STUDY OF A PATIENT WITH SYMPTOMS COMPATIBLE WITH NIEMANN-PICK DISEASE TYPE C BUT WITH INTERMEDIATE LEVELS OF LYSOSPHINGOMYELIN-509 AND A GENETIC CARRIER OF NPC1

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INTRODUCTION

- **Niemann-Pick disease type C (NPC) is a rare autosomal recessive disorder caused by mutations in the *NPC1* or *NPC2* genes.**

- **Patients affected by the disease have impaired lipid metabolism.**

- **This results in their accumulation in cells, causing damage to the brain, liver and other organs.**

- **N-palmitoyl-O-phosphocholineserine (PPCS, also named lysosphingomyelin-509), a recently identified plasma biomarker, is used alongside lysosphingomyelin (lysoSM) for differential diagnosis between NPC and Niemann-Pick A/B and potentially for treatment monitoring.**

Patient for study

- 12-year-old patient with bicytopenia and splenomegaly following and prolonged fever.
- While the splenomegaly resolved, bicytopenia persisted.
- At age 15, the patient was asymptomatic except for expressive language difficulties.
- Neurological, psychological and imaging evaluations were normal.
- A comprehensive biochemical and enzymatic workup ruled out other lysosomal storage disorders such as Gaucher disease, Krabble disease and acid sphingomyelinase deficiency (Niemann-Pick A/B disease).
- PPCS plasma levels were the only observed alteration, which were elevated but not as expected.
- Genetic testing revealed a likely pathogenic heterozygous variant in *NPC1* (c.1436G>A,p(Cys479Tyr), with no second variant identified.

METHODOLOGY

- The serum/plasma sample is deproteinized in methanol including the internal standards
- Lysosphingolipids are analyzed by HPLC/ESI-MS/MS, separated on a reverse phase column, recorded by MRM and quantified interpolated in calibration curves of commercial standards, according to the adaptation of a previously published method (1)

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	60	40
1,25	60	40
3	30	70
5	5	95
6	60	40
10	60	40

Gradient, flow= 0,3 mL/min

Mobile phase A- H_2O+0,1% formic acid
Mobile phase B- Acetonitrile+0,1% formic acid

(1) Pettazzoni M., Froissart R., et al. LC-MS/MS multiplex analysis of lysosphingolipids in plasma and amniotic fluid: A novel tool for the screening of sphinlipidoses and Niemann-Pick type C disease. Plos One. 2017; 12 (7):e0181700

METHODOLOGY

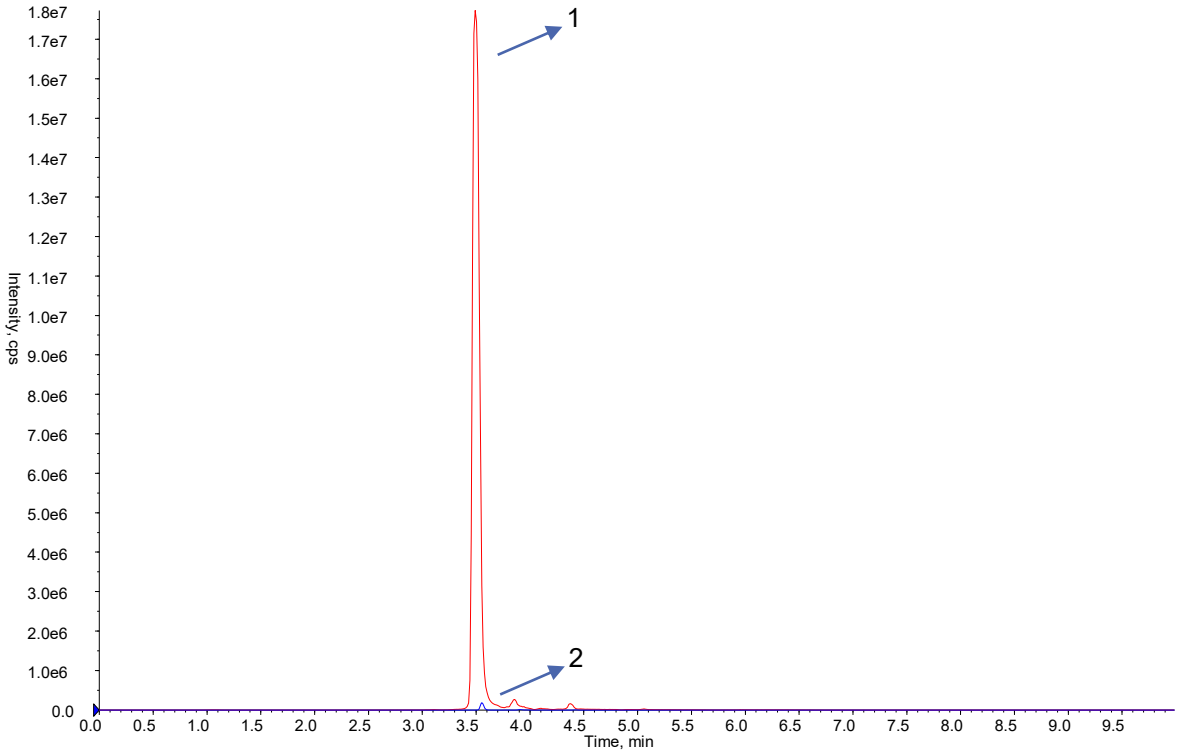
The metabolites are analyzed by the mass spectrometer in positive mode, their MRM being the following:

	Q1	Q3	Disease
Lyso GB3	786	282	**Fabry**
Lysohexosylceramide*	462	282	**Gaucher/Krabble**
Glucopsychosine (plant) (IS)	460	280	
Lysosphingomyelin (LysoSM d18:1)	465	184	**NP A/B**
Lysosphingomyelin (LysoSM d17:1) IS	451	184	
PPCS**	509	184	**NPC**
D9 PPCS	518	193	

*** lysoglucosylceramide+lysogalactosylceramide**

****N-palmitoyl-O-phosphocholineserine**

BIOCHEMICAL RESULTS



PPCS analysis in NPC positive patient (1) and patient for study (2)

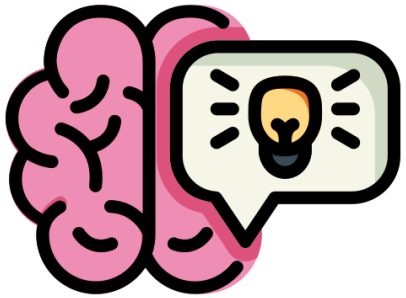
PPCS levels (nM) in plasma from controls, positive patient, patient for study, father and brother of the patient under study

	PPCS (nM)
Controls	67.7 ± 46.5
Positive patient	5458.9
Patient for study	222.4
Father	404.6
Sibling	327.7

FAMILIAR GENETIC STUDY

Family analysis showed the same variant as the father and a sibling.

CONCLUSIONS



- PPCS may help distinguish between Niemann Pick C patients, carriers and healthy individuals.
- While the case under study is now asymptomatic except for dificulty in language expression, his father and sibling are asymptomatic.
- Further genetic and biochemical studies are ongoing to clarify the patient´s status and explore the presence of additional variants if there were any.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THANK YOU