SSIEM ACADEMY 2024

Case Discussions presented by delegates

Session 3

Rapid and reliable LC-MS/MS method to quantify GM1, GM2, GM3 and GD3 molecular species of Gangliosides

Dr. Giulia Dingeo, Pharm.D

Metabolic Biochemistry

Functional Unit Biochemistry of Neurodegenerative and Neurometabolic diseases

University Hospital Pitié-Salpêtrière - APHP - Paris

SSIEM Academy 2024, Amsterdam

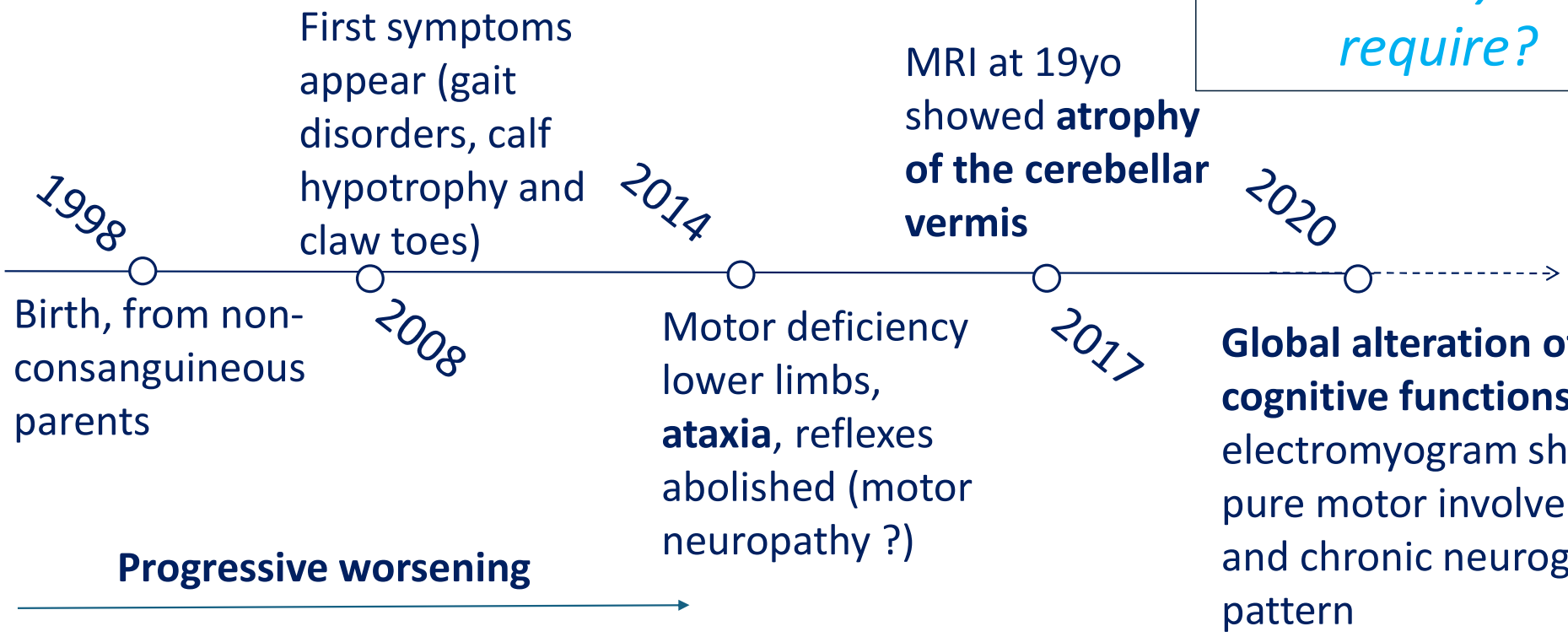
Conflicts of Interest

None to declare

Case study Dr. Giulia Dingeo

Clinical Case

Which diagnostic examination would you require?



Case study Dr. Giulia Dingeo

Biological Results

Hexosaminidase A+B Serum

<u>Normal Values (nmol/L/h):</u>
Hexo A+B =900-2000
Hexo B= 300-1000
%HexoA/HexoB= 50-60%

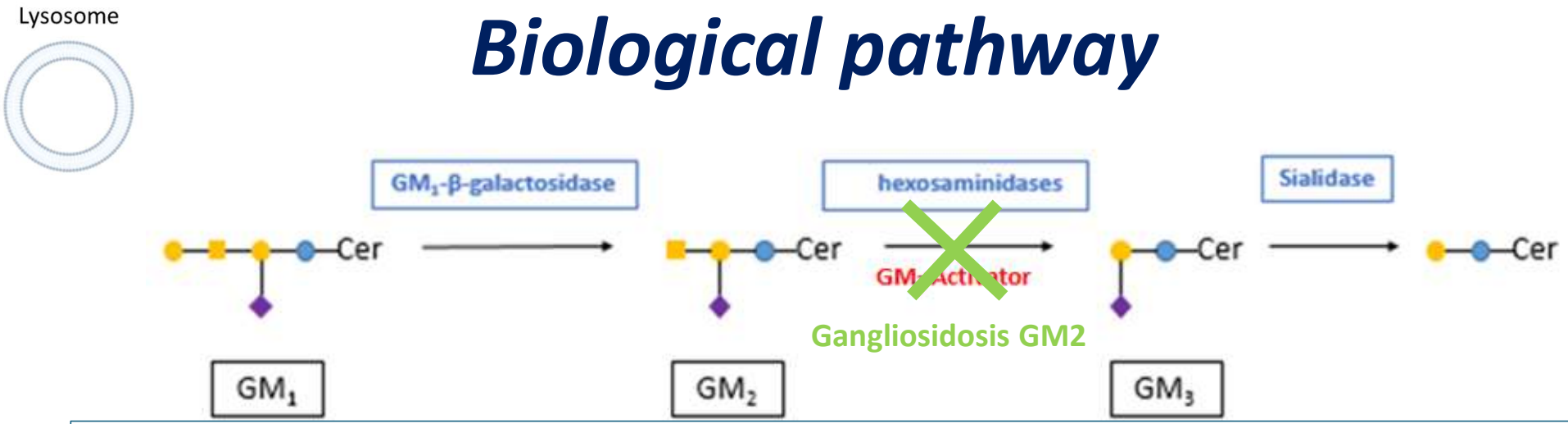
<u>Patient (nmol/L/h)</u>
Hexo A+B = 729,23 / 697,41
Hexo B= 305,56 / 291,22
%HexoA/HexoB= 58,09% / 58,24%

Genetic

NGS panel for hereditary ataxia showed two mutations in GM2A gene:
– c.79A > T:p.Lys27*; nonsense variant
– c.415C > T:p.Pro139Ser; missense variant

Case study Dr. Giulia Dingeo

Biological pathway

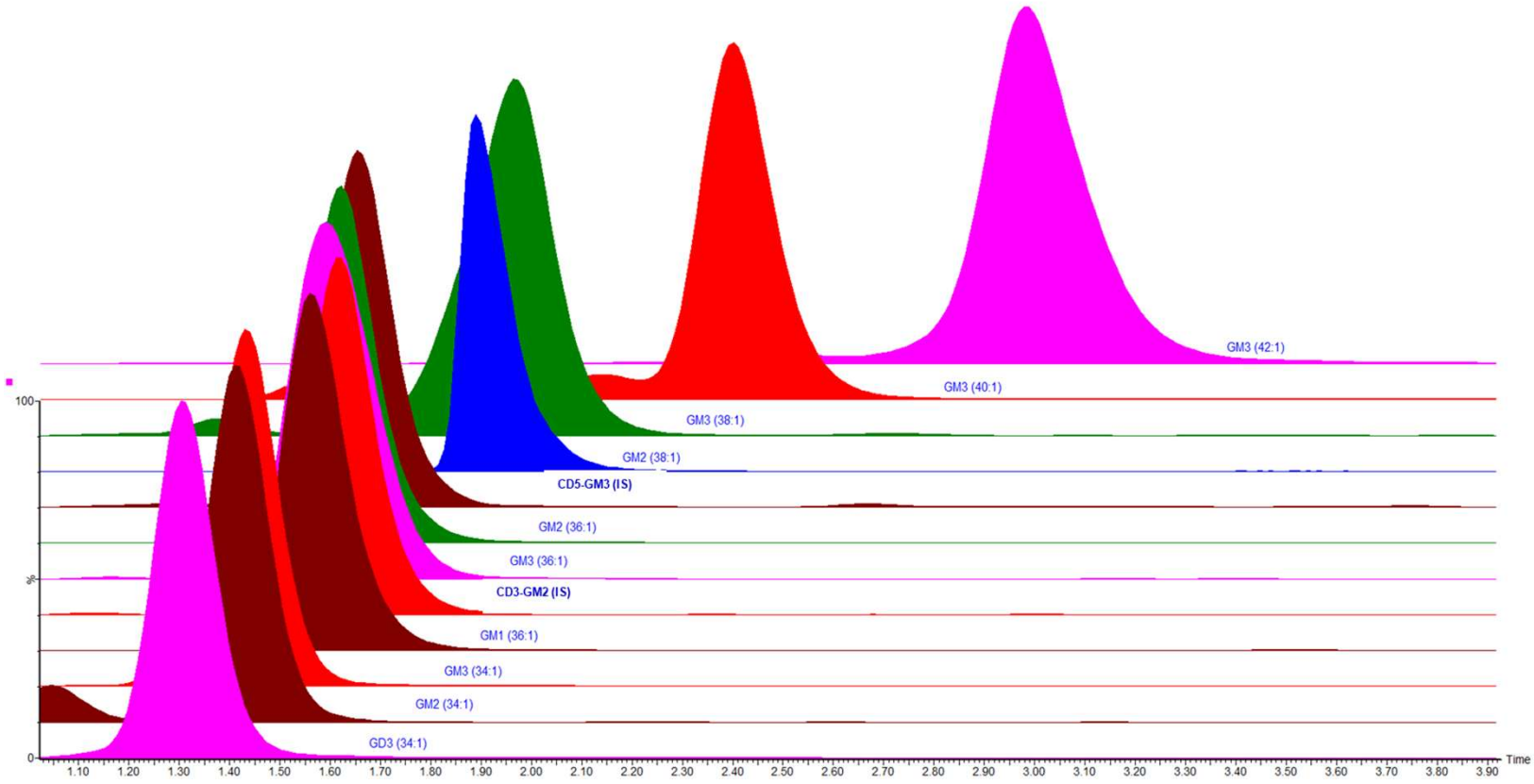


Gangliosidoses GM2:

- Tay-Sachs Disease, Sandhoff Disease, Gangliosidosis GM2 variant AB
- **Accumulation of GM2** in tissues
- Lab. tests: Enzymatic Activity of Hexos and Genetic

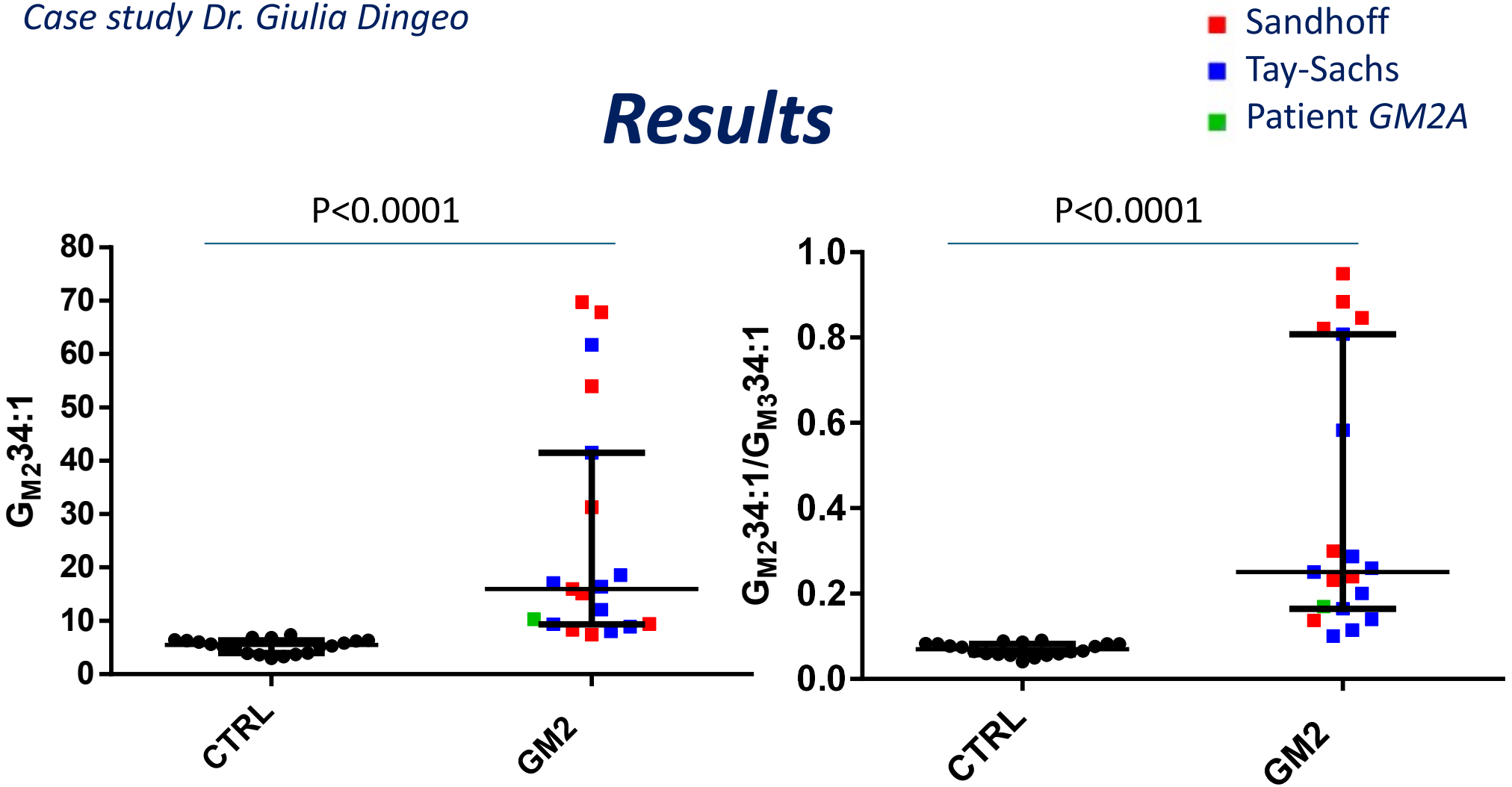
Case study Dr. Giulia Dingeo

LC-MS/MS



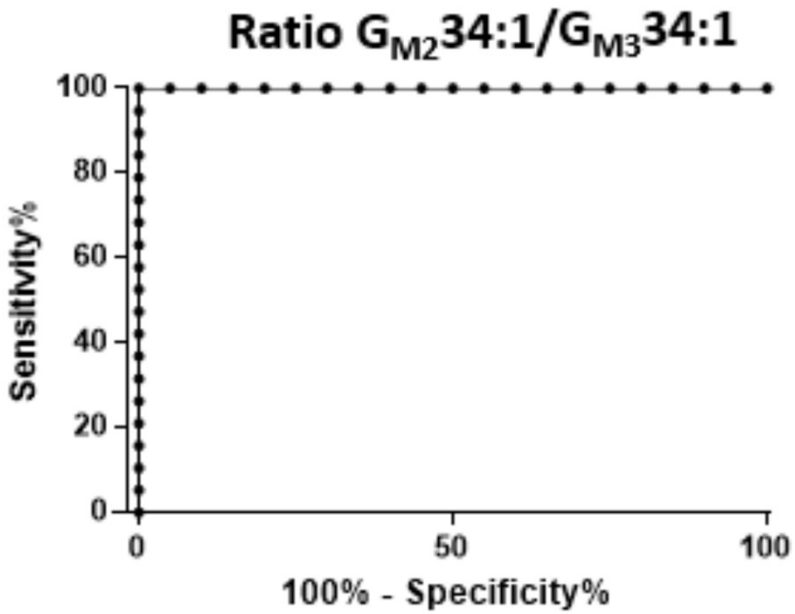
Case study Dr. Giulia Dingeo

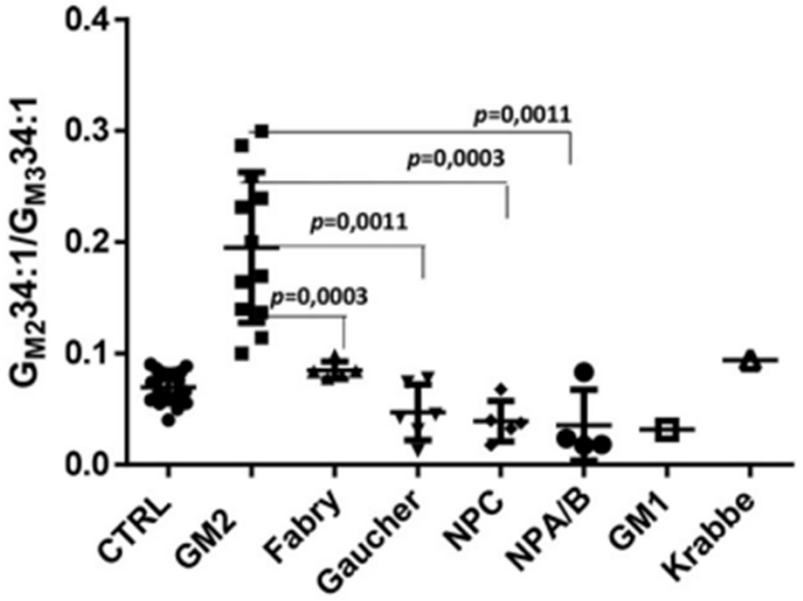
Results



Case study Dr. Giulia Dingeo

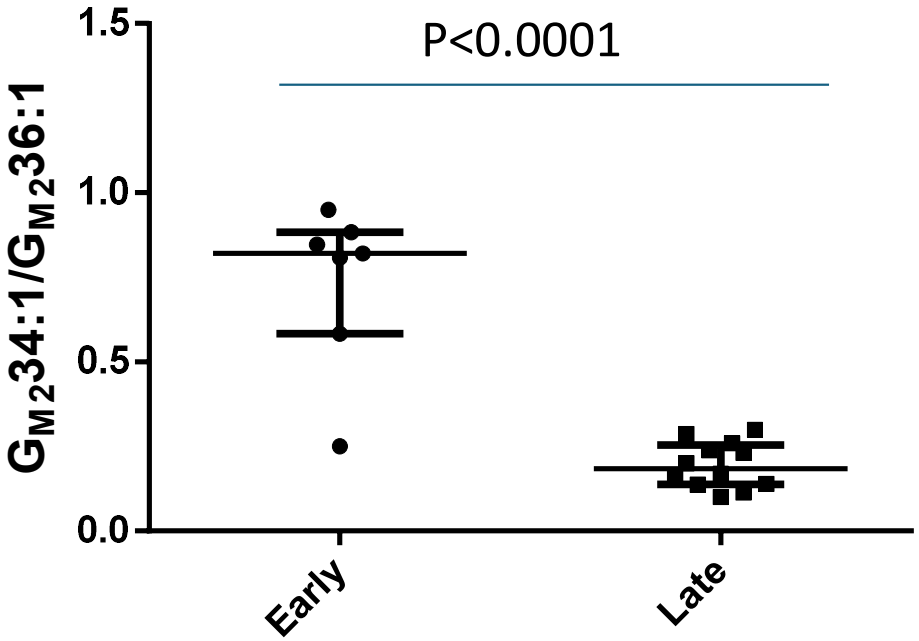
Results





Case study Dr. Giulia Dingeo

Results



Case study Dr. Giulia Dingeo

Conclusion

The importance to have Biological Markers for confirming vus mutations and diagnosis of Gangliosidosis GM2 variant AB.

HÔPITAUX UNIVERSITAIRES
PITIÉ SALPÊTRIÈRE
CHARLES FOIX

Thank You
for your attention


SSIEM
ACADEMY 2024

A 22-Year-Old Female: SeIzures, AtaxIa, and CognItIve DeclIne PresentatIon

Aslı Durmuş, Mehmet Cihan Balcı, Meryem Karaca, Arzu Selamioğlu, Belkıs Ak, Gülden Gökçay

Istanbul University Medical Faculty, Division of Peditaric Nutrition and Metabolism

SSIEM Academy 2024, Amsterdam

Conflicts of Interest

- None to declare

Name: R.G

DOB: 09.01.1999

Age: 25 years old

Gender: Female

DOA: 01.03.2023

- Progressive myoclonic epilepsy
- Cognitive decline
- Ataxia
- Dysarthria

Timeline

2011

Initial Seizure

Fixed gaze and wheezing 15 minutes long

Pediatric Neurology:

VALPROATE initiated

Seizure-free (2 years)

2015

JTC seizure

Marked upward eye deviation, tonic-clonic muscle contractions, loss of consciousness, and urinary incontinence

LAMOTRIGIN added:

6-7 month seizure free period

2020

Generalized seizure with morning jerks

5 seizure in 2 months: JTC, jerks 4-5 minutes long

Brief morning jerking episodes added

LEVETIRACETAM added:

- Generalized seizures→ halted
- Daily jerking episodes couldn't be controlled

her academic performance: impacted

2021

Ongoing seizure activity

Seizure frequency: increased
Quality of life and academy: affected
Hospitalized (17 days)- 5 AED
signs of moderate ataxia emerged
Dysarthria, cognitive decline

PROGRESSIVE MYOCLONIC EPILEPSY (PME)
Lafora disease investigated
Eye examination: Normal findings

2023

Admission to our clinic

History

- Fourth child of a consanguineous marriage with spontaneous vaginal delivery
- Birth weight: 3500 gr

- No postnatal adaptational problem
- Age-appropriate motor milestones development

- Multiple bone fracture with trauma

- Family History:
- Mother: 43 years old, Father: 47 years old
- Both healthy.
- Consanguineous marriage: second-degree cousin marriage
- The patient has three siblings:
  - 27 years old (M), 21 years old (M), 12 years old (M) with no known medical history.
- Medications:

- Levetiracetam 2x250 mg
- Na Valproate 1000mg +500 mg
- Lamotrigine 2x150 mg

Physical Examination:

- The patient exhibited overall good health, with orientation and cooperation observed.
- Respiratory System: Bilateral equal and natural breath sounds; absence of rales or ronchi.
- Cardiovascular System: Heart rate: 52 bpm, rhythmic heartbeat, palpable bilateral pulses, and no murmurs.

- Digestive System: Comfortable abdomen with no tenderness, defense, or rebound. Splenomegaly (SM) 2 cm below the costal margin.
- Neurologic System: Ataxic gait, hyperactive deep tendon reflexes (DTR), slow speech, nystagmus (+)
- No pathological reflexes
- Eye movements are normal in all directions.

Laboratory Investigations

- Complete Blood Count (CBC):
  WBC: 4170/mm3, Neu: 1930/mm3, Lymph: 1850/mm3, Hb: 12.1 gr/dL, Hct: 37.1%, Plt: 77,000/mm3.
- Glucose: 90 mg/dL, Na: 140 mmol/L, K:3.91 mmol/L, Cl: 101 mmol/L,
- AST: 14.5 U/L, ALT: 6.6 U/L, GGT: 44 U/L,
- Ca: 9.54 mg/dL, P: 3.87 mg/dL, ALP: 72 U/L
- Triglyceride: 42.9 mg/dl, Cholesterol: 137 mg/dl, HDL: 66 mg/dl, LDL: 54 mg/dl
- Ferritin: 48.1 ng/mL, Vitamin B12: 450 pg/mL, Folate: 2 ng/mL.
- Coagulation Profile: PT: 14.2 s, INR: 1.14, PTT: 30.7, ESR: 4 mm/h.

- Enzymatic analysis:
- Chitotriosidase: 476,9 mol/L.h (reference value <200)
- LYSO-GB1: 79.58 ng/ml (normal range: 0-14)
- Lyso- SM509: high (not reported quantitatively)
- Psychosine: 0,10 nmol/L (N <2.0)
- Beta-glucosidase: 2,0 nmol/mg.h (>1.0)
- Sphingomyelinase: 65,6 nmol/mg.h (>10)

Radiologic evaluations

- Brain MRI (10/06/2020): Thickening of the nasopharyngeal mucosal tissue and a 9 mm Thornwald cyst without additional pathology.
- Echocardiography (02/2023): Normal
- DEXA (01/03/2023): Z-score -1.2 SD
- Abdominal USG: Normal liver size, spleen size increased to 14 cm with homogeneous parenchyma.

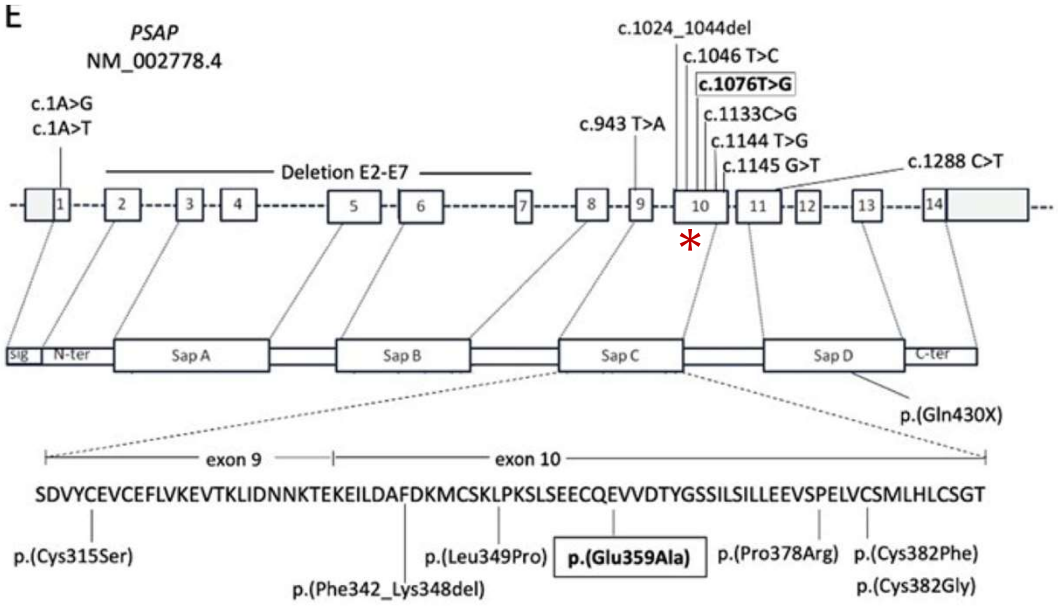
EEG

- Light-sensitive generalized type epileptiform anomaly - accompanied by mild and widespread disorganization and myoclonia
- with epileptogenic foci observed in the occipital regions

Neuropsychological evaluation (WAIS) revealed a borderline intelligence quotient (IQ) of 77, with impaired verbal memory learning, normal delayed recall, difficulty in visual memory learning, impaired delayed free recall, moderately impaired navigation skills, mild reduction in verbal fluency from frontal functions, and moderately impaired working memory.

SUMMARY

- Seizures, variable, progressive with brief morning jerks
- Ataxia, dysarthria, cognitive decline
- Consangineous marriage
- Multiple bone fracture history
- Splenomegaly
- Ataxic gait, hyperactive deep tendon reflexes (DTR), slow speech, nystagmus (+)
- Trombocytopenia
- **Chitotriosidase, Lyso-GB1 significant elevated Lyso- SM509 (high) → Psychosine, beta glucosidase and Sphingomyelinase enzyme levels normal**
- Light-sensitive generalized-type epileptiform anomaly accompanied by mild and widespread disorganization and myoclonia, with epileptogenic foci observed in the occipital regions



Genes **2022**, *13*, 662. https://doi.org/10.3390/genes13040662

VARSOME

Frequency: exomes: **ƒ = 0.0000012** (cov: 64.0)
genomes: **not found** (cov: 31.8)
Conservation Scores
phyloP100: **8.222**
In silico predictor: PP3 Pathogenic Strong

Molecular analysis

Myoclonic epilepsy (CES) panel:

- ***PSAP* gene:** c.1078T>C: p.(Cys360Arg) in exon 10
- a novel homozygous missense variant associated with atypical Gaucher disease (OMIM 610539)
- (not reported in HGMD Professional, ClinVar,GnomAD, G1000, ESP5400, ExAc, not reported also in 144 patients inhouse data)
- Variant was reported as VUS according to ACMG
- NPC1,NPC2: no pathogenic variant

Patient	Gender	Clinical Features	cDNA Variant (Allele 1/Allele 2)	Protein Variant (Allele 1/Allele 2)	Exon	Origin	Reference
1	Female	Hepatosplenomegaly, Seizure	c.1145G>T/ unknown	p.(Cys382Phe)/ unknown	10	Sweden	[6]
2	Male	Hepatosplenomegaly, Seizure, Ataxia, tremor, ophthalmoplegia	c.1144T>G/ c.1288 C>T	p.(Cys382Gly)/ p.(Gln430X)	10, 11	Spain	[7]
3	Female	Hepatosplenomegaly, intellectual decline, epilepsy	c.1A>G/ c.943T>A	p.(Met1Val)/ p.(Cys315Ser)	1, 9	France	[8]
4	Male	Hepatosplenomegaly, osteopenia	c.1A>T/ c.1046 T>C	p.(Met1Leu)/ p.(Leu349Pro)	1, 10	Poland	[5]
5	Female	Hepatosplenomegaly, osteopenia	c.1A>T/ c.1046T>C	p.(Met1Leu)/ p.(Leu349Pro)	1, 10	Poland	[5]
6	Female	Hepatosplenomegaly	c.1024_1044del/ c.1024_1044del	p.(Phe342_Lys348del)/ p.(Phe342_Lys348del)	10, 10	India (Sikh)	[9]
7	Male	Hepatosplenomegaly, thrombocytopenia, anemia, abnormal electroencephalogram	c.1133C>G/ delE2–E7	p.(Pro378Arg)/nonsense mediated mRNA decay	10, delE2–E7	China	[10]
8	Female IV:1	Hepatosplenomegaly, thrombocytopenia, kyphosis, Myopia (late onset), vestibular dysfunction, hearing impairment	c.1076A>C/ c.1076A>C	p.(Glu359Ala)/ p.(Glu359Ala)	10, 10	Pakistan	Present study
9	Female IV:4	Hepatosplenomegaly, thrombocytopenia, kyphosis, vestibular dysfunction, hearing impairment	c.1076A>C/ c.1076A>C	p.(Glu359Ala)/ p.(Glu359Ala)	10, 10	Pakistan	Present study
10	Male IV:5	Hepatosplenomegaly, thrombocytopenia, kyphosis, Myopia (late onset), vestibular dysfunction, hearing impairment	c.1076A>C/ c.1076A>C	p.(Glu359Ala)/ p.(Glu359Ala)	10, 10	Pakistan	Present study
11	Female IV:7	Hepatosplenomegaly, thrombocytopenia, kyphosis, vestibular dysfunction, hearing impairment	c.1076A>C/ c.1076A>C	p.(Glu359Ala)/ p.(Glu359Ala)	10, 10	Pakistan	Present study

- Reported 11 patients with Atypical Gaucher Disease PSAPD
- Female(7), Male (4)
- 12 variant
  - c.1145G>T/unknown
  - c.1144T>C/c.1288C>T
  - c.1A>G/c.943T>A
  - c.1A>T/c.1046T>C (2)
  - c.1024_1044del/c.1024_1044del
  - c.1133C>G/delE2-E7
  - c.1076A>C/c.1076A>C (4)

Our Patient:
PSAP
c.1078T>C/ c.1078T>C
p.(Cys360Arg)
homozygous missense variant

Genes **2022**, *13*, 662. https://doi.org/10.3390/genes13040662

Prof. Dr. Gülden Gökçay, MD, PhD
Prof. Dr. Asuman Gedikbaşı, MD, PhD
Prof.Dr. Fatmahan Atalar
Mehmet Cihan Balcı, MD
Meryem Karaca MD
Dilek Güneş, MD

Fellowship Trainee:
Belkıs Ak, MD
Şebnem Kılıç, MD
Ülkem Çolak Aktaş, MD

SSIEM Academy 2024, Amsterdam


SSIEM
ACADEMY 2024

Manchester University
NHS Foundation Trust
Evelina London
Children's Healthcare

Massive hepatosplenomegaly and weight loss in a 20 month old boy

James Cooper

Willink Biochemical Genetics Lab

Manchester, UK

SSIEM Academy 2024, Amsterdam

J. Cooper – HSM in a 20 month old boy

Conflict of Interests

- *Nothing to declare*

J. Cooper – HSM in a 20 month old boy

Clinical Presentation

- *20 month old male*
- *Bloating and weight loss noted by mum after cold and fever at around 12 months*
- *Initially viral illness diagnosed but weight loss persisted +6m*
- *Referred to primary care by community health visitor*
- *Seen urgently at paediatric liver unit and noted to have massive hepatosplenomegaly*

J. Cooper – HSM in a 20 month old boy

History

- *Second child of non-consanguinous parents*
  - *6 year old male sibling fit and well.*
- *Pregnancy uneventful*
  - *born by NVD at 41.1 weeks*
  - *birth weight 3.72 kg.*
- *Breast fed to 4m then formula feeds.*
- *Fed well and gained weight.*

J. Cooper – HSM in a 20 month old boy

Initial investigations

- *Routine blood tests unremarkable except:*
  - *ALT = 234 (4-59 IU/L)*
  - *Hb 106 (120-140 g/L), RBC = 3.94 (4.1-5.5x10^12), Plt = 192 (150-400 x 10^9)*
  - *Total cholesterol = 7.1 (2.9-5.4 mmol/L), LDL 5.4 (1.3-2.9 mmol/L), TG = 3.34 (0.5-2.5 mmol/L),*
  - *Imaging:*
    - *X-ray chest - Bilateral lung infiltration in reticulo-nodular pattern (interstitial lung disease).*
    - *Upper abdomen – Soft tissue shadowing in bilateral upper quadrants (HSM).*

J. Cooper – HSM in a 20 month old boy

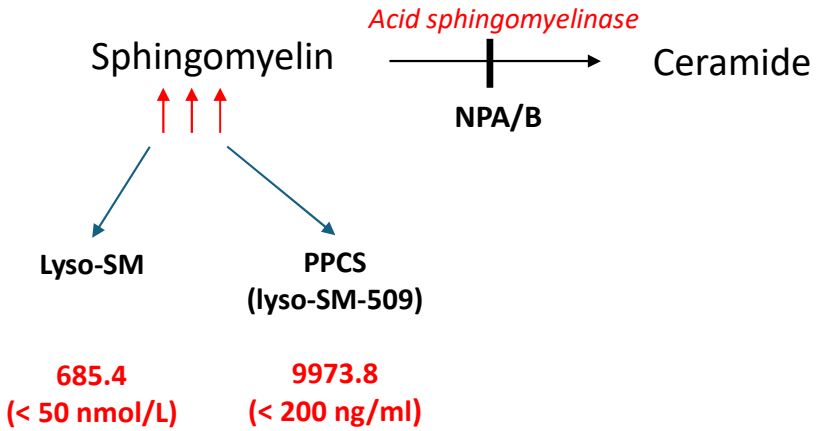
Specialist Investigations

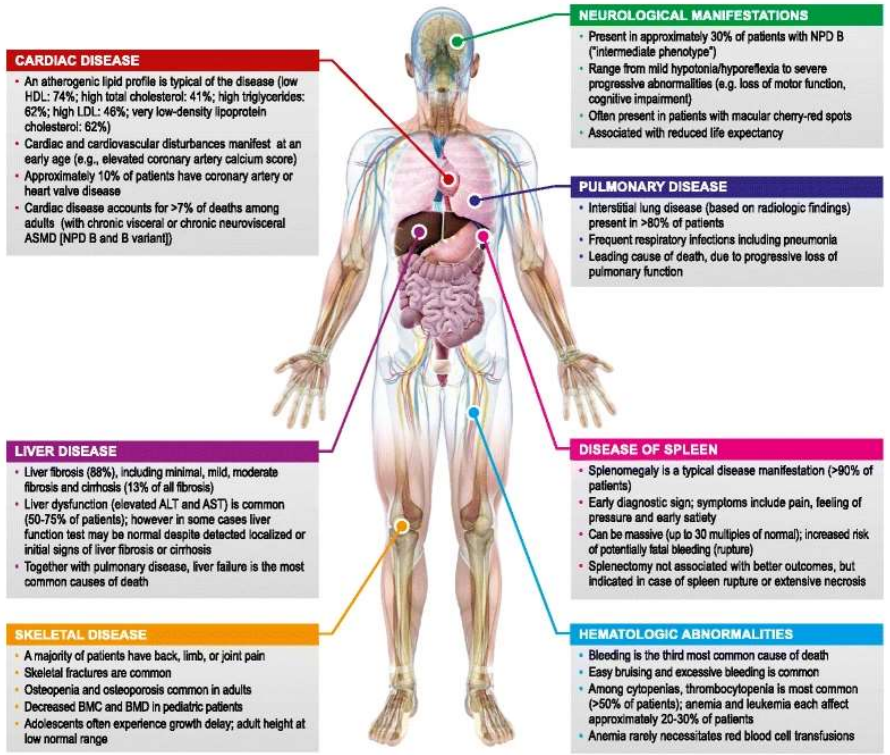
- *Lysosomal enzyme screen (leucocytes)*
  - *Acid sphingomyelinase activity = 0.2 nmol/mg/hr (0.75-7.1)* ↓
  - Beta-glucosidase activity = 26.0 nmol/mg/hr (8.4-32.8) **N**
  - *Chitotriosidase activity (plasma) = 3493 nmol/ml.hr (0-170)* ↑
- *Consistent with a diagnosis of Acid Sphingomyelinase Deficiency (ASMD, Niemann-Pick Disease type A/B)*
- **Age at presentation and absence of neurological features suggests NPB rather than NPA**
- Genotype (SPMD1) .354del p.(Ile119Serfs*7); c.748A>C p.(Ser250Arg)

J. Cooper – HSM in a 20 month old boy

ASMD - Niemann-Pick Disease Type A/B

NPA	NPA/B	NPB
Infantile Neurovisceral	Chronic Neurovisceral	Chronic Visceral



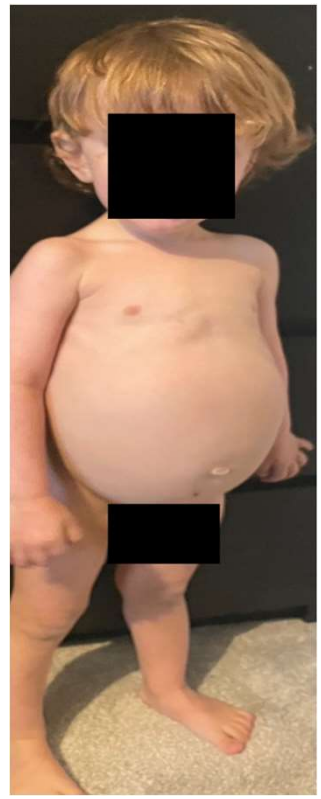


McGovern, M.M., Avetisyan, R., Sanson, BJ. *et al.* Disease manifestations and burden of illness in patients with acid sphingomyelinase deficiency (ASMD). *Orphanet J Rare Dis* **12**, 41 (2017). https://doi.org/10.1186/s13023-017-0572-x

J. Cooper – HSM in a 20 month old boy

Metabolic Review and Follow-up / Treatment

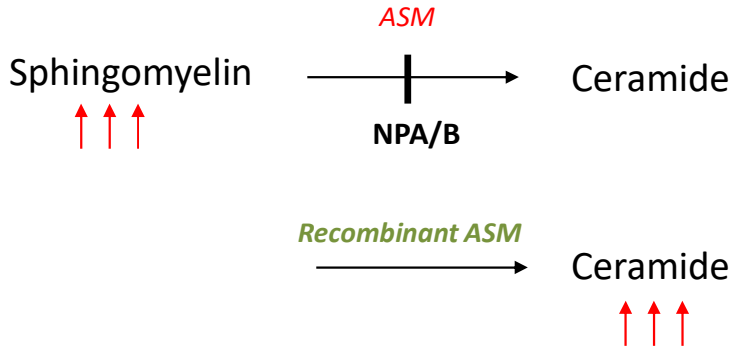
- Eats little and often but varied diet
  - Likes chips, pasta and fruit
- No history of diarrhoea / constipation / recurrent chest infection
- Easy bruising but no mucosal bleeding
- Developmentally normal
- HSM



J. Cooper – HSM in a 20 month old boy

Treatment

- *Xenpozyme (Olipudase-alfa), recombinant enzyme replacement therapy*
  - *EMA/FDA approved for NPB phenotype*
- *Slow dose escalation*
- *Too fast = ceramide mediated inflammatory response ("cytokine storm") with fatal outcome*

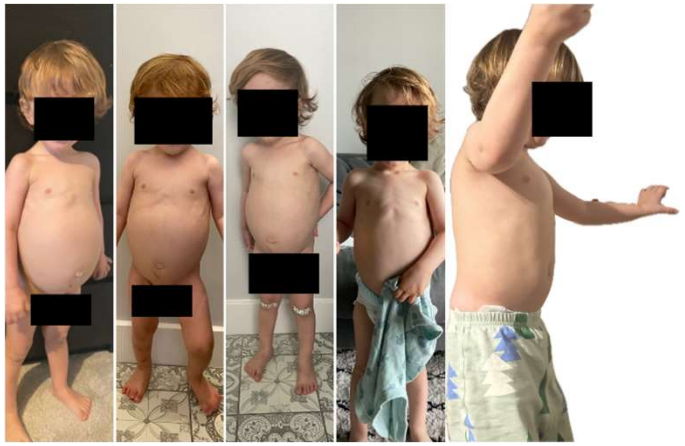


Pediatric patients (0 to <18 years old)	
First dose (Day 1)	0.03 mg/kg
Second dose (Week 2)	0.1 mg/kg
Third dose (Week 4)	0.3 mg/kg
Fourth dose (Week 6)	0.3 mg/kg
Fifth dose (Week 8)	0.6 mg/kg
Sixth dose (Week 10)	0.6 mg/kg
Seventh dose (Week 12)	1.0 mg/kg
Eighth dose (Week 14)	2.0 mg/kg
Ninth dose (Week 16)	3.0 mg/kg (recommended maintenance dose)

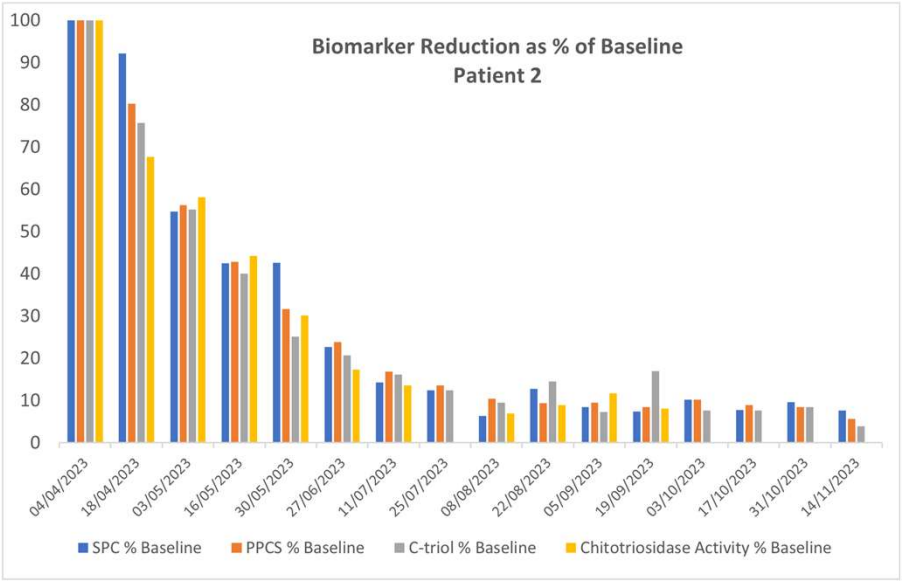
EMA: EU-RISK MANAGEMENT PLAN FOR XENPOZYME® (OLIPUDASE ALFA), version 2.3, 06/11/2023.

J. Cooper – HSM in a 20 month old boy

Response to Treatment



Outcome Measure	Pre-ERT	Post-ERT (+10m)
Weight Z-score	-1.18	+0.78
Height Z-score	-2.23	-1.93
Liver size (cm)	11	3
Spleen size (cm)	Beyond lower pole of R kidney	At lower pole of R kidney
CT chest	Extensive interlobular septal thickening without ground-glass opacification	No obvious change…yet
ALT	213	39
T Cholesterol	4.7	2.3
LDL (mmol/L)	3.2	1.1
Triglycerides	2.45	0.91
Hb	106	116
Plt	192	154



Summary

- *Patient presented at 20 months with HSM*
- *Diagnosis of ASMD achieved by enzymology*
- *Age of onset and clinical phenotype (no neurological involvement) implies NPB*
- *Olipudase-alfa ERT*
  - *Standard dose escalation protocol*
- *Improvement of visceral manifestations*
- *Biomarker response sustained*

J. Cooper – HSM in a 20 month old boy

Acknowledgements

- *Dr Roshni Vara (Evelina Children's Hospital, London)*
- *Dr Alex Broomfield (Great Ormond Street Hospital, London)*
- *Prof. Simon Jones (Willink / University of Manchester)*
- *Willink laboratory team: Liz Smith, Teresa Wu, Karen Tylee, Heather Church, Stephen Dent, Owain Cox, Christine Egerton, Fausto Yeboah.*