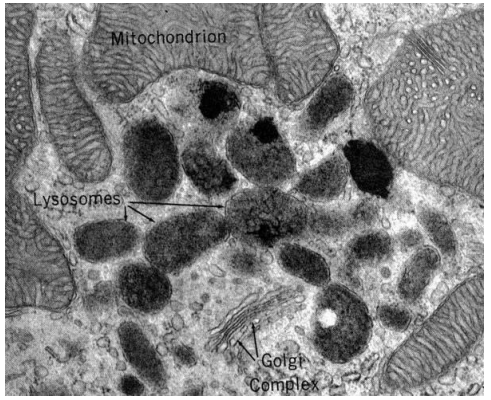
Lysosomal Storage Disorders

Apolline Imbard / James Davison

SSIEM Academy 2024, Amsterdam

The lysosomes

- Ubiquitous intracytoplasmic organelles
- Present in all animal cells (except red blood cells)
- heterogeneous in size (0.2-0.5 µm), morphology, location and number (50 to 1000/cell)
- Discovered in 1955 by C. de Duve, Nobel Prize in 1974



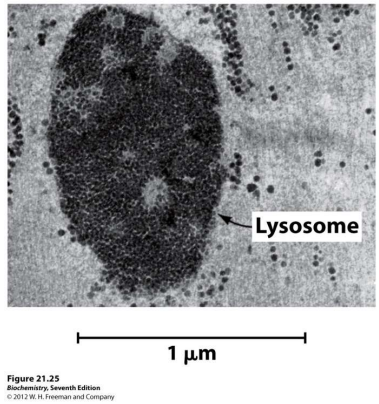
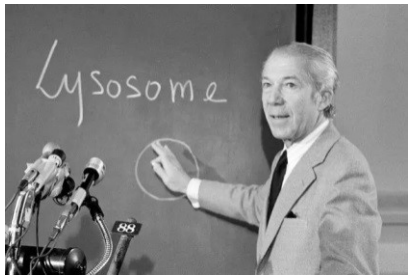


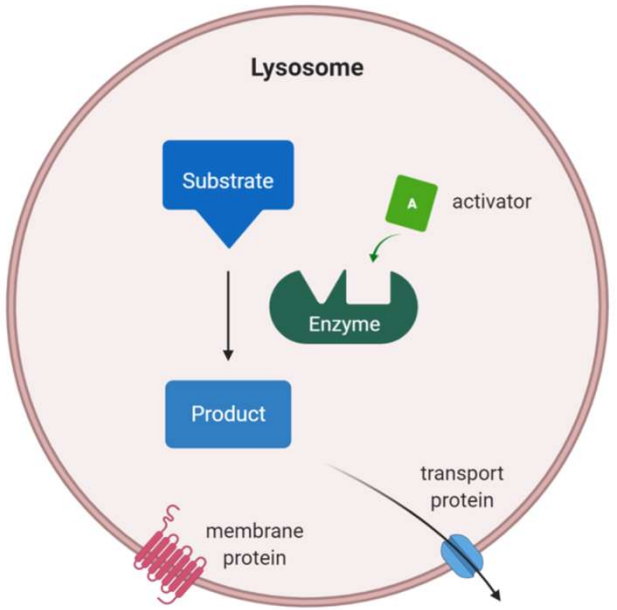
Figure 21.25
Biochemistry, Seventh Edition



The lysosomes

- Degradation and recycling of macromolecules of intra- or extra-cellular origin
- --> continuous turnover of cellular material

- Through the action of some 60 hydrolases active at acidic pH (5.2 to 5.5).



The lysosomes

Lysosomal membrane/membranes associated proteins

- ATP-dependent proton pump (vacuolar-ATPase = v-ATPase)
→maintains intra-luminal acid pH

- LAMP proteins (Lysosome-Associated Membrane Proteins)
→ protection against hydrolases, transport of macromolecules, membrane lipids and ions, interaction with other membranes.

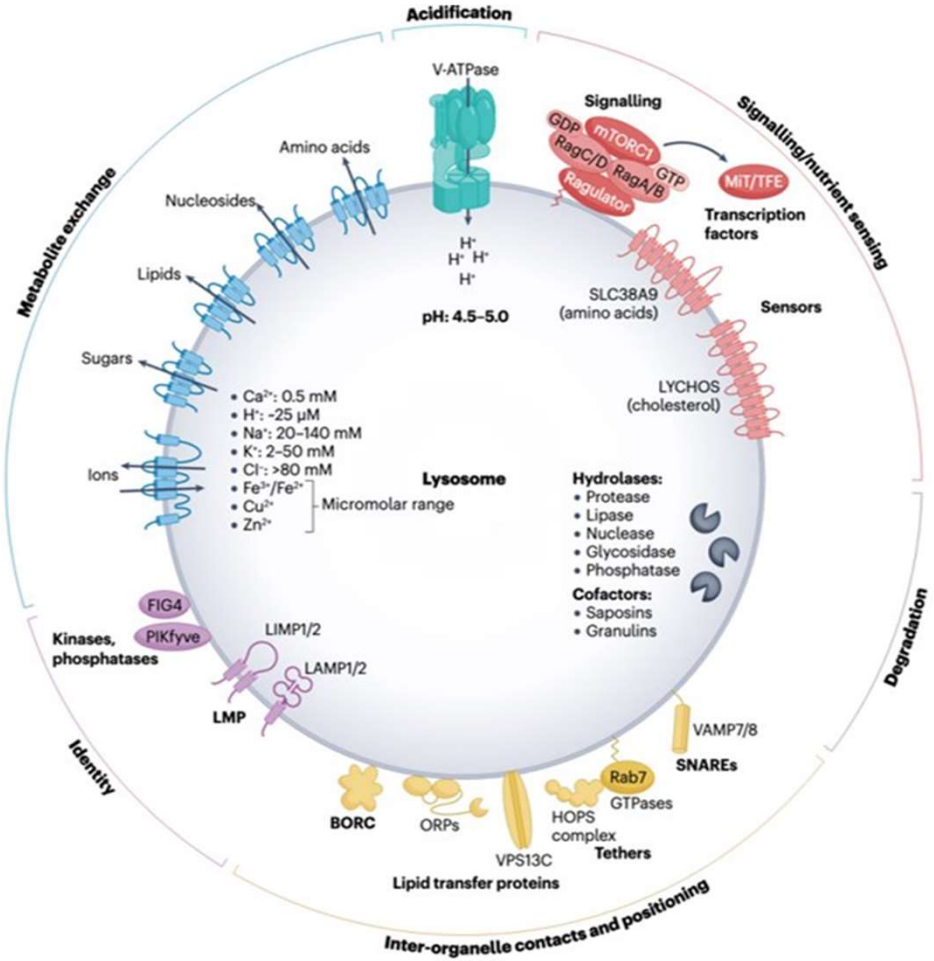
- LIMP (Lysosome-Integral Membrane Proteins) cytosol-facing proteins
→ protein-protein interactions involved in the integration of cellular signals.

- Transporters and channels

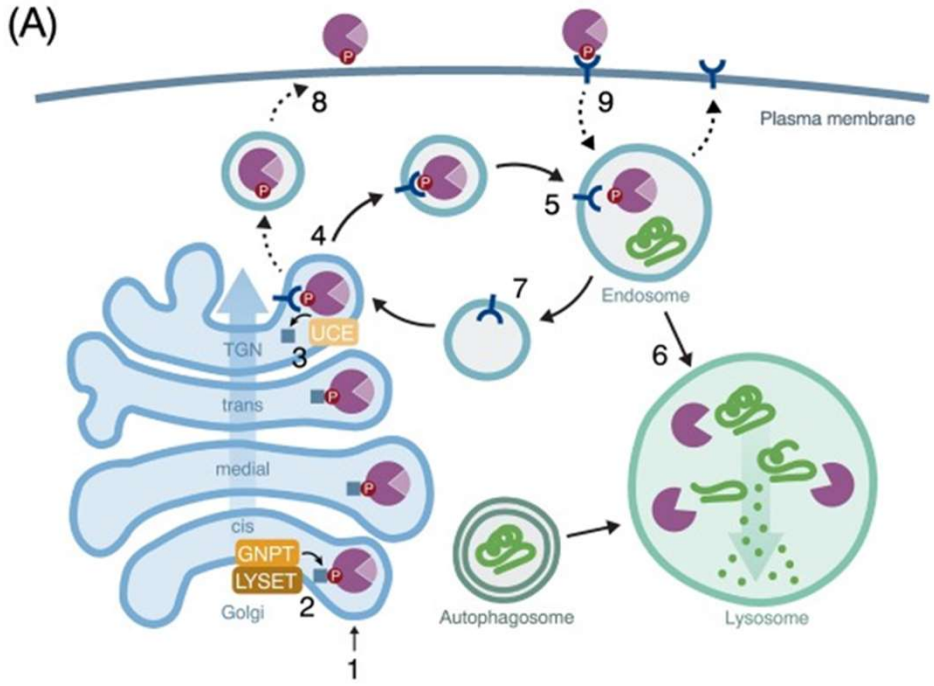
Lysosomal Internal proteins (intra-luminal)

- Acid hydrolases (>60 different)
- Lipases: break down complex lipids
- Glycosidases: break down complex carbohydrates
- Proteases: break down proteins
- Peptidases: break down tripeptides, dipeptides
- Nucleases: degrade nucleic acids
- Enzyme activators and protective factors
- Binding and transport proteins

Settembre et al. 2024, nature reviews molecular cell biology


Acidification
V-ATPase
H+ H+ H+
pH: 4.5–5.0
Signalling
GDP
mTORC1
RagC/D
RagA/B
GTP
Ragulator
MiT/TFE
Transcription factors
Signalling/nutrient sensing
Amino acids
Nucleosides
Lipids
Sugars
Ions
SLC38A9 (amino acids)
Sensors
LYCHOS (cholesterol)
Metabolite exchange
• Ca2+: 0.5 mM
• H+: ~25 µM
• Na+: 20–140 mM
• K+: 2–50 mM
• Cl−: >80 mM
• Fe3+/Fe2+
• Cu2+
• Zn2+
Micromolar range
Lysosome
Hydrolases:
• Protease
• Lipase
• Nuclease
• Glycosidase
• Phosphatase
Cofactors:
• Saposins
• Granulins
Degradation
FIG4
PIKfyve
Kinases, phosphatases
LIMP1/2
LAMP1/2
LMP
VAMP7/8
SNAREs
Rab7
GTPases
HOPS complex
Tethers
BORC
ORPs
VPS13C
Lipid transfer proteins
Identity
Inter-organelle contacts and positioning

The lysosomes



- Majority of lysosomal protein use the M6P pathway to enter the lysosome:
- Newly synthesized lysosome hydrolases are modified with M6P= sorting signal
- N-acetylglucosamine-phosphate transferase (GNPT) catalyses the addition of M6P to lysosome hydrolases
- M6P-modified hydrolases are recognized by the M6P receptors and transported by the endosomal system for delivery to the lumen of the lysosome

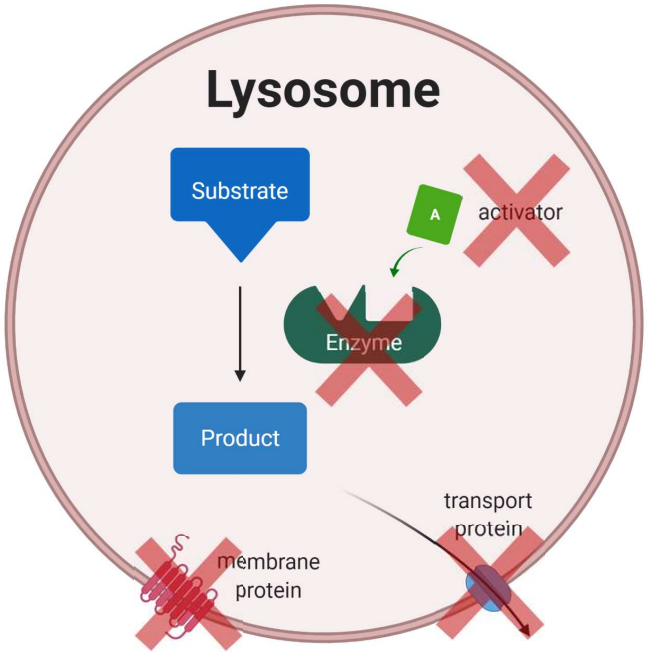
- Not all hydrolases follow this route: eg β-Glucocerebrosidase is transported to the lysosome in an M6PR-independent manner facilitated by lysosomal integral membrane protein 2 (LIMP2)

Braulke et al., Trends Cell Biol, 2024

Lysosomal storage disorder (LSD): Definition

- Any disease caused by a **genetic** deficiency of **lysosomal function**
- **Lysosomal enzyme** ++, transporter, cofactor…

- Accumulation of substrate within the lysosome



Lysosomal storage disorder (LSD): Classification

Lipidosis

Fabry
Gaucher
GM1 gangliosidosis (Landing)
GM2 gangliosidosis (Tay-Sachs, Sandhoff)
Niemann-Pick A/B
Niemann-Pick C
Wolman
Farber
Krabbe
Metachromatic leucodystrophy
Austin

Mucolipidoses (ML)

I cell disease (ML II)
ML III

Mucopolysaccharidosis (MPS)

Hurler-Scheie (MPS I)
Hunter (MPS II)
Sanfilippo A-D (MPS III)
Morquio A-B (MPS IV)
Maroteaux-Lamy (MPS VI)
Sly (MPS VII)

Oligosaccharidosis

Aspartylglucosaminuria
Fucosidosis
Alpha-mannosidosis
Bêta-mannosidose
Sialidosis & galactosialidosis
Schindler-Kanzaki

Transporter deficiency

Cystinosis
Salla
Danon

Ceroid-lipofuscinosis

Glycogen storage disease

Pompe

Other

Pycnodysostosis
Chediak-Higashi
Papillon-Lefèbvre…

Lysosomal storage disorder (LSD): diagnostic

1- Search for overload cells

Peripheral blood or bone marrow smear

2- Accumulated substrates (urine and plasma)

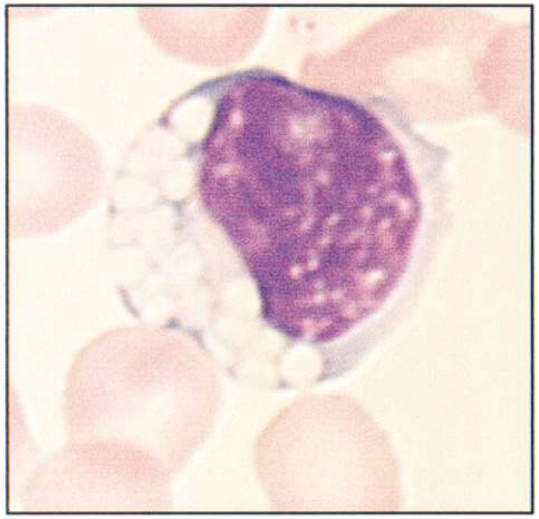
- Non-specific (group diagnosis): urinary mucopolysaccharides, urinary oligosaccharides
- Specific: sulfatides (MLD), lysosphingolipids (lipidosis) including lysoGb3 (Fabry), oxysterols (NPC)
- Other biomarkers: chitotriosidase activity (Gaucher disease)

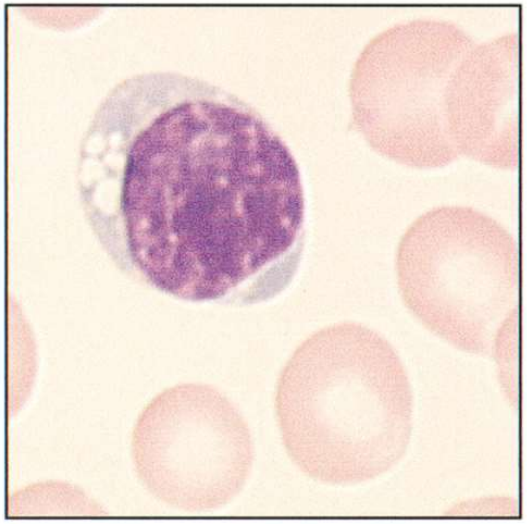
3- Enzymatic activity

4- Genetic study

1- Search for overload cells : peripheral blood

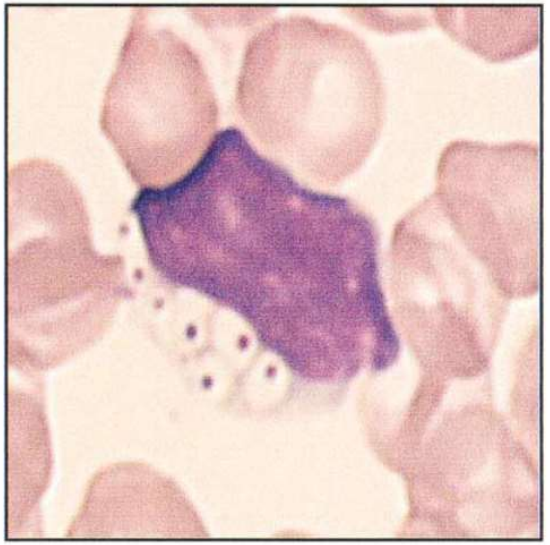
- You must specifically ask your cytologist to look for them!!!





Vacuolated lymphocytes

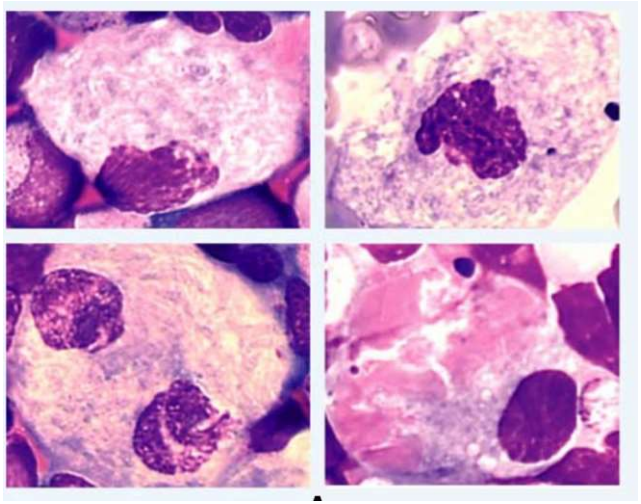
De Lonlay P, Fennetau O, Arch Pédiatr 2002



Gasser cell.

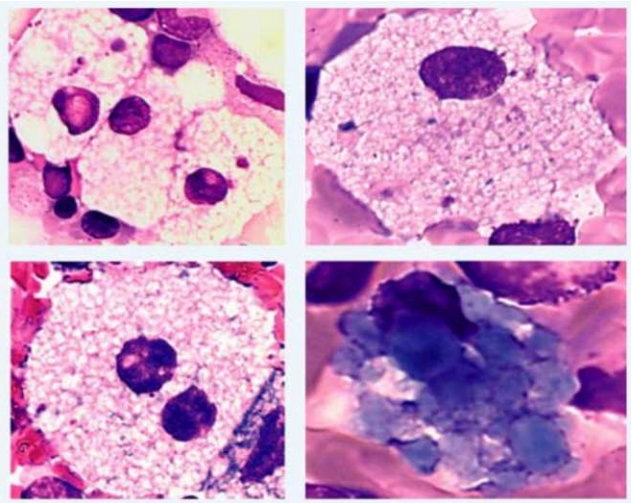
Vacuolated lymphocyte with vacuoles centered by a more or less voluminous inclusion characteristic of mucopolysaccharidoses except type IV

1- Search for overload cells : Bone marrow



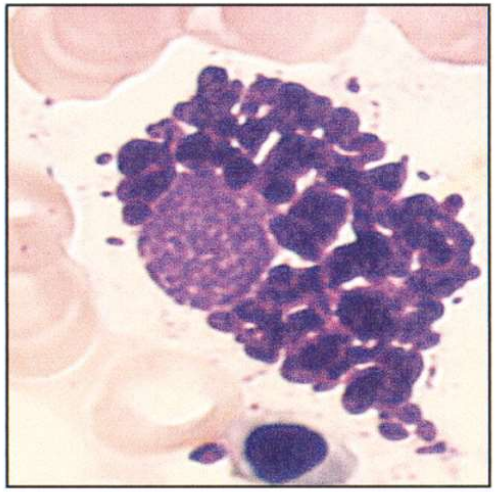
Gaucher disease

suggestive histiocytes with cytoplasm striated with fibrillar and curvilinear greyish to blue inclusions, cells sometimes multinucleated and, in the lower right, may contain phagocytized elements (red blood cells...)



Niemann-Pick

histiocytes with vacuole-filled cytoplasm
vacuoles, sometimes with cytophagic residues, bottom right, in type B and C, also presence of blue histiocytes



MPS

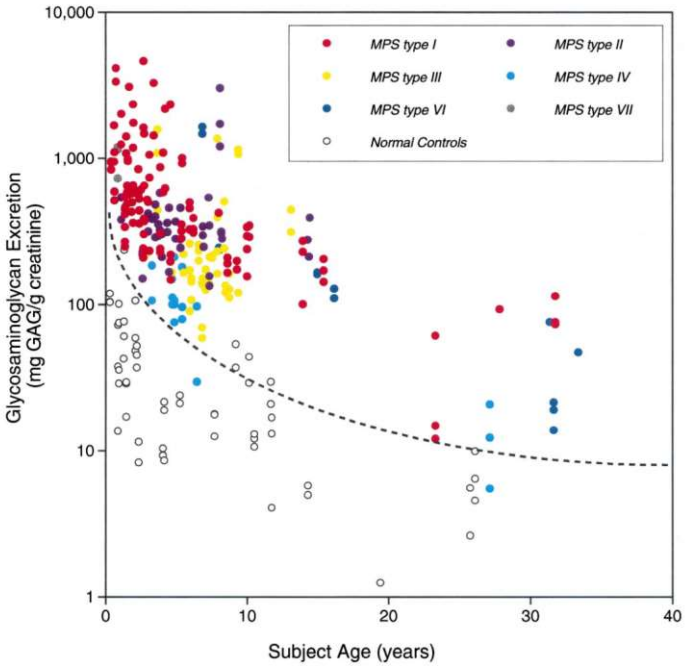
Gasser's histiocytes. Their cytoplasm contains numerous
granules of variable size and a violet-black color

Fenneteau O, Ann Biol Clin 2007

2- Accumulated substrates

Non-specific (group diagnosis): urinary mucopolysaccharides

- **A- quantitative total analysis: DMB test. (dimethylmethylene blue)**



Whitley et al., MGM, 2002

Limitations:

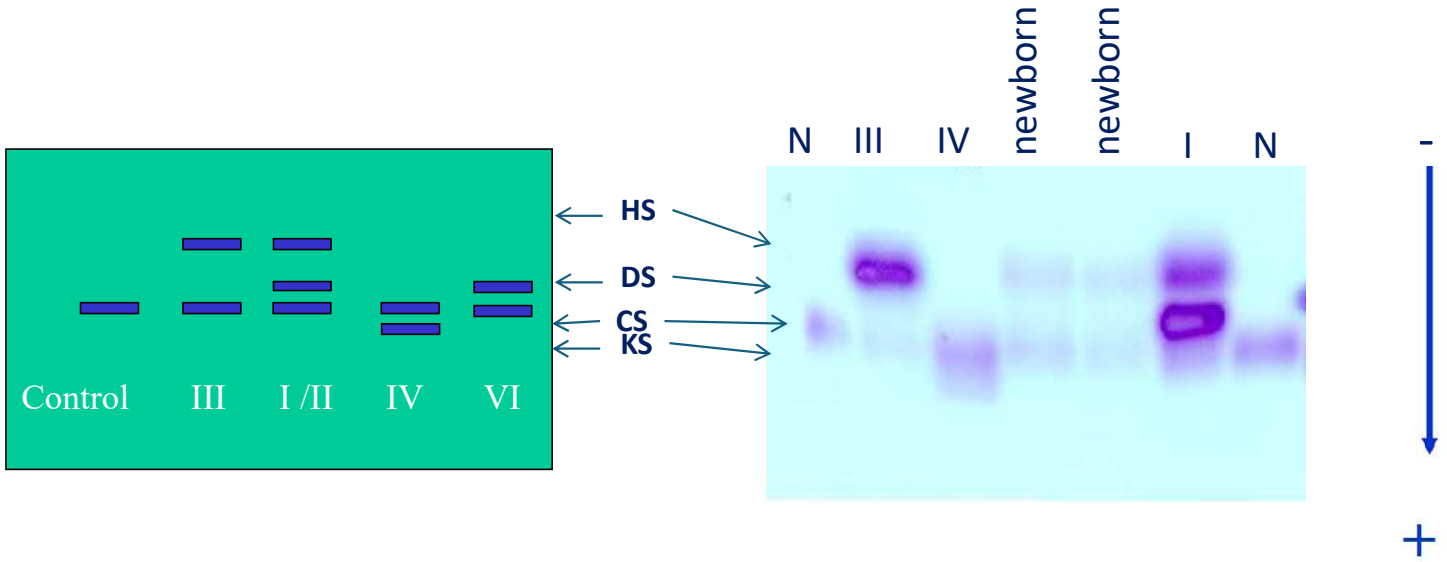
- Not always elevated! Cases reports of MPS III, IV and VII without total GAGs elevation

- No type/sub-type diagnostics

2- Accumulated substrates

Non-specific (group diagnosis): urinary mucopolysaccharides

- **B- qualitative analysis: mucopolysaccharides electrophoresis**



Limitations : Semi-quantitative results, visual assessment, interferences (heparin, dextran sulfate…), electrophoresis plates to be discontinued in 2022!, no definitive diagnosis

2- Accumulated substrates

Non-specific (group diagnosis): urinary mucopolysaccharides

- **Qualitative analysis: mucopolysaccharides analysis by LC-MS/MS**

Numerous methods developed by laboratories

→ You have to ask the limitation to your biologist!!

An improved method for glycosaminoglycan analysis by LC–MS/MS of urine samples collected on filter paper

Christiane Auray-Blais [a,*], Pamela Lavoie [a], Haoyue Zhang [b], René Gagnon [a], Joe T.R. Clarke [a], Bruno Maranda [a], Sarah P. Young [b], Yan An [b], David S. Millington [b]

Clinica Chimica Acta 413 (2012) 771–778

A straightforward, quantitative ultra-performance liquid chromatography-tandem mass spectrometric method for heparan sulfate, dermatan sulfate and chondroitin sulfate in urine: An improved clinical screening test for the mucopolysaccharidoses ☆

Haoyue Zhang [a], Tim Wood [b], Sarah P. Young [a], David S. Millington [a,*]

Molecular Genetics and Metabolism 114 (2015) 123–128

UPLC-MS/MS detection of disaccharides derived from glycosaminoglycans as biomarkers of mucopolysaccharidoses

Christiane Auray-Blais [a,*], Pamela Lavoie [a], Shunji Tomatsu [b], Vassili Valayannopoulos [c], John J. Mitchell [d], Julian Raiman [e], Maxime Beaudoin [a], Bruno Maranda [a], Joe T.R. Clarke [e]

Analytica Chimica Acta 936 (2016) 139–148

LC-MS/MS method for simultaneous quantification of heparan sulfate and dermatan sulfate in urine by butanolysis derivatization

Giulia Forni[a], Sabrina Malvagia[a], Silvia Funghini[a], Emanuela Scolamiero[a,b], Massimo Mura[a], Maria Della Bona[a], Fabio Villanelli[a], Roberta Damiano[a,b], Giancarlo la Marca[a,b,*]

Clinica Chimica Acta 488 (2019) 98–103

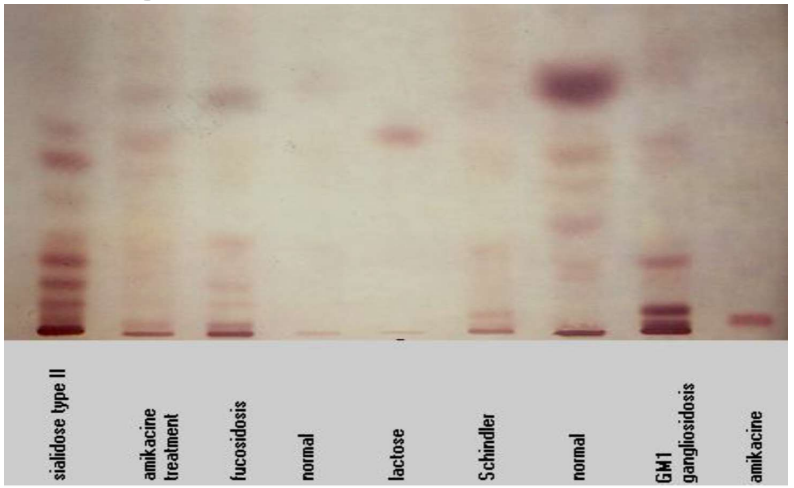
2- Accumulated substrates

Non-specific (group diagnosis): urinary oligosaccharides

A- Oligosaccharides thin layer chromatography

Limitations :

- No quantification: visual assessment only, experience +++.
- β-mannosidosis not detected
- No typical profile
- Interferences: aminoglycosides, milk (enriched with bone polymers or breast-feeding)
- Age: excretion may be difficult to visualize in older patients



B- New LC-MS/MS methods

- More sensitive & specific
- None of the interferences observed with TLC
- + determination of oligosaccharides and free sialic acid in the same technique

Fast urinary screening of oligosaccharidoses by MALDI-TOF/TOF mass spectrometry

Laurent Bonesso[1], Monique Piraud[5], Céline Caruba[1], Emmanuel Van Obberghen[1,2,3,4], Raymond and Charlotte Hinault[1,2,3,4*†]

SSIEM Academy 2024, Amsterdam

Received: 21 December 2016 Revised: 22 March 2017 Accepted: 25 March 2017 Published online in Wiley Online Library

Rapid Commun. Mass Spectrom. **2017**, *31*, 951–963
(wileyonlinelibrary.com) DOI: 10.1002/rcm.7860

Development of a new tandem mass spectrometry method for urine and amniotic fluid screening of oligosaccharidoses

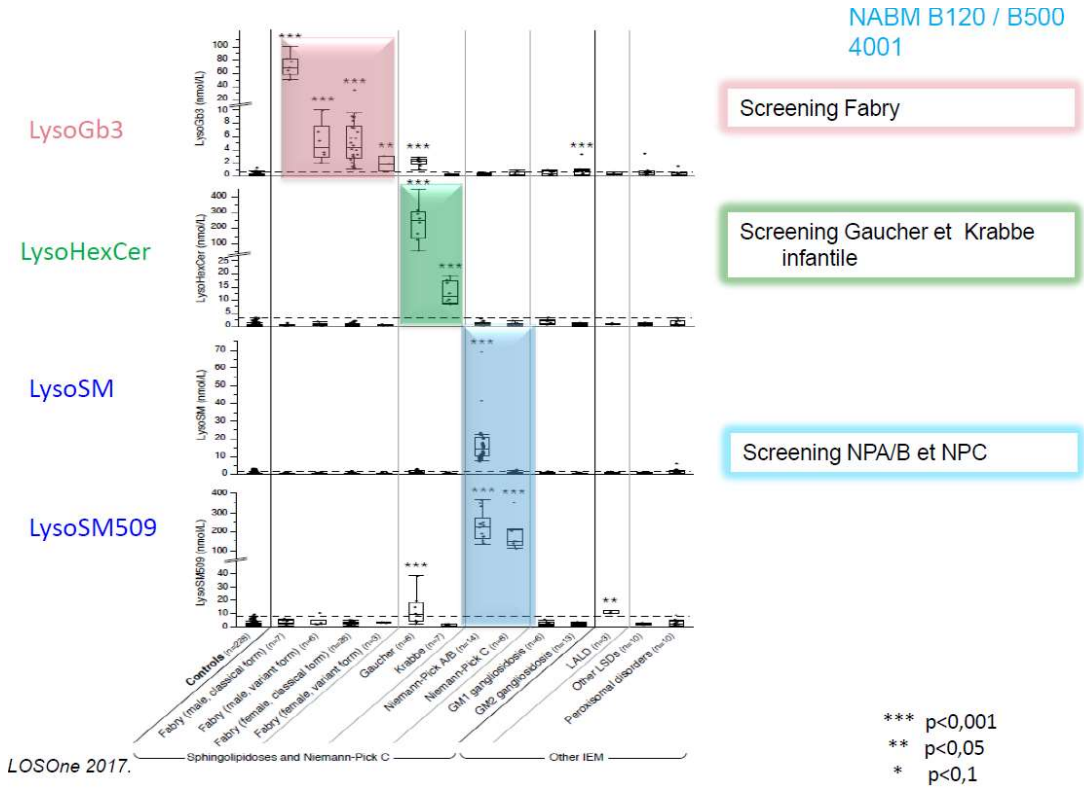
Monique Piraud[1]*, Magali Pettazzoni[1], Louise Menegaut[1,2], Catherine Caillaud[3], Yann Nadjar[4], Christine Vianey-Saban[1,5] and Roseline Froissart[1,6]

[1]Service de Biochimie et Biologie Moléculaire Grand Est, UM Pathologies Métaboliques, Erythrocytaires et Dépistage Périnatal, ologie et de Pathologie Est, Hospices Civils de Lyon, France
de Biochimie Médicale, Centre Hospitalo-Universitaire François Mitterrand, Dijon, France
de Biochimie Métabolomique et Protéomique, Hôpital Universitaire Necker-Enfants Malades, Assistance Publique-Paris, France ; Unité INSERM 1151, Université Paris Descartes, Paris, France

2- Accumulated substrates

- **plasma lysosphingolipides**
- *LC-MS/MS assay*
- *Multiplexing*
- *Applications: diagnosis and monitoring of sphingolipidosis*

- **Other:**
  - *chitotriosidase activity: Gaucher + other LSD (= reflects the lysosomal macrophage function)*
  - *Sulfatides: MLD*



Pettazzoni et al. PlosOne 2017

3- Enzymatic activity

- Enzyme activity expressed in most tissues
  - Leukocytes or lymphocytes
  - Serum
  - Blood spots (DBS, dried blood spot)→ commercial kit
  - Cultured cells (fibroblasts, chorionic villi, amniotic cells)
  - Frozen tissues (liver, ….)
- Suitable for most lysosomal enzymes
- Manual but relatively simple technique
- Performed in a specialized laboratory
- Some analytical pitfalls: pseudo-deficits, activator deficiencies, combined or multiple deficiencies…

4- Genetic study

- **Diagnostic :**
  - Molecular studies must be performed in all patients +++ (prenatal diagnosis, familial diagnosis, confirmation…)
  - Generally in 2nd place to confirm the disease (except CLN…)

- **Therapeutic :**
  - Amenable mutations : Ex. treatment with chaperone molecules in Fabry disease, etc.)
  - CRIM status in Pompe

- **Prognostic**
  - Gaucher disease: "neuroprotective" mutation
  - Pompe : late-onset mutation

LSDs: *Clinical Aspects*

- >70 rare disorders….. ~1:5000 livebirths affected

- **Variable, progressive, multisystem involvement presenting from infancy-adulthood**
  - **Variable** organ involvement depends on normal distribution/expression of the lysosomal enzyme and the local production of substrate
  - **Progressive** accumulation and downstream pathological effects → progressive manifestations of disease
  - **Age of manifestation** relates to rate of accumulation of substrate, dependent on rate of substrate production and degree of residual enzyme activity

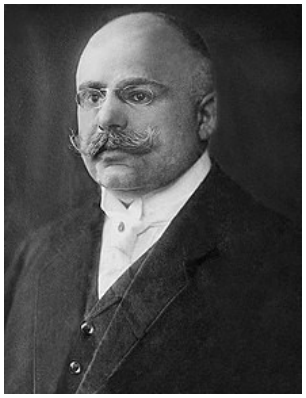
Johannes Fabry

Philippe Gaucher

Knud Krabbe

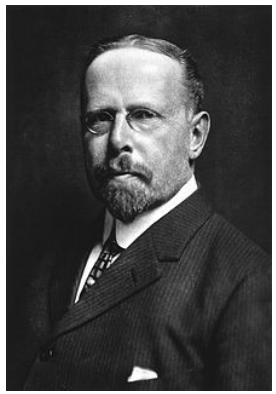
Joannes Pompe

Sylvester Sanfilippo



Ludwig Pick

Warren Tay



Bernard Sachs

William Sly

Norman-Landing

Clinical features of LSD

Progressive disorders, **Varied age** of presentation

Multisystem disorders

	Dysmo-rphism	Skeletal	Neuro	Organo megaly	Cardiac	Resp/ ENT	Skin	Hearing	Eye	Renal	Pain
Prenatal	Hydrops fetalis	Short limbs	Brain anomaly								
Infancy	Dysmorphic at birth: I-cell	Dysostosis multiplex	Macroceph, Dev delay, seizures, hypotonia, **regression,** leuco-dystrophy	HSM	Cardiomyopathy	Apnoea	Ichth-yosis	Impaired hearing	Cherry red spot	Nephro-sialidosis	
Child	Progressive coarsening	Dysostosis multiplex		HSM	Cardiomyopathy, valvular	OSA	Angio-keratoma	Recurrent otitis Impaired hearing	Corneal Retinal		Joints Neuro-pathic
Adult			Myopathy Psychiatric	HSM	Cardiomyopathy, valvular	Failure			Corneal Retinal	Renal failure	Joints Neuro-pathic

Hydrops Fetalis: Metabolic Causes

- Lysososomal storage disorders
  - Mucopolysaccharidosis VII, I, IVa
  - Sialidosis
  - Mucolipidosis II
  - Sphingolipidoses
  - Lipid storage (Niemman Pick C)
- Sterol biosynthesis
  - Smith-Lemli-Opitz
- Peroxisomal (Zellweger Spectrum)
- GSD IV
- Glycosylation disorders
- Mitochondrial, Pearson

Zschocke, Hoffmann. Vademecum Metabolicum

Treatment of LSD Overview

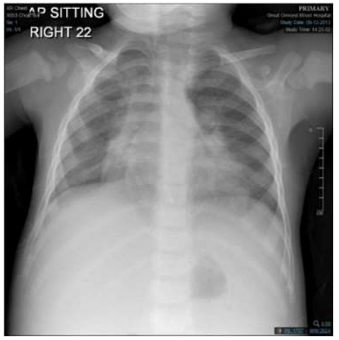
- MDT approach important
- Symptomatic/ supportive treatments are mainstay of good clinical management
- Disease-modifying therapies
  - Correct the Enzyme Deficiency
    - **Enzyme replacement therapy**
    - **Stem cell transplant**
    - Chaperone therapy
    - Gene therapy
  - Decrease substrate
    - Substrate reduction therapies
  - Target pathophysiology

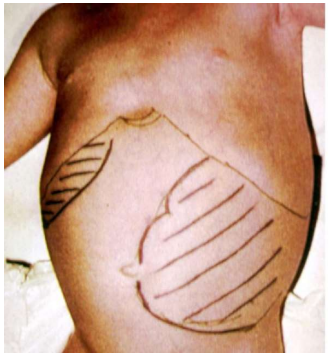
Mannose-6-phosphate receptor system facilitates cellular uptake and trafficking to lysosomal compartment

	ERT	Small molecule	HSCT	Other
Fabry	✓	✓		
Gaucher	✓	✓		
MPS I	✓		✓	
MPS II	✓		(✓)	
MPS IVa	✓			
MPS VI	✓			
MPS VII	✓		✓	
α-mannosidosis	✓		✓	
Pompe	✓			
Acid sphingomyelinase	✓			
Niemann Pick C		✓		
Wolman/CESD	✓		✓	
Metachromatic leukodystrophy			(✓)	*Ex vivo* Gene therapy transplant
Neuronal ceroid lipofuscinosis type 2	✓			

Hepatosplenomegaly

- Male child
- Incidental finding of anaemia at 7 months – given iron supplement
- Unwell with pneumonia at 17 months – incidental finding of **massive hepatosplenomegaly**
- No dysmorphic features
- Microcytic anaemia, thrombocytopaenia





Differential diagnosis of paediatric hepatosplenomegaly....	
Infection	Acute viral hepatitis, EBV, CMV, Tropical (Malaria)
Haematological	Malignancy eg leukaemia/ lymphoma Non malignant eg haemolytic anaemias, haemoglobinopathies
Connective tissue disorders / infiltrative	Sarcoidosis
Chronic liver disease / cirrhosis	
Congestive	Heart failure
Storage disorders	

Hepatosplenomegaly: Metabolic differentials

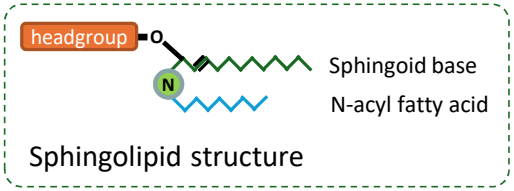
Lysosomal storage disorders	Gaucher Mucopolysaccharidoses Spingolipidoses eg Niemann Pick A/B, C Oligosaccharidoses
Glycogen storage disorders	Hepatic forms eg GSD I, GSD III Pompe disease
Other glucose/fructose pathway disorders	Fanconi Bickel
Congenital disorders of glycosylation	MPI-CDG
Lysinuric protein intolerance	
Mevalonic aciduria	
Polyol pathway disorders	Transaldolase deficiency
Peroxisomal	Zellweger spectrum
Disorders resulting in chronic liver disease	
Disorders with acute liver disease	Fatty acid oxidation, Organic acidurias, galactosaemia

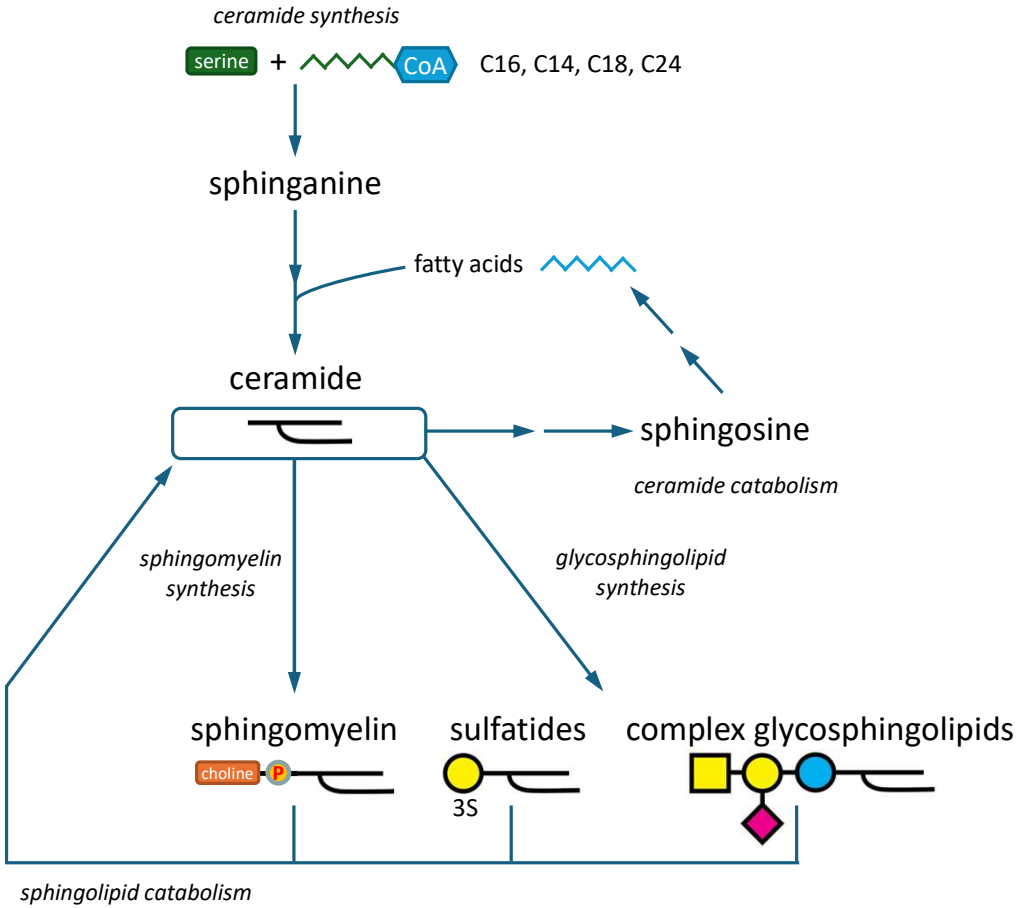
Hepatosplenomegaly investigations

- **Overloaded cells?**
  - Bone marrow aspirate not done
- **Accumulated substrates?**
  - Urine glycosaminoglycans: normal quantification and electrophoresis
  - Urine oligosaccharides
- **Enzyme deficiency?: "Hepatosplenomegaly panel"**
  - Leucocyte β-glucocerebrosidase: 0.52 nmol/hr/mg prt (5.4-16.8)
  - Chitotriosidase: 60202 nmol/hr/ml (0-150)
- **Molecular genetics?:**
  - compound heterozygous pathogenic variants in GBA
- Diagnosis: **Gaucher Disease**

Disease	Enzyme
GM1 gangliosidosis	β-galactosidase
Sialidosis	α-neuraminidase
Galactosialidosis	α-neuraminidase/ β-galactosidase
Gaucher	β-glucosidase
Niemann Pick A&B	Sphingomyelinase
Wolman/ CESD	Acid esterase
Fucosidosis	α-fucosidase
α-mannosidosis	α-mannosidase
I-cell disease	I-cell screen
β-mannosidosis	β-mannosidase
MPS VII	β-glucuronidase

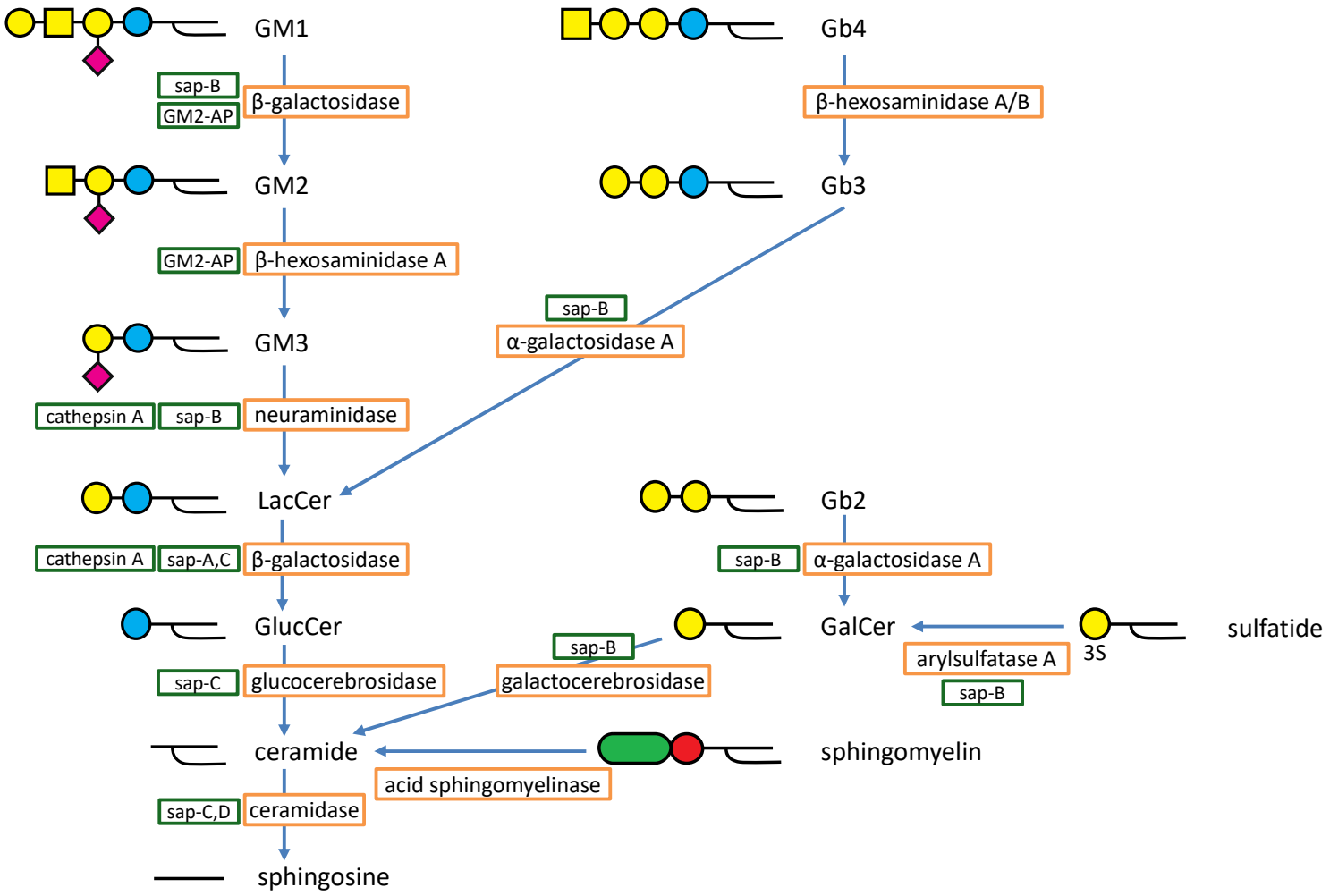
sphingolipid metabolism





sphingolipid catabolism

with cofactors



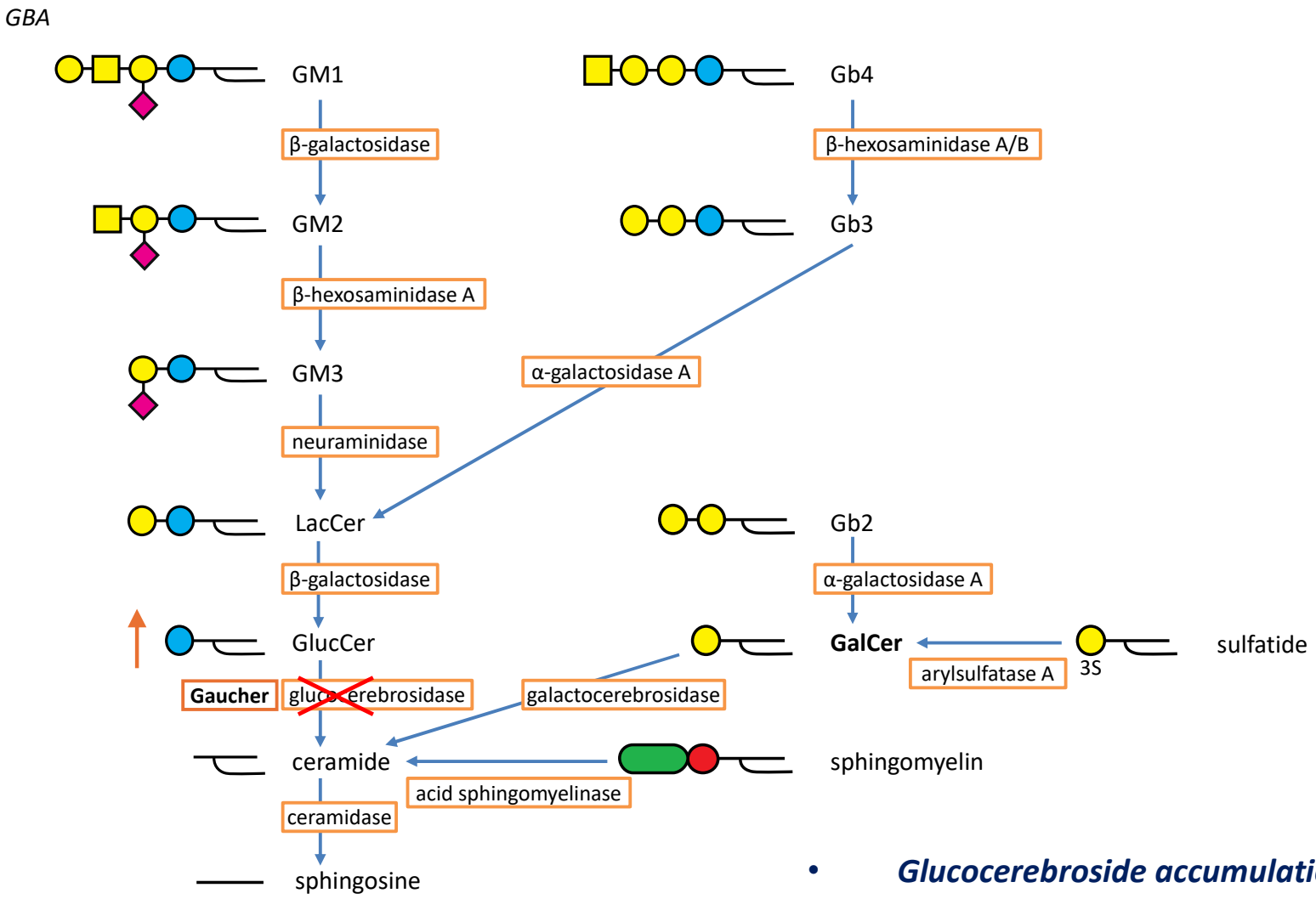
glucose
galactose
N-acetylglucosamine
N-acetylgalactosamine
sialic acid
3-O-sulfated galactose
3S
ceramide
phosphocholine

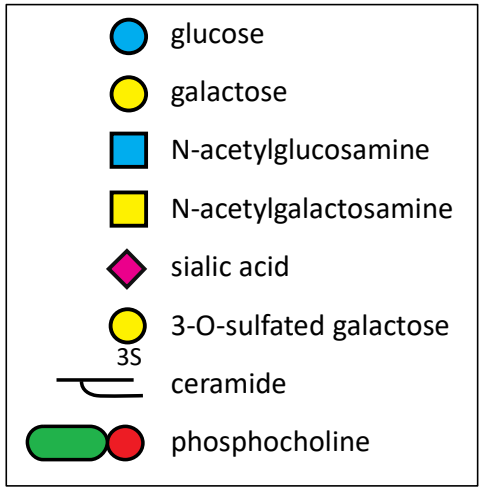
saposin deficiencies lead to various sphingolipidoses

cathepsin A deficiency lead to galactosialidosis

Gaucher disease

glucocerebrosidase deficiency





- ***Glucocerebroside accumulation***
- ***Activated macrophages – Gaucher cells accumulate***

Gaucher Disease

Type 1 non-neuronopathic

- Hepatosplenomegaly
- Haematological abnormalities: anaemia, thrombocytopaenia
- Bone involvement
  - Erlenmeyer flask deformity of distal femur
  - Osteopaenia- osteoporosis
  - Bone crises
- Pulmonary disease

Type 3 chronic neuronopathic

- Supranuclear horiztonal gaze palsy
- Ataxia, tremor
- Variable cognitive involvement
- Seizures

Type 2 acute neuronopathic

- Lethal, severe hypertonic posturing, strabismus, opisthotonus

Biomarkers

- Chitotriosidase (nb deficient in ~10-20% general population)
- Lyso-Gb1 (glucosylsphingosine)
- Angiotensin converting enzyme (ACE)

Treatment

- Enzyme Replacement Therapy
  - Imiglucerase, Velaglucerase alfa, Taliglucerase alfa
- Substrate Reduction Therapy
  - Miglustat, Eliglustat
  - Used in place of ERT for some patients
- Novel Therapies under evaluation
  - Chaperone-mediated enzyme enhancement eg ambroxol
  - Gene therapy

A footballer short of breath…

- 27 year old male investigated due to exercise intolerance, shortness of breath playing football.
- As a teenager he had complained of non-specific abdominal pain, and foot pain after exercise.
- Long-standing high-frequency hearing impairment.
- Uncle (mother's brother) had died in 40's of renal failure.

- Initial investigations:
  - Na 138mmol/L, K 4.5mmol/L, Urea 10.4 umol/L, Creatinine 145 mmol/L
  - Urine protein 3+
  - eGlomerular Filtration rate 58ml/min/1.73m2
- Echo: left ventricular hypertrophy
- Renal biopsy: abnormal **storage material** within glomerular podocytes

Further investigations

- **Accumulated substrate?**
  - Plasma lysoGb3: 64.5 ng/ml (0-1.8)

- **Enzyme deficiency?** - Targeted testing
  - Leucocyte a-galactosidase 5.8 nmol/hr/mg ptn (33-134)
  - B-galactosidase 295 nmol/hr/mg ptn (161-540)

- **Molecular genetics:** hemizygous GLA pathogenic variant c.644A>G, p.

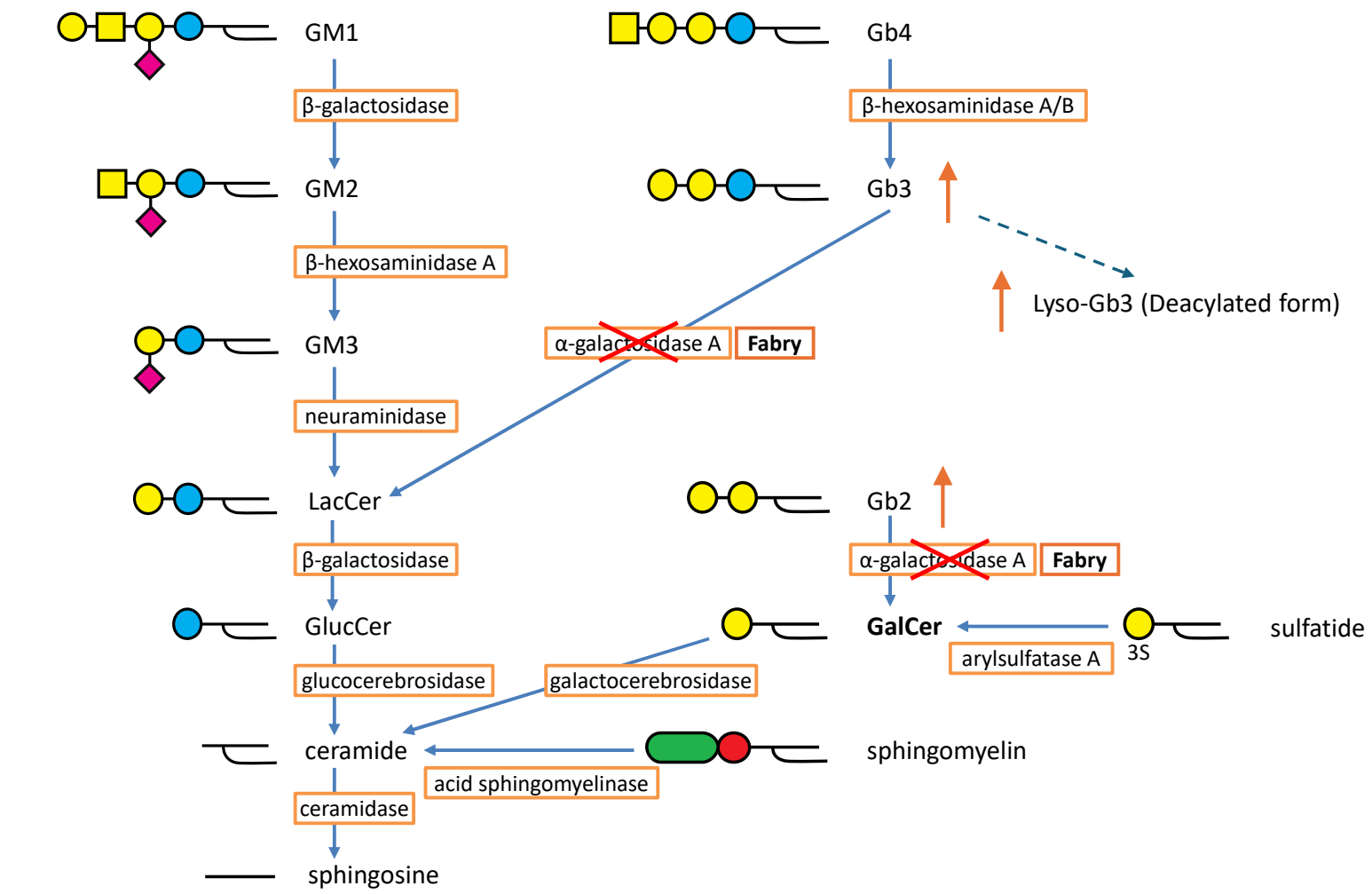
- **Diagnosis: Fabry Disease**

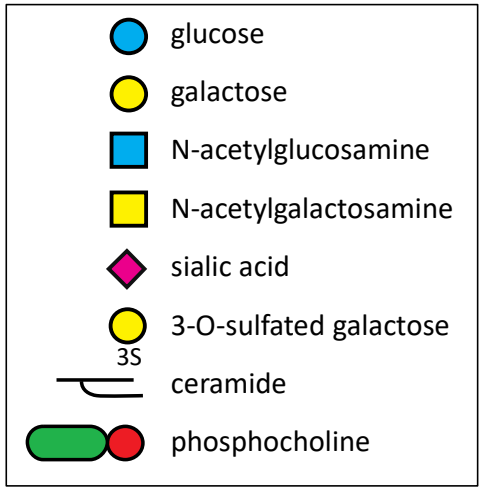
- **Cascade testing:**
  - Best test to screen his younger sisters?
  - What about his 4 year old son?

GALC

Fabry disease

α-galactosidase A deficiency





Fabry Disease

- X-linked α-galactosidase deficiency
- Female manifestations common but variable

	Paediatric Age	**Adulthood**
Skin	Angiokeratomas	
Peripheral nerves	Acroparaesthesia (chronic pain, acute pain crisis)	
Gastrointestinal	GI disturbance (diarrhoea, abdominal pain, constipation)	
Sweating	Hypohidrosis/Anhidrosis	
Ophthalmic	Cornea verticallata Lenticular change Vessel tortuosity (retinal, conjunctival)	
Renal		Proteinuria **End stage renal disease**
Cardiac		Left ventricular hypertrophy Cardiomyopathy **Ischemic heart disease** Arrhythmia
Cerebrovascular/ central nervous system		Transient ischaemic attack **Stroke**
Pulmonary		
CN VIII	High frequency hearing loss	

Fabry Disease Treatments

Symptomatic

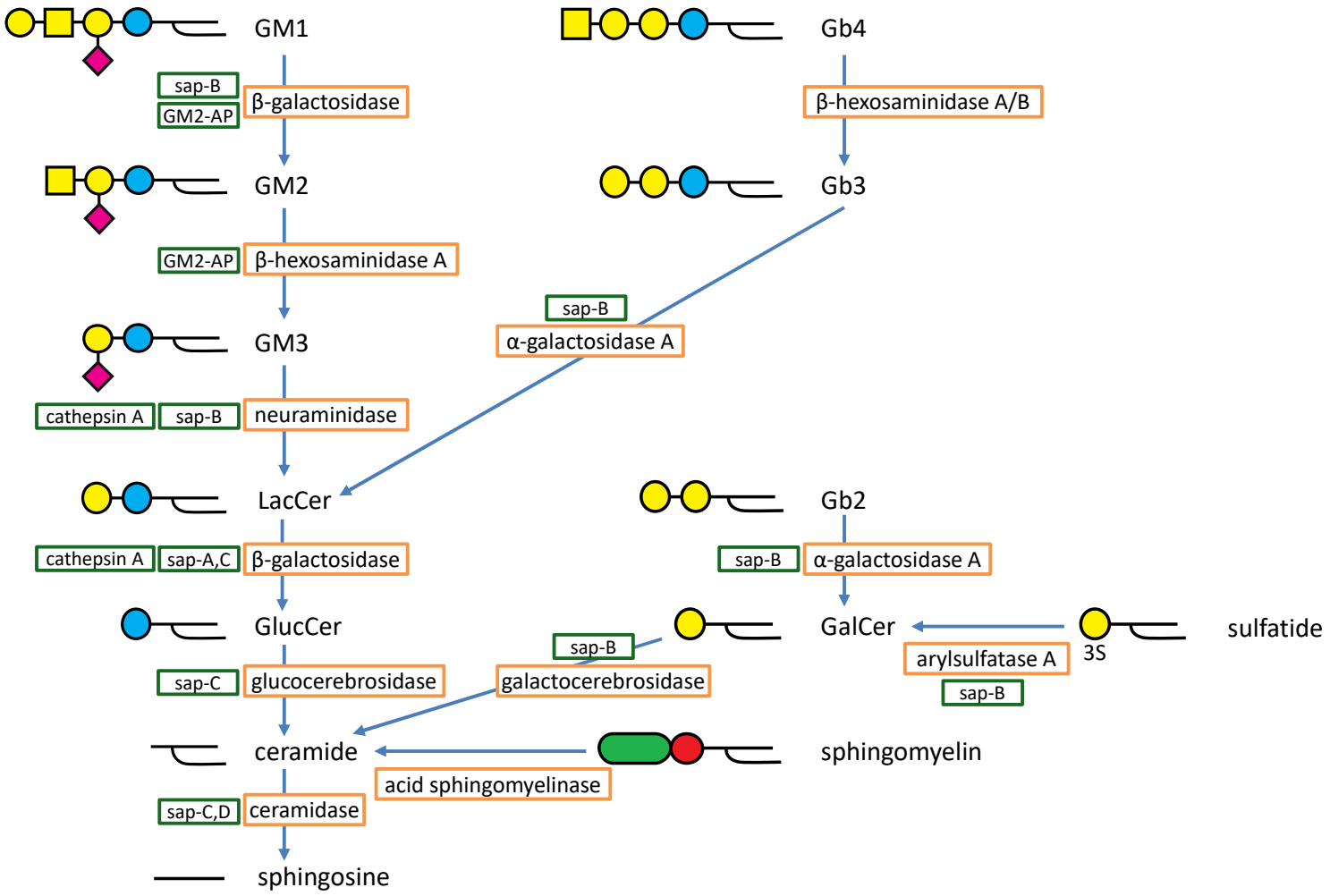
- Pain management
- Hearing aids
- Cardiac
  - Lipid-lowering
  - Blood pressure
  - Arrhythmia
    - Pacemaker/implantable defibrillator
- Renal
  - ACE-Inhibition
  - Renal replacement therapies

Disease-Modifying

- Enzyme replacement therapy
  - Agalsidase alfa, agalsidase beta, Pegunigalsidase alfa
- Chaperone therapy
  - Migalastat for amenable mutations
- Ongoing debate about treatment start/stop criteria, relative efficacy…

sphingolipid catabolism

with cofactors



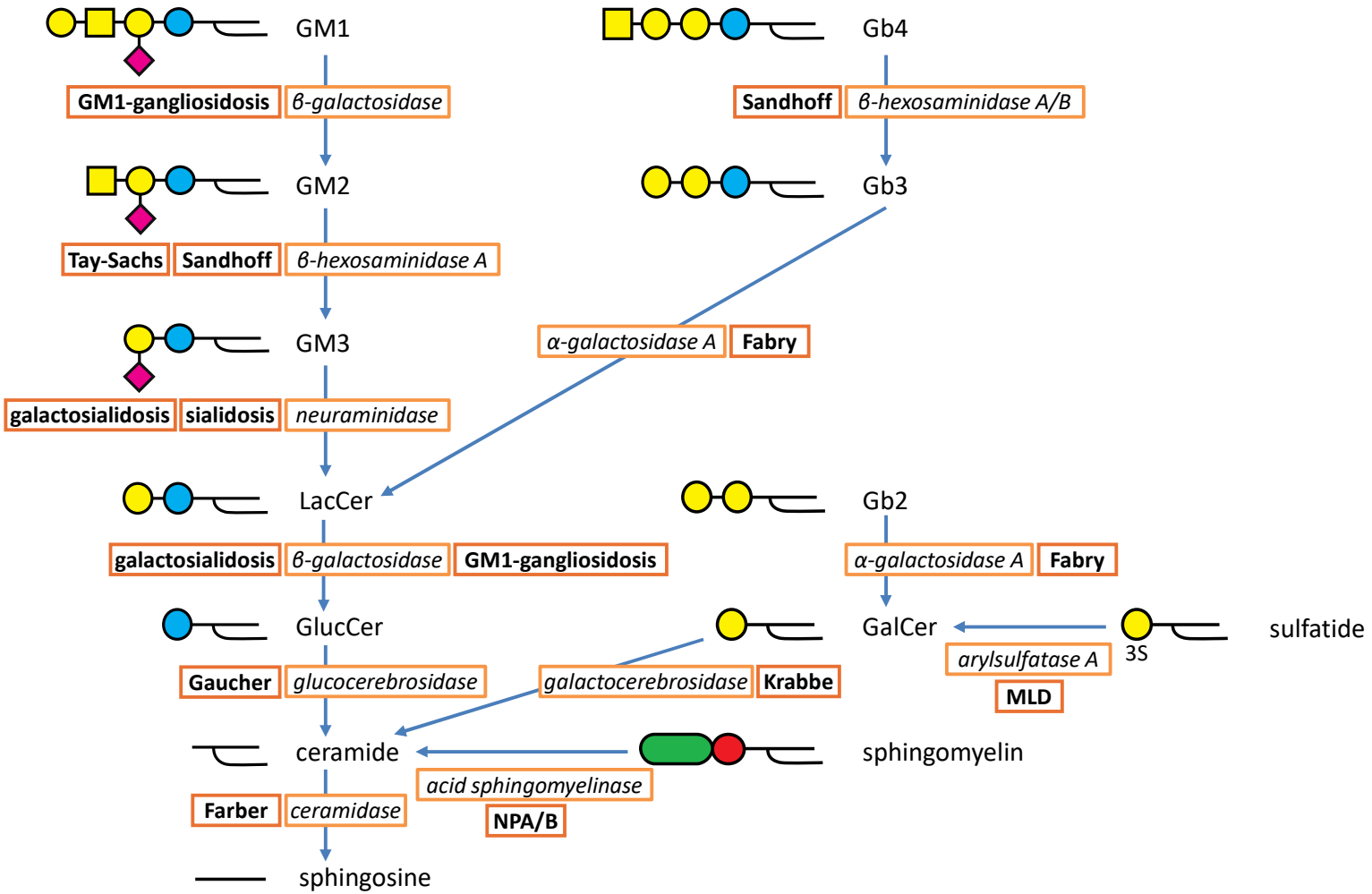
glucose
galactose
N-acetylglucosamine
N-acetylgalactosamine
sialic acid
3-O-sulfated galactose
3S
ceramide
phosphocholine

saposin deficiencies lead to various sphingolipidoses

cathepsin A deficiency lead to galactosialidosis

sphingolipid catabolism

disorders



glucose

galactose

N-acetylglucosamine

N-acetylgalactosamine

sialic acid

3-O-sulfated galactose

3S

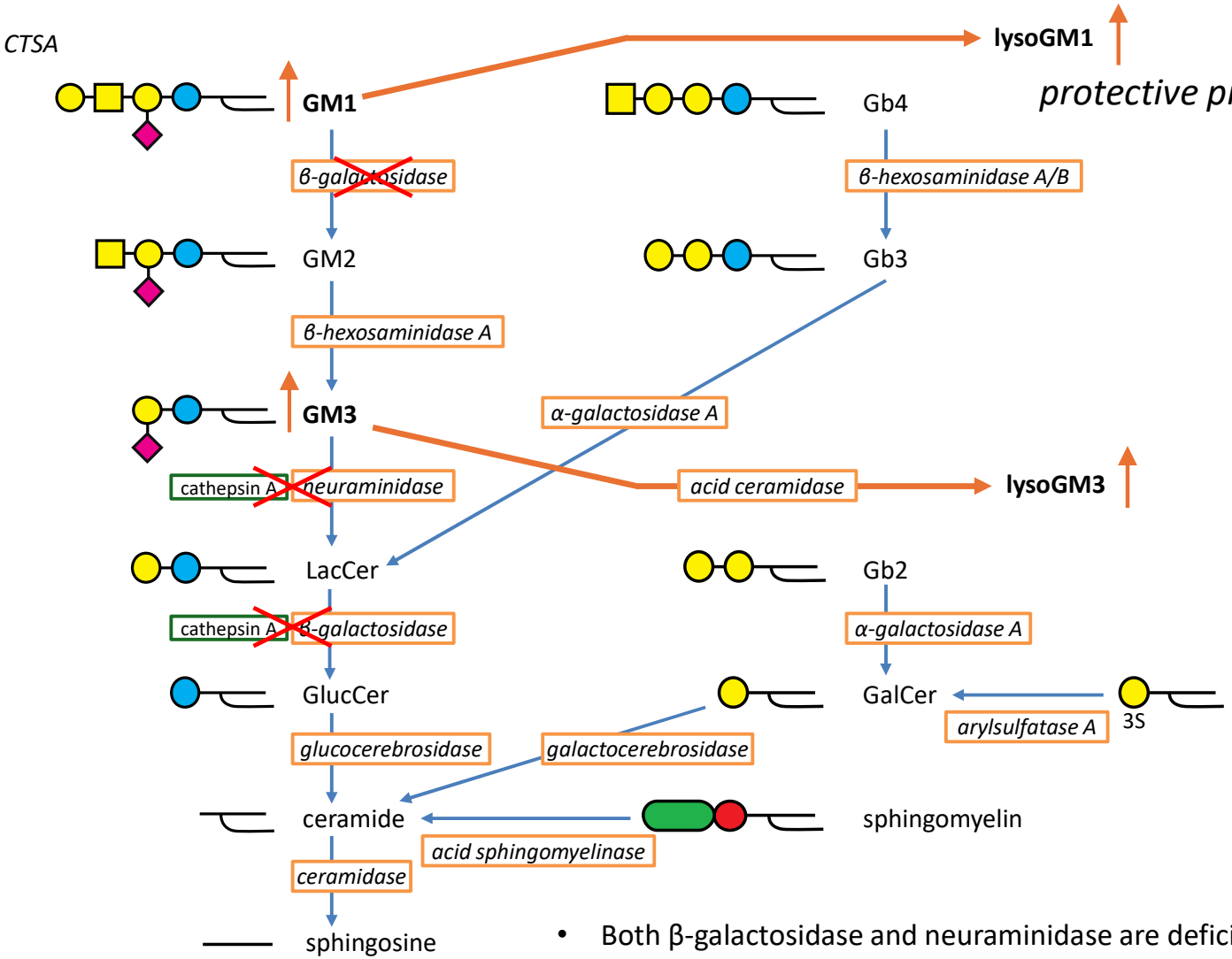
ceramide

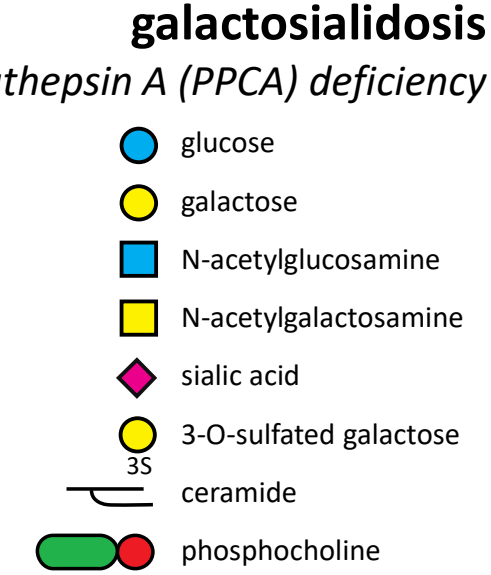
phosphocholine

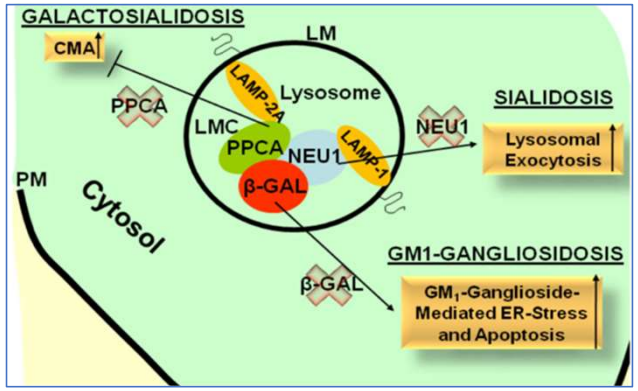
cathepsin A deficiency causes **galactosialidosis**

galactosialidosis

protective protein/cathepsin A (PPCA) deficiency







- Both β-galactosidase and neuraminidase are deficient. This causes ganglioside accumulation (GM1/GM3) but also oligosaccharide (N-glycosylation) and keratan sulfate (GAGs) accumulation

A constellation of features…

- 4 year boy referred with speech delay
- Second child to non-consanguineous parents.

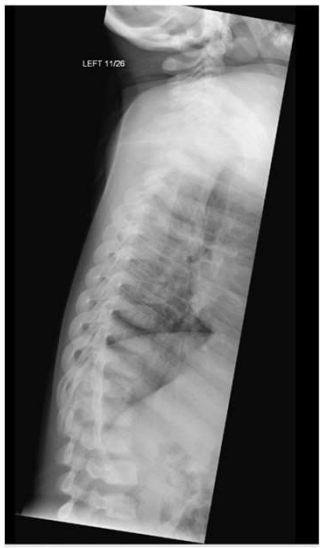
- Early development was normal, walked at 13 months
- First words at 7 months but then progressive concerns about **speech delay**
- By 3 years concern about **hyperactivity**

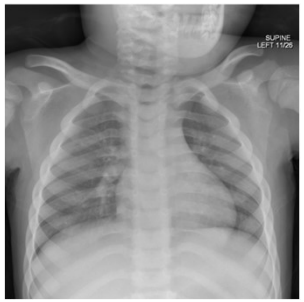
- History of **nasal polyps, frequent otitis media**
- **Umbilical hernia**

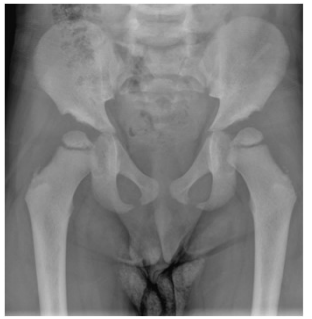
- **Examination**:
  - Relative **macrocephaly**
  - **Coarse facial** appearance with hypertelorism
  - Wide inter-dental spaces
  - Systolic cardiac **murmur** audible

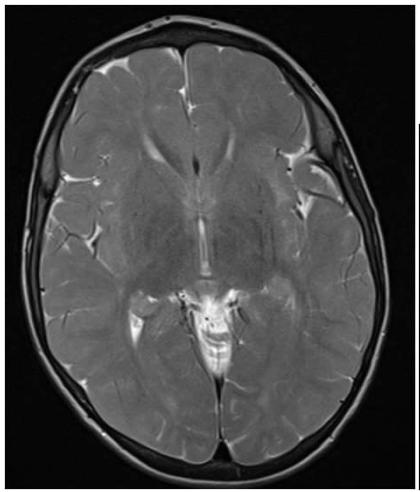
Imaging

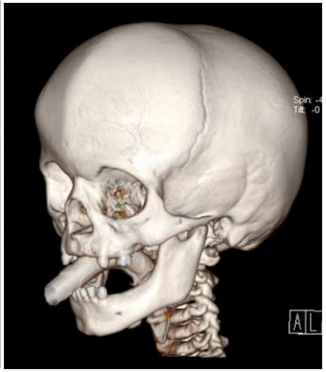
- **Echocardiogram**: thickened and dysplastic mitral valve, good function
- **X-ray skeletal survey**: dysostosis multiplex (moderate) with broad ribs and clavicles, hypoplastic T11 verterba, dysplastic hips
- **MRI brain**: global delay in myelination, abnormal thalamus signal
- **CT head**: sagittal and bilateral lambdoid synostosis





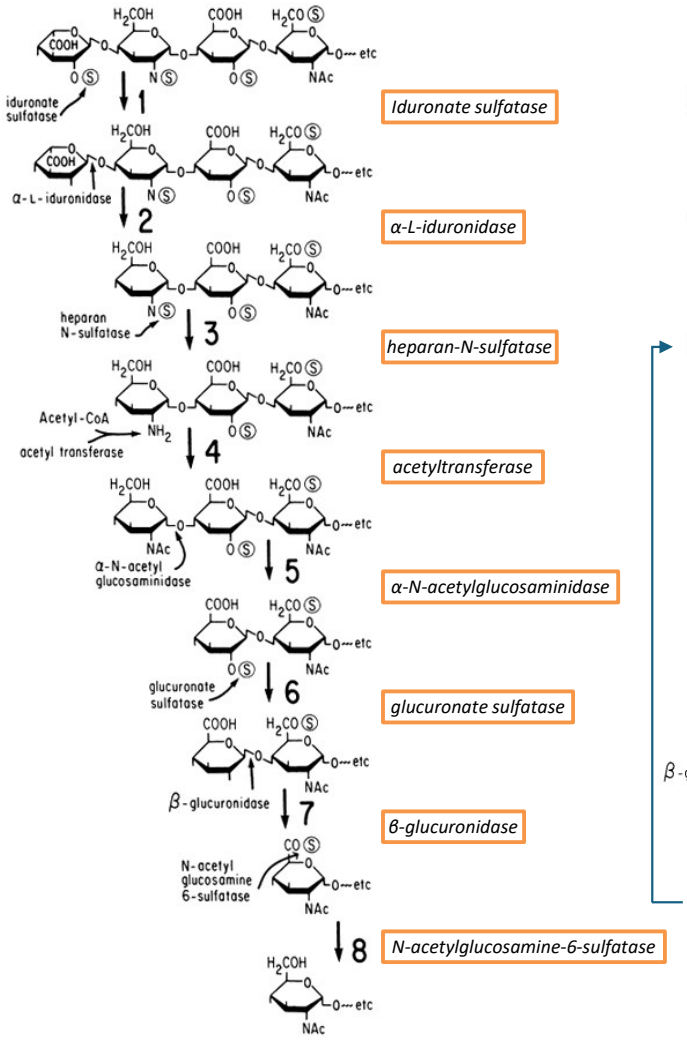






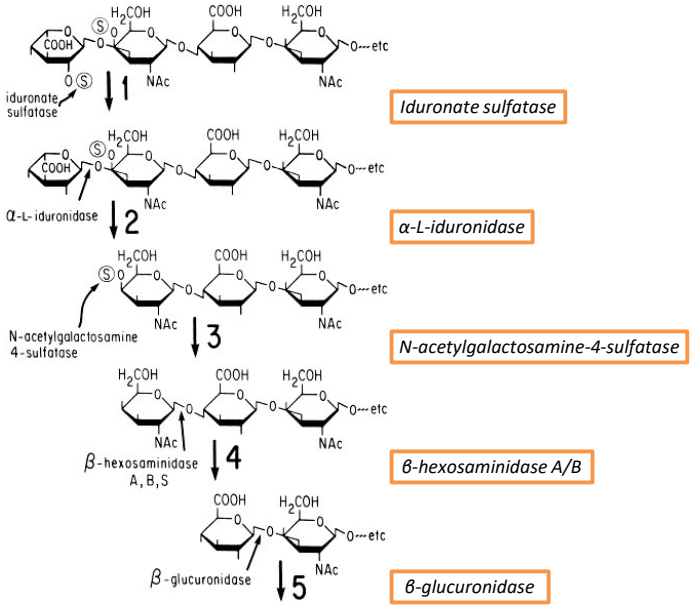
Glycosaminoglycan (GAGs) degradation

heparansulfate (HS)



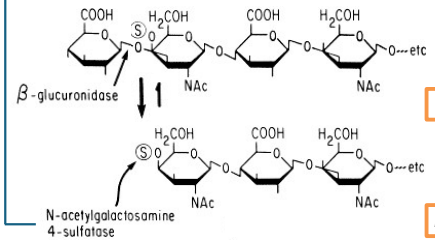
- *Iduronate sulfatase*
- *α-L-iduronidase*
- *heparan-N-sulfatase*
- *acetyltransferase*
- *α-N-acetylglucosaminidase*
- *glucuronate sulfatase*
- *β-glucuronidase*
- *N-acetylglucosamine-6-sulfatase*

dermatansulfate (DS)



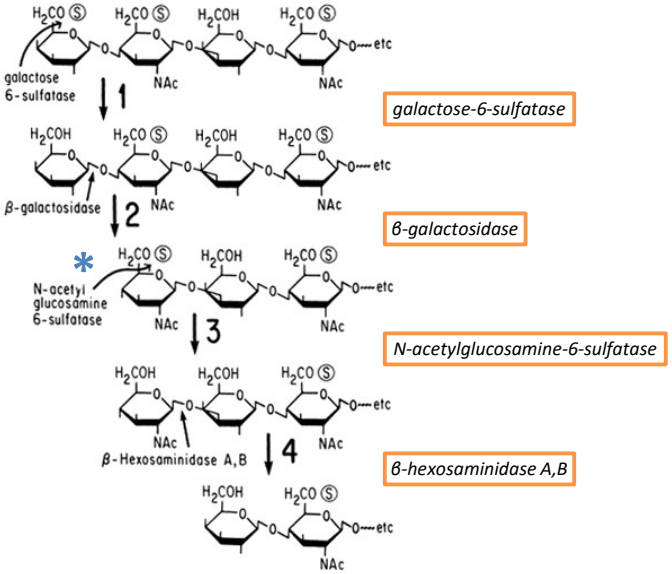
- *Iduronate sulfatase*
- *α-L-iduronidase*
- *N-acetylgalactosamine-4-sulfatase*
- *β-hexosaminidase A/B*
- *β-glucuronidase*

chondroitin sulfate (CS)



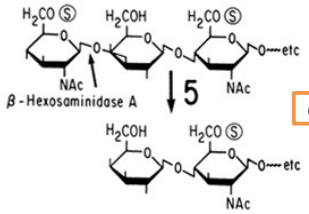
- *β-glucuronidase*
- *N-acetylgalactosamine-4-sulfatase*
- *N-acetylglucosamine-6-sulfatase*

keratansulfate (KS)



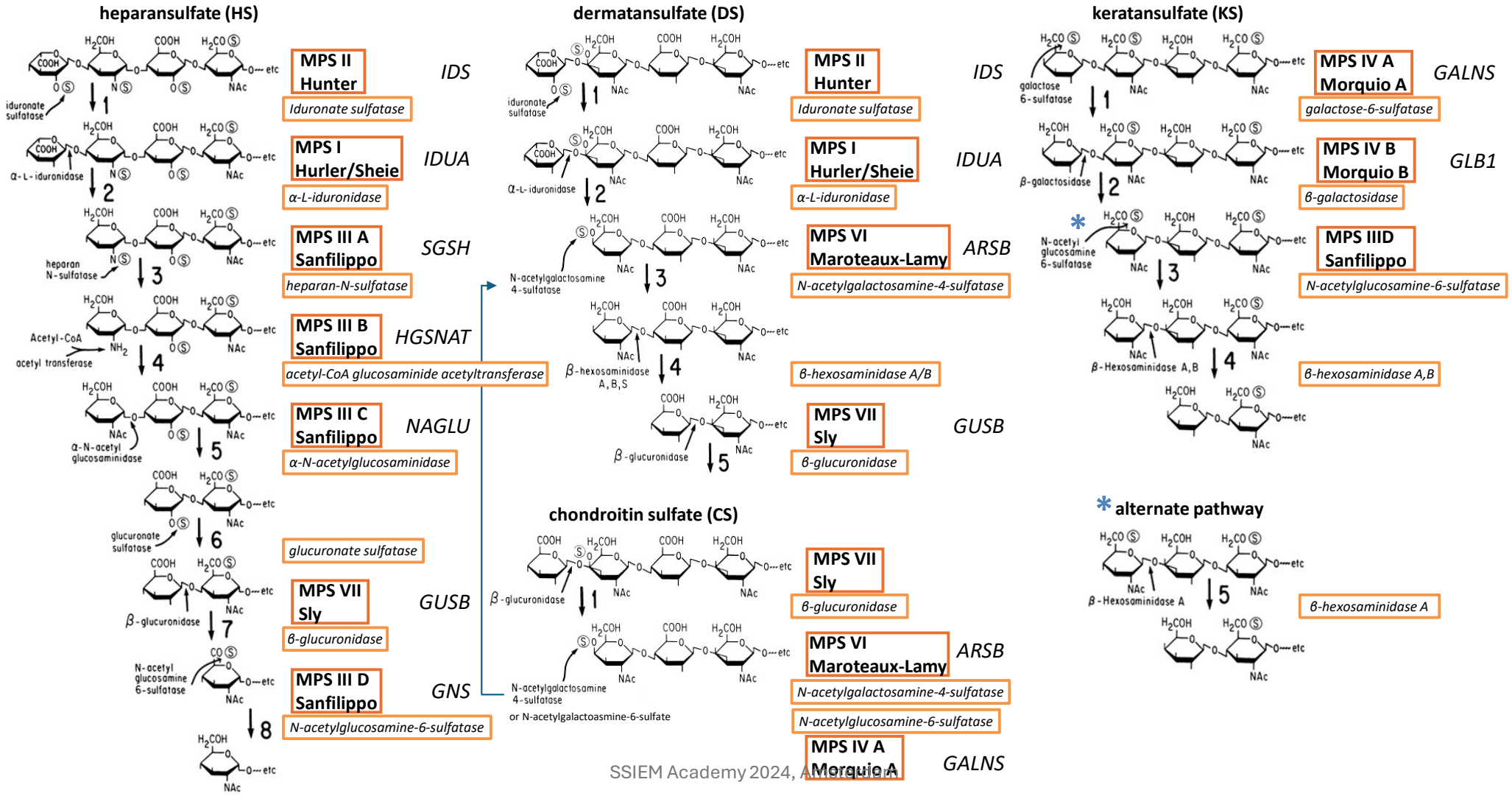
- *galactose-6-sulfatase*
- *β-galactosidase*
- *N-acetylglucosamine-6-sulfatase*
- *β-hexosaminidase A,B*

* alternate pathway



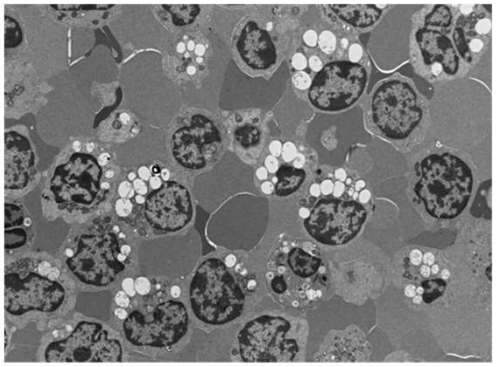
- *β-hexosaminidase A*

Mucopolysaccharidoses



Investigations

- **Overloaded cells?**
  - **Blood film by histopathology**: numerous vacuolated lymphocytes, negative for lipid and glycogen, no Alder granulation. Suggestive of lysosomal storage disorder



x800: lymphocyte vacuolation

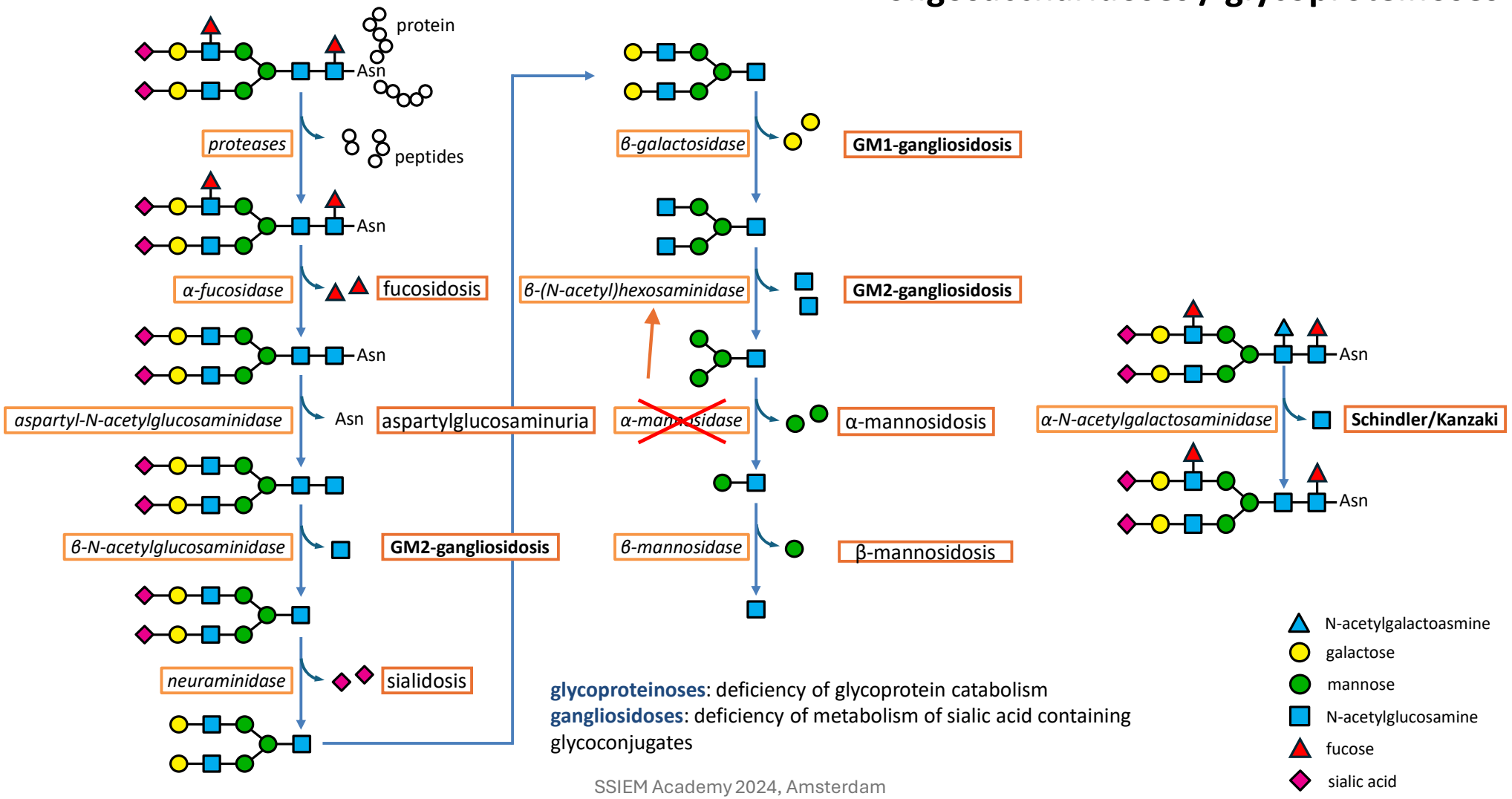
- **Accumulated Substrate?**
  - **Urine glycosaminoglycan**/creatinine ratio: 18 mg/mmol (2-15)
  - 2D Electrophoresis: Chondroitin sulfate, heparan sulfate and trace of dermatan sulfate of unclear significance
  - Repeat urine GAGs: normal
  - **Urine sialic acid**: normal
  - **Urine oligosaccharides**: insufficient sample.

Investigations

- **Enzyme deficiency?** "dysmorphic panel"
- **Molecular genetics?:**
  - *MAN2B1* homozygous pathogenic missense mutation
- **Diagnosis: Alpha Mannosidosis**

Leucocytes		
α-mannosidase	7.2 nmol/hr/mg prn	61-520
β-galactosidase	114 nmol/hr/mg prn	163-378
Plasma		
α-fucosidase	212 nmol/hr/ml	175-1403
β-glucuronidase	48 nmol/hr/ml	27-512
β-mannosidase	167 nmol/hr/ml	65-677
α-mannosidase	1.1 nmol/hr/ml	19-119
Total β-hexosaminidase	0.78 umol/hr/ml	0.41-1.7
Chitotriosidase	254 nmol/hr/ml	0-150

oligosaccharidoses / glycoproteinoses



glycoproteinoses: deficiency of glycoprotein catabolism
gangliosidoses: deficiency of metabolism of sialic acid containing glycoconjugates

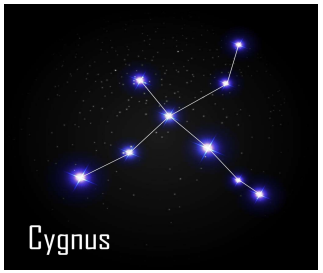
Alpha mannosidosis

- MPS1 Hurler-like phenotype

- **Treatment**
  - Supportive care
  - Immunodeficiency/ infections

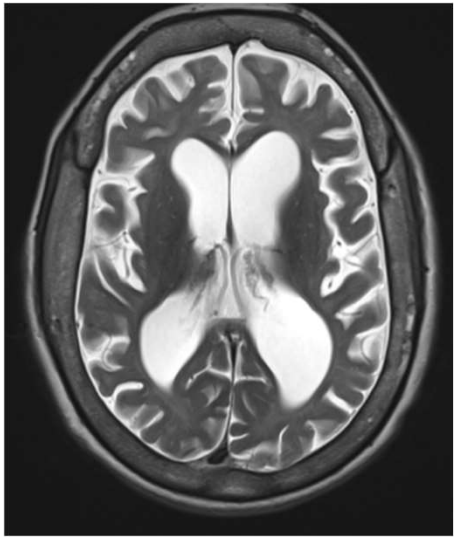
  - Replacing the missing enzyme:
    - Haemoatopoietic stem cell transplant (somatic and neurological treatment)
    - Enzyme replacement therapy (velmanase alpha) for non-neurological aspects

Another constellation…



- 6 year old boy referred to metabolic clinic
  - Born at 31 weeks gestation, preterm labour.
  - Non-consanguineous parents.
  - Followed up in paediatric clinic

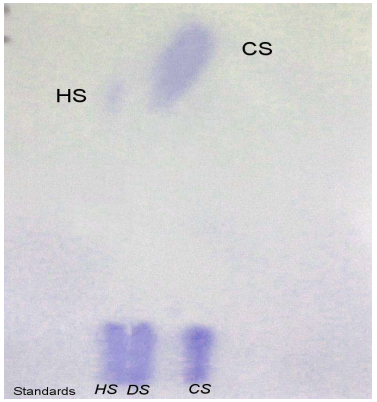
- 22 months: evident **speech and language delay**
- At 3 years
  - emerging **global developmental delay**
  - Recurrent tonsillitis and ear infections
- At 5 years: development at 18 month equivalent
- At 6 years:
  - **regression** in skills, stopped saying family names.
  - **Hyperactivity**
  - Noted to have **hepatomegaly**
  - **Coarse facial features** compared to parents.



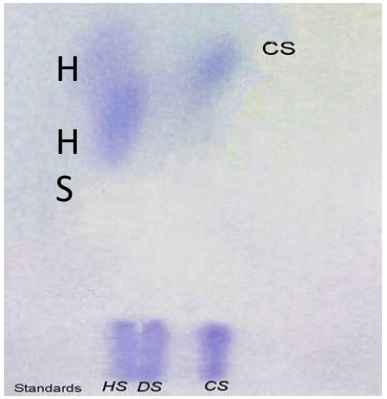
Investigations

- **Accumulated substrate?**
  - Urine GAG 67 mg/mmol creat (2-15)
  - GAG electrophoresis
    - Chondrotin sulfate
    - **Heparan & heparin sulfate**

Normal Urine



Patient Sample



- 2D electrophoresis cellulose acetate with alcian blue detection

- **Enzyme deficiency?**

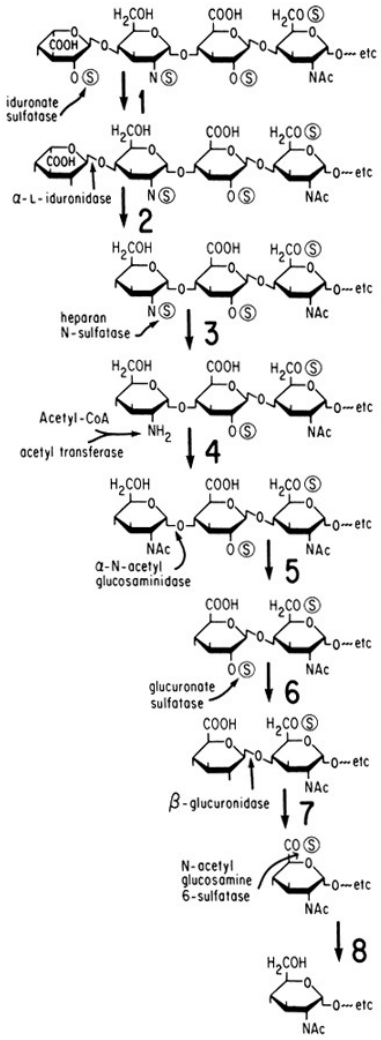
Leucocytes		Control	Affected MPS III
Heparan n sulfatase	None detected nmol/17h/mg ptn	3.2-12	0-0.4
β-galactosidase	252 nmol/hr/mg ptn	131-303	
Plasma			
α-N--acetylglucosaminidase	28 nmol/hr/ml	12.0-73	
Total b-hexosaminidase	1.62 umol/hr/ml	0.41-1.7	

- **Molecular genetics?**
  - ***SGSH***: homozygous likely pathogenic missense variant

Diagnosis: MPS IIIA (Sanfilippo)

MPS III Sanfilippo

heparansulfate (HS)



Iduronate sulfatase

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

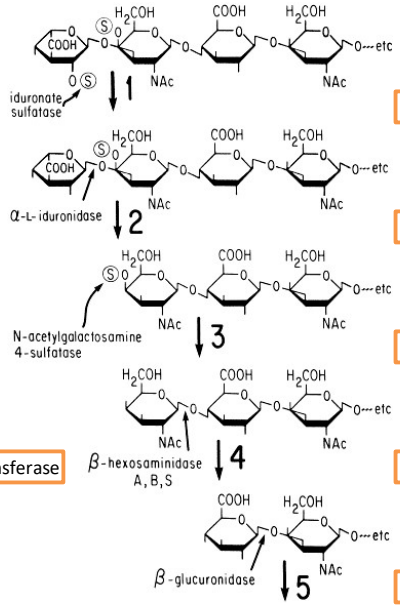
glucuronate sulfatase

β-glucuronidase

MPS III D Sanfilippo *GNS*

N-acetylglucosamine-6-sulfatase

dermatansulfate (DS)



Iduronate sulfatase

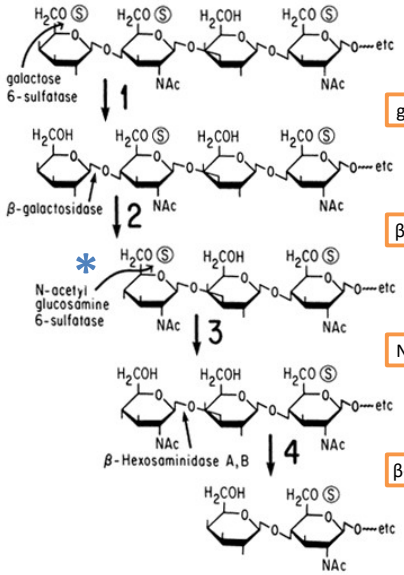
α-L-iduronidase

N-acetylgalactosamine-4-sulfatase

β-hexosaminidase A/B

β-glucuronidase

keratansulfate (KS)



galactose-6-sulfatase

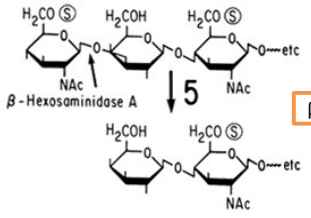
β-galactosidase

MPS III D Sanfilippo *GNS*

N-acetylglucosamine-6-sulfatase

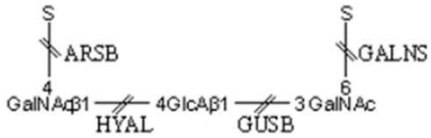
β-hexosaminidase A,B

* **alternate pathway**



β-hexosaminidase A

Chondroitin sulfate



What can you do to help?

- Supportive/symptomatic management

- Challenge of treating the central nervous system
  - "standard" ERT does not penetrate blood brain barrier
  - HSCT non-effective for MPS III

- Novel therapeutics in trial
  - Enzyme replacement therapy
    - CNS-penetrating intravenous
    - ERT via intrathecal / ICV route
  - Substrate reduction therapy
  - Gene therapy
    - In vivo AAV-based
    - Ex vivo lentiviral-based autologous HSCT

Yilmaz B, Davison J, Jones SA, Baruteau J. novel therapies for mucopolysaccharidosis type III. JIMD 2021; 44:129-147

Conclusions:

Diverse, progressive, multisystem rare disorders

Diagnosis

- Spot the constellation…. join the dots
- Look for overloaded cells, accumulated substrate, defective enzymes & gene mutations
  - Important to get the right diagnostic tests done: discuss with the laboratory!
- However… investigations for LSD can give difficult to interpret results.
  - May require repeat testing
  - Some LSD don't have good biomarkers: value of molecular genetic testing
  - Novel biomarkers (especially LS-MS/MS methods) under evaluation
- Novel emerging phenotypes from genetic analyses… need for biochemistry to evaluate VUS

Treatment and Management

- Established and Novel disease modifying treatments for many LSDs
- Holistic MDT approach to management

Lysosomal storage disorders: a practical guide (2nd ed). Mehta & Winchester