


A 9-Month-Old with Skeletal Abnormalities and a Consanguineous Sibling with Mucopolysaccharidosis IVA: The Role of Urinary Glycosaminoglycan Testing in Disease Diagnosis and Treatment Monitoring

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ABSTRACT: Mucopolysaccharidosis IVA (MPS IVA) is a rare autosomal recessive lysosomal storage disorder resulting from N-acetylgalactosamine-6-sulfatase (GALNS) deficiency that occurs in approximately 1 in 76 000 to 1 in 640 000 live births. Given that the diagnosis of MPS IVA relies heavily on the results of initial urine glycosaminoglycan (GAG) screening, cases that present with falsely normal urine GAG concentrations can delay the diagnosis and follow-up care for patients. This case study follows a patient diagnosed with MPS IVA at 9 months of age based on relation to a consanguineous 3-year-old sibling with MPS IVA and the use of direct enzyme activity analysis. Details regarding skeletal presentation and identification of genetic variants are presented along with data on follow-up urinary GAG monitoring during treatment with enzyme replacement therapy and treatment for a growth hormone disorder.

KEYWORDS: Mucopolysaccharidosis IVA, glycosaminoglycan, N-acetylgalactosamine-6-sulfatase (GALNS), keratan sulfate, growth hormone deficiency, enzyme replacement therapy

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Clinical History

A 9-month-old female with flared ribs and kyphosis of the spine was referred for a medical genetics consultation based on a recent diagnosis of mucopolysaccharidosis IVA (MPS IVA) in a consanguineous 3-year-old sibling who exhibited similarly flared ribs and kyphosis as well as platyspondyly, genu valgum, dystrophy of the femoral heads, and short stature in the absence of observable cognitive impairment (Table 1). A sagittal T2 fat-suppressed image obtained from the younger sibling at 20 months of age demonstrating a classical cartilage cap causing mild narrowing at the craniocervical junction is shown in Figure 1a. Typical thoracolumbar spine changes and increased acetabular angles are evident in the lateral spine and pelvis radiographs in Figure 1b and c.

A diagnosis of MPS IVA in the older sibling was supported by elevated quantitative urinary glycosaminoglycan (GAG) concentration (20.4 mg/mmol creatinine; reference range ≤ 16 mg/mmol) without qualitative increases in individual GAGs by qualitative electrophoresis. The MPS IVA diagnosis was finalized based on the results from a direct whole blood N-acetylgalactosamine-6-sulfatase (GALNS) enzyme activity assay demonstrating severely deficient GALNS activity (0.7054 nmol 4MU (4-methylumbelliferone) released/h/mg; reference range 82.9–254.3 nmol 4MU/h/mg). To confirm a preliminary diagnosis of MPS IVA in the younger sibling, a direct enzyme activity assay was conducted using a leukocyte

sample which resulted in a GALNS activity of 1.0 nmol 4MU released/h/mg (reference range 82.9–254.3 nmol 4MU/h/mg), indicating nearly complete loss of GALNS activity. The *GALNS* genetic sequencing results using polymerase chain reaction (PCR) coupled with capillary electrophoresis revealed 14 identical *GALNS* variants (6 of which were intronic) in both siblings, including a heterozygous Gly247Asp variant known to be pathogenic¹ and a heterozygous Tyr240Cys variant predicted to be pathogenic through amino acid substitution analysis.² The patients' other genetic variants were of unknown significance.

The patient was provided supportive treatment as needed for complications of MPS IVA including pain medication and close follow-up by cardiac, pulmonary, ENT, and other specialists. Frequent ear and lung infections were treated as needed, and surgical repair of the eardrum, and surgery for closure of the ductus arteriosus was performed. The patient was enrolled in a Phase 3 enzyme replacement therapy (ERT) clinical trial of elosulfase alfa (Vimizim[®]), a recombinant GALNS enzyme, to reduce the severity of MPS IVA symptoms. The elosulfase alfa dosage and administration were adjusted based on the response to treatment, and the therapeutic regimen included monthly urinary GAG testing using 24-hour urine samples. ERT treatments are continued to date.

Prior to the availability of more sensitive and specific analytical methods, the urine samples were tested in 3 different



Table 1. Patient Demographic Information and Testing Results at Time of Diagnosis and Following MPS IVA Treatment Initiation.

	YOUNGER SIBLING	OLDER SIBLING
Birth length	57.2 cm	58.4 cm
Birth weight	4.8 kg	4.3 kg
Presentation at diagnosis		
Age	9 months	3 y
Height	75.5 cm	100.0 cm
Weight	8.5 kg	19.0 kg
Symptoms	<ul style="list-style-type: none"> Slightly flared ribs and mild to moderate lumbar kyphosis Slightly delayed developmental milestones of sitting upright and bearing weight Neurologically interactive 	<ul style="list-style-type: none"> Inferior rib flaring and marked, moderate spinal kyphosis Normal developmental milestones; slight limping gait with genu valgum Normal cognitive development Shortened neck and extremities Generalized platyspondyly Motor weakness Leg pain with decreased endurance Hyperlaxity of arm and shoulder joints
uGAG	NA*	Initial: 20.4 ng/mmol (≤ 16) Resample (1 mo later): 40.0 ng/mmol (≤ 16)
Enzyme concentrations	<ul style="list-style-type: none"> GALNS: 1.0 nmol 4MU/h/mg protein (82.9-254.3). β-Galactosidase: NA* 	<ul style="list-style-type: none"> GALNS: 0.7 nmol 4MU/h/mg protein (82.9-254.3) β-Galactosidase: 75.1 4MU/h/mg protein (24.4-196.3)
GALNS sequencing (known causative mutations)	<ul style="list-style-type: none"> p. Gly247Asp p. Tyr240Cys 	<ul style="list-style-type: none"> p. Gly247Asp p. Tyr240Cys
Treatment response assessment		
Duration of ERT	7 y	
Duration of GHT	2 y	
Height	8 y old: 131.3 cm 9 y old (1 y after GHT): 137.0 cm	
Weight	8 y old: 25.9 kg 9 y old (1 y after GHT): 30.5 kg	
IGF concentrations	6 y old: 148 ng/dL (45-316) 8 y old: 170 ng/dL (76-424) 9 y old (1 y after GHT): 313 ng/dL (99-432)	

Abbreviations: NA*, test not performed. Reference ranges are shown in parentheses. uGAG, urine glycosaminoglycan; ERT, enzyme replacement therapy; GHT, growth hormone replacement therapy; IGF, insulin growth factor; 4MU, 4-methylumbelliferone.

reference labs with the use of a cetylpyridinium chloride (CPC) with carbazole or a dimethylene blue (DMB) assay (Figure 2). Total urinary GAG concentrations were measured using the DMB method a total of 17 times over a period of 32 months at Reference Lab 1 and a total of 36 times over a period of

73 months at Reference Lab 2 (Figure 2a). As an example of the variability in the performance of the DMB method, the urinary GAG concentration in 1 sample was determined to be within the reference range when analyzed at Reference Lab 1 (15.2 mg/mmol Cr; reference range 5.2-16.7 mg/mmol Cr),

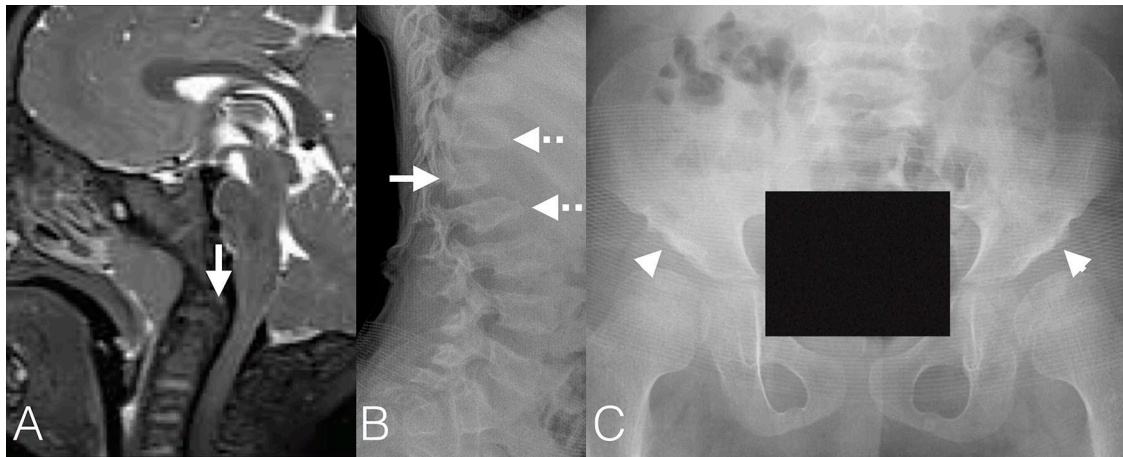


Figure 1. Representative MRI and x-ray images demonstrating classical MPS IVA features. (A) Sagittal T2 fat-suppressed image obtained at 20 months of age demonstrating a classical cartilage cap (arrow) causing mild narrowing at the craniocervical junction. (B) Lateral spine radiograph obtained at 4 years of age demonstrating typical thoracolumbar spine changes including anterior vertebral body beaking (dashed arrows) and a short vertebral body/gibbus deformity (arrow) resulting in acute kyphosis. (C) Pelvis radiograph obtained at 4 years of age demonstrating the classic appearance of increased acetabular angles (arrowheads).

but the concentration was 34% above the reference range when the same sample was analyzed at Reference Lab 2 (21.5 mg/mmol Cr; reference range ≤ 16.0 mg/mmol Cr). Other similar discrepancies in urinary GAG concentrations were observed when comparing the results from the DMB method with the results from the CPC method. In one urine sample measured using the DMB method, the GAG concentration was 23.6 mg/mmol Cr (reference range ≤ 16.0 mg/mmol Cr), whereas the GAG concentration in the same sample analyzed using the CPC method was 1.1 mg/mmol Cr (reference range ≤ 8.7 mg/mmol Cr). All urine specimens tested by the CPC method were also assayed using electrophoresis, and they were noted to have qualitatively increased chondroitin-6-sulfate (C6S) and keratan sulfate (KS) concentrations (Figure 2b). Four of the nine urine samples analyzed using the CPC method had total urinary GAG concentrations within the reference range despite having qualitatively increased C6S and KS concentrations as determined by electrophoresis.

Patient Follow-up

The increasing role of liquid chromatography tandem mass spectrometry (LC-MS/MS) in the clinical laboratory has led to the enhanced analytical sensitivity and specificity of many assays, including those that are used to monitor urinary GAG concentrations. Beginning 6 years (74 months) after the patient's urinary GAG concentrations were initially monitored on a monthly basis using the DMB and/or CPC method, assessment of the patient's urinary GAG concentration has been conducted using LC-MS/MS on an approximately monthly basis (Figure 3). Details of the LC-MS/MS testing methodology are not provided due to the proprietary nature of the method. To date, the dermatan sulfate (DS) and heparan sulfate (HS) concentrations in each urine specimen have been within the respective reference ranges as expected for MPS IVA, whereas the C6S and KS concentrations have been above

the respective reference ranges. Due to the large contribution of C6S to the total GAG concentration, small elevations of KS above the reference range, such as at month 77, could appear normal when evaluating the total GAG concentration whereas evaluation of individual GAG concentrations could identify the abnormally increased KS (Figure 3).

At 8 years of age, the patient was evaluated for growth hormone deficiency (GHD) due to concerns regarding decreased growth velocity (Figure 4) and IGF-1 concentrations (Table 1) that were within the age-related reference ranges but not increasing with approach to puberty. Random measurement of growth hormone was not conducted due to its pulsatile secretion. In the absence of stimulation, measurements of growth hormone concentrations in the blood are difficult to interpret and have little clinical utility. A growth hormone stimulation test was conducted to assess the anterior pituitary's ability to secrete sufficient growth hormone in response to intravenous administration of clonidine and arginine (Figure 5). Growth hormone was measured at 30-minute intervals over a span of 4 hours. The growth hormone stimulation test results showed a peak of 8.6 ng/mL in response to clonidine and a peak of 5.9 ng/mL in response to arginine. A response of <10 ng/mL to both tests was consistent with GHD and the patient was prescribed Somatropin as part of her growth hormone replacement therapy (GHT). Within 1 year of the commencement of GHT, the patient's IGF-1 concentrations increased from 170 ng/dL at 8 years of age (4 months prior to GHT) (reference range: 58–367 ng/dL) to 313 ng/dL at 9 years of age (reference range: 99–432 ng/dL). GHT is continued to date.

The patient is continuing weekly elosulfase alfa infusions which have been well-tolerated based on the absence of fever, chills, productive cough, abnormal vital signs, night sweats, chest pain, decreased appetite, and unexplained weight loss or fatigue. ERT has been generally effective in managing her MPS IVA symptoms. Daily Somatropin injections have also

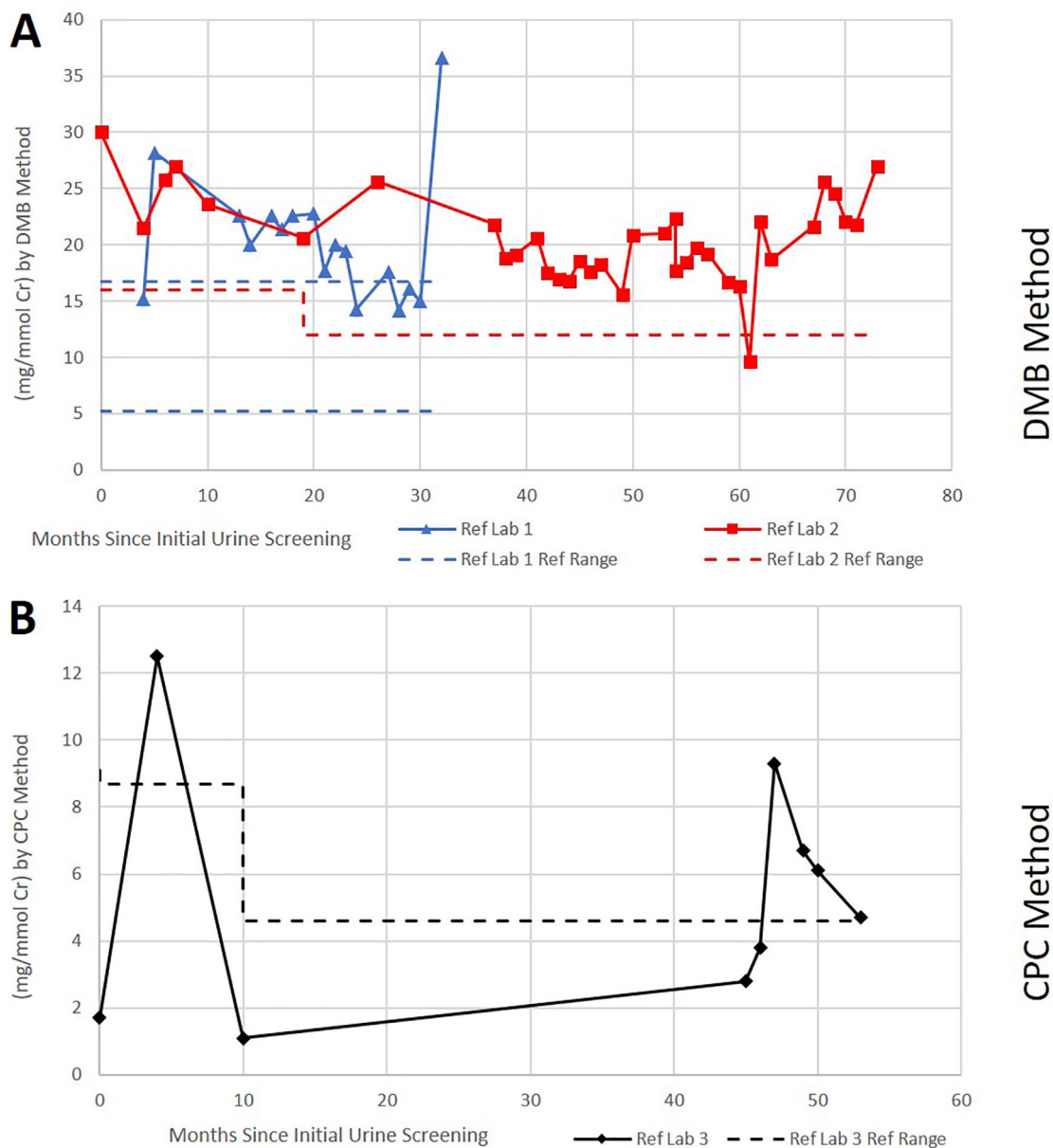


Figure 2. Urine GAG measurements. Urinary GAG concentrations were measured using the DMB (A) and CPC (B) method. The younger sibling was receiving ERT at all times of measurement. Abbreviation: Cr, creatinine.

continued without any adverse medical complications. The prescribed dose of Somatropin is being maximized to coincide with her rapid growth plate maturation during puberty, and her height is within the 40th percentile of healthy females.

Laboratory Role in Diagnosis and Treatment of MPS IVA

Given that MPS IVA is primarily caused by the excess build-up and deposition of KS in cartilaginous tissue, urine screening methods which seek to detect MPS disorders based on total GAG concentration alone may result in false negative MPS IVA diagnoses due to the smaller contribution of KS to the total GAG concentration as opposed to other GAGs such as DS and HS that contribute to the pathophysiology of other

MPS disorders.⁴ In these cases, qualitative tests such as thin-layer chromatography (TLC) or urine electrophoresis facilitate the improved detection of KS in the urine despite normal total GAG concentration. LC-MS/MS has become more frequently used due to the increased accuracy of results as well as its ability to measure individual GAG subclass concentrations as opposed to total GAG concentration. GAG subclass quantification by LC-MS/MS can be conducted using several specimen types including serum, urine, CSF, and tissue. Various LC-MS/MS techniques have been developed for GAG subclass quantification and they differ based on the associated ionization method, sample preparation procedure, accuracy, and turnaround time.^{5,6}

The screening methods for MPS IVA include the use of CPC with carbazole, dye-specific methods such as DMB,

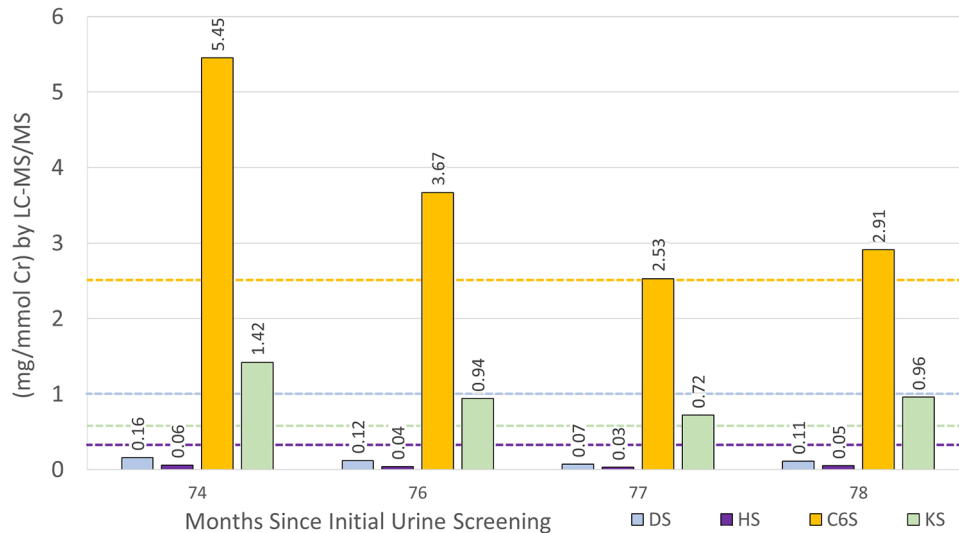


Figure 3. Urinary creatinine-normalized GAG concentrations determined by an LC-MS/MS-based laboratory developed test using enzymatic digestion of GAGs. Creatinine concentrations were determined using an enzymatic method conducted on an automated chemistry analyzer. Dermatan Sulfate (DS), Heparan Sulfate (HS), Chondroitin-6-Sulfate (C6S), and Keratan Sulfate (KS) concentrations of different 24-hour urine specimens were measured by LC-MS/MS. Reference Ranges: DS (≤ 1.00 mg/mmol Cr), HS (≤ 0.25 mg/mmol Cr), C6S (≤ 2.50 mg/mmol Cr), KS (≤ 0.50 mg/mmol Cr). The patient was receiving ERT at all times of measurement. Cr, creatinine.

paper and thin layer chromatography, enzyme-linked immunosorbent assay, capillary electrophoresis, and various mass spectrometry-coupled methods.⁵ The CPC method is based on the precipitation and isolation of GAGs present in a urine specimen and their subsequent reaction with a carbazole reagent to produce a green pigment that is directly proportional to GAG concentration. The reaction is then measured by spectrophotometry. A more recently developed version of the CPC method entails the addition of the precipitated GAGs to a citrate buffer followed by the spectrophotometric measurement of the solution based on turbidity.⁷⁻⁹ However, the CPC method is associated with a high false negative rate of 11% to 30%.¹⁰ The alternative DMB method is a dye-binding method wherein urinary GAGs react with DMB to produce a color change measurable by spectrophotometry.^{8,9,11} The false-negative rate of MPS IVA diagnosis using the DMB method was observed to be as high as 65%.¹² The false-negative results associated with the DMB method are potentially due to macromolecular aggregates that are formed by electrostatic interactions with collagen, glycoproteins, albumin, or other serum proteins which could modify the physicochemical properties of GAGs.¹³ Although the DMB method is the most widely used analytical method for urinary GAG quantification, LC-MS/MS methods are increasingly used in clinical laboratories due to their improved accuracy, sensitivity, and specificity.^{4,14} Irrespective of the method used to quantify GAGs in urine, the interpretation of the results can be complicated by the age-specific reference ranges.¹⁵

The screening methods for diagnosing MPS IVA have less than ideal reliability, but the tests are also challenged by the difficulty in preserving specimen integrity and the consistency between clinical laboratories that use different methods. Dried

whole blood spot patient samples can be sent to reference laboratories for direct enzyme activity assays, but GALNS enzyme activity can appear falsely decreased if the sample is exposed to high heat during transportation to the laboratory where the test is conducted.¹⁶

To confirm a positive urinary GAG screening test or the results from direct testing when evaluating the sibling of an affected individual with a confirmed MPS IVA diagnosis - as was the case for the patient presented in this case report—a GALNS enzymatic activity assay using fibroblasts or leukocytes from a concentrated whole blood sample or skin biopsy is performed. Although fibroblasts and leukocytes are the preferred cell types for measuring GALNS activity, the tissue or blood sample required for the test must be obtained using invasive procedures, which are particularly concerning for young patients.¹⁶ Consequently, GALNS activity assays using these specimens are not among the MPS IVA screening methods. After confirming decreased GALNS activity, molecular sequencing can be used to identify genetic variants that cause the enzyme dysfunction.

Discussion

MPS IVA can be difficult to diagnose due to the broad spectrum in presentation of the disease as well as its similarity to other genetic skeletal disorders.¹⁷ Cases that lack signature features of MPS IVA such as genu valgum or atlantoaxial instability or are not assessed by complete radiographic analysis may have a delayed diagnosis.¹⁷ As a finalized diagnosis of MPS IVA relies on screening for increased KS concentration with no increase in DS or HS concentration, the CPC and DMB methods of analyzing urine are widely used for the preliminary identification MPS IVA. However, the high false negative rates

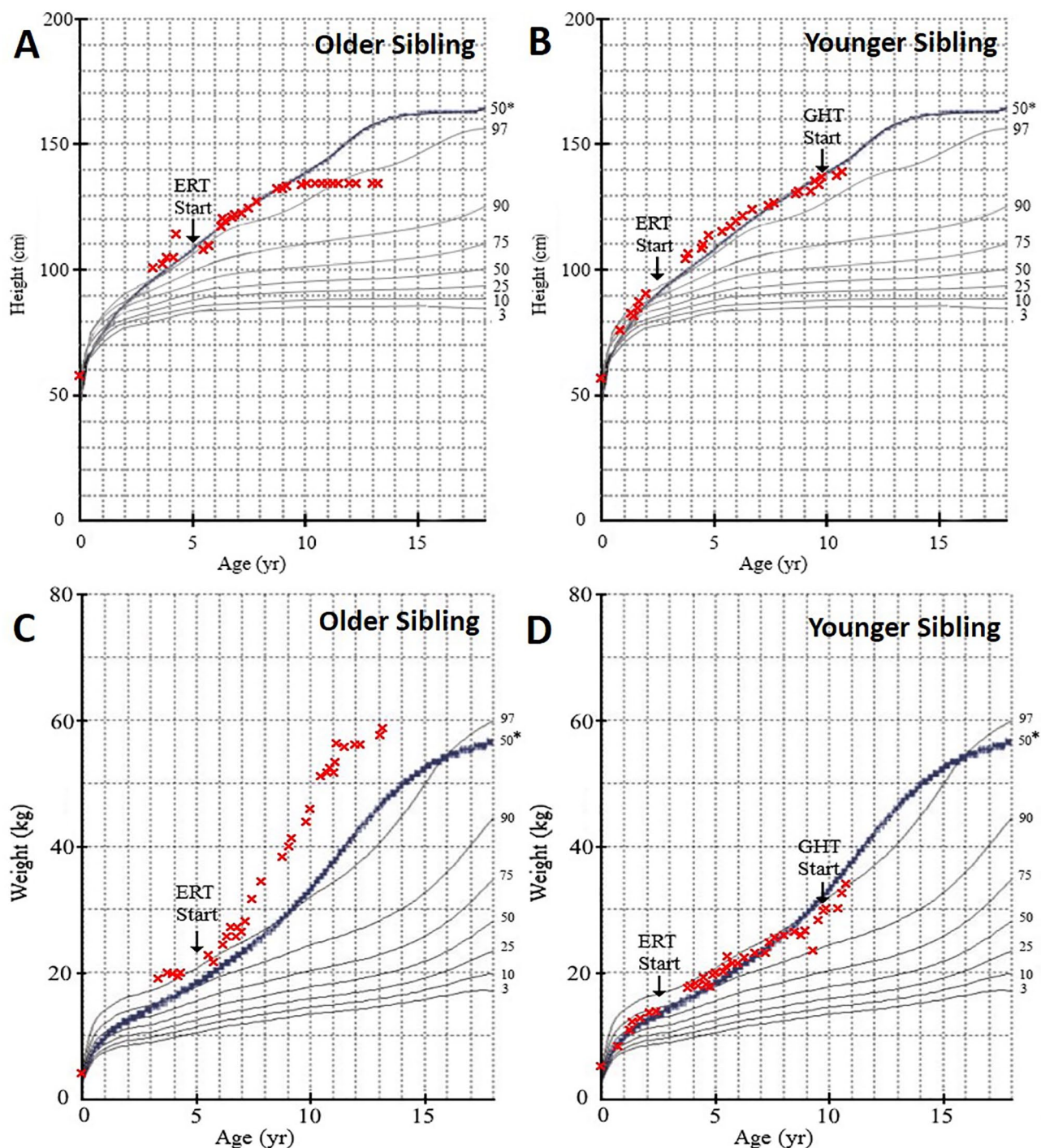


Figure 4. MPS IVA female height (A, B) and weight (C, D) growth charts of the older and younger siblings. 50*, the thicker line in each chart represents the 50th percentile height (A, B) or weight (C, D) of healthy females without MPS IVA. Figure modified with permission from *American Journal of Medical Genetics Part A*.³

of these tests threaten the ability to accurately diagnose MPS disorders. With the increased use of LC-MS/MS due to its adaptability to test multiple sample types and measure GAGs by subclass, the capability to correctly diagnose MPS disorders has increased in recent years.¹⁸

One of the greatest barriers to diagnosing MPS IVA is accurately identifying elevated KS despite its minimal contribution to total GAG quantification. A method proposed by

Lin et al¹⁹ suggests normalizing KS to CS concentration as opposed to creatinine concentrations due to age-, hydration-, renal state-, and muscle mass-based variations in creatinine concentration. However, there is a lack of consensus regarding whether urine KS concentrations are reliable for assessing patient status. KS is regularly stored in chondrocytes and the extracellular matrix, but in MPS patients where KS is not properly degraded, intact KS, instead of its degradation

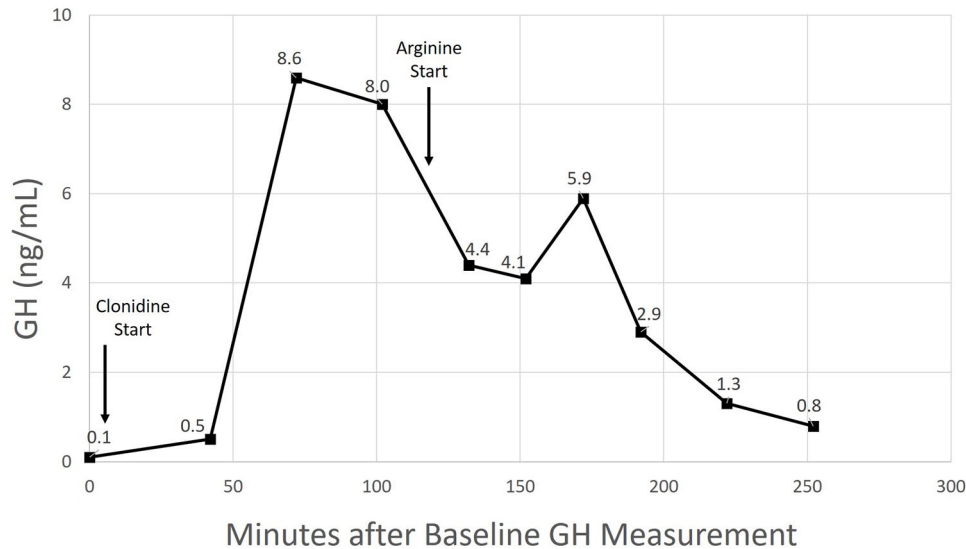


Figure 5. Growth hormone stimulation test results. Peak values < 10 ng/mL after administration of clonidine and after arginine indicate growth hormone deficiency.

Abbreviation: GH, growth hormone.

products, is secreted into the blood at an increased rate. Patients with elongating long bones, particularly patients from ages 5 to 15, have significantly higher blood and urine KS concentrations due to the rapid degradation and replacement of cartilaginous tissue, which explains why individuals outside of this age range are likely to have KS concentrations closer to the reference ranges for healthy individuals.²⁰ There is a poor correlation between blood KS and urine KS concentrations with indications that urine KS concentrations may not be dependent on glomerular filtration and may originate in the kidney as opposed to blood KS origination.²⁰⁻²² For these reasons, blood-based KS quantification has become more widely adopted due to its capability to better represent systemic KS levels.²³ GAGs were only measured in urine samples, and not blood samples, for the patient described in this case study.

C6S is known to accumulate along with KS in MPS IVA patients, but it is not as frequently assayed as other GAGs such as KS due to its *in vivo* degradation by enzymes other than GALNS, its implication in other MPS disorders, and the history of its assay relying on a radio-labeled substrate.⁴ However, C6S is very relevant for distinguishing cases of MPS IVA from MPS IVB, and other applications for C6S quantification have been proposed. Shimada et al²⁴ explored the utilization of a C6S assay to identify cases of MPS IVA, and Lin et al¹⁹ studied the normalization of KS to C6S to improve the consistency of urinary GAG assays that commonly entail normalization to creatinine.

In the case presented here, the diagnosis of the patient was reliant on the correct diagnosis of the older sibling with MPS IVA. The older sibling's quantitative urine GAG screening results were slightly above the reference range and they were associated with ambiguous urinary GAG qualitative electrophoresis results. Confirmatory testing by direct enzyme activity

analysis was performed to ensure a true positive diagnosis of MPS IVA. Although urinary GAG screening was not performed as part of the diagnostic workup of MPS IVA in the younger sibling, a direct GALNS enzyme activity analysis was conducted which confirmed the MPS IVA diagnosis based on severely reduced GALNS activity.

The height of the patient featured in this case report exceeds the average height of MPS IVA patients as seen in Figure 4;³ however, she was diagnosed with a GHD. The incidence of GHD in individuals with MPS IVA is not well reported in the literature,^{25,26} possibly due to the difficulty in evaluating patient height and growth related to the skeletal abnormalities that are associated with MPS IVA or the delayed onset of puberty in MPS IVA patients. For monitoring patients diagnosed with MPS IVA, with the approval of elosulfase alfa to reduce the severity of MPS IVA symptoms, regular urine GAG quantification is commonly used to monitor the effects of ERT in reducing urinary KS concentration. Less severe MPS IVA symptom manifestation could be associated with decreased KS deposition in cartilaginous tissues as evidenced by reduced urinary KS concentrations.¹⁴ During Phase 3 elosulfase alfa ERT clinical trials, physicians noted that all patients produced antibodies, including those of the IgE subclass, in response to this treatment with no significant effect on drug efficacy or patient health,²⁷ but the occurrence of a rare transfusion reaction during intravenous administration of the recombinant enzyme might require specialized assays such as IgE quantification or drug immunogenicity tests.

ERT has been shown to improve the quality of life for MPS IVA patients by decreasing incidences of infection and pain while increasing physical endurance. For the patient who is the focus of this case report, it is difficult to determine whether decreased urine KS concentrations are directly attributable to

ERT as all urine quantifications were performed after the start of ERT. However, some degree of KS decrease is expected based on the conclusions from ERT clinical trials.²⁷ The pharmacodynamics of ERT and its effects on body GAG concentrations have not yet been fully elucidated. Khan et al¹⁴ performed KS quantification using urine and blood samples of patients treated with ERT and they noted a decrease in urine KS concentrations without a concomitant change in blood KS concentrations. The results from another ERT study showed a limited effect of ERT on bone and cartilage metabolism with an increase in PIIANP (type IIa collagen N propeptide) in an early dose-escalation trial.²⁸ The results from subsequent studies of the efficacy of ERT did not indicate any changes in PIIANP or C-terminal cross-linked C- telopeptide concentrations, which were used as indicators of increased bone and cartilage metabolism.

Hematopoietic stem cell transplantation (HSCT) is also worth noting as one of the available treatment options for individuals with MPS IVA.²⁹ Studies conducted by Yabe et al³⁰ have shown a few cases of restoration of complete GALNS activity in patients who underwent allogeneic bone marrow transplant with successful engraftment. However, HSCT is associated with a significant risk of death from graft-versus-host-disease, other transplant complications, and limited effects on pre-existing skeletal dysplasias. ERT remains the standard form of treatment for MPS IVA patients due to its relative safety and minimal risk.


Author Contributions

EG, KD, and SNT analyzed the data. EG, AV, KD, and SNT wrote the manuscript, made critical revisions, and approved the final version.

Informed Consent

Consent from the patients' parents was secured to publish the findings of this case study.

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REFERENCES

- Bunge S, Kleijer WJ, Tylki-Szymanska A, et al. Identification of 31 novel mutations in the N-acetylgalactosamine-6-sulfatase gene reveals excessive allelic heterogeneity among patients with Morquio A syndrome. *Hum Mutat.* 1997;10:223-232. doi:10.1002/(SICI)1098-1004(1997)10:3<223::AID-HUMU8>3.0.CO;2-J
- Chistiakov DA, Savost'yanov KV, Kuzenkova LM, et al. Molecular characteristics of patients with glycosaminoglycan storage disorders in Russia. *Clin Chim Acta.* 2014;436:112-120. doi:10.1016/j.cca.2014.05.010
- Montaño AM, Tomatsu S, Brusius A, Smith M, Orii T. Growth charts for patients affected with Morquio A disease. *Am J Med Genet A.* 2008;146A:1286-1295. doi:10.1002/ajmg.a.32281
- Wood TC, Harvey K, Beck M, et al. Diagnosing mucopolysaccharidosis IVA. *J Inherit Metab Dis.* 2013;36:293-307. doi:10.1007/s10545-013-9587-1
- Khan SA, Mason RW, Kobayashi H, Yamaguchi S, Tomatsu S. Advances in glycosaminoglycan detection. *Mol Genet Metab.* 2020;130:101-109. doi:10.1016/j.yimgme.2020.03.004
- Tomatsu S, Montaño AM, Oguma T, et al. Validation of keratan sulfate level in mucopolysaccharidosis type IVA by liquid chromatography-tandem mass spectrometry. *J Inherit Metab Dis.* 2010;33 Suppl 3:S35-S42. doi:10.1007/s10545-009-9013-x
- Di Ferrante NM. The measurement of urinary mucopolysaccharides. *Anal Biochem.* 1967;21:98-106. doi:10.1016/0003-2697(67)90087-5
- Stone JE. Urine analysis in the diagnosis of mucopolysaccharide disorders. *Ann Clin Biochem.* 1998;35:207-225. doi:10.1177/000456329803500204
- Whitley CB, Ridnour MD, Draper KA, Dutton CM, Neglia JP. Diagnostic test for mucopolysaccharidosis. I. Direct method for quantifying excessive urinary glycosaminoglycan excretion. *Clin Chem.* 1989;35(3):374-379.
- de Jong JG, Wevers RA, Laarakkers C, Poorthuis BJ. Dimethylmethylene blue-based spectrophotometry of glycosaminoglycans in untreated urine: a rapid screening procedure for mucopolysaccharidoses. *Clin Chem.* 1989;35:1472-1477.
- de Jong JG, Heijs WM, Wevers RA. Mucopolysaccharidoses screening: dimethylmethylene blue versus alcian blue. *Ann Clin Biochem.* 1994;31:267-271. doi:10.1177/000456329403100309
- Lin HY, Lee CL, Lo YT, et al. The relationships between urinary glycosaminoglycan levels and phenotypes of mucopolysaccharidoses. *Mol Genet Genomic Med.* 2018;6:982-992. doi:10.1002/mgg3.471
- Piraud M, Maire I, Mathieu M. Pitfalls of screening for mucopolysaccharidoses by the dimethylmethylene blue test. *Clin Chem.* 1993;39:163-164.
- Khan SA, Mason RW, Giugliani R, et al. Glycosaminoglycans analysis in blood and urine of patients with mucopolysaccharidosis. *Mol Genet Metab.* 2018;125:44-52. doi:10.1016/j.yimgme.2018.04.011
- Dave MB, Chawla PK, Dherai AJ, Ashavaid TF. Urinary glycosaminoglycan estimation as a routine clinical service. *Indian J Clin Biochem.* 2015;30:293-297. doi:10.1007/s12291-014-0456-y
- Camelier MV, Burin MG, De Mari J, Vieira TA, Marasca G, Giugliani R. Practical and reliable enzyme test for the detection of mucopolysaccharidosis IVA (Morquio Syndrome type A) in dried blood samples. *Clin Chim Acta.* 2011;412:1805-1808. doi:10.1016/j.cca.2011.06.001
- Bhattacharya K, Balasubramanian S, Choy YS, et al. Overcoming the barriers to diagnosis of Morquio A syndrome. *Orphanet J Rare Dis.* 2014;9:192. doi:10.1186/s13023-014-0192-7
- Oguma T, Tomatsu S, Montano AM, Okazaki O. Analytical method for the determination of disaccharides derived from keratan, heparan, and dermatan sulfates in human serum and plasma by high-performance liquid chromatography/turbo ionspray ionization tandem mass spectrometry. *Anal Biochem.* 2007;368:79-86. doi:10.1016/j.ab.2007.05.016
- Lin HY, Lo YT, Wang TJ, et al. Normalization of glycosaminoglycan-derived disaccharides detected by tandem mass spectrometry assay for the diagnosis of mucopolysaccharidosis. *Sci Rep.* 2019;9:10755. doi:10.1038/s41598-019-46829-x
- Ding VC, Tomatsu S, Montaño AM, et al. Mucopolysaccharidosis IVA: correlation between genotype, phenotype and keratan sulfate levels. *Mol Genet Metab.* 2013;110:129-138. doi:10.1016/j.yimgme.2013.06.008
- Hintze JP, Tomatsu S, Fujii T, et al. Comparison of liquid chromatography-tandem mass spectrometry and sandwich ELISA for determination of keratan sulfate in plasma and urine. *Biomark Insights.* 2011;6:69-78. doi:10.4137/BMI.S7451
- Erickson RP, Sandman R, Epstein CJ. Lack of relationship between blood and urine levels of glycosaminoglycans and lysosomal enzymes. *Biochem Med.* 1975;12:331-339. doi:10.1016/0006-2944(75)90064-2
- Saville JT, McDermott BK, Fuller M. Glycosaminoglycan fragments as a measure of disease burden in the mucopolysaccharidosis type I mouse. *Mol Genet Metab.* 2018;123:112-117. doi:10.1016/j.yimgme.2017.12.007
- Shimada T, Tomatsu S, Yasuda E, et al. Chondroitin 6-Sulfate as a novel biomarker for mucopolysaccharidosis IVA and VII. *JIMD Rep.* 2014;16:15-24. doi:10.1007/8904_2014_311
- Cattoni A, Motta S, Masera N, Gasperini S, Rovelli A, Parini R. The use of recombinant human growth hormone in patients with Mucopolysaccharidoses and growth hormone deficiency: a case series. *Ital J Pediatr.* 2019;45:93. doi:10.1186/s13052-019-0691-1
- Nakamura-Utsunomiya A, Nakamae T, Kagawa R, et al. A case report of a Japanese boy with Morquio A syndrome: effects of enzyme replacement therapy initiated at the age of 24 months. *Int J Mol Sci.* 2020;21:989. doi:10.3390/ijms21030989
- Hendriks CJ, Burton B, Fleming TR, et al. Efficacy and safety of enzyme replacement therapy with BMN 110 (elosulfase alfa) for Morquio A syndrome (mucopolysaccharidosis IVA): a phase 3 randomised placebo-controlled study. *J Inherit Metab Dis.* 2014;37:979-990. doi:10.1007/s10545-014-9715-6
- Lyseng-Williamson KA. Elosulfase Alfa: a review of its use in patients with mucopolysaccharidosis type IVA (Morquio A syndrome). *BioDrugs.* 2014;28:465-475. doi:10.1007/s40259-014-0108-z
- Tomatsu S, Sawamoto K, Alméciga-Díaz CJ, et al. Impact of enzyme replacement therapy and hematopoietic stem cell transplantation in patients with Morquio A syndrome. *Drug Des Devel Ther.* 2015;9:1937-1953. doi:10.2147/DDDT.S68562
- Yabe H, Tanaka A, Chinen Y, et al. Hematopoietic stem cell transplantation for Morquio A syndrome. *Mol Genet Metab.* 2016;117:84-94. doi:10.1016/j.yimgme.2015.09.011