RECENT AND FUTURE CHANGES IN LABORATORY DIAGNOSIS OF MUCOPOLYSACCHARIDOSES

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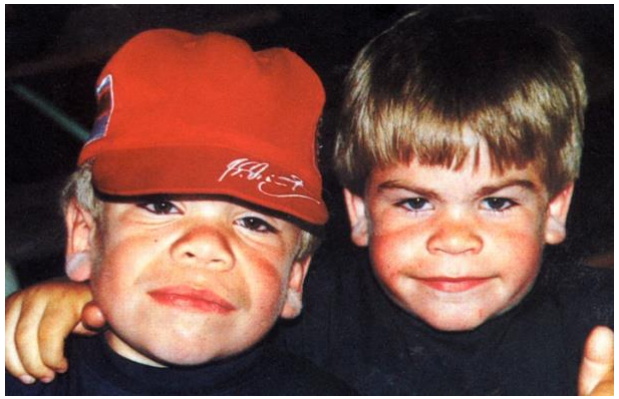
Medical University of Graz, Austria

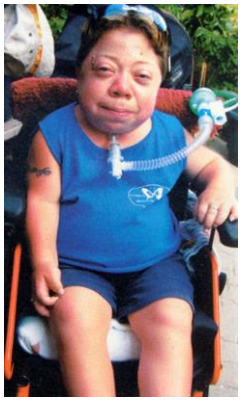
20. MPS konferencia
2014. október 3-5. Gödöllő-Máriabesnyő

Mukopolysaccharidoses

Hurler (MPS I H)

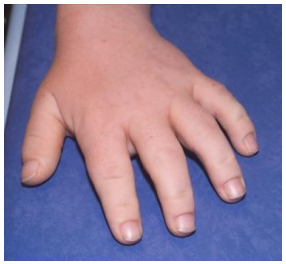


Hunter Disease (MPS II)

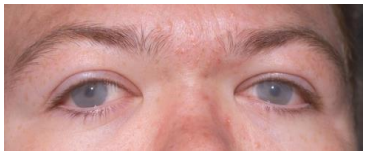


Maroteaux-Lamy (MPS VI)

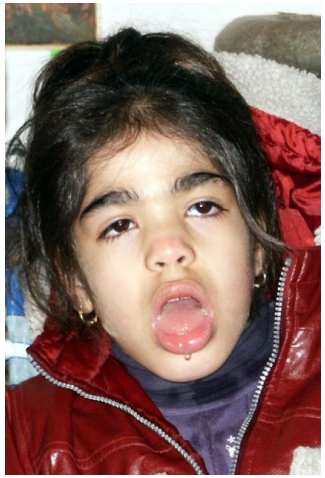
Morquio A (MPS IVA)



Joint contractures



Corneal clouding



Sanfilippo A (MPS IIIA)

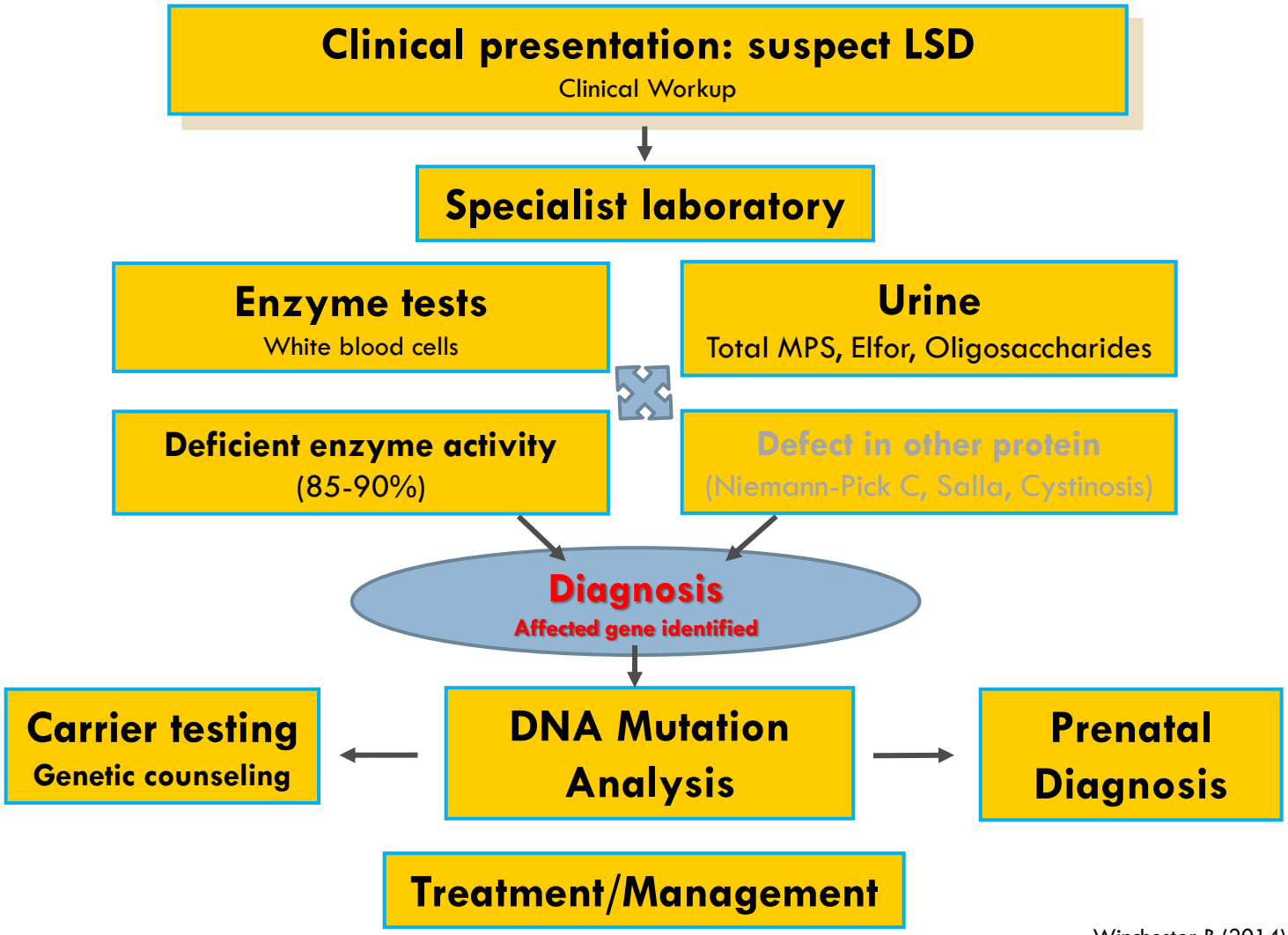
Mukopolysaccharidoses

heterogenous „natural histories“

M.Hurler (MPS I H)	„Hurler/Scheie compound“ (MPS I H/S)	M.Scheie (MPS I S)
	⟷	
Central nervous system	**+++**	**normal**
Bone	**+++**	**(+)**
Lung	**+++**	**(+)**
Ears	**+++**	**(+)**
Eyes	**+++**	**+**
Gastrointestinal Functions	**++**	**(+)**
Heart and Circulation	**+++**	**++**
Life span	**<10J**	**normal**

The Classical Strategy

for the Diagnosis of Mucopolysaccharidoses



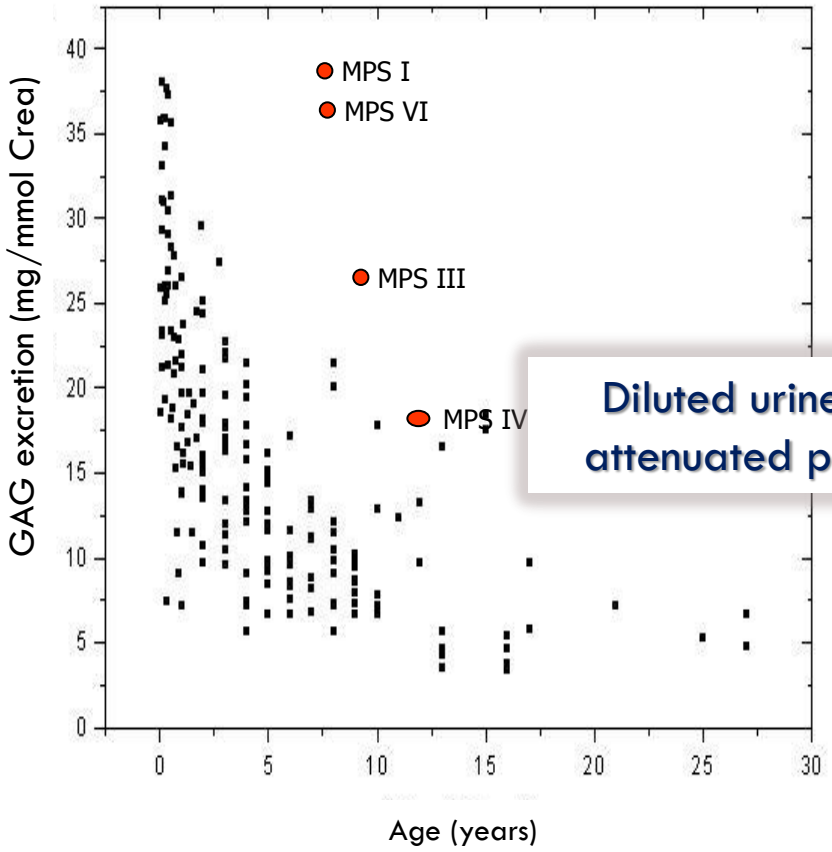
Winchester B (2014), JIMD 37: 599-608

Routine Analysis of Urinary Metabolites

Spontaneous Urine sample, [dried urine on filter paper], manual methods

Total GAG excretion (DMB-Assay)

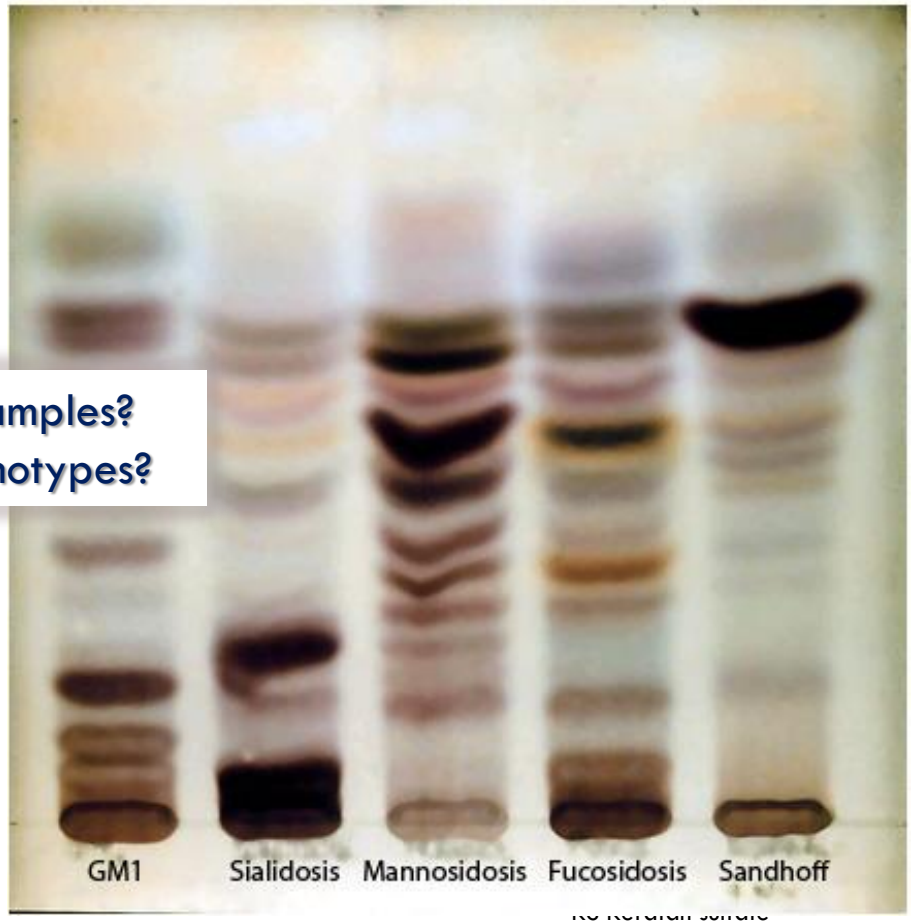
(de Jong JG et al, Clin Chem. 1989)



Diluted urine samples?
attenuated phenotypes?

TLC of Urinary Oligosaccharides

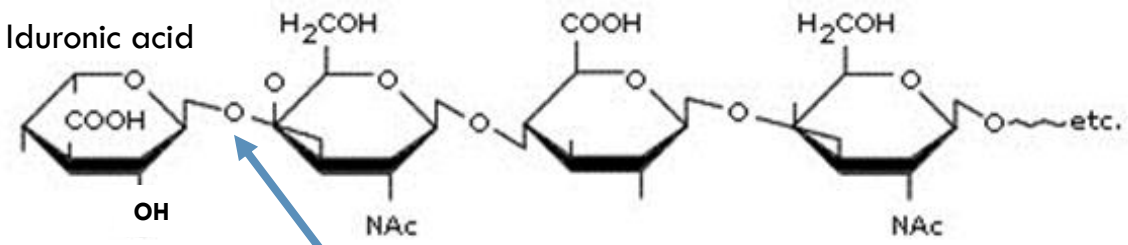
(Sewell et al (1980)



Routine Enzyme Assays

in Leucocytes (serum, fibroblasts, chorionic villi…)

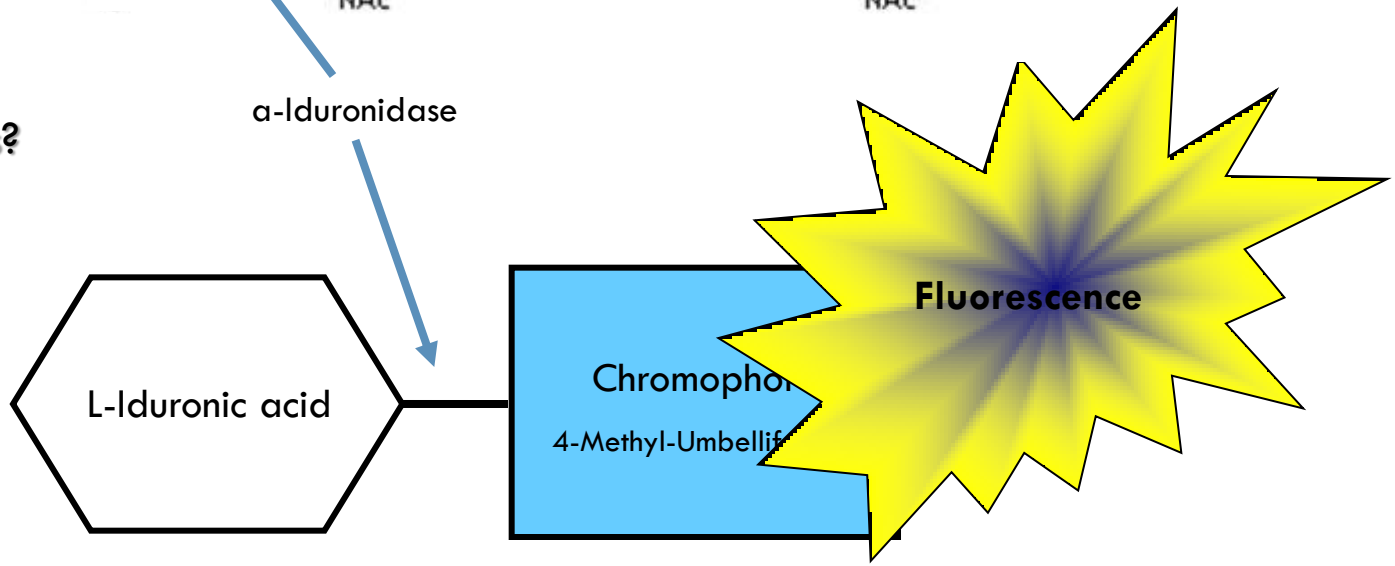
„natural“ substrate



Heterozygote carriers?

a-Iduronidase

„synthetic“ substrate



pH10

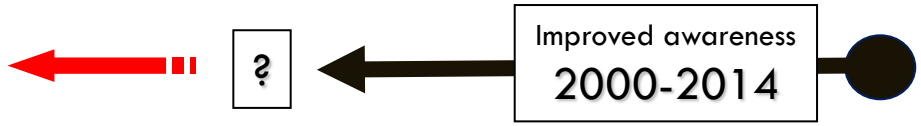
DNA Analysis

Current Routine Procedures

- If the disease-causing gene has been successfully identified, all its exons are sequenced („Sanger sequencing“)
- Mutations are detected and confirmed by restriction analysis
- Novel missense mutations are checked for pathogenicity
  - parental segregation,
  - occurrence in >100 WT DNA samples
  - in silico analysis and protein alignment (e.g. Polyphen-2, SIFT)
- Genetic couseling
  - carrier testing
  - prenatal diagnosis in CVS or cultured chorionic villi cells
- Hundreds of mutations/gene, genotype-phenotype relations are unclear, only few genotypes are predicitive

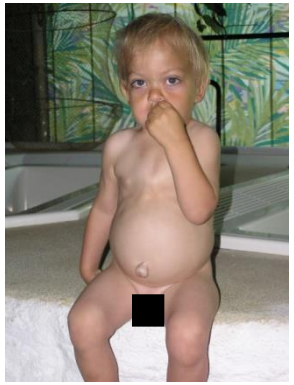
Therapy demands early or presymptomatic diagnosis

to improve the outcome



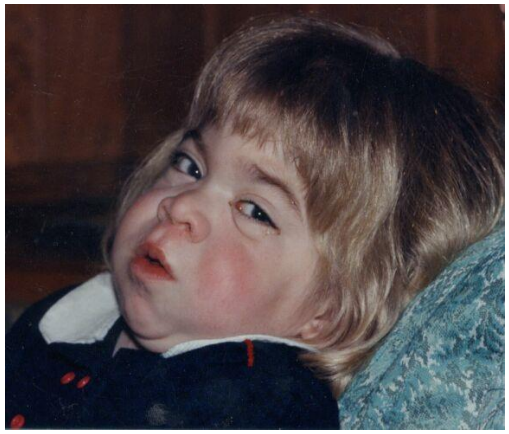
1 week

1.8 years



Hernias,
Hepatomegaly,
retarded speech
development

5.5 years



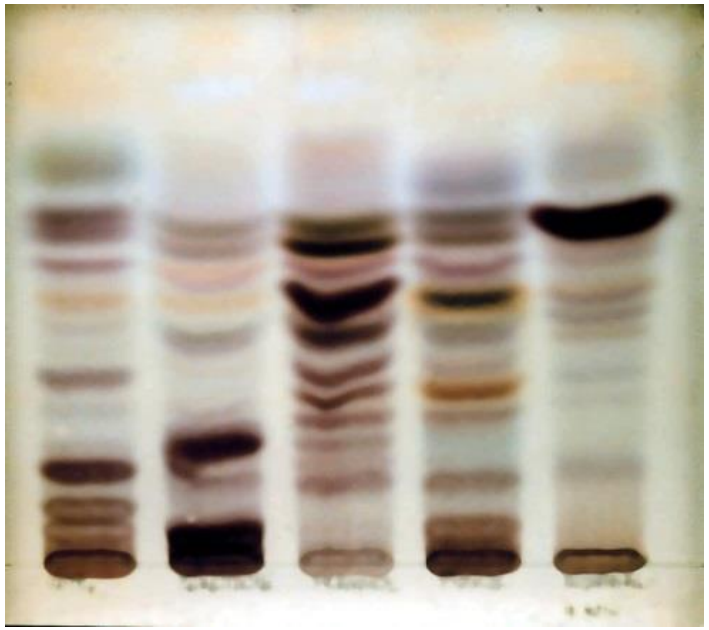
Remarkable facies,
Joint problems,
Cardiomyopathy,
Developmental arrest

Preymptomatic diagnosis?
Newborn screening?

Mass Spectrometry for Analysis of Storage Products

Recent Achievements for Oligosaccharides

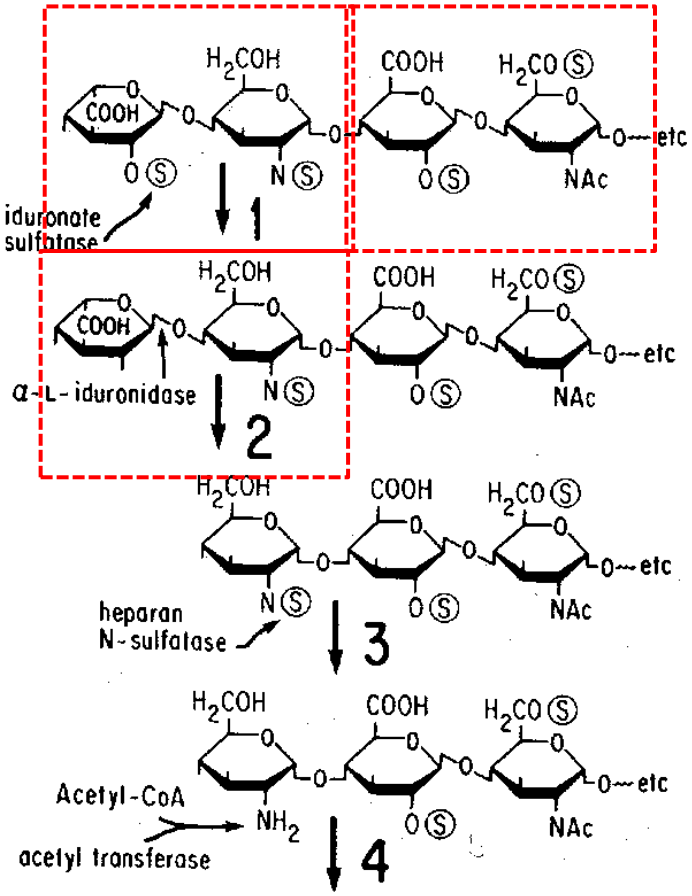
- **Oligosaccharides (e.g. MALDI-TOF/TOF)**
  (Xia B Clin Chem 59/9; 1357-1368 (2013))
  - High-throughput quantitative screening with permethylated free urinary oligosaccharides detects sialyloligosaccharides and glycoaminoacids (AGU)
  - Characteristic, quantitative patterns for
    - a-Mannosidosis
    - Pompe
    - Galactosialidosis
    - Sialidosis
    - MLII/III
    - Fucosidosis
    - AGU
    - Gaucher
    - GM1/GM2 Gangliosidosis



Mass Spectrometry for Analysis of Storage Products

Recent Achievements for Glycosaminoglycans

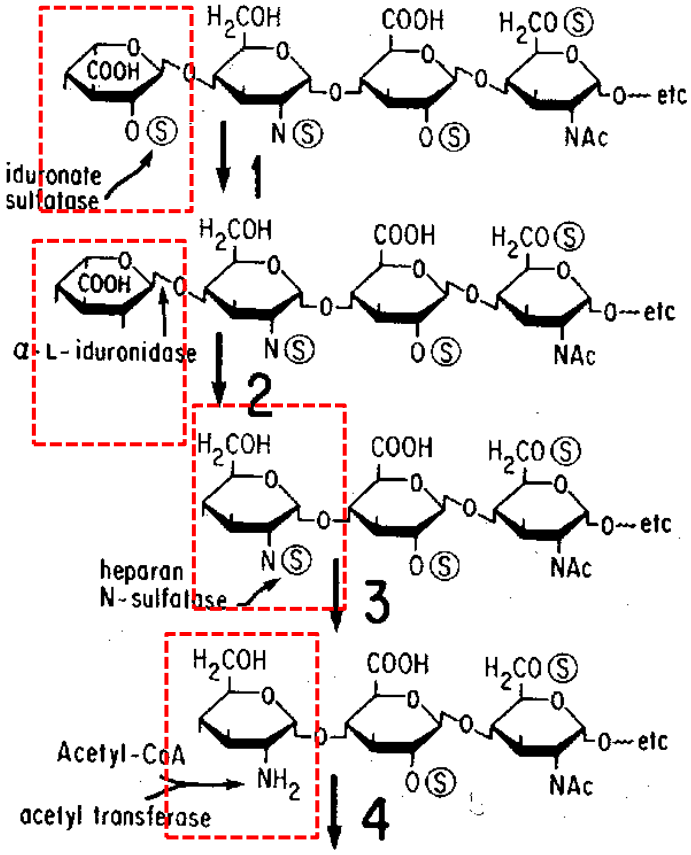
- Glycosaminoglycan Subtypes
  - Specific internal standards,
  - depolymerization of GAGs with bacterial disaccharidases or by methanolysis
  - Quantitation of disease-specific GAG-fragment
    - MPS I, II and III (Ruijter, 2012)
    - MPS I,II and VII (Tomatsu, 2013)
    - MPS I, II and VI (Auray-Blais, 2012)



Mass Spectrometry for Analysis of Storage Products

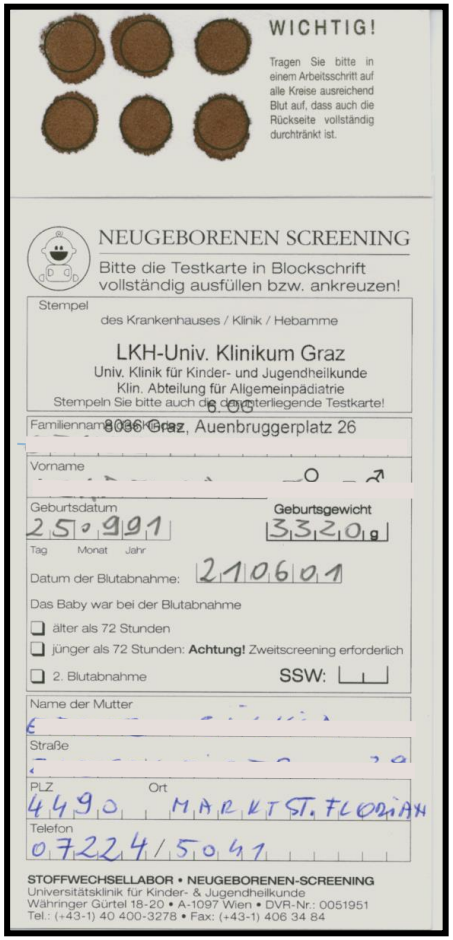
Recent Achievements for Glycosaminoglycans

- Analysis of non-reducing ends (NRE) of GAGs
  - NREs are specific for enzyme deficiency
  - NREs can be identified and quantified after tagging with aniline and HPLC separation (Lawrence 2012)
  - Applicable for urine, blood CSF, cultured cells, DBS
  - Detects MPS I, II, IIIA and IIIB in newborns
  - **Enzyme-specific diagnosis of MPS types in urine samples!**



Early Diagnoses improve the outcome of treatment

Dry blood spots



Chamoles NA et al

Hurler-like Phenotype: Enzymatic Diagnosis in Dried Blood Spots on Filter Paper

Clinical Chemistry 47:12 2098–2102 (2001)

- Lysosomal enzyme are stabilized upon drying of blood spots on filter paper
- Can be shipped over long distances
- If stored under appropriate conditions the activities are stable for months
- Conventional fluorimetric assays available for many enzyme activities

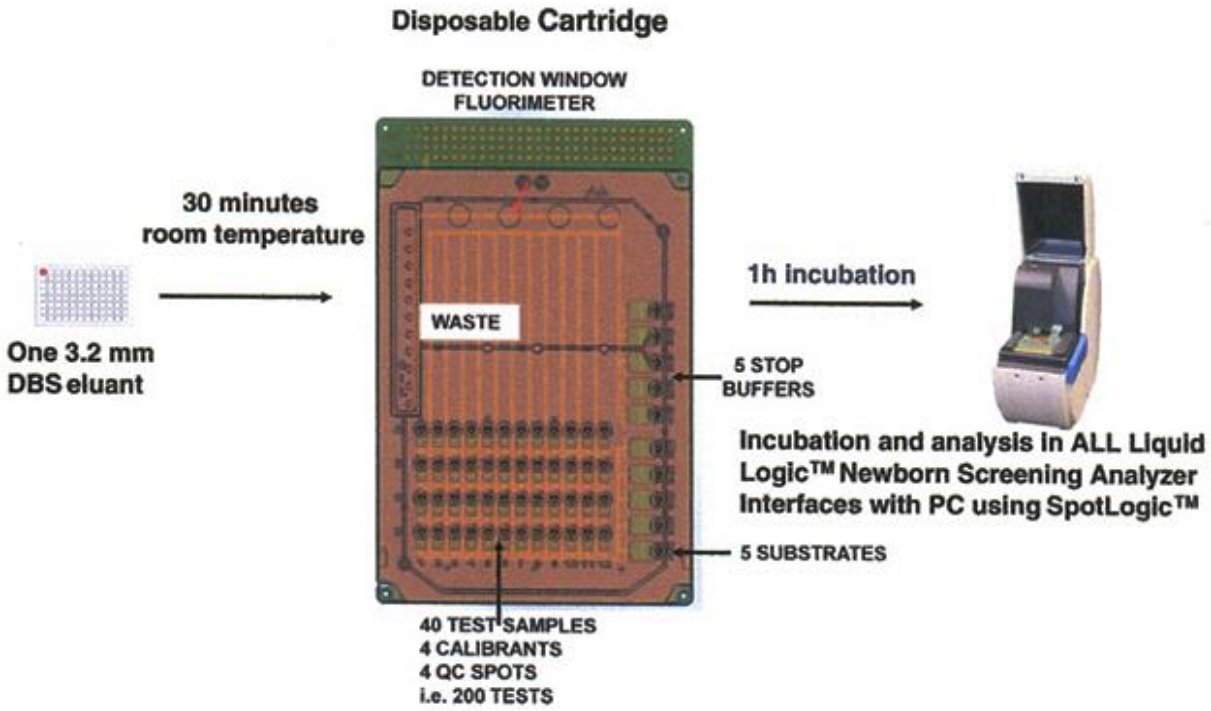
Problems:

- Only 1-2 enzymes could be assayed from 1 DBS
- Long incubation times
- Some enzymes (Sulfamidase/MPS IIIA, GalNac-6-Sase/MPS IVA) cannot be measured

Novel methods for Diagnosis

conventional 4-MU-Substrates using **microfluidics technology („Lab on a Chip“)**

Newborn screening for **Fabry, Gaucher, MPS I** und **II** and **Pompe disease**



Submicroliter volumes (tiny drops) of the assay components are transported by an electric field produced by an array of electrodes.

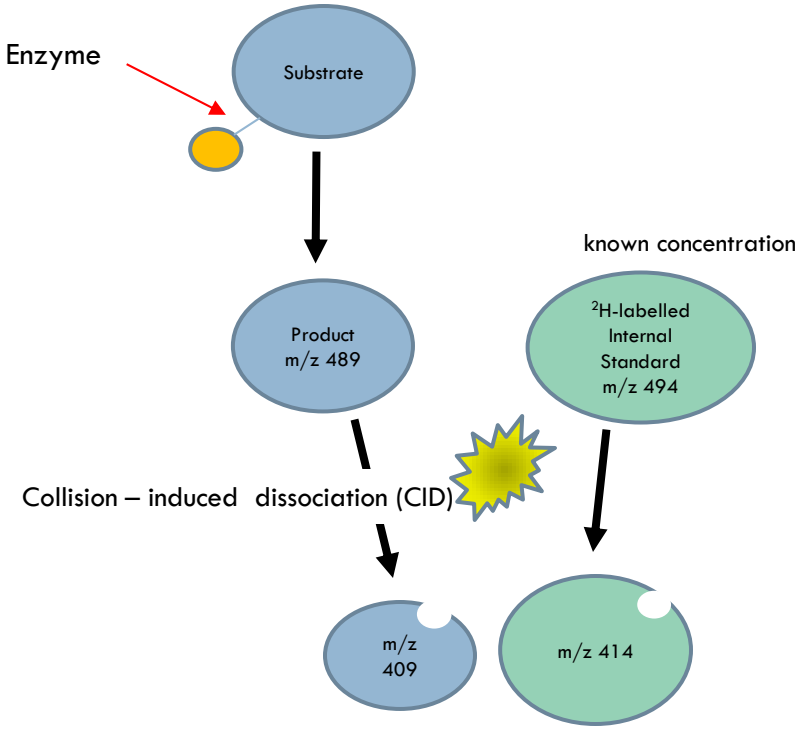
Droplets can be moved, merged, split, mixed and dispensed by varying the patterns of voltage activation in a dispensable cartridge under full software control.

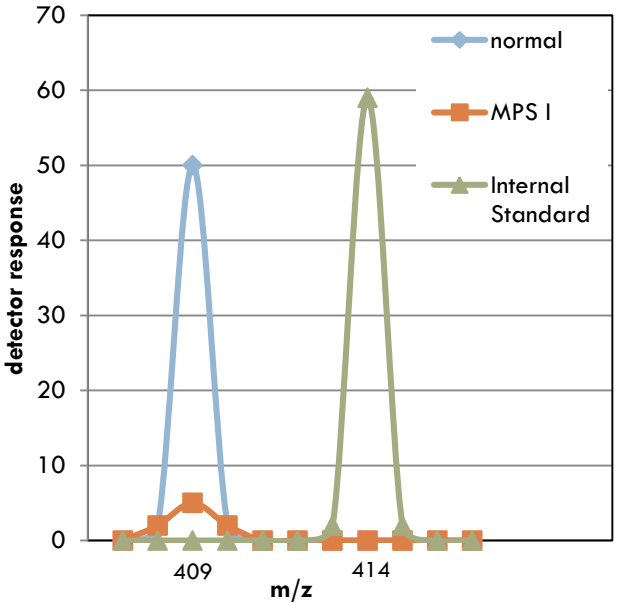
Required time is less than 3 hours for 44 samples

http://www.liquid-logic.com/;
Winchester B (2014), JIMD 37: 599-608

Mass Spectrometry for Newborn screening

Novel substrates are detected by mass/charge ratio



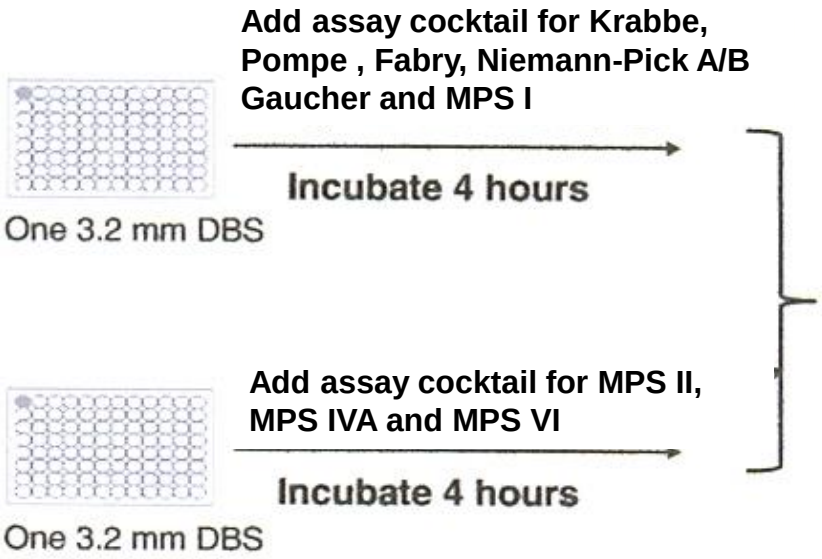


Gelb MH et al
Direct multiplex assay of enzymes in dried blood spots
J Inherit Metab Dis (2006) 29:397–404

Novel methods for Newborn screening

Spacil Z et al. High throughput assay of 9 lysosomal enzymes for newborn Screening Clin Chem 59 (2013) 501-11 2013

Winchester B (2014), JIMD 37: 599-608



Lancet. 2012 Jan 28;379(9813):335-41

Neonatal screening for lysosomal storage disorders: feasibility and incidence from a nationwide study in Austria.

Mechtler TP[1], Stary S, Metz TF, De Jesús VR, Greber-Platzer S, Pollak A, Herkner KR, Streubel B, Kasper DC.

- 34,736 dried blood spots from newborn babies analysed for

  Gaucher, Fabry, Pompe Niemann-Pick A/B

  by electrospray ionisation tandem mass spectrometry
  and confirming mutation analyses

- Low enzyme activities were detected in 38 babies, 15 of them confirmed by mutation analysis

- Fabry's disease (1 per 3859 births) vs. (>1 : 40.000 in selective screening)
- Pompe's disease (1 per 8684) vs. (>1: 40000)
- Gaucher's disease (1 per 17,368) vs. (1:160000 in Western Europe)

- How can we predict severity and organ affection?

DNA analysis and bioinformatics

new techniques are currently introduced

Whole exome sequencing (WES) entails

- selective capture, amplification and sequencing of the entire exome (ca. 1% of total genome)
- identification of potentially disease-causing variants
- Ranking for pathogenicity using bioinformatic algorithms (VAR-MD; Sincan et al 2012)
- WES has been applied successfully to the solution of several diagnostic problems in the LSDs but will not identify all mutations with equal efficiency (Fajardo et al 2012)

DNA analysis and bioinformatics

new techniques are currently introduced

19year-old female,

progressive motor and cognitive decline within12 years,
Diagnosis failed, including lysosomal enzyme screen

↓

Whole exome sequencing
133,155 variants in proband's family

↓ *Filtering to **52** relevant genes*

21 homozygous variants,
31 compound heterozygous variants

↓ *Predict pathogenicity and association with clinical syndromes, Ranking of variants*

Two variants in **ß-galactosidase (GLB1)** gene,
c.602G>A, **p.R201H**; c.785G>T, **p.G262V**

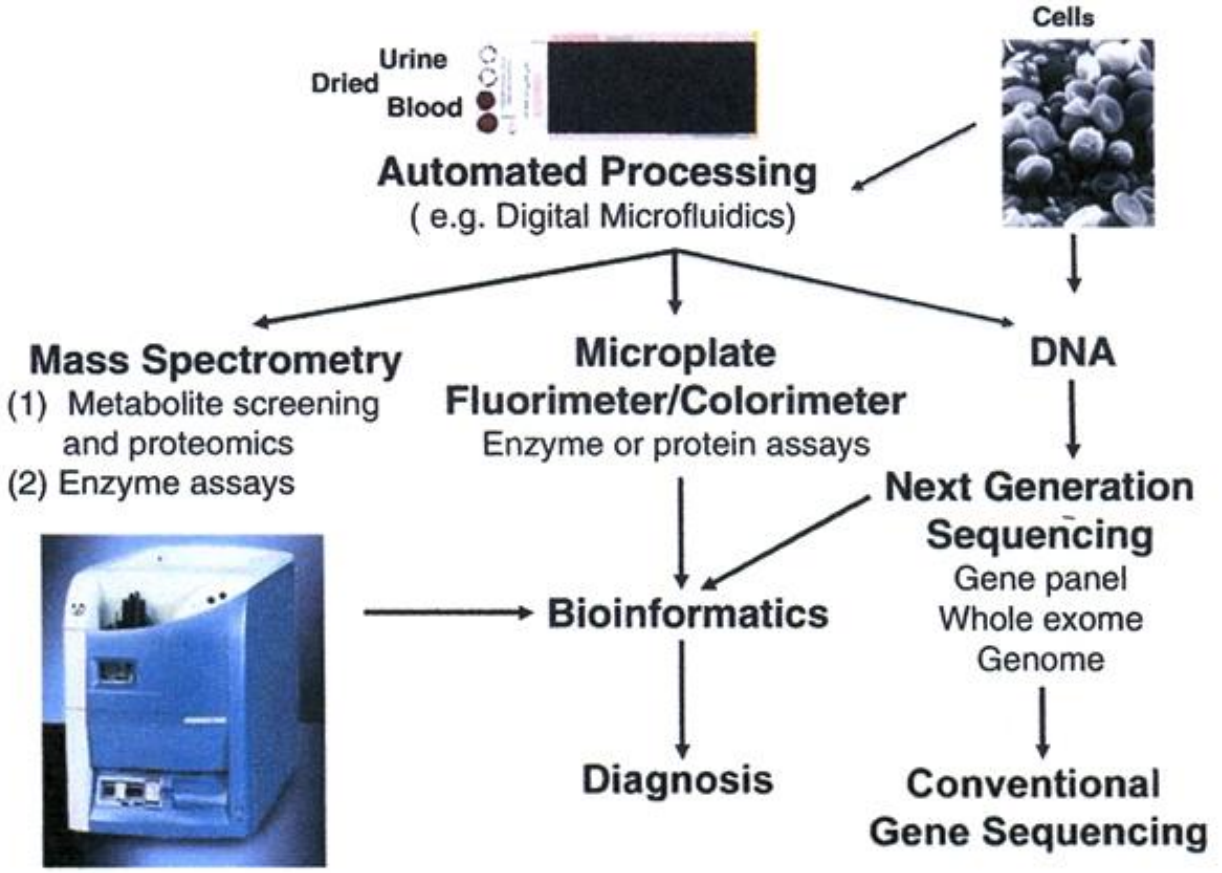
↓

Juvenile GM1-Gangliosidosis

confirm by conventional methods:
oligosaccharides in urine,
defective **enzyme activity**,
conventional **gene sequencing**

Sincan M et al Hum Mut 33: 593-98; Winchester B (2014), JIMD 37: 599-608

LSD diagnosis in the future?



- Blood and Urine dried on filter paper
- Newborn screening

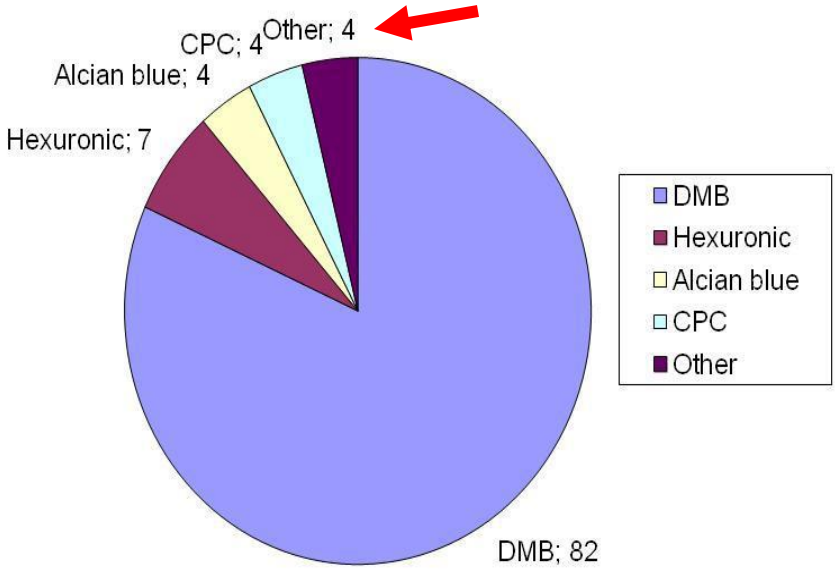
  or
- WES?
  - Automated analysis starting with WES?
  - Followed by single gene sequencing
  - Enzyme assays,
  - Metabolites
  - Specific Protein detection

Winchester B (2014), JIMD 37: 599-608

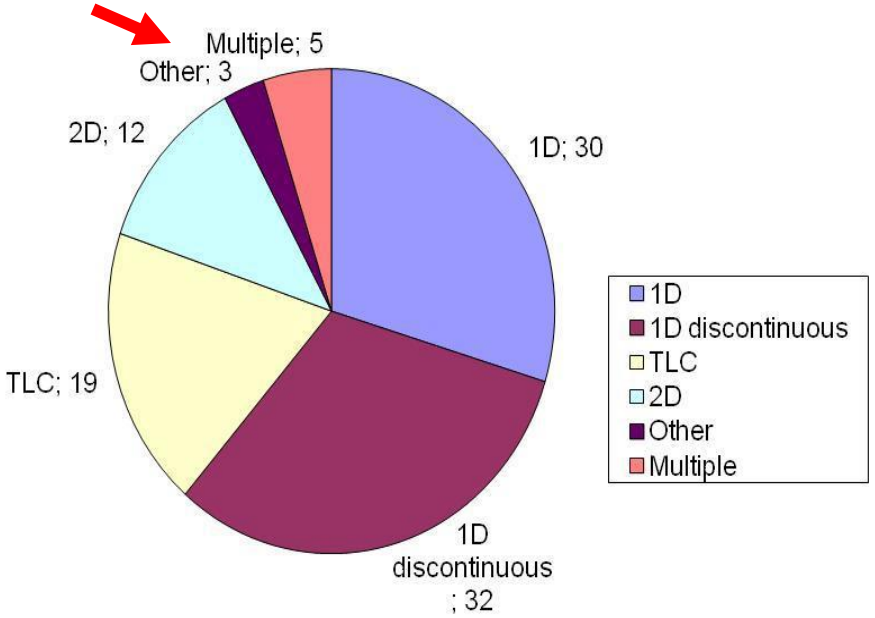
ERNDIM Report 2013 for MPS Diagnosis

the new era has not yet started

Quantitative GAG assay in urine



GAG electrophoresis



Summary

- During the last 20 years, the diagnosis of MPS was achieved using clinical workup of symptomatic patients, and analysis of urinary excretion of glycosminoglycans, enzyme analysis in leucocytes and fibroblasts and DNA analysis from blood samples
- With the advent of treatment **early diagnosis** has become essential for appropriate clinical management of patients. Therefore the introduction of **high-throughput screening methods** in newborns have been proposed.
- Numerous novel developments including automation of assays, analytics of glycoconjugates and clinical enzymology using mass spectrometry, the novel technology of „next generation“-DNA analysis and bioinformatics have successfully been devolped and will form an entirely new tool kit
- Manual routine methods for the laboratory diagnosis of lysosomal storage disease have now been in use for more that 20 years. A profound change has to be expected within the next years.

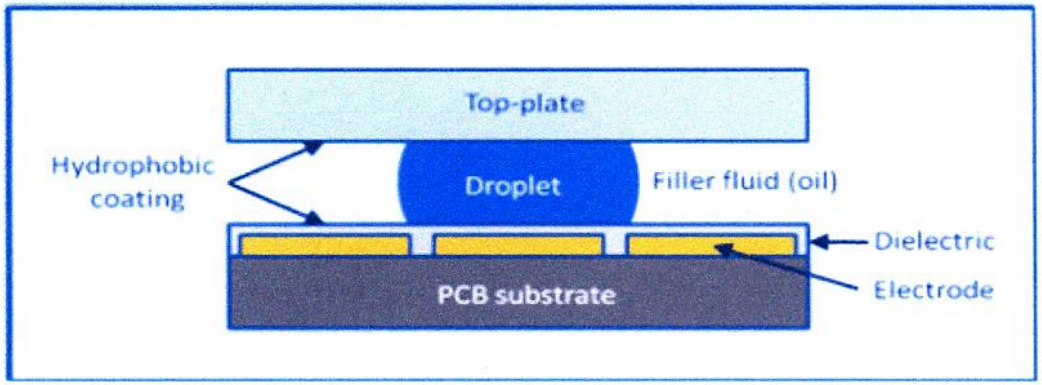
Early Diagnosis is essential

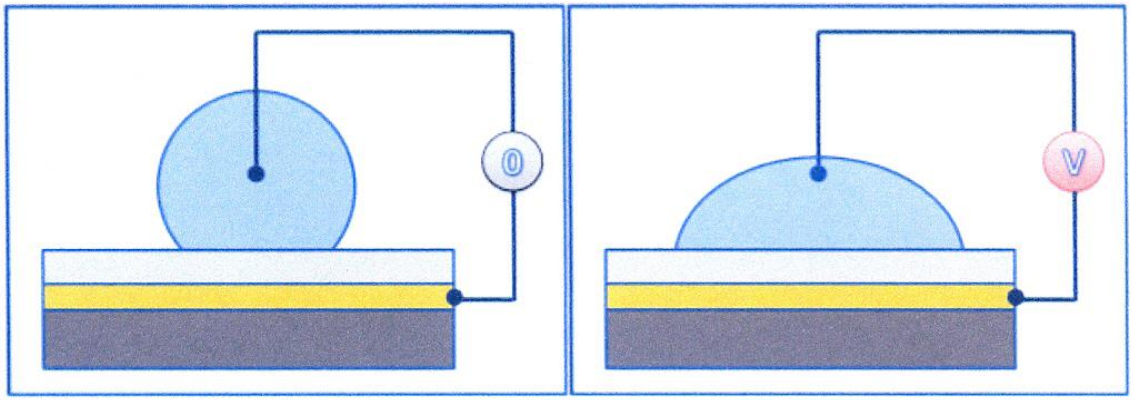
Newborn screening?

Large trials were **sucessful**

- **Conventional fluorimetric DBS assay**
  - Fabry (Spada 2006)
  - Pompe (Chiang 2012)
- **Microfluidics, automated fluorimetric assay**
  - Fabry, Pompe, Gaucher
    (Sista, Clin Chim Acta, 2012)
- **Mass spectrometry from a single DBS**
  - Krabbe (Orsini 2009)
  - Gaucher, Fabry, Pompe, Niemann-Pick (Mechtler 2012)

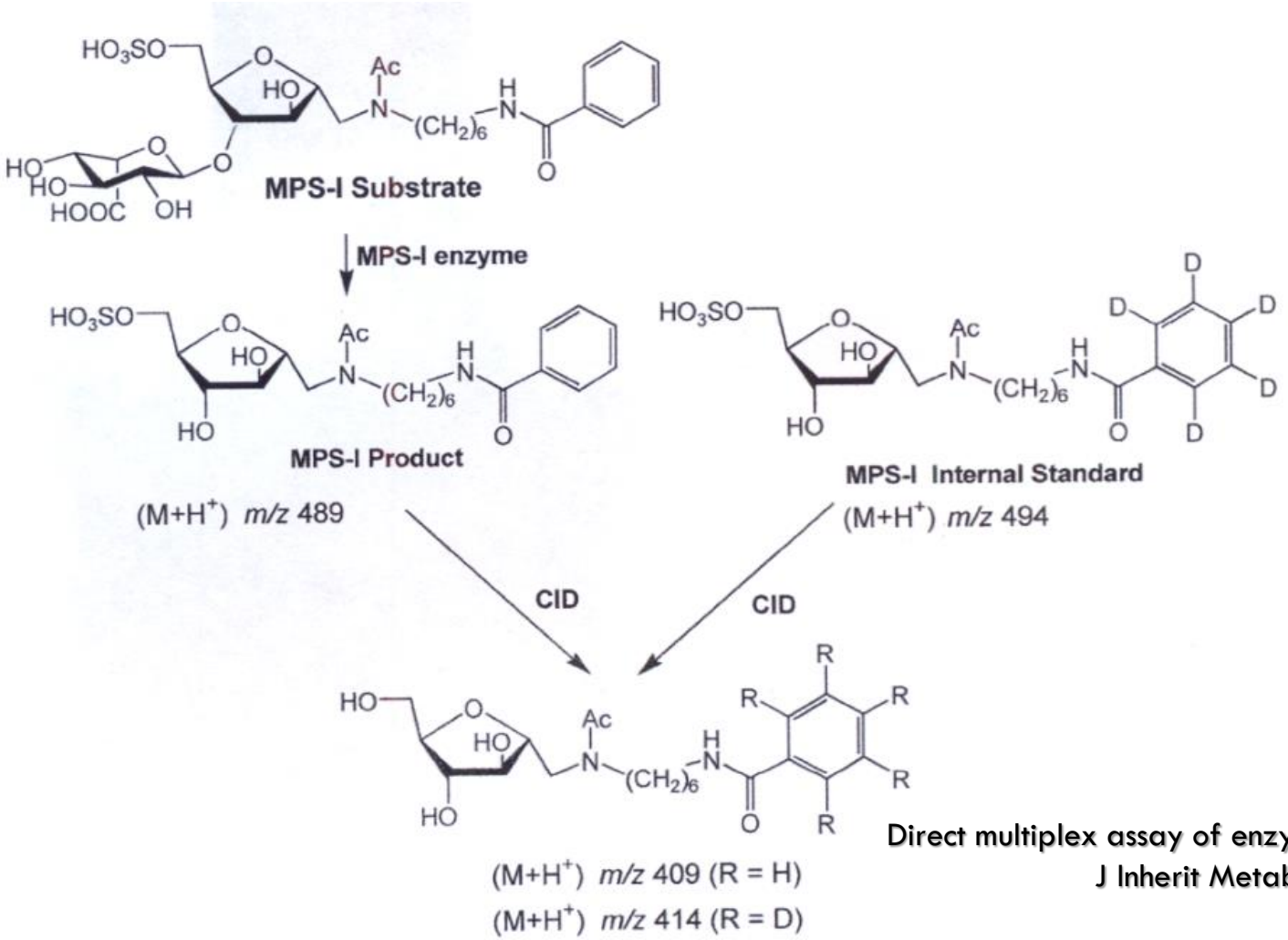
Electrowetting





Novel methods for Newborn screening

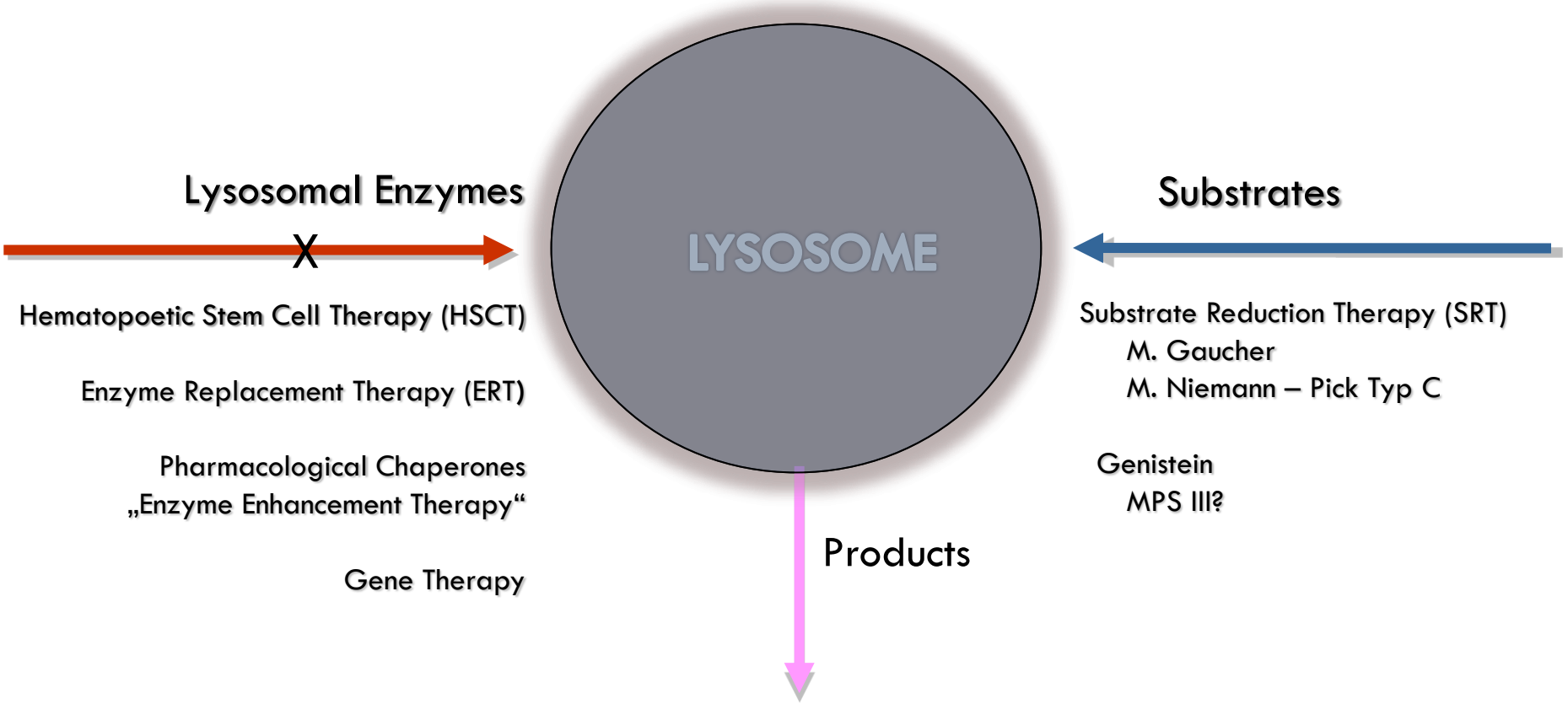
Novel substrates using mass spectrometry



Gelb MH et al
Direct multiplex assay of enzymes in dried blood spots
J Inherit Metab Dis (2006) 29:397–404

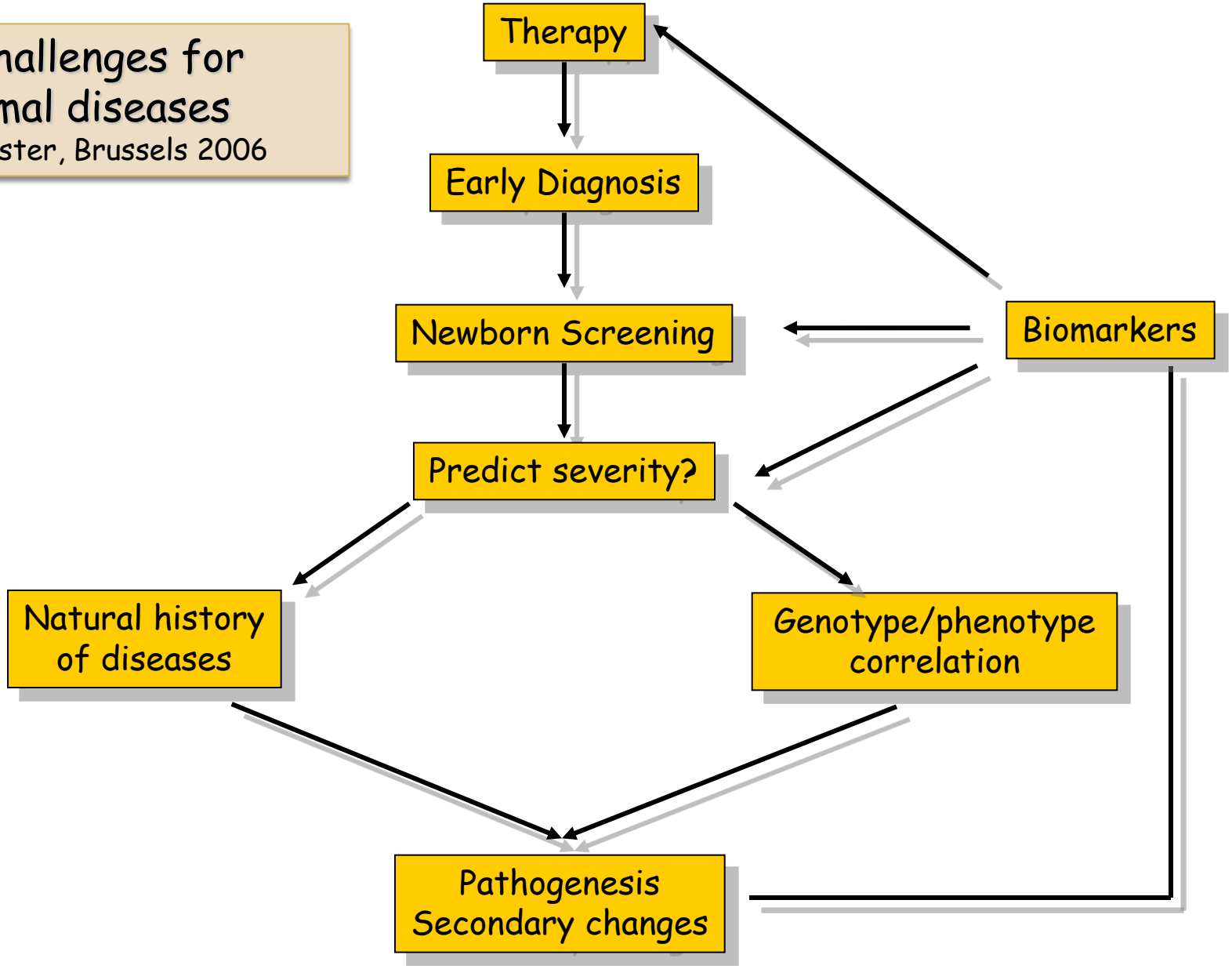
Available Causal Therapies

(1990-2014)



New challenges for lysosomal diseases

B.Winchester, Brussels 2006



Current biomarkers in MPS Diseases

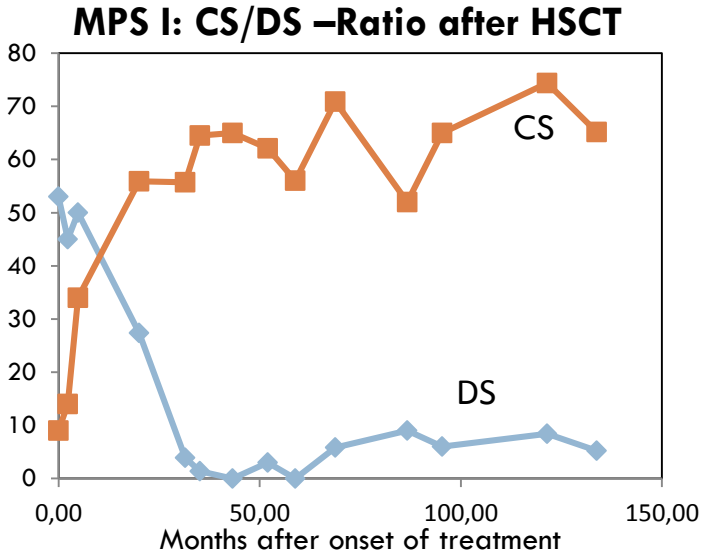
	parameter	method	disease	proposed aim
blood				
	heparin cofactor II-thrombin complex	ELISA	MPS I	monitoring
	keratan sulfate	ELISA	MPS	diagnosis
	heparan sulfate	ELISA	MPS	diagnosis
	GAG	TMS	MPS I, II, III, VI	diagnosis
urine				
	glycosaminoglycan fragments	TMS	MPS I, II, III, VI	diagnosis

Biomarker for MPS diseases

Langford-Smith et al. J Inherit Metab Dis. 2011 Apr;34(2):499-508

- **Urinary DS:CS ratio** in MPS I, II, VI
- **Heparin cofactor II-thrombin complex (HCII-T)** in serum or dried blood spots (DBS)

- **Serum HCII-T** is elevated approximately **25-fold** in DS-storing MPS diseases and distinguish untreated **MPS I, II and VI patients clearly** from unaffected age-matched controls, but only 4-fold elevated in MPSIII
- HCII-T responds **rapidly** to perturbations in treatment, whilst DS:CS ratio responds more **slowly.**
- For MPS diseases storing HS alone the observed elevation is smaller.



Heparin cofactor II-Thrombin Complex in MPS I

Randall DR, Sinclair, GB, Colobong KE et al (2006) Mol Genet Metabolism 88: 235-243

HCII: a lysosomal serin protease related to connective tissue re-modelling at inflammation sites, is enhanced by dermatan, but not by heparan sulfate

HCII-T ELISA in serum and plasma samples of MPS I patients, MPS I mice, and controls

Sample (age in brackets)	Serum [HCII-T] (pM ± SD)	Plasma [HCII-T] (pM ± SD)
Control (10 years F)	115.1	17.92
Control (10 years M)	398.0	9.91
Control (30 years M)	384.7	6.27
MPS 1H (10 months, Patient A)	174,700	30.15
MPS 1H (12 months, Patient B)	182,400	Not tested
MPS 1H (14 months, Patient C)	208,600	98.37
MPS 1H/S (8 years, Patient D)	46,000	Not tested
Idua$^{+/+}$ ($n = 5$)	75.46 ± 4.99	3.77 ± 1.20
Idua$^{-/-}$ ($n = 3$)	628.1 ± 163.2	79.50 ± 38.9

Prediction of Neuropathology in MPS I?

Fuller et al. (2005) Mol Genet Metab 84,18

- **Oligosaccharides derived from heparan and dermatan sulfate** in fibroblasts from **MPS I** patients
  - patients with and without CNS disease were grouped for two trisaccharides
  - Ratio of alpha-l-iduronidase activity to these trisaccharides discriminates between MPS I patients with and without CNS pathology.

Holley RJ et al JBC, 286, 43 37515-37524 (2011)

- **Structure of accumulated heparansulfate and N-sulfotransferase activity in MPS I mice**
  - Excess HS colocalized to the Golgi secretory pathway regulates HS-sulfation and increases the N-sulfotransferase of HS modifying enzymes
  - Can tissue-specific differences in HS modification be used to predict neuropathology?

Kingma S et al Orphanet Journal of Rare Diseases 2013, 8, 99

- **Algorithm to predict severity from genetic, biochemical and clinical data**

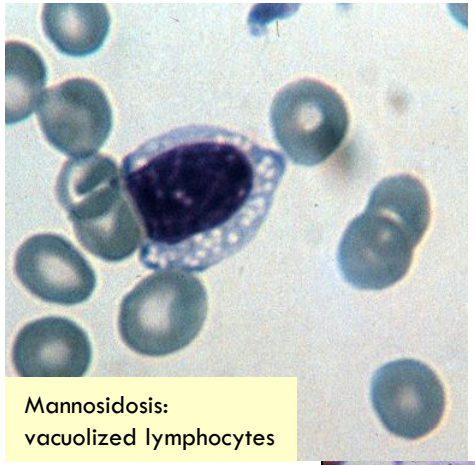
Mucopolysaccharidoses

are multisystem diseases

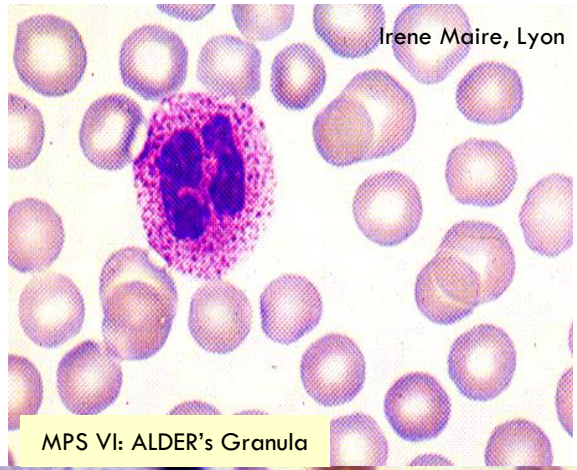
Disease	Bone	visceral	Eyes	CNS	Urinary GAGs	amount
MPS I H	+++	++	+++	+ - +++	HS, DS	+++
MPS I S	+	n	+	n	HS, DS	+
MPS II	+ - +++	n - ++	n	+++	HS, DS	++
MPS III A-D	n - ++	n - ++	++ - +++	+++	HS	+ - ++
MPS IVA, B	++ - +++	n	n	n	CS, KS	+ - ++
MPS VI	++ - +++	n	(+)	n	DS	+++
MPS VII	+ - +++	+	++	n - ++	CS	n - +
MPS IX	+	n	n	n	HA	

n: normal

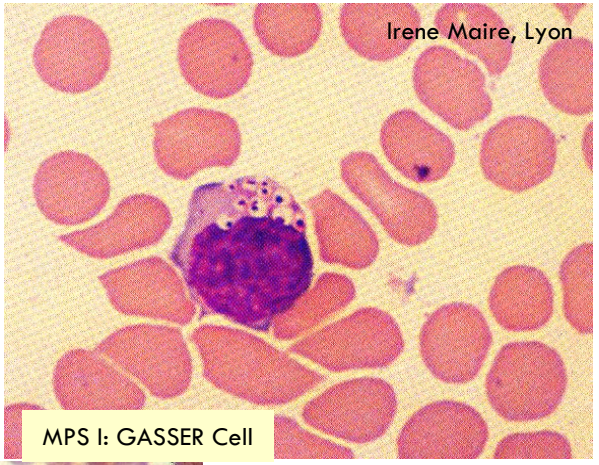
Hematological Symptoms



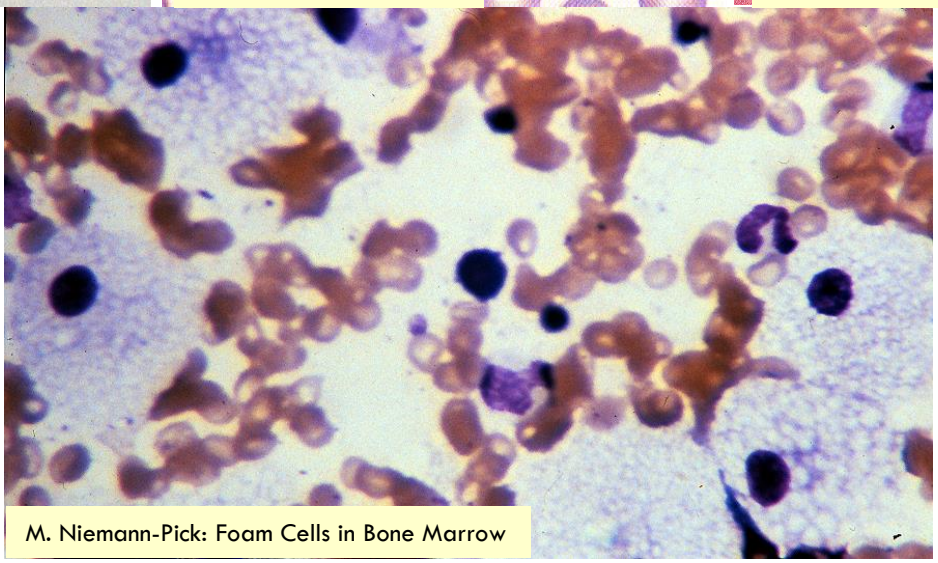
Mannosidosis:
vacuolized lymphocytes



MPS VI: ALDER's Granula



MPS I: GASSER Cell

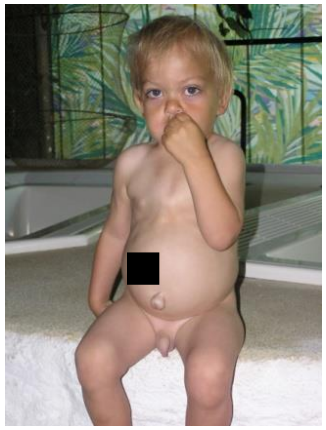


M. Niemann-Pick: Foam Cells in Bone Marrow

Natural history of MPS IH

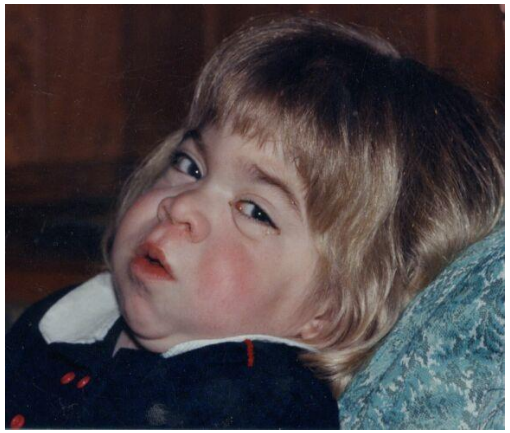
1 week

1.8 years



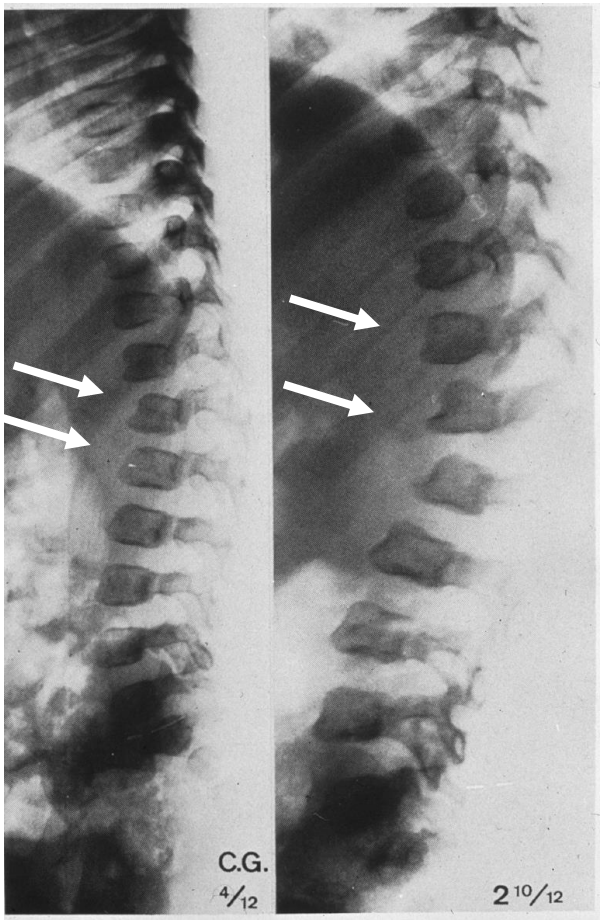
Hernia,
Hepatomegaly
retarded speech development

5.5 years



Course facies,
jount problems,
cardiomyopathie
developmental arrest

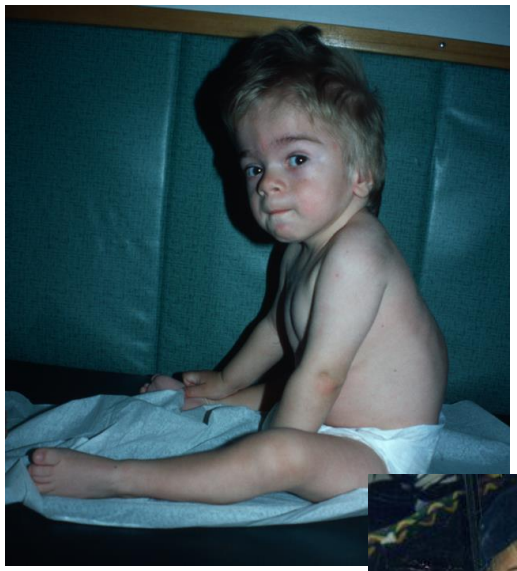
8 years

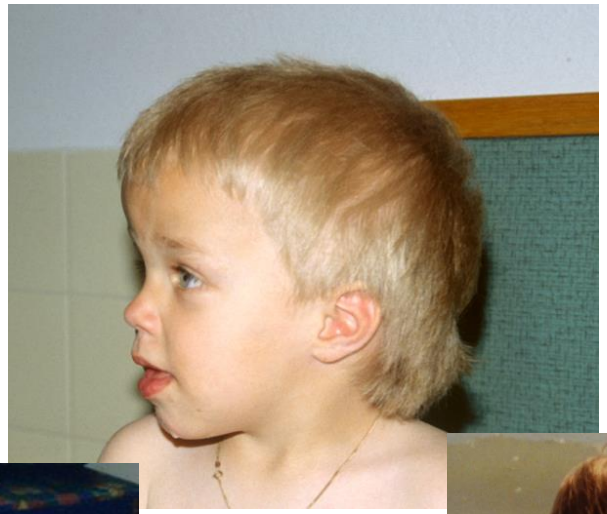


Dysostosis multiplex,
progressive deformation of vertebral bodies

Early Diagnosis

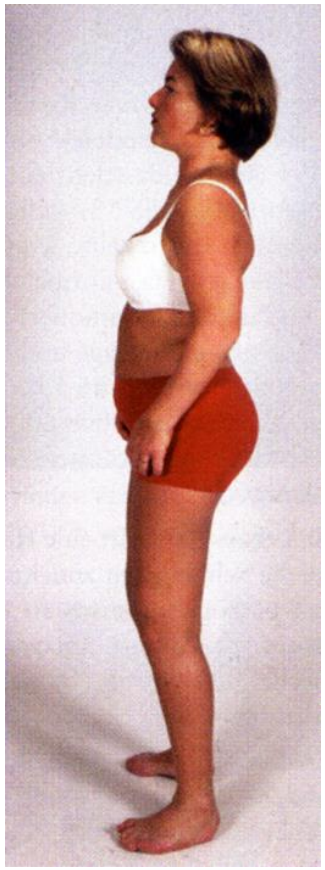
is a prerequisite for genetic counseling and therapy





Attenuated Phenotypes

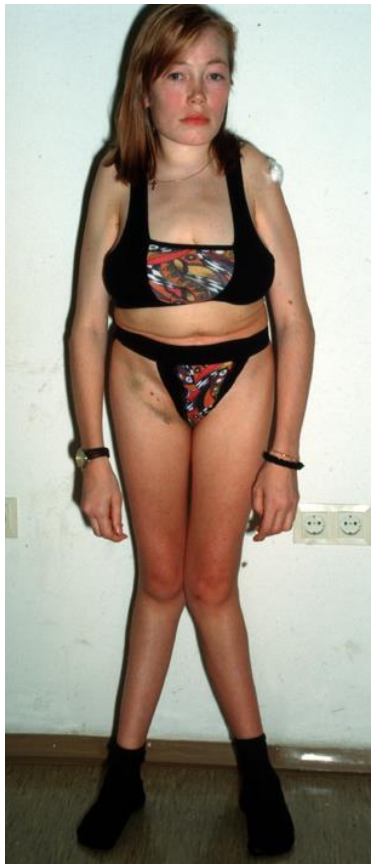
are not „mild diseases“



MPS I-S

Late manifestation

attentuated course



MPS IV B

Approved Enzyme replacement therapies

Disease	enzyme deficiency	product	company, approval
Gaucher disease	ß-glucocerebrosidase	Cerenzyme®	Genzyme, 1997
Fabry disease	a-galactosidase	Fabrazyme® Replagal®	Genzyme, 2001 Shire (TKT), 2001
MPS I	a-iduronidase	Aldurazyme®	Biomarin, 2003
MPS VI	N-acetylgalactosamine-4-sulfatase	Nagalazyme®	Biomarin, 2006
MPS II	iduronate-2-sulfatase	Eleprase®	Shire, 2006
Pompe disease	a-glucosidase	Myozyme®	Genzyme, 2006
MPS IV A (Morquio A)	N-Acetyl galactosamin-6-sulfatase	Vimizyme®	Biomarin, 2014

Newborn screening?

Problems

- Mutation frequencies
  - Non-symptomatic, affected newborns >> manifest cases
- MPS have a very variable course
  - Manifestation in the newborn or in the adult age?
  - Will the central nervous system be affected or not?
  - How to predict severity?
- Newborn screening detects all patients, but the brain is currently not treatable
  - Can one refuse to treat patients with CNS affection despite a possible benefit for visceral functions?
  - How to deal with attenuated forms?
    Shall we tell the parents of a newborn, that it will suffer from MPS in 10 or 15 years?