Clinical features, disease course, and effects of enzyme therapy in Pompe disease

Nadine van der Beek

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CLINICAL FEATURES, DISEASE COURSE, AND EFFECTS OF ENZYME THERAPY IN POMPE DISEASE

Klinische kenmerken, ziektebeloop, en effecten van enzymtherapie bij de ziekte van Pompe

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1

General introduction & scope of the thesis

adapted from:

Pompe disease (glycogen storage disease type II): clinical features and enzyme-replacement therapy

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A.T. van der Ploeg, A.J.J. Reuser, and P.A. van Doorn

Acta Neurologica Belgica 2006; 106:82-86

Pompe disease, also known as acid maltase deficiency or glycogen storage disease type II, is an autosomal recessive metabolic disorder whereby mutations in the *GAA* gene lead to the partial or total absence of the lysosomal enzyme acid α-glucosidase. This chapter not only outlines its historical perspectives, incidence, genetics, mode of inheritance, clinical features and natural course, but also describes diagnostic procedures, differential diagnosis, and the therapeutic options currently available.

HISTORICAL PERSPECTIVE

Pompe disease was named after the Dutch pathologist Dr. J.C. Pompe – the first to recognize, in 1932, the defect in glycogen metabolism in a seven-month-old girl who had died from what was initially thought to be pneumonia, but whose heart was "enormous".[1] In the same year, a similar case was reported by Dr. M. Putschar.[2] In 1963, it was discovered by Dr. H.G. Hers that Pompe disease was caused by a partial or total absence of the enzyme acid α-glucosidase.[3] The lysosomal localization of this enzyme was established in the same year,[4] whereby Pompe disease became the first proven lysosomal storage disorder.

Lysosomes are intracytoplasmic, membrane-bound vesicles. They contain hydrolytic enzymes, which are involved in the degradation of a wide variety of macromolecules such as proteins, glycosaminoglycans, sphingolipids, glycogen, nucleic acids, oligosaccharides and complex lipids. The degradation products are reutilized in biosynthetic processes. Acid α-glucosidase is required for the degradation of glycogen that has entered the lysosome through autophagy. Its shortage leads lysosomal glycogen to accumulate in almost all tissues.

Currently, over sixty lysosomal storage disorders are known. In them, lysosomal dysfunction leads to the progressive accumulation of undigested macromolecules within the cell.[5] The prevalence of individual disorders is thought to vary between 1 in 20,000 – 100,000 live births; collectively, the entire group affect at least 1 in 5,000 – 10,000 newborns.[6,7] While most of these disorders are caused by the lack of a single lysosomal protein (e.g. Pompe disease, Fabry disease and Gaucher disease), a number of other causes is known: defective mannose 6-phosphate phosphorylation, resulting in defective trafficking of the lysosomal enzymes to the lysosomes as in mucolipidosis II and III; a defective lysosomal membrane transporter as in cystinosis and Salla disease; defects in a lysosomal membrane protein as in Danon disease; or the lack of a lysosomal activator protein as in GM_2 activator protein deficiency.[8]

INCIDENCE

The reported incidence of Pompe disease varies between ethnic groups and geographic areas;[6,7,9-11] in the Netherlands, its predicted frequency is 1 in 40,000 life births: 1 in 138,000 for the classic infantile phenotype, and 1 in 57,000 for the "milder" phenotypes.[9] At present, some 125 living patients are known in the Netherlands: eight have the classic infantile phenotype, 16 were diagnosed during childhood, and 99 in adulthood.

GENETICS AND INHERITANCE

Pompe disease is an autosomal recessive trait (Online Mendelian Inheritance in Man [OMIM] number 232300). It is caused by pathogenic sequence variations in the gene coding for acid α-glucosidase (*GAA*), which is located on chromosome 17q25.2-q25.3, resulting in partial or total absence of the lysosomal enzyme acid α-glucosidase. Both copies of the *GAA* gene need to harbour a pathogenic sequence variation before the disease manifests itself. Acid α-glucosidase is synthesized as a catalytically inactive 110-kDa precursor, which is further processed into two mature lysosomal forms of 76 and 70 kDa.[12] To date, over 300 pathogenic variants in the *GAA* gene have been identified (www.pompecenter.nl). Most mutations are private, but some are common within an individual ethnic group.

By far the most common mutation is c.-32-13T>G (IVS1), which is found in most Caucasian children and adults with Pompe disease.[13-23] In 80-90 percent of splicing events, this mutation leads to a non-functional *GAA* messenger, but in the remaining 10-20 percent it leads to a normal transcript resulting in the production of a substantial proportion of functionally normal acid α-glucosidase in patients carrying the mutation. After screening of 3,075 anonymous newborns in the Netherlands, the allele frequency of this mutation was reported to be 0.0033.[9]

Three other common mutations among Caucasians are c.2481+102_2646+31del (delexon18; p.Gly828_Asn882del); c.525delT (delT525; p.Glu176fsX45); and c.925G>A (p. Gly309Arg).[15-17,24-26] Two common mutations among other ethnic groups are c.2560C>T (p.Arg854X) in African Americans;[27,28] and c.1935C>A (p.Asp645Glu) in Asians.[26]

CLINICAL SPECTRUM AND NATURAL COURSE

Even among experts, there is no consensus on the nomenclature of the various subtypes of Pompe disease.[29] The literature reflects the large variety of terms used to subdivide the clinical spectrum, including classic infantile, infantile, infantile-onset, non-typical infantile, childhood, juvenile, adult, adult-onset, late-onset, later-onset, classic, non-classic, muscular variant, and adolescent variant. The disease is now considered to encompass a continuous spectrum of phenotypes,[30,31] ranging from a rapidly progressive infantile phenotype characterized by hypertrophic cardiomyopathy[1,32,33] to more slowly progressive forms in children and adults (Figure 1).[30,34,35] The clinical phenotype is determined primarily by the amount of residual acid α-glucosidase activity resulting from different mutations and combinations of mutant alleles.

For the purpose of this thesis, the clinical spectrum is divided into 1) the classic infantile phenotype, in which the symptoms are seen shortly after birth and are combined with cardiomegaly; and 2) phenotypes that become clinically manifest either during childhood – with onset of symptoms between birth and adolescence, but without progressive cardiac hypertrophy – or in adulthood. Though the studies in this thesis focus on patients with this second phenotype, the classic infantile phenotype is also discussed briefly for the sake of completeness.

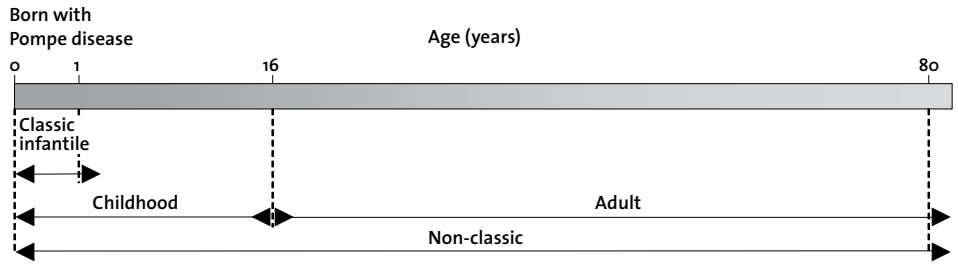

FIGURE 1

The spectrum of Pompe disease

This figure was adapted from Güngör and Reuser. How to describe the clinical spectrum in Pompe disease? Am J Med Genet part A 2013; 9999:1-2, by permission of John Wiley and Sons.

CLASSIC INFANTILE POMPE DISEASE

Due to a set of two fully deleterious mutations in the acid α-glucosidase gene, affected neonates with the classic infantile form of Pompe disease have virtually no residual α-glucosidase activity. The median age of onset of symptoms ranges from 1.6 to 2.0 months.[32,33,36] Failure to thrive and poor motor development are generally noticed first: affected infants are extremely hypotonic ("floppy") and do usually not reach major motor milestones such as rolling over, sitting, and standing.

Characteristically, the heart is affected; usually, the tongue is also enlarged, and the liver moderately enlarged. Due to the improved survival brought by enzyme-replacement therapy, hearing loss,[37-41] facial muscle weakness, and speech disorders[42,43] are being increasingly recognized as important causes of morbidity. If patients with the classic infantile phenotype remain untreated, cardiac and respiratory failure reduce their average life expectancy to less than one year (median 7.7 to 8.7 months).[32,33]

POMPE DISEASE IN CHILDREN AND ADULTS

In most patients, Pompe disease progresses more slowly. The first symptoms can manifest themselves at any age between early infancy – even within the first years of life – and late adulthood, sometimes as late as the seventh decade of life (Figure 2).[44,45] In these patients, the disease is dominated by a slowly progressive limb-girdle myopathy and weakness of respiratory muscles.[14,19,21,30,34,35,44-48] The initial symptoms usually involve impaired skeletal-muscle function, such as difficulty in running, performing sports, walking, climbing stairs, or rising from a seated or supine position. Approximately twenty percent of patients first complain of fatigue and pain;[35,49] approximately ten percent of patients first complain of a respiratory complaint such as shortness of breath or morning headache, while about two percent report acute respiratory failure requiring mechanical ventilatory support.[21,35,50] In general, while the degree of pulmonary dysfunction and skeletal-muscle weakness are related, there may be severe pulmonary involvement even in patients with minor mobility problems.[46,49]

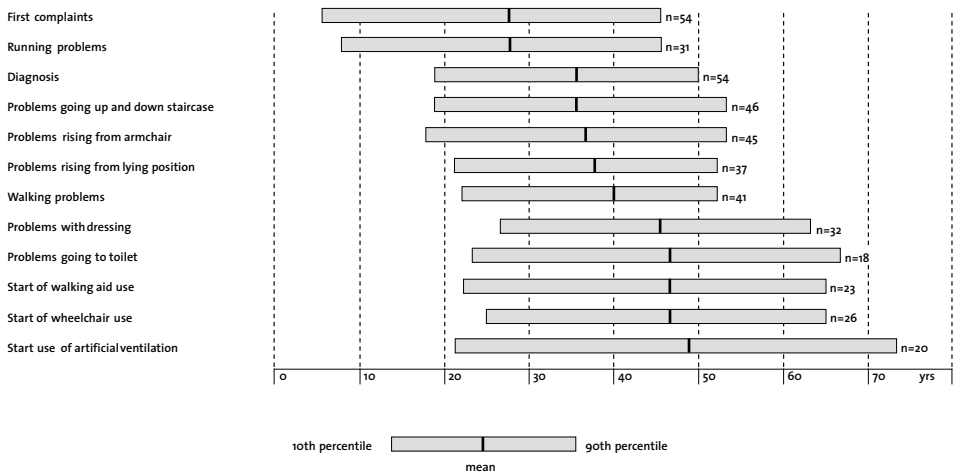

FIGURE 2

Age distribution for specific events in the course of the disease for 54 late-onset Pompe patients. The number behind each bar indicates how many patients provided information on the time of these events. This figure was taken from Hagemans et al. Clinical manifestation and natural course of late-onset Pompe disease in 54 Dutch patients. Brain 2005; 128 (Pt 3):671–677, by permission of Oxford University Press.

More recently, features that are sometimes symptomatic of other neuromuscular disorders were described in a considerable number of patients with Pompe disease; they include ptosis,[51-54] severe scapular winging,[55,56] and bulbar muscle weakness.[51,56] [see also Chapter 3.2]

The course of the disease varies substantially, even among affected individuals from the same family.[21,47,48] Ultimately, over fifty percent of patients become wheelchair-bound and require artificial ventilation.[35,57] Disease course seems to be more serious in a subset of children under the age of 15, who require intensive respiratory, ambulatory and nutritional support at an early age.[57]

Children and adults with Pompe disease have a lower life expectancy than the general population, respiratory failure being the commonest cause of death.[58]

As well as describing the clinical disease spectrum in detail, Chapters 3.1 through 3.5 of this thesis describe its natural course, and prognostic factors for disease outcome.

DIAGNOSTIC PROCEDURES

Besides neurological investigation, the diagnostic procedures in patients with suspected Pompe disease include routine laboratory measurements such as measurement of serum creatine kinase (CK) activity. More specific procedures include muscle biopsy, measurement of acid α-glucosidase activity in white blood cells or cultured skin fibroblasts; screening of blood films for the presence of periodic acid-Schiff (PAS)-positive lymphocyte vacuoles, or DNA analysis.

ENZYME ASSAYS

A demonstrated lack of acid α-glucosidase activity is a prerequisite for diagnosis. Acid α-glucosidase activity can be measured in fibroblasts, leukocytes, lymphocytes, dried blood spots, muscle tissue, and amniotic cells or chorionic villi, using the natural substrate glycogen or the artificial substrate 4-methylumbelliferyl-alpha-D-glucopyraniside (4-MU). The amount of residual acid α-glucosidase activity is most reliably determined in cultured fibroblasts obtained by skin biopsy.

The interference of maltase-glucoamylase used to mean that the measurement of acid α-glucosidase activity in leukocytes was prone to error. Thanks to the development of a new diagnostic assay using acarbose to minimize the maltase-glucoamylase activity, it became possible to diagnose Pompe disease reliably in total leukocytes.[59] In recent years, new methods for detecting acid α-glucosidase in dried blood spots have been developed, the underlying idea being that it can be used in newborn screening programs.[60-63]

MUSCLE PATHOLOGY

In the early stages of Pompe disease, small, glycogen-filled lysosomes are located between intact myofibrils – minor changes that can easily be missed in standard light-microscopic examination using hematoxylin and eosin (HE) or periodic acid-Schiff (PAS) staining. However, staining for acid phosphatase as a non-specific marker for increased lysosomal activity may be positive.

As the disease progresses, the glycogen-filled lysosomes increase in size and number. When the lysosomes have reached a critical size, they may rupture and release lytic enzymes into the cytoplasm – so-called "suicide bags".[64] This causes cellular damage, loss of myofibrils, and hence loss of contractile force.[65,66] However, as well as such lysosomal pathology, the autophagic pathway is disturbed, leading to centrally located autophagic clusters of debris ("non-contractile" material), which also interfere with the contractile function.[65,67] Excessive amounts of desmin and a local loss of titin have also been reported, both contributing to the deterioration of muscle quality. Enhanced muscle oxidative stress, which is reflected in the appearance of lipofuscin, further leads to muscle wasting and thus loss of muscle force.[68,69]

CLASSIC INFANTILE POMPE DISEASE

Diagnosis of the classic infantile phenotype of Pompe disease on the basis of its characteristic clinical features may be aided by a number of procedures. Cardiomegaly – one of the hallmarks of classic infantile Pompe disease – can be detected by chest X-ray and echocardiography. Measurement of serum levels of CK, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) will generally reveal moderately increased values (three to five times the upper limit of normal). Sometimes, however, these values lie within the normal range.[70] Recently, it was also shown that quantification of glycogen-filled vacuoles in peripheral blood lymphocytes was a reliable and simple screening method of supporting a diagnosis of Pompe disease.[71]

A further common biochemical characteristic is the presence of the tetrasaccharide Glc_4 in urine, which is thought to result from intravascular degradation of glycogen released into the circulation.[72,73] Although its additional value for diagnosing Pompe disease

TABLE 1

Differential diagnosis in patients with Pompe disease

Classic infantile Pompe disease	Pompe disease in children and adults
Spinal Muscular atrophy type I (Werdnig-Hoffman disease)	Limb-girdle muscular dystrophies (LGMD)
Congenital muscular dystrophy	Spinal Muscular Atrophy type III and IV
Glycogen storage diseases type III (debrancher deficiency / Cori or Forbes disease) type IV (branching enzyme deficiency / Anderson disease)	Glycogen storage diseases type V (muscle phosphorylase deficiency / McArdle disease) type VII (muscle phosphofructokinase deficiency / Tauri disease)
Mitochondrial / respiratory chain disorders	Myasthenia gravis
Deficiencies in lipoprotein metabolism	Scapuloperoneal syndromes
Fatty-acid oxygenation disorders (e.g. VLCADD)	Mitochondrial myopathies
Danon disease	Rigid spine syndrome
Idiopathic hypertrophic cardiomyopathy	Polymyositis/dermatomyositis
Peroxisomal disorders	Danon disease
Myocarditis	
Congenital myopathy (e.g. Nemaline myopathy)	

VLCADD= Very long chain acyl-coA dehydrogenase deficiency

is disputed, it has been proposed as a biomarker for monitoring the therapeutic effect of enzyme-replacement therapy.[73,74]

Muscle biopsies in classic infantile Pompe disease show pronounced vacuolization in nearly all muscle fibres. These vacuoles contain massive amounts of glycogen, and stain positively with PAS. Acid phosphatase histochemistry also shows positive staining in all vacuolated fibres, indicating lysosomal dysfunction. In end-stage disease, the muscle architecture is severely disturbed and the contractile filaments are replaced by empty spaces.[30,75,76] The lack of acid α-glucosidase can be demonstrated in leukocytes, fibroblasts, or muscle tissue. Typically, residual acid α-glucosidase activity in cultured skin fibroblasts in infants with Pompe disease is under 1%.[30] Mutation analysis is the final step in the diagnosis.

POMPE DISEASE IN CHILDREN AND ADULTS

The varied nature of Pompe disease in children and adults means that many patients are diagnosed only after a great delay. The following diagnostic procedures may facilitate the diagnosis of patients with limb-girdle weakness who are suspected of it.

In general, attention should be paid to pulmonary status through pulmonary-function testing, plus, when necessary, blood-gas analysis to diagnose hypoventilation (including hypoventilation only at night).

Approximately 90 percent of patients have moderately increased serum CK.[14,35,77] Whereas this seldom lies above ten times the upper limit of normal, serum CK is normal in

about ten percent of patients. As in infants with Pompe disease, quantification of glycogen-filled vacuoles in peripheral blood lymphocytes can be a simple screening method for supporting a diagnosis.[71] Measurement of the urinary tetrasaccharide content is unreliable.[72]

Electromyography may reveal non-specific myopathic changes and spontaneous activity, mainly in the paraspinal musculature.[14,78]

Unlike in infants with Pompe disease, muscle biopsies show vacuolization in only 10-50% of muscle fibres.[75] Ultrastructurally, these vacuoles contain granular glycogen and/or autophagic debris, and stain positive for PAS and acid phosphatase. Initially, the intralysosomal accumulation of glycogen causes loss of muscle-fibre force through the misalignment of myofibrils, while further accumulation leads to severe muscle-fibre damage and the replacement of muscle fibres by connective tissue and fat. A review in 225 patients reported that a muscle biopsy showed neither glycogen accumulation nor other morphologic abnormalities in about 20 percent of adults with Pompe disease.[35] Although positive staining for acid phosphatase may provide a clue to the diagnosis in these patients, immunostaining techniques showed recently that all muscle biopsies of children and adult patients have evident muscle damage.[79]

As in classic infantile Pompe disease, the definitive diagnosis is made through DNA analysis and by demonstrating shortage of acid α-glucosidase in leukocytes or fibroblasts. Residual acid α-glucosidase activity ranges between 1% and 30% of average normal activity.[30]

DIFFERENTIAL DIAGNOSIS

The diagnosis of Pompe disease often poses a diagnostic dilemma, due partly to the rarity of the disease, and partly to its relatively non-specific clinical features, especially in children and adults. In infancy, diseases presenting with hypotonia, cardiomegaly, or myopathy should be considered in the differential diagnosis; in older children and adults, diseases manifesting as limb-girdle myopathies are the main diagnostic candidates (table 1). As Pompe disease is now a treatable condition, its early diagnosis has become even more important. For this reason, the disease is now high in the decision tree of the diagnostic algorithm for limb-girdle weakness in children and adults used by the Dutch Neuromuscular Research Centre (ISNO; Interuniversitair Steunpunt Neuromusculair Onderzoek).[80]

CURRENT THERAPEUTIC APPROACHES

In the first place, supportive/symptomatic treatment such as physiotherapy should be considered. To ensure that the patients remain ambulatory as long as possible, walking aids can be used. If there is severe scoliosis or shortened Achilles tendons, corrective surgery should be considered. Respiratory insufficiency should be treated by installing proper ventilatory support using non-invasive or invasive ventilation, which may help to improve the patient's quality of life and to prevent incidents of acute respiratory failure.[50]

DIETARY TREATMENT

One therapeutic approach attempted in the management of Pompe disease is dietary treatment, either through a high-protein diet or through a diet supplemented with

branched-chain amino acids. One cause of muscle wasting and weakness in Pompe disease is increased muscle-protein breakdown, which is reflected in greater resting energy expenditure.[81-83] A high-protein diet is thought to increase the pool of amino acids available for protein synthesis, and thereby to counteract net muscle-protein breakdown. Similarly, supplementation of l-alanine may be beneficial, as it reduces the catabolism of branched-chain amino acids for energy production, thereby conserving muscle protein and muscle function.[81,84]

There is some debate on the effects of dietary treatment for Pompe disease. In classic infantile Pompe disease, dietary therapy does not seem to be effective.[85] In older children and adults with Pompe disease, results have been inconclusive: while some studies report improvement in respiratory or skeletal-muscle function,[82-84,86-94] others do not.[95-98] However, most studies involved single case reports or a small number of cases, and no randomized controlled trials have been performed.

The largest study was carried out by Slonim et al.[99] who reported on the effect of combining a high-protein diet with submaximal exercise therapy in 34 adult patients. The progression of muscle weakness halted in the 22 patients who were fully compliant with the protocol, six of whom improved slightly. The non-compliant patients continued to deteriorate. The effect on respiratory function was less clear. As this study exemplified, compliance with a high-protein diet is often poor due to the large quantities of protein necessary and the high caloric intake (with consequent weight gain).

Low-carbohydrate diets, fructose, vitamin-A supplementation and ketogenic diets did not benefit patients with Pompe disease.[31,34,87,100]

ENZYME-REPLACEMENT THERAPY (ERT)

As early as the 1960s, enzyme-replacement therapy was attempted with enzyme preparations from *Aspergillus niger* [101-103] and human placenta.[104,105] It was not beneficial: dosing was insufficient and the enzyme preparations were impure and immunogenic.

Since 1984, our group at Erasmus MC University Medical Center has focused on the development of enzyme-replacement therapy for Pompe disease. In 1988, we cloned the GAA gene, and between 1990 and 1998 we successfully explored methods for the biotechnological production of recombinant human alpha-glucosidase in the milk of transgenic rabbits and in Chinese Hamster Ovary (CHO) cells in close collaboration with our industrial partners (Pharming, Leiden, the Netherlands; and Genzyme, Cambridge MA, USA). After demonstrating the feasibility of ERT in a knock-out mouse model of Pompe disease, we finally performed the first clinical trial in patients in 1999.[12,106-112]

In 2006, alglucosidase alfa (Myozyme®) was approved for all patients with Pompe disease by the European and American regulatory authorities (EMA and FDA). Therewith, Pompe disease became the first genetic neuromuscular disease for which a disease-specific treatment was available. At the same time, the EMA noted that the efficacy of enzyme therapy still needed to be established in older children and adults with Pompe disease. By the end of 2006 the Dutch health authorities decided to reimburse enzyme therapy for all patients with Pompe disease under the Dutch regulation for orphan diseases ("Beleidsregel weesgeneesmiddelen").

Enzyme-replacement therapy in classic infantile Pompe disease

Between 1999 and 2004, six patients with classic infantile Pompe disease were treated with recombinant human α-glucosidase derived from the milk of transgenic rabbits.[39,113-115] On a weekly dose of 20 to 40 mg/kg, all patients survived well beyond 2 years of age. Their cardiac hypertrophy improved, and those who were in relatively good clinical condition at baseline gained muscle strength and muscle function.

In 2001, the first results were published on the effect of CHO-cell-derived recombinant human α-glucosidase. It concerned three infants who were treated for the first months with a dose of 5 mg/kg twice weekly;[116] later, two of these infants received substantially higher doses of 10 mg/kg 2-5 times weekly.[117,118] Although all three infants survived without heart failure beyond the age of one, one of them developed a nephrotic syndrome after two years of treatment at this very high dose.[119] One of the three infants is still alive. In 2006[38,66] and 2007[120] the results of two larger studies were published, the first describing eight patients, the second describing eighteen patients, all of whom were treated with doses ranging from 10 mg/kg weekly to 20 or 40 mg/kg every other week. Although most patients survived beyond one year of age, one-third became ventilator dependent, and only two-thirds showed a clear improvement in motor function. The 40 mg/kg dose brought no clear advantage over the 20 mg/kg dose.

Although these studies plainly demonstrated the benefits of treatment, it also became clear that not all patients benefited equally from enzyme-replacement therapy. Factors that were thought to account for this include the patient's condition at the start of treatment, the severity of structural skeletal muscle-fibre damage,[76] the amount of glycogen storage, and the level of antibody response. More recent studies have shown that therapeutic outcome in infants is negatively affected by the absence of Cross Reactive Immunogenic Material (CRIM), which is due only partly to the higher chance of antibody formation.[40,121,122]

Enzyme-replacement therapy in children and adults with Pompe disease

In 2004, at the start of our nationwide study on the natural course of Pompe disease and the effect of enzyme-replacement therapy in children and adults, data on the effects of enzyme therapy in this patient group were even scarcer than in infants with the disease. Since then, especially since the approval of Myozyme® by the EMA and FDA, many case reports and several larger studies have been published, including one randomized controlled trial. Table 2 gives an overview of the results of the clinical studies involving ≥ 3 patients.

The first clinical study in children and adults involved three patients who started treatment with recombinant human α-glucosidase from rabbit milk in 1999 and were switched to the enzyme produced in CHO-cells in 2002. At the start of treatment, all patients were wheelchair-bound, and two of them used mechanical ventilatory support. While the least-affected patient showed an incredible response – after 96 weeks of treatment he could walk, run, and play football – the other two severely affected patients showed minimal improvements in muscle strength and pulmonary function.[123,124] In 2009, results were published on 44 adults who had been treated with enzyme-replacement therapy for one year,[125] and in 2012 the same group published the 3-year

TABLE 2

Summary of clinical trials of enzyme-replacement therapy in children and adults with Pompe disease[a,b]

Author	Year of publication	No. of patients	Duration of ERT (months)	Age at start ERT (years)	Effect on[c]:			
					Muscle strength	Muscle function	Pulmonary function	Fatigue / Handicap / Quality of life
Winkel et al.‡	2004	3[d,e]	36	11,16,32	MRC: improvement (2), decline (1) HHD: improvement (2), stable (1)	GMFM: stable (1), improvement (2) PEDI: improvement (3)	FVC: improvement (3)	–
Rossi et al.	2007	3[f]	17, 30 and 5	3,2,19	MRC: stable within normal range (1), decline (1)	GMFM: improvement (2) Walton-Gardner-Medwin scale: stable (2) PEDI: stable within normal range (1), improvement (1)	less infections (1)	–
van Capelle et al.‡	2008	3[g]	72 (after initial 36 months described by Winkel et al. 2004)	11,16,32	HHD: improvement (2), stable (1)	GMFM: no further change		RHS: improvement (3) FSS: improvement (3) SF-36: improvement (3)
Strothotte et al.†	2009	44	12	49 (range 21-69)	MRC: stable	Arm function test: improvement/stable Walton-Gardner-Medwin scale: stable 6MWT: improvement/stable Timed tests: improvement/stable	FVC: stable	SF-36: stable
Angelini et al. ∞	2009	11	3-18	42 (range 22-66)	–	6MWT: improvement/stable	FVC: stable/improvement	SF-36: improvement (performed in 3 pts only)
Merk et al.	2009	4	6	39,41,61,68	–	6MWT: improvement (1), stable (1)	FEV_1: improvement (2), stable (2) MIP: improvement (3)	SF-36: improvement
van der Ploeg et al.# [Chapter 4.2]	2010	90 (60 alglucosidase alfa, 30 placebo)	18	Alglucosidase alfa: 45 (range 15-70) Placebo: 43 (range 10-68)	QMT: no difference between groups	6MWT: difference in favour of Alglucosidase alfa	FVC sitting: difference in favour of Alglucosidase alfa MIP/MEP: difference in favour of Alglucosidase alfa	SF-36: no difference between groups
Bembi et al.∞	2010	24	>36	Juveniles 12 (SD 3.3) Adults 48 (SD 10.7)		6MWT: improvement Walton-Gardner-Medwin scale: improvement	VC: stable FEV_1: stable	–
Ravaglia et al.∞	2010	11	>24	54 (SD 11.2)	HHD: improvement/stable	6MWT: improvement	FVC: improvement	–
Kobayashi et al.	2010	4	>12	17,28,36,44	MRC: improvement	Walton-Gardner-Medwin scale: stable	Subjective improvement respiratory complaints	–
Yang et al.	2011	13	42 (range 5-59)	26 (range 14-45)		Walton-Gardner-Medwin scale: stable	FVC: improvement/stable after 1 year (5), improvement after 4 years (2)	–
van Capelle et.al [Chapter 4.3]	2011	5	36	11 (range 5-15)	MRC: improvement (5) HHD: improvement (5)	6MWT: improvement (1), stable (4) Timed tests: improvement (5) QMFT: improvement (5)	FVC sitting: improvement (2), stable (3) FVC supine: improvement (3), stable (2)	–
Furusawa et al.	2011	5	24	32,38,44,55,66	MRC: stable	GMFM: improvement/stable Barthel index: stable	(F)VC: improvement/stable	–

Author	Year of publication	No. of patients	Duration of ERT (months)	Age at start ERT (years)	Effect on[c]:			
					Muscle strength	Muscle function	Pulmonary function	Fatigue / Handicap / Quality of life
Orlikowski et al.	2011	5	12	28,40,48,61,62,	QMT: stable	MFM: improvement (3), decline (2) Arm function test: stable Leg function test: stable Walton-Gardner-Medwin scale: improvement/stable	VC sitting: improvement (2), decline (3) VC supine: improvement/ stable (2), decline (1) MIP: improvement/stable (4), decline (1) MEP: improvement (3), decline (2)	SF-36: improvement (3) FSS: improvement
Angelini et al. ∞	2012	74	12-54	43 (SD 15.4; range 7-72)		6MWT: improvement Walton-Gardner-Medwin scale: improvement/stable	FVC: stable	–
Regnery et al.†	2012	38	24 (after initial 12 months described by Strothotte et al. 2009)	51 (range 23-68)	MRC: stable	6MWT: improvement Arm function test: stable Walton-Gardner-Medwin scale: stable Timed tests: stable	FVC sitting: stable	SF-36: stable
van der Ploeg et al.#	2012	55	6-12 (after initial 18 months described by van der Ploeg et al. 2010)	45 (range 15-70)	QMT: improvement	6MWT: no further change	FVC sitting: no further change MIP: improvement MEP: improvement	–
de Vries / van der Beek et al. [Chapter 4.1]	2012	69	23 (range 5-47)	52 (range 26-76)	MRC: improvement HHD: improvement	QMFT:stable	FVC stable FVC supine: decline	–

No.=number; ERT=Enzyme-Replacement Therapy; MRC=Medical Research Council; HHD=Hand-Held Dynamometry; QMFT=Quick Motor Function Test; PEDI=Pediatric Evaluation of Disability Inventory; RHS=Rotterdam Handicap Scale; FSS=Fatigue Severity Scale; FEV_1: Forced Expiratory Volume in 1 second, MFM=Motor Function Measure, QMT=Quantitative Muscle Testing, SF-36=Medical Outcomes Study 36-item short-form health survey

[a] Studies with more than 3 patients are mentioned, [b] dosing regimen enzyme-replacement therapy: 20 mg/kg every 2 weeks unless otherwise specified, [c] for the studies with more than 5 patients the effect at group level is presented, otherwise the number of patients showing improvement or deterioration is mentioned between brackets, [d] rabbit derived recombinant human acid α-glucosidase, [e] dosing regimen: initially 10/mg/kg/week, later on 20 mg/kg/ week with a transition period of 15 mg/kg/week, [f] dosing regimen: 1 patient initially 10 mg/kg/2 weeks, gradual increase to 40 mg/kg/2 weeks by week 96, [g] dosing regimen 30-40 mg/kg/2 weeks,

‡ all patients are included in both studies, †a subset of patients is included in both studies, ∞ a subset of patients is included in more than one of these studies, # a subset of patients is included in both studies

follow-up results on 38 of these patients.[126] During the first and second year of treatment, the distance walked in six minutes had improved significantly. However, during the third year, a decline in walking ability was noted, although the distance walked was still better than at baseline. Improvements were also noted in the time needed to rise from supine to standing positions. While serum CK levels decreased, there were no changes in muscle strength, pulmonary function, or quality of life.

In 2010, Bembi et al.[127] published the results of enzyme therapy in seven juvenile and 17 adult patients who had been treated for 36 months. This was followed in 2012 by a report by Angelini et al.[128] describing the results of a multicentre open-label study in 74 Italian patients. This found a significant increase in the distance walked in six minutes that was independent of the degree of muscle-function impairment. Respiratory function also stabilized, even in severely affected patients. Importantly, the juvenile patients continued to improve during the follow-up period, while the adult patients reached a plateau after the first year of treatment.

The best proof of efficacy came from a randomized, placebo-controlled trial in 90 children and adults with Pompe disease (age range 10-70 years), in which, relative to the same variables in the placebo-group, walking distance and pulmonary function in upright position both improved during treatment with alglucosidase alfa (recombinant human acid α-glucosidase).[13,129] The results of this study are presented in more detail in Chapter 4.2. All these studies have shown that – as in classic infantile Pompe disease – the response to

enzyme therapy is variable. Since enzyme-replacement therapy was introduced only relatively recently, its effects in the long-term need further investigation. In this context, the recent finding that enzyme-replacement therapy positively affects survival in adult patients is very important.[130]

As well as the results obtained in the double-blind, placebo-controlled trial in children and adults [Chapter 4.2], this thesis also includes the results of a nationwide study in the Netherlands in 69 adult patients that compared the effect of enzyme-replacement therapy with the natural course of the disease before treatment [Chapter 4.1]. Additionally, Chapter 4.3 presents the results of an open-label study in five children aged 5–15 years.

SCOPE OF THE THESIS

Our understanding of the spectrum of disease presentation, disease severity, health status, fatigue, and handicap in children and adults with Pompe disease was clearly improved by the IPA/Erasmus MC Pompe Survey, an international survey based on self-reported outcome measures, which was initiated in 2002 as a cooperation between Erasmus MC University Medical Center and patient organizations affiliated with the International Pompe Association.[44,45,131-134] Despite this, data on important clinical aspects was still missing – particularly the distribution and severity of muscle weakness, the degree of pulmonary dysfunction, the presence of cardiac involvement or hearing impairment, and the rate of disease progression.

In 2004, this was an excellent reason to initiate a nationwide prospective observational study on the natural course of Pompe disease and the effects of enzyme therapy in children and adults with the disease. Patients with the classic infantile type of Pompe disease are excluded from participation. All patients are seen at regular intervals at the Center for Lysosomal and Metabolic Diseases at Erasmus MC University Medical Center. With a patient cohort of over 125, this single-centre study of patients with Pompe disease is the largest of its type worldwide.

The studies described in this thesis had the following objectives: 1) to investigate the clinical spectrum in children and adults with Pompe disease, including its "non limb-girdle" features; 2) to provide more detailed insight in the natural course of Pompe disease; 3) to identify prognostic factors associated with faster disease progression; 4) to evaluate the effect of enzyme-replacement therapy in children and adults; 5) to investigate whether enzyme therapy alters the natural course of the disease; 6) to identify prognostic factors for response to enzyme treatment; and 7) to compose new measurement scales for examining muscle function, and for evaluating patient's ability to carry out daily life activities and social participation.

Chapter 2 describes the study design and provides an overview of the clinical assessments and self-reported outcome measures used. Chapter 3 delineates the natural disease course and the clinical spectrum of Pompe disease in children and adults, focusing on skeletal-muscle weakness, pulmonary function, cardiac involvement, and hearing impairment. Chapter 4 presents the results of enzyme-replacement therapy in children and adults, and discusses potential predictive factors for treatment efficacy. Chapter 5 presents two new measurement scales for assessing muscle function and evaluating activities and social participation, which were designed specifically for use in patients with Pompe disease. Finally, Chapter 6 summarizes the main findings, discusses their significance and clinical implications, and makes suggestions for future research.

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2

STUDY DESIGN

Prospective study on the natural course and effects of enzyme-replacement therapy in children and adults with Pompe disease

STUDY DESIGN AND PARTICIPANTS

The Erasmus MC study on the natural course of Pompe disease and the effects of enzyme-replacement therapy (ERT) in children and adults with the disease is an ongoing, nationwide, prospective study which was initiated in 2004. All patients are seen at regular intervals at Erasmus MC University Medical Center, the designated centre of expertise for Pompe disease in the Netherlands. Participation is open to all children and adults diagnosed with Pompe disease. Patients with classic infantile Pompe disease are not included in this study.

Patients are recruited either through neuromuscular centres in the Netherlands and Belgium or through the Dutch neuromuscular patient organization (Spierziekten Nederland), or are referred to our Center by their treating physicians in the Netherlands or abroad. All patients or their parents/guardian gave written informed consent for participation in the study. By February 2013, 109 adults and 30 children were taking part in the study.

Since the end of 2006, alglucosidase alfa (Myozyme®) has officially been reimbursed in the Netherlands; from that time on, patients who had been participating in the study gradually began treatment with ERT. As the Dutch government had decided that the Center for Lysosomal and Metabolic Diseases at Erasmus MC University Medical Center would be the only treatment centre in the Netherlands, it was not possible to start treatment in all patients simultaneously. A special committee comprising neurologists, paediatricians, internal medicine specialists and geneticists was therefore instituted to decide which patients needed treatment most urgently. This decided that the most severely affected patients – meaning those who were mechanically ventilated and wheelchair-bound – should be treated first, followed by patients whose pulmonary function and muscle strength were declining rapidly. Patients in whom the disease was progressing slowly were started on ERT at a later stage. For children, the need for enzyme therapy was discussed on a case-by-case basis.

Of the Dutch patients participating in our study, 82 adults and 12 children are now being treated with enzyme therapy. Treatment is always started at our Center, with all patients receiving alglucosidase alfa at a dose of 20 mg/kg every other week. If no severe side-effects are apparent within six to twelve months, patients can receive treatment at home or at a nearby hospital. As of February 2013, 63 adults and 11 children are receiving home treatment, while 10 adults receive ERT at a hospital other than Erasmus MC University Medical Center.

Figure 1 presents a schematic overview of the various studies described in this thesis; Table 1 shows an overview of the number of patients included in the analyses per specific topic. As this is an ongoing study, new data were still being collected when the data from the first groups of patients had already been analyzed for a specific topic. This explains the different numbers of patients described in the different chapters.

FIGURE 1

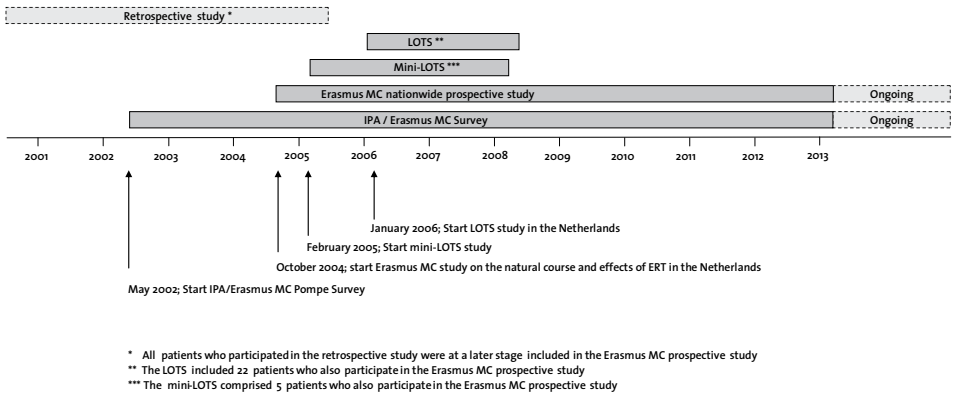

TABLE 1

Overview of the number of patients included in the analyses per specific topic in this thesis

Study Topic	Chapter	Total No. of patients	Adults	Children
Retrospective long-term follow-up of natural disease course	3.1	16	16	–
Clinical disease spectrum and disease course in adults	3.2	94	94	–
Pulmonary outcome in children and adults	3.3	92	75	17
Cardiac evaluation in children and adults	3.4	68	51	17
Hearing evaluation in adults	3.5	58	58	–
Effect of ERT compared to the natural disease course in adults	4.1	69	69	–
Randomized controlled trial with ERT in "late-onset" disease (LOTS)	4.2	90	86	4
Effect of ERT in juvenile patients (Mini-LOTS)	4.3	5	–	5
Rasch-built Pompe-specific Activity (R-PAct) scale	5.1	186 [a,b]	186 [a,b]	–
Quick Motor Function Test (QMFT)	5.2	91	72	19

No.=number of patients, ERT=Enzyme-replacement Therapy.
[a] ≥ 16 years of age, [b] patients were recruited either through neuromuscular centres in the Netherlands and Belgium, or through patient organizations affiliated with the International Pompe Association (IPA) in Canada, the Netherlands, the United Kingdom, and the United States.[42]

CLINICAL ASSESSMENTS AND SELF-REPORTED OUTCOME MEASURES

During the natural-course phase of the study, patients were assessed every six months. After the start of treatment, they were assessed every three months. Tables 2 and 3 give a schematic overview of the measurements performed during the study. The main investigations are described below.

MUSCLE STRENGTH AND MUSCLE FUNCTION

The first symptoms in children and adults with Pompe disease usually involve mobility and limb-girdle weakness. A slowly progressive limb-girdle myopathy is a hallmark of the disease.

Muscle strength

Twenty-five different muscle groups were measured by manual muscle testing using the Medical Research Council (MRC) grading scale, where grade 5 represents normal muscle strength and grade 0 represents paralysis of the muscle group tested.[1] The following muscle groups were examined: neck extensors, neck flexors, abdominal muscles, trunk muscles, and bilateral sternocleidomastoid muscle, trapezius muscle, shoulder adductors, shoulder abductors, shoulder exorotators, shoulder endorotators, elbow flexors, elbow extensors, wrist extensors, wrist flexors, finger extensors, finger flexors, finger abductors, hip extensors, hip flexors, hip abductors, hip adductors, knee flexors, knee extensors, ankle dorsal flexors, and ankle plantar flexors. In the double-blind, placebo-controlled trial of ERT in 90 adults and children [Chapter 4.2], and in the open-label study investigating the effect of ERT in five juvenile patients [Chapter 4.3], we used a modified 11-point version of the MRC scale.[2]

Hand-held dynamometry (HHD) (Cytec dynamometer, Groningen, the Netherlands)[3] – which measures maximum voluntary contraction – was used to examine the following muscle groups: neck extensors, neck flexors and bilateral shoulder abductors, elbow flexors, elbow extensors, wrist extensors, hip flexors, hip abductors, knee flexors, knee extensors, ankle dorsal flexors, and ankle plantar flexors.

In a subset of patients, quantitative muscle testing (QMT) was performed.[4,5] During an isometric contraction, force was measured in the following muscle groups: bilateral shoulder adductors, elbow flexors, elbow extensors, hip adductors, knee extensors, knee flexors. Grip strength was also measured.

Chapter 3.2 presents our results on the distribution and severity of muscle weakness, and on the involvement of individual muscle groups over time.

Muscle function

The Quick Motor Function Test (QMFT) was developed specifically to measure functional impairments in children and adults with Pompe disease [Chapter 5.2].[6] Comprising 16 motor skills related to daily activities that require use of the shoulder-girdle musculature, trunk muscles, and pelvic-girdle/proximal lower limb muscles, this test is based on the Gross Motor Function Measure[7,8] and the IPA/Erasmus MC Pompe Survey.[9,10]

As a measure of functional endurance, we used the six-minute walk test (6MWT), which is widely regarded as an objective measure for reflecting a patient's performance

TABLE 2

Measurements performed during the natural course phase of our study in patients with Pompe disease

Procedure/measurement	t = baseline	t = every 6 months
Informed consent	X	
Inclusion/exclusion criteria	X	
General and neurological examination	X	X
Muscle strength and muscle function testing		
Manual muscle testing (MRC score)	X	X
Hand-Held Dynamometry	X	X
QMFT	X	X
AIMS[a]	X	X
Griffiths developmental scale[b]	X	X
Video monitoring of functional outcome measures	X	X
Pulmonary function testing		
Spirometry (VC, FVC, FEV_1)	X	X
Respiratory muscle strength (MIP, MEP)	X	X
Capnography	X	X
Ventilator use assessment[c]	X	X
Cardiac evaluation		
Electrocardiogram[d]	X	
24-hour Holter ECG[d]	X	
Echocardiogram[d]	X	
Audiometry		
Pure-tone audiogram[d]	X	
Tympanography[d]	X	
BAEP[e]	X	
Blood sample collection		
CK, AST, ALT, LDH	X	X
Acid α-glucosidase activity in leukocytes	X	X
Blood film (PAS-positive lymphocyte vacuoles)	X	X
DNA mutation analysis	X	
Urine sample collection		
Oligosaccharides	X	X
Skin biopsy (fibroblast culture)[f]		
acid α-glucosidase activity	X	
Self-reported outcome measures		
Fatigue Severity Scale	X	X
Rotterdam Handicap Scale	X	X
SF-36 / TACQOL	X	X
R-PAct	X	X
HADS	X	X
Health-economic questionnaire	X	X

◂ *MRC=Medical Research Council; QMFT=Quick Motor Function Test; AIMS=Alberta Infant Motor Scale; VC=Vital Capacity; FVC=Forced Vital Capacity; FEV_1=Forced Expiratory Volume in 1 second; MIP=Maximum Inspiratory Pressure; MEP=Maximum Expiratory Pressure; ECG=electrocardiogram; BAEP=Brainstem Auditory Evoked Potentials; CK=creatine kinase, AST=aspartate aminotransferase; ALT=alanine aminotransferase; LDH=Lactate dehydrogenase; PAS=Periodic Acid Schiff; DNA=Deoxyribonucleic acid; SF-36=Medical Outcomes Study 36-item short-form health survey; TACQOL=TNO-AZL Child Quality of Life Questionnaire; R-PAct=Rasch-built Pompe-specific Activity scale; HADS=Hospital Anxiety and Depression Scale.*

[a] In patients ≤ 18 months of age, [b] In patients ≤ 8 years, [c] If applicable,

[d] If abnormalities are found at baseline, follow-up will be continued at the next visits,

[e] In patients ≤ 5 years, [f] If not performed prior to this study.

in the activities of daily living (ADL).[7,8,11-13] We recorded the distance walked in six minutes. In patients who were unable to complete the full six-minute walk, we recorded the time they were able to walk.

To further evaluate the impact of muscle weakness on a patient's ability to perform functional activities in daily life, we performed three additional timed tests: 1) walking 10 meters; 2) climbing four stairs; and 3) getting up from a supine position on the floor.

In children under 18 months, motor milestones were documented using the Alberta Infant Motor Scale (AIMS).[14] In children under eight years, the Griffith developmental scale was used to score motor and intellectual development and the development of speech and language.[15]

PULMONARY FUNCTION

As well as skeletal-muscle weakness, respiratory dysfunction is one of the main features of children and adults with Pompe disease. Severe pulmonary dysfunction may occur even in patients with minor mobility problems. We used a Lilly type pneumograph (Viasys Healthcare, Würzburg, Germany) or the KoKo spirometry system (Ferraris Respiratory, Louisville, USA) to measure vital capacity (VC), forced vital capacity (FVC), and forced expiratory volume in one second (FEV_1). Measurements were performed when the patient was in supine and upright seated positions.[16-18]

To obtain the maximum force that can be exerted by the respiratory muscles, we recorded maximum static inspiratory (MIP) and expiratory (MEP) pressures using a differential pressure transducer (Viasys Healthcare, Würzburg, Germany).[19]

For information on hypercapnia and alveolar hypoventilation, the carbon dioxide fraction in the expired gas was measured.[20] If patients experienced symptoms indicating nocturnal hypoventilation, they were referred to a centre for home ventilation, where further investigations could determine whether mechanical ventilatory support should be started.

Our results regarding pulmonary involvement are presented in Chapter 3.3.

TABLE 3

Measurements performed during the treatment phase of our study in patients with Pompe disease

Procedure / measurement	Baseline	t = 0 weeks	t = every 2 weeks	t = 4 weeks	t = 8 weeks	t = every 3 months	t = every 6 months	t = 2 years
Informed consent	X							
Inclusion/exclusion criteria	X							
General and neurological examination	X			X	X	X		
Weight	X					X		
Infusion with alglucosidase alfa		X	X					
Vital signs	X	X	X					
Adverse events assessment		X	X					
Concomitant medication assessment		X	X					
Muscle strength/muscle function testing								
Manual muscle testing (MRC score)	X					X		
Hand-Held Dynamometry/QMT	X					X		
Six-minute walk test	X					X		
Timed tests	X					X		
QMFT	X					X		
AIMS[a]	X					X		
Griffiths developmental scale[b]	X					X		
Video monitoring of functional outcomes	X					X		
Pulmonary function testing								
Spirometry (VC, FVC, FEV_1)	X					X		
Respiratory strength (MIP, MEP)	X					X		
Ventilator use assessment[c]	X		X					
Cardiac evaluation								
Electrocardiogram[d]	X			X	X	X		
Echocardiogram[e]	X			X	X	X		
Hearing testing								
Tone audiogram[e]	X						X	
Tympanography[e]	X						X	
BAEP[e]	X						X	
Blood sample collection								
CK, AST, ALT, LDH	X			X	X	X		
α-glucosidase activity in leukocytes	X			X	X	X		
PAS-positive lymphocyte vacuoles	X			X	X	X		
DNA mutation analysis[f]	X							
Antibodies against α-glucosidase		X		X	X	X		
PK-analysis[g]		X						
Urine sample collection								
Oligosaccharides	X			X	X	X		
Skin biopsy (fibroblast culture)[f]	X							
acid α-glucosidase activity	X					X		
X-ray spine[h]	X							
DEXA scan[i]	X							X
CT/MRI muscle[i]	X							X
Muscle biopsy[i]	X							X
Self-reported outcome measures[j]	X						X	
Fatigue Severity Scale	X						X	
Rotterdam handicap Scale	X						X	
SF-36/TACQOL[k]	X						X	
R-PAct	X						X	
HADS	X						X	
Health-economic questionnaire	X						X	

MRC=Medical Research Council; QMT=Quantitative Muscle Testing; QMFT=Quick Motor Function Test; AIMS=Alberta Infant Motor Scale; VC=Vital Capacity; FVC=Forced Vital Capacity; FEV_1=Forced Expiratory Volume in 1 second; MIP=Maximum Inspiratory Pressure; MEP=Maximum Expiratory Pressure; BAEP=Brainstem Auditory Evoked Potentials; CK=creatine kinase, AST=aspartate aminotransferase; ALT=alanine aminotransferase; LDH=Lactate dehydrogenase; PAS=Periodic Acid Schiff; DNA=Deoxyribonucleic acid; PK=pharmacokinetics; DEXA=Dual-energy X-ray absorptiometry; CT=Computed Tomography; MRI=Magnetic Resonance Imaging; SF-36=Medical Outcomes Study 36-item short-form health survey; TACQOL=TNO-AZL Child Quality of Life Questionnaire; R-PAct=Rasch-built Pompe-specific Activity scale; HADS=Hospital Anxiety and Depression Scale.

[a] In patients ≤ 18 months of age, [b] In patients ≤ 8 years, [c] If applicable, [d] If not performed prior to this study, or when abnormalities were found at a prior investigation, [e] In patients ≤ 5 years, [f] If not performed prior to this study, [g] Only in patients ≥ 18 years. PK analysis will be performed at week 0 and week 2, [h] In children or adults with signs of scoliosis in whom corrective surgery is considered, [i] Not compulsory, [j] In patients ≥ 16 years unless otherwise indicated, [k] In patients ≥ 6 years and ≤16 years.

CARDIAC EVALUATION

In patients with the classic infantile phenotype, the heart is typically affected. While cardiac involvement has been described incidentally in patients with more slowly progressive phenotypes,[21-23] it is unknown whether it occurs in a substantial number of patients or is just an exceptional finding in this patient group. To gather more information on this subject, we performed the following procedures in a subset of patients: an electrocardiogram (ECG), 24-hour Holter ECG, two-dimensional echocardiography, low-dose dobutamine stress echocardiography, and tissue Doppler imaging [Chapter 3.4].

HEARING ASSESSMENT

While hearing loss is increasingly recognized as an important cause of morbidity in patients with the classic infantile phenotype,[24-28] hearing problems affect only a few older children and adults with Pompe disease.[28-30] At the start of our study, it was unknown whether hearing impairments are more common in adults with Pompe disease than in the general population. In some of the study cohort we therefore performed pure-tone audiometry and tympanometry [Chapter 3.5].

SELF-REPORTED OUTCOME MEASURES

Rotterdam 9-items Handicap Scale (RHS)

The RHS was originally developed to measure handicap in patients with immune-mediated polyneuropathies;[31] recently it was also proven to be useful for the evaluation of patients with Pompe disease.[10] Suitable for patients age 16 years and older, the scale comprises nine questions on the following topics: mobility indoors and outdoors; kitchen tasks; domestic tasks indoors and outdoors; leisure activities indoors and outdoors; travelling; and work/study. The total score ranges from 9 to 36, with higher values representing a lower level of handicap.

Fatigue Severity Scale (FSS)

The FSS comprises nine items for determining the severity of fatigue and the impact of fatigue on a patient's life.[32] In recent years, it has become clear that fatigue is highly prevalent among patients with Pompe disease, independently of the severity or duration of the disease.[33] The total FSS score is the average of the 9 item scores and ranges from 1 ("no signs of fatigue") to 7 ("most disabling fatigue"). Scores of 4 and higher indicate fatigue, and scores of 5 and higher indicate severe fatigue. Fatigue was assessed in patients aged 16 years and older.

Quality of life

Over the last decade, greater emphasis has been placed on "quality of life" as a measure which should be included in modern studies. Quality of life in patients over 16 years was measured using the Medical Outcomes Survey short-form 36 Health Survey (SF-36; see below); in children aged between six and sixteen years, it was measured using the TNO-AZL Child Quality of Life Questionnaire (TACQOL; see below) or the Child Health Questionnaire (CHQ-10; see below).

The SF-36 is widely used in various health conditions, including lysosomal storage disorders.[34-36] It comprises 36 items addressing four physical health domains (physical functioning, role limitations due to physical problems, bodily pain, and general health perceptions); and four mental health domains (vitality, social functioning, role limitations due to emotional problems, and mental health). Items are summed per domain and subsequently transformed into scores between 0 and 100, with higher values representing better functioning. Its usefulness for evaluating the burden of Pompe disease was demonstrated recently.[37]

The TACQOL is a generic instrument that includes items representing the following concepts: physical complaints, motor functioning, autonomous functioning, social functioning, cognitive functioning, positive moods, and negative moods.[38]

The CHQ-10 survey includes items representing the following concepts: physical functioning, role, bodily pain, general behavior, mental health, self-esteem, general health perceptions, change in health, parental impact, and family activities and family cohesion.[39]

Hospital Anxiety and Depression Scale (HADS)

the HADS was developed by Zigmond and Snaith to identify anxiety disorders and depression among patients in non-psychiatric hospital clinics.[40] It is divided into an anxiety subscale (HADS-A) and a depression subscale (HADS-D), each containing seven intermingled items. All symptoms of anxiety or depression that also related to physical disorder, such as dizziness, headaches, insomnia and fatigue were excluded. The HADS has been widely used in different disorders.[41] In our study, it was used to distinguish the fatigue caused by Pompe disease from that induced by anxiety or depression.

Rasch-built Pompe-specific activity (R-pact) scale

Pompe disease strongly affects patients' functioning in daily life. Although the various aspects of this are difficult to measure, it is important to quantify them, both to manage individual patients and to evaluate the possible effects of enzyme-replacement therapy or any other future treatment modality. We therefore constructed a patient-based interval scale using Rasch analysis that is specifically suited to quantifying the effects of Pompe disease on a patient's ability to carry out daily-life activities [Chapter 5.1 and Appendix A]. For this specific part of the study, our study cohort was extended with an international patient cohort participating in the IPA/Erasmus MC Pompe Survey.[42]

Health-economic questionnaire

the direct and indirect costs of treated and untreated Pompe disease were established through a self-applicable questionnaire, which included elements from the PROductivity and DISease Questionnaire (PRODISQ)[43], EuroQol-5D[44], and Vragenlijst Mantelzorg (Department of Public Health, Erasmus MC). We quantified seven aspects of the costs associated with medical care: living conditions, home adjustments, use of health care facilities, professional and non-professional care (e.g. partner or family), artificial ventilation, wheelchair use, and work or loss of productivity. All costs were evaluated against the background of the anticipated costs of enzyme-replacement therapy.

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3

CLINICAL DISEASE SPECTRUM AND NATURAL COURSE

3.1

RATE OF DISEASE PROGRESSION DURING LONG-TERM FOLLOW-UP OF PATIENTS WITH LATE-ONSET POMPE DISEASE

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ABSTRACT

To determine the rate of disease progression in patients with late-onset Pompe disease, we collected longitudinal data on pulmonary function and skeletal muscle strength in 16 patients whose symptoms had started in childhood or adulthood. The mean duration of follow-up was 16 years (range 4-29 years). During the follow-up period, eight patients (50%) became wheelchair bound and three (19%) became ventilator dependent. At a group level, pulmonary function deteriorated by 1.6% per year, and proximal muscle weakness progressed gradually. At the individual level, however, the rate and extent of progression varied highly between patients. In two thirds of patients, pulmonary function and muscle strength declined simultaneously and to the same extent. The remaining one third of patients showed a variable, sometimes rapidly progressive course, leading to early respirator or wheelchair dependency. These individual differences, especially in pulmonary dysfunction, indicate the need for regular monitoring every 6-12 months depending on the rate of disease progression.

INTRODUCTION

Pompe disease (glycogen storage disease type II or acid maltase deficiency) is characterized by intra-lysosomal glycogen storage caused by acid α-glucosidase deficiency. The estimated frequency of the disease is 1 in 40,000 newborns.[1-3] The clinical spectrum is broad, and disease severity is related primarily to the degree of enzyme deficiency. Patients with the classic infantile phenotype manifest generalized muscle weakness and a hypertrophic cardiomyopathy shortly after birth, and usually die within the first year of life due to cardiorespiratory failure.[4,5] Patients with milder phenotypes usually present in the first to sixth decade of life with a slowly progressive proximal myopathy or, occasionally, with respiratory failure.[6-8]

Enzyme-replacement therapy (ERT) with recombinant human α-glucosidase has recently been approved as long-term treatment. In infants with Pompe disease, treatment prolongs survival.[9-14] Variable effects on respiratory function and muscle have been reported both in infantile patients and in severely affected patients with late-onset disease.[10,15] The results obtained so far indicate that the effect on clinical outcome is better when treatment is started early in the course of the disease, before irreversible muscle damage has occurred. However, experience is still limited, especially in older children and adults. To optimize the effect of ERT and determine its long-term effects, accurate knowledge of the rate of disease progression in untreated patients is crucial.

As yet, there is little information available on the long-term clinical course in patients with late-onset Pompe disease.[16-19] We therefore, assessed the rate of deterioration of pulmonary function and skeletal muscle strength through longitudinal clinical follow-up of a group of untreated patients.

PATIENTS AND METHODS

Clinical data were obtained from medical records of 16 Dutch patients with late-onset Pompe disease from 13 families. All were diagnosed between 1975 and 2002 and seen regularly at the University Medical Center Utrecht (n=8) or the Erasmus MC University Medical Center Rotterdam (n=8). The diagnosis was confirmed in all patients through mutation analysis and measurement of acid α-glucosidase deficiency in leukocytes, muscle tissue or fibroblasts. All measurements of pulmonary function and muscle strength were performed between March 1977 and May 2006. The data was analyzed in 2006. In 1995, seven patients have previously been reported in a cross-sectional study on genotype-phenotype correlation.[20]

Data collected comprised gender, current age, age at first complaints, age at diagnosis, wheelchair use, use of respiratory support, disease duration, type of mutation, proximal skeletal muscle strength and pulmonary function. Muscle strength was determined by manual muscle testing using the Medical Research Council (MRC) grading scale (range 0–5; only full grades were used). In most cases, manual muscle testing was performed by the same neurologist (PAVD or JHJW). A sumscore was calculated of the following muscle pairs: shoulder abductors, elbow flexors, hip flexors and knee extensors. This MRC sumscore ranges from 0 (“total paralysis”) to 40 (“normal strength”). Vital capacity (VC) in sitting and

supine position, and the forced expiratory volume in one second (FEV_1) were used as measures of pulmonary function.

STATISTICAL ANALYSIS

Variables (age, age at diagnosis, age at first complaints, follow-up time, disease duration, use of wheelchair and respiratory support) were summarized using descriptive statistics, comprising mean, SD and range using SPSS for Windows (version 15, SPSS Inc., Chicago, IL). Longitudinal analysis of MRC sumscore and pulmonary function was performed using random coefficient models for repeated measurements, allowing for irregularly measured data, with the SAS PROC MIXED statistical program (SAS Institute Inc., Cary, NC).

RESULTS

CLINICAL CHARACTERISTICS

Sixteen patients with Pompe disease were followed for a prolonged period of time. Ten of these 16 patients were female. All patients were ambulant at the time of their first visit. One patient used artificial ventilation at night. The mean age at first symptoms was 24 ± 11 years (range 1-40 years) and at diagnosis 27 ± 12 years. Four patients were diagnosed before the age of 18 (age at diagnosis 8, 11, 15 and 15 years). One patient was diagnosed pre symptomatically after measurement of an elevated CK value. The mean age of the patients at the end of follow-up was 45 ± 12 years (range 27-69 years). In all patients, the initial complaints were related to limb-girdle muscle weakness. The most often mentioned complaints were difficulty running/taking part in other sports, walking, climbing stairs or rising from a sitting or supine position. None of the patients had experienced difficulties in breathing as a first complaint. The mean duration of follow-up was 16 ± 7 years (range 4-29 years). During this follow-up period eight patients (50%) became wheelchair dependent, and three also needed respiratory support (19%). The time between first complaints and wheelchair use was on average 18 years (range 2-30 years), and between first symptoms and ventilator dependency 19 years (range 5-30 years). Two patients died during the follow-up period, one due to pneumonia complicated by respiratory failure (age 55) and one due to carcinoma of the pancreas (age 43). Further details are given in Table 1.

GENETIC ANALYSIS

Fifteen patients carried the c.-32-13T>G (IVS1-13T>G) mutation on one allele and a fully deleterious mutation on the other. The other patient (patient 12 in table 1) had genotype c.1634C>T/c.525del.

PULMONARY FUNCTION

Within the study group, repeated measurements of pulmonary function were performed in 13 patients. In these patients a total of 95 measurements of VC in sitting position (mean 6.5 per patient, range 2-16) were made with a mean follow-up duration of 9 years. In the other three patients, pulmonary function was not measured (n=1) or measured only once (n=2). The first VC measurement was performed on average 9 years after symptom onset. For the whole group, mean first measured VC was 3.08 l (range 0.70-5.05 l). The corresponding mean percentage of predicted values was 82% (range 24-111%). VC showed a variable, but in most cases progressively deteriorating course, with a mean rate of decline of 1.6% per year ($p=0.002$) (Figure 1A). Still, individual variability was large. In three patients, VC remained stable throughout the follow-up period, five patients had a rather rapid decline in pulmonary function of more than 3% per year. The most rapid decline was seen in patient 11. In five years time pulmonary function deteriorated from 70 to 34% (annual decline per year of 7.2%), leading to respiratory failure and initiation of ventilatory support. In the next 10 years, a further decline of 12% was seen and he progressed to an end stage disease in which he was fully dependent on invasive ventilatory support.

FEV_1 recordings were obtained in 10 patients at the time of first visit. Mean FEV_1 was 2.75 l, corresponding with a predicted value of 79%.

In 10 patients VC was measured during the follow-up period in sitting as well as supine position. In all but one patient, VC decreased in supine position compared to the VC measured in sitting position. The mean difference in VC between sitting and supine position was -13% (range -34 to +4 %). In addition, two patients were unable to perform testing in the supine position due to the already severely compromised VC in sitting position (patient 3 and 11).

SKELETAL MUSCLE STRENGTH

By manual muscle testing a sumscore (range 0-40) was calculated of bilateral shoulder abductors, elbow flexors, hip flexors and knee extensors. Examinations were performed on two or more occasions in all 16 patients. In total, 120 measurements were available (mean 7.5 per patient, range 2-22) with a mean follow-up duration of 8 years. Mean first measured MRC sumscore was 36 (range 18-40). Two patients remained stable during the follow-up period, the other 14 patients all showed a gradual progression of weakness, with a mean decrease in MRC score of 0.5 points per year ($p<0.001$) (Figure 1B). In three patients (patient 9, 13 and 15), there seemed to be an initial phase of approximately six years in which no skeletal muscle weakness was present. After this period, weakness progressed in a linear way.

VARIABILITY IN CLINICAL COURSE

Multiple measurements of respiratory and muscle function were available in 13 patients, allowing us to individualize the course of the disease by comparing the rate of decline of both parameters. In nine patients, the decline in pulmonary function and muscle strength occurred more or less simultaneously and to the same extent. Two patients (patient 6 and 8) who had normal respiratory function and limb-girdle muscle strength at first investigation, showed a fairly stable condition. During the follow-up period of 13 and 19 years, little or no decline in respiratory function or muscle strength was detected. One patient (patient 10) showed only deterioration of muscle strength without decline in respiratory function. After a rapid decline in pulmonary function, aggravated by a progressive scoliosis (annual decline of 7.2%), patient 11 developed respiratory insufficiency at the age of 15. Shortly after corrective surgery, ventilatory support was initiated, while only mild proximal muscle weakness was present. Only seven years later he lost the ability to walk.

TABLE 1

Clinical characteristics and changes in pulmonary function and muscle strength during follow-up in patients with Pompe disease.

Pat. No.	Sex	Current age (yr)	Age first symptoms (yr)	Age at diagnosis (yr)	First complaints	Wheelchair use (age start)	Respiratory support (age start)	First measured VC (% predicted)	Last measured VC (% predicted)	Observation period VC (yrs)	Annual decline VC (%/yr)	First measured MRC score	Last measured MRC score	Observation period MRC score (yrs)	Annual decline MRC score (points/yr)
1	female	69	40	45	Difficulty running/doing sports and rising from a chair	Yes (64)	Yes (68)	86	47	12	-3.3	22	16	3	-2.0
2	male	66	31	43	Difficulty running/doing sports, climbing stairs and rising from a chair	Yes (61)	No	88	67	13	-1.6	37	26	18	-0.6
3	female	Died at age 54	22	24	Difficulty running/doing sports, climbing stairs and rising from a chair	Yes (44)	Yes (44)	23	13	10	-1.0	22	12	28	-0.6
4*	female	52	36	37	Difficulty running, climbing stairs, fatigue and muscle soreness	Yes (38)	No	111	54	15	-3.8	35	24	12	-0.9
5	female	51	31	36	Difficulty doing sports, walking, climbing stairs and rising from a chair	Yes (49)	No	96	77	13	-1.5	36	32	15	-0.3
6	female	49	34	34	Difficulty rising from supine position, sleeping difficulty, fatigue and muscle cramps	No	No	87	90	11	0.3	40	40	13	0
7*	male	47	40	43	Difficulty doing sports, climbing stairs, rising from supine position and fatigue	No	No	83	–	–	–	38	36	4	-0.5
8	female	Died at age 43	21	24	Fatigue, muscle soreness	No	No	87	96	10	0.9	40	38	19	-0.1
9**	female	40	23	33	Difficulty walking, fatigue and muscle cramps/soreness	Yes (35)	No	69	53	6	-2.7	40	30	17	-0.6
10*	female	40	24	25	Difficulty running, climbing stairs, fatigue and muscle soreness	No	No	105	115	15	0.7	38	32	15	-0.4
11	male	40	10	11	Difficulty running/doing sports, rising from supine position and raise the head	Yes (22)	Yes (15)	70	22	15	-3.2	33	18	22	-0.7
12	female	35	1	8	Difficulty running/doing sports, climbing stairs and raise the head	Yes (23)	Yes (20)	24	15	12	-0.7	32	18	14	-1.0
13	male	32	17	19	Difficulty walking, running, climbing stairs and fatigue	No	No	–	–	–	–	40	34	12	-0.5
14**	male	31	23	26	Difficulty riding a bike, muscle soreness	No	No	98	78	6	-3.3	40	36	10	-0.4
15***	male	30	23	15	Difficulty running, climbing stairs, rising from supine position and fatigue	No	No	92	78	2	-7.0	40	33	14	-0.5
16***	female	27	15	15	Muscle soreness, back ache	No	No	105	–	–	–	40	40	8	0

VC=vital capacity. Related patients are marked with one, two or three asterisks.

INTRAFAMILIAL VARIABILITY

In our study group we had three families with more than one affected member (patient 4, 7 and 10, patient 9 and 14, and patient 15 and 16 are siblings). Within these families, the clinical course of the disease was not remarkably different (Table 1).

DISCUSSION

Late-onset Pompe disease is known as a slowly progressive myopathy with or without respiratory involvement. However, information about the rate of disease progression in untreated patients, evaluated by physical examination, is largely lacking. In this study we longitudinally assessed the rate of deterioration in pulmonary function and proximal muscle strength in a group of 16 affected patients.

At a group level, we found a decline in respiratory function of 1.6% per year and a gradual progression of weakness of proximal skeletal musculature. Still, the rate and extent of disease progression varied. Although the yearly decline seems small, eight patients (50%) became wheelchair bound, three (19%) became respirator dependent, and two died during the follow-up period. Recently, we described a significant increase in level of handicap and the number of hours of respiratory support over a 2-year-period in a similar group of patients based on patient reported questionnaires.[17] The present study confirms these findings using long-term follow-up through physical assessments.

A recent study in German adolescents and adults with Pompe disease reported wheelchair dependency in 6 out of 18 patients (30%) and respirator dependency in 10 out of 18 patients (56%) during a similar long observation period of 15 years.[19] The mean age at onset and diagnosis was somewhat lower in our group (24 and 27 years compared to 31 and 40 years). This may be explained by the greater awareness of Pompe disease in the Netherlands as a result of longstanding interest in the condition, which was first described in 1932 by the Dutch pathologist Dr. J.C Pompe.[21] The time between symptom onset and wheelchair or respirator use was alike (18 and 19 years compared to 18 and 15 years). In addition to these general results, our study quantifies the rate of progression of the disease and emphasizes individual differences in the course of the condition.

Two thirds of patients showed a simultaneous decline in MRC score and respiratory function. One third of patients had a more variable, sometimes extremely fast, disease course. This was demonstrated by one of our patients who had a rapid deterioration of pulmonary function leading to respiratory insufficiency and use of artificial ventilation, five years before suffering marked loss of strength in proximal limb muscles. This independent deterioration of pulmonary function without muscular weakness has been described earlier.[16, 18] In fact, respiratory insufficiency is reported as presenting symptom in 2% of all patients with late-onset Pompe disease.[7, 22, 23] This stresses the importance of regularly monitoring pulmonary function in all patients with Pompe disease, even if there are only mild locomotor problems.

Diaphragmatic weakness, manifested as a difference between pulmonary function in sitting and supine position of more than 20% ("postural drop"), is an important feature in Pompe disease.[23, 24] Two of our patients showed a postural drop of more than 20%, while five other patients had a decrease in VC between 10% and 20%. Follow-up of respiratory

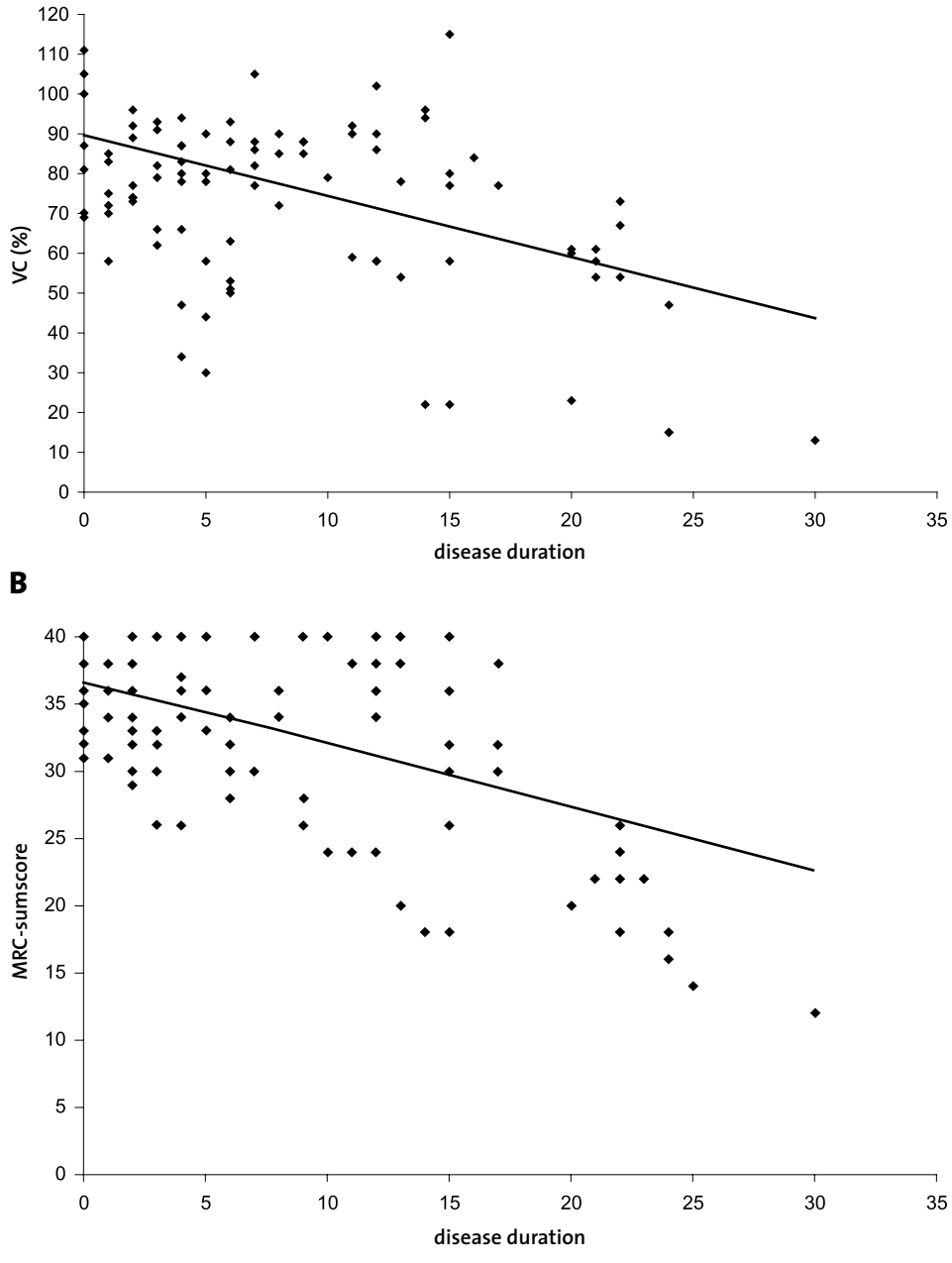

FIGURE 1

Rate of decline in vital capacity (percentage predicted) (A) and MRC sumscore (B) related to disease duration measured from time of diagnosis in 16 patients with late-onset Pompe disease. Squares represent measured values. The line represents the mean regression line at a group level.

function should therefore include measurement of pulmonary function in supine position to detect whether there is a need for (nightly) ventilation. Based upon the results of this study and earlier reports,[16, 23] we recommend annual measurement of pulmonary function in patients with a VC ≥ 80%, and measurement every six months in patients with a VC < 80% or a rapidly progressive weakness. Timely initiation of noninvasive ventilation may help to maintain the physical functioning of the patients and to prevent acute respiratory failure.

There are some methodological aspects which may affect the estimated rate of decline. First, to evaluate proximal skeletal muscle strength, manual muscle testing was performed. Because the MRC scale is not a linear scale, and especially because MRC grade 4 represents a wide range of actual strength, the decline may have been underestimated in the group of patients we studied. To assess muscle strength in more detail, quantitative muscle testing should be considered in future follow-up studies. In addition, to investigate the impact of weakness on the physical functioning of the patient, it would also be useful to assess muscle function. Second, two patients (patient 3 and 12) already had a severely compromised pulmonary function when first investigated in our centre. During the follow-up period of about 10 years, these two patients showed an annual decline of approximately 1%. It is plausible that the initial decline will have been faster. This may cause a small underestimation of the progression rate at a group level. Third, all patients except one had the c.-32-13T>G (IVS1-13T>G) mutation in combination with a null allele. The c.-32-13T>G mutation is the most common mutation in patients with late-onset Pompe disease, occurring in 68-90% of all patients with milder phenotypes.[25-28] The estimated rate of disease progression may have been influenced by the composition of the study group, since patients with different genotypes might have a different rate of decline. Nevertheless, we found considerable variation in the onset of symptoms and in clinical severity, suggesting once more that the phenotype is modulated by secondary factors.[26, 27]

Due to the retrospective nature of the study, the number of measurements and the follow-up period varied per patient. Given the rate of decline, we decided to monitor all patients with Pompe disease every 6 to 12 months depending on the patient's condition.

At this moment, data on the effect of ERT in late-onset Pompe disease is limited, and the extent to which pulmonary function and skeletal muscle strength can be restored is unknown. Accurate follow-up before and after start of treatment will help to evaluate the long-term effects of ERT in patients with different stages of disease.

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3.2

Clinical features and predictors for disease progression in adults with Pompe disease: a nationwide prospective observational study

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ABSTRACT

Background Due partly to physicians' unawareness, many adults with Pompe disease are diagnosed with great delay. Besides, it is not well known which factors influence the rate of disease progression, and thus disease outcome. We delineated the specific clinical features of Pompe disease in adults, and mapped out the distribution and severity of muscle weakness, and the sequence of involvement of the individual muscle groups. Furthermore, we defined the natural disease course and identified prognostic factors for disease progression.

Methods We conducted a single-centre, prospective, observational study. Muscle strength (manual muscle testing, and hand-held dynamometry), muscle function (quick motor function test), and pulmonary function (forced vital capacity in sitting and supine positions) were assessed every 3–6 months and analyzed using repeated-measures ANOVA.

Results Between October 2004 and August 2009, 94 patients aged between 25 and 75 years were included in the study. Although skeletal muscle weakness was typically distributed in a limb-girdle pattern, many patients had unfamiliar features such as ptosis (23%), bulbar weakness (28%), and scapular winging (33%). During follow-up (average 1.6 years, range 0.5–4.2 years), skeletal muscle strength deteriorated significantly (mean declines of -1.3% point/year for manual muscle testing and of -2.6% points/year for hand-held dynamometry; both $p<0.001$). Longer disease duration (>15 years) and pulmonary involvement (forced vital capacity in sitting position <80%) at study entry predicted faster decline. On average, forced vital capacity in supine position deteriorated by 1.3% points per year ($p=0.02$). Decline in pulmonary function was consistent across subgroups. Ten percent of patients declined unexpectedly fast.

Conclusions Recognizing patterns of common and less familiar characteristics in adults with Pompe disease facilitates timely diagnosis. Longer disease duration and reduced pulmonary function stand out as predictors of rapid disease progression, and aid in deciding whether to initiate enzyme-replacement therapy, or when.

BACKGROUND

Pompe disease is a rare autosomal recessive metabolic disorder, whereby mutations in the *GAA* gene lead to partial or total absence of the lysosomal enzyme acid α-glucosidase. The disease presents as a spectrum of phenotypes, ranging from a rapidly fatal phenotype in infants[1] to slower progressive phenotypes in older children and adults.[2,3] Many adults with Pompe disease are diagnosed late in life, when they are already in an advanced stage of the disease. While this may be due to clinicians' unawareness, it may also be explained by similarities in clinical presentation with other 'limb-girdle' diseases such as the limb-girdle muscular dystrophies (LGMD), Becker muscular dystrophy, or metabolic myopathies.[4] Our first objective was to optimize future diagnosis in this patient group by classifying their specific clinical features, and by delineating the distribution and severity of muscle weakness and the sequential involvement of individual muscle groups during the course of the disease.

Since 2006, enzyme-replacement therapy (ERT) with recombinant human acid α-glucosidase has been approved for the treatment of Pompe disease. In infants, treatment generally improves cardiorespiratory function and motor function, and prolongs survival.[5-8] In older children and adults ERT was shown to improve or stabilize skeletal muscle strength, muscle function and respiratory function. However, the magnitude of the therapeutic response varies between individual patients.[9-14] To fully assess the effects of enzyme therapy, and to decide whom to treat and when to start treatment, it is necessary to be optimally informed about the course of disease prior to treatment, and about factors influencing disease progression. We thus prospectively studied the natural disease course, and aimed to identify prognostic factors for faster disease progression and poor outcome in a large cohort of adult Pompe patients.

METHODS

PARTICIPANTS AND STUDY DESIGN

We performed a single-centre, prospective, cohort study, in which participation was open to all adults diagnosed with Pompe disease who had not yet received treatment with enzyme-replacement therapy. Their diagnosis was confirmed by acid α-glucosidase assay in leukocytes or fibroblasts and by mutation analysis. All patients were seen between October 2004 and August 2009 at Erasmus MC University Medical Center, the designated centre of expertise for Pompe disease in the Netherlands. The interval between visits was three to six months. Patients were recruited either through neuromuscular centres in the Netherlands and Belgium, through the Dutch neuromuscular patient organization, or were referred to our Center by their treating physicians. The research protocol was approved by the Central Committee on Research Involving Human Subjects in the Netherlands (CCMO). All patients provided written informed consent.

Seven patients participated in the placebo arm of the randomized, placebo controlled trial on the safety and efficacy of alglucosidase alfa in late-onset Pompe disease.[13] Data on these patients collected during this period are included in the present analyses. We have previously reported long-term retrospective data on muscle strength and pulmonary

function in 16 patients with Pompe disease.[15] While 12 of these patients participated in the current study, the present analyses were based solely on new, prospectively obtained data.

PROCEDURES

We gathered information on the following: 1) the nature of first symptoms and the age at which these had presented; 2) the age at which the diagnosis had been made; 3) duration of disease since onset of first symptoms; 4) the presence of specific clinical features such as scoliosis, bulbar involvement (defined as weakness of muscles involved in speech, chewing and swallowing), winging of the scapula (defined as a clearly visible protrusion of the scapula when the patient was in a resting position or was lifting the arms anteriorly or sidewards), muscle atrophy, or ptosis; 5) the use of a wheelchair or walking aids; 6) skeletal muscle strength; 7) the use of mechanical ventilatory support; 8) the number of hours of ventilatory support per day; 9) pulmonary function; 10) cardiac function; 11) acid α-glucosidase activity in leukocytes and fibroblasts; 12) serum creatine kinase (CK); and 13) type of *GAA* mutation.

Skeletal muscle strength and muscle function

By manual muscle testing using the Medical Research Council (MRC) grading scale[16] (range 0–5; all patients were examined by NvdB or JdV), we measured 25 different muscle groups throughout the body to define the distribution of skeletal muscle weakness and the severity of involvement of the separate muscle groups. We calculated a sumscore (range 0–130) for the muscle groups that were involved most: neck extensors, neck flexors, and bilateral shoulder adductors, shoulder abductors, shoulder exorotators, shoulder endorotators, elbow flexors, elbow extensors, hip extensors, hip flexors, hip abductors, hip adductors, knee flexors and knee extensors. This score was subsequently converted to a percentage of the maximum possible score. Although the abdominal muscles and trunk muscles were frequently involved, we did not include these muscle groups in the MRC sumscore since they were difficult to grade.

Hand-held dynamometry (HHD) (Cytec dynamometer, Groningen, the Netherlands) was used as a second measure of muscle strength to examine 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. The value (Newton) measured in each muscle group was expressed as a percentage of the median strength of healthy females and males,[17] and then combined into a sumscore by averaging these for all 16 muscle groups, producing a score between 0 and 100 percent.

Muscle function was assessed using the Quick Motor Function Test (QMFT).[18] A total score (range 0–64) was obtained by adding the scores of all items. This was then expressed as a percentage of the maximum score.

Pulmonary function

Forced vital capacity (FVC) was measured using a Lilly type pneumograph (Viasys Healthcare, Würzburg, Germany) or the KoKo spirometry system (Ferraris Respiratory, Louisville, USA) with the patient in upright seated and supine positions, according to ATS/ERS standards.[19] Results were expressed as a percentage of predicted normal values.[20]

A measured value below 80% of the predicted value was considered to be abnormally low. Seven male patients who were invasively ventilated were artificially allotted a FVC value of 10% – just below the least observed value – since their omission might have led to biased results. These seven patients were however excluded from the longitudinal analysis.

STATISTICAL ANALYSIS

Baseline characteristics are summarized using descriptive statistics. Differences between males and females, and between patients with and without scapular winging, were assessed using χ^2 tests (wheelchair use, use of mechanical ventilation, and presence of scoliosis, bulbar muscle weakness, scapular winging or ptosis) or Mann-Whitney tests (strength of individual muscle groups, MRC sumscore, HHD sumscore, QMFT sumscore, and FVC measured in sitting and supine positions). We used the Spearman's rank correlation coefficient (ρ) to calculate the relationships between residual enzyme activity and rate of decline in muscle strength and pulmonary function, and between serum CK activity and age, muscle atrophy and disease duration. Longitudinal analysis of muscle strength and pulmonary function was performed using repeated measures ANOVA (random coefficient models). The annual changes are expressed in absolute percentage points (pp/y). For subgroup analyses, patients were divided into groups on the basis of gender (male, female); wheelchair use (yes, no); use of mechanical ventilation (yes, no); age at first study visit (<50 or ≥50 years; taking the median as the cut-off point); disease duration (<15 or ≥15 years; taking the median as the cut-off point); MRC/HHD sumscore at study entry (categorization in tertiles); and FVC in sitting position at study entry (<80 or ≥80 % predicted). 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

STUDY POPULATION

In order of referral, we included 91 adult Pompe patients from the Netherlands – representing virtually all known patients in the Netherlands – and three patients from Belgium. On average, there was a seven-year delay between the first noted symptoms of Pompe disease and the actual diagnosis. The characteristics of the study population are summarized in table 1.

BASELINE MEASUREMENTS

Characteristic clinical features

A substantial number of patients had less familiar features of Pompe disease, such as bulbar muscle weakness (28%), prominent scapular winging (33%, Figure 1b), or ptosis – not accompanied by external eye-movement disturbances (23%; Figure 1c). Seventy-one percent of patients with scapular winging had bulbar muscle weakness, against 37% without scapular winging (p=0.001).

TABLE 1

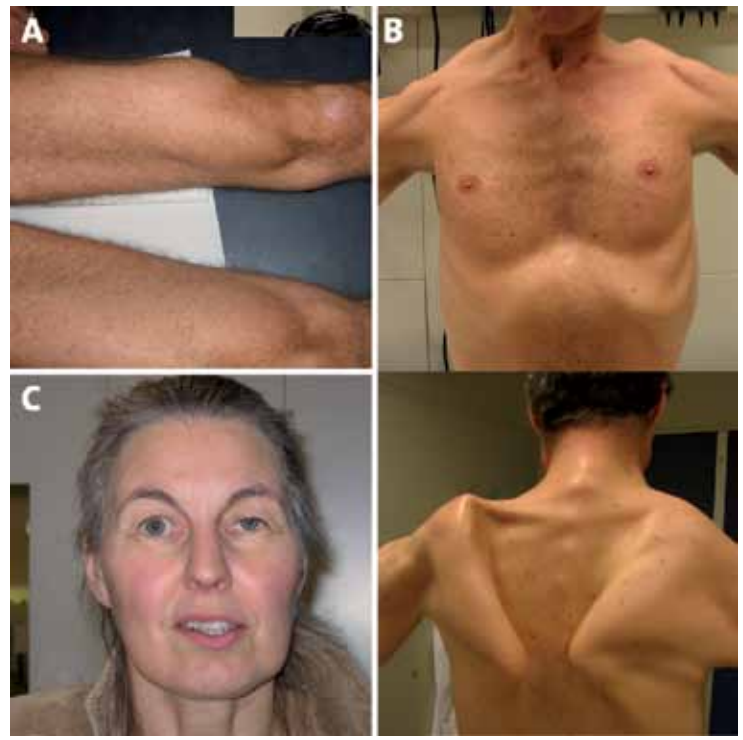
Characteristics of the study population (n=94)[a]

General characteristics	
Gender (males)	48 (51%)
Age at first study visit (years)	51.1 (38.3–60.6)
Age at onset of symptoms (years)	32.0 (25.5–40.0)
< 18 years	10 (11%)
≥ 18 years	84 (89%)
Age at diagnosis (years)	40.2 (32.7–50.2)
Disease duration since onset of first symptoms at first study visit (years)	15.3 (7.7–24.7)
Time since diagnosis at first study visit (years)	9.2 (0.6–16.0)
0 to 5 years	41 (44%)
5 to 10 years	15 (16%)
10 to 15 years	10 (11%)
> 15 years	28 (30%)
Use of walking aids	14 (15%)
Wheelchair use	30 (32%)
Age at start of wheelchair use (years)	49.0 (43-56)
Use of mechanical ventilation[b]	27 (29%)
Age at start of mechanical ventilation (years)	48 (38.5–57.5)
First symptoms noted[c]	
Skeletal muscle weakness	93 (99%)
Difficulty running	30 (32%)
Difficulty performing sports	22 (23%)
Difficulty climbing stairs	24 (26%)
Difficulty walking	15 (16%)
Difficulty rising from an armchair	11 (12%)
Difficulty rising from a lying position	9 (10%)
Fatigue	17 (18%)
Muscle soreness / cramps	16 (17%)
Respiratory failure	1 (1%)
Clinical features	
Ptosis	22 (23%)
Bulbar muscle weakness[d]	26 (28%)
Scapular winging[d]	31 (33%)
Scoliosis	22 (23%)
Increased lumbar lordosis	62 (66%)
Prominent muscle atrophy	53 (56%)
Shoulder girdle / upper arms	25 (27%)
Trunk muscles	27 (29%)
Pelvic girdle / Upper leg (Figure 1a)	40 (43%)

Laboratory parameters	
CK (U/l)	
Males	449 (279–1040)
Females	493 (237–715)
α-glucosidase activity in leukocytes (nmol glucose/h/mg protein)[e]	1.2 (0.4–2.2)
α-glucosidase activity in fibroblasts (nmol 4-MU/h/mg protein)[f]	13.0 (11.0–15.0)
Genotype	
c.-32-13T>G / very severe or potentially less severe pathogenic mutation	92 (98%)
c.671G>A / c.525del	1 (1%)
unknown	1(1%)

4-MU=4-methylumbelliferyl-α-D-glucopyranoside

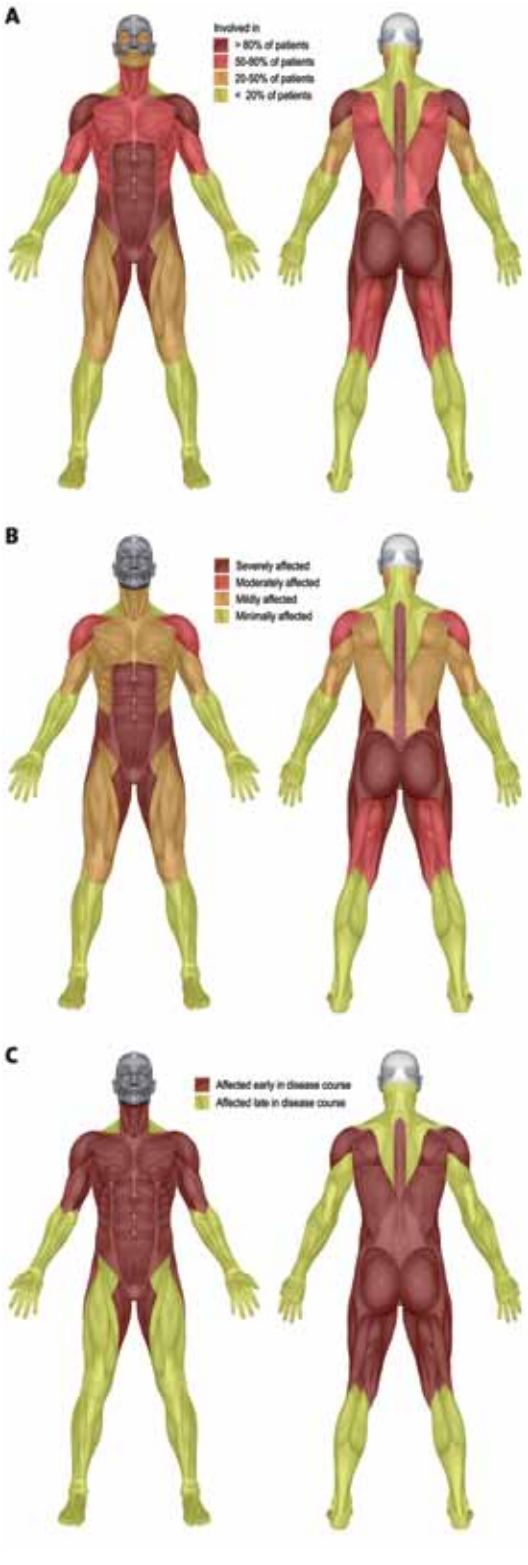
[a] Data are number (%) or median (IQR). [b] More men then women used mechanical ventilation (p=0.009). [c] Two or more complaints were counted if these occurred within the same year. [d] More men than women had scapular winging and bulbar muscle weakness (p=0.001 and p=0.05). [e] control range 48 to 215 nmol glucose/h/mg protein. [f] control range 45 to 180 nmol 4-MU/h/mg protein.



◂ FIGURE 1

Clinical features in Pompe disease

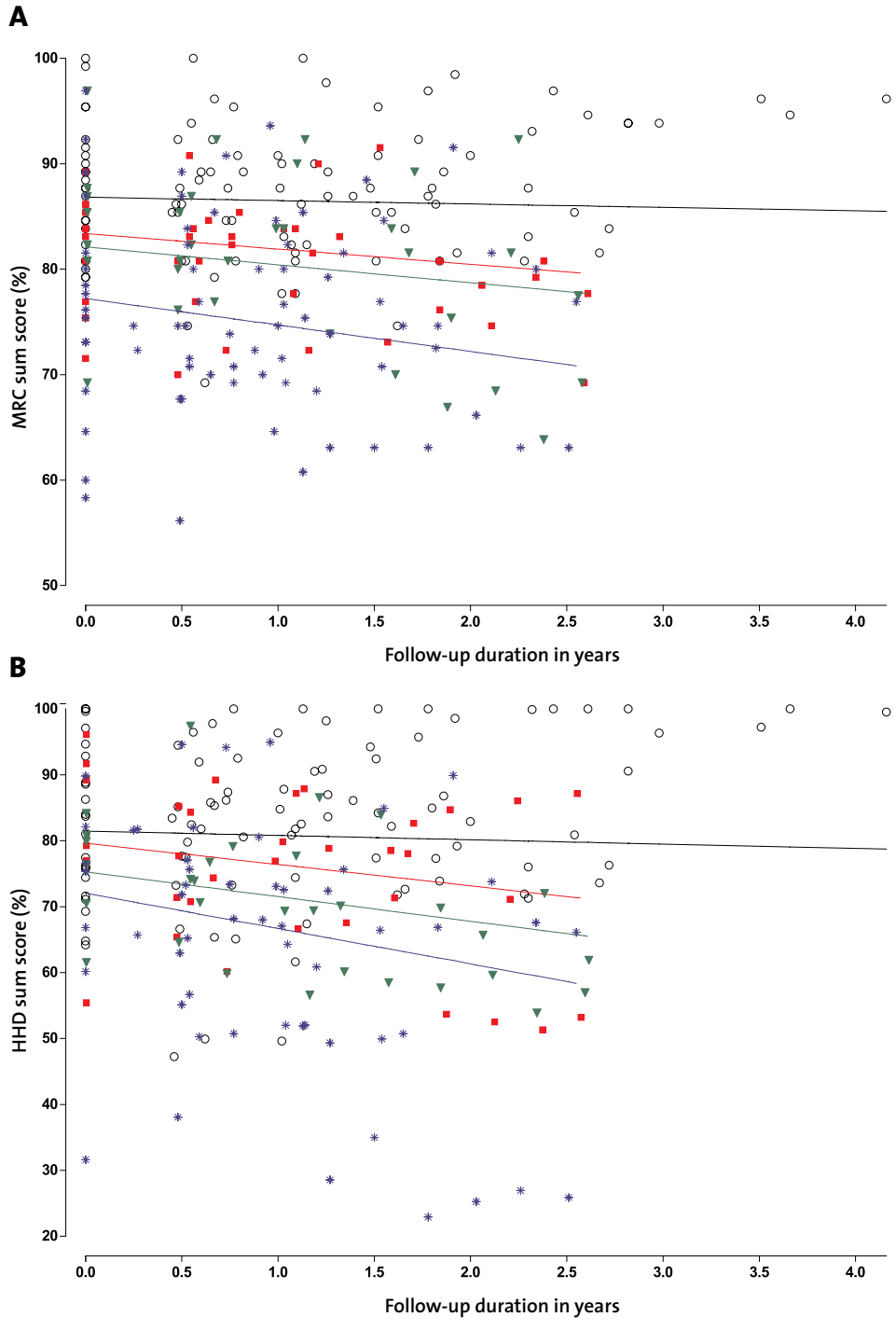
Atrophy of the quadriceps muscle (A), scapular winging (B), and ptosis (C) as notable clinical features in adults with Pompe disease. Photographs are printed with permission of the patients.



◂ FIGURE 2

Muscle weakness in adults with Pompe disease

Distribution of skeletal muscle weakness (A), severity of muscle weakness of the individual muscle groups (B), and involvement of the individual muscles over time (C) in 94 adults with Pompe disease.



► FIGURE 3

Longitudinal changes in muscle strength

Rate of disease progression measured by manual muscle testing (MRC sumscore) (A) and hand-held dynamometry (HHD sumscore) (B) related to follow-up duration measured from time of inclusion in the study for 66 adults with Pompe disease. The figure shows the measured values and regression lines at group level for the following subgroups: 1) Patients with normal pulmonary function (FVC ≥80% predicted) and disease duration <15 years (circles, black line); 2) patients with normal pulmonary function (FVC ≥80% predicted) and disease duration ≥15 years (red squares, red line); 3) patients with abnormal pulmonary function (FVC <80% predicted) and disease duration <15 years (green triangles, green line); and 4) patients with abnormal pulmonary function (FVC <80% predicted) and disease duration ≥15 years (blue asterisks, blue line).

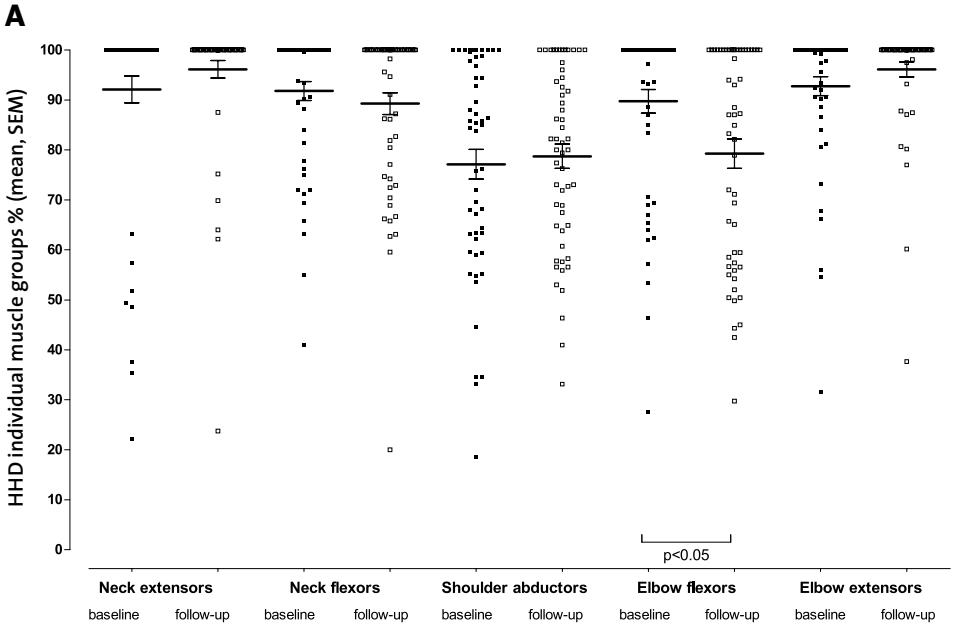


FIGURE 4

Muscle strength in individual muscle groups measured by hand-held dynamometry

Values for individual patients are shown at baseline (black squares) and during follow-up (last measured value) (open squares). Mean and standard error of the mean are given for each muscle group.

Clinical distribution and severity of muscle weakness, and sequence of muscle involvement during the disease course

Shoulder abductors, abdominal muscles, paraspinal muscles, hip flexors, hip extensors, hip adductors, and hip abductors were affected in more than 80% of all patients (Figure 2a). The strength of the quadriceps muscle was reduced in only 55% of patients. The muscles of the hands and feet were relatively spared, being affected in under 10% of patients. Abdominal muscles, paraspinal muscles – with exception of the neck extensors and neck flexors –, hip flexors, hip extensors, hip adductors and hip abductors were the most severely affected muscles (Figure 2b). The pattern of muscle weakness was symmetrical in 95% of patients, and the distribution of weakness did not differ between males and females. Relative to patients without scapular winging, patients with scapular winging had more severe involvement of the shoulder girdle musculature (trapezius muscle, deltoid muscle, pectoral muscle, shoulder exorotators, and shoulder endorotators; all $p<0.02$). The 'limb-girdle' and trunk muscles were affected early in the course of the disease, while the distal muscle groups – if they were involved at all – were affected late in the course of the disease (Figure 2c).

Cardiac evaluation

In a subset of 51 patients cardiac evaluation was performed, comprising electrocardiography, Holter monitoring, two-dimensional echocardiography, low-dose dobutamine stress echocardiography, and tissue Doppler imaging. One patient had a mild hypertrophic cardiomyopathy, while four other patients had minor cardiac abnormalities that could be attributed to advanced age, hypertension or preexisting cardiac pathology unrelated to Pompe disease.[21,22]

Laboratory parameters

Most patients had moderately increased serum CK; in two patients it was more than 10 times the upper limit of normal, while 10 had a normal serum CK. Serum CK activity was moderately inversely associated with age ($\rho=-0.71$, $p<0.001$), disease duration ($\rho=-0.45$, $p<0.001$), and the presence of muscle atrophy ($\rho=-0.53$, $p<0.001$).

PROSPECTIVE FOLLOW-UP

General aspects

Prospective follow-up data for a period longer than six months were available for 66 patients (median 1.6 years, range 0.5–4.2 years), 52 of whom were followed for longer than one year, and 22 of whom for longer than two years. Within the follow-up period, one patient became wheelchair bound, mechanical ventilation was initiated in four patients, and eight patients needed to increase their number of hours of ventilation per day. One severely affected patient died due to pneumonia complicated by respiratory failure.

Disease progression and predictors for disease outcome

Muscle strength and muscle function

At baseline, the MRC sumscore ranged from 39.2% to 100% (median 84.7%, one patient had the maximum possible score). During follow-up, the MRC sumscore deteriorated by 1.3% points per year on average ($p<0.001$; Figure 3a). Baseline values for hand-held dynamometry

ranged from 31.6% to 100% (median 77.0%, three patients had the maximum score). Within the observed follow-up period, HHD sumscore deteriorated by 2.6% points per year (p<0.001; Figure 3b). With regard to the individual muscle groups, strength declined significantly in the elbow flexors, hip abductors, knee extensors and knee flexors, ranging from -6.9 pp/y in elbow flexors to -2.5 pp/y in knee extensors (Figure 4). Subgroup analysis (table 2) revealed that the decline was faster in patients with a reduced pulmonary function at baseline (FVC <80%) than in those with normal pulmonary function (-2.2 pp/y against -0.6 pp/y for MRC sumscore (p=0.01), and -4.5 pp/y against -1.4 pp/y for HHD sumscore (p<0.01)), and in patients who had had the disease for longer than 15 years compared to those who had been ill less long (-2.1 pp/y against -0.7pp/y for MRC sumscore (p=0.04), and -4.2 pp/y against -2.0 pp/y for HHD sumscore (p<0.01)). Baseline QMFT scores ranged from 16.7% to 100% (median 63.7%). The changes that were found in muscle

TABLE 2

Disease progression in 66 adults with Pompe disease: annual changes in muscle strength and pulmonary function across various subgroups

	MRC sumscore			HHD sumscore			QMFT score			FVC sitting position			FVC supine position		
Total group	**n**	**Annual change (%/year)**	**95% CI**	**n**	**Annual change (%/year)**	**95% CI**	**n**	**Annual change (%/year)**	**95% CI**	**n**	**Annual change (%/year)**	**95% CI**	**n**	**Annual change (%/year)**	**95% CI**
	65	-1.30	-1.95 to -0.66	55	-2.6	-3.72 to -1.45	62	0.05	-0.76 to 0.86	59	-1.04	-2.14 to 0.06	54	-1.30	-2.42 to -0.19
Subgroups	**n**	**Annual change (%/year)**		**n**	**Annual change (%/year)**		**n**	**Annual change (%/year)**		**n**	**Annual change (%/year)**		**n**	**Annual change (%/year)**	
Gender															
Female	36	-1.41		31	-2.97		37	-0.22		36	-1.38		33	-2.27	
Male	29	-1.08		24	-1.99		25	0.57		23	-0.45		21	0.35	
Age at study entry[a]															
< 50 years	34	-1.39		31	-2.06		33	-0.17		33	-1.34		31	-2.22	
≥ 50 years	31	-1.21		24	-3.29		29	0.30		26	-0.69		23	-0.08	
Disease duration at study entry[a]															
< 15 years	34	**-0.70**[c]		31	**-1.95**[e]		34	-0.07		35	-1.36		33	-1.37	
≥ 15 years	31	**-2.09**[c]		24	**-4.18**[e]		28	0.17		24	-0.60		21	-1.16	
FVC (sitting) at study entry															
≥ 80% predicted	33	**-0.62**[d]		32	**-1.43**[f]		33	-0.21		33	-1.09		31	-1.59	
< 80% predicted	32	**-2.21**[d]		23	**-4.52**[f]		29	0.31		26	-0.89		23	-0.92	
Ventilation at study entry															
No	49	-1.12		46	-2.50		50	0.24		50	-1.30		47	-1.50	
Yes	16	-1.83		9	-3.55		12	-0.70		9	1.07		7	0.32	
Wheelchair use at study entry															
No	47	-0.94		44	-2.28		47	-0.39		47	-0.98		43	-0.99	
Yes	18	-2.36		11	-3.92		15	1.44		12	-1.22		11	-2.57	
MRC/HHD sum score at study entry[b]															
< 33rd percentile	18	-1.27		10	-2.06		20	0.49		12	-0.30		9	-1.59	
33rd to 66th percentile	23	-1.31		22	-4.34		23	-0.06		23	-2.40		21	-2.01	
≥ 66th percentile	24	-0.98		23	-1.56		19	-0.41		24	-0.34		24	-0.63	

MRC=Medical Research Council, HHD=Hand-Held Dynamometry, QMFT=Quick Motor Function Test, FVC= Forced Vital Capacity, CI=confidence interval. [a] the median was taken as the cut-off point. [b] categorization in tertiles. n represents the number of patients for whom data were available for each specific measurement. For muscle strength, no sumscore was calculated if measurements for three or more individual muscle groups were missing (e.g. due to severity of muscle weakness or injury). For seven invasively ventilated patients no reliable measurements of FVC could be performed. Neither could testing in the supine position be endured by 5 patients whose pulmonary function was already severely restricted in sitting position.

Data shown are mean annual changes (percentage points per year) as calculated by repeated measures ANOVA. For MRC sumscore and HHD sumscore there were significant differences between groups based on disease duration and FVC at study entry: c) p=0.04, d) p=0.01, e) p<0.01, and f) p<0.01.

strength were however not reflected in changes in the QMFT (annual change 0.05% points, p=0.9). In no subgroups was a significant decline observed.

Pulmonary function

At baseline, FVC measured in sitting position was reduced (<80% of the predicted value) in 56 patients (60%). The reduction in FVC was more prominent in males than in females (mean FVC 57.5 % predicted against 80.3 % predicted, p<0.001). Patients with scapular winging had significantly lower FVC than those without scapular winging (mean FVC 50.7% predicted against 84.5% predicted, p<0.001). In supine position, 76 patients (80%) had a reduced FVC. Changing from a sitting to a supine position, FVC fell in 21 patients by over 25%, indicating possible diaphragmatic weakness. Neither was testing in the supine position attempted in 12 patients whose pulmonary function was already severely restricted in sitting position. We have recently reported more detailed data on pulmonary function in some of the study cohort.[23] The mean yearly change in FVC measured in supine position was 1.3% points (p=0.02), and for FVC in upright position -1.0% points (p=0.06) (Figure 5). The rate at which pulmonary function declined was consistent across subgroups.

There was no significant association between the change in muscle strength or pulmonary function and residual enzyme activity (Spearman's rho for MRC sumscore -0.21, p=0.14; for HHD sumscore -0.51, p=0.74; for FVC in upright position 0.07, p=0.65; and for FVC in supine position -0.32, p=0.84).

Disease course variability

In 59 patients progress of muscle weakness and pulmonary dysfunction could be compared. In nine patients (15%; 4 males, 5 females), pulmonary function and muscle strength did not decline during the prospective follow-up period. Relative to the total group, they had a shorter disease duration from onset of symptoms (7.3 years against 15.5 years, p=0.03). At baseline, the following had all been higher, though not significantly: FVC in sitting position (88.1% against 78.6%); FVC in supine position (80.4% against 61.1%); MRC sumscore (87.2% against 83.4%); and HHD sumscore (86.2% against 76.3%). In 28 patients (47%), pulmonary function and skeletal muscle strength declined at a similar rate. In 22 patients (37%) the course of pulmonary function and muscle weakness followed different courses, one deteriorating while the other remained stable, or one deteriorating faster than the other. Of the 50 patients who deteriorated during the follow-up period, eight (2 males, 6 females) had a relatively fast decline – more than 5 percentage points per year – in muscle strength, while a rapid decline in pulmonary function was seen in five patients (1 male, 4 females). There were no significant differences between these groups with regard to sex, age, age at onset of symptoms, duration of disease, length of prospective follow-up, level of residual enzyme activity, and the severity of pulmonary or skeletal muscle involvement at the start of the study.

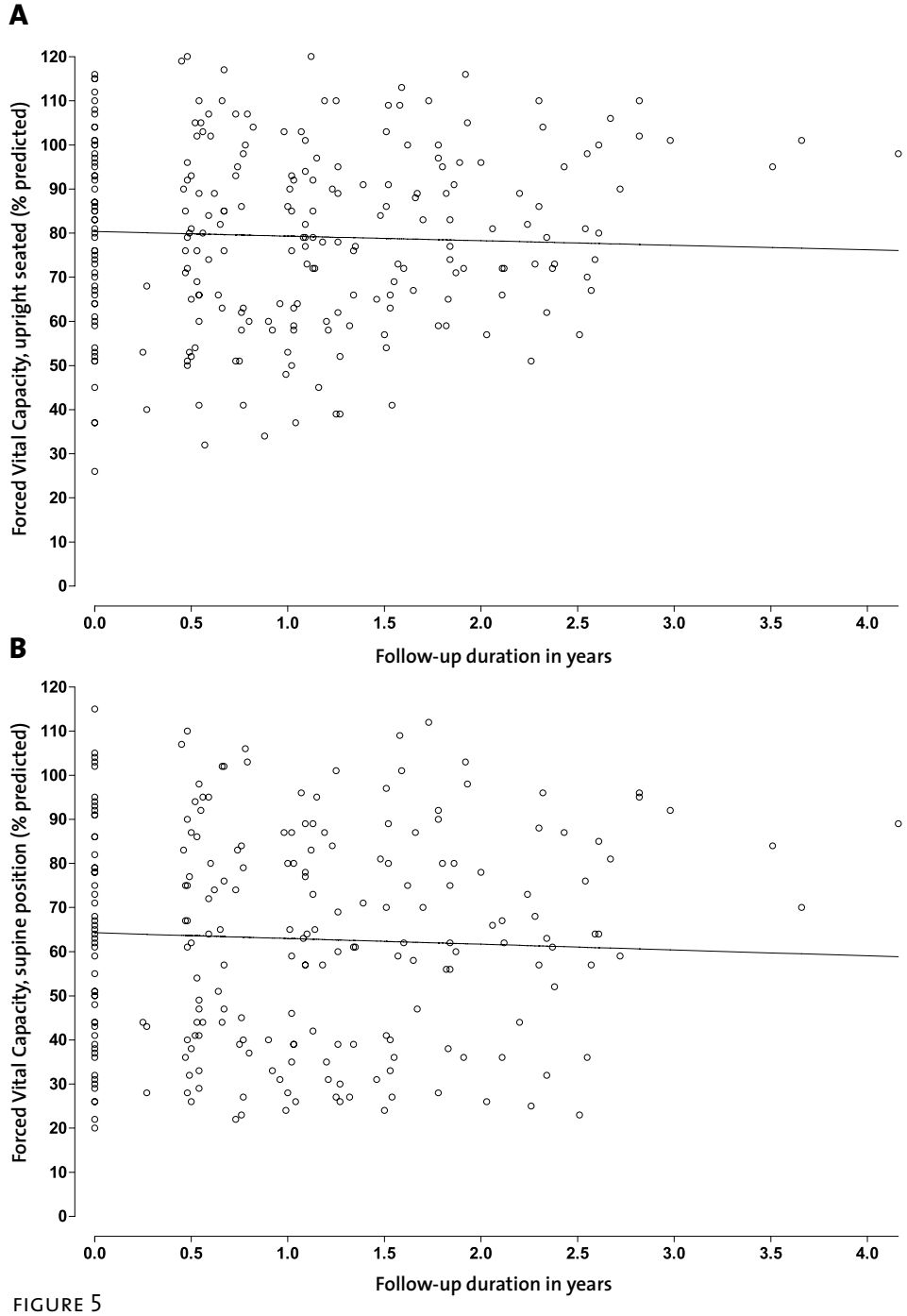


FIGURE 5

Longitudinal changes in pulmonary function

Decline in pulmonary function in the upright seated (A) and supine (B) positions related to follow-up duration. Circles represent the measured values, the line represents the mean regression line at a group level.

DISCUSSION

CHARACTERISTIC CLINICAL FEATURES AND PATTERN OF MUSCLE WEAKNESS

Our study in 94 adults with Pompe disease – which included virtually all known adult patients in the Netherlands – shows that, generally speaking, muscle weakness typically fits a pattern of limb-girdle myopathy. Interestingly, our findings about the distribution of muscle weakness, based on clinical examinations, match those of CT and MRI studies.[24-26] We found weakness of the quadriceps muscle in 55% of patients only. This may have been due to our method of measuring muscle strength: whereas the advantage of the MRC score and HHD lie in the fact that they are easy for the clinician to use, quantitative muscle testing using QMT[13,27] may be more reliable in patients with only minor loss of strength.

Besides limb-girdle weakness, many patients had less familiar features, which are sometimes symptomatic of other neuromuscular diseases. Though ptosis has been reported in the literature in no more than seven adult patients to date,[28-31] it was found in almost one quarter of our patients. Notably, while one might expect myogenic ptosis to be bilateral, it was unilateral in two-thirds of our patients.

Despite the fact that bulbar muscle weakness is reported only occasionally in adult Pompe patients,[28,32] twenty-eight percent of the patients in our cohort had distinct bulbar muscle weakness. These patients are at risk of pulmonary complications.

One third of patients had prominent scapular winging, which is in line with several recent reports.[32,33] Many of these patients also had bulbar muscle involvement and severer weakness of the shoulder-girdle muscles than patients without scapular winging; these features are reminiscent of FSHD. If patients with such a "pseudo-FSHD" phenotype are overlooked, diagnosis may be delayed. However, the asymmetrical distribution of muscle weakness, the relatively common involvement of the distal muscles of the lower limbs, and the absence of severe or relatively severe pulmonary involvement in FSHD will generally allow the two diseases to be diagnosed correctly.[34]

Serum CK activity was normal in 10 patients, confirming that a normal serum CK does not rule out Pompe disease.[35-37] In early stages of the disease, however, normal serum CK is quite uncommon. Although we did not systematically perform electromyography or muscle biopsies, it is well known that these may reveal no or only non-specific abnormalities in a considerable number of patients.[38,39] Therefore, measurement of acid α-glucosidase activity and mutation analysis remain essential to confirm the diagnosis.

NATURAL DISEASE COURSE

Although the average follow-up duration of 19 months seems rather short for a chronic disorder such as Pompe disease, it is the longest prospective follow-up ever carried out in "late-onset" Pompe disease. Moreover, as many patients are now treated with enzyme-replacement therapy, prospective data on the natural course of the disease over a longer period of time are now impossible to obtain.

We found a significant worsening of muscle strength, reflected by declines in MRC sumscore (mean decline -1.3 pp/y) and HHD sumscore (mean decline -2.6 pp/y). Although we found significant changes in muscle strength and some patients became wheelchair or ventilator dependent during follow-up, the QMFT did not indicate a deterioration in limb-girdle muscle function. There may be two main reasons for this. First, the decline in muscle strength may have been too small to cause changes in functional daily activities within the observed time-span. Second, although the QMFT showed a good discriminative ability and good responsiveness to change after start of enzyme-replacement therapy, it may not be sensitive enough to reflect minor changes in strength in functional changes during the natural course.[18]

Mechanical ventilation became necessary in four of the 67 hitherto un-ventilated patients, eight patients needed to increase their number of hours of ventilation, and one patient died due to respiratory complications. Despite this indication that pulmonary function clearly worsened during the time-span of the study, our findings regarding the yearly decline seem somewhat lower than those of other studies.[13,27] This may have been due to our method of patient selection: in the present study participation was open to all adult patients with a confirmed diagnosis of Pompe disease, including those with very limited pulmonary function who were invasively ventilated 24 hours a day. Although this reflects the spectrum of disease encountered in daily practice, one would expect further deterioration of pulmonary function in these patients to be minimal, thereby partly obscuring decline in the total group. Secondly, when enzyme-replacement therapy became commercially available, we decided first to treat the most severely affected patients and patients with a rapid decline in pulmonary function and muscle strength, while patients with a slow disease course were started on ERT at a later stage. As a result, the length of prospective follow-up for the most severely affected patients was somewhat shorter (1.1 years on average) than for the least affected patients (1.7 years on average, the longest follow-up being 4.2 years). Another factor that could have influenced the estimated rate of decline is the fact that data on seven patients who participated in the placebo arm of the randomized controlled trial on the safety and efficacy of alglucosidase alfa in late-onset Pompe disease were included in the present analyses. However, a second analysis, excluding these patients to overcome a possible placebo effect, led to similar estimates of the rate at which muscle weakness and pulmonary dysfunction progress (not shown).

It should be noted that all patients except one had the c.-32-13T>G (IVS1-13T>G) mutation in combination with a null allele. Although this is the most common genotype in adult Pompe patients – present in 68-93% of patients[27,40,41] – the estimated rate of disease progression may not apply to patients with different genotypes.

PREDICTORS FOR DISEASE OUTCOME AND DISEASE COURSE VARIABILITY

Longer disease duration (>15 years) and pulmonary dysfunction (FVC <80%) were shown to be associated with a faster decline in muscle strength. Our results thus support those of the only other prospective study in adult Pompe patients, which found baseline status and duration of illness to be the most important predictors of disease severity and disease progress.[27]

A subset of patients with shorter average disease duration and better baseline status did not deteriorate during follow-up, indicating that there might be a stable phase of several years before a gradual decline inevitably occurs. This raises an interesting dilemma regarding the best time to start ERT.[42] On the one hand, in patients who are only mildly affected and whose condition is stable, one might advocate that this – costly – lifelong

treatment be postponed. On the other hand, studies measuring the effect of ERT show a trend toward better outcome in patients who were in a relatively good condition at the start of treatment.[11,13,14] On the basis of our results, we suggest to start ERT in all patients with pulmonary dysfunction and in patients with progressive muscle weakness, whereas in patients with minimal weakness, or in patients with solely elevated laboratory parameters treatment may be postponed, provided that they are monitored regularly. At our Center, all patients undergo evaluation of muscle strength, pulmonary function, cardiac function, and hearing at referral, and evaluation of progression of muscle weakness and pulmonary dysfunction every three months thereafter.

CONCLUSIONS

In summary, since the approval of enzyme-replacement therapy in Pompe disease, early recognition of affected individuals has gained importance. The typical limb-girdle type muscle weakness – including prominent involvement of the trunk musculature – combined with early and disproportionate pulmonary involvement relative to the degree of skeletal muscle weakness should raise the suspicion of Pompe disease. Although these are the most salient characteristics, less familiar features such as ptosis, scapular winging and bulbar weakness are far more common than generally thought. If these are recognized properly, timely diagnosis will be facilitated. Longer disease duration and reduced pulmonary function stand out as the most important factors for a rapid decline in muscle strength, which may aid in deciding whom to treat and when.

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3.3

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 organization (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.378_379del (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]

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.

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/m2			
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_{ED} dimension LV ejection fraction 48%	Unlikely

VC=vital capacity, DM=diabetes mellitus, LV=left ventricular, LV_{ED}=left ventricular end-diastolic, WPW=Wolff-Parkinson-White. [a] All patients carried the c.-32-13T>G mutation in combination with a null mutation on the second allele.

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 fibres compared to cardiomyocytes may lead to more extensive lysosomal glycogen accumulation in the skeletal muscles through autophagy, resulting in more muscle fibre 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.

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3·4

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-centre, 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 centres in the Netherlands and Belgium, or through the Dutch neuromuscular patient organization, or were referred to our centre 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,CO_2}). In the absence of ventilation irregularities, the P_{EE,CO_2} approximates the arterial carbon dioxide pressure (P_{a,CO_2}). A daytime P_{EE,CO_2} 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 χ^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

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 Non-invasive (nose hood or face mask) Invasive (tracheostomy tube)	19 (38)[a] 13 6	7 (17) 7 –
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 (χ^2 4.13; p=0.042).

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 centre 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.

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).

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).

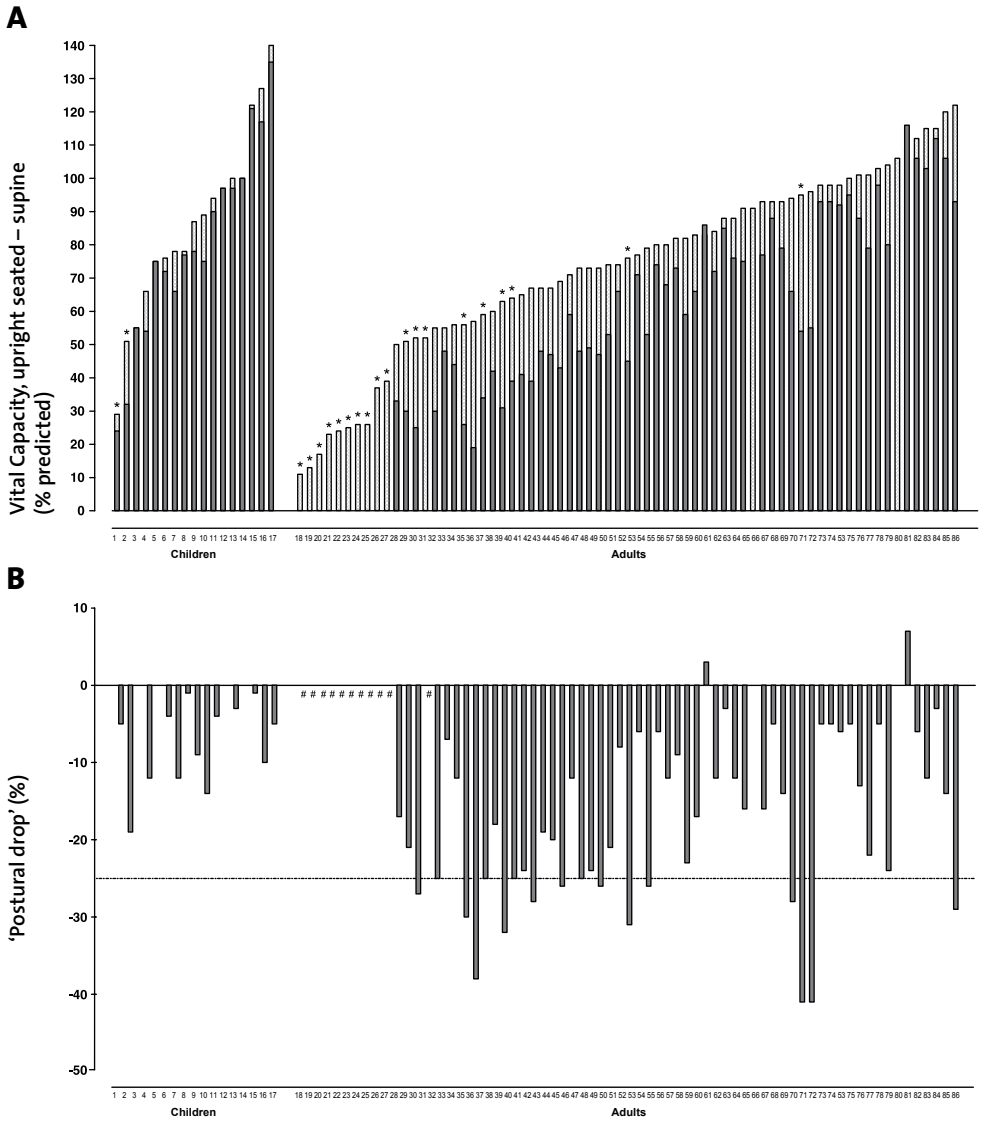

FIGURE 1

(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.

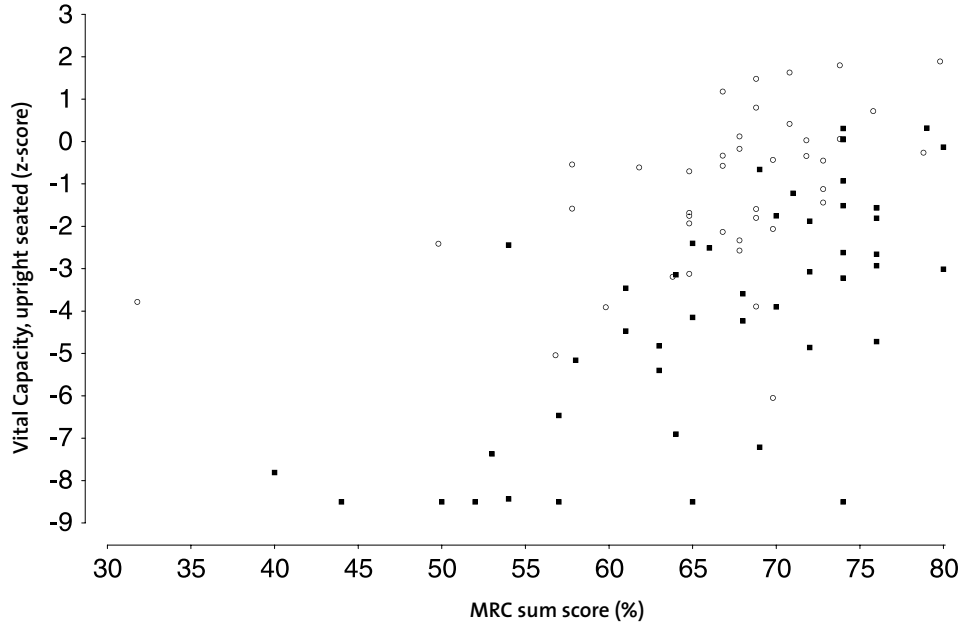

FIGURE 2

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).

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. 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.

Capnography

Capnography was performed in 65 adult patients, ten of whom had P_{EE,CO_2} 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

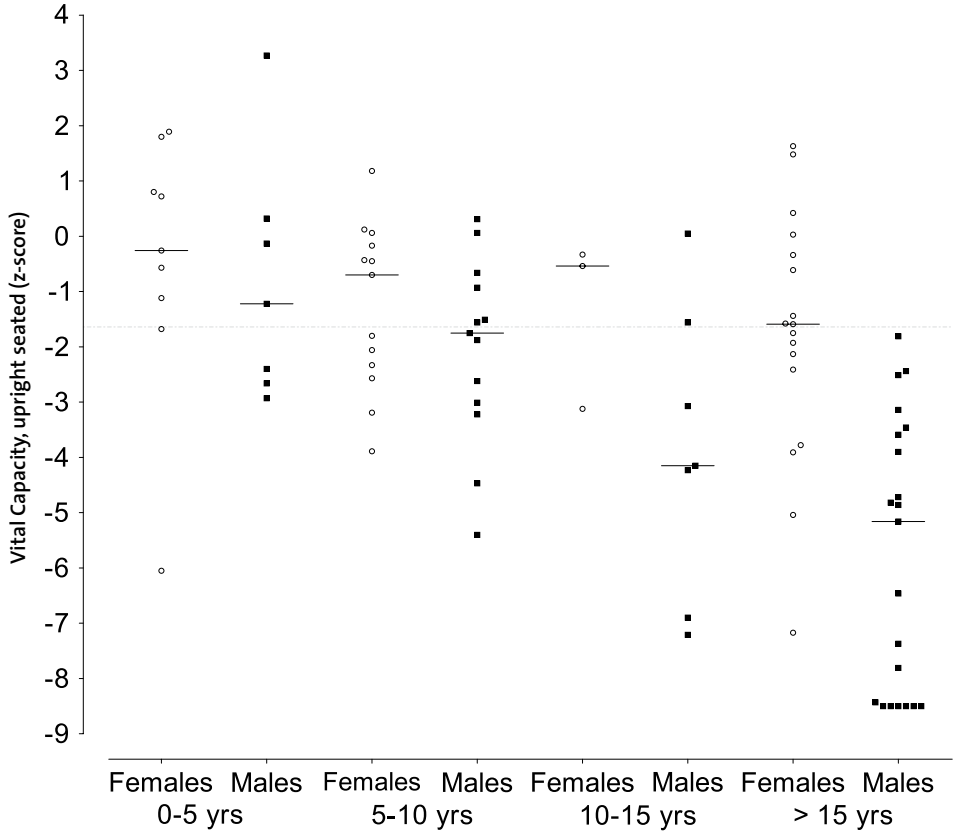

FIGURE 3

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.

part of the day. They were referred to their centre 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 centre 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,CO_2} being only marginally elevated in one (P_{EE,CO_2} 6.06 kPa). These two patients had no respiratory complaints.

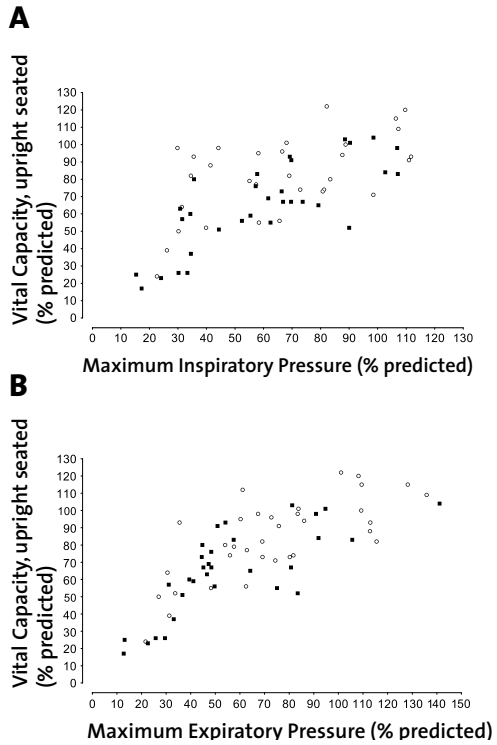

FIGURE 4

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.

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.

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

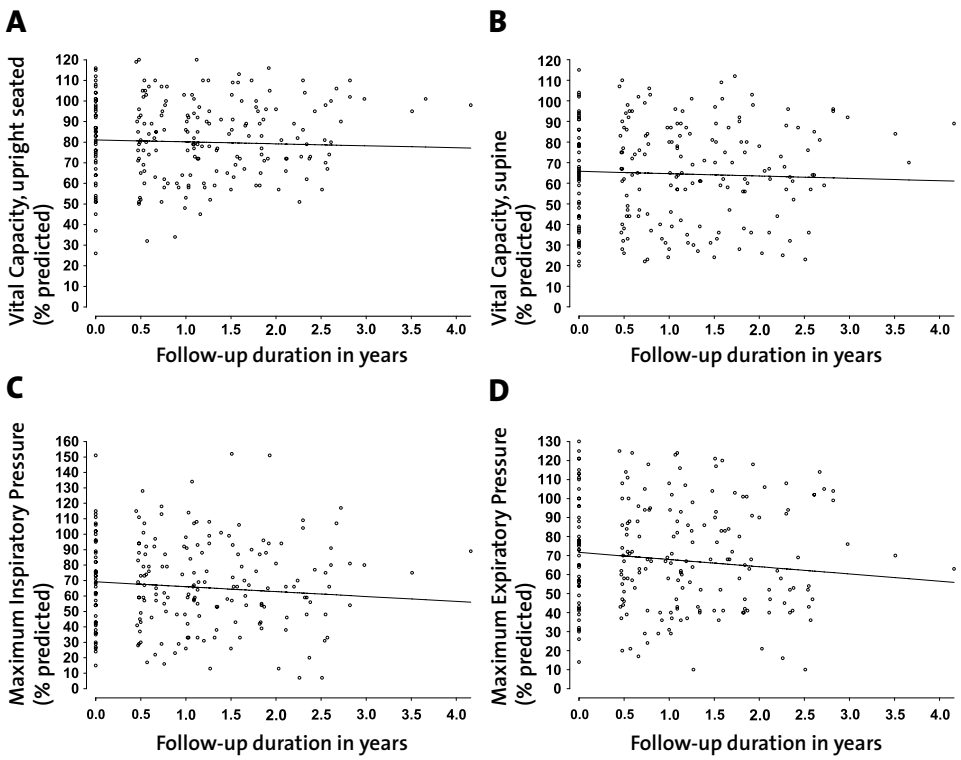

FIGURE 5

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.

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 centre, 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.

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3.5

HEARING IN ADULTS WITH POMPE DISEASE

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ABSTRACT

Hearing loss has been recognized as an important cause of morbidity in infants with Pompe disease, a metabolic disorder caused by deficiency of acid α-glucosidase. It is unknown whether hearing is also affected in adult Pompe patients. We have studied the prevalence, severity, and type of hearing loss in 58 adult patients using tympanometry and pure-tone audiometry. Compared to normative data (International Organization for Standardisation standard 7029), 72% of patients had impaired hearing thresholds at one or more frequencies in at least one ear. All measured frequencies were equally affected. All patients had a sensorineural type of hearing loss, pointing to cochlear or retrocochlear pathology. Categorized according to the standards of the World Health Organization 21% of patients had a clinically relevant hearing loss (16% slight, 3% moderate, 2% profound). Though this suggests that hearing loss occurs in a considerable number of patients with Pompe disease, this prevalence is similar to that in the general population. Therefore, we conclude that hearing loss is not a specific feature of Pompe disease in adults.

INTRODUCTION

Pompe disease (also called Glycogen Storage Disease type II or acid maltase deficiency) (OMIM 232300) is a lysosomal storage disorder caused by deficiency of the enzyme acid α-glucosidase (EC 3.2.1.3). This deficiency results in lysosomal glycogen accumulation, leading to a multi-system disorder affecting virtually all body tissues in patients with the classic infantile phenotype, or to a more slowly progressive disease mainly affecting skeletal and respiratory muscle function in older children and adults.[1-4]

Hearing deficits have been reported in several lysosomal storage diseases, including Fabry disease, Gaucher disease and the Mucopolysaccharidoses.[5-11] In Pompe disease, hearing loss is increasingly recognized as an important cause of morbidity in patients with the classic infantile phenotype. So far, auditory function has been described in the literature for 22 classic infantile Pompe patients. Sixteen of these 22 patients had a hearing deficit.[12-16] The observation of glycogen storage in the cells of the organ of Corti in knock-out mice with Pompe disease suggests that cochlear pathology is the main cause of the hearing impairment.[16] In older children with Pompe disease, hearing problems are sporadically present.[15] In two related studies on late-onset/adult-onset Pompe disease four patients were mentioned to have hearing impairment, but the problem was not studied systematically, and it is as yet unknown whether hearing impairment occurs more frequently in adults with Pompe disease than in the general population.[17,18] It is quite possible that hearing problems develop only after a prolonged period of time in patients who have residual enzyme activity.

Since the introduction of enzyme-replacement therapy with alglucosidase alfa (Myozyme®, Genzyme Corporation) the perspective of patients with Pompe disease has changed.[19-25] Today, the focus is not exclusively on the most limiting aspects of the disease such as reduced mobility and impaired respiration, but it has also shifted towards other aspects that may influence the quality of life. Increased awareness can lead to earlier detection of hearing loss and timely hearing rehabilitation. Therefore, we studied the prevalence, severity, and type of hearing impairment in a cohort of 58 Dutch adults with Pompe disease who are followed in our Center.

PATIENTS AND METHODS

PATIENTS AND PROCEDURES

Data were obtained between October 2004 and August 2009 as part of an ongoing nationwide study in the Netherlands on the natural course of Pompe disease. All patients were seen at Erasmus MC University Medical Center. Patients were recruited through neuromuscular centres within the Netherlands, or through the Dutch neuromuscular patient organization. The study was approved by the Central Committee on Research Involving Human Subjects in the Netherlands (CCMO). All patients provided written informed consent. At the time of the investigation, none of the patients received enzyme-replacement therapy.

HEARING ASSESSMENT

The hearing examination comprised tympanometry and pure-tone audiometry. Pure-tone audiometry was recorded using a Madsen OB822 clinical audiometer (Copenhagen, Denmark) and standard TDH 39 earphones with MX41-AR cushions. Air conduction thresholds and bone conduction thresholds were obtained for each ear at 0.25, 0.5, 1, 2, 4 and 8 kHz according to a standardized protocol (International Organization for Standardisation (ISO) 8253-1).[26] All audiometric tests were performed by an experienced audiologist. Hearing thresholds were considered abnormal if they were below the 95th percentile of the predicted value using normative data from the ISO standard 7029 for comparison.[27] Conductive hearing loss was considered to be present in case of an air-bone gap of more than 7.5 dB averaged over the frequencies 0.5 to 2 kHz.

To indicate expected difficulty with conversational speech, the guidelines of the World Health Organization (WHO) on physical functioning, disability and health were used to grade the severity of the hearing impairment. Hearing loss was defined as a pure-tone average (PTA) of thresholds at 0.5, 1, 2 and 4 kHz greater than 25 dB hearing level. The ears were classified as better-hearing or worse-hearing depending on this average score. The severity of hearing loss was categorised as slight (26–40 dB), moderate (41–60 dB), severe (61–80 dB) or profound (>81 dB) based on this pure-tone average.[28]

ADDITIONAL CLINICAL INFORMATION

The following information was available: age at symptom onset, age at diagnosis of Pompe disease, disease duration, use of walking devices or wheelchair, use of ventilatory support, and type of mutation. The presence of contributing medical, genetic or environmental conditions such as noise exposure, exposure to chemicals, or use of ototoxic medication was evaluated by means of a self-reported questionnaire.

TABLE 1

Prevalence of hearing loss in 58 Dutch adults with Pompe disease compared to the general population, stratified by age.

		Dutch adult Pompe patients						General population[29-31]						
		Better-hearing ear[b]			Worse-hearing ear[c]				Better-hearing ear[b]			Worse-hearing ear[c]		
Age group	n	≥25–45 dB[a]	≥45–65 dB	≥65 dB	≥25–45 dB	≥45–65 dB	≥65 dB	n	≥25–45 dB	≥45–65 dB	≥65 dB	≥25–45 dB	≥45–65 dB	≥65 dB
20–50 yrs	25	1 (4%)	1 (4%)	-	3 (12%)	1 (4%)	-	1869	1.6–3.6%	0–0.8%	0–0.3%	5.2–10.6%	0–2.8%	0–0.8%
51–60 yrs	17	2 (12%)	1 (6%)	-	6 (35%)	1 (6%)	-	1000	11.3–18.9%	0.7–4.0%	0–0.9%	22.6–33.8%	2.8–12.2%	0.6–5.2%
61–70 yrs	13	4 (31%)	-	-	5 (38%)	1 (8%)	-	810	36.8–48.3%	2.5–7.4%	0–2.3%	51.2–61.3%	10.4–19.0%	1.9–7.5%
71–80 yrs	3	2 (67%)	-	1 (33%)	2 (67%)	-	1 (33%)	542	60.3–73.8%	17.6–51.6%	1.8–3.3%	71.6–86.0%	33.2–41.2%	6.3–12.6%
Overall	**58**	**9 (16%)**	**2 (3%)**	**1 (2%)**	**16 (28%)**	**3 (5%)**	**1 (2%)**	**4224**	**16.1–16.9 %**	**2.8–3.9%**	**0.2–1.1%**	**22.2–26.3%**	**6.2–9.3%**	**1.0–3.5%**

[a] The hearing impairment as reported in this table is graded according to the WHO guidelines. It is measured as dB (decibel) hearing level averaged over 0.5, 1.2 and 4 kHz: 25–45 dB signifies slight hearing loss, 45–65 dB signifies moderate hearing loss, and >65 dB signifies profound hearing loss. [b] These columns report the hearing loss in the better-hearing ear of patients with bilateral hearing loss. [c] These columns report the hearing loss in the worse-hearing ear of patients with either bilateral (12 of the 20 patients) or unilateral hearing loss (8 of the 20 patients).

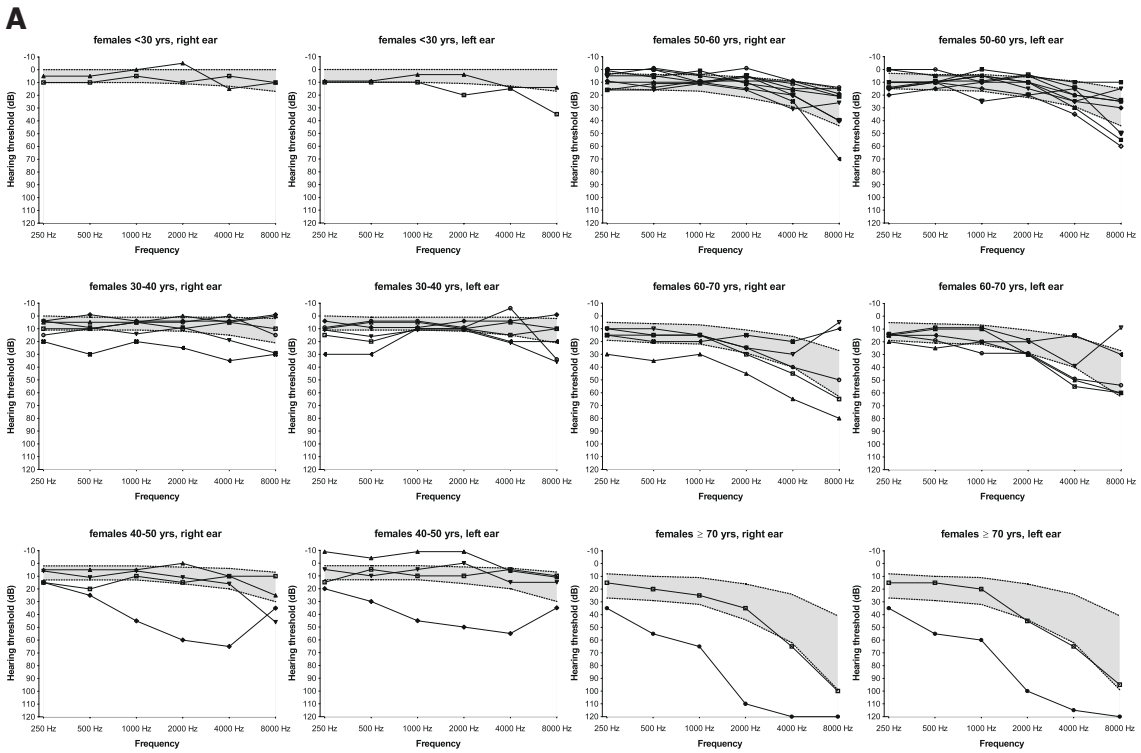
STATISTICAL ANALYSIS

Continuous variables are presented using mean, median, standard deviation or range. For categorical variables percentages or frequencies are given. Differences in contributing factors between the groups of patients with and without hearing impairment were tested by χ^2 analyses. Analyses were performed with SPSS for windows (version 15.0, SPSS Inc., Chicago, IL). A p-value of ≤ 0.05 was considered significant.

RESULTS

STUDY POPULATION

Fifty-eight adults with Pompe disease (29 males, 29 females) were included in the study. The mean age at the time of investigation was 50.5 years (SD 12.5, range 26–76 years). The mean age at which first neuromuscular symptoms had manifested, considered as disease onset, was 31.7 years (SD 11.1), and the mean age at diagnosis was 40.7 years (SD 12.8). Disease duration at the time of the investigation ranged from 1 to 48 years (median 18.9 years). Six patients had experienced their first symptoms in childhood or adolescence, at the ages of 1, 10, 11, 15, 16, or 18 years, of whom only the patient who had symptoms in the first year of life had been diagnosed before adulthood. At the time of the hearing investigation, this patient, now 29 years of age, has only mild skeletal muscle weakness and does not need ventilatory support. Eight patients (14%) used walking devices, 21 (36%) were wheelchair dependent, and 18 (31%) used mechanical ventilatory support. Three patients (5%) had hearing aids. Fifty-seven patients had the common c.-32-13T>G (IVS1-13T>G; leaky splice) mutation, combined with a second pathogenic mutation on the other allele. One patient had genotype c.671G>A/c.525delT (p.Arg224Gln/p.Glu176fsX45).



HEARING ASSESSMENT BY PURE-TONE AUDIOMETRY AND TYMPANOMETRY

Sixteen of the 58 patients (28%) had normal hearing levels for both ears at all frequencies, as measured by pure-tone audiometry. We found abnormal hearing levels in 42 patients (72%): 22 patients had abnormal hearing thresholds in both ears, and 20 patients in one ear. All measured frequencies were equally affected (p=0.63). Figures 1A and 1B show the hearing thresholds at all frequencies for the individual patients compared to the normative values for age, stratified by gender.

With regard to functional hearing impairment, as assessed by means of self-reported questionnaires, one of the 16 patients with normal hearing thresholds reported difficulty hearing. Of the 42 patients with abnormal hearing thresholds, 14 reported hearing difficulties.

Tympanometry was abnormal in 20 patients (34%): nine had slightly negative middle ear pressures, 11 had abnormal static admittance. However, a significant contribution (air-bone gap of more than 7.5 dB averaged over the frequencies 0.5 to 2 kHz) of these conductive abnormalities to the total amount of hearing impairment was present in three patients only: one patient had a perforated eardrum, one had otitis media, and the other one had stiffening of the bony middle-ear structures. The other 39 patients (93%) had a pure sensorineural type of hearing impairment.

GRADING OF HEARING IMPAIRMENT ACCORDING TO THE WHO GUIDELINES

Since abnormalities at one or two of the measured frequencies might not be of clinical importance to the patient, we also graded the hearing impairment according to the WHO guidelines, which better reflects the severity of the hearing loss (Table 1). Regarding the better-hearing ear of each patient, 12 of the 58 patients (21%) had hearing loss: 16% of these patients had slight hearing loss, 3% moderate hearing loss, and 2% profound hearing loss. Each of these 12 patients had bilateral hearing loss. Based on assessments of the worse-hearing ear, 20 of the 58 patients had some level of hearing loss (34%). Among these 20 patients are the 12 patients with bilateral hearing loss and an additional eight patients with unilateral hearing loss. For comparison, Table 1 also shows the frequency of hearing loss in the general population, as graded according to the WHO guidelines, stratified by age.

CONTRIBUTING FACTORS

Twenty of the 42 patients with abnormal hearing thresholds reported medical, genetic or environmental conditions that may have contributed to the degree of their hearing abnormality. Reported were: noise exposure (8 patients), frequent middle ear infections

◂ FIGURE 1
Hearing thresholds in 29 females (A) and 29 males (B) with Pompe disease stratified by age. The shaded area represents the 95 percent confidence interval for normal hearing (ISO 7029). The connected lines represent the pure-tone audiograms of the individual patients for the right and left ear; each patient within an age group is represented by a different symbol (×, △, ▲, ▽, ▼, ◆, ◇, ●, ○, ■, or □).

during childhood (7 patients), familial occurrence of hearing loss (6 patients), use of intravenous aminoglycoside antibiotics (5 patients), diabetes mellitus (2 patients), and use of acetylsalicylic acid (2 patients). The proportion of patients reporting possible contributing factors did not differ between the group of patients with abnormal hearing thresholds and the group of patients with normal hearing thresholds (p=0.70).

DISCUSSION

We studied the prevalence, severity, and type of hearing loss in adults with Pompe disease to see whether it is an important cause of morbidity in adults, as it is in infants with Pompe disease.

With regard to the prevalence of hearing loss, we found that abnormal hearing thresholds were frequently present among adults with Pompe disease, but that a clinically relevant hearing loss was present in 21% of patients only. This percentage seems high, but it appears comparable to the prevalence in the general population, matched for age and gender.[29-31] The severity of hearing loss was categorised as slight in 16%, moderate in 3%, and profound in 2%. Fifty percent of the patients with abnormal hearing thresholds, reported factors such as concomitant disease, noise exposure or use of ototoxic medication, which may be responsible for a certain degree of hearing loss. Unfortunately, it could not be determined to which extent these factors contributed to the observed hearing impairment. Of the patients with normal hearing, the same proportion reported exposure to these conditions.

In all patients in whom we observed hearing loss, we found a sensorineural type of hearing impairment. Only three patients (7%) also had significant conductive abnormalities, despite the fact that one third of patients had slightly impaired middle ear function. Therefore, the impact of middle ear dysfunction on the overall severity of hearing loss is presumably small.

Hearing loss in Pompe disease was first recognized in patients with the classic infantile phenotype, when it became evident that these patients could survive beyond the age of one year, if they were treated with recombinant human acid α-glucosidase.[20,22,32] Information on the precise cause of the hearing loss in Pompe disease is scarce. One study showed raised auditory brainstem responses thresholds in nine of 11 infantile patients, whereas mild abnormalities in inter-peak latency times were seen in five.[15] Another study reported on flat oto-acoustic emission (OAE) and abnormal wave latencies in brainstem auditory evoked potentials (BAEP) in some infants, pointing to inner ear or auditory nervous system pathology or to both.[12] A study in knock-out mice with Pompe disease led to the discovery of glycogen deposition in the inner hair cells and the outer hair cells of the cochlea, the supporting cells, the stria vascularis, and the spiral ganglion cells.[16] These findings suggest that cochlear pathology rather than involvement of the central auditory nervous system could be the main cause of the hearing loss. In the present study, we did not investigate auditory brainstem responses. However, since the underlying metabolic defect in adults and infants is the same, the presence of retrocochlear pathology as the primary cause of the hearing impairment in adults with Pompe disease is less likely. The observation that profound hearing impairment is present in nearly every patient with classic infantile Pompe disease, but only sporadically in older children and adults is probably due to the higher amount of residual α-glucosidase activity in the latter groups of patients, preventing generalized glycogen storage.

Due to the development of innovative therapeutic measures and higher standard of care, the perspective of patients with Pompe disease has changed over the last years.[21,23] It is to be expected that the focus will not exclusively be on the most threatening aspects of the disease such as severe muscle weakness and respiratory failure, but will shift towards other aspects that influence the quality of life of patients with Pompe disease. In light of these changing perspectives it is important to conclude that, unlike in infants, clinically relevant hearing loss is not a hallmark of Pompe disease in adults. Therefore, standard screening for hearing impairment does not seem indicated in adult-onset Pompe disease.

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4

ENZYME-REPLACEMENT THERAPY

4.1

EFFECT OF ENZYME THERAPY AND PROGNOSTIC FACTORS IN 69 ADULTS WITH POMPE DISEASE: AN OPEN-LABEL SINGLE-CENTER STUDY

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ABSTRACT

Background Enzyme-replacement therapy (ERT) in adults with Pompe disease, a progressive neuromuscular disorder, is of promising but variable efficacy. We investigated whether it alters the course of disease, and also identified potential prognostic factors.

Methods Patients in this open-label single-centre study were treated biweekly with 20 mg/kg alglucosidase alfa. Muscle strength, muscle function, and pulmonary function were assessed every 3–6 months and analyzed using repeated-measures ANOVA.

Results Sixty-nine patients (median age 52.1 years) were followed for a median of 23 months. Muscle strength increased after start of ERT (manual muscle testing 1.4 percentage points per year (pp/y); hand-held dynamometry 4.0 pp/y; both p<0.001). Forced vital capacity (FVC) remained stable when measured in upright, but declined in supine position (-1.1 pp/y; p=0.03). Muscle function did not improve in all patients (quick motor function test 0.7 pp/y; p=0.14), but increased significantly in wheelchair-independent patients and those with mild and moderate muscle weakness.

Relative to the pre-treatment period (49 patients with 14 months pre-ERT and 22 months ERT median follow-up), ERT affected muscle strength positively (manual muscle testing +3.3 pp/y, p<0.001 and hand-held dynamometry +7.9 pp/y, p<0.001). Its effect on upright FVC was +1.8 pp/y (p=0.08) and on supine FVC +0.8 (p=0.38). Favorable prognostic factors were female gender for muscle strength, and younger age and better clinical status for supine FVC.

Conclusions We conclude that ERT positively alters the natural course of Pompe disease in adult patients; muscle strength increased and upright FVC stabilized. Functional outcome is probably best when ERT intervention is timely.

BACKGROUND

Pompe disease (OMIM number 232300) is an autosomal recessive metabolic myopathy caused by deficiency of the lysosomal enzyme acid α-glucosidase. This deficiency impairs lysosomal glycogen breakdown, leading to glycogen accumulation in several tissues.[1-4] The disease covers a broad clinical spectrum, ranging from a rapidly progressive infantile phenotype that results in death within the first year of life, to more slowly progressive forms in children and adults.[5-11] In adults, the disease generally presents as a limb-girdle myopathy. As well as the skeletal muscles, respiratory muscles – including the diaphragm – are affected.[1,12,13] As the disease progresses, most patients lose ambulation and require ventilatory support.[5,10,11,14,15]

Although Pompe disease used to be untreatable, patients' prospects changed in 2006 upon the introduction of enzyme-replacement therapy (ERT) with recombinant human acid α-glucosidase. Initial studies in infants showed that ERT improved survival and motor outcome.[16-23] Several studies focusing on adult patients have been published since registration, but most report data in relatively small numbers of patients, or have a short follow-up.[24-29] Proof of efficacy was provided by the 18-month randomized-placebo controlled trial in 90 patients, 60 of whom received alglucosidase alfa. This showed that walking distance improved and pulmonary function in upright position stabilized.[29] As mild and severely affected patients had been excluded, the trial involved a selected group of patients.

The aims of the current study were therefore 1) to determine whether ERT alters the progressive course of Pompe disease in a broader adult patient population ranging from very severely affected to mildly affected; 2) to determine how much ERT alters the course of the disease relative to that reflected in pre-treatment data; and 3) to identify prognostic factors for response to treatment.

METHODS

PATIENTS AND STUDY DESIGN

This single-centre, prospective, open-label cohort study on the use of ERT was conducted from January 2005 to August 2009 at the Center for Lysosomal and Metabolic Diseases, Erasmus MC University Medical Center, Rotterdam, which is the Dutch national referral centre for Pompe patients.

Patients were eligible for inclusion if 1) they were over 18 years of age; 2) their diagnosis had been confirmed by enzyme analysis in leukocytes or fibroblasts[30-32] and by mutation analysis;[33] 3) they had not previously been treated with recombinant human acid α-glucosidase; 4) they had been treated for a minimum of 5 months; and 5) they were symptomatic, i.e. had measurable muscle weakness and/or diminished pulmonary function.

Patients received intravenous infusions with 20 mg/kg alglucosidase alfa every two weeks. Clinical assessments were performed every three to six months before the start of ERT and every three months thereafter.

The study protocol was approved by the Medical Ethical Committee at Erasmus MC

University Medical Center. All patients provided written informed consent.

Twenty of the 69 patients participated in the randomized-placebo controlled trial on the safety and efficacy of alglucosidase alfa in late-onset Pompe disease; 13 in the treatment arm and seven in the placebo arm.[29] Data on FVC in upright position of these patients collected during the 18 months study period were included in the current analyses.

SKELETAL MUSCLE STRENGTH AND FUNCTION

Skeletal muscle strength was measured by manual muscle testing using the Medical Research Council (MRC) grading scale (range 0–5) and hand-held dynamometry (HHD) (Cytec dynamometer, Groningen, the Netherlands).[34,35] The following muscle groups were tested: neck extensors, neck flexors, shoulder abductors, elbow flexors, elbow extensors, hip flexors, hip abductors, knee flexors, and knee extensors. The MRC grade was also assessed for shoulder adductors, exorotators and endorotators, hip extensors, and hip adductors. A MRC sumscore was derived by adding the grades for all 26 muscles and expressing the sum as a percentage of the maximum possible score. HHD values (Newton) of each muscle group were first expressed as a percentage of the median strength of healthy males or females,[35] and then combined into a sumscore by averaging these for all 16 muscle groups. If values for three or more muscle groups were missing, no sumscores were calculated for either method.

Muscle function was assessed using the Quick Motor Function Test (QMFT);[36] this consists of 16 motor skills related to daily activities that require the use of muscles of the shoulder girdle, trunk, pelvic girdle and/or proximal lower limbs. Each item was scored on a 5-point ordinal scale, with 0 representing "cannot perform task" and 4 "can perform task with no effort". Adding all items gives a total score between 0 and 64. The actual sumscore was expressed as a percentage of the maximum score.

The use of wheelchair and walking aids was registered at each visit.

PULMONARY FUNCTION

Forced vital capacity (FVC) in upright and supine positions was measured using spirometry as described earlier.[10,37] Results were expressed as a percentage of the predicted normal value.[38,39] Values lower than 80% of predicted normal values were considered to be abnormal. The use of ventilatory support and hours of use per day was registered at each visit.

SAFETY ASSESSMENTS AND LABORATORY INVESTIGATIONS

Vital signs and adverse events were recorded at each visit. Electrocardiograms, a physical examination, and hematological, biochemical and urine analyses were made at regular intervals.

STATISTICAL ANALYSIS

Longitudinal analysis of the outcome parameters (MRC, HHD and QMFT sumscores and FVC in upright and supine positions) was performed using repeated-measures ANOVA (random coefficient models). This method allows for irregular measurement times and different treatment durations. First, we assessed the mean annual change in the outcome parameters during the treatment period. Mean annual changes were expressed in absolute percentage points (pp/y). Analyses were also stratified by subgroups described in previous studies: gender, age (<45 years and ≥45 years old), disease duration (<15 years and ≥15 years), wheelchair use, ventilation use, FVC in upright position (≥80% and <80%) and MRC sumscore (in tertiles).[12,29]

Second, for the patients with pre-treatment and treatment data we established the extent to which the course of disease altered after starting ERT. To be included in this analysis, patients should have had at least one measurement done a minimum of 4 months before ERT. We included a linear effect of time before and during ERT in the repeated-measures ANOVA. Per individual, the two linear segments connect with each other at the time of start of ERT. This method is also known as the "broken-stick method" or as "piece-wise linear regression".

Third, we investigated prognostic factors for treatment response. As well as performing the subgroup analysis, we assessed the association between patients' characteristics and their individual response to ERT. Patients were classified into three groups: 1) non-responders, i.e. people in whom the course of disease (measured by one of the outcome parameters) was the same or worse during ERT than before; 2) good responders, whose disease course improved more than the median improvement of responders; and 3) moderate responders. Each patient's response was represented by the individual change in regression lines calculated in the "broken-stick" analysis. In patients without pre-treatment data, the natural course slope was imputed. Associations were tested using the Spearman test for continuous variables and the Chi-square trend test for categorical variables.

Analyses were performed with SPSS for Windows (version 17, SPSS Inc., Chicago, IL) or SAS (version 9.2, SAS Institute Inc., Cary, NC). A p-value lower than or equal to 0.05 (two-sided) was considered significant.

RESULTS

PATIENTS

In total, 71 adult Pompe patients started ERT within the study period. Two were excluded because they had received ERT for less than five months: one died two months after starting ERT due to a dissection of the aortic arch; the second withdrew from the study after four months and died six months later due to respiratory insufficiency.

Table 1 shows the patients' characteristics at start of ERT. The median age at the start of ERT was 52.1 years, 52% were male, 40% used a wheelchair, and 37% used mechanical ventilation. The median treatment duration was 23 months (range 5–47 months). Of the 69 patients, 13 were treated for more than 3 years and six patients for less than 1 year, respectively for 5, 6, 7, 10, and two for 11 months.

All but one patient carried the common c.-32-13T>G (IVS1-13T>G) mutation on one allele in combination with another pathogenic mutation on the second allele. Of the patients carrying the c.-32-13T>G (IVS1-13T>G) mutation, 14 different mutations were found on the second allele. Forty-eight percent carried the c.525delT and 16% the c.2481+102_2646+31del mutation. The other mutations found were the c.-32-13T>G + c.1076-22T>G, c.1115A>T, c.1396G>T, c.1548G>A, c.172C>T, c.1799G>A, c.2314T>C, c.378_379del, c.461_469del, c.701C>A,

c.896T>C and c.925G>A mutation. Of those mutations, 74% was indicated as very severe and 26% as potentially less severe (www.pompecenter.nl). For three patients the result of the mutation analysis could not be tracked, because these were performed by another centre.

For 49 patients, pre-treatment data were available in addition to the treatment follow-up. These were slightly younger and less severely affected. Their median follow-up was 14 months before (range 4–33), and 22 months during ERT (range 6–41).

TABLE 1

Patients' characteristics and baseline values of clinical outcome measures at start of ERT

Characteristic	Total study population (N=69)	Patients with pre-ERT and ERT follow-up (N=49)	p-value[a]
Age at start of ERT in years, median (range)	52.1 (26.2–76.3)	50.1 (26.2–74.0)	0.83
Age at onset of symptoms in years, median (range)	30.8 (1.4–62.0)	32.0 (1.4–62.0)	0.63
Age at diagnosis in years, median (range)	39.6 (1.4–63.8)	40.9 (1.4–63.0)	0.97
Duration of disease at start of ERT in years, median (range)	9.3 (0.2–31.2)	8.3 (0.5–31.2)	0.74
MRC sumscore at start of ERT in percentage, median (range)	77.7 (48.3–92.3)	79.2 (60.8–92.3)	0.52
HHD sumscore at start of ERT in percentage, median (range)	69.3 (25.9–94.1)	69.3 (25.9–94.1)	0.97
QMFT score at start of ERT in percentage, median (range)	55.6 (10.0–89.1)	59.5 (21.7–89.1)	0.42
Time of mechanical ventilation at start of ERT per day in hours, median (range)	11.5 (0.0–24.0)	10.5 (0.0 –24.0)	0.81
FVC in upright position at start of ERT in percentage, median (range)	68.3 (11.3–106.9)	71.9 (26.5–106.9)	0.43
FVC in supine position at start of ERT in percentage, median (range)	46.8 (23.0–99.0)	52.4 (23.0–99.0)	0.71
Gender, No. of patients (%)			
Male	36 (52)	21 (43)	0.43
Female	33 (48)	28 (57)	
Wheelchair at start of ERT, No. of patients (%)			
No wheelchair use	42 (61)	33 (67)	0.22
Partial wheelchair use	8 (12)	8 (16)	
Permanent wheelchair use	19 (28)	8 (16)	
Mechanical ventilation at start of ERT, No. of patients (%)			0.72
No mechanical ventilation	44 (64)	36 (74)	
Non-invasive mechanical ventilation	19 (28)	10 (20)	
Invasive mechanical ventilation	6 (9)	3 (6)	

ERT=Enzyme-Replacement Therapy, MRC sumscore=Medical Research Council sumscore, HHD sumscore=Hand-Held Dynamometry sumscore, QMFT score=Quick Motor Function Test score, FVC=Forced Vital Capacity, N/No.=Number of patients.

[a] Differences between the total group (N=69) and the group with pre-ERT and ERT follow-up (N=49) were calculated using Chi-square tests for the categorical variables, and using Mann-Whitney tests for continuous variables.

SKELETAL MUSCLE STRENGTH

During ERT, the MRC sumscore among all 69 patients rose by an average of 1.4 pp/y, and the HHD sumscore by 4.0 pp/y (both p<0.001; Table 2). All individual muscle groups contributed to the effect (p≤0.02 for all muscle groups). Subgroup analyses showed that the mean annual increase in muscle strength during ERT was greater for women than for men (2.6 pp/y vs. 0.4 pp/y; difference between groups p<0.001 for MRC; and 6.3 pp/y vs. 2.0 pp/y, difference between groups p=0.05 for HHD). For the other subgroups investigated, there

TABLE 2

Clinical outcome measures during enzyme-replacement therapy and relative to the natural course of disease

Clinical Outcome Measure	Natural course mean pp/y (95% CI)	p-value	Treatment course mean pp/y (95% CI)	p-value	Difference[a] mean pp/y (95% CI)	p-value
MRC sumscore						
Total study population (N=69, M=558)			1.4 (0.8 to 2.1)	<0.001		
Patients with pre-ERT + ERT follow-up (N=49, M=523)	-1.2 (-2.1 to -0.4)	0.006	2.1 (1.2 to 3.0)	<0.001	3.3 (1.9 to 4.7)	<0.001
HHD sumscore						
Total study population (N=64, M=503)			4.0 (2.5 to 5.6)	<0.001		
Patients with pre-ERT + ERT follow-up (N=42, M=435)	-2.8 (-4.2 to -1.3)	<0.001	5.1 (3.0 to 7.3)	<0.001	7.9 (5.0 to 10.7)	<0.001
QMFT score						
Total study population (N=69, M=553)			0.7 (-0.2 to 1.7)	0.14		
FVC in upright position						
Total study population (N=62, M=475)			0.1 (-1.0 to 1.1)	0.92		
Patients with pre-ERT + ERT follow-up (N=46, M=480)	-2.0 (-3.1 to -0.8)	0.001	-0.2 (-1.6 to 1.2)	0.76	1.8 (-0.2 to 3.7)	0.08
FVC in supine position						
Total study population (N=54, M=411)			-1.1 (-2.1 to -0.1)	0.03		
Patients with pre-ERT + ERT follow-up (N=42, M=436)	-1.8 (-2.9 to -0.7)	0.002	-1.0 (-2.3 to 0.3)	0.12	0.8 (-0.9 to 2.4)	0.38

ERT=Enzyme-Replacement Therapy, pp/y=percentage points per year, 95% CI=95% Confidence Interval, MRC sumscore=Medical Research Council sumscore, QMFT score=Quick Motor Function Test score, HHD sumscore=Hand-Held Dynamometry sumscore, FVC=Forced Vital Capacity, N=Number of patients, M=Number of Measurements. Data shown are mean changes in percentage points per year as calculated by repeated-measures ANOVA.

[a] Difference between the course of disease during treatment and the natural course.

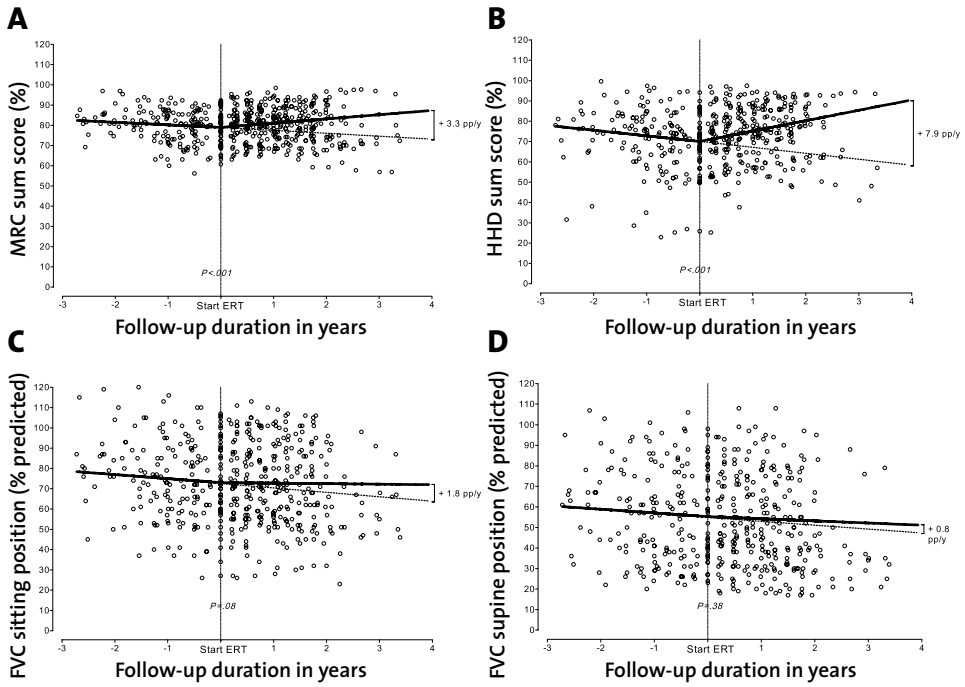

FIGURE 1

The clinical course of disease before and after the start of enzyme-replacement therapy (ERT) in the 49 patients with ERT and pre-ERT follow-up data for A) MRC sumscore; B) hand-held dynamometry (HHD) sumscore; C) forced vital capacity (FVC) in upright position; and D) FVC in supine position.

The figure shows all the individual observations for the different outcome measures (shown as dots) and the solid lines represent the estimated mean trend in these observations as calculated by the "broken stick" repeated-measures ANOVA. The dotted lines represent the extrapolated natural course slopes.

were no significant differences in the increase in muscle strength during ERT.

Before the 49 patients with pre-treatment and treatment data started ERT, their muscle-strength sumscores declined significantly (-1.2 pp/y for MRC, p=0.006; -2.8 pp/y for HHD, p<0.001). Treatment data produced a significant improvement, the differences being +3.3 pp/y for the MRC sumscore and +7.9 pp/y for the HHD sumscore (both p<0.001; Table 2, Figure 1A and B).

SKELETAL MUSCLE FUNCTION

Although QMFT scores for all 69 patients increased by an average of 0.7 pp/y during ERT, this was not significant (p=0.14). Subgroup analyses showed that while muscle function improved in patients with mild muscle weakness (2.1 pp/y, p=0.01) and moderate muscle weakness (1.6 pp/y, p=0.05), it fell by 1.4 pp/y (p=0.08) in patients with severe muscle weakness (difference between groups p=0.004). In line with this, QMFT scores rose in wheelchair-independent patients (1.7 pp/y, p=0.008), but did not improve in those who were wheelchair-dependent (-0.6 pp/y, p=0.39; difference between groups p=0.02).

The number of patients who were partially or fully wheelchair dependent at the last follow-up visit was the same as at the start of ERT. Nevertheless, two of the 27 patients who used walking aids at the start of ERT regained the ability to walk independently during ERT. Two other patients became dependent on walking aids.

PULMONARY FUNCTION AND USE OF MECHANICAL VENTILATION

During treatment with ERT, FVC in upright position remained stable (0.1 pp/y, p=0.92), while FVC in supine position declined (-1.1, p=0.03) (Table 2). Subgroup analyses showed that FVC in supine position did remain stable in patients under 45 years old (0.0 pp/y, p=1.0), but declined in those 45 years and over (-2.1 pp/y, p=0.002) (difference between groups p=0.03). There were no differences between subgroups for FVC in upright position.

Before start of ERT, FVC in upright and supine positions both declined significantly (-2.0 pp/y, p=0.001 and -1.8 pp/y, p=0.002, respectively). Compared to this, FVC in upright position improved during ERT, albeit at borderline statistical significance (+1.8 pp/y, p=0.08), while FVC in supine position did not (+0.8, p=0.38) (Table 2 and Fig ure 1C and D).

For the whole group there was no change in the median number of hours of ventilation per day between the start of ERT and the last treatment visit (Wilcoxon signed-rank test

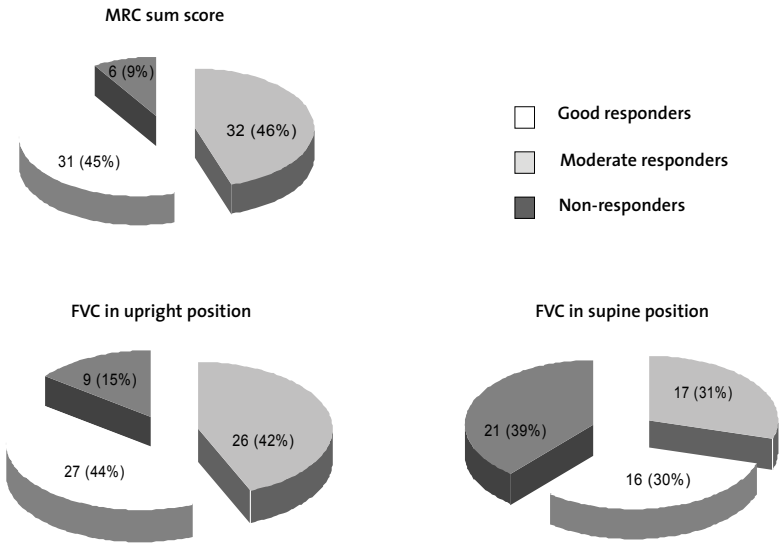

FIGURE 2

Individual response groups for skeletal muscle strength and pulmonary function.

The number and percentage of good responders, moderate responders and non-responders are shown for MRC sumscore, and forced vital capacity (FVC) in upright and supine positions.

TABLE 3

Characteristics of individual response groups with regard to skeletal muscle strength and pulmonary function

	Non-responders	Moderate Responders	Good responders	p-value
Response groups for MRC sumscore, No. of patients (%)	**6 (9)**	**32 (46)**	**31 (45)**	
Female, No. of patients (%)	*1 (17)*	*11 (34)*	*21 (68)*	*0.002*
Age at start of ERT in years, median (range)	51.6 (38.0–66.2)	50.0 (26.2–67.0)	52.7 (29.2–76.3)	0.47
Disease duration at start of ERT in years, median (range)	12.1 (3.6–22.7)	11.1 (0.9–31.2)	9.0 (0.2–29.1)	0.55
MRC sumscore at start of ERT in percentage, median (range)	80.8 (60.8–86.9)	80.0 (48.3–91.5)	74.6 (53.3–92.3)	0.12
Wheelchair use at start of ERT, No. of patients (%)	2 (33)	9 (28)	16 (52)	0.11
FVC in upright position at start of ERT in percentage, median (range)	61.5 (34.4–79.3)	69.9 (11.3–105.0)	69.3 (16.4–106.9)	0.48
Ventilation use at start of ERT, No. of patients (%)	1 (17)	12 (38)	12 (39)	0.49
Response groups for FVC in upright position, No. of patients (%)	**9 (15)**	**26 (42)**	**27 (44)**	
Female, No. of patients (%)	5 (56%)	14 (54%)	13 (48%)	0.64
Age at start of ERT in years, median (range)	52.6 (31.9–69.2)	51.2 (35.7–71.7)	43.3 (26.2–76.3)	0.16
Disease duration at start of ERT in years, median (range)	8.3 (0.6–31.2)	14.1 (0.9–27.0)	4.7 (0.2–29.1)	0.06
MRC sumscore at start of ERT in percentage, median (range)	74.6 (63.1–91.5)	77.7 (48.3–90.0)	80.8 (67.5–92.3)	0.26
Wheelchair use at start of ERT, No. of patients (%)	4 (44%)	10 (38%)	6 (22%)	0.15
FVC in upright position at start of ERT in percentage, median (range)	66.6 (51.4–100.5)	68.3 (11.3–106.9)	69.9 (16.4–105.0)	0.95
Ventilation use at start of ERT, No. of patients (%)	2 (22)	8 (31)	8 (30)	0.76
Response groups for FVC in supine position, No. of patients (%)	**21 (39)**	**17 (31)**	**16 (30)**	
Female, No. of patients (%)	10 (48)	10 (59)	10 (63)	0.36
Age at start of ERT in years, median (range)	*51.7 (31.9–67.0)*	*47.9 (26.2–62.9)*	*40.4 (29.2–74.0)*	*0.007*
Disease duration at start of ERT in years, median (range)	10.7 (0.6–31.2)	4.7 (0.5–21.0)	5.0 (0.9–29.1)	0.12
MRC sumscore at start of ERT in percentage, median (range)	*76.9 (60.8–91.5)*	*80.8 (69.2–90.8)*	*81.9 (69.2–92.3)*	*0.02*
Wheelchair use at start of ERT, No. of patients (%)	*8 (38)*	*2 (12)*	*2 (13)*	*0.05*
FVC in upright position at start of ERT in percentage, median (range)	*66.6 (45.4–106.9)*	*65.7 (41.5–105.8)*	*81.0 (62.6–105.0)*	*0.02*
Ventilation use at start of ERT, No. of patients (%)	*4 (19)*	*6 (35)*	*0 (0)*	*0.01*

ERT=Enzyme-replacement Therapy, MRC sumscore=Medical Research Council sumscore, FVC=Forced Vital Capacity, No.=Number of patients.

p=0.88). Mechanical ventilation time could be reduced by one or more hours per day in nine of the 25 patients who had been using mechanical ventilation at start of ERT. One of these was able to discontinue the use of his ventilator. In 14 patients, ventilation times remained the same, while ventilation was intensified in two. Nocturnal ventilation was initiated in two others.

INDIVIDUAL RESPONSE WITH REGARD TO SKELETAL MUSCLE STRENGTH AND PULMONARY FUNCTION

With regard to MRC sumscore, 31 patients (45%) were good responders, 32 (46%) moderate, and six (9%) non-responders (Figure 2). More women than men were good responders (p=0.002, Table 3). Twenty-seven of the 62 patients with FVC data in upright position (44%) responded well, 26 (42%) moderately, and nine (15%) poorly. The characteristics between these FVC responder categories did not differ significantly.

Although FVC in supine position declined in the whole group during ERT, almost two-thirds of patients improved after starting ERT: 16 of 54 patients (30%) responded well, 17 (31%) moderately and 21 (39%) did not respond (Figure 2). Better responses were associated with younger age (p=0.007), less severe muscle weakness (p=0.02), wheelchair independence (p=0.05) and better pulmonary function in upright position (p=0.02) (Table 3). None of the good responders were dependent on artificial ventilation.

Responses for muscle strength correlated poorly with responses for FVC. The correlation between response categories for FVC in upright and supine was moderate (Spearman's ρ=0.56, p<0.001).

SAFETY ASSESSMENTS AND LABORATORY INVESTIGATIONS

Laboratory safety parameters and ECGs remained unchanged during ERT. In total, 12 patients (17%) developed one or more infusion-associated reaction (IARs). These were similar to the IARs described in the randomized-placebo controlled study; most could be controlled by slowing infusion rates and/or giving premedication.[29] To prevent further IARs, seven patients received antihistamines as pre-medication, and five a combination of antihistamines and corticosteroids. At the end of the study three patients still had IARs, three patients were using antihistamines as premedication and two a combination of corticosteroids and antihistamines. Enzyme-replacement therapy was discontinued in three patients who experienced IARs. In only one patient this was for safety reasons: this patient had a medical history of multiple auto-immune diseases and drug-induced allergies. In one of the other two patients IARs co-occurred with a very high antibody titer and a poor response to ERT, as described previously.[40]

Two severely affected patients died. Their causes of death (sepsis after severe decubitus and chronic respiratory insufficiency) were considered to be unrelated to ERT.

DISCUSSION

This study describes a large cohort of adult Pompe patients receiving treatment with alglucosidase alfa. It reflects a unique situation in which most patients were also prospectively followed before starting therapy, thereby extending median follow-up to

3 years (14 months before starting ERT and 23 months afterwards). We found that ERT significantly altered the natural course of disease in adult Pompe patients. Muscle strength increased significantly after they started ERT, and FVC in upright position stabilized. Even though, at group level, FVC in supine position and muscle function did not improve during ERT, there were improvements in certain subgroups of patients.

Like previous studies, we found that muscle strength deteriorated significantly before the start of therapy.[10,11] However, the improvement in muscle strength after the start of ERT was greater than that reported in other studies.[25-27,29,41] There may be various reasons for this. Our study was restricted to adult patients, but included patients across the entire disease spectrum. We tested more muscle groups, included more patients, and followed a longer treatment period than other studies, thereby producing over 500 measurements in total.

One new finding of this study is that women benefit more from ERT with respect to muscle strength than males. At the same dosage of 20 mg/kg bodyweight, it is possible that the relative dose of alglucosidase alfa received per gram of muscle-fibre tissue is higher in women than in men. Men generally have a higher lean body mass than women, and thus a somewhat higher muscle mass per kg.[42] As women also have smaller muscle fibres than men, they have a higher ratio of muscle-fibre surface to muscle-fibre volume. Consequently, they may have relatively more mannose 6-phosphate surface receptors, which mediate the uptake of alglucosidase alpha.[42,43] Other factors that may underlie the greater benefit women derive from ERT include muscle-fibre types, activity patterns, and hormonal influences.

Our study did not incorporate the six-minute walk test, a measure of functional endurance used in other studies.[24,27,29] Instead, we assessed motor skills related to daily activities, using the QMFT, which was recently validated for use in patients with Pompe disease.[36] Although there was no change in muscle function across the entire group, there were significant improvements in wheelchair-independent patients and those with less pronounced muscle weakness. This finding indicates that timely intervention with ERT may be crucial to improving muscle function. The same is suggested by the results of the subgroup analysis in the trial of late-onset patients.[29]

The stabilization of FVC in upright position in our patients was similar to that recorded in the trial and in other studies;[24,27,29] the decline in FVC before ERT was similar to that observed in the placebo arm of the trial and natural course studies.[10-12,29]

This is the first study to report on the effect of ERT on the FVC in supine position. In the whole study population, this measure continued to deteriorate despite ERT, but individual results showed an improvement in almost two-thirds of patients. Patients were more likely to improve if they were younger, were independent of artificial ventilation, had a better FVC in upright position, and had less severe muscle weakness at the start of treatment. Again, this suggests that starting ERT early in the disease course may be beneficial.

Because ERT has been available since 2006, we performed an open-label study rather than a clinical trial, assessing the effect of ERT in all adult patients – from mild to severely affected – for many of whom we had also collected pre-treatment data prospectively. As this is an observational study, we could not correct for residual confounding. The small sample size inherent to rare disorders meant that we could not apply a full multivariate model to identify prognostic factors.

CONCLUSIONS

In summary, by improving muscle strength and stabilizing pulmonary function in upright position, treatment with alglucosidase alfa positively altered the natural course of Pompe disease in adults. As well as finding that female gender is potentially a favorable prognostic factor for the effect of ERT on muscle strength, we found that younger age and better clinical status are favorable prognostic factors for pulmonary function. This suggests that it is important to start treatment early in the course of disease. Prognostic variables may help to identify patients with the best chances of benefiting from treatment.

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4.2

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 centres 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 centre (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 centre. 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, multicentre 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 centre 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.

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.

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.

TABLE 2

Results of Analysis of Covariance for Changes from Baseline to Week 78 for Primary and Secondary End Points.[a]

End Point	Alglucosidase Alfa Group (N=60)	Placebo Group (N=30)	Difference between Groups	p value
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[b]				
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

[a] Plus–minus values are means ±SD. CI denotes confidence interval. [b] 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.

TABLE 3

Serious Adverse Events during the Treatment Period.[a]

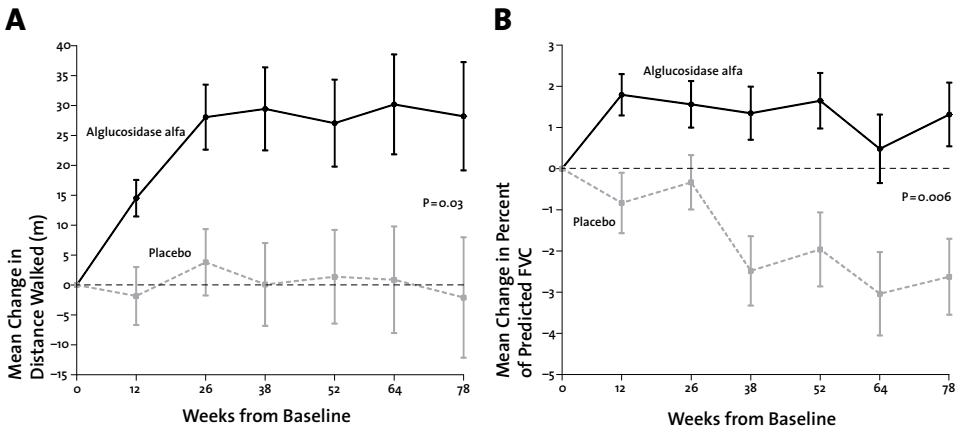
Adverse Event	Alglucosidase Alfa Group (N=60)	Placebo Group (N=30)
	no. of patients (%)	*no. of patients (%)*
Any event	13 (22)	6 (20)
Infections	2 (3)	1 (3)
Diverticulitis	0	1 (3)
Gastroenteritis	1 (2)	0
Pneumonia	1 (2)	0
Cardiac disorders	2 (3)	0
Coronary artery disease	1 (2)	0
Supraventricular tachycardia	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)
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.

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.



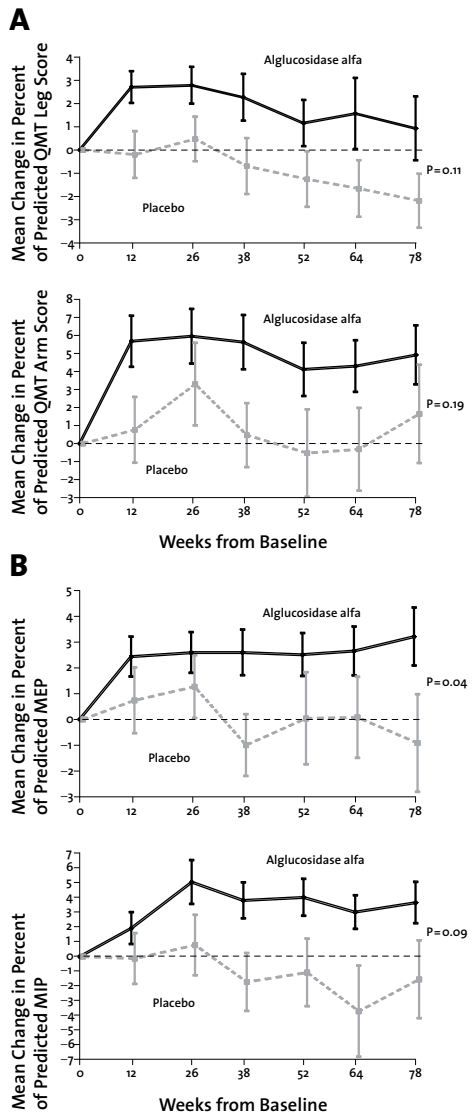
► FIGURE 2

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.

Infusion-associated reactions occurred in 28% of 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.

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 fibres 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.

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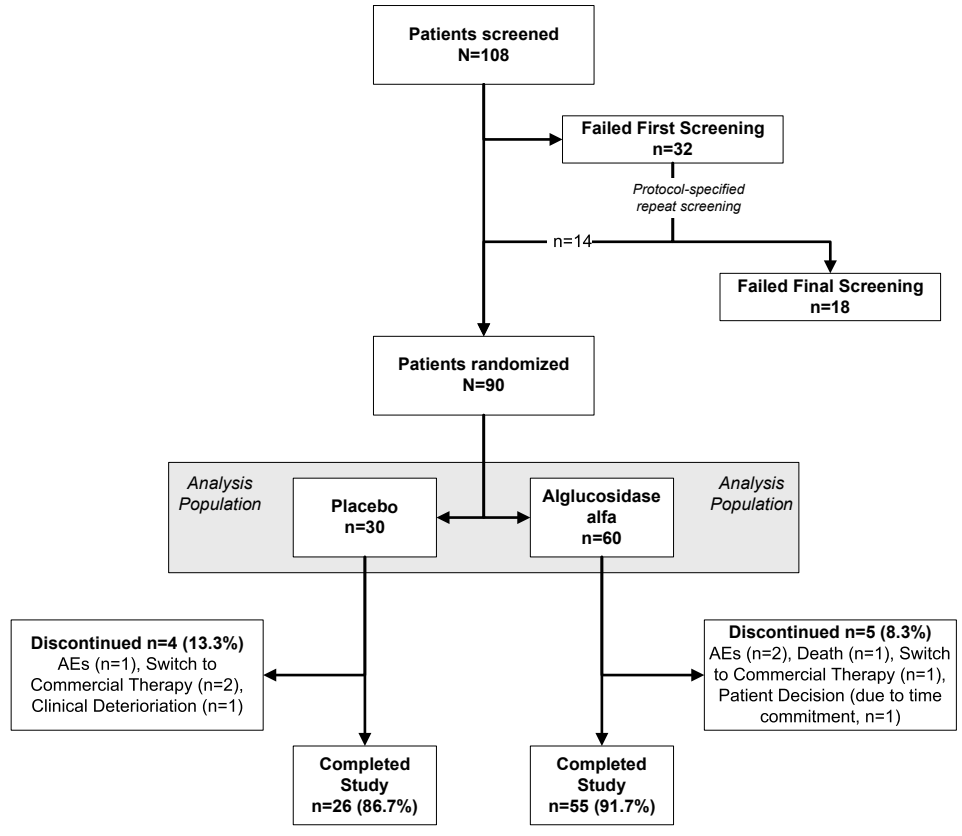
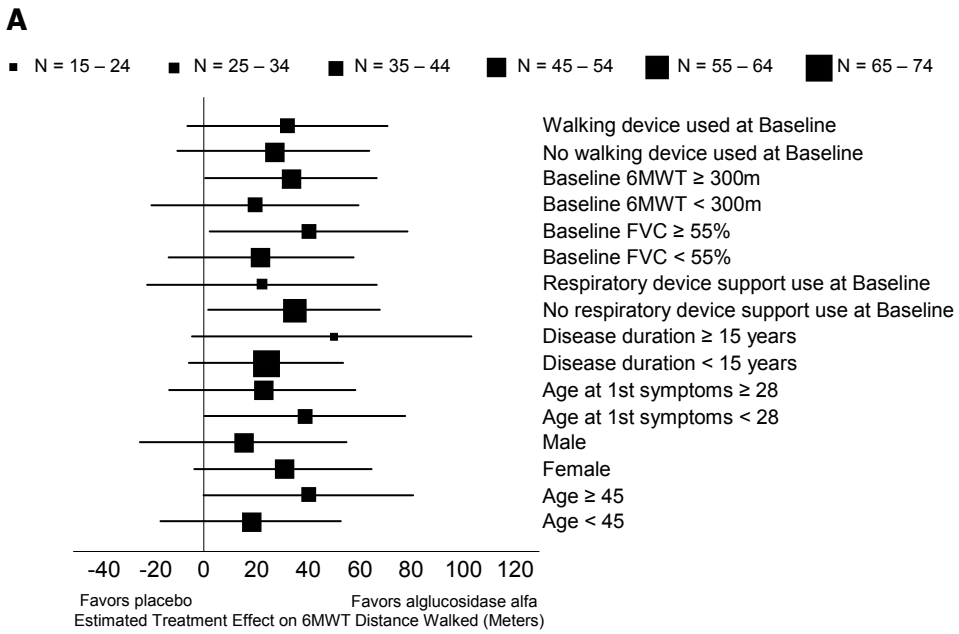
SUPPLEMENTARY APPENDIX

SUPPLEMENTARY FIGURES

SUPPLEMENTARY FIGURE 1
Patient flow



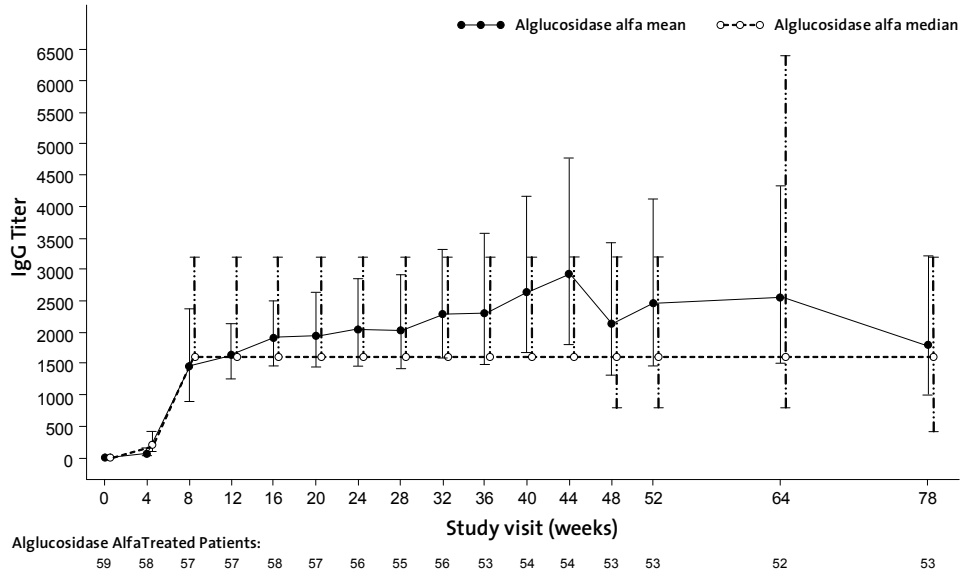


SUPPLEMENTARY FIGURE 2

Treatment Effect of Alglucosidase alfa on Walking Distance (A) and Pulmonary Function (B) across Various Patient Subgroups*. Squares indicate the estimated difference in 6MWT distance walked (a) or % predicted FVC (b) between the alglucosidase alfa treatment group and the placebo group for various patient subgroups. The horizontal line is the 95% confidence interval. Estimates to the right of the vertical line (greater than 0) favor alglucosidase alfa. The squares are scaled to the sample size within each subgroup, as shown in the legend.*

SUPPLEMENTARY FIGURE 3

Geometric Mean and Median IgG Titer Values over Time for All Alglucosidase alfa-treated Patients.



Note: Antibody titers observed in a cross-reactive immunologic material-negative (CRIM-negative) population studied by Kishnani et al. ranged from 0 to 1/1,638,400.By contrast, antibody titers in the current patient population ranged from 1/200 to 1/819,200. It is important to note that patients with late-onset Pompe disease are CRIM-positive, meaning they have some residual GAA protein. This probably explains why patients with late-onset Pompe disease do not tend to mount the high and sustained antibody response seen in CRIM-negative individuals, who have little to no residual protein.

SUPPLEMENTARY TABLES

SUPPLEMENTARY TABLE 1

Results of Sensitivity Analyses Comparing Six-minute Walk Test and Percent Predicted FVC Change from Baseline to Week 78 between Alglucosidase alfa- and Placebo-treated Patients

Endpoint	Method	Imputation Technique	Statistics	Results	
Six-minute Walk Test distance walked, meters	Mixed model for repeated measures	None	Estimate (95% CI)	32.0 m	(7.2, 56.8)
			p-value	0.01	
	Nonparametric Wilcoxon-Mann-Whitney	LOCF	p-value	0.029	
		Multiple imputation	p-value	0.028	
		Worst-rank	p-value	0.04	
FVC (% predicted)	Mixed model for repeated measures	None	Estimate (95% CI)	3.6 %	(1.4, 5.8)
			p-value	0.001	
	Nonparametric Wilcoxon-Mann-Whitney	LOCF	p-value	0.003	
		Multiple imputation	p-value	0.002	
		Worst-rank	p-value	0.002	

SUPPLEMENTARY TABLE 2

Summary of Treatment-emergent Adverse Events Considered Related to Treatment Occurring in at Least 5% of Patients by Treatment Group

System Organ Class Preferred Term	Number of Patients Receiving Alglucosidase alfa n (%)	Number of Patients Receiving Placebo n (%)
Any Adverse Events	32 (53.3)	17 (56.7)
General disorders and administration site conditions	15 (25.0)	7 (23.3)
Fatigue	3 (5.0)	4 (13.3)
Chest discomfort	4 (6.7)	1 (3.3)
Asthenia	0	2 (6.7)
Nervous system disorders	10 (16.7)	8 (26.7)
Headache	5 (8.3)	6 (20.0)
Dizziness	4 (6.7)	2 (6.7)
Skin and subcutaneous tissue disorders	13 (21.7)	4 (13.3)
Urticaria	5 (8.3)	0
Hyperhidrosis	5 (8.3)	0
Gastrointestinal disorders	9 (15.0)	4 (13.3)
Nausea	5 (8.3)	3 (10.0)
Vomiting	3 (5.0)	0
Musculoskeletal and connective tissue disorders	8 (13.3)	2 (6.7)
Muscle twitching	4 (6.7)	1 (3.3)

SUPPLEMENTARY TABLE 2, CONTINUED

System Organ Class Preferred Term	Number of Patients Receiving Alglucosidase alfa n (%)	Number of Patients Receiving Placebo n (%)
Myalgia	3 (5.0)	1 (3.3)
Eye disorders	6 (10.0)	2 (6.7)
Cataract	4 (6.7)	1 (3.3)
Ear and labyrinth disorders	4 (6.7)	2 (6.7)
Hypoacusis	2 (3.3)	2 (6.7)
Vascular disorders	4 (6.7)	2 (6.7)
Flushing	3 (5.0)	0
Investigations	4 (6.7)	0
Blood pressure increased	3 (5.0)	0

SUPPLEMENTARY TABLE 3

Summary of Infusion-associated Reactions Occurring in at Least 5% of Patients by Treatment Group

System Organ Class Preferred Term	Number of Patients Receiving Alglucosidase alfa n (%)	Number of Patients Receiving Placebo n (%)
Any Infusion-associated Reactions	17 (28.3)	7 (23.3)
Nervous system disorders	9 (15.0)	6 (20.0)
Headache	5 (8.3)	5 (16.7)
Dizziness	4 (6.7)	2 (6.7)
General disorders and administration site conditions	10 (16.7)	2 (6.7)
Chest discomfort	4 (6.7)	0
Gastrointestinal disorders	8 (13.3)	3 (10.0)
Nausea	5 (8.3)	3 (10.0)
Vomiting	3 (5.0)	0
Skin and subcutaneous tissue disorders	10 (16.7)	0
Urticaria	5 (8.3)	0
Hyperhidrosis	3 (5.0)	0
Vascular disorders	3 (5.0)	1 (3.3)
Flushing	3 (5.0)	0
Investigations	3 (5.0)	0
Blood pressure increased	3 (5.0)	0

SUPPLEMENTARY TABLE 4

Summary of Safety and Efficacy in Alglucosidase alfa-treated Patients by Peak IgG Titer Quartiles

Parameter	Peak IgG Titer Category for Alglucosidase alfa Patients Who Seroconverted (N=59)			
	Quartile 1 200-1600	Quartile 2 3200-3200	Quartile 3 6400-12800	Quartile 4 25600-819200
Number of Patients n (%)	17 (28.8)	12 (20.3)	16 (27.1)	14 (23.7)
Number of Patients with any AE, n (%)	17 (100.0)	12 (100.0)	16 (100.0)	14 (100.0)
Number of Patients with any SAE, n (%)	5 (29.4)	2 (16.7)	4 (25.0)	1 (7.1)
Number of Patients with any IAR, n (%)	6 (35.3)	2 (16.7)	5 (31.3)	3 (21.4)
6MWT change in meters walked from Baseline to last observation	6.1±53.67 median 5.0	16.0±24.98 median 9.0	34.8±76.60 median 16.5	49.1±79.91 median 19.5
FVC change in % predicted from Baseline to last observation	0.8±5.68 median 0.0	1.8±5.29 median 3.0	1.5±5.73 median 0.5	1.1±5.95 median 0.0

SUPPLEMENTARY TABLE 5

Inhibitory and IgG Antibodies for Patients Receiving Alglucosidase alfa Who Improved ≥25 M Compared with the Rest of Treated Patients

	Patients who improved ≥25 M N=27	Patients who did not improve ≥25 M N=30	p-value*
Inhibitory antibody status: Positive, N (%)	7 (26)	10 (33)	0.58
Peak IgG titer:			
N	27	30	
Median	6400	4800	0.85
Min, Max	1600, 20480	200, 819200	
IgG titer at Week 24:			
N	26	29	
Median	1600	1600	0.63
Min, Max	400, 25600	200, 51200	

** Inhibitory antibody status was tested using Fisher's exact test. IgG titer data are tested using Wilcoxon test.*

4.3

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 fibres. 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-centre, 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.

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).

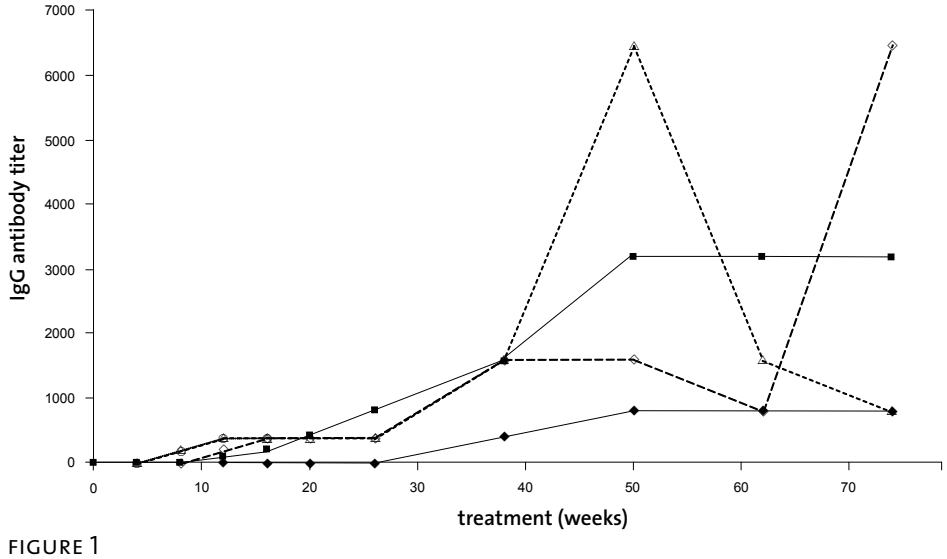

FIGURE 1

Anti-recombinant human α-glucosidase IgG antibody titers over 74 weeks of treatment.

◊ = patient 1, ■ = patient 2, ♦ = patient 3, △ = patient 4, ○ = patient 5.

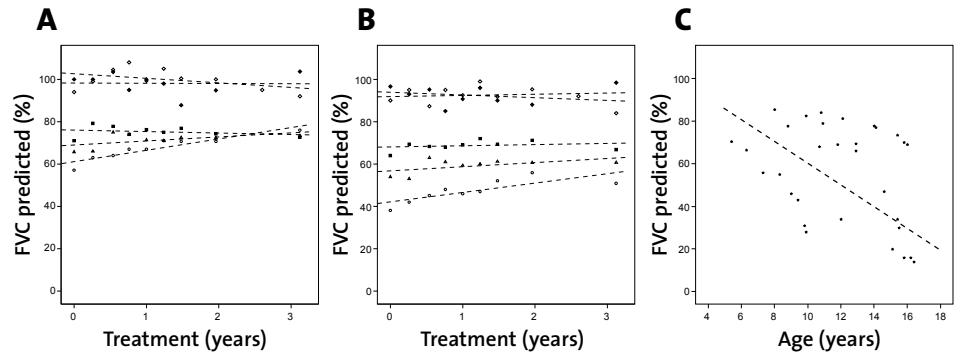

FIGURE 2

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.

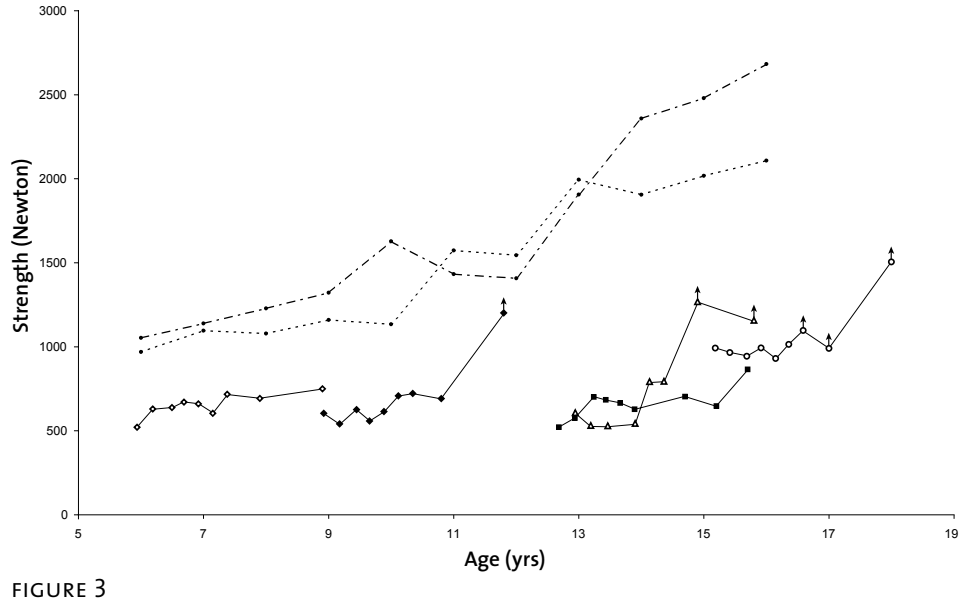

FIGURE 3

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.

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).

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 sumscores 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.

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.

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-month 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.

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

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).

at diagnosis was 27±12 years. All 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 2C) 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.

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5

DEVELOPMENT OF NEW MEASUREMENT SCALES

5.1

THE RASCH-BUILT POMPE-SPECIFIC ACTIVITY (R-PACT) SCALE

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ABSTRACT

We constructed a patient-based interval scale using Rasch analysis, specifically suited to quantify the effects of Pompe disease on patient's ability to carry out daily life activities and their social participation: Rasch-built Pompe-specific Activity scale. Between July 2005 and April 2011, 186 patients aged 16 or older, participated to develop this scale. External construct validity was determined through correlations with the MRC sumscore and Rotterdam Handicap Scale. Furthermore, test-retest reliability was determined in a subgroup of 44 patients. Finally, individual person-level responsiveness was used to determine the proportion of patients demonstrating significant improvement or deterioration during their natural disease course, or during treatment with enzyme-replacement therapy. Of the original 49 items, 31 were removed after investigation of model fit, internal reliability, threshold examination, item bias, and local dependency. The remaining 18 items were ordered on a linearly weighted scale and demonstrated good discriminative ability (Person Separation Index 0.96), external construct validity (intraclass correlation coefficient (ICC) for MRC sumscore 0.82, and for the Rotterdam Handicap Scale 0.86), reliability of person's location (ability comparison: ICC 0.95), and responsiveness. We therefore conclude that the R-PAct scale enables us to accurately detect limitations in activities and social participation throughout the entire disease spectrum in patients with Pompe disease.

INTRODUCTION

Pompe disease – also named glycogen storage disease type II – is an inherited metabolic disorder in which partial or total absence of the enzyme acid α-glucosidase causes intra-lysosomal accumulation of glycogen in many tissues.[1] The clinical spectrum ranges from the rapidly progressive classic infantile phenotype, which – when not treated with enzyme-replacement therapy – leads to death within the first year of life,[2,3] to a more slowly progressive phenotype that primarily affects skeletal and respiratory muscles.[4-6] Progressive muscle weakness eventually leads to wheelchair and ventilator dependency in a substantial amount of patients.

As a consequence, Pompe disease strongly affects patients' ability to carry out daily life activities and influences their social participation. Quantifying these aspects is important for the management of individual patients and for evaluating effects of enzyme-replacement therapy (ERT)[7,8] or future treatment modalities. At present, limitations in activities and social participation are often assessed by non-specific functional tests such as the 10-meter walk test and the six-minute walk test,[7,8] or ordinal measurement scales such as the Rotterdam Handicap Scale or the Gross Motor Function Measure.[9-11] It is now realised that these ordinal scales are prone to differential sensitivity – meaning that a one-point change in score at the centre of the scale may not be the same as a one-point change at the extremes.[12,13] Therefore, for health evaluation, a modern scientific approach transforming ordinal scores into a linearly weighted measure is required.

We thus developed a patient-based interval scale (Rasch-built Pompe-specific Activity scale: R-PAct scale) using Rasch analysis.[14,15] Subsequently, we evaluated its validity, reliability and responsiveness. It is to be expected that this measurement scale, based on patients' experiences of limitations in daily life, will have a high discriminatory capacity and will be able to measure changes in functional status, which aids in follow-up of the natural disease course or in the evaluation of therapeutic efficacy throughout the entire spectrum of disease severity.

PATIENTS AND METHODS

STUDY POPULATION AND PROCEDURES

Between July 2005 and April 2011, 186 patients aged 16 or older, participated to develop the R-PAct scale. Patients were recruited though patient organizations affiliated with the International Pompe Association (IPA) in Canada, the Netherlands, the United Kingdom, and the United States.[6] In addition, patients were recruited through neuromuscular centres in the Netherlands and Belgium. Forty-four of the Dutch patients completed the scale a second time 2–4 weeks later for test-retest reliability studies. To evaluate responsiveness of the R-PAct scale, patients were followed longitudinally up to 36 months during the natural disease course or during treatment with enzyme-replacement therapy. Patients from the international patient cohort completed the scale every year, while the Dutch and Belgian patients completed the scale every 3–6 months. The studies were approved by the Medical Ethics Committee of Erasmus MC University Medical Center. All patients gave written informed consent.

SCALE DEVELOPMENT

Preliminary R-PAct questionnaire

A preliminary R-PAct questionnaire was developed taking the following steps:

1) To cover the widest range of physical functioning, activities, and participation skills important for patients with Pompe disease, data from an international patient survey among 263 patients with Pompe disease were used as the basis for construction of the current questionnaire.[6] In this survey, patients provided information about their disease history and current status by means of self-reported questionnaires. Firstly, an inventory was made of the answers on the following open question from this patient survey: "what are your most important (limiting, annoying) problems?" Secondly, answers to the question "can you describe your walking problems?" were used to refine the items on walking. Thirdly, patients were asked to indicate other items that were important for their daily life but that were not discussed in the questionnaires.
2) Additional items based on the Rotterdam Handicap Scale (1 item)[10] and Paediatric Evaluation of Disability Inventory (8 items),[16] thought to be of importance by expert judgement, were included.
3) This preliminary list comprising 136 items was classified according to the International classification of functioning, disability and health (ICF) into the domains impairment, activities, and participation.[17]
4) A panel of experts consisting of senior staff members from our departments of neurology, paediatrics, internal medicine, and clinical genetics, all involved in research projects and treatment of Pompe disease, discussed the items and their classification. Changes were implemented according to their suggestions: items that were almost identical were merged, and items that were mentioned only occasionally were left out. After reaching consensus, a list of 49 activity and participation items was selected for the purpose of the current study. All items had five response options: (0) unable to perform; (1) able to perform, but with great difficulty; (2) able to perform, but with some difficulty; (3) able to perform, but with little difficulty; (4) easy to perform, without difficulty; or "not applicable".
5) The questionnaire was tested in a group of ten healthy subjects. Based on their comments, changes were made to prevent overlap and to improve clarity.
6) For use in English speaking patient groups, this final questionnaire was translated and back-translated by two independent certified translators according to published guidelines.[18]

The items of the preliminary R-PAct questionnaire are listed in Appendix A.

Final R-PAct scale

Rasch analysis

Since a sample size of approximately 250 is needed to adequately estimate item difficulty,[19] we decided to stack the data of the first (n=186) and second (n=44) assessments, controlling for "time factor" as possible confounding factor,[20] leading to a total number of 230 records to be examined. In the model construction, items scored as "not applicable" were interpreted as missing data. Items with more than 10% missing values and questionnaires with more than 10% unanswered items were omitted as a quality control procedure. Thereafter, the remaining response values of the preliminary R-PAct were analyzed using Rasch unidimensional measurement models (RUMM 2030).[21] Through Rasch analysis, ordinal scores are transformed into interval measures, placing both item and person parameter estimates on the same log-odds units (logit) scale, which allows for linear transformation of the raw scores.[14,15] A detailed description of the statistical modelling procedures has been provided elsewhere, also specifically for neurologists.[22-24] Briefly, the following Rasch model requirements were checked:

A) *Fit statistics and fit residuals*: To test whether the data meet the model expectations, three overall fit statistics were considered. Two are item-person interaction statistics, expressed as z-scores: if the items and persons fit the model, a mean around zero and a standard deviation of 1 would be expected. The third is an item-trait interaction statistic, reported as chi-square (χ^2): a non-significant chi-square reflects the required property of invariance. Additionally, individual person-fit statistics and item-fit statistics were examined as residuals, and by using a chi-squared statistic. Residuals between ±2.5 are considered adequate fit to the model, whilst a significant χ^2 points to misfit.
B) *Internal reliability*: This was measured by the Person Separation Index (PSI). A value of ≥0.7 indicates that the scale is able to differentiate at least two groups of patients, and is generally considered to be acceptable.[25]
C) *Threshold examination*: The point between two adjacent response categories where both responses are equally probable is called the "threshold". Difficulty to discriminate between response options – for example due to too many possibilities – may lead to disordered thresholds. The overall model fit may improve by merging of categories.
D) *Item bias*: This was assessed by means of differential item functioning (DIF).[26] DIF occurs when the probability of responding to an item is systematically different between groups with equal levels of disability but differences in another characteristic (e.g. age). This was examined by analysis-of-variance for the following arbitrarily chosen personal factors, aiming for an equal distribution of patients among the categories: 1) age (<40 years, 40-50 years, 50-60 years, or ≥60 years); 2) gender (male or female); 3) duration of symptoms (<5 years, 5-10 years, 10-20 years, or ≥20 years); 4) country of assessment (Belgium, Canada, the Netherlands, UK, or USA); and 5) language (Dutch or English).
E) *Local dependency*: Local dependency arises when items are linked. For example, a patient who is unable to walk 100 m, will also be unable to walk 1 km. An inter-item residual correlations ≥0.3 indicates local dependency.[27]
F) *Unidimensionality*: This was tested by a principal component analysis of the residuals, needed to support the assumption of local independence and, consequently, the unidimensionality of the scale.[23]

Throughout the analyses, we continuously monitored whether Rasch model criteria were met: items that did not fulfil these requirements were removed, or adjusted to fit the model, while monitoring changes and fit statistics of the individual remaining items and the overall model fit. As a last step, the calculated Rasch person locations (in logits) were transformed into a more understandable centile metric ranging from 0 (most severe activity and participation restrictions) to 100 (no activity limitations and participation restrictions).

Validity
The external construct validity was assessed by correlations between the final R-PAct scale and the Medical Research Council (MRC)[28] sumscore and Rotterdam Handicap Scale (RHS).[10] To obtain the most appropriate graphical model fit, regression analysis with restricted cubic spline functions was performed on the summed raw R-PAct scores: the intraclass correlation coefficient (ICC) is reported.[29]

Medical Research Council (MRC) score
Through manual muscle testing using the MRC grading system,[28] we assessed the degree of skeletal muscle weakness in the 88 patients participating in the ongoing study on the natural course of Pompe disease in the Netherlands. Since, in patients with Pompe disease, muscle weakness is present predominantly in the proximal muscle groups ("limb-girdle" weakness), leaving the distal muscle groups relatively unaffected until the late stages of the disease, a sumscore was calculated for the following muscles or muscle groups: neck extensors, neck flexors, and bilateral shoulder abductors, elbow flexors, elbow extensors, hip flexors, hip abductors, knee flexors and knee extensors (range 0 (paralytic) to 80 (normal strength)).

Rotterdam Handicap Scale (RHS)
The RHS was developed originally for measuring handicap in patients with immune-mediated polyneuropathies,[10] and was proven to be useful for examining limitations in activities and participation in patients with Pompe disease.[9] The scale consists of nine questions on the topics of mobility indoors and outdoors, kitchen tasks, domestic tasks indoors and outdoors, leisure activities indoors and outdoors, travelling, and work/study. The total score ranges from 9 to 36, with higher values representing a lower level of handicap.

Reliability
To investigate whether the hierarchy of patients' ability location was consistent over time, test-retest reliability studies were performed.[30] Reliability was quantified by calculating the intraclass correlation coefficient using a one-way random effects analysis-of-variance (ANOVA) model for group comparison.

Responsiveness
Responsiveness was calculated at the individual person-level, since modern clinimetric methods have demonstrated that the standard error (SE) around an individual patients' ability level, and therewith the clinical importance of changes within a patient over time, may vary across the range of an outcome measure.[31,32] As a measure for individual responsiveness, the minimal clinically important difference-standard error (MCID-SE: individual change / standard error of difference (SE_{diff})) was calculated for every participating patient at each assessment.[33] To obtain individual SEs, all data were subjected to the RUMM 2030 model first, hereby creating the location of each patient (in logits) with the corresponding SE (also in logits). The cut-off value for a clinically important change – improvement or deterioration – was defined at ±1.96 x SE. Subsequently, Kaplan-Meier curves were applied to estimate the cumulative proportion of patients demonstrating significant improvement or significant deterioration over time (3, 6, 9, 12,... up to 36 months), stratified for "treatment" (natural disease course against enzyme-replacement therapy). The log-rank test was used to examine possible group differences.

STATISTICS AND SOFTWARE
Rasch analyses were performed with the partial credit model as default (RUMM2030).[21] Further analyses were undertaken using Stata Statistical Software for Windows XP (version 11.0, StataCorp, Texas, USA). Throughout the analyses, Bonferroni corrections were applied to adjust the p-values for multiple testing.[34]

RESULTS

STUDY POPULATION
The study population comprised patients from the Netherlands (n=94), the USA (n=65), the UK (n=18), Canada (n=6), and Belgium (n=3). Fifty-one percent was female. The median age at which patients had been diagnosed was 37 years (range 1 to 67 years). Median age at first investigation was 50 years (range 23 to 85 years), and median disease duration 11 years (range 0 to 33 years). Thirty-seven percent of patients were fully ambulatory, 16% used walking devices, and 47% of all patients was either partially or permanently wheelchair dependent. Forty-five percent of patients used mechanical ventilatory support. Patients from the United States, United Kingdom or Canada had a longer disease duration ($p<0.01$) and were more frequently ventilator dependent than the Dutch or Belgian patients ($p<0.01$).

RASCH ANALYSIS
Initial analysis on the preliminary R-PAct questionnaire
The preliminary R-PAct questionnaire (see Appendix A for full list of items) did not meet all Rasch model expectations (Table 1, initial analysis).

Data handling to fit the Rasch model
1) Thirty items of the preliminary R-PAct questionnaire demonstrated disordered thresholds, particularly in the mid-response area (response options 1–3, Figure 1). The remaining 19 items had thresholds very adjacent to each other in the mid-response area. Based on these observations, we decided to rescore all items into three response categories as follows: (0) = (0) unable to perform; (1–3) = (1) able to perform, but with difficulty; and (4) = (2) able to perform without difficulty.
2) Inspection of individual item-fit and individual person-fit statistics showed that 14 items demonstrated misfit to the model, 13 having a significant chi-square probability, and one having fit residuals exceeding -2.5. These items were removed one by one, continuously checking the class intervals, statistical control panel and possible changes on other Rasch requirements.
3) Thirteen items showed item-bias: six items with regard to the personal factor "country", three for "language", two for "age", and two for "duration of symptoms". These items

TABLE 1

Summary statistics of Rasch analysis during construction of the R-PAct scale for Pompe patients

Analysis	Item Fit Residuals		Person Fit Residuals		Item-trait χ^2 interaction		PSI	Unidimensionality t-tests (95% CI)
	Mean	SD	Mean	SD	DF	p-value		
Initial[a] (preliminary R-PAct)	-0.254	1.780	-0.146	0.982	147	<0.0001	0.98	0.22 (0.187–0.243)
Final (R-PAct)	-0.353	0.826	-0.318	0.839	54	0.82	0.96	0.061 (0.032–0.090)

χ2=Chi Square, DF=Degrees of Freedom, SD=Standard Deviation, CI=Confidence Interval, PSI=Person Separation Index.

[a] Thirty-one items were removed from the original preliminary R-PAct questionnaire. The remaining 18 items as part of the final R-PAct scale fulfilled all Rasch model expectations.

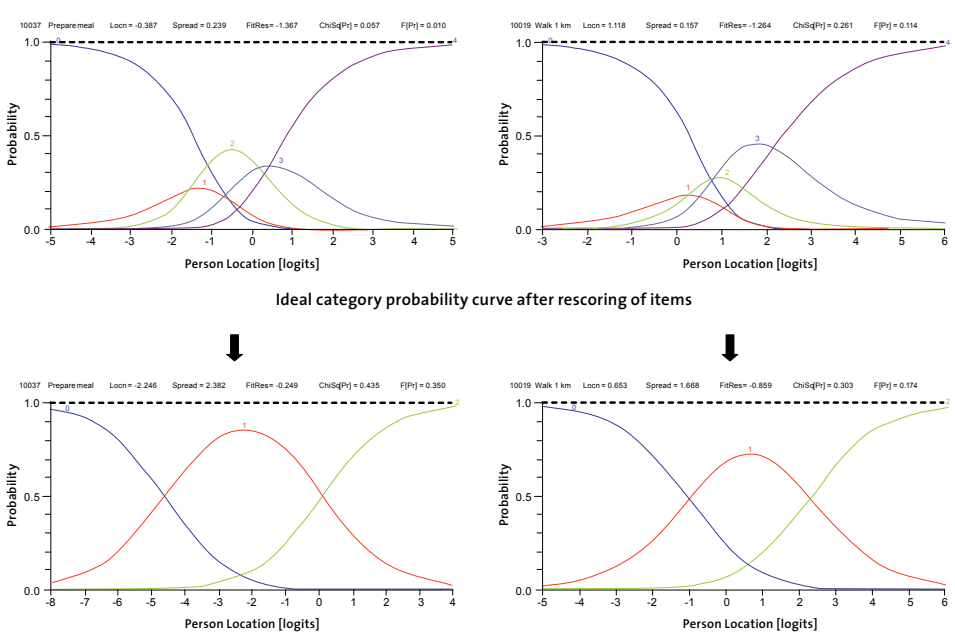
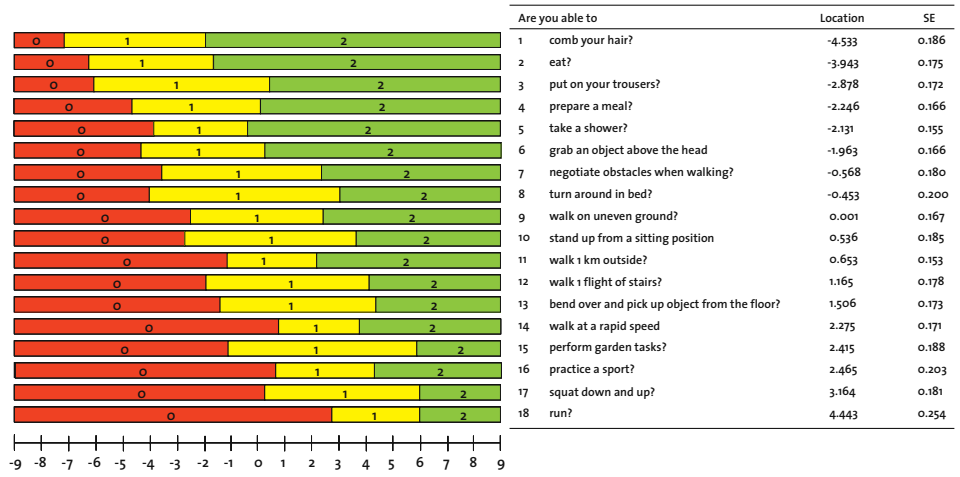


FIGURE 1

Upper panels: two items (left: prepare a meal, right: walk 1 km) are presented as examples of reversed thresholds. Response categories, particularly in the mid response area (from 1 to 3) were not equally probable, indicating the inability of the patients to discriminate between these response options. Thirty of the initial items demonstrated this pattern. Lower panels: ideal probability curves after rescoring the items from 5 to 3 response options.



	Are you able to	Location	SE
1	comb your hair?	-4.533	0.186
2	eat?	-3.943	0.175
3	put on your trousers?	-2.878	0.172
4	prepare a meal?	-2.246	0.166
5	take a shower?	-2.131	0.155
6	grab an object above the head	-1.963	0.166
7	negotiate obstacles when walking?	-0.568	0.180
8	turn around in bed?	-0.453	0.200
9	walk on uneven ground?	0.001	0.167
10	stand up from a sitting position	0.536	0.185
11	walk 1 km outside?	0.653	0.153
12	walk 1 flight of stairs?	1.165	0.178
13	bend over and pick up object from the floor?	1.506	0.173
14	walk at a rapid speed	2.275	0.171
15	perform garden tasks?	2.415	0.188
16	practice a sport?	2.465	0.203
17	squat down and up?	3.164	0.181
18	run?	4.443	0.254

FIGURE 2

Threshold distribution map and fit statistics for the 18-item final R-PAct scale, fulfilling all Rasch model expectations. Items are ordered by increasing difficulty. The easiest item turned out to be "are you able to comb hair", the most difficult one "are you able to run". Red sections (0) = unable to perform, yellow sections (1) = able to perform, but with difficulty, green sections (2) = able to perform, without difficulty.

were removed from the analyses.

4) To identify possible local dependency, all pairs of items with correlations above 0.28 were evaluated, starting with the highest correlations. Of each item-pair, the item showing the least clinical relevance (i.e. face validity and content validity), and the most over-discrimination or under-discrimination on its category probability curve was removed. In this way, four items were removed.

After completing these procedures, the final R-PAct scale, comprising 18 items, met all Rasch model expectations (Table 1, final analysis). In the final R-PAct scale, the item "are you able to comb your hair?" was the easiest to perform whereas the item "are you able to run?" turned out to be the most difficult task. Item difficulty ranged from -4.53 to 4.44 logits and patient ability level from -8.33 to 7.79 logits. Figure 2 shows the threshold distribution map and the fit statistics for the 18 items of the final R-PAct scale. Table 2 provides a nomogram allowing the translation of the R-PAct summed raw scores to the calculated Rasch person location (in logits), and to an understandable centile metric. Fourteen patients (6%) could not perform any task at all (floor effect), and 1 patient (0.4%) was able to perform all activities without any difficulty (ceiling effect).

EXTERNAL CONSTRUCT VALIDITY

The R-PAct scale demonstrated strong correlations with the MRC sumscore (ICC 0.82) and RHS (ICC 0.86), reflecting good construct validity (Figure 3). The discriminatory capacity of

TABLE 2

Nomogram allowing translation of summed raw scores of the final 18-items R-PAct scale (range 0 to 36) to an estimate of the Rasch person location (in logits) and a convenient centile metric (range 0 to 100). The corresponding logits in relation to the summed raw scores are provided by the RUMM software. [a] *The nomogram can only be used when all questions have been completed by the patient.*

R-PAct summed raw score[a]	Rasch Person location (Logit)	Centile metric
0	-8.33	0
1	-7.25	7
2	-6.37	12
3	-5.66	17
4	-5.05	20
5	-4.53	24
6	-4.07	26
7	-3.65	29
8	-3.25	32
9	-2.88	34
10	-2.52	36
11	-2.18	38
12	-1.85	40
13	-1.54	42
14	-1.23	44
15	-0.92	46
16	-0.61	48
17	-0.31	50
18	0.00	52
19	0.31	54
20	0.63	56
21	0.95	58
22	1.29	60
23	1.63	62
24	1.99	64
25	2.34	66
26	2.69	68
27	3.04	70
28	3.40	73
29	3.76	75
30	4.14	77
31	4.55	80
32	5.00	83
33	5.50	86
34	6.09	89
35	6.84	94
36	7.79	100

the R-PAct scale is clearly illustrated in Figure 4. Patients with more disability, measured by the use of walking devices or wheelchair use, scored significantly lower on the R-PAct scale. The same pattern was seen when comparing patients using mechanical ventilation against those with no need for ventilation.

RELIABILITY

The Person Separation Index was 0.96, demonstrating good internal consistency reliability. The test-retest reliability for person location was good as well: patient locations at the first and second assessment were nearly always within the 95% confidence intervals, reflecting ideal invariance (intraclass correlation coefficient 0.95) (Figure 5).

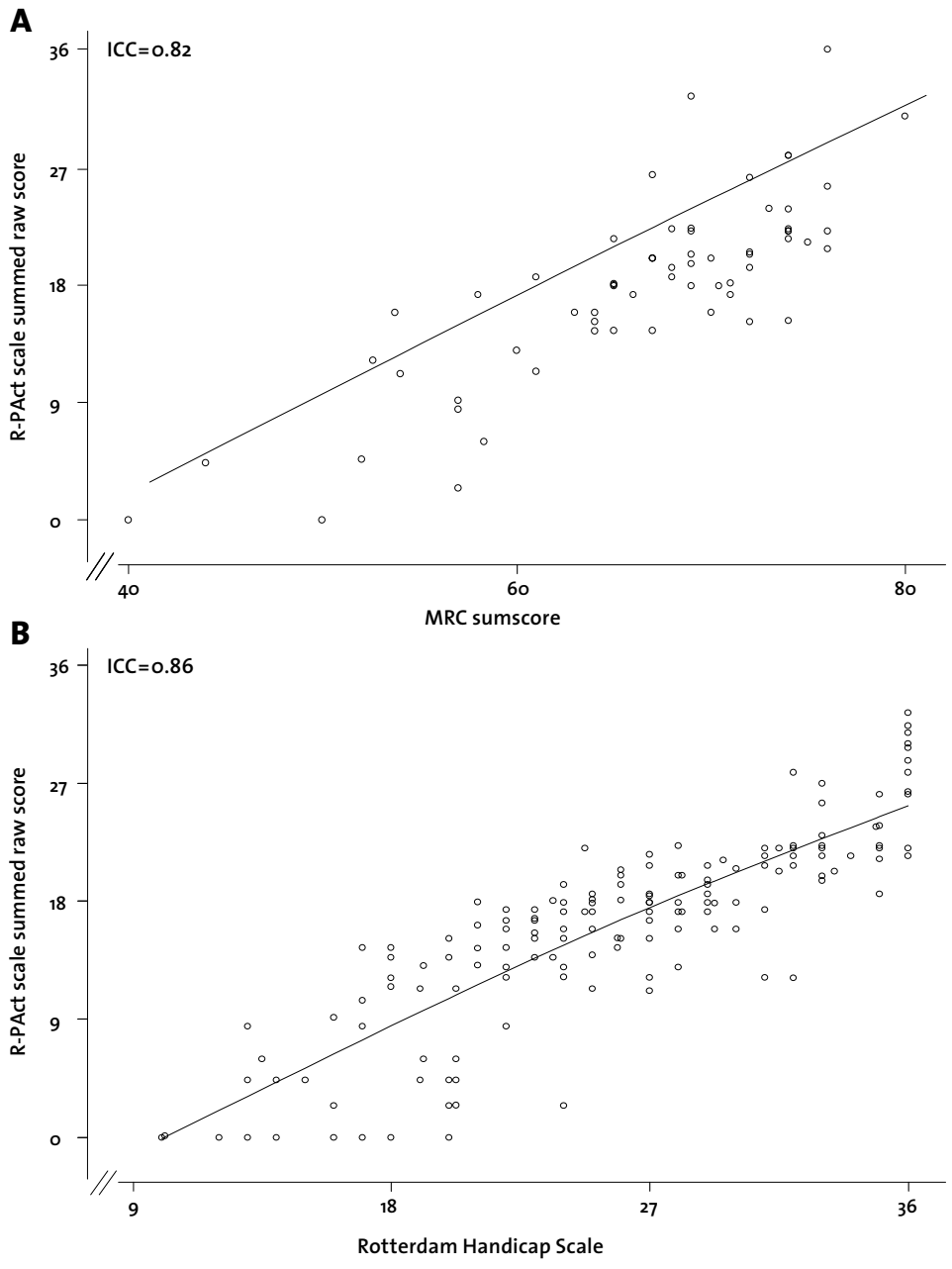
RESPONSIVENESS

Longitudinal data on the natural disease course were available for 101 patients, while for 111 patients follow-up data following treatment with enzyme-replacement therapy were evaluated. At 36 months, 21% of patients who were being treated with enzyme-replacement therapy demonstrated a clinically important improvement at the *individual person-level*, against 7% of patients who were not being treated (i.e. natural disease course) ($p<0.01$). By comparison, 33% of patients who did not receive enzyme-replacement therapy demonstrated a clinically important deterioration compared to entry, while 7% patients who were being treated with enzyme therapy showed a clinically meaningful deterioration ($p<0.01$). Figure 6 shows the corresponding Kaplan-Meier curves.

DISCUSSION

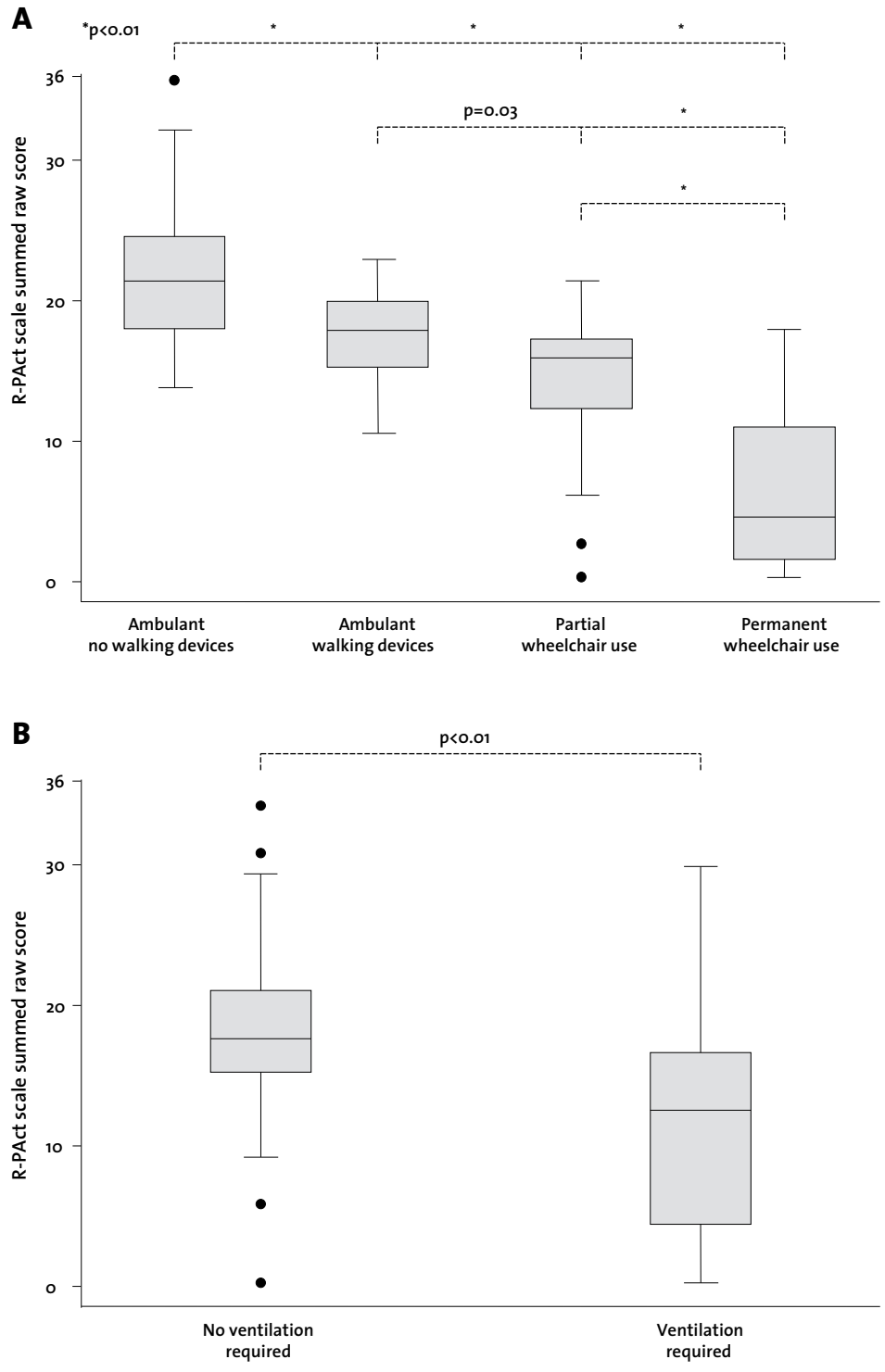
We developed and validated a new, self-reported, questionnaire designed specifically for use in patients with Pompe disease, based upon experiences from patients about their most important and limiting aspects in daily life. The final 18-items R-PAct scale was able to measure across the spectrum of very severely to mildly affected patients without any relevant floor or ceiling effects, and showed good test-retest reliability. Furthermore, the final scale showed no item bias or local dependency and demonstrated acceptable unidimensionality. The high Person Separation Index, which indicates a strong ability of the scale to differentiate between patients with various degrees of ability, is proof of good internal consistency reliability. External construct validity was demonstrated by good correlations with the MRC sumscore and RHS, indicating that the R-PAct scale is capable of indirectly capturing physical impairments leading to problems in daily and social functioning.[17] Similar patterns between impairment, disability and handicap have been reported in patients with chronic immune-mediated neuropathies[35] and myotonic dystrophy.[22]

This study contributes to the movement from classical test theory to modern test theory in the creation and evaluation of outcome measures in chronic neurological conditions. Incorporating MCID techniques will help in better understanding of the clinical relevance of changes in scores (e.g. defining responders *against* non-responders), rather than concentrating on statistical significance alone.[36,37] Traditional responsiveness indicators do not always provide information on the magnitude and direction of change (improvement, stable situation, or deterioration) for each individual patient. The Rasch



▲ FIGURE 3
Correlation between the R-PAct scale summed raw scores and MRC sumscore (A) and Rotterdam Handicap Scale (RHS) (B). ICC=Intraclass Correlation Coefficient.

► FIGURE 4
Correlation between the R-PAct scale summed raw scores and mobility (A) and use of ventilatory support (B) illustrating the discriminatory capacity of the R-PAct scale. ICC=intraclass correlation coefficient.



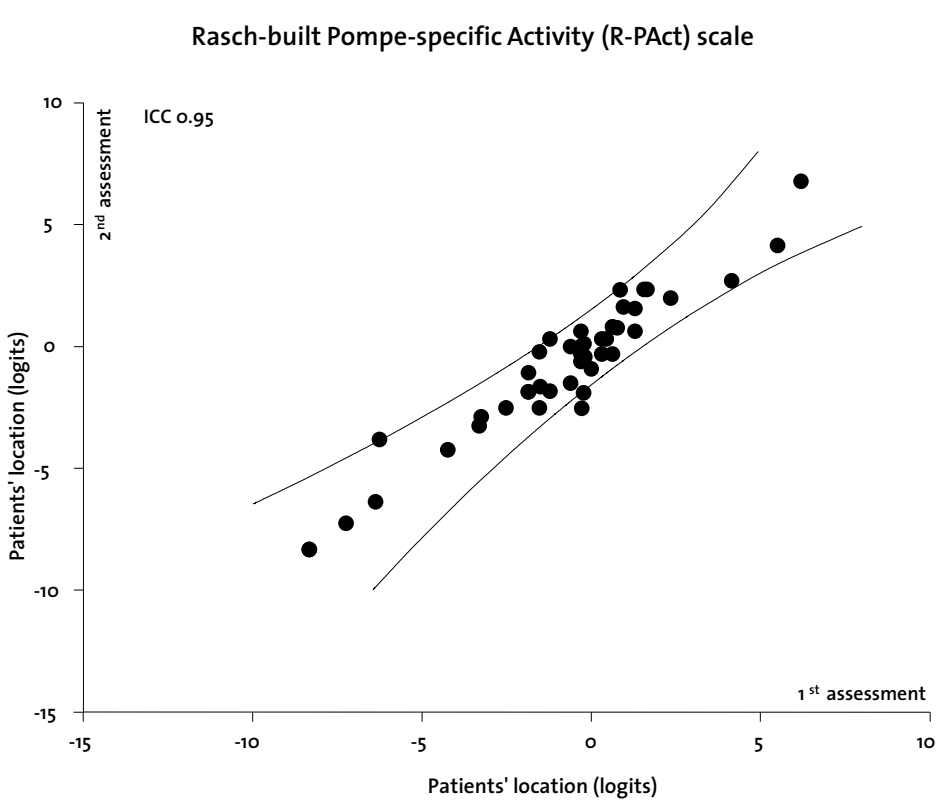
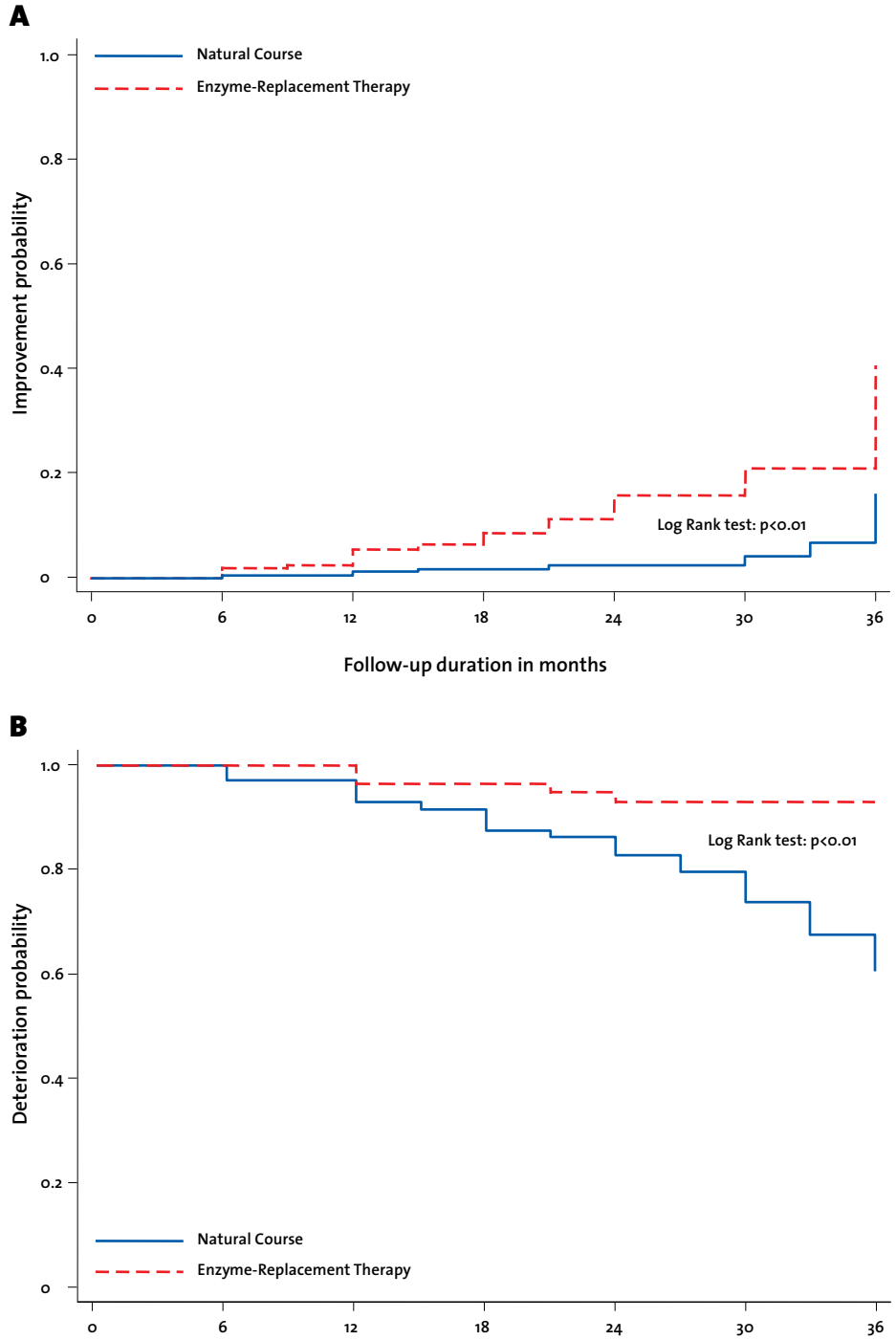


FIGURE 5

Test-retest reliability studies between patients' ability location measured at the first and second assessment and the 95% confidence intervals (solid lines) of the ideal invariance. ICC=Intraclass Correlation Coefficient.

► FIGURE 6

Kaplan-Meier curves illustrating the proportion of Pompe patients demonstrating clinically meaningful improvement (A) or deterioration (B) during the natural disease course or during treatment with enzyme-replacement therapy, applying the concept of minimal clinically important change (MCID) using a cut-off value of ±1.96 x SE.



method makes it possible to define "response" at the individual person-level, taking into account individual standard errors changing according to patient's ability level, and may therefore have major implications also for future trials. Taking a cut-off value for MCID-SE of ±1.96, the R-Pact scale was shown to detect clinically meaningful changes over time. This will be valuable in estimating the rate of disease progression, determining the best moment to initiate treatment, and evaluating therapeutic efficacy.

In recent years, several Rasch built measures of limitations in activities and participation have been constructed for patients with neuromuscular disorders (ACTIVLIM),[38] myotonic dystrophy type I (DM1-Activ),[22] immune-mediated neuropathies (R-ODS),[24] and also for children and adolescents with Pompe disease (Pompe-PEDI).[39] Whereas the ACTIVLIM is a generic activity measure for patients with neuromuscular disorders, the DM1-Activ, R-ODS and Pompe-PEDI are disease-specific measurement instruments. The rationale for development of yet another scale was that we expected that a measurement instrument based on patients' own experiences would be the most appropriate to the patient group under study. It can be argued that for different patient groups selection of items would be different, or that the difficulty of the selected items is different. For example, items that are relevant for patients with a proximal myopathy do not necessarily apply to patients with pronounced distal skeletal muscle weakness. In part the current measurement scale overlaps with the existing measurement instruments: eight items of the final R-PAct scale are the same as in the DM1-Activ, and five items are shared with the ACTIVLIM. However, the estimated item difficulty differs substantially between the patient groups examined. Therefore, a disease-specific scale is more suited to estimating the impact of Pompe disease on daily life. In contrast to the Pompe-PEDI, which was developed specifically for children and adolescents with Pompe disease and especially takes the domains of mobility and self-care into account, the R-PAct scale is designed for use in patients of 16 years or older and also addresses aspects of social participation. We believe that the newly constructed scale gives more insight in the disabling impact of this disorder. Nevertheless, for a comprehensive overview of patients' functionality it should be used complementary to clinical evaluation methods measuring impairment, such as manual muscle testing, quantitative muscle testing, muscle function tests or pulmonary function testing.

Some limitations of the study need to be addressed. Ideally, a sample size of approximately 250 patients is needed to provide accurate model stability. This could only be reached by stacking of the data. Secondly, we used the MRC sumscore and RHS for establishing the external construct validity of the newly constructed scale, which are in fact ordinal summed scores. Recently, a revised MRC scoring system was developed, which is considered a substantial improvement in evaluating muscle strength.[40] It should now be determined whether this modified MRC scoring system is more appropriate for use in patients with Pompe disease.

In conclusion, the R-PAct scale enables us to accurately measure limitations in activities and restrictions in social participation throughout the whole spectrum of disease severity in patients with Pompe disease older than 16 years, and is able to capture clinically important changes over time. We therefore expect the R-PAct to be useful in future studies evaluating the natural disease course or therapeutic efficacy.

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5.2

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.

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).

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).

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

TABLE 1

Clinical characteristics of the 91 study patients

Sex (male/female)	**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

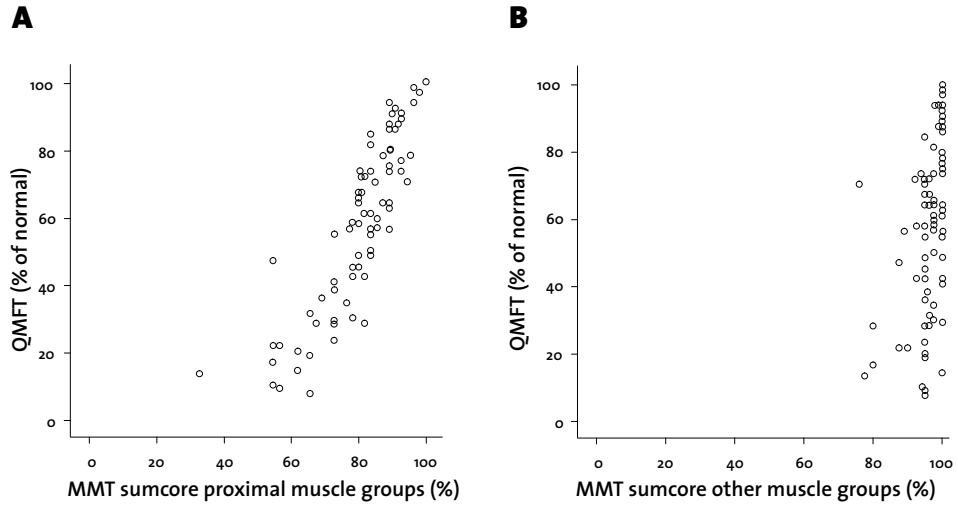
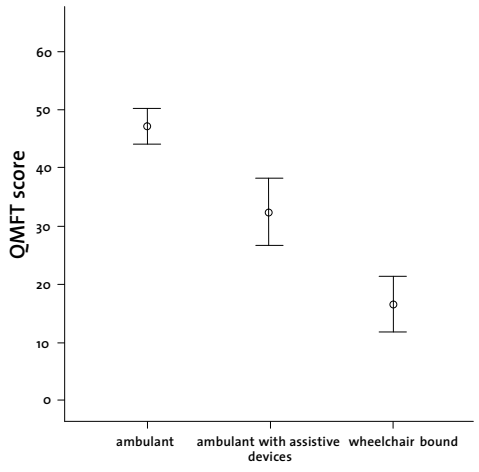


FIGURE 1

Relationship of the Quick Motor Function Test (QMFT) score to manual muscle testing (MMT): A) sumscore of proximal muscle groups; B) sumscore of other muscle groups.

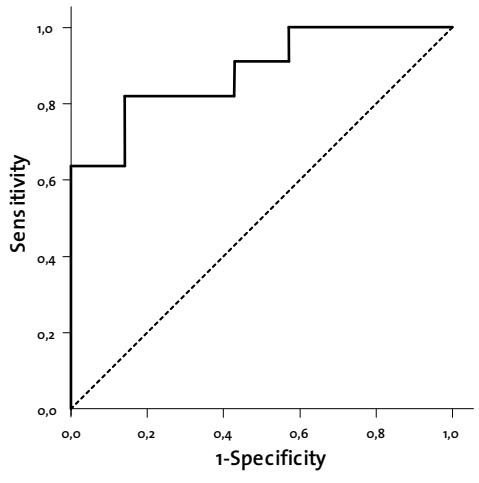
▾ FIGURE 2

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



▾ 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.



(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 ($p<0.05$).

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.

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GENERAL DISCUSSION

GENERAL DISCUSSION

Until recently, Pompe disease was an incurable and untreatable disease. But when continued efforts over several decades finally resulted in a disease-specific treatment using recombinant human acid α-glucosidase, it became the first treatable genetic neuromuscular disease. This changed the perspective of patients affected by Pompe disease. Today, the main focus in the management of patients with Pompe disease no longer lies exclusively on the most limiting aspects of the disease, such as reduced mobility and impaired respiratory function, but extends to other factors that influence the quality of life.

A primary aim of this thesis was to establish the clinical disease spectrum in a large group of children and adults with Pompe disease – excluding patients with the classic infantile phenotype – and to improve our insight into the disease course by identifying prognostic factors for disease outcome. The second main aim was to evaluate the effects of enzyme-replacement therapy and, by identifying predictors of therapeutic efficacy, to support decisions on whom to treat and when to start treatment. Thirdly, to improve the assessment of patients' functional ability and social participation, we developed two new measurement scales.

Today, more than 125 patients are participating in our study. Between them, they are currently the largest series of patients with Pompe disease followed at a single centre worldwide (Figure 1).

This chapter summarizes the main findings of our studies, and discusses their significance and implications for clinical practice. It also examines a number of methodological issues and presents some perspectives for future research.

STUDY POPULATION

In rare diseases such as Pompe disease it is a challenge to obtain high-quality, long-term data. The search can be aided by international patient registries, which collect data on a broad, worldwide, patient population – unlike clinical studies/trials, whose extrapolation of results to the overall patient population is limited by the small numbers of patients included, by the strict eligibility criteria, and by the limited duration of follow-up. But despite the advantages of gathering information on a broad patient cohort, the quality of data collected through a registry is variable. As their assessments are not standardized, data is often collected at inconsistent time points based on routine patient care. Data may therefore be incomplete or missing, and also prone to patient-selection bias.[1,2] The first results published using data from the (industry driven) Pompe registry showed that the amount of missing data ranged from seven percent (onset of symptoms) to as much as fifty percent (genotype).[1,3] In addition, in the two years prior to data analysis for this publication, no follow-up data were entered for over twenty percent of patients, excluding those who had died.

The major advantage of our nationwide, prospective study lies in the fact that it is performed at a single centre, the Center for Lysosomal and Metabolic Diseases at Erasmus MC University Medical Center, which was designated by the Dutch government as the centre of expertise for Pompe disease in the Netherlands. Since then, nearly all patients diagnosed with Pompe disease have been referred there, thereby minimizing selection bias. All patients have undergone the same set of evaluations at standardized time points, and as the number of missing or incomplete data has been minimal, the data is robust.

Ideally, we would have preferred to have at least two years of follow-up data on natural disease course for each patient before the start of enzyme therapy, but this became impossible when enzyme-replacement therapy was approved for clinical use in 2006. From then on, starting with those affected most severely, all patients with progressive disease and evident pulmonary involvement or muscle weakness were started on ERT. As a result, the length of prospective follow-up over the natural course of the disease was shorter for the most severely affected patients than for the other patient groups. This may have slightly influenced the estimated rate of decline, and may have impeded the identification of more predictive factors for disease outcome in untreated patients. But even though an average of 19 months follow-up may seem rather short for a chronic disorder such as Pompe disease, it is the longest prospective follow-up ever carried out in "late-onset" Pompe disease. And since many patients are now treated with enzyme-replacement therapy, it is no longer possible to obtain prospective data on the natural course of the disease over a longer period of time in large numbers of patients.

It should be noted that 97% of adult patients and 77% of the children in our patient cohort had the c.-32-13T>G (IVS1) mutation in combination with a "null" allele. Although this is the most common genotype – it is present in 68-93% of patients[4-6] – our conclusions apply mainly to this patient group, and cannot be extrapolated without any further consideration to patient groups with other genotypes.

FIGURE 1
Distribution of patients participating in our nationwide prospective cohort study on Pompe disease in the Netherlands and Belgium.

MAIN FINDINGS

CLINICAL SPECTRUM

Disease onset and diagnosis

In this thesis we focused on the phenotypic spectrum of Pompe disease manifesting during childhood or adulthood, excluding neonates with the classic infantile phenotype. It is well-known from the literature that even among siblings this patient group is very variable regarding the age at first clinical presentation, the nature of the first symptoms, and the course of the disease. In our cohort, too, this clinical heterogeneity was clearly evident: some patients were identified in early childhood – even within the first year of life – while the symptoms in others did not become clinically manifest until the patients were in their sixties.

A point worthy of further attention is the fact that, on average, there was a seven-year delay between the first noted symptoms of Pompe disease and the actual diagnosis. This delay has not shortened over the past years. In this respect, there is still much to be gained, as was emphasized in a recent study investigating the health and functional status of patients with Pompe disease at the time of diagnosis.[7] This found that, at the time of diagnosis, 80% of patients experienced problems with domestic tasks outdoors, almost fifty percent experienced limitations in performing their work or study, and over twenty percent of adult patients were already wheelchair-bound or dependent on mechanical ventilatory support. This stresses the consequences of delayed diagnosis.

Although it seems contradictory, the persistent delay with respect to diagnosing Pompe disease may be due to increased awareness: many patients who were symptomatic for a long time, but up to now remained without a diagnosis, are now recognized as having Pompe disease, which has increased the mean time-lag between onset of first symptoms and diagnosis. This increased awareness of Pompe disease is reflected in the fact that three affected children and adults were diagnosed annually in the Netherlands between 1990 and 2000, against five to eight patients in the subsequent 12 years. This is due to the combined efforts of neurologists in the Netherlands, the Dutch neuromuscular patient organization (Spierziekten Nederland), and also of many patients. With Pompe disease now placed high in the diagnostic algorithm for limb-girdle weakness of the Dutch Neuromuscular Research Center (ISNO; Interuniversitair Steunpunt Neuromusculair Onderzoek), it is even more likely that it will be considered in virtually all patients with limb-girdle weakness.

Characteristic clinical features

In nearly all children and adults, signs of respiratory dysfunction were preceded by skeletal muscle weakness. Typically, the distribution of muscle weakness fitted a limb-girdle type myopathy that included prominent involvement of the trunk musculature. Our findings on the pattern of muscle weakness match those in CT and MRI studies.[8-10] Although respiratory complaints seldom featured among the presenting symptoms of Pompe disease, most patients had a degree of pulmonary dysfunction at a later stage of the disease, leading to ventilator dependency in over half of the patients who had been symptomatic for more than 15 years. Our studies showed that diaphragmatic weakness is a common finding in Pompe disease,[6,11] emphasizing the need for regular pulmonary function testing in sitting *and* supine positions (e.g. every six months) so proper ventilatory support can be initiated as soon as necessary.

As previously noted,[12,13] the degree to which skeletal muscle or respiratory muscles are involved may be well out of proportion in individual patients. Muscle weakness nonetheless proved to be one of the main predictors of the severity of pulmonary dysfunction. Rather surprisingly, we found that pulmonary function was affected more

severely in males than in females. Later on, we found that more men than women had bulbar involvement, and that they had more severe weakness of the shoulder-girdle muscles than women.

We found also that some rather unfamiliar features – such as ptosis, severe scapular winging and bulbar muscle weakness – were far more common than generally thought. These findings have been corroborated by other recent studies.[1,14-18] In particular bulbar involvement and dysphagia, which are also increasingly being recognized in patients with classic infantile Pompe disease treated with enzyme-replacement therapy,[19,20] are likely to lead to morbidity such as aspiration pneumonia. Timely diagnosis can be achieved only if these characteristics are also recognized as part of the clinical disease spectrum.

Cardiac involvement

In only two cases, cardiac evaluation revealed conduction disturbances or cardiac enlargement that may have been related to Pompe disease, confirming that cardiac involvement is rare in older children and adults with the common c.-32-13T>G (IVS1) mutation. The occurrence of conduction disturbances has also been reported by other authors.[21,22] While these disturbances may be due to alterations in the composition of the atrio-ventricular conduction system resulting from glycogen storage, it should be realized when interpreting these results that altered atrio-ventricular conduction is also quite common in the general population (up to three percent).[23]

In the randomized trial on the effect of alglucosidase alfa, left ventricular mass was mildly increased in 5% of patients.[21] However, as no information was supplied on the patients' genotypes, and as no data were collected on possible comorbidities (e.g. hypertension), further analysis to ascertain the potential relationships is precluded.

Nonetheless, several other observations also indicate increased cardiovascular morbidity in adult Pompe patients: 1) reports of increased aortic stiffness[24] and dilated thoracic arteriopathy;[25] and 2) reports that the occurrence of cerebral aneurysms is higher than in the general population.[26-34] Both may result from of glycogen accumulation within the vascular smooth muscle cells. In our own patient cohort, one patient died due to a dissection of the aortic arch two months after starting enzyme-replacement therapy, and one patient who complained of headaches appeared to have a dolichoectatic basilar artery on CT angiography (unpublished data). As yet, however, no study has been performed to systematically investigate these vascular abnormalities. Their real incidence therefore remains unknown.

On the basis of our findings, we recommend that, upon diagnosis, an electrocardiogram is performed in all patients with Pompe disease, and that echocardiography is performed only in patients with abnormal electrocardiographic findings, a history of cardiac disease, or evident cardiac symptoms. It may also be useful to screen for a dilative arteriopathy in patients suffering from unusual headaches or stroke symptoms, particularly in the absence of cardiovascular risk factors.

Hearing

Now that infants with Pompe disease are being treated with enzyme therapy, hearing impairment has been shown to be one of the causes of considerable comorbidity in long-term infant survivors of the disease.[35-39] However, only a few older children were found

to have hearing problems,[39] and neither did our analysis of 58 adult patients with Pompe disease give us reason to believe that hearing loss is one of the main features of Pompe disease in the "milder" phenotypes.

While we therefore concluded that standard screening for hearing loss is not indicated in older children and adults with Pompe disease, our opinion was the opposite of that in a recent study on 5 juvenile and 15 adult patients in Italy.[40] While this found hearing abnormalities greater than 25 dB hearing level in 12 patients (60 percent), our own study found hearing abnormalities in 20 out of 58 patients (34 percent) – equalling the prevalence observed in the general population matched for age and gender.
We cannot yet resolve these differences, despite two main dissimilarities between the studies: 1) a relatively high number of patients had conduction abnormalities (5 out of 12 patients with hearing loss), indicating middle-ear dysfunction rather than cochlear pathology, which is thought to be the main cause of hearing impairment in Pompe disease; and 2) the mean age of the Italian patients was lower than in our patient group, which may suggest a more severe phenotype. However, patients in both studies had similar mutation profiles – nearly all had the common c.-32-13T>G (IVS1) mutation – and the clinical characteristics also seem comparable. In the absence of a conclusive explanation for these differences, the new findings therefore show that we should remain alert to signs that might indicate hearing problems, especially because earlier detection of hearing loss will lead to the timely rehabilitation of hearing.

Main conclusions with regard to the clinical spectrum of Pompe disease in children and adults

- The delay between onset of first symptoms and diagnosis is still too long: timely diagnosis will be facilitated by better understanding of the patterns of the disease –common and less common characteristics alike.
  - Pompe disease should be suspected whenever one encounters the characteristic limb-girdle pattern of muscle weakness, which includes the prominent involvement of the trunk musculature, especially if it is combined with pulmonary involvement.
  - It should be realized that less familiar features such as ptosis, scapular winging and bulbar weakness are far more common than generally thought.
- Pulmonary involvement is more severe in males than in females.
- Because cardiac involvement is uncommon in children and adults with the common c.-32-13T>G genotype, an electrocardiogram is sufficient as a first screening tool. Further cardiac investigations should be performed if there are abnormal electrocardiographic findings, a history of cardiac disease, or evident cardiac symptoms.
- Because hearing may be impaired more in people with Pompe disease than in the general population, patients with suspected hearing problems should undergo pure-tone audiometry.

NATURAL DISEASE COURSE AND ENZYME-REPLACEMENT THERAPY

Outcome measures

Before discussing the natural course of the disease and the effects of enzyme-replacement therapy, some remarks are in order on the measurement scales we used.

Because many different outcome measures have been used to assess disease progression or therapeutic efficacy in Pompe disease, it is difficult to compare the results obtained in different studies. Besides the diversity in outcome measures, many of these are based on ordinal – Likert – scales: it is assumed that differences in scores have equal

meanings in patients with different degrees of functionality, but this is highly unlikely.

One of the measures we used to assess muscle strength in our studies was the Medical Research Council (MRC) grading scale. While this is the most widely used measure of muscle strength in daily neurological practice, it has often been criticized for the unequal width of its categories.[41-45] Despite this limitation, the scale was able to reflect change during the follow-up of the natural disease course and during treatment with enzyme-replacement therapy. Recently, a revised MRC scoring system was developed on the basis of Rasch methodology, which is considered to evaluate muscle strength better.[46] It should now be determined whether this modified MRC scoring system is more appropriate for clinical use in patients with Pompe disease.

Hand-held dynamometry was used as a second, linear, measure of muscle strength. While the muscle strength of untreated patients was found to deteriorate significantly during follow-up, it improved significantly during treatment with enzyme therapy. The changes measured for the individual muscle groups matched those measured earlier using quantitative muscle testing (QMT) as reported by Wokke *et al*.[6] Although this suggests that the two methods seem to be interchangeable for measuring muscle strength, QMT may be more reliable in patients with only minor weakness, since the use of hand-held dynamometry obtained many right-censored observations for the stronger muscles. Although it is possible to extend the scale beyond 250 Newton to 300 or 350 Newton, these forces are too high for the average examiner to be able to make measurements carefully.[47] However, QMT also has its disadvantages: it is time consuming, requires specialized equipment, and can test only a limited number of muscles important to patients with Pompe disease. Ideally, if optimal information on a patient's muscle weakness is to be provided, all these measures of muscle strength should be combined. While this can be achieved in clinical trials, it is not feasible in daily clinical practice.

To assess the clinical relevance of – small – changes in muscle strength, it is also important to investigate muscle function. For this purpose, the six-minute walk test (6MWT) has been widely used in recent years. It evaluates all the systems involved during exercise, including pulmonary and cardiovascular systems, systemic and peripheral circulation, neuromuscular units, and muscle metabolism, and is regarded as an objective measure which reflects patient's performance in the activities of daily living (ADL).[48-52] However, as it was developed originally for patients with chronic bronchitis, and *not* for measuring response in patients with neuromuscular disorders, it does not provide specific information on limb-girdle muscle function.

To evaluate the effects of changes in muscle strength on skeletal muscle function in greater detail, we developed the Quick Motor Function Test (QMFT).[53] Comprising 16 motor skills related to daily activities that require use of the shoulder-girdle musculature, trunk muscles, and pelvic-girdle/proximal lower limb muscles, this test is based on the clinical expertise of physicians involved in the daily care of Pompe patients, experience obtained with the Gross Motor Function Measure[50,51] and patient-reported information from the IPA/Erasmus MC Pompe Survey.[12,54] Unfortunately, when we followed-up the natural disease course, the group-level changes in muscle strength were not reflected in changes in limb-girdle muscle function. However, treatment with enzyme therapy brought significant improvements in wheelchair-independent patients and in those whose muscle weakness was less pronounced. To further refine this functional outcome measure, the application of Rasch methodology may be justified.

Rasch methodology

Over recent years, Rasch methodology has been used in the development of new measurement scales. This methodology is based upon the assumption that a patient who is less affected (i.e. one whose level of ability is higher) will have a greater chance of completing a more difficult task than a patient who is more disabled.[55-58] Rasch allows the summed scores generated by ordinal rating scales to be transformed into linear interval measures. These Rasch-built measurement scales may better reflect true disease impact, differences between individuals and groups, and treatment effects.

A second advantage is that the Rasch method makes it possible to define "response" at the *individual person-level*, taking account of individual standard errors that change according to an individual patient's ability level. Rather than concentrating on statistical significance alone,[59-62] this may improve our understanding of the clinical relevance of changes in scores – how we define responders against non-responders, for example – and may therefore have major implications in clinical practice and in future clinical trials for assessing the effect of a particular treatment. For all these reasons, we therefore constructed a patient-based interval scale using Rasch analysis: the Rasch-built Pompe-specific Activity scale (R-Pact).[63] The rationale for development of yet another scale was that we expected this measurement scale – based as it was on patients' own experiences – to be more suitable for estimating the impact of Pompe disease on daily life than any existing Rasch-built measurement scale (e.g. ACTIVLIM,[64] DM1-Activ,[65] or R-ODS[66]).

The R-PAct scale proved to be highly discriminatory, and was shown to be able to detect clinically meaningful changes over time. This is valuable when estimating the rate of disease progression, determining the best moment to initiate treatment, and evaluating therapeutic efficacy. In this context, it will be most interesting to use this scale in future studies investigating therapeutic interventions and long-term clinical follow-up in patients with Pompe disease.

Main conclusions with regard to the outcome measures used

- Although the MRC scale is the most widely used measure of muscle strength in daily neurological practice, its major drawback is the lack of linearity. Future studies should establish the value of the modified MRC scoring system developed recently on the basis of Rasch methodology.
- To be optimally informed on a patient's muscle weakness, linear measures of muscle strength such as hand-held dynamometry and quantitative muscle testing are of additional value.
- To assess the clinical relevance of – small – changes in muscle strength, it is also important to investigate muscle function, either using non-specific function tests (e.g. 6MWT, timed tests) or using tests developed specifically to measure limb-girdle muscle function in Pompe disease (e.g. QMFT).
- The Quick Motor Function Test is able to reliably rate clinical severity and motor function in children and adults with Pompe disease.
- By using Rasch-built linear interval scales, it is possible to define "response" at the *individual person-level*. This may improve our understanding of the clinical relevance of changes in scores (minimum clinically important difference).
- The R-PAct scale proved to be highly discriminatory, and is able to detect clinically meaningful changes over time, which is valuable when estimating the rate of disease progression, determining the best moment to initiate treatment, and evaluating therapeutic efficacy.

Natural disease course

Through manual muscle testing using the MRC grading scale, and also through hand-held dynamometry, we found significant decreases in muscle strength. Similarly, decreases in FVC in sitting and supine positions and reductions in maximum inspiratory and maximum expiratory pressures reflected loss of pulmonary function. Although the average yearly declines seem small, many patients eventually became severely restricted in their daily functioning, or required respiratory support.

In about half of the patients, the declines in pulmonary function and muscle strength occurred simultaneously and at a similar rate. In the remaining patients, the disease followed a more varied course, effectively falling into three subgroups: 1) patients in whom pulmonary function and muscle weakness followed different courses, one deteriorating while the other remained stable, or one deteriorating faster than the other (about 30 percent of patients); 2) patients who remained stable during follow-up (about 10 percent); and 3) patients who declined relatively fast (about 10 percent).

We found that a faster decline in muscle strength was associated with longer disease duration and with pulmonary involvement at baseline. Even though our cross-sectional analyses showed males to have more severe pulmonary involvement than females, gender did not emerge as a predictor for faster pulmonary decline. This may be because the follow-up cohort comprised fewer males than females; by definition, these were males who were affected relatively mildly, since all patients with severe pulmonary involvement or a rapid disease course had been started on ERT early in the study.

Enzyme-replacement therapy

Three of the studies in this thesis measured the effect of enzyme-replacement therapy in children and adults with Pompe disease. The most prominent effects of ERT were found on muscle strength and muscle function: over 90% of patients showed a positive response compared to the natural disease course, while only 9% of patients showed a continued decline during treatment. The effects on pulmonary function were more modest. In juvenile patients, pulmonary function remained stable or improved slightly in upright seated position and in supine position. In adults, patient's pulmonary function remained stable in upright seated position, but it continued to decline in the supine position – although less rapidly than before start of ERT. This overall finding of ongoing decline in pulmonary function in the supine position was largely accounted for by a subgroup of 40 percent of patients, who were mostly older, were dependent on mechanical ventilation, and had more severe muscle weakness at the start of treatment. This finding, which suggests that the pathologic changes in respiratory muscle are more severe than in skeletal muscle, or that loss of diaphragmatic muscle function is more difficult to reverse than loss of function in skeletal muscles, corroborates findings in knock-out mice in which the restoration of tissue morphology was less pronounced in the diaphragm than in than in skeletal muscles.[67] While the exact mechanisms still need to be unravelled, they may involve age-related changes or differences in myofibre type distribution and regenerative capacity.

Surprisingly, with respect to muscle strength, women seemed to benefit more from treatment than men. This may be due to differences in muscle composition,[68,69] activity patterns or hormonal influences. But it may also be secondary to the fact that since males

usually have a higher lean body mass, they may, per gram of muscle tissue, receive a smaller relative dose of recombinant human acid α-glucosidase. In hindsight, the subgroup analyses in the randomized placebo-controlled trial also suggest a better response in females than in males, though treatment efficacy was not *significantly* different between sexes.

Though the treatment effects seem modest – especially compared to the phenomenal effects on survival and cardiorespiratory function in infants – it was clear that the use of ERT altered natural disease course for the better. While a similarly positive effect on survival was shown by a prospective cohort study in 283 adult patients,[70] thereby underlining the beneficial effect of enzyme therapy, it must still be established whether such effects are sustained. There is some evidence that the effect of treatment may level off after the the first six to twelve months of treatment,[71-74] especially in adult patients. In juvenile patients, the improvements seem to continue over several years,[71,75,76] possibly reflecting a different degree of muscular damage and another margin of regenerative capacity. However, in a disease that is relentlessly progressive and leads, if untreated, to severe impairments in daily functioning, the maintenance of patients' level of ability can be regarded as a positive outcome.

As our experience with ERT increases, it is now recognized that not all patients derive equal benefit from it. That said, the patients involved in the first studies were mainly those who were severely affected, and it is plausible that less affected patients may benefit more from treatment. Subgroup analyses throughout the different studies also point in this direction: better efficacy is expected when treatment is started early in the course of the disease, before irreversible muscle damage has occurred.

This raises the question of the best time to start enzyme therapy: should treatment be initiated upon diagnosis, even in pre-symptomatic individuals? A recent study, in which patients were identified through a neonatal screening program and were followed over the years, showed that, during the clinically asymptomatic phase of the disease, an increasing number of myocytes developed histopathologic changes.[77] While muscle MRI has also shown muscle involvement in asymptomatic patients, especially of the trunk musculature,[78,79] some cases have shown that Pompe disease may be clinically silent over decades.[80] In our own nationwide study in adult patients, too, a subgroup (approximately 10 percent of patients) remained stable for many years without receiving ERT.

On the basis of these observations and of the predictive factors identified in our studies, it seems advisable to start enzyme-replacement therapy at an early stage in males, children, and patients who, at diagnosis, already have severe skeletal muscle weakness and pulmonary dysfunction. In pre-symptomatic or minimally affected patients, muscle imaging may be helpful to visualize muscle damage, and, possibly, to aid decisions on whom to treat and when. This needs to be evaluated in future clinical studies.

The ultimate goal of treatment of Pompe disease is to identify at the earliest possible stage patients whose outcome is expected to be poor. To this end, prognostic models are needed that can be used to individualize therapy in accordance with the expected outcome. Over the last years, several models to predict outcome have been constructed, such as in Guillain-Barré syndrome and traumatic brain injury.[81-86] If this is also to be accomplished in such a rare disease as Pompe disease, international collaboration is crucial.

Main conclusions on natural disease course and enzyme-replacement therapy

- Despite the small annual declines in MRC score, hand-held dynamometry and pulmonary function, over half of all patient patients with Pompe disease eventually become wheelchair dependent or require ventilatory support in a later stage of the disease.
- Although, for most patients, the natural course of the disease involves a gradual decline, 10% remain stable for a long time, and another 10% decline unexpectedly fast.
- Longer disease duration and reduced pulmonary function stand out as predictors of rapid disease progression.
- Enzyme-replacement therapy alters the natural course of Pompe disease positively.
  - Muscle strength and muscle function may be easier to restore than pulmonary dysfunction.
  - Women may benefit more from treatment than men.
- Due to the variable effect of enzyme treatment and the limited duration of follow-up, it is difficult to decide whom to treat, and when treatment should start. Further nationwide and international collaboration is necessary to develop prognostic models which, at the earliest possible stage, can identify patients whose outcome is likely to be poor.

FUTURE PERSPECTIVES

POTENTIAL FUTURE THERAPIES

Enzyme-replacement therapy using recombinant human acid α-glucosidase is currently the only therapy available clinically. Although it has significantly altered patients' perspectives, it has several limitations: 1) recombinant enzymes are large molecules that do not freely diffuse across membranes, but that depend on the mannose or mannose 6-phosphate receptor pathways for delivery to the lysosomes. And because it is currently not possible to specifically target the exogenous enzyme on the tissues affected most (i.e. heart, skeletal muscle, diaphragm), the major fraction of the infused enzyme is captured by the liver and spleen.[87] 2) The repair of muscle damage appears to be particularly challenging: skeletal muscle comprises approximately 40% of body mass and is therewith the largest organ of the human body; levels of mannose 6-phosphate receptors on the cell surface of muscle fibres, used as target for ERT, are relatively low; type II fibres might be refractory to ERT compared to type I fibres (although this is contested in humans[88,89]); and the time needed to remodel muscle fibers and restore muscle function is long . 3) Some of the enzyme internalized by muscle cells does not seem to reach the lysosomes due to autophagic build-up.[90,91] 4) The repeated intravenous infusions of recombinant human acid α-glucosidase may lead to an immune response against the administered enzyme, thereby negatively influencing therapeutic efficacy in some of the patients.[38,92,93] 5) The effectiveness of treatment depends on repeated and life-long intravenous infusions, imposing quite a burden on the patient's quality of life. Finally, 6) the production, purification, and intravenous administration of recombinant human acid α-glucosidase in sufficient amounts is associated with high costs. Annual treatment for a single adult patient (based upon weight in kg) may cost as much as several hundred thousand Euros.

Together with the fact that not all patients derive equal benefit from enzyme-replacement therapy, these limitations have underlain continued improvements of the enzyme currently used, and the search for possible alternative approaches such as the following: modifying the immune response raised against the exogenously administered enzyme; exercise training to improve therapeutic efficacy; chaperone therapy; gene therapy; and transplantation of gene-modified autologous hematopoietic stem cells. These alternative strategies are discussed below.

Glycoengineering of the enzyme currently used

The uptake of exogenous recombinant human acid α-glucosidase by the skeletal muscles is mediated primarily by the cation-independent mannose-6-phosphate receptor (M6PR).[94-96] The current enzyme contains however relatively low numbers of mannose-6-phosphate (M6P) residues, which are important recognition markers for the M6PR.[97] The binding of the enzyme to the receptor and its subsequent uptake by cells in culture is improved by the addition of M6P moieties to the recombinant enzymes, either by enzymatic engineering (HP-GAA) or by the chemical conjugation of synthetic oligosaccharides bearing M6P residues (neo-rhGAA/oxime-neo-rhGAA).[97-100]

However, due to the non-productive sequestration of the infused enzyme by mannose receptors on endothelial cells and macrophages,[97] the hyper-mannose-6-phosphorylated variant (HP-GAA) was no more effective than the unmodified enzyme at clearing tissue glycogen in knock-out mice. Administration of neo-rhGAA/oxime-neo-rhGAA to Pompe mice resulted in greater clearance of glycogen from all affected muscles than achieved by the drug currently available.[98,100] While the results of these pre-clinical studies are promising, improved efficacy of the modified enzyme has yet to be proven in humans.

Other approaches to improve the uptake of intravenously administered recombinant human acid α-glucosidase include glycosylation-independent lysosomal targeting (GILT),[101] and production of the enzyme in genetically-modified yeast, in which the structure of the carbohydrate side-chains can be manipulated, thereby increasing the mannose 6-phosphate content of the enzyme. [102]

Modifying the immune response raised against the exogenously administered enzyme

Nearly all patients develop antibodies to the recombinant enzyme. Clinical studies in infants have shown that patients with high IgG antibody titres, respond more poorly to enzyme therapy than patients with relatively low antibody titres.[93,103]

In patients who do not have the infantile phenotype, the association between treatment outcome and antibody development seems less evident. In the recent randomized controlled trial in children and adults with Pompe disease, all patients developed anti-alglucosidase alfa IgG antibodies, and 31% of these patients showed *in vitro* inhibition of enzyme uptake. However, there was no consistent association between the height of the serum IgG antibody titre and the primary outcome measures.[72] But as we showed recently, antibodies may nonetheless play an important role in individual patients;[92] one adult patient from our Dutch cohort developed extremely high antibody titres similar to those found in infantile patients, and deteriorated despite treatment with alglucosidase alfa. About forty percent of the intravenously administered enzyme in this patient was bound to circulating antibodies, inhibiting enzyme uptake by the target organs, and thereby negatively affecting his clinical outcome.

It has also been shown *in vitro* and *ex vivo* that, as well as forming IgG and IgE antibodies to the purified protein which is administered, enzyme treatment may induce a pro-inflammatory T-cell response that interferes with treatment efficacy.[104] Still, the exact

mechanisms remain to be elucidated.

In general, patients receiving enzyme-replacement therapy become tolerant after prolonged treatment. If they do not, immune tolerization protocols may help to neutralize the detrimental effects of antibody formation. In humans, successful immune tolerization using rituximab combined with methotrexate and intravenous immunoglobulin (IVIg) was reported in a CRIM-negative infantile patient.[105] Another study showed that treatment with omalizumab successfully reduced antibody IgE response in a CRIM-negative patient.[38] Similarly, a gradual dose-escalation regimen successfully desensitized an adult patient with a history of anaphylaxis to the enzyme that was administered.[106]

Improving the efficacy of enzyme therapy through exercise training

Uptake of the intravenously administered recombinant enzyme may also be improved by exercise training, which, at least in theory, prevents physical de-conditioning and muscle wasting,[107] and has a positive influence on the aerobic and anaerobic capacity of muscle. However, over-exertion might cause more rapid disease progression. While studies in other neuromuscular disorders show that moderate-intensity strength training is not harmful, there is insufficient evidence in most diseases to conclude that it is beneficial.[108-113]

There is little evidence to suggest that exercise is beneficial in Pompe disease. Even though a positive effect on muscle strength and pulmonary function was reported in a study in 34 patients who received a high-protein/low-carbohydrate diet combined with submaximal aerobic training, this combined regimen made it impossible to conclude that exercise alone was responsible.[114] While Terzis *et al* [115] showed recently that muscular strength and endurance improved significantly in five patients who participated in a 20-week training programme combined with enzyme therapy, no positive effect on forced vital capacity was shown by another study, which focused on the combined effect of respiratory-muscle-strength training and enzyme-replacement therapy.[116]

At our centre, we recently performed a training study in 25 patients with Pompe disease who were treated with enzyme therapy for at least one year. These results are awaited in 2013.

Chaperone therapy

Chaperones are innate proteins (molecular chaperones) or small molecule drugs (pharmacological chaperones) that assist in protein folding and the maintenance of native conformation, and that improve trafficking between the endoplasmatic reticulum and the lysosome. Lysosomal storage disorders – including Pompe disease – can be seen as excellent candidates for chaperone-mediated therapy. There are at least three reasons for this. 1) It is believed that a small increment in lysosomal enzyme activity may be sufficient to reduce the rate of substrate accumulation.[117] 2) As well as having better cellular and tissue distribution than enzyme-replacement therapy, chaperones can cross the blood-brain barrier. 3) Since they can be ingested orally and do not require life-long infusions, their impact on patients' quality of life is less pronounced.

Some of the pathogenic mutations in the acid α-glucosidase gene lead to forms of the enzyme that are either trapped and degraded in the endoplasmatic reticulum, are poorly transported to the lysosome, or are unstable in the lysosomal environment. Patients carrying these mutations may benefit from chaperone treatment. Paradoxically, many small molecule chaperones reported in the literature – mostly iminosugars and their alkylated derivatives – are also inhibitors of the enzyme.

It was shown by *in vitro* studies that the pharmacological chaperones 1-deoxynojirimycin (DNJ) and N-butyldeoxynojirimycin (NB-DNJ) promoted the transport of the mutant α-glucosidase species from the endoplasmatic reticulum to the lysosome: in the presence of these chaperones, the amount of intralysosomal α-glucosidase increased.[118-122]

Although, unfortunately, a phase 2 clinical trial of DNJ as monotherapy treatment in adults with Pompe disease was placed on hold after two patients experienced increased muscle weakness, it was shown recently that enzyme-replacement therapy combined with chaperones improved the delivery of α-glucosidase to lysosomes, enhanced enzyme maturation, and increased enzyme stability in *GAA* knockout mice.[121] These results may greatly extend the applications of chaperone-mediated therapy, possibly as an adjuvant therapy to all patients with Pompe disease treated with ERT.

Recently some potential new chaperones were discovered (such as N-acetylcysteine, bortezemib) that are not active-site directed and do not therefore interfere with the catalytic activity of acid α-glucosidase itself.[123,124]

Gene therapy

The rationale underlying gene therapy is to transfer an intact copy of acid α-glucosidase cDNA to the patients' somatic cells, thereby creating a permanent enzyme source. In 2012, Glybera® (alipogene tiparvovec), an adeno-associated viral vector engineered to express lipoprotein lipase in muscle, was approved by the European Medicines Agency (EMA) for the treatment of lipoprotein lipase deficiency.[125] Currenly, there is no effective gene therapy for Pompe disease.

The feasibility of gene therapy in Pompe disease was first shown in *in vitro* studies using retroviral and adenoviral vectors expressing human *GAA*. The human gene was highly expressed in cultured fibroblasts, myoblasts, and myotubes derived from patients with Pompe disease. This resulted in *de novo* synthesis of acid α-glucosidase, uptake of the 110 kDa precursor enzyme by recipient cells, and clearance of lysosomal glycogen.[126-130]
For *in vivo* gene therapy in *GAA* knockout mice, two gene-transfer systems have been used: vectors based on Adenoviruses (Ad), and vectors based on Adeno-associated viruses (AAV). Although skeletal or cardiac muscle seems an obvious site for the vector transduction, the intramuscular or intramyocardial injection of these viral vectors, which encode human acid α-glucosidase, into adult knockout mice was effective only at the injection site, but not in other distant muscle groups.[127,131,132]

Unlike skeletal muscle, liver was shown to be an excellent target tissue for transduction. High levels of acid α-glucosidase expression in transduced hepatocytes, achieved by intravenous administration of the viral vectors, resulted in efficient production, secretion, and uptake of the enzyme by skeletal muscle.[126] However, the long-term efficacy of this approach is impeded by the onset of anti-hGAA antibodies within days of vector injection,[131,133-139] and by an immediate "innate" immune response.[140-142]

In order to improve the efficacy of the viral vectors and to minimize the immune response, several approaches have been used: modification of the *GAA* cDNA sequence,

application of different promoters, and the use of different AAV serotypes.[143,144] Remarkably, even the extremely high levels of enzyme activity in plasma achieved by means of gene therapy – levels which cannot be realistically obtained with ERT – did not result in full reversal of pathology in skeletal muscle of mice. This goes against the prevailing idea that a small increase in residual acid α-glucosidase activity is sufficient to reverse the glycogen accumulation.

An open label, phase I/II study to assess the safety of administration of a recombinant adeno-associated virus acid α-glucosidase (rAAV1-CMV-GAA) gene vector via direct intramuscular injection to the diaphragm of Pompe patients is currently ongoing (ClinicalTrials.gov: NCT00976352).

Transplantation of gene-modified autologous hematopoietic stem cells

Hematopoietic stem-cell transplantation (HSCT) was the first treatment to be introduced into the management of lysosomal storage disorders in the mid-1980s.[145] The therapy aims to replace enzyme deficient cells through infiltration of healthy donor cells into various tissues. In theory, these donor cells can serve as a permanent source of the missing enzyme, and correct neighbouring cells through enzyme secretion and re-uptake by enzyme-deficient host cells.[146]

The few attempts at HSC transplantation for Pompe disease have not met with success.[147,148] However, several studies have shown that it may be worth pursuing gene-modified autologous hematopoietic stem-cell therapy using Lentiviral vectors as a future therapeutic option.[149-151] Transplantation of genetically engineered hematopoietic stem cells using a competitive repopulation strategy resulted in stable partial chimerism, in which approximately 35 percent of hematopoietic cells overexpressed acid α-glucosidase.[150,151] This led to reconstitution of enzyme activity in the target tissues, and resulted in glycogen clearance from the heart, skeletal muscle, diaphragm, spleen, and liver. Overexpression of acid α-glucosidase did not affect overall hematopoietic cell function. The first trials in patients with Pompe disease are awaited.

NEWBORN SCREENING

Over recent years, screening for a wider array of inborn errors of metabolism has been implemented. As treatments are now available for a number of lysosomal storage disorders, some advocate their addition to the newborn-screening programmes. However, the difficulty in Pompe-disease screening programmes is that they cannot differentiate between the severe classic infantile phenotype and milder phenotypes.

Between 2005 and 2009, a large screening programme in Taiwan identified 19 patients with Pompe disease among 344,056 newborns; six were classified as having infantile Pompe disease, and 13 were classified as having "later-onset" disease.[152-154] As soon as their diagnosis had been confirmed by additional testing, the patients with infantile Pompe disease were treated with enzyme-replacement therapy. All survived, remained free of mechanical ventilation, had normal growth for the duration of the study (14 to 31 months), and had improvements in motor skills that resulted in their attaining normal motor development by approximately 1 year of age. The other 13 patients were followed every 3 to 6 months. In the course of four years, four of them were treated for hypotonia, muscle weakness, delayed developmental milestones/motor skills, or elevated creatine kinase levels, which had started at the respective ages of 1.5, 14, 34, and 36 months. Further follow-up will show whether these children attain the same motor skills as their peer group. Two remarks should be made before these results are generalized to the patient population worldwide. First, whereas all of the infantile patients reported were CRIM-positive, other populations may have a higher proportion of patients with CRIM-negative mutations, who may derive less benefit from treatment.[93,103,155,156] Second, many of the patients with "later-onset" phenotypes in the Taiwanese population will have onset of symptoms in adolescence, and will become wheelchair or respirator dependent in their twenties; in the Caucasian population, disease onset is much more variable.[12,30,157] Currently, several other countries are also exploring the feasibility of newborn screening.[158-161]

One consequence of expanded screening programmes is the emergences of new ethical questions, such as stigmatization, social and economic impact, and the impacts of uncertainty and knowledge.[162] The term "patient-in-waiting" was recently illustrated by a case report on a patient who had been diagnosed at the age of two years, but was still asymptomatic at the age of 21.[80] Medicine as it is currently practiced is ill-equipped to support and advise individuals who are "affected", but still well – who have been diagnosed, but still have no disease.[163] A study published last year measured the opinion of the Dutch general public on neonatal screening for Pompe disease. It found that there was fairly high support for neonatal screening; to most respondents, the expected annual numbers of false positives and late-onset cases were acceptable. When the benefits of such unintended screening outcomes were weighed against their potential harm, the balance favoured the short-term and long-term benefits to the child.[164]

FINAL REMARKS

Over recent years, considerable progress has been made towards understanding the natural history of Pompe disease in children and adults, and the availability of a registered therapy has changed the lives of many patients.

Only through close national and international collaboration between clinicians, basic scientists, patient organizations and the pharmaceutical industry, will it be possible to further advance our knowledge of the long-term effects of treatment, make prognostic models for individualizing therapy in accordance with the expected outcome, and explore other options for treatment.

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7

SUMMARY | SAMENVATTING

SUMMARY

Pompe disease is an autosomal recessive metabolic disorder whereby mutations in the *GAA* gene lead to the partial or total absence of the lysosomal enzyme acid α-glucosidase.

Chapter 1 – the general introduction – summarizes the historical perspectives and pathophysiology of Pompe disease, the current state of knowledge regarding its clinical features and natural course, and the therapeutic measures currently taken in its treatment. Chapter 2 describes the study design of the nationwide study on Pompe disease in children and adults in the Netherlands, the recruitment of patients, and the measurement scales that were used.

Chapter 3 focuses on its clinical disease spectrum and natural course in children and adults. First, **Section 3.1** discusses its long-term natural course. Sixteen patients were followed over an average of 16 years, a period in which muscle strength declined significantly (-0.5 points in MRC sumscore per year), as did pulmonary function in the upright seated position (-1.6% points of the predicted value yearly). It was also found that the course of the disease could vary substantially among patients. While two patients remained stable for many years, muscle strength and pulmonary function declined progressively in the other 14, one-third of whom declined rapidly, leading to early wheelchair or ventilator dependency.

Section 3.2 presents the results of a nationwide prospective study on the spectrum of Pompe disease in adults, focusing on their clinical features, the natural course of the disease, and predictors for disease progression. There were 91 patients from the Netherlands – nearly all the patients known to have the disease in the Netherlands – and three from Belgium. Many patients were found to have unfamiliar features, such as ptosis (23%), bulbar weakness (28%), and scapular winging (33%), all of which may delay diagnosis if not recognized properly. During the follow-up period of this study, muscle strength declined significantly (-1.3% points/year for manual muscle testing and -2.6% points/year for hand-held dynamometry), as did pulmonary function in the supine position. Longer disease duration (>15 years) and the presence of pulmonary involvement (forced vital capacity in sitting position <80%) were found to be associated with more rapid muscle weakening. The rate of decline in pulmonary function was consistent among subgroups.

Section 3.3 focuses in greater detail on pulmonary involvement in a cohort of 92 patients (age-range 5–76 years). Seventy-four percent of all patients were found to have some degree of respiratory dysfunction, and 38% to have severe or relatively severe diaphragmatic dysfunction. Importantly, pulmonary involvement was more severe in males than in females; other important predictors of poor respiratory outcome were severe skeletal muscle weakness and long disease duration. During an average prospective follow-up period of 1.6 years, there were significant declines in pulmonary function in supine position and in inspiratory and expiratory respiratory muscle strength. We concluded that respiratory dysfunction is a serious threat to patients with Pompe disease.

Section 3.4 describes the findings of extensive cardiac evaluation in 17 children and 51 adult patients carrying the common c.-32-13T>G genotype. Two patients had cardiac abnormalities that may have been related to Pompe disease: one 8-year-old girl had a Wolff-Parkinson-White (WPW) pattern on electrocardiography, while one adult patient had mild hypertrophic cardiomyopathy. Four other adult patients had minor cardiac abnormalities which were attributed to advanced age, hypertension, or pre-existing cardiac pathology unrelated to Pompe disease. It was concluded that electrocardiography seems to be an appropriate first screening tool, and that elaborate cardiac screening should be performed only when electrocardiography is abnormal, or when the patient has evident cardiac symptoms or a history of cardiac disease.

In **Section 3.5**, the final part of Chapter 3, we present the findings of hearing evaluation among 58 adults with Pompe disease. Although 72% had abnormal hearing thresholds, only 21% had clinically relevant hearing loss. As this prevalence is similar to that in the general population, it was concluded that, unlike in infants, hearing loss is not a salient feature of Pompe disease in adults.

Chapter 4 is dedicated to the effects of enzyme-replacement therapy (ERT) in children and adults with Pompe disease. First, **Section 4.1** focuses on its effects in our nationwide prospective study in adults with the disease, in which 69 patients were treated for an average of 23 months. Muscle strength improved significantly: the MRC sumscore showed a mean annual increase of 1.4% points per year, and the HHD sumscore of 4.0% points. The increase in muscle strength was greater in females than in males. At group level, pulmonary function in seated position remained stable, while pulmonary function in the supine position declined further (-1.1% points per year). This overall finding of ongoing decline in pulmonary function in the supine position was largely accounted for by a subgroup of 40 percent of patients, who were mostly older, and were more severely affected at the start of treatment. Comparison of the rate of disease progression in 51 patients before and after the start of ERT showed that enzyme-replacement therapy effectively changes the course of the disease.

In **Section 4.2** we report the results of a randomized, placebo-controlled study of alglucosidase alfa in 90 children and adults with Pompe disease. Over an 18-month period, treatment was shown to be associated with a greater walking distance in the six-minute walk test and with stabilization of pulmonary function relative to placebo. The effect of treatment became apparent early after the start, reaching a plateau after 26 weeks of treatment. Although the treatment effect seemed more pronounced in patients who were in a better condition at baseline, no subgroups were identified in which outcome was *significantly* better. Five percent of patients who received the active study drug had experienced anaphylactic reactions, leading to discontinuation of treatment. All patients developed IgG antibodies, but these had no consistent – adverse – effects on outcome.

Finally, **Section 4.3** shows the results of an open-label study in five children aged 5 to 15 years. After three years of treatment with ERT, pulmonary function had remained stable in four patients, and had improved in one patient. Muscle strength had improved in all patients, one of whom caught up with her healthy peers. Evidence for the safety of treatment was provided by the fact that none of the children had had infusion-associated reactions.

Chapter 5 reports on the construction of two new measurement instruments for use in Pompe disease. **Section 5.1** describes the development of the Rasch-built Pompe-specific

Activity scale (R-PAct), a patient-based, self-reported measurement scale that uses modern clinimetric methods (Rasch methodology) to measure limitations in activities and social participation. On the basis of patients' most limiting problems in daily life, a 18-item linear interval measure was constructed which was shown to be valid, reliable, and able to measure responsiveness at the individual person level throughout the whole spectrum of disease severity.

Section 5.2 describes the construction and validation of a new functional measurement instrument for rating clinical severity and motor function: the Quick Motor Function Test (QMFT). The final measure consists of 16 motor skills related to daily activities requiring use the shoulder-girdle musculature, trunk muscles, and pelvic girdle/proximal lower limb muscles. This Test proved to be capable of adequately assessing disease severity and of detecting changes in motor function over time.

Finally, **Chapter 6** summarizes our main findings, discusses their significance and clinical implications, and makes suggestions for future research.

SAMENVATTING

De ziekte van Pompe is een autosomaal recessief erfelijke metabole aandoening die wordt veroorzaakt door mutaties in het *GAA* gen, welke resulteren in volledige of gedeeltelijke afwezigheid van het lysosomale enzym zure α-glucosidase.

In **hoofdstuk 1** – de algemene introductie – wordt achtergrondinformatie gegeven over het historisch perpectief en de pathofysiologie van de ziekte van Pompe, de huidige kennis over de klinische kenmerken en het natuurlijk beloop van de ziekte, en de huidige behandelingsmogelijkheden. In **hoofdstuk 2** wordt de opzet van onze landelijke studie naar de ziekte van Pompe bij kinderen en volwassenen besproken, evenals het werven van de deelnemende patiënten, en de meetmethodes en meetschalen die we tijdens de studie gebruikt hebben.

Hoofdstuk 3 richt zich op het klinisch spectrum en het natuurlijk beloop van de ziekte van Pompe bij kinderen en volwassenen. Allereerst worden in **sectie 3.1** de lange-termijn uitkomsten van de ziekte besproken (natuurlijk beloop). Zestien patiënten zijn gedurende een periode van gemiddeld 16 jaar vervolgd. Gedurende deze periode vonden we een significante achteruitgang in spierkracht (-0.5 punten op de MRC somscore per jaar) en in longfunctie in zittende positie (-1.6% van de voorspelde waarde per jaar). Er werd ook aangetoond dat het ziektebeloop sterk kan verschillen tussen patiënten: twee patiënten bleven gedurende vele jaren stabiel, terwijl de andere 14 patiënten een duidelijke achteruitgang lieten zien in spierkracht en longfunctie. Eenderde van deze 14 patiënten had een snelle achteruitgang, met als gevolg dat zij al vroeg in het ziektebeloop afhankelijk werden van een rolstoel of beademing.

In **sectie 3.2** worden de resultaten beschreven van een landelijke prospectieve studie naar het spectrum van de ziekte van Pompe bij volwassenen, waarbij voornamelijk gefocust wordt op de klinische kenmerken, het natuurlijke ziektebeloop (zonder enzymtherapie), en factoren die van invloed kunnen zijn op het ziektebeloop. Eenennegentig Nederlandse patiënten – dit zijn vrijwel alle patiënten die gediagnosticeerd zijn met de ziekte van Pompe in Nederland – en drie Belgische patiënten namen deel aan deze studie. Het bleek dat veel patiënten kenmerken hebben waarvan het tot nu toe niet algemeen bekend was dat deze bij de ziekte van Pompe kunnen voorkomen, zoals een afhangend ooglid (23%), zwakte van spieren die betrokken zijn bij spreken, eten en slikken (28%), en afstaande schouderbladen (33%). Als deze kenmerken niet herkend worden als karakteristieken van de ziekte van Pompe kan het lang duren voor de correcte diagnose wordt gesteld. Tijdens de follow-up periode van deze studie werd een significante achteruitgang vastgesteld van de spierkracht (-1.3% per jaar van de MRC somscore, en -2.6% per jaar van de somscore gemeten met de hand-held dynamometer). Ook werd een significante daling van de longfunctie in liggende houding gevonden. Het bleek dat een langere ziekteduur (>15 jaar) en de aanwezigheid van pulmonale betrokkenheid (geforceerde vitale capaciteit in zittende houding <80% van de voorspelde waarde) geassocieerd waren met een snellere daling van de spierkracht. Er werden geen factoren gevonden die van invloed waren op de snelheid van achteruitgang in longfunctie.

Sectie 3.3 richt zich meer in detail op de pulmonale betrokkenheid in een cohort van 92 kinderen en volwassenen in de leeftijd tussen 5 en 76 jaar. Het bleek dat in 74% van alle patiënten de longen in meer of mindere mate zijn betrokken bij het ziekteproces, en dat 38% van alle patiënten ernstige zwakte heeft van het middenrif. Ook bleek dat bij mannen de longfunctie ernstiger is aangedaan dan bij vrouwen. Andere belangrijke voorspellende factoren voor een slechte pulmonale uitkomst zijn ernstige spierzwakte en een lange ziekteduur. Gedurende de follow-up periode van gemiddeld 1.6 jaar werd een significante achteruitgang gevonden in de longfunctie gemeten in liggende houding, en in de kracht van de in- en uitademingsspieren. Hieruit werd door ons geconcludeerd dat respiratoire insufficiëntie een serieuze bedreiging is voor patiënten met de ziekte van Pompe.

In **sectie 3.4** worden de bevindingen beschreven van uitgebreide cardiale screening in 17 kinderen en 51 volwassenen met het meest voorkomende genotype: c.-32-13T>G. Het bleek dat 2 patiënten cardiale afwijkingen hadden die mogelijk gerelateerd zijn aan de ziekte van Pompe: een meisje van 8 jaar had een Wolff–Parkinson–White (WPW) patroon op het hartfilmpje, terwijl een volwassen man een milde verdikking van de hartspier had. Daarnaast werden er bij 4 patiënten enkele kleine afwijkingen gevonden die toe te schrijven waren aan een hoge leeftijd, hoge bloeddruk, of pre-existente hartproblemen niet gerelateerd aan de ziekte van Pompe. Er werd geconcludeerd dat het maken van een hartfilmpje een goede eerste screeningsmethode is voor het opsporen van hartproblemen, en dat uitgebreidere screening alleen noodzakelijk is wanneer het hartfilmpje afwijkingen toont, of wanneer patiënten al eerder hartproblemen hebben gehad.

In het laatste deel van dit hoofdstuk, **sectie 3.5**, worden de resultaten gepresenteerd van het gehooronderzoek bij 58 volwassen patiënten. Bij 72% van alle patiënten werden voor sommige toonhoogtes abnormale gehoordrempels gevonden, maar slechts 21% van alle patiënten had ook daadwerkelijk een – in het dagelijks leven – relevant gehoorverlies. Dit percentage bleek gelijk aan de frequentie van voorkomen van gehoorproblemen in de algemene bevolking, waaruit de conclusie getrokken werd dat gehoorverlies niet behoord tot een van de belangrijkste kenmerken van de ziekte van Pompe bij volwassenen, in tegenstelling tot bij baby's met de ziekte.

Hoofdstuk 4 gaat in zijn geheel over de effecten van enzymtherapie bij kinderen en volwassenen met de ziekte van Pompe. Allereerst richt **sectie 4.1** zich op het effect van enzymtherapie in onze landelijke prospectieve studie bij volwassenen met de ziekte van Pompe, waarin 69 patiënten werden behandeld gedurende gemiddeld 23 maanden. De spierkracht nam significant toe: de MRC sumscore toonde een verbetering van 1.4% per jaar, en de HHD sumscore verbeterde 4.0% per jaar. Bij vrouwen was er een grotere toename van de spierkracht dan bij mannen. Op groepsniveau bleef de longfunctie in zittende positie stabiel, terwijl de longfunctie in liggende positie verder bleef dalen (-1.1% per jaar). Deze bevinding wordt grotendeels verklaard door een subgroep van 40% van de patiënten, die gemiddeld ouder waren, en een slechtere klinische conditie hadden bij start van de therapie dan de andere patiënten. Vergelijking van de snelheid van ziekteprogressie voor en na de start van enzymtherapie toonde dat enzymtherapie het natuurlijk beloop van de ziekte significant ten goede verandert.

In **hoofdstuk 4.2** rapporteren we de resultaten van een gerandomiseerde placebogecontroleerde studie naar het effect van alglucosidase alfa in 90 kinderen en volwassenen met de ziekte van Pompe. Na 18 maanden was duidelijk dat behandeling met enzymtherapie gepaard ging met een significante verbetering van de afstand die patiënten konden afleggen op de 6 minuten looptest en met stabilisatie van de longfunctie in vergelijking met placebo. Het effect van de behandeling werd al snel na het starten zichtbaar en bereikte een plateau na 26 weken behandeling. Het effect van de behandeling leek beter bij patiënten met een relatief goede klinische toestand bij aanvang van de therapie, maar er waren geen subgroepen waarbij het effect op de uitkomst *significant* beter was. Vijf procent van de patiënten die behandeld werden met alglucosidase alfa kreeg ernstige allergische reacties waardoor de behandeling gestopt moest worden. Alle patiënten ontwikkelden IgG antilichamen tegen alglucosidase alfa, maar deze hadden geen consistent – negatief – effect op de uitkomst.

Als laatste beschrijft **sectie 4.3** de resultaten van een open-label studie in 5 kinderen tussen de 5 en 15 jaar. Na 3 jaar behandeling met enzymtherapie was de longfunctie stabiel gebleven in 4 patiënten en duidelijk verbeterd in 1 patiënt. De spierkracht was in alle patiënten was toegenomen. Bij 1 patiënt was de spierkracht zelfs tot op het niveau van gezonde leeftijdsgenoten toegenomen. Dat behandeling met enzymtherapie veilig is bleek uit het feit dat geen van de kinderen infusie gerelateerde bijwerkingen had gedurende de studie.

In **hoofdstuk 5** beschrijven we de ontwikkeling van 2 nieuwe meetinstrumenten voor de ziekte van Pompe. **Sectie 5.1** beschrijft de ontwikkeling van een vragenlijst die geschikt is voor het meten van beperkingen in dagelijkse activiteiten en sociale participatie, gebruik makend van moderne klinimetrische methodes (Rasch methodologie): R-PAct. Deze lineaire interval meetschaal, bestaande uit 18 items, is gebaseerd op de meest beperkende factoren in het dagelijks leven van patiënten met de ziekte van Pompe. We hebben aangetoond dat deze meetschaal valide en betrouwbaar is, en in staat is om respons op individueel patiëntenniveau te kunnen meten.
In **sectie 5.2** wordt de ontwikkeling en validatie van een nieuwe functionele meetschaal voor het graderen van de ernst van motorische beperkingen beschreven: de Quick Motor Function Test (QMFT). De uiteindelijke meetschaal bestaat uit 16 motorische vaardigheden, gerelateerd aan activiteiten in het dagelijks leven waarvoor de spieren van de schouders, romp, bekkengordel, en bovenbenen zijn vereist. Deze test bleek bleek in staat om op een adequate manier de ziekte-ernst te meten, en ook veranderingen in motorische functie in de loop van de tijd te kunnen detecteren.

Tot slot bediscussiëren we in **hoofdstuk 6** onze voornaamste bevindingen en hun significantie en klinische implicaties, en doen we suggesties voor toekomstig onderzoek.

8

APPENDICES

APPENDIX A: RASCH-BUILT POMPE-SPECIFIC ACTIVITY SCALE

PRELIMINARY R-PACT QUESTIONNAIRE

	Are you able to:	No (0)	Yes, but with great difficulty (1)	Yes, but with some difficulty (2)	Yes, but with little difficulty (3)	Yes, without difficulty (4)
1	Write?					
2	Open a tube of toothpaste (with a screw cap)?					
3	Close a zipper?					
4	Fasten buttons on your shirt?					
5	Reach for and grasp an object above your head (such as something from the top shelf of a closet)?					
6	Pour a drink from a carton or can?					
7	Pick up a small object from the table (such as a pen)?					
8	Stand up from a supine position (lying on your back)?					
9	Turn over in bed?					
10	Sit up straight without support?					
11	Stand up from a seated position?					
12	Bend at the knee and then stand up again?					
13	Bend over to pick something up off the ground and then stand up again?					
14	Get in and out of a car?					
15	Mount a bicycle and dismount?					
16	Stand?					
17	Walk approximately 10m?					
18	Walk approximately 100m?					
19	Walk more than 1km?					
20	Walk on an uneven surface (such as cobblestones)?					
21	Walk at a rapid rate?					
22	Run (for example to catch a train)?					
23	Step over a threshold or negotiate obstacles in your path?					
24	Walk up and down the stairs halfway (approx. 7 steps)?					
25	Walk up and down a complete set of stairs (approx. 14 steps)?					
26	Walk up and down several sets of stairs?					
27	Walk up a ramp or slope?					
28	Can you wash your face at the sink?					
29	Take a shower?					
30	Dry yourself off?					
31	Put on a sweater or T-shirt?					
32	Pull on a pair of trousers (without closures)?					
33	Comb your hair?					
34	Brush your teeth?					

	Are you able to:	No (0)	Yes, but with great difficulty (1)	Yes, but with some difficulty (2)	Yes, but with little difficulty (3)	Yes, without difficulty (4)
35	Use the toilet without assistance?					
36	Eat (swallow, chew)?					
37	Prepare a meal?					
38	Carry out your duties at work or your studies?					
39	Carry out household tasks (vacuuming, mopping the floor, ironing, washing windows)?					
40	Garden or carry out tasks in and around your yard?					
41	Buy groceries?					
42	Ride a bicycle?					
43	Drive a car?					
44	Travel on public transportation?					
45	Carry out your hobbies?					
46	Practice a sport?					
47	Use the telephone or computer?					
48	Easily enter shops and the post office?					
49	Visit family members or friends?					

	Are you able to:	No (0)	Yes, but with difficulty (1)	Yes, without difficulty (2)
1	Comb your hair?			
2	Eat (swallow, chew)?			
3	Pull on a pair of trousers (without closures)?			
4	Prepare a meal?			
5	Take a shower?			
6	Reach for and grasp an object above your head?			
7	Step over a threshold or negotiate obstacles in your path?			
8	Turn over in bed?			
9	Walk on an uneven surface?			
10	Stand up from a seated position?			
11	Walk more than 1km?			
12	Walk up and down a complete set of stairs?			
13	Bend over to pick something up off the ground and then stand up again?			
14	Walk at a rapid rate?			
15	Garden or carry out tasks in and around your yard?			
16	Practice a sport?			
17	Bend at the knee (squat) and then stand up again?			
18	Run (for example to catch a train)?			

APPENDIX B: 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

9

EPILOGUE

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ABBREVIATIONS

4-MU	4-methylumbelliferyl-alpha-D-glucopyranoside
6MWT	Six-Minute Walk Test
AAV	Adeno-associated viruses
ACTIVLIM	Rasch-built measure of activity limitations in children and adults with neuromuscular disorders
Ad	Adenoviruses
ADL	Activities of daily living
AIMS	Alberta Infant Motor Scale
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BAEP	Brainstem Auditory Evoked Potentials
β-hCG	Beta-human chorionic gonadotropin
CCMO	Central Committee on Research Involving Human Subjects in the Netherlands
cDNA	Complementary deoxyribonucleic acid
CHO-cells	Chinese hamster ovary-cells
CHQ	Child Health Questionnaire
CI	Confidence interval
CK	Creatine kinase
COPD	Chronic obstructive pulmonary disease
CRIM	Cross reactive immunogenic material
CT	Computed tomography
CVZ	College voor zorgverzekeringen
dB	Decibel
DEXA	Dual-energy X-ray absorptiometry
DM1-Activ	Rasch-built myotonic dystrophy type 1 activity and participation scale
DNA	Deoxyribonucleic acid
DNJ	1-deoxynojirimycin
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EuroQol-5D	European Quality of Life-5 Dimensions
ERT	Enzyme-replacement therapy
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in 1 second
FSS	Fatigue Severity Scale
FVC	Forced vital capacity
GAA	Gene coding for acid α-glucosidase
GILT	Glycosylation-independent lysosomal targeting
Glc4	Tetraglucose oligomer Glcα1-6Glcα1-4Glcα1-4Glc
HADS	Hospital Anxiety and Depression Scale.
HE	Hematoxylin and eosin staining
HHD	Hand-held dynamometry
HP-GAA	Hyper-mannose-6-phosphorylated variant of recombinant human acid alpha glucosidase generated by enzymatic engineering
HSCT	Hematopoietic stem-cell transplantation
IAR	Infusion-associated reaction
ICF	International classification of functioning, disability and health
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IPA	International Pompe Association
IQR	Inter quartile range
ISNO	Interuniversitair Steunpunt Neuromusculair Onderzoek (the Dutch Neuromuscular Research Center)
ISO	International Organization for Standardization
LDH	Lactate dehydrogenase
LOTS	Late-onset treatment study
kDa	Kilo Dalton
kHz	Kilo Herz
kPa	Kilo Pascal
M6P(R)	Mannose-6-phosphate (receptor)
MCID	Minimal clinically important difference
MEP	Maximum expiratory pressure
MFM	Motor Function Measure
MIP	Maximum inspiratory pressure
MMT	Manual muscle testing
MPS	Mucopolysaccharidosis
MRC	Medical Research Council
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NB-DNJ	N-butyldeoxynojirimycin
neo-rhGAA	Hyper-mannose-6-phosphorylated variant of recombinant human acid alpha glucosidase generated by chemical conjugation of synthetic oligosaccharides bearing M6P residues
OAE	Oto-acoustic emission
OMIM	Online Mendelian Inheritance in Man
P_{a,CO_2}	Arterial carbon dioxide pressure
P_{EE,CO_2}	Carbon dioxide fraction in the expired gas at maximum expiration
PAS	Periodic acid-Schiff
PFT	Pulmonary function testing
PK	Pharmacokinetics
PKU	Phenylketonuria
Pompe-PEDI	Pediatric Evaluation of Disability Inventory adapted for children and adolescents with Pompe disease
PRODISQ	PROductivity and DISease Questionnaire
PTA	Pure-tone average
QMFT	Quick Motor Function Test
QMT	Quantitative muscle testing
rhGAA	Recombinant human acid alpha-glucosidase
RHS	Rotterdam 9-items Handicap Scale
R-ODS	Rasch-built Overall Disability Scale
R-PAct	Rasch-built Pompe-specific Activity scale
SD	Standard deviation
SF-36	Medical Outcomes Study 36-item short-form health survey
TACQOL	TNO-AZL Child Quality of Life questionnaire
VC	Vital capacity
VLCADD	Very long chain acyl-coA dehydrogenase deficiency
(V)SN	(Vereniging) Spierziekten Nederland
WHO	World Health Organization

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onderzoek alle patiënten straks een gepast trainingsschema aanbieden waardoor we het effect van enzymtherapie kunnen optimaliseren. Veel succes met de afronding van je onderzoek en je opleiding. Deniz en Merel, bedankt voor de gezelligheid tijdens de WMS aan het strand. Stephan, jouw onderzoek vordert inmiddels ook alweer gestaag, ik ben ervan overtuigd dat de internationale samenwerking veel nuttige informatie gaat opleveren waar patiënten in de toekomst mee gebaat zullen zijn. We zullen ook de komende jaren tijdens onze opleiding nog veel samen kunnen werken. Esther, jij kwam recent ons team versterken. Heel veel succes! Alle anderen veel succes, maar vooral ook veel plezier met jullie verdere onderzoek.
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Mijn paranimfen Marloes en Juna. Wat mag ik mezelf gelukkig prijzen met 2 van zulke fantastische dames aan mijn zijde. Marloes; we hebben samen heel wat afgereisd.
Jij was het die tegen me zei toen ik begon: "dit onderzoeksproject is nooit saai". En dat was het inderdaad nooit. In de loop der jaren hebben er vele onverwachte wendingen plaatsgevonden, maar altijd keerde de rust weer (even) terug. Ik weet nog dat we onszelf voornamen nooit de dag van tevoren nog aan onze presentaties te moeten werken. Uiteindelijk bleek het vliegveld in Brussel best een goede plek voor een last-minute. Je bent qua carrière inmiddels een nieuwe weg ingeslagen, maar gelukkig zien we elkaar nog regelmatig in de speeltuin of in het park in Breda samen met onze kinderen. Juna wat ben ik blij dat jij mijn opvolger op het project bent geworden. Niet alleen ben je een uitermate fijne collega, maar ook nog eens een super vriendin. Lief en leed hebben we de afgelopen jaren gedeeld, en nu ben je voor de 2e keer mijn steun en toeverlaat op een bijzondere dag! Dank voor alle 'kantoor-Breda' dagen, wellicht kan ik in de nabije toekomst mijn 'schuld' enigszins inlossen.

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'Lest best'
Anne, de wereld is niet mooi, maar jij kan haar een beetje mooier kleuren...
Daan, onze wereldkampioen!
Lieve Geert, jouw aandeel in dit proefschrift reikt veel verder dan het design van de 'spierenman' en is eigenlijk niet in woorden uit te drukken. Dank voor alles. Of, zoals we ooit na één van onze trektochten door Noorwegen in het logboek van Tomtehytta schreven:
Tusen takk!!

ABOUT THE AUTHOR

Nadine van der Beek was born on July 26th, 1976 in Veldhoven, the Netherlands. In 1994, she graduated from the Anton van Duinkerkencollege in Veldhoven (grammar school), after which she began her medical training at the Rijksuniversiteit Limburg (nowadays Maastricht University). During her study she joined a research project on balance disturbances at the clinical vestibulology section of the Ear, Nose and Throat surgery department (prof. dr. H. Kingma). She obtained her medical degree in 2000.

From 2000 until 2002 she worked as a medical doctor at the emergency department and the intensive care unit of the St. Anna hospital in Geldrop. From 2002 until 2003 she worked as a resident at the department of neurology at the Maxima Medisch Centrum in Veldhoven, and in 2003 she started working as a resident in neurology at the Erasmus MC University Medical Center in Rotterdam (head: prof. dr. P.A.E. Sillevis Smitt). In august 2004 she started the research underlying this thesis at the Pompe-research team at Erasmus MC (since 2010 named Center for Lysosomal and Metabolic Diseases) under the supervison of prof. dr. P.A. van Doorn, prof. dr. A.T. van der Ploeg, and dr. A.J.J. Reuser. From August 2008 she is continuing her training as a neurologist at the Erasmus MC University Medical Center in Rotterdam.

She is married to Geert Willems, and they have two children: Anne and Daan.

OVER DE AUTEUR

Nadine van der Beek werd geboren op 26 juli 1976 in Veldhoven. In 1994 behaalde zij haar VWO-diploma aan het Anton van Duinkerkencollege in Veldhoven, waarna zij begon met de opleiding geneeskunde aan de Rijksuniversiteit Maastricht (nu Maastricht University). Tijdens haar studie deed zij onderzoek naar evenwichtsstoornissen op de afdeling keel-, neus-, en oorheelkunde (sectie vestibulologie) onder begeleiding van prof. dr. H. Kingma. Ze behaalde haar artsexamen in 2000.

Na haar artsexamen werkte zij tot 2002 op de afdeling spoedeisende hulp en de afdeling intensive care van het St. Anna ziekenhuis in Geldrop. Van 2002 tot 2003 werkte zij als assistent neurologie in het Maxima Medisch Centrum te Veldhoven. Aansluitend begon zij als assistent neurologie in het Erasmus MC Universitair Medisch Centrum (afdelingshoofd: prof. dr. P.A.E. Sillevis Smitt). In augustus 2004 startte zij haar onderzoek naar de ziekte van Pompe onder supervisie van prof. dr. P.A. van Doorn, prof. dr. A.T. van der Ploeg, en dr. A.J.J. Reuser (Centrum voor Lysosomale en Metabole Ziekten). Vanaf augustus 2008 vervolgt zij haar verdere opleiding tot neuroloog aan het Erasmus MC Universitair Medisch Centrum te Rotterdam.

Nadine is getrouwd met Geert Willems en ze hebben samen 2 kinderen: Anne en Daan.

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† *Equal contributors*

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PHD PORTFOLIO: SUMMARY OF PHD TRAINING AND TEACHING

	Year	Workload (ECTS)
PhD training		
General courses		
Classical methods for data-analysis	2005	5.7
Modern statistical methods	2007	4.3
Regression analysis	2007	1.9
Repeated measurements	2008	1.4
Missing values in clinical research	2008	0.7
Biomedical English writing and communication	2008	3.0
International conferences		
Association for Glycogen Storage Diseases, London (oral presentation)	2005	1.0
International Congress on Neuromuscular Diseases, Istanbul (oral presentation)	2006	1.5
World Muscle Society, Bruges (poster presentation)	2006	0.5
World Muscle Society, Giardini Naxos (3 poster presentations)	2007	1.5
World Muscle Society, Newcastle (2 poster presentations)	2008	1.0
World Muscle Society, Geneva (chair poster session; 2 poster presentations)	2009	1.5
World Muscle Society, Almancil (poster presentation)	2011	0.5
Symposium on lysosomal storage diseases, Athens	2005	0.3
Symposium on lysosomal storage diseases, Berlin	2006	0.3
Symposium on lysosomal storage diseases, Vienna	2007	0.3
Symposium on lysosomal storage diseases, Nice (poster presentation)	2007	0.5
Seminars and workshops		
Belgisch-Nederlandse neuromusculaire studieclub, Utrecht	2004	0.2
Belgisch-Nederlandse neuromusculaire studieclub, Utrecht (oral presentation)	2006	0.5
Belgisch-Nederlandse neuromusculaire studieclub, Utrecht	2007	0.2
In depth courses		
Pompe disease expert day, Rotterdam (2 oral presentations)	2006-2008	2.0
Research conference on Pompe disease, Boston	2004,2008	2.0
Concluding symposium of the Late-Onset Treatment Study, Boston	2008	1.0
Boerhaave Prinses Beatrix Fonds symposium neuromusculaire ziekten	2007-2013	1.0
Cursorium kinderneurologie: neurologische leer- en gedragsstoornissen	2010	0.2
Other		
Design, organization, and coordination of the nationwide prospective study on Pompe disease in the Netherlands	2004-2008	6.0
Teaching activities		
Reviewing papers for peer reviewed journals	2006-2012	2.0
Supervising Master's thesis (students physical therapy Hoogeschool Leiden)	2008	0.5