Workshop 3 – Diagnosis and management of lysosomal storage disorders

Iron deficiency after Thailand trip?

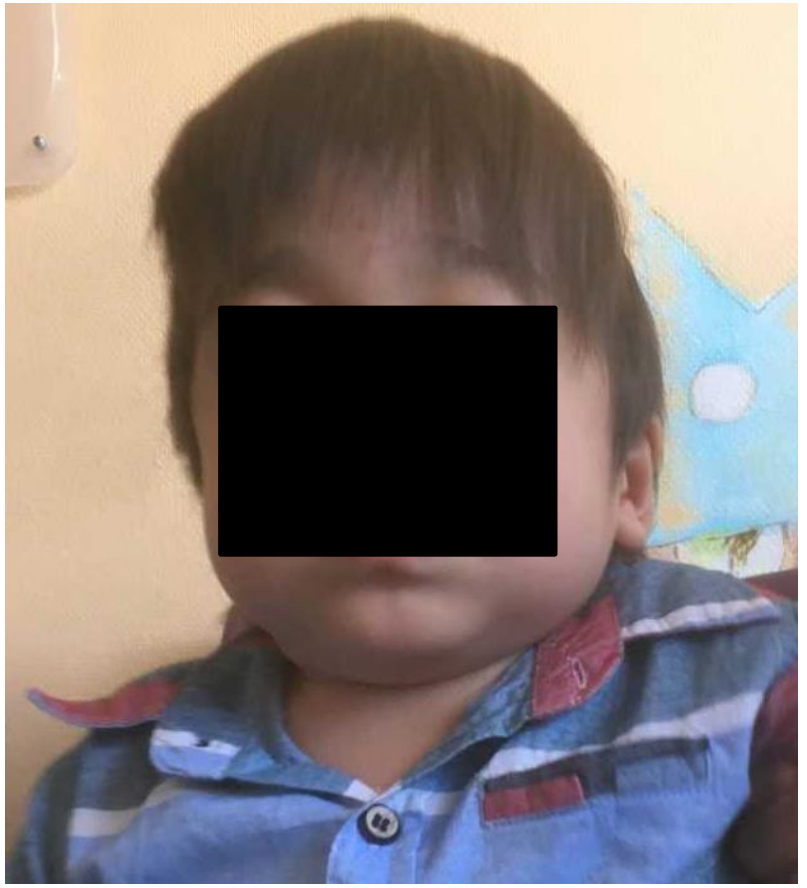
9 month old boy

- *First child of non-consanguineous parents of Asian origin*
- *Comes back from a long trip to Thailand, often tired*
- *Paediatrician: weight and height below 3rd percentile*
- *Haemoglobin: 49 g/L (3.0 mmol/L), severe iron depletion*
- *ASAT: 82 U/L (9-45), ALAT: 102 U/L (9-38)*
- *Hospital: transfusion, transient feeding by nasogastric tube*

What do you think about this situation?

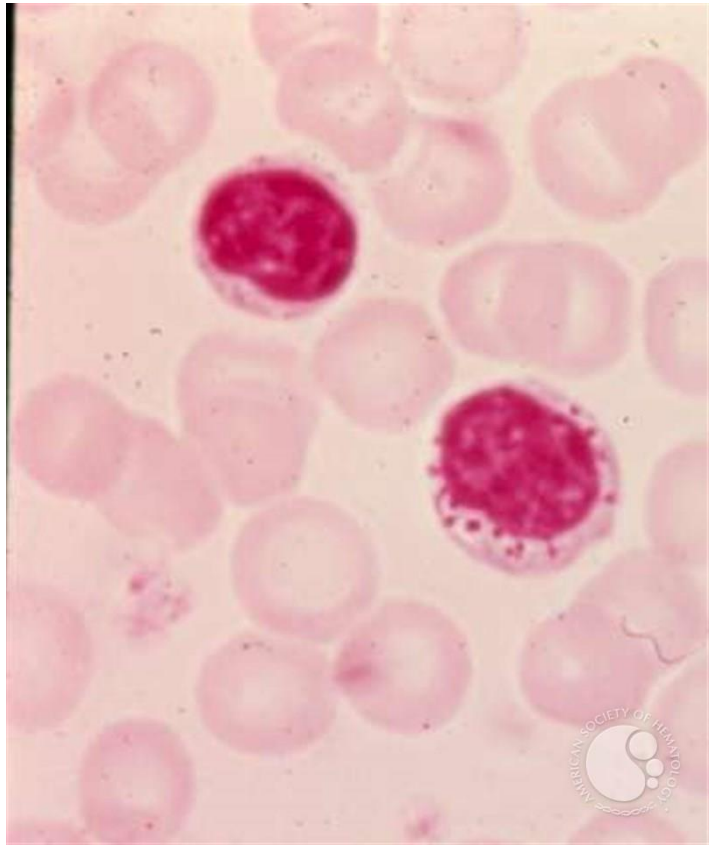
3 year old boy

- *Cognitive and language delay*
- *Small stature (P3)*
- *Behaviour: hyperactivity, fits of rage*
- *Hepatosplenomegaly, confirmed by US*
- *ASAT: 48 U/L (9-45), ALAT: 39 U/L (9-38), Blood smear: inclusions in leucocytes*



What would you do now?

Blood smear: Alder Reilly anomaly



cytoplasmic metachromatic inclusions with circumferential clearing

ASH Image Bank, Author: Teaching collection Vicky Smith

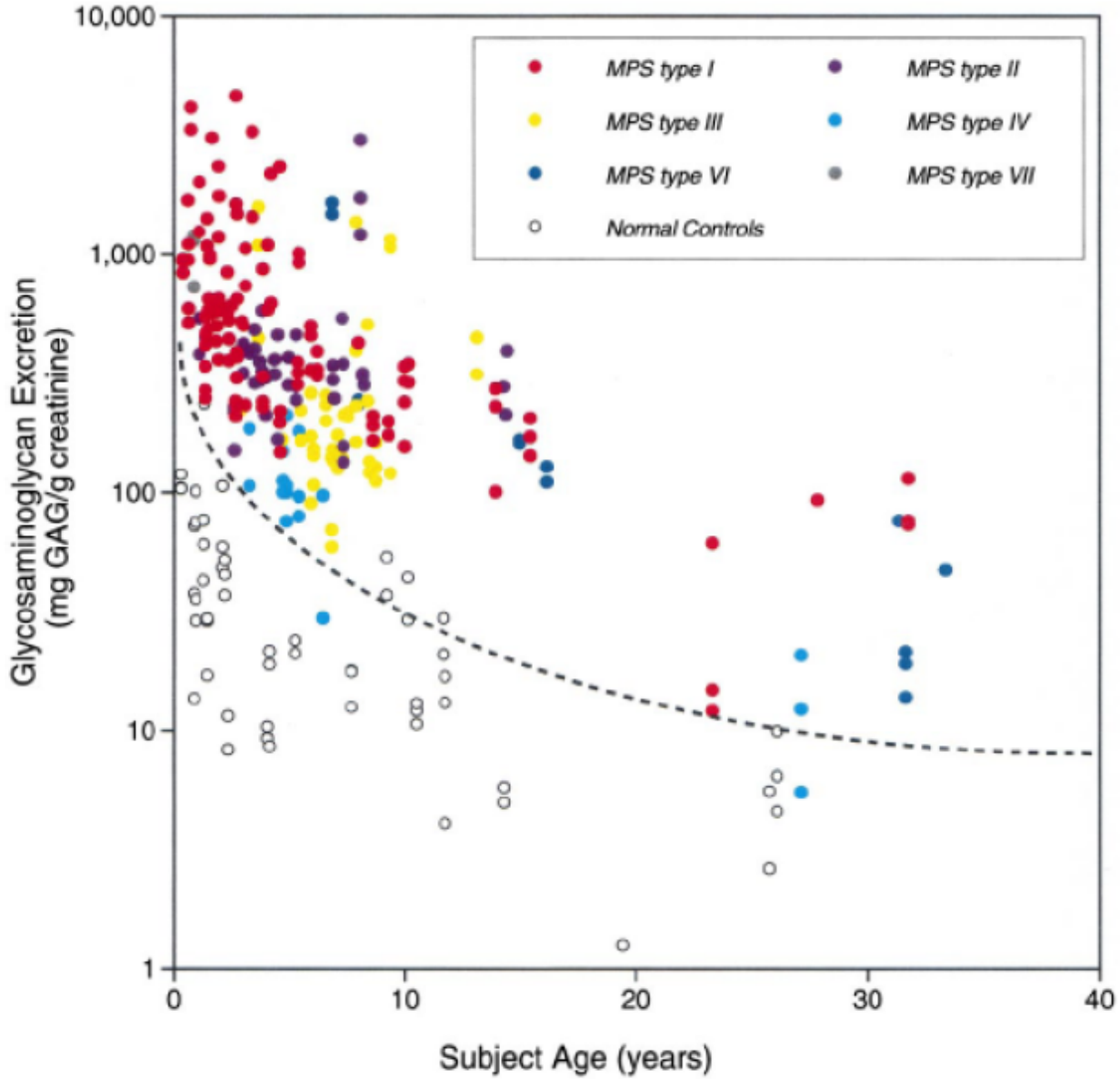
Urinary Glycosaminoglycans

- *GAGs quantification: 73.2 mg/mmol creatinine (n < 36)*
- *GAGs electrophoresis: dermatan and heparan sulfate*

What is the differential diagnosis?

MPS I or MPS II

What is next diagnostic step?

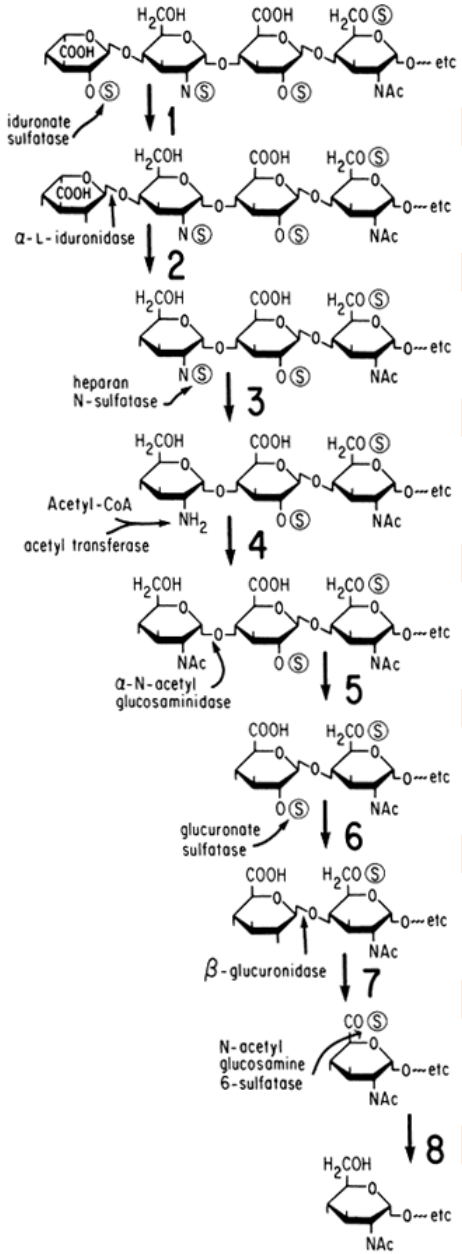


Urinary GAGs: Age-dependent excretion

Whitley et al *Molecular Genetics and Metabolism* **75**, 56–64 (2002)

Mucopolysaccharidoses

heparansulfate (HS)



MPS II Hunter — *IDS*
Iduronate sulfatase

MPS I Hurler/Sheie — *IDUA*
α-L-iduronidase

MPS III A Sanfilippo — *SGSH*
heparan-N-sulfatase

MPS III B Sanfilippo — *HGSNAT*
acetyl-CoA glucosaminide acetyltransferase

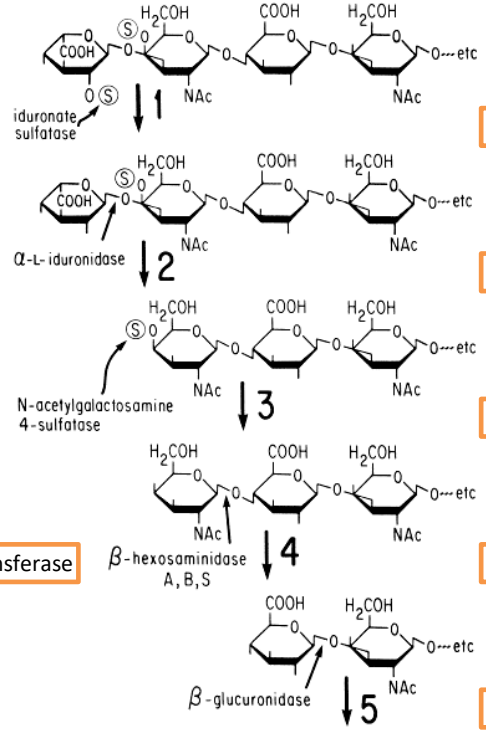
MPS III C Sanfilippo — *NAGLU*
α-N-acetylglucosaminidase

glucuronate sulfatase

MPS VII Sly — *GUSB*
β-glucuronidase

MPS III D Sanfilippo — *GNS*
N-acetylglucosamine-6-sulfatase

dermatansulfate (DS)



MPS II Hunter — *IDS*
Iduronate sulfatase

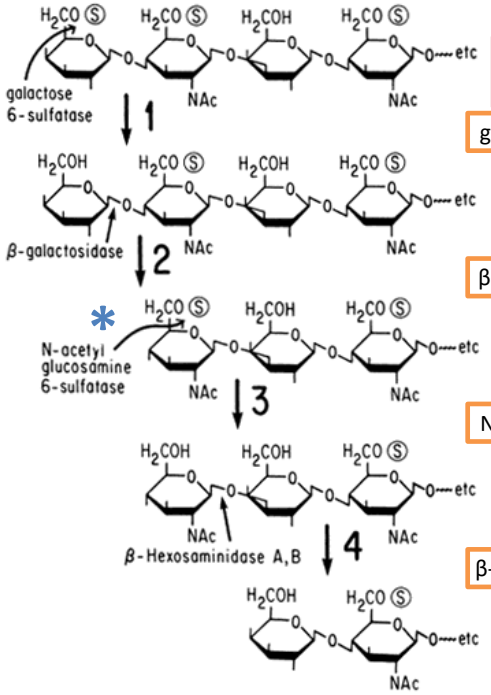
MPS I Hurler/Sheie — *IDUA*
α-L-iduronidase

MPS VI Maroteaux-Lamy — *ARSB*
N-acetylgalactosamine-4-sulfatase

β-hexosaminidase A/B

MPS VII Sly — *GUSB*
β-glucuronidase

keratansulfate (KS)



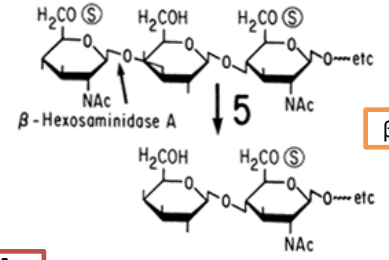
MPS IV A Morquio A — *GALNS*
galactose-6-sulfatase

MPS IV B Morquio B — *GLB1*
β-galactosidase

MPS III D Sanfilippo — *GNS*
N-acetylglucosamine-6-sulfatase

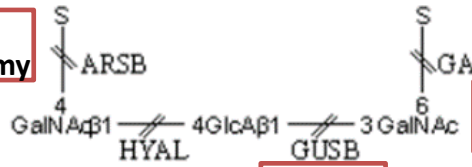
β-hexosaminidase A,B

* alternate pathway



β-hexosaminidase A

Chondroitin sulfate

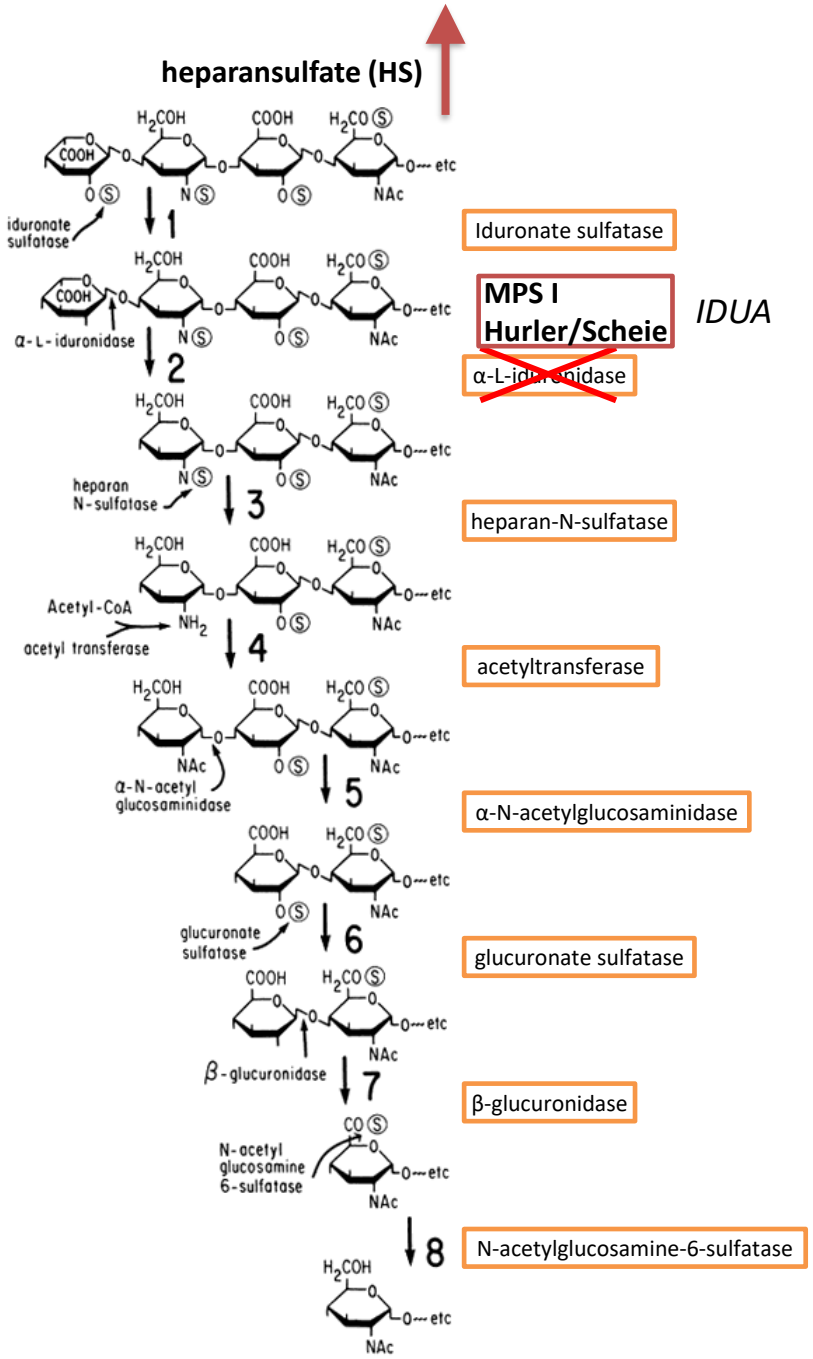


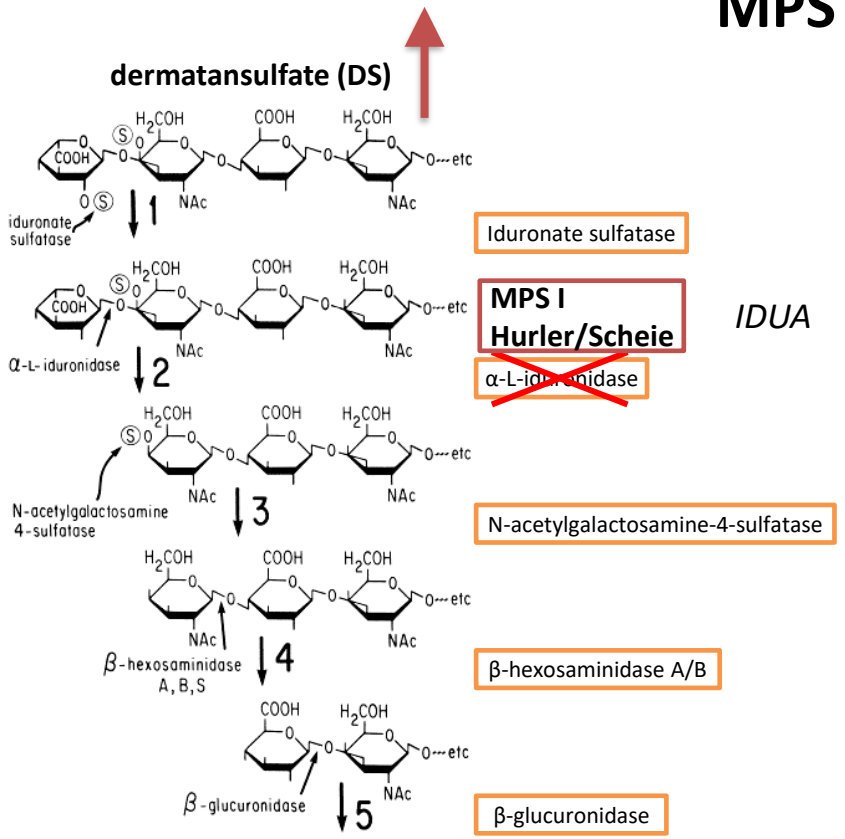
MPS VI Maroteaux-Lamy

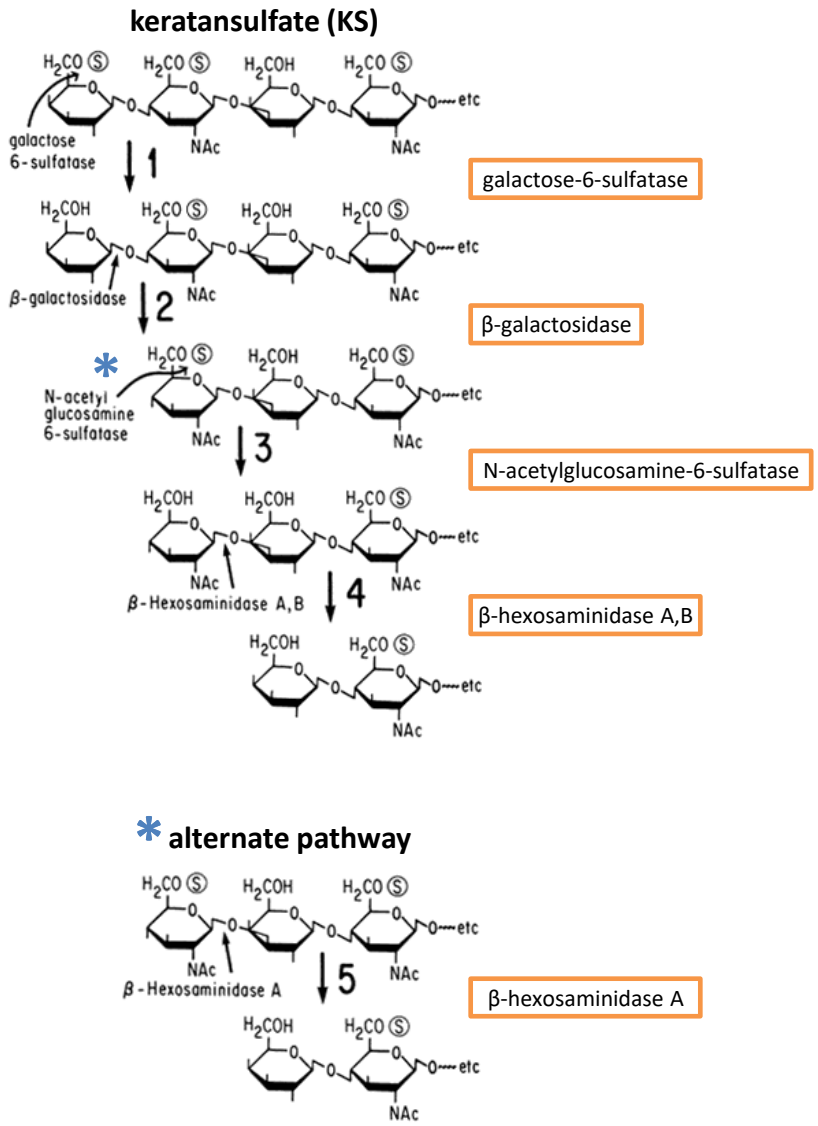
MPS IV A Morquio A

MPS VII Sly

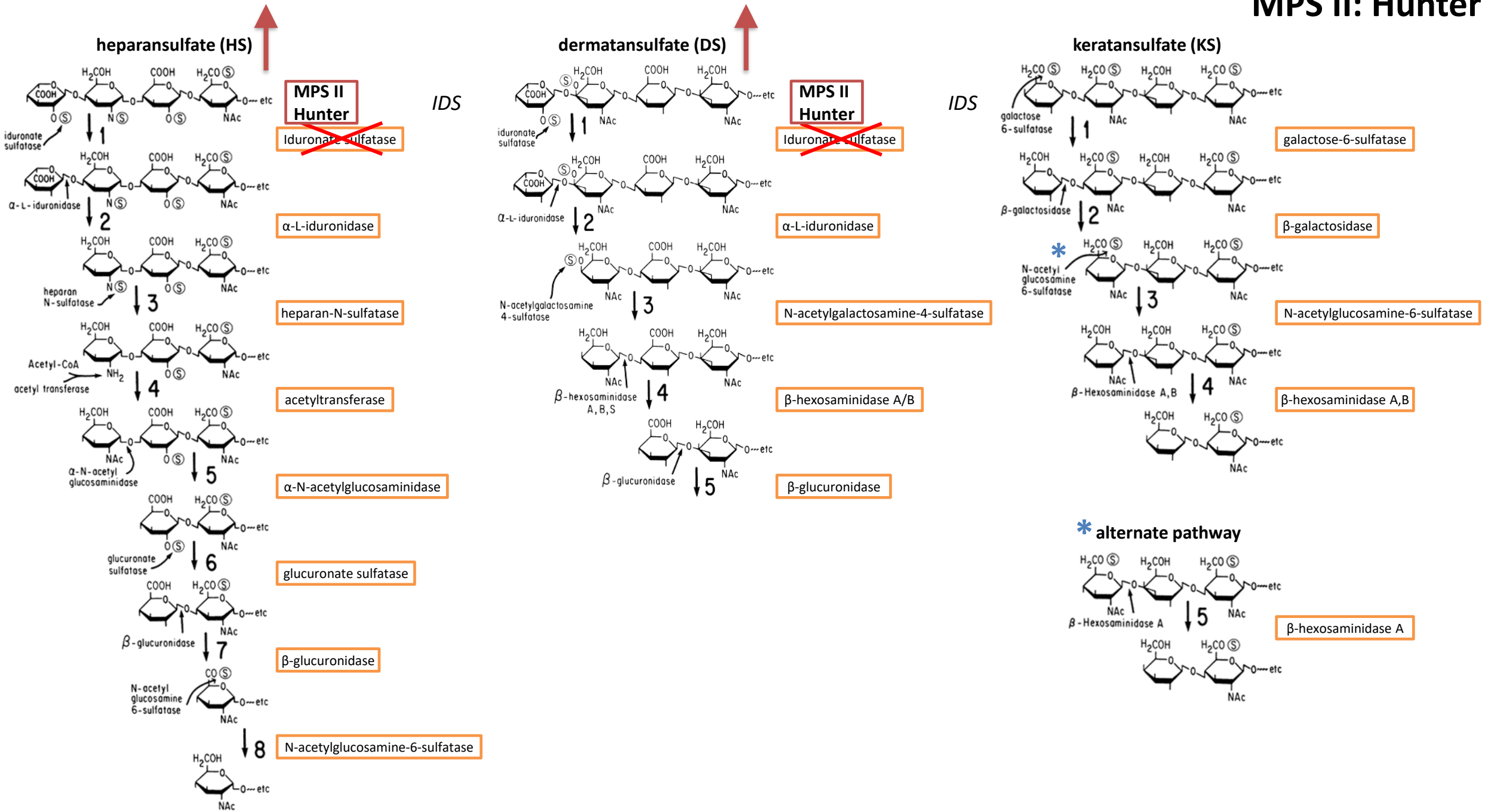
MPS I: Hurler, Hurler-Scheie, Scheie







MPS II: Hunter



Enzyme activity measurement

- *Alpha-L-iduronidase in leucocytes:*
  0.22 nmol/min/mg protein (0.15-0.52)
- *Iduronate-2-sulfatase in leucocytes:*
  0.04 nmol/4h/mg protein (7.18-35.75)

What is your diagnosis?

Mucopolysaccharidosis type II (Hunter)

Is there another differential diagnosis?

Multiple Sulfatase Deficiency (at least one other sulfatase)

How do you confirm the diagnosis?

Molecular Analysis of IDS gene

What do you expect to find?

- *IDS gene is localized on the X-chromosome*
- *Patient is hemizygous for p.Arg468Gln in IDS*
- *Mother is a carrier*

What are the treatment options for MPS II?

Treatment options for MPS II

- *Enzyme replacement therapy with recombinant Idursulfase (Elaprase®), weekly infusions, only corrects some of the peripheral symptoms (hepatosplenomegaly, walking distance, respiratory function)*
- *Hematopoietic stem-cell transplantation (HSCT): only in young patients (<2 years), variable effects on different disease aspects*
- *Trials for brain-penetrating ERTs*

Which treatment would you suggest for this patient?
Anything else to be taken into consideration?

Family history

- *5-month-old brother, clinically normal*
- *Laboratory: ASAT: 43 U/L (9-45), ALAT: 41 U/L (9-38)*
- *Iduronate-2-sulfatase in leucocytes: 0.05 nmol/4h/mg prot. (7.18-35.75)*
- *US abdomen: liver at upper limit of normal, no splenomegaly*

How would you treat the brother?

Outcome

- *Older brother (7 y, under ERT): severe cognitive impairment, small stature, profound deafness, repeated infections, sleep disorder, behavioural problems (autism-like)*

- *Younger brother (4 y, after HSCT at 8 months): language delay, low-normal intelligence, normal growth, normal behaviour, no major hearing problem*

Take home messages

- *MPS don't tend to cause dysmorphism in early infancy*
- *Many LSD do not cause clear symptoms in early infancy*
- *Liver transaminase elevations in MPS can be mild*
- *Clinical symptoms can be inconsistent in early stage*
- *Early diagnosis is critical for outcome in several LSD*
- *Quantitative urinary GAGs are a screening test, qualitative GAG analysis (electrophoresis, LCMS) needs to be added*
- *Confirmation requires enzymatics and/or genetics*

Confusing coincidental findings

- *5 year old girl admitted with gastroenteritis*
- *No past history of note, parents second cousins*
- *Height & weight <0.4th centile, hepatomegaly (3cm below ribs)*
- *ALT 340 iu/L (normal <50), Bilirubin, Alk Phos, GGT, clotting normal*
- *Ultrasound: enlarged echobright liver. Liver biopsy: microvesicular steatosis*
- *No weakness but CK 1050 iu/L (normal <320)*
- *ECG normal, echocardiography – mild left ventricular hypertrophy*
- *Thyroid function & growth hormone stimulation tests normal*

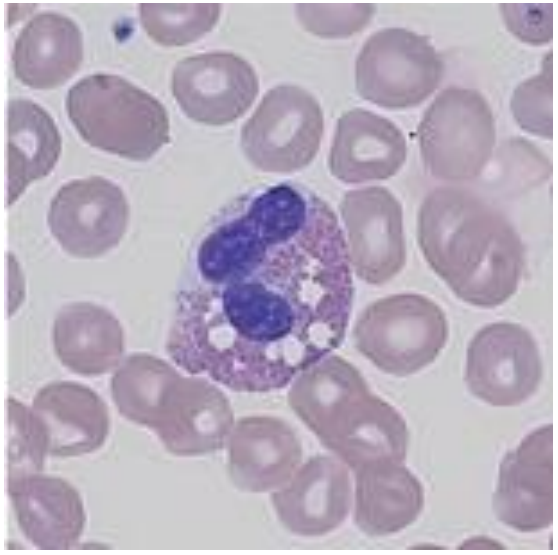
What further tests would you do?

- *Acylcarnitines & Organic acids normal*
- *Cholesterol 5.5mmol/L, Triglycerides 0.9 mmol/L*
- *Liver gene panel: no AGL mutations – i.e. not GSD III*

One heterozygous PNPLA2 mutation - biallelic mutations cause Neutral Lipid Storage Disease with Myopathy ± cardiomyopathy & liver steatosis

- *Blood count & film normal, no Jordan's anomaly*

Jordan's anomaly (vacuoles with lipid seen in Neutral Lipid Storage Diseases)



What further tests would you do?

- *Whole genome sequencing: homozygous VUS in GAA c.2015G>A (p.Arg672Gln) → Late-onset Pompe disease*
- *Lymphocyte α-glucosidase activity undetectable (assay with acarbose)*
- *Assay can also be done on fibroblasts or bloodspots*

Why is the assay done with acarbose?

- *Acarbose inhibits the cytoplasmic α-glucosidase isoenzyme*

Would a muscle biopsy show any abnormalities?

- *Most patients have a vacuolar myopathy (often leads to diagnosis in adults)*

Are there any biomarkers for Pompe disease?

- *Urine glucose tetrasaccharide 97 umol/mmol creatinine, normal <5*

Patient aged 10 years by time of diagnosis

- *No muscle symptoms but running slow & hands used on climbing stairs*
- *Mild leg weakness on examination*
- *Height <0.4th centile, weight 2nd centile*
- *Cardiovascular examination normal*
- *Hepatomegaly (2cm below ribs)*
- *CK 1490 iu/L (normal <320)*
- *Lung function test: FEV1 & FVC 70% predicted*
- *Echocardiography: LV thickness at upper limit of normal*

Are all this girl's findings explained by Pompe disease?

Hepatic steatosis & short stature are probably not due to Pompe disease

How would you manage this girl?

- *Fortnightly ERT started (Avalglucosidase)*
- *Portacath inserted to facilitate infusions*

How would treatment differ in a 3 month-old with cardiomyopathy?

- *If no Cross Reactive Immunological Material (CRIM) is present, immune modulation needs to be given, starting before first infusion, to prevent neutralising antibodies*
- *Usually rituximab & methotrexate with 1st 3 infusions & IV immunoglobulins monthly x 5*
- *CRIM status is often predictable from mutations, otherwise assessed in fibroblasts or lymphocytes*

Take home messages

- *Infantile-onset Pompe disease presents with hypertrophic cardiomyopathy*
- *Late-onset forms present with myopathy in children or adults*
- *The myopathy is proximal (including scapular winging) & affects the diaphragm (& so breathing)*
- *α-glucosidase can be assayed in fibroblasts, lymphocytes or blood spots*
- *ERT can help but immunomodulation is needed in CRIM negative cases*
- *In consanguineous families there may be dual pathology*

A 14-year-old boy with ataxia

14-year-old boy

- *Seen at the neurology department with dysarthria and ataxia in extremities*
- *History of abdominal pain since birth, frequent diarrhoea, normal appetite*
- *Splenomegaly at 8 years of age (considered to be Epstein Barr Virus)*
- *Eye examination: vertical gaze palsy*
- ***Which tests would you consider?***

Videos

Metabolic lab results

- *Initial metabolic screening: normal*
- *Lysosomal enzymes in fibroblasts and plasma, all normal except:*

Enzyme	**Value**	**Ref values**	
Sphingomyelinase	45	55-81 nmol/17uur/mg prot	fibroblasts
Chitotriosidase	169	4-97 nmol/h/ml plasma	Plasma

Which diagnoses do you consider?

Be aware of chitotriosidase deficiency (6%)

J. Inher. Metab. Dis. 18 (1995) 717–722

Table 2 Plasma chitotriosidase activity in 24 lysosomal disorders[a]

Disease	*Plasma chitotriosidase activity*	
	Abnormal/total	*Elevated activity* (nmol/h per ml)
Aspartyl glucosaminuria	0/3	
Fabry	0/8	
Gaucher type I[a,b]	20/21	5580–51 800
Glycogen storage disease II	2/8	360; 420
GM1-gangliosidosis[b]	7/13	380; 720–1420
GM2-gangliosidosis	0/11	
Krabbe disease[b]	7/11	610–1670
α-Mannosidosis	1/3	300
β-Mannosidosis	0/2	
Metachromatic leukodystrophy	1/29	550
Mucopolysaccharidoses (I; II; IIIA; B, C; IVA, B)	2/63	600; 400
Mucolipidosis II/III	0/3	
α–NAGA deficiency	0/2	
Niemann-Pick A/B[b]	13/15	250; 602–2800
Niemann-Pick C[b]	6/11	263; 304–940
Sialic acid storage disorders	0/1	
Sialidosis	0/1	
Total	58/205	(28%)

[a]The patients in this table were diagnosed in the authors' laboratories (Rotterdam, Nijmegen and Leiden) and reflect the relative frequencies of the various diseases. The number of Gaucher patients is therefore different from the high number in Table 1

[b]Diseases where majority of patients have elevated chitotriosidase activity

Filipin staining on cultured fibroblasts

Historical gold standard for NPC

Filipin binds free cholesterol, detection by fluorescence microscopy

Results:
Positive (classic phenotype)

DNA diagnostics

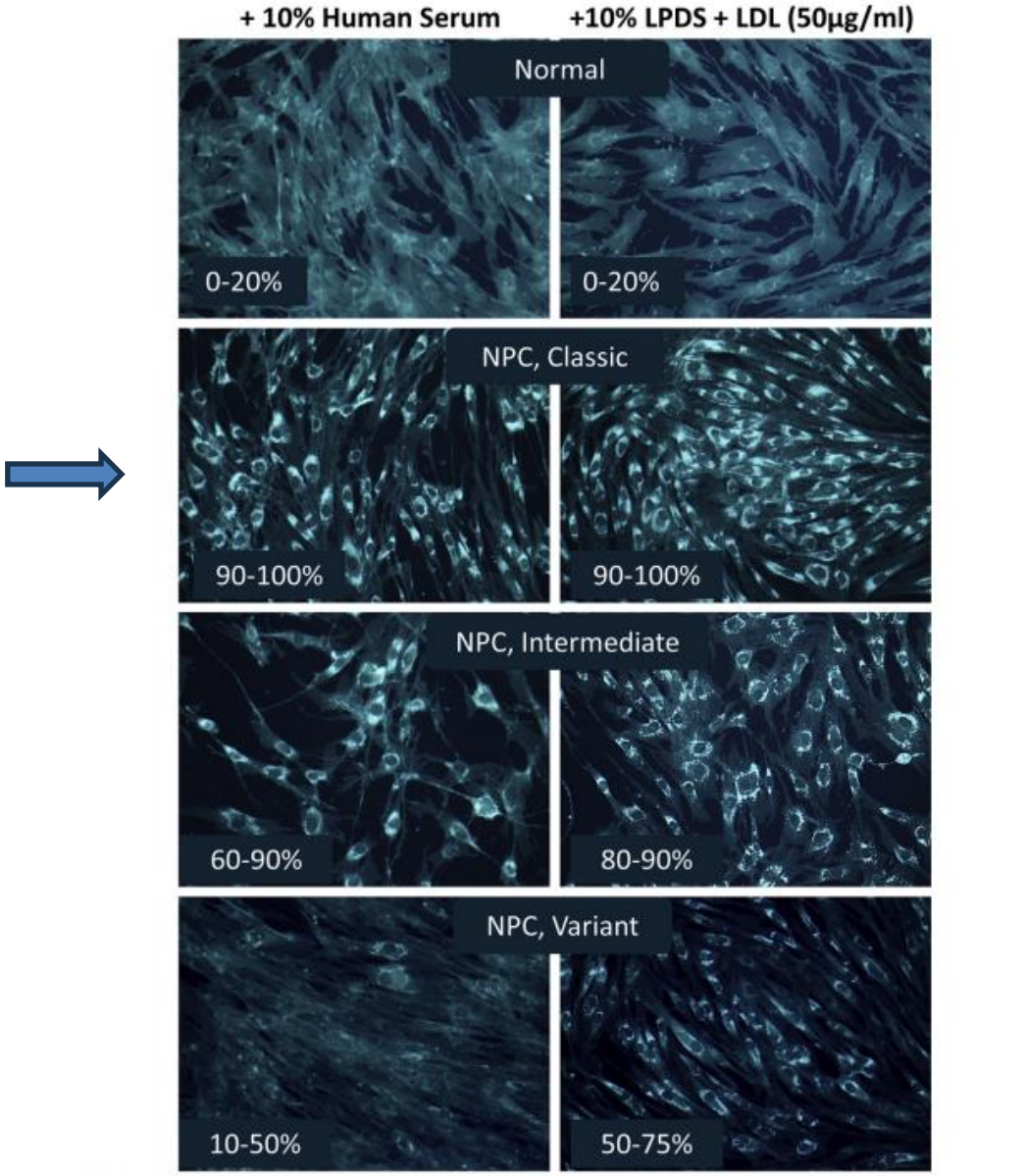


FIGURE 2 Illustration of the range of variation (classic, intermediate, and variant phenotypes) observed for the filipin test in fibroblasts from Niemann–Pick type C patients (one representative cell line per phenotype), comparatively to normal cells.

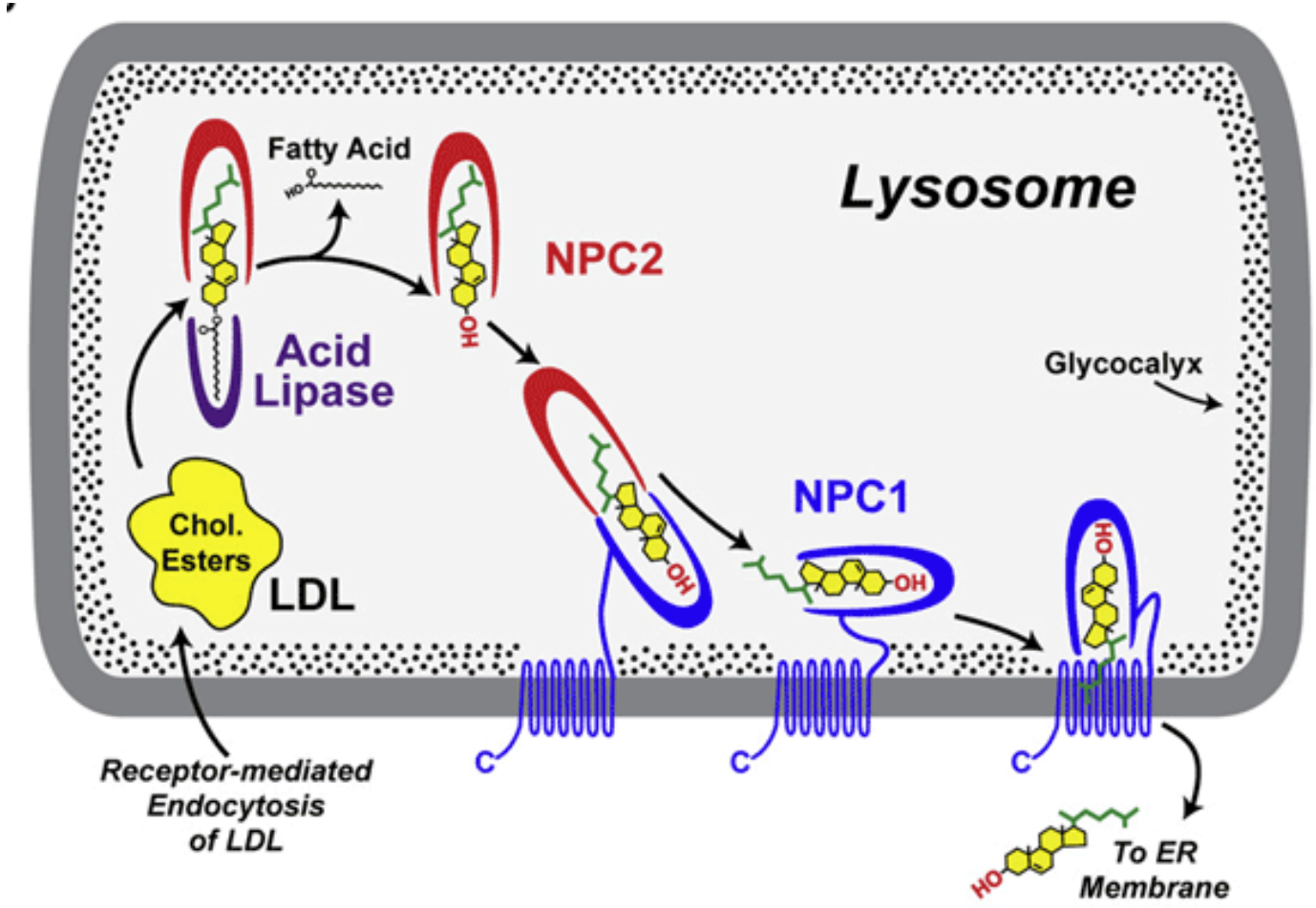
Vanier et al. Methods in Cell Biol 2015;126:357-375

Niemann-Pick C

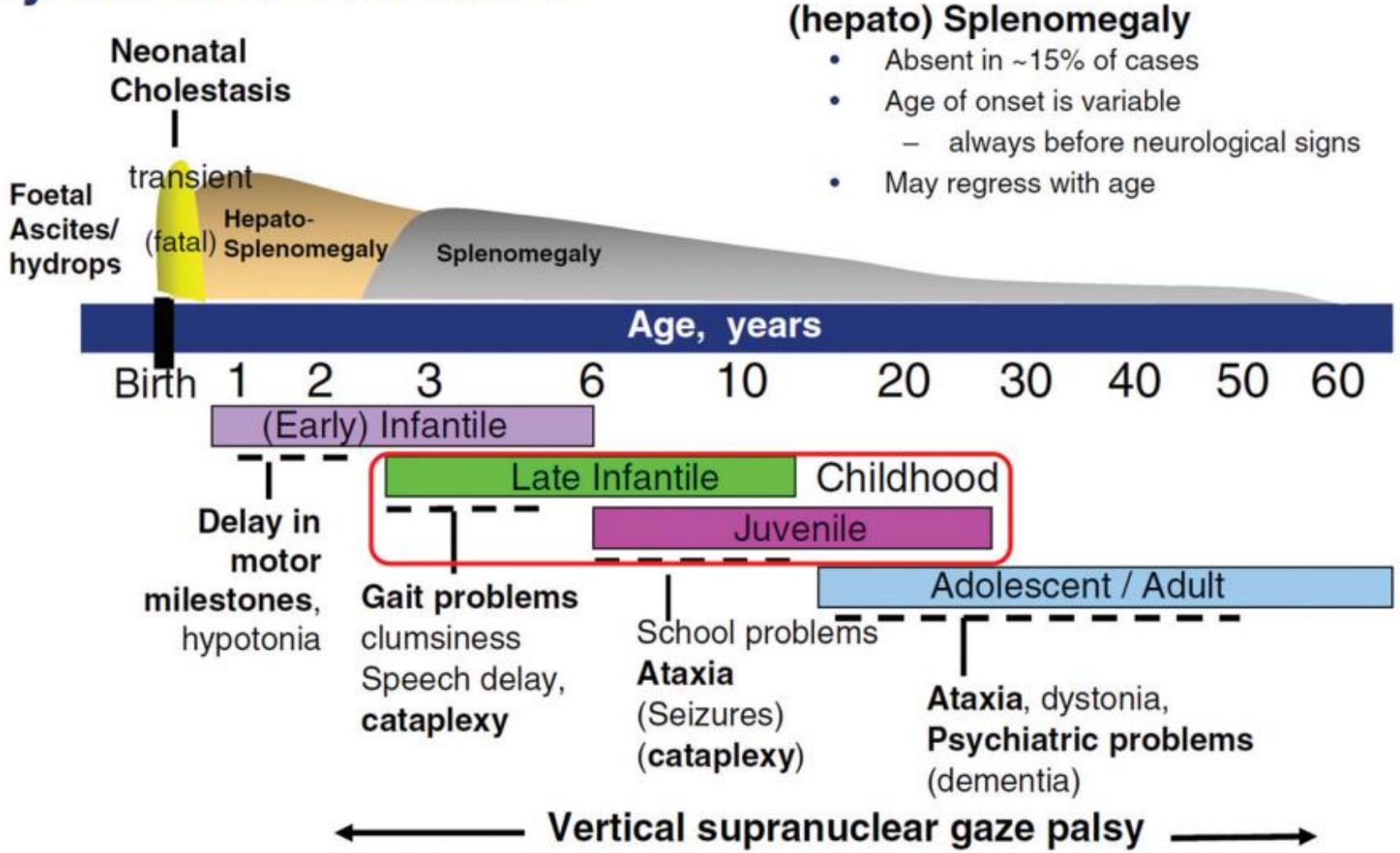
Lysosomal storage disease caused by defective transport of free cholesterol and other lipids from lysosomes and late endosomes.

NPC1 variants: 95%

NPC2 variants: 5% *(often severe)*



Systemic involvement



(hepato) Splenomegaly

- Absent in ~15% of cases
- Age of onset is variable
  - always before neurological signs
- May regress with age

Neurological involvement

Period of onset — Duration

Vanier *Orphanet Journal of Rare Diseases* 2010, 5:16

Which other biochemical tests for Niemann-Pick type C do you know

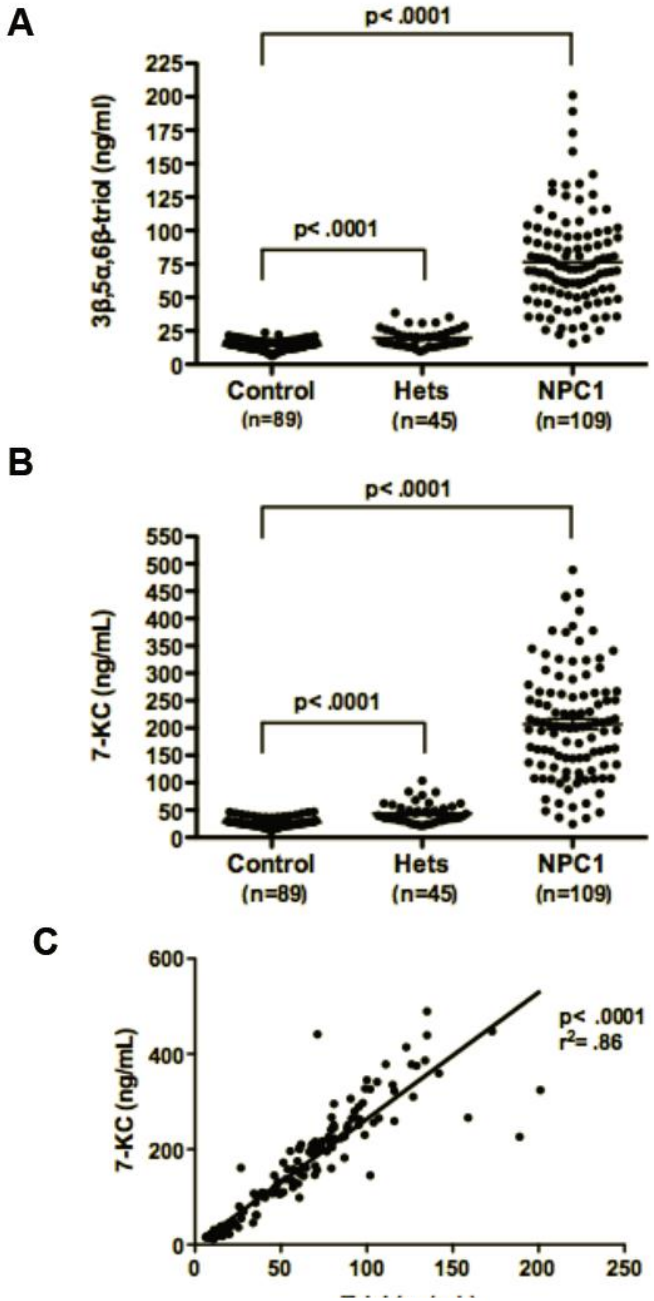
Oxysterols are biomarkers of NPC

7-ketocholesterol
Cholestane-3β,5α,6β-triol

Both also elevated in:

- *NP-A and NP-B*
- *Acid lipase deficiency*
- *Cerebrotendinous xantho-matosis*
- *Smith-Lemli-Opitz syndrome*

Cholestane-3β,5α,6β-triol is more specific



Jiang. J. Lipid Res 2011

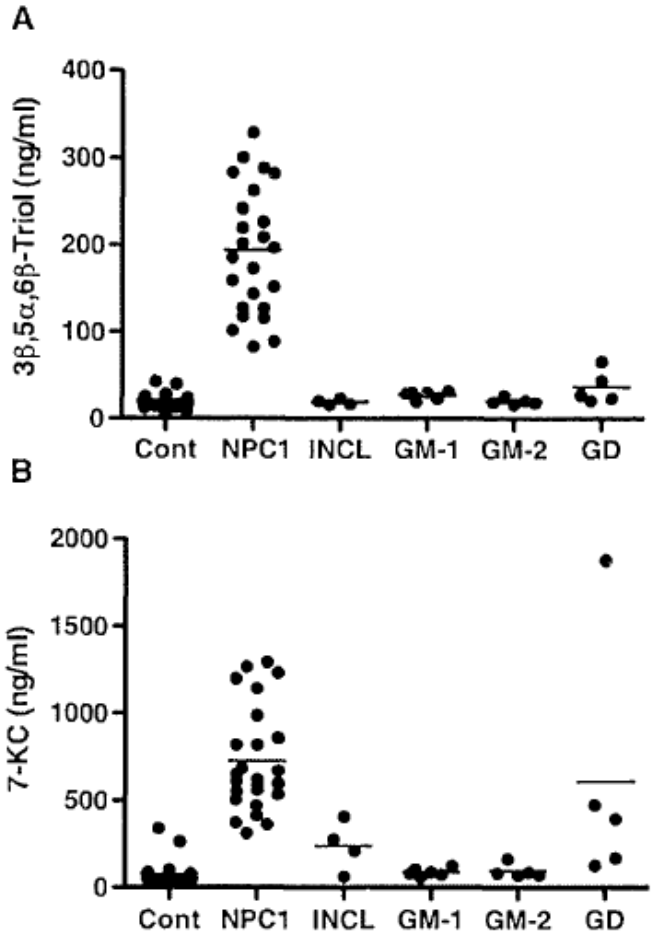


Fig. 6. Comparison of plasma oxysterol concentrations in NPC1 disease and other lysosomal storage diseases. (**A** to **C**) 3β,5α,6β-triol (A), 7-KC (B), and 24(*S*)-HC (C) concentrations in fasting plasma samples from control (Cont), NPC1, infantile neuronal ceroid lipofuscinosis (INCL), GM-1 gangliosidosis (GM-1), GM-2 gangliosidosis (GM-2), and Gaucher disease (GD) subjects. For 3β,5α,6β-triol, $P < 0.001$ for NPC1 versus INCL, GM-1, GM-2, and GD; for 7-KC, $P < 0.001$ for NPC1 versus GM-1 and GM-2, and $P < 0.01$ for NPC1 versus INCL.

Forbes D. Porter, *et al.*
Sci Transl Med **2**, 56ra81 (2010);

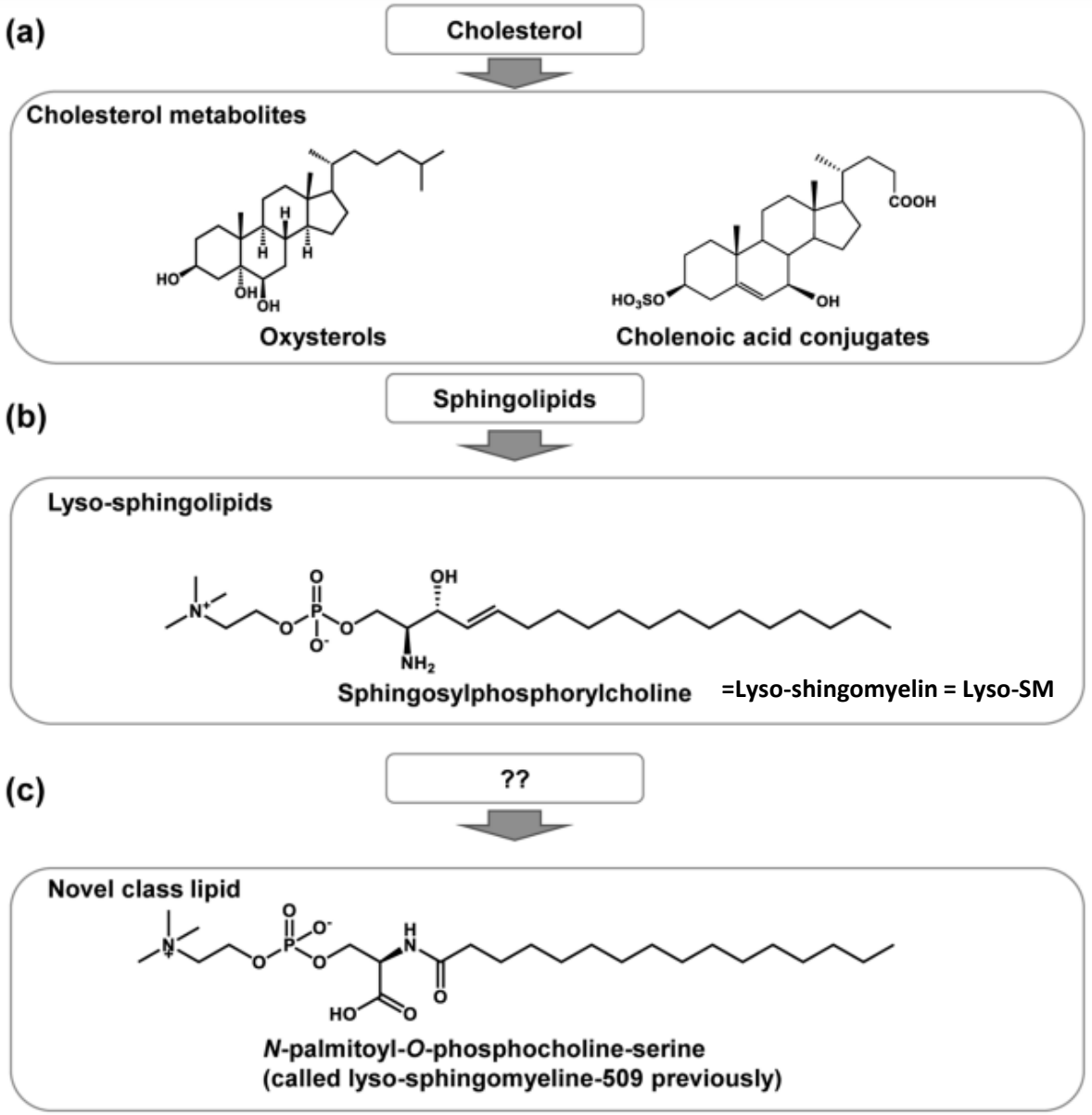


Fig. 2. Recently reported lipid metabolites as NPC biomarkers: (a) cholesterol metabolites: oxysterols, cholenoic acids, and the conjugates; (b) lyso-sphingolipids: sphingosylphosphorylcholine; (c) novel class lipid: *N*-palmitoyl-*O*-phosphocholine-serine (previously known as lyso-sphingomyeline-509).

Other NPC biomarkers

Bile acids are more sensitive and specific (also ↑ in NP-A/NP-B)

Lyso-SM and Lyso-SM-509 also ↑ in NP-A/NP-B, High lyso-SM-509/lyso-SM seems NPC-specific

Biomarkers are now first-line tests for NPC
Place of Filipin staining nowadays

Mass Spectrom (Tokyo) 2022; 11(1): A0111

Therapeutic options

Miglustat (FDA and EMA approved medication)

Other under investigation:

- *Cyclodextrin (mobilizes and reduces stored cholesterol in a dose-dependent manner independent of NPC1/2)*
- *Gene therapy*
- *N-acetyl-L-leucine (ataxia only)*
- *Other*

EXPERT OPINION ON PHARMACOTHERAPY
2023, VOL. 24, NO. 11, 1229–1247

Take home message

- *Ataxia, vertical gaze palsy, (hepato)splenomegaly: think of Niemann-Pick type C*
- *Adult NP-C can be very slowly progressive*
- *Psychiatric features can predominate (long diagnostic delay)*
- *Several rapid, non-invasive biomarkers available, but all have certain limitations in terms of sensitivity and specificity*

Abdominal swelling in a 27-year-old man

Clinical presentation

- 27-year-old man
- Referred to the adult inherited metabolic disease clinic with abdominal swelling
- Other issues
  - Early satiety
  - Recurrent respiratory infections
- On examination
  - Hepatomegaly – 3 cm below the costal border
  - Splenomegaly – extending beyond the umbilicus

Question

- What are the possible causes in an adult of hepatosplenomegaly?

Gaucher disease
Lysosomal acid lipase deficiency
Mucopolysaccharidosis (type 1)
Acid sphingomyelinase deficiency (former: Niemann-Pick type B disease)
Glycogen storage disorders

Chronic liver disease with portal hypertension
Haematological disease e.g. lymphoma, leukaemia, myeloproliferative, haemolytic anaemias
Infection e.g. viral hepatitis, EBV, CMV
Infiltration e.g. amyloidosis, sarcoidosis

Background

- First presented at the age of 3 years with abdominal swelling
- Gradually progressed – partial splenectomy at 15 years (1 kg of spleen removed!)
- Stable for about 7 years then increasing splenomegaly again
- No nose bleeds
- No easy bruising
- Prolonged bleeding after dental extraction
- Married, working full-time

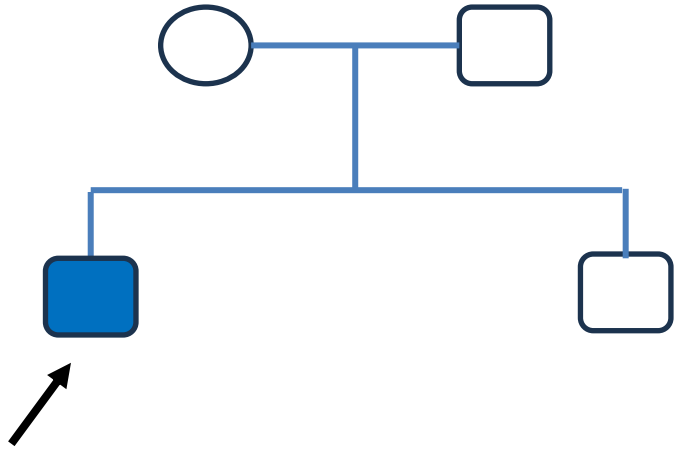
Question

- **What other information would you ask?**

Normal birth and development
Normal schooling – no education support

Medications:
Simvastatin 40 mg daily
Antibiotic prophylaxis

No alcohol, no smoking
No foreign travel, no fevers, no weight loss



Question

- **What investigations you would request?**

CT / MR imaging of the abdomen
CT imaging of the thorax
Pulmonary function tests
DEXA bone density scan

FBC, blood film
Renal, liver, bone profiles
Coagulation studies
Infection screen
Serum ACE
Enzyme activities – for the lysosomal storage disorders

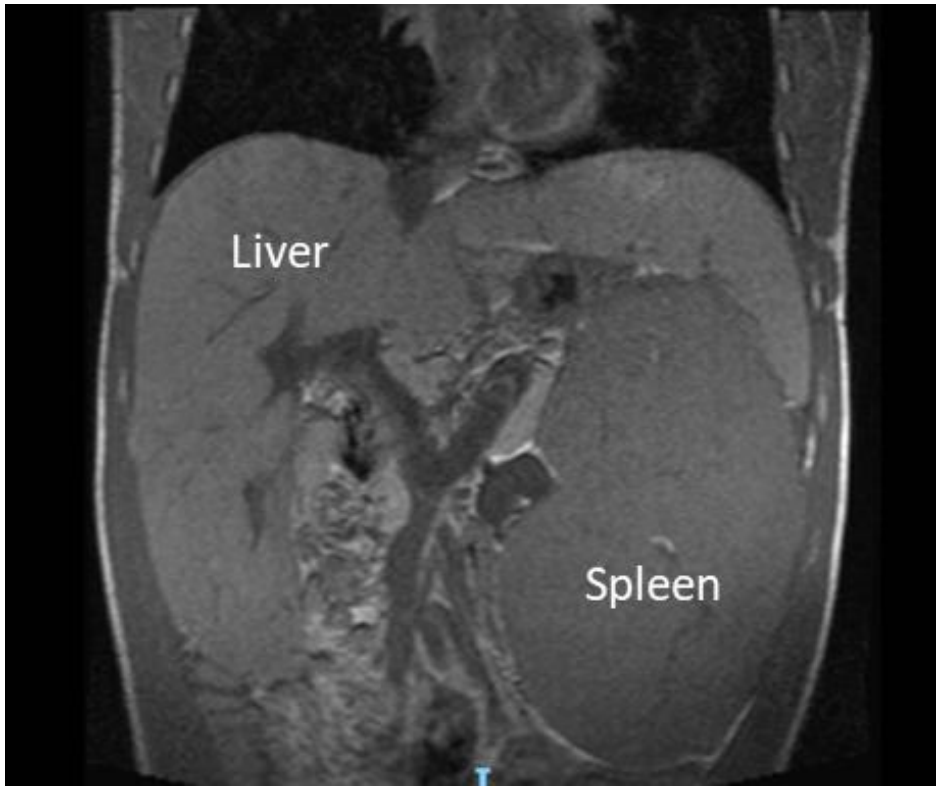
Imaging

CT Thorax

- Striking thickening of the interlobular septi with diffuse ground-glass shadowing.

MR abdomen

- The liver has a slightly irregular contour with relative hypertrophy of the left lobe and caudate in keeping with cirrhosis. There are also multiple bands of periportal fibrosis. The splenic size is 20 cm in length (normal 12- 13.5 cm), volume is 1800 mL (normal: 236 ± 77 mL). The spleen contains several likely Gamna-Gandy bodies.



Results: Discuss

	Result	Reference range
Hb	145	130-170 g/L
Platelets	**87**	150-400 X10^9/L
ALT	**84**	10-50 IU/L
ALP	87	40-129 IU/L
Total cholesterol	4.6	2.5-5.0 mmol/L
HDL-cholesterol	**0.3**	0.9-1.5 mmol/L
Triglycerides	**4.1**	0.4-2.3 mmol/L
TL_{CO} (Gas transfer) %predicted	**68 %**	100 %
Liver span	**21.6**	< 16 cm
Spleen span	**21.5**	< 14 cm

Question

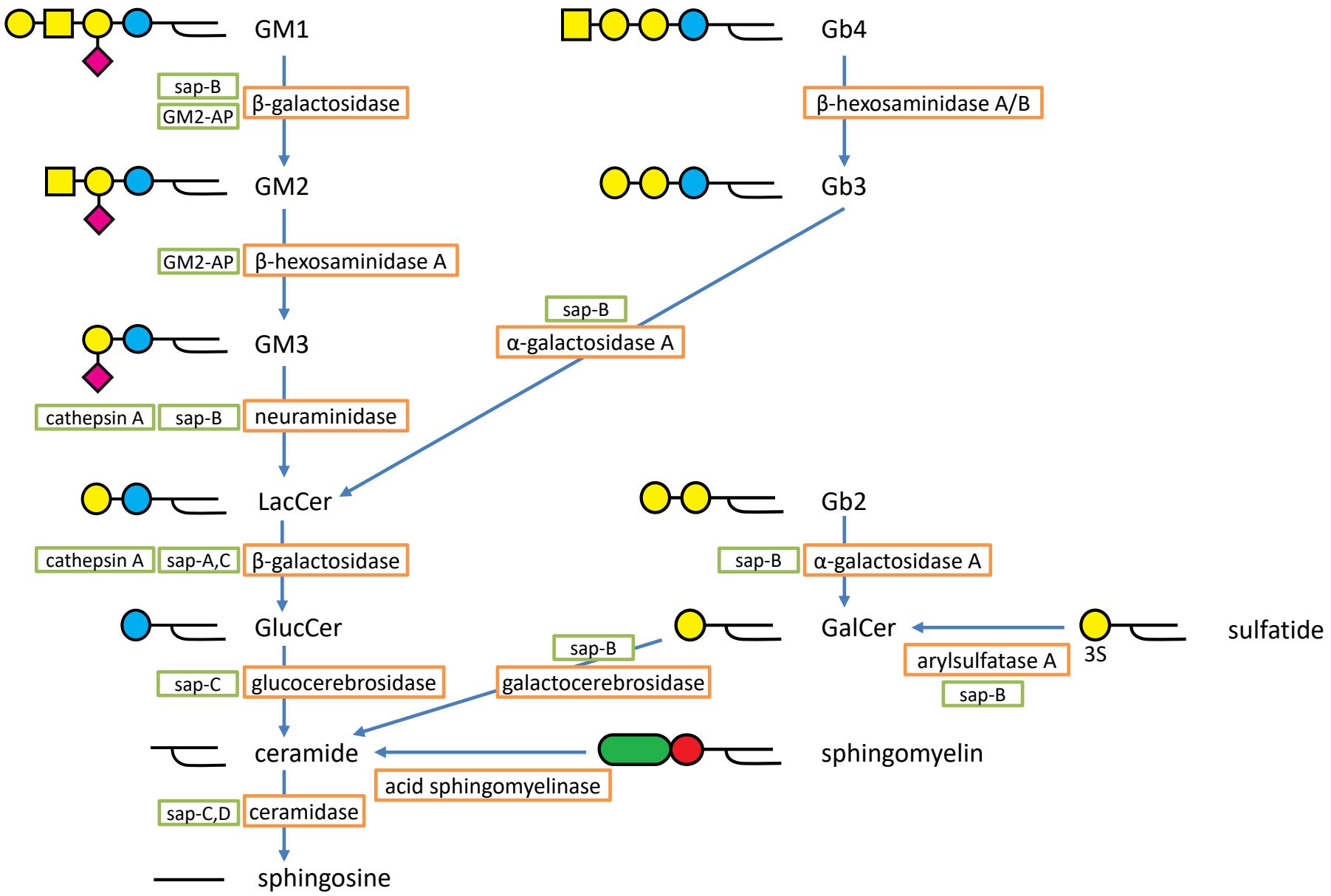
- What specific enzyme(s) would you like to measure?

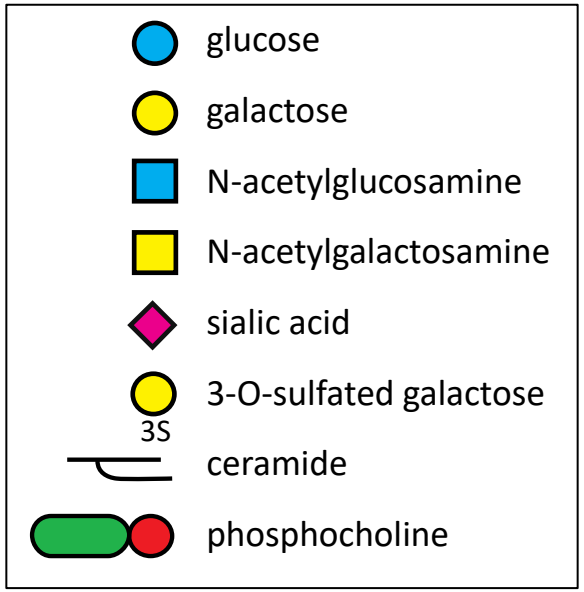
Acid sphingomyelinase activity **0.07 nmol/hr/mg ptn** (Normal: 0.86-2.8 nmol/hr/mg ptn)

Acid sphingomyelinase deficiency (ASMD, former: Niemann-Pick type B disease)

sphingolipid catabolism

with cofactors



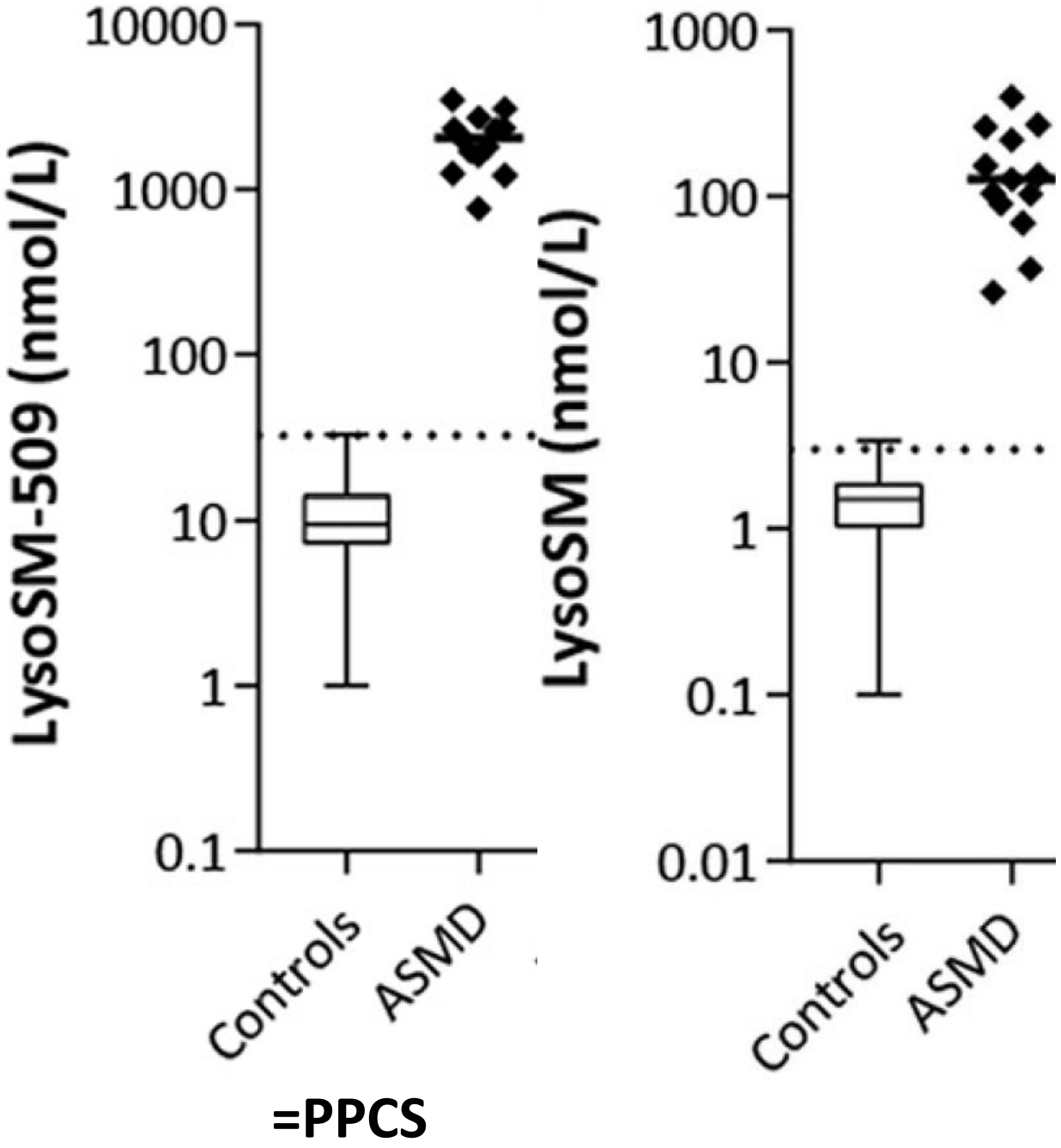


saposin deficiencies lead to various sphingolipidoses

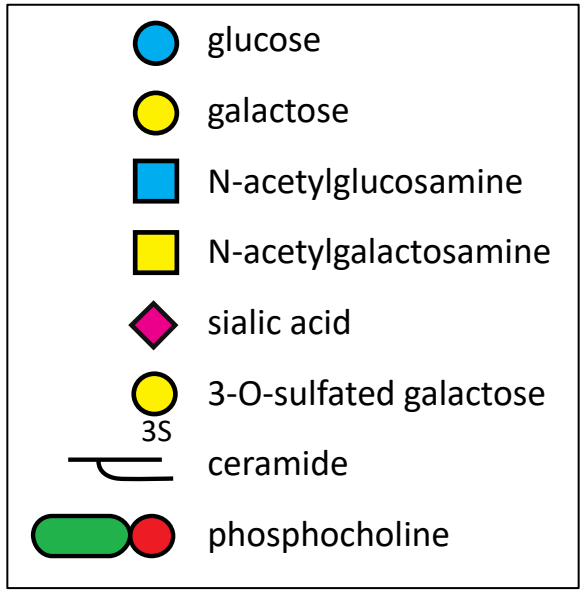
cathepsin A deficiency lead to galactosialidosis

Acid sphingomyelinase deficiency

Niemann-Pick A/B



=PPCS



- **cholesterol** also accumulates in the lysosome

Question

- *What treatment would you offer him?*

Olipudase alfa – intravenous enzyme replacement therapy (clinical trial from 2014) (Now funded in Germany, Austria, Italy, Cyprus and Hungary)

- What do you need to watch out for when starting therapy?

Risk of acute phase reaction-type reactions - elevated inflammatory biomarkers (C-reactive protein, interleukin-8, and calcitonin) and constitutional symptoms (fever, pain, nausea, and/or vomiting). Due to catabolism of accumulated sphingomyelin.

Need to titrate the dose upwards slowly over months.

Outcome

	Baseline	Most Recent	Reference range
Hb	145	155	130-170 g/L
Platelets	**87**	**114**	150-400 X10^9/L
ALT	84	23	10-50 IU/L
ALP	87	53	40-129 IU/L
Total cholesterol	4.6	4.0	2.5-5.0 mmol/L
HDL-cholesterol	**0.3**	**0.7**	0.9-1.5 mmol/L
Triglycerides	**4.1**	2.0	0.4-2.3 mmol/L
TL_{CO} (Gas transfer) %predicted	**68 %**	**89 %**	100 %
Liver span	**21.6**	13.9	< 16 cm
Spleen span	**21.5**	**14.7**	< 14 cm

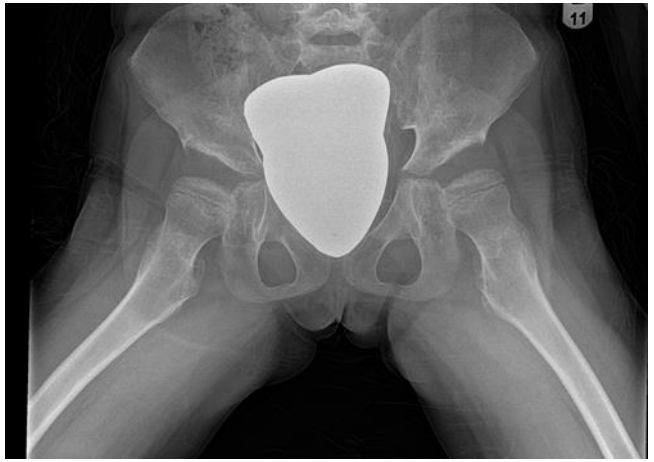
ASMD: Take home messages

- *Hepatosplenomegaly can be massive (hard to turn over in bed; bend down to tie shoelaces etc)*
- *Significant reversibility (of non-neurological disease) with olipudase alfa ERT*
- *Avoid total splenectomy as is associated with worsening of pulmonary involvement*
- *Continuum of phenotypes:*
  - severe early-onset infantile neurovisceral phenotype (Niemann-Pick disease type A, or NPD-A)
  - intermediate chronic neurovisceral phenotype (*former NPD-A*/B)
  - chronic visceral ASMD (*former NPD-B*)

Dysplastic hips

Workshop 3, Case 5.

History



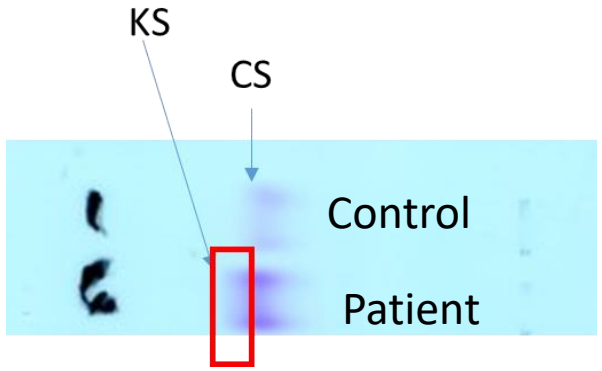
- *8 year old female*
  - *18 month history of leg/knee/hip pain*
  - *Developed "waddling" gait*
  - *Normal height/ weight centiles (50th)*
  - *Pelvic X-ray arranged by General Practitioner:*
    - *"femoral capital epiphyses are small, flattened and slightly sclerotic bilaterally."*
    - *Suspected to have "**Multiple Epiphyseal Dysplasia**"*
    - *MED Genetic panel initiated:*
      - *no variants identified*

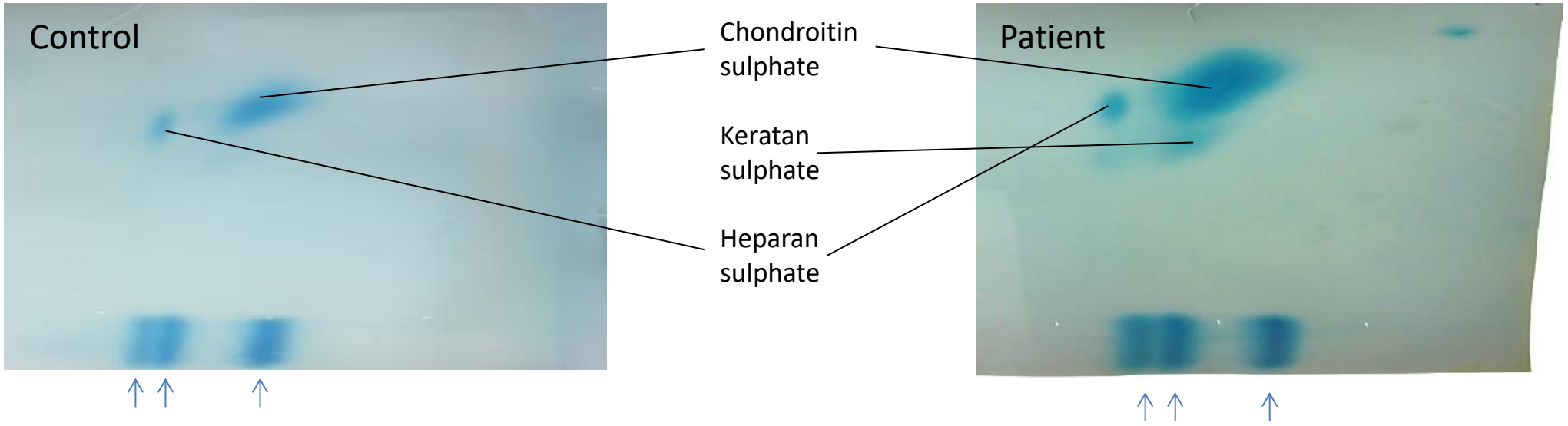
COMP	EDM1	Cartilage oligomeric matrix protein
COL9A2	EDM2	Collagen type IX A2
COL9A3	EDM3	Collagen type IX A3
SLC26A2 (DTDST)	EDM4	Diastrophic dysplasia sulfate transporter
MATN3	EDM5	Matrilin 3
COL9A1	EDM6	Collagen type IX A1

What other tests might you do?

Urine GAG

- *Urine glycosaminoglycans:*
  - *GAG/creatinine 19 mg/mmol (reference 2-12)*
- *Glycosaminoglycans 1D and 2D electrophoresis:*

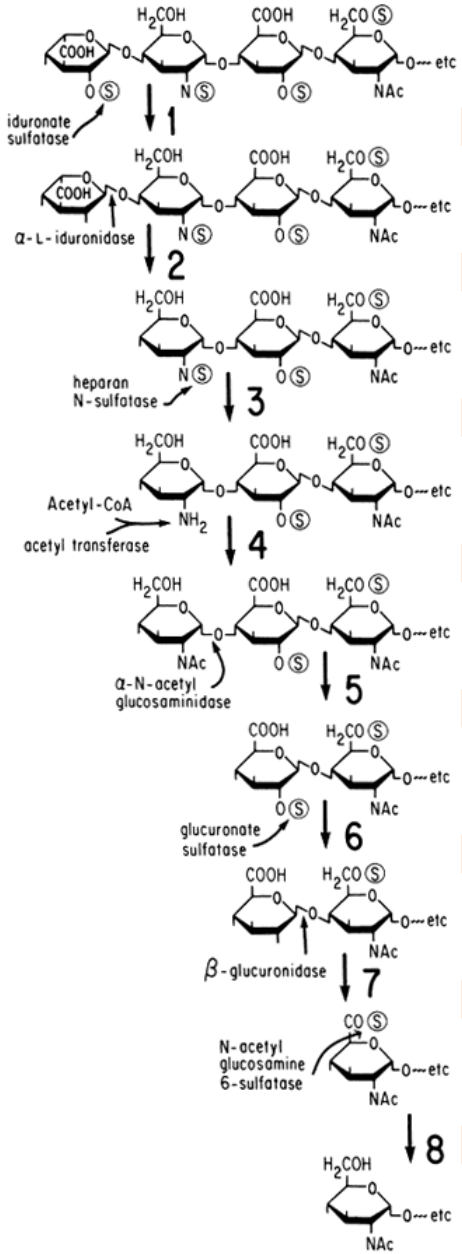




What diagnosis do you consider and how will you confirm this?

MPS IV: Morquio

heparansulfate (HS)



Iduronate sulfatase

α-L-iduronidase

heparan-N-sulfatase

acetyl-CoA glucosaminide acetyltransferase

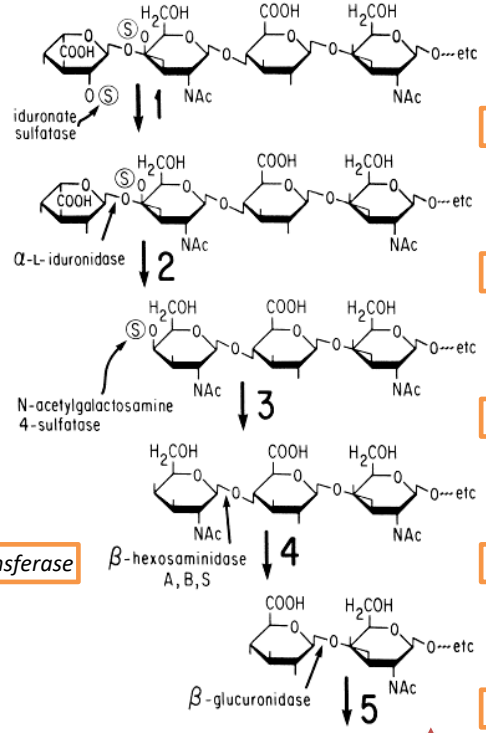
α-N-acetylglucosaminidase

glucuronate sulfatase

β-glucuronidase

N-acetylglucosamine-6-sulfatase

dermatansulfate (DS)



Iduronate sulfatase

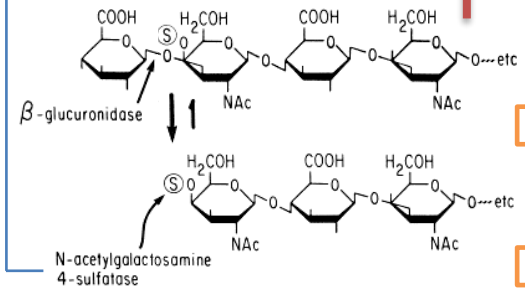
α-L-iduronidase

N-acetylgalactosamine-4-sulfatase

β-hexosaminidase A/B

β-glucuronidase

chondroitin sulfate (CS)



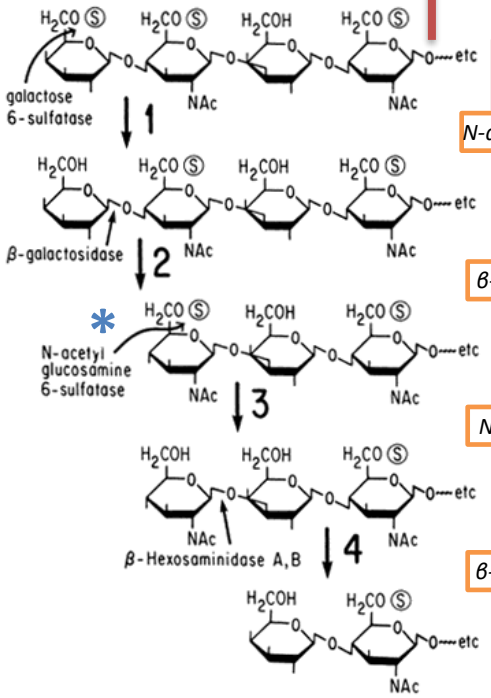
β-glucuronidase

N-acetylgalactosamine-4-sulfatase

~~*N-acetylgalactosamine-6-sulfatase*~~

MPS IV A Morquio A *GALNS*

keratansulfate (KS)



MPS IV A Morquio A *GALNS*

~~*N-acetylgalactosamine 6 sulfatase*~~

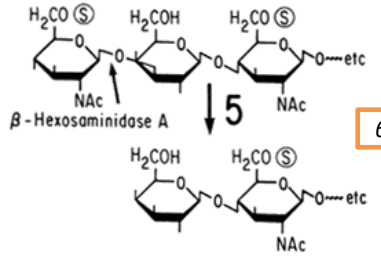
MPS IV B Morquio B *GLB1*

~~*β-galactosidase*~~

N-acetylglucosamine-6-sulfatase

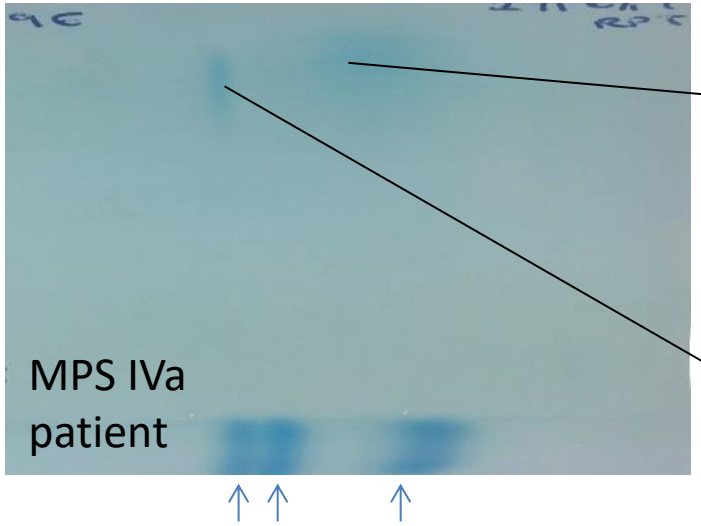
β-hexosaminidase A,B

*** alternate pathway**



β-hexosaminidase A

NB: Keratan sulphate can be difficult to detect



Chondroitin sulphate

No keratan sulphate!

Heparan sulphate

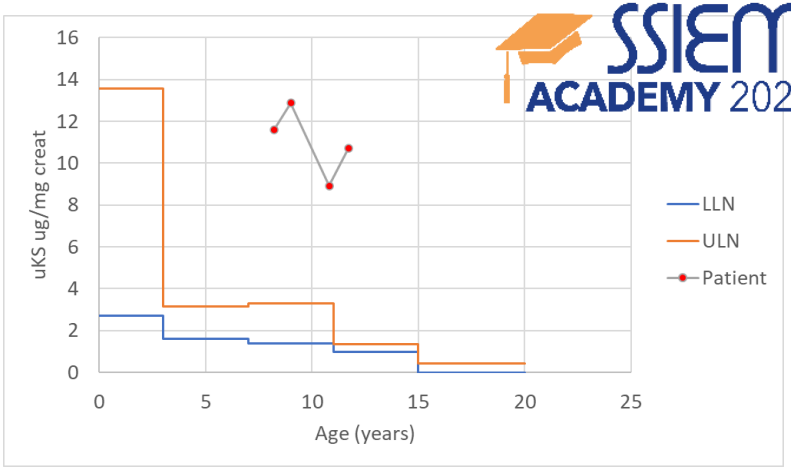
So what tests you do next?

Fig. 5 Challenging to interpret urinary GAG 2D electrophoresis results from MPS IVA patients illustrating the difficulty in discerning the presence of keratan sulfate. All patients were subsequently confirmed to have MPS IVA by enzyme activity analysis

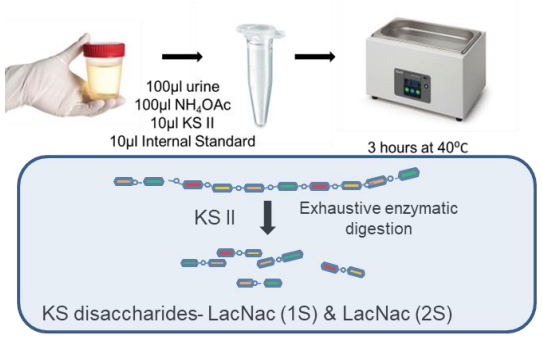
J Inherit Metab Dis 2013;36:293-307

- ***Quantitative** urine keratan sulfate (mass spec)*
  - *uKS **11.59** µg/mg Creatinine*
    - *Age-related reference ranges:*

0-3 years	*2.7 - 13.57*
3-7 years	*1.62 - 3.13*
7-11 years	*1.37 - 3.31*
11-15 years	*0.99 - 1.33*
> 15 years	*< 0.41*

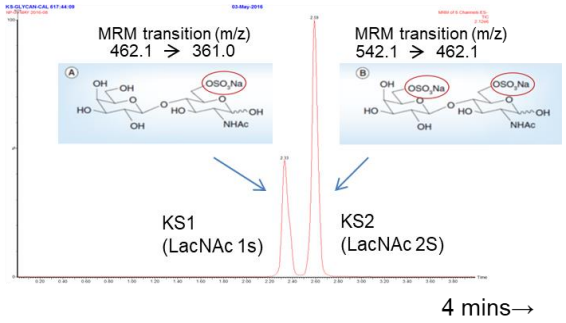


- ***Enzyme assay:** Leucocyte galactose-6-sulphatase*
  - *1.0 nmol/hr/mg protein (3.9-45.9)*
    - *"Lower than reference interval but not as low as usually seen in Morquio A"*
    - *B-galactosidase control enzyme within normal range, not Morquio B*
    - *(NB MPS IVb: may have abnormalities on urine oligosaccharides)*



- ***Molecular genetics:** GALNS sequencing*
  - *Single heterozygous pathogenic variant identified*

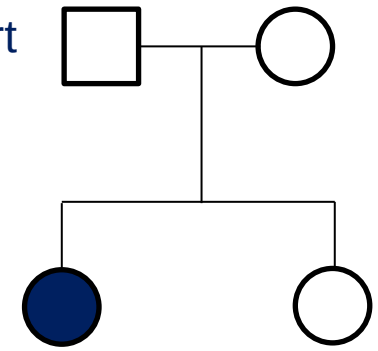
- ***What could you do next?***



Pedigree studies

GALNS wt/wt
Enzyme: 22 nmol/hr/mg prt
uGAG/Cr 2 mg/mmol
uKS 0.18 µg/mg Creat

GALNS wt/path
Enzyme: 28 nmol/hr/mg prt
uGAG 3 mg/mmol
uKS 0.66 µg/mg Creat



GALNS wt/path
Enzyme: 1.0 nmol/hr/mg prt
uGAG 19 mg/mmol
uKS 11.59 µg/mg Creat

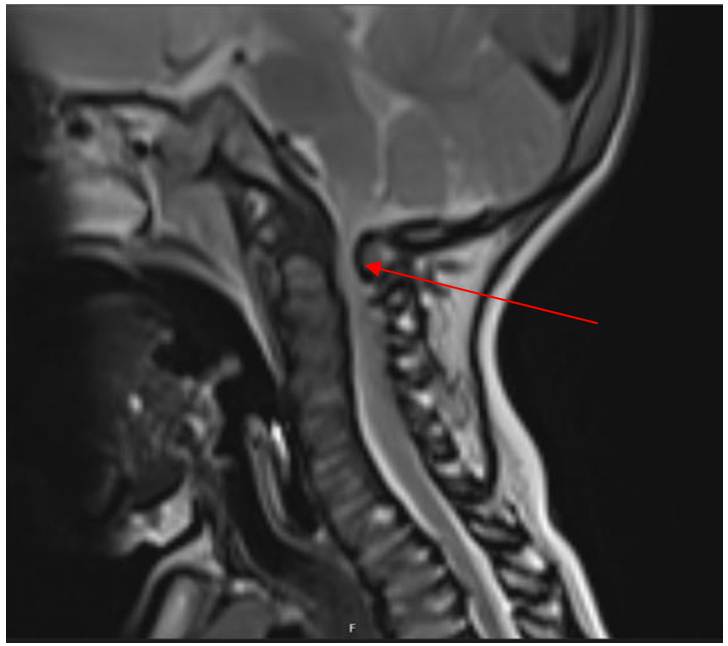
GALNS wt/path
Enzyme: 0.4 nmol/hr/mg prt
uGAG 19 mg/mmol
uKS 14.62 µg/mg Creat

- **Presumed unidentified paternally-inherited second *GALNS* variant (eg intronic)**
- **Sisters both have "attenuated" MPS IVa phenotype**

What further clinical evaluations and assessments would you do?

Multisystem evaluation

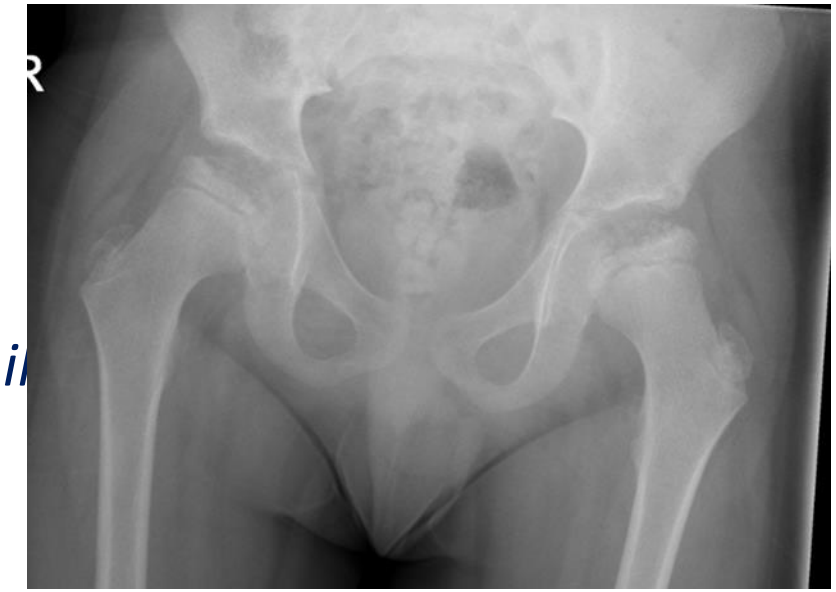
- *Skeletal imaging*
- *C-spine X-ray, MRI*
- *Cardiac evaluation*
- *Respiratory status*
- *Neurological evaluation*
- *Growth*
- *ENT*
- *Ophthalmological*
- *Anaesthetic assessment…*



Prominent craniovertebral stenosis

Akyol MU et al. Recommendations for the management of MPS IVA: systematic evidence- and consensus-based guidance. Orphanet J Rare Dis 2019;14:137.

History (v2)



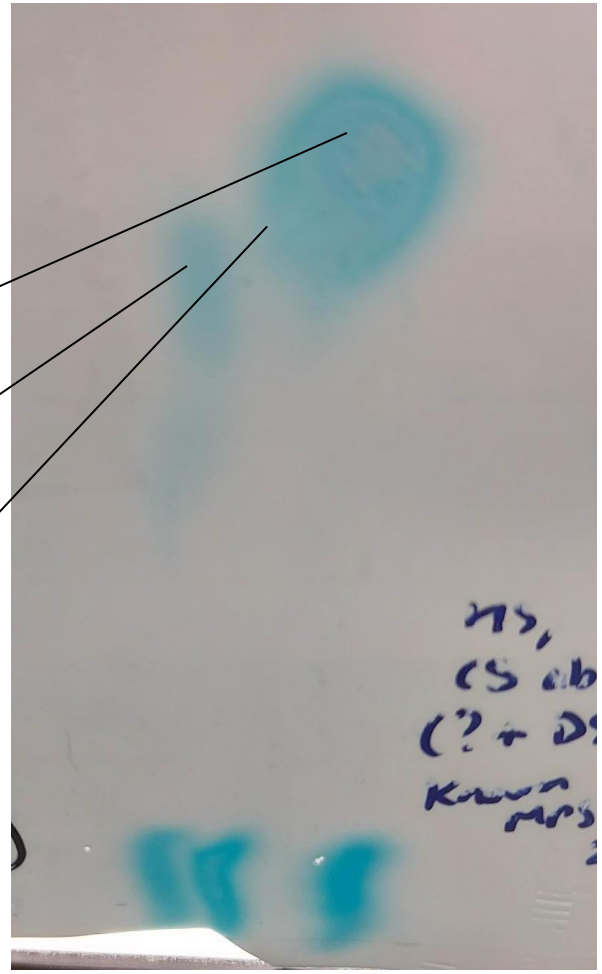
- *8 year male*
- *4 year history of progressive hip pain, waddling gait*
- *Imaging shows femoral head epiphyseal dysplasia, mi spinal changes.*

- *Urine GAG 36mg/mmol creatinine (0-24)*
- *1D GAG electrophoresis normal and "MPS excluded"*

- ***What would you do next?***

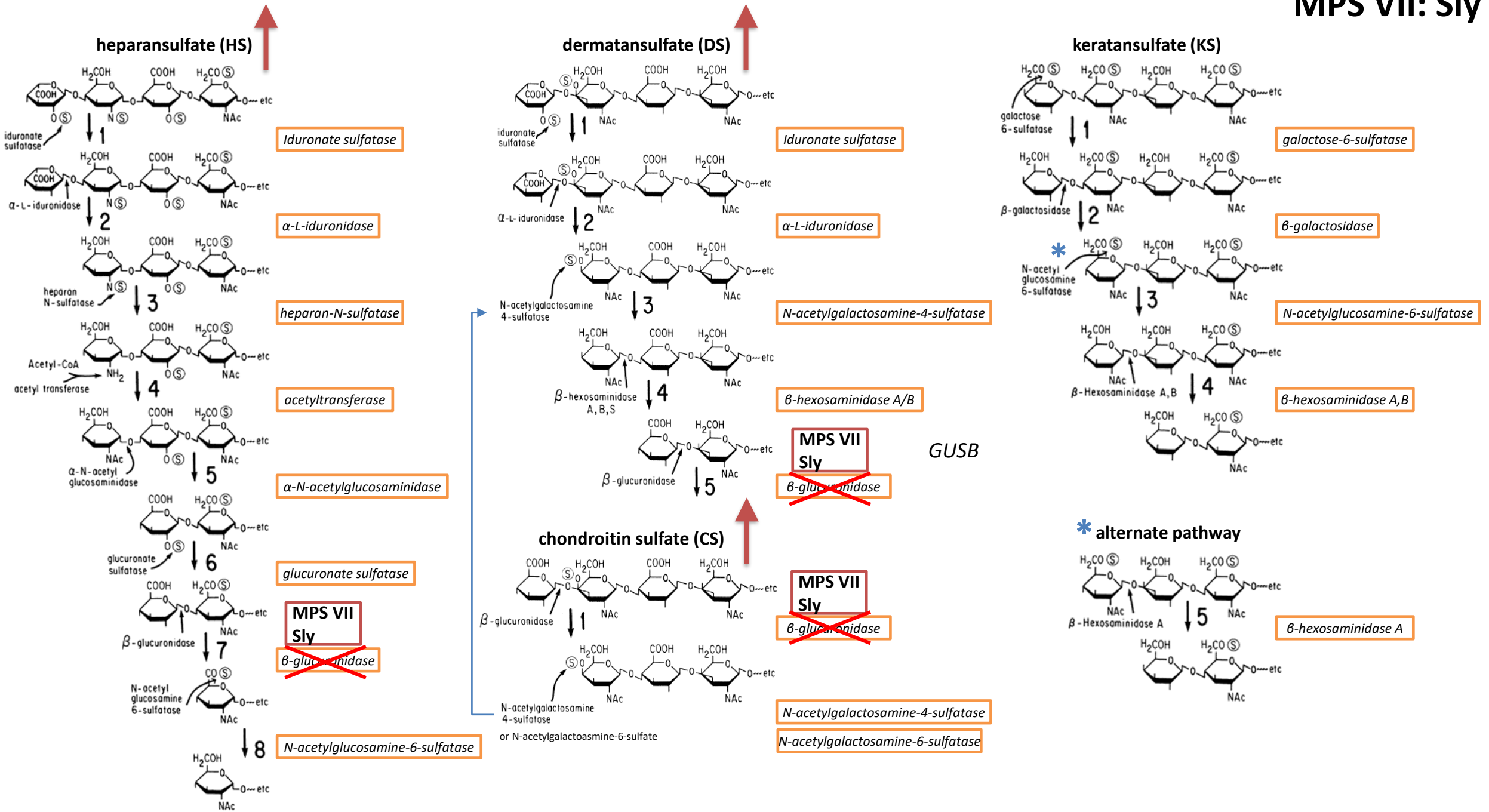
Investigations

- *Trio WGS*
  - *compound heterozygous GUSB pathogenic variants*
- *2D GAG electrophoresis: ABNORMAL*
  - *Dermatan sulphate*
  - *Increased chondroitin sulphate*
- *Leucocyte β-glucuronidase 15 nmol/hr/mg ptn (250-813)*
- *Plasma β-glucuronidase undetectable*
- ***Diagnosis: MPS VII (Sly Syndrome)***



Don't ignore elevated quantitative GAG
Careful interpretation of 2D GAG electrophoresis required

MPS VII: Sly


heparansulfate (HS)
Iduronate sulfatase
α-L-iduronidase
heparan-N-sulfatase
acetyltransferase
α-N-acetylglucosaminidase
glucuronate sulfatase
MPS VII Sly
β-glucuronidase
N-acetylglucosamine-6-sulfatase
dermatansulfate (DS)
Iduronate sulfatase
α-L-iduronidase
N-acetylgalactosamine-4-sulfatase
β-hexosaminidase A/B
MPS VII Sly
β-glucuronidase
GUSB
chondroitin sulfate (CS)
MPS VII Sly
β-glucuronidase
N-acetylgalactosamine-4-sulfatase
or N-acetylgalactoasmine-6-sulfate
N-acetylgalactosamine-6-sulfatase
keratansulfate (KS)
galactose-6-sulfatase
β-galactosidase
N-acetylglucosamine-6-sulfatase
β-hexosaminidase A,B
* alternate pathway
β-hexosaminidase A

Take Home Message

- *Wide phenotype spectrum in MPS IVa and MPS VII*
  - *Attenuated/ later-presenting forms with hip dysplasia*
  - *Fetal hydrops frequent in MPS VII*
  - *All MPS disorders can have attenatued phenotype – relating to residual enzyme activity.*

- *Careful interpretation of urine glycosaminoglycans needed to avoid missed diagnosis*
  - *Keratan sulphate hard to detect*
  - *Quantification and GAG species determination required*
  - *2D electrophoresis and/or quantitative mass spec techniques optimal*

Baby boy with hepatosplenomegaly

Background

- Boy born at term, uncomplicated pregnancy
- Multiple incidents with cyanosis and vomiting, frequent diarrhea
- At 2 months of age re-evaluation because of frequent vomiting and dystrophy
- Physical exam: hepatosplenomegaly (both +6 cm)
- Echo: adrenal calcifications

Which laboratory investigations do you consider?

First-line investigations

Test	Result	Ref range
Full blood count	Hb: 5.1 MCV: 71 WBC: 10.8 PLT: 307	5.9-8.4 mmol/L 80-105 fL 5-23 10E9/L 150-350 10E9/L
Clotting screen	PT: 14.7 sec aPTT: normal	9-12 sec
LDH	2056	<400 U/L
CRP	95	<5 mg/L
Ferritin	>13000	<400 ug/L

Test	Result	Ref range
Triglycerides	4.56	0.59 – 2.63 mmol/L
Cholesterol	4.2	<5.3 mmol/L
Bilirubin	11	<21 µmol/L
Alkaline phosphatase	192	142 – 335 IU/L
Alanine aminotransferase Aspartate aminotransferase	29 224	<40 IU/L <40 IU/L

What further investigations would you suggest?

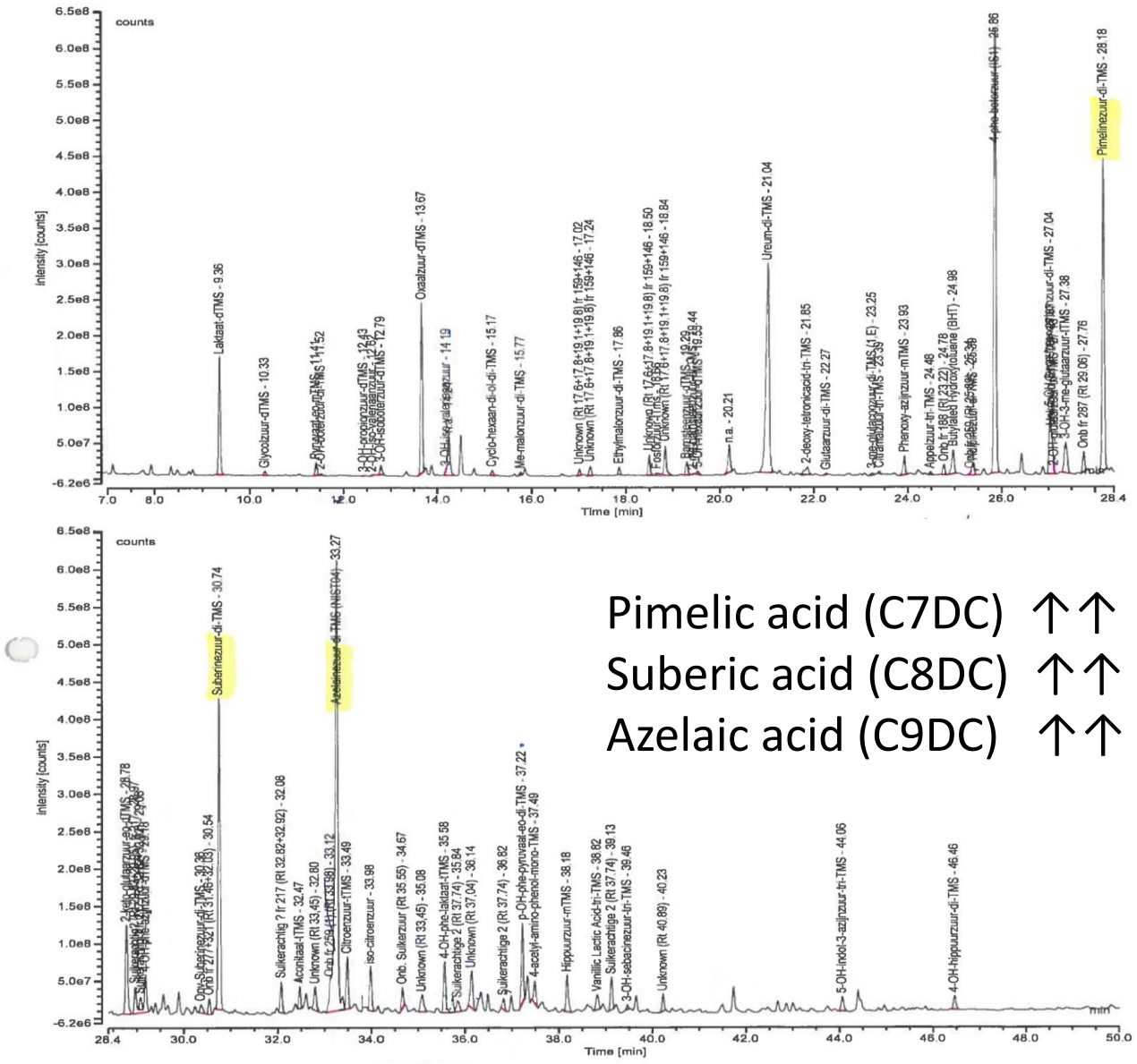
Urinary organic acids: *see figure*

Plasma amino acids: *normal*

Plasma chitotriosidase: *1187*

(5-97 nmol/h/ml)

What are the possible diagnoses?
Any further investigations?



Pimelic acid (C7DC) ↑↑
Suberic acid (C8DC) ↑↑
Azelaic acid (C9DC) ↑↑

Figure 1. First urine organic acid chromatogram of the patient revealing the three dicarboxylic acids; pimelic acid (at 28.18 min), suberic acid (at 30.74 min) and azelaic acid (at 33.27 min).

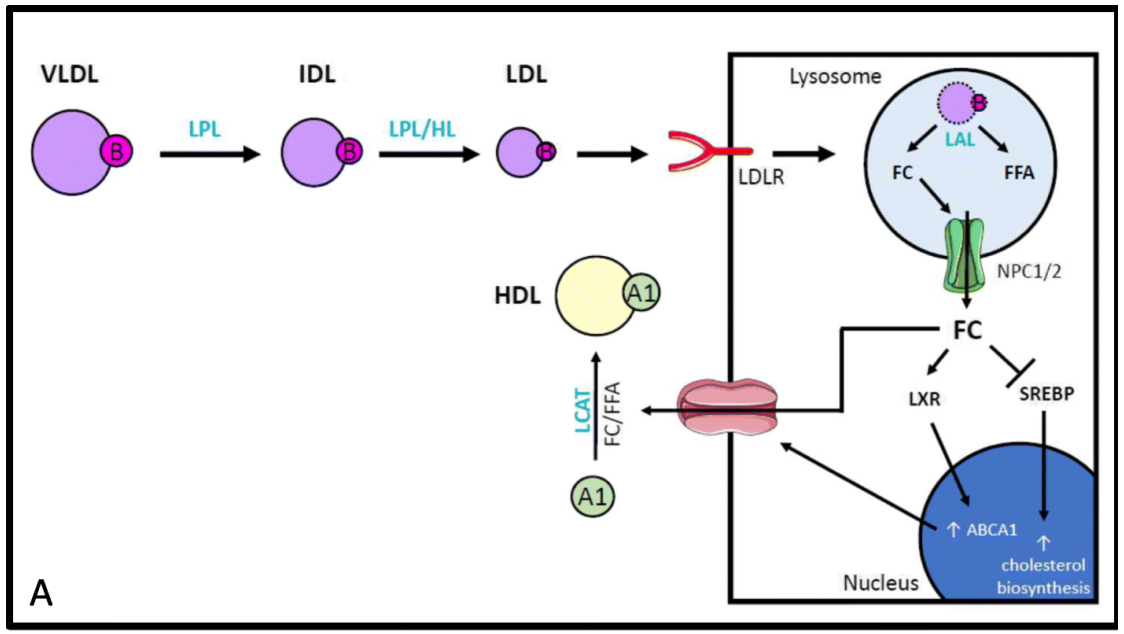
Further tests

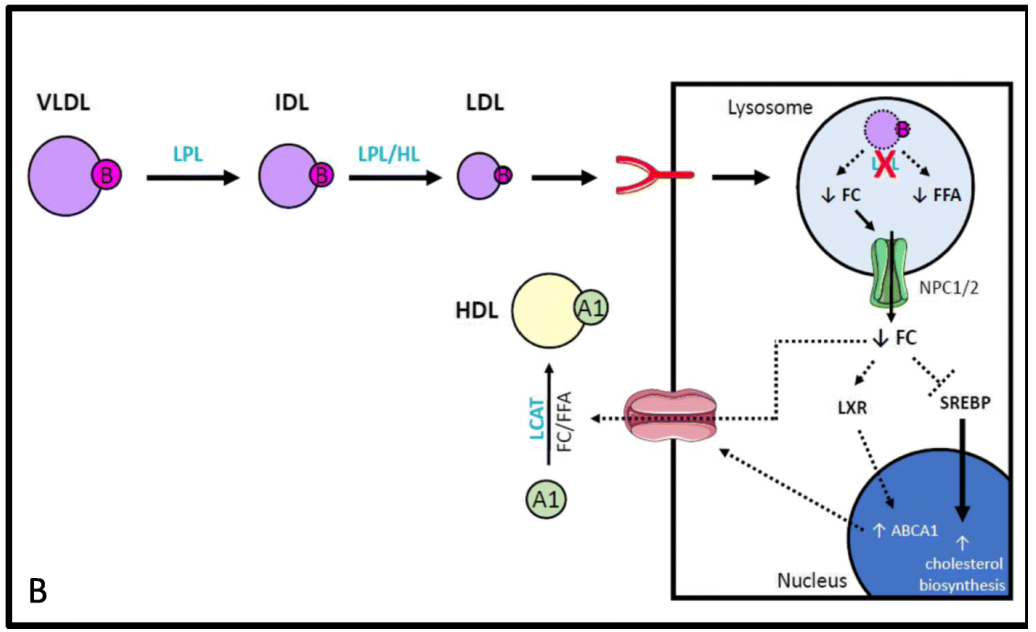
- Leukocyte lysosomal acid lipase: 24 nmol/h/mg protein
  (ref 287-855, residual activity 3%)

  => Lysosomal acid lipase deficiency (Wolman Disease)

- Genetic analysis:
  Homozygous pathological variant in *LIPA*: c.894+1G>A splice mutation

Biochemistry of LAL





a) LAL hydrolyzes the cholesteryl esters and triglycerides in LDL to FFA and Free Cholesterol. FC prevents SREBP pathway activation, thereby decreasing de novo cholesterol biosynthesis.

b) Decreased FC generation results in increased SREBP pathway activation, which increases de novo cholesterol biosynthesis. Accumulation of the FC and FFAs in the lysosome causes hepatic, adrenal, and intestinal toxicity.

Therapeutic options

- *Enzyme replacement therapy*
  - *Parenteral nutrition frequently needed initially*
  - *Sebelipase alfa i.v.*
  - *Diet with fat restriction once tolerated*
  - *Risk of ERT-neutralizing anti-drug antibodies*
- *Allogenic stem cell transplantation*
  - *Risk due to underlying liver disease and developmental delay*

Adult slide

- LAL deficiency causes cholesterol ester storage disease
- Presents late childhood or adulthood
- Slow disease progression
- Features
    - Hepatosplenomegaly
    - Premature atherosclerosis
    - Hypercholesterolaemia (↑LDL-C, ↓HDL-C, normal TG)
- Enzyme replacement therapy

Take-home messages

- *LIPA* variants cause lysosomal acid lipase deficiency
- Spectrum of disease severity:
  - Wolman disease – severe neonatal presentation
  - CESD (cholesterol ester storage disease) – late-onset, milder disease
- Suspect LAL deficiency in patients with
  - Hepatosplenomegaly with ↑transaminases
  - ↑LDL-C, ↓HDL-C
- Lipids may be normal/variable in Wolman disease
- ↑ C7DC – C9DC reported on organic acids ?cause