Workshop 4:
Diagnosis and Management of Metabolic Neurodegenerative Disorders

Regression & irritability aged 1 year

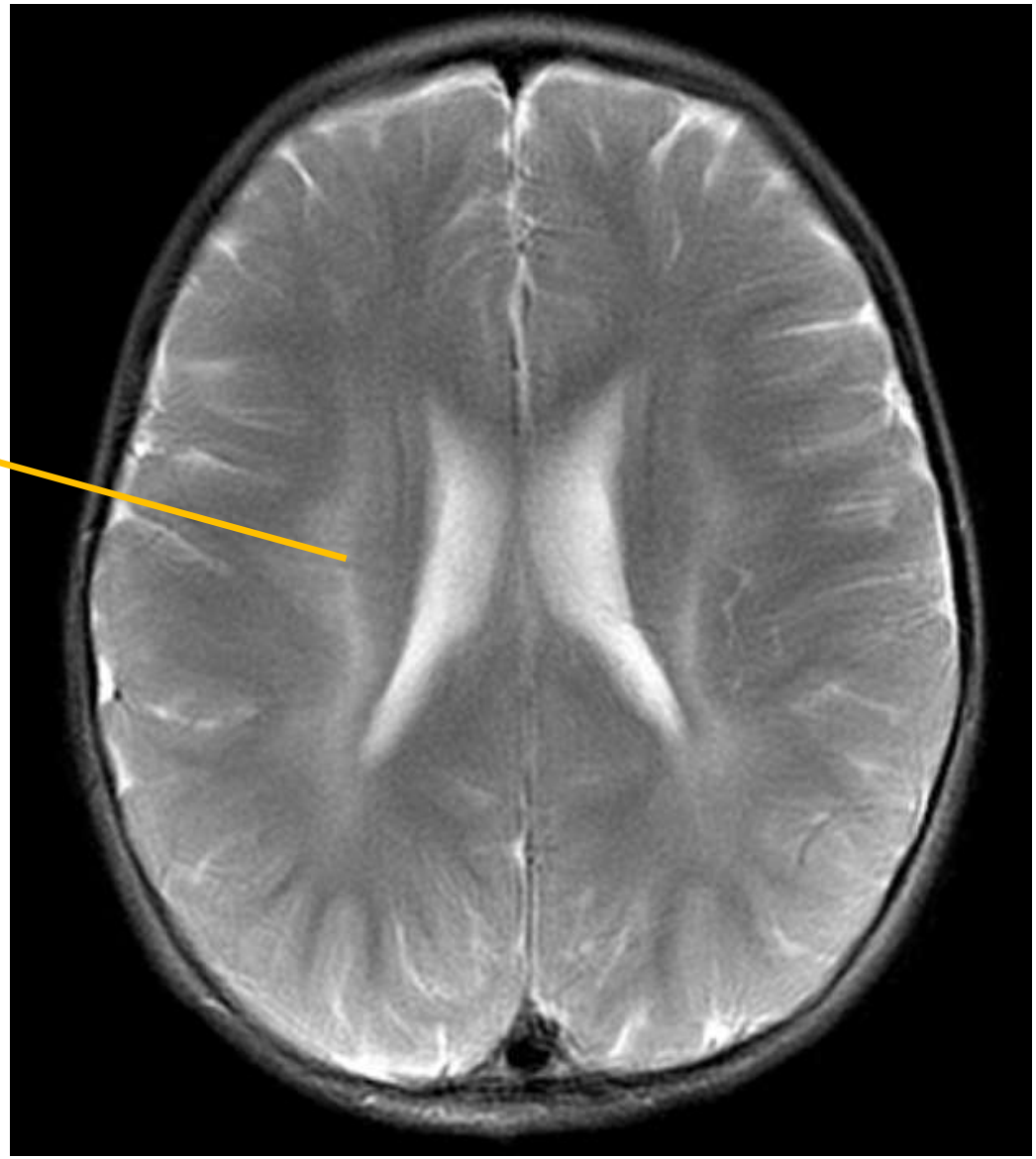
- 1st daughter of healthy non-consanguineous parents
- Normal delivery at term, 3.4kg, no neonatal problems
- Normal initial development: vocalising, transferring hand to hand & sitting without support by 7 months, commando crawling at 9 months
- At 12-15 months, stopped crawling, feeding deteriorated, vomiting
- At 18 months, unable to sit without support, drooling, excessive crying
- Head circumference <0.4%
- Truncal hypotonia, limbs hypertonic, scissoring of legs

MRI aged 20 months

T2 weighted image:

Increased signal in periventricular white matter with sparing of U fibres.

Non-specific & relatively subtle



What investigations would you do?

- Blood count, ferritin, folate, B12 - normal
- Urea & electrolytes, bone chemistry, liver function tests, creatine kinase, thyroid function, lactate, urate – normal
- Plasma amino acids & total homocysteine – normal
- Urine organic acids – normal
- Plasma VLCFAs – normal
- Serum transferrin isoelectric focusing – normal

Leukocyte & plasma lysosomal enzyme screen

- Leukocyte galactocerebrosidase* 0.1 nmol/mg/h ref interval 0.8-4.0
- Plasma chitotriosidase 262 nmol/mg/h ref interval 4-120
- Other enzymes normal

*measured using artificial substrate & detection of fluorescent product 6-hexadecanoyl-4-methylumbelliferone (6-H-4-MU)

What diagnosis does this suggest?

- Krabbe disease, Globoid cell leukodystrophy

Why was chitotriosidase raised?

- Plasma chitotriosidase is a non-specific marker of macrophage activation e.g. due to lysosomal storage

Is this diagnosis entirely secure?

- Artificial substrates can give misleading results – ‘pseudodeficiencies’

Repeat assay using natural substrate

- Leukocyte galactocerebrosidase 0.0 nmol/mg/h ref interval 0.4-4.0
- Leukocyte β-galactosidase 118 nmol/mg/h ref interval 100-400

Molecular genetic analysis of *GALC*:

Compound heterozygous for pathogenic deletion of exon 12 – exon 17

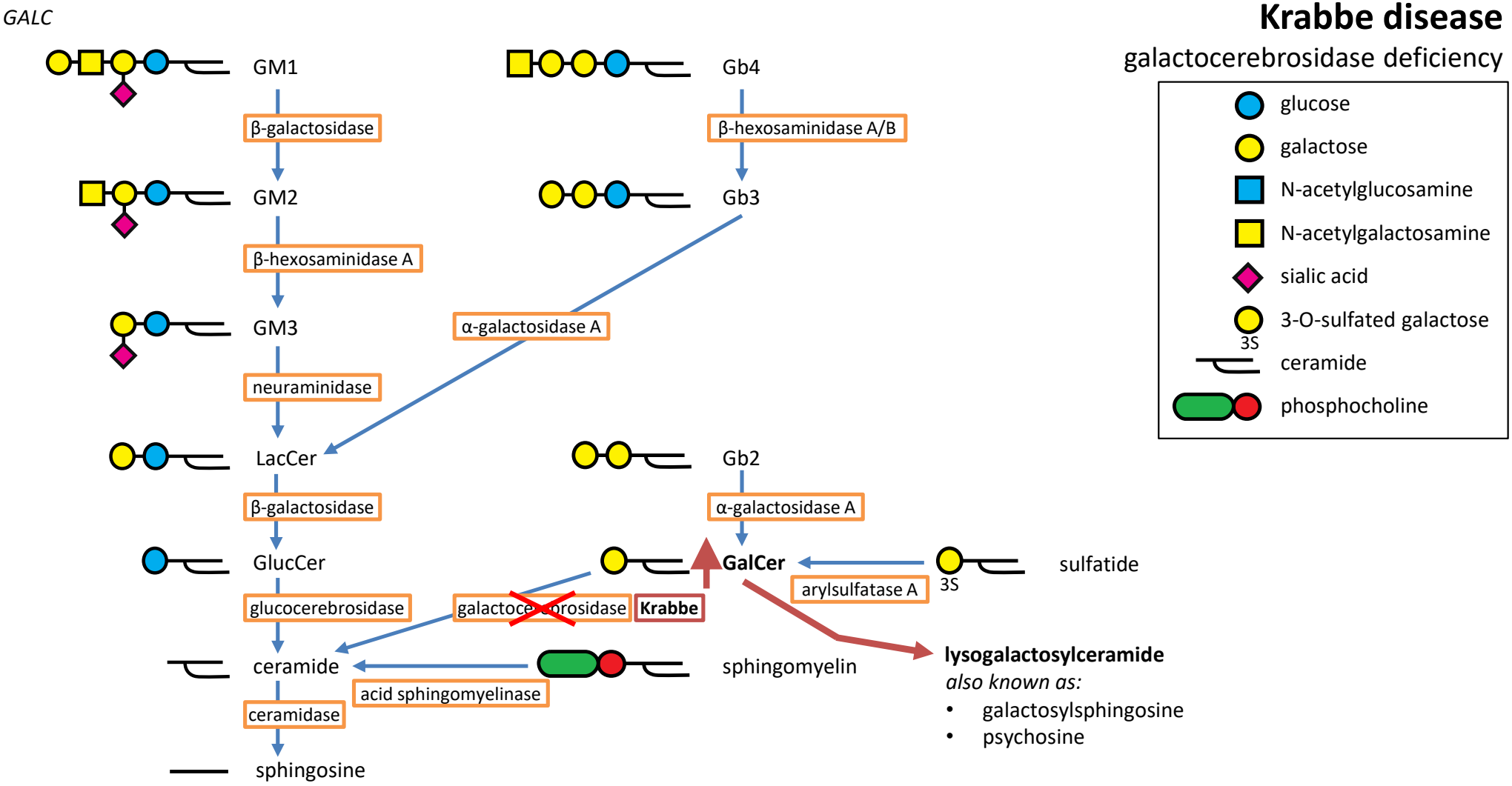
& likely pathogenic variant c.916G>A; p.(Ala306Thr)

- This patient had ‘late infantile’ onset Krabbe disease
- Most patients have early infantile onset (by 6 months) & death by 1 year
- Juvenile & adult forms usually present with abnormal gait due to spasticity ± peripheral neuropathy. Variable mental deterioration ± visual loss

GALC

Krabbe disease

galactocerebrosidase deficiency



Management & progress

- Haematopoietic stem cell transplantation can slow progression but is not a cure, even in pre-symptomatic patients. Not done in this patient
- Diazepam & baclofen for spasticity
- Hyoscine patches for drooling
- Fundoplication & gastrostomy to prevent vomiting & facilitate feeding
- Generalised seizures from 3 years, managed with sodium valproate
- At 4.5 years, less responsive & sometimes hypothermic but relatively stable

Take home messages

Disease spectrum for Krabbe disease:

- Infantile: irritability, feeding problems, fevers, then spasticity, seizures
- Late-infantile: regression, spasticity, then seizures
- Juvenile & adult: spastic/ataxic gait ± peripheral neuropathy, regression, visual loss

- Haematopoietic stem cell transplantation may help late-onset cases, especially if presymptomatic

- Beware of pseudodeficiencies due to artificial substrates

A girl with short stature and stiff joints

Workshop 4, Case 3.

History

- *4 year old girl previously well*
  - *Slowing height growth trajectory, now on 0.4th Centile*
  - *Joint stiffness/ pain*

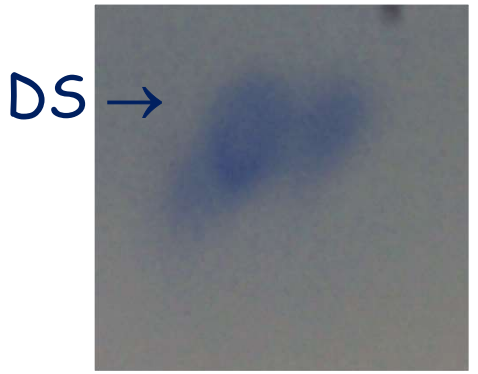
- *Urine glycosaminoglycans:*
  - *Quantification 12 mg/mmol creatinine (5-20)*
  - *GAG electrophoresis normal*
  - *Urine oligosaccharides normal*

- *10 years age:*
  - *Moderate kyphoscoliosis, femoral epiphyseal dysplasia*
  - *Learning difficulties at school*
  - *Mild corneal clouding noted but normal visual function*

History

- *Urine glycosaminoglycans:*
  - *Quantification 10.2 mg/mmol creatinine (1-10)*
  - *GAG electrophoresis chondroitin sulfate & small amount of dermatan sulfate*
  - *Urine oligosaccharides normal*

What is your differential diagnosis?



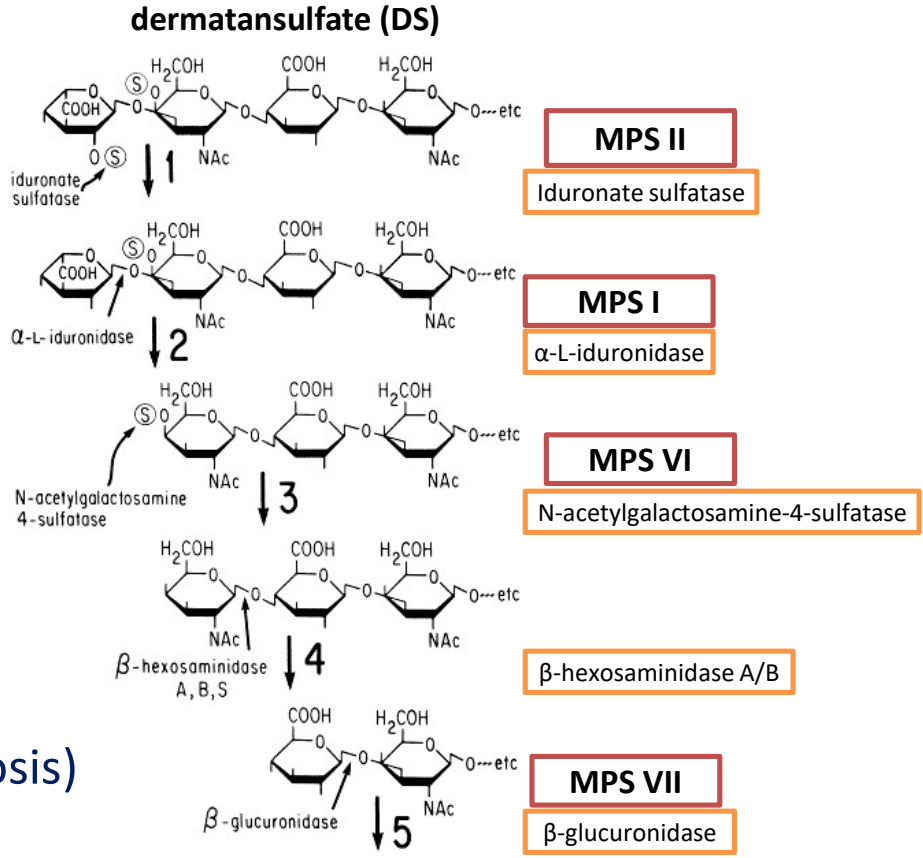
Investigations

Dermatan sulphate – differential diagnosis

- Abnormal component
- Large amounts – MPS I, II, VI or VII
- Small amount with KS – MPS IV
- Non-MPS disorders
  - Mucolipidosis II or III
  - Sialidosis or Galactosialidosis
  - Multiple sulphatase deficiency

What further tests do you want to do?

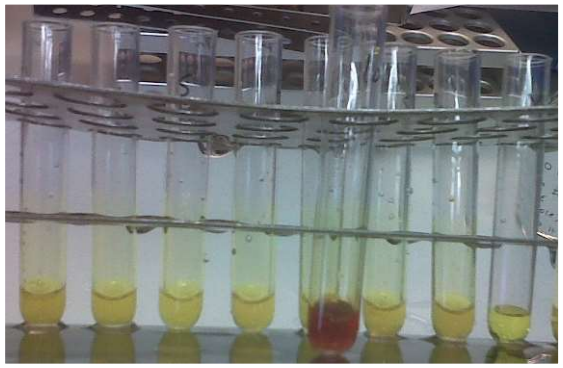
- Repeat urine GAG (LC-MS/MS Dermatan test?)
- Blood for lysosomal enzymology
- Blood for vacuolated lymphocytes ((galacto)sialidosis)
- Molecular genetics



Investigations

Lab received only a small plasma sample, insufficient for leucocyte pellet. No urine. What do you do?

	Patient (nmol/hr/ml)
I-cell screen	POSITIVE
Hexosaminidase	15134 (438-2047)
B-glucuronidase	3169 (30-534)



- I-cell screen = qualitative arylsulphatase assay (yellow to red colour change with excess enzyme in plasma)

What further tests do you want to do?

Cultured fibroblasts (nmol/h/mg protein)

		β-gal	Hex	Hex A	Control enzyme glucocerebrosidase
Patient		102	1358	131	559
Normal	1	1438	17258	1828	413
	2	1231	15495	1809	324
	3	1007	16358	1623	223

All samples assayed at the same time.

Gaucher enzyme
Trafficking via mannose receptor
not mannose 6 phosphate system

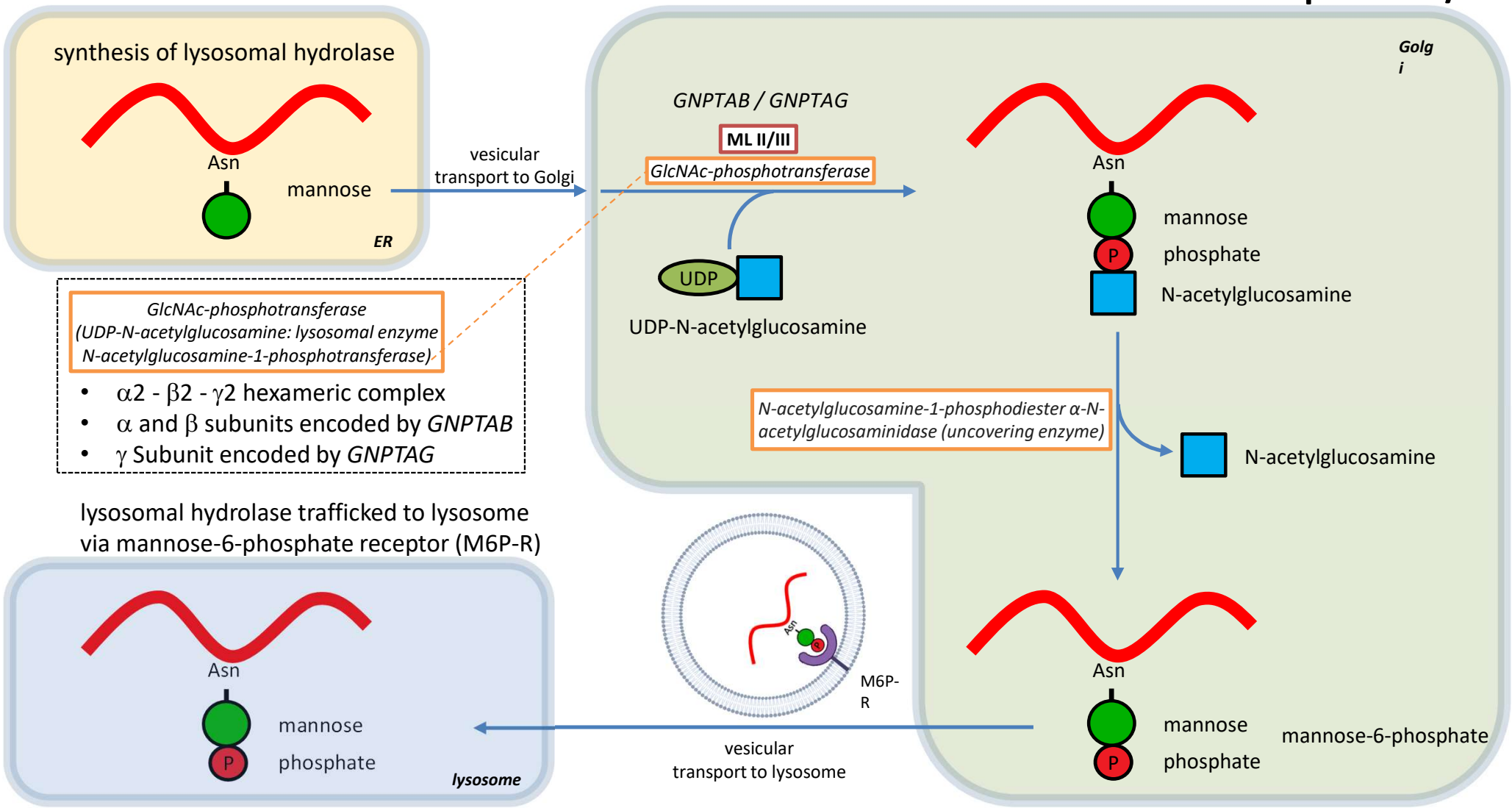
What do these results mean?

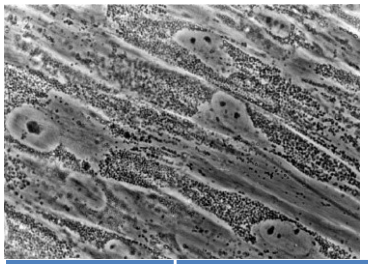
All lysosomal enzyme activities assayed in cultured fibroblasts are deficient, except for the glucocerebrosidase activity which is within normal limits (Why?).

Confirms the diagnosis of Mucolipidosis type II or type III:

Failure to traffic hydrolases to lysosome → multiple deficiencies, with high plasma levels

Mucolipidosis II/III





Phase-contrast microscopic view of "I-cells" in skin fibroblast culture

	Synonyms	Enzyme/protein	Gene	
ML I	Sialidosis	Neuraminidase	*NEU1*	Sialylated glycoconjugates
ML II	**(Inclusion) I-cell disease Pacman Dysplasia**	**N-acetylglucosamine-1-phosphotransferase**	***GNPTAB* (ML II A/B)**	**Multiple**
ML III	**Pseudo-Hurler polydystrophy**	**N-acetylglucosamine-1-phosphotransferase**	***GNPTAB* (ML III A/B)** ***GNPTAG* (ML III-γ)**	**Multiple**
ML IV	Sialolipidosis	Lysosomal calcium channel	*MCOLN1*	

https://www.ncbi.nlm.nih.gov/books/NBK1828/

https://www.dailymail.co.uk/health/article-4815698

Take home message

- *Plasma enzyme screen is important part of LSD testing*
- *Normal urine GAG quantification doesn't rule out an LSD*
  - *Qualitative GAG species evaluation helpful if high index of suspicion by electrophoresis of Mass Spec Method*

- *ML II – ML III represent spectrum of deficiency of the N-acetylglucosamine-1-phosphotransferase*

  - *Clinical presentation of ML II in neonatal period*
  - *ML III presentation later in childhood*

- *Pathology results from failure to traffic multiple lysosomal hydrolases to the lysosome*

Girl with difficulty walking

Background

- 2½-year-old girl with difficulty walking and falls
- Review by neurologist
  - Normal pregnancy and delivery
  - First child of unrelated parents
  - Regression in balance and mobility
  - Unsteady tip-toe gait, requiring assistance
  - High tone in all limbs, brisk reflexes

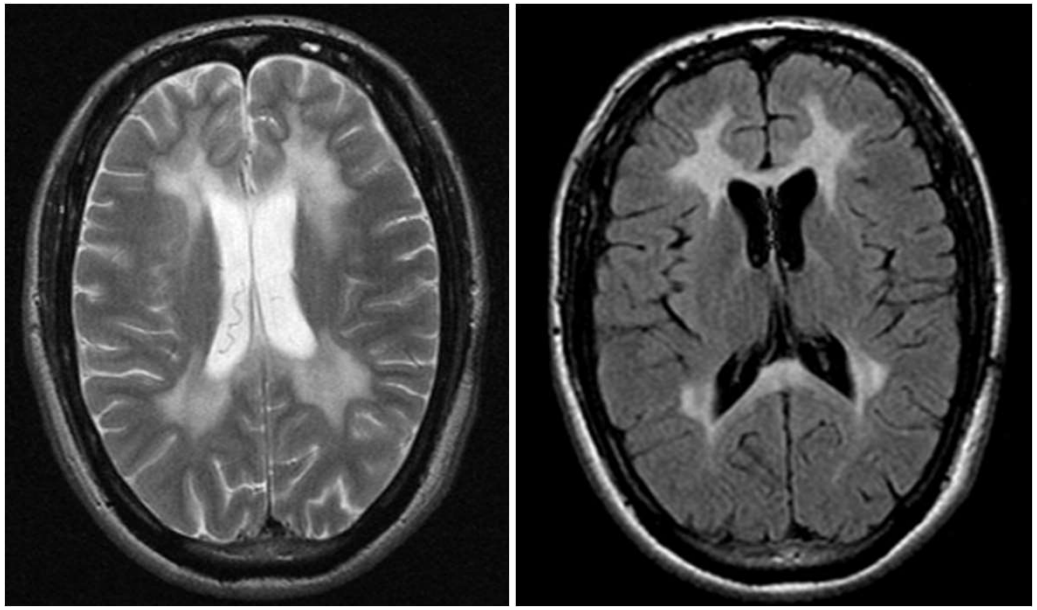
Which investigations do you consider?

First-line laboratory investigations

- Liver, renal and full blood count, vitamin D, B12, folate
- Ammonia, lactate
- Amino acids, acylcarnitines, organic acids, biotinidase
- Glycosaminoglycans (GAGs)

- No significant abnormality

Brain MRI



- Extensive white matter T2 hyperintensity with sparing of the subcortical U fibres.
- There is also sparing of the periventricular regions resulting in tigroid pattern on T2-weighted sequence.
- Subtle hypointensity within thalami.
- Appearances suggestive of leukodystrophy

Images from [Radiology Reference Article | Radiopaedia.org]

Which further laboratory investigations do you consider?

Leukodystrophy Investigations

- Plasma very long chain fatty acids – normal
- Lysosomal enzymes (leukodystrophy panel)

Enzyme	Disorder	Result mol/mg/hr	Ref range
β-galactosidase	Control enzyme	189	100 - 400
Galactocerebrosidase	Krabbe disease	1.3	0.8 – 4.0
Arylsulphatase A	Metachromatic leukodystrophy	6	45 - 250

N.B. Low arylsulphatase A may be due to pseudo deficiency, a benign variant found in 1 – 2% of the population

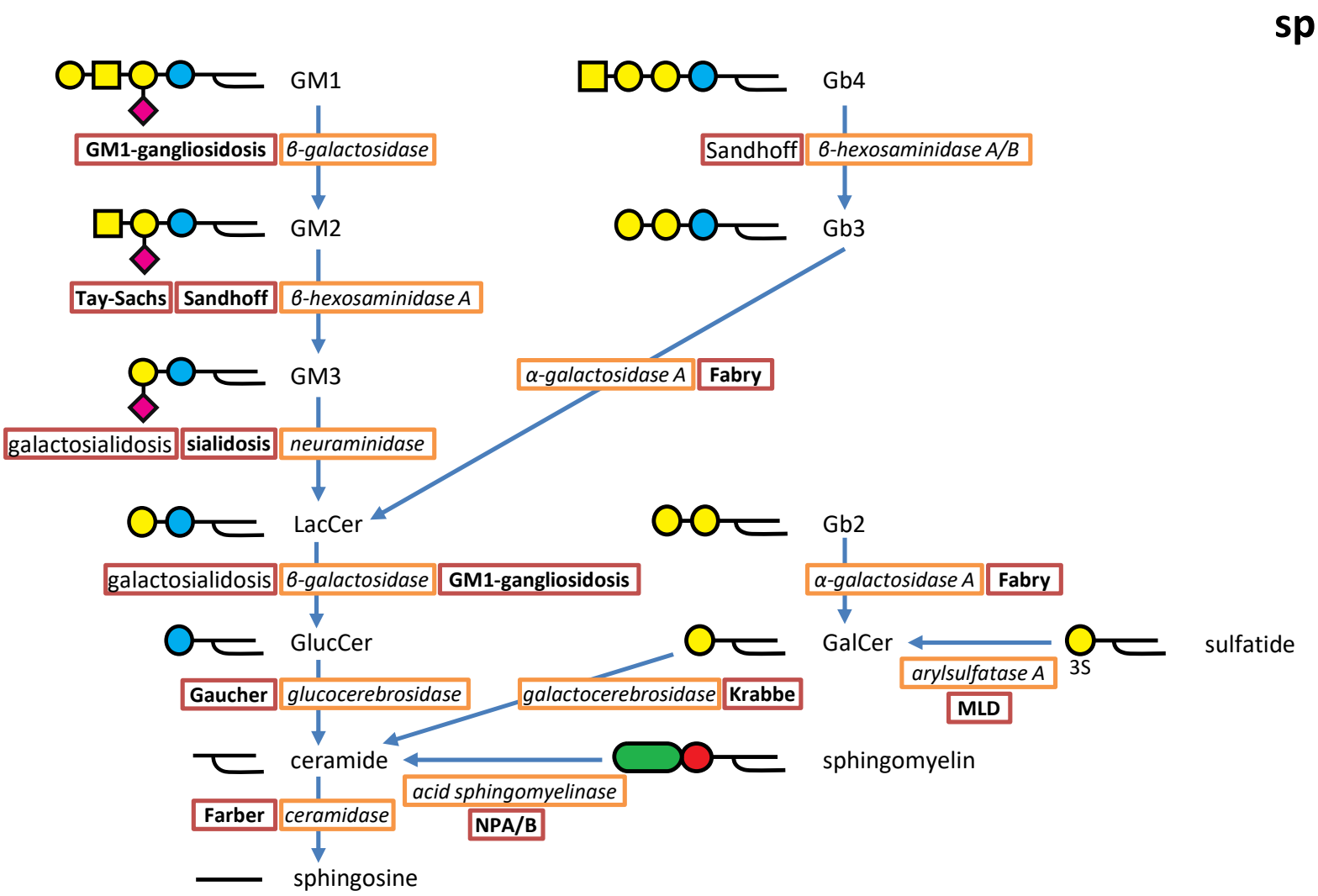
Does the patient have pseudo deficiency?

Genetic test results

- Compound heterozygous likely pathogenic *ARSA* missense variants, detected *in trans*

  c.131C>T, p.(Pro44Leu); c.1177_1178delinsGG, p.(Thr393Gly)

- Confirms diagnosis of metachromatic leukodystrophy (Arylsulphatase A deficiency)



sphingolipid catabolism

disorders

glucose

galactose

N-acetylglucosamine

N-acetylgalactosamine

sialic acid

3-O-sulfated galactose

3S

ceramide

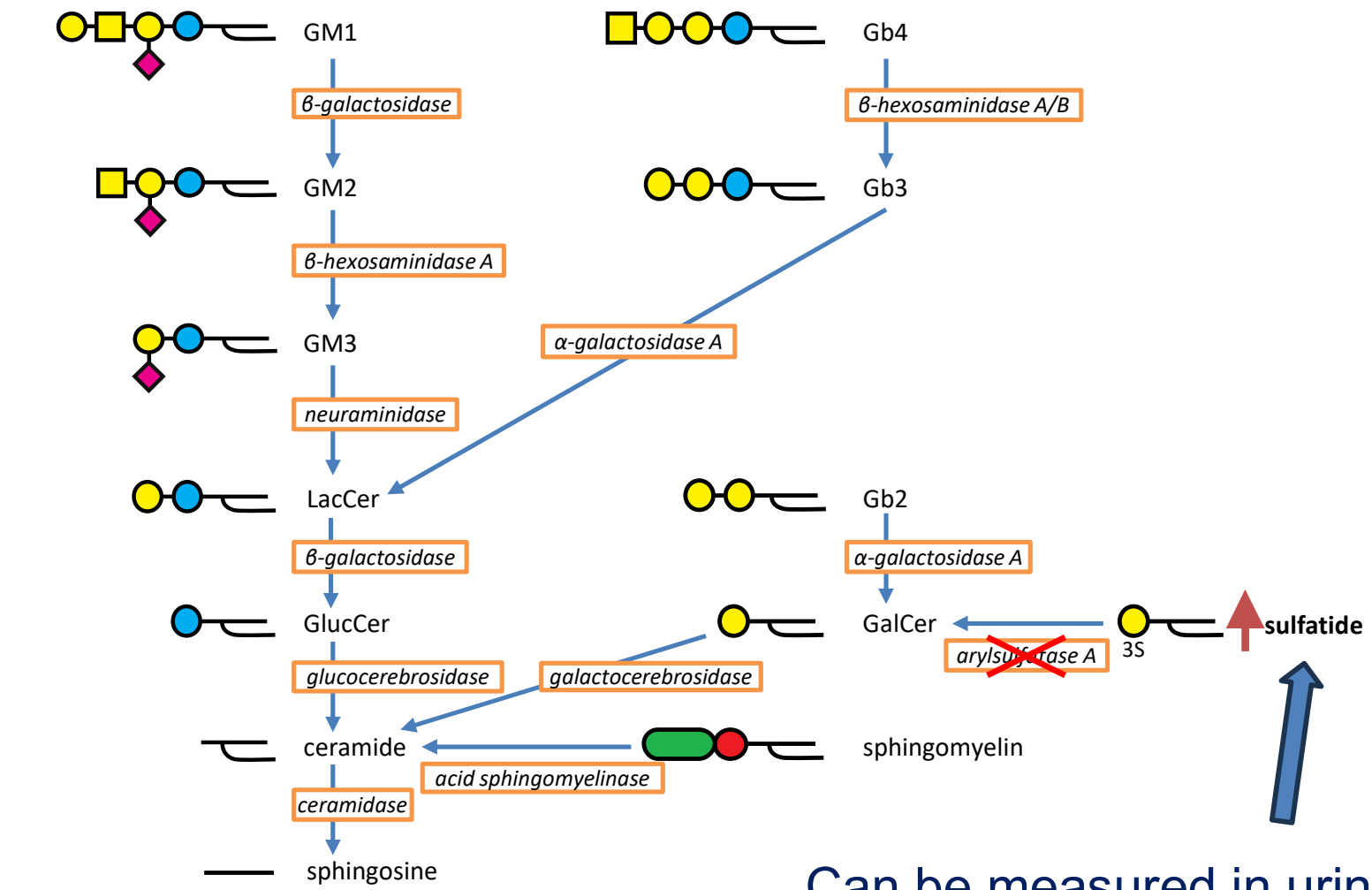
phosphocholine

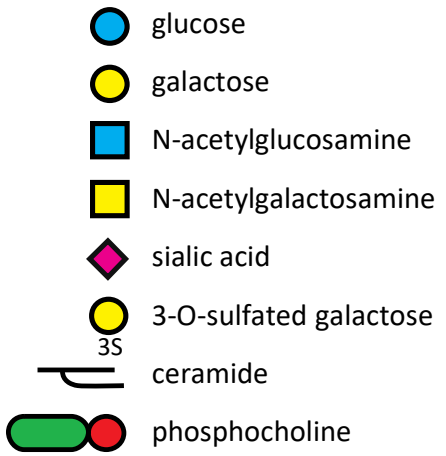
cathepsin A deficiency causes galactosialidosis

ARSA

metachromatic leukodystrophy

arylsulfatase A deficiency





Can be measured in urine! Biomarker!

Follow-up

- Treatment of symptoms
- Supportive therapy

- Younger sister, aged 8 months, no symptoms

What investigations would you recommend?

Results

- Lysosomal enzymes

Enzyme	Disorder	Result mol/mg/hr	Ref range
β-galactosidase	Control enzyme	165	100 - 400
Galactocerebrosidase	Krabbe disease	1.8	0.8 – 4.0
Arylsulphatase A	Metachromatic leukodystrophy	5	45 - 250

- Genetic variants confirmed the diagnosis of MLD
- Brain MRI normal

Treatment

- Gene therapy (Libmeldy) *ex vivo* lentiviral autologous gene therapy transplant at 12 months of age
- Good developmental progress
- No signs of MLD at 3 years of age
- Case reported widely in UK media

  NHS England » First baby receives life-saving gene therapy on NHS
- HSCT can be considered for a late-onset juvenile/adult case

Take home messages

- Metachromatic leukodystrophy is a lysosomal storage disorder with slowly progressive motor disease
- MRI brain shows characteristic abnormalities
- There is no treatment to reverse neurological symptoms
- Gene therapy has produced good results if given before symptoms develop
- Low Arylsulphatase A requires careful interpretation as it may be due to partial deficiency (mistakenly called pseudo deficiency)

Seizures & developmental delay/regression

Case report

- 3.5 year-old girl presented with generalised tonic clonic seizures, eyes deviating to right
- First 2 episodes with fever, others afebrile, duration up to 8 minutes
- Also isolated jerks, brief drop attacks & 30 second vacant episodes for 1 yr
- Delayed speech: 1st words aged 2 yrs, joining words together at 3.5 yrs
- Walked at 12 months but deteriorating balance, increasing falls

- Examination: normal except hyperactive, delayed development & ataxia

Initial investigations

- Blood count, urea & electrolytes, bone chemistry, liver function tests, lactate: normal
- EEG: bursts of generalised spike & wave and of irregular slow wave activity
- MRI: prominent cerebellar folia, suggesting cerebellar atrophy

- Seizures much improved on Sodium valproate & Levetiracetam
- At 4 years, continuous twitching of mouth for 2 weeks
- EEG: no accompanying discharges

What is the likely cause of these twitches (& her isolated jerks)?

- Myoclonus resolved when Clonazepam added

What metabolic investigations would you request?

- Plasma biotinidase, amino acids & total homocysteine – normal
- Urine organic acids – normal

- Leukocyte & plasma lysosomal enzyme screen – normal
- Plasma N-palmitoyl-0-phosphocholine-serine – normal (against NPC)

- Genetic epilepsy, mitochondrial & lysosomal panels requested
- LP for CSF: plasma glucose & CSF lactate planned

- Leukocyte Tripeptidyl peptidase 1 (CLN2): 13 nmol/hr/mg protein (ref range 42-339)
- Palmitoyl-protein thioesterase 1 (CLN1): 65 nmol/hr/mg protein (ref range 17-139)
- Genetics: homozygous *TPP1* c.509-1G>C (common mutation in CLN2)

Neuronal ceroid lipofuscinoses

Neurodegenerative disorders with

- Combinations of regression, seizures, myoclonus & loss of vision
- Defects in 13 genes, different mutations in each causing variable phenotypes

Gene	Lysosomal function	Usual onset	Regression	Seizures, myoclonus	Visual loss	Other features
CLN1	PPT1 enzyme	Infantile 6-18 months	1st			Acquired microcephaly
CLN2	TPP1 enzyme	Late infantile 1.5-4 years	1st	1st		Ataxia
CLN3	Trans-membrane	Juvenile 4-8 years			1st	Psychiatric problems

Management & progress

- Rapid regression: at 5 years little speech & unable to walk
- Partial gastrostomy feeding from 5.5 years as weight static
- Intracerebroventricular ERT (Cerliponase) fortnightly from 6 years using a Rickham® reservoir

- Regression slowed but unable to crawl by 7 years
- Spasticity managed with botulinum toxin injections in gastrocnemius
- Deteriorating vision – only light perception by 9 years
- Currently 13 years, still on ERT but tube fed, stiff & limited interaction

Adults

Take home message

- “Classical” lysosomal investigation in plasma and urine do not allow diagnosis of neuronal ceroid lipofuscinoses
- Laboratory investigation
  - Vacuolised lymphocytes: CLN3
  - Measurement of enzyme activities (leukocytes, fibroblasts, DBS): CLN1, CLN2, CLN10 (total number at least 13 conditions)
  - Electron microscopy: lysosomal accumulation of autofluorescent material in neurons and other cell types (specialised laboratories)
  - Molecular genetic testing: all neuronal ceroid lipofuscinoses
- ERT is now available for CLN2 defects

The girl with macrocephaly

Patient

3 months	global developmental delay with regression and irritability along with feeding difficulties and poor sleep
Examination:	weight and length in the lower normal range relative macrocephaly normal eye examination, but not fixing and following
	increasing head circumference with crossing percentiles over next few months
6 months	head circumference above the 99.6th percentile

What are your differential diagnoses?

Which tests would you consider?

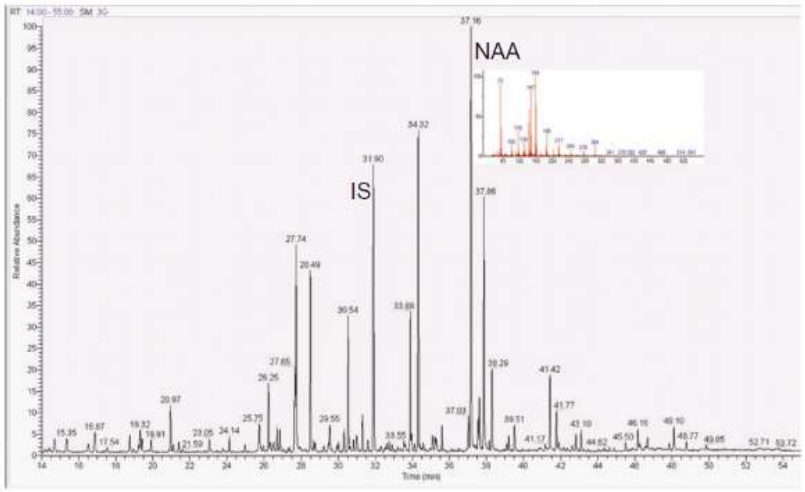
Diagnostics

Normal results for

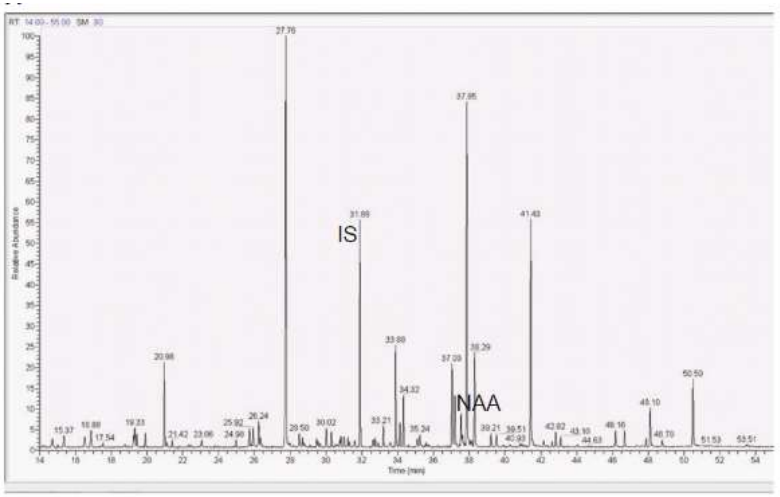
- Plasma amino acids
- Urinary glycosaminoglycans
- Urinary oligosaccharides
- Plasma/white blood cell lysosomal enzymes

Urinary Organic Acids

Patient



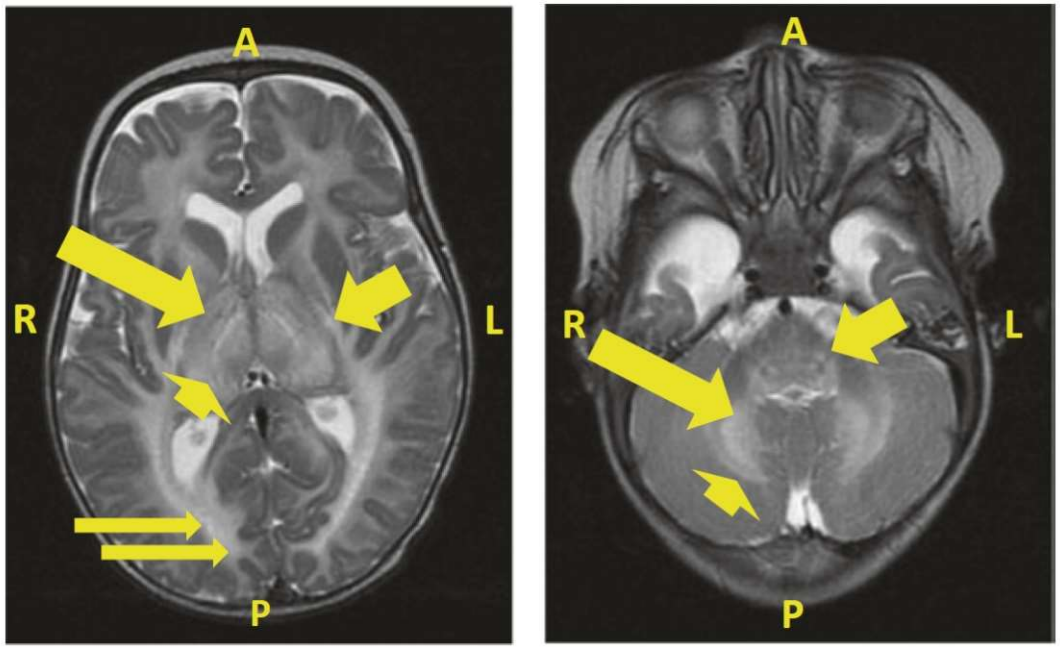
Healthy Control



Urinary *N*-Acetylaspartic acid ↑↑↑
(also in a repeat sample)

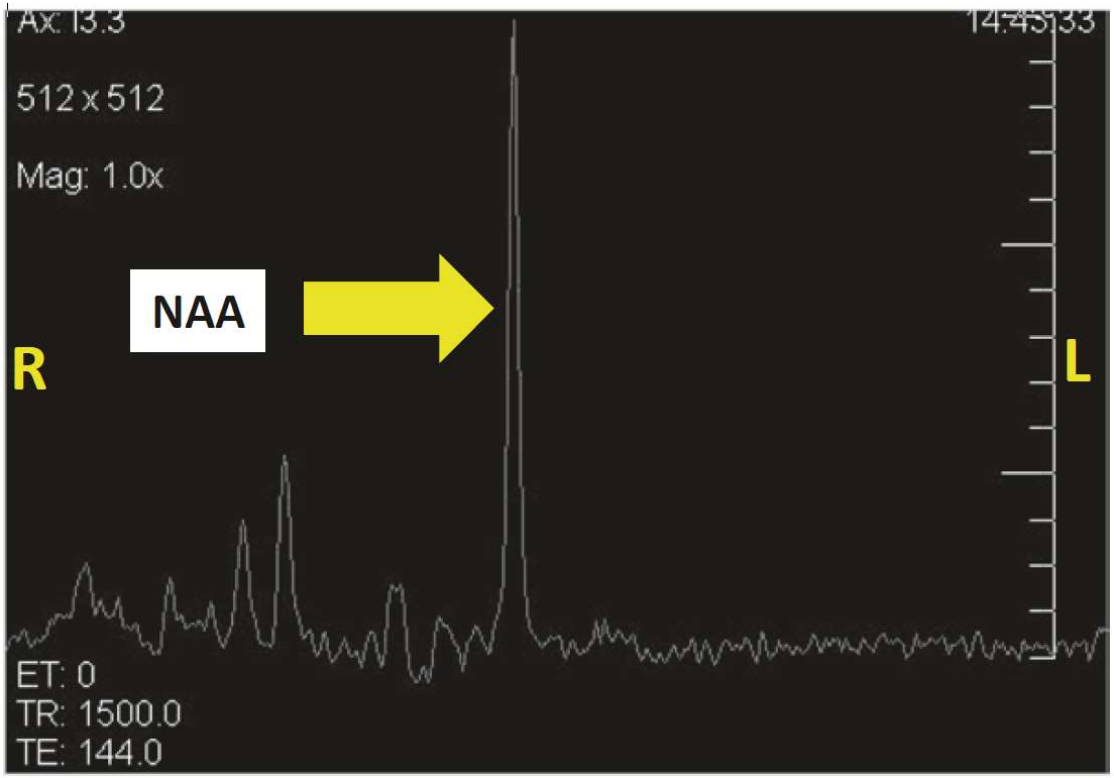
What is the likely diagnosis?

Brain MRI



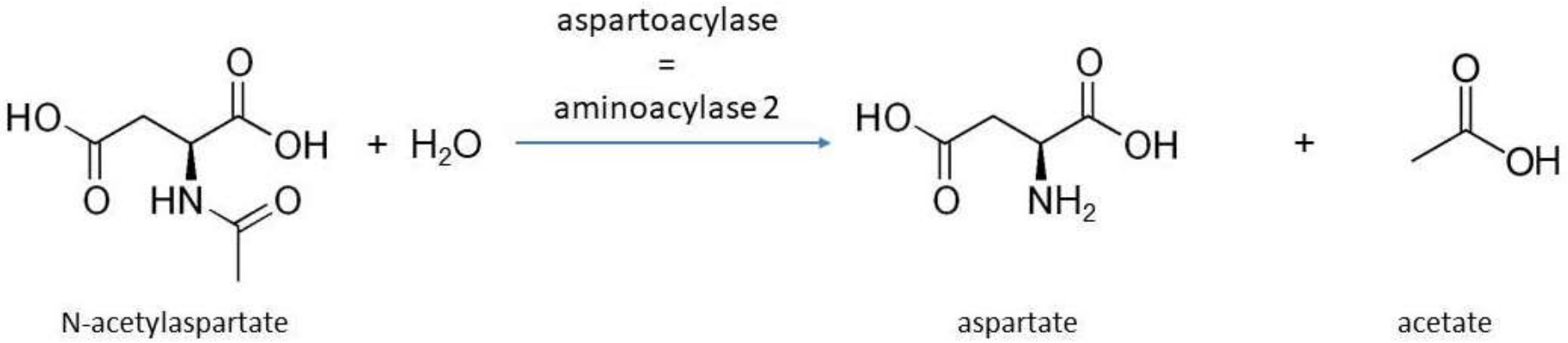
Diffuse white matter changes, e.g., of supratentorial white matter and also involvement of the cerebellum. Abnormal signal in the thalamus (arrowhead) and globus pallidus (arrow) bilaterally as well as focally in the posterior limbs of the internal capsules (short arrow) and the posterior deep white matter (double arrow). Characteristic sparing of putamina, claustrum, caudate, corpus callosum, and most of theinternal capsules

MR Spectroscopy



strong increase of the *N*-acetylaspartate (NAA) peak

Pathway



- Symptoms especially due to enzyme deficiency in oligodendrocytes => leukodystrophy
- Underlying pathophysiology is still in part enigmatic

Enzyme and genetic testing

Aspartoacylase deficiency demonstrated both in immortalized lymphocytes and in cultured skin fibroblasts.

Mutation analysis (including MLPA) did not reveal an *ASPA* mutation.

Further Course on Therapy

- Development of epileptic seizures, predominantly tonic
- Well controlled by antiepileptic treatment (phenobarbital and later valproic acid)
- Benzodiazepine treatment helpful for management of the patient's irritability
- Overall reduced muscle tone and head lag
- Episodes of stiffness with an increase in peripheral tone and progressive spasticity
- Tube feeding required

Adult presentation

- Milder form with juvenile or adult onset and only mild developmental delay with or without macrocephaly
- Vision impairment
- Only few cases reported
- Individuals with atypical Canavan disease may survive to adulthood

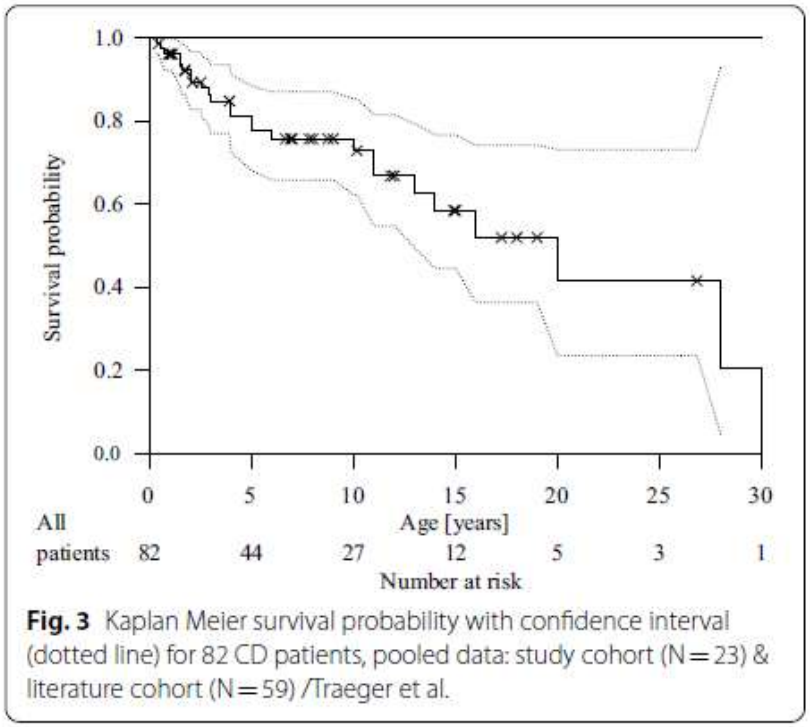


Fig. 3 Kaplan Meier survival probability with confidence interval (dotted line) for 82 CD patients, pooled data: study cohort (N = 23) & literature cohort (N = 59) /Traeger et al.

Bley et al., Orphanet J Rare Dis. 2021 19;16(1):227

Discussion/ Take home messages

- Head circumference at birth may not be increased; enlarging head may cross percentiles in the first year of life
- *N*-acetylated aspartic acid is identified in the analysis of urinary organic acids, but usually NOT in amino acid analysis
- Lack of identified DNA sequence variants does not rule out Canavan disease
- Eye examination may be normal in very young patients despite poor vision, but optic atrophy is a frequent finding over the course of the disease, and nystagmus may be apparent
- Distinctive white matter findings, usually symmetric and progressive, reflecting abnormal development and destruction of the brain myelin
- No published clinical practice guidelines, no curative therapy

Source

Case description based on

Jörn Oliver Sass, Ina Knerr
Aspartoacylase Deficiency (Canavan Disease, *N*-Acetylaspartic Aciduria)
In: Oohashi, Tsukahara et al. (Eds.):
Human Pathobiochemistry: From Clinical Studies to Molecular Mechanisms,
Singapore: Springer, 2019, pp 15-21

Pathophysiological Aspects

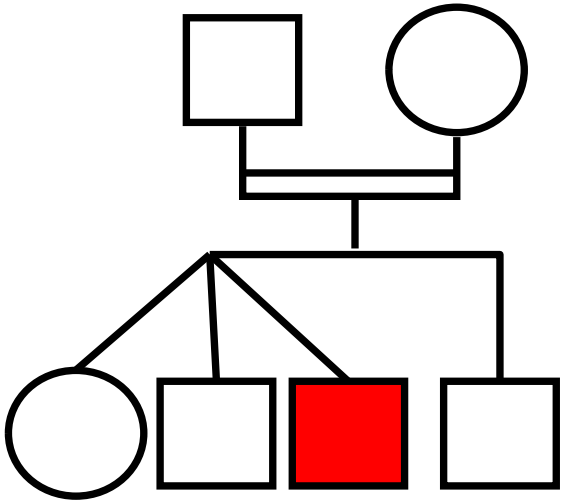
- High concentrations of *N*-acetylaspartylglutamate in the brain, secondary to high concentrations of NAA, could have detrimental effects by disturbing *N*-methyl-D-aspartate (NMDA) receptor dependent processes or by causing accumulation of glutamate (Burlina et al. 1999)
- NAA functions as a molecular water pump in myelinated neurons and that NAA accumulation may lead to osmotic dysregulation in the brain which can subsequently result in dysmyelination and subcortical vacuolation observed in CD patients (Baslow 2003).
- Aspartoacylase may be involved in the epigenetic regulation of genes relevant to myelin and genes responsible for the differentiation of oligodendrocytes, cells that express much aspartoacylase (Kumar et al. 2009)

20-month-old boy with spastic paraplegia

20-month-old boy

- *Seen in neuropediatric clinic for spastic paraplegia*
- *Triplet pregnancy (trichorial, triamniotic)*
- *Born at 34 weeks by elective C-section, length 43 cm (<P3)*
- *4 months: plagiocephaly*
- *5 months: surgery for hydrocele*
- *9 months: diagnosis of tracheomalacia*
- *14 months: convergent strabismus*
- *18 months: chronic cough*

What else do you want to know?



Differential diagnosis of spastic paraplegia

- *Brain damage, mostly white matter damage*
- *Abnormal brain development*

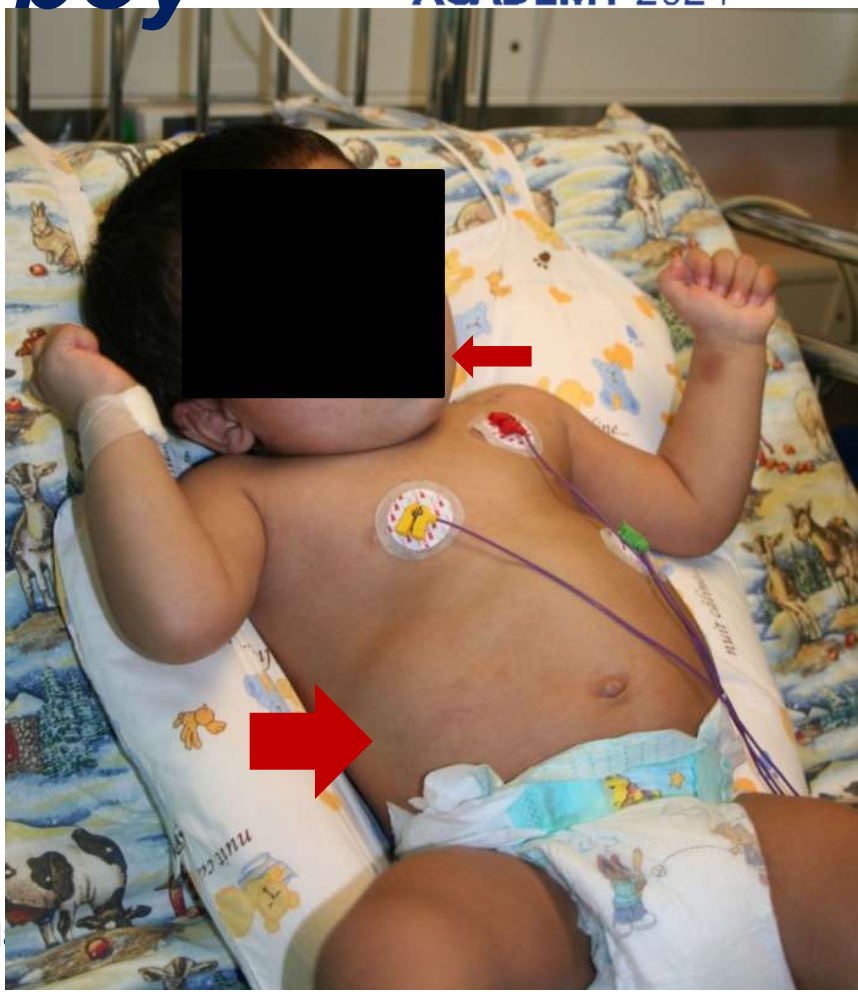
May be due to:

- *Prematurity, foetal infections or stroke, bleeding, hypoxia, hyperbilirubinemia (kernicterus)*
- *Maternal infections or medical conditions*
- *Exposure to toxins*
- *Genetic disorders including many inborn metabolic diseases: e.g. CDG, LSD (oligosacch., sphingolipidosis), NCL mitochondrial disorders*

20-month-old boy

- *Open mouth*
- *Teleangiectasias*
- *Hepatomegaly*

Which further clinical investigations would you do?

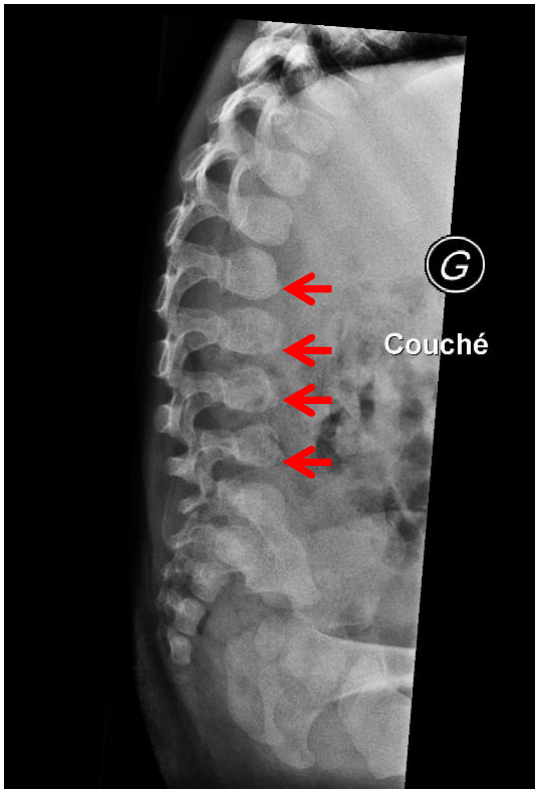


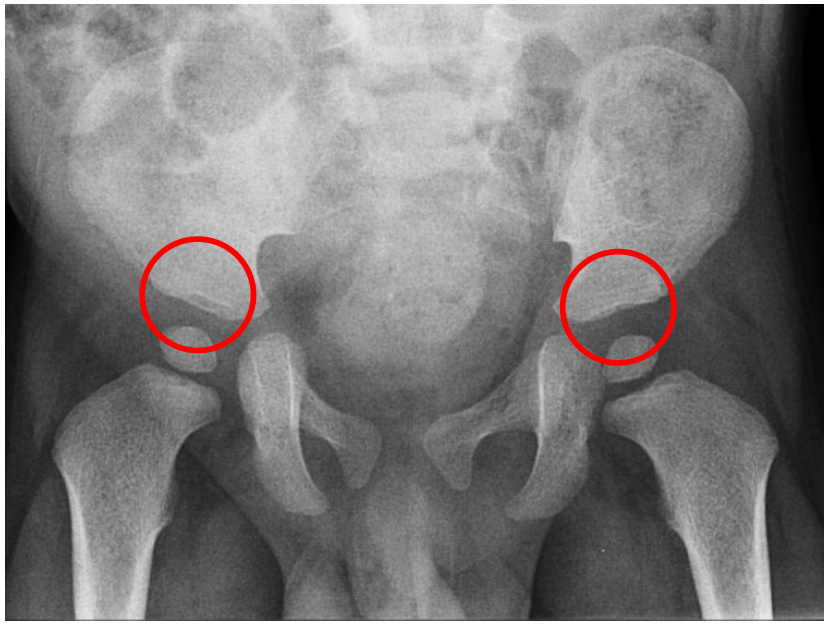
Investigations

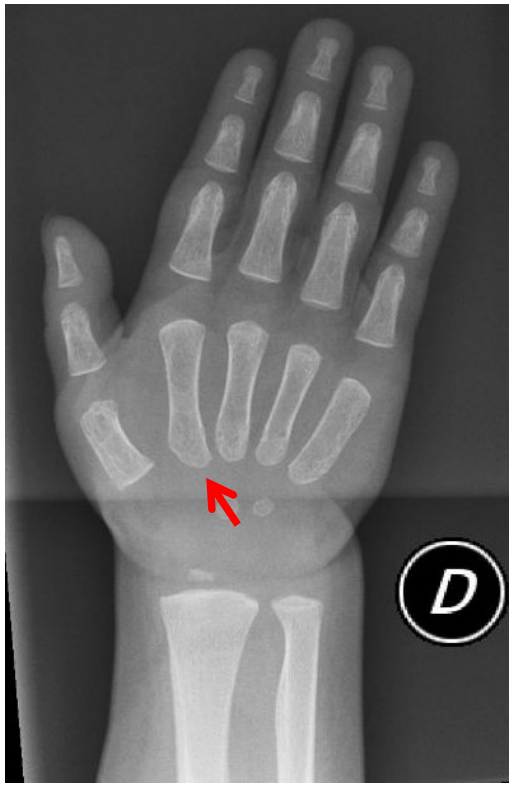
- *Plasma amino acids, blood gases, lactate, urinary organic acids: normal*
- *Sialotransferrins (CDG): normal*
- *Purines/pyrimidines in urine: normal*

- *Blood smear:* ***vacuolated lymphocytes, only slightly periodic acid-Schiff (PAS) positive***

X-rays: spine, pelvis, hand





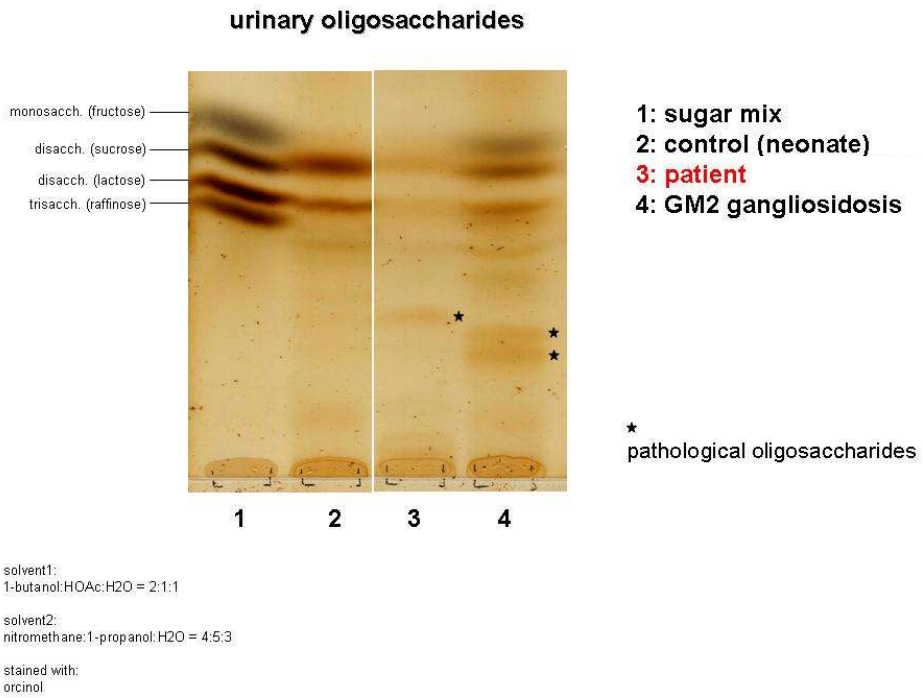


Signs of dysostosis multiplex

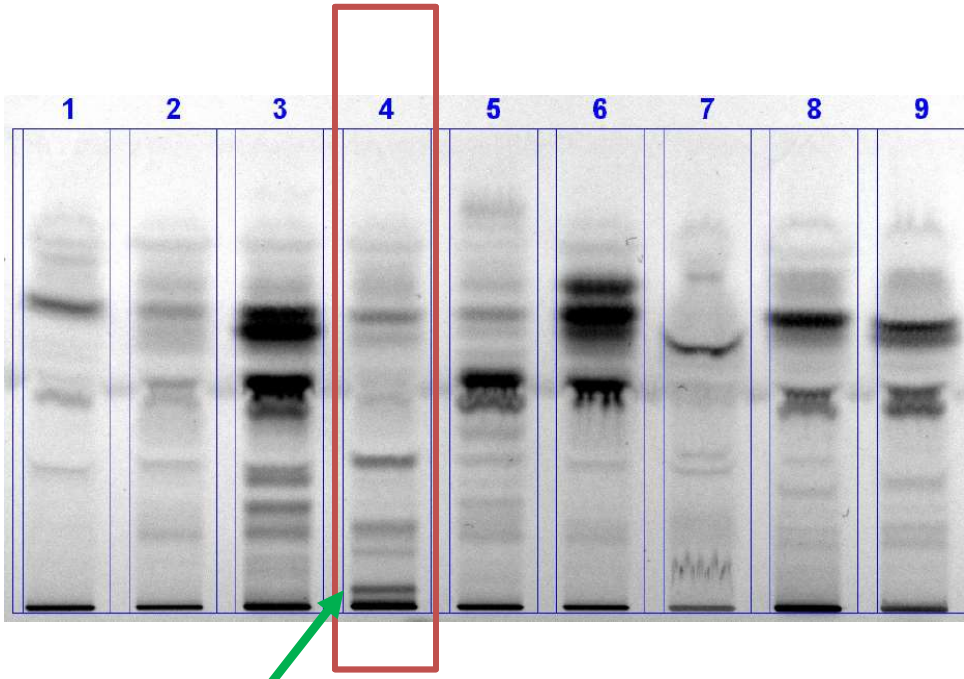
Which investigations would you do?

Urine GAGs and oligosaccharides

- Glycosaminoglycans normal

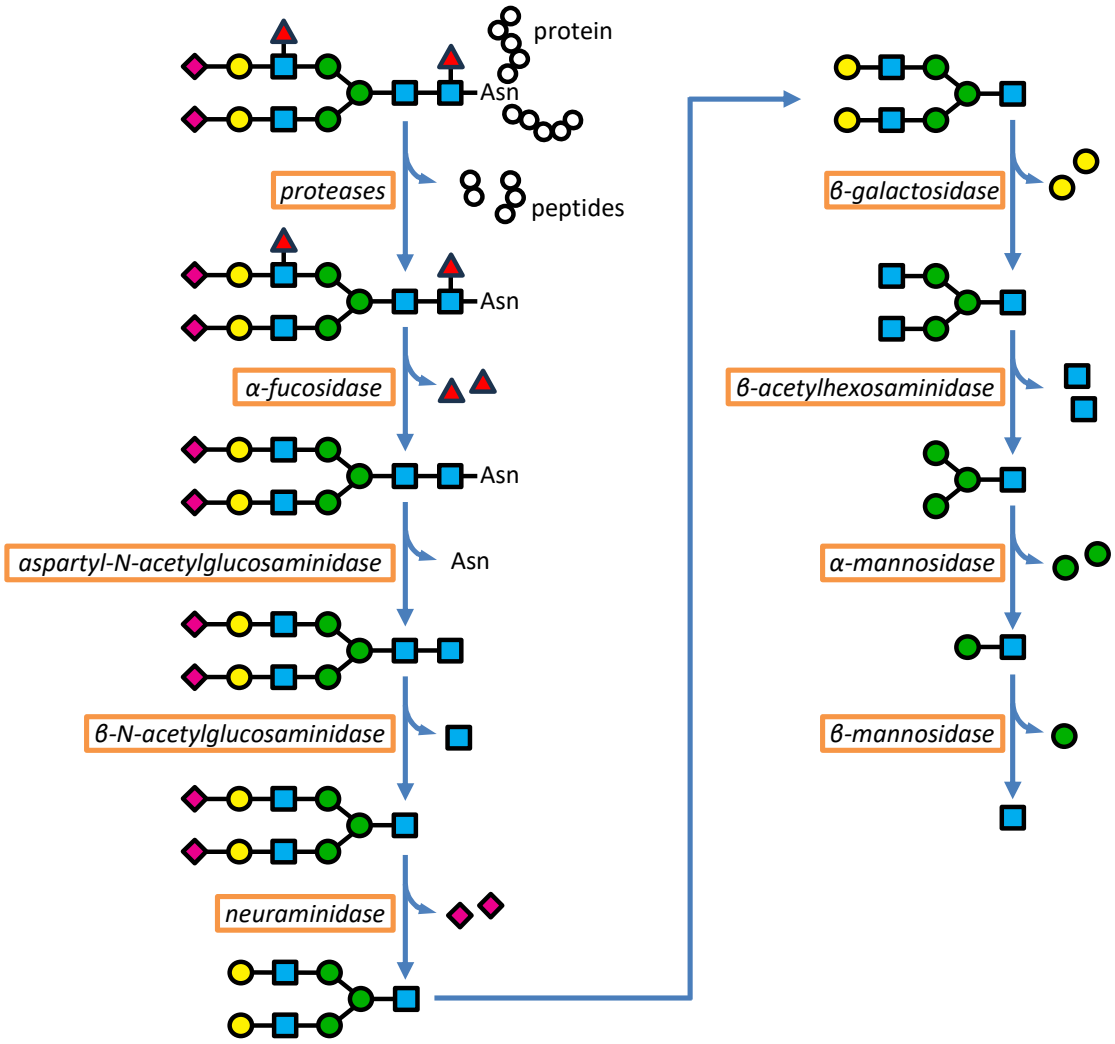


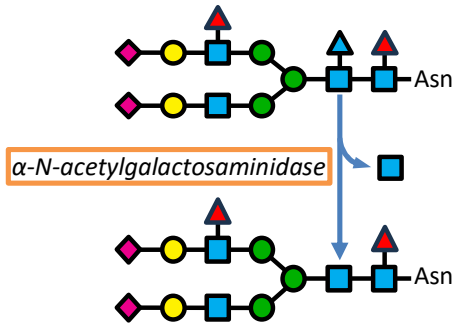
More typical profile

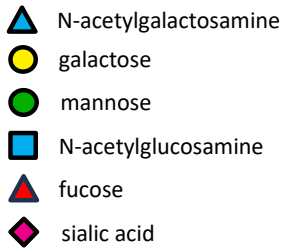


Diagnosis: fucosidosis

oligosaccharide catabolism

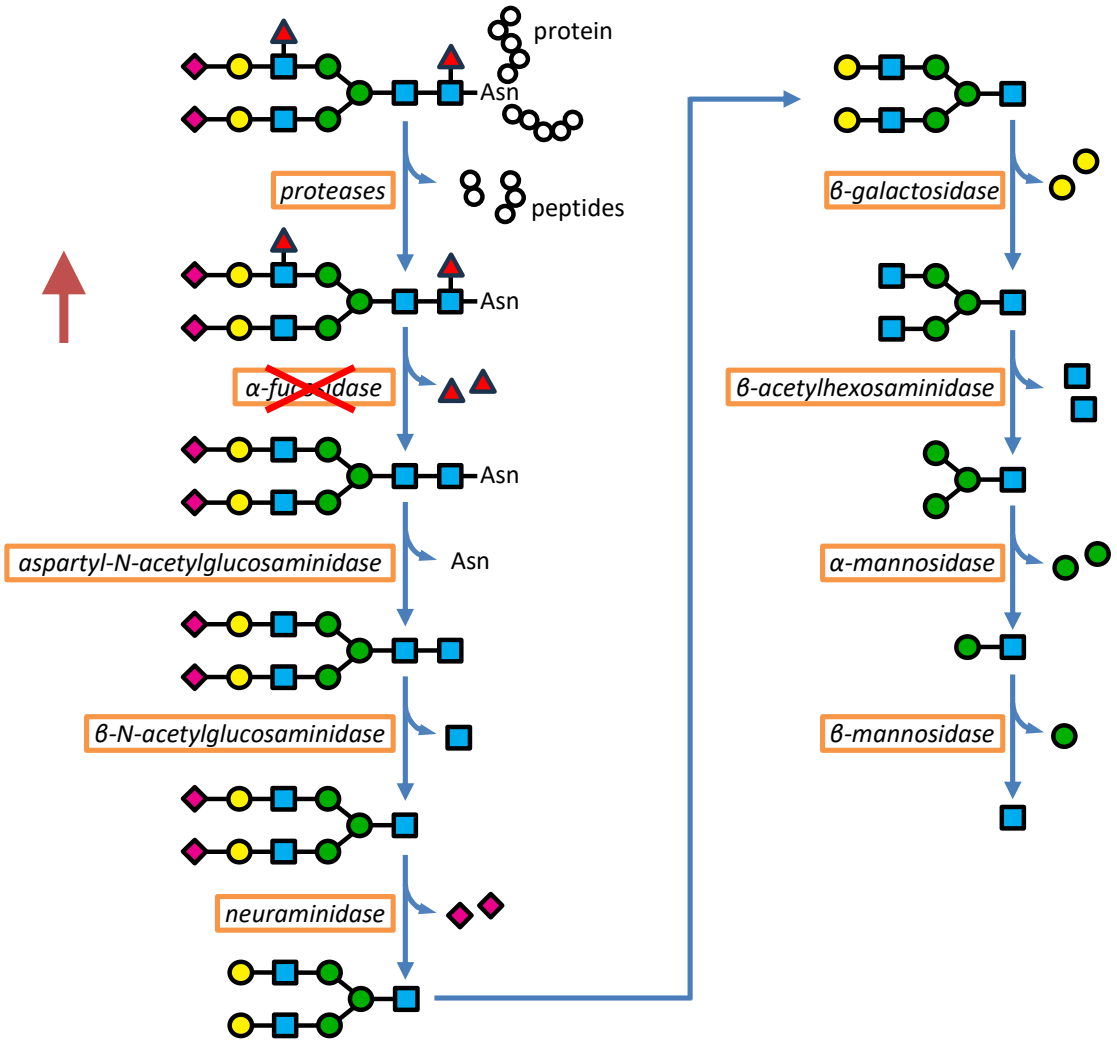


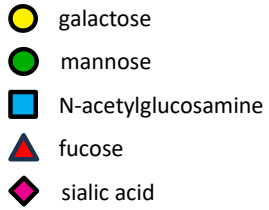




fucosidosis

α-fucosidase deficiency





Confirmational diagnostics

Fucosidase activity in leukocytes, serum or fibroblasts:
Substrate: 4-methylumbelliferyl α-L-fucopyranoside
Fluorometry: ex 355 nm, em 460 nm

Fucosidase in leucocytes: 0.06 mU/mg protein (n 0.34-1.06)
Fucosidase in fibroblasts: 0.2 nmol/h/mg protein (n 30 – 134)

Genetics: homozygous for c.389C>G in FUCA1 (CAVE: pseudogene FUCA1P)

What should you do next?

ERNDIM proficiency testing 2016

- Urine oligosaccharidosis screening using TLC is challenging
  - 7/19 labs correctly interpreted results
  - ERNDIM oligosaccharidosis panel available

Diagnosis	most likely	other possible
Fucosidosis	7	-
GM1 gangliosidosis	5	3
Sialidosis/galactosialidosis	3	2
Mucolipidosis type II (III)	1	4
MPS IVB	1	2
MPS	2	-
Aspartylglucosaminuria	1	-
Oligosaccharidosis	-	1

- GAG, e.g. keratansulphate, may be elevated in fucosidosis
  - Keratan-sulphate contains a terminal fucose

    Greiling et al. J Clin Chem Clin Biochem 1978;16:329

Urine oligosaccharide screening can be more straightforward

LC - MS/MS or LC - High Res MS

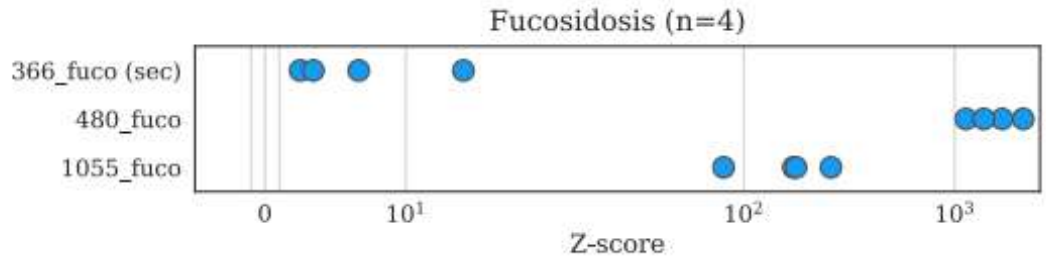


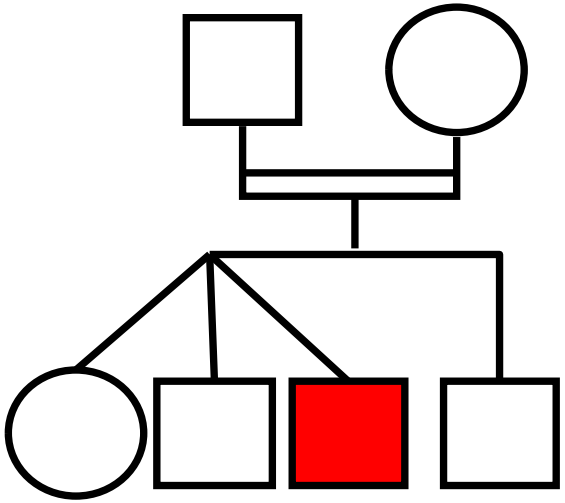
TABLE 2 Oligosaccharide-species detected by UPLC/HRAM mass spectrometry in patient urine samples.

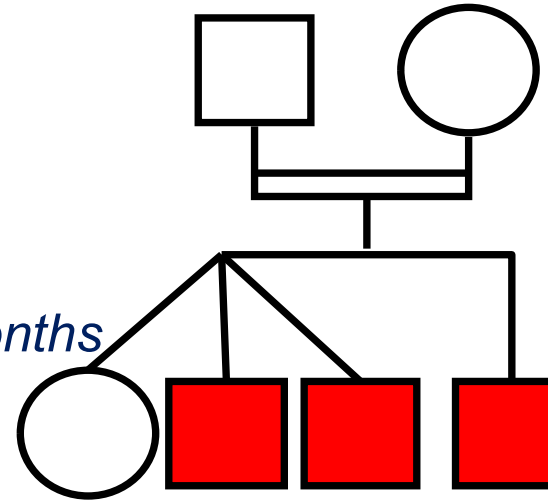
IEM	Rt (min)	Component	m/z	Adduct	Chemical formula	Oligosaccharides	ID	Reference
Fucosidosis ($n = 4$)	2.66	366_fuco (sec)	366.1400	[M-H]-	C14H25NO10	GlcNAc-Fuc	3	Ramsay et al.[16]
	3.39	480_fuco	480.1835	[M-H]-	C18H31N3O12	Asn-GlcNAc-Fuc	3	Bonesso et al.[9]
	3.37	1055_fuco	1055.3784	[M-H]-	C40H68N2O30	GlcNAc-Man-Man-GlcNAc(-Fuc)-Gal	3	Bonesso et al.[9]

LC-MS/MS
MoM: Multiple of medians
UMCG, Groningen,
The Netherlands

Hagemeijer et al. J Inherit Metab Dis 2023;46:206-219

Family screening





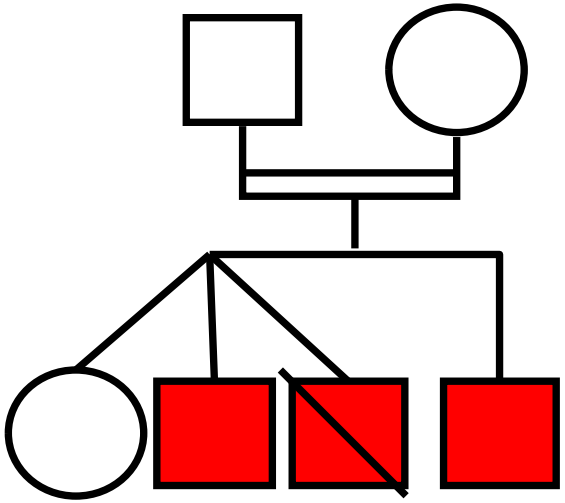
Twin brother and younger brother also affected!

How would you treat these patients?

Treatment options

Symptomatic, no approved treatments
Clinical trials with hematopoietic stem cell transplantation (HSCT)

Outcome



- *Index case died*
- *Both affected brothers got stem cell transplantation at age 25 and 5 months*

At 16 and 14 y:
Both have small stature and mental retardation, the older one doesn't speak, both suffer from severe pain (chronic treatment with morphine)

Take home messages

Two clinical forms:
Type I: *rapidly progressive, leading to decerebration and death before the age of 10 years*
Type II: *milder course, possible survival into adulthood, angiokeratoma corporis diffusum*

Diagnosis can be easily missed on urinary oligosaccharide analysis (depending on method)

HSCT seems to be of limited effect, even if performed very early