Clinical Approach to Diagnosis of Lysosomal Storage Diseases

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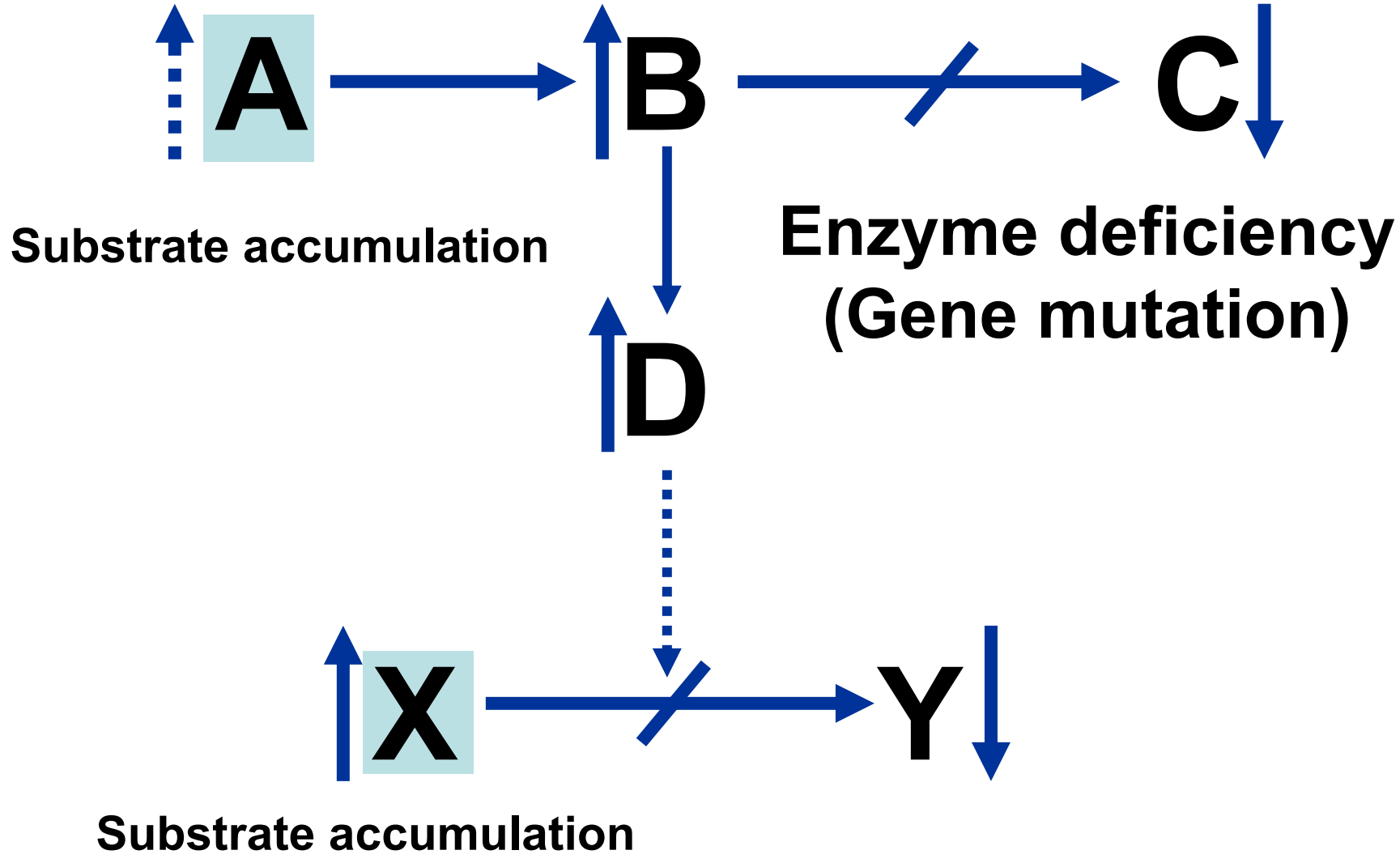
Lysosomal storage disorders …

- 45>; expanding number
- Individually rare
- as a group 1:1500-7000[1]
- high risk of recurrence (AR and XR)
- highly variable
- some are treatable
- diagnostically challenging

[1] Prevalence of lysosomal disorders, JAMA, 1999, Vol 281;3; 249-254


A
B
C
D
X
Y
Substrate accumulation
Enzyme deficiency
(Gene mutation)
Substrate accumulation

Classification

- Glycosaminoglycans
  - Mucopolysaccharidoses
- Glycoproteins
  - Oligosaccharidoses
- Glycolipids
  - Sphingolipidoses
- Lipids
  - Niemann-Pick C, Wolman
- Multi-enzyme; Trafficking; Complex

Metabolic studies for LSD

Analysis of metabolites

Blood: Chitotriosidase
Urine:
Glycosaminoglycans qn/ql
Toluidin
Thin layer chromatography

Analysis of gene product

Blood: Enzyme activity

Molecular genetic analysis

Blood/tissue:
Mutation screening

Metabolite 1: Chitotriosidase (ChT)

- Fully active chitinase expressed by activated macrophages
- Elevation in Gaucher disease and in various lysosomal disorders, malaria, thalassaemia, atherosclerosis, etc
- Anti-fungal

Chitotriosidase in LSD

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

Yufeng G et al *J. Inher. Metab.Dis.* 18 (1995)

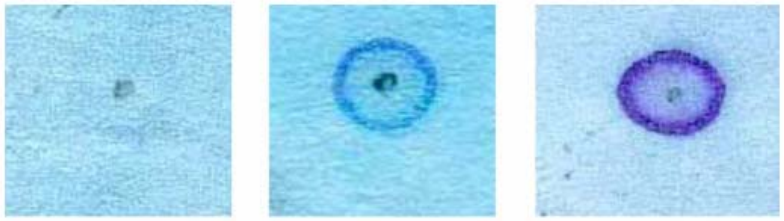
ChT activity deficiency

Table 1. Frequencies of wild-type and mutant alleles in different ethnic groups.

Population	Genotype			Reference
	Wild type	Heterozygote mutant	Homozygote mutant	
Dutch	58.5%	35.1%	6.4%	[16]
Ashkenazi Jewish	60.3%	33.8%	5.9%	[16]
Portuguese	60.0%	37.3%	2.7%	[39]
Spanish	54.3%	39.6%	6.03%	[7]
Sicilian	51.01%	44.54%	5.45%	[42]
Sardinian	65.56%	32.71%	3.73%	[42]
Benin	100%	0%	0%	[42]
Burkina Faso	98%	2%	0%	[42]

L. Malaguarnera *Cell. Mol. Life Sci*. 63 (2006) 3018–3029

Metabolite 2: Berry spot test, semiquantitative Glycosaminoglycan analysis



Negative Mild positive Positive

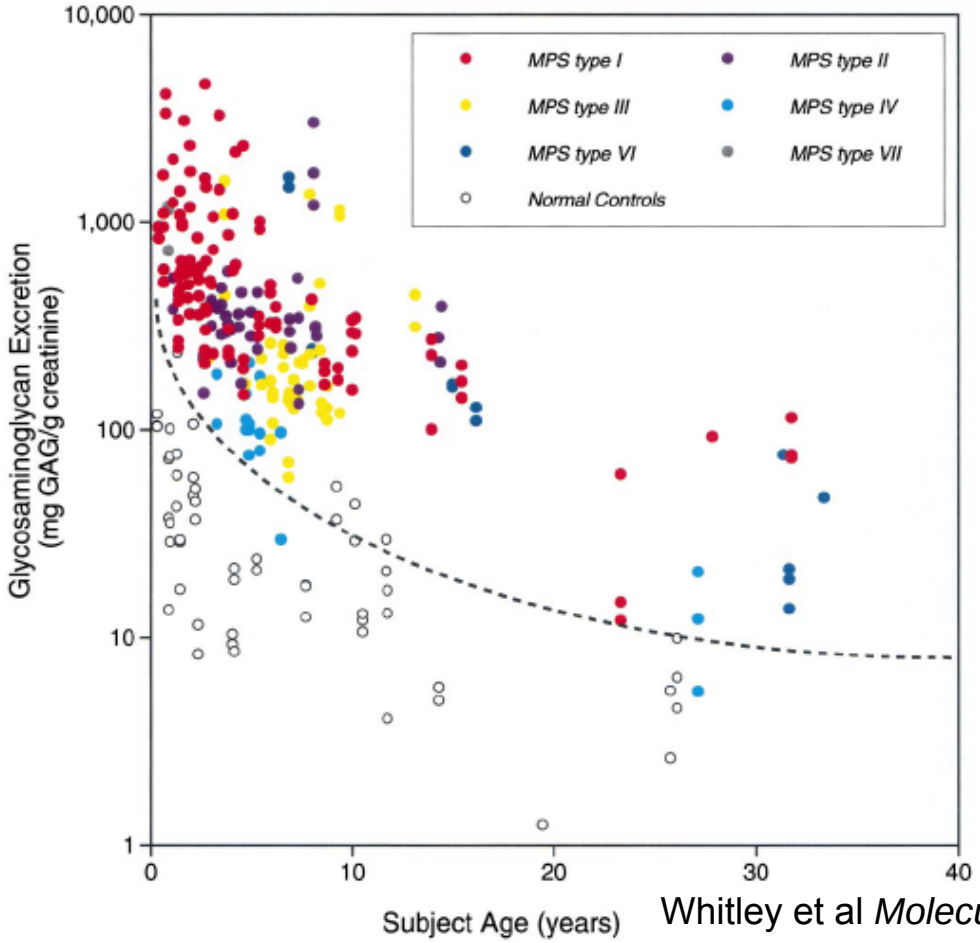
Table 1
Sensitivity and specificity of the MPS urine screening through DMB test, Berry spot test (BST) and both tests

	DMB test	BST	DMB + BST[a]
No. of false-negative samples	0/31	2/31	
%	0	6.4	
Sensitivity (%)	100	93.6	
No. of false-positive samples	52/204	94/204	32/204
%	25.5	46.1	15.7
Specificity (%)	74.5	53.9	84.3

[a] Considering as abnormal an alteration of both tests.

P. Mabe et al. / Clinica Chimica Acta 345 (2004) 135–140

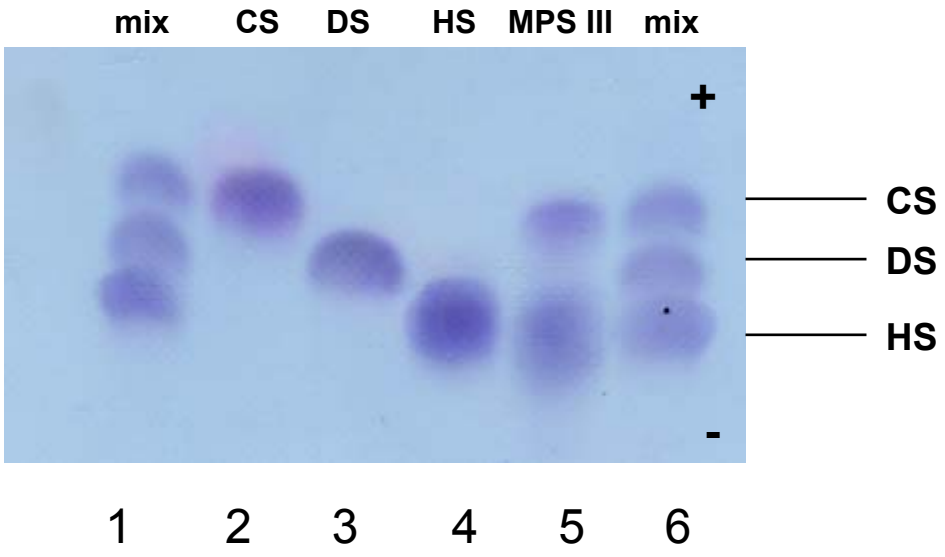
Metabolite 3: Urinary Glycosaminoglycans qn



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

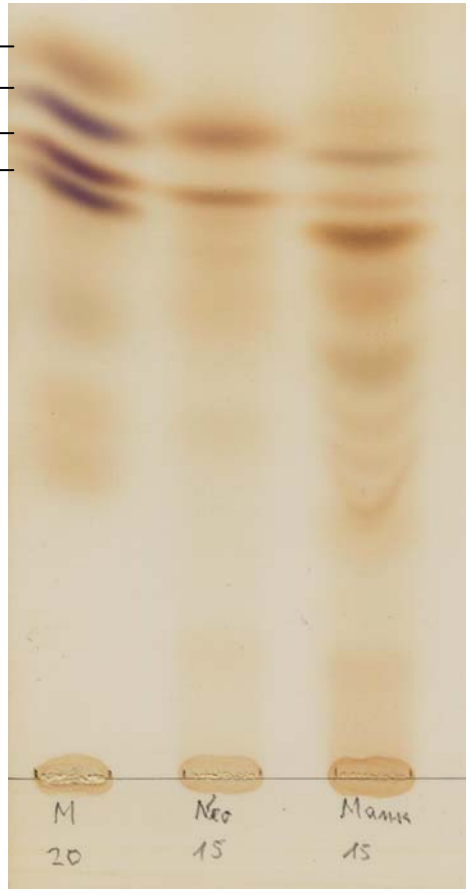
Metabolite 4: GAG electrophoresis

Electrophoresis of GAGs followed by toluidin staining



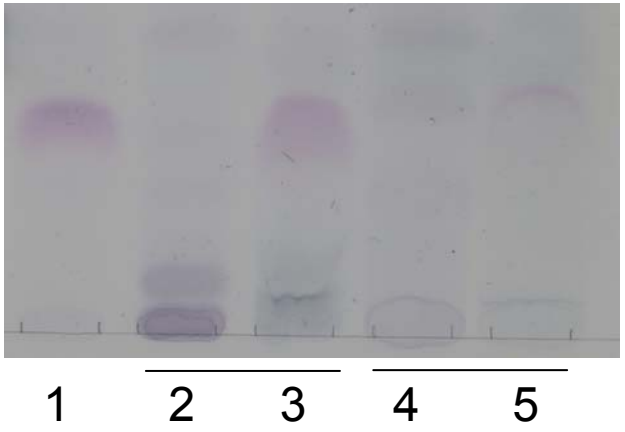
Metabolite 5: Thin layer Chromatography

Oligosaccharides



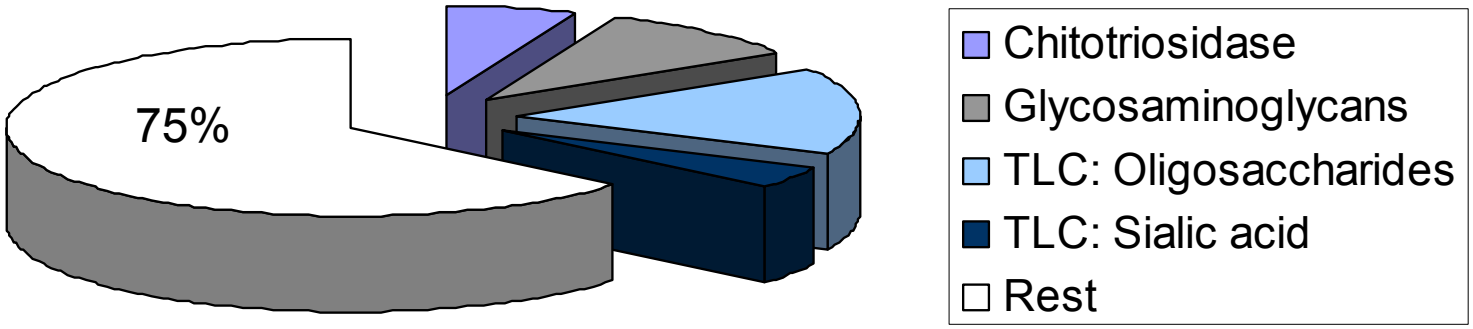
1: sugar marker
2: neonate, healthy
3: mannosidosis patient

Sialic Acid

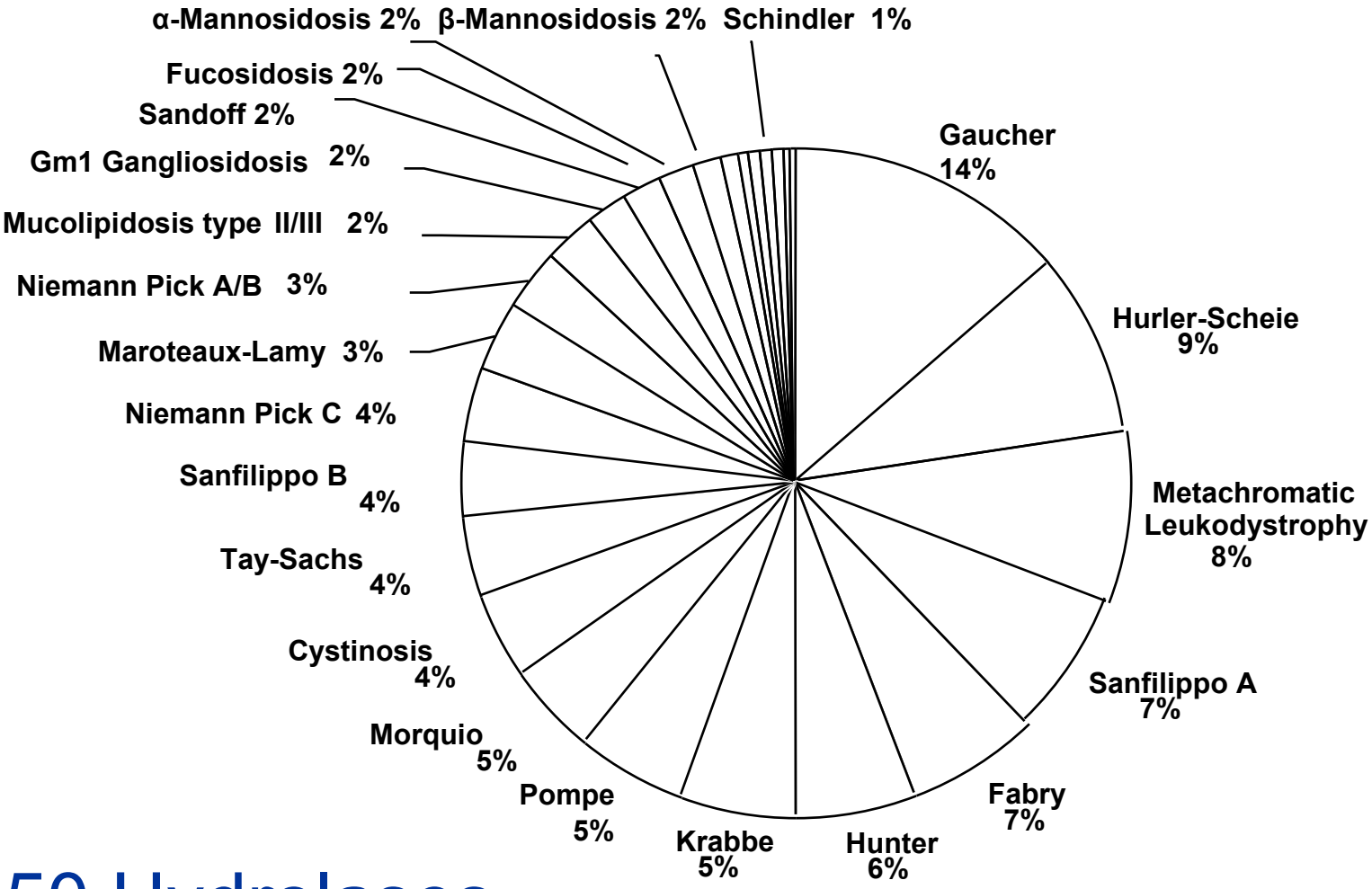


1: sialic acid (marker)
2: Galactosialidosis patient
3: Galactosialidosis patient; acidic hydrolysis
4: control
5: control ; acidic hydrolysis

LSDs detected by metabolites



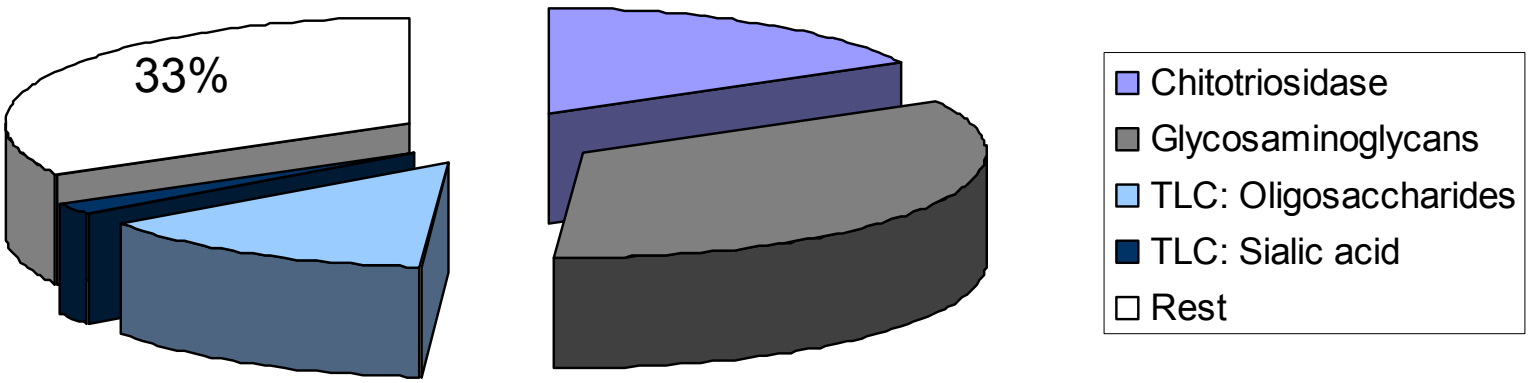
Prevalence of LSDs



> 50 Hydrolases

For Australia1980-1996; *JAMA* 281 p249 '99

Frequency of the disorder



Enzyme assay = Gene product

- Clinical features overlapping
- > 50 enzymes involved
- Exceptions: specific clinic
  - Pompe disease
  - Fabry disease
  - Gaucher disease adults

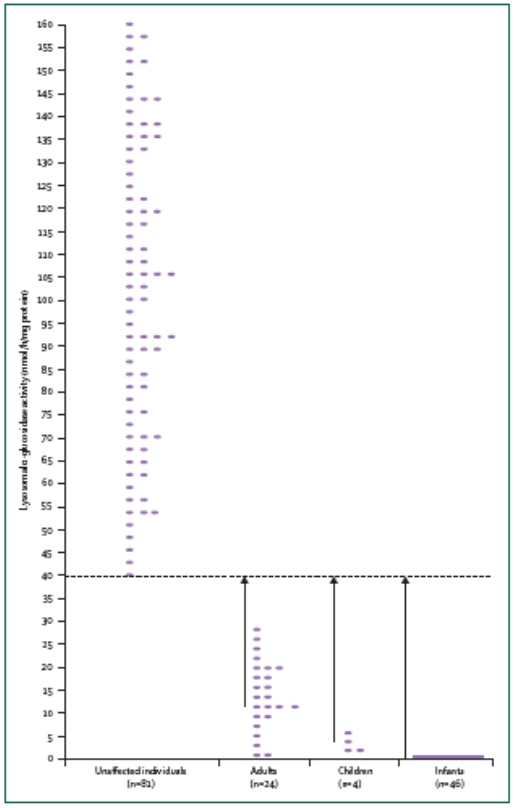
Enzyme assay challenges:

X- chromosomal:
Fabry disease

"Enzyme Activity for Determination of Presence of Fabry Disease in Women Results in 40% False-Negative Results"

Journal of the American College of Cardiology
Vol. 51, No. 21, 2008

Infantile versus adult onset:
Pompe disease

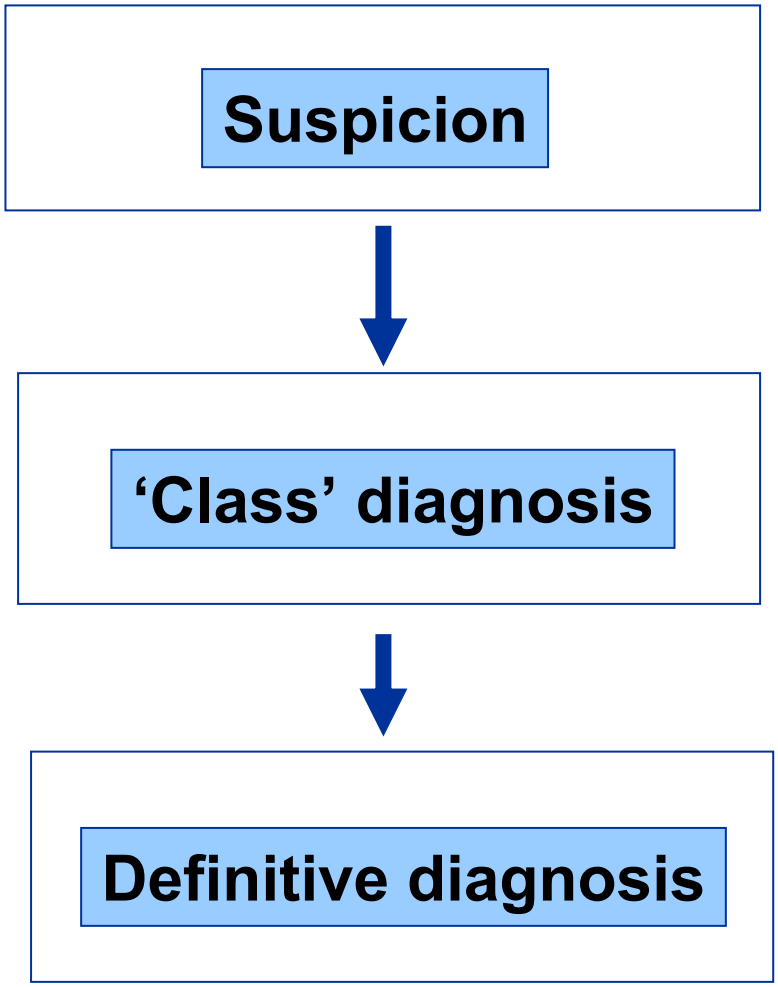


The Lancet. Vol 372 October 11, 2008

Mutation analysis

- **Confirmation**
- **Challenges:**
  - **nature of mutation, new mutations**
  - **X linked disorders, carrier detection**
  - **special problems**
    - reagents: unique
    - reaction conditions
    - allelic heterogeneity
    - polymorphisms
    - pseudogenes

Stepwise approach

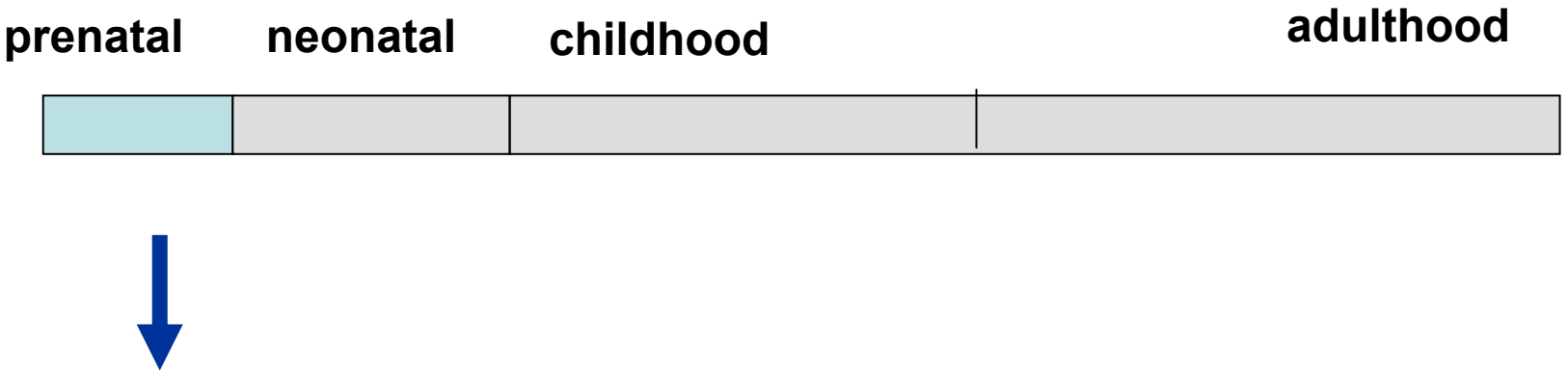


Clinical

Screening
„metabolites"

Enzyme
DNA

Clinical approach based on the time of suspicion



Non-immune hydrops fetalis

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Hydrops fetalis

Table 2
Hereditary metabolic diseases found in association with HF

Lysosomal storage diseases	Non-lysosomal diseases
Mucopolysaccharidoses:	Glycogenoses:
Mucopolysaccharidosis I (Hurler) [18]	Glycogenosis type IV (Anderson disease) [20]
Mucopolysaccharidosis IVA (Morquio A) [6]	
Mucopolysaccharidosis VII (β-glucuronidase deficiency) [7]	
Oligosaccharidoses:	Fatty acid oxidation defects:
Galactosialidosis [2]	Long-chain hydroxyacyl CoA dehydrogenase deficiency [21]
Sialidosis [27]	
GM_1-gangliosidosis [2]	
Lysosomal transport defects:	Cholesterol biosynthesis defects:
Sialic acid storage disease [7]	Smith–Lemli–Opitz syndrome [22]
	3β-hydroxysterol-Δ^{14}-reductase deficiency [23]
Sfingolipidoses:	Congenital disorders of glycosylation:
Gaucher type 2 [19]	CDG Ix [24]
Niemann-Pick A [18]	
Niemann-Pick C [2]	
Lipogranulomatosis (Farber) [2]	
Wolman [18]	
Mucolipidoses:	Others:
Mucolipidosis II (I-cell disease) [2]	Citric acid cycle-defect [25]
Others:	Hereditary hemochromatosis [13]
Multiple sulfatase deficiency	

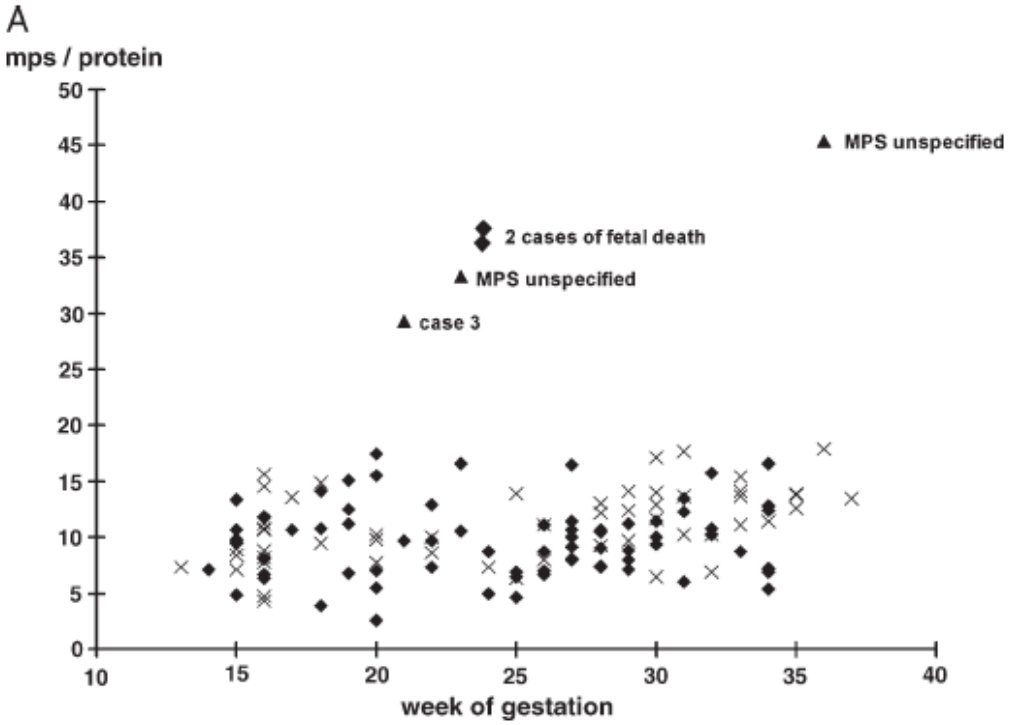
Glycosaminoglycans

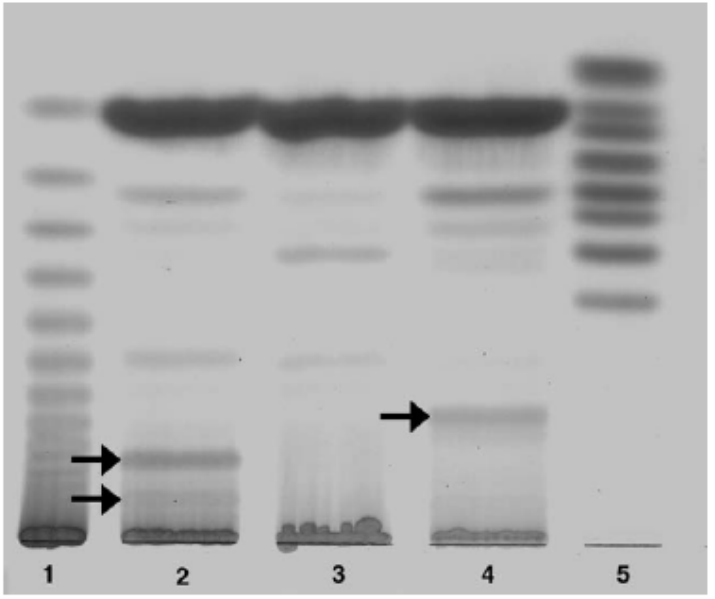
Oligosaccharides

Enzyme assay in cultured amniocytes

Clinica Chimica Acta 371 (2006) 176–182000

Prenatal GAGs and Oligos

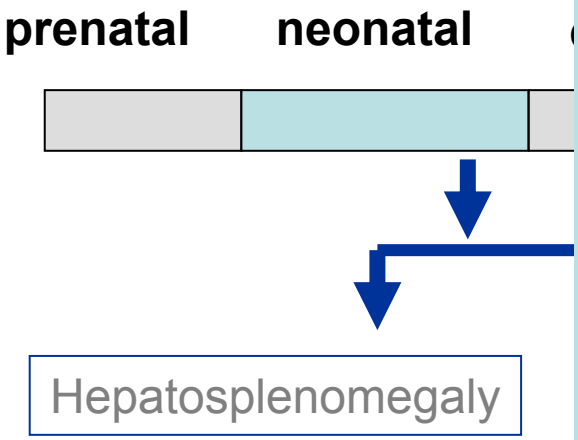




2: GM1-gangliosidosis
3: Normal amniotic fluid
4: Galactosialidosis

Clinica Chimica Acta 371 (2006) 176–182

Clinical approach based on the time of suspicion



Gaucher Type 2
Niemann Pick C
Wolman

- Sphingolipid storage disease:
  - Farber, Gaucher 2, GM1, NP A/B, Krabbe
- Mucopolysaccharide storage disease:
  - MPS I, MPS IV, MPS VII
- Glycogen storage disease:
  - Pompe
- Glycoprotein storage disease
  - Schindler, Sialidosis
- Complex storage disease
  - Wolman
- Multienzyme storage disease
- Transport and trafficking disorders
  - Sialuria, Salla

Pediatrics; Volume 123, April 2009, 1191-1270

Neonatal

Hepatosplenomegaly	Cardiomyopathy	Neurology
Gaucher Type 2 Niemann Pick C Wolman	Pompe	Pompe Gaucher Type 2 Krabbe Gangliosidoses
M: Chitotriosidase (Fillipin stain) (Cholesterol)	E: α-Glycosidase	M: Chitotriosidase TLC Oligos E: Galactocerebro-sidase

Clinical approach based on the time of suspicion

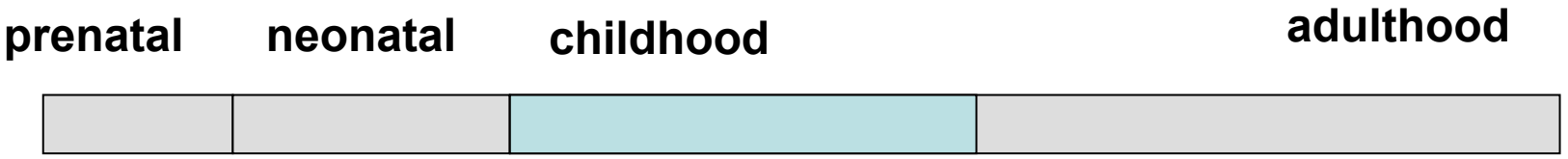


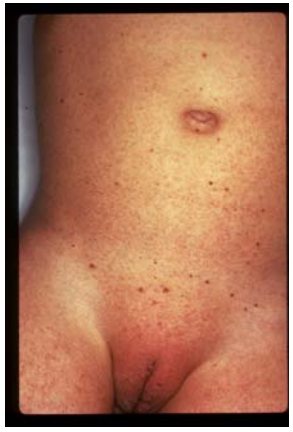
Table 1 Key clinical features of a selection of lysosomal storage disorders

Disease	Protein defect	Main clinical signs and symptoms	Chromosomal localization	OMIM
Defects in glycosaminoglycan degradation (mucopolysaccharidoses)				
MPS I (Hurler, Scheie)	α-Iduronidase	Disproportionate dwarfism, coarse facial features, visceromegaly, mental retardation in variable degree	4p16.3	607015
MPS II (Hunter)	Iduronate sulphatase	Disproportionate dwarfism, coarse facial features, visceromegaly, mental retardation in variable degree	Xq28	309900
MPS IIIA (Sanfilippo A)	Heparan *N*-sulphatase	Mental retardation ending in a vegetative state	17q25.3	252900
MPS IIIB (Sanfilippo B)	*N*-Acetylglucosaminidase	Mental retardation ending in a vegetative state	17q21	252910
MPS IIIC (Sanfilippo C)	Acetyl-CoA transferase	Mental retardation ending in a vegetative state	8p11.1	252930
MPS IIID (Sanfilippo D)	*N*-Acetylglucosamine-6-sulphatase	Mental retardation ending in a vegetative state	12q14	252940
MPS IVA (Morquio A)	*N*-Acetylgalactosamine-6-sulphatase	Severe skeletal dysplasia, spinal stenosis at the cranio-cervical junction, hearing loss, normal intelligence	16q24.3	253000
MPS IVB (Morquio B9	β-Galactosidase	Skeletal dysplasia, spinal stenosis at the cranio-cervical junction, hearing loss, normal intelligence	3p21.33	230500
MPS VI (Maroteaux–Lamy)	*N*-Acetylgalactosamine-4-sulphatase	Disproportionate dwarfism, coarse facial features, visceromegaly, normal intelligence	5q11-13	253200
MPS IX	Hyaluronidase	Short stature, joint swelling and deformities, normal intelligence	3p21.3	601492
Defects in glycoprotein degradation (oligosaccharidoses)				
α-Mannosidosis	α-Mannosidase	Slow progression, disproportionate dwarfism, coarse facial features, hearing loss, mental retardation	19q12	248500
β-Mannosidosis	β-Mannosidase	Slow progression, disproportionate dwarfism, coarse facial features, hearing loss, mental retardation	4q22	248510
α-Fucosidosis	α-Fucosidase	Severe mental retardation, skeletal dysplasia, visceromegaly, often angiokeratoma	1q34	230000
Sialidosis	α-Sialidase	Skeletal dysplasia, coarse facial features, visceromegaly, mental retardation, myoclonic seizures, cherry-red spot	6p21.3	608272
Galactosialidosis	Cathepsin A	Skeletal dysplasia, coarse facial features, visceromegaly, mental retardation, seizures, cherry-red spot	20q13.1	256540
Aspartylglucosaminuria	Aspartylglucosaminidase	Mild skeletal dysplasia, visceromegaly, mental retardation	4q32	208400
Schindler disease, Kanzaki disease	α-Acetylglucosaminidase	Very heterogeneous: neuroaxonal dystrophy with rapid neurodegenerative course in some cases, in other cases angiokeratoma being the only sign	22q13.1	104170
Defects in glycolipid degradation				
GM_1-gangliosidosis	β-Galactosidase	Neurodegenerative course, visceromegaly, skeletal dysplasia, cherry-red spot	3p21.33	230500
GM_2-gangliosidosis (Tay–Sachs)	α-Subunit of β-Hexosaminidase	Neurodegenerative course, startle reaction, cherry-red spot, no visceromegaly, no skeletal dysplasia	15q23	606869
GM_2-gangliosidosis (Sandhoff)	β-Subunit of β-Hexosaminidase	Neurodegenerative course, startle reaction, cherry-red spot, visceromegaly, mild skeletal dysplasia	5q13	606873
GM_2-gangliosidosis (variant AB)	GM_2 activator protein	Neurodegenerative course, startle reaction, cherry-red spot, no visceromegaly, no skeletal dysplasia	5q31	272750
Gaucher disease	β-Glucocerebrosidase	Visceromegaly, anemia, thrombocytopenia, bone disease. Involvement of the central nervous in type II and III	1q21	606463
Fabry disease	α-Galactosidase	Multisystemic disorder involving heart, kidney, central nervous system. Angiokeratoma are a specific sign	Xq22.1	301500
Defects in lipid degradation				
Niemann–Pick type A and B	Sphingomyelinase	Visceromegaly, neurodegenerative course (type A), visceromegaly, lung involvement (type B)	11p15.2	607808
Farber disease	Acid ceramidase	Joint swelling and deformities, hoarseness, mental retardation in variable degree	8q22	228000

M. Beck, *Hum Genet* (2007) 121:1–22

Childhood

prenatal | neonatal | childhood | adulthood

- Skin
  - 
  - α-Fucosidosis
  - Fabry → M: Oligosaccharides; E: α-Galactosidase A and gene analysis
- Progressiv neurology and mental deterioration +/- Ataxia, +/- Hypotonia
- Coarse/ Hepatosplenomegaly Skeletal
  - Mucopolysaccharidoses
  - Oligosaccharidoses
  - → M: Glycosaminoglycans; TLC: Oligosaccharides
- Ophthalmology

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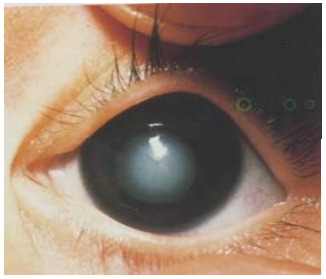
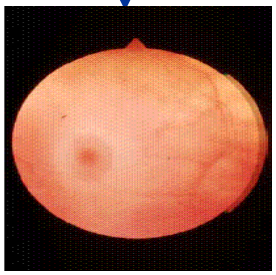
Ophthalmology



Cataracts

Sialidosis
α-Mannosidosis

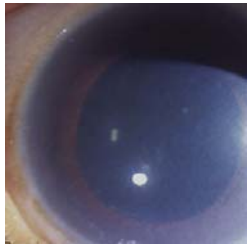
M: TLC



Cherry red spot

Galactosialidosis
GM1, GM2
α-Mannosidosis
Sialidosis

M: TLC Oligosaccharides
E: β-hexosaminidase A/B



Corneal Clouding

MPS I/IV/VI
α-Mannosidosis

M: GAG,
TLC Oligos

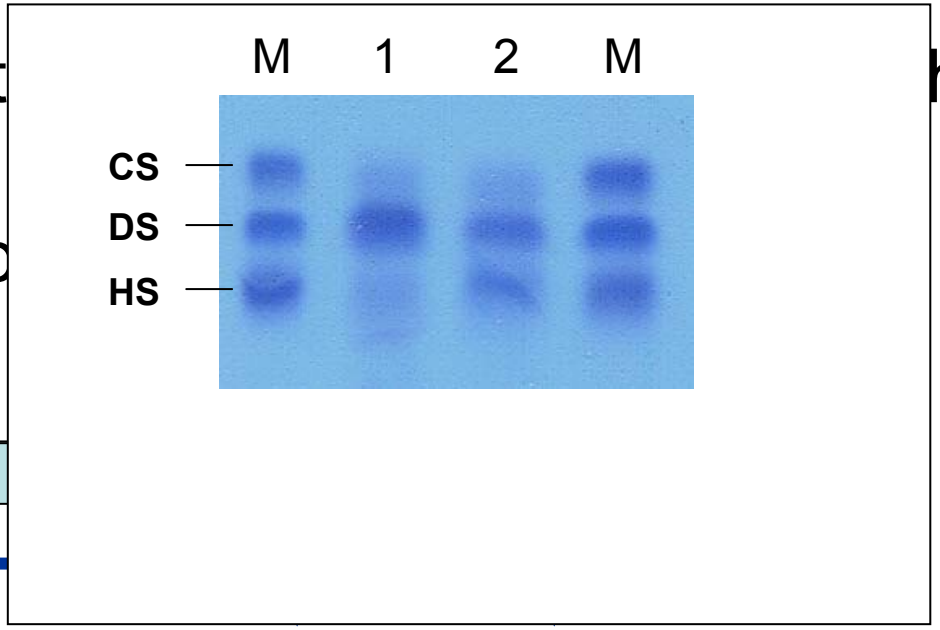
Childhood

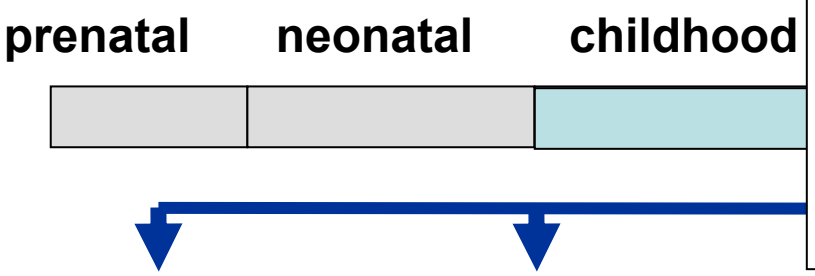
Progressiv neurology and mental deterioration

Skeletal	Hepatosplenomegaly	Ataxia or Hypotonia	Psychic dysfunction
Mannosidosis Fucosidosis Galactosialidosis Siladisosis	Niemann Pick C	GM1 GM2 Salla Sialic acid storage	Niemann Pick C Schindler
M: TLC E: Confirmation	M: ChT Filippin staining G: Mutation	M: TLC Oligosaccharides + Sialic acid E: Confirmation	M: ChT (Filippin staining) TLC E: Confirmation

Clinical case 1

4.5 year old boy, recent … h
restricted movement,
mild mental retardatio





Skin

Progressiv neurology and mental deterioration +/- Ataxia

Coarse/ Hepatosplenomegaly/ skeletal

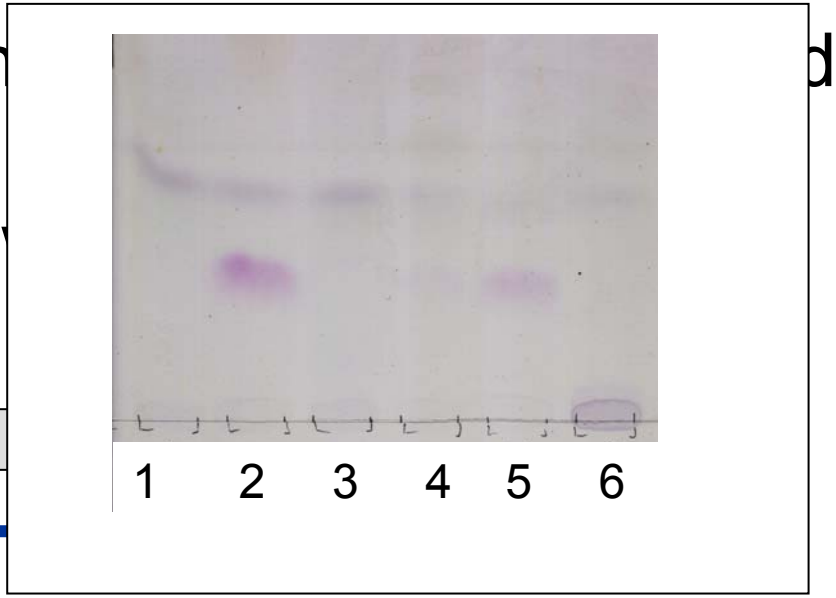
Ophthalmology

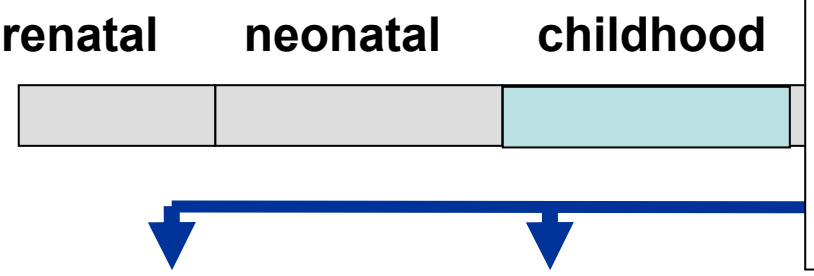
M: GAG‘s qn elevated
GAG electrophoresis

MPS II

Clinical case 2

6 year old girl with promin d
mild mental retardation
Only able to speak a fe





Skin

Progressiv neurology and mental deterioration +/- Ataxia +/- hypotonia

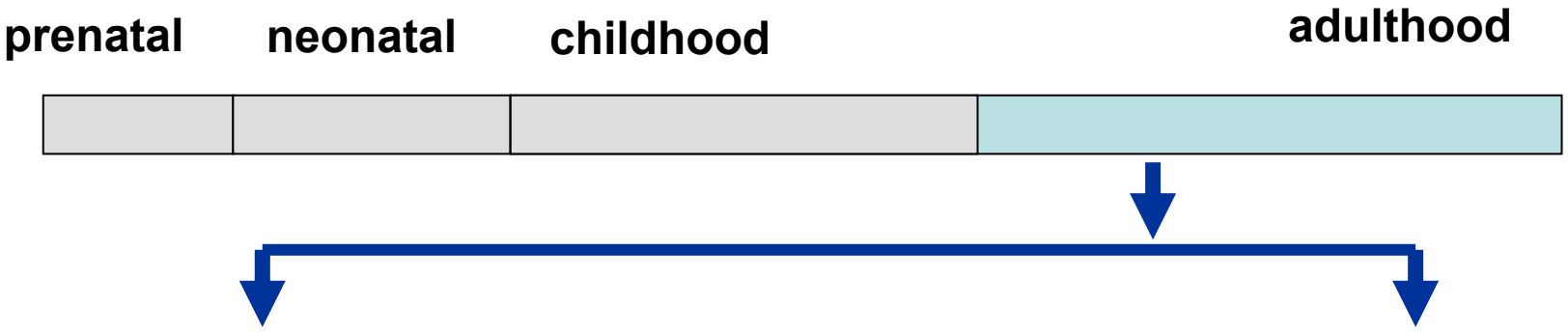
Coarse/ Hepatosplenomegaly/ skeletal

Ophthalmology

M: TLC for Oligosaccharides/ Free Sialic Acid

Salla disease

Adulthood



Renal/Cardiology

Fabry

E: in male
M: in female

Progressiv neurology

Ataxia and Dementia

MLD
GM1/GM2
Niemann Pick C
Neuronal ceroid lipofuscinosis

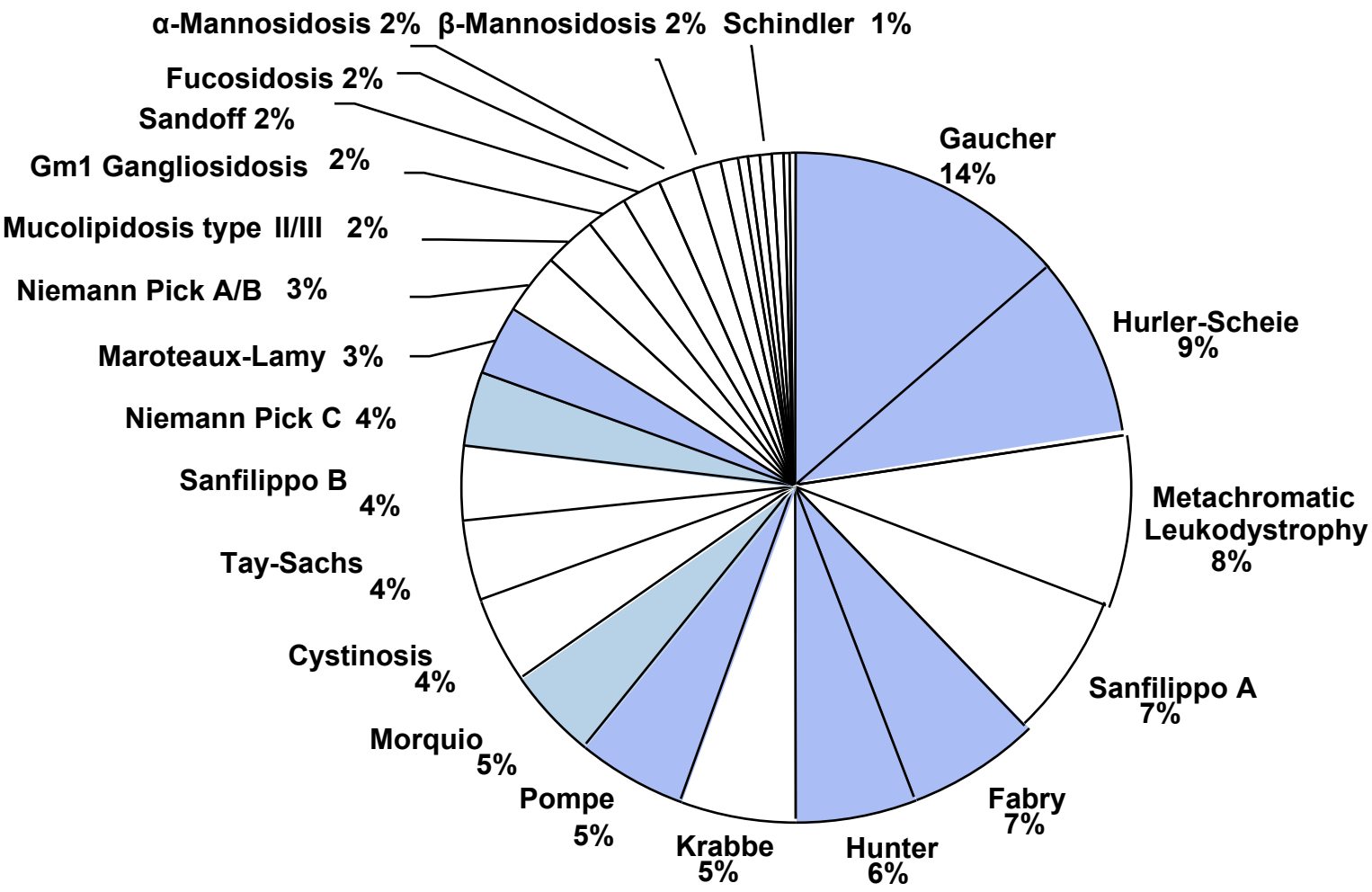
M: Oligosaccharides; CTh
Fingerprints in biopsies
E: Arylsulfatase A

Without mental deterioration

Late onset Krabbe

E: Galacto-cerebrosidase

Treatable LSD



Conclusion

- > 45 lysosomal storage disoders
- Specific diagnosis is challenging
- Critically important to patient management
- Some are treatable
- Clinical features
- Stepwise approach: Metabolite, Gene product, Mutation analysis