Survey of diagnosis of lysosomal storage disorders

Milan Elleder
Institute of Inherited Metabolic Disorders
Charles University, 1st Faculty of Medicine and University Hospital Prague

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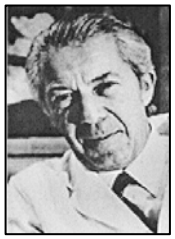
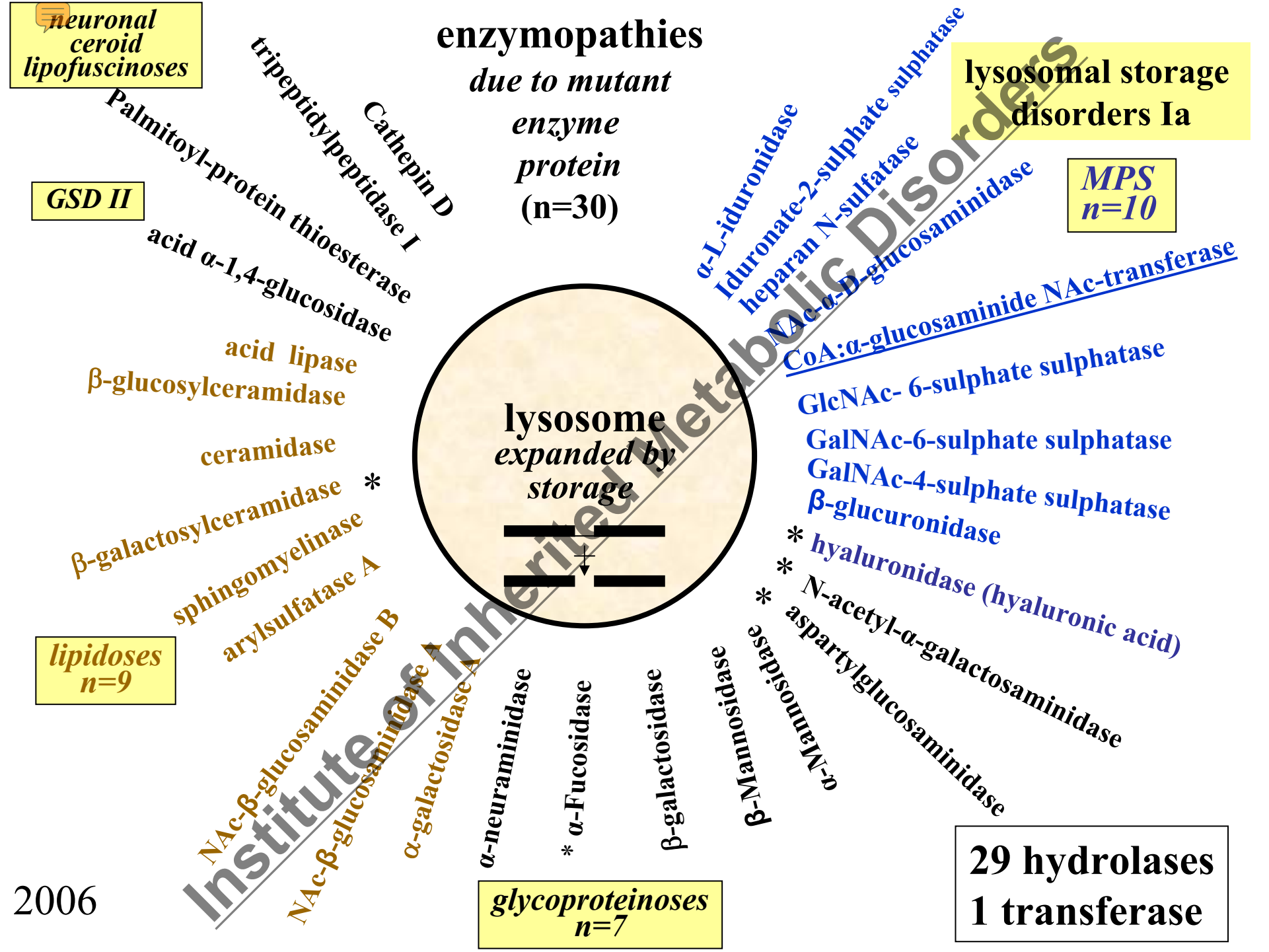
prehistory – empirical part of the story
clinical reports by Tay (1881), Gaucher (1882) and Sachs (1896)
and by others

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)


neuronal ceroid lipofuscinoses
tripeptidylpeptidase I
Cathepin D
Palmitoyl-protein thioesterase
GSD II
acid α-1,4-glucosidase
enzymopathies
due to mutant enzyme protein
(n=30)
α-L-iduronidase
Iduronate-2-sulphate sulphatase
heparan N-sulfatase
NAc-α-D-glucosaminidase
lysosomal storage disorders Ia
MPS n=10
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
α-Mannosidase
β-Mannosidase
β-galactosidase
* α-Fucosidase
α-neuraminidase
α-galactosidase A
NAc-β-glucosaminidase
NAc-β-glucosaminidase B
acid lipase
β-glucosylceramidase
ceramidase
β-galactosylceramidase
sphingomyelinase *
arylsulfatase A
lysosome
expanded by storage
lipidoses n=9
glycoproteinoses n=7
29 hydrolases
1 transferase
2006
Institute of Inherited Metabolic Disorders

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: αGalNAc- [Fucα]- βgal-;)
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 !!

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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 – mucolipidosis 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, αGal*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

2006

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 !!

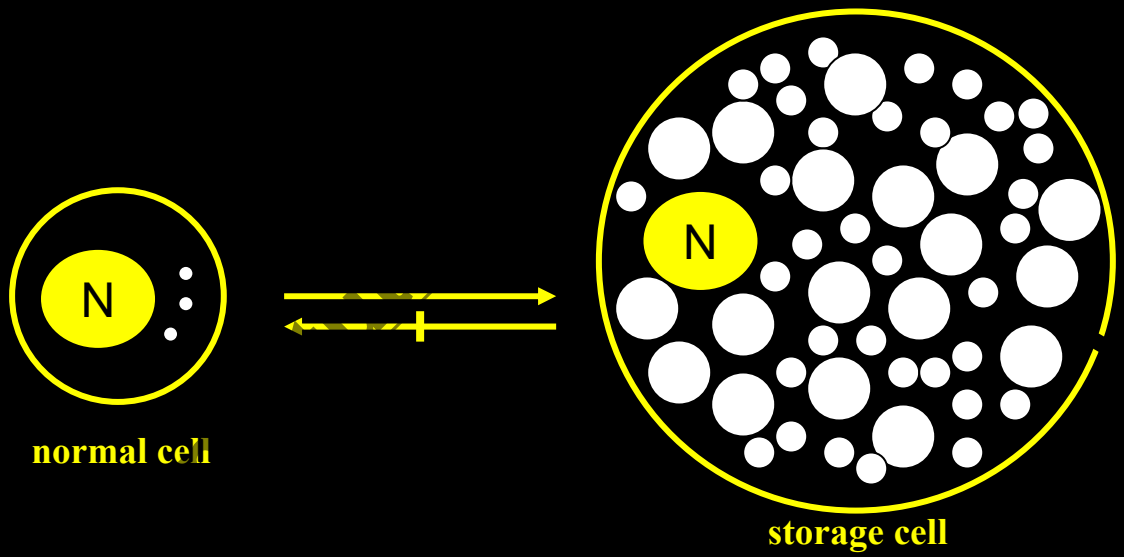
I. A, B (n=38) deficiencies in breakdown catalysis

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

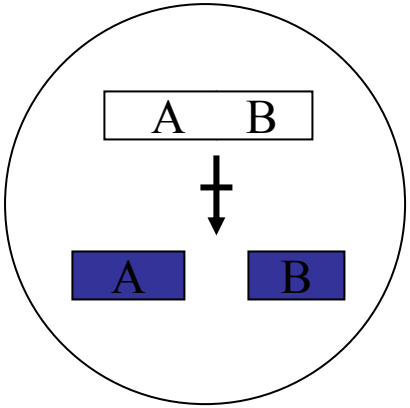
48 entities

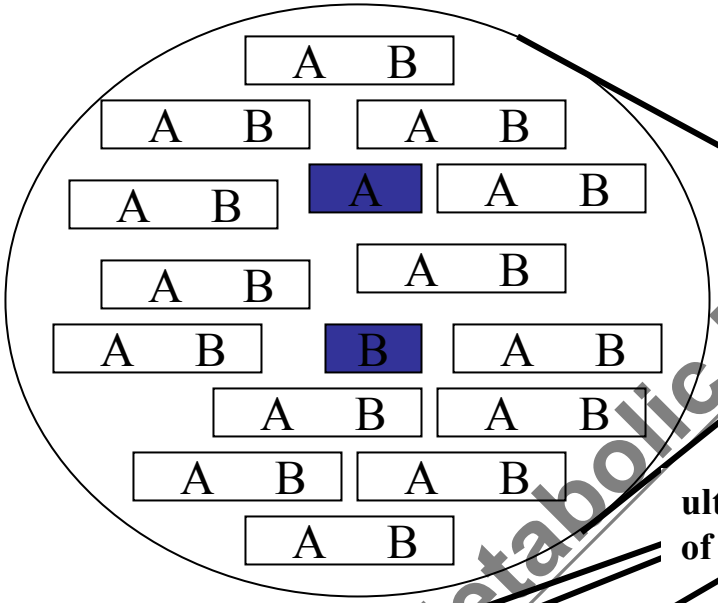
hall mark: „storage“- overfilling and gradual lysosomal expansion by:

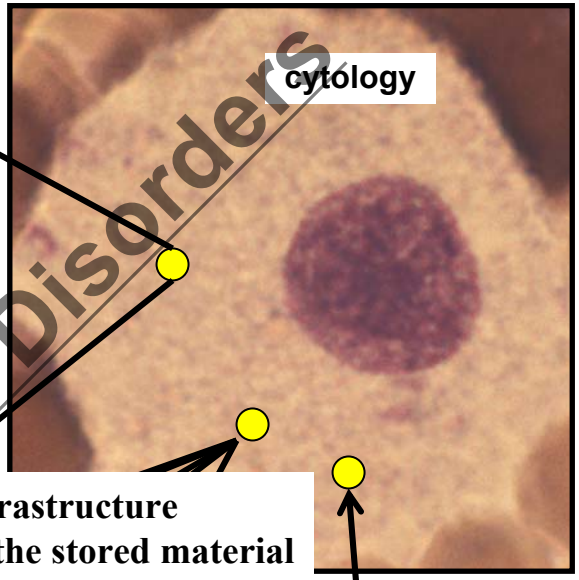
- ***undegraded*** **enzyme substrates**
- ***unremovable*** **enzyme products**
- ***misshandeled*** **heterogenous compounds**



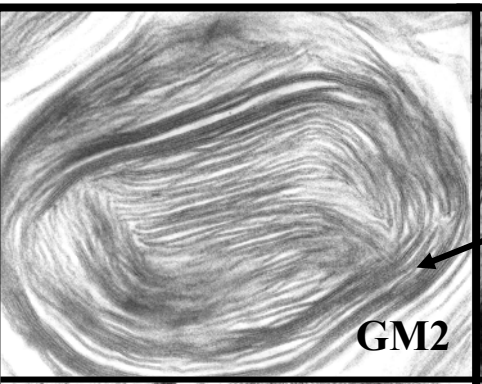
Lysosomal enzymopathy cytology - EM

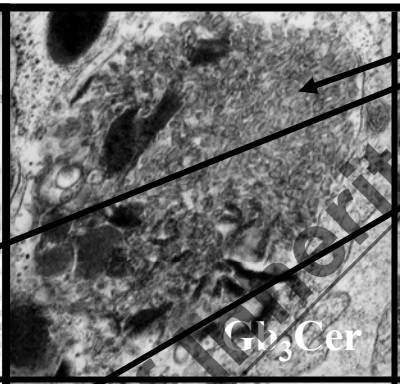


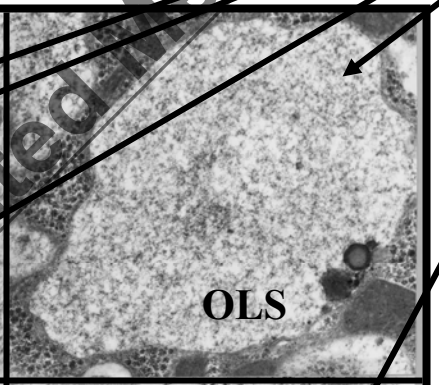


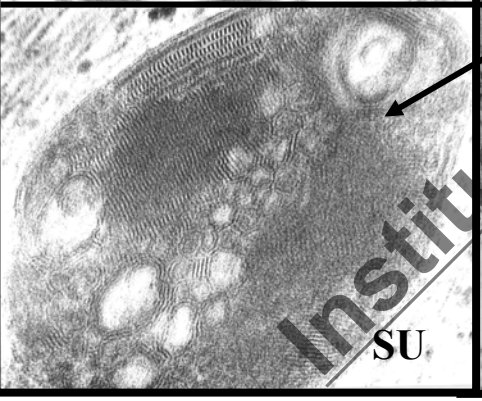


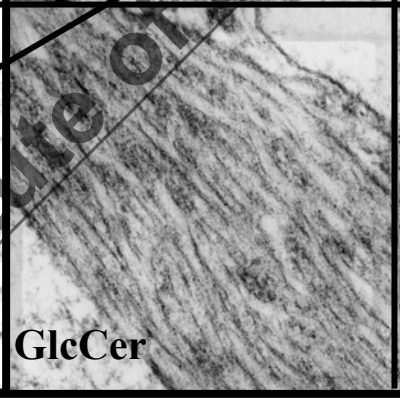
ultrastructure
of the stored material

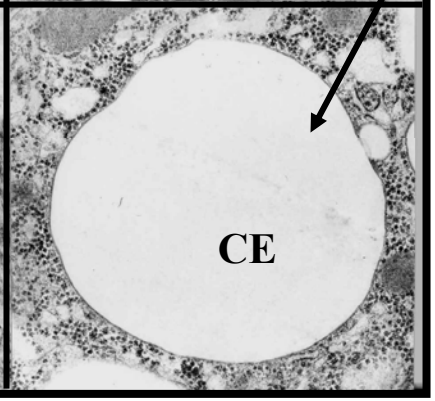


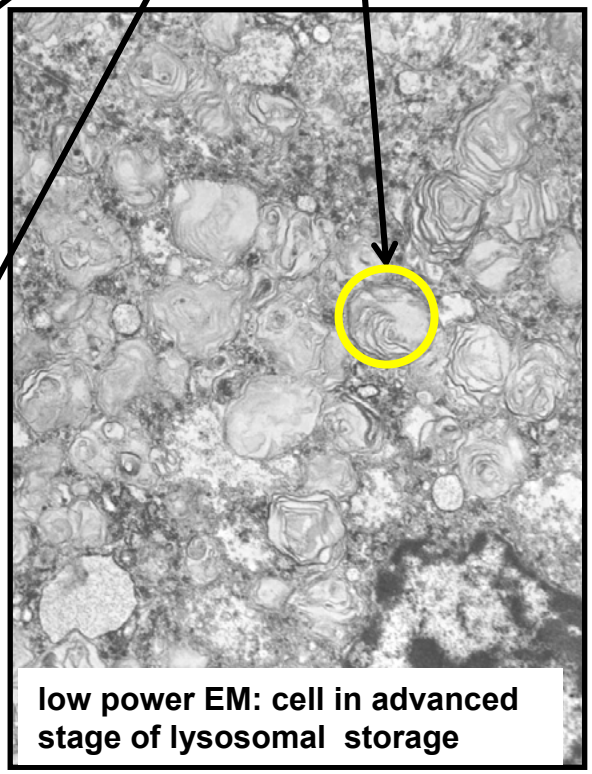




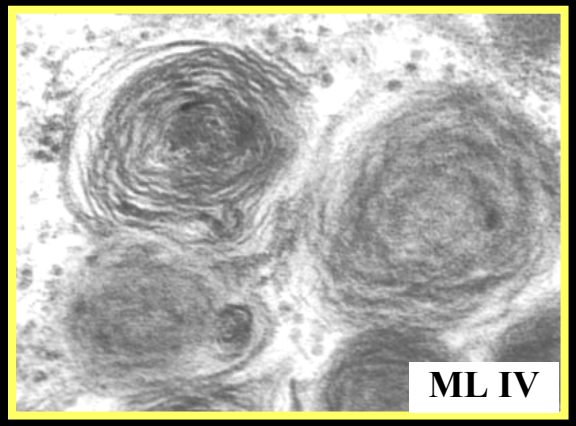




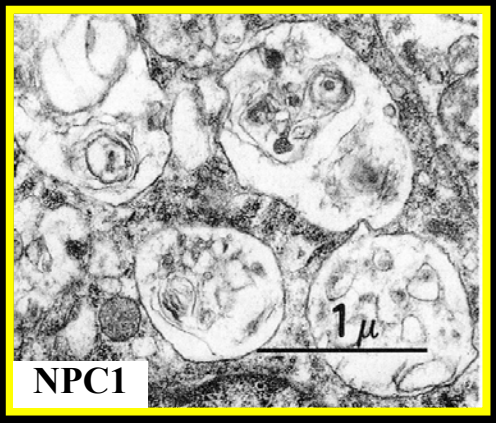




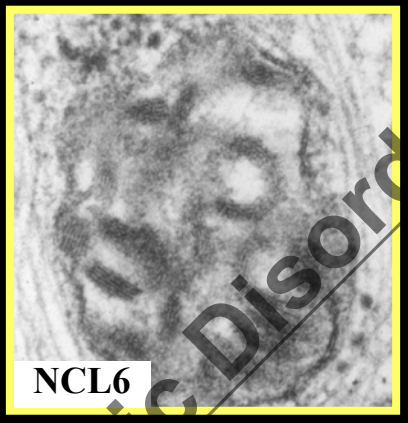
low power EM: cell in advanced stage of lysosomal storage



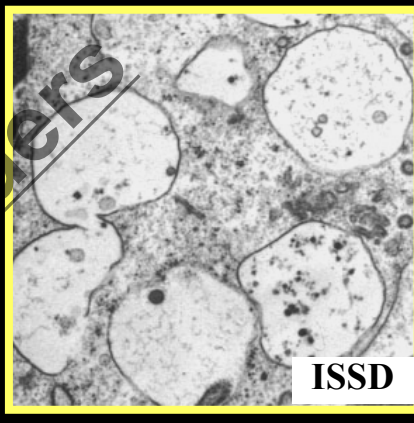
loss of mucolipin function
(function unknown)
mixture of lipids stored



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

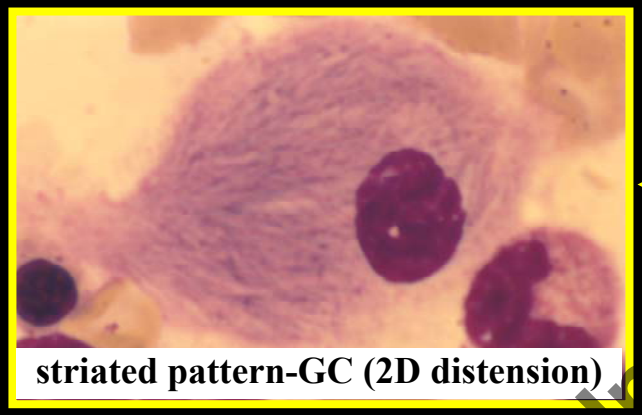


loss of NCL 6 protein
(function unknown)
SCMAS storage



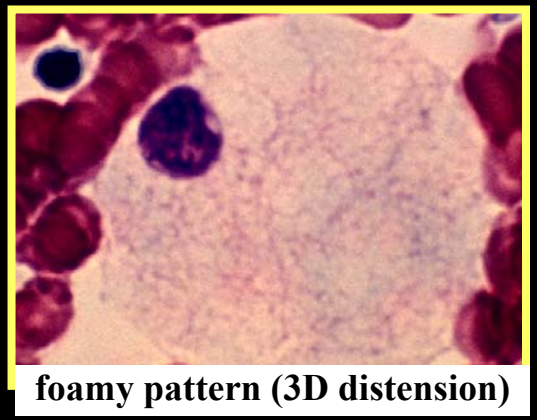
loss of SA transporter
retention of sialic acid

EM patterns in group II LSDs



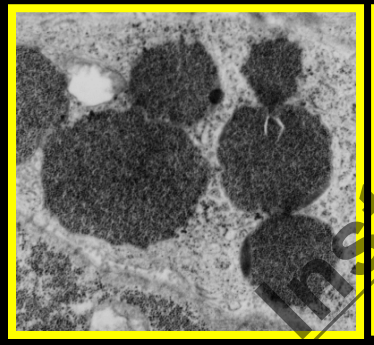
striated pattern-GC (2D distension)

basic cytological patterns of lysosomal storage



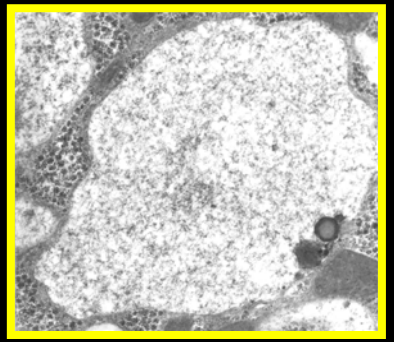
foamy pattern (3D distension)

EM patterns in uncleaved substrate accumulation

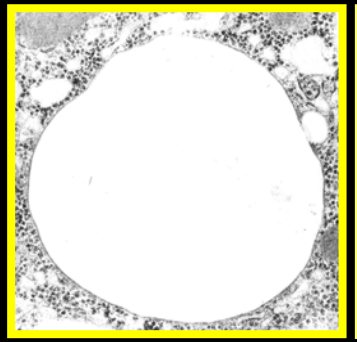


GSD II (glycogen)

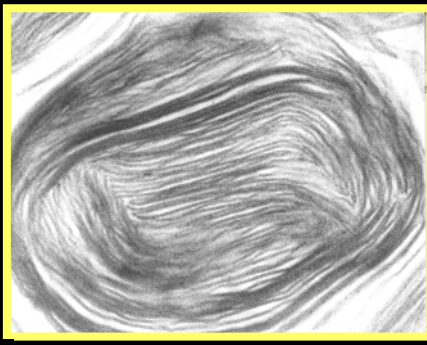
Gaucher (GlcCer)



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

decisions at the clinical level (phenotype analysis) →

decisions at the tissue level
- ***storage cell analysis (biopsy)***
- ***results of urine analysis***

→ **decisions at the biochemical/molecular levels (48 entities !!)**

Intermediate auxiliary direction indicator „at a crossroad“

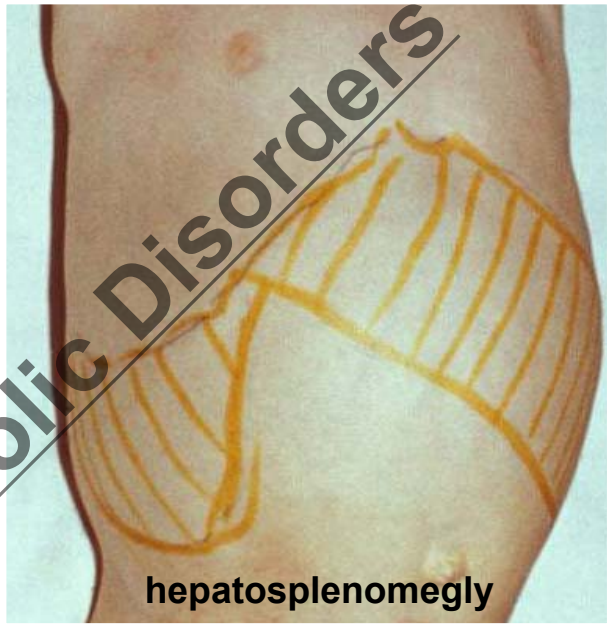
enzyme deficiency (and its mechanism!)

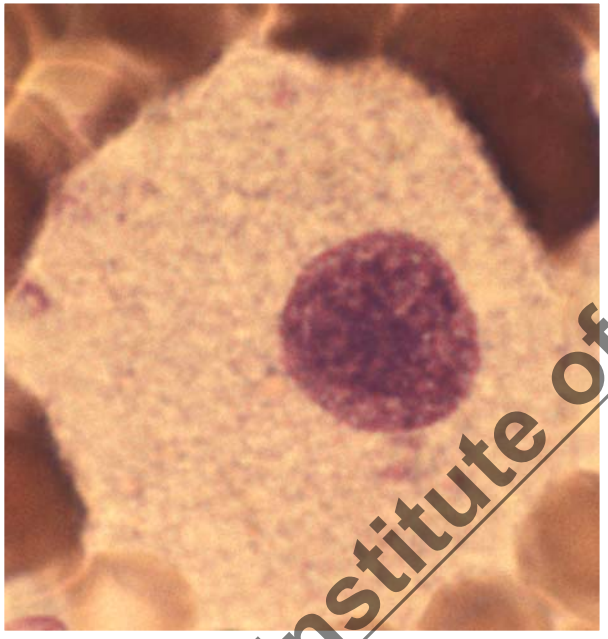
deficient lysosomal noncatalytic membrane protein

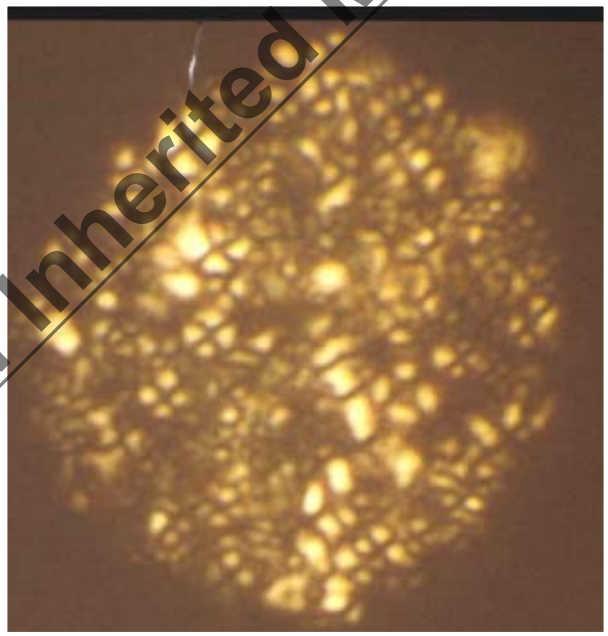
specific storage pattern at the cell level:
uniform storage of SM liquid crystals
in a b.m. histiocyte

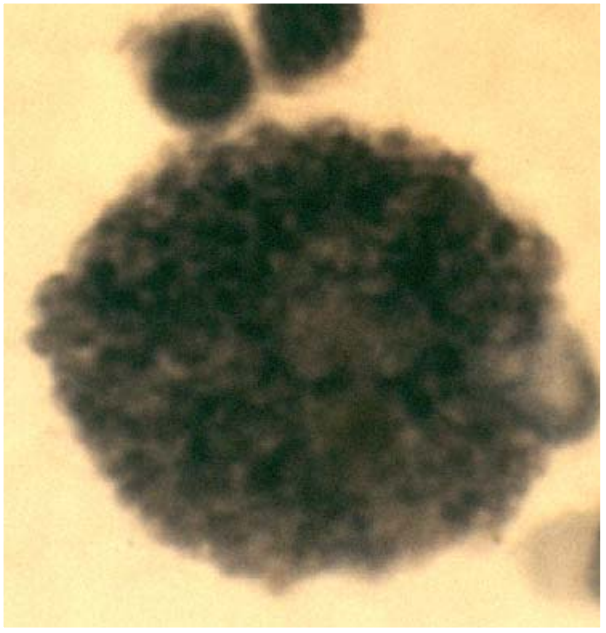
recommendation:
ASM activity evaluation

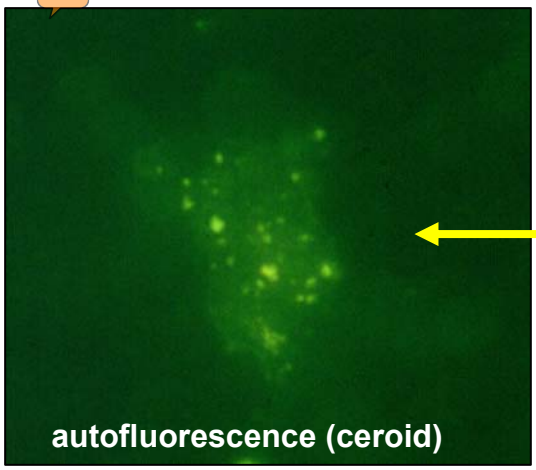
Niemann-Pick type A

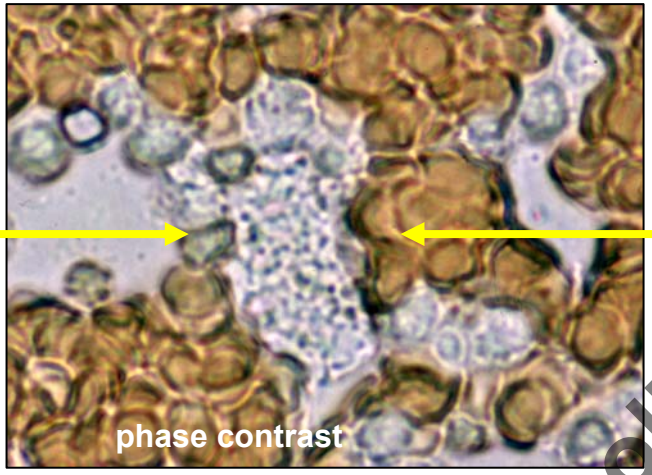

hepatosplenomegly

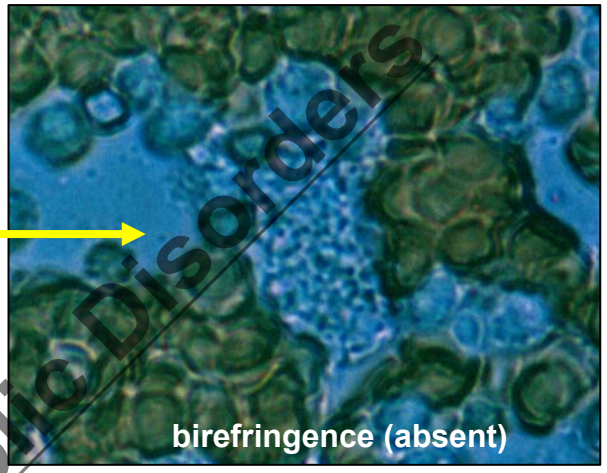

foamy storage pattern


birefringence of SM liquid crystals


uniform staining
for phospholipids

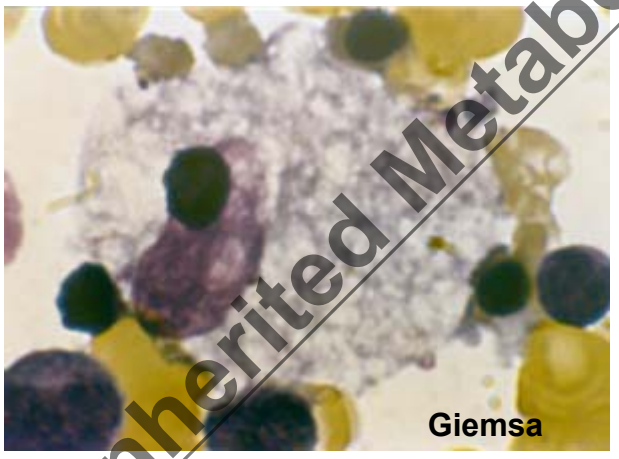






bone marrow storage pattern in NPC

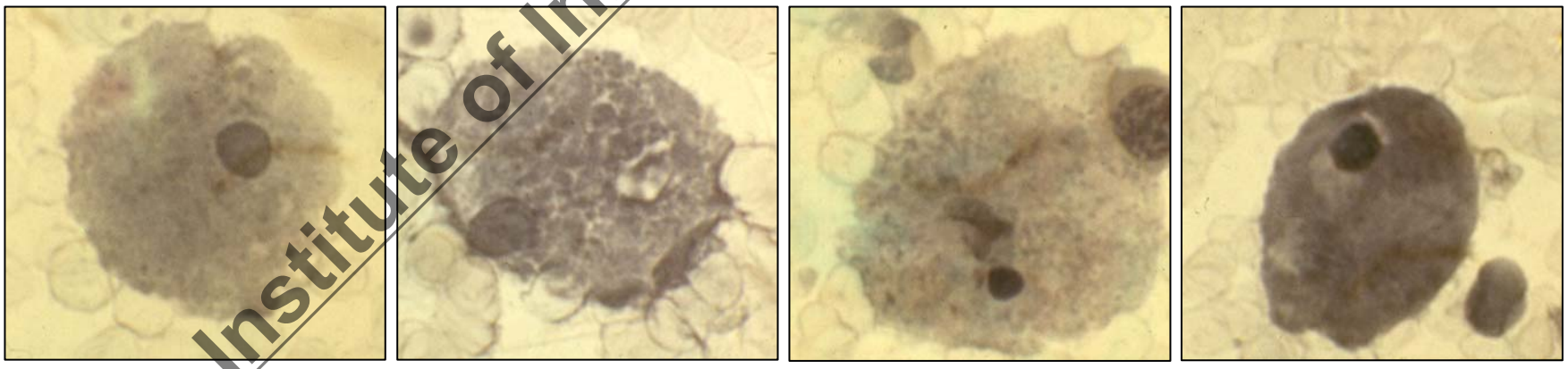
cytology and staining



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

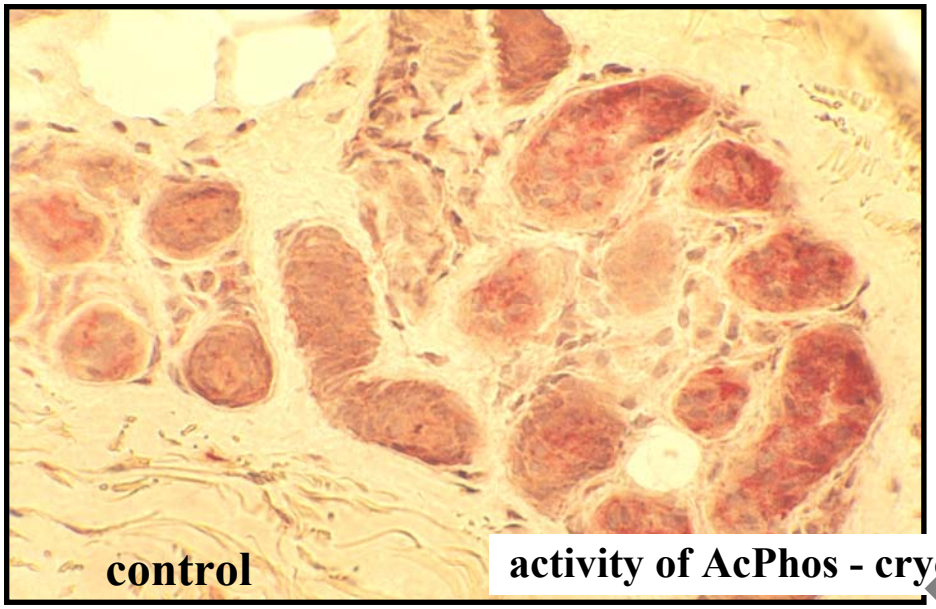
↓

Filipin test – *NPC1,2* gene

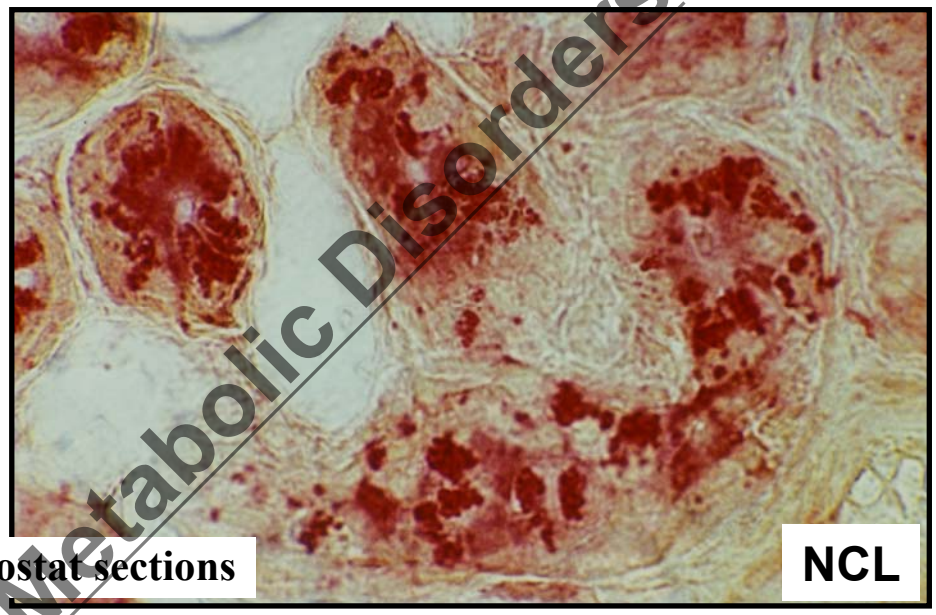


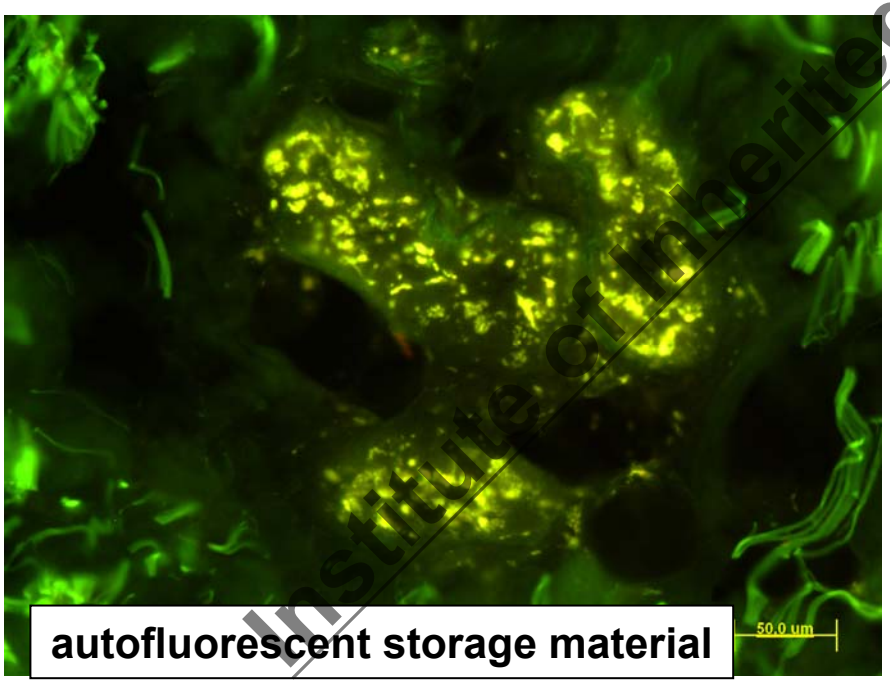
irregular accumulation of phospholipids (ferric hematoxylin)

storage pattern in NCL2 (TPP I deficiency)

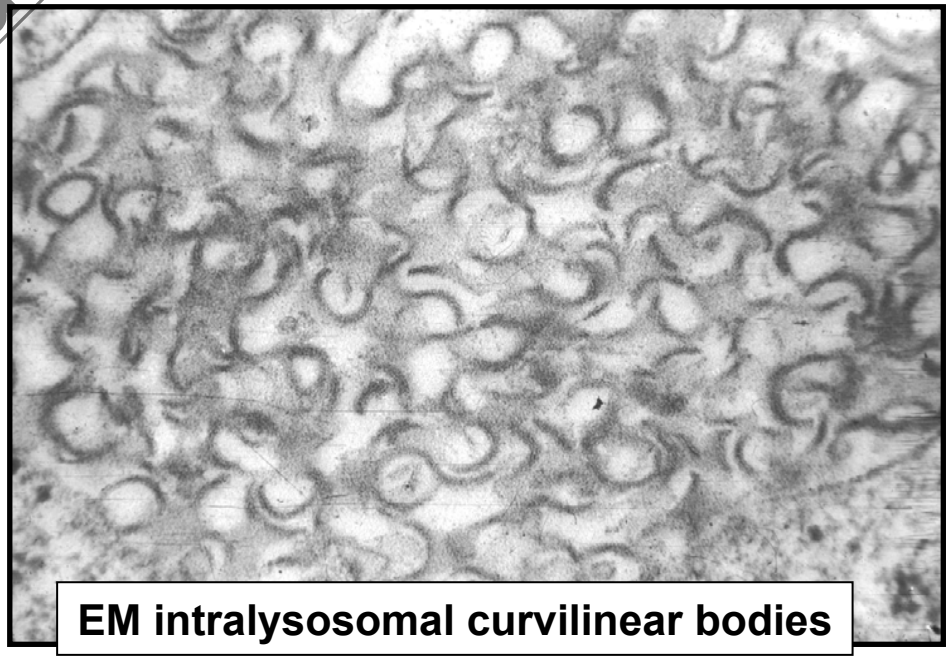


activity of AcPhos - cryostat sections



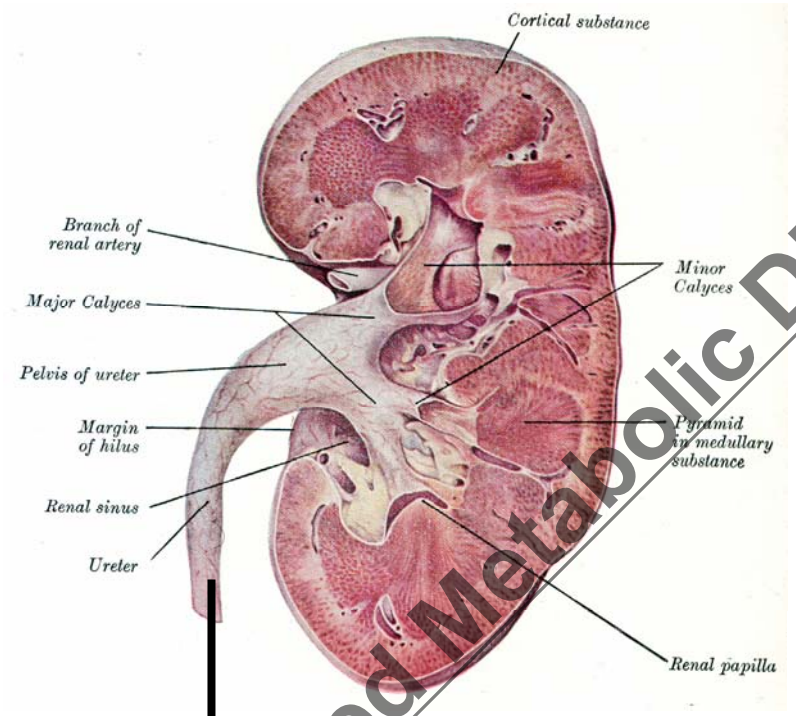


autofluorescent storage material



EM intralysosomal curvilinear bodies

Grays Anatomy, TB Johnston et al., eds.,1958



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

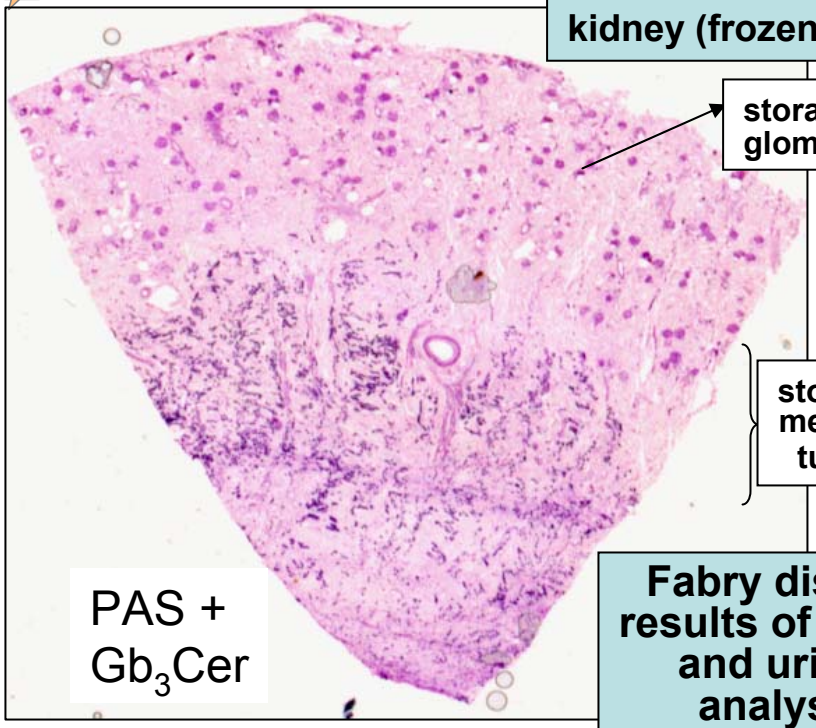
Gaucher	negative
Krabbe	negative

Lipidoses with positive findings in the urine
(currently not utilized for screening)

NPA/B	sphingomyelin/cholesterol
CESD/Wolman	cholesteryl esters

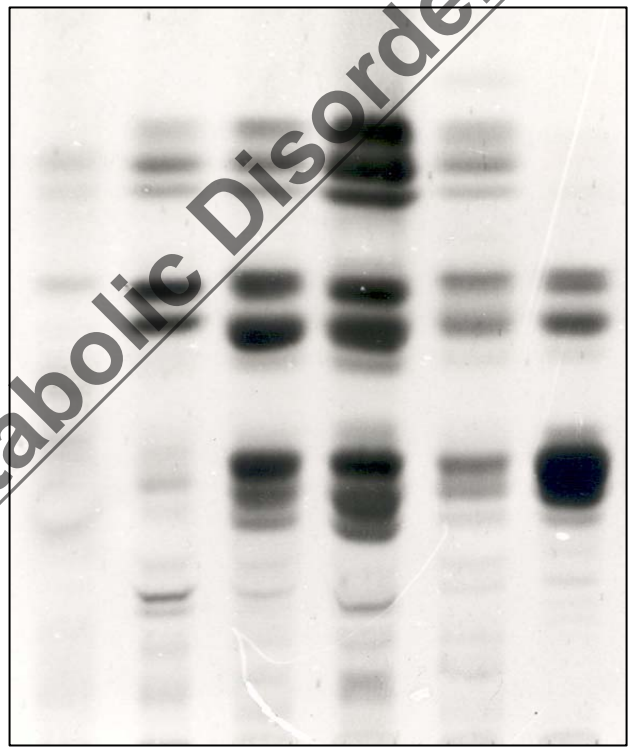
Lipidoses with positive findings in the urine
(recommended for screening)

Fabry (incl. SapB def.)	Gb_3Cer
MLD (incl. SapB def.)	sulphatide
Farber	ceramide
GM1 gangliosidosis	βgal - OLS
GM2 gangliosidosis	GlcNAc – OLS

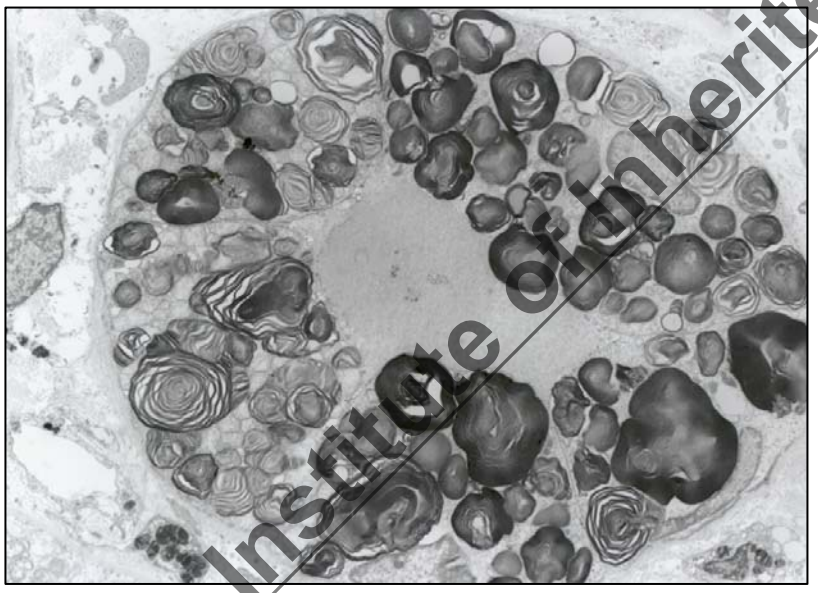


Fabry disease results of kidney and urinary analysis

glycolipids in the urinary sediment

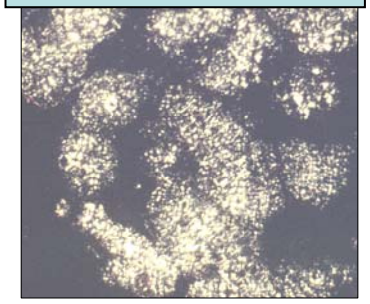


N normal control; C carriers; H hemizygote



EM – storage in tubular epithelium

section of the kidney

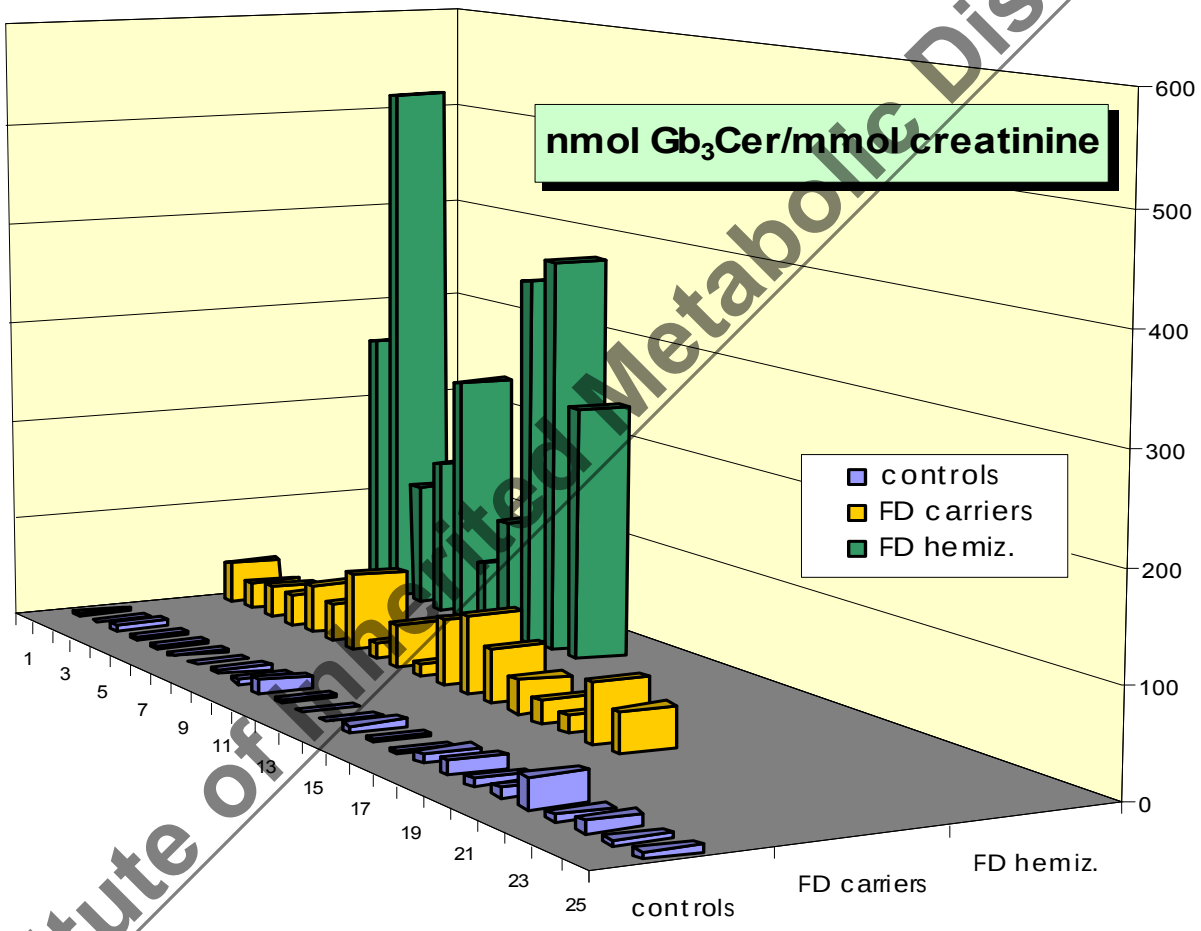


birefringence of in situ tubular cell storage

u.sediment

desquamated storage cell

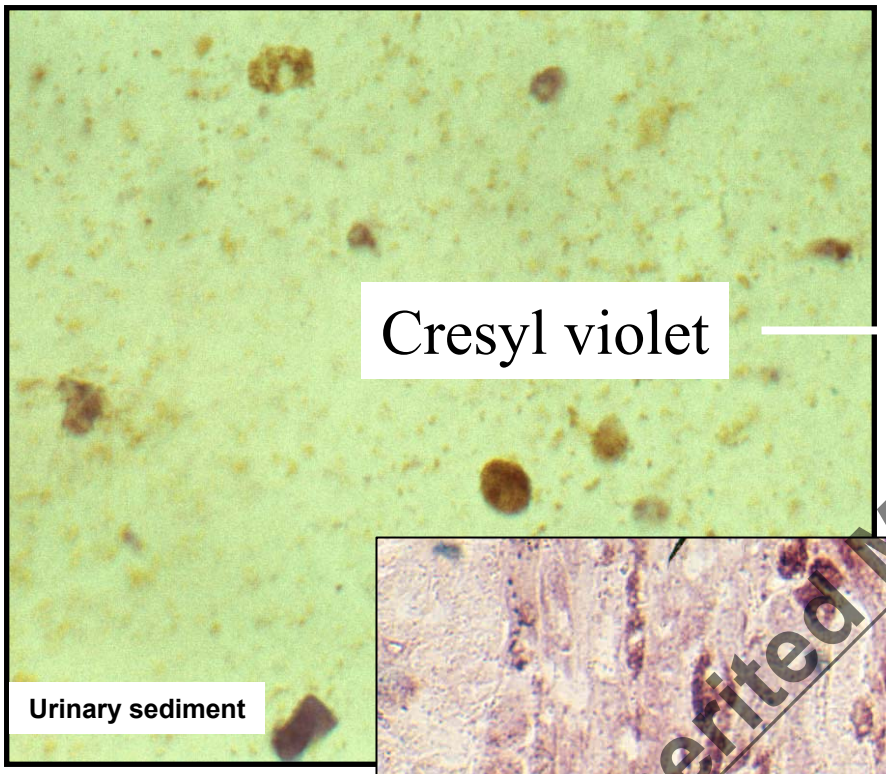
Gb_3Cer in urines of Fabry (FD) hemizygotes, female carriers and controls.

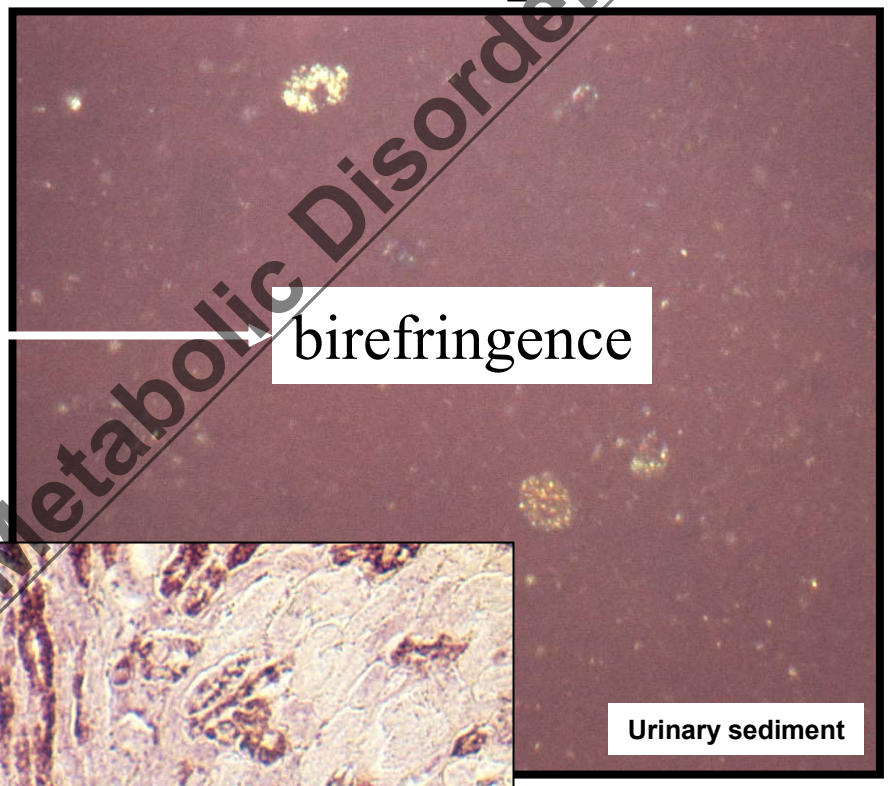


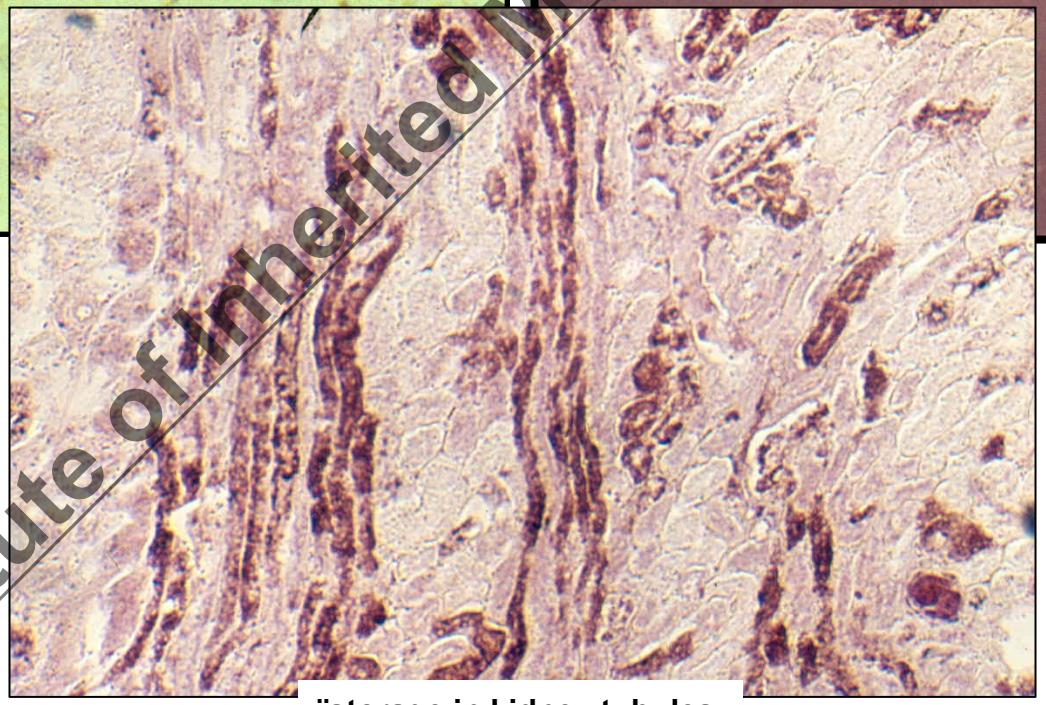
METHOD: Extraction of total urinary lipids – reversed-phase chromatography → **Lipid separation – HPTLC → orcinol detection → quantification densitometrically (CAMAG II)**

Berná L. et al.,Anal Biochem, 269, 304-311 (1999)

kidney tubules and urinary sediment in sulphatidosis

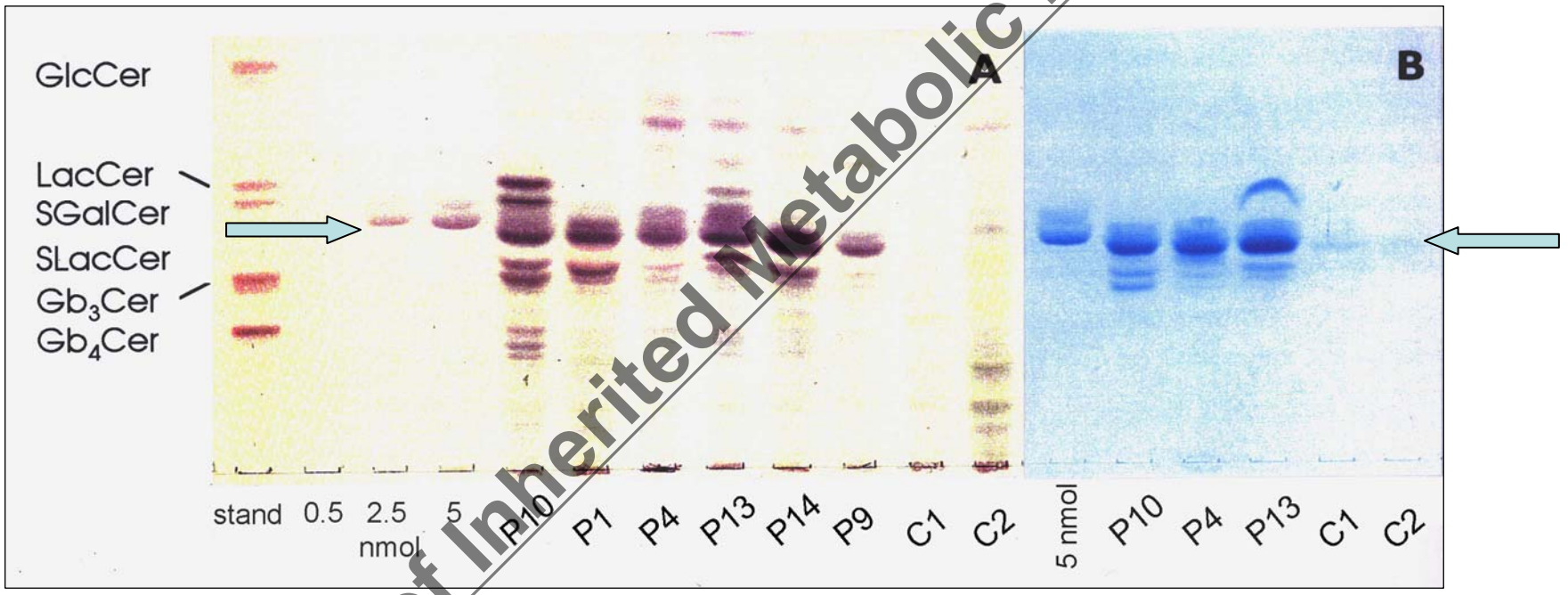






¨storage in kidney tubules

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



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

Berná L. et al.,Anal Biochem, 269, 304-311 (1999)

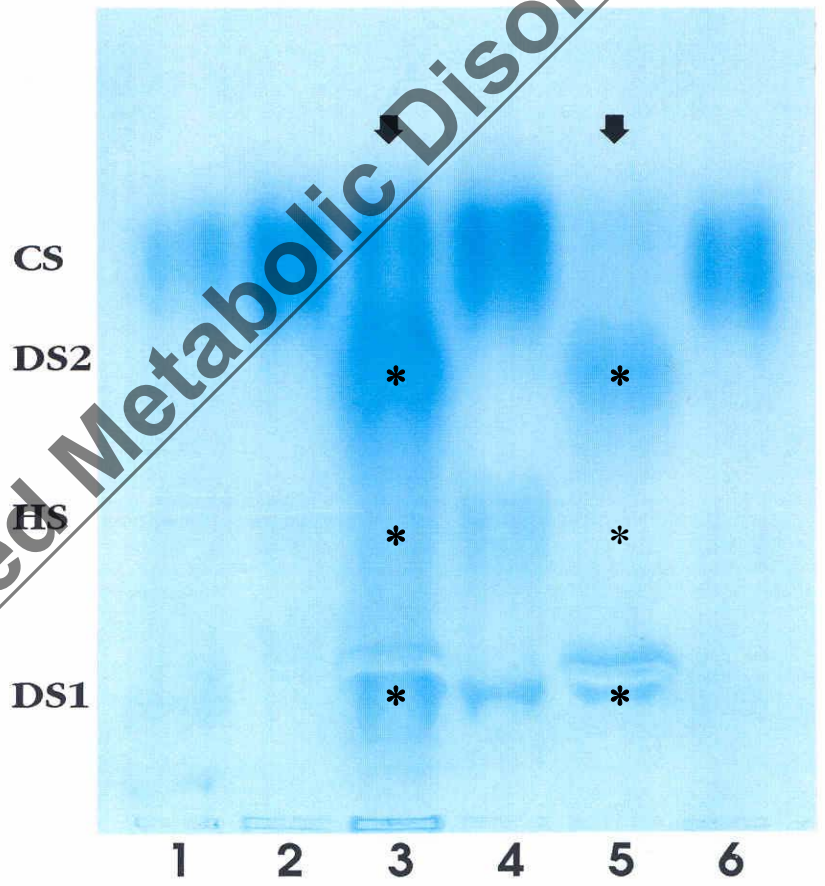
urine in mucopolysaccharidoses (GAGs)

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

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

MPS I – Hurler disease (α –iduronidase deficiency)

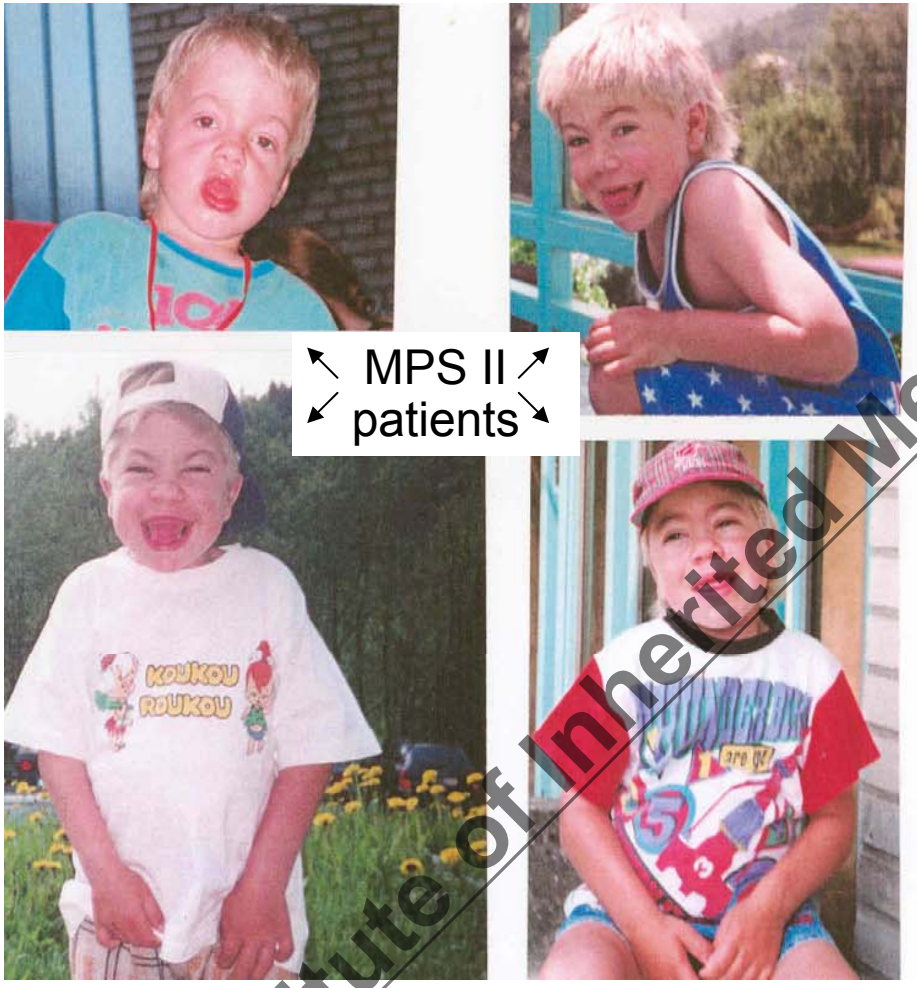
MPS I in a 6-year-old girl



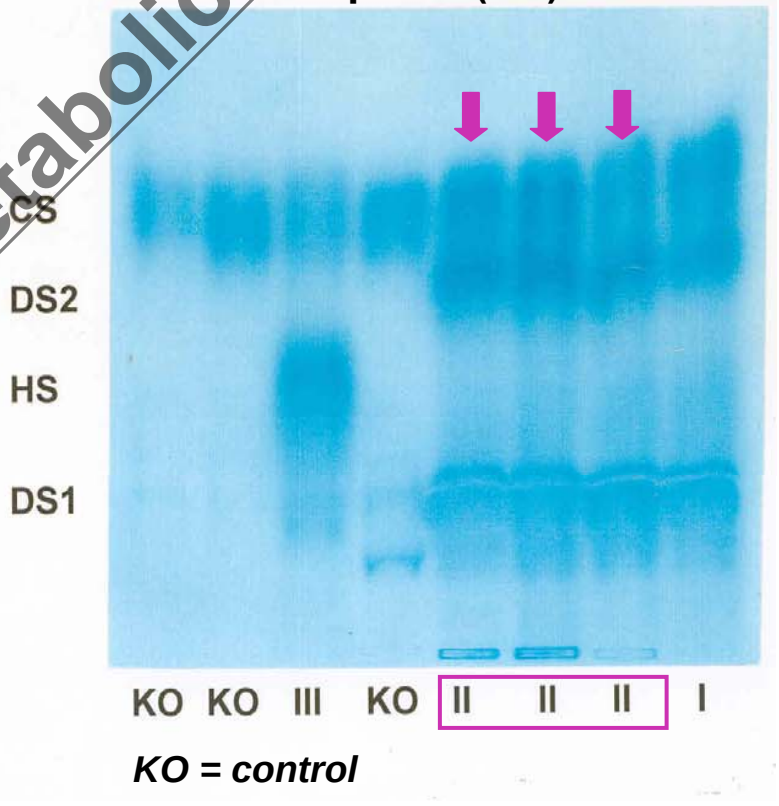
Electrophoresis of urinary GAGs

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



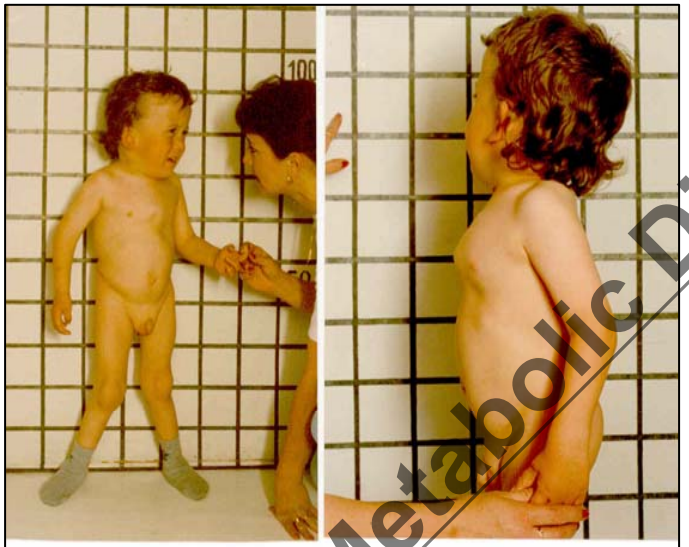
Urinary excretion of dermatan sulphate (DS1,2) and heparan sulphate (HS)

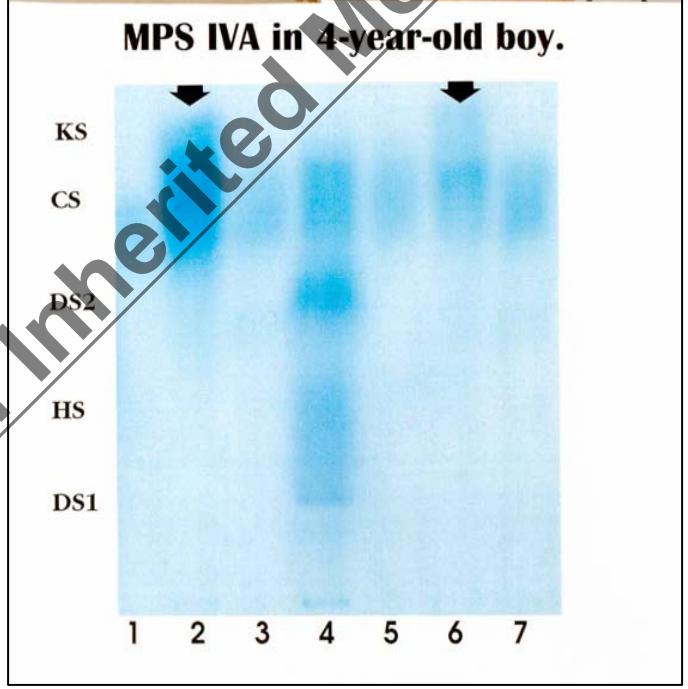


MPS II: iduronate-2-sulphate sulphatase deficiency, X – linked disorder

MPS IVA

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





2,6 = MPS IVA (see arrows)

4 = MPS I

1,3,5,7 = controls

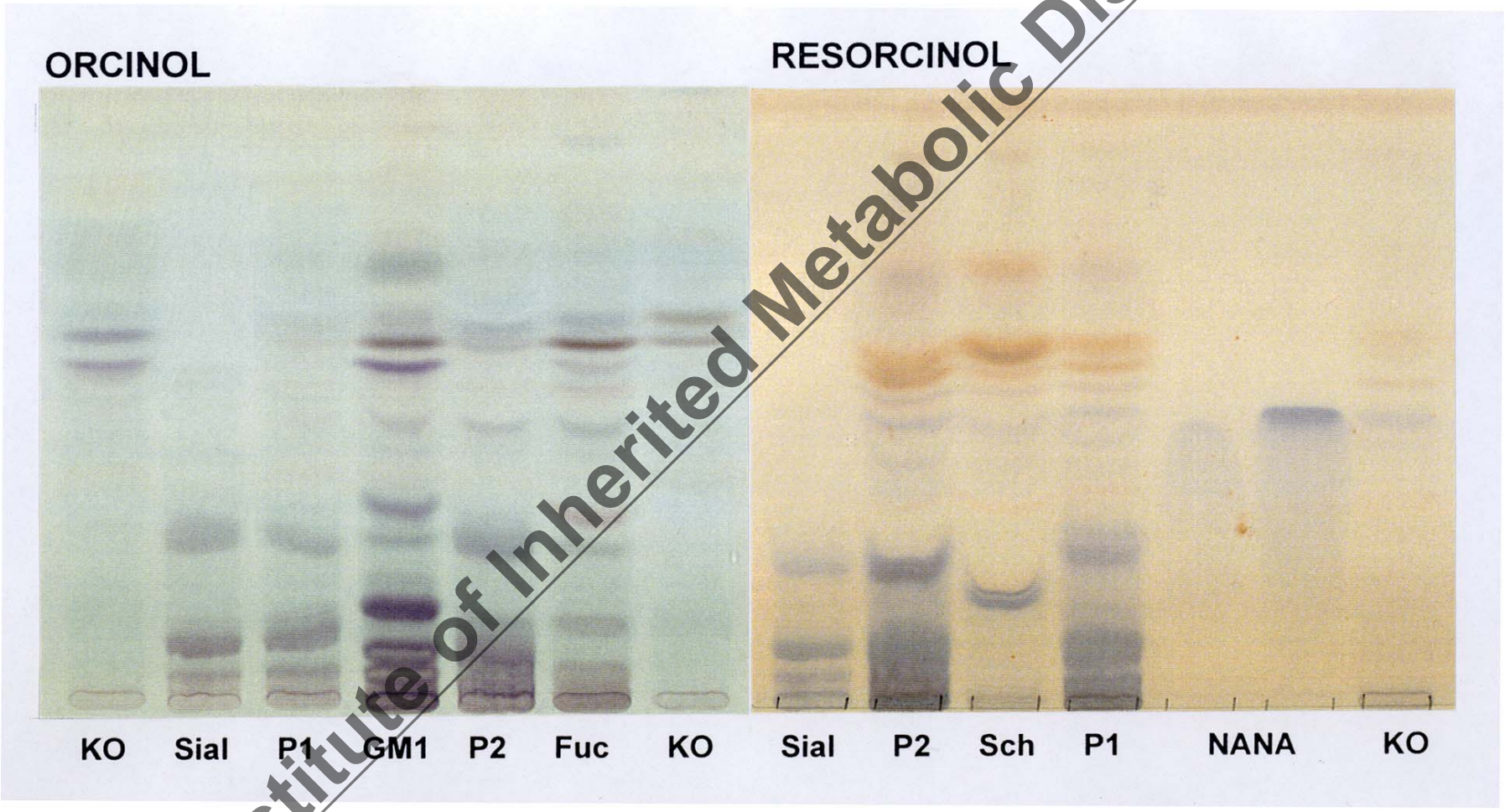
Urine in glycoproteinoses and related disorders

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

GM1 gangliosidosis ..... βgal-OLS
α-mannosidosis ..... αmann- OLS
β-mannosidosis ..... βmann- OLS
α-Fucosidosis ..... αfuco - OLS
Sialidosis ..... sialyl - OLS
Galactosialidosis ..... gal- and sialyl – OLS
AGU ..... aspartylglucosamine
(+ other glycoasparagines)
ISSD (SALLA dis.) ..... free sialic acid
Schindler disease ..... sialyl – OLS
GSD II ..... *occasionally* $(Glc)_4$ *

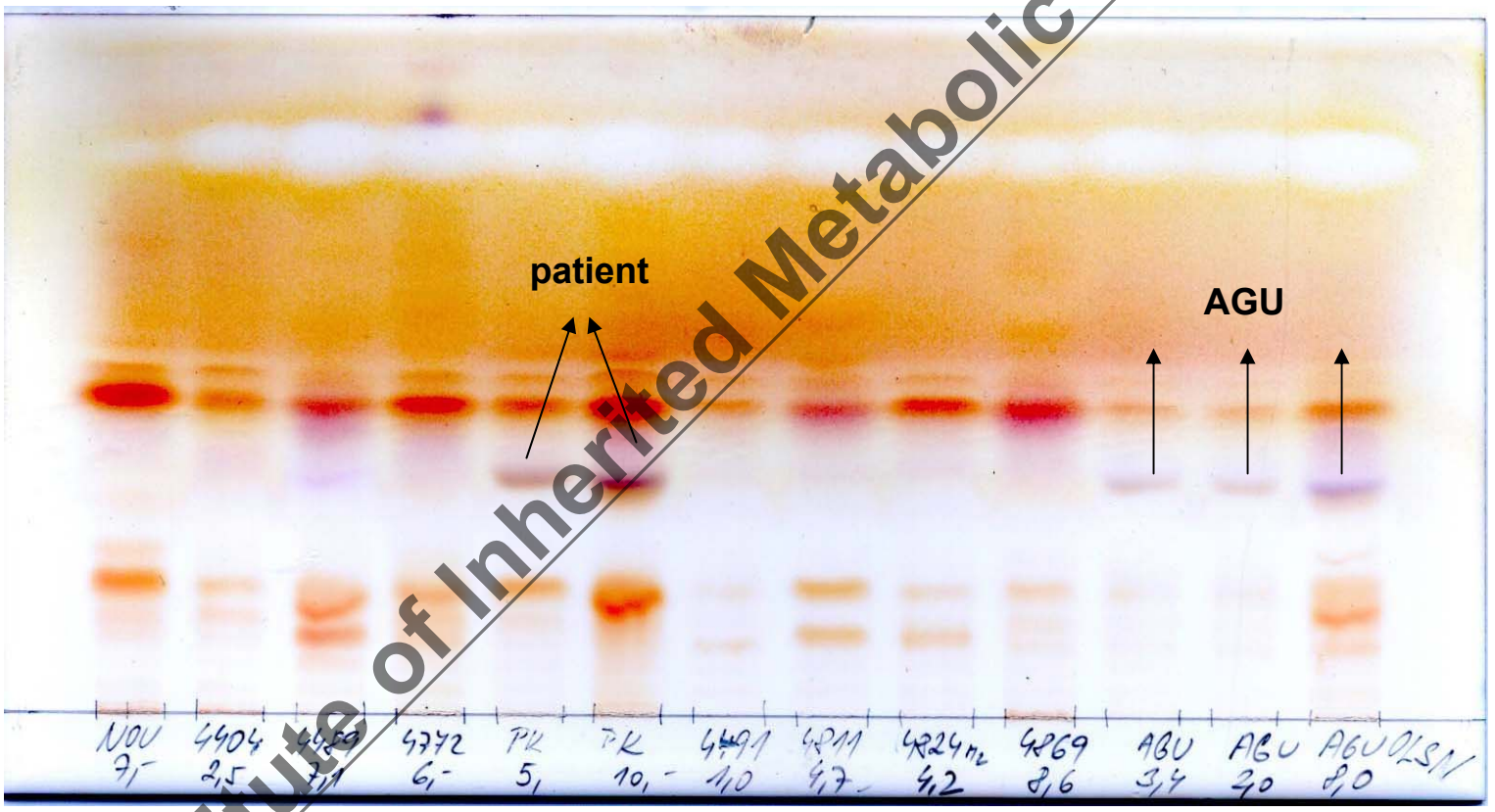
*

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 unsignificant findings)

NPCs	**negative**
NCLs	**negative (**or SCMAS)
Danon dis.	**not studied**
ML IV	**phospholipids**
Cystinosis	**generalized AAU**

Clinical findings :

dysmorphia; dysostosis

neurology; visceromegaly;

corneal clauding (absent in MPS II)

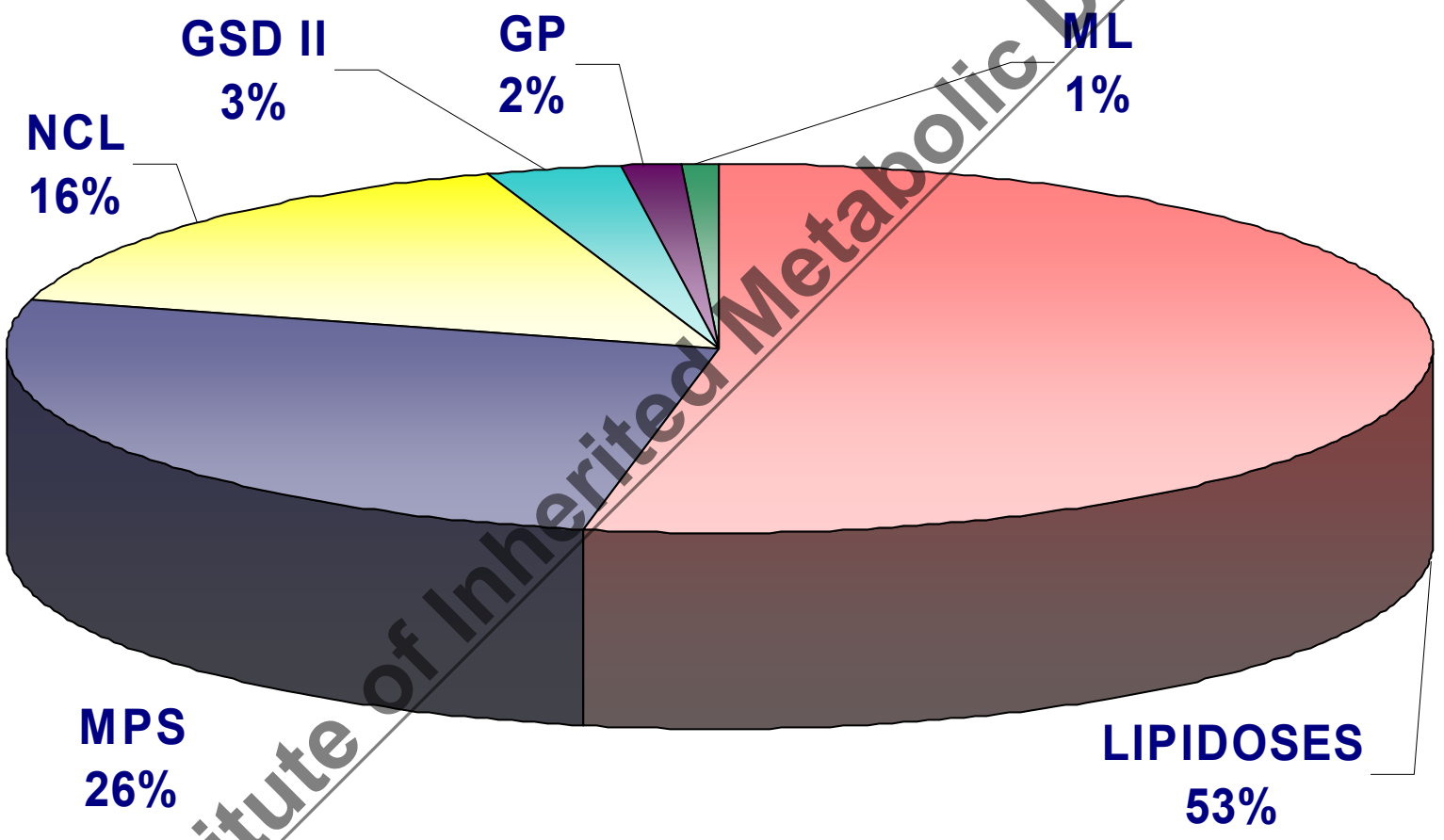
heart valvular disease;

storage vacuoles in lymphocytes incl. Alder-Reily granules

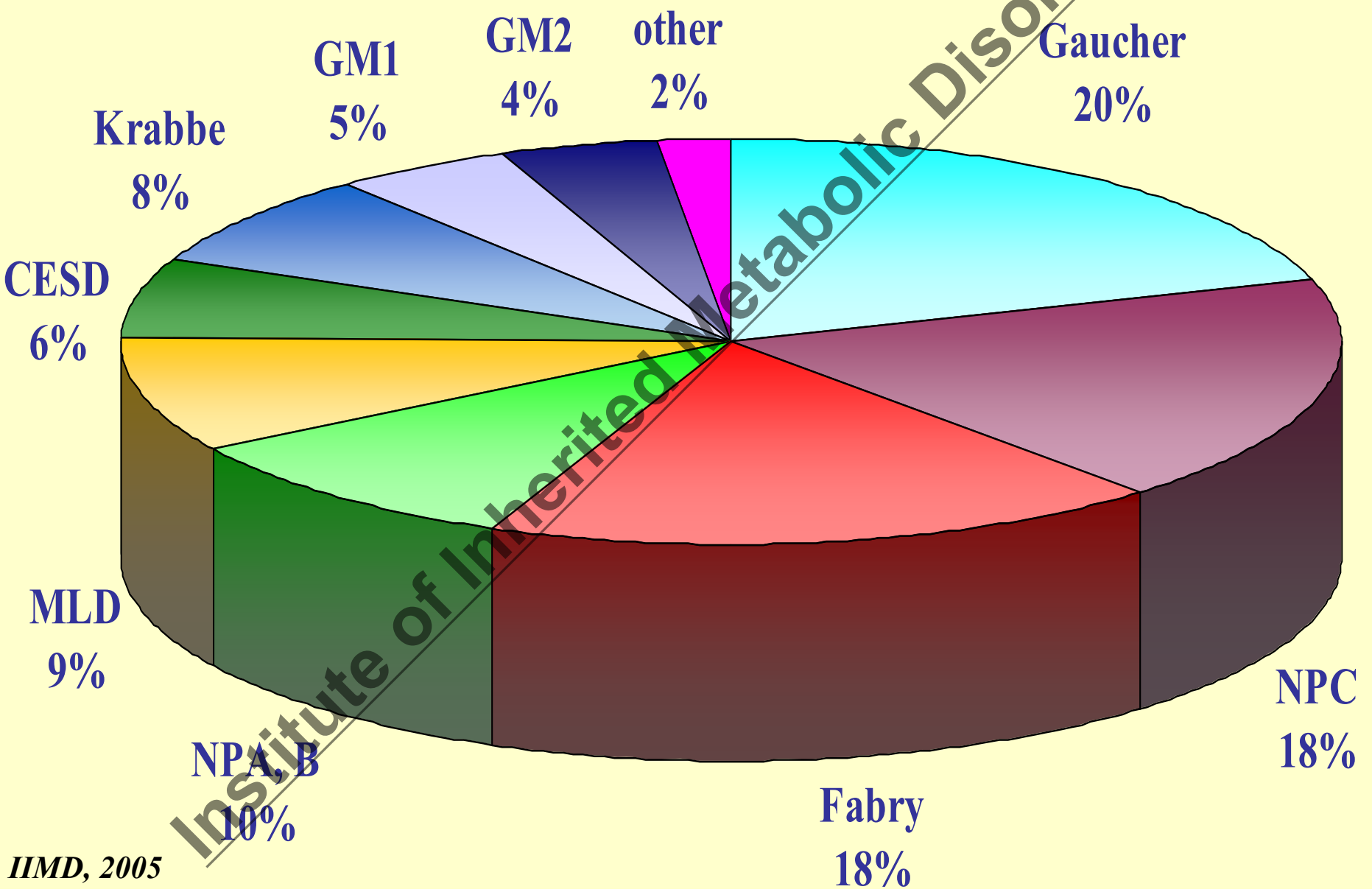
Urine analysis:

- **GAGs**
- **OLS**
- **free silaic acid**
- **AGU**

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

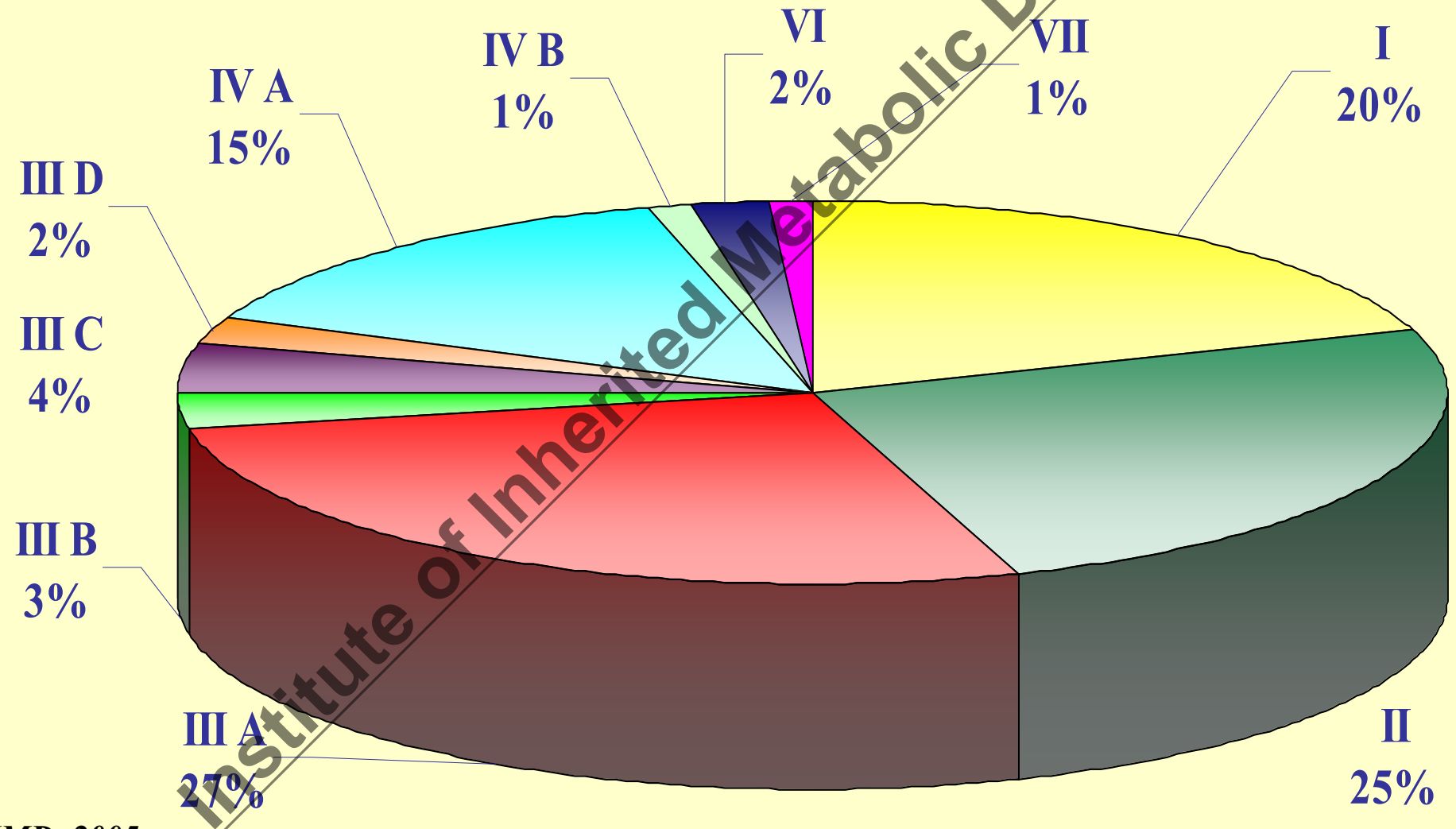


LIPIDOSES IN THE CZECH AND SLOVAK REPUBLICS
1975-2005 (n=279)



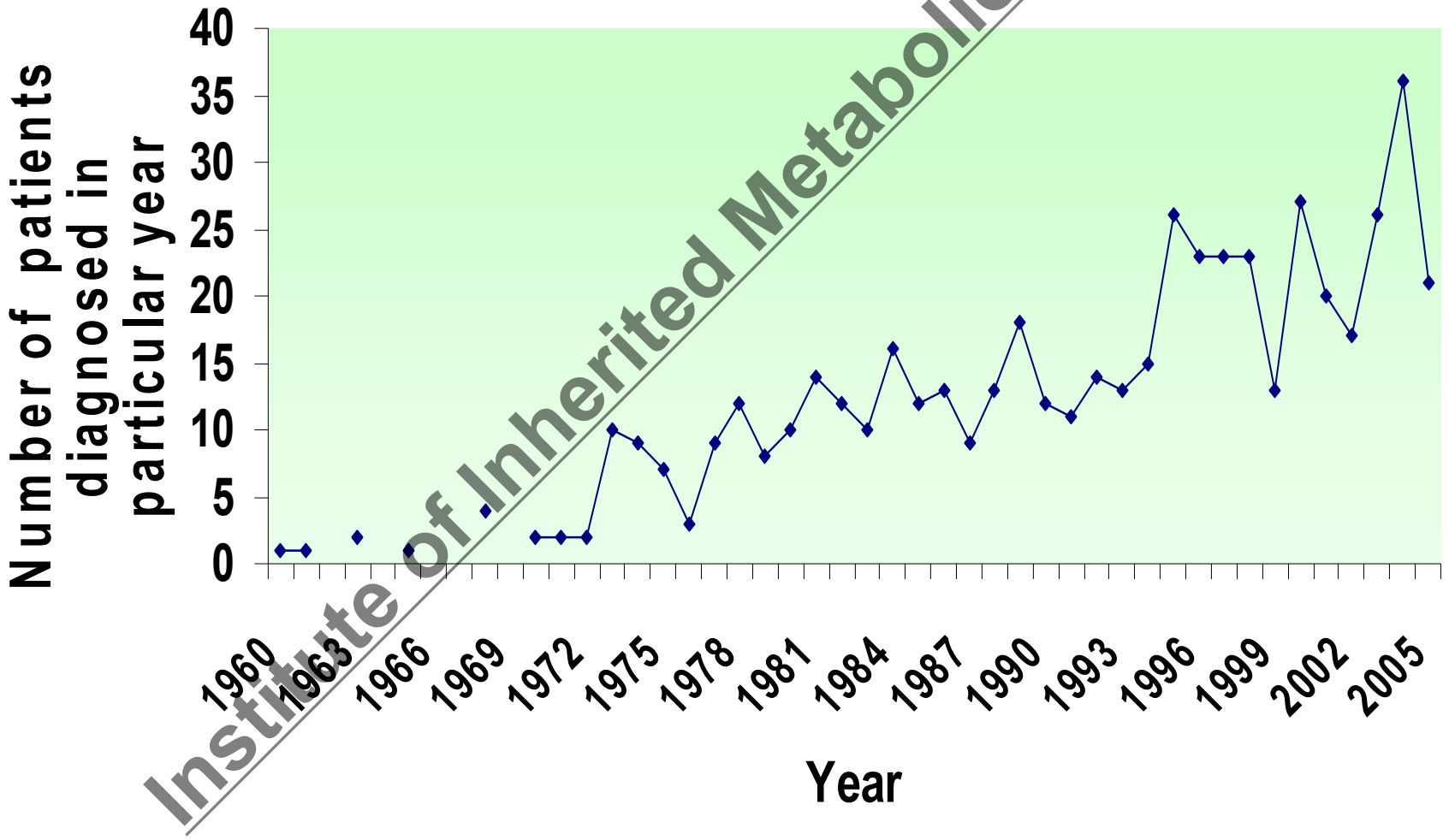
IIMD, 2005

MUCOPOLYSACCHARIDOSES 1975 - 2005 (n=103)

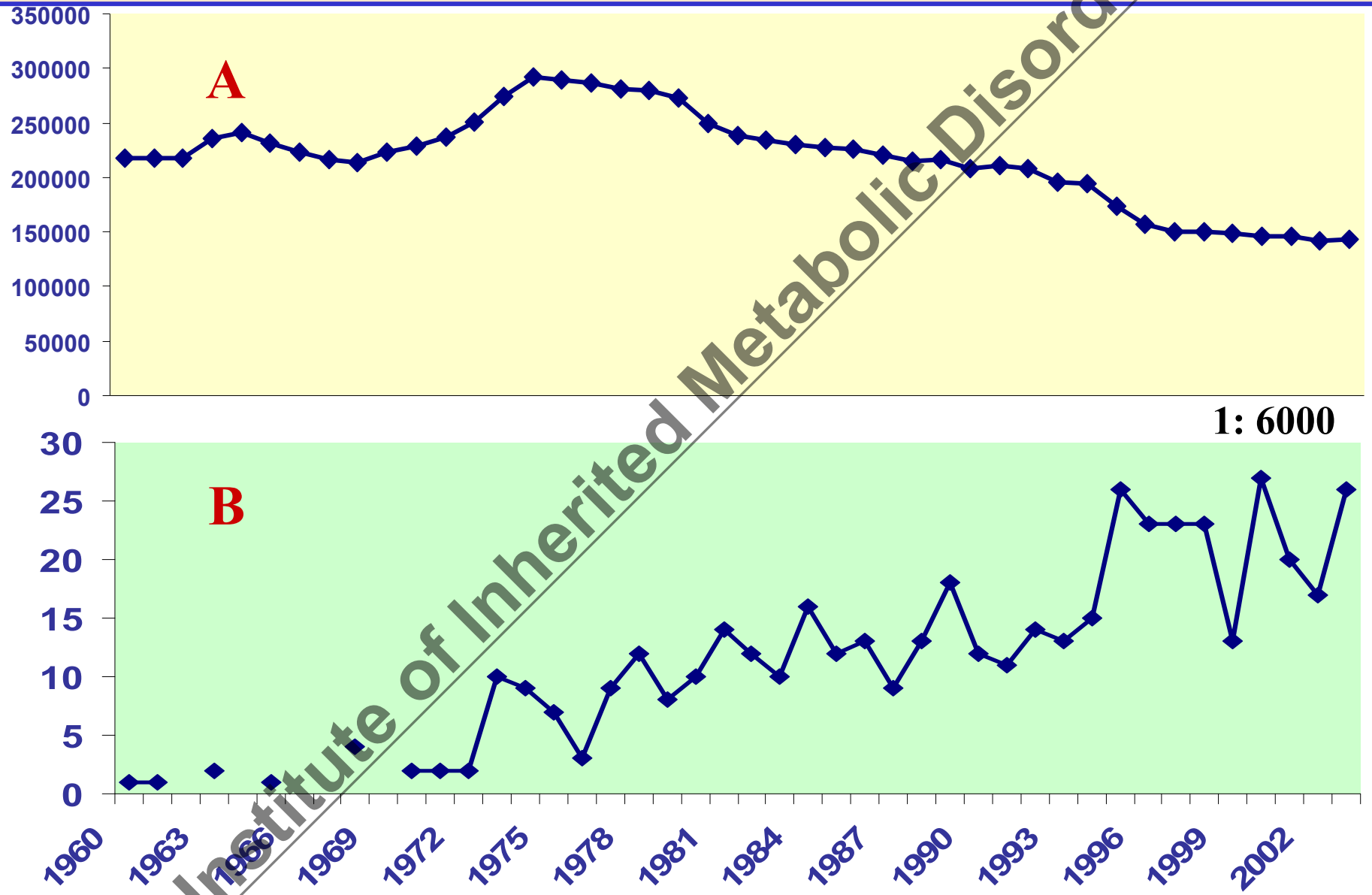


IIMD, 2005

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

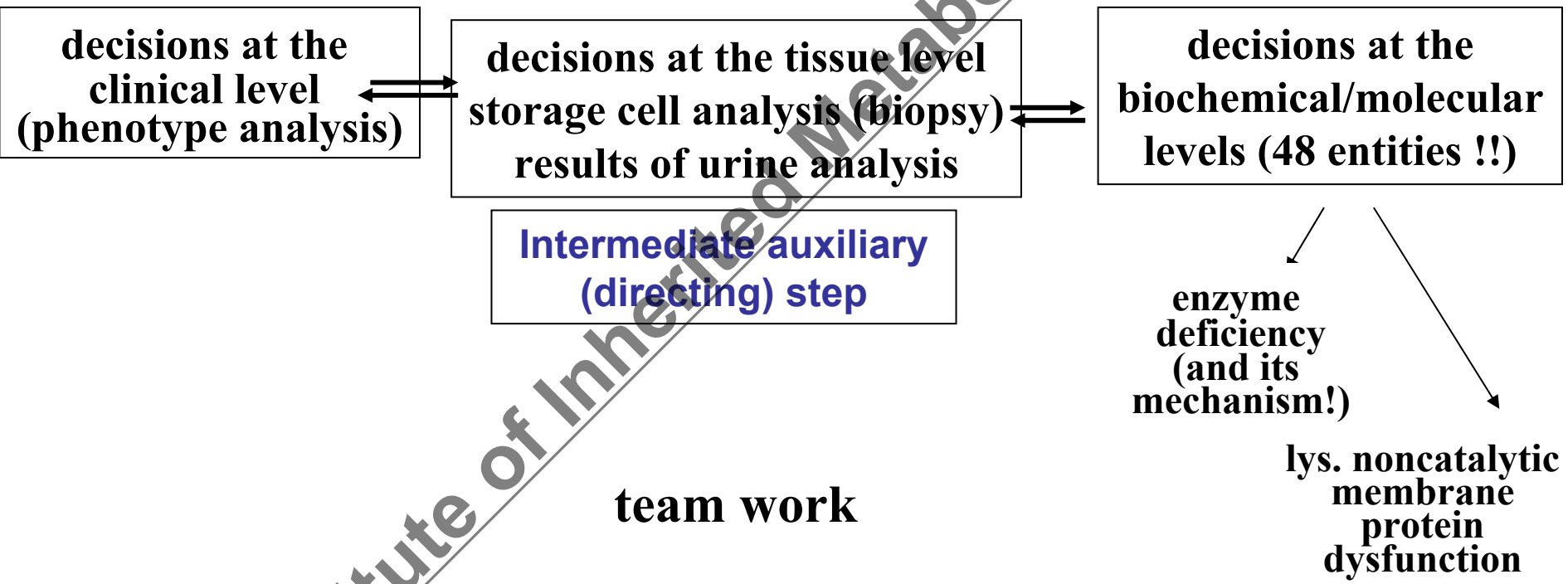


No. of live births (A), postnatal and prenatal diagnoses of LSDs (B) in the Czech and Slovak Republics (1960 - 2003)



Institute of Inherited Metabolic Disorders
Prague, March 2004

The diagnostic procedures



team work

Selected syndromes suggestive of LSD

systemic disorder in childhood mainly

dysmorphy + 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 (αGal 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 disciplins

(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 neurolopidoses)

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-, βMannosidosis, PSD, Farber)

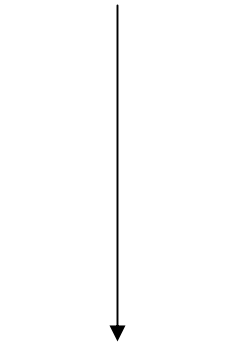
hematology (Gaucher, NPA/B, NPC, ect)

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



The end

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histology, EM, histochemistry: M. Elleder, H. Hůlková (div. B)