Enzyme-replacement Therapy in Mucopolysaccharidoses with a Specific Focus on MPS VI

Enzym vervangende therapie in de mucopolysaccharidosen met specifieke aandacht voor MPS VI

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Inhoudsopgave

Chapter 1

General introduction

General introduction

History of MPS

Before the discovery of the lysosome, already three out of the seven currently known mucopolysaccharidoses (MPSs) were described in medical literature. In 1917, Hunter syndrome was the first to be described in two brothers ("they are as alike as two peas") by Charles Hunter [1]. Gertrud Hurler published her two case reports about MPS I in 1919, and MPS IV was outlined in 1929 by the Uruguayan dr. Morquio [2, 3]. All described young children with or without mental impairment, with profound bone abnormalities.

In 1955, the Belgian scientist Christian de Duve discovered a new intra-cellular organelle that he named the lysosome. Eventually, this brought light to more than 50 inherited diseases all concerning a defect in the lysosomal system. The group of mucopolysaccharidoses belonged to these lysosomal disorders and all types proved to be characterized by the deficiency of a lysosomal enzyme. New types of MPSs were discovered since then.

In 1963 two French physicians described a new form of dysostosis mupltiplex, MPS VI, and the syndrome was named after them: Maroteaux-Lamy's syndrome [4]. In the same year dr. Sanfilippo described for the first time a MPS III patient with mental impairment and excretion of heparan sulphate [5]. Later, Sanfillippo disease was subdivided in type A, B, C, and D because between 1972 and 1980 different enzyme deficiencies were discovered in each of the four subtypes. Finally, in 1973, MPS VII was described by the American dr. Sly [6]. MPS V and MPS VIII, first thought to be novel diseases, later turned out to be already known types of MPS and are not separate entities anymore. In 1996 Natowicz et al reported the first patient with MPS IX [7]. Till now, only one other family has been reported with this disease [8].

Pathophysiology and clinical features

The word mucopolysaccharidosis (MPS) literally means "a disease in which viscous polysaccharides are being stored" [9]. Mucopolysaccharides are nowadays termed glycosaminoglycans (GAGs). GAGs are essential constituents of the extracellular matrix of connective tissues. They are composed of long sugar chains, containing highly sulfated, repeating units of disaccharides with alternating uronic acid and hexosamine residues. The polysaccharide chains are linked to a specific core protein thereby forming complex macromolecular structures called proteoglycans (Figure 1). Depending on the composition of the repeating units, several glycosaminoglycans are known. GAG degradation takes place inside the lysosomes and requires concerted action of several acid hydrolases. Deficiencies of these enzymes are responsible for the different types of MPSs [10].

There are seven major clinical types of MPSs, involving eleven enzyme deficiencies (Table 1). The types of MPSs share many clinical features, varying from dysostosis multiplex, hepatosplenomegaly, neurological abnormalities, cardiac abnormalities, and

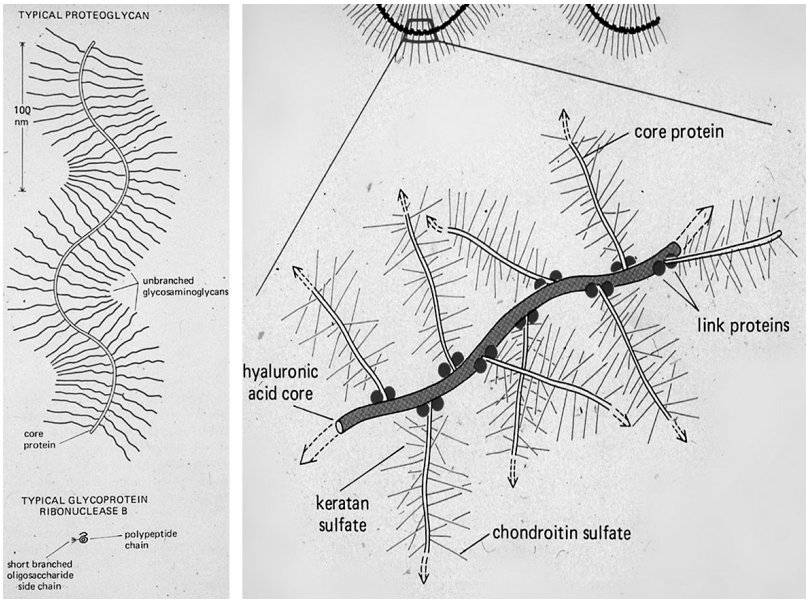

Figure 1: Aggrecan is the complex of proteoglycans that all are connected to a central long chain of hyaluronic acid via link proteins. The proteoglycans in the large aggrecan complex are feather-like structures composed of a number of regularly spaced glycosaminoglycan chains, such as keratin sulfate and chondroitin sulfate, which are covalently linked to a protein core. *From: Alberts et al., Essential Cell Biology (2003 2nd ed.).*

Table 1: Different types of mucopolysaccharidoses

	Eponym	Missing enzyme	Current therapy options	Mental retardation
MPS I	Hurler/ Hurler-Scheie/ Scheie	alpha-L-iduronidase	- Enzyme replacement therapy (ERT) - Bone marrow transplantation (HSCT; for Hurler phenotype <2.5 years)	Hurler: Yes Scheie: No
MPS II	Hunter	iduronate-2-sulfatase	ERT	Often, but not always
MPS III	Sanfilippo type: A-B-C-D	A: heparin N-sulfatase B: α-N-acetylglucosaminidase C: acetyl coenzyme A: α-glucosaminide acetyltransferase D: N-acetylglucosamine-6-sulfatase	No ltreatment available	Often, but not always
MPS IV	Morquio	A: N-acetylgalactosamine-6-sulfate sulfatase, B: β-galactosidase	No licensed treatment available yet	No
MPS VI	Maroteaux-Lamy	N-acetylgalactosamine 4-sulfatase	ERT	No
MPS VII	Sly	β-glucuronidase	No treatment available	Often, but not always
MPS IX		hyaluronoglucosaminidase 1	No treatment available	No

developmental delay. All represent a clinical spectrum, with on one end the severely affected patient with a substantially reduced lifespan and neurodegenerative disease, and on the other end the patient with an attenuated type of disease, presenting primarily with bone and cartilage disease.

All MPSs are caused by genetic deficiency of a different lysosomal enzyme involved in the degradation of these polysaccharides or glycosaminoglycans (GAGs). All genes involved in the pathophysiology of the different types of MPSs are known and hundreds of mutations have already been described. Fenotype-genotype correlations are under close investigation in all types of MPSs, but are not fully elucidated for any type of MPS.

Pathophysiology of Mucopolysaccharidosis type VI

Mucopolysaccharidosis type VI (MPS VI, Maroteaux-Lamy syndrome, OMIM #253200) is an autosomal recessive disorder caused by mutations in the ARSB (arylsulfatase B) gene located on chromosome 5q13-q14. The mutation causes a deficiency of the lysosomal enzyme arylsulfate B (also called N-acetylgalactosamine 4-sulfatase) and leads to accumulation of the glycosaminoglycan dermatan sulfate. The storage of dermatan sulphate disrupts normal cellular and extra-cellular matrix functions which eventually causes the clinical symptoms (Figure 2).

The ARSB protein composed of 533 amino acids is synthesized on membrane-bound ribosomes. The nascent polypeptide is translocated into the lumen of the endosplasmatic reticulum (ER) where the 39 amino acid long signal peptide is cleaved off. The 55.8 kDa ARSB polypeptide is glycosylated and the lysosomal targeting signal mannose 6-phophatase is acquired. During transport through the ER and the Golgi complex, ARSB attains a molecular mass of 66 kDa, but is subsequently processed to 57 kDa. The 57 kDa species is cleaved into an amino-terminal 43 kDa species, a central 7 kDa species and a carboxyl-terminal 8 kDa

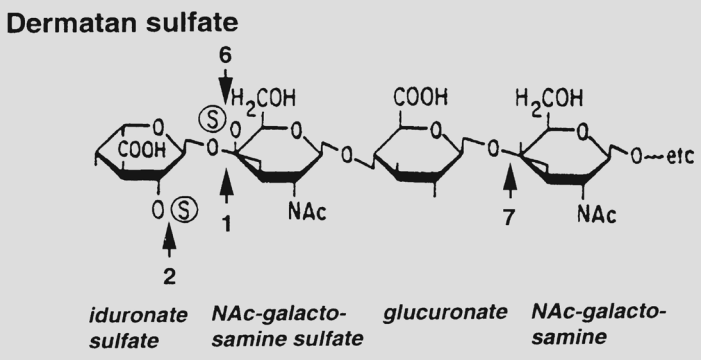

Figure 2: Main repeating units in dermatan sulfate and location of the enzyme defects in the mucopolysaccharidoses. NAc, N-acetyl; S, sulfate. 1, α-iduronidase (MPS I: Hurler and Scheie disease); 2, iduronate-2-sulfatase (MPS II: Hunter disease); *From: Saudubray et al. Inborn Metabolic Diseases. Diagnosis and Treatment (2012, 5th ed.).*

species during transport to and residence within the lysosome [11]. Mutations in the ARSB gene can affect this process of synthesis, transport and proteolytic maturation in several ways and thus lead to a gradation of ARSB deficiencies with an associated spectrum of clinical phenotypes.

Clinical features of MPS VI

MPS VI is a rare disease with a birth prevalence estimated between 1 in 43,261 and 1 in 1,505,160; in the Netherlands it was estimated at 1 in 667,000 [12]. Like many other lysosomal storage disorders, MPS VI manifests as a clinical spectrum. Within this spectrum, MPS VI patients are classified according to the rate of disease progression. The height of urinary GAG excretion seems to divide the spectrum in two broad categories: patients with a rapid type of disease have GAG values >200 µg/mg kreatinine whereas patients with a slowly progressive disease have GAG values <200 µg/mg kreatinine [13]. Though this classification helps to monitor patients, one should bear in mind that the disease spectrum is a continuum.

The rapidly progressive phenotype presents itself with clinical features before the age of five years. Patients have profound bone abnormalities, cardiac disease, including specific features of the face. Spinal cord compression occurs more frequent in rapidly progressive patients [14]. The slowly progressive patients present later in life, and usually have bone and cartilage problems as their main symptom. Although the patients with a slow disease progression usually have a later onset of symptoms, they are likely to develop the same serious manifestations of MPS VI later in life: markedly reduced growth, skeletal and joint abnormalities, coarse facial features, obstruction of the upper airways, sleep apnea, recurrent ear and airway infections, an enlarged liver and spleen and corneal clouding [15]. Furthermore, a hydrocephalus, enlarged heart, valvular disease, cardiopulmonary problems, blindness and spinal cord compression may be present. Although many types of MPSs share similar clinical features, one of the main distinctive features of MPS VI is the absence of prominent neurocognitive problems. Nevertheless, case reports about mental development disorders in MPS VI have been published, but it was not clear whether this had anything to do with confounding factors [16].

Survival studies in MPS VI have not been reported; the oldest patient with MPS VI described so far reached the age of 56 years [13].

Due to the multi-organ involvement in MPS VI a multidisciplinary follow-up of patients is essential. The rarity of the disease also calls for centralized care. Guidelines for the systematic management of patients with MPS VI have been formulated [17].

Treatment

Haemotopoietic stem cell treatment in MPS

No curative treatment is yet available for any type of lysosomal storage disorder. Two types of therapy are currently available for patients with MPSs. Hematopoietic stem cell

transplantation (HSCT) has been used since the beginning of the 1980s as a treatment for severe MPS I Hurler [18]. Since then, long-term follow-up of transplanted children has resulted in a recommendation to perform HSCT before 2.5 years of age [19, 20]. Significant success has been reported in Hurler patients after HSCT (e.g. improvements in the upper extremity joint mobility, stabilized hearing and cognition), provided the transplantation was performed early in life. The success of HSCT has been limited by donor availability, high rates of graft failure, mixed chimerism, and treatment-related morbidity and mortality [21].

Haemotopoietic stem cell treatment (HSCT) in MPS VI

Although initially promising, bone marrow or stem cell transplantation has not been successful in MPS VI. The biochemical parameters such as urinary GAG levels and ARSB activity improved, but clinical features such as dysostosis multiplex were not easy targets for this type of treatment. Moreover, a recently published review of the 45 patients who received HSCT over the past 25 years showed a 1-year survival of 62% which is too low given the poor clinical effects of HSCT in MPS VI [22]. Why HSCT is successful in only MPS I and not in the other MPSs is still unclear.

Enzyme-replacement therapy

The first trials assessing efficacy of ERT were in Gaucher patients in the early 1990s [23]. This opened the door for the same therapeutic approach in other lysosomal storage disorders. Since the registration of ERT for Gaucher disease in 1991 other types of ERT have been developed, such as for Pompe disease and Fabry disease. MPS I was the first MPS specific ERT and was introduced on the European market in 2003. MPS II and MPS VI followed in 2006 and 2007. All types of ERT for MPS proved to be effective on several clinical endpoints, such as range of motion, pulmonary function, cardiac function and endurance.

Enzyme-replacement therapy in MPS VI

The first phase I clinical trial on the efficacy and safety of enzyme-replacement therapy in MPS VI was published in 2004 [24]. One-weekly infusions of galsulfase (recombinant human ARSB) proved to decrease GAG storage, measured in six patients as a decrease in urinary glycosaminoglycan excretion. The following phase II (n=10) and phase III (n=39) studies showed the first clinical response, with a significant increase on the 12-minute walk test (12MWT) and the 3-minute stair-climb test (3MSC) after 24 and 48 weeks of treatment [25, 26]. Extension studies also showed an increase in pulmonary function and growth [27, 28]. Later on, smaller observational studies confirmed these effects and showed additional effects on range of motion and liver size [29, 30]. Effects observed so far on cardiac parameters are scarce, although it has been reported that valve regurgitation seems refractory to treatment [31].

ERT is not able to cross the blood-brain barrier. Consequently, intravenous treatment of ERT is not able to correct the severe spinal cord compression which can occur due to storage of GAGs in the meninges. Intrathecal treatment was the logical step to overcome this problem. Although effective in MPS VI cats, intrathecal medication in humans showed no to be a safe alternative so far, since instability of the spinal cord can induce severe neurological complications [32].

Antibodies to ERT

With the introduction of ERT, new questions arose in managing patients. Although major adverse events are rare, publications on the development of antibodies against the foreign protein already appeared in 1999 [33]. In course of time antibody formation was reported in all available ERTs [34]. The immune system apparently recognizes the recombinant human enzyme as a foreign protein due to the patient's inability to produce a functional enzyme. The current opinion is that antibody formation can affect the outcome of ERT in a negative way [35, 36]. The height of the antibody response may depend on whether the patient does not produce enzyme at all, a miss-folded enzyme that is prematurely degraded, or an enzyme that reaches the lysosomes but does not function. Synthesis, transport and maturation defects result from mutations in the disease specific gene, and the patient's morbid ARSB genotype may thus determine in part the height of the immune response. Classic infantile Pompe disease serves as a proof of principle to this theory. In this lysosomal glycogen storage disorder patients have been classified as CRIM-positive for those who produce too little or non-functional enzyme and as CRIM-negative for those who do not produce any enzyme, whereby CRIM stands for cross-reactive immunologic material. Major clinical events such as survival were reported to be determined by this CRIM status and its related immunologic response [35]. Although there is considerable evidence that antibodies affect ERT in MPS I dogs [3,7], the data are still scarce in other MPSs including MPS VI.

Monitoring treatment

After concluding the phase of randomized-controlled trials, information about the effectiveness of ERT can only be collected in observational studies. Site-specific, observational and investigator driven studies have been reported for all types of ERT in MPSs. A disadvantage of these types of studies is the small sample size due to the rarity of the disease. In an attempt to overcome this problem, information about patients with MPSs is currently being gathered and published through several European and worldwide Registries (MPS I, II, IV and VI) available for the different diseases [38-40]. These Registries are funded and managed by the pharmaceutical companies that supply the enzyme replacement therapy. They certainly have an important function in data collection, but do

not cover the whole picture. The disadvantage of these Registries is that data are entered by physicians while the patient's point of view is hardly taken into account.

Patient-reported outcome measures (PROMs) provide a means of gaining insight into the way patients perceive their health and the impact that treatments or adjustments to lifestyle may have on their quality of life (university of Oxford, www. http://phi.uhce.ox.ac.uk/home.php). These instruments can be completed by the patient itself or by others on their behalf. In other lysosomal storage disorders such as Pompe disease, PROMs have contributed to the knowledge we nowadays have about, for instance, disability and quality of life [41]. In MPSs, PROMs were rarely used so far. The neurological involvement of many MPSs patients hampers the application of this instrument because one is always dependent on a "proxy report". However, PROMs that can be entered by proxies (parents or caregivers) have been developed in the last few years and enable the follow-up of patients with MPSs.

Scope of this thesis

With the introduction of enzyme-replacement therapy for MPS I, II and VI in The Netherlands the question arose if the reported effects of these therapies in the phase III trials would still be obtained after long-term treatment. Functioning as an expert center for lysosomal and metabolic diseases we were able to optimize standard care for these patients and provide a thorough follow-up programme. We were also able to gather more information on the natural course of the disease while caring for yet untreated patients. The studies described in this thesis discuss the long-term effects of ERT in mainly MPS VI patients. In cooperation with the Dutch patient organisation (VKS) we compared the perception of pain in several types of MPSs, with or without ERT. Besides recording the clinical parameters, we also investigated the phenotype-genotype profile of patients in relation with immunological response and effect of ERT.

Chapter 2 reviews all previously published clinical effects of ERT in MPS VI. In the first part of **Chapter 3** we looked more specifically into the cardiac response to ERT in MPS I, II and VI since cardiac disease is one of the main causes of death in MPSs patients. The second part of this chapter presents a case report about a young girl with MPS VI who underwent cardiac valve surgery. The emphasis here is on the underlying mechanism of cardiac valve pathology and the relative resistance of the valve to ERT. **Chapter 4** reports the clinical results of long-term follow-up of patients receiving ERT, while **Chapter 5** describes the phenotype-genotype correlation in 12 patients with MPS VI and explores the antibody response to galsulfase in these patients. **Chapter 6** addresses specifically the long-term cognitive development in MPS VI patients. As a result of studies conducted in collaboration with the Dutch patient association VKS, **Chapter 7** at last categorizes the frequency and amount of pain that patients with all types of MPSs experience, since pain can be very invalidating.

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Chapter 2

Enzyme-replacement therapy for mucopolysaccharidosis type VI (Maroteaux-Lamy): A systematic review of the literature

Marion Brands, Iris Plug, Marloes Hagemans, Audrey Vollebregt, Esmee Oussoren, Arnold Reuser, Ans van der Ploeg

Ready for Submission

Summary

Mucopolysaccharidosis type VI (MPS VI, Maroteaux-Lamy syndrome) is an autosomal recessive disorder caused by the deficiency of the lysosomal enzyme N-acetylgalactosamine 4-sulfatase (arylsulfatase B), leading to accumulation of the glycosaminoglycan (GAG) dermatan sulfate. This accumulation leads to various symptoms, mainly affecting the cartilage and bones. Growth retardation, skeletal and joint abnormalities, coarse facial features, restrictive pulmonary disease and cardiac disease are prominent features of this disease. Enzyme-replacement therapy (ERT) is currently the only treatment option for this disease.

We performed a systematic review of the literature to summarize the effects of ERT (galsulfase) in patients with MPS VI in order to provide insight in the existing evidence for this treatment. Inclusion criteria were prospectively designed clinical studies evaluating ERT with quantifiable endpoints, including more than three patients. We included randomized controlled trials and open label studies. We selected the most frequent chosen endpoints of these studies to make a qualitative synthesis.

A total of 1 RCT and 9 open label studies were identified for inclusion. The most commonly studied endpoints were pulmonary function, endurance (12-minute-walk-test, 3-minute-stair-case-test) cardiac response, urinary GAG excretion, growth, size of liver and spleen and range of motion (ROM).

In conclusion, although ERT in MPS VI patients has promising results on endpoints like pulmonary function, endurance, excretion of GAGs, growth and cardiac disease it seems that the correction of bone and cartilage disease remains a challenge.

Introduction

Mucopolysaccharidosis type VI (MPS VI, Maroteaux-Lamy syndrome) is an autosomal recessive disorder caused by the deficiency of the lysosomal enzyme N-acetylgalactosamine 4-sulfatase (arylsulfatase B), leading to accumulation of the glycosaminoglycan dermatan sulfate [1]. MPS VI is a rare disease with an estimated birth prevalence in the Netherlands of 1 in 667,000 [2]. The progressive dermatan sulphate storage leads to a variety of symptoms, all concerning the dysfunction of the extracellular matrix. Markedly reduced growth, skeletal and joint abnormalities, coarse facial features, obstruction of the upper airways, sleep apnea, recurrent ear and airway infections, an enlarged liver and spleen and corneal clouding are all frequently reported symptoms of MPS VI. Furthermore, an enlarged heart, valvular disease, cardiopulmonary problems, blindness and spinal cord compression may be present. In contrast to other types of MPS, mental retardation is not a part of the clinical picture in MPS VI. MPS VI is considered to be a clinical spectrum with a variable progression [3].

Enzyme-replacement therapy (galsulfase, Naglazyme®, Biomarin) for MPS VI is available since 2007 after EU approval by the FDA (2005) and the EMA (2006), and aims at reduction of glycosaminoglycan storage and subsequent amelioration or elimination of symptoms. Patients receive the approved dosage of 1mg/kg galsulfase every week intravenously.

Objective

The objective of this review is to summarize the effects of ERT (galsulfase) in patients with MPS VI in order to provide insight in the existing evidence for this treatment. We focus on the most frequently used clinical endpoints namely respiratory function, endurance, organ size, range of motion, growth and urinary GAG excretion.

Efficacy endpoints

The efficacy of galsulfase has been measured against a variety of endpoints. In most studies subclinical parameters such as organ function tests, (e.g. pulmonary function), organ size (liver and spleen) and Range of Motion (ROM) of the joints were included. Clinical parameters were mostly focused on endurance, assessed by the 6-minute-walk-test (6MWT), 12-minute walk test (12MWT) or the three-minute stair climb test (3MSC). In almost all clinical studies the excretion of urinary glycosaminoglycans (GAGs) has been used as a marker to evaluate efficacy of therapy. Information on the impact of ERT on survival, the endpoint considered most relevant has not been published yet.

Methods of literature review

We searched and reviewed PubMed (MEDLINE) on November 17th 2012. We used the search terms 'Maroteaux-Lamy (MeSH)', 'Mucopolysaccharidosis type VI', 'enzyme-replacement therapy', 'galsulfase', 'Naglazyme' and combinations of these terms.

Inclusion criteria for the systematic review were prospectively designed clinical studies evaluating ERT in humans with quantifiable endpoints. We included open-label studies as well, since no additional randomized-controlled trials were conducted after the approval of ERT and since open label studies also provide information on effectiveness of treatment in clinical practice. Review articles were excluded, we also excluded case reports, cases studies with less than 3 patients, and letters to the editor.

Results

Literature

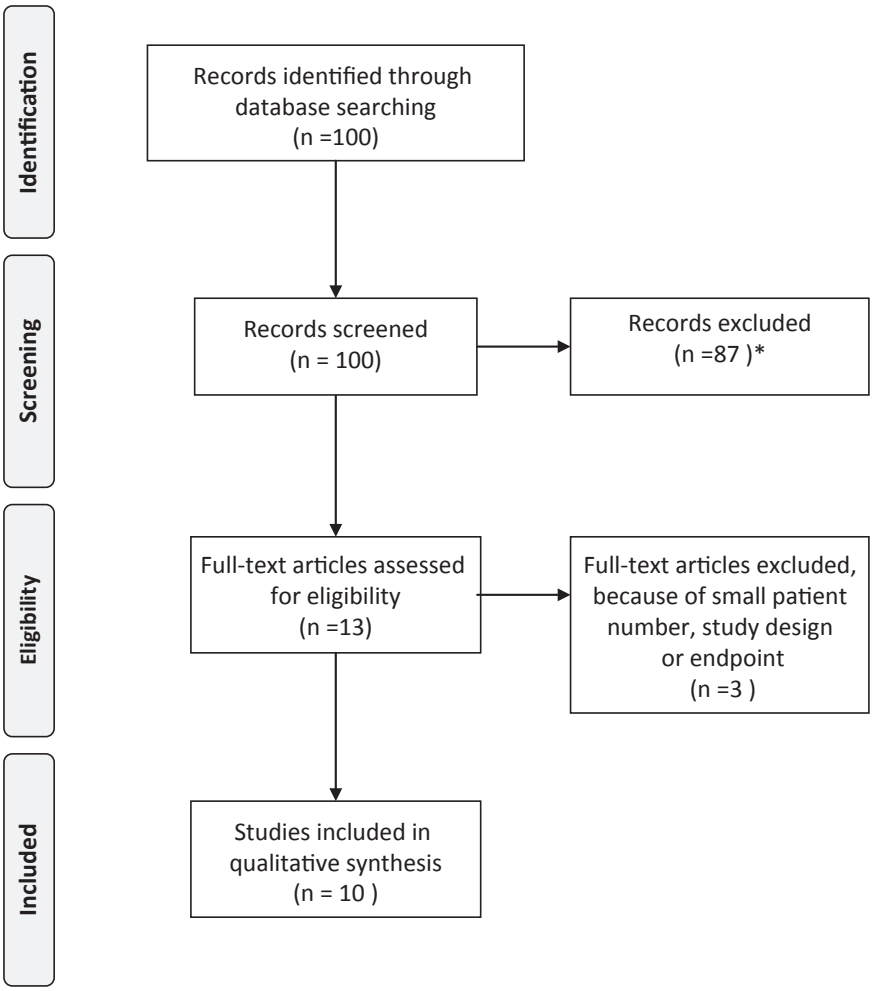
Based on the literature search we identified 100 records of which 83 were excluded because these did not report on effects of ERT in human subjects or were literature reviews. We excluded five full text articles due to the small patient numbers or study

Table 1 Randomized controlled trials of enzyme-replacement therapy in patients with mucopolysaccharidosis type VI

Study, year [ref]	Patients		Intervention and control	Duration	Efficacy endpoints
	Number (age range)	Characteristics			
Harmatz, 2006 [4]	39 (5-39 y)	≥ 7 years, who could walk unaided for at least 5 meters and no more than 270 meters in the first 6 minutes; or no more than 400 meters in total in 12 minutes.	galsulfase 1,0 mg/kg/ week versus placebo (19 pt : 20 pt)	- 24 weeks RCT - followed by 24 weeks open label extension study	Primary: - 12MWT Secondary: - 3MSC - urinary GAG excretion Tertiary - joint pain/joint stiffness - ROM - coin pick-up test Other: - Respiratory function test - Cardiac function - Health resource use

12MWT=12-minute walk test; 3MSC= 3-minute stair climb test; GAG= glycosaminoglycan; ROM= range of motion

Figure 1: PRISMA flow chart. Included were one randomized, controlled trials and 9 open label studies.



* Studies were excluded if they were reviews or did not involve effects of ERT in human subjects

design. Ten studies were eligible to include in the qualitative synthesis (Figure 1). This included one randomized controlled trial which was followed by an open label extension study; we also included the phase I and II studies. Nine open-label studies were included (Table 1 and 2) of which three studies described the same patient cohort of the phase I, II and III studies. Seven to 56 patients were included per study. Two studies focused on solely cardiac response to ERT.

Table 2: Open label studies in MPS VI patients receiving 1.0 mg/kg/week ERT (galsulfase)

Study, year	Patient		Drug	Duration	Efficacy endpoints
	Number (age range)	Characteristics			
Harmatz, 2004 [5]	7 (7-16 y)	Clinical manifestation of MPS VI	Randomisation in either 0.2 mg/kg/week and 1.0 mg/kg/week galsulfase, followed by 24 weeks open label extension with the same dosages	48 wk of which 24 weeks were blinded	- urinary GAG excretion - 6MWT - ROM - FVC and FEV1 - monitoring physical movements of Denver developmental examination - ophthalmological examination - echocardiogram and ECG - lumbar bone density - polysomnography - Childhood Health Assessment Questionnaire (CHAQ)
Harmatz 2005 [6]	10 (6-22 y)	Rapidly advancing disease; ability to walk unaided at least 1 m but <250m in 6 minutes	galsulfase 1,0 mg/kg/week	48 wk	- 12MWT - 3MSC - ETGG - ROM - FVC and FEV1 - Grip and pinch strength - coin pick-up test - CHAQ - monitoring physical movements of Denver developmental examination - ophthalmological examination - echocardiogram and ECG - liver size - lumbal bone density
Harmatz, 2008 [7]	56 (5-29 y)	Patients from the phase I, I/II and III study	galsulfase 1,0 mg/kg/wk (in phase I, 3 pt who received 0,2 mg/kg/week)	Patients I/II study: 240 wk Phase II: 144 wk Phase III: 96 wk	- 6MWT - 12MW - 3MSC - urinary GAG excretion

Scarpa, 2009 [8]	9 (4-10 y)	Patients who participated in the phase III study of the MPS VI registry	galsulfase 1,0 mg/kg/wk	0,5-4,5 y	- liver and spleen size - 6 MWT or 12 MWT - 3 MSC - ROM - Hearing problems - Eye problems - Valvular heart disease - brain MRI - spine MRI - neurological problems - breathing problems - growth
Harmatz, 2010 [9]	56 (5-29 y)	Combination of inclusion criteria phase I,II and III	galsulfase 1,0 mg/kg/wk (in phase I,3 pt who received 0,2 mg/kg/ week)	240 w, analysis possible till wk 96	- pulmonary function relative to growth - FEV 1 - FVC - MVV
Decker, 2010 [10]	56 (5-29 y)	Combination of inclusion criteria phase I,II and III	galsulfase 1,0 mg/kg/wk	96 w	- growth - urinary GAG excretion - stage of puberty according to Tanner
Hsiang-Yu 2010 [11]	9 (1.4-21.1 y)	Taiwan patients	galsulfase 1,0 mg/kg/wk	2-6 y	- urinary GAG excretion - length and weight - 6 MWT or 12 MWT - 3 MSC - ROM - FVC and FEV1 - CHAQ - coin pick up test - sleep study
Braunlin 2012 [12]	11.8±5.4 (6-29 y)	Ultrasound data from phase I/II/III study	galsulfase 1,0 mg/kg/wk	96-240 w	- LVED - LVES - IVSd - LVPWd
Brands 2012 [13]	10 (2-16 y)	Dutch patients with MPS VI	galsulfase 1,0 mg/kg/wk	0-154 w	- LVMI - IVSd - LVPWd - LVIDd - SF - Valve pathology

3MSC= 3-minute stair climb test; 6MWT=6-minute walk test; 12MWT=12-minute walk test; (C)HAQ=(Childhood) Health Assessment Questionnaire; ETGG=Expanded timed get-up-and-go test; FEV1= forced expiratory volume in 1 seconde; FVC=Forced Vital Capacity; GAGs=glycosaminoglycanen; MVV=maximum voluntary ventilation

Table 3 Absolute Forced Vital Capacity (FVC) and/or predicted FVC predicted (%FVC) assessed in clinical trials of ERT in MPS VI patients

Study	Duration	N	Baseline	Endpoint	p-value	overall result
Randomized controlled trials				**Galsulfase vs placebo**		
Harmatz	24w	39	Data not shown	Data not shown	Data not shown	↔
Open label studies				**Galsulfase**		
Harmatz 2005	48w	10	Data not shown	Data not shown	Data not shown	10%↑ in 5 pt
Harmatz 2010 (L, mean, range)	144-240w	53	0.56 (0.16-1.74)	0.10±0.03	<0.001*	↑
Liu (L, mean±SD)	2-6 y	4	0.54±0.21	0.64±0.16	Data not shown	↑

*p-values are calculated using a linear longitudinal mixed-effects model with pooled data
↑=increase, ↔=stable, ↓=decrease

Pulmonary function: Forced vital capacity, predicted Forced Vital Capacity

The RCT included pulmonary function, measured as FVC and predicted %FVC as an outcome measure to allow long-term evaluation of ERT. No improvement was reported after 48 weeks of treatment [4] (Table 3). The 10 patients included in a phase I/II open label study were so severely affected that only two of them were able to perform a pulmonary function test. In one patient an increase of 11% was observed [5]. The following phase II study in 10 patients (age 6-22 years) showed a clinically relevant improvement in 5 patients. Clinically relevance was defined as an increase in FVC of 10%. This change was mainly found after 24-48 weeks of therapy [6].

One open label study evaluated the pooled long-term data from the phase I/II/III trials in comparison with a Survey study to assess the effects of pulmonary function specifically [9]. The FVC and FEV1 were analysed in 56 patients after 240 weeks of treatment. Data could be analysed till 96 weeks. The authors observed a significant increase in absolute FVC and FEV1 after 2 years of ERT. A longitudinal linear mixed effects model was developed in which the included patients were incorporated, together with untreated patients from a patient Survey [14] and patients who received placebo in the first 24 weeks of the RCT. In this manner the authors were able to assess a change in FVC post ERT compared to ante ERT. In the untreated patients a negligible increase in FVC was found of 0.01L per year; in the treatment group an increase of 0.1L/years was found ($p<0.001$). In the age group<12 years, as well as >12 years, the increase in absolute FVC and FEV1 was significant. An increase in height as a possible cause of increase in lung capacity was less likely, since the lung capacity in the older patients also increased.

A case series from Taiwan in 10 patients showed an increase in FEV1 and FVC in 4 patients who could perform a pulmonary function test [11].

In summary, absolute FVC improved after long term treatment with ERT. These effects were observed in the largest cohort after 2 years of treatment. In case series with shorter

follow-up, an improvement was also noted, but no statistics were performed in these small sample sizes. Predicted FVC did not improve during ERT (Table 3).

Endurance: 6-minute walk test (6MWT), 12-minute walk test (12MWT) and the 3-minute stair climb test (3MSC)

The 12MWT was the primary outcome measure in the phase III trial and showed a significant improvement after 48 weeks of treatment compared to placebo [4]. After 24 weeks, the treatment group walked a mean of 92 meters further compared to the placebo group. After 24 weeks, the placebo group was switched to galsulfase, and an increase of 66 meters was measured compared to the number of meters patients were able to walk at the 24 weeks point (p=0.007). Additionally, the phase III study included the 3-minute stair climb test (3MSC) as a measure of endurance. The 3MSC is a non-standardized test, in which patients were instructed to climb as many steps as possible in a 3-minute period and were allowed to rest and use handrails during the test. At week 24, the difference between the treated and the placebo group was 5.7 stairs (p=0.053). After 24 weeks, the placebo group switched to active treatment and also showed a significant improvement of 5.7 stairs after 48 weeks, compared to start of therapy [4].

The 6MWT was used as an outcome measure in the first open label study on the effects of ERT in patients with MPS VI [5]. The second phase II study included a 12MWT to provide a wider dynamic range for capturing improvements in endurance relative to 6-minute time point during the same walk [6]. The phase I/II trial showed a significant improvement on the 6MWT after 48 weeks of treatment; the three patients who received 1.0 mg/kg/wk of galsulfase walked 61 meter more after 48 weeks of treatment (p=0.04) compared to baseline [5]. The following phase II trial in 10 patients reported a mean increase of 211 meters on the 12MWT after 48 weeks of treatment (p=0.002) [6]. In the same study, these patients walked a mean of 91 meters further at the 6-minute point of the 12MWT compared to baseline (p=0.002). Also, the 3MSC was included in this study, showing a wide range of 20 to 92 stairs at baseline. All patients showed an improvement after 48 weeks of therapy, with a mean improvement of 60.8 stairs (range 3-136 stairs; p=0.005) (Table 5) [6].

In the phase I/II/III extension study, the effect on the 6MWT and 12MWT persisted after long-term follow-up. The phase I/II study shows a trend in improvement (p=0.0625, n=4) after 240 weeks; by that time patients were able to walk 130 meters further than at baseline. The phase II (144 weeks) and phase III study (96 weeks) showed a significant improvement on the 12MWT (p=0.004 and p<0.001, respectively). A trend in improvement was seen in the 3MSC outcome measure in the phase II group after 144 weeks of treatment (improvement of 71.2 stairs). In addition, the phase III extension group showed a significant improvement at the 3MSC after 96 weeks of follow-up (p<0.001).

The Italian cohort showed an improvement on either the 6MWT or the 12MWT in 3 of 7 patients (crude figures are not given) [8]. The 3MSC improved in 2 of the 4 patients who were able to perform this test.

The Taiwan study showed on the 6MWT as well as the 12MWT an improvement of 27.3% and 39.9%, respectively, compared to baseline. In the 7 patients who performed a 3MSC, an improvement of 35% after 2 years of treatment was observed [11].

In summary, the phase I/II/III studies including the extension studies, which included the 6MWT or the 12MWT as outcome measure reported an improved endurance. The observational studies also showed improvement, but only in individual patients, an improvement was not always observed in individual cases (Table 4).

Table 4: 6-minute walk test (6MWT or 12MWT) assessed in clinical trials of ERT in MPS VI patients

Study	Duration	N	Baseline (m)	Endpoint (m)	p-value	overall result
Randomized controlled trials (12MWT)				**Galsulfase (vs placebo)**		
Harmatz	24w	39	227±170 vs 381±202	336±227 399±217	0.025*	↑
Open label studies (12MWT)				**Galsulfase**		
Harmatz 2005	48w	10	264±170.4	475.1±206.9	0.002	↑
Harmatz 2008 (summary)	phase I: 240 w (mean±SD)	Data not shown	Data not shown	Data not shown		
	phase II: 144 w (mean±SD)	10	264±79	537±273	0.004**	↑
	phase III 96 w placebo/galsulfase: (fitted data±SE)	19	400±49	516±49	<0.001***	↑
	phase III extension 96w (fitted data±SE)	19	214±49	397±50	<0.001****	↑
Scarpa	4-4.5 y	4	Data not shown.	Data not shown		2pt↑ 2pt -
Liu	2-6 y	6	574.0±230.3	802.9±222.1		39.9%↑
Open label (6MWT)						
Harmatz 2004 (mean±SD)	48 w	6	1.0 mg/kg/wk 176±29	Δ61m	0.04**	
Harmatz 2005 (mean±SD)	48 w	10	152.35±79.1	243.6±103.3	0.002***	
Harmatz 2008 (summary)	phase I: 240 w (mean±SD)	5	202±138	332±126	0.0625**	
	phase II: 144 w (mean±SD)	10	152±79	279±134	0.0028***	
	phase III 96 w placebo/galsulfase: (fitted data±SE)	19	209±23	270±23	<0.001****	
	phase III extension 96w (fitted data±SE)	19	128±24	208±24	<0.001****	

* comparison galsulfase with placebo using a repeated measures linear model
** p-value of the total group using Wilcoxon signed rank test for small groups
*** p-value using paired t-test
**** using longitudinal repeated measures model

Table 5: 3 minute stair climb test (3MSC) assessed in clinical trials of ERT in patients with MPS VI

Study	Duration	N	Baseline	Endpoint (m)	p-value	overall result
Randomized controlled trials				**Galsulfase (vs placebo)**		
Harmatz (stairs/min; mean±SD)	24 w	39	19.4±12.9 vs 31.0±18.1	26.9±16.8 vs 32.6±19.6	0.025*	↑
Open label studies				**Galsulfase**		
Harmatz 2005 (stairs/min; mean±SD)	48 w	10	50.0±29.5	110.8±65.2	0.005	↑
Harmatz 2008 (summary)	phase I: 240 w (stairs/min; mean±SD)	Data not shown				
	phase II: 144 w (stairs/min; mean±SD)	10	50.0±29.5	124.4±70.9	0.006**	↑
	phase III 96 w placebo/galsulfase: (stairs/min, fitted data±SE)	20	92.1±11.9	114.9±11.9	<0.001***	↑
	phase III extension 96w (stairs/min, fitted data±SE)	19	58.3±11.4	92.1±11.4	<0.001***	↑
Scarpa	4.0-4.5 y	4	Data not shown			2/4 patients improved
Lin et al. (stairs/min; mean±SD)	2-6 y	7	130.9±40.8	186.0±46.1	Data not shown	35.7% improvement in comparison with baseline

* using longitudinal repeated measures model
** p-value using paired t-test
*** using longitudinal repeated measures model
↑=increase, ↔=stable, ↓=decrease

Range of Motion

The RCT included range of motion (ROM), measured as as tertiary outcome measure and no significant improvement was observed.

In the phase I/II study an improvement of the ROM of the shoulder was measured in 5 of 6 patients (treated with different dosages) after 48 weeks of treatment. Restriction in shoulder flexion decreased significantly from baseline to week 48 for the total population (right shoulder 84 to 68 degrees, p=0.04; left shoulder 83 to 67 degrees p=0.04). In the elbow and knee these differences were smaller and not significant [5].

The phase II study in 10 patients showed a small improvement of 10 degrees in active and passive shoulder flexion, extension and lateral rotation after 48 weeks of treatment. The Italian observational case series showed an improvement of joint mobility in 5 out of 7 patients [8].

The Taiwan study reported an improvement in shoulder mobility in all nine patients, but crude data were not provided [11].

In conclusion the range of motion of the shoulder improved with a mean of around 10 degrees in the phase I/II and II study. Also in the observational studies improvement in range of motion of the shoulder was measured, but crude data were not provided (Table 6).

Table 6: Range of Motion (ROM) assessed in clinical trials of ERT in MPS VI patients

Study	Duration	N	Baseline	Endpoint	p-value	overall result
Randomized controlled trials				**Galsulfase vs placebo**		
Harmatz 2005	24 w	39	Data not shown	Data not shown		↔
Open label studies (shoulder flexion/restriction)				**Galsulfase**		
Harmatz 2004^	48 w	6	Right shoulder: 84º restriction left shoulder: 83º restriction	Right shoulder: 68º restriction left shoulder: 67º restriction	0.04	↑
Harmatz 2005	48 w	10	Data not shown			↔
Scarpa	0.5-4.5 y	9	Data not shown	Data not shown		5/9 patients improved
Liu	2-6 y	9	Data not shown			all improved

*No statistical analysis was performed due to the small sample size

^shoulder restriction in flexion

↑=increase, ↔=stable, ↓=decrease

Urinary GAG excretion

All studies showed a substantial decrease in urinary GAG excretion of 51-79% compared to baseline (Table 7). P-values ranged from p=0.04 after 48 weeks of therapy to p<0.001 after 44 months of therapy. The urinary GAG excretion in the phase I study was borderline significant (p=0.0625) after 240 weeks, but the range in GAG reduction was still between 62-88% [7].

Liver size

Two observational studies reported a decrease of liver size in MPS VI patients (Table 8). Harmatz 2005 observed no change in liver size [6]. Scarpa et al found a decrease in 5 out of 9 patients [8].

Growth

Decker et al analysed growth after a periode of 96 weeks of ERT in 56 patients who were included in the phase I/II/III studies [10]. Growth rate was analyzed for approximately

Table 7: Urinary glycosaminoglycan (GAG) excretion assessed in clinical trials of ERT in MPS VI patients

Study	Duration	N	Baseline	Endpoint	p-value	overall result
Randomized controlled trials			**Galsulfase vs placebo**			
Harmatz (µg / mg creatinine; mean±SD)	24 w	39	346±128 vs. Data not shown	Δ85±36 vs Data not shown	<0.001*	↓
Open label studies				**Galsulfase**		
Harmatz 2004	48 w	6	Data not shown	Reduction of 63% in 1.0/mg/kg group and 51% in 0.2 mg/kg/wk group	0.04	↓
Harmatz 2005	48 w	10	Data not shown	76% reduction		↓
Harmatz 2008 (summary)	phase I: 240w (% reduction, range)	5	Data not shown	79 (62-88)	0.0625**	
	phase II: 144w (% reduction, range)	10	Data not shown	72 (45-84)	<0.001***	
	phase III placebo/ galsulfase: 96w (% reduction, range)	19	Data not shown	71 (52-87)	<0.001****	
	phase III extension 96w (% reduction, range)	19	Data not shown	72 (42-82)	<0.001****	

*p-value using ANOVA
** p-value of the total group using Wilcoxon signed rank test for small groups
*** p-value using paired t-test
**** using longitudinal repeated measures model
† Due to the small sample size, no statistical calculations were performed
↑=increase, ↔=stable, ↓=decrease

2 years prior to and following the start of ERT using longitudinal modelling. In the total group, the change in height pre-ERT was 0.65 cm. After a year of ERT, mean height increased about 2.6 cm on average (p<0.001). The largest impact was observed in patients under 12 years of age. Baseline urinary GAG excretion did not affect the rate of growing. The Italian observational cohort showed an improvement in growth after start of ERT, but none of the patients reached the third percentile [8]. In summary, ERT seemed to positively affect linear growth, especially in children younger than 12 years of age (Table 9).

Cardiac abnormalities

The RCT included cardiac size as a variable, but it is unclear whether an effect was observed. Four observational studies reported on cardiac size. In the phase I study no difference was observed in cardiac size while Scarpa et al reported a worsening in valve

Table 8: Size of liver assessed in clinical trials of ERT in patients with MPS VI

	Duration	N	Baseline	Endpoint	p-value	overall result
Randomized controlled trials: not assessed						
Open label studies				**Galsulfase**		
Harmatz et al. 2005	48 w	10	Data not shown			↔
Scarpa et al. 2009	0.5-4.5 y	9	Data not shown	Data not shown	5/9 showed a reduction in liver size	↓

Table 9: Growth velocity assessed in clinical trials of ERT in patients with MPS VI

Study	Duration	N	Baseline	Endpoint	p-value	overall result
Randomized controlled trials: not assessed						
Open label studies				**Galsulfase**		
Scarpa 2009	0.5-4.5 y	9	Data not shown			?
Decker et al. 2010 growth velocity (cm/year)	96 w	54	Estimated 0.65 (SE 0.25)	2.6 (SE 0.22)	<0.001*	↑

* change in growth velocity was measured with a longitudinal mixed-effects model with pooled data

Table 10: Cardiac mass assessed in clinical trials in patients with MPS VI

Study	Duration	N	Baseline	Endpoint	p-value	overall result
Randomized controlled trials						
Harmatz 2006	24 w	39	Data not shown			?
Open label studies				**Galsulfase**		
Harmatz 2004	48 w	6	Data not shown			↔
Harmatz 2005	48 w	10	Data not shown			↔
Scarpa 2009 (no. of pt with valve disease)	0.5-4.5 y	8		3/8 worsened 5/8 stable		↔↑
Brands 2011 LVMI	48-154 w	8	3/8 Z-score>2	2 patients improved	0.009 0.043	↑
Braunlin IVSd (Z-score, mean±SD)	96 w	52	1.57±0.95	0.97±0.89	<0.0001	↓
BraunlinLVPWd (Z-score, mean±SD)	96 w	52	1.86±0.99	1.64±0.94	0.551	↔

?= no effect was mentioned
LVMI=left ventricular mass index
IVSd= interventricular septum diameter in diastole
LVPWd =left ventricular posterior wall in diastole
↑=increase, ↔=stable, ↓=decrease

pathology. Two papers specifically reported on cardiac pathology. Brands et al reported a significant decrease in left ventricular mass index (LVMI) in two patients [13]. Braunlin et al found a significant decrease in interventricular septum diameter (IVSd) and LVPWd in 52 patients after 96 weeks of treatment (Table 10) [12].

Discussion

In this systematic review we evaluated the available evidence on the efficacy of enzyme replacement therapy with galsufase in patients with mucopolysaccharidosis type VI. We focused on the most frequently used clinical endpoints. The qualitative synthesis included one randomized controlled trial and 10 open-label studies (in which the phase I and II studies were included). Except for the phase I study, the studied dosage was 1 mg/kg of weekly intravenously administered galsulfase. All studies showed an improvement in almost all included endpoints: pulmonary function (FVC, %FVC), endurance (6MWT, 12 MWT and 3MSC), excretion of urinary GAGs, size of liver and spleen, growth and cardiac dimensions. MPS VI presents as a clinical spectrum in which the disease progresses more rapidly in some patients than in others. Based on current available literature we have not been able to assess if the observed effects of ERT are similar throughout the complete spectrum.

Pulmonary function was most accurately assessed in the overview of phase I, II and III studies up to a period of 96 weeks published by Harmatz et al in 2010 in which they were able to perform follow-up on 56 included patients and compared the outcome to earlier obtained data on untreated patients [9]. Significant effect on pulmonary function could be measured after 2 years on both the absolute FVC and the predicted FVC. They concluded that growth did not have an additional influence on the increase in pulmonary function in older patients. However this conclusion is based on height change and not on height-dependent normal-values. The effect of ERT on pulmonary function seemed to diminish after 96 weeks of treatment in the older patient group, unfortunately the number of patients included in the long-term analysis was too small to further elaborate on this finding. One can argue if the eventually measured effect of 0.1L FVC gain per year is a clinical relevant finding.

A considerable effect on endurance was found, with an increase of almost 255 meters walking after 144 weeks of follow-up in the 9 phase II patients and an additional 117 to 183 meters improvement after 96 weeks in the RCT included patients. Unfortunately, the 12MWT (and 6MWT) has no age-dependent normal values, which complicates the interpretation of certain figures. One should take into account that a healthy child will also improve on the 12MWT with increasing age. However it is plausible that an increase of 255 meters, is attributable to ERT.

The 12MWT is often used as a measurement of endurance. Since patients with MPS VI have severe joint abnormalities this assessment can be subject to variable interpretations: the patient is able to walk a limited distance, but one cannot discern whether this is due to limited endurance, joint abnormalities, pain, or a combination of these three factors. The same holds for the 3MSC, an endpoint which significantly improved as well in several studies. The clinical relevance and interpretation of these assessments should be discussed in these manuscripts.

Range of motion did not show major improvements with only 10 degrees improvement in the phase II study. The other studies that we reviewed confirmed this finding as no improvement in range of motion were observed. It seems that ERT does not correct the joint deformities. A possible explanation could be that the accessibility of the chondrocytes is restricted by slow diffusion of the relatively large therapeutic enzymes through the molecular structure of the matrix [15]. Since bone deformities are a major feature of MPS VI, innovative developments for therapy are necessary to overcome this problem. A first study of the effect of gene therapy in MPS VI cats shows promising findings [16].

The difficulty to correct bone disease might also explain the poor effects of ERT on growth. Growth was an endpoint in 2 studies. The largest was performed by Deckers et al and showed a significant improvement in growth, both in pre-pubertal aged 5-16 years patients (1-3 cm/year) as well as in post-pubertal patients aged 16 years or older (2 cm/year). Again, it would have been more insightful/informative if these figures would have been compared to normal growth figures, instead of solely assessing the group data. For instance, the normal growth velocity of a 10 years old Dutch child is 6 cm/year [17]. The Italian group reported percentile growth and showed that none of the patients reached the 3rd percentile despite growth increase during ERT [8].

Urinary excretion of glycosaminoglycans was an important endpoint in all studies. This endpoint has been used as a surrogate biomarker for the effectiveness of ERT. However, it is still not clear if the urinary GAG content reflects GAG deposition in the entire body or only the plasma. Therefore, it remains unclear whether the reduction of the GAG content of the urine can be considered a reliable endpoint for measuring the effect of ERT.

Since cardiac disease is a life threatening feature of MPS VI, the results on cardiac response are at first sight promising since there was a significant decrease of the IVSd and the LVIDd observed in 52 patients after 96 weeks of treatment [12]. However, both studies also report that the heart valves are refractory to ERT. This might be due to the fact that the heart walls are very well vascularized to constantly supply the cardiomyocytes with nutrients, but the myofiboblasts composing the valves and the chordea tendineae are supplied with oxygen mainly by diffusion from the valve surface and therefore no easy targets for the relatively large therapeutic enzymes [18]. Valve regurgitation eventually results in dilated cardiomyopathy. Since ERT does not seem to correct valve pathology, one has to bear in mind that although ERT has significant effects on cardiac dimensions, the cardiomyopathy cannot be stopped.

In conclusion, although ERT in MPS VI patients has promising results on endpoints like pulmonary function, endurance, excretion of GAGs, growth and cardiac disease it seems that the correction of bone and cartilage disease remains a challenge. Furthermore, the identification of new biomarkers, next to endpoints on clinical symptom level, would be a major contribution to this field in order to obtain objective endpoints. With this in mind, quality of life data would be helpful, so we are able to see the clinical improvement in the light of clinical relevance and the effect on the wellbeing of the patient. Finally, international collaboration is needed to produce evidence on a larger population and provide insight in the effect of ERT over the complete clinical spectrum.

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Chapter 3

MPS and Cardiology Part 3.1

Mucopolysaccharidosis: Cardiologic features and effects of enzyme-replacement therapy in 24 children with MPS I, II and VI

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Summary

We determined the cardiologic features of children with MPS I, II and VI, and evaluated the effect of enzyme-replacement therapy (ERT) on cardiac disease.

Twenty-four children aged 1-18 years with MPS I, II or VI were prospectively evaluated with echocardiogram and electrocardiogram from the start of enzyme-replacement therapy up to 6 years of treatment. At start of therapy, 66% had abnormal cardiac geometric features. Left-ventricular mass index (LVMI) was increased in half of the patients, due mainly to concentric hypertrophy in MPS I and II and to eccentric hypertrophy in MPS VI. Regurgitation was most severe in a subgroup of young MPS VI patients (<5 years) at the mitral valve. At baseline, all patients had abnormal valves. The ECG showed no clear rhythm or conduction abnormalities; neither, in most patients, did it reflect the hypertrophy. After ERT, the LVMI Z-score normalized in 70% of the patients who had a Z-score>2. LVMI Z-scores decreased significantly in patients with MPS I and MPS II ($p=0.04$ and $p=0.032$). Despite ERT, valve regurgitation increased in 60% of the patients. We conclude that all our MPS patients have cardiac abnormalities. The most severe cardiac disease was observed in a subgroup of young MPS VI patients. While ERT had an effect on LVMI, it apparently had little or none on valve regurgitation.

Introduction

The mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders, each caused by deficiency in one or more specific lysosomal enzymes involved in degrading glycosaminoglycans [1]. The resulting intralysosomal storage of glycosaminoglycans causes progressive disease that involves multiple organs, including the heart. All MPS are rare diseases; in the Netherlands their combined birth prevalence is estimated to be 1 in 22,000 [2]. Cardiac involvement has been reported for all forms of MPS, the most documented abnormalities being regurgitation, stenosis and morphologic changes of the cardiac valves, and cardiac hypertrophy [3-6]. Recently, enzyme-replacement therapy (ERT) was introduced for MPS types I, II and VI. While this has alleviated several aspects of the disease, such as joint-mobility, endurance, and lung function [7-10], it is unclear whether it affects the cardiac abnormalities [11, 12]. In a cohort of patients with MPS I, II and VI we therefore studied these abnormalities before the start of ERT, and the effect of ERT upon them.

Materials and Methods

We prospectively studied 24 children with MPS I Hurler syndrome (OMIM 607014), MPS I Scheie syndrome (OMIM 607016), MPS II Hunter syndrome (OMIM 309900) and MPS VI Maroteaux-Lamy syndrome (OMIM 253200). The diagnosis of MPS was confirmed in all patients by mutation analysis and enzyme assay on leukocytes and fibroblasts.

Patients with MPS I received 0.58 mg/kg/week intravenous laronidase (Aldurazyme ®, Genzyme Corporation); MPS II patients received 0.5 mg/kg/week intravenous idursulfase (Elaprase ®, Shire Pharmaceutical Inc); and MPS VI patients received 1.0 mg/kg/week intravenous galsulfase (Naglazyme ®, BioMarin Pharmaceutical Inc). None of these patients had undergone hematopoietic stem cell transplantation. The institutional review board approved the study and all patients provided written informed consent before participation.

A standardized assessment program was initiated to prospectively investigate cardiovascular abnormalities and function using a 12-lead electrocardiogram (ECG) and detailed echocardiogram. Cardiologic assessments were performed before the start of ERT and yearly thereafter, or more frequently if cardiologic abnormalities were severe.

Echocardiographic studies were performed by an experienced sonographer (JP) using a Philips iE33 xMAtrix Echocardiography System, Philips Medical Systems, Andover, MA, USA. Data were digitally stored and subsequently analyzed by two researchers (MB, IF). The following parameters were measured by 2D-guided M-mode tracing: end-diastolic left-ventricular internal-cavity dimension (LVIDd); inter-ventricular septum thickness in diastole (IVSd); left-ventricular posterior wall thickness in diastole (LVPWd); and shortening fraction (SF). These values were compared with normal values according to Kampmann et

al [13]. The left-ventricular mass index (LVMI) was calculated using the Devereux formula and indexed by body surface area with normal values according to Poutanen et al [14].

Diastolic filling was established using the E/A ratio by measuring mitral-inflow as determined by pattern-peak early filling (E) and late filling (A) velocities, and systolic function using the shortening fraction [15]. An E/A ratio<1 was considered abnormal. Specific attention was paid to the morphology and thickness of the valves, and also to valve regurgitation, which was determined according to the recommendations of the American Society of Echocardiography [16]. Trivial valve regurgitation was not considered abnormal.

The change over time in the thickness of the aortic and mitral valve was established during ERT, the first and last echocardiogram of every patient being studied by three experienced cardiologists and echocardiographists (IF, MvO, JP). The tricuspid and pulmononary valve could not be evaluated for changes in thickness over time since the imaging quality of these valves during follow-up was inconsistent. To evaluate the valves during ERT, one additional patient was excluded from the analysis, since his obesity impaired the echo window.

Due to the inability to lie quietly at time of the echocardiogram, there were no complete baseline echocardiograms of three patients; for each, we therefore used an echocardiogram made before enzyme therapy as baseline value. The effects of enzyme therapy could be reliably evaluated in 20 patients, with a median follow-up of 104 weeks (range of treatment 48-312 weeks of therapy). Four patients were excluded from the analysis of the effect of ERT: two had received ERT for less than one year; one had no reliable follow-up echocardiograms due to behavioural problems; and one had received a mitral graft which was considered to be a co-intervention bias.

Twelve-lead standard ECG's were recorded using a Mortara ELI 350, Mortara instrument Inc, Milwaukee, USA. ECGs were analyzed by hand by a single observer from a cardiologic core laboratory (Cardialysis BV). Pediatric reference values were obtained from Park et al. and corrected for age and heart rate. QT duration was corrected for heart rate using Bazett's formula: $QTc = QT / \sqrt{(RR\text{-interval})}$ [17].

Every echocardiographic value obtained was transformed into a Z-score-calculated as the difference between the observed value for the patient and the mean reference value divided by the standard deviation from the reference value. Z-scores >2 were considered abnormal. For every individual and at group level, change over time was analyzed using multiple linear regression with weeks of treatment as the independent variable. A p-value<0.05 was considered significant. All statistical analyses were conducted using SPSS version 15.0.

Results

Twenty-four patients were included in the study: 8 MPS I patients (5 Hurler and 3 Scheie), 6 MPS II patients, and 10 MPS VI patients. Fourteen patients (56%) were Caucasian, 4 Arab (16%), 4 Turkish (16%), 1 Asian and 1 African. Most patients in the MPS VI group (70%) were non-Caucasian. Table 1 shows the first and latest main clinical and cardiologic features of the echocardiograms per patient. All abnormal values (Z >2) are printed in bold. The median age at diagnosis was 2.5 years (range 0-16 years); at start of therapy it was 5.3 years (range 1-18 years). In 3 patients, all of whom had MPS VI, the cardiac signs led to the diagnosis; these were also the only patients who were using cardiac medication (diuretics and ACE-inhibitors) at baseline. Due to respiratory failure after a respiratory infection, one MPS I Hurler patient treated with ERT died during follow-up at the age of 11 years.

At baseline, 16 of these 24 patients (66%) had an abnormal Z-score for one of the geometric features (LVMI, LVPWd, IVSd, LVIDd), on echocardiogram. In MPS I and II, concentric hypertrophy was noted with an increase in IVSd and to a lesser extent an increase in LVPWd. In MPS VI, eccentric hypertrophy was observed in three of ten patients with related increased intraventricular dimensions. In one of these patients the shortening fraction was decreased. LVIDd was increased only in MPS VI patients.

The LVMI ranged from 57 – 268 grams/m2. Twelve of the 24 patients showed a LVMI Z score >2. One MPS I Scheie patient had an increase in LVMI without any other increased parameters. Figures 1A and 1B shows an overview of the Z-scores of the LVMI and the IVSd of all patients by type of MPS.

At start of therapy, all patients had abnormal valves. An echocardiogram had been performed in one patient shortly after birth (at the age of 3 months) and 42 weeks before the start of enzyme-replacement therapy. In retrospect, it is now clear that slight echodense abnormalities were already visible in the mitral valve.

Fifteen patients (63%) had a mild to severe regurgitation at one or more cardiac valves. The median age of this group was 5.3 years, whereas the median age of the nine patients without regurgitation was 2.4 years. Most regurgitation occurred at the mitral and aortic valve. There was regurgitation at the mitral valve in 7 patients (3 MPS I and 4 MPS VI), at the aortic valve in 7 patients (2 MPS I, 3 MPS II and 2 MPS VI), and at the tricuspid valve and pulmonary valve in 2 patients (both MPS VI patients). The most severe regurgitation was in MPS VI patients. All four MPS VI patients with mitral valve regurgitation had been diagnosed at a young age (median 2.8 years); in two of them, MPS VI had been diagnosed after presentation with severe valve regurgitation. In one of these patient severe mitral regurgitation resulted in severe congestive heart failure. At 8 years old (one year after the start of enzyme therapy), she underwent a successful implantation of an artificial valve in mitral position and a valvuloplasty of the tricuspid valve. Five patients had a combination of

Table 1: Baseline clinical and cardiologic features of 24 mucopolysaccharidosis patients.

Pt	Age at baseline (yrs)	Sex	MPS type	Age at diagnosis (yrs)	IVSd start ERT	IVSd after ERT*	LVIDd start ERT	LVIDd after ERT	LVPWd start ERT	LVPWd after ERT
1	5	F	IH	2	**5.20**	0.38	1.93	2.97	-0.13	-0.19
2	1.3	F	IH	0	1.37	-0.13	-0.04	-0.40	0.93	-0.27
3	4.4*	M	IH	1	**8.00**	***	-3.05	***	**2.24**	***
4	4.8	M	IH	1	**3.47**	1.56	0.93	-0.69	**2.00**	0.91
5	9.3†	M	IH	1	**4.00**	**6.67**	0.60	-0.58	**4.27**	4.82
6	4.9	M	IS	5	**3.30**	**-0.13**	-0.07	0.95	**2.13**	-0.59*
7	2.4	F	IS	1	1.60	0.67	0.37	0.95	1.60	0.12
8	8.4	M	IS	5	**2.56**	**1.55**	1.03	1.13	1.55	-0.79
9	10.8	M	II	3	0.11	0.74	-0.41	-0.12	0.00	0.25
10	8.3	M	II	2	0.33	**1.11**	-0.03	-0.69	-0.27	0.09
11	5.3	M	II	3	**3.56**	**2.22**	-0.36	0.10	1.55	0.23
12	1.0	M	II	p.n	**2.88**	-0.56*	-0.36	0.30	-0.93	1.00
13	2.3	M	II	2	1.87	***	0.50	***	1.33	***
14	9.5	M	II	6	**4.0**	0.78	0.75	0.57	**2.00**	-0.23*
15	8	F	VI	8	**2.00**	1.56	0.71	1.06	0.29	0
16	2.8*	M	VI	2	1.60	-0.40	1.85	**5.50^**	**2.40**	-0.80
17	7.5	M	VI	7	1.89	0.42	0.44	-1.57	1.90	1.67
18	18.3	M	VI	16	0.56	1.36	-2.85	-1.85	0.07	0.80
19	7.6	F	VI	7	0.75	-0.33	0.06	1.28	-1.14	0.27
20	5.9	M	VI	5	0.38	1.00	1.68	1.68	-0.19	0.29
21	1.9	F	VI	1	1.12	-0.67	**5.00**	**3.00**	0.80	-0.67
22	5.3*	M	VI	5	**2.40**	0,67	**2.18**	1.61	0.47	0.82
23	2.3	M	VI	2	1.47	***	1.85	***	0.80	***
24	6.6*	F	VI	3	**4.00**	***	**6.93**	***	6.93	***

M=Male F=Female; IVSd=interventricular septum dimension at diastole; LVIDd=left-ventricular internal dimension at diastole; LVPWd=left-ventricular posterior wall diameter at diastole; LVMI=left-ventricular mass index; SF=shortening fraction; p.n.=prenatal diagnostic procedure. 0=absent. Abnormal values are printed in bold.

* first echocardiogram of these patients was inoperable due to uncooperative behaviour. The echocardiogram of patient number 3 was made at 26 weeks of ERT; that of patient number 16 was made at 12 weeks of ERT; that of patient number 24 was made 20 weeks before ERT; and that of patient number 22 at 27 weeks before ERT.

** the follow-up time was identical to the period that the patients were treated with ERT.

*** No follow-up echocardiogram available due to short follow-up time, behavioural problems or co-intervention bias

LVMI start ERT	LVMI after ERT	Hypertrophy start ERT	E/A ratio start ERT	SF start ERT	Follow-up (weeks)**
6.18	2.50*	Concentric	1.30	26	312
-0.28	-1.54	0	1.10	36	311
1.87	***	0	0.74	43	104
4.28	-0.13*	Concentric	1.10	42	302
6.68	**6.94**	Concentric	0.80	52	81
2.89	-0.90	0	1.30	41	93
2.38	1.37	0	1.30	34	87
1.62	1.71	0	1.70	32	259
-0.62	-0.10	0	0.97	40	149
-0.32	-0.61	0	1.20	36	104
3.01	0.97	Concentric	1.20	36	116
0.10	-0.03	0	1.40	35	159
1.62	***	0	1.40	45	0
3.92	-0.23	Concentric	1.20	33	115
0.75	1.20	0	1.20	45	79
5.04	3.90	Eccentric	1.30	23	92
1.50	-0.47	0	1.70	46	104
-1.59	-0.52	0	1.30	34	92
-0.81	0.36	0	1.50	35	53
1.23	1.52	0	1.90	36	52
7.32	1.24*	Eccentric	2.00	30	48
4.28	1.91*	0	1.80	32	154
3.01	***	0	0.81	34	0
26.4	***	Eccentric	1.70	36	144

aortic and mitral valve regurgitation: 1 MPS I Scheie patient, 3 MPS II patients, and 1 MPS VI patient.

Systolic function was impaired in one Hurler patient (FS 26%) and two Maroteaux-Lamy patients (FS 30% and FS 23%). At baseline, the MPS VI patient who required mitral graft surgery had had a normal shortening fraction of 36%. E/A ratio as a measure of diastolic function was abnormal in four patients.

Table 2 shows the baseline characteristics of the ECGs, 50% of which showed one or more abnormality. ECG abnormalities were generally of minor clinical importance.

The effects of enzyme therapy could be reliably evaluated in 20 patients (see Methods). Follow-up time was the longest in the patients with MPS I Hurler (81-312 weeks). At start of

Table 2: Baseline characteristics on electrocardiography in 24 MPS patients.

	Baseline overall	MPS I (H/S)	MPS II	MPS VI
ECG Data				
Rhythm				
Sinus	24 (100%)	8 (100%)	6 (100%)	10 (100%)
Sinus tachycardia	1 (4%)	1 (13%)	0 (0%)	0 (0%)
Heart rate (bpm)	107 [78-155]	120 [100-155]	118 [80-150]	93 [78-125]
PR interval (ms)	140 [110,180]	130 [120,150]	145 [110,180]	140 [120,180]
Prolonged PR	3 (13%)	0 (0%)	2 (33%)	1 (10%)
QRS duration (ms)	80 [70,100]	80 [70,80]	80 [80-00]	80 [70,100]
Prolonged QRS	3 (13%)	0 (0%)	2 (%)	1 (10%)
QTc interval (ms)	415 [378,453]	402 [390, 417]	440 [412,453]	422 [378, 441]
Prolonged QTc	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Incomplete bundle branch block	2 (8%)	0 (0%)	1 (17 %)	1 (10%)
Left fascicular block	2	1 (13%)	0 (0%)	1 (10%)
Left atrial enlargement	2 (8%)	0 (0%)	1 (17 %)	1 (10%)
Right-ventricular hypertrophy	1 (4%)	1 (13 %)	0 (0%)	0 (0%)
Left-ventricular hypertrophy	1 (4%)	0 (0%)	0 (0%)	1 (10%)
Repolarisation disorder	2 (8%)	0 (0%)	1 (17%)	1 (10%)

MPS I (H/S) = MPS I Hurler and MPS I Scheie patients. Bpm=beats per minute. ms=milliseconds

therapy, 10 patients had been younger than 5 years: 3 Hurler patients, 2 Scheie patient, 2 Hunter patients, and 3 Maroteaux-Lamy patients. Table 1 shows the first and latest results of the echocardiograms per patient.

A normal value of IVSd was achieved in 8 of the 10 patients with an IVSd Z-score above 2. At group level, the decrease in IVSd was significant for the group of MPS II patients (-0.36 Z-score/year) (p=0.05). In one MPS I patient the IVSd Z-score increased over 81 weeks of therapy. This patient died after 81 weeks of enzyme therapy.

The LVIDd decreased in one patient (MPS VI) with a Z-score for LVIDd above 2, but deteriorated in two patients who had a normal diameter at baseline; in whom an increase in mitral regurgitation was likely to contribute to the increase in LVIDd.

LVPWd decreased in 4 of 5 patients with a Z-score above 2. The patient who did not respond on IVSd did not respond on LVPWd either.

Over time, Z-scores could be established reliably in 10 of the 12 patients who had had an LVMI Z-score above 2 at baseline. Z-scores normalized in 7 of these 10 patients. The decrease in Z-scores was statistically significant in the MPS I group (0.31 Z-score/year, p=0.04) and the MPS II group (0.26 Z-score/year, p=0.032). In 4 individual MPS I and VI patients the decrease in Z-score was significant with values ranging from 0.002 to 0.043. Figure 2 shows their regression lines.

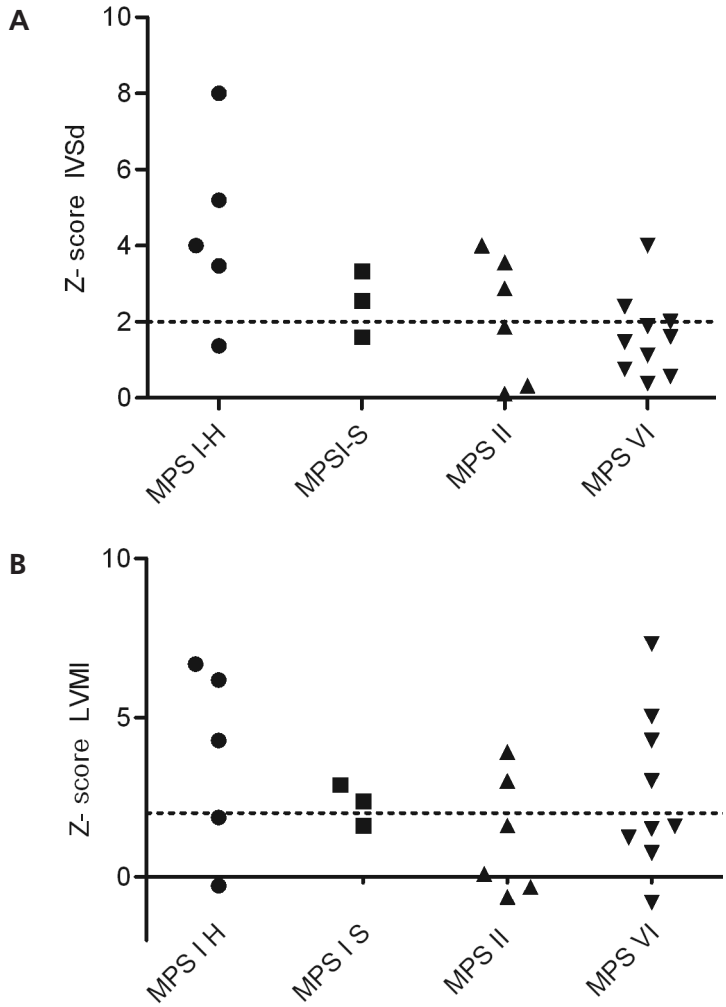

Fig 1A and 1B: Z-score for interventricular septum diameter in diastole (IVSd) and left-ventricular mass index (LVMI) in the different types of MPS. The dotted line represents the upper limit of the normal range (=2). One patient (number 24) fell outside the axis range because she had an LVMI of 26.4 grams/m^2.

Regurgitation increased after start of ERT in 60% of the patients and was mostly, in 40% of the cases, seen at the mitral valve. In most cases regurgitation increased from none to mild. In one MPS VI patient mitral valve regurgitation increased from moderate to severe; aortic regurgitation increased from mild to moderate in two patients, one MPS VI and one MPS I patient. In 15% of the patients an improvement in regurgitation was observed. Patients with moderate to severe regurgitation did not ameliorate.

Thickness of the mitral valve decreased in six or twenty patients (1 MPS IH, 1 MPS IS, 2 MPS II and 3 MPS VI), who had been treated with enzyme therapy for a median of 86 weeks (range of 52-159 weeks). Eight or twenty patients had an increase in mitral valve

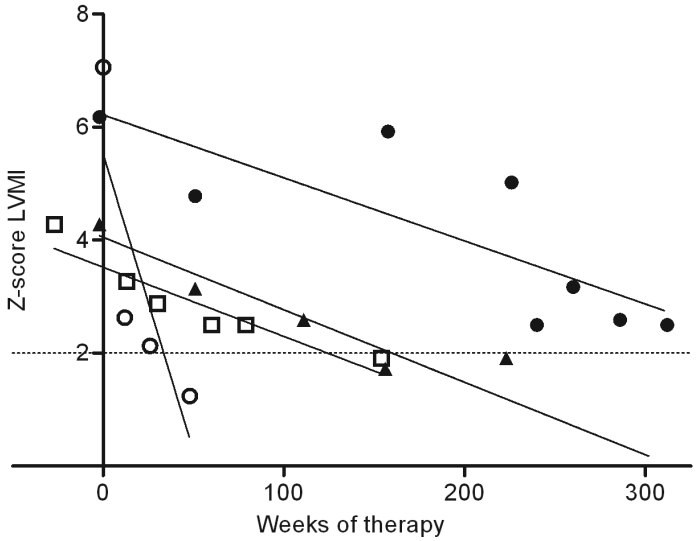

Fig 2: Regression lines of patients with significant improvement in left-ventricular mass index (LVMI). Z-score LVMI= Z-score of the left-ventricular mass index.
●=patient number one (p=0.015); ▲=patient number four (p=0.002); ○=patient number 21 (p=0.009); □=patient number 22 (p=0.043).

thickness (3 MPS I-H, 1 MPS I-S, 2 MPS II and 2 MPS VI); three of these patients also had an increase in aortic valve thickness, and had been treated for a median of 110 weeks (range of 48-312 weeks).

The thickness of the aortic valve decreased in 5 patients (2 Hunter and 3 Maroteaux-Lamy patients, range of therapy 52-159 weeks) and increased in three. There was no clear relationship between the increase in thickness of the valves and the development of regurgitation during ERT.

Left ventricular shortening fraction normalized in two of three patients (FS 35% and 39% at last assessment in patients 16 and 21), but remained abnormal in the third (patient 1). The same patients with an impaired diastolic function at start of therapy continued to have an E/A ratio <1 at follow-up, except for one patient.

No clinically important changes were found at the follow-up ECGs. We report only the changes found at follow-up. One patient had atrial beats on the follow-up electrocardiograms. The increased PR-interval present at baseline in 2 MPS II patients was absent later. The QRS-complex increased in duration in the patient who already had a prolonged QRS at baseline. This was probably due to the left-ventricular hypertrophy, which was also seen on the follow-up echocardiogram. Hypertrophy disappeared on ECG in the only patient who had shown signs of hypertrophy on ECG at baseline; this was consistent with the findings on echocardiogram.

Discussion

We studied cardiac abnormalities and the effects of enzyme therapy in children with MPS I, II and VI. As the current literature points out, cardiologic abnormalities are a common finding in patients with MPS [3-6, 12, 18-20]. At start of therapy, all our patients had cardiologic abnormalities, which is remarkable, as they were all relatively young.

At start of therapy, 66% had abnormal cardiac geometric features caused either by increased cardiac-wall diameter (IVSd) or by cardiac dilatation. Interestingly, while most MPS I and II patients had hypertrophic cardiomyopathy – findings that are consistent with studies reporting the occurrence of increased IVSd and left-ventricular hypertrophy in MPS I and II patients [3, 19] - eccentric hypertrophy was seen solely in the patients with MPS VI. Reports of ventricular dilatation in MPS VI are still limited [18, 21, 22]. Dilatation in our MPS VI patient group was explained mainly by mitral-valve regurgitation. Primary regurgitation causes the left-ventricular remodeling and dilatation. Generally in MPS, we hypothesize that the increase in geometric features is partly due to the compensatory mechanism caused by the mitral regurgitation and partly because of the accumulation of storage material. The asymmetric hypertrophy in the wall diameters with an increased IVSd in MPS I and MPS II and an increased LVIDd in MPS VI indicates this.

While heparan sulphate and dermatan sulphate accumulate in MPS I and II, storage in MPS VI is confined to dermatan sulphate. Leal et al. hypothesized that storage of dermatan sulphate is the main cause of valve deformation and dysfunction in MPS [18]. We in turn hypothesize that storage of heparan sulphate is an additional cause of increased thickness of the walls, particularly in the septum, while the storage of dermatan sulphate leads to valve impairment and consequent eccentric hypertrophy.

The presence of affected valves in all our patients from the age of 3 months shows that the deposition of glycosaminoglycans in the heart can occur at a very early age, and may already start in utero; a similar conclusion was drawn from studies in MPS VII mice [23]. The mitral and aortic valves were the most affected, which is consistent with earlier reports [5, 6]. There was no clear relationship between thickened valves and regurgitation.

Although the most severe valve regurgitation and cardiac disease occurred in the group of MPS VI patients, this group also had the least cardiac involvement. Apparently these patients can be subdivided into two categories: one in which patients present before the age of 5 years, with cardiac problems as the main presenting feature; and another in which clinical features such as joint pains and mobility problems lead to the diagnosis later in life. While the literature has concluded that the severity of cardiologic abnormalities in MPS VI is either minor [12, 19] or severe [3, 6], our results support both conclusions. These differences may be explained by further genotype-phenotype studies [24].

Although 50% of ECGs showed abnormalities, none showed clinically important rhythm or conduction abnormalities. Generally, ECGs did not reflect the hypertrophy found in patients, possibly because glycosaminoglycans have a low electric conductance [5, 25]. We

therefore conclude that ECG is not a reliable tool for identifying cardiologic abnormalities in MPS, and that echocardiography should be mandatory in all patients. Although conduction abnormalities were not found in our group of children with MPS, they may occur at later age [26]. Therefore ECG should remain part of the follow-up program of MPS patients.

There have been few reports on the effects of enzyme-replacement therapy on cardiologic abnormalities in MPS [11, 12, 27, 28]. After ERT, the Z-score normalized in 70% of our patients who had had a Z-score >2 for LVMI; in four patients this was significant. This suggests that ERT has some effect on GAG accumulation in cardiac tissue. Earlier Wraith et al, Braunlin et al. and Okuyama et al. reported positive or mildly positive effects on the cardiac diameters in MPS I and II patients [9, 11, 29].

Effects on cardiac valve regurgitation seem to be limited. In 60% of patients, valve regurgitation increased, and mitral valve regurgitation increased in 40%. On the other hand, 15% of patients showed minor improvements in mitral valve regurgitation. There was no clear relationship between the increase in thickness of the valves and the development of regurgitation during ERT. In some patients, the thickness of the valves decreased, while in others it increased or stabilized. We should note, however, that 2D imaging is not the best way of assessing morphologic change. For assessment of valve morphology, 3D echo cardiography currently is the technique of choice.

Although it is unknown whether the percentage of patients with dysmorphology and deterioration of valve regurgitation is similar to that of patients not receiving ERT [12, 19], our findings suggest overall that the cardiac valves are only slightly accessible to ERT. This might be explained by the ultra structure of the cardiac valves. While microvasculature can be present in the heart valves, the myofiboblasts composing the valves are supplied with oxygen mainly by diffusion from the valve surface [30] and therefore no easy targets for the relatively large therapeutic enzymes that need to enter the cells via receptor mediated endocytosis. As a consequence, the correction of MPS storage in the heart valves remains challenging. While other studies agree that ERT has a limited effect on the cardiac valves in MPS I and MPS II [12], Scarpa et al. described an improvement in valve disease in one MPS VI patient in an Italian cohort [27].

In our group, 1 MPS I Hurler patient died after a respiratory infection during follow-up. This, notably, was one of the few patients in whom ERT had no effect on any of the cardiologic parameters. Further research is required to investigate the role of prognostic factors.

In conclusion, all MPS I, II and VI patients included in our study had abnormalities of the valves, 63% had valve regurgitation, and 66% had one or more abnormal left ventricular geometric features at an early age. While ERT seemed to have little or no effect on valve regurgitation, it had some effect on cardiac dimensions. Cardiac disease was most severe in a subset of young MPS VI patients.

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Chapter 3

MPS and Cardiology Part 3.2

Macrophage involvement in mitral valve pathology in mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome)

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Summary

Maroteaux-Lamy syndrome (mucopolysaccharidosis type VI) is a rare lysosomal storage disorder in which the pathologic storage of glycosaminoglycans in various tissues can lead to severe symptoms, including cardiomyopathy. We report a child with Maroteaux-Lamy syndrome whose cardiac condition deteriorated and eventually led to cardiac failure at the age of 7 years due to severe mitral regurgitation. She received a mitral valve replacement and tricuspid repair with successful outcome. Histologic examination of the mitral valve showed abundant "clear" cells in both the leaflets and chordae tendineae. In Hurler disease (MPS I), similar cells have been identified as activated valvular interstitial cells (VICs, a myofibroblast like cell type). Here we report that the "clear"cells are CD68 positive, a frequently used marker of macrophage lineage. The "clear"cells remained unstained with the more specific macrophage marker CD14 while persistent staining of other cells demonstrated macrophage infiltration. From these observations, we infer that macrophages are involved in mitral valve pathology in MPS VI.

Introduction

The mucopolysaccharidoses (MPSs) are a group of lysosomal storage disorders caused by the deficiency of a specific enzyme required for the stepwise degradation of glycosaminoglycans (GAGs), also known as mucopolysaccharides [1]. In mucopolysaccharidosis type VI (MPS VI), Maroteaux-Lamy syndrome (OMIM 253200), the lysosomal enzyme N-acetylgalactosamine 4-sulfatase (arylsulfatase B) is deficient and leads to accumulation of dermatan sulfate. The clinical hallmarks of MPS VI include profound skeletal deformities, short stature, hearing loss, corneal clouding, restrictive pulmonary function and cardiac anomalies. The severity of the disorder varies considerably between patients [2]. Enzyme-replacement therapy is available for MPS VI with positive results on pulmonary function, endurance, urinary GAG excretion, and cardiac dimensions [3,4].

We recently showed that severe cardiac features such as mitral valve regurgitation and dilated cardiomyopathy can be the key presenting symptom in children under 5 years old [5]. We report a severely affected MPS VI child who received a mitral valve replacement. The tissue specimens that were collected during the procedure enabled us to further elaborate on the pathophysiology of valve dysfunction in MPS VI.

Clinical report

A 3-year-old girl was referred to our hospital with severe mitral regurgitation, a dysmorphic mitral valve, moderate tricuspid regurgitation, and mild aortic regurgitation. Besides her cardiac problems, she had profound skeletal deformities with small stature, corneal clouding and hepatosplenomegaly. On the basis of the cardiologic abnormalities and her coarse facial features, she was suspected to have Maroteaux-Lamy syndrome. She was born to consanguineous Pakistani parents. Both of the parents were heterozygous for a novel pathogenic splice site mutation c.1142+2T>C (exon 5) in the *ARSB* gene. The patient was found to be homozygous for this mutation concordant with a deficiency of arylsulfate B activity in cultured skin fibroblasts (84.8 nmol/h.mg in the patient versus 379-980 nmol/h.mg in healthy controls).

Over the next 3 years, the patient's mitral regurgitation and congestive heart disease worsened. Furosemide, spironolactone, captopril and carvedilol were used as heart-failure medications. At the age of 6 years she became the first patient in the Netherlands to receive weekly enzyme replacement therapy (ERT) for MPS VI. After a few months her fatigue improved and she was more flexible in her joints. Even though she missed only two infusions in this period, her cardiac parameters did not improve: the dilatation of the left side of the heart and the valvular regurgitation progressed. The mitral valve leaflets showed no co-optation and had thickened further. The anterior mitral valve leaflet showed

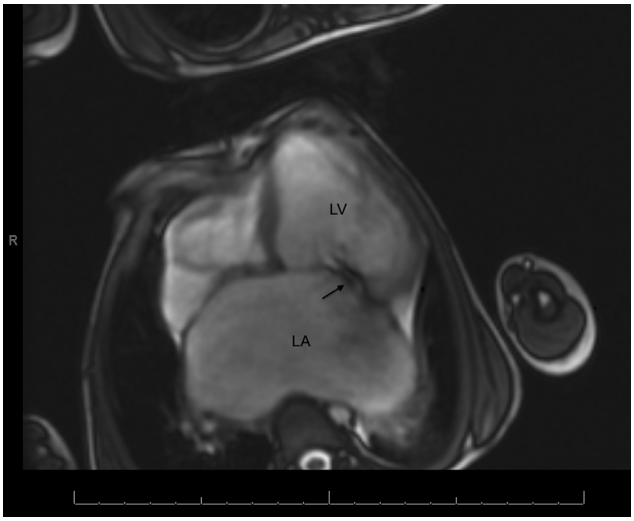

Fig 1: Magnetic resonance image (4-chamber view; obtained with steady-state free-precession MR sequence). Note thickened mitral valve leaflets (arrow), massively enlarged left atrium (LA) and enlarged left ventricle (LV) resulting from mitral regurgitation.

moderate prolapse. Figure 1 shows the severely enlarged left atrium and left ventricle, and severe mitral regurgitation on cardiac MRI.

At the age of 7 years she presented with life-threatening progressive heart failure, probably triggered by an upper airway infection. She became oxygen dependent and could not be stabilized with additional cardiac medication. As there were no alternatives, it was decided to intervene surgically. The mitral valve was replaced by a 25mm St. Jude mechanical prosthesis, and tricuspid valve repair was performed. After surgery, she recovered well: the left ventricular end-diastolic diameter (LVEDD) decreased from 11.2 to 3.4 (Z-score), the left ventricular end-systolic diameter (LVESD) decreased from 12 to 3.7 (Z-score, measured by M-mode echocardiography), and the right ventricular pressure normalized.

The surgically removed valve displayed prominent abnormalities. Macroscopically, the leaflets were thickened and the architecture distorted. Microscopically, there were numerous vacuolated fibroblasts, also called “clear” cells or “Hurler” cells. They were mainly present in the rather loose connective tissue of the leaflets and in the dense connective tissue of the chordae tendineae, and less so in the adjacent cardiac muscle tissue of the chordae insertion sites (Fig 2). The CD68 marker of macrophage lineage showed strong staining of macrophages that were spread throughout the tissue as well as diffuse staining of the “clear” cells (Fig 2, panel E). CD68 is a member of the lysosomal-associated membrane glycoprotein family (LAMP), thus stains the GAG-loaded lysosomes of the “clear” cells. To distinguish the macrophages from the “clear” cells we subsequently applied the more specific macrophage marker CD14, whereby the “clear” cells were negative while the macrophages were positive (Fig. 2, panel F).

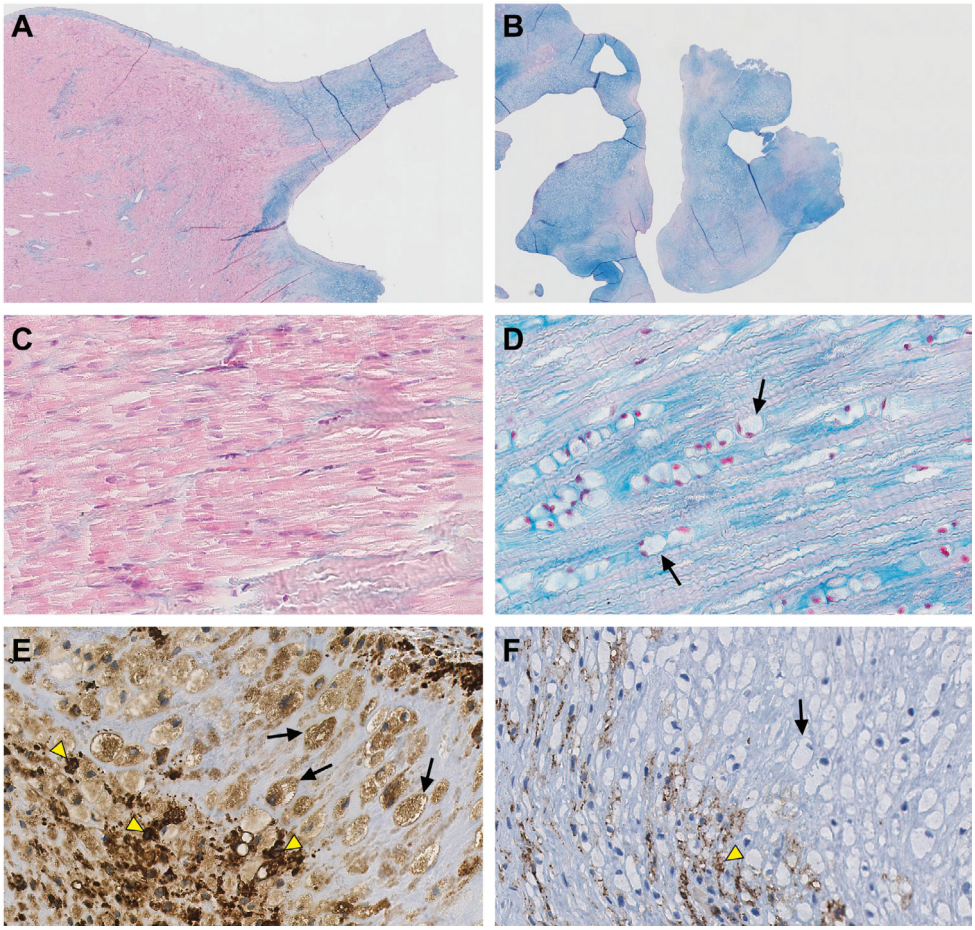

Fig 2: Microscopic images of the surgically resected mitral valve Panels A and B (overview: alcian blue staining, original magnification 12.5x) show the junction of the chordea tendineae and the contractile cardiac tissue (panel A), and pieces of the valve leaflets (panel B). Panels C and D (alcian blue staining, original magnification 200x) show an area with cardiomyocytes in panel C and the presence of "clear" cells in the chordea tendineae in panel D. Panel E (200x magnification) shows staining of both the "clear" cells (arrows) as well the macrophages (arrow heads) in the valve leaflet with antibody marker CD68, while panel F (200x magnification) shows staining of only the macrophages (arrow heads), but not the fibroblasts (arrows) in the same area with antibody marker CD14.

Discussion

Heart valve regurgitation is common in MPS VI, and the mitral valve seems to be most susceptible [6, 7]. In healthy subjects, dermatan sulfate is the dominant GAG in heart valve connective tissue matrix [8]. It has therefore been speculated that in MPS types wherein dermatan sulfate accumulates (MPS I, II, VI and VII), the cardiac abnormalities are more severe than in other types of MPS [9, 10]. The cardiac problems of this reported

patient manifested at a very young age and became life-threatening. The early onset and severity of symptoms are likely explained by the severity of the novel splice site mutation c.1142+2T>C [11, 12].

Histologic examination of the heart valve provided insight in the pathophysiology of MPS VI cardiac valvular disease. The "clear" cells that were abundantly present in the leaflets and the chordae tendineae were first described in 1976 by Renteria and colleagues in a case of Hurler syndrome [13]. Recently, they were demonstrated to be activated VICs (myofibroblasts) expressing vimentin and smooth muscle actin [14]. Using the macrophage lineage marker CD68 to visualize infiltration of pathologic tissue specimens, we unexpectedly observed staining of almost all cells in the valve leaflet (Fig 2 E). This can be explained by the CD68 protein being a member of the lysosomal-associated membrane protein family (LAMP) that would be expected to be abundant in "clear" cells that are packed with GAG-loaded lysosomes [15]. Using CD14 (directed against a cell surface protein of macrophage lineage) as alternative and more specific marker of macrophage lineage we were able to distinguish macrophage infiltration from GAG storage in "clear" cells (activated VICs) [16]. Our results are at variance with those of Braunlin et al, 2011 who did not obtain CD68 staining in atrioventricular valves of infants with MPS I despite very similar valve pathology characterized by abundant "clear cells" that they characterized as activated VICs.

Based on the combination of findings, it is plausible that glycosaminoglycans accumulate in the lysosomes of VICs that synthesize and recycle GAGs, eventually depriving these cells of the capacity to maintain the valvular matrix. The ensuing tissue damage attracts macrophages that cause even more damage by lack of repair processes due to dysfunctional VICs. The presence of macrophages supports the theory that GAG storage induces inflammation through activation of the Toll-like receptor 4 signaling pathway [17, 18]. Over time, the normal configuration of the heart valves is lost and valve regurgitation occurs.

In the case of this patient, treatment with ERT (recombinant human N-acetylgalactosamine-4-sulfatase, galsulfase) led to an improvement of her clinical condition, which favored surgical treatment of the heart failure. Several studies have shown that ERT in MPS VI positively affects endurance, joint range of motion, and pulmonary function [19]. Though the disease process seems to halt, the cardiac valves are rather refractory to ERT even though the structures are surrounded by blood and freely exposed to galsulfase upon intravenous infusion [3], thus diffusion of galsulfase through the valvular tissue must be limited [20]. The microscopic sections from this patient's mitral valve confirm that significant amounts of GAGs were still present after 12 months of ERT using recommended dosing schedules per protocol.

Usually, cardiac surgeons refrain from valve replacement in severely affected children with MPS VI because of the poor prognosis. The successful outcome of mitral valve replacement and tricuspid repair in this case is an important experience that may guide others in the treatment of MPS VI and related MPS disorders.

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Chapter 4

Up to five years experience with 11 mucopolysaccharidosis type VI patients

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Mol Genet Metab. 2013 May;109(1):70-6.

Summary

Maroteaux-Lamy disease (mucopolysaccharidosis type VI, MPS VI) is a rare progressive metabolic disorder characterized by coarse facial features, hepatosplenomegaly, restrictive pulmonary function, cardiac abnormalities and stiff joints. The disease is caused by a deficiency of the lysosomal enzyme N-acetylgalactosamine 4-sulfatase which leads to glycosaminoglycan (GAG) storage in various tissues. It presents as a clinical spectrum with varying disease progressions and severity. While phase I/II/III studies proved the effectiveness of enzyme-replacement therapy (ERT) with recombinant human arylsulfatase B, long-term data are still scarce.

Over treatment periods ranging from 1.3 to 5.4 years, this prospective open-label follow-up study in 11 Dutch MPS VI patients (age 2-18 years) showed that ERT had significant positive effects on cardiac-wall diameters (IVSd and LVMI), left and right shoulder flexion ($p<0.001$), liver size and spleen size ($p<0.001$), urinary GAG excretion ($p<0.001$), and scales of quality of life (motor functioning and body functioning). ERT did not affect cardiac valve regurgitation or hearing function; HRQoL decreased slightly in two domains ('anxiety' and 'negative emotions'), and patients with the rapid and slow progressive forms of the disease differed with regard to baseline GAG excretion and GAG decrease during treatment.

In conclusion, ERT had an effect on several clinical parameters. This effect was established in an open cohort of young MPS VI patients.

Introduction

Mucopolysaccharidosis type VI (MPS VI) or Maroteaux-Lamy syndrome (OMIM 253200) is a rare lysosomal storage disorder whose reported birth prevalence worldwide ranges between 1 in 43,261 and 1 in 1,505,160; in the Netherlands it is 1 in 667,000 [1, 2]. The disease is caused by a deficiency of the lysosomal enzyme N-acetyl galactosamine 4-sulfatase (arylsulfatase B). This impairs the stepwise degradation of the glycosaminoglycan (GAG) dermatan sulphate, which accumulates in various tissues, ultimately leading to multi-organ dysfunction [3].

As the severity of organ involvement and progression of the disease varies considerably between patients [2], MPS VI is generally regarded as a clinical spectrum in which the disease progresses more rapidly in some patients than in others. Patients in whom it progresses rapidly develop profound bone abnormalities, short stature, cardiac abnormalities, hearing problems and sometimes mild cognitive impairment at a relatively young age. Although those in whom it progresses more slowly have a later onset of symptoms, they are likely to develop serious manifestations of MPS VI later in life [4]. To date, there have been no survival studies of patients with MPS VI; the oldest patient with MPS VI described so far reached the age of 56 [5].

Enzyme-replacement therapy (ERT) with galsulfase (Naglazyme®) has been reimbursed in the Netherlands since 2007. Before this treatment was registered, the only treatment option was allogeneic or haematopoietic stem-cell transplantation (HSCT); outcome varied, and mortality was high.

Although clinical trials have shown that ERT positively affects endurance, pulmonary function and urinary GAG excretion [6-8], there is still limited little information on long-term follow-up. We therefore conducted an open-label study to establish the effects of ERT on clinical parameters and health-related quality of life (HRQoL) in all Dutch MPS VI patients (n=11) who have been treated so far at the national expertise center, Erasmus MC University Medical Hospital. At the time of writing, they had been treated for periods of up to 5.4 years. Three had started therapy very early (before 5 years of age).

Materials and Methods

Setting

This study has been conducted as a single-centre, open-label study since 2007. The study protocol was approved by the Medical Ethical Committee at Erasmus MC University Medical Center. In the Netherlands, treatment and standardized clinical follow-up of MPS VI patients receiving ERT take place at a single national expertise centre (Erasmus MC University Medical Center Rotterdam).

Patients

We included all patients with a diagnosis of MPS VI confirmed by the deficiency of arylsulfatase B activity in fibroblasts and DNA analysis. Patients were treated with 1 mg/kg recombinant human arylsulfatase-B (galsulfase, Naglazyme®, Biomarin Corporation).

Assessments

Pulmonary function

Forced vital capacity

Forced vital capacity (FVC) was measured twice a year using a Lilly-type pneumograph (Viasys Healthcare, Würzburg, Germany) with the patients in an upright position. For analysis, we used the maximum value of three reproducible tests.

Polysomnography

Overnight sleep respiratory recording were captured using the Embletta Portable Diagnostic System and analysed with Somnologica for Embletta software 3.3 ENU (Medcare Flaga, Reykjavic, Iceland). Thoracic and abdominal movements were followed using circumferential impedance elastic trace belts (X act). Nasal airflow was measured with a flow transducer attached to a nasal cannula (Salter Labs, Arvin, USA). Pulse oximetry haemoglobin oxygen saturation (SpO_2) and heart rate (HR) were recorded using an infant or paediatric sensor (Nellcor, Pleasanton, USA). Obstructive apnoeas and hypopnoeas were scored according to the AASM criteria of 2007 [9]. OAHI (obstructive apnea and hypopnea index) was defined as the number of obstructive apnoeas and obstructive hypopnoeas divided by total sleep time. In case of absent nasal flow, obstructive events were considered if there was a decrease in thoracic or abdominal signal (paradoxal movement), all in combination with an oxygen desaturation. Central apnoeas were not taken into account. A haemoglobin oxygenation-desaturation index (ODI) was calculated as the number of desaturations (≥3% from baseline) per hour of sleep time. Obstructive sleep apnoea syndrome (OSAS) was present when the OAHI was greater than 1 per hour.

Cardiac evaluation

We set up a standardized assessment program to prospectively investigate cardiovascular abnormalities and function using a 12-lead electrocardiogram (ECG) and detailed echocardiogram. Cardiologic assessments were performed before the start of ERT and yearly thereafter, or more frequently if cardiologic abnormalities were characterized as severe.

Echocardiographic studies were performed by a single experienced sonographer (JP) using a Philips iE33 xMAtrix Echocardiography System, Philips Medical Systems, Andover, MA, USA. The following parameters were measured by 2D-guided M-mode tracing:

end-diastolic left-ventricular internal-cavity dimension in diastole (LVIDd); inter-ventricular septum thickness in diastole (IVSd); left-ventricular posterior wall thickness in diastole (LVPWd); and shortening fraction (SF). These values were compared with normal values according to Kampmann et al [10]. Left-ventricular mass index (LVMI) was calculated using the Devereux formula and indexed by body-surface area with normal values according to Poutanen et al [11].

Size of liver and spleen

Every three months, the size of the liver and spleen was assessed manually by two physicians. Liver size was expressed as the number of centimetres the liver edge was felt beneath the costal margin in the right midclavicular line. Spleen enlargement was established on the basis of Castell's sign and palpation of the left costal margin.

Joint mobility

At start of therapy and every 3-6 months thereafter, two researchers used a goniometer to assess range of motion of the shoulder (flexion), elbow (extension), hip (flexion) in standing position and knee (extension). To measure active deflection of the joints, patients were asked to actively raise their shoulder or extend their knees. A measurement of 175-180 degrees was considered as normal. Joint mobility was measured for the left and right sides of each joint and a combined score was calculated on the basis of the mean of the left and right joint mobilities.

Hearing function

Pure-tone audiograms were made twice a year with a Madsen OB 822 audiometer (Copenhagen, Denmark) in a sound-proof room. Pure-tone threshold testing was performed for both air conduction and bone conduction. The average hearing threshold at 1,000, 2,000, and 4,000 Hz frequencies (high Fletcher index) was used to classify the type of hearing loss (conductive component in case of an air-bone gap >15 dB). Hearing loss was classified according to the Goodman classification, 25-40 dB being considered as mild, 40-55 dB as moderate, and 55-90+ dB as severe to profound [12].

Urinary glycosaminoglycan (GAG) level

Total urinary GAGs were quantitatively assessed every three months using the dimethylmethylene blue (DMB) method [13]. GAG concentrations were normalized to urinary creatinine concentrations. GAG concentrations (mg/L) were routinely expressed in mg GAG/mmol creatinine, but converted to microgram GAG/mg creatinine by multiplying the value by 8.8 (molecular weight creatinine =113.1 g/mol) in order to facilitate comparison with other data reported in the literature. GAG urinary samples were taken, prior to start of infusion of ERT.

Health Related Quality of Life (HRQoL)

TAPQOL/TACQOL

The child's and/or parent's perception of a child's health-related quality of life were measured using the Dutch version of the TNO-AZL Child Quality of Life Questionnaire Child Form for children (TACQOL) and the TNO-AZL Questionnaire for Preschool Children's Health-Related Quality of Life (TAPQOL) Questionnaire [14, 15]. The TACQOL questionnaire can be used for parents of patients from 6-15 years old, and consists of 7 domains, such as 'body', which measures problems/limitations concerning general physical functioning; and 'peers', which measures problems/limitations in social contacts with parents and peers. The TAPQOL questionnaire is suitable for parents of patients from the ages of 6 months to 6 years, and consists of 12 domains such as 'positive mood' and 'motor functioning'. All parents were asked to complete the parent form. As HRQoL is defined as a multidimensional construct, no total score is calculated. The values of the TACQOL were transposed to a 0-100 score; a higher score indicates a better HRQoL. The values were compared with those of a reference group, i.e. a sample of healthy Dutch peers of the same age [14, 15]. Parents were asked to fill in the questionnaire twice a year.

Antibodies

Serum samples were taken every 3 months prior to each infusion. The presence of IgG antibodies to galsulfase was assessed using enzyme-linked immunosorbent assays (ELISA) with serum dilutions from from 50 to 3,906,250 fold in dilution buffer.

Statistical analysis

All values obtained with echocardiogram of the heart and forced vital capacity (FVC) were transformed into a Z-score calculated as the difference between the value estimated for the patient and the mean reference value divided by the standard deviation from the reference value. Z-scores > 2 were considered abnormal. In the other outcome measures (e.g. size of liver and spleen and GAGs values), the absolute number was used. The progression over time for GAG values, the size of liver and spleen, echocardiographic outcome and goniometric outcome was calculated with a univariate linear regression model in which change over time was assessed using weeks of treatment as the independent variable. A p-value<0.05 was considered significant. All statistical analyses were conducted using SPSS 15.0 and SAS 9.2.

Results

Patient characteristics at start of therapy

Eleven patients with MPS VI were included in the study. Among them were two pairs of siblings. Five patients were Dutch, 4 Turkish, 1 Pakistani and 1 Guinean. Table 1 provides the patients' baseline characteristics. The patients' age at start of ERT ranged from 2 to 18.3 years. Patients were followed up for 1.3-5.4 years.

Four of the ten patients were considered to have a rapidly progressive form of the disease, presenting with a cardiomyopathy, dysmorphic facial features or severe joint limitations before the age of 5 years. Three of these four patients with a rapidly progressive form started ERT before the age of 5 (at 2, 2.3 and 2.9 years).

Table 1 Baseline characteristics of 11 Dutch Maroteaux-Lamy patients

No. Of patients, n (%)	11 (100)
Sex	
- Male, n (%)	7 (63.6)
- female, n (%)	4 (36.4)
Current age in years, median (range)	11.5 (4.9-21.7)
Age diagnosis in years, median (range)	5.1 (0.7-10.2)
Age at start of ERT	6.8 (2.0 -18.3)
ERT treatment, n (%)	11 (100)
Treatment duration in years, median (range)	3.7 (1.3-5.4)
Presenting symptoms:	
- Joint pain and/or dysmorphic features, n (%)	5 (45.5)
- Cardiomyopathy, n (%)	3 (27.3)
- Positive sibling, n (%)	2 (18.1)
- Trigger fingers, n (%)	1 (9.1)
Wheelchair use at baseline, n (%)	3 (27.3)
Impaired FVC <2 SD, n (%)	1 (9.1)
Cardiomyopathy, n (%)	3 (27.3)

FVC=forced vital capacity; ERT=enzyme-replacement therapy; SD=standard deviation

3.2. Pulmonary function and polysomnography

At baseline, none of the patients required respiratory support. At start of therapy, all eight patients over five years of age were able to perform a pulmonary function test; the others (N=3) were too young. FVC was normal in 5 of these 8 patients, Z-scores ranging from of -1.86 to +1.52, and staying within normal ranges during follow-up. Three patients had an FVC Z-score below the cut-off level of -2; one had a test result (Z-score- 2.24) that

was considered unreliable due to poor technique. At baseline, another 11-year-old patient had a borderline normal FVC Z-score of -2.16 (81% of normal); at the time of writing, this patient had received one year's ERT treatment, and had remained stable so far. The last patient had an FVC Z-score that was clearly reduced, being -4.77 (40% of normal). Over 5 years of therapy, this patient's FVC Z-score has improved to -2.29 (70% of normal).

Polysomnography was performed in 10 patients at baseline. In one patient no reliable registration was available. At start of therapy 1 child was diagnosed with a moderate OSAS. In 8 children a repeated polysomnography was available (range 2.1-4 years of therapy). Six children without OSAS at start of therapy showed no changes in respiratory variables at the second measurement. The patient with moderate OSAS at start of therapy, showed an OAHI decrease from 8.4 to 1.2 after 2.8 years of therapy. In one patient who had no OSAS at start of therapy, the OAHI increased from 0.3 to 4.4 after 4 years of therapy, which indicated mild OSAS. No patient had had an adenoidectomy or tonsillectomy.

Cardiac parameters

At baseline, five of the 11 patients (45%) had a Z-score for LVMI of above 2. The LVMI in these patients ranged from 57.3 grams/m^2 to 268 grams/m^2 (Z-score 3.0-26.4). In three of these patients – the only patients who used cardiac medication at baseline – this was because LVIDd was increased by dilated cardiomyopathy. In one other patient the LVMI was increased because of an increased Z-score for LVPWd and in another because of an increased IVSd.

Over the study period, LVMI Z-scores for the group as a whole decreased by -0.35 Z-score/ per year ($p=0.035$). For patients with abnormal LVMI values, the Z-scores declined to 2.8 or less over a follow-up period of 2.5-4.1 years. By the end of the study, LVMI had normalized only in the patient with the longest follow up. This was due to decreases in both LVIDd and IVSd values. Normalization in this particular patient was explained by a decrease of both LVIDd and IVSd values.

At baseline, 4 of the 11 patients had a Z-score for IVSd of 2. In the group as a whole, Z-scores for IVSd decreased significantly by -0.25/year ($p<0.024$). At the end of follow-up, all patients with an abnormal Z-score for IVSd at baseline had normal scores.

Although ERT improved cardiac geometry/dimensions in several ways, it did not seem to have a clear effect on valve regurgitation or the dysmorphic features of the valves. Valve regurgitation was most profound for the mitral valve; in three patients, mitral valve regurgitation increased. Increased mitral valve regurgitation explained the significant increase in one patient's LVIDd z-scores. Progressive mitral valve regurgitation in another patient led to life-threatening cardiac failure. The life of this patient – who had had a Z-score of 26.4 – was saved by the combination of a successful mitral graft replacement with tricuspid valve repair. This patient was subsequently excluded from further statistical analyses on the effects of ERT.

Clinically important ECG conduction abnormalities were not observed at baseline or during follow-up.

Size of liver and spleen

At baseline, liver size ranged from 0-7.0 cm (median 4.0 cm) below the costal margin. Spleen size ranged from 0-6.0 cm (median 1.0 cm) below the costal margin, and had increased in 7 patients. After start of ERT, liver and spleen size decreased in all patients. At group level, liver size decreased by an average of 0.6 cm/year ($p<0.001$); in 3 out of 8 patients, it became normal. Similarly, spleen size fell by 0.3 cm/year ($p<0.001$) at group level and became normal in 4 out of 7 patients.

Joint mobility

At start of therapy, all patients had impaired shoulder flexion and hip flexion. Shoulder flexion was limited to 94 -152 degrees, and hip flexion in standing position to 144 to 168 degrees. Five patients had a slight impairment in the extension of the elbow (extension limited to 162-174 degrees); in nine patients knee extension was limited to 155 to 173 degrees. During treatment, shoulder flexion improved by 2.3 degrees per year ($p<0.001$). The increase in the range of motion of the hip, knee and elbow was not significant.

Hearing

At the start of therapy, reliable audiometry results could be obtained for 8 patients. Two patients were too young to perform audiometry. In another, behavioural problems interfered with the proper conduct of the test.

Hearing losses at baseline ranged in the high Fletcher index from 7-43 dB in the right ear and 2-53 dB in the left ear. According to the Goodman criteria, three patients were classified as having either mild or moderate hearing loss. All hearing losses were of a conductive nature.

During the ERT period, hearing loss decreased in two of three patients who had had mild or moderate hearing loss at baseline. After 4 years of ERT, one 7-year-old patient improved from a mild hearing loss of 23/25 dB to 8/8 dB (right/left ear) without the need for middle-ear tubes. After 3 years of therapy, the second patient with moderate hearing loss improved from 43/35 dB to 18/23 dB (right/left ear) at the age of 5 years. This patient received middle-ear tubes after 2 years of ERT. The third patient with moderate hearing loss of 43/53 dB at baseline was already using hearing-aids at start of therapy. After 5 years of therapy, when she was 12, the hearing loss had worsened to 45/67 dB. After 4.5 years of therapy, a 10-year-old patient whose hearing had been normal at baseline had conductive hearing loss of 52/42 dB (right/left ear); he recently received middle ear tubes. The other patients remained stable with normal hearing.

Urinary GAG levels

At start of therapy, all patients had elevated GAG levels in their urine, levels ranging from 106 to 1287 µg GAG / mg creatinine (median 231 µg GAG / mg creatinine). At group level, all values decreased significantly by 40 µg GAG /mg creatinine per year ($p<0.001$). The largest reduction in GAG excretion was observed during the first 6 months.

Interestingly, the 4 patients who were considered to have a rapidly progressive form of the disease presented with higher GAG levels at start of therapy (range 554 – 1287 µg GAG / mg creatinine) than the rest of the group (range 106-231 µg GAG / mg creatinine). Although GAG levels did not normalize completely in any of these four patients, they did normalize in five of the seven patients with the lower values. The two patients in the lower GAG-value group who did not normalize reached borderline normal values with a maximum of 13 µg GAG / mg creatinine above the normal range. However, GAG values in the rapidly progressive patients (n=4) deviated from normal values with a range of 19-125 µg GAG

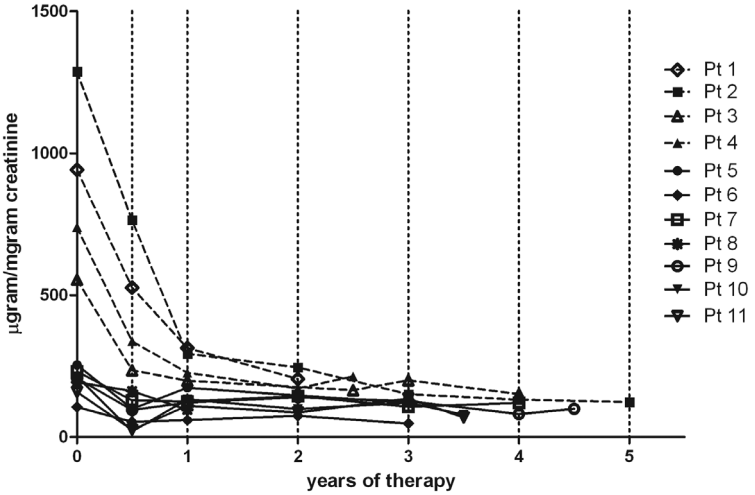

Figure 1 Glycosaminoglycan levels in 11 patients with Maroteaux-Lamy disease during treatment with galsulfase. The dashed line (---) represents rapidly progressive patients.

/ mg creatinine; in one of these patients, they returned to the same levels as at start of therapy. The GAG levels during therapy per individual are shown in Figure 1.

Quality of Life: TAPQOL/ TACQOL

At baseline, the parents of the 4 children under 6 years of age filled in the TAPQOL questionnaire; the TACQOL questionnaire was completed by 7 parents. At baseline, the median score of the 4 patients in the TAPQOL questionnaire was at least 25 points below the reference value for healthy peers in 7 domains ('lung problems', 'sleeping problems',

'liveliness, 'positive mood', 'social functioning', 'motor functioning' and 'communication'). The greatest deviation from the values of normal peers lay in 4 domains ('lung problems', 'social functioning', 'motor functioning' and 'positive mood'), the study group having median scores of 65-67 points, against 93-100 for healthy peers. Patients scored closer to the reference group in 5 domains ('stomach problems', 'skin problems', 'appetite', 'behaviour' and 'anxiety'), the maximum difference being 8 points. On the TACQOL questionnaire, the median score of the 7 patients at baseline lay at least 25 points below the reference value for healthy peers on 2 domains: 'body' and 'motor'.

Figure 2 shows the development of the TACQOL and TAPQOL scores relative to those in the reference group. The median follow-up was 2.5 years in the TAPQOL group (n=2)

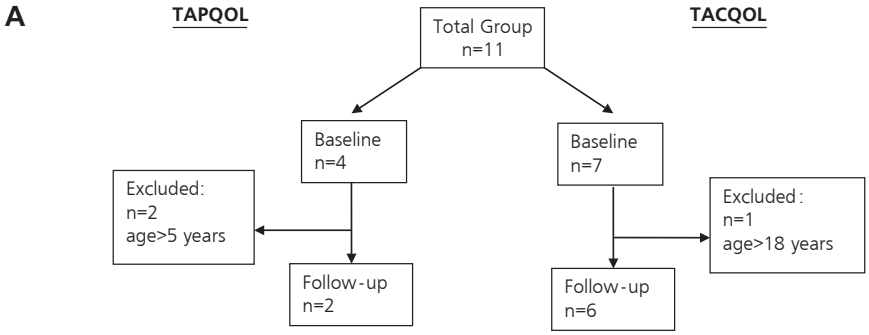

Figure 2A: Flowchart of the TAPQOL and TACQOL with the age-related exclusion of patients during follow-up.

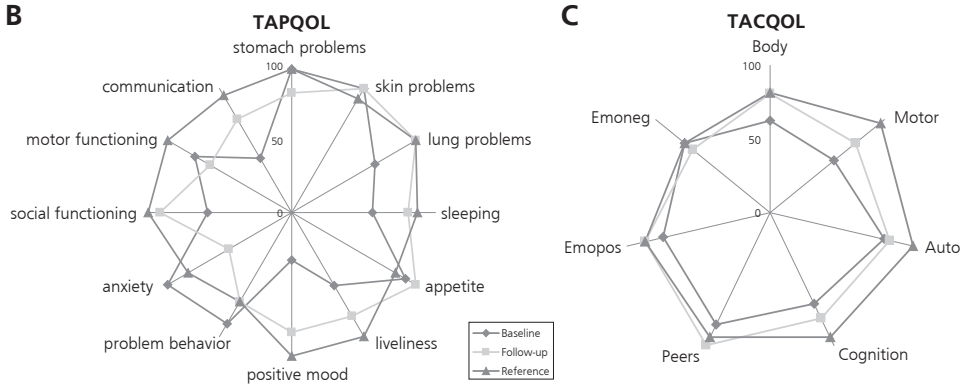

Figure 2B and 2C: HRQoL measured with the TAPQOL and the TACQOL at baseline, and follow-up relative to that in a Dutch group of healthy peers. Only patients were included in the analysis if both baseline and follow-up assessments were available. Values are median scores for the different domains. The centre of the graph represents the lowest possible score on each scale. Emoneg=experience of negative emotions; emopos=experience of positive emotions; auto=autonomy.

(2.1-3.1 years), against 3.6 years (1.0-4.5 years) in the TACQOL group (n=6). Figure 2A shows the flow chart of the included patients into follow-up.

The greatest progress in the TAPQOL (>25 points) was made in the following 6 domains: 'lung problems' (median start vs. end: 66.7 vs. 100); 'sleeping' (median start vs. end 56.3 vs. 81.3); 'liveliness' (median start vs. end 58.3 vs. 83.3); 'positive mood' (median start vs. end 33.3 vs. 83.3); 'social functioning' (median start vs. end 58.3 vs. 91.7); and 'communication' (median start vs. end 43.8 vs. 75). A decrease of over 25 points was seen in one domain, 'anxiety' (100 vs. 50) (Fig 2B). The largest increase in scores in the TACQOL questionnaire were seen in two domains 'body' (62.5-81.3) and 'motor' (56.3-75). Only the 'negative emotions' domain decreased slightly from 75 to 68 points (Fig 2C).

Antibodies

All patients developed antibodies against galsulfase after 24 weeks of therapy with titers ranging from 1:250 to 1:31,250. Because of the small sample size it was not possible to assess if effect of ERT were antagonised by antibody status.

Discussion

In this prospective single-centre open label study presenting follow-up periods of up to 5.4 years, we studied the effect of enzyme replacement therapy on clinical parameters and HRQoL in all Dutch patients diagnosed with MPS VI. Enzyme-replacement therapy had a positive effect on 11 patients with MPS VI with regard to flexion of the shoulder, size of the liver and spleen, pulmonary function (n=1), certain domains of the HRQoL questionnaire, and the level of GAGs in the urine. Although ERT improved cardiac scales, it did not seem to have a definite effect on valve regurgitation or the dysmorphic features of the valves [16]. The study also confirms the earlier observation that urinary GAG levels are correlated with disease severity [5].

The results of our study are in line with those published on the pivotal clinical trials of MPS VI patients [8, 17, 18]. One important difference is that our study includes three children under 5 years of age at start of therapy. Consequently, our patients group of patients were generally younger, with a median of 6.8 years (2-18.3 years) against the phase-I median age of 11 years (range 7-16 years), the phase-II median age of 12.0 years (range 6-22 years), and the phase-III treatment-group median age of 12.0 years (range 8-29 years) [8, 17, 18]. Our median follow-up time (3.7 years; range 1.3-5.4 years) in a cohort of 11 patients is relatively long. To our knowledge, only a Taiwan open-label study reports a follow-up of 6 years in one patient [19].

The decrease in liver and spleen size we saw in our study is a well-known effect of ERT in MPS VI and other types of MPS [20-22]. Because liver cells and spleen cells are in close contact with the bloodstream, they are easily accessible to enzymes with a high molecular

weight, and thus a perfect target for ERT. This has also been seen in other lysosomal storage disorders such as Gaucher's disease [23].

Shoulder flexion increased significantly by 2.8 degrees/year. Although the extent of this improvement may seem limited, it also proved to be important in daily life: some parents indicated that their children were now able to grasp objects that they had not previously been able to hold. Improvement in shoulder function has been reported for other long-term follow-up studies of the effects of ERT in MPS VI [19, 24]. Studies on the effect of ERT in other types of mucopolysaccharidosis also report improvements in shoulder flexion [22].

Hip extension did not seem to improve significantly, although like the shoulder, the hip is a ball-and-socket joint. We therefore hypothesize that many factors may make the shoulder more susceptible to the effects of ERT. First, the surface area of the glenoid is smaller than that of the hip. Since the glenoid is made of cartilage (including glycosaminoglycans), it is possible that the damage caused by lysosomal storage is confined to a smaller area, and that improvements with ERT are achieved more easily. Second, the shoulder is less affected by weight-bearing forces than the hip. As a result, the hip has probably sustained more cartilage damage than the shoulder, and the inevitable weight-bearing forces limit functional improvements [25]. Thirdly and importantly, the poorer response of the hip joint to ERT may lie in the fact that the anatomical structure of the acetabulum is abnormal at an early age in MPS VI [26].

Pulmonary function can be impaired in untreated MPS VI. At start of therapy, it was clearly reduced in one of our 11 patients, but improved enormously over the 5 years of follow-up – an outcome that may also have been influenced by a successful mitral graft for severe mitral-valve regurgitation in this patient. Harmartz et al. showed that the effects of ERT on pulmonary function could be established only after 96 weeks of therapy, and not earlier [6]. Only one patient showed signs of OSAS at start of therapy which improved during ERT. Another patient developed mild OSAS, despite ERT. The remaining patients did not show any signs of OSAS. Earlier studies reported much higher frequencies of OSAS (85%) among patients with MPS VI [27, 28] – a difference in findings that once again highlights the clinical spectrum of MPS VI.

We found an effect of ERT on cardiac geometry (LVMI and IVSd). The change in LVMI was due largely to the significant decrease of the ventricular septum, which was reported earlier in a cohort of MPS VI patients [29]. Even though the valves are surrounded by blood and freely exposed to galsulfase upon infusion, galsulfase had only limited effects on cardiac valves and regurgitation. The explanation might lie in the relatively poor vascularization of the valves: because myofibroblasts composing the valves are supplied with oxygen and nutrients mainly by diffusion from the valve surface [30], they are no easy targets for the relatively large therapeutic enzymes that need to enter the cells through receptor-mediated endocytosis. The effects of ERT on cardiac parameters were reported in more detail in a former paper concerning 24 children with MPS I, II and VI [16]. In this paper the follow-up is extended with another 1.5 years compared to the former. The positive effects of ERT on

cardiac geometry have also been observed for other types of MPS, as has the limited effect on the valves [22].

When they started therapy, three of our relatively young patients had conductive hearing loss. In the two youngest patients, this disappeared during ERT treatment, due possibly to the same ERT-related decrease in upper airway infections that has been reported in other types of MPS [31]. Although we observed no sensorineural hearing loss in our patient group, it remains unclear whether hearing loss has a congenital basis or is acquired secondary to deposition of glycosaminoglycans in the inner ear or central nervous system [32]. A recent report in MPS II mice suggests that although early initiation of ERT might prevent hearing loss, sensorineural loss might not be prevented in the long run [33]. A study reporting sensorineural loss in MPS VII mice suggests that, due to alterations in the mass and stiffness of cochlear structures, or to impaired sensory-cell function, this form of hearing loss occurs later in life [34]. This issue can be resolved only after longer follow-up.

The decline in GAG levels within 6 months is similar to that specified in other studies that report GAG values as an outcome measure [7, 19, 24]. The relationship between clinical severity and urinary GAG excretion in untreated patients reported by Swiedler also seemed to be the case with our patients: those who presented with the severest disease (with severe cardiac disease, for example) all had high urinary GAG levels (> 500 µg GAG / mg creatinine) at start of therapy, which was significantly higher than for the rest of the group [5]. This confirms that the level of GAG in urine at baseline might be a good indicator of disease severity and might anticipate the pattern of later symptoms. We also observed that the GAG values did not fully normalize in this rapidly progressive patient group. This raises the question of whether patients with a severe phenotype would be benefited by a higher dose of ERT.

To our knowledge, we are the first to report on HRQoL in MPS VI patients. During treatment with ERT, our patients' HRQoL improved, especially in seven domains in the younger patients ('lung problems', 'social functioning', 'sleeping', 'liveliness', 'positive mood', 'social functioning' and 'communication'), and in two domains ('body' and 'motor') in patients over 5 years of ages. In these older patients, HRQoL decreased slightly in two domains ('anxiety' and 'negative emotions'), representing a potential increase in negative emotions and anxiety.

The lower HRQoL in the different domains once again emphasizes the great impact of a metabolic disorder on young patients – and also on their parents [35]. Until 6 years of age, children measured with the TAPQoL had lower HRQoL values in 7 of the 11 domains at baseline. Some of these low scores directly reflected the symptoms of the disease. The 'lung problems' domain probably reflected the recurrent upper airway infections; the improvement seen during ERT in this domain might reflect the parent- reported decreases in these upper airway infections/respiratory infections. The lower score for 'motor functioning' reflects the delay in gross motor functions, and did not change during ERT. In the older age group, the scores in the 'body' and 'motor' domains were lower at baseline; the scores in

these domains also increased the most. The improvement we measured in shoulder function and the decrease of hepatosplenomegaly may have contributed to this improvement.

Although improvements in activities of daily living were also reported in other types of MPS, the questionnaire used (Childhood Health Assessment Questionnaire) focused on daily functioning and disability, and not on true HRQoL [36], thus making comparison difficult. During treatment, there was a notable decrease in HRQoL in the 'anxiety' domain in the TAPQOL and a slight decrease in the 'negative emotions' domain in the TACQOL – a finding that has not previously been reported in relation to start of ERT. The relevance of the latter finding needs further investigation and may highlight the need to pay more attention to the impact of ERT on children's psychosocial status.

The rarity of MPS VI means that our findings relate to a small patient group. The relatively small sample size limited our options for studying differences in treatment effects between the rapidly progressive and slowly progressive patients.

When ERT was introduced in the Netherlands in 2007, only two patients had been diagnosed with MPS VI. However, since the introduction of ERT, 9 new patients have been diagnosed. This suggests that the introduction of ERT has raised awareness of the disease among health-care practitioners, and that this disorder is much more prevalent than expected. With the increasing number of patients and longer follow-up, this might provide a basis for further studies on the effects of ERT on clinical endpoints such as patient survival and the progression of disabilities under ERT. More international collaboration might help to increase the study cohort of MPS VI patients.

While we are fully aware of the shortcomings of an observational study in reporting the effects of ERT, the phase of randomized-controlled trials has now passed, and we are dependent on observational studies to report on these effects.

In conclusion, ERT positively affected mobility of the shoulder joint, the size of the liver and spleen, cardiac parameters (LVMI and IVSd), pulmonary function, certain domains of HRQoL, and the level of GAGs in the urine. This effect was established in an open cohort of young MPS VI patients.

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Chapter 5

Mucopolysaccharidosis type VI phenotypes-genotypes and antibody response to galsulfase

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Summary

Background: Mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome; MPS VI) is an autosomal recessive lysosomal storage disorder in which deficiency of N-acetylgalactosamine 4-sulfatase (arylsulfatase B; ARSB) leads to the storage of glycosaminoglycans (GAGs) in connective tissue. The genotype-phenotype correlation has been addressed in several publications but the picture is not complete. Since 2007, enzyme-replacement therapy (ERT) has been available for patients with MPS VI in the Netherlands. The purpose of our study was to learn more about the genotype-phenotype correlations in MPS VI and the antibody response to ERT with galsulfase (recombinant human arylsulfatase B).

Methods: We identified ARSB mutations in 12 patients and used site-directed mutagenesis to study their effect. Antibody levels to galsulfase were measured using ELISA and a semi-quantitative immunoprecipitation method. We assessed the *in vitro* inhibitory effect of antibodies on galsulfase uptake and their effect on clinical outcome.

Results: Five patients had a rapidly progressive phenotype and seven a slowly progressive phenotype. In total 9 pathogenic mutations were identified including 4 novel mutations (N301K, V332G, A237D, and c.1142+2T>C) together composing 8 pathogenic genotypes. Most mutations appeared not to affect the synthesis of ARSB (66kD precursor), but to hamper its maturation (43kD ARSB). Disease severity was correlated with urinary GAG excretion. All patients developed antibodies to galsulfase within 26 weeks of treatment. It was demonstrated that these antibodies can inhibit the uptake of galsulfase *in vitro*.

Conclusions: The clinical phenotypes and the observed defects in the biosynthesis of ARSB show that some of the mutations that we identified are clearly more severe than others. Patients receiving galsulfase as enzyme-replacement therapy can develop antibodies towards the therapeutic protein. Though most titers are modest, they can exceed a level at which they potentially affect the clinical outcome of enzyme-replacement therapy.

Background

Mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome; MPS VI; MIM 253200) is a lysosomal storage disorder in which the deficiency of N-acetylgalactosamine 4-sulfatase leads to the storage of glycosaminoglycans (GAGs) in connective tissue. It is an autosomal recessive disease caused by mutations in the ARSB (arylsulfatase B) gene, which is located on chromosome 5q14.1. More than 130 mutations have been identified, the vast majority being missense mutations [1]. Although the genotype-phenotype correlation has been described for several mutations, it is far from complete [2]. Patients with MPS VI present with connective-tissue pathology of varying severities; the main symptoms include restrictive pulmonary disease, cardiomyopathy, hepatosplenomegaly, corneal clouding, and bone disease with short stature. The variability in disease covers a clinical spectrum which is common in lysosomal storage disorders [1]. Since 2007, enzyme replacement therapy (ERT) has been available for patients with MPS VI in the Netherlands, where treatment and standardized care are concentrated at the national expertise centre at Erasmus MC University Medical Center. The effects of ERT on endurance, pulmonary function, hepatosplenomegaly, and GAG excretion were described in previous publications [3-5]. Antibody formation is common in patients receiving ERT for lysosomal storage disorders [6]. In Fabry disease and Pompe disease, for instance, it is a well-documented problem [7, 8]. Due to the patient's enzyme deficiency, the immune system apparently treats the recombinant human enzyme as a foreign protein. The extent of the antibody response may depend on whether ARSB is not produced at all, is misfolded and prematurely degraded, or finds its way to the lysosomes but lacks function, all of which depends on the mutations in the ARSB gene.

Here, we report on the genotypes and phenotypes of 12 Dutch patients with MPS VI, and on the correlation between genotypes, antibody response, and therapeutic effect of ERT.

Methods

Patients

Dutch patients with MPS VI are diagnosed, treated and followed-up by the Center for Lysosomal and Metabolic Diseases at Erasmus MC. Since 2007, twelve patients were referred to our center. Their diagnosis was based on a combination of clinical findings, arylsulfatase B deficiency in fibroblasts, and the presence of a pathogenic mutation in each of the two ARSB alleles. ERT treatment for MPS VI consists of 1 mg/kg recombinant

human arylsulfatase B (galsulfase, Naglazyme®, BioMarin Corporation), administered by infusion once a week.

ARSB activity

The ARSB activity in fibroblasts was spectrophotometrically measured on the basis of the conversion of p-nitrocatecholdisulfate to paranitrocatechol, as previously described, and is expressed in nmol paranitrocatechol liberated per mg cellular protein per hour [9].

DNA analysis and site-directed mutagenesis

DNA was isolated from leukocytes or skin fibroblasts using routine procedures. After amplification, the exons and flanking intron regions of the ARSB gene were sequenced using an ABI 3730 DNA Analyser under standard GLP conditions. NM_32373.2 was used as reference sequence for the coding regions, whereby the 'A' nucleotide of the ATG translation-initiation codon at nucleotide position 560 constitutes +1 numbering of the cDNA sequence. The ATG-codon represents +1 for the amino acid numbering according to the galsulfase preprotein sequence NP_000037.2. The positions of intronic sequence variations were assigned on the basis of the cDNA sequence and the genomic contig sequence NC_000005.9, whereby the 'A' of the ATG start codon is at genomic position 78073032. The full length ARSB cDNA sequence cloned in expression vector pcDNA3 (p.V358; p.S384) was used as template for site-directed mutagenesis [10]. To introduce all mutations except splice-site mutations and established polymorphic sequence variations such as p.S384N into the wild-type ARSB cDNA, we used the QuickChange XL Site-Directed Mutagenesis Kit (Stratagene Life Technologies Co., La Jolla, CA, USA) for site-directed mutagenesis. In each case, the integrity of the resulting mutant constructs was confirmed by full-length sequencing of the ARSB cDNA insert.

Two independent colonies of each mutation were used to transfect COS-7 cells. The wild-type ARSB cDNA construct in pcDNA3 served as positive control, and a similar plasmid construct with the hexosaminidase-β cDNA as negative control. COS-7 cells were seeded into 6-well plates and grown overnight in Dulbecco's modified Eagle medium (DMEM) (Lonza, Verviers, Belgium) supplemented with 10% fetal bovine serum (FBS), 50 U/ml penicillin, and 50 μg/ml streptomycin. Cells at more than 90% confluence were transfected with 2.0 μg plasmid using lipofectamine 2000 (InvitroGen). The cells were harvested 48h and 72 hr later, and the ARSB activity was measured in the cell homogenates using paranitrocatecholdisulfate as substrate. The synthesis of ARSB was analysed by SDS-PAGE and immunoblotting using a polyclonal rabbit antiserum against recombinant human arylsulfatase B (galsulfase, Naglazyme®). In this assay, different molecular mass species reflect the synthesis of ARSB as 66 kD precursor, which is post-translationally modified to mature enzyme of 43 kD [11].

Antibodies

Immuno-precipitation

Blood samples for antibody titer determination were drawn just before the start of galsulfase infusions. Serum was prepared and stored at -80 °C. Starting with a 25-fold dilution, two-fold serial dilutions were made in acetate buffer pH 6.0 (0.5M sodium acetate, 10mM barium acetate and 0.02% sodium azide) containing bovine serum albumin (BSA, Sigma) in a concentration of 0.2 mg/ml. A 10 µl solution of a 250-fold dilution of galsulfase (total activity approximately 0.3 µmol/hr) in acetate buffer was mixed with 10 µl diluted serum and 60 µl of a 1:6 suspension of Protein A Sepharose CL-4B beads in PBS. The mixture was incubated under continuous agitation for 1 h at room temperature. The beads were then removed by centrifugation (14,000 g), and the activity of galsulfase in the supernatant was measured with paranitrocatecholdisulfate.

Pharmacokinetic (PK) analysis

Blood was drawn before the start of galsulfase infusion, and 1h, 2h and 2.5h thereafter. It was collected again 15 min before the end of galsulfase infusion, at the end of the infusion, and 15, 30, 60 and 120 min thereafter. Plasma was prepared and stored at -80 ºC until use. To measure the percentage of antibody-bound galsulfase we used Sepharose beads, with and without Protein A bound to it, for precipitation as described in the preceding paragraph.

ELISA

ELISA was used as a third method to determine antibody formation in response to ERT with galsulfase. A 96-well plate (Nunc, F96 Maxisorp, Denmark) was coated with 50 µL/well galsulfase in a concentration of 5 µg/mL diluted in phosphate-buffered saline (PBS, pH 7.4), and incubated for 2 hours at room temperature while shaking. Plates were blocked with 250 µL/well BSA/PBS (1 gram BSA/100 ml PBS from Sigma A7030), and incubated overnight at 4 °C. After incubation, the plates were rinsed six times at room temperature with 200 µL washing buffer (0.5 mL Tween-20 per liter PBS). Plates were incubated with 50 µL of 5-fold serial dilutions of patients' sera for one hour while shaking. Samples were diluted in dilution buffer (1 gram BSA and 50 µL Tween-20 per 100 mL PBS) in dilutions that ranged from 50-fold to 3,906,250-fold. A healthy person's serum was used as a negative control, and rabbit antiserum prepared against galsulfase was used as a positive control. After washing, 50 µL conjugate was added to each well. For patients' sera, we used polyclonal anti-human-[IgG, IgA and IgM]-HRP (Acris) in a 20,000-fold dilution. For polyclonal rabbit anti-serum, we used anti-rabbit-IgG-HRP (Sigma) in a 10,000 fold dilution. After washing, 100 µL Tetramethylbenzidine Microwell Peroxidase substrate (Kirkegaard and Perry Laboratories, Maryland) was added, and the plates were incubated for 10 minutes. The colorimetric reaction was stopped by adding 100 µL 1M phosphoric

acid (H_3PO_4). Absorbance was measured at 450 nm using a spectrophotometer (Thermo Electron corporation, Vantaa, Finland). The titer was determined as the maximal dilution at which absorbance was at least twice the absorbance of the negative control.

Inhibition of galsulfase uptake

To assess the interference of antibodies with the uptake of galsulfase by human fibroblasts we added 160 μl plasma from patient 2, collected at peak-rate galsulfase infusion, to 640 μl culture medium (Ham's F10 supplemented with 3 mM Pipes) in a 6-well-tissue-culture plate containing fibroblasts of this patient. In parallel, we added to other wells galsulfase in an amount equivalent to 2400 nmol/h ARSB activity in combination with either polyclonal rabbit anti-galsulfase antiserum or serum from a healthy individual. Uptake of galsulfase by the cells was measured 48 hours later.

Clinical assessments

To evaluate the effect of ERT, we selected three variables: joint mobility (shoulder flexion), urinary GAGs and pulmonary function (Forced Vital Capacity, FVC). All assessments were performed according to standard protocol.

Table 1. ARSB genotypes and phenotypes of 12 MPS VI patients

Pt	Age at start of ERT	Age at diagnosis (years)	Main presenting symptom at diagnosis (years)	GAG at start of ERT (μg/mg creatinine)	ARSB activity fibroblasts (nmol/h*mg)
1.	2.1	1.8	Cardiomyopathy	941.6	85,7
2.	6.8	3.4	Cardiomyopathy	1286.6	84,8
3.	2.9	2.8	Cardiomyopathy	554.4	32,3
4.	2.3	1.9	macrocephalia	739.2	57,6
5.	8.3	7.8	joint abnormalities dysmorphic features	254.3	79,9
6. α	18.3	10.1	joint abnormalities	105.6	46,7
7. α	7.6	0.7	positive sibling	230.6	61,9
8.	10.6	10.2	joint abnormalities	192.7	50
9.	5.9	5.1	joint abnormalities dysmorphic features	206.8	38
10. β	7.8	7.4	trigger fingers	213.8	40,7
11. β	6.1	5.8	positive sibling	158.4	32,3
12.#	n.a.	2.8	joint abnormalities	712.8	57

Pt patient; α, β siblings; GAG glycosaminoglycans; ARSB Arylsulfatase B; ARSB activity, normal range 379-980 nmol/mg/hr; ^NCBI Reference sequence: NM_000046; * S384N has previously been described as a polymorphic change; novel mutations are marked in bold; #Pt 12 did not receive ERT in our hospital and is therefore only included in the genotype-phenotype section.

Results

Patients

We describe 12 patients with mucopolysaccharidosis type VI in 10 unrelated families (Table 1). Five of the twelve patients had a rapidly progressive phenotype and presented before the age of 5 years with cardiomyopathy, dysmorphic facial features and/or severe joint limitations. The remaining seven patients had a slowly progressive phenotype. The diagnosis of all twelve patients was confirmed by the finding of ARSB deficiency in cultured skin fibroblasts, elevated GAG levels in their urines, and pathogenic mutations in both ARSB alleles. GAG values appeared to correlate with disease status. The pathogenic sequence variations comprised 7 missense mutations, 1 nonsense mutation and 1 mutation in the splice-donor site of exon 6. Four of these nine mutations were not previously reported. Eight of the 12 patients were the children of consanguineous marriages (third degree or higher) and were homozygous for the given mutations (Table 1).

Except for patient 12, all patients are currently receiving ERT (galsulfase, Naglazyme®, 1mg/kg weekly); three of them started before the age of five years. Only patient 2 has had infusion-associated reactions (IAR); these consisted of urticaria and general malaise.

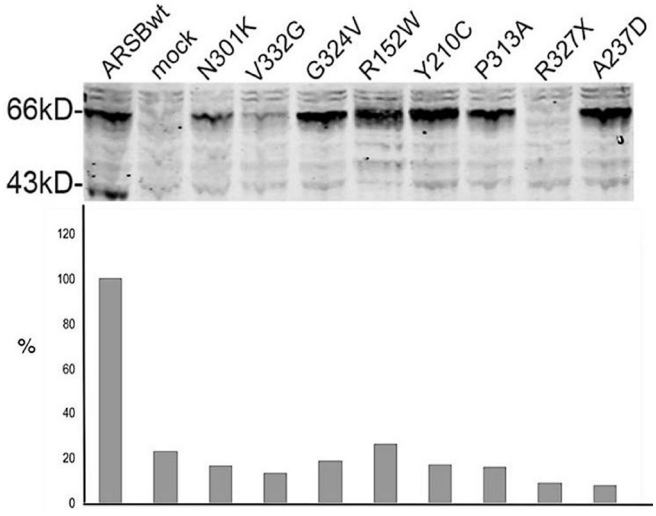
Allele 1		Allele 2		Progression	Ethnicity
DNA^	protein	DNA^	protein		
c.903C>G	**p.N301K**	**c.903C>G**	**p.N301K**	Rapid	Turkish
c.1151G>A*	**p.S384N***	**c.1151G>A***	**p.S384N***		
c.1142+2T>C		**c.1142+2T>C**		Rapid	Pakistani
c.995T>G	**p.V332G**	**c.995T>G**	**p.V332G**	Rapid	Marrocan
c.971G>T	p.G324V	c.971G>T	p.G324V	Rapid	Guinean
c.454C>T	p.R152W	c.454C>T	p.R152W	Slow	Turkish
c.454C>T	p.R152W	c.454C>T	p.R152W	Slow	Turkish
c.454C>T	p.R152W	c.454C>T	p.R152W	Slow	Turkish
c.629A>G	p.Y210C	c.937C>G	p.P313A	Slow	Dutch
c.629A>G	p.Y210C	c.979C>T	p.R327X	Slow	Dutch
c.629A>G	p.Y210C	c.979C>T	p.R327X	Slow	Dutch
c.629A>G	p.Y210C	c.979C>T	p.R327X	Slow	Dutch
c.710C>A	**p.A237D**	**c.710C>A**	**p.A237D**	Rapid	Iranian

Biosynthesis of ARSB

ARSB is synthesized as a polypeptide of 533 amino acids that is co-translationally delivered into the lumen of the endoplasmic reticulum (ER) [12]. The 39 amino acids long N-terminal signal peptide is cleaved off, and the protein is glycosylated and folded. It attains a relative molecular mass of 66 kD, but is subsequently processed to a lysosomal form of approximately 43 kD with a central domain of 7 kD and a carboxyl-terminal domain of 8 kD [13].

To study the effect of the mutations in the ARSB gene, we attempted to visualize the synthesis and post-translation modification of ARSB in the patients' fibroblasts by SDS-PAGE followed by Western blotting. However, due to the low intensity of the ARSB signal and the presence of 'background' staining, we failed to obtain clear results.

As an alternative approach, we then used site-directed mutagenesis to introduce the mutations in the wild-type ARSB cDNA, and expressed these constructs transiently in COS-7 cells to gain an impression of how the mutations affected the biosynthesis of ARSB (Fig 1). None of the 7 missense mutations led to total loss of ARSB protein synthesis. The 66 kD precursor was detectable in all cases, but its amount varied from very little (V332G), to clearly less than normal (N301K), to near normal (G324V, R152W, Y210C, P313A, and A237D). By contrast, introduction of the nonsense mutation R327X led to total loss of ARSB production (Fig. 1). Notably, expression of none of the missense mutations led to



F**igure 1. Transient expression of wildtype and mutant ARSB cDNA constructs** COS-7 cells were transfected with wildtype (wt) and mutant cDNA constructs. 'mock' indicates that the cells were transfected with a cDNA construct containing beta-hexosaminidase cDNA. SDS-PAGE followed by immunoblotting was performed and polyclonal rabbit anti-galsulfase serum was used to visualize ARSB. Equal amounts of protein were loaded per lane. The figure at the top shows the result of a representative experiment obtained at 72 hours after transfection. The figure at the bottom shows the ARSB activity as percentage of wild type ARSB activity as measured in cell homogenates at 48 hours after transfection.

the formation of mature 43 kD ARSB protein, which was in our system best visualized at 72 hours after transfection (Fig. 1 top, for ARSBwt), whereas the highest ARSB activity was measured at 48 hrs after transfection (Fig. 1, bottom). At this time point the activity of R152W was barely above the ARSB activity in mock transfected cells, while all other mutants had less activity.

Immune response to ERT

Within the first half year of ERT, all patients developed antibodies against galsulfase with an ELISA titer of at least 1:250 (range of 1:250 to 1:31,250) (Fig. 2). After approximately three years of therapy, patient 2, who was homozygous for the splice-site mutation c.1142+2T>C, had the highest antibody titer (1:156,250). Patient 4 had the second highest titer (1:31,250). Most patients had titers ranging from 1:1,250 to maximally 1:6,250, which remained fairly stable from one year after start of therapy until the end of follow-up.

As an alternative method of estimating the height of the antibody titer, we performed an immunoprecipitation assay in which a given amount of galsulfase was precipitated from solution by serial dilutions of the patient's serum. In this assay, only patient 2 had a measurable antibody titer (shown for patient 2 in Fig. 3). This figure shows that the titer was highest after 26 weeks of therapy and then gradually declined over 4.5 years of follow-up. The result was somewhat unexpected, since the ELISA titer remained more or less constant over the same period (Fig. 2).

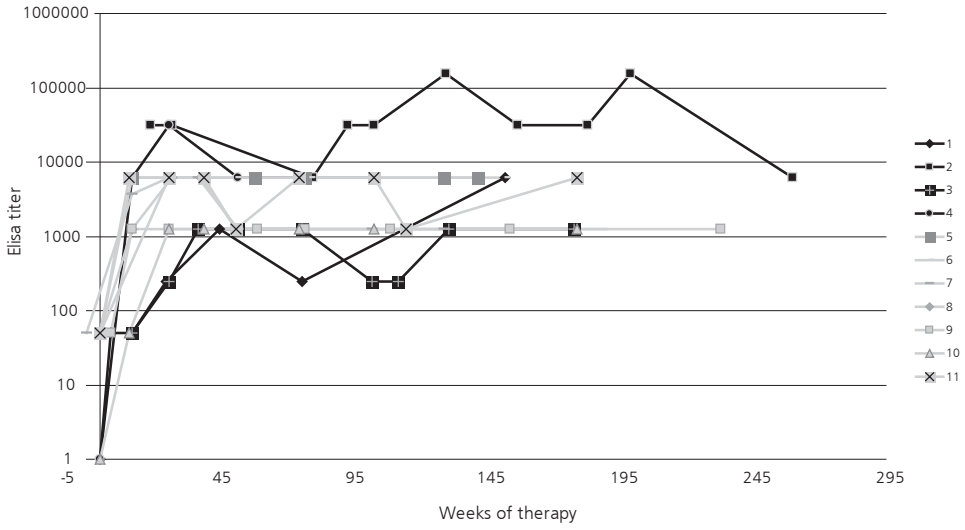


Figure 2. ELISA titers of patients receiving ERT The black lines represent the patients with rapid disease progression; the grey lines represent the patients with a slower disease progression.

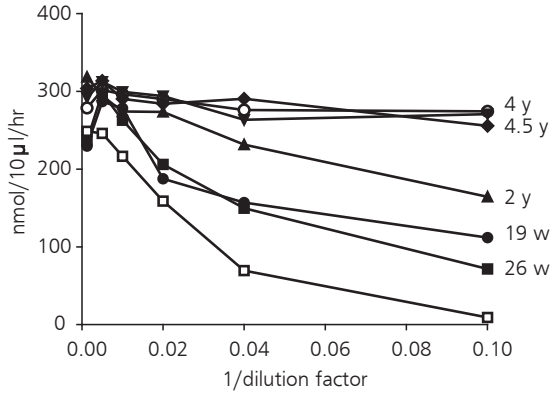


Figure 3. Antibody titer of patient 2 by immunoprecipitation A fixed amount of galsulfase was incubated with serially diluted serum of patient 2. X-axis: 1/dilution factor (for example, 0.02 represents a dilution factor of 50). The antibody-bound galsulfase was precipitated using Protein A Sepharose beads, and the activity remaining in the supernatant was measured with paranitrocatecholdisulfate. The numbers at the right side of the curves represent the ERT duration in weeks (w) or years (y). The ELISA titers at these time points were 1:31,250 except at the 4y point (1:156,250). The open squares show the precipitation of galsulfase by rabbit anti-galsulfase serum.

In addition to these two methods of determining the antibody titer, we also collected blood samples at regular intervals during the 5 hours of enzyme infusion in order to measure the capture of galsulfase by circulating antibodies. In patient 2 we performed this assay at 4.5 years after start of ERT, when her ELISA titer was 1:31,250; in patient 6, we performed it after 3.2 years of ERT, when his titer was 1:6,250. Figure 4 shows two curves for each of these patients. The curves with open symbols represent the total ARSB activity that was measured in the plasma, whereas the curves with closed symbols represent the galsulfase activity remaining after precipitation of antibody-bound galsulfase mediated by Protein A Sepharose. Thus, in patient 2, approximately 50% of galsulfase was antibody-bound after 2.5 hours (150 minutes) of infusion. The proportion of antibody-bound enzyme slightly decreased during infusion, and was 31% at the end of the infusion (300 minutes). In patient, 6 the amount of antibody-bound galsulfase was approximately 15% throughout infusion.

Uptake of galsulfase by fibroblasts

To investigate the adverse effect that antibody-binding can have on the efficacy of ERT, we measured the uptake of galsulfase by cultured ARSB deficient fibroblasts. To this end we collected blood-plasma from patient 2 during infusion and selected the sample with the highest galsulfase activity. An aliquot of this sample was added to the cell culture medium. To other wells we added galsulfase together with either rabbit anti-galsulfase antiserum (rabbit serum) or serum from a healthy individual (normal serum).

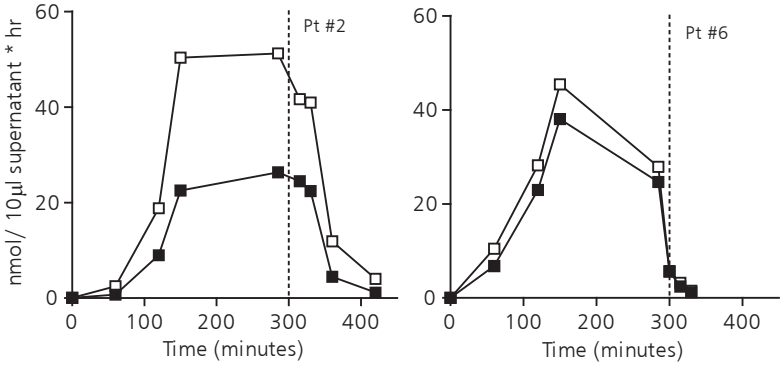


Figure 4. Antibody binding of galsulfase during infusion Blood-plasma samples were collected from patient 2 and patient 6 just before start of galsulfase infusion (0 min) and at time points thereafter. The open symbols present the total galsulfase activity measured in the plasma; the closed symbols present the amount of activity remaining after Protein-A-Sepharose-mediated immunoprecipitation of antibody-bound ARSB. The difference between the curves represents the proportion of antibody-bound enzyme. The activity in the supernatant was measured with paranitrocatecholdisulfate and is expressed in nmol/10µl per hour.

After 48 hours, the galsulfase activity in the medium had dropped to 50% using normal serum and to 63% using the patient's sample. Addition of rabbit serum resulted in a 50% drop of galsulfase activity within the first 15 minutes, but no further drop in activity was measured in the following 48 hours. At this time point, the cells were harvested and the intracellular galsulfase activity was measured. Uptake of galsulfase from the patient's sample was 42% less than uptake of galsulfase combined with normal serum. The presence of rabbit antiserum inhibited the uptake of galsulfase almost completely (98%).

Immune response and clinical outcome

Since the study group was very small and heterogeneous, we could not perform statistical analysis. Because analysis was further restricted by the limited availability and variability of clinical parameters, we could only investigate the overall correlation between immune response and clinical outcome. To this end, we compared the effect of ERT in two patients who had rather different immune responses: patient 2, whose antibody titers ranged from 1:31,250 to 1:156,250; and patient 6, whose titer was more or less constant at 1:6,250. Figure 5 shows the responses of both patients on FVC, shoulder flexion and GAG excretion. In both patients GAG values decreased significantly. GAG values remained slightly above the upper limit of normal in patient 2 over a period of 4.5 years. In patient 6, whose urinary GAG content had been lower at baseline than in patient 2, it normalized within 2.5 years of ERT.

Patient 2 had restrictive pulmonary disease at baseline (FVC -5.5 SD). During ERT, this improved from -4.8 SD -2.4 SD, though her respiratory capacity must also have been improved by the mitral valve replacement she underwent in the same period. In patient

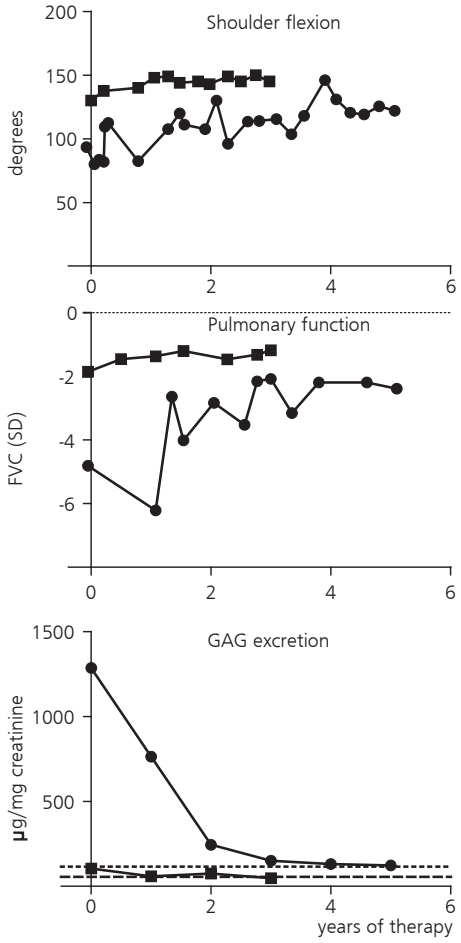


Figure 5. Shoulder flexion, pulmonary function and glycosaminoglycan excretion During ERT, shoulder flexion (degrees), pulmonary function (FVC) and urinary glycosaminoglycan (GAG) excretion were measured in patient 2 (●) and patient 6 (■). Shoulder flexion: normal range 175-180. Pulmonary function (FVC) is expressed as a Z-score of the predicted value. GAG excretion: the dashed lines denote the normal value for age (115 µg/mg creatinine for patient 2, and 55 µg/mg creatinine for patient 6).

6, FVC was -1.8 SD at baseline and -1.2 SD at last assessment. At start of therapy, both patients had severe joint restriction. In patient 2, the degree of shoulder flexion (starting from a 94 degrees restrictive shoulder flexion at baseline) improved by 30%; in patient 6, it improved by 12% (starting from 130 degrees restriction; normally 175-180 degrees). While the hepatomegaly and the splenomegaly both improved significantly under ERT in patient 2, no comparison was possible with patient 6, in whom these signs had not been evident at baseline. Neither could the effects of ERT on cardiac parameters be compared, as patient 2 received a mitral graft at the age of 7 years. Also the mild aortic regurgitation in patient 6 did not improve during ERT.

Discussion

Since many pathogenic sequence variations have been identified in the ARSB gene, a genotype-phenotype correlation is slowly emerging [14-19] – a picture to which this description of our twelve patients and their genotypes now contributes. Five of our patients presented with rapidly progressive disease; progression in the other seven was slower. In total, we identified 9 different pathogenic mutations, 4 of which had not been published previously. Together they compose 8 different pathogenic genotypes. MPS VI is indeed very heterogeneous [13].

Genotypes and phenotypes

Like others, we found that the level of residual ARSB activity in cultured fibroblasts using rather non-specific artificial substrates is a poor means of differentiating between patients with different clinical phenotypes. The radiolabeled three-saccharide substrates perform better [20, 21]. By contrast, urinary GAG content seems to help indicating the degree of disease severity [12, 22]. In our patient group too, a high urinary GAG content (mainly dermatan sulphate) of 300 µgram / mg creatinine or higher was associated with a severe phenotype.

With regard to our patients' genotypes, 4 patients were homozygous for missense mutations and one was homozygous for a splice-site mutation. The advantage of homozygous genotypes over compound heterozygous genotypes is that the impact of the genotype does not need to be gathered from the additive effects of the two individual mutant alleles. Karageorgos et al. were able to correlate the genotype, urinary GAG excretion and phenotype of several patients with homozygous missense mutations [19].

Three of our patients were homozygous for p.R152W. The fact that they were the least severely affected patients in our study group is consistent with the literature indicating that patients with this mutation are not amongst the most severely affected. One publication about a 38-year-old woman describes this mutation as being associated primarily with a 'cardiac phenotype' [23]. While our three patients with this mutation do not have prominent cardiac signs, the oldest –who is currently 21 years old– does have mild aortic regurgitation. By contrast, four of the other missense mutations that we identified (p.N301K, p.V332G, p.G324V, and p.A237D) are severely pathogenic; this is suggested by the rapidly progressive phenotypes of the homozygotes in which they were found. Only p.G324V has been described previously, but in a compound heterozygote, meaning that its effect was still obscure [19]. Its great severity is indicated by the fact that our patient who is homozygous for it had macrocephalia, hepatosplenomegaly, severe joint deformities and eye problems at a very young age (2y old). As patient 2, who is homozygous for 1142+2T>C, also had a rapidly progressive phenotype we conclude that this newly discovered mutation must be severe too. Moreover, this mutation affects the invariant splice donor sequence and is therefore expected to completely disrupt splicing [24]. Homozygosity for c.1142+1G>T in

a patient with a very high GAG content in urine –like our patient– also resulted in total loss of ARSB protein [19]. Among the remaining patients, we identified four compound heterozygotes carrying the common p.Y210C mutation in combination with either p.P313A (patient 8) or p.R327X (patients 9, 10 and 11). p.Y210C is known to be associated with an intermediate MPS VI phenotype, which was also the case in our patients with this mutation [25]. Compound heterozygosity for p.Y210C and p.P313A was earlier described by Karakeorgos et al in association with a slowly progressive phenotype [19].

The effect of 8 mutations was investigated by site directed mutagenesis and transient expression studies. Introduction of the non-sense mutation p.R327X in the wild type ARSB cDNA construct led to total absence of ARSB protein. The seven missense mutations had variable effects on the biosynthesis of ARSB; some resulted in (much) less than the normal amount of 66 kD precursor while others did not seem to have a dramatic effect on the amount of 66kD ARSB as judged from several independent experiments. The low amount of 66kD precursor in the respective cases might point to aberrantly folded proteins that are partly degraded by endoplasmic reticulum-associated degradation (ERAD) [12, 26, 27]. The expression of none of the 7 missense mutations in COS-7 cells led to the formation of both the 66 kD precursor as well as the 43 kD processed form of ARSB and none resulted in clearly measurable ARSB activity. This outcome agreed with the very low ARSB activity in fibroblasts of the patients. The lack of 43 kD ARSB by expression of p.Y210C was unexpected since Brooks et al. demonstrated in a pulse-chase experiment that 67% of p.Y210C-substituted ARSB is processed to mature 43 kD enzyme. However, the apparent discrepancy can relate to the fact that different methods and different antibodies were used (steady-state detection as opposed to pulse-chase labelling) [28].

Antibody response to galsulfase

Within 26 weeks from the start of galsulfase infusions all patients showed seroconversion, which is consistent with observations during the clinical trials [4]. The ELISA titers differed between patients during ERT, varying from relatively low in some patients (1,250) to relatively high (1:156,250) in others.

Patient 2 consistently had the highest antibody titers and was the only patient who experienced infusion associated reactions (IARs). Notably, due to homozygosity for c.1142+2T>C, the patient is not expected to have any level of endogenous ARSB expression, and is therefore immunologically naive to ARSB. This might underlie the strong immune response. Her high antibody titer as measured by ELISA was confirmed by immuno-precipitation. While the ELISA titer remained constantly high after 26 weeks of treatment, the titer obtained by immuno-precipiation declined. This would imply that the pool of antibodies directed against immobilized galsulfase (ELISA) remained constant while the pool of antibodies against native galsulfase decreased. The assay in which the pool of antibody-bound galsulfase is measured during enzyme infusion probably mimics the actual situation best; from it, we inferred that approximately 50% of galsulfase administered to

patient 2 was captured by antibodies at the time that she had an ELISA titer of 1:31,250. In patient 6, whose ELISA titer was 1:6,250, the percentage of antibody-bound galsulfase was much lower.

With regard to the effect of antibody binding, we demonstrated for patient 2 that it can inhibit the uptake of galsulfase by cultured fibroblasts. It is plausible that a similar effect occurs *in vivo* and can limit the effect of ERT. In such a case, the adverse effect of antibody formation can possibly be overcome by higher dosing, unless higher dosing would lead to higher antibody titers or severe infusion-associated reactions.

We have no clinical indications that antibody formation in MPS VI has a very strong impact on the effect of ERT. Despite the much higher percentage of antibody-bound galsulfase during infusion in patient 2 compared to patient 6, both patients had similar increases in shoulder flexion, FVC and decrease in urinary GAG. It is known from other lysosomal storage diseases that immune response to ERT can reduce therapeutic outcome. In Pompe disease, for instance, antibody formation has been associated with loss of milestones and decreased pulmonary function [8]. In MPS VI, however, clinical changes in response to ERT are more subtle and the adverse effect of antibodies may go unnoticed [5]. We have investigated the effects of enzyme-replacement therapy in this patient group in a separate paper [29].

Conclusions

In summary, our characterisation of the MPS VI patients currently receiving ERT in the Netherlands reflects considerable genetic heterogeneity. The clinical phenotypes and the defects we observed in the biosynthesis of ARSB show that some of the mutations we identified –known and novel alike– are clearly more severe than others. As in other lysosomal storage diseases, the antibody response to ERT can differ greatly between patients and may to some extent relate to the genotype [30]. Although antibody formation seems to have little impact on clinical outcome, we have demonstrated that the antibody concentration in the blood can reach a level at which it potentially affects the efficacy of ERT by inhibiting uptake of enzyme by the target tissues.

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Chapter 6

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

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In preparation

Summary

Introduction: Maroteaux-Lamy syndrome or mucopolysaccharidosis type VI is a lysosomal storage disease affecting various tissues. While enzyme replacement therapy (ERT) has demonstrated to elicit positive effects, its inability to cross the blood-brain barrier is a limitation. The aim of our study was to assess cognitive development in the Dutch patient population before and after start of ERT and to relate it to findings obtained with brain Magnetic Resonance Imaging (MRI) and other factors like genetic background and environmental factors that might affect cognitive outcome.

Methods: We prospectively assessed cognitive functioning in a series of 11 children with MPS VI who had been treated with ERT since 2007 and were followed for a period up to 4.8 years. To get insight in potential other genetic background and environmental factors we also assessed the cognitive development of their siblings and collected data on the educational levels of the parents.

Results: The patients scores ranged between normal and mildly delayed. Six of the 11 patients scored above the critical threshold of normal intelligence > 84 (55%) and did so at all time-points. During follow-up, cognitive development remained stable over time in all, except in one patient who showed clear deterioration. Many patients needed special education services (80%). Generally, intelligence scores of the patients were in line with the test scores of their siblings and also with the parental educational level. Native Dutch speaking patients seemed to have higher intelligence test scores than non-native Dutch speaking patients. In addition, patients with high glycosaminoglycan (GAG) levels at baseline more often had mild to severe developmental delays (75%) when compared to patients with low GAG levels (29%). Several mild structural brain abnormalities were found. Virchow Robin spaces, patchy white matter lesions and a thin corpus callosum were more frequently seen in the more severely affected patients. Interestingly, the white matter abnormalities became less prominent over time.

Conclusions: We found intelligence in patients with MPS VI to be normal or mildly delayed. Cognitive development remained stable over time in 90% of our population. Genetic background and environmental factors were clearly of influence, but also high GAG levels and a more severe phenotype/genotype seemed to increase the risk on poorer cognitive functioning. We conclude that long-term cognitive development in children with MPS VI differs substantially from other types of mucopolysaccharidosis in which profound and progressive mental retardation is a hallmark and that many factors determine the cognitive outcome of MPS VI patients.

Introduction

Maroteaux-Lamy (MPS VI, OMIM #253200) is a lysosomal storage disease caused by a deficiency of the lysosomal enzyme N-acetylgalactosamine 4-sulfatase or arylsulfatase B leading to storage of dermatan sulphate (a glycosaminoglycan, GAG) in various tissues. The disease is rare with a frequency of 1:600,000. Characteristic clinical features are short stature, bone abnormalities, coarsening of facial features, sensory perception disorders, corneal clouding, umbilical or inguinal hernias, cardiac problems, hepatosplenomegaly, and reduced life expectancy. Other problems that may occur are carpal tunnel syndrome, and spinal cord compression [1-6].

The disease comprises a spectrum of disease presentations ranging from an early onset, rapidly progressive form typically presenting before the age of five years to milder variants with later onset of symptoms. It has been suggested that there is a relationship between the severity of the disease and GAG excretion in the urine (>300 µg/mg creatinine) [2, 7, 8]. The first therapeutic option that became available was bone marrow transplantations, which was later replaced by hematopoietic stem cell transplantations. The latter therapies had relatively high mortality risks. In 2007 enzyme replacement therapy (ERT) with recombinant human arylsulfatase B (Naglazyme®, galsulfase) was registered as treatment for MPS VI. ERT is now considered to be the first choice of therapy for MPS VI. The therapy has shown to elicit positive effects on endurance, pulmonary function, liver and spleen size, joint mobility and levels of glycosaminoglycans [8-11], but it is assumed that ERT cannot pass the blood-brain barrier because of the large size of the therapeutic molecules [12].

While progressive mental retardation is a common feature in other types of MPS, like MPS I-Hurler, Hunter's Disease and the Sanfilippo syndromes [3], there are several reports indicating that MPS VI does not affect cognition and that patients may even achieve above average scores on cognitive tests [4, 13, 14]. Other investigators have reported that intellectual disabilities may occur in MPS VI patients [13, 15]. Pathology studies in animals with MPS VI and magnetic resonance imaging (MRI) of the brain of patients with this disease have demonstrated that mild structural central nervous system abnormalities may occur [16-22], but the relationship between these abnormalities and mental outcome has not been studied in depth. Very recently Azevedo et al. (2012) reported on a cross-sectional study on mental development in relation to MRI findings in a group of Brazilian patients with MPS VI [13]. Some of these patients received ERT; others were untreated. They found that 31% of the 16 patients that underwent complete evaluation had mental retardation, but they also indicated that the test results might not be fully reliable in all patients since some had severe visual and/or hearing deficits. Many of their patients showed brain abnormalities.

We studied the mental development and MRI findings in the Dutch MPS VI patient population at start of ERT and up to 4.8 years after start. We also examined the siblings and asked for the educational level of the parents. In addition, we performed mutation analysis

of the patients and determined GAG levels in their urine with the following aims 1. To assess long-term cognitive outcome of individual patient. 2. To get some insight in potential influence of genetic background and environmental factors on intelligence. 3. To relate cognitive outcome to the severity of mutations in the arylsulfatase B gene and the level of GAG excretion and 4. To get insight in the potential effect of structural abnormalities of the brain on intelligence.

Methods

Participants

Patients diagnosed with Maroteaux-Lamy participated in a long-term standardized follow-up study on the effects of ERT. The study was performed at the Center of Lysosomal and Metabolic Diseases at Erasmus MC University Medical Center in Rotterdam and started in 2007. The diagnosis of MPS VI was made by GAG analysis in the urine, followed by enzyme assay on leukocytes and fibroblasts and mutation analyses. All patients and siblings participating in this study and/or their parents gave written informed consent. Study protocols had been approved by the Institutional Review Board. Patients were treated with the registered dose of 1 mg/kg/weekly recombinant human arylsulfatase B (galsulfase, Naglazyme®, Biomarin Corporation). None of the patients had received hematopoietic stem cell transplantation.

Intelligence

On a yearly basis, patients underwent standardized cognitive assessment. For this purpose, we used the Griffith Mental Developmental Scales (Griffith; 0-72 months) [23] Bayley Scales of Infant Development, and the Wechsler Intelligence Scales (Wechsler Intelligence Scales Children – third edition; 6 - 16 years or Wechsler Adult Intelligence Scales – third edition; > 16 years) [24, 25]. For children with impaired hearing, we used the Snijders Oomen Nonverbal Intelligence test-Revised (SON-R 2½-7)[26]. To get some insight in the potential influence of genetic background and environmental factors, we compared the most recent intelligence test score of the patient with that of their sibling(s). 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 highest educational level, the patients' ethnic background, and school performance of patients and siblings.

Brain MRI

Magnetic resonance brain imaging (MRI) was performed on a yearly basis if feasible. Baseline MRI and most recent MRI were used to determine change in brain abnormalities over time. The choice of MRI follow-up parameters was based on literature review and in

particular deduced from the combined information obtained from four articles that had addressed brain abnormalities in patients with MPS VI more specifically before [13, 27-29]. Two observers evaluated all MRI's: a physician assistant (M.M.M.G. B.) specifically trained for this job and an expert pediatric neuroradiologist (16 years of experience) (M.H. L.). MRI's were scored on the presence or absence of the following parameters:

1) Virchow robin (VR) spaces on T2 and FLAIR sequence. A distinction was made between VR in the basal nuclei, white matter, or corpus callosum (CC). VR were scored for all three locations separately. If absent, they were rated as 0; if present as 1. If large VR (≥ 8 mm diameter) were present patients received one additional score (0 = absent, 1 = present).
2) White matter signal changes on T2, FLAIR sequence. Separate scores were made for the presence of patchy and diffuse lesions a) patchy lesions 0 = absent, 1 = present and b) diffuse lesions 0 = absent, 1 = present.
3) Ventricular enlargement on T2, FLAIR sequence. For this purpose the frontal and occipital horn width ratio (FOHWR index) was used [30]. We used a control group of normal children (n = 20) as a reference group (*normal values in press*). FOHWR scores of two standard deviations or more above the mean of this control group was considered a deviant score (0 = normal, 1 = deviant).
4) Increased flow void was visually assessed at the level of sinus rectus. A widened sinus rectus was scored as 1; a non-dilated sinus rectus scored as 0.
5) Brain atrophy as measured by dilated subarachnoid spaces (SS) on T2, FLAIR sequence. For this purpose both the width of the Sylvian fissure and the interhemispheric fissure (measured at foramen of Monro level) were measured. If the size of the spaces was < 3 mm dilatation and none of the sulci were involved, it was considered to be absent scored as 0; if the width of the space was ≥ 3 mm dilatation in the Sylvian fissure or interhemispheric fissure, but without widening of other fissures and sulci, it was considered mild and scored as 1. Cortical atrophy was defined as significant and scored as 2 as widening in Sylvian fissure and interhemispheric fissure by ≥ 3 mm and of all fissures and sulci involved (or with definite loss of cortex and white matter).
6) Mild compression of the spinal cord at the level of cranio-cervical junction. Absent or not significant compression of the spinal cord was 0, mild compression of the spinal cord (taping) was scored as 1.
7) Thinner corpus callosum on T2, FLAIR sequence 0 = normal, 1 = thinner.

Statistics

The performances on the psychological tests were compared to 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. A deviation of more than two standard deviations (>30 IQ points) between total intelligence test scores within one patient, was considered a difference. We compared baseline clinical features to baseline intelligence test scores, to rule out the influence of ERT on clinical parameters such as GAG values. All analyses were performed with SPSS for Windows (version 20, SPSS Inc., Chicago, IL).

Results

Participants characteristics

Eleven children with MPS VI were included in this study (7 males and 4 females); among them were two sibling couples (patients 1 and 10, and patients 7 and 8). Patients were from Dutch (4), Turkish (4), Moroccan (1), Pakistani (1) and Guinean (1) ancestry. Seven

Table 1. Patient characteristics

Patient	Gender	Age first symptoms (years)	Age at diagnosis (years)	Age at start treatment (years)	Allel 1	Allel 2
1 *	M	5.0	0.7	7.6	454 C>T (p.R152W), exon 2	454 C>T (p.R152W), exon 2
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
3	M	.6	1.9	2.3	971 G>T (p.G324V), exon 5	971 G>T (p.G324V), exon 5
4	M	1.2	2.8	3.0	995 T>G (p.V323G), exon5	995 T>G (p.V323G), exon5
5	F	.4	3.4	6.7	c.1142 + 2T>C, exon 5	c.1142 + 2T>C, exon 5
6	M	.8	5.1	5.9	629 A>G (p.Y210C), exon 3	979C>T (p.R327X)
7*	M	3.0	5.8	6.2	629 A>G (p.Y210C), exon 3	979C>T (p.R327X)
8	F	3.0	7.4	7.8	629A>G (p.Y210C), exon 3	979C>T (p.R327X)
9	F	2.0	7.8	8.3	454C>T (p.R152W), exon 2	454C>T (p.R152W), exon 2
10	M	7.0	10.1	18.3	454 C>T (p.R152W), exon2	454 C>T (p.R152W), exon2
11	M	9.0	10.2	10.7	629 A>G (p.Y210C)	937 (C>G) (p.P313A)

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 * diagnosed after the diagnosis of sibling (patient 1 sib patient 10; Patient 7 sib patient 8). ** No reliable first hearing assessment. ^ mild hearing problems for which the patient used a wireless listening system in school. Hearing problems disappeared after a while. *** Developed hearing loss during enzyme-replacement therapy

patients had consanguineous parents. Main patient characteristics are summarized in Table 1.

The median age at which first symptoms presented was 2.0 years (range 0.4 - 9.0). Median age at diagnosis was 5.1 years (range 0.7-10.2) and median age at start of therapy was 6.8 years (range 2.0 – 18.3). Median follow-up with enzyme therapy was 2.9 years (range 0 – 4.8).

In total, 7 out of the 11 patients needed glasses during the course of therapy: all patients were hyperopic. In eight patients reliable hearing tests were performed at baseline; 3 patients could not be assessed due to young age and behavior problems. Four patients developed mild to moderate conductive hearing loss (range 7-43 dB right ear; 2-53 bD left ear) [8]. Conductive hearing loss disappeared in one of them. Hearing aids were needed in four patients. Hearing and vision problems were sufficiently compensated at time of IQ testing. In none of the patients decreased vision or hearing difficulties interfered with reliable testing.

Rapid/slow progressive	GAG-level baseline	Hearing aids	Glasses	Native speaker (y/n)	Education mother	Education father
Slow	231	N	Y	N	1	1
Rapid	942	**/Y	Y	N	4	1
Rapid	739	**	Y	N	1	2
Rapid	554	**/Y^	Y	N	4	4
Rapid	1287	Y	Y	N	1	2
Slow	207	Y***	N	Y	4	4
Slow	158	N	Y	Y	6	5
Slow	214	N	N	Y	6	5
Slow	254	N	Y	N	3	2
Slow	106	N	N	N	1	1
Slow	193	N	N	Y	6	5

Other comorbidities

During follow-up, 55% (6/11) of the patients received a carpal tunnel release (one or both hands). One patient (patient 6) received a ventriculoperitoneal shunt for increased intracranial pressure at the age of five.

Cognitive development at baseline and during follow-up.

Forty-two intelligence tests were performed in 11 patients between 2007 and 2012 (age range 2 to 20 years). Figure 1a shows the total IQ scores of the patients expressed against duration of enzyme therapy. Six patients scored above the critical threshold of normal intelligence (> 84); they did so at all time points (54%). At baseline, scores ranged from mildly delayed to above average development; the median total intelligence test score of the group was normal (median = 87, range 73-129, n = 11). During follow-up cognitive development remained stable in time (one year therapy median = 88, range 59-123, n =10; two years therapy median = 89, range 52-123, n = 10; three years therapy median = 94, range 60-131, n = 7). The median test scores at four and five years after start of ERT were not calculated, since only 3 and 1 patients respectively were assessed at this time point.

Figure 1b shows the same test scores, but now expressed against chronological age. It is shown that the scores remain more or less stable for all age categories over time. Only one patient, age 1.9 at baseline, showed a clear deterioration of more than 30 points (2 standard deviations) over a time interval of 1.3 years. This patient also had a significant increase in skull size until age 1.8 year (from 0 SD toward +2,5 SD), prominent ventricles and his behaviour was diagnosed as Autistic Disorder by a child psychiatrist.

Disharmonic profiles were found in all patients at some point during the course of therapy and in 25 of the 42 tests performed; only one patient had disharmonic profiles in all test assessments. There was no consistency in disharmonic profiles.

Education

Ten chilren were at school age at the time of assessments; one patient, a young adult, finished secondary education without special assistance and had a part-time job (patient 10). Two of the ten school aged children were attending regular schools without needing extra assistance (patient 2 and 8). They fulfilled the curricular requirements of their peer-group, in accordance with their normal intelligence test scores. The other eight patients needed special education services (80%). Three of the eight children followed regular classes as aged matched peers, but required extra support (remedial teaching) by a specialized Dutch institution to keep up with their peers. Two of them were diagnosed with dyslexia (patient 6 and 7), the other had mildly delayed IQ scores (patient 1). The five other patients attended special schools for sick children (n = 3, patients 4, 5 and 9,

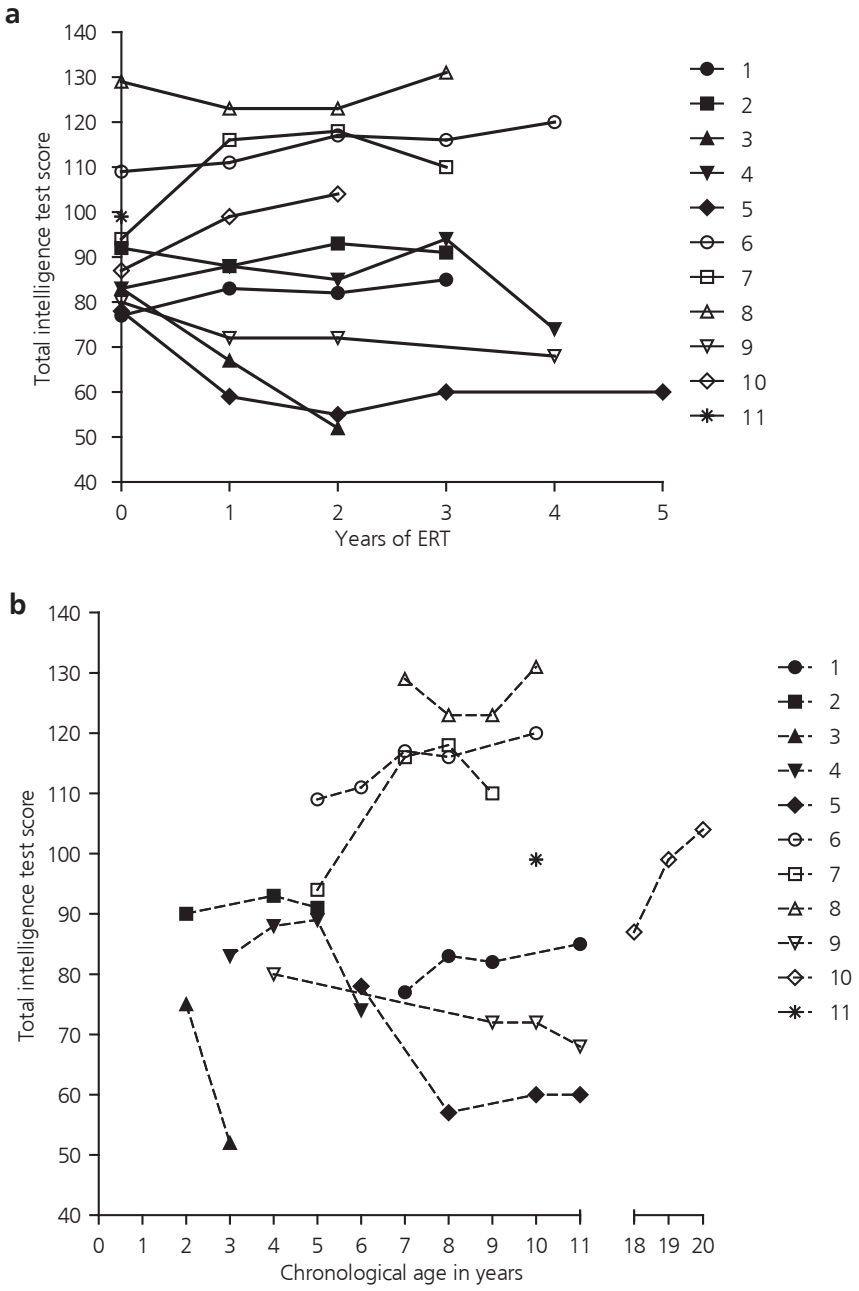

Figure 1. Long-term cognitive development in 11 patients with Maroteaux-Lamy (1a) expressed against duration of enzyme replacement therapy (1b) expressed against chronological age. The first assessment of patient 5 was based on one sub scale score (the Performance scale); the other sub scales were unreliable due to shyness and withdrawn behaviour. Patient 7 his first assessment was unreliable; he was highly distractible. Also, patient 10 had higher test scores at last assessment when compared to baseline due to differences in type of test.

patient 4 was diagnosed with Attention Deficit Hyperactivity Disorder) or for children with motor disabilities (n = 2, patients 3 and 11).

Genetic background and environmental factors

Seven healthy siblings of seven patients agreed to participate in the study. Two patients had no siblings, and the siblings of two other patients declined to participate, but the latter patients agreed upon inquiry of their school data.

The overall test scores of the siblings compared well with the total test score of the patients (Mann Whitney *U* Test: patients median total intelligence 104 range 68 – 131; verbal 96 range 74 – 125, performal 104 range 66 – 128, n = 7; siblings median total intelligence 88 range 77-139; verbal 94 range 75 – 135, performal 84, range 81 – 150, n = 7)(Figure 2).

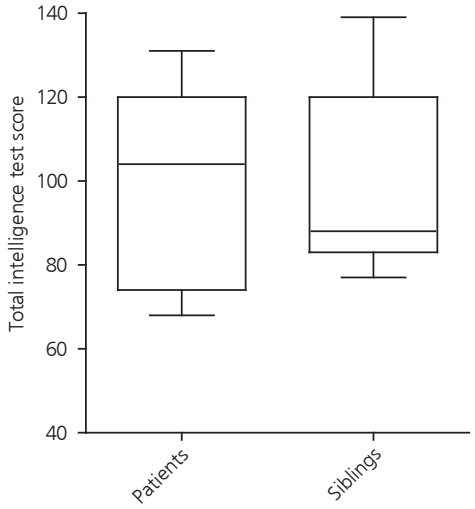

Figure 2. Total intelligence test scores of the patients compared with their siblings (most recent test scores of the patients, which were closest to the assessment of the siblings).

Five healthy siblings had harmonic profiles. The sixth sibling had a higher score on the verbal domain than on the performal domain. The seventh sibling was a toddler having higher scores on the performance domain and a lower score on the gross motor domain.

Unfortunately, the siblings of the two patients with the lowest IQ's (patient 3 and 5) refused testing. Inquiry on their school results revealed that these healthy siblings attended regular schools without needing special assistance. They had never failed promotion to the next grade so far. Based on these school results, it was concluded that their intelligence was likely to be within the normal range (TIQ ≥ 85), while the scores of their affected siblings were severely delayed.

Next, we obtained the highest parental education level, both of the mothers and the fathers, in order to relate this marker of the intelligence of the parents with the IQ scores of their children. Parent's education levels ranged from finishing primary school only to

obtaining a University degree. We found an association between the level of education of the parents and the height of the test scores of the MPS VI children and their siblings. Children of parents with higher educational level generally had higher intelligence test scores. Children of parents with lower education level generally had lower intelligence test scores (An example of this association is presented in Figure 3).

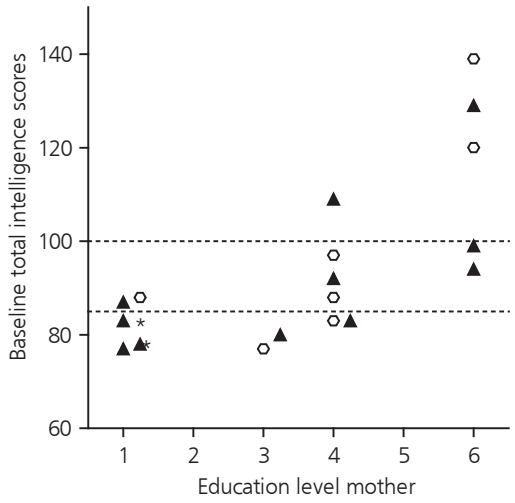

Figure 3. Total intelligence test scores of both patients and siblings related to their parental education level. * = Two patients defined as having the mild form of the disease, had intelligence test scores similar to that of their sibling. Triangle=patient; circle=sibling.

Seven out of the eleven children had non-native speaking parents and were raised bilingual (patients 1 – 5, 9 and 10). Patients of native speaking parents had on average higher test scores (n = 4, range 94-131, 4/4 patients had normal to above normal intelligence at all times) compared to children with non-native speaking parents (n = 7, range 52-104, 2/7 patients had normal test scores at all times).

Age of diagnosis, GAG levels, genetic mutations and IQ scores

Four patients were considered to have a more severe disease course than the others (patients 2-5). They presented at young age (diagnosis age range 1.8 – 3.4 year). Three of the four presented with a severe cardiomyopathy. These four were also the patients who excreted the highest of GAG levels in their urine at baseline (>300 µg/mg creatinine).

In Figure 4A the GAG levels are plotted against the total IQ of the patient. Of the patients with high GAG levels (>300 µg/mg creatinine), 75% (3/4 patients) had mild or severe developmental delay (IQ < 85), which was a higher percentage when compared to children with a low GAG excretion (29%; 2/7 patients had IQ < 85).

Patients diagnosed at early age (<5 years of age) had a higher percentage of mild or severe developmental delay (80%, 4/5 patients had IQ < 85), when compared to patients diagnosed after age 5 (17%, 1/6 patients had IQ < 85). Note that one patient was diagnosed when he was still asymptomatic (< 5 years of age) because he had a symptomatic older sibling with MPS VI.

Next, we subdivided the patients in three categories on the basis of their mutations. Group one compromised three patients from Turkish ancestry. They had the same genotype and were homozygous for the p.R152W mutation (patient 9 and sibling couple 1 and 10). The second group comprised four patients from Dutch ancestry. They all carried the common p.Y210C mutation and expressed either the p.P313A (patient 11), or p.R327X (patient 6 and sibling couple 7 and 8) on the other allele. The third groups comprised the patients with other mutations and the most severe disease course. Children with the p.Y210C mutation had higher total intelligence test scores than patients in the other two groups (Figure 4B).

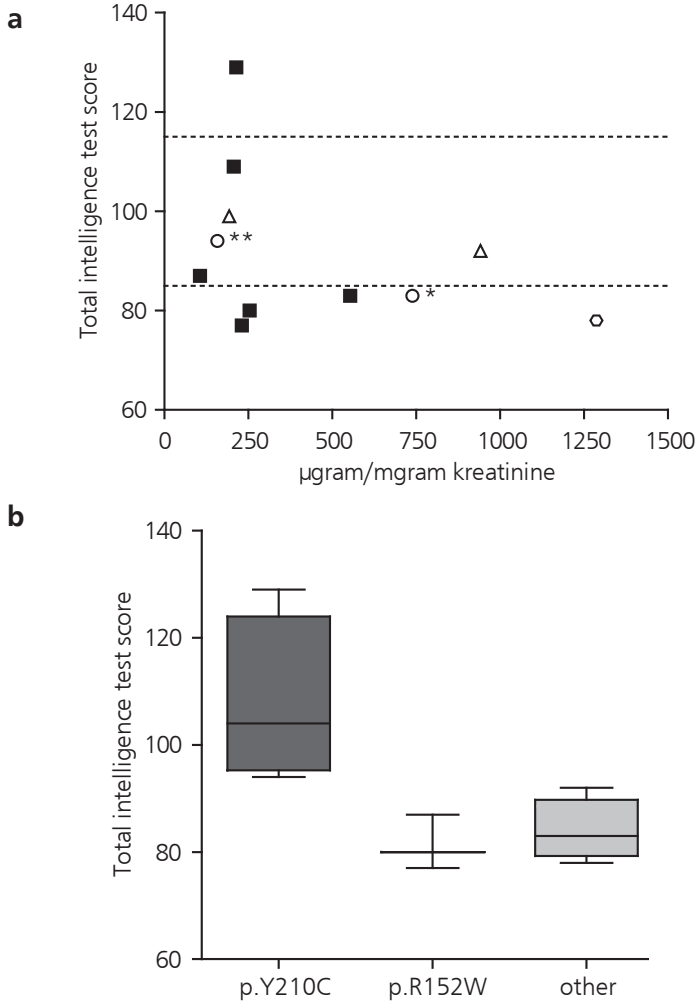

Figure 4. (4a) Total intelligence test score of the patients at baseline versus GAG level, related to the IQ scores of the siblings. * = this child had a neurologic complication. ** = the sibling of this child had an extraordinary high test score (IQ = 139). Square=similar/higher than siblling. Circle=lower than siblings. Triangle=no siblings. (4b) Total intelligence test scores related to mutation analysis.

Brain abnormalities

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

Common findings at baseline were enlarged virchow robin spaces in the basal ganglia (5/11 patients 45% MRI 1; 6/11 patients 55% MRI 2) and in the white matter (3/11 patients 27% MRI 1; 5/11 patients 45% MRI 2); patchy white matter abnormalities (5/11 patients 45% MRI 1; 3/11 patients 27% MRI 2, especially in the occipital area, but also in the frontal and periventricular area); widened sinus rectus (5/11 patients 45% MRI 1; 8/11 patients 73% MRI 2), a thinner corpus callosum (5/11 patients 45% both MRI's), and mild compression of the spinal cord (5/11 patients 45% MRI 1; 6/11 patients 55% MRI 2).

Noteworthy was that the patchy and diffuse white matter abnormalities became less intense over time and sometimes even disappeared in time (Figure 5).

It was noted that brain abnormalities were more frequently found in the severely affected patient population than the relatively mildly affected patients (Virchow Robin in

Table 2: Results baseline MRI and most recent MRI.

Domain		Rapid progressive		Slow progressive	
		MRI 1	MRI 2	MRI 1	MRI 2
Enlarged VR	Basal nuclei	2/4 (50%)	2/4 (50%)	3/7 (43%)	4/7 (57%)
	White matter	3/4 (75%)	4/4 (100%)	0/7 (0%)	1/7 (14%)
	CC	1/4 (25%)	2/4 (50%)	0/7 (0%)	0/7 (0%)
	Large lesions	1/4 (25%)	1/4 (25%)	0/7 (0%)	0/7 (0%)
White matter	Patchy lesion	4/4 (100%)	2/4 (50%)*	1/7 (14%)	1/7 (14%)
	Diffuse lesion	0/4 (0%)	0/4 (0%)	1/7 (14%)	1/7 (14%)*
Venticular englargement	FOHWR	0/3 (0%)	1/4 (25%)	1/7 (14%)	1/7 (14%)
Intracranial pressure	Widened sinus rectus	3/4 (75%)	3/4 (75%)**	2/7 (29%)	5/7 (71%)***
Atrophy	All fissures and sulci involved****	0/4 (0%)	0/3 (0%)	0/7 (0%)	0/7 (0%)
Thinner Corpus callosum		3/4 (75%)	3/4 (75%)	2/7 (29%)	2/7 (29%)
Mild compression of the spinal cord		3/4 (75%)	3/4 (75%)	2/7 (29%)	3/7 (43%)
Total abnormalities		20/48 (42%)	21/48 (44%)	12/84 (14%)	18/84 (21%)

* white matter abnormalities in these patients decreased slightly as well, but were still visuable

** no increase in time

*** 2 patient remained stable, one showed a slight increase, which potentially could be explained by differences in technique

**** All patients with the rapid progressive form of the disease had a widened Sylvian or intrahemispheric fissure (≥3 mm) at at least one MRI, but none of the patients had involvement of other sulci or fissures. Therefore, they were not considered to have brain atrophy.

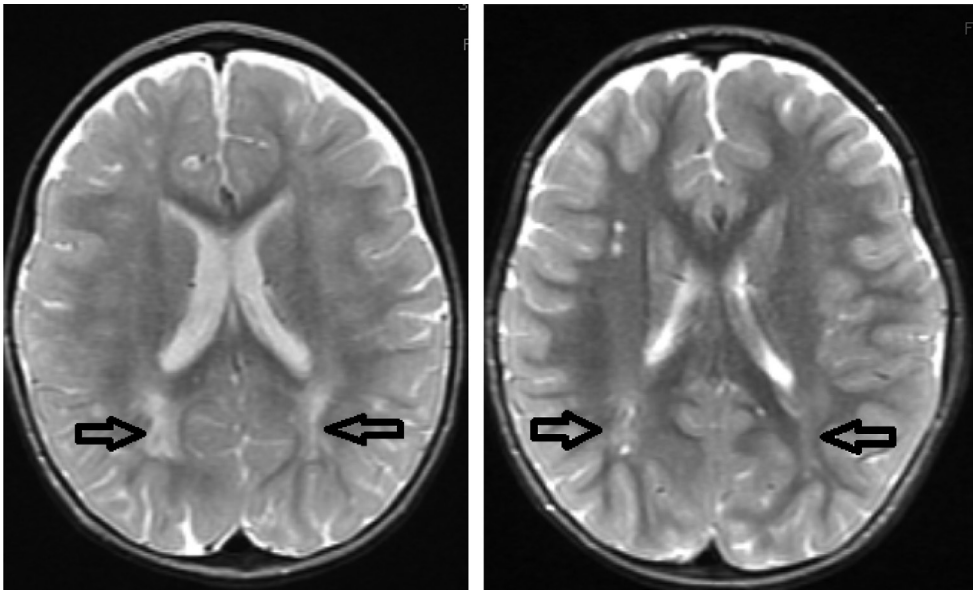

Figure 5. Longitudinal brain MRI. The patient was 2 years of age at first MRI and 4,5 years of age at the second MRI. The arrows indicate a delay in myelinisation in the occipital region. Also note the presence of enlarged VR spaces in the white matter in the second MRI (not present in the first MRI).

white matter 4/4 of the severely affected patients, 100% vs 1/7 patients 14% of the mildly affected patients (MRI 2); patchy white matter signal changes 4/4 patients 100% vs 1/7 patients 14% (MRI 1); thin corpus callosum in 3/4 patients 75% vs 2/7 patients 29%, both MRI's). In 2 patients an arachnoid cyst was found, which did not lead to clinical symptoms.

Discussion

Our study shows that the cognitive functioning of patients with MPS VI is determined by various factors. The cognitive development of the patients in our study ranged between above normal and severe developmental delay and remained rather stable over time in 90% of our patients. Patients with the most severe phenotypes/genotypes, but also the educational level of the parents and the cognitive functioning of the siblings were in most cases good predicators for the mental outcome of the patients. Patients with the p.Y210C mutation performed best. In patients with severe phenotypes/genotypes and the highest GAG levels structural abnormalities like VR spaces, patchy white matter lesions and a thin corpus callosum were more frequently seen on MRI than in the other patients.

In our study we investigated the cognitive development and MRI findings in the Dutch MPS VI population and we tried to identify factors influencing cognition in MPS VI. The

first question we tried to answer concerned the long-term cognitive development in the Dutch MPS VI patient population. We found that just above 50% (6/11) of the patients had normal to above normal intelligence test scores at baseline and during follow-up. We found all patients to remain more or less stable in their intelligence test scores over time, except for one patient who declined in test scores. We conclude that long-term cognitive development in children with MPS VI substantially differs from other types of mucopolysaccharidosis, such as MPS I-Hurler, Hunter's Disease and Sanfilippo syndrome in which profound and progressive mental retardation occur at early age [3]. Similar to our test results, other cross-sectional studies on cognition in patients with MPS VI indicate a large range in test scores from above normal development to severe mental delay [4, 13, 15]. Since this was the first long-term follow-up cohort study we could not compare our data with other reports.

Secondly, in addition to previous reports, we tried to identify factors influencing cognitive outcome. We gathered information on 1. native language, 2. the parental highest education level and 3. the IQ scores of siblings, since cognition is affected by these genetic background and environmental factors [24, 31, 32]. We found cognitive outcome of patients with MPS VI to be influenced by these factors, since 1. native Dutch speaking patients tend to have higher test scores than non-native Dutch speaking patients, 2. patients and siblings with parents having lower education levels tend to have lower intelligence test scores when compared to parents with higher education levels. Note however, that parental educational levels might be somewhat biased, because many parents were educated abroad and it is difficult to compare educational levels between different countries and cultures, 3. intelligence test scores of the patients compared well with that of their siblings. There were two sticking exceptions; these patients had severe developmental delay, and their siblings were considered to be of normal intellect. Note that one of these two patients deteriorated in intelligence test scores after having additional neurologic and behaviour problems (patient 3). He will be discussed in more detail below. We advise in further research on cognition in children with MPS VI to include genetic and environmental factors as a factor influencing cognition.

Third, we tried to identify medical factors influencing cognitive development. We found both normal test scores and mental delays in patients with low GAG levels as well as in patients with high GAG values. On the other hand, we also found that children with high GAG levels more often had mental delays (IQ < 85)(3/4) when compared to patients with low GAG levels (2/7). Note that in this last group the two patients with low GAG values and mild developmental delays had a sibling with similar intelligence test scores and parents with low education levels; their intelligence test score could also be explained by genetic make-up and environmental background. Of the three patients with high GAG values and mental delays (IQ <85), one patient could also be explained by environmental background and genetic make-up since he had similar test scores when compared to his sibling. The two remaining patients with high GAG values, early diagnoses, and severe developmental delay were considered to have lower intelligence test scores than their siblings; one of

these two had additional behaviour and neurologic problems (patient 3). In general, these findings indicate that although genetic background and environmental factors influences the cognition of children with MPS VI, we cannot rule out that in children with high GAG levels it is an additional factor determining cognition. This explanation is in accordance with several reports indicating a relation between GAG levels and severity of the disease [6-8]. On the other hand, Azevedo et al. (2012) suggested that the results of IQ tests are not related to disease severity, and Vedolin et al. (2007) did not find a correlation between GAG levels and brain abnormalities [13, 33]. More research is needed to explore the possible relation between GAG levels, brain pathological abnormalities and the consequence it might have on cognitive functioning.

Another medical factor we investigated was mutation analysis. We found that all children with the p.Y210C mutation (heterozygote) had normal to above average intelligence test scores and children with the p.R152W mutation (homozygote) had intelligence scores between normal and mild developmental delay. Also, the two patients with severe developmental delay had genetic mutations which were considered to be severe in literature (homozygous for mutations p.G324V and c.1142 + 2T> C) [34, 35].

The fourth question we tried to answer concerned structural brain abnormalities as observed by MRI and possible changes in MRI over time in relation to IQ. Interestingly, several brain abnormalities were more common in patients with high GAG values when compared to patients with low GAG values. Some abnormalities were only found in patients with high GAG values, such as large VR lesions (> 8mm) and patchy white matter abnormalities.

In almost all patients we found enlarged VR spaces. We found both normal and abnormal intelligence in patients with enlarged VR spaces. VR spaces are a common finding in the normal population, but enlarged VR spaces in a typical appearance can be used to differentiate between various diseases such as lacunar infarctions, cystic periventricular leukomalacia, cryptococcosis, and also MPS [36]. Some reports find an association between enlarged VR spaces and cognitive impairment [37, 38], which might suggest enlarged VR spaces also affects cognition in patients with MPS VI. On the other hand, Thorne et al. (2001) describe several patients with MPS VI with normal intellectual abilities (no test assessments) and dilated Virchow-Robin spaces (and ventriculomegaly) [20].

Interestingly, we found that the white matter abnormalities decreases in time in 5/6 patients; this may suggest that the periventricular white matter abnormalities, seen on the baseline MRI, present hypomyelination which normalizes in time. In literature, 2 cases of patients with MPS VI were found who also showed a decrease in white matter abnormalities; one patient after bone marrow transplantation [29] and one patient assessed before the age of 2 (which could show a normal myelination process. So far, no long-term follow-up cohort study of brain MRI in patients with MPS VI is performed, focussing on the myelination process in time. Therefore, to our knowledge this has not been systematically investigated before. On the other hand, cross-sectional studies show that white matter abnormalities

are a common finding in MPS VI [13, 19, 20, 33, 39], possibly white matter abnormalities might again occur at a later stage of the disease progression and may present demyelination or leucomalacia. In accordance to this hypothesis, Azevedo et al. (2012) found a correlation between normalized white matter lesion load and age of the patient, and they suggested that white matter lesions are progressive in MPS VI disease. Also Vedolin et al. (2007) found that white matter lesions become more extensive as disease duration increases in patients with several types of MPS (a cross sectional study). In order to differentiate between irreversible white matter damage and hypomyelination, we advise to perform a follow-up MR at a regular base.

Literature systematically combining brain MRI and cognitive assessments in patients with MPS VI is limited. We found one recent published report in children with MPS VI by Azevedo et al. (2012); they did not find a correlation between IQ scores and brain MRI findings in patients with MPS VI (not on quantitative nor qualitative measurements) [13].

Two patients will be discussed in more detail because of their neurologic complications. The first patient (patient 4) complained of persistent headaches at age 5. He did not have papilledema, his ventricles were only slightly prominent and he had an increased flow void on MR at the sinus rectus level. The patient started complaining about nausea in the morning. After evaluating his cranial cervical junction, three lumbar punctions were performed. He had an elevated intracranial pressure for which he received a ventriculoperitoneal shunt. His symptoms disappeared. Hydrocephalus with enlargement of the ventricles and papilledema have been described before in patients with MPS VI [13, 19, 40-46]; our case illustrates that caution should be taken of elevated intracranial pressure when patients with MPS VI complain of persistent headaches, even when they have relatively normal ventricle size and no papilledema.

Also, another patient (patient 3) was referred to and diagnosed at our centre at age 2. Retrospective he had an increase of his skull between 2 and 3 months of age and at age 1.7 years from 0 SD to +2,5 SD. He also had a frontal bossing and a dolichocephalic skullshape. X-ray of his skull did not indicate craniosynostosis. Although he had prominent ventricles, he did not have other clinical signs of hydrocephalus (no papilledema, no headaches and no nausea). Therefore no lumbar punction was performed. He was first tested on his cognition at age two and he presented a cognitive decline of more than 2 standard deviations in 1 year and 4 months. His behaviour was diagnosed as Autistic Disorder by a child psychiatrist.

Above mentioned results combined, we found that children with MPS VI usually have relative stable intelligence. Therefore, children with MPS VI generally differ in their cognitive development from patients with MPS IH, MPS II, and MPS III in which progressive mental retardation occur at early age. We found the highest intelligence test scores in patients of Dutch ancestry with the p.Y210C mutation, having parents with normal to high education levels. In patients with mild developmental delay it was usually associated with genetic make-up and environmental background. There were two exceptions of patients with severe developmental delay, high GAG values, early diagnoses which were not likely to

be explained by environmental factors or genetic make-up. One of these two could be explained by additional neurologic problems, and one could not be explained. GAG is likely to store in the brain, but it is not likely to have a major effect on intelligence. Perhaps more subtle cognitive deficits occur, which cannot be detected by standard intelligence tests.

Children with MPS VI were in high need for special education services (73% compared to 5% in the normal population), because of their medical problems, lower intelligence, dyslexia, and school absence related to treatment. This high percentage also suggest that although children may have normal intelligence, children could be at risk for more subtle deficits. We therefore recommend that all children have regular in depth neuropsychological examination to detect subtle learning deficits early and to give adequate interventions. In conclusion, cognitive outcome in patients with MPS VI is usually normal. In children with mild developmental delay it seemed influenced by environmental factors. Although we found brain abnormalities on MRI (probably caused by GAG storage) and we cannot rule out that this may lead to subtle cognitive deficits, we did not find a major impact on intelligence. Since this disease is rare and a limited amount of patients was included in this study, we recommend to repeat this investigation international to study possible predictors for cognitive development in a larger cohort.

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Chapter 7

Pain: a prevalent feature in patients with mucopolysaccharidosis
Results of a cross-sectional national survey

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To be submitted

Summary

Background: While clinical observations suggest that many patients with mucopolysaccharidosis (MPS) experience chronic pain, few studies have assessed its extent and impact. We therefore investigated its prevalence in patients with all types of MPS in the Netherlands. We also examined the association between pain and Health Related Quality of Life (HRQoL) and other clinical variables. **Methods:** We conducted a nationwide MPS survey that used questionnaires on MPS and disease-related symptoms (MPS-specific questionnaire), Developmental Level (Vineland Screener 0-6 years), Quality of Life (PedsQl and SF-36), and Disability (Childhood Health Assessment Questionnaire). Depending on their age and developmental level, patients or their parents were asked to assess pain by keeping a pain diary for 5 consecutive days: either the Non-communicating Children's Pain Checklist – Revised (3-18 years intellectually disabled and children < 8 years), the VAS-score (> 18 years), or the Faces Pain Scale – Revised (8-18 years). **Results:** Eighty-nine MPS patients were invited, 55 of whom agreed to participate (response rate 62%; median age 10.9 years, range 2.9-47.2 years). They covered a wide spectrum in all age groups, ranging from no pain to severe pain. Forty percent scored above the cut-off value for pain. Most reported pain sites were the back and hips. While the MPS III group experienced the highest frequency of pain (52.9%), 50% of patients with an intellectual disability seemed to experience pain, versus 30% of patients with a normal intelligence. MPS patients scored much lower (i.e. more pain) than a random sample of the Dutch population on the bodily pain domain of the SF-36 scale and the PedsQl. **Conclusion:** With or without intellectual disabilities, many MPS patients experience pain. We recommend that standardized pain assessments are included in the regular follow-up program of patients with MPS.

Introduction

Mucopolysaccharidoses (MPS) comprise a group of rare lysosomal storage disorders caused by deficiencies of the lysosomal enzymes involved in the degradation of glycosaminoglycans (GAGs). There are several types of MPS, which are numbered historically from MPS I to MPS IX (see Table 1). The storage of GAGs leads to a variety of symptoms, such as bone deformities, carpal tunnel syndrome, glaucoma, hepatosplenomegaly, and, in some types of MPS, intellectual disability (ID). Although the clinical features of these mucopolysaccharidoses are largely comparable, there are differences in the level of involvement of the different organ systems [1]. Enzyme-replacement therapy is available as a therapeutic option for MPS I, II, and VI. Hematopoietic stem cell therapy (HSCT) is the first therapeutic choice for MPS I Hurler aged under 2.5 years [2].

Clinical observations strongly suggest that patients with MPS have chronic pain, due possibly to the painful disorders that evolve from GAG storage, such as carpal tunnel syndrome and glaucoma and, probably most prominently, from the consequences of the musculoskeletal disease which complicates all types of MPS [3].

It is widely accepted that, overall, pain in adults and children has a negative impact on Health Related Quality of Life (HRQoL) [4, 5]. For conditions resembling MPS, such as rheumatoid arthritis, studies have indeed shown an association between pain and lower HRQoL [6, 7].

Table 1: Different types of mucopolysaccharidoses

	Eponym	Enzyme deficiency	Current therapy options	Mental impairment
MPS I	Hurler/ Hurler-Scheie/ Scheie	alpha-L-iduronidase	- ERT - HSCT/BMT; for Hurler phenotype <2.5 years)	MPS I-H MPS I H/S
MPS II	Hunter	iduronate-2-sulfatase	ERT	Severe phenotypes
MPS III	Sanfilippo type: A-B-C-D	A: heparin N-sulfatase B: a-N-acetylglucosaminidase C: acetyl coenzyme A: a-glucosaminide acetyltransferase D: N-acetylglucosamine-6-sulfatase	No registered therapy	Most
MPS IV	Morquio	A: N-acetylgalactosamine-6-sulfatase , B: β-galactosidase	No registered therapy	Rare
MPS VI	Maroteaux-Lamy	N-acetylgalactosamine 4-sulfatase	ERT	Rare
MPS VII	Sly	β-glucuronidase	No registered therapy	Most
MPS IX		hyaluronidase	No registered therapy	No

Although pain has been documented in detail for other lysosomal storage disorders – such as Fabry and Gaucher disease, in which the pathophysiologic origin of pain is more evident [8, 9] – we know of few studies of pain in MPS. To date, these have used pain mainly to study the effects of enzyme-replacement therapy in specific types of MPS [10, 11]; none included intellectually disabled patients or patients who underwent HSCT.

To improve our understanding of pain in mucopolysaccharidosis, we investigated its prevalence in patients with MPS in the Netherlands. We also assessed the association between pain and HRQoL and other clinical variables. Pain in intellectually disabled patients was measured through the parents or caregivers. This study is part of a large national MPS Survey conducted in collaboration with the Dutch patient's organizations for MPS.

Methods

Study design

The Mucopolysaccharidosis Survey, a cross-sectional observational study of patients with all types of mucopolysaccharidosis, was conducted by the Center for Lysosomal and Metabolic diseases at Erasmus MC University Hospital, the Netherlands. It was performed in collaboration with the Dutch patient organizations for adults and children with metabolic diseases and for patients with MPS IV (Morquio syndrome). After approval of the study by the Medical Ethics Review Committee (METC) at Erasmus MC, members of the patient organizations and patients who had been treated at the Center for Lysosomal and Metabolic diseases were asked by mail to participate. They were sent an informed consent form in which they or a parent could enter the age and state whether or not the patient had an intellectual disability. If parents or patients choose not to participate, they were asked to enter their reason for declining. All participants were asked to fill out questionnaires that had been validated and were appropriate for the patient's age and mental development.

Pain

Parents or patients were asked to complete a pain diary for 5 consecutive days at the same time of day. For children aged under 8 years and those with an intellectual disability, the Non-communicating Children's Pain Checklist – Revised (NCCPC-R) was used. The NCCPC-R is designed for children aged 3 to 18 years who are unable to speak because of intellectual impairments or disabilities [12]. The revised version of the NCCPC [13] consists of a list of 30 items divided into 7 categories (subdomains): vocal, eating and sleeping, social, facial, activity, body and limb, physiological signs. On a scale of 0–3 according to the frequency of its occurrence in a specific time period, the observer provides a score for

each item: 0 = not at all, 1 = just a little, 2 = fairly often, 3 = very often. This questionnaire has proven its reliability and validity [13, 14].

Patients aged 8-18 years with no cognitive impairment filled in the six-face Faces Pain Scale – Revised (FPS-R), a well-validated and consistently described instrument for pain assessment that uses 6 faces to indicate pain: face 1=no pain (score 0), face 6= very much pain (score 100) [15]. Patients were asked to point to the face that best epitomized the pain they had experienced in the past two hours. Patients over 18 years with no cognitive impairment were asked to register their pain on a VAS-score [12], i.e., a 100-mm scale consisting of a single horizontal black line whose extremes were labeled `no pain' and `very much pain'. Each patient was asked to mark the line at the position that best corresponded with the amount of hurt or pain he or she had experienced over the past two hours. The VAS was scored 0±100, 1 unit being equivalent to 1 mm.

A mean pain score was derived from the total crude scores of the pain diaries over the 5 consecutive days. The validated threshold crude score of 7 was used as the cut-off score for the presence of pain in the NCCPC-R [13]. The NCCPC-R does not provide a pain severity scale; it only defines the presence (score≥ 7) or absence (score <7) of pain. In the cases of the FPS-R and the VAS, we assessed the presence of pain on the basis of a cut-off score of 45 (moderate pain) according to Jensen et al. [16]. We chose moderate pain as the cut-off point, as moderate pain or more than moderate pain has been reported to substantially influence daily life [17, 18].

Other variables

At our center we developed an MPS questionnaire consisting of general questions about diagnosis, first symptoms, current symptoms, family history, breathing, sleeping, behavior, school type or job, and treatment. This also contained a question on the 'occurrence of joint pain', in which patients could enter the amount of pain and where it was located. Before the questionnaire was distributed to the total group, it was tested in a group of five patients with different types of MPS.

Mental development was assessed with the Vineland Screener 0-6 years in patients under 6 years and in those with intellectual disabilities. The Vineland screener encompasses 72 items divided into 4 sub-domains relating to features important to adaptive behavior: communication skills, social skills, daily skills and motor skills [19]. The end score is considered to be rated as the adaptive behavioral age.

To assess quality of life, the PedsQl was used in patients <18 years. The 23-item PedsQL Generic Core domains were designed to measure the core dimensions of health as delineated by the World Health Organization, as well as role functioning. The 4 Multidimensional domains are: physical functioning, emotional functioning, social functioning, and school functioning. Scores were compared with data available from healthy peers with a range of 0-100 [20]. Patients older than 5 years who were not mentally disabled filled in the PedsQl child self-report. For adult patients, we used the SF-36v2, a generic health-related quality-

of-life questionnaire consisting of 36 items assigned to nine domains: physical functioning, physical role functioning, emotional role functioning, social functioning, bodily pain, mental health, vitality, general health perception and change in health [21, 22]. Items are summed per scale and transformed into scores between 0-100, with higher values representing better function. Scores were calculated using Dutch reference values by Aaronson et al [23]. A physical component score (PCS) and a mental component score (MCS) were calculated from the different domains and expressed as a norm based score (mean=50, SD=10).

Physical Disability was assessed with the Health Assessment Questionnaire (HAQ) and the Childhood Health Assessment Questionnaire (CHAQ). The HAQ or CHAQ is widely used to assess the disabilities of patients with arthritis [24, 25]. The HAQ disability index (HAQ DI) is divided into eight different domains (dressing, arising, eating, walking, hygiene, reach, grip and activities), and is scored on a scale from 0-3 (0= best score). It is calculated as the unweighted average of the eight scale scores, and yields a disability score between 0 (no disability) and 3 (most severe disability). The CHAQ was used in patients younger than 18 years and the HAQ was used in adult patients.

Statistics

All variables were summarized using descriptive statistics that included median, ranges, percentages and/or frequencies.

Spearman's rank correlation coefficients were used to evaluate the correlations between crude pain scores and the following: age at study entry, disease duration from time of diagnosis, disease duration from time of first symptoms, the adaptive behavioral age produced by the Vineland Screener, the CHAQ disability index of the CHAQ, and the quality of life scores. The Mann-Whitney U test or the Kruskall-Wallis test were used to compare mean crude scores at start of ERT between groups categorized on the basis of gender, intellectual disability, wheelchair use and age.

HRQoL measurement in the SF-36v2 and the PedsQL were transposed to a 0-100 score, and Z-scores were calculated according to the data of Aaronson et al and Varni et al [20, 23]. If child-reported data were available, we analyzed these rather than parent-reported data.

Data analyses were performed using SPSS for Windows (version 17, SPSS Inc., Chicago, IL). A p-value of ≤ 0.05 was considered statistically significant.

Results

Study population

Eighty-nine patients were asked to participate (Table 2), and 55 patients gave written informed consent and returned the questionnaire (response rate 62%). All reported having a confirmed diagnosis of mucopolysaccharidosis. Figure 1 shows the flow chart of the inclusion process. The questionnaire was completed by the caregivers for 35 patients (64%), either because

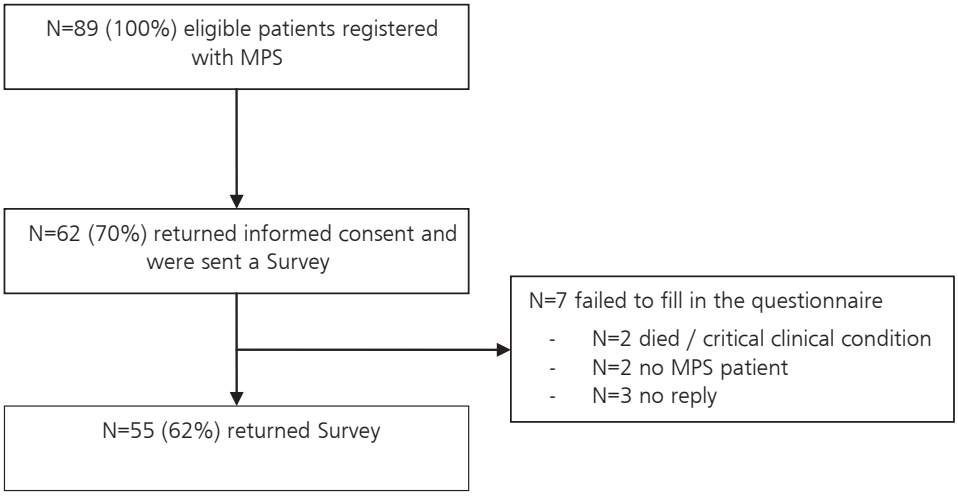
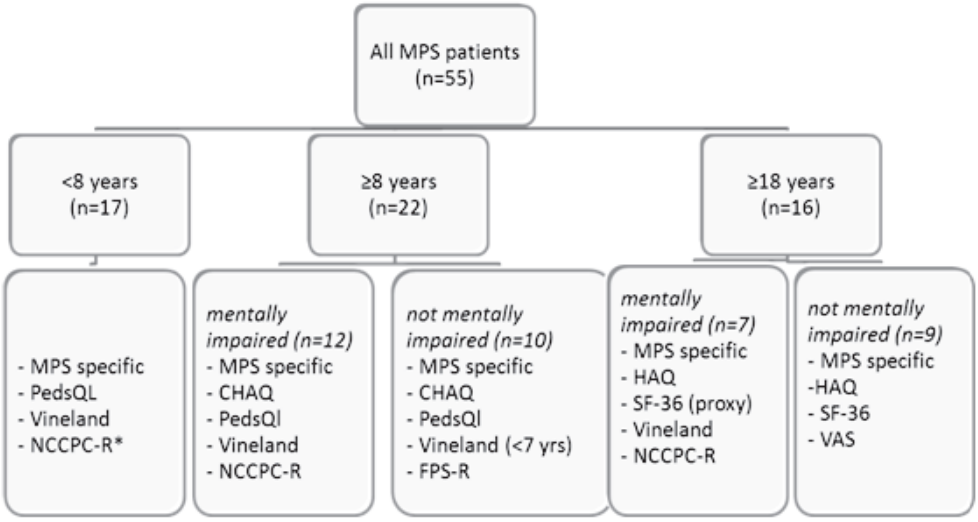


Figure 1: Flow chart of the inclusion process



a patient was mentally impaired and/or because a child was under 8 years of age. Two of the 55 patients were excluded from the analysis regarding pain scores: one MPS IV patient who had not completed the pain diary and one MPS IV patient aged 6.5 years who filled out the FPS-R instead of the NCCPC-R. Figure 2 shows the distribution of questionnaires according to age and developmental level.

General characteristics

General characteristics were available for all 55 patients (35 males, 64%, Table 2). The median age at the time of data collection was 11.3 years (range 2.9-47.2 years). A majority of patients had either MPS I (21.8%) or MPS III (30.9%). The median disease duration from age of diagnosis was 7.2 yrs (range 0-42.2 yrs). At time of the study, thirty-two patients were being treated with either ERT (n=25: 6 MPS I patients, 8 MPS II patients, and 11 MPS VI patients) or had undergone haematopoietic stem-cell transplantation (n=7). One MPS III patient participated in the MPS III trial for intrathecal administration of ERT. Four patients reported using pain medication (one MPS I, one MPS II, one MPS IV and one MPS VI patient).

Table 2: General characteristics of patients participating in the MPS Survey.

Characteristics	**Resp (%)**	**Non resp (%)**
- Total	55 (100)	27 (100)
- MPS I	12 (22)	3 (11)
- MPS II	9 (16)	1 (4)
- MPS III	17 (31)	12 (44)
- MPS IV	6 (11)	6 (22)
- MPS VI	11 (20)	0 (0)
- MPS type unknown	0 (0)	5 (19)
	Responders	
Gender male (%)	35 (64)	
Age<18	39 (71)	
Mental impairment	28 (51)	
Use of wheelchair	37 (67)	
ERT treatment	25 (46)	
Haemopoietic stem cell therapy	7 (13)	
	Median (range)	
Age	11.3 (2.9-47.2)	
Age at diagnosis (year)	4 (0-15)	
Disease duration from symptom onset (years)	10.2 (1.0-42.2)	
Disease duration from diagnosis (years)	7.2 (0-42.2)	

Pain prevalence

A. Joint pain

As part of the MPS-specific questionnaire, 69% of the participants reported that they 'currently' experienced joint pain. Of all patients, 9.1% reported that joint pain was almost 'always present', 14.5% 'often' experienced it, and 41.8% 'sometimes' experienced it.

The frequency with which joint pain was experienced was unknown in 3.6%. The most commonly reported joint pains were back pain (25.9%) and hip pain (27.8%). In 9 cases, caregivers reported the presence of joint pain, but could not locate the pain due to their child's intellectual disability.

B. Pain reported through diaries

A pain diary was kept by 54 participants (either patients or caregivers), with 35 completing the NCCPC-R, 11 the Faces-Pain Scale revised, and 8 the VAS-score. Figure 3 shows the median scores and ranges obtained on the different scales. Forty percent of the participants reported a mean pain score above the cut-off value for pain, indicating that pain was present.

The NCCPC-R was filled out by the caregivers, with 16 of the 35 caregivers (45.7%) reporting a score above the cut-off value for pain. Total NCCPC-R Scores ranged from 0-36 (maximum score 90; cut off value for pain 7). The highest scores were obtained on the 'social' subscale of the NCCPC-R.

The FSP-R and VAS were completed by the patients. On the FSP-R, two out of eleven patients (18.1%) reported a pain score above the cut-off value; one had a pain score of 56, the other of 92. Scores for the total group ranged from 0-92; the cut-off value for moderate pain was 45; the maximum score was 100.

The highest pain scores were obtained on the VAS-score. Three out of 8 patients scored above the cut-off value for pain. These patients' pain scores were 48, 69 and 88. The scores for the total group ranged from 10-88; the cut-off value for moderate pain was 45; the maximum score was 100.

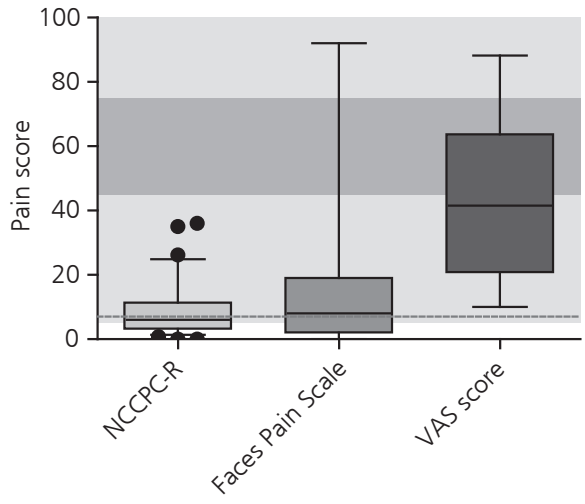


Figure 3. Median pain scores (whiskers 10-90 percentile) in different pain questionnaires. The shades of grey indicate mild pain (5-44), moderate pain (45-74) and severe pain (75-100) for the Faces pain scale and VAS score according to Jensen et al. The dotted line at score 7 represents the cut-off value for pain in the NCCPC-R.

C. Discrepancies

While five patients reported experiencing pain in their pain diary, they reported no joint pain in the MPS-specific questionnaire. In all these cases, the NCCPC-R pain questionnaire had been used. Additionally, three patients reported that they 'often' or 'always' had joint pain, while they did not score above the cut-off score for pain on their pain diaries (1 NCCPC-R and 2 FPS-R).

Relation between pain and patient/disease characteristics

Table 3 shows the characteristics of patients with and without pain, between whom no differences were observed regarding disease duration and level of disability. Half of the patients who were mentally disabled scored above the cut-off value for pain, versus 30% of patients with normal intelligence. For patients tested with the Vineland Screener, the adaptive behavioral age of the patients with pain (median developmental age 21 months; actual age median 146 months) tended to be slightly lower than that of those without pain (median developmental age 33.5; actual median age 120 months) (p=0.08).

The highest frequency of pain was observed in the MPS III group (52.9%), followed by 41.7% of the MPS I , 40% of the MPS IV, 33.3% of the MPS II, and 27.3% of the

Table 3: Characteristics of patients with and without pain.

	Pain*	N	Median (range)	p-value #
Disease duration from symptom onset (median, years)**	no	31	9.9 (1.0-39)	0.35
	yes	21	10.7 (2.5-42.2)	
Disability Index (median)	no	21	1.9 (0.3-3.0)	0.59
	yes	12	2.0 (1.0-3.0)	
Adaptive age Vineland (median, months)	no	18	33.5 (2.0-58.0)^120 (48-480)	0.08
	yes	14	21.0 (2.0-53.0)^^146 (36-564)	

*Patients who had a mean pain score above the cut-off value for their pain diary, measured over 5 consecutive days. ** One patient did not fill in the pain diary, two patients did not indicated whether they used a wheelchair or not; ^ actual age of patients with no pain; ^^ actual age of patients with pain; # As assessed with Mann-Whitney test

MPS VI patient groups. The differences were not statistically significant, nor were there any significant differences in reported pain between patients who were wheelchair bound (41.7%) or ambulant (35.5%), or between patients who had received either HSCT or ERT (38%) or neither of these therapies (46%).

The scores obtained on the NCCPC-R, the FPS-R and the VAS did not correlate with disease duration, with the adaptive age calculated from the Vineland Screener, or with the (C)HAQ disability index.

Relation between pain and quality of life

Overall, quality of life was clearly reduced in patients with MPS. Sixteen patients aged over 18 years completed the SF-36. Three cases were excluded as too many values were missing to calculate a reliable end score. The scale which deviated most from that of healthy peers was the 'physical component summary score' (PCS). As part of the PCS the ´bodily pain´ domain deviated clearly from the reference group with a median score of 41 (range 0-100) for the total group. Although the scores were lower in patients with pain compared to patients without pain, the difference was not statistically significant. This may be due to the small sample size.

Thirty-five participants completed the PedsQl. As with the SF-36, the largest deviation from healthy subjects was seen in the mean score of the PedsQl physical score, on which parents and children reported similar scores: parents reported a median score of 51.2 (23.5 SD) and children of 50.7 (23.8 SD). These scores were clearly lower than obtained in a sample of healthy children (85.6; 13.7 SD) and their parents (78.5; 22.3 SD) [20]. In 13 cases, the PedsQL was completed by parents and the children at the same time. Higher NCCPC-R scores correlated with lower scores on the PedsQl psychosocial scale (ρ=-0.47, p=0.02), indicating that patients scored lower on the psychosocial scale if they had more pain.

Table 4: Quality of life of patients with and without pain

	Pain*	N	Median	Range	p-value^	Mean score (SD) reference population%
PedsQl physical score**	no	23	57.1	9.4-93.8	0.76	parents: 78.5 (22.3)
	yes	12	53.1	9.4-87.5		children: 85.2 (13.7)
PedsQl psychosocial score**	no	23	71.1	25.0-95.0	0.17	parents: 76.1 (16.3)
	yes	11	59.6	34.6-75.0		children: 78.2 (14.9)
SF-36 physical component summary score (NBS)	no	5	29	21-42	0.94	50 (10)
	yes	7	30	21-59		
SF-36 mental health component summary score(NBS)	no	5	43	31-63	0.94	50 (10)
	yes	7	48	22-63		
SF-36 bodily pain	no	5	41	0-42	0.77	80.5 (24.4)
	yes	8	36.5	0-100		

*Patients who had a mean pain score above the cut-off value for their pain diary, measured over 5 consecutive days.** Combined scores of parent and children. If a child form was available this was used instead of the parental form. ^p-value comparing patients with and without pain using Mann-Whitney test; NBS= norm base score; mean population score=50, SD=10

Discussion

In this nationwide survey of MPS patients, we found pain to be prevalent in many children and adults with mucopolysaccharidoses, 40% of whom reported a pain score above the critical cut-off value for significant pain. To our knowledge, this is the first study to quantify pain in patients with MPS.

Most of the patients in our study population were children (71%), whose median age was 10.9 years. Although few studies have investigated pain in normal children, a large pain survey among school children in the Netherlands (n=5423, age range 0-18 years) reported a 25% prevalence of chronic pain [26]. The prevalence of pain in MPS patients thus seems higher than in the normal population.

The few previous studies quantifying pain in MPS focused mainly on the effect of ERT on pain scores at group level. A study by Clarke and co-workers showed that Pain Index scores in MPS I patients were significantly lower after long-term use of enzyme-replacement therapy [11]. Similarly, in a phase II trial of enzyme-replacement therapy in 10 MPS VI patients, Harmatz et al. found that pain had decreased after 24 weeks of treatment. Their trial, which used the VAS-score as part of the Childhood Health Assessment Questionnaire, showed that pain had been reduced by 63±41% (p=0.002) by the end of the study period [10]. As in our study, the mean VAS-score of 41 lay below the critical threshold for pain (45), and was comparable to our VAS score (median score 41.5). Apparently some patients experience much more pain than others.

The highest frequency of pain in our study was observed in the MPS III group (52.9%), followed by 41.7% of the MPS I group, 40% of the MPS IV group, 33.3% of the MPS II group and 27.3% of the MPS VI group. Some patients had extremely high scores. One, for example, had a mean score of 88 on the VAS scale. Another scored 92 on the FPS-R. Both of these scores lay only slightly below the maximum score of 100. Overall, the MPS IV patients appeared to experience the severest pain, as their scores were the highest. While this group was small, we conclude that because some patients seem to experience extreme pain, pain should definitely be addressed, partly through pain management. In this context, it is noteworthy that only 4 MPS patients used pain medication and these were not the patients with the highest scores.

Another interesting finding was the high frequency of pain among patients with intellectual disabilities. Half of this group of patients seemed to experience pain, against 30% of patients with normal intelligence. None of the very few previous studies that assessed pain in MPS included patients with an intellectual disability. Like other researchers [27, 28], we realize that it is extremely difficult to recognize and accurately assess pain in patients with intellectual disabilities. However, while several studies have examined this issue and have shown that the experience of pain of patients with intellectual disabilities does not differ from the general population, they also state that pain in this patient group is generally underestimated in daily practice [13, 29]. Although our results should be interpreted

with caution, so far they indicate that the presence of pain in intellectually disabled MPS patients deserves further attention. The frequency of pain in MPS patients was comparable to that found by Breau et al. in other patients with severe cognitive impairments [29].

If the right approach is taken to pain treatment, it may provide a key to distinguishing between pain-related behavior and MPS-related behavior in patients with intellectual disabilities. Behavioral problems often arise in mentally impaired MPS patients, potentially explaining some of the higher scores on certain items of the NCCPC-R (such as item 15, 'jumping around, agitated'; or item 5, 'irritable') [30] – behavior that may have led to overestimated pain scores. While five of our patients did indeed have high scores on the NCCPC-R, their parents did not report any pain on the MPS questionnaire. Nonetheless, reports that behavioral problems such as agitation and aggression in dementia patients were explained by pain suggest that it would be useful to develop instruments that assess pain more selectively in patients with a combination of intellectual disabilities and behavioral problems [31]. An alternative option might be found in Batten's Observational Pain Scale, which was developed only recently for mentally retarded patients with Juvenile Neuronal Ceroid Lipofucsinosis [32].

Our study shows that MPS clearly has an impact on quality of life in children and adults. The low physical component summary score of the SF-36 and the PedsQL physical score affirm this finding. Earlier studies in other chronic pain syndromes such as rheumatoid arthritis [7] and osteoarthritis showed similar results [33]. As part of the quality of life assessment with the SF-36 bodily pain was measured. With a median score of 41.0 MPS patients scored much lower on the bodily pain item of the SF-36 scale than a random sample of the Dutch population, whose mean score was 80.5 (24.4 SD).

While scores on physical functioning and bodily pain were lower for patients with pain scores above the critical pain threshold than for those without, our study population was too small to show significant differences. We remind our readers that a lower score for bodily pain means more pain.

Our study could not identify the exact origin of the pain. In the MPS-specific questionnaire, 68% of patients indicated that they had bone pain. The most-reported joint pains were back pain (25.9%) and hip pain (27.8%). The pathophysiology of pain in patients with mucopolysaccharidosis is probably multifactorial. For example, it has been shown in animal models that glycosaminoglycan storage induces inflammation by activating toll-like receptor 4 and increasing levels of cytokines such as TNF-α [34, 35]. A similar mechanism is seen in painful other conditions such as rheumatoid arthritis. Another possible cause of inflammation may lie in the weight-bearing forces on the affected bones, which might eventually lead to pain [36]. As early osteoarthritis has been described in MPS, this might add to the severity of pain in this patient group [36-38]. As well as joint problems, MPS patients have other complications that might lead to pain, two of them being carpal tunnel syndrome and glaucoma [39, 40]. However, as patients with MPS have been reported to lack

the strikingly painful symptoms of these disorders, it will not be easy to find the origin of pain, especially in patients with intellectual disability [41] .

The recruitment of patients for our study was performed in collaboration with the patient organizations, who represent the vast majority of MPS patients in the Netherlands. This may have underlain the relatively high response rate of 62%. Though most of the patients who declined were MPS III patients, a significant number of MPS III patients nonetheless took part in the study.

In conclusion, we found that pain is a prominent feature in MPS patients with and without intellectual disabilities. Although, at group level, it was generally mild to moderate, 40% of patients scored above the critical threshold for significant pain. We note that pain medication was hardly used in our population, even though some patients had extremely high pain scores. Our results also showed that pain in patients with intellectual disabilities may be much more common than currently realized – an issue that should be addressed in further studies.

Overall, we conclude that pain is underestimated in MPS and that its management and treatment deserves more attention. We recommend that standardized pain assessments are included in the regular follow-up program of patients with MPS.

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Chapter 8

Discussion

In 1991, after more than two decades of random and unsuccessful attempts to treat lysosomal storage disorders by enzyme replacement therapy (ERT) with limited amounts of enzyme from different human and non-human sources, ERT came suddenly within reach after the successful introduction of ERT in Gaucher disease with human glucocerebrosidase purified from human placenta. This first clinical success was attributable to i) the availability of large stocks of enzyme for repeated intravenous injections, ii) to the accessibility of the target cells –glycolipid storing resident macrophages in the liver and the spleen-, and iii) to the modification of the carbohydrate chains of glucocerebrosidase allowing the enzyme to bind to the mannose receptor on the main target cells of Gaucher disease (macrophages) thereby enabling efficient endocytosis. Since the first introduction of ERT in Gaucher disease similar therapeutic approaches have been developed for the treatment of several other lysosomal storage disorders (LSDs). The cloning of the genes involved in those disorders was crucial to enable large scale production of recombinant human lysosomal enzymes needed for ERT. Also the enzyme for Gaucher disease is nowadays produced via biotechnological methods. ERT is currently available for Gaucher disease, Fabry disease, MPS I, II, VI and Pompe disease and under development for a small number of other lysosomal diseases. ERT in all other LSDs than Gaucher disease is targeted to the mannose 6-phosphate receptor, which is exposed by many different cell types. Despite the undeniable success of ERT as a treatment for otherwise incurable diseases the initial great enthusiasm about the effects of ERT in a wide variety of lysosomal storage diseases has somewhat abated.

This thesis covers multiple aspects of MPS VI and the application of ERT. In Chapter 5 we identified the genotypes of the 12 patients that we care for in our clinic improving our understanding of the cause of clinical diversity, the response to ERT, and the immune response to ERT would this occur. Chapter 2 reviews the 8 years world wide experience with ERT in MPS VI, while Chapter 4 evaluates our own experience. The cardiac problems in MPS I, II and VI and the cardiac response to ERT in MPS VI are highlighted in Chapter 3.1 while the case report in Chapter 3.2 describes the pathophysiology of the mitral valve in MPS VI in a tissue specimen that became available after surgical replacement of the mitral valve. At last, Chapters 7 and 8 describe findings regarding cognition and pain, two less well studied clinical issues in MPS VI. Our major findings are discussed in the broader context of the following paragraphs.

ERT in MPS VI

The patient cohort

The introduction of ERT for MPS I, MPS II, and MPS VI in Europe, in the years 2003, 2006, and 2007, has brought relief to the MPS patient society. Patients who till then were treated only symptomatically could hope for a better life since ERT had proven to combat

some of their symptoms effectively. Besides access to treatment, the patients received from then on frequent attention and optimal care, and they entered into a program of standardized follow-up aimed at studying the effects of ERT. In the Netherlands, the Center for Lysosomal and Metabolic diseases was assigned by the government as expert center for MPS I (in conjunction with AMC Amsterdam), MPS II and MPS VI, and was allotted the task to evaluate the natural course of these diseases, the effects of ERT, and the health economic aspects of treatment with very expensive drugs. The problem with performing some of these tasks was the lack of systematically collected data on the natural course of these diseases, nationally as well internationally; once ERT has started the opportunity to collect data on the natural course ends. For instance, in MPS VI the only historical information available at the time that ERT was approved by the EMA was a cross-sectional study that was published in 2005 and had been performed by BioMarin, the pharmaceutical company that had developed galsulfase [1]. In MPS II, data collection on the natural course had started just 2 years prior to the marketing approval of idursulfase in a Registry (Hunter Outcome Survey; HOS) driven by the pharmaceutical company Shire Therapeutics who developed ERT for this disease. The lack of data collected in a standardized fashion from an untreated patient group makes it hard to document the results of ERT. One has to rely on the fact that these diseases are progressive so that any improvement of clinical parameters can be marked as a probable result of ERT.

Previous studies and chosen endpoints

The scarcity of historic data, the rarity of the disease and the lack of a systematic standardized follow-up could be the major reason that only few studies have been published since 2007. As our review of the literature shows (data were collected till November 2012; Chapter 2), there were only 7 studies (including more than three patients) published since the introduction of ERT till November 2012 [2-8]. For MPS VI only 7 reports were published since the introduction of ERT, and the data in four of these reports were derived from the same cohort [2-4, 6-9]. Another reason for the limited number of publications can be that the historically chosen parameters to evaluate the natural course of these diseases did not always match the clinical endpoints chosen to prove the effect of ERT as the most salient clinical features may be difficult to assess. Furthermore, if patients present at a young age or have are severely affected by the disease, assessments aren't always possible. In MPS II for instance, the ability to gather standardized clinical information on the patient group with mainly cognitive impairment is a big challenge, to put it mildly. Thus, for practical reasons, the clinical trials for MPS II were directed towards measuring improvement of endurance and pulmonary capacity while patients with neurologic involvement were excluded. This situation emphasizes the need of informative biomarkers that reflect disease progression irrespective of neurologic involvement so that information from a larger group of patients can be lumped. Also,

the truly measurable endpoints are sometimes questionable: In MPS VI for instance, an important clinical endpoint is the 12 minute-walk test. This test, derived from the 6 minute walk test, was developed to measure endurance. However, since patients with MPS VI have severe joint abnormalities this test can be subject to variable interpretations: the patient is able to walk a limited distance, but one cannot discern whether this is due to limited endurance, joint abnormalities, pain, or a combination of these three factors. For present day decision taking on the reimbursement of expensive medication true endpoints are required to prove the efficacy of therapy and this might require the setting of new measures.

The published data showed that ERT in MPS VI had a positive effect on pulmonary function, growth, endurance, urinary GAG excretion and mobility. The next chapters discuss our findings on the effects of ERT in the Dutch MPS VI patient group.

Cardiac response

Since cardiac disease is one of the main causes of death in patients with MPSs, we first evaluated the effects of ERT on cardiac parameters [10]. We found in our cohort of MPS I, II and VI patients different types of cardiac abnormalities. Several MPS I and II patients presented with a hypertrophic cardiomyopathy, whereas dilated cardiomyopathy was only seen in MPS VI patients. This might be due to the different storage compounds in these diseases: whereas both heparan sulfate and dermatan sulfate store in MPS I and MPS II the storage in MPS VI is confined to dermatan sulfate. According to our initial observations (Chapter 3.1) ERT had a significant effect on the left ventricular mass index (LVMI) Z-scores in patients with MPS I (312 weeks of follow-up) and MPS II (159 weeks of follow-up), but not in patients with MPS VI (154 weeks of follow-up). With a two year longer follow-up of patients with MPS VI (Chapter 4), we were able to document significant results on cardiac parameters (IVSd and LVMI) in this group of patients as well. Importantly, our finding is supported by other publications on this subject [2]. Unfortunately, ERT does not seem to have a clinical effect on valve pathology in the MPSs. This might be due to the near absence of vascularization of the heart valves that are nurished mainly by diffusion from the valve surface [11]. The affected myofibroblasts are therefore not an easy target for the relatively large therapeutic enzymes. The dilated cardiomyopathy that ultimately arises can be explained as the result of chronic valve regurgitation. Since the currently applied ERT does not seem to correct the valve pathology one has to bear in mind that the life threating cardiomyopathy is not eradicated by ERT, while ERT does have significant effects on the cardiac dimensions.

Studying a case of MPS VI (Chapter 3.2) in which the mitral valve was surgically replaced we reached the conclusion that deficieny of repair processes combined with macrophage involvement are likely factors causing persistent valve pathology. Our findings suggest that GAG storage induces inflammation whereby the macrophages contribute to the tissue

damage by lack of repair processes. Our observations are in line with Simonaro's findings that the Toll-like receptor 4/TNF-alpha pathway is activated in MPS VI rats [12]. In this context it was suggested that anti-TNF-alpha therapy could possibly be a valuble adjuvant to the treament of MPSs with ERT [13].

ERT in MPS VI: Additional clinical variables and how to interpret quality of life

Besides the cardiac response subject of Chapter 3, Chapter 4 describes the positive results of ERT in our cohort of MPS VI patients on the pulmonary function, the size of the liver and the spleen, and the urinary GAG excretion. Our findings are therewith in line with previously reported effects of ERT [4, 6, 8, 9, 14-16]. As the severity of organ involvement and the rate of disease progression vary considerably between patients [17], MPS VI is generally regarded as a clinical spectrum. Our patient group covers the entire spectrum, but was too small to analyze the effect of ERT in patients with a rapidly progressive course versus those with attenuated disease. Based on our experience with using different doses of ERT in Pompe disease we find it intriguing to know whether better results can be obtained with higher doses of ERT in the most severe MPS VI cases.

In Chapter 4 we also describe the effect of ERT on quality of life, a key issue for patients that was nevertheless not given much attention in the scientific literature on MPS VI. Quality of life (QoL) is an important Patient Reported Outcome Measure (PROM), but also a difficult variable to analyze since the measured quality of life before and after treatment is influenced by the patients' high expectations about the effect of certain therapies [18]. In Chapter 7 we found that the low scores of Quality of Life in MPS patients is mainly due to the severe physical burdens they encounter; mental health is generally good as measured with the standardized QoL sacles. It has been previously suggested that patients score higher Quality of Life than one would expect as an outsider or caregiver. The frame-shifts are not entirely unknown in Quality of Life-research, especially if one regards Quality of Life as a measurement of the adjustments to a patient's expectations and objectives that flow from the reality they experience [18]. With such a frame-shift in mind, patients might consider their health to improve at start of therapy, because of their high expectations, but in later course of therapy their expectations might not live up to the improvements they perceived. Thus, it is of crucial importance to measure the QoL at the right time points. We cannot exclude that our results, pointing to an increase in negative emotions might have been influenced by the patients' expectations, but they may also indicate that the weekly treatments with ERT are experienced as a burden that comes on top of their disease.

With the addition of our data to the already existing studies about ERT in MPS VI patients, it is fair to conclude that ERT certainly has its effect on many chosen clinical endpoints,

but we also concluded that there is still room for improvement to reach a better clinical outcome in the future.

Future perspectives: focus on MPS VI

Optimizing data collection

Collaboration and sharing information are key elements for expanding knowledge on the natural course of rare diseases and the effects of enzyme-replacement therapy. Data collections of different expert centers should be combined. The current Registries, set up and maintained by the pharmaceutical companies, are a valuable initiative but they do not suffice the demand for clear answers on all key questions regarding rare diseases. The voluntary nature of these Registries does not provide a solid basis to build on. Collaborations should be initiated by the expert centres themselves, ideally with funding of the European Union. Larger cohorts of patients and longer follow-up are needed to ascertain the effects of ERT. The role of expert centers are essential to reach this goal. The Dutch scenario in which patients are treated in one expert center enables a thorough and systematic follow-up of a rare disease like MPS VI. It would be recommendable to have this expert center regulation in every European country in order to obtain a large cohort with a similar systematic follow-up, for all rare diseases.

Last year, this issue became eminent when the Dutch Health Insurance Board (College voor Zorgverzekeringen) advised to stop the reimbursement of ERT for patients with Pompe disease and Fabry disease by claiming marginal effects [19]. After a debate between scientists, politicians, patients and members of the Health insurance Board, reimbursement was temporarily extended. It was felt that intense collaboration in the broader European context could possibly complement the missing information on therapeutic efficacy and lead to an agreement on start and stop criteria for these orphan drugs [20]. Notably, the efficacy of ERT in Pompe disease (n=123) has been proven in ten times more patients than participating in our clinical trials on safety and efficacy of ERT in MPS VI (n=11). Thus, European collaboration is certainly needed in the field of MPS VI. No party ignores that cost-effectiveness is at the basis of all debate; had the drugs used for ERT been much cheaper, the reimbursement issue would not have been raised.

Modifying the immune response

Like in other lysosomal storage disorders treated with ERT, the development of antibodies against galsulfase in patients with MPS VI was more or less anticipated. Indeed, we could demonstrate antibody formation in the 12 MPS VI patients that we followed upon start of ERT, but the number of patients was too small to proof that these antibodies had any effect on their clinical course (Chapter 5). So much was clear, however, that the

patient with the most severe set of MPS VI mutations had the highest antibody titer and that in this patient almost half of the intravenously administered enzyme was bound to circulating antibodies. It is generally believed that a strong immune response counteracts the effect of ERT [21, 22], but more research is needed to elucidate the role of neutralizing antibodies and clinical outcome in MPS VI.

Early detection by neonatal screeningto improve therapy outcome

In January 2007 the Dutch newborn screening programme was extended with 15 diseases, 13 of which were metabolic disorders. All these 15 diseases fulfilled the criteria of Wilson and Jungner rating the availability of a treatment and the availability of a suitable test as the most important factors [36]. With respect to MPS I, II and VI the availability of DBS tests is assured [37-40] while the availability of a curative treatment or at least a treatment that delays or prevents disease progression is best met for MPS I (Hurler phenotype) wherein hematopoietic stem cell transplantation is most effective before 2.5 years of age. However, prediction of the phenotype can be very challenging in all types of MPS by scarcity of methods to measure the true level of residual enzyme activity and the seemingly poor correlation between that measured level and the clinical phenotype. We experienced the same problem in our patient cohort with MPS VI (Chapter 5). Apart from this problem, the best results of ERT in all types of MPSs were obtained in patients who were treated early in life, and early treatment can be achieved by neonatal screening [41, 42]. To illustrate this, MPS VI cats who received ERT from birth on responded better on neurological parameters as well as on bone length than their littermates that started ERT at 3-5 months after birth [43]. A case report about two siblings with MPS VI also showed the benefit of early treatment. The younger sibling who had started receiving ERT at 8 weeks after birth did not develop all the same symptoms as the older brother who had started receiving ERT at the age of 3.6 years. In contrast with the older sibling, the younger sibling did not develop scoliosis nor facial dysmorphology, and his joint movement and cardiac valve function were preserved. Despite these encouraging effects of early treatment, the onset of corneal clouding could not be prevented nor the progression of skeletal deformities [44]. At present, the arguments for including MPS VI in the new-born screening program based on the Wilson and Jungner criteria are likely too slim. One would prefer collecting more data on the effects of early versus later treatment of MPS VI patients and in time be better informed about the long term effects of ERT. At present, the longest follow-up of MPS VI patients treated with ERT is only 6 years [8].

Alternative treatment strategies

The great challenge in the treatment of MPS VI and other MPSs with ERT is to correct the joint and bone malformations that probably exist from birth on [23]. ERT in its current entity is not sufficient to entirely correct these symptoms. It is disputable if the current dosage

of 1 mg/kg per week is adequate enough to correct all abnormalities. It is accepted that all organs have a different uptake of enzyme, with for example a great uptake in the liver but a scarce uptake in muscle and in particular bones and cartilage. Cartilage is not vascularized. Cartilage of the synovial joints, for instance, is fed by nutrients supplied by the capillaries in the synovial membrane that reach the chondrocytes via diffusion through the cartilage matrix. Bone by itself is very well vascularized, and every osteocyte is directly connected with the blood flow. The problem with correcting bone abnormalities is that most bone is initially laid out as cartilage that is replaced by bone during embryonal growth. Thus, any abnormality in the cartilage 'anlage' may affect bone formation and have its effect later in life. For example, wrong positioning of the bones forming a joint can lead to early artrosis, and the growth of long bones can be hampered by structural and functional abnormalities in the growth plate consisting of cartilage. Once formed, the remodeling of bones is a slow process even if ERT would do very well in correcting the osteocytes [23]. A higher dosage of enzyme would be worthwhile trying to see if the enzyme uptake by chondrocytes can be improved. Skeletal abnormalities dominate the clinical picture of MPS VI, and new strategies have been developed to overcome the shortcoming of ERT.

Intra-articular enzyme-replacement therapy

An obvious approach would be to treat the joints of MPS VI patients more effectively by injecting the missing enzyme directly into the synovial fluid. A study in cats showed a significant reduction of storage material in the articular chondrocytes and in the synovial membrane. One month after injection, a slight reaccumulation of storage compounds was observed near the surface of the joint, but it took two months before the effect of treatment was no longer visible. With these promising results in mind, a feasibility study in Australia included two patients in which intra-articular ARSB was administered every two months in addition to weekly intravenous injections. The final results have not been published yet [24], but the first reported results were promising: improved walking endurance and less joint pain.

Intrathecal enzyme-replacement therapy

MPS VI patients can develop severe spinal cord compression with concurrent compressive cervical myelopathy. Accumulation of lysosomal storage in the dura mater and supporting structures in combination with the vertebral bone deformities contribute to this clinical feature [25-27]. Due to the blood-brain barrier and absence of mannose 6-phosphate receptor at this barrier, an insufficient amount of intravenously administered enzyme reaches the dura mater, and lysosomal storage material continues to accumulate. Treatment of the dura mater could be preventive for the development of cervical myelopathy. Therefore intrathecal administration of ARSB was tried in MPS

VI cats[25]. Two MPS VI cats were treated four times in a period of four weeks with intrathecal injections of ARSB. After treatment they had less oligosaccharide fragments in their cerebrospinal fluid and less vacuolation in the dura mater. However, treatment of spinal cord compression isn't without risks. A case report about a 7 year old MPS VI child with pachymeningitis showed that intrathecal ERT improved the spinal cord compression caused by the meninges, with small improvements on motor function and sensibility. Nevertheless, even when intravenous ERT was combined with intrathecal ERT, this child developed a generalized hypotonia, and a life-saving surgical fixation of the neck had to be performed. The severe adverse event experienced by this child raises some concern about the effect of GAGs clearance from the paravertebral structures: increasing the range of vertebral movements and possibly exposing the cord to an unstable spine. If a cervical subluxation, bony stenosis or similar external pathology is the major cause of cord compression, the patient would not likely benefit from intrathecal ERT and neurosurgery should be the first treatment of choice.

Other treatment options: Hemopoetic stem cell treatment (HSCT)

HSCT has been successful in patients by increasing the arylsulfatase B activity. Also evidence has been presented that the overall clinical condition of these patients improves. For instance, long-term improvements in facial dysmorphism, hepatosplenomegaly, joint mobility, and cardiac manifestations have been reported in MPS VI patients following HSCT [28, 29]. However, MPS VI patients are not characterized by cognitive impairment due to central nervous system GAG storage and their profound skeletal abnormalities persist or progress despite HSCT [30, 31]. In MPS I, HSCT is the only method currently available to treat the cognitive impairment (neurological involvement) of these patients. MPS VI patients, however, are not characterized by cognitive impairment, and it is generally believed that the risks of HSCT (graft versus host disease, rejection or low donor chimerism) outweigh the benefits [32].

Other treatment options: Gene therapy

Recently, progress has been made in treating MPS VI cats with gene therapy. If one would succeed to express the ARSB transgene in the liver of the patient, the liver could then in theory serve as a life time factory of ARSB so that weekly intravenous ERT is no longer required [33, 34]. The feasibility of this approach has been proven in cats. Gene therapy in new born cats and resulted in increased bone length and reduction of heart valve pathology, but not all skeletal problems were resolved[33,35]. The procedure in cats did not bear safety risks so that there seems all reason to further develop the technology for application in humans.

Concluding remarks

In 1963 MPS VI was recognized and described as clinical entity [45]. The underlying arylsulfatase B deficiency was discovered in the beginning of the '70s [46, 47], adding MPS VI to the still growing list of lysosomal storage disorders caused by lysosomal enzyme deficiencies. The gene coding for MPS VI was cloned in 1990[48], and this was followed by the identification of the disease causing mutations [49-51]. Since 2007 our Center for Lysosomal and Metabolic Diseases at Erasmus MC University Medical Hospital got involved in the treatment of MPS VI by enzyme replacement therapy. In few years' time the care for MPS VI patients and their treatment with ERT has become a key function of our center, next to the diagnostic services that have a much longer history within Erasmus MC. The past 6 years we found that ERT certainly has its effect on clinical endpoints in patients with MPS VI, but there is still room for improvement. The work described in this thesis covers our center's first five years' time experience with ERT in MPS VI and contributes to the broader understanding of the disease in all its complexity.

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Addendum

Summary

Summary

The mucopolysaccharidoses (MPSs) are a group of lysosomal storage disorders, each caused by the deficiency of one specific lysosomal enzymes involved in the degradation of one or more glycosaminoglycans (GAGs). Mucopolysaccharidosis type VI (MPS VI, Maroteaux-Lamy syndrome, OMIM #253200) is one of the seven major types of MPSs. It is a rare autosomal recessive disorder caused by mutations in the ARSB (arylsulfatase B) gene located on chromosome 5q13-q14. The mutations lead to arylsulfate B defiency and thereby results in the accumulation of dermatan sulfate. The storage of dermatan sulfate eventually causes the characteristic clinical multi-system symptoms. After the marketing approval of enzyme replacement therapy (ERT) for MPS VI in 2007, based on the promising results of the pivotal trials, ERT became available in The Netherlands , and the challenge started to prove its long-term effects. This thesis describes the first 5 years of experience with ERT in MPS VI in the Netherlands and deals with several different aspects of the disease that are addressed in the various chapters of this thesis following a short general introduction into the field (**Chapter 1**)

Chapter 2 reviews all articles about the effects of ERT in patients with MPS VI that were published before November 2012, but does not include reports about less than 3 patients. All in all we found one article reporting the outcome of the randomized-controlled trial and 9 articles reporting the outcome of open-label studies; including the phase I/II studies. All these articles agreed on the positive effect of ERT on pulmonary function, endurance, cardiac parameters and urinary GAG excretion.

Chapter 3 focuses on the cardiac problems of patients with MPS I, II and VI and the response to ERT. **Section 3.1** presents the results of 24 patients, aged 1-18 years, who were prospectively evaluated with echocardiogram and electrocardiogram from the start of enzyme-replacement therapy up to 6 years of treatment. We found an increased left ventricular mass index (LVMI) in half of the patients, due mainly to concentric hypertrophy in MPS I and II and to eccentric hypertrophy in MPS VI. Regurgitation together with a dilated cardiomyopathy was most severe in a subgroup of young MPS VI patients (<5 years). After ERT, the LVMI Z-score normalized in 70% of the patients who had a Z-score>2. Valve disease was refractory to ERT. **Section 3.2** is devoted to cardiac valve pathology on the basis of a case report of a mitral valve replacement in an MPS VI patient. The mitral valve showed abundant GAG storing “clear” cells in both the leaflets and chordae tendineae. A new finding was the involvement of macrophages in the pathophysiologic process of valve malfunction.

In **Chapter 4** we investigated in a prospective follow-up study the effects of ERT in 11 Dutch MPS VI patients (age 2-18 years). ERT had significant positive effects on cardiac-wall diameters, left and right shoulder flexion, liver size and spleen size, urinary GAG excretion and scales of health related quality of life (HRQoL). ERT did not improve cardiac valve regurgitation nor hearing function. Patients with rapidly progressive disease had higher baseline urinary GAG values than those with slower progressive forms. Furthermore, the GAG-values in the rapid progressive patients group declined faster, but tended not to normalize completely.

Chapter 5 relates to the genotype-phenotye correlation in 12 MPS VI patients, and to the development of antibodies against ERT. Four novel mutations in this patient group were identified, together composing 8 pathogenic genotypes. Site-directed mutagenesis showed that most mutations appeared not to affect the synthesis of ARSB (66kD precursor), but to hamper its maturation (43kD ARSB). All patients developed antibodies to galsulfase within 26 weeks of treatment. It was demonstrated that these antibodies can inhibit the uptake of galsulfase *in vitro*. It is still unclear whether these antibodies affect the clinical outcome.

In **chapter 6** we looked at the neurocognitive development of 11 children with MPS VI. Although MPS VI has been defined as a disease without cognitive impairment, we found 5 patients in our patient group with an IQ of less than 84. Three of these patients had a rapidly progressive course. When we weighed these findings against the normal distribution of IQ, family impact and cultural background, there was not enough power to decide whether the low IQ was disease related or the result of other genetic and/or environmental factors. Mental development differed completely from MPS I, II and III in which mental impairment is a hallmark feature.

Chapter 7 describes the prevalence of pain in patients with all types of MPSs. These are the first results of a nationwide MPS Survey. With a response rate of 62%, we found that 40% of patients indicated that they experienced pain. They covered a wide spectrum in all age groups, ranging from no pain to severe pain. Most reported pain sites were the back and hips. It seemed that patients with an intellectual disability experienced more pain than patients with a normal intelligence. MPS patients had much more pain than a random selection of the Dutch population on the QoL questionnaires.

Finally, **Chapter 8** places the findings described in this thesis in broader perspective by discussing them in the light of the currently available literature. This chapter also discusses the design of future studies and treatment options.

Samenvatting

Samenvatting

De mucopolysaccharidosen (MPS) behoren tot de groep van lysosomale stapelingsziekten. Er zijn 7 verschillende MPS ziektebeelden bekend en bij elk van deze ziektebeelden is de oorzaak gelegen in het ontbreken van een specifiek enzym dat verantwoordelijk is voor de afbraak van glycosaminoglycanen (GAGs). Mucopolysaccharidose type VI, ook wel het syndroom van Maroteaux-Lamy genoemd (OMIM #253200), is een van deze ziektebeelden. Het is een zeldzame autosomaal recessieve aandoening die veroorzaakt wordt door mutaties in het arylsulfatase B gen (ARSB gen). Deze mutaties zorgen voor een tekort aan arylsulfatase B activiteit hetgeen vervolgens leidt tot de stapeling van dermatan sulfaat. De stapeling van dermatan sulfaat leidt uiteindelijk tot de karakteristieke symptomen passend bij deze multi-systeem ziekte.

In 2007 werd enzym vervangende therapie (enzyme-replacement therapy; ERT) voor MPS VI goedgekeurd, nadat klinische trials veelbelovende resultaten hadden laten zien. Vrij spoedig daarna kwam ERT beschikbaar in Nederland , waarbij we ons de vraag stelden wat de lange-termijn effecten van ERT voor patiënten met MPS VI zouden zijn.

Dit proefschrift beschrijft de eerste 5 jaar waarin wij ervaring hebben opgedaan met ERT voor MPS VI in Nederland. **Hoofdstuk 1** biedt een korte introductie vervolgens zullen meerdere aspecten van het ziektebeeld komen aan bod komen.

Hoofdstuk 2 geeft een overzicht van alle literatuur tot november 2012 over de effecten van ERT voor MPS VI. Er werden alleen artikelen geëvalueerd waarin meer dan 3 patiënten werden onderzocht. Wij vonden in totaal 1 publicatie over een gerandomiseerde trial. Daarnaast vonden we nog 9 andere open-label studies inclusief de fase I en fase II studies. Al deze artikelen rapporteerden positieve effecten van ERT op longfunctie, uithoudingsvermogen, verschillende cardiale parameters, en de uitscheiding van glycosaminoglycanen in de urine. Het relatieve kleine aantal patiënten, de gekozen eindpunten en het soms ontbreken van een gestandaardiseerde follow-up maakt het moeilijk om een harde conclusie te kunnen trekken over de effectiviteit van ERT in MPS VI.

Hoofdstuk 3 concentreert zich op de cardiale problemen die voorkomen bij patiënten met MPS I, II en VI en de effecten van ERT daarop. In **Deel 3.1** worden 24 patiënten beschreven, in de leeftijd van 1-18 jaar, die prospectief werden gevolgd door middel van echocardiogram en electrocardiogram vanaf de start van ERT tot 6 jaar behandeling. De helft van deze patiënten had een vergrootte linker hartkamer hetgeen wordt uitgedrukt in left ventricular mass index (LVMI). Bij MPS I en II is er voornamelijk sprake van een eccentrische hypertrofie, terwijl het bij MPS VI meestal een gedilateerde hypertrofie betreft. In een subgroep van jonge kinderen met MPS VI (<5 jaar) treedt klepinsufficientie het meest op de voorgrond waardoor er een gedilateerde cardiomyopathie ontstaat.

Als deze patiënten behandeld worden met ERT normaliseert de LVMI in 70% van de gevallen. ERT had geen invloed op de karakteristieke klepafwijkingen. **Deel 3.2** is gewijd aan de hartkleppathologie in MPS VI aan de hand van een casus van een MPS VI patiënt die een mitraalklepvervaning onderging. In de mitraalklep werden heel veel "clear cells" waargenomen, die GAGs stapelen, zowel in de klepbladen als in de pezen waarmee de klep aan de hartwand vastzit (chorda tendinae). Een nieuwe bevinding was de betrokkenheid van macrofagen in het pathofysiologische proces van de disfunctionerende klep.

In **hoofdstuk 4** beschrijven we de resultaten van een prospectieve follow-up studie van 11 Nederlandse patiënten met MPS VI die behandeld werden met ERT (leeftijd 2-18 jaar). ERT had positieve effecten op de dikte van de hartwand, de schouderflexie, grootte van lever en milt, excretie van glycosaminoglycanen in de urine, en op bepaalde schalen die de kwaliteit van leven meten. ERT had geen effect op de hartklep-insufficiëntie noch op het gehoor. Bij start van therapie was de GAG excretie in de urine het hoogst bij patiënten met een snel progressieve vorm van MPS VI.

Hoofdstuk 5 behandelt de genotype-fenotype correlatie bij 11 MPS VI patiënten. Daarnaast gaat dit hoofdstuk ook over de ontwikkeling van antilichamen tegen galsulfase dat gebruikt wordt als ERT voor MPS VI. In deze patiëntengroep ontdekten wij vier nieuwe ARSB genmutaties die in verschillende combinaties met ook andere mutaties leidden to 8 pathogene genotypes. Gedetailleerd onderzoek naar de aard van de 4 nieuw ontdekte mutaties liet zien dat ze de veelal niet de synthese van ARSB beïnvloedden maar wel een nadelig effect hadden op de verdere processen (zoals transport en structurele veranderingen) die de uiteindelijke functie van het enzym bepalen. Alle patiënten die wij met ERT behandelden ontwikkelden binnen 26 weken na start van de therapie antilichamen tegen galsulfase. We toonden aan dat deze antilichamen de opname van galsulfase *in vitro* remmen. Het is vooralsnog onduidelijk of deze antilichamen van klinisch belang zijn.

In **hoofdstuk 6** komt de neurocognitieve ontwikkeling van 11 kinderen met MPS VI aan bod. Hoewel MPS VI gedefinieerd wordt als een ziekte waarbij geen cognitieve beperking voorkomt, troffen wij in onze populatie van 12 patiënten toch 5 kinderen aan met een IQ onder de 84. Drie van deze patiënten hadden een snel progressieve vorm van MPS VI. Als we deze bevindingen beschouwen in het licht van de normale verdeling van het IQ in de populatie, en ook niet MPS gerelateerde genetische factoren in aanmerking nemen, kunnen we niet direct een duidelijke relatie leggen tussen de gevonden lage IQs en het feit dat deze kinderen MPS VI hebben. De neurocognitieve ontwikkeling in patiënten met MPS VI verschilt compleet met bijvoorbeeld MPS I (Hurler), II en III, waarin mentale retardatie op de voorgrond staat.

Hoofdstuk 7 beschrijft het vóórkomen van pijn bij patiënten met alle typen MPS. Het betreft de eerste resultaten van een nationale MPS Survey. Met dit onderzoek deed 62% van de aangeschreven patiënten mee. In totaal gaven 40% van de patiënten in hun 'pijndagboek' aan pijn te hebben. De heup en de rug bleken de meest pijnlijke locaties te zijn. Het leek alsof patiënten met een cognitieve beperking meer pijn hadden dan patiënten met een normale intelligentie. Ook in de pijnschaal, een onderdeel van de vragenlijst over kwaliteit van leven, gaven MPS patiënten aan meer pijn te ervaren dan een doorsnee van de Nederlandse bevolking.

Hoofdstuk 8 is een slotbeschouwing die de inhoud van de hoofdstukken van dit proefschrift en het onderliggende werk nog eens op een rij zet. In dit hoofdstuk wordt ook gespeculeerd over toekomstige onderzoeks- en behandel opties.

Dankwoord

Dankwoord

Het zit erop. Vier jaar Centrum voor Lysosomale en Metabole Ziekten (toen ik begon heette het nog gewoon 'Pompe-team'). Ik heb veel geleerd, veel gelachen en ben de mensen dankbaar die mij hebben geholpen bij de totstandkoming van dit proefschrift.

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Prof. dr. A.T. van der Ploeg, beste Ans, mijn promotor. Ik wil je bedanken dat je mij vier jaar geleden op dit project hebt aangenomen. Je hebt me geleerd hoe belangrijk translationeel onderzoek kan zijn en hoe je je meer dan volledig moet inzetten om daar te komen waar je wil. De afgelopen jaren zijn soms veelbewogen geweest; het leek jou niet af te remmen om het beste voor de patiëntenzorg voor elkaar te krijgen. Jouw kritische blik op mijn artikelen zorgde altijd dat de kwaliteit verbeterde. Het Centrum is inmiddels denk ik verviervoudigd, ik hoop dat het alleen nog maar groter zal worden.

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Portfolio

	Year	Workload (ECTS)
General Academic Skills		
General courses		
Integrity in Medical Research	2009	2.0
Basiscursus regelgeving en management (BROK)	2010	0.9
Biomedical English writing and communication	2012	3.0
Research skills		
Principles of Research in Medicine	2010	0.7
Clinical Decision Analysis	2010	0.7
Methods of Public Health Research	2010	0.7
Clinical Trials	2010	0.7
Study Design	2010	4.3
Pharmaco-epidemiology	2010	0.7
Markers and Prognostic Research	2010	0.7
Classical Methods for Data Analysis	2010	5.7
Quality of Life Measurement	2010	0.9
Clinical Epidemiology	2010	5.7
Bayesian Statistics	2011	1.1
Missing Values in Clinical Research	2011	0.7
Methodologic Topics in Epidemiologic Research	2011	1.4
Modern Statistical Methods	2011	4.3
Topics in Meta-analysis	2012	0.7
Conceptual foundation of Epidemiological Study Design	2012	0.7
Introduction to Global Public Health	2012	0.7
Epidemiologic Research: an Avant-garde Introduction	2012	0.7
History of Epidemiologic Ideas	2012	0.7
Social Epidemiology	2012	0.7
Courses for the Quantative Researcher	2012	1.4
Development Research Proposal (Master Clinical Epidemiology)	2012	2.5
Research Period (Master Clinical Epidemiology)	2012	29.5
Presentations and International conferences		
9th International Symposium on Lysosomal Storage Diseases, Frankfurt (poster presentation)	2009	0.5
10th International Symposium on Lysosomal Storage Disorders Prague (oral presentation)	2010	1.0
11th International Symposium on Mucopolysaccharide and Related Diseases, Adelaide (poster presentation)	2010	0.5

Annual Symposium of the Society for the Study of Inborn Errors of Metabolism, Istanbul (poster presentation)	2010	0.5
Annual Symposium of the Society for the Study of Inborn Errors of Metabolism Geneva (poster presentation)	2011	0.5
Annual Symposium of the Society for the Study of Inborn Errors of Metabolism Birmingham (poster presentation)	2012	0.5
MPS patient day Amersfoort (oral presentation)	2011	1.0
Voorjaarsvergadering Erfelijke Stofwisselingsziekten in het Nederlands Taalgebied (oral presentation)	2012	1.0
12th International Symposium on Mucopolysaccharide and Related Diseases, Noordwijkerhout (poster presentation)	2012	0.5
In depth courses		
Mucopolysaccharidosis and the eye, Venice	2010	0.5
Pompe disease expert day, Rotterdam	2010	0.5
Teaching activities		
Supervising medical student 'introduction to research'	2011	0.5
Lecture Erasmus MC medical students	2012	1.0

Curriculum vitae

Curriculum vitae

Marion Brands is geboren op 7 september 1981 in Heerlen. Daar doorliep ze ook haar schoolcarrière op het Bernardinuscollege. Hier haalde ze in 1999 cum laude haar gymnasium diploma. Na haar jeugd in Heerlen, vertrok ze in 1999 naar Amsterdam om daar geneeskunde te gaan studeren aan de Universiteit van Amsterdam. Tussen haar geneeskunde studie door heeft ze ook een propedeuse Nederlandse Taal en Cultuur gehaald. Na haar studie geneeskunde is zij ANIOS geworden in het Flevoziekenhuis op de afdeling kindergeneeskunde. Hier heeft zij van 2007 t/m 2008 met veel plezier gewerkt. Maar het promoveren trok aan en eind 2008 is zij daarom gestart met haar promotietraject aan het Erasmus MC Sophia bij prof. dr. A.T. van der Ploeg en dr. A.J.J. Reuser. Hiervan hebben jullie het resultaat nu in handen. Tijdens haar promotie heeft ze een master in Clinical Epidemiology aan het Institute for Health Sciences (NIHES, Erasmus Universiteit Rotterdam) behaald.

Vanaf januari 2013 is zij in opleiding tot kinderarts aan het AMC Amsterdam onder supervisie van dr. D. Bosman.

List of publications

List of publications

Brands MM, Roelants J, Krijger RR, Bogers AJ, Reuser AJ, van der Ploeg AT, Helbing WA. Macrophage involvement in mitral valve pathology in mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome). *In press. American Journal of Medical Genetics Part A.*

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Brands MM, Frohn IM, Hagemans ML, Hop WC, Oussoren E, Helbing WA, van der Ploeg AT. Mucopolysaccharidosis: Cardiologic features and effects of enzyme-replacement therapy in 24 children with MPS I, II and VI. *J Inherit Metab Dis. 2013 Mar;36(2):227-34*

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Ebbink BJ, **Brands MM**, van den Hout JM, Lequin MH, Oussoren E, Coebergh van den Braak RR, van de Weitgraven RL, Plug I, Aarsen FK, van der Ploeg AT. Long-term cognitive follow-up in children treated for Maroteaux-Lamy syndrome. *In preparation.*

Brands MM, Plug I, Hagemans ML, Vollebregt A, Oussoren E, Reuser A, van der Ploeg AT. Enzyme-replacement therapy for mucopolysaccharidosis type VI (Maroteaux-Lamy): A systematic review of the literature. *Ready for submission.*