in babies with classic infantile Pompe disease could improve prognosis.[5,12,13] An important disadvantage of the currently available blood-spot based tests is that at present they cannot distinguish between classic infantile Pompe disease and later-onset phenotypes. This distinction can only be made by clinical follow-up and more extensive laboratory testing, which means that a positive bloodspot test could inform parents not only about the fact that their newborn child will develop classic infantile disease but also about the fact that their newborn child is likely to develop symptoms of Pompe disease at an unpredictable time-point later in life.[14] The early detection of adult-onset diseases is not a goal of current neonatal screening programs, although in the case of Pompe disease timely diagnosis could enable doctors to carefully follow their patients and install ERT promptly when indicated.

To explore the potential of a screening test, analytic validity, clinical validity, clinical utility and ethical, legal and social implications need to be reviewed.[15] It is already known that including Pompe disease in neonatal screening programs without being able to filter for classic infantile Pompe disease raises many social, ethical and legal issues.[16,17] Analytic validity of various methods is being compared elsewhere[18,19] and information on clinical utility will evolve from experiences with implemented programmes. An important step in investigating the clinical utility of neonatal screening for Pompe disease is to get better insight into the health damage which could potentially be averted or delayed by earlier diagnosis.

This report aims to quantify the health and functional status of both patients with classic infantile Pompe disease and patients with a less progressive phenotype at the time of diagnosis. It discusses to what extent patients with Pompe disease may be prone to health and functional damage for various domains (e.g. clinical parameters and limitations in activities) in order to explore the potential benefits of neonatal screening and subsequent early interventions for future patients with Pompe disease.

2 | METHODS

In this study a retrospective inventory was made of the documented health and functional status of patients with Pompe disease within a year after diagnosis. Prospectively collected data from previously conducted studies on natural course and effectiveness of ERT (e.g. [4,7,20,21]) were used to select relevant information. These data include clinical data from patient records and results from the IPA/Erasmus MC Pompe survey, an international patient reported questionnaire study.[4] A confirmed diagnosis of Pompe disease by enzyme activity assay and/or DNA analysis was the main inclusion criteria. Patients with the classic infantile form characteristically had virtually no enzyme activity and completely deleterious mutations on both alleles, while children and adults with late onset variants expressed at least one milder mutation. Further inclusion criteria for the present analysis were i) date of diagnosis between 1999 and 2009 and ii) less than 1 year between date of diagnosis and date of first visit at the Erasmus MC University Medical Center. These criteria were chosen to ensure adequate availability of patient records. Data were excluded when collected or referring to a moment more than a year after the year of diagnosis (for children and adults) or a moment more than 2 weeks after start of ERT (for classic infantile patients). Outcome measures of this study were chosen on the basis of the most apparent phenotypical characteristics of Pompe disease, which lie in the physical health domains of quality of life.[22] The selected data were used to describe patients' body function and structure (impairments), activity (limitations), participation (restrictions) and some contextual factors, following the International Classification of Functioning, Disability and Health.[23]

2.1 | Body function and structure impairments

The cardiac function and cardiac dimension of patients with classic infantile Pompe disease were examined by 2D M-mode echocardiography according to recommendations of the American Society of Echocardiography. The left ventricular mass index (LVMI) was used as a measure for hypertrophic cardiomyopathy and was expressed as the number of standard deviations from normal mean (z-scores).[24]

The hearing loss of patients with the classic infantile variant was evaluated by auditory brainstem-evoked responses, oto-acoustic emissions, and impedance audiometry as previously described.[21]

To determine the degree of glycogen accumulation in the muscles and muscle damage of patients with classic infantile Pompe disease muscle biopsies from the M. quadriceps were

used. Semi-quantitative data were obtained by histological examination with PAS-staining and evaluating the muscle fibers for their number of contractile elements. Muscle fibers were considered normal when surface area contained more than 75% contractile elements (see also [25]).

Skeletal muscle strength of children and adults was measured by manual muscle testing using the Medical Research Council (MRC) grading scale (range 0 – 5, 20 muscle groups tested (see for details Figure 3))[26] and Hand-Held Dynamometry (HHD) (13 muscle groups tested (see for details Figure 2)) (Cytec dynamometer, CIT Technics, Haren, the Netherlands). The value measured by HHD (Newton) of each muscle group was expressed as percentage of the age- and sex-matched reference values (for children no reference value available for neck extensors).[27-29]

The pulmonary function at the time of diagnosis was assessed by analysing data on forced vital capacity (FVC). FVC was measured in upright seated and supine position using a Lilly type pneumograph (Viasys Healthcare, Würzburg, Germany) or the KoKo spirometry system (Ferraris Respiratory, Louisville, CO, USA). Measurements were performed according to ATS/ERS standards[30] and reference values were derived from published data.[31] Results were expressed as percentage of the predicted normal value.

2.2 | Activity limitation

Activity limitation of classic infantile patients was assessed by the Alberta Infant Motor development Scale (AIMS).[32] The AIMS score is a percentile score of the gross motor development, which depicts the child's development.

To assess the muscle function of older children and adults, the Quick Motor Function Test (QMFT) was used. This test describes to what extent patients experience difficulty with sixteen different actions (e.g. sit-ups, jumping etc.). Scores per action can range from 0 (not possible, or not executed for other reason) to 4 (completed action).[33]

2.3 | Participation restriction

The Rotterdam Nine Items Handicap Scale (RHS) is used to assess the self-perceived ability to perform certain everyday tasks. Scores per task can range from 0 (not applicable) or 1 (not able to perform task) to 4 (perceiving no difficulty with task). When calculating the percentage of patients having problems with a certain task, scores of zero are excluded.[34]

2.4 | Contextual factors

Information on the use of walking devices, ventilators, or feeding support was collected from the patients' medical files and the IPA/ Erasmus MC Pompe survey.[4]

2.5 | Statistical analysis

Data were analyzed using descriptive statistics in SPSS for Windows (version 15.0, SPSS inc., Chicago, IL, USA).

3 | RESULTS

Data of 11 patients with classic infantile Pompe disease (median age at diagnosis: 1 month, range: 3 – 180 days), 13 affected children (median age at diagnosis: 10 years, range 0 – 16 years) and 29 adult patients (median age at diagnosis: 43 years, range: 24 – 68 years) met the inclusion criteria (see Table 1). There was an even gender distribution in this study population (not shown). Median time between diagnosis and date of collection of the data of patients with classic infantile Pompe disease was 12 days. Median time between first symptoms and diagnosis of the children in this study was 1 year (range 0 – 13) and in the adult patient group 8 years (range 0 – 43). Not all the selected parameters were available for all patients. Table 1 provides an overview of the age of the patients and number of patients included per test.

3.1 | Classic infantile patients (n=11)

At the time of diagnosis the left ventricular mass index (LVMI) of the 11 patients with classic infantile Pompe disease ranged from 98 to 599 g/m2 with a median of 231 g/m2, with reference values of 59.2 g/m2 (normal mean), SD 7.9.[24] This shows that even the lowest LVMI found in the study population already deviates 4.9 times the standard deviation from normal mean. Systolic function was depressed in four patients.

Data on hearing from before or within a maximum of 10 days after start of ERT were available for all eleven patients with classic infantile Pompe disease. Nine out of the 11 patients described around diagnosis had sensoneuronal hearing loss, with estimated hearing thresholds ranging from 10 – 90 dB. The nature of the hearing loss was mainly cochlear, although some patients also showed impaired middle-ear function, resulting in variable degrees of conductive hearing loss.[21]

Table 1 | Available patient records: number and age of patients per test.

Patient group	Classic infantile	Affected children	Affected adults
n	**11**	**13**	**29**
Age: median (range)[a]	1 (0.1-6.0) m	10 (0-16) y	43 (24-68) y
Test			
Heart dimension (LVMI)	**11** 1 (0.1 – 6.0)		
Hearing loss (BERA)	**11** 1 (0.1 – 6.0)		
Muscle condition (biopsy)	**9** 1 (0.3 – 6.0)		
Motor development (AIMS)	**11** 1 (0.1 – 6.0)		
Muscle strength (HHD)		**8** 13 (8 – 16)	**29** 43 (24 – 68)
Muscle strength (MMT)		**5** 12 (4 – 14)	**29** 43 (24 – 68)
Pulmonary function (FVC)		**8**[b] 14 (10 – 16)	**29** 43 (24 – 68)
Muscle function (QMFT)		**8** 13 (4 – 16)	**29** 43 (24 – 68)
Handicaps (RHS)			**22** 44 (34 – 64)

[a] For classic infantile patients the median age and age range in the group is described in months (m), for children and adults in years (y).

[b] FVC in sitting position. In supine position FVC was measured of 7 children (mean age: 13 y, range: 7 – 16)

Muscle biopsies of nine infants with classic infantile Pompe disease were taken at the time of diagnosis. Analysis of the biopsies revealed that the samples contained 3% – 77% normal fibers when evaluated for the preservation of contractile elements, with a median of 49%. Figure 1A depicts the percentage-wise distribution of normal muscle fibers in the biopsies.

Motor development of all 11 infants was assessed at the time of diagnosis and all of them showed severe hypotonia and head lag. The percentile scores of the AIMS of the patients ranged from p1 to 50, with a median percentile score of 2. Five patients showed severe motor development delay (percentile score ≤5). The distribution of the percentile scores is depicted in Figure 1B.

At the time of diagnosis 4 of the 11 infants required supplemental oxygen and 7 required (partial) nasogastric tubefeeding.

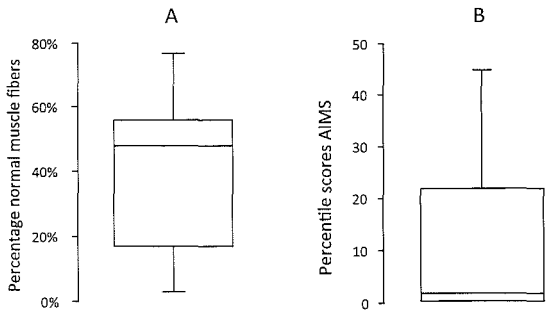

Figure 1 | Percentage normal muscle fibers in biopsies (A) (n=11)* and AIMS percentile scores (B) (n=11) of patients with classic infantile Pompe disease at the time of diagnosis.

* Fibers with more than 75% surface area covered by contractile elements are considered normal.

3.2 | Children (n=13) and adults (n=29)

The youngest child with Pompe disease without cardiac involvement was diagnosed directly after birth, due to an affected sibling. Of this patient only an AIMS-score (at the age of 3 months) was available from the tests selected for this research. The percentile score of this child was 5, which indicates a delay in motor development.

Data on hand-held dynamometry (HHD) around the time of diagnosis were available for 8 children (age 7 – 16 years) and all 29 adults (age 24 – 68 years). In 2 children the first HHD was performed more than 1 year after diagnosis and these data were therefore excluded. For 3 children (aged 0 – 2 years) no HHD data were available because they were too young to perform the test. The median HHD scores per muscle group were ≤80% of the reference value for 4 muscle groups (hip abductor, knee flexor, hip flexor and shoulder abductor). The HHD scores for the individual muscle groups are depicted in Figure 2 as percentages of age- and sex-matched reference values (100%).

The manual muscle test (MMT) was performed in 29 adult patients and 5 affected children (age 4 – 14 years) at the time of diagnosis. Using the MRC scale, more than 50% of the patients had lost strength in the shoulder abductors, adductors and exorotators as well as the hip abductors, adductors and extensors (Figure 3). The lowest MRC score that was reported for a muscle group in our study population was 2 (only horizontal movement possible).

Data on the forced vital capacity (FVC) in both sitting and supine position at the time of diagnosis were available of 7 children and all 29 adults. FVC of one additional child was measured

only in sitting position. Data of 2 children were excluded because pulmonary function was measured more than 1 year after diagnosis. No data were available on the pulmonary function of five children for various reasons, mainly because they were too young to perform the test. In sit- ting position the median FVC at diagnosis was 92% of normal (range 16% – 140%), while the median FVC in supine position was 77% (range 20% – 124%). In supine position the FVC was 80% or below (pathological threshold) for 18 adults and 3 children (58.3% of the tested patients).

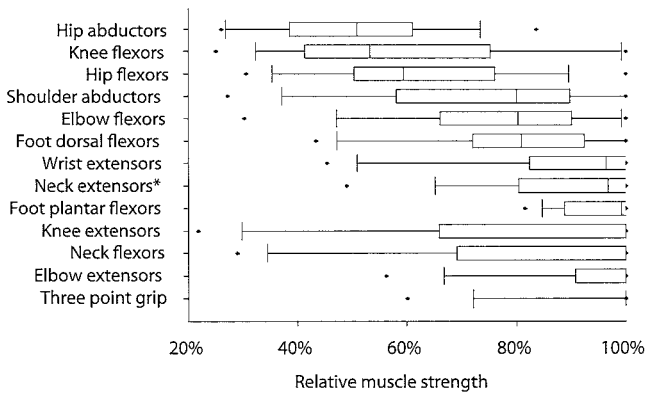

Figure 2 | HHD scores per muscle group of 8 children and 29 adults with Pompe disease at the time of diagnosis.

Percentage of normal value of age- and sex-matched normal population. (Dots depict minimum and maximum percentages). * For children no reference value for neck extensors was available.

Data from the Quick Motor Function Test (QMFT) performed at the time of diagnosis of 8 children and all 29 affected adults with Pompe disease were available. Not included were data of 3 children (aged, 2, 5 and 8 years) because the QMFT was only documented from measurements more than 1 year after diagnosis. Of two children with Pompe disease no data on the QMFT were available. The data show that at diagnosis more than 80% of them had difficulty with executing a sit-up from lying position or succeeded only partly. More than 50% of them had difficulties with or could only partly execute the following actions (scores under 4): getting up from a squat, or from one knee, squatting, jumping, stretching the legs in supine position, getting up from a chair, raising the torso from prone position, walking ten meters, picking up an object from the ground when standing or flexing the hip or knee in supine position. More than 40% of the patients described had difficulty with or could not stand on one leg and/or walk stairs. An overview of the results of the QMFT is depicted in Figure 4.

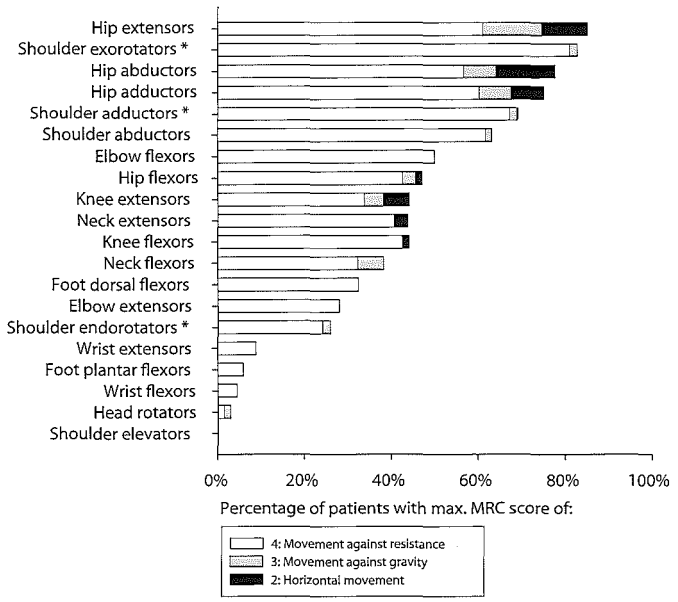

Figure 3 | Percentage of patients with loss of strength in different muscle groups at the time of diagnosis. MRC was measured in 20 muscle groups in adults and in 17 muscle groups in children.

* Shoulder adductors and shoulder endo- and exorotators have not been evaluated in children.

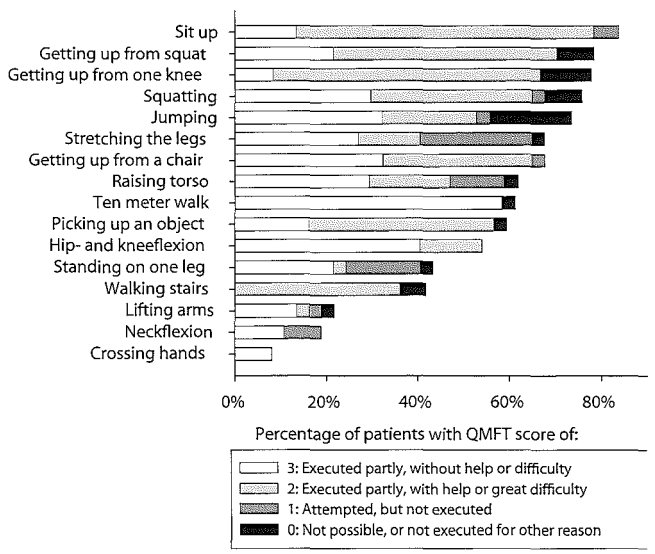

Figure 4 | Percentage of affected children and adult patients with any difficulty executing different tasks at the time of diagnosis.

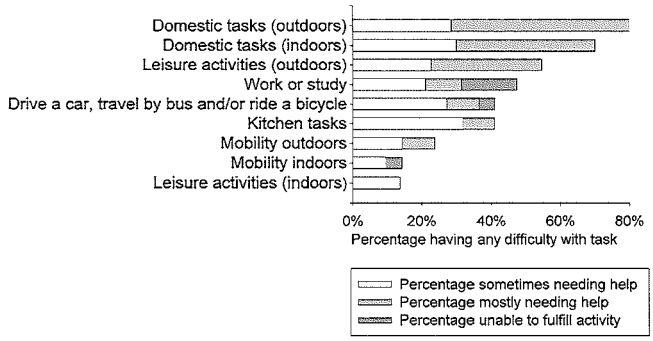

Figure 5 | Rotterdam Handicap Scale results of adults at the time of diagnosis.

Data of 22 adults on the Rotterdam Handicap Scale were available and met the inclusion criteria (Figure 5). More than 70% of them at least sometimes needed help with domestic tasks indoors and outdoors. More than 40% reported that they had difficulty with driving a car, traveling by bus and/or riding a bicycle and/or needed help with leisure activities outdoors; 15.8% of the patients (aged 34 – 64 years, median 44) reported that they were unable to fulfill their work or study at the time of diagnosis.

At time of diagnosis two adult patients (aged 43 and 64 years) were using a wheelchair and four adult patients (aged 36 – 64 years) were using non-invasive ventilation.

4 | DISCUSSION

The delay in diagnosing Pompe disease was until now only described in terms of time i.e. number of years.[3,4,10] Our study reporting on the condition of the patients at the time of diagnosis provides insight into the damage, which might be averted by early diagnosis and effective intervention. The data presented in this report reveal that patients with Pompe disease are significantly impaired in body function and structure, limited in activities, restricted in participation and in some cases dependent on respiratory, walking and/or feeding support already at the time of diagnosis.

The reported substantial loss of muscle fibers retaining normal contractile elements in classic infantile patients and low AIMS scores are particularly noteworthy, because the effect of ERT depends on preservation of intact muscle fibers and consequently residual muscle function at the start of treatment.[8,24,35] Also the life-threatening cardiomyopathy found in

these patients at diagnosis, which is known to normalize when treated promptly[13] and the hearing loss which is irreversible[21] emphasize the need for earlier diagnosis in patients with classic infantile Pompe disease. This study however also shows significant loss of muscle strength at the time of diagnosis of children and adults with Pompe disease and consequently the loss of muscle function. Eighty percent of the patients had problems with executing a sit-up, 50% with getting up from a chair and 40% had difficulty with walking stairs at the time of diagnosis. The functional limitations had also significant consequences for daily functioning. At the time of diagnosis 80% experienced problems with domestic tasks outdoors, while almost 50% experienced limitations in performing their work or study. The fact that more than 20% of the adult patients were already either wheelchair bound and/or required respiratory support stresses the consequences of the delayed diagnosis. Part of these problems might have been prevented if patients were diagnosed and treated with ERT earlier.[7,20] Given the progressive nature of Pompe disease, rapid in patients with classic infantile Pompe disease and slower in other patients, early diagnosis is in all cases expected to be associated with less tissue pathology, better preserved muscle function and fewer limitations in daily life at the start of treatment. Altogether the results of this study form a strong argument to advocate for earlier diagnosis for the whole spectrum of Pompe disease.

The patients described in this study include all patients seen in the Erasmus MC University Medical Center diagnosed between 1999 and 2009 who met the inclusion criteria. The median age at diagnosis of patients with classic infantile Pompe disease in this study was 30 days (range: 3 – 180 days). Since the literature[3] describes a longer diagnostic delay than in our study-group this may mean that awareness has been raised during recent years resulting in earlier diagnosis or that our results may not be representative for all patients with classic infantile Pompe disease at diagnosis. However, because the reason for the earlier diagnosis in the current group is unknown, our results could be an under- or overestimate of the health and functional status of patients with classic infantile Pompe disease in general. Six children in our study group did not perceive any neuromuscular symptoms before being diagnosed. They were tested for Pompe disease following the diagnosis of a sibling or acting on findings from tests for other purposes indicating muscle or liver pathology. Therefore the time between first symptoms and diagnosis in this group (of 13 children) is rather broad and ranges between 0 and 13 years, with a median of 1 and a mean of 2.5 years. The diagnostic delay in our adult patient group (median delay 8 years, range 0 – 43 years) is similar to what has been described in the literature.[4,10] Most missing data are assumed to be missing at random, so we presume they do not bias our conclusions. Unfortunately data on pulmonary and muscle function and

muscle strength are missing for three of the five affected children under 5 years of age without cardiomyopathy, due to their inability to adequately perform the selected tests at a young age.

Neonatal screening seems an obvious choice to ensure the earliest diagnosis, mainly because of the bloodspot screening system that is already in place for other diseases in most developed countries. However, the currently available screening techniques for Pompe disease on blood spots do not discriminate between patients with classic infantile Pompe disease and less progressive variants of the disease.[14,19] Consequently, not only babies who need immediate treatment will be identified, but also seemingly healthy infants who will manifest symptoms at unpredictable age. For the latter patients and their parents this implies a constant awareness of a pre-symptomatic stage of the disease, while some cases may remain asymptomatic until the age of 70. Possible consequences for these so-called "patients in waiting"[36] have been discussed elsewhere[16,17] as well as views of the public on this dilemma.[37] Most of the current neonatal screening programs are aimed at detecting conditions that require immediate treatment. In this respect finding late-onset cases would be an unsought effect of the screening. This study, however, suggests that not only future patients with disease manifestations at very young age but also patients who would experience the first signs of disease much later in life might benefit from earlier diagnosis, as it allows for earlier intervention and presumably better treatment results. Consequences of early diagnosis for patients at the opposite ends of the clinical spectrum clearly need to be further explored.

5 | CONCLUSION

This study quantifies the health and functional status of patients with Pompe disease and clearly shows that this is already severely impaired at the time of diagnosis. This is a strong argument to advocate for earlier diagnosis and to further explore the potential of neonatal screening.

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CHAPTER 10

General discussion and future perspectives

Pompe disease is a neuromuscular disorder that was fatal prior to enzyme-replacement therapy (ERT). Since ERT eradicates the life threatening cardiomyopathy and minimizes other symptoms it prolongs survival and poses the new challenge of how to optimally care for the long-term survivors. Not all infants respond equally well to ERT and virtually all treated infants exhibit various extents of residual disease. New features informative for understanding the pathophysiological process reveal themselves. Prognostic factors that improve the therapeutic outcome need to be identified, and possibilities to improve the effect of ERT need to be explored.

The aims of this thesis were 1) to delineate the long-term outcome of patients with classic infantile Pompe disease treated with ERT, 2) to identify factors predictive for therapeutic outcome, and 3) to explore the effect of a higher and more frequent dosing regimen and early start of treatment by neonatal screening.

The most important findings in patients with classic infantile Pompe disease are:

- As demonstrated before in many peer reviewed publications from many different centers, our experience also is that ERT prolongs the life of patients with classic infantile Pompe diseases significantly.
- ERT reverses the life threatening cardiomyopathy.
- Emerging features in long-term survivors are: delayed motor development, impaired motor function, facial-muscle weakness (including ptosis), speech disorders, and dysphagia. Severe ptosis, extra-ocular motility disorder, and myopia were collectively identified in one long-term survivor.
- The emerging musculoskeletal phenotype of treated infants differs from the phenotype of children and adults with Pompe disease.
- The cognitive development of treated infants ranges between normal and mildly delayed, the oldest patient being assessed at 12 years of age.
- Antibody formation to ERT is common in classic infantile Pompe disease, and high antibody titers counteract the effect of treatment. A CRIM-negative status itself seems associated with a poor treatment outcome.
- Higher and more frequent dosing (40 mg/kg weekly) than recommended for ERT is well tolerated and seems to improve ventilator-free survival and motor outcome.
- Early start of ERT in classic infantile Pompe disease improves the therapeutic outcome; most Pompe patients have a severely impaired health status at the time of diagnosis.

In this **Chapter**, the main findings of our studies are reviewed and their implications for clinical practice are discussed along with future perspectives.

6.1 | The emerging phenotype of classic infantile Pompe disease

The beneficial effects of ERT in classic infantile Pompe disease have been widely addressed,[1-9] while the extent and consequences of residual disease received less attention. As the treatment period and the number of long-term survivors increases, new features of the disease are gradually emerging. We have focused our attention on the extent and consequences of residual muscle weakness, and on the cognitive development of long-term survivors assuming that ERT does not cross the blood-brain barrier,[10] and the storage of glycogen in the CNS may proceed.

From the data presented in **Chapter 3**, **4**, and **5** we conclude that all patients show signs of residual skeletal muscle weakness including the facial and bulbar muscles. ERT enabled the vast majority of the patients in our cohort to achieve previously unmet motor milestones, including walking. However, their motor development was often delayed and significant motor coordination difficulties were present in all school aged children. In general, after the first two to three years of life, their performance declined over time, whereas healthy age-matched peers perform better and better with age. Some patients lost motor skills after severe respiratory infections. The motor skills of others deteriorated probably due to an imbalance between their muscle strength and an increase of body mass and height. The latter is often encountered in neuromuscular disorders. Typically, at the time of diagnosis, the muscle weakness was more pronounced in the proximal muscles than in the distal muscles. Over time, however, distal muscle weakness became more marked. Profound weakness of the anterior tibialis with complete absence of ankle dorsi-flexion has also been reported by others.[11] The legs are typically more involved than the arms, but some treated infants developed substantial weakness of the finger extensors.

Facial-muscle weakness, speech disorders and dysphagia appeared to be common in classic infantile Pompe patients receiving ERT, even in patients with an otherwise good response. These findings have been corroborated by other recent studies[12-15] and have a significant impact for the patients. Reduced facial expression and disordered speech impair the patients' social life. Dysphagia and aspiration increase the risk for aspiration pneumonias. Bilateral ptosis was also frequently encountered and might be so severe that surgical correction is required. Extraocular motility can also be disordered, as was illustrated by the combination of ptosis, extraocular motility disorder and myopia in a 4.5-year old patient with classic infantile Pompe disease.

Language delays have also been reported, but tend to improve with time.[12] It was suggested that delays in language development may be related to delays in myelination, and that improved myelination over time might contribute to the subsequent improvement in language.[12,16] This suggestion requires further investigation. Other factors, such as hearing problems, disordered speech, and some degree of environmental poverty due to chronic illness may also contribute to the observed language delays.

The pattern of muscle weakness in treated infants is remarkable: while the weakness of the facial and bulbar muscles and the foot dorsoflexors is striking, the weakness of the proximal muscles is less pronounced. In children and adults with Pompe disease, ptosis and bulbar muscle weakness were previously incidentally reported, but recent studies have shown that these features are present in approximately 25% of patients, although less severe.[17,18] The severe distal muscle weakness in treated infants is very different from the typical limb-girdle distribution observed in children and adults with Pompe disease; in the latter patients the muscles of the hands and feet are affected in less than 10% of cases.[18]

The exact cause for the observed distribution pattern of muscle weakness in classic infantile Pompe patients treated with ERT remains unknown, and requires further investigation. Several factors might play a role including variation in response to ERT by different muscle-fiber types and potential neurologic involvement due to glycogen storage in the nervous system.

Because widespread glycogen storage is found in the CNS of untreated classic infantile Pompe patients,[19-21] and because ERT is not expected to cross the blood-brain barrier,[10] we have been concerned about the cognitive development of treated infants since the first trial with ERT in 1999. Our findings in **Chapter 6** suggest that the impact of glycogen storage on the function of the CNS seems to be limited. Although brain imaging revealed mild white matter abnormalities in infants treated with ERT, which was also described by other authors,[16,22-24] cognitive development at school age ranged between normal and mildly delayed in our long-term survivors; the oldest patient being assessed at 12 years of age. This corroborates another study in seven patients with classic infantile Pompe disease treated with ERT for 6.8 years on average, whose cognition developed at the lower end of the normal range.[25] In reports on cognition in treated infants up to four years of age the majority of patients showed a progression in mental development, although the range of developmental scores was wide.[2,4,5,7,8,26,27] It should be noted that in patients younger than 5 years cognition tests should be interpreted with caution, as in this patient group cognition is easily underestimated due to poor motor functioning and hearing deficits interfering with oral-motor and fine-motor skills.

Similar to the findings of Spiridigliozzi et al.,[25] some patients had abnormalities in processing speed, which may be explained in part by the mild white matter changes on the MRIs that we reported. The white matter changes became more profound during treatment (our study and [24]), but did not increase. Continued attention to cognition and other aspects of CNS-mediated behavior, and neuroimaging is warranted to evaluate the effect of glycogen accumulation in the CNS in long-term survivors. Due to the various physical limitations of the long-term survivors, many children attend special schools or require special education support. Regular neuropsychological assessments are recommended to determine the best education level and supportive measures.

Altogether, classic infantile Pompe disease has been shown to differ substantially from other lysosomal storage disorders like mucopolysaccharidosis type I, II, and III, in which progressive storage in the CNS results in profound mental retardation at a young age.

6.2 | Antibody formation and CRIM status

The first trials in 1999 already indicated that not all patients derive equal benefit from ERT.[1] Since then, several factors have been identified that influence treatment outcome. The studies in this thesis have confirmed the need for early initiation of ERT, before irreversible damage has occurred. The patients that started ERT at an advanced stage of the disease had the poorest clinical outcome.

As antibody formation is a natural response to foreign invading proteins, and as antibodies to ERT might affect the safety and efficacy of treatment,[28,29] the formation of antibodies has been monitored closely from the first trials onwards. By now, it has become evident that nearly all patients develop antibodies to high or low extent.

The results presented in **Chapter 7** illustrate that a high level of antibodies is associated with a poor motor outcome in patients with classic infantile Pompe disease. In a larger study comprising 34 infants with classic infantile Pompe disease, the survival differed dramatically between patients with high sustained antibody titers and patients with low antibody titers; all patients with high titers were either deceased or invasively ventilated by 33.8 months of age.[30]

On the basis of immuno-precipitation curves and clinical responses, our study suggests that the negative effect of antibody formation starts at an antibody titer of approximately 1:31,250, which corresponds remarkably well with the cut-off value of 1:51,200 chosen by Banaguria et al.[30] As even a mild immune response may reduce the efficacy of ERT in critical organs if sustained for a long period of time,[31] it is advised to monitor the immune-response regularly and to identify all patients at risk for a diminished response to ERT.

The greatest challenge at present is to identify those infants who are prone to develop a strong immune response. In general, CRIM-negative patients who do not produce any amount of acid α-glucosidase develop a stronger immunological response than those who are CRIM-positive,[32] but our study and other recent reports have shown that this relation is far from strict.[30,31,33] In part, this might be due to the imprecise definition of the CRIM-positive status that does not describe the amount, the conformation or the location of the endogenous acid α-glucosidase. Additionally, in certain cases it has proven difficult to determine the CRIM status by western blot (our study and [34]).

Consequently, knowledge of the patients' genotype and associated CRIM status helps predict patients' immunological response to ERT at a group level, but individual responses remain unpredictable. Surprisingly, in our study the immunological response seemed to be associated to the age at the initiation of ERT. A recent case report described two siblings with the same genotype who developed a different level of antibody titers; the authors also speculated that different immunological response might be attributed to the difference in age at initiation of ERT.[33] In response to that paper Prater et al. reported that early commencement of ERT does not necessarily explain low(er) or no antibody formation, although only 4 out of 28 patients under 31 days old at the start of ERT developed peak antibody titers of 1:51,200 or higher.[34] Early start of treatment may be achieved by neonatal screening. This way, the use of immunomodulatory protocols, which include significant side effects and prolonged administration, might become unnecessary. Further identification of the characteristics of patients at risk of developing high antibody titers is required.

The number of studies reporting immune tolerance induction to prevent the immune response is increasing. Different protocols have been used.[23,35-40] The ideal regimen for immunomodulation would consist of agents that allow for brief administration, prolonged tolerance and limited side effects. It still has to be determined which protocol is the most beneficial and which patients will benefit most. The combination of rituximab and methotrexate, with or without intravenous gamma globulins, is often used; a recently reported algorithm using this combination has shown improved effect of ERT in CRIM-negative patients.[39] Altogether, immunomodulation should be considered particularly in patients who start ERT at an advanced age and in CRIM-negative patients. The negative effect of high antibody titers might also be overcome by dose adjustment (see **Chapter 7**).

A CRIM-negative status itself also seemed associated with a poorer response to treatment, irrespective of the level of antibodies ([31] and this thesis). However, as a recently described CRIM-negative sibship developed low antibody titers and responded well to ERT,[33] relying solely on CRIM status to predict the clinical response to ERT might be too strict.

In children and adults with Pompe disease, the relation between antibody titers and treatment outcome is less clear. It is more difficult to assess the impact of antibodies in these patients due to slower disease progression, clinical heterogeneity and greater difficulty in defining clear end-points.[41] In the recent randomized controlled trial in children and adults with Pompe disease, all patients developed antibody titers, which were in general relatively low, and no consistent association between the antibody level and the primary efficacy outcomes was found.[42] However, when focusing on individual adult patients the association of high antibody titers and poor treatment outcome cannot be ignored.[41,43] As all children and adults produce some amount of acid α-glucosidase, these studies reaffirm that the CRIM status does not predict the immunological response alone.

6.3 | Potential ways to improve the effect of enzyme-replacement therapy

Dose augmentation

As it stands, much is still unknown about the optimal dose and dosing regimen of ERT in Pompe disease. The data of earlier studies evaluating the effect of ERT in patients with classic infantile Pompe disease and in Pompe knockout mice revealed that: 1) the treatment response to ERT is suboptimal with a biweekly dosing regimen of either 20 or 40 mg/kg eow,[6,7] and 2) patients should be treated with an adequate dose from the initiation of ERT due to the difficulty to reverse muscle pathology.[5,8,44] Our theoretical model illustrating the consequences of different doses and dosing regimens of ERT in **Chapter 8** predicts that weekly infusions have a better effect than dosing regimens with longer time intervals. However, weekly administration will increase the burden for the patients and will increase the costs of treatment. The model also illustrates that higher dosing leads to higher enzyme activity levels, but does not predict how high the dose needs to be to have a constantly curative effect. This depends on the level of acid α-glucosidase that is actually reached in the skeletal muscle, on the half-life of the enzyme inside the lysosomes, and on the rate of glycogen re-accumulation.

Our findings in **Chapter 8** suggest that a dosing regimen of 40 mg/kg/week improves ventilator-free survival and motor outcome compared to the currently recommended dose of 20 mg/kg eow. We therefore recommend using this higher dose for all infants, particularly in those with poor prospects. Of note, also when this higher dose is applied, residual disease remains.

It should however be noted that there is variation in response between patients treated with the same dose. This is illustrated by one of the patients in our study treated with 20 mg/kg eow who performed extremely well. The reason why this patient performed so well

remains unclear. Indeed, ERT was initiated at an early age, the patient was CRIM-positive, and antibody levels were low, but the outcome of two similar patients treated with the same dose was obviously poorer. The observed difference in response might be caused by epigenetic and environmental factors.

Now that ERT has become a registered treatment, experience in clinical practice has to reveal whether higher and more frequent dosing regimens than recommended are more beneficial. Notably, many patients are currently receiving higher doses of ERT than recommended due to suboptimal effects with this dose.[15] The rarity of the disease limits the performance of extensive clinical trials and dose finding studies. We studied the effect of dose augmentation in an ongoing single-center, prospective, open-label cohort study as randomization to different dosing regimens was not feasible. Ideally, the study population should be larger and more homogeneous, and additional outcome measures should be included, more suitable to detect small differences in treatment response.

Although dose augmentation may cause problems, such as the development of nephrotic syndrome in a patient receiving 10 mg/kg five times per week for 10 months,[38] a higher risk of infusion-associated reactions,[6] and higher antibody production,[6] in our experience dose augmentation was safe.

In children and adults with Pompe disease, the experience with different dosing regimens of ERT is also limited. In one 17-year old child with Pompe disease, improvement in muscle function and ptosis was observed within 6 months after dose augmentation from 20 mg/kg eow to 40 mg/kg eow.[45] Another study reports stabilization of disease through the use of a low-frequency maintenance regimen of ERT; a 33-year old patient who initially received 20 mg/kg eow, received 20 mg/kg once every four weeks after a 12-month period.[46] Although such a regimen may allow for greater patient convenience and lower treatment cost, lowering the dose or frequency of ERT should be performed with extreme caution, as earlier clinical and preclinical studies as well as our hypothetical model indicate that a lower than the recommended dose is probably insufficient and certainly not recommended for all patients.

Potential benefit from newborn screening

From the clinical trials with ERT we have learned that treatment needs to be started before irreversible tissue damage has occurred. As diagnosis is often delayed,[47,48] we assessed the potential gain of earlier diagnosis by newborn screening.

At the time of diagnosis, the health status of patients with classic infantile Pompe disease is severely impaired, as described in **Chapter 8**. Muscle pathology was severe, motor

development was significantly delayed, and all patients had a hypertrophic cardiomyopathy, some in an advanced stage. As these factors have been associated with a poor response to ERT,[2,49,50] earlier initiation of ERT enabled by neonatal screening might improve the patients' outcome. At present, neonatal screening is implemented in Taiwan and in a few states in the USA,[51] and pilot studies have been performed in Japan,[52] Austria,[53] northern Germany,[54] Italy,[55] Korea,[56] and Hungary.[57]

For patients with classic infantile Pompe disease, clinical benefit of neonatal screening has been reported in Taiwan. Newborn screening resulted in earlier diagnosis compared to diagnosis based on clinical symptoms (<1 month versus three to six months of age), and thus enabled earlier start of ERT.[58] All five patients who were diagnosed through newborn screening and treated shortly thereafter – for 14 to 32 months – demonstrated normalization of cardiac size and muscle pathology with normal physical growth and age-appropriate gains in motor development.[59] Survival was significantly improved as compared to an untreated historical cohort, but not compared to treated patients who were clinically diagnosed.[59] It is important to note that the clinical results obtained in this study may not be entirely predictive of expected results in other populations due to the fact that all patients detected by screening were CRIM-positive. Taiwan has a high incidence of mutations consistent with a CRIM-positive status, whereas most other populations have a higher incidence of CRIM-negative patients.

The current screening techniques cannot distinguish between patients with classic infantile or other forms of Pompe disease. Therefore, one of the biggest concerns regarding the implementation of newborn screening for Pompe disease is that the majority of diagnosed newborns will have the non-classic form of the disease, meaning that clinical symptoms may manifest at any time from childhood to late adulthood.[53] For these patients, the benefit of a newborn screening program is not evident. **Chapter 8** shows that the health status of most of these patients is also severely impaired at diagnosis: for example, more than 20% of the patients was already wheelchair bound and/ or required respiratory support. Part of these problems might have been prevented if these patients had been diagnosed and treated with ERT earlier, which advocates for earlier diagnosis for the whole spectrum of Pompe disease. The advantages of newborn screening in these 'patients in waiting' for their disease[60] should be carefully weighed against the disadvantages of long-term uncertainty of when the disease will expose itself.

The cornerstone of newborn screening is to detect disorders that are treatable, but not clinically evident in the newborn period, and for which early treatment significantly improves the outcome.[61] Over recent years, the criteria for newborn screening are being expanded and

screening for broad-phenotype diseases such as Pompe is universally being considered.[62] A recent study that measured the support for newborn screening for Pompe disease in the general public as well as in (parents of) patients with Pompe disease in the Netherlands showed that both groups support screening.[63] Close follow-up of children and adults with Pompe disease detected through screening is required. It will be a challenge to find the best strategy for follow-up and management of these patients.[64-66] It is anticipated that the results of the several pilot programs currently ongoing will provide the data necessary to recommend universal newborn screening for Pompe disease.[67]

6.4 | Clinical implications and future perspectives

The perspective for patients with classic infantile Pompe disease has changed considerably since the availability of ERT. However, the long-term outcome is hampered by residual disease, few prognostic factors are known, and as the clinical outcome is often still suboptimal ways to improve the effect of ERT are being explored.

Residual disease and prognostic factors

Prior to the introduction of ERT as a treatment for classic infantile Pompe disease, little was known about the development of this condition due to the aggressive nature of the disease and associated early mortality. Treatment with ERT has led to an emerging phenotype of long-term survivors. In addition to the residual disease described in this thesis, several new clinical issues have been reported, including hearing loss,[68,69] osteoporosis and increased fracture risk,[70] risk of cardiac arrhythmias,[71-73] increased anesthesia risk,[72] low anal sphincter tone,[74] and basilar artery aneurysm.[75] The detection of residual disease and previously unknown complications of the disease due to early death is important, as it has significant consequences for clinical practice: for instance, the detection of residual facial and bulbar muscle weakness has revealed the high risk for aspiration pneumonias due to dysphagia, which necessitates regular assessment of swallowing function. Delayed motor development and impaired motor function require the development of appropriate exercise programs and musculoskeletal management. Further identification of residual disease and complications that may influence clinical outcome is needed to develop appropriate supportive measures to improve outcome, for prognostic purposes, and to increase insight in disease pathology thereby possibly revealing new avenues to improve therapy. Additionally, more data on long-term outcomes with ERT are required, as there is still a paucity of data although the first clinical trials started 15 years ago. Now that the first cohort of treated infants reaches adolescence, close monitoring

for possible complications in older patients such as scoliosis is recommended. Altogether, a multidisciplinary approach is required for the treatment of patients with classic infantile Pompe disease.

Ideally, the outcome-measures for evaluating the effect of ERT in older surviving patients with classic infantile Pompe disease and children and adults with Pompe disease should be the same. However, as the emerging phenotype of treated patients with classic infantile Pompe disease clearly differs from that of affected children and adults, it might be necessary to develop additional and more specific outcome-measures. It is intriguing why the phenotype of surviving classic infantile Pompe patients differs from that of patients born with childhood forms of the disease.

For prognostic purposes, it is of crucial importance to identify reliable and readily available prognostic markers. Despite the fact that several prognostic factors for the effect of ERT have been identified, it remains as yet impossible to predict which infants will respond best. Knowledge of these factors might enable patient-tailored treatment: it might help in deciding whether or not treatment should be initiated, but also whether or not additional treatments, such as immunomodulation, are required. Surely a prognostic model would be of great help, but will be difficult to construct due to the rarity of the disease.

The future of ERT for Pompe disease

Although ERT undoubtedly gives patients new perspectives, its effect can still be improved: 1) The uptake of the recombinant human enzyme by skeletal muscle is low compared to uptake of the enzyme by other tissues. The reason why skeletal muscle tissue is hard to target is actually not known, but several factors seem to play a role, including: i. low abundance of the mannose 6-phosphate (M6P) receptor required for efficient endocytosis ([76,77] and **Chapter 1**), ii. the large mass of skeletal muscle (up to 40% body weight), and iii. the difficulty to reach the skeletal muscle tissue. 2) The effect of ERT might also depend on the muscle-fiber types affected; especially type II muscle fibers appeared difficult to correct in mice,[78] although this seems less so the case in humans.[79,80] 3) The effect of ERT is partly counteracted by the formation of antibodies (**Chapter 3** and [30]). 4) A portion of administered enzyme might not reach the lysosomes due to autophagic build-up, which impairs intracellular vesicular trafficking.[81] 5) ERT cannot prevent glycogen storage and potentially associated pathology in the CNS, as the blood-brain barrier prevents the access of large molecules like alglucosidase alfa.[10] 6) Patients might experience ERT as a burden, as repeated and lifelong infusions are needed. Finally, 7) ERT is accompanied by high costs.

These limitations have stimulated researchers to put a lot of effort in the improvement of ERT. New developments have arisen over the past few years, such as immunomodulation to limit the immunological response to ERT, the application of higher dosages, changing the 'formulation' of the recombinant enzyme to improve uptake by skeletal muscle (glycoengeneering of currently applied alglucosidase alfa, application of GILT technology, and alternative production technology[82-84]), and chaperone assisted ERT.[85] Notably, gene therapy, either delivered locally or systemically, is also being explored (**Chapter 2**).

Early initiation of ERT appears crucial. Neonatal screening seems an obvious way to ensure the earliest diagnosis, as screening for Pompe could easily be integrated into neonatal bloodspot screening programs. On May 17th 2013 the Secretary's Discretionary Advisory Committee on Heritable Diseases in Newborns and Children (USA) approved a recommendation to the Health and Human Services Secretary to add Pompe to the Recommended Uniform Screening Panel.[86]

The reimbursement issue

Altogether, ERT is effective across the entire spectrum of Pompe disease phenotypes. ERT is lifesaving in classic infantile Pompe patients and has a positive effect on survival, motor function, and pulmonary function in children and adults. It is easily overlooked that also prevention of clinical decline through stabilization of pulmonary and motor function is a significant clinical response in a progressive disease like Pompe disease.

Pompe disease is an orphan disease which makes the development of a therapy complex and costly. The annual costs of ERT for a ten-year old child is in the order of 200,000 euros when a dose of 20 mg/kg eow is applied. This coupled to the need for lifelong administration has led to a debate on the reimbursement of ERT in several countries around the world, particularly with respect to the treatment of children and adults. However, the spectrum of Pompe disease is continuous; a strict separation of patients in different subtypes is disputable on scientific and clinical grounds. Moreover, continued experience with treating Pompe disease will open new avenues for developing innovative therapies for other lysosomal storage disorders and neuromuscular diseases.

FINAL REMARKS

The introduction of ERT has changed the life of infants with Pompe disease and their families. A new phenotype emerges in long-term survivors. This leads to new challenges with respect to clinical management and therapeutic approaches.

Combined efforts are required to increase the knowledge on long-term effects of ERT and residual disease, to identify biomarkers and to develop prognostic models to enable patient-tailored treatment, and to increase the insight in disease pathology, as this might prompt the development of new and ultimately curative therapies.

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CHAPTER 11

Summary | Samenvatting

Summary

Pompe disease is an autosomal recessive lysosomal storage disorder, in which a deficiency of the enzyme acid α-glucosidase results in the accumulation of glycogen within lysosomes, ultimately resulting in tissue destruction and loss of function. Pompe disease can become apparent at any age. Children and adults with Pompe disease have a partial deficiency of the enzyme that causes a slowly progressive phenotype involving mainly skeletal muscle tissue. Complete enzyme deficiency leads to the very aggressive and rapidly progressive classic infantile form, characterized by onset of symptoms within the first months of life, hypertrophic cardiomyopathy, generalized muscle weakness, respiratory problems, and feeding difficulties. Cardiac failure and respiratory insufficiency eventually lead to death, typically in the first year of life.

Until recently, Pompe disease was untreatable. Registration of enzyme-replacement therapy (ERT) in 2006 has significantly improved the prospect for affected patients. At present, the oldest patients with classic infantile Pompe disease are 15 years old thanks to the beneficial effects of ERT. With their life extension, new clinical features of Pompe disease have emerged and require attention.

The studies described in this thesis aimed to delineate the emerging phenotype of children with classic infantile Pompe disease treated with ERT, to identify factors predictive for therapeutic outcome, and to explore methods for improving the treatment outcome. All patients described in this thesis participated in a nationwide prospective observational cohort study evaluating the safety and efficacy of ERT.

Chapter 1 provides introductory information on Pompe disease and numerates the current knowledge on the effects and challenges of ERT. The aims of the studies described in this thesis are formulated at the end of this chapter. **Chapter 2** reviews the pros and cons of different treatment options for lysosomal storage disorders, including ERT, stem-cell therapy, gene therapy, chaperone based therapy, and substrate reduction therapy. Although considerable progress has been made in establishing different therapies for lysosomal storage disorders, none of the current options is completely curative yet. All are complicated by the difficulty in targeting all affected tissues, in reaching sufficiently high enzyme levels in the target tissues, and by high costs. The pathways leading from the genetic defect in each disease to the clinical symptoms should be better understood, as this might prompt the development of new and ultimately curative therapies.

Chapters 3, 4, 5, and **6** focus on residual muscle weakness and long-term cognitive outcome in patients with classic infantile Pompe disease treated with ERT. **Chapter 3** describes the motor development, motor function, and muscle strength of 11 infants who were at least 1.5 years of age at the end of the study (age-range 1.5 – 14.9 years). ERT enabled all patients to reach previously unmet motor milestones. At the same time, motor development was often delayed or started to show abnormalities over time. Patients showed difficulties in specific motor functions, such as neck flexion, sit-up, walking stairs, jumping, and standing on one leg. Over time, some loss of muscle function was observed, and patients developed a characteristic gait. The distal muscles became gradually more affected whereas initially proximal muscle weakness was more prominent. This finding was unexpected as the pattern of muscle weakness is not the same as in untreated children and adults with non-classic Pompe disease. The findings emphasize the need for appropriate musculoskeletal management and exercise programs as supportive tools next to the application of ERT in classic infantile Pompe disease.

Chapter 4 focuses on the frequency and consequences of facial-muscle weakness, speech disorders and dysphagia in 11 long-term survivors (age-range at study end 7.7 months to 12.2 years). Residual weakness of the facial and bulbar muscles was found to be common and was accompanied by disordered speech and dysphagia in the majority of patients. To improve speech and reduce the risk for aspiration related complications, early treatment by a speech therapist and regular swallowing assessments are recommended. All patients developed a myopathic facies, sometimes accompanied by ptosis. The case report in **Chapter 5** illustrates that ptosis can be so severe that surgery is required. It shows that a combination of ptosis, extraocular motility disorder, and myopia may occur in long-term survivors. It is recommended to include ophthalmic examination in the routine follow-up of patients with classic infantile Pompe disease.

Chapter 6 describes the cognitive functioning in 10 patients with classic-infantile Pompe disease treated with ERT. Six patients also underwent brain imaging. Although patients with classic-infantile Pompe disease store glycogen in the CNS, and although ERT is unlikely to cross the blood-brain barrier, the cognitive development of five patients at school age ranged from normal to mildly delayed; the oldest patient in this study was 12 years old. This suggests that the consequences of glycogen accumulation in the CNS are limited. In patients younger than 5 years cognition is easily underestimated due to poor motor functioning and hearing deficits. Some patients showed abnormalities in processing speed, which might be explained by the observed white matter changes.

The studies described in **Chapter 7** were aimed to examine the patients' immunologic response to ERT (antibody formation) in relation to the endogenous production of acid α-glucosidase by the patient (CRIM-status). Eleven patients participated in this study. They were treated with ERT for a median duration of 5.6 years. They all developed an immunological response to ERT. The antibody titers were not strictly related to the patients' CRIM status, but high antibody titers were associated with a poor motor outcome. Notably, the CRIM-negative status itself seems associated with early demise, irrespective of the height of the immune response to ERT. The finding that all patients who started ERT before 2 months of age had low antibody titers underlines the need to start ERT in classic infantile Pompe disease as soon as possible.

In **Chapter 8** and **9** dose augmentation and newborn screening are explored as ways to improve the effect of ERT. In **Chapter 8** we compared the safety and efficacy of the recommended dose of 20 mg/kg eow (n=6) with that of a higher and more frequent dose of 40 mg/kg/week (n=4). Four of the six patients who received 20 mg/kg eow either died (n=2), became ventilator dependent (n=2), or did not learn to walk (n=2). There were no apparent differences between the two dose groups in terms of number of infusion associated reactions (IARs) and extent of immune response to ERT. As treatment with 40 mg/kg/week is well tolerated and seems to improve ventilator-free survival and motor outcome as compared to treatment with 20 mg/kg eow, it is recommend using the higher dose for all affected infants with poor prospects.

Chapter 9 presents the results of a cross-sectional study of 53 clinically diagnosed patients with Pompe disease within a very broad age range (0 – 64 years old). This study focuses on the health and functional status at the time of diagnosis. All patients with classic infantile Pompe disease had cardiac hypertrophy at the time of their diagnosis and their motor function was considerably impaired. Most children and adults with Pompe disease had advanced muscle weakness and impaired respiratory function at the time of their diagnosis. The finding of severely impaired health status at the time of clinical diagnosis provides a strong argument to aim for earlier diagnosis and to explore the option of newborn screening.

Chapter 10 discusses the main findings of this thesis and their implications for present day and future clinical practice.

Samenvatting

De ziekte van Pompe is een autosomaal recessieve lysosomale stapelingsziekte, veroorzaakt door een tekort van het enzym zure α-glucosidase. Dit leidt tot stapeling van glycogeen in lysosomen, wat uiteindelijk leidt tot weefselschade en functieverlies van organen. De ziekte van Pompe kan zich op allerlei leeftijden presenteren. Kinderen en volwassenen met de ziekte van Pompe hebben een gedeeltelijk tekort van het enzym: dit leidt tot een relatief langzaam progressief ziektebeeld waarbij met name de skeletspieren zijn aangedaan. Een compleet verlies van het enzym leidt tot de snel progressieve klassiek infantiele vorm van de ziekte: bij deze vorm treden de symptomen al in de eerste levensmaanden op. Karakteristieke symptomen zijn een hypertrofische cardiomyopathie, gegeneraliseerde spierzwakte, ademhalingsproblemen en voedingsproblemen. Toenemend hartfalen en respiratoire achteruitgang leidt ertoe dat deze kinderen bijna altijd in het eerste levensjaar overlijden.

Tot kortgeleden was de ziekte van Pompe onbehandelbaar. De registratie van enzymvervangingstherapie (ERT) in 2006 heeft het vooruitzicht voor patiënten met de ziekte van Pompe sterk verbeterd. Door behandeling met ERT is de oudste patiënt met de klassiek infantiele vorm van de ziekte nu 15 jaar oud. Doordat deze kinderen nu langer leven komen steeds meer nieuwe kenmerken van de ziekte tot uiting die extra aandacht vragen.

De onderzoeken in dit proefschrift hadden als doel om 1) de nieuwe kenmerken van kinderen met de klassiek infantiele vorm van de ziekte die behandeld worden met ERT te beschrijven, 2) prognostische factoren voor het effect van ERT te identificeren en 3) mogelijkheden om het effect van ERT te vergroten te onderzoeken. Alle patiënten die beschreven worden in dit proefschrift nemen deel aan een nationale prospectieve observationele cohort studie waarin de effecten en de veiligheid van ERT worden bestudeerd.

Hoofdstuk 1 geeft achtergrondinformatie over de ziekte van Pompe en bespreekt wat er bekend was over de effecten en beperkingen van ERT bij aanvang van de studies in dit proefschrift. Aan het eind van dit hoofdstuk wordt het doel van de onderzoeken van dit proefschrift beschreven. **Hoofdstuk 2** bespreekt de voor- en nadelen van verschillende behandelingsmogelijkheden bij lysosomale stapelingsziekten, waaronder ERT, stamceltherapie, gentherapie, chaperone therapie en substraat reductie therapie. Hoewel het ontwikkelen van deze therapieën geleid heeft tot aanzienlijke vooruitgang in de behandeling van lysosomale stapelingsziekten leidt geen van deze behandelingen op dit moment nog tot volledige genezing van de patiënt. De

behandelingen die op dit moment beschikbaar zijn hebben moeite om de aangedane weefsels in voldoende mate te bereiken en om ervoor te zorgen dat er voldoende enzym wordt opgenomen in de weefsels. Daarnaast zijn de huidige therapieën erg duur. Het inzicht in de manier waarop het genetische defect leidt tot de klinische symptomen bij de verschillende ziektebeelden moet worden vergroot, omdat dit kan leiden tot aangrijpingspunten voor het ontwikkelen van nieuwe en mogelijk curatieve behandelingen.

Hoofdstuk 3, **4**, **5** en **6** bespreken de spierzwakte bij patiënten met de klassiek infantiele vorm van de ziekte van Pompe die behandeld worden met ERT en cognitie van deze kinderen op de lange termijn. **Hoofdstuk 3** beschrijft de motorische ontwikkeling, spierfunctie en spierkracht van 11 kinderen die ouder waren dan 1,5 jaar (spreiding 1,5 – 14,9 jaar). ERT leidde er bij al deze kinderen toe dat zij motorische mijlpalen bereikten die zij zonder behandeling nooit bereikt zouden hebben. De motorische ontwikkeling was wel vaak vertraagd en werd in de loop van de tijd afwijkend. Patiënten hadden moeite met bepaalde spierfuncties, waaronder het buigen van de nek, het gaan zitten vanuit rugligging, traplopen, springen en op één been staan. In de loop van de tijd gingen sommige spierfuncties achteruit en ontwikkelden de patiënten een karakteristiek looppatroon. De spierzwakte was aanvankelijk het grootst in de proximale spieren, maar doordat de spierzwakte in de distale spieren geleidelijk toenam kwam de distale spierzwakte steeds meer op de voorgrond. Deze verdeling van de spierzwakte was opvallend, omdat het duidelijk anders is dan het patroon dat gezien wordt bij onbehandelde kinderen en volwassenen met de niet-klassieke vorm van de ziekte van Pompe. De bevindingen van deze studie benadrukken de noodzaak van adequate behandeling van problemen van het bewegingsapparaat en het opstellen van geschikte trainingsprogramma's bij patiënten met de klassiek infantiele vorm van de ziekte van Pompe in aanvulling op de behandeling met ERT.

Hoofstuk 4 bestudeert de frequentie en de gevolgen van gezichtspierzwakte en spraak- en slikproblemen in 11 patiënten met de klassiek infantiele vorm van de ziekte van Pompe die langdurig behandeld zijn met ERT (spreiding van de leeftijd bij het eind van de studie 7.7 maanden tot 12.2 jaar). Spierzwakte van het gezicht en de bulbaire spieren kwam bij deze patiënten vaak voor en ging in de meerderheid van de patiënten gepaard met een afwijkende spraak en slikproblemen. Om de spraak te verbeteren en de kans op een aspiratiepneumonie te verminderen is het belangrijk om vroeg te starten met logopedie en om de slikfunctie regelmatig te onderzoeken. Alle patiënten ontwikkelden een facies myopathica (spierzwakte van de gelaatspieren) en sommigen ontwikkelden een ptosis (afhangend bovenooglid). De patiënt beschreven in **hoofdstuk 5** laat zien dat de ptosis zo ernstig kan worden dat een

operatieve behandeling noodzakelijk is. Deze casus laat ook zien dat de combinatie van ptosis, verminderde oogbewegingen en bijziendheid voor kan komen in lang behandelde patiënten. Dit alles wijst erop dat oogheelkundig onderzoek opgenomen zou moeten worden in de standaard follow-up van patiënten met de klassiek infantiele vorm van de ziekte van Pompe.

Hoofdstuk 6 beschrijft de cognitieve ontwikkeling van 10 patiënten met de klassiek infantiele vorm van de ziekte van Pompe; de oudste patiënt was 12 jaar. Bij zes patiënten werd ook beeldvorming verricht. Patiënten met de klassiek infantiele vorm van de ziekte van Pompe stapelen ook glycogeen in het centraal zenuwstelsel; behandeling met ERT kan deze stapeling niet verminderen doordat het niet door de bloed-hersen barrière kan. Toch bleek uit onze studie dat de cognitieve ontwikkeling van de patiënten op schoolgaande leeftijd varieerde van normaal tot licht vertraagd. Dit suggereert dat de stapeling van glycogeen in de hersenen weinig consequenties heeft voor de cognitieve ontwikkeling van de patiënten. De cognitie van kinderen onder de vijf jaar werd vaak onderschat door de verminderde spierfunctie en gehoorproblemen van de patiënten. Sommige patiënten hadden een vertraagde verwerkingssnelheid, wat verklaard zou kunnen worden door de geobserveerde witte stof afwijkingen.

In **hoofdstuk 7** wordt de immunologische respons (vorming van antilichamen) van de patiënten als reactie op het krijgen van ERT onderzocht in relatie tot de endogene productie van zure α-glucosidase door de patiënt (CRIM status). Elf patiënten met de klassiek infantiele vorm van de ziekte van Pompe namen deel aan deze studie. De mediane behandelingsduur met ERT was 5.6 jaar. Alle patiënten ontwikkelden een immuunrespons tegen ERT. De hoogte van de antilichaam titers was niet duidelijk gecorreleerd aan de CRIM status van de patiënt, maar hoge antilichaam titers waren wel geassocieerd met een slechtere motorische uitkomst. Opvallend was dat een CRIM-negatieve status op zichzelf geassocieerd leek met een slechte klinische uitkomst, onafhankelijk van het niveau van de antilichamen van de patiënt. De bevinding dat alle patiënten die voor de leeftijd van twee maanden waren gestart met ERT lage antilichaam titers hadden bevestigt dat het belangrijk is om zo vroeg mogelijk te starten met ERT in patiënten met de klassiek infantiele vorm van de ziekte.

In **hoofdstuk 8** en **9** worden twee mogelijkheden onderzocht om het effect van ERT te verbeteren: Verhoging van de dosering en vroege start van behandeling door neonatale screening. In **hoofdstuk 8** hebben we de veiligheid en het effect van behandeling met de aanbevolen dosering (20 mg/kg om de week, n=6) vergeleken met een hogere en frequentere

dosering (40 mg/kg/week, n=4). Vier van de zes patiënten die behandeld werden met 20 mg/kg om de week overleden (n=2), werden beademingsbehoeftig (n=2) of leerden nooit lopen (n=2). Er was geen duidelijk verschil in het aantal infusie geassocieerde reacties en de hoogte van de antilichaam titers tegen ERT tussen de twee doseringen. Behandeling met 40 mg/kg/week lijkt veilig en lijkt de beademingsvrije overleving en de motorische uitkomst van patiënten te verbeteren; daarom raden wij aan om patiënten met de klassiek infantiele vorm van de ziekte te behandelen met deze hogere dosering.

Hoofdstuk 9 beschrijft de resultaten van een cross-sectionele studie van 53 klinisch gediagnosticeerde patiënten met de ziekte van Pompe met een grote spreiding in leeftijd (0 – 64 jaar). Dit onderzoek richt zicht op de gezondheid en functionele status van de patiënten op het moment van diagnose. Op het moment van diagnose hadden alle patiënten met de klassiek infantiele vorm van de ziekte een hypertrofische cardiomyopathie en een verminderde spierfunctie. De meeste kinderen en volwassenen met de ziekte van Pompe hadden ernstige spierzwakte en een verminderde longfunctie bij diagnose. De bevinding dat de gezondheid van de patiënten op het moment van de klinische diagnose al ernstig is verminderd pleit er voor om de mogelijkheden van neonatale screening verder te onderzoeken.

Hoofdstuk 10 bediscussieert de belangrijkste bevindingen van dit proefschrift en bespreekt de klinische consequenties van deze bevindingen en mogelijke implicaties voor de toekomst.

Abbreviations

Dankwoord

Curriculum Vitae

Publications

PhD Portfolio

List of abbreviations

6MWT	6-Minute walk test
AAV	Adeno-associated virus
AIMS	Alberta Infant Motor Scale
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BAEP	Brainstem Auditory Evoked Potentials
BBB	Blood-brain barrier
BM	Bone marrow
BSA	Bovine serum albumin
BSID-II	Bayley Scales of Infant Development II
CK	Creatine kinase
CB	Cord blood
CHO-cells	Chinese hamster ovary cells
CNS	Central nervous tissue
CRIM	Cross reactive immunological material
CT	Computed Tomography
DEXA	Dual-energy X-ray absorptiometry
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EMG	Electromyogram
eow	every other week
ER	Endoplasmic reticulum
ERT	Enzyme-replacement therapy
FCS	Fetal calf serum
FDA	US Food and Drug Administration
FEES	Fiberoptic endoscopic evaluation of swallowing
FVC	Forced Vital Capacity
GAA	gene coding for acid α-glucosidase
GAGs	Glycosaminoglycans
GILT	Glycosylation independent lysosomal targeting
Griffiths	Griffiths Mental Development Scales
HADS	Hospital Anxiety and Depression Scale

HHD	Hand-held dynamometry
HLA	Human leukocyte antigen
HSCT	Hematopoietic stem-cell therapy
IAR	Infusion-associated reaction
IGF-II	Insulin-like growth factor II
IPA	International Pompe Association
iPS cells	Induced pluripotent stem cells
kDa	Kilo Dalton
LAMP-2	Lysosomal-associated membrane protein
LDH	Lactate dehydrogenase
LIMP-2	Lysosomal integral membrane protein 2
LSDs	Lysosomal storage disorders
LVMI	Left ventricular mass index
M6P	Mannose 6-phosphate
M6PR	Mannose 6-phosphate receptor
M-ABC	Movement Assessment Battery for Children
MMT	Manual muscle testing
MLD	Metachromic leukodystrophy
MPS	Mucopolysaccharidosis
MRC	Medical Research Council grading scale
MRI	Magnetic Resonance Imaging
MU	4-methylumbelliferon
MUGlc	4-methylumbelliferyl-α-D-glucopyranoside
NGT	Nasogastric tube feeding
NPC	Nieman Pick disease type C
PAS	Periodic acid-Schiff
PB	Peripheral blood
PBS	Phosphate-buffered saline
PK	Pharmacokinetic
QMFT	Quick Motor Function Test
RHS	Rotterdam nine items Handicap Scale
SF-36	Medical Outcomes Study 36-item short-form health survey
SON-R	Snijders Oomen Nonverbal Intelligence Test–Revised
SRT	Substrate-reduction therapy
TACQOL	TNO-AZL Child Quality of Life Questionnaire
TT	Timed tests
WISC-III	Wechsler Intelligence Scales for Children, Third Edition

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Curriculum Vitae

Carin van Gelder was born in Bergen op Zoom on April 21, 1982. In 2000 she graduated from high school (Stedelijk Gymnasium Arnhem), and started her medical training at Katholieke Universiteit Leuven, Belgium. From 2002, she continued her medical training at University of Utrecht. During her medical training, she participated in several committees of the Medical Factorial Society of both universities. In the year 2004-2005 she was member of the board of the Medical Factorial Society of University of Utrecht. In 2006 she did internships in Public Health and Pediatrics (elective) at Igogwe Hospital, Igogwe, Tanzania. In 2007 she did an internship in Obstetrics/Gynecology at Rotunda Hospital, Dublin, Ireland. After obtaining her medical degree in 2008, she started working as a resident at the Department of Pediatrics in Meander Medisch Centrum in Amersfoort. In April 2009 she started as a PhD-student at the Center for Lysosomal and Metabolic Diseases at Erasmus MC University Medical Center under supervision of Prof. dr. A.T. van der Ploeg and dr. A.J.J. Reuser. In February 2013, she will start her pediatric trainingship in UMC St Radboud in Nijmegen under supervision of dr. J.M.T. Draaisma. Carin lives in Utrecht with her husband Joost and son Luuk.

Publications

Wens SCA, **van Gelder CM**, Kruijshaar ME, de Vries JM, van der Beek NAME, Reuser AJJ, van Doorn PA, van der Ploeg AT, Brusse E. Phenotypical variation within 22 families with non-classic Pompe disease. *(submitted)*

Van Gelder CM, van der Giessen LJ, van Rijs M, Plug I, van den Hout JMP, van der Ploeg AT. Motor development, motor function, and muscle strength in patients with classic infantile Pompe disease treated with enzyme therapy; an open-label, single-center study. *(in prep)*

Van Gelder CM, Plug I, Hoogeveen-Westerveld M, Reuser AJJ, van der Ploeg AT. A higher dose of alglucosidase alfa in classic infantile Pompe disease positively affects ventilator-free survival and motor outcome: an open-label single-center study. *(submitted)*

Van Gelder CM, Hoogeveen-Westerveld M, Kroos MA, Plug I, van der Ploeg AT, Reuser AJJ. Antibody titers in relation to CRIM status: Effects on enzyme therapy in classic infantile Pompe disease. *(submitted)*

Rigter T, Weinreich SS, van El CG, de Vries JM, **van Gelder CM**, Güngör D, Reuser AJJ, Hagemans MLC, Cornel MC, van der Ploeg AT. Severely impaired health status at diagnosis of Pompe disease: a cross-sectional analysis to explore the potential utility of neonatal screening. *Mol Genet Metab* 2012; **107**: 448–55.

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Sluiter W, van den Bosch JC, Goudriaan DA, **van Gelder CM**, de Vries JM, Huijmans JGM, Reuser AJJ, van der Ploeg AT, Ruijter GJG. Rapid ultraperformance liquid chromatography-tandem mass spectrometry assay for a characteristic glycogen-derived tetrasaccharide in Pompe disease and other glycogen storage diseases. *Clin Chem* 2012; **58**: 1139–47.

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Slingerland NWR, Polling JR, **van Gelder CM**, van der Ploeg AT, Bleyen I. Ptosis, extraocular motility disorder, and myopia as features of pompe disease. *Orbit* 2011; **30**: 111–3.

Van Gelder CM, Hagemans MLC, van den Hout JMP, Smeitink JAM, de Coo IFM, van der Ploeg AT. Tijdige diagnostiek en behandeling van de ziekte van Pompe kan irreversibele orgaanschade voorkomen [Dutch]. *Tijdschr Kindergeneeskd* 2010; **78**: 81-88.

PhD portfolio – Summary of PhD training and teaching

Erasmus MC Department:	**Pediatrics, Center for Lysosomal and Metabolic Diseases**
Research school:	**Nihes**
PhD period:	**2009-2013**
Promotor:	**Prof. dr. A.T. van der Ploeg**

	Year	Workload (ECTS)
General academic skills		
Good Clinical Practice	2010	0.9
English Biomedical Writing and Communication	2010-2011	4.0
Research Integrity	2012	2.0
Research skills		
Introduction to clinical research	2010	0.9
Biostatistics for clinicians	2010	0.9
Regression analysis for clinicians	2010	1.4
Survival analysis for clinicians	2010	1.9
Repeated measurements	2011	1.4
Biweekly journal club, Center for Lysosomal and Metabolic Diseases	2009-2012	1.0
Biweekly research meeting, Center for Lysosomal and Metabolic Diseases	2009-2013	2.0
Seminars and workshops		
Dag voor de jonge onderzoekers, NVK, Veldhoven	2009	0.5
Office applicatie (access), DienstenCentrum Onderwijs, Rotterdam	2009	0.5
Talent class Oral Presentation, Netherlands Organisation for Scientific Research (NWO)	2010	0.3
Talent class Concise Presentation, Netherlands Organisation for Scientific Research (NWO)	2010	0.3
In depth courses		
6th Pompe disease expert day, Rotterdam	2009	1.0
Steps Forward in Pompe Disease, Munich, Germany	2009	1.0
International postgraduate course on lysosomal storage disorders, Nierstein, Germany	2010	1.2
Presentations and international conferences		

Steps Forward in Pompe Disease, Londen, England (poster presentation)	2010	1.0
Society for the Study of Inborn Errors of Metabolism Symposium (SSIEM), Istanbul, Turkey (poster presentation)	2010	1.0
Najaarssymposium Vereniging Erfelijke Stofwisselingsziekten Nederland, Zeist (oral presentation)	2010	1.0
9th Pompe disease expert day, Rotterdam (2 oral presentations)	2011	1.0
5th Steps Forward in Pompe Disease Symposium, Budapest, Hungary (oral presentation)	2011	2.0
Spierziektendag Vereniging Spierziekten Nederland (VSN), Lunteren (oral presentation)	2011	1.0
SSIEM, Geneva, Swiss (poster presentation)	2011	1.0
World Muscle Society, Algarve, Portugal (poster presentation)	2011	1.0
10th Pompe disease expert day, Rotterdam (oral presentation)	2012	1.0
SSIEM, Birmingham, England (oral presentation)	2012	2.0
Steps Forward in Pompe Disease, Berlin, Germany (2 poster presentations)	2012	1.0
Spierziektendag VSN, Veldhoven (poster presentation)	2012	0.5
11th Pompe disease expert day, Rotterdam (oral presentation)	2013	1.0
12th Pompe disease expert day, Rotterdam (oral presentation)	2013	1.0
International GSD Conference, Heidelberg, Germany (oral presentation)	2013	1.0
Teaching activities		
Lecture pediatric residents, Erasmus MC	2010	0.6
Supervising Master's thesis	2011-2012	3.0
Other		
Clinical work Division of Metabolic Diseases	2009-2012	
Reviewing paper for peer reviewed journal	2011	0.4