LYSOSOMAL STORAGE DISORDERS
DIAGNOSTIC TESTS OVERVIEW

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Pettazzoni M, Froissart R, Ruet S, Pagan C, Cheillan D, Latour P, Vianey-Saban C

LYSOSOMAL STORAGE DISORDERS

Defect of a lysosomal enzyme (or of a lysosomal protein)
Accumulation of undegraded substrates

MUCOPOLYSACCHARIDOSES
- Glycosaminoglycans (U)
- Enzyme activities
- Gene

OLIGOSACCHARIDOSES
- Oligosaccharides (U)
- Enzyme activities
- Gene

SIALIC ACID STORAGE DISORDERS
- Sialic acid (U)
- Gene

SPHINGOLIPIDOSES
- Gb3, sulfatides (U)
- Oxysterols, Lysosphingolipids *(P)*
- Enzyme activities
- Gene

OTHERS

Glycogenosis type 2 (Pompe disease)
- Acid alpha glucosidase
- Glc4 (Urine)
- Gene

Cystinosis
- Cystine (leucocytes)
- Gene

Ceroid lipofuscinosis
- Enzyme activities (type 1 and 2)
- Gene

…

- **Common clinical signs**
- or **Variable clinical signs** (sphingolipidoses)
- **Some are involved in *hydrops fetalis***

How MS/MS can be useful in routine labs ?

TANDEM MASS SPECTROMETRY

- **MS/MS analyzer** (Triple quadrupole, or else…)
- **Ionisation efficiency**
- **Sample preparation / Liquid chromatography**
- **Internal standards (IS)**
  isotope labeled or homologous

e.g. API 4500/Qtrap, Sciex

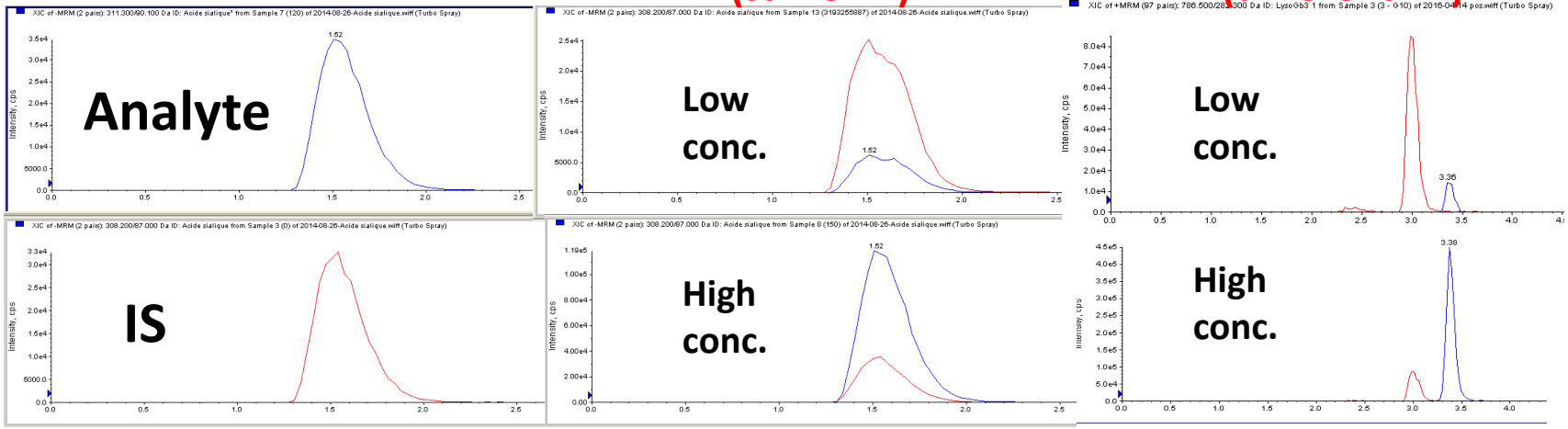
Analytes in complex mixtures, according to specific fragmentation of each molecule

= pairs of parent ion/product ion = transition or MRM (Multiple Reaction Monitoring)
Positive or negative ion mode

Very sensitive, very specific measurement technique

Isotope labeled IS (same RT)

Homologous IS (different RT)



Measurement of free sialic acid
Urine, amniotic fluid, cultured cells

- **TLC** silicagel plates
- revelation chlorhydric orcinol
- heating at 150°C

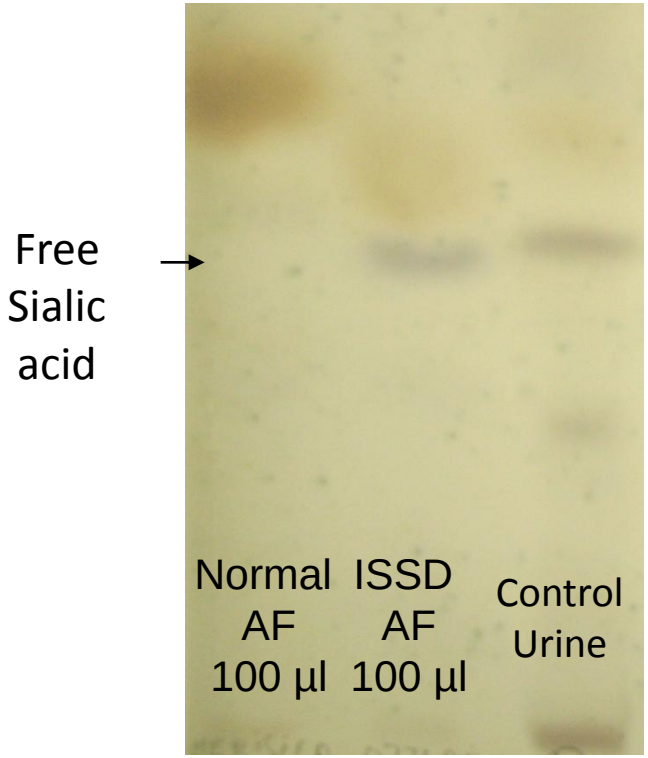
Diagnosis of Infantile Sialic acid Storage Disease (ISSD)

Commercially available IS (D_3 sialic acid)

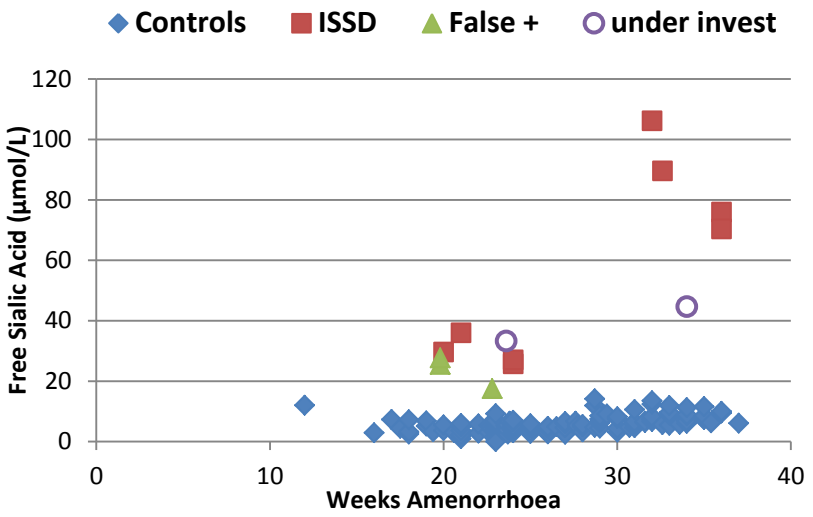
van der Ham M *et al.,* J Chromatogr B Analyt Technol Biomed Life Sci. 2010 May 1;878(15-16),

2011 : from TLC and Warren method to MS/MS

AMNIOTIC FLUID



MS/MS Quantification



Confirmation by measuring sialic acid content (free and total) in cultured cells (fibroblasts, amniotic cells) by MS/MS.

Oligosaccharidoses, diagnosis

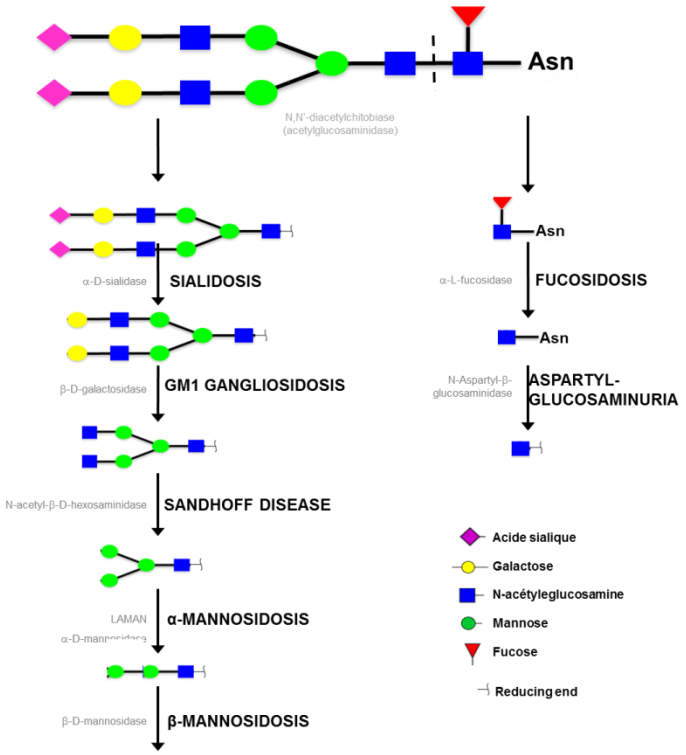
Clinica Chimica Acta, 60 (1975) 143—145

CCA 6841

OLIGOSACCHARIDES IN URINE OF PATIENTS WITH GLYCOPROTEIN STORAGE DISEASES

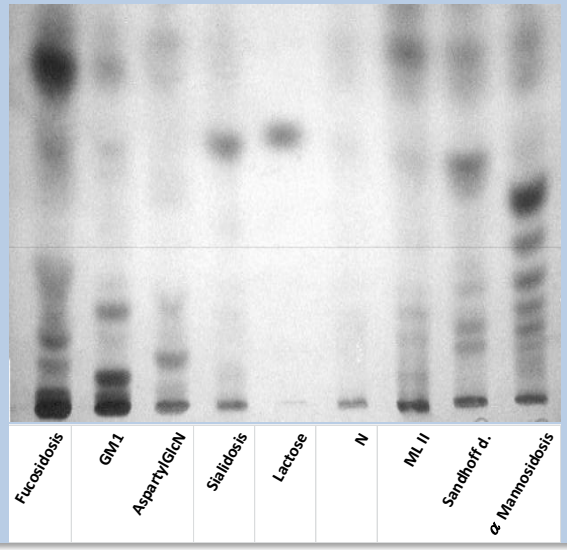
I. RAPID DETECTION BY THIN-LAYER CHROMATOGRAPHY

R. HUMBEL and M. COLLART
State Pediatric Clinic, Luxembourg (Luxembourg)
(Received September 13, 1974)



Courtesy of Dr C Hinault, Nice University

Thin Layer Chromatography (TLC, urine)
Humbel and Collart method (1974)



- **Qualitative method**
- 1 oligosaccharidosis : 1 **characteristic** pattern
- **Difficult interpretation**
- **Interference** breast feeding, amikacine, Ca glubionate, Dotarem® (MRI gadolinium)
- **False negative results :** adult, moderate forms

Bonesso *et al. Orphanet Journal of Rare Diseases* 2014, **9**:19
http://www.ojrd.com/content/9/1/19

ORPHANET JOURNAL OF RARE DISEASES

RESEARCH Open Access

Fast urinary screening of oligosaccharidoses by MALDI-TOF/TOF mass spectrometry

Laurent Bonesso[1], Monique Piraud[5], Céline Caruba[1], Emmanuel Van Obberghen[1,2,3,4], Raymond Mengual[1†] and Charlotte Hinault[1,2,3,4*†]

7 oligosaccharidoses studied:

Fucosidosis
α-Mannosidosis
Sandhoff disease (GM2)
Mucolipidosis type II
Aspartyglucosaminuria
GM1 Gangliosidosis
Sialidosis

Identification of parent ions and product ions of UNDERIVATIZED oligosaccharides, characteristic of oligosaccharidoses.

TRANSPOSITION to a TRIPLE QUADRUPOLE analyzer (API 4500, ABSciex)

Identification and optimisation of many MRM (pairs of parent ion/product ion) **characteristic of oligosaccharidoses after urine infusion.**

Positive mode: $[M+H]^+$ ou $[M+Na]^+$
Negative mode: $[M-H]^-$

IS: Glc_7 (maltoheptaose)

DEVELOPMENT of LC

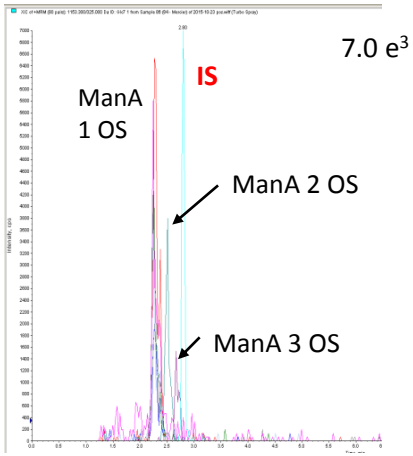
NH2 column (Uptisphere 5 µm, 120 Å, 50 mm x 2.1 mm, Interchrom©, Interchim)

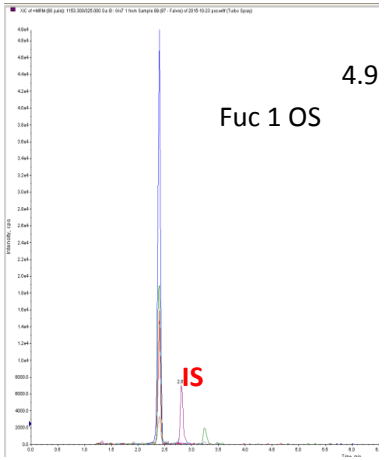
- Excellent **specificity**
- Excellent **sensitivity**
- Quantification not validated (lack of specific IS for each oligosaccharide)
- **Results normalized to creatinine and IS area, then expressed in MoM (Multiple of Median)** of simultaneous control urines

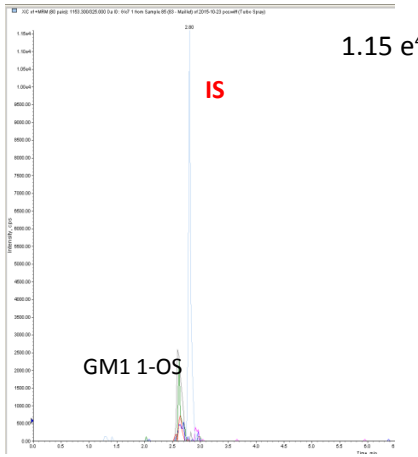
ex: **MRM specific for oligosaccharidoses**

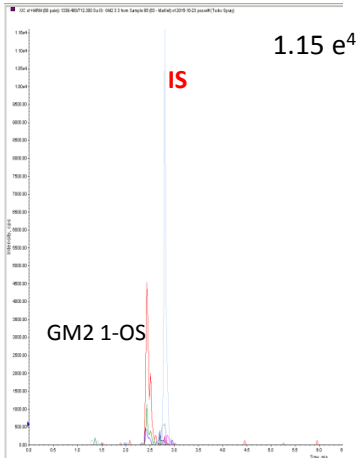
IS : Glc_7 (maltoheptaose)

Control urine









pos. mode

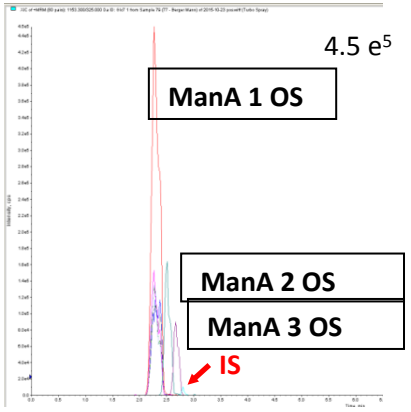
α-Mannosidosis (n = 10) 48 MoM

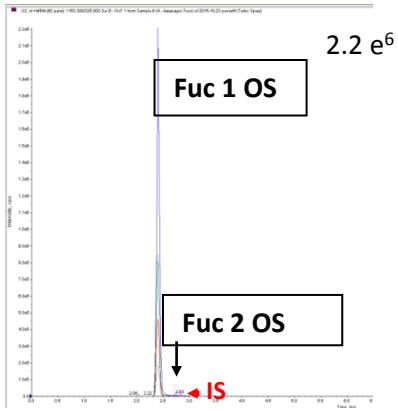
Fucosidosis (n = 6) 280 MoM

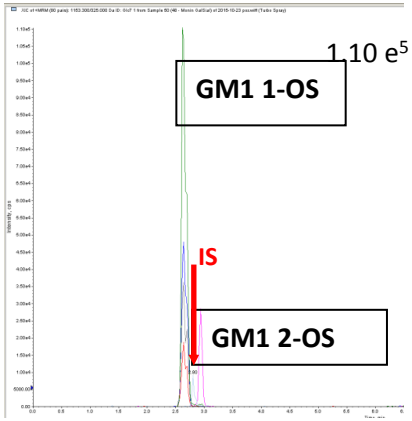
GM1 Gangliosidosis (n = 20) 1013 MoM, Galactosialidosis (n = 5) 108 MoM

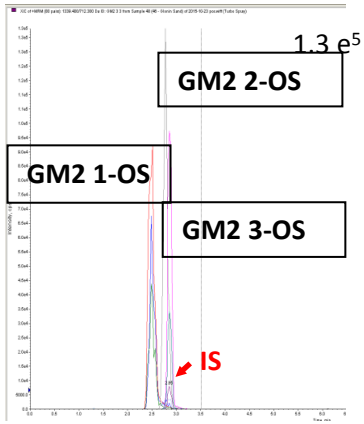
Sandhoff disease (n = 11) 120 MoM

Patient's urine









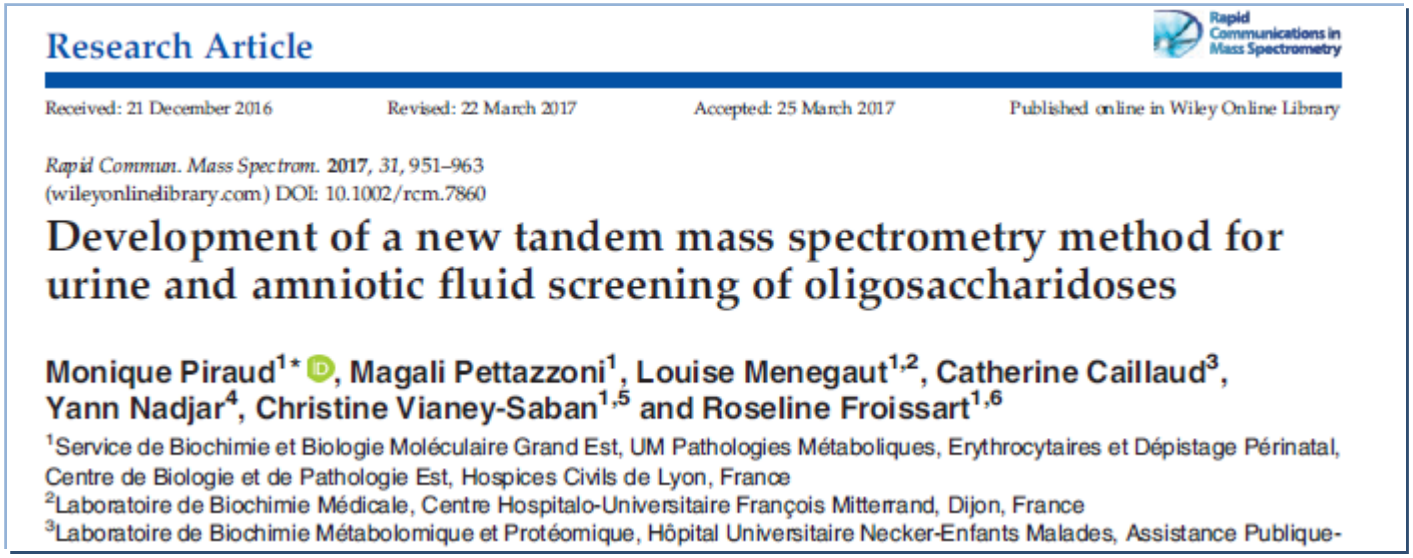
MoM = multiple of median

Oligosaccharides : results in MoM

mean (range)

Urine of patient affected with	mode n=	MRM specific of: Sialyl-OS neg 1200.4 > 1099.4	Glycoasparaginyl-OS neg 334.0 > 195.9	Glycoasparaginyl-OS pos 336.2 > 133.1	Fucosyl-OS pos 504.2 > 289.2	Mannosyl-OS neg 568 > 244	Galactosyl-OS pos 933.5 > 388.3	Galactosyl-OS pos 1460.6 > 1095.5	NAcGlucosaminyl-OS pos 771.3 > 568.4	NAcGlucosaminyl-OS pos 1136.3 > 933.4	Hex-HexNAc pos 406.0 > 244.0	Glycopeptides neg 307> 59	Glycopeptides pos 323> 39	
Sialidosis (infantile)	6	**1287** (103 - 15776)	1 (0 - 7)	1 (0 - 3)	2 (1 - 5)	2 (1 - 15)	23 (6 - 455)	5 (1 - 48)	4 (0 - 26)	2 (0 - 8)	2 (0-6)	0 (0-1)	1 (0-4)	Expected increase
Sialidosis (late-onset)	5	**221** (25 - 3619)					13 (1- 106)	7 (0 - 25)						
Mucolipidosis type II	9	**116** (20 - 799)	1 (0 - 1)		4 (1 - 9)	14 (2 - 30)	**59** (5 - 124)	**45** (3 - 59)	6 (1 - 18)	4 (1 - 21)	6 (1-13)	1 (0-2)	2 (1-5)	
Galactosialidosis	5	**1689** (401 -3159)			2 (1 - 7)	2 (0 - 7)	**108** (93 - 212)	**18** (10 - 24)	5 (1 - 16)	1 (0 - 3)				Interference (in-source fragmentation ?)
Aspartylglucosaminuria	7	0 (0 - 1)	**46** (28 - 55)	**62** (61 - 110)	2 (1 - 3)		1 (0 - 3)	2 (0 - 2)	0 (0 - 1)	0 (0 - 1)	0 (0-1)	0 (0-1)	1 (0-2)	
Fucosidosis	6	1 (0 - 3)	2 (0 - 5)		**280** (62 - 509)		9 (1 - 24)	1 (0 - 2)	3 (1 - 14)	1 (0 - 1)	2 (1-3)	1 (1-1)	2 (0-5)	
α-Mannosidosis	10	2 (0 - 5)	0 (0 - 1)		0 (0 - 3)	**48** (21 - 401)	1 (0 - 5)	1 (0 - 3)	0 (0 - 3)	0 (0 - 2)	1 (0-3)	0 (0-1)	1 (0-1)	
GM1 gangliosidosis (infantile)	15	7 (1 - 32)	1 (0 - 3)	1 (0 - 3)	2 (1 - 5)	2 (0 - 10)	**1013** (187 - 1916)	**1180** (244 - 2526)	57 (11 - 105)	2 (0 - 6)	2 (0-9)	0 (0-1)	0 (0-1)	
GM1 gangliosidosis (late-onset)	5	2 (1 - 4)					**108** (93 - 212)	**18** (10 - 24)	5 (2 - 8)	0 (0 - 1)				
MPS IV B	2	2 - 3			3 – 1	3 – 1	**464 - 68**	**416 - 55**	4 - 32	1 - 0				
GM2 gangliosidosis (Sandhoff)	11	2 (1 - 8)	2 (0 - 7)	1 (0 - 3)	2 (0 - 4)	1 (0 - 5)	5 (0 - 51)	2 (1 - 84)	**120** (65 - 229)	**302** (145 - 537)	2 (1-29)	1 (0-1)	2 (0-4)	
β-Mannosidosis	1	1	0	0	0	1	0	1	2	0	**169**	1	3	
Schindler-Kanzaki	6 (4 patients)	1 (0-13)	1 (0-8)	1 (0-8)	1 (0-4)	1 (0-7)	1 (0-7)	1 (0-10)	1 (0-5)	1 (1-14)	1 (0-5)	**16** (8 - 129)	**28** (2 - 632)	
MPS type I, II, IV A and VI	57	**186** (9 - 1295)	N	N	N	N	N	N	N	N	N	N	N	Interference

Oligosaccharides



Research Article

Rapid Communications in Mass Spectrometry

Received: 21 December 2016 Revised: 22 March 2017 Accepted: 25 March 2017 Published online in Wiley Online Library

Rapid Commun. Mass Spectrom. **2017**, *31*, 951–963
(wileyonlinelibrary.com) DOI: 10.1002/rcm.7860

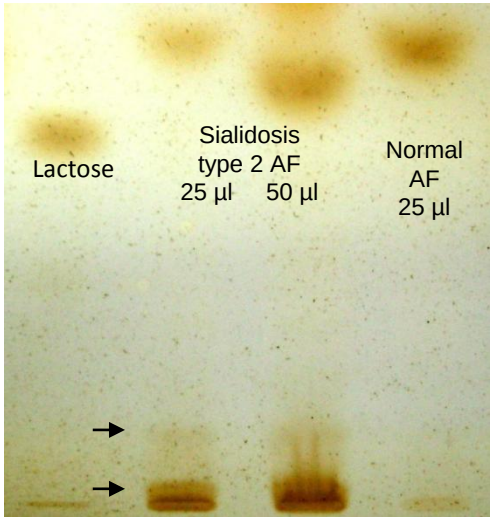
Development of a new tandem mass spectrometry method for urine and amniotic fluid screening of oligosaccharidoses

Monique Piraud[1]*, Magali Pettazzoni[1], Louise Menegaut[1,2], Catherine Caillaud[3], Yann Nadjar[4], Christine Vianey-Saban[1,5] and Roseline Froissart[1,6]

[1]Service de Biochimie et Biologie Moléculaire Grand Est, UM Pathologies Métaboliques, Erythrocytaires et Dépistage Périnatal, Centre de Biologie et de Pathologie Est, Hospices Civils de Lyon, France
[2]Laboratoire de Biochimie Médicale, Centre Hospitalo-Universitaire François Mitterrand, Dijon, France
[3]Laboratoire de Biochimie Métabolomique et Protéomique, Hôpital Universitaire Necker-Enfants Malades, Assistance Publique-

- VERY **SPECIFIC** AND **SENSITIVE** METHOD, diagnosis of **MODERATE FORMS**
- **EASY PREPARATION** (50 µl urine + IS: Glc_7 and *sialic acid)
- Replaces TLC
- None of the interference observed with TLC
- **ONE SINGLE METHOD** : oligosaccharides + free sialic acid
- **EXTENSION** to Mucolipidosis type II, Sandhoff disease and β-mannosidosis

Oligosaccharides in amniotic fluid



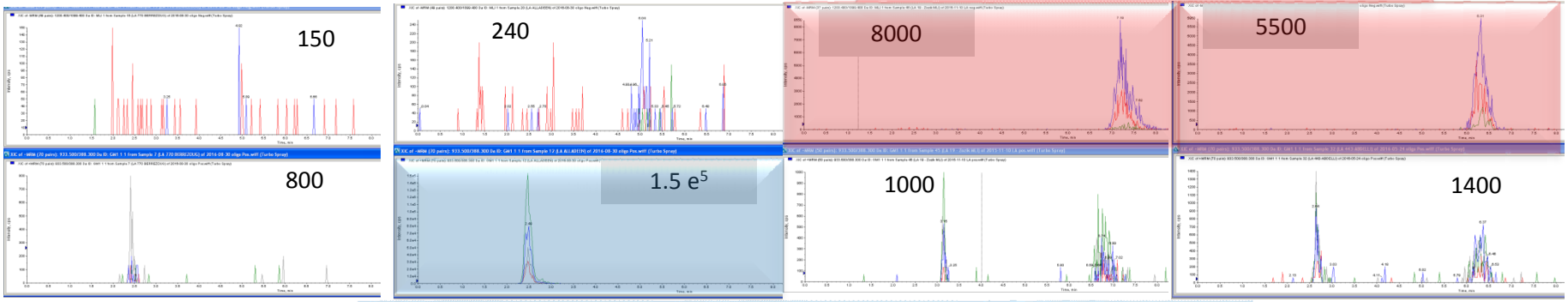
Abnormal oligosaccharides in *hydrops fetalis*

(>16 WA)

- **Sialidosis** (n = 3)
- **Galactosialidosis** (n = 7)
- **GM1-gangliosidosis** (n = 6)

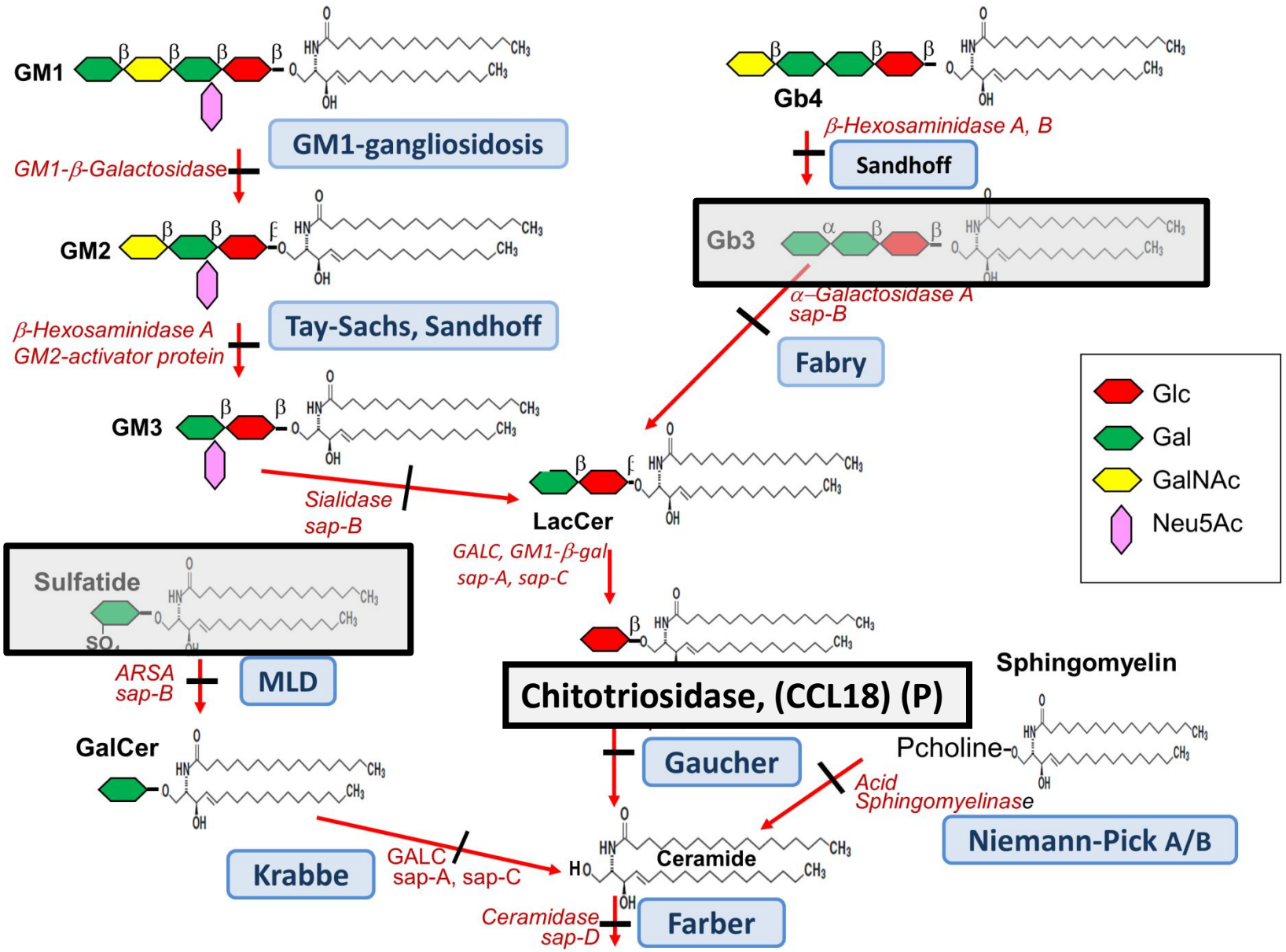
Control AF | **GM1 Gangl AF** | **Sialidosis AF** | **Galactosialidosis AF**

MRM Sialyl-OS, neg mode



MRM Galactosyl-OS, pos mode

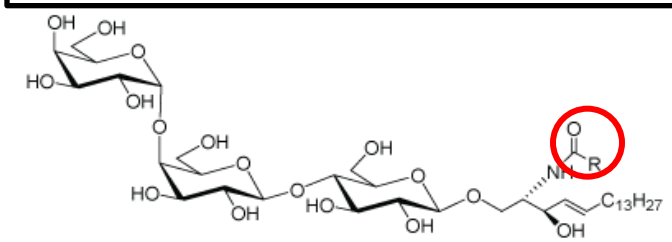
Sphingolipidoses biomarkers



Courtesy of Dr M-T. Vanier, Lyon France

Measurement of Gb3

Globotriaosylceramide



Fatty acid

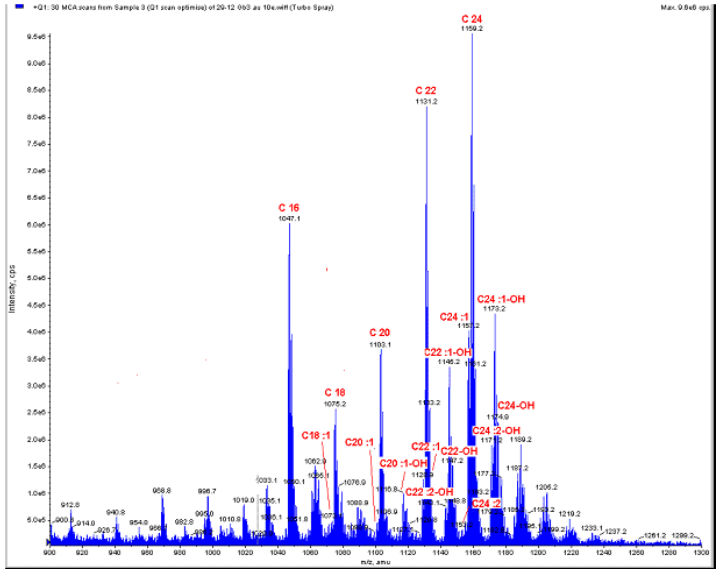
(C16 to C24)

Gal-Gal-Glc

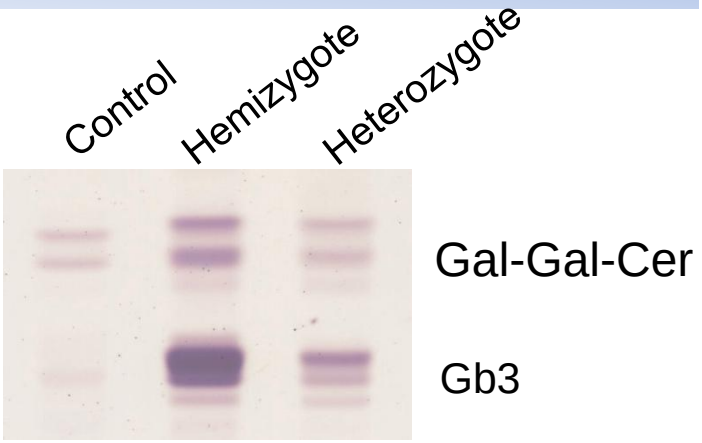
Sphingosine

MS/MS measurement: isoforms

Gb3 is the total of the different isoforms



Accumulated in Fabry disease



HPTLC analysis

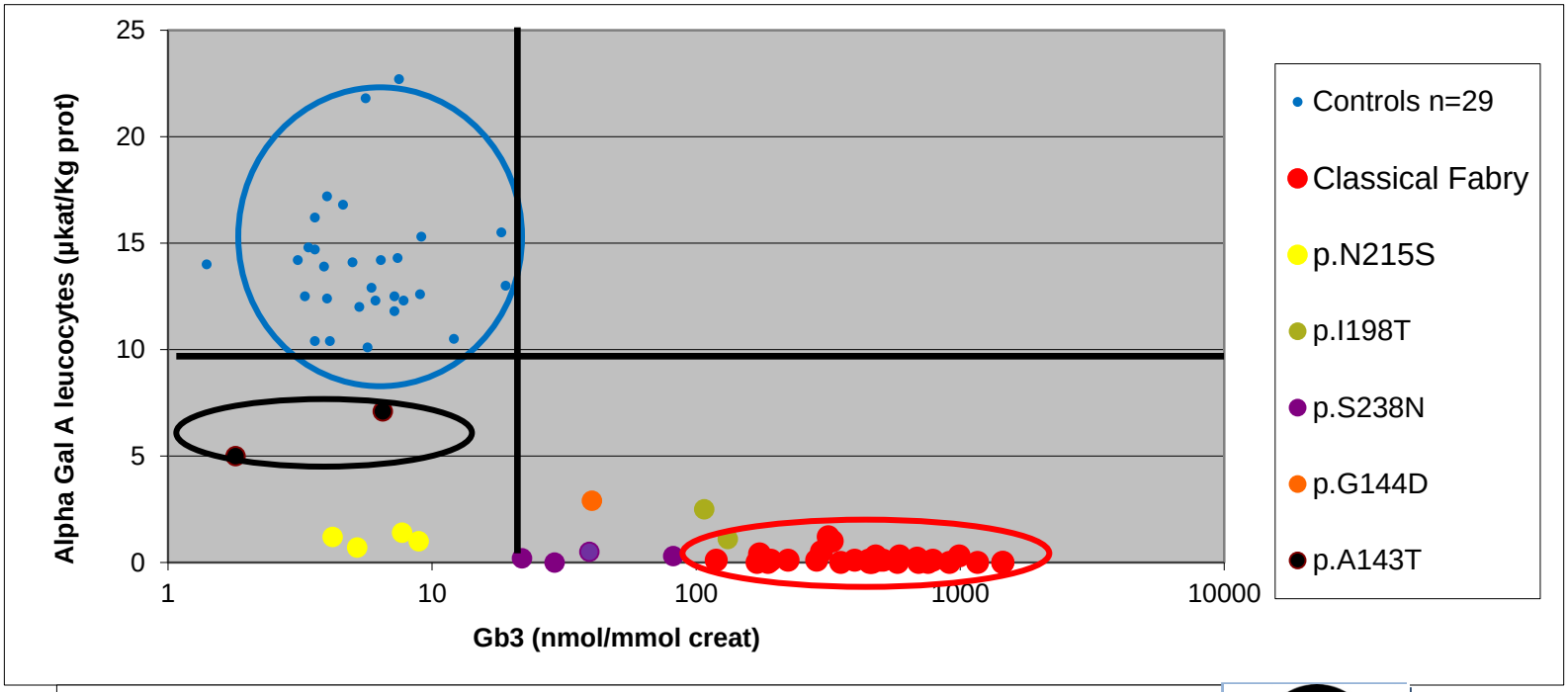
Courtesy Dr MT. Vanier

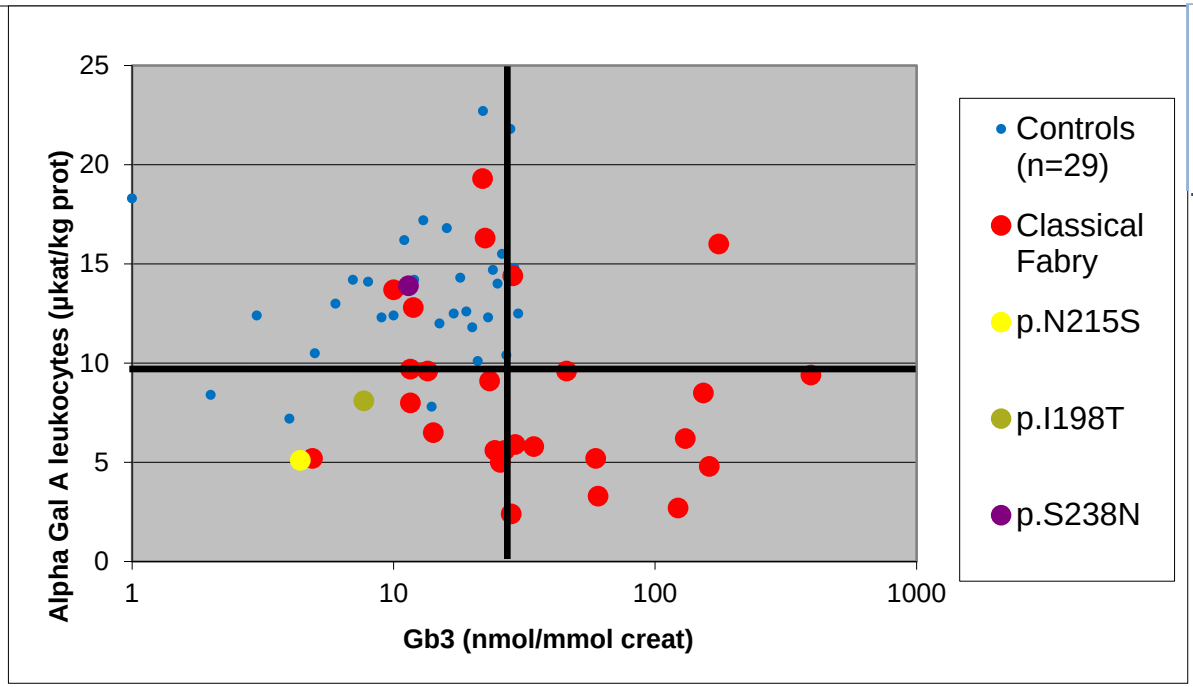
Originating from desquamation of renal tubular cells

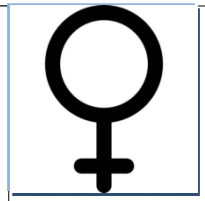
Sampling conditions

Diuresis or complete urine micturition, including urinary sediment

Similar method for the measurement of sulfatides for MLD diagnosis



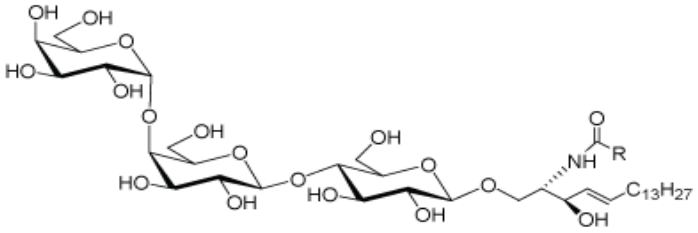




Measurement of urinary Gb3

Sphingolipids/*Lyso*Sphingolipids

Globotriaosylceramide



Acide gras

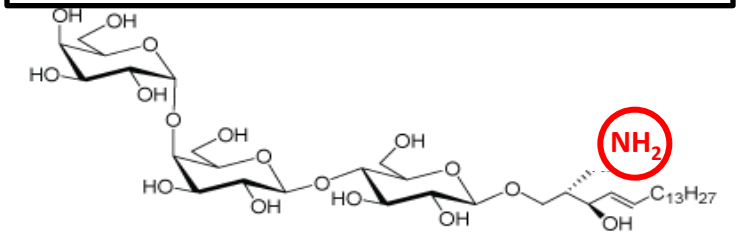
(C16 à C24)

Gal-Gal-Glc

Sphingosine

Very slightly increased in plasma

***Lyso*Globotriaosylceramide**



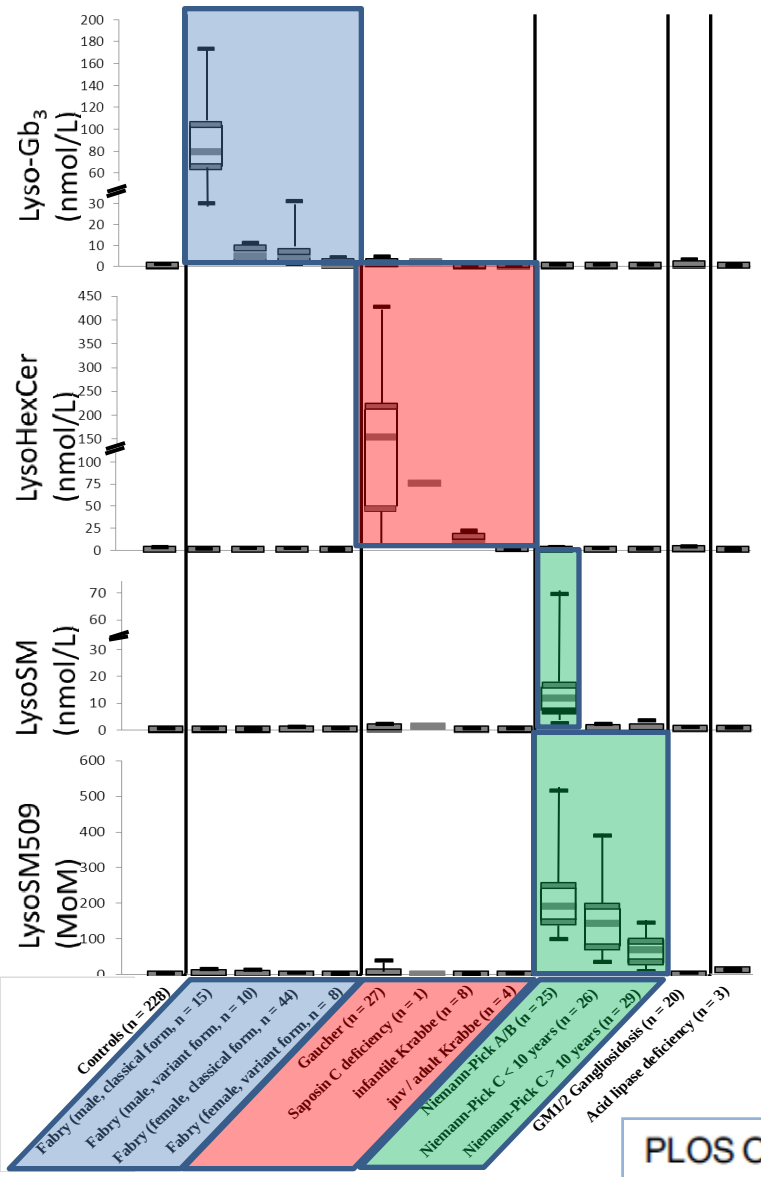
Gal-Gal-Glc

Sphingosine

- **Lysosphingolipid (LysoSL) = deacylated form of sphingolipids with a free amino group. Role in pathophysiology ?**

- **Emerging plasma biomarkers for diagnosis of sphingolipidoses and follow-up of patients**
  (Fabry, Gaucher, Krabbe, Niemann-Pick A/B and C, and GM1/2 gangliosidoses).

Lysosphingolipids



RESEARCH ARTICLE

LC-MS/MS multiplex analysis of lysosphingolipids in plasma and amniotic fluid: A novel tool for the screening of sphingolipidoses and Niemann-Pick type C disease

Magali Pettazzoni[1]*, Roseline Froissart[1,2], Cécile Pagan[1], Marie T. Vanier[3,4], Séverine Ruet[1], Philippe Latour[5], Nathalie Guffon[6], Alain Fouilhoux[6], Dominique P. Germain[7], Thierry Levade[8], Christine Vianey-Saban[1,9], Monique Piraud[1☯], David Cheillan[1,9☯]

PLOS ONE | https://doi.org/10.1371/journal.pone.0181700 July 27, 2017

Oxysterols : screening of NPC

A sensitive and specific LC-MS/MS method for rapid diagnosis of Niemann-Pick C1 disease from human plasma[S]

Xuntian Jiang,* Rohini Sidhu,* Forbes D. Porter,† Nicole M. Yanjanin,† Anneliese O. Speak,§ Danielle Taylor te Vruchte,§ Frances M. Platt,§ Hideji Fujiwara,* David E. Scherrer,* Jessie Zhang,* Dennis J. Dietzen,** Jean E. Schaffer,* and Daniel S. Ory†,*

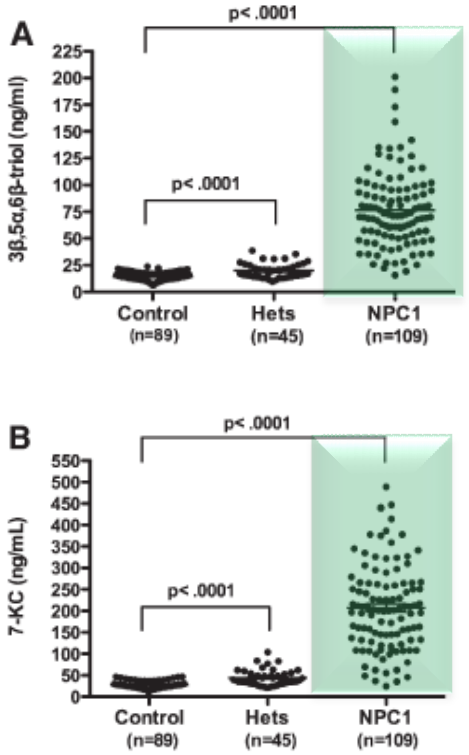
Diabetic Cardiovascular Disease Center* and Department of Pediatrics,**Washington University School of Medicine, St. Louis, MO; Program in Developmental Endocrinology and Genetics,† *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, the National Institutes of Health, Department of Health and Human Services, Bethesda, MD; and Department of Pharmacology,§ University of Oxford, Oxford, UK

Journal of Lipid Research Volume 52, 2011

Sci Transl Med. 2010 November 3; 2(56): 56ra81. doi:10.1126/scitranslmed.3001417.

Cholesterol oxidation products are sensitive and specific blood-based biomarkers for Niemann-Pick C1 disease

Forbes D. Porter[1], David E. Scherrer[2], Michael H. Lanier[2], S. Joshua Langmade[2], Vasumathi Molugu[2], Sarah E. Gale[2], Dana Olzeski[2], Rohini Sidhu[2], Dennis J. Dietzen[3], Rao Fu[1], Christopher A. Wassif[1], Nicole M. Yanjanin[1], Steven P. Marso[4], John House[4], Charles Vite[5], Jean E. Schaffer[2], and Daniel S. Ory[2,*]



Cholestane-3ß-5α-6ß-Triol

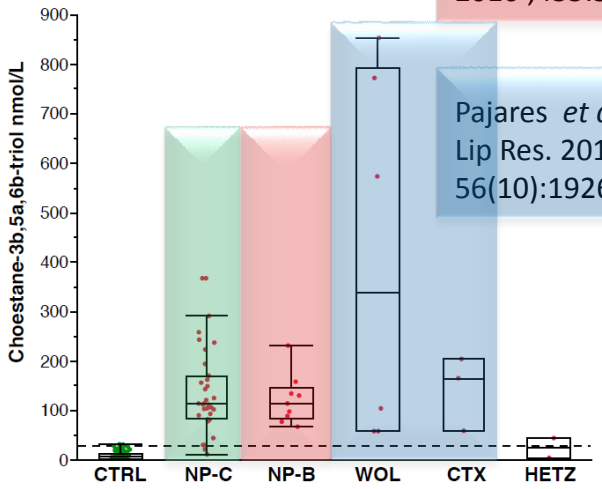
more discriminant than

7-Keto-cholesterol

Cholestane-3β-5α-6β-Triol
(results C. Pagan, Lyon)

Romanello *et al.*, Clin Chim Acta. 2016 ;455:39-45.

Pajares *et al.*, J Lip Res. 2015; 56(10):1926-35.



CTRL, controls; CTX, Cerebro-Tendinous-Xanthomatosis; HETZ, Heterozygotes NPC ; NP-B, Niemann-Pick disease Type B; NP-C, Niemann-Pick disease Type C; WOL, Wolman disease

Urinary Glycosaminoglycans analysis for mucopolysaccharidoses

QUANTITATIVE TESTS: normalization to creatinine and age

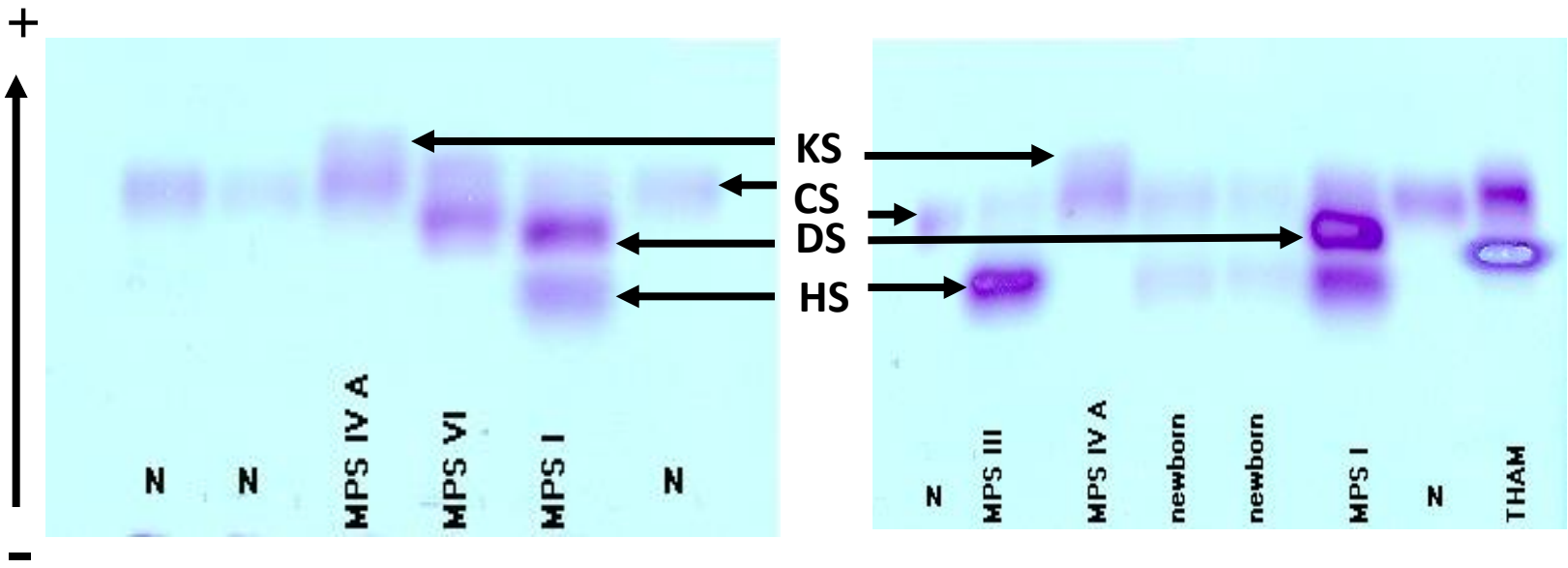
- DMB test (dimethylmethylene blue test)
- Measurement of uronic acids (harmine…)

But several false negative results

CS: Chondroïtin sulfate
DS : Dermatan sulfate
HS : Heparan sulfate
KS : Keratan sulfate

QUALITATIVE TESTS

- electrophoresis on cellulose acetate in baryum buffer



MS/MS analysis of native oligosaccharides from GAGs

0031-3998/04/5605-0733
PEDIATRIC RESEARCH
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Vol. 56, No. 5, 2004
Printed in U.S.A.

Disease-Specific Markers for the Mucopolysaccharidoses

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Lysosomal Diseases Research Unit [M.F., T.R., S.L.R., J.J.H., P.J.M.], Department of Genetic Medicine, Women's and Children's Hospital, North Adelaide, 5006, South Australia, Australia; and Department of Paediatrics [M.F., J.J.H., P.J.M.], University of Adelaide, Adelaide, 5005, South Australia, Australia

- Fuller et al., 2004: MS/MS analysis of short MW native oligosaccharides present in urine of MPS patients.
- **Derivatization with PMP (phenyl-methyl-pyrazolone)**
- Patterns specific of each MPS
- Decrease with ERT

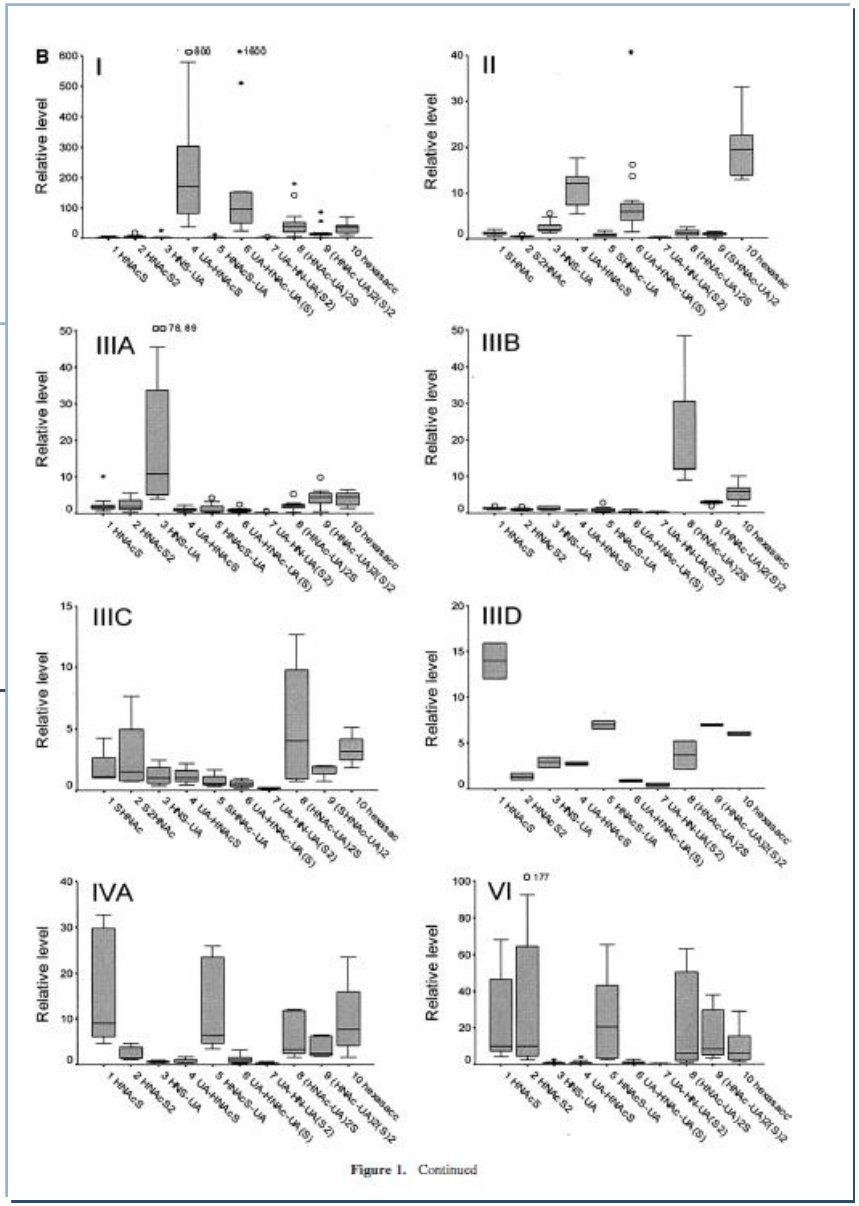


Figure 1. Continued

LC-MS/MS analysis of GAGs disaccharides after enzymatic hydrolysis

- Multiplex assay
- **Commercially available enzymes** (keratanase II, heparitinase and chondroitinase B)
- **LC-MS/MS**

- 2001 Oguma et al., CS/DS
- 2007 Oguma et al., DS/HS/KS
- 2014 Tomatsu et al., DS/HS/KS

- Patterns specific of each MPS
- Decrease with ERT

BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **15**: 356–362 (2001)
DOI: 10.1002/bmc.74

ORIGINAL RESEARCH

Analytical method of chondroitin/dermatan sulfates using high performance liquid chromatography/turbo ionspray ionization mass spectrometry: application to analyses of the tumor tissue sections on glass slides

Toshihiro Oguma,* Hidenao Toyoda, Toshihiko Toida and Toshio Imanari
Faculty of Pharmaceutical Sciences, Chiba University, Inage, Chiba, Japan

Received 11 August 2000; revised 26 September 2000; accepted 27 September 2000

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

Toshihiro Oguma [a,*], Shunji Tomatsu [b], Adriana M. Montano [b], Osamu Okazaki [a]

[a] *Drug Metabolism and Pharmacokinetics Research Laboratory, Shinagawa-ku, Tokyo 140-8710, Japan*
[b] *Department of Pediatrics, Saint Louis University Pediatric Research Institute, St. Louis, MO 63130, USA*

Received 21 February 2007
Available online 24 May 2007

Analytical Biochemistry 368 (2007) 79–86

J Anal Bioanal Tech. ; 2014(Suppl 2): 006–. doi:10.4172/2155-9872.S2-006.

Assay for Glycosaminoglycans by Tandem Mass Spectrometry and its Applications

Shunji Tomatsu[1,*], Tsutomu Shimada[1], Robert W Mason[1], Joan Kelly[2], William A LaMarr[2], Eriko Yasuda[1], Yuniko Shibata[3], Hideyuki Futatsumori[3], Adriana M Montaño[4], Seiji Yamaguchi[5], Yasuyuki Suzuki[6], and Tadao Orii[7]

LC-MS/MS analysis of GAGs disaccharides after enzymatic hydrolysis

- Multiplex assay
- **Genetic engineering home prepared enzymes**
- 2013 : analysis of DS and HS (de Ru et al.)
- 2015 : analysis of DS, HS and KS (Langereis et al.)

J Inherit Metab Dis (2013) 36:247–255
DOI 10.1007/s10545-012-9538-2

ORIGINAL ARTICLE

Plasma and urinary levels of dermatan sulfate and heparan sulfate derived disaccharides after long-term enzyme replacement therapy (ERT) in MPS I: correlation with the timing of ERT and with total urinary excretion of glycosaminoglycans

Minke H. de Ru · Linda van der Tol · Naomi van Vlies · Brian W. Bigger · Carla E. M. Hollak · Lodewijk IJlst · Wim Kulik · Henk van Lenthe · Muhammad A. Saif · Tom Wagemans · Willem M. van der Wal · Ronald J. Wanders · Frits A. Wijburg

RESEARCH ARTICLE

A Multiplex Assay for the Diagnosis of Mucopolysaccharidoses and Mucolipidoses

Eveline J. Langereis[1], Tom Wagemans[1,2], Wim Kulik[2], Dirk J. Lefeber[3], Henk van Lenthe[2], Esmee Oussoren[4], Ans T. van der Ploeg[4], George J. Ruijter[5], Ron A. Wevers[3], Frits A. Wijburg[1]*, Naomi van Vlies[1,2]

1 Department of Pediatric Metabolic Diseases, Emma Children's Hospital and Amsterdam Lysosome Center 'Sphinx', Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, 2 Laboratory for Genetic Metabolic Diseases, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, 3 Translational Metabolic Laboratory, Departments of Neurology & Laboratory Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands, 4 Department of Pediatrics, Center for Lysosomal and Metabolic Diseases, Erasmus Medical Center, Rotterdam, The Netherlands, 5 Department of Clinical Genetics, Center for Lysosomal and Metabolic Diseases, Erasmus Medical Center, Rotterdam, The Netherlands

PLOS ONE | DOI:10.1371/journal.pone.0138622 September 25, 2015

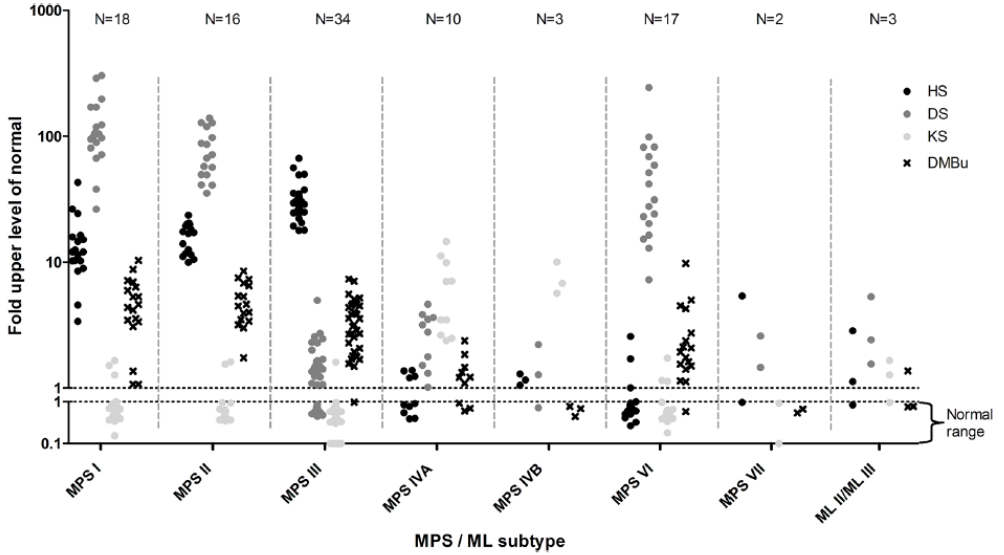
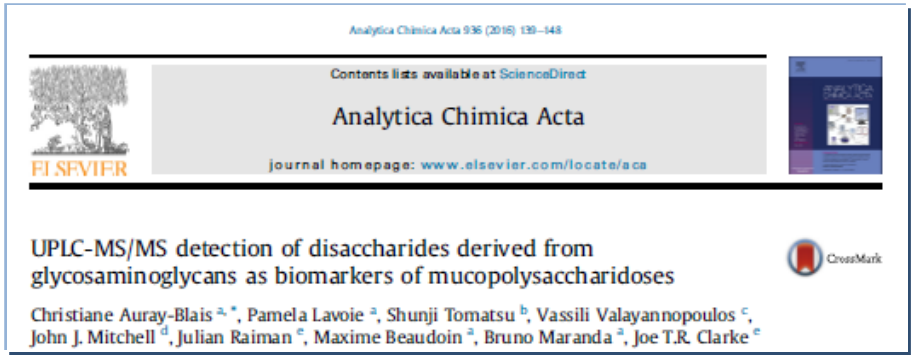


Fig 2. Concentrations of HS, DS and KS measured by multiplexed LC-MS/MS in confirmed MPS and ML patients. Values represent the fold-ULN.

doi:10.1371/journal.pone.0138622.g002

LC-MS/MS analysis of GAGs disaccharides after methanolysis

- Multiplex assay
- C Auray-Blais 2011 : analysis of DS, HS in urine
- C Auray-Blais 2012 : analysis of DS, HS in urine collected on filter paper
- Chuang et al. 2013 : analysis of DS and HS
- C Auray-Blais 2016 : KS



Analytica Chimica Acta 936 (2016) 139–148

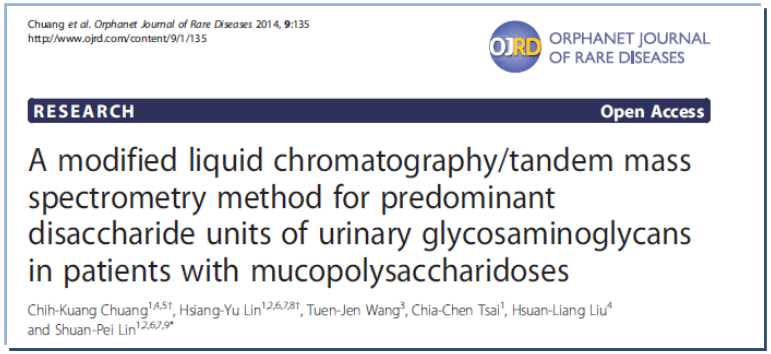
Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

UPLC-MS/MS detection of disaccharides derived from glycosaminoglycans as biomarkers of mucopolysaccharidoses

Christiane Auray-Blais [a, *], Pamela Lavoie [a], Shunji Tomatsu [b], Vassili Valayannopoulos [c], John J. Mitchell [d], Julian Raiman [e], Maxime Beaudoin [a], Bruno Maranda [a], Joe T.R. Clarke [e]



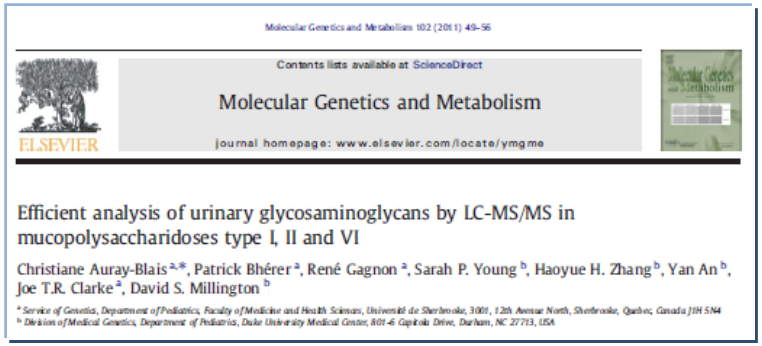
Chuang et al. Orphanet Journal of Rare Diseases 2014, 9:135
http://www.ojrd.com/content/9/1/135

ORPHANET JOURNAL OF RARE DISEASES

RESEARCH Open Access

A modified liquid chromatography/tandem mass spectrometry method for predominant disaccharide units of urinary glycosaminoglycans in patients with mucopolysaccharidoses

Chih-Kuang Chuang[1,4,5†], Hsiang-Yu Lin[1,2,6,7,8†], Tuen-Jen Wang[3], Chia-Chen Tsai[1], Hsuan-Liang Liu[4] and Shuan-Pei Lin[1,2,6,7,9*]



Molecular Genetics and Metabolism 102 (2011) 49–56

Contents lists available at ScienceDirect

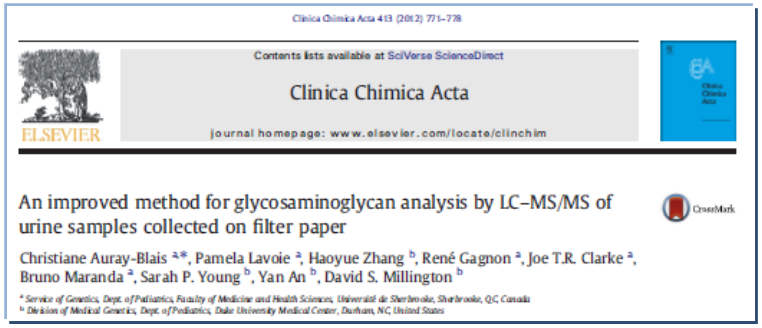
Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Efficient analysis of urinary glycosaminoglycans by LC-MS/MS in mucopolysaccharidoses type I, II and VI

Christiane Auray-Blais [a,*], Patrick Bhérer [a], René Gagnon [a], Sarah P. Young [b], Haoyue H. Zhang [b], Yan An [b], Joe T.R. Clarke [a], David S. Millington [b]

[a] Service of Genetics, Department of Pediatrics, Faculty of Medicine and Health Sciences, Université de Sherbrooke, 3001, 12th Avenue North, Sherbrooke, Quebec, Canada J1H 5N4
[b] Division of Medical Genetics, Department of Pediatrics, Duke University Medical Center, 801-6 Capitola Drive, Durham, NC 27713, USA



Clinica Chimica Acta 413 (2012) 771–778

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

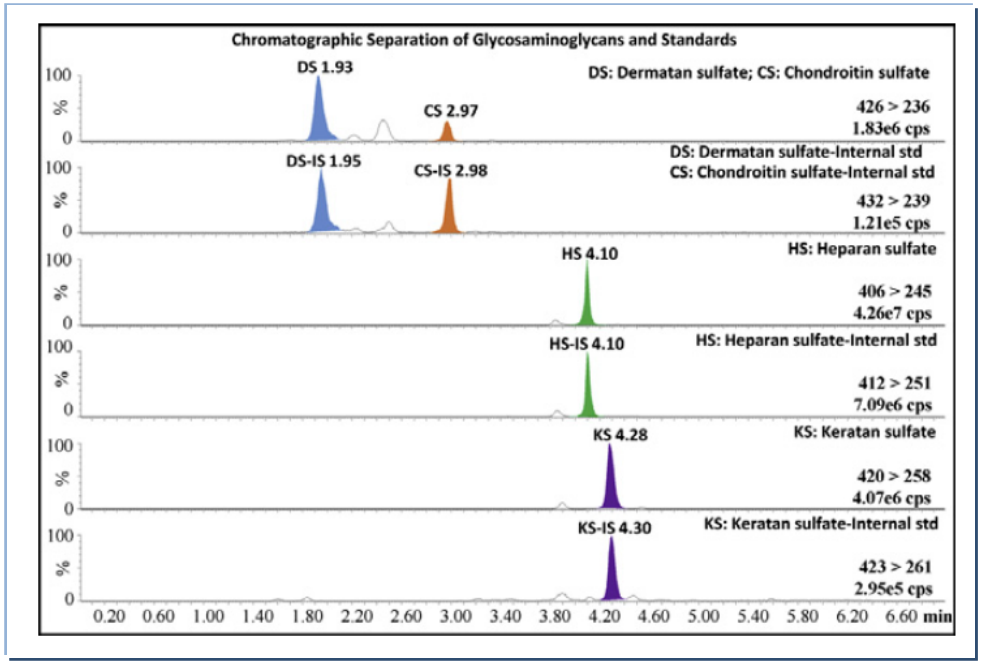
journal homepage: www.elsevier.com/locate/clinchim

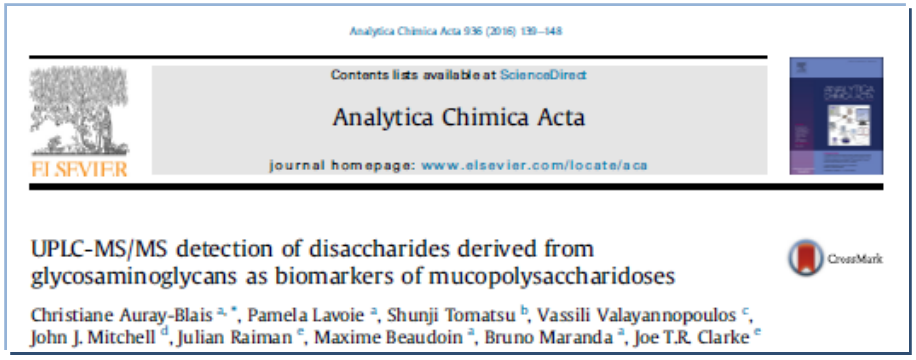
An improved method for glycosaminoglycan analysis by LC–MS/MS of urine samples collected on filter paper

Christiane Auray-Blais [a,*], Pamela Lavoie [a], Haoyue Zhang [b], René Gagnon [a], Joe T.R. Clarke [a], Bruno Maranda [a], Sarah P. Young [b], Yan An [b], David S. Millington [b]

[a] Service of Genetics, Dept. of Pediatrics, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, QC, Canada
[b] Division of Medical Genetics, Dept. of Pediatrics, Duke University Medical Center, Durham, NC, United States

LC-MS/MS analysis of GAGs disaccharides after methanolysis

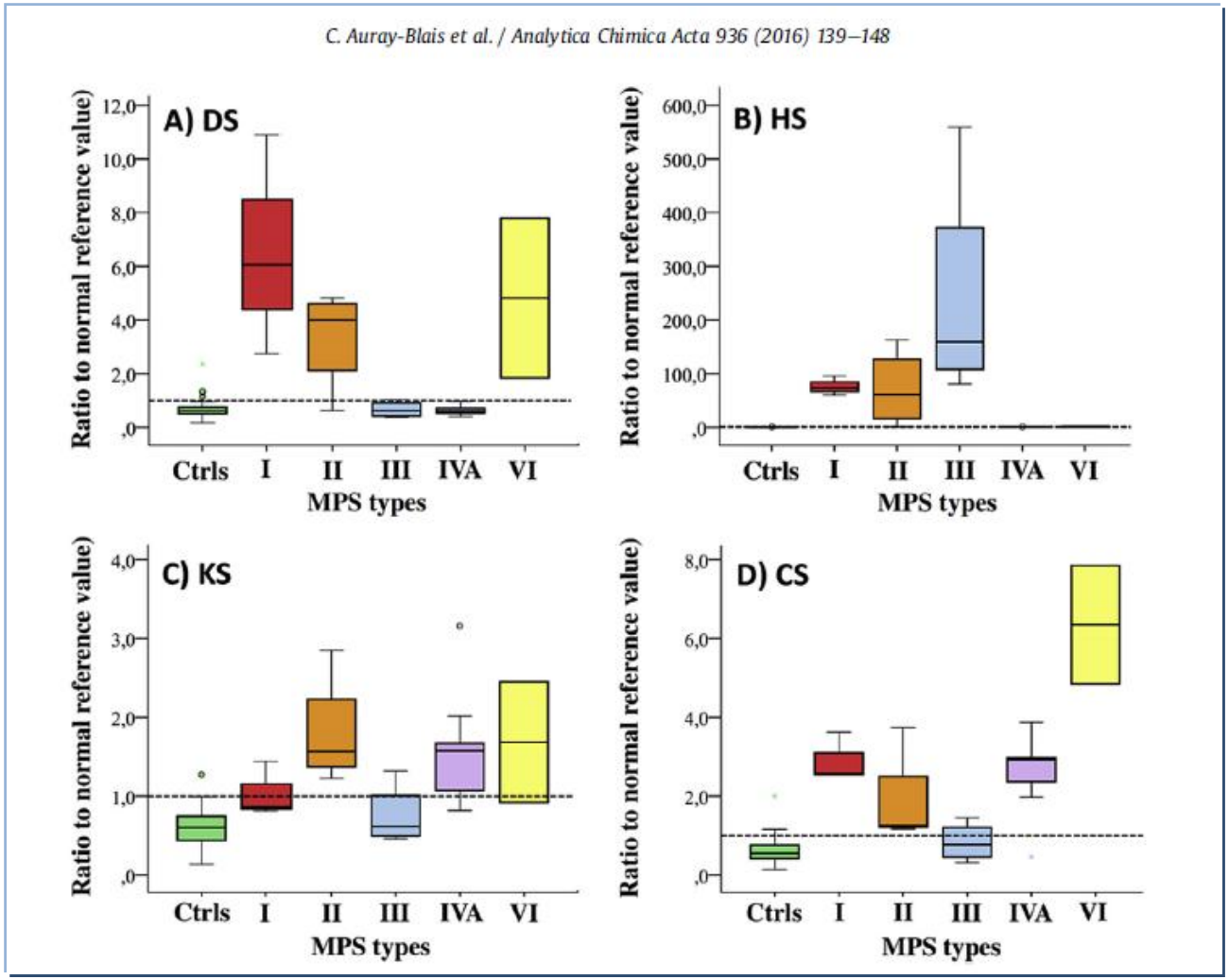




MPS Type	Disease Name	Deficient enzyme	Expected GAG elevation DS	HS	CS	KS	Hyaluronan
MPS IH	Hurler	α-Iduronidase	●	●			
MPS IHS	Hurler-Scheie	α-Iduronidase	●	●			
MPS IS	Scheie	α-Iduronidase	●	●			
MPS II	Hunter	Iduronate sulfatase	●	●			
MPS IIIA	Sanfilippo A	Heparan-N-sulfatase		●			
MPS IIIB	Sanfilippo B	α-N-acetylglucosaminidase		●			
MPS IIIC	Sanfilippo C	AcetylCoA α-glucosamine acetyltransferase		●			
MPS IIID	Sanfilippo D	N-acetylglucosamine 6-sulfatase		●			
MPS IVA	Morquio A	Galactosamine-6-sulfate sulfatase			●	●	
MPS IVB	Morquio B	β-Galactosidase				●	
MPS VI	Maroteaux-Lamy	N-acetylgalactosamine 4-sulfatase	●				
MPS VII	Sly	β-Glucuronidase	●	●			
MPS IX	Natowicz	Hyaluronidase 1					●

IS prepared by deuterio-methanolysis

LC-MS/MS analysis of GAGs disaccharides after methanolysis



Enzyme measurements

Large literature since 2001 with improvements

MS/MS measurement of the product of the enzymatic reaction, more hydrophobic than the substrate

SPE/LLE/LC

Simultaneous incubations

Deuterated IS

Largely developed in **DBS** (Gelb group, Seattle):

- symptomatic diagnosis
- targeted large scale screening (*i.e.* HCM in Fabry, SM in Gaucher
- newborn screening
- …

Possible in **leuko/fibro/lympho**

One commercialized kit (Perkin-Elmer): Fabry, Gaucher, Pompe, Krabbe, NPAB, MPS I

ENZYMATIC ACTIVITIES in DRIED BLOOD SPOTS (or leukocytes)

- Gaucher,
- Fabry,
- Pompe,
- Krabbe,
- Niemann-Pick A/B
- MPS I,
- MPS II,
- MPS III A-D,
- MPS IV A,
- MPS VI
- CLN1, CLN2,
- …

NEWBORN SCREENING

Gelb *et al*., Clin Chem. 2015;61:335-46.
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Elliott *et al*., Mol Genet Metab. 2016;118:304-9.
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Ombrone et al., Mass Spectrom Rev. 2016;35:71-84.
Peake and Bodamer J Pediatr Genet. 2017;6:51-60

Conclusions

- **SMALL SAMPLE SIZE** plasma, urine (pediatrics +++)

- **New developments**
  - Global screening of sphingolipidoses
  - Screening of NPA/B and NPC

- **Great improvement in sensitivity and specificity**
  - Diagnosis of adult/moderate forms
  - Better screening of Fabry variants/females
  - A tool for evaluating pathogenicity of the mutation (Fabry,…)

- ***Hydrops fetalis*** (amniotic fluid):
  - More reliable screening of ISSD and oligosaccharidoses
  - New screening of Gaucher disease

- **Changing the diagnosis strategy ?**
  - High throughput methods – diagnosis - screening –
  - Enzyme measurements: 1st tier ?
  - Plasma screening of NPA/B and NPC
  - …

Acknowledgements

Service des Maladies Héréditaires du Métabolisme
Centre de Biologie et de Pathologie Est

David Cheillan
Roseline Froissart
Cécile Pagan
Magali Pettazzoni
Christine Vianey-Saban
Séverine Ruet

Philippe Latour
Marie-T. Vanier

Hospices Civils de Lyon

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