Children with Pompe disease: clinical characteristics, peculiar features and effects of enzyme replacement therapy

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Children with Pompe Disease: Clinical Characteristics, Peculiar Features and Effects of Enzyme Replacement Therapy

Kinderen met de ziekte van Pompe: klinische karakteristieken, bijzondere kenmerken en effecten van enzymtherapie

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Maarten, Jelte en Hidde

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General introduction and outline of this thesis

Pompe disease is a metabolic myopathy. Since the first description of the disease in 1932 by J.C. Pompe,[1] tremendous progress has been made from discovering the biochemical and genetic basis of the disease to developing enzyme replacement therapy (ERT).

With this therapy, the management of Pompe disease has moved from supportive care alone, to a disease-specific intervention aimed at correcting the underlying enzymatic defect. While in the past research mainly concentrated on elucidating the biochemical pathways and pathophysiology of Pompe disease, nowadays focus has shifted towards documenting the natural history of the disease and studying the effect of the new treatment.

A structured follow-up of a large number of patients is difficult in a rare disorder like Pompe disease. The establishment of an expert center for Pompe disease at the Erasmus MC University Medical Center has helped us to systematically study all Pompe patients living in the Netherlands. At present, we follow 149 patients, of which 18 patients are diagnosed with classic-infantile Pompe disease; and 20 children and 111 adults with less progressive forms of the disease.

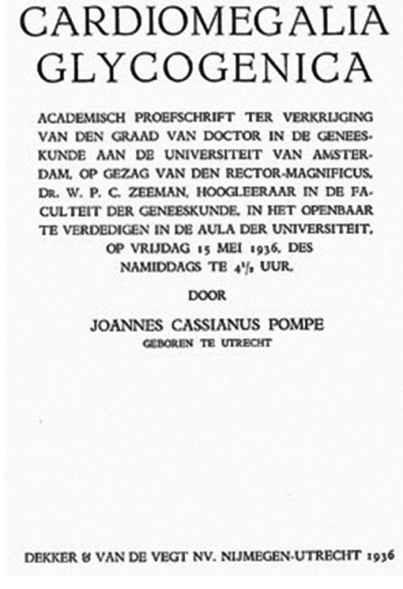
The studies in this thesis have focused on children with Pompe disease. Our aim was to delineate the first presentation and clinical characteristics of the disease and to study the long-term effects of enzyme replacement therapy with recombinant human alpha-glucosidase.

POMPE DISEASE

Pompe disease (OMIM #232300), also called glycogen storage disease type 2 or acid maltase deficiency, is a rare lysosomal storage disorder that was named after the Dutch pathologist JC Pompe (Figure 1A). In 1932 he presented the case of a 7-month old infant who allegedly had died of pneumonia but showed a massively enlarged heart on post-mortem investigation. Initially the patient was diagnosed to have died from idiopathic hypertrophy of the heart and a bronchopneumonia but on further examination Dr. Pompe not only found large amounts of vacuolar glycogen storage in the heart, he also found glycogen in virtually all tissues he

examined. This made him suggest that idiopathic hyperthrophy of the heart was part of a systemic disorder of glycogen metabolism rather than the result of a diffuse tumor (rhabdomyoma) or water accumulation as was suggested before.[1] Dr. Pompe's first publication led to his thesis called *Cardiomegalia Glycogenica* which he finished in 1936 (Figure 1B). In 1939 he started working as a pathologist in a hospital (currently the Onze Lieve Vrouwe Gasthuis) in Amsterdam where he used his laboratory to hide people and station an illegal radio transmitter in collaboration with the Dutch resistance movement in World War II. Unfortunately the radio transmitter was discovered and Dr. Pompe was arrested. In 1945 he was executed as retaliation for the destruction of a railway bridge by the resistance near St Pancras.[2] Although the name Pompe was given to the disease later defined as glycogen storage disease type II, two comparable cases were independently described in the same year as Pompe did, by Putschar and Bischoff.[3,4]

A


CARDIOMEGALIA GLYCOGENICA

ACADEMISCH PROEFSCHRIFT TER VERKRIJGING VAN DEN GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE UNIVERSITEIT VAN AMSTERDAM, OP GEZAG VAN DEN RECTOR-MAGNIFICUS, Dr. W. P. C. ZEEMAN, HOOGLEERAAR IN DE FACULTEIT DER GENEESKUNDE, IN HET OPENBAAR TE VERDEDIGEN IN DE AULA DER UNIVERSITEIT, OP VRIJDAG 15 MEI 1936, DES NAMIDDAGS TE 4½ UUR.

DOOR

JOANNES CASSIANUS POMPE

GEBOREN TE UTRECHT

DEKKER & VAN DE VEGT NV. NIJMEGEN-UTRECHT 1936

B

Figure 1. (**A**) Johannes Cassianus Pompe (1901–1945). (**B**) Dr. Pompe's thesis called Cardiomegalia Glycogenica. Both pictures were taken from Onze Lieve Wetenschap 2013; 1:4-5.

Several major discoveries confirmed Dr. Pompe's observations. After Drs. Gerty and Carl Cori clarified the normal pathway of glycogen metabolism, they focused on the so called 'glycogen storage disorders'.[5] In 1957 Gerty Cori classified 'Pompe disease' as glycogen storage disease type II, but could not discover a defect in the pathway of glycogen degradation as was found for the other glycogen storage disorders.
Around the same time Christian de Duve and colleagues revealed a new cell organelle containing hydrolytic enzymes that were active at acidic pH: the lysosome. These membrane-bound organelles appeared to be responsible for the intracellular digestion of macrocolecules.[6] For this discovery he received the Nobel Price in Physiology or Medicine in 1974. Eight years later Dr. Henri G. Hers and colleagues demonstrated that the lysosomal enzyme acid alpha-glucosidase was deficient in tissues from Pompe patients.[7,8] With this observation they officially identified the first lysosomal storage disorder, of which to date more than 50 have been described.[9]

Clinical spectrum

Pompe disease presents as a continuous spectrum of closely related clinical phenotypes in which progressive muscle weakness is the main clinical manifestation.[10,11]
At the most severe end of the spectrum is the classic-infantile form of the disease.[1,12,13] Skeletal, cardiac and smooth muscles are severely involved and patients show a rapid progression of muscle weakness and usually die within their first year of life. At the other end of the spectrum are patients with more slowly progressive phenotypes; they present with limb-girdle muscle weakness.[14] Age at presentation and rate of disease progression vary. Deterioration of the strength of the limb-girdle and trunk muscles eventually results in wheelchair dependency, respirator need, and shortened life expactancy.[10,11,15-22]

Classic-infantile Pompe disease

The classic-infantile form is relatively homogeneous in presentation and disease course. Patients show their first symptoms at a median age of 1.6 to 2.0 months.[12,13,15,23] Presenting symptoms include feeding difficulties, failure to thrive,

respiratory distress, recurrent respiratory infections, cardiac problems, generalized hypotonia, or paucity of movements. Physical examination typically shows a patient with generalized hypotonia characterized by slipping through, a prominent head lag and frog like position of the legs. Other frequent findings are enlargement of the tongue, decreased tendon reflexes, hepatomegaly, or heart murmur (45–90% of the patients).[12,13,15,23] A severe concentric hypertrophic cardiomyopathy is present in all patients with classic-infantile Pompe disease, is rapidly progressive and may lead to systolic and diastolic dysfunction.[12,13,24-26] Characteristic electrocardiogram (ECG) changes are a short PR interval,[27] increased QT dispersion, and large QRS voltages. Patients are at risk for tachyarrhythmia and sudden death.[28-31]
Motor development is delayed and motor milestones are not achieved or quickly lost. Respiratory infections frequently occur, and cardio-respiratory failure is the main cause of death. Patients usually die within the first year of life at a median age of 6.0 to 8.7 months.[12,13]

Atypical infantile Pompe disease
Closely related to the classic-infantile form of Pompe disease is a group of "atypical" infantile patients. These patients have a similar clinical presentation as those with classic-infantile Pompe disease, but usually present a few months later (mean age at presentation 4.8±2.9 months).[23] Their cardiac hypertrophy is less severe, and they show a more slowly progressive disease course. They survive well beyond the first year of life, as long as they have assisted ventilation and nutritional support (ages at time of description ranged from 1.5 to 13 years of age, n=9). Main cause of death in these patients is respiratory insufficiency.[23,32]

Patients with attenuated forms of Pompe disease
Children and adults with later onset and less progressive forms of Pompe disease represent the majority of patients under care. They are variably referred to as patients with childhood, juvenile, adult or late-onset forms of Pompe disease.[33] The presentation and disease course varies widely between patients[10,11,14-22,34] and sometimes varies even within families.[35]

Several studies were undertaken to delineate the natural history of these forms of Pompe disease[10,11,14-22,34] and showed that the clinical spectrum was much broader than initially recognized. Onset of symptoms can present at any age, ranging from infancy to late adulthood. First symptoms are most often mobility problems caused by limb-girdle muscle weakness, such as difficulties in climbing stairs, rising from a chair, rising from supine position, walking, running, or playing sports. Fatigue,[14,36,37] myalgia[38] and less familiar features such as ptosis,[18,39-43] bulbar weakness[18,39,44-46] and scapular winging[15,18,46] have also been reported. Respiratory muscle weakness has been found in the majority of Pompe patients. Respiratory insufficiency may present at any age, even when patients have mild muscular problems[18,19,47-52] and is the main cause of death.[14,15] Some recent publications indicate that glycogen accumulation in vascular smooth muscle cells can cause dilatative arteriopathy leading to cerebral or thoracic aneuryms that can become another life-threatening complication of Pompe disease.[14,53-63]

Cardiac hypertrophy or conduction problems are rarely found in adult patients,[64,65] although a post-mortem investigation in such a case did reveal glycogen accumulation in cardiomyocytes[66] It has been speculated that this glycogen accumulation may lead to conduction disorders such as Wolff-Parkinson-White syndrome.[34,64]

In general, the muscle weakness in patients with non-classic forms of Pompe disease progresses slowly and the rate of disease progression varies widely.[14,16,18,22,32,51,67] Hagemans showed that approximately 50% of patients have become wheelchair bound or ventilator dependent within 10 to 15 years after diagnosis.[32] In her study, the median survival after diagnosis was 27 years [68] and the disease severity seemed to be related to the disease duration and not to age. Exception to this was a subset of patients under the age of 15 years who showed an extremely rapid disease course.[32] Close follow-up and timely intervention is necessary for these patients.

Pathology

Pompe disease is caused by acid alpha-glucosidase deficiency, an enzyme that catalyzes the hydrolysis of the glycogen that is stored within lysosomes. Partial or total deficiency leads to the accumulation of lysosomal glycogen in all cells of the body, but the pathological changes are most notable in skeletal muscle (Figure 2).[11]

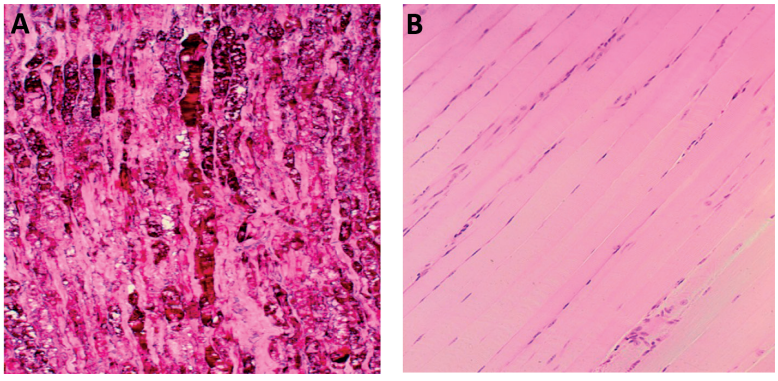


Figure 2. Longitudinal sections of skeletal muscle were stained with Periodic Acid-Schiff (PAS). Panel **A** shows a muscle biopsy of a patient with Pompe disease which contains massive glycogen storage, vacuolar myopathy and myofibrillar loss. Panel **B** shows a muscle biopsy of a healthy control.

Little is known about the exact mechanism through which glycogen accumulation in lysosomes leads to muscle weakness and destruction of skeletal muscle. The process has been extensively studied in both mice and humans.[69-76] It appeared that large clusters of enlarged, glycogen-filled, lysosomes obstruct the longitudinal force transmission of muscle fibers leading to muscle weakness,[75,77] and that additional formation of centrally localized autophagic vacuoles contributes substantially to the cellular damage.[78,79] Continuous accumulation of glycogen may even cause rupture of the lysosomal membrane whereby autolytic enzymes are released into the cytoplasm.[69]

In recent studies in Pompe knock-out (KO) mice, the secondary pathogenic mechanism involving the autophagic pathway was demonstrated.[78-82] Autophagy is a process involving the lysosomal degradation of the cell's own organelles and proteins. Autophagosomes sequester part of the cytoplasm and organelles. They fuse with endosomes (the so-called amphisomes) and lysosomes after which their content is degraded.[83,84] Fukuda et al. described abnormal vesicular trafficking along both the endocytic and the authophagic pathways resulting in expansion of the

endosomal-autophagosomal compartments and defective lysosomal acidification in a Pompe KO mouse model.[81]

The resulting massive accumulation of autophagic debris not only significantly attributes to the muscle damage, but may also pose a serious problem for the delivery of exogenous enzymes to lysosomes, the principle mechanism of enzyme replacement therapy.

Two studies performed in patients with Pompe disease showed that the authophagic process was equally disturbed as in mice.[85,86] But specific involvement of type 2 fibers, as was seen in KO mice, was less evident.[80,85,86] A recent study showed that both views on the pathogenesis of muscle damage may apply: in untreated classic-infantile Pompe disease lysosomal rupture seems to be the predominant cause of muscle damage while accumulation of autophagic material seems to be the major cause of muscle damage in untreated adults.[87]

It is still unknown what triggers the extensive autophagic build-up. Some authors have suggested that oxidative stress, hypoglycaemia, or the generation of substrates for cellular remodelling during muscle differentiation may induce authophagy [85]. As for now, these are just hypotheses, but it is obvious that the acid alpha-glucosidase deficiency and the ensuing lysosomal glycogen storage are at the beginning of the pathological process.

Genetic heterogeneity

Pompe disease is inherited as an autosomal recessive trait and is caused by pathogenic mutations in the gene (OMIM 606800) that codes for lysosomal acid alpha-glucosidase (GAA). The gene is localized on chromosome 17q25.2-25.3.[88-92] It contains 19 coding exons in 20 kb of genomic DNA. At present the Pompe Disease Mutation Database, a database that collects all reported nucleotide variations in the GAA gene (http://www.pompecenter.nl), is listing 372 published sequence variations, of which 248 are proven pathogenic mutations.[93]

The type of mutations and the combination of mutant alleles largely explain the Pompe disease phenotype. Different mutations in the acid α-glucosidase gene lead to different degrees of acid α-glucosidase deficiency, and consequently account for different phenotypes. A combination of two mutations that completely eliminate

enzyme activity are associated with the severe classic-infantile phenotype.[94] This phenotype is quite homogeneous. A combination of mutations that encodes for residual enzyme activity is usually associated with later onset and slowly progressive disease.

Although there seems to be a tendency of finding lower alpha-glucosidase activities in more severely affected patients and higher levels of alpha-glucosidase activities in later presenting cases, the correlation between enzyme activity and phenotype is not strict in this group of patients.[23,94-100] The absence of a genotype-phenotype correlation within groups of patients with identical genotypes and even within families[35,101-103] suggests the involvement of secondary genetic or environmental factors that modulate the phenotype.

While most pathogenic mutations are unique, a subset occurs at higher frequency in certain ethnic groups. The most common mutation found in Caucasians is c.-32-13T>G (also known as IVS1). This mutation was found in more than 70% of children and adults with first symptoms at a relatively late age.[19,21,34,98,102-104]

Other more frequently found mutations are for example c.525delT, delexon 18 (c.2481+102_2646+31del) , and c.925G->A among Caucasians;[105,106] the c.2560C->T (p.R854X) mutation among Africans, African Americans and Brazilians of African descent;[107] the c.1935 C->A (p.D645E) mutation among Asians;[108,109] the c.377G>A mutation among Argentineans;[110] and the c.1905C->A, the c.-32-3C->A, and the c.2560C->T mutations among Brazilians.[111]

Diagnosis

Due to the rarity of Pompe disease and the fact that many signs and symptoms are non-specific and can mimic other musculoskeletal disorders, diagnosing Pompe disease can be challenging. Table 1 shows the differential diagnosis for both the classic-infantile as well as the other phenotypes (adapted from[112]). Now that Pompe disease has become a treatable condition, timely diagnosis can improve patients outcome. Diagnostic delays ranging from 5 to 30 years, as described in the literature[14,16,34] should therefore be avoided.

Table 1. Differential diagnosis of Pompe disease

Classic infantile Pompe disease	Non-Classic Pompe disease (milder phenotypes)
Spinal Muscular Atrophy type I (Werdnig Hoffman disease)	Limb girdle muscular dystrophies (LGMD)
Congenital Muscular dystrophy	Duchenne and Becker Muscular Dystrophies
Glycogen storage diseases III (Cori or Forbes disease) and IV (Anderson disease)	Spinal Muscular Atrophy type II, III and IV
Mitochondrial/respiratory chain disorders	Glycogen storage diseases type V (McArdle disease) and VII (Tauri disease)
Deficiencies in lipoprotein metabolism	Myasthenia Gravis
Fatty acid oxygenation disorders (e.g. VLCADD)	Scapuloperoneal syndromes
Danon disease	Mitochondrial myopathies
Idiopathic hypertrophic cardiomyopathy	Rigid spine syndrome
Peroxismal disorders	Polymyositis/dermatomyositis
Myocarditis	Danon disease
Congenital myopathy (e.g. Nemaline myopathy)	
Hypothyreoidism	
Endocardial fibroelastosis	

Overall, patients suspected of having Pompe disease will first undergo a clinical evaluation including physical examination, muscle strength tests, pulmonary function tests (in sitting and supine position), ECG and echocardiogram, , EMG, and chest x-ray and assessment of motor development in infants. Routine laboratory measurements such as serum CK, ALT, AST and LDH can be elevated, but they have limited diagnostic significance since normal values have been found in patients diagnosed with Pompe disease.[12,14,18,52,113,114] A muscle biopsy, often performed early in the diagnostic work-up of patients with suspected myopathy, may show characteristic vacuoles that contain glycogen and stain positive for PAS and acid phosphatase.[11]

In classic-infantile Pompe disease, marked vacuolization is found in nearly all muscle fibers. In patients with milder phenotypes, on average only 10–50% of the muscle fibers reveal vacuolization.[86] A study performed in 225 child and adult patients with less progressive forms of Pompe disease showed that 20% of them had normal

muscle glycogen content, and that 3% of the muscle biopsies showed no pathology at all.[14] To avoid false-negative results, Pompe disease should be diagnosed by demonstrating partial or complete deficiency of the lysosomal enzyme acid alpha-glucosidase or by mutation analysis.[115,116]

Measurement of alpha-glucosidase activity can be performed in muscle, fibroblasts, leukocytes, lymphocytes, amniotic cells, chorion villi or dried bloodspots. The assay in cultured skin fibroblasts has long been and still is the gold standard.[117] Using the artificial substrate 4-methylumbelliferyl-alpha-D-glucopyranoside (4-MUGluc), the assay is very sensitive at detecting low levels (2% of average normal) of alpha-glucosidase activity and is able to distinguish classic-infantile patients from patients with milder forms of the disease.[11,97] Performing a skin biopsy, on the other hand, is by some clinicians considered invasive and the assay takes several weeks, which significantly delays diagnosis. This is one of the reasons why the focus has shifted towards using blood samples for diagnosing the disease. While the measurement of acid alpha-glucosidase in leukocytes or lymphocytes used to be unreliable,[118,119] Pompe disease can nowadays be diagnosed in whole-blood samples, given that acarbose is added to the assay to inhibit the activity of interfering neutral alpha-glucosidases. The use of glycogen over 4-MUGluc as substrate is preferred since it provides a more precise discrimination between patients and healthy subjects.[120]

Recently, techniques have been developed that can measure alpha-glucosidase activity in dried blood spots using tandem mass spectrometry or fluorimetric procedures. The advantage of dried blood spots is that they can easily be collected and are therefore not only suitable for newborn screening but also provide a rapid first-line test to diagnose Pompe disease.[121-125] It is generally agreed that the outcome of a dried blood spot test needs to be confirmed by more conventional types of testing.[115,126]

Epidemiology

Pompe disease is a rare condition with an estimated frequency of one per 14.000 to one per 300.000 newborns depending on the geographic region that is studied.[17,127-130] The prevalence of Pompe disease varies between different clinical forms and between ethnic groups.

In the Netherlands, Pompe disease is the most common single lysosomal storage disorder with a birth prevalence of 2–3 per 100.000 live births.[131,132] This is different than in other countries. For example, Gaucher disease is the most common single lysosomal storage disorder in Australia (1.8 per 100,000 births),[130] whereas GM2 Gangliosidosis variant B is the most prevalent lysosmal storage disorder in Portugal (3.1 per 100,000 births).[133]
Although prevalence rates of individual LSDs show these diseases to be rare, taken as a group they are quite common with a birth prevalence ranging from one per 4000 to one per 9000 live births.[130,132,133]

TREATMENT OF PATIENTS WITH POMPE DISEASE

In 2006 both the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) granted marketing approval for the drug Myozyme (alglucosidase alfa).[134,135] With this approval a major milestone was reached and alglucosidase alfa became the first treatment ever for Pompe disease. In fact, it is the first therapy ever approved for an inherited muscle disorder.[136]
In healthy human beings, endogenous alpha-glucosidase is synthesized in the rough endoplasmic reticulum (RER) and, after passing through the RER, enters the Golgi complex at the cis-side, binds to the mannose-6-phosphate (M6P) receptor at the trans-side and continues its way to the lysosomes.[137,138] The M6P receptors deliver the enzyme to the late endosomes after which the enzyme ends up in the lysosomes. The M6P receptor recycles back to the trans-Golgi network.[139] Approximately 90% of the M6P receptors are located in the Golgi apparatus or in the endosomal system.[140] The remaining percentage is present at the plasma membrane, where they can bind extracellular enzymes and mediate their transport to the lysosomes. Endocytosis of extracellular enzyme at the plasma membrane is the rationale behind enzyme replacement therapy with alglucosidase alfa.[141]
In 1964, Christian de Duve suggested that lysosomal storage disorders could be treated by replacing the defective enzyme: “In our pathogenic speculations and in our therapeutic attempts, it may be well to keep in mind that any substance

which is taken up intracellularly in an endocytotic process is likely to end up within lysosomes. This obviously opens up many possibilities for interaction, including replacement therapy".[142] The first attempts with exogenous enzymes derived from Aspergillis Niger,[143-145] followed by enzyme preparations from human placenta[146] were unsuccessful. It was not until Fratantoni and Neufeld[147] succeeded in correcting the enzyme defect of cultured fibroblasts from Mucopolysaccharidosis patients via cross-correction that the importance of receptor mediated endocytosis via the M6P receptor was discovered by Sly and colleagues.[148,149]

These findings led to the initial studies in which M6P-containing acid alpha-glucosidase was administered to cultured fibroblasts and skeletal muscle from Pompe patients and knock out mice with Pompe disease.[150-153] After positive results, two methods to produce recombinant acid alpha-glucosidase for the treatment of Pompe disease were almost simultaneously developed. One involved the production of enzyme in the milk of transgenic rabbits, in which genomic DNA constructs were used;[154-156] the other involved Chinese hamster ovary cell lines expressing high levels of recombinant enzyme, in which cDNA constructs were used.[157,158] Both enzyme preparations were tested in patients with Pompe disease. After the initial studies the production of recombinant human alpha-glucosidase from rabbit milk was discontinued as large-scale production appeared to be easier in CHO cells.[159]

Enzyme replacement therapy: outcome in classic-infantile Pompe disease

The first clinical trials studying the effect of enzyme replacement therapy started in 1999.[160-164] The initial studies mainly focused on the classic-infantile phenotype, while only very few patients with less severe phenotypes participated in trials. The experiences gathered in these initial studies are described in the following paragraphs.

Nine infants with Pompe disease were included in the first three pilot studies. One of these eight patients did not have classic-infantile Pompe disease but a variant phenotype.[161] They received recombinant alpha-glucosidase derived from the milk of transgenic rabbits in a weekly dose of 20–40 mg/kg (159[161-163,165] or CHO-cell derived recombinant human alpha-glucosidase initially 5 mg/kg twice weekly, later

10 mg/kg 2–5 times a week.[166-168] The children varied in age from 2.5 to 8 months at start of treatment. Study duration varied between 1.4 to 3 years. All eight patients with classic-infantile Pompe disease survived beyond the first year of life although five with ventilator support. One patient died at the age of four years. A significant improvement in cardiac dimension and function, obtained in all patients, almost certainly contributed to their prolonged survival. Muscle strength and motor function showed a more variable response to treatment; 2/8 patients learned to walk, and 2/8 patients were able to sit without support. The other patients (4/8) showed minor or no motor gains. The most impressive improvements were found in those patients that were least affected at baseline.

It was not until 2006 and 2007 that the results of two open label, multicenter, multinational studies were published.[169,170] The first study included eight infants with Pompe disease. Some of them had classic-infantile Pompe disease and others were atypical variants. The second study included 18 patients and all of them had the classic-infantile phenotype. Their age at baseline ranged from 1 to 14.6 months. Patients received weekly infusions of 10 mg/kg to 20 mg/kg or 40 mg/kg recombinant human alpha-glucosidase from CHO cells. The results of these studies corroborated the initial results. All but one patient showed improvements in cardiac size. Although most patients survived beyond the first year of life, 12 of 26 patients died at a median age of 31 months (range 14.7 to 44 months), the main cause of death being respiratory insufficiency caused by severe respiratory tract infections. Almost 60% of the patients was ventilator-free at study end, while 62% of the patients could sit or even walk.

Infusion associated reactions (IARs) occurred in all clinical trials. Most frequently reported IARs were rashes, urticaria, fever, tachycardia, malaise, blood pressure changes, or bronchospasm. In general, IARs were well managed by slowing or temporarily stopping the infusions, or by administering antihistaminica or corticosteroids. None of the patients discontinued treatment because of unmanageable IARs.

Although these studies showed significant improvements in survival and cardiac size and function, respiratory and motor results were more variable. At present, the causes of variable response remain largely unknown. Possible factors suggested

are patients' condition at baseline, extent of disease-related muscle pathology, and inhibitory antibody formation which may hamper the uptake of the administered enzyme into the muscle cells.

Enzyme replacement therapy: outcome in children and adults

At the time that the studies presented in this thesis started, very little was known about the effect of ERT in children and adults. One pilot study in two juvenile patients (11 and 16 years old at baseline, respectively) and one adult patient (32 years old at baseline), who had received therapy with enzyme from rabbit milk for three years (initially 10 mg/kg/week and after 12 weeks 20 mg/kg/week), had demonstrated positive results.[164] All three patients were wheelchair bound at baseline. The youngest patient had normal respiratory function, but the older two were invasively ventilated. The oldest patient was severely affected. He was bedridden for most of the day. After three years of treatment, the pulmonary function had stabilized in all patients. The patients were less fatigued, which enabled the oldest patient to sit in a wheelchair for most of the day and to participate in family activities. The youngest patient showed the most impressive response. He gained sufficient muscle strength to abandon his wheelchair and to walk after 72 weeks of treatment.

An observational study in three patients, who started enzyme replacement therapy between the ages of 2.8 and 19.9 years,[171] and two case studies, reporting about five adult patients (age at baseline 39–68 years),[172,173] supported these results and showed that ERT can lead to improvements of motor and respiratory functions, especially when treatment is started before significant muscle damage has occurred.[171]

SCOPE AND AIMS OF THIS THESIS

The pilot studies probing the effect of enzyme replacement therapy in classic-infantile Pompe disease had provided a first insight in the possible benefits of this type of treatment. Although more than a decade has passed since those first studies were started, long-term treatment effects are still largely unknown. Now that these patients survive far beyond their first year of life, the most important question is whether the striking regression of cardiac hypertrophy, closely related to decreased morbidity and mortality, sustains. New aspects of the disease may emerge as children survive and grow older.

Most information on presenting symptoms and disease progression in patients with Pompe disease comes from studies that were predominantly performed in adult patients. In retrospect approximately 58% of these adult patients remember that they already had mild muscular problems during childhood,[16] but specific information on disease presentation in children with milder forms of the disease is scarce. In addition, long-term effects of ERT in this group of patients are largely unknown.

The studies described in this thesis had the following objectives: 1) delineate the clinical spectrum of Pompe disease in children with more slowly progressive phenotypes of Pompe disease; 2) evaluate the effect of enzyme-replacement therapy in these children; 3) investigate the long-term outcome of patients with classic-infantile Pompe disease treated with ERT and compare it with the clinical phenotype found in children with less progressive forms; 4) compose a new measurement scale to better assess the motor function of children with Pompe disease.

Most patients described in this thesis participated in a nationwide prospective observational study evaluating the safety and efficacy of ERT in children and adults with Pompe disease. All patients were monitored at regular intervals at the Center for Lysosomal and Metabolic Diseases at the Erasmus MC University Medical Center.

Part I of this thesis focuses on the clinical characteristics and natural course of children with more slowly progressive phenotypes of Pompe disease. **Chapter 2** provides clinical and genetic characteristics of children with more slowly progressive phenotypes of Pompe disease. **Chapter 3** describes the severity of pulmonary involvement and the rate of its progression in children and adults with Pompe disease. The presence and extent of cardiac involvement in children and adults with the common c.-32-13T>G genotype is described in **Chapter 4**.

Part II focuses on the effect of ERT in children and adults with Pompe disease and includes the results of eight years of treatment in two children and one adult with Pompe disease (**Chapter 5**) followed by the results of a three year open-label study investigating the effect of treatment with alpha-glucosidase in five juvenile patients (**Chapter 6**). **Chapter 7** describes the results of the "LOTS" study: a randomized, placebo-controlled multi-center trial in patients with late-onset forms of Pompe disease including 90 patients aged 8 years and older.

Part III focuses on classic-infantile Pompe disease. **Chapter 8** is a case-report of one of the first treated and one of the longest survivors of classic-infantile Pompe disease. This patient is currently 15 years old and developed massive gingival overgrowth at the age of 3 years. The results of long-term ERT on facial muscle function, speech and swallowing are described in **Chapter 9**. The effect of ERT on hearing in **chapter 10,** and on cardiac dimension and function in **Chapter 11**.

In **part IV**, which contains **Chapter 12,** the construction and validation of the quick motor function test (QMFT) is reported: a functional motor scale specifically designed for Pompe disease and used in clinical practice for monitoring disease progression and evaluating therapeutic efficacy.
At the end, the results of the studies presented in this thesis are discussed **in Chapter 13** and future perspectives are given.

REFERENCES

1. Pompe JC. Over idiopathische hypertrofie van het hart. *Ned Tijdsch Geneesk.* 1932;76:304-311.
2. van Schilfgaarde M. J.C. Pompe: OLVG-er, inspirerend patholoog en verzetsheld. *Onze lieve Wetenschap.* 2013 (1):4-5.
3. Putschar W. Uber angeborne Glycogenspeicherkrankheit des herzens:Thesaurismosis glycogenica (v.Gierke). *Beitr Pathol Anat.* 1932; 90:222-232.
4. Bischoff G. Zum klinischen Bild der Glykogen-Speicherungskrankheit (Glykogenose). *Zeitschrift für Kinderheilkunde.* 1932; 52(6):722-726.
5. Cori GT. Glycogen structure and enzyme deficiencies in glycogen storage disease. *Harvey Lectures.* 1954; 8:145.
6. De Duve C, Pressman BC, Gianetto R, Wattiaux R, Appelmans F. Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. *Biochem. J.* 1955; 60:604-617.
7. Hers HG. alpha-Glucosidase deficiency in generalized glycogen storage disease (Pompe's disease). *Biochem. J.* 1963; 86:11-16.
8. Lejeune N, Thinès-Sempoux D, Hers HG. Tissue fractionation studies: Intracellular distribution and properties of α-glucosidases in rat liver. *Biochem. J.* 1963; 86:16-21.
9. Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic and molecular bases of inherited disease.* 8th ed: McGraw-Hill, Inc.; 2001; No. III.
10. van der Ploeg AT, Reuser AJ. Pompe's disease. *Lancet.* Oct 11 2008; 372(9646):1342-1353.
11. Hirschhorn R, Reuser AJJ. Glycogen Storage Disease Type II: acid alpha-glucosidase (acid maltase) deficiency. In: Scriver CR, Beaudet AL, Valle D, Sly WS, eds. *The Metabolic and Molecular Bases of Inherited Disease*. 8 ed. NY: McGraw-Hill; 2001: 3389-3420.
12. Van den Hout HM, Hop W, Van Diggelen OP, et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics.* 2003; 112(2):332-340.
13. Kishnani PS, Hwu WL, Mandel H, Nicolino M, Yong F, Corzo D. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *The Journal of pediatrics.* May 2006; 148(5):671-676.
14. Winkel LP, Hagemans ML, van Doorn PA, et al. The natural course of non-classic Pompe's disease; a review of 225 published cases. *J Neurol.* Aug 2005; 252(8):875-884.
15. Byrne BJ, Kishnani PS, Case LE, et al. Pompe disease: Design, methodology, and early findings from the Pompe Registry. *Molecular genetics and metabolism.* May 2011; 103(1):1-11.
16. Hagemans ML, Winkel LP, Van Doorn PA, et al. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. *Brain.* Mar 2005; 128(Pt 3):671-677.
17. Kishnani PS, Steiner RD, Bali D, et al. Pompe disease diagnosis and management guideline. *Genetics in medicine : official journal of the American College of Medical Genetics.* May 2006; 8(5):267-288.
18. van der Beek NA, de Vries JM, Hagemans ML, et al. Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study. *Orphanet journal of rare diseases.* Nov 12 2012; 7(1):88.
19. Laforet P, Nicolino M, Eymard PB, et al. Juvenile and adult-onset acid maltase deficiency in France: genotype-phenotype correlation. *Neurology.* 2000; 55(8):1122-1128.
20. Engel AG, Gomez MR, Seybold ME, Lambert EH. The spectrum and diagnosis of acid maltase deficiency. *Neurology.* 1973; 23(1):95-106.

21. Wokke JH, Ausems MG, van den Boogaard MJ, et al. Genotype-phenotype correlation in adult-onset acid maltase deficiency. *Annals of neurology.* Sep 1995; 38(3):450-454.
22. Wokke JH, Escolar DM, Pestronk A, et al. Clinical features of late-onset Pompe disease: A prospective cohort study. *Muscle Nerve.* Oct 2008; 38(4):1236-1245.
23. Slonim AE, Bulone L, Ritz S, Goldberg T, Chen A, Martiniuk F. Identification of two subtypes of infantile acid maltase deficiency. *The Journal of pediatrics.* 2000; 137(2):283-285.
24. Seifert BL, Snyder MS, Klein AA, O'Loughlin JE, Magid MS, Engle MA. Development of obstruction to ventricular outflow and impairment of inflow in glycogen storage disease of the heart: serial echocardiographic studies from birth to death at 6 months. *American Heart Journal.* 1992; 123(1):239-242.
25. Ehlers KH, Hagstrom JW, Lukas DS, Redo SF, Engle MA. Glycogen-storage disease of the myocardium with obstruction to left ventricular outflow. *Circulation.* 1962; 25:96-109.
26. Di Sant'Agnese PA, Andersen DH, Mason HH. Glycogen storage disease of the heart.II Critical review of the literature. *Pediatrics.* 1950; 6(4):607-624.
27. Gillette PC, Nihill MR, Singer DB. Electrophysiological mechanism of the short PR interval in Pompe disease. *American Journal of Diseases of Children.* 1974; 128(5):622-626.
28. Ansong AK, Li JS, Nozik-Grayck E, et al. Electrocardiographic response to enzyme replacement therapy for Pompe disease. *Genetics in medicine : official journal of the American College of Medical Genetics.* May 2006; 8(5):297-301.
29. Desena HC, Brumund MR, Superneau D, Snyder CS. Ventricular Fibrillation in a Patient with Pompe Disease: A Cautionary Tale. *Congenit Heart Dis.* Mar 10 2011.
30. McDowell R, Li JS, Benjamin DK, Jr., et al. Arrhythmias in patients receiving enzyme replacement therapy for infantile Pompe disease. *Genetics in medicine : official journal of the American College of Medical Genetics.* Sep 18 2008.
31. Huang PK, Wang CC, Chiu SN, et al. Torsade de pointes ventricular tachycardia during elective intubation in a patient with Pompe disease. *Paediatr Anaesth.* Apr 2008; 18(4):346-348.
32. Hagemans ML, Winkel LP, Hop WC, Reuser AJ, Van Doorn PA, Van der Ploeg AT. Disease severity in children and adults with Pompe disease related to age and disease duration. *Neurology.* Jun 28 2005; 64(12):2139-2141.
33. Gungor D, Reuser AJ. How to describe the clinical spectrum in Pompe disease? *Am J Med Genet A.* Feb 2013; 161A(2):399-400.
34. Muller-Felber W, Horvath R, Gempel K, et al. Late onset Pompe disease: clinical and neurophysiological spectrum of 38 patients including long-term follow-up in 18 patients. *Neuromuscular disorders : NMD.* Oct 2007; 17(9-10):698-706.
35. Wens SC, van Gelder CM, Kruijshaar ME, et al. Phenotypical variation within 22 families with Pompe disease. *Orphanet journal of rare diseases.* Nov 19 2013; 8(1):182.
36. de Vries JM, Hagemans ML, Bussmann JB, van der Ploeg AT, van Doorn PA. Fatigue in neuromuscular disorders: focus on Guillain-Barre syndrome and Pompe disease. *Cell Mol Life Sci.* Nov 16 2009.
37. Hagemans ML, van Schie SP, Janssens AC, van Doorn PA, Reuser AJ, van der Ploeg AT. Fatigue: an important feature of late-onset Pompe disease. *J Neurol.* Mar 12 2007.
38. Gungor D, Schober AK, Kruijshaar ME, et al. Pain in adult patients with Pompe disease: A cross-sectional survey. *Molecular genetics and metabolism.* Jun 7 2013.
39. Barnes D, Hughes RAC, Spencer GT. Adult-onset acid maltase deficiency with prominent bulbar involvement and ptosis. *J R Soc Med.* 1993; 86(1):50.

40. De Wilde F, D'Haens M, Smet H, Martin JJ, Tassignon MJ. Surgical treatment of myogenic blepharoptosis. *Bulletin de la Societe Belge d Ophtalmologie.* 1995; 255:139-146.
41. Groen WB, Leen WG, Vos AM, Cruysberg JR, van Doorn PA, van Engelen BG. Ptosis as a feature of late-onset glycogenosis type II. *Neurology.* Dec 26 2006; 67(12):2261-2262.
42. Ravaglia S, Bini P, Garaghani KS, Danesino C. Ptosis in Pompe disease: common genetic background in infantile and adult series. *J Neuroophthalmol.* Dec 2010; 30(4):389-390.
43. Slingerland NW, Polling JR, van Gelder CM, van der Ploeg AT, Bleyen I. Ptosis, extraocular motility disorder, and myopia as features of pompe disease. *Orbit.* Mar 2011; 30(2):111-113.
44. Hobson-Webb LD, Jones HN, Kishnani PS. Oropharyngeal dysphagia may occur in late-onset Pompe disease, implicating bulbar muscle involvement. *Neuromuscular disorders : NMD.* Jan 15 2013.
45. Maggi L, Salerno F, Bragato C, et al. Familial adult-onset Pompe disease associated with unusual clinical and histological features. *Acta myologica : myopathies and cardiomyopathies : official journal of the Mediterranean Society of Myology / edited by the Gaetano Conte Academy for the study of striated muscle diseases.* Oct 2013; 32(2):85-90.
46. Schuller A, Wenninger S, Strigl-Pill N, Schoser B. Toward deconstructing the phenotype of late-onset Pompe disease. *Am J Med Genet C Semin Med Genet.* Feb 15 2012; 160(1):80-88.
47. Mellies U, Lofaso F. Pompe disease: A neuromuscular disease with respiratory muscle involvement. *Respir Med.* Jan 6 2009.
48. Mellies U, Ragette R, Schwake C, Baethmann M, Voit T, Teschler H. Sleep-disordered breathing and respiratory failure in acid maltase deficiency. *Neurology.* 2001; 57(7):1290-1295.
49. Mellies U, Stehling F, Dohna-Schwake C, Ragette R, Teschler H, Voit T. Respiratory failure in Pompe disease: treatment with noninvasive ventilation. *Neurology.* Apr 26 2005; 64(8):1465-1467.
50. Pellegrini N, Laforet P, Orlikowski D, et al. Respiratory insufficiency and limb muscle weakness in adults with Pompe's disease. *The European respiratory journal.* Dec 2005; 26(6):1024-1031.
51. Van der Beek NA, Hagemans ML, Reuser AJ, et al. Rate of disease progression during long-term follow-up of patients with late-onset Pompe disease. *Neuromuscular disorders : NMD.* Dec 10 2008.
52. Ausems MG, Lochman P, van Diggelen OP, Ploos van Amstel HK, Reuser AJ, Wokke JH. A diagnostic protocol for adult-onset glycogen storage disease type II. *Neurology.* 1999; 52(4):851-853.
53. Braunsdorf WE. Fusiform aneurysm of basilar artery and ectatic internal carotid arteries associated with glycogenosis type 2 (Pompe's disease). *Neurosurgery.* 1987; 21(5):748-749.
54. El-Gharbawy AH, Bhat G, Murillo JE, et al. Expanding the clinical spectrum of late-onset Pompe disease: Dilated arteriopathy involving the thoracic aorta, a novel vascular phenotype uncovered. *Molecular genetics and metabolism.* May 5 2011.
55. Goeber V, Banz Y, Kaeberich A, Carrel T. Huge aneurysm of the ascending aorta in a patient with adult-type Pompe's disease: histological findings mimicking fibrillinopathy. *Eur J Cardiothorac Surg.* Sep 3 2012.
56. Hobson-Webb LD, Proia AD, Thurberg BL, Banugaria S, Prater SN, Kishnani PS. Autopsy findings in late-onset Pompe disease: A case report and systematic review of the literature. *Molecular genetics and metabolism.* May 18 2012.
57. Kretzschmar HA, Wagner H, Hubner G, Danek A, Witt TN, Mehraein P. Aneurysms and vacuolar degeneration of cerebral arteries in late-onset acid maltase deficiency. *Journal of the neurological sciences.* 1990; 98(2-3):169-183.

58. Laforet P, Petiot P, Nicolino M, et al. Dilative arteriopathy and basilar artery dolichoectasia complicating late-onset Pompe disease. *Neurology.* May 27 2008; 70(22):2063-2066.
59. Lilleker J, Roberts M, Boothman B. Dilative vasculopathy and cerebral haemorrhage as a presentation of late-onset pompe disease. *Journal of neurology, neurosurgery, and psychiatry.* Nov 2013; 84(11):e2.
60. Sacconi S, Bocquet JD, Chanalet S, Tanant V, Salviati L, Desnuelle C. Abnormalities of cerebral arteries are frequent in patients with late-onset Pompe disease. *J Neurol.* Jun 18 2010.
61. Matsuoka Y, Senda Y, Hirayama M, Matsui T, Takahashi A. Late-onset acid maltase deficiency associated with intracranial aneurysm. *J Neurol.* 1988; 235(6):371-373.
62. Refai D, Lev R, Cross DT, Shimony JS, Leonard JR. Thrombotic complications of a basilar artery aneurysm in a young adult with Pompe disease. *Surgical neurology.* Jan 18 2008.
63. Makos MM, McComb RD, Adickes ED, Bennett DR. Acid maltase deficiency and basilar artery aneurysms: a report of a sibship. *Neurology.* 1985; 35 Suppl 1:193-194.
64. van der Beek NA, Soliman OI, van Capelle CI, et al. Cardiac evaluation in children and adults with Pompe disease sharing the common c.-32-13T>G genotype rarely reveals abnormalities. *Journal of the neurological sciences.* Aug 29 2008.
65. Forsha D, Li JS, Smith PB, van der Ploeg AT, Kishnani P, Pasquali SK. Cardiovascular abnormalities in late-onset Pompe disease and response to enzyme replacement therapy. *Genetics in medicine: official journal of the American College of Medical Genetics.* May 3 2011.
66. van der Walt JD, Swash M, Leake J, Cox EL. The pattern of involvement of adult-onset acid maltase deficiency at autopsy. *Muscle & Nerve.* 1987; 10(3):272-281.
67. Hagemans ML, Hop WJ, van Doorn PA, Reuser AJ, van der Ploeg AT. Course of disability and respiratory function in untreated late-onset Pompe disease. *Neurology.* 2006; 66:581-583.
68. Gungor D, de Vries JM, Hop WC, et al. Survival and associated factors in 268 adults with Pompe disease prior to treatment with enzyme replacement therapy. *Orphanet journal of rare diseases.* Jun 1 2011; 6(1):34.
69. Griffin JL. Infantile acid maltase deficiency. II. Muscle fiber hypertrophy and the ultrastructure of end-stage fibers. *Virchows Archiv. B, Cell Pathology Including Molecular Pathology.* 1984; 45(1):37-50.
70. Cardiff RD. A histochemical and electron microscopic study of skeletal muscle in a case of Pompe's disease (glycogenosis II). *Pediatrics.* 1966; 37(2):249-259.
71. Hug G, Garancis JC, Schubert WK, Kaplan S. Glycogen storage disease: types II, III, VII and IX. *Am J Dis Child.* 1966; 11:457-474.
72. Martin JJ, De Barsy T, Van Hoof F, Palladini G. Pompe's disease: an inborn lysosomal disorder with storage of glycogen. A study of brain and striated muscle. *Acta Neuropathol.* 1973; 23(3):229-244.
73. Garancis JC. Type II Glycogenosis; Biochemical and electron microscopic study. *Americ J Med.* 1968; 44:289-299.
74. Thurberg BL, Lynch Maloney C, Vaccaro C, et al. Characterization of pre- and post-treatment pathology after enzyme replacement therapy for pompe disease. *Laboratory investigation; a journal of technical methods and pathology.* Oct 30 2006; 86(12):1208-1220.
75. Hesselink RP, Schaart G, Wagenmakers AJ, Drost MR, van der Vusse GJ. Age-related morphological changes in skeletal muscle cells of acid alpha-glucosidase knockout mice. *Muscle Nerve.* Apr 2006; 33(4):505-513.

76. Hesselink RP, Gorselink M, Schaart G, et al. Impaired performance of skeletal muscle in alpha-glucosidase knockout mice. *Muscle Nerve.* 2002; 25(6):873-883.
77. Hesselink RP, Wagenmakers AJ, Drost MR, Van der Vusse GJ. Lysosomal dysfunction in muscle with special reference to glycogen storage disease type II. *Biochim Biophys Acta.* Mar 20 2003; 1637(2):164-170.
78. Raben N, Hill V, Shea L, et al. Suppression of autophagy in skeletal muscle uncovers the accumulation of ubiquitinated proteins and their potential role in muscle damage in Pompe disease. *Hum Mol Genet.* Sep 9 2008.
79. Raben N, Roberts A, Plotz PH. Role of autophagy in the pathogenesis of Pompe disease. *Acta myologica.* Jul 2007; 26(1):45-48.
80. Fukuda T, Roberts A, Ahearn M, et al. Autophagy and Lysosomes in Pompe Disease. *Autophagy.* Oct 5 2006; 2(4).
81. Fukuda T, Ewan L, Bauer M, et al. Dysfunction of endocytic and autophagic pathways in a lysosomal storage disease. *Annals of neurology.* Apr 2006; 59(4):700-708.
82. Shea L, Raben N. Autophagy in skeletal muscle: implications for Pompe disease. *Int J Clin Pharmacol Ther.* 2009; 47 Suppl 1:S42-47.
83. Stromhaug PE, Berg TO, Fengsrud M, Seglen PO. Purification and characterization of autophagosomes from rat hepatocytes. *The Biochemical journal.* Oct 15 1998; 335 (Pt 2):217-224.
84. Liou W, Geuze HJ, Geelen MJ, Slot JW. The autophagic and endocytic pathways converge at the nascent autophagic vacuoles. *The Journal of cell biology.* Jan 13 1997; 136(1):61-70.
85. Raben N, Takikita S, Pittis MG, et al. Deconstructing Pompe Disease by Analyzing Single Muscle Fibers: To See a World in a Grain of Sand...''. *Autophagy.* Jun 15 2007; 3(6).
86. Schoser BG, Muller-Hocker J, Horvath R, et al. Adult-onset glycogen storage disease type 2: clinico-pathological phenotype revisited. *Neuropathol Appl Neurobiol.* Oct 2007; 33(5):544-559.
87. Raben N, Ralston E, Chien YH, et al. Differences in the predominance of lysosomal and autophagic pathologies between infants and adults with Pompe disease: implications for therapy. *Molecular genetics and metabolism.* Aug 7 2010.
88. Halley DJ, Konings A, Hupkes P, Galjaard H. Regional mapping of the human gene for lysosomal alpha-glucosidase by in situ hybridization. *Human genetics.* 1984; 67(3):326-328.
89. Martiniuk F, Ellenbogen A, Hirschhorn K, Hirschhorn R. Further regional localization of the genes for human acid alpha glucosidase (GAA), peptidase D (PEPD), and alpha mannosidase B (MANB) by somatic cell hybridization. *Human genetics.* 1985; 69(2):109-111.
90. Martiniuk F, Mehler M, Tzall S, Meredith G, Hirschhorn R. Sequence of the cDNA and 5'-flanking region for human acid alpha-glucosidase, detection of an intron in the 5' untranslated leader sequence, definition of 18-bp polymorphisms, and differences with previous cDNA and amino acid sequences. *DNA and cell biology.* 1990; 9(2):85-94.
91. Hoefsloot LH, Hoogeveen-Westerveld M, Kroos MA, van Beeumen J, Reuser AJ, Oostra BA. Primary structure and processing of lysosomal alpha-glucosidase; homology with the intestinal sucrase-isomaltase complex. *EMBO Journal.* 1988; 7(6):1697-1704.
92. Hoefsloot LH, Hoogeveen-Westerveld M, Reuser AJJ, Oostra BA. Characterization of the human lysosomal alpha-glucosidase gene. *Biochem. J.* 1990; 272(2):493-497.
93. Kroos M, Hoogeveen-Westerveld M, Michelakakis H, et al. Update of the pompe disease mutation database with 60 novel GAA sequence variants and additional studies on the functional effect of 34 previously reported variants. *Human mutation.* Aug 2012; 33(8):1161-1165.

94. Kroos M, Hoogeveen-Westerveld M, van der Ploeg A, Reuser AJ. The genotype-phenotype correlation in Pompe disease. *Am J Med Genet C Semin Med Genet.* Feb 15 2012; 160(1):59-68.
95. Hermans MM, De Graaff E, Kroos MA, et al. The effect of a single base pair deletion (delta T525) and a C1634T missense mutation (pro545leu) on the expression of lysosomal alpha-glucosidase in patients with glycogen storage disease type II. *Human Molecular Genetics.* 1994; 3(12):2213-2218.
96. Hermans MM, van Leenen D, Kroos MA, et al. Twenty-two novel mutations in the lysosomal alpha-glucosidase gene (GAA) underscore the genotype-phenotype correlation in glycogen storage disease type II. *Human mutation.* Jan 2004; 23(1):47-56.
97. Reuser, Kroos MA, Hermans MMP, et al. Glycogenosis type II (acid maltase deficiency). *Muscle Nerve.* 1995; 3(9):S61-S69.
98. Pittis MG, Filocamo M. Molecular genetics of late onset glycogen storage disease II in Italy. *Acta myologica: myopathies and cardiomyopathies: official journal of the Mediterranean Society of Myology / edited by the Gaetano Conte Academy for the study of striated muscle diseases.* Jul 2007; 26(1):67-71.
99. Bali DS, Tolun AA, Goldstein JL, Dai J, Kishnani PS. Molecular analysis and protein processing in late-onset pompe disease patients with low levels of acid alpha-glucosidase activity. *Muscle Nerve.* May 2011; 43(5):665-670.
100. Kroos, Kirschner J, Gellerich FN, et al. A case of childhood Pompe disease demonstrating phenotypic variability of p.Asp645Asn. *Neuromuscular disorders.* Jun 2004;14(6):371-374.
101. Kroos MA, Van der Kraan M, Van Diggelen OP, Kleijer WJ, Reuser AJ. Two extremes of the clinical spectrum of glycogen storage disease type II in one family: a matter of genotype. *Human mutation.* 1997; 9(1):17-22.
102. Kroos, Pomponio RJ, Hagemans ML, et al. Broad spectrum of Pompe disease in patients with the same c.-32-13T->G haplotype. *Neurology.* Jan 9 2007; 68(2):110-115.
103. Huie ML, Chen AS, Tsujino S, et al. Aberrant splicing in adult onset glycogen storage disease type II (GSDII): molecular identification of an IVS1 (-13T->G) mutation in a majority of patients and a novel IVS10 (+1GT->CT) mutation. *Human Molecular Genetics.* 1994; 3(12):2231-2236.
104. Montalvo AL, Bembi B, Donnarumma M, et al. Mutation profile of the GAA gene in 40 Italian patients with late onset glycogen storage disease type II. *Human mutation.* Oct 2006; 27(10):999-1006.
105. Van der Kraan M, Kroos MA, Joosse M, et al. Deletion of exon 18 is a frequent mutation in glycogen storage disease type II. *Biochemical & Biophysical Research Communications.* 1994; 203(3):1535-1541.
106. Kroos MA, Van der Kraan M, Van Diggelen OP, et al. Glycogen storage disease type II: frequency of three common mutant alleles and their associated clinical phenotypes studied in 121 patients. *J Med Genet.* 1995; 32(10):836-837.
107. Becker JA, Vlach J, Raben N, et al. The African origin of the common mutation in African American patients with glycogen-storage disease type II. *American journal of human genetics.* 1998; 62(4):991-994.
108. Shieh JJ, Wang LY, Lin CY. Point mutation in Pompe disease in Chinese. *Journal of inherited metabolic disease.* 1994; 17(1):145-148.
109. Shieh JJ, Lin CY. Frequent mutation in Chinese patients with infantile type of GSD II in Taiwan: evidence for a founder effect. *Human mutation.* 1998; 11(4):306-312.

110. Palmer RE, Amartino HM, Niizawa G, Blanco M, Pomponio RJ, Chamoles NA. Pompe disease (glycogen storage disease type II) in Argentineans: Clinical manifestations and identification of 9 novel mutations. *Neuromuscular disorders: NMD.* Oct 20 2006; 17(1):16-22.
111. Oba-Shinjo SM, da Silva R, Andrade FG, et al. Pompe disease in a Brazilian series: clinical and molecular analyses with identification of nine new mutations. *J Neurol.* Jul 9 2009; 256(11):1881-1890.
112. van der Beek NA, Hagemans ML, van der Ploeg AT, Reuser AJ, van Doorn PA. Pompe disease (glycogen storage disease type II): clinical features and enzyme replacement therapy. *Acta Neurol Belg.* Jun 2006; 106(2):82-86.
113. Di Fiore MT, Manfredi R, Marri L, Zucchini A, Azzaroli L, Manfredi G. Elevation of transaminases as an early sign of late-onset glycogenosis type II [letter]. *European journal of pediatrics.* 1993; 152(9):784.
114. Toscano A, Schoser B. Enzyme replacement therapy in late-onset Pompe disease: a systematic literature review. *J Neurol.* Aug 28 2012.
115. Reuser, Verheijen F, Kroos M, et al. Enzymatic and molecular strategies to diagnose Pompe disease. *Expert Opin Med Diagn.* Jan 2010; 4(1):79-89.
116. Vissing J, Lukacs Z, Straub V. Diagnosis of Pompe disease: muscle biopsy vs blood-based assays. *JAMA neurology.* Jul 2013; 70(7):923-927.
117. Heukels-Dully MJ, Niermeijer MF. Variation in lysosomal enzyme activity during growth in culture of human fibroblasts and amniotic fluid cells. *Experimental cell research.* Feb 1976; 97(2):304-312.
118. Dreyfus JC, Poenaru L. Alpha glucosidases in white blood cells, with reference to the detection of acid alpha 1-4 glucosidase deficiency. *Biochemical and biophysical research communications.* Nov 29 1978; 85(2):615-622.
119. Whitaker CH, Felice KJ, Natowicz M. Biopsy-proven alpha-glucosidase deficiency with normal lymphocyte enzyme activity. *Muscle Nerve.* Mar 2004; 29(3):440-442.
120. Okumiya T, Keulemans JL, Kroos MA, et al. A new diagnostic assay for glycogen storage disease type II in mixed leukocytes. *Molecular genetics and metabolism.* May 2006; 88(1):22-28.
121. Li Y, Scott CR, Chamoles NA, et al. Direct Multiplex Assay of Lysosomal Enzymes in Dried Blood Spots for Newborn Screening. *Clin Chem.* Aug 3 2004; 50(10):1785-1796.
122. Chamoles NA, Niizawa G, Blanco M, Gaggioli D, Casentini C. Glycogen storage disease type II: enzymatic screening in dried blood spots on filter paper. *Clin Chim Acta.* Sep 2004; 347(1-2):97-102.
123. Umapathysivam K, Hopwood JJ, Meikle PJ. Determination of Acid alpha-Glucosidase Activity in Blood Spots as a Diagnostic Test for Pompe Disease. *Clin Chem.* 2001; 47(8):1378-1383.
124. Zhang H, Kallwass H, Young SP, et al. Comparison of maltose and acarbose as inhibitors of maltase-glucoamylase activity in assaying acid alpha-glucosidase activity in dried blood spots for the diagnosis of infantile Pompe disease. *Genetics in medicine : official journal of the American College of Medical Genetics.* May 2006; 8(5):302-306.
125. Shigeto S, Katafuchi T, Okada Y, et al. Improved assay for differential diagnosis between Pompe disease and acid alpha-glucosidase pseudodeficiency on dried blood spots. *Molecular genetics and metabolism.* Jan 22 2011.
126. Pompe Disease Diagnostic Working G, Winchester B, Bali D, et al. Methods for a prompt and reliable laboratory diagnosis of Pompe disease: report from an international consensus meeting. *Molecular genetics and metabolism.* Mar 2008; 93(3):275-281.

127. Lin CY, Hwang B, Hsiao KJ, Jin YR. Pompe's disease in Chinese and prenatal diagnosis by determination of alpha-glucosidase activity. *Journal of inherited metabolic disease.* 1987; 10(1):11-17.
128. Ausems MG, Verbiest J, Hermans MP, et al. Frequency of glycogen storage disease type II in The Netherlands: implications for diagnosis and genetic counselling. *European journal of human genetics : EJHG.* Sep 1999; 7(6):713-716.
129. Martiniuk F, Chen A, Mack A, et al. Carrier frequency for glycogen storage disease type II in New York and estimates of affected individuals born with the disease. *American journal of medical genetics.* Aug 27 1998; 79(1):69-72.
130. Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. *JAMA: the journal of the American Medical Association.* Jan 20 1999; 281(3):249-254.
131. Ausems MG, ten Berg K, Kroos MA, et al. Glycogen storage disease type II: birth prevalence agrees with predicted genotype frequency. *Community Genet.* 1999; 2(2-3):91-96.
132. Poorthuis BJ, Wevers RA, Kleijer WJ, et al. The frequency of lysosomal storage diseases in The Netherlands. *Human genetics.* 1999; 105(1-2):151-156.
133. Pinto R, Caseiro C, Lemos M, et al. Prevalence of lysosomal storage diseases in Portugal. *European journal of human genetics: EJHG.* Feb 2004; 12(2):87-92.
134. FDA. FDA approves first treatment for Pompe disease. 2006; http://www.fda.gov/newsevents/newsroom/pressannouncements/2006/ucm108645.htm.
135. EMA. Myozyme. 2006.
136. Wagner KR. Enzyme replacement for infantile Pompe disease: the first step toward a cure. *Neurology.* Jan 9 2007; 68(2):88-89.
137. Ashwell G, Morell AG. The role of surface carbohydrates in the hepatic recognition and transport of circulating glycoproteins. *Advances in enzymology and related areas of molecular biology.* 1974; 41(0):99-128.
138. Kaplan A, Achord DT, Sly WS. Phosphohexosyl components of a lysosomal enzyme are recognized by pinocytosis receptors on human fibroblasts. *Proc. Natl. Acad. Sci. USA.* 1977;74(5):2026-2030.
139. Kornfeld S. Structure and function of the mannose 6-phosphate/insulinlike growth factor II receptors. *Annu Rev Biochem.* 1992; 61:307-330.
140. Hebert E. Mannose-6-phosphate/insulin-like growth factor II receptor expression and tumor development. *Bioscience reports.* Feb 2006; 26(1):7-17.
141. Stahl P, Schwartz AL. Receptor-mediated endocytosis. *The Journal of clinical investigation.* Mar 1986; 77(3):657-662.
142. De Duve C. From cytosis to lysosomes. *Fed. Proc. Am. Soc. Exp. Biol.* 1964; 23:1045-1049.
143. Baudhuin P, Hers HG, Loeb H. An electron microscopic and biochemical study of type II glycogenosis. *Lab. Invest.* 1964; 13:1139-1152.
144. Hug G, Schubert WK. Lysosomes in type II glycogenosis. Changes during administration of extract from Aspergillus niger. *The Journal of cell biology.* 1967; 35(1):C1-6.
145. Lauer RM, Mascarinas T, Racela AS, Diehl AM, Brown BI. Administration of a mixture of fungal glucosidases to a patient with type II glycogenosis (Pompe's disease). *Pediatrics.* 1968; 42:672-676.
146. de Barsy T, Jacquemin P, Van Hoof F, Hers HG. Enzyme replacement in Pompe disease: an attempt with purified human acid alpha-glucosidase. *Birth Defects: Original Article Series.* 1973; 9(2):184-190.

147. Fratantoni JC, Hall CW, Neufeld EF. Hurler and Hunter syndromes: mutual correction of the defect in cultured fibroblasts. *Science.* Nov 1 1968; 162(3853):570-572.

148. Hickman S, Shapiro LJ, Neufeld EF. A recognition marker required for uptake of a lysosomal enzyme by cultured fibroblasts. *Biochemical and biophysical research communications.* Mar 15 1974; 57(1):55-61.

149. Sly WS, Kaplan A, Achord DT, Brot FE, Bell CE. Receptor-mediated uptake of lysosomal enzymes. *Progress in clinical and biological research.* 1978; 23:547-551.

150. Reuser AJ, Kroos MA, Ponne NJ, et al. Uptake and stability of human and bovine acid alpha-glucosidase in cultured fibroblasts and skeletal muscle cells from glycogenosis type II patients. *Experimental cell research.* 1984; 155(1):178-189.

151. van der Ploeg AT, Kroos M, van Dongen JM, et al. Breakdown of lysosomal glycogen in cultured fibroblasts from glycogenosis type II patients after uptake of acid alpha-glucosidase. *Journal of the neurological sciences.* 1987; 79(3):327-336.

152. Van der Ploeg AT, Bolhuis PA, Wolterman RA, et al. Prospect for enzyme therapy in glycogenosis II variants: a study on cultured muscle cells. *Journal of Neurology.* 1988; 235(7):392-396.

153. Van der Ploeg AT, Loonen MC, Bolhuis PA, Busch HM, Reuser AJ, Galjaard H. Receptor-mediated uptake of acid alpha-glucosidase corrects lysosomal glycogen storage in cultured skeletal muscle. *Pediatric research.* 1988; 24(1):90-94.

154. Bijvoet AG, Kroos MA, Pieper FR, et al. Expression of cDNA-encoded human acid alpha-glucosidase in milk of transgenic mice. *Biochimica et Biophysica Acta.* 1996; 1308(2):93-96.

155. Bijvoet AG, Kroos MA, Pieper FR, et al. Recombinant human acid alpha-glucosidase: high level production in mouse milk, biochemical characteristics, correction of enzyme deficiency in GSDII KO mice. *Human Molecular Genetics.* 1998; 7(11):1815-1824.

156. Bijvoet AG, Van Hirtum H, Kroos MA, et al. Human acid alpha-glucosidase from rabbit milk has therapeutic effect in mice with glycogen storage disease type II. *Human Molecular Genetics.* 1999; 8(12):2145-2153.

157. Van Hove JL, Yang HW, Wu JY, Brady RO, Chen YT. High-level production of recombinant human lysosomal acid alpha-glucosidase in Chinese hamster ovary cells which targets to heart muscle and corrects glycogen accumulation in fibroblasts from patients with Pompe disease. *Proceedings of the National Academy of Sciences of the United States of America.* 1996; 93(1):65-70.

158. Fuller M, Van der Ploeg A, Reuser AJ, Anson DS, Hopwood JJ. Isolation and characterisation of a recombinant, precursor form of lysosomal acid alpha-glucosidase. *European Journal of Biochemistry.* 1995; 234(3):903-909.

159. Reuser AJ, Van Den Hout H, Bijvoet AG, Kroos MA, Verbeet MP, Van Der Ploeg AT. Enzyme therapy for Pompe disease: from science to industrial enterprise. *European journal of pediatrics.* 2002; 161 Suppl 1:S106-111.

160. Van den Hout H, Reuser AJ, Vulto AG, Loonen MC, Cromme-Dijkhuis A, Van der Ploeg AT. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients. *Lancet.* 2000; 356(9227):397-398.

161. Amalfitano A, Bengur AR, Morse RP, et al. Recombinant human acid alpha-glucosidase enzyme therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2001; 3(2):132-138.

162. Klinge L, Straub V, Neudorf U, et al. Safety and efficacy of recombinant acid alpha-glucosidase (rhGAA) in patients with classical infantile Pompe disease: results of a phase II clinical trial. *Neuromuscular disorders.* Jan 2005; 15(1):24-31.

163. Klinge L, Straub V, Neudorf U, Voit T. Enzyme replacement therapy in classical infantile pompe disease: results of a ten-month follow-up study. *Neuropediatrics.* Feb 2005; 36(1):6-11.

164. Winkel LP, Van den Hout JM, Kamphoven JH, et al. Enzyme replacement therapy in late-onset Pompe's disease: a three-year follow-up. *Annals of neurology.* Apr 2004; 55(4):495-502.

165. Van den Hout JM, Reuser AJ, de Klerk JB, Arts WF, Smeitink JA, Van der Ploeg AT. Enzyme therapy for pompe disease with recombinant human alpha- glucosidase from rabbit milk. *Journal of inherited metabolic disease.* 2001; 24(2):266-274.

166. Reuser A. Inconsistent reporting about dosing, dosing regimen, and immunomodulation therapy in Pompe disease. *Genetics in medicine: official journal of the American College of Medical Genetics.* Aug 9 2012.

167. Reuser AJ. Enzyme therapy in Pompe disease: questions remain. *Molecular genetics and metabolism.* May 25 2012.

168. Banugaria SG, Patel TT, Mackey J, et al. Persistence of high sustained antibodies to enzyme replacement therapy despite extensive immunomodulatory therapy in an infant with Pompe disease: Need for agents to target antibody-secreting plasma cells. *Molecular genetics and metabolism.* Apr 2012; 105(4):677-680.

169. Kishnani PS, Corzo D, Nicolino M, et al. Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology.* Jan 9 2007; 68(2):99-109.

170. Kishnani PS, Nicolino M, Voit T, et al. Chinese hamster ovary cell-derived recombinant human acid alpha-glucosidase in infantile-onset Pompe disease. *The Journal of pediatrics.* Jul 2006; 149(1):89-97.

171. Rossi M, Parenti G, Della Casa R, et al. Long-term enzyme replacement therapy for pompe disease with recombinant human alpha-glucosidase derived from chinese hamster ovary cells. *J Child Neurol.* May 2007; 22(5):565-573.

172. Ravaglia S, Danesino C, Pichiecchio A, et al. Enzyme replacement therapy in severe adult-onset glycogen storage disease type II. *Adv Ther.* Aug 12 2008.

173. Merk T. Enzyme replacement therapy in late-onset type II glycogenosis. *Eur J Neurol.* Jul 2009; 16(7):e124.

PART I

Natural course

2

Childhood Pompe disease:

clinical spectrum and genotype in 31 children

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ABSTRACT

Introduction

Pompe disease is a lysosomal storage disorder that presents as a progressive myopathy. The aim of the present study was to gain knowledge on the presentation of Pompe disease in children with a non-classic presentation of the disease and to describe their specific clinical characteristics and genotypes.

Methods

We conducted a single-center, cross-sectional study. All patients younger than 18 years of age that were referred to our clinic between 1975-2012 were included in this study. Patients were excluded if they were diagnosed with the classic-infantile form of Pompe disease. Information considering first symptoms, diagnosis and the use of a wheelchair or respirator was collected. Muscle strength, pulmonary function, and cardiac function were assessed and mutation analyses and blood tests including CK, LDH, and transaminases, were performed.

Results

31 juvenile Pompe patients participated in this cross sectional study. First symptoms presented at a median age of 2.3 years and diagnosis was made at a median age of 4 years. Most common first problems were related to delayed motor development and limb-girdle weakness. Most prominent muscle weakness was found in the neck flexors, neck extensors and flexors of the hip. Muscle strength, measured by HHD, ranged from 0.3% to 91% of normal. Pulmonary function was decreased in 50% of the patients in a sitting position and in 59% of the patients in supine position. 68% of the patients carried the c.-32-13T>G mutation. These patients were predominantly male.

Conclusion

Our study demonstrates that disease severity varies in childhood but that a substantial part of patients is already severely affected at young age. Disease presentation, distribution of muscle weakness and occurrence of specific symptoms like ptosis can be different from adult patients. Patients with other mutations than the c.32-13T>G mutation were in general more severely affected. Patients with the c.-32-13T>G mutation/null genotype were predominantly male.

INTRODUCTION

Pompe disease, also known as acid maltase deficiency or glycogen storage disease type 2 (OMIM 232300), is a lysosomal storage disorder that presents as a progressive myopathy. Deficiency of the enzyme acid α-glucosidase (EC 3.2.1.20) causes glycogen to accumulate in lysosomes, and ultimately leads to cell destruction.[1-3] In 1932, J.C. Pompe first described the classic-infantile form of the disease.[4] These patients present with generalized and severe muscle weakness, hypertrophic cardiomyopathy, and usually die within their first year of life.[5,6] Later, other forms were reported and Pompe disease appeared to be a continuous spectrum with the severe classic-infantile form at one end, and milder presentations, also referred to as childhood, juvenile, adult, and late-onset phenotypes, at the other end. The latter patients usually do not have hypertrophic cardiomyopathy and present with a more slowly progressive limb-girdle muscle weakness, which eventually results in wheelchair dependency, respirator need, and shortened life expectance.[2,7-17]

Numerous publications can be found in literature describing the natural history of the disease in classic-infantile and adult Pompe patients.[5,6,10-12,16] But information on the disease presentation of children not fulfilling the criteria of classic-infantile Pompe disease is scarce.[18]

The aim of the present study was to gain knowledge on the presentation of Pompe disease in children and to describe their specific clinical characteristics. For this purpose we collected information on disease symptoms, distribution and severity of muscle weakness, involvement of respiratory function, cardiac structure and function, physical limitations, and genotypes of 31 children diagnosed with a non-classic presentation of Pompe disease. We compared our findings to Pompe patients presenting at adult age.

METHODS

Subjects

All patients younger than 18 years of age that were diagnosed or referred to our center between 1975 and 2010, either coming from the Netherlands or abroad, were included in this observational study. Patients were diagnosed by measurement of α-glucosidase activity in cultured fibroblasts, leukocytes or muscle biopsy specimens, and mutation analysis. Patients were excluded if they were diagnosed with classic-infantile Pompe disease.

Thirty-one patients participated in this cross-sectional study. Patients were evaluated as part of an Institutional Review Board approved study (n=28) or as part of routine clinical evaluation (n=3). The study was approved by the Institutional review Board. A medical history was obtained at first visit. For each patient, the following data were collected: gender, current age, geographic origin, first symptoms, age at first symptoms, age at diagnosis, wheelchair use, respiratory support, specific clinical findings (e.g. facial muscle weakness, bulbar muscle weakness, scoliosis, contractures, muscle atrophy), functional impairments, and weight and height. Low body weight was defined as weight corrected for height that was below -2SD for peers. Blood tests included measurement of creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH). All patients underwent clinical and neurological examination, performed by a paediatrician, and child neurologist.

Muscle strength testing

Muscle strength was assessed by both manual muscle strength testing (MMT) (n=24) and hand held dynamometry (HHD) (n=24).[19,20] The following muscle groups were tested with HHD: neck flexors, shoulder abductors, elbow flexors, wrist extensors, hip flexors, hip abductors, knee extensors, knee flexors, foot dorsal flexors. HHD scores (Newton) were expressed as percentages of the reference values (50th percentile) for healthy peers.[20] All percentages were cumulated and divided by 9 to obtain a total HHD sum score expressed in percentage of normal (ranging from 0 to 100%).

MMT was performed according to the Medical Research Council guidelines[21] for the following muscle groups: neck flexors, neck extensors, deltoid muscles, biceps, triceps, wrist extensors, hip flexors, hip extensors, hip abductors, hip adductors, knee flexors, knee extensors, and foot dorsal and plantar flexors.

Pulmonary function testing

Pulmonary function testing was performed by spirometry in both upright seated (n=28), and supine position (n=23) according to ATS/ERS standards.[22] The maximum value of three reproducible tests was used for the analysis. The results were expressed as percentage predicted values.[23] Three patients were too young to reliably perform spirometry. Pulmonary function of five other patients was compromised to the extent that they were unable to endure pulmonary function testing in supine position.

Cardiac assessment

Conventional Doppler, and 2D M-mode tracings were performed in all patients according to the recommendations of the American Society of Echocardiography by an experienced sonographer (JP) (Sonos 5500 ultrasound system, Philips, Best, the Netherlands). In addition, standard 12 lead electrocardiograms were made and analyzed by a paediatric cardiologist.

Enzymatic and molecular assays

The acid α-glucosidase activity was measured in leukocytes[24] and cultured skin fibroblasts[15] according to standard procedures, and was expressed in nmol/hr/mg protein. The protein concentrations of cell homogenates was measured as previously described.[25]

Genomic DNA was isolated from blood or cultured fibroblasts and mutation analysis was performed according to standard procedures.[15,26] The mutations were rated by severity using the format of Kroos et al.[27] To determine the severity of the various mutations, this format takes into account both the quantity and quality of acid α-glucosidase (GAA) expression, and the functional effect of the mutation on enzyme activity.[26] For splice-site mutations, the effect of the mutations was

examined by real-time PCR, to determine the quality and efficiency of mutant GAA-mRNA.[27]

Statistics

Demographic and clinical data were summarized using descriptive statistics including mean, SD, median, ranges, and percentages. Differences between groups were analyzed using a Mann-Whitney test because the data were not normally distributed. P-values <0.05 were considered statistically significant. All statistical analyses were performed using SPSS for Windows version 17.0.

RESULTS

Symptom onset and diagnosis

Thirty-one children participated in this cross-sectional study. Table 1 shows the patient characteristics and genotype. Twenty-two patients were male, nine were female. Seventeen patients were from the Netherlands, 4 from Belgium, 5 from Germany, 3 from Greece, 1 from Great Britain, and 1 from the United States.

The median age at which patients experienced their first symptom was 2.3 years. Median age of the patients at time of diagnosis was 4.0 years. The most common presenting symptoms were delayed motor development (nine patients), and symptoms related to limb-girdle weakness like frequent falling, difficulty climbing stairs, and problems with running and performing sports. Fatigue, persistent diarrhoea and problems in raising the head in supine position were other first complaints. Median time span between symptom onset and diagnosis was 1.0 year (range 0 to 5.8 years).

Ten patients were diagnosed presymptomatically. In six of them the diagnosis was made after elevated CK and transaminase serum levels were found during a hospital admission for unrelated matters. The other four patients were diagnosed because they had a sibling with Pompe disease. Five of these ten patients developed symptoms between diagnosis and first examination in our hospital (see for details Table 1).

Clinical findings

All 31 patients were evaluated in our hospital. The age at time of examination ranged from 0.1 to 17.3 years. Table 2 shows the findings on clinical examination. Low or absent reflexes, a myopathic face, and scoliosis were found in over 50 percent of the 31 patients. Facial muscle weakness was generally mild and did not lead to speech difficulties or dysphagia. One exception was a patient (patient 23 in Table 1) who was fed by percutaneous endoscopic gastrostomy catheter and had severe dysarthria. This patient was wheelchair bound and ventilator dependent since the age of 6 years.

Flexion contractures were present in 19% of the patients and were most frequently found in the ankles. Three patients also had contractures of the hips and knees. Several patients underwent corrective surgery for either contractures (n=4, patients 1, 6, 10, 29 in Table 1), or scoliosis (n=4, patients 7, 14, 23, 30 in Table 1).

Noteworthy, thirty percent of the patients were underweight; their weight corrected for height was: -4.3 to -2.0 standard deviations below healthy peers.

In all patients, a standardized neurological examination was performed. This examination revealed physical limitations in 70% of the patients. In more than 50% of the children difficulties were observed with standing up from supine position and flexing the neck in supine position (Table 2). Other important limitations were problems with standing up from sitting on heels, climbing stairs, and rising from a chair.

Distribution of muscle weakness

Figure 1 shows the severity of muscle weakness and the frequency in which the various muscle groups were affected. The most prominent weakness was found in the neck flexors, which were affected in 75% of the patients. Other muscles that were frequently affected were the gluteus maximus (extension of the hip), the ileopsoas (flexion of the hip), and the deltoid muscle. The triceps, wrist extensors, and foot plantar flexors were relatively spared. In patients with far advanced disease, all muscles were affected.

Table 1. Patient characteristics. Patients are listed by age of onset and are subdivided in two groups: those who carry the c.-32-13T>G mutation and those who do not.

Pt	Sex	Onset (y)[1]	Diagnosis (y)[1]	Examination (y)[1]	Wheelchair (y)	Ventilator (y)	allele 1	allele 2
1[*]	M	0.5	2.5	8.1	Yes (11)	No	c.-32-13T>G (pm)	c.525delT (vs)
2[@,!]	F	0.8	0	0.1	No	No	c.-32-13T>G (pm)	c.2135T>C (ls)
3	F	0.8	1.1	8.9	No	No	c.-32-13T>G (pm)	c.923A>C (pls)
4[@]	M	0.8	2	2.4	No	No	c.-32-13T>G (pm)	c.2135T>C (ls)
5	M	0.9	2.3	9.5	No	No	c.-32-13T>G (pm)	c.525delT (vs)
6	M	1	2	8.2	Partially (4)	No	c.-32-13T>G (pm)	c.1051delG (vs)
7	M	1	2	15.2	No	At night (12)	c.-32-13T>G (pm)	c.525delT (vs)
8	M	1.5	2	13.3	No	No	c.-32-13T>G (pm)	c.2481+102_2646+31del (vs)
9[*,!]	M	2	1	9	No	No	c.-32-13T>G (pm)	c.525delT (vs)
10	M	2.5	3	13	No	No	c.-32-13T>G (pm)	c.2331+2T>A (vs)
11[&]	M	5	10.8	10.8	No	No	c.-32-13T>G (pm)	c.525delT (vs)
12	F	6	7.8	7.8	No	No	c.-32-13T>G (pm)	c.2331+2T>A (vs)
13[!]	M	6.5	2	7.6	No	At night (5)	c.-32-13T>G (pm)	c.1062C>G (pls)
14	M	7	10	10.7	Yes (22)	At night (16)	c.-32-13T>G (pm)	c.1548G>A (pls)
15[!]	M	8	4	15.8	No	No	c.-32-13T>G (pm)	c.1441T>C (pls)
16[!]	M	12	8	14.6	No	No	c.-32-13T>G (pm)	[c.307T>G + c.271G>A] (pls)
17	M	13	14	14.3	No	No	c.-32-13T>G (pm)	c.1933G>A (pls)
18[!]	M	no symptoms	4	6.4	No	No	c.-32-13T>G (pm)	c.2481+102_2646+31del (vs)
19[&,!]	M	no symptoms	13.1	13.1	No	No	c.-32-13T>G (pm)	c.525delT (vs)

Pt	Sex	Onset (y)[1]	Diagnosis (y)[1]	Examination (y)[1]	Wheelchair (y)	Ventilator (y)	allele 1	allele 2
20[$,!]	M	no symptoms	14	15.2	No	No	c.-32-13T>G (pm)	c.307T>G (pls)
21[$,!]	M	no symptoms	16	17.1	No	No	c.-32-13T>G (pm)	c.307T>G (pls)
22	M	0.5	1	1.3	Yes (4)	died (10†)	c.1798C>T (ls)	c.525delT (vs)
23	F	1	1.9	13.3	Yes (6)	Yes (6)	c.875A>G (pm)	unknown/ r.0?
24	M	2	2.9	2.9	Yes (6)	died (6†)	unknown	c.1645G>A (pm)
25	M	2.7	3.5	5.9	No	No	c.1634C>T (ls)	c.2481+102_2646+31del (vs)
26[#]	F	4	4	8.1	No	No	c.-32-3C>G (ls)	c.1551+1G>A (vs)
27[#]	M	5	5	10.1	No	No	c.-32-3C>G (ls)	c.1551+1G>A (vs)
28	F	6	7	9.9	Partially (9)	At night (8)	c.1829C>T (ls)	c.1912G>T (pls)
29	F	6.5	11.6	12.7	No	No	unknown (r.spl 2%)	c.525delT (vs)
30	F	10	11	12	Yes (16)	Yes (12)	c.-32-3C>A (ls)	c.877G>A+c.271G>A (pls)
31[!]	F	no symptoms	15	15.9	No	No	c.861C>T (r.spl=3%)	c.925G>A (pls)

*,#,$, &,@ : Siblings; ! patients that were diagnosed presymptomatically; [1] Age at onset, diagnosis, examination expressed in years (y); † both patients died at the age of 6, and 10 years from respiratory failure; severity of the mutation is indicated by (vs) very severe; (pls) potentially less severe; (ls) less severe; (pm) potentially mild; see for more information www.pompecenter.nl.

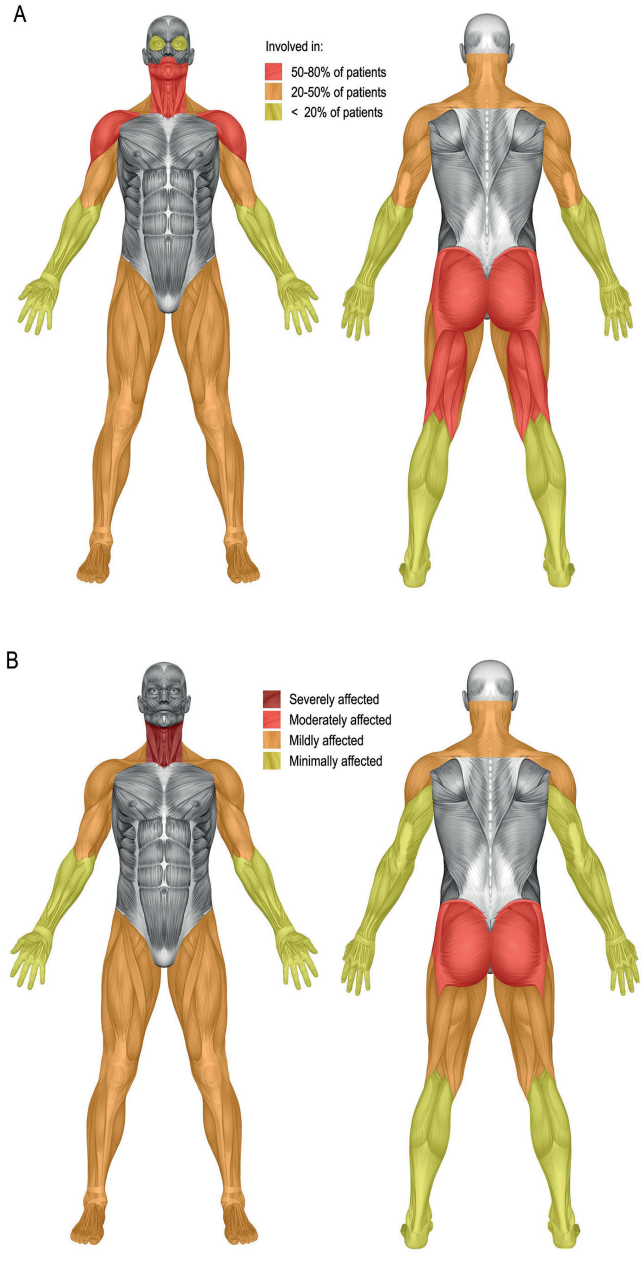

Figure 1. (**A**) the frequency in which the various muscles were affected. (**B**) the severity of involvement of the separate muscle groups.

All patients, including those who had no symptoms at the time of examination had a lower total HHD sum score than age related peers. Total muscle strength ranged from 0.3–91 % of normal (median 55.4%). Four patients were wheelchair bound at the time of investigation; four others became wheelchair dependent in the period thereafter. At the moment these eight patients became wheelchair dependent their age ranged from 4 to 22 years (n=8, median 7.5 years). The median time period between first symptoms and loss of ambulation was 4.5 years (range 3 to 15 years, n=8).

Table 2. Results of clinical and neurological examination. Deep tendon reflexes that were tested were the biceps reflex, the triceps reflex, the knee jerk reflex, and the ankle jerk reflex

	Number of patients (Total 31)
Clinical findings	
Low/absent reflexes	22 (71%)
Weakness facial muscles	16 (52%)
Scoliosis	16 (52%)
Muscle tone decreased	13 (42%)
Scapular winging	12 (39%)
Muscle atrophy	11 (35%)
Scapular winging	11 (35%)
Contractures	9 (29%)
Low body weight	9 (29%)
Ptosis	0 (0%)
Physical limitations	
Standing up from supine position	18 (58%)
Flexing the neck in supine position	17 (55%)
Standing up from sitting on heels	13 (42%)
Climbing stairs	13 (42%)
Rising from a chair	10 (32%)
Erecting back in prone position	8 (26%)

Pulmonary function testing

In sitting position, 14 of the 29 patients (50%) had a decreased pulmonary function (<80% according to the ATS guidelines). Seventeen of the 29 patients (59%) had a decreased Forced Vital Capacity (FVC) in supine position; in five of these patients pulmonary function was too compromised to obtain a reliable value. FEV1/VC measurements were normal in all patients, indicating a restrictive pattern of pulmonary dysfunction.

The median difference in FVC between sitting and supine position (postural drop) was -6.0% (range 0 to -20%). Eight patients had a postural drop between -10 and -20%. Five patients were ventilator dependent at the time of first evaluation, and one patient became ventilator dependent during follow-up (see Table 1 for details); Two additional patients died from respiratory failure at the ages of 6 and 10 years, when it was decided not to start respiratory support. The median time period from first symptoms to any kind of ventilation or death by respiratory insufficiency was 5.5 years (range 2 to 11 years, n=8). The median age at start of ventilation or death was 9 years (range 5 to 16 years, n=8).

Cardiac evaluation

Six patients showed abnormalities on cardiac evaluation. In three patients the findings were considered to be related to Pompe disease. Two patients had a hypertrophic cardiomyopathy without outflow tract obstruction (patients 22, 23 in Table 1) In one patient, this was first noticed at the age of one year, and in the other patient at the age of two years. Their ECGs showed high amplitude QRS complexes and repolarisation disturbances consistent with their hypertrophic cardiomyopathy. In addition, their ECGs and that of a third patient (patient 3 in Table 1) showed a short PR interval and a delta wave suggestive for Wolff-Parkinson-White syndrome. Minor abnormalities of the cardiac valves were noted in three patients. The abnormalities included a quadricuspid aortic valve, a minor deformity of the tricuspid valve leading to minimal tricuspid regurgitation, and minimal insufficiency of both atrioventricular valves. These abnormalities were all considered to be accidental findings and not related to Pompe disease.

Enzymatic and molecular diagnosis

Table 1 shows the genotype of the patients and the severity of their mutations. All mutations were previously described in the literature (www.pompecenter.nl). Twenty-three patients (74%) carried a potentially mild mutation on one *GAA* allele and a severe mutation on the other. Twenty-one of these patients (68%) carried the common c.-32-13T>G splice site mutation. Since none of the mutations in the other 8 patients was considered potentially mild, the genotype of these patients was considered more severe.

Laboratory parameters

At first visit 29 of 31 patients had elevated CK levels (median 979 U/l, range 358 to 3078 U/l; normal values below 230 U/l)). Both patients, who had normal CK levels, were symptom free at the time of investigation. One of them was diagnosed because of an accidental finding of elevated transaminase levels during admission for a gastroenteritis; the other was diagnosed because of a sibling with Pompe disease.

Noteworthy, in all patients, including the symptom free patients, the transaminase levels were elevated; AST ranged from 82 to 972 U/l (normal values below 51 E/l), ALT from 71-487 U/l (normal values below 39 U/l). LDH levels were elevated in 22 of 30 patients and ranged from 797 to 2828 U/l (normal values below 765 U/l).

Comparison of patients with the c.-32-13T>G/null genotype (IVS1)and those with other genotypes (non-IVS1)

Comparison of the twenty-one patients with the c.-32-13T>G/null genotype to the ten patients with other mutations showed no significant differences in age at first symptoms and age at diagnosis (Table 3). The median age of the patients at the time they were examined was similar. It was noted that patients in the non-IVS1 group had lower muscle strength and pulmonary function in sitting and supine position than those in the IVS1 group. This was in line with the observation that more non-IVS1 than IVS1 patients became wheelchair bound and ventilator dependent at a relatively young age. In addition, the two patients with a hypertrophic cardiomyopathy belonged to the non-IVS1 group. Another interesting observation

was that eighteen of the twenty-one (86%) patients with the c.-32-13T>G/null genotype were male, while this was 40% in the non-IVS1 group.

Table 3. Comparison of the patients with the c.-32-13T>G and patients with other mutations at the time of examination.

	All	c.-32-13T>G	other mutations	P-value
Patients	31	21	10	–
M/F	22/9	18/3	4/6	–
Age first symptom (median)	2.5 (0.5–13)	1.3 (0.5–13)	4.0 (0.5–10)	0.4
Age diagnosis (median)	4.0 (0–16)	3.0 (0–16)	4.5 (1–15)	0.3
Age at examination (median)	10.2 (0.1–17)	10.8 (0.1–17.3	9.6 (1.3–16.3)	0.3
Disease duration[1] (median)	3.9 (0–12.7)	3.2 (0–12.7)	4.0 (0.8–11.5)	0.7
Diagnosed pre-symptom	10	9	1	–
Still symptom free	5	4	1	–
HHD sumscore	52	58.3	34.1	0.004
FVC pred sitting (%)	78	86.8	61.4	0.08
FVC pred supine (%)	72	80.6	57.5	0.04

[1] Disease duration is calculated as time between the presentation of first symptoms and first examination in our hospital.

DISCUSSION

Information on the clinical presentation of children with non-classic forms of Pompe disease is scarce. In the present study we evaluated the clinical and molecular characteristics of 31 children. The results emphasize that the disease may already cause a significant burden of disease in childhood and add to the understanding that Pompe disease presents as a broad spectrum of clinical phenotypes.

The presentation of Pompe disease can be variable. In our patient population, children typically presented with weakness of the limb-girdle muscles and/or delayed motor development; in all symptomatic patients CK levels were elevated,

approximately half of the children had a compromised pulmonary function, and two patients with a very rapid deterioration of muscle function had a hypertrophic cardiomyopathy. Nevertheless, Pompe disease should also be considered in children with less familiar findings such as disproportional weakness of the neck flexors, unexplained fatigue, persistent diarrhoea, and an (isolated) elevation of transaminase levels. An elevation of ALT and AST was found in all patients participating in the present study, whether they showed symptoms of the disease or not.

Although respiratory problems did not precede proximal muscle weakness in any of the patients, pulmonary function was already significantly reduced in more than half. In 33% of the patients respiratory insufficiency led to the requirement of ventilator support or death during childhood. This confirms that pulmonary function should be closely monitored in children, as was previously reported for adult patients.[14,28] The fact that eight patients had postural drops suggestive for diaphragm weakness, a well-known feature in Pompe disease and the main cause of nightly hypoventilation,[28,29] stresses the importance to perform pulmonary function tests in both sitting and supine position. Following a recent study in children with neuromuscular disorders that found a poor correlation between daytime lung function and nocturnal hypoxemia, we suggest the use of systematic sleep studies as an additional tool to identify children at risk for nocturnal hypoventilation.[30]

Seventy-one percent of the 31 patients that we investigated were male, which interestingly included 18 of the 21 patients (86%) with the common c.-32-13T>G/null genotype compared to only 4 of the 10 patients (40%) with other mutations. Two studies that focused on disease variation among children and adult patients with the c.-32-13T>G/null genotype, found an equal male to female distribution (55% and 58% males respectively). In both studies, however, no attention was given to a potential difference in age of onset between male and female patients.[15,16] Our study shows that such a gender difference exists. Earlier we also found a male predominance (67%) in patients under 18 years when we analysed 225 published case reports about children and adults with Pompe disease.[17] Earlier studies also showed that pulmonary function was more affected in men,[14] and that more men than women had bulbar involvement and shoulder-girdle muscle weakness.[14] A

study comparing phenotypes in siblings with Pompe disease also confirmed that males were more severely affected than females.[31]

Since Pompe disease inherits as an autosomal recessive trait, there is no good explanation why males with the same genotype would present at an earlier age than females other than that secondary gender related factors might play a role in the clinical expression of this autosomal recessive disease. Gender differences have also been reported for other neuromuscular disorders like fascioscapulohumeral muscular dystrophy and some subtypes of limb-girdle muscular dystrophy.[32-34] The only difference between men and women found so far was a better capacity in muscle fiber atrophy regeneration in women.[34] This may also apply for Pompe disease. Although other causes, like differences in genetic and epigenetic factors have been suggested, the exact mechanism remains elusive.

Our findings are fully in line with the broad spectrum of clinical phenotypes associated with the c.-32-13T>G/null genotype.[15,16] Comparison of these patients with patients carrying other mutations showed a similar age of disease onset, age at diagnosis and actual age. In both groups several patients became wheelchair bound and/or ventilator dependent during childhood. Hence, it can be concluded that significant morbidity at an early age can occur in patients carrying other genotypes as well as in patients carrying the c.-32-13T>G/null genotype.

On a group level, patients in the non-IVS1 group seemed to be more severely affected. This is illustrated by specific cases. Two patients in the non IVS1 group had a hypertrophic cardiomyopathy. They became fully wheelchair bound at 4 and 6 years of age respectively. One died from respiratory failure at the age of 10 years, and the other patient became completely ventilator dependent when she was 6 years old. These two patients express a phenotype that was earlier called the “atypical infantile form” of Pompe disease by Slonim et al.[18] Overall, mutations identified in the non- IVS1 group were more severe (see Table 1 and www.pompecenter.nl). Some of the genotypes have been described in literature. For example patient 30 (Table 1) had genotype c.-32-3C>A in combination with c.877G>A+c.271G>A. This genotype has been described before by Oba-Shinjo et al. The patient in our study presented with first symptoms at the age of 10 years and showed respiratory insufficiency leading to night-time ventilation at the age of 12

year, while the patient from literature presented at the age of 7 years and died of respiratory failure at the age of 15 years.[35] Two case reports considering genotype c.1634C>T in combination with c.2481+102_2646+31del as found in patient 25 were described before.[12] One patient presented at the age of one, became dependent on respiratory support at the age of 20 years, and wheelchair bound at the age of 23 years. The other patient was diagnosed at the age of 16 years, began to use a walking stick at the age of 19 years. Pulmonary function worsened severely from age 17–19 years on and dropped to 26% of normal at the age of.[36] These case reports show substantial similarities and both point to the fact that our patient, who was only six years old at time of examination, awaits severe respiratory and mobility problems at young age. This kind of information may become relevant when it comes to decision making on when to start enzyme replacement therapy.

Comparing our results in children with those in adults, we find both similarities as well as differences. The wide variation in disease presentation and disease progression, as well as the involvement of both respiratory and proximal skeletal muscles are similar in children and adults.[8-12,15,16] The distribution of muscle weakness also shows a limb-girdle pattern in both cases, but there are differences. While the neck flexors are by far the most severely affected muscle group in children this does not seem to be the case in adults.[9,12] Although neck flexors were shown to be affected in the majority (50–80%) of adult patients (n=94) t, muscle strength of the neck flexors was only mildly reduced.[37] Relative mild involvement was also shown by a recent MRI study performed in 20 adult patients by Carlier et al.[38] Another difference is the relative sparing of the quadriceps muscle in adult patients.[37,38] In the current population of 31 children with Pompe disease, the muscles of the thigh were more heterogeneously affected, and the quadriceps muscle were not spared. Additionally, we did not find ptosis in any of our patients, while this has recently been described to be present in 14.7 to 23% of adult Pompe patients.[37,39,40] It should be noted that ptosis was often found in an early stage of the disease, even as a presenting symptom in adult Pompe patients. While van der Beek et al found difficulties with speech, chewing or swallowing, suggestive for bulbar weakness in 28% of patients, we found bulbar weakness in only one patient. On the contrary, scoliosis was present in 52% of our patients, compared

to only 21 to 23% of adult Pompe patients.[17,37] In several children the scoliosis was so severe that it interfered with mobility or influenced respiratory function, four patients needed surgical correction. A cross-sectional analysis of data from the Pompe Registry, a large multinational observational program, found scoliosis to be present in 57% of patients with disease onset in childhood.[41]

Some limitations of our study should be mentioned. A selection bias can be present since our centre serves as an (inter)national referral centre for Pompe disease. This probably has caused the referral of more severely affected patients. Nevertheless, ten of the thirty-one patients were diagnosed pre-symptomatically, of which five are still symptom-free. A second limitation was that the study was cross-sectional. The approval of enzyme replacement therapy in 2006 interfered with the collection of longitudinal follow-up data since all children that manifested significant symptoms of the disease started to receive enzyme replacement therapy at some time during follow-up.

In conclusion, several studies among adult Pompe patients reported the occurrence of mild symptoms during childhood.[8,99,11] Our study, on the contrary, demonstrates that although the course of childhood Pompe disease varies widely, a substantial proportion of patients manifests serious problems during their youth. We stress that Pompe disease should also be considered in the differential diagnosis of patients with less familiar signs such as disproportional weakness of the neck flexors, unexplained fatigue, persistent diarrhoea, and an isolated elevation of transaminase levels. Disease presentation, distribution of muscle weakness, and the occurrence of specific symptoms such as bulbar muscle weakness or ptosis, appear to be different from adult patients. Patients with other mutations than the c.-32-13T>G mutation were in general more severely affected, which is in line with more severe genotypes present in these patients. Children with a c.-32-13T>G/null genotype presenting in childhood appeared to be predominantly males.

REFERENCES

1. Hirschhorn R, Reuser AJJ. Glycogen Storage Disease Type II: acid alpha-glucosidase (acid maltase) deficiency. In: Scriver CR, Beaudet AL, Valle D, Sly WS, eds. *The Metabolic and Molecular Bases of Inherited Disease*. 8 ed. NY: McGraw-Hill; 2001:3389-3420.
2. van der Ploeg AT, Reuser AJ. Pompe's disease. *Lancet.* Oct 11 2008; 372(9646):1342-1353.
3. Engel AG, Hirschhorn R, Huie ML. Acid maltase deficiency. In: Engel A, ed. *Myology*. NY: McGraw-Hill; 2004:1559-1586.
4. Pompe JC. Over idiopathische hypertrofie van het hart. *Ned Tijdsch Geneesk.* 1932; 76:304-311.
5. Van den Hout HM, Hop W, Van Diggelen OP, et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics.* 2003; 112(2):332-340.
6. Kishnani PS, Hwu WL, Mandel H, Nicolino M, Yong F, Corzo D. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *The Journal of pediatrics.* May 2006; 148(5):671-676.
7. Engel AG, Gomez MR, Seybold ME, Lambert EH. The spectrum and diagnosis of acid maltase deficiency. *Neurology.* 1973; 23(1):95-106.
8. Hagemans ML, Winkel LP, Van Doorn PA, et al. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. *Brain.* Mar 2005; 128(Pt 3):671-677.
9. Laforet P, Nicolino M, Eymard PB, et al. Juvenile and adult-onset acid maltase deficiency in France: genotype-phenotype correlation. *Neurology.* 2000; 55(8):1122-1128.
10. Wokke JH, Escolar DM, Pestronk A, et al. Clinical features of late-onset Pompe disease: A prospective cohort study. *Muscle & Nerve.* Oct 2008; 38(4):1236-1245.
11. Muller-Felber W, Horvath R, Gempel K, et al. Late onset Pompe disease: clinical and neurophysiological spectrum of 38 patients including long-term follow-up in 18 patients. *Neuromuscular disorders.* Oct 2007; 17(9-10):698-706.
12. Van der Beek NA, Hagemans ML, Reuser AJ, et al. Rate of disease progression during long-term follow-up of patients with late-onset Pompe disease. *Neuromuscular disorders.* Dec 10 2008.
13. Pellegrini N, Laforet P, Orlikowski D, et al. Respiratory insufficiency and limb muscle weakness in adults with Pompe's disease. *The European Respiratory Journal.* Dec 2005; 26(6):1024-1031.
14. van der Beek NA, van Capelle CI, van der Velden-van Etten KI, et al. Rate of progression and predictive factors for pulmonary outcome in children and adults with Pompe disease. *Molecular genetics and metabolism.* Jun 24 2011.
15. Kroos, Pomponio RJ, Hagemans ML, et al. Broad spectrum of Pompe disease in patients with the same c.-32-13T->G haplotype. *Neurology.* Jan 9 2007; 68(2):110-115.
16. Montalvo AL, Bembi B, Donnarumma M, et al. Mutation profile of the GAA gene in 40 Italian patients with late onset glycogen storage disease type II. *Human mutation.* Oct 2006; 27(10):999-1006.
17. Winkel LP, Hagemans ML, van Doorn PA, et al. The natural course of non-classic Pompe's disease; a review of 225 published cases. *J Neurol.* Aug 2005; 252(8):875-884.
18. Slonim AE, Bulone L, Ritz S, Goldberg T, Chen A, Martiniuk F. Identification of two subtypes of infantile acid maltase deficiency. *The Journal of pediatrics.* 2000; 137(2):283-285.

19. Van der Ploeg RJ, Oosterhuis HJ, Reuvekamp J. Measuring muscle strength. *Journal of Neurology.* 1984(231):200-203.
20. Beenakker EA, van der Hoeven JH, Fock JM, Maurits NM. Reference values of maximum isometric muscle force obtained in 270 children aged 4-16 years by hand-held dynamometry. *Neuromuscular disorders : NMD.* Jul 2001; 11(5):441-446.
21. *Medical Research Council. Aids to examination of the peripheral nervous system. Memorandum no. 45.* London: Her Majesty's Stationary Office; 1976.
22. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *The European respiratory journal.* Aug 2005; 26(2):319-338.
23. Lung function testing: selection of reference values and interpretative strategies. American Thoracic Society. *The American review of respiratory disease.* Nov 1991; 144(5):1202-1218.
24. van Diggelen OP, Oemardien LF, van der Beek NA, et al. Enzyme analysis for Pompe disease in leukocytes; superior results with natural substrate compared with artificial substrates. *Journal of inherited metabolic disease.* Apr 19 2009.
25. Van Diggelen OP, Zhao H, Kleijer WJ, et al. A fluorimetric enzyme assay for the diagnosis of Morquio disease type A (MPS IV A). *Clin Chim Acta.* Feb 28 1990; 187(2):131-139.
26. Hermans MMP, van Leenen D, Kroos MA, Reuser AJJ. Mutation detection in glycogen storage-disease type II by RT-PCR and automated sequencing. *Biochemical and biophysical research communications.* 1997; 241(2):414-418.
27. Kroos M, Pomponio RJ, van Vliet L, et al. Update of the Pompe disease mutation database with 107 sequence variants and a format for severity rating. *Human mutation.* Jun 2008; 29(6):E13-26.
28. Mellies U, Ragette R, Schwake C, Baethmann M, Voit T, Teschler H. Sleep-disordered breathing and respiratory failure in acid maltase deficiency. *Neurology.* 2001; 57(7):1290-1295.
29. Mellies U, Lofaso F. Pompe disease: A neuromuscular disease with respiratory muscle involvement. *Respir Med.* Jan 6 2009.
30. Bersanini C, Khirani S, Ramirez A, et al. Nocturnal hypoxaemia and hypercapnia in children with neuromuscular disorders. *The European respiratory journal.* May 2012; 39(5):1206-1212.
31. Wens SC, van Gelder CM, Kruijshaar ME, et al. Phenotypical variation within 22 families with Pompe disease. *Orphanet journal of rare diseases.* Nov 19 2013; 8(1):182.
32. Zatz M, Marie SK, Cerqueira A, Vainzof M, Pavanello RC, Passos-Bueno MR. The facioscapulohumeral muscular dystrophy (FSHD1) gene affects males more severely and more frequently than females. *American journal of medical genetics.* May 1 1998; 77(2):155-161.
33. Tonini MM, Passos-Bueno MR, Cerqueira A, Matioli SR, Pavanello R, Zatz M. Asymptomatic carriers and gender differences in facioscapulohumeral muscular dystrophy (FSHD). *Neuromuscular disorders : NMD.* Jan 2004; 14(1):33-38.
34. Fanin M, Nascimbeni AC, Angelini C. Gender difference in limb-girdle muscular dystrophy: a muscle fiber morphometric study in 101 patients. *Clinical neuropathology.* Mar 12 2014.
35. Oba-Shinjo SM, da Silva R, Andrade FG, et al. Pompe disease in a Brazilian series: clinical and molecular analyses with identification of nine new mutations. *J Neurol.* Jul 9 2009; 256(11):1881-1890.
36. Korpela MP, Paetau A, Lofberg MI, Timonen MH, Lamminen AE, Kiuru-Enari SM. A novel mutation of the GAA gene in a Finnish late-onset pompe disease patient: Clinical phenotype and follow-up with enzyme replacement therapy. *Muscle & nerve.* May 26 2009.

37. van der Beek NA, de Vries JM, Hagemans ML, et al. Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study. *Orphanet journal of rare diseases.* Nov 12 2012; 7(1):88.
38. Carlier RY, Laforet P, Wary C, et al. Whole-body muscle MRI in 20 patients suffering from late onset Pompe disease: Involvement patterns. *Neuromuscular disorders : NMD.* Jul 29 2011.
39. Ravaglia S, Bini P, Garaghani KS, Danesino C. Ptosis in Pompe disease: common genetic background in infantile and adult series. *J Neuroophthalmol.* Dec 2010; 30(4):389-390.
40. Groen WB, Leen WG, Vos AM, Cruysberg JR, van Doorn PA, van Engelen BG. Ptosis as a feature of late-onset glycogenosis type II. *Neurology.* Dec 26 2006; 67(12):2261-2262.
41. Roberts M, Kishnani PS, van der Ploeg AT, et al. The prevalence and impact of scoliosis in Pompe disease: Lessons learned from the Pompe Registry. *Molecular genetics and metabolism.* Aug 16 2011.

3

Rate of progression and predictive factors for pulmonary outcome in children and adults with Pompe disease

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ABSTRACT

Respiratory insufficiency is a serious threat to patients with Pompe disease, a neuromuscular disorder caused by lysosomal acid alpha-glucosidase deficiency. Innovative therapeutic options which may stabilize pulmonary function have recently become available. We therefore determined proportion and severity of pulmonary involvement in patients with Pompe disease, the rate of progression of pulmonary dysfunction, and predictive factors for poor respiratory outcome.

In a single-center, prospective, cohort study, we measured vital capacity (VC) in sitting and supine positions, as well as maximum inspiratory (MIP) and expiratory (MEP) mouth pressures, and end expiratory CO_2 in 17 children and 75 adults with Pompe disease (mean age 42.7 years, range 5–76 years).

Seventy-four percent of all patients, including 53% of the children, had some degree of respiratory dysfunction. Thirty-eight percent had obvious diaphragmatic weakness. Males appeared to have more severe pulmonary involvement than females: at a group level, their mean VC was significantly lower than that of females ($p<0.001$), they used mechanical ventilation more often than females ($p=0.042$) and the decline over the course of the disease was significantly different between males and females ($p=0.003$). Apart from male gender, severe skeletal muscle weakness and long disease duration were the most important predictors of poor respiratory status. During follow-up (average 1.6 years, range 0.5–4.2 years), three patients became ventilator dependent. Annually, there were average decreases in VC in upright position of 0.9% points ($p=0.09$), VC in supine position of 1.2% points ($p=0.049$), MIP of 3.2% points ($p=0.018$) and MEP of 3.8% points ($p<0.01$).

We conclude that pulmonary dysfunction in Pompe disease is much more common than generally thought. Males, patients with severe muscle weakness, and those with longer disease duration seem most at risk.

INTRODUCTION

Pompe disease is a rare inherited metabolic disorder[1-4] caused by deficiency of the lysosomal enzyme acid α-glucosidase. The spectrum of phenotypes is continuous, but in clinical practice two subtypes can be recognized: 1) the classic infantile phenotype, in which the disease manifests shortly after birth, leading to generalized muscle weakness, cardiorespiratory failure and death within the first year of life;[5,6] and 2) a more slowly progressive phenotype predominantly affecting skeletal and respiratory muscles, in which cardiac involvement is only sporadically present.[7,8] Symptoms in this latter group of patients can become manifest at any age, from as early as the first year of life to as late as the sixth decade.[9-12] The course of the disease can vary substantially between patients,[13] and the severity of respiratory involvement is not always related to the degree of skeletal-muscle weakness.[14,15]

Due to the disproportionate involvement of the diaphragm, respiratory insufficiency is a serious threat to patients with Pompe disease;[16,17] this is also seen in several other neuromuscular disorders such as Duchenne muscular dystrophy or facioscapulohumeral dystrophy. As the disease progresses, many patients ultimately become dependent on mechanical ventilatory support, and respiratory failure is a major cause of death.[7,12] However, most studies have investigated only a small number of patients, or only a selected group; the actual percentage of patients with respiratory dysfunction, who are thus at risk for developing respiratory failure, is therefore not exactly known.[11,14,15,17] Neither is it known which factors are associated with poor pulmonary outcome.

In April 2010, a placebo controlled trial showed that pulmonary function in patients older than eight years may be stabilized by treatment with recombinant human α-glucosidase. Early identification of respiratory problems may thus be important for the timely initiation not only of mechanical ventilation, but also of enzyme therapy.[18,19]

To establish the proportion of patients with pulmonary involvement, and also the severity of pulmonary dysfunction and the rate of deterioration, we conducted a prospective cohort study in 92 untreated children and adults with Pompe disease. We also aimed to identify predictive factors for poor respiratory outcome.

MATERIALS AND METHODS

Study population and study design

Ninety-two patients (17 children and 75 adults) were included in an ongoing prospective cohort study on the natural course of Pompe disease. Participation was open to all patients who did not have the classic infantile type of Pompe disease. Diagnosis in all patients was confirmed through mutation analysis and by measuring acid α-glucosidase deficiency in leukocytes, muscle tissue or fibroblasts. All patients were examined at Erasmus MC University Medical Center between August 2003 and August 2009. They were recruited either through neuromuscular centers in the Netherlands and Belgium, or through the Dutch neuromuscular patient organization, or were referred to our center of expertise by their treating physicians. Throughout the study, none of the patients received enzyme replacement therapy. The research protocol was approved by the Central Committee on Research Involving Human Subjects in the Netherlands (CCMO). All patients or their parents provided written informed consent.

Pulmonary function tests

Vital capacity (VC) and forced expiratory volume in one second (FEV_1) were measured using a Lilly type pneumograph (Viasys Healthcare, Würzburg, Germany) according to ATS/ERS standards.[20] Patients were tested in upright seated or supine position while wearing a nose clip. Three repeated flow volume curves were made; in case of a non-characteristic curve, an extra measurement was performed. The best effort, determined as the measurement with the highest sum of VC and FEV_1, was used in further analyses. Values were expressed as percentage of predicted normal values (based on able-bodied persons of the same age, gender, and height) or as z-scores, calculated as the difference between the observed and predicted value divided by the standard deviation from the reference value. Z-scores <-1.64 (5^{th} percentile of the reference population) were considered abnormally low. Reference values were derived from published data.[21,22] For vital capacity a further subdivision was made to categorize the severity of lung function impairment: mild (z-score -3 to -1.64), moderate (z-score -4 to -3) and severe (z-score <-4). A drop

in percentage predicted VC upon changing posture from the upright to the supine position of more than 25% was considered as diaphragmatic weakness.[23-25]

Maximum static inspiratory (MIP) and expiratory (MEP) pressures were recorded using a differential pressure transducer (Viasys Healthcare, Würzburg, Germany) according to ATS/ERS standards.[23] Patients were comfortably seated, wearing a nose clip. Pressures were measured against an obstructed mouthpiece with a small leak to prevent glottic closure during the MIP maneuver and to reduce the use of buccal muscles during the MEP maneuver. In addition, the patient held the cheeks during the MEP maneuver. MIP was measured at residual volume after maximal expiration and MEP at total lung capacity after maximal inspiration. Pressures had to be maintained for at least one second. Maneuvers were repeated until three reproducible measurements were recorded. At least 1 min was taken between consecutive measurements. The highest value obtained was taken for analysis. Reference values were taken from published data.[26] MIP below the lower limit of the normal predicted value was interpreted as diaphragmatic weakness.

The carbon dioxide fraction in the expired gas was measured with a capnograph (ms-capno, Viasys Healthcare, Würzburg, Germany) at maximum expiration ($P_{EE,CO2}$). In the absence of ventilation irregularities, the $P_{EE,CO2}$ approximates the arterial carbon dioxide pressure ($P_{a,CO2}$). A daytime $P_{EE,CO2}$ over 6,0 kPa suggests hypercapnia and chronic alveolar hypoventilation.[27]

Additional clinical information

Information was gathered on the following: 1) age at symptom onset, 2) age at diagnosis, 3) disease duration since first symptoms, 4) height, 5) weight, 6) gender, 7) use of wheelchair or walking aids, 8) muscle strength, 9) use of ventilatory support, 10) number of hours of ventilatory support per day, 11) presence of sleep disorders, 12) presence of scoliosis and scoliosis surgery, 13) smoking habit, 14) concomitant diseases such as chronic obstructive pulmonary disease or asthma, and 15) family history of pulmonary disease.

Muscle strength was graded through manual muscle testing using the Medical Research Council (MRC) grading scale[28] (range 0–5; all patients were assessed by the same examiner (NvdB) without having access to the pulmonary function data).

A muscle sumscore was calculated for the following muscle groups: neck extensors, neck flexors and bilateral shoulder abductors, elbow flexors, elbow extensors, hip flexors, hip abductors, knee flexors and knee extensors. This sumscore ranges from 0 ("total paralysis") to 80 ("normal strength").

Statistical analyses

Continuous variables are presented using median and range. For categorical variables, percentages or frequencies are given. Pulmonary function testing could not be performed in six patients who were ventilated 24 h a day through a tracheostomy tube. In the statistical analyses these patients were considered to have the most severely affected pulmonary function, and were artificially given a VC of -8.5SD (just below the least observed value), since their omission might have led to biased results.

Baseline differences between males and females were assessed using X^2 tests (wheelchair use and ventilator use) or Mann-Whitney tests (age, disease duration, mobility, age at first symptoms, age at diagnosis and MRC sumscore).

The relationships between disease duration, MRC sumscore, mobility, gender, MIP, MEP and vital capacity were calculated using the Spearman's rank correlation coefficients (ρ).

Multiple linear regression analysis was used to further explore the relationship of VC versus gender and disease duration, with adjustment for age, MRC sumscore and mobility.

Longitudinal analysis of pulmonary function was performed using random coefficient models for repeated measurements, allowing for irregularly measured data. For subgroup analyses, patients were divided into groups on the basis of disease duration (<5, 5 to 10, 10 to 15, or ≥15 years); mobility (no walking aids, walking aids, partial wheelchair use, fully wheelchair dependent); vital capacity at study entry (normal, mild to moderately reduced, severely reduced); and muscle strength at baseline (severely affected, moderately affected, mildly affected).

All analyses were performed with SPSS for Windows (version 15, SPSS Inc., Chicago, IL) or SAS (version 9.1, SAS Institute Inc., Cary, NC). A p-value of ≤0.05 was considered statistically significant.

RESULTS

Baseline measurements

Clinical characteristics

The study population comprised 92 patients with Pompe disease: 17 children aged between 5 and 17 years, and 75 adults. Fifty-four percent of the patients were male. Disease duration at the time of the initial investigation ranged from 0 to 47 years. The median age at which patients had experienced their first symptoms was 30 years (range 1 to 62 years). Seven patients (six children and one adult) had been diagnosed within the first two years of life. None of these patients had a hypertrophic cardiomyopathy, a typical feature of the classic infantile phenotype. Sixty-nine adults and 10 children (86% of all patients) expressed the common c.-32-13T>G (IVS1-13T>G) mutation combined with a second pathogenic mutation on the other allele.

Twenty-one of the 75 adults (28%) and eight of the 17 children (47%) had a mild scoliosis. One child had a severe scoliosis requiring surgery. Twenty-four of the 75 adults (32%) used mechanical ventilation either non-invasively (n=18) or invasively (n=6; all males). Two of the 17 children (12%) used nocturnal non-invasive ventilation (one boy, age 13 years, age at start of ventilation 13 years; one girl, age 9 years, age at start of ventilation 8 years). In one patient, respiratory insufficiency had been the first symptom of Pompe disease. Six patients (four adults and two children) who used ventilatory support were able to walk without needing walking devices. Table 1 shows the clinical characteristics of the patient group.

Pulmonary function tests

Spirometry

In upright seated position, 45 of the 75 adults (60%) had a decreased VC. VC was mildly decreased in 15, moderately decreased in 11 and severely decreased in 19 (including the six patients being ventilated through a tracheostomy tube). The FEV_1/VC ratio was >80% in all but one patient, indicating a restrictive nature of the compromised pulmonary function. Of the 17 children, eight (47%) had a decreased VC in sitting position. Vital capacity was mildly decreased in three, moderately

decreased in one and severely decreased in four. The FEV_1/VC ratio was >80% in all children. The youngest patient in whom a diminished pulmonary function was measured was seven years old. At a group level, mean VC was significantly lower in the patients who had a scoliosis compared to those without a scoliosis (mean z-score -3.1 (65.3% predicted) against mean z-score -1.7 (85.1% predicted), p=0.02). Of the group of 92 patients, three had COPD (all adults), four had asthma (three adults, one child), and none had first-degree relatives with pulmonary disease. Twelve adult patients were current smokers, while 27 had smoked in the past. Comparing distributions of VC between these subgroups of patients, no significant differences were found.

Measured in supine position, VC was diminished in 59 adult patients (79%) and in 10 children (59%). When patients moved from a sitting to supine position, VC fell in 18 (20%) by more than 25% (so called “postural drop”), indicating possible diaphragmatic weakness. Testing in the supine position was not attempted in 11 patients whose pulmonary function was already severely restricted in seated position. Two patients whose VC was normal or mildly reduced when they were seated nonetheless required ventilatory support when supine. Figure 1 shows a) the VC in sitting and supine positions expressed as percentage of the predicted value, and b) the magnitude of the difference when the patient changed position. This is indicated as “postural drop”.

Twenty of the 66 patients who received no ventilatory support complained of frequent dyspnoea while at rest or in supine position, or of morning headache, sleep disturbances or difficulty concentrating. Pulmonary function was severely impaired in ten of these patients (<-4SD or <50% of the predicted normal value), only two of whom had already been monitored for nightly hypoventilation by a center for home ventilation; the other eight were referred by us. In three, nocturnal ventilation had to be initiated. In addition, seven patients who did not have any respiratory complaints were found to have a severely reduced pulmonary function in the supine position. Evaluation of their respiration during sleep did not lead to installation of mechanical ventilation so far. It is noteworthy that VC had never previously been measured in 13 of these 17 patients whose pulmonary function appeared to be severely reduced, or at least not in supine position.

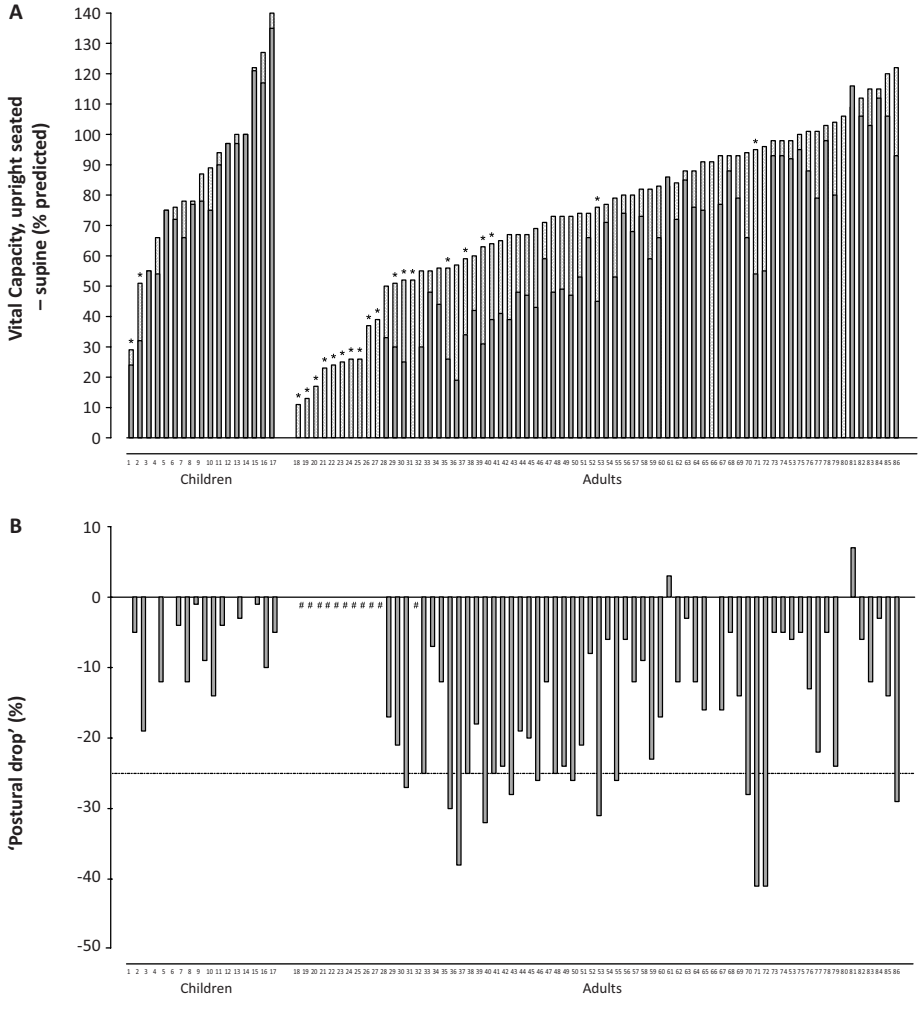

Figure 1. (**A**) Vital capacity in upright seated position compared to vital capacity in supine position in 17 children and 69 adults with Pompe disease. The light gray represents vital capacity in upright position; the dark gray represents vital capacity in supine position. Asterisks identify patients using ventilatory support. The six adult patients who are ventilated invasively 24 hours a day are not included in the graph. (**B**) Difference between vital capacity in sitting and supine positions, expressed in percentage (i.e. "postural drop"). The dotted line represents a difference between vital capacity in sitting and supine positions of 25%, defined as diaphragmatic weakness. # identifies patients whose pulmonary function was already so severely restricted when seated, that they could not perform the test when supine. For patients 3, 5, 12, 14, 65 and 80, vital capacity was identical in sitting and supine positions.

The severity of pulmonary dysfunction was significantly but moderately associated with the degree of skeletal muscle weakness measured by MRC sumscore (ρ=0.55, p<0.001, Figure 2), mobility (ρ=0.50, p<0.001) and disease duration (ρ=-0.46, p<0.001).

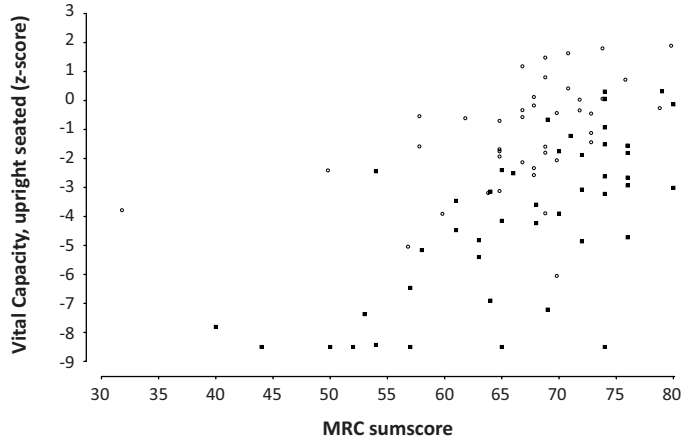

Figure 2. Relationship between vital capacity and MRC sumscore in 88 patients with Pompe disease. This muscle sumscore was composed by adding the grades (0–5) for the following muscle groups: neck extension, neck flexion and bilateral shoulder abduction, elbow flexion, elbow extension, hip flexion, hip abduction, knee flexion and knee extension (range 0–80). Circles represent females, squares represent males. MRC sumscore did not differ significantly between males and females (p=0.64).

An important finding was that there appeared to be a difference in the degree of pulmonary dysfunction between males and females. Firstly, at a group level, VC in male patients was significantly lower than in females (mean z-score -3.6 (69.6% predicted) against mean z-score -1.3 (82.8% predicted), p<0.001). Secondly, more males than females used ventilatory support (p=0.042, Table 1). Thirdly, all patients who were ventilated 24 h a day, whether invasively or otherwise, were male. Regression analyses showed that the difference in severity of pulmonary involvement between males and females increased with disease duration. In

females, z-score decreased by 1.5 points (p=0.15) from the category with a disease duration less than 5 years compared to the category with a disease duration of more than 15 years, while in males the corresponding decrease was 6.9 points (p<0.001; Figure 3). This difference in decline of vital capacity between males and females over the course of the disease was significant (p=0.001) and remained significant after adjustment for age, MRC sumscore and mobility (p=0.003). For the latter factors, it was found that older age, a higher MRC sumscore, and a better mobility score were associated with a higher VC (all p≤0.01).

Table 1. Clinical characteristics of the study population (n=92)

	Males (n=50)	**Females (n=42)**
Age at first study visit, y	45 (5–76)	47 (8–71)
Age first complaints, y	30 (1–61)	33 (1–62)
Age at diagnosis, y	36 (1–67)	36 (1–63)
Disease duration, y	12 (0–48)	10 (0–41)
Use of walking aids, n	6 (12)	5 (12)
Wheelchair use, n	16 (32)	11 (26)
Age at start of wheelchair use, y	49 (3–76)	50 (35–67)
Disease duration at start of wheelchair use, y	21 (2–46)	16 (5–26)
Use of ventilatory support, n	19 (38)[a]	7 (17)
Non-invasive (nose hood or face mask)	13	7
Invasive (tracheostomy tube)	6	–
Number of hours ventilatory support per day	12 (8–24)	9 (8–14)
Age at start of ventilatory support, y	45 (13–66)	46 (8–68)
Disease duration at start of ventilatory support, y	6 (0–36)	15 (1–28)

Continuous variables are presented as median (range); categorical data are presented as number (%).
[a] Ventilatory support was used more frequently by male patients (X^2 4.13; p=0.042).

Respiratory muscle strength

Respiratory muscle strength was assessed in 62 adults. MIP was reduced in 24 patients (39%; mean value 66.0±29.6% predicted, range 15–155% predicted)

and MEP in 38 patients (61%; mean value 64.7±30.9% predicted, range 13–141% predicted). Inspiratory and expiratory muscle strength were both affected in 24 patients. One patient had isolated inspiratory muscle weakness, and 14 patients had isolated expiratory muscle weakness.

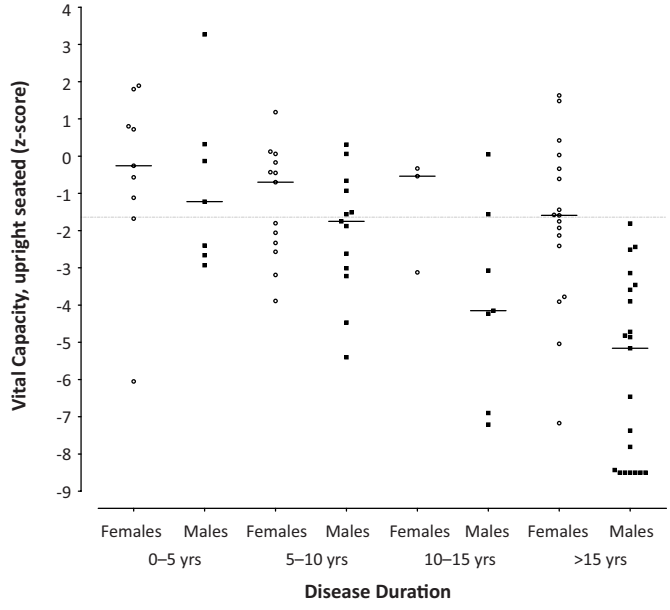

Figure 3. Relationship between disease duration, gender and vital capacity in 92 patients with Pompe disease. The observed values and median value for each category are presented. The dotted line represents the fifth percentile of the predicted vital capacity.

Overall, VC was strongly correlated with MIP and MEP (ρ=0.75 (MIP) and ρ=0.79 (MEP), both p<0.001; Figure 4). Despite this, inspiratory and/or expiratory respiratory muscle strength were reduced in four patients, while VC measured in upright and supine positions was completely normal.

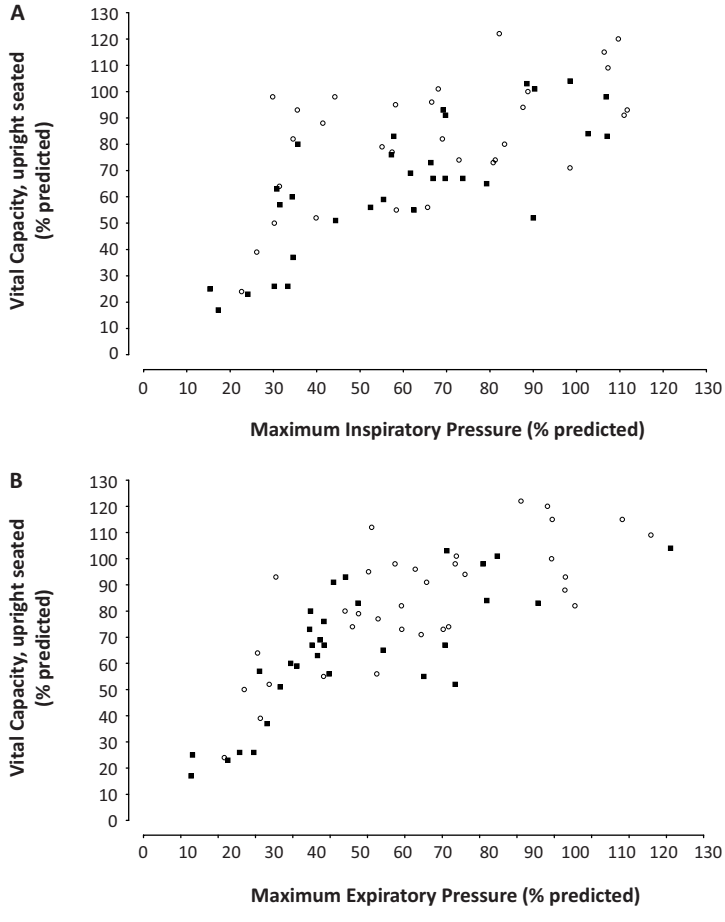

Figure 4. Relationship between vital capacity and maximum inspiratory pressure (**A,** n=60) and maximum expiratory pressure (**B**, n=62) in patients with Pompe disease. Circles represent females, squares represent males.

Capnography

Capnography was performed in 65 adult patients, ten of whom had $P_{EE,CO2}$ values of more than 6.0 kPa (patient range 6.06 to 7.41 kPa). Five of these patients, who also had clinical symptoms of hypercapnia, were already being mechanically ventilated

at night and during part of the day. They were referred to their center for home ventilation to see whether adjustments had to be made to their ventilation settings. Three of the other five patients had a combination of severely reduced VC in supine position, impaired respiratory muscle strength and complaints such as morning headache, falling asleep in the daytime, or difficulty concentrating. They belonged to the group of patients that had been referred to a center for home ventilation. Subsequently, mechanical ventilatory support was started in one.

The last two patients had normal or only mildly reduced VC without signs of respiratory muscle weakness, $P_{EE,CO2}$ being only marginally elevated in one ($P_{EE,CO2}$ 6.06 kPa). These two patients had no respiratory complaints.

Prospective longitudinal follow-up

General aspects

Prospective follow-up data were available for 53 adult patients (21 males, 32 females). Duration of follow-up ranged from 0.5 to 4.2 years (median 1.6 years). Within this period, mechanical ventilation was started in three patients. Five patients increased the number of hours of ventilation per day. One patient, who was severely affected and dependent on wheelchair use and ventilatory support, died of respiratory failure at the age of 55.

Pulmonary function during longitudinal follow-up

At a group level, VC in upright seated position, expressed as percentage of the predicted value, deteriorated by 0.9% points per year (p=0.094). The average rate of decline in VC measured in supine position was 1.2% points per year (p=0.049). MIP deteriorated by 3.2% points per year (p=0.018), and MEP by 3.8% points per year (p<0.01) (Figure 5).

In five individual patients, the disease progressed very rapidly, with pulmonary function in seated position declining by more than 10 percentage points per year. We did not find any significant differences in characteristics (age at first symptoms, gender, mobility, MRC sumscore, wheelchair use, use of ventilation), between these patients and the larger group of patients whose disease progressed less rapidly.

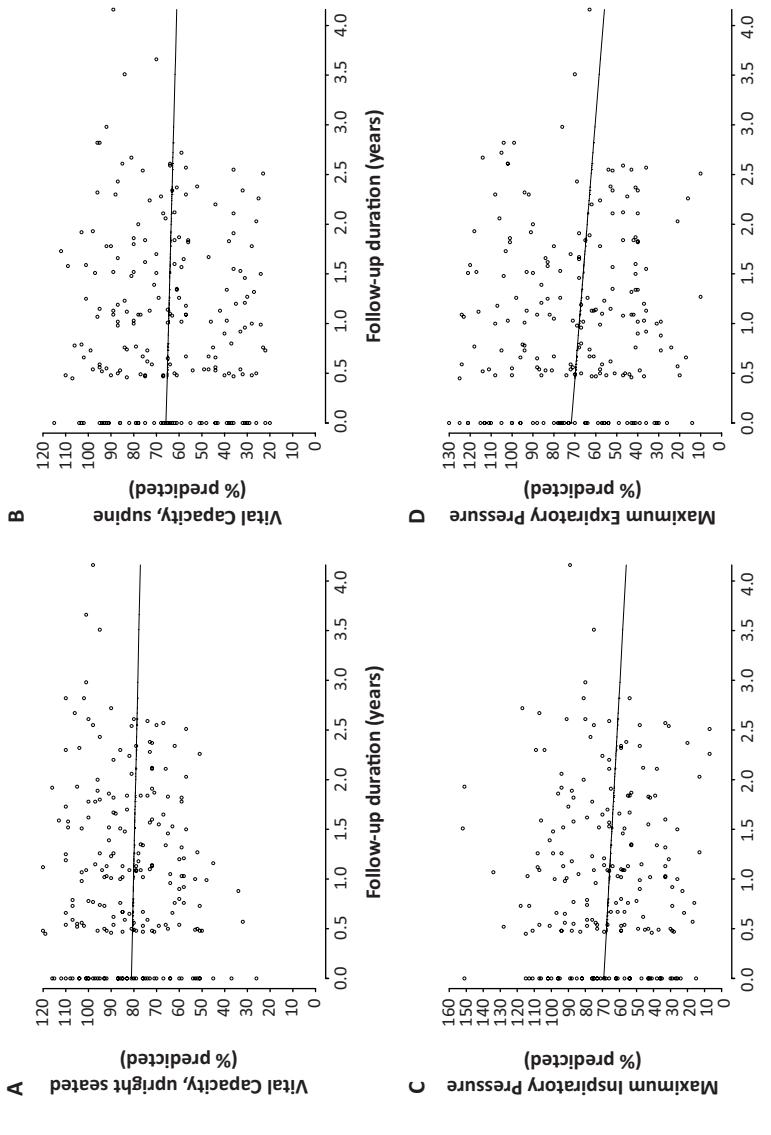

Figure 5. Rate of decline in vital capacity in upright seated position (**A**); vital capacity in supine position (**B**); maximum inspiratory pressure (**C**); and maximum expiratory pressure (**D**) related to follow-up duration measured from study onset in 53 adult patients with Pompe disease. Circles represent measured values. The line represents the mean regression line at a group level.

Although we noted during the prospective follow-up phase of the study that some of the specified subgroups (gender, mobility, current age and disease duration) seemed to influence the estimated rate of decline in pulmonary function, the differences in rate of progression between subgroups were not consistent throughout.

DISCUSSION

The purposes of our study were to determine the proportion and severity of pulmonary involvement in patients with Pompe disease, the rate of deterioration of pulmonary function, and to identify predictive factors for poor respiratory outcome.

With regard to the proportion of patients with pulmonary involvement, we found that 79% of the adults and 59% of the children, had some degree of pulmonary dysfunction.

With regard to severity, 42 patients, 26 of whom used mechanical ventilation, had a severely reduced pulmonary function in sitting or supine position (<-4SD or <50% of the predicted normal value). Pulmonary function was already moderately to severely decreased in seven of the children, three of whom were under the age of ten. Two of these seven children required ventilatory support from a young age (8 and 13 years). This indicates that the disease progresses more rapidly in a subset of children, and supports the findings by an international patient-oriented survey that our group has reported earlier.[12]

Thirty-five of the 92 patients (38%) in our study cohort had signs of diaphragmatic weakness, manifested by an inability to endure testing in supine position, a large drop in VC upon changing posture, or decreased MIP. Two patients required night-time ventilation due to respiratory insufficiency when supine, despite having normal or only slightly reduced VC when upright. As this group of patients may progress insidiously to respiratory failure when pulmonary function is measured only in upright seated position, they are in particular need of specific attention.

In 36 patients, VC had never been tested before, or at least not when they were supine. Of these, 13 (20% of all patients who were not ventilated) appeared to have a severely reduced pulmonary function in supine position. Three of these patients required the initiation of nocturnal mechanical ventilation. As only half of them had clinical signs indicating potential pulmonary dysfunction, it is clear that clinical respiratory complaints do not always predict the presence or severity of pulmonary dysfunction, and should not be relied on when selecting patients for pulmonary function testing.

Despite the fact that vital capacity was strongly correlated to MIP and MEP, a small number of patients whose VC was normal had evident respiratory muscle weakness. Future follow-up will show whether this might be a first indication of respiratory insufficiency.

Ten patients had abnormal end expiratory CO_2 values, two of whom had normal or only mildly reduced VC without signs of respiratory muscle weakness. Further follow-up will show whether their hypercapnia precedes decline in pulmonary function. It has been reported that the severity of hypercapnia may be disproportionate to that of respiratory muscle weakness in patients with neuromuscular disorders. One possible explanation is that pulmonary microatelectasis or rib cage abnormalities (such as scoliosis) increase elastic load.[29-31] Since nearly all patients with pulmonary dysfunction were identified by measurement of VC in sitting and supine positions and measurement of respiratory pressures, it is unclear if standard measurement of end expiratory CO_2 adds to the regular assessment of Pompe patients, other than in evaluating ventilatory settings in patients already using mechanical ventilation.

We identified three factors that were associated with more severe pulmonary involvement.

First, pulmonary function seems to decline faster in men than in women. This could not be explained by differences in age or disease duration; neither could it be explained by the severity of skeletal muscle weakness or the presence of scoliosis. This is the first time a possible gender difference has been found. We have no clear explanation for it: smoking habit and body mass index were not significantly different, nor had any of the patients experienced environmental exposures known

to be associated with reduced pulmonary function. In sleep disorders, gender differences have also been reported in which males are affected more severely than females. Two hypotheses have been put forward to explain these differences: 1) higher upper airway resistance caused by different body-fat distributions, and 2) differences in central ventilatory control induced by sex hormones.[32,33] Similar explanations may apply to patients with Pompe disease. For the time being, the mechanisms underlying these gender differences are uncertain and our findings need further investigation.

The second factor associated with poor pulmonary outcome was the severity of skeletal muscle weakness measured by MRC sumscore and mobility. In fact, these factors showed the best correlation with pulmonary involvement, though still moderate. This is in line with what was reported previously in smaller or selected groups of patients.[14,15] There nonetheless remains a subgroup of patients in whom either pulmonary function or skeletal muscle strength is substantially more severely affected than the other.

The third factor associated with poor pulmonary outcome has also been observed by other authors: that longer disease duration leads to more advanced pulmonary involvement.[12,17]

Regarding the rate of progression, we found a significant decline in vital capacity measured in the supine position, and also in maximum inspiratory pressure and maximum expiratory pressure during the prospective follow-up phase of this study; the decline in vital capacity measured when seated upright was not significant. Relative to that found in other recent studies,[13,17,34] the annual rate of decline we report seems rather small. We can think of two possible explanations which may have led us to slightly underestimate the actual rate of decline.

First, the introduction of enzyme-replacement therapy for patients with Pompe disease in 2006 provided a therapeutic option for this previously untreatable disease. At our center, we decided first to treat children and severely affected adults, while less affected patients started to receive ERT at a later stage. As a result, prospective follow-up data on the natural course are therefore available for only 53 adult patients from our study cohort, and the patients who were affected relatively mildly were followed for longer.

A second, related, issue is the fact that the follow-up cohort comprised fewer males than females, and only those males who were affected relatively mildly. This may also explain the fact that although we found a difference in the severity of pulmonary involvement between males and females when studying the baseline cohort, our prospective analyses did not show any differences in the rate of progression between the two sexes.

In conclusion, even though awareness of Pompe disease is high in the Netherlands, pulmonary function testing was not routinely performed upon diagnosis, and even cases of severely reduced pulmonary function were sometimes overlooked. Because it was possible to identify pulmonary dysfunction in nearly all patients by measuring VC in the upright and supine positions, we believe that the regular assessment of all patients with Pompe disease should at least include these measurements.

With regard to poor respiratory outcome, male sex, severe skeletal muscle weakness, and advanced disease duration were the most important predictors. These findings may help to identify patients at risk for developing respiratory failure, so that supportive measures such as night-time ventilation can be initiated as soon as they are needed.

REFERENCES

1. Ausems MG, Verbiest J, Hermans MP et al. Frequency of glycogen storage disease type II in The Netherlands: implications for diagnosis and genetic counselling. *Eur J Hum Genet* 1999; 7:713-716.
2. Martiniuk F, Chen A, Mack A et al. Carrier frequency for glycogen storage disease type II in New York and estimates of affected individuals born with the disease. *Am J Med Genet* 1998; 79:69-72.
3. Meikle PJ, Hopwood JJ, Clague AE, and Carey WF. Prevalence of lysosomal storage disorders. *JAMA* 1999; 281:249-254.
4. Lin CY, Hwang B, Hsiao KJ, and Jin YR. Pompe's disease in Chinese and prenatal diagnosis by determination of alpha-glucosidase activity. *J Inherit Metab Dis* 1987; 10:11-17.
5. Kishnani PS, Hwu WL, Mandel H et al. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *J Pediatr* 2006; 148:671-676.
6. van den Hout HM, Hop W, van Diggelen OP et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics* 2003; 112:332-340.
7. Müller-Felber W, Horvath R, Gempel K et al. Late onset Pompe disease: clinical and neurophysiological spectrum of 38 patients including long-term follow-up in 18 patients. *Neuromuscul Disord* 2007; 17:698-706.
8. van der Beek NA, Soliman OI, van Capelle CI et al. Cardiac evaluation in children and adults with Pompe disease sharing the common c.-32-13T>G genotype rarely reveals abnormalities. *J Neurol Sci* 2008; 275:46-50.
9. Engel AG, Gomez MR, Seybold ME, and Lambert EH. The spectrum and diagnosis of acid maltase deficiency. *Neurology* 1973; 23:95-106.
10. Hirschhorn R and Reuser AJ Glycogen storage disease type II : acid a-glucosidase (acid maltase) deficiency. In: Scriver CR, Beaudet AL, Sly W, and Valle D eds. *The Metabolic and Molecular Bases of Inherited Disease*, 8th ed. New York: McGraw-Hill, 2001:3389-3420.
11. Winkel LP, Hagemans ML, van Doorn PA et al. The natural course of non-classic Pompe's disease; a review of 225 published cases. *J Neurol* 2005; 252:875-884.
12. Hagemans ML, Winkel LP, Hop WC et al. Disease severity in children and adults with Pompe disease related to age and disease duration. *Neurology* 2005; 64:2139-2141.
13. van der Beek NA, Hagemans ML, Reuser AJ et al. Rate of disease progression during long-term follow-up of patients with late-onset Pompe disease. *Neuromuscul Disord* 2009; 19:113-117.
14. Laforêt P, Nicolino M, Eymard PB et al. Juvenile and adult-onset acid maltase deficiency in France: genotype-phenotype correlation. *Neurology* 2000; 55:1122-1128.
15. Pellegrini N, Laforêt P, Orlikowski D et al. Respiratory insufficiency and limb muscle weakness in adults with Pompe's disease. *Eur Respir J* 2005; 26:1024-1031.
16. Mellies U, Ragette R, Schwake C et al. Sleep-disordered breathing and respiratory failure in acid maltase deficiency. *Neurology* 2001; 57:1290-1295.
17. Wokke JH, Escolar DM, Pestronk A et al. Clinical features of late-onset Pompe disease: a prospective cohort study. *Muscle Nerve* 2008; 38:1236-1245.

18. van Capelle CI, Winkel LP, Hagemans ML et al. Eight years experience with enzyme replacement therapy in two children and one adult with Pompe disease. *Neuromuscul Disord* 2008; 18:447-452.
19. Strothotte S, Strigl-Pill N, Grunert B et al. Enzyme replacement therapy with alglucosidase alfa in 44 patients with late-onset glycogen storage disease type 2: 12-month results of an observational clinical trial. *J Neurol* 2010; 257:91-97.
20. Miller MR, Hankinson J, Brusasco V et al. Standardisation of spirometry. *Eur Respir J* 2005; 26:319-338.
21. Quanjer PH, Tammeling GJ, Cotes JE et al. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993; 16:5-40.
22. Zapletal A, Samanek M, and Paul T. Lung function in children and adolescents. Methods, reference values. *Progress in Respiratory Research* 1987; 22:113-218.
23. ATS/ERS Statement on respiratory muscle testing. *Am J Respir Crit Care Med* 2002; 166:518-624.
24. Allen SM, Hunt B, and Green M. Fall in vital capacity with posture. *Br J Dis Chest* 1985; 79:267-271.
25. Fromageot C, Lofaso F, Annane D et al. Supine fall in lung volumes in the assessment of diaphragmatic weakness in neuromuscular disorders. *Arch Phys Med Rehabil* 2001; 82:123-128.
26. Wilson SH, Cooke NT, Edwards RH, and Spiro SG. Predicted normal values for maximal respiratory pressures in caucasian adults and children. *Thorax* 1984; 39:535-538.
27. Annane D, Orlikowski D, Chevret S, Chevrolet JC, and Raphael JC. Nocturnal mechanical ventilation for chronic hypoventilation in patients with neuromuscular and chest wall disorders. *Cochrane Database Syst Rev* 2007;CD001941.
28. Medical Research Council. *Aids to examination of the peripheral nervous system*. Memorandum no. 45. London: Her Majesty's Stationary Office: 1976.
29. Misuri G, Lanini B, Gigliotti F et al. Mechanism of CO(2) retention in patients with neuromuscular disease. *Chest* 2000; 117:447-453.
30. Baydur A. Respiratory muscle strength and control of ventilation in patients with neuromuscular disease. *Chest* 1991; 99:330-338.
31. Braun NM, Arora NS, and Rochester DF. Respiratory muscle and pulmonary function in polymyositis and other proximal myopathies. *Thorax* 1983; 38:616-623.
32. Zhou XS, Shahabuddin S, Zahn BR, Babcock MA, and Badr MS. Effect of gender on the development of hypocapnic apnea/hypopnea during NREM sleep. *J Appl Physiol* 2000; 89:192-199.
33. Trinder J, Kay A, Kleiman J, and Dunai J. Gender differences in airway resistance during sleep. *J Appl Physiol* 1997; 83:1986-1997.
34. van der Ploeg AT, Clemens PR, Corzo D et al. A randomized study of alglucosidase alfa in late-onset Pompe's disease. *N Engl J Med* 2010; 362:1396-1406.

4

Cardiac evaluation in children and adults with Pompe disease sharing the common c.-32-13T>G genotype rarely reveals abnormalities

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ABSTRACT

Background and objective

Pompe disease is an inherited metabolic disorder caused by deficiency of acid α-glucosidase. All affected neonates have a severe hypertrophic cardiomyopathy, leading to cardiac failure and death within the first year of life. We investigated the presence and extent of cardiac involvement in children and adults with Pompe disease with the common c.-32-13T>G genotype to determine the usefulness of cardiac screening in these patients with relatively 'milder' phenotypes.

Methods

Cardiac dimensions and function were evaluated through echocardiography, electrocardiography and Holter monitoring. The total group comprised 68 patients with Pompe disease, of whom 22 patients had disease onset before the age of 18.

Results

Two patients (3%) had cardiac abnormalities possibly related to Pompe disease: Electrocardiography showed a Wolff-Parkinson-White pattern in an 8-year-old girl, and one severely affected adult patient had a mild hypertrophic cardiomyopathy. This hypertrophy did not change during treatment with recombinant human α-glucosidase. In addition, four adult patients showed minor cardiac abnormalities which did not exceed the prevalence in the general population and were attributed to advanced age, hypertension or pre-existing cardiac pathology unrelated to Pompe disease.

Conclusions

Cardiac involvement is rare in Pompe patients with the common c.-32-13T>G genotype. The younger patients were not more frequently affected than the adults. Electrocardiographic evaluation appears to be appropriate as initial screening tool. Extensive cardiac screening seems indicated only if the electrocardiogram is abnormal or the patient has a history of cardiac disease.

INTRODUCTION

Pompe disease (glycogen storage disease type II or acid maltase deficiency) is a progressive metabolic myopathy with an estimated frequency of 1 in 40,000 births.[1,2] Deficiency of the lysosomal enzyme acid α-glucosidase leads to accumulation of glycogen in a variety of tissues.[3] The clinical spectrum is broad, ranging from a rapidly progressive phenotype characterized by generalized muscle weakness, hypertrophic cardiomyopathy and death usually within the first year of life in infants,[4,5] to a more slowly progressive proximal myopathy with or without respiratory involvement in older children, adolescents and adults.[6,7]

In infants with the classic infantile phenotype, severe cardiac involvement is present in all cases. The electrocardiogram often shows a shortened PR-interval and high voltage QRS-complexes. Echocardiography reveals hypertrophy of both ventricles and an increased left ventricular (LV) mass index. Ultimately, hypertrophy may lead to reduction of the ventricular lumen and LV outflow obstruction, resulting in cardiorespiratory failure.[5,8]

It is well known that cardiac involvement can be an important feature in patients with other neuromuscular disorders like myotonic dystrophy and Duchenne and Becker muscular dystrophy.[9] It is also a feature in other lysosomal storage disorders such as Fabry disease[10] and mucopolysaccharidoses.[11] Furthermore, glycogen storage disorders are frequently mentioned in the differential diagnosis of hypertrophic cardiomyopathy.[12]

In contrast to the severe cardiomyopathy in infants, we recently found that cardiac involvement is rare in adults with the c.-32-13T>G genotype,[13] which is present in the majority of all patients with milder phenotypes.[14] However, a recent study indicated that a more severe phenotype may exist in a subgroup of patients under 15 years of age.[15] The aim of the current study was to compare the occurrence and severity of cardiac abnormalities in children and adults with Pompe disease sharing the common c.-32-13T>G genotype.

PATIENTS AND METHODS

This study is part of an ongoing research project on the natural course of late-onset Pompe disease. Sixty-eight patients were evaluated in our hospital between April 1998 and April 2008. Patients were recruited through the neuromuscular centres within the Netherlands and Belgium and via the Dutch neuromuscular patient organisation (Vereniging Spierziekten Nederland) or referred to our hospital by their treating physicians. The clinical diagnosis was confirmed in all patients through mutation analysis and measurement of decreased acid α-glucosidase activity in leukocytes or fibroblasts. The research protocol was approved by the Central Committee on Research Involving Human Subjects in the Netherlands (CCMO) and written informed consent was obtained from all patients or their parents.
Part of the data found in the adult patients was recently described.[13]
Information was gathered on gender, current age, age at first complaints, age at diagnosis, use of wheelchair or walking aids, pulmonary function, use of respiratory support and cardiac risk factors. Disease duration was calculated as the time between diagnosis and date of investigation.

GAA genotype

All patients were compound heterozygote and had the common c.-32-13T>G (IVS1-13T>G) mutation on one allele. The mutations found on the second allele were: c.525delT (n=26), c.2481+102_2646+31del (n=11; also known as delexon 18), c.925G>A (n=4), c.1548G>A (n=4), c.379_380del (n=3), c.307T>G (n=3), c.1799G>A (n=3), c.2135T>C (n=2), c.461_469del, c.896T>C, c.923A>C, c.2331+2T>A, c.2314T>C, c.1933G>A, c.172C>T, c.1441T>C, c.1396G>T, c.1115A>T (all n=1). Of these second mutations only c.461_469del does not entirely abolish the acid α-glucosidase activity. All other second mutations are fully deleterious. In two patients the second mutation could not be found.

Electrocardiography

Standard 12-lead electrocardiography recordings were examined for LV pre-excitation, increased LV voltage and rhythm or conduction disturbances. In a subset of 21 adult patients, 24-h Holter monitoring was performed.

Echocardiography

All patients underwent a detailed echocardiographic evaluation (Sonos 5500 or 7500 ultrasound system, Philips, Best, The Netherlands), performed by an experienced sonographer. The following data were acquired from M-mode recordings: left atrial dimension, LV end-diastolic and LV end-systolic dimension, LV septal and posterior wall thickness. As a measure of systolic function, fractional shortening or LV ejection fraction was used. LV mass index was calculated using the modified Devereux formula and indexed by body surface area.[16]

Statistical analysis

All variables were summarized using descriptive statistics comprising mean, SD, and range.

RESULTS

Patient demographics and clinical features

A total of 68 patients (41 males, 27 females) from 58 families were included in this study. All patients except one were from Caucasian origin. The mean age of the patients at the time of the investigation was 38±19 years (range 3 months to 71 years) and at the time of diagnosis 29±18 years (range several days to 63 years). Ten patients were diagnosed under 2 years of age. Twenty-three patients experienced symptoms of limb-girdle muscular weakness or respiratory problems before the age of 18. Within this group, delayed motor milestones were mentioned as first symptoms in five patients, while another seven patients were diagnosed presymptomatically due to elevated liver enzymes, an enlarged liver or an already known affected sibling. Other presenting symptoms in this group included feeding

difficulties, extreme fatigue and difficulty doing sports. In the patients with onset of symptoms in adulthood frequently mentioned first symptoms were: difficulty running/doing sports, difficulty climbing stairs, fatigue and muscle soreness or muscle cramps.

Twenty-seven patients (40%) had moderate to severe ambulatory problems for which either walking aids (n=8) or a wheelchair (n=19) were necessary. Fifteen patients (22%) used artificial ventilation: three patients were invasively ventilated 24-h a day, 12 patients used non-invasive ventilation only at night or when they were in a supine position during the day. Eight patients used a wheelchair as well as respiratory support. The mean age at start of wheelchair use was 44 years (range 3 to 67 years) and at start of using respiratory support 47 years (range 13 to 68 years).

Cardiac evaluation

Electrocardiography

Rhythm abnormalities or impaired conduction were present in one 8-year-old child and five adult patients. The electrocardiographic and echocardiographic parameters of the whole group are summarized in Table 1. The 8-year-old girl (patient 1 in Table 2) had an intermittent sinus rhythm and atrial rhythm, a δ-wave and a non-specific interventricular conduction block. Of the adult patients, one (patient 2) had an atrial rhythm with a short PR-interval (114 ms; normal range 120–200 ms) without evidence of a δ-wave. Three patients (patients 3, 4 and 5) had a prolonged QRS duration due to right or left bundle branch block, and one (patient 6) showed permanent atrial fibrillation which had been treated by His-bundle ablation and pacemaker implantation. None of the patients had increased QRS-voltages consistent with left ventricular hypertrophy.

Holter monitoring

Holter monitoring was performed in a subset of 21 adult patients. All patients except one showed a sinus rhythm during the 24-h monitoring. One patient (patient 6) had permanent atrial fibrillation, with 100% pacemaker rhythm. Nine patients showed rhythm alterations during monitoring. A low frequency of premature atrial

beats (<15/1000 beats) was seen in seven patients. Three patients had sporadic (<10/24 h) short lasting (<10 beats) atrial tachycardias. Transient nocturnal type I second degree atrio-ventricular block was noted in three patients. Apart from sporadic premature ventricular complexes in three patients, no ventricular arrhythmias were seen. All findings are within the normal range seen in the general population.[21]

Table 1. Electrocardiographic and echocardiographic parameters

	Age 0–9 years[a] (n=8) Mean±SD (range)	**Age 10–18 years (n=9) Mean±SD (range)**	**Age>18 years (n=50) Mean±SD (range)**
Electrocardiography			
PR-interval, ms	129±10 (116–146)	151±17 (130–186)	152±19 (**114**–196)
QRS duration, ms	75±15 (55–**104**)	93±8 (82–110)	98±18 (74–160)
Echocardiography			
Left atrial size, mm			32±4 (24–41)
LV end-diastolic dimension, mm	34±7 (21–43)	49±3 (45–53)	47±6 (29–58)
LV end-systolic dimension, mm	22±4 (14–27)	32±5 (23–37)	31±5 (20–44)
LV posterior wall, mm	5±1 (4–6)	8±2 (6–10)	9±1 (7–**15**)
LV interventricular septum, mm	6±1 (5–9)	8±1 (6–10)	9±2 (7–**16**)
LV systolic function			
Ejection fraction, %			65±9 (**48**–84)
Fractional shortening, %	35±4 (30–42)	36±7 (26–50)	
LV mass index, g/m^2			
Females	54±8 (48–60)	-	73±9 (58–95)
Males	32±18 (47–98)	79±15 (55–101)	81±25 (47–134)

The values indicated in bold are outside the normal range according to age.[16-20]
[a] Age at the time of investigation.

Table 2. Characteristics of the Pompe patients with abnormal electrocardiography or echocardiography findings (n=6)

Patient[a]	Gender	Age first symptoms (years)	Current age (years)	Mobility	Pulmonary function	Cardiovascular risk factors	ECG abnormalities	Echocardiographic abnormalities	Relation to Pompe disease
1	Female	9 months	8	Ambulant, no walking aids	VC 2.35 l (100%)	–	WPW pattern	–	Possible
2	Female	33	34	Ambulant, no walking aids	VC 3.66 l (109%)	Smoking	Atrial rhythm PR interval 114 ms	–	Unlikely
3	Male	35	45	Ambulant, no walking aids	VC 3.39 l (65%)	–	Left anterior hemi block	–	Unlikely
4	Female	31	50	Partial wheelchair use	VC 2.68 l (80%)	–	Left bundle branch block	–	Unlikely
5	Male	33	57	Permanent wheelchair use	Invasive ventilation 24 h/day	DM type II Smoking Hypertension	Right bundle branch block Left anterior hemi block	Septum 16 mm Posterior wall 15 mm	Possible
6	Male	20	67	Ambulant, walking aids	VC 3.11 l (69%)	DM type II	Atrial fibrillation	↑ LV end-diastolic dimension LV ejection fraction 48	Unlikely

VC=vital capacity, DM=diabetes mellitus, LV=left ventricular.

[a] All patients carried the c.-32-13T>G mutation in combination with a null mutation on the second allele.

Echocardiography

In all patients with symptom onset before the age of 18, atrial and ventricular dimensions, LV systolic function and LV mass index were normal. In one 15-year-old boy cardiac ultrasound revealed a congenital anomaly (quadricuspid aortic valve). Two patients with onset in adult life had an abnormal echocardiogram. One (patient 5) had mild right and left ventricular hypertrophy (septum 16 mm, LV posterior wall 15 mm), without signs of LV outflow obstruction. A second ultrasound 1.5 years after the start of enzyme replacement therapy with recombinant human α-glucosidase did not show any alterations. The patient known with atrial fibrillation and pacemaker implantation (patient 6) had an increased end-diastolic LV dimension and reduced LV systolic function (LV ejection fraction=48%). One patient was excluded from the analysis due to the poor quality of the echocardiographic images.

The characteristics of the six patients in whom we found abnormalities during cardiac evaluation are presented in further detail in Table 2.

DISCUSSION

We evaluated cardiac dimensions and electro-physiological properties in a cohort of 68 children and adults with Pompe disease sharing the common c.-32-13T>G genotype. Cardiac abnormalities were present in one child and five adults.

One 8-year-old child (patient 1) had an intermittent sinus rhythm and atrial rhythm, a δ-wave and a non-specific interventricular conduction block corresponding with a Wolff-Parkinson-White (WPW) pattern. This girl had no clinical manifestations of arrhythmias. An atrial rhythm and short PR-interval, but no δ-wave or other abnormalities indicating cardiac disease, was also seen in one adult patient (patient 2). Recently, in a study in 38 German adolescents and adults with Pompe disease, the frequent occurrence of symptomatic Wolff-Parkinson-White syndrome (in 8% of patients) was attributed to Pompe disease.[22] However, altered atrio-ventricular conduction is also quite common in the general population (reported frequency up to 3%[23]) and disappearance of the δ-wave later in life can occur due to changes in autonomic tone, maturation of the conduction system and aging.[24] The WPW-

pattern in the young girl could well be related to Pompe disease since alterations in the composition of the atrio-ventricular conduction system may lead to accelerated conduction.[25] Further clinical follow-up is necessary. The short PR-interval in the adult patient is most likely caused by the atrial rhythm (atrial ectopy originating close to the atrio-ventricular node).

One severely affected patient (patient 5), who was wheelchair bound and ventilator dependent for 24 h a day, had a mild hypertrophic cardiomyopathy and conduction disturbances in both bundle branches. This patient had a history of hypertension which may in part explain the left ventricular hypertrophy. Furthermore, pulmonary hypertension as a result of chronic hypoxemia may lead to right ventricular hypertrophy. However, right ventricular hypertrophy was not found in any of the other (invasively) ventilated patients. Unfortunately, it was difficult to obtain a clear tissue doppler signal in this patient and we are therefore unable to exclude the effect of the patient's pulmonary status on the right ventricular hypertrophy. A second echocardiography performed 1.5 years after start of enzyme replacement therapy did not show any decrease of right and left ventricular wall thicknesses. This is in contrast to the response observed in severely affected infants treated with enzyme therapy, in whom the massively enlarged heart in most cases diminishes to (almost) normal values.[26-28] The lack of response to treatment could be due to the prolonged existence of the hypertrophy leading to fibrosis, other secondary changes or an unrelated genetic cause for the cardiomyopathy. For the moment it remains unclear whether this patient's hypertrophic cardiomyopathy is related to Pompe disease.

Two more patients (patient 3 and 4) showed conduction disturbances in the bundle branches in the absence of structural echocardiographic abnormalities. These patients had no history of cardiac disease or any known cardiac risk factors, but showed signs of hypertension at the time of investigation. The frequency of conduction disturbances in the bundle branches in our study group (4%) does not exceed that in the general population (reported prevalence 2.5–8.5%).[27-30]

The last patient (patient 6) had permanent atrial fibrillation, which had been treated by His bundle ablation and pacemaker implantation at the age of 59 years. Apart from atrial fibrillation, this patient had reduced systolic function and

increased LV end-diastolic dimensions, probably related to the before mentioned cardiac pathology and unrelated to Pompe disease.

All patients in our study group carried the c.-32-13T>G mutation on one allele. This mutation is present in 68–90% of all patients with milder phenotypes[14,31,32] Despite this homogeneous genotype, our patient showed a wide variety in clinical severity, age at first symptoms and disease duration. Eight patients were both wheelchair and respirator dependent and 17 patients had a disease duration of more than 15 years. Therefore, the low frequency of cardiac abnormalities is not likely due to the lack of severely affected patients in our study group. In the large group of patients we studied, the patients with disease onset under 18 years of age were neurologically not more severely affected than the adult patients, suggesting that the subgroup of younger patients with a more severe phenotype[15] does not have the c.-32-13T>G genotype. Therefore we cannot draw conclusions on the occurrence of cardiac involvement in patients who do not express the c.-32-13T>G mutation.

The observed cardiomyopathy in one of our patients was relatively mild, in contrast to the severe hypertrophic cardiomyopathy resulting in cardiac failure seen in neonates. The difference in the prevalence and severity of cardiac involvement in infants compared to adults is likely due to the higher amount of residual α-glucosidase activity in adults.[33] A low level of enzyme activity seems sufficient to prevent intralysosomal accumulation of glycogen in cardiomyocytes, but not in skeletal muscle, since all patients showed skeletal muscle weakness, ranging from mild to severe involvement. This may be due to differences in storage capacity and metabolism of heart and skeletal muscle.[34] The more abundant presence of cytoplasmic glycogen in skeletal muscle fibers compared to cardiomyocytes may lead to more extensive lysosomal glycogen accumulation in the skeletal muscles through autophagy, resulting in more muscle fiber damage.[35,36]

In spite of this apparent low frequency of cardiac involvement within our study group, several observations suggest an increased cardiovascular morbidity in adults with Pompe disease. We recently reported the presence of increased aortic stiffness in a subset of patients from this study compared to controls, possibly due to glycogen deposition in the smooth muscle cells of the aortic wall.[37] In addition,

there are several reports on the occurrence of aneurysms in Pompe disease which maybe related to the same phenomenon.[38,39] At present, the clinical consequences of these findings are not fully clear and need further research.

In conclusion, cardiac evaluation in 68 children and adults with Pompe disease sharing the common c.-32-13T>G genotype showed abnormalities possibly related to Pompe disease in only two cases (one child, one adult). Since all patients with abnormal cardiac parameters were detected by electrocardiography, we advise to perform an electrocardiogram at least once in routine clinical follow-up. Additional echocardiography seems indicated only in those patients with abnormal electrocardiographic findings, a history of cardiac disease or evident cardiac symptoms.

REFERENCES

1. Ausems MG, Verbiest J, Hermans MP et al. Frequency of glycogen storage disease type II in The Netherlands: implications for diagnosis and genetic counselling. *Eur J Hum Genet* 1999; 7:713-716.
2. Martiniuk F, Chen A, Mack A et al. Carrier frequency for glycogen storage disease type II in New York and estimates of affected individuals born with the disease. *Am J Med Genet* 1998; 79:69-72.
3. Hirschhorn R and Reuser AJ. Glycogen storage disease type II: acid a-glucosidase (acid maltase) deficiency. In: Scriver CR, Beaudet AL, Sly W, and Valle D eds. *The Metabolic and Molecular Bases of Inherited Disease*, 8th ed. New York: McGraw-Hill, 2001:3389-3420.
4. Kishnani PS, Hwu WL, Mandel H et al. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. J *Pediatr* 2006; 148:671-676.
5. van den Hout HM, Hop W, van Diggelen OP et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics* 2003; 112:332-340.
6. Hagemans ML, Winkel LP, van Doorn PA et al. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. *Brain* 2005; 128:671-677.
7. Winkel LP, Hagemans ML, van Doorn PA et al. The natural course of non-classic Pompe's disease; a review of 225 published cases. *J Neurol* 2005; 252:875-884.
8. Seifert BL, Snyder MS, Klein AA et al. Development of obstruction to ventricular outflow and impairment of inflow in glycogen storage disease of the heart: serial echocardiographic studies from birth to death at 6 months. *Am Heart J* 1992; 123:239-242.
9. Bushby K, Muntoni F, and Bourke JP. 107th ENMC international workshop: the management of cardiac involvement in muscular dystrophy and myotonic dystrophy. 7th-9th June 2002, Naarden, the Netherlands. *Neuromuscul Disord* 2003; 13:166-172.
10. Kampmann C, Baehner F, Ries M, and Beck M. Cardiac involvement in Anderson-Fabry disease. *J Am Soc Nephrol* 2002; 13 Suppl 2:S147-S149.
11. Rigante D and Segni G. Cardiac structural involvement in mucopolysaccharidoses. *Cardiology* 2002; 98:18-20.
12. Arad M, Maron BJ, Gorham JM et al. Glycogen storage diseases presenting as hypertrophic cardiomyopathy. *N Engl J Med* 2005; 352:362-372.
13. Soliman OI, van der Beek NA, van Doorn PA et al. Cardiac involvement in adults with Pompe disease. *J Intern Med* 2008; 264:333-339.
14. Kroos MA, Pomponio RJ, Hagemans ML et al. Broad spectrum of Pompe disease in patients with the same c.-32-13T->G haplotype. *Neurology* 2007; 68:110-115.
15. Hagemans ML, Winkel LP, Hop WC et al. Disease severity in children and adults with Pompe disease related to age and disease duration. *Neurology* 2005; 64:2139-2141.
16. Devereux RB, Alonso DR, Lutas EM et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol* 1986; 57:450-458.
17. Mason JW, Ramseth DJ, Chanter DO et al. Electrocardiographic reference ranges derived from 79,743 ambulatory subjects. *J Electrocardiol* 2007; 40:228-234.
18. Otto CM. *Textbook of clinical echocardiography*, 3rd ed. Philadelphia: Elsevier Saunders, 2004.

19. Poutanen T and Jokinen E. Left ventricular mass in 169 healthy children and young adults assessed by three-dimensional echocardiography. *Pediatr Cardiol* 2007; 28:201-207.
20. Rijnbeek PR, Witsenburg M, Schrama E, Hess J, and Kors JA. New normal limits for the paediatric electrocardiogram. *Eur Heart J* 2001; 22:702-711.
21. Brodsky M, Wu D, Denes P, Kanakis C, and Rosen KM. Arrhythmias documented by 24 hour continuous electrocardiographic monitoring in 50 male medical students without apparent heart disease. *Am J Cardiol* 1977; 39:390-395.
22. Müller-Felber W, Horvath R, Gempel K et al. Late onset Pompe disease: clinical and neurophysiological spectrum of 38 patients including long-term follow-up in 18 patients. *Neuromuscul Disord* 2007; 17:698-706.
23. Rosner MH, Brady Jr WJ, Kefer MP, and Martin ML. Electrocardiography in the patient with the Wolff-Parkinson-White syndrome: diagnostic and initial therapeutic issues. *Am J Emerg Med* 1999; 17:705-714.
24. Klein A, Lebreton A, Lemoine J et al. Identification of urinary oligosaccharides by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Chem* 1998; 44:2422-2428.
25. Bharati S, Serratto M, DuBrow I et al. The conduction system in Pompe's disease. *Pediatr Cardiol* 1982; 2:25-32.
26. Kishnani PS, Corzo D, Nicolino M et al. Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology* 2007; 68:99-109.
27. van den Hout H, Reuser AJ, Vulto AG et al. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients. *Lancet* 2000; 356:397-398.
28. van den Hout JM, Kamphoven JH, Winkel LP et al. Long-term intravenous treatment of Pompe disease with recombinant human alpha-glucosidase from milk. *Pediatrics* 2004; 113:e448-e457.
29. de Bacquer D, de Backer G, and Kornitzer M. Prevalences of ECG findings in large population based samples of men and women. *Heart* 2000; 84:625-633.
30. Kreger BE, Anderson KM, and Kannel WB. Prevalence of intraventricular block in the general population: the Framingham Study. *Am Heart J* 1989; 117:903-910.
31. Huie ML, Chen AS, Tsujino S et al. Aberrant splicing in adult onset glycogen storage disease type II (GSDII): molecular identification of an IVS1 (-13T–>G) mutation in a majority of patients and a novel IVS10 (+1GT–>CT) mutation. *Hum Mol Genet* 1994; 3:2231-2236.
32. Montalvo AL, Bembi B, Donnarumma M et al. Mutation profile of the GAA gene in 40 Italian patients with late onset glycogen storage disease type II. *Hum Mutat* 2006; 27:999-1006.
33. Reuser AJ, Kroos MA, Hermans MM et al. Glycogenosis type II (acid maltase deficiency). *Muscle Nerve* 1995; 3:S61-S69.
34. Merin RG. Myocardial metabolism for the toxicologist. *Environ Health Perspect* 1978; 26:169-174.
35. Kuma A, Hatano M, Matsui M et al. The role of autophagy during the early neonatal starvation period. *Nature* 2004; 432:1032-1036.
36. van der Ploeg AT, Kroos M, van Dongen JM et al. Breakdown of lysosomal glycogen in cultured fibroblasts from glycogenosis type II patients after uptake of acid alpha-glucosidase. *J Neurol Sci* 1987; 79:327-336.
37. Nemes A, Soliman OI, Geleijnse ML et al. Increased aortic stiffness in glycogenosis type 2 (Pompe's disease). *Int J Cardiol* 2007; 120:138-141.

38. Matsuoka Y, Senda Y, Hirayama M, Matsui T, Takahashi A. Late-onset acid maltase deficiency associated with intracranial aneurysm. *J Neurol* 1988; 235:371-3.
39. Kretzschmar HA, Wagner H, Hubner G et al. Aneurysms and vacuolar degeneration of cerebral arteries in late-onset acid maltase deficiency. *J Neurol Sci* 1990; 98:169-183.

PART II

Effects of enzyme replacement therapy

5

Eight years experience with enzyme replacement therapy in two children and one adult with Pompe disease

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ABSTRACT

Pompe disease (type 2 glycogenosis, acid maltase deficiency) is a disorder affecting skeletal and cardiac muscle, caused by deficiency of acid α-glucosidase. In 2006 enzyme therapy with recombinant human α-glucosidase received marketing approval based on studies in infants. Results in older children and adults are awaited.

Earlier we reported on the 3-year follow-up data of enzyme therapy in two adolescents and one adult. In the present study these patients were followed for another 5 years.

Two severely affected patients, wheelchair and ventilator dependent, who had shown stabilization of pulmonary and muscle function in the first 3 years, maintained this stabilization over the five-year extension period. In addition patients became more independent in daily life activities and quality of life improved.

The third moderately affected patient had shown a remarkable improvement in muscle strength and regained the ability to walk over the first period. He showed further improvement of strength and reached normal values for age during the extension phase. The results indicate that both long term follow up and timing of treatment are important topics for future studies.

INTRODUCTION

Pompe disease (acid maltase deficiency, glycogen storage disease type II) (OMIM 232300) is an autosomal recessive neuromuscular disorder, caused by a deficiency of the enzyme acid α-glucosidase. This deficiency results in lysosomal glycogen accumulation in skeletal muscle and other tissues.[1,2] The disease encompasses a wide spectrum of presentations.[3-6]

The classic infantile form is at the severe end of this spectrum. The patients show a rapidly progressive course of disease with muscle weakness, a hypertrophic cardiomyopathy and death before the first year of age.[1,5,6] Acid α-glucosidase activity is virtually absent. A more slowly progressive myopathy affecting mobility and respiratory function is found in children and adults.[1] The time of onset and progression of symptoms is heterogeneous[7,8] and patients with this form of disease can express up to 30% residual α-glucosidase activity.[9]

Since Spring 2006, a registered therapy has been available (recombinant human acid a-glucosidase, Myozyme™, Genzyme Corporation). The initial clinical trials focused mainly on infants with the classic infantile form of Pompe disease.[10-17]

The results of an 18-month randomized placebo controlled trial in a large cohort of older children and adults are currently awaited.

In 1999, we started treatment in two older children and one adult. Over 3 years, these patients received α-glucosidase derived from the milk of transgenic rabbits (rabbit-AGLU). After this period they were switched to the product registered in 2006, i.e. recombinant human acid α-glucosidase derived from Chinese hamster ovary cells (CHO-AGLU). The results of the initial 3 years were described in ref.[18]

By now, after eight years of therapy, each patient has received approximately 380 infusions. All patients have reached adult age. This report provides the results of their eight years of therapy, focusing especially on the last five years.

PATIENTS AND METHODS

After patients had completed the first 3-year study period they entered an extension phase during which the safety and efficacy of recombinant human α-glucosidase was continued to be followed. Study assessments were performed in a standardized manner essentially as described before.[7,18-20] The extension protocol was approved by the Institutional Review Board and all patients (and parents if necessary) gave their written informed consent.

At start of the extension period, patients were transitioned from rabbit-AGLU (20 mg/kg/week, see[18] for details) to CHO-AGLU. CHO-AGLU was introduced in a stepwise manner starting with a dose of 10 mg/kg/week (week 1–12), 20 mg/kg/biweekly (week 13–26) and 30–40 mg/kg/biweekly thereafter. The following infusion rates were applied: 0.1–0.2 mg/kg/h for the first hour, 0.7–0.8 mg/kg/h for the second hour and 15–17 mg/kg/h until the end of the infusion. The total infusion duration was 4 h.

Information on the diagnosis of the three patients, including enzyme analysis and genotyping as well as a detailed description of the medical history can be found in ref.[18]

Muscle biopsy

Muscle sections taken at initiation of therapy and 6 months thereafter were available for all three patients. For patient 3 extra muscle biopsies were taken from the quadriceps muscle at start of the extension phase (3 years after start of therapy) and after a total treatment period of 5.5 years using a standard open surgical procedure. The samples were prepared for histological examination as described in.[2,21]

Statistical analysis

The relation between various outcomes and treatment duration for the different patients was evaluated using least-squares regression. In case of non-linear relations, Spearman's correlation coefficients were used. P-values <0.05 were considered significant.

RESULTS

Patients

Patient 1 was 16 years old when enzyme replacement therapy (ERT) was started. She had a severe scoliosis, was wheelchair and ventilator dependent. Muscle function and pulmonary function had shown a rapid decline over the previous 4 years (vital capacity decreased on average 4% per year).

During the first 3 years of treatment pulmonary function stabilized (Figure 1) and the number of mechanical non-invasive ventilation hours was reduced from 18 to 11–12 h per day (Table 1). Total muscle strength showed no significant change. Muscle function improved slightly (Table 1, Gross Motor Function Measure (GMFM)). The patients' scoliosis was surgically corrected during this period.

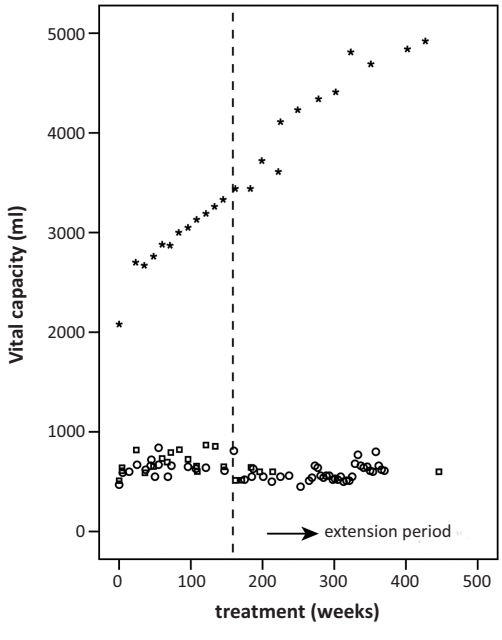

Figure 1. Effect of enzyme therapy on expiratory vital capacity over 8 years. Different symbols represent different patients (o=patient 1, □=patient 2, *=patient 3). The vertical dotted line marks the start of the 5-year extension phase.

Table 1. Overview of test results at baseline, 3 and 8-year follow-up including reference values for two children and one adult with Pompe disease

	Baseline	3-year ERT	8-year ERT	Ref. values
Patient 1				
Age (y)	16	19	24	–
Ventilation hours	16–18	11–12	11–12	–
HHD (Newton)	751	848	1371	3356[a]
GMFM (sitting)	47%	80%	83%	100%
GMFM (lying)	67%	78%	73%	100%
GMFM (crawling)	21%	33%	36%	100%
FSS	–	5.6	3.9	2.9[b]
	–	–	–	5.2[c]
RHS	–	16	25	–
Patient 2				
Age (y)	32	35	41	–
Ventilation hours	24	23.5	23.5	–
HHD (Newton)	199	305	349	4759[a]
GMFM (total)	12%	13%	12%	100%
FSS	–	6	4.2	2.9[b]
	–	–	–	5.2[c]
RHS	–	18	20	–
Patient 3				
Age (y)	11	14	20	–
HHD (Newton)	255	4310	4823	4759[a]
GMFM (total)	56%	100%	100%	100%
Running 10m (s)	–	3	2.3	–

HHD=Hand Held Dynamometry.[27,28] HHD values represent the sum score of the following muscle groups: neck flexion, neck extension, shoulder abduction, elbow flexion, elbow extension, wrist extension, hip flexion and abduction, knee extension and flexion, ankle dorsiflexion and plantar flexion. GMFM=Gross Motor Function Measurement.[29] FSS=Fatigue Severity Scale.[19] RHS=Rotterdam 9-item Handicap Scale.[7]
[a] Reference values for age at 8 years ERT. [b] General population. [c] Untreated patients.

Over the last 5 years pulmonary function and ventilation hours have remained stable (Figure 1, Table 1). Muscle strength increased with approximately 500 N spread over 6 muscle groups: shoulder abductors left, and elbow flexors ($p<0.02$);

hip flexors left ((p<0.05); knee flexors left, foot dorsal flexors and foot plantar flexors (p<0.01) (Figure 2). Scores on the GMFM and PEDI questionnaire did not change (Table 1). The overall level of handicap decreased as reflected by higher scores on the following functional activities of the Rotterdam 9 item handicap scale (RHS): mobility indoors, domestic tasks indoors and outdoors and the ability to perform work and study (Table 1). After the patient had resumed her education in 2001, she graduated from college and a Masters degree program and started Law School in 2007. She became less fatigued as reflected by lower score on the Fatigue Severity Scale (FSS) (Table 1). Quality of life assessed via the SF-36 showed improvements on the physical health and to a lesser extent on the mental health domains.

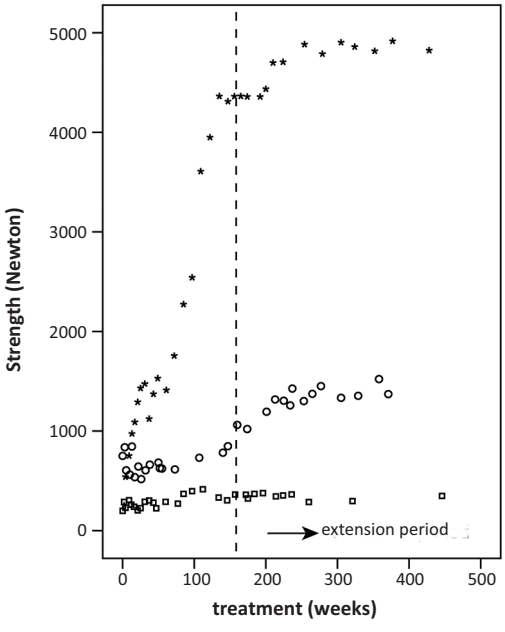

Figure 2. Effect of enzyme therapy on muscle strength as measured by Hand Held Dynamometry over 8 years. The y-axis represents the sum score of the following muscle groups: neck flexion, neck extension, shoulder abduction, elbow flexion, elbow extension, wrist extension, hip flexion and abduction, knee extension and flexion, ankle dorsiflexion and plantar flexion. Different symbols represent different patients (o=patient 1, □=patient 2, *=patient 3). The vertical dotted line marks the start of the 5-year extension phase.

Patient 2 was 32 years old when ERT was initiated. He was virtually quadriplegic and was bedridden for 21 h per day. From the age of 8 years onwards, he had lost, on average, 2.6% of vital capacity per year and had become completely dependent of invasive ventilation.

During the first 3 years of ERT, respiratory function stabilized (Figure 1). He could be weaned from the ventilator for 30 minutes per day (Table 1). Total muscle strength showed a minimal, but significant improvement (Figure 2). GMFM scores did not change (Table1). The patient performed slightly better on the self-care items of the PEDI questionnaire. At the end of the 3-year period he was able to stay up for 13 h per day.

Over the last 5 years pulmonary function (Figure 1), mechanical ventilation hours, muscle strength (Figure 2) and muscle function assessed by the GMFM (Table 1) and the PEDI questionnaire have remained stable.

The overall level of handicap (RHS) decreased slightly and the patient is now able to perform domestic activities outdoors and leisure activities independently (Table 1). He became less fatigued (FSS) (Table 1), leads an active life, and he experiences no limitations in participating in social activities. Quality of life (SF-36) improved, especially on the domains bodily pain and general health.

Patient 3 was 11 years at initiation of ERT. He had become wheelchair dependent 2 years earlier but could bear some weight on his legs. His pulmonary function was normal for age and height (FVC: 94% of normal).

During the first 3 years of treatment the patient showed an enormous improvement in muscle strength and function. Muscle strength (Figure 2) increased significantly in all muscle groups tested ($p<0.01$) except for the elbow extensors, but the total muscle sum score remained below the average of the general population (Figure 3). Muscle function assessed by GMFM reached a 100% score, but it was noted that he had difficulties rising from a squatting position.

Over the last 5 years muscle strength has reached normal values for age for all muscle groups (Figure 3) and rising from a squatting position is no longer difficult. Cycling against resistance, a measure for endurance, improved slightly (180 to 220 W). Currently there are no limitations in sports and other social activities. The patient works as a gardener. Pulmonary function remained within normal limits (Figure 1).

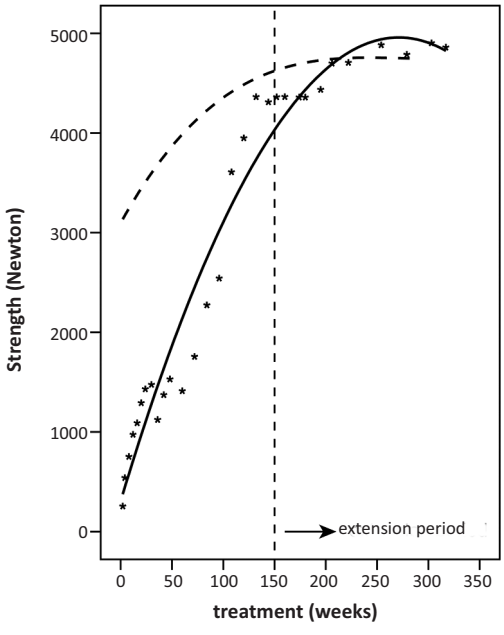

Figure 3. Effect of enzyme therapy on muscle strength as measured by Hand Held Dynamometry in patient 3. Age related reference values[27] are plotted for comparison. ----- =reference values —— =patient 3
The y-axis represents the sum score of the following muscle groups: neck flexion, shoulder abduction, elbow flexion, wrist extension, hip flexion and abduction, knee extension and flexion and ankle dorsiflexion.

Muscle biopsies

The baseline muscle biopsy of patient 3 showed a homogeneous pattern of abnormalities (Figure 4A and C). Most muscle fibers contained small PAS positive deposits of glycogen over the entire fiber length, but the gross muscle morphology was well preserved. In addition, fibers stained positive for acid phosphatase. After 3 (start extension phase) and 5.5 years of treatment, acid phosphatase reactive foci had decreased in number (Figure 4D and E). Also the PAS-stained section obtained after 5.5 years of treatment showed diminished staining intensity (Figure 4B). Fiber

size increased with age (Figure 4C-E) and cross-striation was normal (Figure 4B). In comparison to patient 3, muscle biopsy sections from patient 1 and 2 taken at start of therapy showed more severe pathology. Most fibers contained multiple vacuoles, numerous glycogen deposits (PAS positive material) and stained strongly positive for acid phosphatase. The muscle pathology varied substantially between fibers. The pathology after 6 months of treatment had not changed.

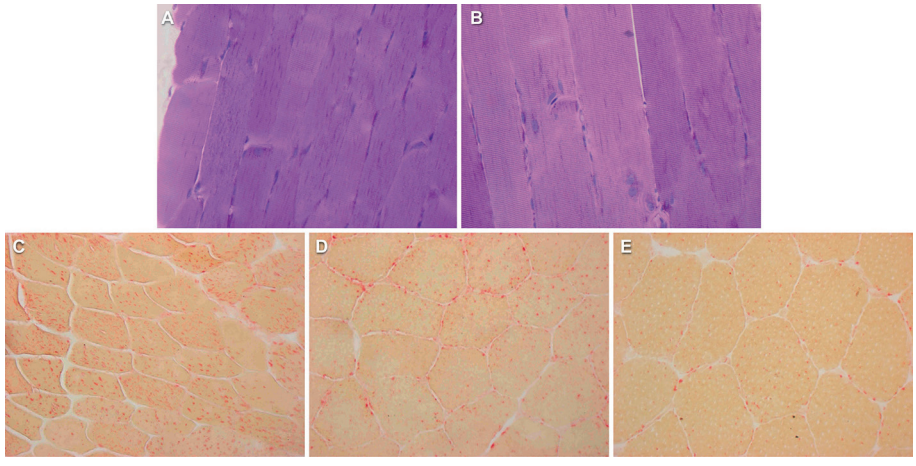

Figure 4. Effect of enzyme therapy on muscle morphology for patient 3. The upper two panels are stained with Periodic Acid Schiff reagent (PAS), the lower three panels for lysosomal acid phosphatase activity. (**A** and **C**) Sections obtained at baseline; (**D**) section obtained after 3 years of treatment; (**B** and **E**) sections obtained after 5.5 years of treatment.

Safety

Treatment with recombinant human α-glucosidase derived from CHO cells was tolerated well during the 5-year extension phase. In the first few months after transition, one patient experienced mild infusion related reactions, namely chills, during part of the infusion. No prophylactic medication was required and side effects disappeared gradually. Two patients are currently receiving the enzyme infusions at home.

DISCUSSION

The present study shows the long-term effects of enzyme therapy in patients with an attenuated form of Pompe disease. Three patients with different severities of disease who completed a 3-year pilot study were followed for another 5 years. All three patients had substantially more residual a-glucosidase activity than measured in classic infantile Pompe patients and two of the three patients had the c.-32-13T-->G mutation in combination with a null allele. The latter genotype is most common among children (53%) and adults (77%) with a protracted course of disease,[22-24] and not found in patients with the classic infantile form.

At initiation of therapy, two of our patients (patient 1 and 2) were severely affected with respect to motor function and pulmonary function. During the first 3 years of treatment, further loss of muscle function was prevented and pulmonary function stabilized. Both patients showed some decrease in mechanical ventilation hours. This situation was maintained during the 5-year follow-up. Most remarkable was that patients became more confident about their physical condition and more independent in daily life activities. This was reflected by a better score on the Rotterdam 9-item Handicap Scale, the SF-36 and the Fatigue Severity Scale.

The third and least affected patient, who had shown significant improvements in muscle strength and function over the first 3 years of therapy, needed 2 more years of enzyme therapy to catch up with healthy peers.

The functional gains of the three patients were reflected by changes in muscle morphology. Unlike the severely affected patients, most of the glycogen deposits of the best responder disappeared during treatment. The fact that minimal residual lysosomal staining remained present in this patient, illustrates the continuous balance between glycogen storage and clearance.

In untreated patients with the attenuated form of Pompe disease, muscle strength and pulmonary function deteriorate over time,[7,8,25,26] the odds for wheelchair use increase by 13%, and for respiratory support by 8% per year since diagnosis.[8] In a 2-year follow-up study four out of 52 adult patients died at a relatively young age.[7] The three patients described here had followed a similarly progressive course prior to treatment. Two of them had shown a steady decrease of 2.6% and 4% FVC per

year. All three had become wheelchair dependent and one patient had progressed to end stage disease, he was bed ridden and completely ventilator dependent. The long-term stabilization and sustained improvement of the three patients after the start of enzyme therapy is therefore a significant finding.

None of the patients experienced severe side effects during the enzyme infusions and two are currently receiving enzyme therapy at home. During the 5-year extension period they received 30–40 mg/kg recombinant human a-glucosidase every other week. This dose was based on the amount of rabbit-AGLU that was administered every week during the first 3 years of treatment and is higher than the registered dosage of 20 mg/kg every other week. The results of this study indicate that even a higher dose may have limits in changing the clinical course of severely affected patients.

Obviously, our limited observations cannot be taken as proof that enzyme therapy exerts long-term beneficial effects in children and adults with Pompe disease, but they provide an important guideline for the broader application of enzyme replacement therapy.

The results of an 18-months randomized placebo-controlled trial in a large cohort of older children and adults are awaited soon. Our report indicates that follow-up should be continued for several years to assess the full effect of therapy.

REFERENCES

1. Hirschhorn R, Reuser AJJ. Glycogen storage disease type II: acid a-glucosidase (acid maltase) deficiency. In: Scriver CR BA, Sly WS (ed) The metabolic and molecular bases of inherited disease. New York: McGraw-Hill, 2001: 3389-3420.
2. Engel AG, Hirschhorn R. Acid maltase deficiency. In: Engel A, Franzini-Armstrong C (eds) Myology: Basic and Clinical. New York: McGraw-Hill, 1994: 1533-1553.
3. Winkel LP, Hagemans ML, van Doorn PA, et al. The natural course of non-classic Pompe's disease; a review of 225 published cases. *J Neurol* 2005; 252:875-884.
4. Hagemans ML, Winkel LP, Van Doorn PA, et al. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. *Brain* 2005; 128:671-677.
5. van den Hout HM, Hop W, van Diggelen OP, et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics* 2003; 112:332-340.
6. Kishnani PS, Hwu WL, Mandel H, Nicolino M, Yong F, Corzo D. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. J Pediatr 2006; 148: 671-676.
7. Hagemans ML, Hop WJ, Van Doorn PA, Reuser AJ, Van der Ploeg AT. Course of disability and respiratory function in untreated late-onset Pompe disease. *Neurology* 2006; 66:581-583.
8. Hagemans ML, Winkel LP, Hop WC, Reuser AJ, Van Doorn PA, Van der Ploeg AT. Disease severity in children and adults with Pompe disease related to age and disease duration. *Neurology* 2005; 64:2139-2141.
9. Reuser AJ, Kroos MA, Hermans MM, et al. Glycogenosis type II (acid maltase deficiency). *Muscle Nerve* 1995; 3:S61-69.
10. Van den Hout JM, Reuser AJ, de Klerk JB, Arts WF, Smeitink JA, Van der Ploeg AT. Enzyme therapy for pompe disease with recombinant human alpha-glucosidase from rabbit milk. *J Inherit Metab Dis* 2001; 24:266-274.
11. Van den Hout JM, Kamphoven JH, Winkel LP, et al. Long-term intravenous treatment of Pompe disease with recombinant human alpha-glucosidase from milk. *Pediatrics* 2004; 113:e448-457.
12. Klinge L, Straub V, Neudorf U, Voit T. Enzyme replacement therapy in classical infantile pompe disease: results of a ten-month follow-up study. *Neuropediatrics* 2005; 36:6-11.
13. Klinge L, Straub V, Neudorf U, et al. Safety and efficacy of recombinant acid alpha-glucosidase (rhGAA) in patients with classical infantile Pompe disease: results of a phase II clinical trial. *Neuromuscul Disord* 2005; 15:24-31.
14. Amalfitano A, Bengur AR, Morse RP, et al. Recombinant human acid alpha-glucosidase enzyme therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial. *Genet Med* 2001; 3:132-138.
15. Van den Hout H, Reuser AJ, Vulto AG, Loonen MC, Cromme-Dijkhuis A, Van der Ploeg AT. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients. *Lancet* 2000; 356: 397-398.
16. Kishnani PS, Nicolino M, Voit T, et al. Chinese hamster ovary cell-derived recombinant human acid alpha-glucosidase in infantile-onset Pompe disease. *J Pediatr* 2006; 149:89-97.

17. Kishnani PS, Corzo D, Nicolino M, et al. Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology* 2007; 68:99-109.
18. Winkel LP, Van den Hout JM, Kamphoven JH, et al. Enzyme replacement therapy in late-onset Pompe's disease: a three-year follow-up. *Ann Neurol* 2004; 55:495-502.
19. Hagemans ML, van Schie SP, Janssens AC, van Doorn PA, Reuser AJ, van der Ploeg AT. Fatigue: an important feature of late-onset Pompe disease. *J Neurol* 2007; 254:941-945.
20. Hagemans ML, Janssens AC, Winkel LP, et al. Late-onset Pompe disease primarily affects quality of life in physical health domains. *Neurology* 2004; 63:1688-1692.
21. Winkel LP, Kamphoven JH, van den Hout HJ, et al. Morphological changes in muscle tissue of patients with infantile Pompe's disease receiving enzyme replacement therapy. *Muscle Nerve* 2003; 27:743-751.
22. Kroos MA, Pomponio RJ, Hagemans ML, et al. Broad spectrum of Pompe disease in patients with the same c.-32-13T->G haplotype. *Neurology* 2007; 68:110-115.
23. Huie ML, Chen AS, Tsujino S, et al. Aberrant splicing in adult onset glycogen storage disease type II (GSDII): molecular identification of an IVS1 (-13T-->G) mutation in a majority of patients and a novel IVS10 (+1GT-->CT) mutation. *Hum Mol Genet* 1994; 3:2231-2236.
24. Montalvo AL, Bembi B, Donnarumma M, et al. Mutation profile of the GAA gene in 40 Italian patients with late onset glycogen storage disease type II. *Hum Mutat* 2006; 27:999-1006.
25. Mellies U, Ragette R, Schwake C, Baethmann M, Voit T, Teschler H. Sleep-disordered breathing and respiratory failure in acid maltase deficiency. *Neurology* 2001; 57:1290-1295.
26. Mellies U, Stehling F, Dohna-Schwake C, Ragette R, Teschler H, Voit T. Respiratory failure in Pompe disease: treatment with noninvasive ventilation. *Neurology* 2005; 64:1465-1467.
27. Thurberg BL, Lynch Maloney C, Vaccaro C, et al. Characterization of pre- and post-treatment pathology after enzyme replacement therapy for Pompe disease. *Lab Invest* 2006; 86:1208-1220.
28. Beenakker EA, van der Hoeven JH, Fock JM, Maurits NM. Reference values of maximum isometric muscle force obtained in 270 children aged 4-16 years by hand-held dynamometry. *Neuromuscul Disord* 2001; 11:441-446.
29. van der Ploeg RJO. Hand-held dynamometry. In: Department of Neurology. Rijksuniversiteit Groningen, Groningen,1992 p 92
30. Russel D RP, Gowland C et al. Gross Motor Function Measure Manual. Hamilton: McMaster University, 1993.

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Effect of enzyme therapy in juvenile patients with Pompe disease: A three-year open-label study

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ABSTRACT

Pompe disease is a rare neuromuscular disorder caused by deficiency of acid α-glucosidase. Treatment with recombinant human α-glucosidase recently received marketing approval based on prolonged survival of affected infants. The current open-label study was performed to evaluate the response in older children (age 5.9–15.2 years). The five patients that we studied had limb-girdle muscle weakness and three of them also had decreased pulmonary function in upright and supine position. They received 20 mg/kg recombinant human α-glucosidase every two weeks over a 3-year period. No infusion-associated reactions were observed. Pulmonary function remained stable (n=4) or improved slightly (n=1). Muscle strength increased. Only one patient approached the normal range. Patients obtained higher scores on the Quick Motor Function Test. None of the patients deteriorated. Follow-up data of two unmatched historical cohorts of adults and children with Pompe disease were used for comparison. They showed an average decline in pulmonary function of 1.6% and 5% per year. Data on muscle strength and function of untreated children were not available. Further studies are required.

INTRODUCTION

Pompe disease (glycogenosis type II, acid maltase deficiency) (OMIM 232300) is a rare neuromuscular disorder caused by deficiency of the lysosomal enzyme acid α-glucosidase. As a result, glycogen accumulates in lysosomes of many cell types, but predominantly in skeletal muscle fibers. The process is progressive and finally destroys the muscle architecture and function.[1-4] The disease encompasses a clinical spectrum.[5-8] The classic infantile form is characterized by progressive cardiac hypertrophy and rapid loss of muscle function. Symptoms manifest shortly after birth and patients usually die within the first year of life.[1,3,6,7] Childhood, juvenile and adult phenotypes may present any time from infancy to late adulthood. The disease course is less progressive and cardiomyopathy is usually absent. Patients eventually become wheelchair and ventilator dependent. Respiratory failure is the major cause of early demise.[9-11] An intermediate non-typical infantile variant with cardiac hypertrophy and respiratory failure in early childhood has been described as well.[12] The nature of the acid α-glucosidase gene mutations is largely decisive for the degree of enzyme deficiency and clinical severity.[1,13]

Until recently there was no therapy for patients with Pompe disease other than supportive care. This has changed with the introduction of Enzyme Replacement Therapy. So far clinical trials with recombinant human acid α-glucosidase have mainly focused on infants and there have been incidental reports on effects in adults.[14-22] Treatment of infants was shown to increase survival, to diminish cardiac hypertrophy and to improve motor outcome. Based on positive results recorded in these trials, enzyme therapy with recombinant human acid α-glucosidase was approved for all patients, but it was explicitly stated that the safety and efficacy of the therapy still had to be proven across the clinical spectrum. The present study was designed to test the safety and efficacy of enzyme therapy in juvenile patients over a three-year treatment period.

MATERIALS AND METHODS

Study design

This study was conducted as an 18-month single-center, open-label, phase II study followed by an 18-month extension period and was approved by the Institutional Review Board of the Erasmus MC-Sophia Children's Hospital. Informed consent was obtained from patients and parents.

The endpoints of the study were exploratory and included safety, and the effect of treatment on pulmonary function, muscle strength and function. All assessments were performed at baseline and every three months thereafter.

Inclusion and exclusion criteria

Inclusion criteria were:

- Confirmed diagnosis of Pompe disease documented by deficient α-glucosidase activity in fibroblasts and/or DNA analysis
- Age between 5 and 18 years
- Demonstrable muscle weakness by manual muscle testing
- Able to provide 3 reproducible FVC measurements in sitting position (within 5% of one another)
- Able to walk 10 m

Patients were excluded if they required invasive ventilation or non-invasive ventilation whilst awake or in upright position. None of the patients had previously received enzyme therapy. Patient characteristics are described in Table 1.

Treatment

Patients received every other week, intravenously, 20 mg/kg recombinant human α-glucosidase from Chinese hamster ovary cells (Genzyme Corporation, Cambridge) in a step-wise manner: 0.2, 0.8, and 3.5 mg/kg/h each for 30 min and 10 mg/kg/h for the remainder of the infusion. Total duration of the infusion was approximately 3.5 h.

Safety variables

Physical examination, vital signs, and adverse event recording were performed at every visit. Echocardiograms and standard 12 lead electrocardiograms (ECG) were performed at baseline and at regular intervals thereafter along with safety laboratory measurements (complete blood count with differential, blood urea nitrogen, creatinine, glucose, uric acid, calcium, phosphorus, albumin, total protein, sodium, potassium, chloride, serum glutamic oxaloacetic transaminase/aspartate transaminase (SGOT/AST), serum glutamic pyruvic transaminase/alanine transaminase (SGPT/ALT), alkaline phosphatase, total bilirubin, creatine kinase (CK), creatine kinase with MB fraction (CK-MB), and urinalysis). Anti-recombinant human α-glucosidase IgG antibodies were measured from week 0 through week 74.

Pulmonary function

Pulmonary function (Forced vital capacity (FVC)) was assessed by spirometry[23] in the upright and supine position. The maximum value of three reproducible tests was used for analysis. The effect of therapy on pulmonary function in patients with an FVC <80% predicted at baseline was compared with two cohorts of untreated patients. Historical cohort 1 comprised 8 untreated children with Pompe disease that had an FVC <80% predicted at their first visit to our hospital. Historical cohort 2 consisted of 16 adult patients that were followed for a mean duration of 16±7 years (published in part by[24]).

Muscle strength

Muscle strength was assessed by Manual Muscle Testing (MMT)[25] and Hand-Held Dynamometry (HHD).[26-28] MMT was scored by an 11-point modified version of the Medical Research Council (MRC) scale.[29] HHD was conducted using a hand-held dynamometer (CT3001, C.I.T. Technics, Groningen, the Netherlands). Muscle groups tested by HHD and MMT were: 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 to calculate a total score for MMT (maximum score 45) and for HHD (Newton). The HHD sumscore was compared with reference values of age related peers.[26]

Muscle function

A 6-min walk test (6MWT) was performed according to the guidelines of the American Thoracic Society.[30] The maximum walking distance achieved in 6 min was measured at comfortable pace and at fast speed. Functional activity assessments included two timed tests: 10 meter running and rising from supine position to standing position.[29] The Quick Motor Function Test, a test that was specifically designed and validated for Pompe patients, was performed on regular intervals.[31] The scale consists of 16 specific motor function items. A total score is achieved by summing the scores for each item.

Patient and parent reports

All patients and their parents were interviewed at baseline and every three months thereafter. The interviews were scheduled before the different assessments and consisted of relevant issues such as mobility, fatigue, muscle pain, and self-reported changes from baseline.

Statistical analysis

The individual relationships between various outcomes and treatment duration for the different patients were evaluated using least-squares regression. In case of non-linear relations, Spearman's correlation coefficients' were used. For each patient, HHD% predicted values were estimated by linear interpolation of the reference data. Mean values of FVC % predicted according to age in an untreated historical control group was calculated by repeated measures ANOVA. On group level the various repeated measurements were analyzed by mixed model ANOVA (random coefficients models) (SAS PROC MIXED 8.2). p-values <0.05 were considered significant.

RESULTS

Patient characteristics study group

Five juvenile patients, three males and two females, were enrolled in the study (Table 1). They ranged in age from 5.9 to 15.2 years. All presented with mobility problems early in life (0.8–6.5 years). They were diagnosed between 1.1 and 11.6 years of age. The diagnosis was confirmed by mutation analysis (see Table 1 for details), and deficient α-glucosidase activity in cultured fibroblasts (range 2.8–17.9 nmol/mg/h). The α-glucosidase activity was clearly below the normal range (45–160 nmol/mg/h).

Safety

Patients were treated with 20 mg/kg α-glucosidase every two weeks. The three years of treatment were well tolerated. No infusion-associated reactions occurred during 390 infusions in total. None of the patients received premedication with antihistamines or corticosteroids. All patients developed IgG antibodies against the recombinant human enzyme between week 8 and week 38. The highest titers were observed between week 38 and week 74 and ranged from 800 to 6400 units (Figure 1). Lab safety parameters remained stable. There were no apparent changes in the condition of the patients in the two weeks between sequential infusions.

Pulmonary function

At baseline, two of the five patients (patient 1 and 3) had normal pulmonary function in both sitting and supine position (Figure 2). Their 'postural drop', defined as the difference between the forced vital capacity in sitting and supine position (ΔFVC), was 4% and 3%.

The other three patients (patient 2, 4 and 5) had a decreased pulmonary function (Forced Vital Capacity (FVC) of <80% predicted) at baseline (Figure 2), with a postural drop of 9.9%, 18.0%, and 33.3%. According to the ATS/ERS guidelines a postural drop of >25% is indicative for weakness of the diaphragm.[32,33] Patient 5 required nocturnal non-invasive ventilation (Bi-level Positive Airway Pressure).

Table 1. Diagnostic and baseline characteristics of the study patients

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age at diagnosis (y)	3.5	11.6	1.1	3	2
Age at first symptoms (y)	2.7	6.5	0.8	2.5	1
First symptoms	Episodes with falling and not able to take support on the legs	Difficulties with running during sports and while climbing stairs	Delayed motor milestones and hypotonia	Frequent episodes of falling	Floppy child, delayed motor milestones
Age at start therapy (y)	5.9	12.7	8.9	12.9	15.2
Respiratory support at baseline	None	None	None	None	BIPAP at night
Genotype[a]	c.2481+102_2646+31del (s) c.1634C>T (i)	c.525delT (s) unknown	c.-32-13T>G (m) c.923A>C (s)	c.-32-13T>G (m) c.2331+2T>A (s)	c.-32-13T>G (m) c.525delT (s)
α-Glucosidase activity in fibroblasts (nmol/h/mg)[b]	2.8	8.4	13.3	8.6	17.9

[a] Effect of the mutations: severe (s), intermediate-severe (i), and mild (m) (see for details www.pompecenter.nl). [b] Normal range: 45–160 (nmol/h/mg)

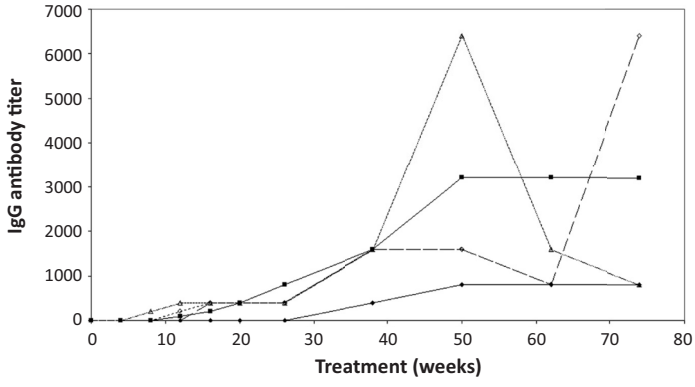

Figure 1. Anti-recombinant human α-glucosidase IgG antibody titers over 74 weeks of treatment. ◊=patient 1, ■=patient 2, ♦=patient 3, △=patient 4, ○=patient 5.

During treatment, pulmonary function of patients 1 and 3 remained within normal limits (Figure 2A and B). The FVC predicted remained stable in patients 2 and 4 and increased significantly in patient 5 ($p=0.01$, sitting and $p<0.01$, supine, Figure 2A and B). The postural drop remained unchanged.

Muscle strength

At baseline, muscle weakness was more pronounced in the proximal muscles than the distal muscles and more in the lower extremities than the upper extremities. Hip muscles (flexors, extensors and abductors) and neck flexors were most affected. Table 2 shows the results of the individual patients obtained over three years therapy. On a group level, both muscle strength assessed by MMT and HHD increased significantly (MMT 0.07% (=0.08 MMT point)/week ($p=0.007$), HHD 3.0 Newton/week ($p=0.01$)).

However, whilst all patients reached near-normal sum scores applying MMT, muscle strength measured with HHD remained below that of healthy peers. One patient showed significant catch-up growth towards normal values (Figure 3), the other patients did not but did not deteriorate either.

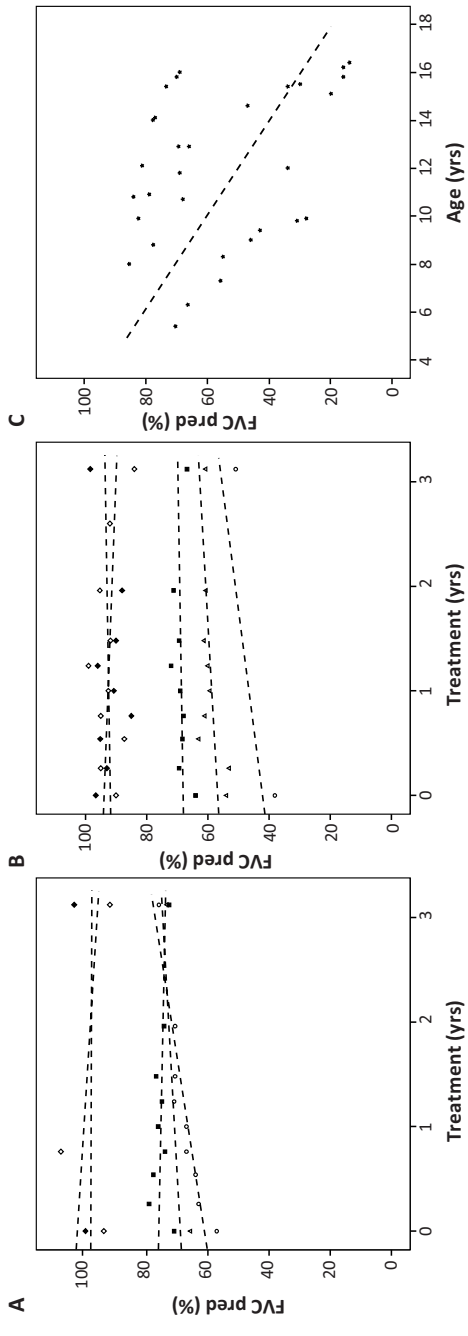

Figure 2. Effects of enzyme therapy on pulmonary function. (**A**) Predicted forced vital capacity (FVC) during treatment in sitting position. (**B**) Predicted forced vital capacity (FVC) during treatment in supine position. ◊=patient 1, ■=patient 2, ◆=patient 3, △=patient 4, ○=patient 5. (**C**) Mean predicted FVC in sitting position of historical cohort 1 comprising 8 untreated patients.

Table 2. Results of the individual patients at baseline and after three years of treatment.

		Baseline	3 year ERT	
Patient 1	HHD sumscore (Newton)	521.5	750	p=0.008
	MMT sumscore (%)	86	92	p=0.015
	6MWT (km/hr)	3.4	5.3	p=0.006
	Rising (sec)	4.4	3.94	n.s.
	Running (sec)	4.1	4.0	n.s.
	QMFT (%)	70.3	95.3	p=0.001
Patient 2	HHD sumscore (Newton)	521	865	p=0.004
	MMT sumscore (%)	79	93	p=0.07
	6MWT (km/hr)	4.7	5.8	p=0.07
	Rising (sec)	5.1	4.13	n.s.
	Running (sec)	4.2	4.0	n.s.
	QMFT (%)	73.4	92.2	p=0.002
Patient 3	HHD sumscore (Newton)	605	1202	p=0.002
	MMT sumscore (%)	87	100	p=0.001
	6MWT (km/hr)	5.2	5.9	n.s.
	Rising (sec)	4.4	3.0	p=0.06
	Running (sec)	3.9	3.0	p=0.005
	QMFT (%)	89.1	100	p=0.006
Patient 4	HHD sumscore (Newton)	608	1158	p=0.006
	MMT sumscore (%)	79	100	p=0.06
	6MWT (km/hr)	4.0	5.7	p=0.06
	Rising (sec)	6.2	3.2	p=0.01
	Running (sec)	4.5	3.8	p=0.037
	QMFT (%)	67.2	92.2	p<0.001
Patient 5	HHD sumscore (Newton)	992	1505	p=0.01
	MMT sumscore (%)	83	96	p=0.016
	6MWT (km/hr)	5.5	6.5	n.s.
	Rising (sec)	3.91	2.8	p=0.011
	Running (sec)	3.59	2.9	p=0.024
	QMFT (%)	79.7	92.2	p<0.001

All assessments were performed at baseline and at three months intervals thereafter. The individual changes over the three-year treatment period were evaluated using least-squares regression. All data points gathered every three months over three years time were used in this analysis. ERT=enzyme replacement therapy, HHD=Hand-Held Dynamometry, MMT=Manual Muscle Testing, 6MWT=Six-Minute Walk Test, QMFT=Quick Motor Function Test, n.s.=not significant.

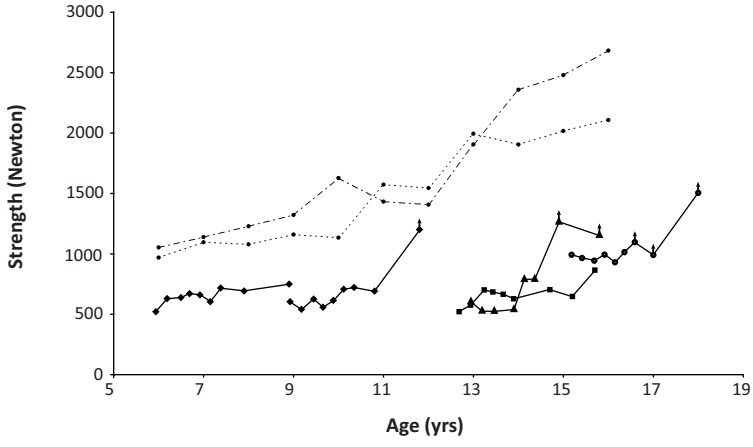

Figure 3. Effects of enzyme therapy on muscle strength measured by Hand-Held Dynamometry. Results of nine muscles were grouped together to calculate a total sum score. The sum score was plotted for all patients: ◊=patient 1 (boy), ■=patient 2 (girl), ♦=patient 3 (girl), △=patient 4 (boy), ○=patient 5 (boy). Age related reference values are plotted for comparison [27]. Reference values boys: –•–•–•–•–•–•. Reference values girls - - - - - -. Patients' muscle strength was measured with an upper limit of testing of 180 Newton per muscle group. The resulting outcomes for these patients therefore underestimate the true outcome (right-censored values). Data points representing right-censored values are represented by arrows.

Muscle function

Walking at comfortable pace appeared insufficiently challenging for the children and the results did not show any consistency over the different assessment days. This was different for the 6MWT at fast pace. At baseline the patients ran with an average speed of 3.4 to 5.5 km/h (see Table 2 for individual data). At the end of the study they managed to increase their distance with 64–184 m (mean increase 120 m). This increase was significant on group level (0.7 m/week (p=0.045)).

Unfortunately there are very few data available to compare with. One study reported healthy children between 12 and 14 years of age to run with mean velocities of 11.8 km/hour (range 10.3–13.4 km/h)[34] during a 6 min test. Three of our patients were in or above this age category.

At baseline, rising from supine to standing position took on average 4.4 times longer for the patients compared to healthy peers (see[35] for reference values age 5–12 years and Table 2 for individual data). On a group level, the results showed a trend towards significance (p=0.07 for rising and 0.096 for running).

Quick motor function test (QMFT)

Patients were regularly tested on 16 motor items that were specifically difficult for patients with Pompe disease.

Before start of treatment, patients had difficulty with most motor items tested, except for reaching hands over midline in supine position and stretching both arms simultaneously upward in sitting position.

Over three years of therapy, there was a significant increase in QMFT score on a group level (p=0.04, Table 2). Improvements were predominantly found in lifting head 45° in supine position (patients 1, 2, and 4), flexing hips through full range in supine position (all patients), doing a sit-up from supine position (patients 1, 2, 3, and 4), attaining standing position through half knee on the other knee (patients 1, 2, 4, and 5) and climbing four steps (patients 1, 2, 4, and 5).

Cardiac evaluation

Cardiac evaluation showed no signs of hypertrophic cardiomyopathy. Cardiac dimensions and diastolic and systolic function were normal. Cardiac ultrasound revealed a quadricuspid aortic valve in one patient. A second patient showed minor deformations of the tricuspid valve with a slight prolapse of the anterior leaflet, leading to minimal tricuspid regurgitation. A third patient showed the following ECG abnormalities: an intermittent sinus and atrial rhythm, a delta wave and a non-specific interventricular conduction block. These findings did not change during the study.

Patient and parent reports

Parents reported that their children had become more active during the day. They were able to participate more easily in activities such as running, playing sports, playing outdoors and cycling and had more energy left in the evening. Regular

headaches, muscle pain and fatigue present at start of therapy subsided. Two patients reported that frequent loose stools no longer occurred.

Surgical interventions

During the study, two patients (patient 2 and 4) received a unilateral Achilles tendon release. For patient 4, this was performed 6 months after start of treatment and for patient 2, 1.5 years after start of treatment. They recovered well without sequelae. To judge the significance of our findings we compared the FVC data of the study cohort with those of two untreated historical cohorts. Follow-up data on muscle strength and function of untreated children with Pompe disease were insufficiently available.

Patient characteristics historical cohort 1

Eight patients with Pompe disease, six males and two females, who did not receive treatment comprised historical cohort 1 (Table 3). All patients in this cohort had a decreased FVC (less than 80%) when first seen in our hospital. Age range of the patients at their first pulmonary function test was 5.4 to 14.1 years. In total 30 FVC measurements were performed in sitting position. Patients were ambulant and presented with mobility problems between 0.8 and 13 years (mean 5.8). They were diagnosed between 1.1 and 14 years. Two of the patients required respiratory support at night. Mutations and α-glucosidase activities are shown in Table 3.

Patient characteristics historical cohort 2

Sixteen untreated patients with Pompe disease, ten females and 6 males comprised historical cohort 2. Full details of this cohort have been published in [24]. Fifteen of these patients were compound heterozygotes (c.-32-13T>G in combination with a severe mutation (c.525delT (n=8), c.1548G>A (n=2), c.1115A>T (n=2), c.172C>T (n= 2), c.925G>A (n=1))). The genotype of the remaining patient was c.1634C>T (intermediate) /c.525delT (severe) functionally comparable to genotype c.1634C>T/ delexon18 of patient 1 of the study group. Cohort 2 included patients with normal and decreased pulmonary function at first assessment. Mean age at first symptoms was 24±11 years (range 1–40 years); mean age at diagnosis was 27±12 years. All

Table 3. Diagnostic characteristics of the patients of historical cohort 1

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4
Age at diagnosis (y)	11	7	1	4
Age at first symptoms (y)	10	6	0.5	8
First symptoms	Difficulty climbing stairs	Difficulty running	Delayed motor milestones	Difficulty performing sports
Age at first assessment (y)	12	9.4	9	8
Respiratory support at first assessment	BIPAP at night	BIPAP at night	None	None
Genotype [a]	c.-32-3C>A (s/i) c.877G>A + c.271G>A (s)	c.1829C>T (i) c.1912G>T (s)	c.1798C>T (i) c.525delT (s)	c.-32-13T>G (m) c.1441T>C (s)
α-Glucosidase activity in fibroblasts (nmol/h/mg) [b]	3.4	0.3	1.7	7.8
Characteristics	Patient 5	Patient 6	Patient 7	Patient 8
Age at diagnosis (y)	3	2	10	14
Age at first symptoms (y)	2.5	1	7	13
First symptoms	Frequent falling	Delayed motor milestones	Difficulty lifting head supine position	Severe fatigue
Age at first assessment (y)	10.8	5.4	10.7	14.1
Respiratory support at first assessment	None	None	None	None
Genotype [a]	c.-32-13T>G (m) c.2331+2T>A (s)	c.-32-13T>G (m) c.1051delG (s)	c.-32-13T>G (m) c.1548G>A (s)	c.-32-13T>G (m) c.1933G>A (s)
α-Glucosidase activity in fibroblasts (nmol/h/mg) [b]	8.6	4.2	9.1	6.2

[a] Effect of the mutations: severe (s), intermediate-severe (i), and mild (m) (see for details www.pompecenter.nl). [b] Normal range: 45–160 (nmol/h/mg).

were ambulant at their first visit, and one patient required respiratory support at night. Four patients were diagnosed before they were 18 years old. Age range at the first pulmonary function test was 11–57 years. In total 95 measurements of vital capacity (VC) in sitting position were performed. Mean follow-up duration of pulmonary function was 9 years (range 2–15 years).
For only one patient pulmonary function measurements were available before the age of 18 years. The first test was performed when the patient was 11 years old. VC at that time was 70% of predicted. Over the next 5 years, his VC decreased to 32%. The patient with genotype c.1634C>T/c.525del, had a VC of 24% predicted at the first available measurement when she was 22 years old. These patients became ventilator dependent at the age of 15, and 20 years.

Comparison of pulmonary function
The average decline of FVC predicted was 5% per year for historical cohort 1 (Figure 1C) and 1.6% per year for cohort 2. This was significantly different from the study group.

DISCUSSION

The present study assessed safety and efficacy of three years treatment with recombinant human α-glucosidase in five juvenile Pompe patients. All patients were ambulant and free of ventilator support during the day.
Treatment with recombinant human α-glucosidase was well tolerated. In a recent study on enzyme therapy in 18 patients with classic infantile Pompe disease, infusion-associated reactions were reported to occur in approximately 60% of the cases.[16] In the present study none of the patients experienced infusion-associated reactions, even though all five patients developed antibodies. The antibody titers did not reach the high levels that were observed in some infants. These differences might be explained by residual α-glucosidase activity expressed in juvenile patients, as opposed to the virtual absence of enzyme activity in classic infantile patients.

The presence of residual activity also explains the more slowly progressive disease course of the study patients prior to start of therapy.

Insight in the natural course of Pompe disease is essential to evaluate effects of treatment. There are several reports on pulmonary function in untreated adults with Pompe disease. They all indicate that pulmonary function declines with disease duration.[10,36-38] Limited data have been published on children. For that reason we compared the pulmonary function of the patients in this study with two historical cohorts. Both cohorts were unmatched. Historical cohort 1 consisted of eight children who all had an FVC predicted of <80% at their first visit. Mean age at first symptoms, age at diagnosis, time lag between diagnosis and age at first assessment were comparable with the study cohort. Five of 8 patients in cohort 1 compared to 3 of 5 patients in the study group had the common c.-32-13T>G/null genotype, that is found in 53% of children over 1 year of age and 77% of adults.[39,40] The other genotypes of patients in cohort 1 and the study group are more severe and clinical effects difficult to compare. It can therefore not be ruled out that the 5% predicted FVC decline per year in untreated children is slightly overestimated. On the other hand, historical cohort 2, which consisted of 15 patients with the milder c.-32-13T>G/null genotype and only one patient with a combination of a severe and intermediate mutation, showed an average decline of 1.6% predicted FVC per year. Also this course was significantly different from the study cohort.

All five patients in our study group had moderate muscle weakness at baseline. During treatment, improvements in strength were recorded with MMT and HHD. All children reached near-normal scores on MMT. Muscle strength assessed with HHD remained below the strength of healthy peers and confirms that MMT is less reliable and sensitive than HHD to give full information about the strength of muscles.[41-44] In particular this is the case for MMT grade 4 that covers a wide range of forces (10–250 Newton).[42] Applying HHD, one child significantly caught up with healthy peers, while the others did not. Earlier we found that a moderately affected 11-year-old patient needed five years of enzyme therapy before he reached normal strength.[45] This may indicate that long-term treatment may be required to obtain full effects.

Despite the fact that muscle strength remained below normal values, several functional improvements were observed in the patients. They were able to run longer distances in 6 min, or were able to rise faster from the floor. In addition, all patients performed significantly better according to QMFT scores. Part of these improvements may be explained by growth or by the Achilles tendon release operation that was performed in two patients. The fact that several patients learned to lift their head from the surface or to do a sit-up without use of hands, skills particularly difficult for untreated Pompe patients[5], cannot be explained by growth or surgery.

The results of the present study extend previously reported effects of treatment in classic infantile patients,[14-18,20,22] but should be interpreted with caution.

Limitations of our study are the small number of patients and the fact that our study was not placebo controlled. To overcome the latter problem we used two historical cohorts that only partly matched the treated patients. Cohort 2 mainly comprised adults. Historical data of untreated children with Pompe disease were only available for pulmonary function and not for muscle strength and function. We could therefore not fully rule out that untreated children might also have shown improvements of muscle strength and function over a certain period of time, for example with onset of puberty.

We found it encouraging, that none of the patients deteriorated over a three-year period. Some patients showed moderate improvements. All patients tolerated the enzyme infusions well. Long term follow-up studies with more patients are required.

REFERENCES

1. Engel A and Hirschhorn R. Acid maltase deficiency. In: Engel A and Franzini-Armstrong C eds. *Myology*, 2nd ed. New York: McGraw-Hill, 1994: 1533-1553.
2. Fukuda T, Ewan L, Bauer M et al. Dysfunction of endocytic and autophagic pathways in a lysosomal storage disease. *Ann Neurol* 2006; 59:700-708.
3. Hirschhorn R and Reuser AJ. Glycogen storage disease type II : acid a-glucosidase (acid maltase) deficiency. In: Scriver CR, Beaudet AL, Sly W, and Valle D eds. *The Metabolic and Molecular Bases of Inherited Disease*, 8th ed. New York: McGraw-Hill, 2001: 3389-3420.
4. Thurberg BL, Lynch MC, Vaccaro C et al. Characterization of pre- and post-treatment pathology after enzyme replacement therapy for Pompe disease. *Lab Invest* 2006; 86:1208-1220.
5. Hagemans ML, Winkel LP, van Doorn PA et al. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. *Brain* 2005; 128:671-677.
6. Kishnani PS, Hwu WL, Mandel H et al. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *J Pediatr* 2006; 148:671-676.
7. van den Hout HM, Hop W, van Diggelen OP et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics* 2003; 112:332-340.
8. Winkel LP, Hagemans ML, van Doorn PA et al. The natural course of non-classic Pompe's disease; a review of 225 published cases. *J Neurol* 2005; 252:875-884.
9. Hagemans ML, Winkel LP, Hop WC et al. Disease severity in children and adults with Pompe disease related to age and disease duration. *Neurology* 2005; 64:2139-2141.
10. Hagemans ML, Hop WJ, van Doorn PA, Reuser AJ, and van der Ploeg AT. Course of disability and respiratory function in untreated late-onset Pompe disease. *Neurology* 2006; 66:581-583.
11. Laforêt P, Nicolino M, Eymard PB et al. Juvenile and adult-onset acid maltase deficiency in France: genotype-phenotype correlation. *Neurology* 2000; 55:1122-1128.
12. Slonim AE, Bulone L, Ritz S et al. Identification of two subtypes of infantile acid maltase deficiency. *J Pediatr* 2000; 137:283-285.
13. Reuser AJ, Kroos MA, Hermans MM et al. Glycogenosis type II (acid maltase deficiency). *Muscle Nerve* 1995; 3:S61-S69.
14. Amalfitano A, Bengur AR, Morse RP et al. Recombinant human acid alpha-glucosidase enzyme therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial. *Genet Med* 2001; 3:132-138.
15. Kishnani PS, Nicolino M, Voit T et al. Chinese hamster ovary cell-derived recombinant human acid alpha-glucosidase in infantile-onset Pompe disease. *J Pediatr* 2006; 149:89-97.
16. Kishnani PS, Corzo D, Nicolino M et al. Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology* 2007; 68:99-109.
17. Klinge L, Straub V, Neudorf U, and Voit T. Enzyme replacement therapy in classical infantile pompe disease: results of a ten-month follow-up study. *Neuropediatrics* 2005; 36:6-11.
18. Klinge L, Straub V, Neudorf U et al. Safety and efficacy of recombinant acid alpha-glucosidase (rhGAA) in patients with classical infantile Pompe disease: results of a phase II clinical trial. *Neuromuscul Disord* 2005; 15:24-31.

19. Strothotte S, Strigl-Pill N, Grunert B et al. Enzyme replacement therapy with alglucosidase alfa in 44 patients with late-onset glycogen storage disease type 2: 12-month results of an observational clinical trial. *J Neurol* 2010; 257:91-97.
20. van den Hout H, Reuser AJ, Vulto AG et al. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients. *Lancet* 2000; 356:397-398.
21. van den Hout JM, Reuser AJ, de Klerk JB et al. Enzyme therapy for pompe disease with recombinant human alpha-glucosidase from rabbit milk. *J Inherit Metab Dis* 2001; 24:266-274.
22. van den Hout JM, Kamphoven JH, Winkel LP et al. Long-term intravenous treatment of Pompe disease with recombinant human alpha-glucosidase from milk. *Pediatrics* 2004; 113:e448-e457.
23. American Thoracic Society. Lung function testing: selection of reference values and interpretative strategies. *Am Rev Respir Dis* 1991; 144:1202-1218.
24. van der Beek NA, Hagemans ML, Reuser AJ et al. Rate of disease progression during long-term follow-up of patients with late-onset Pompe disease. *Neuromuscul Disord* 2009; 19:113-117.
25. Medical Research Council. *Aids to examination of the peripheral nervous system*. Memorandum no. 45. London: Her Majesty's Stationary Office, 1976.
26. Beenakker EA, van der Hoeven JH, Fock JM, and Maurits NM. Reference values of maximum isometric muscle force obtained in 270 children aged 4–16 years by hand-held dynamometry. *Neuromuscul Disord* 2001; 11:441-446.
27. van der Ploeg RJ, Fidler V, and Oosterhuis HJ. Hand-held myometry: reference values. *J Neurol Neurosurg Psychiatry* 1991; 54:244-247.
28. van der Ploeg RJ. *Hand-held dynamometry. Thesis.* Groningen: 1992.
29. Brooke MH, Griggs RC, Mendell JR et al. Clinical trial in Duchenne dystrophy. I. The design of the protocol. *Muscle Nerve* 1981; 4:186-197.
30. American Thoracic Society. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 2002; 166:111-117.
31. van Capelle CI, van der Beek NA, de Vries JM et al. The quick motor function test: a new tool to rate clinical severity and motor function in Pompe patients. *J Inherit Metab Dis* 2012; 35:317-323.
32. American Thoracic Society/European Respiratory Society. ATS/ERS Statement on respiratory muscle testing. *Am J Respir Crit Care Med* 2002; 166:518-624.
33. Vilke GM, Chan TC, Neuman T, and Clausen JL. Spirometry in normal subjects in sitting, prone, and supine positions. *Respir Care* 2000; 45:407-410.
34. van Mechelen W, Hlobil H, and Kemper HC. Validation of two running tests as estimates of maximal aerobic power in children. *Eur J Appl Physiol Occup Physiol* 1986; 55:503-506.
35. Beenakker EA, Maurits NM, Fock JM, Brouwer OF, and van der Hoeven JH. Functional ability and muscle force in healthy children and ambulant Duchenne muscular dystrophy patients. *Eur J Paediatr Neurol* 2005; 9:387-393.
36. Mellies U, Stehling F, Dohna-Schwake C et al. Respiratory failure in Pompe disease: treatment with noninvasive ventilation. *Neurology* 2005; 64:1465-1467.
37. Pellegrini N, Laforet P, Orlikowski D et al. Respiratory insufficiency and limb muscle weakness in adults with Pompe's disease. *Eur Respir J* 2005; 26:1024-1031.
38. Wokke JH, Ausems MG, van den Boogaard MJ et al. Genotype-phenotype correlation in adult-onset acid maltase deficiency. *Ann Neurol* 1995; 38:450-454.

39. Kroos MA, Pomponio RJ, Hagemans ML et al. Broad spectrum of Pompe disease in patients with the same c.-32-13T->G haplotype. *Neurology* 2007; 68:110-115.
40. Montalvo AL, Bembi B, Donnarumma M et al. Mutation profile of the GAA gene in 40 Italian patients with late onset glycogen storage disease type II. *Hum Mutat* 2006; 27:999-1006.
41. Munsat TL. Development of measurement techniques. *Neurology* 1996; 47:S83-S85.
42. van der Ploeg RJ and Oosterhuis HJ. Physical examination – measurement of muscle strength. *Ned Tijdschr Geneeskd* 2001; 145:19-23.
43. Schwartz S, Cohen ME, Herbison GJ, and Shah A. Relationship between two measures of upper extremity strength: manual muscle test compared to handheld myometry. *Arch Phys Med Rehabil* 1992; 73:1063-1068.
44. Escolar DM, Henricson EK, Mayhew JE et al. Clinical evaluator reliability for quantitative and manual muscle testing measures of strength in children. *Muscle Nerve* 2001; 24:787-793.
45. van Capelle CI, Winkel LP, Hagemans ML et al. Eight years experience with enzyme replacement therapy in two children and one adult with Pompe disease. *Neuromuscul Disord* 2008; 18:447-452.

7

A randomized study of alglucosidase alfa

in late-onset Pompe's disease

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ABSTRACT

Background

Pompe's disease is a metabolic myopathy caused by a deficiency of acid alpha-glucosidase (GAA), an enzyme that degrades lysosomal glycogen. Late-onset Pompe's disease is characterized by progressive muscle weakness and loss of respiratory function, leading to early death. We conducted a randomized, placebo-controlled trial of alglucosidase alfa, a recombinant human GAA, for the treatment of late-onset Pompe's disease.

Methods

Ninety patients who were 8 years of age or older, ambulatory, and free of invasive ventilation were randomly assigned to receive biweekly intravenous alglucosidase alfa (20 mg per kilogram of body weight) or placebo for 78 weeks at eight centers in the United States and Europe. The two primary end points were distance walked during a 6-minute walk test and percentage of predicted forced vital capacity (FVC).

Results

At 78 weeks, the estimated mean changes from baseline in the primary end points favored alglucosidase alfa (an increase of 28.1±13.1 m on the 6-minute walk test and an absolute increase of 3.4±1.2 percentage points in FVC; p=0.03 and p=0.006, respectively). Similar proportions of patients in the two groups had adverse events, serious adverse events, and infusion-associated reactions; events that occurred only in patients who received the active study drug included anaphylactic reactions and infusion-associated reactions of urticaria, flushing, hyperhidrosis, chest discomfort, vomiting, and increased blood pressure (each of which occurred in 5 to 8% of the patients).

Conclusions

In this study population, treatment with alglucosidase alfa was associated with improved walking distance and stabilization of pulmonary function over an 18-month period. (ClinicalTrials.gov number, NCT00158600.)

BACKGROUND

Pompe's disease is a rare, autosomal recessive, progressive neuromuscular disease caused by a deficiency of acid α-glucosidase (GAA), which degrades lysosomal glycogen. In patients with the classic infantile form, the deposition of glycogen in the heart, skeletal, and respiratory muscles causes severe cardiomyopathy, hypotonia, and respiratory failure, typically leading to death within the first year of life.[1-5] Children and adults, in contrast, have variable rates of disease progression. Glycogen deposition is confined mainly to skeletal and respiratory muscles, causing progressive limb-girdle myopathy and respiratory insufficiency.[2,5-9] Respiratory failure is a major cause of death.[6,10,11]

No disease-specific treatment was available for Pompe's disease until 2006, when enzyme-replacement therapy with alglucosidase alfa (Myozyme, Genzyme) was approved for all patients with Pompe's disease in the United States and the European Union, on the basis of open-label studies of infantile-onset Pompe's disease.[12] Trials involving infants showed improvements in survival and motor outcomes as compared with untreated historical controls.[12-16] Preliminary studies showed positive effects in children and adults but were small and not controlled.[11,17-19] We report the results of a randomized, controlled trial of alglucosidase alfa in late-onset Pompe's disease.

METHODS

Study design

The protocol was designed by Genzyme, with input from the authors and an independent statistical center (Cytel). The protocol and all amendments were approved by local review boards, ethics committees, and health authorities. Genzyme employees analyzed the data in accordance with the statistical plan and with additional suggestions from the investigators. Study conduct was monitored by an independent data and safety monitoring board. Primary efficacy analyses were ratified by the independent statistical center. All the authors collected the

data, had access to the data, and decided to submit the manuscript for publication. The first author and the coauthors wrote the manuscript, with the assistance of medical writers at Genzyme, and the first author determined the final content of the manuscript. All authors vouch for the completeness and veracity of the data and analyses.

This was a randomized, double-blind, placebo-controlled, multicenter study of the safety and efficacy of alglucosidase alfa in 90 patients with late-onset Pompe's disease. The study began in early September 2005 and was completed at the end of September 2007. Patients were screened and, after providing written informed consent (by patients 18 years of age or older and by guardians for younger patients), underwent a full baseline evaluation. Those who qualified were randomly assigned in a ratio of 2:1 to receive biweekly infusions of alglucosidase alfa (20 mg per kilogram of body weight) or placebo. The Pocock and Simon minimization algorithm[20] was used to balance the baseline distance walked on a 6-minute walk test (<300 or ≥300 m) and the baseline percentage of the predicted forced vital capacity (FVC) in an upright position (<55 or ≥55%) between study groups at each site.

Patients

All eligible patients had a confirmed diagnosis of Pompe's disease (GAA deficiency and two *GAA* gene mutations); were 8 years of age or older; were able to walk 40 m on the 6-minute walk test (with assistive devices permitted); had a percentage of the predicted FVC within the range of 30% to less than 80% in the upright position, with a postural drop in FVC (in liters) of 10% or more (from upright to supine); and had evidence of muscle weakness in the lower extremities, defined as bilateral knee extension less than 80% of predicted performance, as measured by quantitative muscle testing (QMT). Patients were excluded if they required any invasive ventilation or if they required noninvasive ventilation while awake and upright (see the Supplementary Appendix).

Assessments of clinical efficacy

Coprimary efficacy end points were meters walked on the 6-minute walk test and percentage of the predicted FVC in the upright position. Secondary and tertiary efficacy end points included changes in the percentage of the predicted QMT leg score and QMT arm score, maximum inspiratory pressure, and maximum expiratory pressure. Changes in walking distance on the 6-minute walk test were evaluated according to American Thoracic Society guidelines.[21]

Spirometric and manometric assessments of pulmonary function and respiratory muscle strength were performed according to American Thoracic Society and European Respiratory Society guidelines.[22-24]

The quantitative measurement system of the Cooperative International Neuromuscular Research Group was used to perform QMT to assess muscle force production during maximal voluntary isometric contraction of bilateral shoulder and hip adductors, elbow and knee flexors and extensors, and grip.[25,26] Data were reported as composite QMT leg and arm scores (i.e., the average of the percentage of predicted scores for bilateral knee flexors and extensors and bilateral elbow flexors and extensors).[25]

The Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) was administered to patients 14 years of age or older. The scores for the Physical Component Summary are reported.[27]

Antibody monitoring

Serum samples were obtained every 4 weeks for the first 52 weeks and again at weeks 64 and 78. IgG antibodies to alglucosidase alfa were assessed by means of the enzyme-linked immunosorbent assay (ELISA), and results were confirmed on radioimmunoprecipitation, as described previously.[28] Patients who tested positive for IgG antibodies were evaluated for antibodies that inhibit enzyme activity or uptake into cells.[29] Twofold dilution series of serum samples were preincubated with a fixed amount of enzyme. These samples were then analyzed to determine whether the antibodies interfered with the enzyme-substrate interaction. Similarly diluted samples were also preincubated with fluorescence-labeled enzyme and analyzed by means of flow cytometry to determine their ability to interfere with

enzyme internalization into fibroblasts (an easily grown cell type that expresses mannose-6-phosphate receptors, which mediate enzyme uptake). The last serum dilution that inhibited enzyme activity relative to the established assay cutoff point was recorded as the titer.

Safety assessments

All adverse events, serious adverse events, and infusion-associated reactions were recorded. The site investigator and the study sponsor determined whether an adverse event was related to the study drug.

Statistical analysis

We calculated that a minimum sample of 63 patients would be required to detect a treatment difference of 0.75 SD with 80% power (on the basis of a two-sample t-test with a significance level of 5% and a 2:1 ratio for randomization). Enrollment of at least 72 patients was planned, assuming a 10 to 15% dropout rate. The planned model for the primary efficacy analysis was a linear mixed-effects model with random intercepts and slopes. The estimated treatment effect was the absolute difference in the linear slopes of change between the alglucosidase alfa and placebo groups.

An adaptive design was implemented (under a protocol amendment) in which the initial 52-week treatment period could be extended by 3 or 6 months on the basis of an interim estimate of the standard error of the treatment effect on the 6-minute walk test; the estimate was used to determine the length of follow-up required to ensure adequate power for assessment of this end point. Because only the interim estimate of the standard error was used, no adjustment of the type I error rate was needed (see the Supplementary Appendix).[30] An interim analysis of the data on the 6-minute walk test was performed by an independent statistical center after all patients had completed week 38. On the basis of this interim analysis, the data and safety monitoring board recommended that the study be extended to 78 weeks; there were no interruptions in the study regimens during the 78-week trial. Neither the study sponsor nor the investigators had access to the interim results until the conclusion of the study.

The efficacy analysis was performed for the intention-to-treat population, defined as all patients randomly assigned to either alglucosidase alfa or placebo. A fixed-sequence testing procedure was used to account for multiple testing and to preserve the overall significance level of 5% for both coprimary end points. Formal testing for a treatment effect on FVC in the upright position was performed only after the significance of the treatment effect on the 6-minute walk test had been shown by means of a two-sided test. Prespecified testing of the assumptions for the linear mixed-effects model indicated that use of this model was not warranted; therefore, the primary efficacy analysis was an analysis of covariance (ANCOVA) for the change from baseline to week 78. The last-observation-carried-forward method was used for the ANCOVA model, with adjustment for randomization strata and baseline scores. Treatment effects were also estimated in predefined subgroups, and a post hoc sensitivity analysis with the use of mixed models for repeated measures and nonparametric tests was conducted to assess the robustness of the efficacy findings (see the Supplementary Appendix). Secondary and tertiary end points were analyzed by means of ANCOVA. The reported p values are two-sided and were not adjusted for multiple testing.

RESULTS

Characteristics of the patients

A total of 90 patients between 10 and 70 years of age were randomly assigned to either alglucosidase alfa (60 patients) or placebo (30 patients). Of this group, 81 completed the study; 5 in the alglucosidase alfa group and 4 in the placebo group dropped out (see Figure 1 in the Supplementary Appendix). The demographic and baseline characteristics of the patients are summarized in Table 1. In the alglucosidase alfa group, there were more men, the patients were slightly older, and fewer patients used a walking device at baseline. The only significant difference between the groups in disease-related characteristics was age at symptom onset (p=0.02). In both groups, the mean SF-36 Physical Component Summary scores were more than 1.5 SD below the norm for the U.S. general population (50±10), indicating that baseline physical health status was substantially diminished.

Table 1. Demographic and baseline characteristics of the study population[a]

Characteristic	Alglucosidase Alfa Group (n=60)	Placebo Group (n=30)	P value
Age at first infusion, years			
Mean	45.3±12.4	42.6±11.6	0.32
Range	15.9–70.0	10.1–68.4	
Sex, no. (%)			
Male	34 (57)	11 (37)	0.12
Female	26 (43)	19 (63)	
Race, no. (%)[b]			
White	57 (95)	27 (90)	0.40
Other	3 (5)	3 (10)	
Age at onset of symptoms, years			
Mean	30.3±12.3	23.9±11.0	0.02
Range	5.3–58.6	2.7–42.6	
Duration of disease, years			
Mean	9.0±6.3	10.1±8.4	0.48
Range	0.3–24.8	0.5–31.3	
Normal GAA activity, %			
Mean	10.8±8.2	10.1±7.8	0.71
Range	0–47.4	0–32.2	
Use of walking device, no. (%)	23 (38)	16 (53)	0.19
Use of ventilatory support, no. (%)	20 (33)	11 (37)	0.82
Score on SF-36 Physical Component Summary	34.33±8.93	34.91±7.26	0.23
Performance on 6-min walk test			
Distance walked, m			
Mean	332.2±126.7	317.9±132.3	0.62
Range	77.0–626.0	41.0–608.0	
% of predicted value			
Mean	52.5±19.0	50.3±20.5	0.61
Range	9.8–82.2	6.2–99.0	
FVC, % of predicted value			
Mean	55.4±14.4	53.0±15.7	0.47
Range	31.0–78.0	30.0–78.0	

[a] Plus-minus values are means ±SD. Fisher's exact test was used for comparisons of binary variables, and Student's t-test for comparisons of continuous variables. FVC denotes forced vital capacity, GAA acid α glucosidase, and SF-36 Medical Outcomes Study 36-Item Short-Form Health Survey.
[b] Race was reported by the patient.

Efficacy

By 78 weeks, treatment with alglucosidase alfa had significantly increased both the distance walked on the 6-minute walk test and the percentage of the predicted FVC (Table 2 and Figure 1). The alglucosidase alfa group had a mean increase of 25.1 m on the 6-minute walk test (the average baseline was 332.2 m), whereas the placebo group had a decrease of 3.0 m (the average baseline was 317.9 m), for an estimated differential treatment effect of 28.1 m (p=0.03). The estimated change in FVC, expressed as a percentage of each patient's predicted value, was an increase of 1.2 percentage points for the patients who received alglucosidase alfa and a decrease of 2.2 percentage points for the patients who received placebo, for an estimated treatment effect of 3.4 percentage points (p=0.006).

For each subgroup evaluated, the patients who received alglucosidase alfa had numerically better results (Figure 2 in the Supplementary Appendix). Subgroup analyses showed a greater difference between the study groups among patients with better baseline status – that is, patients whose baseline distance on the 6-minute walk test was 300 m or greater and whose baseline FVC was 55% or more of the predicted value. In addition, sensitivity analyses with the use of alternative statistical methods showed that the results were consistent and robust across analytic methods (Table 1 in the Supplementary Appendix).

The pattern of response with respect to QMT leg and arm scores and the percentage of the predicted maximum expiratory and inspiratory pressures support the findings for the two coprimary end points, although only the change in the percentage of the predicted maximum expiratory pressure differed significantly between the groups (Table 2 and Figure 2).

Safety

Patients in the two groups had similar frequencies of adverse events, serious adverse events, treatment-related adverse events, and infusionassociated reactions. Most adverse events were mild or moderate in severity and were not considered to be related to the study drug (Table 3, and Table 2 in the Supplementary Appendix). The most frequently reported events (falls, nasopharyngitis, and headache) were similar between groups. Infusion-associated reactions occurred in 28% of

Table 2. Results of analysis of covariance for changes from baseline to week 78 for primary and secondary end points*

	Alglucosidase Alfa Group (n=60)	Placebo Group (n=30)	Difference between Groups	P Value
End Point				
Distance walked on 6-min walk test – m				
Baseline	332.2±126.7	317.9±132.3		
Week 78	357.9±141.3	313.1±144.7		
Change (95% CI)	25.13 (10.07 to 40.19)	-2.99 (-24.16 to 18.18)	28.12 (2.07 to 54.17)	0.03
Forced vital capacity – % of predicted				
Baseline	55.4±14.4	53.0±15.7		
Week 78	56.7±16.3	50.7±14.9		
Change (95% CI)	1.20 (-0.16 to 2.57)	-2.20 (-4.12 to -0.28)	3.40 (1.03 to 5.77)	0.006
Quantitative muscle testing, leg – % of predicted				
Baseline	37.7±18.9	32.5±18.2		
Week 78	39.1±21.8	30.4±20.5		
Change (95% CI)	1.18 (-1.07 to 3.42)	-2.00 (-5.16 to 1.17)	3.18 (-0.73 to 7.08)	0.11
Quantitative muscle testing, arm – % of predicted				
Baseline	55.9±20.4	56.9±18.2		
Week 78	60.9±21.7	58.3±20.9		
Change (95% CI)	5.05 (1.91 to 8.18)	1.47 (-2.92 to 5.87)	3.57 (-1.83 to 8.97)	0.19
Maximum inspiratory pressure – % of predicted				
Baseline	40.0±19.7	42.6±21.0		
Week 78	43.7±21.0	41.7±19.3		
Change (95% CI)	3.48 (0.91 to 6.04)	-0.35 (-3.95 to 3.25)	3.83 (-0.60 to 8.26)	0.09
Maximum expiratory pressure – % of predicted				
Baseline	32.0±12.1	30.8±12.0		
Week 78	35.1±13.3	30.5±13.1		
Change (95% CI)	3.24 (1.19 to 5.29)	-0.56 (-3.43 to 2.31)	3.80 (0.27 to 7.33)	0.04
Score on SF-36 Physical Component Summary†				
Baseline	34.3±8.9	34.9±7.3		
Week 78	35.1±9.8	36.5±9.6		
Change (95% CI)	0.80 (-1.22 to 2.82)	1.16 (-1.64 to 3.97)	0.37 (-3.83 to 3.09)	0.83

* Plus-minus values are means ±SD. CI denotes confidence interval.
† The Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) consists of an interview and self-administered questionnaire designed to assess generic health-related quality of life in healthy and ill adult populations.

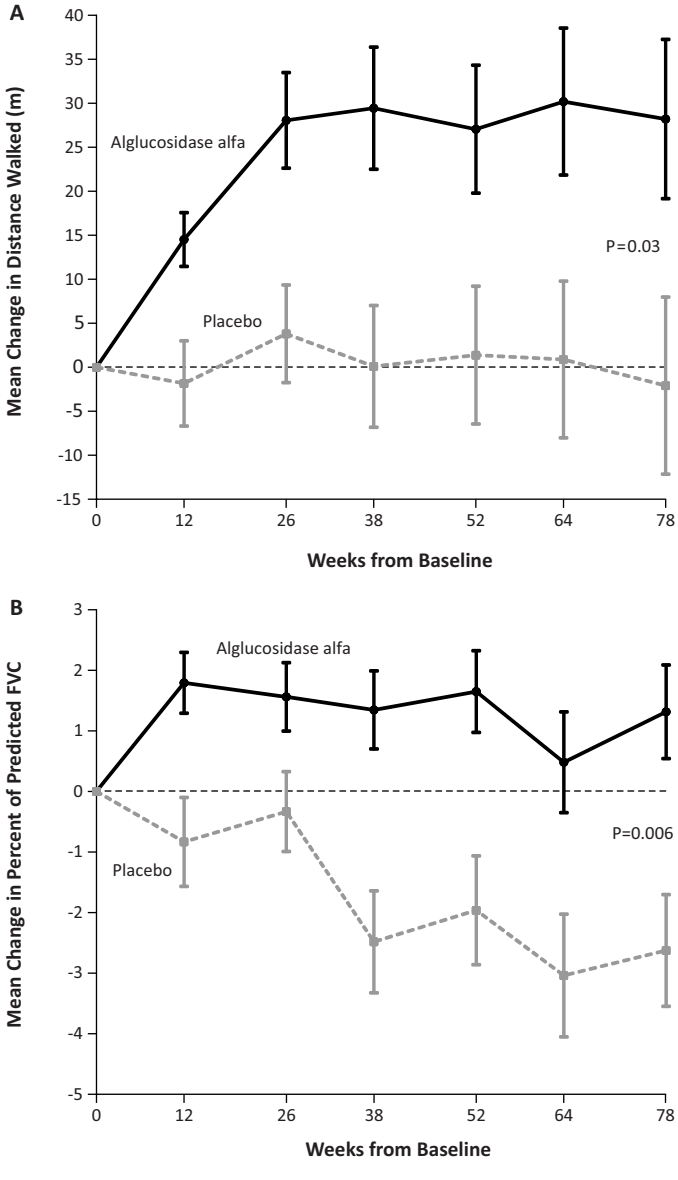

Figure 1. Changes from baseline in distance walked and in Forced Vital Capacity, according to study group.

The graphs show the changes from baseline to week 78 for the two study groups. On the 6-minute walk test (**Panel A**), the alglucosidase alfa group had an increase of 25 m, whereas the placebo group had a decrease of 3 m – a difference of 28 m. The percentage of predicted forced vital capacity (FVC) (**Panel B**) increased by 1.2% in the alglucosidase alfa group but decreased by 2.2% in the placebo group – a difference of 3.4%. These values represent estimates of the mean on analysis of covariance.

alglucosidase alfa recipients and 23% of placebo recipients. Most of the reactions were not serious or were mild to moderate in severity and resolved with no need to withdraw the study treatment (Table 3 in the Supplementary Appendix). Anaphylactic, allergic, and infusion-associated reactions that involved urticaria, flushing, hyperhidrosis, chest discomfort, vomiting, and increased blood pressure occurred in 5 to 8% of the patients treated with alglucosidase alfa but were not reported in the placebo group. Of the 60 patients in the alglucosidase alfa group, three (5%) had anaphylactic reactions, two of whom tested positive for IgE antibodies to alglucosidase alfa; two had respiratory and cutaneous reactions, and the third had severe tongue edema. Two of these three patients withdrew from the study. One patient in the placebo group withdrew owing to headaches. During the study, one patient in the alglucosidase alfa group who was receiving clinical care for two broad-based basilar-artery aneurysms died from brain-stem ischemia due to basilar-artery thrombosis.

Anti-alglucosidase alfa IgG antibodies developed in all 59 patients in the treatment group who underwent at least one post-treatment assessment, with a median time to seroconversion of 4 weeks (range 3.6 to 12). After seroconversion, the median time to the peak titer was 12 weeks; the median peak titer was 6400, and the median final titer (last sample or sample at week 78) was 1600. The geometric mean titer of anti-alglucosidase alfa IgG antibodies on ELISA increased from baseline through week 44 (2925) and declined slightly through week 78 (1607) (Figure 3 in the Supplementary Appendix). In 36 of 59 patients (61%) with one or more post-treatment assessments, there was a trend toward decreasing titers by a factor of two or more, whereas titers in the remaining patients plateaued. No consistent association was found between the serum IgG antibody titer and the coprimary efficacy end points or the incidence of adverse events, serious adverse events, and infusion-associated reactions (Tables 4 and 5 in the Supplementary Appendix).

No patients tested positive for inhibition of enzyme activity. Of the 59 patients who were positive for anti-alglucosidase alfa IgG antibodies, 18 (31%) tested positive for inhibition of enzyme uptake. The mean time to the first detection of inhibitory antibodies was 36 weeks after the first infusion.

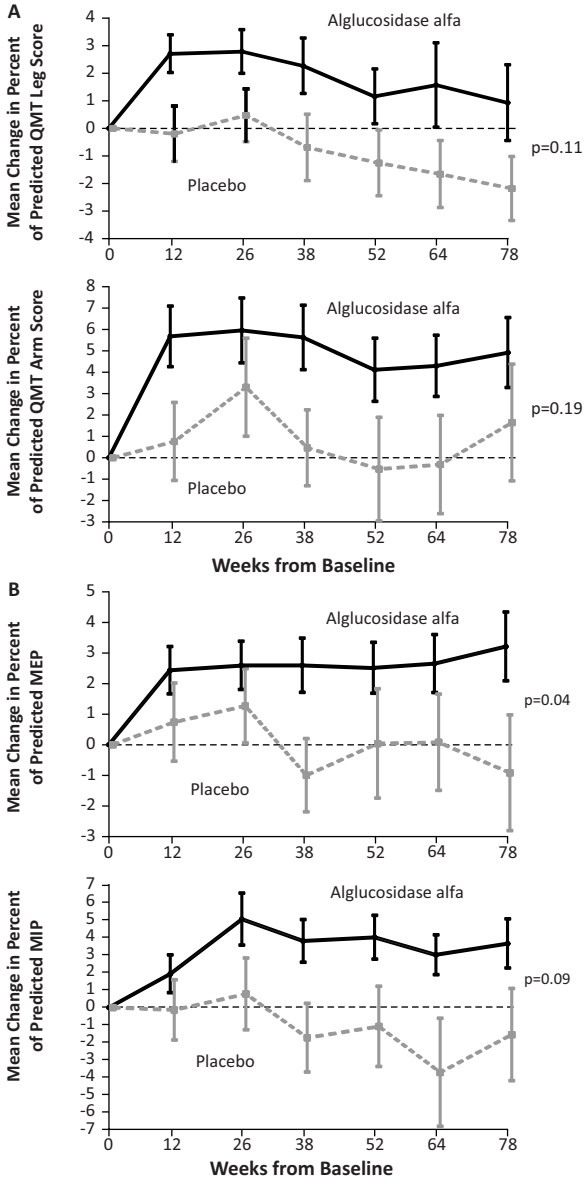

Figure 2. Changes from baseline in Quantitative Muscle Testing (QMT) arm and leg scores and Maximum Expiratory and Inspiratory Pressures.

The graphs show changes in the percentage of predicted values from baseline to week 78 for the alglucosidase alfa group and the placebo group. On QMT (**Panel A**), the changes in leg scores were 1.2% for the alglucosidase alfa group and -2.0% for the placebo group; the corresponding values for the arm scores were 5.1% and 1.5%. In **Panel B**, the changes in maximum expiratory pressure (MEP) were 3.2% for the alglucosidase alfa group and -0.6% for the placebo group; the corresponding changes in maximum inspiratory pressure (MIP) were 3.5% and -0.4%. These values represent estimates of the mean on analysis of covariance.

Table 3. Serious adverse events during the treatment period[a]

Adverse Event	Alglucosidase Alfa Group (n=60)	Placebo Group (n=30)
	No. of patients (%)	
Any event	13 (22)	6 (20)
Infections	2 (3)	1 (3)
Diverticulitis	0	1 (3)
Gastroenteritis	1 (2)	0
Pneumonia	1 (2)	0
Cardiac disorders	2 (3)	0
Coronary artery disease	1 (2)	0
Supraventricular tachycardia	1 (2)	0
Immune system disorders	2 (3)	0
Hypersensitivity	2 (3)	0
General disorders and conditions at site of administration	2 (3)	0
Chest discomfort	1 (2)	0
Noncardiac chest pain	1 (2)	0
Respiratory, thoracic, and mediastinal disorders	2 (3)	0
Lung disorder	1 (2)	0
Throat tightness	1 (2)	0
Injury, poisoning, and complications of procedure	1 (2)	1 (3)
Fall	1 (2)	1 (3)
Fracture (humerus)	1 (2)	1 (3)
Musculoskeletal and connective-tissue disorders	1 (2)	1 (3)
Intervertebral disk protrusion	1 (2)	0
Flank pain	0	1 (3)
Gastrointestinal disorders	1 (2)	1 (3)
Generalized abdominal pain	1 (2)	0
Upper abdominal pain	0	1 (3)
Nervous system disorders	1 (2)	1 (3)
Brain-stem ischemia	1 (2)	0
Headache	0	1 (3)

Adverse Event	Alglucosidase Alfa Group (n=60)	Placebo Group (n=30)
	No. of patients (%)	
Skin and subcutaneous-tissue disorders	1 (2)	1 (3)
Angioedema	1 (2)	0
Septal panniculitis	0	1 (3)
Metabolism and nutritional disorders	1 (2)	0
Dehydration	1 (2)	0
Vascular disorders	1 (2)	0
Aneurysm	1 (2)	0

[a] Patients may have had more than one adverse event.

DISCUSSION

In this randomized, controlled trial of alglucosidase alfa in patients with late-onset Pompe's disease, significant differences were observed at 78 weeks between the alglucosidase alfa and placebo groups in the distance walked on the 6-minute walk test and in the percentage of the predicted FVC. Alglucosidase alfa treatment was associated with improvements in walking distance and stabilization of pulmonary function; therefore, the coprimary end points of the study were met. Treatment effects were supported by the consistently favorable pattern of response in proximal and respiratory muscle strength among the patients who received alglucosidase alfa. Of these secondary and tertiary end points, the percentage of predicted maximum expiratory pressure (a surrogate marker of expiratory muscle strength) differed significantly between the study groups. These results indicate that alglucosidase alfa has a positive effect on the complex process that leads to impaired ambulation and respiratory failure in late-onset Pompe's disease. Whether alglucosidase alfa exerts a differential effect on the various respiratory muscles (diaphragm or intercostal muscles) requires further investigation.

Natural history studies of late-onset Pompe's disease indicate that it is defined by progressive deterioration in proximal arm, leg, and respiratory muscle strength and function.[2,6,7,9,31-33] Two recent natural history studies showed mean annual declines

of 4.6% and 1.7% in the percentage of predicted FVC, measured in the upright position;[9,33] these findings are consistent with the 2.2% decline that occurred over a period of 18 months in the placebo group in our study. Important clinical benefits can be provided if further deterioration in pulmonary and motor function can be prevented, and the patient's independence can be maintained by preventing the need for a ventilator or a wheelchair.

The estimated treatment response to alglucosidase alfa as compared with placebo, although variable in its magnitude, was consistently positive for all subgroups. Hypotheses about the nature and progression of muscle damage in Pompe's disease led us to speculate that patients with less baseline impairment would benefit more from treatment. Subgroup analyses of the changes in the 6-minute walk test and the percentage of the predicted FVC suggest a more pronounced treatment effect in patients with better clinical status at baseline (all estimated treatment effects >0) (Figure 2 in the Supplementary Appendix). However, individual patients' responses did not consistently show this effect, nor did the subgroup analyses identify any consistent predictor of a treatment response.

The effect of alglucosidase alfa treatment became apparent early; the greatest improvement in all end points in the treated group occurred during the first 26 weeks, with those gains then generally being maintained. This response pattern may be due to the limited capacity to repair muscle tissue that has sustained substantial damage. Functional recovery may then be explained by the uptake of exogenous alglucosidase alfa and subsequent lysosomal glycogen clearance from muscle tissue that has not yet sustained endstage damage.[34] The overall clinical response observed in our study may represent the balance between more mildly affected muscle fibers and those with potentially irreversible damage and might suggest that prevention of further loss of muscle tissue and function is an important treatment goal. Longer-term study of alglucosidase alfa in children and adults with Pompe's disease would be needed to understand fully the potential of treatment.

Adverse events occurred in both groups of patients in our study. Anaphylactic reactions occurred in three of the 60 patients treated with alglucosidase alfa; two of these reactions were IgE-mediated. One patient who tested positive for IgE underwent a successful rechallenge with the use of a modified regimen and

remained in the study. After discontinuing the study, the second IgE-positive patient was successfully rechallenged with alglucosidase alfa and was able to continue treatment. IgG antibodies to alglucosidase alfa were detected in all the patients who received alglucosidase alfa, with a trend toward decreasing levels with continued treatment. Although we found no consistent effect of these antibodies on clinical response or safety variables, such an effect may emerge over time. Anaphylactic reactions are a serious potential complication of treatment with any recombinant human protein and have previously been reported to occur with alglucosidase alfa.[12] Antibodies, particularly neutralizing antibodies, have a negative effect on clinical response in some diseases treated with infused proteins, but this effect has been inconsistent across patient populations.[29] Patients treated with alglucosidase alfa who have persistently high antibody titers should be followed closely until the effect of the antibodies is more fully understood.

Our study has several limitations. Although 90 patients is a large population for a clinical trial designed to study an orphan disease, the number is relatively small when the goal is to judge the progression of a clinically heterogeneous disease. Before the start of this trial, no longitudinal data were available on changes in the 6-minute walk test over time in patients with untreated Pompe's disease, and the mean decline in the distance walked was minimal in the patients in our study who received placebo. Longer follow-up will be needed to confirm our results, given the variable presentation and rate of deterioration among the patients in our study and the possible effect of the degree of muscle destruction at baseline on their response to treatment.

In summary, our data indicate that alglucosidase alfa treatment, as compared with placebo, has a positive, if modest, effect on walking distance and pulmonary function in patients with late-onset Pompe's disease and may stabilize proximal limb and respiratory muscle strength.

REFERENCES

1. Chen YT and Amalfitano A. Towards a molecular therapy for glycogen storage disease type II (Pompe disease). *Mol Med Today* 2000; 6:245-251.
2. Hirschhorn R and Reuser AJ. Glycogen storage disease type II : acid a-glucosidase (acid maltase) deficiency. In: Scriver CR, Beaudet AL, Sly W, and Valle D eds. *The Metabolic and Molecular Bases of Inherited Disease*, 8th ed. New York: McGraw-Hill, 2001; 3389-3420.
3. Kishnani PS, Hwu WL, Mandel H et al. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *J Pediatr* 2006; 148:671-676.
4. van den Hout HM, Hop W, van Diggelen OP et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics* 2003; 112:332-340.
5. van der Ploeg AT and Reuser AJ. Pompe's disease. *Lancet* 2008; 372:1342-1353.
6. Hagemans ML, Winkel LP, van Doorn PA et al. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. *Brain* 2005; 128:671-677.
7. Laforêt P, Nicolino M, Eymard PB et al. Juvenile and adult-onset acid maltase deficiency in France: genotype-phenotype correlation. *Neurology* 2000; 55:1122-1128.
8. Müller-Felber W, Horvath R, Gempel K et al. Late onset Pompe disease: clinical and neurophysiological spectrum of 38 patients including long-term follow-up in 18 patients. *Neuromuscul Disord* 2007; 17:698-706.
9. Wokke JH, Escolar DM, Pestronk A et al. Clinical features of late-onset Pompe disease: a prospective cohort study. *Muscle Nerve* 2008; 38:1236-1245.
10. Mellies U, Stehling F, Dohna-Schwake C et al. Respiratory failure in Pompe disease: treatment with noninvasive ventilation. *Neurology* 2005; 64:1465-1467.
11. Winkel LP, van den Hout JM, Kamphoven JH et al. Enzyme replacement therapy in late-onset Pompe's disease: a three-year follow-up. *Ann Neurol* 2004; 55:495-502.
12. Kishnani PS, Corzo D, Nicolino M et al. Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology* 2007; 68:99-109.
13. Amalfitano A, Bengur AR, Morse RP et al. Recombinant human acid alpha-glucosidase enzyme therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial. *Genet Med* 2001; 3:132-138.
14. Kishnani PS, Corzo D, Leslie ND et al. Early treatment with alglucosidase alpha prolongs long-term survival of infants with Pompe disease. *Pediatr Res* 2009; 66:329-335.
15. Nicolino M, Byrne B, Wraith JE et al. Clinical outcomes after long-term treatment with alglucosidase alfa in infants and children with advanced Pompe disease. *Genet Med* 2009; 11:210-219.
16. van den Hout H, Reuser AJ, Vulto AG et al. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients. *Lancet* 2000; 356:397-398.
17. Angelini C, Semplicini C, Tonin P et al. Progress in Enzyme Replacement Therapy in Glycogen Storage Disease Type II. *Ther Adv Neurol Disord* 2009; 2:143-153.
18. Strothotte S, Strigl-Pill N, Grunert B et al. Enzyme replacement therapy with alglucosidase alfa in 44 patients with late-onset glycogen storage disease type 2: 12-month results of an observational clinical trial. *J Neurol* 2010; 257:91-97.

19. van Capelle CI, Winkel LP, Hagemans ML et al. Eight years experience with enzyme replacement therapy in two children and one adult with Pompe disease. *Neuromuscul Disord* 2008; 18:447-452.
20. Pocock SJ and Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. *Biometrics* 1975; 31:103-115.
21. American Thoracic Society. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 2002; 166:111-117.
22. American Thoracic Society/European Respiratory Society. ATS/ERS statement on respiratory muscle testing. *Am J Respir Crit Care Med* 2012; 166:518-624.
23. Hankinson JL, Odencrantz JR, and Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* 1999; 159:179-187.
24. Black LF and Hyatt RE. Maximal respiratory pressures: normal values and relationship to age and sex. *Am Rev Respir Dis* 1969; 99:696-702.
25. The National Isometric Muscle Strength (NIMS) Database Consortium. Muscular weakness assessment: use of normal isometric strength data. *Arch Phys Med Rehabil* 1996; 77:1251-1255.
26. Mayhew JE, Florence JM, Mayhew TP et al. Reliable surrogate outcome measures in multicenter clinical trials of Duchenne muscular dystrophy. *Muscle Nerve* 2007; 35:36-42.
27. Ware JEJ, Kosinski M, Bjorner JB et al. *User's manual for the SF-36v2 health survey.*, 2nd ed. Lincoln, RI: QualityMetric Incorporated, 2007.
28. Kishnani PS, Nicolino M, Voit T et al. Chinese hamster ovary cell-derived recombinant human acid alpha-glucosidase in infantile-onset Pompe disease. *J Pediatr* 2006; 149:89-97.
29. Wang J, Lozier J, Johnson G et al.*Nat Biotechnol* 2008; 26:901-908.
30. Mehta CR and Tsiatis AA. Flexible sample size considerations using informationbased interim monitoring. *DIA Journal* 2000; 35:1095-1112.
31. Hagemans ML, Janssens AC, Winkel LP et al. Late-onset Pompe disease primarily affects quality of life in physical health domains. *Neurology* 2004; 63:1688-1692.
32. Hagemans ML, Winkel LP, Hop WC et al. Disease severity in children and adults with Pompe disease related to age and disease duration. *Neurology* 2005; 64:2139-2141.
33. van der Beek NA, Hagemans ML, Reuser AJ et al. Rate of disease progression during long-term follow-up of patients with late-onset Pompe disease. *Neuromuscul Disord* 2009; 19:113-117.
34. Thurberg BL, Lynch MC, Vaccaro C et al. Characterization of pre- and post-treatment pathology after enzyme replacement therapy for Pompe disease. *Lab Invest* 2006; 86:1208-1220.

PART III

Peculiar features and response to therapy

8

Gingival overgrowth in Pompe disease, a case report

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ABSTRACT

Pompe disease or glycogen storage disease type 2, is a rare inheritable metabolic disease caused by a deficiency of the lysosomal enzyme acid a-glucosidase. Patients with the classic infantile form of Pompe disease present with symptoms during the first three months after birth, and most will die within their first year. Recently, enzyme replacement therapy (ERT) with recombinant human a-glucosidase became commercially available for Pompe disease. This is a case report of an 8-year-old girl with the infantile form of Pompe disease who is one of the longest survivors through ERT. The patient was tetraplegic when she started ERT. At age 3 years, she developed massive gingival overgrowth and could not close her mouth, prompting a reduction of the gingival overgrowth surgically. We expected that massive accumulation of glycogen would explain the gingival overgrowth. However, histopathology of the gingiva tissue showed marked glycogen accumulation in smooth muscle cells of the arteries, but the glycogen content in fibroblasts did not exceed that of control individuals. Further, there was an increase of immature collagen in the connective tissue, and signs of a mild chronic inflammation. We concluded that glycogen storage is not a direct causative factor of gingival overgrowth in our patient. Chronic inflammation, dryness of the gingiva, or even the minimal glycogen accumulation in the fibroblasts may have played a role.

INTRODUCTION

Pompe disease, also known as glycogen storage disease type 2 or acid maltase deficiency is a rare, inheritable metabolic disease.[1] Its transmission is autosomal recessive, with a similar occurrence in men and women. Mutations in the a-glucosidase gene, located on chromosome 17, cause a total or partial deficiency of the lysosomal enzyme acid a-glucosidase. Deficiency of this enzyme leads to accumulation of glycogen in cells throughout the body, but most notably in skeletal and cardiac muscles.[2-4]

The incidence of Pompe disease is estimated to be 1:40.000 births in the Western world.[5,6] The first description of the classic infantile form of Pompe disease was made by the Dutch pathologist JC Pompe.[7] Classic infantile Pompe patients present early in life, at a median age of 1.6 months, with hypertrophic cardiomyopathy and severe generalized hypotonia. Without treatment, most patients die within their first year of life.[8,9] Children and adults with Pompe disease present with a more slowly progressive proximal myopathy, usually without cardiac involvement. The age of onset and disease progression varies widely in this group of patients. Patients eventually need respiratory support, a percutaneous gastrostomy, and they become wheelchair dependent. The main cause of death is respiratory failure.[2,4,9-11]

Since 2006, enzyme replacement therapy (ERT) with recombinant human a-glucosidase has become commercially available for Pompe disease. This treatment has been shown to have an effect on survival, hypertrophic cardiomyopathy, and motor function.[12-20]

An 8-year-old girl with classic infantile Pompe disease who survived because of ERT is presented in this case study. During the course of treatment, she developed hypertrophic masses of the gingiva (30–45 mm).

Case Report

The patient was admitted at the age of 3 months for evaluation of severe hypotonia, hepatomegaly and cardiomegaly. She was diagnosed with classic infantile Pompe disease. At the age of 7 months, she was enrolled in the first ERT study with recombinant human α-glucosidase.[17] At the time of her first infusion, the patient

was in the end stage of Pompe disease. She was tetraplegic, completely dependent on invasive ventilation, and had symptoms of cardiac failure.

After the start of therapy, the cardiac hypertrophy improved, but the patient remained tetraplegic and completely ventilator dependent. She appeared to have normal mental development and attended school daily. Because of complete paralysis of the facial musculature, she was enterally fed via percutaneous gastrostomy. She could not close her mouth, was unable to speak, and could only move her mandible laterally for 2 to 3 millimeters. This small movement of her mandible was mandatory to control her wheelchair. She used the following medications: budesonide, cotrimoxazole, furosemide, spironolactone, and recombinant human a-glucosidase.

At the age of 3 years, an enlargement of the palatal gingiva was noted, and the patient was referred to the department of oral and maxillofacial surgery. Intraoral examination revealed generalized gingival overgrowth in the molar region of the palatal aspect of the maxilla and of the lingual and buccal sides of the mandibula. The patient had class III malocclusion, a small maxilla, and impaired eruption of the molars. The temporomandibular joint was in place. She was referred to a pediatric dentist and dental hygienist to improve oral hygiene.

At the age of 5 years, the patient returned to our department with progression of the masses in all 4 quadrants. Although the masses interfered with occlusion, the patient did not experience any discomfort. In addition, oral hygiene was good, and permanent teeth eruption was normal. On the basis of the patient's condition and the risk for anesthesia, as well as in consideration of her well-being, surgical intervention was not performed, and the patient remained under supervision of the pediatric dentist and dental hygienist.

At the age of 8 years, progression of the masses made it impossible for the patient to move her mouth, and hence she was unable to control the wheelchair. The four masses that ranged from 30 mm to 45 mm were therefore excised under general anesthesia, and sent for histopathological examination (Figures 1, 2).

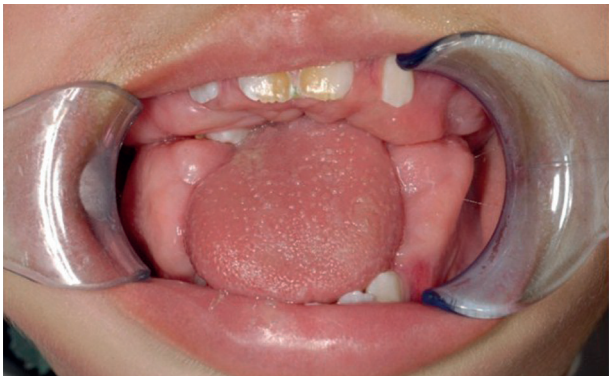

Figure 1. Gingival overgrowth in all 4 quadrants.

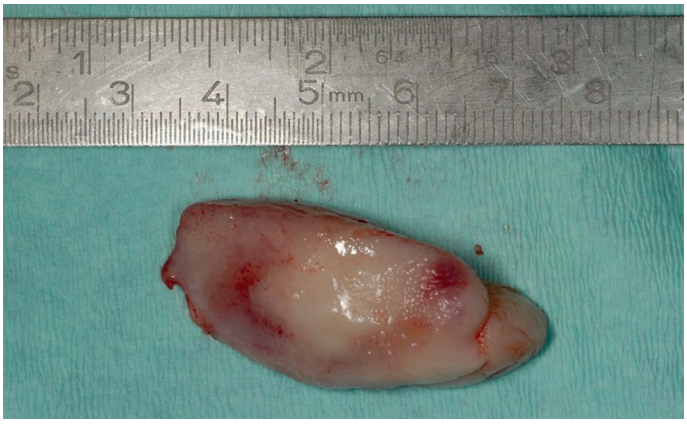

Figure 2. Excised mass.

Histopathology

Resection and biopsy specimens from the patient and control cases were fixed with buffered formalin for 12 to 24 hours and processed for routine sections from paraffin- embedded tissue. Stainings for haematoxylin and eosin (HE), periodic acid-Schiff (PAS), PAS after diastase digestion, and alcian blue (AB) were carried out according to standard protocols. PAS staining was performed to visualize

glycogen and compared with PAS after diastase digestion, which removes glycogen. AB staining was applied to visualize myxoid changes of the collagenous matrix of the connective tissue. The resection specimens of the patient showed acanthotic nonkeratinizing squamous epithelium with elongated rete ridges. The submucosal connective tissue contained marked lymphoid and plasma cell infiltrations. The connective tissue had a loose appearance with large quantities of Alcian blue-positive myxoid matrix (Figure 3). PAS-stained sections showed marked glycogen accumulation in arterial smooth muscle cells (Figure 4). Some glycogen could also be noted in fibroblasts (Figure 5). Glycogen was absent in the sections stained with PAS after diastase digestion. We compared the sections of our patient with HE, PAS, PAS after diastase digestion, and AB-stained gingiva sections of 6 patients seen in our hospital for inflammatory papillary hyperplasia, irritation fibroma (2 patients), chronic gingivitis (2 patients), and a gingival cyst. In the sections of the latter patients, glycogen was also found in fibroblasts, but not in arterial smooth muscle cells. In addition, no excessive myxoid extracellular matrix was found.

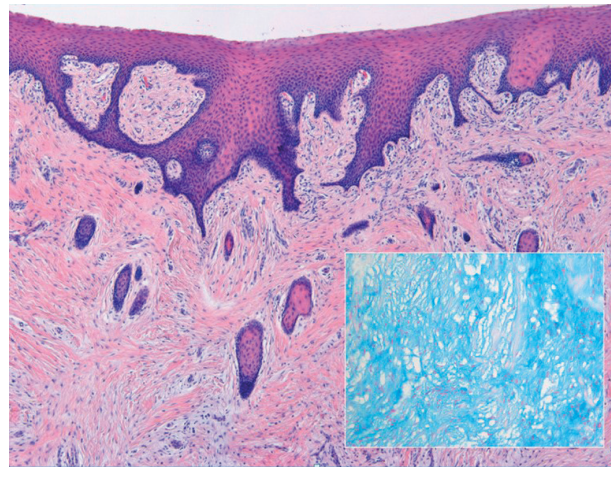

Figure 3. Gingival hypertrophy: expansion of the submucosal connective tissue has evoked a pseudoepitheliomatous hyperplasia of the nonkeratinizing squamous epithelium (hematoxylin and eosin, x40). Inset: abundant myxoid matrix in between collagen bundles (alcian blue, x40).

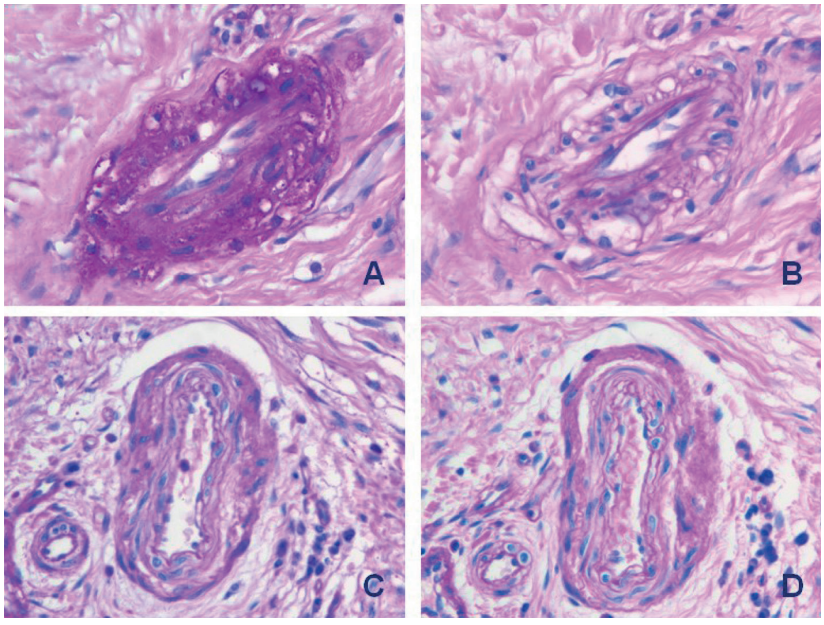

Figure 4. (**A**) artery of patient showing deposition of glycogen in vacuolated smooth muscle cells of the wall (periodic acid-Schiff [PAS], x400); (**B**) same artery after diastase digestion (PAS after diastase digestion, x400); (**C**) artery of control patient with chronic inflammation without glycogen deposition and no vacuolization of muscle cells (PAS, x400); (**D**) same artery after diastase digestion (PAS after diastase digestion, x400).

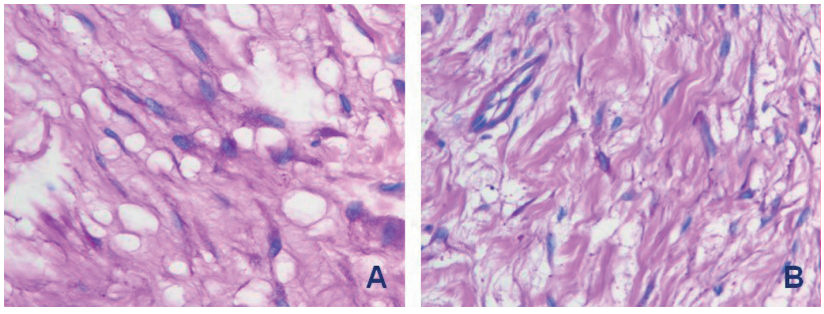

Figure 5. (**A**) fibroblasts of patient showing some deposition of glycogen (periodic acid-Schiff [PAS], x400); (**B**) fibroblasts of control patient with similar amount of glycogen (PAS, x400).

DISCUSSION

Before enzyme therapy became available, patients with infantile Pompe disease died before the age of 1 year.[4,8] In this case report, we present one of the longest survivors with classic infantile Pompe disease, achieved through enzyme therapy. The patient started to receive therapy in the end stage of the disease when she was completely tetraplegic and her skeletal muscles severely damaged. This explains, in retrospect, why the effect of enzyme therapy on motor performance was poor. Paralysis also included facial muscle weakness, and the patient could barely open and close her mouth. From the age of 3 years, she started to develop gingival overgrowth. Gingival overgrowth has not been described in patients diagnosed with Pompe disease thus far but has been found in other lysosomal storage disorders, such as mucopolysaccharidosis,[21,22] aspartylglucosaminuria,[23] mucolipidosis type 2,[24,25] and lysosomal glycogen storage disease type 1b.[26] For the mucopolysaccharidoses, gingival hyperplasia was shown to be related to the presence of non-degradable glycosaminoglycans.[27] In the other cases, chronic inflammation of the gingiva was a frequent finding.

We expected that the excessive gingival overgrowth in our patient with Pompe disease was caused by glycogen storage. However, we found that the amount of glycogen in fibroblasts was not different from that of patients with gingival inflammation who did not have Pompe disease. The marked glycogen accumulation in the arterial smooth muscle cells was insufficient to explain the occurrence of the gingival hypertrophy. Diffuse gingival overgrowth has also been described for diseases other than lysosomal storage disorders. The most common causes known in children are drug-induced gingival hyperplasia, hereditary gingival fibromatosis and neurofibromatosis type 1, and inflammatory gingival enlargement or as part of a systemic disease such as leukemia.[28] These causes could be excluded in our patient. Because prominent infiltration of inflammatory cells was observed in the excised gingival masses of our patient, chronic inflammatory gingivitis may explain, in part, the gingival overgrowth. The inflammatory response was confined to the submucosal connective tissue and did not affect the deeper connective tissue; furthermore, mild inflammation is not uncommon in healthy persons. Therefore, it

is unlikely that inflammation was the only cause of the abnormalities. The patient's oral hygiene has been closely monitored by a specialized dental hygienist over the years. There was no history of bleeding or redness of the gingiva. In conclusion, we cannot fully explain the gingival overgrowth in our patient. We have ruled out massive storage of glycogen as a direct cause. The fact that glycogen was abundantly present in smooth muscle cells of the arterial walls indicates that ERT did not clear all tissues of glycogen storage, but the quantities of storage material were insufficient to fully explain the gingival overgrowth. The abuncance of immature collagen in the extracellular matrix suggests that the homeostasis of collagen synthesis and degradation was disturbed. We hypothesize that a combination of chronic inflammation, dryness of the gingiva, and even the minimal glycogen accumulation in the fibroblasts may have played a role in the etiology of the patient's gingival overgrowth. Whatever the cause, we wish to focus attention on the fact that gingival overgrowth may be a complication in long survivors with infantile Pompe disease who show a poor motor response to enzyme therapy.

REFERENCES

1. van der Ploeg AT, Reuser AJ. Pompe's disease. *Lancet* 2008; 372:1342-53.
2. Engel AG, Gomez MR, Seybold ME, et al. The spectrum and diagnosis of acid maltase deficiency. *Neurology* 1973; 23:95-106.
3. Hers HG. Alpha-glucosidase deficiency in generalized glycogen storage disease (Pompe's disease). *Biochem J.* 1963; 86:11-6.
4. Hirschhorn K, Reuser AJ, editors. Glycogen storage disease type II: acid alpha-glucosidase (acid maltase) deficiency, in Scriver CR, Beaudet AL, Valle D, Sly WS (eds): The Metabolic and Molecular Bases of Inherited Disease (ed 8). New York: McGraw-Hill; 2001, p 3389.
5. Ausems MG, Verbiest J, Hermans MP, et al. Frequency of glycogen storage disease type II in The Netherlands: implications for diagnosis and genetic counselling. *Eur J Hum Genet*. 1999; 7:713-6.
6. Martiniuk F, Chen A, Mack A, et al. Carrier frequency for glycogen storage disease type II in New York and estimates of affected individuals born with the disease. *Am J Med Genet.* 1998; 79:69-72.
7. Pompe J. Over idiopathische hypertrophie van het hart. *Nederlands Tijdschrift Voor Geneeskunde* 1932;76:304.
8. van den Hout HM, Hop W, van Diggelen OP, et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics* 2003; 112:332-40.
9. Kishnani PS, Hwu WL, Mandel H, Nicolino M, Yong F, Corzo D. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *J Pediatr.* 2006; 148: 671-6.
10. Van der Beek NA, Hagemans ML, Reuser AJ, et al. Rate of disease progression during long-term follow-up of patients with late-onset Pompe disease. *Neuromuscul Disord.* 2008; 19:113.
11. Hagemans ML, Winkel LP, Hop WC, et al. Disease severity in children and adults with Pompe disease related to age and disease duration. *Neurology* 2005; 64:2139-41.
12. van Capelle CI, Winkel LP, Hagemans ML, et al. Eight years experience with enzyme replacement therapy in two children and one adult with Pompe disease. *Neuromuscul Disord*. 2008; 18:447-52.
13. Amalfitano A, Bengur AR, Morse RP, et al. Recombinant human acid alpha-glucosidase enzyme therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial. *Genet Med.* 2001; 3:132-8.
14. Kishnani PS, Corzo D, Nicolino M, et al. Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology* 2007; 68:99-109.
15. Klinge L, Straub V, Neudorf U, et al. Enzyme replacement therapy in classical infantile pompe disease: results of a ten-month follow-up study. *Neuropediatrics* 2005; 36:6-11.
16. Rossi M, Parenti G, Della Casa R, et al. Long-term enzyme replacement therapy for Pompe disease with recombinant human alpha-glucosidase derived from Chinese hamster ovary cells. *J Child Neurol.* 2007; 22:565-73.
17. Van den Hout H, Reuser AJ, Vulto AG, er al. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients. *Lancet* 2000; 356:397-8.

18. Winkel LP, Van den Hout JM, Kamphoven JH, et al. Enzyme replacement therapy in late-onset Pompe's disease: a three-year follow-up. *Ann Neurol*. 2004 ; 55:495-502.
19. Kishnani PS, Nicolino M, Voit T, et al. Chinese hamster ovary cell-derived recombinant human acid alpha-glucosidase in infantile-onset Pompe disease. *J Pediatr*. 2006; 149:89-97.
20. Van den Hout JM, Kamphoven JH, Winkel LP, et al. Long-term intravenous treatment of Pompe disease with recombinant human alpha-glucosidase from milk. *Pediatrics* 2004; 113:e448-57.
21. Alpoz AR, Coker M, Celen E, et al. The oral manifestations of Maroteaux-Lamy syndrome (mucopolysaccharidosis VI): A case report. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006; 101:632-7.
22. Gardner DG. The oral manifestations of Hurler's syndrome. *Oral Surg Oral Med Oral Pathol.* 1971; 32:46-57.
23. Arvio P, Arvio M, Kero M,et al. Overgrowth of oral mucosa and facial skin, a novel feature of aspartylglucosaminuria. *J Med Genet.* 1999; 36:398-404.
24. Melo MD, Obeid G. Radiolucent lesions of the maxillofacial complex in a patient with mucolipidosis type II (MLSII): Case report. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2007; 104:e30-3.
25. Tsuruyama K, Sugano A, Miyamoto M, et al. Gingivectomy for gingival enlargement in a child with I-cell disease: A report of a case. *Pediatric Dent J.* 2005; 15:5.
26. Mortellaro C, Garagiola U, Carbone V, et al. Unusual oral manifestations and evolution in glycogen storage disease type Ib. *J Craniofac Surg*. 2005; 16:45-52.
27. Cawson RA. The oral changes in gargoylism. *Proc R Soc Med.* 1962; 55:1066-70.
28. Delaney JE, Keels MA, Griffin A. Soft tissue lesions of the oral cavity in children. Up-to-date online 2007 (http;//www.utdol.com) edition 16.1.

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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 (EC 3.2.1.20).[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 judgment, 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 analyze 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 fiberoptic endoscopic evaluation of swallowing (FEES).[19] First, the masticatory pattern was investigated. Then, after the fiberscope 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.

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.

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).

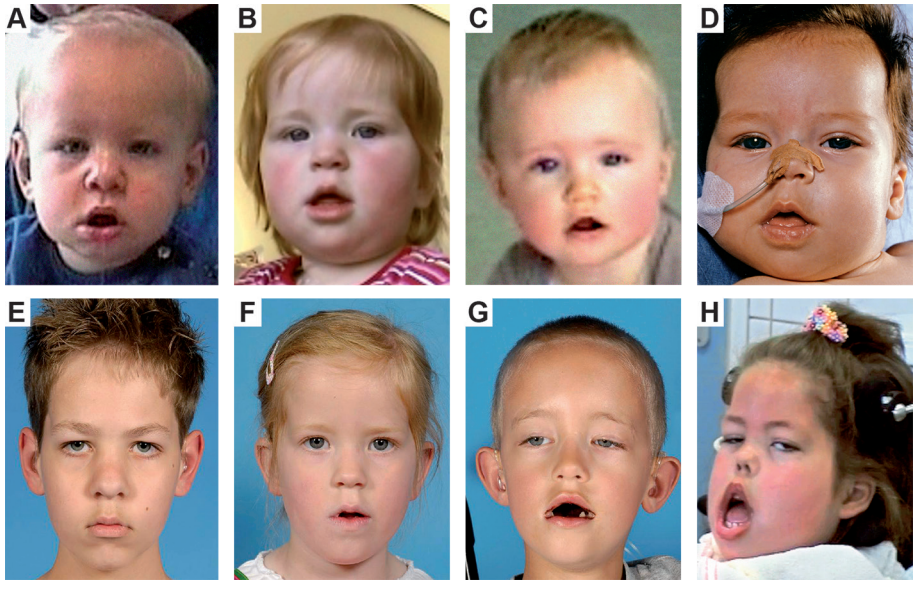

Figure 1. Development of facial muscle weakness over time in four patients with classic infantile Pompe disease treated with ERT.

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**).

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).

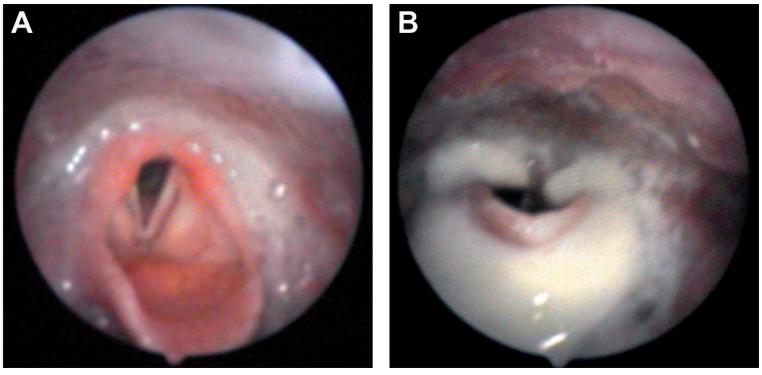

Figure 2. FEES examination in a 6-year old boy with classic infantile Pompe disease treated with ERT.

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**).

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.

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.

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 behavioral exercises. Other options to improve speech include a palatal lift prosthesis, or surgical interventions such as pharyngoplasty or a pharyngeal flap.[24] But as these may also increase swallowing difficulties or cause obstructive sleep apnea, 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-fiber types. It was shown in mice with Pompe disease that type 2 muscle fibers were largely resistant to ERT.[32] In humans, type 1 and type 2a muscle fibers 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.

REFERENCES

1. Van der Ploeg AT, Reuser AJJ. Pompe's disease. *Lancet* 2008; 372:1342-53.
2. Hirschhorn R, Reuser AJJ. Glycogen storage disease type II: acid alpha-glucosidase (acid maltase) deficiency. In: Scriver C, Beaudet A, Valle D, Sly W, eds. The Metabolic and Molecular Bases of Inherited Disease. New-York, McGraw-Hill, 2001: 3389-420.
3. Pompe JC. Over idiopathische hypertrofie van het hart [Dutch]. *Ned Tijdschr Geneeskd* 1932; 76: 304-11.
4. Van den Hout HMP, Hop W, van Diggelen OP, et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics* 2003; 112:332-40.
5. Kishnani PS, Hwu W-L, Mandel H, Nicolino M, Yong F, Corzo D. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *J Pediatr* 2006; 148: 671-6.
6. Van den Hout H, Reuser AJJ, Vulto AG, Loonen MCB, Cromme-Dijkhuis A, van der Ploeg AT. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients. *Lancet* 2000; 356: 397-8.
7. Van den Hout JMP, Kamphoven JHJ, Winkel LP, et al. Long-term intravenous treatment of Pompe disease with recombinant human alpha-glucosidase from milk. *Pediatrics* 2004; 113:e448-57.
8. Kishnani PS, Corzo D, Leslie ND, et al. Early treatment with alglucosidase alpha prolongs long-term survival of infants with Pompe disease. *Pediatr Res* 2009; 66:329-35.
9. Nicolino M, Byrne B, Wraith JE, et al. Clinical outcomes after long-term treatment with alglucosidase alfa in infants and children with advanced Pompe disease. *Genet Med* 2009; 11: 210-9.
10. Muller CW, Jones HN, O'Grady G, Su AH. Language and speech function in children with infantile Pompe disease. *J Pediatr Neurol* 2009; 7:147-56.
11. Kishnani PS, Corzo D, Nicolino M, et al. Recombinant human acid α-glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology* 2007; 68:99-109.
12. Kishnani PS, Nicolino M, Voit T, et al. Chinese hamster ovary cell-derived recombinant human acid alpha-glucosidase in infantile-onset Pompe disease. *J Pediatr* 2006; 149:89-97.
13. Klinge L, Straub V, Neudorf U, et al. Safety and efficacy of recombinant acid alpha-glucosidase (rhGAA) in patients with classical infantile Pompe disease: results of a phase II clinical trial. *Neuromuscul Disord* 2005; 15:24-31.
14. Klinge L, Straub V, Neudorf U, Voit T. Enzyme replacement therapy in classical infantile pompe disease: results of a ten-month follow-up study. *Neuropediatrics* 2005; 36:6-11.
15. Amalfitano A, Bengur AR, Morse RP, et al. Recombinant human acid alpha-glucosidase enzyme therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial. *Genet Med* 2001; 3:132-8.
16. Chakrapani A, Vellodi A, Robinson P, Jones S, Wraith JE. Treatment of infantile Pompe disease with alglucosidase alpha: the UK experience. *J Inherit Metab Dis* 2010; 33:747-50.
17. Dubowitz V. Muscle disorders in childhood. London and Philadelphia, Sauders, 1978.
18. Darley FL, Aronson AE, Brown JR. Differential diagnostic patterns of dysarthria. *J Speech Hear Res* 1969; 12:246-69.

19. Langmore SE, Schatz K, Olsen N. Fiberoptic endoscopic examination of swallowing safety: a new procedure. *Dysphagia* 1988; 2:216-9.
20. Langmore SE. Endoscopic evaluation and treatment of swallowing disorders. New York, Thieme Medical Publishers, 2001.
21. Van Capelle CI, Goedegebure A, Homans NC, Hoeve HLJ, Reuser AJ, van der Ploeg AT. Hearing loss in Pompe disease revisited: results from a study of 24 children. *J Inherit Metab Dis* 2010; 33: 597-602.
22. 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.
23. Darley FL, Aronson AE, Brown JR. Clusters of deviant speech dimensions in the dysarthrias. *J Speech Hear Res* 1969; 12:462-96.
24. Rudnick EF, Sie KC. Velopharyngeal insufficiency: current concepts in diagnosis and management. *Curr Opin Otolaryngol Head Neck Surg* 2008; 16:530-5.
25. Sakurai I, Tosaka A, Mori Y, Imura S, Aoki K. Glycogenosis type II (Pompe). The fourth autopsy case in Japan. *Acta Pathol Jpn* 1974; 24:829-46.
26. Kobayashi H, Shimada Y, Ikegami M, *et al.* Prognostic factors for the late onset Pompe disease with enzyme replacement therapy: from our experience of 4 cases including an autopsy case. *Mol Genet Metab* 2010; 100:14-9.
27. Bijvoet AG, Van Hirtum H, Kroos MA, et al. Human acid alpha-glucosidase from rabbit milk has therapeutic effect in mice with glycogen storage disease type II. *Hum Mol Genet* 1999; 8:2145-53.
28. Gambetti P, DiMauro S, Baker L. Nervous system in Pompe's disease. Ultrastructure and biochemistry. *J Neuropathol Exp Neurol* 1971; 30:412-30.
29. Mancall EL, Aponte GE, Berry RG. Pompe's disease (diffuse glycogenosis) with neuronal storage. *J Neuropathol Exp Neurol* 1965; 24:85-96.
30. Martin JJ, Barsy T, Hoof F, Palladini G, de Barsy T, van Hoof F. Pompe's disease: an inborn lysosomal disorder with storage of glycogen. A study of brain and striated muscle. *Acta Neuropathol* 1973; 23:229-44.
31. Begley DJ, Pontikis CC, Scarpa M. Lysosomal storage diseases and the blood-brain barrier. *Curr Pharm Des* 2008; 14:1566-80.
32. Raben N, Fukuda T, Gilbert AL, *et al.* Replacing acid alpha-glucosidase in Pompe disease: recombinant and transgenic enzymes are equipotent, but neither completely clears glycogen from type II muscle fibers. *Mol Ther* 2005; 11:48-56.
33. Drost MR, Schaart G, van Dijk P, et al. Both type 1 and type 2a muscle fibers can respond to enzyme therapy in Pompe disease. *Muscle Nerve* 2008; 37:251-5.
34. Hoh JFY. Laryngeal muscle fibre types. *Acta Physiol Scand* 2005; 183:133-49.
35. Kent RD. The uniqueness of speech among motor systems. *Clin Linguist Phon* 2004; 18:495-505.
36. Sciote JJ, Horton MJ, Rowlerson AM, Link J. Specialized cranial muscles: how different are they from limb and abdominal muscles? *Cells Tissues Organs* 2003; 174:73-86.

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)

	Initial assessment (reassessment)						
Patient	**1**	**6**	**7**	**9**	**10**	**11**	**Total**
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.

10

Hearing loss in Pompe disease revisited:

results from a study of 24 children

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ABSTRACT

Little information is available regarding the auditory function in Pompe patients. Hearing loss has been reported in classic infantile patients, but it is still unknown whether central nervous system involvement interferes with auditory function and whether enzyme replacement therapy can improve hearing loss. Auditory function has not been studied in children with milder forms of the disease.

We analyzed repetitive auditory brainstem response measurements and pure tone audiometry in 24 children with Pompe disease.

Only one of 13 patients with milder phenotypes showed recurrent conductive hearing loss, while ten out of eleven classic infantile patients had sensorineural hearing defects. These patients also had a high prevalence of conductive hearing loss. Five patients showed evidence of mild retrocochlear pathology, suggestive of glycogen accumulation in the central nervous system. Hearing loss persisted during therapy in all patients. The results emphasize the need for careful monitoring of auditory function in classic infantile Pompe patients, and for early implementation of hearing aids to protect speech and language development.

INTRODUCTION

Hearing deficits have been found in several lysosomal storage disorders including Gaucher disease,[1-3] Fabry disease,[4-7] and Mucopolysaccharidoses.[8-10] Previously, we have detected hearing loss (HL) in four classic infantile Pompe patients as a new finding.[11]

Pompe disease (also called glycogen storage disease type II or acid maltase deficiency) (OMIM 232300) is caused by deficiency of the lysosomal enzyme acid alpha-glucosidase (EC 3.2.1.20). This leads to progressive glycogen storage in several tissues, among which skeletal and cardiac muscle are the most important.[12,13] The disease occurs in infants, children, and adults with a variable degree of disease severity and progression of muscle weakness. The clinical condition of patients with the so-called classic infantile form rapidly deteriorates, leading to death before the age of one year.[14,15] Patients with other phenotypes show a more slowly progressive disease course.[16] In the past, no treatment other than supportive care was available, but the recent introduction of enzyme replacement therapy has changed the prospect for patients.[17-21]

Although hearing loss is now increasingly recognized in classic infantile patients, the exact prevalence and type of hearing loss has never been investigated in a large group of patients, especially not in patients with more slowly progressive forms of the disease.

In this study, we analyzed repetitive auditory brainstem response measurements (ABRs) and pure tone audiometries before and after start of enzyme therapy in a cohort of 24 children: 11 patients with the classic infantile form and 13 patients with a less severe, juvenile form of the disease.

PATIENTS AND METHODS

Subjects

Twenty-four patients, eleven classic infantile and thirteen juvenile patients, were enrolled in the present study. All patients were diagnosed with Pompe disease by detecting enzyme deficiency in leukocytes or fibroblasts, and by mutation analysis. Inclusion criteria for the infantile group were: symptoms of muscle weakness within three months after birth, hypertrophic cardiomyopathy at diagnosis, and severe GAA (the gene encoding acid a-glucosidase) mutations on both alleles. Inclusion criteria for the juvenile group were: all other children with Pompe disease having one GAA mutation on one allele, at least one of which not severe. Eleven of the juvenile patients and all classic infantile patients were enrolled in a prospective study investigating the safety and efficacy of enzyme replacement therapy with recombinant human acid a-glucosidase (20–40 mg/kg). The other two juvenile patients were diagnosed pre-symptomatically, and were enrolled in an observational study that monitored disease progression in untreated patients. Both studies were approved by the Institutional Review Board of the Erasmus Medical Center, and all patients and parents gave written informed consent. All hearing assessments were performed before start of enzyme therapy and at regular intervals thereafter, if applicable.

Hearing assessments

In the juvenile patients, routine pure-tone audiometry was performed. When abnormalities were detected, the test was repeated at regular intervals, and patients were seen by an ENT (ear, nose and throat) specialist.

In the classic infantile patient group, auditory brainstem evoked responses, oto-acoustic emissions, and impedance audiometry were performed before the start of treatment and at regular intervals thereafter. An experienced audiologist evaluated the test results.

Auditory tests

Pure tone audiograms were made with a Madsen OB 822 audiometer (Copenhagen, Denmark) in a sound-proof room. Pure-tone threshold testing was performed for both air conduction and bone conduction. The average hearing threshold at frequencies 500, 1000, 2000 and 4000 Hz was used to classify the type of hearing loss (conductive component in case of an air-bone gap >15 dB) and to grade the amount of hearing loss according to the WHO definition of hearing impairment.[22]

Auditory brainstem evoked responses were determined using the Euphra-1 system, and a Jaeger-Toennies pre-amplifier. Click stimuli were presented at different levels with a repetition rate of 23/s via a TDH 49 headphone. The waveform was analyzed by an experienced audiologist, defining peak latencies of waves I (distal cochlear part of the VIIIth nerve), III (in between cochlear nucleus and the superior olivary complex) and V (between the superior olivary complex and the inferior colliculus). The amount of hearing loss was estimated from the detection threshold of peak V. The time interval between peaks I, III and V at stimulus level of 90 dB was used to identify retrocochlear dysfunction.

To assess cochlear function, transient evoked otoacoustic emissions (OAE) were carried out using the ILO288 (Otodynamics, UK). Otoacoustic emissions are vibrations presumably produced by the outer hair cells in the cochlea. Presence of otoacoustic emissions indicates a normal reponse to auditory stimuli of the middle ear, and cochlea. To assess middle ear function, impedance audiometry was performed with a standard clinical impedance meter (AT260, Interacoustics, USA). An abnormal result indicates middle ear dysfunction. The origin of the hearing deficit (conductive, cochlear, retrocochlear) was estimated by the combined interpretation of the ABR, otoacoustic emissions, and impedance audiometry.

Statistical analysis

ABR waveform latencies and inter-peak time intervals were compared to reference values obtained in 150 children with normal hearing in the Erasmus MC Sophia Children's hospital, and were expressed as z-scores. A z-score represents the number of standard deviations from the mean. Waveform latencies and inter-peak time intervals were considered abnormal if the measured value was above a z-score

of 1.96. The individual relationship between the different outcome measures and treatment time were analyzed using least-squares regression.
All data analyses were performed using SPSS for Windows version 15.

RESULTS

Classic infantile Pompe patients

Hearing loss

Eleven classic infantile Pompe patients were included in the present study. Table 1 summarizes the clinical features of each of the patients. Table 2 tabulates the type of hearing loss found in each individual patient before start of treatment and after 1 to 6 years of treatment. Hearing loss was detected immediately after birth in some patients. There was no significant correlation between the age of the patients at their first hearing test and the severity of hearing loss. Baseline measurements were performed before start of enzyme therapy and revealed a sensorineural hearing loss in nine of the eleven patients (for details see Table 2). In all these patients, latencies of peaks I and V as function of stimulus level suggested a cochlear origin of the hearing loss. Absent OAEs with normal middle ear function confirmed the cochlear dysfunction in six patients, while abnormal tympanograms prohibited a reliable interpretation of the OAE results in two patients. Two patients had normal cochlear function but showed evidence of retrocochlear pathology, as inter-peak latencies between waves I and V were significantly prolonged.
Ten patients were followed for a maximum period of six years. One patient died at the age of 8 months. During the period that patients received enzyme therapy, the sensorineural hearing loss persisted in all with the exception of one patient, who had normal hearing at baseline. In addition, variable degrees of conductive hearing loss ranging from 15 to 60 dB were repeatedly found in nine patients (Figure 1). Insertion of ventilation tubes in two patients did not improve their hearing levels. All patients (except patient 8) received hearing aids and/or speech therapy.

Neurological involvement

Waves I, III, and V were identified in all patients. Baseline nerve conduction patterns appeared to be close to normal for most of the patients. Inter-peak

latency between peak I and peak V (IPL_{I-V}) was prolonged in 3 patients (Table 2A, patients 6, 8, and 9).

During follow-up assessments, IPL_{I-V} remained prolonged in these three patients. Two other patients, who both had normal values at baseline, showed prolongation of IPL_{I-V} at the age of 1.5 years (patients 3 and 7). One of these patients started receiving enzyme therapy in the first month of life, the other at the age of 8 months when she was in an end stage of the disease. In both patients, IPL_{I-V} remained prolonged during the entire follow-up period. Further examination showed that a prolongation of the inter-peak latency was mainly found between peaks III and V.

Juvenile Pompe patients

Age of the patients at their baseline hearing test ranged from 3 to 16.4 years (mean 11.4). Age at first symptoms ranged from 6 months to 10 years, and age at diagnosis ranged from 0.8 to 11.6 years. Baseline characteristics are presented in Table 3.

Pure-tone audiometry showed conductive hearing losses in patients 2 and 5. No hearing deficits were found in the other patients. Patient 2 had a mild unilateral conductive hearing loss of 33.3 dB. This patient exhibited mobility problems in early childhood, and was diagnosed with Pompe disease at the age of 3.5 years. At the age of 6 years, his auditory function was measured for the first time. At that moment, the patient was able to walk, and his pulmonary function was within normal limits. During follow-up examinations, the conductive hearing loss subsided, and test results became normal.

Patient 5 showed mild bilateral hearing loss during the first assessment in our hospital, at the age of 13 years. The patient was diagnosed with Pompe disease at the age of 2 years. In the following years, she followed a rapidly progressive disease course, became completely wheelchair bound, and was invasively ventilated at the age of 6. Severe weakness of the facial muscles caused dysarthria and dysphagia, for which she received feeding by percutaneous endoscopic gastroscopy tube. There was a medical history of persistent otitis media, for which she had received ventilation tubes at the age of 3 years. Additional examinations revealed a recurrent bilateral otitis media, and the hearing loss of 35 dB persisted during follow-up assessments.

Table 1. Characteristics of 11 classic infantile Pompe patients

Patient	Age first assessment (months)	Gender (M/F)	Motor status at first assessment	Motor status at final assessment	Age at final ABR (years)
1	3	M	Axial hypotonia, head-lag, extremity movement +	Walks unsupported	6
2	7	F	Paralysis legs, paresis arms	Tetraplegic	6
3	8	F	Paralysis legs, paresis arms	Tetraplegic	2
4	2.5	F	Axial hypotonia, head-lag, extremity movement +/-	Sits unsupported, † 4.2 years	4
5	2	M	Axial hypotonia, head-lag, extremity movement +	Walks unsupported, † 4.2 years	4
6	1	M	Axial hypotonia, head-lag, extremity movement +	Walks unsupported	5
7	0.5	F	Axial hypotonia, head-lag, extremity movement +	Walks unsupported	5
8	0.1	M	Axial hypotonia, head-lag, extremity movement +	Walks unsupported	3
9	2	M	Axial hypotonia, head-lag, extremity movement +/-	Sits unsupported	3
10	0.2	F	Axial hypotonia, head-lag, extremity movement +	Walks unsupported	1
11	3.5	F	Axial hypotonia, head-lag, extremity movement +/-	No gains, †8 months	-

ABR=auditory brainstem response. †Died

Table 2. Summary of audiometric results of the 11 classic infantile patients at baseline, and after a maximum period of 6 years of enzyme replacement therapy with recombinant human alpha-glucosidase

Patient	Age	Right ear					Left ear				
		EHT	Tymp	OAE	I-V	III-V	EHT	Tymp	OAE	I-V	III-V
Baseline											
1	3 m	30	normal	abnormal	normal	N.A.	60	normal	abnormal	normal	N.A.
2	7 m	70	normal	abnormal	normal	N.A.	90	abnormal	abnormal	normal	N.A.
3	8 m	40	normal	abnormal	normal	N.A.	40	normal	abnormal	normal	N.A.
4	2.5 m	40	abnormal	abnormal	normal	N.A.	60	abnormal	abnormal	normal	N.A.
5	2 m	80	normal	abnormal	normal	N.A.	60	normal	abnormal	normal	N.A.
6	1 m	20	normal	normal	abnormal	2.2	20	normal	normal	abnormal	2.7
7	0.5 m	50	normal	abnormal	normal	N.A.	40	normal	abnormal	normal	N.A.
8	0.1 m	10	normal	normal	abnormal	3.6	10	normal	normal	abnormal	2.5
9	2 m	60	?	?	abnormal	3.3	60	normal	abnormal	normal	N.A.
10	2.5 m	40	normal	abnormal	normal	N.A.	40	normal	abnormal	normal	N.A.
11	3.5 m	70	abnormal	abnormal	normal	N.A.	70	abnormal	abnormal	normal	N.A.
After maximum 6 years of enzyme replacement therapy											
1	6 y	80	abnormal	abnormal	normal	N.A.	80	abnormal	abnormal	normal	N.A.
2	6 y	70	normal	abnormal	normal	N.A.	70	normal	abnormal	normal	N.A.
3	2 y	60	abnormal	abnormal	abnormal	2.3	80	abnormal	abnormal	abnormal	3.3
4	4 y	80	abnormal	abnormal	normal	N.A.	90	abnormal	abnormal	normal	N.A.
5	4 y	80	normal	abnormal	normal	N.A.	90	abnormal	abnormal	normal	N.A.
6	5 y	40	abnormal	abnormal	abnormal	2.3	50	abnormal	abnormal	abnormal	3.4
7	5 y	70	abnormal	abnormal	abnormal	2.2	50	abnormal	abnormal	abnormal	1.0
8	3 y	20	normal	normal	abnormal	2.3	20	normal	normal	abnormal	2.3
9	3 y	80	abnormal	abnormal	abnormal	2.3	80	abnormal	abnormal	abnormal	2.9
10	1 y	40	abnormal	abnormal	normal	N.A.	40	abnormal	abnormal	normal	N.A.
11	died										

m:Months, y:years, EHT estimated hearing threshold (dB HL, decibels hearing level), Tymp:tympanogram result, OAE:otoacoustic emission result, I-V:auditory brainstem response inter-peak interval I to V, III-V:auditory brainstem response inter-peak interval III to V (data are represented by z-scores, >1.96 is considered abnormal), N.A. not applicable.

Table 3. Characteristics of 13 children with Pompe disease, and the amount of hearing loss

Patient	Age at hearing test (years)	Gender (M/F)	Motor status [a]	Respiratory function [a]	Hearing loss (dB)[a]	
					(right ear)	(left ear)
1	13.1	M	Ambulant, prox weak	Normal	nh	nh
2	6.0	M	Ambulant, prox weak	Normal	33.3 cond	nh
3	12.7	F	Ambulant, prox weak	Diminished	nh	nh
4	11.9	M	Partially wheelchair dep.	Normal	nh	nh
5	12.7	F	Tetraplegic	Invasive ventilation	35 cond	35 cond
6	5.2	M	Ambulant	Normal	nh	nh
7	3	M	Ambulant, prox weak	Normal	nh	nh
8	16	M	Ambulant	Diminished	nh	nh
9	13	M	Ambulant, prox weak	Diminished	nh	nh
10	9	F	Ambulant, prox weak	Normal	nh	nh
11	15.2	M	Ambulant, prox weak	Diminished, BIPAP	nh	nh
12	16.4	F	Paresis legs, scoliosis	Invasive ventilation	nh	nh
13	9.2	F	Partially wheelchair dep.	Diminished, BIPAP	nh	nh

dB:Decibels, nh:normal hearing, cond:conductive hearing loss, prox weak:proximal weakness, BIPAP:bi-level positive airway pressure. [a] Before start of enzyme replacement therapy.

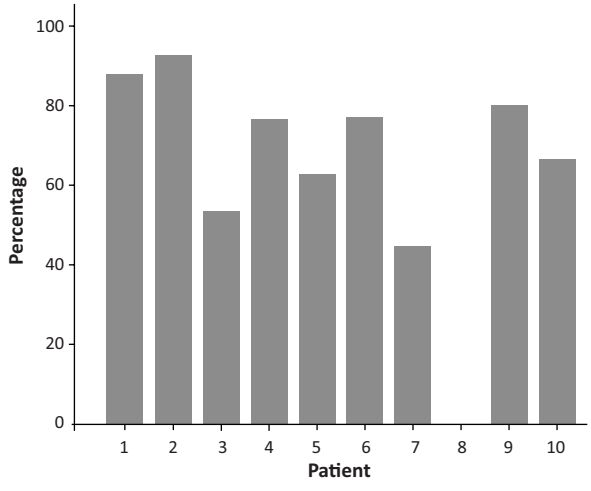

Figure 1. Percentage of auditory brainstem responses (ABR) in which conductive hearing losses were found per patient. The number of ABRs performed per patient ranged from 1 (patient died at 5 months of age) to 18 and was on average 9.

DISCUSSION

The present study confirms our previous finding that hearing disorders are a major concern in patients with the classic infantile form of Pompe disease.[11] These problems are rarely found in children with milder forms of the disease. The reason why hearing loss selectively occurs in classic infantile patients is not fully understood, but may be explained by the less severe mutations and higher levels of residual alpha-glucosidase activity in juvenile patients.

The hearing deficits that we detected in ten of eleven classic infantile patients ranged from 30 to 90 dB. As reported earlier, cochlear dysfunction was frequently found. But we also discovered additional conductive hearing losses in 75% of all hearing tests performed (in 90% of the patients). This is suggestive of chronic middle ear dysfunction. The etiology of chronic otitis media is multifactorial, and includes infectious, allergic, and immunologic factors, resulting in Eustachian tube

dysfunction and middle ear effusion.[23,24] Our patients did not have clinical evidence for allergic or infectious middle ear problems, but we found severe weakness of the facial muscles in all classic infantile patients[25] (personal communication). The tensor veli palatini muscle is the principal muscle involved in opening the Eustachian tube. Weakness of this muscle has been reported to cause a pressure drop in the middle ear and may very well be the cause of the high incident of the conductive hearing loss found in infantile patients with Pompe disease.

To elucidate whether retrocochlear pathology was present in our classic infantile patient group, we studied repetitive ABRs. We found prolongations of inter-peak latencies in five patients. The most frequent finding was prolongation of between peaks III and V. This is suggestive for pathology of the central part of the auditory pathway.

It was anticipated that glycogen storage in the brain and other parts of the central nervous system might cause sensorineural hearing loss,[11] since glycogen storage has been found in nuclei of the brain stem, thalamus, anterior horn cells, and neurons of the spinal ganglia. Glycogen storage was also reported to be present in several parts of the brain of a patient that died during treatment with enzyme therapy. This finding was not unexpected since the intravenously administered enzyme cannot pass the blood-brain barrier.[26]

Of note, three of the five patients with a prolongations of the inter-peak latencies belonged to the best responders to enzyme therapy. They were able to walk and had adequate cognitive abilities. Compared to other lysosomal storage disorders, like Gaucher type 3, the abnormalities in inter-peak latencies were relatively mild. It is therefore suggested that glycogen storage, although undoubtedly present in brains of infants with Pompe disease, only minimally interferes with auditory functioning.

Hearing deficits were present shortly after birth. The extent of hearing loss did not decrease over time. This suggests the storage of glycogen in the cochlea already commences during gestation and that enzyme therapy does not have an effect on it. It should be noted, however, that there were variable degrees of conductive hearing loss, which interfered with the test results and prevented us from estimating the exact degree of sensorineural hearing loss.

In conclusion, our data emphasize that hearing problems are a major concern for patients with classic infantile Pompe disease, and are only sporadically present in children with milder forms of the disease. We therefore advise the monitoring of auditory function on a regular basis in infants with the disease, while regular examinations of auditory function are not required in older children. Retrocochlear pathology may be present, but does not seem to be a major contributor to the hearing loss. In addition to the previously reported cochlear hearing loss in infants, we found frequent conductive hearing losses in our patients. It is our experience that insertion of ventilation tubes does not consistently improve the hearing deficit. We therefore recommend early implementations of hearing devices and speech therapy, in order to save precious time, especially since the first two years of life are most important for speech and language development.[27]

REFERENCES

1. Bamiou DE, Campbell P, Liasis A, et al. Audiometric abnormalities in children with Gaucher disease type 3. *Neuropediatrics.* Jun 2001; 32(3):136-141.
2. Kaga M, Azuma C, Imamura T, Murakami T, Kaga K. Auditory brainstem response (ABR) in infantile Gaucher's disease. *Neuropediatrics.* Nov 1982; 13(4):207-210.
3. Lacey DJ, Terplan K. Correlating auditory evoked and brainstem histologic abnormalities in infantile Gaucher's disease. *Neurology.* Apr 1984; 34(4):539-541.
4. Hajioff D, Hegemann S, Conti G, et al. Agalsidase alpha and hearing in Fabry disease: data from the Fabry Outcome Survey. *European journal of clinical investigation.* Sep 2006; 36(9):663-667.
5. Hegemann S, Hajioff D, Conti G, et al. Hearing loss in Fabry disease: data from the Fabry Outcome Survey. *European journal of clinical investigation.* Sep 2006; 6(9):654-662.
6. Palla A, Hegemann S, Widmer U, Straumann D. Vestibular and auditory deficits in Fabry disease and their response to enzyme replacement therapy. *J Neurol.* Oct 2007; 254(10):1433-1442.
7. Schachern PA, Shea DA, Paparella MM, Yoon TH. Otologic histopathology of Fabry's disease. *The Annals of otology, rhinology, and laryngology.* May 1989; 98(5 Pt 1):359-363.
8. Komura Y, Kaga K, Ogawa Y, Yamaguchi Y, Tsuzuku T, Suzuki JI. ABR and temporal bone pathology in Hurler's disease. *International journal of pediatric otorhinolaryngology.* Mar 1 1998; 43(2):179-188.
9. Peck JE. Hearing loss in Hunter's syndrome--mucopolysaccharidosis II. *Ear and hearing.* Jul-Aug 1984; 5(4):243-246.
10. Schachern PA, Cureoglu S, Tsuprun V, Paparella MM, Whitley CB. Age-related functional and histopathological changes of the ear in the MPS I mouse. *International journal of pediatric otorhinolaryngology.* Feb 2007; 71(2):197-203.
11. Kamphoven JH, de Ruiter MM, Winkel LP, et al. Hearing loss in infantile Pompe's disease and determination of underlying pathology in the knockout mouse. *Neurobiol Dis.* Jun 2004; 16(1):14-20.
12. van der Ploeg AT, Reuser AJ. Pompe's disease. *Lancet.* Oct 11 2008; 372(9646):1342-1353.
13. Engel AG, Hirschhorn R, Huie ML. Acid maltase deficiency. In: Engel A, ed. *Myology*. NY: McGraw-Hill; 2004: 1559-1586.
14. Van den Hout HM, Hop W, Van Diggelen OP, et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics.* 2003; 112(2):332-340.
15. Kishnani PS, Hwu WL, Mandel H, Nicolino M, Yong F, Corzo D. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *The Journal of pediatrics.* May 2006; 148(5):671-676.
16. Winkel LP, Hagemans ML, van Doorn PA, et al. The natural course of non-classic Pompe's disease; a review of 225 published cases. *J Neurol.* Aug 2005; 252(8):875-884.
17. Kishnani PS, Corzo D, Nicolino M, et al. Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology.* Jan 9 2007; 68(2):99-109.
18. Kishnani PS, Corzo D, Leslie ND, et al. Early Treatment with Alglucosidase Alfa Prolongs Long Term Survival of Infants with Pompe Disease. *Pediatric research.* Jun 17 2009.

19. Strothotte S, Strigl-Pill N, Grunert B, et al. Enzyme replacement therapy with alglucosidase alfa in 44 patients with late-onset glycogen storage disease type 2: 12-month results of an observational clinical trial. *J Neurol.* Jan 2010; 257(1):91-97.
20. van Capelle CI, Winkel LP, Hagemans ML, et al. Eight years experience with enzyme replacement therapy in two children and one adult with Pompe disease. *Neuromuscular disorders.* May 26 2008.
21. Van den Hout JM, Kamphoven JH, Winkel LP, et al. Long-term intravenous treatment of Pompe disease with recombinant human alpha-glucosidase from milk. *Pediatrics.* May 2004; 113(5):e448-457.
22. WHO. International classification of impairments, disabilities and handicaps: a manual of classification related to the consequences of disease. Geneva 1980.
23. Mathew GA, Kuruvilla G, Job A. Dynamic slow motion video endoscopy in eustachian tube assessment. *American journal of otolaryngology.* Mar-Apr 2007; 28(2):91-97.
24. Tos M. Importance of eustachian tube function in middle ear surgery. *Ear, nose, & throat journal.* Sep 1998; 77(9):744-747.
25. Jones HN, Muller CW, Lin M, et al. Oropharyngeal dysphagia in infants and children with infantile Pompe disease. *Dysphagia.* Dec 2010; 25(4):277-283.
26. Thurberg BL, Lynch Maloney C, Vaccaro C, et al. Characterization of pre- and post-treatment pathology after enzyme replacement therapy for pompe disease. *Laboratory investigation.* Oct 30 2006; 86(12):1208-1220.
27. Zumach A, Gerrits E, Chenault MN, Anteunis LJ. Otitis media and speech-in-noise recognition in school-aged children. *Audiology & neuro-otology.* 2009; 14(2):121-129.

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Cardiac outcome after 13 years of treatment with acid alpha-glucosidase in classic-infantile Pompe disease

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ABSTRACT

Introduction: Cardiorespiratory failure before the age of one year is the main cause of death in untreated patients with classic-infantile Pompe disease, an inheritable metabolic myopathy with a characteristic progressive hypertrophic cardiomyopathy. Since the introduction of enzyme replacement therapy (ERT), survival has increased significantly; mainly by reduction of cardiac hypertrophy and improvement cardiac function. But it's insufficiently known whether these effects sustain. Therefore the aim of our study was to investigate the long term effects of ERT on cardiac parameters and function.

Methods: Fourteen patients who had started ERT between 1999 and 2012 and who had been treated for at least 12 months were included in this single-center observational study. Cardiac dimensions, cardiac function, and conduction and rhythm disturbances were evaluated at baseline and regular intervals thereafter using echocardiography, electrocardiography and Holter monitoring.

Results: The median treatment duration was 4.8 years (range 1.1 to 13.9 years) at study end. All patients had an increased left ventricular mass index (LVMI) at baseline (median 225 g/m^2, range 98 to 599 g/m^2). Six patients showed an increase of LVMI during the first four weeks of treatment, thereafter LVMI started to reduce. Normalization of LVMI was observed in 13 patients after a median follow-up period of 38 weeks (range 4 to 724 weeks). In one patient cardiac size never reached normal values and in another, after initial LVMI normalization, LVMI started to increase again during a period of clinical deterioration leading to invasive ventilation. Both patients died at the age of four years. PR-interval was below normal values in five patients at baseline and in 9 patients at study end. Six patients showed a delta wave pattern on ECG, which resulted in documented periods of SVT in 2 patients requiring medication and surgery.

Conclusion: In the present study it was shown that cardiac improvements were maintained over 14 years of treatment. Time to normalization seemed to be related to clinical status at baseline. LVMI may deteriorate during treatment in patients that show clinical decline and become ventilator dependent The risk of tachyarrhythmias deserves attention, particularly in those in whom ERT had been initiated relatively late. Based on our results we advise to perform cardiac evaluations at regular intervals.

INTRODUCTION

Prior to the introduction of enzyme replacement therapy, cardiorespiratory failure was the main cause of death in patients with the classic-infantile form of Pompe disease (OMIM 232300), a lysosomal storage disorder caused by deficiency of the enzyme acid alpha-glucosidase (EC 3.2.1.20). Progressive accumulation of glycogen in different tissues throughout the body, but predominantly in skeletal and cardiac muscle, is the hallmark of the disease. Pompe disease presents as a spectrum of clinical phenotypes. Complete deficiency of alpha-glucosidase results in the most severe "classic-infantile" form. Patients present with a progressive generalized myopathy and cardiac hypertrophy. Motor milestones are not achieved and patients rarely survive beyond one year of age. Cardiac failure is the major cause of death.[1-4]

Since the introduction of enzyme replacement therapy (ERT) with recombinant human alpha-glucosidase (rhGAA), survival has significantly improved, mainly by reducing cardiac hypertrophy and improving cardiac function. Nevertheless, not all patients respond equally well. Results of ERT on respiratory function and muscle strength are more variable.[5-8] Now that classic-infantile patients survive far beyond their first year of life, it is important to determine whether the previously described benign cardiac effects are sustained over a longer period of time.[9] The present report describes the effect of enzyme replacement therapy on cardiac size, cardiac function, and conduction pattern in fourteen classic-infantile patients over a maximum period of thirteen years of treatment.

MATERIAL AND METHODS

This study is part of an ongoing clinical study, investigating safety and efficacy of ERT with rhGAA in classic-infantile Pompe patients. Between 1999 and 2012, seventeen patients were enrolled and evaluated in our hospital. All patients were diagnosed with classic-infantile Pompe disease, confirmed by deficiency of endogenous α-glucosidase in fibroblasts <1% of the normal mean, mutation analysis, and the

presence of a hypertrophic cardiomyopathy. Since we were mainly interested in long-term effects, only patients that received ERT for at least 12 months at the end of the study period (December 31st, 2012) were enrolled. Fourteen patients fulfilled this criteria. Two were still too young and one died after 3 months of therapy due to insufficient response of therapy both on cardiac and other parameters.
Patients received enzyme replacement therapy with rhGAA. The dose ranged from 20 mg/kg every other week to 40 mg/kg weekly.

Study design

The study was performed at the Erasmus Medical Center Sophia Children's Hospital Rotterdam, the Netherlands, and was approved by the Institutional Review Board. Written informed consent was obtained from parents or legal guardians.
Standardized assessments were performed before the start of ERT and every three months thereafter. Cardiac assessments are described below.

Echocardiography

Conventional echocardiography including trans-thoracic M-Mode and two-dimensional echocardiography as well as conventional echo-Doppler measurements were performed by an experienced sonographer (JP) using a Philips iE33 xMAtrix Echocardiography System (Philips Medical Systems, Andover, MA, USA). Recordings were performed according to the recommendations of the American Society of Echocardiography[10] at baseline and every three months thereafter.
The following parameters were documented: end-diastolic left ventricular internal cavity dimension in diastole (LVIDd), inter-ventricular septum thickness in diastole (IVSd), left ventricular posterior wall thickness in diastole (LVPWd), left and right ventricular pre-ejection periods (LVPEP and RVPEP), left and right ventricular ejection times (LVET and RVET), and trans-mitral and tricuspid E and A wave peak velocities.. These values were compared with normal reference values according to Kampmann et al.[11] Left ventricular mass index (LVMI) was calculated by the Devereux formula and indexed by body surface area.[12] Left ventricular hypertrophy was considered to be present if the LVMI was above the p95 for age related peers.[11,13]

Systolic function was examined by calculating the shortening fraction (SF). Values between 28–44% were considered normal.[14] Diastolic function was assessed by examining the E/A ratio by measuring the peak early (E) and late (A) transmitral filling velocities with conventional Doppler imaging. An E/A ratio <1 was considered abnormal. Relative wall thickness (RWT) was calculated as RWT = ((IVSd + LVPWd)/ LVIDd). A cut-off value of 0.42 has been proposed to divide LV hypertrophy into concentric (RWT >0.42) and eccentric hypertrophy (RWT <0.42).[14] Cases without LV hypertrophy were divided into either normal (RWT <0.42) or concentric remodelling (RWT >0.42).

Electrocardiography

Standardized 12-lead electrocardiograms (ECGs) were made using a Mortara ELI 350, Mortara instrument inc. Milwaukee, USA. ECGs were obtained for all patients at baseline and every three months thereafter to determine PR-interval, QT interval corrected for heart rate, LV voltages and rhythm or conduction disturbances. The QT interval was measured in lead II, V5, or V6. The corrected QT interval was determined by dividing the measured QT interval by the square root of the RR interval (Bazett's formula). LV voltages were calculated by the sum of the R wave in lead V6 and the S wave in lead V1. Pediatric reference values were obtained from Rijnbeek et al and were corrected for age and heart rate.[15] 24-hour Holter monitoring was performed in a subset of 3 patients. Two investigators examined all ECGs and Holter ECGs (CvC, IF).

Statistical analysis

All values obtained by echocardiography were transformed into a *Z*-score calculated as the difference between the measured value and the mean reference value divided by the standard deviation from the reference value. *Z*-scores>2 were considered abnormal. Variables were summarized using descriptive statistics comprising median and range. The Wilcoxon rank signed test was used to evaluate differences in cardiac dimensions over time. p-values <0.05 were considered statistically significant. Statistical analyses were performed using SPSS version 21.0.

RESULTS

Patients

A total of fourteen patients were included. Baseline characteristics of the patients are summarized in Table 1. Age at start of treatment ranged from 3 days to 8.3 months (median age 2.7 months). Duration of ERT treatment ranged from 1.1 to 13.9 years (median 4.8 years). Two patients died during follow up at the ages of 4.4 and 4.3 years (patients 3 and 5). At study end eight patients had received ERT for more than 4 years (range 4.1 to 13.9 years).

Concentric left ventricular (LV) hypertrophy was present in all patients at start of therapy (Table 1). Two patients had LV outflow tract obstruction, and two other patients had accelerated mid-ventricular velocity. Four patients (patients 2, 3, 4 and 12) experienced symptoms of congestive heart failure. At start of therapy only four out of fourteen patients did not receive any cardiac medication (patients 1, 8, 9 and 11), four patients were treated with diuretics (patient 2, 3, 4 and 14), two patients with beta-blockers (6 and 10) and one patient with an ACE-inhibitor (patient 7). The remaining three patients received a combination of these drugs.

The general clinical condition varied between patients at start of treatment. Two patients (patient 2 and 4) had profound generalized hypotonia and could hardly lift their arms from the surface in supine position and were considered to have end stage disease. The other patients could all move their limbs against gravity to some extent. Nine of 14 patients scored below p5 for motor development as assessed by the Alberta Infant Motor Scale (AIMS, patients 2, 3, 4, 6, 10, 11, 12, 13 and 14). Four patients required oxygen via nasal prongs (patients 4, 7, 8 and 13). One became ventilator-dependent before start of treatment (patient 2). At the study end two patients had died and three others were ventilator dependent.

Echocardiography

At baseline, LVMI was profoundly elevated in all patients (median 225 g/m^2, range 98 to 599 g/m^2) (Table 1). There was a good response to therapy (Fig 1). However, during the first four weeks of treatment cardiac size initially increased in six patients. Median increase in LVMI was 79.0 g/ m2 (range 12.4–149 g/m^2) in

these patients. Thereafter LVMI started to decline also in these patients. At final assessment the median LVMI was 70.4 g/ m^2 (range 48.2 to 119.6 g/m^2).
In thirteen patients cardiac dimensions normalized (Table 2). Median time to normalization was 38 weeks (range 4 to 724 weeks). One patient never reached normal values and died at the age of 4.3 years. In another patient normal LVMI values were initially reached, but thereafter a significant increase in cardiac size and decline in motor function was observed after he became ventilator dependent following a severe pneumonia at the age of two. This patient eventually died at the age of 4.4 years due to respiratory failure.
For two patients (patients 2 and 4) it took 13 years of treatment before LVMI was within normal limits.

Systolic and Diastolic function

Four patients showed a decreased SF (patients 3, 4, 12, 14), in the other ten patients systolic function was normal at baseline (Table 2). During treatment SF normalized in all.
At baseline, diastolic function as measured by E/A ratio was normal in all patients (Table 2). At follow-up, two patients showed abnormalities in diastolic function. Patient 3 showed a decline of diastolic function after her clinical condition deteriorated. Patient 2, who started ERT at the age of 7.2 months, had abnormal E/A ratios in most measurements (median 1.20, range 0.63 to 1.90), with the last MV E/A ratio being 1.70. E/A ratios are shown in Table 2.

Electrocardiography

During follow-up a total of 118 ambulant ECGs from 14 patients and 5 Holter ECGs from 3 patients were evaluated. At baseline, 9 patients had a normal PR interval and 5 patients had a shortened PR interval (median PR interval 80 ms, range 60 to 100 ms) (Table 2). At study end, the PR interval was within normal limits in 5 patients: in 2 patients the PR interval remained normal (patients 5 and 6), in 3 other patients the PR interval normalized (patient 9, 11 and 12), and in 7 patients the PR interval shortened after having a normal interval at baseline (patients 1, 4, 7, 8, 10, 13 and 14) (Table 2). The median PR-interval remained below normal values at 80 ms (range 50 to 250 ms).

Table 1. Clinical features

Pt	Gender	Age at diagnosis (months)	Age at start ERT (months)	Age at end of study (years)	Mutations	CRIM status	ERT dose (mg/kg)	Baseline cardiac medication	Start Invasive ventilation (years)	Baseline LVMI (*Z*-score)
1	M	0.7	3.8	14.2	c.2481+102_2646+31del c.1799G>A	Positive	40/week	None	-	171 (14.2)
2	F	3.6	7.2	14.5	c.1115A>T c.525delT	Positive	40/week	Diuretics	0.6§	203 (18.2)
3	F	0.6	3.0	4.3*	c.525delT c.525delT	Negative	40/week	Diuretics	2.2	308 (31.5)
4	F	6.2	8.3	14.5	c.1913G>T c.1548G>A	Positive	40/week	Diuretics, ACE-inhibitor and Digoxin	0.9	599 (68.3)
5	M	0.2	1.9	4.4*	c.2741delinsCAG c.2741delinsCAG	Negative	20/eow	Diuretics	2.0	296 (30.0)
6	M	0.7	1.2	8.6	c.del525T c.1933G>T	Positive	40/week	Beta-blocker	-	191 (16.7)
7	F	0.2	0.5	8.2	c.2481+102_2646+31del c.2481+102_2646+31del	Positive	20/eow	ACE-inhibitor	-	231 (21.7)
8	M	0.1	0.1	5.8	c.1460T>C c.1460T>C	Positive	40/week	None	2.7	98 (4.9)
9	M	2.0	2.2	5.3	c.525delT c.2481+102_2646+31del	Positive	40/week	None	-	140 (10.2)
10	F	2.3	2.4	4.3	c.2481+102_2646+31del c.2481+102_2646+31del	Positive	40/week	Beta-blocker	-	237 (22.5)

Pt	Gender	Age at diagnosis (months)	Age at start ERT (months)	Age at end of study (years)	Mutations	CRIM status	ERT dose (mg/kg)	Baseline cardiac medication	Start Invasive ventilation (years)	Baseline LVMI (*Z*-score)
11	F	0.1	0.3	2.2	c.525delT c.1933G>A	Positive	40/week	None	-	110 (6.4)
12	F	4.4	4.6	2.2	c.2104C>T c.379_380del	Positive	40/week	Diuretics and ACE-inhibitor	-	263 (25.8)
13	M	3.8	3.8	2.0	c.2481+102_2646+31del c.525delT	Positive	40/week	Diuretics and beta-blocker	-	220 (20.3)
14	M	2.9	3.0	1.4	c.2104C>T c.2481+102_2646+31del	Positive	40/week	Diuretics	-	238 (22.7)

§ = Invasive ventilation started before start of ERT. * = deceased, CRIM= cross reactive immunological material

Table 2. Cardiac parameters at baseline and at final assessment

Pt	LVMI at baseline (*Z*-score)	LVMI at last assessment (*Z*-score)	Shortening fraction at baseline (%)	Shortening fraction at last assessment (%)	MV E/A ratio at baseline	MV E/A ratio at last assessment	PR interval at baseline	PR interval at last assessment	Maximal motor milestone
1	**171** (14.2)	70.4 (0.4)	55	41	1.31	4.00	0.1	**0.08**	Persistent walker
2	**203** (18.2)	119.6 (1.8)	41	49	1.17	**1.70**[#]	**0.08**	**0.1**	Tetraplegic
3	**308** (31.5)	**109** (4.4)*	**25**	46	1.03	**0.62**	**0.07**	**0.08**	Sitting
4	**599** (68.3)	N	**12**	41	1.24	NA	0.1	**0.08**	Tetraplegic
5	**296** (30.0)	**78** (2.4)*	64	57	NA	1.30	0.1	0.12	Walking‡
6	**191** (16.7)	67.8 (0.4)	35	37	1.20	1.80	0.09	0.25	Persistent Walker
7	**231** (21.7)	71.1 (0.8)	44	32	1.10	1.70	0.08	**0.05**	Persistent Walker
8	**98** (4.9)	57.6 (-0.6)	39	46	1.30	1.60	0.08	**0.08**	Walking‡
9	**140** (10.2)	74.3 (1.1)	55	37	1.00	1.60	**0.08**	0.12	Bumscoots
10	**237** (22.5)	63.3 (1.0)	37	38	1.40	1.80	0.08	**0.08**	Persistent Walker
11	**110** (6.4)	48.2 (-1.4)	38	36	1.40	1.50	**0.06**	0.09	Persistent Walker
12	**263** (25.8)	74.1 (1.9)	**25**	35	1.40	1.10	**0.07**	0.08	Persistent Walker
13	**220** (20.3)	64.5 (0.7)	32	39	2.30	2.40	0.08	**0.08**	Persistent Walker
14	**238** (22.7)	65.7 (0.8)	**20**	32	1.20	1.50	0.08	**0.08**	Pulls to stand

* = deceased, ‡ = Lost the ability to walk after they became ventilator dependent, [#]abnormal E/A ratio in most of her measurements, Abnormal values are marked in bold, NA = not available, N = normal dimension.

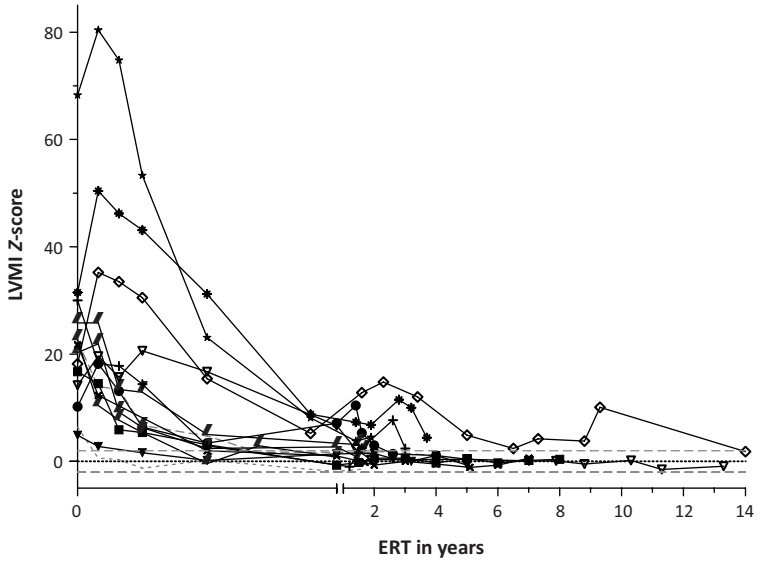

Figure 1. Left Ventricular Mass Index (LVMI) z-score during a maximum period of 13.9 years of enzyme replacement therapy (ERT).

LV voltages were substantially increased at baseline (median 4.8 mV, range 2.2 to 8.6 mV) and showed a significant reduction after start of ERT (median 3.9 mV, range 1.7 to 6.2 mV, p=0.023). All patients showed repolarisation disturbances (strain) at baseline. These disturbances normalized in all patients except patient 2. Four patients (patient 1, 9, 10 and 13) had an incomplete right bundle branch block. At baseline, the corrected QT-interval was normal in 10 patients (median: 390 ms, range 340 to 430 ms). In 4 patients the corrected QT-interval was shortened (patients 1,4, 13 and 14). At study end corrected QT-interval was normal in 10 patients, shortened in 3 patients (patients 4, 5 and 11) and increased in 1 patient (patient 2 had an increase to 520 ms).

Delta waves, suggestive for Wolff-Parkinson-White (WPW) pattern, were found in six patients (patient 2, 4, 6, 7, 9 and 10). In the years after start of therapy,

arrhythmias were documented in two patients (patients 2 and 4) as multiple episodes of supraventricular tachycardia. The tachyarrhythmias were successfully converted by adenosine and rhythm control was established by Sotalol in patient 2 and by Atenolol and Flecainide in patient 4. Because of frequent relapses patient 4 underwent ablation of three aberrant pathways. The most recent Holter ECG of patient 2 revealed severe sinus bradycardia with a minimum of 35 per minute. More than 6000 pauses were recorded over 24 hours, with a maximum duration of 3.5 seconds. Because of the poor clinical condition of the patient the decision was made to refrain from implanting a pacemaker.

DISCUSSION

Now that patients with classic-infantile Pompe disease treated with ERT survive far beyond their first year of life, an important question is whether the short-term positive effects on cardiac structure and function, closely related to prolonged survival and decreased morbidity, sustain.[6,8,16,17] The present study in 14 patients with classic-infantile Pompe disease confirms that enzyme replacement therapy (ERT) significantly reduces left ventricular mass index and improves cardiac function. Moreover, these beneficial effects were maintained over at least 13.9 years. This is in line with the results of a recently published study in 11 patients with classic-infantile Pompe disease followed for a maximum period of 12 years[9] with the exception that LVMI in two of our patients did not remain within normal limits. These patients first showed improvements of cardiac size but after they clinically deteriorated and became ventilator dependent, LVMI worsened. Both patients died at the age of 4 years. Two other patients, who did not reach normal values of LVMI until 13 years of treatment, were both in an end-stage of the disease when treatment was initiated (paresis of the arms and paralyzed legs, ventilator-dependent). Both had recurrent SVTs, and patient 2 showed episodes of severe bradycardia on Holter monitoring and had an abnormal diastolic function. These results may suggest that a relation exists between LVMI and clinical status, though

other previously published studies did not find an association between motor outcome and LVMI.[7,18,19] Whether the deterioration of LVMI despite ERT in the previously described patients was influenced by a limited effect of the treatment itself, or by patient related factors such as immune responses, remains unknown. Both patients did not have higher peak antibody titers than the other patients.[20] Of note, these patients were the only CRIM negative patients in this study.

Despite ERT, rhythm disturbances remained present throughout treatment. The short PR interval that is characteristic for Pompe disease and is also found in other storage diseases such as Fabry disease, Danon disease and patients with PRKAG2 mutations, was below normal values in 60% of our patients at study end.[21,22] This is in contrast to a study by Ansong et al., in which 19 Pompe patients showed normalization of PR interval during treatment.[23] The mechanism that causes the short PR interval is not completely understood; an interesting hypothesis was raised by Bharati et al. in 1982, who correlated electrophysiological abnormalities to pathological post mortem findings in the conduction system of untreated patients with classic-infantile Pompe disease. They found marked glycogen infiltration, vacuolization and an increase in cell size of the Purkinje cells and suggested that the short PR interval could reflect enhanced conduction caused by de deposition of glycogen.[24] The electrophysiological finding that the atrium-His interval was shortened and the His-ventricular interval was normal, while enlarged cells filled with glycogen were found in all parts of the conduction system including the bundle of His, challenges this hypothesis. A more applicable theory was reported by Arad et al. They studied a mouse model with a human PRKAG2 mutation, which leads to excessive glycogen accumulation in myocytes. They observed that the annulus fibrosis was disrupted by glycogen-filled myocytes, allowing atrioventricular activation by bypassing the AV node. [25,26] A non- reversible disruption of the annulus fibrosis might explain that the PR interval remains shortened despite treatment, as found in most of the patients presented in this study. It might also explain the WPW pattern that is often found in classic-infantile Pompe disease, including 6 of 14 patients in the present study. Yet, neither of the two hypotheses can completely explain the variability of the PR interval between the patients in our study.

The fact that two of our patients showed recurrent supraventricular tachyarrhythmia (SVT) despite ERT should be noted. Several publications report that arrhythmias, including fatal arrhythmia, may occur in patients with classic-infantile Pompe disease, even though ERT improves cardiac size and function.[23,27-31] While Wang et al described a correlation between arrhythmias and elevated LVMI, Ansong et al did not. In our study both patients with recurrent SVTs were already severely affected at start of treatment. Both became ventilator dependent within their first year of life and are currently tetraplegic. Cardiac hypertrophy reduced, but the heart did not regain a normal size during most of the study period.

In the present study we did not find diastolic dysfunction at baseline, although pseudo-normalization of E/A ratio cannot be ruled out since at the start of the study measurement of mitral annular motion by TDI was not performed regularly. A recently published study by Chen et al, which followed 9 patients with classic-infantile Pompe disease for a maximum period of 3.2 years, found impaired global LV function and synchronicity with a close relationship between LVMI and diastolic dyssynchrony at baseline.[32] Since TDI appears to be more sensitive in detecting abnormalities in systolic and diastolic function than conventional echocardiography, we added this measurement to our follow-up protocol. The 9 patients described by Chen et al. showed normalization of LV function after start of treatment, while in the present study two patients had abnormal diastolic functioning after 4 and 13 years of treatment respectively. The relatively young age at start of treatment in the patient group followed by Chen et al. and the shorter follow-up period of these patients may explain the differences between the study results. To detect early signs of deterioration of cardiac function, TDI measurements may play a pivotal role.

Since the introduction of ERT many advances have been made in the treatment of Pompe disease. The present study shows that cardiac improvements are maintained over 14 years of treatment. In conclusion, LVMI reached normal values in all but one patient. Time to normalization seemed to be related to clinical status at baseline. LVMI may deteriorate during treatment in patients that show clinical decline and become ventilator dependent. On the ECG, PR interval remained shortened in 60%

of the patients whereas 6 of the 14 patients had a delta wave. A delta wave was also found in mildly affected patients, even in one of the best responders to ERT. The possible occurrence of cardiac arrhythmias remain a threat to these patients, particularly in those in whom ERT has been initiated relatively late. Cardiac follow-up, including regular ECGs and Holter assessment, would therefore be advised.

REFERENCES

1. Hirchhorn RR, AJJ. Glycogen storage disease type II: acid alpha-glucosidase (acid maltase) deficiency. *The Metabolic and Molecular Bases of Inherited Disease.* 2001:3389-3420.
2. van den Hout HM, Hop W, van Diggelen OP, et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics.* Aug 2003; 112(2):332-340.
3. van der Ploeg AT, Reuser AJ. Pompe's disease. *Lancet.* Oct 11 2008; 372(9646):1342-1353.
4. Kishnani PS, Hwu WL, Mandel H, et al. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *J Pediatr.* May 2006; 148(5):671-676.
5. Van den Hout JM, Kamphoven JH, Winkel LP, et al. Long-term intravenous treatment of Pompe disease with recombinant human alpha-glucosidase from milk. *Pediatrics.* May 2004; 113(5):e448-457.
6. Van den Hout JM, Reuser AJ, de Klerk JB, Arts WF, Smeitink JA, Van der Ploeg AT. Enzyme therapy for pompe disease with recombinant human alpha-glucosidase from rabbit milk. *J Inherit Metab Dis.* Apr 2001; 24(2):266-274.
7. Kishnani PS, Corzo D, Nicolino M, et al. Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology.* Jan 9 2007; 68(2):99-109.
8. Klinge L, Straub V, Neudorf U, et al. Safety and efficacy of recombinant acid alpha-glucosidase (rhGAA) in patients with classical infantile Pompe disease: results of a phase II clinical trial. *Neuromuscular disorders.* Jan 2005; 15(1):24-31.
9. Prater SN, Banugaria SG, DeArmey SM, et al. The emerging phenotype of long-term survivors with infantile Pompe disease. *Genet Med.* Sep 2012; 14(9):800-810.
10. Lai WW, Geva T, Shirali GS, et al. Guidelines and standards for performance of a pediatric echocardiogram: a report from the Task Force of the Pediatric Council of the American Society of Echocardiography. *Journal of the American Society of Echocardiography.* Dec 2006; 19(12):1413-1430.
11. Kampmann C, Wiethoff CM, Wenzel A, et al. Normal values of M mode echocardiographic measurements of more than 2000 healthy infants and children in central Europe. *Heart.* Jun 2000; 83(6):667-672.
12. Devereux RB. Left ventricular mass in children and adolescents. *J Am Coll Cardiol.* Sep 1988; 12(3):709-711.
13. Poutanen T, Jokinen E. Left ventricular mass in 169 healthy children and young adults assessed by three-dimensional echocardiography. *Pediatr Cardiol.* May-Jun 2007;28(3):201-207.
14. Park MK. Pediatric Cardiology. 2008;5th edition.
15. Rijnbeek PR, Witsenburg M, Schrama E, Hess J, Kors JA. New normal limits for the paediatric electrocardiogram. *European heart journal.* Apr 2001; 22(8):702-711.
16. Amalfitano A, Bengur AR, Morse RP, et al. Recombinant human acid alpha-glucosidase enzyme therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2001; 3(2):132-138.
17. Kishnani PS, Nicolino M, Voit T, et al. Chinese hamster ovary cell-derived recombinant human acid alpha-glucosidase in infantile-onset Pompe disease. *The Journal of pediatrics.* Jul 2006; 149(1):89-97.

18. Levine JC, Kishnani PS, Chen YT, Herlong JR, Li JS. Cardiac Remodeling After Enzyme Replacement Therapy with Acid alpha-Glucosidase for Infants with Pompe Disease. *Pediatr Cardiol.* Jul 26 2008.
19. Nicolino M, Byrne B, Wraith JE, et al. Clinical outcomes after long-term treatment with alglucosidase alfa in infants and children with advanced Pompe disease. *Genetics in medicine.* Mar 2009; 11(3):210-219.
20. van Gelder CM, Hoogeveen-Westerveld M, Kroos MA, Plug I, van der Ploeg AT, Reuser AJ. Enzyme therapy and immune response in relation to CRIM status: the Dutch experience in classic infantile Pompe disease. *Journal of inherited metabolic disease.* Apr 9 2014.
21. Jastrzebski M, Bacior B, Dimitrow PP, Kawecka-Jaszcz K. Electrophysiological study in a patient with Fabry disease and a short PQ interval. *Europace.* Dec 2006; 8(12):1045-1047.
22. Arad M, Maron BJ, Gorham JM, et al. Glycogen storage diseases presenting as hypertrophic cardiomyopathy. *The New England journal of medicine.* Jan 27 2005; 352(4):362-372.
23. Ansong AK, Li JS, Nozik-Grayck E, et al. Electrocardiographic response to enzyme replacement therapy for Pompe disease. *Genetics in medicine.* May 2006; 8(5):297-301.
24. Bharati S, Serratto M, DuBrow I, et al. The conduction system in Pompe's disease. *Pediatr Cardiol.* 1982; 2(1):25-32.
25. Arad M, Moskowitz IP, Patel VV, et al. Transgenic mice overexpressing mutant PRKAG2 define the cause of Wolff-Parkinson-White syndrome in glycogen storage cardiomyopathy. *Circulation.* Jun 10 2003; 107(22):2850-2856.
26. Patel VV, Arad M, Moskowitz IP, et al. Electrophysiologic characterization and postnatal development of ventricular pre-excitation in a mouse model of cardiac hypertrophy and Wolff-Parkinson-White syndrome. *Journal of the American College of Cardiology.* Sep 3 2003; 42(5):942-951.
27. Cook AL, Kishnani PS, Carboni MP, et al. Ambulatory electrocardiogram analysis in infants treated with recombinant human acid alpha-glucosidase enzyme replacement therapy for Pompe disease. *Genetics in medicine.* May 2006; 8(5):313-317.
28. Desena HC, Brumund MR, Superneau D, Snyder CS. Ventricular fibrillation in a patient with Pompe disease: a cautionary tale. *Congenit Heart Dis.* Jul-Aug 2011; 6(4):397-401.
29. McDowell R, Li JS, Benjamin DK, Jr., et al. Arrhythmias in patients receiving enzyme replacement therapy for infantile Pompe disease. *Genet Med.* Oct 2008; 10(10):758-762.
30. Wang LY, Ross AK, Li JS, et al. Cardiac arrhythmias following anesthesia induction in infantile-onset Pompe disease: a case series. *Paediatr Anaesth.* Aug 2007;17(8):738-748.
31. Desena HC, Brumund MR, Superneau D, Snyder CS. Ventricular Fibrillation in a Patient with Pompe Disease: A Cautionary Tale. *Congenit Heart Dis.* Mar 10 2011.
32. Chen CA, Chien YH, Hwu WL, et al. Left ventricular geometry, global function, and dyssynchrony in infants and children with pompe cardiomyopathy undergoing enzyme replacement therapy. *J Card Fail.* Nov 2011; 17(11):930-936.

PART IV

Outcome measures

12

The quick motor function test: a new tool to rate clinical severity and motor function in Pompe patients

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ABSTRACT

Pompe disease is a lysosomal storage disorder characterized by progressive muscle weakness. With the emergence of new treatment options, psychometrically robust outcome measures are needed to monitor patients' clinical status. We constructed a motor function test that is easy and quick to use. The Quick Motor Function Test (QMFT) was constructed on the basis of the clinical expertise of several physicians involved in the care of Pompe patients; the Gross Motor Function Measure and the IPA/Erasmus MC Pompe survey. The test comprises 16 items. Validity and test reliability were determined in a cohort of 91 Pompe patients (5 to 76 years of age). In addition, responsiveness of the scale to changes in clinical condition over time was examined in a subgroup of 18 patients receiving treatment and 23 untreated patients. Interrater and intrarater reliabilities were good (intraclass correlation coefficients: 0.78 to 0.98 and 0.76 to 0.98). The test correlated strongly with proximal muscle strength assessed by hand held dynamometry and manual muscle testing (ρ=0.81, ρ=0.89), and showed significant differences between patient groups with different disease severities. A clinical-empirical exploration to assess responsiveness showed promising results, albeit it should be repeated in a larger group of patients. In conclusion, the Quick Motor Function Test can reliably rate clinical severity and motor function in children and adults with Pompe disease.

INTRODUCTION

Pompe disease (OMIM #232300) is a rare neuromuscular disorder caused by deficiency of the lysosomal enzyme acid α-glucosidase. This deficiency induces glycogen to accumulate in the lysosomes of many tissues, albeit mainly in skeletal muscle. Its major clinical manifestation is progressive muscle weakness, which eventually impairs motor and respiratory function.[1,2] The disease manifests across a spectrum of severity, and affects infants, children and adults.[3-5] Patients with the classic infantile form present with severe generalized hypotonia and a hypertrophic cardiomyopathy shortly after birth; the disease progresses rapidly, and the patients usually die in their first year of life from cardiorespiratory failure. Childhood, juvenile, and adult forms of the disease are characterized by a more slowly progressive proximal myopathy. Respiratory muscles are affected as well. In these patients, onset of symptoms, disease severity and rate of disease progression varies. Cardiomyopathy rarely occurs. The majority of patients eventually become wheelchair and respirator dependent.[6-8]

In our centre we follow more than 100 children and adults with Pompe disease. The disease severity of these patients shows large differences. Some are ambulant and others completely wheelchair dependent. Currently there is no functional scale that has been standardized for Pompe disease and is capable to rate differences in muscle function sufficiently. This has become even more important since marketing approval was given to recombinant human alpha-glucosidase as enzyme replacement therapy for Pompe disease.

The aim of the present study was to construct a functional motor scale specific for Pompe disease that is easy to apply and sufficiently sensitive to assess disease severity and to detect clinically important changes over time, so that it can be used both in clinical practice to monitor disease progression and to evaluate therapeutic effectiveness. For this purpose, we constructed and psychometrically tested the scale in a large cohort of children and adults with Pompe disease.

METHODS

Construction of the test Motor function items that were difficult specifically for patients with Pompe disease were derived from the clinical expertise of several neurologists, paediatricians, and physical therapists involved in the treatment and care of Pompe patients; the Gross Motor Function Measure,[9] an 88-item motor function test that has been validated for Cerebral Palsy; and the IPA/Erasmus MC Pompe survey, an international questionnaire study performed in over 300 Pompe patients.[10,11]

The final version of the test consisted of 16 items and was named the Quick Motor Function Test (Appendix B). Administering this test takes approximately 10 to 15 minutes. An evaluator observes the performance of a patient and scores the items separately on a 5-point ordinal scale (ranging from 0 to 4). If items can be performed on both left and right extremities, the right side is taken. A total score is obtained by adding the scores of all items. The total score ranges between 0 and 64 points.

Psychometric testing

Subjects

A total of 91 child and adult patients with Pompe disease who had attended the Erasmus Medical Center between February 2005 and February 2008 were included in the present study. All patients were diagnosed with Pompe disease through the measurement of acid α-glucosidase activity in cultured fibroblasts or leukocytes, and mutation analysis. They were enrolled in either one of two observational studies. One study investigated the rate of disease progression in untreated patients; the other study monitored the disease course after start of treatment with recombinant human alpha-glucosidase. Both studies were approved by the Institutional Review Board of the Erasmus MC. All patients (and/or parents if necessary) gave signed informed consent.

Design

In both studies, the newly constructed Quick Motor Function Test (QMFT) was part of a standardized follow-up protocol. The following assessments were performed at baseline and every 3 months thereafter: hand-held dynamometry (HHD),[12,13] manual muscle testing (MMT),[14] pulmonary function testing in sitting and supine position,[15] and the QMFT. A physical examination was performed at every visit.

The QMFT was administered by two pediatricians and two neurologists. Beforehand, all physicians were trained by testing at least five patients following standardized instructions, while being observed by one of the senior physicians who originally developed the test. The test was performed in a separate examination room and all assessments were videotaped. The scores were recorded on an QMFT scoring sheet.

Reliability

The internal consistency[16] was measured by Cronbach's α. To estimate intrarater reliability,[17] three evaluators were shown the videotapes of the baseline assessments of 20 of their patients more than one year after the assessments and were asked to rescore the QMFT. The patients were randomly selected and the evaluators were blinded for their initial scoring.

To measure the interrater reliability,[17] videotapes of assessments from 60 randomly selected patients were scored by all four evaluators.

Test-retest reliability[17] was assessed in 24 patients. As we assumed that little change in functional performance had occurred in this period of time, each patient was evaluated at baseline and approximately three months thereafter. The evaluators had no access to their initial scoring.

Validity

Validity is defined as the extent to which an instrument measures the concept it is intended to measure.[17] If no gold standard exists to compare the instrument, criterion validity[16,18] may be assessed. Since Pompe disease predominantly presents as a proximal myopathy, we examined whether the QMFT would correlate with other tests that are used to measure proximal muscle weakness. For this purpose,

the strength of proximal muscle groups as assessed by both manual muscle testing[14] and hand held dynamometry[12,13] were compared with the QMFT score. The following proximal muscle groups were tested: neck flexors, shoulder abductors, elbow flexors, elbow extensors, hip flexors, hip abductors, knee extensors, and knee flexors. To demonstrate that the QMFT score correlated less well with the strength of other muscles, the following muscle groups were tested and compared with the QMFT: neck extensors, wrist extensors, wrist flexors, foot dorsal flexors, and foot plantar flexors. Finally, differential validity was assessed by comparing the QMFT scores of patients with different severities of disease. To this end, the patients were classified into three groups based on their ability to walk: patients who were completely ambulant, patients who were able to walk with aids, and patients who were completely wheelchair bound.

Responsiveness

To assess the responsiveness of the QMFT, a clinical-empirical exploration was performed. This analysis included a sub-sample of 18 patients who had been treated with recombinant human α-glucosidase for more than one year, and also a sub-sample of 23 untreated patients who were followed for more than one year. As no gold standard exists, several strategies for assessing responsiveness have been suggested.[19-21] We therefore used three different methods to explore the responsiveness of the QMFT.

First, we compared the change in score at 12 months follow-up between the untreated and the treated group. Second, we calculated sensitivity to change using the standardized response mean,[21,22] an effect size statistic that is equal to the mean of the change in score of treated patients divided by the standard deviation of the change score. Third, two physicians were asked to judge whether the motor function of individual patients in the treated group had to their opinion improved, remained stable, or deteriorated after 12 months of treatment.[20,23] These physicians were involved in the care of either children or adults with Pompe disease but had not participated in the construction and administration of the QMFT.

Statistical analysis

All continuous variables are described as mean ± standard deviation, or as median and range; categorical data are presented as percentages. Internal consistency of the test was measured using Cronbach's α. A Cronbach's α>0.80 was considered good. Intrarater reliability, interrater reliability, and test-retest reliability of the test and the separate items were determined by calculating intraclass correlation coefficient (ICC) with a random effect ANOVA model. An ICC value of less than 0.40 was considered poor; an ICC value between 0.40 and 0.80 was considered fair and an ICC value greater than 0.80 was considered excellent. Pearson correlation coefficients were used to determine criterion validity; Spearman rank correlation coefficients were used in case of non-normal distributions. For differential validity, group differences were analyzed by one-way analysis of variance followed by a Bonferroni post hoc correction for multiple testing. P-values <0.05 (two-tailed) were considered statistically significant.

Responsiveness was investigated by calculating the standardized response mean. A score between 0.50 and 0.80 was considered moderate, and a score greater than 0.80 represents high responsiveness.[22] A Mann-Whitney test was used to test differences in QMFT scores between the treated patients and the untreated patients. A ROC curve was made by plotting the true-positive rate (sensitivity) against the false-positive rate (1-specificity). The area under the curve represents the ability of the test to correctly discriminate between improved and non-improved patients. This area ranges from 0.5 (no discriminating ability) to 1.0 (perfect discriminating ability).

Statistical analysis was performed using SPSS version 15.0.

RESULTS

Study population

Clinical characteristics of the 91 patients enrolled in the study are shown in Table 1. Nineteen patients were younger than 18 years. The mean QMFT score at baseline

assessment was 36.9±16.6 (median 38.5, range 3 to 64), on a possible score range from 0 to 64.

Table 1. Clinical characteristics of the 91 study patients

Sex (m/f)	52/39
Age (median)	46.3 years (range 5–76 years)
Age groups	Number of patients
5–20 years	19
21–40 years	17
41–60 years	36
61–80 years	19
Motor status	% of patients
Wheelchair bound	23
Using assistive devices	18
Fully ambulant	59
Respiratory status	% of patients
Ventilation use	29.7
No ventilation	70.3

Reliability

The internal consistency of the QMFT was excellent: Cronbach's α was 0.94. There were no substantial floor and ceiling effects: none of the patients reached the lowest possible score, and only two of the patients reached the highest possible score of 64. Both patients were diagnosed presymptomatically.

The intraclass correlation coefficient for *intrarater* reliability was 0.95 for the total scale. The ICCs for the separate items of the scale ranged from 0.78 to 0.98. The intraclass correlation coefficient for *interrater* reliability was 0.91 for the total test. The ICCs for the separate items of the scale ranged from 0.76 to 0.98.

The intraclass correlation coefficient for *test-retest* reliability was 0.98 for the total test. The ICCs for the separate items of the scale ranged from 0.84 to 1.00.

Validity

The total QMFT score correlated strongly with the strength sum scores of proximal muscle groups assessed by MMT and HHD (ρ (MMT)=0.89 (p<0.001), ρ (HHD)=0.81, (p<0.01)). In sharp contrast, much lower correlations were found between the QMFT and strength sum scores of other muscle groups (ρ (MMT)=0.05 (p=0.33), ρ (HHD)=0.33 (p<0.01)) (Figure 1).

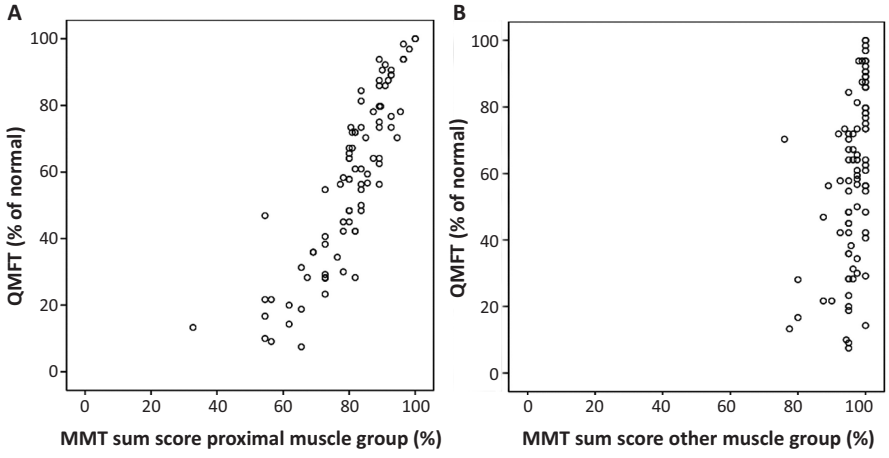


Figure 1. Relationship of the QMFT score to manual muscle testing: (**A**) sumscore of proximal muscle groups; (**B**) sumscore of other muscle groups.

Differential validity was supported by significant differences between the three groups with different severities of disease (F (2,84)=66.29, p<0.001). Mean QMFT scores significantly decreased with the ability to walk: scores were highest in the group that was fully ambulant (47.1±10.7, p<0.01), followed by those for patients who were able to walk with aids (32.4± 11.0, p<0.01), and those for patients who were completely wheelchair bound (16.6±10.6, p<0.01) (Figure 2).

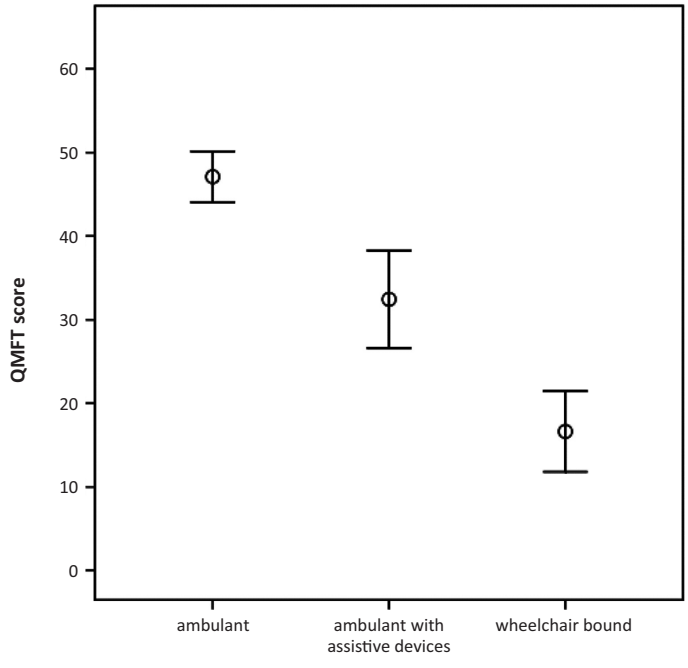


Figure 2. Mean scores (95% CI) on the Quick Motor Function Test of the patients related to three grades of disease severity.

Responsiveness

Responsiveness was tested in 41 patients (18 treated; 23 untreated). Median age of the 18 patients that received recombinant human alpha-glucosidase was 51.5 years (range 5 to 76 years). Eight of these patients were wheelchair bound, and ten patients were dependent on ventilation. Median age of the 23 untreated patients was 52.1 years (range 34 to 72 years). Two patients were wheelchair bound, and three used ventilation.

The QMFT scores of the treated patients (median change 4.15) showed a significant difference over one year period compared to the QMFT scores of the untreated patients (median change 0), ($p<0.01$). The standardized response mean was high (0.81). Figure 3 shows the ROC curve of the Quick Motor Function Test with an area under the curve of 0.88 ($p<0.05$).

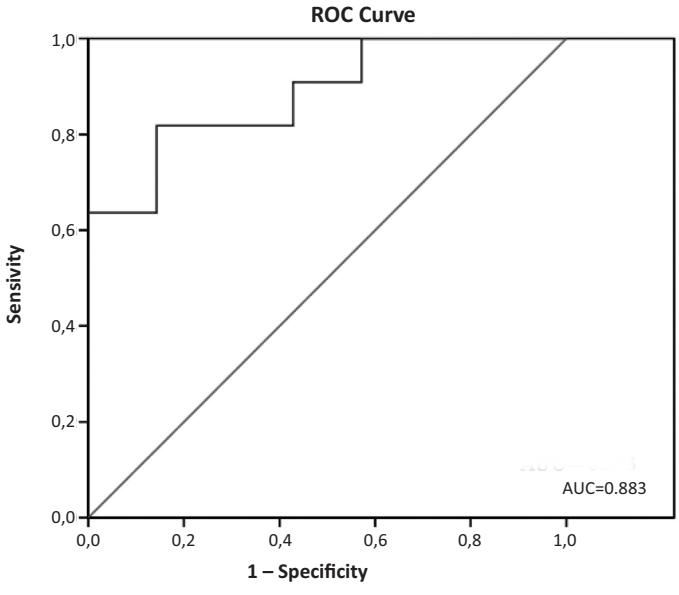


Figure 3. Receiver operating characteristic (ROC) curve to determine the responsiveness of the Quick Motor Function Test (QMFT), with clinical judgment as the external factor. Sensitivity was defined by dividing the number of patients, who had been identified by the QMFT to have changed, by the number of patients who had truly undergone change as based on the judgment of two physicians. Specificity was defined as the number of patients who had been identified by the QMFT to not have changed, divided by the number of patients who had not changed, based on the judgment of the same physicians.

DISCUSSION

This study shows that the Quick Motor Function Test is a reliable and valid test for assessing motor function in patients with Pompe disease. It is the first muscle function test designed and validated specifically for Pompe patients. The test had good psychometric properties, including good internal consistency, and good intrarater and interrater reliabilities over the entire test and the separate items. The QMFT score strongly correlated with proximal muscle strength as measured

by HHD and MMT, and significantly differentiated between patients with different levels of mobility. The test was evaluated in patients between 5 and 76 years of age, and was easy and quick to administer.

According to the World Health Organization, assessment of health should have a multi-dimensional approach. The International Classification of Functioning, Disability and Health (ICF)[24] provides such an interdisciplinary framework and measures consequences of disease in three domains: impairments of body functions and body structures, activity limitations (individual level), and participation restrictions (societal level).

In Pompe disease, the approach towards evaluating disease severity and effect of treatment has become increasingly multi-dimensional over the past years. Measurement tools have been designed and validated for their use in Pompe patients. Currently, a battery of tests is used in the long-term follow-up of Pompe patients. For example, muscle strength, pulmonary function tests, echocardiography, timed tests and the 6-minute walk test are used to evaluate disease consequences and effect of treatment on the level of body functions and body structures. The Pompe Pediatric Evaluation of Disability Inventory (Pompe PEDI), SF-36, and the Rotterdam Handicap Scale are used to assess the level of participation restrictions and quality of life. However, a validated tool to measure activity limitations on an individual level is currently lacking.

In clinical practice, muscle *strength* tests have often been applied to assess muscle *function*. However, although closely related, muscle strength and muscle function represent two different entities of the muscle system, and correspond to different levels of the ICF.[24] Both parameters should therefore be evaluated separately by valid and reliable assessment tools.

In a recent placebo-controlled clinical trial in 90 juvenile and adult patients with Pompe disease, primary outcome measures were the 6 minute walk test (distance walked in 6 minutes), and Forced Vital Capacity in seated position.[25] Muscle function was not assessed, because a reliable motor function test validated for Pompe disease did not exist. Although it would have been possible to use scales that were designed for other neuromuscular disorders[9,26-29] or a composite disease severity score that covers various domains of the ICF, as developed by Lue et al. for Duchenne Muscular Dystrophy,[30] none of these scales were validated for Pompe

disease. Some were designed for children, while Pompe disease affects all age groups. Our study demonstrates that the QMFT can be used in both children and adults, with different levels of disease severity.

Another quality of the QMFT is that it is easy and quick to perform. The test takes approximately 15 minutes, does not require specialized equipment, and can be performed by a physician in a clinical setting. As opposed to other scales, that frequently need to be performed by a physical therapist. It is a practical tool that can be used in all patients including those who are confined to a wheelchair or dependent on artificial ventilation. The overall responsiveness of the QMFT appears to be good: the test accurately detected change when it had occurred and remained stable when no change had occurred. It also discriminated between varying levels of disease. This indicates that the QMFT can serve as a tool to estimate disease severity, but also as a longitudinal assessment tool to detect changes in motor function over time. This is useful, as the emergence of new treatment modalities such as enzyme replacement therapy and possibly chaperone therapy will make the (long-term) evaluation of therapeutic effects essential.

Four issues need further attention. First, while responsiveness to change, which was assessed in a subgroup of 18 treated and 23 untreated patients, showed promising results, it is recommended to perform a large scale empirical study. The current study was insufficient to demonstrate whether the changes observed over time were related to enzyme replacement therapy or not. Second, the test was validated for patients between 5 and 76 years of age. In the youngest and oldest patients, motor development and age-related motor limitation might have interfered with the test results. Therefore, reference values for age should be obtained. Third, to ensure tester reliability we recommend annual recertification of the physicians who perform the QMFT. Fourth, the present study validated the QMFT in Pompe patients, but the test may also be useful for other neuromuscular disorders, especially those with proximal muscle weakness.

In conclusion, this study shows that the Quick Motor Function Test has good psychometric properties and excellent clinical utility. Our findings indicate that this test can be used to assess motor function and response to treatment in children and adults with different levels of disease severity. The applicability of the test for other neuromuscular disorders deserves further investigation.

REFERENCES

1. Engel A and Hirschhorn R. Acid maltase deficiency. In: Engel A and Franzini-Armstrong C eds. *Myology*, 2nd ed. New York: McGraw-Hill, 1994; 1533-1553.
2. van der Ploeg AT and Reuser AJ. Pompe's disease. *Lancet* 2008; 372:1342-1353.
3. Kishnani PS, Hwu WL, Mandel H et al. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *J Pediatr* 2006; 148:671-676.
4. van den Hout HM, Hop W, van Diggelen OP et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics* 2003; 112:332-340.
5. Winkel LP, Hagemans ML, van Doorn PA et al. The natural course of non-classic Pompe's disease; a review of 225 published cases. *J Neurol* 2005; 252:875-884.
6. Hagemans ML, Hop WJ, van Doorn PA, Reuser AJ, and van der Ploeg AT. Course of disability and respiratory function in untreated late-onset Pompe disease. *Neurology* 2006; 66:581-583.
7. Laforêt P, Nicolino M, Eymard PB et al. Juvenile and adult-onset acid maltase deficiency in France: genotype-phenotype correlation. *Neurology* 2000; 55:1122-1128.
8. Wokke JH, Ausems MG, van den Boogaard MJ et al. Genotype-phenotype correlation in adult-onset acid maltase deficiency. *Ann Neurol* 1995; 38:450-454.
9. Russell DJ, Rosenbaum PL, Cadman DT et al. The gross motor function measure: a means to evaluate the effects of physical therapy. *Dev Med Child Neurol* 1989; 31:341-352.
10. Hagemans ML, Winkel LP, van Doorn PA et al. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. *Brain* 2005; 128:671-677.
11. Hagemans ML, Laforêt P, Hop WJ et al. Impact of late-onset Pompe disease on participation in daily life activities: evaluation of the Rotterdam Handicap Scale. *Neuromuscul Disord* 2007; 17:537-543.
12. van der Ploeg RJ. *Hand-held dynamometry. Thesis. Rijksuniversiteit Groningen*. Groningen: 1992.
13. Beenakker EA, van der Hoeven JH, Fock JM, and Maurits NM. Reference values of maximum isometric muscle force obtained in 270 children aged 4–16 years by hand-held dynamometry. *Neuromuscul Disord* 2001; 11:441-446.
14. Brooke MH, Griggs RC, Mendell JR et al. Clinical trial in Duchenne dystrophy. I. The design of the protocol. *Muscle Nerve* 1981; 4:186-197.
15. American Thoracic Society / European Respiratory Society. ATS/ERS Statement on respiratory muscle testing. *Am J Respir Crit Care Med* 2002; 166:518-624.
16. Nunnally JC. *Psychometric theory.* New York: McGraw-Hill, 1978.
17. Hobart JC, Lamping DL, and Thompson AJ. Evaluating neurological outcome measures: the bare essentials. *J Neurol Neurosurg Psychiatry* 1996; 60:127-130.
18. Cronbach LJ and Meehl PE. Construct validity in psychological tests. *Psychol Bull* 1955; 52:281-302.
19. Husted JA, Cook RJ, Farewell VT, and Gladman DD. Methods for assessing responsiveness: a critical review and recommendations. *J Clin Epidemiol* 2000; 53:459-468.
20. Liang MH. Evaluating measurement responsiveness. *J Rheumatol* 1995; 22:1191-1192.

21. Wright JG and Young NL. A comparison of different indices of responsiveness. *J Clin Epidemiol* 1997; 50:239-246.
22. Liang MH, Fossel AH, and Larson MG. Comparisons of five health status instruments for orthopedic evaluation. *Med Care.* 1990; 28:632-642.
23. Deyo RA and Centor RM. Assessing the responsiveness of functional scales to clinical change: an analogy to diagnostic test performance. *J Chronic Dis* 1986; 39:897-906.
24. World Health Organization. *International classification of functioning, disability and health*. Geneva: WHO, 2001.
25. van der Ploeg AT, Clemens PR, Corzo D et al. A randomized study of alglucosidase alfa in late-onset Pompe's disease. *N Engl J Med.* 2010; 362:1396-1406.
26. Bérard C, Payan C, Hodgkinson I, Fermanian J, and MFM Collaborative Study Group. A motor function measure for neuromuscular diseases. Construction and validation study. *Neuromuscul Disord* 2005; 15:463-470.
27. Lovell DJ, Lindsley CB, Rennebohm RM et al. Development of validated disease activity and damage indices for the juvenile idiopathic inflammatory myopathies. II. The Childhood Myositis Assessment Scale (CMAS): a quantitative tool for the evaluation of muscle function. The Juvenile Dermatomyositis Disease Activity Collaborative Study Group. *Arthritis Rheum* 1999; 42:2213-2219.
28. Main M, Kairon H, Mercuri E, and Muntoni F. The Hammersmith functional motor scale for children with spinal muscular atrophy: a scale to test ability and monitor progress in children with limited ambulation. *Eur J Paediatr Neurol* 2003; 7:155-159.
29. Scott OM, Hyde SA, Goddard C, and Dubowitz V. Quantitation of muscle function in children: a prospective study in Duchenne muscular dystrophy. *Muscle Nerve* 1982; 5:291-301.
30. Lue YJ, Su CY, Yang RC et al. Development and validation of a muscular dystrophy-specific functional scale. *Clin Rehabil* 2006; 20:804-817.

13

General discussion

Shortly after J.C. Pompe's thesis *Cardiomegalia glycogenica* was published in 1936,[1] G.O.E. Lignac, a professor of Pathology, wrote a critique in the Nederlands Tijdschrift voor Geneeskunde wherein he stated that Dr. Pompe clearly defined in his thesis what was known till then about this disease, but that he was even more specific on what was still unknown [2]. At that time both Lignac and Pompe could not have foreseen that more than 1500 publications would follow about the ins and outs of 'Pompe disease' over the next 80 years or so. Even at present, old questions remain and new have arisen since the introduction of enzyme replacement therapy. This thesis tries to answer some of these questions as it 1) describes the clinical spectrum of children with non-classic forms of Pompe disease and compares these children with adult Pompe patients; 2) evaluates the effect of enzyme-replacement therapy in these children; 3) investigates the long-term outcome of patients with classic-infantile Pompe disease treated with ERT and compares the response of children with classic and non-classic forms of Pompe disease; 4) introduces a new measurement scale to better assess the motor function of patients with Pompe disease.

This chapter evaluates the main findings and discusses their significance in the context of previously published studies. Recommendations for clinical practice as well as suggestions for future research are presented in the format of final conclusions.

MAIN FINDINGS

1. Children with non-classic forms of Pompe disease

Presentation and clinical features

Pompe disease is regarded as a broad spectrum of clinical phenotypes, with a wide range in age of onset and clinical progression.[3,4] Distinct clinical subtypes are not clearly defined with the exception of classic-infantile Pompe disease.[5] Disease severity appears not to be related to the actual age of the patient, but to the disease duration counting from the onset of symptoms. Though this holds in general, a subgroup of patients diagnosed under the age of 15 years has a rapid

disease progression and becomes wheelchair bound and/or respiratory assisted before adulthood.[6]

Our cross-sectional study including 31 affected children ranging in age from 0.1 till 17.3 years, all with non-classic forms of Pompe disease, (**Chapter 2**) confirms these findings. The clinical severity varied profoundly in this patient group: five of the thirty-one patients were symptom-free, seven patients had become wheelchair dependent during childhood, six patients had started using a ventilator and two patients had died of respiratory failure at the age of 6 and 10 years. In over 50% of children difficulties were observed with standing up from supine position and flexing the neck in supine position, and 47%–50% had an abnormally low Forced Vital Capacity (FVC) in sitting position *versus* 59% of the patients in supine position (**Chapter 2** and **3**). The c.-32-13T>G/'null' genotype was the most prevalent genotype amongst these 31 children, whereby 'null' stands for any *GAA* mutation leading to total loss of acid α-glucosidase activity. It is the most common pathogenic *GAA* genotype in the Netherlands (95% of all adult patients and 68% of all children with less progressive forms of Pompe disease). Some children with the c.-32-13T>G/null genotype had a rapid deterioration of muscle and respiratory function while others presented a very slowly progressive disease, which adds to the previous findings that this genotype is associated with a broad spectrum of phenotypes [7,8]. The clinical variation among children with other *GAA* genotypes was also substantial. Overall, children with these other genotypes were more severely affected than those with the common c.-32-13T>G/null genotype.

These results underline that Pompe disease is a true clinical spectrum and emphasize that the disease may already cause a significant burden of disease in childhood. In our group of 31 patients, 10 children stood out because of their rapid disease progression, similarly as a group of patients previously described by Hagemans et al.[6] Two of our 10 children had a cardiomyopathy and actually conform to the definition of 'atypical infantile variant of Pompe disease' as introduced by Slonim et al.[9] In these atypical patients a similar age at onset of symptoms was found as in classic-infantile Pompe disease, but the survival was well beyond the first year of life, and the degree of hypertrophic cardiomyopathy was less severe.

To our surprise, we found the majority (89%) of affected children with the c.-32-13T>G/null genotype to be male (**Chapter 2**), as if the gender would somehow contribute to the phenotype. In addition, pulmonary function appeared to be more affected in males than in females (**Chapter 3**). In another study, Van der Beek et al found that more men than women had bulbar muscle involvement and that the shoulder-girdle muscles were more severely affected in males than in females.[10] A study comparing the phenotypes of siblings with Pompe disease also revealed that males are more severely affected than females.[11] Such a gender difference had not been previously described. Since Pompe disease inherits as an autosomal recessive trait, there is no immediate explanation why males would present symptoms earlier than females or would be more severely affected than females other than that gender related factors play a role in the clinical expression of Pompe disease.

Effects of ERT

Since the first three patients with non-classic forms of Pompe disease, then 11, 16 and 32 years old, started to be treated with recombinant human α-glucosidase from rabbit milk in 1999,[12] more than 20 clinical studies have been published describing the effects of ERT in these types of patients.[13] Three of these studies are incorporated in this thesis (**Chapter 5**, **6**, **7**).

The most extensive study and the only placebo-controlled trial (LOTS) ever performed in Pompe disease is described in **Chapter 7**. The average age of the patients in this study was 44.4 years. All but three patients in this study were adults, while the youngest patient was 10 years old when she entered into the trial. This study named LOTS showed for the first time that ERT had a significant positive effect on distance walked in six minutes time and on pulmonary function as measured by FVC. A recently published follow-up study reported that positive effects were maintained over a total period of 104 weeks,[14] whereas the disease is relentlessly progressive if left untreated. For example, the natural history of Pompe disease in adults teaches that approximately 50% of untreated patients become wheelchair bound or ventilator dependent within 10–15 years after diagnosis.[6] Furthermore, a recent observational study in 268 adults with Pompe disease has shown that untreated patients have a higher mortality rate than the general population.[15] For

these reasons, stabilization of disease progression should be considered a positive outcome of treatment.

The effect of ERT in children with non-classic forms of Pompe disease is documented less well since only ten of the 98 patients that participated in the three studies included in this thesis were children and other reports on the effects of ERT in children are scarce. Nevertheless, there are some indications that younger patients benefit the same or more from ERT than adults. Six of the children described in **Chapters 5** and **6** showed a continuous improvement of muscle strength over 3 to 8 years, while several studies, including the LOTS study, suggest that some adult patients may reach a plateau after a few years of treatment.[14,16,17] A three-year treatment study in 24 Italian patients supports this notion.[16] The positive effect of ERT in children compared to adults with non-classic forms of Pompe disease may reflect a difference in muscle growth or a higher regenerative capacity of the damaged muscle in children compared to adults.[18,19]

It is generally recognized that not all patients respond equally well to ERT. A recent systematic literature review including all published studies on enzyme replacement therapy in non-classic forms of Pompe disease showed that one third of patients do not show improvements of muscle strength or pulmonary function during treatment.[13] Similarly, de Vries et al found a poor response to ERT in 9% of the 69 adult patients with regard to muscle strength, in 15% of the patients with regard to FVC in sitting position and in 39% of the patients with regard to FVC in supine position.[20] At present, it is not clear what causes this variation in response; phenotypic, genetic, epi-genetic, and age-related factors have been suggested. Gender related factors may also influence treatment potential: van der Beek et al. from our group, found a better response to treatment with respect to muscle strength in female patients than in males.[20] All these aspects should be taken into account when considering the best timing to start treatment. The invasive character and the high costs of the treatment frequently withholds clinicians from starting enzyme therapy in a pre-symptomatic state of the disease.[21] However, the findings that even asymptomatic patients have some degree of trunk muscle atrophy on MRI[22] and a considerable number of myocytes with irreversible histopathologic changes,[23] make it clear that additional and longer follow-up studies are necessary

to better define starting criteria for enzyme replacement therapy and prognostic factors to identify patients at risk for a poor outcome.

2. Patients with classic-infantile Pompe disease

Many studies have demonstrated that enzyme replacement therapy evidently corrects the cardiomegaly and increases survival in classic-infantile Pompe disease. The effects of ERT on muscle strength and pulmonary function are more variable. Now that treated infants live longer, new aspects of the disease emerge and require attention. The following paragraph discusses to what extent patients with classic-infantile Pompe disease treated with ERT mimic untreated children with non-classic forms of Pompe disease.

The heart

Patients with classic-infantile Pompe disease present with a marked cardiomegaly. Echocardiography shows an increased thickness of both ventricles and the inter-ventricular septum. The hypertrophy is already present at birth, increases steadily after birth commonly leading to left ventricular outflow obstruction, cardiac failure and eventually death. Histological examination reveals prominent glycogen deposits in the cardiac muscle and a characteristic loss of myofibrils within cardiomyocytes. In contrast to classic-infantile Pompe disease, the heart of children and adults with non-classic forms of Pompe disease is rarely affected. The vast majority of these patients do not develop a cardiomyopathy. A shortened PR interval and Wolff-Parkinson-White pattern are commonly reported (up to 10%) in this group of patients.[24-26] The study described in **Chapter 4** corroborates these findings and shows that cardiac involvement is rare in children and adults with only 2/68 patients showing conduction abnormalities/anomalies (WPW pattern) or ventricular hypertrophy possibly related to Pompe disease, but very young and severely affected children were not included in that study.

Chapter 2 is more informative about the cardiac manifestations in children as it describes the results of a cross-sectional study in 31 untreated children with non-classic forms of Pompe disease. Two of the 10 patients with severe *GAA* genotypes survived significantly longer than patients with classic-infantile Pompe disease

but presented with hypertrophic cardiomyopathy, and both these patients as well as a third patient had a delta-wave suggestive for WPW syndrome. According to the terminology of Slonim et al., the two patients with the hypertrophic cardiomyopathy would fit the description of 'atypical infantile Pompe patient'.[6,9] The finding of intermediate phenotypes between classic-infantile Pompe disease and non-classic childhood, juvenile and adult- cases highlights the continuity of the clinical spectrum.[4] The presence or absence of cardiac involvement seems to be determined by the level of residual α-glucosidase activity[4] (**Chapter 4**).

The preferential involvement of skeletal muscle over cardiac muscle in Pompe disease may be due to organ specific differences in metabolism. In healthy human beings, cytoplasmic pools of glycogen are far more pronounced in liver and skeletal muscle than in cardiomyocytes. The liver uses large amounts of glycogen to maintain the caloric need of the whole body, while skeletal muscles need glycogen for quick action. In contrast, the heart mainly uses fatty acids (60–90%) as substrate for oxidation and only a small amount of ATP is derived from carbohydrate metabolism (10–40%). In fact, high levels of fatty acids inhibit glycolysis and glucose oxidation.[27] In a rat model it was found that only a small part of the total glucose uptake by the heart is converted to glycogen,[28,29] leading to small glycogen pools in cardiac muscle (appr. 30 mmol/g wet weight) in contrast to skeletal muscle (appr. 150 mmol/g wet weight).

It is not until 7–10 days after birth that fatty acids become the main energy source of the heart. The fetal heart derives 60% of energy supply by lactate oxidation and the environment of the fetal myocardium is highly enriched in lactate compared to the newborn and adult milieu.[30] The other 40% of energy is supplied by glucose oxidation. Although the circulating glucose levels in newborns and adults are almost equal, the rate of glucose influx into the fetal cardiomyocyte is substantially different from the rate of influx after birth. GLUT-1, the fetal isoform of the glucose transporter, is not dependent on insulin, but is mainly regulated by plasma glucose levels.[30] This might explain the large amount of glucose present in the cardiomyocytes.[31] The predominant use of glucose and lactate in the cardiomyocyte during fetal life and the high content of glycogen in cardiomyocytes[27,32] might lead to the marked cardiomegaly with pronounced glycogen accumulation present in classic-infantile patients at birth.

It has been described that in response to cardiac hypertrophy, cardiac metabolism is reprogrammed to a fetal-like metabolic profile. This profile is characterized by an increased use of glucose metabolism and decreased use of fatty acid oxidation as energy source in the heart.[33] We hypothesize that the above described reprogramming and the suggestion that the fetal-like metabolic profile is maladaptive for sustaining myocardial energetics and function in cardiac hypertrophy, might both explain the quick progression of cardiac hypertrophy towards heart failure in untreated classic-infantile Pompe patients.[34-37]

The first clinical trials performed in classic-infantile Pompe disease showed that the cardiac hypertrophy responds very well to enzyme replacement therapy as expressed by reduction of the left ventricular mass and an improvement of cardiac function.[38-41] Although respiratory function and motor development showed a more variable response to ERT, patients with classic-infantile Pompe disease nowadays survive far beyond their first year of life. Hence, it is important to determine whether the earlier described therapeutic effects on cardiac mass and function are sustained over a longer period of time.

The study presented in **chapter 11** describes the effects of enzyme replacement therapy on cardiac size, cardiac function and conduction pattern in 14 classic-infantile patients. Eight of these 14 patients received treatment for more than 4 years to a maximum period of 13.9 years. The results confirm that ERT significantly reduces the left ventricular mass and improves cardiac function, which is in line with a recently published study showing that effects of ERT are sustained over a longer period of time.[42] Nevertheless, not all of our patients showed normalization of LVMI as was described by Prater et al who followed 11 children up to 12 years. Two patients reached normal values after 13 years of treatment, and an increase of LVMI during treatment was seen in two other patients. However, it is important to note that all these patients were either in a severe clinical condition with paresis of the arms and paralysis of the legs at the time they started to receive enzyme replacement therapy, were in need of ventilator support, or deteriorated during treatment and became ventilator dependent.

ERT seems to be less effective in preventing rhythm disturbances. The short PR interval that is characteristic for Pompe disease and is also found in other

storage diseases such as Fabry disease, Danon disease and patients with PRKAG2 mutations,[43] remained below normal values in most of our patients until study end. This is in contrast to a study by Ansong et al., in which all patients showed a progressive increase of PR interval during treatment.[44] The mechanism that causes the short PR interval is not completely understood; one study that correlated electrophysiological abnormalities to pathologic post mortem findings in the conduction system of patients with classic- infantile Pompe disease found marked glycogen infiltration, vacuolization and an increase in cell size in the Purkinje cells. They suggested that the short PR interval could reflect enhanced conduction caused by the deposition of glycogen.[45] The electrophysiological finding that the atrium-His interval was shortened and the His-ventricular interval was normal, while enlarged cells filled with glycogen were found in all parts of the conduction system including the bundle of His, challenges this hypothesis. A result that fits the above described electrophysiological findings was reported by Arad et al. They studied a mouse model with a human PRKAG2 mutation, which leads to excessive glycogen accumulation in myocytes. They observed that the annulus fibrosis was disrupted by glycogen-filled myocytes, as a consequence allowing atrioventricular activation by bypassing the AV node.[46,47] A non-reversible disruption of the annulus fibrosis would be in line with the PR interval that remained below normal values despite treatment as found in most of the patients presented in **chapter 11**. It may also explain the Wolff-Parkinson-White pattern that is often found in patients with classic-infantile Pompe disease. Nevertheless, since some of our patients and all patients in the study published by Ansong et al showed an increase of their PR interval during treatment, the hypothesis that reduction of glycogen within the conduction system may result in normalization of conduction speed must also be considered.

The fact that two of our patients showed recurrent supraventricular tachyarrhythmia (SVT) despite ERT should be noted. Several publications report that arrhythmias, including fatal arrhythmia, may occur in patients with classic-infantile Pompe disease, even though ERT improves cardiac size and function.[44,48-51] While Wang et al described a correlation between arrhythmias and LVMI, Ansong et al. did not. In our study, both patients with recurrent SVTs were already severely

affected before start of treatment. Both became ventilator dependent within their first year of life and are currently tetraplegic. The cardiac hypertrophy reduced, but the heart did not regain a normal size during most of the study period.

Hearing, cognitive functioning and facial muscle weakness

Now that patients with classic-infantile Pompe disease are treated with enzyme replacement therapy, they survive longer and new aspects of the disease become apparent. One of these clinical findings is hearing deficits. Presence of hearing loss was first described in 2004 in four patients included in the initial pilot study investigating the effects of ERT.[52] Kamphoven et al. found that hearing loss was mainly of cochlear nature and tried to get insight into the pathogenic process by studying a knockout mouse model of Pompe disease. They found glycogen storage in the cells forming the organ of Corti: the inner and outer hair cells of the cochlea, the supporting cells, and the stria vascularis. In addition, glycogen storage was found in the spiral ganglion cells, a group of nerve cells that sends impulses from the organ of Corti to the brain. In our study presented in **Chapter 10** we revisited the issue by including 11 patients with classic-infantile Pompe disease and compared the results with those obtained in 13 children diagnosed with non-classic forms of Pompe disease. We found hearing deficits in 10 of the 11 classic-infantile patients. In addition to the earlier described cochlear dysfunction, conductive hearing loss was present in 75% of all hearing tests performed. These findings were confirmed by other studies.[38,39,53] Hearing deficits are thus a major concern for these patients. They are already present at birth and seem to remain rather stable over time. Early implementation of hearing devices and speech therapy are important to protect speech and language development.

Hearing deficits were only sporadically identified (n=1, conductive hearing loss) in children with non-classic forms of Pompe disease (**Chapter 10**). A study performed in 58 adults led to the same conclusion i.e. that standard screening for hearing loss is not indicated in children and adults with non-classic forms of Pompe disease.[54] The latter study found that 21% of these patients had a clinically relevant hearing loss according to the standards of the World Health Organization, which is in agreement with the prevalence of hearing loss observed in the general population.

Two recently published studies concluded differently. A German study performed in 11 adult patients reported mild hearing loss in 36% of the patients, which slightly exceeds the normative data of the general population.[55] An Italian study performed in 20 adult Pompe patients found a substantial number of patients (53%) to have mild hearing loss.[56] It should be noted that a third of these cases were of conductive nature indicating middle-ear pathology rather than cochlear dysfunction. Combining the results of these three studies 29 out of a total of 89 patients had sensorineural hearing defects (33%). Since this percentage slightly exceeds the overall prevalence of hearing impairment above 25 dB hearing threshold level described in the general population (27.2% Norway, 26.1% Great Britain, 22.2% South Australia),[57-59] the hearing deficits in these Pompe patients might not be entirely attributed to physiological aging of the auditory system. As the underlying metabolic defect in adults and infants with Pompe disease is the same, cochlear pathology may also be present in non-classic forms of Pompe disease. Since less than half of the patients in which we measured hearing loss actually reported hearing problems, we suggest performing audiological assessments on a regular basis in all patients with Pompe disease.

Because widespread glycogen storage was found in the brains of untreated classic-infantile Pompe patients and the therapeutic enzyme cannot pass the blood-brain barrier,[60] we were interested in the neurological development of these patients. In order to monitor neuronal function, we performed repetitive auditory brainstem evoked responses in 11 treated patients (**Chapter 10**). We found prolongation of inter-peak latencies in five patients. The most frequent finding was prolongation between peaks III and V. This is suggestive for pathology in the central part of the auditory pathway. The clinical meaning of these abnormalities were recently explored by studying regular psychological assessments and brain imaging in the same group of patients as described in **Chapter 10**.[61] The data on early development were in concordance with other reported cases of classic-infantile Pompe disease and showed a wide range of scores.[39,53,62,63] However, developmental tests used in young children are influenced by motor skills. Hence, residual muscle weakness in this group of patients may have affected the test results and should therefore be interpreted with caution.[61,62] Cognitive development at school age was reliably

assessed and appeared to range from normal to mildly delayed. Abnormalities in processing speed were found in some children, which may be explained by the mild white matter changes detected on brain imaging. Another study in 7 classic-infantile patients, followed for a median period of 7 years, found IQ scores within the lower range of normal with no evidence of a cognitive decline over time.[64] In the latter study they also found a relative weakness in processing speed, but brain imaging was not performed in this group of patients.

White matter abnormalities were also reported in two other studies.[53,65] In addition, one of these studies found brain myelination delays, especially in a patient that did not respond favorably to ERT.[65] Few autopsy reports have been published, none of which described myelination defects in classic-infantile Pompe disease. Since these were all untreated patients, they may have lived too short to show this defect.

The main findings of the few autopsy studies that have been published to date were severe glycogen storage in the anterior horn cells of the spinal cord, the neurons in the brain stem, the thalamus, the cerebellum, and to a lesser extent the cerebral cortex.[66-71] This pathologic storage may explain some of the clinical features that were recently described in long-term survivors of classic-infantile Pompe disease. For example, glycogen storage in motor neurons of the spinal cord may be a component contributing to residual muscle weakness;[72] glycogen storage in neurons driving the respiratory function may contribute to respiratory dysfunction;[73] storage in the hypothalamic para-ventricular nuclei may have added to the etiology of an intractable fever that was described in three Pompe patients before they died (personal communication and[66]); and the severe storage of glycogen in the motor nuclei of the brain stem, found by Martin et al., may lead to bulbar palsy with dysphagia (**Chapter 9**); involvement of the third cranial nerve nucleus may add to the ptosis and extra-ocular motility dysfunction as described in one of our classic-infantile patients.[74]

Progressive glycogen accumulation in the lower motor neurons as described above together with weakness of the bulbar muscles are probably responsible for the profound facial muscle weakness (including ptosis), speech disorders and dysphagia that we encountered in our classic-infantile Pompe patients (**Chapter 9**) despite receiving ERT. These findings have been supported by others[42,74-77] and

raise important clinical concerns. The effect of aspiration on pulmonary function is of particular concern, since respiratory insufficiency is the main cause of death in these patients. Poor facial expression and disordered speech can hinder a patients' social interaction. A bilateral ptosis may become so severe that surgical intervention may be necessary.

If we compare these findings in classic-infantile Pompe disease with our study in 31 children with non-classic forms of the disease (**Chapter 2**), ptosis was not encountered, and bulbar weakness was found in only one patient. Thus, it appears that treated patients with classic-infantile Pompe disease develop a different phenotype than naturally less affected children with non-classic forms of Pompe disease.

3. Outcome measures

The approach towards evaluating disease severity and effect of treatment has become increasingly multi-dimensional over the past years, in agreement with the International Classification of Functioning, Disability and Health (ICF, World health organization). However, a core set of standardized outcome measures has not yet been implemented on an international basis. A recent literature study showed that direct comparisons between different studies is difficult because of the different outcome measures used in these studies.[13] The clinical studies in adults and children with non-classic forms of Pompe disease presented in this thesis used the following outcome measures: FVC in sitting and supine position as a measure of pulmonary function; the Medical Research Council (MRC) grading scale, hand held dynamometry, and quantitative muscle testing as measure of muscle strength; the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36), the Rotterdam 9-item Handicap Scale (RHS) and the Pompe Pediatric Evaluation of Disability Inventory (Pompe PEDI) to assess the level of participation, restrictions, and quality of life. In the past, muscle strength tests have often been applied to assess muscle function, but both should be evaluated separately by valid and reliable assessment tools. For this purpose the Quick Motor Function Test (QMFT) was developed as described in **Chapter 12**. It is a functional motor scale specific for Pompe disease that appears to be sufficiently sensitive to assess disease severity

and to detect clinically important changes over time. The test is conveniently quick as it comprises a mere 16 items and can be applied by a physician in the outpatient clinic. However, it is not widely used as yet. In **Chapter 6**, the test is used for studying the effect of enzyme replacement therapy in adolescents over a period of three years' time. Furthermore, it was used in a nationwide prospective observational study to evaluate the natural course in 91 adults with Pompe disease,[10] and in an open-label therapy study in 69 adult Pompe patients.[20] While the QMFT showed a significant improvement of muscle function during ERT, which correlated with an improvement of muscle strength, the test results did not reflect the group-level changes in muscle strength found in the natural course study over a median period of 1.6 years. The discrepancy is difficult to explain other than that the changes in muscle strength might have been too small to result in a change in muscle function. The test itself seems to satisfy since its responsiveness to change appeared to be good, as shown by a high standardized response mean of 0.81 and an area under the ROC curve of 0.88 ($p<0.05$)[78] (**Chapter 12**). Whether the QMFT is not sensitive enough, indeed, to detect small changes in muscle function may be answered by performing a larger study. The suggestion made by van der Beek to refine this outcome measure by Rasch methodology seems justified.[79]

During our studies we encountered some difficulties while using the six minute walk test (6MWT) and Hand Held Dynamometry (HHD) in children. The 6MWT, a frequently used scale to assess motor function in neuromuscular disorders and one of the primary endpoints in the LOTS study, was originally developed to assess functional capacity in patients with moderate to severe heart or lung disease. It evaluates the integrated responses of all the systems involved in exercise: including the pulmonary and cardiovascular systems, systemic and peripheral circulation, blood, neuromuscular units, and muscle metabolism. It does not provide specific information on the neuromuscular unit, and therefore cannot be used as a measure of limb-girdle muscle function.[80] Although the 6MWT has recently been applied in different chronic pediatric conditions,[81] including Duchenne muscular dystrophy,[82] we (**Chapter 6**) and others were unable to obtain any consistency over different assessment days when applied to children. The test appeared to be insufficiently challenging for our patients. Geiger et al. also stated that a lack of motivation and

understanding for the need of a 6MWT may affect performance in children.[83] To improve motivation we asked our patients to run. These results appeared to be more consistent, but no reference values exist for 6 minutes running, which makes the test difficult to use in a childhood Pompe population.

We encountered another difficulty by using Hand Held Dynamometry in our childhood studies. When interpreting the test results, we used published reference values by Beenakker et al.[84] To our surprise, some patients that were diagnosed pre-symptomatically showed muscle strength results below normal values, while all other tests showed maximum scores (**Chapter 2**). When we compared the reference values to those established for adult Pompe patients, there appeared to be a large difference and the percentage of normal scores for strength were approximately 10% lower using the adult reference values[85] instead of those obtained for children. Since the study that obtained the reference values in children was performed in 270 subjects and no clear methodological inconsistencies could be identified, we cannot explain these differences. In 1989 another study which contains muscle force values obtained in a control group of children between 3.5 and 15 years of age, was published by Bäckman et al.[86] Reference values obtained by Bäckman et al were approximately 10% lower than those obtained by Beenakker et al. and seem more in line with those obtained for adults. Unfortunately, Bäckman et al. assessed muscle strength in only 10 muscle groups and did not provide reference values for some muscle groups that are clearly affected in Pompe patients like the neck flexors. To be able to reliably interpret follow-up of muscle strength in children with Pompe disease and other neuromuscular disorders, we encourage the construction of a new set of reference values for HHD.

MAIN CONCLUSIONS

The studies presented in this thesis illustrate that Pompe disease presents as a true clinical spectrum whereby childhood phenotypes bridge the classic-infantile and adult onset phenotypes. While some patients with a non-classic forms of Pompe disease present in adulthood with a slowly progressive disease course, a

substantial number of patients becomes wheelchair bound or respirator dependent already during childhood. Childhood Pompe disease presents with delayed motor development or limb-girdle weakness, but disproportional weakness of the neck flexors, unexplained fatigue or solitary elevation of transaminases should also raise suspicion for the disease. Children with the common c.-32-13T>G/null genotype are predominantly male, and patients with other mutations than the c.-32-13T>G/null genotype presenting during childhood generally are more severely affected. ERT initiated when a child is still ambulant mostly results in significant improvements of muscle strength and muscle function as well as in stabilization of pulmonary function. In patients with a more advanced stage of the disease at start of therapy, ERT merely stabilizes muscle strength and pulmonary function, while quality of life improves.

The emerging phenotype of treated patients with classic-infantile Pompe disease clearly differs from the phenotype of children with less progressive forms of the disease. Treated classic-infantile patients, as opposed to non-classic patients, appear prone to manifest new problems like facial muscle-weakness, speech disorders, dysphagia, arrhythmias, hearing loss, and distal muscle weakness. Additionally, although less profound than in other lysosomal storage disorders like the Mucopolysaccharidoses, lysosomal storage is found in the CNS, which might be responsible for delayed processing speed, while these abnormalities are not observed in children with non-classic forms of Pompe disease.

FUTURE PERSPECTIVES

The outcome of patients with Pompe disease has clearly improved since the introduction of ERT. The studies presented in this thesis demonstrate that the process has come a long way: patients live longer, muscle strength, cardiac size, pulmonary function, and quality of life has clearly improved. However, the studies reveal several hurdles that still need to be taken. Not all patients respond equally well to treatment, and patients with classic-infantile Pompe disease who receive ERT are prone to develop new problems. Thus, a lot of work remains

ahead for improving the currently available enzyme replacement therapy and searching for possible alternatives. Potential improvements lay in structurally optimizing the therapeutic enzyme so that it reaches and enters the muscle cells more effectively[87-91] or, along a completely different line, trying to reduce the lysosomal glycogen accumulation by interfering with the autophagic pathway.[92] The prevention of antibody formation towards the therapeutic enzyme[53,93,94] may enhance[95,96] the efficacy of ERT in specific cases, and so may the co-administration of chaperones.[97-100] Besides, the long-term investments in the development of gene therapy[101,102] might finally come to fruition.

FINAL REMARKS

In the footsteps of 'Cardiomegalia glycogenica', the first thesis on Pompe disease written by Dr. J.C. Pompe himself, the current thesis is the 13th dissertation on Pompe disease at the Erasmus MC University Medical Center in Rotterdam, The Netherlands. The studies were aimed to contribute to the ever expanding knowledge on Pompe disease so to improve patient care and treatment, and could not have been completed without the close collaboration between clinicians, basic scientists, patients, and patient associations.

REFERENCES

1. Pompe JC. *Cardiomegalia glycogenica*. Nijmegen-Utrecht, University of Amsterdam; 1936.
2. Lignac GOE. Cardiomegalia glycogenica. *Nederlands Tijdschrift voor Geneeskunde.* 1936; 80:3747.
3. van der Ploeg AT, Reuser AJ. Pompe's disease. *Lancet.* Oct 11 2008; 372(9646):1342-1353.
4. Hirschhorn R, Reuser AJJ. Glycogen Storage Disease Type II: acid alpha-glucosidase (acid maltase) deficiency. In: Scriver CR, Beaudet AL, Valle D, Sly WS, eds. *The Metabolic and Molecular Bases of Inherited Disease*. 8 ed. NY: McGraw-Hill; 2001: 3389-3420.
5. Winkel LP, Hagemans ML, van Doorn PA, et al. The natural course of non-classic Pompe's disease; a review of 225 published cases. *J Neurol.* Aug 2005; 252(8):875-884.
6. Hagemans ML, Winkel LP, Hop WC, Reuser AJ, Van Doorn PA, Van der Ploeg AT. Disease severity in children and adults with Pompe disease related to age and disease duration. *Neurology.* Jun 28 2005; 64(12):2139-2141.
7. Kroos, Pomponio RJ, Hagemans ML, et al. Broad spectrum of Pompe disease in patients with the same c.-32-13T->G haplotype. *Neurology.* Jan 9 2007; 68(2):110-115.
8. Montalvo AL, Bembi B, Donnarumma M, et al. Mutation profile of the GAA gene in 40 Italian patients with late onset glycogen storage disease type II. *Human mutation.* Oct 2006; 27(10):999-1006.
9. Slonim AE, Bulone L, Ritz S, Goldberg T, Chen A, Martiniuk F. Identification of two subtypes of infantile acid maltase deficiency. *The Journal of pediatrics.* 2000; 137(2):283-285.
10. van der Beek NA, de Vries JM, Hagemans ML, et al. Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study. *Orphanet journal of rare diseases.* Nov 12 2012; 7(1):88.
11. Wens SC, van Gelder CM, Kruijshaar ME, et al. Phenotypical variation within 22 families with Pompe disease. *Orphanet journal of rare diseases.* Nov 19 2013; 8(1):182.
12. Winkel LP, Van den Hout JM, Kamphoven JH, et al. Enzyme replacement therapy in late-onset Pompe's disease: a three-year follow-up. *Annals of neurology.* Apr 2004; 55(4):495-502.
13. Toscano A, Schoser B. Enzyme replacement therapy in late-onset Pompe disease: a systematic literature review. *J Neurol.* Aug 28 2012.
14. van der Ploeg AT, Barohn R, Carlson L, et al. Open-label extension study following the Late-Onset Treatment Study (LOTS) of alglucosidase alfa. *Molecular genetics and metabolism.* Sep 17 2012.
15. Gungor D, de Vries JM, Hop WC, et al. Survival and associated factors in 268 adults with Pompe disease prior to treatment with enzyme replacement therapy. *Orphanet journal of rare diseases.* Jun 1 2011; 6(1):34.
16. Bembi B, Pisa FE, Confalonieri M, et al. Long-term observational, non-randomized study of enzyme replacement therapy in late-onset glycogenosis type II. *Journal of inherited metabolic disease.* Dec 2010; 33(6):727-735.
17. Regnery C, Kornblum C, Hanisch F, et al. 36 months observational clinical study of 38 adult Pompe disease patients under alglucosidase alfa enzyme replacement therapy. *Journal of inherited metabolic disease.* Jan 31 2012.

18. Corbu A, Scaramozza A, Badiali-DeGiorgi L, et al. Satellite cell characterization from aging human muscle. *Neurological research.* Feb 2010; 32(1):63-72.
19. Lexell J, Taylor CC, Sjostrom M. What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. *Journal of the neurological sciences.* Apr 1988; 84(2-3):275-294.
20. de Vries JM, van der Beek NA, Hop WC, et al. Effect of enzyme therapy and prognostic factors in 69 adults with Pompe disease: an open-label single-center study. *Orphanet journal of rare diseases.* Sep 26 2012; 7(1):73.
21. Cupler EJ, Berger KI, Leshner RT, et al. Consensus treatment recommendations for late-onset pompe disease. *Muscle Nerve.* Oct 21 2011.
22. Alejaldre A, Diaz-Manera J, Ravaglia S, et al. Trunk muscle involvement in late-onset Pompe disease: Study of thirty patients. *Neuromuscular disorders.* Oct 1 2012; 22 Suppl 2:S148-154.
23. Chien YH, Lee NC, Huang HJ, Thurberg BL, Tsai FJ, Hwu WL. Later-Onset Pompe Disease: Early Detection and Early Treatment Initiation Enabled by Newborn Screening. *The Journal of pediatrics.* Jan 11 2011; 158(6):1023-1027 e1021
24. van der Beek NA, Soliman OI, van Capelle CI, et al. Cardiac evaluation in children and adults with Pompe disease sharing the common c.-32-13T>G genotype rarely reveals abnormalities. *Journal of the neurological sciences.* Aug 29 2008.
25. Muller-Felber W, Horvath R, Gempel K, et al. Late onset Pompe disease: clinical and neurophysiological spectrum of 38 patients including long-term follow-up in 18 patients. *Neuromuscular disorders.* Oct 2007; 17(9-10):698-706.
26. Francesconi M, Auff E, Ursin C, Sluga E. [WPW syndrome combined with AV block 2 in an adult with glycogenosis (Type II)] WPW-Syndrom, kombiniert mit AV-Block 2 bei einer adulten Form einer Glykogenose Typ II. *Wiener Klinische Wochenschrift.* 1982; 94(15):401-404.
27. Lopaschuk GD, Collins-Nakai RL, Itoi T. Developmental changes in energy substrate use by the heart. *Cardiovascular research.* Dec 1992; 26(12):1172-1180.
28. van den Hout HM, Hop W, van Diggelen OP, et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics.* Aug 2003; 112(2):332-340.
29. Henning SL, Wambolt RB, Schonekess BO, Lopaschuk GD, Allard MF. Contribution of glycogen to aerobic myocardial glucose utilization. *Circulation.* Apr 15 1996; 93(8):1549-1555.
30. Goodwin GW, Arteaga JR, Taegtmeyer H. Glycogen turnover in the isolated working rat heart. *J Biol Chem.* Apr 21 1995; 270(16):9234-9240.
31. Castello A, Rodriguez-Manzaneque JC, Camps M, et al. Perinatal hypothyroidism impairs the normal transition of GLUT4 and GLUT1 glucose transporters from fetal to neonatal levels in heart and brown adipose tissue. Evidence for tissue-specific regulation of GLUT4 expression by thyroid hormone. *The Journal of biological chemistry.* Feb 25 1994; 269(8):5905-5912.
32. Razeghi P, Young ME, Alcorn JL, Moravec CS, Frazier OH, Taegtmeyer H. Metabolic gene expression in fetal and failing human heart. *Circulation.* Dec 11 2001;104(24):2923-2931.
33. Kolwicz SC, Jr., Purohit S, Tian R. Cardiac metabolism and its interactions with contraction, growth, and survival of cardiomyocytes. *Circulation research.* Aug 16 2013;113(5):603-616.
34. Akki A, Smith K, Seymour AM. Compensated cardiac hypertrophy is characterised by a decline in palmitate oxidation. *Molecular and cellular biochemistry.* Apr 2008; 311(1-2):215-224.

35. Allard MF, Schonekess BO, Henning SL, English DR, Lopaschuk GD. Contribution of oxidative metabolism and glycolysis to ATP production in hypertrophied hearts. *The American journal of physiology.* Aug 1994; 267(2 Pt 2):H742-750.
36. Barger PM, Kelly DP. Fatty acid utilization in the hypertrophied and failing heart: molecular regulatory mechanisms. *The American journal of the medical sciences.* Jul 1999; 318(1):36-42.
37. Young ME, Laws FA, Goodwin GW, Taegtmeyer H. Reactivation of peroxisome proliferator-activated receptor alpha is associated with contractile dysfunction in hypertrophied rat heart. *The Journal of biological chemistry.* Nov 30 2001; 276(48):44390-44395.
38. Klinge L, Straub V, Neudorf U, et al. Safety and efficacy of recombinant acid alpha-glucosidase (rhGAA) in patients with classical infantile Pompe disease: results of a phase II clinical trial. *Neuromuscular disorders.* Jan 2005; 15(1):24-31.
39. Kishnani PS, Nicolino M, Voit T, et al. Chinese hamster ovary cell-derived recombinant human acid alpha-glucosidase in infantile-onset Pompe disease. *The Journal of pediatrics.* Jul 2006; 149(1):89-97.
40. Van den Hout H, Reuser AJ, Vulto AG, Loonen MC, Cromme-Dijkhuis A, Van der Ploeg AT. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients. *Lancet.* 2000; 356(9227):397-398.
41. Amalfitano A, Bengur AR, Morse RP, et al. Recombinant human acid alpha-glucosidase enzyme therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial. *Genetics in medicine.* 2001; 3(2):132-138.
42. Prater SN, Banugaria SG, Dearmey SM, et al. The emerging phenotype of long-term survivors with infantile Pompe disease. *Genetics in medicine.* Apr 26 2012.
43. Arad M, Maron BJ, Gorham JM, et al. Glycogen storage diseases presenting as hypertrophic cardiomyopathy. *The New England journal of medicine.* Jan 27 2005; 352(4):362-372.
44. Ansong AK, Li JS, Nozik-Grayck E, et al. Electrocardiographic response to enzyme replacement therapy for Pompe disease. *Genetics in medicine.* May 2006; 8(5):297-301.
45. Bharati S, Serratto M, DuBrow I, et al. The conduction system in Pompe's disease. *Pediatric Cardiology.* 1982; 2(1):25-32.
46. Arad M, Moskowitz IP, Patel VV, et al. Transgenic mice overexpressing mutant PRKAG2 define the cause of Wolff-Parkinson-White syndrome in glycogen storage cardiomyopathy. *Circulation.* Jun 10 2003; 107(22):2850-2856.
47. Patel VV, Arad M, Moskowitz IP, et al. Electrophysiologic characterization and postnatal development of ventricular pre-excitation in a mouse model of cardiac hypertrophy and Wolff-Parkinson-White syndrome. *Journal of the American College of Cardiology.* Sep 3 2003; 42(5):942-951.
48. Wang LY, Ross AK, Li JS, et al. Cardiac arrhythmias following anesthesia induction in infantile-onset Pompe disease: a case series. *Paediatr Anaesth.* Aug 2007; 17(8):738-748.
49. Desena HC, Brumund MR, Superneau D, Snyder CS. Ventricular Fibrillation in a Patient with Pompe Disease: A Cautionary Tale. *Congenit Heart Dis.* Mar 10 2011.
50. McDowell R, Li JS, Benjamin DK, Jr., et al. Arrhythmias in patients receiving enzyme replacement therapy for infantile Pompe disease. *Genetics in medicine.* Sep 18 2008.

51. Cook AL, Kishnani PS, Carboni MP, et al. Ambulatory electrocardiogram analysis in infants treated with recombinant human acid alpha-glucosidase enzyme replacement therapy for Pompe disease. *Genetics in medicine.* May 2006; 8(5):313-317.
52. Kamphoven JH, de Ruiter MM, Winkel LP, et al. Hearing loss in infantile Pompe's disease and determination of underlying pathology in the knockout mouse. *Neurobiol Dis.* Jun 2004; 16(1):14-20.
53. Rohrbach M, Klein A, Kohli-Wiesner A, et al. CRIM-negative infantile Pompe disease: 42-month treatment outcome. *Journal of inherited metabolic disease.* Sep 30 2010.
54. van der Beek NA, Verschuure H, Reuser AJ, van der Ploeg AT, van Doorn PA, Poublon RM. Hearing in adults with Pompe disease. *Journal of inherited metabolic disease.* Mar 2012; 35(2):335-341.
55. Hanisch F, Rahne T, Plontke SK. Prevalence of hearing loss in patients with late-onset Pompe disease: Audiological and otological consequences. *Int J Audiol.* Dec 2013; 52(12):816-823.
56. Musumeci O, Catalano N, Barca E, et al. Auditory system involvement in late onset Pompe disease: A study of 20 Italian patients. *Molecular genetics and metabolism.* Aug 17 2012.
57. Borchgrevink HM, Tambs K, Hoffman HJ. The Nord-Trondelag Norway Audiometric Survey 1996-98: unscreened thresholds and prevalence of hearing impairment for adults >20 years. *Noise & health.* Jul-Sep 2005; 7(28):1-15.
58. Davis AC. The prevalence of hearing impairment and reported hearing disability among adults in Great Britain. *International journal of epidemiology.* Dec 1989; 18(4):911-917.
59. Wilson DH, Walsh PG, Sanchez L, et al. The epidemiology of hearing impairment in an Australian adult population. *International journal of epidemiology.* Apr 1999; 28(2):247-252.
60. Begley DJ, Pontikis CC, Scarpa M. Lysosomal storage diseases and the blood-brain barrier. *Current pharmaceutical design.* 2008; 14(16):1566-1580.
61. Ebbink BJ, Aarsen FK, van Gelder CM, et al. Cognitive outcome of patients with classic infantile Pompe disease receiving enzyme therapy. *Neurology.* May 8 2012; 78(19):1512-1518.
62. Van den Hout JM, Kamphoven JH, Winkel LP, et al. Long-term intravenous treatment of Pompe disease with recombinant human alpha-glucosidase from milk. *Pediatrics.* May 2004; 113(5):e448-457.
63. Hamdan MA, Almalik MH, Mirghani HM. Early administration of enzyme replacement therapy for Pompe disease: Short-term follow-up results. *Journal of inherited metabolic disease.* Dec 12 2008.
64. Spiridigliozzi GA, Heller JH, Kishnani PS. Cognitive and adaptive functioning of children with infantile Pompe disease treated with enzyme replacement therapy: long-term follow-up. *Am J Med Genet C Semin Med Genet.* Feb 15 2012; 160(1):22-29.
65. Chien YH, Lee NC, Peng SF, Hwu WL. Brain Development in Infantile-Onset Pompe Disease Treated by Enzyme Replacement Therapy. *Pediatric research.* Jul 20 2006; 60(3):349-352.
66. Martini C, Ciana G, Benettoni A, et al. Intractable fever and cortical neuronal glycogen storage in glycogenosis type 2. *Neurology.* 2001; 57(5):906-908.
67. Thurberg BL, Lynch Maloney C, Vaccaro C, et al. Characterization of pre- and post-treatment pathology after enzyme replacement therapy for pompe disease. *Laboratory investigation; a journal of technical methods and pathology.* Oct 30 2006; 86(12):1208-1220.
68. Martin JJ, De Barsy T, Van Hoof F, Palladini G. Pompe's disease: an inborn lysosomal disorder with storage of glycogen. A study of brain and striated muscle. *Acta Neuropathol.* 1973; 23(3):229-244.

69. Gambetti P, DiMauro S, Baker L. Nervous system in Pompe's disease. Ultrastructure and biochemistry. *J Neuropathol Exp Neurol.* 1971; 30(3):412-430.
70. Mancall EL, Aponte GE, Berry RG. Pompe's disease (diffuse glycogenosis) with neuronal storage. *J Neuropath Exp Neurol.* 1965; 24:85-96.
71. Teng YT, Su WJ, Hou JW, Huang SF. Infantile-onset glycogen storage disease type II (Pompe disease): report of a case with genetic diagnosis and pathological findings. *Chang Gung Med J.* May 2004; 27(5):379-384.
72. Chien YH, Lee NC, Huang PH, Lee WT, Thurberg BL, Hwu WL. Early pathologic changes and responses to treatment in patients with later-onset Pompe disease. *Pediatr Neurol.* Mar 2012; 46(3):168-171.
73. Fuller DD, Elmallah MK, Smith BK, et al. The respiratory neuromuscular system in Pompe disease. *Respir Physiol Neurobiol.* Jun 21 2013.
74. Slingerland NW, Polling JR, van Gelder CM, van der Ploeg AT, Bleyen I. Ptosis, extraocular motility disorder, and myopia as features of pompe disease. *Orbit.* Mar 2011; 30(2):111-113.
75. Muller CW, Jones HN, O'Grady G, Su AH. Language and speech function in children with infantile Pompe disease. *J Pediatr Neurol.* 2009; (7):147-156.
76. Yanovitch TL, Banugaria SG, Proia AD, Kishnani PS. Clinical and histologic ocular findings in pompe disease. *J Pediatr Ophthalmol Strabismus.* Jan-Feb 2010; 47(1):34-40.
77. Jones HN, Muller CW, Lin M, et al. Oropharyngeal Dysphagia in Infants and Children with Infantile Pompe Disease. *Dysphagia.* Sep 10 2009.
78. Liang MH, Fossel AH, Larson MG. Comparisons of five health status instruments for orthopedic evaluation. *Medical care.* Jul 1990; 28(7):632-642.
79. van der Beek NAME. *Clinical features, disease course, and effects of enzyme therapy in Pompe disease*. Breda, Erasmus University Rotterdam; 2013.
80. Laboratories ATSCoPSfCPF. ATS statement: guidelines for the six-minute walk test. *American journal of respiratory and critical care medicine.* Jul 1 2002; 166(1):111-117.
81. Bartels B, de Groot JF, Terwee CB. The six-minute walk test in chronic pediatric conditions: a systematic review of measurement properties. *Physical therapy.* Apr 2013; 93(4):529-541.
82. Kempen JC, Harlaar J, van der Kooi AJ, et al. Reliability of the walking energy cost test and the six-minute walk test in boys with Duchenne muscular dystrophy. *Neuromuscular disorders : NMD.* Mar 2014; 24(3):216-221.
83. Geiger R, Strasak A, Treml B, et al. Six-minute walk test in children and adolescents. *The Journal of pediatrics.* Apr 2007; 150(4):395-399, 399 e391-392.
84. Beenakker EA, van der Hoeven JH, Fock JM, Maurits NM. Reference values of maximum isometric muscle force obtained in 270 children aged 4-16 years by hand-held dynamometry. *Neuromuscular disorders.* Jul 2001; 11(5):441-446.
85. van der Ploeg RJ, Fidler V, Oosterhuis HJ. Hand-held myometry: reference values. *Journal of neurology, neurosurgery, and psychiatry.* Mar 1991; 54(3):244-247.
86. Backman E, Odenrick P, Henriksson KG, Ledin T. Isometric muscle force and anthropometric values in normal children aged between 3.5 and 15 years. *Scandinavian journal of rehabilitation medicine.* 1989; 21(2):105-114.

87. Zhu Y, Li X, Kyazike J, et al. Conjugation of mannose 6-phosphate-containing oligosaccharides to acid alpha-glucosidase improves the clearance of glycogen in pompe mice. *The Journal of biological chemistry.* Nov 26 2004; 279(48):50336-50341.

88. Zhu Y, Jiang JL, Gumlaw NK, et al. Glycoengineered Acid alpha-Glucosidase With Improved Efficacy at Correcting the Metabolic Aberrations and Motor Function Deficits in a Mouse Model of Pompe Disease. *Molecular therapy.* Mar 10 2009.

89. Maga JA, Zhou J, Kambampati R, et al. Glycosylation-independent lysosomal targeting of acid alpha-glucosidase enhances muscle glycogen clearance in pompe mice. *The Journal of biological chemistry.* Jan 18 2013; 288(3):1428-1438.

90. Tiels P, Baranova E, Piens K, et al. A bacterial glycosidase enables mannose-6-phosphate modification and improved cellular uptake of yeast-produced recombinant human lysosomal enzymes. *Nat Biotechnol.* Nov 18 2012.

91. McVie-Wylie AJ, Lee KL, Qiu H, et al. Biochemical and pharmacological characterization of different recombinant acid alpha-glucosidase preparations evaluated for the treatment of Pompe disease. *Molecular genetics and metabolism.* Aug 2008; 94(4):448-455.

92. Spampanato C, Feeney E, Li L, et al. Transcription factor EB (TFEB) is a new therapeutic target for Pompe disease. *EMBO Mol Med.* Apr 18 2013.

93. Messinger YH, Mendelsohn NJ, Rhead W, et al. Successful immune tolerance induction to enzyme replacement therapy in CRIM-negative infantile Pompe disease. *Genetics in medicine.* Jan 2012; 14(1):135-142.

94. Lipinski SE, Lipinski MJ, Burnette A, Platts-Mills TA, Wilson WG. Desensitization of an adult patient with Pompe disease and a history of anaphylaxis to alglucosidase alfa. *Molecular genetics and metabolism.* Jul 7 2009.

95. Amalfitano A, McVie-Wylie AJ, Hu H, et al. Systemic correction of the muscle disorder glycogen storage disease type II after hepatic targeting of a modified adenovirus vector encoding human acid-alpha-glucosidase. *Proceedings of the National Academy of Sciences of the United States of America.* 1999; 96(16):8861-8866.

96. Fraites TJ, Jr., Schleissing MR, Shanely RA, et al. Correction of the Enzymatic and Functional Deficits in a Model of Pompe Disease Using Adeno-associated Virus Vectors. *Molecular therapy.* 2002; 5(5 Pt 1):571-578.

97. Porto C, Ferrara MC, Meli M, et al. Pharmacological Enhancement of alpha-Glucosidase by the Allosteric Chaperone N-acetylcysteine. *Molecular therapy.* Sep 18 2012.

98. Flanagan JJ, Rossi B, Tang K, et al. The pharmacological chaperone 1-deoxynojirimycin increases the activity and lysosomal trafficking of multiple mutant forms of acid alpha-glucosidase. *Human mutation.* Sep 4 2009.

99. Parenti G, Zuppaldi A, Gabriela Pittis M, et al. Pharmacological Enhancement of Mutated alpha-Glucosidase Activity in Fibroblasts from Patients with Pompe Disease. *Molecular therapy.* Mar 2007; 15(3):508-514.

100. Porto C, Cardone M, Fontana F, et al. The Pharmacological Chaperone N-butyldeoxynojirimycin Enhances Enzyme Replacement Therapy in Pompe Disease Fibroblasts. *Molecular therapy.* Mar 17 2009.

101. Smith BK, Collins S, Conlon T, et al. Phase I/II trial of AAV1-GAA gene therapy to the diaphragm for chronic respiratory failure in Pompe disease: initial safety and ventilatory outcomes. *Hum Gene Ther.* Apr 9 2013.
102. Falk DJ, Mah CS, Soustek MS, et al. Intrapleural Administration of AAV9 Improves Neural and Cardiorespiratory Function in Pompe Disease. *Molecular therapy.* Jun 4 2013.

14

Summary

Samenvatting

Summary

Pompe disease is a rare metabolic disease, caused by an inherited deficiency of the enzyme acid α-glucosidase. This enzyme is required for the breakdown of glycogen in the lysosomes. Lysosomes are membrane-bound organelles that are responsible for the intracellular digestion of macromolecules. Partial or total deficiency of acid α-glucosidase leads to the accumulation of lysosomal glycogen in all cells of the body, but the pathological changes are most notable in skeletal muscle.

Pompe disease presents as a continuous spectrum of clinical phenotypes in which progressive muscle weakness is the main manifestation. Patients with the classic-infantile form of this disease have virtually no residual enzyme activity and are at the severe end of the spectrum, while patients with a certain level of residual enzyme activity present milder phenotypes at the other end of the spectrum. Patients with classic-infantile Pompe disease present shortly after birth with severe generalized muscle weakness and a progressive hypertrophic cardiomyopathy; before a treatment became available, these patients usually died before the age of one year. Patients with less progressive phenotypes present with a slowly progressive proximal myopathy, eventually leading to wheelchair dependency and use of ventilator support.

The first results of enzyme replacement therapy with recombinant human α-glucosidase were promising since most patients with the classic-infantile form of Pompe disease survived far beyond the first year of life. But, there is still little known about the long-term treatment effects in this group of patients. Much less is known about the natural course of disease in children with less progressive phenotypes and how they respond to enzyme replacement therapy. This thesis describes the clinical spectrum of children with Pompe disease, compares them with adult Pompe patients, and evaluates the effects of enzyme replacement therapy in these childhood Pompe patients. Furthermore, it describes the long-term outcome of enzyme replacement therapy in patients with classic-infantile Pompe disease, for a maximum period of 14 years, and compares the functioning of these treated infants with that of children with less progressive forms of Pompe disease.

Chapter 1 provides a general introduction into the field of Pompe disease by giving a short historical background, by describing the clinical spectrum, the pathology, the genetic heterogeneity, diagnostic procedures, and the current stage of enzyme replacement therapy.

Part I of this thesis focuses on the clinical characteristics and natural course of children with slowly progressive phenotypes of Pompe disease. **Chapter 2** provides information on the initial presentation and the clinical and genetic characteristics of 31 children with less progressive forms of Pompe disease. These children usually present with delayed motor development or limb-girdle weakness, but the disease should also be considered in the differential diagnosis of less familiar signs such as disproportional weakness of the neck flexors, unexplained fatigue, persistent diarrhea, and an elevation of transaminase levels. Disease presentation, distribution of muscle weakness, and the occurrence of specific symptoms such as bulbar muscle weakness or ptosis appear to be different from adult patients. Patients in this group with other mutations than c.-32-13T>G were more severely affected than those with the c.-32-13T>G mutation, in line with their more severe genotypes, but, the clinical course varied substantially in both groups. Children with a c.-32-13T>G/null genotype presenting in childhood appeared to be predominantly males. **Chapter 3** focuses in more detail on pulmonary function in 17 children and 75 adults with Pompe disease. Seventy-four percent of all patients, including 53% of the children, had some degree of respiratory dysfunction. Forty-seven percent of the children had a decreased vital capacity in sitting position and 59% in supine position. The youngest patient in whom a diminished pulmonary function was measured was seven years old. Pulmonary function was severely diminished in four of the childhood patients. Male patients appeared to have more severe pulmonary involvement than female patients. During an average follow-up period of 1.6 years, there were significant declines in vital capacity in upright and supine position. We concluded that pulmonary dysfunction in Pompe disease is much more common than generally thought. The presence and extent of cardiac involvement in children and adults with the common c.-32-13T>G genotype is described in **Chapter 4**. Sixty-eight patients were evaluated, of which 23 had

symptom onset before the age of 18 years. Two patients had cardiac abnormalities possibly related to Pompe disease. In one 8-year old patient a Wolff-Parkinson-White pattern was found. A severely affected adult patient had a mild hypertrophic cardiomyopathy that did not change during enzyme replacement therapy. It was concluded that cardiac involvement is rare among Pompe patients with the c.-32-13T>G/null genotype. Since all patients with cardiac abnormalities were detected by electrocardiography, an electrocardiogram should be performed as first level of screening in this group of patients.

Part II focuses on the effect of ERT in children and adults with Pompe disease and includes the results of eight years of treatment in two children and one adult with Pompe disease (**Chapter 5**). In two severely affected patients, ventilator dependent and wheelchair bound, further loss of muscle function was prevented and respiratory function stabilized. Both became more confident about their physical condition and more independent in daily life activities. A third and less severely affected patient that regained the ability to walk during the first three years of treatment, showed further improvements and caught up with healthy peers regarding muscle strength. None of the patients showed severe side effects during the enzyme infusions. The results indicate that long-term follow-up and timing of treatment are important topics for future studies. **Chapter 6** shows the results of an open-label study that included five children aged 5.9 to 15.2 years that were treated with enzyme replacement therapy for three years. Pulmonary function remained stable in four patients and improved in one patient, while two unmatched historical cohorts of untreated patients with Pompe disease showed an average decline of pulmonary function of 1.6 to 5% per year. Muscle strength improved in all patients, and one of the patients approached the normal range. Muscle function as measured by the Quick Motor Function Test improved in all patients. No-infusion associated reactions were observed. The small number of patients and the fact that the study was not placebo controlled made it difficult to draw firm conclusions, but we found it encouraging that none of the patients deteriorated over a three-year period. In **Chapter 7** we report the results of a randomized, placebo-controlled trial of alglucosidase alpha in 90 patients, older than 8 years of age. Over an 18-month period, treatment was associated with a

significant increase in walking distance in the 6-minute walk test and stabilization of pulmonary function compared to placebo. The greatest improvement in the treatment group occurred during the first 26 weeks and was maintained over the following 12 months. Although subgroup analyses suggested a more pronounced treatment effect in patients with better clinical status at baseline, this was not confirmed and the treatment response appeared to be consistently positive for all subgroups. Three of the 60 patients treated with alglucosidase alpha experienced anaphylactic reactions, and their treatment had to be discontinued. All patients developed IgG antibodies, but no consistent effect on clinical outcome was detected.

Part III reports on the long-term outcome of patients with classic-infantile Pompe disease. **Chapter 8** describes the occurrence of gingival overgrowth in one of the longest survivors of enzyme replacement therapy who is currently 15 years old. Histopathology of the gingiva showed marked glycogen accumulation in smooth muscle cells of the arteries but the glycogen content in fibroblasts did not exceed that of controls. We concluded that glycogen storage is not a direct cause of gingival overgrowth in this patient, rather chronic inflammation in combination with dryness of the gingiva. **Chapter 9** shows that facial muscle weakness, speech disorders and dysphagia are common in patients with classic infantile Pompe disease treated by ERT. Eleven patients were included in the study. Age at final assessment ranged from 7.7 months to 12.2 years (median 4.3 years). All patients developed facial muscle weakness before the age of 15 months. Speech assessments showed that articulation was disordered with hyper nasal resonance and reduced speech intelligibility. More important swallowing was ineffective and patients appeared to be at risk for aspiration and related complications. Therefore early treatment by a speech therapist and regular assessments of swallowing was recommended. In **Chapter 10** hearing was analyzed in a group of 24 children with Pompe disease using repetitive auditory brainstem response measurements and pure tone audiometry. Only 1 out of 13 children with non-classic forms of Pompe disease showed recurrent conductive hearing loss, while 10 out of 11 patients with classic-infantile Pompe disease had sensorineural hearing loss. These infants

also had a high prevalence of conductive hearing loss. Five patients showed evidence of mild retro cochlear pathology, suggestive of glycogen accumulation in the central nervous system. Auditory function should be carefully monitored in patients with classic-infantile Pompe disease and early implementation of hearing aids is pivotal to preserve adequate speech development. The results of almost 14 years of treatment with alglucosidase alpha on cardiac structure and function are described in **Chapter 11**. The study shows that significant reduction of cardiac size and improvement of cardiac function is maintained over this period of time. This is an important finding as the reduction of cardiac mass is associated with decreased morbidity and mortality in this group of patients. Arrhythmias remain a threat to these patients and close cardiac follow-up is required.

Part IV, which includes **Chapter 12** reports the construction and validation of the quick motor function test (QMFT): a functional motor scale specifically designed for Pompe disease. The test comprises 16 items, which can be easily applied in clinical practice. The QMFT appeared to be a reliable and valid test for assessing motor function in children and adults with Pompe disease between 5 and 76 years of age. The test proved to be sufficiently sensitive to assess disease severity and to detect clinically important changes in motor function over time.
Finally, **Chapter 13** summarizes the main findings and discusses their significance and clinical implications.

Samenvatting

De ziekte van Pompe is een erfelijke stofwisselingsziekte die wordt veroorzaakt door een tekort aan het lysosomale enzym zure α-glucosidase. Dit enzym zorgt bij gezonde mensen voor de afbraak van glycogeen in het lysosoom. Een lysosoom is een organel waarin allerlei grote moleculen afkomstig uit het intra-cellulaire en het extracellulaire milieu gerecycled worden. Een tekort aan het enzym zure α-glucosidase leidt tot stapeling van glycogeen in het lysosoom en uiteindelijk tot weefselschade. Dit gebeurt in vrijwel alle lichaamscellen, maar de schade is het duidelijkst merkbaar in spiercellen.

De ziekte van Pompe kan zich op alle leeftijden manifesteren. Bij patiënten met de klassiek-infantiele vorm van de ziekte van Pompe is er totaal geen zure α-glucosidase aanwezig. Dit ziektebeeld heeft een snel progressief beloop, waarbij de eerste symptomen al kort na de geboorte optreden. Baby's vertonen gegeneraliseerde spierzwakte en hebben een ernstige hypertrofische cardiomyopathie (verdikking van de hartspier). Zij sterven vrijwel altijd voor het eerste levensjaar ten gevolge van hartfalen en ademhalingsproblemen. Bij kinderen en volwassenen met een gedeeltelijk tekort van zure α-glucosidase zijn vooral de skeletspieren van de romp, de schouder- en bekkengordel betrokken en niet het hart. Het ziektebeeld is minder progressief, maar de toenemende spierzwakte leidt uiteindelijk tot rolstoelafhankelijkheid en noodzaak tot beademing.

Tot 2006 was de ziekte van Pompe een onbehandelbare ziekte waarvoor alleen ondersteunende behandeling mogelijk was. De ontwikkeling van enzymtherapie, waarbij het ontbrekende enzym wordt toegediend met een infuus, heeft het toekomstbeeld van de patiënten sterk verbeterd. Patiënten met de klassiek infantiele vorm van de ziekte komen niet meer voor hun eerste levensjaar te overlijden. De oudste patiënten met deze vorm van de ziekte zijn inmiddels 15 jaar oud, maar de effecten op heel lange termijn zijn nog onbekend. Ook is er behoefte aan meer inzicht in het natuurlijk beloop van minder ernstige vormen van de ziekte van Pompe en de effecten van enzymtherapie in deze groep patiënten. Daarover is nog nauwelijks literatuur verschenen. De onderzoeken die in dit proefschrift

beschreven worden hadden dan ook als doel om 1) het ziektebeeld van kinderen met minder progressief verlopende vormen van de ziekte te beschrijven, 2) de effecten van enzymtherapie in deze groep patiënten te evalueren en 3) de lange termijn effecten van de therapie bij patiënten met de klassiek infantiele vorm te onderzoeken waarbij wij deze patiënten, inmiddels bijna 14 jaar oud, hebben vergeleken met kinderen met minder progressieve vormen van de ziekte.
In **Hoofdstuk 1** wordt achtergrondinformatie gegeven over de ziekte van Pompe. Dit hoofdstuk geeft een historische achtergrond en beschrijft de pathofysiologie van de ziekte. Verder worden het klinische beeld beschreven, de manier waarop de diagnose wordt gesteld, de huidige kennis over het natuurlijk beloop en de huidige behandelingsmogelijkheden.

Deel I van dit proefschrift richt zich voornamelijk op de klinische kenmerken en het natuurlijk beloop van de ziekte van Pompe bij kinderen met langzaam progressieve vormen van de ziekte. **Hoofdstuk 2** beschrijft dan ook hoe de ziekte zich presenteert en wat de specifieke klinische en genetische kenmerken zijn van 31 kinderen, die bij dit onderzoek betrokken waren. Het eerste symptoom van de ziekte was meestal vertraagde motorische ontwikkeling of spierzwakte van de romp en bekkengordel. Het bleek echter dat de ziekte van Pompe ook moet worden overwogen bij de volgende kenmerken: een buitenproportionele spierzwakte van de flexoren van de nek, onbegrepen vermoeidheidsklachten, aanhoudende diarree of een verhoging van leverenzymen in het bloed. Een aantal symptomen van deze kinderen bleek te verschillen van die van aangedane volwassenen zoals de verdeling van de spierzwakte en het hebben van een afhangend ooglid, en zwakte van de spieren die gebruikt worden bij het spreken, eten en slikken. Patiënten met andere mutaties in het α-glucosidase gen dan de meest voorkomende c.-32-13T>G base verandering waren over het algemeen ernstiger aangedaan dan patiënten die deze mutatie wel hadden en vertoonden een snellere achteruitgang. Overigens was de klinische variatie binnen de groepen ook groot. In de groep aangedane kinderen met de veel voorkomende c.-32-13T>G mutatie hadden er opvallend veel het mannelijk geslacht. **Hoofdstuk 3** richt zich op de longfunctie in een cohort van 17 kinderen en 75 volwassenen met de ziekte van Pompe. Vierenzeventig procent van de

patiënten en 53% van de kinderen bleek een verminderde longfunctie te hebben. In zittende houding was de longfunctie verminderd bij 47% van de kinderen en in liggende houding bij 59%. De jongste patiënt waarbij een verminderde longfunctie werd gevonden was 7 jaar oud. Vier kinderen hadden een ernstig verminderde longfunctie. Mannen bleken ernstiger te zijn aangedaan dan vrouwen. Gedurende de vervolgperiode van gemiddeld 1.6 jaar werd een significante achteruitgang gezien van longfunctie in zittende en liggende houding. Hieruit concluderen wij dat een verminderde longfunctie bij patiënten met de ziekte van Pompe vaker voorkomt dan werd verwacht. In **Hoofdstuk 4** worden de bevindingen beschreven van cardiaal onderzoek bij kinderen en volwassenen met de veel voorkomende c.-32-13T>G mutatie. Achtenzestig patiënten werden geëvalueerd. Drieëntwintig van deze patiënten vertoonden reeds voor het 18e levensjaar symptomen van de ziekte van Pompe. In twee patiënten werden cardiale afwijkingen gevonden die kunnen passen bij de ziekte van Pompe. Een meisje van 8 jaar oud had een Wolff-Parkinson-White (WPW) patroon op het elektrocardiogram en een volwassen man die reeds ernstig door de ziekte was aangedaan bleek een milde verdikking van de hartspier te hebben. Deze verdikking veranderde niet na start van enzymtherapie. Er werd geconcludeerd dat cardiale afwijkingen zelden voorkomen bij patiënten met de c.-32-13T>G mutatie en dat een elektrocardiogram een goede eerste screeningsmethode is voor het opsporen van cardiale afwijkingen in deze groep patiënten.

Deel II van dit proefschrift beslaat een drietal studies waarin de effecten van enzymtherapie bij kinderen en volwassenen met de ziekte van Pompe worden beschreven. Allereerst rapporteren we de resultaten van een studie waarin twee kinderen en één volwassene gedurende 8 jaar met enzymtherapie werden behandeld (**Hoofdstuk 5**). De therapie zorgde ervoor dat de overgebleven spierkracht van twee ernstig aangedane patiënten, beiden afhankelijk van een rolstoel en beademingsapparaat, niet verder achteruitging en dat hun longfunctie stabiliseerde. Hun kwaliteit van leven verbeterde en zij werden zelfstandiger op het gebied van ADL (algemene dagelijkse levensverrichtingen). De jongste patiënt was het minst ernstig aangedaan toen de therapie startte. Tijdens de

eerste drie jaar van behandeling heeft hij weer leren lopen en tijdens de overige 5 jaar verbeterde zijn spierkracht verder tot normaalwaarden werden bereikt. Er werden geen ernstige bijwerkingen gezien tijdens de enzym infusies. Deze resultaten tonen aan dat lange termijn follow-up en timing van behandeling belangrijke aandachtspunten zijn voor vervolg onderzoek. **Hoofdstuk 6** beschrijft de resultaten van een open-label studie betreffende 5 kinderen die tussen 5 en 15 jaar oud waren bij start van de behandeling en gedurende 3 jaar enzymtherapie ontvingen. Gedurende deze periode verbeterde de longfunctie van één patiënt en bleef deze stabiel in de overige 4 patiënten, terwijl 2 historische niet gematchte cohorten van onbehandelde patiënten met de ziekte van Pompe een gemiddelde achteruitgang van longfunctie lieten zien van 1.6 tot 5% vitale capaciteit per jaar. De spierkracht verbeterde bij alle patiënten, waarbij de spierkracht van één patiënt bijna normaliseerde. Spierfunctie werd onderzocht middels de “Quick Motor Function Test” en verbeterde bij alle patiënten. Er werden geen bijwerkingen gezien tijdens de enzyminfusies. Het is lastig om harde conclusies te trekken gezien het kleine aantal patiënten en het feit dat de studie niet placebo gecontroleerd was. Een positieve bevinding was dat geen van de patiënten achteruitgang vertoonde gedurende de drie jaar behandeling. **Hoofdstuk 7** beschrijft de resultaten van een gerandomiseerde placebogecontroleerde studie naar de effecten van alglucosidase alfa in 90 kinderen en volwassenen die ouder waren dan 8 jaar. Na 18 maanden therapie werd een significante verbetering gevonden van de afstand die patiënten konden afleggen in een 6 minuten looptest vergeleken met de groep die placebo kreeg. De duidelijkste verbetering werd in de eerste 26 weken gezien. Deze verbetering werd gehandhaafd gedurende de overige 12 maanden behandeling. Globaal genomen leek het effect van behandeling beter te zijn bij patiënten met een relatief goede klinische conditie bij start van enzymtherapie, maar in een subgroep analyse kon geen statistisch significant verschil worden aangetoond. Er traden bij 3 van de 60 patiënten die met het enzym werden behandeld anafylactische reacties op waarbij 2 patiënten niet verder konden worden behandeld. Bij alle patiënten werden verhoogde IgG antilichamen gemeten, maar deze leken geen effect te hebben op de klinische uitkomst.

Deel III van dit proefschrift beschrijft de lange termijn effecten van de behandeling met enzymtherapie bij patiënten met de klassiek infantiele vorm van de ziekte van Pompe. In **Hoofdstuk 8** beschrijven we het voorkomen van tandvleeshypertrofie bij één van de langst overlevende patiënten met de klassiek infantiele vorm van de ziekte van Pompe. Zij is inmiddels 15 jaar oud. Histopathologie van het tandvlees toonde glycogeen stapeling in gladde spiercellen van de vaten maar de hoeveelheid glycogeen in fibroblasten was vergelijkbaar met controle patiënten die niet de ziekte van Pompe hebben. We concludeerden dat glycogeen stapeling waarschijnlijk niet de directe oorzaak is van de tandvleeshypertrofie, maar dat dit mogelijk veroorzaakt wordt door een chronische ontsteking en uitdroging van het tandvlees. De resultaten gepresenteerd in **Hoofdstuk 9** tonen aan dat spierzwakte van het gelaat, spraakproblemen en dysfagie frequent voorkomen bij patiënten met de klassiek infantiele vorm van de ziekte van Pompe al dan niet behandeld met enzymtherapie. Elf patiënten werden geïncludeerd in deze studie. De leeftijd ten tijde van de laatste metingen varieerde van 7.7 maanden tot 12.2 jaar (gemiddeld 4.3 jaar). Gezichtsspierzwakte werd in alle patiënten gevonden, reeds voor de leeftijd van 15 maanden. Bij de evaluatie van de spraak bleek dat er sprake was van afwijkende articulatie met hypernasale resonantie. De slikfunctie bleek ineffectief te zijn wat het risico op aspiratie en gerelateerde complicaties vergroot. Er werd dan ook aanbevolen om vroegtijdige begeleiding door een logopedist te starten en frequente controles van de slikfunctie uit te voeren. In **Hoofdstuk 10** wordt het gehoor van 24 kinderen met de ziekte van Pompe geanalyseerd met herhaaldelijke “auditory brainstem response” metingen en toonaudiometrie. In slechts 1 van de 13 kinderen met een niet klassieke vorm van de ziekte van Pompe werd bij herhaling conductief gehoorverlies gevonden, terwijl 10 van de 11 patiënten met de klassiek infantiele vorm van de ziekte een sensorineuraal gehoorverlies hadden. Een conductief gehoorverlies kwam ook veelvuldig voor bij deze laatste groep patiënten. Vijf patiënten toonden retro cochleaire pathologie, welke suggestief is voor glycogeen stapeling in het centrale zenuwstelsel. We concluderen dat het belangrijk is om in de groep patiënten met de klassiek infantiele vorm van de ziekte van Pompe het gehoor regelmatig te testen en tijdig gehoorapparaten aan te meten om adequate taal- en spraakontwikkeling te garanderen. Het effect van

bijna 14 jaar behandeling op het hart wordt in **Hoofdstuk 11** beschreven. Deze studie toont aan dat de enorme afname van de hypertrophie en de verbetering van de hartfunctie die in het begin van behandeling bereikt werden doorzetten tijdens de volgende langdurige behandelperiode. Dit is een belangrijke bevinding omdat verbetering van de hartfunctie een significante invloed heeft op de vermindering van morbiditeit en mortaliteit in deze groep patiënten. Ondanks de goede effecten van enzymtherapie op het hart blijven hartritmestoornissen helaas voorkomen bij deze patiënten. Dit maakt nauwgezette cardiale controle noodzakelijk.

Deel IV bevat **Hoofdstuk 12** en rapporteert over de samenstelling en de validatie van de Quick Motor Function Test (QMFT): een spierfunctie test die speciaal is ontworpen voor de ziekte van Pompe. Deze test bestaat uit 16 items en kan door een arts in de kliniek worden uitgevoerd. De QMFT bleek een betrouwbare en valide test te zijn om spierfunctie te meten in kinderen en volwassenen met de ziekte van Pompe tussen 5 en 76 jaar. De test bleek voldoende sensitief te zijn om de ernst van de ziekte te bepalen en om klinisch relevante veranderingen van spierfunctie te meten.
Hoofdstuk 13 bediscussieert de belangrijkste bevindingen en bespreekt de klinische consequenties van de bevindingen en de implicaties.

15

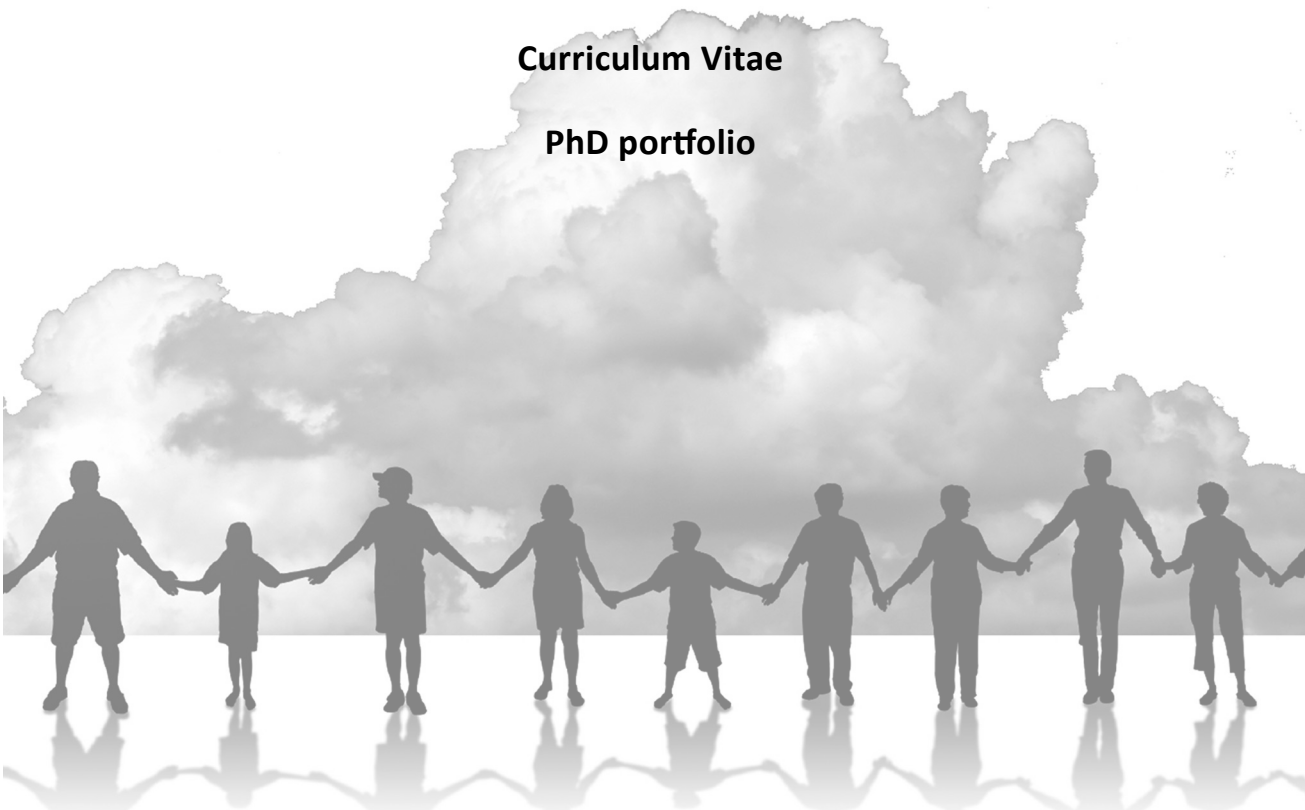
Appendix

Dankwoord

Publications

Curriculum Vitae

PhD portfolio



Appendix: Quick Motor Function Test

1. Raising the torso

Starting position: prone with arms by sides. Examiner may hold the patient's legs.
Movement: the torso must be completely raised from the mat without using the arms.

- □ initiates no neck extension
- □ initiates neck extension but cannot raise head from mat
- □ raises head from mat but torso remains on mat
- □ partially raises head and torso from the mat
- □ completely lifts head and torso from the mat (approx. 45°)

2. Neck flexion

Starting position: supine position, preferably with head in midline and arms by sides.
Movement: raises head to 45°.

- □ initiates no neck flexion
- □ initiates neck flexion (some movement of the head that indicates neck flexion such as lifting or retracting the chin) but does not raise head
- □ raises head <45°
- □ raises head to 45° with difficulty
- □ raises head to 45° or more with no difficulty

3. Hand across midline

Starting position: supine position, preferably with head in the midline and arms by sides. Examiner holds hand at level of patient's chest on L/R sides of the midline. Asks patient to reach towards hand.
Movement: reaches with R/L arm and crosses the midline.

- □ makes no attempt to reach towards the midline
- □ makes attempt to reach towards the midline
- □ reaches with R/L arm, hand does not cross the midline

- □ reaches with R/L arm, hand crosses the midline with difficulty (slowly, requiring effort)
- □ reaches with R/L arm, hand crosses the midline without difficulty

4. Hip and knee flexion

Starting position: supine position, preferably with head in the midline, legs extended and arms by sides.

Movement: flexes R/L hip and knee through full range of motion.

- □ unable to initiate flexion in R/L hip and knee
- □ initiates flexion in R/L hip and knee, but does not move hip more than 10°
- □ flexes R/L hip and knee through part of full range of motion(<90°)
- □ flexes R/L hip and knee through full range of motion but with difficulty (slowly, with effort)
- □ without difficulty flexes R/L hip and knee through full range of motion

5. Extending the legs

Starting position: supine position, preferably with head in the midline, legs stretched and arms by sides.

Movement: extends and raises both legs simultaneously.

- □ does not attempt to raise legs
- □ attempts to raise legs but neither leg leaves the mat (tightens abdominal/ leg muscles), or raises 1 leg
- □ raises legs from mat but does not extend them, or uses arms
- □ with difficulty extends both legs and lifts them from the mat (e.g. very briefly)
- □ extends both legs and lifts them from the mat without difficulty

6. Sit up

Starting position: supine position, preferably with head in the midline, legs in comfortable position, arms by sides or crossed over the chest.

Movement: sit up without support.

- □ does not attempt to sit up (or initiate neck flexion)
- □ attempts, but does not achieve sit up (even using the arms)
- □ does a sit up, but using the arms
- □ does a sit up without using arms but with difficulty
- □ does a sit up without using arms without difficulty (quick and controlled movement)

7. Extending the arms

Starting position: comfortable sitting position (seated on a chair, not leaning on back of the chair), arms by sides.

Movement: raises both arms upwards along the body (180°).

- □ does not attempt to raise arms
- □ attempts to raise arms but they do not come above shoulder level
- □ raises both arms above shoulder level but arms do not quite reach 180°
- □ raises both arms along the body and hands touch above the head but with difficulty (arms are not completely stretched)
- □ raises both arms along the body and hands touch above the head without difficulty (arms remain extended)

8. Standing up from a chair

Starting position: seated in a chair, arms by sides not leaning on the back of the chair.

Movement: stands up from chair without using arms.

- □ makes no attempt to stand up from chair
- □ attempts to stand up from chair but is not able to (even using arms)
- □ stands up from chair using arms
- □ stands up from chair without using arms but with difficulty (slowly, with effort, number of attempts are necessary)
- □ stands up from chair without using arms without difficulty

9. Standing up from half-knee

Starting position: kneeling without arm support.

Movement: stands by means of half-knee position using L/R knee without using arms.

- □ makes no attempt to stand, OR: non-applicable
- □ attempts to stand up but is not able to (even using arms)
- □ stands by means of half-knee position using L/R knee and using arms
- □ stands by means of half-knee position using L/R knee without using arms but with difficulty
- □ stands by means of half-knee position using L/R knee without using arms and without any difficulty

10. Squatting

Starting position: standing.

Movement: squats without using arms.

- □ does not initiate squat, or: non-applicable
- □ initiates squat but is unable to bend legs to 90° (even using arms or a support)
- □ able to squat using arms or holding on
- □ able to squat without using arms, but with difficulty (quickly falls over, cannot easily maintain position)
- □ squats with no difficulty without using arms

11. Standing up from a squatting position

Starting position: squatting:

Movement: stands without using arms.

- □ unable to stay in squatting position without help, OR: non-applicable
- □ attempts to get up from squatting position but is unable to stand (also not when using arms)
- □ able to go from squatting to standing using arms
- □ able to go from squatting to standing without using arms but with difficulty
- □ goes from squatting to standing without using arms with no difficulty

12. Picking up an object

Starting position: standing without arm support.

Movement: able to pick up an object from the floor and stand up again without arm support.

- □ makes no attempt to pick up an object from the floor, OR: non-applicable
- □ attempts to pick up an object from the floor, put does not pick up the object
- □ picks up object from the floor and stands up again using arms (uses arms for balance, both on the floor and on the body)
- □ able to pick up an object from the floor without arm support and stand up again with difficulty
- □ able to pick up an object from the floor without arm support and stand up again without difficulty (fast, controlled movement)

13. Standing on one leg

Starting position: standing without arm support

Movement: standing without arm support, lift L/R foot up for 10 seconds (and remains standing on the same leg).

- □ lifts L/R foot up without arm support, OR: non-applicable
- □ stands without arm support, lift L/R foot up for <3 seconds
- □ stands without arm support, lifts L/R foot up for 3–9 seconds
- □ stands without arm support, lifts L/R foot up for 10 seconds with difficulty
- □ stands without arm support, lifts L/R foot up for 10 seconds without difficulty

14. Walking ten metres

Starting position: standing without arm support.

Movement: walks forward for 10 metres without arm support.

- □ does not attempt to walk, OR: non-applicable
- □ attempts to, but cannot walk for 10 m, even with support (hands, wall)
- □ walks 10 m but uses hands or wall for support
- □ walks 10 m without support of hands or wall, but with abnormal gait (e.g. staggering)
- □ walks 10 m without difficulty

15. Jumping

Starting position: standing without arm support.

Movement: jumps forward with both feet simultaneously.

- □ does not attempt to jump forwards, OR: non-applicable
- □ jumps forwards <10 cm with both feet simultaneously (or falls on jumping or landing)
- □ jumps forwards between 10 and 40 cm with both feet simultaneously
- □ jumps forwards between 40 and 100 cm with both feet simultaneously
- □ jumps forwards more than 100 cm with both feet simultaneously and without effort

16. Walking up steps

Starting position: standing without arm support.

Movement: walks up 4 steps using alternating feet without arm support.

- □ does not attempt to walk up 4 steps, OR: non-applicable
- □ walks (alternating or non-alternating feet), up 1 or more step using railing
- □ walks (alternating or non-alternating feet), up 4 steps using railing
- □ walks up 4 steps using non-alternating feet without arm support
- □ walks up 4 steps using alternating feet without arm support

Dankwoord

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Publications

Van Capelle CI, van der Meijden JC, van Gelder CM , van den Berg LEM, van den Hout JMP, Jaeken J, Kroos MA, Reuser AJJ, van der Ploeg AT. Pompe disease in children: clinical spectrum and genotype in 31 children with milder phenotypes. (*Submitted*)

Van Capelle CI, Poelman E, Frohn-Mulder IM, van den Hout JMP, Ponsen J, Regal L, Helbing WA, van der Ploeg AT. Cardiac outcome after 13 years of treatment with acid alpha-glucosidase in classic infantile Pompe disease. (*in preparation).*

Van Gelder CM, **van Capelle CI,** Ebbink BJ, Moor-van Nugteren I, van den Hout JMP, Hakkesteegt MM, van Doorn PA, de Coo IFM, Reuser AJJ, de Gier HH, van der Ploeg AT. Facial muscle weakness, speech disorders, and dysphagia are common in patients with classic infantile Pompe disease treated with enzyme therapy. *J Inherit Metab Dis* 2012; 35:505-11.

Van Capelle CI, van der Beek NAME, de Vries JM, van Doorn PA, Duivenvoorden HJ, Leshner RT, Hagemans ML, van der Ploeg AT. The Quick Motor Function Test: a new tool to rate clinical severity and motor function in Pompe patients. *J Inherit Metab Dis* 2012; 35:317-23.

de Gijt JP[#], **van Capelle CI[#],** Oosterhuis JW, van der Ploeg AT, van der Wal KG. Gingival overgrowth in Pompe disease: a case report. *J Oral Maxillofac Surg* 2011; 69:2186-90.[#]*equal contributors*

van der Beek NA, **van Capelle CI**, van der Velden-van Etten KI, Hop WC, van den Berg B, Reuser AJ, van Doorn PA, van der Ploeg AT, Stam H. Rate of progression and predictive factors for pulmonary outcome in children and adults with Pompe disease. *Mol Genet Metab* 2011; 104:129-36.

van Capelle CI, van der Beek NA, Hagemans ML, Arts WF, Hop WC, Lee P, Jaeken J, Frohn-Mulder IM, Merkus PJ, Corzo D, Puga AC, Reuser AJ, van der Ploeg AT. Effect of enzyme therapy in juvenile patients with Pompe disease: a three-year open-label study. *Neuromuscul Disord* 2010; 20:775-82.

Van den Berg LE, Zandbergen AA, **van Capelle CI**, de Vries JM, Hop WC, van den Hout JM, Reuser AJ, Zillikens MC, van der Ploeg AT. Low bone mass in Pompe disease: muscular strength as a predictor of bone mineral density. *Bone* 2010; 47:643-9.

van Capelle CI, Goedegebure A, Homans NC, Hoeve HL, Reuser AJ, van der Ploeg AT. Hearing loss in Pompe disease revisited: results from a study of 24 children. *J Inherit Metab Dis* 2010; 33:597-602.

Van der Ploeg AT, Clemens PR, Corzo D, Escolar DM, Florence J, Groeneveld GJ, Herson S, Kishnani PS, Laforet P, Lake SL, Lange DJ, Leshner RT, Mayhew JE, Morgan C, Nozaki K, Park DJ, Pestronk A, Rosenbloom B, Skrinar A, **van Capelle CI**, van der Beek NAME, Wasserstein M, Zivkovic SA. A randomized study of alglucosidase alfa in late-onset Pompe's disease. *N Eng J Med* 2010; 362:1396-406.

Hagemans ML, Stigter RL, **van Capelle CI**, van der Beek NA, Winkel LP, van Vliet L, Hop WC, Reuser AJ, Beishuizen A, van der PLoeg AT. PAS-positive lymphocyte vacuoles can be used as diagnostic screening test for Pompe disease. *J Inherit Metab Dis* 2010; 33:133-9.

Van der Beek NA, Soliman OI, **van Capelle CI**, Geleijnse ML, Vletter WB, Kroos MA, Reuser AJ, Frohn-Mulder IM, van Doorn PA, van der Ploeg AT. Cardiac evaluation in children and adults with Pompe disease sharing the common c.-32-13T>G genotype rarely reveals abnormalities. *J Neurol Sci* 2008; 275:46-50.

van Capelle CI, Winkel LP, Hagemans ML, Shapira SK, Arts WF, van Doorn PA, Hop WC, Reuser AJ, van der Ploeg AT. Eight years experience with enzyme replacement therapy in two children and one adult with Pompe disease. *Neuromuscul Disord* 2008; 18:447-52.

Drost MR, Schaart G, van Dijk P, **van Capelle CI**, van der Vusse GJ, Delhaas T, van der Ploeg AT, Reuser AJ. Both type 1 and type 2a muscle fibers can respond to enzyme therapy in Pompe disease. *Muscle Nerve* 2008; 37:251-5.

Curriculum Vitae

Carine van Capelle werd geboren op 26 augustus 1978 in Heemstede. Nadat zij in 1996 haar diploma had gehaald aan het Stedelijk Gymnasium te Haarlem startte zij, nadat zij was uitgeloot voor de studie geneeskunde, met de studie medisch technische informatica aan de Universiteit Utrecht. Aansluitend studeerde zij een jaar sociale wetenschappen waarna zij in 1998 werd ingeloot voor de studie geneeskunde aan dezelfde universiteit. Tijdens deze studie deed zij een wetenschappelijke stage in het Centre for Cardiovascular Science aan de University of Edinburgh, Schotland. Twee van haar co-schappen heeft zij volbracht in het St. Elisabeth Hospitaal in Willemstad, Curaçao. Een keuze co-schap cardiologie werd gevolgd in het St. Antonius Ziekenhuis in Nieuwegein. Na haar artsexamen werkte zij eerst een jaar als arts-assistent kindergeneeskunde in het Meander Medisch Centrum te Amersfoort, waarna zij in 2005 startte met het promotieonderzoek dat heeft geleid tot dit proefschrift onder supervisie van prof. dr. A.T. van der Ploeg en dr. A.J. Reuser. In 2009 begon zij met de opleiding tot kinderarts in het Erasmus MC Sophia Kinderziekenhuis (opleider prof. dr. M. de Hoog). Tijdens het perifere gedeelte van haar opleiding werkte zij in het Reinier de Graaf Gasthuis te Delft (opleider dr. N. van der Lely). In januari 2015 zal zij haar opleiding tot kinderarts voltooien. Carine woont samen met Maarten Vink en zij hebben 2 zoons: Jelte en Hidde.

PhD Portfolio

	Year	Workload
General Academic Skills and Research Skills		
Classical methods for data-analysis	2006	5.7
Methodology of patient oriented research and preparation for subsidy application	2006	1.0
Biomedical English writing and communication	2008	3.0
Weekly research meeting, Center for Lysosomal and Metabolic Diseases	2005-2009	4.0
In depth courses		
Pompe disease expert days 2006-2009, Rotterdam	2006	2.0
International postgraduate course on lysosomal storage disorders, Nierstein, Germany	2006	2.0
Presentations and International Conferences		
Selbsthilfegruppe Glycogenose Deutschland, Duderstadt, Germany (oral presentation)	2005	1.0
International Symposium on Lysosomal Storage Disorders, Budapest, Hungary (poster presentation)	2006	1.0
International Symposium on Lysosomal Storage Disorders, Berlin, Germany	2006	0.3
6th International Symposium on Lysosomal Storage Diseases, Stockholm, Sweden	2006	0.3
First Pompe disease expert day, Rotterdam (oral presentation)	2006	1.0

	Year	Workload
SSIEM, Annual Symposium, Hamburg, Germany (poster presentation)	2007	1.0
Second Pompe expert day, Rotterdam (oral presentation)	2007	1.0
7th Congres of the European Paediatric Neurology Society, Turkey (oral presentation)	2007	1.5
International Symposium: Steps forward in Pompe disease, Nice, France (oral presentation)	2007	1.0
4th Symposium on Lysosomal Storage Disorders, Vienna, Austria	2007	0.3
7th International Symposium of Lysosomal Storage Diseases Rome, Italy	2007	0.3
Najaarssymposium Vereniging Erfelijke Stofwisselingsziekten Nederland, Zeist (oral presentation)	2008	1.0
3rd Europaediatrics 2008, Istanbul, Turkey (oral presentation)	2008	1.5
5th Symposium on Lysosomal Storage Disorders, Paris, France	2008	0.3
World Muscle Society, Geneva, Austria (poster presentation)	2009	0.5
International Symposium: Steps forward in Pompe disease, London, United Kingdom (oral presentation)	2010	1.0
Teaching activities		
Supervising Master's thesis	2007	0.5