

Survey of diagnosis of lysosomal storage disorders

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prehistory – empirical part of the story

clinical reports by Tay (1881), Gaucher (1882) and Sachs (1896)
and by others

modern history of the lysosomes

their discovery: *C. de Duve et al.*
(*Biochem. J.* 60, 604, 1955)

Nobel Prize 1974



modern history of the lysosomal storage

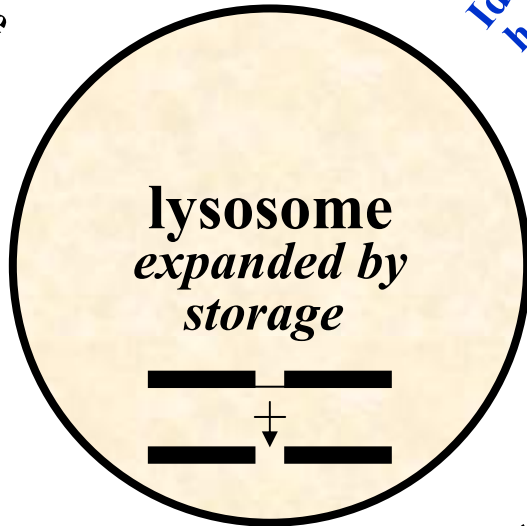
- *H.G. Hers et al. (1963) Acid glucosidase deficiency in GSD II*
- *Austin et al. (1963) Arylsulphatase deficiency in MLD*

**present state of the art (2006) – 48 defined entities
of different molecular basis (groups Ia,b and II)**

enzymopathies
due to mutant
enzyme
protein
(n=30)

lysosomal storage disorders Ia

MPS
n=10



- α-L-iduronidase*
- Iduronate-2-sulphate sulphatase*
- heparan N-sulfatase*
- NAC-α-D-glucosaminidase*
- CoA:α-glucosaminide NAc-transferase*
- GlcNAc- 6-sulphate sulphatase*
- GalNAc-6-sulphate sulphatase*
- GalNAc-4-sulphate sulphatase*
- β-glucuronidase*
- * hyaluronidase (hyaluronic acid)*
- * N-acetyl-α-galactosaminidase*
- * aspartylglucosaminidase*
- β-Mannosidase*
- α-Mannosidase*

neuronal ceroid lipofuscinoses

GSD II

- tripeptidylpeptidase I*
- Cathepin D*
- Palmitoyl-protein thioesterase*
- acid α-1,4-glucosidase*
- acid lipase*
- β-glucosylceramidase*
- ceramidase*
- β-galactosylceramidase **
- sphingomyelinase **
- arylsulfatase A*

lipidoses
n=9

- NAC-β-glucosaminidase B*
- NAC-β-glucosaminidase A*
- α-galactosidase A*
- α-neuraminidase*
- * α-Fucosidase*
- β-galactosidase*

glycoproteinoses
n=7

29 hydrolases
1 transferase

I.A lysosomal enzymopathies caused by mutation of the enzyme protein

mutated enzymes degrading lipids, GPs, proteins, and glycogen

1. ceramidase (N-Acyl-sfingoid-Cer)
2. β -glucosylceramidase (GlcCer)
3. β -galactosylceramidase (GalCer)
4. arylsulphatase A (SGalCer)
5. NAc- β -glucosaminidase A (GM2)
6. NAc- β -glucosaminidase B (GM2,GP)
7. β -galactosidase (GM1, OLS, GP, KS)
8. α -galactosidase A (Gb3Cer)
9. sphingomyelinase (P-cholin-cer)
10. acid lipase (CE, TAG)
11. α -neuraminidase (GP, gangliosides?)
12. N-acetyl- α -galactosaminidase (GP, blood gr. A: α GalNAc- [Fuca]- β gal-;)
13. acid α -1,4-glucosidase (glycogen)
14. α -Mannosidase (GP)
15. β -Mannosidase (GP)
16. α -Fucosidase (GP)
17. aspartylglucosaminidase (GP)
18. tripeptidylpeptidase I – TPP (prot.)
19. palmitoyl-protein thioesterase-PPT

20. cathepsin D

mutated enzymes degrading GAGs

21. α -L-iduronidase (DS, HS)
22. Iduronate-2-sulphate sulphatase(DS,HS)
23. heparan N-sulphatase (HS)
24. NAc- α -D-glucosaminidase (HS)
25. CoA: α -glucosaminid NAc-transferase (HS)
26. GlcNAc- 6-sulphate sulphatase (HS)
27. GalNAc-6-sulphate sulphatase (KS,C6S)
28. GalNAc-4-sulphate sulphatase (DS)
29. β -glucuronidase (DS,HS,C4S,C6S)
30. hyaluronidase (hyaluronic acid)

30 lysosomal enzymes

29 hydrolases +1 transferase

30 proteins 30 genes 30 entities

memento – there is more than 40 lysosomal enzymes known !!

I.B deficient enzyme associated functions (n=8)

deficient posttranslational processing (n= 2)

- *abnormal targeting* of lys. enzymes extracellularly (deficient synthesis of Mannose-6P label – mucopolipidosis II/III)
- *deficient synthesis of active site* in a group of sulphatases (special enzyme in ER: FGE formylglycine generating enzyme or SUMF1 sulphatase modifying factor 1): *cystein* → *formylglycine* (*PSD = MPS + sulphatidosis, ect*)

**deficient protection=increased degradation (galactosialidosis)
(protection by cathepsine A) (n=1)**

deficient lysosomal enzyme activators (n=5)

- *SAPs A-D* (pSap deficiency): A (GALC*ase*); B (ASA, α Gal*ase*);
C (GC*ase*); D (ceramidase)
- *hexosaminidase activator* (alternat. Tay-Sachs)

total 38 enzymopathic entities to be diagnosed
prime importance: enzyme activity assay

*mechanism of the deficient
activity should be specified*

*DNA analysis is of
secondary importance*

II. lysosomal disorders due to deficiency of noncatalytic membrane components (n = 10)

A. transporters across the lysosomal membrane(n=2)

- cystinosis**
- sialic acid storage disease**

B. mutant lysosomal membrane proteins operating in the membrane lipid trafficking and by so far unknown (albeit essential) mechanisms (n<8)

NCL3, NCL5, NCL6, NCL 8 (neuronal ceroid lipofuscinoses) proteins, ML IV, NPC1, NPC2 (Niemann-Pick disease type C), LAMP 2 (Danon disease)

***total 10 entities to be diagnosed at the protein/metabolite levels
final diagnosis the DNA level***

(gene coding the dysfunctional protein)

!! activities of all the lysosomal enzymes are in the normal range !!

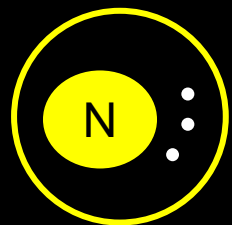
**I. A, B (n=38)
deficiencies
in breakdown
catalysis**

**II. (n=10)
deficiencies of noncatalytic
lysosomal membrane
functions**

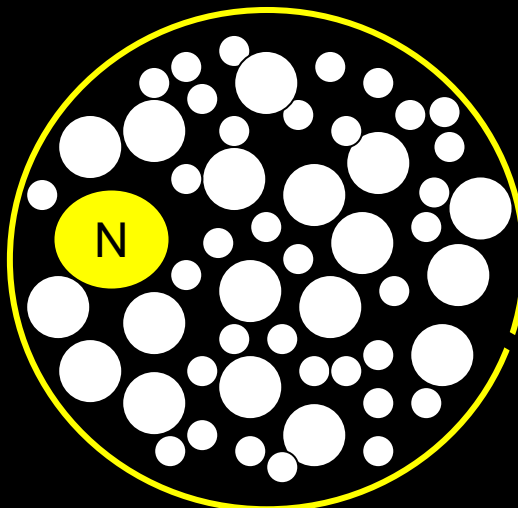
**48
entities**

**hall mark: „storage“- overfilling
and gradual lysosomal expansion by:**

- *undegraded enzyme substrates*
- *unremovable enzyme products*
- *misshandeled heterogenous compounds*

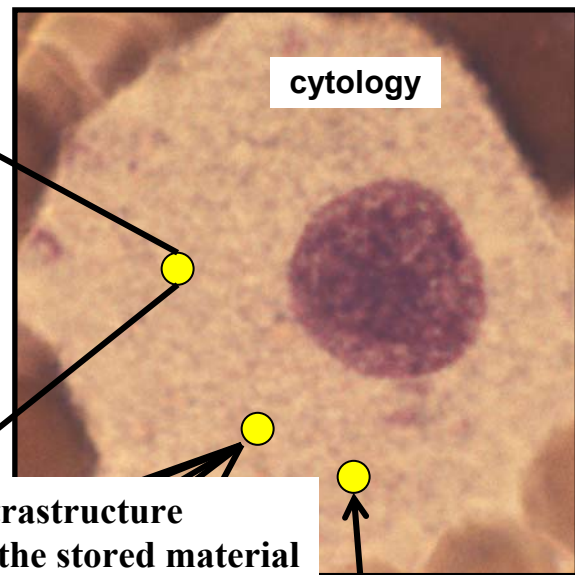
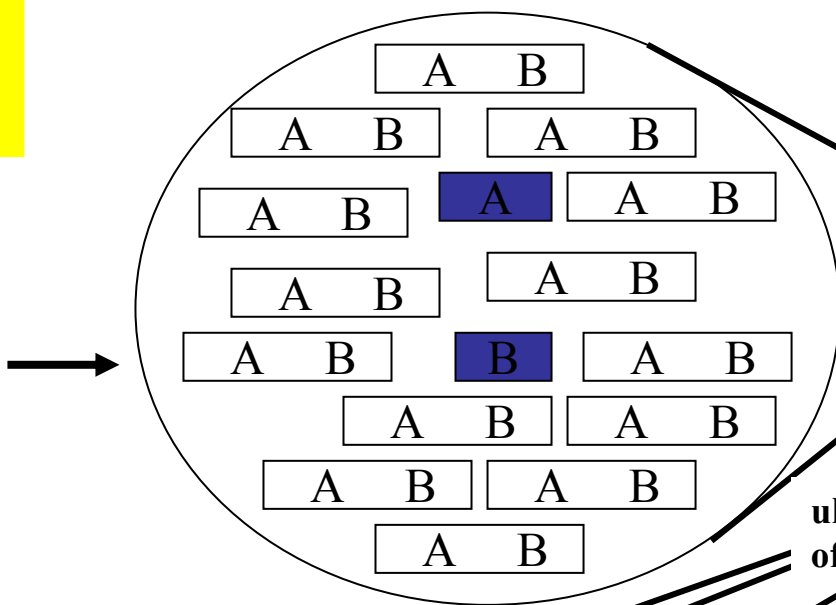
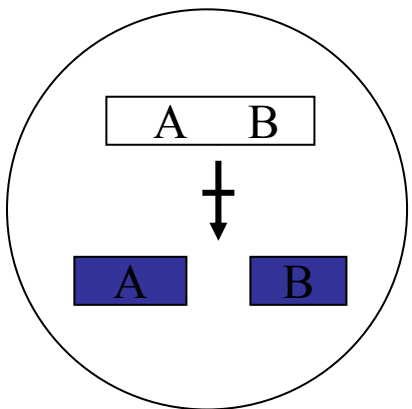


normal cell

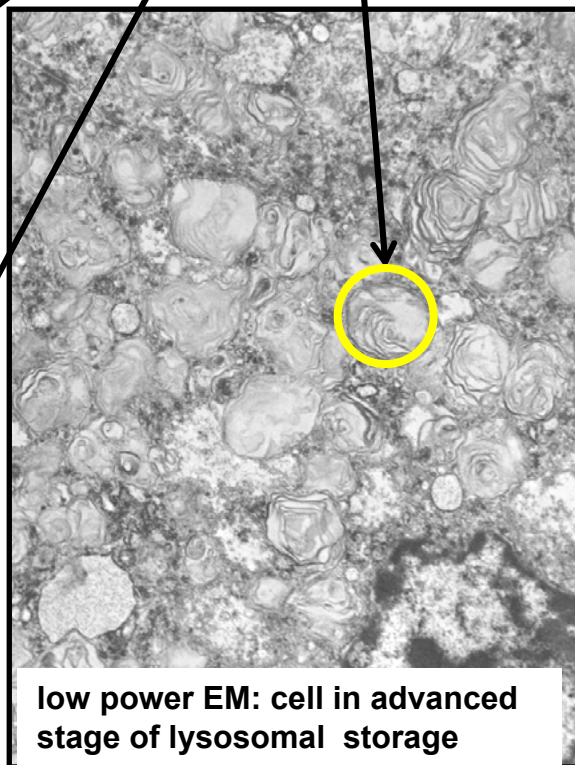
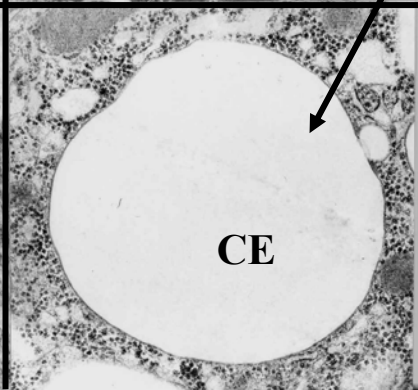
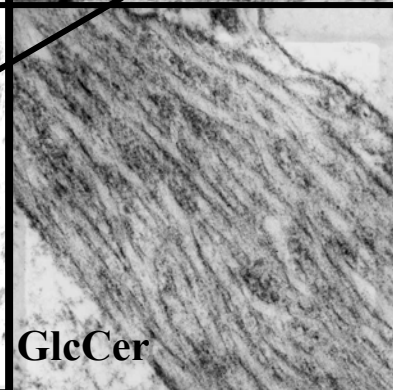
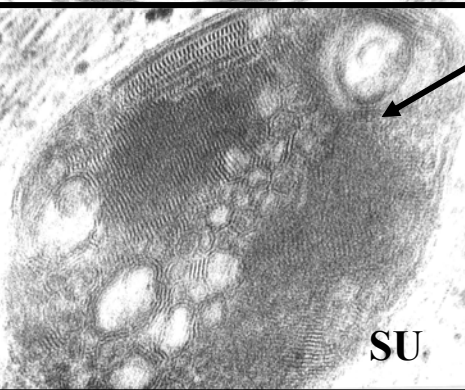
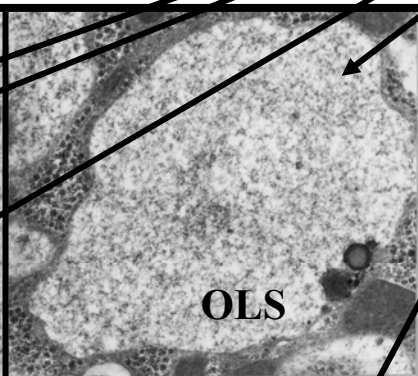
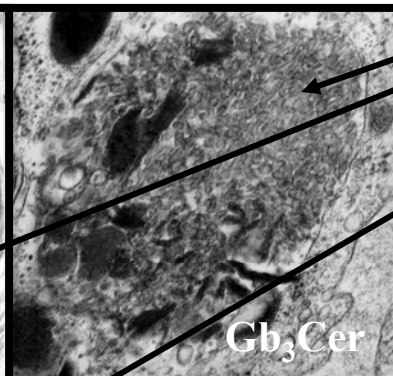
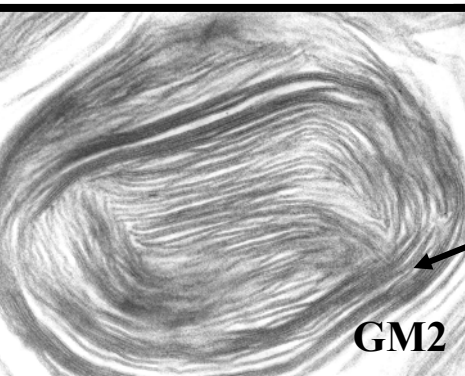


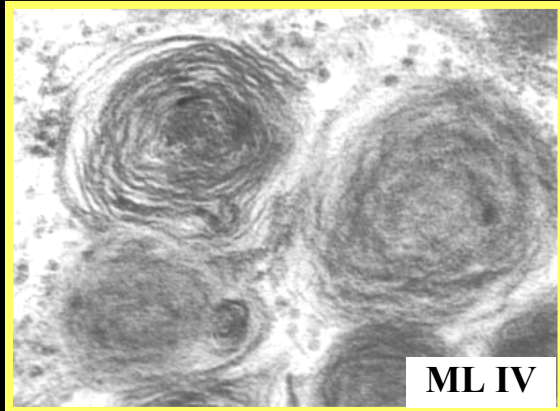
storage cell

Lysosomal enzymopathy cytology - EM

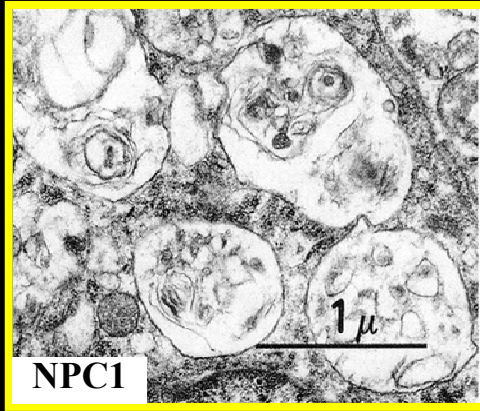


ultrastructure
of the stored material

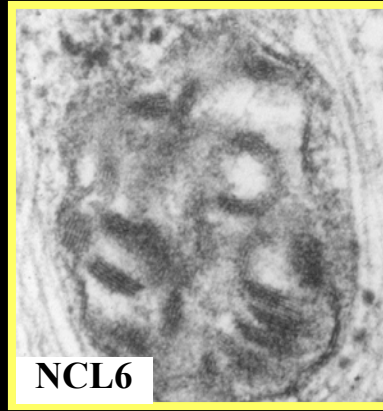




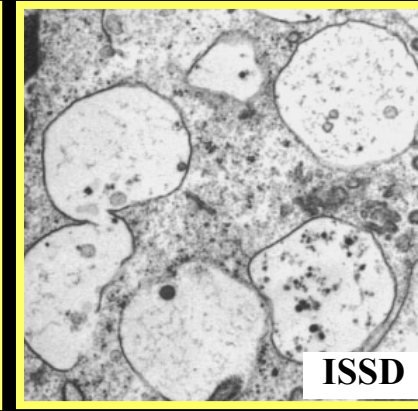
ML IV



NPC1



NCL6



ISSD

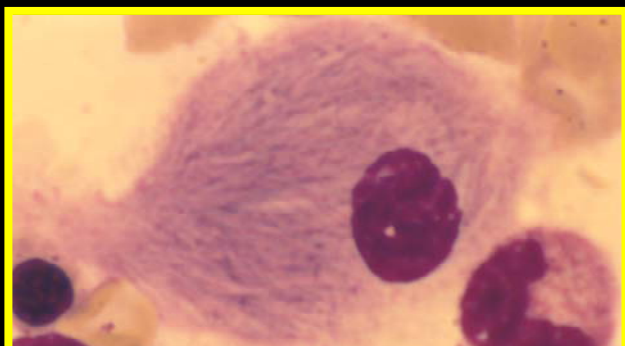
**loss of mucopolin function
(function unknown)
mixture of lipids stored**

**loss of NPC1 protein function
(altered lipid trafficking)
mixture of lipids stored**

**loss of NCL 6 protein
(function unknown)
SCMAS storage**

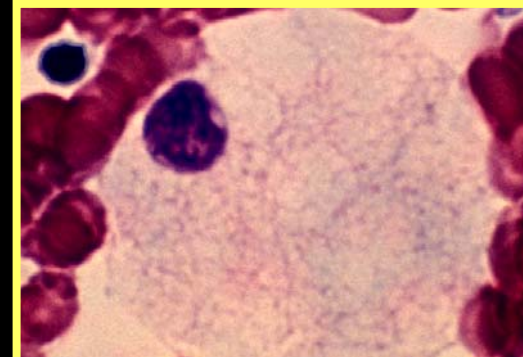
**loss of SA transporter
retention of sialic acid**

EM patterns in group II LSDs



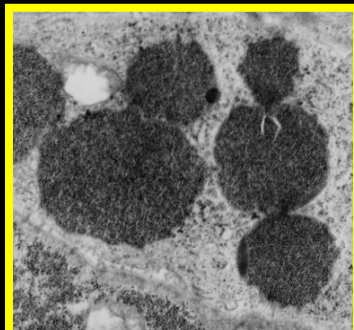
striated pattern-GC (2D distension)

**basic cytological patterns
of lysosomal storage**

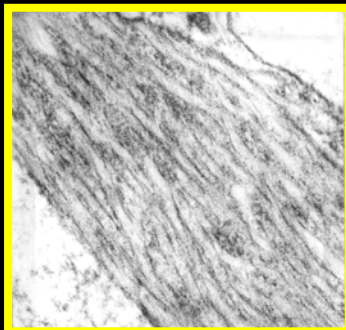


foamy pattern (3D distension)

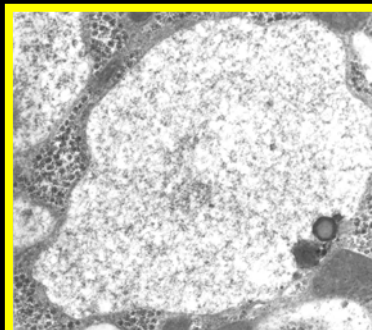
**EM patterns in uncleaved
substrate accumulation**



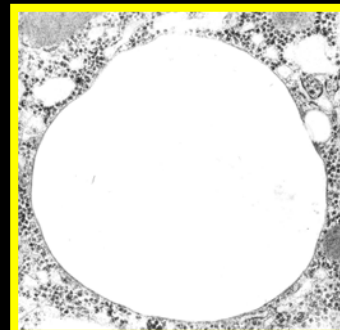
GSD II (glycogen)



Gaucher (GlcCer)



MPS (GAGs)



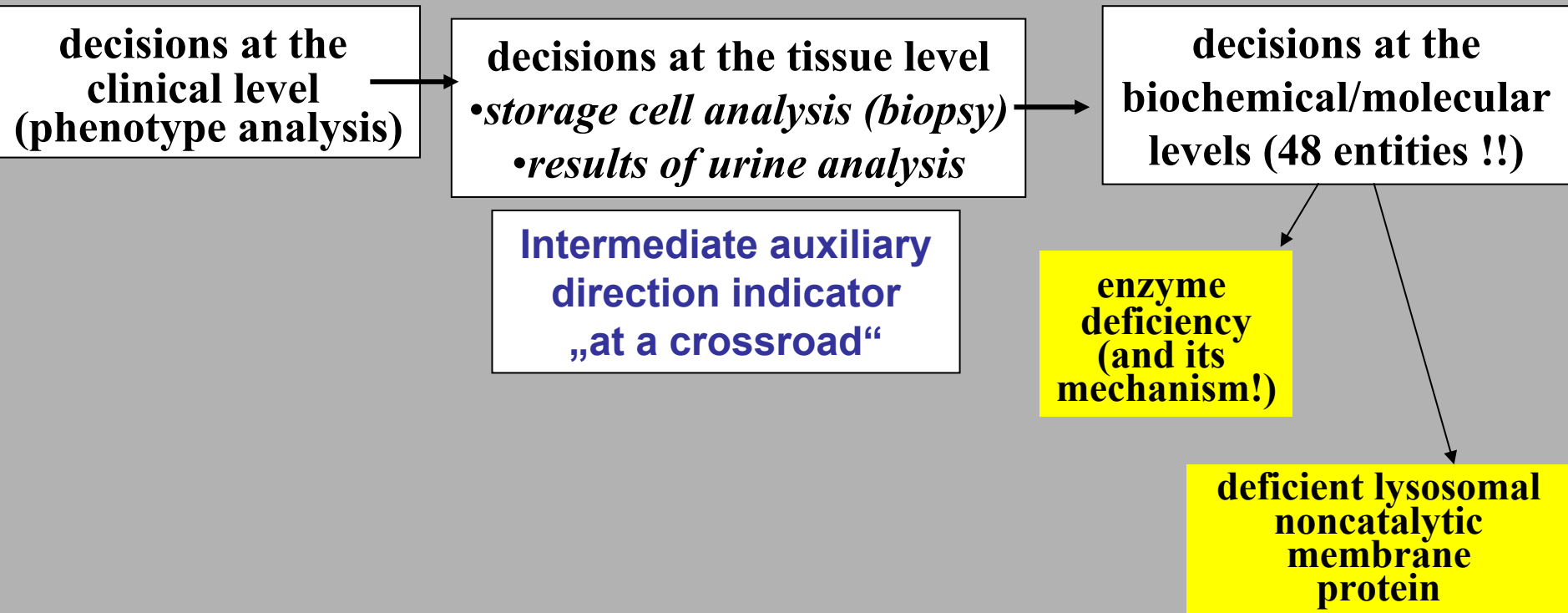
CESD (CE storage)



Tay-Sachs (GM2)

each of the molecular defects is a specific biological entity informing indirectly about the level of a critical lysosomal function (e.g. degradative, trafficking) in normal human (eukaryotic) tissues

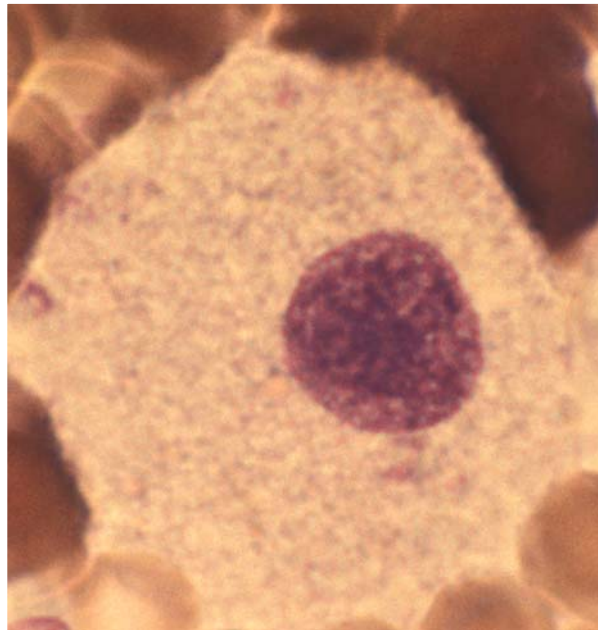
lysosomal storage disorders *the diagnostic approach*



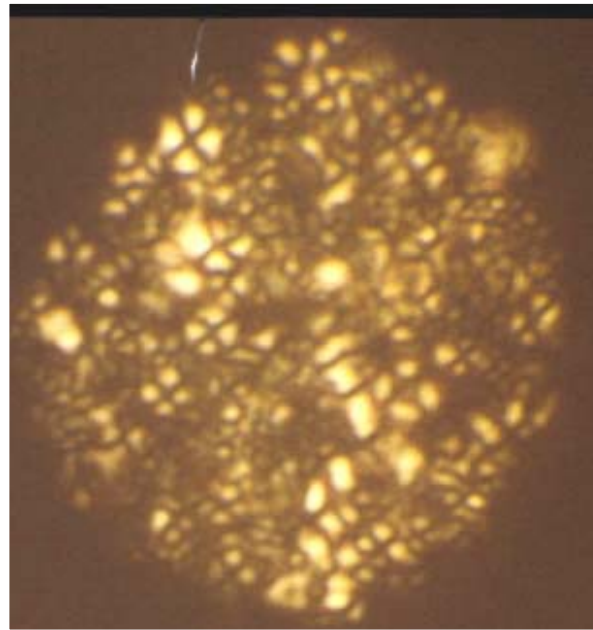
**specific storage pattern at the cell level:
uniform storage of SM liquid crystals
in a b.m. histiocyte**

↓
**recommendation:
*ASM activity evaluation***

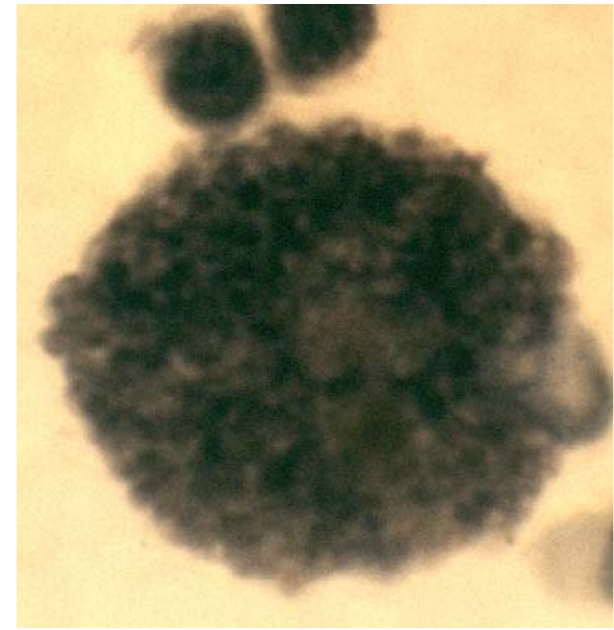
↓
Niemann-Pick type A



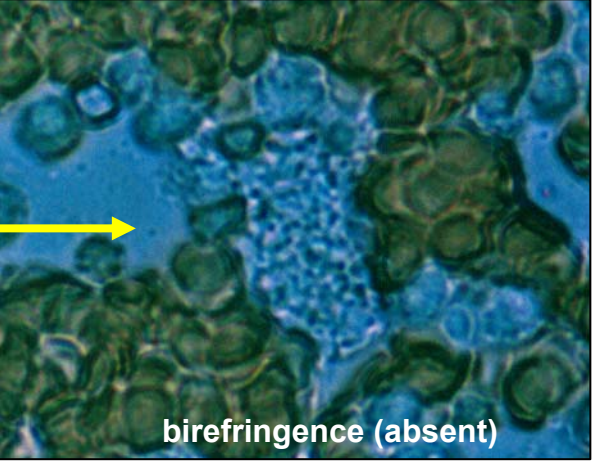
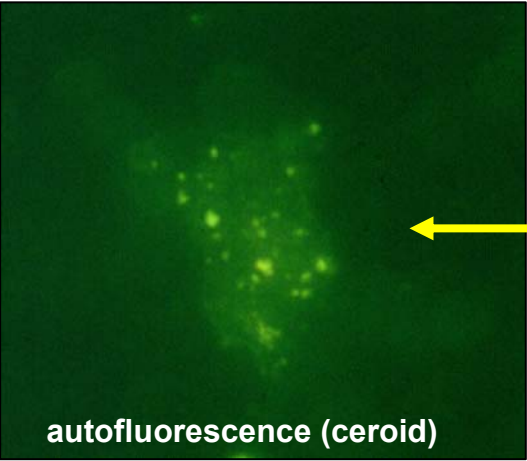
foamy storage pattern



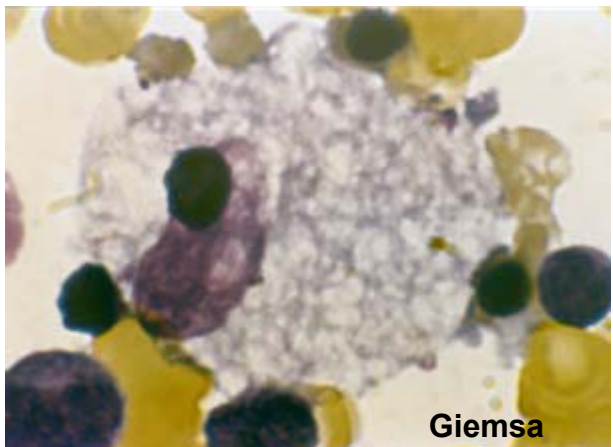
birefringence of SM liquid crystals



**uniform staining
for phospholipids**



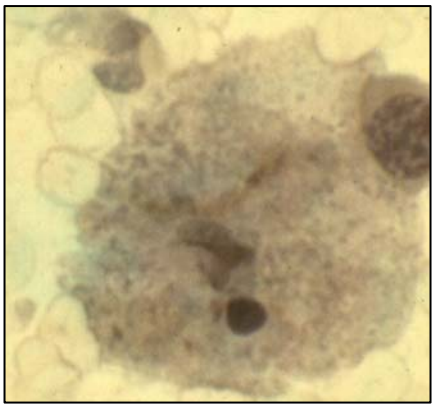
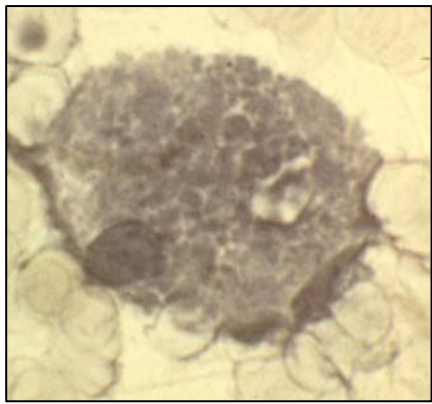
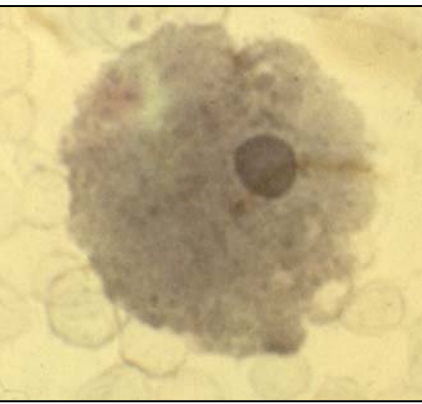
**bone marrow storage
pattern in NPC
cytology and
staining**



*variable storage
of phospholipids
In isotropic state
(admixture of ceroid)*

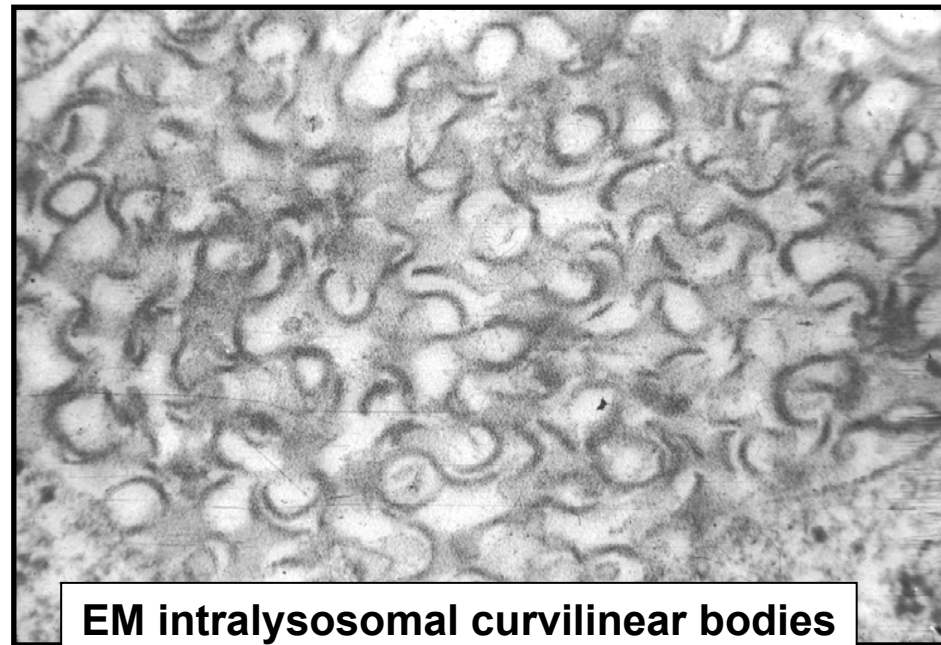
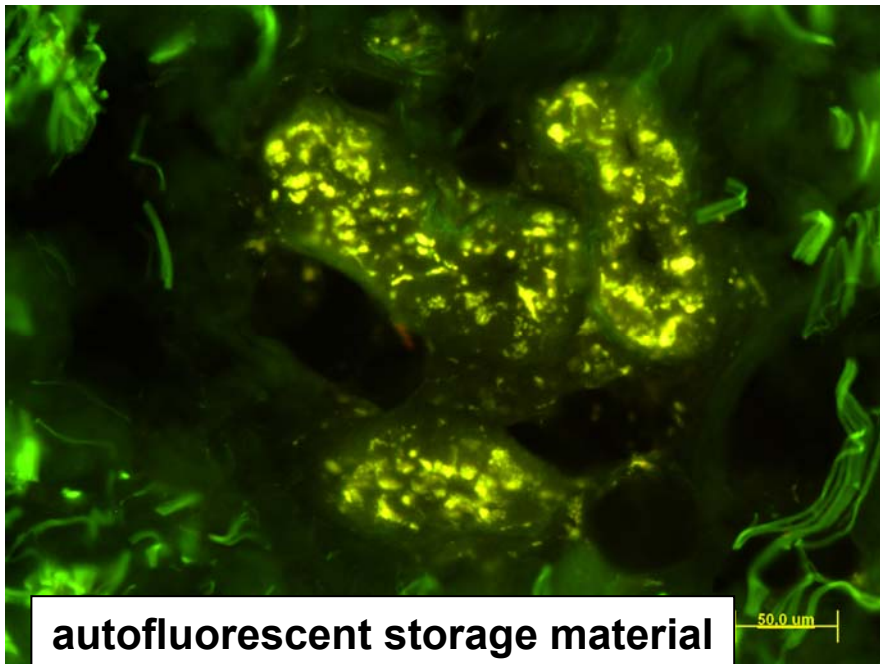
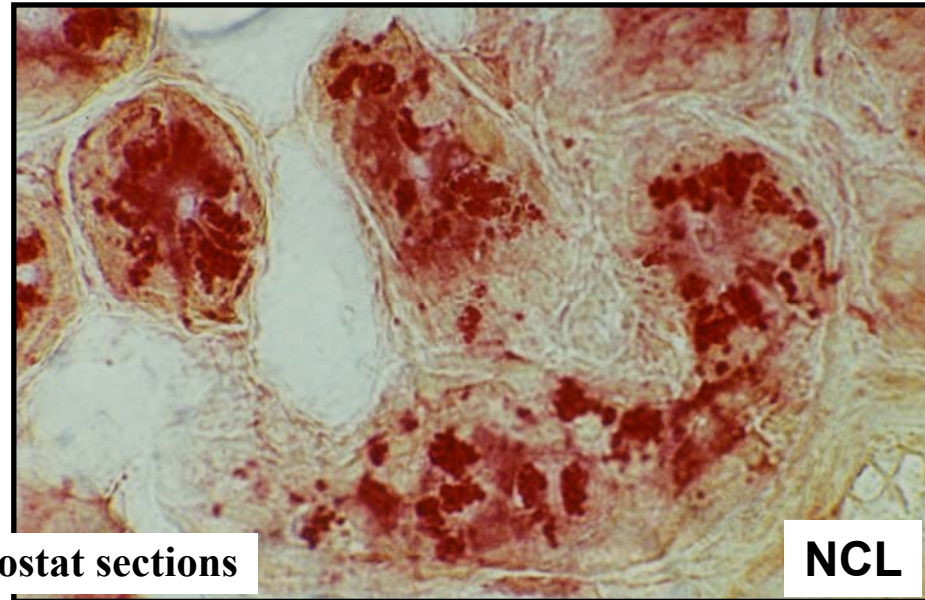


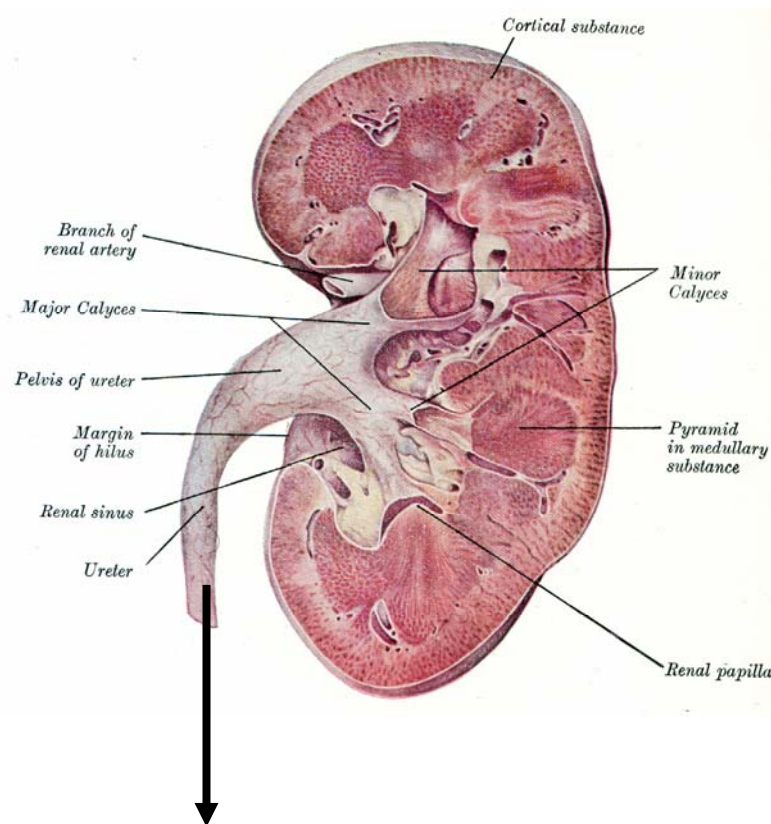
Filipin test – NPC1,2 gene



irregular accumulation of phospholipids (ferric hematoxylin)

storage pattern in NCL2 (TPP I deficiency)





urine analysis = „chemical biopsy of the kidney“
positive finding means **presence of lysosomal storage in the tubular and glomerular cells**; detached storage cells should be the main source of the diagnostic stored compound

Lipidoses free of urinary findings

Gaucher	negative
Krabbe	negative

Lipidoses with positive findings in the urine

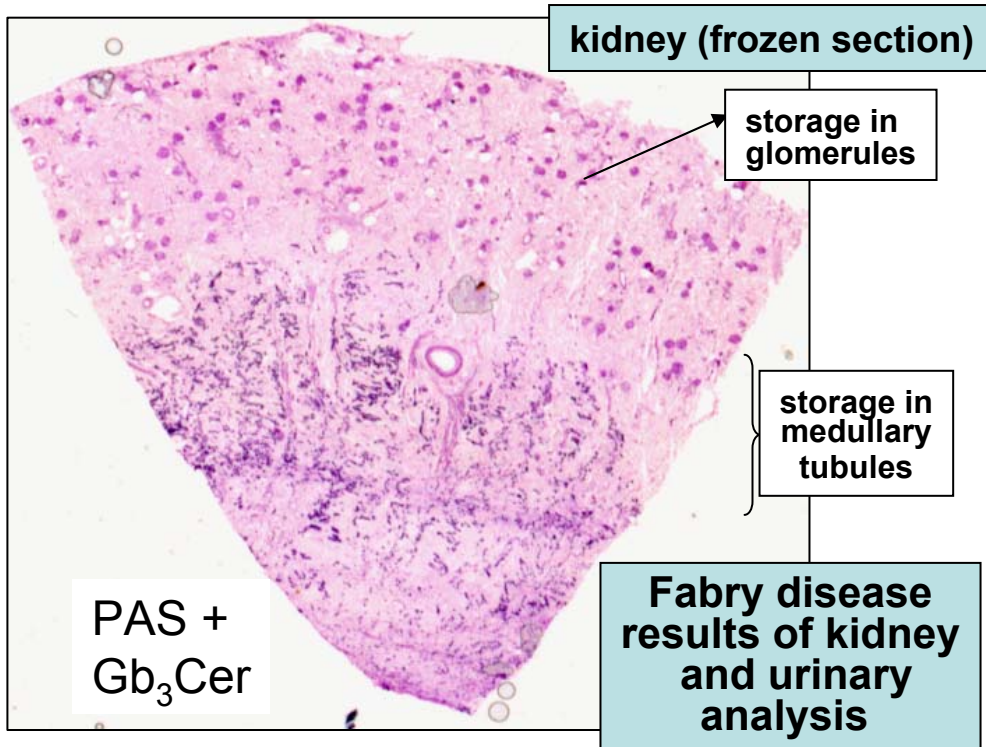
(currently not utilized for screening)

NPA/B	sphingomyelin/cholesterol
CESD/Wolman	cholesteryl esters

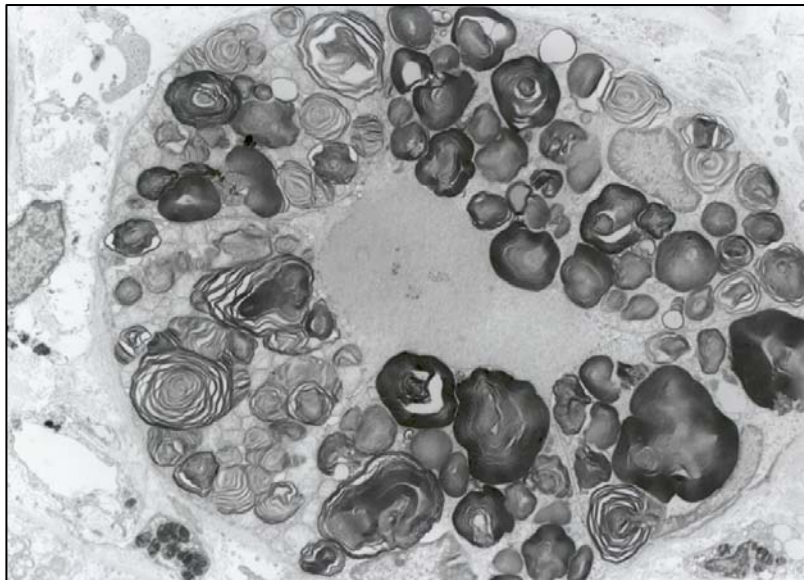
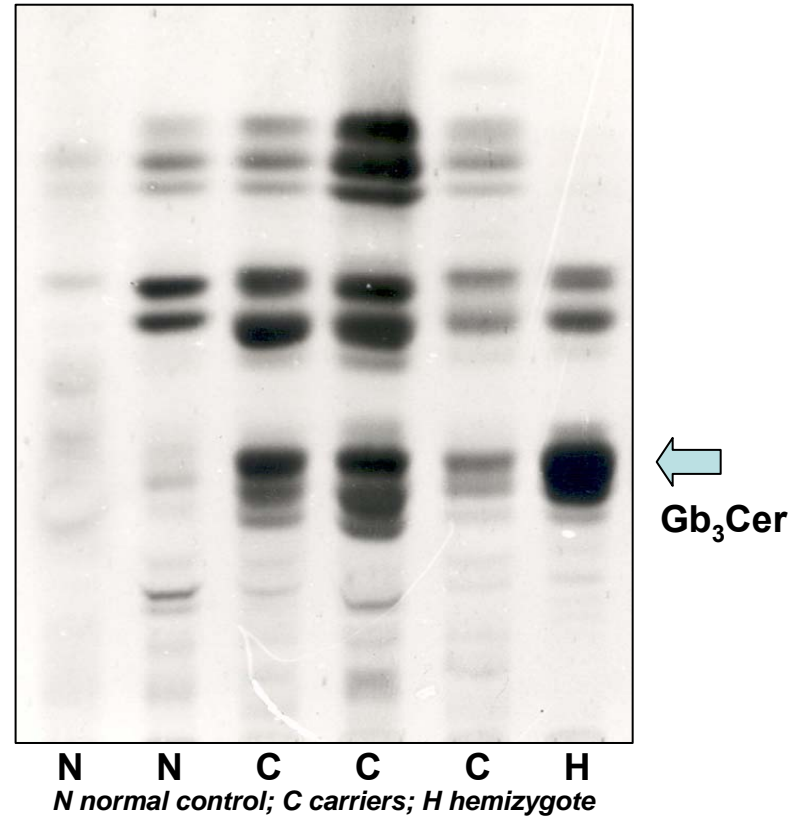
Lipidoses with positive findings in the urine

(recommended for screening)

Fabry (incl. SapB def.)	Gb ₃ Cer
MLD (incl. SapB def.)	sulphatide
Farber	ceramide
GM1 gangliosidosis	βgal - OLS
GM2 gangliosidosis	GlcNAc – OLS



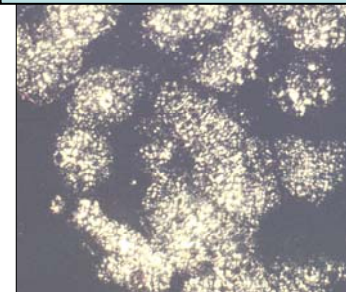
glycolipids in the urinary sediment



EM – storage in tubular epithelium



section of the kidney



birefringence of in situ tubular cell storage

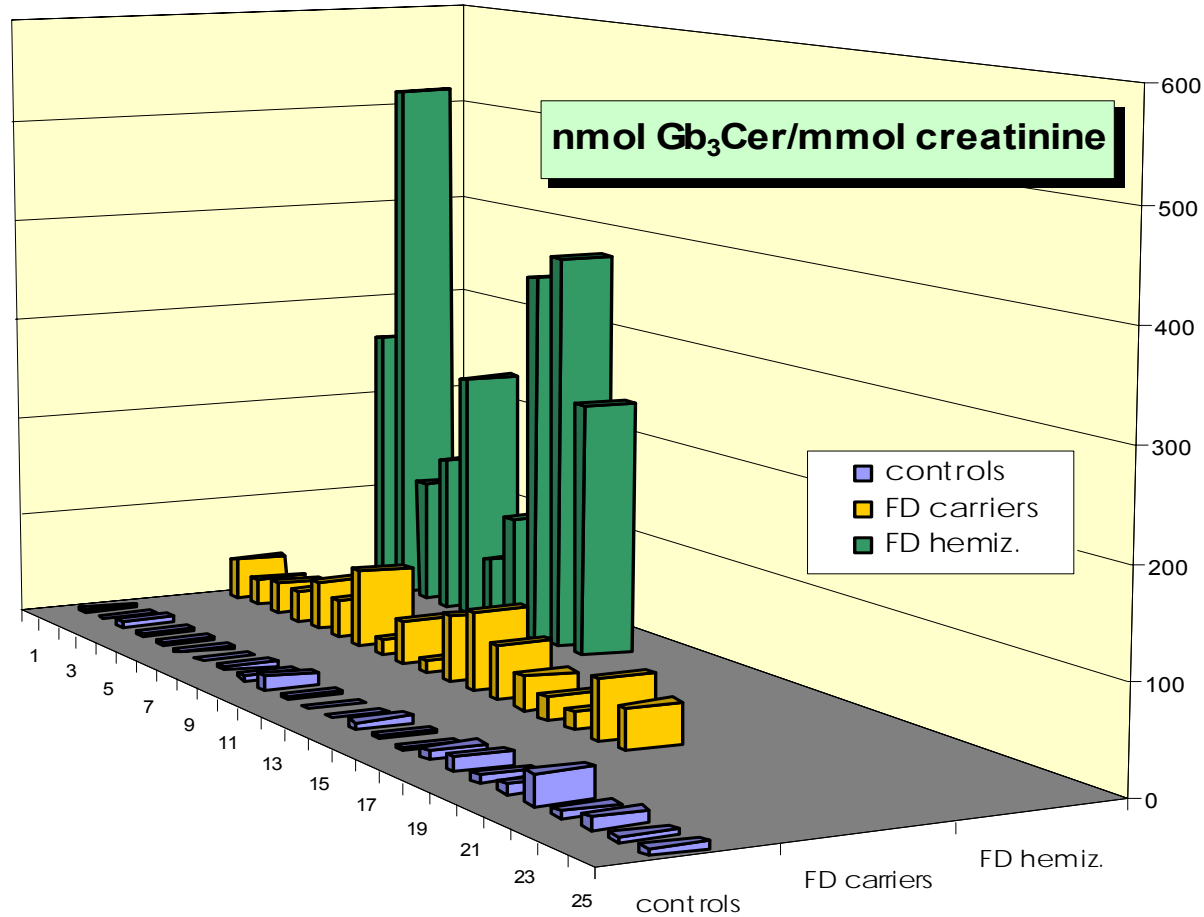


u.sediment



desquamated storage cell

Gb₃Cer in urines of Fabry (FD) hemizygotes, female carriers and controls.

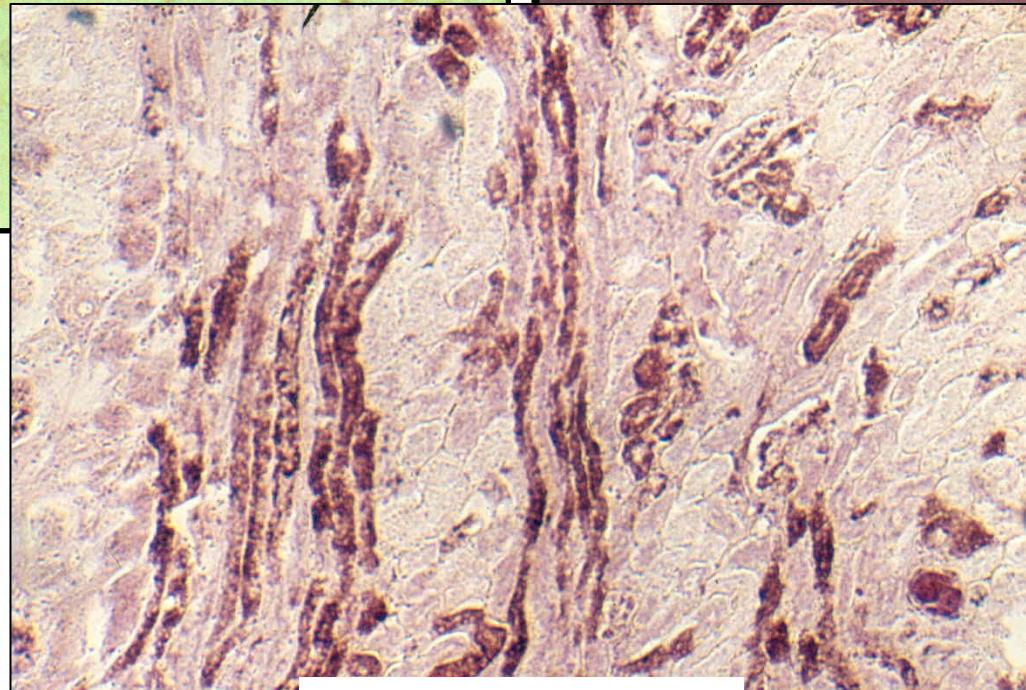
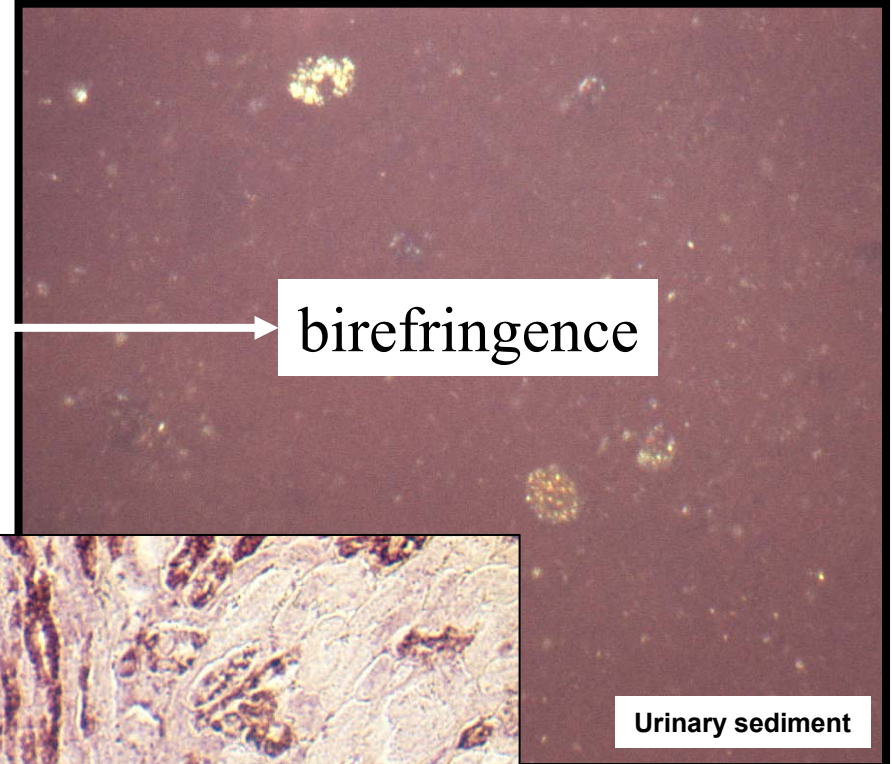
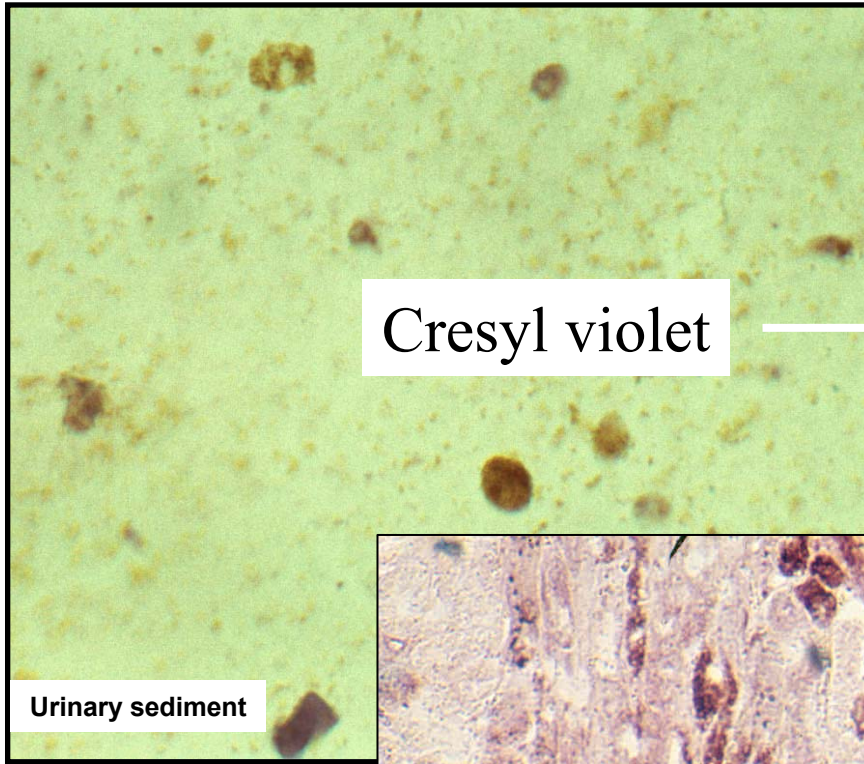


METHOD: Extraction of total urinary lipids - reversed-phase chromatography

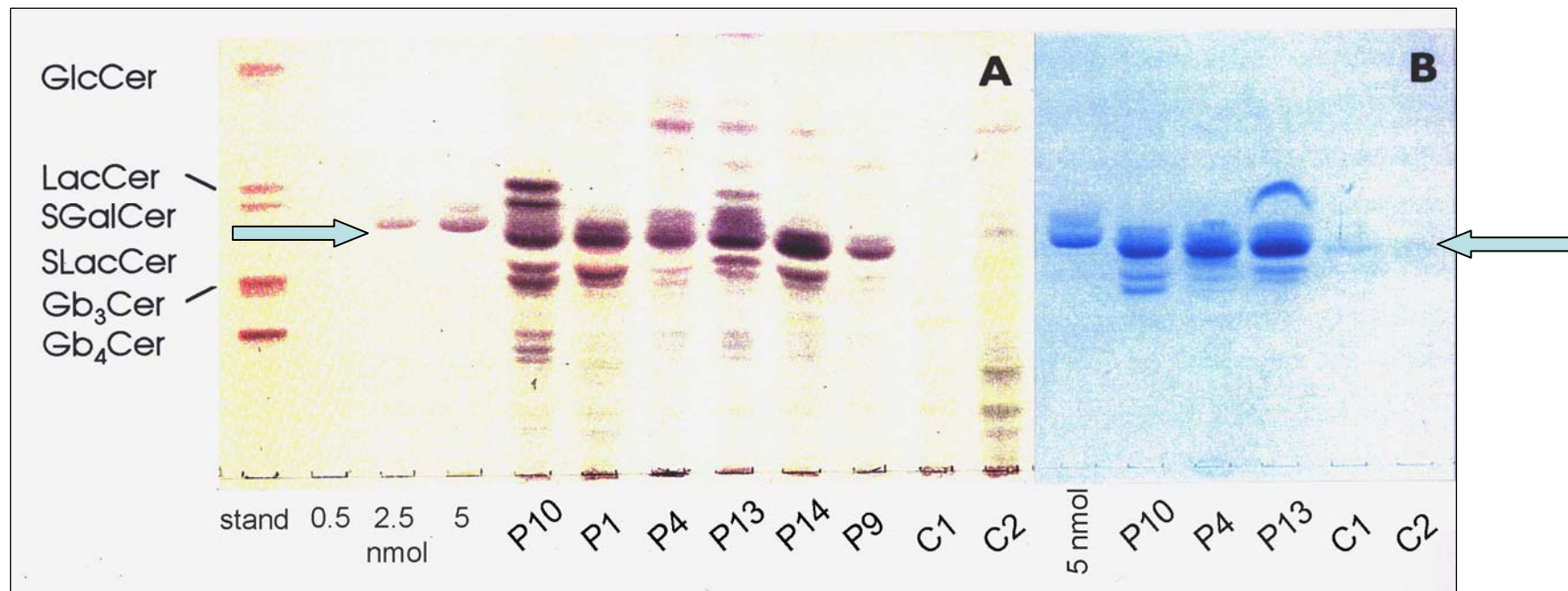


Lipid separation - HPTLC ➔ orcinol detection ➔ quantification densitometrically (CAMAG II)

kidney tubules and urinary sediment in sulphatidosis



Sulfatides (SGalCer) in urines of patients (P) with sulfatidosis (C1,2=controls)



A. Orcinol detection, B. Azur A detection (specific), Stand.= sulfatide and GL standard

urine in mucopolysaccharidoses (GAGs)

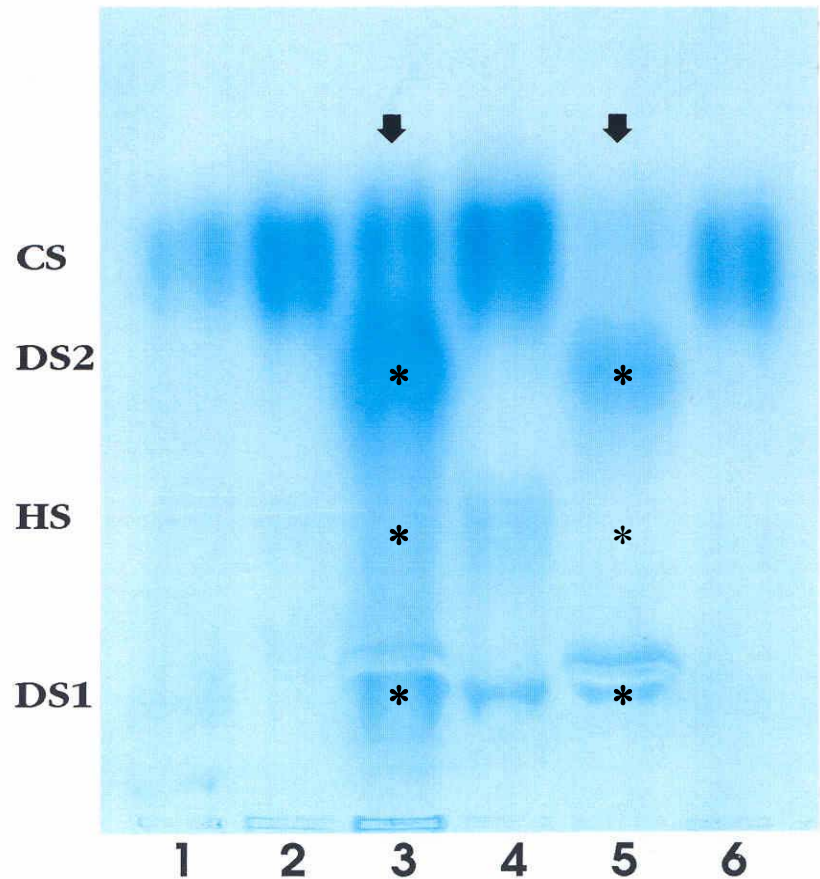
	KS	CS	DS2	HS	DS1	Hep
CONTROL	-	+	-	-/ <u>±</u>	-/ <u>±</u>	-
MPS I	-	+	+	+ variable	+	-
MPS II	-	+	+	+ variable	+	-
MPS III	-	+	-	+ !!	-	+
MPS IVA	+	++	-	-	-	-
MPS IV B	-/ <u>±</u>	+	-	-	-	-
MPS VI	-	+	+!!	-	+!!	-
MPS VII	-	+++	+!!	+	+!!	-

*KS – keratan sulphate; DS – dermatansulphate;
CS – chondroitinsulphate; HS – heparan sulphate; Hep - heparin*

MPS I – Hurler disease (α -iduronidase deficiency)



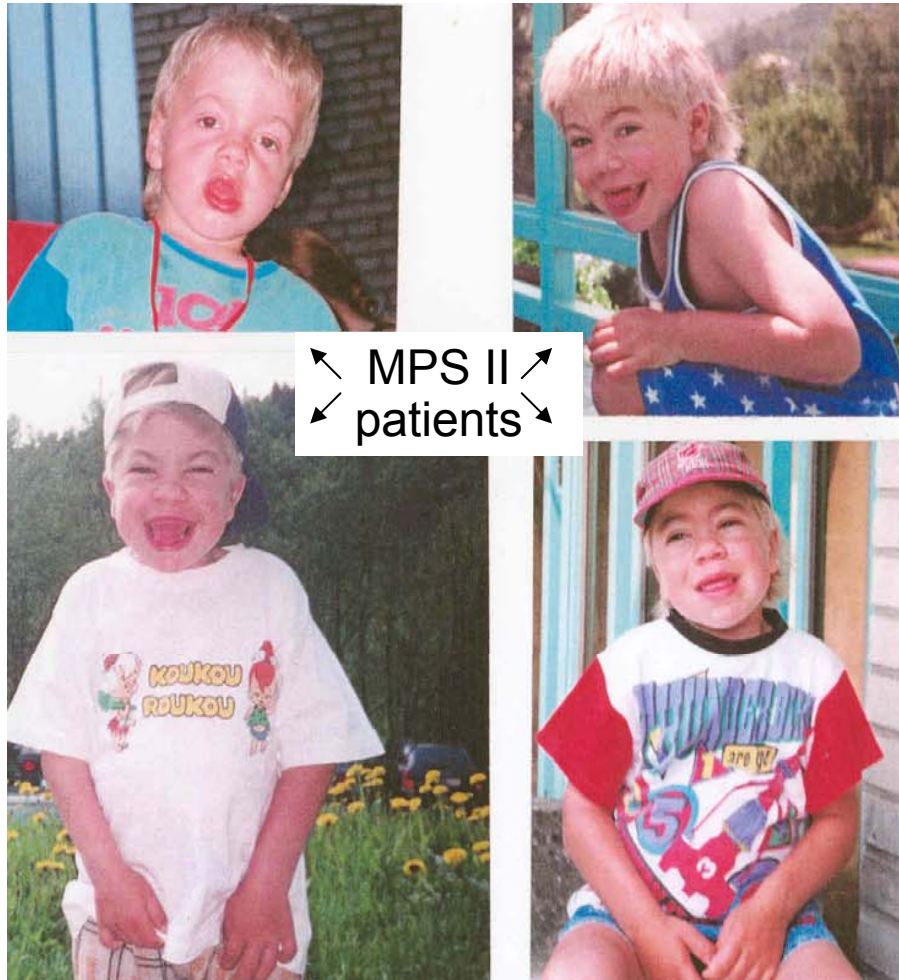
MPS I in a 6-year-old girl



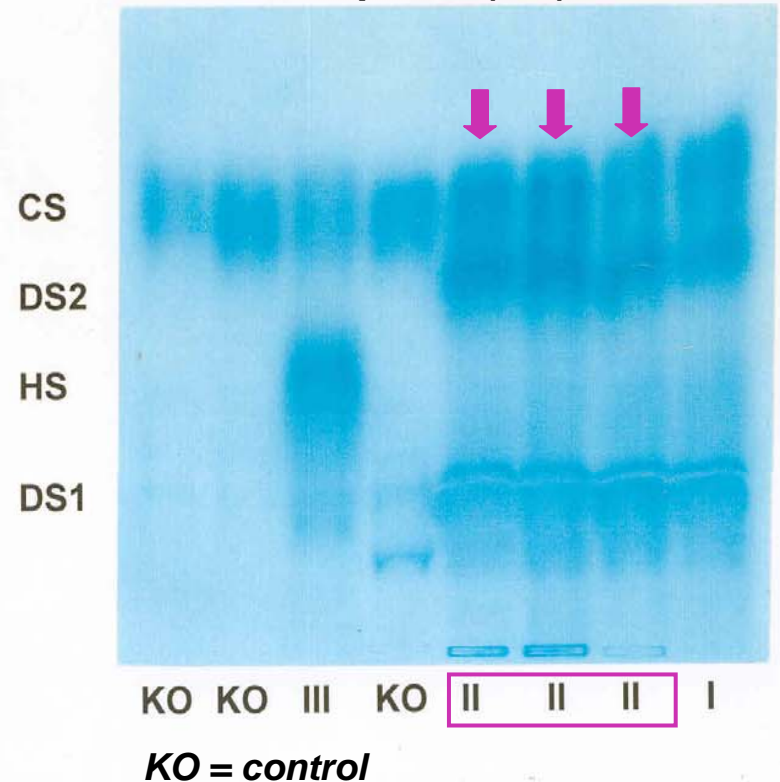
Electrophoresis of urinary GAGs

3,5 = MPS I (excretion of dermatan sulphate/DS and heparan sulphate/HS, see arrows), **4 = neonatal control** (traces of HS and DS1), **1,2,6 = controls**

Urine GAG ELFO in patients with MPS II, and its comparison with MPS I a MPS III



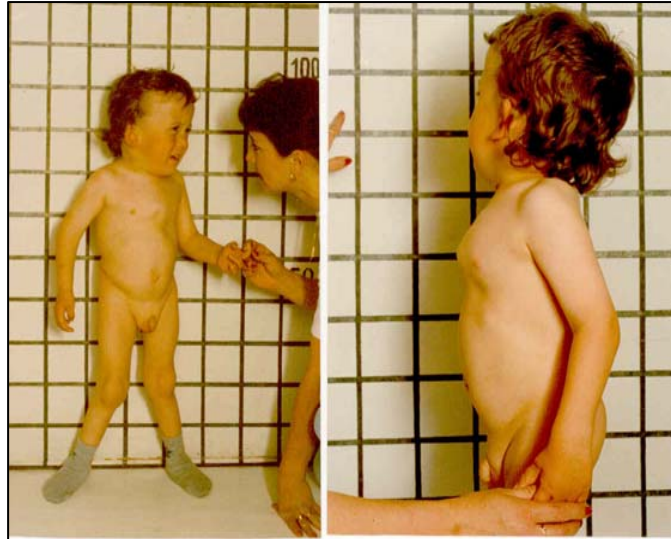
Urinary excretion of dermatan sulphate (DS1,2) and heparan sulphate (HS)



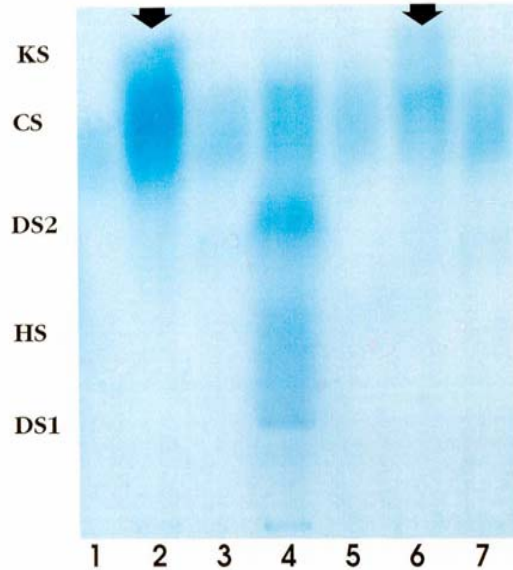
MPS II: iduronate-2-sulphate sulphatase deficiency, X – linked disorder

MPS IVA

deficiency of
GalNAc-6-sulphate
sulfatase (excretion of
keratan sulfate,
chondroitin-6-sulphate)



MPS IVA in 4-year-old boy.



2,6 = MPS IVA (see arrows)

4 = MPS I

1,3,5,7 = controls

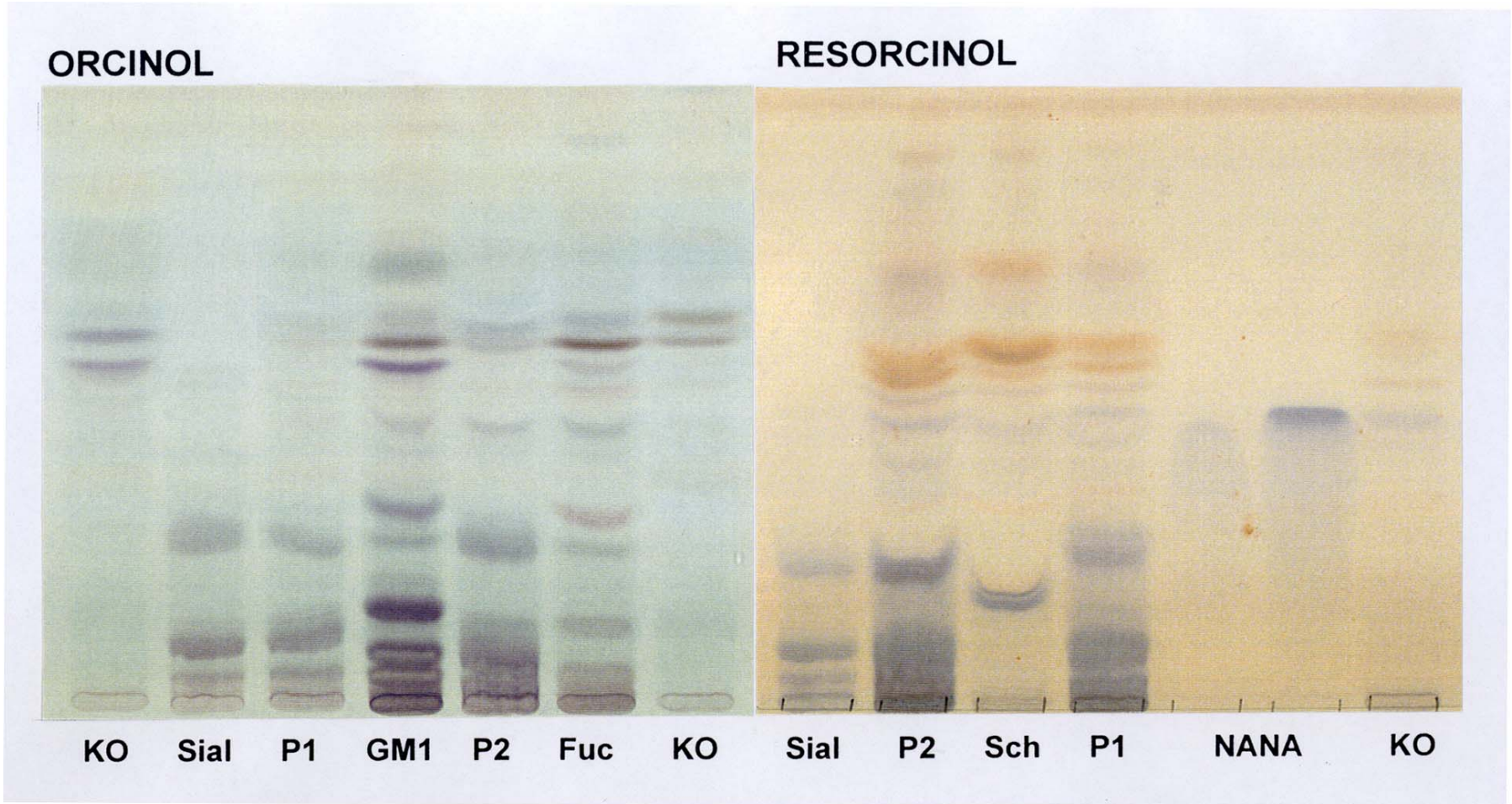
Urine in glycoproteinoses and related disorders

OLS (oligosaccharides) – low mol.w. glycoconjugates reflecting incomplete degradation of glycoproteins

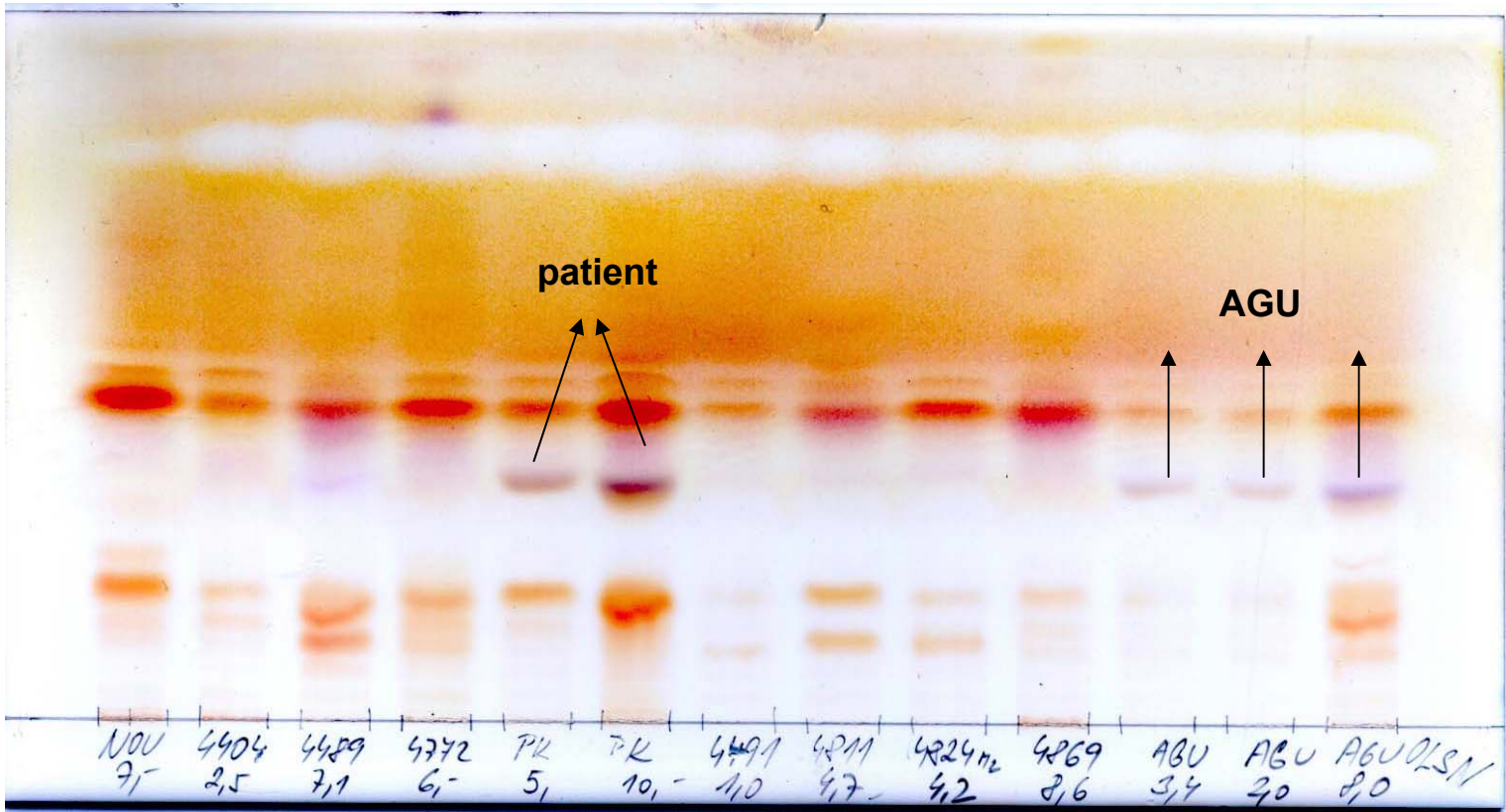
<i>GM1 gangliosidosis</i>	βgal-OLS
<i>α-mannosidosis</i>	αmann- OLS
<i>β-mannosidosis</i>	βmann- OLS
<i>α-Fucosidosis</i>	αfuco - OLS
<i>Sialidosis</i>	sialyl - OLS
<i>Galactosialidosis</i>	gal- and sialyl – OLS
<i>AGU</i>	aspartylglucosamine (+ other glycoasparagines)
<i>ISSD (SALLA dis.)</i>	free sialic acid
<i>Schindler disease</i>	sialyl – OLS
<i>GSD II</i>	occasionally (Glc)₄*

*

Glycoproteinoses – HPTLC of urinary oligosaccharides



P1, P2 = susp.sialidosis confirmed later by enzymology (sample applied in two concentrations), Sial=sialidosis(archived pathologic control), GM1=GM1 gangliosidosis, Fuc= fucosidosis, Sch=Schindler disease, NANA= N-acetylneuraminic acid (standard), Ko=control urine



ninhydrine detection – urine in AGU

Urine in other LSDs

(negative or insignificant findings)

NPCs

NCLs

Danon dis.

ML IV

Cystinosis

negative

negative (or SCMAS)

not studied

phospholipids

generalized AAU

Clinical findings :

dysmorphia; dysostosis

neurology; visceromegaly;

corneal clouding (absent in MPS II)

heart valvular disease;

storage vacuoles in lymphocytes incl.

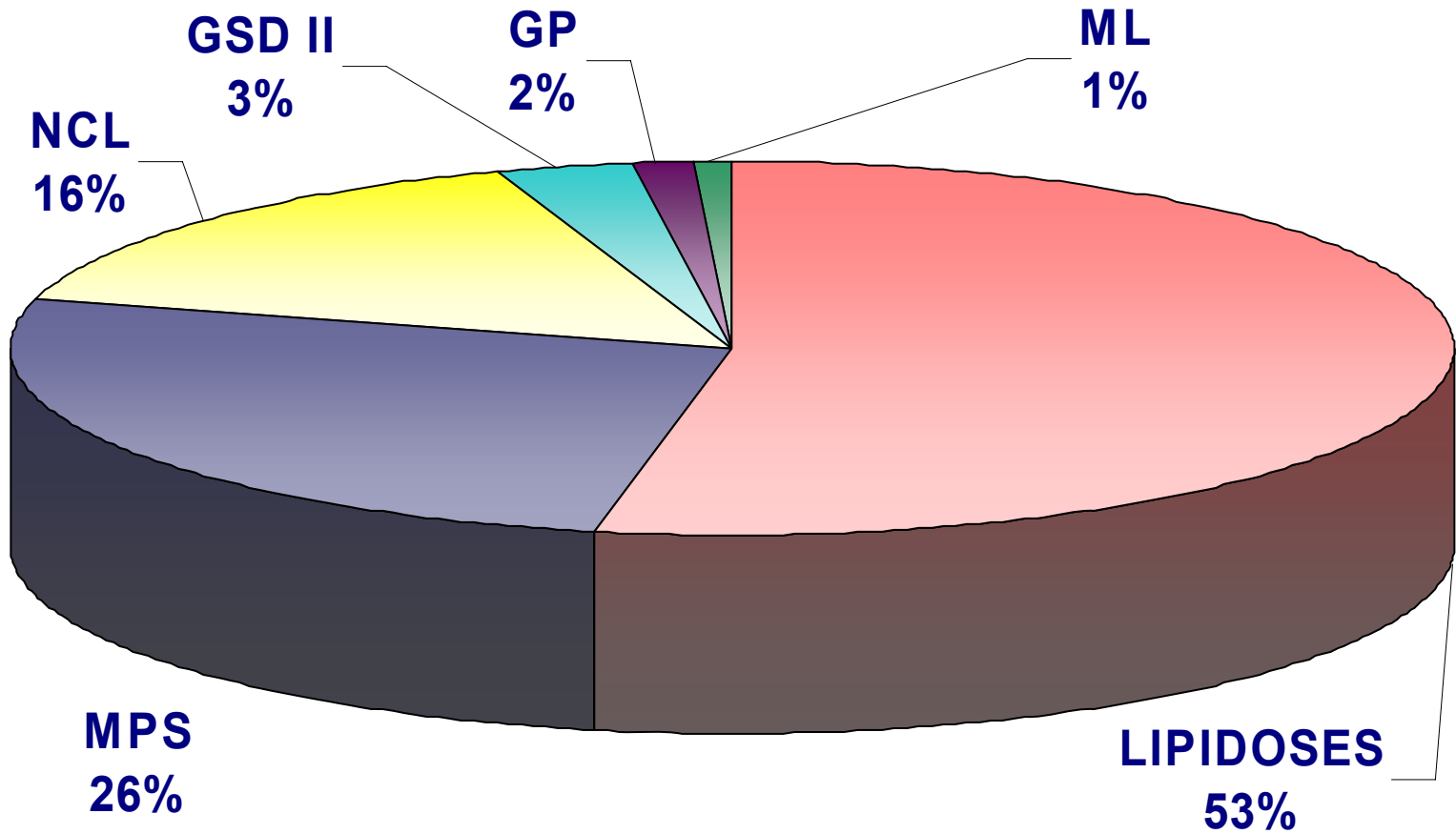
Alder-Reilly granules



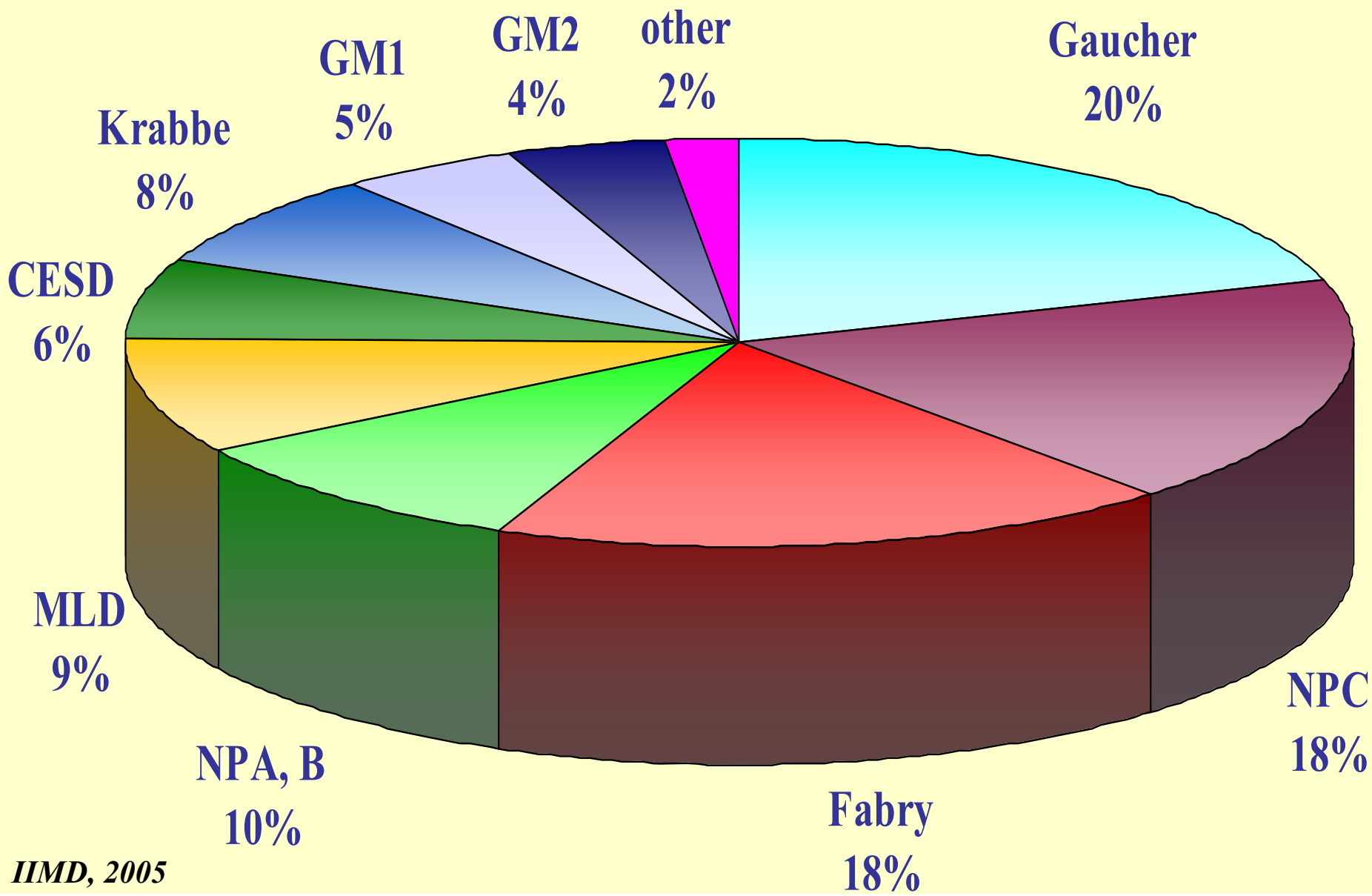
Urine analysis:

- **GAGs**
- **OLS**
- **free silicic acid**
- **AGU**

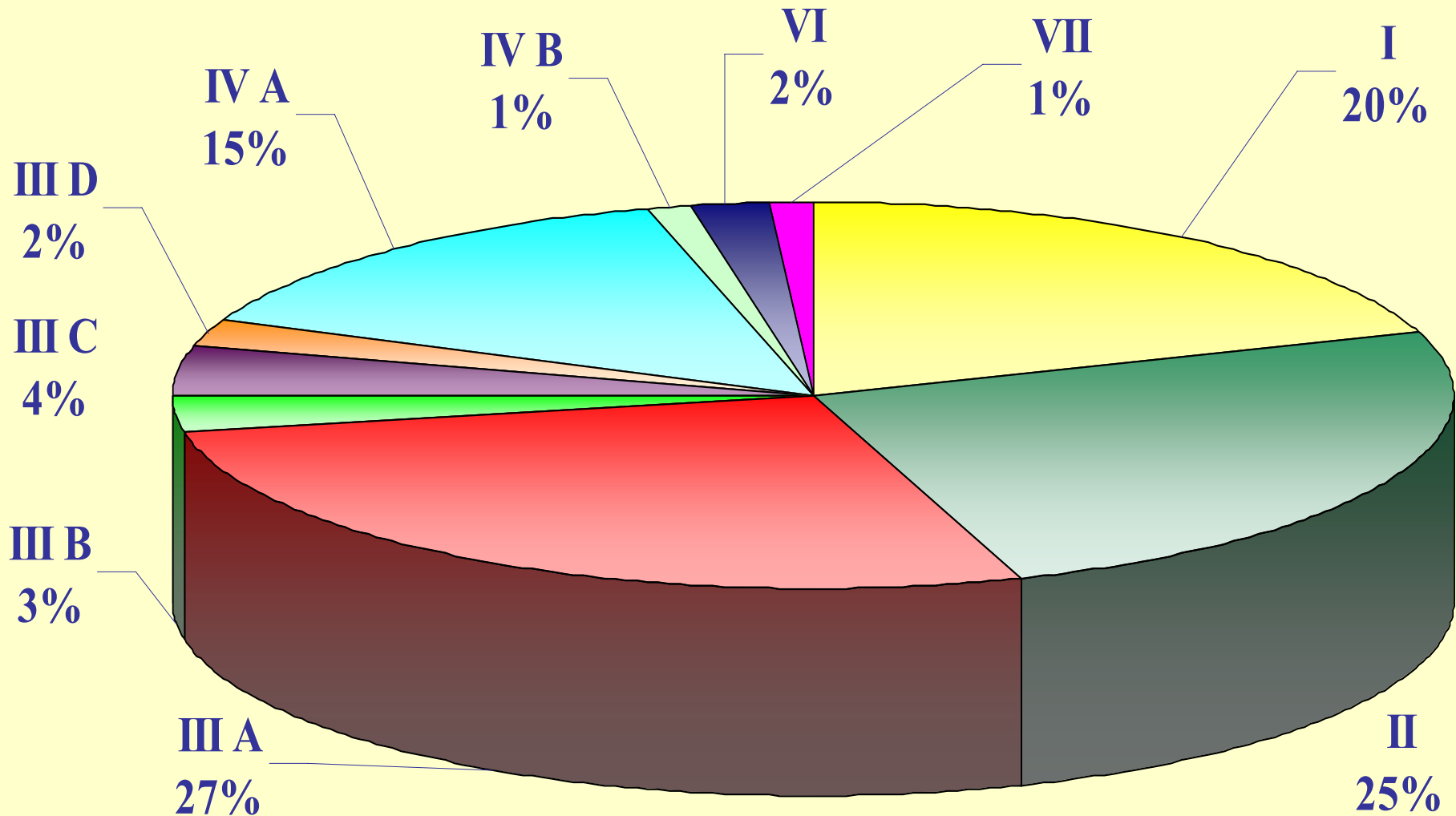
LYSOSOMAL STORAGE DISORDERS IN THE CZECH and SLOVAK REPUBLICS 1975-2005 (n=525)



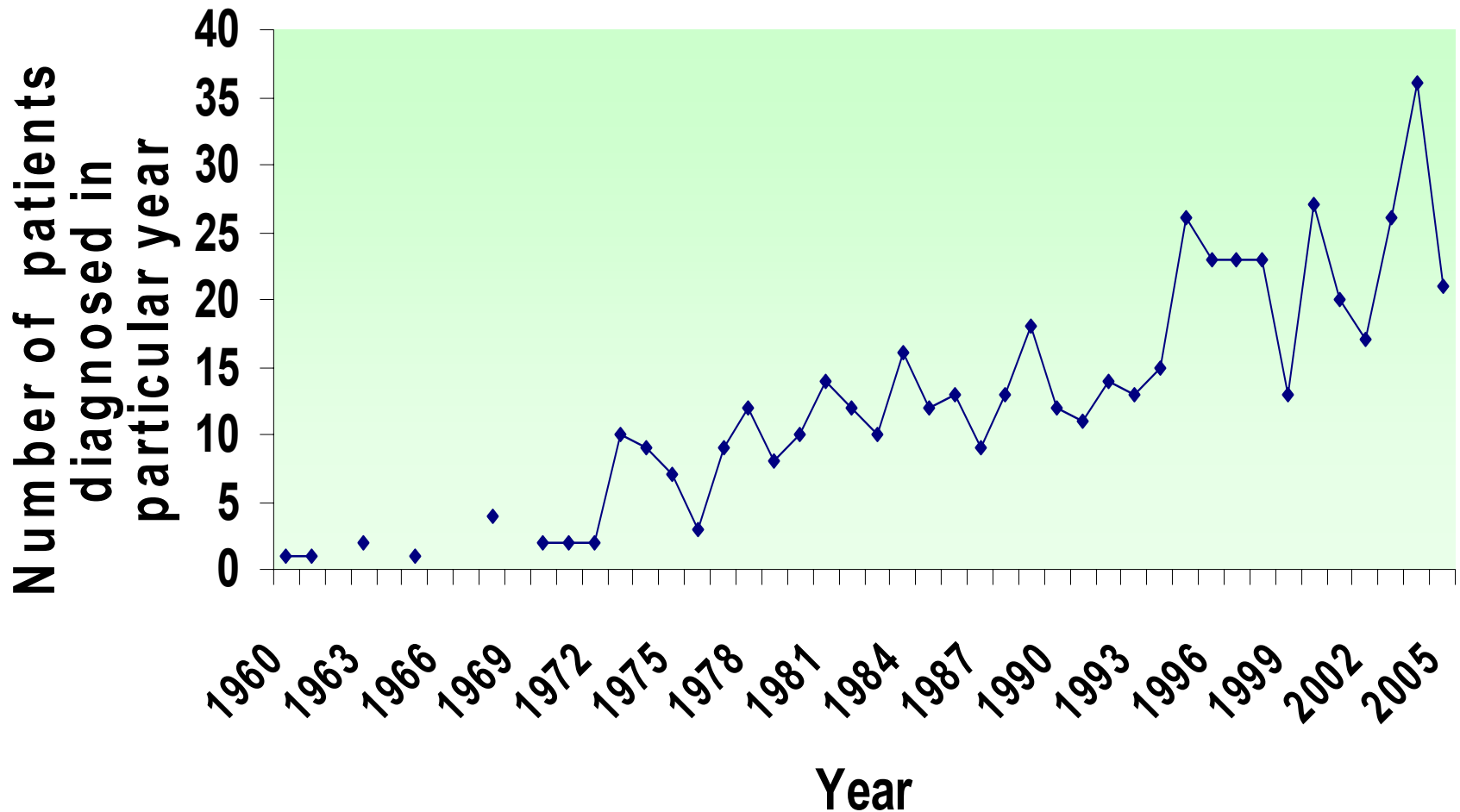
LIPIDOSES IN THE CZECH AND SLOVAK REPUBLICS 1975-2005 (n=279)



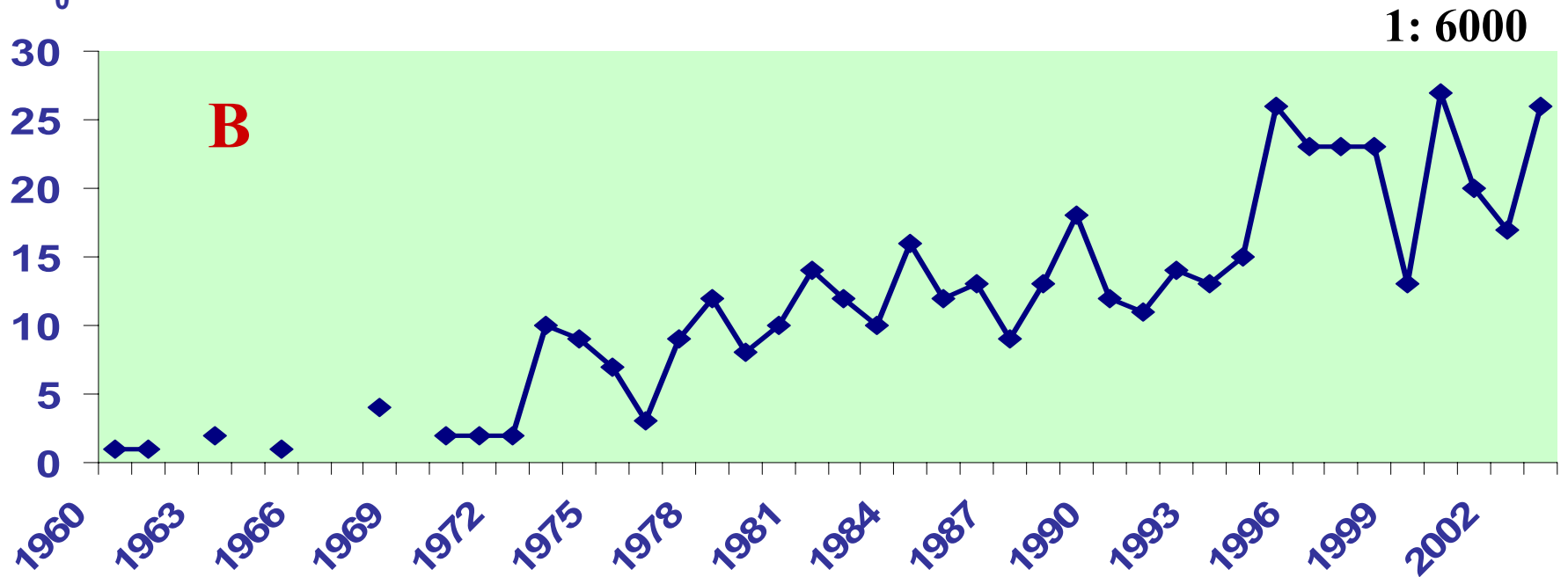
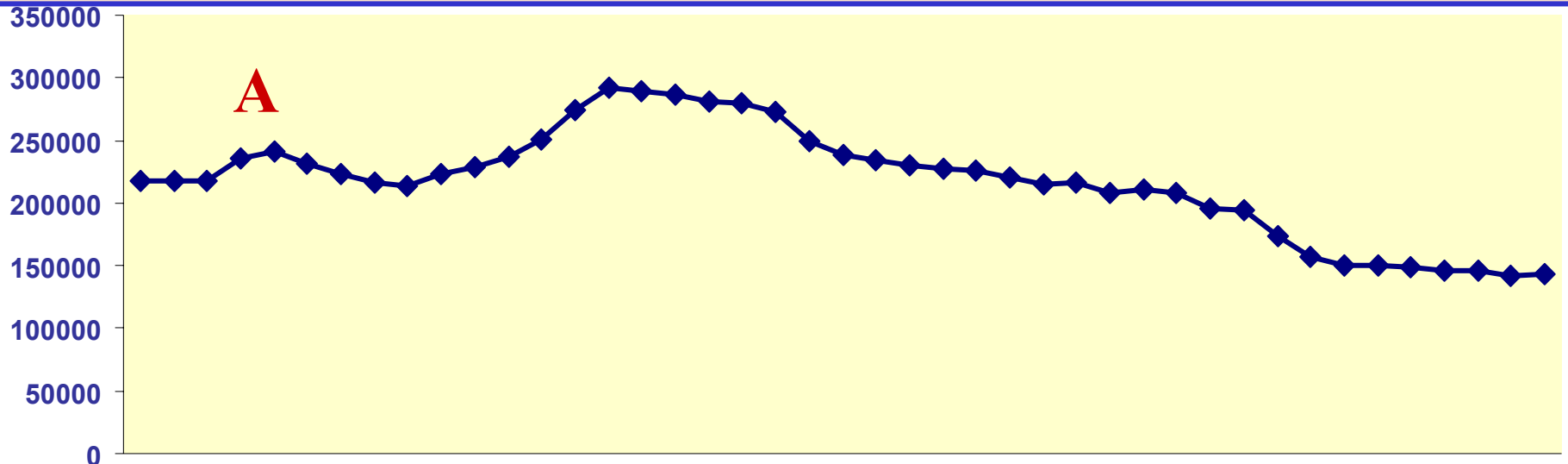
MUCOPOLYSACCHARIDOSES 1975 - 2005 (n=103)



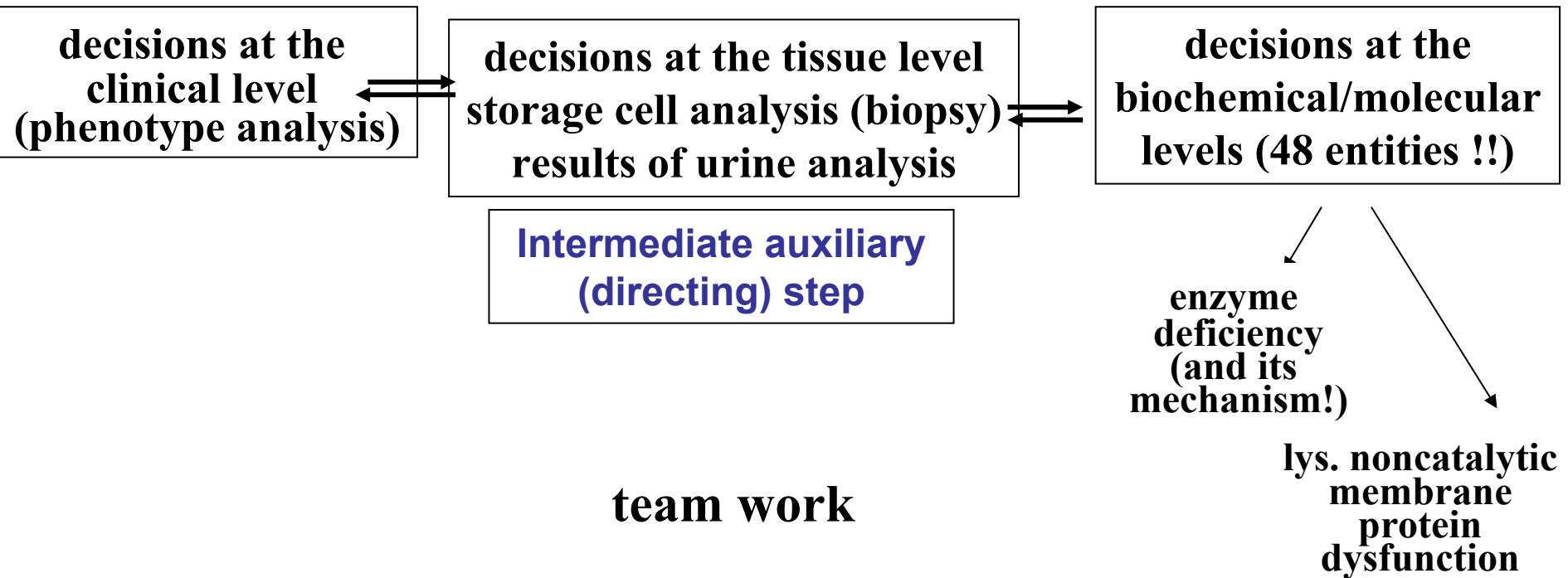
Postnatal and prenatal diagnoses of LSDs in our Institute (1960 - 2005)



No. of live births (A), postnatal and prenatal diagnoses of LSDs (B) in the Czech and Slovak Republics (1960 - 2003)



The diagnostic procedures



Selected syndromes suggestive of LSD

systemic disorder in childhood mainly

**dysmorphism + dysostosis + corneal clouding + valvular heart
disease + neurology
(MPS, GP, GM1, PSD)**

adolescence - adulthood

**progressive nephropathy + cardiomyopathy +
angiokeratomas + neuropathy (sensitive)
Fabry disease**

early childhood

**progressive neurol. disturbance + retinopathy (blindness)
neuronal ceroidlipofuscinoses**

childhood - adolescence

**neurological disturbance + VSO + splenomegaly (even mild)
Niemann-Pick type C**

selected syndromes suggestive of LSD cont.

adulthood (adolescence)

isolated splenomegaly + hypersplenism
splenomegaly + unexplained femoral head necrosis
Gaucher's disease (glucocerebrosidase deficiency)

myoclonous epilepsy + cherry red spot
ML I (sialidase deficiency)

isolated hypertrophic CMP
cave! *Fabry disease (α Gal deficiency)* (inter alia)

isolated hepatomegaly with slightly altered LFTs
serum cholesterol increased in LDL (decreased in HDL)
risk of accelerated atherosclerosis
CESD (acid lipase deficiency)

LSDs project into majority of clinical disciplines

(only significant involvement is included)

ophthalmology (NCL, GM1-2, NPA, Fabry, MPS, GP, ML IV)

neurology (GM1-2, NCL1,2, NCL3,5,6,8, NPCs, Krabbe, MLD, MPS, GP, GD)

psychiatry (adult neurolipidoses)

pneumology (Gaucher, NPA/B, NPC2)

cardiology (Fabry, MPS, GP, GSD II)

angiology (Fabry, CESD, MPS)

hepatology (CESD, NPC infantile, MPS, GSD II, GP)

nephrology (Fabry, nephrosialidosis)

dermatology (Fabry, Fuco-, β Mannosidosis, PSD, Farber)

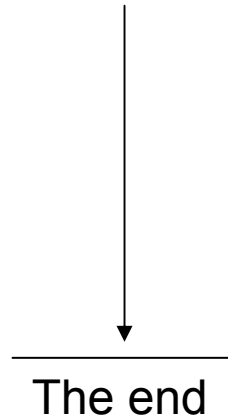
hematology (Gaucher, NPA/B, NPC, ect)

orthopedy/osteology (MPS, GP, GD, ML II/III)

stomatology (MPS, GP, ML II/III)

ORL (Fabry, MPS, GP)

GP glycoproteinoses, CESD acid lipase deficiency, PSD polysulphatase deficiency, GSD II Pompe disease
MPS mucopolysaccharidoses; GD Gaucher disease, Farber dis. = ceramidase deficiency



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