Multiplex LC-MS/MS lysosphingolipids analysis in plasma for the screening of sphingolipidoses and Niemann-Pick type C disease

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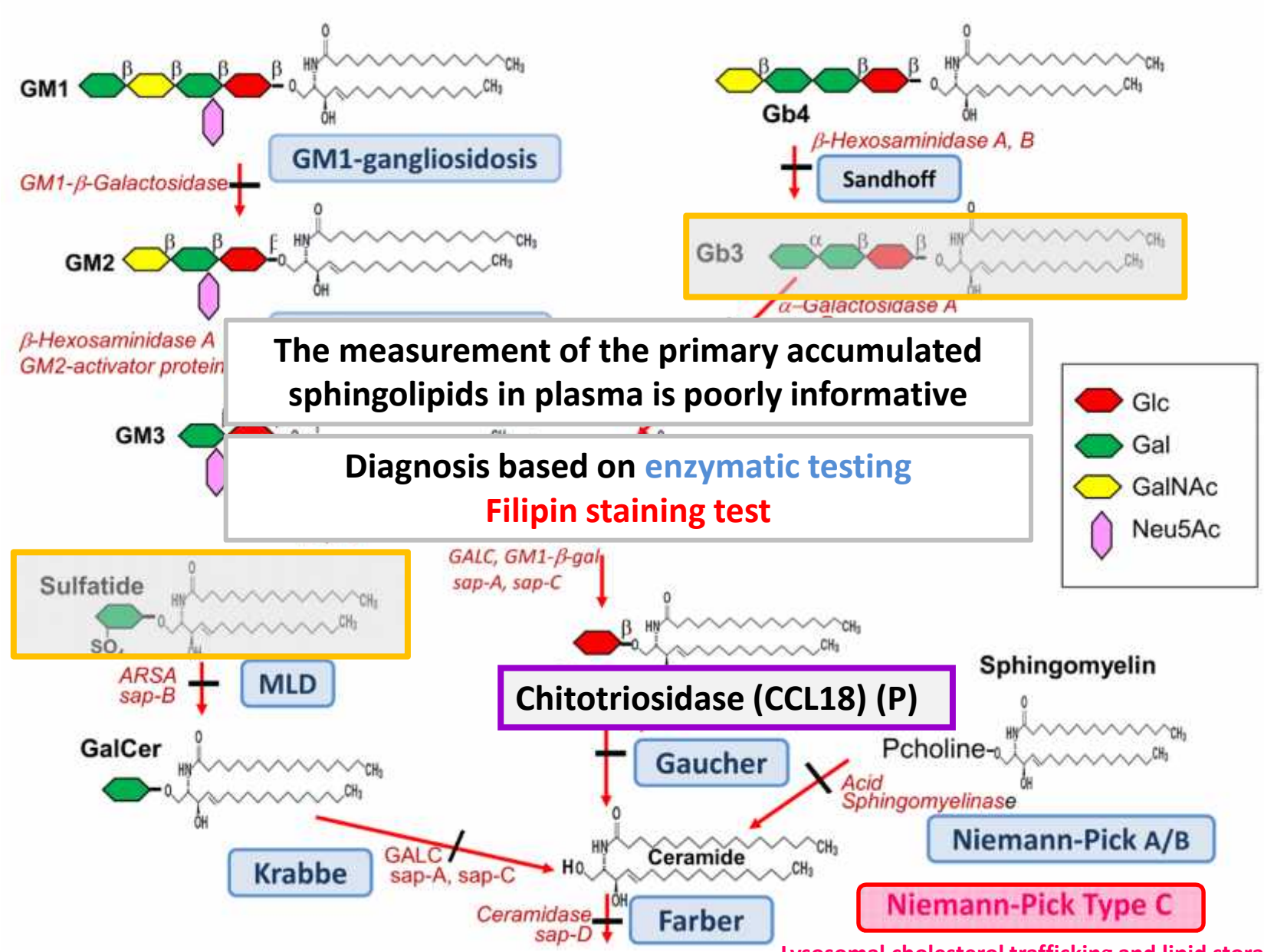
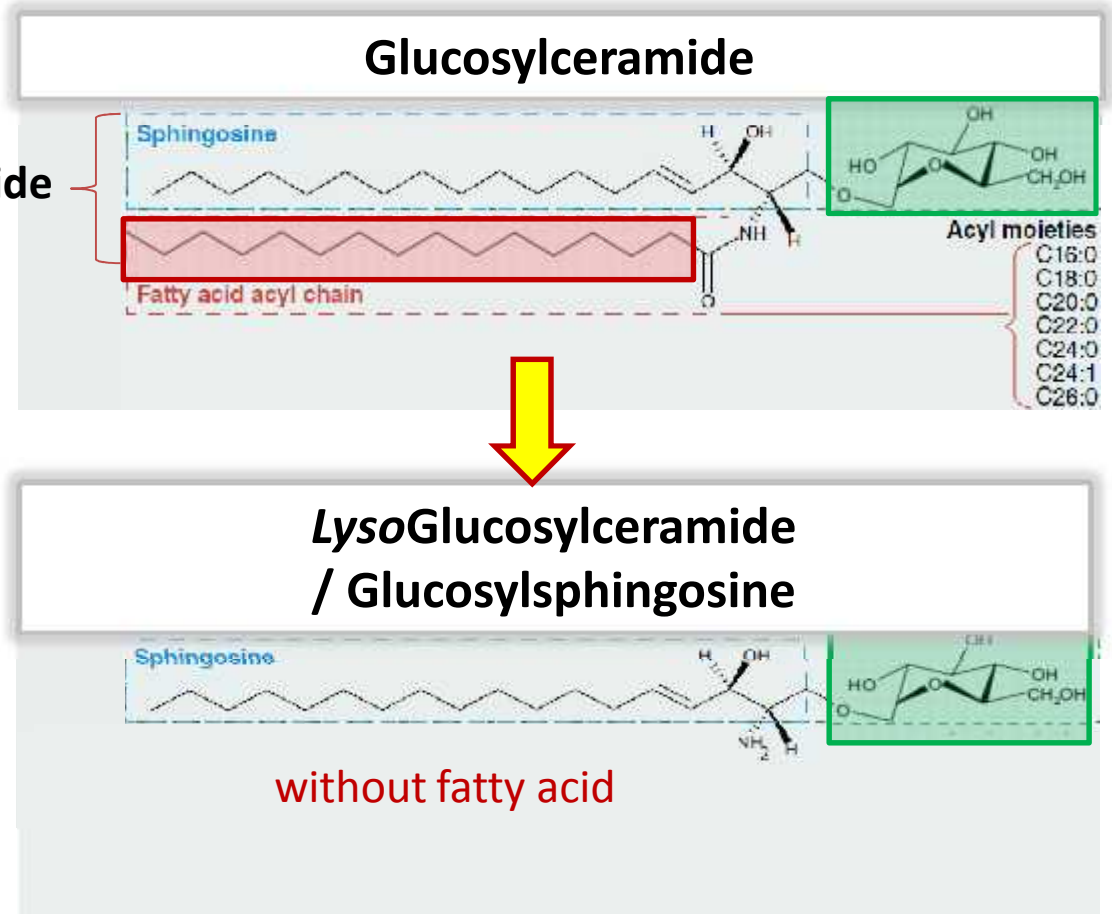
Sphingolipidoses



Courtesy of Dr M-T. Vanier, Lyon France

Sphingolipids/LysoSphingolipids in plasma



From Sun and Zhang, Advances in Gaucher Disease: Basic and Clinical Perspectives 260 pages, August 2013. Future Medicine Ltd.

- Lysosphingolipids (LysoSL) = deacylated form of the sphingolipids
- Role in pathophysiology ?
- With the use of MS/MS Emerging biomarkers **in plasma**

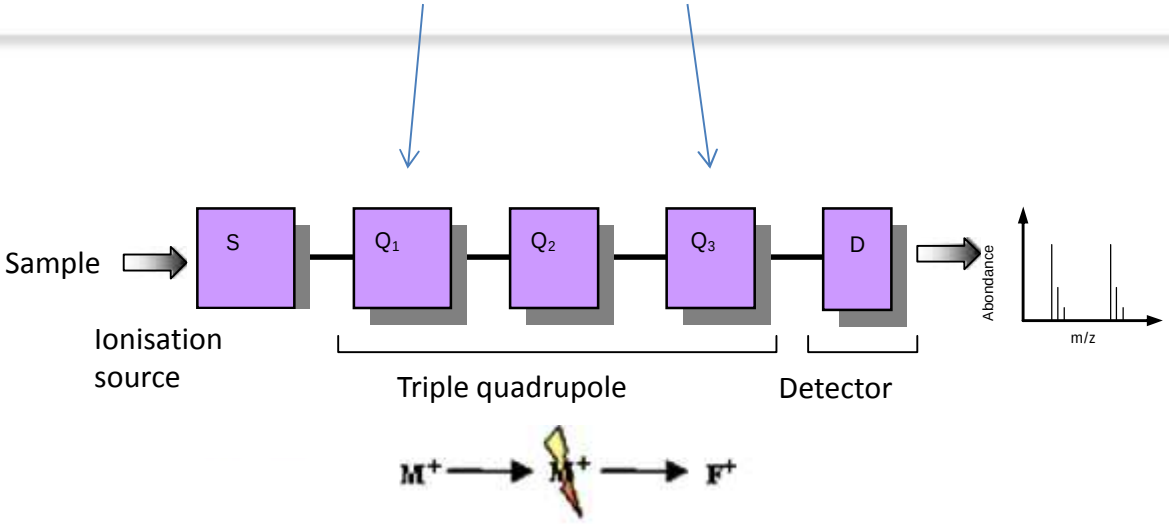
for screening of sphingolipidoses and Niemann-Pick type C

Tandem Mass Spectrometry

Very sensitive,
very specific technique

Allows **the simultaneous measurement** of numerous compounds **all together**, in complex mixtures, according to **specific fragmentation of each molecule**

« **TRANSITION** » = pair of (precursor ion)/(product ion)



LC-MS/MS LysoSLs measurement

LysoGlobotriaosylceramide (LysoGb$_3$) **Fabry**	***Aerts et al.* Proc Natl Acad Sci USA, 2008** (not MS/MS) **Boutin et al. Clin Chim Acta, 2012**
Lysogalactosyl-ceramide (LysoGalCer) **Krabbe**	*Chuang et al. Clin Chim Acta 2013*
Lysoglucosyl-ceramide (LysoGlcCer) **Gaucher**	*Dekker et al. Blood 2011*
Lysosphingomyelin (LysoSM) **Niemann-Pick A/B**	*Chuang et al . Mol Genet Metab 2014*
LysoSM analogue 509 (LysoSM509) **Niemann-Pick A/B and C**	*Giese et al.* Orphanet J Rare Dis 2015
~~**Lysosulfatide** (LysoSulf) **Metachromatic leukodystrophy**~~	*Mirzaian et al. Blood cell Mol Dis 2015*


DE GRUYTER

Open Access

Giulia Polo, Alessandro P. Burlina, Thilini B. Kolamunnage, Michele Zampieri, Carlo Dionisi-Vici, Pietro Strisciuglio, Martina Zanetti, Mario Plebani and Alberto B. Burlina*

Diagnosis of sphingolipidoses: a new simultaneous measurement of lysosphingolipids by LC-MS/MS

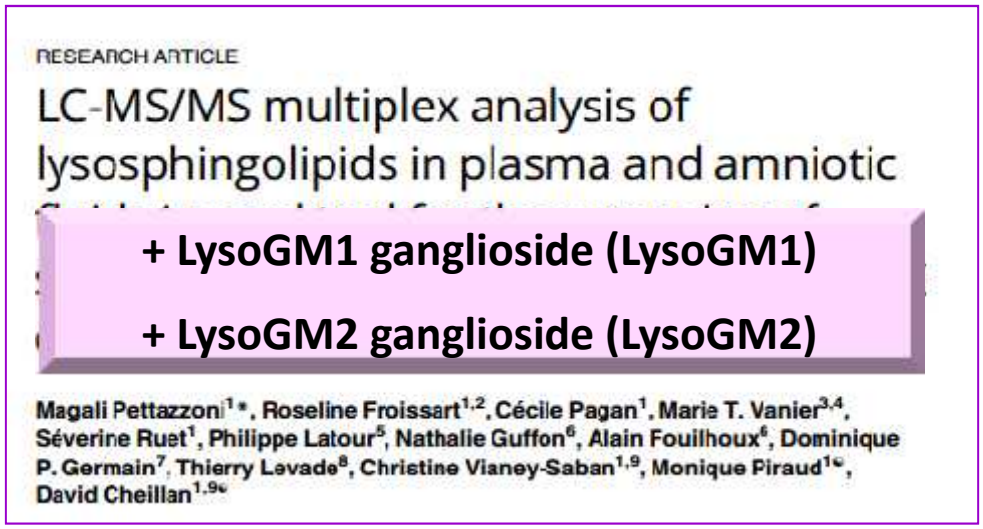
Polo et al. Clin Chem Lab Med 2016


Clinica Chimica Acta

Simultaneous quantitation of sphingoid bases by UPLC-ESI-MS/MS with identical ^{13}C-encoded internal standards

M. Mirzaian, P. Wisse, M.J. Ferraz, A.R.A. Marques, P. Gaspar, S.V. Oussoren, K. Kytidou, J.D.C. Codée, G. van der Marel, H.S. Overkleeft, J.M. Aerts

Mirzaian et al. Clin Chim Acta 2017


RESEARCH ARTICLE

LC-MS/MS multiplex analysis of lysosphingolipids in plasma and amniotic

+ LysoGM1 ganglioside (LysoGM1)

+ LysoGM2 ganglioside (LysoGM2)

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]☯

Pettazzoni M et al. PLoS One. 2017

Method

Extraction/Purification

200 µL of EDTA plasma + IS + MeOH

SPE (Oasis MCX, 30 mg, 60 µm, Waters Corp)

Liquid chromatography

C8 column (Uptisphère. Interchim©)

Gradient of elution(Phase A: H2O 0.2% FA, Phase B ACN 0.2% FA)

MS/MS– Multiple Reaction Monitoring Mode (MRM)

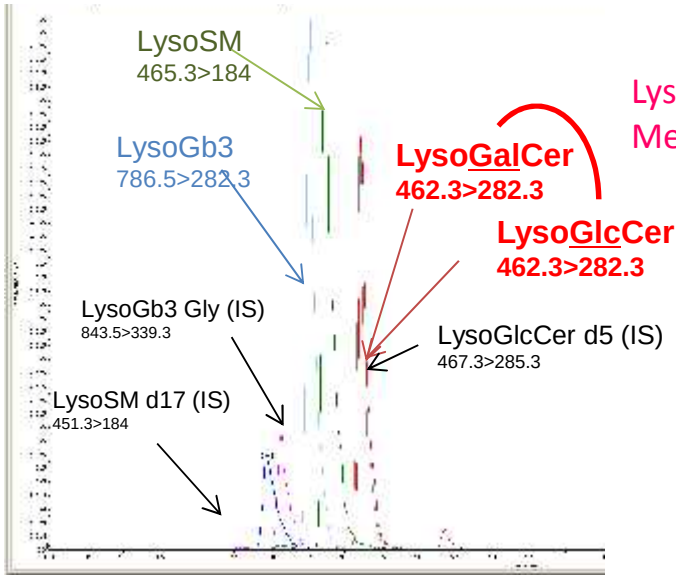
API 4500 Q-Trap
(AbSciex©)

Analyte	STANDARDS	Internal Standard **	MODE	TRANSITION		
Lyso Gb3	Lyso Gb_3	Glycinated lysoGb$_3$	Positive	786.5	>	282.3
Lyso HexCer	Lyso GlcCer	Lyso GlcCer d5	Positive	462.3	>	282.3
Lyso SM	Lyso SM d18:1	Lyso SM d17 :1	Positive	465.3	>	184
Lyso SM 509	Lyso SM d18:1	Lyso SM d17 :1	Positive	509.3	>	184
Lyso GM1	Lyso GM1	S1P d17 :1	Negative	1278.6	>	290.1
Lyso GM2	/	S1P d17 :1	Negative	1116.6	>	290.1*

* No standard; calculated from mass/structure

** Commercially available

Positive mode



LysoGLUCOSYLceramide and lysoGALACTOSYLceramide: not separated
Measured as lysohexosylceramide « LysoHexCer »

Quantitative validation
Except LysoGM1 and LysoGM2

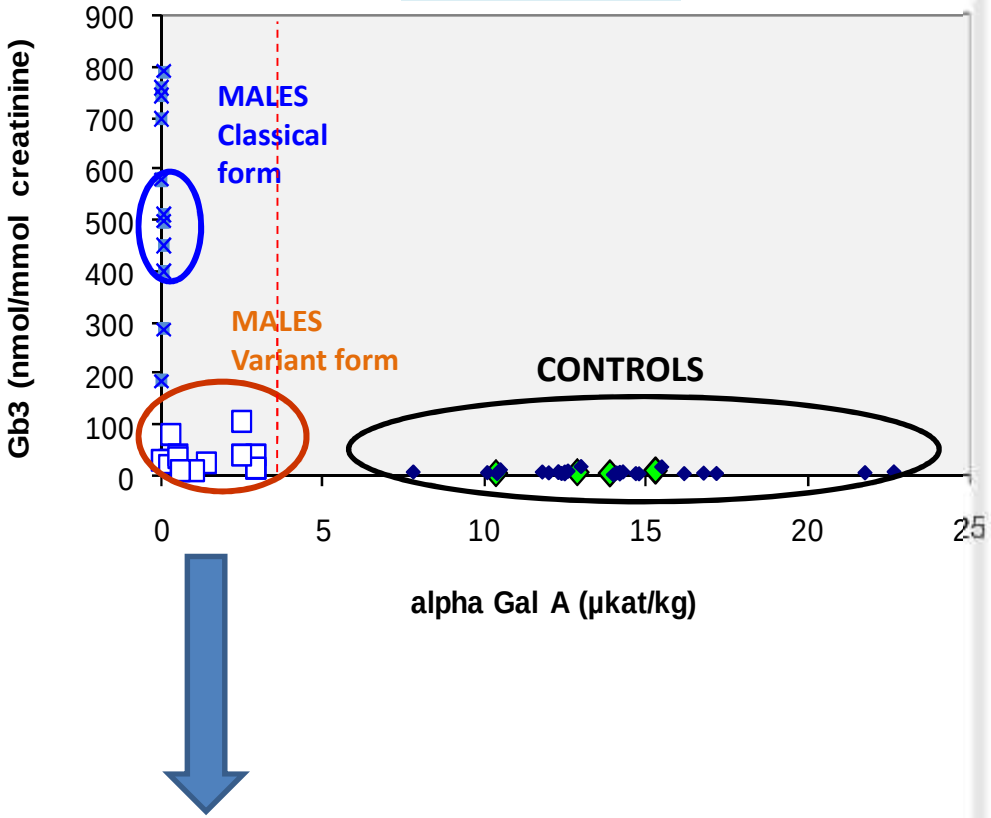
LysoSL multiplex measurement:
Routine experience in Lyon
for the screening of sphingolipidoses and NPC

Fabry disease (X-linked)

Mutations on *GLA gene*

Classical forms / Variant forms with specific variant mutations on *GLA* gene

Fabry males



MALES DIAGNOSIS

α Gal A activity (always deficient)

Fabry females

Heterozygote females

- ***Normal*** **α Gal A: 30% cases**
- ***Normal*** **(U)Gb_3 :**

  18% cases (classical forms)

  most of cases (variant forms)

FEMALES DIAGNOSIS

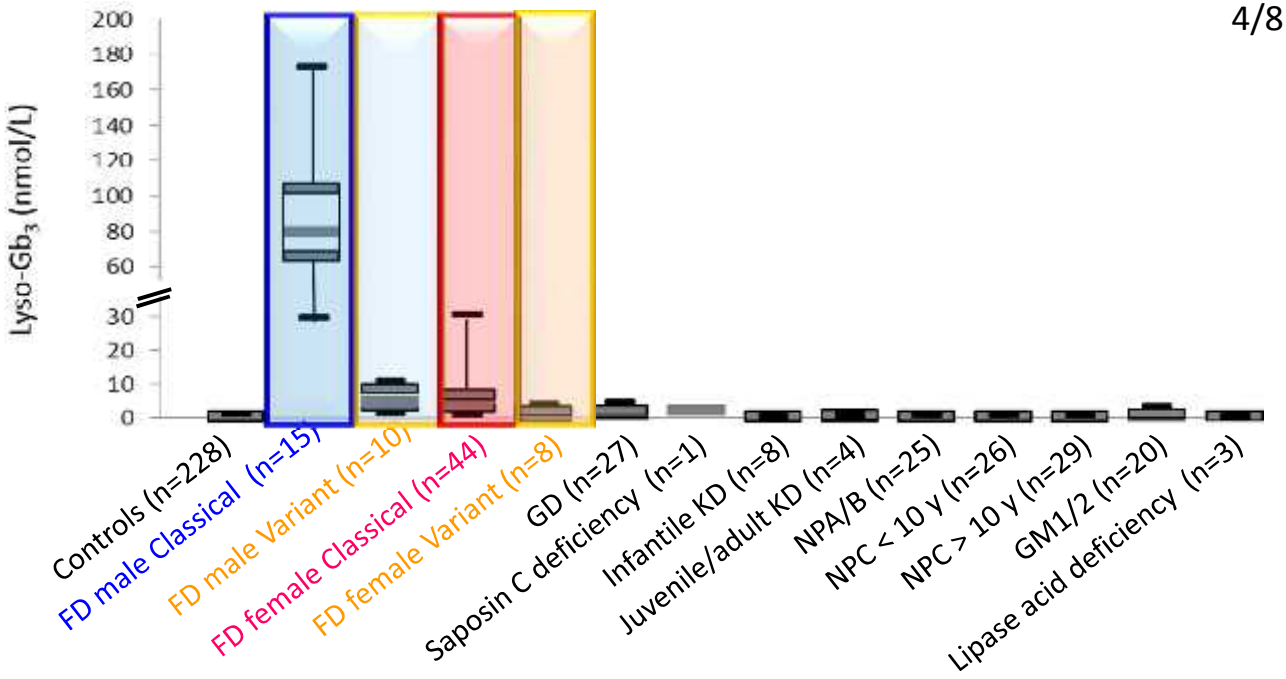
- α Gal A activity
- (U) Gb_3
- *GLA* gene

Fabry disease: Plasma $LysoGb_3$

Results LYON, France

		Males Classical form	Males Variant form *	Females Classical form	Females Variant form **	Controls
	N = 77	n = 15	n = 10	n = 44	N = 8	N = 228
$LysoGb_3$ (nmol/L)	Mean	91	5.8	5.7	1.4	< 0.6 (99^{th} perc.)
	Range	30 - 173	1.8 - 11.1	1.1-31.2	0.3-4	0-1.1

Normal in 4/8 cases



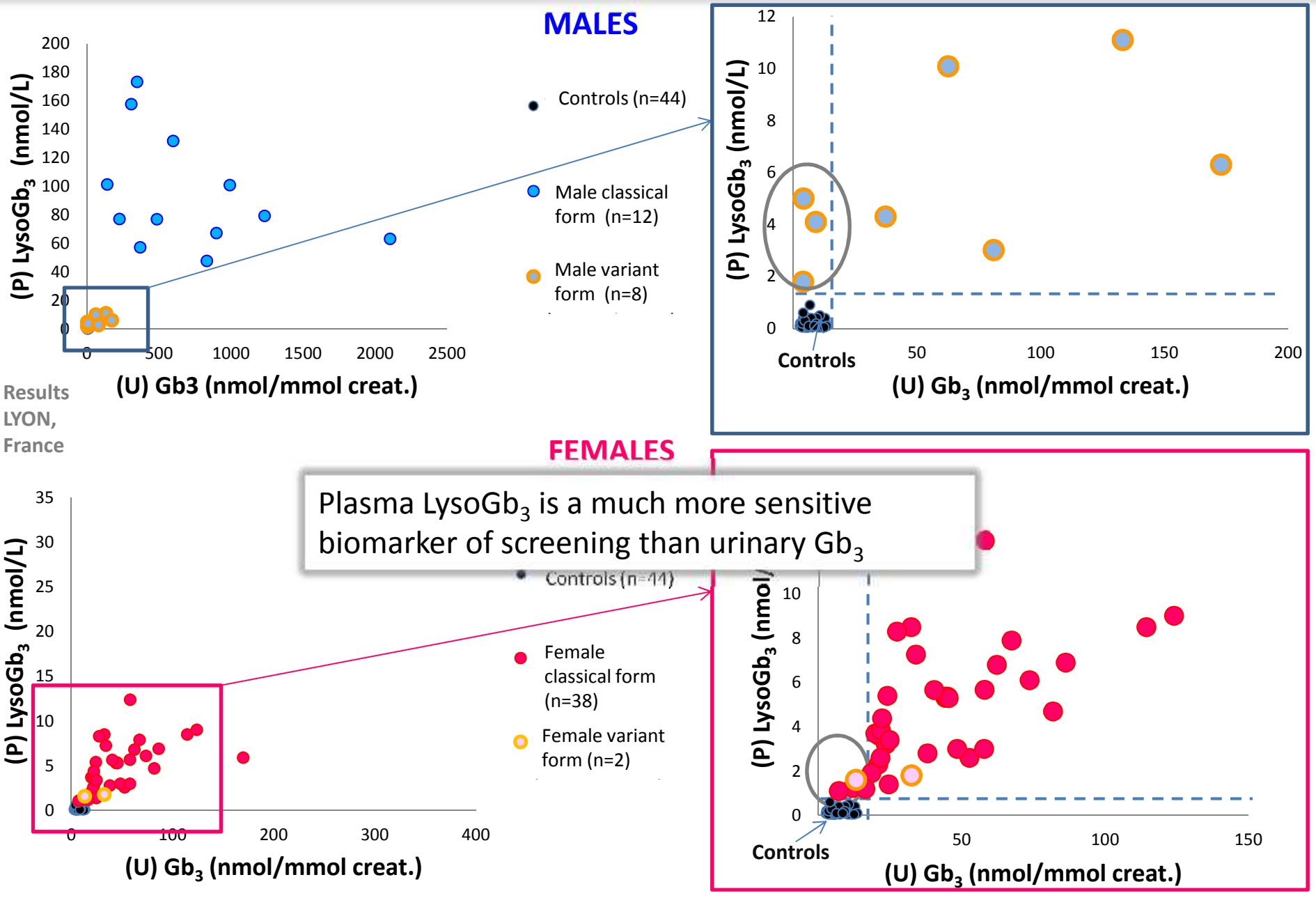
*** Variant *GLA* mutations in males**

- p.Ile232Thr
- p.Met296Val
- p.Arg301Gln
- p.Arg363His
- p.Asn215Ser (n=2)
- p.Asn215Ser
- p.Ile198Thr
- p.Phe113Leu (n=3)

**** Variant *GLA* mutations in females**

- p.Arg363His (n=3)
- p.Asn215Ser
- p.Phe113Leu (n=4)

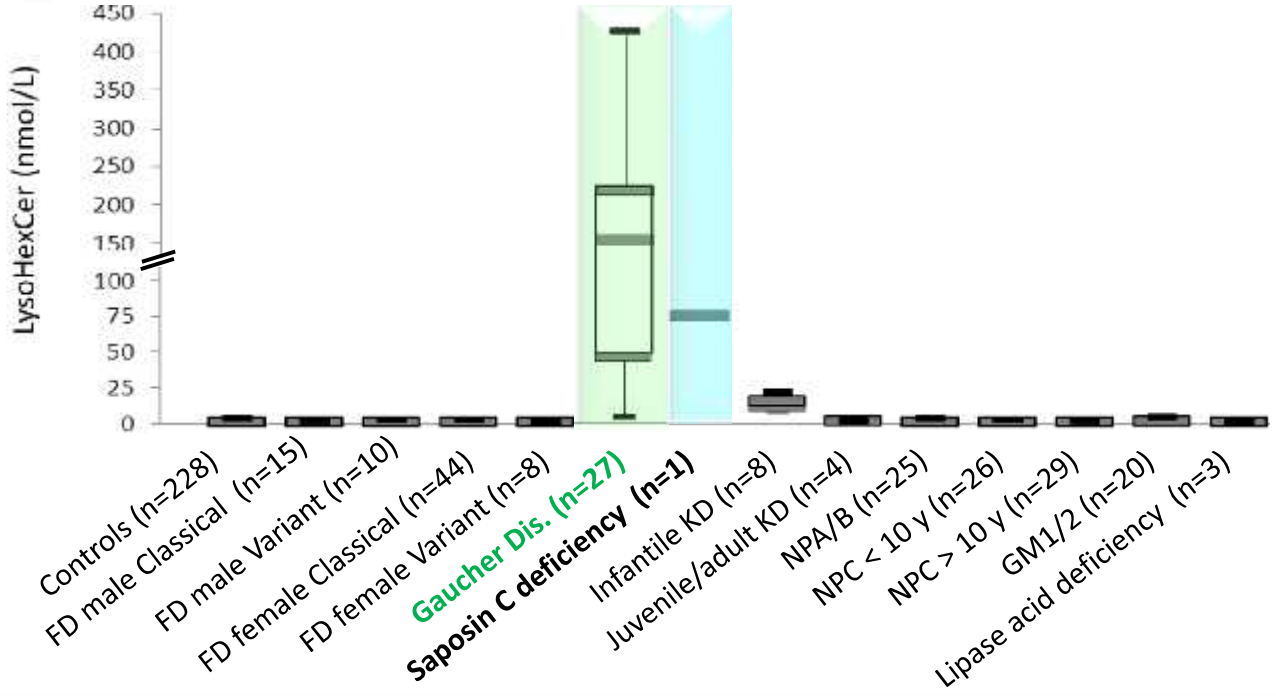
Fabry disease: Correlation (U)Gb_3 / (P) $LysoGb_3$



Gaucher disease: LysoHexCer

Results LYON, France

		GAUCHER	Saposin C deficiency *	KRABBE (infantile)	KRABBE (juvenile / adult)	Controls
		n = 27	n = 1	n = 8	n = 4	n = 228
LysoHexCer (nmol/L)	Mean	160	76	13	1.2	< 3.3 (99th perc.)
	Range	5 - 427	-	9 - 22	0.9 – 1.4	0.1-3.5



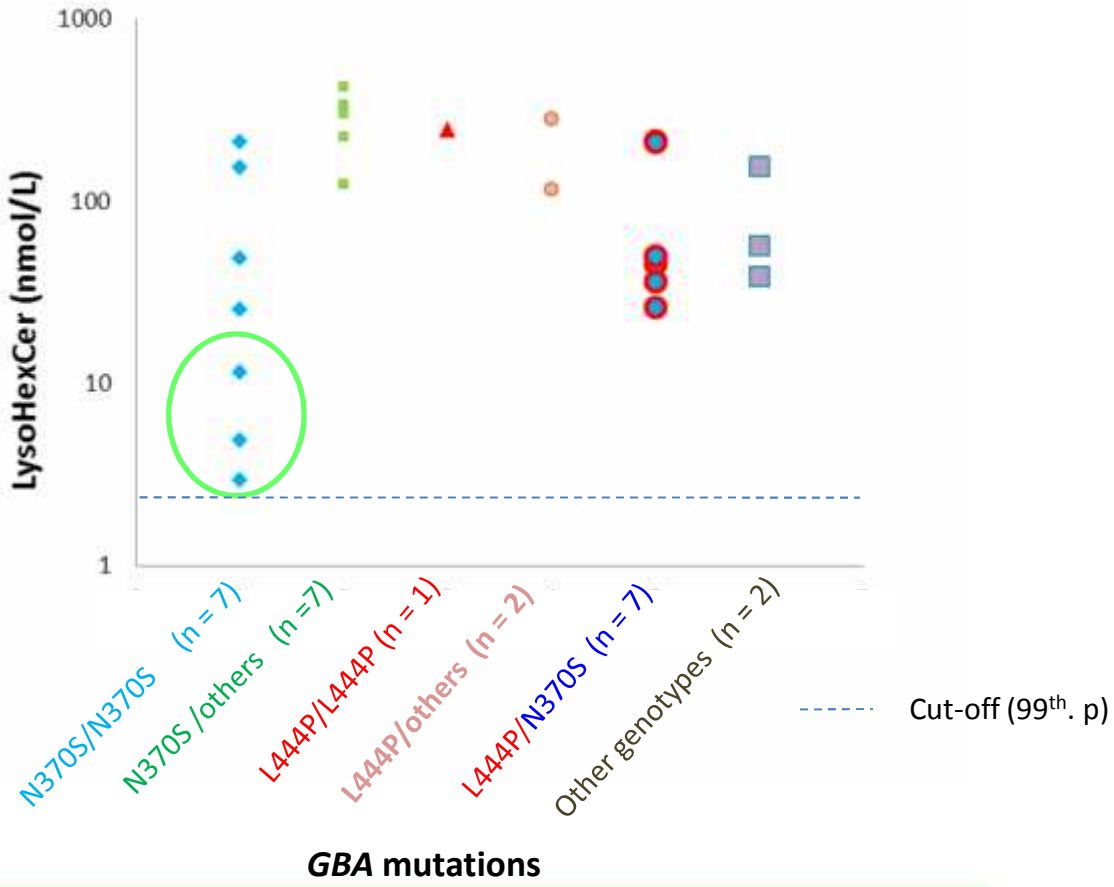
* Treated by SRT

- Highly elevated in GD, large range of values, can be very moderately elevated in some cases
- Elevated in case of saposin C deficiency
- In GD: Correlation with chitotriosidase and CCL18 (= indirect and non specific biomarkers)

Gaucher disease: LysoHexCer/*GBA* Genotype correlation

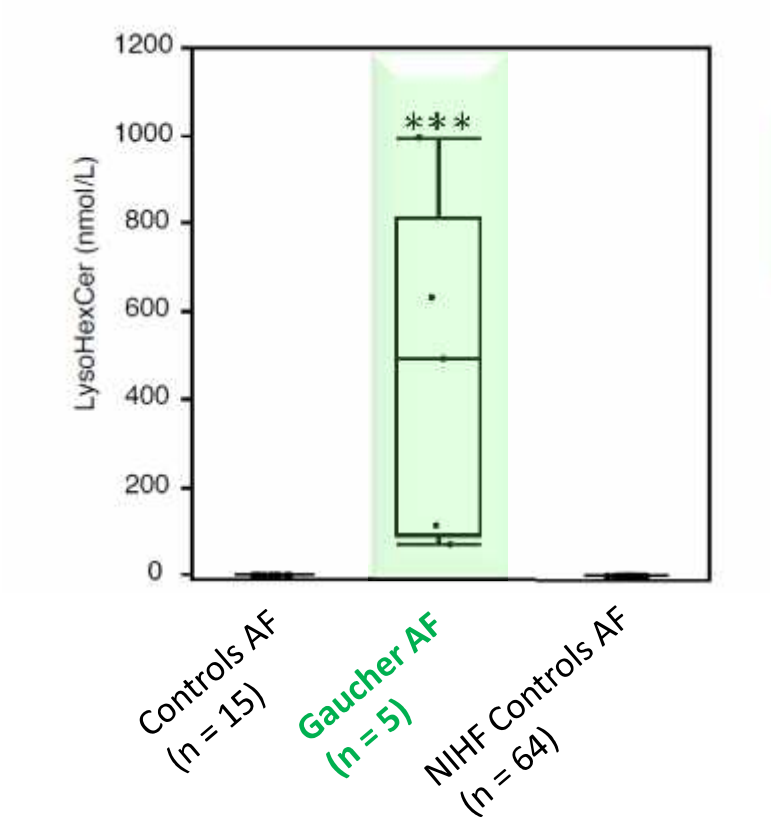
Biological variability of LysoHexCer levels +++ for a same genotype

Results of the French cohort, untreated Gaucher patients



Lower LysoHexCer values in patients homozygous for N370S mutation of *GBA* gene

Gaucher disease: LysoHexCer in Amniotic Fluid (AF)



In case of non-immune hydrops fœtalis (NIHF)
New powerful biomarker in AF of fetal GD

		Controls	Gaucher AF	NIHF Controls AF
		n = 15	n = 5	n = 64
LysoHexCer (nmol/L)	**Mean**	< 0.7	460 ***	< 0.7
	Range		68 - 996	

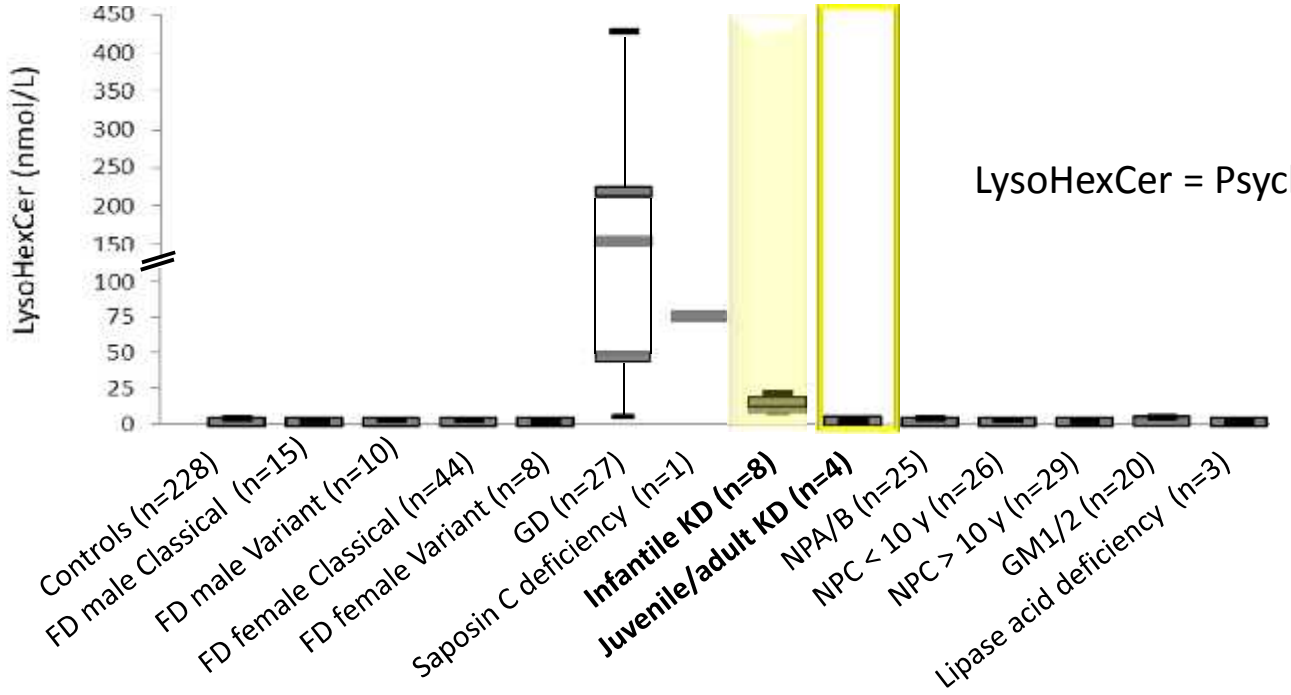
Pettazzoni M et al. PLoS One. 2017

*** p<0.001

Krabbe disease: LysoHexCer

Results LYON, France

		GAUCHER	KRABBE (infantile)	KRABBE (juvenile / adult)	Controls
		n = 27	n = 8	n = 4	n = 228
LysoHexCer (nmol/L)	Mean	160	13	1.2	< 3.3 (99th perc.)
	Range	5 - 427	9 - 22	0.9 – 1.4	0.1-3.5



LysoHexCer = Psychosine

Moderate increase in infantile KD
In normal range in juvenile and adult forms
Saposin A deficiency ? To be evaluated

Niemann-Pick diseases

Niemann-Pick type A/B

Sphingomyelinase deficiency

- Tedious enzyme activity measurement

Niemann-Pick type C

Lysosomal cholesterol trafficking and lipid storage disorder

- Filipin staining test on cultured fibroblasts
- Genetic testing *NPC1* or *NPC2* gene

Chitotriosidase

Moderate but inconstant elevation

6 % of patients are homozygous for 24 bp duplication in *CHIT1* gene and 30 – 40 % heterozygotes

MS/MS

Oxysterols plasma (2010)

Cholestane-3β,5α,6β-triol (and 7-ketocholesterol)

LysoSM (2014)


Molecular Genetics and Metabolism

Short Communication

Lyso-sphingomyelin is elevated in dried blood spots of Niemann–Pick B patients

Wei-Lien Chuang, Joshua Pacheco, Samantha Cooper, Margaret M. McGovern, Gerald F. Cox, Joan Keutzer, X. Kate Zhang

LysoSM « 509 » = LSM + 44 (2015)


ORPHANET JOURNAL OF RARE DISEASES

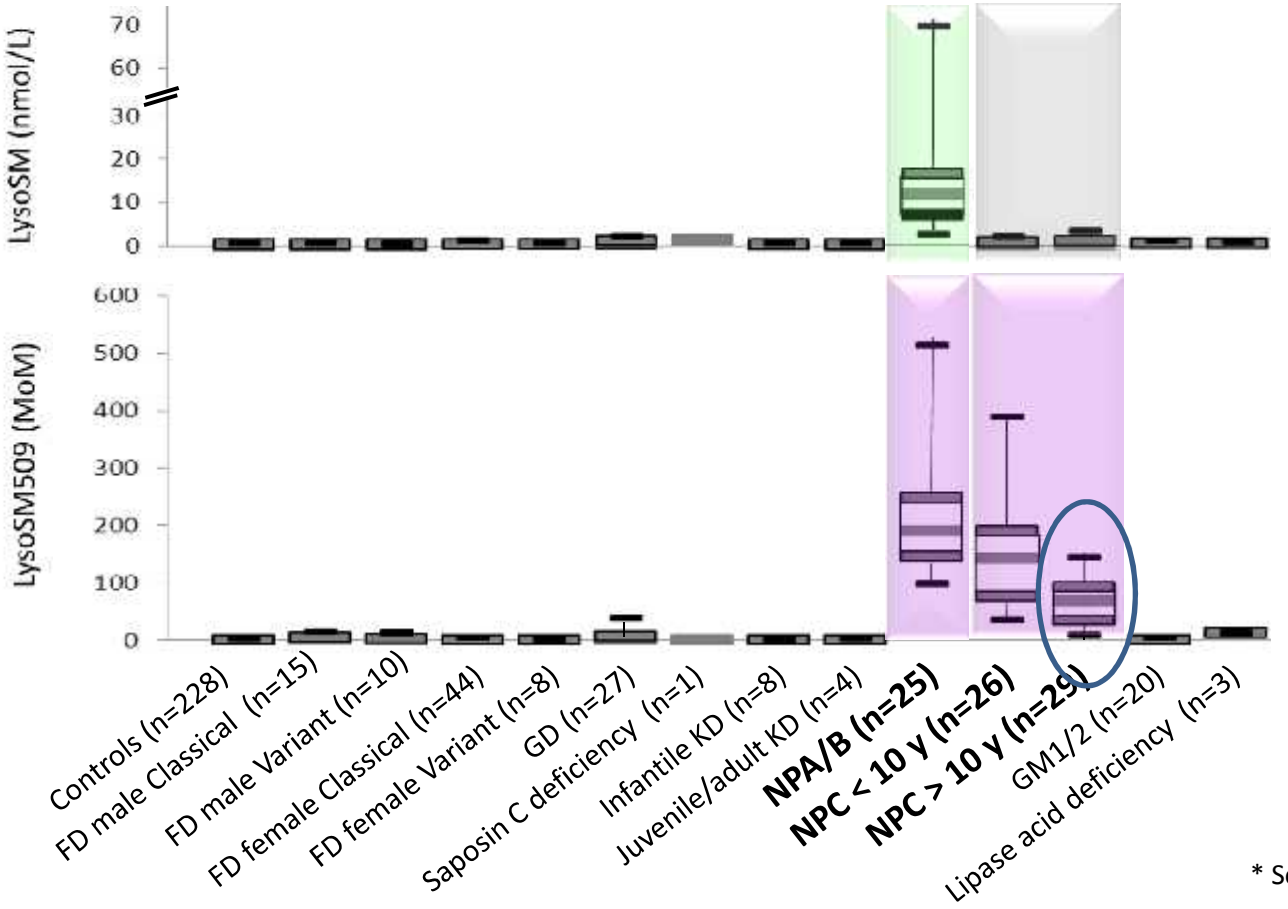
RESEARCH Open Access

A novel, highly sensitive and specific biomarker for Niemann-Pick type C1 disease

NPA/B and NPC: LysoSM and LysoSM509 in plasma

Results LYON, France

		NPA/B	NPC* < 10 y	NPC* > 10 y	Controls
		n = 24	n = 26	N = 29	n = 228
LysoSM (nmol/L)	Mean	16.5	0.7	1.0	< 1.9 (99th perc.)
	Range	2.4 - 69.6	0.2 – 2.1	0.2 – 3.5	0.1-2.0
LysoSM509 (MoM)	Mean	214	149	63	< 4.3 (99th perc.)
	Range	98-515	34 - 390	7 - 144	0-9.1

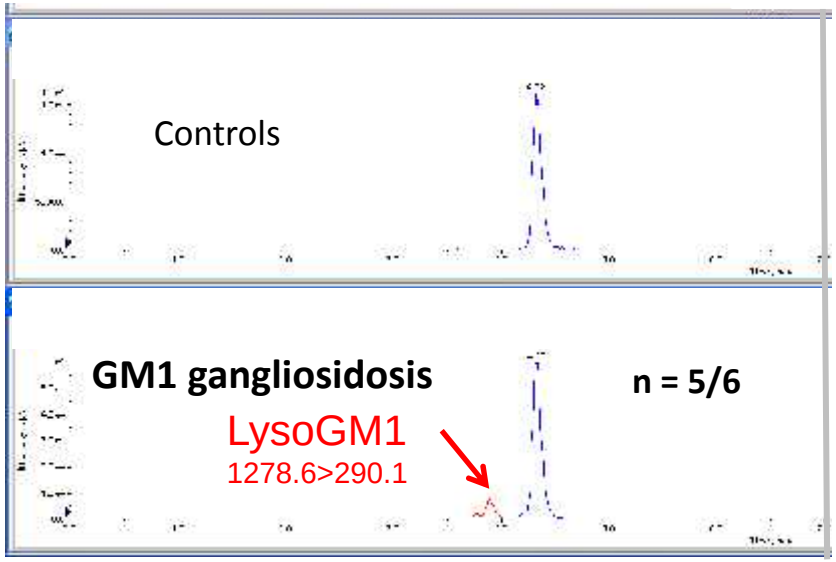


* Some patients are treated

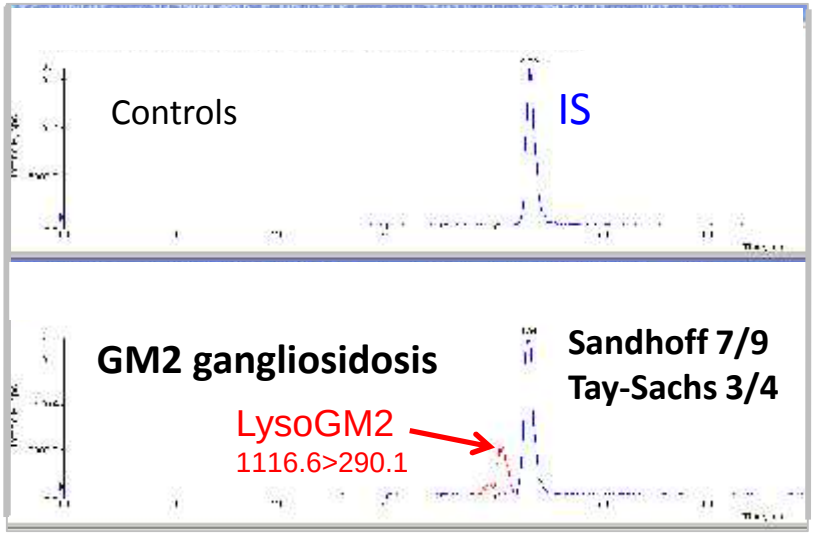
GM1 & GM2 gangliosidoses: LysoGM1/GM2 in plasma

IS: d17 Sphingosine-1P

LysoGM1



LysoGM2



In spite of poor recovery results (lack of specific IS) **Good specificity**

Not detected in controls

Abnormal presence of lysoGM1 and lysoGM2

in GM1 gangliosidosis (5/6 cases)

in Sandhoff diseases (7/9 cases)

in Tay-Sachs diseases (3/4 cases)

Lack of sensitivity for mild and adult forms

Conclusion

Our LysoSLs multiplex assay: 6 lysoSLs including LysoGM1 and LysoGM2

- **Efficient and rapid biochemical screening tool**
  - ✓ Small plasma sample volume (pediatrics +++)
  - ✓ Better screening of FD males with variant form, and females
  - ✓ More sensitive screening of NPC than oxysterols
  - ✓ Differential screening of NPC, NPA/B and GD in the same run
  - ✓ Specific screening of fetal GD
  - ✓ Useful for the monitoring of patients (FD, GD)

- **Positive results to be confirmed by enzymatic measurement, and molecular studies**
- **Adults and mild phenotype: possibly normal screening**

- **Other analogues of LysoSL** (mass variation on the sphingosine moiety): could be interesting biomarkers to be evaluated ?
  (C Auray-Blais, Sherbrooke, Canada)

Acknowledgments

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