Long-term Neuropsychologic Outcome in Children Diagnosed with a Lysosomal Storage Disease

Berendine J. Ebbink

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Lange termijn neuropsychologische gevolgen bij kinderen gediagnosticeerd met een lysosomale stapelingsziekte.

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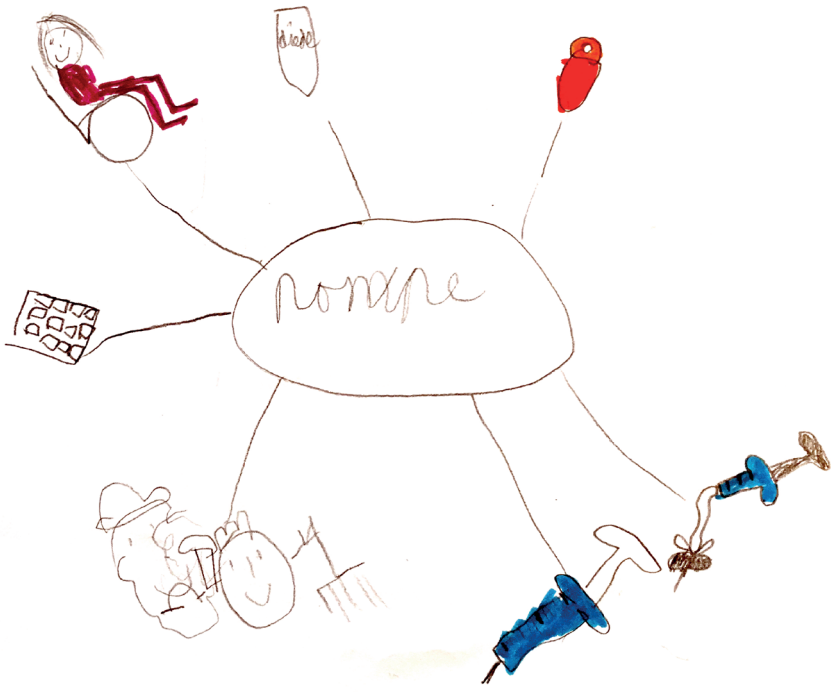

GENERAL INTRODUCTION AND SCOPE OF THE THESIS.

Lysosomal storage diseases

At this moment over 600 different metabolic diseases are known. For many of these disorders still no treatment exists. Metabolic diseases are categorized in several ways, e.g. according to the dysfunction of a metabolic pathway involved (e.g. urea cycle defects), according to the organelles involved (for example lysosomal storage diseases or mitochondrial diseases) and/or the storage product involved (e.g. glycogen storage disorders; mucopolysacchidoses)[1, 2]

This thesis focuses on lysosomal storage disorders (LSDs). Lysosomes are intra-cellular organelles containing over 50 different types of enzymes.[3] The function of these enzymes is to degrade specific macromolecules that have entered the lysosomes by autophagy or endocytosis. Thereby the lysosome preserves the homeostasis and function of a cell. It should however be noted that the lysosome has many functions. It is part of the innate immune system by killing multifarious micro-organisms in leucocyte. It plays an important role in the turnover of hormones and liberation of essential nutrients from carrier proteins and it is the target in receptor mediated endocytosis. In addition, lysosomes are involved in remodeling of tissues as cartilage and bone.[4, 5]

The amino acid sequence of lysosomal enzymes and lysosomal transporters is encoded by the DNA. Variations in the DNA sequence may lead to partial or total deficiency of the activity of a lysosomal enzyme. The human body is composed of many tissues and organs, which all have their own function and specific macromolecules to deal with. The cells of which these tissues and organs are composed, synthesize their own lysosomal enzymes. Although deficiency of a particular lysosomal enzyme includes all cells in the human body, it may affect some tissues more than others based on the macromolecules that cannot be degraded. The tissue specific consequences of storage explain the disease specific signs and symptoms of the different lysosomal storage disorders (LSD) (e.g., connective tissue, cartilage and bone is specifically affected in MPS -because glycosaminoglycans cannot be degrade-; muscle is specifically affected in Pompe disease -because the lysosomal degradation of glycogen is insufficient-). As the activity of an enzyme can be total or partially deficient, the severity of the LSD can vary from severe to mild. Thus many LSD's comprise a spectrum of severity with a variation in onset of the disease and severity of the symptoms.[6,2]

LSDs are classified according to the substance that stores in the lysosomes, e.g. lysosomal storage disorders in which glycosaminoglycans or mucopolysaccharides store are classified as mucopolysaccharidoses.

Enzyme replacement therapy

During the last decades, innovative treatment options have become available for several LSDs, such as haematopoietic stem cell transplantation, substrate reduction therapy, chaperones and enzyme replacement therapy (ERT). Currently gene therapy is under development for various disorders.[6] In this thesis we focus on ERT.

In 1955 De Duve discovered the lysosome.[3] In the years thereafter he described the processes of endocytosis, phagocytosis and autophagy.[7-9] He depicted how proteins and other substances entering the cell by endocytosis were automatically targeted to the lysosomal apparatus. He envisaged that lysosomal storage disorders were therefore amenable to therapy and that the availability of ERT was just a matter of time. He received the Nobel Prize for his findings in 1974. In 1968 Fratantoni et al.,[10] showed that enzyme deficient cells of different types of LSDs (MPS I and II) could mutually correct each other's deficiency when co-cultured together. The identification of the lysosome and the description of the excretion and recapture process further strengthened the feasibility of enzyme replacement therapy.

The first ERT that eventually became successful was for Gaucher disease.[11-15] Gaucher disease is caused by a deficiency of the lysosomal enzyme beta-glucosidase (beta- glucocerebrosidase) and mainly affects the macrophages, including the Kupffer cells of the liver. Storage of glucocerebrosides leads among others to hepatosplenomegaly, thrombocytopenia and anemia. Macrophages expose mannose receptors on their cell surface. Enzyme purified from placenta contains a high amount of mannose. Macrophages and Kupffer cells are in close contact with the blood stream and appeared to be an easy target for the enzyme purified from human placenta. Thus, the first ERT from a human natural source became available in 1991. Since most other cell types do not expose mannose receptors on the cell surface, human placenta appeared unfit as source of ERTs for other LSDs.[16-18]

In 1977 Kaplan et al.[19] and Sando and Neufield[20] revealed the function of the mannose-6-phosphate receptor (M6P/IGFII). This receptor appeared to play a major role in the intracellular transport of newly synthesized endogenous lysosomal enzymes from the trans Golgi network to the lysosomes, and appeared also to be exposed on the cell surface where it could capture exogenous lysosomal enzymes containing M6P. Thereby the receptor forms the gateway to the lysosomes for exogenous enzymes containing mannose-6-phosphate. It lasted until 1984[21-25] when the potential of this receptor was recognized for the application of ERT in LSDs. Preclinical tests were performed for Pompe disease with enzymes purified from bovine testis and human urine.[21, 25] It was demonstrated that the enzymes purified form natural sources reached the lysosomes of cultured fibroblasts and skeletal muscle cells of patients and degraded stored glycogen. In addition, the enzymes were also taken up by skeletal muscles of mice, and it could be demonstrated that the intravenous administered

precursor form of alpha-glucosidase was processed to the mature form that resides in the lysosomes.[26] However, in case of Pompe disease natural sources were not capable of delivering sufficient amounts of alpha-glucosidase for human use. Cloning of the GAA gene was a crucial next step and made it possible to explore biotechnological production of recombinant human alpha-glucosidase.[26-28]

The first clinical test for Pompe disease was performed in 1999 at Erasmus MC with recombinant human alpha-glucosidase from rabbit milk.[29, 30] Four infants participated in this first successful pilot trial.[31] ERT with recombinant human alpha-glucosidase for Pompe disease was finally registered in 2006. The registered enzyme was produced in Chinese Hamster Ovary (CHO) cells.

At the same time ERTs targeted to the M6P/IGFII receptor were developed and registered for other LSDs[6] such as for MPS I (2003),[32] MPS II (2006),[33] MPS VI (2007),[34] Fabry disease (2001)[35] and MPS IV (2013).[36] For several other LSDs such as alpha-mannosidosis,[37] NCL2[38, 39] and AGU,[40] ERT is under development. The various ERTs have shown to have beneficial effects for patients. One of the limitations is that the ERT molecules cannot pass the blood-brain barrier, which is a major limitation when it comes to lysosomal storage disorders that also effect the brain.[41]

The brain in lysosomal storage disorders

Brain involvement is observed in various LSDs.[42] As the disease spectrum ranges from mild to severe, the consequences of the storage in the brain may vary from mild involvement to severe and progressive intellectual disability, sometimes accompanied by behavioral problems.[43] The course of the disease and the risk of cognitive deterioration cannot always be predicted. Brain involvement puts a considerable stress on patients, parents, siblings and the patients' surroundings.

Accurate prediction of brain involvement and thorough knowledge of the natural course can help parents reduce stress and help them find the right guidance and education for their children. Equally important, this knowledge is crucial as bench mark for upcoming new therapy's which are aiming on passing the blood brain barrier and treat the brain.

In this thesis we aimed to get insight in brain involvement and the neuropsychological consequences in four types of lysosomal storage diseases: Pompe disease, MPS II, MPS VI, and alpha-mannosisodosis.

Pompe disease (progressive metabolic myopathy)

Pompe's disease (acid maltase deficiency, OMIM # 232300) is a progressive metabolic myopathy. In 1932 the Dutch pathologist J.C. Pompe described the first patient, a 7 months old girl with a hypertrophic cardiomyopathy due to massive glycogen accumulation.[44] More than 30 years later, Hers[45, 46] discovered the cause of Pompe disease, by demonstrating a deficiency of acid alpha-glucosidase (GAA, EC 3.2.1.20) in five infantile Pompe patients. In 1990, Hoefsloot[27] and Martiniuk[47] unraveled the sequence of the human lysosomal alpha-glucosidase-gene. Variations in this gene lead to a deficiency of the enzyme alpha-glucosidase causing (excessive) accumulation of glycogen in the lysosomes. Since this occurs especially in muscle tissue, this leads to a progressive myopathy.[48]

The disease is autosomal recessively inherited and has a frequency of 1:40.000.[49, 50] Pompe disease comprises a spectrum from mildly affected non-classic Pompe patients with some residual activity of the enzyme (alpha-glucosidase) to severely affected classic infantile patients with variations in the GAA gene leading to complete inactivity of the enzyme[51] (Figure 1).

Figure 1. Clinical spectrum in Pompe disease[156]

In the non-classic Pompe variant partial deficiency of enzyme activity results in a slowly progressive disease, which may present at any moment in life during childhood or adulthood.[52, 53] Patients have a gradual developing proximal myopathy mostly without involvement of the heart. The muscle weakness leads to mobility problems, such as difficulties in climbing stairs, standing up from a chair, walking and combing hair.[52, 54] Since the respiratory muscles are also affected, respiratory difficulties occur frequently, which present first during sleep when in supine position, due to impairment of the diaphragm.[55] The complaints of muscle weakness and the respiratory problems are frequently accompanied by fatigue.[56, 57] Most patients ultimately need aids such as a wheelchair and a respirator. Quality of life is diminished.[58, 59]

Patients with the classic infantile form of the disease have a complete enzyme deficiency.[60] They present in the first year of life with a fast progressive generalized hypotonic myopathy (muscle weakness) and a hypertrophic cardiomyopathy, which is already present at birth. The proximal muscle weakness is impressive and patients clinically present as "floppy infants". Symptoms such as a head lag and slipping through are found in all. Patients never achieve any motor milestone and suffer from repetitive airway infections leading to respiratory problems, feeding difficulties and failure to thrive. Untreated, infants die within the first year of life.[61]

The ErasmusMC has a long history of research on Pompe disease ranging from the description of the cDNA[27] sequence alpha-glucosidase to production and modification of alpha-glucosidase,[62,63] the elucidation of the variability of the disease,[51,60,64-70] (prenatal) diagnosis,[71] description of the natural course[72] and the development of treatment.[26,73]

In classic infantile Pompe patients ERT improved survival, normalized the heart, increased muscle strength and enabled the achievement of motor milestones such as walking.[29,74-79] However, glycogen is known to store in the central nervous system in classic infantile Pompe patients as well.[80-89] Due to the limited lifespan of untreated patients with classic infantile Pompe disease no clinical consequences of the glycogen storage in the brain had been observed initially. Over time it has become increasingly evident that the CNS is not spared. Therefore, the brain becomes an important region of interest. Studies on the (potential) long-term neuropsychological consequences were therefore part of this thesis. These potential cognitive consequences of brain involvement may make the brain be the next challenge in the treatment of classic infantile Pompe disease and the target of innovative initiatives/therapies.

Mucopolysaccharidosis (MPS)

Mucopolysaccharidosis (MPS) is a collective name for several diseases caused by at least 10 different enzyme deficiencies (Table 2 summarizes the most relevant ones).[43,90] In the case of MPS, the process of the degradation of mucopolysaccharides (glycosaminoglycans, or GAGs) is disrupted, affecting predominantly connective tissue, cartilage and bone and frequently also the brain.[43] As part of this thesis the consequences for the CNS in MPS II and MPS VI were studied in more detail. In 2007 Erasmus MC became the only national Dutch expert center on implementation of ERT for those two diseases.

Table 1. Types of Mucopolysaccharidosis

Type	Name	Inheritance	Enzyme deficiency	Storage product	CNS
MPS I	Hurler, Hurler-Scheie, Scheie	Autosomal recessive	a-L-iduronidase	Dermatan sulphate and heparan sulphate	IH: Yes IS: No IH/S: intermediate
MPS II	Hunter	Sex linked recessive	iduronate-2-sulphatase	Dermatan sulphate and heparan sulphate	Spectrum Neuronopathic: yes Non-neuronopathic: No
MPS III (A,B,C,D)	Sanfilippo	Autosomal recessive	A: Heparan N-sulphatase B: N-acetyl-a-D-glucosaminidase C: a-glucosamine-N-acetyl transferase D: N-acetyl-glucosamine-6-sulphate Sulphatase	Heparan sulphate	Yes, often, but not always
MPS IV (A,B)	Morquio	Autosomal recessive	A:Galactosamine 6-sulphate B: β-galactosidase	Keratan sulphate	No
MPS VI	Maroteaux-Lamy	Autosomal recessive	arylsulfatase B	Dermatan sulphate	No
MPS VII	Sly	Autosomal recessive	β-glucuronidase	Dermatan sulphate Heparan sulphate	Yes
MPS IX	Natowicz syndrome	-	hyaluronidase 1	Hyaluronan	No

MPS II (Hunter Syndrome)

Mucopolysaccharidosis type II (or Hunters syndrome) was first described by Charles Hunter in 1917.[91] He described the first two patients as "being alike as two peas". He reported symptoms such as being undersized and having extremely large heads, curiously shaped and harsh hear, abnormal appearance of the face, short neck, and abnormal joints. As MPS II is an X-linked disorder, it mainly affects males. It is caused by a deficiency of the enzyme iduronate-2-sulphatase, leading to lysosomal storage of dermatan sulphate and heparan sulphate.[90] The disease' prevalence ranges between 1:140.000-156.000 male live births.[92] It comprises a spectrum from non-neuronopathic patients with symptom onset and/or diagnosis during childhood or adulthood to neuronopathic, severely affected patients with symptom onset and/or diagnosis in infancy or early childhood. [93-98] The severe neuronopathic patients mostly die during the second decade of life,[99] while the non-neuronopathic patients can survive well into their adulthood.[100] MPS II affects mainly connective tissue and cartilage and bone, but also other cell types. Typical clinical findings are cardiac-valve abnormalities, dysostosis multiplex, coarse facial features, contractures of the joints, carpal tunnel syndrome, hepatosplenomegaly, impaired lung-function, tracheomalacia, hearing deficits, and myelopathy.[99, 101, 102]

Brain involvement is an important characteristic feature of the neuronopathic form of the disease, leading to intellectual disabilities.[97, 98, 103-107] Reports on brain MRI of neuronopathic and non-neuronopathic patients describe abnormalities such as atrophy, widening of ventricles, white matter abnormalities, and enlarged virchow robin spaces.[101, 108-114] The following developmental course of the neuronopathic variant can be deduced form the literature so far: until the age of approximately 4 years development is normal to delayed. Thereafter, children reach a plateau in their development, which is followed by a decline in intellectual abilities. The combined symptoms eventually lead to early death in their teenage years.[101] Associated with this severe form of the disease are behavioral problems such as hyperactivity, disturbed sleep and aggression. [115-118] This combination of symptoms highly disrupts family life. The disease comprises a spectrum. Approximately two-third of the patients are reported to develop the neuronopathic form of the disease.[95, 96, 111] Intermediate disease variants may occur as well. No prediction on the neuropsychological disease course can be made beforehand so far. This creates a need for studies that identify early prognostic signs on e.g. brain MRI or in the neuropsychological profile that enable prediction of the cognitive outcome.

Since 2006, ERT has been commercially available as treatment option.[33] The therapy has improved several clinical aspects of the diseases such as joint mobility, lung function and walking distance.[119, 120] However, as in other lysosomal storage disorders the molecular size of the ERT proteins is not able to pass the blood-brain barrier, leaving the cognitive deterioration and behavioral problems untouched.[41] Novel treatment modalities, such as intrathecal enzyme replacement therapy[121] and enzyme replacement therapy with a fusion protein targeted to the insulin receptor [122-124] with the aim to cross the blood-brain barrier, are currently under investigation. To appreciate the effect of these upcoming therapies, it is important to have thorough knowledge on the course of the disease with the current treatment option (ERT), which does not improve the CNS symptoms.

MPS VI (Maroteaux-Lamy Syndrome)

Maroteaux-Lamy syndrome (MPS VI) was first described in 1963 by the French physicians Maroteaux and Lamy.[125] The disease is an autosomal recessive lysosomal storage disorder, caused by mutations in the gene encoding the enzyme arylsulfatase B (also called arylsulfatase beta, EC 3.1.6.12). Deficiency causes accumulation of dermatan sulphate in the lysosomes. The disease has a prevalence of 1: 248.000-1.300[92, 126-128]. As in Hunter's disease, Maroteaux-Lamy syndrome mainly affects connective tissues, cartilage and bone. Clinical characteristics are short stature, bone abnormalities, sensory perception disorders, corneal clouding, carpal tunnel syndrome, spinal cord compression, cardiac involvement and reduced life expectancy.[90, 129-133] Many of these symptoms occur in Hunter syndrome as well.

The disease presents as a clinical spectrum, which is divided into two broad categories, a rapidly progressive and slowly progressive form as defined by the excretion of glycosaminoglycans (GAGs).[130, 133-136] Patients with the rapidly progressive form of the disease present early in life (diagnosis under the age of 5 years) with severe symptoms, and the life expectancy is limited to the 2nd or 3rd decade of life.[133] Patients with a severe disease severity usually have high GAG levels (> 200 µg/mg kreatinine) in the urine. Although their symptoms develop later and are potentially milder, the impact and burden of disease may eventually be large as well especially when it comes to myelum compression, respiratory problems and skeletal abnormalities.[137] Enzyme replacement therapy became available in 2007 and has shown to have beneficial effects on joint range of motion, pulmonary function, cardiac function and endurance.[135, 138-140] As in the other LSDs, ERT cannot pass the blood-brain barrier. However, an important difference with Hunter syndrome is that Maroteaux-Lamy syndrome is generally considered not to affect cognitive development.[43, 90] Nevertheless, brain abnormalities in the white matter are found, and enlarged virchow robin spaces have been described before. Additionally, mega cisterna magna, subarachnoid space dilation, cervical junction/spinal cord compression, skull dysplasia, thickening of the dura mater and hydrocephalus can occur.[141-147] Literature on cognitive development in Maroteaux-Lamy syndrome is limited.[131, 141, 148] So far no long-term follow-up on intelligence and brain MRI were performed in this progressive LSD.

To extend the studies on CNS involvement in LSDs in this thesis beyond Pompe disease and MPS, we also studied Alpha-mannosidosis in more detail.

Alpha mannosidosis

Alpha-mannosidosis is a rare, autosomal recessive lysosomal storage disorder caused by deficiency of the lysosomal enzyme is alpha-mannosidase (EC 3.2.1.24) caused by mutations in the MANB1 gene. The accumulating substance is glycogen. The disease has a prevalence between 1:300.000-1:500.000 (Malm et al 2014). The clinical spectrum is wide and divided in three distinct phenotypes (Type I, II and III), based on severity. Type I is rapidly progressive with severe infections leading to early death. Type II is less severe and includes a slowly progressive disease course. Characteristic symptoms are delayed speech, hearing impairment, ataxia, coarse facial appearance, skeletal deformities, central nervous system involvement with psychiatric disorders. Patients survive into adulthood.[149-151] The limited neuropsychological follow-up studies of untreated patients with alpha-mannosidosis present conflicting results.[37, 152-154] There is a need for additional studies, especially since the effect of enzyme replacement therapy for alpha-mannosidase is currently under study.[155]

Aim of study

In this thesis we focus on the brain and the neuropsychological sequelae in patients with LSDs. Up till now studies on the consequences of brain involvement for neuropsychological development in patients with LSDs is limited. Although most LSDs have in common that they are caused by a single lysosomal enzyme deficiency, the storage products differ and as a consequence the effect on the brain and cognitive development varies between the different types of LSDs. Studies on the neuropsychological profile of patients with the different LSDs help to reveal the commonalities and differences and the new insights may be instrumental for a better understanding of the pathophysiological mechanisms leading to the differential effects on cognition. A better understanding on the neuropsychological profile is also needed to support patients, their parents and families, physicians, other caretakers and teachers. Finally, knowledge on neuropsychological involvement in patients with LSDs is a prerequisite to appreciate the effect of potentially new treatment modalities targeting the brain.

All of these considerations led us to study the neuropsychological consequences of lysosomal storage diseases in–four very different LSDs; Pompe disease, Alpha-mannosidosis, Hunter Syndrome (MPS II), and Maroteaux-Lamy Syndrome (MPS VI). This thesis can be subdivided in 4 parts.

PART 1. POMPE DISEASE.

This part of the thesis focuses on the classic infantile form of Pompe disease **(chapter 2 t/m 6).** Since enzyme replacement therapy has become available, the prospects for patients with classic infantile Pompe disease have improved significantly. The first treated patients with this previously lethal disease have survived and are now on the edge of adulthood. However a limitation is that ERT does not pass the blood brain barrier, while some of the glycogen also stores in the brain.

With this in mind, we studied the MRI findings and long-term neuropsychological development of patients with classic infantile Pompe disease treated with ERT from early infancy to adulthood. The studies provide unique information since they include the first patients treated worldwide with ERT.

PART 2. OTHER LYSOSOMAL STORAGE DISEASES

Part 2 focuses on other three other LSDs

Chapter 7 describes the long-term neuropsychological follow-up and MRI findings of an untreated patient with alpha-mannosidosis from infancy to adulthood. Our attention became focused on alpha-mannosidosis when we noticed that the neuropsychological consequences of the disease were quite different from the lysosomal storage disorders that we studied before (M. Pompe, MPS II and VI).

In **Chapter 8 and 9** we studied (and compared) the neurocognitive development and MRI findings of patients with two different types of mucopolysaccharidosis (MPS II and MPS VI). MPS II (Hunter syndrome) which typically affects the brain and is considered to lead to neurocognitive deficits in at least two third of the patients, and MPS VI (Maroteaux Lamy syndrome) which was considered a disease without cognitive impairment.

Lysosomal storage diseases are rare diseases. In order to evaluate the effect of novel therapies on central nervous system manifestations in these diseases, international consensus on how to evaluate the effects is necessary. **Chapter 10** describes the process and outcome of an international consensus meeting on cognitive endpoints in patients with MPS I, II and III.

PART 3. GENERAL DISCUSSION AND FUTURE PERSPECTIVES (chapter 11).

In this part the results of the studies performed as part of this thesis are discussed in the light of current and future developments.

PART 4. EPILOGUE (chapter 12)

This part reflects on the value of a neuropsychologist in a multidisciplinary team treating patients with complex lysosomal storage disorders.

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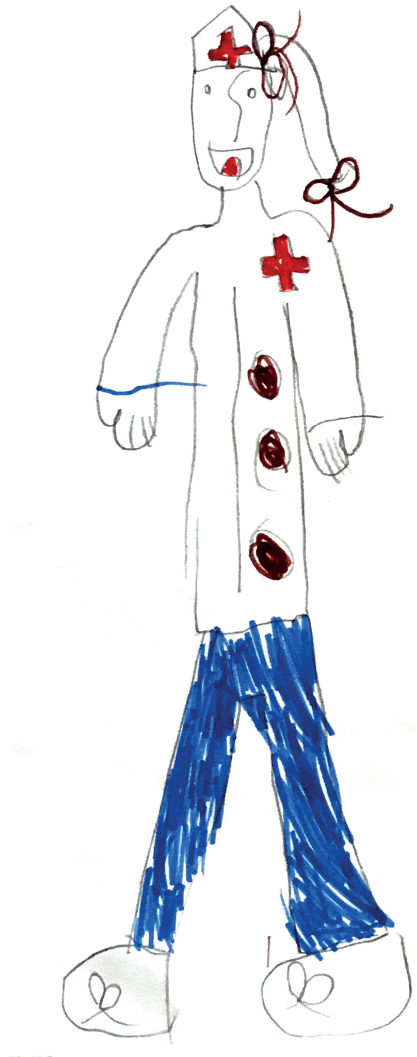
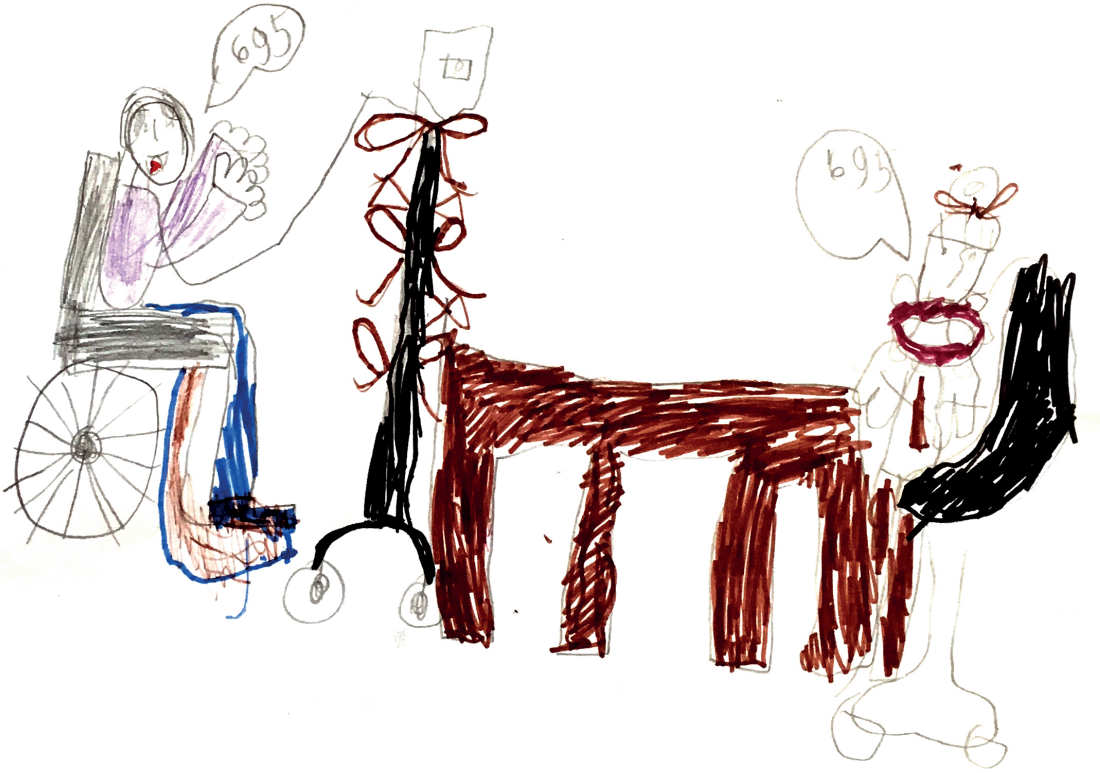


PART 1

Pompe Disease



Chapter 2

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

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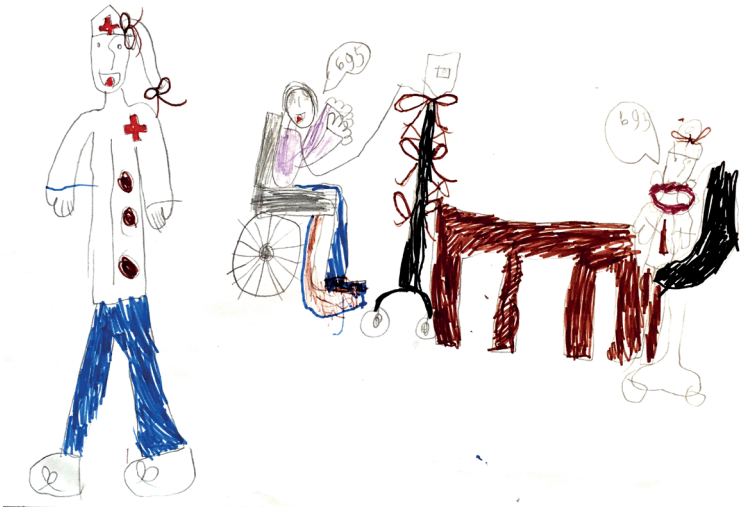


ABSTRACT

Objective

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

Methods

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

Results

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

Conclusions

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

INTRODUCTION

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

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

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

PATIENTS AND METHODS

Patients

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

Standard Protocol Approvals, Registrations, and Patient Consents

Study protocols were approved by the Institutional Review Board, and written informed consent was obtained from the participating children's parents.

Methods

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

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

Statistics

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

RESULTS

Patient characteristics

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

Table 1. Patient characteristics.

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

ERT = Enzyme-replacement therapy, F = Female, M = Male, Y = Yes, N = No, [a] = age in months at which invasive ventilation was started, [b] = Died at age 4 years, MMF = Minimal Motor Function, [c] = Lost virtually all motor milestones after becoming ventilator dependent, -[d] = not performed

Median age at diagnosis was 0.7 months (range 0.1 - 6.2 months). When ERT started (at a median age of 2.3 months [range 0.1 - 8.3 months]), all patients had signs of muscle weakness. Two children aged 7.2 and 8.3 months were in the end stage of disease; they had lost virtually all muscle function and became ventilator dependent before age one. At last assessment, they were still tetraplegic at ages of 11 and 12 years. The other eight children gained motor skills and learned to sit or walk unsupported as therapy proceeded. Three of these eight patients also became ventilator-dependent at age 2 (patient 3, 5 and 8). Thereafter they lost all motor skills; patients 3 and 5 finally died at age 4, but patient 8 improved when the ERT dose was raised from 20 mg/kg/every other week to 40 mg/kg weekly; at last assessment he was ventilator-dependent only during sleep, and had regained his motor functions to the extent that he could sit up independently, and also ride a tricycle. To date, however, he has not regained the ability to walk.

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

Cognitive development during the first four years of life

Forty psychological assessments were performed in 10 children (age range 2 months - 4 years). Table 2 groups test results by five different time points during the first three years of therapy: before the start of ERT (median age 5 months), at six months of ERT (median age

8 months); at one year of ERT (median age 14 months); at two years of ERT (median age 27 months); and at three years of ERT (median age 37 months).

Table 2. Total test scores per patient per assessment.

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

BSID-II = Bayley Scales of Infant Development, Second Edition, Griffith = Griffiths Mental Development Scales; SON-R = Snijders Oomen Nonverbal Intelligence Test-Revised; WISC-III = Wechsler Intelligence Scales for Children, Third Edition. <70 severe developmental delay; 70-84 mild developmental delay; 85-115 normal development; >115 advanced development. [a] = disharmonic profile, [b] = After this time point hearing aids were fitted, [c] = Raven (disharmonic profiles could not be calculated).[d] = died at age 4, [e] = At this time point the patient suffered from a serious respiratory infection

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

Disharmonic profiles were found in 7 out of 10 patients and in 21 of the 30 tests performed in these 7 patients. At each time point after the start of therapy, the percentage of patients

with disharmonic profiles was higher than that of their healthy peers ($p < 0.05$). The disharmonic profiles were due to lower gross motor functioning (15 of 21 Griffiths or BSID II) and, to a lesser extent, also to higher Personal-Social scores (5 of 21), lower MDI versus PDI scores (2 of 21 BSID-II), higher Eye and Hand Coordination scores (1 of 21), higher Performance scores (2 of 21), lower Performance scores (1 of 21) and higher Hearing and Language scores (2 of 21). Some patients had an imbalance in their test profile for more than one subscale. Median subscale scores per measurement are presented in Table 3.

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

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

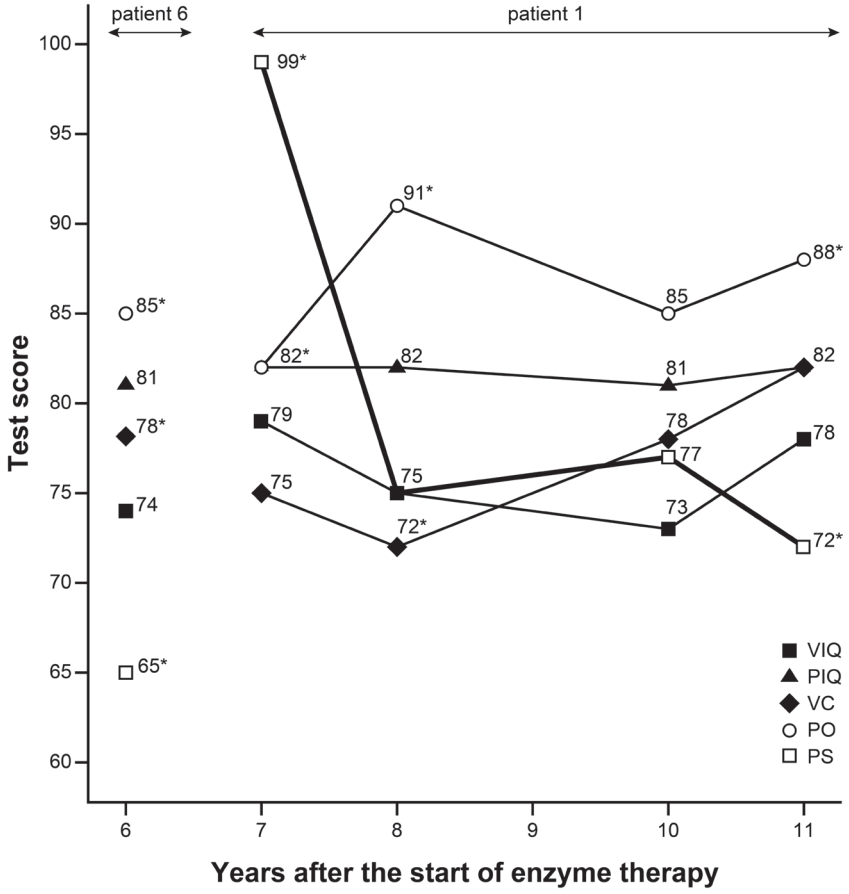
Cognitive development up to twelve years of age

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

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

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

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



TIQ = total intelligence quotient; VIQ = verbal intelligence quotient; PIQ = performance intelligence quotient; VC = verbal comprehension factor; PO = perceptual organisation factor; PS = processing speed factor. * = Significant differences between the subscales conform the Dutch norms at a given time point.[23]

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

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

Education

All five children aged 5 and older were attending school (patients 1, 2, 4, 6, and 7). One child attended a regular school (patient 7) and one child (patient 4) was educated at home, where she used an Internet link to attend classes at a special school for children with motor disabilities. Both patients fulfilled the curricular requirements for their age group, in accordance with their normal intelligence test score. The other three (patients 1, 2 and 6) needed special education, mainly because of their motor disabilities, but also because of their learning disabilities. The overall educational levels of patients 1, 2 and 6 were below their intelligence score. Patients 1 and 2 had specific learning disabilities in mathematics and language comprehension (patient 2). At age 5, patient 2, who was completely paralyzed and was only able to communicate with eye movements and grunting, received a speech computer. Her learning disabilities may have been at least partly attributable to her limited exposure to the world outside.

Parents' highest educational level

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

Adaptive skills

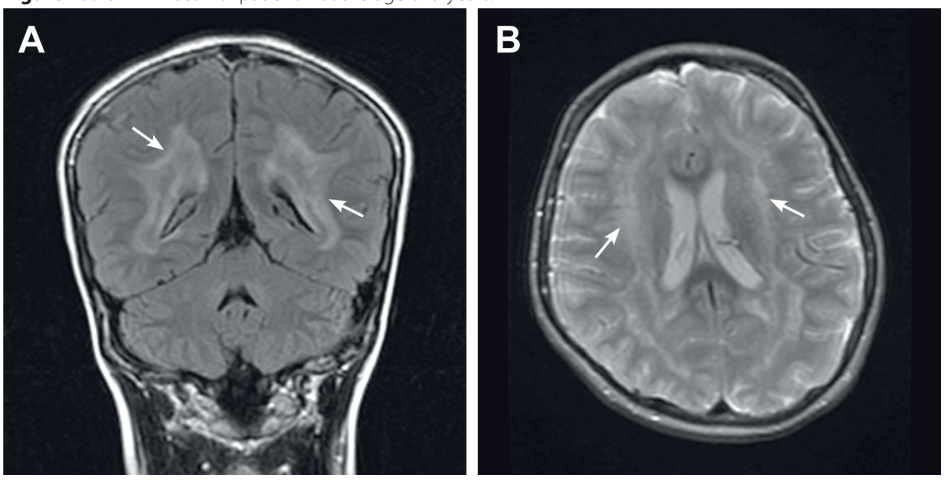
Except for the two tetraplegic patients, children had relatively normal adaptive skills. For example, they could eat independently (except for patients receiving tube feeding), have hobbies such as horse-back riding and swimming, go on errands to nearby shops, and make friends. Some children had difficulties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

Brain imaging

Ultrasounds of the brains were made of six patients at ages ranging from 9 days to 8 months (median age 2 months). Except for minor asymmetry of the lateral ventricles (patient 3) and a double contour in the right-sided choroid plexus (patient 2), there were no detectable abnormalities. One CT and five MRIs were made of the brains of five children between the ages of 6 months and 9 years (Table 1). No abnormalities were observed in the youngest patient at the age of 6 months (patient 2, MRI). Four patients showed periventricular white-matter abnormalities (MRI, patients 1, 3, 4, and 5) (Figure 2). Other observations included a thinner corpus callosum (patients 3 and 5, MRI), and white matter tract changes in the internal

capsula (patient 3, CT), the cerebral peduncles (patient 1, MRI), and the mesencephalon/pons area (patient 3, MRI). Patient 3 had hyperthermia during scanning.

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



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

DISCUSSION

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

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

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

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

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

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

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

Problems with hearing, speech and language should also be addressed when assessing cognitive functioning in these children. Earlier we reported that relatively many patients with classic infantile Pompe disease had hearing problems.[37] For optimal cognitive functioning, these patients' hearing should be tested regularly, and hearing aids should be fitted

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

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

Before ERT became available in 1999, children with classic infantile Pompe disease would die before age 1. Since then we have had a unique opportunity to study cognition in the longest survivors of this disease. Despite the strong evidence that glycogen stores in the brain of these children, the impact on the function of the CNS seems to have been limited. Here we show that cognitive development at school age ranged from normal to mildly delayed in our children with infantile Pompe disease, of whom the oldest was 12 years. Some children older than 5 had abnormalities in processing speed, which may be explained partly by mild white matter changes. Most developmental delays in young children were caused by muscle weakness and hearing and speech difficulties, making it easy to underestimate true levels of mental development. Because patients who started enzyme therapy early had the best motor outcome and the highest scores on early cognitive development, it is mandatory that enzyme therapy should start early.

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Chapter 3

WriteClick. Cognitive outcome of classic infantile Pompe patients receiving enzyme therapy.

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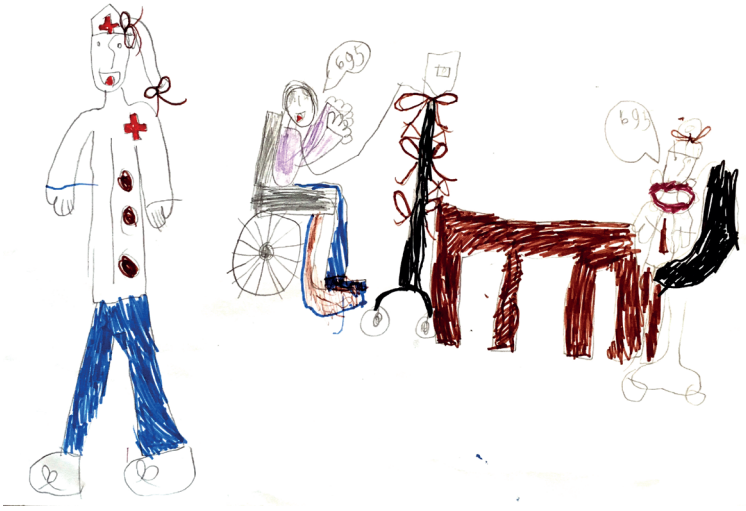
Neurology 2013;80;1173



WRITECLICK

G.A. Spiridigliozzi, J.H. Heller, P. S. Kishnani

We would like to inquire whether glycogen impairs cognitive function. In addition, since enzyme replacement therapy (ERT) does not cross the blood–brain barrier, does cognitive function decline over time? The data of Ebbink et al.[1] suggest that glycogen in the CNS does not significantly impair cognitive function. This finding is consistent with an earlier report of 13 infants with infantile Pompe who responded positively to the first year of ERT and showed stable cognitive function at the lower end of the normal range.[2] Additionally, stable function was reported within the lower end of the normal range for 7 children treated with long-term ERT for 6.75 years, on average.[3] Scores on a standardized measure of adaptive functioning, negatively affected by motor performance, were below IQ scores. Both research teams identified a particular weakness in processing speed and the need for developmental and educational support services, long-term cognitive surveillance, and neuroimaging studies.

AUTHOR RESPONSE

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Our study[1] confirmed Spiridigliozzi et al.[2,3] findings of normal or mildly delayed IQ scores in children with classic infantile Pompe disease treated with enzyme replacement therapy (ERT) (Ebbink et al.[1] submitted July 2011; revisions accepted January 17, 2012). This is important because ERT cannot cross the blood-brain barrier. The longest follow-up reported by Spiridigliozzi et al.[2] was 9 years and 11 months; ours was 12 years and 3 months.[1]

Though Spiridigliozzi et al.[3] found a correlation between cognitive and motor development, test limitations prevented them from determining whether lower cognition was caused by motor disabilities or weak cognition. Our more suitable nonmotor intelligence tests of two tetraplegic teenagers revealed the influence of severe motor disabilities on developmental scores. These teenagers had had the lowest possible mental development scores during their first 4 years, but now scored normal or mildly delayed.

Similar to the findings of Spiridigliozzi et al.,[2] we also found delayed processing speed. Conceivably, these delays are explained by white-matter changes like those on the MRIs we reported.

Although mild delays may develop over time, infantile Pompe disease differs substantially from other lysosomal storage diseases, where progressive storage in the CNS and profound mental retardation occur at an early age.

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Chapter 4

Cognitive decline in classic infantile Pompe disease: an underacknowledged challenge.

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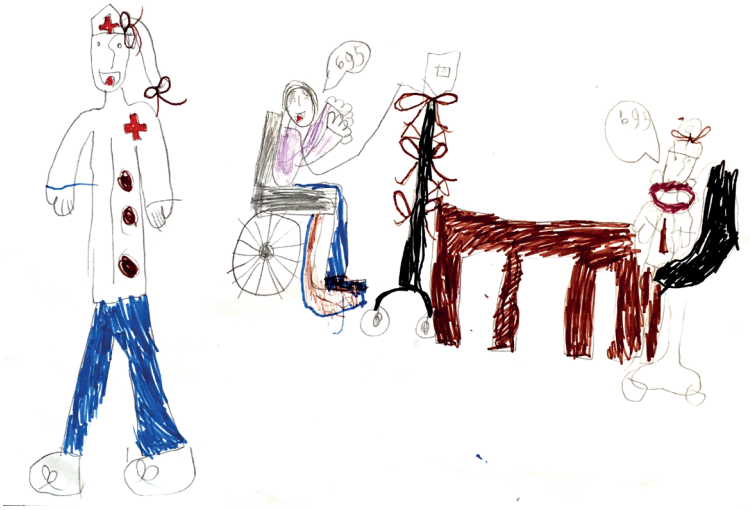


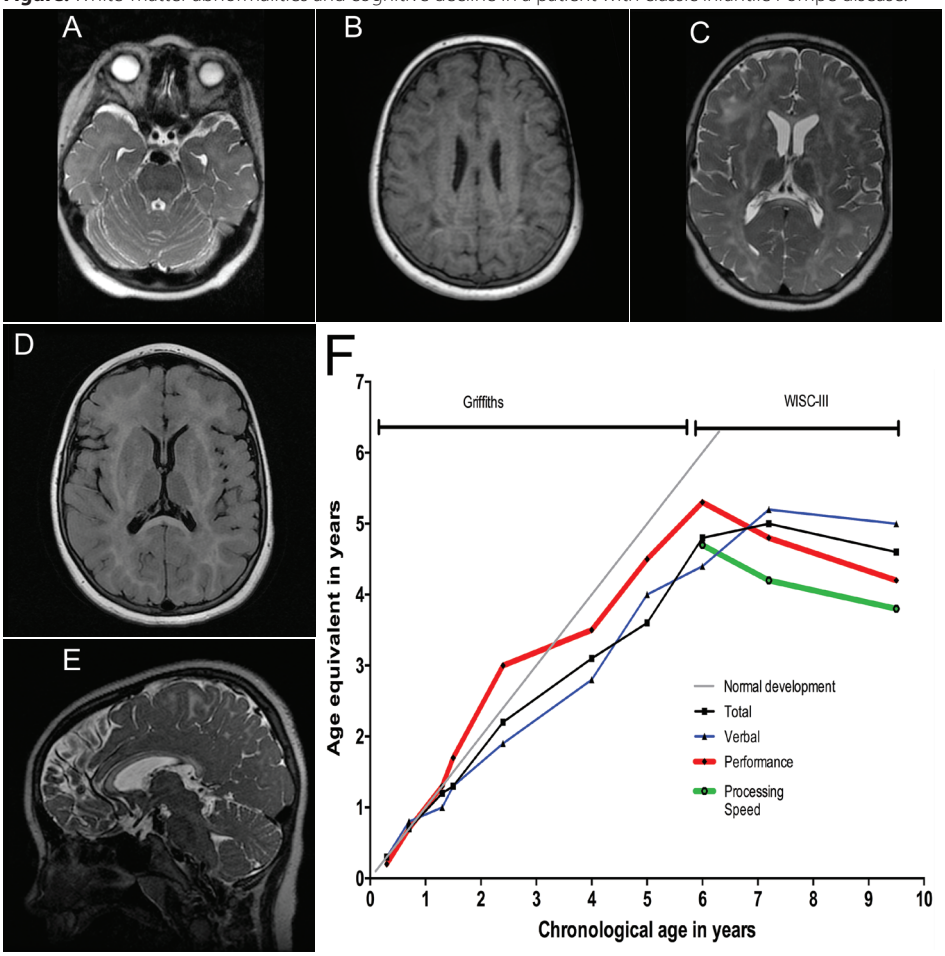
CASE REPORT

Classic infantile Pompe disease is a progressive lysosomal glycogen storage disorder, which, if untreated, leads to severe skeletal muscle weakness, inability to achieve any motor milestones, and death in the first year. In 1999, we reported the first successful use of enzyme replacement therapy (ERT).[1] Since then, patients' outcome with respect to survival, cardiac function, and motor performance has improved significantly. The oldest patient is currently 16 years old. Although glycogen storage occurs in the brain as well, and ERT cannot pass the blood-brain barrier, so far clinical studies show normal to mildly delayed cognitive development.[2,3]

A nine-year-old patient with classic infantile Pompe disease presented with increasing behavioral problems. He had presented at birth with persistent oxygen need and a hypertrophic cardiomyopathy (cardiac ultrasound left ventricular mass index 176.7 gr/m2; Z-score 16.7). At five weeks of age, we confirmed the diagnosis. Blood tests showed an alpha-glucosidase deficiency in leukocytes and two mutations in the *GAA* gene (c.525delT and c.1933G>T, cross-reactive immunologic material-positive on immunoblotting, highest measured antibody was low, titer 1:6,250). Immediately after his diagnosis, he started ERT at a dose of 20 mg/kg every two weeks. The cardiac hypertrophy disappeared (age 9.9 months) and he learned to walk (age 16.8 months). He had moderate hearing loss of 50-70 dB compensated sufficiently by hearing aids. Between three and five years of age, he developed signs of residual muscle weakness such as ptosis, an extraocular motility disorder, and weakness of the dorsal flexors of the feet, hands, and finger extensors. Therefore we increased his ERT dose to 40 mg/kg weekly at age 5.5 years. From age six he experienced difficulties with walking. At age nine, he could walk short distances without support.

Neuropsychological development was normal to mildly delayed until age six, as described previously.[2] At age nine years, we repeated the neuropsychological tests due to behavioral problems, like uncorrectable teasing and wanton behavior at school. These tests showed scores in the range of moderate intellectual disability (Wechsler Intelligence Scale for Children-III total IQ 48), with a specific decline in his processing speed and on the performance intelligence. Brain MRI (Figure) demonstrated severe symmetrical white matter abnormalities extending deep into the subcortical white matter also involving the capsula interna and externa. Additional biochemical analyses ruled out other neurodegenerative or leucodystrophic disorders. In retrospect, we conclude that a mismatch between the cognitive expectations of the patient's surroundings and his own cognitive abilities explained this child's behavioral problems.

Figure. White-matter abnormalities and cognitive decline in a patient with classic infantile Pompe disease.



From top left to bottom right: (***A)*** axial T2 (3 mm), (***B)*** axial T1 (3mm), (***C)*** axial T2 (3 mm), (***D***) axial T2 fluid-attenuated inversion recovery (4 mm), and (***E***) sagittal T2 (3mm) show signal abnormalities of the white matter, both periventricular and subcortical, with sparing of the U-fibers, and with involvement of the capsula interna, externa, claustrum, corpus callosum, and coriticospinal tracts in the brainstem. The cerebellar peduncle superior and the decussation at the mesencephalon level are also involved. (***F***) Decline in cognitive development in a patient with classic infantile Pompe disease. Griffiths = Griffiths Mental Development Scales; WISC-III = Wechsler Intelligence Scales for Children, Third Edition, Dutch version; Total = Total Intelligence; Verbal = Verbal Intelligence; Performance = Performance Intelligence; Processing Speed = Processing Speed Index.

DISCUSSION

Earlier reports on the occurrence of periventricular white matter abnormalities in classic infantile Pompe disease[2,4,5] suggested that these abnormalities were restricted and fairly stable over time,[2,4] and that cognitive development in school-aged children ranged from

normal to mildly delayed.[2,3] Extensive white matter changes were reported once in literature with a cognitive follow-up limited to 44 months. This patient had stable developmental delay (Bayley at 32 months developmental age of 22 months, Snijders-Oomen nonverbal intelligence test at 44 months showed developmental age of 35 months).[5]

Halting of cognitive development combined with extensive white matter abnormalities as found in our patient is a potential new feature of classic infantile Pompe disease. The deterioration of the processing speed and performance intelligence suggests that the process in the white matter might have been progressive in this moderately affected patient. We want to alert physicians that the consequence of the glycogen storage in the brain on cognition may be larger than expected before. We therefore advice to monitor cognitive development closely in classic infantile Pompe disease. This underacknowledged challenge of the brain may have important consequences for the development of next-generation therapeutic strategies that are currently under development.

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Chapter 5

Classic infantile Pompe disease: As pioneering patients approach adulthood, the next puzzle is the brain.

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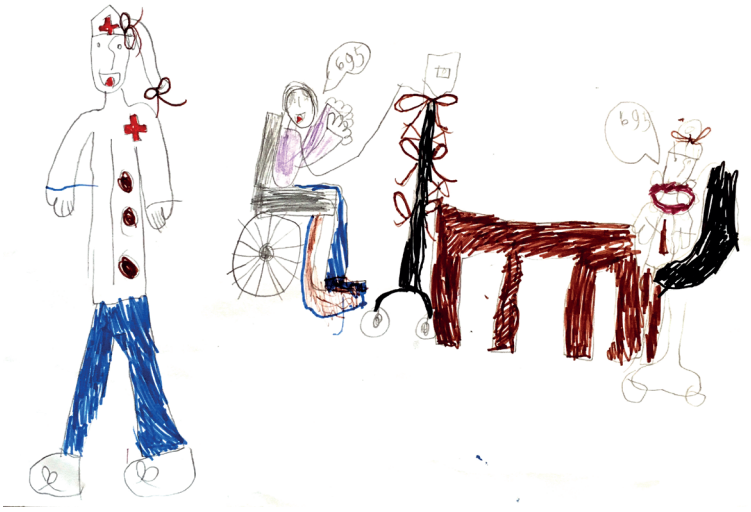


ABSTRACT

Objection

Although the development of enzyme replacement therapy (ERT) for classic infantile Pompe disease has drastically improved patients' prospects, the long-term consequences of glycogen storage in the CNS are still unknown.

Methods

Using neuropsychological tests and brain MRIs, we prospectively assessed a cohort of 11 classic infantile Pompe patients aged up to 17 years.

Results

From approximately age 2 onwards, brain MRIs showed involvement of the periventricular white matter and centrum semiovale. After age 8, additional white-matter abnormalities occurred in the corpus callosum, internal and external capsule, and subcortical areas. From age 11, white-matter abnormalities were also found in the brainstem. Although there seemed to be a characteristic pattern of involvement over time, there were considerable variations between patients, which are reflected by variations neuropsychological development. Cognitive development ranged from stable and normal to declines that lead to intellectual disabilities.

Conclusions

As our patient population with classic infantile Pompe disease matures towards adulthood (as a result of long term treatment with ERT), white-matter abnormalities are becoming increasingly evident, affecting the neuropsychological development. In our view, the central nervous system should be an additional target in the development of next-generation therapeutic strategies.

INTRODUCTION

Classic infantile Pompe disease is a progressive inheritable myopathy caused by a deficiency of the lysosomal enzyme alpha-glucosidase. This leads to an accumulation of glycogen that mainly affects skeletal muscles and the heart. As patients have severe mutations in the GAA gene, enzyme activity is less than 1% in cultured fibroblasts. Characteristically, they present before the age of six months with a hypertrophic cardiomyopathy, progressive generalized muscle weakness, and respiratory problems. Untreated infants die before the age of one year [1]. In 1999, the first patients with classic infantile Pompe disease were treated with recombinant human alpha-glucosidase. In 2006 ERT (enzyme replacement therapy) was registered [2]. Over the years, ERT has been demonstrated to significantly improve survival, cardiac, and motor outcome [2-5]. The first surviving infants treated in our center are now on the threshold of adulthood.

One limitation of ERT is that it cannot pass the blood-brain barrier. However, small amounts of glycogen also store in the brain [6-12]. To date, studies on the consequence of this storage have been limited. Reported intelligence ranged from normal to mildly delayed. Early development was easily underestimated if motor functioning was poor [5, 13-15]. Brain MRIs (magnetic resonance imaging) showed predominantly periventricular white-matter abnormalities [13, 16-18].

As no studies have related long-term neuropsychological follow-up results to the brain abnormalities in patients up to adulthood, we had two objectives: (i) to use brain MRI and neuropsychological tests to study the long-term consequences of glycogen storage on the CNS, and (ii) to relate imaging results of to our findings on neuropsychological functioning in the oldest surviving patients.

METHODS

Patients

ERT with recombinant human alpha-glucosidase started with four patients in 1999. For the current follow-up study on the effects of this therapy we included the 11 oldest patients (start of ERT before 2009). Four initially received recombinant human alpha-glucosidase form rabbit milk. Since 2003 all patients were treated with ERT derived from CHO cells. The dose ranged from 20 mg/kg every other week to 40 mg/kg/week. Inclusion criteria were: GAA activity of <1%; severe mutations in the GAA gene; start of symptoms before six months; and hypertrophic cardiomyopathy at diagnosis.

Study protocols had been approved by the Institutional Review Board. Written informed consent was obtained from the children's parents.

MRI

We performed magnetic resonance imaging (MRI) of the brain at least once per patient using a 1.5T system, or a 3 T system (EchoSpeed; GE Healthcare, Milwaukee, WI), and a dedicated 8-channel head coil. MRIs were scanned according to a standardized protocol including T1-weighted, T2-weighted, and FLAIR images. MRIs were scored by assessing white matter changes in several anatomical regions, including (i) the supratentorial region: frontal and occipital periventricular white matter, the centrum semiovale, corpus callosum, external capsule, posterior and anterior limb of the internal capsule (PLIC and ALIC); (ii) subcortical white matter, and U-fibers, and (iii) the infratentorial region: decussation, and cortical spinal tract at the brain-stem level. We also assessed ventricle size (lateral ventricles and fourth ventricles), and abnormal signal intensity and/or volume loss of the nucleus dentatus, basal nuclei and thalami. All MRIs were rated by two independent evaluators: a pediatric neuro-radiologist (M.H.L), and a pediatric neurologist (J.M.P. vd H.). If opinions diverged, consensus was reached after deliberation.

Neuropsychological functioning

As early developmental tests in classic infantile Pompe patients can be highly influenced by motor functioning, [13, 14] we focused on the neuropsychological test in patients over the age of five years. Patients underwent regular neuropsychological assessments. These were intended to assess the following:

1. Age five years: early development: Griffiths Mental Developmental Scales
2. From six years onwards: intelligence; the most recent Dutch version of the Wechsler Intelligence Scales for Children – Third edition (WISC-III). The WISC-III-NL is divided into two scales or into three factors. The scales are verbal intelligence and performance intelligence. The factors are verbal comprehension, performance organization, and processing speed. For tetraplegic children, we used the Raven Progressive Matrices.

In a subgroup of patients we tested memory (Rey Auditory-Verbal Learning Test (RAVLT), Memory for Designs (NEPSY - Second Edition), language (Boston Naming Test, Comprehension of Instructions (NEPSY-II)), attention (Dot Cancellation Test, Inhibition (NEPSY-II)), executive functioning (Verbal Fluency (NEPSY-II), Digit Span (WISC-III-NL)), visual spatial functioning (Geometric Puzzles (NEPSY-II), Design Copying (NEPSY-II), Rey Complex Figure Test) processing speed (Processing Speed Index (WISC-III-NL)), behavior (Child Behavior Checklist (CBCL)). All tests were administered in their most recent normed and validated Dutch versions at time of assessment. The children were assessed by two pediatric neuropsychologists (F.A.; B.E). Two patients were tested outside our hospital, once each. One patient due to an MRSA infection. This patient was tested with the most recent Dutch

version of the Wechsler Non-verbal Scales (WNV-NL). The other patient due to initiation of therapy in Germany (assessed with the most recent Dutch version of the Snijders Oomen Nonverbal Intelligence test-Revised (SON-R 2½-7)). As these two time-points were important to determine development over time, we decided to include these into the data set.

Statistics

Patients' test results were compared with the normative data for the Dutch population. The mean score for the intelligence tests is 100, with a standard deviation (SD) of 15 points. An Intelligence Quotient (IQ) above 85 indicates normal development, a score between 84-70 indicates mild developmental delay, and a score below 70 indicates intellectual disability. A disharmonic intelligence profile was defined according to the appropriate table of the instruction manual (using the 95% confidence interval). A significant decline in IQ was defined as a loss of more than 30 IQ points (>2 SD) or a decline in raw scores, and a declining tendency as a loss of more than 15 IQ points (>1 SD) [19]. For purposes of comparison, all neuropsychological tests were converted into Z-scores**.** A neuropsychological test score of more than 1.5 SD below a child's total IQ represents a weaker domain [19].

RESULTS

Patients

Table 1 summarizes the patient characteristics. The current age of the patients ranged from 7.6 to 17.7 years. Motor outcome varied. Six of the eleven patients achieved the ability to walk, two of whom were still able to do so at the last evaluation. Three patients had minimal motor functioning and were respirator dependent. Three patients died at the respective ages of 4.3, 4.4 and, 15.6 years.

Brain MRI

In total, 21 brain MRIs were performed in 11 patients. The patients' ages at the time of MRI ranged from 0.6 – 17.1 years. Five patients (age range 0.6 – 8.5 years) had one MRI. Six patients (age range 2.7 – 17.1 years) had two to four MRIs. The interval between the first and last MRI was 1.8 - 8.7 years. Table 2 presents the scores of all brain MRIs.

At all ages, all patients had white-matter abnormalities in the centrum semiovale.

Hyperintensities in the frontal and occipital periventricular areas started to appear at the age of 2.7 years. A tigroid hyperintensity-pattern developed in the occipital region at various ages.

Table 1. Patient characteristics

Patient	Age at start of ERT	Current age	Invasive ventilation (age onset)	Mutations	CRIM status	Last LVMI Z-score <2	Hearing aids #	Impaired vision #	Best motor milestone	Last motor function
1	0.3	17.4	-	c.2481+102_2646+31del538 c.1799G>A	P	Yes	Yes	Yes	Walking	Walking
2	0.6*	17.7	0.6	c.1115A>T c.525delT	P	Yes	Yes	Yes	MMF	MMF
3	0.2	4.3†	2.1	c.525delT c.525delT	N	No	Yes	No	Sitting	Sitting
4	0.7*	17.7	0.9	c.1913G>T c.1548G>A	P	No	Yes	Yes	MMF	MMF
5	0.2	4.4†	2.0	c.2741delinsCAG c.2741delinsCAG	N	Yes	Yes	No	Walking	Sitting
6	0.1	11.8	-	c.del525T c.1933G>T	P	Yes	Yes	Yes	Walking	Sitting
7	0.04	11.3	-	c.2481+102_2646+31del538 c.2481+102_2646+31del538	P	Yes	Yes	Yes	Walking	Walking
8	0.001	9.0	2.7	c.1460T>C c.1460T>C	P	Yes	No	Yes	Walking	Sitting
9	0.2	8.6	-	c.525delT c.2481+102_2646+31del538	P	Yes	Yes	Yes	Standing	Sitting
10	0.2	7.6	-	c.2481+102_2646+31del538 c.2481+102_2646+31del538	P	Yes	Yes	Yes	Walking	Sitting
11	0.2	15.6†	3.2	U	U	Yes	Yes	Yes	Sitting	MMF

All ages are stated in years (see also Ebbink et al., 2012); ERT = enzyme replacement therapy; CRIM = cross-reactive immunological material, LVMI = left ventricle mass index, P = positive; N= negative; U = unknown; MMF = minimal motor function;

†= deceased;

* = Patients 2 and 4 started therapy in the end stage of the disease; both had minimal motor function, and became ventilator dependent before the age of one year.

Hearing and vision were sufficiently compensated at time of neuropsychological testing.

After the age of 8.8 years (11 MRIs, five patients) MRIs started to show additional white-matter abnormalities in the subcortical areas, with sparing of the U-fibers (all patients and all MRIs), and with involvement of the external capsule (5/5 patients, 10/11 MRIs), of the PLIC (4/5 patients, 8/11 MRIs), and of the corpus callosum (4/5 patients, 9/11 MRIs). In the corpus callosum, hyperintensities were first observed in the splenium, and later in the genu and truncus. Abnormalities in the basal ganglia were found in three out of these five patients.

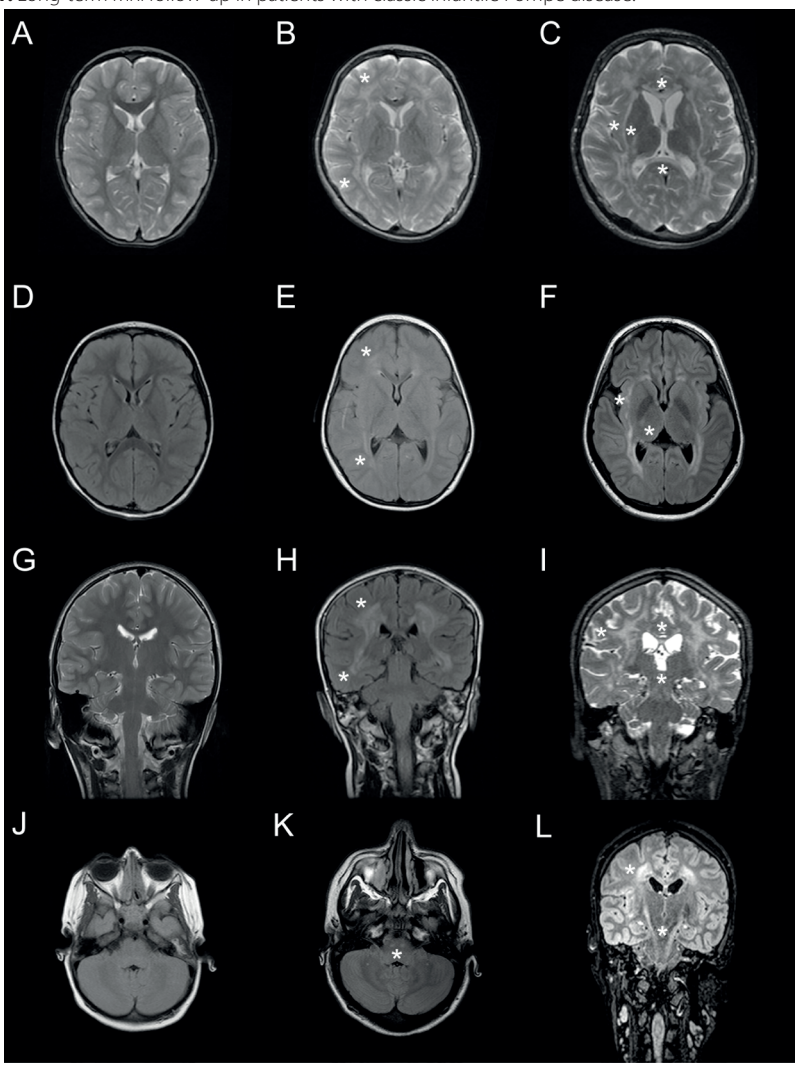
Table 2. Results of the standardized scoring of MRI abnormalities in patients with classic infantile Pompe disease.

Patient	Age	PWM centrum semiovale	PWM frontal/ occipital	Corpus callosum	PLIC	ALIC	External capsule	Subcortical	U fibers	Deccusation	Corticospinal	Widened lateral ventricles	Widened 4th ventricle	Basal ganglia
2	0.6	1	0	0	0	0	0	0	0	0	0	0	0	0
5	1.5	1	0	0	0	0	0	0	0	0	0	0	0	0
10	2.7	1	1	0	0	0	0	0	0	0	0	1*	0	0
3	4.3	1	1	"1"	1	0	0	0	0	0	0	1	0	0
4	5.1	1	1	0	0	0	0	0	0	0	0	0	0	0
10	5.5	1	1	0	0	0	0	0	0	0	0	1*	0	0
4	5.8	1	1	0	0	0	0	0	0	0	0	0	0	0
10	7.3	1	1	0	0	0	0	0	0	0	0	1*	0	0
9	7.5	1	1	0	0	0	0	0	0	0	0	0	0	0
8	8.5	1	1	0	0	0	"1"	0	0	0	0	0	0	0
4	8.8	1	1	0	0	0	1	"1"	0	0	0	0	0	0
7	9.1	1	1	1	1	0	1	"1"	0	0	1	0	0	0
6	9.5	1	1	1	1	0	1	1	0	1	0	0	0	1
1	9.8	1	1	1	0	0	0	1	0	0	0	"1"	0	0
7	11.1	1	1	1	1	0	1	1	0	0	1	0	0	0
6	11.3	1	1	1	1	0	1	1	0	1	1	"1"	0	1
11	13.7	1	1	1	1	0	1	1	0	1	1	1	1	1
4	13.8	1	1	0	0	0	1	"1"	0	1	1	1	0	0
11	15.3	1	1	1	1	0	1	1	0	1	1	1	1	1
1	15.5	1	1	1	1	0	1	1	0	1	0	1	0	1
1	17.1	1	1	1	1	0	1	1	0	1	1	1	1	1

Age = age in years. PWM = periventricular white matter, PLIC = posterior limb of internal capsule, ALIC = anterior limb of internal capsule, *left ventricle. The shades of grey reflect the severity of involvement: from light (restricted) to dark (widespread). 0 = normal, "1" = slightly abnormal, 1 = abnormal.

From the age of 11.3 years onwards (four patients, six MRIs), hyperintensities were noticed in the decussation (4/4 patients, 6/6 MRIs) and the corticospinal tracts (4/4 patients; 5/6 MRIs). In addition, all patients had widening of the lateral ventricles (4/4 patients; 6/6 MRIs). The fourth ventricle was widened in only two patients (2/4 patients; 3/6 MRIs). To illustrate the progressive nature of brain involvement in classical infantile Pompe disease, figure 1 shows the sequential MRIs of the two patients with the longest follow-up.

Figure 1. Long-term MRI follow-up in patients with classic infantile Pompe disease.



A. Axial T2-weighted image of a healthy control subject aged 6 years ***B.*** Axial T2-weighted image of patient 1, aged nine years. The asterisk indicates periventricular white-matter abnormalities. ***C.*** Axial T2-weighted image of patient 1, aged 17 years. The asterisk indicates additional involvement of the capsula externa, posterior limb of internal capsule (PLIC) and corpus callosum. Note that the ventricles are also mildly widened. ***D.*** Axial Flair-weighted image of a healthy control subject, aged 6 years. ***E.*** Axial Flair-weighted image of patient 4, aged 5 years. The asterisk indicates periventricular white-matter abnormalities. ***F.*** Axial Flair-weighted image of patient 4, aged 13 years. Asterisk shows the spread of white-matter abnormalities towards the capsula externa. The PLIC is slightly involved as well. ***G.*** Coronal T2-weighted image of a healthy control subject aged 9 years. ***H.*** Coronal Flair-weighted image of patient 1 (aged 9 years), showing involvement of the periventricular white matter. ***I.*** Coronal reformatted T2-weighted image of patient 1, aged 17 years. Asterisk indicates spread towards the subcortical areas and corticospinal tracts. ***J, K, L*** Two transversal images and one coronal Flair-weighted image of patient 4 at the ages of 5 years (J) and 13 years (K,L). Image K and L show involvement of the corticospinal tracts, which is not seen on image J.

Intelligence

Supplemental table 1 (see below) presents the total IQ scores and the scores on subscales of all patients from the age of five years onwards. A total of 34 intelligence tests were performed in nine patients. The age at the latest assessment ranged from 6.0 to 16.2 years. The total intelligence scores over the period ranged from <45 to 121.

Due to the various ages and varying degrees of motor disability, it was not possible to test all patients with the same instrument. Two tetraplegic patients were tested with the RAVEN (patient 2 at ages five, ten and 13 years; patient 4 at ages 12 and 14 years).

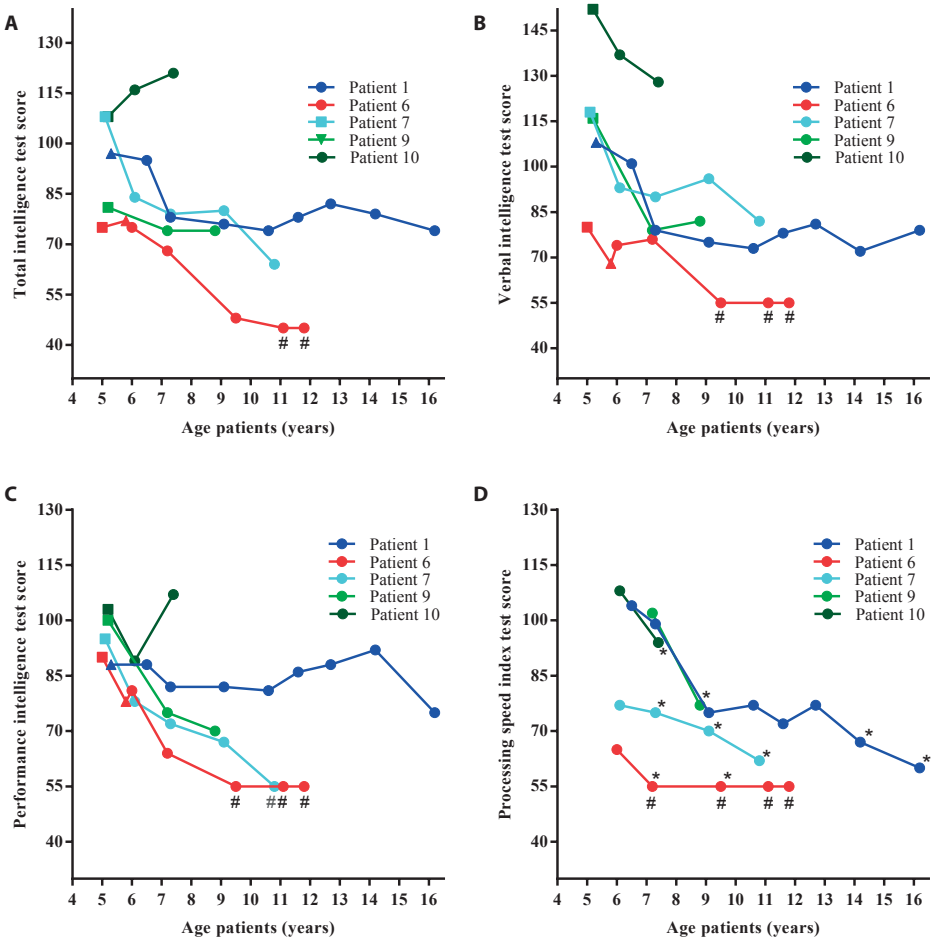
During follow-up, development was stable in both; one had a normal intelligence and the other a mild developmental delay. Two other patients (patients eight and eleven) were tested with the Snijders Oomen Nonverbal Intelligence Test - Revised and the Wechsler Nonverbal Scale of Ability (age six and seven years). Their total intelligence scores lay in the range of intellectual disability.

Five patients (patients 1, 6, 7, 9, and 10) underwent multiple testing with the WISC-III-NL between the age of 5 and 16 years. Figure 2 shows the total IQ (TIQ); total verbal (VIQ); total performance IQ (PIQ); and processing-speed index (PSI) over time. The TIQ, VIQ and PIQ of the two youngest patients remained stable over time (patient 9 and 10); one patient had a normal development and the other a mild delay. The three patients with the longest follow up declined in their IQ-scores. Patient 1 had a declining tendency in TIQ and VIQ from normal to mildly delayed. Patient 6 had a significant decline in TIQ and a declining tendency on VIQ and PIQ from mildly delayed to intellectual disability. Patient 7 had a declining tendency on TIQ and PIQ from mildly delayed to intellectual disability. All five patients declined significantly in their processing-speed index. Patients 1, 7 and 10 had disharmonic profiles at the age of 6 years (VIQ > PIQ). Patient 1 became harmonic after a decline in VIQ, and patients 7 and 10 remained disharmonic.

Follow-up MRI and IQ (patients 1, 6, 7. 9, 10)

To study the relationship between MRI and IQ, the IQs of the patients assessed with the Griffiths and WISC were related to brain MRIs performed at similar ages (time between MRI and IQ assessment < 1 year, range 0.5-9 months). In patients with involvement of the centrum semiovale and periventricular white matter only, intelligence was normal to mildly delayed (patients' ages 2.7 - 7.5 years). In those with additional white-matter abnormalities, the TIQ scores indicated problems ranging from mild developmental delay to intellectual disabilities (ages of patients 9 - 17 years). In these patients, a slight increase in white-matter involvement co-occurred with a declining tendency in their TIQ and PIQ (patient 7), VIQ (patient 6), and PSI (patients 1, 6 and 7).

Figure 2. Intelligence-test scores of patients with classic infantile Pompe disease between the ages of 5 and 16 years.



▲ = SON-R 2 ½ -7; ■ = Griffiths Mental Developmental Scales; ● = WISC-III-NL.
* = decline in raw scores in processing-speed index; # = floor effect.

Additional neuropsychological domains

Additional neuropsychological evaluations were administered in seven patients above the age of five years. Attention and visual-spatial integration were tested in the five patients who were able to perform the WISC III-NL (patients 1, 6, 7, 9 and 10). All five patients had problems in their sustained attention, but not in their selective attention. Visual-spatial integration problems were found in three patients (3/5).

Working memory, memory, language, and executive functioning were tested in six patients. These tasks, most of which were verbal, could also be performed in one fully tetraplegic patient. Working memory was abnormal in five patients (5/6). Two patients had memory

problems (2/6). No specific problems were found with regard to language (0/6) and executive functioning (0/6). The Child Behavior Checklist was administered in the parents of three patients. Social problems were found in two of these three patients.

DISCUSSION

With now over 17 years of experience with ERT in patients with classic infantile Pompe disease insights in long-term outcome are gradually expanding. As a result of enzyme replacement therapy, survival and motor performance have improved significantly, and cardiac hypertrophy has resolved in most patients. One of the limitations of limitations of ERT is that it cannot cross the blood brain barrier. With time our maturing patient population shows that the disease is not only a muscle disease, but also affects the brain.

Brain

We noted a characteristic three-stage pattern of white-matter involvement that evolves from periventricular to subcortical and from superior to inferior. For explicatory reasons, we subdivided the process, which is likely to be gradual, in three stages. In stage one (starting around age two), all the patients we evaluated had periventricular white-matter involvement at the level of the centrum semiovale. In stage two (from age eight onwards) the white-matter abnormalities expanded to the subcortical areas and internal and external capsule. In stage three (from age 11 onwards), infratentorial white-matter areas also became involved.

Although there seemed to be a characteristic pattern of involvement over time the extent of the abnormalities, and the rate of progression from stages one to three varied between patients. For example, brain MRIs in five patients aged between 8.5 and 9.8 showed that some had more abnormalities than others at the same age. Brain involvement appeared to be independent of motor functioning. For example, one patient who learned to walk within the normal age limits showed more abnormalities on the MRI than a patient who became tetraplegic before the age of one.

On the basis of all of these findings we conclude that the white-matter abnormalities on MRI in classic infantile Pompe patients indicate a varied but slowly progressive pattern of white-matter involvement.

This finding is new. To date, predominantly periventricular white-matter abnormalities were described that are consistent with our stage one [13, 16, 17, 20]. The lack of description of progression towards more extensive white-matter involvement in earlier reports is explained by

the absence of reports on MRI in children after the age of nine years, which is when we observed progression towards stages two and three.

Nonetheless, we note variations in the rate at which the white-matter affliction progresses. These explain the three cases reported (one by our own group) that indicate more extensive white-matter disease at a relatively young age (similar to stage two) [21-23].

Neuropsychological profile

The variations in brain involvement are reflected by the wide range of intelligence-test scores, i.e., from normal intelligence to intellectual disabilities. This range is now wider than in earlier studies. The development over time varies from stable to neuropsychological decline. These findings contrast with those of our previous study and of four other studies on intelligence in classic infantile Pompe disease, where cognitive development was stable, ranging from normal to mildly delayed [13, 14, 16, 24]. Only one case report on a four-year old patient and a recent case report on a nine-year-old expressed concerns about 'a not yet fully described CNS phenotype' [22].

Prompted by the potential effect of the disease on intellectual performance shown by MRI, we performed additional neuropsychological tests, whose results showed a consistent neuropsychological profile. Due to visuospatial problems, patients appeared to be at risk of a disharmonic intelligence profile. All patients also had a lower processing speed and problems in their sustained attention, and several had problems with their working memory and social interaction.

The question remains whether neuropsychological outcome can theoretically be related to the white-matter involvement we found in our Pompe patients. Firstly, the white-matter abnormalities in our patients included the frontal and parietal areas. A recent conceptual model related intelligence to the interaction between the prefrontal and parietal cortex, which relies greatly on its white-matter connectivity [25]. This fronto-parietal integration network is believed to be involved not only in intelligence but also in other neuropsychological functions, such as attention, working memory, and processing speed [19]. As the white-matter abnormalities in our patients develop within this network, it is conceivable that intelligence and these specific neuropsychological functions are affected in our patients.

A limitation of our study is that the group of patients is relatively small although it can be considered to be large for a rare disease like classic infantile Pompe disease. A strength of the study is that we could include data from the four patients who were pioneered ERT in 1999 and of whom 3 are still alive and 7 others that started ERT before 2009.

CONCLUSION

As our patient population with classic infantile Pompe disease matures into adulthood, knowledge of this initially fatal muscle disease is broadening. As it seems the brain is now becoming the next puzzle in the treatment. We advise to expand follow-up programs to capture potential CNS/brain involvement in other populations and also to include the brain as an additional target in the development of next-generation therapeutic strategies for classic infantile Pompe disease.

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Supplemental Table 1. Subtest scores of intelligence tests in patients with classic infantile Pompe disease

Patient \ Age		5	5.8	6	7	9	10	11	12	13	14	16
1	TIQ	97[a]		95	78	76[e]	74	78	82		79[e]	74
	TPIQ	88[a]		88	82	82[e]	81	86	88		92[e]	75
	POI				82	91[e]	85	93	93			82
	TVIQ	108[a]		101	79	75[e]	73	78	81		72[e]	79
	VCI				75	72[e]	78	83	80			80
	PSI			104	99	75	77[e]	72	77		67[h]	60[h]
2	TIQ	80[d]					76[d]			75[d]		
3	TIQ											
4	TIQ								92[d]		93[d]	
	TVIQ								87[e]		93[e]	
5	TIQ											
6	TIQ	75[g]	77[a]	75	68	48[f]	<45[f]	<45[f]				
	TPIQ	90[g]	78[a]	81	64	<55[f]	<55[f]	<55[f]				
	POI			85	71	49	<48	<48				
	TVIQ	80[g]	68[b]	74	76	<55[f]	<55[f]	<55[f]				
	VCI			78	80	56	<54	<54				
	PSI			65	55[h]	<55[f,h]	<55[f]	<55[f]				
7	TIQ	108[g]		84[e]	79	80	64					
	TPIQ	95[g]		78[e]	72	67	<55[f]					
	POI			83[e]	79	71	53					
	TVIQ	118[g]		93[e]	90	96	82					
	VCI			93[e]	92	97	80					
	PSI			77	75[h]	70[h]	62[h]					
8	TIQ				65[c]							
9	TIQ	81[g]			74	74						
	TPIQ	100[g]			75	70						
	POI				73	73						
	TVIQ	116[g]			79	82						
	VCI				82	88						
	PSI				102	77[h]						
10	TIQ	108[g]		116	121							
	TPIQ	103[g]		89	107							
	POI			85	112							
	TVIQ	152[g]		137	128							
	VCI			144	128							
	PSI			108	94[h]							
11	TIQ			54[a]								

Age = age in years, TIQ = total intelligence test score, TPIQ = total performance intelligence, POI = perceptual organization index, TVIQ = total verbal intelligence, VCI = verbal comprehension index, PSI = processing speed index. a = Sneijders Oomen Nonverbal Intelligence Test- Revised[26], b=WPPSI-III-NL (short version), VIQ, c = Wechsler Nonverbal Scale of Ability[27], d = Raven Progressive Matrices (short version)[28], e = short version of WISC-III-NL[27](based on 8 subtests), f= floor effect, g= Griffiths Mental Developmental Scales[29], h = decline in raw scores on PSI.

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Chapter 6

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

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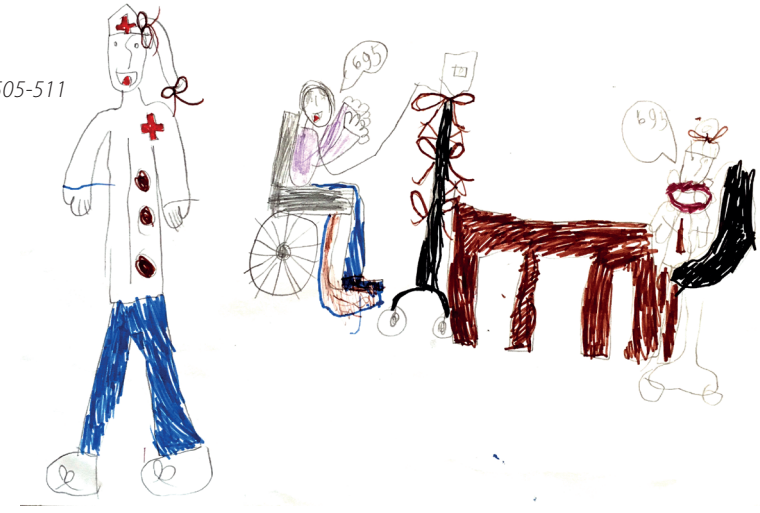
MM Hakkesteegt
PA van Doorn
IFM de Coo
AJJ Reuser
HHW de Gier
AT van der Ploeg

J Inherit Metab Dis 2012;35(3):505-511



ABSTRACT

Objective

Classic infantile Pompe disease is an inherited generalized glycogen storage disorder caused by deficiency of lysosomal acid α-glucosidase. If left untreated, patients die before one year of age. Although enzyme-replacement therapy (ERT) has significantly prolonged lifespan, it has also revealed new aspects of the disease.

Methods

For up to 11 years, we investigated the frequency and consequences of facial-muscle weakness, speech disorders and dysphagia in long-term survivors. Sequential photographs were used to determine the timing and severity of facial-muscle weakness. Using standardized articulation tests and fibreoptic endoscopic evaluation of swallowing, we investigated speech and swallowing function in a subset of patients.

Results

This study included 11 patients with classic infantile Pompe disease. Median age at the start of ERT was 2.4 months (range 0.1 - 8.3 months), and median age at the end of the study was 4.3 years (range 7.7 months - 12.2 years). All patients developed facial-muscle weakness before the age of 15 months. Speech was studied in four patients. Articulation was disordered, with hypernasal resonance and reduced speech intelligibility in all four. Swallowing function was studied in six patients, the most important findings being ineffective swallowing with residues of food (5/6), penetration or aspiration (3/6), and reduced pharyngeal and/or laryngeal sensibility (2/6).

Conclusions

We conclude that facial-muscle weakness, speech disorders and dysphagia are common in long-term survivors receiving ERT for classic infantile Pompe disease. To improve speech and reduce the risk for aspiration, early treatment by a speech therapist and regular swallowing assessments are recommended.

INTRODUCTION

Pompe disease (glycogen storage disease type II, acid maltase deficiency, OMIM # 232300) is a rare autosomal recessive lysosomal storage disorder caused by mutations in the gene-encoding acid α-glucosidase (EC 3.2.1.20).[1,2] Severe mutations cause complete enzyme deficiency, resulting in the classic infantile form of Pompe disease, which was first described by Pompe in 1932.[3] Symptoms are caused by glycogen accumulation, mainly in skeletal, cardiac and smooth muscle, but also in other tissues, including the central and peripheral nervous system. In the first months of life, patients present with progressive muscle weakness, hypertrophic cardiomyopathy, respiratory problems and feeding difficulties. If untreated, this leads to death before the age of one year.[4,5]

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

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

PATIENTS AND METHODS

Patients

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

Facial-muscle weakness

To examine the onset of facial-muscle weakness, we collected photographs of the face taken over a period of 24 months from the start of ERT. For this we used standardized photographs and videos taken every three months. The photographs were ordered arbitrarily

and evaluated independently by three neurologists. The evaluators stated whether facial-muscle weakness was present, and, whether it was mild or severe. Facial-muscle weakness was defined as an expressionless face with an open drooping or tent-shaped mouth.[17] To accept any judgement, the agreement of at least two evaluators was needed. If this was impossible, the evaluation was considered not to be applicable.

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

Speech and swallowing function

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

Speech

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

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

Swallowing function

The speech therapist obtained a feeding history from all parents.

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

screened for deviant anatomy, reduced pharyngeal squeeze, and impaired laryngeal function.

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

Associated clinical outcome measures

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

RESULTS

Patients

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

Facial muscle weakness

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

Between the ages of 1.0 and 15.0 months (Table 1, median 6.6 months), all patients developed evident signs of facial-muscle weakness, even when ERT was started very early. When such weakness was first observed, its severity in most patients (9/11) was considered to be mild. The main characteristics were sunken cheeks (8/11) and a drooping lower lip (9/11). While the absence of the nasolabial fold and forehead lines were difficult to judge, four patients clearly had diminished nasolabial folds. Only one patient had ptosis.

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

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

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

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

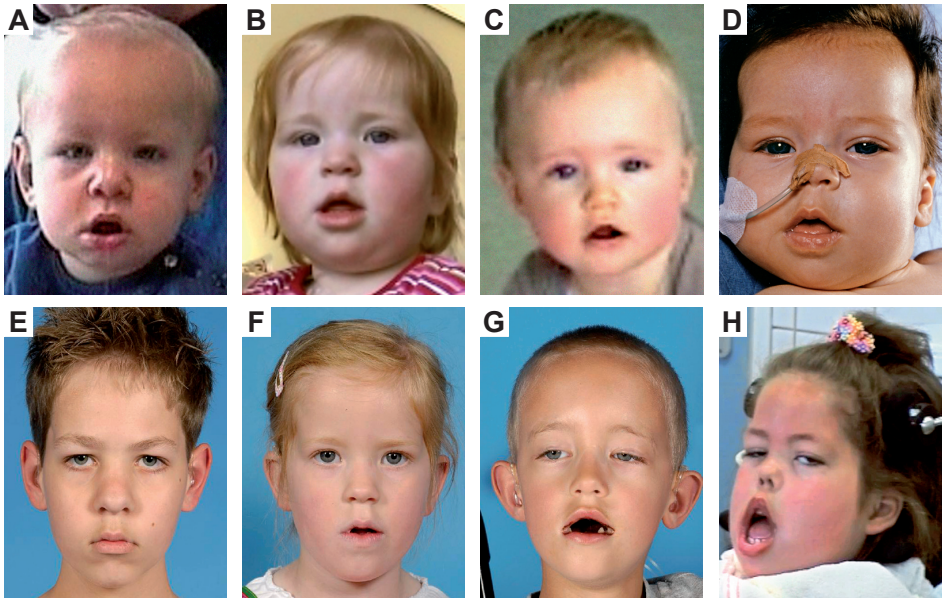
Speech and swallowing function

Speech

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

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

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



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

Swallowing function

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

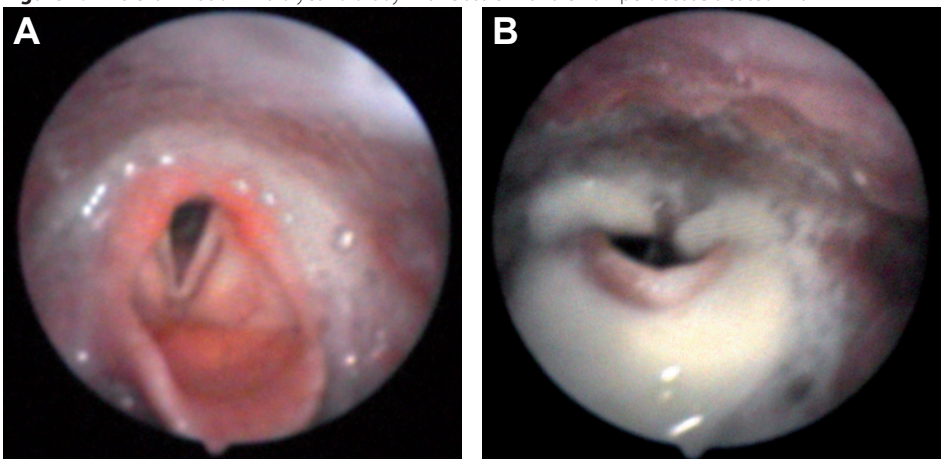
Fibreoptic endoscopic examinations of swallowing showed that five of the six patients had varying extents of dysphagia; only the youngest had no swallowing abnormalities. Reduced velopharyngeal closure was found in four patients, and caused nasal regurgitation in two. Although the anatomy of the swallowing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

Swallowing of various textures of food could be examined in four patients, whereas swallowing function of two patients who refused to eat during the examination (9 and 10) was evaluated on the basis of dry swallows. In all patients with insufficient muscle contraction of the pharynx (5/6), residues of food or saliva remained present at the valleculae, pharyngeal

wall, pyriform sinuses and postcricoid. Because patients used no protective reflexes such as coughing or swallowing to try to clear the food, it was clear that the sensory reaction of the pharynx was impaired (2/6). In three patients, pharyngeal residues resulted in penetration of food or saliva (Fig. 2b). In two of the six patients, the sensory reaction of the larynx was impaired.

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

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



Pooled secretions in the pharynx containing saliva and remnants of previously eaten food at FEES examination (A), and pharyngeal food residue with penetration and aspiration directly after food intake (B).

Associated clinical outcome measures

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

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

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

DISCUSSION

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

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

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

We found no clear relationship between the age at start of ERT and the point at which facial-muscle weakness developed, although the severest facial muscle weakness was found in patients who started ERT late - at 7 and 8 months of age. ERT seemed to reduce feeding difficulties in some patients. During enzyme therapy, nasogastric tube feeding could be discontinued in three of eight patients who needed NGT at start. At the end of the study, five patients in age ranges from 2 - 12 years were completely orally fed. Still, four of these patients showed some signs of dysphagia. It is noteworthy that the patients who were fed

orally were the best performers. Four of these five patients learned to walk and were still walking at the end of the study. ERT could not prevent disordered speech, although the severest speech problems were observed in those with the poorest motor outcome.

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

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

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

Together, these findings suggest that residual muscle pathology of the bulbar muscles almost certainly plays a major role in the speech and swallowing problems described in this study. It cannot be excluded that a role is also played by glycogen storage in the nervous system. Autopsies of untreated patients with classic infantile Pompe disease have shown glycogen accumulation in the glial cells of the cortex, thalamus, brainstem, and spinal anterior motor horns.[28–30] Since ERT cannot cross the blood-brain barrier, ERT is unlikely to affect the glycogen storage in the central nervous system.[31]

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

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

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

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

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

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

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

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PART 2

Other Lysosomal Storage Diseases

Chapter 7

Neuropsychological profile in a patient with alpha-mannosidosis: a longitudinal follow-up from infancy until adulthood.

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Submitted

ABSTRACT

Alpha-mannosidosis is a rare lysosomal storage disorder resulting from deficiency of alpha-mannosidase which affects the Central Nervous System (CNS). Cross sectional studies of individual patients have shown intelligence ranging from mild developmental delays to profound intellectual disability. Studies on long-term cognitive development of alpha-mannosidosis patients are limited. Here, we present the long-term follow-up of a patient who was assessed regularly from the age of 1 year to adulthood, starting even before the diagnosis was made. At first assessment the patient presented with a mild developmental delay, which remained rather stable over time (median IQ 76; 8 measurements between age 1.9 and 17.8 years). At last assessment his mental age was 8.0 years. Three additional in depth neuropsychological tests showed difficulties in attention, memory, fine motor skills and behavior (age 9, 14 and 17 years). Noteworthy, memory deteriorated over time and problems in his fine motor skills and behavior increased. Brain MRI at age 16 showed mild atrophy of the cerebrum and cerebellum and diminished myelination of the periventricular white matter, which had not been noticed at the age of 1 year. In conclusion, while IQ remained rather stable over time, reflecting stable mild developmental delay, behavioral and fine motor problems and memory deteriorated over time, as did findings on MRI. Our study provides new insights of the long-term CNS consequences for patients with milder forms of alpha-mannosidosis. This information is relevant in light of the new therapeutic developments and shows that targeting of the brain remains mandatory in both severe and milder forms of alpha-mannosidosis.

INTRODUCTION

Alpha-mannosidosis is a rare lysosomal storage disorder resulting from a deficiency of the enzyme alpha-mannosidase (AM: EC 3.2.1.76) caused by pathogenic variations in the MAN2B1 gene. Patients mostly have a long odyssey before they are diagnosed. Symptoms such as delayed speech and language, hearing impairment, motor function disturbance, coarse facial appearance and skeletal deformities are rarely recognized as being part of one disease.[1-3] Presently no treatment is registered for alpha-mannosidosis. However research on the effect of enzyme replacement therapy (ERT) is ongoing.[4]

Literature on neuropsychological development in alpha-mannosidosis is limited. In 2015, a cross sectional study of 35 untreated patients with alpha-mannosidosis was published.[5] Development ranged between mildly (IQ 81) to severely delayed (IQ 30). Longitudinal follow-up studies are too scarce to predict long-term consequences for individual patients.[6, 7] We had the unique opportunity to combine data of several neuropsychological tests and MRI findings of a patient with alpha-mannosidosis who had been carefully monitored from start of symptoms (at age 1 year) even before the diagnoses was made, until adulthood.

RESULTS

Early development of the patient was characterized by mild dysmorphic features, such as a large head, and prominent forehead, several upper airway infections, and a delayed psychomotor development (sitting at 12 months, walking 18 months, use of two-word sentences at 3,5 years). At the age of 4 years hearing aids were prescribed to compensate a slowly progressive hearing loss (maximal loss 52 dB right ear, 48 dB left ear). From age 3.5 to 17 years the patient was treated with speech therapy due to speech and language problems. Subnormal muscle strength, mild fine motor problems, and a visuoconstructive dyspraxia were observed at the age of 10 years. The above-mentioned symptoms finally let to a diagnosis of alpha-mannosidosis at age 11,5 years (alfa-mannosidose activity 22 nmol/hour/mg/protein in leucocytes, normal values 150-650). Variations in the MAN2B1 gene were 2248C>T (arg750TRP) and 2887delG.[8] During puberty the patient developed a mild scoliosis (age 13) and a mild ataxia (age 14). Fatigue was a distinct symptom during childhood, which increased during puberty.

Intelligence

The patient underwent eight intelligence assessments from age of one year to age 17 years (Table 1). The median IQ was 76 (range 64-102). The patient's mental capacities were mildly delayed and remained stable over time, with the exception of a normal IQ score at the age of

4.5 years (SON-R). The latter can be explained by the fact that the SON-R uses concrete items at this age. This may have influenced the results, as his following test at the age of 4.9 years with the more abstract WPPSI-R showed results in the range of mild developmental delay again. At last assessment at age of 17.8 years his mental age was 8 years. His educational level was in accordance with his intelligence.

Table 1. Psychological test results obtained between the age of 1 and 17 years. Different psychological tests were used, according to age and suitability.

Test	Measures	CA (y)	MA (y)	Score (z-score)
BOS	Psychomotor development	1.9	1.4	71* (-1.9)
BOS	psychomotor development	2.5	2.1	75* (-1.7)
SON-R 2.5-7*	Non-verbal intelligence	4.5	4.6	102 (0.13)
WPPSI-R	Intelligence	4.9	<4	78 (-1.5)
SON-R 2.5-7	Non-verbal intelligence	5.9	4.9	81 (-1.3)
WISC-III**	Intelligence	10.1	7.1	64 (-2.4)
SON-R 5.5-17***	Non-verbal intelligence	13.9	8.8	76 (-1.6)
SON-R 6-40	Non-verbal intelligence	17.8	8.0	71 (-1.9)

CA = chronological age, MA = mental age, y = years, BOS = Bayley Developmental Scales, SON-R = Sneijders Oomen Non-verbal Scales- Revised, WPPSI-R = Wechsler Preschool and Primary Scales of Intelligence Revised, WISC-III = Wechsler Intelligence Scales for Children

* SON-R items were concrete; at older age items become more abstract. ** The patient was not motivated. *** short version of the SON-R.

Note: Between age three and four, four assessments to monitor his mental development were performed due to dysmorphic appearance, motor delay and to determine his school choice. Assessment at ages five and ten years were performed to monitor his school progress. The last two intelligence tests were performed to monitor his disease process after the diagnosis of alpha-mannosidosis. Disharmonic profiles were found in two of the eight assessments. There was no consistency in disharmonic intelligence profiles.

Additional neuropsychological evaluations

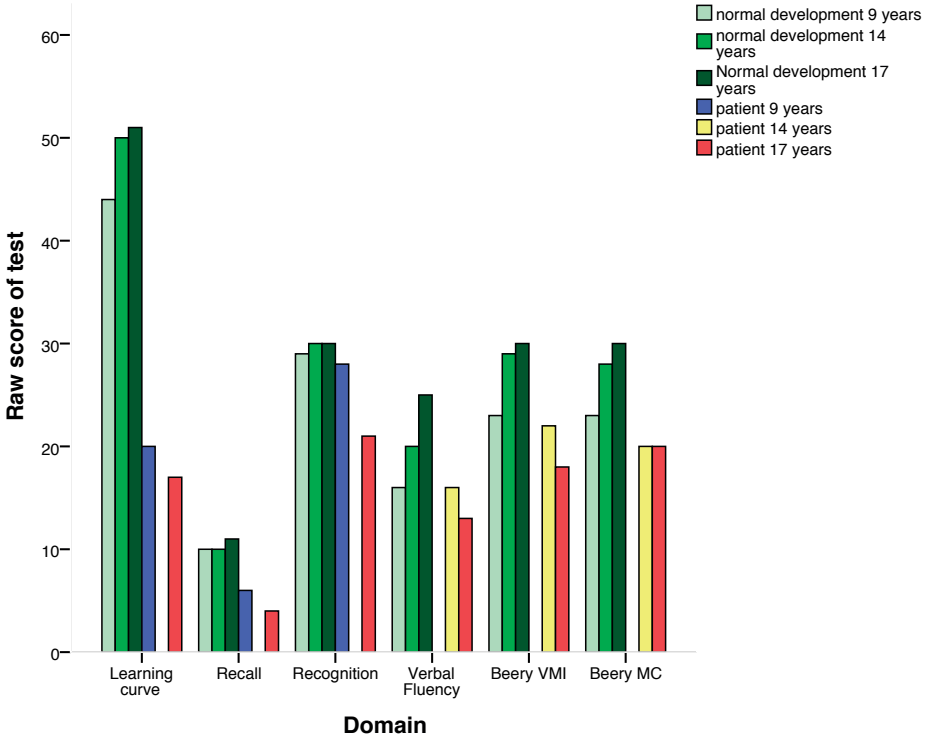
Three additional more in depth neuropsychological assessments were performed at the ages of 9, 14, and 17 years (Figure 1); the test scores were corrected for his mental age.

Attention was normal until the age of 5 years. From age 6 onwards, it was noted that he had a short attention span, and had difficulties in sustained attention. These attention problems were confirmed by The Cancellation Test at the ages of 9, 14 and 17 years and remained stable over time.

At the age of 9 and 17 years his verbal short and long-term memory was assessed (Rey Auditory Verbal Learning Test). At age 9 years the patient had difficulties with all aspects of verbal memory, except for verbal recognition. At the age of 17 the patient lost acquired skills and therefor declined in all raw scores of the verbal memory test (Figure 1). His verbal fluency declined as well. At the same time, the scores on the visual-motor integration test declined

(Beery VMI), while visual perception skills continued to develop (Beery Visual Perception). Therefore, the decline on the Beery VMI was related to progressive fine motor problems.

Figure 1. Results of neuropsychological assessments between 9 and 17 years of age.



Neuropsychological assessments of the California Verbal Learning Test (5 trials –learning curve-, the recall trial and recognition trial), Verbal Fluency, Beery Visual Motor Integration (VMI) test, and the Beery Motor Coordination (MC) test at ages 9, 14 and 17 years.

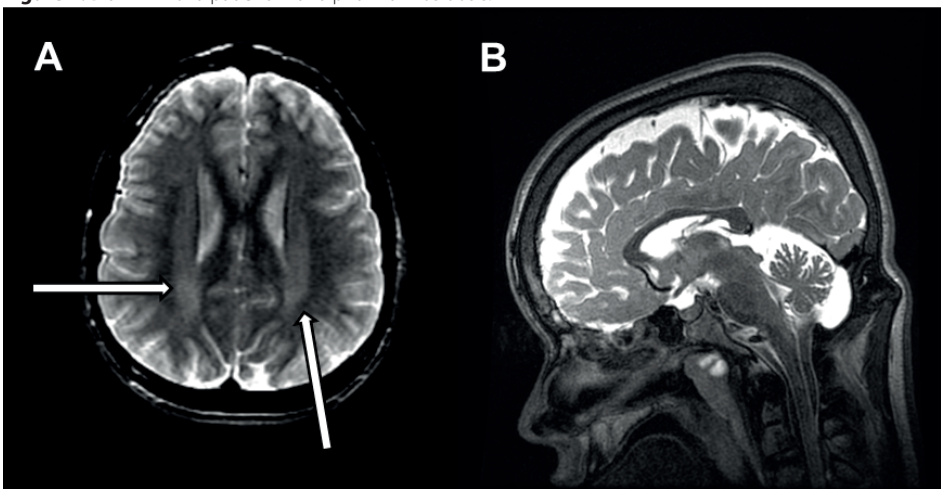
Behavior

Generally, the patient' character was described as cheerful, and humoristic. From age 4 onwards, symptoms of an Autism Spectrum Disorder were described. He had a highly restricted, fixated interests in animals, abnormal in intensity or focus, a strong need for restricted patterns and clear rules, and deficits in social-emotional reciprocity. In time, his behavioural problems increased. At age 14, affective problems were reported by the parents (Child Behavior Checklist, CBCL, Affective problems in the clinically high score when compared to peers). Three years later, he had anxiety problems as well (CBCL anxiety domain clinically high score CBCL). At this point social and emotional problems were reported by the parents on the Strength and Difficulties Questionnaire, Children's Social and Behaviour Questionnaire, CSBQ.

Brain MRI

The patient had two brain MRI's; the first MRI was normal (age 1,5 years), but on the second MRI (age 16 years) several abnormalities were observed including symmetric atrophy of the cerebellar hemispheres and vermis, diminished myelination of the periventricular white matter, and mild abnormalities of the basal ganglia (see for details Figure 2).

Figure 2. Brain MRI of a patient with alpha-Mannosidosis.



At the age of 16 years, symmetric atrophy of the cerebellar hemispheres and vermis was found. He had periventricular white matter abnormalities and mild abnormalities of the basal ganglia. The patient had a normal corpus callosum and normal ventricles size. The brain stem was slightly underdeveloped. The central canal was somewhat prominent at the cervical myelum. a. White arrow indicates the periventricular white matter abnormalities. b. Atrophy of the cerebellar hemispheres and vermis and underdevelopment of the brain stem.
The patient had a brachycephaly.

DISCUSSION

In this case report, we describe the neuropsychological profile of an untreated patient with Alpha-mannosidosis from infancy to adulthood. Compared to other cases described so far, the intelligence of our patient is relatively high.[5-7, 9-17]

Long-term neuropsychological follow-up is scarce[6, 7, 10, 11, 13, 15-17] The studies reporting an intellectual decline did not present age equivalents, which complicates the distinction between stagnation of mental development or intellectual deterioration. In our patient both intelligence test scores and age equivalents indicated that the patient continued to develop over time, but at a slower pace when compared to peers.

In contrast to his stable intelligence, memory deteriorated in time. This finding is new. At 14 years, it was shown that the patient was capable to store new information in the brain (e.g. normal recognition), but that he had difficulties actively retrieving it (short and long term recall). Three years later, he had deteriorated in all aspects of verbal memory, including verbal recognition. In addition, his decline in verbal fluency could be attributed to his verbal memory loss. Several factors may have influenced the progressive memory loss. First of all, patients with alpha-mannosidosis can decline in hearing, speech[2, 10] and language skills.[10] However, hearing was timely compensated with hearing aids in our patient and language problems cannot fully explain his verbal memory loss, since the patients declined in his verbal recognition as well. Memory problems were described before in a cross-sectional study in 35 generally more severely affected patients with alpha-mannosidosis.[5] Additional, Borgwardt et al.[5] described neuropsychological problems in attention, visual function, reasoning and visuo-spatial skills. Our patient also presented problems in attention and a decline in fine motor skills, while his visual-spatial abilities remained stable in time. Noll et al.[6] also reported delayed, but stable visual spatial abilities, using the same test.

Another specific neuropsychological finding in our patient was his typical social interaction, with characteristics commonly seen in children with an autistic spectrum disorder. Although in alpha-mannosidosis behavioral problems were described before, these were restricted to psychiatric symptoms such as states of anxiety, depression, confusion, delusions, and hallucinations.[14, 21] It should be noted that the rate of autism spectrum disorders is strikingly high in patients with psychosis,[22] which is characterized by delusions and hallucinations. We advice to pay specific attention of autistic traits to patients with alpha-mannosidosis.

Can we relate this neuropsychological profile to the abnormalities found in his brain?
The brain MRI of our patient showed atrophy in the cerebellar hemispheres and the vermis, as observed before in patients with alpha-mannosidosis.[15, 23, 24 9, 13, 25] The cerebellum plays a major role in the coordination of movement. Recent studies, however, indicate that the cerebellum also influences higher order cognitive functioning,[26] through its reciprocal cerebro-cerebellar connections. Therefore, the abnormalities in the cerebellum may have influenced the functioning of neuropsychological domains such as memory, and attention. Interestingly, psychosis and behavioral problems both can be related to abnormalities in the cerebellum and vermis as well.[26] Although many brain regions are involved in memory functioning, the key role is played by the hippocampus. Histopathological studies of alpha-mannosidosis knock-out mice and patients with alpha-mannosidosis show vacuolization in the hippocampus. No macroscopic brain abnormalities were found on the patient's brain MRI, but this does not rule out microscopic brain pathology..

In conclusion, here we describe a mildly affected patient with alpha-mannosidosis having a specific neuropsychological developmental course, which can be related to his cerebellar brain abnormalities. His neuropsychological profile was characterized by a stable mild developmental delay, and specific problems in fine motor skills, sustained attiontion, and memory. We noted problems reflecting an autism spectrum disorder. Although intelligence and attention remained stable in time, his fine motor skills and memory declined and behavioral problems increased, leading to severe impairment in everyday functioning. This information is important both for counseling parents and patients, as well as for the evaluation of the effect of therapies.

ACKNOWLEDGEMENT

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Chapter 8

Long-term cognitive follow-up in children treated for Maroteaux-Lamy Syndrome.

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J Inherit Metab Dis 2016;39(2):285-292

ABSTRACT

Objective

It remains unclear to what extent the brain is affected by Maroteaux-Lamy syndrome (MPS VI), a progressive lysosomal storage disorder. While enzyme replacement therapy (ERT) elicits positive effects, the drug cannot cross the blood-brain barrier. We therefore studied cognitive development and brain abnormalities in the Dutch MPS VI patient population treated with ERT.

Methods

In a series of 11 children with MPS VI (age 2 to 20 years), we assessed cognitive functioning and brain magnetic resonance imaging prospectively at the start of ERT and at regular times thereafter up to 4.8 years. We also assessed the children's clinical characteristics, their siblings' cognitive development, and their parents' educational levels.

Results

The patients' intelligence scores ranged from normal to intellectual disabilities (range test scores 52-131). In 90%, their scores remained fairly stable during follow-up, generally lying in the same range as their siblings' test scores (median for patients = 104, median for siblings = 88) and comparing well with the parental educational levels. Native-speaking patients had higher intelligence test scores than non-native-speaking patients. Two patients, both with high baseline glycosaminoglycan levels in their urine and severe mutations in the arylsulfatase B gene, scored clearly lower than expected. Patients with pY210C performed best. Brain abnormalities were aspecific, occurring more in patients with severe symptoms.

Conclusions

Our study shows that cognitive development in MPS VI patients is determined not only by familial and social-background factors, but, in patients with a severe form of the disease, also by the disease itself. Therefore in patients with severe disease presentation cognition should be monitored carefully.

INTRODUCTION

Maroteaux-Lamy syndrome (MPS VI, OMIM #253200) is a lysosomal storage disease caused by a deficiency of the enzyme arylsulfatase B, which leads to storage of dermatan sulphate (a glycosaminoglycan, GAG). Its characteristic clinical features include short stature, bone abnormalities, sensory perception disorders, corneal clouding, carpal tunnel syndrome, spinal cord compression, and reduced life expectancy.[1-6] The disease spectrum varies from a rapidly progressive form, to milder variants with later onset of symptoms. Disease severity is related to urinary GAG levels.[2, 6-9] In 2007, enzyme replacement therapy (ERT) with recombinant human arylsulfatase B was registered as a treatment for MPS VI. Although this has positive effects on various tissues,[8, 10-12] it is assumed that ERT cannot pass the blood-brain barrier. While progressive mental retardation is common in other types of mucopolysaccharidoses,[3] reports on cognition in MPS VI patients are inconsistent.[4, 13, 14] Several reports have indicated that cognition in MPS VI patients is normal. A recent cross-sectional study among Brazilian patients reported mental retardation in one third of the patients, but also indicated that severe visual and/or hearing deficits might have influenced the test results.[13] Many patients had brain abnormalities.

We prospectively studied the mental development and MRI findings in the Dutch MPS VI patient population to increase insight into 1.) long-term cognitive outcome, 2.) the potential influence of social and familial background on intelligence, 3.) cognitive outcome relative to the urinary GAG levels and gene mutations, and 4.) the potential relationship between structural abnormalities of the brain and cognition.

METHODS

Patients

All Dutch patients with Maroteaux-Lamy syndrome treated with ERT participated in this long-term standardized follow-up study, which was performed at the Center of Lysosomal and Metabolic Diseases at Erasmus MC University Medical Center in Rotterdam. MPS VI was diagnosed on the basis of GAG analysis in the urine followed by enzyme assay on leukocytes and fibroblasts, and then by mutation analyses in the Arylsulfatase B gene.[7] Patients were treated with the registered dose of 1 mg/kg/weekly recombinant human arylsulfatase B (galsulfase, Naglazyme®, Biomarin Corporation). None had received hematopoietic stem-cell transplantation.

Study protocols were approved by the Institutional Review Board. Written informed consent was provided by all patients and siblings participating in this study, if necessary in conjunction with their parents.

Intelligence

Each year, patients underwent standardized cognitive assessments, for which we used the following: the Griffith Mental Development Scales;[15, 16] the Bayley Scales of Infant Development;[17] and the Wechsler Intelligence Scales (Wechsler Intelligence Scales Children-third edition; 6-16 years, or Wechsler Adult Intelligence Scales-third edition; > 16 years).[18] For children with impaired hearing, we used the Snijders-Oomen Nonverbal Intelligence test-Revised (SON-R 2½-7).[19]

To investigate social and familial background, we compared each patient's intelligence test score with that of their sibling or siblings. Patients and siblings were tested by three pediatric neuropsychologists (F.K.A., B.J.E., and R.L.v.d.W.). We also collected information on the parents' native language and highest educational level, and on patients' and siblings' school performance.

Brain MRI

Brain magnetic resonance imaging (MRI) was performed regularly, and the baseline MRI and most recent MRI were used to determine change over time. The choice of parameters was based on literature review, being deduced particularly from four articles on brain abnormalities in patients with MPS VI (see supplement).[13, 20-22] Two observers evaluated all MRIs on T2, FLAIR sequence: a physician assistant trained for this job (M.M.G. B.), and a pediatric neuroradiologist (M.H. L.).

Statistics

Performances in the psychological tests were compared against the normative data of the Dutch population. The mean score for each of these tests is 100, with a standard deviation (SD) of 15 points. A score above 84 reflects normal development, a score between 84-70 indicates mild developmental delay, and a score below 70 severe developmental delay. Per patient, a difference was defined as a deviation of more than 1.5 standard deviations between total intelligence test scores.[23] The Mann-Whitney *U* test to determine differences between groups. All analyses were performed with SPSS for Windows (version 20, SPSS Inc., Chicago, IL).

RESULTS

Patients characteristics

Eleven children with MPS VI were included in this study; among them were two sibling couples. Patients were of Dutch (4), Turkish (4), Moroccan (1), Pakistani (1) and Guinean (1) ancestry. Seven had consanguineous parents.

Patient characteristics are summarized in Table 1.

The median age at presentation of symptoms was 2.0 years; at diagnosis it was 5.1 years, and at baseline (start of enzyme therapy) it was 6.8 years. One patient was assessed at baseline only (patient 11). The other patients had at least a follow-up of two years (three assessments) or longer, range 0 – 4.8 years.

Cognitive development at baseline and during follow-up.

Forty-two intelligence tests were performed in the 11 patients (age range 2 to 20 years). Figure 1a shows the patients' total IQ (TIQ) scores expressed against duration of enzyme therapy; figure 1b shows the total IQ scores expressed against chronological age. Six patients scored above the critical threshold of normal intelligence (> 84) at all time points. At baseline, the test scores reflected levels of development that ranged from mildly delayed to above average; the group's median intelligence test score was normal (87, range 73-129, n = 11). During follow-up, cognitive development remained stable (one year therapy median = 88, range 59-123, n =10; two years = 89, range 52-123, n = 10; three years = 94, range 60-131, n = 7). Expressed against chronological age, the scores also remained stable for the various age categories. However, there was one exception: over a period of 17 months from baseline, patient 3 (aged 1.9 at baseline) deteriorated over 30 points (2 standard deviations). His behavior was diagnosed as Autistic Disorder. Until age 20 months, his skull size increased significantly (from 0 SD toward +2,5 SD) and he had frontal bossing and a dolichocephalic skull shape. While MRI showed prominent ventricles, lumbar puncture did not show raised intracranial pressure. Disharmonic profiles were found in all patients and in 25 of the 42 tests performed; there was no consistency in disharmonic profiles.

Education

Six of the eleven patients were attending or had successfully completed regular schools, three of whom required extra support (remedial teaching), due either to dyslexia (n=2) or to mildly delayed IQ scores. The five other patients attended special schools for sick children (n = 3) or for children with motor disabilities (n = 2).

Table 1. Patient characteristics

Patient	Gender	Age at first symptoms (years)	Age at diagnosis (years)	Age at start of treatment (years)	Allele 1	Allele 2	Rapid/slow Progressive	GAG-level at baseline (upper limit of normal for age)	Hearing aids [2]	Glasses [3]	Native speaker	Mother's education	Father's education father	Consanguine	Carpal tunnel release
1[1]	M	5.0	0.7	7.6	454 C>T (p.R152W), exon 2	454 C>T (p.R152W), exon 2	Slow	231 (318)	N	Y	N	1	1	Y	N
2	F	1.5	1.8	2.1	903 C>G (p.N301K) exon 5, S384N, 1152 G>A, exon 6	903 C>G (p.N301K) exon 5, S384N, 1152 G>A, exon 6	Rapid	942 (254)	Y	Y	N	4	1	N	Y
3	M	.6	1.9	2.3	971 G>T (p.G324V), exon 5	971 G>T (p.G324V), exon 5	Rapid	739 (254)	N	Y	N	1	2	N	Y
4	M	1.2	2.8	3.0	995 T>G (p.V323G), exon5	995 T>G (p.V323G), exon5	Rapid	554 (194)	Y	Y	N	4	4	Y	N
5	F	.4	3.4	6.7	c.1142 + 2T>C, exon 5	c.1142 + 2T>C, exon 5	Rapid	1287 (194)	Y	Y	N	1	2	Y	Y
6	M	.8	5.1	5.9	629 A>G (p.Y210C), exon 3	979C>T (p.R327X)	Slow	207 (145)	Y	N	Y	4	4	N	Y
7[1]	M	3.0	5.8	6.2	629 A>G (p.Y210C), exon 3	979C>T (p.R327X)	Slow	158 (145)	N	Y	Y	6	5	Y	N
8	F	3.0	7.4	7.8	629A>G (p.Y210C), exon 3	979C>T (p.R327X)	Slow	214 (124)	N	N	Y	6	5	Y	N
9	F	2.0	7.8	8.3	454C>T (p.R152W), exon 2	454C>T (p.R152W), exon 2	Slow	254 (124)	N	Y	N	3	2	Y	N
10	M	7.0	10.1	18.3	454 C>T (p.R152W), exon2	454 C>T (p.R152W), exon2	Slow	106 (107)	N	N	N	1	1	Y	Y
11	M	9.0	10.2	10.7	629 A>G (p.Y210C)	937 (C>G) (p.P313A)	Slow	193 (107)	N	N	Y	6	5	N	N

M = male, F = Female, N = No, Y = Yes, 1 = Elementary School, 2 and 3 = Junior High School, 4 = Senior High School, 5 = Bachelor of Science, 6 = Master of Science.

[1] diagnosed after the diagnosis of sibling (patient 1 is the sibling of patient 10; patient 7 is the sibling of patient 8)

[2] conductive hearing loss (range 7-43 dB right ear; 2-53 dB left ear) (Brands et al., 2013)

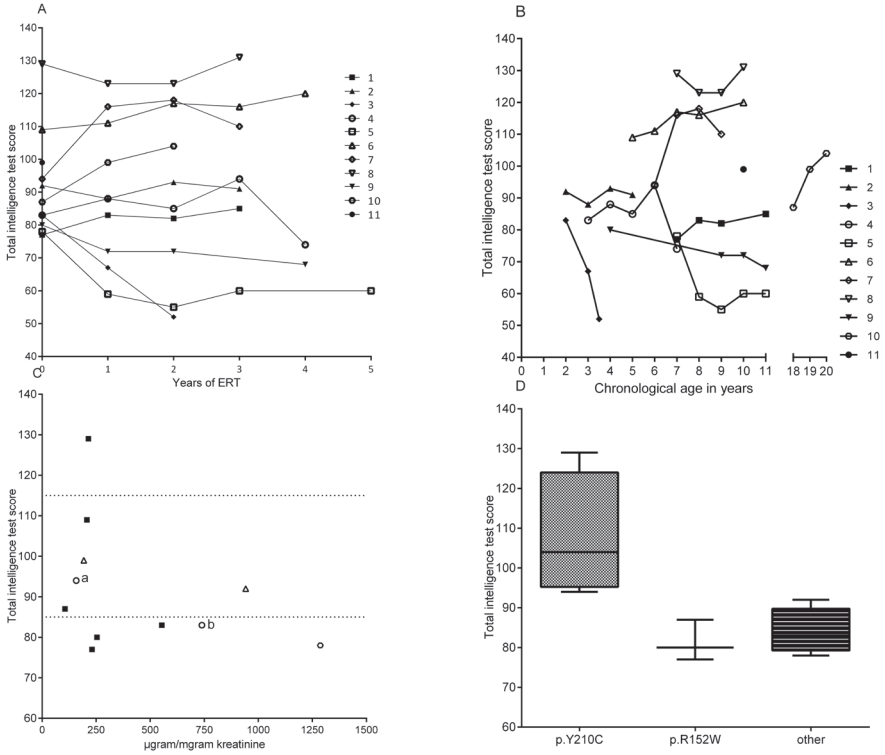
[3] hyperopia

Social and familial background

Siblings

Seven healthy siblings of seven patients agreed to be tested by a neuropsychologist as part of this study. The siblings of two other patients agreed only to inquiry of their school data. The remaining two patients had no siblings. The intervals between the patients' tests and those of their sibling/siblings ranged between 5 and 9 months.

Figure 1. Cognitive development in 11 patients with Maroteaux-Lamy syndrome.



(1a) Long-term cognitive development expressed against duration of ERT, and (1b) expressed against chronological age. The first assessment of patient 5 was based on one subscale score (the Performance scale); due to shyness and withdrawn behavior, the other subscales were unreliable. (1c) Patients' total intelligence test scores at baseline versus urinary GAG level at baseline, relative to the siblings' IQ scores. ■ = scores similar to siblings', o = scores below siblings', Δ = no siblings. a = this child's sibling had an extraordinarily high test score (IQ = 139). b = this child was patient 3 in Figures 1a and 1b. (1d) Total intelligence test scores related to mutation analysis –grouped by identified mutations.

The siblings' overall intelligence test scores were largely similar to the patients' total test scores (Mann Whitney *U* Test: patients median 104 range 68 – 131; verbal 96 range 74 – 125, performal 104 range 66 – 128, n = 7; siblings median 88 range 77-139; verbal 94 range 75 – 135, performal 84, range 81 – 150, n = 7). Five healthy siblings had harmonic profiles. The sixth sibling had a higher score in the verbal domain than in the performal domain; in the seventh it was the other way around.

Unfortunately, the siblings of the two patients with the lowest IQs (patients 3 and 5) were the ones who did not want to be tested. However, inquiry after their school results showed that these healthy siblings attended regular schools without needing special assistance; to

date, they had never failed to be promoted to the next grade. On the basis of these school results, it was concluded that their intelligence was likely to be within the normal range (TIQ ≥ 85), unlike that of their affected siblings, whose scores indicated severe delays.

Parents

All parents' highest parental education levels were obtained. These ranged from finishing primary school to obtaining a university degree. We found an association between the parents' educational level and the level of the patients' and their siblings' test scores, most children of parents with a higher educational level having higher intelligence test scores and vice versa.

Seven of the eleven children had non-native speaking parents and were being raised bilingually. On average, patients of native Dutch-speaking parents had higher test scores (4/4 patients had normal to above normal intelligence at all times; range 94-131) than children with non-native-speaking parents (2/7 patients had normal test scores at all times; range 52-104).

Age of diagnosis, GAG levels, genetic mutations

The disease was considered to be more severe in four patients (patients 2-5). They had presented at a young age (range of age at diagnosis 1.8 – 3.4 years) and excreted the highest of GAG levels in their urine at baseline (>300 µg/mg creatinine). Severe cardiomyopathy was present in three of the four patients. Figure 1c plots the patients' GAG levels against their total IQ. Three of the four patients with high GAG levels (>300 µg/mg creatinine) had a mild or severe developmental delay (IQ < 85), against two of the seven patients with low GAG excretion.

Fig 1d categorizes the patients according to their mutations. The first group consisted of patients who were homozygous for the p.R152W mutation (n=3, all of Turkish Ancestry). The second group had a p.Y210C mutation combined with either p.P313A or p.R327X (n=4, all of Dutch Ancestry). The third comprised the patients with other mutations. Patients with the p.Y210C mutation had the highest total intelligence test scores.

Brain abnormalities

During follow-up, all children had at least two brain MRI scans. Five had their first MRI before the start of ERT. Median age at the first MRI was 7.4 years (range 1.8 – 18.0) and median age at the second was 10.8 years (range 4.1 – 20.5). Median time between the two MRIs was 2.6 years (range 1.2 – 5.7). See Table 2 for a detailed description of the results.

Table 2. Results of baseline MRI and most recent MRI

		Rapid progressive		Slow progressive	
Domain		MRI 1	MRI 2	MRI 1	MRI 2
Enlarged Virchow Robin	Basal nuclei	2/4	2/4	3/7	4/7
	White matter	3/4	4/4	0/7	1/7
	Corpus Callosum	1/4	2/4	0/7	0/7
	Large lesions	1/4	1/4	0/7	0/7
White matter	Patchy lesion	4/4	2/4 [1]	1/7	1/7
	Diffuse lesion	0/4	0/4	1/7	1/7
Ventricular enlargement	FOHWR	0/3	1/4	1/7	1/7
Intracranial pressure	Widened sinus rectus	3/4	3/4	2/7	3/7 [2]
Atrophy	All fissures and sulci involved	0/4	0/3	0/7	0/7
Thinner corpus callosum		3/4	3/4	2/7	2/7
Mild compression of the spinal cord		3/4	3/4	2/7	3/7
Total abnormalities		20/43	21/43	12/77	16/77

[1] White-matter abnormalities disappeared in two patients. In the remaining two patients, white-matter abnormalities decreased, but were still visible.
[2] 1/7 showed a slight increase, possibly due to differences in technique.

Common findings at baseline were enlarged Virchow Robin spaces (in the basal ganglia and in the white matter); patchy white matter abnormalities (especially in the occipital area, but also in the frontal and periventricular area); widened sinus rectus, a thinner corpus callosum, and mild compression of the spinal cord.

It is noteworthy that the patchy and diffuse white matter abnormalities became less intense over time and sometimes even disappeared (Figure 2).

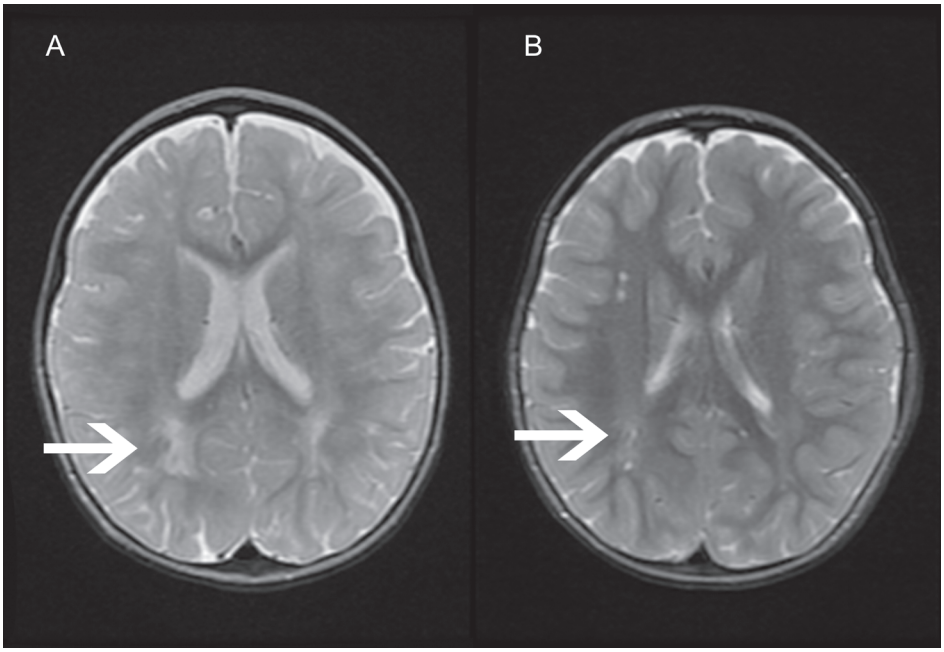
Brain abnormalities were found in a higher number of severely affected patients than in patients who were relatively mildly affected: 4/4 of the severely affected patients had Virchow Robin spaces in white matter vs. 1/7 of the mildly affected patients (MRI 2); 4/4 had patchy white-matter signal changes vs. 1/7 (MRI 1); and 3/4 had thin corpus callosum vs. 2/7 (both MRIs).

Miscellaneous

All patients visited an ophthalmologist and underwent hearing tests once and twice a year. Seven out of 11 patients needed glasses, all for hyperopia (4/4 were severely affected patients and 3/7 mildly affected patients). Four patients developed mild to moderate conductive hearing loss, and hearing adds were fitted and adjusted if necessary (range 7-43 dB right ear; 2-53 dB left ear) (Brands et al., 2013; three of these patients were severely affected). At time of IQ testing, hearing and vision problems were sufficiently compensated (Table 1).

Patients were regularly examined for carpal tunnel syndrome and five patients underwent carpal tunnel release. At the age of 5, patient 4 received a ventriculoperitoneal shunt for increased intracranial pressure. All patients required hospital admissions for medical procedures requiring general anaesthesia. The number of interventions ranged from 1 to 11, with a median of 5. The two most severely affected patients underwent 11 and 7 procedures.

Figure 2. Longitudinal brain magnetic resonance imaging (MRI). (2a) The patient was 2 years of age at first MRI (2b)



The patient was 4.5 years old at the second MRI. The arrows indicate a delay in myelination in the occipital region (slices are at slightly different levels, due to the quality of MRI). Please note the presence of enlarged Virchow Robin spaces in the white matter in the second MRI (not present in the first MRI).

DISCUSSION

To date, the extent to which MPS VI affects mental outcome has remained unclear. Our study shows that cognitive development in these patients is determined not only by familial and social-background factors, but, in patients with a severe form of the disease, also by the disease itself.

Based on the accumulated data of the literature and our study,[4, 13, 14] we conclude that, just as in other types of Mucopolysaccharidoses,[3] cognitive development in MPS VI presents as

a spectrum from normal to delayed. However, the spectrum in MPS VI seems milder than in other mucopolysaccharidoses that affect the brain, such as MPS I, II and III.

Whereas previous studies were cross sectional, our prospective approach found that cognition remained fairly stable in 90% of our patients during follow-up periods that lasted up to 4.8 years. Progressive mental retardation was noted in only one of our 11 patients.

A number of variables influenced cognitive development in our patients with MPS VI. The most important factors appeared to concern social and family background. These patients were drawn from various cultural backgrounds and reflected a wide variation in parents' educational level. Patients with non-native speaking parents generally had lower intelligence test scores when compared to those with native speaking parents. Also, as five of the 11 patients scored below average, three of whom had siblings with similar test scores and parents with relatively low educational levels, we conclude that social and familial background factors largely contributed to their relatively low cognitive level. In healthy children, cognition is known to be associated with parental educational level,[24] inheritance,[25] and native and or non-native-speaking environments.[18] Our study shows that any interpretation of cognitive test results should take account of social and familial background factors, especially when the patient population is small and diverse as in ultra-rare diseases such as MPS VI.

With regard to these social and familial background factors, we found that two patients clearly had lower test scores than expected. Both presented before the age of 5 years with severe symptoms and high GAG excretion in the urine (>300 µg/mg creatinine). While we concluded that MPS VI-related factors probably played a role in determining these children's cognitive level, the picture was not clear-cut: at least one child with high GAG levels and early disease onset performed reasonably well and continued to do so during follow-up. We conclude that it is only in children with severe disease presentation, characterized by high urinary GAG excretion, that there seems to be a risk of low cognitive levels.

With regard to the effect of the genotype on cognition, we note that the group of patients with the p.Y210C mutation performed best.[26] These patients had parents who were all native-speakers of the national language, and who all had a high educational level. Similarly, they had siblings whose intellectual performance was in the highest range. Earlier, it was reported by Swiedler et al (2005) that the p.Y210C mutation was associated with low GAG excretion and better growth. In three of the four children, p.Y210C was combined with p.R327X, a nonsense mutation leading to no production of the arylsulfatase B protein. This indicates that the second mutation did not influence the milder phenotype. Patients 3 and 5, who had the lowest intelligence test scores, both had severe mutations. One of these

patients was homozygous for 1142+2T>C, a mutation affecting the invariant splice donor sequence that is therefore expected to completely disrupt splicing; the other was homozygous for p.G324V, which leads to reduced synthesis of arylsulfatase B protein (Brands et al 2013).

Despite the seemingly limited influence of MPS VI on cognition at group level, there was a considerable need for special education services (73% versus 5% in the normal population). In part, this was no doubt explained by physical limitations such as limited joint mobility, bone deformities, pain, cardiac problems, and fatigue – medical problems that also substantially increased school absence. Also, the frequent hospitalizations and surgical procedures may have influenced cognitive ability. It is unsure whether educational potential was influenced by subtle neuropsychological deficits that had not been detected by standard intelligence tests. Although hearing problems and poor eye sight more often occur in severely affected patients, vision or hearing deficits were not a main contributor. At the time of testing, they appeared to have been sufficiently compensated by glasses and hearing aids.

The structural abnormalities detected on our patients' brain MRIs were generally mild, fairly aspecific, and similar to those previously reported.[13, 27-32] Patients with rapidly progressive and attenuated disease presentations had similar types of abnormality. Those with severe disease had slightly more abnormalities, particularly with regard to the frequency of white matter lesions (patchy lesions, thinner corpus callosum and Virchow Robin spaces in the white matter). The white matter abnormalities found in our patient group were different from those in other lysosomal storage diseases such as Metachromatic Leukodystrophy[33] and Hunter Disease[34], in which such abnormalities are clearly progressive.

CONCLUSION

Cognitive development in MPS VI presents in a spectrum that ranges from normal to delayed. Our study shows that cognitive development in MPS VI patients is determined not only by familial and social-background factors, but, in patients with a severe form of the disease, also by the disease itself. Cognition of patients with these hallmarks should be monitored closely. Furthermore, cognition testing in rare diseases such as MPS VI should include examination of social and familial background factors such as parental educational level, and testing of siblings' cognitive levels. Such an approach is especially necessary in small patient populations from varied cultural backgrounds.

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Supplement 1.. MRI protocol

Supplement
MRIs were scored for the presence or absence of the following parameters:

1.) Virchow robin spaces (VR ≤ 8 mm diameter) in the basal nuclei, white matter, and/or corpus callosum (CC) or large VR (≥ 8 mm diameter) in any location. Scores: 0 = absent; 1= present.

2.) Patchy and/or diffuse lesions in the white matter. Scores: 0 = absent; 1 = present.

3.) Ventricular enlargement. Scores: 0 = normal; 1 = deviant if the FOHWR index (frontal and occipital horn width ratio) was two or more standard deviations above the mean (Jamous et al 2003). Reference data were obtained from a control group of 20 healthy children analyzed at Erasmus MC (unpublished results).

4.) Increased flow void. Scores: 0 = non-dilated sinus rectus; 1= widened sinus rectus.

5.) Brain atrophy as measured by dilated subarachnoid spaces (SS). For this purpose, we measured the width of the Sylvian fissure and the interhemispheric fissure (measured at foramen of Monro level). Scores: 0 = brain atrophy absent if the width of the subarachnoid spaces was < 3 mm and none of the sulci were widened; 1= mild brain atrophy if the width of the spaces was ≥ 3 mm in the Sylvian fissure or interhemispheric fissure, but without widening of other fissures and sulci; 2 = significant cortical atrophy if the Sylvian fissure and interhemispheric fissures and other fissures and sulci were ≥ 3 mm and/or if there was significant loss of cortex and white matter.

6.) Compression of the spinal cord at the level of cranio-cervical junction. Scores 0 = absent or insignificant compression of the spinal cord; 1 = mild compression of the spinal cord (taping).

7.) Corpus callosum. Scores: 0 = normal; 1 = thinner than normal corpus callosum.

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Chapter 9

Can sequential cerebral MRIs predict the neuronopathic phenotype of MPS II?

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ABSTRACT

Objective

This long-term prospective follow-up study was intended to improve understanding of the relationship between IQ and MRI findings in mucopolysaccharidosis (MPS) II patients. The ultimate goal was to enable earlier prediction of neurocognitive development.

Methods

Neuropsychological tests were conducted by experienced neuropsychologists. Cerebral MRIs were rated using a standardized protocol. MRI sumscores were calculated for atrophy, white-matter abnormalities (WMA) and Virchow-Robin spaces (VRS). A random effects repeated measurement model was designed to correlate IQ scores with three MRI sumscores. Patients with an IQ below 70 were classified as neuronopathic.

Results

We analyzed MRIs (n=48) and IQ scores (n=78) of 19 patients, whose age at assessment ranged from 0-47.5 years. At group level, the linear mean decline in IQ was 6.7 points per year. Initially, loss of IQ was rather rapid, which slowed down in time due to a floor effect. Ten patients were neuronopathic and nine non-neuronopathic. VRS were found in the majority of MPS II patients. VRS sumscores showed no relationship with age and disease severity. The severity of atrophy and WMA progressed over time. Both sum scores were correlated with IQ decline (r =-0.9 for atrophy and -0.69 for WMA).

Conclusion

We developed a three-dimensional model, which combines scores of MRI, IQ and age to predict early cognitive decline. Abnormal atrophy scores were present before IQ dropped below the threshold of intellectual disability (<70) and served as a better early marker of detecting the neuronopathic phenotype of MPS II than WMA sumscore.

INTRODUCTION

Mucopolysaccharidosis type II (MPS II, OMIM #30990) is an X-linked, lysosomal storage disorder caused by a deficiency of iduronate-2-sulphatase that leads to storage of heparan and dermatan sulphate.[1] Patients with MPS II represent a broad clinical spectrum. At the severe end of the spectrum are patients with the neuronopathic phenotype. They develop progressive intellectual disability and behavioral problems.[2, 3] Most neuronopathic patients do not survive into adulthood.[4, 5] At the milder end of the spectrum are patients with the non-neuronopathic phenotype, with close to normal cognitive development, and survival into adulthood. About-two thirds of MPS II patients develop the neuronopathic phenotype. To varying extents, patients with neuronopathic and non-neuronopathic forms all develop somatic features such as cardiac-valve abnormalities, dysostosis multiplex, coarse facial features, contractures of the joints, carpal tunnel syndrome, hepatosplenomegaly, impaired lung-function, tracheomalacia, hearing deficits, and myelopathy. Since 2007, enzyme replacement therapy (ERT) with recombinant human iduronidate-2-sulfatse (Elaprase, Cambridge, MA) has been available for MPS II patients. Although ERT improved the prospects of patients with positive effects on joint mobility, walking distance and lung function,[6, 7] it is not expected to halt disease progression in the brain, as the intravenously administered enzymes cannot pass the blood-brain barrier.[3, 8, 9] New therapies such as intrathecal ERT and gene therapy are under development, and are intended to target the brain. This makes it even more important to identify patients with the neuronopathic phenotype at an early stage, i.e. before cerebral damage has occurred. However, Iduronate-2-sulphatase activity seems to be equally deficient in both the neuronopathic and non-neuronopathic group, and baseline GAG levels are not usually predictive for the severity of the phenotype.[1] Although the phenotype seems to be rather consistent for some mutations, suggesting that the mutation can be predictive of the MPS II phenotype, in most patients the prediction on biochemical or molecular grounds is not possible at the time of diagnosis[10]. In young children, very little information is available with regard to IQ data, especially in combination with MRI analysis. For this reason, after prospectively analyzing long-term IQ and cerebral MRI follow-up data collected on MPS II patients, we correlated IQ with the brain abnormalities found on MRI. Our ultimate goal was to find a predictive tool for neurocognitive outcome in young children with MPS II.

METHODS

The Center for Lysosomal and Metabolic Diseases at Erasmus MC (Rotterdam, the Netherlands) serves as the Dutch national reference center for MPS II. We included all MPS II patients referred to the Center before February 1, 2016. Diagnosis was based on mutation analysis,

elevated urinary glycosaminoglycans (uGAG), and iduronate-2-sulphatase deficiency in leukocytes and/or fibroblasts.

Standard Protocol Approvals, Registration and Patient Consents

The study protocol was approved by the Medical Ethical Committee at Erasmus MC, and written informed consent was obtained from patients and/or their parents.

Neuropsychological testing

Annual neuropsychological testing was performed in patients younger than 21 years. Such tests were not performed regularly in non-neuronopathic adult patients, who, on the basis of their social functioning in daily life were not considered to have prominent intellectual disabilities. Neuropsychological tests were conducted by two experienced neuropsychologists at our center (B.E. and F.A.). The Griffiths Mental Developmental Scales (Griffiths) was used for patients whose estimated developmental age lay below 6 years, the Dutch third edition of the Wechsler Intelligence Scales for Children (WISC-III-NL) for those whose estimated developmental age was 6-16 years, and the Wechsler Adult Intelligence Scales (WAIS-VI-NL) for patients whose estimated developmental age over 16 years. The Snijders-Oomen Non-verbal Intelligence test-Revised (SON-R 2 ½ -7) was used for patients with uncompensated hearing loss. In rare cases, IQ testing was performed by other evaluators associated with the children's educational environment. These tests consisted either of the WISC-III-NL, the Griffiths, the second edition of the Bayley Scales of Infant and Toddler Development (BSID II), or the Kaufmann-Assessment Battery for Children (K-ABC).

The subscales of all neuropsychological tests were analyzed for disharmony. A disharmonic profile was defined as a deviation on a subscale score of more than one SD from the mean score. Patients were classified as neuronopathic if their IQ at the latest assessment had been below 70.

Imaging

In patients younger than 21 years, magnetic resonance imaging of the brain was performed regularly, usually once a year. In patients older than 21, we aimed to perform an MRI at least once. Patients were scanned on a 1.5T system, with a dedicated 8-channel head coil (EchoSpeed; GE Healthcare, Milwaukee, Wis.). T1-weighted, T2-weighted, and FLAIR sequences were included in the scanning protocol. All available brain MRIs were reviewed using a protocol specifically designed for this study. For full details of the scoring system used and calculation of the composite sumscores, see supplementary table 1. Instrumental to its design was the combined information of four articles on brain abnormalities in MPS II patients.[3, 11-13] The MRIs were scored independently by two observers (J. vd H. and A.V.) who had been trained by an experienced pediatric neuroradiologist (M.L).

To reach consensus, scoring differences were discussed (for more details, see supplementary table 1). The following items were evaluated: 1) the size and number of Virchow-Robin spaces (VRS) in five different areas (the basal ganglia, amygdala, corpus callosum, subcortical white matter, and periventricular white matter); 2) the size and location of white-matter abnormalities (WMA) in two areas (occipital and frontal); 3) to assess cerebral atrophy, the diameter of the lateral, third and fourth ventricles and, the width of the fissure and sulci; and furthermore (4) we evaluated corpus-callosum size, the presence of an increased flow void, the optic nerve sheath diameter, hyperostosis of the skull, sella turcica enlargement, sella turcica shape, skull shape, mega cisterna magna, and Chiari malformation.
To use these scores for further statistical analysis, we composed three different sumscores of the most relevant MRI items (supplementary table 1); the Virchow-Robin spaces sumscore (sumscore range: 0-20); the white-matter abnormality sumscore (sumscore range 0-12); and the cerebral atrophy sumscore (sumscore range 0-7).

Statistical analysis

Statistical analysis was performed using *R* (version 3.2.1) with *nlme*.[14] To analyze the change in IQ and MRI sumscores over time, while also accounting for the correlations in the repeated measurements of each patient we utilized the framework of linear mixed effects models. MRI an IQ scores were analyzed at group level by generating a linear mixed-effect model based on all individual linear models. Time was expressed as years after the start of ERT. The linear model used was corrected for the patients' age. To statistically investigate the correlation of IQ and MRI scores, we used three bivariate mixed models in which we the IQ scores was analyzed jointly with (1) the Virchow Robin spaces sumscore, (2) the white-matter abnormality sumscore; and (3) the cerebral atrophy sumscore. The correlation between these outcomes was measured via the random effects variance covariance matrix.

RESULTS

Patients

Nineteen male MPS II patients were enrolled in this study (Table 1), 18 of them received ERT, and, unless indicated otherwise, continued to receive it until their latest evaluation. During the course of the study, three neuronopathic patients died at the ages of 15, 16 and 20 years. The patients' ages at latest assessment ranged between 5 and 50 years (median = 9 years). Figure 1 shows the ages at which MRI and IQ tests were performed per individual patient.

Table 1. Patient characteristics.

Patient no.	Age at diagnosis	Age at start ERT	Age at latest visit	*IDS* variant coding DNA	*IDS* variant protein	Latest IQ
1	4	4	5	c.673T>G	p.Tyr225Asp	96
2	2	3	5	c.998C>T	p.Ser333Leu	50
3	3	4	6	c.410T>C	p.Phe137Ser	107
4	3	4	6	c.410T>C	p.Phe137Ser	116
5	4	4	7	Total IDS deletion	No protein	25
6	4	$	8	Total IDS deletion	No protein	13
7	2	2	8	c.544del	p.Leu182Cysfs*31	32
8	5	5	8	c.1511delG	p.Gly504Valfs*8	73
9	0^	1	10	c.349_c.351delTCC	p.Ser117del	17
10	3	6	14	c.998C>T	p.Ser333Leu	11
11	2	9	15#	c.1375G>T	p.Glu459*	15
12	3	10	16#	c.1561G>A	p.Glu521Lys	2
13	6	11	19	c.257C>T	p.Pro86Leu	9
14	3	5	20#	c.1047C>A	p.Ser349Arg	14
15	3	25	30	c.1122C>T	p.Gly373Gly	N/A
16	9	25	33	c.1265G>A	p.Cys422Tyr	N/A
17	*	37α	45	c.182C>A	p.Ser61Tyr	N/A
18	44	45	46	c.1024C>T	p.His342Tyr	112
19	*	47β	50	c.806A>T	p.Asp269Val	N/A

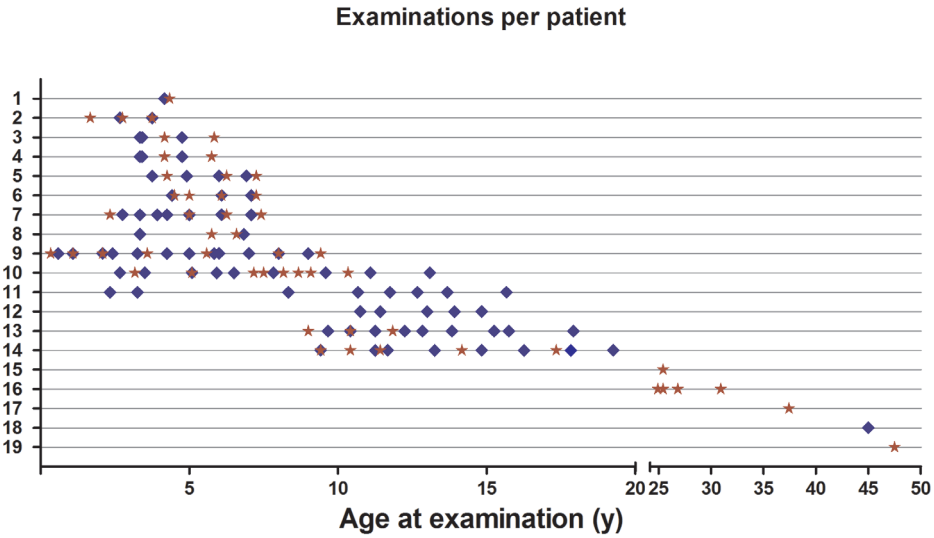
Patients are classified according to their age at latest visit. ERT=enzyme replacement therapy; ^ diagnosed prenatally; N/A Not applicable; $ Did not start ERT; # deceased at this age; α ERT stopped after 18 months; β ERT stopped after 12 months.

MRI

A total of 47 cerebral MRIs were performed in 16 patients (age range 1.7 - 47.5 years). More than one MRI was performed in 12 patients, the actual number ranging from 2 to 7, and the time span between the first and last MRI ranging from 0.8 to 7.9 years (median time span 2.9 years).

Overall, the following abnormalities were observed on the MRIs of the MPS II patients at some point during follow-up: VRS (in 87% of patients); enlargement of the lateral ventricle (in 73%) and of the third ventricle (in 60%); widening of the fissures (in 67%); white-matter lesions (in 67%); mega cisterna magna (in 60%); and enlarged optic-nerve sheath (in 53%). No Chiari malformation was observed. In addition, the following bone abnormalities were found: hyperostosis (in 73% of patients); abnormal skull shape (in 60%); and abnormally shaped sella turcica (in 40%).

Figure 1. Overview of the frequency of MRI and neuropsychological assessment.



The numbers on the y-axis correspond to the patient numbers shown in Table 1. A red stars represent a single MRIs and a blue diamonds represent an IQ test. In supplementary figure 1, IQ test are further defined.

Follow-up of MRI's in individual patients showed that Virchow-Robin spaces barely had changed over time (Figures 2 A, B, and C). Most VRS were located periventricularly (in 87% of patients), subcortically (in 87%), and in the corpus callosum (in 80%); to a lesser extent, they were also located in the basal ganglia (in 60%).

White-matter abnormalities progressed over time. Initially, small white-matter lesions were observed around the occipital horn of the lateral ventricles; later, they also increased around the frontal horns. The small solitary white-matter lesions progressed slowly towards areas of large lesions that merged. Eventually these formed large areas of abnormal white matter that extended from the occipital, lateral and frontal periventricular white matter to the subcortical u-fibers (Fig 2D, E and F).

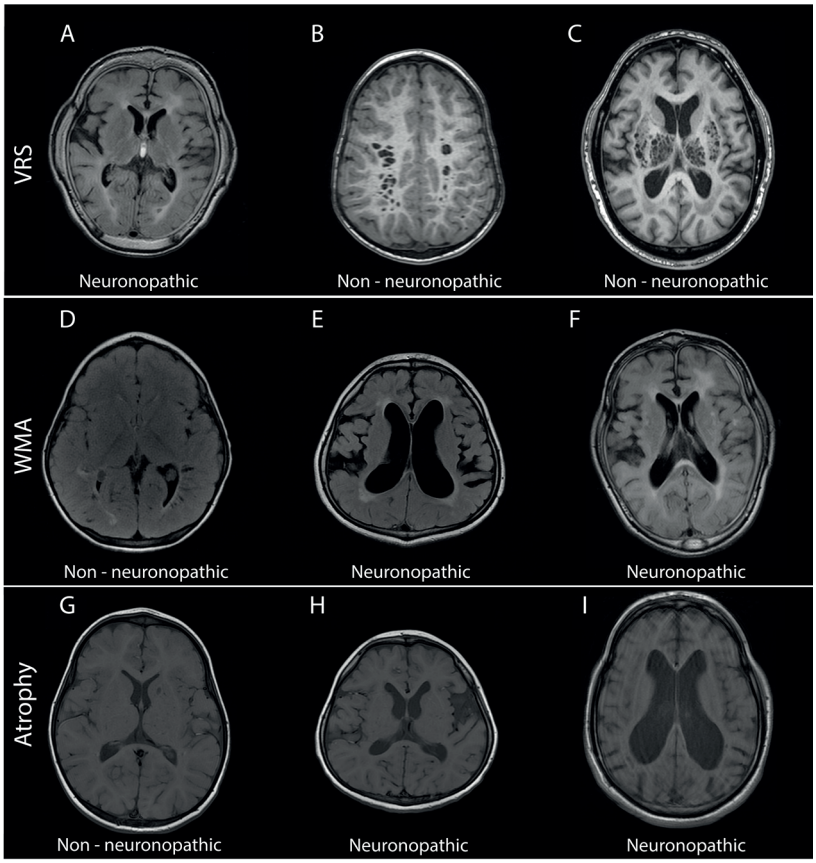
Brain atrophy also progressed over time. This was generalized and not restricted to specific areas of the brain (Figure 2 G, H, and I).

IQ

Fifteen of the 19 patients underwent IQ testing. A total of 78 neuropsychological tests were performed (see supplementary Figure 2 for more details). At first assessment, the patients' age range was 0.6 to 45 years (Figure 1). Per patient, the follow-up period ranged from 1 to 13.3 years (median follow-up 4.3 years). At the latest assessment, ten patients were classified as neuronopathic (patients 2, 5, 6, 7, and 9-14; IQ range 2-50; age at latest assessment

range 5-20 years). Eight were classified as non-neuronopathic (patients 1, 3, 4 and 15-19; IQ range 96-112; age range at latest assessment 5-50 years). One patient scored just above the threshold for intellectual disability (patient 8, IQ=73; age at latest assessment 8 years) and for this reason was also included in the non-neuronopathic group (Table 1). On the basis of their social functioning and educational level, adult patients for whom no IQ data were available (patients 15,16,17 and 19) were also classified as non-neuronopathic. Disharmony of IQ profiles was evaluated in all patients for whom IQ was available (data not shown). No consistency in profiles was found.

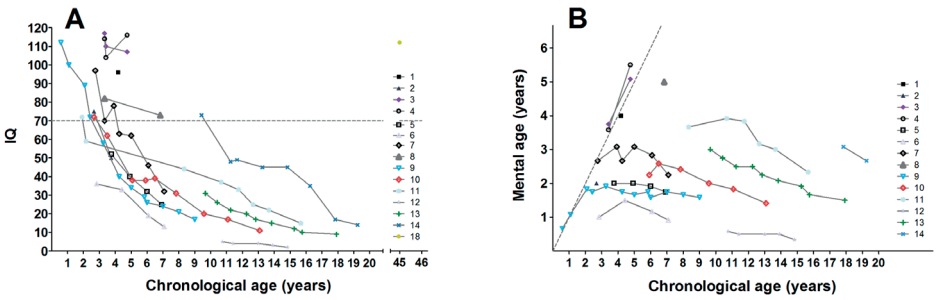
Figure 2. Magnetic Resonance Images of MPS II patients.



Examples of MRI images arranged from mild (left) to severe (right). A-C show Virchow-Robin spaces (VRS), D-F show white-matter abnormalities (WMA), and G-I show atrophy.
A. T2-FLAIR image of a neuronopathic patient at the age of 10.2 years; B. processed image of a non-neuronopathic patient at the age of 4.1 years; C. processed image of a non-neuronopathic patient at the age of 46.9 years. D. T2-FLAIR image of a non-neuronopathic patient at the age of 4.1 years; E. T2-FLAIR image of a neuronopathic patient at the age of 7.2 years; F. T2-FLAIR image of a neuronopathic patient at the age of 8.0 years; G. T1 image of a non-neuronopathic patient at the age of 2.3 years; H. T1 image of a neuronopathic patient at the age of 4.2 years; I. T1 image of a neuronopathic patient at the age of 14.2 years.

The repeated measurements of IQ over time (IQ vs. chronological age; see Fig. 3A), were further analyzed using a random-effects repeated-measurement model, which showed a mean group decline of 6.7 IQ points per year. More specifically, in neuronopathic patients loss of IQ was initially rather rapid, which slowed down in time due to a floor effect (Figure 3A). The maximal mental-age equivalent for neuronopathic patients was 4 years (Figure 3B). It should be noted that plateauing in mental age actually represented a strong decline in IQ. Since IQ scores are age related the gap between patients and age related peers grows in time (Fig 3A & 3B). The IQ of the borderline non-neuronopathic patient (patient 8) declined by 9 IQ points in 3.5 years. The other non-neuronopathic patients for whom IQ was available all had an IQ above 96 and continued to do so over a follow-up period of 1.5 years.

Figure 3. Neuropsychological follow-up



The numbers in the legends correspond to the patient numbers shown in Table 1; A. IQ data derived on the basis of all available neuropsychological tests used; B. Mental age determined on the basis of the Griffiths mental-developmental scales. Figure 3B does not include neuropsychological tests other than the Griffiths mental developmental scales. No neuropsychological data was available for patients 15, 16, 17, and 19.

IQ and cerebral MRI

Figures 4 A-C comprise a 3D chart integrating the following three variables: age in years (x-axis), IQ scores (y-axis) and MRI sumscores of brain atrophy (Figure 4A), white-matter abnormalities (Figure 4B) and Virchow-Robin spaces (Figure 4C). See supplementary table 1 for more details on the calculation of sumscores. The size of the bubbles indicates the magnitude of the MRI sumscores (Figure 4 A-C). Since we had no combined IQ and MRI data for the adult patients, these are plotted below the x-axis of Figure 4A-C. Fig. 4A shows the increase of the cerebral atrophy sumscore for neuronopathic patients over time. The non-neuronopathic patients had little or no atrophy on MRI. Overall, the atrophy sumscore showed a strong inverse correlation with IQ scores (correlation= -0.90).

Like atrophy, the white-matter abnormalities generally progressed over time in neuronopathic patients (Figure 3B). This is indicated by the inverse correlation of the WMA sumscore with IQ scores (correlation= -0.69). However, this correlation was not as strong as for atrophy,

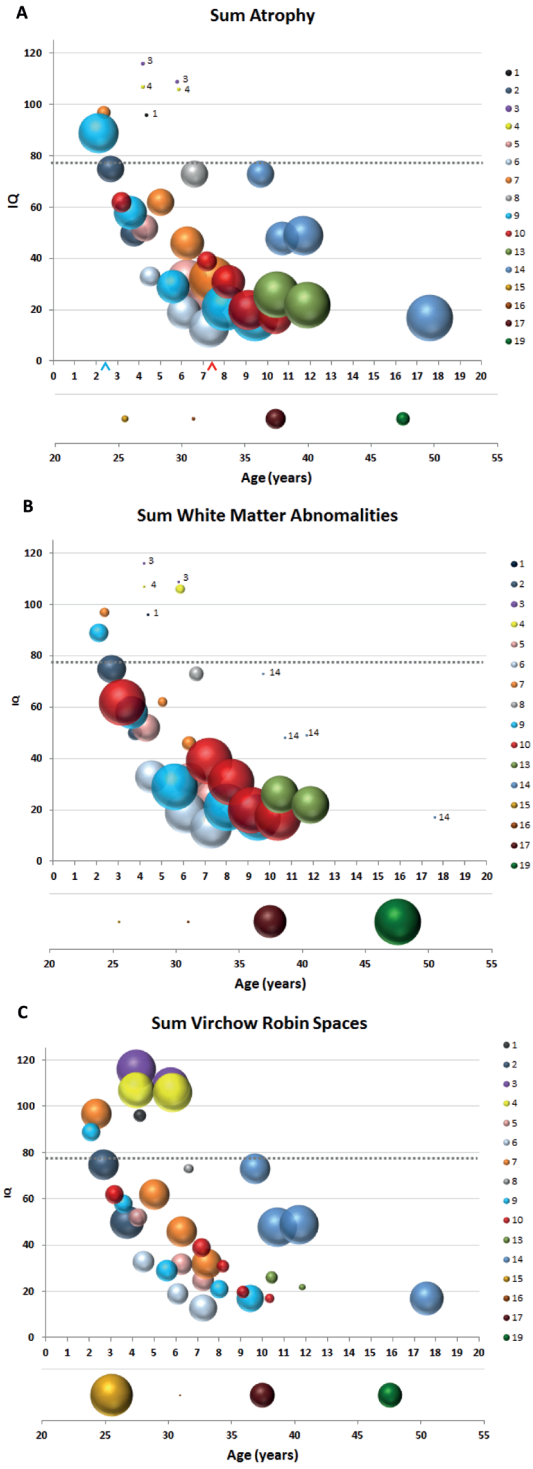


Figure 4. Integrated results of IQ, and MRI sumscores

This figure presents the combined data of our IQ and MRI results related to age. MRI and IQ scores were obtained at about the same time point with a median time difference between MRI scan and IQ assessment of 3.1 months (standard deviation of time difference = 2.4 months). As no IQ score was determined for patients 15, 16, 17, and 19, data for these patients are presented below the main figure. No MRIs were available for patients 11,12, and 18.

A. 3D chart of cerebral atrophy sumscore. This indicates the magnitude of the MRI sumscores, ranging from 0= smallest bubble to 7= largest bubble. The arrows indicate the VP drain placement of patients 9 (blue) and 10 (red).

B. 3D chart of white-matter abnormalities sumscore. This indicates the magnitude of the MRI sum scores, ranging from 0= smallest bubble to 10= largest bubble

C. 3D chart of Virchow-Robin spaces sumscore. This indicates the magnitude of the MRI sumscores, ranging from 0= smallest bubble to 14= largest bubble. No patient had a VRS sumscore above 14.

illustrated by patient 14, who had a neuronopathic presentation, and no white-matter lesions at 17 years of age, whereas some of the non-neuronopathic patients did show clear white-matter lesions and high WMA sumscores (Fig. 4B).

Virchow-Robin spaces were seen in both neuronopathic and non-neuronopathic patients (Figure 4C). However, unlike the scores for atrophy and white-matter abnormalities, the VRS scores did not seem to progress over time. Due to the wide range of scores and the lack of change in these scores over time, no statistically significant model could be fitted and no correlation between VR and IQ could be calculated.

DISCUSSION

In this prospective follow-up study of MPS II patients, we quantified brain atrophy, white-matter abnormalities, and the number and size of Virchow-Robin spaces, and related them to IQ scores. Our results are based on data of patients with a wide age range (from under 1 year to 47.5 years); a long follow-up; a large number of assessments (47 MRIs and 78 neuropsychological tests); and both neuronopathic and non- neuronopathic patients. The IQ of patients in this study declined by 6.7 points per year, based on the full cohort. The decline in IQ score was strongly correlated with an increase in brain atrophy. The IQ decline and progression of white-matter lesions were also related, but less strongly. In contrast to that, VRS sumscore could not be related to IQ scores and did not progress strongly over time.

IQ

Approximately two-thirds of MPS II patients develop a neuronopathic phenotype.[2, 3] In our cohort, a majority of patients (10 patients), were classified as neuronopathic.

In neuronopathic MPS II patients, cognitive development has been reported to diverge from that of peers at the chronological age of 4-4.5 years.[2, 4] These authors identified three phases in cognitive development in neuronopathic MPS II patients. In the first years of life, cognitive development was normal or close to normal. Thereafter, in the plateauing phase, these patients grew into deficit, leading to stagnation in mental age. Finally, the loss of skills resulted in a decrease in mental age. Our study indicates stronger variations in the (1) initiation and (2) duration of the plateauing phase. Patients in our cohort dropped below the IQ threshold of 70 at ages between 3 and 10 years. This variation is further illustrated by two patients: patient 8 of our cohort, who was classified as borderline non-neuronopathic at the age of 6 years, when he had a mental age of 5; and patient 14, who was classified as neuronopathic but still had IQ scores above 70 at the age of 9 years. Also, the three youngest non-neuronopathic patients in our cohort (patients 1,3 and 4), had IQ scores ranging

from 96-116 when in the 5-6 year age range. Although they performed notably better on neuropsychological testing than their neuronopathic peers at the same age, we do not rule out future neurocognitive decline. This indicates the importance of including other markers that may predict the neuronopathic phenotype. The marker we used in our study was MRI.

MRI

Our ultimate goal was to find a predictive tool for neurocognitive outcome in young children with MPS II. Our findings indicate that atrophy and white-matter changes are important hallmarks of neuronopathic MPS II. This is in agreement with earlier studies [3, 12, 15-17].

In our study, the sumscore that showed the strongest correlation to IQ was atrophy. Importantly, in contrast to the non-neuronopathic patients, none of our neuronopathic patients had normal atrophy scores (score 0) at any point. The earliest MRI, in a patient who had been diagnosed prenatally, was performed at the age of 2 years and already showed abnormal atrophy scores. When this MRI was performed, he had an IQ of 89, and showed no abnormal behavior. It should be noted that high MRI atrophy sumscores could not only be caused by a reduced amount of brain tissue as we assume in this study, but also by hydrocephalus All patients in our study with an atrophy sum score of 3 or more also had wide fissures and sulci. Since hydrocephalus requires surgical intervention it is important but difficult to discriminate between the two. On the basis of our results, we can conclude that, whatever the cause, a high atrophy sumscore in a young MPS II patient is a sign that the development of a neuropathic phenotype is imminent.

Various studies concluded that neuronopathic patients presented with more severe WMA. [3, 12, 15, 16] Our own study is in partial agreement with this. Although we, too, found a correlation between IQ and WMA (-0.69), it was not as strong as the correlation between IQ and atrophy. This weaker correlation is further illustrated by two individuals from our cohort. First, in a non-neuronopathic patient, patient 19 in our cohort, we found extensive WMA abnormalities with little atrophy at the age of 46 years. Second, despite his progressive atrophy, patient 14 in our cohort—who had a neuronopathic phenotype—did not show WMA during the full 10 years of follow-up. Therefore, we conclude that atrophy may occur without WMA, and that WMA are not inevitably related to the development of severe intellectual disability. The causal relationship between neuronal degeneration and WMA visible on MRI is still not fully elucidated. In a recent study, Zalfa et al. investigated the causal relationship in mice and men between WMA, neuronal degeneration and pathological features at cellular level. The authors identified three stages of the progression of MPS II in the brain. It started with neuro-inflammation, was followed by glial degeneration and eventually resulted in neuronal degeneration. [18] It has even been shown that abnormalities at the cellular level may already

occur during fetal life.[19, 20] However, it is not entirely clear at which stage this sequence of events eventually produces WMA visualized by MRI.

The vast majority of patients showed VRS on MRI in both adults and children. As in other studies, most were located periventricularly (in 87% of patients); subcortically (in 87%); in the corpus callosum (in 80%); and in the basal ganglia (in 60%). Many other studies have indicated that VRS occur in both neuronopathic and non-neuronopathic patients.[11, 13] As we showed recently for MPS VI, VRS are also found in other MPS types that are not directly associated with intellectual disability.[21] It should be noted that VRS surround the walls of vessels as a 'sleeve' connected with the subarachnoid space, and are therefore located outside the blood-brain barrier.[22] Although ERT did not seem to dissolve the VRS during the study period of our cohort, it is still unclear whether the stabilization in size and amount of VRS can be attributed to ERT. The VRS score was not related to IQ scores, and the size and the number of VRS did not change over time. We may therefore conclude that VRS should be ruled out as an indicator for IQ decline.

The protocol we used is a qualitative method for structurally analyzing cerebral MRIs, which was derived from four previously described protocols. [3, 11-13] Lee et al., was the first to demonstrate that a standardized MRIs scoring system could be useful for comparing individuals with MPS. Subsequently, Manara et al. and Seto et al. showed that this approach was also useful for objectifying individual MRI changes over time by using repeated MRIs measurements. In the current study, we not only used our protocol to compare individuals, or to describe changes over time, but we additionally used the protocol for calculating sum scores on atrophy, white-matter abnormalities and Virchow-Robin spaces. This enabled us to correlate the course of IQ with the course of three different MRI sumscores on group level. In future, it might be of interest to compare or combine our approach with the one recently published by Yund et al. on a computerized method to quantify corpus callosum, gray-matter and white-matter volumes in non-neuronopathic patients. As the authors indicated, a potential pitfall of using on a computerized method alone might be that VRS interfere with volume quantification. This would require further investigation.

CONCLUSION

The ultimate goal of our study was to find a predictive tool for neurocognitive development in young children with MPS II. We therefore analyzed the long-term follow-up of IQ and MRI data. We found that the increase in atrophy sumscores strongly correlate with decline in IQ. Abnormal atrophy sumscores are already present before IQ drops below the threshold of

intellectual disability (<70). Atrophy sum scores may therefore serve as an early marker of the neuronopathic phenotype of MPS II.

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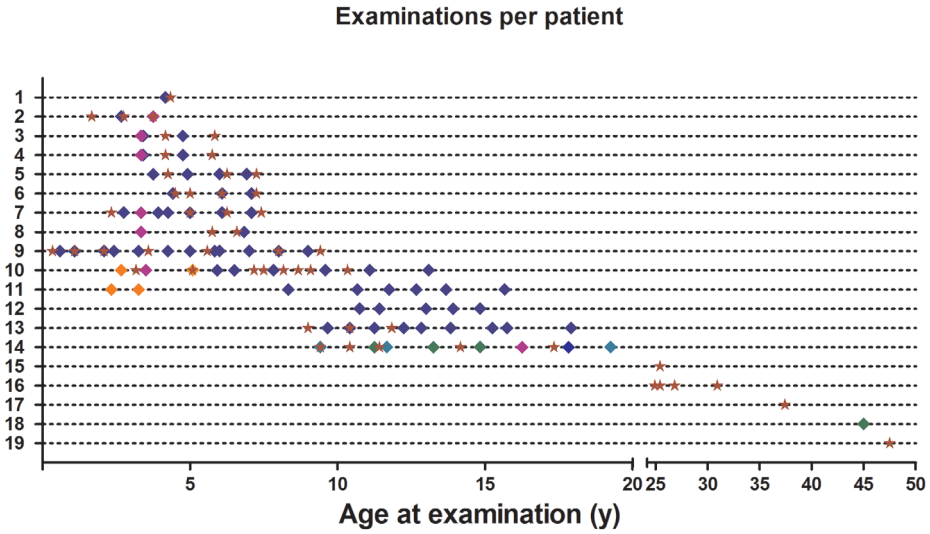
Supplement 1. MRI protocol

MRI Protocol			
1. Virchow-Robin Spaces	Basal nuclei	0 = absent 1 = <10 VRS, <3mm 2 = >10 VRS, <3mm 3 = <10 VRS, ≥ 3 mm 4 = >10 VRS, ≥ 3 mm	VRS-sumscore.
	Amygdala	0 = absent 1 = <10 VRS, <3mm 2 = >10 VRS, <3mm 3 = <10 VRS, ≥ 3 mm 4 = >10 VRS, ≥ 3 mm	
	Corpus Callosum	0 = absent 1 = <10 VRS, <3mm 2 = >10 VRS, <3mm 3 = <10 VRS, ≥ 3 mm 4 = >10 VRS, ≥ 3 mm	
	Subcortical white matter	0 = absent 1 = <10 VRS, <3mm 2 = >10 VRS, <3mm 3 = <10 VRS, ≥ 3 mm 4 = >10 VRS, ≥ 3 mm	
	Periventricular	0 = absent 1 = <10 VRS, <3mm 2 = >10 VRS, <3mm 3 = <10 VRS, ≥ 3 mm 4 = >10 VRS, ≥ 3 mm	Maximun sumscore: 20
2.White-matter abnormalities	Occipital lesions with increased white-matter signal	0 = absent 1 = periventricular 2 = subcortical	WMA-sumscore.
		0= absent 1= <3mm 2 = 3-10 mm 3 = > 10 mm	
	Frontal lesions with increased white-matter signal	0 = absent 1 = periventricular 2 = subcortical	
		0= absent 1= <3mm 2 = 3-10 mm 3 = > 10 mm	Maximun sumscore: 10

Supplement 1. MRI protocol (continued)

MRI Protocol			
3.Atrophy	Third ventricle	0 = normal aspect 1 = mild widening 2 = severe widening	Cerebral atrophy sumscore
	Lateral ventricles	0 = normal aspect 1 = mild widening, symmetric 2 = mild or intermediate widening, asymmetric 3 = severe widening, symmetric or asymmetric	
	Fissures/sulci	0 = normal aspect 1 = mild widening of fissures/sulci 2 = severe widening of all fissures and sulci	Maximum sumscore: 7
4. Other	Corpus callosum size	0 = normal 1 = atrophic	
	Optic nerve sheath diameter	0 = normal, ≤ 5.7 mm 1 = unilateral enlargement, > 5.7 mm 2 = bilateral enlargement, > 5.7 mm	
	Hyperostosis	0 = No 1 = Yes	
	Sella turcica enlarged	0 = No 1 = Yes	
	Sella turcica shape	0 = normal 1 = J- or W-shaped	
	Shape of the skull	0 = normal 1 = abnormal Type: □ Scaphocephalic □ Plagiocephalic □ Trigonocephalic □ Brachycephalic	
	Mega cysterna magna	0 = No 1 = Yes	
	Chiari malformation	0 = absent 1 = type I (tonsils) 2 = type 2 (tonsils, cerebellum, brainstem involved)	

Supplement 2. Overview of MRI and neuropsychological assessment



The numbers on the y-axis correspond to the patient numbers shown in Table 1. A red stars represent a single MRIs. The diamonds represent IQ measured with either Griffith (blue), WISC/WAIS (green), SON-R (purple), BSID-II (orange), and the K-ABC (blue-green). Tests measured outside our centre were: 1 WAIS-IV, 2 K-ABC, 3 WISC-III, 4 BSID-II, and 7 SON-R tests.

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Chapter 10

Cognitive endpoints for therapy development for neuronopathic mucopolysaccharidoses: results of a consensus procedure.

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ABSTRACT

The design and conduct of clinical studies to evaluate the effects of novel therapies on central nervous system manifestations in children with neuropathic mucopolysaccharidoses is challenging. Owing to the rarity of these disorders, multinational studies are often needed to recruit enough patients to provide meaningful data and statistical power. This can make the consistent collection of reliable data across study sites difficult. To address these challenges, an International MPS Consensus Conference for Cognitive Endpoints was convened to discuss approaches for evaluating cognitive and adaptive function in patients with mucopolysaccharidoses. The goal was to develop a consensus on best practice for the design and conduct of clinical studies investigating novel therapies for these conditions, with particular focus on the most appropriate outcome measures for cognitive function and adaptive behavior. The outcomes from the consensus panel discussion are reported here.

INTRODUCTION

Mucopolysaccharidoses are inborn errors of metabolism characterized by the progressive accumulation of glycosaminoglycans in tissues throughout the body.[1] There are currently eleven known mucopolysaccharidoses, each caused by a different lysosomal enzyme deficiency.

Mucopolysaccharidoses vary in their prevalence and presentation, although most include extensive somatic involvement affecting the heart, lungs, airway, bones, joints, vision, hearing, and gastrointestinal system.[1] In the most severe forms of mucopolysaccharidosis (MPS) types I, II and III, this is accompanied by central nervous system (CNS) dysfunction or decline, becoming evident in the second or third year of life and ultimately resulting in the loss of attained skills. The CNS manifestations of these conditions are devastating to parents, relentless in their decline, and result in premature death. CNS manifestations are also observed in MPS VII; an ultra-rare disease that is not discussed in this article.

Treatments for MPS I and II have been available for several years in the form of hematopoietic cell transplantation (HCT) and enzyme replacement therapy (ERT). Although both have been found to have benefits in addressing and preventing progression of many of the somatic features of these disorders,[2-16] only HCT has been found to have any effect on CNS decline owing to the inability of ERT to cross the blood–brain barrier.[16-20] Currently, there are several potential disease-modifying products in pre-clinical and clinical development to address the CNS manifestations of MPS I, II and III, with the ultimate aim of preventing or halting the neurologic decline characteristic of these disorders. The design and conduct of clinical studies to evaluate the effects of novel therapies on CNS manifestations in children with neurodegenerative diseases is challenging. The most appropriate measures of the effects of novel therapies on the CNS are changes in cognitive function and adaptive skills (i.e. the ability to engage in day-to-day activities). Until now there have been a great variety of approaches taken to evaluate cognition and adaptive behavior in patients with mucopolysaccharidoses, which is perhaps understandable given the plethora of psychometric measurement instruments available for these purposes. To enable clinicians, investigators, regulatory bodies and caregivers to fully understand the relative effectiveness of treatments for mucopolysaccharidoses, it is essential that standard protocols are applied consistently to ensure reliable measurement of cognitive outcomes and adaptive behavior in clinical trials. The importance of this was emphasized at a workshop convened by the Food and Drug Administration on cognitive assessment in inborn errors of metabolism and in guidelines developed by the National Institutes of Health (NIH; www.nlm.nih/gov/cde).[21,22] Owing to the rarity of mucopolysaccharidoses, multinational studies are often needed to recruit enough patients to provide meaningful data and achieve statistical power. However, this

brings with it diversity of testing languages and cultures. The availability of the most up-to-date versions of tests also varies between countries, meaning that older versions of a psychometric measurement instrument may be used by some countries within the same study.

To address these challenges, an International MPS Consensus Conference for Cognitive Endpoints took place on 2–3 December 2016, organized by the US and UK MPS Societies and supported by industry. During this meeting an international panel of experts was convened to discuss approaches for evaluating cognitive function in patients with mucopolysaccharidoses. The goal was to achieve consensus on best practice for the design and conduct of clinical studies investigating novel therapies for these conditions, with a focus on the most appropriate outcome measures for cognitive function and adaptive behavior. The outcomes from the consensus panel discussion are reported here.

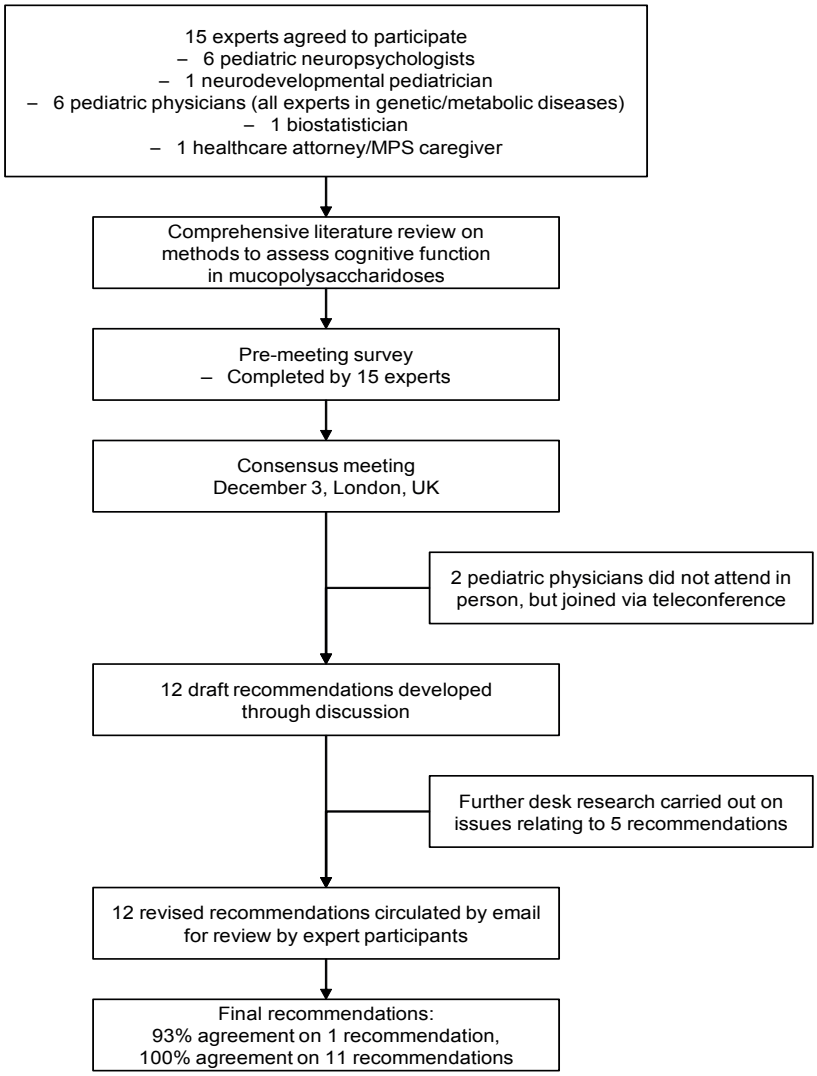
METHODS

A modified Delphi technique was used to reach consensus on best practice for evaluating cognitive and adaptive function in patients with mucopolysaccharidoses. This methodology, developed by the Rand Corporation/University of California, Los Angeles (UCLA), CA, USA,[23] is based on the original Delphi process[24] which has been widely used to achieve consensus on a specific issue and is increasingly used for the developing of clinical guidelines when there is insufficient evidence.[20,25,26] An overview of the consensus process is shown in Figure 1.

In consultation with the UK Society for Mucopolysaccharide Diseases and US National MPS Society, an 18-member steering committee was formed and chaired by Elsa Shapiro, PhD. A comprehensive literature review was performed by a member of the steering committee – a psychologist with expertise in another inborn error of metabolism (DJ) – to consolidate the best available published information on the methods used to assess cognitive function and adaptive behavior in patients with MPS diseases, including psychometric properties, usefulness in various settings, and use and sensitivity to change in MPS diseases. Full details and findings from the literature review can be found in Janzen *et al.* elsewhere in this issue of *Molecular Genetics and Metabolism*.[27] The steering committee discussed and determined the composition of an expert panel to participate in a Delphi consensus process. The final composition of the expert panel included four pediatric neuropsychologists with expertise in mucpolysaccharidoses, two pediatric neuropsychologists with expertise in other neurological conditions, one neurodevelopmental pediatrician with expertise in psychological assessment in mucopolysaccharidoses, six pediatric physicians with expertise in mucopolysaccharidoses, a statistician, and a healthcare attorney/MPS caregiver. All participating

clinicians and psychologists have authored peer-reviewed publications on mucopolysaccharidoses, with the exception of two pediatric neuropsychologists who have published extensively on neurocognitive testing in their respective fields.

Figure 1. Flow chart of the consensus process.



The expert panel convened for a 1-day face-to-face meeting in London, UK. The meeting was facilitated by an independent clinical epidemiologist with experience of conducting Delphi-style consensus panels. The focus of the meeting was approaches for evaluating cognitive function and adaptive behavior in patients with MPS I, II or III. Methods for assessing behavior were not included in the discussion.

Before the meeting the panel members were provided with information about 14 measurement instruments previously used to evaluate cognitive outcomes in patients with mucopolysaccharidoses; these tools are discussed in detail in Janzen *et al.* elsewhere in this issue of *Molecular Genetics and Metabolism*.[27] Having reviewed this information, panel members with asked to answer an e-mail survey about:

- Whether they would want to consider these instruments during the consensus meeting, and
- Their assessment of the importance of 14 measurement characteristics on an 11-point scale (0, not important; 10, very important). Measurement characteristics included the psychometric properties of the instruments (e.g. reliability, sensitivity, validity), feasibility, and cross-cultural relevance.

During the consensus meeting, all panel members participated in setting the agenda; deciding the topics for discussion and areas for recommendation. With regard to choice of which measurement instrument to use in each disease and age group, panel members were first asked to eliminate those that were not relevant or deemed not suitable for use, and then to rank those measurement instruments that remained. Subsequently, a draft statement for each topic was proposed by the moderator or by one of the panel members and discussed by all panel members. The formulation of each statement was adapted during the discussion until there was consensus. When considered necessary, the literature review and survey results were consulted to provide evidentiary support and to remind panel members of their own priorities.

For some statements, it was decided during the meeting that additional information from the literature was needed, and several panel members agreed to provide that information after the meeting. This information was incorporated into the statements before they were presented to the panel members for final approval.

Following the consensus meeting and supplementary research, a full draft of all consensus statements was sent by email to the panel members for comment. In instances where the position of the panel was not clear, further surveys were sent to all panel members to further explore the topic in question and to formulate potential recommendations. Suggested amendments were discussed and agreed via email and incorporated into the final statements presented here.

RESULTS

In the pre-meeting survey, six members (all experts in psychological testing) of the 15-person panel provided input into which of the 14 measurement instruments should be considered during the consensus meeting. The results are shown in Table 1. The instruments that all respondents indicated they would consider were the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III); the Kaufman Assessment Battery for Children, Second Edition (KABC-II); and the Vineland Adaptive Behavior Scales, Third Edition (Vineland-3). The Stanford Binet Intelligence Scales, Fifth Edition (SB5) was the least considered; only one respondent wanted to include this instrument in discussions. Full descriptions of each of the instruments discussed in this article and an explanation of technical terms can be found in Janzen *et al.* elsewhere in this issue of *Molecular Genetics and Metabolism*.[27]

Table 1. Proportion of respondents stating that a particular measurement instrument should be considered for discussion.

Category	Instrument	Proportion of positive respondents (n = 6)
Test for children <6 years of age	Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) [28]	100%
	Mullen Scales of Early Learning (MSEL) [29]	50%
	Griffiths Scales of Child Development, Third Edition (Griffiths-III) [30]	33%
	Differential Ability Scales, Second Edition (DAS-II) [31]	67%
	Wechsler Preschool and Primary Scales of Intelligence, Fourth Edition (WPPSI-IV) [32]	67%
	Stanford Binet Intelligence Scales, Fifth Edition (SB5) [33]	17%
	Kaufman Assessment Battery for Children, Second Edition (KABC-II) [34]	100%
	Leiter International Performance Scale, Third Edition (Leiter-3) [35]	83%
Observer-reported outcomes, adaptive Skills	Vineland Adaptive Behavior Scales, Third Edition (Vineland-3) [36]	100%
	Scales of Independent Behavior, Revised (SIB-R) [37]	50%
	Adaptive Behavior Assessment System, Third Edition (ABAS-3) [38]	50%
>6 years of age	Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI-II) [39]	67%
	Wechsler Intelligence Scale for Children, Fifth Edition (WISC-V) [40]	67%
	Wechsler Adult Intelligence Scale, Fourth Edition (WAIS-IV) [41]	67%

Thirteen of the 15 panel members submitted their assessment of the importance of the 14 measurement characteristics of the tools to be discussed (Table 2). "Sensitivity to change" and "feasibility for the specific disease" were rated the most important characteristics for measurement tools. "Ease of administration" received the lowest score; although there was not much variability between the scores given each characteristic. Median scores ranged between 8 and 10. Two further characteristics were added, each by one respondent: "ability of reflect actual

cognitive ability and not merely behavioral aspects of the child on that day", which received a score of 8, and "acceptability to the participant", which received a score of 7.

Table 2. Assessment of the importance of measurement characteristics (scale, 1–10).

	Importance score (n = 13)				
Characteristic	**Mean**	**SD**	**Median**	**Min.**	**Max.**
Sensitivity to change	9.08	0.93	9	7	10
Feasibility for the specific disease	9.08	1.26	10	5	10
Applicability to a range of functional levels	8.92	1.56	9	7	10
Development characteristics of the test	8.46	1.94	8	7	10
Cross-cultural validity	8.23	1.74	8	4	10
Error of measurement	8.08	1.83	8	7	10
Availability of modern normative data	7.77	0.95	8	6	10
Concurrent validity	7.77	1.36	8	5	10
Content of face validity	7.54	1.44	8	5	10
Availability and familiarity	7.54	1.04	8	5	10
Construct validity	7.46	1.24	8	4	10
Interpretability – MCID*	7.23	1.98	8	5	9
Ease of administration	7.15	1.13	8	4	10
Additions by responders (n = 1 for each)					
Ability of reflect actual cognitive ability and not merely behavioral aspects of the child on that day	8.00	-	8	8	8
Acceptability to the participant	7.00	-	7	7	7

*MCID, minimal clinically important difference

At the conclusion of the consensus meeting, a series of 12 initial consensus recommendations was presented to the group. Following the meeting, further background research and discussion and revision of the draft statements via email led to 93% consensus (14 of 15) on the first statement and full consensus, indicated by expression of agreement by all expert panel members, on the remaining 11 statements. All are described below.

1. For trials evaluating the effect of treatment in children with MPS I, II or III aged up to 3 years (age equivalent), the recommended instrument to measure cognitive outcomes is the Bayley-III

Rationale

Of the available tools for evaluating cognitive function in this age group, both the Bayley-III (and earlier versions of this measure) and Mullen Scales of Early Learning (MSEL) have been used extensively in clinical studies of mucopolysaccharidoses in the past,[6-9,14,15,42-61] and both have been shown to be feasible and sensitive to change in patients with these

conditions.[8,12,42-45] Although earlier versions of the Griffiths Scales of Child Development have also been used to study the characteristics, natural history and treatment outcomes in mucopolysaccharidoses, experience with the latest iteration is limited. Furthermore, the absence of validated translated versions of the Griffiths-III and the requirement for intensive training on the test indicate that it is not recommended for use in clinical trials currently, although this may change in the future.

The availability of validated translations of each measurement tool is particularly important given the multinational nature of most trials for mucopolysaccharidoses. The Bayley-III has been translated and validated in multiple languages, making it an attractive option for use in multicenter studies, whereas the MSEL has been translated in only a few countries. Another distinguishing factor between the Bayley-III and MSEL is the recency of the underlying normative data (Bayley-III, 2004; MSEL, 1980s).[62] Generally, performance may be overestimated in tests that have normative data more than a decade old due to the Flynn effect of increasing IQ over time.[63]

A show of hands from panel members indicated that the Bayley-III was used most often in patients under 3 years in this group, although it is not yet available in all countries. It was acknowledged that the relatively narrow development age range of the Bayley-III (1–42 months) compared with other instruments (MSEL, 0–68 months; Griffiths-III, Birth–6 years) may necessitate transition to an alternative instrument in long-term studies.

With these factors in mind, there was 93% consensus among panel members that the Bayley-III may be the most suitable instrument for widespread use in clinical trials evaluating cognitive function in patients with MPS I, II or III, based on the widespread availability of validated translations of this instrument and the recency of its normative data. One panel member did not agree with this recommendation, preferring a statement recommending both the Bayley-III and the MSEL; the choice depending on the clinical trial design and the feasibility of use in the population being studied.

2. For trials evaluating the effect of treatment in children with MPS I, II or III of all ages, the recommended instrument to measure adaptive behavior is the Vineland, using the extended interview format

Rationale

Measures of adaptive behavior help to put scores of cognitive function into context. A show of hands by panel members indicated that the majority used the Vineland-2 most often when evaluating children with MPS I, II or III. The Vineland-3 would also be appropriate, but has only recently been introduced. The Scales of Independent Behavior, Revised (SIB-R) was

viewed as out of date and is rarely used by this group. The Adaptive Behavior Assessment System, Third Edition (ABAS-3) is never used by this group. These comments are consistent with the number of published studies that have used the Vineland-2 (27 studies), SIB-R (9 studies) and ABAS-3 or prior versions (0 studies) in patients with mucopolysaccharidoses.[7,9,12,14,43-46,48,50,51,55,56,64-79]

The recently updated Vineland-3 benefits from modern normative data (2014–2015); especially compared with the SIB-R (1990s), which is viewed as out of date. The Vineland-3 is also well correlated to changes in cognitive function compared with the SIB-R and ABAS-3,[80] and it can be used to determine which battery of cognitive tests is suitable based on developmental age.[68] With these factors in mind, the Vineland-3 is recommended as an instrument for evaluating adaptive behavior in patients of all ages with mucopolysaccharidoses.

The panel noted, however, that to be fully informative, the extended interview format (approximately 45 minutes) of the Vineland-3 must be used. Wherever possible when using this format, the same caregiver should be the informant on every visit to ensure consistency across time points, and examiners must receive training on how to administer patient/caregiver interviews most effectively. A disadvantage of the Vineland is the limited availability of the latest version (3) in non-English languages. However, strong correlation between the second edition (Vineland-2) and the Vineland-3 means that the earlier version of this instrument, which is widely available in non-English languages, can still be used in clinical studies as an alternative. The newer version also has an improved parent rating form, which may provide a useful alternative assessment during follow-up visits or when trained interviewers are not available.

3. For (multinational) trials evaluating the effect of treatment in children with MPS I aged 3 years and over (age equivalent), the recommended instruments to measure cognitive outcome are the Wechsler tests. We recognize the utility of the Kaufman Assessment Battery for Children, Second Edition (KABC-II) and the Differential Ability Scales, Second Edition (DAS-II) in particular populations because of their reduced fine motor demand and less emphasis on speed of performance compared with the Wechsler tests

Rationale

Of the instruments available for evaluating cognitive function in patients with a developmental age of 3 years and over, two were not recommended for use in patients with MPS I. The Leiter-3 was originally designed for use in deaf children and, although the non-verbal nature of the instrument may have some benefits in this population, its lower reliability and validity compared with other available instruments meant that this test was not deemed

the strongest candidate for use in MPS I clinical studies. Similarly, the SB5 has a large verbal component that makes it suitable for use in older patients (e.g. 5–6 years), but not in children as young as 3 years with MPS I.

The DAS-II has been used successfully to assess the longitudinal effects of HCT in children with MPS I.[12] It was noted that the instrument can take a long time to administer (approximately 20–40 minutes) and includes a number of tests that rely on previous learning, which can be challenging for severely affected patients. The DAS-II is only available in English, although there is a Spanish-language supplement available in the early years version. Naturally, this will limit its utility in multinational clinical trials; however, the wide developmental age range suitable for the DAS-II (2.6–18.0 years) makes it an attractive option for longitudinal study within English- or Spanish-speaking populations.

The Wechsler Preschool and Primary Scales of Intelligence, Fourth Edition (WPPSI-IV) is available and validated in most languages and earlier versions of this measure have been used successfully to assess the longitudinal effects of bone marrow transplantation in children with MPS I.[8,15] Some patients with low developmental ages may struggle with the fine motor demand and greater emphasis on speed of performance of the WPPSI-IV compared with the DAS-II and KABC-II. On the other hand, the WPPSI-IV can be useful when monitoring children who have been treated successfully and continue to increase their cognitive skills. Importantly, minimally clinically important difference (MCID) values have been calculated for the WPPSI-IV. A potential limitation is the narrow age range of the WPPSI-IV (2.6–7.7 years), which would require patients to transition to another test once the ceiling has been reached. However, it is unlikely that many patients with MPS I will show an increase of more than 4 age-equivalent years during a clinical trial; plus, similarities in design mean that transition from the WPPSI-IV to the Wechsler Intelligence Scale for Children, Fifth Edition (WISC-V; age, 6.00–16.11 years) is likely to be straightforward.

The panel acknowledged the utility of the KABC-II non-verbal scale owing to its wide age range for use (3–18 years), good reliability and validity. It also benefits from ease of administration in children with hearing problems and in older patients with low levels of cognitive function. However, it was deemed to be less sensitive to change than the Wechsler tests in patients who are capable of performing the tasks required in the WPPSI-IV and WISC-V, and may be most useful in older low-function children with MPS I. Thus, the consensus among panel members was that age-appropriate Wechsler tests should be used in patients with MPS I whenever possible, unless the clinical trial design benefits from alternative measures.

4. For (multinational) trials evaluating the effect of treatment in children with MPS II or III aged 3–18 years (age equivalent), the recommended instruments to measure cognitive outcome are either the DAS-II or the KABC-II. The use of only the non-verbal domain/index may be appropriate if this is necessary to ensure consistent application between trial sites across multiple countries. Other factors to consider in the selection of the measure and on whether to use the entire test or only the non-verbal domain are: the need for verbal interaction; the time required to administer the test; fine motor requirements; availability of normative data; availability of translations.

Rationale

The SB5 and Leiter-3 were not considered appropriate for use in children with MPS II or III for the same reasons outlined above for MPS I.

As has been mentioned above, the WPPSI-IV and WISC-V can be challenging for patients with low development ages owing to task demands on fine motor skills and sustained attention span, and the emphasis placed on speed of performance. With this in mind, the panel felt that the Wechsler tests are not suitable for the assessment of cognitive function in children with MPS II or III, who often exhibit behavioral abnormalities and have low levels of cognitive function. If treatment is sufficiently effective to allow the child to increase the rate of skill development, as is observed in HCT in MPS I, and in all attenuated forms of mucopolysaccharidoses, then the Wechsler tests may be preferred.

The DAS-II has a large verbal component, which may pose a challenge to some patients with MPS II or III; although it is possible to calculate a cognitive function score based on the non-verbal subtests only. The DAS-II has been widely used in patients with MPS II and takes 20–40 minutes to complete, depending on the age and developmental capacity of the child.[44,45,48,71,81]

The KABC-II has been found to be sensitive to change in patients with MPS III,[43] and it is available and validated in multiple languages. Its non-verbal components take approximately 30 minutes to complete and can be done in pantomime; enabling the administrator to demonstrate to the patient how each task should be completed. This is particularly useful in patients with low developmental ages. For such patients, use of the non-verbal components only may be acceptable in some clinical trial settings with sufficient justification, especially in multinational studies or when patients must cross borders to take part in such initiatives.

Considering the above, the consensus of the panel is that the DAS-II or the KABC-II should be used in patients with MPS II or III with a developmental age of 3 years or older.

5. In a set of trials within the same program, we recommend using the same test protocol for all trial sites worldwide; including, if possible, the same test editions or, if not, the most recent

Rationale

Minimizing inter-rater variability is crucial to providing reliable data in multisite clinical trials,[82] so it is important that the same test protocol – including the specific measurement instrument, choice of subtests and method of administration – is applied to consistently high standard across all trial sites in a single study, but also across an entire program of clinical trials for a given treatment or disease. Importantly, the study protocol should be feasible for use in multiple centers and across multiple languages and cultures, and the risk of loss of accuracy and reliability of data in multicenter studies should be offset through the use of precise and simple measurements obtained by experienced, highly trained examiners.[68,83]

Wherever possible, the same version of the chosen measurement instrument should be used across all sites. Depending on the test being used, there can be great differences between versions, both in terms of the normative data underlying them and in their reliability and applicability in the specific patient population being studied, which can threaten trial data validity. In multinational studies this may present a challenge owing to the variable availability of translated and validated versions of some instruments in non-English-speaking countries.

In cases where the most recent version of a measurement instrument is not available in the local non-English language, it may be acceptable to make use of an earlier version of the same instrument across all sites when the two editions correlate well.

6. We acknowledge the usefulness and value of historical data that elucidate the natural history of MPS I, II and III, including standardized cognitive and developmental outcome measures other than those recommended in these consensus statements

Rationale

Although these recommendations provide guidance on the most robust and widely available instruments to use to evaluate cognitive function in patients with mucopolysaccharidoses in future multinational clinical trials, this does not discount the value and use of natural history data for these disorders that were generated using other cognitive outcome measures.

For example, the MSEL have been used extensively in the study of cognitive function in patients with MPS I, II and III,[9,12,14,44,45,48,50,57] and the Leiter-3 has played an important role in enhancing current understanding of the natural history of cognitive decline in patients with MPS III.[50] The panel strongly recommends that the value of these data be recognized and utilized as a basis for further study of the neurodegenerative changes in patients with mucopolysaccharidoses.

7. We strongly recommend building and sustaining an infrastructure to share natural history data

Rationale

As with many rare conditions, natural history data relating to cognitive function in patients with mucopolysaccharidoses are scarce. The geographic spread of patients with these conditions makes it likely that data are available for small cohorts of patients at individual treatment centers, with a variety of measurement instruments being used. These data provide valuable insights into the natural history of mucopolysaccharidoses, and the cumulative value of these findings is likely to be greater than the sum of their parts.

The panel strongly recommends the development of an infrastructure by which investigators around the globe can easily share and review each other's natural history data in a collaborative fashion. This will of course rely on the willingness of both industry members and investigators to collaborate. Recommendations for the design and implementation of such an initiative are beyond the scope of this article, but warrant further in-depth discussion.

8. We acknowledge that in multinational trials it may be necessary and appropriate to use one set of psychometrically sound normative data; however, this is only recommended for a non-verbal outcome measure. If a specified tool has not been validated in a country, we recommend the parallel use of a country-specific instrument to establish concurrent validity

Rationale

Ideally, all instruments used in a multinational trial will have been translated and validated for use in each country and language that is taking part in the study, taking into account cultural variations. This includes validation of normative data to ensure its appropriateness for use in the host country. If a fully translated and validated measurement instrument is not available for use across all study sites, the choice of normative data against which to evaluate changes in cognitive function becomes an important factor in the potential success of the trial.

In the absence of a validated translation of the measurement instrument, an additional country-specific test should be used in parallel to the main assessment to establish the concurrent validity of the two tests in each country. This procedure will help to calibrate the findings of the primary measurement instrument in each country.

9. We recommend the use of a standard written translation of the measurement instrument, including the administration instructions, produced by a professional translator with experience with standardized tests. Such a professional translation should always be accompanied by a back-translation. We also recommend cross-cultural adaptation. Lastly, we recommend that a local psychologist/psychometrician should review the fidelity of the translation and of the cross-cultural adaptation

Rationale

Translation of cognitive measurement instruments to non-English languages and cultures is a precise task that must be carried out in advance of a trial by a skilled professional translator with knowledge of psychological tests.[84] The translation should be provided as a standardized written script for use by all assessors when administering the test. Back-translations should always be carried out by a third party who is a native speaker to ensure the appropriateness of the translation. Where possible, the translation should also be reviewed by a local psychologist/psychometrician to evaluate the wording and the cultural suitability of the translated test.

We do not recommend that the assessor translate the instructions as the test is administered. Similarly, we do not recommend that verbal instructions to patients are translated by an interpreter in the room. These approaches may de-standardize the instructions provided to study participants, therefore introducing unnecessary variability.

For non-native patients attending a study site in another country, it is acceptable to provide a translator for the testing session to give non-test-related instructions and assistance. However, the translator must have received appropriate training so as not to inadvertently interfere with standardized test administration.

10. Assessors must be qualified in administering neurodevelopmental measurement instruments and have experience in their use, preferably with the disease being evaluated. Assessors need to be trained in person to perform the specific measurements in the protocol and should be subject to periodic quality control and auditing of scoring

Rationale

As has been mentioned already in this article, poor inter-rater reliability, along with poor interview quality and rater bias, can significantly impact the validity and statistical power of clinical studies.[82,85] The skilled administration of neurodevelopmental measurement instruments is therefore essential to the generation of reliable and robust data.[68]

The behavioral challenges that manifest in some patients with mucopolysaccharidoses mean that familiarity with these conditions is extremely beneficial when administering cognitive assessments.[83] In particular, assessors should have enough clinical experience with patients who have the disorder being evaluated at all levels of development to recognize and judge the severity of each cognitive symptom rated in the scale, and to determine the level of difficulty of tasks that a child with that condition is likely to be able to perform within the protocol.[68] Furthermore, all assessors must be trained in the use of the test as per the study protocol, including what constitutes an appropriate environment for assessment and how to recognize when a test result is not valid and data cannot be used.

We acknowledge that there are challenges in maintaining reliable and valid cognitive assessment for mucopolysaccharidoses. There is no current, established, published standard for training, supervision and ongoing maintenance of the quality of cognitive assessment, scoring, and data management in clinical trials. However, it is imperative that even experienced assessors receive regular re-training and are subject to quality control and monitoring of test administration.

11. We recommend analyzing and reporting age-equivalent scores in all trials, and standard scores where possible

Rationale

When considering endpoints for cognitive function in clinical trials there are three potential metrics that could be used: age-equivalent scores (i.e. the test score achieved by a healthy individual of that age), developmental quotient (i.e. ratio of age-equivalent scores to chronological age), and standard scores derived from test normative data.

Standard scores have traditionally been used as the primary measure of cognitive function in psychological tests; however, their use is problematic in patients with low levels of cognitive function.[68] The standard scores in most tests have a high floor that many patients with mucopolysaccharidoses will score below, making the scores insensitive to any change. Age-equivalent scores have been used in patients with mucopolysaccharidoses to examine treatment effects using both cognitive and adaptive behavior scales.[7-9,86] They are easily interpretable and, when used in a longitudinal context in neurodegenerative diseases, provide information about whether a child is developmentally progressing, stagnant or declining. Standard scores and developmental quotients do not provide this context, but do provide valuable information about the discrepancy between the patient's development and that of typically developing peers [68]. Thus, it is preferable to obtain both age-equivalent scores and standard scores or developmental quotients when possible.

12. When transitioning from one test to another because of developmental or chronological age, we recommend administering the two tests concurrently at least once during the same visit (on separate days) to compare the test results

Rationale

There is no single test that provides precise measures of all developmental domains from infancy to adulthood, making it necessary to use different tests across age boundaries. By overlapping two psychometric tests it is possible to cross-validate the test findings, to see what the discrepancy is and adjust for that discrepancy in the trajectory.[87] The choice of initial and subsequent tests, the age-equivalent when the transition should occur, and the approach to transition between them, should be defined in the study protocol and/or statistical analysis plan.

DISCUSSION

The promise of novel therapies that address the varied CNS manifestations of mucopolysaccharidoses is encouraging for both families affected by these devastating conditions and the clinicians who treat them. However, before these treatments can be made available to patients, it is essential that reliable and consistent data are obtained so that we can fully understand and feel confident of the effects they have on neurocognitive development. This consensus document has been developed to provide a clear set of considered recommendations based on all available evidence and decades of experience of designing and administering neuropsychological studies in patients with mucopolysaccharidoses and related conditions.

Avoiding missteps in the design and implementation of clinical studies to evaluate natural history and treatment effects on cognition and adaptive behavior requires an in-depth knowledge of the relative strengths and weaknesses of the available measurement tools and when it is appropriate to use them. Investigators must also consider which scoring system to use to ensure that improvement is detectable, and also have sufficient practical experience of the conditions being studied to predict and circumvent potential methodological challenges associated with the somatic and behavioral features of the disease. Until now, the formal guidance available to study sponsors and clinicians working the field of mucopolysaccharidoses has been limited and varied.[46,68,88,89] with much of the advice provided by the authors of this article. By bringing together recognized experts in the field and facilitating consensus development through a structured Delphi-based process, we believe the recommendations listed here will provide much-needed clarity to a complex yet important area of study.

It was not possible to address here every issue associated with the study of neurocognition and adaptive behavior, as some aspects are beyond the scope of this consensus development process. For example, it is desirable to calculate MCID values for each of the cognitive tests discussed, but this is not currently possible without further research. Similarly, there needs to be extensive and dedicated discussion of how to develop and maintain an infrastructure for the sharing of natural history data among investigators. Both issues warrant investigation and action as a matter of priority.

This is the first example of such a consensus panel on pediatric cognitive and adaptive measures as endpoints in clinical trials in lysosomal or other neurodegenerative diseases. As new treatments are developed, such a disease-specific consensus may be a model for conditions other than mucopolysaccharidoses. It should be noted that the recommendations described here reflect current understanding and experience with the instruments available. New tools and new editions and translations of current tests will inevitably become available in the future. With this in mind, it will be important that this guidance is reviewed and updated regularly (e.g. every 3 years) by a panel of appropriate qualified experts.

5. CONCLUSION

The modified Delphi process described here was successful in generating 12 expert consensus recommendations relating to best practice for the design and conduct of clinical studies for novel therapies for MPS I, II and III. It is hoped that the guidance provided in this article will contribute to the development of robust clinical programs and study protocols that will help accelerate the development of novel therapies to address the neurologic impact

of these devastating conditions, and enable clinicians, regulatory bodies and patients/caregivers to derive a clear unbiased understanding of the relative benefits of the treatment options available to them.

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PART 3

Chapter 11

General discussion and future perspectives

DISCUSSION

In this thesis we aimed to acquire knowledge on neuropsychological functioning in several lysosomal storage diseases, specifically in classic infantile Pompe disease, MPS II and VI, and alpha-mannosidosis. In this discussion section, we will first summarize the main findings on neuropsychological outcome and brain abnormalities in these patient populations (part 1). Subsequently we will reflect on the potential relation between the neuropsychological outcome and the abnormalities seen on brain MRI per disease (part 2). Thereafter we will discuss the effect of the lysosomal storage on the brain, and possible mechanisms involved for each LSD (part 3). Finally we will present in part 4 the similarities and differences in these lysosomal storage diseases, while integrating part 1, 2 and 3, and give recommendations for further research (part 5).

Part 1. Long-term neuropsychological outcome and brain abnormalities.

Classic infantile Pompe's disease

In classic infantile Pompe disease, glycogen mainly stores in muscles leading to death within the first year of life.[1, 2] To a lesser extent glycogen accumulates in the brain as well.[3-11] With the introduction of enzyme replacement therapy, the perspective of patients with this lethal disease drastically improved, making a long-term future within reach.[12-15] At start of this thesis, the cognitive development in patients with classic infantile Pompe disease was described in only a few studies with very young children no older than three years, mostly as case reports.[14, 16-19] Their developmental outcome varied between normal to severely delayed. With survivors now being on the edge of adulthood, topics such as neuropsychological functioning and understanding the impact of glycogen storage on the brain become increasingly important.

In the longitudinal follow-up in classic infantile Pompe patients we found an initially normal neuropsychological development, but also mild delays (TIQ>70).[20] In young classic infantile Pompe patients cognition could easily be underestimated because of severe muscle weakness. Early developmental tests highly rely on fine motor performance (for example the pegboard). Follow-up of these patients into their adolescence, showed that intelligence ranged from normal to intellectual disabilities (WISC TIQ<70).[21] Although especially the youngest patients remained stable in time, others declined in test scores. A particular neuropsychological profile seemed to emerge. Initially this profile is characterized by a slow decline in processing speed, followed by difficulties in visuo-spatial functioning, and working memory and finally a gradual global intellectual deterioration. Other emerging features were dysarthria, facial-muscle weakness, severe ptosis, extra-ocular motility disorder, myopia and distal muscle weakness.[22] Longitudinal brain MRI showed a characteristic

pattern of white-matter involvement that evolves from periventricular abnormalities of the deep white matter to a generalized subcortical white matter affliction. At start (visible at MRIs from toddlers onwards), all the patients had white-matter involvement at the level of the centrum semiovale. Later the white-matter abnormalities expanded to the subcortical areas, the corpus callosum, and internal and external capsule (seen in our patients around the middle childhood). Eventually (mostly in the teenage years), infratentorial white-matter areas also became involved. Although there seems to be a characteristic pattern of involvement over time the rate of progression varied between patients and was independent of motor functioning.

Mucopolysaccharidosis

MPS II: Hunter disease

Neuropsychological outcome in MPS II consists of a spectrum from patients with relatively normal cognitive development and survival into adulthood (non-neuropathic) to patients with rapidly progressive intellectual disabilities and death in their teenage years (neuronopathic). The developmental pattern of the neuronopathic variant has been studied, as well as the brain abnormalities in patients with Hunter disease.[23-36] However, as yet, it is difficult to predict the phenotype, leaving some parents with great uncertainties about future perspective.[37] No study investigated the relation between brain abnormalities and intelligence in a standardized long-term follow-up cohort. We tried to predict the disease course of the patients with a predetermined protocol by combining the brain MRI and neuropsychological functioning.

The most important findings in neuronopathic MPS II patients were a normal to delayed cognitive development in the first 2 years of life, followed by a rapid slowing down of development and a plateau phase with a maximum mental age of approximately four years. Thereafter patients loose cognitive abilities during childhood and teenage years. Cognitive decline was global, and no specific neuropsychological pattern of progression was found. Death occurred in their teenage years in some of the oldest patients. This disastrous mental deterioration was accompanied by severe behavioral problems.[28] These behavioral problems could disrupt family's lives for months or even years.[38, 39] It was noted that after this episode of behavioral problems cognitive abilities had declined (personal communication). Typical behavior patterns were similar to symptoms of Attention Deficit Hyperactivity Disorder, with hyperactivity and impulsivity, which could sometimes be interpreted as physical aggression (hitting, throwing as a consequence of a deficiency in impulse regulation). Also symptoms of Autism Spectrum Disorders were found, deficits in social-emotional reciprocity, deficits in non-verbal communicating behavior, stereotype repetitive motor movements, excessive resistance to change, highly restricted fixated interest (for instance in food). An-

other behavioral problem was periods of sleeping difficulties. These were often persistent and very difficult to treat with psychotherapy (classic conditioning) or with medication. Non-neuronopathic MPS II patients were considered to have normal intelligence and no behavior problems, although attention problems may occur.[26] Long-term follow-up of brain MRI showed that the increase in atrophy sumscores strongly correlate with decline in IQ in MPS II patients. Abnormal atrophy sumscores are already present before IQ drops below the threshold of intellectual disability (<70). Atrophy may therefore serve as an early marker of the neuronopathic phenotype of MPS II.

We used our protocol to compare individuals, and to describe changes over time. We additionally used the protocol for calculating sum scores on group level.

MPS VI: Maroteaux-Lamy Syndrome

Neuropsychological outcome in MPS VI patients is considered normal, and therefor largely differs from neuronopathic MPS I, II and III. However, intellectual disabilities have been described as well.[40-42] No long-term follow-up study has been done so far in MPS VI. This is the first standardized long-term follow-up study in MPS VI on neuropsychological functioning and brain MRI.[43]

Our group of patients diagnosed with MPS VI presented with a large variation in intelligence from above normal to intellectual disabilities. Intelligence remained stable in all patients, except for one patient, who had Autism Spectrum Disorder, and was suspicious for having had increased intracranial pressure. Social and familial background factors were considered as the main contributor to intellectual outcome. Severe disease presentations, characterized by high GAG excretions, seem to elevate the risk of low cognitive levels. Findings on brain MRI were generally mild. Common findings were enlarged Virchow Robin spaces (in the basal ganglia and in the white matter), patchy white matter abnormalities, which could disappear in time, widened sinus rectus, a thinner corpus callosum, and compression of the spinal cord. Patients with severe disease had slightly more abnormalities, especially concerning the white matter.

Alpha-mannosidosis

Alpha-mannosidosis is a rare lysosomal storage disorder resulting from a deficiency of the enzyme alpha-mannosidase (AM: EC 3.2.1.76).[44, 45] Only recently, enzyme replacement therapy became available in an ongoing study.[46] Thorough knowledge is needed on the natural course to evaluate the effect of this therapy. In 2015 a cross sectional study on the neuropsychological development of 35 alpha-mannosidosis patients was published. Studies on long-term follow-up of cognition in patients with alpha mannosidosis are limited.

We studied the long-term neuropsychological follow-up and findings on brain MRI in an untreated patient with alpha-mannosidosis from age 1 year until adulthood.

The most important findings in this patient with alpha-mannosidosis were: A stable mild developmental delay with specific problems in speech/language, motor functioning, attention, verbal memory, and behavior. A deterioration over time was noted in fine motor skills, memory, and behavior. Brain MRI showed symmetric atrophy of the cerebellar hemispheres and vermis, diminished myelination of the periventricular white matter, and mild abnormalities of the basal ganglia.

Part 2. Neuropsychological outcomes related to the abnormalities on brain MRI

Classic infantile Pompe disease

The main finding on brain MRI in patients with classic infantile Pompe disease was slowly progressive white matter abnormalities. The patient with the most severe brain abnormalities presented with the most severe cognitive outcome. In patients with milder brain abnormalities (and younger patients) cognition was less impaired. Could these abnormalities be related to the cognitive outcome? Two theoretical neuropsychological concepts could possibly apply here. First, the slowly progressive white matter involvement could theoretically be related to the neuropsychological outcome by a conceptual model called the parieto-fronto integration theory (P-FIT)[47, 48]. This extensive neuronal network connects parietal, temporal, frontal areas and cingulate cortices of the brain. The integrity of this network might be related to the global functioning of the brain and therefor intelligence. This theory postulates that the network (the white matter) is more important for intelligence than domain-specific processes (the grey matter).

Secondly, the white matter abnormalities, which develop in classic Infantile Pompe disease, show similarities with the white matter involvement in another lysosomal storage disorder, Metachromatic Leucodystrophy (MLD). Patients with MLD present with a cognitive profile similar to the characteristics of NLD.[49] Non-verbal learning disorder (NLD)[50] is characterized by verbal strengths, and difficulties in visual-spatial functioning, motor skills, and social behavior.

Furthermore, brain MRI did not show brain atrophy. In line, we did not find neuropsychological higher order deficits such an aphasia, amnesia, agraphia, neglect or apraxia, associated with grey matter involvement. The absence of epilepsy is in line with this.

However, brain MRI did show abnormalities in the brain stem, and the basal ganglia, which may also have a potential effect on brain functioning, such as attention, speed, and control of movement, motivational behavior and reward behavior.

MPS II, MPS VI

Neuronopathic MPS II

In our study we found that in the neuronopathic MPS II patients atrophy was the most distinct characteristic of the MRI. White matter abnormality was present in some, but not in all neuronopathic patients and therefor did not seem to be a prerequisite for the development of cognitive decline (submitted). It is not surprising that cortical neuronal involvement leading to brain atrophy on MRI is a distinct characteristic in neuronopathic MPS II patients with severe and progressive intellectual disabilities. Other diseases in children with severe and progressive brain atrophy such as Hurlers Disease (MPS I), Sanfilippo (MPS III),[51] diseases show progressive cognitive deterioration as well. The atrophy was general, and the cognitive decline was general as well, lacking a specific neuropsychological profile.

Non-neuronopathic MPS II and MPS VI

Neuropsychological tests of native Dutch speaking patients with MPS VI showed a normal range of intelligence and non-neuronopathic MPS II were considered having normal intelligence as well. However MRI studies did show risks of having enlarged perivascular spaces, and white matter abnormalities in these patient populations. Although this could indicate that these mild brain abnormalities did not affect general intelligence, these abnormalities may potentially lead to subtle neuropsychological deficits, not detected by general intelligence tests. Yund et al., [26] found an association between the volume of the corpus callosum (white matter) and attention difficulties in non-neuronopathic adults with MPS II. Problems with attention also have been reported in some of our patients with non-neuronopathic MPS II and MPS VI patients (personal communication). It is unclear what the future perspective will be in these patients.

Alpha-mannosidosis

Our patient with alpha-mannosidosis presented with brain abnormalities common in these patients, such as atrophy in the cerebellar hemisphere and the vermis. He presented with a specific neuropsychological profile, with a stable mild developmental delay, and specific problems in his fine motor skills, speech/language, attention, and declining memory and behavior. Although the cerebellum plays a major role in the coordination of movements,[52] recent increasing evidence is found on the influence of the cerebellum in higher order cognitive functioning, due to it reciprocal cerebro-cerebellar connections.[53, 54] A distinct feature

was his decline in memory. Vacuolization in the hippocampus was found in the brains of both alpha mannosidosis patients and knock-out mice[55-57]

Part 3. Storage product and possible mechanisms involved.

Above we described the neuropsychological sequelae and brain MRI findings in patients with several types of lysosomal storage diseases. In order to understand the relationship between the storage product of a particular lysosomal storage disease and the outcome on the brain MRI and neuropsychological tests, we looked at brain autopsies. Below we describe abnormalities found in several brain autopsy studies and thoughts about the underlying mechanisms, which may be related to the abnormalities seen on brain MRI.

Pompe

Storage product

The storage product in Pompe disease is glycogen. Glycogen storage mainly occurs in muscles where it serves as a source of glucose. The brain by nature is not a glycogen-accumulating organ, since neurons have a continuous need for glucose. Therefore, storage of glycogen in the brain seems an unexpected finding. However, autopsy studies describe glycogen storage in the central nervous system in untreated patients with classic infantile Pompe disease. Here we discuss nine reports.[3-11]

Severe glycogen storage was mainly found in the neurons of the anterior motor horn cells of the spinal cord, the brain stem, the thalamus, basal ganglia, hippocampus, cerebellum, and dentate nucleus. The cerebral cortex and Purkinje cells were usually regarded as affected to a lesser extent, or not at all. Glycogen storage was also found in the smooth muscle cells of the walls of the vessels and arteries. Comprehensive glycogen storage was found in the perikaryon of glial cells in the entire central nervous system; mostly astrocytes and microglia were reported to be affected whereas oligodendrocytes tended to be involved to a lesser extent. Pronounced and diffuse glycogen storage in the white matter and subependymary cells and the choroid plexus was described as well. In addition, a proliferation of astrocytes in damaged areas of the central nervous system was found in neuronal areas and white matter.

Possible mechanisms of brain involvement in classic infantile Pompe disease.

The main characteristic on brain MRI was white matter abnormalities. No pronounced and progressive cerebral atrophy was reported. Indeed, autopsy studies did not report extensive abnormalities in the cerebral cortex.

When trying to understand the underlying mechanisms, we start with one of the main findings in autopsy studies, glycogen storage in astrocytes (1). Functions of the astrocyte

are diverse, for instance the supply of nutrients from blood vessels to neurons, removing neuronal carcasses (and causing fibrous gliosis) and maintenance of homeostasis.[58] Oligodendrocytes are particularly sensitive to slight disruptions of the interior milieu of the brain. Thus disturbance of the function of the astrocyte may lead to disturbance of homeostasis, amongst others, which then may lead to disruption of oligodendrocyte function. Since the function of the oligodendrocyte is to produce myelin, this may lead to white matter abnormalities. Alternatively, a primary effect on oligodendrocytes, which store little amounts of glycogen as well, may occur (2). Additionally, glycogen storage was found in the smooth muscle cells of the walls of blood vessels. So far no infarctions were reported. However, some brain aneurysms were described.[59-61]

Finally, gliosis may occur as a general reaction to lysosomal glycogen storage. Above mentioned hypotheses are merely hypotheses on underlying mechanisms and the pathophysiological process still needs to be elucidated. Other and more complex processes may occur.

MPS

Storage product.

The storage products in MPS II are the glycosaminoglycans Heparan Sulphate and Dermatan Sulphate. In MPS VI Dermatan Sulphate only accumulates. Below we present common characteristics in several autopsy studies of humans and animals with MPS II.[62-69]. Remarkably no autopsy studies were found in literature on patients with MPS VI.

Generally high GAG levels were reported in the brain.[68, 69] Autopsy studies showed neuronal involvement in the cerebral cortex, thalamus, cerebellum, the brain stem and spinal cord.[63-66, 69] Storage in the white matter was described infrequently. Wiesmann et al described astrocytes containing few vacuoles.[64] Several authors described storage in the endothelial cells of the capillaries and perivascular spaces.[63, 64, 67] Fibrilairy gliosis was described in MPS II as well.[63]

Possible mechanisms of brain involvement in Mucopolysaccharidosis

The main finding on brain MRI in neuronopathic MPS II patients was severe and progressive cerebral atrophy, which was consistent with the extensive cerebral neuronal involvement on brain autopsies. Several hypotheses are postulated on the occurrence of neuronal involvement, which all include a cascade of events.

Neuronal atrophy occurs in MPS I, MPS II and MPS III, while it is far less common in MPS IV and MPS VI.[43] The common storage product in MPS I, II and III is heparan sulphate, while this product is not accumulating in MPS IV and VI.[70, 71] The first hypothesis indicates that heparan sulphate might be a main contributor to the atrophy and potentially can start a

cascade of events, which eventually leads to intellectual disabilities. The second hypothesis attributing to neurodegeneration in MPS II patients involves a decrease in β-galactosidase activity possibly driven by inhibition of beta-galactosidase activity by for example storage products.[64] This subsequently may lead to an accumulation of gangliosides (GM3, GM2, and TD4)[63, 64, 66, 68], known to have a potential toxic effect on neurons. The third hypothesis involves storage in and around the endothelial cells of the capillaries. Reports indicated that GAG storage was found in the walls of blood vessels throughout the entire nervous system,[63, 64, 66] and in the perivascular tissue, which may cause impairments in the blood supply to neurons, resulting in intellectual disabilities. Contradictive to this hypothesis is the finding of enlarged virchow robin spaces (perivascular spaces) in both neuronopathic as well as in non-neuronopathic MPS II patients as well as in MPS VI patients which may not always have consequences for intelligence.[43]

A fourth hypothesis involves the white matter. A recent study by Zalfa[62] hypothesized that inflammatory reactions are caused by damage to astrocytes, which in turn results in gliosis and neuronal damage. This is however in contrast with our findings of a teenager with neuronopathic MPS II disease, whom did not have any white matter abnormalities, whereas all older non-neuronopathic patients did develop white matter abnormalities.

Finally, Purpura and Suzuki (1976)[72] postulated the distortion of neuronal geometry and formation of aberrant synapses by accumulation of storage products.

Alpha-mannosidosis

The storage products in alpha-mannosidosis are N-linked oligosaccharides. Autopsy studies of the brain in patients with alpha-mannosidosis showed some widening of the gyri in several regions.[73] Ballooning of the cytoplasm of nerve cells was found throughout the cerebral cortex, brainstem, and spinal cord. Diffuse loss of nerve cells was shown and a concomitant gliosis were found in the cerebral cortex, together with a diffuse gliosis of the white matter.[74] The entire white matter had an abnormal consistency[74] with diffuse loss of myelin with preservation of the frontal and callosal body.[74] The lateral ventricles were enlarged.[73, 74]. No abnormalities were seen in the basal ganglia. The cerebellar hemispheres were atrophic, especially at the vermis, and histological examination showed a significant, widespread loss of Purkinje neurons and granular neurons in the cerebellum.[73, 74] The medulla was broaded.[74] The substantia nigra was pale, but pigmented.[73].

Part 4. Similarities and differences of brain functioning in these lysosomal storage diseases

Similarities and differences

In this thesis we focused on four different lysosomal storages diseases, Pompe Disease, Hunter Syndrome, Maroteaux-Lamy Syndrome and Alpha-mannosidosis.

The above-mentioned diseases have in common that the deficiency of a lysosomal enzyme leads to build up of macromolecules within the cells. However, the enzyme and the accumulating substance differ per disease, causing a specific phenotype. Apparently, the deficiency of a particular enzyme and storage product causes specific brain abnormalities, and a disease specific neuropsychological outcome. For example, when we compare the autopsy studies in classic infantile Pompe disease with those in MPS, we notice differences in the preferential tissue with histopathological changes. In MPS II most storage is described in neurons and in the (peri-)vascular tissue whereas in Pompe disease most storage occurs in glia cells and to a far lesser extent in neurons. On the MRI, neuronopathic MPS II is characterized by progressive and severe atrophy, often but not always accompanied by white matter abnormalities. Patients with classic infantile Pompe disease typically present with slowly progressive white matter abnormalities. This is in line with the histopathological findings. Neuropsychological evaluation in neuronopathic MPS II patients showed severe and rapid progressive intellectual disabilities with disruptive behavior problems. In the longitudinal follow-up in classic infantile Pompe patients we found an initially normal neuropsychological development, which after years slowly changes towards a specific profile of neuropsychological deficits. Although both diseases are LSD's, the tissue in the brain most involved differs both on MRI and on autopsy studies of the brain. This may point to different pathophysiological consequences of lysosomal glycogen storage versus lysosomal glycosaminoglycan storage in the brain.

Part 5. Recommendations for future research

General recommendations

The recent development of treatment options in Pompe's disease,[13, 14, 75] MPS II,[76] and MPS IV[77] increases life expectancy and quality of life. Since the current form of enzyme replacement therapy cannot pass the blood-brain-barrier in these diseases,[78] knowledge on neuropsychological functioning becomes increasingly important. The essential challenge is to develop an effective treatment, which alters brain disease.[79] To enable appraisal of the effect of these therapies thorough knowledge of the natural course is needed. In this thesis, we studied the natural course of cognitive development in Pompe disease, MPS II, MPS VI and alpha-mannosidosis. This research was performed in patients with rare diseases. It is

inevitable that patient populations are small and that the findings need to be confirmed in other studies. More studies with international collaboration are anticipated in order to fully understand the disease progression and to monitor effects of treatment.[80] One of the main challenges for international collaboration on neuropsychological functioning is consensus on which tests to use internationally, in order to adequately compare patients globally.[81, 82] The international consensus meeting on cognitive endpoints in MPS I, II and III is an excellent example of overcoming this hurdle.[83]

Secondly, the cause of brain involvement in patients with a lysosomal storage disorders and its consequences are far from clear and are likely to be related to a cascade of events that needs to be elucidated further. Both clinical studies using MRI (e.g. volumetry, Spect, DTI) and neuropsychological tests as well as laboratory studies on knock out animals and neuronal cells may help in clarifying the exact cascade of events and the cell types involved. Understanding the underlying mechanism involved is important, since this could potentially stimulate the development of efficient therapeutic strategies targeting the brain and also help to better understand the metabolism of the healthy brain. Long-term follow-up of other lysosomal storage diseases with brain involvement could help to elucidate these underlying mechanisms, as well.

Third, how can the variation in the neuropsychological course be explained within a disease? Is this driven by a variation of residual activity, (which we are not yet able to measure in MPS)? Alternative, are there epigenetic factors? In addition, what are the predicting factors of the variability in the disease courses in neuronopathic Hunter Syndrome and classic infantile Pompe disease?

Fourth, from a psychological perspective, questions remain as well. How do patients socially and emotionally cope growing up with the limitations caused by their disease (e.g. motor difficulties, hearing, and speech difficulties, vision problems, intellectual problems, contractures, visually abnormal appearance, and often a combination of these factors). Could these difficulties potentially lead to risks for mood or anxiety disorders? How do these patients cope with the social consequences of the intensive treatment with the weekly infusions of enzyme replacement therapy and uncertain future perspectives? How much stress do parents experience in raising their child with a lysosomal storage disease, compared to other parents with a chronically ill child? What is the family burden/stress of having a child, brother or sister with a lysosomal storage disorder? Further research is needed to study the social-emotional consequences of this disease.

Disease specific recommendations

Classic infantile Pompe disease

Besides the above mentioned general recommendations, disease specific questions remain. Below we present several:

1.Social-emotional development

A topic not yet investigated is the social-emotional development of patients with classic infantile Pompe disease. For example, what is the influence of having a child with an initially life-treating disease to the parents' attachment style in raising their child towards an independent life? In addition, do patients with classic infantile Pompe patients have difficulties in expressing or communicating their emotions in social interaction, since they are at risk of having facial muscle weakness, ptosis, extraocular motility disorders, hearing difficulties, severe speech disorders, and neuropsychological problems? Furthermore, it was noted that some children have difficulties in their ability to interact socially with peers to an extent that might not solely be explained by their physical disabilities.

2. Neuropsychological evaluation

In addition to the lack of research on social-emotional development in classic infantile Pompe disease, little in depth neuropsychological evaluations (e.g. language, executive functioning, memory, attention, visuo-spatial functioning) have been published so far.[84] It is plausible that patients with classic infantile Pompe disease have a specific neuropsychological profile with strengths and weaknesses, related to the brain abnormalities. Also, possibly the neuropsychological deficits could be clustered into symptoms of a child psychiatric disorder such as Attention-deficit/hyperactivity disorder.

MPS

Several questions remain related to patients with MPS. Below we describe the most relevant at this time.

1.Prediction of future disease course

It is very difficult to predict the disease course in young children with a normal or slightly delayed development. Therefor parents need to deal with uncertain future perspectives. With the ongoing and future therapeutically trials aimed to target the brain more specifically the prediction of disease course is of great importance for the interpretation of the study results as well. Escolar presented several potential predictive factors, such as sleep disturbance, increased activity, behavior difficulties, seizure-like behavior, perseverative chewing behavior, and inability to achieve bowel training and bladder training. More research is needed to enable us to build a predictive model for MPS II using these aspects.

2.Long-term follow-up of non-neuropathic MPS II patients.

Although patients with non-neuropathic MPS II were considered to have a normal development, specific neuropsychological problems can occur, such as attention problems. In addition, brain abnormalities were found in these patients.[26] More research is needed to understand the long-term neuropsychological development of these patients.

3.Adaptive functioning.

Intellectual disabilities are diagnosed on the basis of intellectual testing, as well as the documentation of daily functioning and adaptive skills, as stated in the recent edition of the Diagnostic and Statistic Manual of Mental Disorders V. As for now research has focused on formal neuropsychological testing. The next step will be the documentation of daily functioning and adaptive skills using for example the most recent version of the Vineland Adaptive Behavior Scales.[85, 86]

4.Understanding behavior problems

Typical behavior patterns were described[34, 37-39, 87] and observed (personal communication) in neuronopathic patients with MPS II. For example, severe and disruptive sleeping problems, hyperactivity, impulsivity, intense focus of interest (such as for food), stereotype movements, perseverations, and difficulties in the social interaction occur at some time point and disappear at a later stage.[34] The behavior patterns of these patients resemble child psychiatric diagnosis such as Attention Deficit Hyperactivity Disorders or Autism Spectrum Disorders.[88] Formal psychiatric evaluation is needed to investigate its incidence and course of the behavior patterns. These severe behavior problems observed in MPS II also interfere with reliable testing, and a trained and experienced psychologist should always perform the tests in these children.[83] It is important to understand the risks factor of expressing behavioral problems, at what ages they may occur, and how long they will last (until what age) in order to give proper advice to parents.

5.Treatment of behavior problems

Behavioural problems are severely disruptive for the MPS patients, and their families. Treatment, however, is challenging. Per patient there is a quest for effective behavioral medicine. Psychotherapeutic strategies are often limited to classical conditioning only, due to the moderate to profound intellectual disabilities. Pharmacological treatment is not evidence based due to the lack of scientific studies. There is an intense need to understand these invalidating problems and for the development of effective treatment strategies.

MPS VI

Several topics in MPS VI need further investigation, such as:

1.Tiredness

Several patients with MPS VI complained about invalidating tiredness, especially during puberty, causing a high rate of absence from school (personal communication). Although physical factors will undoubtedly contribute, the question remains if neuropsychological problems occur and whether they add to the complains of tiredness.

2. Lower intelligence

The lower intelligence found in some in patients with MPS VI can be caused by severe disease presentation, and familial and social background. However, the elevation of intracranial pressure due to a combination of craniosynostosis, blockage of liquorcirculation and hampering of the re-absorption of liquor could also be a factor influencing cognition and needs further investigation.

CONCLUSION AND FUTURE PERSPECTIVE

Over two third of the fifty lysosomal storage diseases are characterized by brain involvement. With this thesis we aimed to describe the neuropsychological consequences and differences of 4 lysosomal storage disorders. By studying patients with these diseases we have obtained many new insights. However much more remains to be unraveled concerning the consequences of lysosomal storage diseases for the brain. How can we predict the diseases course? What sequelae for cognition, behavior, social and emotional functioning can occur during the disease and how can we treat these? What are the driving mechanisms behind these diseases and how can future therapies such as intrathecal enzyme replacement therapy, gene therapy, or stem cell transplantation, use these mechanisms to treat the brain? To enable us to shed more light on these problems and give better support to patients and their family's thorough knowledge on the neuropsychological aspects is essential. As lysosomal diseases are orphan diseases, international collaboration between expert centers is needed. Large patient cohorts with follow-up in a standardized way with consensus on tests used to compare results is the most efficient way to take a next step forward.

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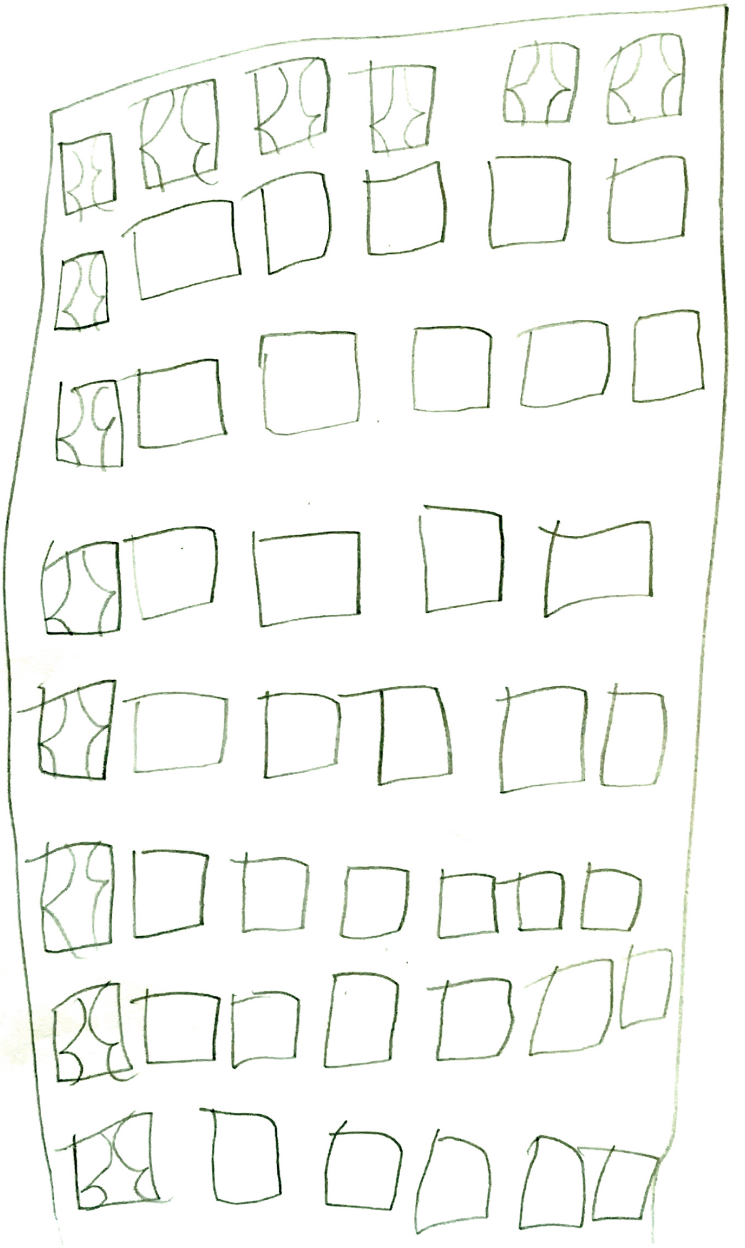
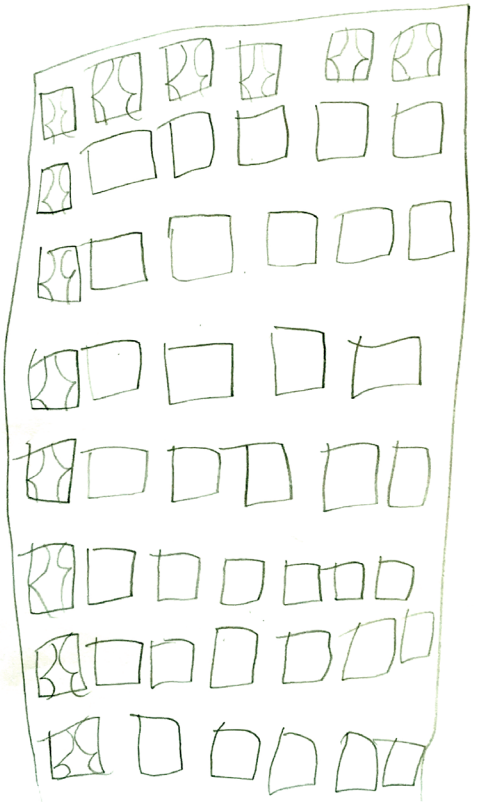
PART 4



Chapter 12

Epilogue



EPILOGUE - ROLE OF A PEDIATRIC NEUROPSYCHOLOGIST IN A METABOLIC EXPERT CENTER[1-5]

A neuropsychologist is interested in the relationship between the functioning of the central nervous system, and the behavioral consequences in daily living. A neuropsychological assessment can be used to evaluate cognition, behavior, social and emotional functioning of the patient, compared to peers. Results are used to inform, and advice patients and their parents on the consequences of the neuropsychological problems for daily living and/or academic performances and if indicated, to start psychotherapeutic treatment. Alternatively, neuropsychological assessments can be used for research, to increase insights on neuropsychological development of a particular patient population. Which in turn by using the obtained knowledge may improve guidance of patients and families. Below we describe these roles in more detail.

Neuropsychologist within an expert center: Research

Publications on neuropsychological evaluations in patients with lysosomal storage diseases are limited. They are often restricted to the clinical impressions of the physician. If neuropsychological evaluations are performed, they are limited by:

1. the use of a wide variety of developmental tests
2. lack of description of mental ages of patients with intellectual disabilities, which complicates the distinction between a stagnation of mental development, or intellectual deterioration
3. complete absence of in depth neuropsychological evaluations, while more detailed information on the neuropsychological profile could indicate the involvement of particular brain areas.
4. lack of prospective cohort studies due to the rarity of the disease.

In order to achieve more knowledge about the (long term) outcome of patients rare LSDs this should be optimized, and evaluations should preferably be done in expert centers.

Extensive neuropsychological knowledge on LSDs is important for several reasons. First of all, improving the understanding of the disease on a general and individual level will provide prognostic information and enable individual advice to patients and parents and individualized psychotherapy. Secondly, standardized neuropsychological follow-up can be instrumental to delineate the natural course of the disease, thus creating a benchmark for the evaluation of the effects of upcoming new therapeutic strategies targeting the brain. Third, new therapeutic strategies may lead to an increase in survival and, along with it, new insights on possible long-term neuropsychological consequences of the disease. Fourth, in surviving patients, neuropsychological functioning becomes more important since good

functioning is an important driver behind quality of life and adaptation to daily life. Fifth, improved knowledge on the neuropsychological developmental course of LSDs could be useful in improving diagnostics, since these diseases are often difficult to recognize due their rarity and nonspecific symptoms.

Above-mentioned factors stress the need of standardized neuropsychological follow-up by a specialized neuropsychologist in expert centers for patients with LSDs. Preferably these follow-up programs are based on international consensus, while international collaboration between expert centers (for example in the recently installed European Reference Networks endorsed by the European commission) will further improve knowledge needed to take next steps forward in these rare diseases.

Neuropsychologist within an expert center: Patient care

Sometimes the search for a right diagnosis takes a long time in patients with a rare disease. When a child is (finally) diagnosed with a LSD, the parents often worry about the future perspective. Besides questions involving life expectancies and complex physical problems caused by LSDs, parents are concerned about their child's cognitive performances and quality of life. From diagnosis onwards families are supported in their new reality, in which patients and parents have to deal with uncertainty about future disease courses. Some answers about the disease course can be provided by regular neuropsychological evaluations. Evaluations take place in a standardized, reproducible follow-up setting, applying neuropsychological tests that are age-appropriate for the evaluation of the development of a patient compared to healthy peers. In addition, the results can also be compared to previous assessments of the patient to delineate the patient's own developmental course. These insights will be used to adjust expectations of the patients' development at home and school. Experience with patients with lysosomal diseases is of essential for the selection of the most appropriate test. For example, children with intellectual disabilities and severe behavior problems, such as in neuronopathic MPS II, often have short attention span, are impulsive and unpredictable in their behavior. To enable the administration of a full test, experience and training with this patient category is needed.

Besides diagnostics, a neuropsychologist can treat patients in helping them dealing with psychological problems. For instance, in a hospital setting, common topics of treatment are for example acceptance of being chronically ill or having functional losses, or anxiety for medical treatments (MRIs, infusions). A neuropsychologist can also help parents in the treatment of disruptive behavioral problems of their children, such as excessive eating, severe sleeping problems including insomnia, hyperactivity, inattention or social problems. For example, in MPS II sleeping problems are often long lasting and highly disruptive to

family life. Psychotherapeutic treatments based on classical conditioning can be used by a neuropsychologist to minimize the sleeping difficulties.

In conclusion, the presence of a neuropsychologist in an expert center adds to the creation of an unique environment integrating medical and paramedical care. Due to the rarity of the diseases centralization of care of patients in expert centers is a prerequisite to enable excellent care. The Rotterdam Center for Lysosomal and Metabolic Disease is an expert center for LSDs. The long lasting day-by-day collaboration of all disciplines required for the care of patients with lysosomal disorders has led to an increase in knowledge and experience in the treatment of these disorders. This has created a unique home for patients with these rare disorders as well as a unique opportunity to explore these diseases from a multidisciplinary perspective.

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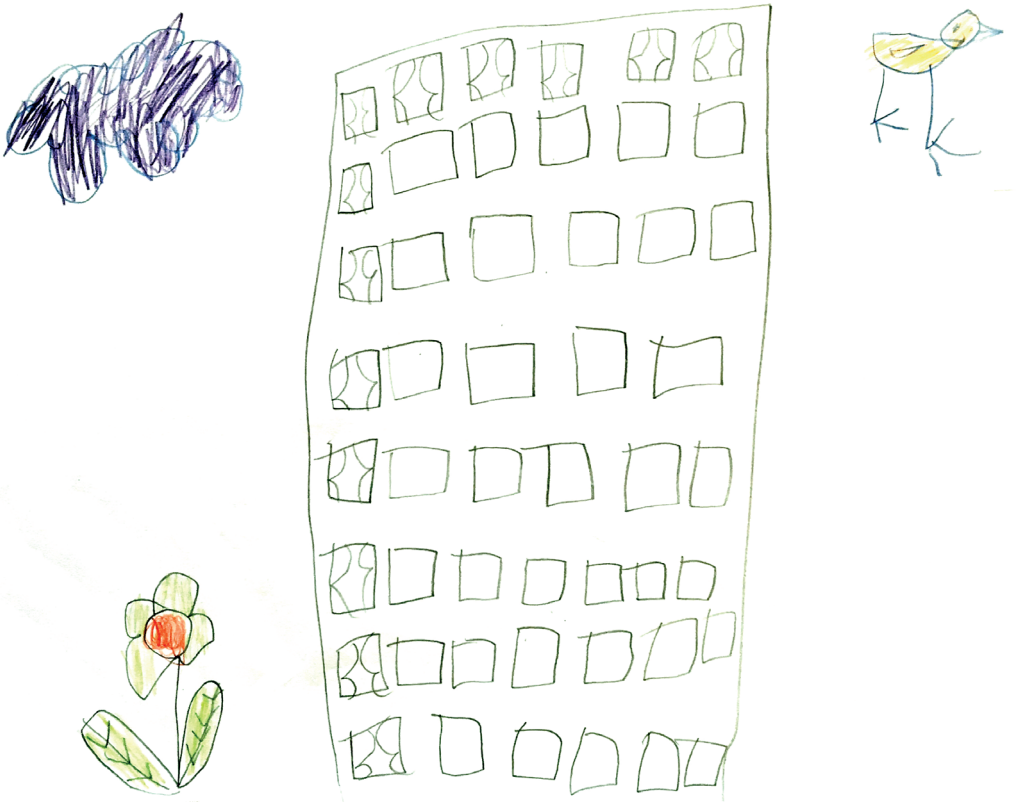
Addendum



SUMMARY

At this moment over 600 different metabolic diseases are known. This thesis focuses on lysosomal storage disorders (LSDs). Lysosomes contain over 50 different types of enzymes. These enzymes degrade macromolecules that have entered the lysosomes. Variations in the DNA sequence may lead to partial or total deficiency of the activity of a particular lysosomal enzyme. Subsequently, this leads to lysosomal accumulation of specific storage products in distinct tissues causing characteristic disease symptoms. Lysosomal storage disorders are rare with a combined birth prevalence of 14 per 100.000 live births. The last decades innovative treatments, such as enzyme replacement therapy have become available for several LSDs. As life expectancy improves, long-term outcome becomes more important. Brain involvement is observed in various LSDs, but studies on the brain and the long-term neuropsychological outcome are limited. Enzyme replacement therapy cannot pass the blood-brain barrier, therefor the cascade of events resulting from progressive storage in the central nervous system are not treated. This thesis focuses on the long-term neuropsychological consequences of four very different lysosomal storage diseases.

The introduction (Chapter 1) describes the basic mechanisms of lysosomal storage diseases and focuses in particular on the four different LSD's whom are investigated in more detail as part of this thesis: Pompe's Disease, Mucopolysaccharidosis Type II (Hunter Syndrome), Mucopolysaccharidosis Type VI (Maroteaux-Lamy Syndrome), and Alpha-Mannosidosis. The aims of the studies and outlines of the thesis are formulated at the end of Chapter 1.

Part 1 (chapter 2 – 5) describes the long-term cognitive follow-up and brain MRI findings of patients with classic infantile Pompe disease. Classic infantile Pompe disease is a metabolic myopathy in which glycogen stores predominantly in muscles. Autopsy studies of the brain show small amounts of glycogen storage as well. Untreated patients rarely survive beyond their first year of life due to cardiorespiratory failure. In 1999, we pioneered enzyme replacement therapy improving cardiac hypertrophy, motor development and increasing life expectancy. Currently, the oldest patients worldwide have reached adulthood. Enzyme replacement therapy cannot pass the blood-brain-barrier, therefor we carefully followed the cognitive development. At start of this thesis cognitive outcome was available in toddlers only. **Chapter 2** presents our long-term prospective follow-up study on cognitive outcome in 10 classic infantile Pompe patients from infancy to age 12 years. We found that cognitive outcome at school age ranged from normal to mild developmental delay (IQ 74-108; age range 5-12 years). Cognition was easily underestimated under the age of five years due to poor motor functioning. Processing speed was delayed in two patients. Brain imaging in 4/5 patients revealed periventricular white matter abnormalities, which seemed to become

more evident in time, but did not increase in extent. This outcome suggested that the consequences of glycogen accumulation in the CNS were limited. The outcome of our study and a similar study of Spiridigliozi et al (2012) is discussed further in **Chapter 3. Chapter 4** describes the alarming finding of a cognitive decline in a nine-year-old patient with classic infantile Pompe disease. The patient presented with behavioral problems, like uncorrectable teasing and wanton behavior. Follow-up of cognitive functioning showed a decline from normal development in early childhood towards moderate intellectual disabilities at age 9 years (IQ 48). The decrease in his processing speed and performance intelligence was most striking. He had symmetrical white-matter abnormalities, which were more extensive than we found in our earlier study. The deterioration in processing speed and performance intelligence over time suggested a progressive process in his white matter. This prompted us to perform a more in depth study on long-term cognitive follow-up and brain MRI findings in our population now reaching adulthood (**Chapter 5**). In total, 21 brain MRIs were performed in 11 patients. Six patients had consecutive MRIs. Five patients had follow-up assessments with the WISC-III-NL. Cognitive evaluations now ranged between stable and normal development to declines leading to intellectual disabilities. Brain MRI showed slowly progressive white matter involvement, which was independent of motor functioning. The rate of progression varied per patient. This shows that the central nervous system should be an important additional target in the development of next-generation therapeutic strategies.

An additional feature of classic infantile Pompe disease was described in **Chapter 6.** It highlights the frequency and consequences of facial-muscle weakness, speech disorders and dysphagia in the 11 oldest survivors. All patients developed facial-muscle weakness before the age of 15 months (n =11). Speech was studied in four patients, and articulation was disordered. Swallowing function was ineffective increasing the risk of aspiration. To improve speech and reduce the risk for aspiration, early treatment by a speech therapist and regular swallowing assessments are recommended.

In conclusion, with the introduction of enzyme replacement therapy, children with classic infantile Pompe disease now survive this initially lethal disease. Many accomplishments have been made since then. The next hurdle to overcome is the brain. Future research is focusing on the differentiation between muscle and central nervous system pathology. Simultaneously, research will be focusing on increasing the effect of the therapy and the ability to reach the brain effectively.

Part 2 describes cognitive follow-up and brain MRI findings in three other lysosomal storage diseases. **Chapter 7** presents a mildly affected patient with Alpha-mannosidosis. Longitudinal follow-up studies on the neuropsychological consequences of alpha-mannosidosis are

limited and conclusions are inconsistent. We had the unique opportunity to combine data of several neuropsychological tests and MRI findings of a patient with alpha-mannosidosis who had been carefully monitored from start of symptoms at age 1 year until adulthood. His intellectual development remained stable over time in the range of a mild developmental delay. He had a specific neuropsychological profile, with problems in fine motor skills, sustained attention, memory and behavior. His memory declined over time, and his problems in fine motor skills and behavior increased. A main finding on brain MRI was a mild atrophy of the cerebellum. Our study provides new insights of the long-term consequences of milder forms of alpha-mannosidosis for individual patients. This information is also relevant in light of the new therapeutic developments for alpha-mannosidosis as it indicates that targeting of the brain is also mandatory in patients with milder forms of alpha-mannosidosis.

Chapter 8 presents the long-term cognitive follow-up of patients with Mucopolysaccharidosis Type VI (Maroteaux-Lamy Syndrome). This disease is considered to have preserved cognition. However, there are no study reports on long-term cognitive follow-up in these patients. In chapter 8, eleven patients with MPS VI were evaluated (age range 2-20, follow-up up to 4.8 years). Cognition ranged from normal to intellectual disabilities (range test scores 52-131). Intelligence remained rather stable in 90% of the patients. Intelligence test scores of the patients compared well with test scores of their siblings and education level of their parents. Native speaking patients had higher test scores than non-native speaking patients. Patients with the p.Y210C mutation performed best. Two patients with high GAG levels and severe mutations had intellectual disabilities, which was clearly lower than their parents' educational level and siblings school functioning. Brain abnormalities were aspecific, but occurred more in severely affected patients. Intellectual development differs from MPS IH, neuronopathic MPS II and MPS III in which progressive intellectual disabilities are a hallmark of the disease.

Chapter 9 presents patients with Mucopolysaccharidosis Type II (Hunter Syndrome). Two-third of these patients present with progressive, intellectual disabilities and die within their teenage years. One third has relatively normal intelligence and survives into adulthood. At diagnosis the disease course cannot be predicted. We studied long-term follow-up with brain MRI and IQ (age range 0-47,5 years) in nineteen patients. We aimed at understanding the relationship between IQ and MRI with the ultimate goal of being able to predict neurocognitive development at an early age. At a group level, the mean decline in IQ was 6.7 points per year. Initial loss was rapid, which slowed down in time due to a floor effect. Virchow Robin Spaces had no relationship with age and disease severity. Atrophy and white matter abnormalities progressed over time. Both were correlated with IQ decline. Abnormal atrophy was present before IQ dropped below 70 and may therefore serve as an early marker of the neuronopathic phenotype of MPS II. This is important for counseling

parents and caretakers, and the adequate prediction of the phenotype is a prerequisite for the interpretation of the effect of innovative therapies targeting the brain and for a timely start of therapy.

As lysosomal storage disorders are rare, studies are restricted by small patient numbers. Multinational studies are needed to recruit enough patients to provide meaningful data and to achieve enough statistical power. **Chapter 10** presents the results of an international consensus meeting on the design and conduct of clinical studies in children with rare neurodegenerative diseases, such as MPS I, II, and III, with a focus on the most appropriate outcome measures for cognitive functions and adaptive behavior. This is an important step forward for clinical studies evaluating the effect of novel therapies on the central nervous system.

Part 3 (Chapter 11) places the findings described in a broader perspective by reflecting on the relationship between the neuropsychological outcome and the brain abnormalities per disease. Similarities and differences between the diseases are presented and the possible pathophysiological brain mechanisms involved in each LSD are discussed. Recommendations are given for further research.

Finally, **Part 4 (Chapter 12)** reflects on the value of a neuropsychologist in a multidisciplinary team treating patients with complex lysosomal storage disorders.

SAMENVATTING

Op dit moment zijn er meer dan 600 verschillende metabole ziekten bekend. Dit proefschrift richt zich op lysosomale stapelingsziekten (LSD's). Lysosomen bevatten meer dan 50 verschillende soorten enzymen. Deze enzymen breken macromoleculen af nadat deze opgenomen zijn in de lysosomen. Variaties in de DNA-sequentie kunnen leiden tot een gedeeltelijk of volledig tekort aan activiteit van een bepaald enzym. Dit leidt tot stapeling van specifieke stapelingsproducten in bepaalde weefsels, hetgeen karakteristieke ziekte specifieke symptomen veroorzaakt. Lysosomale stapelingsziekten zijn zeldzaam en hebben een gemiddelde prevalentie van 14 per 100.000 geboortes. De laatste decennia zijn innovatieve behandelingen ontwikkeld voor meerdere LSDs, bijvoorbeeld enzymvervangende therapie. Deze behandelingen verbeteren de levensverwachting aanzienlijk waardoor de lange termijn uitkomst belangrijker wordt. Verschillende stapelingsziekten treffen het brein. Desondanks zijn er weinig studies die de lange termijn gevolgen voor het brein en het neuropsychologisch functioneren, bestuderen. De huidige enzymvervangende therapieën bereiken de hersenen niet. De gevolgen voor het centrale zenuwstelsel worden dus niet behandeld. Dit proefschrift richt zich op de lange termijn neuropsychologische gevolgen van lysosomale stapelingsziekten in vier zeer uiteenlopende LSD's.

De introductie (hoofdstuk 1) beschrijft de basismechanismen van lysosomale stapelingsziekten met een focus op de vier verschillende LSD's die in dit proefschrift zijn bestudeerd: de ziekte van Pompe, Mucopolysaccharidose Type II (De ziekte van Hunter), Mucopolysaccharidose Type VI (De ziekte van Maroteaux-Lamy) en Alpha-Mannosidose. Aan het eind van dit hoofdstuk wordt het doel van de onderzoeken van dit proefschrift beschreven.

Deel 1 (hoofdstukken 2 - 5) beschrijft de lange termijn gevolgen voor de cognitieve ontwikkeling van patiënten met de klassiek infantiele vorm van de ziekte van Pompe en de veranderingen op MRI's van de hersenen. De klassiek infantiele vorm van de ziekte van Pompe is een metabole myopathie, waarbij glycogeenstapeling met name plaatsvindt in de spieren. Autopsie studies wijzen echter uit dat kleine hoeveelheden glycogeen ook stapelen in de hersenen. Door cardiorespiratoir falen overlijden patiënten onbehandeld voor hun eerste levensjaar. In 1999 startten we de enzym-vervangingstherapie bij de eerste patiënten ter wereld. De therapie verbeterde het hart, de motorische ontwikkeling en de overleving. Momenteel bereiken deze oudste patiënten ter wereld de volwassenheid. Vanwege de bloed-hersenbarrière kan enzymvervangingstherapie het brein niet bereiken. In verband hiermee hebben we vanaf de start van de behandeling van de eerste patiënten de cognitieve ontwikkeling zorgvuldig vervolgd. Bij de start van dit proefschrift was deze slechts beschreven in enkele studies naar baby's en peuters met de klassiek infantiele vorm van de ziekte van Pompe. **Hoofdstuk 2** presenteert de resultaten van onze prospectieve

follow-up studie naar de cognitieve ontwikkeling van 10 klassiek infantiele Pompe patienten tot de leeftijd van 12 jaar. De intelligentie van de patiënten op de school leeftijd varieerde van normale ontwikkeling tot moeilijk lerend niveau (Intelligentie Quotiënt; IQ 74-108, leeftijdsgroep 5-12 jaar). Door de slechte motoriek werd de ontwikkelingscore gemakkelijk onderschat bij kinderen jonger dan vijf jaar. Bij 2 patiënten werd een vertraagde verwerkingssnelheid gevonden. Vier van de 5 patiënten hadden afwijkingen in de periventriculaire witte stof op hersen MRI's. Deze afwijkingen werden duidelijker zichtbaar in de tijd, maar breidden zich niet uit. Op basis van dit onderzoek leken de gevolgen van glycogeen stapeling in het CNS beperkt. Het resultaat van deze studie en een vergelijkbare studie van Spiridigliozi et al (2012) wordt verder besproken in **Hoofdstuk 3**. **Hoofdstuk 4** beschrijft de zorgwekkende bevinding van cognitieve achteruitgang bij een negenjarige patiënt met de klassiek infantiele vorm van de ziekte van Pompe. Deze patiënt presenteerde zich met gedragsproblemen, zoals niet te corrigeren plagen en onbehoorlijk gedrag. Zijn cognitieve ontwikkeling liet een achteruitgang zien van een normale ontwikkeling in zijn vroege kindertijd tot een matig verstandelijke beperking op de leeftijd van 9 jaar (IQ 48). De afname van zijn verwerkingssnelheid en performale intelligentie was het meest opvallend. Hij had symmetrische witte stof afwijkingen, die uitgebreider waren dan wij in onze eerdere studie hadden gevonden. De verslechtering van de verwerkingssnelheid en performale intelligentie in de tijd, suggereert een progressieve witte stof aandoening. Naar aanleiding van deze bevinding startten wij een lange termijn vervolgstudie naar de effecten van de klassiek infantiele vorm van de ziekte van Pompe op de hersenen van onze bijna volwassen patiënten met behulp van neuropsychologisch onderzoek en hersen MRI's. **(Hoofdstuk 5)**. In totaal werden 21 hersen MRI's geanalyseerd van 11 patiënten. Zes patiënten hadden opeenvolgende MRI's. Vijf patiënten waren langere tijd vervolgd met de WISC-III-NL. Uitkomsten van deze cognitieve testen varieerden van een normale en stabiele ontwikkeling tot een daling van de intelligentie tot het niveau van een licht verstandelijke beperking. Hersen MRI's vertoonden langzaam progressieve afwijkingen in de witte stof. De snelheid van de progressie varieerde per patiënt en leek onafhankelijk van het motorisch functioneren van de patiënt. Ons onderzoek laat zien dat de volgende generatie therapeutische strategieën zich ook moet richten op het centrale zenuwstelsel.

Een opvallend kenmerk van de klassiek infantiele vorm van de ziekte van Pompe wordt beschreven in **Hoofdstuk 6**. Hierin beschrijven we een studie naar de frequentie en gevolgen van spierzwakte in het gezicht, spraakstoornissen en dysfagie bij de 11 oudste patiënten (bij einde van deze studie was oudste patiënt 12.2 jaar). We vonden dat alle patiënten spierzwakte in het gezicht (n = 11) ontwikkelden vóór de leeftijd van 15 maanden Sommigen ontwikkelden een ptosis. Spierzwakte van de bulbaire spieren kwamen vaak voor en ging in de meerderheid van de patiënten gepaard met afwijkende spraak en slikproblemen. Om de

spraak te verbeteren en het risico op aspiratiepneumonie te verminderen, raden wij vroegtijdige behandeling door een logopedist en geregeld onderzoek van de slikfunctie aan.

Concluderend, met de introductie van enzymvervangingstherapie veranderde het perspectief van patienten met de klassiek infantiele vorm van de ziekte van Pompe aanzienlijk van een dodelijke aandoening naar een ziekte met een aanzienlijke overlevingskans. Sindsdien is er veel bereikt. De volgende uitdaging is het brein. Toekomstig onderzoek richt zich op de ontraffeling van de oorzaken van de hersenafwijkingen en de differentiatie tussen de gevolgen van spier en hersenpathologie. Gelijktijdig focust onderzoek zich op het verbeteren van de effecten van het medicijn.

Deel 2 beschrijft het cognitieve beloop en de bevindingen op hersen MRI's bij drie andere lysosomale stapelingsziekten. **Hoofdstuk 7** presenteert een patiënt met een milde vorm van alfa-mannosidose. Het aantal studies naar de neuropsychologische gevolgen bij patiënten met alfa-mannosidose op de lange termijn is beperkt. Wij hadden de unieke gelegenheid om de gegevens van verschillende neuropsychologische testen en MRI-bevindingen van een patiënt met alpha-mannosidose te combineren. Deze patiënt was zorgvuldig vervolgd in de tijd vanaf het begin van de symptomen op de leeftijd van 1 jaar tot aan de volwassenheid. Zijn intellectuele ontwikkeling bleef stabiel in de tijd op moeilijk lerend niveau. Hij had een specifiek neuropsychologisch profiel, met problemen in de fijne motoriek, volgehouden aandacht, geheugen en het gedrag. Er werd een achteruitgang in zijn geheugen geconstateerd en zijn problemen in de fijne motoriek en het gedrag namen toe. Op de hersen MRI werd een milde atrofie van het cerebellum gezien. Deze studie geeft nieuwe inzichten in de lange-termijn consequenties van alpha-mannosidosis voor individuele patienten. Deze informatie is tevens relevant in het licht van de nieuwe therapeutische ontwikkelingen voor alpha-mannosidose. De patiënt laat zien dat er ook bij milde varianten van alpha-mannosidose cognitieve problemen kunnen ontstaan.

Hoofdstuk 8 presenteert een studie naar de cognitieve gevolgen op de lange termijn bij patiënten met Mucopolysaccharidosis Type VI (Maroteaux-Lamy Syndrome). De cognitieve ontwikkeling bij deze patiënten wordt over het algemeen beschouwd als niet afwijkend, maar er zijn geen studies uitgevoerd die de cognitie gedurende langere tijd in kaart hebben gebracht. Elf patiënten met MPS VI werden geëvalueerd (spreiding leeftijd patiënten 2-20 jaar, follow-up tot 4,8 jaar). De cognitie varieerde van een normale ontwikkeling tot een licht verstandelijke beperking (IQ 52-131). De intelligentie bleef bij 90% van de patiënten vrij stabiel in de tijd. Intelligentietest scores van de patiënten kwamen overeen met de test scores van hun broers en zussen en de opleidingsniveaus van hun ouders. Nederlands sprekende patiënten hadden hogere test scores dan tweetalig sprekende patiënten. Patiënten met de p.Y210C mutatie hadden de hoogste scores op de intelligentietest. Twee patiënten met

hoge GAG-waarden en ernstige mutaties hadden beide een licht verstandelijke beperking, wat duidelijk lager was dan het opleidingsniveau van hun ouders en het schoolniveau van hun broers en zussen. De afwijkingen in de hersenen waren aspecifiek, maar kwamen vaker voor bij ernstig aangedane patiënten. De intellectuele ontwikkeling van patiënten met MPS VI verschilt van die van patiënten met MPS IH, neuronopathische MPS II en MPS III waarbij een progressieve verstandelijke achteruitgang een kenmerk is van het ziektebeeld.

Hoofdstuk 9 presenteert een studie bij patiënten met Mucopolysaccharidosis Type II (Hunter Syndrome). Tweederde van deze patiënten presenteert zich met een progressieve, verstandelijke achteruitgang en zal in de tienerjaren overlijden. Eenderde van de patiënten heeft een relatief normale intelligentie en overleeft tot in de volwassenheid. Bij diagnose kan het ziektebeloop niet voorspeld worden. Negentien patiënten met MPS II werden vervolgd in de tijd middels cognitieve testen en hersen MRI's (leeftijd 0-47,5 jaar) om zo de relatie tussen IQ en MRI te begrijpen met als doel vroegtijdig het neurocognitieve beloop te kunnen voorspellen.

Op groepsniveau was de gemiddelde daling in IQ 6,7 punten per jaar. Aanvankelijk ging de achteruitgang in IQ snel, later vertraagde deze door een bodemeffect. Virchow Robin Spaces hadden geen relatie met leeftijd en ernst van de ziekte. Atrofie en witte stofafwijkingen namen toe in de tijd. Beiden waren gecorreleerd met een afname van IQ. Atrofie was reeds aanwezig voordat het IQ onder de 70 daalde en zou daarom kunnen dienen als een vroege voorspeller van het neuronopathische fenotype van MPS II. Dit is belangrijk aangezien een voorspelling van het fenotype nodig is voor de juiste interpretatie van het effect van innovatieve therapieën gericht op de hersenen en ook om tijdig te starten met de therapie. De studie geeft belangrijke inzichten om ouders en verzorgers zo goed mogelijk te adviseren en begeleiden.

Studies op het gebied van LSD's zijn beperkt door kleine patiënten-aantallen door de zeldzaamheid van de ziekten. Internationale samenwerking is nodig om voldoende patiënten te werven om solide conclusies te trekken en voldoende statistische power te behalen. **Hoofdstuk 10** presenteert de resultaten van een internationale consensusmeeting over de opzet en uitvoering van klinische studies met kinderen met zeldzame neurodegeneratieve aandoeningen zoals MPS I, II en III. De belangrijkste uitkomst van deze bijeenkomst was een advies ten aanzien van de meest geschikte uitkomstmaten voor cognitief en adaptief functioneren. Dit was een belangrijke stap voorwaarts voor klinische studies die het effect van nieuwe therapieën op het centrale zenuwstelsel evalueren.

Deel 3 (hoofdstuk 11) plaatst de bevindingen van de voorafgaande hoofdstukken in een breder perspectief, door te reflecteren op de relatie tussen de neuropsychologische uit-

komsten en de hersenafwijkingen per ziektebeeld. Overeenkomsten en verschillen tussen de ziektebeelden worden beschreven. Mogelijke pathofysiologische mechanismen die een rol spelen in de hersenen bij de verschillende LSD's worden besproken. Afsluitend worden aanbevelingen gegeven voor verder onderzoek.

Ten slotte beschrijft **deel 4 (hoofdstuk 12)** de waarde van een neuropsycholoog in een multidisciplinair team betrokken bij de behandeling van patiënten met complexe lysosomale stapelingsziekten.

DANKWOORD

In de eerste plaats wil ik alle patiënten en hun ouders bedanken voor hun participatie. Ik hoop we met dit onderzoek een stap verder zetten in het begrijpen van het beloop van de neuropsychologische ontwikkeling van verschillende lysosomale stapelingsziekten. Op persoonlijk vlak hebben jullie mij op vele manieren geraakt. Ik ben dankbaar voor jullie openheid in het delen van jullie ervaringen. Ik ben onder de indruk van de veerkracht die ik gezien heb in het omgaan met moeilijke momenten en het genieten van vreugdevolle momenten.

Mijn bijzondere dank gaat uit naar mijn promotor, Prof. Dr. Ans T. van der Ploeg en copromotoren Dr. Femke Aarsen en Dr. Hannerieke van den Hout.

Allerbeste Ans. Ik wil je heel graag bedanken voor deze kans en de buitengewoon prachtige reis die het heeft opgeleverd. Er is veel veranderd in de 8 jaar waarin we hebben samengewerkt. Fantastisch om mee te maken hoe het Centrum voor Lysosomale en Metabole Ziekten is uitgegroeid tot een (inter)nationaal expertisecentrum. De "kinderziektes" zijn voorbij en het wordt steeds meer een "geoliede machine". Het is inspirerend te zien met hoeveel energie en doorzettingsvermogen jij vecht voor je patiënten, zowel in de zorg, op het gebied van onderzoek (zowel in de kliniek als in het laboratorium) en zelfs op beleids- en financieel niveau. Wat ik vooral krachtig vond, was dat je op al deze niveaus waarop je zorg levert, altijd de hulpbehoefte voor de individuele patiënt centraal liet staan ('hoe help je de patiënt hiermee?'). Je hebt een prachtig team samengebracht dat met veel enthousiasme jouw visie deelt om topklinische zorg te leveren en op nationaal en internationaal niveau het expertisecentrum op de kaart te zetten. Ik ben trots dat ik bij jou heb mogen promoveren.

Beste, lieve Femke. Wat heb ik veel van je geleerd, zowel op neuropsychologisch gebied, werk gerelateerd, maar ook privé. Ik ben je dankbaar dat je mij dit prachtige project, waar jij in eerste instantie aan bent begonnen, hebt toevertrouwd. Ook ben ik je dankbaar voor al je begeleiding tijdens dit traject, zowel op klinisch als op wetenschappelijk vlak. Je bent mijn grote voorbeeld als klinisch kinderneuropsycholoog. Razendsnel zie jij de grote lijn en kun je verbanden leggen. Dit maakt ook dat je heel goed bent in teksten schrappen. Dat kwam goed uit, want ik ben vrij wollig in mijn taalgebruik. Ik vond het erg leuk met je te kunnen discussiëren over de (soms ingewikkelde) neuropsychologische profielen van de patiënten. Ik ga je op veel gebieden enorm missen nu je naar het Prinses Maxima Centrum voor Kinderoncologie gaat. Hopelijk kunnen we in de toekomst nog veel met elkaar van gedachten wisselen, in ieder geval tijdens onze intervisie/diner avonden. Bedankt voor alles!

Beste, lieve Hannerieke, geweldige mensendokter en onuitputtelijk werkpaard. Jij hebt de belangrijke gave om moeilijke boodschappen duidelijk over te brengen en desondanks mensen toch met een rechte rug de kamer uit te laten gaan. Ik vind het knap hoe je complexe problematiek in begrijpelijke taal kunt uitleggen. Gezamenlijke gesprekken waarbij we zowel de neurologische als neuropsychologische kant uitleggen aan ouders of patiënten waren voor mij heel waardevol. Wat betreft het werkpaard in je: ontelbare keren heb ik 's avonds laat (zelfs 's nachts) of 's ochtends heel vroeg revisies van je gehad. Op weg naar het vliegtuig, bij de gates, in het vliegtuig, reizend naar het hotel, altijd stond de computer open en was je druk aan het typen. Ik heb enorm genoten van onze sessies samen achter de computer. 'Nee, deze zin loopt nog niet lekker, even nog een keer vanaf het begin van de alinea teruglezen' is denk ik de meest gehoorde zin tijdens mijn promotietraject. Onze gedeelde passie in neuropsychologie en neurologie, gecombineerd met onze kritische maar enthousiaste houding naar elkaar, bleek een vruchtbare basis voor een uitstekende werkrelatie. Ook onze reisjes naar Rome en Barcelona zal ik niet snel vergeten. Een tas in het hotel laten liggen, een vliegtuig dat om 03.00 uur 's nachts uitweek en op Schiphol landde (en je reis naar Londen de volgende ochtend)…! Ik ben je zeer dankbaar voor deze fantastische jaren.

Ik wil de kleine commissie bestaande uit Prof. Dr. Shapiro, Prof. Dr. Utens en Prof.Dr. van Doorn bedanken voor het plaatsnemen in deze commissie.

Dear Prof.Dr. Shapiro. Thank you very much for attending the inner committee. I was very pleased to hear that you are able to sit in on the defense. You are a big inspiration for me (and no doubt for many neuropsychologists). Recently, you organized an international consensus conference for cognitive endpoints in MPS disorders. This was an important step forward in research on MPS disorders and a good example for other lysosomal storage diseases. Hopefully we will discuss more on this topic in the future.

Geachte Prof.Dr. Utens, lieve Lisbeth, ik wil je graag bedanken dat je plaats wilde nemen in de kleine commissie. Voor mij is dat heel bijzonder. In de afgelopen jaren heb ik je mogen leren kennen als Universitair Hoofddocent waarbij je vanaf de zijlijn mijn promotietraject gevolgd hebt. Ik heb zeker onze gesprekken hierover ter harte genomen, al ben ik klaarblijkelijk sterker in het maken van een planning, dan er aan vasthouden. Ik vond het geweldig te horen dat je, in de welverdiende hoedanigheid als recent beëdigde professor, in de kleine commissie wilde plaatsnemen. Dank je wel (en ook dank aan je dochter!).

Geachte Prof.Dr. van Doorn, hartelijk dank voor het plaatsnemen in de kleine commissie. Ik vond het erg interessant om met u samen na te denken over de hersenen bij patiënten met de klassiek infantiele vorm van de ziekte van Pompe. Ook heb ik het leerzaam en interessant

gevonden om samen met u na te denken over het optimaliseren van een (inter)nationaal expertisecentrum tijdens de CLMZ dag.

Dan wil ik graag de overige leden, Prof. Dr. Willemsen, Dr. Wolf, Dr. Catsman-Berrevoets, van de grote commissie bedanken voor het plaatsnemen in deze commissie.

Geachte Prof. Dr. Willemsen en Dr. Wolf, heel hartelijk dank dat u wilde plaatsnemen in de grote commissie, waarbij u op basis van uw expertise in neurometabole en neurodegeneratieve ziekten van gedachten wilde wisselen over dit de inhoud van dit proefschrift.

Geachte Dr. Catsman-Berrevoets. Heel hartelijk dan voor het plaatsnemen in de grote commissie. Mijn werkzaamheden als neuropsycholoog ben ik gestart in directe samenwerking met u, op de kinderneurologie. Destijds vond ik de samenwerking erg plezierig. Hartelijk dank dat u 8 jaar later met mij van gedachten wil wisselen tijdens mijn promotie.

Ook al mijn andere mede-auteurs (Maarten Lequin, Carin van Gelder, Marion Brands, Esther Poelman, Audrey Vollebregt, Esmee Oussoren, Iris Plug, Nadine van der Beek, Carine van Capelle, Pieter van Doorn, Arnold Reuser, Rianne van de Weitgraven, Dimitris Rizopoulos, Ilon Moor- van Nugteren, Marieke Hakkesteegt, Jet de Gier, Renée de Coo, Nienke Weisglas-Kuperus, Jaak Jaeken, Willem-Frans Arts, Luc Regal, Carten Muentjes, Robert Coebergh van den Braak, Hanneke van der Lee, Jonathan Morton, Heather Adams, Lorne Clarke, Maria Escolar, Roberto Giugliani, Paul Harmatz, Melissa Hogan, Simon Jones, Shauna Kearney, Joseph Muenzer, Stewart Rust, Margaret Semrud-Clikeman, Frits Wijburg, Zi-fan Yu, Darren Janzen Elsa Shapiro) wil ik bedanken voor al hun inzet ten aanzien van hun aandeel in het artikel. Speciale dank daarbij gaat naar Maarten Lequin. Maarten, heel hartelijk dank voor het beoordelen van de MRI's en alle uitleg die je daarbij gaf!

Hartelijk dank Chris van der Meijden, Tom de Vries Lentsch en Laurens Groenendijk voor jullie hulp bij de opmaak van de figuren.

Mijn paranimfen Isabelle Streng en Daphne van Vliet ben ik eveneens dankbaar .

Isabelle, je hebt me zo veel geleerd en vertrouwen gegeven. Ondanks je eigen drukte had je altijd tijd en ruimte voor een luisterend oor. Daar ben ik je dankbaar voor. Je bent een inspiratie voor mij in de wijze waarop jij met betrokkenheid en aandacht je patiënten behandeld. Ik vind het een eer dat je mijn paranimf wil zijn.

Lieve Daph, mijn liefste vriendin. Ik heb je zo gemist de laatste tijd! Nu is het tijd voor gezelligheid onder het genot van een goed glas wijn! Ook gaan we weer dates maken met onze

kleuters, want die hebben elkaar ook gemist! Maar bovenal gaan we dat jaarlijks uitgestelde weekendje weg nu eens echt boeken! Fijn dat jij dit moment als paranimf met mij wil delen.

Beste collega's van het Centrum voor Lysosomale en Metabole Ziekten. Ik wil jullie bedanken voor de prettige samenwerking en de interesse die jullie in het onderzoek hebben getoond. Voor de steun bij frustratie, irritatie en het delen van hoogtepunten zoals een publicatie! Lieve Ca, Marionsky, Deniz en Lin, wat hebben we veel gedeeld in de tijd dat wij tegelijkertijd aan onze promoties werkten. We deelden zowel leuke dingen (zoals bijzondere groupon ervaringen, Bob, vuilniszakken op de ramen, luidruchtige kamergenoten, geboortes van onze kinderen), als minder leuke dingen (zoals in de gezins- of familiesfeer). Ik ben dan wel de hekkensluiter van ons, maar die etentjes houden we erin! Lieve, grappige, betrokken Esmee, wat hebben we veel leuke momentjes gedeeld, bedankt voor de fijne samenwerking! Beste Monique en Hidde, bedankt voor de fijne samenwerking tot nu toe en op naar de volgende projecten. Beste Audrey, Esther, Chris, Marein, Jeroen en Rachel, volgende generatie CLMZ promovendi, bikkels en doorzetters. Ik wens jullie veel succes bij jullie promoties en alle goeds bij jullie vervolgopleidingen. Beste Wilma, bedankt voor al je hulp met het inplannen en soms weer wijzigen van de afspraken en alles wat daarbij komt kijken! En natuurlijk voor je luisterend oor. Beste Rineke, Jacq, Asia en Anneke, bedankt voor jullie interesse en de fijne samenwerking!

Beste Iris, Michelle, Nathalie, Dorothee en Marianne, bedankt voor jullie organisatorische hulp en luisterend oor! Beste Lianne, bedankt voor de fijne samenwerking rondom de patiëntenzorg!

Lieve vrienden, lieve Schuimpjes! Ik heb een tijd lang onder een grote promotiesteen gelegen, maar nu ben ik er weer langzaam onderuit gekropen. Op naar de etentjes, borrels, (aankomende) baby's ontmoeten etc.

En tot slot de belangrijkste mensen uit mijn leven. Lieve (schoon)familie. Mijn ouders Berend en Ria Ebbink, mijn schoonouders Wil en Toos van Hooijdonk, zwager Remon en zijn vriendin Lisette, mijn zus Annelien, zwager Herbert en hun kinderen Lieke en Renske, mijn zusje Margriet en natuurlijk tante Wes. Alle steun die ik van jullie heb ontvangen was onontbeerlijk voor het slagen van dit project. Jullie hebben me gesteund in praktische zin, maar zeker ook in emotionele zin. Jullie waren er altijd voor mij en voor de kinderen. Duizend maal dank voor de vele uurtjes oppassen en wasjes draaien en jullie interesse in dit onderzoek.

En dan de allerbelangrijkste mensen van mijn leven, de liefste mannen van mijn leven, Jeroen, Reinhard en Julius. Lieve Rein en Juul, elke dag als ik thuis kom, is het een feest om jullie te zien. Jullie vrolijkheid en liefde geeft me zoveel energie. Ik geniet elke dag van jullie.

Liefste Jeroen. Niemand heeft mij in de afgelopen jaren meer gesteund dan jij. Je hebt het soms (zwaar ;-)) te verduren gehad en ik dank je dat je dit altijd kon relativeren. Jouw heerlijk nuchterheid, blik naar de toekomst, liefde en humor heeft mij enorm geholpen om dit allemaal te doen. Zeker het laatste jaar, waarbij het staartje van de promotie gecombineerd werd met een fulltime GZ-opleiding, vraagt veel van je. Ik voel me gelukkig dat je er voor me bent en ja, nu is het echt klaar!

PHD PORTFOLIO

Summary of PhD training and teaching activities

Name PhD student:	Berendine J. Ebbink
PhD period:	2009-2017
ErasmusMC Department:	Pediatrics, Center for Lysosomal and Metabolic Diseases
Promotor:	Prof. Dr. A.T. van der Ploeg
Copromotoren:	Mevr. Dr. F.K. Aarsen, Mevr. Dr. J.M.P. van den Hout

PhD training	Year	Workload (ECT)
General courses and workshops		
Basiscursus regelgeving en organisatie voor klinisch onderzoekers (BROK)	2010	0.9
Herregistratie BROK	2014	0.1
Biomedical English Writing and Communication	2010-2011	4.0
Neuroanatomy and neuroradiology	2013	1.0
Workshop Systematic Literature Retrieval in PubMed part 1	2014	0.6
Workshop Systematic Literature Retrieval inneuropsychologische PubMed part 2	2014	0.6
Workshop Systematic Literature Retrieval in other databases	2014	0.6
Workshop Endnote	2014	0.6
SKION dagen	0.3	
Biweekly journal club, Center for Lysosomal and Metabolic Diseases	2009-2012	1.0
Biweekly researchmeeting, Center for Lysosomal and Metabolic Diseases	2009-2015	2.0
Researchmeeting psychosociale zorg (6x per jaar)	2013-2014	0.6
Cursusdag scientific integrity	2016	0.2
(Inter) national conferences		
Hogrefe: Executieve functies: hype of handvat	2009	0.3
Hogrefe: Executieve functies in de praktijk	2010	0.3
Scem: Neuropsychologie: vragen en tekens	2010	0.3
Samen nog beter 2	2010	0.6
Second meeting of the Federation of European Societies of Neuropsychology	2010	1.0
Pearson: Neurodag: het lerende brein	2011	0.3
Mid-year meeting interenational neuropsychological society	2013	1.5
Second International Paediatric Psychology Conference in Europe	2014	0.6

Teaching		
Erasmus MC: Minor kinderoncology (medical students)	2010,2013	-
Erasmus MC: Minor kinderoncology (Nurse students	-	-
Erasmus MC: Stichting Ziek en Onderwijs Kinderoncologie	2011,2014	-
In depth (inter) national conference		
11th Pompe Expert Day, Rotterdam	2013	0.5
In depth presentations and international conferences		
MPS patient day, Amersfoort (oral presentation)	2010	1.0
ErasmusMC: Genzyme meeting (oral presentation)	2011,2016	0.6
Steps Forward in Pompe Disease, Berlin, Germany (1e price poster presentation)	2012	0.5
Voorjaarsvergadering Erfelijke Stofwisselingsziekten in het Nederlands Taalgebied (oral presentation)	2012	1.0
Genzyme: Nursemeeting, Oslo, Norway (oral presentation)	2012	1.0
ESN Amsterdam (poster presentation)	2012	0.5
SOV researchday (poster presentation)	2012	0.3
Erasmus MC: Nurse meeting (oral presentation)	2013	0.3
World Symposium, Orlando, USA (poster presentation)	2014	0.5
European Symposium on Lysosomal Storage Diseases Barcelona, Spain (oral presentation)	2015	1.0
Erasmus MC: Genzyme Quarterly meeting RSA	2016	0.3
World Symposium, San Diego, USA (poster presentation)	2016	0.5
World Symposium, San Diego, USA (poster presentation)	2017	0.5
Pompe day for parents/caretakers	2016	1.0
SSIEM, Rome, Italy (oral presentation)	2016	1.0
International MPS Consensus Conference of Cognitive Endpoints, Delphi panel, Londen, England	2016	2.0
Supervising		
Supervising internship Master students neuropsychology	2012, 2014, 2017	2.0
Supervising bachelor thesis HvA students (2 students)	2014	1.0
Supervising minor thesis student psychology/genetics	2014	0.3
Other		
Clinical work Division of Metabolic Diseases	2009-2015	
Clinical work Division Child Oncology (0,3 fte)	2009-2016	-
Reviewing paper for peer reviewed journal	2010	0.3
Advisory Board Meeting, Shire, Barcelona, Spain	2017	1.0

LIST OF PUBLICATIONS

2017 J.H. van der Lee, J. Morton, H.R. Adams, L. Clarke, **B.J.Ebbink**, M.L. Escolar, R. Giugliani, P. Harmatz, M. Hogan, S. Jones, S. Kearney, J Muenzer, S. Rust, M. Semrud-Clikeman, F.A. Wijburg, Z.F. Yu, D. Janzen, E. Shapiro. Cognitive endpoints for therapy development for neuronopathic mucopolysaccharidoses: Results of a consensus procedure. Molecular Genetics Metabolism 2017;121(2):70-79

2016 **B.J. Ebbink**, E. Poelman, I. Plug, M.H. Lequin, P.A. van Doorn, F.K. Aarsen, A.T. van der Ploeg, J.M.P. van den Hout. Cognitive decline in classic infantile Pompe disease: an underacknowledged challenge. Neurology 2016;86(13):1260-1261

2016 **B.J. Ebbink**, M.M.M.G. Brands, J.M.P. van den Hout, M.H. Lequin, R.R. Coebergh van den Braak, R.L. van de Weitgraven, I. Plug, F.K. Aarsen, A.T. van der Ploeg. Long-term cognitive follow-up in children treated for Maroteaux-Lamy syndrome. Journal of Inherited and Metabolic Diseases 2016;39(2):285-292

2013 A.T. van der Ploeg, **B.J. Ebbink**, F.K. Aarsen, C.M. van Gelder, K.M.P van den Hout. Authors response to Cognitive outcome of patients with classic infantile Pompe disease receiving enzyme therapy. Neurology 2013;80(12):1173

2012 **B.J. Ebbink,** F.K. Aarsen, C.M. van Gelder, J.M.P. van den Hout, N. Weisglas-Kuperus, J. Jaeken, M.H. Lequin, W.F.M. Arts, A.T. van der Ploeg. Cognitive outcome of patients with classic infantile Pompe disease receiving enzyme therapy. Neurology 2012;78(19):1512-1518

2012 C.M. van Gelder, C.I. van Capelle, **B.J. Ebbink,** I. Moor, J.M.P. van den Hout, M.M. Hakkesteegt, P.A. van Doorn, I.F.M. de Coo, A.J.J. Reuser, H.H.W. de Gier, A.T. van der Ploeg. Facial-muscle weakness, speech disorders and dysphagia are common in patients with classic infantile Pompe disease treated with enzyme therapy. Journal of Inherited Metabolic Diseases 2012;35(3):505-511

CURRICULUM VITAE

Berendine Johanne (Johanneke) Ebbink werd als boerendochter geboren op 17-08-1983 te Warsnveld. Na haaar eindexamen VWO (Isendoorn College) in september 2001 vertrok zij voor 9 maanden naar Los Angeles, Amerika om daar een jaar Engels te studeren, en te reizen. In september 2002 begon zij haar studie psychologie aan de Vrije Universiteit te Amsterdam. In 2006 behaalde zij haar bachelor klinische neuropsychologie met aanvullende vakken in de kinder- en jeugdpsychologie. In 2008 behaalde zij haar master Klinische Neuropsychologie. In hetzelfde jaar behaalde zij de basisaantekening psychodiagnostiek NIP en de registratie als kinder- en jeugdpsycholoog NIP. Sinds 2016 staat zij geregistreed in het Stichting Kwaliteitsregister Jeugd. Johanneke begon haar carrière als neuropsycholoog in 2008 in het Erasmus Medisch Centrum, Sophia kinderziekenhuis middels een zwangerschapsvervanging van Femke Aarsen op de kinderneurologie, waar zij daarvoor haar stage had afgerond. Vervolgens heeft zij een zwangerschapsvervanging gedaan van Marlies van Harmelen-Bouman op de afdeling Neonatologie. Daarna begon zij in 2009 haar promotietraject bij het Centrum voor Lysosomale en Metabole Ziekten. In hetzelfde jaar startte zij tevens op de afdeling kinderoncologie in het Sophia kinderziekenhuis om onder begeleiding van Femke Aarsen en Isabelle Streng neuropsychologische diagnostiek en behandelingen uit te voeren.

Het onderzoekstraject in het Centrum voor Lysosomale en Metabole Ziekten gaf de unieke mogelijkheid om de integratie tussen klinische onderzoek en de klinische praktijk te maken. Zij heeft zich ingezet om samenwerking tussen medici en paramedici te intensiveren rondom de zorg voor patiënten.

Daarnaast kocht zij in 2010 samen met haar vriend, Jeroen van Hooijdonk, een appartement in de Jordaan in Amsterdam en in mei 2012 werd hun eerste kind Reinhard geboren. In 2014 trouwde zij met Jeroen. Eind oktober 2015 verhuisden zij naar Rotterdam en in december 2015 werd hun tweede zoon Julius geboren. In september 2016 startte zij met de opleiding tot Gezondheidszorg (GZ-) Psycholoog op de polikliniek Kinder- en Jeugdpsychiatrie en Psychologie/Adolescentenkliniek, terwijl zij betrokken bleef bij het opzetten van nieuwe studies naar de effecten van enzym vervangende therapie bij lysosomale stapelingsziekten.