

A capillary electrophoresis procedure for the screening of oligosaccharidoses and related diseases

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Abstract The most widely used method for the biochemical screening of oligosaccharidoses is the analysis of the urinary oligosaccharide pattern by thin-layer chromatography on silica gel plates. However, this method is not always sensitive enough, and it is extremely time-consuming and laborious. In this work, the analysis of the urine oligosaccharide pattern was standardized for the first time by using capillary electrophoresis with laser-induced fluorescence (CE-LIF) detection (Beckman P/ACE MDQ) with a 488-nm argon ion laser module. All of the analyses were conducted using the Carbohydrate Labeling and Analysis Kit (Beckman-Coulter), which derivatizes samples with 8-aminopyrene-1,3,6-trisulfonate. Urine samples from 40 control subjects (age range, 1 week to 16 years) and from ten patients diagnosed with eight different lysosomal diseases (six of them included in the

Educational Oligosaccharide Kit from ERNDIM EQA schemes) were analyzed. Two oligosaccharide excretion patterns were established in our control population according to age (younger or older than 1 year of age). Abnormal peaks with slower migration times than the tetrasaccharide position were observed for fucosidosis, α -mannosidosis, GM1 gangliosidosis, GM2 gangliosidosis variant 0, Pompe disease, and glycogen storage disease type 3. In conclusion, the first CE-LIF method to screen for oligosaccharidoses and related diseases, which also present oligosacchariduria, has been standardized. In all of the cases, the urine oligosaccharide analysis was strongly informative and showed abnormal patterns that were not present in any of the urine samples from the control subjects. Only urine from patients with aspartylglucosaminuria and Schindler disease displayed normal results.

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Introduction

Oligosaccharidoses are rare diseases characterized by increased urinary excretion of oligosaccharides due to a deficiency in the lysosomal enzymes responsible for the degradation of the oligosaccharide chain of glycoproteins. Diagnosis of oligosaccharidoses relies on oligosaccharide analysis of urine. Early clinical and biochemical recognition are crucial to patients prognosis because the window of an effective intervention may be very brief after the onset of symptoms. Moreover, prenatal diagnosis may be of paramount importance [1]. For the biochemical screening of oligosaccharidoses, the most widely used method is the analysis of the urinary oligosaccharide pattern by thin-layer chromatography (TLC) on silica gel plates

[2]. However, this method is not always sensitive enough, and it is extremely time-consuming, laborious, and not automatable, making it impossible to screen a high number of patients. Other more sensitive analytical procedures with higher resolution have recently been developed, such as HPLC with pulsed amperometry [3], tandem mass spectrometry [4, 5], MALDI-TOF [6], capillary high-performance anion-exchange chromatography with mass spectrometry detection [7], and MALDI-TOF/TOF [8].

Capillary electrophoresis might also be suitable for the analysis of urinary oligosaccharides. It has a high resolution efficiency, and when it is coupled to a laser-induced fluorescence detector system (CE-LIF), it offers a good analytical sensitivity. Because oligosaccharides do not exhibit native fluorescence, derivatization is necessary before LIF detection. Some methods based in CE-LIF have been developed to analyze N-linked oligosaccharides enzymatically released from glycoproteins [9–12]. In these glycomic studies, high sensitivity, good reproducibility, and the resolution of very closely related oligosaccharide structures were obtained. However, these procedures have not been applied to urine samples for the screening of human oligosaccharidoses.

In the present work, we have described, for the first time, a CE-LIF method for the analysis of urinary oligosaccharides as a tool for the diagnosis of diseases that may present oligosacchariduria. Urine samples from control subjects and from patients with different oligosaccharidoses were analyzed, and their urinary oligosaccharide patterns were described.

Materials and methods

Reagents

All of the analyses were performed using the Carbohydrate Labeling and Analysis Kit (Beckman-Coulter, Fullerton, CA, USA). This kit is composed of the derivatization reagent 8-aminopyrene-1,3,6-trisulfonate (APTS), a 15 % acetic acid solution, a 25-mmol/L sodium acetate run buffer with a pH of 4.75 containing 0.4 % of polyethylene oxide, an N-CHO-coated capillary, and a dextran ladder standard (see manufacturer insert for further information). The ladder is prepared by partial acid hydrolysis of dextran. It is comprised of linear glucose polymers from G1 to G15 (5 mg in 80 μ L of distilled water). Sodium cyanoborohydride (1.0 mol/L NaBH₃CN solution in tetrahydrofuran) was obtained from Sigma-Aldrich (Steinheim, Germany).

Samples

Urine samples were obtained from 40 healthy control subjects (age range, 7 days to 16 years; median age, 3.5 years) and from ten patients diagnosed with eight different lysosomal diseases: four urine samples from patients diagnosed in our

hospital and six urine samples included in the Educational Oligosaccharide Kit from ERNDIM EQA schemes. The urine samples included in this study were from patients with fucosidosis (age, 3.8 years), glycogen storage disease type 3 (age, 2.5 years), and Sandhoff disease ($n=2$; ages, 1.0 and 3.7 years). The urine samples included in the Educational Oligosaccharide Kit from ERNDIM EQA schemes were from patients with GM1 gangliosidosis (age, 3 months), Pompe disease (age, 3 months), α -mannosidosis (age, 7 years), Schindler disease (age, 3.7 years), aspartylglucosaminuria (age, 8.5 years), and Sandhoff disease (age, 1.6 years). All of the samples were centrifuged (1500 \times g, 10 min) and stored at -20 °C until they were analyzed. The samples from the patients were obtained in accordance with the Helsinki Declaration of 1964, as it was revised in 2000. The ethical committee of the Hospital Sant Joan de Déu approved the study.

Derivatization procedure

A 200-mmol/L solution of APTS was prepared by dissolving 5 mg of APTS in 48 μ L of a 15 % acetic acid solution. Urinary creatinine was determined on the Architect c8000 analyzer using a commercial kit (Jaffé acid-picrate method; Abbott Laboratories, Illinois, USA). Prior to the analysis, the urine volume containing 150 nmol creatinine was calculated for all patients and controls (urine volume (μ L)=150 nmol/creatinine concentration (nmol/ μ L)). To increase the oligosaccharide concentration, the urine volume was concentrated by heating at 50 °C in an oven for 5 h. Then, 5 μ L of concentrated urine samples or the dextran ladder standard solution were mixed with 2 μ L of the APTS solution and 2 μ L of 1 mol/L sodium cyanoborohydride in tetrahydrofuran. The mixed solutions were reacted in the dark at 37 °C overnight in a heating block. After derivatization, these solutions underwent 40-fold dilution in deionized water before they were injected onto the capillary electrophoresis apparatus for analysis.

Instrumentation and analysis

The capillary electrophoresis experiments were performed using a Beckman P/ACE MDQ system equipped with a 488-nm argon ion laser module (Beckman-Coulter, Fullerton, CA, USA). The compounds were separated in neutrally coated N-CHO capillaries with a 50- μ m I.D., a 60.2-cm total length, and a 50-cm length to the detector. The capillary cartridge was kept at 20 °C by liquid coolant. All new capillaries were conditioned with deionized water for 10 min and run buffer for 2 min. Before each injection, the capillary was rinsed with deionized water and run buffer for 30 and 60 s, respectively. All of the rinses were performed at 20 psi. The sample was injected by a pressure of 0.5 psi for 3 s. Samples were stored at 10 °C, while they awaited injection. A separation voltage of 30 kV was applied with reverse polarity,

which resulted in an electrophoretic current of 14 μ A. The vials of buffer were replaced with fresh buffer after 10 runs.

Results

Typical electropherograms of urinary APTS-oligosaccharides are shown in Electronic Supplementary Material Fig. S1. The lower trace in the electropherograms shows the separation of the APTS-labeled maltooligosaccharide ladder standard. This standard was the product of a partial digestion of dextran, and therefore, it was composed of a mixture of glucose and linear oligosaccharides (from disaccharide G2 to G15). The peaks of the ladder standard were used as size reference markers and represented the polymerization degree. The huge peak at 7 min corresponded to the excess reagent used for the derivatization. After this peak, the APTS-monosaccharides were detected at approximately 8 min, and the APTS-disaccharides were observed between 8.5 and 9.5 min. Oligosaccharides were eluted from 9.5 to 25 min. To study the reproducibility of the separations, a ladder solution and a pathological urine sample were analyzed for 15 different days. The variation of migration time of the hexasaccharide observed in the fucosidosis sample relative to the glucose from the dextran ladder was calculated. The obtained relative migration time was very stable, with an inter-day coefficient of variation of 2.3 %.

Non-pathological urine samples

Urine samples from the control subjects were analyzed to establish a normal excretion pattern. Examples of non-pathological urine samples are given in Electronic Supplementary Material Fig. S1. Usually, non-pathological urine samples do not contain oligosaccharides. Electropherograms showed a great excretion of monosaccharides at a migration time of 8 min, and several disaccharides were found with

migration times between 8.5 and 9.5 min. In addition to these peaks, a peak with an electrophoretic mobility similar to the mobility of the maltotetraose in the ladder standard (with four glucose units) was usually observed. We studied non-pathological urine samples from subjects of different ages, and we observed that in very young children (age <1 month), several peaks in the trisaccharide to pentasaccharide area were detected in most of the cases (Electronic Supplementary Material Fig. S1a). These peaks were present only in the few urine control samples of children between 1 month and 1 year, and they disappeared in the profiles of children older than 1 year. Therefore, the oligosaccharide excretion pattern from children older than 1 year lacked these peaks and exhibited only the excretion of monosaccharides, disaccharides, and a small amount of a tetrasaccharide (Electronic Supplementary Material Fig. S1b). Correlation of tetrasaccharide excretion (area under the peak) with the age was studied in 24 control subjects (Fig. 1), and a significant negative correlation was observed (Pearson's test: $r=-0.645$; $p=0.0007$).

Oligosaccharidoses urine samples

Urine samples were analyzed from patients with different oligosaccharidoses and related diseases that also present oligosacchariduria including the following: fucosidosis, α -mannosidosis, GM1 gangliosidosis, GM2 gangliosidosis variant 0 (Sandhoff disease), glycogen storage disease type 2 (Pompe disease), glycogen storage disease type 3, aspartylglucosaminuria, and Schindler disease. Figure 2 and Electronic Supplementary Material Figs. S2 and S3 show the oligosaccharide excretion pattern for each disease compared with an age-matched control. In all of the cases, except for aspartylglucosaminuria and Schindler disease, abnormal peaks with slower migration times than the tetrasaccharide position were observed. Proposed structures of abnormal oligosaccharides are commented in the "Discussion" and

Fig. 1 Correlation between urine tetrasaccharide (Glc4) excretion (area under the peak) and the age of control subjects (Pearson's test: $r=-0.645$; $p=0.0007$)

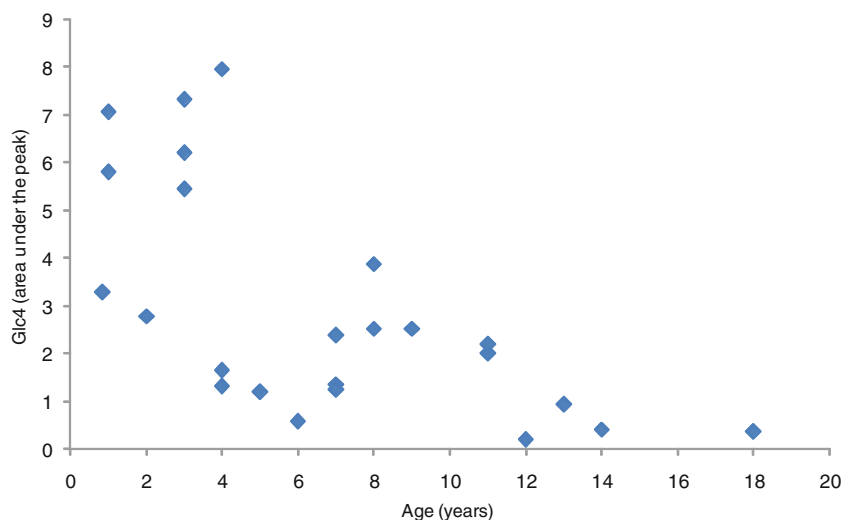
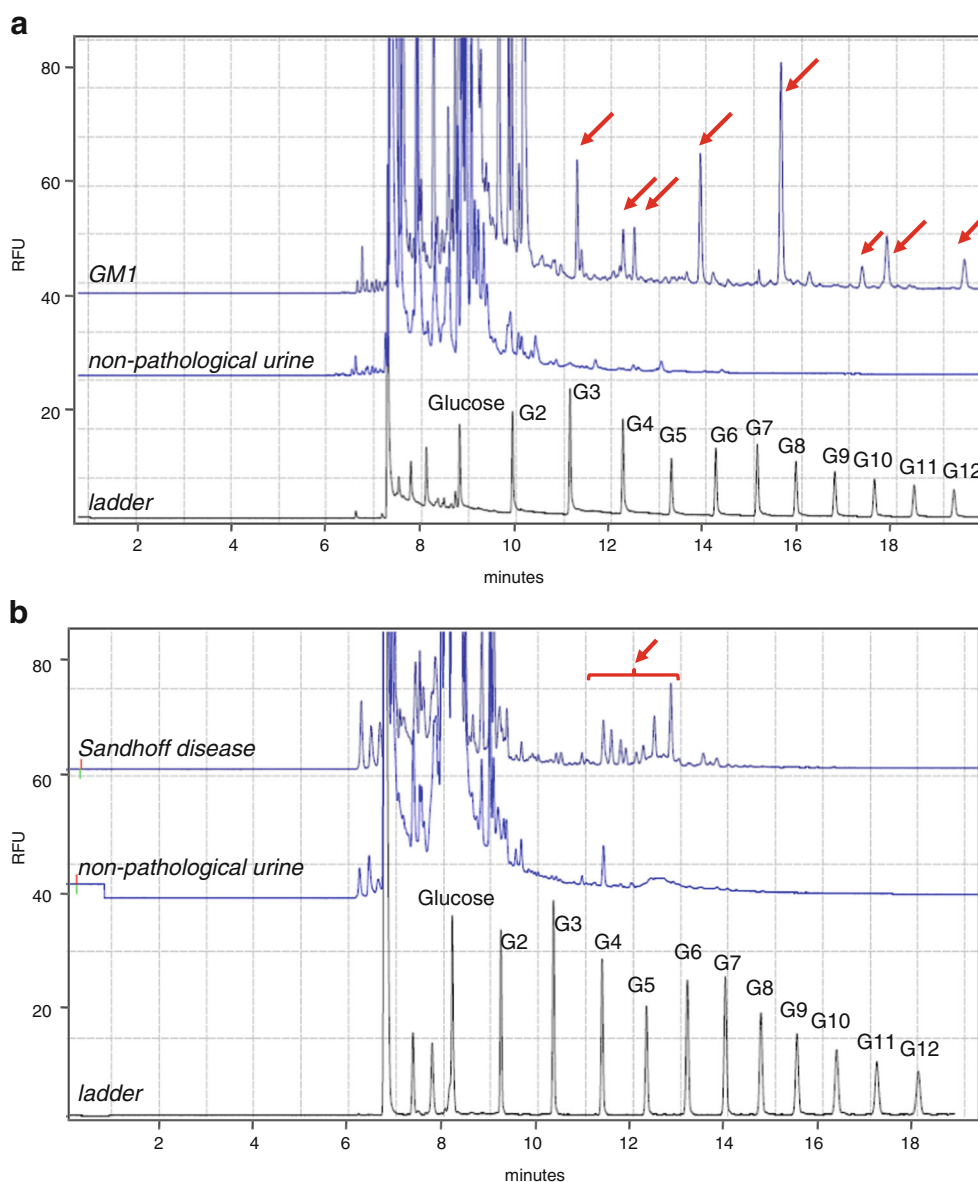


Fig. 2 Urinary oligosaccharide profiles from patients with gangliosidosis (*upper trace*). The oligosaccharide excretion pattern for each disease is compared with an age-matched control (*middle trace*). *Arrows* show abnormal oligosaccharides. The *lower trace* in the electropherograms shows the separation of the APTS-labeled maltooligosaccharide ladder standard; the *G numbers* indicate the glucose residues in the corresponding ladder oligosaccharide (G2, G3, ..., Gn). **a** GM1 (age, 3 months) and **b** Sandhoff disease (age, 1 year)



common monosaccharide acronyms are described in Electronic Supplementary Material Table S1. Generally, more peaks were detected by CE in oligosaccharidoses samples as compared to TLC, and these peaks were better resolved. Retention time values relative to glucose of the oligosaccharides detected in oligosaccharidoses urine samples are stated in Table 1. The polymerization degree of the pathological oligosaccharides was assigned by comparing with the nearest migration time of the ladder oligosaccharides.

Discussion

The CE-LIF detection procedures have a high separation efficiency and a high sensitivity, which are necessary for glycomic studies. These methods are even able to resolve

isomers, which are indistinguishable by tandem mass spectrometry or MALDI-TOF procedures. Despite these advantages, CE-LIF procedures had not been applied for the screening of oligosaccharidoses in human urine samples. In the present work, we have adapted a commercially available kit designed for the study of glycoproteins by CE-LIF for the analysis of urinary oligosaccharides. Regarding the derivatization procedure, mild labeling conditions were chosen to avoid losing the sialic acid residues from the oligosaccharide chains [13]. For this purpose, we left the reaction mixture at 37 °C overnight. This derivatization conditions are common in reductive amination of glycans [6]. In most of the MS procedures for glycan analysis, derivatization is often necessary since oligosaccharides display poor ionization efficiency [4, 5]. Besides this derivatization reaction, solid-phase extraction is frequently required in MS procedures [6–8]. Thus, the

Table 1 Retention times relative to glucose (t_R) of abnormal oligosaccharides detected by CE in oligosaccharidosis urine samples (left column)

Disease	t_R	Nearest ladder oligosaccharide		
		Polymerization degree	t_R	
Fucosidosis	0.59	6	0.60	
α -Mannosidosis	0.76	3	0.78	
	0.70	4	0.71	
	0.65	5	0.65	
	0.85	3	0.86	
GM1	0.72	4	0.72	
	0.71	4	0.72	
	0.63	6	0.62	
	0.56	8	0.55	
	0.51	10	0.50	
	0.49	10	0.50	
	0.45	12	0.46	
	Sandhoff disease	0.72	4	0.73
		0.71	4	0.73
		0.70	4	0.73
0.69		5	0.67	
0.68		5	0.67	
0.67		5	0.67	
0.66		5	0.67	
Pompe disease	0.64	6	0.63	
	0.70	4	0.70	
	0.64	5	0.64	
GSD-III	0.60	6	0.59	
	0.73	4	0.73	
	0.68	5	0.68	
	0.63	6	0.63	
	0.61	7	0.60	
	0.60	7	0.60	
	0.58	8	0.57	
	0.57	8	0.57	

The nearest ladder oligosaccharides to each abnormal peak with its relative retention time are also shown (right column)

time and labor required for sample preparation and labeling for the CE procedure is similar when compared to other MS procedures.

Several urine samples from control subjects were analyzed, and we established two normal excretion patterns according to the age of the subject. For the reference profiles from younger children (age ≤ 1 year), there were increases in some of the oligosaccharides (especially in those children younger than 1 month old). Human breast milk and some infant formulas with added galactooligosaccharides may contain a large variety of oligosaccharides. The presence of these molecules in the urine depends on the lactational stage and the Lewis antigen system of the mother [14, 15]. Urine oligosaccharides

from breastfed infants have been analyzed, and the excretion of oligosaccharides with diverse degrees of polymerization, from tri- to heptasaccharides, has been described [13]. Moreover, a peak at the tetrasaccharide migration time is usually present in the analyses of control urine samples from individuals of any age. In our control population, its excretion decreased with increasing age, as has been described in other studies [16]. This peak might correspond to the previously described oligosaccharide 6- α -D-glucopyranosyl-maltotriose (Glc4), which is derived from the intravascular degradation of glycogen.

Fucosidosis (Mendelian Inheritance in Man (OMIM) 230000) is an autosomal recessive lysosomal storage disease caused by defective α -L-fucosidase activity as a consequence of mutations in the *FUCA1* gene. In tissues and urine, fucosidosis patients accumulate fucosylated glycoconjugates from the incomplete catabolism of *N*- and *O*-glycoproteins, glycolipids, and proteoglycans. The major glycopeptide, a glycoasparagine Fuc-GlcNAc-Asn, present in the urine corresponds to the linkage region of the oligosaccharide [17]. However, this glycopeptide is not a reducing oligosaccharide, and therefore, it cannot be labeled with APTS and was not detected by this procedure. These patients accumulate other reducing oligosaccharides, which are also excreted in urine and can be detected. The profile obtained by CE-LIF (Electronic Supplementary Material Fig. S2a) shows an abnormal peak with an electrophoretic mobility corresponding to hexaheptasaccharide in the ladder standard. This peak might correspond to the previously described hexasaccharide Fuc-Gal-GlcNAc-Man₂-GlcNAc excreted in the urine of fucosidosis patients [5, 8].

α -Mannosidosis (OMIM 248500) is an autosomal recessive lysosomal storage disease caused by mutations in the *MAN2B1* gene, which codes for the enzyme α -mannosidase. A deficiency of this enzyme leads to the accumulation of high-mannose-containing oligosaccharides (Man_{*n*}-GlcNAc, $n \geq 2$), which are excreted in urine [17]. The pattern obtained by CE-LIF (Electronic Supplementary Material Fig. S2b) was similar to patterns previously described by other procedures [6, 8] and had a prominent peak in the trisaccharide area, which corresponded to Man₂-GlcNAc as well as peaks of Man₃₋₅-GlcNAc that showed decreasing excretion with an increasing degree of polymerization.

GM1-gangliosidosis (OMIM 230500) is an autosomal recessive lysosomal disease caused by mutations in the *GLB1* gene, which codes for the hydrolase β -galactosidase. This enzyme is involved in the cleavage of galactose in GM1 ganglioside catabolism. In addition to this reaction, β -galactosidase is implicated in the degradation pathway of the glycan portion of other glycolipids, glycoproteins, and mucopolisaccharides. An increase in the excretion of galactosylated oligosaccharides is characteristic of this deficiency [18]. The profile obtained by CE-LIF is shown in

Fig. 1a. Eight abnormal oligosaccharides, having 3 to 12 monosaccharide units, were clearly detected. This profile was similar to previously described profiles obtained by MS procedures, in which oligosaccharides were described with a degree of polymerization from five to ten, with a major excretion of the octasaccharide Gal₂-GlcNAc₂-Man₃-GlcNAc [8].

Sandhoff disease (OMIM 268800) is an autosomal recessive lysosomal disease caused by mutations in the *HEXB* gene. This gene encodes the beta subunit of two enzymes: β -hexosaminidase A and β -hexosaminidase B. Thus, hexosaminidases A and B are both deficient in patients with Sandhoff disease. The enzyme hexosaminidase B is not only involved in degradation of the oligosaccharide portion of the GM2 ganglioside but also in the cleavage of GlcNAc during glycoprotein degradation [18]. The characteristic urine oligosaccharide pattern from these patients was clearly detected by CE-LIF (Fig. 1b). Eight different oligosaccharides were separated with migration times between tetra- to hexasaccharides. In mass spectrometry procedures, there have only been three oligosaccharides described with a polymerization degrees of tetra-, penta-, and hexasaccharides [5, 7, 8], whereas more peaks were obtained by CE-LIF analysis. These differences in the excretion profiles can be due to the capacity of capillary electrophoresis to separate positional isomers with the same weight.

Glycogen storage disease type 2 (Pompe disease, OMIM 232300) is caused by mutations in the gene encoding the lysosomal enzyme acid α -1,4-glucosidase (acid maltase). Due to the deficiency of this enzyme, glycogen accumulates in the lysosomes of these patients, and the urinary excretion of the tetrasaccharide 6- α -D-glucopyranosyl-maltotriose (Glc4) derived from the intravascular degradation of glycogen is increased. Urinary Glc4 has been reported to be a useful biomarker for the monitoring of the progression of the disease and the therapeutic response to enzyme replacement therapy because this oligosaccharide correlates strongly with the skeletal muscle glycogen content [16, 19]. The profile obtained by CE-LIF showed an elevated excretion of Glc4, which was 5-fold higher when compared with an age-matched control. Other small amounts of oligosaccharides with greater degrees of polymerization (penta- to heptasaccharides) were also detected, which corresponded to diverse glucose polymers (Electronic Supplementary Material Fig. S3a).

Glycogen storage disease type 3 (OMIM 232400) is caused by a defective glycogen debrancher enzyme as a consequence of mutations in the *AGL* gene. This defect is associated with an accumulation of abnormal glycogen with short outer chains. In this disorder, a high excretion of Glc4 has also been described [16]. This tetrasaccharide was markedly increased in the profile obtained by CE-LIF (Electronic Supplementary Material Fig. S3b). Other oligosaccharides with greater degrees of polymerization (penta- to octasaccharides) were also detected, which corresponded to diverse glucose polymers. This profile was clearly abnormal and was similar to the

patterns previously described for other glycogen storage diseases [16].

Because of the very low incidence of these defects, there was not an opportunity to evaluate other diseases in this work, and further analyses of urine samples from patients with other diseases seem necessary for the complete validation of this procedure. It should be noted that the pathological oligosaccharides usually have branched structures, while the ladder oligosaccharides have linear structures. This causes slight discrepancies in their relative migration times (Table 1) since the coiling tendency of the linear structures may result in faster migration times as compared with the branched oligosaccharides [20]. However, it is plausible that most of the oligosaccharidoses and related diseases can be detected by CE-LIF.

It is important to note that those defects that excrete nonreducing glycoconjugates in urine, such as aspartylglucosaminuria, cannot be detected using this procedure, which can in turn be detected by conventional ion-exchange chromatography procedures for the analysis of amino acids. Concerning Schindler disease, the profile obtained by CE-LIF was practically normal and difficult to discriminate from the control samples (data not shown). This observation may be explained by the fact that an α -N-acetylgalactosaminidase deficiency leads to a high urine excretion of glycopeptides containing GalNAc-O-Ser/Thr [17], which are not reducing compounds, and therefore, they cannot be derivatized by the APTS reagent. High excretion of the reducing trisaccharide GalNAc-Fuc-Gal has also been described [17], but it is only present in patients with blood type A.

In summary, in this study, the first CE-LIF procedure for the screening of oligosaccharidoses and related diseases that also present oligosacchariduria has been standardized. Characteristic patterns of urinary oligosaccharides in patients with different diseases have been described. In all of the cases, the oligosaccharide profiles were strongly informative and showed abnormal peaks that were not present in any of the urine samples from the control subjects. Only the urine analysis of individuals with aspartylglucosaminuria and Schindler disease displayed normal results.

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References

1. Leroy JG (2007) Oligosaccharidoses and allied disorders. In: Rimoin DL, Connor JM, Pyeritz RE, Korf BR (eds) Principles and Practice of Genetics, 5th edn. Churchill Livingstone, Philadelphia

2. Humbel R, Collart M (1975) Oligosaccharides in urine of patients with glycoprotein storage diseases. I. Rapid detection by thin-layer chromatography. *Clin Chim Acta* 60:143–145
3. Peelen GO, de Jong JG, Wevers RA (1994) HPLC analysis of oligosaccharides in urine from oligosaccharidosis patients. *Clin Chem* 40:914–921
4. Sowell J, Wood T (2011) Towards a selected reaction monitoring mass spectrometry fingerprint approach for the screening of oligosaccharidoses. *Anal Chim Acta* 686:102–106
5. Ramsay SL, Meikle PJ, Hopwood JJ, Clements PR (2005) Profiling oligosaccharidurias by electrospray tandem mass spectrometry: quantifying reducing oligosaccharides. *Anal Biochem* 345:30–46
6. Klein A, Lebreton A, Lemoine J, Périni JM, Roussel P, Michalski JC (1998) Identification of urinary oligosaccharides by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Chem* 44:2422–2428
7. Bruggink C, Poorthuis BJ, Deelder AM, Wuhrer M (2012) Analysis of urinary oligosaccharides in lysosomal storage disorders by capillary high-performance anion-exchange chromatography-mass spectrometry. *Anal Bioanal Chem* 403:1671–1683
8. Xia B, Asif G, Arthur L, Pervaiz MA, Li X, Liu R, Cummings RD, He M (2013) Oligosaccharide analysis in urine by maldi-tof mass spectrometry for the diagnosis of lysosomal storage diseases. *Clin Chem* 59:1357–1368
9. Guttman A, Chen FT, Evangelista RA (1996) Separation of 1-aminopyrene-3,6,8-trisulfonate-labeled asparagine-linked fetuin glycans by capillary gel electrophoresis. *Electrophoresis* 17:412–417
10. Chen FT, Evangelista RA (1998) Profiling glycoprotein n-linked oligosaccharide by capillary electrophoresis. *Electrophoresis* 19:2639–2644
11. Mechref Y, Muzikar J, Novotny MV (2005) Comprehensive assessment of *N*-glycans derived from a murine monoclonal antibody: a case for multimethodological approach. *Electrophoresis* 26:2034–2046
12. Ruhaak LR, Hennig R, Huhn C, Borowiak M, Dolhain RJ, Deelder AM, Rapp E, Wuhrer M (2010) Optimized workflow for preparation of APTS-labeled *N*-glycans allowing high-throughput analysis of human plasma glycomes using 48-channel multiplexed CGE-LIF. *J Proteome Res* 9:6655–6664
13. Chen FT, Evangelista RA (1995) Analysis of mono- and oligosaccharide isomers derivatized with 9-aminopyrene-1,4,6-trisulfonate by capillary electrophoresis with laser-induced fluorescence. *Anal Biochem* 230:273–280
14. Rudloff S, Pohlentza G, Borscha C, Lentze MJ, Kunz C (2012) Urinary excretion of in vivo ¹³C-labelled milk oligosaccharides in breastfed infants. *Br J Nutr* 107:957–963
15. De Leoz ML, Wu S, Strum JS, Niñonuevo MR, Gaerlan SC, Mirmiran M, German JB, Mills DA, Lebrilla CB, Underwood MA (2013) A quantitative and comprehensive method to analyze human milk oligosaccharide structures in the urine and feces of infants. *Anal Bioanal Chem* 405:4089–4105
16. Sluiter W, van den Bosch JC, Goudriaan DA, van Gelder CM, de Vries JM, Huijmans JG, Reuser AJ, van der Ploeg AT, Ruijter GJ (2012) Rapid ultraperformance liquid chromatography-tandem mass spectrometry assay for a characteristic glycogen-derived tetrasaccharide in Pompe disease and other glycogen storage diseases. *Clin Chem* 58:1139–1147
17. Michalski JC, Klein A (1999) Glycoprotein lysosomal storage disorders: α - and β -mannosidosis, fucosidosis and α -*N*-acetylgalactosaminidase deficiency. *Biochim Biophys Acta* 1455:69–84
18. Sandhoff K, Harzer K (2013) Gangliosides and gangliosidoses: principles of molecular and metabolic pathogenesis. *J Neurosci* 33:10195–10208
19. Young SP, Zhang H, Corzo D, Thurberg BL, Bali D, Kishnani PS, Millington DS (2009) Long-term monitoring of patients with infantile-onset Pompe disease on enzyme replacement therapy using a urinary glucose tetrasaccharide biomarker. *Genet Med* 11:536–541
20. Guttman A, Pritchett T (1995) Capillary gel electrophoresis separation of high-mannose type oligosaccharides derivatized by 1-aminopyrene-3,6,8-trisulfonic acid. *Electrophoresis* 16:1906–1911