Title

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Biomarkers of Glycosaminoglycans (GAG) accumulation in patients with mucopolysaccharidosis type VI—LeukoGAG, Corneal Opacification (COM) and Carotid Intima Media Thickening (CIMT)

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ABSTRACT

Mucopolysaccharidosis type VI (MPS VI) is an autosomal recessive lysosomal storage disorder characterized by deficient activity of arylsulfatase B enzyme (ASB) resulting in cellular accumulation of dermatan sulfate (DS) and chondroitin sulfate (CS) that leads to cell injury. Urinary glycosaminoglycans (GAG) are often used as a biomarker in MPS diseases for diagnosis and to monitor treatment efficacy. This study evaluated leukocyte GAGs (leukoGAG) and skin GAGs as alternate biomarkers representing intracellular GAG changes in patients with MPS VI and treated with enzyme replacement therapy (ERT). In addition, we evaluated corneal opacification measurements (COM) and carotid intima media thickness (CIMT) as indicators of GAG accumulation and tissue injury. The study was performed in a serial two-step design in a single center. A quantitative method to measure leukoGAG levels in leukocytes was developed in Study 1 to compare the GAG levels between MPS VI patients and a control group and to assess correlations between leukoGAG and urineGAG. Study 2 validated the leukoGAG measurement, assessed the effect of ERT infusion on leukoGAG and ASB activity in leukocytes, identified correlations between leukoGAG and other biomarkers, and assessed differences in GAG accumulation between MPS VI patients and control subjects. In Study 1, leukoCS and leukoDS levels were significantly higher in the MPS VI group than the control group (leukoCS: 37.9 ± 10.2 and 2.9 ± 1.5 μg/μg protein, respectively, $p = 0.005$; leukoDS: 0.26 ± 0.2 and 0.0 ± 0.0 μg/μg protein, respectively, $p = 0.028$) with positive correlations between leukoCS and urine CS and leukoDS and urineDS. In Study 2, leukoCS (32.0 ± 11.8 vs 6.9 ± 3.1 μg/mg protein, $p = 0.005$) and leukoDS (0.4 ± 0.1 and 0.2 ± 0.1 μg/mg protein, $p = 0.020$) were significantly higher compared with control subjects. Thus, these results highlight the potential of leukoGAG as a new biomarker representing intracellular GAG accumulation in MPS VI patients and may be valuable for patient management.

1. Introduction

Mucopolysaccharidosis type VI (MPS VI), or Maroteaux-Lamy syndrome, is an autosomal recessive lysosomal storage disorder, caused by pathogenic *ARSB* gene variants that results in a deficiency of the arylsulfatase B enzyme (ASB) and cellular accumulation of dermatan

Abbreviations: MPS VI, mucopolysaccharidosis type VI; ASB, arylsulfatase B enzyme; DS, dermatan sulfate; CS, chondroitin sulfate; GAG, glycosaminoglycans; COM, corneal opacification measurements; CIMT, carotid intima media thickness.

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sulfate (DS) and chondroitin sulfate (CS) leading to cell injury [1,2]. The clinical features include characteristic skeletal dysplasia manifested as short stature, dysostosis multiplex, and degenerative joint disease, facial coarseness, corneal clouding, recurrent otitis media, hearing loss, sleep apnea, cardiac valve disease, reduced pulmonary function, hepatosplenomegaly, and inguinal and/or umbilical hernia. Although intellectual deficit is generally absent in MPS VI, central nervous system findings may include cervical cord compression caused by cervical spinal instability, meningeal thickening and/or bony stenosis, communicating hydrocephalus, optic nerve atrophy, and blindness [1,3]. The time of onset, rate of progression, and extent of MPS VI disease varies widely among affected individuals [2,3]. Patients with rapidly progressing MPS VI experience an early onset of symptoms, usually before the age of 2 years. Although symptoms may appear later in life in those with slowly progressing disease, these patients nonetheless demonstrate severe morbidity and early mortality by the third to fifth decade of life. Patients often require clinical interventions related to dysfunction in one or more organs such as corneal transplants, cardiac valve replacement, hip replacement or spinal cord decompression surgery by their late teen to adult years [3,4].

Enzyme replacement therapy (ERT) with galsulfase has been available to treat MPS VI since 2005 in the United States and 2006 in Europe. Results from clinical trials demonstrate that ERT improves walking ability, endurance, and pulmonary function [5–7]. However, ERT is not curative and fails to resolve many pathological changes, in particular when present prior to initiation of therapy with ERT [8]. These limitations are mainly due to poor penetration of the recombinant enzymes across blood-brain and blood-ocular barriers and into poorly vascularized tissues such as cartilage and bone [9,10]. Cardiac valve disease is not improved with initiation of ERT in the majority of patients with valve disease [11,12]. Despite ERT, MPS VI disease progresses over time, and symptoms continue to hamper many patient functions including mobility, vision, and respiratory and cardiac function.

Over the past decade, the amount of total glycosaminoglycans (GAGs) and the specific components of GAGs (CS and DS especially in MPS VI) in urine has been used as a biomarker in the diagnosis of MPS diseases, for monitoring disease activity, and for assessing treatment efficacy as a surrogate for the extent of GAG accumulation [1]. However, urinary GAG levels are not considered an ideal efficacy biomarker as they do not directly reflect the disease burden of affected patients and improvement after therapy [13,14]. The amount of urinary GAGs is insufficient to reflect the extent of GAG accumulation in specific cells or tissues, because quantities of GAG accumulation vary among different tissues or organs owing to differences in penetration of intravenous (IV) enzyme in those treated with ERT. Furthermore, biomarkers would be useful for identifying new drugs with different mechanisms of action for treating patients with MPS VI. For example, odiparcil is an oral small molecule that results in the synthesis of odiparcil-linked GAGs to reduce accumulation of DS and CS GAG in tissues and increase urinary levels of GAG (uGAG). Therefore, a need exists for new biomarkers that detect the intracellular or tissue accumulation and changes in GAGs.

This study aimed to investigate new biomarkers representing intracellular and tissue-specific GAG accumulation and changes with ERT in patients with MPS VI. However, evaluating intracellular levels in tissues mainly affected by MPS VI such as bones, lungs, eyes, and myocardium is invasive and not feasible in most patients. A need exists to identify other biomarkers that correlate with disease burden that are readily accessible and less invasive. GAG levels in circulating leukocytes (leukoGAG) was hypothesized as an alternative biomarker of intracellular activity from data in mammalian models of MPS VI such as cats [15,16] and rats [17]. This study was performed to evaluate and validate the feasibility of leukoGAG and skin GAG as biomarkers representing intracellular GAG changes in patients with MPS VI. In addition, we evaluated corneal opacification measurements (COM) and carotid intima media thickness (CIMT) as indicators of GAG accumulation and injury in specific tissues such as cornea and carotid vessel wall.

2. Methods

2.1. MPS VI patient and control groups

Inclusion criteria were male or female patients, at least 7 years of age with a confirmed diagnosis of ASB deficiency based upon biochemical and/or genetic analysis. Patients had to be treated with ERT for a minimum of 3 months prior to enrollment. Participants in any investigational drug study in the 3 months preceding the screening, those unable to provide blood and urine samples for any reason or those with previous hematopoietic stem cell transplant were excluded. A participant in the control group was eligible if no diagnosis of any type of MPS was identified.

2.2. Study design

Two observations studies were conducted. Different cohorts were used for control subjects in Study 1 and Study 2. Study 1 consisted of three visits at baseline and Week 1 and 2. The objectives were to develop a quantitative method to measure GAG levels in leukocytes, to compare the GAG levels between MPS VI patients and a control group, and to assess the correlations between leukoGAG and urineGAG.

Study 2 was performed approximately 2 years after completion of Study 1 and consisted of seven visits over approximately 30 days. Study 2 aimed to validate the leukoGAG measurement, to assess the effect of ERT infusion on leukoGAG and ASB activity in leukocytes, to identify correlations between leukoGAG and other biomarkers (urine GAG and skin GAG), and to assess the potential differences of GAG accumulation in specific tissues (skin, cornea, and carotid artery wall) between MPS VI patients and control subjects.

The protocols of the study and the informed consent form were reviewed and approved by the institutional review board of the University of California San Francisco Benioff Children's Hospital, Oakland, California. Written informed consent and/or assent from the participants and the parents or legal guardian were obtained according to local ethics committee regulations.

2.2.1. Study 1

Study 1 was conducted from April 2017 to Sep 2017. At baseline, participants in both groups had blood and urine samples collected for measurement of leukoGAG and urine GAG. The MPS VI patient group had two more consecutive sample collections at weekly intervals with a scheduled ERT infusion to assess changes of GAGs with ERT. All blood and urine samples were collected prior to ERT infusion in the MPS VI group.

2.2.2. Study 2

Study 2 was conducted from Aug 2019 to Oct 2019. The participants in both groups had blood and urine samples collected at baseline. Skin punch biopsy was performed for measurement of skin GAG and histologic analysis in both groups. The levels of components of GAG in leukocytes (leukoCS, leukoDS, and leukoHS) and skin (skinGAG) were analyzed and compared between MPS VI patients and the control group. To evaluate changes of leukoGAG and ASB enzyme activity in leukocytes during the ERT infusion, blood samples were collected before the ERT infusion and 1, 4, 24, and 48 h after ERT infusion in the MPS VI group. Correlations between leukoGAG, urine GAG, and skinGAG levels in both groups also were analyzed. The accumulation of GAG in corneal and carotid tissues was compared between groups by measuring COM (baseline visit) and CIMT (up to 30 days after Day 9 for those with MPS or up to 30 days after Day 6 for those without MPS), respectively.

2.3. Study assessments

2.3.1. TotalGAG and components in leukocytes and urine

LeukoGAG and urine GAG were measured in isolated leukocytes

from blood samples using a colorimetric (1,9-dimethylmethlyene blue (DMB) staining method. Measurement of CS, DS, and heparan sulfate (HS) in leukocyte and urine was performed using ultra-performance liquid chromatography (UPLC) and analyzed by electrospray ionization tandem mass spectrometry (MS/MS) (Greenwood Genetic Center, Greenwood, SC). The specimen was treated with methanol to degrade large GAG species (CS, DS, and HS) into uronic or iduronic acid *N*-acetylhexosamine or iduronic acid-N-sulfoglucosamine dimers which were mixed with internal standards derived from deuteriomethanolysis of GAG standards. Specific dimers derived from CS, DS, and HS were separated by UPLC MS/MS using selected reaction monitoring for each GAG dimer.

2.3.2. Histologic analysis of skin biopsy samples

GAG levels in epidermis, papillary dermis, and total dermis layers of the skin were measured by immunostaining by Alcian blue pH 1 and also by colloidal iron. Levels of GAG for each staining were assessed at three thresholds: 1) a low threshold (weak) that allowed detection of GAG background for which no much difference between MPS VI patients and control subjects was expected; 2) an intermediate threshold (moderate) that allowed the detection of fine GAG particles for which a trend toward an increase might be observed in MPS VI patients compared with control subjects; and 3) a high threshold (strong) that allowed the detection of GAG aggregate for which an increase in MPS VI patients compared with control subjects was expected.

2.3.3. ASB enzyme activity in leukocytes

Measurement of arylsulfatase B (ASB) enzyme activity in leukocytes was performed using a validated method (Greenwood Genetic Center). A fixed concentration of internal standard that was structurally similar to the product formed by the enzymatic reaction was included in each reaction. Six enzyme assays were performed as a multiplex reaction and analyzed by UPLC-MS/MS to simultaneously measure the amount of product formed by each of six different lysosomal enzymes by the hydrolysis of enzyme-specific substrates using selected reaction monitoring for each enzyme-specific product and internal standard.

2.3.4. Corneal opacification measurements

For corneal opacification measurements (COM), images were analyzed to obtain an overall COM score; this method has been validated and shown to have good repeatability [18]. The semiautomated COM score calculates the corneal opacity at each individual pixel and provides a mean and standard deviation, which demonstrates the spread of the corneal opacity pixel measurements throughout the image.

2.3.5. Carotid intima media thickness

CIMT was measured using the methodology of Wang et al., 2014 [19]. Using digital calipers, the maximum end-diastolic far wall CIMT was measured 1 cm proximal to the bifurcation of the common carotid artery. The measurements were performed bilaterally, with three scanning angles (anterior, lateral, and posterior), and the six values were averaged to produce the mean CIMT measurement.

2.4. Statistical analysis

For Study 1, no formal determination of sample size was performed. Six MPS VI patients treated with ERT, and 6 control subjects not affected by MPS were considered appropriate for the achievement of study objectives. For Study 2, the sample size was estimated from observing the *p*-values obtained for urine analysis in 12 participants in Study 1 to identify differences in uGAG (Total, HS, DS) between the MPS VI and control groups. Assuming a similar mean and SD for leukoCS in both groups, the power to detect a significant difference was 88.5% with 6 patients per group; 56.2% and 42.8% respectively for leukoDS and total leukoGAG.

Summary statistics are presented for total GAG levels and HS/CS/DS levels in leukocytes, for each age stratum independently, and by patients suffering from MPS VI and treated with ERT and a reference control group of subjects without MPS.

For Study 1, the correlation between uGAG, leukoGAG, and/or ARSB enzyme activity, a Pearson correlation was calculated for each endpoint (uGAG, leukoGAG or ARSB activity) if hypotheses of normality were accepted. Data were stratified for age 7–15 years and 16 years or more. If hypotheses of normality were unaccepted for any of these endpoints, a Spearman correlation were calculated.

For Study 2, descriptive statistics were presented by variables at each time point when assessed, overall and for each age stratum independently (ages <18 years / ≥18 years old) and by cohort (MPS VI patients / control subjects). The effect of ERT on leukoGAG and leukoASB was assessed in the MPS VI patients by comparing these endpoints between pre-ERT time point and at each post-ERT time points. Differences in skinGAG, uGAG, and leukoGAG was assessed by comparing the HS/DS/CS levels of these endpoints between control subjects and MPS VI patients using Wilcoxon test or Fisher's exact test. A Rank test was used to measure the change from baseline for leukoASB.

A linear multiple regression model was used to assess relationships with adjustment for age for 1) total GAG level in leukocytes by total GAG level in urine; 2) HS/DS/CS level in leukocytes by HS/DS/CS level in urine; 3) total GAG level in leukocytes by leukoARSB activity; 4) HS/DS/CS level in leukocytes by leukoARSB activity; and 5) leukoARSB activity by total GAG level in urine 6) leukoARSB activity by HS/DS/CS level in urine. Correlations between uGAG, skinGAG and leukoGAG also were assessed using Spearman correlation coefficients. The Type 1 error rate was set at 0.05, and comparisons were two-sided. Statistical analyses were performed using SAS® software Version 9.4 or later (SAS Institute, Cary, NC, USA).

3. Results

3.1. Study 1

3.1.1. Patients

A total of 12 participants including 6 MPS VI patients and 6 control subjects were screened and enrolled in the study (Table 1). Across groups, patients had comparable age (mean ± SD: 17.0 ± 6.7 vs 17.2 ± 7.4 years), gender ratio (4 and 5 male patients in each group, respectively). Height and weight were significantly lower in the MPS VI group than the control group (111.6 ± 9.1. vs 160.3 ± 18.1 cm, 30.3 ± 6.1 vs 52.9 ± 18.8 kg, respectively). Disease characteristics of MPS VI patients group are summarized in Table 2. MPS VI was diagnosed at age 3.8 ± 5.3 years (median: 1.7 years; range: 0.5 to 14.3 years). ERT was initiated at 7.0 ± 5.6 years, and the duration of ERT before the study was 10.1 ± 3.0 years.

3.1.2. LeukoGAG

Total leukoGAG was higher in the MPS VI group (48.8 ± 9.1 μg/mg protein) than the control group (21.9 ± 19.1 μg/mg protein). However, total leukoGAG values were close to the lower limit of quantification (LLOQ) in both groups. LeukoCS and leukoDS levels were significantly higher in the MPS VI group than the control group (leukoCS: 37.9 ± 10.2 and 2.9 ± 1.5 μg/mg protein, respectively, $p = 0.005$, Wilcoxon test; leukoDS: 0.26 ± 0.2 and 0.0 ± 0.0 μg/mg protein, respectively, $p = 0.028$, Wilcoxon test). LeukoHS was comparable between the MPS VI and control groups (1.5 ± 0.4 vs. 2.2 ± 1.4 μg/mg protein, respectively, $p = 0.298$, Wilcoxon test). No significant differences were observed by age < 16 or ≥ 16 years.

3.1.3. Repetitive measurement of LeukoGAG in MPS VI group

Repeat LeukoGAG levels were obtained at a minimum of two consecutive ERT visits. Total leukoGAG levels were not informative as data from some patients were close or below the LLOQ. Prior to ERT infusion and for all MPS VI patients, leukoCS levels remained stable at

Table 1
Baseline demographics.

	Study 1		Study 2	
	MPS VI group (N = 6)	Control group (N = 6)	MPS VI group (N = 6)	Control group (N = 6)
Age, years*	17.0 ± 6.7	17.2 ± 7.4	19.4 ± 6.7	19.2 ± 6.0
<16 years, N (%) Individual age	3 (50.0%) (10,11,12)	3 (50.0%) (22, 22, 25)	NA	NA
≥16 years, N (%)	3 (50.0%)	3 (50.0%)	NA	NA
<18 years, N (%) Individual age	NA	NA	3 (50.0%) (24, 24, 27)	3 (50.0%)
≥18 years, N (%) Individual age	NA	NA	3 (50.0%) (12, 13, 14)	3 (50.0%)
Male, N (%)	4 (66.7%)	5 (83.3%)	4 (66.7%)	5 (83.3%)
Female, N (%)	2 (33.3%)	1 (16.7%)	2 (33.3%)	1 (16.7%)
Race				
Asian, N (%)	0	2 (33.3%)	0	3 (50.0%)
White, N (%)	5 (83.3%)	4 (66.7%)	4 (66.7%)	0
Other, N (%)	1 (16.7%)	0	2 (33.3%)	3 (50.0%)
Height, cm*	111.6 ± 9.1	160.3 ± 18.1	113.7 ± 10.3	165.4 ± 11.2
<16 years	116.1 ± 9.4 N = 3	151.8 ± 23.7	NA	NA
≥16 years	107.1 ± 7.6 N = 3	168.8 ± 6.8	NA	NA
<18 years	NA	NA	119.6 ± 10.8 N = 3	158.0 ± 12.1
≥18 years	NA	NA	107.8 ± 6.5 N = 3	172.8 ± 1.36
Weight, kg*	30.3 ± 6.1	52.9 ± 18.8	34.2 ± 7.4	60.3 ± 16.7
<16 years, N = 3	28.8 ± 6.6	43.6 ± 19.1	NA	NA
≥16 years, N = 3	31.8 ± 6.4	62.2 ± 16.1	NA	NA
<18 years, N = 3	NA	NA	35.1 ± 9.6	47.7 ± 14.6
≥18 years, N = 3	NA	NA	33.3 ± 6.4	73.0 ± 2.3

* mean ± standard deviation; ERT: enzyme replacement therapy, MPS VI: mucopolysaccharidosis type VI, N: number of subjects, NA: not available; Study 1 and Study 2 MPS VI group were the same patients; Study 2 was undertaken 2 years after Study 1.

each visit (37.9 ± 10.2, 35.1 ± 13.3 and 34.1 ± 14.4 μg/mg protein). LeukoDS levels were similar at each visit (0.26 ± 0.20, 0.37 ± 0.07 and 0.44 ± 0.14 μg/mg protein). LeukoHS levels also remained stable at each visit (1.5 ± 0.4, 1.7 ± 0.9 and 1.7 ± 1.2 μg/mg protein).

3.1.4. UrineGAG

Total urine GAG levels were significantly higher in the MPS VI group compared to control (12.2 ± 3.8 versus 5.3 ± 3.0 mg/mmol creatinine, respectively, $p = 0.013$) indicating that ERT did not reduce urine GAG levels to normal levels. UrineCS tended to be higher in the MPS VI group compared to control (5.8 ± 2.8 versus 2.6 ± 1.9 mg/mmol creatinine, respectively, $p = 0.128$). In contrast, urineDS was significantly higher in the MPS VI group compared to control (6.5 ± 1.2 versus 2.2 ± 0.3 mg/mmol creatinine, respectively, $p = 0.005$). Urine HS was slightly higher in the MPS VI group compared to control (0.7 ± 0.1 versus 0.5 ± 0.1 g/mol creatinine, respectively, $p = 0.013$).

3.1.5. Repetitive measurement of urineGAG in MPS VI group

Total urine GAG levels were 12.2 ± 3.8, 12.2 ± 3.7 and 11.7 ± 3.6 mg/mmol creatinine at each study visit. Urine CS levels were 5.8 ± 2.8, 6.0 ± 3.2, and 6.1 ± 2.9 mg/mmol creatinine, respectively, over time. UrineDS levels were 6.5 ± 1.2, 6.9 ± 2.0 and 6.6 ± 1.7 mg/mmol creatinine over time. UrineHS levels were 0.7 ± 0.1, 0.7 ± 0.2 and 0.8 ± 0.1 mg/mmol creatinine over time. These results indicate stability over time.

Table 2
Patient characteristics at baseline, by age group – MPS VI group.

	Study 1			Study 2		
	<16 years N = 3	≥16 years N = 3	Total N = 6	<18 years N = 3	≥18 years N = 3	Total N = 6
Age at diagnosis, years*	2.1 ± 1.7	5.4 ± 7.7	3.8 ± 5.3	2.1 ± 1.7	5.4 ± 7.7	3.8 ± 5.3
Time since diagnosis, years*	9.1 ± 8.9	17.6 ± 9.1	13.2 ± 7.6	11.3 ± 2.6	19.9 ± 9.0	15.6 ± 7.6
Age at ERT start, years*	2.3 ± 1.6	11.7 ± 3.2	10.0 ± 3.1	3.1 ± 1.3	11.7 ± 3.2	8.2 ± 5.2
Duration of ERT, years*	8.7 ± 2.4	11.5 ± 3.4	10.1 ± 3.0	10.0 ± 2.1 (n = 2)	13.7 ± 3.4 (n = 3)	12.2 ± 3.4 (n = 5)
Urine GAG at diagnosis	Not available	Not available	10.4 ± 4.2	241.5 ± 190.8	66.0	183.0 ± 168.7

* Mean ± standard deviation; ERT: enzyme replacement therapy, GAG: glycosaminoglycan, MPS VI: mucopolysaccharidosis type VI, N or n: number of subjects.

3.1.6. Correlations between leukoGAG and urineGAG

Despite long-term ERT, MPS VI patients had significantly higher total urineGAG (12.2 ± 3.8 vs. 5.3 ± 3.0 mg/mmol creatinine, respectively, $p = 0.013$, Wilcoxon test), urine HS (0.7 ± 0.14 vs. 0.5 ± 0.0.14 g/mol creatinine, respectively, $p = 0.005$, Wilcoxon test), and urineDS (6.5 ± 1.2 vs. 2.2 ± 0.3 g/mol creatinine, respectively, $p = 0.013$, Wilcoxon test) compared to control subjects. Although no significant difference was observed, urineCS was higher in MPS VI patients (5.8 ± 2.8 vs 2.6 ± 1.9 g/mol creatinine, respectively, $p = 0.128$, Wilcoxon test). Values of leukoGAG, total urineGAG, urineCS, urineDS, and urineHS levels remained stable in MPS VI patients at two consecutive ERT visits. When correlations were analyzed independent of age, positive correlations were observed between leukoCS and urineCS in the whole population (correlation coefficient: 0.66, $p = 0.018$; Spearman coefficient). LeukoDS and urineDS also were significantly correlated in the whole population when analyzed independent of age (correlation coefficient: 0.59, $p = 0.044$; Spearman coefficient).

3.2. Study 2

3.2.1. Patients

Six MPS VI patients and 6 control subjects were screened and enrolled in the study (Table 1). Participants in MPS VI and control groups had comparable age (mean ± SD: 19.4 ± 6.7 and 19.2 ± 6.0 years, respectively). Three participants in each group were ages <18 years, and 4 and 5 participants in the MPS VI and control groups were male, respectively. Patients aged ≥18 years were treated with ERT for 13.7 ± 3.5 years, and the last uGAG level before enrollment was 5.5 ± 2.4 μg/mg creatinine.

3.2.2. LeukoGAG

As total leukoGAG was inconclusive in Study 1, only components of leukoGAG (leukoCS, leukoDS, and leukoHS) were measured in Study 2. In MPS VI patients, leukoCS and leukoDS were significantly higher compared with control subjects: leukoCS (32.0 ± 11.8 vs 6.9 ± 3.1 μg/mg protein, $p = 0.005$, Wilcoxon test) and leukoDS (0.4 ± 0.1 and 0.2 ± 0.1 μg/mg protein, $p = 0.020$, Wilcoxon test), respectively, compared with control subjects (Fig. 1). LeukoHS was similar in both groups (2.6

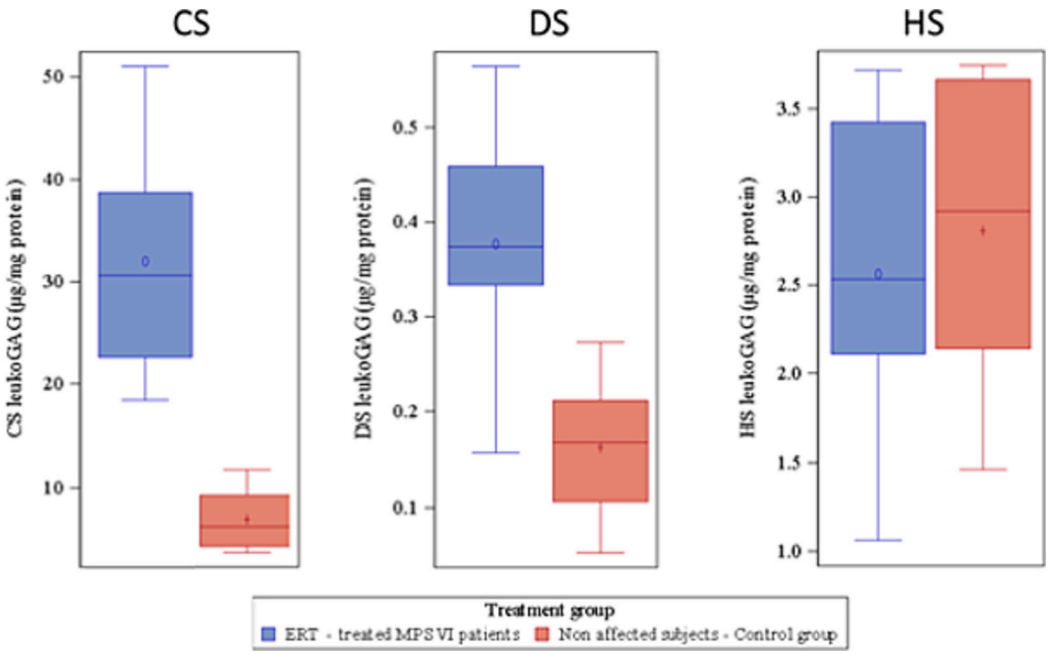

Fig. 1. Levels of CS, DS, and HS in leukocytes.
In MPS VI patients, CS and DS leukoGAG were significantly higher compared with control subjects (32.0 ± 11.8 and 6.9 ± 3.1 µg/mg protein, $p = 0.005$) and (0.4 ± 0.1 and 0.2 ± 0.1 µg/mg protein, $p = 0.020$), respectively, compared with control subjects. HS in leukocytes was similar in both groups (2.6 ± 1.0 and 2.8 ± 1.0 µg/mg protein, respectively).
CS: chondroitin sulfate, DS: dermatan sulfate, ERT: enzyme replacement therapy, GAG: glycosaminoglycan, HS: heparan sulfate, leukoGAG: GAG contained in leukocytes, MPS VI: mucopolysaccharidosis type VI.

± 1.0 and 2.8 ± 1.0 µg/mg protein, respectively, $p = 0.575$, Wilcoxon test).

3.2.3. UrineGAG

Urine GAG levels in Study 1 at diagnosis were 183.0 µg/mg creatinine, and in Study 2 pre-treatment were 7.3 µg/mg creatinine. In individuals aged <18 years, total uGAG was slightly higher in MPS VI patients compared to control subjects (10.7 ± 6.5 and 5.4 ± 6.0 µg/mg creatinine, $p = 0.38$, Wilcoxon test). In contrast in individuals aged ≥18 years, total uGAG was 6-fold higher in MPS VI patients compared with control subjects (9.6 ± 10.3 and 1.5 ± 0.8 µg/mg creatinine, $p = 0.08$). Components of uGAG were significantly higher in MPS VI patients compared with control subjects. Levels were 2-fold higher for urineCS (70.1 ± 33.9 and 25.5 ± 22.8 µg/mg creatinine, respectively, $p = 0.03$, Wilcoxon test), 3-fold higher for urineDS (60.5 ± 19.0 and 18.4 ± 2.2 µg/mg creatinine, respectively, $p = 0.005$), and 2-fold times higher for urineHS (12.6 ± 3.3 and 4.8 ± 0.7 µg/mg creatinine, respectively, $p = 0.005$). Results with CS and DS are consistent with the pathophysiology of MPS VI. For urineCS, the levels decreased with age in MPS VI patients (96.3 ± 10.7 and 43.9 ± 26.3 µg/mg creatinine in patients aged <18 years and > 18 years, respectively) as well as in control subjects (42.0 ± 21.9 and 9.0 ± 0.84 µg/mg creatinine in subjects aged <18 years and > 18 years, respectively).

3.2.4. Effect of ERT on ASB activity and component GAG in leukocytes

ERT administration induced a significant and transient 7-fold increase in leukoASB activity. The peak response was observed 1 h post infusion (16.9 ± 8.8 to 129.7 ± 63.9 nmol/h/mg protein, $p = 0.03$, rank test). Thereafter, the increase was progressively reduced at 4 h (6-fold, 106.6 ± 66.2 nmol/h/mg protein, $p = 0.03$, rank test), 24 h (5-fold, 85.6 ± 55.2 nmol/h/mg protein, $p = 0.03$, rank test), and at 48 h (3-fold, 59.9 ± 39.1 nmol/h/mg protein, $p = 0.03$, rank test). Over 48 h after infusion, ASB activity had not returned to pre-infusion levels. Despite the dynamic changes of ASB enzyme activity, ERT did not induce a change for CS, DS, and HS leukoGAG in the MPS VI group at any time point through 48 h after administration ($p > 0.05$, rank test) (Fig. 2).

3.2.5. GAG levels in skin

Although levels of CS and DS in skin were higher in the MPS VI group compared with the control group (0.08 ± 0.01 and 0.43 ± 0.08), all components of skinGAG showed no significant differences between the two groups; skinCS (0.10 ± 0.02 and 0.08 ± 0.01 µg/mg tissue, $p = 0.383$, Wilcoxon test), skinDS (0.51 ± 0.10 and 0.43 ± 0.08 µg/mg tissue, $p = 0.633$, Wilcoxon test) and skinHS (0.03 ± 0.01 and 0.03 ± 0.01 µg/mg tissue, $p = 1.00$, Wilcoxon test).

3.2.6. GAG levels in skin measured through histologic analysis

The thickness of epidermis, papillary dermis, and total dermis layers measured on histology sections showed no significant difference in any of the layers in MPS VI patients compared control subjects. In papillary dermis, a higher level of GAG with Alcian blue staining was observed in MPS VI patients compared with control subjects with a high threshold of detection (5.8 ± 3.7 vs 1.9 ± 1.0, respectively, $p = 0.151$). In total dermis, a higher level of GAG also observed with a moderate (3.3 ± 0.8 vs 1.8 ± 0.9, respectively, $p = 0.096$) and a high threshold (0.8 ± 0.6 vs 0.2 ± 0.1 µg/mg tissue, respectively, $p = 0.145$).

3.2.7. Correlations between leukoGAG, urinary GAG, and skin GAG

The correlations were evaluated between the levels of each component GAG (CS, DS, and HS) in leukocytes with those in urine, those in leukocytes with those in skin and those in urine with those in skin. Significant correlations were observed between leukoGAG and CS uGAG ($R^2 = 0.89$, $p = 0.0188$).

3.2.8. Corneal opacity measurement

No corneal opacification was observed in any of the control subjects (all COM scores of both eyes were 0.0). In MPS VI patients who had a corneal transplantation (all were bilateral transplants), no corneal opacification was observed. COM scores were significantly higher in

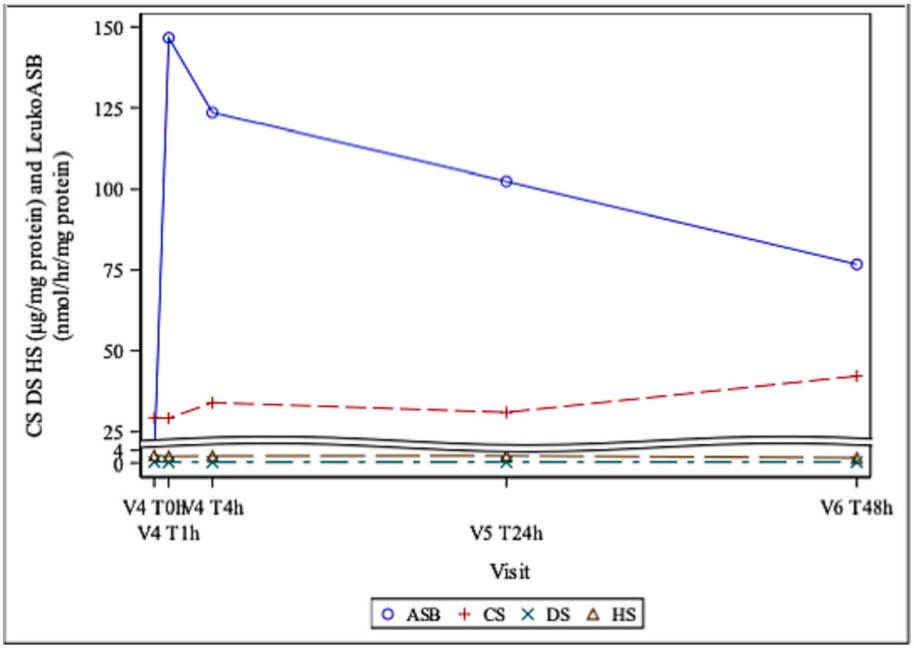

Fig. 2. Evolution of mean CS, DS, and HS leukoGAG, and leukoASB over 48 h after ERT infusion in MPS VI patients.
The y-axis is divided in two portions (from 0 to the threshold of 4 and from the threshold of 24 to 150) in order to represent all values in the graph. Two black lines identify the two thresholds.
ASB: arylsulfatase B enzyme, CS: chondroitin sulfate, DS: dermatan sulfate, ERT: enzyme replacement therapy, GAG: glycosaminoglycan, HS: heparan sulfate, leukoASB: ASB activity in leukocytes, leukoGAG: GAG in leukocytes, MPS VI: mucopolysaccharidosis type VI.

MPS VI patients not receiving corneal transplantation compared with control subjects (left eye: 12.5 ± 8.7 and 0.0 ± 0.0 mm, respectively, $p = 0.0057$, right eye: 12.4 ± 8.2 and 0.0 ± 0.0 mm, respectively, $p = 0.0057$, Wilcoxon test) (Table 3).

3.2.9. *Carotid intima media thickness*

CIMT to measure GAG accumulation in vascular tissue was significantly ($p = 0.0192$, Wilcoxon test) higher in MPS VI patients compared with control subjects (0.592 ± 0.087 mm and 0.425 ± 0.072 mm, respectively) (Fig. 3).

4. Discussion

The primary objectives of this study were to investigate new biomarkers representing intracellular and tissue-specific GAG accumulations in patients with MPS VI. In Study 1, leukoGAG was evaluated as an alternative biomarker representing intracellular GAG accumulation by comparing the levels between MPS VI patients and control group. Study 1 demonstrated that leukoGAG levels, especially leukoCS and leukoDS, were significantly higher in MPS VI patients compared to control subjects, which is compatible with disease pathogenesis. In addition, significant correlations were observed between leukoCS and urineCS and between leukoDS and urineDS. These results suggested that leukoGAG could further be qualified as a biomarker for MPS VI. Therefore, Study 2 was conducted to identify if leukoGAG could be qualified as a biomarker of intracellular GAG accumulation in MPS VI patients. SkinGAG also was evaluated as an intracellular biomarker in Study 2. The impact of GAG accumulation in tissues severely affected by MPS VI and not penetrated by ERT such as cornea and vascular tissues (carotid) was evaluated with COM and CIMT, but will require further investigation to determine their value as biomarkers. Interestingly, a significant positive correlation was observed between levels of leukoCS and leukoDS and levels of urineCS and urineDS in Study 1, but no correlation was observed in Study 2. Study 2 validated the possibility of leukoGAG as an alternative biomarker by reproducing the significant differences in leukoCS and leukoDS levels between MPS VI patients and control groups.

Table 3
Corneal opacity measurement in each group – Study 2.

Mean COM score between right and left eyes	MPS VI patients with no transplanted cornea N = 4	MPS VI patients with transplanted cornea (bilateral) N = 2	Control group N = 6
Mean (standard deviation)	12.43 ± 7.85	0.46 ± 0.65	0.00 ± 0.00
median [minimum, maximum]	13.35 [1.16, 22.18]	0.00 [0.00, 0.65]	0.00
p-value (Fisher's exact test)	0.033		

COM: corneal opacity measure, MPS VI: mucopolysaccharidosis type VI, N: number of subjects.

While all patients in the MPS VI group had been on long-term ERT, higher leukoCS and leukoDS levels suggest the opportunity for further improvement and the potential to reduce these levels with a new treatment. New treatment modalities including small molecules could be an additional treatment option for those patients currently on ERT but not adequately controlled. Furthermore, to monitor the efficacy of new treatments, new biomarkers that are compatible with the mechanism of new drugs are necessary. LeukoGAG could be a useful alternative biomarker to reflect intracellular GAG accumulation and to monitor the efficacy of new therapies with a different mechanism of action from ERT.

An important limitation of this study is the lack of a treatment-naïve MPS VI patient group. However, it was impossible to recruit treatment-naïve patients during the short study periods given the rarity of MPS VI (estimated incidence of 0.36–1.3/100,000 live births) [1]. In Study 2, we assessed changes in intracellular GAG accumulation and enzyme activity by measuring leukoGAG and ASB activity in leukocytes at each time point from initiation of the enzyme infusion to 48 h after infusion. ERT induced a transient increase in leukoASB enzyme activity at 1 h (7-fold increase compared to pre-infusion level), which decreased over 48 h

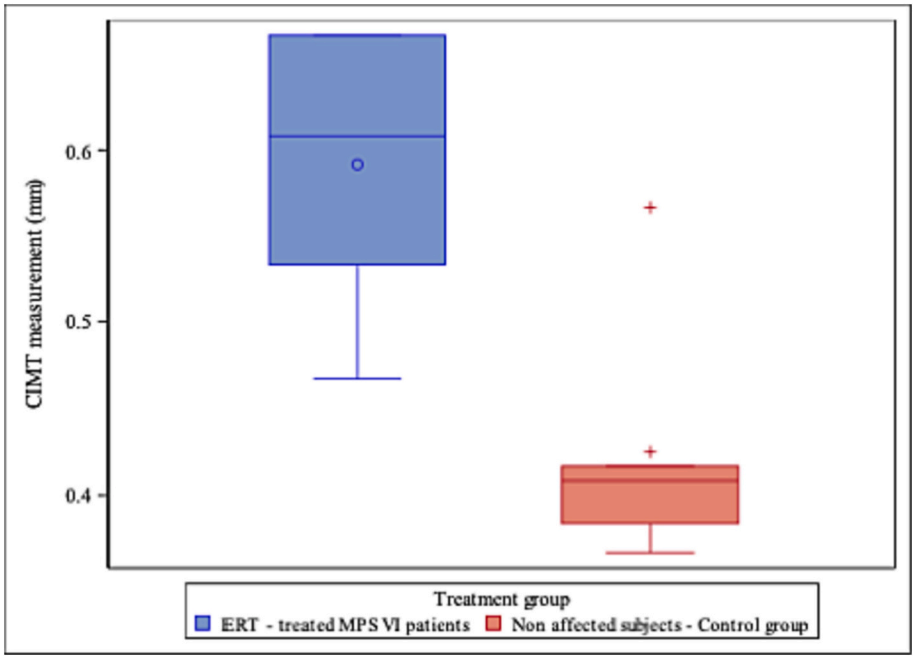

Fig. 3. Carotid intima media thickness in each group.
CIMT was significantly higher in MPS VI patients compared with control subjects (0.592 ± 0.087 mm and 0.425 ± 0.072 mm, respectively, $p = 0.0192$, Wilcoxon test).
CIMT: carotid intima media thickness, ERT: enzyme replacement therapy, MPS VI: mucopolysaccharidosis type VI.

(3-fold increase compared to pre-infusion level) even though it did not affect the level of any leukoGAG component over 48 h after administration. However, further study in treatment-naïve patients is warranted. In addition, none of the components of skinGAG were increased in MPS VI patients compared with control subjects. However, this may have occurred because all patients in the MPS VI group had been on long-term ERT given that skin is a "well-penetrated" tissue with ERT. To evaluate the usefulness of skinGAG as a biomarker, further study in a treatment-naïve patient cohort also is warranted.

Corneal clouding typically is the first ocular manifestation of MPS VI and can result in visual impairment with disease progression [20]. The cause of corneal opacification is GAG accumulation in the corneal stroma affecting keratocyte size and disrupting the network of collagen fibrils in the stroma [21]. Although ERT is a current standard treatment for MPS VI, it could not prevent the progressive corneal clouding leading to loss of vision, because the cornea is a poorly vascularized tissue. To date, corneal transplantation is the only available treatment option for vision restoration in MPS VI patients [20]. However, corneal transplantation is not curative because GAG re-accumulation occurs in transplanted cornea [20,22].

Recently, orally administered odiparcil, a new small molecule therapy for metabolizing GAG, was effective for improving corneal phenotype by reducing the GAG accumulation and restoring corneal structure in a MPS VI murine model [20]. Other studies have confirmed beneficial effects of odiparcil in animal models of MPS VI [15,17,23]. Evidence for improvement in COM was observed in a Phase 2a study of odiparcil in patients with MPS VI [24]. To apply the new therapy in the clinical setting, a quantitative measurement is necessary to evaluate and monitor the severity of corneal clouding in MPS VI patients. The COM score using iris recognition camera technology is one objective quantitative measurement system used for evaluating the severity of corneal opacification [18,25]. COM scores were significantly higher in MPS VI patients who had not received corneal transplantation compared with control subjects (Table 3). This indicates that corneal opacification was not effectively corrected despite long-term ERT, and the COM score could be a useful clinical parameter for evaluation and monitoring the severity of corneal clouding in patients with MPS VI.

Cardiovascular diseases including valvular insufficiency, ventricular dysfunction, sudden cardiac death, and coronary intimal medial proliferation frequently cause mortality in patients with MPS disorders despite ERT or hematopoietic stem cell transplantation (HSCT) [26–30]. Arterial luminal stenosis in MPS patients caused by intimal medial proliferation is recognized as an underlying cardiovascular pathology [31], and we evaluated the CIMT as a clinical parameter for MPS VI patients. The CIMT was significantly higher in MPS VI patients compared with control subjects (Fig. 3). This finding was in good agreement with previous studies demonstrating higher CIMT in various types of MPS patients compared with controls [19,30,32]. In the Phase 2 study, improvement in CIMT was observed in patients treated with odiparcil [24]. CIMT is correlated with carotid pathology and has been used as a marker predictive of cerebrovascular accidents in other chronic conditions including diabetes mellitus and hypertension [32–34]. CIMT measured using non-invasive carotid artery ultrasonography could be a feasible in vivo marker reflecting GAG accumulation and intimal medial proliferation in MPS VI patients. In addition, the significantly higher CIMT in our MPS VI patients who were on long-term ERT, indicate the unmet needs for new therapeutic modalities targeting cardiovascular disease. Any new therapy should improve treatment outcomes by reaching poorly vascularized tissues and reduce tissue GAG [30].

The limitations of this study include small numbers of patients, absence of treatment-naïve patients, and use of a cross-sectional study design. Evaluation of the usefulness of leukoGAG and skinGAG as novel biomarkers in treatment-naïve patients in comparison with a control group would be necessary to draw a firm conclusion. Longitudinal follow-up studies also are needed to monitor the changes in leukoGAG and skinGAG levels from the time at diagnosis and throughout any treatment in a larger treatment-naïve patient cohort.

In conclusion, this study highlights the potential of leukoGAG as well as COM and CIMT as biomarkers representing intracellular GAG accumulation in MPS VI patients.

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Declaration of Competing Interest

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Data sharing statement

Data from this study are available from the study protocol, statistical analysis plan, and manuscript. The sponsor will review requests for additional data upon which this manuscript is based and may provide these data for review purposes.

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References

[1] P. Harmatz, R. Shediac, Mucopolysaccharidosis VI: pathophysiology, diagnosis and treatment, Front. Biosci. (Landmark Ed) 22 (2017) 385–406 (In eng), https://doi.org/10.2741/4490.

[2] E.F. Neufeld, J. Muenzer, The mucopolysaccharidoses, in: C. Scriver, A. Beaudet, W. Sly (Eds.), The Metabolic and Molecular Bases of Inherited Disease, McGraw Hill, New York, 2001, pp. 3421–3452.

[3] V. Valayannopoulos, H. Nicely, P. Harmatz, S. Turbeville, Mucopolysaccharidosis VI, Orphanet J. Rare Dis. 5 (2010) 5 (In eng), https://doi.org/10.1186/1750-1172-5-5.

[4] R. Giugliani, P. Harmatz, J.E. Wraith, Management guidelines for mucopolysaccharidosis VI, Pediatrics 120 (2) (2007) 405–418 (In eng), https://doi.org/10.1542/peds.2006-2184.

[5] P. Harmatz, W.G. Kramer, J.J. Hopwood, J. Simon, E. Butensky, S.J. Swiedler, Pharmacokinetic profile of recombinant human N-acetylgalactosamine 4-sulphatase enzyme replacement therapy in patients with mucopolysaccharidosis VI (Maroteaux-Lamy syndrome): a phase I/II study, Acta Paediatr. Suppl. 94 (447) (2005) 61–68, discussion 57. (In eng), https://doi.org/10.1111/j.1651-2227.2005.tb02115.x.

[6] P. Harmatz, R. Giugliani, I. Schwartz, et al., Enzyme replacement therapy for mucopolysaccharidosis VI: a phase 3, randomized, double-blind, placebo-controlled, multinational study of recombinant human N-acetylgalactosamine 4-sulfatase (recombinant human arylsulfatase B or rhASB) and follow-on, open-label extension study, J. Pediatr. 148 (4) (2006) 533–539 (In eng), https://doi.org/10.1016/j.jpeds.2005.12.014.

[7] P. Harmatz, R. Giugliani, I.V.D. Schwartz, et al., Long-term follow-up of endurance and safety outcomes during enzyme replacement therapy for mucopolysaccharidosis VI: final results of three clinical studies of recombinant human N-acetylgalactosamine 4-sulfatase, Mol. Genet. Metab. 94 (4) (2008) 469–475 (In eng), https://doi.org/10.1016/j.ymgme.2008.04.001.

[8] J. Muenzer, Early initiation of enzyme replacement therapy for the mucopolysaccharidoses, Mol. Genet. Metab. 111 (2) (2014) 63–72 (In eng), https://doi.org/10.1016/j.ymgme.2013.11.015.

[9] D. Auclair, J.J. Hopwood, D.A. Brooks, J.F. Lemontt, A.C. Crawley, Replacement therapy in Mucopolysaccharidosis type VI: advantages of early onset of therapy, Mol. Genet. Metab. 78 (3) (2003) 163–174 (In eng), https://doi.org/10.1016/s1096-7192(03)00007-6.

[10] T. Ruane, M. Haskins, A. Cheng, et al., Pharmacodynamics, pharmacokinetics and biodistribution of recombinant human N-acetylgalactosamine 4-sulfatase after 6months of therapy in cats using different IV infusion durations, Mol. Genet. Metab. 117 (2) (2016) 157–163 (In eng), https://doi.org/10.1016/j.ymgme.2015.10.006.

[11] M.M. Brands, I.M. Frohn-Mulder, M.L. Hagemans, et al., Mucopolysaccharidosis: cardiologic features and effects of enzyme-replacement therapy in 24 children with MPS I, II and VI, J. Inherit. Metab. Dis. 36 (2) (2013) 227–234 (In eng), https://doi.org/10.1007/s10545-011-9444-z.

[12] E. Braunlin, H. Rosenfeld, C. Kampmann, et al., Enzyme replacement therapy for mucopolysaccharidosis VI: long-term cardiac effects of galsulfase (Naglazyme®) therapy, J. Inherit. Metab. Dis. 36 (2) (2013) 385–394 (In eng), https://doi.org/10.1007/s10545-012-9481-2.

[13] F. D'Avanzo, A. Zanetti, C. De Filippis, R. Tomanin, Mucopolysaccharidosis Type VI, an updated overview of the disease, Int. J. Mol. Sci. 22 (24) (2021), https://doi.org/10.3390/ijms222413456 (In eng).

[14] T.J. Lehman, N. Miller, B. Norquist, L. Underhill, J. Keutzer, Diagnosis of the mucopolysaccharidoses, Rheumatology (Oxford) 50 (Suppl. 5) (2011) v41–v48 (In eng), https://doi.org/10.1093/rheumatology/ker390.

[15] P.F. Jezyk, M.E. Haskins, D.F. Patterson, W.J. Mellman, M. Greenstein, Mucopolysaccharidosis in a cat with arylsulfatase B deficiency: a model of Maroteaux-Lamy syndrome, Science 198 (4319) (1977) 834–836 (In eng), https://doi.org/10.1126/science.144321.

[16] J. Alroy, G.O. Freden, V. Goyal, S.S. Raghavan, K.L. Schunk, Morphology of leukocytes from cats affected with alpha-mannosidosis and mucopolysaccharidosis VI (MPS VI), Vet. Pathol. 26 (4) (1989) 294–302 (In eng), https://doi.org/10.1177/030098588902600402.

[17] M. Yoshida, J. Noguchi, H. Ikadai, M. Takahashi, S. Nagase, Arylsulfatase B-deficient mucopolysaccharidosis in rats, J. Clin. Invest. 91 (3) (1993) 1099–1104 (In eng), https://doi.org/10.1172/jci116268.

[18] T.M. Aslam, S. Shakir, J. Wong, L. Au, J. Ashworth, Use of iris recognition camera technology for the quantification of corneal opacification in mucopolysaccharidoses, Br. J. Ophthalmol. 96 (12) (2012) 1466–1468 (In eng), https://doi.org/10.1136/bjophthalmol-2011-300996.

[19] R.Y. Wang, E.A. Braunlin, K.D. Rudser, et al., Carotid intima-media thickness is increased in patients with treated mucopolysaccharidosis types I and II, and correlates with arterial stiffness, Mol. Genet. Metab. 111 (2) (2014) 128–132 (In eng), https://doi.org/10.1016/j.ymgme.2013.11.001.

[20] E. Entchev, S. Antonelli, V. Mauro, et al., MPS VI associated ocular phenotypes in an MPS VI murine model and the therapeutic effects of odiparcil treatment, Mol. Genet. Metab. 135 (2) (2022) 143–153 (In eng), https://doi.org/10.1016/j.ymgme.2021.07.008.

[21] K.T. Fahnehjelm, J.L. Ashworth, S. Pitz, et al., Clinical guidelines for diagnosing and managing ocular manifestations in children with mucopolysaccharidosis, Acta Ophthalmol. 90 (7) (2012) 595–602 (In eng), https://doi.org/10.1111/j.1755-3768.2011.02280.x.

[22] S. Ferrari, D. Ponzin, J.L. Ashworth, et al., Diagnosis and management of ophthalmological features in patients with mucopolysaccharidosis, Br. J. Ophthalmol. 95 (5) (2011) 613–619 (In eng), https://doi.org/10.1136/bjo.2010.179937.

[23] E. Entchev, I. Jantzen, P. Masson, S. Bocart, B. Bournique, J.M. Luccarini, A. Bouchot, O. Lacombe, J.L. Junien, P. Broqua, M. Tallandier, Odiparcil, a potential glycosaminoglycans clearance therapy in mucopolysaccharidosis VI-Evidence from in vitro and in vivo models, PLoS One 15 (5) (2020), e0233032, https://doi.org/10.1371/journal.pone.0233032. PMID: 32413051.

[24] N. Guffon, P. Chowdary, E.L. Teles, D. Hughes, J.B. Hennermann, P. Huot-Marchand, E. Faudot-Vernier, O. Lacombe, A. Fiquet, M.P. Richard, J.L. Abitbol, M. Tallandier, C.J. Hendriksz, Oral treatment for mucopolysaccharidosis VI: outcomes of the first phase IIa study with odiparcil, J. Inherit. Metab. Dis. 45 (2) (2022) 340–352, https://doi.org/10.1002/jimd.12467.

[25] K. Sornalingam, A. Javed, T. Aslam, et al., Variability in the ocular phenotype in mucopolysaccharidosis, Br. J. Ophthalmol. 103 (4) (2019) 504–510 (In eng), https://doi.org/10.1136/bjophthalmol-2017-311749.

[26] H.Y. Lin, S.P. Lin, C.K. Chuang, M.R. Chen, B.F. Chen, J.E. Wraith, Mucopolysaccharidosis I under enzyme replacement therapy with laronidase – a mortality case with autopsy report, J. Inherit. Metab. Dis. 28 (6) (2005) 1146–1148 (In eng), https://doi.org/10.1007/s10545-005-0211-x.

[27] L. van den Broek, A.P. Backx, H. Coolen, et al., Fatal coronary artery disease in an infant with severe mucopolysaccharidosis type I, Pediatrics 127 (5) (2011) e1343–e1346 (In eng), https://doi.org/10.1542/peds.2009-2047.

[28] M. Aldenhoven, R.F. Wynn, P.J. Orchard, et al., Long-term outcome of hurler syndrome patients after hematopoietic cell transplantation: an international multicenter study, Blood 125 (13) (2015) 2164–2172 (In eng), https://doi.org/10.1182/blood-2014-11-608075.

[29] H.Y. Lin, C.K. Chuang, Y.H. Huang, et al., Causes of death and clinical characteristics of 34 patients with Mucopolysaccharidosis II in Taiwan from 1995-2012, Orphanet J. Rare Dis. 11 (1) (2016) 85 (In eng), https://doi.org/10.1186/s13023-016-0471-6.

[30] R.Y. Wang, K.D. Rudser, D.R. Dengel, et al., The carotid intima-media thickness and arterial stiffness of pediatric mucopolysaccharidosis patients are increased compared to both pediatric and adult controls, Int. J. Mol. Sci. 18 (3) (2017), https://doi.org/10.3390/ijms18030637 (In eng).

[31] E. Braunlin, P.J. Orchard, C.B. Whitley, L. Schroeder, R.C. Reed, J.C. Manivel, Unexpected coronary artery findings in mucopolysaccharidosis. Report of four cases and literature review, Cardiovasc. Pathol. 23 (3) (2014) 145–151 (In eng), https://doi.org/10.1016/j.carpath.2014.01.001.

[32] R.Y. Wang, K.K. Covault, E.M. Halcrow, et al., Carotid intima-media thickness is increased in patients with mucopolysaccharidoses, Mol. Genet. Metab. 104 (4) (2011) 592–596 (In eng), https://doi.org/10.1016/j.ymgme.2011.09.004.

[32] M.W. Lorenz, H.S. Markus, M.L. Bots, M. Rosvall, M. Sitzer, Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis, Circulation 115 (4) (2007) 459–467 (In eng), https://doi.org/10.1161/circulationaha.106.628875.

[33] A. Ayuna, K.M. Stepien, C.J. Hendriksz, M. Balerdi, A. Garg, P. Woolfson, Cardiac rhythm abnormalities - an underestimated cardiovascular risk in adult patients with Mucopolysaccharidoses, Mol. Genet. Metab. 130 (2) (2020) 133–139, https://doi.org/10.1016/j.ymgme.2020.03.005.

[34] K.M. Stepien, E.A. Braunlin, Unmet cardiac clinical needs in adult mucopolysaccharidoses, Front. Cardiovasc. Med. 9 (2022), 907175, https://doi.org/10.3389/fcvm.2022.907175 (PMID: 35757333).