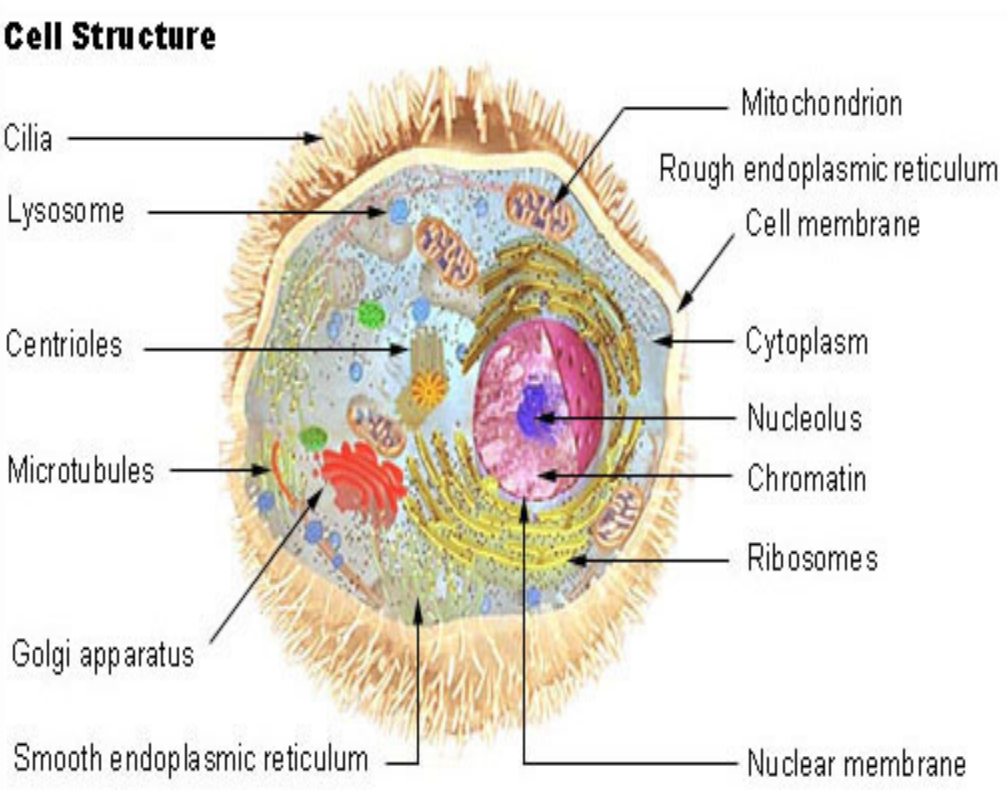
Lysosomal Storage Diseases

Virginia Kimonis, MD, MRCP
Professor, Division of Genetics & Genomic Medicine
Dept of Pediatrics, Neurology, Pathology
Univ. of California, Irvine, & CHOC Hospital

vkimonis@uci.edu

949-824-0571 (O)
949-266-7946 (M)

LYSOSOME

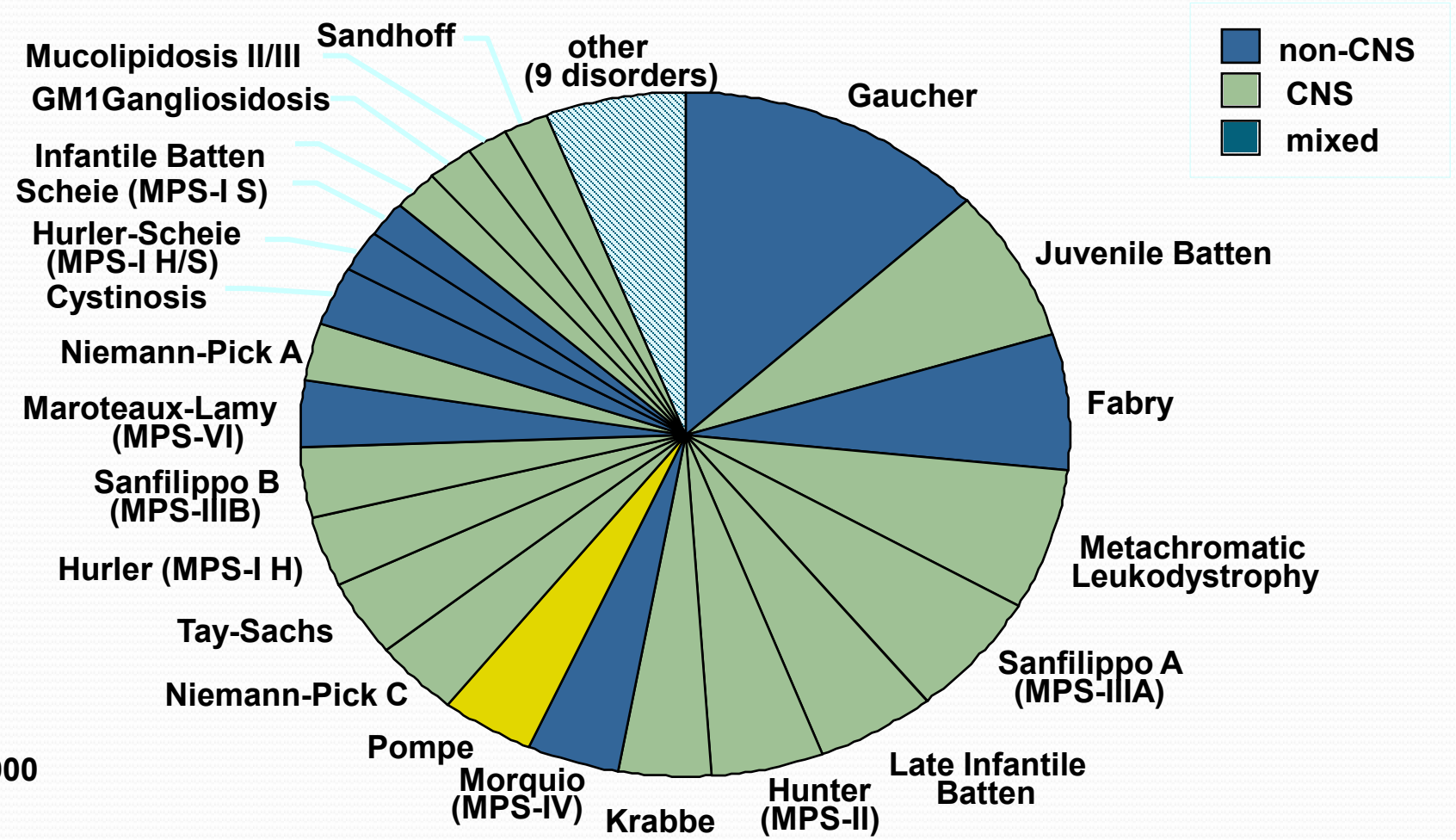


- Lysosomes are organelles that contain digestive enzymes
- They digest excess or worn-out organelles, food particles, and engulfed viruses or bacteria.
- The membrane surrounding a lysosome allows the digestive enzymes to work at 4.5 pH
- Lysosomes are created by the addition of hydrolytic enzymes to early endosomes from the Golgi apparatus
- Lysosomes fuse with vacuoles and dispense their enzymes into the vacuoles digesting their contents

Lysosomal Storage Disorders (LSDs)

- LSDs result from different acid hydrolase deficiencies in lysosomes
- LSDs include a family of 40 disorders classified according to type of substrate stored, e.g.
  - Sphingolipidoses
  - Glycogenoses
  - Mucolipidoses
  - Mucopolysaccharidoses/Oligosaccharidoses
- Prevalence of >1 per 7700 live births
- Autosomal recessive except for Fabry and MPS II (Hunter)-X-linked recessive

Incidence of Lysosomal Storage Disorders



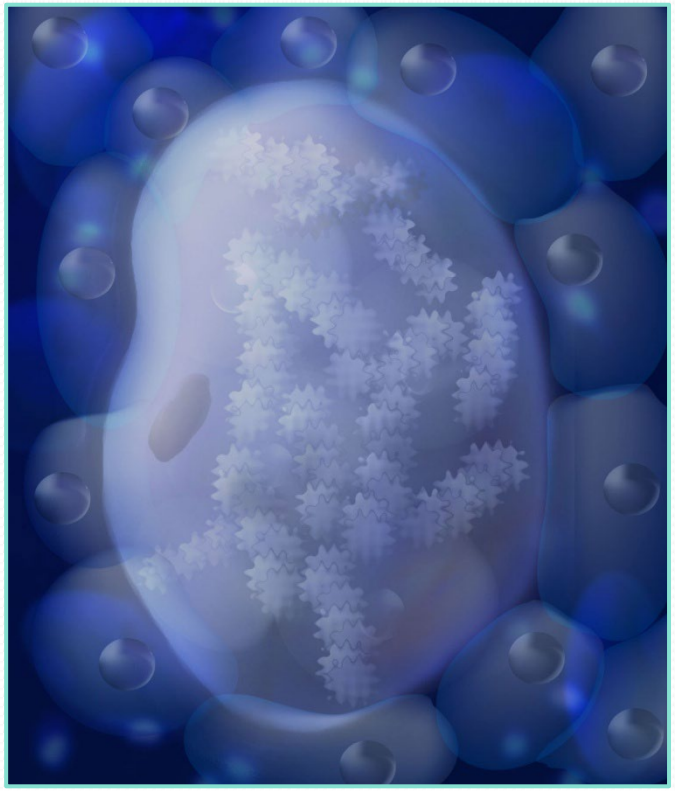
Ashkenazi
Jewish
-Gaucher 1/855
-Tay Sachs 1/3,900

Meikle et al, JAMA 281:249-254, 1999

Lysosomal Storage Disorders

Accumulation of incompletely degraded glycosphingolipids in intracellular lysosomes leads to characteristic pathology:

- Splenomegaly
  - Pancytopenia
  - Bleeding/bruising
- Hepatomegaly
  - Liver dysfunction
  - Cirrhosis
- Growth, Short stature
  - Bony Involvement
- Pulmonary disease
  - Dyspnea
  - Recurrent infections



Lysosomal Storage Diseases Treated by Enzyme Replacement Therapy

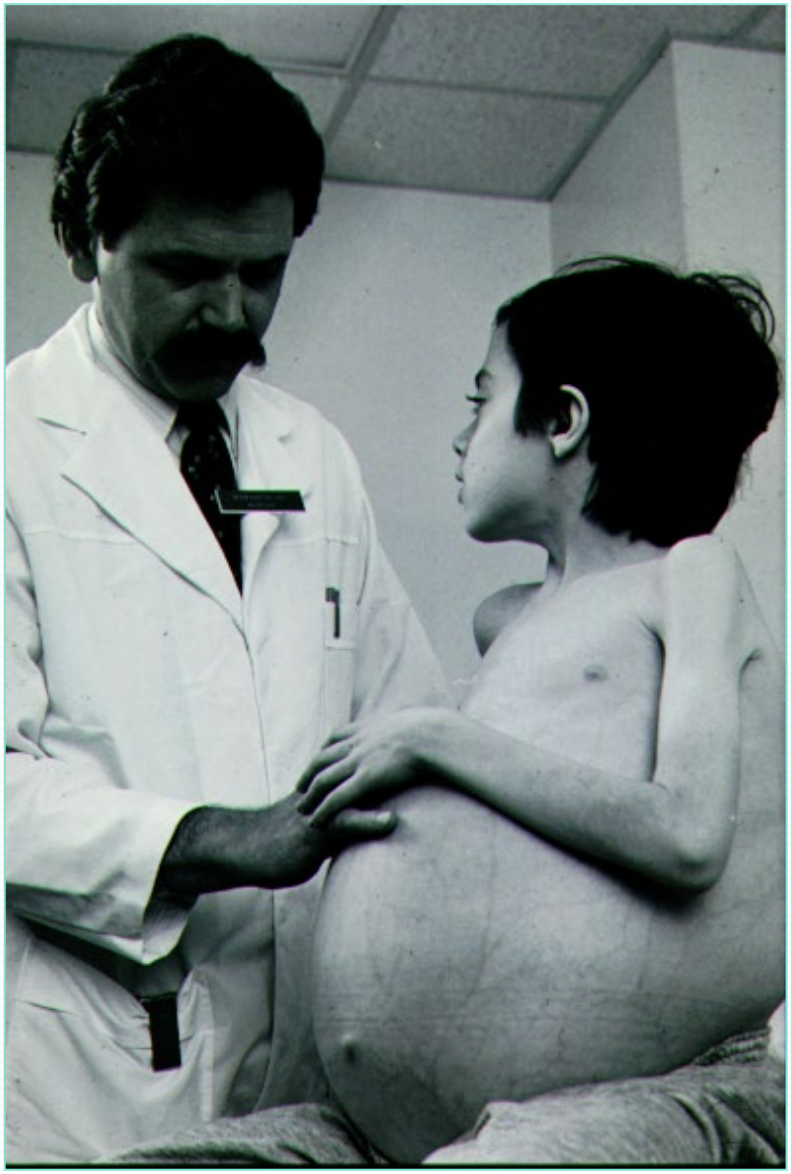
- Gaucher
- Fabry
- MPS (Mucopolysaccharidoses I, II, IV, VI
- Pompe (GSD Type II)
- Niemann-Pick B
- Wolman

Case 1: MH 60 y

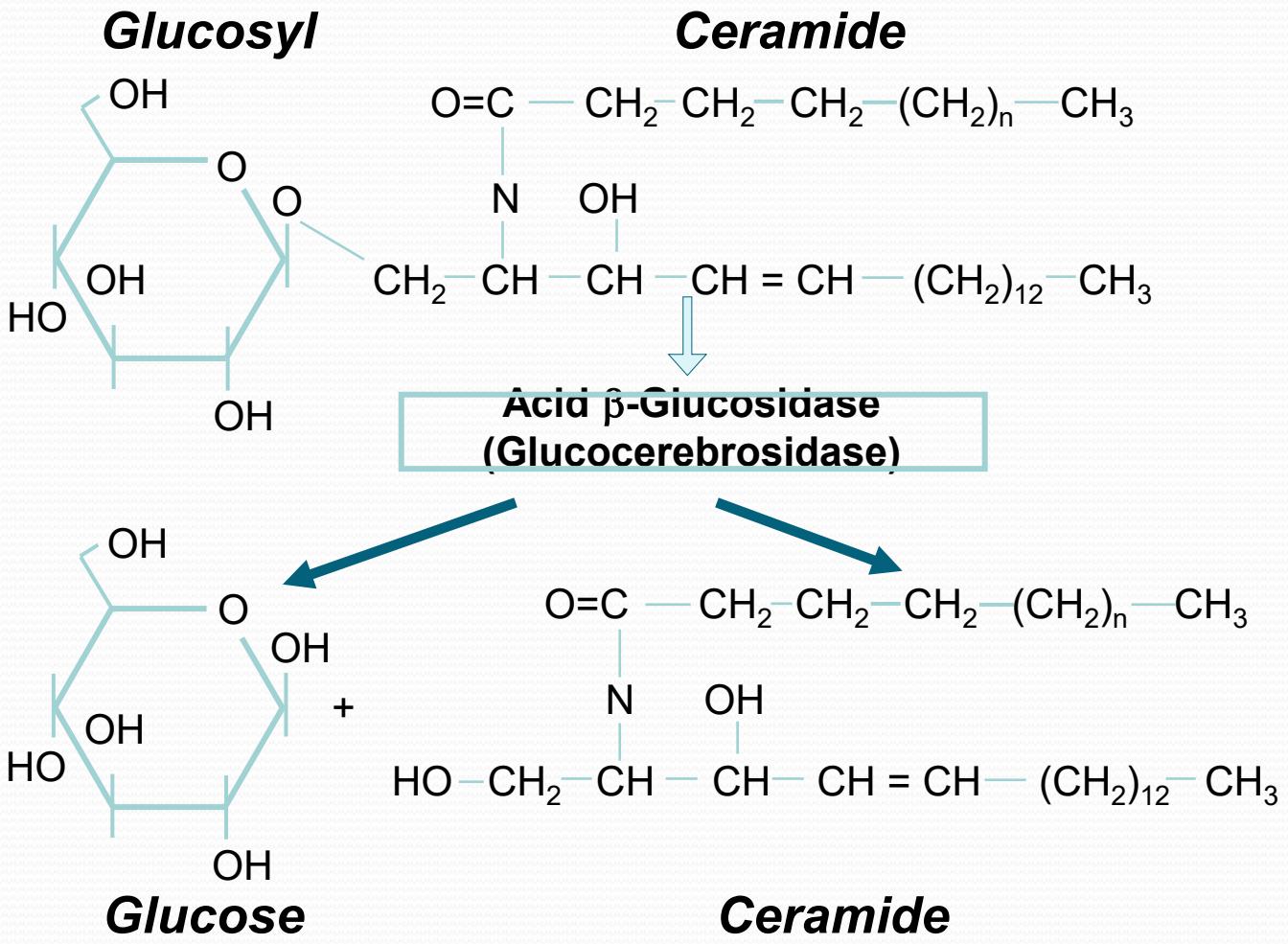
- Tiredness
- Muscle aches and pains
- Pallor, Anemia
- Hepatosplenomegaly
- Peripheral neuropathy

Gaucher Disease

- Most prevalent lysosomal storage disorder
- Autosomal recessive - deficiency of glucocerebrosidase

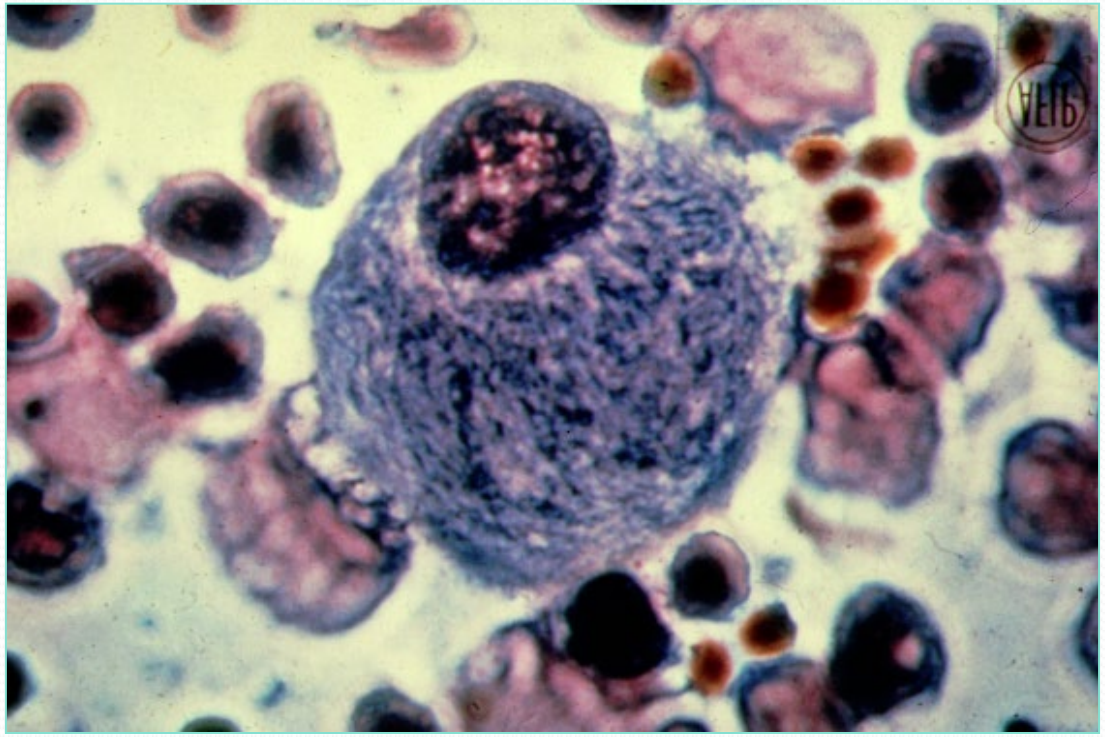


The Enzymatic Defect in Gaucher Disease



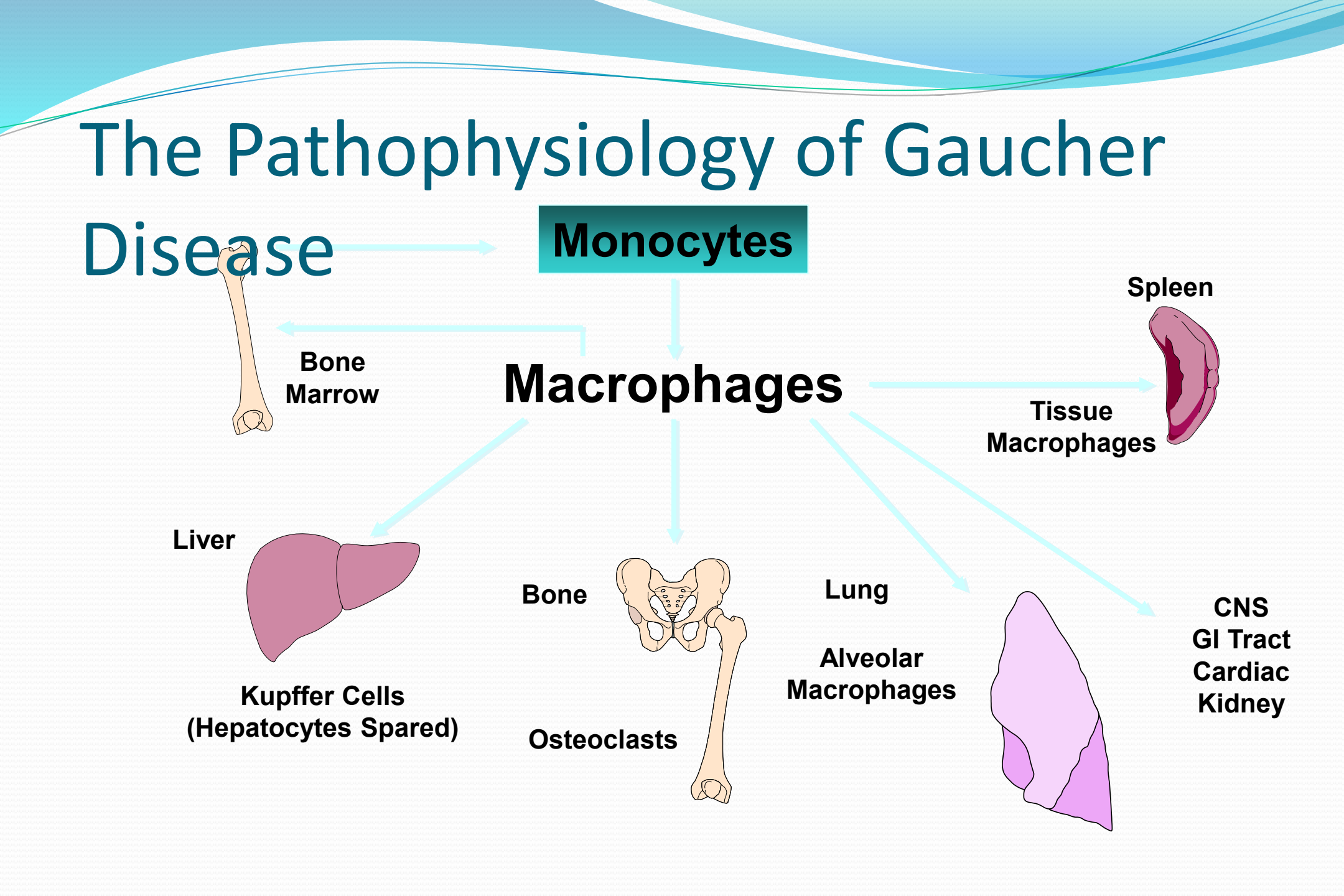
Gaucher Disease:

A multi-system disorder consisting of progressive visceral enlargement and gradual replacement of the haematopoietic system with lipid-laden macrophages



The Gaucher Cell

The Pathophysiology of Gaucher Disease



Gaucher Disease: Clinical Diagnosis

Common Presenting Signs

- Asymptomatic hepato-splenomegaly
- Thrombocytopenia and/or anemia with bruising or bleeding
- Fatigue
- Bony lesions with or without symptoms
- Aseptic necrosis of hip
- Growth retardation in children or delayed puberty in adolescence

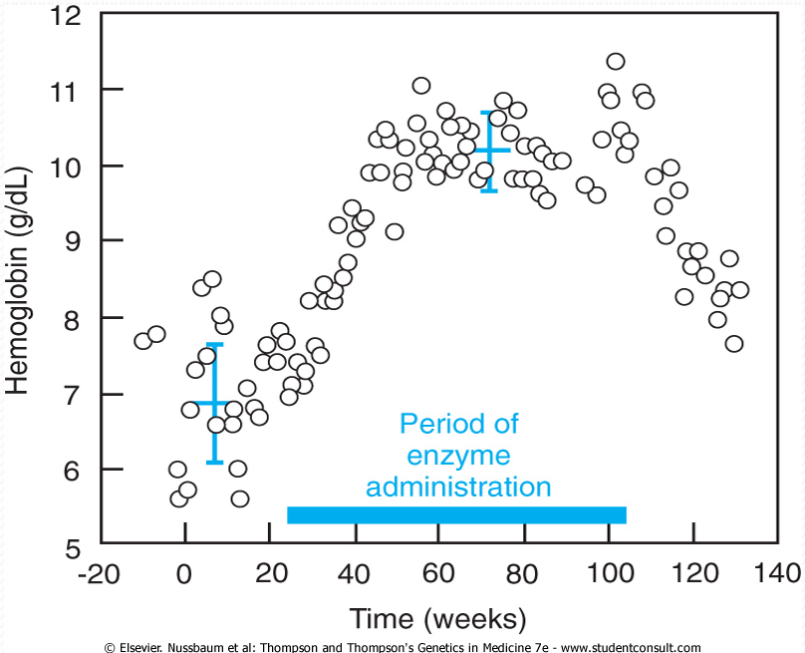
Enzymatic Diagnosis

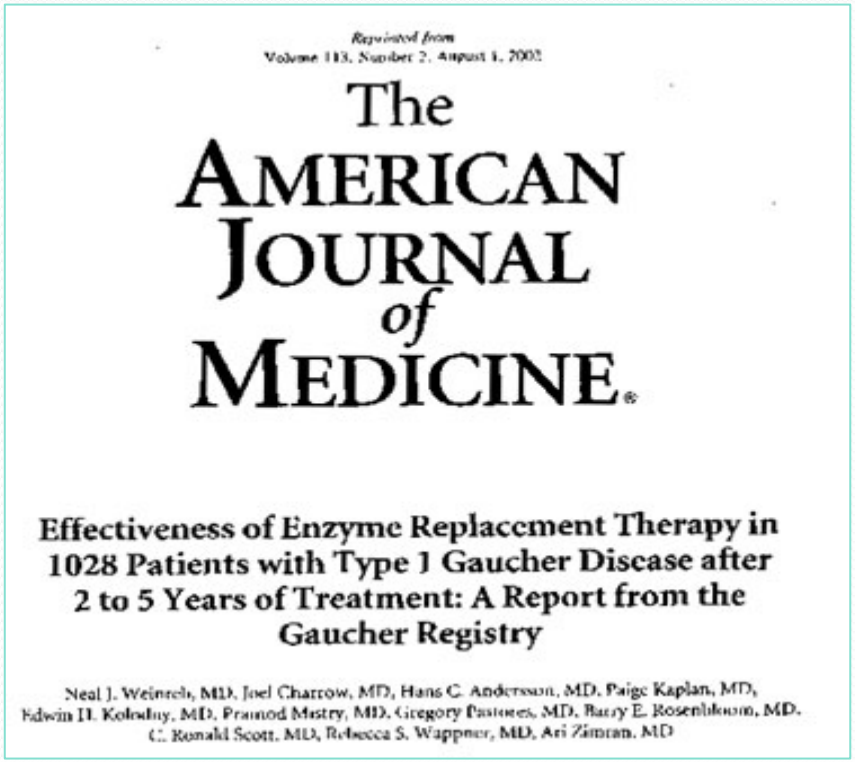
- Confirmed Diagnosis of Gaucher Disease by assay of enzyme activity (glucocerebrosidase)
  - Blood sample (leukocytes or white blood cells
  - Skin biopsy (cultured fibroblasts)
  - Mutation analysis
    - 97% Jewish mutations in panel (N370S, L444P)

Enzyme Replacement Therapy

ERT reverses the anemia, thrombocytopenia and organomegaly in the first 12 mths

Clears the storage of glucocerebroside from the bone marrow in 24 months.

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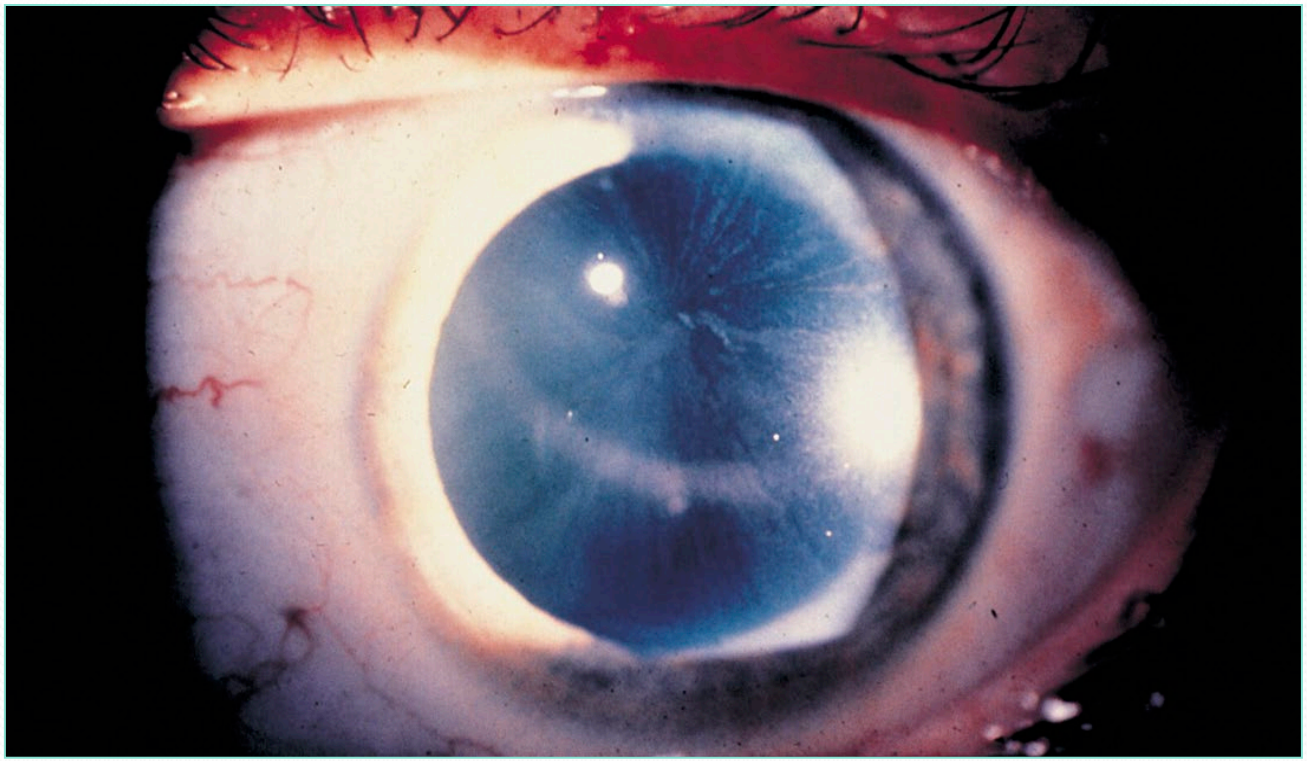
Effectiveness of Enzyme Replacement Therapy in 1028 Patients with Type 1 Gaucher Disease after 2 to 5 Years of Treatment: A Report from the Gaucher Registry

Neal J. Weinreb, MD, Joel Charrow, MD, Hans C. Andersson, MD, Paige Kaplan, MD, Edwin H. Kolodny, MD, Pramod Mistry, MD, Gregory Pastores, MD, Barry E. Rosenbloom, MD, C. Ronald Scott, MD, Rebecca S. Wappner, MD, Ari Zimran, MD

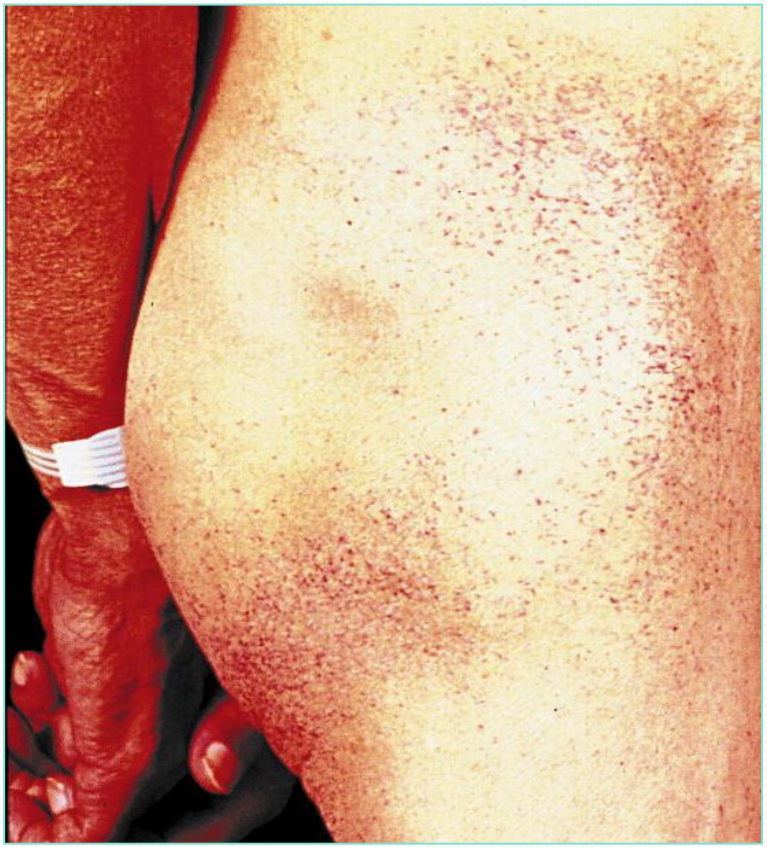
Case 2: RM 40 Y

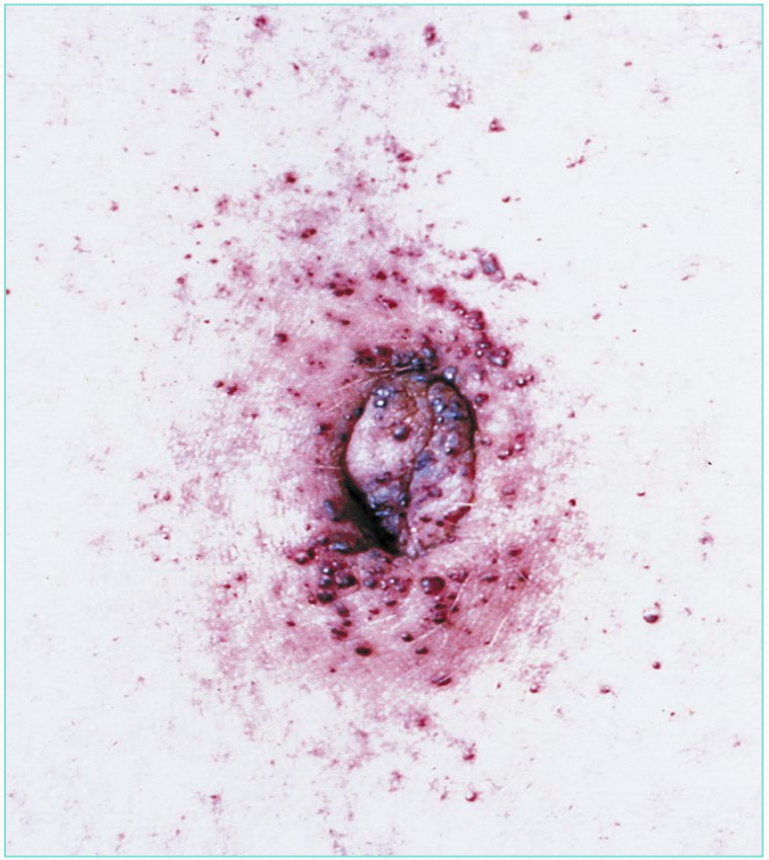
- Several year history of:
- Fatigue
- Swelling of feet
- Anxiety
- Irritable bowel syndrome

Corneal Opacities

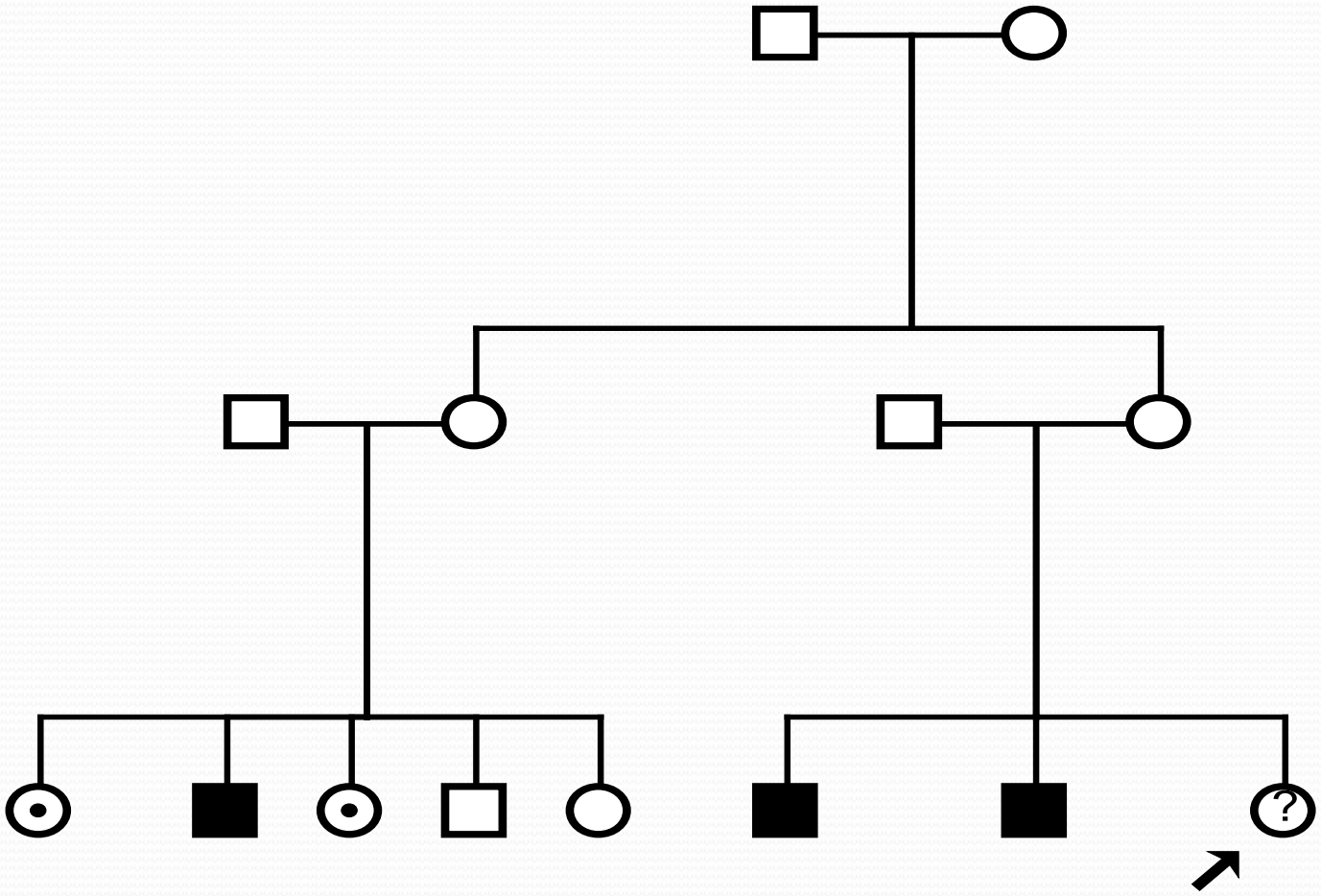


Angiokeratomas





RM's Family



Fabry Disease

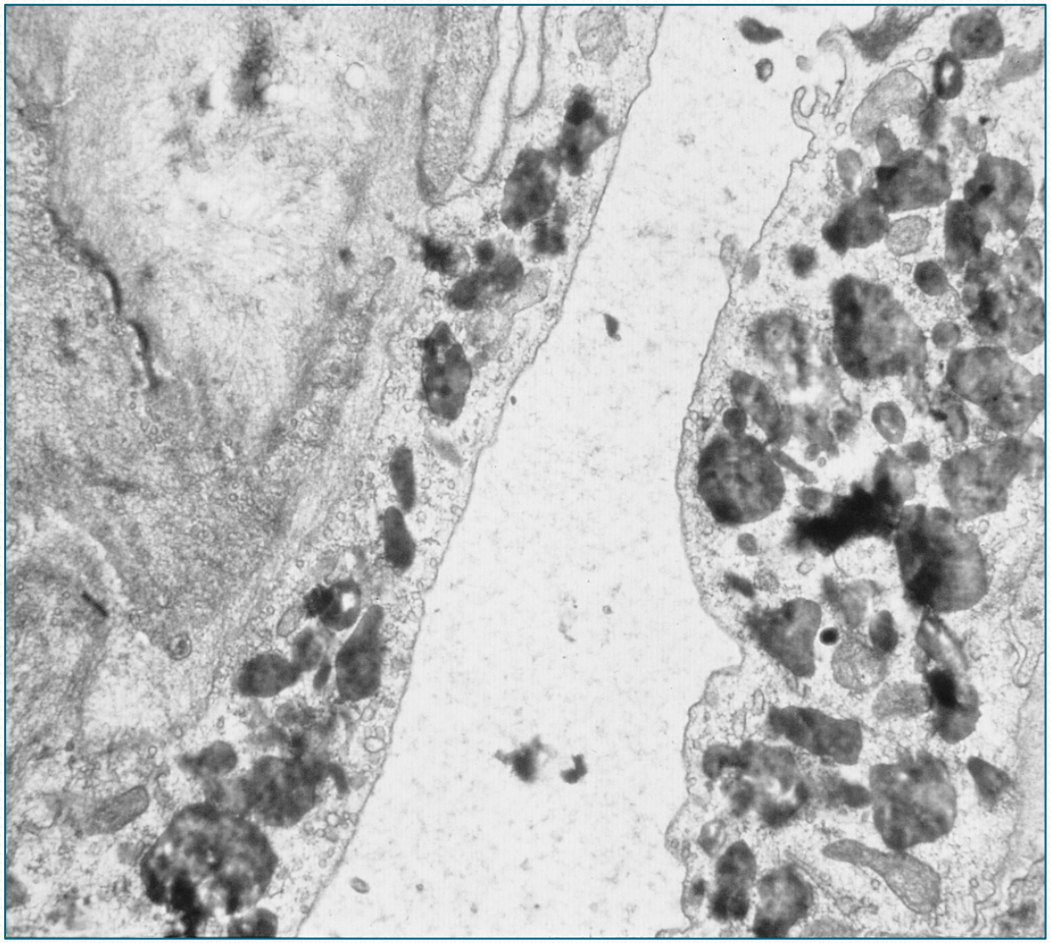
- X-linked inborn error of metabolism
- U.S prevalence estimated at 2000-4000
- Deficient α-Galactosidase A (α-GAL) activity
- Progressive globotriaosylceramide (GL-3) accumulation in tissue leading to end organ impairment
- Key pathology: vascular endothelial deposition

Diagnosis of Fabry Disease

- Presumptive diagnosis
  - Observation of symptoms and laboratory findings
  - Family history/medical pedigree
- Definitive diagnosis
  - Enzyme assay in plasma, leukocytes, tears, or biopsied tissue (Males)
  - Gene mutation analysis (Females)

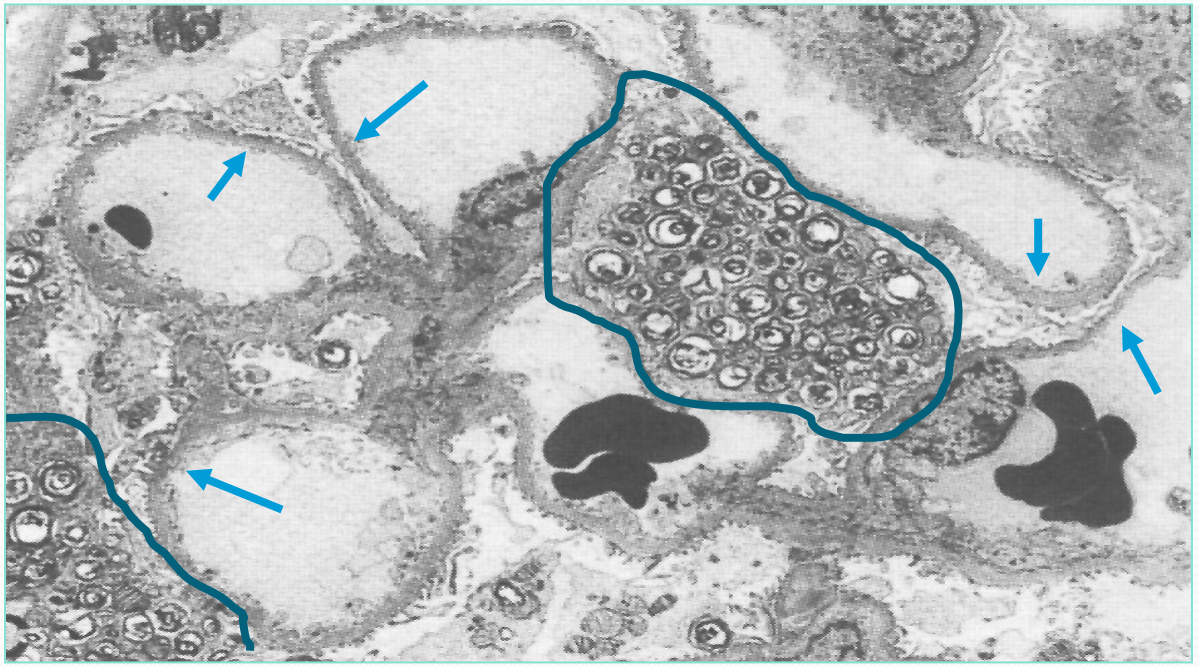
Fabry Disease

- Resembles the progression of atherosclerosis rather than a Lysosomal Storage Disorder



Fabry Disease: Progressive Accumulations

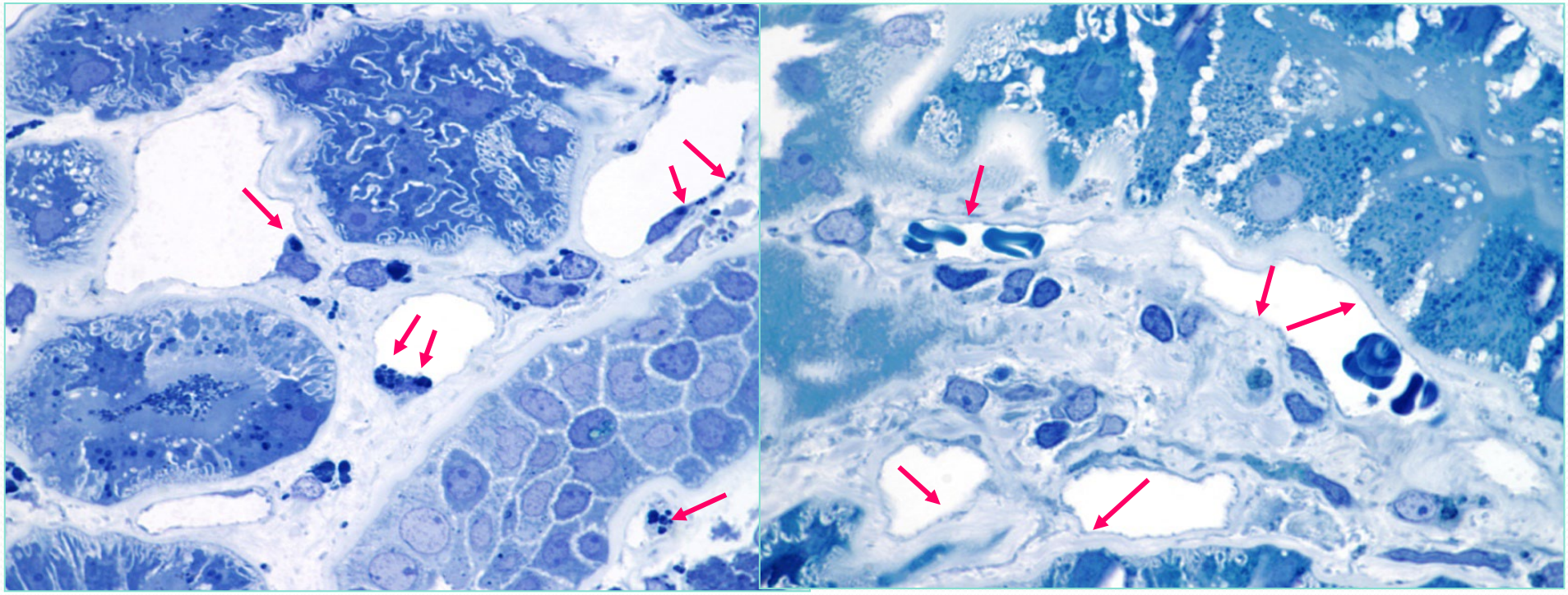
Renal insufficiency is the result of decades of progressive nephron loss and early strokes.
Figure illustrates the destructive consequences of the GL-3 accumulation



Alpha GAL treatment

- Alpha GAL clears the GL-3 substrate to normal or near normal levels
  - Kidney, Heart, skin but not the corneal whorls

Primary Endpoint GL-3 Is Cleared From Peritubular Capillary Endothelium



Pre-treatment

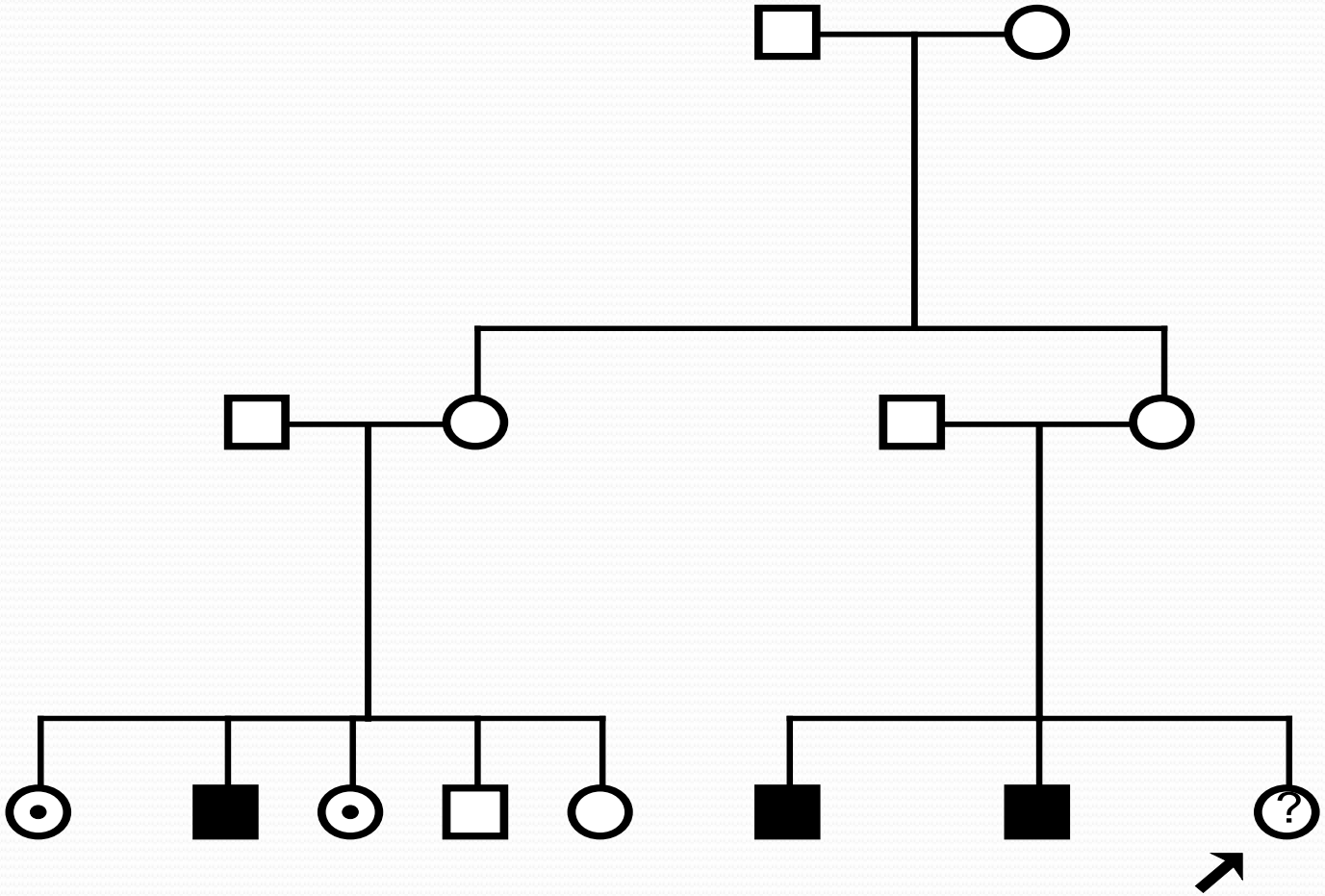
Post-treatment

RM's Px:

Fabrazyme, aspirin and lisinopril

- Amelioration of ankle swelling
- Sweating noted for the first time
- Anxiety, paranoia improved
- Sex life improved
- GI symptoms improved
- Microalbuminuria persistent
- Angiokeratoma persistent

RM's female relatives have milder symptoms



Fabry disease should be considered in males and females with these signs:

- Periodic crises of severe pain in the extremities (acroparesthesias)
- Vascular cutaneous lesions (angiokeratomas)
- Hypohidrosis
- Characteristic corneal and lenticular opacities
- Stroke
- Left ventricular hypertrophy
- Renal insufficiency of unknown etiology

Case 3: JW 60 y

- 20s waddling gait
- 30 back pain
- 30s weakness, walks with sway back
- Proximal limb girdle weakness
- PFTs-diaphragmatic dysfunction

Case 3: JW 60 y (con't)

- Brother diagnosed with Pompe recently
- 2 mutations
  - Deletion GG c.1951-1952 --> termination codon
  - C. 336-13 T>G change in first intron
    - (affects splicing exon 2, seen in 36% to 90% of late-onset Pompe disease and not associated with the infantile-onset form, leads to a leaky splice site resulting in diminished, but not absent, GAA enzyme activity)

Pompe Disease GSD II

Acid maltase deficiency acid a-glucosidase (GAA) catalyzes α-1,4- and α-1,6-glucosidic linkages at acid pH.

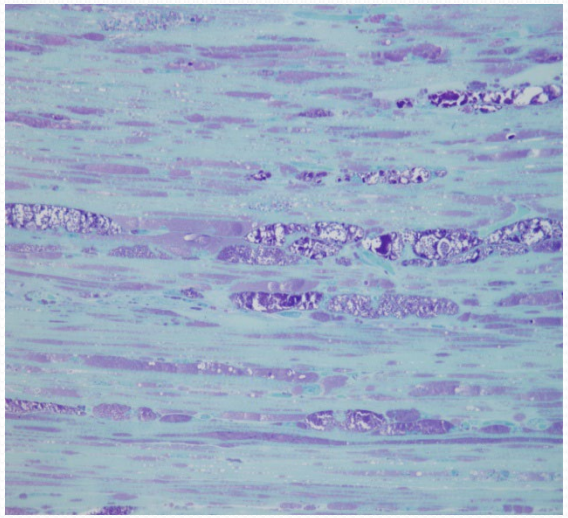
Autosomal recessive inheritance (European descent, 1:100 K infantile onset, 1:40-60K late onset)

Accumulation of glycogen in tissue

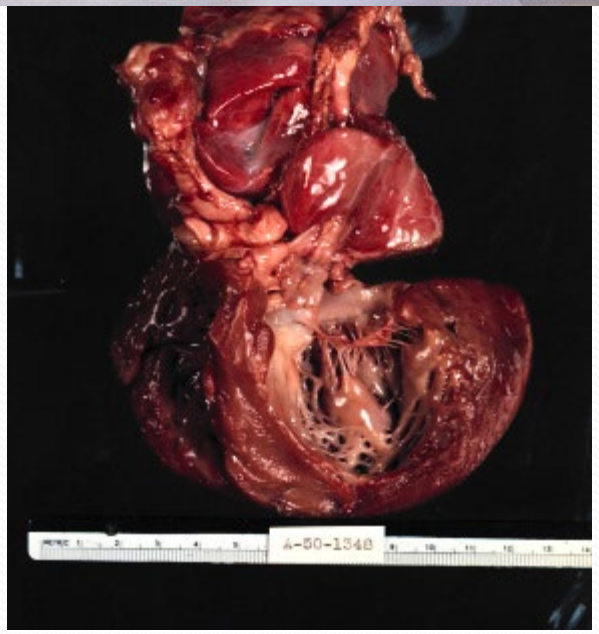
No/minimal CNS involvement

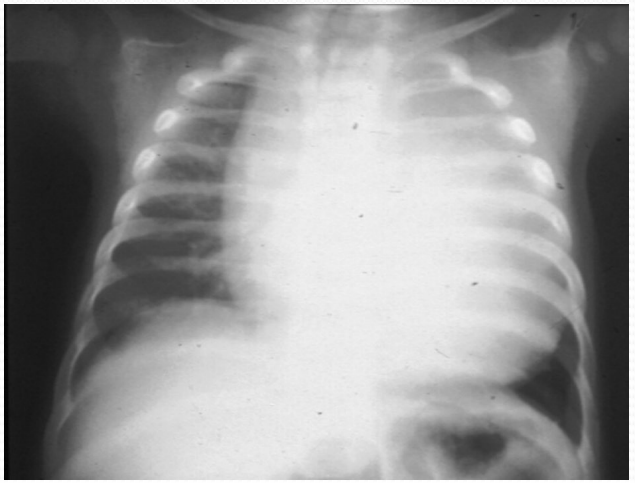
- Classical Infantile Form (<1% GAA activity)
  - –death in first year of life
  - –severe hypertrophy cardiomyopathy with cardiomegaly
  - –severe myopathy (hypotonia, muscle weakness)
- Juvenile (5-10% GAA activity) & Adult Forms (10-30 % GAA). Later onset; no cardiac involvement, mild-moderate myopathy, many become ventilator-dependent

Pompe Disease: Infants



Myopathy

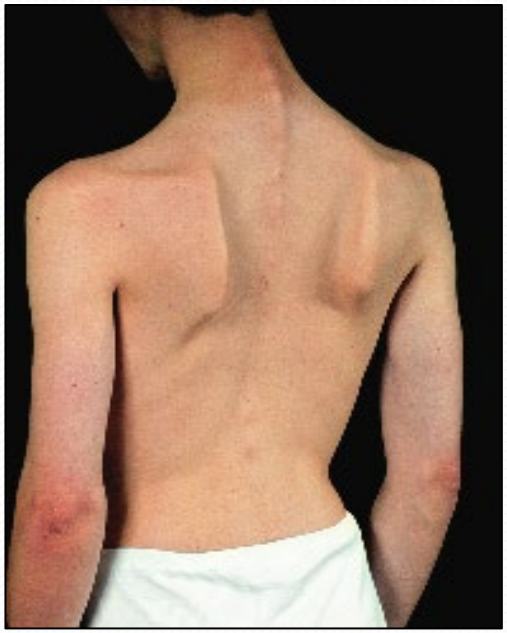




Hypertrophic Cardiomyopathy

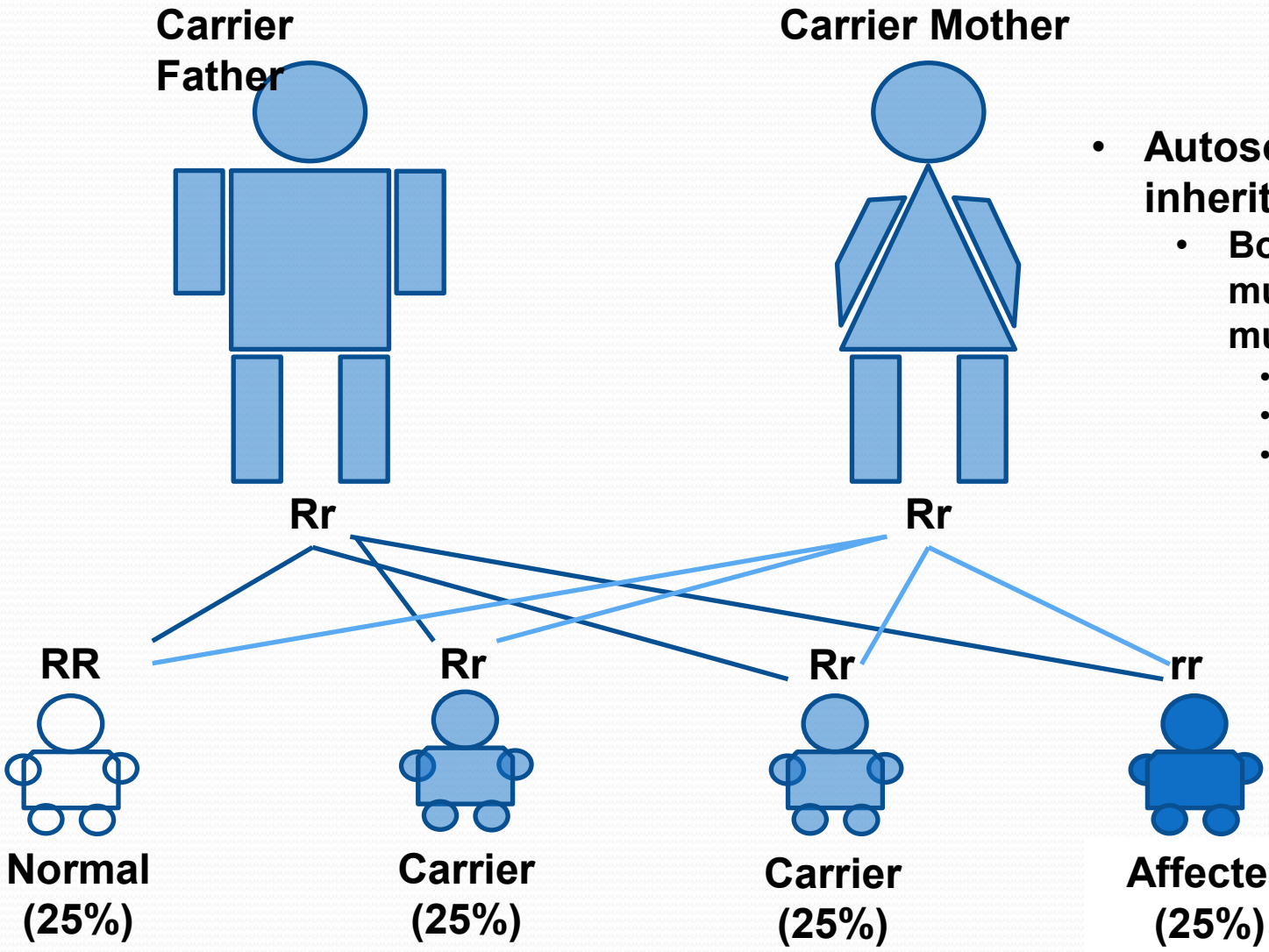
Pompe Disease: Older Patients

Skeletal Muscle Weakness



Respiratory Muscle Weakeness

Autosomal Recessive

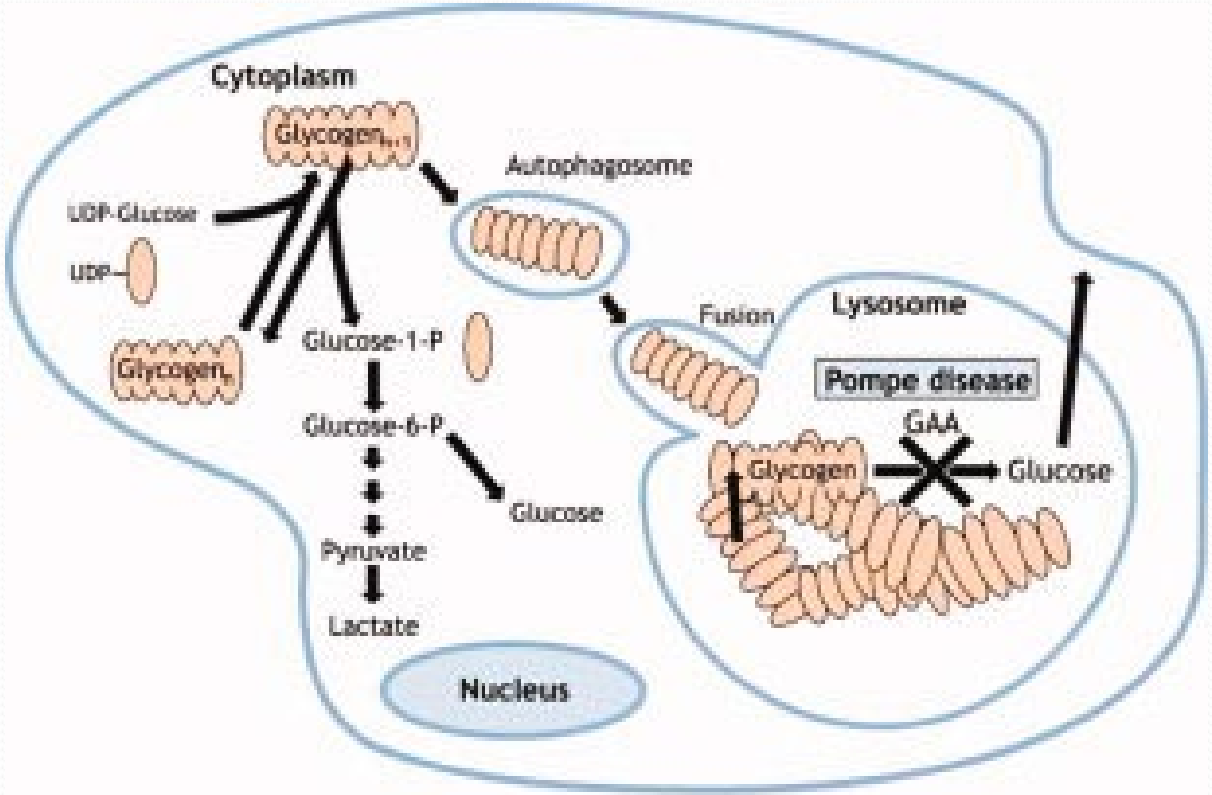


- Autosomal recessive inheritance
  - Both parents of a child must be carriers of a mutant gene
    - 25% chance affected
    - 25% chance unaffected
    - 50% chance carrier

Diagnosis of Pompe

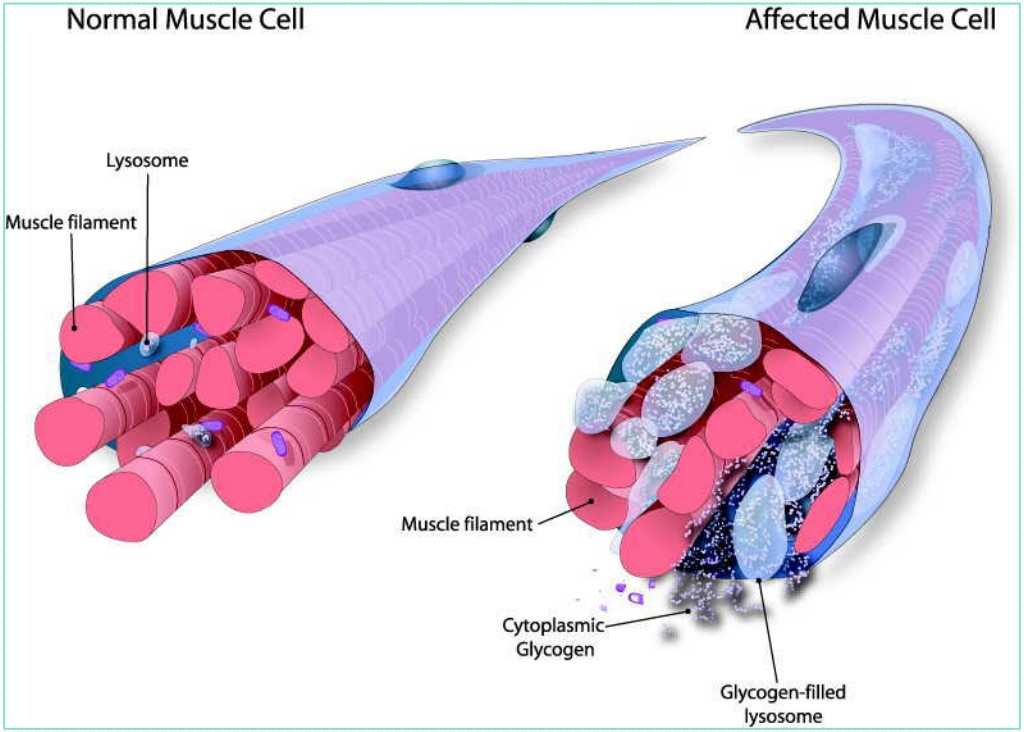
- **Serum creatine kinase (CK) concentration** is uniformly elevated (as high as 2000 IU/L; normal: 60-305 IU/L) in classic infantile-onset Pompe disease and in the childhood and juvenile variants, but may be normal in adult-onset disease
- **Urinary oligosaccharides.** Elevation of urinary glucose tetrasaccharide
- **Acid alpha-glucosidase (GAA) enzyme activity.** GAA enzyme activity analysis on dried blood spots
- **Targeted mutation analysis.** Individual could be tested first for one of the three common mutations p.ASP645Glu, p.Arg854X and common adult c. -36-13 T>G — before proceeding to full sequence analysis.

Pathophysiology of Pompe disease. GAA, acid alpha-glucosidase; UDP, uridine diphosphate.



GAA = acid alpha-glucosidase; UDP = uridine diphosphate.

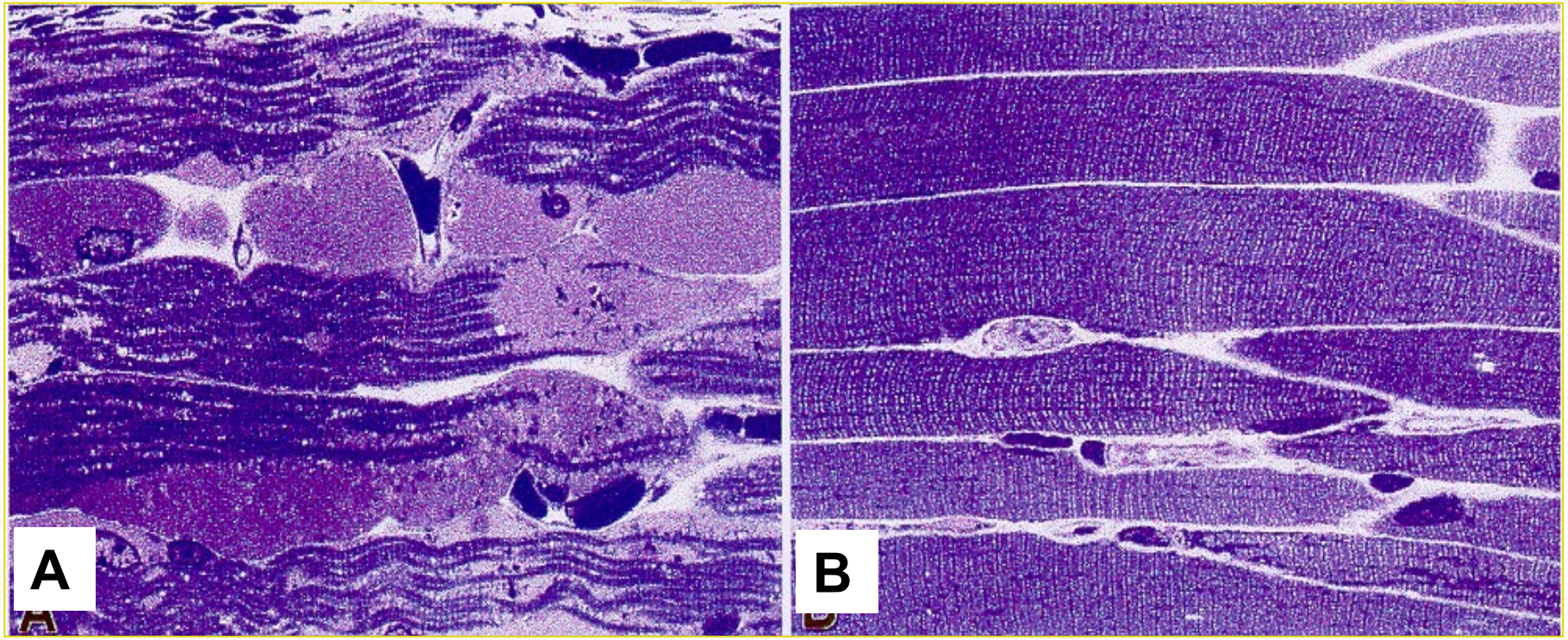
Accumulation of glycogen in a muscle cell



Clinical Presentation

	Classical Infantile	Non-classical Infantile	Juvenile	Adult
Age of Onset (yrs)	<0.5	<1	2 - 15	>15
Age of Death (yrs)	<1	1st decade	2 - 30	>15
Enzyme Activity (%)	<1	1-5	5 - 10	5 - 30
% of Patients	4:1		75%	
Clinical Symptoms	Hypertrophic Cardiomyopathy		Respiratory Failure Proximal Muscle Weakness	

Histopathology: Patient 103 CRIM (+)



Pre-treatment Post-treatment

Semithin sections (0.5 micron) of quadriceps muscle stained with toluidine blue, X 198.

A: Pre-treatment biopsy of Patient 103.

B: Biopsy of same patient following 13 weeks treatment with rhGAA.

ERT Studies in Infantile Pompe
Clinical Summary: Infantile Patients

- 15 / 16 alive
  - Age: 25 months (9 - 42)
  - Duration of treatment: 21 months (4 - 35)
- Robust myocardial response
  - Rapid and uniform improvement of hypertrophic cardiomyopathy
  - No cases of cardiac failure / medications
- Respiratory status
  - 10 / 16 ventilator-free

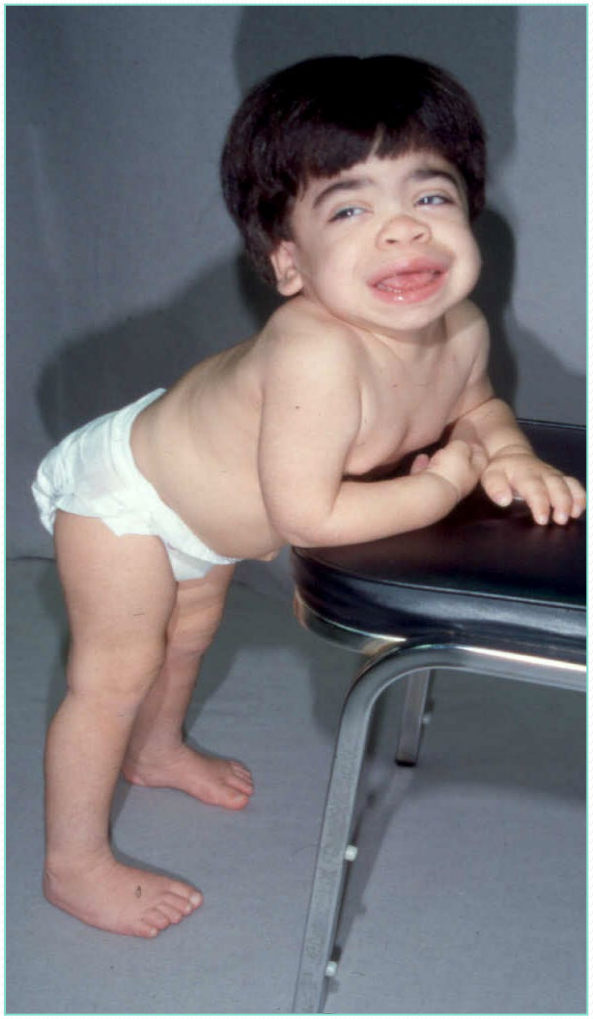
Response to Treatment depends on a variety of factors: severity at time of treatment, genetic background

Pre-operation months

Post-operation months

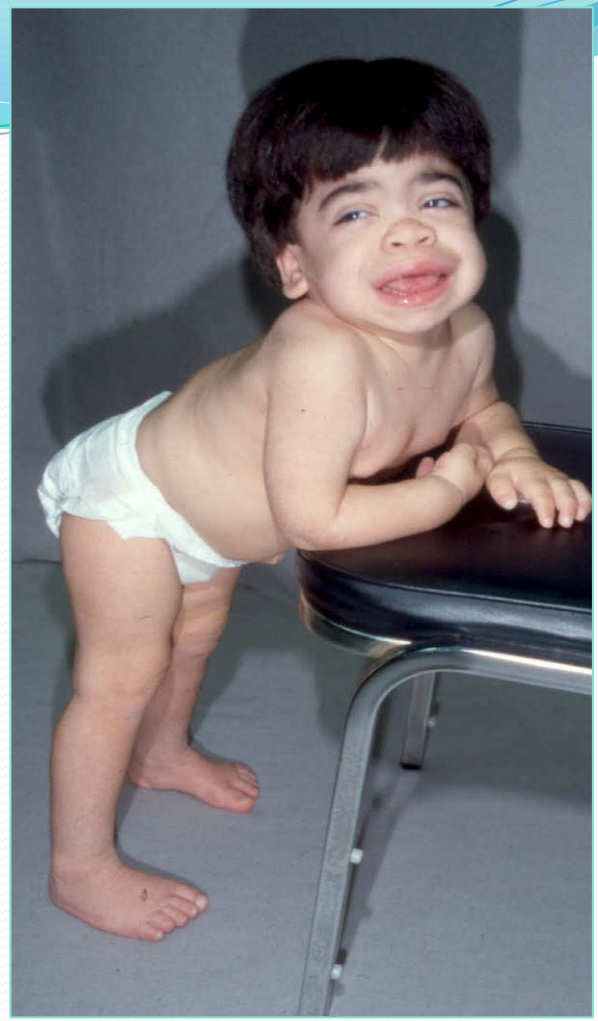
Case 3

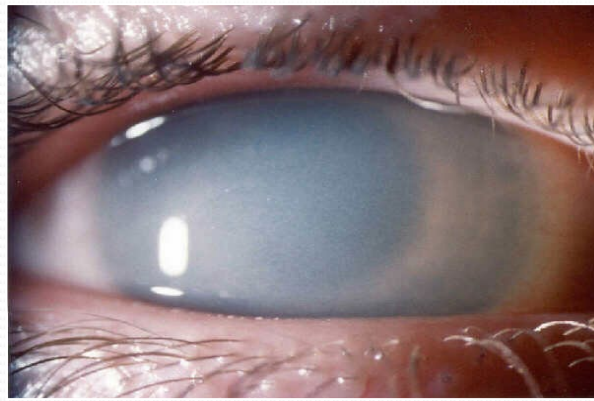
- Normal at birth
- URTIs
- Developmental delay
- Coarse features
- Large protuding tongue
- Cloudy corneas
- Hepatosplenomegaly
- Gibbus



Mucopolysaccharidosis I (MPS I)

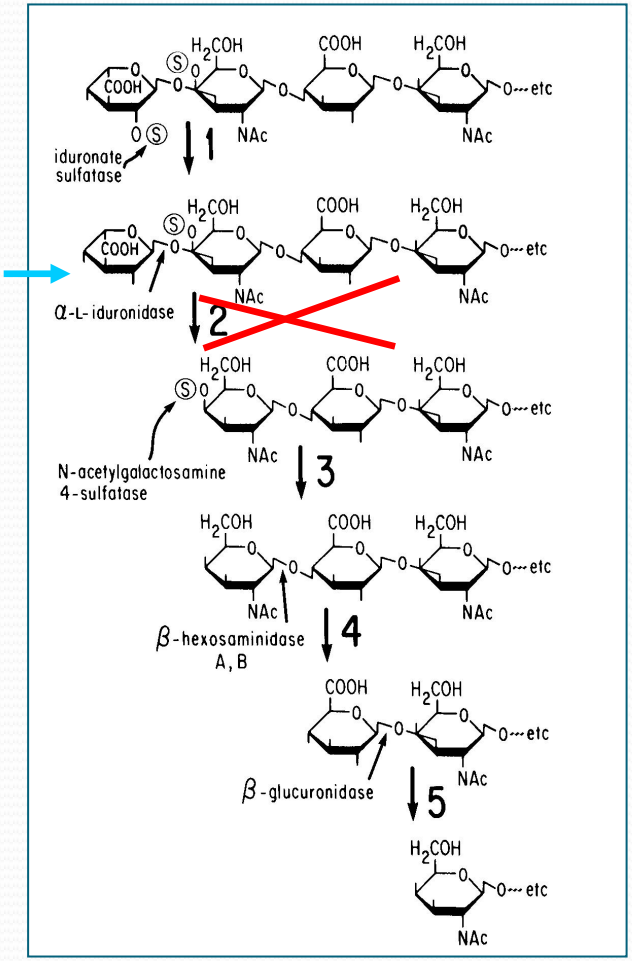
- Deficiency of α-L-iduronidase
- Progressive accumulation of glycosaminoglycans (dermatan sulphate)
- Multi-systemic, heterogeneous
- Severe morbidity and early mortality
- Rare (est. incidence 1:100,000)





MPS Disorders Biochemistry

MPS I



Iduronidase deficiency causes a block in the sequential breakdown steps of glycosaminoglycans e.g. dermatan sulfate degradation

The Mucopolysaccharidoses (MPS)

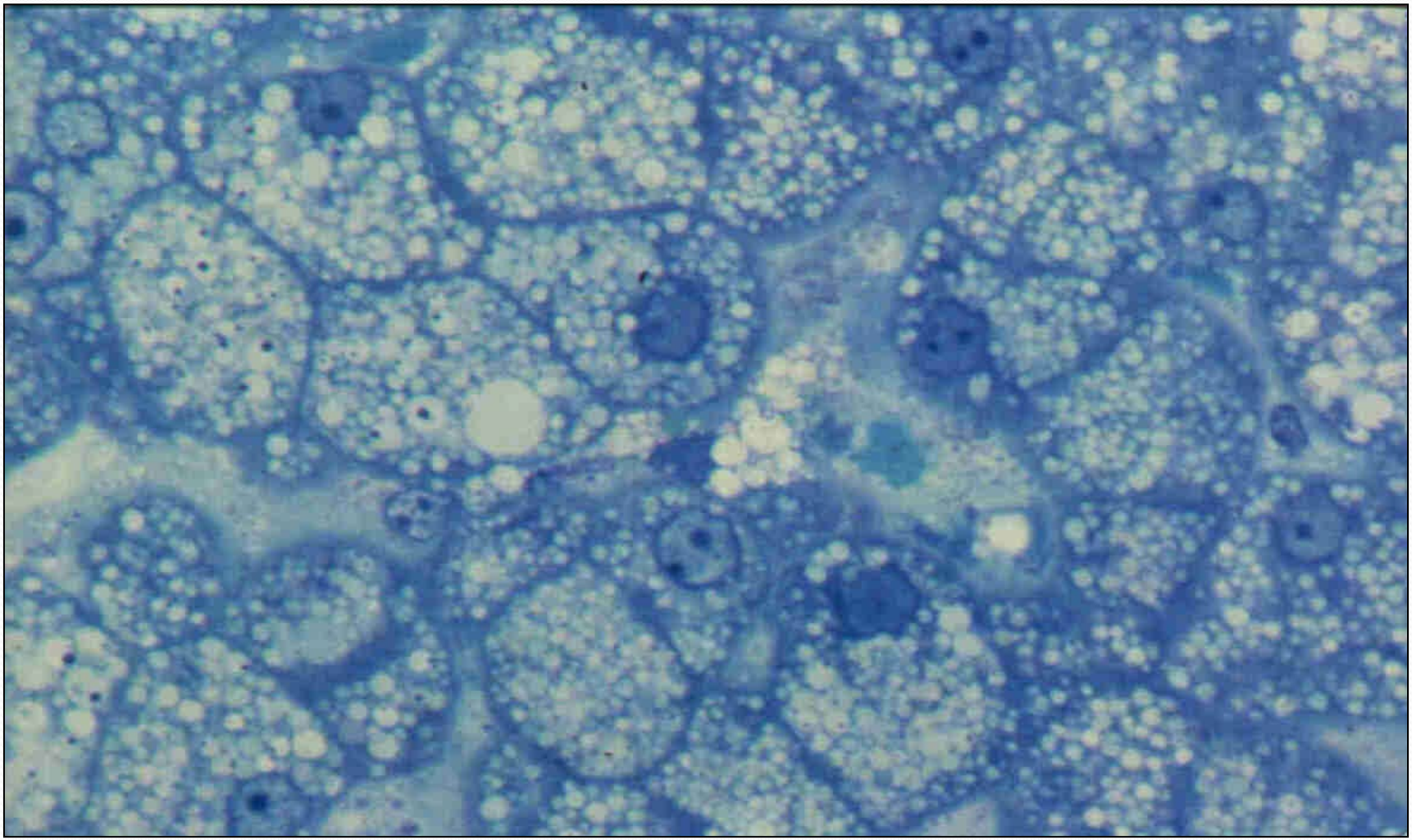
- Caused by deficiency of enzymes catalyzing the degradation of mucopolysaccharides
- Glycosaminoglycans accumulate to very high levels in lysosomes
- Lysosomes dysfunction/break resulting in impaired cell function and/or death.

The Mucopolysaccharidoses

Disorder	Eponym	Defective enzyme	Gene localization	Metabolite
MPSI-H	Hurler	α–iduronidase	4p16.3	DS, HS
MPS I-S	Scheie (former V)	α–iduronidase	4p16.3	DS, HS
MPS-H/S	Variants	α–iduronidase	4p16.3	DS, HS
MPS II	Hunter	Iduronate-2-sulphatase	Xq28	DS, HS
MPS III A	Sanfilippo A	Sulphamidase		HS
MPS IIIB	Sanfilippo B	α-N-Acetylglucosaminidase	17q21	HS
MPS IIIC	Sanfilippo C	Acetyl CoA:α-glucosamidase acetyltransferase	8p11	HS
MPS IIID	Sanfilippo D	N-Acetylyglucosamine 6-sulphatase	12q14	HS
MPS IV A	Morquio A	Galactose 6 sulphatase	16q24	KS, CS6
MPS IV B	Morquio B	β-galactosidase	3p22.3	KS
MPS VI	Maroteax-Lamy	N-Acetylgalactosamine 4-sulphatase	5q13	DS
MPS VII	Sly	β-glucoronidase	7q21-q22	DS, HS, $CS_{4, 6}$

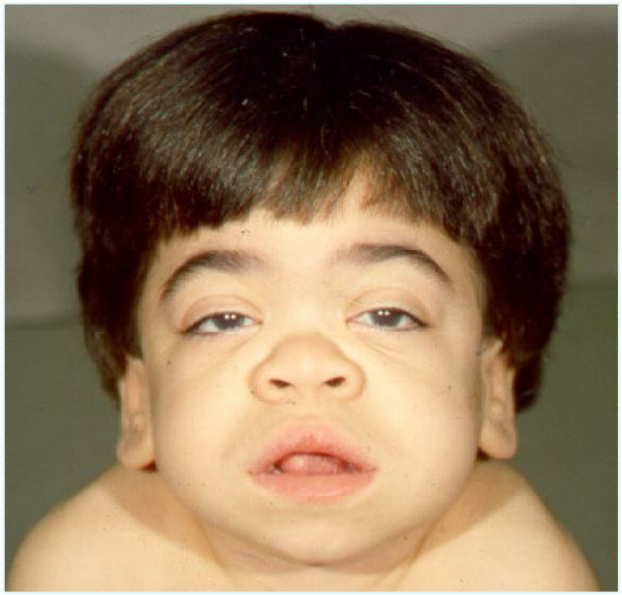
DS dermatan sulphate, HS heparan sulphate, CS6 chondroitin-6-sulfate

Liver Storage in MPS I: foamy vacuoles

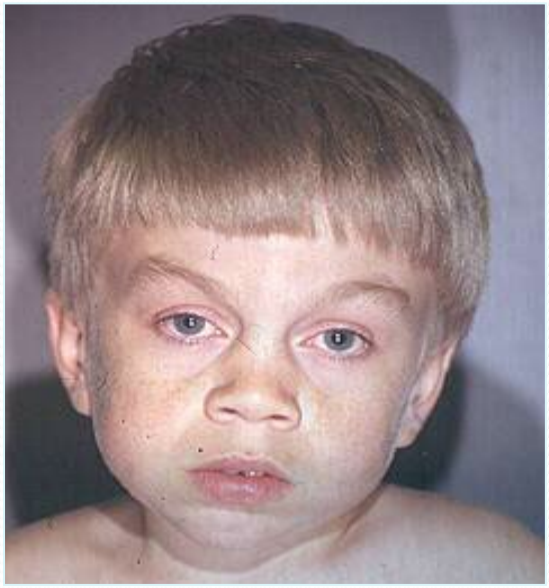


MPS I Clinical Spectrum

Hurler



Hurler-Scheie



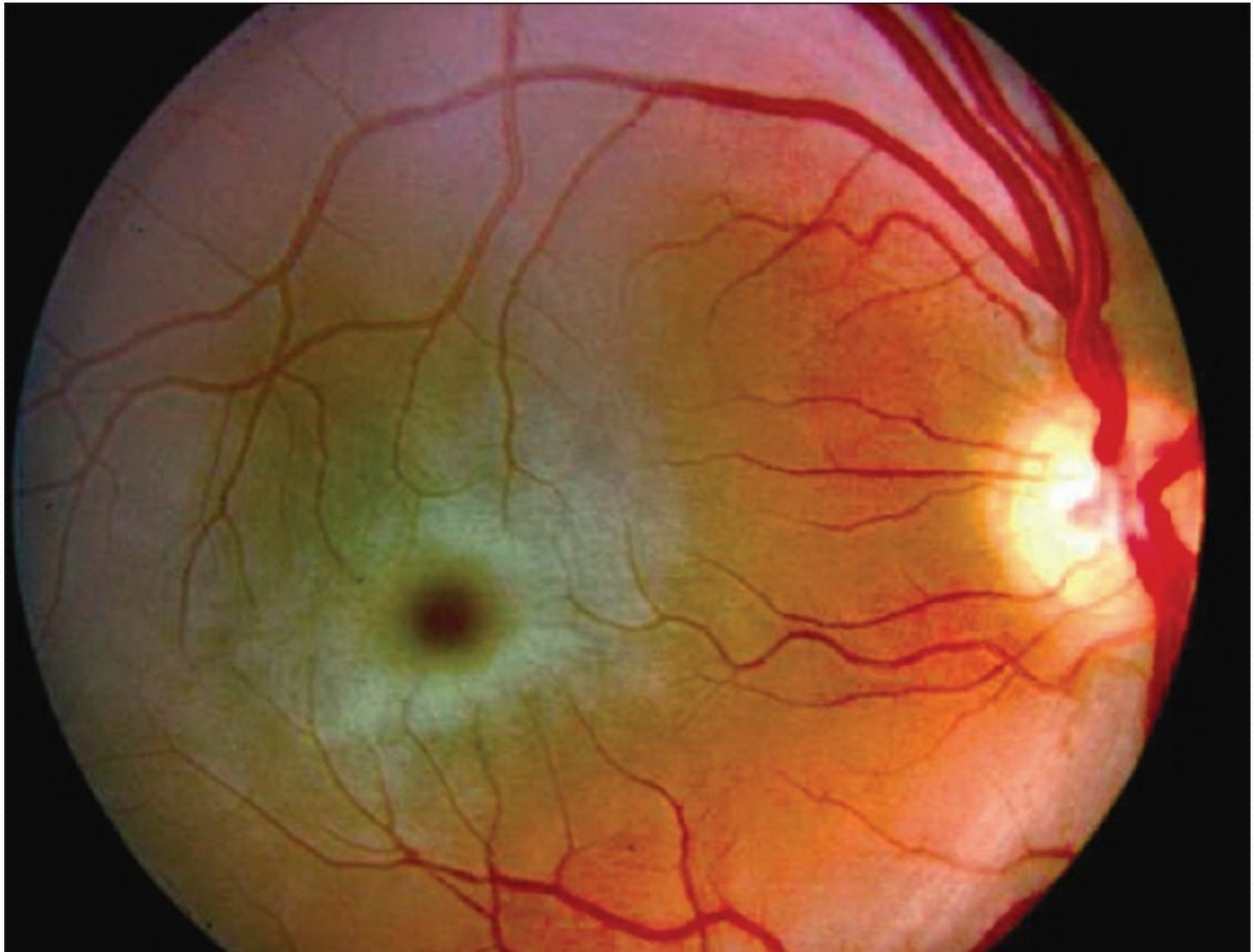
Scheie

All patients typically have <1% of normal enzyme levels

Spectrum of Disease

Hurler MPS I H	Hurler-Scheie MPS I H/S	Scheie MPS I S
• Severe mental retardation • More progressive • Severe respiratory disease • Obstructive airway disease • Death before age 10 years	• Little or no intellectual defect • Respiratory disease • Obstructive airway disease • Cardiovascular disease • Joint stiffness/contractures • Skeletal abnormalities • Decreased visual acuity • Death in teens and 20's	• Normal intelligence • Less progressive • physical problems • Corneal clouding • Joint stiffness • Valvular heart diseas • Death in later decade

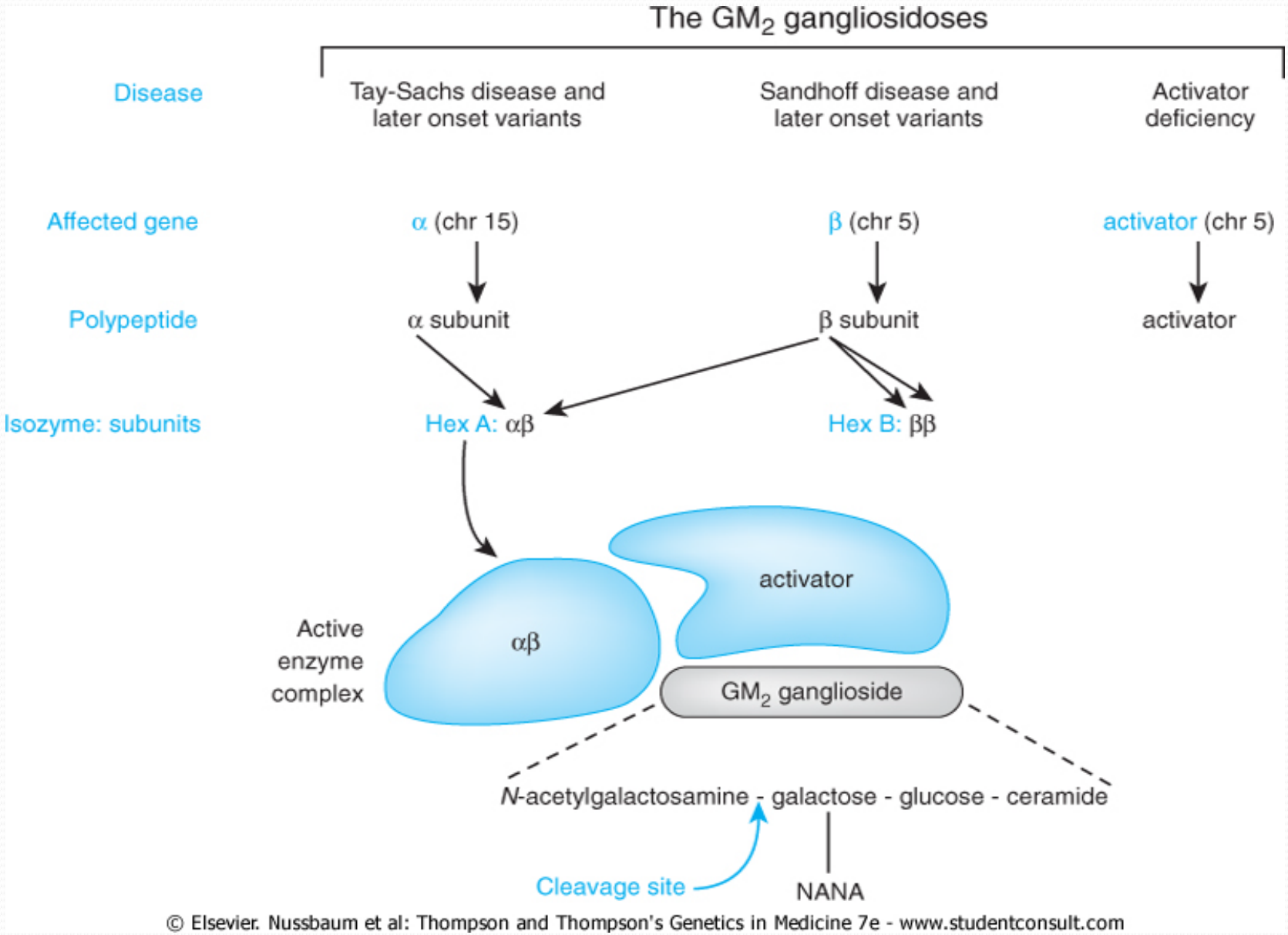
Case 4. What is the diagnosis?



Springolipidoses: Tay Sachs

- Hexosaminidase A deficiency: Intralysosomal storage of glycosphingolipid GM2 ganglioside
- Weakness and loss of motor skills at 3-6 months
- Decreased attentiveness, increased startle response
- Cherry-red spot of the fovea centralis of macula
- Normal-sized liver and spleen
- Progressive neurodegeneration- seizures, blindness, spasticity, death before 4 years
- Symptomatic treatment

3-gene system of hexosaminidase A activity



Testing

- Mutation analysis of the 3 genes for hexosaminidase A enzyme
  - HEXA, HEXB, activator protein
  - Need to distinguish pseudodeficiency from disease-causing alleles
  - 3 common mutations account for 94% of heterozygotes in Ashkenazi Jewish population
  - Carrier Freq population 1/250, Ashkenazi 1/30

Treatment of LSDs
Enzyme replacement therapy

Disease	Current Status
• Gaucher Disease	Approved 1991
• Fabry Disease	Approved 2001
• Hurler, Mucopolysaccharidosis (MPS) I	Approved 2003
• Maroteaux-Lamy, MPS VI	Approved 2005
• Hurler MPS II	Approved 2006
• Pompe Disease	Approved 2006
• Niemann-Pick B Disease	Trial

Cost of Enzyme Replacement Therapy

Cerezyme avg cost $138,00 per year

Fabrazyme avg cost $180,000 per year

Aldurazyme avg cost $150,000 per year

Myozyme avg cost >$200,000 per year

- **96% of the cases in US are covered by insurance**
- **Orphan Drug Act-Compassionate Drug Program**
  - **Finances should never restrict therapy**

UC Irvine Lysosomal Storage Diseases Program

Number of patients:

- 3 Gaucher
- 23 Pompe
- 1 Hunter
- 39 Fabry
- 2 Wolman

Newborn Screening for LSD

- California NBS for LSDs https://www.cdph.ca.gov/Programs/CFH/DGDS/Pages/nbs/NBS-Disorders-Detectable.aspx

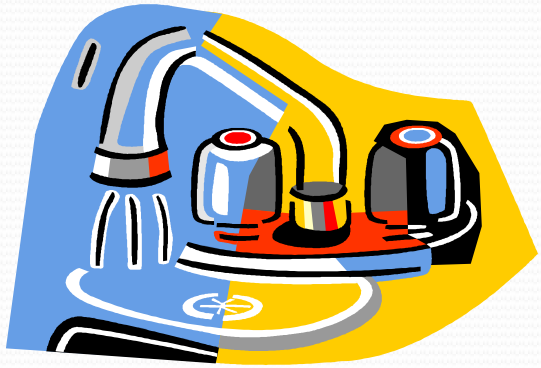
- Glycogen Storage Disease (Pompe)
- Mucopolysaccharidosis Type I

Gene Therapy in Genetic Disorders

LSD Research at UCI

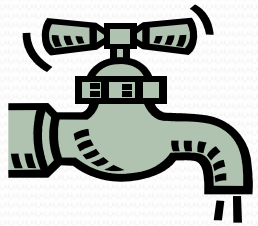
Substrate Inhibition in the Lysosomal Disorders

ERT versus Substrate Inhibition



Gaucher Disease: Clogs up the drain

ERT: Unclogs the drain

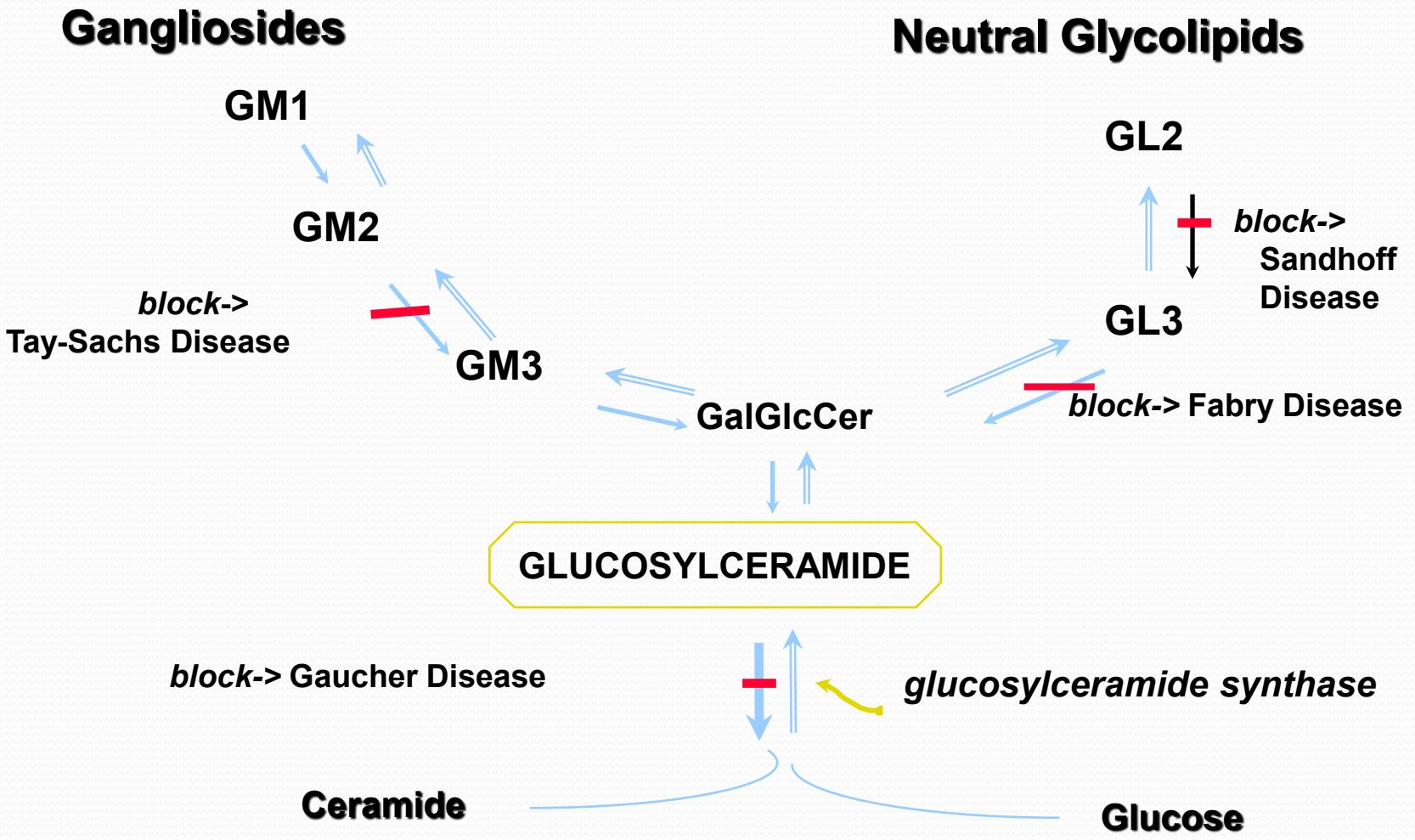


Normal equilibrium:

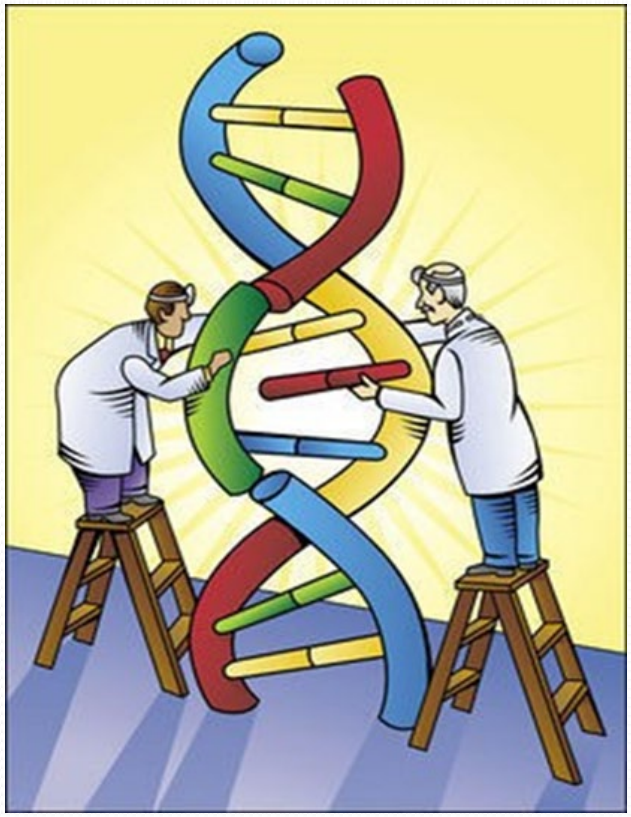
Faucet running with sink draining

Substrate Inhibition: Turns Down the Faucet

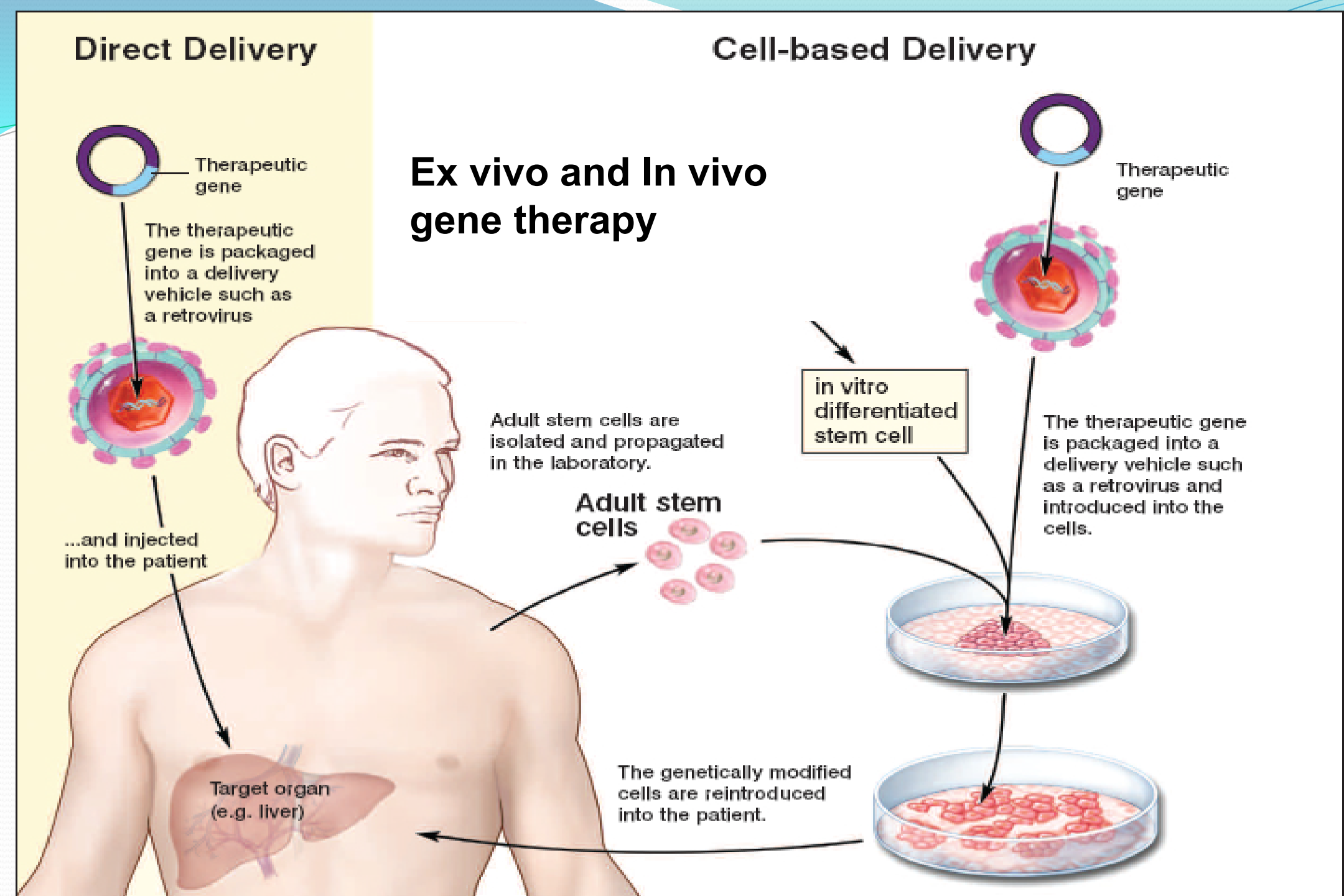
Glycosphingolipid Pathways



Gene Therapy



- An approach of treating diseases by:
- Replacing a mutated gene that causes disease with a healthy copy of the gene.
- Inactivating, or “knocking out,” a mutated gene that is functioning improperly.
- Introducing a new gene to combat disease.


Direct Delivery
Cell-based Delivery
Ex vivo and In vivo gene therapy
Therapeutic gene
The therapeutic gene is packaged into a delivery vehicle such as a retrovirus
...and injected into the patient
Target organ (e.g. liver)
Adult stem cells are isolated and propagated in the laboratory.
Adult stem cells
in vitro differentiated stem cell
Therapeutic gene
The therapeutic gene is packaged into a delivery vehicle such as a retrovirus and introduced into the cells.
The genetically modified cells are reintroduced into the patient.

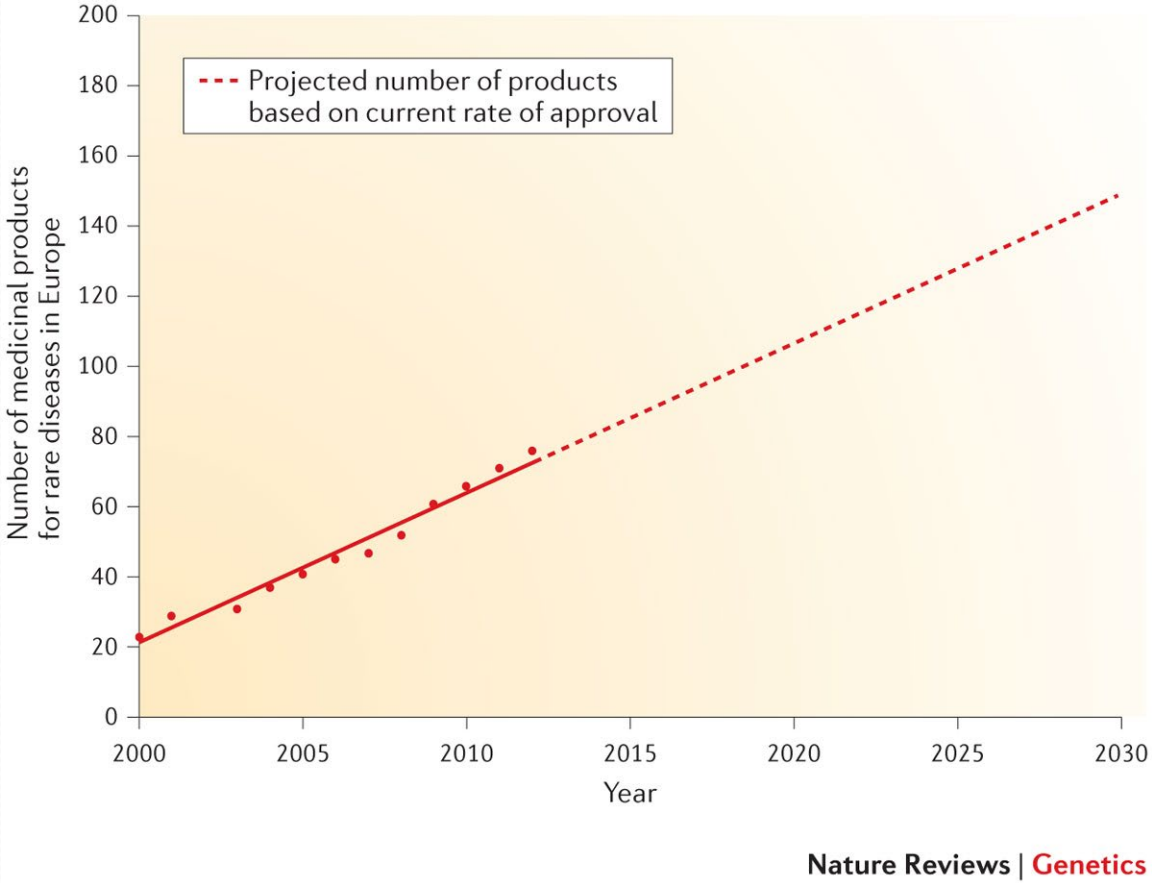
Gene Therapy

Over expressing genes serves as a biological enzyme pump that delivers the normal enzyme

Pre-clinical and clinical studies in several LSDs:

- Pompe
- Fabry
- MPS-I/II
- Gaucher
- Niemann Pick

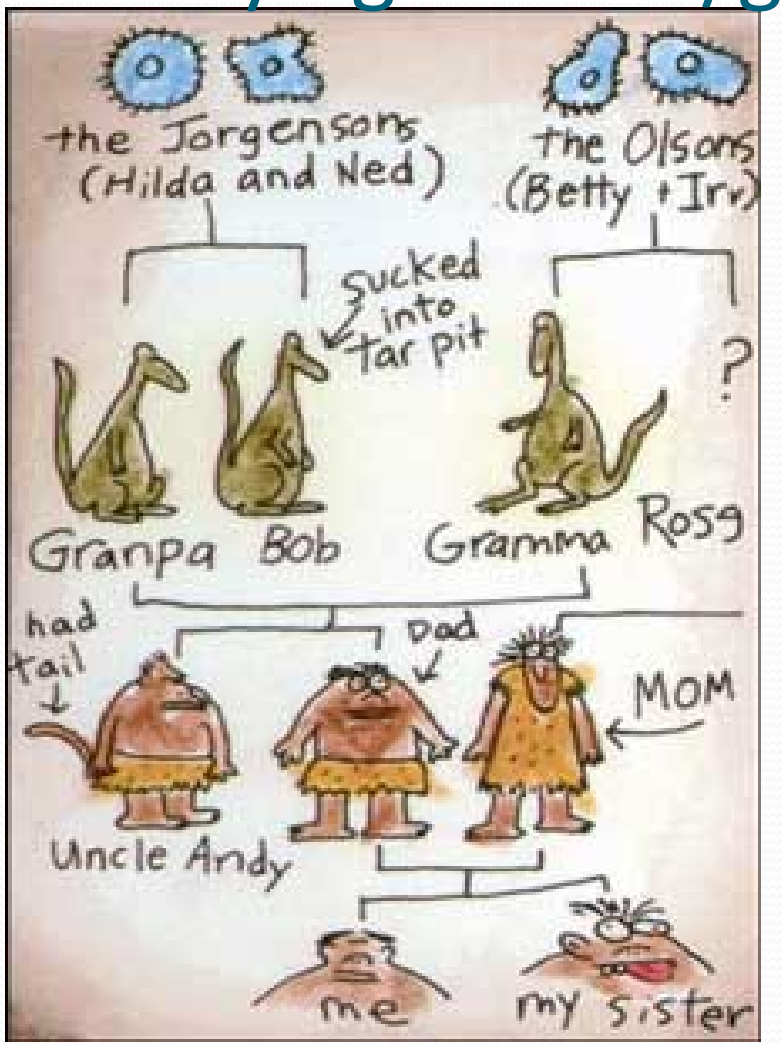
Take Home Messages



- Identify diseases early so they are amenable to Px
- Increasing molecular insight inevitably suggests novel therapeutic avenues

New strategies for treating genetic disorders

Keep taking those pedigrees! HCPs have a unique role in identifying familial/genetic disorders



LYSOSOMAL DISEASES QUESTIONS

Question 1

Fabry disease is characterized by the following clinical features except:

A. Increased sweating

B. Hypertrophic cardiomyopathy

C. Kidney failure

D Strokes

E Acroparasthesis

Question 2

A gentleman has a long history of tiredness, pallor and anemia. Suspicious for Gaucher disease. What are the features that are suggestive of this diagnosis:

a) Hepatosplenomegaly

b) Muscle weakness

c) Skin angiokeratomas

d) Cardiomyopathy

Question 5

Pompe disease:

A. Is associated with storage of lipid
B. Affects cognitive function
C. Therapy is with vitamin replacement
D. Is associated with a cherry red spot
E. Is associated with a myopathy

Reference:

Genereviews

http://www.ncbi.nlm.nih.gov/books/NBK1261/

http://trailers.apple.com/trailers/independent/extraordinarymeasures/

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