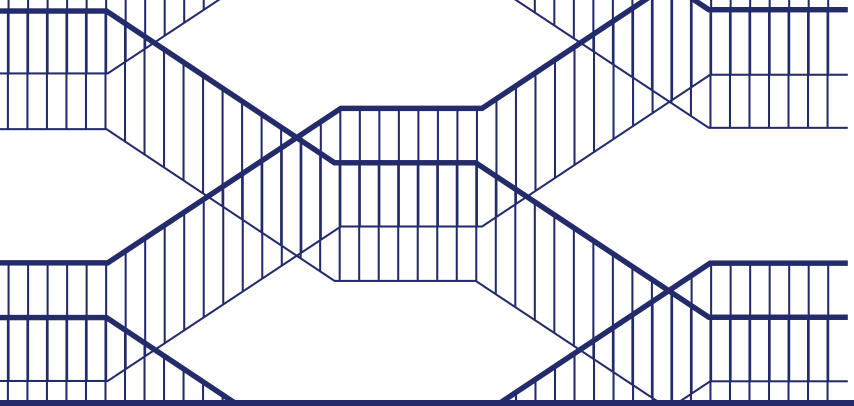
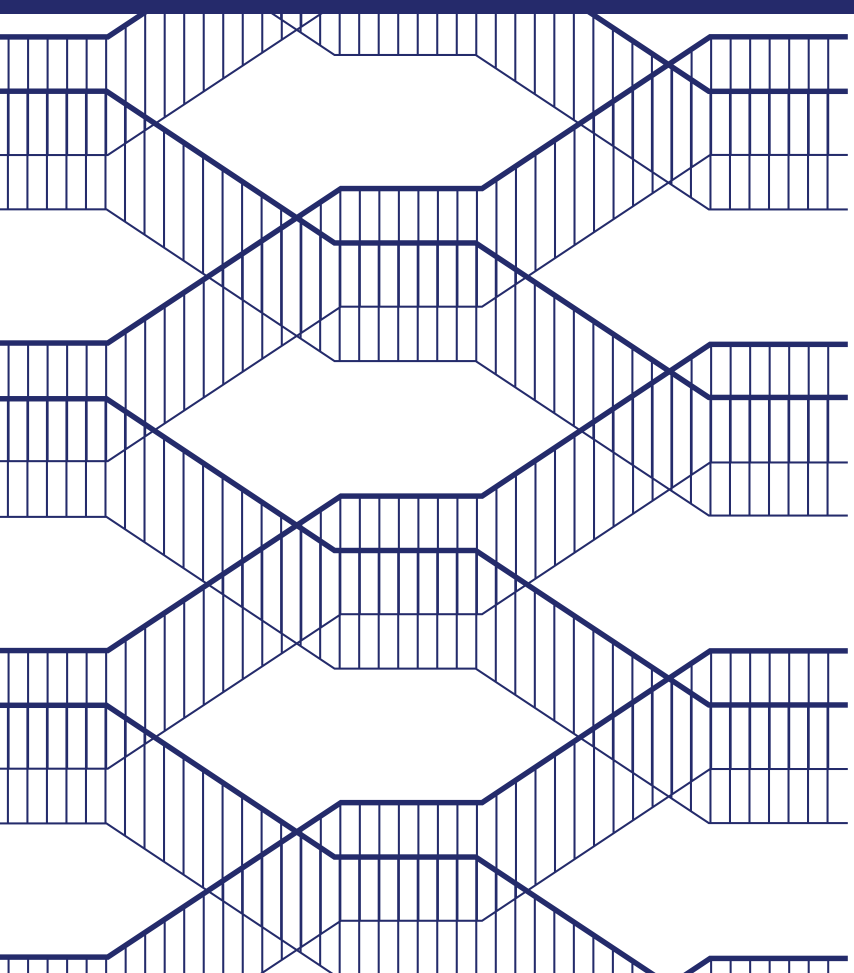


Pompe Disease in Children and Adults: Further Insights into the Clinical Presentation and Long-Term Effects of ERT

An example of sustainable data collection in rare diseases



J.C. van der Meijden

Pompe Disease in Children and Adults: Further Insights into the Clinical Presentation and Long-Term Effects of ERT

An example of sustainable data collection in rare diseases

J.C. van der Meijden

The research presented in this thesis was financially supported by ZonMw – the Netherlands Organization for Health Research and Development [projectnumbers 152001005], "Prinses Beatrix Spierfonds" [projectnumber W.OR13-21,W.OR15-10, W.OR16-07], TKI – Health Holland [projectnumber LSHM16008], the European Union, 7th Framework Programme ["EUCLYD" a European consortium for lysosomal disorders], TiPharma [T6-208], the International Pompe patient Association, and Sanofi Genzyme.

Cover design: Sophia van der Meijden

Lay-out and printing: Ridderprint BV, Ridderkerk

ISBN: 978-94-6299-772-1

2017© J.C. van der Meijden

© Copyright of the published articles is with the corresponding journal or otherwise with the author. No parts of this thesis may be reproduced in any form by print, photo print, microfilm or any other means without permission from the author or the corresponding journal.

Pompe Disease in Children and Adults: Further Insights into the Clinical Presentation and Long-Term Effects of ERT

An example of sustainable data collection in rare diseases

De ziekte van Pompe bij kinderen en volwassenen: Verdere inzichten in de klinische presentatie en lange termijn effecten van ERT

Een voorbeeld van duurzame data verzameling bij zeldzame ziektes

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus
Prof.dr. H.A.P. Pols
en vervolgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
dinsdag 12 december 2017 om 15:30 uur
door

Jan Christiaan van der Meijden
geboren te Hardinxveld-Giessendam

Erasmus University Rotterdam

Promotiecommissie

Promotor:

Prof.dr. A.T. van der Ploeg

Overige leden:

Prof.dr. P.A. van Doorn

Prof.dr. G. Parenti

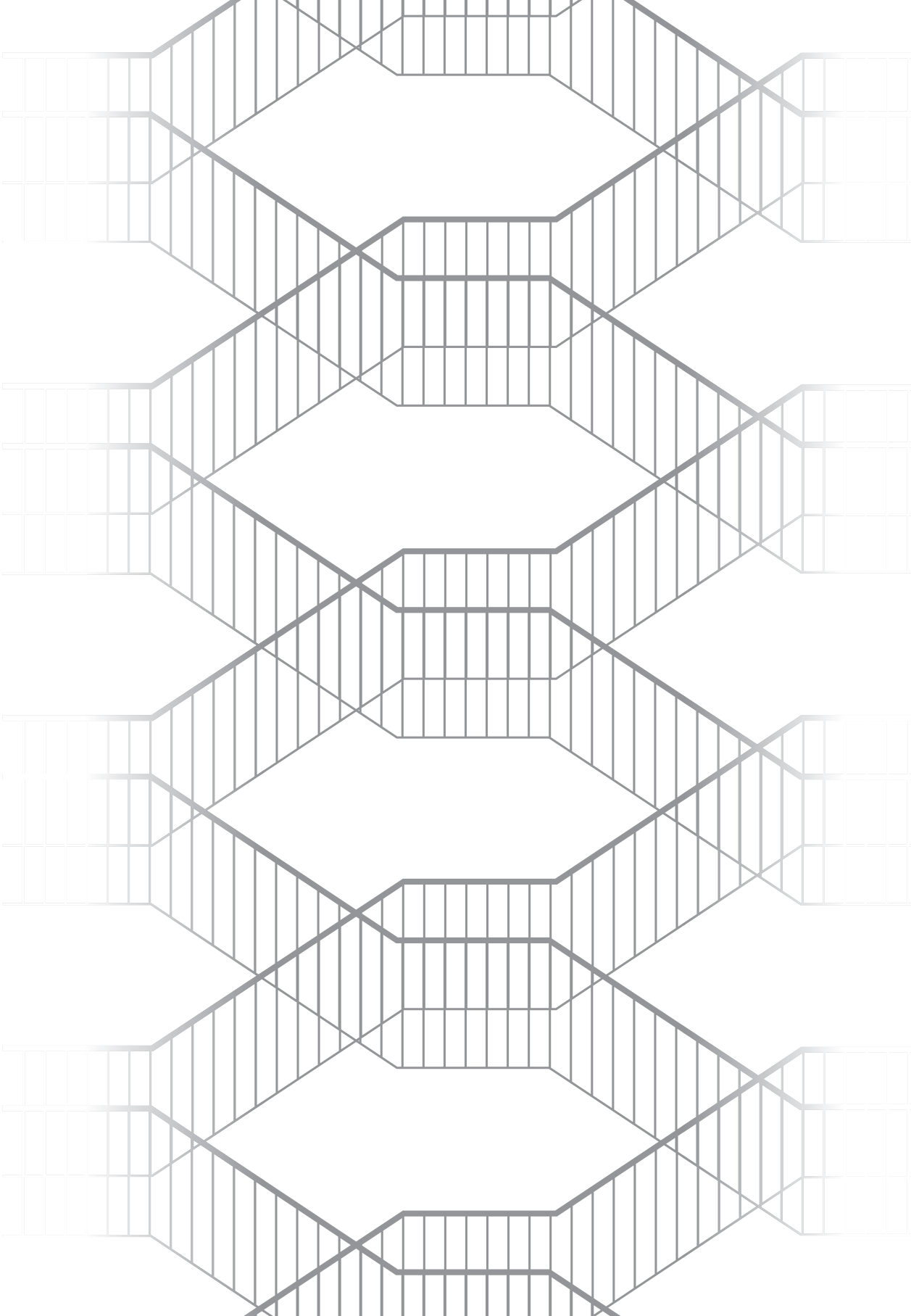
Prof.dr. H.A.W.M. Tiddens

Copromotor:

Dr. M.E. Kruijshaar

Table of contents

Chapter 1	General introduction and outline of this thesis	7
Chapter 2	Childhood Pompe disease: clinical spectrum and genotype in 31 patients *Orphanet Journal of Rare Diseases 2016;11:65*	33
Chapter 3	Long-term follow-up of 17 patients with childhood Pompe disease treated with enzyme replacement therapy *Submitted*	53
Chapter 4	Long-term benefit of enzyme-replacement therapy in Pompe disease: a 5-year prospective study *Neurology 2017; in print*	73
Chapter 5	Association of muscle strength and walking performance in adult patients with Pompe disease *Submitted*	89
Chapter 6	Differences in disease severity and response to enzyme replacement therapy in Pompe patients with the common c.-32-13C>T GAA gene variant cannot be explained by the ACE I/D polymorphism *In preparation*	103
Chapter 7	Ten years of the international Pompe survey: patient reported outcomes as a reliable tool for studying treated and untreated children and adults with non-classic Pompe disease *Journal of Inherited Metabolic Diseases 2015;38:495-503*	121
Chapter 8	Enzyme replacement therapy reduces the risk for wheelchair dependency in adult Pompe patients *Submitted*	139
Chapter 9	General discussion and future perspectives	151
Addendum	Summary	175
	Samenvatting	179
	Acknowledgements | Dankwoord	183
	About the author	187
	List of publications	189
	PhD Portfolio	191
	Abbreviations	193



General introduction and outline of this thesis

1

Pompe disease (OMIN #232300), also known as glycogen storage disease type II or acid maltase deficiency, is an autosomal recessive, lysosomal storage disease caused by mutations in the *GAA* gene. The disease is rare, with an estimated incidence of 1 in 40.000 births.[1,2] It is characterized by partial or total deficiency of the lysosomal enzyme *acid α-glucosidase* and results in glycogen buildup in the lysosome. Patients with this disease have progressive muscle weakness.[3,4] Since 2006 enzyme replacement therapy (ERT) is available for this disease.

This chapter gives an overview of the function of the lysosome and synthesis of lysosomal enzymes, the glycogen metabolism pathway, the pathophysiology of Pompe disease and its clinical spectrum. Furthermore, we discuss the current knowledge of the effects of ERT, the use of patient reported outcomes (PROs) and factors that modify the disease and the efficacy of ERT. Finally, the outline and the scope of this thesis are given.

The lysosome and its enzymes

The function of the lysosome

The lysosome is a subcellular organelle that contains many hydrolytic enzymes that can break down different molecular structures and polymers, such as proteins, lipids, and polysaccharides. It was discovered by Christian de Duve in 1955,[5] who was awarded the Nobel Prize in 1974 for his work. One of the functions of lysosomes is to break down material taken up from outside the cell. Such extracellular material is taken up into the cell by a process of endocytosis, whereby vesicles are formed by inward folding of the cell membrane. These vesicles fuse with early endosomes. Early endosomes mature to the late endosome by acidification and the formation of intraluminal vesicles.[6] These merge with the lysosome, where the materials are degraded.[7,8] Membrane components that were internalized during endocytosis, such as the manose-6-phosphate (M6P) receptor, are not degraded but recycled and transported back to the plasma membrane or Golgi system. Another example of recycling in the endocytosis pathway is the separation of the LDL-receptor from its cholesterol complex in the endosomal/lysosomal compartment.

Lysosomes are also involved in phagocytosis. Phagocytosis takes place in specialized cells such as macrophages. Large particles, like bacteria, old cells and cell debris are taken up and degraded by these cells through phagosomes (large phagocytic vacuoles). While this process resembles endocytosis, phagosomes are a thousand times bigger than endosomes. These phagosomes merge with lysosomes in order to degrade the material in the phagosome.[9,10]

Besides material from the outside the cell, the lysosome also digests material from inside the cell through autophagy. During this process, large double membraned vesicles called autophagosomes form around damaged cell organelles, unused cellular components, or other substrates, and enclose it. These autophagosomes eventually merge with late endosomes and lysosomes in order to degrade and recycle the materials.[7,9,11] Some materials cannot be digested by the lysosome. Over time, these accumulate in lysosomes and impair its function. An example is lipofuscin and its accumulation has been suggested to be related to aging.[12] While the name "autophagy" was first suggested by Christian de Duve in 1963, the genes and exact mechanism involved in this process were elucidated in the 1990s by the Japanese researcher Yoshinori Oshumi. He received a Nobel Prize for his work in 2016.[13,14]

Production of lysosomal enzymes

The lysosome contains about 60 enzymes that can degrade different polymers such as lipids, polysaccharides, proteins, DNA and RNA. The synthesis of lysosomal enzymes starts with transcription of the DNA, i.e. of the gene coding for a specific enzyme, to messenger RNA (mRNA). mRNA is translated into protein by ribosomes that are attached to the Rough Endoplasmic Reticulum (RER). Newly synthesized lysosomal enzymes are then phosphorylated, folded and processed during transport through the RER and the Golgi network to the endosomes and lysosomes. For example, en route to the lysosome the enzyme *acid α-glucosidase*, which is deficient in Pompe disease, matures from a 110kDa precursor, via a 95kDA intermediate form, to one of two active forms, the 70kDa and 76kDa forms. Both N-terminal and C-terminal processing occurs during the maturation of this enzyme.

In the Golgi network, lysosomal enzymes are sorted to endosomes and lysosomes. Lysosomal enzymes can also be sorted to the cell surface and excreted. From there they reach the endosomes and lysosomes through endocytosis. In both cases, transport of the lysosomal enzymes inside the cell is predominantly by clathrin-coated vesicles.[9,15] M6P residues play an important role in the correct sorting and transport of lysosomal enzymes. When lysosomal enzymes are synthesized in the RER they become glycosylated on selected asparagine residues. In the Golgi network these oligosaccharides are processed by two enzymes called *GlcNac-1-phosphotransferase* and *α-N-acetyl-glucosaminidase* to expose M6P residues. These bind to M6P receptors that help to package the lysosomal enzymes into clathrin-coated vesicles that bud from the Golgi network. The vesicles subsequently deliver their contents to the late endosomes and lysosomes and, to a smaller degree, the plasma membrane (Figure 1).[16-20] The importance of the M6P receptors for adequate transport of lysosomal enzymes from the Golgi network to the endosomes and lysosomes is shown in

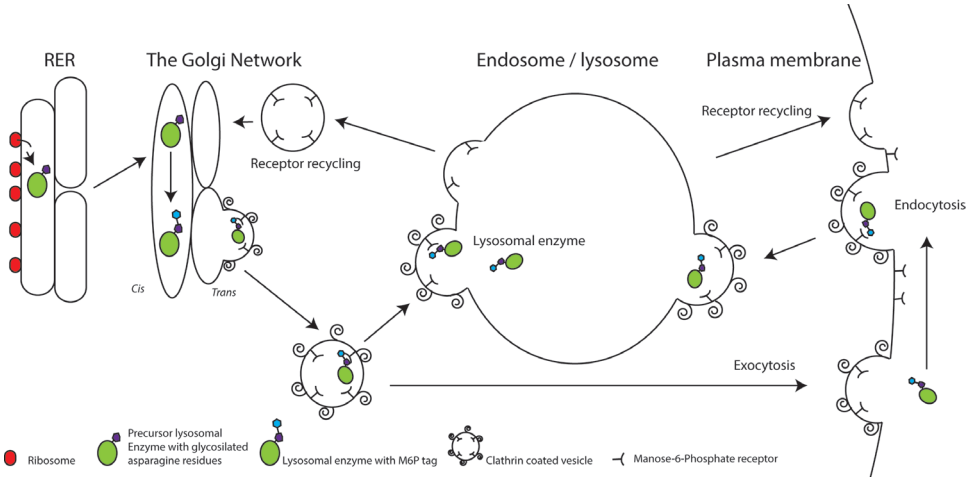


Figure 1 | Pathways of synthesis and transport of lysosomal enzymes.

I-cell disease. Mutations in the gene coding for the enzyme *GlcNAc 1-phosphotransferase*, needed to generate M6P residues, results in most lysosomal enzymes being excreted outside the cell instead of being transported to the lysosomes.[21] M6P -receptors are not only present in the Golgi network and endosomes, but also on the plasma membrane of the cell, allowing the uptake of secreted enzymes. The M6P receptor also has affinity for other ligands such as IGF-II and its presence on the cell surface form the basis of ERT.[22,23]

Lysosomal storage diseases

Diseases such as Pompe disease and mucolipidosis (deficiency of *GlcNAc-1-phosphotransferase*) are lysosomal storage diseases. In general, lysosomal storage diseases (LSDs) are diseases in which the lysosomal function is impaired. Around 50 lysosomal storage disorders are currently known. Most have an autosomal recessive pattern of inheritance. Different types of lysosomal storage diseases are: mucopolysaccharidoses, oligosaccharidoses, sphingolipidoses, mucolipidoses, lipid storage diseases, glycogen storage disorder type II, and lysosomal transporter defects.[24]

Glycogen metabolism

Glycogen and its synthesis

In Pompe disease, glycogen accumulates in the lysosome due to a defect in the lysosomal enzyme *acid α-glucosidase* (*GAA*). Glycogen is a polysaccharide of glucose that serves as a form of energy storage. Glycogen can consist of thousands of glucose repeats, and forms a

large structure or 'granule' in the cytoplasm of the cell, as shown in Figure 2. These can have a diameter ranging from 10 to 40 nm. Glycogen in synthesized by the enzyme *glycogen synthase*. This enzyme converts UDP-glucose into glycogen (UDP-glycose is synthesized from glucose by *UDP glucose phosphorylase)*. At the core of every *glycogen* granule the enzyme *glycogenin* is present. *Glycogenin* acts as a primer for the synthesis of glycogen and is needed to bind the first glucose molecules. *Glycogen synthase* requires at least four glycose residues in the glycogen chain to perform its function thereafter.[25] Glucose residues are linked through α-1,4-glycosidic bonds by *glycogen synthase*. G*lycogen branching enzyme* creates branches by forming α-1,6-glycosidic bonds (figure 3).

Glycogen is an important source of energy since it can be broken down to glucose in a fast and controlled manner. As such, it is an important buffer to maintain blood glucose levels and an important energy source in muscle tissue, especially when sudden activity requires additional energy. The main storage sites of glycogen are the liver and skeletal muscle. Glycogen stored in the liver is mainly used to maintain blood-glucose levels, while in the muscle it is solely used for the energy requirements of the muscle itself.[25,26]

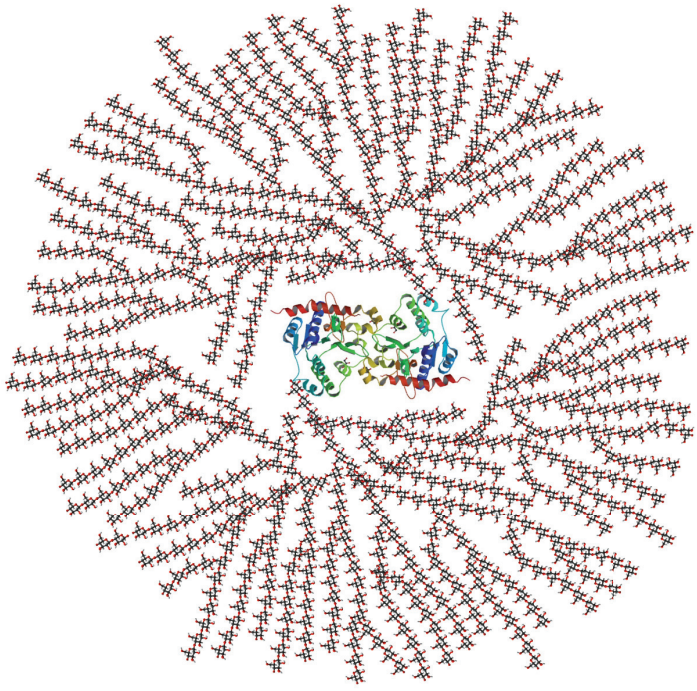


Figure 2 | Schematic overview of a glycogen granule. *Glycogenin* is displayed as the enzyme in the middle. *By Mikael Häggström, used with permission.*

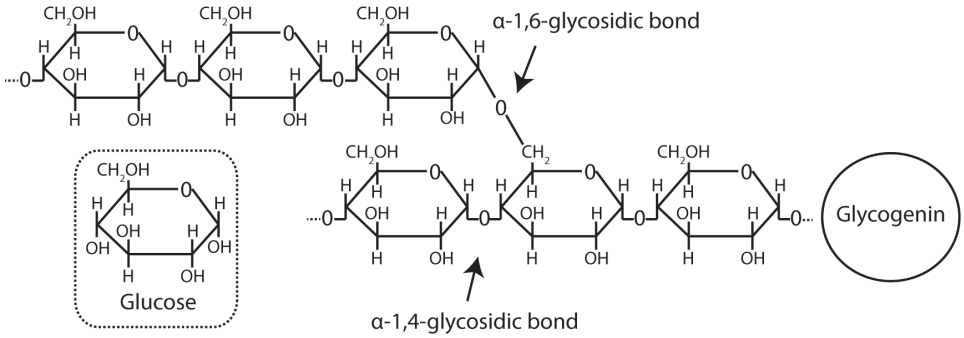


Figure 3 | Molecular structure of glycogen.

Breakdown of glycogen in the cytoplasm

Glycogenolysis is the breakdown of glycogen to glucose. The main enzyme involved in this process is *glycogen phosphorylase*. This enzyme cleaves the α-1,4-glycosidic bond of the terminal glucose residue resulting in a glucose-1-phosphate molecule. When four glucose groups are left on a glycogen branch three groups are transferred to a different branch by *glycogen debranching enzyme*. Next, the enzyme *amylo-α-1,6-glucosidase* cleaves the last glucose group at the α-1,6-glycosidic bond, removing the branch entirely and resulting in the production of a glucose molecule. Figure 3 shows the two different glycosidic bounds which are cleaved. The glucose molecule resulting from cleavage by *amylo-α-1,6-glucosidase* can be phosphorylated to glucose-6-phosphate by the enzyme *hexokinase*. The glucose-1-phosphate molecule resulting from cleavage by *glycogen phosphorylase*, is converted to glucose-6-phosphate by *phosphoglucomutase* (figure 4).

Glucose-6-phosphate can be used for various processes. It can be used in the pentose phosphate pathway, lipogenesis, used for aerobic or anaerobic respiration, for glucose synthesis in the liver, or again more in glycogen synthesis. The main pathways are displayed in figure 4. Diseases resulting from mutations in the enzymes involved in the breakdown (and in some cases the synthesis) of glycogen are known as glycogen storage diseases.[16,25-28]

Breakdown of glycogen in the lysosome

In addition to the breakdown of glycogen in the cytoplasm, which is the main pathway for glycogenolysis, glycogen can also be broken down in the lysosome. Glycogen enters the lysosome through autophagy.[29-31] Autophagosomes enclose glycogen granules and fuse with lysosomes to degrade the content. Once in the lysosome, the enzyme *acid α-glucosidase* (*GAA*) breaks down glycogen to glucose molecules. *GAA* can cleave both the α-1,4 and α-1,6 bounds of the glycogen polymer. The degradation of glycogen in the lysosome is displayed in Figure 4.

Pompe disease

Pompe disease is a lysosomal storage disease and a glycogen storage disease. It was first described in 1932 by the Dutch pathologist Johannes Cassianus Pompe in his publication titled "*Over idiopathische hypertrophie van het hart*" in the Dutch journal "*Nederlands Tijdschrift voor Geneeskunde*". In this paper he described his findings from an autopsy performed on a 7-month old girl showing an enlarged heart. After further examination he identified vacuolar deposits of glycogen in the heart and other tissues, and hypothesized that these findings were part of a systemic disorder of glycogen metabolism.[32] A defect in the breakdown of glycogen is characteristic for glycogen storage diseases. The pathophysiological mechanism of the disease remained unknown until Henri G. Hers, a colleague of dr. de Duve who discovered the lysosome, identified that the lysosomal enzyme *acid α-glucosidase* was responsible for the breakdown of glycogen in the lysosome. Deficiency of this enzyme causes glycogen to build up in the lysosome, making Pompe disease both a glycogen storage disorder as well as a lysosomal storage disease.[33] Pompe disease is not the only disease that is both a lysosomal storage disease and glycogen storage disease. In Danon disease the fusing of the autophagosome and the lysosome is impaired due to a deficiency in the *lysosomal associated membrane protein-2 (LAMP-2)* gene. This disease resembles Pompe disease as it is characterized by large intercellular vacuoles and leads to muscle weakness and cardiomyopathy.[34]

Acid α-glucosidase deficiency

Accumulation of glycogen in lysosomes is mainly seen in muscle tissue, but can take place in other tissues as well.[35,36] As a result of this accumulation the size and number of lysosomes increase. With increasing size, the lysosomal membrane is prone to rupture causing degradative enzymes to be released into the cytoplasm and resulting in cell damage.[37,38] The subsequent muscle damage may not only be caused by rupturing of enlarged lysosomes. It has been suggested that the autophagic and/or endocytic pathways are involved as well.[39] Mouse studies have shown defects in the acidification of endosomes, a large increase of vesicles involved in the endocytic and/or autophagic pathways, and a slowdown of trafficking in the overcrowded cell.[40] These findings show that the endocytic and/or authophagic pathways become impaired. Impairment of these pathways leads to problems with all metabolic processes in the cell, as organelles and other structures are not properly recycled. Furthermore, a buildup of vesicles and/or of products that should be broken down or recycled, also called autophagic buildup, potentially interferes with the architecture of the muscle and metabolic processes. All in all, the processes described above lead to impairment of the cells function and ultimately destruction of, mainly, muscle tissue.[41]

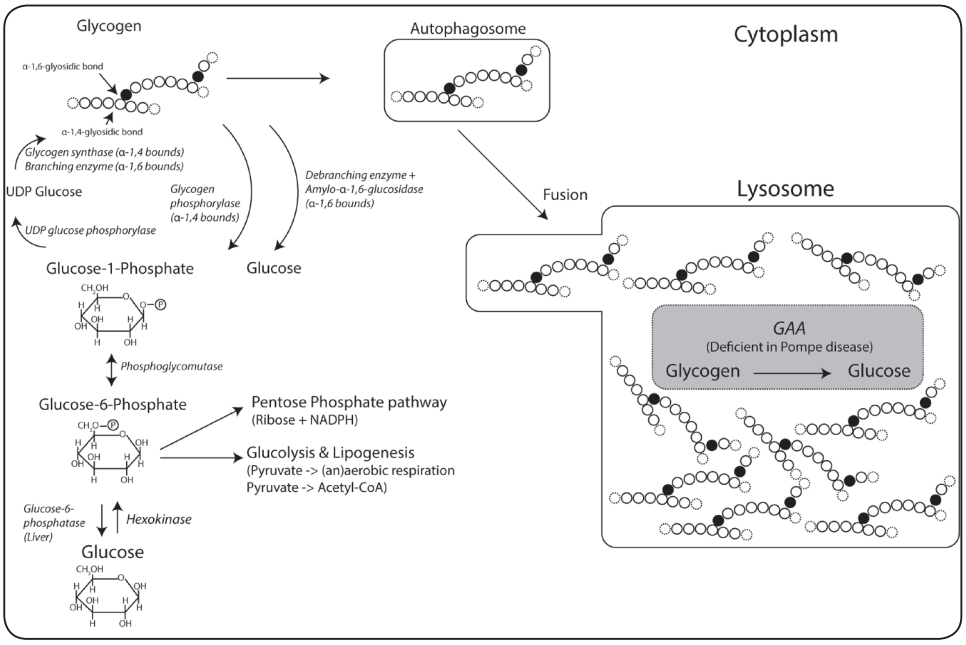


Figure 4 | Cytoplasmic and lysosomal glycogen metabolism.

This figures displays both glycogen synthesis and breakdown in the cytoplasm and glycogen breakdown in the lysosome. On the left is displayed how glycogen is synthesized or degraded and eventually enters different pathways in the cytoplasm. On the right, lysosomal degradation of glycogen is displayed. Glycogen is taken up by autophagosomes, which fuse with lysosomes. Normally glycogen gets degraded to glucose in the lysosome by *acid a-glucosidase* (GAA). When this enzyme is deficient glycogen accumulates in the lysosome as shown by the multiple glycogen polymers depicted in the lysosome.

Genetics of Pompe disease

Pompe disease results from pathological sequence variations in the gene coding for the enzyme acid α-glucosidase (*GAA*), which is located on chromosome 17q25.2-q25.3 and encodes 20 exons. Pompe disease inherits in an autosomal recessive way. For the disease to manifest, both alleles need to carry a pathogenic variant in the GAA gene.[42] As of May 2016, more than 500 variants of the GAA gene have been identified, the majority of which are pathogenic (www.pompecenter.nl).

In the Dutch population, the three most common pathological variants are c.-32-13T>G (also referred to as 'IVS1'), c.2481+102_2646+31del (deletion of exon 18) and c.525delT.[43,44] In total, 63% of Dutch patients carry one or two of these mutations.[45] The c.2481+102_2646+31del mutation embodies the deletion of the entire exon 18. This is an 'in-frame' deletion, which implies that it does not change the DNA reading frame. As a consequence a shorter enzyme is produced which is not functional and not transported to the lysosome.[46,47] The deletion

of a single nucleotide at location c.525 causes a frameshift, which results in a premature stop codon that ends transcription of DNA. This gives rise to unstable messenger RNA and therefore no production of protein takes place at all.[48] When both alleles carry mutations that produce no functional enzyme at all (so called 'null' mutations), the patient has the most severe form of the disease which is referred to as the classic-infantile phenotype (see below for further description).[44,46-50]

The IVS1 mutation, on the other hand, results in a milder phenotype and is never associated with the classic infantile phenotype. This mutation, located in intron 1, affects the splicing of messenger RNA (mRNA). Splicing is the process during the transcription of DNA to mRNA, in which intronic (i.e. non-coding) regions are skipped so that only exons are transcribed to mRNA. The IVS1 variant results in exon 2 being incorrectly skipped in 80 to 90% of the mRNA created. Nevertheless, since 10-20% of the mRNA is formed correctly a small proportion of functional enzyme is produced. While too low, these patients have some residual *acid α-glucosidase* activity left resulting in a less severe phenotype.[51-56]

When no GAA enzyme nor a precursor of it is formed, a patient's immune system may recognize GAA as foreign. Such patients are said to be cross reactive immunological material (CRIM) negative and are for example those with homozygote c.525delT mutations. In patients who produce some functional enzyme (e.g. those with IVS-1), and those in whom an, albeit inactive, precursor protein is generated (e.g. the deletion of exon 18) the immune system can recognize GAA as non-foreign, and these are referred to as CRIM positive. However, also CRIM positive patients may elicit antibody formation when ERT with recombinant human *acid α-glucosidase* is applied. CRIM status is determined by the combination of GAA mutations of the patient. Of all classic-infantile patients around 1/3th are CRIM negative. All patients with milder forms of Pompe disease are per definition CRIM positive.[57-59]

Clinical spectrum of Pompe disease

Clinical spectrum

As mentioned above, not all mutations result in a total deficiency of acid α-glucosidase. Patients who do not produce any functional enzyme are at the most severe end of the clinical spectrum and are referred to as having 'classic-infantile' Pompe disease. Patients with some residual enzyme activity (below 20-30% of normal) have a milder phenotype, which was first described by dr. Engel in 1973 as acid maltase deficiency.[60] This phenotype is now referred to as non-classic or late-onset and has a broad age range of disease onset. The full spectrum of Pompe disease is displayed in figure 5.[61] The combination of mutations determines whether a patient has complete absence of functional enzyme, or whether low

levels of functional enzyme are produced. Besides this, no clear-cut genotype-phenotype relations exist to fully explain the broad clinical variation observed in non-classic patients.

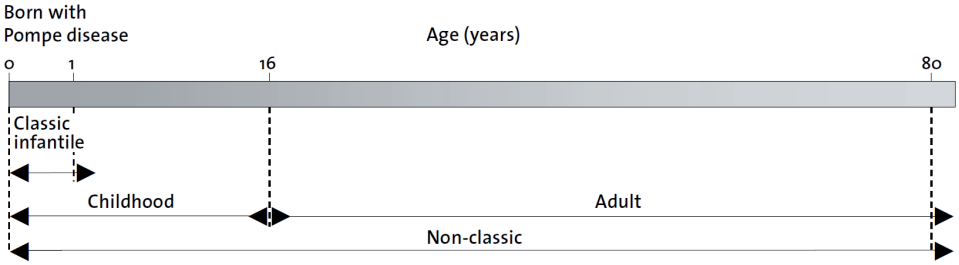


Figure 5 | Clinical spectrum of Pompe disease (adapted from Güngör and Reuser[61]).

Clinical characteristics of classic-infantile Pompe disease

Patients with the classic-infantile form of the disease are characterized by a rapidly progressive phenotype. These children develop symptoms within the first months of life, commonly consisting of feeding problems, failure to thrive, and general hypotonia. Hypertrophic cardiomyopathy is a key characteristic observed in these patients and the majority die within the first year of life due to cardiorespiratory failure.[62-64] Patients are unable to achieve motor milestones (being able to sit or stand) and frequently have decreased weight despite interventions like feeding through a nasogastric tube.[62,64]

Clinical characteristics of children and adults with non-classic forms of Pompe disease

In patients who have some residual enzyme activity disease progression is slower compared to classic-infantile patients.[65-67] The disease can manifest at any age, from birth to late adulthood. Characteristic symptoms in these children and adults are (limb-girdle) muscle weakness, walking problems, and respiratory problems.[66,68-70] Fatigue is also commonly reported. Other identifying features, seen in some 25 to 66% of patients are ptosis, bulbar muscle weakness, scapular wining, scoliosis, and increased lumbar lordosis. Over time, muscle weakness progresses and patients start having problems to participate in daily life situations, have reduced quality of life, and become dependent on a wheelchair and/or ventilator.[69,71-77] Furthermore, compared to the general population, patients with Pompe disease have poorer survival.[78]

A small group of these patients develop symptoms similar to the 'classic-infantile' phenotype, but have a slightly later disease onset. They can present in the first year of life and some of

these patients also have hypertrophic cardiomyopathy. Without treatment they do survive past the age of 1 year, but not beyond childhood. Slonim et al. have described these patients as 'atypical' infantile patients.[79]

Enzyme Replacement Therapy

The first efforts to develop a treatment for Pompe disease started in the 1960s when *acid a-glucosidase* extracted from Aspergillus Niger was administered to a patient.[80-82] This attempt and subsequent ones using human sources of the enzyme were not successful.[83] Produced enzymes proved to be too immunogenic and not enough enzyme could be produced to achieve adequate doses. Around 1980 the focus shifted to bone marrow transplantations, but this was unsuccessful too.[84-86] In 1984 the quest to find a treatment for Pompe disease made a leap forward when studies showed that adding phosphorylated mannose residues to *acid a-glucosidase* could increase the cellular uptake targeting the M6P receptor.[87] The M6P receptor was discovered by Elizabeth Neufeld when studying I-cell disease (mucolipidosis).[88] She discovered that this disease is caused by a deficiency of the enzyme that uncovers M6P groups on lysosomal enzymes. The lack of these M6P groups results in these enzymes being secreted out of the cell instead of being transported to the lysosome. Most cells have M6P receptors on its plasma membrane to facilitate the uptake of lysosomal enzymes (as discussed above in the section on lysosomal enzymes) and will therefore also facilitate the uptake of Enzyme Replacement Therapy (ERT) tagged with M6P.[17-20]

In 1984 researchers from the Erasmus MC started to explore the potential of the M6P receptor as target for ERT in Pompe disease. In 1988 the GAA gene was cloned which opened the way for biotechnological production of *recombinant human GAA* (*rhGAA*). This discovery was followed by their generation of a knock-out mouse model, production of *rhGAA* in the milk of transgenic mice and rabbits and, in 1999, the first trials in humans.[87,89-106] Parallel research in other universities using enzyme derived from Chinese Hamster Ovaria cells (CHO)[107] and collaboration with industry ultimately led to the market approval of alglucosidase alfa in 2006. Pompe disease thereby became the first treatable metabolic myopathy.

Effects of enzyme replacement therapy in classic-infantile Pompe disease

The first trials with ERT were performed in patients with the classic-infantile phenotype. Positive effects were observed: hypertrophic cardiomyopathy reduced or resolved, survival and ventilator-free survival improved, and patients started to achieve motor milestones.[89,95,108-114] Also, a reduction of glycogen storage and improvement of the muscle architecture was observed in muscle biopsies of these patients.[89,95,96]

Despite these large improvements, there are still a number of infants who die whilst being treated with ERT. Also, long-term survivors are not free of symptoms: about half of the classic-infantile patients who are treated become ventilator-dependent, a large number does not learn to walk, and most retain some level of residual muscle weakness.[89,96,97,109,110,114-116] Several studies have focused on elucidating the reasons behind this and on improving the response to treatment. Some of these report that starting ERT at a younger age improves its effect,[109,117-119] while others have tried to achieve this by reducing the immune response to ERT and increasing the dose of ERT.[120]

Enzyme replacement therapy in children and adults

Since the registration of ERT in 2006, many studies have been performed and published on the effects of ERT in children and adults with Pompe disease. The first and only randomized clinical trial was performed in 60 patients receiving ERT and 30 receiving a placebo for 78 weeks. Starting in 2005, this trial demonstrated ERT to improve lung function and distance walked during the 6 Minute Walk Test (6MWT) compared to placebo.[121] Many observational studies have since found similar positive effects in patients. Around 50 studies have been published so far, mostly from Europe. With a few exceptions, follow-up did not exceed 3-4 years.[122-130] Most of these studies concern adult patients only, or include a few children. Studies on the effects of ERT specifically in children are rare.[127,131-136] In the discussion we will further discuss the results of these studies in the light of our findings of longer-term treatment with ERT.

Modifying factors for the natural course of disease and response to ERT

Studies on the effects of ERT, both in infants as well as in children and adults, indicate that some patients benefit more from treatment than others. While on group level muscle strength improves, some patients still have declining muscle strength.[130] Also without treatment there is a large variation in terms of disease progression and how severely patients are affected by the disease, especially in those with the non-classic phenotype. This variation cannot be explained by the combination of *GAA* mutations. Multiple factors may underlie these variations and it is important to gain insight into these factors as this can help to understand the disease better as well as improve treatment.

For classic-infantile patients, it has so far been suggested that an early start treatment plus a positive CRIM status combined with the absence of antibodies against the treatment are the most important factors predicting treatment success.[58,59,111,119,137-142] Another important factor of influence on the outcome of patients is the dosing of ERT. An increased dose has shown to have positive effects in patients.[120]

Patients with the non-classic phenotype have a very broad disease spectrum. The variation in disease onset and severity, observed within these patients, cannot be explained on the basis of the mutation alone. Other factors must therefore play a role in the progression and severity of the disease.[65,68,143-146] One of these factors is the disease duration, i.e. time since onset of symptoms or diagnosis. This has been shown to be more important determinant of disease severity than the age of the patient.[72] Furthermore, one of our studies suggest that male gender, along with longer disease duration and muscle weakness, is associated with a poorer lung function and faster decline.[73] This gender difference was also described in a study on phenotypic variation within families carrying the c.-32-13T>G/'null' mutations, where male patients were often more severely affected than their female siblings.[147] Nevertheless, even given these suggestions It is still poorly understood why there is broad phenotypical variation in Pompe disease.

Also the effect of treatment can vary greatly between 'non-classic' patients.[130] Since these patients are per definition CRIM positive, antibodies are usually not a problem.[134,148] It has been suggested that an early start of treatment is associated with an better outcome.[149] As in infants, a higher dose is likely to be more effective, but other factors remain to be elucidated. Identification of these factors could be the basis for personalization of the treatment for Pompe disease.

It is likely that genetic background factors may play a role in the observed variation. Factors that have been suggested to influence both the phenotype and effects of ERT in Pompe patients are polymorphisms in the angiotensin-converting-enzyme (ACE), alpha actinin 3 (ACTN3) and peroxisome proliferative activated receptor alpha (PPARα).[150] After an initial study the ACE and ACTN3 polymorphism seemed most promising to have an influence on disease severity and the efficacy of a treatment.[150-153] The studies on the effect of these polymorphisms so far are not conclusive and therefore more research is needed.

Importance of patient reported outcomes and the IPA survey

The use of patient reported outcome measures (PROs) is becoming increasingly important in studying the impact a disease has on patients and the effects of treatments. While clinical outcome measures like laboratory tests or the 6MWT are obtained through healthcare professionals, PROs are directly obtained from the patient. They can therefore provide in-depth information on various aspects of the patients' lives and functioning.[154] Regulatory bodies like the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) recognize the value of PROs in the process of registration of new drugs.[155]

In 2002, the Erasmus MC and the International Pompe Association (IPA) initiated a survey amongst Pompe patients in 13 different countries. The aim of this survey was to collect information directly from the patients to study the natural course of disease. When ERT became available this could also be used to study its effects. By recruiting patients directly through the national patient organizations, this survey was able to reach a large number of patients. At present, the survey has included a total of 458 patients (Figure 6) from 13 countries who have been followed for a median period of 5.2 years (range 0 - 15 years). As such, the cohort has become much larger than in any of the clinical studies. This survey is unique in the research on Pompe disease and resulted in a large, consistent, dataset and multiple publications.

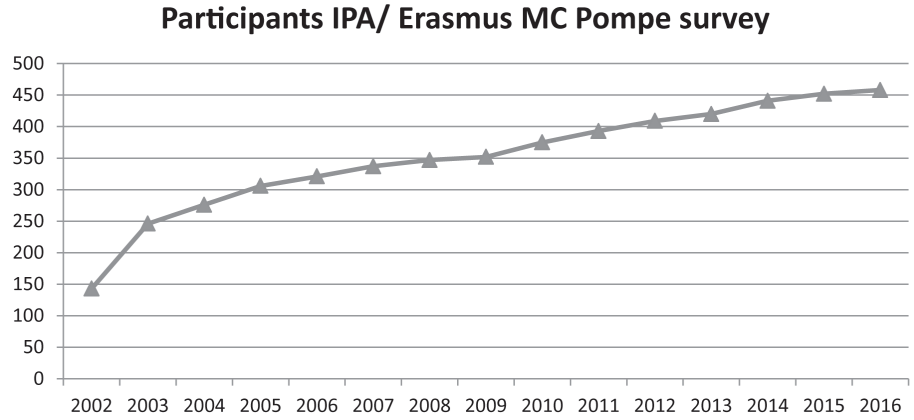


Figure 6 | Cumulative number of subjects participating in the IPA survey.

Scope and outline of the thesis

Over the years great progress has been made in elucidating the natural course of Pompe disease, in developing ERT, and in studying its effects. The Erasmus MC is one of the international centers with the longest track record on studies on Pompe disease and has played a large role in the development of ERT for this disease. By following patients both with clinical outcome measures and with PROs through the "IPA/ Erasmus MC Pompe survey", this center has had a major contribution towards the current knowledge on the disease and its treatment. Yet, there are still remaining questions that need answers. For example, much of the research so far has focused on classic-infantile and adult patients, while there is only limited information on patients who present with the disease during childhood, both in terms of their natural disease progression and the effects of ERT. Also, little is known about factors that modify disease progression or the effect of treatment. This research often requires larger numbers of patients than those seen in many of the individual treatment centers.

In this thesis we aim to further the understanding of Pompe disease and its treatment, and seek answers on some of the important questions that have so far remained unanswered. Furthermore we share our experience on the long term use of the "IPA/ Erasmus MC Pompe survey" so it can serve as an example to be applied in other rare diseases.

This thesis had multiple aims.

1. To provide information on the natural course of patients who present with Pompe disease during childhood.
2. To describe the long-term effects of ERT both in adult patients and children, and determine whether ERT can reduce the need for a wheelchair or respiratory support.
3. To study whether the ACE polymorphism influences disease severity and/or the effect of ERT in children and adults with Pompe disease, and investigate what factors influence patients' walking performance.
4. To share our experience of using the "IPA/ Erasmus MC Pompe survey" and its contribution to the research on Pompe disease and discuss how it can serve as an example for other rare diseases.

Chapter 2 describes the clinical status of untreated patients who presented with Pompe disease during childhood. The long-term effect of ERT in children with Pompe disease is investigated in **chapter 3**, while **chapter 4** discusses the long-term effects in adult patients. In **chapter 5** we study factors that influence walking performance in untreated patients, including muscle force, BMI and invasive ventilator use. In **chapter 6** we investigate the

influence of the ACE polymorphism on disease severity and the effect of ERT. **Chapter 7** provides an overview of the information obtained so far through the "IPA/ Erasmus MC Pompe survey" regarding the natural course of disease and the effects of ERT**.** In **chapter 8** we study how ERT affects the risk of patients becoming wheelchair and ventilator dependent. Finally, in **chapter 9** we discuss the results of these studies, placing them in a broader perspective. Also in this chapter we discuss our experience of using the "IPA/ Erasmus MC Pompe survey" in a sustainable way for over 15 years and make recommendations on how such PROs can contribute to other rare diseases. Finally, we provide our conclusions and future perspectives for research in Pompe disease in this chapter.

References

1. Martiniuk F, Chen A, Mack A, Arvanitopoulos E, Chen Y, Rom WN, Codd WJ, Hanna B, Alcabes P, Raben N *et al*. Carrier frequency for glycogen storage disease type II in New York and estimates of affected individuals born with the disease. *Am J Med Genet* 1998;79(1):69-72.
2. Ausems MG, ten Berg K, Kroos MA, van Diggelen OP, Wevers RA, Poorthuis BJ, Niezen-Koning KE, van der Ploeg AT, Beemer FA, Reuser AJ *et al*. Glycogen storage disease type II: birth prevalence agrees with predicted genotype frequency. *Community Genet* 1999;2(2-3):91-96.
3. Engel AG, Hirschhorn R, Huie ML: Acid maltase deficiency. In: *Myology.* edn. Edited by Engel AG. New York: McGraw-Hill; 2004.
4. Hirschhorn R, Reusser AJ: Glycogen storage disease type II: acid alphaglucosidase (acid maltase) deficiency. In: *The metabolic and molecular bases of inherited disease.* 8th edn. Edited by Scriver CR, Beaudet AL, Sly WS, Valle D. New York: McGraw-Hill; 2001: 3389–3420.
5. de Duve C. The lysosome turns fifty. *Nat Cell Biol* 2005;7(9):847-849.
6. Piper RC, Katzmann DJ. Biogenesis and function of multivesicular bodies. *Annu Rev Cell Dev Biol* 2007;23:519-547.
7. Scott CC, Vacca F, Gruenberg J. Endosome maturation, transport and functions. *Semin Cell Dev Biol* 2014;31:2-10.
8. Xu H, Ren D. Lysosomal physiology. *Annual review of physiology* 2015;77:57-80.
9. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P: Molecular Biology of the Cell, 4th edn. New York: Garland Science; 2002.
10. Freeman SA, Grinstein S. Phagocytosis: receptors, signal integration, and the cytoskeleton. *Immunol Rev* 2014;262(1):193-215.
11. Yorimitsu T, Klionsky DJ. Autophagy: molecular machinery for self-eating. *Cell Death Differ* 2005;12 Suppl 2:1542-1552.
12. Sergiev PV, Dontsova OA, Berezkin GV. Theories of Aging: An Ever-Evolving Field. *Acta Naturae* 2015;7(1):9-18.
13. Takeshige K, Baba M, Tsuboi S, Noda T, Ohsumi Y. Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. *J Cell Biol* 1992;119(2):301-311.
14. Baba M, Takeshige K, Baba N, Ohsumi Y. Ultrastructural analysis of the autophagic process in yeast: detection of autophagosomes and their characterization. *J Cell Biol* 1994;124(6):903-913.
15. Staudt C, Puissant E, Boonen M. Subcellular Trafficking of Mammalian Lysosomal Proteins: An Extended View. *Int J Mol Sci* 2016;18(1).
16. Lodish H, Berk B, Zipursky SL, Matsudaira P, Baltimore D, Darnell J: Molecular Cell Biology, 4th edn. New York: Freeman, W.H.; 2000.
17. Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, C.R. B, Hart GW, Etzler M: Essentials of glycobiology - P-type Lectins. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009.
18. Pohlmann R, Nagel G, Hille A, Wendland M, Waheed A, Braulke T, von Figura K. Mannose 6-phosphate specific receptors: structure and function. *Biochemical Society transactions* 1989;17(1):15-16.
19. Le Borgne R, Hoflack B. Mannose 6-phosphate receptors regulate the formation of clathrin-coated vesicles in the TGN. *J Cell Biol* 1997;137(2):335-345.
20. Duncan JR, Kornfeld S. Intracellular movement of two mannose 6-phosphate receptors: return to the Golgi apparatus. *J Cell Biol* 1988;106(3):617-628.
21. Braulke T, Pohl S, Storch S. Molecular analysis of the GlcNac-1-phosphotransferase. *J Inherit Metab Dis* 2008;31(2):253-257.
22. Barranger JA, Cabrera-Salazar MA: Lysosomal Storage Disorders. New York: Springer; 2007.
23. Tsuji A, Omura K, Suzuki Y. Intracellular transport of acid alpha-glucosidase in human fibroblasts: evidence for involvement of phosphomannosyl receptor-independent system. *J Biochem* 1988;104(2):276-278.
24. Greiner-Tollersrud OK, Berg T: Madame Curie Bioscience Database. In., edn. Austin (TX): Landes Bioscience; 2013.
25. Berg JM, Tymoczko JL, Styer L: Biochemistry, 5th edn. New York: WH Freeman; 2002.

26. Adeva-Andany MM, Gonzalez-Lucan M, Donapetry-Garcia C, Fernandez-Fernandez C, Ameneiros-Rodriguez E. Glycogen metabolism in humans. *BBA clinical* 2016;5:85-100.
27. Fernandes J, Chen YT: Inborn Metabolic Diseases - Glycogen Storage Diseases. In., edn. New York: Springer; 2013: 69-84.
28. Hicks J, Wartchow E, Mierau G. Glycogen storage diseases: a brief review and update on clinical features, genetic abnormalities, pathologic features, and treatment. *Ultrastructural pathology* 2011;35(5):183-196.
29. Raben N, Ralston E, Chien YH, Baum R, Schreiner C, Hwu WL, Zaal KJ, Plotz PH. Differences in the predominance of lysosomal and autophagic pathologies between infants and adults with Pompe disease: implications for therapy. *Mol Genet Metab* 2010;101(4):324-331.
30. Raben N, Roberts A, Plotz PH. Role of autophagy in the pathogenesis of Pompe disease. *Acta Myol* 2007;26(1):45-48.
31. Raben N, Baum R, Schreiner C, Takikita S, Mizushima N, Ralston E, Plotz P. When more is less: excess and deficiency of autophagy coexist in skeletal muscle in Pompe disease. *Autophagy* 2009;5(1):111-113.
32. Pompe JC. Over idiopathische hypertrofie van het hart. *Ned Tijdschr Geneeskd* 1932;76:304-311.
33. Hers HG. alpha-Glucosidase deficiency in generalized glycogenstorage disease (Pompe's disease). *Biochem J* 1963;86:11-16.
34. Rowland TJ, Sweet ME, Mestroni L, Taylor MR. Danon disease - dysregulation of autophagy in a multisystem disorder with cardiomyopathy. *J Cell Sci* 2016;129(11):2135-2143.
35. Wisselaar HA, Kroos MA, Hermans MM, van Beeumen J, Reuser AJ. Structural and functional changes of lysosomal acid alpha-glucosidase during intracellular transport and maturation. *J Biol Chem* 1993;268(3):2223-2231.
36. Reuser AJ, Kroos M, Oude Elferink RP, Tager JM. Defects in synthesis, phosphorylation, and maturation of acid alpha-glucosidase in glycogenosis type II. *J Biol Chem* 1985;260(14):8336-8341.
37. Thurberg BL, Lynch Maloney C, Vaccaro C, Afonso K, Tsai AC, Bossen E, Kishnani PS, O'Callaghan M. Characterization of pre- and post-treatment pathology after enzyme replacement therapy for Pompe disease. *Lab Invest* 2006;86(12):1208-1220.
38. Reuser AJ, Drost MR. Lysosomal dysfunction, cellular pathology and clinical symptoms: basic principles. *Acta Paediatr Suppl* 2006;95(451):77-82.
39. Lim JA, Li L, Raben N. Pompe disease: from pathophysiology to therapy and back again. *Front Aging Neurosci* 2014;6:177.
40. Fukuda T, Ahearn M, Roberts A, Mattaliano RJ, Zaal K, Ralston E, Plotz PH, Raben N. Autophagy and mistargeting of therapeutic enzyme in skeletal muscle in Pompe disease. *Mol Ther* 2006;14(6):831-839.
41. van der Ploeg AT, Reuser AJ. Pompe's disease. *Lancet* 2008;372(9646):1342-1353.
42. Taglia A, Picillo E, D'Ambrosio P, Cecio MR, Viggiano E, Politano L. Genetic counseling in Pompe disease. *Acta Myol* 2011;30(3):179-181.
43. Kroos MA, Van der Kraan M, Van Diggelen OP, Kleijer WJ, Reuser AJ, Van den Boogaard MJ, Ausems MG, Ploos van Amstel HK, Poenaru L, Nicolino M *et al.* Glycogen storage disease type II: frequency of three common mutant alleles and their associated clinical phenotypes studied in 121 patients. *J Med Genet* 1995;32(10):836-837.
44. Ausems MG, ten Berg K, Sandkuijl LA, Kroos MA, Bardoel AF, Roumelioti KN, Reuser AJ, Sinke R, Wijmenga C. Dutch patients with glycogen storage disease type II show common ancestry for the 525delT and del exon 18 mutations. *J Med Genet* 2001;38(8):527-529.
45. Ausems MG, Verbiest J, Hermans MP, Kroos MA, Beemer FA, Wokke JH, Sandkuijl LA, Reuser AJ, van der Ploeg AT. Frequency of glycogen storage disease type II in The Netherlands: implications for diagnosis and genetic counselling. *Eur J Hum Genet* 1999;7(6):713-716.
46. Van der Kraan M, Kroos MA, Joosse M, Bijvoet AG, Verbeet MP, Kleijer WJ, Reuser AJ. Deletion of exon 18 is a frequent mutation in glycogen storage disease type II. *Biochem Biophys Res Commun* 1994;203(3):1535-1541.
47. Ausems MG, Kroos MA, Van der Kraan M, Smeitink JA, Kleijer WJ, Ploos van Amstel HK, Reuser AJ. Homozygous deletion of exon 18 leads to degradation of the lysosomal alpha-glucosidase precursor and to the infantile form of glycogen storage disease type II. *Clin Genet* 1996;49(6):325-328.

48. Hermans MM, De Graaff E, Kroos MA, Mohkamsing S, Eussen BJ, Joosse M, Willemsen R, Kleijer WJ, Oostra BA, Reuser AJ. The effect of a single base pair deletion (delta T525) and a C1634T missense mutation (pro545leu) on the expression of lysosomal alpha-glucosidase in patients with glycogen storage disease type II. *Hum Mol Genet* 1994;3(12):2213-2218.
49. Hermans MM, Graaff E, Kroos MA, Mohkamsing S, Eussen BJ, Joosse M, Willemsen R, Kleijer WJ, Oostra BA, Reuser AJ. The effect of a single base pair deletion (delta T525) and a C1634T missense mutation (pro545leu) on the expression of lysosomal alpha-glucosidase in patients with glycogen storage disease type II. *Human molecular genetics* 1994;3.
50. Hirschhorn R, Huie ML. Frequency of mutations for glycogen storage disease type II in different populations: the delta525T and deltaexon 18 mutations are not generally "common" in white populations. *J Med Genet* 1999;36(1):85-86.
51. Raben N, Nichols RC, Martiniuk F, Plotz PH. A model of mRNA splicing in adult lysosomal storage disease (glycogenosis type II). *Hum Mol Genet* 1996;5(7):995-1000.
52. Bergsma AJ, In 't Groen SL, Verheijen FW, van der Ploeg AT, Pijnappel WP. From Cryptic Toward Canonical Pre-mRNA Splicing in Pompe Disease: a Pipeline for the Development of Antisense Oligonucleotides. *Mol Ther Nucleic Acids* 2016;5:e361.
53. Huie ML, Chen AS, Tsujino S, Shanske S, DiMauro S, Engel AG, Hirschhorn R. Aberrant splicing in adult onset glycogen storage disease type II (GSDII): molecular identification of an IVS1 (-13T-->G) mutation in a majority of patients and a novel IVS10 (+1GT-->CT) mutation. *Hum Mol Genet* 1994;3(12):2231-2236.
54. Bergsma AJ, Kroos M, Hoogeveen-Westerveld M, Halley D, van der Ploeg AT, Pijnappel WW. Identification and characterization of aberrant GAA pre-mRNA splicing in pompe disease using a generic approach. *Hum Mutat* 2015;36(1):57-68.
55. Boerkoel CF, Exelbert R, Nicastri C, Nichols RC, Miller FW, Plotz PH, Raben N. Leaky splicing mutation in the acid maltase gene is associated with delayed onset of glycogenosis type II. *Am J Hum Genet* 1995;56(4):887-897.
56. Kroos MA, Pomponio RJ, Hagemans ML, Keulemans JL, Phipps M, DeRiso M, Palmer RE, Ausems MG, Van der Beek NA, Van Diggelen OP *et al*. Broad spectrum of Pompe disease in patients with the same c.-32-13T->G haplotype. *Neurology* 2007;68(2):110-115.
57. Bali DS, Goldstein JL, Banugaria S, Dai J, Mackey J, Rehder C, Kishnani PS. Predicting cross-reactive immunological material (CRIM) status in Pompe disease using GAA mutations: lessons learned from 10 years of clinical laboratory testing experience. *Am J Med Genet C Semin Med Genet* 2012;160C(1):40-49.
58. van Gelder CM, Hoogeveen-Westerveld M, Kroos MA, Plug I, van der Ploeg AT, Reuser AJ. Enzyme therapy and immune response in relation to CRIM status: the Dutch experience in classic infantile Pompe disease. *J Inherit Metab Dis* 2015;38(2):305-314.
59. Berrier KL, Kazi ZB, Prater SN, Bali DS, Goldstein J, Stefanescu MC, Rehder CW, Botha EG, Ellaway C, Bhattacharya K *et al*. CRIM-negative infantile Pompe disease: characterization of immune responses in patients treated with ERT monotherapy. *Genet Med* 2015;17(11):912-918.
60. Engel AG, Gomez MR, Seybold ME, Lambert EH. The spectrum and diagnosis of acid maltase deficiency. . *Neurology* 1973;23:95-106.
61. Gungor D, Reuser AJ. How to describe the clinical spectrum in Pompe disease? *Am J Med Genet A* 2013;161A(2):399-400.
62. van den Hout HM, Hop W, van Diggelen OP, Smeitink JA, Smit GP, Poll-The BT, Bakker HD, Loonen MC, de Klerk JB, Reuser AJ *et al*. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics* 2003;112(2):332-340.
63. Kishnani PS, Hwu WL, Mandel H, Nicolino M, Yong F, Corzo D, Infantile-Onset Pompe Disease Natural History Study G. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *J Pediatr* 2006;148(5):671-676.
64. Kishnani PS, Howell RR. Pompe disease in infants and children. *J Pediatr* 2004;144(5 Suppl):S35-43.
65. Wokke JH, Ausems MG, van den Boogaard MJ, Ippel EF, van Diggelene O, Kroos MA, Boer M, Jennekens FG, Reuser AJ, Ploos van Amstel HK. Genotype-phenotype correlation in adult-onset acid maltase deficiency. *Ann Neurol* 1995;38(3):450-454.
66. Winkel LP, Hagemans ML, van Doorn PA, Loonen MC, Hop WJ, Reuser AJ, van der Ploeg AT. The natural course of non-classic Pompe's disease; a review of 225 published cases. *J Neurol* 2005;252(8):875-884.

67. Hagemans ML, Hop WJ, Van Doorn PA, Reuser AJ, Van der Ploeg AT. Course of disability and respiratory function in untreated late-onset Pompe disease. *Neurology* 2006;66(4):581-583.
68. Laforet P, Nicolino M, Eymard PB, Puech JP, Caillaud C, Poenaru L, Fardeau M. Juvenile and adult-onset acid maltase deficiency in France: genotype-phenotype correlation. *Neurology* 2000;55(8):1122-1128.
69. van der Beek NA, de Vries JM, Hagemans ML, Hop WC, Kroos MA, Wokke JH, de Visser M, van Engelen BG, Kuks JB, van der Kooi AJ *et al*. Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study. *Orphanet J Rare Dis* 2012;7:88.
70. Muller-Felber W, Horvath R, Gempel K, Podskarbi T, Shin Y, Pongratz D, Walter MC, Baethmann M, Schlotter-Weigel B, Lochmuller H *et al*. Late onset Pompe disease: clinical and neurophysiological spectrum of 38 patients including long-term follow-up in 18 patients. *Neuromuscul Disord* 2007;17(9-10):698-706.
71. Hagemans ML, Winkel LP, Doorn PA, Hop WJ, Loonen MC, Reuser AJ, Ploeg AT. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. *Brain* 2005;128.
72. Hagemans ML, Winkel LP, Hop WC, Reuser AJ, Van Doorn PA, Van der Ploeg AT. Disease severity in children and adults with Pompe disease related to age and disease duration. *Neurology* 2005;64(12):2139-2141.
73. van der Beek NA, van Capelle CI, van der Velden-van Etten KI, Hop WC, van den Berg B, Reuser AJ, van Doorn PA, van der Ploeg AT, Stam H. Rate of progression and predictive factors for pulmonary outcome in children and adults with Pompe disease. *Mol Genet Metab* 2011;104(1-2):129-136.
74. Hagemans ML, Laforet P, Hop WJ, Merkies IS, Van Doorn PA, Reuser AJ, Van der Ploeg AT. Impact of late-onset Pompe disease on participation in daily life activities: evaluation of the Rotterdam Handicap Scale. *Neuromuscul Disord* 2007;17(7):537-543.
75. Hagemans ML, Janssens AC, Winkel LP, Sieradzan KA, Reuser AJ, Van Doorn PA, Van der Ploeg AT. Late-onset Pompe disease primarily affects quality of life in physical health domains. *Neurology* 2004;63(9):1688-1692.
76. Hagemans ML, van Schie SP, Janssens AC, van Doorn PA, Reuser AJ, van der Ploeg AT. Fatigue: an important feature of late-onset Pompe disease. *J Neurol* 2007;254(7):941-945.
77. Beek NA, Vries JM, Hagemans ML, Hop WC, Kroos MA, Wokke JH, Visser M, Engelen BG, Kuks JB, Kooi AJ. Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study. *Orphanet J Rare Dis* 2012;7.
78. Gungor D, de Vries JM, Hop WC, Reuser AJ, van Doorn PA, van der Ploeg AT, Hagemans ML. Survival and associated factors in 268 adults with Pompe disease prior to treatment with enzyme replacement therapy. *Orphanet J Rare Dis* 2011;6:34.
79. Slonim AE, Bulone L, Ritz S, Goldberg T, Chen A, Martiniuk F. Identification of two subtypes of infantile acid maltase deficiency. *J Pediatr* 2000;137(2):283-285.
80. Baudhuin P, Hers HG, Loeb H. An Electron Microscopic and Biochemical Study of Type Ii Glycogenosis. *Lab Invest* 1964;13:1139-1152.
81. Hug G, Schubert WK. Lysosomes in type II glycogenosis. Changes during administration of extract from Aspergillus niger. *J Cell Biol* 1967;35(1):C1-6.
82. Lauer RM, Mascarinas T, Racela AS, Diehl AM, Brown BI. Administration of a mixture of fungal glucosidases to a patient with type II glycogenosis (Pompe's disease). *Pediatrics* 1968;42(4):672-676.
83. de Barsy T, Jacquemin P, Van Hoof F, Hers HG. Enzyme replacement in Pompe disease: an attempt with purified human acid alpha-glucosidase. *Birth Defects Orig Artic Ser* 1973;9(2):184-190.
84. Harris RE, Hannon D, Vogler C, Hug G. Bone marrow transplantation in type IIa glycogen storage disease. *Birth Defects Orig Artic Ser* 1986;22(1):119-132.
85. Watson JG, Gardnermedwin D, Goldfinch ME, Pearson ADJ. Bone-Marrow Transplantation for Glycogen-Storage-Disease Type-Ii (Pompes Disease). *New Engl J Med* 1986;314(6):385-385.
86. Howell JM, Dorling PR, Shelton JN, Taylor EG, Palmer DG, Di Marco PN. Natural bone marrow transplantation in cattle with Pompe's disease. *Neuromuscul Disord* 1991;1(6):449-454.
87. Reuser AJ, Kroos MA, Ponne NJ, Wolterman RA, Loonen MC, Busch HF, Visser WJ, Bolhuis PA. Uptake and stability of human and bovine acid alpha-glucosidase in cultured fibroblasts and skeletal muscle cells from glycogenosis type II patients. *Exp Cell Res* 1984;155(1):178-189.
88. Neufeld EF: The receptor for lysosomal enzymes on the surface of cultured fibroblasts. In: *Receptor-Mediated Binding and Internalization of Toxins and Hormones.* edn. New York: Academic Press; 1981.

89. Van den Hout H, Reuser AJ, Vulto AG, Loonen MC, Cromme-Dijkhuis A, Van der Ploeg AT. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients. *Lancet* 2000;356(9227):397-398.
90. van der Ploeg AT, Bolhuis PA, Wolterman RA, Visser JW, Loonen MC, Busch HF, Reuser AJ. Prospect for enzyme therapy in glycogenosis II variants: a study on cultured muscle cells. *J Neurol* 1988;235(7):392-396.
91. van der Ploeg AT, van der Kraaij AM, Willemsen R, Kroos MA, Loonen MC, Koster JF, Reuser AJ. Rat heart perfusion as model system for enzyme replacement therapy in glycogenosis type II. *Pediatr Res* 1990;28(4):344-347.
92. Van der Ploeg AT, Kroos MA, Willemsen R, Brons NH, Reuser AJ. Intravenous administration of phosphorylated acid alpha-glucosidase leads to uptake of enzyme in heart and skeletal muscle of mice. *J Clin Invest* 1991;87(2):513-518.
93. Bijvoet AG, Kroos MA, Pieper FR, Van der Vliet M, De Boer HA, Van der Ploeg AT, Verbeet MP, Reuser AJ. Recombinant human acid alpha-glucosidase: high level production in mouse milk, biochemical characteristics, correction of enzyme deficiency in GSDII KO mice. *Hum Mol Genet* 1998;7(11):1815-1824.
94. Martiniuk F, Chen A, Donnabella V, Arvanitopoulos E, Slonim AE, Raben N, Plotz P, Rom WN. Correction of glycogen storage disease type II by enzyme replacement with a recombinant human acid maltase produced by over-expression in a CHO-DHFR(neg) cell line. *Biochem Biophys Res Commun* 2000;276(3):917-923.
95. Amalfitano A, Bengur AR, Morse RP, Majure JM, Case LE, Veerling DL, Mackey J, Kishnani P, Smith W, McVie-Wylie A *et al*. Recombinant human acid alpha-glucosidase enzyme therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial. *Genet Med* 2001;3(2):132-138.
96. Van den Hout JM, Reuser AJ, de Klerk JB, Arts WF, Smeitink JA, Van der Ploeg AT. Enzyme therapy for pompe disease with recombinant human alpha-glucosidase from rabbit milk. *Journal of inherited metabolic disease* 2001;24(2):266-274.
97. Reuser AJ, Van Den Hout H, Bijvoet AG, Kroos MA, Verbeet MP, Van Der Ploeg AT. Enzyme therapy for Pompe disease: from science to industrial enterprise. *Eur J Pediatr* 2002;161 Suppl 1:S106-111.
98. van der Ploeg AT, Kroos M, van Dongen JM, Visser WJ, Bolhuis PA, Loonen MC, Reuser AJ. Breakdown of lysosomal glycogen in cultured fibroblasts from glycogenosis type II patients after uptake of acid alpha-glucosidase. *J Neurol Sci* 1987;79(3):327-336.
99. Van der Ploeg AT, Loonen MC, Bolhuis PA, Busch HM, Reuser AJ, Galjaard H. Receptor-mediated uptake of acid alpha-glucosidase corrects lysosomal glycogen storage in cultured skeletal muscle. *Pediatr Res* 1988;24(1):90-94.
100. Bijvoet AG, Van Hirtum H, Kroos MA, Van de Kamp EH, Schoneveld O, Visser P, Brakenhoff JP, Weggeman M, van Corven EJ, Van der Ploeg AT *et al*. Human acid alpha-glucosidase from rabbit milk has therapeutic effect in mice with glycogen storage disease type II. *Hum Mol Genet* 1999;8(12):2145-2153.
101. Bijvoet AG, Kroos MA, Pieper FR, de Boer HA, Reuser AJ, van der Ploeg AT, Verbeet MP. Expression of cDNA-encoded human acid alpha-glucosidase in milk of transgenic mice. *Biochim Biophys Acta* 1996;1308(2):93-96.
102. Bijvoet AG, van de Kamp EH, Kroos MA, Ding JH, Yang BZ, Visser P, Bakker CE, Verbeet MP, Oostra BA, Reuser AJ *et al*. Generalized glycogen storage and cardiomegaly in a knockout mouse model of Pompe disease. *Hum Mol Genet* 1998;7(1):53-62.
103. Bijvoet AG, Van Hirtum H, Vermey M, Van Leenen D, Van Der Ploeg AT, Mooi WJ, Reuser AJ. Pathological features of glycogen storage disease type II highlighted in the knockout mouse model. *J Pathol* 1999;189(3):416-424.
104. Hoefsloot LH, Hoogeveen-Westerveld M, Reuser AJ, Oostra BA. Characterization of the human lysosomal alpha-glucosidase gene. *Biochem J* 1990;272(2):493-497.
105. Hoefsloot LH, Willemsen R, Kroos MA, Hoogeveen-Westerveld M, Hermans MM, Van der Ploeg AT, Oostra BA, Reuser AJ. Expression and routeing of human lysosomal alpha-glucosidase in transiently transfected mammalian cells. *Biochem J* 1990;272(2):485-492.
106. Hoefsloot LH, Hoogeveen-Westerveld M, Kroos MA, van Beeumen J, Reuser AJ, Oostra BA. Primary structure and processing of lysosomal alpha-glucosidase; homology with the intestinal sucrase-isomaltase complex. *EMBO J* 1988;7(6):1697-1704.
107. Van Hove JL, Yang HW, Wu JY, Brady RO, Chen YT. High-level production of recombinant human lysosomal acid alpha-glucosidase in Chinese hamster ovary cells which targets to heart muscle and corrects glycogen accumulation in fibroblasts from patients with Pompe disease. *Proc Natl Acad Sci U S A* 1996;93(1):65-70.

108. Klinge L, Straub V, Neudorf U, Voit T. Enzyme replacement therapy in classical infantile pompe disease: results of a ten-month follow-up study. *Neuropediatrics* 2005;36(1):6-11.
109. Kishnani PS, Nicolino M, Voit T, Rogers RC, Tsai AC, Waterson J, Herman GE, Amalfitano A, Thurberg BL, Richards S *et al*. Chinese hamster ovary cell-derived recombinant human acid alpha-glucosidase in infantile-onset Pompe disease. *J Pediatr* 2006;149(1):89-97.
110. Kishnani PS, Corzo D, Nicolino M, Byrne B, Mandel H, Hwu WL, Leslie N, Levine J, Spencer C, McDonald M *et al*. Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology* 2007;68(2):99-109.
111. Kishnani PS, Corzo D, Leslie ND, Gruskin D, Van der Ploeg A, Clancy JP, Parini R, Morin G, Beck M, Bauer MS *et al*. Early treatment with alglucosidase alpha prolongs long-term survival of infants with Pompe disease. *Pediatr Res* 2009;66(3):329-335.
112. Nicolino M, Byrne B, Wraith JE, Leslie N, Mandel H, Freyer DR, Arnold GL, Pivnick EK, Ottinger CJ, Robinson PH *et al*. Clinical outcomes after long-term treatment with alglucosidase alfa in infants and children with advanced Pompe disease. *Genet Med* 2009;11(3):210-219.
113. Winkel LP, Kamphoven JH, van den Hout HJ, Severijnen LA, van Doorn PA, Reuser AJ, van der Ploeg AT. Morphological changes in muscle tissue of patients with infantile Pompe's disease receiving enzyme replacement therapy. *Muscle Nerve* 2003;27(6):743-751.
114. Van den Hout JM, Kamphoven JH, Winkel LP, Arts WF, De Klerk JB, Loonen MC, Vulto AG, Cromme-Dijkhuis A, Weisglas-Kuperus N, Hop W *et al*. Long-term intravenous treatment of Pompe disease with recombinant human alpha-glucosidase from milk. *Pediatrics* 2004;113(5):e448-457.
115. Broomfield A, Fletcher J, Davison J, Finnegan N, Fenton M, Chikermane A, Beesley C, Harvey K, Cullen E, Stewart C *et al*. Response of 33 UK patients with infantile-onset Pompe disease to enzyme replacement therapy. *J Inherit Metab Dis* 2016;39(2):261-271.
116. Hahn A, Schmidt D, Hagel KJ, Neubauer BA, Katz N. Monitoring cardiac function by B-type natriuretic peptide (BNP) in patients with infantile Pompe's disease treated with recombinant alpha-glucosidase. *Clin Lab* 2006;52(11-12):615-619.
117. Chakrapani A, Vellodi A, Robinson P, Jones S, Wraith JE. Treatment of infantile Pompe disease with alglucosidase alpha: the UK experience. *J Inherit Metab Dis* 2010;33(6):747-750.
118. Chien YH, Lee NC, Chen CA, Tsai FJ, Tsai WH, Shieh JY, Huang HJ, Hsu WC, Tsai TH, Hwu WL. Long-term prognosis of patients with infantile-onset Pompe disease diagnosed by newborn screening and treated since birth. *J Pediatr* 2015;166(4):985-991 e981-982.
119. Yang CF, Yang CC, Liao HC, Huang LY, Chiang CC, Ho HC, Lai CJ, Chu TH, Yang TF, Hsu TR *et al*. Very Early Treatment for Infantile-Onset Pompe Disease Contributes to Better Outcomes. *J Pediatr* 2016;169:174-180 e171.
120. van Gelder CM, Poelman E, Plug I, Hoogeveen-Westerveld M, van der Beek NA, Reuser AJ, van der Ploeg AT. Effects of a higher dose of alglucosidase alfa on ventilator-free survival and motor outcome in classic infantile Pompe disease: an open-label single-center study. *J Inherit Metab Dis* 2016;39(3):383-390.
121. van der Ploeg AT, Clemens PR, Corzo D, Escolar DM, Florence J, Groeneveld GJ, Herson S, Kishnani PS, Laforet P, Lake SL *et al*. A randomized study of alglucosidase alfa in late-onset Pompe's disease. *N Engl J Med* 2010;362(15):1396-1406.
122. de Vries JM, van der Beek NA, Hop WC, Karstens FP, Wokke JH, de Visser M, van Engelen BG, Kuks JB, van der Kooi AJ, Notermans NC *et al*. Effect of enzyme therapy and prognostic factors in 69 adults with Pompe disease: an open-label single-center study. *Orphanet J Rare Dis* 2012;7:73.
123. Regnery C, Kornblum C, Hanisch F, Vielhaber S, Strigl-Pill N, Grunert B, Muller-Felber W, Glocker FX, Spranger M, Deschauer M *et al*. 36 months observational clinical study of 38 adult Pompe disease patients under alglucosidase alfa enzyme replacement therapy. *J Inherit Metab Dis* 2012;35(5):837-845.
124. Strothotte S, Strigl-Pill N, Grunert B, Kornblum C, Eger K, Wessig C, Deschauer M, Breunig F, Glocker FX, Vielhaber S *et al*. Enzyme replacement therapy with alglucosidase alfa in 44 patients with late-onset glycogen storage disease type 2: 12-month results of an observational clinical trial. *J Neurol* 2010;257(1):91-97.
125. Angelini C, Semplicini C, Ravaglia S, Bembi B, Servidei S, Pegoraro E, Moggio M, Filosto M, Sette E, Crescimanno G *et al*. Observational clinical study in juvenile-adult glycogenosis type 2 patients undergoing enzyme replacement therapy for up to 4 years. *J Neurol* 2012;259(5):952-958.

126. Anderson LJ, Henley W, Wyatt KM, Nikolaou V, Waldek S, Hughes DA, Lachmann RH, Logan S. Effectiveness of enzyme replacement therapy in adults with late-onset Pompe disease: results from the NCS-LSD cohort study. *J Inherit Metab Dis* 2014;37(6):945-952.

127. Bembi B, Pisa FE, Confalonieri M, Ciana G, Fiumara A, Parini R, Rigoldi M, Moglia A, Costa A, Carlucci A *et al*. Long-term observational, non-randomized study of enzyme replacement therapy in late-onset glycogenosis type II. *J Inherit Metab Dis* 2010;33(6):727-735.

128. Stepien KM, Hendriksz CJ, Roberts M, Sharma R. Observational clinical study of 22 adult-onset Pompe disease patients undergoing enzyme replacement therapy over 5years. *Mol Genet Metab* 2016;117(4):413-418.

129. van der Ploeg AT, Barohn R, Carlson L, Charrow J, Clemens PR, Hopkin RJ, Kishnani PS, Laforet P, Morgan C, Nations S *et al*. Open-label extension study following the Late-Onset Treatment Study (LOTS) of alglucosidase alfa. *Mol Genet Metab* 2012;107(3):456-461.

130. van der Ploeg AT, Kruijshaar ME, Toscano A, Laforet P, Angelini C, Lachmann RH, Pascual Pascual SI, Roberts M, Rosler K, Stulnig T *et al*. European consensus for starting and stopping enzyme replacement therapy in adult patients with Pompe disease: a 10-year experience. *Eur J Neurol* 2017;24(6):768-e731.

131. Deroma L, Guerra M, Sechi A, Ciana G, Cisilino G, Dardis A, Bembi B. Enzyme replacement therapy in juvenile glycogenosis type II: a longitudinal study. *Eur J Pediatr* 2014;173(6):805-813.

132. Ishigaki K, Murakami T, Nakanishi T, Oda E, Sato T, Osawa M. Close monitoring of initial enzyme replacement therapy in a patient with childhood-onset Pompe disease. *Brain Dev* 2012;34(2):98-102.

133. Porta F, Pagliardini V, Roasio L, Biamino E, Spada M. Playing competitive basketball in face of late-onset pompe disease. *Muscle Nerve* 2015;51(2):302-303.

134. van Capelle CI, van der Beek NA, Hagemans ML, Arts WF, Hop WC, Lee P, Jaeken J, Frohn-Mulder IM, Merkus PJ, Corzo D *et al*. Effect of enzyme therapy in juvenile patients with Pompe disease: a three-year open-label study. *Neuromuscul Disord* 2010;20(12):775-782.

135. van Capelle CI, Winkel LP, Hagemans ML, Shapira SK, Arts WF, van Doorn PA, Hop WC, Reuser AJ, van der Ploeg AT. Eight years experience with enzyme replacement therapy in two children and one adult with Pompe disease. *Neuromuscul Disord* 2008;18(6):447-452.

136. Winkel LP, Van den Hout JM, Kamphoven JH, Disseldorp JA, Remmerswaal M, Arts WF, Loonen MC, Vulto AG, Van Doorn PA, De Jong G *et al*. Enzyme replacement therapy in late-onset Pompe's disease: a three-year follow-up. *Ann Neurol* 2004;55(4):495-502.

137. Chien YH, Lee NC, Huang HJ, Thurberg BL, Tsai FJ, Hwu WL. Later-onset Pompe disease: early detection and early treatment initiation enabled by newborn screening. *J Pediatr* 2011;158(6):1023-1027 e1021.

138. Chien YH, Hwu WL, Lee NC. Pompe disease: early diagnosis and early treatment make a difference. *Pediatr Neonatol* 2013;54(4):219-227.

139. Hamdan MA, Almalik MH, Mirghani HM. Early administration of enzyme replacement therapy for Pompe disease: short-term follow-up results. *J Inherit Metab Dis* 2008;31 Suppl 2:S431-436.

140. Abbott MA, Prater SN, Banugaria SG, Richards SM, Young SP, Rosenberg AS, Kishnani PS. Atypical immunologic response in a patient with CRIM-negative Pompe disease. *Mol Genet Metab* 2011;104(4):583-586.

141. Messinger YH, Mendelsohn NJ, Rhead W, Dimmock D, Hershkovitz E, Champion M, Jones SA, Olson R, White A, Wells C *et al*. Successful immune tolerance induction to enzyme replacement therapy in CRIM-negative infantile Pompe disease. *Genet Med* 2012;14(1):135-142.

142. Patel TT, Banugaria SG, Case LE, Wenninger S, Schoser B, Kishnani PS. The impact of antibodies in late-onset Pompe disease: a case series and literature review. *Mol Genet Metab* 2012;106(3):301-309.

143. Dardis A, Zanin I, Zampieri S, Stuani C, Pianta A, Romanello M, Baralle FE, Bembi B, Buratti E. Functional characterization of the common c.-32-13T>G mutation of GAA gene: identification of potential therapeutic agents. *Nucleic Acids Res* 2014;42(2):1291-1302.

144. Kroos MA, Pomponio RJ, Hagemans ML, Keulemans JL, Phipps M, DeRiso M, Palmer RE, Ausems MG, Beek NA, Diggelen OP. Broad spectrum of Pompe disease in patients with the same c.-32–13T->G haplotype. *Neurology* 2007;68.

145. Ausems MG, Wokke JH, Reuser AJ, van Diggelen OP. Juvenile and adult-onset acid maltase deficiency in France: genotype-phenotype correlation. *Neurology* 2001;57(10):1938.

146. Kroos M, Hoogeveen-Westerveld M, van der Ploeg A, Reuser AJ. The genotype-phenotype correlation in Pompe disease. *Am J Med Genet C Semin Med Genet* 2012;160C(1):59-68.

147. Wens SC, van Gelder CM, Kruijshaar ME, de Vries JM, van der Beek NA, Reuser AJ, van Doorn PA, van der Ploeg AT, Brusse E. Phenotypical variation within 22 families with Pompe disease. *Orphanet J Rare Dis* 2013;8:182.

148. de Vries JM, Kuperus E, Hoogeveen-Westerveld M, Kroos MA, Wens SC, Stok M, van der Beek NA, Kruijshaar ME, Rizopoulos D, van Doorn PA *et al*. Pompe disease in adulthood: effects of antibody formation on enzyme replacement therapy. *Genet Med* 2017;19(1):90-97.

149. Toscano A, Montagnese F, Musumeci O. Early is better? A new algorithm for early diagnosis in late onset Pompe disease (LOPD). *Acta Myol* 2013;32(2):78-81.

150. De Filippi P, Saeidi K, Ravaglia S, Dardis A, Angelini C, Mongini T, Morandi L, Moggio M, Di Muzio A, Filosto M *et al*. Genotype-phenotype correlation in Pompe disease, a step forward. *Orphanet J Rare Dis* 2014;9:102.

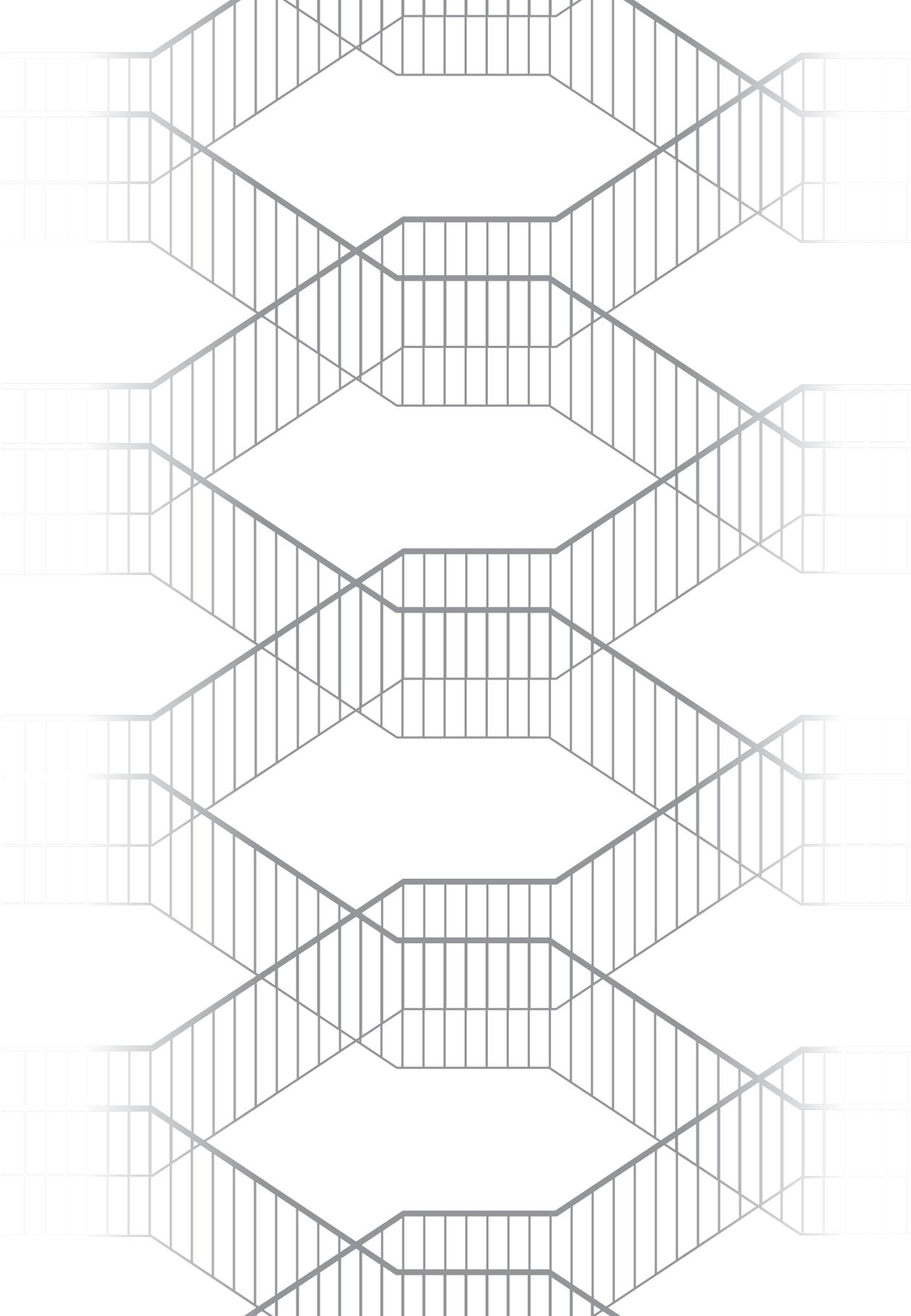
151. Baek RC, Palmer R, Pomponio RJ, Lu Y, Ma X, McVie-Wylie AJ. The influence of a polymorphism in the gene encoding angiotensin converting enzyme (ACE) on treatment outcomes in late-onset Pompe patients receiving alglucosidase alfa. *Mol Genet Metab Rep* 2016;8:48-50.

152. de Filippi P, Ravaglia S, Bembi B, Costa A, Moglia A, Piccolo G, Repetto A, Dardis A, Greco G, Ciana G *et al*. The angiotensin-converting enzyme insertion/deletion polymorphism modifies the clinical outcome in patients with Pompe disease. *Genet Med* 2010;12(4):206-211.

153. Ravaglia S, De Filippi P, Pichiecchio A, Ponzio M, Saeidi Garaghani K, Poloni GU, Bini P, Danesino C. Can genes influencing muscle function affect the therapeutic response to enzyme replacement therapy (ERT) in late-onset type II glycogenosis? *Mol Genet Metab* 2012;107(1-2):104-110.

154. Willke RJ, Burke LB, Erickson P. Measuring treatment impact: a review of patient-reported outcomes and other efficacy endpoints in approved product labels. *Control Clin Trials* 2004;25(6):535-552.

155. Markic J, Polic B, Stricevic L, Metlicic V, Kuzmanic-Samija R, Kovacevic T, Ivkosic IE, Mestrovic J. Effects of immune modulation therapy in the first Croatian infant diagnosed with Pompe disease: a 3-year follow-up study. *Wien Klin Wochenschr* 2014;126(3-4):133-137.



Childhood Pompe disease: clinical spectrum and genotype in 31 patients

J. C. van der Meijden†, C. I. van Capelle†
J. M. P. van den Hout, J. Jaeken, M. Baethmann
T. Voit, M. A. Kroos, T. G. J. Derks
M. E. Rubio-Gozalbo, M. A. Willemsen
R. H. Lachmann, E. Mengel, H. Michelakakis
J. C. de Jongste, A. J. J. Reuser, A. T. van der Ploeg

†Contributed equally

Orphanet Journal of Rare Diseases 2016;11:65

2

Abstract

Background

As little information is available on children with non-classic presentations of Pompe disease, we wished to gain knowledge of specific clinical characteristics and genotypes. We included all patients younger than 18 years, who had been evaluated at the Pompe Center in Rotterdam, the Netherlands, between 1975 and 2012, excluding those with the classic-infantile form. None were treated with enzyme replacement therapy at the time of evaluation. We collected information on first symptoms, diagnosis, use of a wheelchair and/or respirator, and enzyme and mutation analysis and assessed muscle strength, pulmonary function, and cardiac parameters.

Results

Thirty-one patients participated. Median age at symptom onset was 2.6 years (range 0.5-13y) and at diagnosis 4.0 years. Most first problems were delayed motor development and problems related to limb-girdle weakness. Fatigue, persistent diarrhea and problems in raising the head in supine position were other first complaints. Ten patients were asymptomatic at time of diagnosis. Five of them developed symptoms before inclusion in this study. Over 50 percent of all patients had low or absent reflexes, a myopathic face, and scoliosis; 29% were underweight. Muscle strength of the neck flexors, hip extensors, hip flexors, and shoulder abductors were most frequently reduced. Pulmonary function was decreased in over 48% of the patients; 2 patients had cardiac hypertrophy. Patients with mutations other than the c.-32-13T>G were overall more severely affected, while 18 out of the 21 patients (86%) with the c.-32-13T>G/'null' genotype were male.

Conclusions

Our study shows that Pompe disease can present with severe mobility and respiratory problems during childhood. Pompe disease should be considered in the differential diagnosis of children with less familiar signs such as disproportional weakness of the neck flexors, unexplained fatigue and persistent diarrhea and unexplained high CK/ASAT/ALAT. Disease presentation appears to be different from adult patients. The majority of affected children with *GAA* genotype c.-32-13T>G/'null' appeared to be male.

Background

Pompe disease, also known as acid maltase deficiency or glycogen storage disease type 2 (OMIM 232300), is a lysosomal storage disorder that presents as a progressive myopathy in which deficiency of the enzyme acid α-glucosidase (EC 3.2.1.20) causes glycogen to accumulate in lysosomes. Ultimately, this leads to cell destruction.[1-3] In 1932, J.C. Pompe first described the classic-infantile form of the disease.[4] Classic infantile patients characteristically present shortly after birth with generalized and severe muscle weakness and with hypertrophic cardiomyopathy. They do not reach major milestones like walking and usually die within their first year of life.[5, 6] Later, other forms were reported and Pompe disease appeared to be a continuous spectrum of closely related phenotypes with the classic-infantile form at the most severe end of the spectrum. In the literature, milder phenotypes are referred to as childhood, juvenile, adult and late-onset. Patients with these non-classic variants of Pompe disease usually have no hypertrophic cardiomyopathy and present with a more slowly progressive limb-girdle muscle weakness, which eventually results in wheelchair dependency, respirator need, and shortened life expectancy.[3, 7-17]

While many publications have described the natural history of the disease in classic-infantile and adult Pompe patients,[5, 6, 12, 14-16] there is little information on presenting signs and symptoms in children who do not fulfill the criteria of classic-infantile Pompe disease.[18]

To gain knowledge of the presentation of Pompe disease in children and to describe their specific clinical characteristics, we set up an observational study to collect information on disease symptoms, the distribution and severity of muscle weakness, physical limitations, lung function, cardiac structure and function, and genotypes of 31 children diagnosed with a non-classical presentation of Pompe disease.

Methods

Subjects

This observational study included all patients under the age of 18 years who had been diagnosed at or referred to the Pompe Center at Erasmus MC University Medical Center between 1975 and 2012. These patients came from the Netherlands and abroad, and had been diagnosed by measurement of the acid α-glucosidase activity in cultured fibroblasts, leukocytes or muscle biopsy specimens, and by mutation analysis. Patients with classic-infantile Pompe disease were not included in this study.

Thirty-one patients participated in this cross-sectional study. They were evaluated as part of studies approved by the Institutional Review Board (n=28) or as part of routine clinical evaluation (n=3). Medical history was obtained at first visit. Per patient, the following data were collected: gender, current age, geographic origin, first symptoms, age at first symptoms, age at diagnosis, wheelchair use, respiratory support, specific clinical findings (e.g. facial muscle weakness, bulbar muscle weakness, scoliosis, contractures, or muscle atrophy); functional impairments, spirometry and weight and height. Low body weight was defined as weight corrected for height if under 2SD for peers. Blood tests included measurement of creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH). A pediatrician and child neurologist performed clinical and neurological examinations in all patients. Only natural course data were included. None of the patients had been treated with enzyme replacement therapy.

Muscle-strength testing

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

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

Lung function testing

Lung function testing was performed by spirometry with the patients in upright-seated position (n=28), and supine position (n=23) according to ATS/ERS standards.[22] The highest outcome of three reproducible tests was used for analysis. The results were expressed as percentage of predicted and as a z-score, due to the paradigm shift towards z-scores, based on reference values corrected for age, length, gender and race.[23] A percentage lower than 80% of predicted and a z-score below -1.64 were considered abnormal. Two patients were too young for reproducible spirometry to be reliable. Because of poor lung function six other patients were unable to perform testing in a supine position and one of them in either sitting or supine position.

Cardiac assessment

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

Enzymatic and molecular assays

Acid α-glucosidase activity was measured in leukocytes[24] and in cultured skin fibroblasts[13] according to standard procedures, and was expressed in nmol/hr/mg protein. The protein concentrations of cell homogenates was measured as described previously.[25]

Genomic DNA was isolated from blood or cultured fibroblasts, and mutation analysis of the *GAA* gene was performed according to standard procedures.[13, 26] The severity of the mutations were rated using the format of Kroos *et al.*[27], and were examined for their effect on enzyme activity, quantity, and quality, in transfected cells after site directed mutagenesis.[26] In case of splice site mutations, the effect of the mutation was examined using real-time PCR in mRNA, isolated from the patients' fibroblasts.[27]

Statistics

Demographic and clinical data were summarized using descriptive statistics including mean, SD, median, ranges, and percentages. As the data were not normally distributed, differences between groups were analyzed using a Mann-Whitney test. P-values < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS for Windows version 21.

Results

Symptom onset and diagnosis

Thirty-one children participated in this cross-sectional study. Table 1 shows the patient characteristics and genotype. Twenty-two patients were male, nine were female. There were 17 patients from the Netherlands, 4 from Belgium, 5 from Germany, 3 from Greece, 1 from Great Britain, and 1 from the United States.

Table 1 | Patient characteristics.

Pt	Sex	Onset(y)[1]	Diagnosis(y)1	Examination(y)[1]	Wheelchair(y)	Ventilator(y)	FVC z-score sitting (%pred)
1*	M	0.5	2.5	10.04	Yes (11)	No	-0.25 (97%)
2@[P]	F	0.8	0	0.1	No	No	Too young
3	F	0.8	1.1	8.9	No	No	0.21 (102%)
4@	M	0.8	2	2.4	No	No	Too young
5	M	0.8	2.3	9.5	No	No	-1.53 (82%)
6	M	1	2	8.2	Partially (4)	No	-3.47 (59%)
7	M	1	2	13.7	No	At night (12)	-4.08 (54%)
8	M	1.5	2	13.3	No	No	-0.91 (90%)
9*[P]	M	2	1	6.6	No	No	-0.97 (89%)
10	M	2.5	3	13	No	No	-3.01 (66%)
11&	M	5	10.8	10.8	No	No	0.2 (102%)
12	F	5	7.8	7.8	No	No	0.62 (108%)
13[P]	M	5	2	7.6	No	At night (5)	-1.77 (78%)
14	M	7	10	10.7	Yes (22)	At night (16)	-2.22 (75%)
15[P]	M	8	4	15.8	No	No	-2.65 (70%)
16[P]	M	12	8	14.6	No	No	-2.43 (71%)
17	M	13	14	14.3	No	No	-1.68 (81%)
18[P]	M	no symptoms	4	5.2	No	No	-1.41 (82%)
19&[P]	M	no symptoms	13.1	13.1	No	No	-0.69 (92%)
20$[P]	M	no symptoms	14	15.2	No	No	3 (135%)
21$[P]	M	no symptoms	16	17.1	No	No	1.29 (115%)
22	M	0.5	1	1.3	Yes (4)	Died (10†)	-4.77 (45%) ~
23	F	1	1.9	12.5	Yes (6)	Yes (6)	Ventilator
24	M	2	2.9	2.9	Yes (6)	Died (6†)	-5.29 (33%) ~
25	M	2.7	3.5	5.9	No	No	0.34 (104%)
26#	F	4	4	8.1	No	No	0.01 (100%)
27#	M	5	5	10.1	No	No	-1.38 (84%)
28	F	6	7	9.9	Partially (9)	At night (8)	-6.36 (30%)
29	F	6.5	11.6	12.7	No	No	-2.36 (73%)
30	F	10	11	16.4	Yes (16)	Yes (12)	-8.07 (13%)
31!	F	no symptoms	15	15.9	No	No	1.33 (116%)

Patients are listed by age of onset and are subdivided into two groups: those who carry the c.-32-13T>G mutation and those who do not. *, #, $, &, @: Siblings; [P] patients who were diagnosed pre-symptomatically; [1]: Age at onset, age at diagnosis, age at examination expressed in years (y); π: GAA activity was deficient in all patients. Only results obtained in cultured fibroblasts and performed with the same method at Erasmus MC are reported;

FVC z-score supine (%pred)	MRC %	CK U/l	GAA activity (nmol/h/mg) π	Allele 1	Allele 2
-1.12 (87%)	65%	530	9.1	c.-32-13T>G (pm)	c.525delT (vs)
Too young	n.a.	358	Deficient	c.-32-13T>G (pm)	c.2135T>C (ls)
0.1 (101%)	89%	1871	13.3	c.-32-13T>G (pm)	c.923A>C (pls)
Too young	n.a.	1353	13	c.-32-13T>G (pm)	c.2135T>C (ls)
-1.71 (80%)	98%	550	15.4	c.-32-13T>G (pm)	c.525delT (vs)
-4.36 (48%)	79%	3078	4.5	c.-32-13T>G (pm)	c.1051delG (vs)
-5.4 (39%)	87%	548	17.9	c.-32-13T>G (pm)	c.525delT (vs)
-1.76 (80%)	93%	1595	16	c.-32-13T>G (pm)	c.2481+102_2646+31del (vs)
-1.4 (84%)	94%	763	11	c.-32-13T>G (pm)	c.525delT (vs)
-4.09 (54%)	84%	1960	8.6	c.-32-13T>G (pm)	c.2331+2T>A (vs)
-0.29 (97%)	97%	588	11.9	c.-32-13T>G (pm)	c.525delT (vs)
0.35 (104%)	100%	1003	11.6	c.-32-13T>G (pm)	c.2331+2T>A (vs)
-1.72 (79%)	100%	436	Deficient	c.-32-13T>G (pm)	c.1062C>G (pls)
Unable	80%	540	8,9	c.-32-13T>G (pm)	c.1548G>A (pls)
-3.05 (65%)	93%	1424	7.8	c.-32-13T>G (pm)	c.1441T>C (pls)
Unable	82%	1808	Deficient	c.-32-13T>G (pm)	c.307T>G + c.271G>A (pls)
-1.77 (80%)	95%	2935	6.2	c.-32-13T>G (pm)	c.1933G>A (pls)
-1.96 (76%)	97%	677	Deficient	c.-32-13T>G (pm)	c.2481+102_2646+31del (vs)
-1.77 (80%)	100%	614	Deficient	c.-32-13T>G (pm)	c.525delT (vs)
1.72 (120%)	n.a.	1409	Deficient	c.-32-13T>G (pm)	c.307T>G (pls)
-0.25 (97%)	n.a.	1506	Deficient	c.-32-13T>G (pm)	c.307T>G (pls)
Unable	n.a.	586	Deficient	c.1798C>T (ls)	c.525delT (vs)
Ventilator	10%	1381	Deficient	c.875A>G (pm)	unknown/ r.0?
Unable	n.a.	1046	Deficient	unknown	c.1645G>A (pm)
0.04 (100%)	87%	908	2.8	c.1634C>T (ls)	c.2481+102_2646+31del (vs)
-0.63 (92%)	98%	572	2.3	c.-32-3C>G (ls)	c.1551+1G>A (vs)
-2.55 (71%)	n.a.	774	1.7	c.-32-3C>G (ls)	c.1551+1G>A (vs)
-6.88 (25%)	82%	979	0.3	c.1829C>T (ls)	c.1912G>T (pls)
-2.89 (67%)	79%	776	8.4	unknown (r.spl 2%)	c.525delT (vs)
Unable	72%	1560	3.4	c.-32-3C>A (ls)	c.877G>S+c.271G>A (pls)
1.2 (115%)	100%	1040	2.5	c.861C>T (r.spl=<5%)	c.925G>A (pls)

†: both patients died of respiratory failure, one at age 6, the other at age 10; ~: first available lung function measurement (patient still untreated) at the ages of respectively 9 and 5.7 years; severity of the mutation is indicated by (vs) very severe; (pls) potentially less severe; (ls) less severe; (pm) potentially mild (for more information, see www.pompecenter.nl).

The median age at which patients had experienced their first symptom was 2.6 years (range 0.5-13y). At time of diagnosis, their median age was 4.0 years (range 0-16y). The commonest presenting symptoms were delayed motor development (in nine patients), and other symptoms related to limb-girdle weakness, such as frequent falling, difficulty climbing stairs, and problems with running and sports. Fatigue, persistent diarrhea and problems in raising the head in supine position were other first complaints. Median time span between symptom onset and diagnosis was 0.9 years (range 0 to 5.8 years).

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

Clinical findings

All 31 patients were evaluated in the Pompe Center at Erasmus MC University Medical Center. Their ages at the time of examination ranged from 0.1 to 17.1 years. Table 2 shows the findings on clinical examination. Over 50 percent of the 31 patients had low or absent reflexes, a myopathic face, and scoliosis. Facial muscle weakness was generally mild and did not lead to speech difficulties or dysphagia. One exception was a patient (patient 23 in Table 1) that had severe dysarthria and was fed via a percutaneous endoscopic gastrostomy catheter. This patient had been wheelchair bound and ventilator dependent since the age of 6 years.

Nine patients had flexion contractures, mainly in the ankles. Three of the nine patients had contractures of the hips and knees. Several patients had undergone corrective surgery for either contractures (n=4, patients 1, 6, 10, 29 in Table 1), or scoliosis (n=4, patients 7, 14, 23, 30 in Table 1). It is noteworthy that 29% of the patients were underweight; corrected for height, their weight was 4.3 to 2.0 standard deviations below healthy peers.

Standardized neurological examination of all patients showed that 70% had one or more physical limitations. Over 50% of all patients had difficulties standing up from supine position and flexing the neck in supine position (Table 2). Other important limitations were problems with climbing stairs, rising from a chair and standing up from sitting on their heels.

Table 2 | Results of clinical and neurological examination.

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

Four deep tendon reflexes were tested: the biceps reflex, the triceps reflex, the knee-jerk reflex, and the ankle-jerk reflex.

Distribution of muscle weakness

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

All patients had a lower total HHD sum score than age related peers. Total muscle strength ranged from 0 - 79% of normal (median 55%). Four patients were wheelchair-bound at the time of investigation; four others became wheelchair dependent in the period thereafter. The ages of these eight patients when they became wheelchair dependent ranged from 4 to 22 years (n=8, median 7.5 years). The median time period between first symptoms and loss of ambulation was 4.5 years (n=8, range 3 to 15 years).

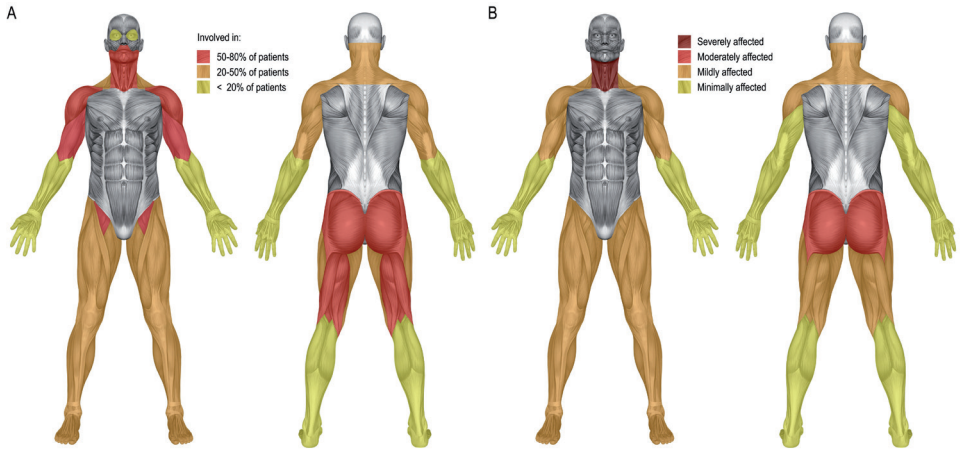


Figure 1 | Distribution of skeletal muscle weakness (A) and severity of weakness of the individual muscles (B).

Lung function testing

In all subjects, FVC values were equal or higher than slow VC. Hence, all analyses were done on FVC. In sitting position, 14 of the 29 patients (48%) had decreased forced vital capacity (FVC) indicated by z-score below -1.64 (13 patients had a percentage of predicted below 80%). In one of these patient the FVC was too low to be measured reliably in sitting position. In supine position, 19 of the 29 (66%) had a FVC z-score below -1.64 (18 patients had a percentage of predicted below 80%). In 6 of these patients, lung volume was too low to be measured reliably in supine position. Lung function measurements are displayed in z-scores and percentage of predicted in figure 2. FEV1/VC ratios were normal in all patients, indicative of a restrictive abnormality.

The median difference in FVC between sitting and supine positions (postural drop) was 6.0% (range 0 to -18%). Five patients were ventilator dependent at the time of first evaluation, and one became ventilator dependent during follow-up (see Table 1 for details). Five of these six patients had a scoliosis. At the ages of 6 and 10 years, two other patients died from respiratory failure, when it was decided not to start respiratory support. The median duration from first symptoms to any kind of respiratory support or death by respiratory insufficiency was 4.5 years (range 0 to 11 years, n=8). The median age at start of ventilation or death was 9 years (range 5 to 16 years, n=8).

Cardiac evaluation

Cardiac evaluation showed abnormalities in six patients; in three, the findings were considered to be related to Pompe disease. Two patients had hypertrophic cardiomyopathy without outflow-tract obstruction (patients 22, 23 in Table 1). In one patient, this had first

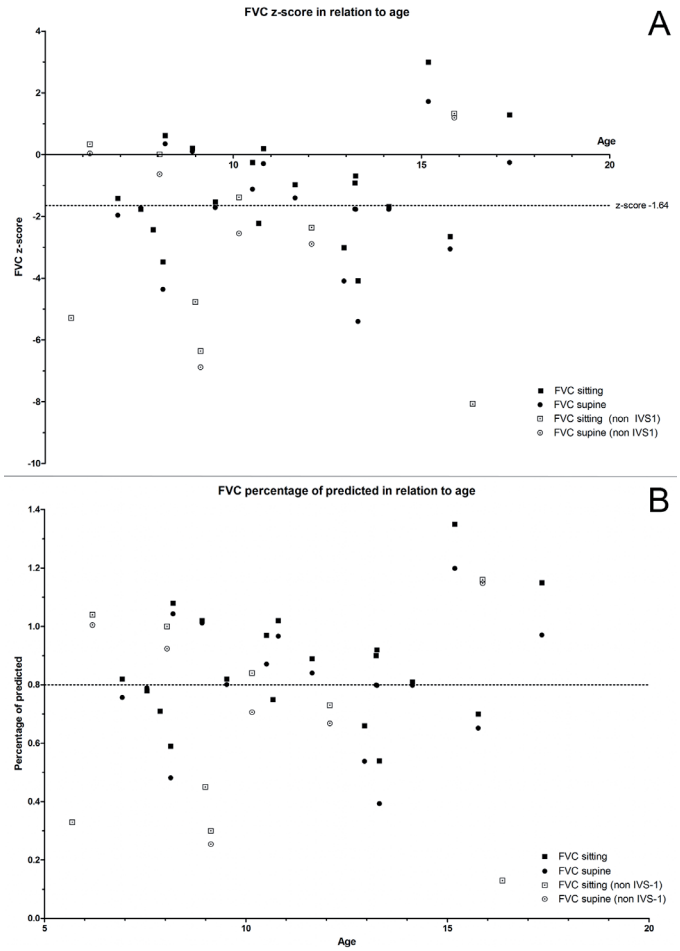


Figure 2 | FVC z-score (a) and percentage of predicted (b) in sitting and supine position.

been noticed at one year of age, and in the other at two. Their ECGs showed high amplitude QRS complexes and repolarization disturbances consistent with their hypertrophic cardiomyopathy. In addition, their ECGs and that of a third patient (patient 3 in Table 1) showed a short PR interval and a delta wave suggestive of Wolff-Parkinson-White syndrome.

Minor abnormalities of the cardiac valves were noted in three patients. The abnormalities included a quadricuspid aortic valve, a minor deformity of the tricuspid valve leading to minimal tricuspid regurgitation, and minimal insufficiency of both atrioventricular valves. These abnormalities were all considered to be coincidental findings and not related to Pompe disease.

Enzymatic and molecular diagnosis

Table 1 shows the patients' genotypes and the severity of their mutations. All mutations have been described previously in the literature (www.pompecenter.nl). Twenty-three patients (74%) carried a potentially mild mutation on one *GAA* allele and a severe mutation on the other. Twenty-one of these patients carried the common c.-32-13T>G splice-site mutation. Since none of the mutations in the other eight patients were considered potentially mild, these patients' genotypes were considered to be more severe. Acid α-glucosidase activity measured in cultured fibroblasts ranged from 0.3 to 17.9 nmol/h/mg protein (n=21, median=7.8; control range 45-160 nmol/h/mg). In patients with the c.-32-13T>G/'null' genotype activity ranged from 4.5 to 17.9 nmol/h/mg (n=14, median=10.4) and from 0.3 to 8.4 nmol/h/mg (n=7, median=2.5) in patients with the non-IVS1 genotype.

Laboratory parameters

At first visit, all patients had elevated CK levels (median 979 U/l, range 358 to 3078 U/l; normal values below 230 U/l)). Transaminase levels were elevated in all 31 patients as well, including those who were symptom-free; AST ranged from 82 to 610 U/l (normal values below 51 U/l), ALT from 71-551 U/l (normal values below 39 U/l). LDH levels were elevated in 21 out of 30 patients, ranging from 449 to 2828 U/l (normal values below 765 U/l).

Comparison of patients with the c.-32-13T>G/'null' genotype (IVS1) and those with other genotypes (non-IVS1)

Comparison of the twenty-one patients with the c.-32-13T>G/'null' genotype and the 10 patients with other mutations showed no significant differences in age at first symptoms and age at diagnosis (Table 3). Their median age at first examination was similar. We noted that patients in the non-IVS1 group tended to have lower muscle strength and a more severely restricted lung function in sitting and supine positions than those in the IVS1 group. This was in line with the fact that more non-IVS1 than IVS1 patients had become wheelchair bound and ventilator dependent at a relatively young age. In addition, the two patients with hypertrophic cardiomyopathy belonged to the non-IVS1 group. Another interesting observation was that 18 out of the 21 patients (86%) with the c.-32-13T>G/'null' genotype were male, against 4 out of 10 in the non-IVS1 group. Other than the possible trend that patients with the c.-32-13T>G/'null' genotype were generally less severely affected compared to patients with other genotypes we weren't able to identify other genotype - phenotype relations in the c.-32-13T>G/'null' genotype group.

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

	All	c.-32-13T>G	other mutations	*p*-value
Patients	31	21	10	-
M/F	22/9	18/3	4/6	
Age first symptom (median)	2.6 (0.5-13)	2 (0.5-13)	4.0 (0.5-10)	0.6
Age diagnosis (median)	4.0 (0-16)	3.0 (0-16)	4.5 (1-15)	0.6
Age at examination (median)	10.1 (0.1-17.1)	10.7 (0.1-17.1)	10 (1.3-16.4)	0.7
Disease duration (median)	5.1 (0.8-12.7)	6.5 (1.3-12.7)	4.1 (0.8-11.5)	0.2
Diagnosed pre-symptom.	10	9	1	-
Still symptom free	5	4	1	-
HHD sumscore (%)	55 (n=24)	58 (n=18)	36 (n=6)	0.02
FVC pred sitting (%) [a]	82	82	58	0.15
FVC pred supine (%) [a]	79	80	55	0.08

Disease duration is calculated as time between the presentation of first symptoms and first examination in our hospital.
[a] For patients who didn't perform lung function testing in supine position the value obtained in sitting position was used. For one patient using 24 h invasive ventilation a value of 0 % was used in the analyses.

Discussion

As little information is available on the clinical presentation of children with non-classic forms of Pompe disease, we evaluated 31 children's clinical and molecular characteristics. Our findings highlight that non-classic Pompe disease can cause a significant burden in childhood and add to the understanding that Pompe disease presents as a broad spectrum of clinical phenotypes.

The presentation of Pompe disease can be variable. Children in our patient population typically presented with weakness of the limb-girdle muscles and/or delayed motor development; lung function was compromised in approximately half of them, and 2 patients whose muscle function deteriorated very rapid also had hypertrophic cardiomyopathy.

Nevertheless, the diagnosis of Pompe disease should also be considered in children whose symptoms are less typical, such as disproportional weakness of the neck flexors, unexplained fatigue, persistent diarrhea, and an elevation of transaminase levels. Independent of whether the children had symptoms or not, all patients participating in the study had elevated CK, ALT and AST values. It should be noted, however, that in rare cases CK, ALT and

AST may be normal, as we very recently encountered in a childhood onset patient (personal communication) and as was earlier described for about 10% of adult patients.[28]

Although respiratory problems did not precede proximal muscle weakness in any of the children, lung function was already significantly restricted in 48% of the cases. In 26% of the affected children, respiratory insufficiency either led to the need for ventilator support or resulted in death during childhood. This finding signifies the importance of early monitoring of lung volume by means of spirometry in children affected by Pompe disease as also advised in adults.[17, 29]

Lung function tests are best performed in both sitting and supine positions, as 9 patients had postural drops suggestive of diaphragm weakness. This is a well-known feature of Pompe disease, and contributes to the onset of nocturnal hypoventilation.[29, 30] Since a recent study in children with neuromuscular disorders found that daytime lung function and nocturnal hypoxemia were poorly correlated, we suggest to regularly perform sleep studies as an additional tool for identifying children with nocturnal hypoventilation.[31]

Twenty-two of the 31 children that we investigated were male. Interestingly, among them were 18 with the c.-32-13T>G/'null' genotype that is most common among adult patients (against only 3 of the 9 female patients). In two studies that focused on disease variation among children and adults with the c.-32-13T>G/'null' genotype, the male-to-female distribution was equal (55% and 58% males, respectively). However, neither study focused on any potential difference in age at onset between male and female patients.[12, 13] The present study indicates the existence of such a gender difference. Our earlier analysis of 225 published case reports on children and adults with Pompe disease also showed a predominance of males (67%) in patients under 18 years old.[11] Previous studies reported that pulmonary function was more affected in males than in females,[17] and that more men than women had bulbar involvement and shoulder-girdle muscle weakness.[17] A study comparing phenotypes in siblings with Pompe disease also confirmed that males were more severely affected than females.[32]

Since Pompe disease is inherited as an autosomal recessive trait, there has been no satisfactory explanation to date why males with the same *GAA* genotype as females would present at an earlier age. This finding seems to suggest that the clinical expression of Pompe disease involves secondary gender-related factors. Gender differences have also been reported for other neuromuscular disorders such as facioscapulohumeral muscular dystrophy and some subtypes of limb-girdle muscular dystrophy.[33-35] One muscle related difference between men and women found so far is that women with limb girdle muscular

dystrophy type 2A and 2B showed less muscle fiber atrophy compared to males.[35] This may also apply in Pompe disease. Though other causes have been suggested, such as differences in genetic and epigenetic factors, the exact mechanism remains elusive.

Our findings are fully consistent with the broad spectrum of clinical phenotypes associated with the c.-32-13T>G/'null' *GAA* genotype.[12, 13] Looking at the genotype-phenotype correlation within our group of children shows a similar age of onset, age at diagnosis, and current age for all different genotypes. Several patients were wheelchair bound and/or ventilator dependent despite of having the c.-32-13T>G/'null' genotype, which is mostly associated with adult onset disease. The c.-32-13T>G, is a leaky splice site mutation that results in the formation of 10-20% of normally processed alpha-glucosidase protein and activity, which explains the later onset non-classic phenotype in patients c.-32-13T>G/'null' genotype. In our group of patients the second mutation (severe, less severe or potentially less severe) did not seem to have an effect on the age of presentation. This is in line with earlier studies of Kroos *et. al.,* Wens *et.al.* and Montalvo *et. al.* also showing a broad variation of phenotypes among patients with the c.-32-13T>G/null genotype. It was hypothesized that epigenetic factors and environmental factors influence the level of disease severity.[12, 13, 32] More research is required.

At a group level, children in the non-IVS1 group seem to be more severely affected than patients with the 32-13T>G/'null' genotype. This is illustrated by two specific patients in the non-IVS1 group who had hypertrophic cardiomyopathy and became fully wheelchair bound; one at the age of 4 years and the other at the age of 6. The first child died from respiratory failure at the age of 10. The other child became completely ventilator dependent when she was 6 years old. These two patients expressed a phenotype that Slonim et al previously called the "atypical infantile form" of Pompe disease.[18]

Overall, the genotypes identified in the non-IVS1 group were more severe than those in the IVS1 group (see Table 1 and www.pompecenter.nl). Some of the genotypes have been described in the literature, such as the genotype c.1634C>T / c.2481+102_2646+31del (our patient 25). The c.1634C>T (Is) in combination with a very severe mutation was previously described in three patients. One of those patients presented at the age of one year, became dependent on respiratory support at the age of 20, and was wheelchair bound at the age of 23.[15] The second patient was diagnosed at 16 years of age, and began to use a walking stick at 19. Pulmonary function worsened at the age of 17-19 years and vital capacity dropped to 26% of predicted and became respirator dependent at the age of 20 years.[36] The third patient presented at the age of 13 years with pronounced limb girdle weakness and died at the age of 18 years.[37] The similarities in clinical course of these previously reported cases

are remarkable, and if the genotype-phenotype correlation holds for our patient, who was only 6 years old at time of examination, he carries a high probability to develop severe respiratory and mobility problems before adulthood. Such information may be relevant when it is time to decide when to start enzyme replacement therapy.

Children and adults share a wide variation of disease presentation and disease progression, and a similar involvement of respiratory and proximal skeletal muscles.[8, 9, 12-16] Although the distribution of muscle weakness shows a limb-girdle pattern in both children and adults, there are also differences. While the neck flexors are by far the most severely affected muscle group in children, they are only mildly affected in adults.[8, 15] A recent MRI study performed in 20 adult patients by Carlier et al also showed relatively mild involvement of the neck flexors in adults.[38]

Another difference is the relative sparing of the quadriceps muscle in adult patients.[28, 38] In the current population of 31 children with Pompe disease, the muscles of the thigh were affected more heterogeneously, and the quadriceps muscles were not spared. Neither did any of our patients have ptosis, despite a recent publication in which ptosis was present in 14.7 to 23% of adult Pompe patients.[28, 39, 40] It should be noted that ptosis has often been found in an early stage of the disease, even as a presenting symptom in adult Pompe patients. While van der Beek et al found that patients had difficulties with speech, chewing or swallowing, which was suggestive of bulbar weakness in 28% of their patients, we found bulbar weakness in only one patient. In contrast, 52% of our children had scoliosis, compared to only 21 to 23% of adult Pompe patients.[11, 28] In several children the scoliosis was so severe that it interfered with their mobility and lung volume; four children needed surgical correction of the spine. A cross-sectional analysis of data from the Pompe Registry, a large multinational observational program, found scoliosis to be present in 57% of patients with childhood disease onset.[41]

Our study had two main limitations. First, since the Pompe Center at Erasmus MC University Medical Center serves as a national and international referral center for Pompe disease, there may be selection bias, due to referral of patients who were more than average severely affected. Nonetheless, ten of the 31 patients had been diagnosed pre-symptomatically, 5 of whom were still symptom-free at time of evaluation. A second limitation is the fact that the study was cross-sectional. As all children manifesting significant symptoms of the disease, started to receive enzyme replacement therapy during follow-up, the approval of this therapy in 2006 interfered with the collection of longitudinal follow-up data.

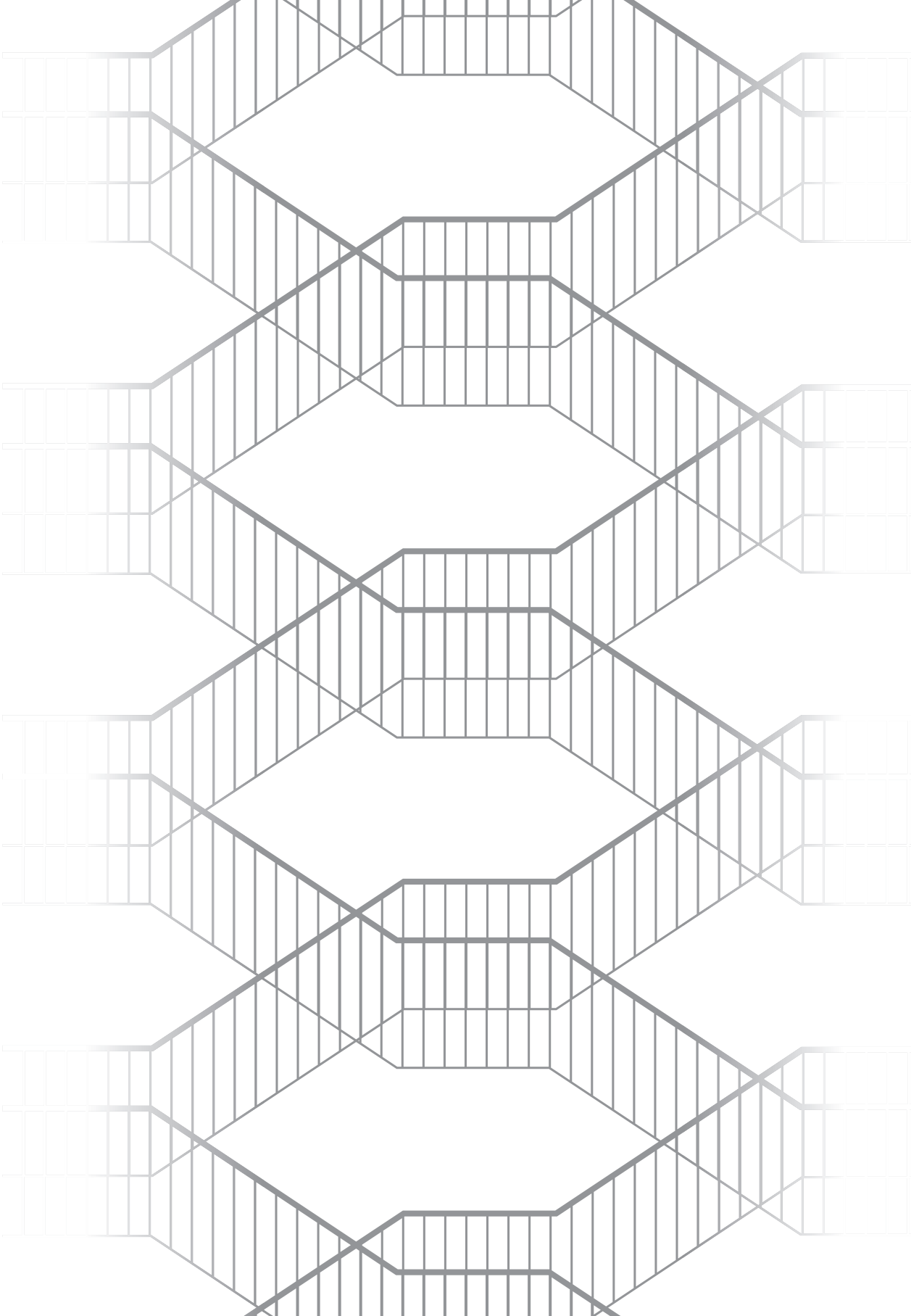
Conclusions

In conclusion, our study shows that the course of childhood Pompe disease varies widely, and patients may manifest serious problems before adulthood. We stress that Pompe disease should be considered in the differential diagnosis of patients with less familiar signs such as disproportional weakness of the neck flexors, unexplained fatigue and persistent diarrhea. Disease presentation, distribution of muscle weakness, and the occurrence of specific symptoms such as bulbar muscle weakness or ptosis all appear to be different from those in adult patients. Regular assessment of lung volume and sleep studies are recommended to identify children at risk for early respiratory insufficiency. Patients with mutations other than the c.-32-13T>G were overall more severely affected, which is consistent with their more severe genotypes. The majority of affected children with *GAA* genotype c.-32-13T>G/'null' appeared to be male.

References

1. Hirschhorn R, Reuser AJJ: Glycogen Storage Disease Type II: acid alpha-glucosidase (acid maltase) deficiency. In: *The Metabolic and Molecular Bases of Inherited Disease*. edn. Edited by Scriver CR, Beaudet AL, Valle D, Sly WS. New York: McGraw-Hill; 2001: 3389-3420.
2. Engel AG, Hirschhorn R, Huie ML: Acid maltase deficiency. In: *Myology*. edn. Edited by Engel AG. New York: McGraw-Hill; 2004: 1559-1586.
3. van der Ploeg AT, Reuser AJ. Pompe's disease. *Lancet* 2008;372(9646):1342-1353.
4. Pompe JC. Over idiopathische hypertrofie van het hart. *Ned Tijdsch Geneesk* 1932;(76):304-311.
5. van den Hout HM, Hop W, van Diggelen OP, Smeitink JA, Smit GP, Poll-The BT, Bakker HD, Loonen MC, de Klerk JB, Reuser AJ *et al*. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics* 2003;112(2):332-340.
6. Kishnani PS, Hwu WL, Mandel H, Nicolino M, Yong F, Corzo D, Infantile-Onset Pompe Disease Natural History Study G. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *J Pediatr* 2006;148(5):671-676.
7. Engel AG, Gomez MR, Seybold ME, Lambert EH. The spectrum and diagnosis of acid maltase deficiency. *Neurology* 1973;23(1):95-106.
8. Laforet P, Nicolino M, Eymard PB, Puech JP, Caillaud C, Poenaru L, Fardeau M. Juvenile and adult-onset acid maltase deficiency in France: genotype-phenotype correlation. *Neurology* 2000;55(8):1122-1128.
9. Hagemans ML, Winkel LP, Van Doorn PA, Hop WJ, Loonen MC, Reuser AJ, Van der Ploeg AT. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. *Brain* 2005;128(Pt 3):671-677.
10. Pellegrini N, Laforet P, Orlikowski D, Pellegrini M, Caillaud C, Eymard B, Raphael JC, Lofaso F. Respiratory insufficiency and limb muscle weakness in adults with Pompe's disease. *Eur Respir J* 2005;26(6):1024-1031.
11. Winkel LP, Hagemans ML, van Doorn PA, Loonen MC, Hop WJ, Reuser AJ, van der Ploeg AT. The natural course of non-classic Pompe's disease; a review of 225 published cases. *J Neurol* 2005;252(8):875-884.
12. Montalvo AL, Bembi B, Donnarumma M, Filocamo M, Parenti G, Rossi M, Merlini L, Buratti E, De Filippi P, Dardis A *et al*. Mutation profile of the GAA gene in 40 Italian patients with late onset glycogen storage disease type II. *Hum Mutat* 2006;27(10):999-1006.
13. Kroos MA, Pomponio RJ, Hagemans ML, Keulemans JL, Phipps M, DeRiso M, Palmer RE, Ausems MG, Van der Beek NA, Van Diggelen OP *et al*. Broad spectrum of Pompe disease in patients with the same c.-32-13T->G haplotype. *Neurology* 2007;68(2):110-115.
14. Muller-Felber W, Horvath R, Gempel K, Podskarbi T, Shin Y, Pongratz D, Walter MC, Baethmann M, Schlotter-Weigel B, Lochmuller H *et al*. Late onset Pompe disease: clinical and neurophysiological spectrum of 38 patients including long-term follow-up in 18 patients. *Neuromuscul Disord* 2007;17(9-10):698-706.
15. Van der Beek NA, Hagemans ML, Reuser AJ, Hop WC, Van der Ploeg AT, Van Doorn PA, Wokke JH. Rate of disease progression during long-term follow-up of patients with late-onset Pompe disease. *Neuromuscul Disord* 2009;19(2):113-117.
16. Wokke JH, Escolar DM, Pestronk A, Jaffe KM, Carter GT, van den Berg LH, Florence JM, Mayhew J, Skrinar A, Corzo D *et al*. Clinical features of late-onset Pompe disease: a prospective cohort study. *Muscle Nerve* 2008;38(4):1236-1245.
17. van der Beek NA, van Capelle CI, van der Velden-van Etten KI, Hop WC, van den Berg B, Reuser AJ, van Doorn PA, van der Ploeg AT, Stam H. Rate of progression and predictive factors for pulmonary outcome in children and adults with Pompe disease. *Mol Genet Metab* 2011;104(1-2):129-136.
18. Slonim AE, Bulone L, Ritz S, Goldberg T, Chen A, Martiniuk F. Identification of two subtypes of infantile acid maltase deficiency. *J Pediatr* 2000;137(2):283-285.
19. van der Ploeg RJ, Oosterhuis HJ, Reuvekamp J. Measuring muscle strength. *J Neurol* 1984;231(4):200-203.
20. Beenakker EA, van der Hoeven JH, Fock JM, Maurits NM. Reference values of maximum isometric muscle force obtained in 270 children aged 4-16 years by hand-held dynamometry. *Neuromuscul Disord* 2001;11(5):441-446.
21. Medical Research Council: Aids to examination of the peripheral nervous system. Memorandum no. 45. London: Her Majesty's Stationary Office; 1978.

22. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P *et al*. Standardisation of spirometry. *Eur Respir J* 2005;26(2):319-338.
23. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Hankinson JL, Ip MS, Zheng J *et al*. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *The European respiratory journal* 2012;40(6):1324-1343.
24. van Diggelen OP, Oemardien LF, van der Beek NA, Kroos MA, Wind HK, Voznyi YV, Burke D, Jackson M, Winchester BG, Reuser AJ. Enzyme analysis for Pompe disease in leukocytes; superior results with natural substrate compared with artificial substrates. *Journal of inherited metabolic disease* 2009;32(3):416-423.
25. van Diggelen OP, Zhao H, Kleijer WJ, Janse HC, Poorthuis BJ, van Pelt J, Kamerling JP, Galjaard H. A fluorimetric enzyme assay for the diagnosis of Morquio disease type A (MPS IV A). *Clin Chim Acta* 1990;187(2):131-139.
26. Hermans MM, van Leenen D, Kroos MA, Reuser AJ. Mutation detection in glycogen storage-disease type II by RT-PCR and automated sequencing. *Biochem Biophys Res Commun* 1997;241(2):414-418.
27. Kroos M, Pomponio RJ, van Vliet L, Palmer RE, Phipps M, Van der Helm R, Halley D, Reuser A, Consortium GAAD. Update of the Pompe disease mutation database with 107 sequence variants and a format for severity rating. *Hum Mutat* 2008;29(6):E13-26.
28. van der Beek NA, de Vries JM, Hagemans ML, Hop WC, Kroos MA, Wokke JH, de Visser M, van Engelen BG, Kuks JB, van der Kooi AJ *et al*. Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study. *Orphanet J Rare Dis* 2012;7:88.
29. Mellies U, Ragette R, Schwake C, Baethmann M, Voit T, Teschler H. Sleep-disordered breathing and respiratory failure in acid maltase deficiency. *Neurology* 2001;57(7):1290-1295.
30. Mellies U, Lofaso F. Pompe disease: a neuromuscular disease with respiratory muscle involvement. *Respir Med* 2009;103(4):477-484.
31. Bersanini C, Khirani S, Ramirez A, Lofaso F, Aubertin G, Beydon N, Mayer M, Maincent K, Boule M, Fauroux B. Nocturnal hypoxaemia and hypercapnia in children with neuromuscular disorders. *Eur Respir J* 2012;39(5):1206-1212.
32. Wens SC, van Gelder CM, Kruijshaar ME, de Vries JM, van der Beek NA, Reuser AJ, van Doorn PA, van der Ploeg AT, Brusse E. Phenotypical variation within 22 families with Pompe disease. *Orphanet J Rare Dis* 2013;8:182.
33. Zatz M, Marie SK, Cerqueira A, Vainzof M, Pavanello RC, Passos-Bueno MR. The facioscapulohumeral muscular dystrophy (FSHD1) gene affects males more severely and more frequently than females. *Am J Med Genet* 1998;77(2):155-161.
34. Tonini MM, Passos-Bueno MR, Cerqueira A, Matioli SR, Pavanello R, Zatz M. Asymptomatic carriers and gender differences in facioscapulohumeral muscular dystrophy (FSHD). *Neuromuscul Disord* 2004;14(1):33-38.
35. Fanin M, Nascimbeni AC, Angelini C. Gender difference in limb-girdle muscular dystrophy: a muscle fiber morphometric study in 101 patients. *Clin Neuropathol* 2014;33(3):179-185.
36. Korpela MP, Paetau A, Lofberg MI, Timonen MH, Lamminen AE, Kiuru-Enari SM. A novel mutation of the GAA gene in a Finnish late-onset Pompe disease patient: clinical phenotype and follow-up with enzyme replacement therapy. *Muscle Nerve* 2009;40(1):143-148.
37. Hermans MM, De Graaff E, Kroos MA, Mohkamsing S, Eussen BJ, Joosse M, Willemsen R, Kleijer WJ, Oostra BA, Reuser AJ. The effect of a single base pair deletion (delta T525) and a C1634T missense mutation (pro545leu) on the expression of lysosomal alpha-glucosidase in patients with glycogen storage disease type II. *Hum Mol Genet* 1994;3(12):2213-2218.
38. Carlier RY, Laforet P, Wary C, Mompoint D, Laloui K, Pellegrini N, Annane D, Carlier PG, Orlikowski D. Whole-body muscle MRI in 20 patients suffering from late onset Pompe disease: Involvement patterns. *Neuromuscul Disord* 2011;21(11):791-799.
39. Groen WB, Leen WG, Vos AM, Cruysberg JR, van Doorn PA, van Engelen BG. Ptosis as a feature of late-onset glycogenosis type II. *Neurology* 2006;67(12):2261-2262.
40. Ravaglia S, Bini P, Garaghani KS, Danesino C. Ptosis in Pompe disease: common genetic background in infantile and adult series. *J Neuroophthalmol* 2010;30(4):389-390.
41. Roberts M, Kishnani PS, van der Ploeg AT, Muller-Felber W, Merlini L, Prasad S, Case LE. The prevalence and impact of scoliosis in Pompe disease: lessons learned from the Pompe Registry. *Mol Genet Metab* 2011;104(4):574-582.



Long-term follow-up of 17 patients with childhood Pompe disease treated with enzyme replacement therapy

J.C. van der Meijden, M.E. Kruijshaar, L. Harlaar
D. Rizopoulos, N.A.M.E. van der Beek, A.T. van der Ploeg

3

Submitted

Abstract

Objectives

Pompe disease is a progressive metabolic myopathy for which enzyme replacement therapy (ERT) was approved in 2006. While various publications have examined the effects of ERT in classic-infantile patients and adults, little has been published on ERT in children with non-classic presentations.

Study design

This prospective study was conducted from June 1999 to May 2015. Seventeen patients from various countries participated. Outcome measures comprised muscle function (6-Minute Walk Test, Quick Motor-Function Test (QMFT)), muscle strength (hand-held dynamometry; manual muscle testing), and lung function (FVC sitting and supine). For each outcome measure, we used linear mixed-effects models to calculate the difference at group level between the start of therapy and 7 years of ERT. Patients' individual responses over time were also evaluated.

Results

Eleven males and six females started ERT at ages between 1.1 and 16.4 years (median 11.9 years); 82% of them carried the common c.-32-13C>T *GAA* gene variant on one allele. At group level, distance walked increased by 7.4 percentage points ($p<0.001$) and QMFT scores increased by 9.2 percentage points ($p=0.006$). Muscle strength scores remained stable. Results on lung function were more variable. Patients' individual data show that the proportion of patients who stabilized or improved during treatment ranged between 56-69% for lung function outcomes and between 71-93% for muscle strength and muscle function outcomes.

Conclusions

We report a positive effect of ERT in patients with childhood Pompe disease. Muscle strength and function seemed to respond better than lung function.

Introduction

Pompe disease (OMIM # 232300) is a progressive metabolic disorder that was first described by the Dutch pathologist J.C. Pompe in 1932.[1] It is caused by deficiency of the lysosomal enzyme acid α-glucosidase (*GAA*). This deficiency results in intracellular glycogen accumulation, mainly in muscle cells, and gives rise to a broad clinical spectrum dominated by skeletal muscle weakness.[2,3]

At the most severe end of the spectrum, classic-infantile patients present with hypertrophic cardiomyopathy and general muscle weakness. Without Enzyme Replacement Therapy (ERT), these infants usually die within the first year of life.[4,5] More slowly progressive forms of Pompe disease can manifest in children and adults, with onset ranging from early infancy until (late) adulthood. These presentations are characterized by limb-girdle and respiratory muscle weakness, resulting in ventilator and/or wheelchair dependency. The heart is rarely involved.[6-11] While there are similarities between children and adults with these non-classic presentations, there are also differences, especially in terms of disease severity. Our recent cross-sectional study of 31 untreated children with non-classic presentations showed that 25% needed a wheelchair during childhood, 48% had decreased pulmonary function, 25% needed respiratory support, and two deceased before reaching adulthood.[12]

In 2006, ERT with alglucosidase alfa was approved for patients with Pompe disease. ERT has been shown to reverse the hypertrophic cardiomyopathy and increase survival in classic-infantile patients.[13,14] In adult patients, positive effects were also demonstrated on endurance, muscle strength, pulmonary function and survival.[15-24] But as not all of these patients respond equally well, it has been speculated that ERT should be started early to provide the best result.[15,17,18,21]

To further our understanding of the long-term effect of ERT, we present the long-term follow-up during ERT (median of 6.8 years) of 17 children with Pompe disease of various severity. The longest follow-up after start of ERT was 15 years.

Methods

Patients and study design

This prospective study included 12 patients from the Netherlands, two from Belgium, one from Germany, and one each from the UK and the USA, all with a confirmed diagnosis of Pompe disease. It was conducted at the Center for Lysosomal and Metabolic Diseases at Erasmus MC University Medical Center in Rotterdam, the Netherlands. It included children

with non-classic presentations of Pompe disease in whom ERT had been initiated before the age of 18 years. Patients with classic-infantile Pompe disease were excluded.

Patients were treated with 20mg/kg alglucosidase alfa every other week. Initially, two patients received recombinant human alpha-glucosidase from transgenic rabbit milk, starting at 10mg/kg weekly and ramping up to 20mg/kg weekly.[25] After approximately three years, they were switched to biweekly infusions of 30 and 40 mg/kg recombinant human alpha-glucosidase derived from Chinese hamster ovarian cells.[26] Only patients with symptoms of skeletal muscle weakness and/or reduced pulmonary function could start ERT.

As part of a standardized protocol, outcome assessments were performed every 3-6 months by trained physical therapists and clinicians. Data were collected prospectively from June 1999 to May 2015. The medical ethical committee approved the protocol. All patients and/or their parents provided informed consent. The study has been carried out in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Motor outcomes

Muscle function was assessed using the 6-Minute Walk Test (6MWT) and Quick Motor-Function Test (QMFT). The 6MWT was performed according to American Thoracic Society (ATS) guidelines.[27] Throughout follow-up, patients 3, 5, 11, 12 and 15 performed a running variant rather than the standard 6MWT.[28] The percentage of predicted meters walked (6MWT-PP) was calculated on the basis of gender-matched and age-matched healthy peers.[29] The QMFT consists of 16 motor tasks that are specifically difficult to perform for Pompe patients and was presented as the percentage of the maximum achievable score.[30]

Muscle strength was assessed by Hand-Held Dynamometry (HHD; CITEC dynamometer, Centre for Innovative Technics, Groningen, the Netherlands) and Manual Muscle Testing (MMT; Medical Research Council grading scale), as described previously.[12,31,32] The following muscle groups were assessed with HHD: neck flexors, shoulder abductors, elbow flexors, elbow extensors, wrist extensors, hip flexors, hip abductors, knee extensors, knee flexors, and foot dorsal flexors. MMT was performed for the same groups, with the addition of neck extensors, hip extensors, hip adductors, and foot plantar flexors.

Lung function

Forced Vital Capacity (FVC) was measured in sitting and supine positions according to European Respiratory Society and ATS guidelines, and was presented as a percentage of

the predicted FVC, corrected for height, gender and ethnicity.[33,34] A percentage below 80% of the predicted value was considered abnormally low.

Statistical analyses

To describe disease progression under treatment, we assessed the development of each of the six outcome measures over time at group level, and also evaluated them for each patient individually. At group level, we report the change in the estimated mean outcome measures between baseline and 7 years of treatment (median follow-up was 6.8 years). To account for the correlations between the repeated measurements of individual patients, we used the framework of linear mixed-effects models. Time was expressed as years after start of ERT. To account for potential non-linear profiles we used natural cubic splines in the fixed-effects part and random-effects part of the model, with a maximum of 3 degrees of freedom. For the random-effects part of the model, we used an unstructured covariance matrix. If they improved the goodness of fit of a model significantly (likelihood-ratio test; $p<0.05$), gender and age at start of ERT were included.

As only one patient had been treated for more than 10 years (pt.9, treated 15.1 years), models were based on all available data until 10 years of ERT. Residual plots were inspected to check the models' assumptions. The nlme package of *R* (version 3.2.1) was used.[35,36] Multiple testing correction was applied according to the Holm method.[37]

For individual patients, plots of the outcome measures over time were assessed by four independent researchers and categorized as improvement, improvement followed by stabilization, stabilization, improvement followed by decline, or decline. Supplemental figure 1 gives examples of plots of individual patients following these different patterns.

Results

Study population

During the study period, 19 children started ERT before their eighteenth birthday. Two were excluded on the basis that they were too young (2 and 3 years old at the end of the study) to perform any of the assessments used as outcome measures in this study. Table 1 provides the characteristics and main clinical findings at start of ERT of the 17 patients included. Eleven patients were male; age at start of ERT ranged from 1.1 to 16.4 years (median 11.9 years); age at first symptoms ranged from 0.5 to 13 years (median 2.5 years); and age at diagnosis ranged from 0.0 to 14.0 years (median 3 years). Fourteen patients carried the common c.-32-13T>G mutation on one allele. Patients had been treated for a median of 6.8 years (range 1.8 to15.1 years of ERT). No patients died during follow-up.

Table 1 | Characteristics of 17 children who were treated with ERT.

Pt.	Age at start ERT (y)	Sex	Clinical status at start ERT					
			Wheel-chair use	Respirator support	Limb Girdle↓	Neck Flexor↓	Lung Function↓	Fatigue↑
1#	1.1	F			X			
2#	2.9	M			X			
3	6.0	M			X	X		
4	8.5	F			X	X		
5	8.9	F			X	X		
6	9.8	M			X	X		
7	10.5	M			X	X		X
8	11.0	M			X	X		X
9@	11.9	M	X (9y)		X	X	X	
10	12.7	F	X (6y)	Cannula (6y)	X	X	X	
11	12.7	F			X		X	X
12	13.0	M			X		X	X
13@	13.1	M			X			
14	14.3	M			X	X		X
15	15.2	M		Nightly BiPAP (12y)	X	X	X	X
16	16.0	M			X	X	X	
17	16.4	F	X (16y)	BiPAP (12y)	X	X	X	X
Overall ~	11.9	11 Male (65%)	N=3 (18%)	N=3 (18%)	N=17 (100%)	N=12 (71%)	N=7 (41%)	N=7 (41%)

Patients are listed by the age at which they started enzyme replacement therapy (ERT).#: siblings; @: siblings; ~for the group overall median ages or numbers of patients (N=) and proportions (%) are given; limb girdle↓ = limb-girdle weakness, neck flexor↓ = neck flexor weakness, lung function↓ = decreased lung function (FVC < 80% sitting

Eight became adults (18+) during the study period; at his last assessment, the oldest and longest-treated patient (pt.9) was 27 years old. ERT was generally well tolerated by patients.

At start of therapy, symptoms ranged from mild motor delays to wheelchair and ventilator dependency. One patient (pt.10) also had hypertrophic cardiomyopathy (left ventricular mass index, LVMI=145 grams/m2 body surface), which normalized within 12 months of treatment (LVMI=69 grams/m2). She was fed via percutaneous gastrostomy and was fully wheelchair and ventilator dependent at start of ERT at the age of 12 years. One further patient was wheelchair and ventilator dependent (pt.17) at start of ERT; one was wheelchair dependent only (pt.9); and one needed respiratory support only (pt.15).

			Mutations	
Main symptoms	Age at Diagnosis (y)	Treatment duration (y)	Allele 1	Allele 2
Delayed motor development	0	6.0	c.-32-13T>G (pm)	c.2135T>C (ls)
Falling, problems walking on stairs	2.0	9.8	c.-32-13T>G (pm)	c.2135T>C (ls)
Problems walking on stairs and running	3.5	9.4	c.1634C>T (ls)	c.2481+102_2646+31del (vs)
Diarrhea, neck flexor weakness	7.8	4.5	c.-32-13T>G (pm)	c.2331+2T>A (vs)
Problems walking on stairs and sit-up	1.1	7.2	c.-32-13T>G (pm)	c.923A>C (pls)
Problems running	2.3	4.0	c.-32-13T>G (pm)	c.525delT (vs)
Problems running	9.4	2.8	c.-32-13T>G (pm)	c.525delT (vs)
Difficulties doing sports	10.8	5.9	c.-32-13T>G (pm)	c.525delT (vs)
Limb-girdle muscle weakness	2.5	15.1[$]	c.-32-13T>G (pm)	c.525delT (vs)
Tetraplegic, PEG for feeding, severely impaired lung function	1.9	6.0	c.875A>G (pm)	unknown/r.0?
Problems walking stairs and sit-up	11.6	9.3	c.-32-13T>G (pm)	c.525delT (vs)
Problems walking stairs, running and with sit-up	3.0	8.9	c.-32-13T>G (pm)	c.2331+2T>A (vs)
Problems walking stairs and weakness in legs	1.0	9.0	c.-32-13T>G (pm)	c.525delT (vs)
Problems running, walking stairs and with sit-up	14.0	7.9	c.-32-13T>G (pm)	c.1933G>A (pls)
Poor lung function, problems with sit-up; scapular winging	2.0	6.8	c.-32-13T>G (pm)	c.525delT (vs)
Problems with sit-up and running	4	1.8	c.-32-13T>G (pm)	c.1441T>C (pls)
Poor lung function; motor problems and scapular winging	11	6.3	c.877G>A+c.271G>A (pls)	c.-32-3C>A (ls)
	3.0	6.8	14 IVS-1 (82%)	

and/or supine, or the use of respiratory support if lung function testing could not be performed); y: years; $: data >10 years not included for modeling of the group mean; (vs) very severe mutation; (pls) potentially less severe; (ls) less severe; (pm) potentially mild; r.spl = effect on splicing; (for more information, see www.pompecenter.nl).

Motor outcomes

Muscle function

Fourteen patients were able to perform the 6MWT at regular intervals. The three others were wheelchair bound. No patients lost the ability to walk during follow-up. One wheelchair-bound patient (pt.9) regained the ability to walk 1.5 years after starting ERT at the age of 13. He was not tested with the 6MWT until he had received 11 years of ERT.[26] Between 11 and 15 years of ERT his walking distance was stable around 600 meters. At start of ERT, the median percentage of the predicted distance walked (6MWT-PP) was 79% (range 32-91%). To describe changes over time at group level, a statistical model was generated. Over 7

years of ERT, the mean 6MWT-PP increased significantly by 7.4%-points (pp) (Figure 1A; 95% Confidence Interval (CI) 2.4pp–12.3pp; p<0.001).

Fifteen patients performed QMFT measurements. At start of ERT, the median QMFT score was 92% (range 44-100%). Mean QMFT scores improved in the first two years of treatment, and then stabilized (Figure 1B). After 7 years of treatment, scores had increased significantly by 9.2pp (CI 1.8pp–16.6pp; p=0.006).

Muscle strength

Fourteen patients performed HHD measurements. At start of treatment, the median HHD score was 57% (range 15 - 70%). Mean HHD scores tended to improve over time (Figure 1C), but were not significantly better at 7 years than at start of ERT (+17.8pp; CI -3.4pp – 39.0pp; p=0.14). The large variations we observed in HHD scores within individual patients may explain why this difference was not statistically significant.

MRC measurements were available for all seventeen patients. At start of ERT, median MRC values were 91.7% (range 10 - 99%). Most patients had close to maximal MRC scores, which they were able to maintain during follow-up, thereby introducing a ceiling-effect. At group level, mean MRC scores were stable over seven years of treatment (Figure 1D: difference of -1.3pp; CI -0.7pp – 3.28pp; p=0.34).

Lung Function

Lung function could be tested reliably in sitting position in sixteen patients, and in supine position in 14 patients. At start of ERT, median FVC scores in sitting position were 87% (range 16-104%) and 85% in supine position (range 39-109%). In sitting position, five of sixteen patients had a reduced FVC (<80%), compared to seven at the last follow-up measurement. Two of these patients could not be tested supine. Five of the remaining fourteen patients had a reduced FVC in supine position at the start and seven at the last follow-up. At start of ERT, three patients needed respiratory support: two used non-invasive ventilation, and one required invasive ventilation and was ventilated for 24hours/day throughout follow-up. No patients started respiratory support during follow-up.

At group level, lung function in sitting position declined significantly over time (Figure 2A; -5.2pp at 7 years of ERT; CI 0.05pp – 10.4pp; p=0.047). In supine position a similar trend was not significant (Figure 2B; -4.7pp; CI -4.5 – 13.9; p=0.34). In sitting position, 331 measurements were available in sixteen patients; in supine position, 253 assessments were available for fourteen patients, reducing statistical power and possibly explaining the difference in statistical significance.

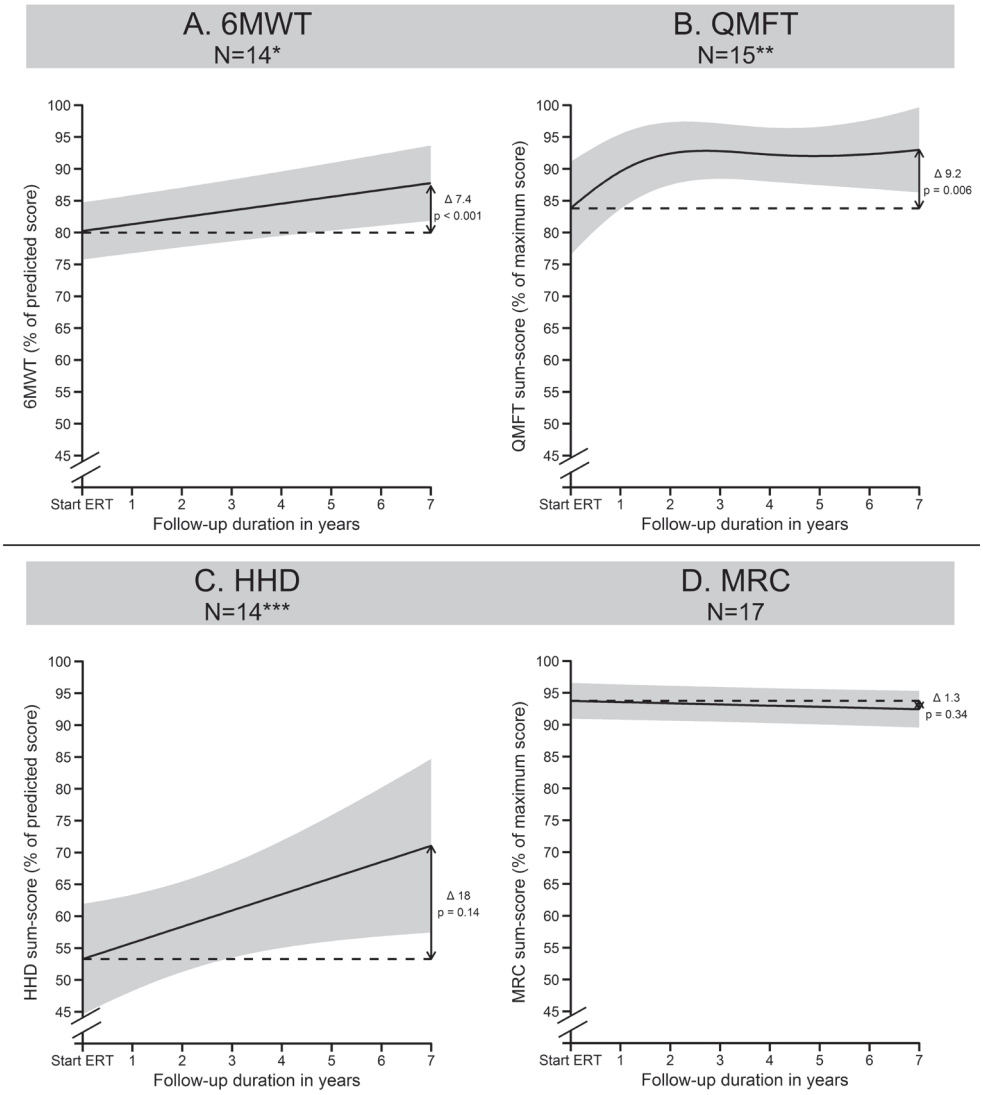


Figure 1 | Predicted group means for motor outcomes over time.

Group mean (black line) of the outcome measures and 95% prediction interval (gray area) obtained using linear mixed-effects models. The difference (Δ) between baseline and 7 years of ERT, and the corresponding p-value, are shown on the right-hand side of the figures. Number of measurements available for analysis of the 6MWT: 199, QMFT: 296, HHD: 221, and MRC: 232. N = number of patients participating in analysis. *: patients 9, 10 and 17 were unable to perform the 6MWT. **: patient 10 was unable to perform the QMFT, and patient 17 did not perform the QMFT.***: patients 2 and 10 could not be tested reliably, and patient 16 did not perform HHD measurements.

Further examination of FVC in sitting position revealed a difference between male and female patients (Figure 2C and D). In males it decreased over time (-8.1pp at seven years ERT; CI 0.9pp–15.3pp; p=0.018), while females remained stable (+1.0pp at seven years of ERT; CI -7.03 – 9.12; p=0.80). This difference was not detected in supine position, possibly due to the lower statistical power.

Individual responses to treatment

In addition to the analyses at group level, we also assessed individual patients' progression on the outcome measures during treatment (Figure 3). In general, patients' motor outcomes responded better than their lung function. Motor outcomes improved or stabilized in 71-93% of patients, whereas lung function improved or stabilized in 56-69%. As these results reflect human judgment, and as follow-up time varied between patients, they should be interpreted with caution. The clinical relevance of an increase or decrease was not included in this assessment, a change from 100-85% FVC, which is still in the normal range, and from 70-50% FVC were both categorized as decline.

In sitting position, 5 patients had an FVC below 80% at start of treatment. Three of these patients improved or stabilized during treatment. For example, patient 15, who required nightly BiPAP, had an initial FVC score in sitting position of 57% which improved to 71% after almost seven years of treatment. On the other hand, five of the eleven patients whose FVC in sitting position was above 80%, deteriorated. For example, patient 3, who started at a relatively young age, improved in the first two years of therapy, but then declined from 115% to 88%. Similarly, patients with good initial motor outcomes were occasionally seen to decline and some with poor initial outcomes improved. In the most severely affected patient (pt.10), who can be classified as an atypical infantile patient [6], ERT only seemed to have an effect on the heart.

Discussion

This study presents the follow-up of 17 patients with non-classic presentations of Pompe disease who had started ERT during childhood. After seven years of treatment, there were group-level improvements in the QMFT and in the distance walked (6MWT), while the MRC, HHD and FVC supine remained stable. For a progressive disorder in which all these outcomes are expected to decline, these results demonstrate that ERT has a positive effect in children.

The improvement in the QMFT is extremely relevant to patients, since this test measures the ability to perform everyday movements that are particularly difficult for Pompe patients, such as squatting, raising the hands above the head, or doing a sit-up. For two reasons, we

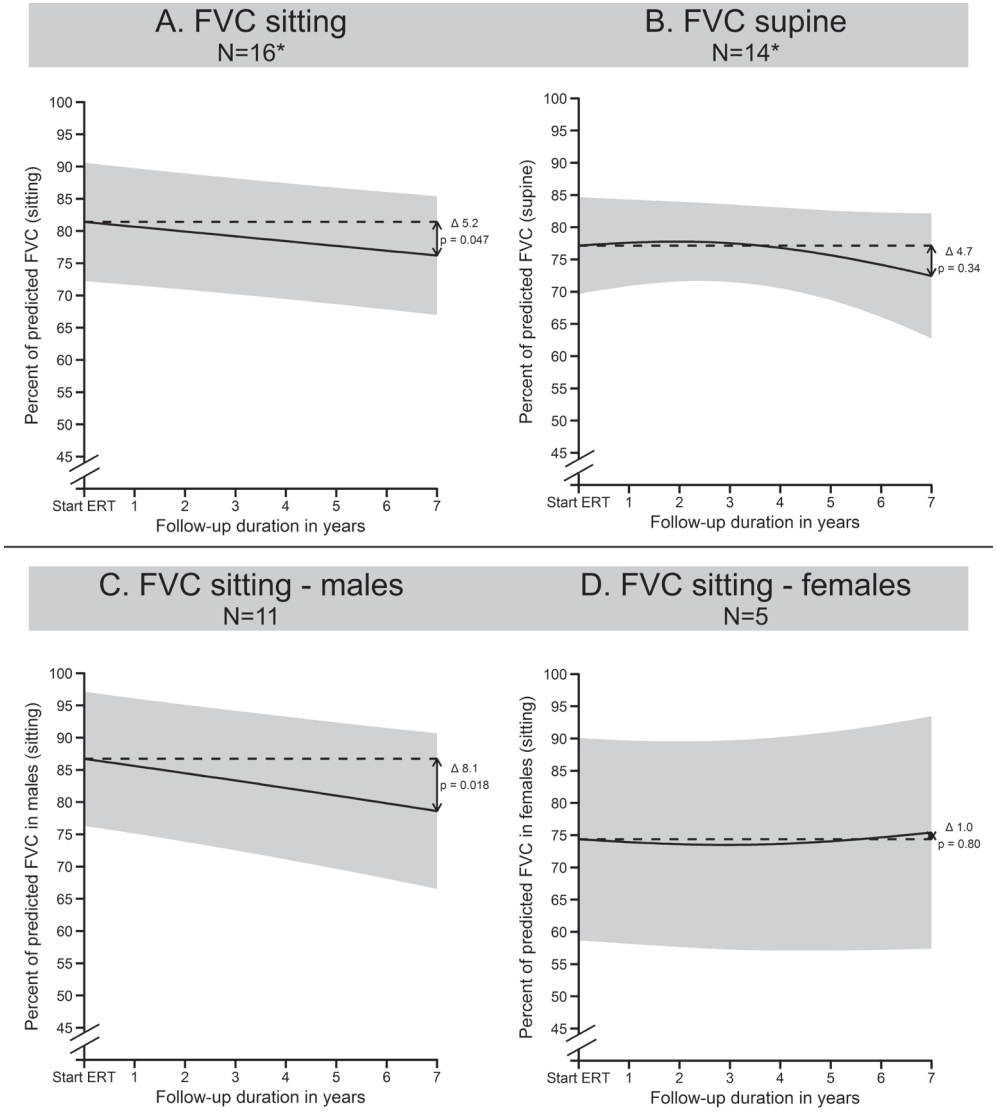


Figure 2 | Predicted mean FVC scores over time.

Group mean (dark line) of the outcome measures and 95% prediction interval (gray area) obtained using linear mixed-effects models. The difference (Δ) between baseline (dotted line) and 7 years of ERT, and the corresponding p-value, are shown on the right-hand side of the figures. The trend of FVC scores in sitting position differed for males and females. They have therefore been plotted separately in panels C and D. Number of measurements available for analysis of FVC in sitting position: 331 and of FVC in supine position: 253. N = number of patients participating in analysis. *: patient 2 and 17 were unable to perform the test supine, and patient 10 was unable to perform the test either sitting or supine.

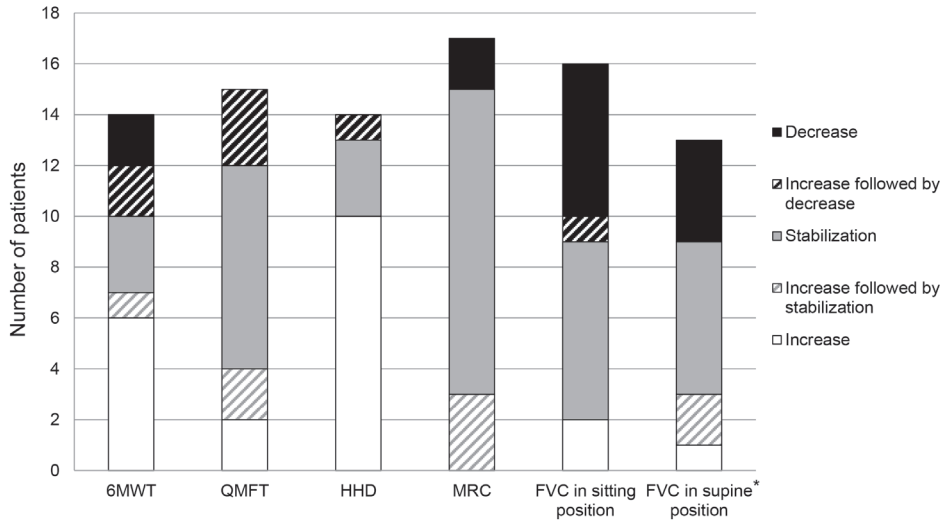


Figure 3 | Individual patients' response on the different outcome measures.

* The individual progressions of FVC scores in supine position were defined for only 13 of 14 patients. Due to her young age, only 2 measurements were available for patient 1.

believe that our results are robust: we had included a relatively large group of children who were followed over a long period, and the observed effects were sustained despite multiple testing correction. While patients' individual response patterns support these results, they also indicate that not all patients benefit equally from treatment.

Generally, patients' motor function and muscle strength responded better compared to their pulmonary function. The poorer response with regard to lung function might be due to the involvement of the diaphragm.[38-42] This involvement is also illustrated by the fact that fewer patients were able to perform lung-function assessments in supine position. Our statistical analyses show that lung function declined significantly in sitting position, and tended to decline in supine position. While these declines were similar in extent (4-5pp over seven years). In untreated adult patients and children, FVC has been reported to decline between 1pp and 5.5pp per year.[10,15,17,28,43,44] This is greater than the decline we observed over 7 years, indicating that progression slows down during ERT.

Our results also suggest that males responded more poorly with regard to lung function than females. However, this difference needs to be interpreted with caution, since there were fewer females than males, and since this difference could be demonstrated in sitting position only. Nevertheless, a similar trend in gender difference was reported in

the multicenter randomized placebo-controlled study in 90 adult patients with Pompe disease.[15] It should be investigated whether such gender differences truly exist.

The individual response to treatment varied considerably between patients, and it would be very relevant to identify its causes. In general, treatment success is believed to be correlated with an early start of treatment, i.e., when patients are still mildly affected. We found that this paradigm does not hold for all patients: some patients with clearly reduced pulmonary and/or motor function improved or stabilized, while some whose initial clinical status was good deteriorated on ERT.

Other factors that could be involved in the response to ERT are the type of mutation in the GAA gene, genetic background factors such as the ACE polymorphisms,[45-47] or antibodies against ERT with alglucosidase alfa. For five of our patients we have three years' data on antibody titers; in all five, these were low.[28] It was also shown in adults that the vast majority of patients had low antibody titers, while a counteracting effect was demonstrated only in incidental cases.[48] All in all, more research is needed to fully understand and predict which patients respond well to treatment and which do not.

Noteworthy, 14 of the 17 children (82%) in our study had the same common genotype, the c.-32-13T>G *GAA* gene variant in combination with a null allele, as found in over 90% of the Caucasian adult population. This highlights that the group of patients with the c.-32-13T>G/'null' *GAA* genotype represent a broad clinical spectrum of which the cause needs further elucidation.

Few studies have been published on the effects of ERT in children. Studies on late-onset patients occasionally include children: a review from 2013 identified 27 children amongst 368 patients in 21 papers.[49] However, most of these studies do not provide separate information on children. Supplemental table 2 summarizes the results of eight publications that report on the outcomes of children treated with ERT.[20,25,26,28,50-53] The number of children included per study ranged from one to eight; follow-up ranged from 1.3 to 8 years (in most studies, follow-up was around 3-4 years). These studies suggest that patients' motor function and muscle strength tend to improve, and their lung function is stable or possibly improves. The present study, which describes long-term findings, is partly in line with this. Upon analysis of the individual follow-up of five of our patients who had previously been described after three years of treatment, we found that, with longer follow-up (up to seven more years in the current study), two had started to decline, while the others had continued to improve or to stabilize.[28] These observations stress the continuing importance of regular long-term patient follow-up.

Conclusion

Pompe disease is a progressively deteriorating disease. We observed that ERT had a clearly positive effect in our cohort of children, most of whose muscle strength and function remained stable or improved during treatment. At group level, response to treatment was better for motor outcomes than for lung function. There were large individual differences between patients in the response to treatment. More research is needed to identify the factors responsible for this.

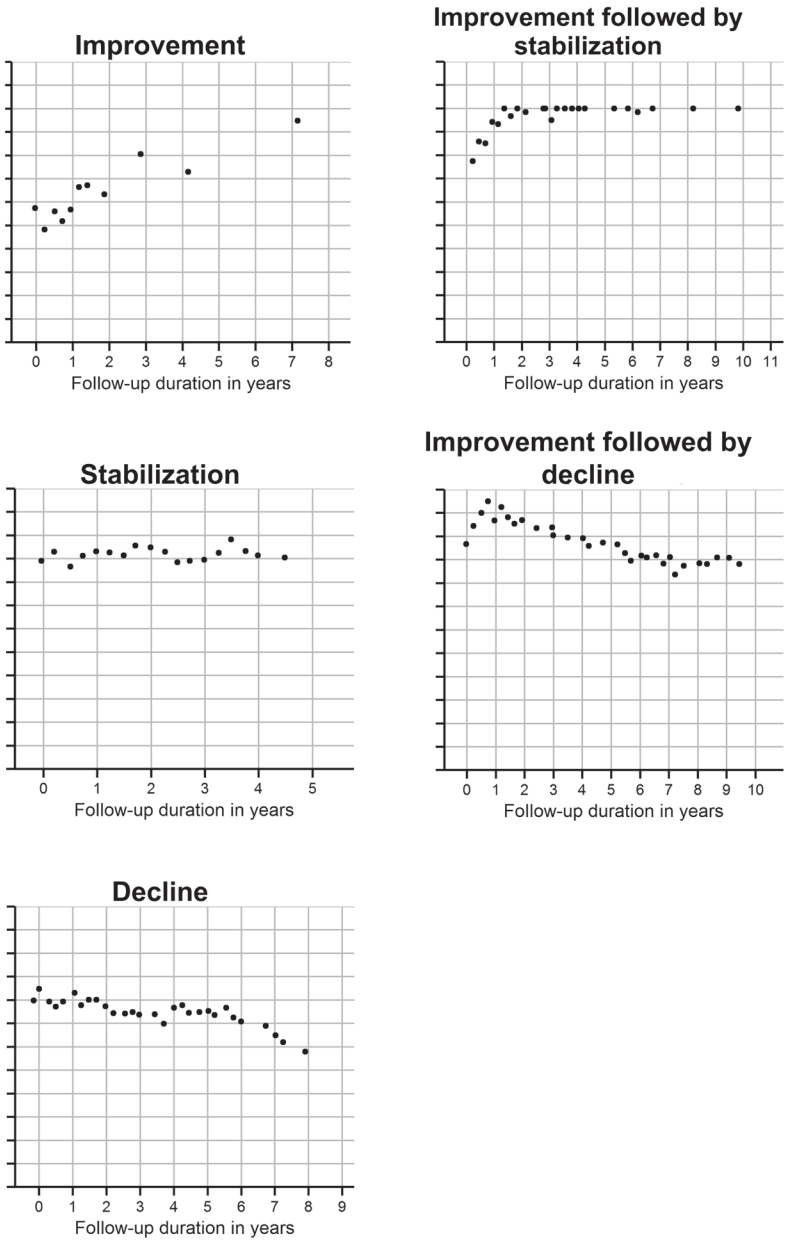
References

1. Pompe JC. Over idiopathische hypertrofie van het hart. *Ned Tijdschr Geneeskd* 1932;76:304-311.
2. Hirschhorn R, Reusser AJ: Glycogen storage disease type II: acid alphaglucosidase (acid maltase) deficiency. In: *The metabolic and molecular bases of inherited disease.* 8th edn. Edited by Scriver CR, Beaudet AL, Sly WS, Valle D. New York: McGraw-Hill; 2001: 3389–3420.
3. van der Ploeg AT, Reuser AJ. Pompe's disease. *Lancet* 2008;372(9646):1342-1353.
4. van den Hout HM, Hop W, van Diggelen OP, Smeitink JA, Smit GP, Poll-The BT, Bakker HD, Loonen MC, de Klerk JB, Reuser AJ *et al*. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics* 2003;112(2):332-340.
5. Kishnani PS, Hwu WL, Mandel H, Nicolino M, Yong F, Corzo D, Infantile-Onset Pompe Disease Natural History Study G. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *J Pediatr* 2006;148(5):671-676.
6. Slonim AE, Bulone L, Ritz S, Goldberg T, Chen A, Martiniuk F. Identification of two subtypes of infantile acid maltase deficiency. *J Pediatr* 2000;137(2):283-285.
7. Gungor D, Reuser AJ. How to describe the clinical spectrum in Pompe disease? *Am J Med Genet A* 2013;161A(2):399-400.
8. Engel AG, Gomez MR, Seybold ME, Lambert EH. The spectrum and diagnosis of acid maltase deficiency. *Neurology* 1973;23(1):95-106.
9. Laforet P, Nicolino M, Eymard PB, Puech JP, Caillaud C, Poenaru L, Fardeau M. Juvenile and adult-onset acid maltase deficiency in France: genotype-phenotype correlation. *Neurology* 2000;55(8):1122-1128.
10. van der Beek NA, van Capelle CI, van der Velden-van Etten KI, Hop WC, van den Berg B, Reuser AJ, van Doorn PA, van der Ploeg AT, Stam H. Rate of progression and predictive factors for pulmonary outcome in children and adults with Pompe disease. *Mol Genet Metab* 2011;104(1-2):129-136.
11. Van der Beek NA, Hagemans ML, Reuser AJ, Hop WC, Van der Ploeg AT, Van Doorn PA, Wokke JH. Rate of disease progression during long-term follow-up of patients with late-onset Pompe disease. *Neuromuscul Disord* 2009;19(2):113-117.
12. van Capelle CI, van der Meijden JC, van den Hout JM, Jaeken J, Baethmann M, Voit T, Kroos MA, Derks TG, Rubio-Gozalbo ME, Willemsen MA *et al*. Childhood Pompe disease: clinical spectrum and genotype in 31 patients. *Orphanet J Rare Dis* 2016;11(1):65.
13. Van den Hout H, Reuser AJ, Vulto AG, Loonen MC, Cromme-Dijkhuis A, Van der Ploeg AT. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients. *Lancet* 2000;356(9227):397-398.
14. Kishnani PS, Corzo D, Nicolino M, Byrne B, Mandel H, Hwu WL, Leslie N, Levine J, Spencer C, McDonald M *et al*. Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology* 2007;68(2):99-109.
15. van der Ploeg AT, Clemens PR, Corzo D, Escolar DM, Florence J, Groeneveld GJ, Herson S, Kishnani PS, Laforet P, Lake SL *et al*. A randomized study of alglucosidase alfa in late-onset Pompe's disease. *N Engl J Med* 2010;362(15):1396-1406.
16. van der Ploeg AT, Barohn R, Carlson L, Charrow J, Clemens PR, Hopkin RJ, Kishnani PS, Laforet P, Morgan C, Nations S *et al*. Open-label extension study following the Late-Onset Treatment Study (LOTS) of alglucosidase alfa. *Mol Genet Metab* 2012;107(3):456-461.
17. de Vries JM, van der Beek NA, Hop WC, Karstens FP, Wokke JH, de Visser M, van Engelen BG, Kuks JB, van der Kooi AJ, Notermans NC *et al*. Effect of enzyme therapy and prognostic factors in 69 adults with Pompe disease: an open-label single-center study. *Orphanet J Rare Dis* 2012;7:73.
18. Strothotte S, Strigl-Pill N, Grunert B, Kornblum C, Eger K, Wessig C, Deschauer M, Breunig F, Glocker FX, Vielhaber S *et al*. Enzyme replacement therapy with alglucosidase alfa in 44 patients with late-onset glycogen storage disease type 2: 12-month results of an observational clinical trial. *J Neurol* 2010;257(1):91-97.
19. Regnery C, Kornblum C, Hanisch F, Vielhaber S, Strigl-Pill N, Grunert B, Muller-Felber W, Glocker FX, Spranger M, Deschauer M *et al*. 36 months observational clinical study of 38 adult Pompe disease patients under alglucosidase alfa enzyme replacement therapy. *J Inherit Metab Dis* 2012;35(5):837-845.
20. Bembi B, Pisa FE, Confalonieri M, Ciana G, Fiumara A, Parini R, Rigoldi M, Moglia A, Costa A, Carlucci A *et al*. Long-term observational, non-randomized study of enzyme replacement therapy in late-onset glycogenosis type II. *J Inherit Metab Dis* 2010;33(6):727-735.

21. Angelini C, Semplicini C, Ravaglia S, Bembi B, Servidei S, Pegoraro E, Moggio M, Filosto M, Sette E, Crescimanno G *et al*. Observational clinical study in juvenile-adult glycogenosis type 2 patients undergoing enzyme replacement therapy for up to 4 years. *J Neurol* 2012;259(5):952-958.
22. Anderson LJ, Henley W, Wyatt KM, Nikolaou V, Waldek S, Hughes DA, Lachmann RH, Logan S. Effectiveness of enzyme replacement therapy in adults with late-onset Pompe disease: results from the NCS-LSD cohort study. *J Inherit Metab Dis* 2014;37(6):945-952.
23. Stepien KM, Hendriksz CJ, Roberts M, Sharma R. Observational clinical study of 22 adult-onset Pompe disease patients undergoing enzyme replacement therapy over 5years. *Mol Genet Metab* 2016;117(4):413-418.
24. Van Der Ploeg AT, Kruijshaar ME, Toscano A, Laforet P, Angelini C, Lachmann R, Ignacio PP, Roberts M, Rösler K, Stulnig T *et al*. European recommendations for starting and stopping enzyme replacement therapy in adult patients with Pompe disease: a ten-year experience. *Submitted* 2017.
25. Toscano A, Schoser B. Enzyme replacement therapy in late-onset Pompe disease: a systematic literature review. *J Neurol* 2013;260(4):951-959.
26. Winkel LP, Van den Hout JM, Kamphoven JH, Disseldorp JA, Remmerswaal M, Arts WF, Loonen MC, Vulto AG, Van Doorn PA, De Jong G *et al*. Enzyme replacement therapy in late-onset Pompe's disease: a three-year follow-up. *Ann Neurol* 2004;55(4):495-502.
27. van Capelle CI, Winkel LP, Hagemans ML, Shapira SK, Arts WF, van Doorn PA, Hop WC, Reuser AJ, van der Ploeg AT. Eight years experience with enzyme replacement therapy in two children and one adult with Pompe disease. *Neuromuscul Disord* 2008;18(6):447-452.
28. van Capelle CI, van der Beek NA, Hagemans ML, Arts WF, Hop WC, Lee P, Jaeken J, Frohn-Mulder IM, Merkus PJ, Corzo D *et al*. Effect of enzyme therapy in juvenile patients with Pompe disease: a three-year open-label study. *Neuromuscul Disord* 2010;20(12):775-782.
29. Deroma L, Guerra M, Sechi A, Ciana G, Cisilino G, Dardis A, Bembi B. Enzyme replacement therapy in juvenile glycogenosis type II: a longitudinal study. *Eur J Pediatr* 2014;173(6):805-813.
30. Rossi M, Parenti G, Della Casa R, Romano A, Mansi G, Agovino T, Rosapepe F, Vosa C, Del Giudice E, Andria G. Long-term enzyme replacement therapy for pompe disease with recombinant human alpha-glucosidase derived from chinese hamster ovary cells. *J Child Neurol* 2007; 22(5):565-573.
31. Porta F, Pagliardini V, Roasio L, Biamino E, Spada M. Playing competitive basketball in face of late-onset pompe disease. *Muscle Nerve* 2015;51(2):302-303.
32. Ishigaki K, Murakami T, Nakanishi T, Oda E, Sato T, Osawa M. Close monitoring of initial enzyme replacement therapy in a patient with childhood-onset Pompe disease. *Brain Dev* 2012;34(2):98-102.
33. A. T. S. Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 2002;166(1):111-117.
34. Ulrich S, Hildenbrand FF, Treder U, Fischler M, Keusch S, Speich R, Fasnacht M. Reference values for the 6-minute walk test in healthy children and adolescents in Switzerland. *BMC Pulm Med* 2013;13:49.
35. van Capelle CI, van der Beek NA, de Vries JM, van Doorn PA, Duivenvoorden HJ, Leshner RT, Hagemans ML, van der Ploeg AT. The quick motor function test: a new tool to rate clinical severity and motor function in Pompe patients. *J Inherit Metab Dis* 2012;35(2):317-323.
36. Beenakker EA, van der Hoeven JH, Fock JM, Maurits NM. Reference values of maximum isometric muscle force obtained in 270 children aged 4-16 years by hand-held dynamometry. *Neuromuscul Disord* 2001;11(5):441-446.
37. Medical Research Council: Aids to the Examination of the Peripheral Nervous System, Memorandum No. 45. London: Her Majesty's Stationary Office; 1978.
38. American Thoracic Society/European Respiratory Society. ATS/ERS Statement on respiratory muscle testing. *Am J Respir Crit Care Med* 2002;166(4):518-624.
39. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Hankinson JL, Ip MS, Zheng J *et al*. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *The European respiratory journal* 2012;40(6):1324-1343.
40. Pinheiro J BD, Debroy S, Sarkar D. {nlme}: Linear and Nonlinear Mixed Effects Models. 2015.
41. R Core Team: R: A Language and Environment for Statistical Computing. In. Vienna, Austria: R Foundation for Statistical Computing; 2015.
42. Holm S. A Simple Sequentially Rejective Multiple Test Procedure. *Scand J Stat* 1979;6(2):65-70.

43. Wens SC, Ciet P, Perez-Rovira A, Logie K, Salamon E, Wielopolski P, de Bruijne M, Kruijshaar ME, Tiddens HA, van Doorn PA *et al*. Lung MRI and impairment of diaphragmatic function in Pompe disease. *BMC Pulm Med* 2015;15:54.
44. Mogalle K, Perez-Rovira A, Ciet P, Wens SC, van Doorn PA, Tiddens HA, van der Ploeg AT, de Bruijne M. Quantification of Diaphragm Mechanics in Pompe Disease Using Dynamic 3D MRI. *PLoS One* 2016;11(7):e0158912.
45. Gaeta M, Barca E, Ruggeri P, Minutoli F, Rodolico C, Mazziotti S, Milardi D, Musumeci O, Toscano A. Late-onset Pompe disease (LOPD): correlations between respiratory muscles CT and MRI features and pulmonary function. *Mol Genet Metab* 2013;110(3):290-296.
46. Pellegrini N, Laforet P, Orlikowski D, Pellegrini M, Caillaud C, Eymard B, Raphael JC, Lofaso F. Respiratory insufficiency and limb muscle weakness in adults with Pompe's disease. *Eur Respir J* 2005;26(6):1024-1031.
47. Prigent H, Orlikowski D, Laforet P, Letilly N, Falaize L, Pellegrini N, Annane D, Raphael JC, Lofaso F. Supine volume drop and diaphragmatic function in adults with Pompe disease. *Eur Respir J* 2012;39(6):1545-1546.
48. Wokke JH, Escolar DM, Pestronk A, Jaffe KM, Carter GT, van den Berg LH, Florence JM, Mayhew J, Skrinar A, Corzo D *et al*. Clinical features of late-onset Pompe disease: a prospective cohort study. *Muscle Nerve* 2008;38(4):1236-1245.
49. van der Beek NA, de Vries JM, Hagemans ML, Hop WC, Kroos MA, Wokke JH, de Visser M, van Engelen BG, Kuks JB, van der Kooi AJ *et al*. Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study. *Orphanet J Rare Dis* 2012;7:88.
50. Baek RC, Palmer R, Pomponio RJ, Lu Y, Ma X, McVie-Wylie AJ. The influence of a polymorphism in the gene encoding angiotensin converting enzyme (ACE) on treatment outcomes in late-onset Pompe patients receiving alglucosidase alfa. *Mol Genet Metab Rep* 2016;8:48-50.
51. De Filippi P, Saeidi K, Ravaglia S, Dardis A, Angelini C, Mongini T, Morandi L, Moggio M, Di Muzio A, Filosto M *et al*. Genotype-phenotype correlation in Pompe disease, a step forward. *Orphanet J Rare Dis* 2014;9:102.
52. Ravaglia S, De Filippi P, Pichiecchio A, Ponzio M, Saeidi Garaghani K, Poloni GU, Bini P, Danesino C. Can genes influencing muscle function affect the therapeutic response to enzyme replacement therapy (ERT) in late-onset type II glycogenosis? *Mol Genet Metab* 2012;107(1-2):104-110.
53. de Vries JM, Kuperus E, Hoogeveen-Westerveld M, Kroos MA, Wens SC, Stok M, van der Beek NA, Kruijshaar ME, Rizopoulos D, van Doorn PA *et al*. Pompe disease in adulthood: effects of antibody formation on enzyme replacement therapy. *Genet Med* 2017;19(1):90-97.

Supplemental tables and figures

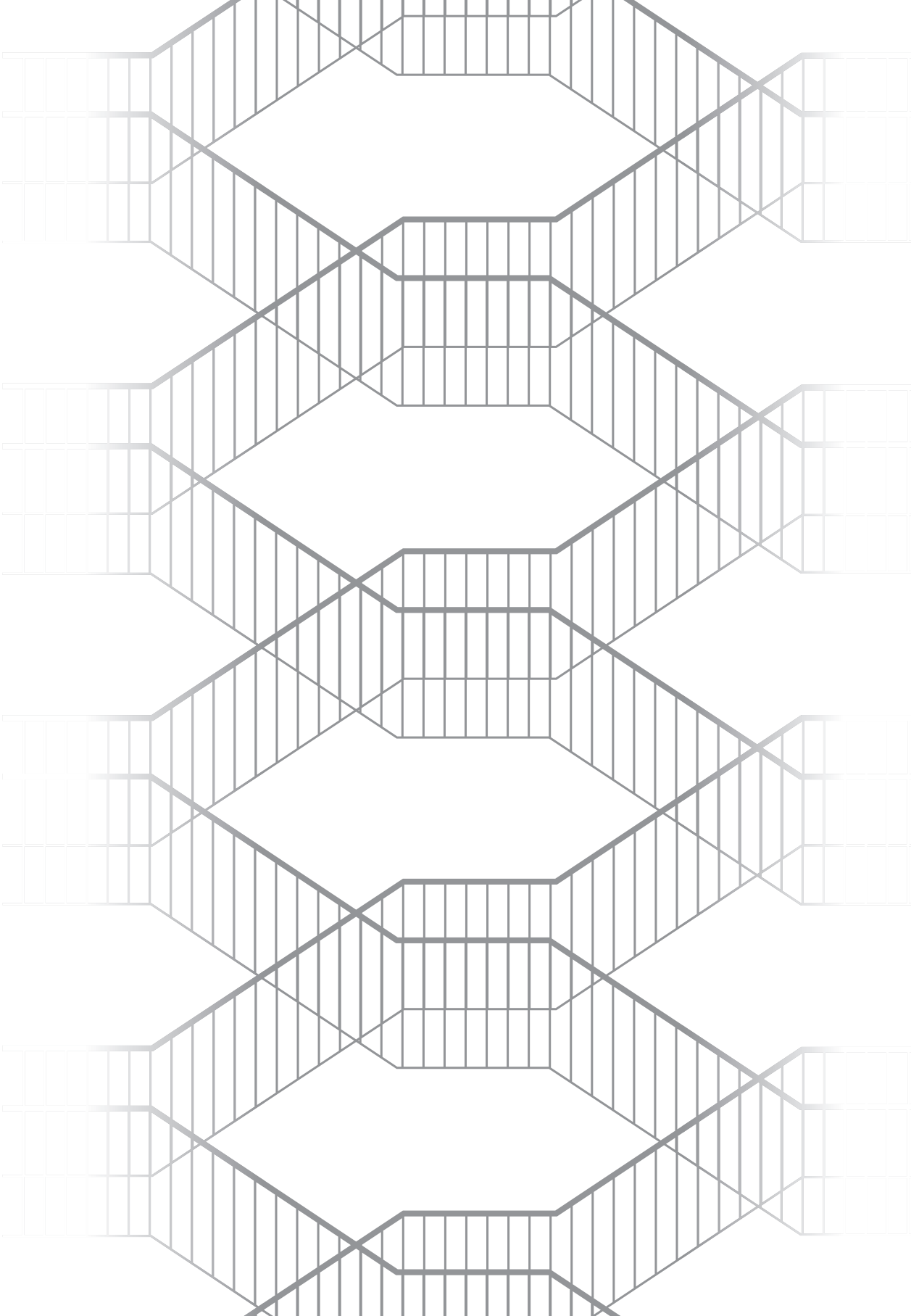


Supplemental figure | Examples of patterns over time observed in the outcome measures for individu.

Supplemental table | Overview of published studies on the effects of ERT in children.

Author	Country	Children/ total patients	Follow-up duration	Age at start ERT	6MWT	QMFT	HHD	MRC	Lung function	Other
Winkel 2004*	NL	2 / 3	3 yr	11 and 16y			Improved	Improved	Improve (1)/ stable (1)	
extension: van Capelle 2008*	NL	2 / 3	8 yr	11 and 16y			Improved		Improve (1)/ stable (1)	GMFM: improve / stable
Rossi 2007	Italy	2 / 3	1.3 and 2.7yr	2.6 and 3.6y						Clinical improvement
Bembi 2010	Italy	7 / 24	3 yr	12y (7-18y)	Improved				Stable	WS: improve
extension: Deroma 2014	Italy	8 / 8	6 yr	11.6y (7.2-15y)	Improved				Improve (4) Stable (2)	WS: stable
van Capelle 2010*	NL	5 / 5	3 yr	12.7y (5.9-15.2y)	Improved	Improved	Improved	Improved	Improve / stable (4) (sitting and supine)	
Ishigaki 2012	Japan	1 / 1	2 yr	10y	Improved			Improved	Improvement followed by deterioration	Initially gained motor function. Started to deteriorate after 8 months of treatment
Porta 2015	Italy	1 /1	4 yr	9y	Improved					

An indent followed by a dash indicates that the same patients participate in more studies; yr = years; wks = weeks; GMFM: gross motor-function test; WS = Walton score.
* Publications describing children included in the present study with approximately 7 years longer follow-up.



Long-term benefit of enzyme-replacement therapy in Pompe disease: a 5-year prospective study

E. Kuperus, M.E. Kruijshaar, S.C.A. Wens, J.M. de Vries
M.M. Favejee, J.C. van der Meijden, D. Rizopoulos
E. Brusse, P.A. van Doorn, A.T. van der Ploeg
N.A.M.E. van der Beek

4

Neurology 2017; in print

Abstract

Objective

As there is little data on the long-term efficacy of enzyme-replacement therapy (ERT) in Pompe disease, we aimed to determine its effect after 5 years, and sought to identify predictors for a favorable response.

Methods

We included 102 adult Pompe patients in a nationwide, prospective, cohort study. We assessed muscle strength (manual muscle testing (MRC grading), hand-held dynamometry (HHD)), muscle function (six-minute walk test (6MWT), quick motor function test (QMFT)), daily life activities (Rasch-build Pompe-specific Activity scale (R-PAct)), and pulmonary function (forced vital capacity (FVC) in upright and supine positions, maximum inspiratory (MIP), and expiratory (MEP) pressures) at 3-6 months intervals before and after start of ERT. Data were analyzed using linear mixed-effects models for repeated measurements.

Results

Median follow-up duration was 6.1 years (range 0.4-7.9 years); of which 5.0 years (range 0.2-7.3 years) were during ERT. Treated patients had better muscle strength (MRC sum-score +6.6 percentage points (pp); HHD sum-score +9.6 pp; both $p<0.0001$), activity levels (R-PAct +10.8 pp, $p<0.002$), and pulmonary function (FVC upright +7.3 pp; FVC supine +7.6 pp; both $p<0.00–03$), than their expected untreated disease course. Walking distance improved (416 *vs.* 376 meters at baseline; $p=0.03$). The largest increase was seen during the first two to three years of treatment. Response to treatment was similar between groups, irrespective of sex, age, or disease duration.

Conclusions

Long-term enzyme-replacement therapy positively affects muscle strength, pulmonary function and daily life activities in adult Pompe patients, with a peak effect around two to three years of treatment.

Introduction

Pompe disease is a progressive myopathy caused by deficient activity of the lysosomal enzyme acid α-glucosidase that leads to glycogen storage in virtually all body tissues.[1] It is the first hereditary myopathy for which a disease-specific treatment (i.e. enzyme-replacement therapy (ERT)) has become available. Shortly after birth, the most severely affected 'classic infantile' patients present with rapidly progressive generalized muscle weakness and cardiac and respiratory failure. Without treatment, this results in death within the first year of life.[2] The phenotype in children and adults with 'non-classic' or 'late-onset' Pompe disease is dominated by a progressive limb-girdle myopathy that leads to severe functional limitations, while respiratory muscle dysfunction limits patients' average life-span.[3,4]

Since 2006, ERT with recombinant human acid α-glucosidase (alglucosidase alfa, Myozyme®) has been registered for the treatment of Pompe disease.[5,6] The only placebo-controlled study of alglucosidase alfa in late-onset Pompe disease, comprising 90 patients studied for 78 weeks, showed improvement of walking distance and stabilization of pulmonary function with treatment.[7] Several observational studies have since underscored this effect, and have also shown improvements in muscle strength, muscle function, survival, and quality of life.[8-14] Nonetheless, experience has shown that not all patients derive equal benefit and very little evidence has been published on the long-term benefits of ERT:[15-18] the average follow-up period in most studies published to date does not exceed 3 years of treatment. We therefore present a large, prospective, nationwide study in which we 1) assessed the long-term effects of ERT on muscle strength, muscle function, pulmonary function, and daily life activities in adult Pompe patients; and 2) aimed to identify potential prognostic factors for treatment efficacy.

Methods

Study design and participants

This study was a nationwide, prospective, open-label, cohort study involving all Dutch patients with a confirmed diagnosis of Pompe disease; this is being conducted at the Center for Lysosomal and Metabolic Diseases, Erasmus MC University Medical Center, Rotterdam, the endorsed center of expertise for Pompe disease in the Netherlands. For the current analyses we included all patients who 1) were aged 18 years or older at first study-assessment; 2) had symptomatic Pompe disease (defined as muscle weakness and/or reduced pulmonary function for which starting ERT was considered desirable); and 3) had not yet received ERT prior to enrollment in the overall study. Clinical assessments took place every three to six months before and after start of ERT. Patients were enrolled since 1 January 2005: for this study, database lock was 31 December 2012. If treated, patients

received 20mg/kg alglucosidase alfa intravenously every other week. Patients who had not yet started ERT at data-closure contributed natural-course data only, and were included to strengthen the follow-up on the natural course of the disease. If ERT was discontinued, data collected thereafter was not included in the analyses.

Procedures

We used the following outcome measures to evaluate the effect of ERT: 1) muscle strength (manual muscle testing using the Medical Research Council (MRC) grading scale[19] and hand-held dynamometry (HHD)[20]); 2) muscle function (six-minute walk test (6MWT)[21] and Quick Motor Function Test (QMFT)[22]); 3) pulmonary function (Forced vital capacity (FVC) in upright seated and supine positions, and maximum static inspiratory (MIP) and expiratory (MEP) pressures)[23,24]; and 4) daily life activities (Rasch-built Pompe-specific Activity (R-PAct) scale.[25] Detailed procedures have been reported previously,[4,7,9] and are described in supplemental data on the *Neurology®* Web site at Neurology.org.

Statistical analysis

Longitudinal analyses of the different outcome measures were performed using linear mixed models to account for correlations in the repeated measurements per patient.[26] The current analyses comprise around 1200 measurements per outcome measure. Time was expressed in years before (i.e. natural-course period) or after start of ERT; for the 14 patients who had not yet started ERT, the date upon which ERT was recommended was designated as "date of start of ERT". Data of these patients were included to strengthen the natural-course analyses.

To compare patients' outcomes under treatment with their situation without treatment, we extrapolated the natural-course data by assuming linear evolutions over time on the basis of data inspection and our previously published results.[9] For data measured during ERT we accounted for potential non-linear profiles by using natural cubic splines in the fixed-effects and random-effects parts of the model. In the specification of the splines, boundary knots were placed at 0 (i.e. start of ERT) and 5 years, and internal knots were placed at 1 and 3.5 years. The use of splines in the random-effects part was also used to flexibly capture the correlation structure among the follow-up visits. The models' assumptions were checked using residuals plots. Observed values at 0.5, 1, and 5 years of treatment were compared to the extrapolated natural disease course. We also compared the outcomes at 5 years of treatment to baseline (i.e. start of ERT). For the 6MWT, we only compared the effects of ERT at 5 years of follow-up with the situation at baseline, since too few data on the natural course of the disease were available to be reliably extrapolated.

We performed subgroup analyses to assess the effect of the most important covariates,

since the dataset-size does not allow a multivariate analysis assessing all variables at the same time. For these analyses, patients were divided into groups, as in previous studies, on the basis of sex; age at start of ERT (< 45 or ≥ 45 years); and disease duration at start of ERT (< 15 or ≥ 15 years; calculated from onset of first symptoms).[7,9] Overall treatment effects were tested using likelihood ratio tests; subgroup analyses were performed using F-tests. The significance level was set at $p \leq 0.05$ ($p \leq 0.006$ if Bonferroni corrections were needed to adjust for multiple testing). Differences were expressed in absolute percentage points (pp), except for the 6MWT, in which the difference in walking distance is given (m). Additionally, for MRC, HHD, FVC and R-PAct we assessed patients' individual response to ERT respective to start of treatment and to the expected untreated disease course (for this purpose the estimated mean decline at group level was used). Their response was classified into three groups: 1) improved or stable compared to start of ERT, 2) better than extrapolated natural disease course but worsened compared to start of ERT, and 3) worse than extrapolated natural disease course. Analyses were performed with SPSS for Windows (version 21, SPSS Inc., Chicago, IL), or R version 3.1.1 (2014-07-10) using package nlme (version 3.1-117).

Standard Protocol Approvals, Registrations and Patient Consents

The study protocol was approved by the Medical Ethical Committee at Erasmus MC University Medical Center. Informed consent was obtained from all participants.

Classification of Evidence

This study provides Class IV evidence for the long-term efficacy of enzyme-replacement therapy in adults with Pompe disease, irrespective of sex, age, or disease duration.

Results

Patients

In total, 102 Dutch adult Pompe patients (age range 24 to 76 years; Table 1) participated in this study, 82 of whom contributed both natural course and ERT data, six of whom contributed ERT data only, and 14 of whom contributed natural-course data only. Median overall follow-up duration was 6.1 years (Interquartile range (IQR) 2.9; range 0.4-7.9 years), comprising 1.1 years (IQR 1.2; range 0.1-7.9) during the natural-course period, and 5.0 years (IQR 2.4; range 0.2-7.3 years) during the treatment period. Forty-five patients were treated with ERT for more than five years, while only seven patients were treated for less than one year. There were no differences in characteristics between treated patients and patients who had not yet started treatment. At start of ERT, 32 patients were fully or partially wheelchair dependent, against 41 at study-end. Twenty-seven patients used mechanical ventilation at ERT-start; by study-end, eight additional patients had become ventilator bound.

Table 1 | Patients' Characteristics.

	Patients (N = 102)
Total follow-up duration in years – median (IQR; range)	6.1 (2.9; 0.4-7.9)
Natural course follow-up duration in years – median (IQR; range) (N = 96)[a]	1.1 (1.2; 0.1-7.9)
ERT follow-up duration in years – median (IQR; range) (N = 88)[a]	5.0 (2.4; 0.2-7.3)
Age at start of ERT in years – median (IQR; range)	52 (20; 24-76)
Age at symptom onset in years – median (IQR; range)	33 (16; 1-62)
Disease duration from symptom onset at start of ERT in years – median (IQR; range)	16 (17; 1-50)
Sex – No. of patients (%)	
Male	53 (52)
Female	49 (48)
Genotype – No. of patients (%)	
c.-32-13T>G / known pathogenic variation	99 (97)
c.1447G>A / c.569G>A	2 (2)
c.671G>A / c.525delT	1 (1)
Wheelchair and/or mechanical ventilation at start of ERT – No. of patients (%)	
Wheelchair and mechanical ventilation	19 (19)
Wheelchair only	17 (17)
Mechanical ventilation only	10 (10)
No wheelchair, no mechanical ventilation	56 (55)
MRC sum-score and FVC in upright position at start of ERT – No. of patients (%)[b]	
MRC < 80% and FVC < 80%	40 (39)
MRC < 80% and FVC ≥ 80%	5 (5)
MRC ≥ 80% and FVC < 80%	25 (25)
MRC ≥ 80% and FVC ≥ 80%	32 (31)

ERT = Enzyme-replacement therapy, MRC sum-score = Medical Research Council sum-score, FVC = Forced vital capacity, N/No. = number of patients. IQR = interquartile range.
[a] There were no differences in baseline characteristics between the 88 patients treated with ERT and the 14 patients who contributed natural course data only (chi-square trend or Wilcoxon rank-sum test).
[b] Due to the use of invasive ventilation, 6 patients were unable to perform pulmonary function tests. Their FVC was considered to be below 80%.

Skeletal muscle strength and muscle function

During treatment, an initial increase in MRC and QMFT score in the first 2 to 3 years was followed by a slight decline, while HHD increased during the first years and stabilized thereafter (figure 1A, 1B, table 2). At the 5-year treatment point, the average MRC and HHD sum-scores were higher than for the extrapolated natural course (+6.6 pp for MRC, and +9.6 pp for HHD; both $p<0.0001$). QMFT sum-scores were similar ($p=0.47$). Relative to start of treatment patients' outcomes had remained stable (MRC and QMFT sum-scores; $p=0.25$ and $p=0.87$), or had improved (HHD sum-score, $p<0.0001$; table 2B). At their last follow-up assessment, 59% of patients had better or similar MRC sum-scores than at start of treatment, while for HHD 89%

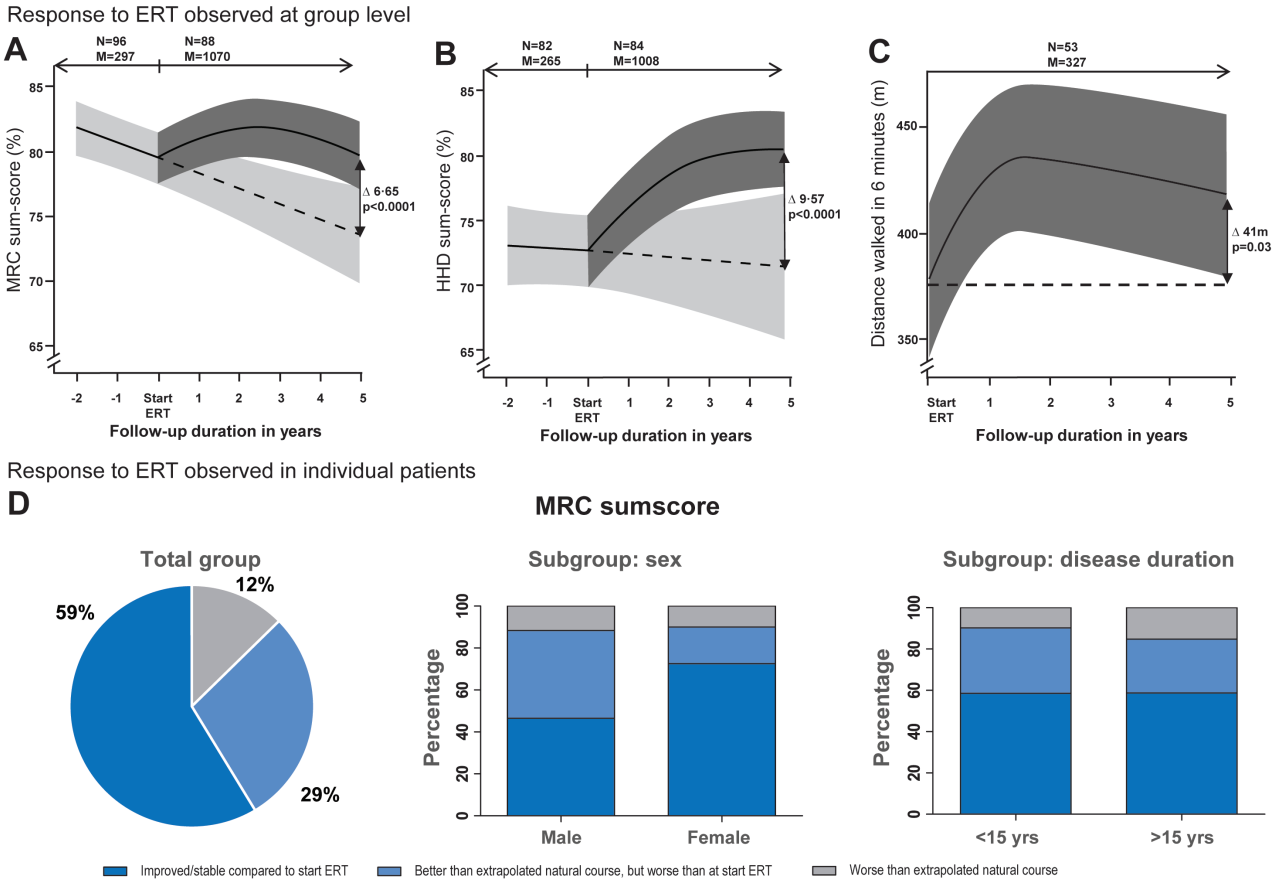


Figure 1 | Effect of enzyme-replacement therapy on muscle strength and muscle function.

The course of disease at group level before and after the start of enzyme-replacement therapy (ERT) for manual muscle testing (MRC sum-score) (**A**), and hand-held dynamometry (HHD sum-score) (**B**). Median natural-course duration was 1.1 years, hence we had sufficient data to calculate the disease course two years pre-ERT. (**C**) Course of disease after start of ERT for the distance walked in 6 minutes (in meters). The solid lines represent the measured natural course of the disease and/or the course during treatment. The dashed line represents the natural course extrapolated on the basis of natural-course data or baseline level. 95% confidence intervals are shown in grey. N=the number of patients and M=the number of measurements included in the analyses before and after start of ERT. Δ=difference between treatment data and extrapolated natural course at 5 years. (**D**) Individual patients' response to treatment on MRC sum-score, shown for the group as a whole or stratified by subgroups (sex, disease duration).

Table 2 | Effect of ERT at 0.5, 1 and 5 years relative to the extrapolated natural disease course (A) and to baseline (B).

	A. Effect of ERT relative to progression in the extrapolated natural disease course							B. Effect of ERT compared to baseline values		
	At 0.5 years of ERT		At 1 year of ERT		At 5 years of ERT			At 5 years of ERT		
	difference	*p*-value	difference	*p*-value	difference	*p*-value	% better than expected if untreated*	difference	*p*-value	% improved/stable compared to baseline*
MRC sum-score	+ 1.7	< 0.0001	+ 2.9	< 0.0001	+ 6.6	< 0.0001	87	+ 0.7	0.25	59
HHD sum-score	+ 2.3	< 0.0001	+ 4.0	< 0.0001	+ 9.6	< 0.0001	89	+ 8.4	< 0.0001	89
FVC upright	+ 1.4	< 0.0001	+ 2.4	< 0.0001	+ 7.3	0.0006	73	- 0.1	0.84	48
FVC supine	+ 1.2	0.0001	+ 2.2	0.0001	+ 7.6	0.0003	75	- 2.9	0.005	37
MIP	+ 4.1	< 0.0001	+ 7.2	< 0.0001	+ 20.8	< 0.0001		- 0.5	0.81	
MEP	+ 3.6	< 0.0001	+ 6.2	< 0.0001	+ 17.3	< 0.0001		+ 2.6	0.18	
QMFT sum-score	+ 1.0	0.0005	+ 1.8	0.0005	+ 1.5	0.47		- 0.2	0.87	
R-PAct	+ 2.7	< 0.0001	+ 4.7	< 0.0001	+ 10.8	0.002	80	+ 3.6	0.004	59
6MWT	NA	NA	NA	NA	NA	NA	69	+40.9	0.03	69

*For the patients who had not yet been treated for 5 years, their last follow-up measurement was taken for comparison.
Effects of ERT are expressed in percentage points (pp), except for the 6MWT, in which the difference is given in meters (m). Except for the 6MWT – which was analyzed in a different manner than the other main outcome measures – Bonferroni corrections were applied to adjust for multiple testing: a p-value <0.006 was considered statistically significant. For the 6MWT a significance-level of p<0.05 was used.
ERT = Enzyme-replacement therapy, NC = natural course, MRC sum-score = Medical Research Council sum-score, HHD sum-score = Hand-held dynamometry, FVC = forced vital capacity, MIP = maximum inspiratory pressure, MEP = maximum expiratory pressure, QMFT sum-score = Quick Motor Function Test sum-score, R-PAct = Rasch-built Pompe-specific Activity scale, 6MWT = 6 minute walk test. NA = not applicable.

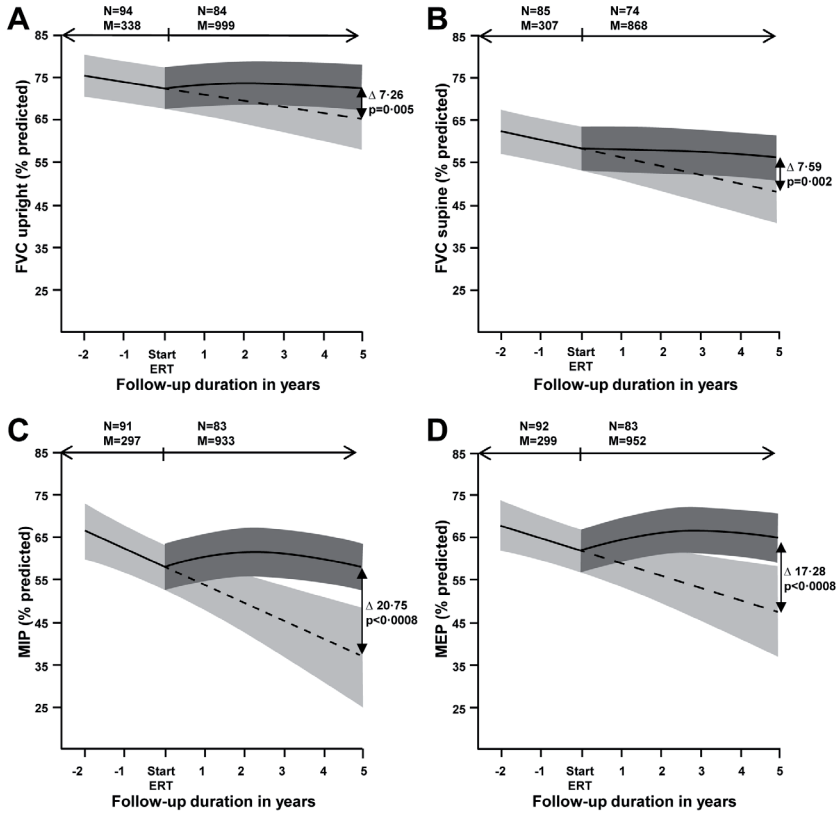


Figure 2 | Effect of enzyme-replacement therapy on pulmonary function.

The course of disease before and after start of enzyme-replacement therapy (ERT) for (**A**) forced vital capacity (FVC) in upright position; (**B**) FVC in supine position; (**C**) maximum inspiratory pressures (MIP); and (**D**) maximum expiratory pressures (MEP). The solid lines represent the measured natural course of the disease and/or the course during treatment. The dashed line represents the natural course extrapolated on the basis of natural-course data. 95% confidence intervals are shown in grey. Median natural-course duration was 1.1 years, hence we had sufficient data to calculate the disease course two years pre-ERT. N=the number of patients and M=the number of measurements included in the analyses before and after start of ERT. Δ=difference between treatment data and extrapolated natural course at 5 years.

of patients performed better than at ERT initiation (table 2B, figure 1D). After an increase in the first years of treatment, the distance walked during the 6MWT declined gradually (figure 1C). Relative to baseline, the median distance walked increased from 376 to 416 meters at the 5-year time point (p=0.03; table 2B): 69% of patients improved their walking ability in comparison to start of ERT. Subgroup analyses showed that the mean increase in HHD sum-score was greater in women than in men (13.6 pp *vs.* 1.9 pp, p=0.003), and was also greater in patients with a disease duration >15 years than in those with a shorter disease duration (15.2 pp *vs.* 4.5 pp, p=0.004; table e-1). For the other subgroups investigated there were no differences.

Pulmonary function

At 5 years, treated patients had better pulmonary function parameters relative to the extrapolated natural course: their FVC in upright position was 7.3 pp higher (p=0.0006), while their FVC in supine position was 7.6 pp higher (p=0.0003; figure 2A, 2B, table 2). Also, MIP and MEP were higher (MIP +20.8 pp, MEP +17.3 pp; both p<0.0001; figure 2C, 2D). The largest increase was found in the first years after start of ERT. Relative to baseline, all pulmonary outcome measures remained stable, except for FVC in supine position, which decreased by 2.9 pp over 5 years (table 2B). Relative to start of ERT, at their last follow-up measurement, 48% of patients had stable or better FVC in upright position, and 37% had better FVC in supine position. Relative to their expected disease course, ERT led to stable or improved FVC in 73% (upright position) and 75% (supine position) of patients. Subgroup analyses showed that MEP improved most in younger patients (+37.5 pp in those aged <45 against -0.3 in those aged ≥45, p<0.0001), and also in patients with a shorter disease duration (+28.8 pp in patients diseased <15 years against +1.8 pp in those diseased ≥15 years, p=0.001; table e-1). The same tendency applied to most of the other pulmonary outcome measures, though not significantly.

Daily life activities and participation

During ERT, the R-PAct score improved during the first 2 to 3 years, and then decreased slightly (figure 3A). Nevertheless, at the 5-year time-point, the R-PAct score was higher than both the extrapolated natural course (+10.8 pp, p=0.002; table 2A) and the baseline values at start of ERT (+3.6 pp, p=0.004; table 2B). Fifty-nine percent of patients maintained or improved their level of functioning while receiving ERT (figure 3B, table 2B).Subgroup analyses showed that the R-PAct score improved more in men than in women (22.4 pp against 3.3 pp, p=0.005; table e-1). No difference was observed in other subgroups.

Safety, infusion-associated reactions and antibody formation

During treatment, 19 patients (22%) had one or more infusion-associated reaction (IAR) similar to those described previously.[7,27] Most IARs could be controlled by slowing down infusion rates and/or giving pre-medication with antihistamines and/or corticosteroids. At the end of the study, four patients who had been treated for a period ranging from 2.6 to 5.4 years still had IARs that were mild but treatable. Although about 60% of patients developed antibodies to the infused enzyme, this did not interfere with the efficacy of ERT, except in one patient (e-table 2).[28] ERT was discontinued in four patients (2, 6, 16, and 32 months after start of treatment). In one patient, this was for safety concerns (i.e. multiple severe IARs). This patient had a history of multiple autoimmune diseases and drug-induced allergies, and died at the age of 45 of autoimmune related hepatitis 3.5 years after discontinuing treatment. In a second patient, this was due to very high antibody titers combined with

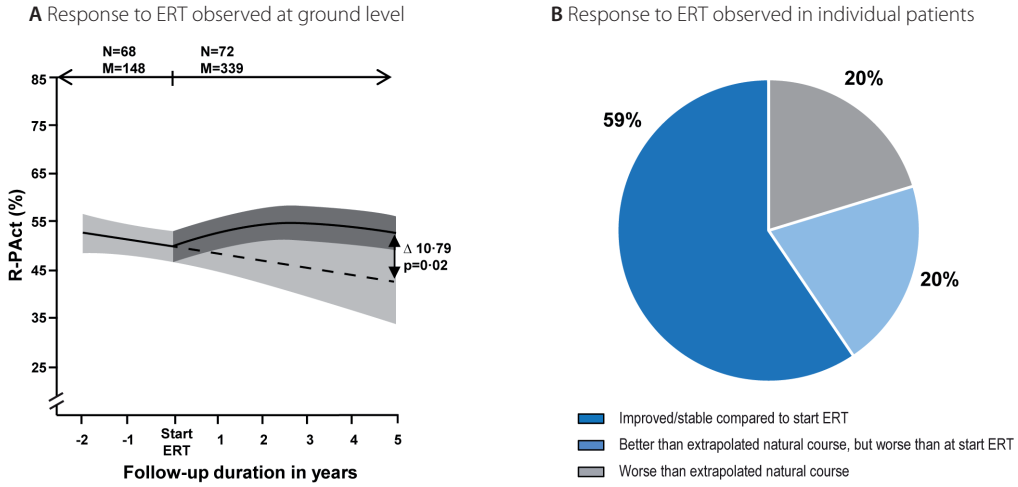


Figure 3 | Effect of enzyme-replacement therapy on daily life activities.

(**A**) The course of disease at group level before and after start of enzyme-replacement therapy (ERT) for the Rasch-Build Pompe-specific Activity scale (R-PAct). The solid lines represent the measured natural course of the disease and/or the course during treatment. The dashed line represents the natural course extrapolated on the basis of natural-course data. 95% confidence intervals are shown in grey. Median natural-course duration was 1.1 years, hence we had sufficient data to calculate the disease course two years pre-ERT. N=the number of patients and M=the number of measurements included in the analyses before and after start of ERT. Δ=difference between treatment data and extrapolated natural course at 5 years. (**B**) Individual patients' response to treatment on R-Pact.

a poor response to ERT.[28] For the two other patients, this was for personal reasons, one of whom died at the age of 56 due to respiratory insufficiency 1.1 years after discontinuing ERT. During this study, six more patients – all receiving ERT – died: one (aged 51) due to respiratory insufficiency, the others (ages ranging from 61 to 77) due to other, non-Pompe-disease related co-morbidities. None of the deaths were considered to be treatment related.

Discussion

In this nationwide, prospective study on the long-term effects of enzyme-replacement therapy in adult Pompe patients we show that after five years of treatment, muscle strength, pulmonary function and daily life ability were better than our expectations would patients have remained untreated. Improvements in clinical outcomes were greatest during the first 2-3 years of treatment, followed by stabilization or secondary decline. The majority of patients still functioned better or at least equal to start of treatment, which – for a progressive disorder such as Pompe disease – is a clear beneficial effect.

Our study encompasses all known Dutch adult Pompe patients, ranging in age from 24 to 76 years, and ranging in severity from mildly to very severely affected. Selection bias was therefore minimal. Another important asset is that we were able to prospectively collect follow-up data on the natural disease course before the start of ERT, allowing us to reliably estimate the natural-course progression using over 300 measurements in 96 patients. When studying the effects of treatment in progressive disorders such as Pompe disease, these natural-course data are extremely important, as this makes it possible to compare patients' outcomes under treatment to their situation without treatment, thereby providing a true estimate of treatment-efficacy.

During the treatment period we saw a remarkable pattern in disease course: a substantial improvement during the first two to three years of treatment, followed by a plateau or slight decline. This pattern was seen across most outcome measures. Some other studies have also hinted that the effect of ERT is greater during the first years of treatment: in the placebo-controlled study, the largest positive effect in walking distance and FVC over placebo was seen during the first year,[7] while a recent study in 22 UK patients suggested that the treatment effect peaks around 2 years, before appearing to decline.[18] It is unclear whether this is because the efficacy of ERT diminishes over time, or whether it is due to other factors. During the first years of treatment, it is conceivable that clearance of glycogen from the lysosomes of the muscles – situated between the sarcomeres – leads to better concerted action of the myofibrils (i.e. contractile function), and thus improved strength.[29-33] Over time, however, this may be outweighed by the negative effects of continued autophagic build-up, compromising the trafficking and processing of the therapeutic enzyme along the endocytic pathway.[34] Age-related decline in muscle mass and contractile function may also contribute – especially above the age of 65 years. Furthermore, we have recently reported that untreated patients with Pompe disease lack robust satellite-cell activation.[35] Whether this is also true in patients receiving ERT needs further investigation. These factors combined may explain ongoing muscle wasting and inadequate muscle regeneration after an initial period of response, and may offer scope for new treatment strategies – which are currently being pursued in several clinical trials or in a preclinical setting.

Although our study shows that ERT has a beneficial effect on pulmonary function, FVC in supine position continued to decline during treatment, although more slowly than we had extrapolated for the natural course of the disease. This ongoing decline was also reflected in the increasing number of patients needing mechanical ventilation. Our recent pulmonary-MRI study under spirometry control confirmed the general belief that the function of the diaphragm in Pompe disease is far more impaired than that of the thoracic musculature.[36,37] It has been suggested that as the diaphragm may be involved early in the disease course,

it may therefore be more difficult to reverse its function. [38] While the exact mechanisms still need to be unraveled, they may involve differences in myofiber distribution and regenerative capacity.

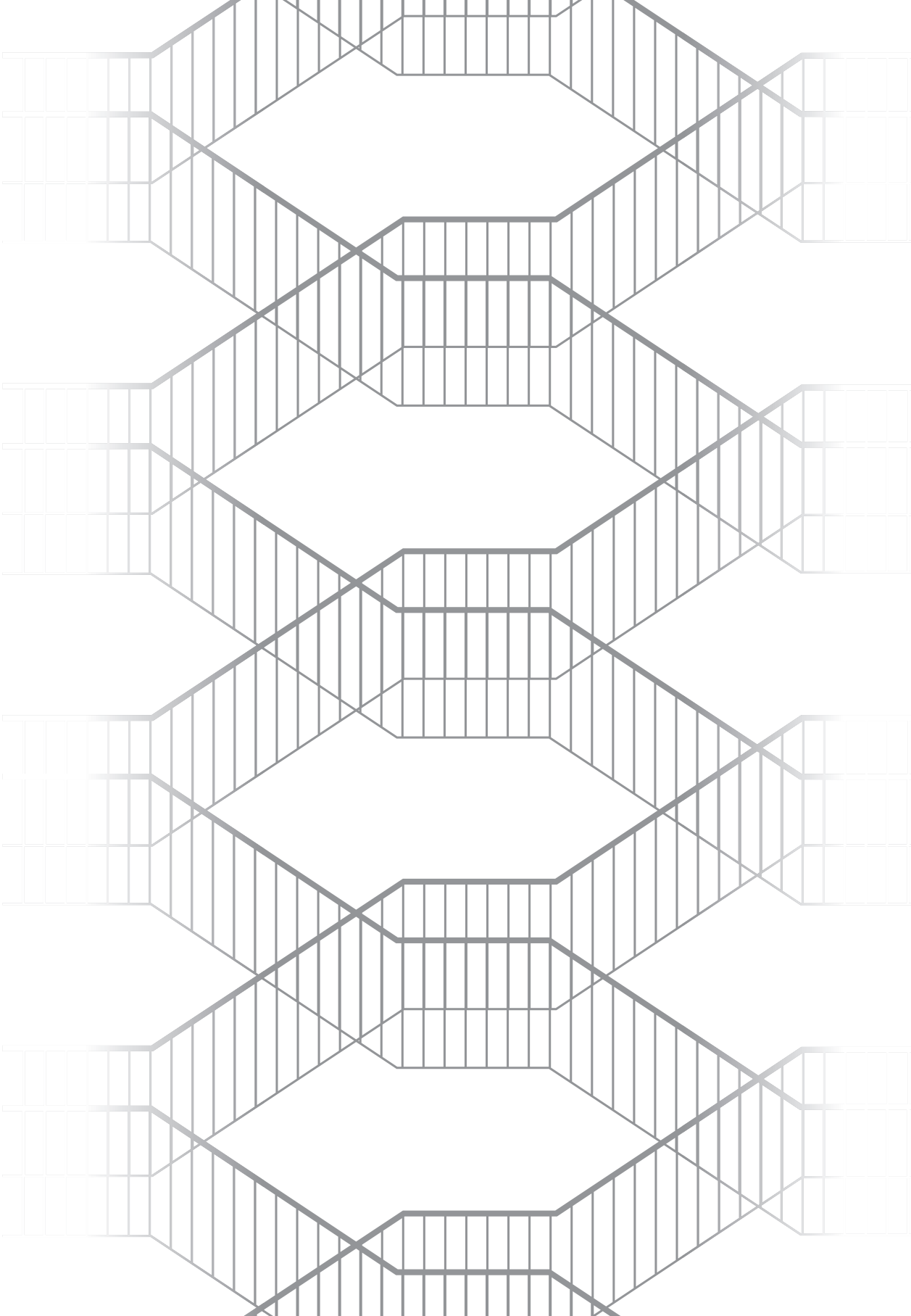
Although this is the largest study to date in adult Pompe patients, and all patients have consistently been examined at regular intervals throughout the years, no consistent differences in the response to ERT were observed between subgroups. We therefore conclude that all patients may benefit from treatment, irrespective of sex, age, or disease severity. Nonetheless, despite the favorable outcomes at group level, a subset of patients became wheelchair and/or ventilator dependent. Mean FVC_{supine} of the newly ventilated patients was 34% at start of ERT – on the edge of ventilator dependency already –, while four patients who started to use a wheelchair in fact had stable or better muscle sum-scores or R-PAct score relative to baseline. We therefore think that the use of these aids may be due partly to better awareness and intensified care, rather than reflect a true deterioration in pulmonary function or motor function. The fact that we did not find robust prognostic factors for treatment outcome cannot be attributed to the relatively small sample size – inherent to orphan disorders – only. Probably yet unknown mechanisms and genetic background factors contribute to the observed differences in response. In order to personalize treatment in accordance with the expected outcome, future – international – studies should search for such factors affecting treatment efficacy.

Our study shows that long-term ERT treatment with alglucosidase alfa positively affects muscle strength, pulmonary function, and daily life activities. Its effect peaks around two to three years of treatment followed by a plateau or secondary decline. As yet, the individual differences in treatment benefit are not well understood.

References

1. Hirschhorn R, Reuser AJ. Glycogen storage disease type II: acid alpha-glucosidase (acid maltase) deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The Metabolic and Molecular Bases of Inherited Disease. New York: McGraw-Hill; 2001:3389-3420.
2. van den Hout HM, Hop W, van Diggelen OP, et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. Pediatrics 2003;112:332-340.
3. Hagemans ML, Hop WJ, van Doorn PA, et al. Course of disability and respiratory function in untreated late-onset Pompe disease. Neurology 2006;66:581-583.
4. van der Beek NA, de Vries JM, Hagemans ML, et al. Clinical features and predictors for disease progression in adults with Pompe disease: a nationwide prospective observational study. Orphanet J Rare Dis 2012;7:88.
5. van den Hout H, Reuser AJ, Vulto AG, et al. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients. Lancet 2000;356:397-398.
6. Kishnani PS, Corzo D, Nicolino M, et al. Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. Neurology 2007;68:99-109.
7. van der Ploeg AT, Clemens PR, Corzo D, et al. A randomized study of alglucosidase alfa in late-onset Pompe's disease. N Engl J Med 2010;362:1396-1406.
8. Bembi B, Pisa FE, Confalonieri M, et al. Long-term observational, non-randomized study of enzyme replacement therapy in late-onset glycogenosis type II. J Inherit Metab Dis 2010;33:727-735.
9. de Vries JM, van der Beek NA, Hop WC, et al. Effect of enzyme therapy and prognostic factors in 69 adults with Pompe disease: an open-label single-center study. Orphanet J Rare Dis 2012;7:73.
10. Regnery C, Kornblum C, Hanisch F, et al. 36 months observational clinical study of 38 adult Pompe disease patients under alglucosidase alfa enzyme replacement therapy. J Inherit Metab Dis 2012;35:837-845.
11. Angelini C, Semplicini C, Ravaglia S, et al. Observational clinical study in juvenile-adult glycogenosis type 2 patients undergoing enzyme replacement therapy for up to 4 years. J Neurol 2012;259:952-958.
12. Güngör D, Kruijshaar ME, Plug I, et al. Impact of enzyme replacement therapy on survival in adults with Pompe disease: results from a prospective international observational study. Orphanet J Rare Dis 2013;8:49.
13. Anderson LJ, Henley W, Wyatt KM, et al. Effectiveness of enzyme replacement therapy in adults with late-onset Pompe disease: results from the NCS-LSD cohort study. J Inherit Metab Dis 2014;37:945-952.
14. Güngör D, Kruijshaar ME, Plug I, et al. Quality of life and participation in daily life of adults with Pompe disease receiving enzyme replacement therapy: 10 years of international follow-up. J Inherit Metab Dis 2016;39:253-260.
15. van Capelle CI, Winkel LP, Hagemans ML, et al. Eight years experience with enzyme replacement therapy in two children and one adult with Pompe disease. Neuromuscul Disord 2008;18:447-452.
16. Andreassen CS, Schlütter JM, Vissing J, Andersen H. Effect of enzyme replacement therapy on isokinetic strength for all major muscle groups in four patients with Pompe disease-a long-term follow-up. Mol Genet Metab 2014;112:40-43.
17. Isayama R, Shiga K, Seo K, et al. Sixty six-month follow-up of muscle power and respiratory function in a case with adult-type Pompe disease treated with enzyme replacement therapy. J Clin Neuromuscul Dis 2014;15:152-156.
18. Stepien KM, Hendriksz CJ, Roberts M, Sharma R. Observational clinical study of 22 adult-onset Pompe disease patients undergoing enzyme replacement therapy over 5 years. Mol Genet Metab 2016;117:413-418.
19. Medical Research Council. Aids to examination of the peripheral nervous system. Memorandum no. 45. London: Her Majesty's Stationary Office, 1976.
20. van der Ploeg RJ, Fidler V, Oosterhuis HJ. Hand-held myometry: reference values. J Neurol Neurosurg Psychiatry 1991;54:244-247.
21. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111-117.
22. van Capelle CI, van der Beek NA, de Vries JM, et al. The quick motor function test: a new tool to rate clinical severity and motor function in Pompe patients. J Inherit Metab Dis 2012;35:317-323.
23. Quanjer PH, Tammeling GJ, Cotes JE, et al. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993;16:5-40.

24. American Thoracic Society/European Respiratory Society. ATS/ERS Statement on respiratory muscle testing. Am J Respir Crit Care Med 2002;166:518-624.
25. van der Beek NA, Hagemans ML, van der Ploeg AT, et al. The Rasch-built Pompe-specific activity (R-PAct) scale. Neuromuscul Disord 2013;23:256-264.
26. Verbeke G, Molenberghs G. Linear mixed models for longitudinal data. New York: Springer, 2000.
27. de Vries JM, Kuperus E, Hoogeveen-Westerveld M, et al. Pompe disease in adulthood: effects of antibody formation on enzyme replacement therapy. Genet Med 2017;19:90-97.
28. de Vries JM, van der Beek NA, Kroos MA, et al. High antibody titer in an adult with Pompe disease affects treatment with alglucosidase alfa. Mol Genet Metab 2010;101:338-345.
29. van der Ploeg AT, Kroos MA, Willemsen R, et al. Intravenous administration of phosphorylated acid alpha-glucosidase leads to uptake of enzyme in heart and skeletal muscle of mice. J Clin Invest 1991;87:513-518.
30. Winkel LP, Kamphoven JH, van den Hout JM, et al. Morphological changes in muscle tissue of patients with infantile Pompe's disease receiving enzyme replacement therapy. Muscle Nerve 2003;27:743-751.
31. Raben N, Danon M, Gilbert AL, et al., Enzyme replacement therapy in the mouse model of Pompe disease. Mol genet metab 2003;80:159-169.
32. Thurberg BL, Malonye CL, Vaccaro C, et al. Characterization of pre- and post-treatment pathology after enzyme replacement therapy for Pompe disease. Lab Invest 2006;86:1208-1220.
33. van der Ploeg AT, Carlier PG, Carlier RY, et al. Prospective exploratory muscle biopsy, imaging, and functional assessment in patients with late-onset Pompe disease treated with alglucosidase alfa: The EMBASSY Study. Mol Genet Metab 2016;119:115-123.
34. Raben N, Roberts A, Plotz PH. Role of autophagy in the pathogenesis of Pompe disease. Acta Myol 2007;26:45-48.
35. Schaaf GJ, van Gestel TJ, Brusse E, et al. Lack of robust satellite cell activation and muscle regeneration during the progression of Pompe disease. Acta Neuropathol Commun 2015;3:65.
36. Wens SC, Ciet P, Perez-Rovira A, et al. Lung MRI and impairment of diaphragmatic function in Pompe disease. BMC Pulm Med 2015;15:54.
37. Mogalle K, Perez-Rovira A, Ciet P, et al. Quantification of diaphragm mechanics in Pompe disease using dynamic 3D MRI. PLoSOne 2016;11:e0158912.
38. Gaeta M, Musumeci O, Mondello S, et al. Clinical and pathophysiological clues of respiratory dysfunction in late-onset Pompe disease: New insights from a comparative study by MRI and respiratory function assessment. Neuromuscul Disord 2015;25:852-858.



Association of muscle strength and walking performance in adult patients with Pompe disease

M. Favejee, J.C. van der Meijden, M.E. Kruijshaar
D. Rizopoulos, A.T. van der Ploeg, J.B.J. Bussmann

5

Submitted

Abstract

Background
The loss of the ability to walk is a most prominent signs of Pompe disease and the associations with muscle strength have not been described.

Objective
To define the associations between walking performance, muscle strength of four specific lower extremity muscle groups and other factors in adult patients with Pompe disease.

Design
Observational study

Methods
Muscle strength (hand-held-dynamometry of hip flexion, -abduction, knee extension and -flexion) and walking performance (unable, able with aids, waddling gait, normal gait) were assessed in 107 adult patients with Pompe disease at their first visit. Relationships between walking performance and muscle strength were studied by multivariate analyses and regression modelling. Age, gender, BMI, disease duration and use of ventilator support were taken into account as potential confounders. We transformed the results into a nomogram to allow the probability of a patient having a certain level of walking performance to be calculated based on the values of the independent variables.

Results
Walking performance declined significantly with decreasing muscle strength of hip flexion, -abduction, knee extension and -flexion. Step-backwards ordered logistic regression analysis resulted in a model including strength of the hip abductor and knee extensor, BMI, age, gender and use of ventilation, explaining 76% of the variance in walking performance.

Limitations
These results are based on cross-sectional data and do not predict future changes.

Conclusions
In adult Pompe patients, walking performance can be explained by muscle strength, BMI, age, gender and ventilation use. Our model gives insight into how a patient is expected to walk based on his risk factors, and serves as a starting point to unravel factors associated with walking performance, and ultimately to develop a prognostic model.

Introduction

Pompe disease is an inheritable metabolic myopathy in which deficiency of the enzyme acid α-glucosidase causes accumulation of lysosomal glycogen, mainly in muscle cells. In adults, Pompe disease is characterized by slowly progressive proximal muscle weakness, which impairs motor and also respiratory function. Eventually patients lose their ability to walk and become wheelchair and respirator dependent.

The loss of the ability to walk is one of the most prominent signs and debilitating effects of Pompe disease and has been shown to be an important factor in determining patients' quality of life.[1] In general, retaining the ability to walk is important to maintain independent from caregivers.[2, 3]

Reduced walking performance has shown to be related to decreased skeletal muscle strength in several populations, including patients with related neuromuscular diseases and elderly.[4-8] However, the associations between walking performance and muscle strength have not been described for Pompe patients. Results from studies in other neuromuscular diseases cannot automatically be generalized to Pompe patients as the distribution of muscle weakness, and thus its effects on walking performance, differs.[8] Also other risk factors, such as age, BMI and respiratory status that might contribute to a reduced walking performance are not well understood in patients with Pompe disease as well. By modeling the relationship between muscle strength and associated risk factors on the one hand, and walking performance on the other, it will be possible to determine a patient's expected position in the spectrum of walking performance compared to a patient's observed walking performance. Both agreement and discrepancies can help therapists to tailor and optimize treatment. In addition, the model can serve as a starting point to further our understanding of the factors that determine walking performance in Pompe disease, and ultimately to develop a prognostic model.

The aim of our study was to better understand and to model the relationships between walking performance, muscle strength of specific lower extremity muscle groups and other factors in adult Pompe patients.

Methods

Participants

Adult patients with a confirmed diagnosis of Pompe disease were included in this study. All patients were first seen between December 2003 and August 2012 at the Center for Lysosomal and Metabolic Diseases of the Erasmus MC University Medical Center, Rotterdam, the national referral center for patients with Pompe disease in the Netherlands. Patients were excluded if they had co-morbidities that affected their walking performance.

On visiting the center, patients were subjected to a standardized set of outcome tests, including assessment of muscle strength and motor function tests encompassing the ability to walk. Data from the patients' first visit to the center was analyzed retrospectively. We also recorded age, gender, height, weight, and disease duration (time since diagnosis) for each patient. All patients signed informed consent.

Outcome measures

Walking performance was classified in four categories (unable to walk, walking with aids, walking without aids but with a waddling gait, or walking without aids and with a normal gait) according to the item "walking 10 meters" of the Quick Motor Function Test (QMFT). This is a valid and reliable motor function test specially designed for Pompe patients[9] consisting of 16 items related to daily activities. The item "walking 10 meters" was assessed by asking patients to walk a 10-m course at their usual pace. Use of aids (canes or walkers) was allowed for this test, but patients were challenged to achieve the maximum performance, i.e., those who used a wheelchair but could still walk 10 meters were asked to do so. Patients were classified as unable to walk when fully wheelchair bound. Partially wheelchair-bound patients who were capable of walking 10 meters were scored as "able to walk with aids". Normal gait was considered as a gait pattern without a Trendelenburg or Duchenne sign or swayback.

Skeletal muscle strength exerted during maximal voluntary isometric contractions was measured by hand-held-dynamometry (HHD) (Cytec dynamometer, Groningen, the Netherlands). The following lower proximal muscle groups were tested using the break test technique[10]: hip flexors, hip abductors, knee flexors and knee extensors. Specifics of the test positions, stabilization and dynamometer placement have been described elsewhere.[11] Muscles were tested separately for each leg and values were averaged for the two legs. The absolute HHD values were used and expressed in Newton. All measurements were carried out by three physicians specially trained to perform HHD and connected to our center.

Data analysis

Median muscle strength values were plotted against the four levels of walking performance using box plots and compared using the Mann Whitney test to assess the relationships of the individual muscles with walking performance.

Ordered logistic regression was used to build a model describing walking performance based on muscle strength of the four lower extremity muscle groups, age, gender, body mass index (BMI), disease duration and use of ventilator support.[2, 12, 13]

A step backward method was used, starting with a full model containing all possible independent variables. Variables that didn't contribute to the model were excluded at a cut-off significance level of 0.20.

Internal validation of the (full and subsequent) models was assessed using the Bootstrap technique with 1000 samples. As measures of predictive ability we use the area under the receiver operating characteristic curve (AUC, discriminative ability), Nagelkerke's R^2 (goodness of fit), and the Brier Score (predictive accuracy). The most parsimonious model that performed satisfactorily in the validation step was reported here.

Next, based on the most parsimonious model, a nomogram was constructed which allows the chance of being in one of the four levels of walking performance to be calculated directly from given specific values of the independent variables.

Statistical analyses were performed using SPSS for Windows (release 21.0; SPSS, Inc., Chicago, IL) and R: A Language and Environment for Statistical Computing, version 3.2.2 using the rms package (rms: Regression Modelling Strategies, Frank E Harrell Jr, R package version 4.4-1, 2015). Visualization was performed using GraphPad Prism (version 5) and the rms package.

Results

Participants

During the study period 108 adult patients with Pompe disease were first seen at the national referral center and examined. One patient was excluded from the analyses because this patient had Pompe disease in combination with spina bifida, both interfering with muscle strength.

Table 1 presents the characteristics of the 107 included patients at their first visit to the center. Patients had a median age of 50 years (range 25-76) and had experienced symptoms for a median of 15 years. 28% were ventilator dependent. In terms of walking performance, 11% were fully wheelchair bound, 28% used walking aids, and 43% walked with an abnormal gait without aids and the remainder (18%) had a normal walking pattern. None had started treatment with ERT at time of examination for this study.

Table 1 | Characteristics of 107 adult patients with Pompe disease (first visit to referral center.

Patient characteristics at first visit	Study population (n=107)
Gender (*male*), no (%)	55 (51.4%)
Age (*years*), median (range)	50 (25-76)
BMI (kg/m^2), median (range)	24.1 (15-48)
Disease duration (*years*), median (range)	15 (2-48)
Walking performance, no (%)	
Unable to walk	12 (11.2%)
Walking aids	30 (28%)
Walking without aids with waddling gait	46 (43%)
Normal gait	19 (17.8%)
Respiratory support at first visit, no (%)	
No ventilator use	77 (72%)
Ventilator dependent	30 (28%)

Association between walking performance and lower proximal muscle strength

Figure 1 shows the differences between each of the consecutive walking categories in strength of the hip flexors, hip abductors, knee extensors and knee flexors. Walking performance declined with decreasing muscle strength. This was most obvious for the hip flexors and hip abductors, where muscle strength differed significantly between each consecutive level of walking performance ($p<0.01$). For knee extension, no significant difference in strength of the knee extensors was found between patients with a waddling gait and those walking normally. For strength of knee flexors there were no differences between any of the consecutive walking categories.

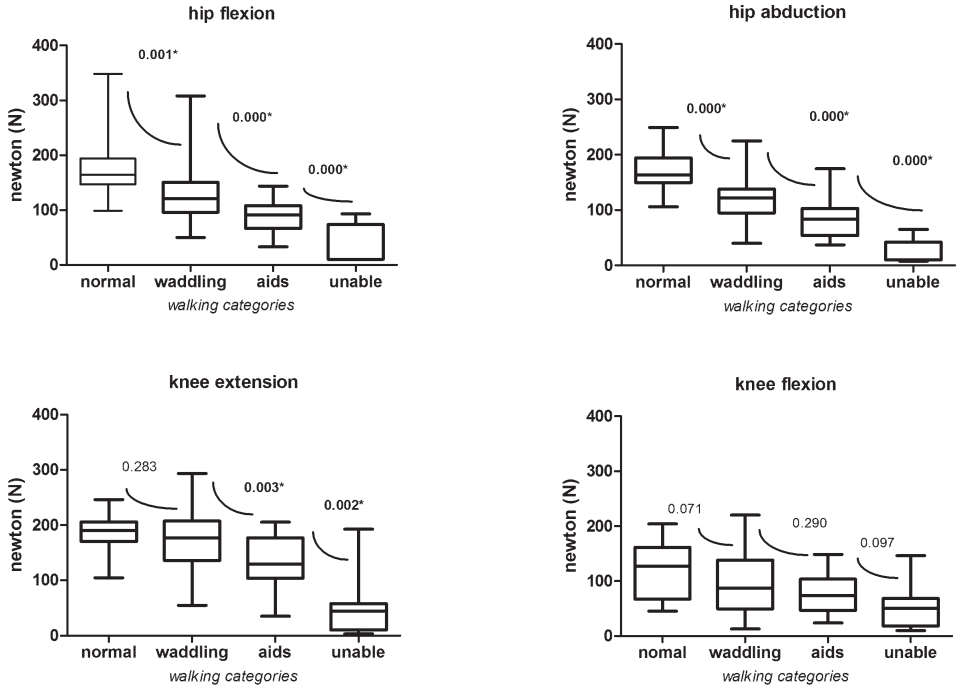


Figure 1 | Relationship between lower extremity muscle strength (expressed in Newton) and walking performance.

Unable: fully wheelchair bound; aids: walking with walking aids; waddling: waddling gait; normal: normal gait pattern.* significant differences.

Table 2 displays the patients' muscle strength values and other risk factors across the four walking categories. Besides reduced muscle strength, patients with more impaired walking performance were older, had been symptomatic for longer and were more often ventilated compared to those with normal / less impaired walking performance. Also higher BMI and male gender seemed to be more frequent in the more impaired patients.

Stepwise backward elimination of the variables in Table 2 ($p<0.20$) resulted in an ordered logistic regression model containing the variables strength of the hip abductors and knee extensors, gender, age, ventilation use and BMI. This was also the most parsimonious model and performed well on internal validation (discriminative ability: 0.76; goodness of fit: 0.66; slope: 0.86; Brier score: 0.14). Higher strength of the hip abductors (OR:1.042; CI [1.026-1.057]) and knee extensors (OR:1.011; CI [1.001-1.022]), lower age (OR:0.968; CI [0.931-1.005]), lower BMI (OR:0.837; CI [0.753-0.930]), female gender (OR:0.365; CI [0.137-0.972]) and not using ventilator assistance (OR:4.540 CI [1.379-14.950]) are associated with the probability of being in a better walking category.

Table 2 | Characteristics and muscle strength across the four walking categories.

	Normal (n=19)	Waddling (n=46)	Aids (n=30)	Unable (n=12)	*P*-value*
Muscle strength (*Newton*)					
Hip flexion	164.5 (99-348)	121.1 (50-308)	91.0 (33-144)	10 (10-93)	0.000*
Hip abduction	163.5 (106-249)	121.8 (40-225)	83.5 (37-175)	10 (7-65)	0.000*
Knee extension	190.5 (105-246)	176.5 (55-293)	129.1 (35-205)	49.0 (3-193)	0.000*
Knee flexion	126.8 (45-204)	87.3 (13-220)	73.4 (24-149)	52.2 (10-147)	0.006*
Characteristics					
Age (*years*)	45.0 (25-72)	46.5 (25-68)	54.0 (26-71)	59.0 (33-76)	0.000*
BMI	23.1 (19-29)	24.2 (17-48)	25.2 (20-38)	23.2 (15-28)	0.074
Disease duration (*years*)	1.0 (0-27)	1.5 (0-19)	13.0 (0-30)	22.0 (0-32)	0.000*
Ventilation *(% yes)*	5.3	15.2	60.0	83.3	0.000*
Gender *(% male)*	36.8	54.3	43.3	83.3	0.059

Figure 2A shows the results of this model transformed into a nomogram. This visualization of the model allows the probability of being in one of the four categories of walking performance to calculate directly based on given values of the independent variables. The range of each independent variable is related to a corresponding number of points on a linear scale. The sum of the corresponding points for each variable (total points) corresponds with the probability for being in each of the four walking categories. Two example calculations, based on two of our patients both with an observed waddling gait, are shown in Figure 2B.

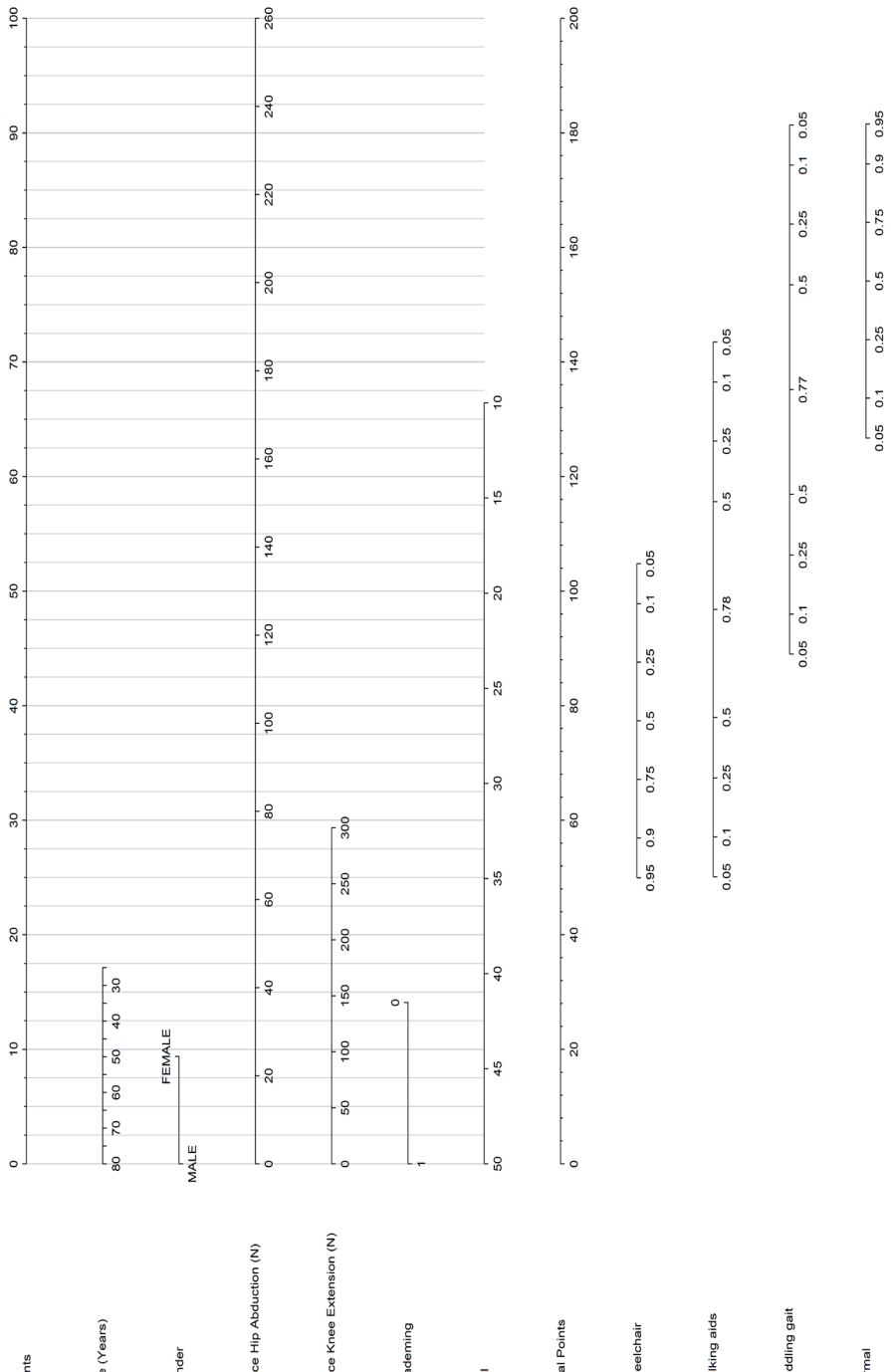


Figure 2A | Nomogram for predicting the chance of being at one of the four categories of walking performance in Pompe patients.

Case report patient I			Case report patient II		
Variables	**Walking category**	**Chances**	**Variables**	**Walking category**	**Chances**
		25%			<5%
42 yr female HA strength 128 N KE strength 147N No ventilation use BMI 22 kg/m^2		70%	50 yr male HA strength 95N KE strength 175N No ventilation use BMI 24 kg/m^2		55%
		<5%			40%
		<5%			<5%

Figure 2B | The probability of being in one of the four categories of walking performance can be calculated by looking up the corresponding points for the values of each independent variable and adding these. The total number of points and corresponding probabilities for being in one of the four walking categories is shown in the bottom part of the graph.

Discussion

In adult patients with Pompe disease walking performance declines with decreasing strength of the lower extremities. This study shows that a patient's chance to have a certain level of walking performance can be calculated based on muscle strength and a number of other risk factors. The model we describe here enables clinicians to compare a patient's actual walking performance to that expected based on his risk factors, and thereby counsel patients on their current disease status and possible supportive measures. It should be further expanded to encompass other factors and ultimately develop a prognostic model.

Walking performance declined most obviously with decreasing strength of the hip flexion and abduction. The strength of knee extension varied significantly between three of the

four walking categories only, while knee flexion didn't distinguish between any of the four consecutive categories. This is consistent with findings based on muscle-driven simulation which showed that gait was most affected by weakness of hip abductors and hip flexors, as well as plantar flexors which are not usually affected in adult Pompe patients.[14] Observed muscle strength values overlapped considerably between the consecutive walking categories, indicating that other patient characteristics, e.g. gender and BMI, may affect walking performance also.[12, 13, 15]. Concerted contraction of muscle groups as a compensation for weakness may play a role as well.[14] Therefore, we developed a multivariate regression model to describe walking performance.

The probability of being in one of the walking categories can be calculated best based on strength of the hip abduction and knee extension, age, gender, BMI and respiratory support. From the four lower extremity muscle strength groups, only hip abduction and knee extension contributed to the final model because of the interdependence between the four groups. Despite disease duration being associated with disease severity,[16] this variable didn't contribute to the model after correcting for age. In our model women had a higher chance of being in a better walking category. These gender differences were also found by De Vries et al.[17]

One of the perspectives of creating a multi-variate model is the application in clinical practice, and therefore the model was transformed into a nomogram. By doing this, a patient's chance to have a certain level of walking performance can be calculated based on his risk factors. While a patient's actual walking performance can be observed, the nomogram allows a clinician to compare this to the probability of being in the four walking performance categories based on the patient's muscle strength and risk factors. This comparison has several potential clinical benefits. For example, in case that the expected (i.e. the walking category with the highest probability) and observed walking performance are the same, there are two options: 1) the probability of the expected walking category might be the clearly highest, or 2) the probability of the expected walking category is quite close to the probability of an adjacent walking category. In the first condition, a patient can be reassured and has not to be prepared on a change to another walking category. In the second condition, a patient can be advised to do strength training and/ or lose weight in order to prolong this particular ambulant status. In case of a discrepancy between observed and expected walking performance, this might draw the attention to the issues of overload (when the observed walking category is "higher" than the expected one) or underload (in case of a "lower" walking category than calculated).

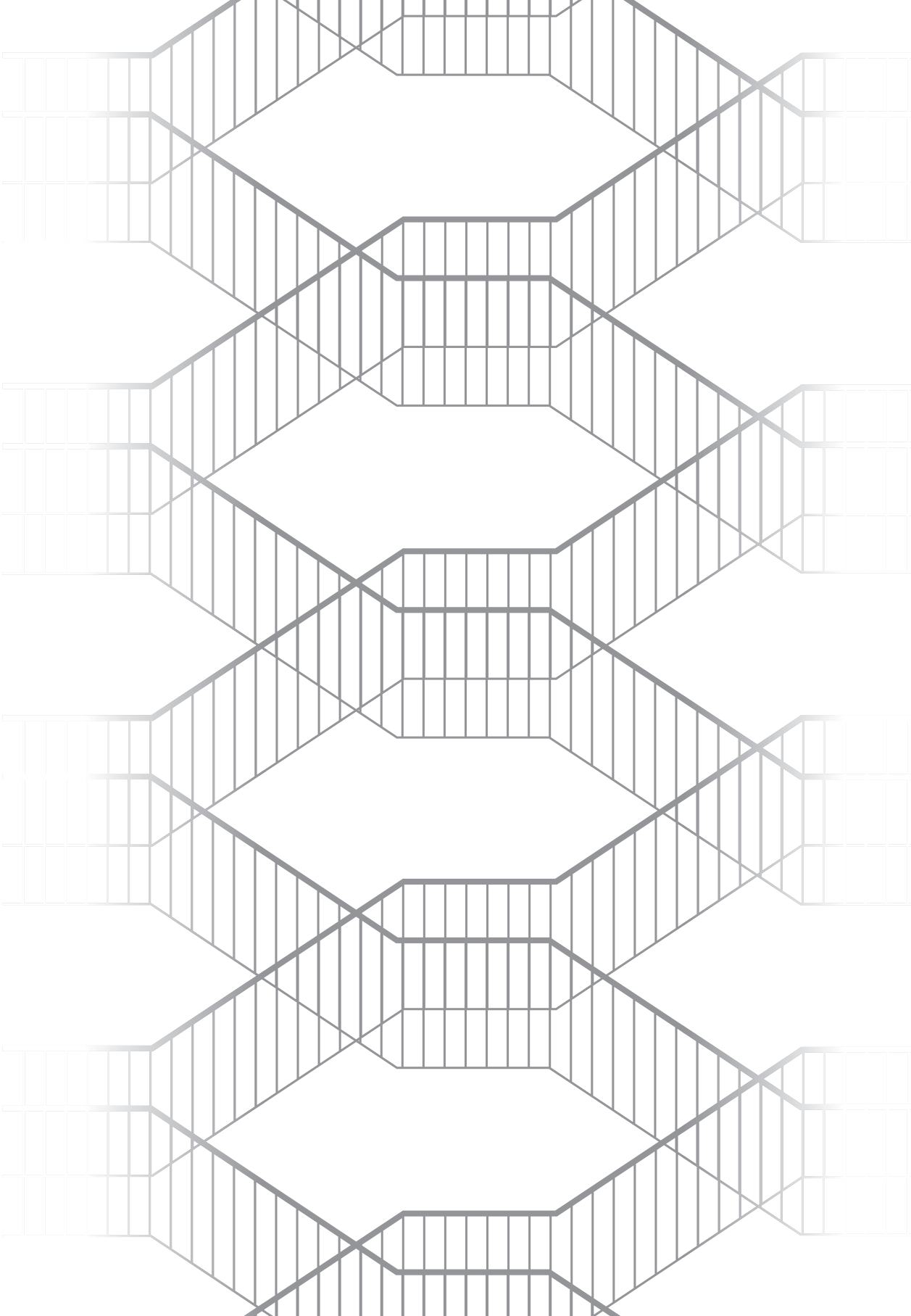
In the current sample, our model explains 76% of the variance in walking performance. Hence, in 24% of the cases the observed walking performance deviated from the model results. Other explanatory factors may play a role as well. For example, one of our patients had a high probability of walking with aids (70% chance), but was observed to walk with a waddling gait (i.e. overloading). Further inspection showed us that this patient had a substantial scapula alata preventing the use of a walking stick. This comes at the price of a high fall risk and alternative solutions to walking aids need to be searched for or a wheelchair recommended. This example illustrates that further factors may need to be incorporated to accurately predict walking performance in a prognostic model.

Our analyses are based on a cohort of more than 100 adult Pompe patients, which is very large given the rarity of the disease. However, to develop an accurate model patient numbers are relatively small. Nevertheless, internal validation of the model yielded a reasonable concordance between actual and estimated walking performance, and our model explained 76% of the variance in walking performance. A lack of external validation can be regarded as a limitation of our study. The model's validity in other populations remains to be studied. Furthermore, the nomogram was based on cross-sectional data rather than longitudinal data, and as a consequence it does not provide information on a patient's prognosis or future walking performance. Another limitation is related to the measurement of muscle strength. HHD values were obtained using standard testing as described and widely used.[11] However, it is known that inter-observer variation exists in HHD assessments, which can limit the applicability of the nomogram. It has to be noted that in our data set data from different (male and female) assessors were already incorporated. Finally, in Pompe disease trunk muscles are frequently involved[13] which affect strength and performance of the lower extremities.[18] Due to the lack of objective measurements of the core strength[18] this could not be included in our model.

This study shows that reduced walking performance in adult patients with Pompe disease is associated with reduced muscle strength of the lower extremities, as well as with higher age, higher BMI, being male and using a ventilator. We developed a model describing the chance to be in one of four walking categories. This model can support a clinician's subjective judgement on whether a patient - based on his risk factors- is capable of more or less than what a patient shows in terms of walking performance. Moreover, it might serve as a first step towards developing a prognostic model.

References

1. Hagemans ML, Winkel LP, Van Doorn PA, Hop WJ, Loonen MC, Reuser AJ, Van der Ploeg AT. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. *Brain* 2005;128(Pt 3):671-677.
2. Shin H, Liu PY, Panton LB, Ilich JZ. Physical performance in relation to body composition and bone mineral density in healthy, overweight, and obese postmenopausal women. *J Geriatr Phys Ther* 2014;37(1):7-16.
3. Kuptniratsaikul V, Tosayanonda O, Nilganuwong S, Thamalikitkul V. The efficacy of a muscle exercise program to improve functional performance of the knee in patients with osteoarthritis. *J Med Assoc Thai* 2002;85(1):33-40.
4. Buchner DM, Larson EB, Wagner EH, Koepsell TD, de Lateur BJ. Evidence for a non-linear relationship between leg strength and gait speed. *Age Ageing* 1996;25(5):386-391.
5. Hasegawa R, Islam MM, Lee SC, Koizumi D, Rogers ME, Takeshima N. Threshold of lower body muscular strength necessary to perform ADL independently in community-dwelling older adults. *Clin Rehabil* 2008;22(10-11):902-910.
6. Jette DU, Slavin MD, Andres PL, Munsat TL. The relationship of lower-limb muscle force to walking ability in patients with amyotrophic lateral sclerosis. *Phys Ther* 1999;79(7):672-681.
7. Slavin MD, Jette DU, Andres PL, Munsat TL. Lower extremity muscle force measures and functional ambulation in patients with amyotrophic lateral sclerosis. *Arch Phys Med Rehabil* 1998;79(8):950-954.
8. Alfano LN, Lowes LP, Flanigan KM, Mendell JR. Correlation of knee strength to functional outcomes in Becker muscular dystrophy. *Muscle Nerve* 2013;47(4):550-554.
9. van Capelle CI, van der Beek NA, de Vries JM, van Doorn PA, Duivenvoorden HJ, Leshner RT, Hagemans ML, van der Ploeg AT. The quick motor function test: a new tool to rate clinical severity and motor function in Pompe patients. *J Inherit Metab Dis* 2012;35(2):317-323.
10. Bohannon RW. Make tests and break tests of elbow flexor muscle strength. *Phys Ther* 1988;68(2):193-194.
11. van der Ploeg RJ, Fidler V, Oosterhuis HJ. Hand-held myometry: reference values. *J Neurol Neurosurg Psychiatry* 1991;54(3):244-247.
12. Auyeung TW, Lee SW, Leung J, Kwok T, Woo J. Age-associated decline of muscle mass, grip strength and gait speed: a 4-year longitudinal study of 3018 community-dwelling older Chinese. *Geriatr Gerontol Int* 2014;14 Suppl 1:76-84.
13. van der Beek NA, de Vries JM, Hagemans ML, Hop WC, Kroos MA, Wokke JH, de Visser M, van Engelen BG, Kuks JB, van der Kooi AJ *et al*. Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study. *Orphanet J Rare Dis* 2012;7:88.
14. van der Krogt MM, Delp SL, Schwartz MH. How robust is human gait to muscle weakness? *Gait Posture* 2012.
15. Serra AJ, de Carvalho Pde TC, Lanza F, de Amorim Flandes C, Silva SC, Suzuki FS, Bocalini DS, Andrade E, Casarin C, Silva JA. Correlation of Six-Minute Walking Performance with Quality of Life is Domain- and Gender-Specific in Healthy Older Adults. *PLoS One* 2015;10(2).
16. Hagemans ML, Winkel LP, Hop WC, Reuser AJ, Van Doorn PA, Van der Ploeg AT. Disease severity in children and adults with Pompe disease related to age and disease duration. *Neurology* 2005;64(12):2139-2141.
17. de Vries JM, van der Beek NA, Hop WC, Karstens FP, Wokke JH, de Visser M, van Engelen BG, Kuks JB, van der Kooi AJ, Notermans NC *et al*. Effect of enzyme therapy and prognostic factors in 69 adults with Pompe disease: an open-label single-center study. *Orphanet J Rare Dis* 2012;7:73.
18. Willson JD, Dougherty CP, Ireland ML, Davis IM. Core stability and its relationship to lower extremity function and injury. *J Am Acad Orthop Surg* 2005;13(5):316-325.



Differences in disease severity and response to enzyme replacement therapy in Pompe patients with the common c.-32-13C>T GAA gene variant cannot be explained by the ACE I/D polymorphism

J.C. van der Meijden†, E. Kuperus†, S.L.M. in 't Groen
M.A. kroos, M. Hoogenveen, D. Rizopoulos
M.Y. Nino Martinez, M.E. Kruijshaar, P.A. van Doorn
N.A.M.E. van der Beek, A.T. van der Ploeg
W.W.M. Pijnappel

†Contributed equally

6

In preparation

Abstract

The majority of children and adults with Pompe disease in the Caucasian population carry the leaky splice site *GAA* gene variant c.-32-13T>G in combination with a fully deleterious *GAA* variant on the second allele. The phenotypic spectrum of this patient group is exceptionally broad, with symptom onset ranging from early infancy to late adulthood. In addition, the response to enzyme replacement therapy (ERT) is heterogeneous. The insertion/deletion (I/D) polymorphism of the angiotensin I-converting enzyme (ACE) has been suggested to be a modifier of disease onset and/or response to ERT.

Here, we have investigated the effect of the ACE I/D polymorphism in a relatively large cohort of 131 children and adults with Pompe disease that were treated with ERT for a median period of 7.5 years of ERT. Outcome measures were the use of wheelchair and mechanical ventilation, muscle strength assessed via manual muscle testing and hand-held dynamometry (HHD), distance walked on the six-minute walk test (6MWT), forced vital capacity (FVC) in sitting and supine position and daily-life activities assessed by R-Pact.

Cross sectional analysis at first visit showed no differences between the ACE groups for age at first symptoms, diagnosis, wheelchair use, or ventilator use. Also response to ERT using linear mixed models showed no significant differences between ACE groups for any of the outcome measures. The patient cohort contained 24 families with 54 siblings.

Differences in ACE genotype could neither explain inter and intra familial differences. We conclude that the ACE I/D polymorphism insufficiently explains the large variation in disease severity and response to ERT observed among Pompe patients with the same c.-32-13T>G *GAA* gene variant.

Introduction

Pompe disease (OMIM 232300) is a metabolic myopathy caused by pathogenic variants in the acid α-glucosidase *(GAA)* gene (OMIM 606800). This results in deficiency of the lysosomal enzyme *GAA*, leading to an impaired breakdown of glycogen.[1] Clinically, a broad disease spectrum can be observed, ranging from a rapidly progressive classic infantile phenotype to a slower progressing disease course in children and adults. Classic infantile patients present shortly after birth with hypertrophic cardiomyopathy and generalized muscle weakness. Without treatment these patients die within the first year of life due to cardiorespiratory insufficiency.[2,3] Children and adults present with a slower progressive limb girdle muscle weakness, while cardiac involvement is rare. Most of these patients become wheelchair and ventilator dependent. Survival is reduced compared to the general population.[4-6]

The majority of Caucasian children and adults with Pompe disease carry the common c.-32-13T>G (IVS1) variant on one *GAA* allele. The IVS1 variant causes aberrant *GAA* pre-mRNA splicing by inducing partial or complete skipping of *GAA* exon 2. A small (10-15%) of leaky wild type splicing occurs.[7-12] The second *GAA* allele in children and adults with Pompe disease is a 'null' allele which does not generate any *GAA* enzymatic activity. A frequently observed 'null' allele in this population is c.525delT.[13] Interestingly, the clinical disease course in patients with the IVS1 variant shows an exceptionally broad spectrum. For example, disease onset can vary from early infancy to late adulthood. Such differences are even observed between patients with the same IVS1/c.525delT *GAA* genotype.[14-16] The variation between siblings is much less broad, suggesting that disease progression can be modified by genetic background factors.[17]

Since 2006, enzyme replacement therapy (ERT) with recombinant human *GAA* (rhGAA), is available for Pompe disease. In children and adults with Pompe disease ERT has shown to improve muscle function and strength and to stabilize pulmonary function. However, individual responses can vary considerably.[18] This is observed irrespective of formation of anti-rhGAA antibodies, suggesting that other factors exist that can modify the response to ERT in this patient group.[19]

It has been suggested that a polymorphism in the angiotensin converting enzyme (ACE) gene – the insertion (I) or deletion (D) of an alu repeat in intron 16 may affect phenotypic variation and response to ERT in patients with Pompe patients.[20-23] Four studies were performed, but different results were obtained.[20-23] The DD genotype was associated with an earlier disease onset in some studies, but not in others, and it was associated with less favorable response to ERT with regard to muscle mass in one study, and with regard

to FVC and 6MWT in another study. The different outcomes may be explained by small group sizes and a short follow up, by different inclusion criteria that eliminated severely affected patients, and by omission of multiple testing correction while calculating statistical differences. This was reason to perform the current nationwide study in a group of 131 children and adults representing the full disease spectrum, all with the same c.-32-13T>G/null gene type. The aim was to further explore the potential influential effect of the ACE polymorphism on age of onset, disease severity and outcome of patients when treated with ERT for a median of 7.4 years.

Materials and methods

Patients and study design

This study was part of an ongoing single-center prospective, open-label study, in which all Dutch children and adults with a confirmed diagnosis of Pompe disease – by enzyme analysis in leucocytes or fibroblasts, and by DNA analysis – participated.[15,24-26] Only patients that carried the c.-32-13 T>G (IVS1) *GAA* variant on one allele and a fully deleterious ("null") *GAA* variant on the other allele were included in this study. Information was collected at the moment of onset of symptoms, diagnosis and wheelchair- of ventilator dependency. Clinical assessments were performed with 3-6 months intervals. Daily life activities were assessed via the Rasch-built Pompe-specific Activity (R-PAct) scale.[27] The assessments and analysis of data were performed without prior knowledge of the ACE polymorphism status. Data were collected from January 1, 1999 through January 1, 2016. The study was conducted according to the Declaration of Helsinki, the Institutional Review Board approved the study protocol, and all patients, or their parents or legal guardians, provided written informed consent.

ACE polymorphism

ACE genotyping was performed based on the methods described by Al-Awadhi et al.[28] In short, genomic DNA from blood or fibroblasts was used in a first PCR flanking the alu insertion in intron 16 using the following primers: fw1: 5'-CTGGAGACCACTCCCATCCTTTCT-3' and rev1: 5'-GATGTGGCCATCACATTCGTCAGAT-3'. PCR was performed using FastStart PCR (Roche) in which 6% DMSO was included in the reaction mixture. Reactions were run on a Bio-rad C1000 Touch Thermal Cycler. When no I allele was found, a second PCR was performed using an alu insertion-specific internal primer fw2: 5'-TGGGATTACAGGCGTGATACAG-3', which was used in a PCR with the same primer rev1 as above. Positive, negative, and blank controls for the ACE genotype were included in all analyses.

Clinical Outcome Measures

The use of wheelchair and mechanical ventilation was registered at each visit. Skeletal muscle strength was assessed using the Medical Research Council (MRC) grading scale and hand-held dynamometry (HHD; Cytec dynamometer, Groningen, The Netherlands).[29-31] The following muscle groups were tested for either method: neck flexors, shoulder abductors, elbow flexors, elbow extensors, hip flexors, hip abductors, knee flexors, and knee extensors. Additionally, an MRC grade was determined for the hip extensors and hip adductors. This was expressed as percentage of the maximum possible score for MRC sum scores, and as a percentage of the median strength of healthy males and females for HHD sum scores. Sum scores were calculated if no more than two muscle groups were missing. Muscle function was assessed using the Quick Motor Function Test (QMFT), consisting of 16 motor skills related to daily life that were particularly difficult for Pompe patients.[32] Muscle endurance was assessed using the six-minute walk test (6MWT) in which the distance walked in 6 minutes was recorded.[33] Forced vital capacity (FVC) was measured in upright and supine positions. Results were expressed as percent of predicted FVC.[34] The R-PAct scale was used to assess patients' self-reported ability to perform daily life activities. A score was calculated as described,[27] but only when all items had been answered. Only adult patients performed this test.

Statistical analysis

Differences in characteristics between the three different ACE genotypes (II, DD and ID) at first visit were calculated as follows. First we tested if any of the ACE groups differed from the others using the Kruskal-Wallis test for numerical data and the chi-square test (2x3) for categorical data. When significant, the Mann-Whitney and chi-square tests (2x2) were used to identify which of the groups (II vs ID, II vs DD or ID vs DD) differed. Since this post-hoc analysis involved 3 extra tests, these 3 tests were corrected for multiple testing using the Holm method.[35]

Longitudinal analyses of the effects of ERT were performed using linear mixed effect models to account for repeated measurements per patient. Models were fitted for each outcome measure using the nlme package of the statistical program R (version 3.2.5).[36,37] Time was expressed as years after start of ERT. To account for potential non-linear profiles we used natural cubic splines (up to 3 degrees of freedom) in the fixed-effects and random-effects parts of the model. For the random-effects part of the model an unstructured covariance matrix was used. Likelihood-ratio-tests were used to asses if there was an interaction between time and ACE polymorphism; e.g. if outcome measures progressed differently during treatment for each ACE polymorphism or if the different polymorphisms had different intercepts; e.g. overall disease severity. Obtained p-values were corrected for

multiple testing using the Holm method.[35] Plots for the group means were generated for the different outcome measures for the first 7.5 years of ERT. Since the median follow-up in this study was 7.4 years, extrapolation of the model beyond 7.5 years would be based on only a few data points and prone to bias. Siblings from various families were identified. For each sibling the age at first symptoms, diagnosis, the start of ERT, the start of wheelchair/ventilator use, and their last follow-up or death were plotted to study if different ACE genotypes explained variation within families. Siblings were classified as varying when an event occurred in one sibling and did not occur within 10 years in the other sibling.

Results

Effect of ACE I/D polymorphism at first clinical examination: cross sectional analysis

A total of 146 Dutch patients with the IVS1/"null" genotype mutation were known in our center at data closure. The ACE genotype could be determined for 131 patients (Table 1). The characteristics of the total patient group (n=131) were as follows. Gender was evenly distributed (50% females) in the total group and the three different ACE groups (II, DD and ID). Most patients (86%) started ERT at adulthood (>18 years of age). Median ages (in years) at symptom onset, diagnosis, first visit, and start of ERT were 31 (0-62); 38 (0-72); 46 (0-75); and 49 (1-76), respectively. At first visit, 31% of patients used a wheelchair, while the median age at which patients started to use a wheelchair was 49 (11-76). For usage of a ventilator, these numbers were 22% and 52 years of age (6-72). The ranges of all parameters were broad, and varied from a minimum of zero to a maximum of 76 years, highlighting the heterogeneity of disease progression of Pompe patients with the IVS1 variant.

The ACE polymorphism genotype was normally distributed within the total patient group (II: 24%; ID: 44%; DD: 31%). The patient group was divided in three ACE genotypes, and the parameters listed in Table 1 were compared between the three groups in a cross sectional analysis. This showed that none of the parameters were different between the groups. The use of a wheelchair at first visit was found to be different with a p-value of 0.047 with the lowest number of wheelchair users in the DD group, but post-hoc testing showed that this was not significant. Slightly more II patients started ERT during childhood, but the differences between ACE groups were not significant either.

Table 1| Patient characteristics and cross-sectional analyses at first visit.

		ACE polymorphism			
		II	DD	ID	*p*-value*
Total group	131 (100%)	32 (24%)	41 (31%)	58 (44%)	
Gender, No. of patients (%)					
Male	65 (50%)	15 (47%)	20 (49%)	30 (52%)	n.s.
Female	66 (50%)	17 (53%)	21 (51%)	28 (48%)	
Start ERT during childhood (<18y), No. of patients (%)					
Yes	18 (14%)	8 (25%)	3 (7%)	7 (14%)	n.s.
No	113 (86%)	24 (75%)	38 (93%)	51 (86%)	
Median age (range) at:					n.s.
Onset of symptoms	31 (0-62)	28 (0-54)	33 (4-61)	30 (0-62)	n.s.
Diagnosis	38 (0-72)	35 (0-69)	42 (0-67)	38 (0-72)	n.s.
First visit	46 (0-75)	41 (2-69)	47 (6-71)	47 (0-75)	n.s.
Start ERT	49 (1-76)	42 (1-68)	50 (14-73)	50 (1-76)	n.s.
Wheelchair use at first visit, No. of patients (%)					
No	91 (69)	22 (69)	23 (56)	46 (79)	n.s.[a]
Yes	40 (31)	10 (31)	18 (44)	12 (21)	
Median age at start wheelchair use (range)	49 (11-76)	43 (11-60)	51 (24-64)	55 (33-76)	n.s.
Ventilation use at first visit, No. of patients (%)					
No	102 (78)	28 (88)	31 (76)	43 (74)	n.s.
Yes	29 (22)	4 (12)	10 (24)	15 (26)	
Median age at Start ventilation use (range)	52 (6-72)	53 (33-61)	48 (6-72)	51 (13-69)	n.s.

[a]Null hypothesis II = ID = DD rejected at the p=0.047 level. Post-hoc testing (II vs ID, II vs DD and ID vs DD) did not show significant differences between the ACE groups.
* *p*-value for the test whether any of the groups differ from each other.

Effect of ACE I/D polymorphism on response to enzyme replacement therapy

A total of 112 patients within the cohort received ERT and were included in longitudinal analysis. Follow-up ranged from 0.18 - 16.1 years (median 7.4 years). We followed the response to ERT by assessment of muscle strength (MRC sumscore, HHD), muscle function (6 minute walking test, QMFT), respiratory function (FVC in sitting and supine positions), and daily life activities (R-PACT). The results were analyzed using linear mixed effects models. For some parameters, a difference was observed between the II and the DD genotype groups, notably for R-PACT, QMFT, and FVC in sitting position, with a more favorable outcome for the II group. However, following multiple testing correction, the differences were not significant.

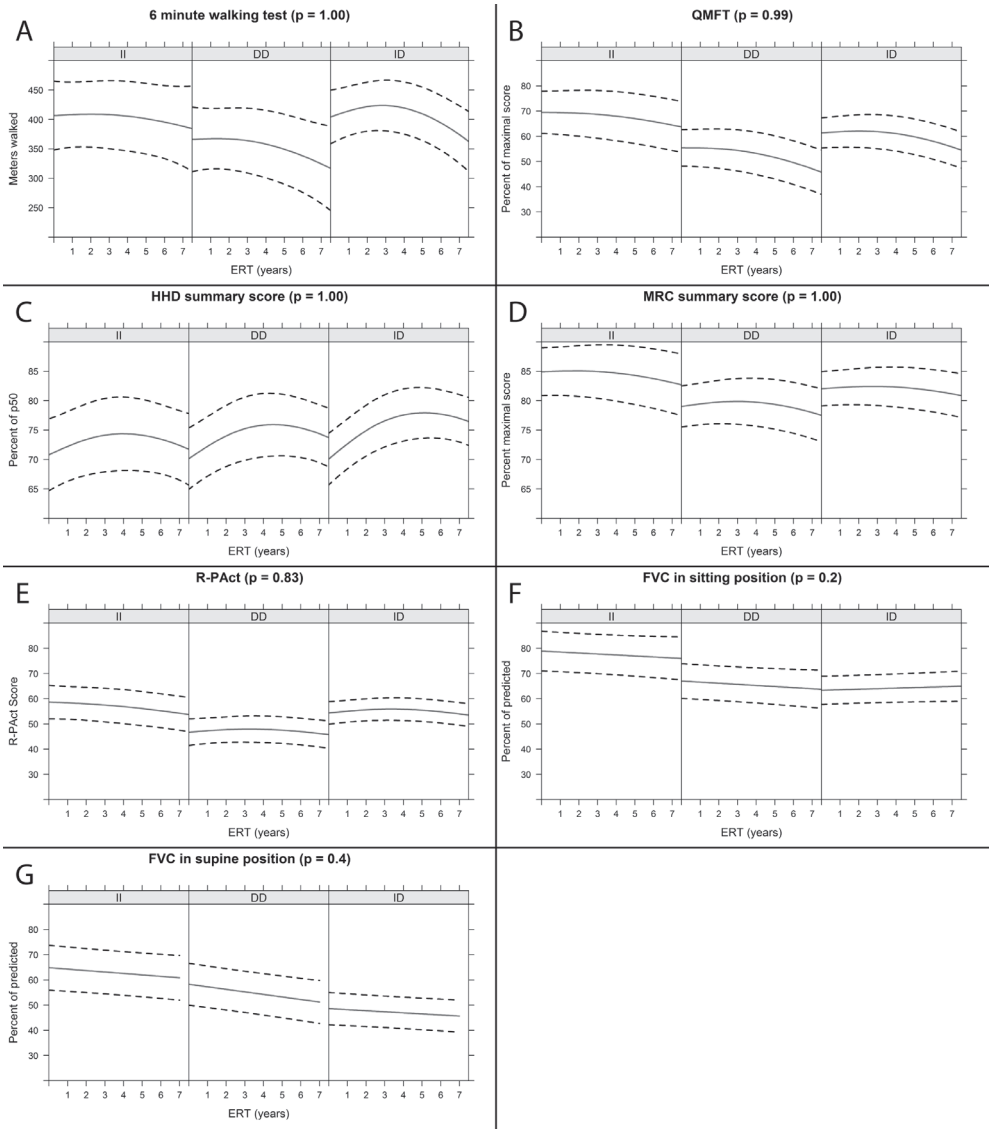


Figure 1 | Response to ERT stratified for ACE genotype. Predicted group mean scores (solid line) over time as analyzed using linear mixed effects models.

(**A**) 6MWT, (**B**) QMFT, (**C**) HHD, (**D**) MRC, (**E**) R-Pact, (**F**) FVC in sitting position, (**G**) FVC in supine position. 95% prediction intervals are indicated as areas between dotted lines.

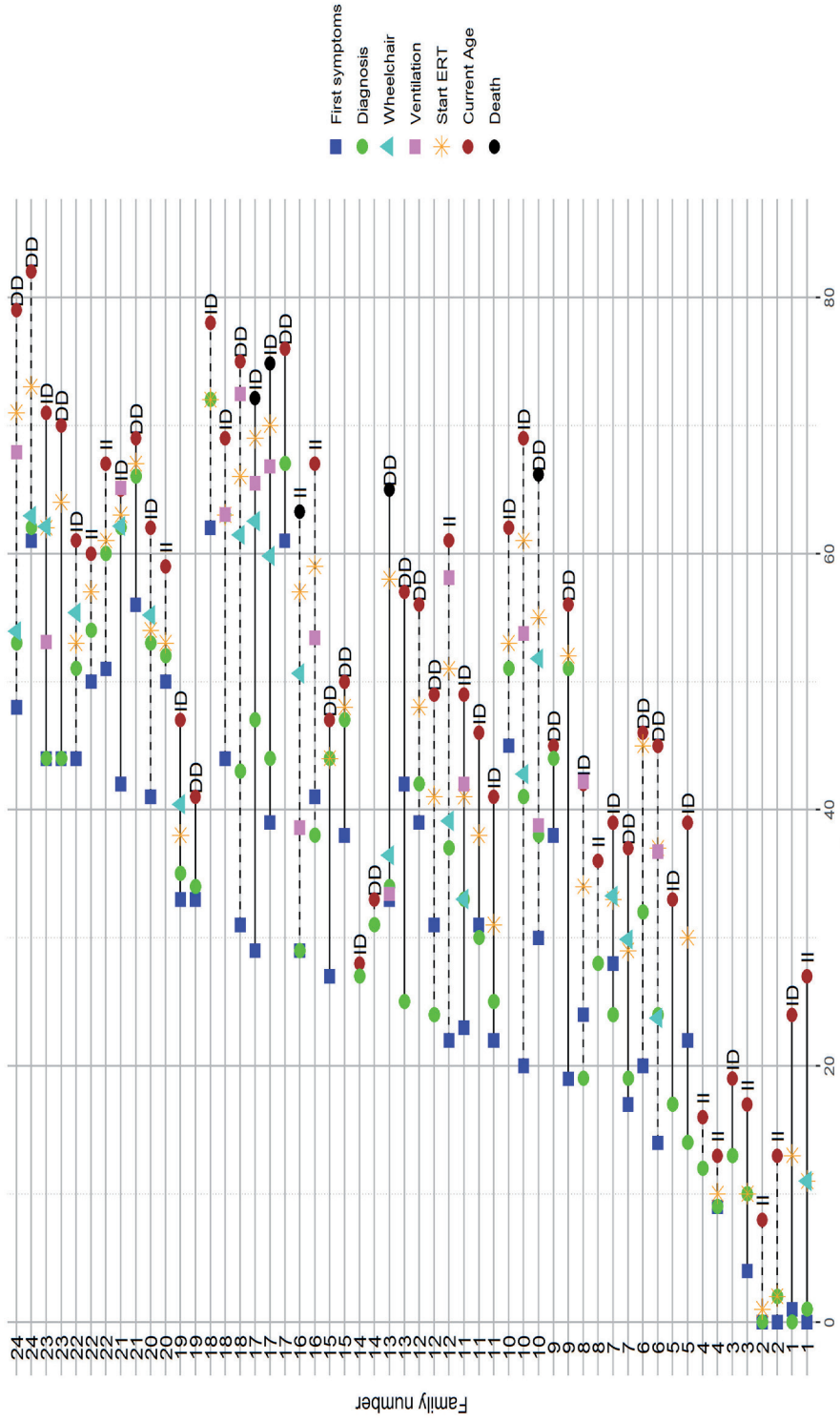


Figure 2 | Overview of the distribution of life-events in families.

Subjects from the same family have the same family number and the same line type (dashed or solid). Families are sorted by the age at first symptoms of youngest family member. Blue squares indicate the age at first symptoms, green circles the age at diagnosis, light blue triangles the age patients started to use a wheelchair, pink squares the age patients started to use ventilator support, orange asterisks the age at which ERT was started, brown circles the current age, and black circles indicate the age at which patients deceased. The ACE genotype of individual patients is notated after their current age or age at death.

ACE polymorphisms within families

Our cohort of patients included 54 siblings from 24 different families (2-3 siblings per family). Age at first symptoms, diagnosis, start of wheelchair use, ventilator use, ERT, survival, and ACE genotype are shown for each patient in Fig 2. Families are ordered by the onset of disease of the youngest family member. In 14 of the 24 families, siblings had discordant ACE polymorphisms, while 10 had the same ACE polymorphism. We found siblings with discordant ACE genotypes but with similar disease courses (8 siblings from 4 families; families 14, 19, 20 and 22), while we also found siblings with the same ACE genotype but very different disease courses (21 siblings from 10 families; families 5, 6, 9, 10, 11, 13, 15, 16, 18 and 24). We conclude that no clear influence of the ACE genotype on onset of disease symptoms can be detected within these families.

Discussion

Pompe patients with the IVS1 variant represent the largest Caucasian patient group with childhood or adult disease onset, and have a particularly large variation in phenotype, with symptom onset ranging from 3 to 60 years of age.[5,15,38] The potential contribution of the ACE I/D genotype as a modifier of Pompe disease has been investigated here. The results of the current study indicate that such contribution, if it exists, is too small to explain the phenotypic heterogeneity in this patient group. This applies both to differences in disease severity and to the response to ERT. Analysis of siblings with the same IVS1 genotype also did not point to a contribution of ACE genotype to clinical variation within families. These results are discussed in the light of other reports and with respect to the statistical power needed to identify a genetic modifier for Pompe disease.

A priori, at least two categories of genetic factors can be envisioned as potential modifiers of Pompe disease. The first is a modifier of splicing, because the IVS1 variant is a splicing variant. For example, in theory it is possible that polymorphisms in splicing factors affect the amount of leaky wild type splicing by the IVS1 variant. So far, this remains a theoretical possibility. If such polymorphism would exist, it would likely be present in a general splicing factor that is shared between fibroblasts and skeletal muscle cells, because these two cell types show similar aberrant splicing patterns.[39] Another category is skeletal muscle function, as skeletal muscle is the main affected tissue in Pompe disease. The ACE I/D genotype falls in this category as it has been associated with performance of top athletes. Depending on the type of sport and its requirement for either endurance or strength, groups of top athletes are either enriched in the I or the D ACE genotype, respectively.[40-46] Considering the exceptionally broad phenotype, a strong modifier is expected to explain the large variation in disease severity. Alternatively, modulation of the phenotype may occur via a combination

of genetic factors with small effects that, when combined, have a strong effect on muscle function.

The first study on the effect of the ACE I/D genotype on the natural course of Pompe patients was published in 2010. This study included 38 patients, 36 of whom contained the IVS1 *GAA* variant.[20] Sixteen parameters were tested in cross sectional analyses. The results suggested significant differences for some parameters, notably an association between the DD genotype and a worse Walton score (which scores disease severity), an earlier disease onset, more muscle pain, and higher CK levels were found. In 2014, the initial study was extended to 85 patients that contained the IVS1 variant.[21] Associations were found between the DD genotype and pain, but in contrast to the previous study, no associations between ACE genotype and Walton score, CK levels or other clinical parameters were found in cross sectional analyses. Another study was published in 2016, in which 58 patients that had previously been included in the LOTS study were analyzed.[23] This showed no association between ACE genotype and any parameter including onset of first symptoms, disease duration, 6MWT, or FVC rating disease severity. It should be noted that the LOTS study represents a selection of Pompe patients. Patients had to fulfill the inclusion criteria and as a result both very mildly affected and severely affected patients were excluded. For example, patients who were not able to walk for more than 40 meters were excluded from this study. In the present study, no inclusion criteria were applied other than the presence of the IVS1 variant and a confirmed diagnosis of Pompe disease. Given the conflicting results published so far, we wished to test the effect of the ACE genotype in a relatively large patient cohort of 131 patients including both children and adults of various ages and different disease severities. This revealed no significant effects of the ACE genotype on any of the parameters tested. It should be noted that initial statistical analysis suggested some significant differences between different ACE genotype groups, but that these turned into non-significant p-values after multiple testing correction. Taken together, the initial idea that the ACE DD genotype may be associated with faster symptom onset and more severe muscle symptoms could not be confirmed in subsequent studies including the present study.

Two previous publications have reported on the effect of the ACE I/D genotype on the response to ERT. In a study on 16 patients with the IVS1 allele that were treated for > 2 years with ERT, the DD genotype (n=3) was associated with reduced muscle mass, while no associations were found with muscle strength, FVC, or 6MWT.[22] In the previously mentioned LOTS study, in which 58 moderately affected patients were treated with ERT for 78 weeks, the DD genotype (n= 17) was associated with a poorer response to ERT with respect to FVC in sitting position.[23] One other parameter was tested, namely the 6MWT, and this showed

a better response in patients with the ID genotype and a trend toward a better response in patients with the II genotype compared to the DD genotype. Other parameters for muscle strength and function were not reported in this study. In the present study, 119 patients were treated with ERT for a period up to 16.1 years (median 7.4 years), and severely affected patients were not excluded. Patients with the DD genotype in general scored lower compared to the II genotype for several parameters, including 6MWT, QMFT, QMFT, FVC, and R-PACT. However, the effects were small and not statistically significant. Altogether, the data available to date do not support the idea that the ACE genotype can explain the heterogeneous response to ERT in juvenile and adult Pompe patients.

The question arises whether we would have been able to detect an effect of the ACE genotype in our patient cohort. This would depend on the number of patients and the degree of variation. To address this, we performed three types of post-hoc power analyses (details are outlined in the supplementary text). In all of these analyses, a single outcome measure has been used to avoid correction for multiple testing. In the first, we assumed that the ACE genotype is the only modifying factor that is responsible for phenotypic variation in patients with the IVS1 variant. As a primary outcome measure, we used symptom onset. In our patient cohort, we calculated that 12 patients would be required to demonstrate a significant effect at a power of 0.96. In the second analysis, we assumed that ACE genotype is not the only modifying factor. To reach a power of 0.86 to reach significance for detecting an effect of ACE genotype on symptom onset based on the distribution observed in our population (see table 1), we would need 930 patients. In the third analysis, we selected FVC in sitting position as an outcome measure for the effect of ERT. When only this outcome measure would be used, we would have a power of 0.83 to detect the small effect of FVC that we measured in our cohort when including 90 patients with a follow up of 5 years of ERT treatment (generating 4 datapoints per year). We conclude from this post-hoc analyses that the effect of the ACE I/D genotype on natural course is too small te be detected with currently available patient cohorts, and that it is not a major modifying factor that explains the heterogeneity between patients. For the response to ERT, only small effects of the ACE genotype could be detected in our cohort, and these are not sufficient to fully explain the variable response. The search for modifying factors that can explain phenotypic variation in Pompe disease should continue.

References

1. Hirschhorn R RA: Glycogen storage disease type II: acid alpha-glucosidase (acid maltase) deficiency. In: *The Metabolic and Molecular Bases of Inherited Disease.* 8th edition edn. Edited by Scriver CR BA, Sly WS, Valle D. New York: McGraw-Hill; 2001: 3389-3420.
2. van den Hout HM, Hop W, van Diggelen OP, Smeitink JA, Smit GP, Poll-The BT, Bakker HD, Loonen MC, de Klerk JB, Reuser AJ *et al*. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics* 2003;112(2):332-340.
3. Kishnani PS, Hwu WL, Mandel H, Nicolino M, Yong F, Corzo D, Infantile-Onset Pompe Disease Natural History Study G. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *J Pediatr* 2006;148(5):671-676.
4. Beek NA, Hagemans ML, Reuser AJ, Hop WC, Ploeg AT, Doorn PA, Wokke JH. Rate of disease progression during long-term follow-up of patients with late-onset Pompe disease. *Neuromuscul Disord* 2009;19.
5. Beek NA, Vries JM, Hagemans ML, Hop WC, Kroos MA, Wokke JH, Visser M, Engelen BG, Kuks JB, Kooi AJ. Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study. *Orphanet J Rare Dis* 2012;7.
6. Laforet P, Nicolino M, Eymard PB, Puech JP, Caillaud C, Poenaru L, Fardeau M. Juvenile and adult-onset acid maltase deficiency in France: genotype-phenotype correlation. *Neurology* 2000;55(8):1122-1128.
7. Boerkoel CF, Exelbert R, Nicastri C, Nichols RC, Miller FW, Plotz PH, Raben N. Leaky splicing mutation in the acid maltase gene is associated with delayed onset of glycogenosis type II. *Am J Hum Genet* 1995;56(4):887-897.
8. van der Wal E, Bergsma AJ, Pijnenburg JM, van der Ploeg AT, Pijnappel W. Antisense Oligonucleotides Promote Exon Inclusion and Correct the Common c.-32-13T>G GAA Splicing Variant in Pompe Disease. *Mol Ther Nucleic Acids* 2017;7:90-100.
9. Chen L, Bush SJ, Tovar-Corona JM, Castillo-Morales A, Urrutia AO. Correcting for differential transcript coverage reveals a strong relationship between alternative splicing and organism complexity. *Mol Biol Evol* 2014;31(6):1402-1413.
10. Jangi M, Sharp PA. Building robust transcriptomes with master splicing factors. *Cell* 2014;159(3):487-498.
11. Lee Y, Rio DC. Mechanisms and Regulation of Alternative Pre-mRNA Splicing. *Annu Rev Biochem* 2015;84:291-323.
12. Bergsma AJ, Kroos M, Hoogeveen-Westerveld M, Halley D, van der Ploeg AT, Pijnappel WW. Identification and characterization of aberrant GAA pre-mRNA splicing in pompe disease using a generic approach. *Hum Mutat* 2015;36(1):57-68.
13. Kroos MA, Van der Kraan M, Van Diggelen OP, Kleijer WJ, Reuser AJ, Van den Boogaard MJ, Ausems MG, Ploos van Amstel HK, Poenaru L, Nicolino M *et al*. Glycogen storage disease type II: frequency of three common mutant alleles and their associated clinical phenotypes studied in 121 patients. *J Med Genet* 1995;32(10):836-837.
14. Kroos M, Hoogeveen-Westerveld M, van der Ploeg A, Reuser AJ. The genotype-phenotype correlation in Pompe disease. *Am J Med Genet C Semin Med Genet* 2012;160C(1):59-68.
15. Kroos MA, Pomponio RJ, Hagemans ML, Keulemans JL, Phipps M, DeRiso M, Palmer RE, Ausems MG, Van der Beek NA, Van Diggelen OP *et al*. Broad spectrum of Pompe disease in patients with the same c.-32-13T->G haplotype. *Neurology* 2007;68(2):110-115.
16. van Capelle CI, van der Meijden JC, van den Hout JMP, Jaeken J, Baethmann M, Voit T, Kroos MA, Derks TGJ, Rubio-Gozalbo ME, Willemsen MA *et al*. Childhood Pompe disease: clinical spectrum and genotype in 31 patients. *Orphanet Journal of Rare Diseases* 2016;11(1):65.
17. Wens SC, van Gelder CM, Kruijshaar ME, de Vries JM, van der Beek NA, Reuser AJ, van Doorn PA, van der Ploeg AT, Brusse E. Phenotypical variation within 22 families with Pompe disease. *Orphanet J Rare Dis* 2013;8:182.
18. van der Ploeg AT, Kruijshaar ME, Toscano A, Laforet P, Angelini C, Lachmann RH, Pascual Pascual SI, Roberts M, Rosler K, Stulnig T *et al*. European consensus for starting and stopping enzyme replacement therapy in adult patients with Pompe disease: a 10-year experience. *Eur J Neurol* 2017;24(6):768-e731.
19. de Vries JM, Kuperus E, Hoogeveen-Westerveld M, Kroos MA, Wens SC, Stok M, van der Beek NA, Kruijshaar ME, Rizopoulos D, van Doorn PA *et al*. Pompe disease in adulthood: effects of antibody formation on enzyme replacement therapy. *Genet Med* 2017;19(1):90-97.

20. de Filippi P, Ravaglia S, Bembi B, Costa A, Moglia A, Piccolo G, Repetto A, Dardis A, Greco G, Ciana G *et al*. The angiotensin-converting enzyme insertion/deletion polymorphism modifies the clinical outcome in patients with Pompe disease. *Genet Med* 2010;12(4):206-211.
21. De Filippi P, Saeidi K, Ravaglia S, Dardis A, Angelini C, Mongini T, Morandi L, Moggio M, Di Muzio A, Filosto M *et al*. Genotype-phenotype correlation in Pompe disease, a step forward. *Orphanet J Rare Dis* 2014;9:102.
22. Ravaglia S, De Filippi P, Pichiecchio A, Ponzio M, Saeidi Garaghani K, Poloni GU, Bini P, Danesino C. Can genes influencing muscle function affect the therapeutic response to enzyme replacement therapy (ERT) in late-onset type II glycogenosis? *Mol Genet Metab* 2012;107(1-2):104-110.
23. Baek RC, Palmer R, Pomponio RJ, Lu Y, Ma X, McVie-Wylie AJ. The influence of a polymorphism in the gene encoding angiotensin converting enzyme (ACE) on treatment outcomes in late-onset Pompe patients receiving alglucosidase alfa. *Mol Genet Metab Rep* 2016;8:48-50.
24. van Diggelen OP, Oemardien LF, van der Beek NA, Kroos MA, Wind HK, Voznyi YV, Burke D, Jackson M, Winchester BG, Reuser AJ. Enzyme analysis for Pompe disease in leukocytes; superior results with natural substrate compared with artificial substrates. *Journal of inherited metabolic disease* 2009;32(3):416-423.
25. Kroos M, Pomponio RJ, van Vliet L, Palmer RE, Phipps M, Van der Helm R, Halley D, Reuser A, Consortium GAAD. Update of the Pompe disease mutation database with 107 sequence variants and a format for severity rating. *Hum Mutat* 2008;29(6):E13-26.
26. Okumiya T, Keulemans JL, Kroos MA, Van der Beek NM, Boer MA, Takeuchi H, Van Diggelen OP, Reuser AJ. A new diagnostic assay for glycogen storage disease type II in mixed leukocytes. *Mol Genet Metab* 2006;88(1):22-28.
27. van der Beek NA, Hagemans ML, van der Ploeg AT, van Doorn PA, Merkies IS. The Rasch-built Pompe-specific activity (R-PAct) scale. *Neuromuscul Disord* 2013;23(3):256-264.
28. Al-Awadhi AM, Hasan EA, Sharma PN, Haider MZ, Al-Saeid K. Angiotensin-converting enzyme gene polymorphism in patients with psoriatic arthritis. *Rheumatol Int* 2007;27(12):1119-1123.
29. Council MR: Aids to examination of the peripheral nervous system, vol. Memorandum no. 45. London: Her Majesty's Stationary Office; 1976.
30. Beenakker EA, van der Hoeven JH, Fock JM, Maurits NM. Reference values of maximum isometric muscle force obtained in 270 children aged 4-16 years by hand-held dynamometry. *Neuromuscul Disord* 2001;11(5):441-446.
31. van der Ploeg RJ, Fidler V, Oosterhuis HJ. Hand-held myometry: reference values. *J Neurol Neurosurg Psychiatry* 1991;54(3):244-247.
32. van Capelle CI, van der Beek NA, de Vries JM, van Doorn PA, Duivenvoorden HJ, Leshner RT, Hagemans ML, van der Ploeg AT. The quick motor function test: a new tool to rate clinical severity and motor function in Pompe patients. *J Inherit Metab Dis* 2012;35(2):317-323.
33. A. T. S. Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 2002;166(1):111-117.
34. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Hankinson JL, Ip MS, Zheng J *et al*. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *The European respiratory journal* 2012;40(6):1324-1343.
35. Holm S. A Simple Sequentially Rejective Multiple Test Procedure. *Scand J Stat* 1979;6(2):65-70.
36. Pinheiro J BD, Debroy S, Sarkar D, {*nlme}*. Linear and Nonlinear Mixed Effects Models. 2015.
37. R Core Team R: A Language and Environment for Statistical Computing. In. Vienna, Austria: R Foundation for Statistical Computing; 2015.
38. Ausems MG, Verbiest J, Hermans MP, Kroos MA, Beemer FA, Wokke JH, Sandkuijl LA, Reuser AJ, van der Ploeg AT. Frequency of glycogen storage disease type II in The Netherlands: implications for diagnosis and genetic counselling. *Eur J Hum Genet* 1999;7(6):713-716.
39. van der Wal E, Bergsma AJ, van Gestel TJM, In 't Groen SLM, Zaehres H, Arauzo-Bravo MJ, Scholer HR, van der Ploeg AT, Pijnappel W. GAA Deficiency in Pompe Disease Is Alleviated by Exon Inclusion in iPSC-Derived Skeletal Muscle Cells. *Mol Ther Nucleic Acids* 2017;7:101-115.
40. Tsianos G, Eleftheriou KI, Hawe E, Woolrich L, Watt M, Watt I, Peacock A, Montgomery H, Grant S. Performance at altitude and angiotensin I-converting enzyme genotype. *Eur J Appl Physiol* 2005;93(5-6):630-633.

41. Tsianos G, Sanders J, Dhamrait S, Humphries S, Grant S, Montgomery H. The ACE gene insertion/deletion polymorphism and elite endurance swimming. *Eur J Appl Physiol* 2004;92(3):360-362.
42. Woods D, Hickman M, Jamshidi Y, Brull D, Vassiliou V, Jones A, Humphries S, Montgomery H. Elite swimmers and the D allele of the ACE I/D polymorphism. *Hum Genet* 2001;108(3):230-232.
43. Williams AG, Rayson MP, Jubb M, World M, Woods DR, Hayward M, Martin J, Humphries SE, Montgomery HE. The ACE gene and muscle performance. *Nature* 2000;403(6770):614.
44. Woods DR, Montgomery HE. Angiotensin-converting enzyme and genetics at high altitude. *High Alt Med Biol* 2001;2(2):201-210.
45. Folland J, Leach B, Little T, Hawker K, Myerson S, Montgomery H, Jones D. Angiotensin-converting enzyme genotype affects the response of human skeletal muscle to functional overload. *Exp Physiol* 2000;85(5):575-579.
46. Vaughan D, Brogioli M, Maier T, White A, Waldron S, Rittweger J, Toigo M, Wettstein J, Laczko E, Fluck M. The Angiotensin Converting Enzyme Insertion/Deletion Polymorphism Modifies Exercise-Induced Muscle Metabolism. *PloS one* 2016;11(3):e0149046.

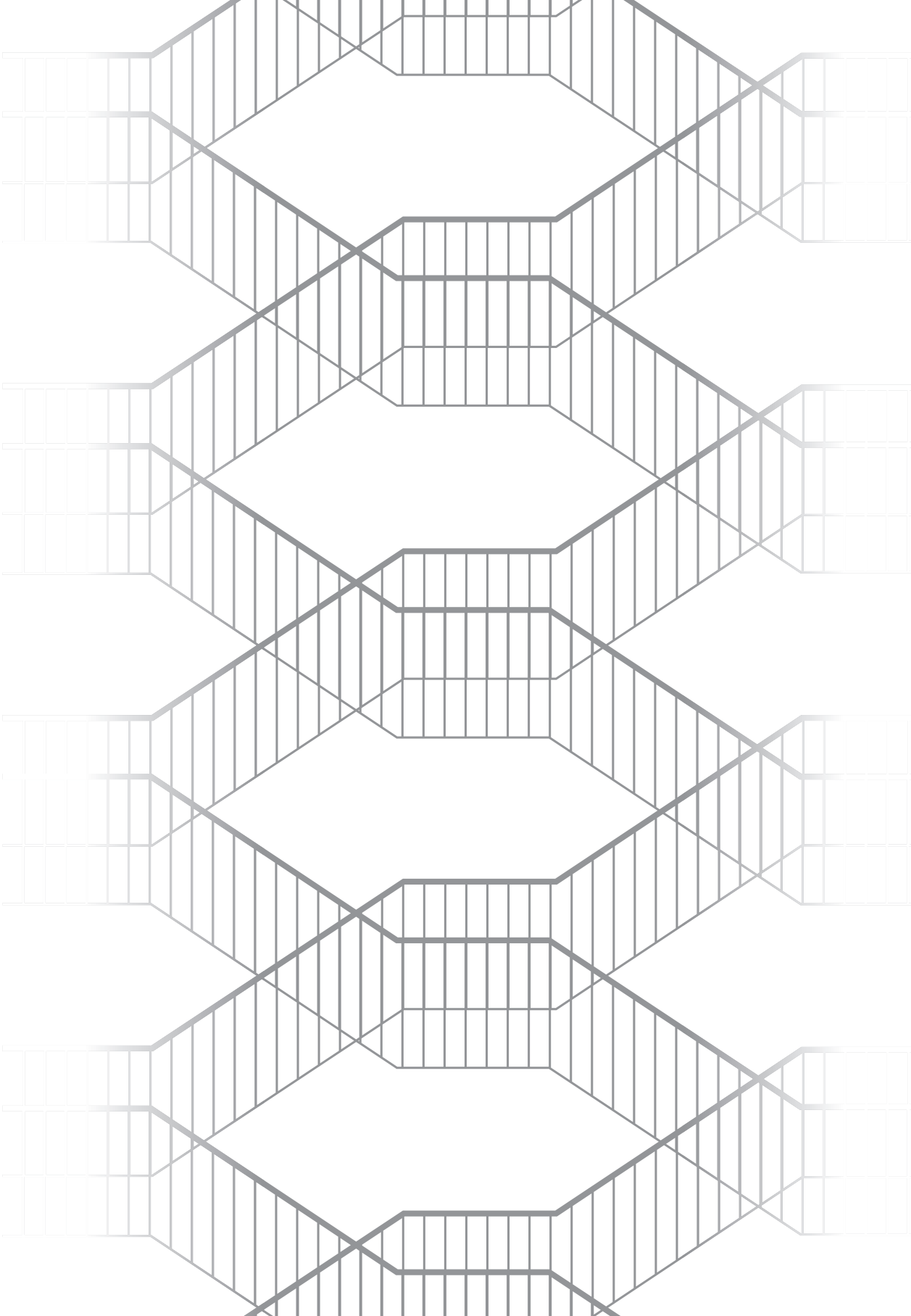
Supplementary text: sample size calculations

We conducted a post hoc power analyses based on our cross-sectional and longitudinal data. The aim of these power analyses are to determine what sample sizes would be needed in future studies in order to detect a potential effect of the ACE I/D genotype on disease severity or the efficacy of ERT.

Cross-sectional power analysis was performed for two different scenarios for the outcome measure age of onset. Age at onset is very variable in patients with the same IVS-1/null variants. Therefore, it is likely that modifying factors influence this outcome. In the first scenario, we assumed that the ACE genotype would fully explain all phenotypical variation between patients. Therefore the medians of the different ACE groups would be the same as the 25^{th}, 50^{th} and 75^{th} percentiles of the age of onset of the overall population. We therefore calculated how many subjects were needed to detect a difference between ACE groups using these theoretical means. In the second scenario we calculated the power to detect differences in age at onset between ACE groups based on the actual observed distribution of age at onset in our dataset.

For both scenario, we investigated the number of patients needed to reach a power of >0.8 to detect a difference between ACE groups. In order to calculate this power to detect a difference for a population with a given number of patients we simulated a thousand 'sample populations' with the given number of patients. The distribution of these 'sample populations' is based on the assumptions of the different scenarios. For each 'sample population' a Kruskal-Wallis test was performed in order to assess if the ACE groups differed from each other. The power to detect a difference between ACE groups, for the given number of patients, is the percentage of significant Kruskal-Wallis tests of the thousand 'sample populations'.

In addition, we calculated the number of subjects needed to detect a difference between ACE groups in response to ERT treatment for the outcome measure FVC in sitting position. For this calculation we assumed a longitudinal study design. Based on the distribution of data in our current study we simulated populations with increasing sizes. All simulated subjects provide 15 measurements over a period of 5 years and were repeated 1000 times. For each simulated population a likelihood ratio test was performed assessing if ACE influences the outcome. The power to detect a difference between ACE groups, for a given sample sizes, was calculated as the proportion of significant likelihood-ratio-test in the 1000 simulated samples.



Ten years of the international Pompe survey: patient reported outcomes as a reliable tool for studying treated and untreated children and adults with non-classic Pompe disease

J. C. van der Meijden, D. Güngör, M. E. Kruijshaar
A. D. J. Muir, H. A. Broekgaarden, A. T. van der Ploeg

7

Journal of Inherited Metabolic Disease 2015; 38:495–503

Abstract

Pompe disease is a rare, progressive lysosomal storage disorder for which enzyme therapy (ERT) became available in 2006. Four years earlier, the IPA/Erasmus MC survey, an international longitudinal prospective survey, was established to collect information on the natural course of the disease and its burden on patients. The survey is a collaboration between Erasmus MC University Medical Center and the International Pompe Association (IPA) and comprises an annual questionnaire that was specifically designed to assess the symptoms and problems of the disease. Here we review our results of over ten years of follow-up, and discuss the survey's contribution to the field. Tracking 408 Pompe patients between 2002 and 2013, the cumulative data reveals the broad range of clinical manifestations that interfere with patients' lives. The survey allowed us to quantify the rate of disease progression and the positive effects of ERT on patients' quality of life, fatigue, and participation in daily life. Furthermore, it showed for the first time that survival is reduced in adult Pompe disease and improved by ERT. Our results show that a patient survey can serve as valuable and reliable tool for obtaining quantifiable information on the natural course of a rare disease and on the effects of therapy in a large cohort over a very long time. Most importantly, by working with patient reported outcomes, the survey provides the data that is truly relevant to the patient and complementary to clinical datasets.

Background

Pompe disease (glycogen storage disease type II, acid maltase deficiency, OMIM # 232300) is a rare, progressive, lysosomal storage disorder with an autosomal recessive pattern of inheritance. Partial or total deficiency of the enzyme acid α-glucosidase (GAA) results in intra-lysosomal glycogen build-up, which leads to cellular damage that is most pronounced in muscles.[1, 2]

The disease has a broad clinical spectrum that ranges from a rapidly progressive classic-infantile phenotype to milder forms that present in children and adults.[3, 4] The first symptoms in classic-infantile patients appear in the first months of life; untreated patients die before the age of one.[5, 6] Patients with non-classic phenotypes have a milder form of the disease that progresses more slowly. They typically suffer from limb-girdle weakness and/or respiratory problems caused by weakness of the respiratory muscles, including the diaphragm.[3, 7-10]

In 2006, enzyme replacement therapy (ERT) with recombinant human α-glucosidase was approved for Pompe disease in Europe and the United States. Market approval was based on studies in infants whose hypertrophic cardiomyopathy and skeletal muscle function had improved and whose survival had increased.[11-18] Studies in children and adults with Pompe disease started only after market approval.[19-25] Before that time, there was little prospectively obtained data on the natural course of disease in these patients.

The estimated frequency of Pompe disease is 1 in 40,000 live births.[26, 27] Due to its rarity, most clinicians see very few Pompe patients. Setting up a clinical follow-up study with sufficient numbers of patients is therefore difficult and time-consuming, requiring the establishment of larger treatment centers (such as regional or national referral centers) and/or data collection through registries. In rare diseases, registries are often managed by pharmaceutical companies and are not started until a feasible therapy appears on the horizon.

To fill this gap in knowledge, the IPA/Erasmus MC Pompe survey (Pompe survey) was started in May 2002 as a collaboration between Erasmus MC University Medical Center and the International Pompe Association (IPA), a federation of Pompe disease patients groups. As several national patient-support groups were already well organized and collaborating internationally as part of the IPA, this made it possible to reach a large number of patients well before treatment became available. Since 2002, this international follow-up study has enrolled over 400 patients around the globe and taken a standardized approach to their

follow-up. Still ongoing, it has evolved into one of the biggest databases for non-classic Pompe patients, making it possible to produce many publications on the natural course of disease and the effect of enzyme replacement therapy.

In this article we review the results of the ten years of follow-up made possible by the Pompe survey. We discuss its contribution to the field of Pompe disease and the role it plays as an example role for the study of other rare diseases.

The Pompe survey

The Pompe survey consists of three generic questionnaires that have previously been used in many other diseases and a questionnaire specifically designed for Pompe patients.

Inclusion of patients

Most patients are included through national support groups affiliated with the IPA, which is an umbrella organization for these groups. When patients join one of these support groups, they are asked to participate in the Pompe survey. Dutch patients are recruited through the outpatient clinic at Erasmus MC University Medical Center, the national referral center for patients in the Netherlands. The inclusion of patients started in 2002 and is still ongoing. All patients provide informed consent. Once enrolled, they are asked to complete follow-up questionnaires every year. Patients with the classic infantile phenotype were excluded from this study.

The Pompe questionnaire

The Pompe-specific part of the survey covers various topics related to patients' medical history and clinical status. At inclusion, a baseline questionnaire assesses 14 topics covering first signs and symptoms; diagnosis and diagnostic procedures; family history, childhood, mobility, specific movements, breathing, sleeping, eating, other complaints, daily activities, education and work, modifications to the home; and treatment, hospital stays and use of care. These topics were selected on the basis of a literature review[7] and the experience of Pompe disease specialists. The questionnaire was piloted by a patient panel, which was also asked whether any topics were missing.

Generic questionnaires

The three generic questionnaires in the Pompe survey are intended to provide information on fatigue, participation, and health-related quality of life.

Before the survey started, it was apparent that patients visiting our outpatient clinic often reported fatigue, which had not been yet investigated systematically as a symptom of the disease.[28] For this reason, the Fatigue Severity Scale (FSS) was included: a self-reported 9-item questionnaire that is short and easy to complete.[29] The score (the mean of the 9 items) ranges from 1-7, with higher values indicating higher levels of fatigue.

Participation in daily life (previously known as handicap) is defined as a person's involvement in daily life situations.[30] It is particularly important to measure participation in Pompe patients, as their wellbeing can be greatly impacted by increasing restrictions in daily life. Measurement using the Rotterdam Handicap Scale (RHS) showed good validity, reliability and responsiveness in patients with immune-mediated polyneuropathies, which are similar to Pompe disease in that the WHO handicap (i.e. participation) dimension of 'orientation' remains unaffected. The RHS contains nine items assessing mobility, domestic tasks and leisure activities (indoors and outdoors, each assessed separately), kitchen tasks, traveling, and work/study. The total score ranges from 9-36, with higher scores indicating better participation.[31-33]

Finally, quality of life was assessed using the Medical Outcomes Survey Short Form-36 Health Survey (SF-36), a 36-item questionnaire designed for a wide range of populations that allows comparison of different patient groups and has demonstrated good psychometric properties. The scale is divided into eight domains: physical functioning, physical role functioning, bodily pain, general health perception, vitality, social role functioning, emotional role functioning, and mental health (which can be further summarized in a physical-component summary score and a mental-component summary score). Scores range from 0-100, with higher scores representing better quality of life.[34, 35]

Patients participating in the Pompe survey

Between May 2002 and March 2013, a total of 408 patients with childhood and adult onset of disease were included into the Pompe survey. The characteristics of these patients are displayed in Table 1 and Figure 1. Per patient, a median follow-up of 5 years (range 0-10) was available.

Table 1 | Characteristics of the 408 child and adult patients participating in the Pompe survey.

Male, n (%)	208 (51)
Median age at first symptoms, years (IQR; full range)*	29 (15-41 ; 0-65)
Median age at diagnosis, years (IQR; full range)	37 (27-47 ; 0-72)
Median age at inclusion, years (IQR ; full range)	47 (37-57 ; 2-81)
Patients under the age of 18 years at inclusion, n (%)	28 (6.9)
Use of a wheelchair at inclusion, n (%)	139 (34)
Use of artificial ventilation at inclusion, n (%)	169 (41)
Median number or hours of ventilation/ day at inclusion, n (%)	10 (2-24)
Median follow-up, years (IQR ; full range) Patients receiving ERT during follow-up, n (%)	5 (1-8 ; 0-10) 265 (65)
Deceased during follow-up, n**	54

* n=394.
** None of the patients who were under 18 at inclusion died.

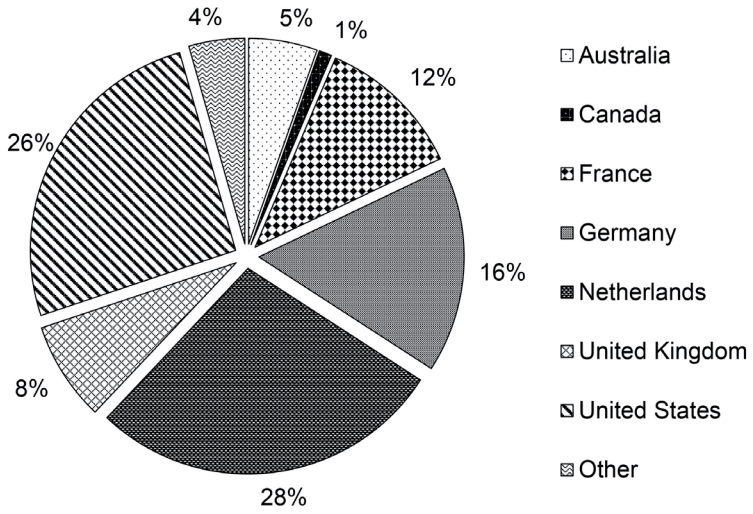


Figure 1| Distribution of the patients' countries of residence (n=408).

Clinical manifestations and progression during the natural course of Pompe disease

Initially, the Pompe survey focused on the natural course of Pompe disease, describing its clinical manifestations, symptom onset, and disease severity, and the course of the disease over time.

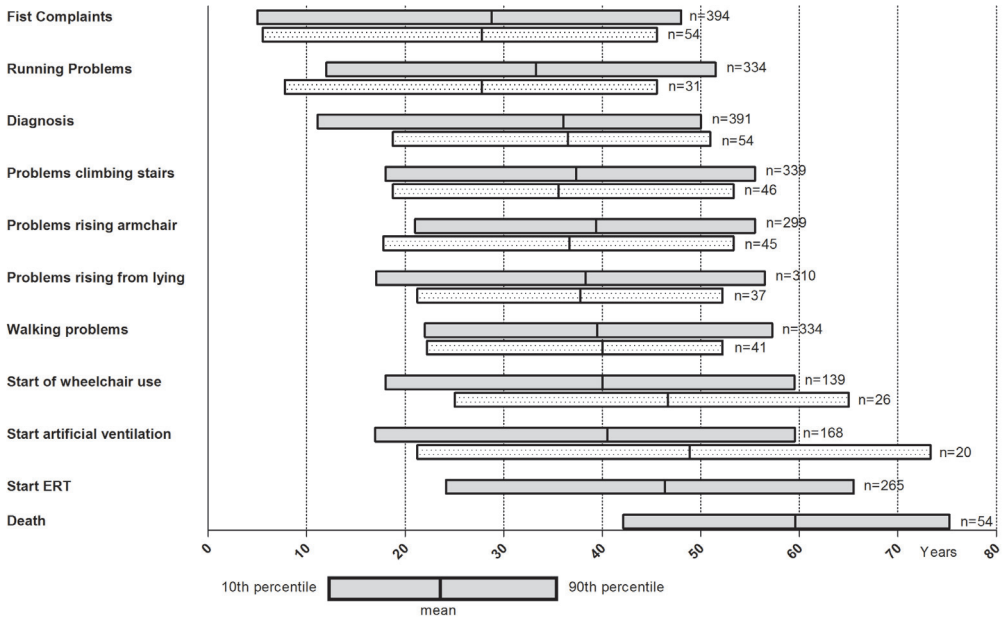


Figure 2 | Age distribution in 2013 for specific events in the course of disease for 408 child and adult Pompe patients (gray bars) relative to that of the original cohort of 54 Dutch patients (dotted bars) that started in 2002.

The number behind each bar indicates how many patients provided information on the age at which these events occurred. In the case of ERT and death the number indicates the number of patients that underwent the event.

Clinical manifestations and symptom onset

One of the very first results, in 2005, described the clinical manifestations of Pompe disease in 54 Dutch patients.[36] Figure 2 shows the age distribution at several events in the course of the disease for the initial 54 Dutch patients enrolled in the survey as well as for the entire childhood and adult-onset population participating in 2013.

As Hagemans *et al* described for the first cohort containing 54 Dutch patients, a general trend towards increasing disease severity with age is evident in the entire international cohort. While patients in the international cohort developed their first symptoms at a median age of 30 (range 0-57.5) years, 18.9% reported symptoms before age 12, and 27.9% before age 18. There was a broad range in the time between first symptoms and diagnosis: 27.5% of patients were diagnosed in the first year after manifesting symptoms, and 27% between 1 and 5 years. In 45.6% of patients, it took 5-30 years, indicating a large diagnostic delay. Age at diagnosis showed a wide range, ranging from 0 years to 72.5 years, with a median age of 37.5 years.

The need to test pulmonary function regardless of motor function was highlighted by striking variations with regard to the age at which mobility and respiratory problems arose and to the order in which they arose. Results for the initial cohort of 54 patients show a similar pattern of heterogeneity in disease severity and long diagnostic delays.[36]

Limb-girdle weakness and reduced motor function were the most common first symptoms. When they completed the questionnaire, 8 out of 10 patients had problems walking and 1 in 3 used a wheelchair. While respiratory problems were not usually reported as first symptom, 2 out of 5 patients were using artificial ventilation at completion of the first questionnaire. Similar figures were found in the initial cohort of 54 Dutch patients.[36]

Course of disability and respiratory function

The rate of disease progression was assessed in 2006 in an initial subgroup of 52 Dutch patients, who were asked to complete follow-up questionnaires at one and two years follow-up. During this follow-up period, four patients died; others lost important motor abilities such as riding a bicycle and jumping; and more became wheelchair dependent. Similarly, more patients started requiring ventilation, while those who were already receiving ventilation significantly increased the number of hours they needed it per day. Over 5 years, RHS scores deteriorated significantly by 2 points.[37]

These previously unknown data provided important information on the rate of progression of the disease, and were later supported in 2012 by clinical assessments in a study of 94 Dutch adult patients by van der Beek *et al.* which included some of the same patients,[38] and thereby confirmed that outcomes reported by patients provide quantifiable information on the development of the disease and the disability associated with it.

Disease severity

In 2005, the bearing of age and disease duration on disease severity was assessed in an international group of 255 adults and children with Pompe disease. Disease severity was measured on the basis of wheelchair use and/or respiratory support; disease duration was defined as the time since diagnosis. Analyses showed that disease severity was significantly associated with disease duration rather than with age. Regardless of the patient's age, the odds for using a wheelchair had increased by 13% with every year since diagnosis, and the odds for using respiratory support by 8%. Between 10 and 15 years after diagnosis, half of the patients were either wheelchair dependent, used a ventilator, or both. Later clinical studies by van der Beek and Wokke, again including several patients who had also participated in the survey, showed similar findings.[8, 38-40]

A small subgroup of children with Pompe disease was identified in whom the disease was more severe and developed more rapidly than in other children, but in whom it was nonetheless milder than in classic infantile patients. This group of children seemed to share similarities with the group of atypical infantile patients described by Slonim *et al.*[40, 41]

Fatigue, participation and quality of life in untreated patients

In 2007, the FSS was used to assess the presence and severity of fatigue in 225 adult Pompe patients. Fatigue was shown to be a common feature: nearly 8 out of 10 adult patients had fatigue (78% FSS score ≥4), and two-thirds had severe fatigue (67% FSS score ≥5). Patients' mean FSS score was also significantly higher than that published for 113 healthy controls in a study on polyneuropathies (5.2 vs 2.9, $p<0.001$). Two-thirds of patients classified fatigue as one of the three most disabling symptoms; even though it was the most pronounced in the most severely affected patients, it was prevalent across all severity levels.[7, 28, 36, 42]

Participation (previously known as handicap) is defined as a person's involvement in life situations.[30, 31] Using the Pompe survey, participation was described for the first time—in 2007—in 257 untreated adult Pompe patients. Relative to the maximum score of 36 that would be scored by an average healthy person, patients had an average score of 25.9 (SD 6.5) points. The mean RHS score correlated significantly with age, disease duration and all SF-36 subscales except the mental health domain; it also differed significantly between patients with and without respiratory support and those with and without a wheelchair. In general, Pompe patients performed best on the items indoor mobility and indoor leisure activities; the items domestic tasks (both indoor and outdoor) and work/study were affected most,[32] reflecting the fact that it is more easy to move indoors than outdoors and perform "light" leisure activities compared to "heavy" domestic tasks.

In 2004, the Pompe survey was also the first to describe quality of life in a large group of 210 adult Pompe patients. Relative to that in the general population, their quality of life was lower in all SF-36 domains except for 'bodily pain', 'mental health' and 'role functioning-emotional'. The domain most affected was 'physical functioning'. Lower SF-36 scores were associated with wheelchair use, respiratory support, longer disease duration. Paradoxically longer disease duration was also associated with better 'role functioning-physical' and 'mental health' scores. This latter phenomenon is probably due to patients having developed better coping mechanisms over time, and thus having developed an acceptance of their impairment. Reductions in quality of life in the physical domains were later confirmed in several other studies.[8, 18, 19, 25, 35, 43, 44]

The effects of ERT on fatigue, participation and quality of life

In 2013 the effect of ERT on fatigue was assessed in 163 treated adult patients. Relative to the levels of fatigue before treatment, which were stable and high, scores fell significantly during treatment (Figure 3). The proportion of patients who were fatigued fell from 85% at baseline to 79% at the last follow-up; in those who were severely fatigued, it fell from 68% to 55%. Fatigue improved mainly in women, patients ≥45 years, and those with a disease duration <15 years. The Pompe survey made it possible to study this subject for the first time, and showed that ERT had a positive effect on fatigue. Given the high burden of fatigue in Pompe patients, these changes are important.[45]

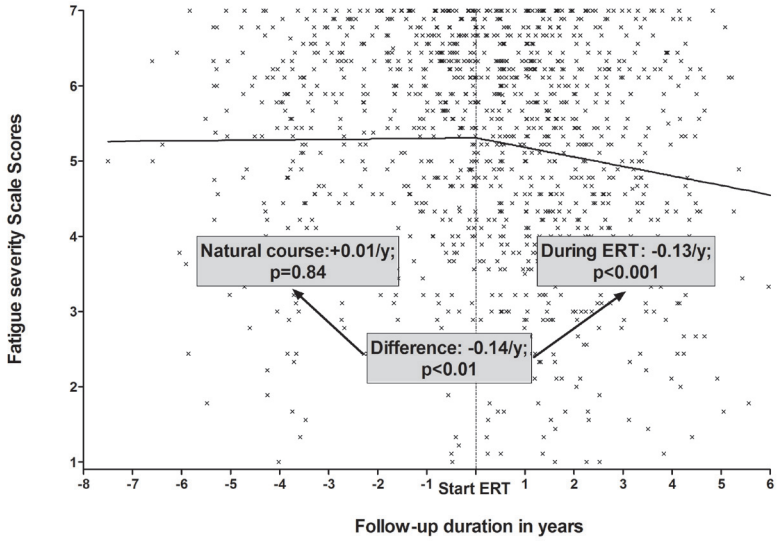


Figure 3 | Change in FSS scores before and after the start of ERT.

The crosses represent individual measurements. The lines represent the mean slopes calculated by the 'broken stick' repeated measures ANOVA (n=163). The mean slope declined significantly after the start of ERT. There was also a significant difference between the period before and after the start of ERT (data was provided by D. Güngör).

Recently we combined ten years international follow-up data obtained by the Pompe survey to study the effects of enzyme replacement therapy on quality of life and participation in daily life activities in adults with Pompe disease.[46] These data suggest that ERT has a positive effect, seemingly halting a gradual decline in both quality of life and participation in daily life.

Survival

Until the results of the Pompe survey were published recently, no information was available on survival in adult Pompe patients. To assess survival in a slowly progressive disease,

sufficiently large numbers of patients have to be followed over a long period of time. While, at the outset, the survey was not set up to measure survival, the consistent follow-up of patients made it possible to provide this information.

In 2011, to gain insight into the life span of untreated adult Pompe patients, survival was evaluated using data on 268 adult patients. The estimated 5-year survival rate from diagnosis was 95%, and the respective 10, 20, and 30-year survival rates were 83, 65, and 40%. Survival was lower in older patients, those using a wheelchair and/or artificial ventilation, and those with lower levels of participation. In a subgroup of Dutch patients, it was also shown that mortality rates in Dutch Pompe patients were higher than in the age- and sex-matched general population.[47]

In 2013, time-dependent survival models were used to assess the effect of ERT on survival in 283 treated and untreated adult Pompe patients. This study was the first to show that ERT improved survival in adult patients. At any given point, a patient on ERT had a 59% smaller chance of dying than an untreated patient. This translates to a gain of approximately one year of life for eight years of treatment. Patients using a wheelchair and ventilator support had a significantly greater chance of dying than those who used none of these support measures.[48]

Other applications of the Pompe survey

In addition to the studies identified above, data collected in the Pompe survey—published and unpublished—has also been used in modeling exercises, for example to highlight potential beneficial effects of newborn screening and to calculate the burden of disease in adult Pompe patients.[49, 50] The items of the Pompe questionnaire were also used as a starting point for a new Pompe-specific standardized questionnaire, the R-Pact, which measures limitations in activities and social participation.[51] Finally, we were also able to use the infrastructure of the patient organizations to invite patients to participate in other research projects, such as that of Güngör *et al*. investigating pain as a feature of Pompe disease.[52]

Discussion

The Pompe survey has provided a large body of new information on children and adults with Pompe disease and the effects of enzyme therapy in this patient population. As well as the broad range of clinical manifestations seen in these patients, it has revealed their heterogeneous disease progression and the relationship between disease severity and disease duration. Relative to the general population, patients were shown to have high

levels of fatigue, poorer quality of life and participation, and higher mortality rates. ERT was shown to have a positive or stabilizing effect on all of these variables.

The most important feature of the Pompe survey has been to quantify the course of disease in patients with Pompe disease. Although the disease was already known to be progressive, the Pompe survey has shown how rapidly patients actually progress. Through its inclusion of severely and mildly affected patients alike, its inclusion of a large number of patients, and its long follow-up, the Pompe survey might even be able to detect changes that regular clinical trials are unable to pick up.

The primary focus of the Pompe survey was to describe the natural course of disease before ERT became available. The timely start of the survey four years before enzyme therapy becoming available was key to the collection of the large body of data on the natural course. Since patients start ERT earlier in their disease, fewer and fewer untreated patients are left in whom the natural course can be studied (Figure 4). The successful mapping of the pre-treatment period not only produced new insights into the natural course of this disease, but also provided a benchmark against which treatment data could be compared.

So far, the Pompe survey has had three main strengths. The first is the large number of patients who have been included and followed consistently over time, both before and after starting ERT. As national or international collaboration is required, a large number of patients with a rare disease can often be hard to obtain. Other national and international registries that have been created for this reason include the Pompe Registry, which is managed by

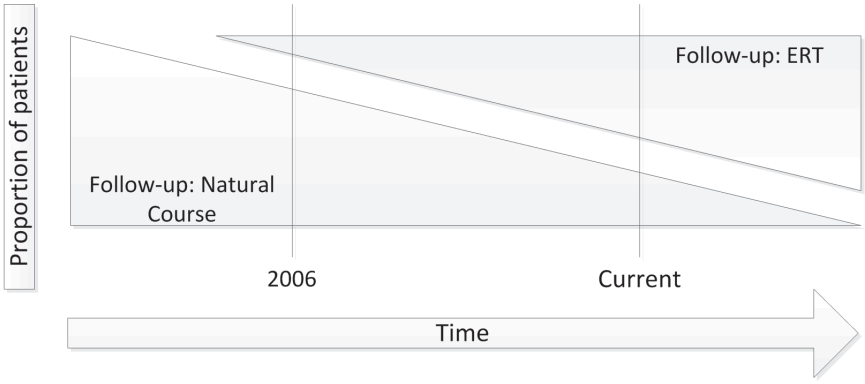


Figure 4 | Schematic overview of the proportion of treated and untreated patients across the follow-up of the survey.

a pharmaceutical company. While this contains the largest number of patients, it does not provide the consistent follow-up that has been acquired in the Pompe survey. Thanks to this consistent follow-up and the direct approach of the patients—who are not only able to report their signs and symptoms, but are usually keen to be involved—we have been able to study changes over time. This has led to the creation of one of the largest databases with the longest and most consistent follow-up of children and adults with Pompe disease worldwide.

The second strength is that the patient-reported outcomes collected in the survey provide information that is additional to clinical outcome measures. While most clinical measures—such as muscle strength, pulmonary function and distance walked—assess functioning at the body level, Lachmann and Schoser question whether these clinical endpoints are clinically significant for the patient.[53] For example, 40% of patients are unable to participating in their work or study, a problem that is not adequately represented by measuring walking distance.[32] We therefore believe that our assessments of quality of life and handicap have allowed us to measure patients' functioning overall and in a social context that truly reflects the impact of the disease on their lives.

The third strength is that our use of specifically tailored questions to assess a broad range of problems and symptoms provides a clear picture not only of the disease's impact on core variables such as the use of a wheelchair or artificial ventilation, but also of almost every other aspect of a patient's life. When these patient-reported outcomes are linked to clinical outcomes, the combined picture can give greater insight into the relevance of clinical outcomes to the patient.

So far, the Pompe survey has also had two main limitations. First, to be approached for participation, patients had to be affiliated to a patient-support group. This may generate selection bias: severely affected patients might be more likely to affiliate with support groups and/or participate in a survey. However, inspection of the survey population shows that the full spectrum of patients has been included—from the most affected to the least affected, and in all age groups. Unlike randomized controlled trials, which include only mildly affected patients, the survey thus provides a more general picture of the disease and the effects of ERT.

The second limitation concerns the recruitment of patients without the involvement of their treating physician and without access to diagnostic data, which means that the diagnosis could not be confirmed in all the patients included. However, as Güngör *et al.* point out, since most patients who have participated in the survey were started on ERT, it is unlikely

that their diagnosis was not confirmed.[52] With the Dutch Pompe patients it is also the case that diagnosis is confirmed through enzyme-activity assessment and mutation analysis.

The approach taken by the Pompe survey can be translated to other rare diseases that struggle to acquire information on large numbers of patients. Two important contributors to the success of this survey have been the process of identifying specific items and topics regarding the disease, and the use of standardized measurement scales. We reiterate that it is important to the ensure availability of natural course data by starting such a survey before therapy is introduced. In order to include a large number of patients, it is also vital to collaborate with patient organizations. If these elements are combined, it is possible to measure robust data on disease progression and treatment.

Conclusion

In summary, the Pompe survey is one of the largest databases providing consistent follow-up of children and adults with Pompe patients worldwide. The survey has enabled us to quantify disease progression in untreated patients, capture the impact on daily life, and quantify the changes elicited by ERT over a long period of time. Patients are trustful partners, and such a partnership should be cherished. In rare diseases, where it is difficult to take a standardized approach to following large groups of patients, this partnership is particularly relevant.

Unlike case reports and clinical trials, the survey was able to quantify the development of disease in treated and untreated patients through the inclusion of mildly and severely affected patients. In this way, the results are representative for the population as a whole, and go beyond mere descriptions of patients' deterioration or improvement. The use of patient-reported outcomes rather than clinical outcome measures has also enabled us to measure patients' functioning in a social context.

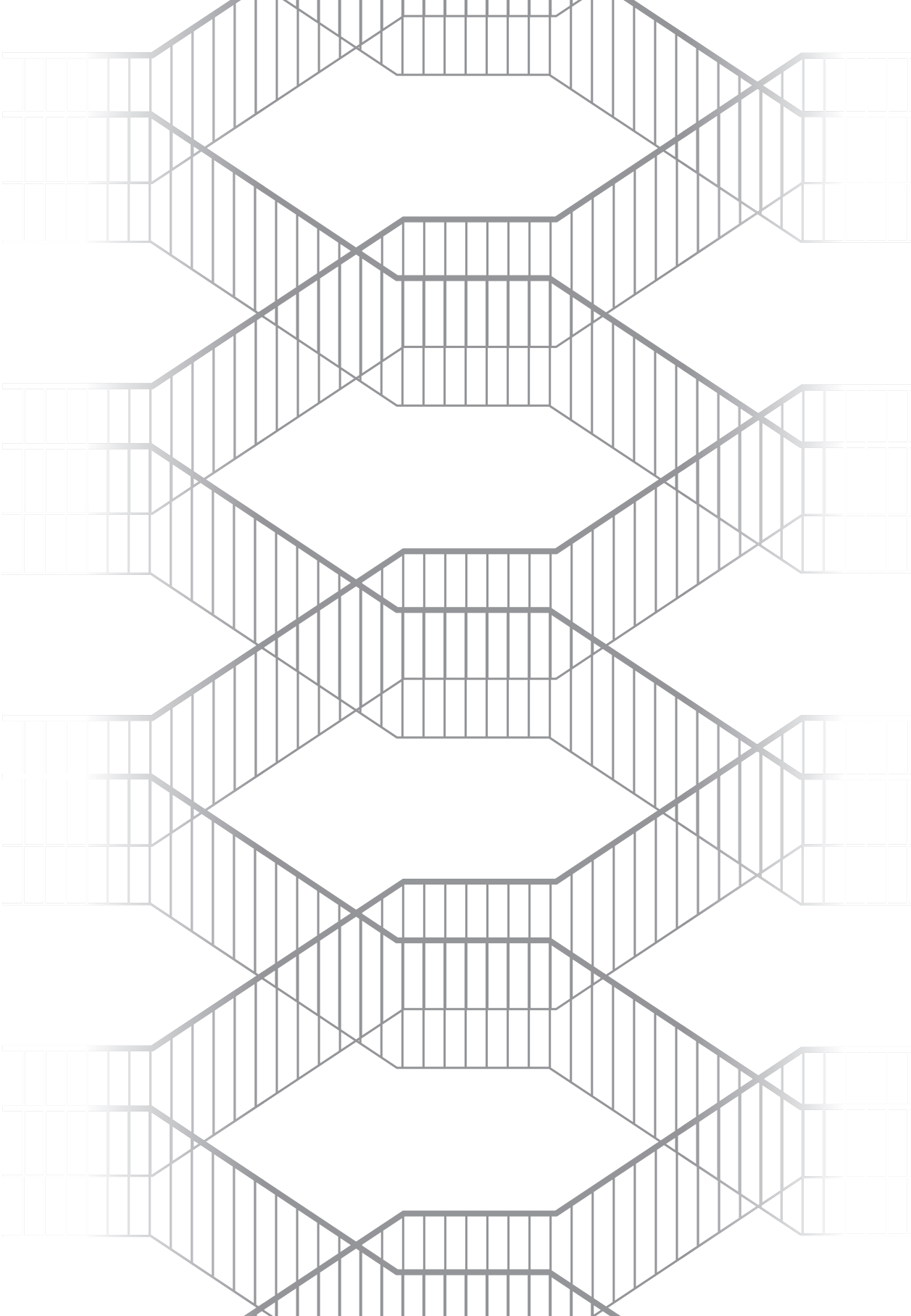
Due to its various application and design, the Pompe survey is a valuable tool to study Pompe disease in untreated and treated patients. It can also be used as a template for collecting patient information in other rare diseases. Such data can be linked to clinical data obtained by physicians.

References

1. Hirschhorn R, Reuser AJ: Glycogen storage disease type II: acid alphaglucosidase (acid maltase) deficiency. In: *The Metabolic and Molecular Bases of Inherited Disease.* 8th edn. Edited by Scriver CR, Beaudet AL, Sly WS, Valle D. New York: McGraw-Hill; 2001: 3389–3420.
2. Pompe JC. Over idiopathische hypertrofie van het hart. *Nederlands tijdschrift voor geneeskunde* 1932;76:304–311.
3. van der Ploeg AT, Reuser AJ. Pompe's disease. *Lancet* 2008;372(9646):1342-1353.
4. Engel AG, Seybold ME, Lambert EH, Gomez MR. Acid maltase deficiency: comparison of infantile, childhood, and adult types. *Neurology* 1970;20(4):382.
5. Van den Hout JM, Hop W, van Diggelen OP, Smeitink JA, Smit GP, Poll-The BT, Bakker HD, Loonen MC, de Klerk JB, Reuser AJ *et al*. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics* 2003;112(2):332-340.
6. Kishnani PS, Hwu WL, Mandel H, Nicolino M, Yong F, Corzo D, Infantile-Onset Pompe Disease Natural History Study G. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *J Pediatr* 2006;148(5):671-676.
7. Winkel LP, Hagemans ML, van Doorn PA, Loonen MC, Hop WJ, Reuser AJ, van der Ploeg AT. The natural course of non-classic Pompe's disease; a review of 225 published cases. *J Neurol* 2005;252(8):875-884.
8. Wokke JH, Escolar DM, Pestronk A, Jaffe KM, Carter GT, van den Berg LH, Florence JM, Mayhew J, Skrinar A, Corzo D *et al*. Clinical features of late-onset Pompe disease: a prospective cohort study. *Muscle Nerve* 2008;38(4):1236-1245.
9. Laforet P, Nicolino M, Eymard PB, Puech JP, Caillaud C, Poenaru L, Fardeau M. Juvenile and adult-onset acid maltase deficiency in France: genotype-phenotype correlation. *Neurology* 2000;55(8):1122-1128.
10. Muller-Felber W, Horvath R, Gempel K, Podskarbi T, Shin Y, Pongratz D, Walter MC, Baethmann M, Schlotter-Weigel B, Lochmuller H *et al*. Late onset Pompe disease: clinical and neurophysiological spectrum of 38 patients including long-term follow-up in 18 patients. *Neuromuscul Disord* 2007;17(9-10):698-706.
11. Amalfitano A, Bengur AR, Morse RP, Majure JM, Case LE, Veerling DL, Mackey J, Kishnani P, Smith W, McVie-Wylie A *et al*. Recombinant human acid alpha-glucosidase enzyme therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial. *Genet Med* 2001;3(2):132-138.
12. Kishnani PS, Corzo D, Nicolino M, Byrne B, Mandel H, Hwu WL, Leslie N, Levine J, Spencer C, McDonald M *et al*. Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology* 2007;68(2):99-109.
13. Van den Hout JM, Reuser AJ, de Klerk JB, Arts WF, Smeitink JA, Van der Ploeg AT. Enzyme therapy for pompe disease with recombinant human alpha-glucosidase from rabbit milk. *Journal of inherited metabolic disease* 2001;24(2):266-274.
14. Van den Hout H, Reuser AJ, Vulto AG, Loonen MC, Cromme-Dijkhuis A, Van der Ploeg AT. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients. *Lancet* 2000;356(9227):397-398.
15. Chakrapani A, Vellodi A, Robinson P, Jones S, Wraith JE. Treatment of infantile Pompe disease with alglucosidase alpha: the UK experience. *J Inherit Metab Dis* 2010;33(6):747-750.
16. Ebbink BJ, Aarsen FK, van Gelder CM, van den Hout JM, Weisglas-Kuperus N, Jaeken J, Lequin MH, Arts WF, van der Ploeg AT. Cognitive outcome of patients with classic infantile Pompe disease receiving enzyme therapy. *Neurology* 2012;78(19):1512-1518.
17. van Gelder CM, van Capelle CI, Ebbink BJ, Moor-van Nugteren I, van den Hout JM, Hakkesteegt MM, van Doorn PA, de Coo IF, Reuser AJ, de Gier HH *et al*. Facial-muscle weakness, speech disorders and dysphagia are common in patients with classic infantile Pompe disease treated with enzyme therapy. *J Inherit Metab Dis* 2012;35(3):505-511.
18. van Capelle CI, Winkel LP, Hagemans ML, Shapira SK, Arts WF, van Doorn PA, Hop WC, Reuser AJ, van der Ploeg AT. Eight years experience with enzyme replacement therapy in two children and one adult with Pompe disease. *Neuromuscul Disord* 2008;18(6):447-452.
19. van der Ploeg AT, Clemens PR, Corzo D, Escolar DM, Florence J, Groeneveld GJ, Herson S, Kishnani PS, Laforet P, Lake SL *et al*. A randomized study of alglucosidase alfa in late-onset Pompe's disease. *N Engl J Med* 2010;362(15):1396-1406.

20. Strothotte S, Strigl-Pill N, Grunert B, Kornblum C, Eger K, Wessig C, Deschauer M, Breunig F, Glocker FX, Vielhaber S *et al*. Enzyme replacement therapy with alglucosidase alfa in 44 patients with late-onset glycogen storage disease type 2: 12-month results of an observational clinical trial. *J Neurol* 2010;257(1):91-97.
21. Regnery C, Kornblum C, Hanisch F, Vielhaber S, Strigl-Pill N, Grunert B, Muller-Felber W, Glocker FX, Spranger M, Deschauer M. 36 months observational clinical study of 38 adult Pompe disease patients under alglucosidase alfa enzyme replacement therapy. *J Inherit Metab Dis* 2012;35(5):837-845.
22. Angelini C, Semplicini C, Ravaglia S, Bembi B, Servidei S, Pegoraro E, Moggio M, Filosto M, Sette E, Crescimanno G *et al*. Observational clinical study in juvenile-adult glycogenosis type 2 patients undergoing enzyme replacement therapy for up to 4 years. *J Neurol* 2012;259(5):952-958.
23. Toscano A, Schoser B. Enzyme replacement therapy in late-onset Pompe disease: a systematic literature review. *J Neurol* 2013;260(4):951-959.
24. de Vries JM, van der Beek NA, Hop WC, Karstens FP, Wokke JH, de Visser M, van Engelen BG, Kuks JB, van der Kooi AJ, Notermans NC *et al*. Effect of enzyme therapy and prognostic factors in 69 adults with Pompe disease: an open-label single-center study. *Orphanet J Rare Dis* 2012;7:73.
25. Orlikowski D, Pellegrini N, Prigent H, Laforet P, Carlier R, Carlier P, Eymard B, Lofaso F, Annane D. Recombinant human acid alpha-glucosidase (rhGAA) in adult patients with severe respiratory failure due to Pompe disease. *Neuromuscul Disord* 2011;21(7):477-482.
26. Ausems MG, Verbiest J, Hermans MP, Kroos MA, Beemer FA, Wokke JH, Sandkuijl LA, Reuser AJ, van der Ploeg AT. Frequency of glycogen storage disease type II in The Netherlands: implications for diagnosis and genetic counselling. *Eur J Hum Genet* 1999;7(6):713-716.
27. Martiniuk F, Chen A, Mack A, Arvanitopoulos E, Chen Y, Rom WN, Codd WJ, Hanna B, Alcabes P, Raben N *et al*. Carrier frequency for glycogen storage disease type II in New York and estimates of affected individuals born with the disease. *Am J Med Genet* 1998;79(1):69-72.
28. Hagemans ML, van Schie SP, Janssens AC, van Doorn PA, Reuser AJ, van der Ploeg AT. Fatigue: an important feature of late-onset Pompe disease. *J Neurol* 2007;254(7):941-945.
29. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch Neurol* 1989;46(10):1121-1123.
30. International classification of functioning, disability and health. *World Health Organisation* 2001.
31. Resnik L, Plow MA. Measuring participation as defined by the international classification of functioning, disability and health: an evaluation of existing measures. *Arch Phys Med Rehabil* 2009;90(5):856-866.
32. Hagemans ML, Laforet P, Hop WJ, Merkies IS, Van Doorn PA, Reuser AJ, Van der Ploeg AT. Impact of late-onset Pompe disease on participation in daily life activities: evaluation of the Rotterdam Handicap Scale. *Neuromuscul Disord* 2007;17(7):537-543.
33. Merkies IS, Schmitz PI, Van Der Meche FG, Samijn JP, Van Doorn PA. Psychometric evaluation of a new handicap scale in immune-mediated polyneuropathies. *Muscle Nerve* 2002;25(3):370-377.
34. Tarlov Ar Fau - Ware JE, Jr., Ware Je Jr Fau - Greenfield S, Greenfield S Fau - Nelson EC, Nelson Ec Fau - Perrin E, Perrin E Fau - Zubkoff M, Zubkoff M. The Medical Outcomes Study. An application of methods for monitoring the results of medical care. (0098-7484 (Print)).
35. Hagemans ML, Janssens AC, Winkel LP, Sieradzan KA, Reuser AJ, Van Doorn PA, Van der Ploeg AT. Late-onset Pompe disease primarily affects quality of life in physical health domains. *Neurology* 2004;63(9):1688-1692.
36. Hagemans ML, Winkel LP, Van Doorn PA, Hop WJ, Loonen MC, Reuser AJ, Van der Ploeg AT. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. *Brain* 2005;128(Pt 3):671-677.
37. Hagemans ML, Hop WJ, Van Doorn PA, Reuser AJ, Van der Ploeg AT. Course of disability and respiratory function in untreated late-onset Pompe disease. *Neurology* 2006;66(4):581-583.
38. van der Beek NA, de Vries JM, Hagemans ML, Hop WC, Kroos MA, Wokke JH, de Visser M, van Engelen BG, Kuks JB, van der Kooi AJ *et al*. Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study. *Orphanet J Rare Dis* 2012;7:88.
39. van der Beek NA, van Capelle CI, van der Velden-van Etten KI, Hop WC, van den Berg B, Reuser AJ, van Doorn PA, van der Ploeg AT, Stam H. Rate of progression and predictive factors for pulmonary outcome in children and adults with Pompe disease. *Mol Genet Metab* 2011;104(1-2):129-136.
40. Hagemans ML, Winkel LP, Hop WC, Reuser AJ, Van Doorn PA, Van der Ploeg AT. Disease severity in children and adults with Pompe disease related to age and disease duration. *Neurology* 2005;64(12):2139-2141.

41. Slonim AE, Bulone L, Ritz S, Goldberg T, Chen A, Martiniuk F. Identification of two subtypes of infantile acid maltase deficiency. *J Pediatr* 2000;137(2):283-285.
42. Merkies IS, Schmitz PI, Samijn JP, van der Meche FG, van Doorn PA. Fatigue in immune-mediated polyneuropathies. European Inflammatory Neuropathy Cause and Treatment (INCAT) Group. *Neurology* 1999;53(8):1648-1654.
43. Angelini C, Semplicini C, Tonin P, Filosto M, Pegoraro E, Soraru G, Fanin M. Progress in Enzyme Replacement Therapy in Glycogen Storage Disease Type II. *Ther Adv Neurol Disord* 2009;2(3):143-153.
44. Vielhaber S, Brejova A, Debska-Vielhaber G, Kaufmann J, Feistner H, Schoenfeld MA, Awiszus F. 24-months results in two adults with Pompe disease on enzyme replacement therapy. *Clin Neurol Neurosurg* 2011;113(5):350-357.
45. Gungor D, de Vries JM, Brusse E, Kruijshaar ME, Hop WC, Murawska M, van den Berg LE, Reuser AJ, van Doorn PA, Hagemans ML *et al*. Enzyme replacement therapy and fatigue in adults with Pompe disease. *Mol Genet Metab* 2013.
46. Gungor D, Kruijshaar ME, Plug I, Rizopoulos D, Kanters TA, Wens SCA, Reuser AJ, van Doorn PA, van der Ploeg AT. Quality of life and participation in daily life activities of adults with Pompe disease receiving enzyme replacement therapy: 10 years of international follow-up. Submitted.
47. Gungor D, de Vries JM, Hop WC, Reuser AJ, van Doorn PA, van der Ploeg AT, Hagemans ML. Survival and associated factors in 268 adults with Pompe disease prior to treatment with enzyme replacement therapy. *Orphanet J Rare Dis* 2011;6:34.
48. Gungor D, Kruijshaar ME, Plug I, D'Agostino RB, Sr., Hagemans ML, van Doorn PA, Reuser AJ, van der Ploeg AT. Impact of enzyme replacement therapy on survival in adults with Pompe disease: results from a prospective international observational study. *Orphanet J Rare Dis* 2013;8(1):49.
49. Kanters TA, Hagemans ML, van der Beek NA, Rutten FF, van der Ploeg AT, Hakkaart L. Burden of illness of Pompe disease in patients only receiving supportive care. *J Inherit Metab Dis* 2011;34(5):1045-1052.
50. Rigter T, Weinreich SS, van El CG, de Vries JM, van Gelder CM, Gungor D, Reuser AJ, Hagemans ML, Cornel MC, van der Ploeg AT. Severely impaired health status at diagnosis of Pompe disease: a cross-sectional analysis to explore the potential utility of neonatal screening. *Mol Genet Metab* 2012;107(3):448-455.
51. van der Beek NA, Hagemans ML, van der Ploeg AT, van Doorn PA, Merkies IS. The Rasch-built Pompe-specific activity (R-PAct) scale. *Neuromuscul Disord* 2013;23(3):256-264.
52. Gungor D, Schober AK, Kruijshaar ME, Plug I, Karabul N, Deschauer M, van Doorn PA, van der Ploeg AT, Schoser B, Hanisch F. Pain in adult patients with Pompe disease: A cross-sectional survey. *Mol Genet Metab* 2013;109(4):371-376.
53. Lachmann R, Schoser B. The clinical relevance of outcomes used in late-onset Pompe disease: can we do better? *Orphanet J Rare Dis* 2013;8(1):160.



Enzyme replacement therapy reduces the risk for wheelchair dependency in adult Pompe patients

J.C. van der Meijden, M.E. Kruijshaar, D. Rizopoulos
P.A. van Doorn, N.A.M.E. van der Beek, A.T. van der Ploeg

8

Submitted

Abstract

Pompe disease is a rare metabolic myopathy. In adult patients, progressive weakness of limb-girdle and respiratory muscles often lead to wheelchair and respirator dependency. Clinical studies have shown enzyme replacement therapy (ERT) to positively affect motor and respiratory outcomes. Here we investigate whether ERT reduces patients' risk of needing a wheelchair or respirator.

Data were collected as part of a prospective international survey, the IPA/Erasmus MC Pompe survey, which was conducted annually between 2002 and 2016. We excluded patients who were already using a wheelchair or respirator, those under 18 at survey entry, and those who had missing information. Time-dependent Cox proportional hazard models were used.

The inclusion criteria for analyzing the risk of wheelchair use were met by 189 patients (median age 47 years; range 18-75). During follow-up, 126 (67%) started ERT. Over 1120 person-years of follow-up (median 5 years), 46 became wheelchair dependent, 16 of whom used ERT. After adjustment for disease duration, sex and country, ERT reduced the risk for wheelchair use (HR 0.39; 95%CI 0.19-0.82). For analyses of respirator use, 177 patients met the inclusion criteria (median age 46 years; range 18-73). Over 1190 person-years of follow-up (median 6 years), 125 patients (71%) were treated and 48 started respiratory support, 28 of whom received ERT. We found no association between ERT and the risk for respirator use (HR 1.23; 95%CI 0.67-2.28).

In conclusion, our study found that ERT reduced the risk for wheelchair dependency. We could not demonstrate an effect on respiratory support.

Introduction

Pompe disease is an autosomal recessive metabolic myopathy for which enzyme replacement therapy (ERT) has been available since 2006.[1, 2] In adults, the disease is characterized by progressive limb-girdle and respiratory muscle weakness. In most cases, this ultimately leads to the use of a wheelchair and/or respiratory support.[3, 4] Wheelchair dependency and the use of respiratory support considerably impact a patient's ability to participate in daily life activities, and reduce quality of life.[5, 6] It should therefore be an important treatment goal to prevent the disease from progressing to the point that a patient becomes dependent on these aids.

Many studies have evaluated the effects of ERT in adult patients with Pompe disease. While these have shown that ERT has a positive effect on motor function and/or lung function,[7-15] its effect on wheelchair dependency and respiratory support has been reported only in a few cases.

Together with the International Pompe Association (IPA), our center has systematically collected data on patients with Pompe disease since 2002, well before the approval of ERT. Over many years, this IPA/ Erasmus MC Pompe survey has consistently followed a large international cohort of patients with Pompe disease both before and during ERT. Earlier findings from the survey include the demonstration of a positive effect of ERT on survival.[16] Using data from the survey, we investigated whether ERT reduces the risk that a patient will need a wheelchair or respiratory support.

Methods

Patients

A detailed description of the survey's design has been published previously.[16] Since 2002, patients have been recruited through national patient organizations in Canada, Germany, France, the Netherlands, the United Kingdom and the United States. Recruitment was independent of patients' disease severity. All patients have provided informed consent.

Annual survey questions included items on the use of a wheelchair and respiratory support. Disease duration was calculated as the number of years since diagnosis. In our analyses we included all questionnaires completed before July 2016.

The current study included patients aged 18 years and above at inclusion in the survey. Patients who already used a wheelchair or respiratory support at survey entry (i.e. who had

already had the "event") were excluded from the analyses, as were those who had completed the survey only once (no follow-up) or had incomplete information on the events or disease duration.

Statistical analysis

Time-dependent Cox proportional hazard models were used to calculate the effect of ERT on the risk of using a wheelchair or respiratory support. Models were developed separately for both outcomes.

Age was used as the time scale of the analysis, each patient being followed from the age at inclusion in the survey, until the date of last follow-up (censoring), or until becoming wheelchair or ventilator dependent. ERT was assessed as a time-dependent variable that switched from 0 to 1 when patients started treatment. This approach allowed patients' to contribute both treated and untreated person-years of follow-up to the analyses. The following covariates were chosen a priori: disease duration, gender and country of residence. Like ERT, disease duration was included as a time-dependent covariate, updating when patients started treatment.

Results are presented as hazard ratios (HRs) with 95% confidence intervals (CIs). The proportional hazards assumption was checked by plotting scaled Shoenfeld residuals and correlating them with the Kaplan Meier estimate of the survival function.

Since both analyses originate from the same population, we used the Holm method to correct for multiple testing.[17]

Statistical tests were performed using R version 3.3.1 including the survival package.[18, 19] A p-value <0.05 was considered significant.

Results

Overall, 458 patients participated in the IPA/ Erasmus MC Pompe survey between 2002 and July 2016. The inclusion flow-chart in Figure 1 shows that 189 patients were eligible for analysis of the effect of ERT on wheelchair dependency and that 177 were eligible for analysis of respiratory support. 125 patients (27%) were excluded as they already used a wheelchair at survey entry; 150 (33%) were excluded because they already required respiratory support.

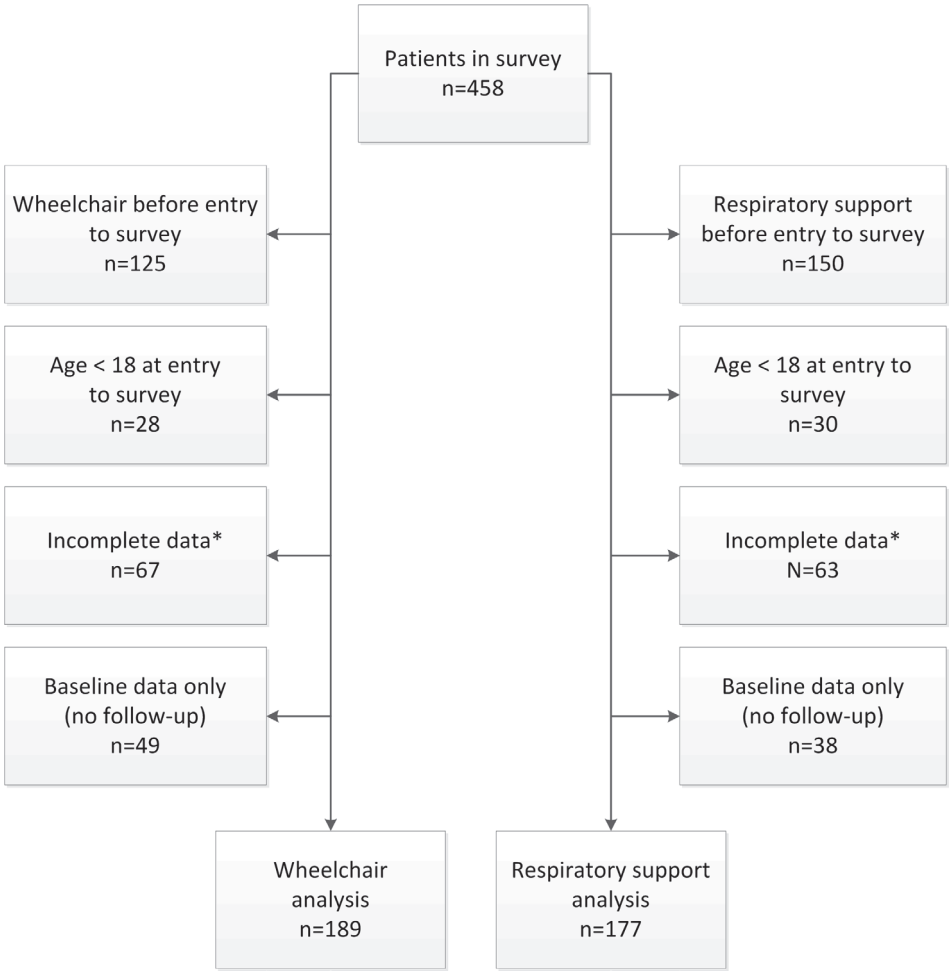


Figure 1 | Inclusion flowchart.

* Data was incomplete if either the age at which the event occurred or the disease duration was unknown.

Table 1 compares the baseline characteristics of patients included in the two analyses with those of all patients participating in the IPA/Erasmus MC survey. Overall, patients who participated in the survey (n=458) were equally distributed between the sexes, and entered the survey at a median age of 47 years. Seventy percent started ERT at some point during their follow-up. Patients who fulfilled the inclusion criteria for our analyses had a shorter disease duration, which might be explained by the fact that they did not require a wheelchair or ventilator at survey entry. Patients not using a ventilator at survey entry were more frequently female than male.

Table 1 | Characteristics of patients participating in the analysis of the effect of ERT on the use of a wheelchair and respiratory support, and of all patients included in the IPA/Erasmus MC Pompe survey.

	Included in analyses		All participants Survey (n=458)
	Risk of using a wheelchair (n=189)	Risk of using respiratory support (n=177)	
Female, n (%)	98 (51.9%)	109 (61.6%)	230 (50%)
Median age at entry into the survey, years (range)	47 (18 - 75)	46 (18 - 73)	47 (2 - 81)
Median age at diagnosis, years (range)	39 (3 - 72)	39 (0 - 72)	38 (0 – 72)
Median disease duration at entry, years (range)	5 (0 - 39)	5 (0 - 31)	7 (0 – 39)
Country of residence, n (%)			
Netherlands	78 (41.3%)	87 (49.2%)	134 (29%)
United Kingdom	9 (4.8%)	9 (5.1%)	36 (8%)
United States	44 (23.3%)	36 (20.3%)	124 (27%)
Germany	26 (13.8%)	25 (14.1%)	66 (14%)
Other	32 (16.9%)	20 (11.3%)	98 (21%)
ERT[&], n (%)	126 (66.7%)	125 (70.6%)	319 (70%)
Median age start ERT, years (range)	48 (13 - 73)	50 (13 - 74)	47 (3 – 77)
Median follow-up duration, years (range)	5 (1 - 14)	6 (1 - 14)	N.A.
Events during follow-up, n (%)	46 (24.3%)	48 (27.1%)	N.A.
Median age at event, years (range)	52 (21 - 76)	50 (24 - 73)	N.A.

Table 2 shows the results of the time-dependent Cox proportional hazard regression models. A total of 1120 person-years of follow-up were available for our analysis of the effect of ERT on wheelchair use (median follow-up 5 years, table 1). Sixteen events occurred over 652 treated-person years and 30 events over 468 untreated-person years. After adjustment for disease duration at survey entry and at start of ERT, and also for sex and country, ERT significantly reduced the risk of becoming wheelchair dependent (hazard ratio of 0.39; CI 0.19 - 0.82, Table 2). In other words, at any point during follow-up, a treated patient had a 61% lower risk for becoming wheelchair dependent than an untreated patient. Disease duration was an important predictor of becoming wheelchair dependent: the risk for becoming wheelchair dependent was over three times higher in those whose disease duration was over 10 years than in those who had been diagnosed for less than 5 years. Country of residence and gender were not significantly associated with the risk for wheelchair use.

Table 2 | Multivariate time-dependent Cox regression analysis of wheelchair use (A) and the need for respiratory support (B).

A. Wheelchair use	Events	Person-years	HR	95% CI	*p*-value
Treatment*					
Untreated (ref)	30	468			
ERT	16	652	0.39	(0.19 – 0.82)	**0.008**
Disease duration*					
<5 years (ref)	10	464			
5-10 years	10	332	1.07	(0.37 – 3.07)	1
>10 years	26	324	3.28	(1.36 - 7.92)	**0.005**
Sex					
Male (ref)	17	583			
Female	29	537	1.69	(0.83 - 3.46)	0.19
Country of residence					
Netherlands (ref)	13	531			
Other	33	589	1.97	(0.90 - 4.31)	0.10

B. Respiratory support	Events	Person-years	HR	95% CI	p-value
Treatment*					
Non-use (ref)	20	529			
ERT	28	661	1.23	(0.67 - 2.28)	0.5
Disease duration*					
<5 years (ref)	11	430			
5-10 years	12	319	1.18	(0.44 – 3.15)	1
>10 years	25	441	2.20	(1.04 – 4.66)	**0.04**
Sex					
Male (ref)	21	420			
Female	27	770	0.68	(0.37 - 1.23)	0.20
Country of residence					
The Netherlands (ref)	18	599			
Other	30	591	1.57	(0.84 – 2.93)	0.16

* Time-dependent covariates, updated at start of ERT.

For the analysis of use of respiratory support, 1190 person-years were accumulated (median follow-up 6 years, table 1). Over 661 treated person-years, 28 patients started using respiratory support, compared to 20 patients over 529 untreated person-years. We detected no association between ERT and the risk for starting respiratory support (HR 1.23; 95%CI 0.67-2.28). Longer disease duration again increased the risk for starting respiratory support, while no significant difference was detected for sex or country of residence.

Discussion

This is the first study to provide evidence that ERT reduces the risk that adult patients with Pompe disease will become wheelchair dependent. Using data from an international cohort of almost 200 adult patients, we show that, at any point in time, a patient who received ERT had a 61% smaller probability of becoming wheelchair dependent than an untreated patient. With regard to the risk for starting respiratory support, no differences could be detected.

To patients, ambulatory and respiratory status are relevant outcomes that have a substantial impact on quality of life.[6, 20] As sometimes decades may pass between a patient's first symptoms and their becoming ventilator or wheelchair dependent, a large cohort and long follow-up are required to study changes in these outcomes. Similarly, any study of the effect of treatment on these outcomes requires data that were obtained before and after start of this treatment. Our survey uniquely meets these requirements.

Since ambulation requires sufficient muscle strength and function, our finding that ERT reduces the risk of becoming wheelchair dependent is in line with studies reporting improvements in muscle strength and function.[7-15] With regard to our inability to demonstrate an effect of ERT on the risk for respiratory support, this may have been due to the smaller effect of ERT on respiratory muscles than on skeletal muscles, which has been reported in several earlier studies.[7, 9-11, 13]

Our analyses were corrected for disease duration, country of residence and gender. Our finding that longer disease duration increased the risk for both wheelchair and respiratory support is in line with the progressive character of the disease, and was also concluded from earlier studies from the IPA/ Erasmus MC Pompe survey and our clinical studies.[21-23] Although the survey detected no differences between country and gender on the risk for wheelchair use and respiratory support, the fact that a larger proportion of men than women were already using respiratory support at inclusion in the survey suggests that there may be gender differences in lung function. This was also suggested by our earlier study in untreated adults, where the decline in lung function was faster in men.[23] More research is needed to elucidate these differences.

The difference between being able to walk and needing a wheelchair is very tangible, and ERT's reduction of the risk of becoming wheelchair dependent is an important improvement. Nevertheless, a proportion of treated patients still become wheelchair dependent at some point in their life. Hence, while ERT shows positive clinical effects in adult patients with Pompe disease, we also conclude that there is still room for improvement.

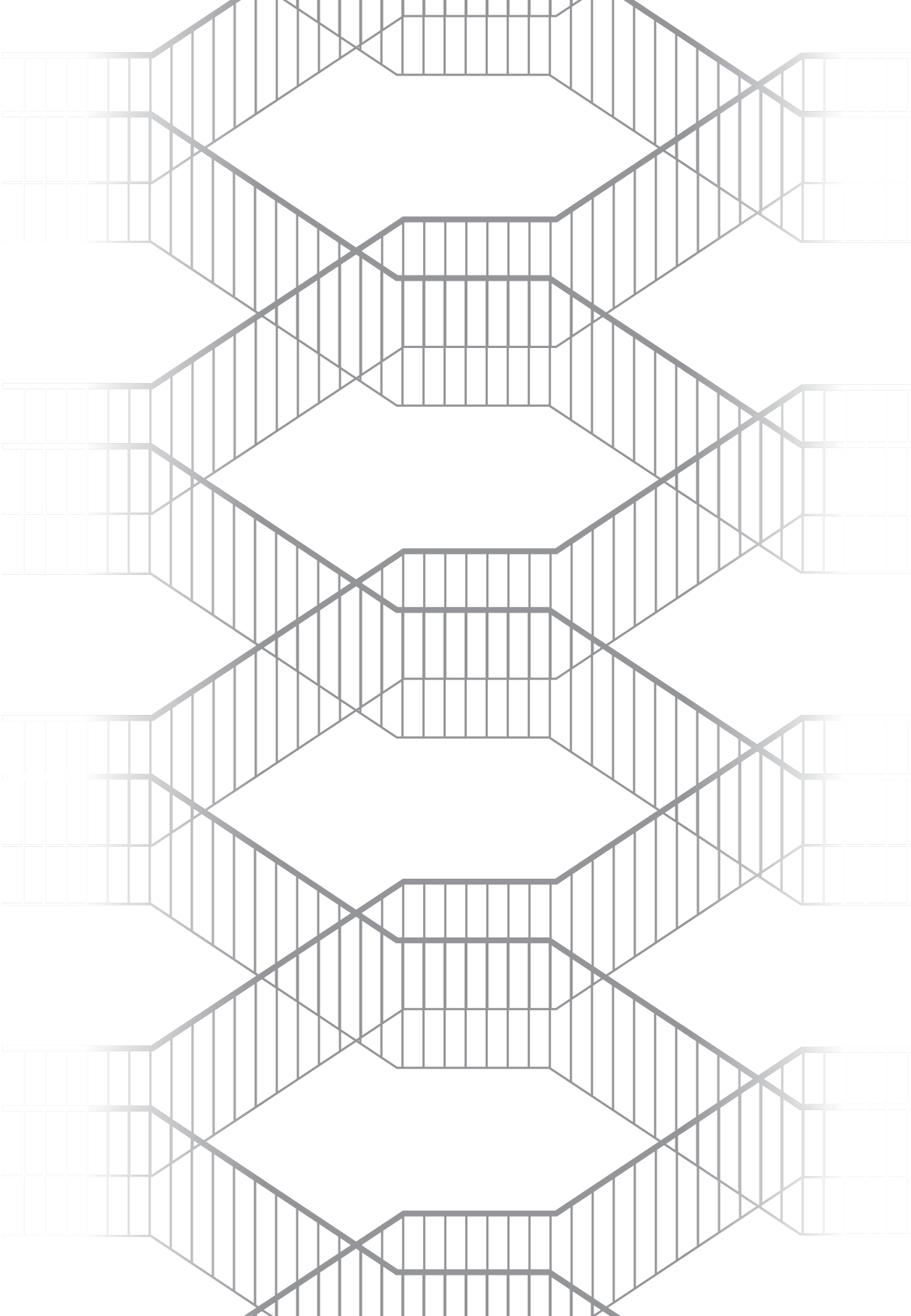
Conclusion

ERT reduces the risk for wheelchair dependency in adult Pompe patients. Since ambulation provides independence, we believe this is of key importance to patients. An effect of ERT on the risk for respiratory support could not be demonstrated.

References

1. Hirschhorn R, Reusser AJ: Glycogen storage disease type II: acid alphaglucosidase (acid maltase) deficiency. In: *The metabolic and molecular bases of inherited disease.* 8th edn. Edited by Scriver CR, Beaudet AL, Sly WS, Valle D. New York: McGraw-Hill; 2001: 3389–3420.
2. Engel AG, Hirschhorn R, Huie ML: Acid Maltase Deficiency. In: *Myology.* 3th edn. Edited by Engel AG. New York: McGraw-Hill; 2004: 1559-1586.
3. Van der Beek NA, Hagemans ML, Reuser AJ, Hop WC, Van der Ploeg AT, Van Doorn PA, Wokke JH. Rate of disease progression during long-term follow-up of patients with late-onset Pompe disease. *Neuromuscul Disord* 2009;19(2):113-117.
4. van der Ploeg AT, Reuser AJ. Pompe's disease. *Lancet* 2008;372(9646):1342-1353.
5. Hagemans ML, Janssens AC, Winkel LP, Sieradzan KA, Reuser AJ, Van Doorn PA, Van der Ploeg AT. Late-onset Pompe disease primarily affects quality of life in physical health domains. *Neurology* 2004;63(9):1688-1692.
6. Hagemans ML, Laforet P, Hop WJ, Merkies IS, Van Doorn PA, Reuser AJ, Van der Ploeg AT. Impact of late-onset Pompe disease on participation in daily life activities: evaluation of the Rotterdam Handicap Scale. *Neuromuscul Disord* 2007;17(7):537-543.
7. Anderson LJ, Henley W, Wyatt KM, Nikolaou V, Waldek S, Hughes DA, Lachmann RH, Logan S. Effectiveness of enzyme replacement therapy in adults with late-onset Pompe disease: results from the NCS-LSD cohort study. *J Inherit Metab Dis* 2014;37(6):945-952.
8. Angelini C, Semplicini C, Ravaglia S, Bembi B, Servidei S, Pegoraro E, Moggio M, Filosto M, Sette E, Crescimanno G *et al*. Observational clinical study in juvenile-adult glycogenosis type 2 patients undergoing enzyme replacement therapy for up to 4 years. *J Neurol* 2012;259(5):952-958.
9. de Vries JM, van der Beek NA, Hop WC, Karstens FP, Wokke JH, de Visser M, van Engelen BG, Kuks JB, van der Kooi AJ, Notermans NC *et al*. Effect of enzyme therapy and prognostic factors in 69 adults with Pompe disease: an open-label single-center study. *Orphanet J Rare Dis* 2012;7:73.
10. Regnery C, Kornblum C, Hanisch F, Vielhaber S, Strigl-Pill N, Grunert B, Muller-Felber W, Glocker FX, Spranger M, Deschauer M *et al*. 36 months observational clinical study of 38 adult Pompe disease patients under alglucosidase alfa enzyme replacement therapy. *J Inherit Metab Dis* 2012;35(5):837-845.
11. van der Ploeg AT, Barohn R, Carlson L, Charrow J, Clemens PR, Hopkin RJ, Kishnani PS, Laforet P, Morgan C, Nations S *et al*. Open-label extension study following the Late-Onset Treatment Study (LOTS) of alglucosidase alfa. *Mol Genet Metab* 2012;107(3):456-461.
12. van der Ploeg AT, Clemens PR, Corzo D, Escolar DM, Florence J, Groeneveld GJ, Herson S, Kishnani PS, Laforet P, Lake SL *et al*. A randomized study of alglucosidase alfa in late-onset Pompe's disease. *N Engl J Med* 2010;362(15):1396-1406.
13. Strothotte S, Strigl-Pill N, Grunert B, Kornblum C, Eger K, Wessig C, Deschauer M, Breunig F, Glocker FX, Vielhaber S *et al*. Enzyme replacement therapy with alglucosidase alfa in 44 patients with late-onset glycogen storage disease type 2: 12-month results of an observational clinical trial. *J Neurol* 2010;257(1):91-97.
14. van der Ploeg AT, Kruijshaar ME, Toscano A, Laforet P, Angelini C, Lachmann RH, Pascual Pascual SI, Roberts M, Rosler K, Stulnig T *et al*. European consensus for starting and stopping enzyme replacement therapy in adult patients with Pompe disease: a 10-year experience. *Eur J Neurol* 2017;24(6):768-e731.
15. Stepien KM, Hendriksz CJ, Roberts M, Sharma R. Observational clinical study of 22 adult-onset Pompe disease patients undergoing enzyme replacement therapy over 5years. *Mol Genet Metab* 2016;117(4):413-418.
16. van der Meijden JC, Gungor D, Kruijshaar ME, Muir AD, Broekgaarden HA, van der Ploeg AT. Ten years of the international Pompe survey: patient reported outcomes as a reliable tool for studying treated and untreated children and adults with non-classic Pompe disease. *J Inherit Metab Dis* 2015;38(3):495-503.
17. Holm S. A Simple Sequentially Rejective Multiple Test Procedure. *Scand J Stat* 1979;6(2):65-70.
18. Therneau TM, Grambsch PM: Modeling Survival Data: Extending the Cox Model. New York: Springer; 2000.
19. R Core Team R: A Language and Environment for Statistical Computing. In. Vienna, Austria: R Foundation for Statistical Computing; 2015.
20. Gungor D, Kruijshaar ME, Plug I, Rizopoulos D, Kanters TA, Wens SC, Reuser AJ, van Doorn PA, van der Ploeg AT. Quality of life and participation in daily life of adults with Pompe disease receiving enzyme replacement therapy: 10 years of international follow-up. *J Inherit Metab Dis* 2016;39(2):253-260.

21. Hagemans ML, Winkel LP, Hop WC, Reuser AJ, Van Doorn PA, Van der Ploeg AT. Disease severity in children and adults with Pompe disease related to age and disease duration. *Neurology* 2005;64(12):2139-2141.
22. van der Beek NA, de Vries JM, Hagemans ML, Hop WC, Kroos MA, Wokke JH, de Visser M, van Engelen BG, Kuks JB, van der Kooi AJ *et al*. Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study. *Orphanet J Rare Dis* 2012;7:88.
23. van der Beek NA, van Capelle CI, van der Velden-van Etten KI, Hop WC, van den Berg B, Reuser AJ, van Doorn PA, van der Ploeg AT, Stam H. Rate of progression and predictive factors for pulmonary outcome in children and adults with Pompe disease. *Mol Genet Metab* 2011;104(1-2):129-136.



General discussion and future perspectives

9

Pompe disease is the first inheritable muscle disorder for which a disease-specific treatment has become available. In 2006 Enzyme Replacement Therapy (ERT) was registered for all Pompe patients in Europe and the US. While this changed the perspectives of patients, research into several aspects of the disease, including the long-term effects of ERT, is ongoing to further improve the lives of patients and their treatment options. At the Erasmus MC more than 150 adults and children with Pompe disease are being followed, and over 400 participate in the "IPA/ Erasmus MC Pompe survey". This data has allowed a number of questions to be answered.

The aim of this thesis was to further elucidate the natural course of Pompe disease in children and effects of ERT and to compare it with the long-term effects of ERT in adults, to investigate factors that can modify the course of the disease and/or response to ERT, and to share our experience using Patient Reported Outcomes (PROs) in this rare disease. The first part of this chapter discusses the main findings of our studies in relation to these aims. In the second part we share our experience with long-term follow up of patients both in the clinic as well as using the "IPA/ Erasmus MC Pompe survey". Finally, the third part of this chapter provides our conclusions and perspectives for future research based on our studies.

Main findings of this thesis

- A third of untreated children with the non-classic phenotype of Pompe disease are already severely affected by the disease before reaching adulthood (Chapter 2).
- Sixty-eight percent of the patients who have their presenting symptoms during childhood carry the c.-32-13T>G mutation, highlighting the broad clinical spectrum caused by this mutation (Chapter 2).
- Children and adults with Pompe disease who receive long-term ERT benefit still from treatment after 5-7 years. The effect of ERT seems to most prominent during the first 2-3 years of treatment (Chapter 3 & 4).
- Enzyme replacement therapy reduces the risk to start using a wheelchair (Chapter 8).
- Reduced muscle strength of the lower extremities, higher BMI, male gender, the use of respiratory support and older age increases the risk for not being able to walk (Chapter 5).
- The ACE polymorphism does not sufficiently explain the broad clinical spectrum nor the differences in the effect of ERT in patients-with Pompe disease (Chapter 6).
- "IPA/ Erasmus MC Pompe survey" has proven to be an effective and fast tool to study the impact of the disease and effect of treatment on the daily life of patients (Chapter 7 and 8).

Main findings

Research on Pompe disease in untreated children and their clinical presentation

Pompe disease has a very broad spectrum where patients can develop symptoms at any age. The majority of patients with the non-classic presentations of the disease are adults, while children are much fewer in numbers. Consequently, little information is available about the development of the disease or the effects of ERT specifically in children.

Thirty-one children visited the Erasmus MC between 1975 and 2012. Findings from their first assessment in our center were studied to improve our understanding of the clinical presentation of Pompe disease in children. None of these children were treated before their first visit to our center, but many were referred to us after they had been diagnosed. The most common first symptoms reported in these children were limb-girdle muscle weakness and delayed motor development. At their first assessment more than 50% had low/absent reflexes, weakness of facial muscles and scoliosis, while 48% had decreased pulmonary function. The majority of children had one or more physical limitations, most commonly in performing tasks that needed limb-girdle muscles, like standing up from supine position. Also, more than half of the children had problems flexing their neck in supine position. Furthermore, before reaching adulthood 32% of our children needed a wheelchair or ventilator, and two died. This shows that children can be severely affected by the disease and have severe mobility and respiratory problems during childhood. A high awareness for Pompe disease in children is needed to identify these patients as early as possible in order to be able to monitor and follow them since the disease can progress fast in these patients.

The results of this study showed that 68% percent of the children had the c.-32-13T>G/null genotype. This genotype is found in over 95% of the adults in the Netherlands (chapter 4) and over 80% of adults in other European countries and the US and points to the broad clinical spectrum in patients with this genotype. Noteworthy, children diagnosed with this mutation were predominantly male in our study (18 of 21 children with this mutation), while in adults this mutation is equally distributed across both sexes. This suggests that male patients with this mutation may develop symptoms earlier than female patients with this mutation. The study also showed that both patients with the c.-32-13T>G/null genotype and other mutations could already present with rather severe disease symptoms at young age such as respiratory failure or severe mobility problems during childhood. The patients who died during childhood were patients with other mutations. Also the results of other outcome measures suggested that patients with other mutations were generally more severely affected.

Comparison of the results of our study in children to those published on adults shows that both similarities and differences exist. Limb-girdle weakness and respiratory involvement are common problems in both adult patients and children.[1,2] However, there are differences in the distribution and severity of muscle weakness. For example, in children, neck flexor muscles were more severely affected than in adults, while weakness of the quadriceps muscle was less prominent. Figure 1 illustrates this further, depicting the distribution and the severity of muscle weakness in our 31 children compared to those in 94 Dutch adult patients reported previously.[2] In addition, also the prevalence of certain clinical findings differs. Scoliosis was more frequently observed in children, while in adults ptosis and bulbar weakness are more often reported.[2] These differences in the distribution and severity of muscle weakness indicate that the disease might progress somewhat differently in children and in adults.

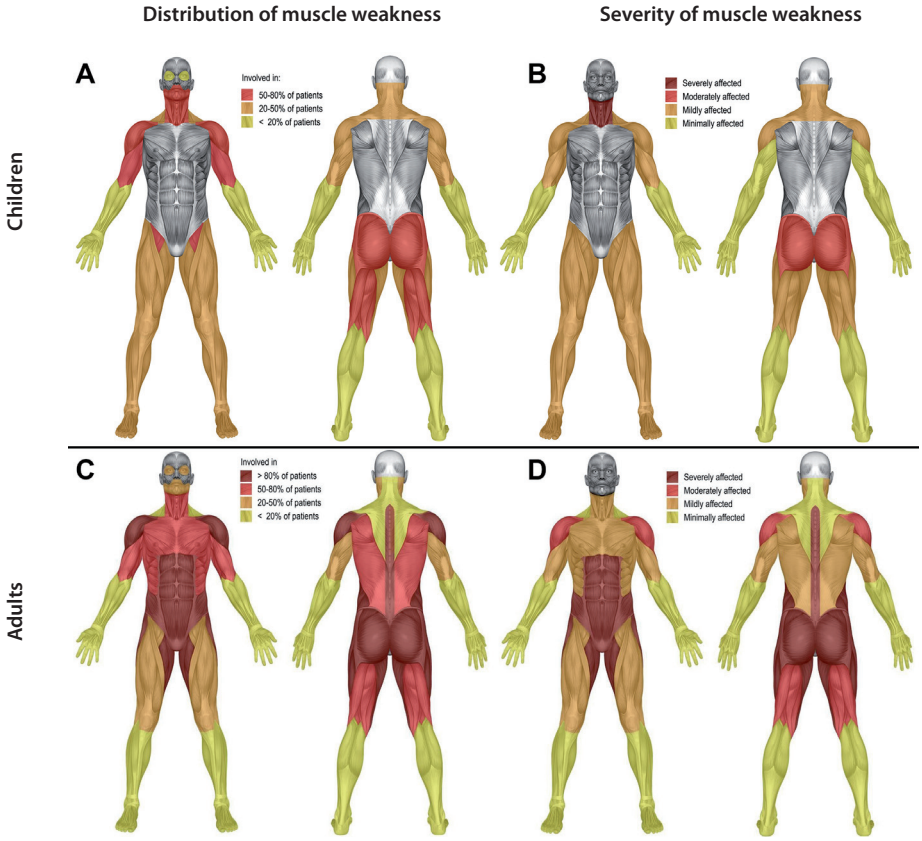


Figure 1 | Distribution of muscle weakness (A+C) and severity (B+D) of muscle weakness in 31 children[3] (top: A+B) and 94 Dutch adults[2] (bottom: C+D).

Long-term effects of ERT in children and adults

Seventeen children were followed for a median of 6.8 years (range 1.8-15.1) while receiving treatment. At group level, muscle function measured by 6MWT and QMFT improved significantly (7.4%-point and 9.2%-point increase, respectively). Muscle strength, measured by HHD and MRC, remained stable during treatment. Lung function responded less well to ERT. In sitting position, it declined by 5.2%-points over 7 years. FVC in sitting position deteriorated particularly in males (8.1%-point) while females remained stable. In supine position the decline was not statistically significant, which might be due to a lack of statistical power, rather than a better response to treatment in supine position. The size of the decline during treatment appears to be slower than without treatment, as studies in untreated adults and children report that FVC scores decline between 1 and 5%-points per year.[4-9] This suggests that despite a continuing decline, ERT still has a positive effect on lung function. The data should be interpreted with caution since the number of patients are relatively small. Earlier it was suggested that early start of treatment might prevent clinical deterioration. Our study shows that this might not be true for all patients.

Our study in 102 adult patients compared the course of disease observed during treatment to the progression of the disease before treatment started. The comparison with pretreatment data allowed us to assess whether ERT effectively slows down or halts the disease progression seen in the untreated situation. This study therefore provides a higher level of evidence than most observational studies on Pompe patients, including our above study on children, which do not make this comparison. Median follow-up was 1.1 years before treatment and 5.0 years during treatment. Most patients were followed both before and during treatment. Some contributed only untreated follow-up (14 patients) or treatment follow-up (6 patients).

At group level, muscle strength, activity levels assessed using the R-PAct scale, and lung function were significantly higher at 5 years of ERT than the projected untreated disease progression. Patients' HHD and MRC were 6.6%-points and 9.6%-points higher at 5 years of treatment compared to the projected untreated disease progression. Muscle function assessed by the QMFT increased in the first 2-3 years, but were similar to the projected untreated disease progression at 5 years of ERT. R-PAct scores increased by 10.8%-point. FVC scores were also significantly higher both in sitting and in supine positions (7.3%-point and 7.6%-point). When supine FVC scores were compared to the start of treatment, on the other hand, they declined (2.9%-point). This, as stated before, highlights the importance of comparison to pretreatment follow-up data when assessing the effect of treatment, and corroborates our suggestion that the decline in FVC in children may be slower than without treatment.

In both our study in adults and children we also examined patients' individual progression on the outcome measures over time and observed that there is a large variation in the response to ERT between patients. Understanding the factors that underlie the variation are important in order to improve the outcome of treatment and personalize care. Also, we found for both adults and children that the response to ERT can change over time, and was most substantial in the first 2-3 years of treatment. Patients who initially respond well can stabilize or, in some cases, decline later during their treatment. This highlights the need for consistent long-term follow up in both children and adults.

Figure 2 compares the individual responses of patients in our two studies (Figure 2A – children; Figure 2B – adults) to those described in literature. For children, eight studies have been published so far (Figure 2C).[10-17] These studies included a total of 20 individual patients, accounting for overlapping populations, who were followed between 1.3-8 years. For adults (figure 2D), the results of a recent literature review was used, encompassing 44 studies on the effects of ERT in adults.[5-7,11,14,16-52] Adjusting for overlap of patients in studies, 586 individual patients were described in these 44 studies, covering 26 different patient populations. The (weighted) median follow-up of these studies was 2.5 years (ranging from 5 months to 8 years), which is considerably shorter than the median follow up of 5 years in our study on adults.

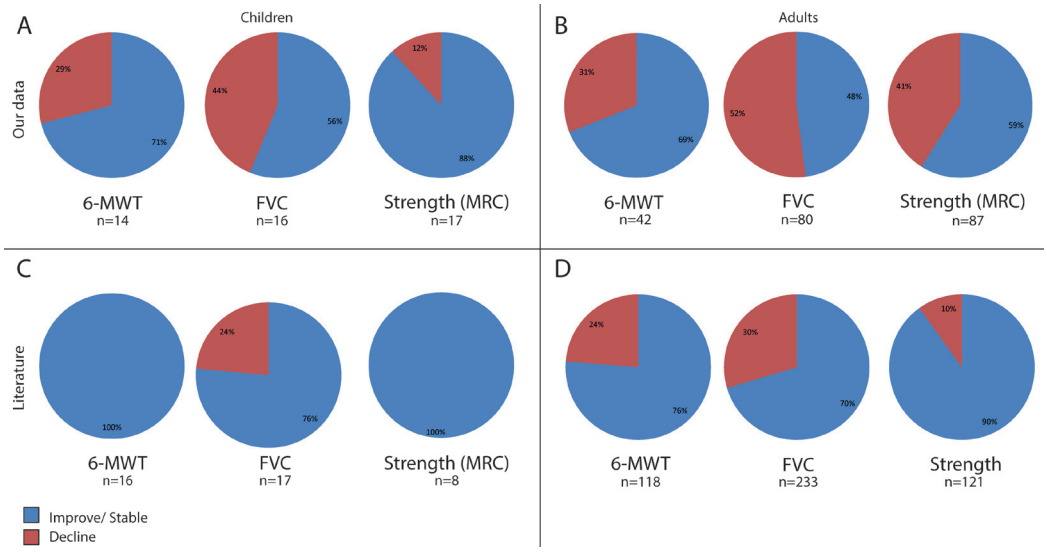


Figure 2 | Individual responses to treatment reported in our studies in children (A) and adults (B) compared to those reported in the literature on children (C[10-17]) and adults (D[18]). All responses are in comparison to baseline (i.e. the start of ERT).

For children and adults, the overall implications of our study are in line with the literature, indicating that patients benefit from treatment in terms of muscle strength and motor function, and to a lesser extent in lung function. Nevertheless, for children the individual responses described in literature suggest that many patients improve or stabilize, while in our study a larger proportion showed a decline. This may be related to a longer follow-up in our study, only one of the published studies followed patients for more than 3 years. Furthermore, some studies reported that patients performed clinically worse, but did not quantify the decline by an assessment. Also for adults, the individual patient responses described in the literature are somewhat more positive than in our five-year study (figure 2). It should be noted that the described decline is compared to baseline. As demonstrated by our study in adults, comparison to the projected untreated disease progression is needed to fully evaluate the effect of treatment.

Preventing the need to use a wheelchair and/or respiratory support is very important to patients. Both aids reduce the quality of life in patients with Pompe disease.[53] As sometimes decades may pass between a patient's first symptoms and their becoming ventilator or wheelchair dependent, a large cohort and long follow-up are required to study changes in these outcomes. We found that ERT reduced the risk for needing a wheelchair significantly. At any point during follow-up, a patient using ERT has a 61% smaller chance to become wheelchair dependent than a patient not receiving treatment. We were unable to demonstrate an effect of ERT on the risk for needing for respiratory support. This may have been due to the smaller effect of ERT on respiratory muscles than on skeletal muscles, which has been reported in several earlier studies and chapter 2 and 3.

All in all, children and adults benefit from treatment with ERT. However, treatment does not fully reverse or prevent symptoms in all patients. In the literature it has been suggested that an early start of therapy results in the best outcome for the patient.[5-7,34] In our study in children we reported that this paradigm does not always hold up. Two children deteriorated on most outcomes despite having a relative early start and being in a good clinical condition. Nevertheless, many factors play a role in the success of the therapy in an individual. Personalizing treatment by, for example, raising the dose in non-responding patients might be beneficial for them.

Factors that can modify the natural course of the disease and/or its response to treatment

Our studies in untreated children and on the effects of ERT resonate that there are factors that influence the severity of the disease and the effects of its treatment. Mutation and gender seem to influence the severity of the disease in untreated children. Gender may

also play a role in the response to treatment. The literature also suggests that the presence of antibodies against ERT may also influence its effect. However, while this is an important predictor for the treatment outcome of in classic-infantile patients, it does not frequently influence the effect of ERT in adults.[54] We believe the same may be true for children. In our previous study assessing 5 children receiving ERT all had low antibodies.[12] Further studies are ongoing to elucidate the role of antibodies in children. Two studies in this thesis focused specifically on factors that can modify disease severity and/or response to ERT. The first tries to elucidate what factors influence a patient's ability to walk. The second investigates whether a polymorphism in the gene coding for the angiotensin converting enzyme (ACE) influences the severity of the disease severity and/or the efficacy of ERT.

Data from 107 adult patients at their first visit to our center were used to study factors that influence a patient's ability to walk. Reduced muscle strength of the lower extremities (hip abductors and knee extensors), higher BMI, older age, male gender, and the use of a ventilator increased a patient's risk for not being able to walk. Some of these factors, such as BMI and, to a lesser degree, muscle strength, can be influenced by life style changes and training and therefore offer potential points for intervention. For example, training has been shown to increase muscle strength in Pompe patients and can therefore potentially improve or prevent loss of motor function.[55,56] All in all, the findings described in our study enable patients to be better informed about their ambulatory status and risk of losing ambulation in the oncoming years. It helps physicians to counsel and decide on the deployment of interventions or supportive measures.

When investigating factors which might explain differences in disease severity and response to treatment, the ACE polymorphism seemed a promising candidate. In the literature the suggestion has been made that ACE influences disease severity and the effects of ERT.[57-60] However, these studies were not conclusive and warranted further research. We investigated whether the ACE polymorphism had any effect on disease severity or on the effect of treatment in 131 children and adults, all carrying c.-32-13T>G/null mutations. Patients with this combination of mutations are known to have a wide variation in terms of onset and severity of the disease and response to treatment, which cannot be explained by their genotype. Despite the large number of patients, we did not find any significant relation between the ACE polymorphism and disease severity, with the effect of treatment, nor could it explain phenotypical variations within families. We therefore concluded that the ACE polymorphism does not play a significant role in these.

Long-term follow-up of Pompe patients - Lessons learnt for research on rare diseases

Research in rare diseases can be challenging due to the small number of patients who are usually scattered across different centers in different countries. This section discusses our long-standing experience of following patients with Pompe disease in the clinic, as well as through the "IPA/ Erasmus MC Pompe survey" in order to share our experiences and draw lessons for research on rare diseases.

IPA/ Erasmus MC Pompe Survey – lesions learned from working with PROs

Development of the survey

When, in 2002, promising results of the first trials with ERT suggested that this is likely to become a treatment for Pompe disease in the near future, the need for information on the natural course of Pompe disease became apparent. A partnership was started between the Erasmus MC and the International Pompe Association to collect data on this directly from patients. This resulted in the development of the "IPA/ Erasmus MC Pompe survey". This new survey asked questions covering all aspects of the life of Pompe patients. Questions were included based on the available literature and then reviewed by expert clinicians and patients. Patient support groups, affiliated to the IPA, reached out to their members in Australia, Canada, France, Germany, the Netherlands, the United Kingdom, the United States, and a few smaller countries and included them in the survey. Each year the survey includes and follows patients.

When we started the "IPA/ Erasmus MC Pompe survey", PROs were not commonly used in the field of metabolic diseases, despite the fact that they were already used quite frequently in other disease areas such as cancer. Nowadays, PROs have become important outcomes for measuring the efficacy of most treatments or interventions. More and more the Food and Drug Administration (FDA) and European Medicines Agency (EMA) require information on PROs in addition to survival and clinical outcomes when assessing the registration of new drugs.[61] As PROs are obtained directly from the patient without interpretation of anybody else they add information on the patient's perspective and his perceived functioning to standard clinical assessments.[62,63] For example, increasing walking distance by a few meters might not be relevant to patients, but if this results in improved quality of life these changes suddenly become very relevant.[64] Furthermore, some effects of a disease or treatment, like fatigue, are only known to a patient and can only be measured using PROs.

Results from the first years of the survey

Over the years the survey has grown into one of the largest databases on Pompe disease with a long and consistent follow-up. Our experience of working with this survey has shown that patients are reliable partners who can tell you exactly how the disease affects them. Also, analyses of the information obtained from this survey can identify changes over time.[65] In our review of the results from the first 10 years of the "IPA/ Erasmus MC Pompe survey" we show that this survey has contributed substantially to our knowledge on the natural course of Pompe disease and on the effects of its treatment, and can serve as an example for other rare diseases.[66]

The first results of the survey focused on elucidating the natural course of the disease and described the clinical manifestations and progressive character of the disease.[67-69] Furthermore, when comparing Pompe patients to the general population we discovered they were more fatigued, had lower participation in everyday life situations, lower quality of life and worse survival.[53,70-72] When ERT became available we learned that ERT reduced fatigue and the need for wheelchair use, and improved participation, quality of life and survival.[20-22] Survival and quality of life are key outcomes for assessment of treatments and important in reimbursement decision. The "IPA/ Erasmus MC Pompe survey" provided key information on this, and was the only study so far to study survival. National or local studies did not have the numbers of patients required and/or the consistent long-term follow-up before and during treatment required for such a study.

Practical issues and sustainability of the "IPA/ Erasmus MC Pompe survey"

One of the most important factors for the success of the "IPA/ Erasmus MC Pompe survey" was the start of data collection several years before ERT became available. Often, systematic data collection starts when a therapy has already been developed. At that point, information on the untreated course of the disease can no longer be collected since it would be unethical to withhold therapy. In the case of the "IPA/ Erasmus MC Pompe survey" we started four years before ERT was approved on the market. This not only served to better understand the natural course of the disease, but was also paramount to test the hypothesis that ERT effectively changes disease progression compared to the untreated situation.

The collaboration with patients and patient organizations proved to be important. During the development of the survey, patients provided valuable input making the survey more relevant for the patients. Through the network of the local patient organizations that are linked to the IPA, we were able to reach a vast number of patients from around the world who would otherwise be unknown to us. Especially in countries where there are no regional or national expert centers and patients are followed in local hospitals it would be hard to

contact all of them.[73] Finally, this collaboration engaged patients in the project and they were highly motivated to participate and contribute to the understanding of their disease.

Another important issue, especially in keeping the survey sustainable, was to use technological advances to improve the survey. Initially, paper questionnaires were sent out and returned via the IPA representatives and were manually entered in a database. In 2009, the survey was digitalized allowing patients to fill out the questionnaire online and reducing the need for data entry and potential data entry errors. The survey website was received with enthusiasm by patients. After several years, this system needed upgrading as well. As part of this PhD project we reevaluated the survey in order to improve patient participation and allow the survey to remain relevant. Firstly, we wanted to be able to change and add questions in the survey, as advances towards new treatments were being made. This was difficult in the old system, as it was outsourced to an IT company. Secondly, new technological options could make the survey more flexible and user-friendly, allowing skip patterns and patients to pause log out of the survey. Also for researchers it became easier to manage invitations and reminders. Work on a new version started in 2015 as part of this thesis. The website was built in collaboration with the IT department of the Erasmus MC using LimeSurvey and was housed internally. The option to complete a paper version was kept for patients who preferred this. In addition, results from the survey were distributed specifically to patients, for example through webinars, newsletters, and on patient congresses to reinvigorate their interest in the survey. Also, patient organizations made special effort to contact those patients who were lost to follow-up in order to re-engage them. These measures seem effective, as since these changes and efforts, response numbers have increased, with patients previously lost to follow-up returning and new patients starting the survey. In the most recent online survey round, which is performed yearly, over 70% continued to participate. This continued participation is remarkable for a survey that started in 2002.

Lessons learnt from clinical research on Pompe disease

The Erasmus MC, national referral center for Pompe disease

The Erasmus MC has a long track-record of research on Pompe disease globally. In 1999 the first trials with ERT produced from rabbit milk started at the Erasmus MC in 4 classic-infantile patients and 3 patients with childhood onset Pompe disease. In addition to this, this center became the national referral center for all patients with Pompe disease in 2004. As a result, the number of patients with Pompe disease seen in the Erasmus MC is relatively high, benefitting our care and allowing our research to be important globally. Centralized care is also advocated by the European Union who recently installed European Reference Network in order to centralize expertise and knowledge on rare diseases.

Sharing data to further research

While there is evidence on the effect of ERT, including from several national datasets including our own, there remains little information on factors that influence the response to treatment. To study this, larger numbers of patients are needed requiring clinical data to be shared internationally. International data is already being collected by in the Pompe registry, set up by the pharmaceutical company which produces ERT.[74] This registry contains data on over 1000 patients with Pompe disease, which are directly reported by physicians. However, a problem that registries like this one are facing is to keep the data entry consistent and complete and to ensure regular follow-up. Furthermore, access to the aggregated data in this registry is subject to constraints for the clinicians and researchers entering the data.

This shows that collecting data internationally is not easy and can be prone to obstacles.[75] It is firstly important that the same outcome measures are obtained using the same underlying definitions. The European Pompe Consortium (EPoC) was founded in 2014 with the aim to further research on Pompe disease. EPoC recently published guidance on a minimal set of outcome assessments to be performed when following patients with Pompe disease.[76] Several of our standardized follow-up assessments have been included in this recommendation.

Secondly, an issue with data sharing is the issue of who controls the data. When a pharmaceutical company sets up a registry like the Pompe Registry, it is often not so easily accessible for other areas of research. When researchers want to share data amongst themselves there is also always a central manager of the database who can access all the data on all patients, which may not always be desirable for example due to (legal) objections.[77] Polymorphic Encryption and Pseudonymisation (PEP) makes it possible to collate data in a central database in which access to patients is restricted to the clinic or physician who treats them. They can grant access to specific information on one or more patients (e.g. a specific outcome measure) for specific projects. Anonymization or pseudonymisation strategies can be applied to prevent linking patients through multiple different projects.[78] Future registries could use PEP, which would allow not only the post marketing requirements to be met, but also allow research groups to share their data internationally without giving up control over their data. This might stimulate researchers to share their data more freely and further the research on Pompe disease.

Analysis of long-term follow-up data

Lysosomal storage diseases (and other rare disorders) are often progressive. For this reason, patients need to be followed over time in order to study the disease and effects of potential treatments. Multiple measurements are taken from each patient over the period

of several years of treatment and sometimes (preferably) also before treatment. To analyze such data, it is firstly important to recognize that data of the same patients are correlated. Specialized statistical methods are required to analyze such data.[79] The simplest way to analyze such data is calculating the difference between the first and last measurement, or percent change from baseline. However, this only uses two data points per patient. To use all available data one could use repeated-Measure Analysis of (Co) Variance (AN(C)OVA), an extension of a linear regression model. This also allows for potential non-linearity of the data as we observed in our long-term follow-up of treated Pompe patients.

However, unlike in a trial where assessments are mandatory and scheduled at precise intervals, the observational follow-up may be irregular in time, and assessments may also be missing. The ANCOVA method does not allow missing data or irregular assessments and therefore only fits data collected in a very rigid structure. Generalized mixed-effects models also take into account all assessments, within-patient correlation and non-linearity, but in addition allows for irregular and missing data. This enables us to fit models that do justice to all available data and are recommended for the analysis of longitudinally obtained data. These models underlie our assessments of the long-term effects of ERT in children and adults.[79]

Conclusions

Our study in untreated children with Pompe disease and the comparison to untreated adults underlines the fact that Pompe disease is a spectrum. A third of our children with Pompe disease were already severely affected at a young age.

Based on our studies in children and adults we conclude that ERT has a positive effect on these patients. The response to treatment changes over time, with some patients starting to deteriorate after 2-3 years of treatment. Nevertheless, on average our patient populations still benefited from ERT after 5-7 years treatment. Treatment effects were most outspoken on muscle strength and function and less pronounced for lung function. Pompe disease is the first treatable inheritable muscle disorder, but we also conclude that next steps need to be made to further improve prospects of patients.

In children, the c.-32-13T>G mutation and female gender may be associated with a milder phenotype. Male patients also responded more poorly to treatment, in children in terms of lung function and in adults on muscle strength, but more research is needed to confirm this. Despite studies suggesting the ACE polymorphism influences disease severity and the effect of ERT, we did not find any evidence for this in our data. We conclude that other

(background) factors play a role in the large variations in disease severity and response to treatment between patients. International collaboration is key to obtain sufficient patient numbers to elucidate these factors.

Finally, we provided our perspective on the use of PROs in rare diseases and our experience in studying Pompe disease in general. The "IPA/ Erasmus MC Pompe survey" proved to be of great importance in discerning the natural course of Pompe disease and studying the effects of ERT. PROs add the patients' perspective to clinical outcomes and provide unique insight in their functioning. We believe that PROs will have an increasingly important role in the research on rare diseases and our survey can serve as an example for other rare diseases.

Future directives

The studies described in this thesis and the lessons learnt emphasize the challenges and opportunities in the research of Pompe disease. While our clinical studies have been performed on relatively large numbers of patients, further international collaboration is needed to advance our knowledge, for example to elucidate predictors. One way to enhance international collaboration would be by using PEP. Furthermore, combining clinical and patient reported outcomes can greatly benefit the efficacy of future projects. An example would be the linkage between the "IPA/ Erasmus MC Pompe survey" and clinical databases.

While patients benefitted from treatment there is still room for improvement. Therefore we believe the treatment of patients with Pompe disease should be more personalized. Patients might respond better to treatment when receiving a higher dose. The development of Antisense Oligonucleotides (AONs) is under investigation for patients carrying the common c.-32-13T>G splice site mutation. This new approach aims to improve splicing and therefore the production of functional enzyme.[80] Since 68% of children and 92% of the Dutch adult patients with non-classic Pompe disease carry the c.-32-13T>G mutation such a treatment would benefit a large proportion of patients.[9]

Efforts in other new therapies is undertaken as well to provide better perspectives for all patients in the future. Innovative treatment strategies which are being investigated by the international scientific community, including our center, are second generation ERT, stem-cell therapy, AAV gene therapy and lentiviral therapy.[80-85]

All in all, 11 years after the registration of the first therapy for Pompe disease we have learned a great deal about the clinical characteristics and effects of treatment in patients with Pompe disease. The main goals for the future are to improve existing therapies and

develop new ones. In order to keep expanding the knowledge on this disease international collaboration should be maintained and intensified. A part of this should be the linkage of the different databases containing PROs and clinical outcome measures. In rare diseases (international) collaboration is the factor leading to success.

References

1. Winkel LP, Hagemans ML, van Doorn PA, Loonen MC, Hop WJ, Reuser AJ, van der Ploeg AT. The natural course of non-classic Pompe's disease; a review of 225 published cases. *J Neurol* 2005;252(8):875-884.
2. Beek NA, Vries JM, Hagemans ML, Hop WC, Kroos MA, Wokke JH, Visser M, Engelen BG, Kuks JB, Kooi AJ. Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study. *Orphanet J Rare Dis* 2012;7.
3. van Capelle CI, van der Meijden JC, van den Hout JM, Jaeken J, Baethmann M, Voit T, Kroos MA, Derks TG, Rubio-Gozalbo ME, Willemsen MA *et al*. Childhood Pompe disease: clinical spectrum and genotype in 31 patients. *Orphanet J Rare Dis* 2016;11(1):65.
4. van der Beek NA, van Capelle CI, van der Velden-van Etten KI, Hop WC, van den Berg B, Reuser AJ, van Doorn PA, van der Ploeg AT, Stam H. Rate of progression and predictive factors for pulmonary outcome in children and adults with Pompe disease. *Mol Genet Metab* 2011;104(1-2):129-136.
5. van der Ploeg AT, Clemens PR, Corzo D, Escolar DM, Florence J, Groeneveld GJ, Herson S, Kishnani PS, Laforet P, Lake SL *et al*. A randomized study of alglucosidase alfa in late-onset Pompe's disease. *N Engl J Med* 2010;362(15):1396-1406.
6. de Vries JM, van der Beek NA, Hop WC, Karstens FP, Wokke JH, de Visser M, van Engelen BG, Kuks JB, van der Kooi AJ, Notermans NC *et al*. Effect of enzyme therapy and prognostic factors in 69 adults with Pompe disease: an open-label single-center study. *Orphanet J Rare Dis* 2012;7:73.
7. Strothotte S, Strigl-Pill N, Grunert B, Kornblum C, Eger K, Wessig C, Deschauer M, Breunig F, Glocker FX, Vielhaber S *et al*. Enzyme replacement therapy with alglucosidase alfa in 44 patients with late-onset glycogen storage disease type 2: 12-month results of an observational clinical trial. *J Neurol* 2010;257(1):91-97.
8. Wokke JH, Escolar DM, Pestronk A, Jaffe KM, Carter GT, van den Berg LH, Florence JM, Mayhew J, Skrinar A, Corzo D *et al*. Clinical features of late-onset Pompe disease: a prospective cohort study. *Muscle Nerve* 2008;38(4):1236-1245.
9. van der Beek NA, de Vries JM, Hagemans ML, Hop WC, Kroos MA, Wokke JH, de Visser M, van Engelen BG, Kuks JB, van der Kooi AJ *et al*. Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study. *Orphanet J Rare Dis* 2012;7:88.
10. Deroma L, Guerra M, Sechi A, Ciana G, Cisilino G, Dardis A, Bembi B. Enzyme replacement therapy in juvenile glycogenosis type II: a longitudinal study. *Eur J Pediatr* 2014;173(6):805-813.
11. van Capelle CI, Winkel LP, Hagemans ML, Shapira SK, Arts WF, van Doorn PA, Hop WC, Reuser AJ, van der Ploeg AT. Eight years experience with enzyme replacement therapy in two children and one adult with Pompe disease. *Neuromuscul Disord* 2008;18(6):447-452.
12. van Capelle CI, van der Beek NA, Hagemans ML, Arts WF, Hop WC, Lee P, Jaeken J, Frohn-Mulder IM, Merkus PJ, Corzo D *et al*. Effect of enzyme therapy in juvenile patients with Pompe disease: a three-year open-label study. *Neuromuscul Disord* 2010;20(12):775-782.
13. Porta F, Pagliardini V, Roasio L, Biamino E, Spada M. Playing competitive basketball in face of late-onset pompe disease. *Muscle Nerve* 2015;51(2):302-303.
14. Rossi M, Parenti G, Della Casa R, Romano A, Mansi G, Agovino T, Rosapepe F, Vosa C, Del Giudice E, Andria G. Long-term enzyme replacement therapy for pompe disease with recombinant human alpha-glucosidase derived from chinese hamster ovary cells. *J Child Neurol* 2007;22(5):565-573.
15. Ishigaki K, Murakami T, Nakanishi T, Oda E, Sato T, Osawa M. Close monitoring of initial enzyme replacement therapy in a patient with childhood-onset Pompe disease. *Brain Dev* 2012;34(2):98-102.
16. Bembi B, Pisa FE, Confalonieri M, Ciana G, Fiumara A, Parini R, Rigoldi M, Moglia A, Costa A, Carlucci A *et al*. Long-term observational, non-randomized study of enzyme replacement therapy in late-onset glycogenosis type II. *J Inherit Metab Dis* 2010;33(6):727-735.
17. Winkel LP, Van den Hout JM, Kamphoven JH, Disseldorp JA, Remmerswaal M, Arts WF, Loonen MC, Vulto AG, Van Doorn PA, De Jong G *et al*. Enzyme replacement therapy in late-onset Pompe's disease: a three-year follow-up. *Ann Neurol* 2004;55(4):495-502.
18. van der Ploeg AT, Kruijshaar ME, Toscano A, Laforet P, Angelini C, Lachmann RH, Pascual Pascual SI, Roberts M, Rosler K, Stulnig T *et al*. European consensus for starting and stopping enzyme replacement therapy in adult patients with Pompe disease: a 10-year experience. *Eur J Neurol* 2017;24(6):768-e731.

19. van der Ploeg AT, Barohn R, Carlson L, Charrow J, Clemens PR, Hopkin RJ, Kishnani PS, Laforet P, Morgan C, Nations S *et al*. Open-label extension study following the Late-Onset Treatment Study (LOTS) of alglucosidase alfa. *Mol Genet Metab* 2012;107(3):456-461.
20. Gungor D, de Vries JM, Brusse E, Kruijshaar ME, Hop WC, Murawska M, van den Berg LE, Reuser AJ, van Doorn PA, Hagemans ML *et al*. Enzyme replacement therapy and fatigue in adults with Pompe disease. *Mol Genet Metab* 2013;109(2):174-178.
21. Gungor D, Kruijshaar ME, Plug I, D'Agostino RB, Hagemans ML, van Doorn PA, Reuser AJ, van der Ploeg AT. Impact of enzyme replacement therapy on survival in adults with Pompe disease: results from a prospective international observational study. *Orphanet J Rare Dis* 2013;8:49.
22. Gungor D, Kruijshaar ME, Plug I, Rizopoulos D, Kanters TA, Wens SC, Reuser AJ, van Doorn PA, van der Ploeg AT. Quality of life and participation in daily life of adults with Pompe disease receiving enzyme replacement therapy: 10 years of international follow-up. *J Inherit Metab Dis* 2016;39(2):253-260.
23. Hundsberger T, Rosler KM, Findling O. Cessation and resuming of alglucosidase alfa in Pompe disease: a retrospective analysis. *J Neurol* 2014;261(9):1684-1690.
24. Regnery C, Kornblum C, Hanisch F, Vielhaber S, Strigl-Pill N, Grunert B, Muller-Felber W, Glocker FX, Spranger M, Deschauer M *et al*. 36 months observational clinical study of 38 adult Pompe disease patients under alglucosidase alfa enzyme replacement therapy. *J Inherit Metab Dis* 2012;35(5):837-845.
25. Vielhaber S, Brejova A, Debska-Vielhaber G, Kaufmann J, Feistner H, Schoenfeld MA, Awiszus F. 24-months results in two adults with Pompe disease on enzyme replacement therapy. *Clin Neurol Neurosurg* 2011;113(5):350-357.
26. Merk T, Wibmer T, Schumann C, Kruger S. Glycogen storage disease type II (Pompe disease)--influence of enzyme replacement therapy in adults. *Eur J Neurol* 2009;16(2):274-277.
27. Andreassen CS, Schlutter JM, Vissing J, Andersen H. Effect of enzyme replacement therapy on isokinetic strength for all major muscle groups in four patients with Pompe disease-a long-term follow-up. *Mol Genet Metab* 2014;112(1):40-43.
28. Korpela MP, Paetau A, Löfberg MI, Timonen MH, Lamminen AE, Kiuru-Enari SMK. A novel mutation of the GAA gene in a finnish late-onset Pompe disease patient: Clinical phenotype and follow-up with enzyme replacement therapy. *Muscle Nerve* 2009;40(1):143-148.
29. Orlikowski D, Pellegrini N, Prigent H, Laforet P, Carlier R, Carlier P, Eymard B, Lofaso F, Annane D. Recombinant human acid alpha-glucosidase (rhGAA) in adult patients with severe respiratory failure due to Pompe disease. *Neuromuscul Disord* 2011;21(7):477-482.
30. Pellegrini N, Laforet P, Orlikowski D, Pellegrini M, Caillaud C, Eymard B, Raphael JC, Lofaso F. Respiratory insufficiency and limb muscle weakness in adults with Pompe's disease. *Eur Respir J* 2005;26(6):1024-1031.
31. Gesquiere-Dando A, Attarian S, Maues De Paula A, Pouget J, Salort-Campana E. Fibromyalgia-like symptoms associated with irritable bowel syndrome: A challenging diagnosis of late-onset Pompe disease. *Muscle Nerve* 2015;52(2):300-304.
32. Papadimas GK, Spengos K, Konstantinopoulou A, Vassilopoulou S, Vontzalidis A, Papadopoulos C, Michelakakis H, Manta P. Adult Pompe disease: clinical manifestations and outcome of the first Greek patients receiving enzyme replacement therapy. *Clin Neurol Neurosurg* 2011;113(4):303-307.
33. Angelini C, Semplicini C. Enzyme replacement therapy for Pompe disease. *Curr Neurol Neurosci Rep* 2012;12(1):70-75.
34. Angelini C, Semplicini C, Ravaglia S, Bembi B, Servidei S, Pegoraro E, Moggio M, Filosto M, Sette E, Crescimanno G *et al*. Observational clinical study in juvenile-adult glycogenosis type 2 patients undergoing enzyme replacement therapy for up to 4 years. *J Neurol* 2012;259(5):952-958.
35. Ravaglia S, Danesino C, Pichiecchio A, Repetto A, Poloni GU, Rossi M, Fratino P, Moglia A, Costa A. Enzyme replacement therapy in severe adult-onset glycogen storage disease type II. *Adv Ther* 2008;25(8):820-829.
36. Ravaglia S, Moglia A, Costa A, Repetto A, Danesino C. Enzyme replacement therapy in late-onset type II glycogenosis. *Eur J Neurol* 2009;16(7):e125.
37. Ravaglia S, Pichiecchio A, Ponzio M, Danesino C, Saeidi Garaghani K, Poloni GU, Toscano A, Moglia A, Carlucci A, Bini P *et al*. Changes in skeletal muscle qualities during enzyme replacement therapy in late-onset type II glycogenosis: temporal and spatial pattern of mass vs. strength response. *J Inherit Metab Dis* 2010;33(6):737-745.

38. Vianello A, Semplicini C, Paladini L, Concas A, Ravaglia S, Servidei S, Toscano A, Mongini T, Angelini C, Pegoraro E. Enzyme replacement therapy improves respiratory outcomes in patients with late-onset type II glycogenosis and high ventilator dependency. *Lung* 2013;191(5):537-544.
39. Marzorati M, Porcelli S, Bellistri G, Morandi L, Grassi B. Exercise testing in late-onset glycogen storage disease type II patients undergoing enzyme replacement therapy. *Neuromuscul Disord* 2012;22 Suppl 3:S230-234.
40. Marzorati M, Porcelli S, Reggiori B, Morandi L, Grassi B. Improved exercise tolerance after enzyme replacement therapy in Pompe disease. *Med Sci Sports Exerc* 2012;44(5):771-775.
41. Crescimanno G, Modica R, Lo Mauro R, Musumeci O, Toscano A, Marrone O. Role of the cardio-pulmonary exercise test and six-minute walking test in the evaluation of exercise performance in patients with late-onset Pompe disease. *Neuromuscul Disord* 2015;25(7):542-547.
42. Kobayashi H, Shimada Y, Ikegami M, Kawai T, Sakurai K, Urashima T, Ijima M, Fujiwara M, Kaneshiro E, Ohashi T *et al*. Prognostic factors for the late onset Pompe disease with enzyme replacement therapy: from our experience of 4 cases including an autopsy case. *Mol Genet Metab* 2010;100(1):14-19.
43. Furusawa Y, Mori-Yoshimura M, Yamamoto T, Sakamoto C, Wakita M, Kobayashi Y, Fukumoto Y, Oya Y, Fukuda T, Sugie H *et al*. Effects of enzyme replacement therapy on five patients with advanced late-onset glycogen storage disease type II: a 2-year follow-up study. *J Inherit Metab Dis* 2012;35(2):301-310.
44. Sugai F, Kokunai Y, Yamamoto Y, Hashida G, Shimazu K, Mihara M, Inoue S, Sakoda S. Use of the muscle volume analyzer to evaluate enzyme replacement therapy in late-onset Pompe disease. *J Neurol* 2010;257(3):461-463.
45. Furusawa Y, Yamamoto T, Oya Y, Miyama K, Suzuki J, Murata M. [Treatment of subcutaneous and mediastinal emphysema with cuirass ventilation in a patient with Pompe disease undergoing non-invasive positive pressure ventilation]. *Rinsho Shinkeigaku* 2010;50(5):306-310.
46. Isayama R, Shiga K, Seo K, Azuma Y, Araki Y, Hamano A, Takezawa H, Kuriyama N, Takezawa N, Mizuno T *et al*. Sixty six-month follow-up of muscle power and respiratory function in a case with adult-type Pompe disease treated with enzyme replacement therapy. *J Clin Neuromuscul Dis* 2014;15(4):152-156.
47. Park JS, Kim HG, Shin JH, Choi YC, Kim DS. Effect of enzyme replacement therapy in late onset Pompe disease: open pilot study of 48 weeks follow-up. *Neurol Sci* 2015;36(4):599-605.
48. de Vries JM, Brugma JD, Ozkan L, Steegers EA, Reuser AJ, van Doorn PA, van der Ploeg AT. First experience with enzyme replacement therapy during pregnancy and lactation in Pompe disease. *Mol Genet Metab* 2011;104(4):552-555.
49. Anderson LJ, Henley W, Wyatt KM, Nikolaou V, Waldek S, Hughes DA, Lachmann RH, Logan S. Effectiveness of enzyme replacement therapy in adults with late-onset Pompe disease: results from the NCS-LSD cohort study. *J Inherit Metab Dis* 2014;37(6):945-952.
50. Stepien KM, Hendriksz CJ, Roberts M, Sharma R. Observational clinical study of 22 adult-onset Pompe disease patients undergoing enzyme replacement therapy over 5years. *Mol Genet Metab* 2016;117(4):413-418.
51. Case LE, Koeberl DD, Young SP, Bali D, DeArmey SM, Mackey J, Kishnani PS. Improvement with ongoing Enzyme Replacement Therapy in advanced late-onset Pompe disease: a case study. *Mol Genet Metab* 2008;95(4):233-235.
52. Patel TT, Banugaria SG, Case LE, Wenninger S, Schoser B, Kishnani PS. The impact of antibodies in late-onset Pompe disease: a case series and literature review. *Mol Genet Metab* 2012;106(3):301-309.
53. Hagemans ML, Janssens AC, Winkel LP, Sieradzan KA, Reuser AJ, Van Doorn PA, Van der Ploeg AT. Late-onset Pompe disease primarily affects quality of life in physical health domains. *Neurology* 2004;63(9):1688-1692.
54. de Vries JM, van der Beek NA, Kroos MA, Ozkan L, van Doorn PA, Richards SM, Sung CC, Brugma JD, Zandbergen AA, van der Ploeg AT *et al*. High antibody titer in an adult with Pompe disease affects treatment with alglucosidase alfa. *Mol Genet Metab* 2010;101(4):338-345.
55. Favejee MM, van den Berg LE, Kruijshaar ME, Wens SC, Praet SF, Pim Pijnappel WW, van Doorn PA, Bussmann JB, van der Ploeg AT. Exercise training in adults with Pompe disease: the effects on pain, fatigue, and functioning. *Arch Phys Med Rehabil* 2015;96(5):817-822.
56. van den Berg LE, Favejee MM, Wens SC, Kruijshaar ME, Praet SF, Reuser AJ, Bussmann JB, van Doorn PA, van der Ploeg AT. Safety and efficacy of exercise training in adults with Pompe disease: evalution of endurance, muscle strength and core stability before and after a 12 week training program. *Orphanet J Rare Dis* 2015;10:87.
57. de Filippi P, Ravaglia S, Bembi B, Costa A, Moglia A, Piccolo G, Repetto A, Dardis A, Greco G, Ciana G *et al*. The angiotensin-converting enzyme insertion/deletion polymorphism modifies the clinical outcome in patients with Pompe disease. *Genet Med* 2010;12(4):206-211.

58. Ravaglia S, De Filippi P, Pichiecchio A, Ponzio M, Saeidi Garaghani K, Poloni GU, Bini P, Danesino C. Can genes influencing muscle function affect the therapeutic response to enzyme replacement therapy (ERT) in late-onset type II glycogenosis? *Mol Genet Metab* 2012;107(1-2):104-110.
59. De Filippi P, Saeidi K, Ravaglia S, Dardis A, Angelini C, Mongini T, Morandi L, Moggio M, Di Muzio A, Filosto M *et al*. Genotype-phenotype correlation in Pompe disease, a step forward. *Orphanet J Rare Dis* 2014;9:102.
60. Baek RC, Palmer R, Pomponio RJ, Lu Y, Ma X, McVie-Wylie AJ. The influence of a polymorphism in the gene encoding angiotensin converting enzyme (ACE) on treatment outcomes in late-onset Pompe patients receiving alglucosidase alfa. *Mol Genet Metab Rep* 2016;8:48-50.
61. Patrick DL, Burke LB, Powers JH, Scott JA, Rock EP, Dawisha S, O'Neill R, Kennedy DL. Patient-reported outcomes to support medical product labeling claims: FDA perspective. *Value Health* 2007;10 Suppl 2:S125-137.
62. Snyder CF, Aaronson NK, Choucair AK, Elliott TE, Greenhalgh J, Halyard MY, Hess R, Miller DM, Reeve BB, Santana M. Implementing patient-reported outcomes assessment in clinical practice: a review of the options and considerations. *Quality of Life Research* 2012;21(8):1305-1314.
63. Matza LS, Patrick DL, Riley AW, Alexander JJ, Rajmil L, Pleil AM, Bullinger M. Pediatric Patient-Reported Outcome Instruments for Research to Support Medical Product Labeling: Report of the ISPOR PRO Good Research Practices for the Assessment of Children and Adolescents Task Force. *Value Health* 2013;16(4):461-479.
64. Johnston BC, Miller PA, Agarwal A, Mulla S, Khokhar R, De Oliveira K, Hitchcock CL, Sadeghirad B, Mohiuddin M, Sekercioglu N *et al*. Limited responsiveness related to the minimal important difference of patient-reported outcomes in rare diseases. *J Clin Epidemiol* 2016;79:10-21.
65. Bronstein MG, Kakkis ED. Patients as key partners in rare disease drug development. *Nat Rev Drug Discov* 2016;15(11):731-732.
66. van der Meijden JC, Gungor D, Kruijshaar ME, Muir AD, Broekgaarden HA, van der Ploeg AT. Ten years of the international Pompe survey: patient reported outcomes as a reliable tool for studying treated and untreated children and adults with non-classic Pompe disease. *J Inherit Metab Dis* 2015;38(3):495-503.
67. Hagemans ML, Winkel LP, Hop WC, Reuser AJ, Van Doorn PA, Van der Ploeg AT. Disease severity in children and adults with Pompe disease related to age and disease duration. *Neurology* 2005;64(12):2139-2141.
68. Hagemans ML, Winkel LP, Van Doorn PA, Hop WJ, Loonen MC, Reuser AJ, Van der Ploeg AT. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. *Brain* 2005;128(Pt 3):671-677.
69. Hagemans ML, Hop WJ, Van Doorn PA, Reuser AJ, Van der Ploeg AT. Course of disability and respiratory function in untreated late-onset Pompe disease. *Neurology* 2006;66(4):581-583.
70. Hagemans ML, Laforet P, Hop WJ, Merkies IS, Van Doorn PA, Reuser AJ, Van der Ploeg AT. Impact of late-onset Pompe disease on participation in daily life activities: evaluation of the Rotterdam Handicap Scale. *Neuromuscul Disord* 2007;17(7):537-543.
71. Hagemans ML, van Schie SP, Janssens AC, van Doorn PA, Reuser AJ, van der Ploeg AT. Fatigue: an important feature of late-onset Pompe disease. *J Neurol* 2007;254(7):941-945.
72. Gungor D, de Vries JM, Hop WC, Reuser AJ, van Doorn PA, van der Ploeg AT, Hagemans ML. Survival and associated factors in 268 adults with Pompe disease prior to treatment with enzyme replacement therapy. *Orphanet J Rare Dis* 2011;6:34.
73. Berlin AC, Eggan ER. Realizing the potential of patient organizations in translational research-delivering treatments for rare diseases. *Acad Med* 2014;89(11):1573.
74. Kishnani PS, Amartino HM, Lindberg C, Miller TM, Wilson A, Keutzer J. Methods of diagnosis of patients with Pompe disease: Data from the Pompe Registry. *Mol Genet Metab* 2014;113(1-2):84-91.
75. Piwowar HA, Becich MJ, Bilofsky H, Crowley RS, ca BIGDS, Intellectual Capital W. Towards a data sharing culture: recommendations for leadership from academic health centers. *PLoS Med* 2008;5(9):e183.
76. Schoser B, Laforet P, Kruijshaar ME, Toscano A, van Doorn PA, van der Ploeg AT, European Pompe C. 208th ENMC International Workshop: Formation of a European Network to develop a European data sharing model and treatment guidelines for Pompe disease Naarden, The Netherlands, 26-28 September 2014. *Neuromuscul Disord* 2015;25(8):674-678.
77. van Panhuis WG, Paul P, Emerson C, Grefenstette J, Wilder R, Herbst AJ, Heymann D, Burke DS. A systematic review of barriers to data sharing in public health. *BMC Public Health* 2014;14:1144.
78. Verheul E, Jacobs B, Meijer C, Hildebrandt M, Ruiter R. Polymorphic Encryption and Pseudonymisation for Personalised Healthcare. *Cryptology ePrint Archive* 2016;(2016):411.

79. Gibbons RD, Hedeker D, DuToit S. Advances in Analysis of Longitudinal Data. *Annu Rev Clin Psycho* 2010;6:79-107.

80. Bergsma AJ, In 't Groen SL, Verheijen FW, van der Ploeg AT, Pijnappel WP. From Cryptic Toward Canonical Pre-mRNA Splicing in Pompe Disease: a Pipeline for the Development of Antisense Oligonucleotides. *Mol Ther Nucleic Acids* 2016;5:e361.

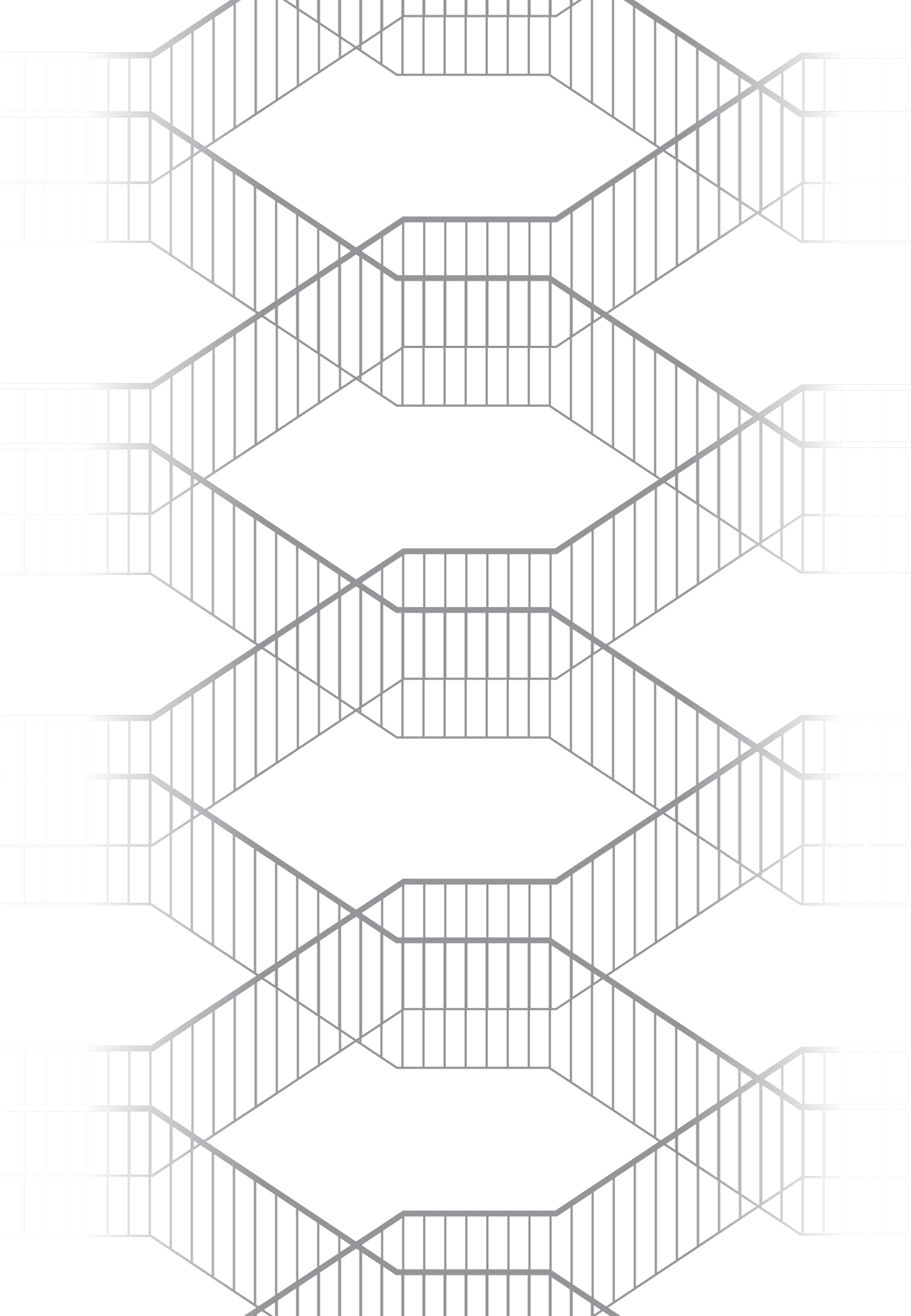
81. van Til NP, Stok M, Aerts Kaya FS, de Waard MC, Farahbakhshian E, Visser TP, Kroos MA, Jacobs EH, Willart MA, van der Wegen P *et al*. Lentiviral gene therapy of murine hematopoietic stem cells ameliorates the Pompe disease phenotype. *Blood* 2010;115(26):5329-5337.

82. Goyenvalle A, Leumann C, Garcia L. Therapeutic Potential of Tricyclo-DNA antisense oligonucleotides. *J Neuromuscul Dis* 2016;3(2):157-167.

83. Higuchi T, Kawagoe S, Otsu M, Shimada Y, Kobayashi H, Hirayama R, Eto K, Ida H, Ohashi T, Nakauchi H *et al*. The generation of induced pluripotent stem cells (iPSCs) from patients with infantile and late-onset types of Pompe disease and the effects of treatment with acid-alpha-glucosidase in Pompe's iPSCs. *Mol Genet Metab* 2014;112(1):44-48.

84. Smith BK, Martin AD, Lawson LA, Vernot V, Marcus J, Islam S, Shafi N, Corti M, Collins SW, Byrne BJ. Inspiratory muscle conditioning exercise and diaphragm gene therapy in Pompe disease: Clinical evidence of respiratory plasticity. *Exp Neurol* 2017;287(Pt 2):216-224.

85. Byrne PI, Collins S, Mah CC, Smith B, Conlon T, Martin SD, Corti M, Cleaver B, Islam S, Lawson LA. Phase I/II trial of diaphragm delivery of recombinant adeno-associated virus acid alpha-glucosidase (rAAaV1-CMV-GAA) gene vector in patients with Pompe disease. *Hum Gene Ther Clin Dev* 2014;25(3):134-163.



Addendum

Summary

Samenvatting

Acknowledgements | Dankwoord

About the author

List of publications

PhD Portfolio

Abbreviations

Summary

Pompe disease is a rare, autosomal recessive, metabolic myopathy caused by mutations in the gene coding for the enzyme *acid α-glucosidase*. The function of *acid α-glucosidase* is to degrade glycogen to glucose in the lysosome. A deficiency in this enzyme therefore causes glycogen to build up in the lysosome, resulting in cell damage and tissue destruction, mainly in the muscles.

Pompe disease presents as a clinical spectrum. Patients with the classic-infantile phenotype of Pompe disease have the most severe form of the disease and die within the first year of life if untreated. Non-classic or 'late-onset' patients have a more slowly progressive disease with limb-girdle and respiratory muscle weakness, leading -eventually- to wheelchair and ventilator dependency. In 2006, enzyme replacement therapy (ERT) became available for all patients with Pompe disease in Europe and the US.

This thesis aimed to describe the clinical presentation of untreated children with Pompe disease, the long-term effects of ERT in children compared to adults, modifying factors of disease severity and ERT, information obtained using Patient Reported Outcomes (PROs) through the "IPA/ Erasmus MC Pompe survey" and how this survey can be used as an example for other diseases.

A general introduction to Pompe disease and ERT was provided in **chapter 1**. Here we summarized background information on the clinical features, pathogenesis and treatment of Pompe disease and introduce patient reported outcomes (PROs).

So far, few studies were available on children with non-classic presentations of Pompe disease. In **chapter 2** we described the clinical characteristics of 31 children with Pompe disease from different countries. We found that 32% of children were already severely affected by the disease during childhood and needed a wheelchair or ventilator or died before reaching adulthood. While there were many similarities between children and adults with regard to the most common symptoms of the disease, also some specific differences were noted. For example neck flexor muscles were more severely affected in children while in adults the quadriceps were more severely affected. Furthermore, ptosis and bulbar muscle weakness were observed less frequently in children, while scoliosis was more common in this population. In our study, children with the c.32-13T>G/null genotype were generally less severely affected than patients with other mutations. They were also predominantly male. Since the male/female ratio of patients with this mutation is equal for adults, this suggests that male patients with this mutation may become symptomatic at an earlier age than females.

In **chapter 3** we studied the effects of ERT in 17 children with a median follow-up of 6.8 years during treatment. Most of these were also included in the study on clinical characteristics described in **chapter 2**. We found that ERT positively affects the course of disease. Muscle function improved and muscle strength stabilized during treatment. Lung function did not respond equally well, and showed a decline over time in male patients. However, this decline does appear to be less steep than the decline reported in studies of untreated patients. Individually, patients' disease progression varied over time, indicating that there are differences in how much patients benefit from treatment. While it has been suggested in literature that an early start of ERT may result in a better response to treatment, we found that also patients who started ERT at a young age and in a good clinical condition can deteriorate.

Our study on the long-term effects of ERT in adult patients is described **chapter 4**. A total of 102 patients were followed for a median of 6.1 years (1.1 pretreatment and 5.0 during treatment). Of these, 88 were followed during treatment, and 96 pre-treatment. At 5 years of ERT, patients performed significantly better on muscle strength and muscle function related outcomes, but also on lung function, compared to their expected natural course. Effects of ERT were largest during the first two to three years of treatment. Also here we observed individual variation in the response to treatment.

In **chapter 5** we explored factors that may influence walking performance in Pompe patients. Based on 107 adult patients we found that higher BMI, male gender, use of invasive ventilation, older age and reduced muscle strength are associated with a poorer ability to walk. Physicians can use these results to counsel patients and suggest potential interventions. These findings can also serve as a starting point to unravel the factors associated with walking performance and ultimately develop a prognostic model.

The aim of the study described in **chapter 6** was to find an explanation for the clinical variation in disease severity between patients and in their response to treatment. In this chapter we investigated whether a polymorphism in the gene coding for the Angiotensin Converting Enzyme (ACE) can explain this variation. The effects of the ACE polymorphism were studied in 131 children and adults with the c.31-13T>G mutation. Using cross-sectional data of the patients' first visit to our center, and longitudinal data of these patients during treatment, no statistical significant influence could be observed of the ACE polymorphism on disease severity or on the effect of ERT. Finally, also in a subgroup of families, the polymorphism could not explain the phenotypical variation between siblings.

Besides the data obtained through clinical assessments in our center we also included the patients' point of view using PROs. Through the "IPA/ Erasmus MC Pompe survey" data was

collected on patients' functioning and quality of life. In **chapter 7** we reviewed the results obtained in the first 10 years of this survey. First studies reported on the progressive character of Pompe disease in untreated patients and demonstrated that these patients were more fatigued, participated less in activities of daily life, had lower quality of life and worse survival compared to the general population. When ERT became available, data from this survey allowed us to demonstrate a positive effect of ERT on fatigue, participation in daily life, quality of life and survival. These results indicate that PROs can have a large impact in the study of rare diseases. The early start of data collection, several years before ERT became available, and the collaboration with patients and patient organizations contributed to the success of the survey. This survey can serve as example for data collection in other rare diseases, especially in those for which a therapy has not yet been developed.

Data from this survey were also used to study the relation between ERT and the risk for wheelchair and ventilator dependency (**chapter 8**). Needing a wheelchair or ventilator has a huge impact on the life of patients and leads to lower quality of life. We found that ERT lowers the risk for needing a wheelchair. We could not demonstrate an effect on the risk for needing respiratory support. These analyses show that the survey is still able to provide new information which could not be studied otherwise.

Finally, **chapter 9** discusses the main findings of our studies, our experience with long-term follow up of patients both in the clinic as well as using the "IPA/ Erasmus MC Pompe survey", and our conclusions and perspectives for future research.

Samenvatting

De ziekte van Pompe is een zeldzame, autosomaal recessieve, spierziekte. De ziekte wordt veroorzaakt door een mutatie in het gen dat codeert voor het enzym *acid α-glucosidase*. Normaal breekt dit enzym glycogeen af tot glucose in het lysosoom. Als dit enzym ontbreekt, stapelt glycogeen zich op in het lysosoom. Dit resulteert in schade aan cellen en weefsels. De schade is het meest uitgesproken in de spieren.

De ziekte van Pompe heeft een breed klinisch spectrum. Patiënten met de klassiek-infantiele vorm van de ziekte zijn het meest ernstig aangedaan. Zij hebben al ernstige spierzwakte en een vergroot hart in de eerste levensmaanden. Zonder behandeling overlijden deze patiënten in hun eerste levensjaar. De ziekte is minder progressief bij patiënten met de niet-klassieke (of 'late-onset') vorm. Deze kinderen en volwassenen hebben toenemende zwakte van de heup- en bekkengordelspieren en de ademhalingsspieren. Zij worden uiteindelijk vaak afhankelijk van een rolstoel en/of hebben ademhalingsondersteuning nodig. Sinds 2006 is in Europa en de VS enzymvervangingstherapie (ERT) beschikbaar voor alle patiënten met de ziekte van Pompe. Er is inmiddels veel gepubliceerd over het natuurlijk beloop en effecten van ERT bij volwassenen en kinderen met de klassiek infantiele vorm van de ziekte. Er zijn weinig studies over kinderen met de niet-klassiek vorm van de ziekte van Pompe.

Het doel van dit proefschrift is om 1) het natuurlijk beloop van kinderen met de niet-klassieke vorm ziekte van Pompe te onderzoeken; 2) de langetermijneffecten van enzymvervangingstherapie bij kinderen en volwassenen met de ziekte van Pompe te bestuderen; 3) factoren te identificeren die van invloed zijn op de ziekte-ernst en/of op het effect van enzymvervangingstherapie; 4) patiënt-gerapporteerde uitkomstmaten verkregen met de "IPA/ Erasmus MC Pompe survey" te bespreken en uiteen te zetten hoe deze vragenlijststudie als voorbeeld kan dienen voor andere zeldzame ziektes.

In **hoofdstuk 1** geven we een algemene introductie met betrekking tot de ziekte van Pompe en ERT. Dit hoofdstuk bevat achtergrondinformatie over de klinische karakteristieken, pathogenese, behandeling van de ziekte van Pompe en het introduceert patiënt-gerapporteerde uitkomstmaten.

In **hoofdstuk 2** beschrijven we de klinische karakteristieken van 31 kinderen afkomstig uit verschillende landen. Eén van onze bevindingen is dat 32% van de kinderen op jonge leeftijd al ernstig is aangedaan door de ziekte. Deze kinderen hebben een rolstoel of ademhalingsondersteuning nodig, of overlijden voordat ze volwassen worden. Symptomen die bij kinderen het meest voorkomen zien we ook het meest bij volwassenen. Echter,

er zijn ook verschillen. Bij kinderen zijn de nek flexie spieren ernstiger aangedaan en de bovenbeenspieren (quadriceps) minder ernstig aangedaan dan bij volwassenen. Verder worden ptosis en zwakte van de bulbaire spieren minder vaak geobserveerd bij kinderen terwijl scoliose vaker geobserveerd wordt. Een andere bevinding is dat kinderen met het c.32-13T>G/'null' genotype minder ernstig door de ziekte zijn aangedaan dan kinderen met andere mutaties. In onze studie/populatie waren kinderen met deze mutaties voornamelijk jongens. Bij volwassen patiënten zien we deze mutatie even vaak bij mannen als bij vrouwen. Dit suggereert dat mannen met deze mutatie eerder symptomen ontwikkelen dan vrouwen.

In **hoofdstuk 3** beschrijven we de effecten van enzymvervangingstherapie bij 17 kinderen (de meesten deden ook mee in de studie beschreven in **hoofdstuk 2**). Deze kinderen zijn voor een mediane duur van 6.8 jaar behandeld. Op groepsniveau zien we dat ERT het ziektebeloop in kinderen positief beïnvloedt. Gemiddeld verbetert spierfunctie en stabiliseert spierkracht tijdens behandeling. De longfunctie reageert minder goed op behandeling en laat een achteruitgang zien bij mannelijke patiënten. Echter, als we onze longfunctie resultaten vergelijken met studies over onbehandelde patiënten lijkt deze achteruitgang minder groot te zijn dan de achteruitgang die gezien wordt bij onbehandelde patiënten. Als we naar het ziektebeloop van de individuele patiënten kijken valt het op dat sommige kinderen beter op ERT reageren dan anderen. In de literatuur wordt geschreven dat vroeg starten met de behandeling tot een betere response kan leiden. In onze patiëntgroep gaat dit niet altijd op. Sommige patiënten gaan toch achteruit terwijl ze gestart waren met ERT op een moment dat ze nog weinig symptomen hadden.

Onze studie naar de langetermijneffecten van ERT bij volwassen patiënten wordt beschreven in **hoofdstuk 4**. In totaal worden 102 patiënten een mediane duur van 6.1 jaar gevolgd (1.1 jaar voor en 5 jaar na start behandeling). We zien na 5 jaar behandeling met ERT een significante verbetering van spierkracht en spierfunctie en van longfunctie ten opzichte van het geëxtrapoleerde beloop zonder behandeling. De effectiviteit van ERT is het meest uitgesproken gedurende de eerste twee tot drie jaar van de behandeling. Ook hier zien we weer dat de response op ERT varieerde tussen individuele patiënten.

In **hoofdstuk 5** bestuderen we factoren die van invloed zijn op het vermogen om te kunnen lopen. We onderzoeken 107 volwassen patiënten en concluderen dat een hoger BMI, het mannelijk geslacht, het gebruik van invasieve ademhalingsondersteuning, een hogere leeftijd en verminderde spierkracht het vermogen om te kunnen lopen negatief beïnvloedt. Clinici kunnen deze resultaten gebruiken om patiënten te counselen en mogelijke interventies te bespreken. Deze resultaten kunnen ook gebruikt worden als startpunt voor het maken van een prognostisch model.

Het doel van de studie die wordt beschreven in **hoofdstuk 6** was om een verklaring te vinden voor de variatie die we zien tussen patiënten wat betreft hun ziekte-ernst en het effect van ERT. In dit hoofdstuk hebben we onderzocht of een polymorfisme in het gen dat codeert voor het *Angiotensin Converting Enzyme* (ACE) deze variatie kan verklaren. We hebben het effect van dit polymorfisme bestudeerd in 131 kinderen en volwassenen met de c.31-13T>G mutatie. Zowel cross-sectioneel (op het eerste bezoek van de patiënt aan onze kliniek) als longitudinaal (tijdens behandeling met enzymvervangingstherapie) kan geen effect van het ACE polymorfisme worden waargenomen: niet op de ziekte-ernst en niet op het effect van ERT. Ook binnen families kan het ACE polymorfisme de variatie tussen familieleden niet verklaren.

Naast het verzamelen van klinische data hebben we binnen ons centrum ook de patiënt gevraagd naar zijn ervaringen met betrekking tot zijn ziekte en functioneren. Dit werd gedaan door middel van een vragenlijstonderzoek, de "IPA/ Erasmus MC Pompe survey", dat jaarlijks patiënt-gerapporteerde uitkomsten van Pompe patiënten uit verschillende landen verzamelt. In **hoofdstuk 7** geven we een overzicht van wat dit vragenlijstonderzoek in de eerste 10 jaar van zijn bestaan aan bevindingen heeft opgeleverd. De eerste studies rapporteerden het progressieve karakter van de ziekte in onbehandelde patiënten en liet zien dat patiënten vermoeider waren, minder participeerden in het dagelijks leven, een lagere kwaliteit van leven hadden en een slechtere overleving in vergelijking met de algemene populatie. Na deze eerste studies werd het vragenlijstonderzoek ook gebruikt om de effecten van ERT te bestuderen. Deze studies lieten een positief effect van ERT zien op vermoeidheid, participatie in het dagelijks leven, kwaliteit van leven en overleving. De resultaten van de "IPA/ Erasmus MC Pompe survey" laten zien dat patiënt gerapporteerde uitkomstmaten van grote waarde kunnen zijn bij het bestuderen van een zeldzame ziekte. De vroege start van de dataverzameling (enkele jaren voordat enzymvervangingstherapie beschikbaar werd) en de samenwerking met patiënten en patiëntenverenigingen hebben een grote bijdrage geleverd aan het succes van deze studie. De "IPA/ Erasmus MC Pompe survey" kan als een voorbeeld dienen voor dataverzameling in andere zeldzame ziekten, vooral voor ziekten waar nog geen therapie voor beschikbaar is.

Gegevens van het vragenlijstonderzoek hebben we ook gebruikt om de relatie te bestuderen tussen enzymvervangingstherapie en het gebruik van een rolstoel of ademhalingsondersteuning (**hoofdstuk 8**). Het nodig hebben van deze hulpmiddelen heeft een grote invloed heeft op de kwaliteit van leven. Onze resultaten laten zien dat enzymvervangingstherapie het risico op het nodig hebben van een rolstoel verlaagd. We kunnen geen effect aantonen van ERT op het nodig hebben van ademhalingsondersteuning. Onze analyses benadrukken dat het onderzoek met de "IPA/ Erasmus MC Pompe survey"

continu nieuwe informatie oplevert. De lange follow-up voor en na ERT en het grote aantal patiënten dat participeert maakt dat met deze studie vragen beantwoord kunnen worden die niet met andere bestaande studies (zoals bijvoorbeeld nationale registers of lokale klinische studies) te beantwoorden zijn.

In **hoofdstuk 9** bespreken we de belangrijkste bevindingen van onze studies en plaatsen we die in een breder kader. Verder bespreken we onze expertise met het volgen van patiënten in de kliniek en met "IPA/ Erasmus MC Pompe survey" en geven we onze visie op toekomstig onderzoek.

Acknowledgements | Dankwoord

Promoveren doe je niet alleen. Zeker als je werkt binnen het vakgebied van een zeldzame ziekte. Tijdens mijn promotiejaren heb ik met heel veel mensen mogen samenwerken, zowel van binnen als buiten het Centrum voor Lysosomale en Metabole Ziekten. Dit heb ik als zeer waardevol ervaren en het heeft me geïnspireerd als onderzoeker, als arts en als mens.

Het onderzoek zoals beschreven in dit boekje was niet mogelijk geweest zonder de bijdrage van de patiënten en hun families. Graag wil ik alle Nederlandse patiënten bedanken. Jarenlang zijn jullie trouw naar Rotterdam gekomen voor de behandelingen en onderzoeken. Zonder jullie continue inspanning zouden we nooit zo ver gekomen zijn als we nu zijn. *I would also like to thank all the patients participating in the "IPA/ Erasmus MC Pompe survey". I'm very grateful for your continuous efforts to provide us with insight into the impact the disease has on your life. The representatives of the International Pompe Association (IPA) played a large role in the organization and success of this survey. Ria Broekgaarden, Wilma Treur, Randal, Marylyn and Tiffany House, Marsha Zimmerman, Allan and Barbra Muir, Thomas Schaller, Guy Ashford-Smith and Raymond Saich, I thank all of you for your continuous efforts and enjoyed our collaboration over the years.*

Vervolgens wil ik mijn promotor Prof. dr. A.T. van der Ploeg en copromotor dr. M.E. Kruijshaar bedanken. Beste Ans, bedankt voor al je steun en vertrouwen. Onder jouw begeleiding heb je me de kans gegeven te groeien als onderzoeker, als arts en als persoon. Je kritische blik en liefde voor je patiënten zijn voor mij een groot voorbeeld geweest. Je hebt mijn passie voor de kindergeneeskunde en specifiek de metabole ziekten laten groeien en me geïnspireerd om mijn wetenschappelijke ambities na te streven. Ik heb er veel bewondering voor hoe je het centrum leidt. Ik ben je heel dankbaar voor alles wat ik van je heb mogen leren.

Beste Michelle, ik ben je heel dankbaar voor de laagdrempelige, persoonlijke en fijne begeleiding die je me gegeven hebt. Ik heb altijd het gevoel gehad dat ik met welke vraag dan ook bij je terecht kon. Door je goede visie en grote precisie zorgde je er altijd voor dat de artikelen 'net weer iets beter' werden. Ook als een project even tegen zat stond je altijd opgewekt klaar om samen het probleem nog een keer van een andere kant te bekijken. Ik heb genoten van onze discussies over statistiek, methodologie, databases, vragenlijsten of gewoon een gezellig gesprek over Twinky en meneer Frits. Zoals Deniz al schreef, je bent echt een topbegeleider die je elke promovendus gunt!

De leden van de kleine commissie, waaronder Prof. dr. P.A. van Doorn, Prof. dr. G. Parenti en Prof. dr. H.A.W.M. Tiddens, wil ik graag bedanken voor hun bereidheid zitting te nemen in

de kleine promotiecommissie en het beoordelen van mijn proefschrift. Beste Pieter, dank voor de fijne manier waarop je bij verschillende projecten betrokken was en sturing hebt gegeven. Ik heb altijd veel bewondering gehad voor je vermogen om een stap terug te nemen en een artikel in bredere zin te analyseren. *Dear professor Parenti, thank you for being part of my PhD committee. I highly appreciate you taking the time out of your busy schedule to assess my thesis and come to Rotterdam.* Geachte professor Tiddens, bij u schreef ik mijn master scriptie. Destijds was dit mijn eerste kennismaking met wetenschap en u heeft mij hiervoor enthousiast gemaakt. Dank hiervoor. De overige leden van de grote commissie wil ik ook bedanken voor hun bereidheid om van gedachte te wisselen over de inhoud van dit proefschrift.

Marein en Esther, ik ben heel blij dat jullie mijn paranimfen willen zijn en mij bij willen staan in deze spannende tijd. Jullie vriendschap is mij zeer waardevol. Marein, wat hebben we het altijd gezellig gehad. Ik heb genoten van het kletsen, sparren, discussiëren of gewoon frustraties uiten onder het genot van een kopje koffie. Je doorzettingsvermogen en enthousiasme zijn bewonderingswaardig. Ik hoop dat er nog heel veel kopjes koffie volgen! Al is het maar om de 'pro-gaming carrière' van je 3 geweldige zonen in de gaten te houden! Esther (K), ik heb genoten van onze samenwerking aan verschillende projecten. Ik heb bewondering voor je kracht, relativerings- en doorzettingsvermogen (en je hardlooptijden). Jij was mijn maatje aan de volwassen kant en ik heb je gemist toen je promotietijd erop zat. Gelukkig volgenden er snel kopjes koffie, etentjes en katten-dates buiten het ziekenhuis met jou en je lieve gezin.

Dimitris, thank you very much for all your statistical advice. I highly appreciate your patience and teaching skills. Our meetings always fueled me with inspiration to learn more about statistics and programming. I am very grateful for your guidance.

Graag wil ik al mijn kamergenoten op kamernummer sb-1770 bedanken. Jarenlang hebben jullie mijn getik en gewiebel zonder klagen getolereerd. Esther (P) en Audrey, ik zag ons altijd als de drie musketiers van het centrum. Ik ben heel dankbaar voor jullie steun, verfrissende visies en alle leuke dingen die we gedaan hebben. Jullie hebben mijn promotie kleur gegeven en ik wens jullie veel succes met de afronding van jullie eigen boekjes. Iris, dank voor je begeleiding. Ik waardeer het erg hoe je altijd de verbinding en harmonie op de kamer (en in het centrum) brengt en je in weet te zetten voor het team. Onze trip naar Milaan was zeker ook een hoogtepunt. Wilma, dank voor je ongelofelijke inzet voor het centrum en de patiënten. Zonder jouw perfecte planningen zouden we ons geen raad weten. Daarnaast heb je ook altijd oog voor het persoonlijk leven van je collega's. Bij lief of leed neem je als eerste het initiatief een kaartje te sturen, dat heb ik erg gewaardeerd. Jeroen, als nieuwe

promovendus neem jij het stokje over. Ik wens je heel veel succes met het onderzoek. Graag wil ik je bedanken voor de gezellige tijd en interessante gesprekken over, onder andere, 'depressieve Russische schrijvers'. Hiernaast mijn complimenten voor je excellente rode wijn smaak. Marloes, Maya en Lois, dank voor de gezelligheid en jullie inzet met betrekking tot de psychosociale patiëntenzorg. Ook alle ex-kamergenoten en oud collega's wil ik hartelijk danken. Johanneke, bij het schrijven van dit dankwoord is het bijna tijd voor jouw promotie. Ik wens je heel veel succes met je verdediging en verdere carrière als psycholoog. Hiernaast wil ik je bedanken voor de fijne tijd en al je inzet. Deniz, van jou nam ik het 'survey stokje' over toen je promotie erop zat. Heel erg bedankt voor al je werk aan dit project en al je adviezen. Carine, heel veel dank voor de fijne samenwerking aan het juveniele stuk. Ik wens je al het beste. Femke, dank voor je nuchterheid en gezelligheid tijdens je jaar op onze kamer. Ik wens je heel veel succes met het staartje van je jaar in Suriname. Carin, Marion, Stefan, Juna, Linda en Rachel, dank voor de fijne tijd en succes met jullie verdere carrières.

De medisch specialisten verbonden aan het Centrum voor Lysosomale en Metabole ziekten wil ik bedanken voor de prettige samenwerking. Hannerieke, bedankt voor de begeleiding van mijn werkzaamheden 'op zaal', je steun en je vertrouwen. Ik heb grote bewondering voor je grenzeloze inzet. Esmee, tijdens onze samenwerking heb ik genoten van je gevoel voor humor en flauwe grappen. Je betrokkenheid bij de patiënten is een voorbeeld voor me. Veel dank voor je begeleiding. Hidde, dank voor je steun, betrokkenheid en begeleiding. Je was een rustige stabiele factor. Monique en Hans, hoewel we minder vaak samen werkten heb ik ook van jullie weer nieuwe dingen over metabole ziekten geleerd. Bedankt!

Dank aan de neurologen en arts-onderzoekers van de 'volwassen kant'. Nadine, heel erg bedankt voor je begeleiding en kritische blik op de verschillende stukken waaraan we hebben samengewerkt. Ik waardeer je grote drive en vriendelijke, doch directe, manier van begeleiden. Esther (B), heel erg bedankt dat ik altijd bij je terecht kon met allerlei verschillende vragen en voor de fijne samenwerking. Laurike en Harmke, heel erg bedankt voor de samenwerking aan verschillende projecten, discussies over methodologie en statistiek, en de gezelligheid. Ik vond het altijd super leuk om met jullie op stap te gaan.

Ook wil ik alle verpleegkundigen, paramedici, analisten en ondersteunend personeel bedanken voor hun overweldigende inzet voor het Pompe onderzoek. Rineke, Jacqueline, Anneke, Asia, Wendy, Tineke en het overige verpleegkundig personeel, bedankt voor jullie inzet en het leveren van hoogwaardige zorg met oog voor de patiënt. Rineke, ik wil jou dubbel bedanken: namelijk ook voor al het werk wat je verzet hebt voor de 'IPA/ Erasmus MC Pompe survey'. Zonder jouw inzet was de survey nooit zo succesvol geworden. Nathalie, Marianne en Dorothee, ik heb altijd enorme bewondering gehad voor hoe jullie de waterval

aan afspraken van Ans in toom houden. Graag wil ik jullie bedanken voor jullie geduld, begrip en steun. David Alexander, *thank you for your critical reading of my manuscripts. Your input was highly valued.* Nicolette en de overige medewerkers van de apotheek, heel veel dank voor het organiseren en bereiden van die duizenden infusen. Ik vond het altijd heel prettig samenwerken en waardeer jullie professionaliteit. Verder wil ik de fysiotherapeuten, in het bijzonder Lianne, Nelleke, Robert en Anton, bedanken voor de ontelbare assessments die ze afgenomen hebben. Ook de longfunctie, ECG/echo laboranten en diëtisten verdienen dank voor hun onuitputbare inzet. Sanne, Bharat en Eva, super veel dank voor het invoeren van alle assessments in de verschillende databases. Jullie hebben ons veel werk uit handen genomen. Veel succes met jullie carrières.

Graag wil ik alle medewerkers van het research- en diagnostisch laboratorium bedanken. Pim, dank voor je begeleiding. We moeten nog een paar slagen aan het ACE stuk slaan, maar we komen er wel! Ik vond het in ieder geval heel met je prettig samenwerken en waardeer je droge gevoel voor humor. Ook wil ik alle onderzoekers en analisten bedanken voor de samenwerking en interessante gezamenlijke meetings. Atze, Gerben, Qiushi, Erik, Monica, Douglas, Rodrigo, Pablo, Joon, Mike, Stijn, Tom, Merel, Marianne en Marian, heel erg bedankt voor alles. *Thank you very much!* Arnold, alhoewel je al een tijdje met pensioen bent wil ik je bedanken voor je enthousiasme en energie. Op de verschillende congressen genoot ik altijd hoe je vol inspiratie en energie de zaal aan je lippen kreeg.

Uiteraard wil ik ook mijn familie en vrienden bedanken voor hun steun tijdens mijn promotie. Zeker in deze laatste periode hebben we weinig tijd gehad om af te spreken. Ik kijk er naar uit om meer tijd met jullie door te brengen. In het bijzonder wil ik mijn ouders, zus en schoonfamilie bedanken voor hun onvoorwaardelijke liefde, steun en begrip. Pa en ma, bedankt dat jullie me altijd gemotiveerd hebben om alles uit het leven te halen en mijn ambities na te streven. Jullie liefdevolle opvoeding en wijze levenslessen hebben me de man gemaakt die ik nu ben. Ik ben jullie dankbaar. Sophia, bedankt dat je al je hele leven mijn maatje bent. Ik ben blij en trots om je als zusje te hebben. Heel erg bedankt voor het ontwerpen van de voorkant van dit boekje. Wat is het mooi geworden. Frank, Jorien, Chris, Rianne en Marte, vanaf het moment dat ik voor het eerst een kopje thee kwam drinken heb ik me welkom gevoeld binnen de familie. Jullie zijn een schoonfamilie uit duizenden en ik ben dankbaar voor jullie steun en medeleven. Lieve vrienden en buren (go Beukers!), dank jullie voor de steun en interesse. Deze is voor mij heel waardevol.

Lieve Marieke, als er iemand dank verdient dan ben jij het. Je was mijn rots in de branding tijdens dit avontuur. Ik ben je dankbaar voor je onvoorwaardelijke liefde en onophoudelijke steun. Wij zijn de toekomst.

About the author

Jan Christiaan van der Meijden was born on 30 May 1986, in Hardinxveld-Giessendam, the Netherlands. In 2004 he graduated from the 'C.S.G. de Oude Hoven' high school in Gorinchem and started his medical training at the Erasmus University in Rotterdam. Shortly after obtaining his medical degree in 2011, he started working as a resident at the department of pediatrics of the Albert Schweitzer Hospital in Dordrecht. One year later he started work on his PhD thesis under supervision of Prof. Dr. A.T. van der Ploeg at the Center for Lysosomal and Metabolic Diseases of the Erasmus University Medical Center in Rotterdam. During this period he managed the international "IPA/ Erasmus MC Pompe survey" and collaborated with representatives from national and international patient organizations.

Chris lives together with his partner Marieke Nijhuis in Rotterdam.

List of publications

Oussoren E, Bessems JHJM, Pollet V, **van der Meijden JC**, van der Giessen LJ, Plug I, Devos AS, Ruijter GJC, van der Ploeg AT, Langeveld M. A long term follow-up study of the development of hip disease in Mucopolysaccharidosis type VI. Molecular Genetics and Metabolism, 2017.

van der Meijden JC, van Capelle CI, van den Hout JM, Jaeken J, Baethmann M, Voit T, Kroos MA, Derks TG, Rubio-Gozalbo ME, Willemsen MA, Lachmann RH, Mengel E, Michelakakis H, de Jongste JC, Reuser AJ, van der Ploeg AT. Childhood Pompe disease: clinical spectrum and genotype in 31 patients. Orphanet Journal of Rare Diseases, 2016 May;18;11(1):65.

van der Meijden JC, Güngör D, Kruijshaar ME, Muir AD, Broekgaarden HA, van der Ploeg AT. Ten years of the international Pompe survey: patient reported outcomes as a reliable tool for studying treated and untreated children and adults with non-classic Pompe disease. Journal of Inherited Metabolic Diseases. 2015 May;38(3):495-503.

Bakker EM, **van der Meijden JC**, Nieuwhof EM, Hop WC, Tiddens HA. Determining presence of lung disease in young children with cystic fibrosis: lung clearance index, oxygen saturation and cough frequency. Journal of Cystic Fibrosis. 2012 May;11(3):223-30. (FLY-studie).

Kuperus E, Kruijshaar ME, Wens SCA, de Vries JM, Favejee MM, **van der Meijden JC**, Rizopoulos D, Brusse E, van Doorn PA, van der Ploeg AT, van der Beek NAME. Enzyme replacement therapy is beneficial after a median of five years of treatment in 88 adult Pompe patients: a nationwide prospective observational cohort study. *Neurology 2017; in print.*

van der Meijden JC, Kruijshaar ME, Rizopoulos D, van der Beek NAME, van der Ploeg AT. Enzyme replacement therapy reduces the risk for wheelchair dependency in adult Pompe patients. *Submitted*

van der Meijden JC, Kruijshaar ME, Rizopoulos D, van den Hout JMP, van der Ploeg AT. Long-term follow-up of 17 patients with childhood Pompe disease treated with enzyme replacement therapy. *Submitted.*

Favejee MM, **van der Meijden JC**, Kruijshaar ME, Rizopoulos D, van der Ploeg AT, Bussmann JB. Association of muscle strength and walking performance in adult patients with Pompe disease: a nomogram. *Submitted.*

van der Meijden JC, Kuperus E, in 't Groen SLM, Kroos MA, Hoogenveen M, Rizopoulos D, van der Beek NAME, Kruijshaar ME, van Doorn PA, van der Ploeg AT, Pijnappel WWM. Differences in disease severity and response to enzyme replacement therapy in Pompe patients with the common c.-32-13C>T GAA gene variant cannot be explained by the ACE I/D polymorphism. *In preparation.*

PhD Portfolio

Activity	Year	ECTS
Training courses		
International Postgraduate Course on Lysosomal Storage Disorders, Nierstein, Duitsland.	2013	1,2
NIHES: Biostatistical Methods I: Basic Principles	2013	5,7
Basiscursus Regelgeving & Organisatie voor Klinisch Onderzoekers (BROK)	2014	1
NIHES: Biostatistical Methods II: Regression Models	2015	4,3
NIHES: Repeated Measurements	2015	1,4
Cursus Wetenschappelijke Integriteit mei 2013	2013	0,3
Oral and poster presentations		
Poster presentation. IPA / Erasmus MC Pompe survey - De resultaten van 10 jaar vragenlijstonderzoek, *Spierziektecongres*, Veldhoven, the Netherlands.	2013	1
Oral presentation. Pain in adult patients with Pompe disease A cross-sectional survey, *The international GSD conference*, Heidelberg, Germany.	2013	1
Plenary lecture. IPA/ Erasmus MC Pompe Survey - the results of 10 years patient reported outcomes, *The international GSD conference*, Heidelberg, Germany.	2013	2
Poster presentation. The Pompe survey - Results of 10 years follow-up, *Steps forward in Pompe disease*, Turin, Italy.	2014	1
Poster presentation. The Pompe survey - Results of 10 years follow-up, *WORLD symposium*, San Diego, United States of America.	2014	1
Oral presentation. IPA/ Erasmus MC Pompe survey - resultaten van 10 jaar onderzoek, *Spierziektecongres,* Veldhoven, the Netherlands.	2014	1
Poster presentation. Childhood Pompe disease: clinical spectrum and genotype in 31 patients (poster), *World muscle symposium*, Brighton, United Kingdom.	2015	1
Poster presentation. Ten years of the international Pompe survey; patient reported outcomes as a reliable tool for studying treated and untreated children and adults with non-classic Pompe disease, *ISPOR 18th Annual European Congress*, Milan, Italy.	2015	1
Poster presentation. Ten years of the international Pompe survey: patient reported outcomes as a reliable tool for studying the natural course of disease and treatment effects in non-classic Pompe patients, *SSIEM congress*, Lyon, France.	2015	1
Plenary lecture. Ten years of the international Pompe survey, *ESN spring symposium*, Rotterdam, the Netherlands.	2015	2
Oral presentation. Ten years of the international Pompe survey, *Sophia Onderzoeksdag*, Rotterdam, the Netherlands.	2015	1
Plenary lecture. Ten years international Pompe survey - Patient reported outcomes as tool to study Pompe disease, *AMDA conference*, San Antonio, United States of America.	2015	2

Activity	Year	ECTS
Poster presentation. Ten years of the international Pompe survey: patient reported outcomes as a reliable tool for studying the natural course of disease and treatment effects, *European conference on rare diseases & orphan products,* Edinburgh, Scotland.	2016	1
Plenary lecture. Effects of ERT in children with non-classic phenotypes, *Steps forward in Pompe disease,* Amsterdam, the Netherlands.	2016	2
Oral presentation. Post-marketing experiences in Pompe disease, *TREAT-NMD meeting*, Leuven, Belgium.	2016	1
Keynote. Childhood Pompe disease: clinical characteristics and the effects of treatment, *Korean National Cross LSD meeting,* Seoul, South Korea.	2017	3
Attended congresses		
Pompe expert day	2013	0,2
Sophia onderzoeksdag	2014 / 2015	0,4
Other activities		
Journal club	2013-2017	1
Research meeting	2013-2017	1
Total ECTS:		**38,5**

Abbreviations

6MWT: 6 minute walking test
6MWT-PP: 6 minute walking test – percent predicted
ACE: Angiotensin converting enzyme
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
ATS: American thoracic society
BiPAP: Biphasic positive airway pressure
CI: Confidence Interval
CK: Creatinine kinase
CRIM: Cross reactive immunological material
EMA: European medicines agency
ERS: European respiratory society
ERT: Enzyme replacement therapy
FDA: Food and drug administration
FEV1: Forced expiratory volume
FSS: Fatigue severity scale
FVC: Forced vital capacity
GAA : Acid a-glucosidase gene
HHD: Hand held dynamometry
HR: Hazard ratio
IAR : Infusion associated reaction
IPA: International Pompe association
IQR: Interquartile range
LDH: Lactate dehydrogenase
LSD : Lysosomal storage disease
LVMI: Left ventricular mass index
M6P: Manose-6-phosphate
mRNA: Messenger RNA
MEP: Maximal expiratory pressure
MIP: Minimal inspiratory pressure
MMT: Manual muscle testing
MRC: Medical research council
PEP: Polymorphic encryption and pseudonymisation
Pp: Percentage point
PRO: Patient reported outcome
QMFT: Quick motor function test
R-Pact: Rasch-build Pompe-specific activity scale
RER: Rough Endoplasmic Reticulum
rh-GAA: Recombinant human acid alpha-glucosidase
RHS: Rotterdam handicap scale
SF-36: Short-form 36 health survey
VC: Vital capacity