MEDICAL BIOCHEMICAL GENETICS
CLINICAL CORE
SEMINAR SERIES

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Lysosomal Storage Diseases Part I

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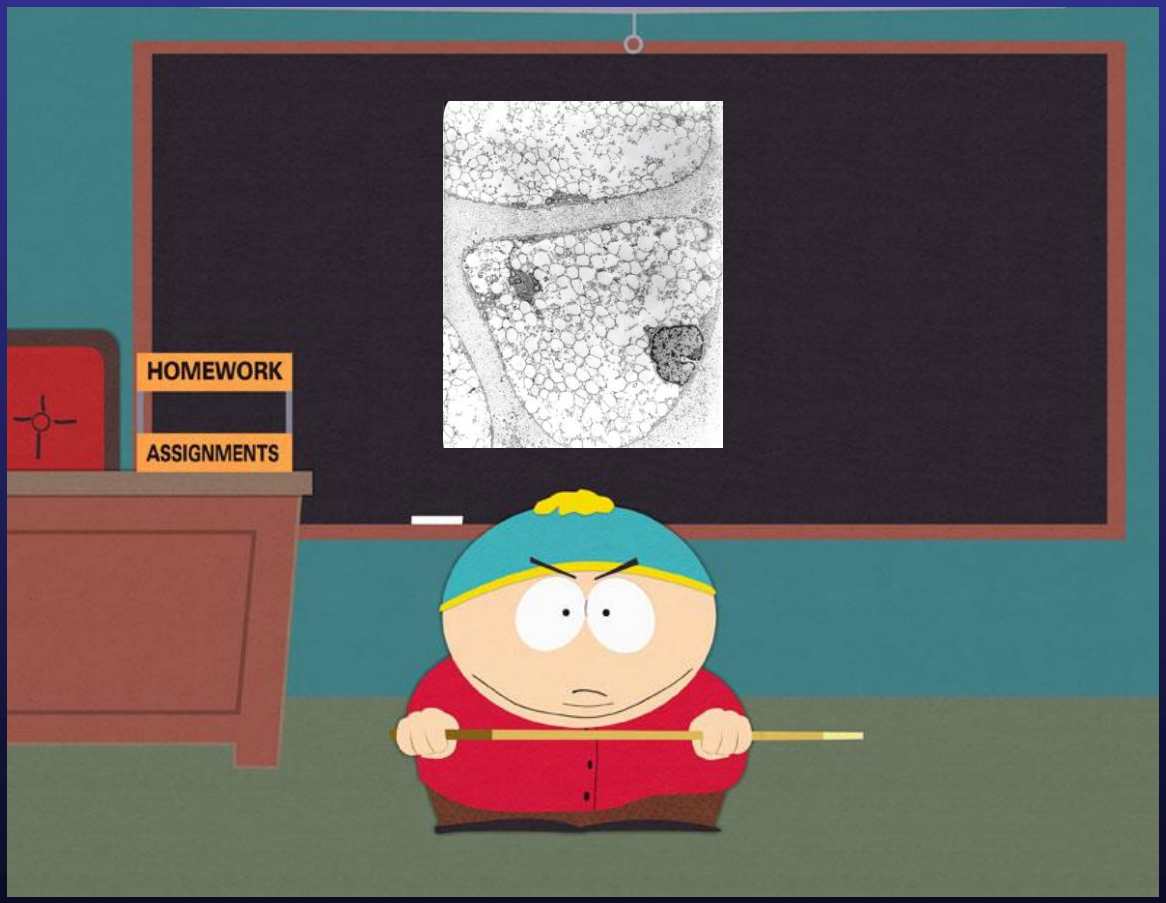
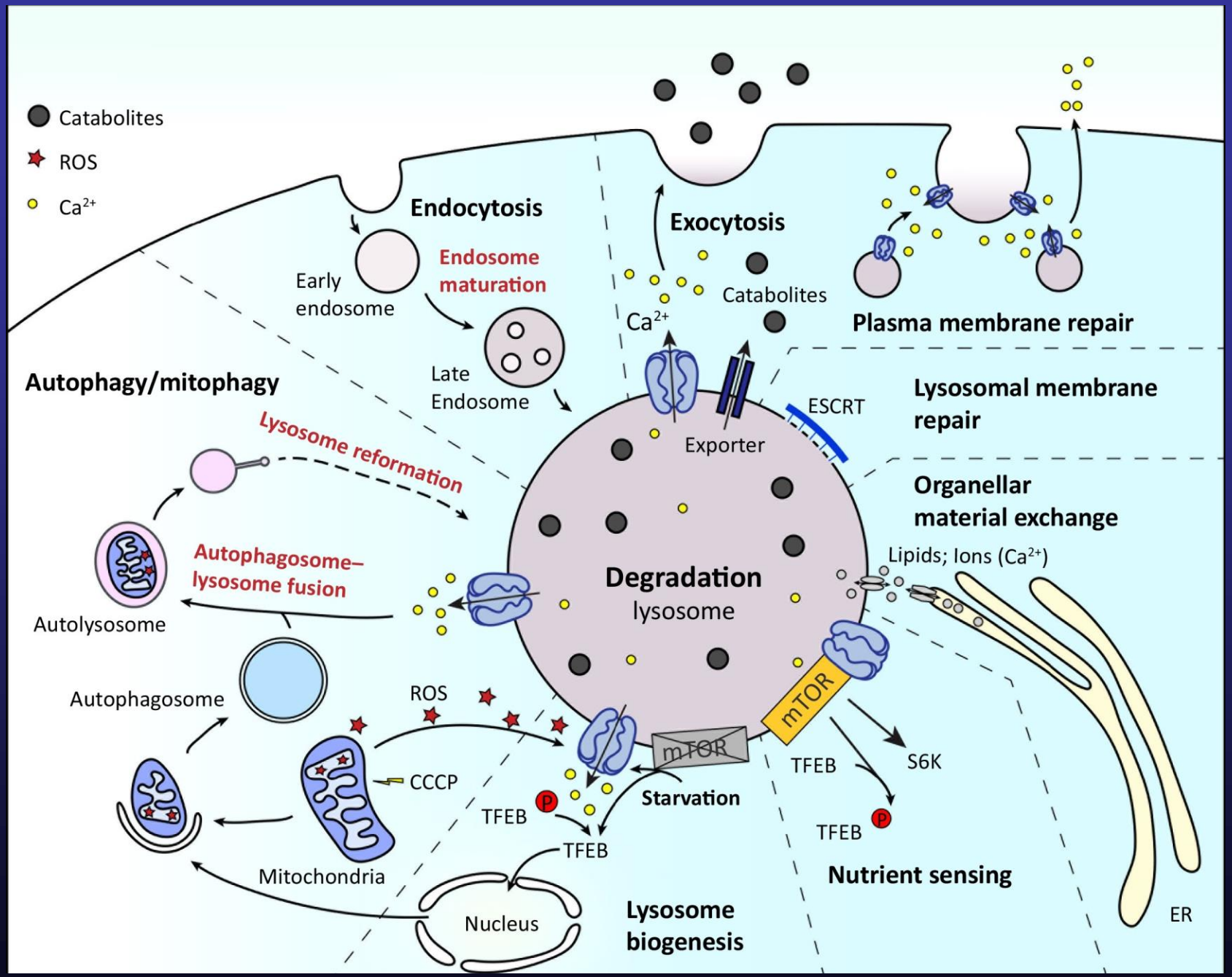
Disclosures:
Advisory Board: Alexion, Chiesi, Sanofi-Genzyme, Takeda, Homology
Consultant: Spark, UniQuire
Clinical trials and registries: Alexion/AstraZeneca, Amicus, BioMarin, 4D Molecular Therapeutics, Chiesi, Orphazyme, Pfizer, Protalix, Sanofi-Genzyme, Sangamo, Takeda, Astellas
Research grants: Amicus, Takeda
Employee and stock: Fulgent diagnostics (spouse)

Let's Learn About Lysosomes



Lysosomal Functions

- Degradation of macromolecules
- Membrane trafficking and homeostasis
- Exocytosis
- Autophagy
- Nutrient sensing
- Calcium signaling



Li P et al, Trends in Biochemical Sciences 44:110, 2019

Lysosomal Storage Diseases

- Over 70 disorders of acid hydrolases and associated proteins, intracellular trafficking, membrane transport and function
- Most are autosomal recessive, 3 are X-linked (Hunter, Fabry, Danon)
- > 1:5,000, most rare
  - Fabry the most common in newborn screening
  - Increased in some populations (e.g. Gaucher, Tay-Sachs in Ashkenazi Jews)

- Heterogeneous clinically
- Most are housekeeping proteins- the pattern of tissue involvement depends on the substrate distribution
- Most involve multiple organ systems, but some predominantly involve the brain or skeleton

Reasons to suspect a LSD

- Coarse facial features
- Neurologic
  - Loss of acquired developmental milestones
  - Behavioral changes, psychosis
  - Seizures
  - Ataxia
  - Neuropathy, neuropathic pain
  - Macrocephaly
- Ophthalmologic
  - Corneal clouding, crystals, verticillata
  - Ophthalmoplegia (vertical)

- ENT
  - Recurrent otitis media, sinusitis
  - Macroglossia
- Pulmonary
  - Obstruction
  - Decreased FVC
- Cardiovascular
  - Cardiomyopathy
  - Valve disease
- GI
  - Hepatomegaly, splenomegaly
  - Large umbilical hernia

- Musculoskeletal
  - Joint stiffness, limited range of motion
  - Madelung deformity
  - Dwarfism, kyphosis, dysostosis multiplex
- Dermatologic
  - Angiokeratomas
  - Excessive Mongolian spots
  - Hypohidrosis
- Hematologic
  - Anemia
  - Thrombocytopenia

General Pathogenic Mechanisms of Lysosomal Storage Diseases

- Mass effect of lysosomal storage in the cell
- Alteration of plasma membrane dynamics and signaling
- Altered autophagy
- Abnormal intracellular trafficking
- Inflammation
- Secretion of toxic secondary breakdown products
- Fibrosis
- Organ enlargement
- Infarction
- Secondary storage from cell breakdown and exosome export

Treatments for Lysosomal Storage Diseases

- Symptomatic and palliative therapies
- Export the stored material
  - cystinosis
- Enzyme replacement therapy (ERT)
  - MPS I, II, VI, VII, Gaucher (x3), Fabry (x2), Pompe (x2), Niemann-Pick B
- Substrate reduction therapy
  - Gaucher (x2), NPC
- Increase residual enzyme activity
  - Fabry (chaperone, migalastat)
- HSCT
  - Severe MPS I, MLD, Krabbe

Therapies in or soon to be in trials

- Next generation ERT (Fabry)
- ERT that can cross the BBB (MPS I, II)
- ERT + chaperone (Pompe)
- Substrate reduction (Fabry, gangliosidoses)
- Increase residual activity (NPC, arimoclomol, increases HSP)
- Gene Therapy
  - *Ex vivo* HSCT (MLD, MPS I, MPS II, Gaucher, Pompe, cystinosis)
  - AAV gene transfer (Fabry, Gaucher, MPS I, MPS II, MPS III, Pompe, Krabbe)
- Improved trafficking (NPC, cyclodextrans)
- Reduce neuroinflammation (NPC, N-acetyl-L-leucine)
- Gene editing

Untreated Cystinosis

- Renal Fanconi with secondary complications
- Renal failure
- Cornea crystals and photophobia
- Hypothyroidism
- Hypohidrosis
- Male hypogonadism
- Myopathy, pulmonary insufficiency, GI, cardiovascular, CNS calcifications
- Variable intellectual disability

Cystinosis

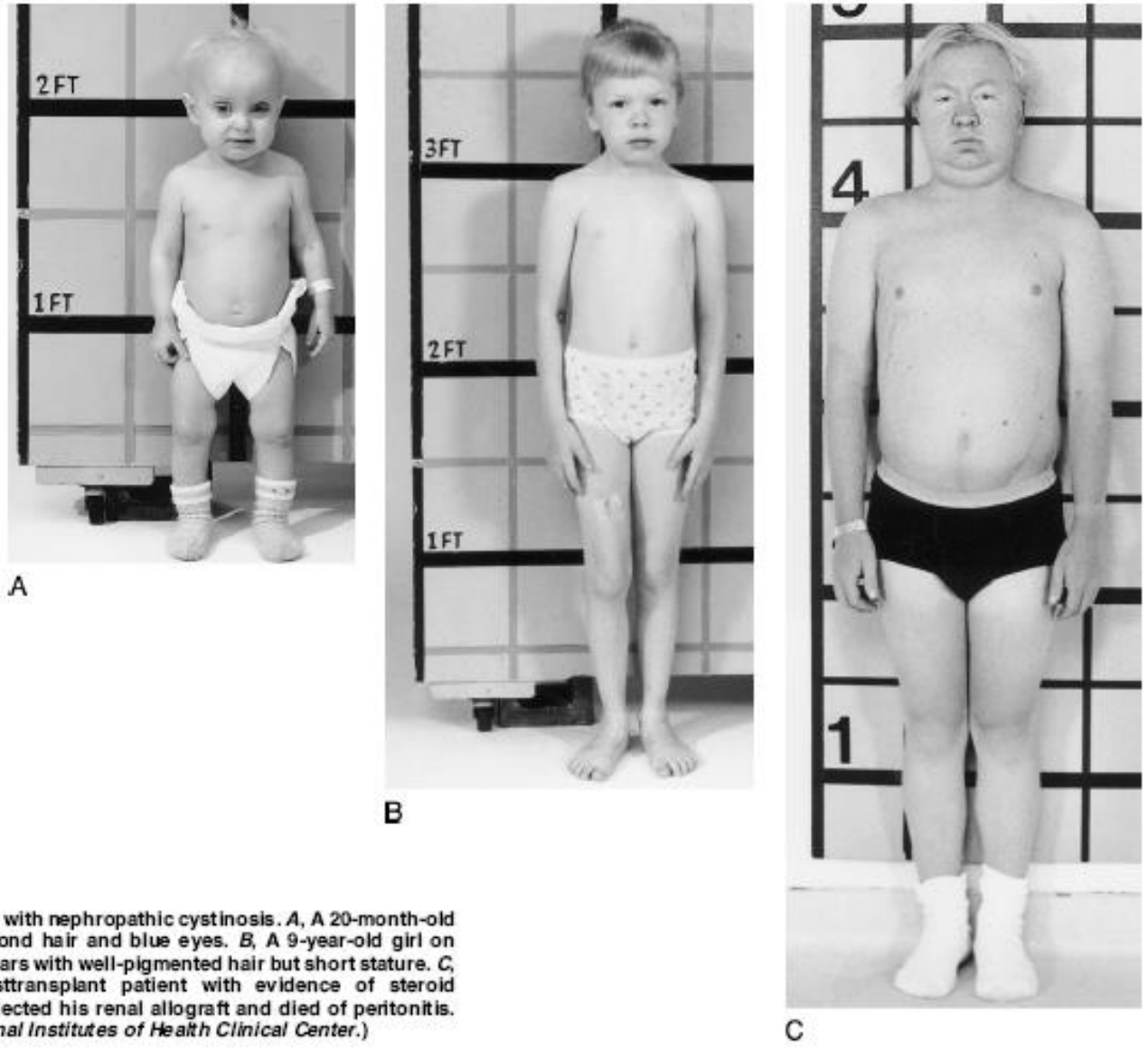
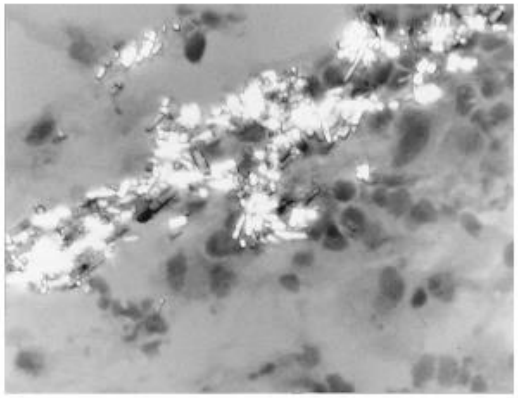
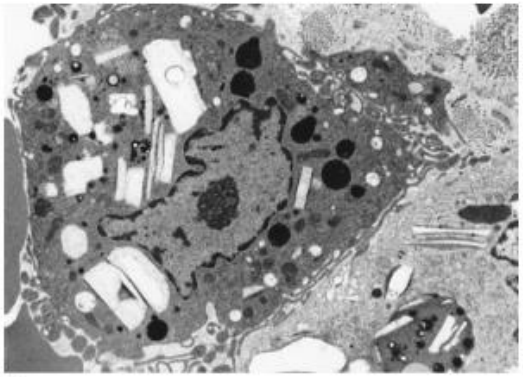


Fig. 199-5 Patients with nephropathic cystinosis. *A*, A 20-month-old girl with sparse blond hair and blue eyes. *B*, A 9-year-old girl on cysteamine for 7 years with well-pigmented hair but short stature. *C*, A 25-year-old posttransplant patient with evidence of steroid effects. He later rejected his renal allograft and died of peritonitis. (*Courtesy of National Institutes of Health Clinical Center.*)

MMBID



A



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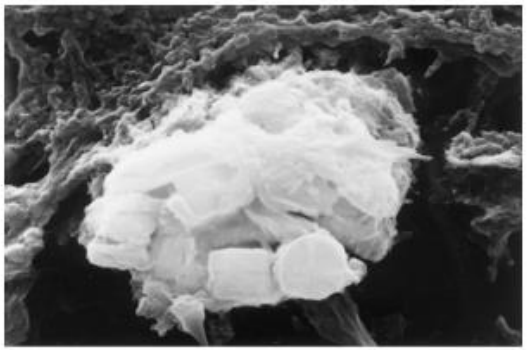

C

Fig. 199-3 Cystine crystals. *A*, Light microscopy showing conjunctival crystals under cross-polarizing light. *B*, Electron micrograph of hexagonal cystine crystals within lysosomes of a Kupffer cell. ×14,500. *C*, Scanning electron micrograph showing crystals protruding from the surface of a Kupffer cell. ×1800. (*B and C courtesy of K.G. Ishak, M.D., Ph.D., Armed Forces Institute of Pathology, Washington, DC.*)

Cystine Crystals in Cystinosis

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Cystinosis Pathology and Treatment

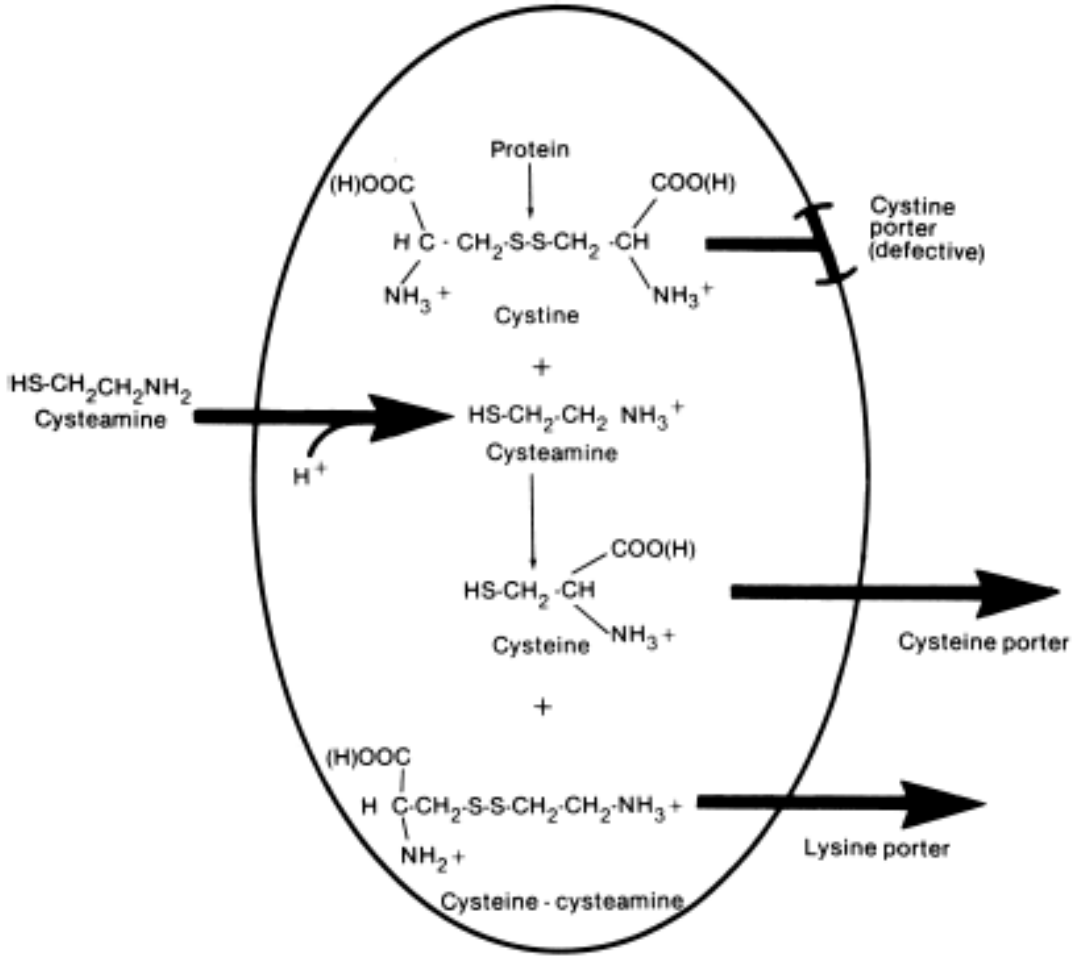


Fig. 199-4 Mechanism of cystine depletion by cysteamine. Cystine is stored inside the cystinotic lysosome because the cystine carrier in the lysosomal membrane is defective. Cysteamine traverses the lysosomal membrane by virtue of its neutral amine group or via a cysteamine carrier. The amine group acquires a positive charge and is "trapped" within the acidic lysosome. Cysteamine then reacts with cystine, producing cysteine and the mixed disulfide cysteine-cysteamine, by disulfide interchange. Cysteine leaves the cystinotic lysosome, perhaps via a cysteine carrier system. The mixed disulfide cysteine-cysteamine is structurally analogous to lysine, and exits the cystinotic lysosome via a lysosomal lysine carrier, which remains functional in cystinosis cells.

Cystinosis- Response of the Cornea

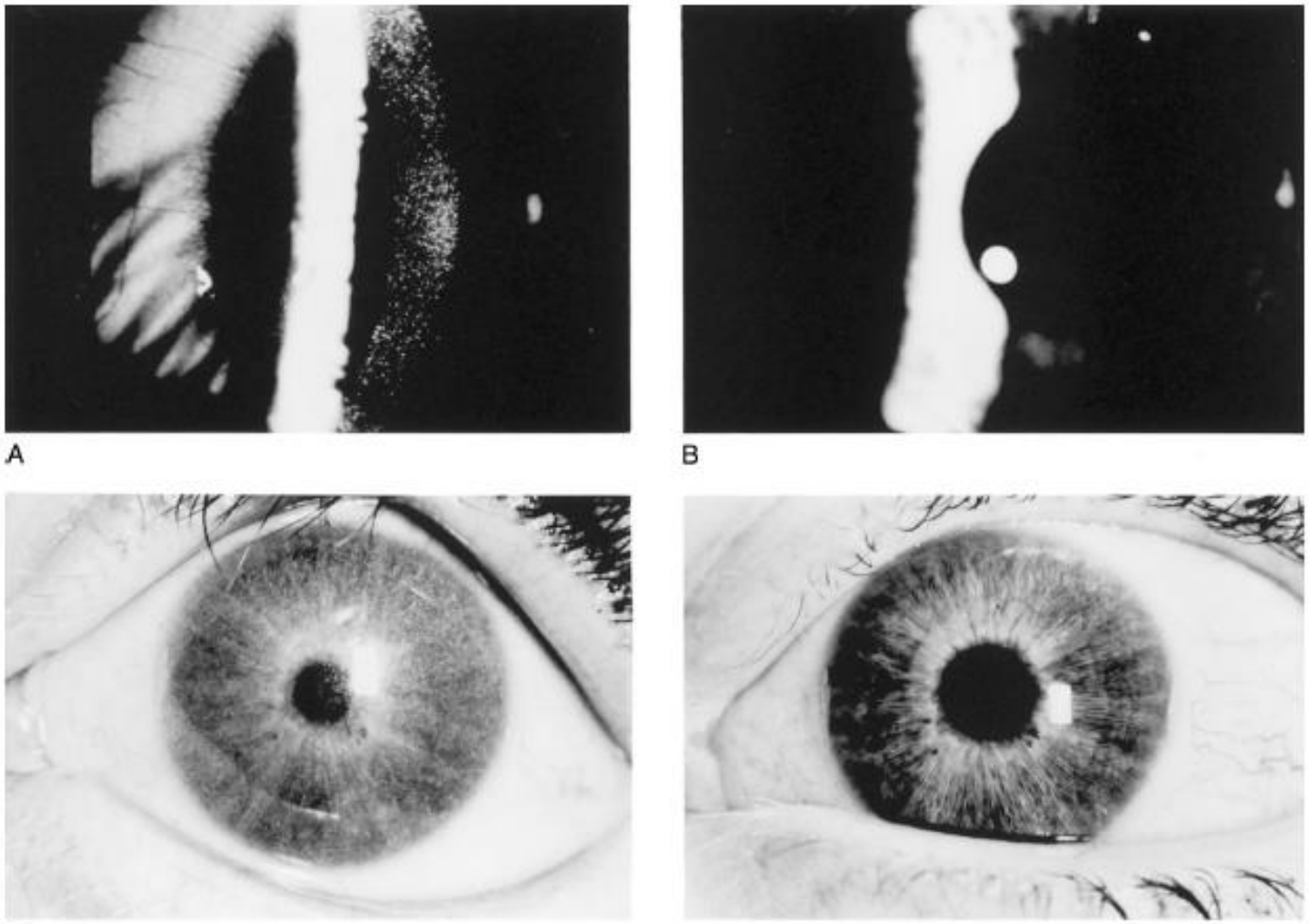
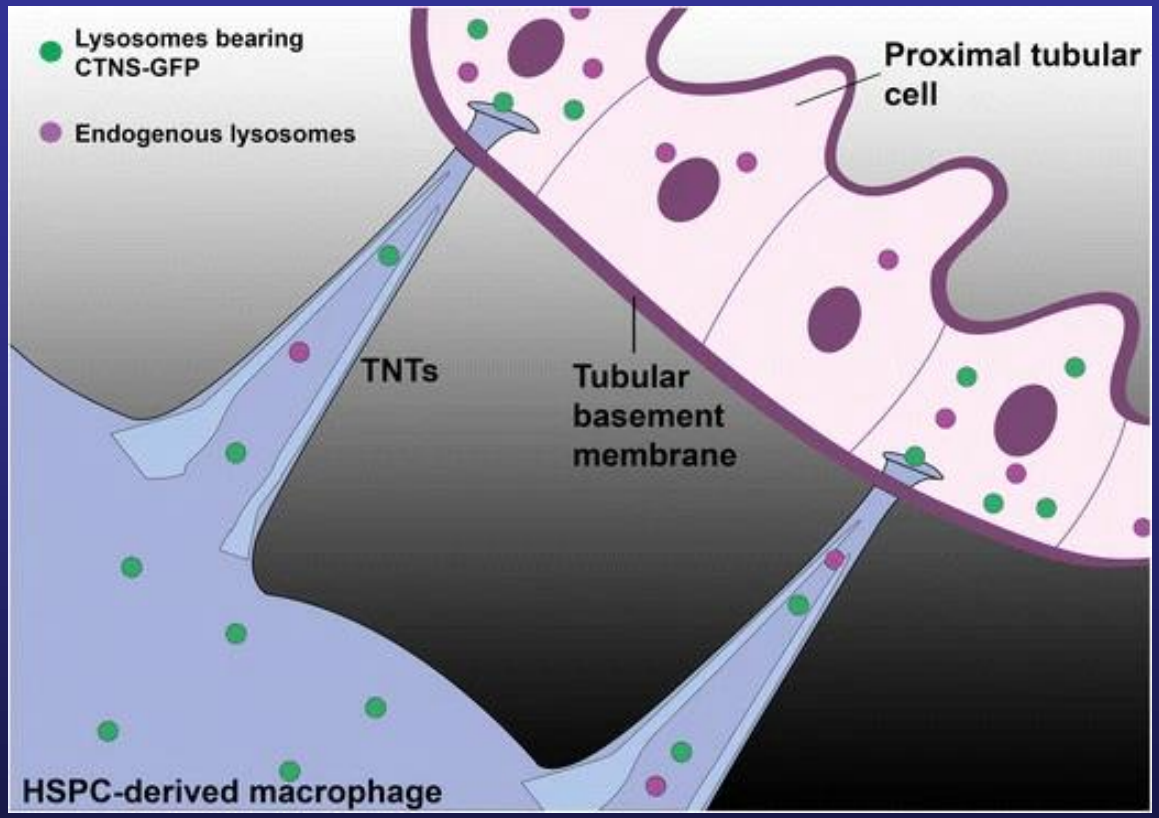


Fig. 199-8 Photographs of corneal cystine crystals before and after cysteamine eyedrop therapy in cystinosis patients. *A*, Right eye of a 28-month-old boy before topical cysteamine therapy, showing abundant crystals. *B*, Same eye as in *A* after 7 months of 0.5% cysteamine eyedrops administered 8 to 12 times per day, with clearing of corneal crystals. *C*, Left eye of 21-year-old woman prior to cysteamine eyedrop therapy. Uniform haziness typifies appearance of cornea at this age. *D*, Same eye as in *C* after several months of diligent therapy with 0.5% cysteamine eyedrops. Cornea is clear to inspection, although crystals remain visible on slit-lamp examination. (*Courtesy of Dr. M.I. Kaiser-Kupfer, National Eye Institute, National Institutes of Health, Bethesda, Maryland.*)

Cystinosis
Long Term Response To Cysteamine Therapy

- If started early enough and patients are compliant (twice a day dosing with delayed release formulation), renal function is preserved
- Eye pain and photophobia is eliminated (eye drops)
- Effect on muscle and brain disease remains to be seen

How would HSCT gene therapy work?



Mechanism of in vivo lysosomal cross-correction via tunneling nanotubes. Transplanted Ctns$^{-/-}$ HSPCs ex vivo transduced with SIN-LV carrying CTNS-GFP repopulate the bone marrow of Ctns$^{-/-}$ mice, migrate into the kidney where they differentiate into macrophages. Affected proximal tubular cells (PTCs) are protected from the extracellular environment by the tubular basement membrane (TBM). The rescue of PTCs requires that macrophages extend tunneling nanotubes (TNTs) crossing the TBM to deliver functional cystinosin-bearing lysosomes and may be take away the endogenous cystine-loaded lysosomes (never shown in vivo) from the PTCs, accounting for the long-term recue of the proximal tubules in Ctns$^{-/-}$ treated by hematopoietic stem and progenitor cell (HSPC) transplantation (Pediatr Neprhol 34:965-973, 2019)

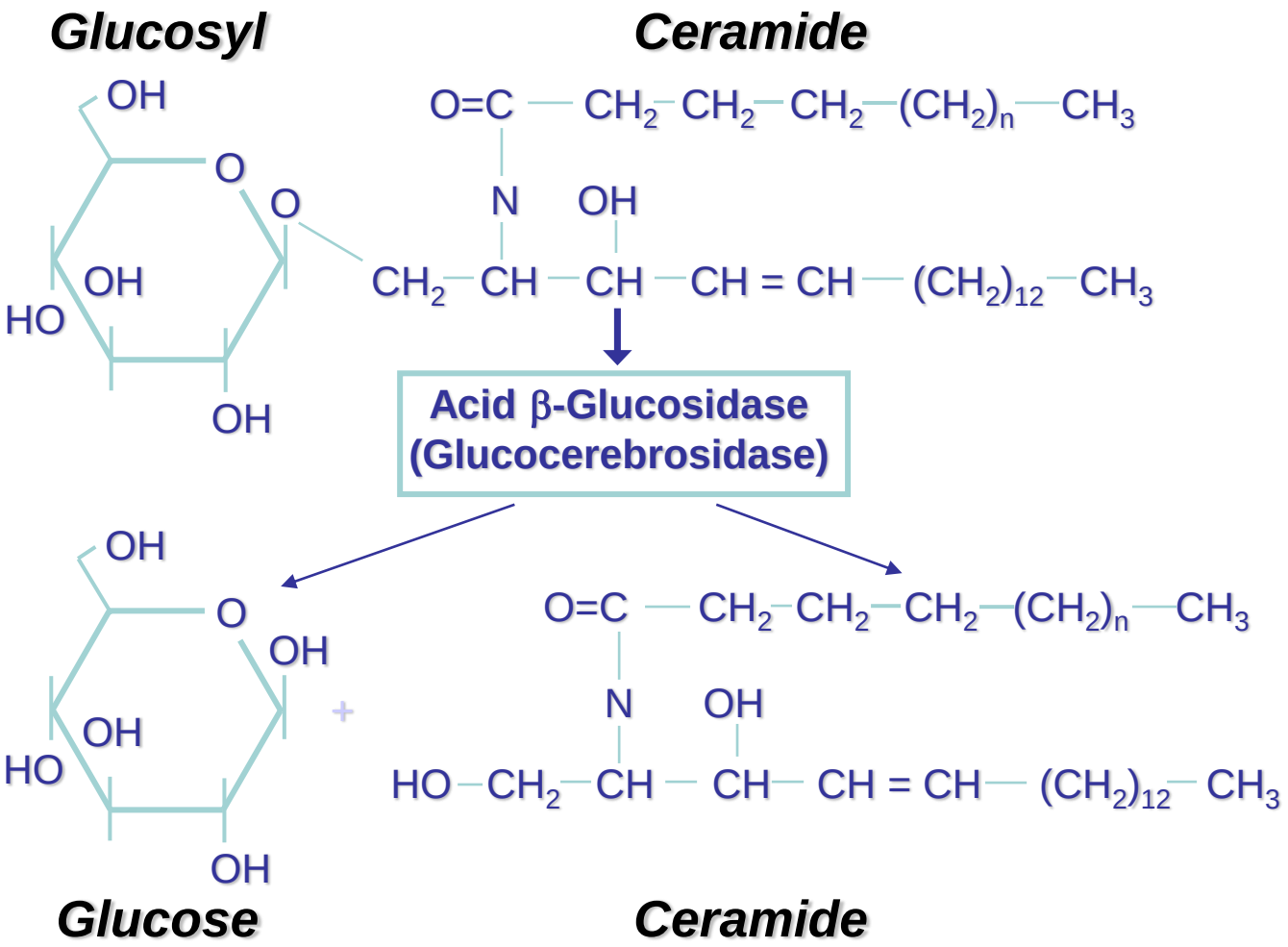
Gaucher Disease

- Glucocerebrosidase deficiency
- Storage primarily in reticuloendothelial system
- Diagnosis: enzyme assay, molecular
- Disease Spectrum
  - perinatal lethal (null)
  - infantile neuronopathic (type II)
  - later neuropathic (type III)
  - non-neuronopathic (type I), can be asymptomatic
- Biomarkers- chitotriosidase (~8% null), ACE, TRAP, lysoGL1 (glucosylsphingosine)

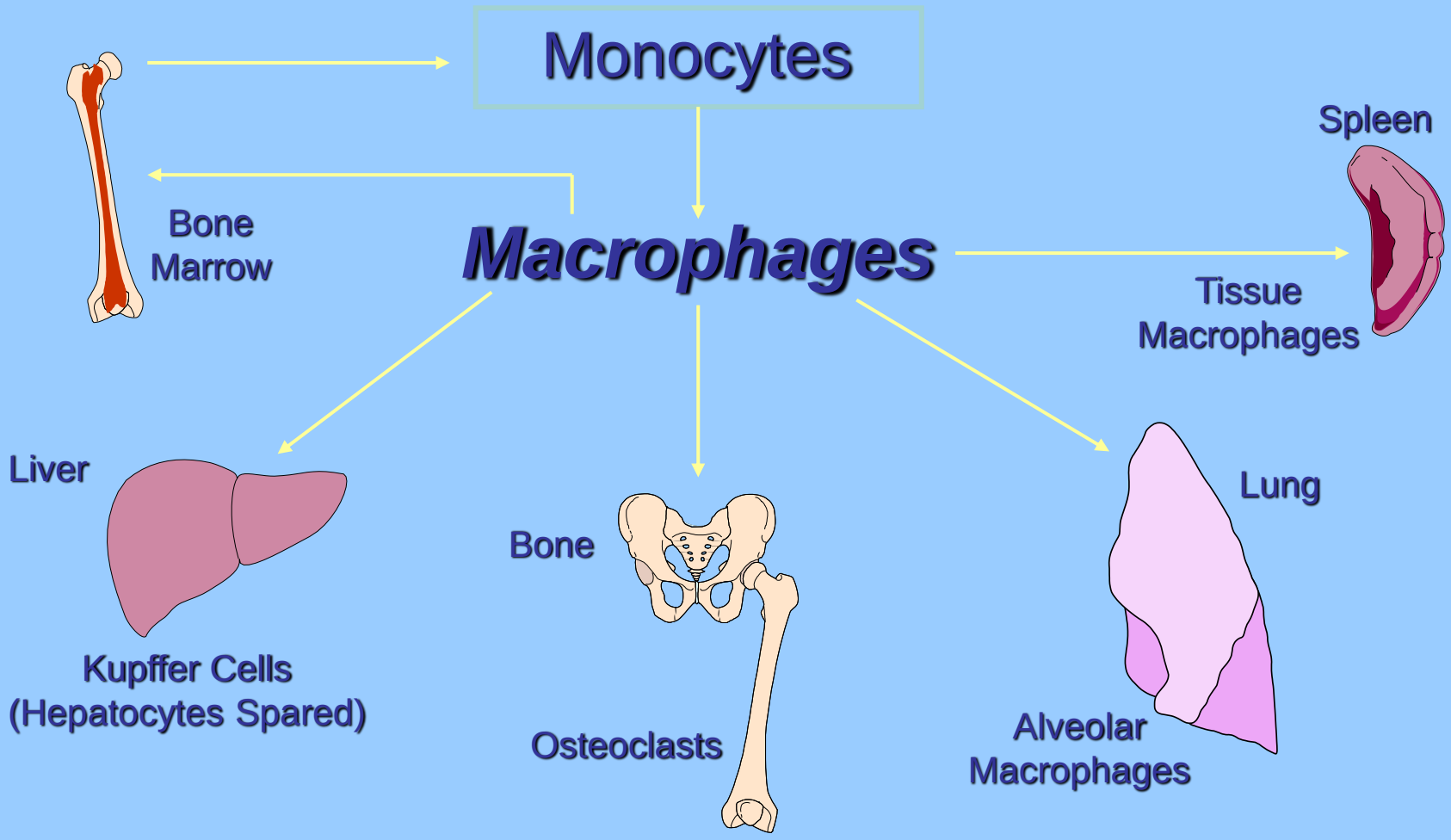
Gaucher Disease Type I

- Incidence
  - General population: 1/40,000
  - Ashkenazi Jewish 1/600 (common mutation p.N370S mildest)
- Onset of clinical symptoms: birth to never
- Common clinical manifestations
  - Splenomegaly +/- hepatomegaly
  - Thrombocytopenia, anemia
  - Osteopenia, osteolysis, bone pain, pathologic fractures
  - Increased risk of Parkinson, MGUS and multiple myeloma

The Enzymatic Defect in Gaucher Disease



The Pathophysiology of Gaucher Disease



Gaucher Cell

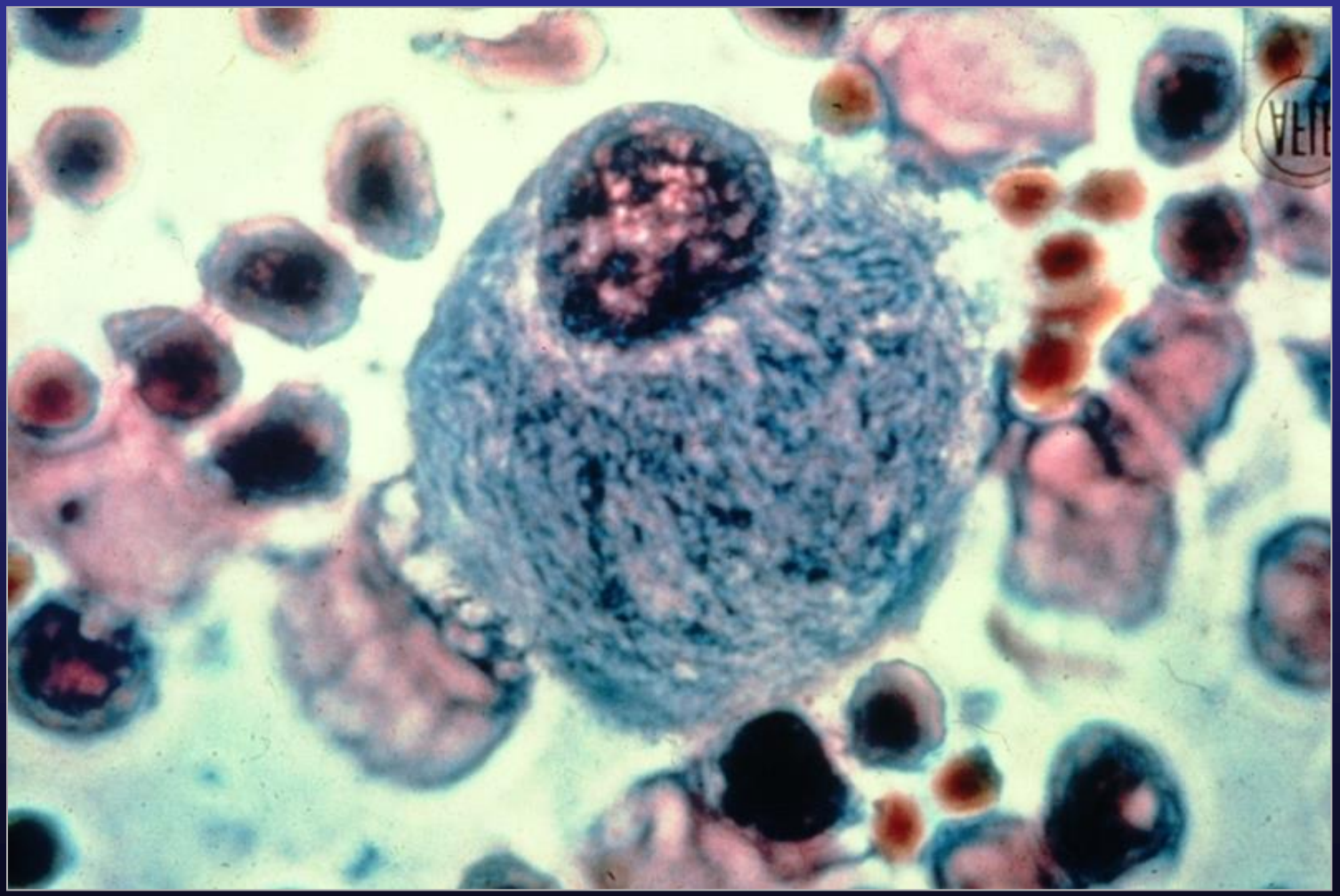
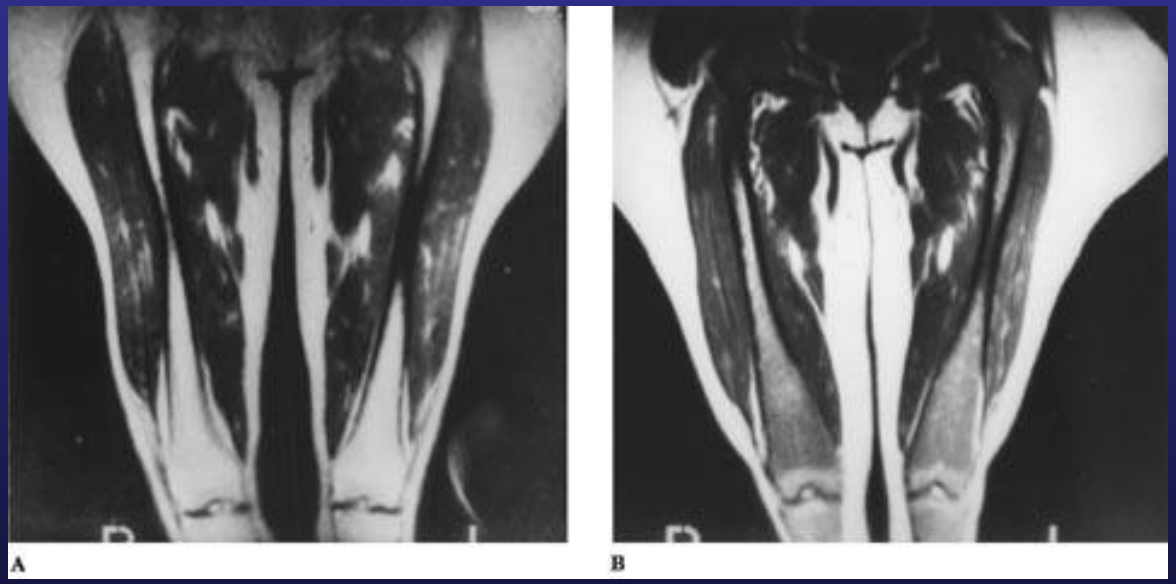
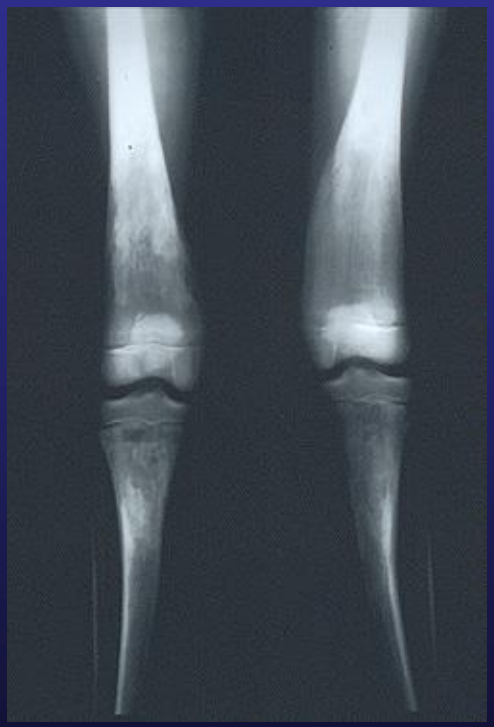


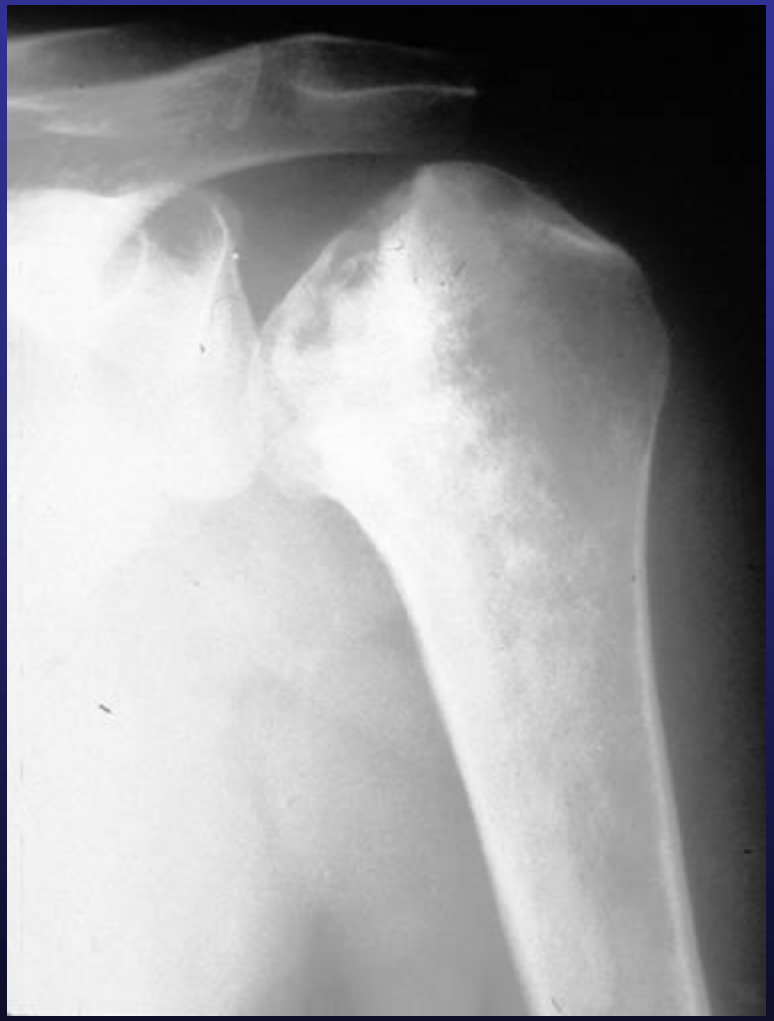
Photo: Property of Genzyme

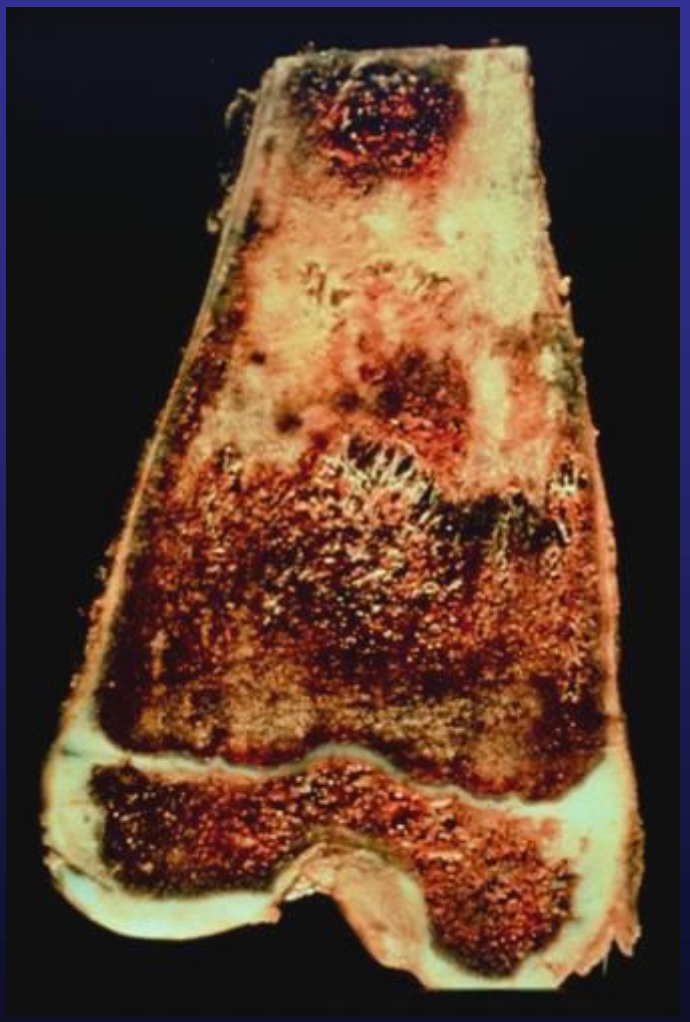
Gaucher Bone Disease



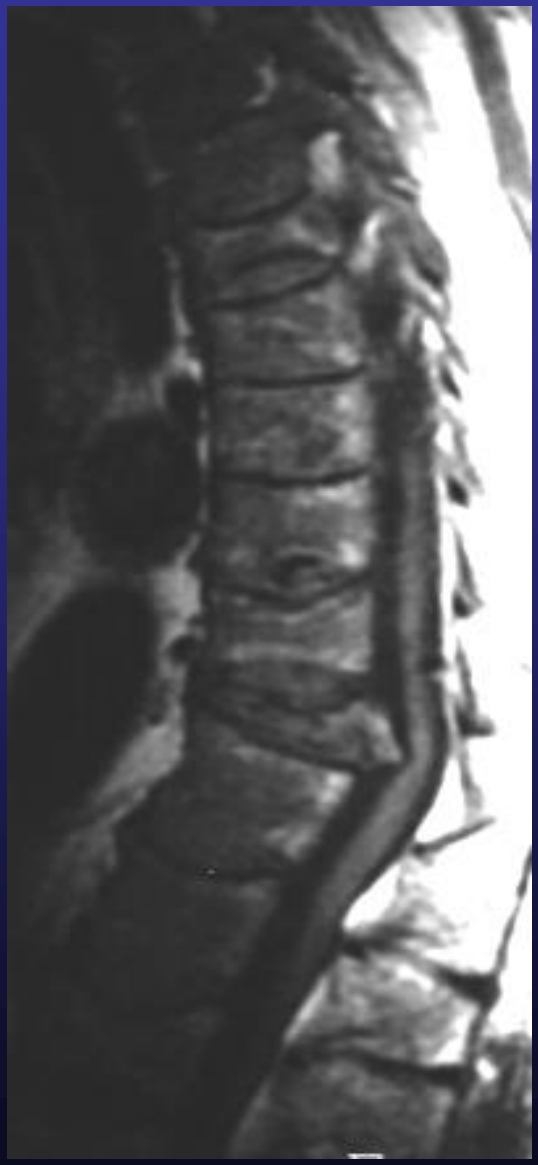


Osteonecrosis

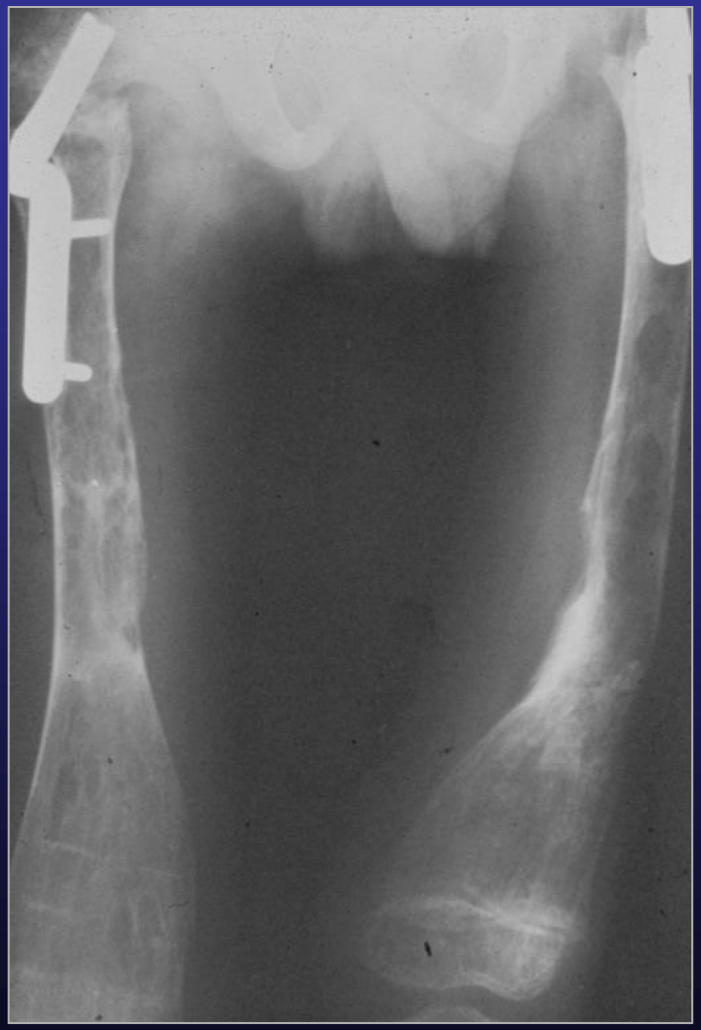




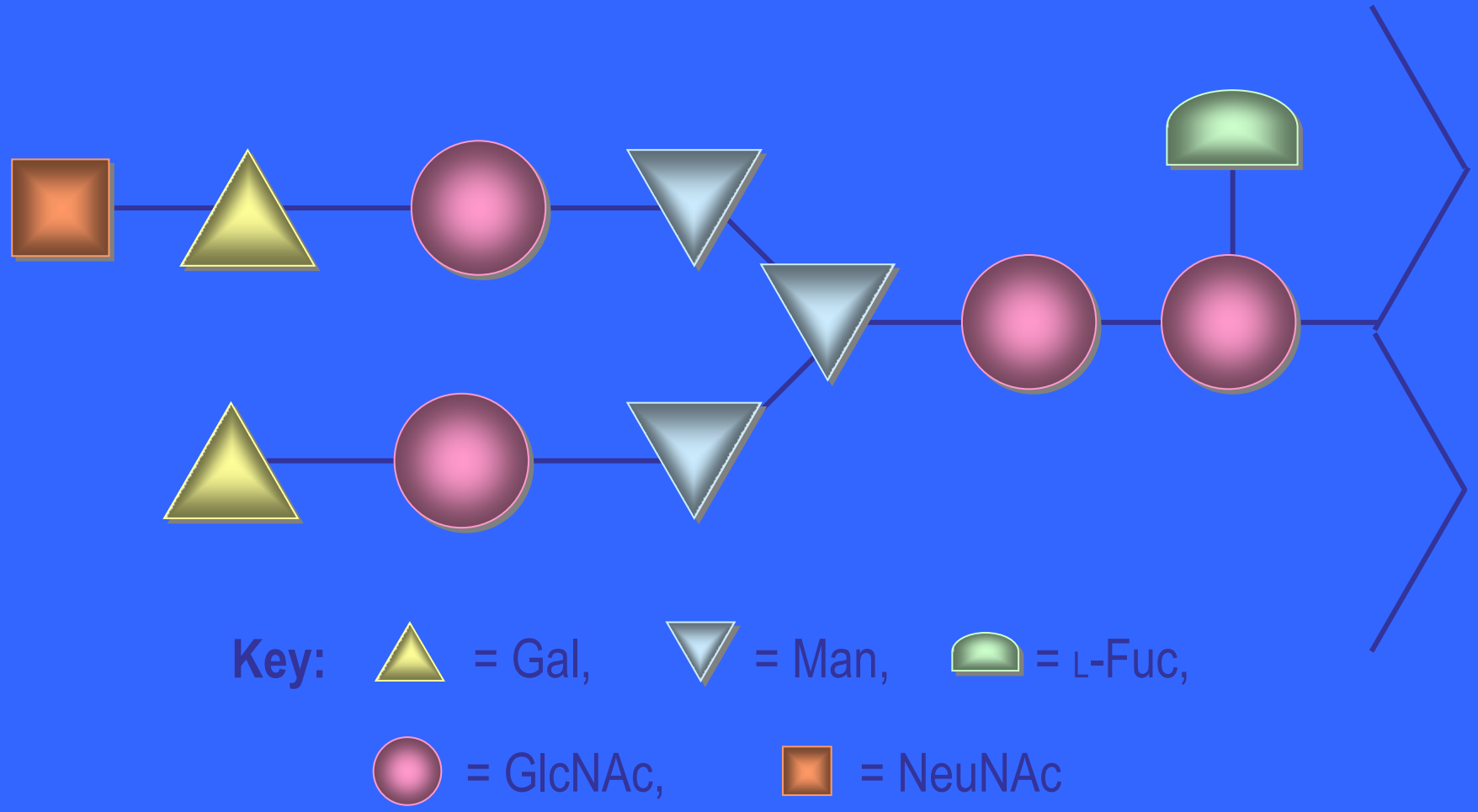
Osteopenia, infarctions, vertebral collapse



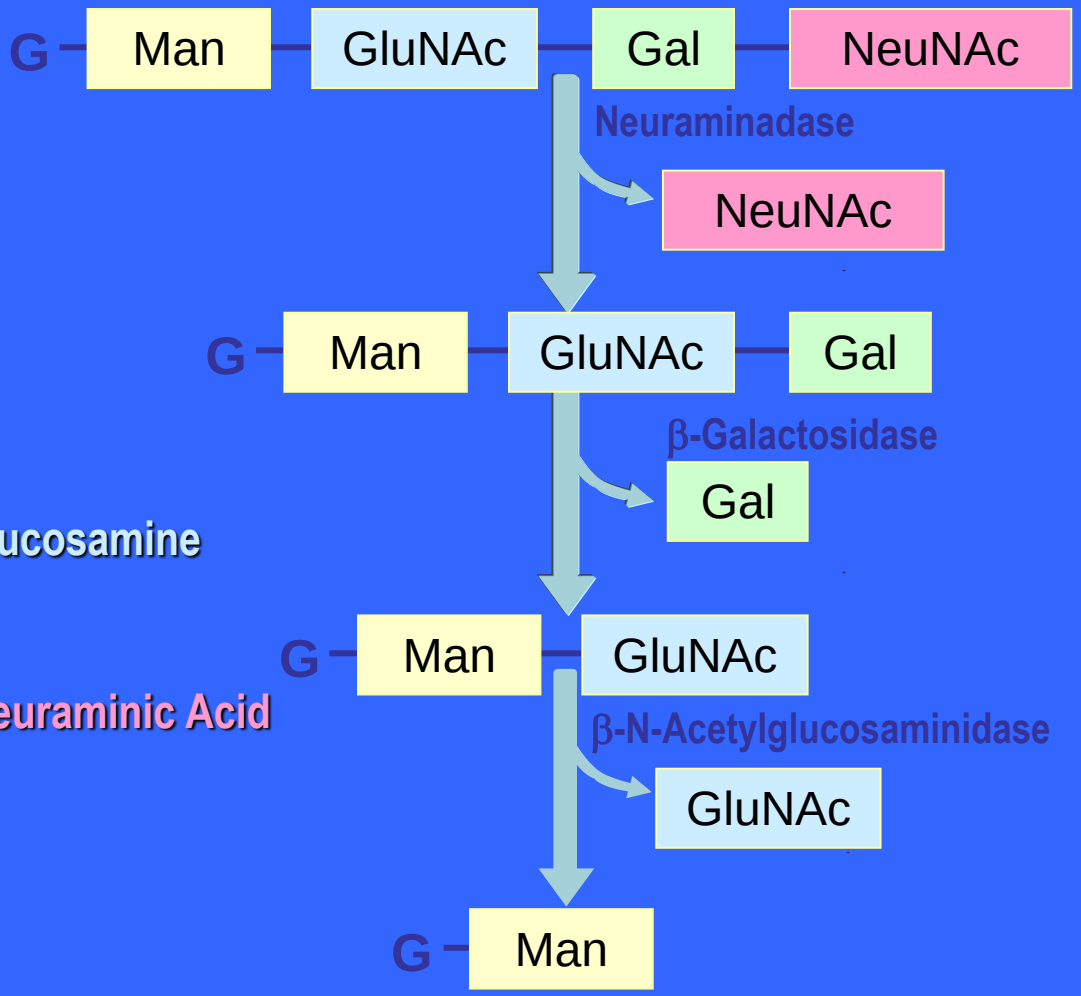
Pathologic Fractures



Carbohydrate Unit of Native Glucocerebrosidase



Enzymatic Modification of Glucocerebrosidase (G)



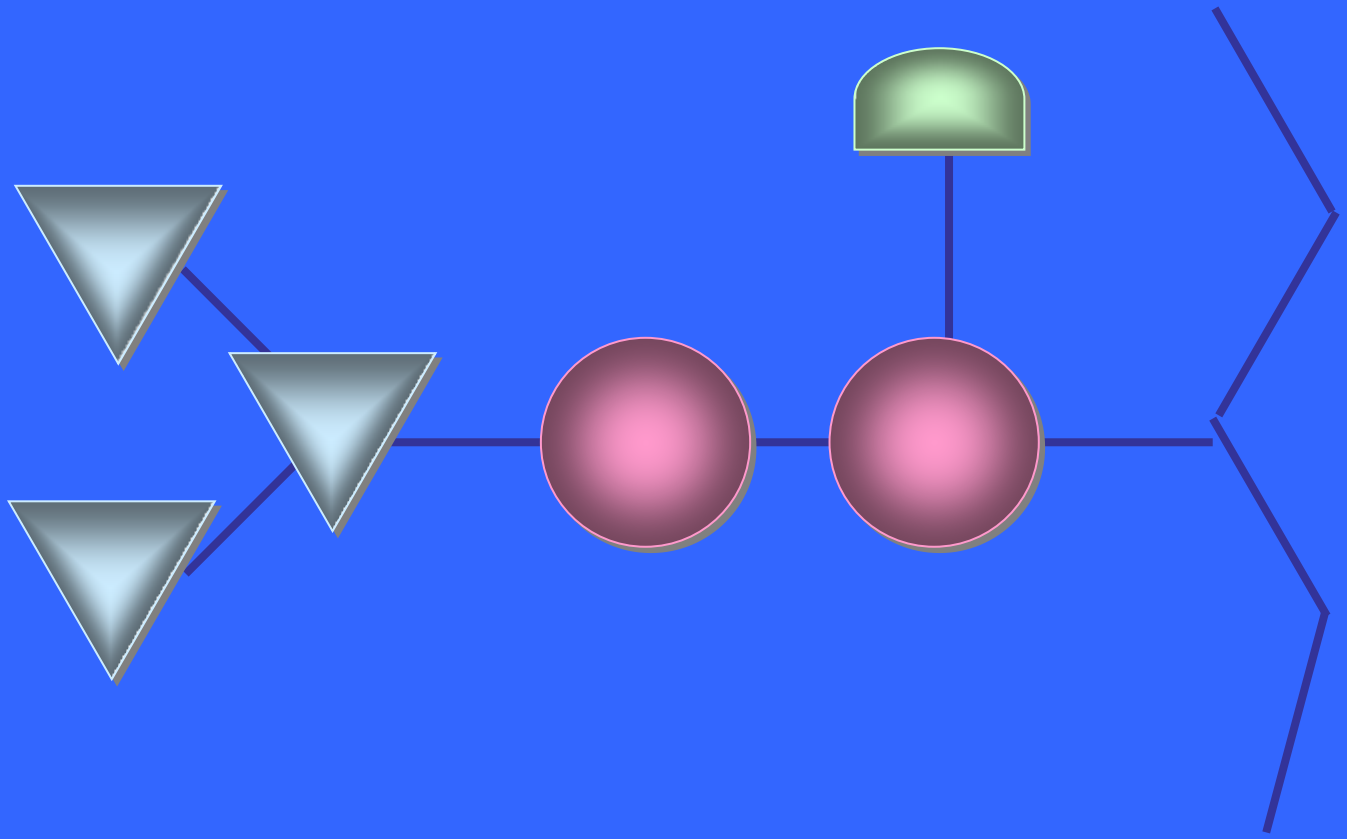
Man = Mannose

GluNAc = N-Acetylglucosamine

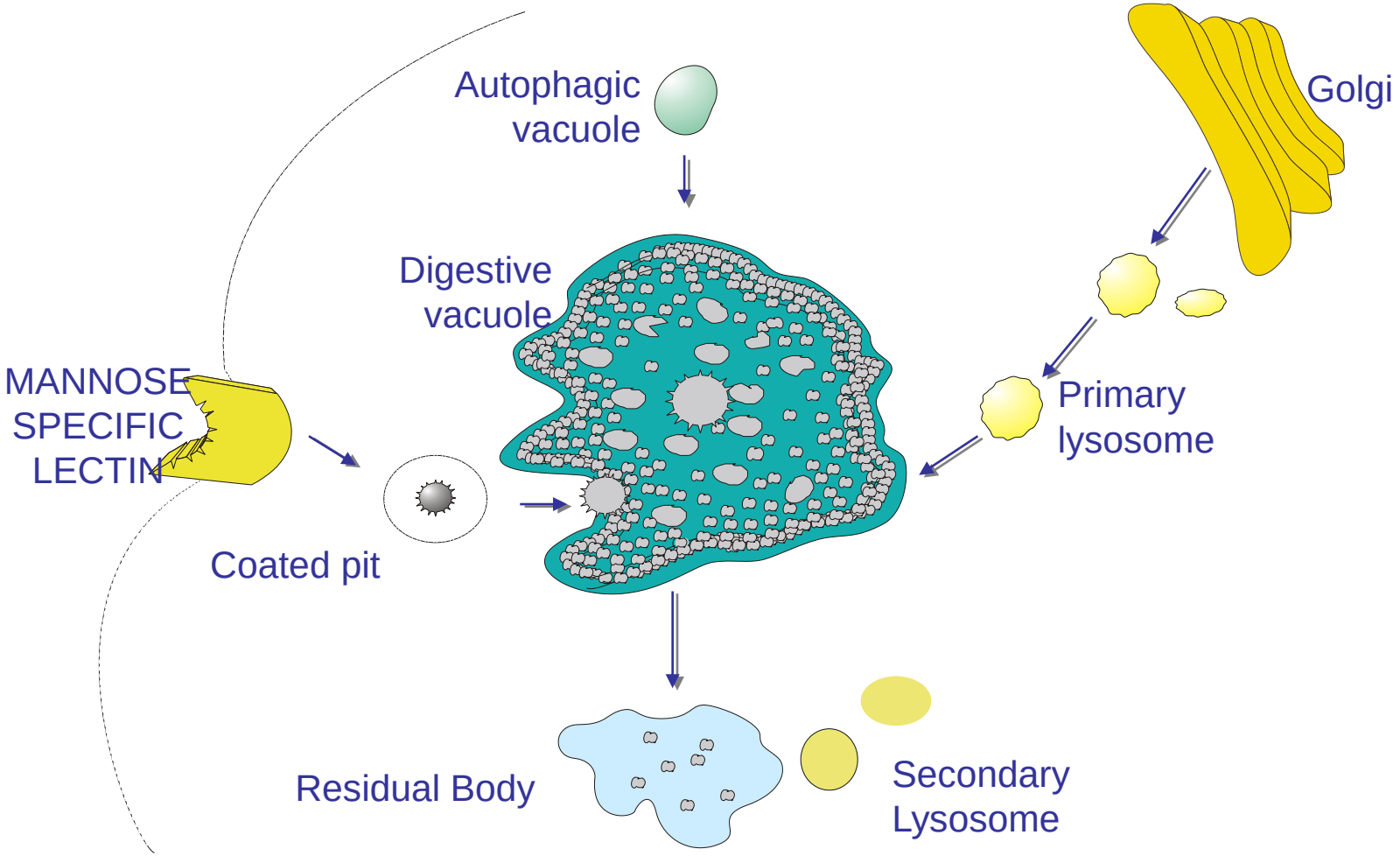
Gal = Galactose

NeuNAc = Nacetyl Neuraminic Acid

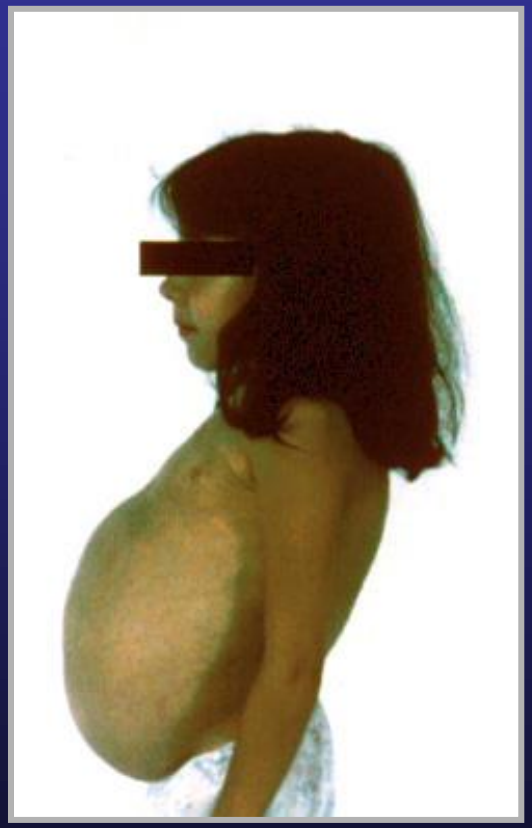
Carbohydrate Unit of Mannose Terminated Glucocerebrosidase



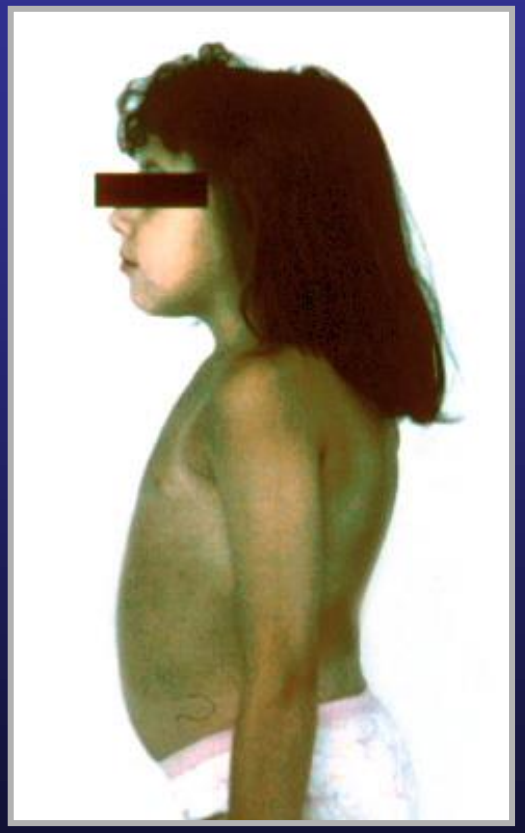
Key: Man, GlcNAc, L-Fuc,


Autophagic vacuole
Golgi
Digestive vacuole
MANNOSE SPECIFIC LECTIN
Primary lysosome
Coated pit
Residual Body
Secondary Lysosome

Patient Response to Enzyme Therapy



Pretreatment
Female; Age 8 Years, 8 Months

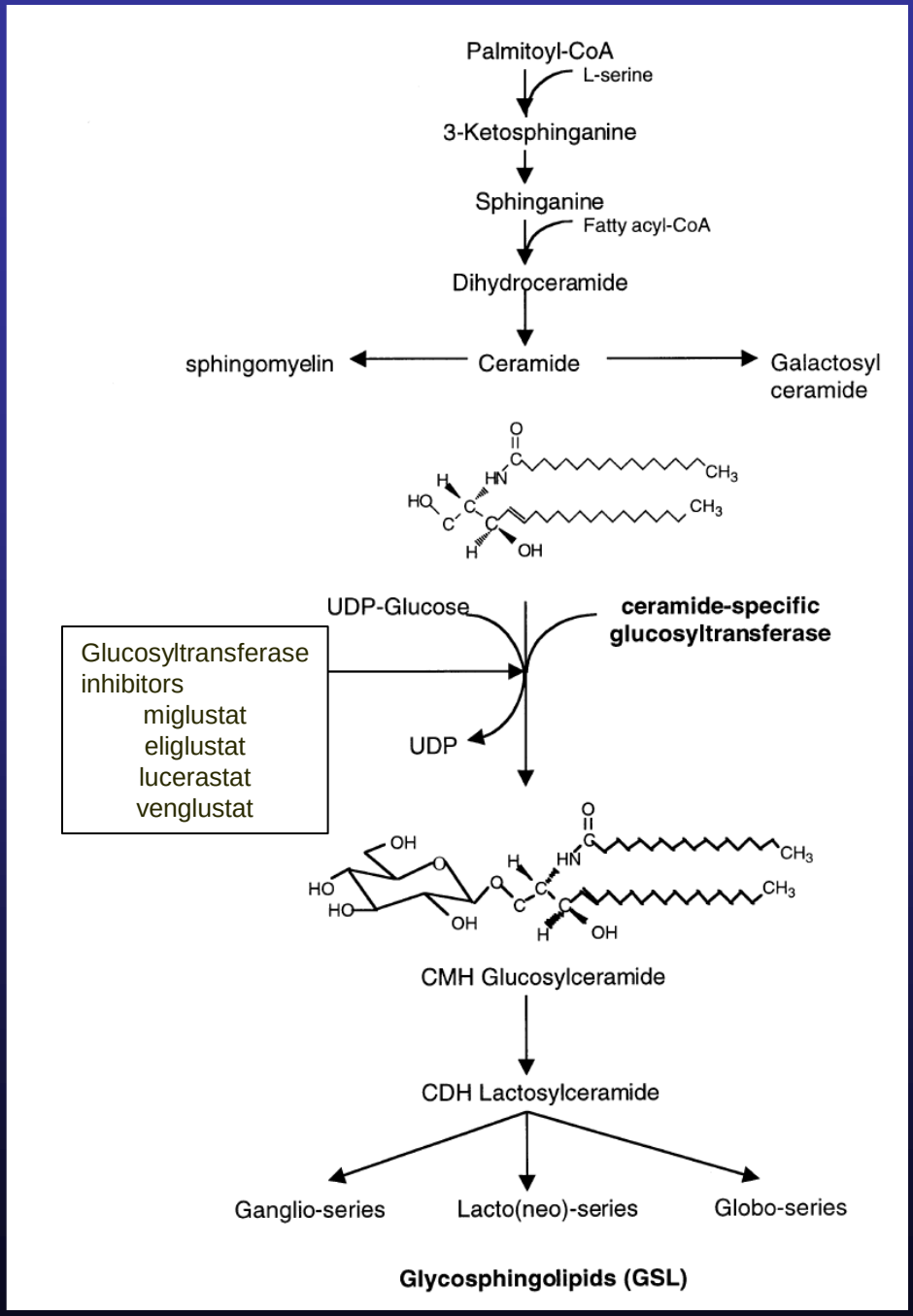


Post-treatment
Female; Age 10 Years, 10 Months

Courtesy of NW Barton. Developmental and Metabolic Neurology Branch of the NINDS.

Substrate Inhibition

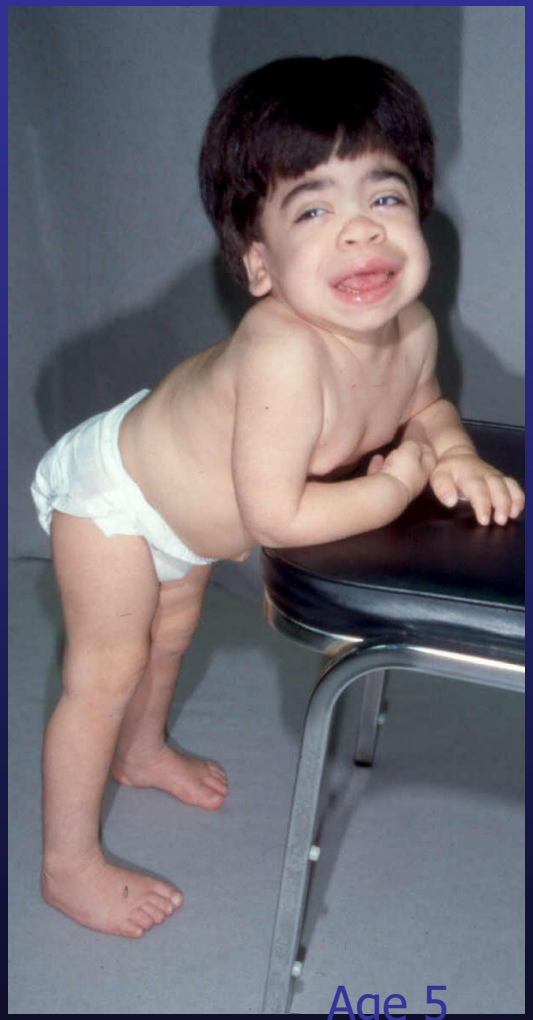
- About as efficacious long-term as ERT
- Miglustat has problems with diarrhea and neuropathy, eliglustat can interfere with the metabolism of other drugs
- Long-term side effects unknown
- Inadvisable during pregnancy



Platt et al, JIMD 24:275, 2001

Mucopolysaccharidosis I (MPS I)

- Deficiency of lysosomal enzyme α-L-iduronidase
- Diagnosed by enzyme assay, suspected by urine MPS analysis
- On the NBS RUSP
- Progressive accumulation of dermatan and heparan sulfate
- Rare (est. incidence 1:100,000)
- Spectrum- Hurler, Hurler-Scheie, Scheie (or classic and attenuated forms)



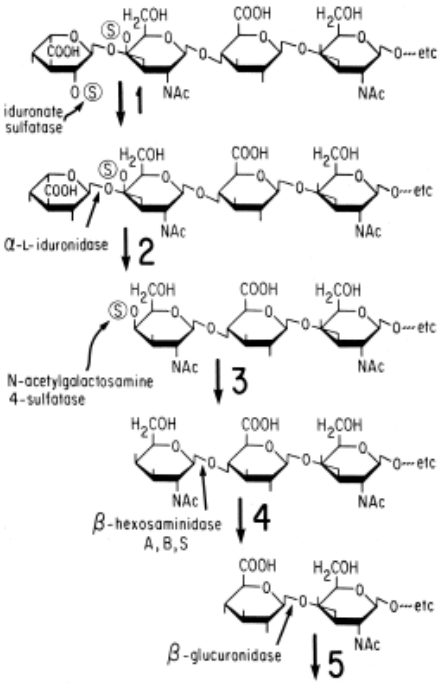


Fig. 136-2 Stepwise degradation of dermatan sulfate. The deficiency diseases corresponding to the numbered reactions are: 1 = MPS II, Hunter syndrome; 2 = MPS I, Hurler, Hurler-Scheie, and Scheie syndromes; 3 = MPS VI, Maroteaux-Lamy syndrome; 4 = Sandhoff disease for β-hexosaminidase A and B; there are knockout mice in which all three isoenzymes are deficient, but no comparable disease in humans; 5 = MPS VII, Sly syndrome. This drawing depicts all structures known to occur within dermatan sulfate, and does not imply that they occur in equal proportion. For instance, L-iduronic acid occurs much more frequently than glucuronic acid and only a few of the α-L-iduronic residues are sulfated.

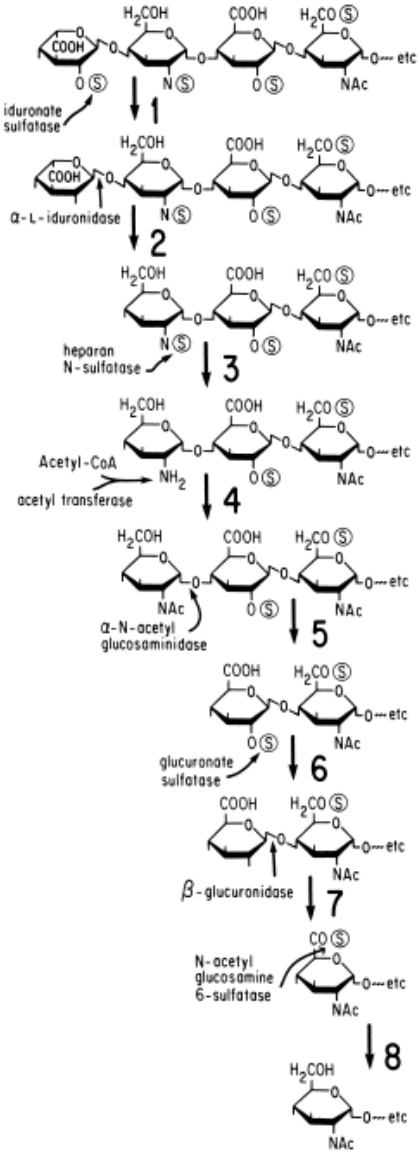


Fig. 136-3 Stepwise degradation of heparan sulfate. The deficiency diseases corresponding to the numbered reactions are: 1 = MPS II, Hunter syndrome; 2 = MPS I, Hurler, Hurler-Scheie, and Scheie syndromes; 3 = MPS III A, Sanfilippo syndrome type A; 4 = MPS III C, Sanfilippo syndrome type C; 5 = MPS III B, Sanfilippo syndrome type B; 6 = no deficiency disease yet known; 7 = MPS VII, Sly syndrome; 8 = MPS III D, Sanfilippo syndrome type D. The drawing depicts all structures known to occur within heparan sulfate, and does not imply that they occur stoichiometrically. For example, very few of the glucuronic acid residues are sulfated.

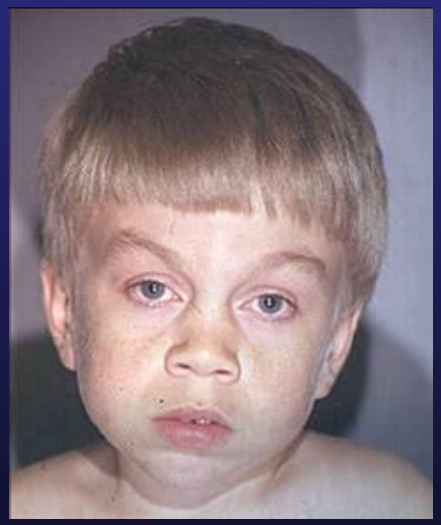
MMBID

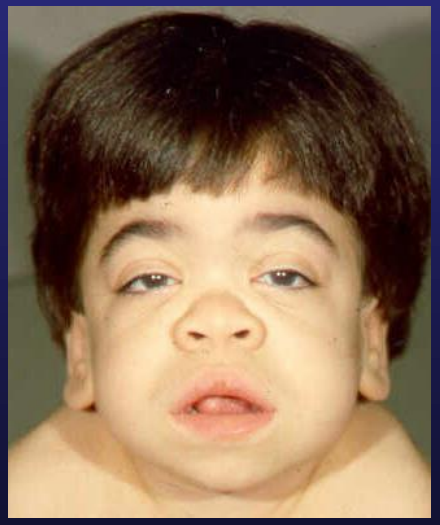
MPS I

Clinical Heterogeneity

Attenuated ⟷ Severe

"Scheie" MPS I S	"Hurler-Scheie" MPS I HS	"Hurler" MPS I H





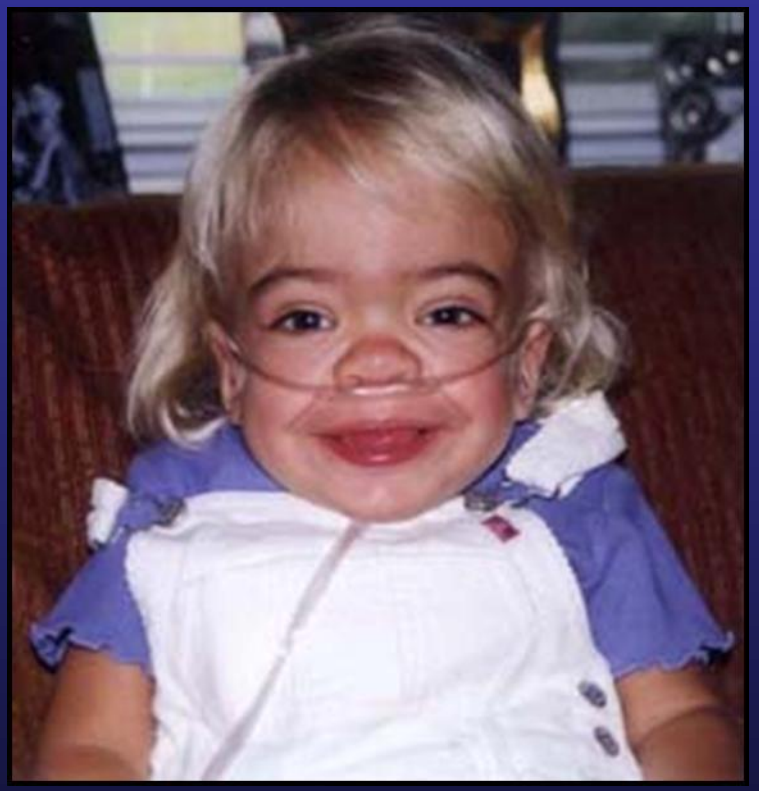
Courtesy of Emil Kakkis, MD.

All patients typically have < 1% of normal enzyme levels, but only MPS I H involves the CNS

Disease Progression

10 months

12 months



39 months

22 months

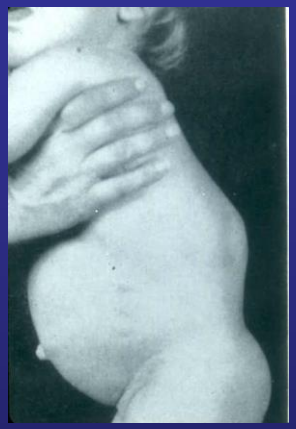
34 months

Photos courtesy of the MPS Society.

Patient with severe MPS I

MPS I

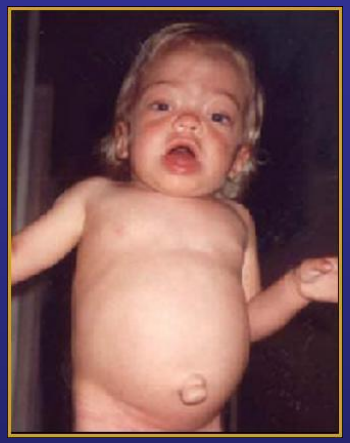
Signs & Symptoms



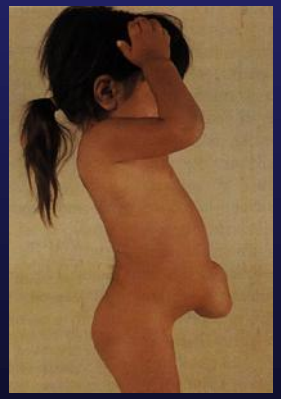
Skeletal deformities (Gibbus)[1]

Carpal tunnel syndrome[2]

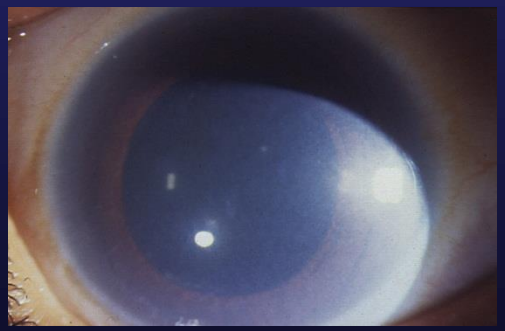
Short stature[2]



Hepatosplenomegaly[2]



Umbilical/ inguinal hernia[3]



Corneal clouding[3]

1. Courtesy of Emil Kakkis, MD.
2. Courtesy of MPS Society.
3. Nyhan and Ozand, 1998. Photo reproduced by permission of Hodder/Arnold Publishers.

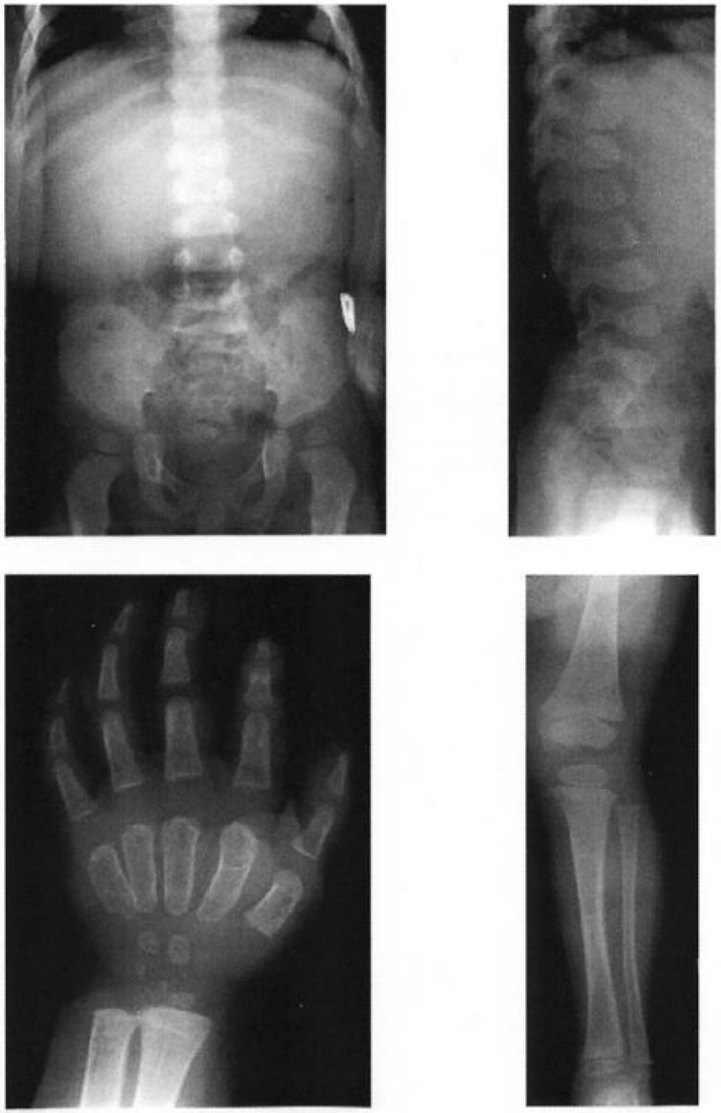
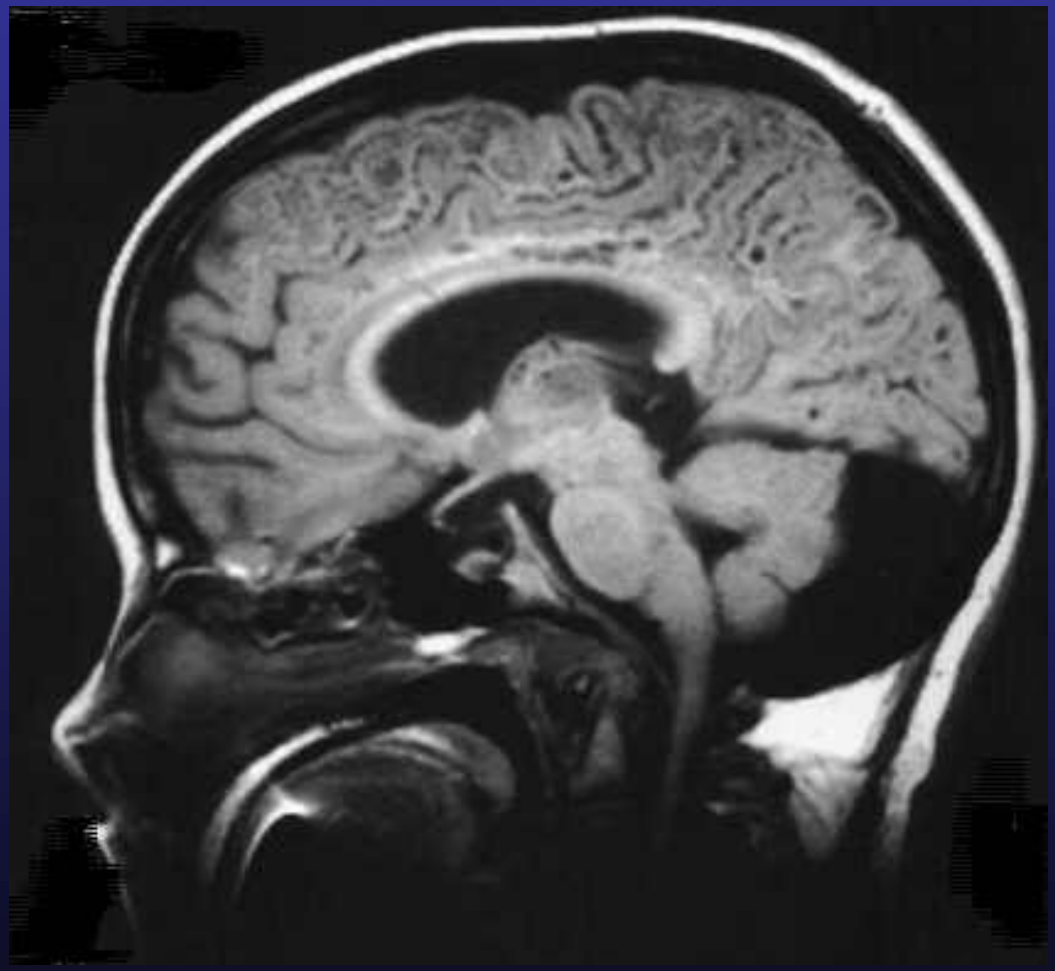
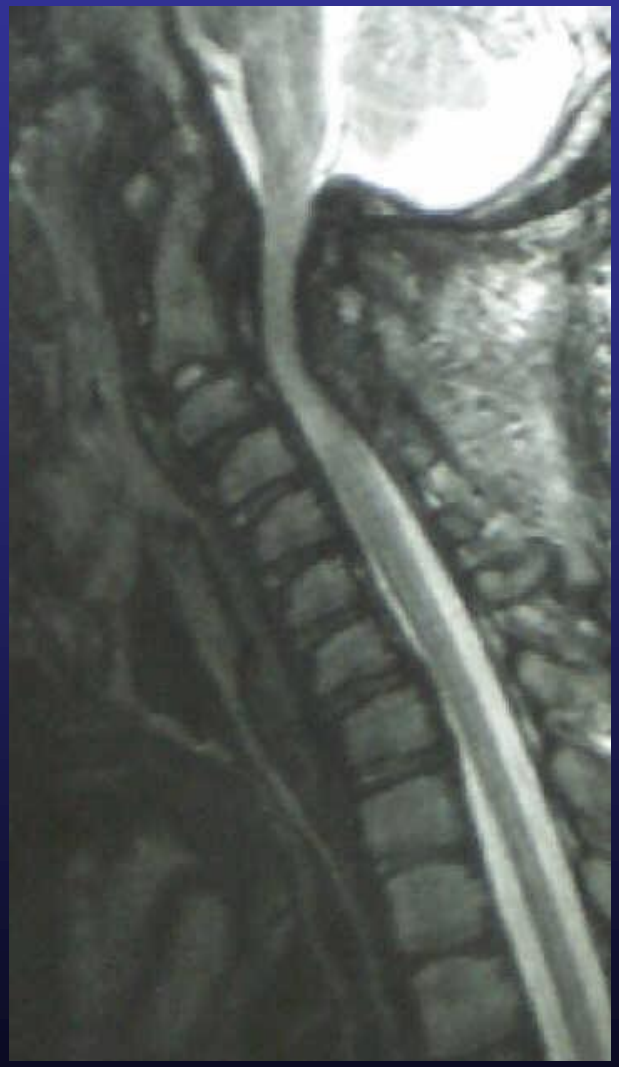


Fig 3. Skeletal abnormalities (dysostosis multiplex). Dysostosis multiplex in a 3-year-old child with severe MPS I (Hurler). Note the spatulate oar-shaped ribs, coxa valga, vertebral body rounding, irregular metaphyses, wide diaphyses and small epiphyses, and wide, bullet-shaped metacarpals and phalanges with proximal pointing of the metacarpals. Printed with permission from Hodder Arnold.

J Peds 144:S3-S14, 2004

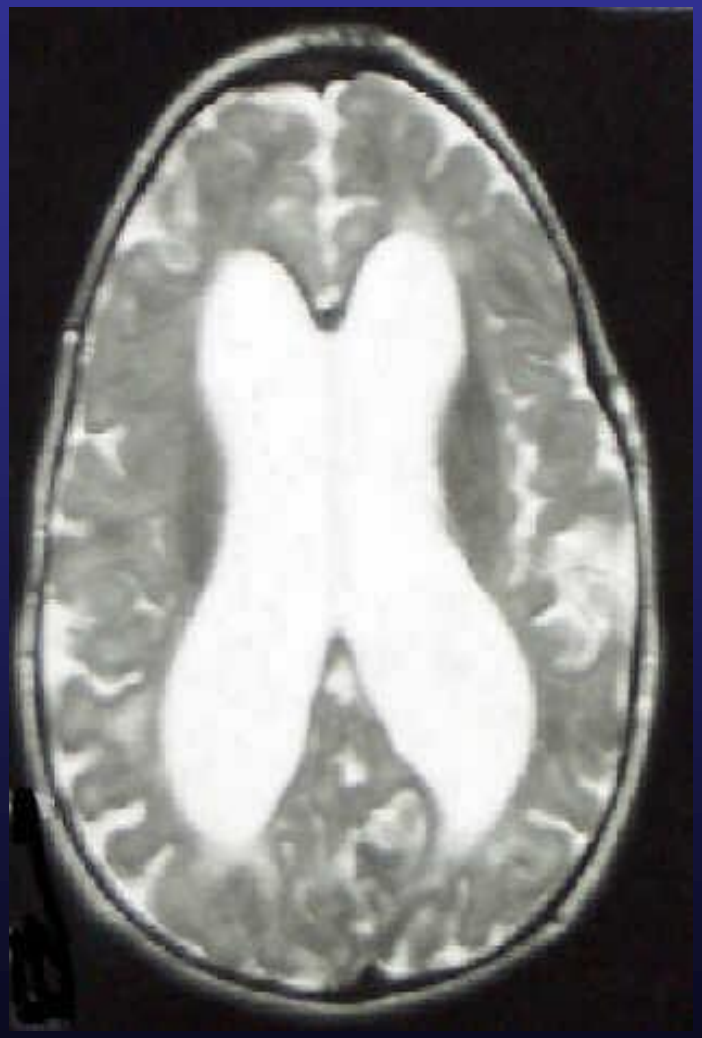
CNS Disease in MPS I





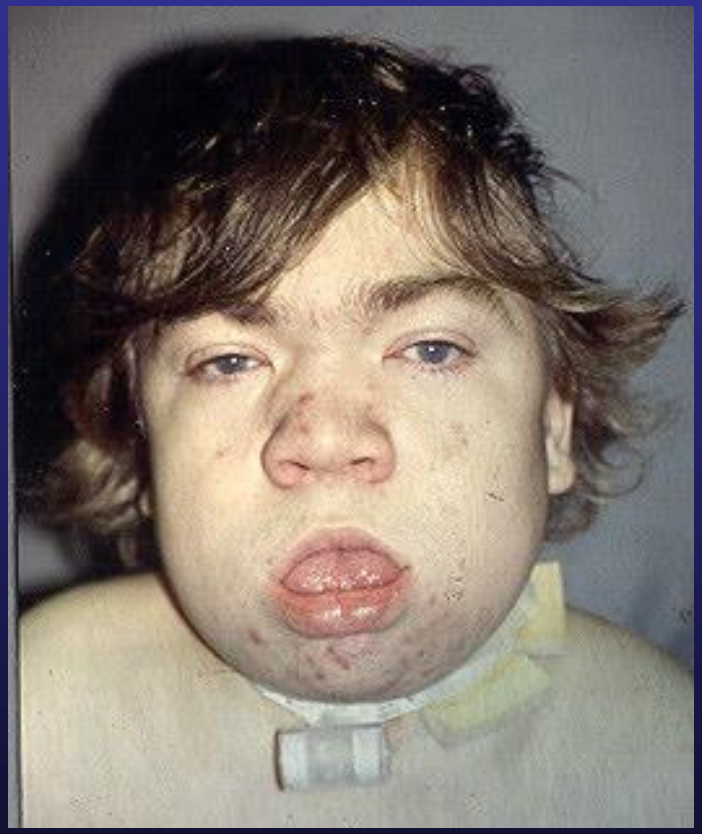
With Permission, Biomarin

Hydrocephalus in MPS I



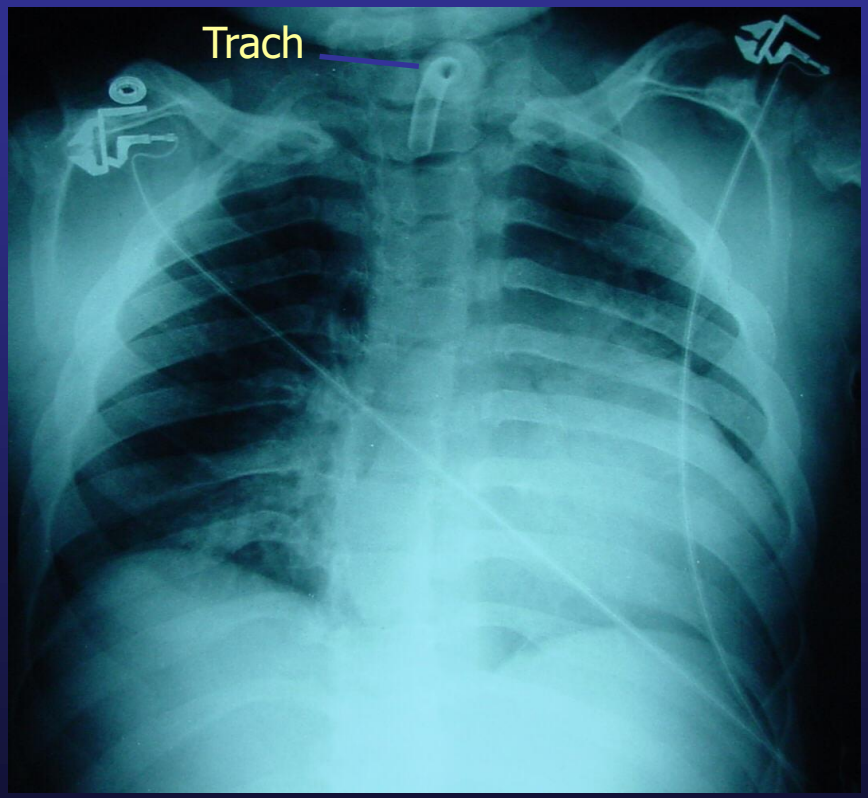
With Permission, Biomarin

Upper Airway Obstruction



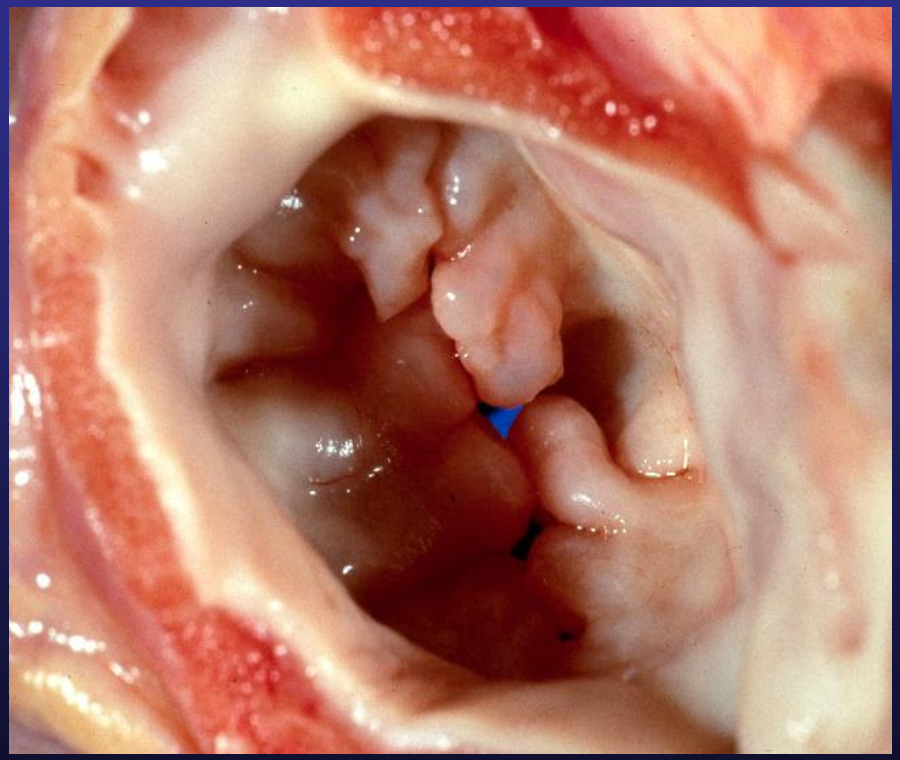
17 year old Hurler-Scheie patient

Pulmonary Disease

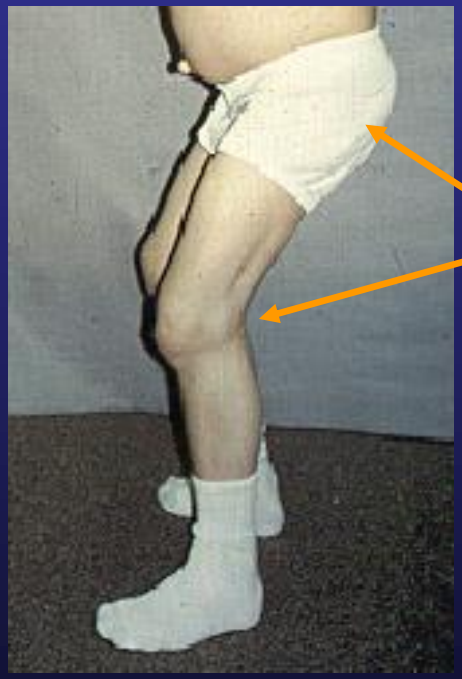


Abnormal oar-shaped ribs,
curved clavicles and scoliosis

Cardiac Disease in MPS I

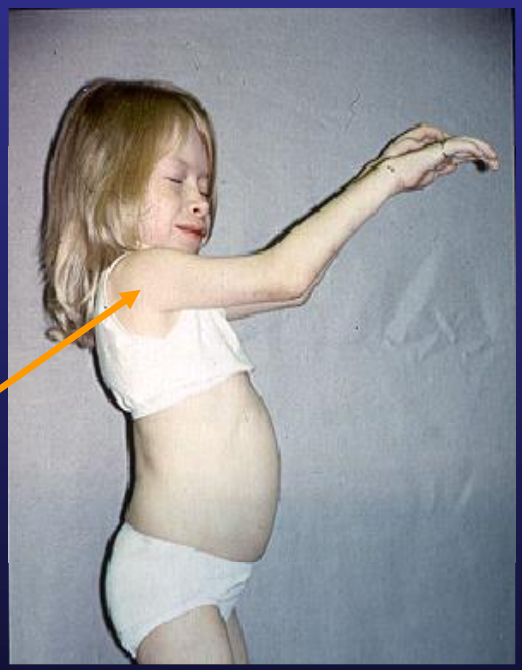


Joint Restriction and Stiffness



Hip and Knee restriction and contractures

Shoulder restriction and contractures



Age 17

Age 12

MPS I Treatment

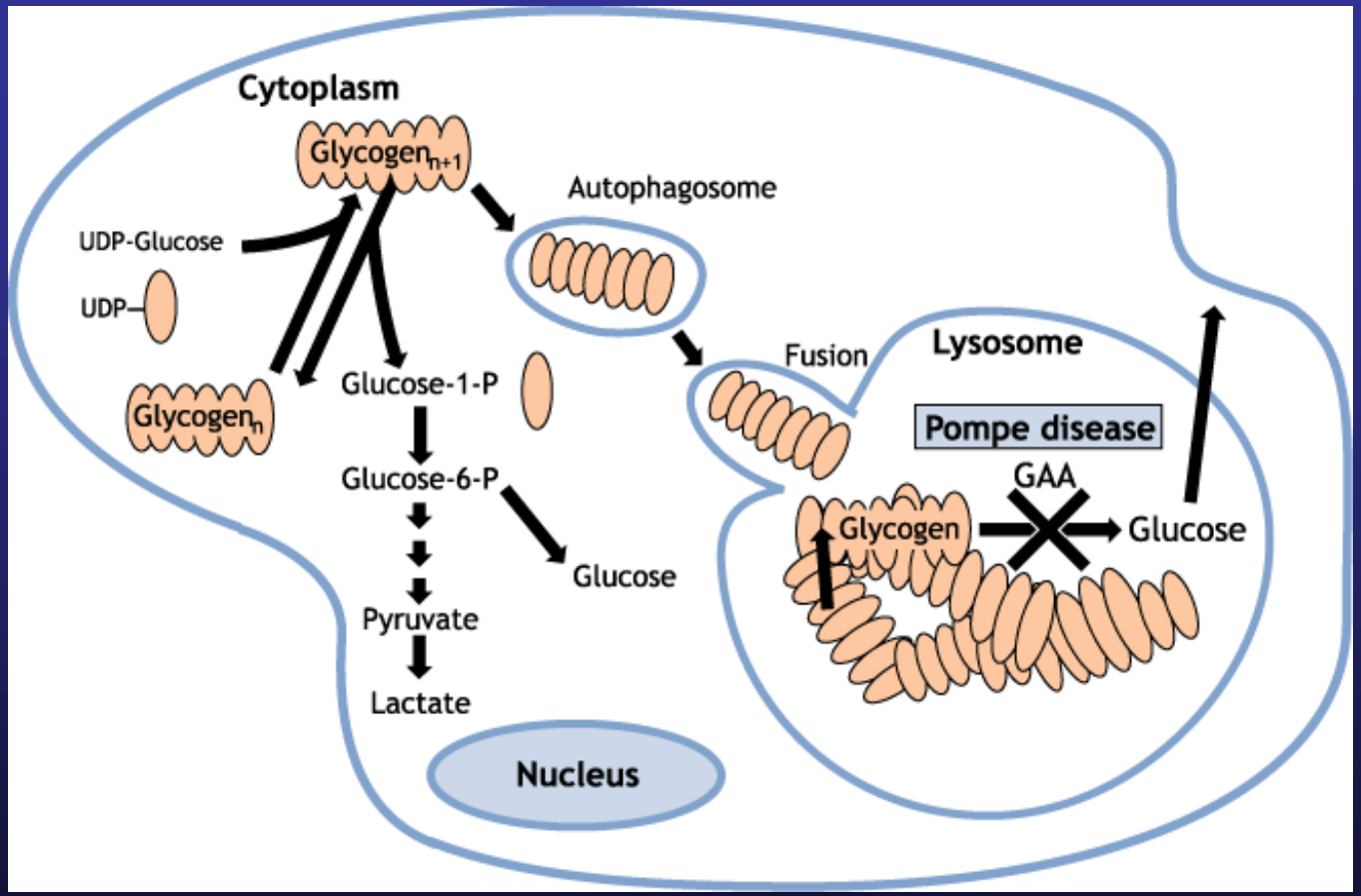
- ERT
  - Improves mobility, organomegaly, airway, ?prevents cardiac valve damage
  - Doesn't help CNS, skeleton (maybe if started very early), cornea, carpal tunnel
- HSCT
  - Doesn't help skeleton, cornea, carpal tunnel
  - Needs to be done early for the CNS
  - Associated morbidity and mortality
- Future-
  - *ex vivo* gene therapy autologous HSCT
  - CNS gene therapy
  - Fusion protein to use a transporter to enter the CNS

Salvador Dalí, “Desoxyribonucleic Acid Arabs”

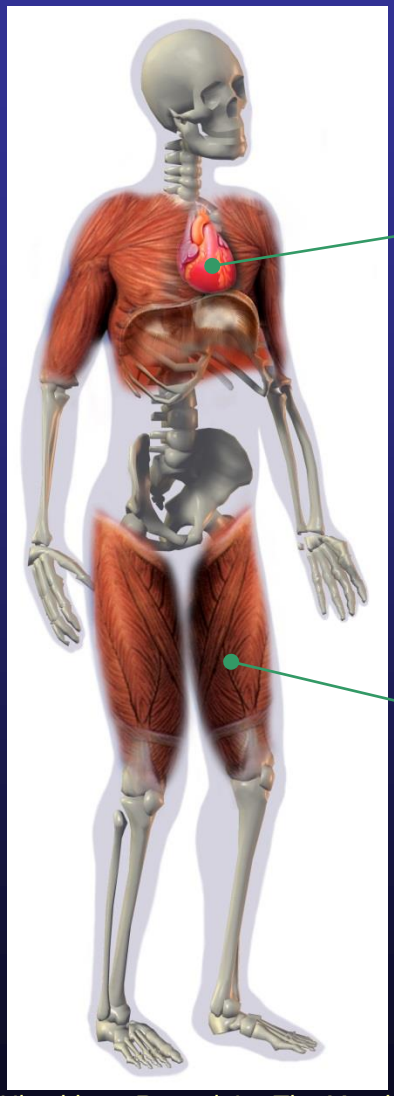
Pompe Disease (Glycogenosis type II)

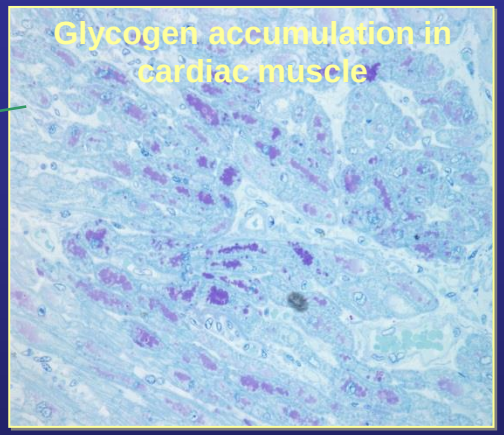
- Deficiency of lysosomal acid alpha-glucosidase (GAA)
- Diagnosis: enzyme assay followed by molecular
- On the NBS RUSP
- Progressive muscle weakness, respiratory failure
- Cardiomyopathy in infantile cases
- Biomarkers- CK, urine Hex4
- Treated with ERT (two approved)

Pompe Disease Pathogenesis

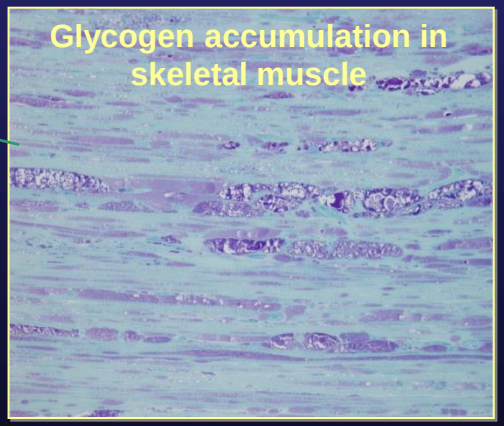


Raben N, et al. *Curr Mol Med.* 2002;2:145-166.





Data on file, Genzyme Corporation.

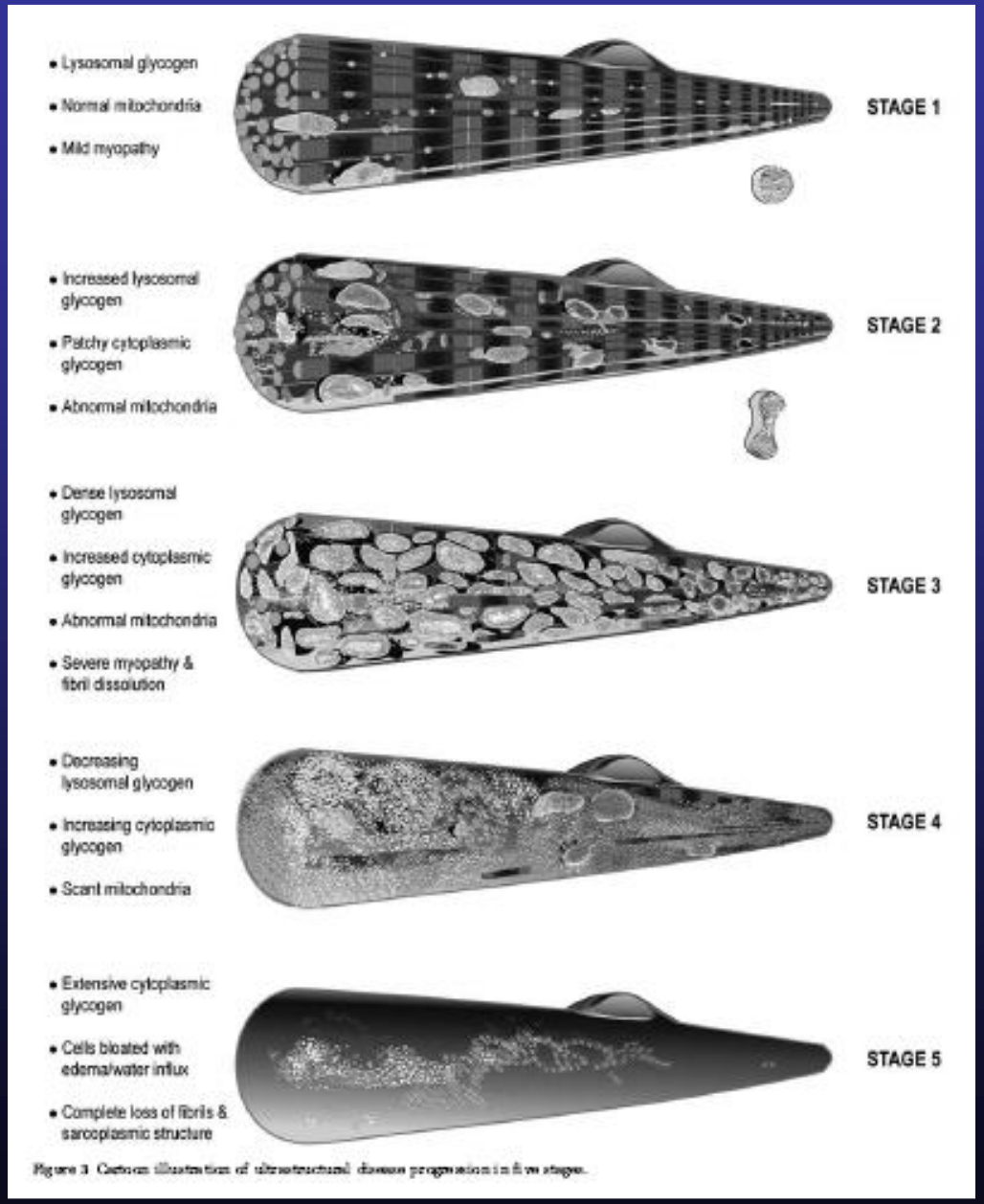


Data on file, Genzyme Corporation.

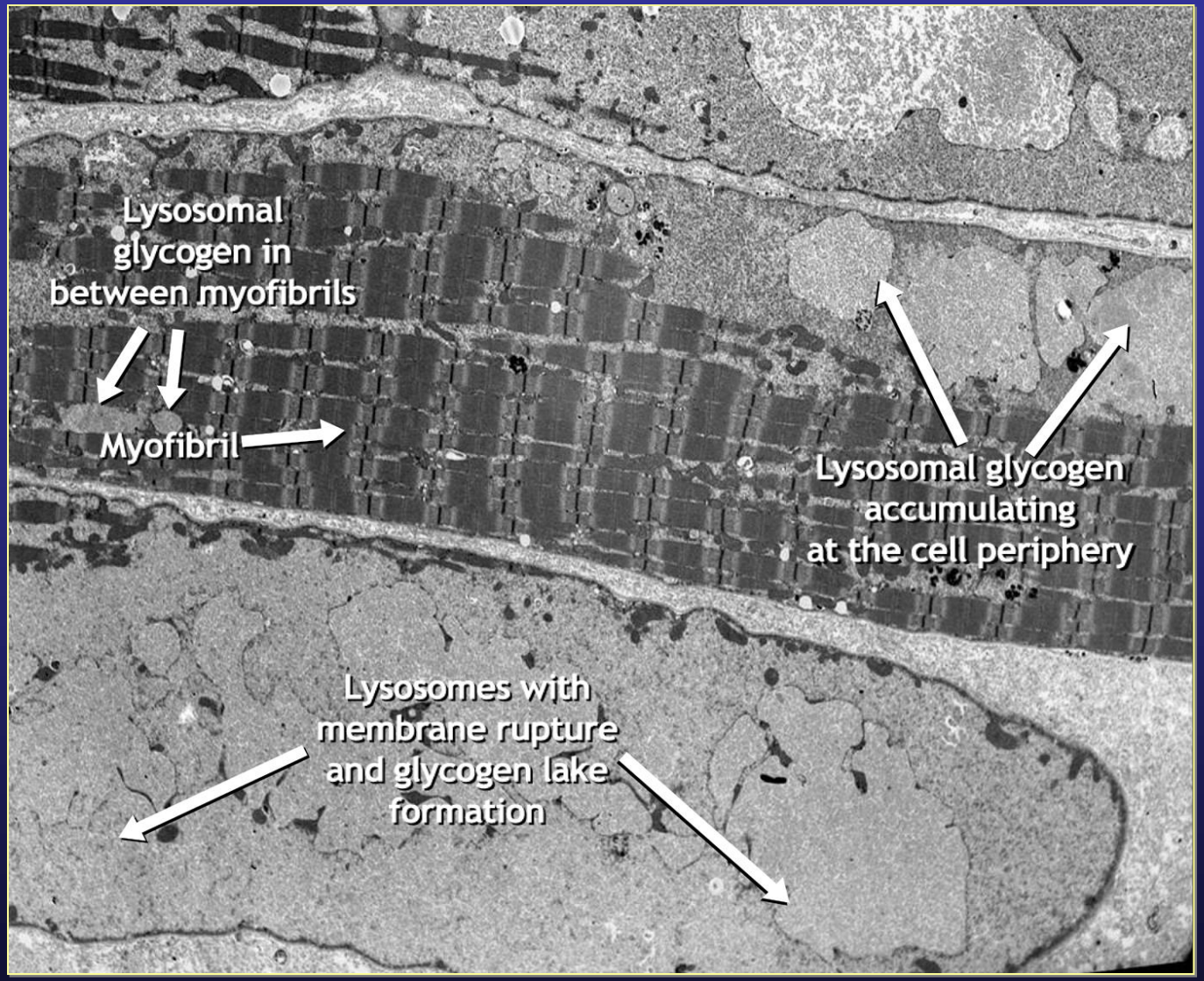
- Glycogen storage primarily affects muscle tissue
- Major muscle groups
  - Cardiac muscle (infantile)
  - Proximal skeletal muscle (esp. in trunk and lower limbs)
  - Respiratory muscles

Hirschhorn R, et al. In: *The Metabolic and Molecular Bases of Inherited Disease*. 2001:3389-3420.

Pompe Disease- Progression of Muscle Damage

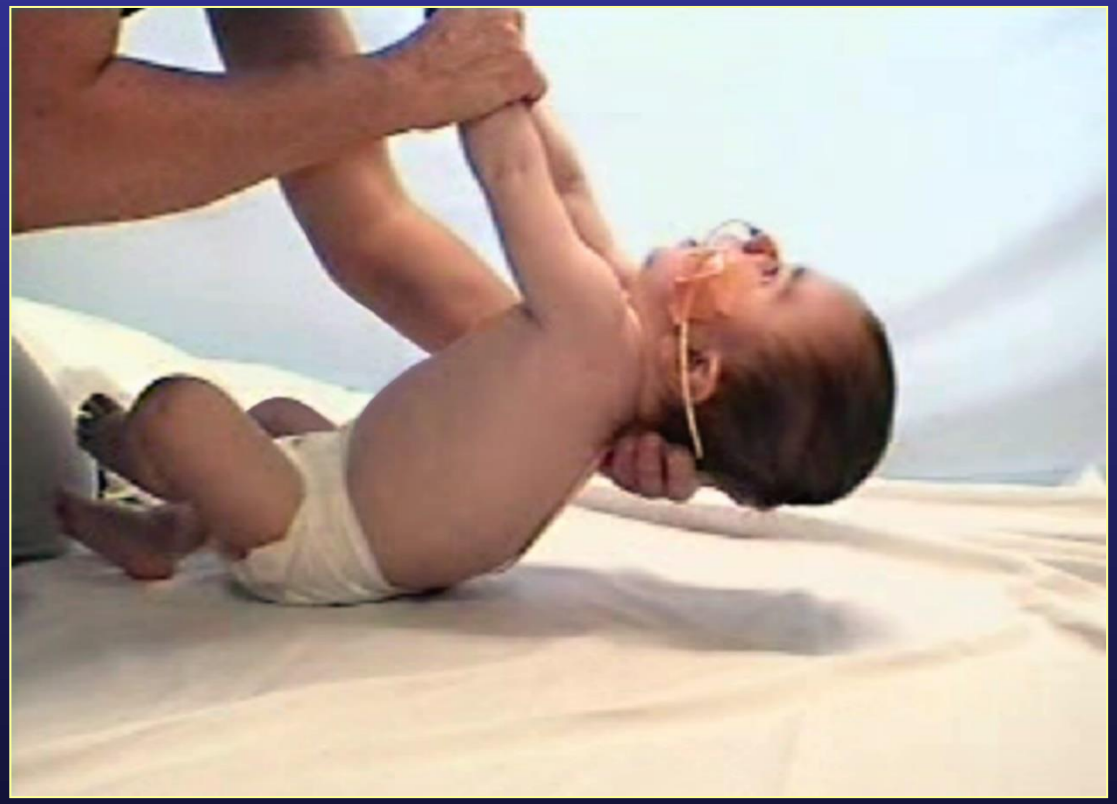


Thurberg et al., Lab Invest. 86:1208-1220, 2006



Raben N, et al. *Curr Mol Med.* 2002;2:145-166.

Infantile-onset Pompe Disease

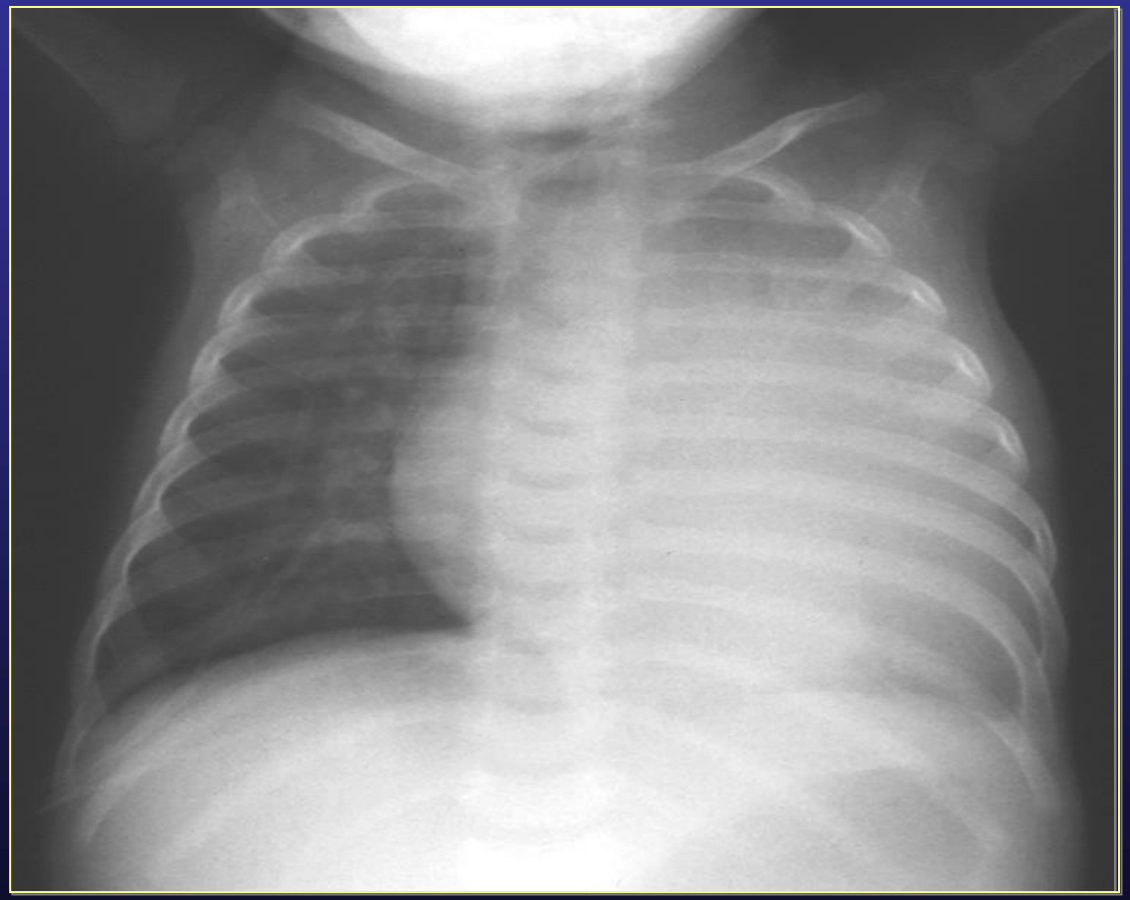


Data on file, Genzyme Corporation.

- Musculoskeletal
  - Profound and rapidly progressive muscle weakness
- Cardiac
  - Marked cardiomegaly/ cardiomyopathy
- Respiratory
  - Progression to respiratory insufficiency

Hirschhorn R, et al. In: *The Metabolic and Molecular Bases of Inherited Disease*. 2001:3389-3420.

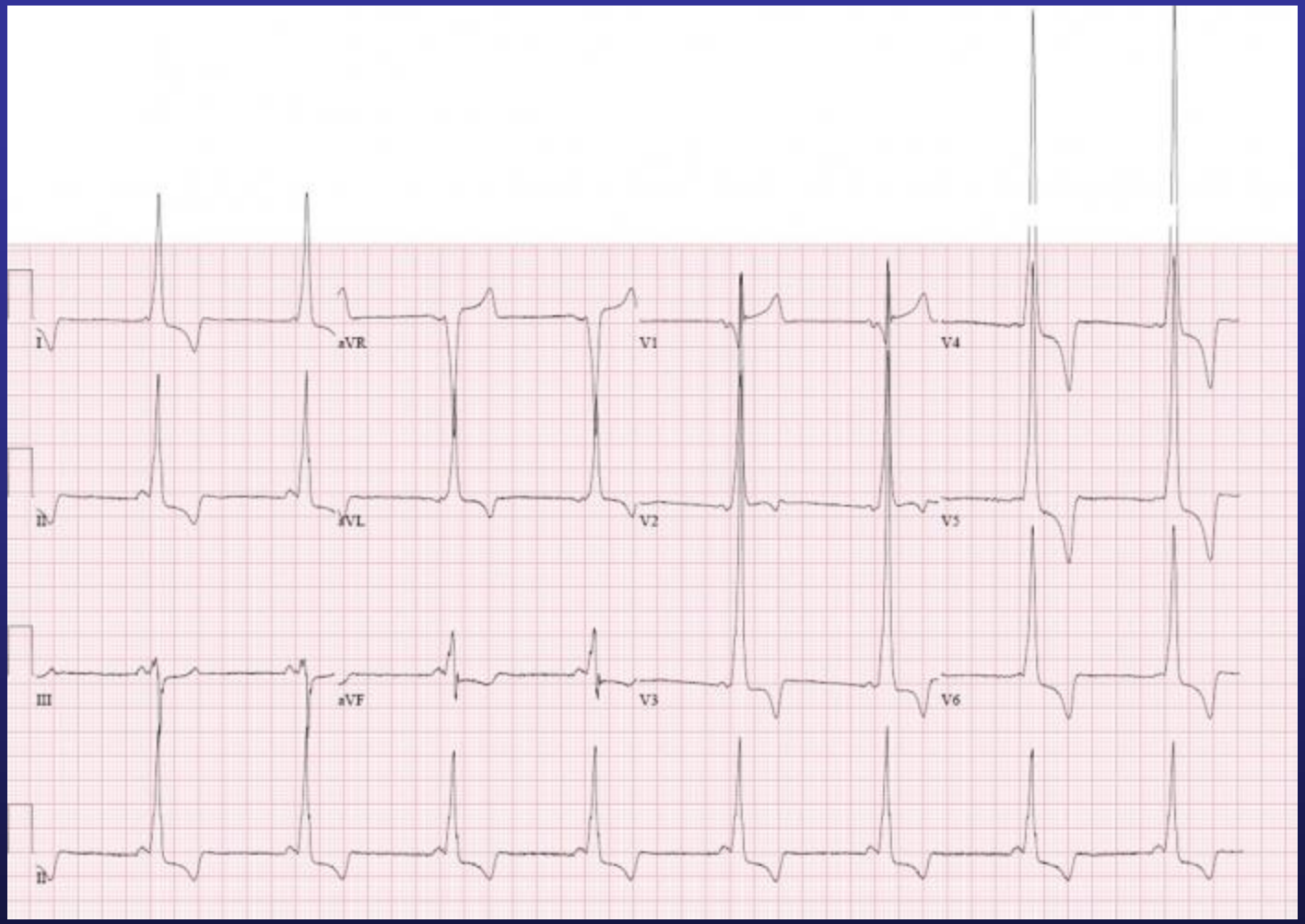
Infantile-onset Pompe Disease



With permission from B. Byrne, MD

- Cardiomegaly
  - Chest x-ray
- Cardiomyopathy
  - Echocardiogram
- Progression to cardiac failure
- EKG abnormalities
  - Short PR interval
  - Tall QRS complexes

Hirschhorn R, et al. In: *The Metabolic and Molecular Bases of Inherited Disease*. 2001:3389-3420.



www.lysosomalstorageresearch.ca
Joe Clarke, MD
Sick Kids Toronto

Infantile-onset Pompe Disease

Data on file, Genzyme Corporation.

- Respiratory distress/ insufficiency
  - Arterial blood gas
  - Sleep studies
- Infections
- Ventilator support

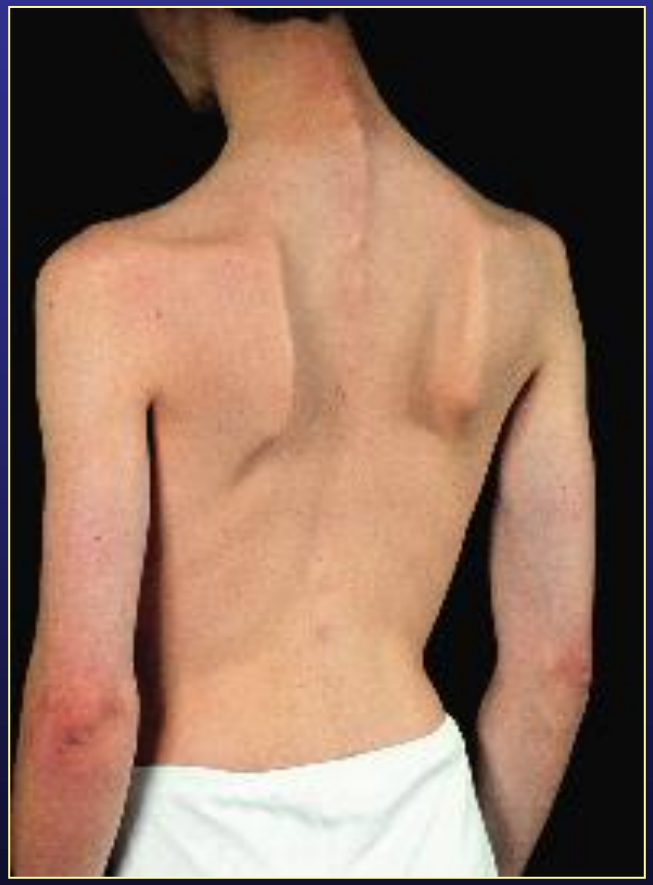
Hirschhorn R, et al. In: *The Metabolic and Molecular Bases of Inherited Disease*. 2001:3389-3420.
Raben N, et al. *Curr Mol Med*. 2002;2:145-166.

Late-onset Pompe Disease

- Weakness in the pelvic girdle muscles demonstrated by positive Gower's maneuver

Hirschhorn R, et al. In: *The Metabolic and Molecular Bases of Inherited Disease*. 2001:3389-3420.

Late-onset Pompe Disease

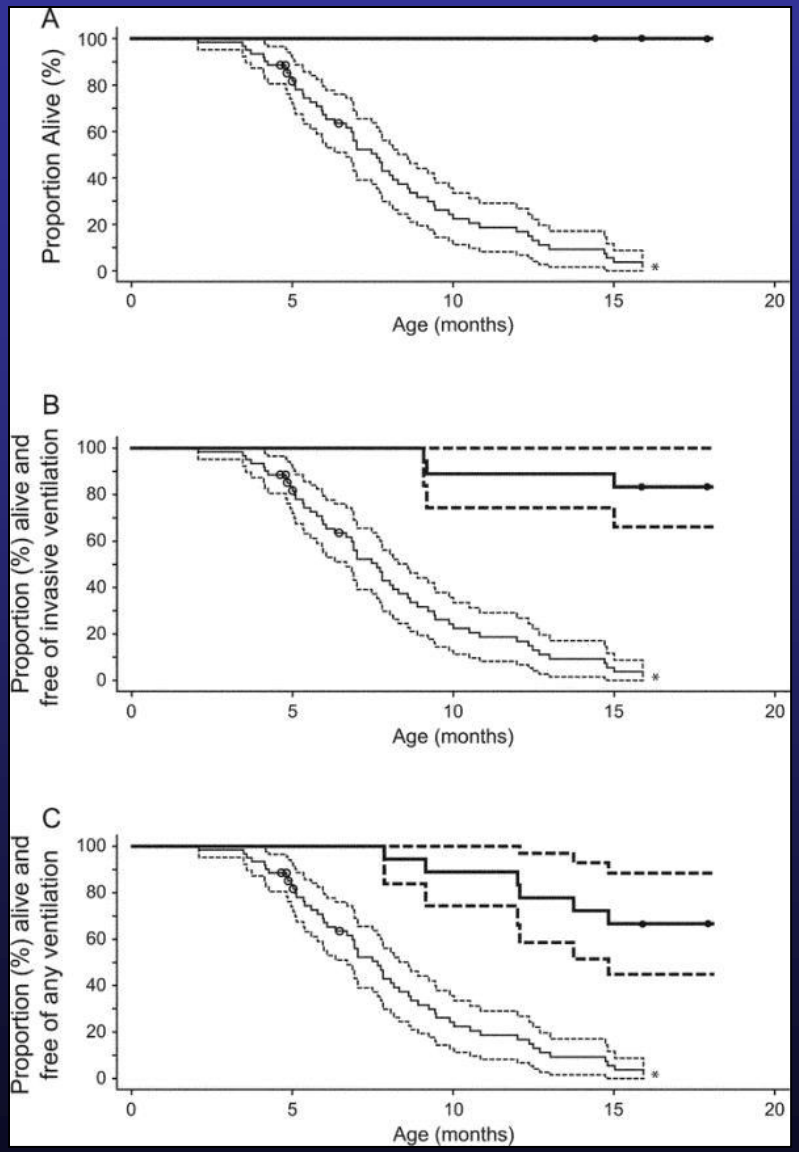


Courtesy of Dr Herman F.M. Busch,
Erasmus MC, Rotterdam, The Netherlands.

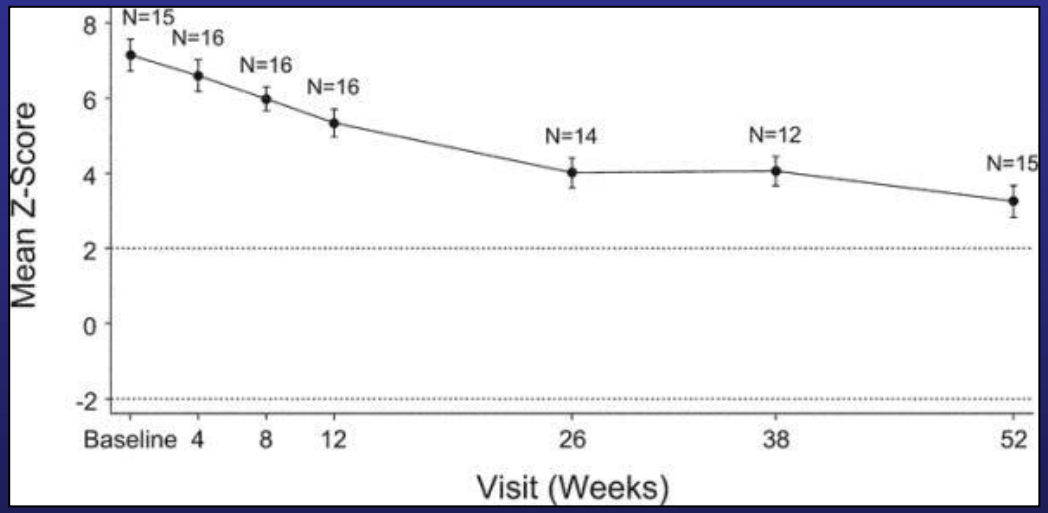
- Atrophy of scapular and paraspinal muscles
- Scapular winging

Hirschhorn R, et al. In: *The Metabolic and Molecular Bases of Inherited Disease*. 2001:3389-3420.

Pompe Disease ERT Results



Cardiac mass



Kishnani et al. Neurology 68:99-109, 2007

Pompe Disease- Variable ERT Response

Good Response

No Response

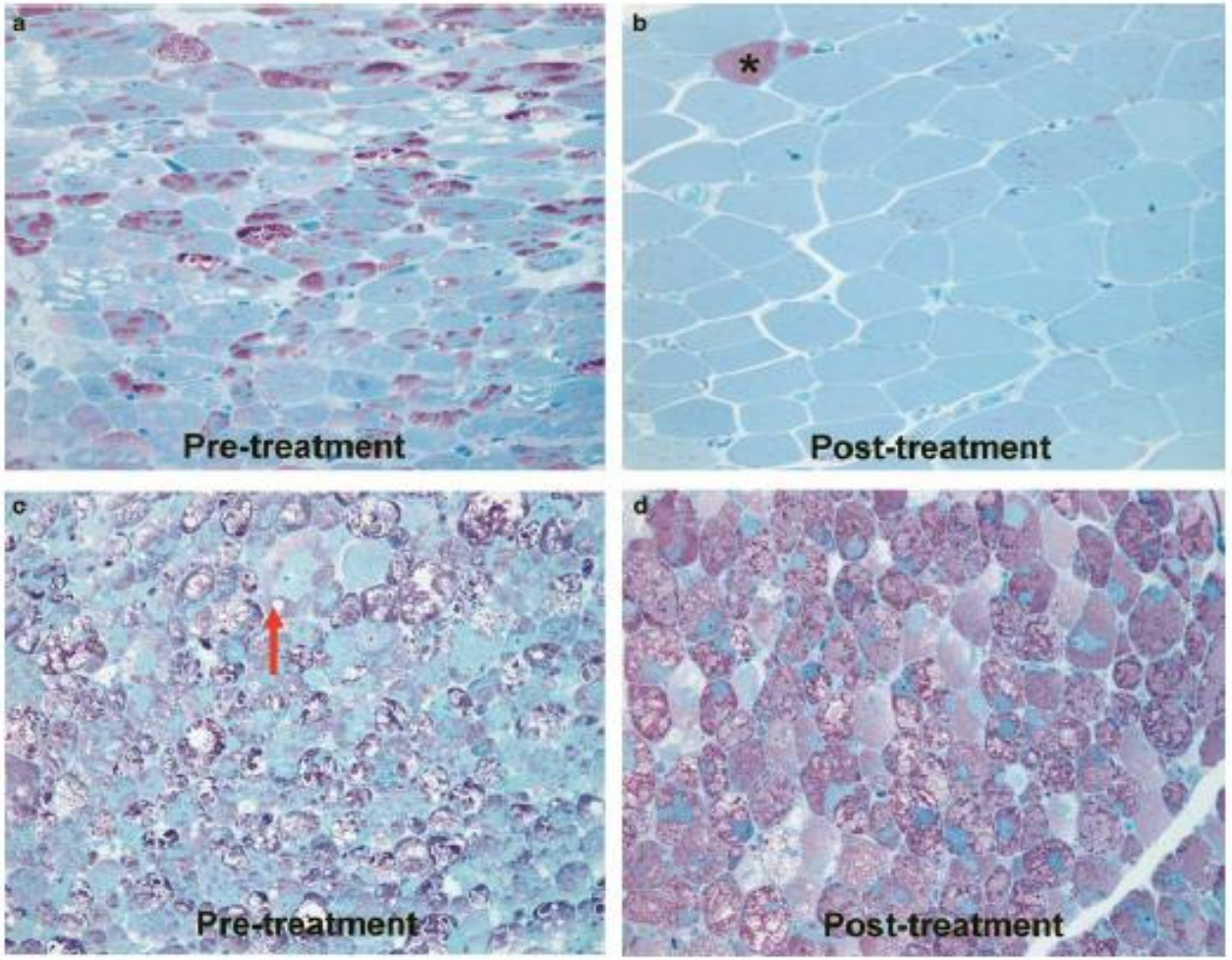


Figure 1 Light microscopic examination of quadriceps biopsies demonstrates that patient biopsies differ in the histologic response to enzyme replacement therapy after 52 weeks of treatment. The glycogen accumulation in patient A at baseline (a) has been cleared in the majority of myocytes after 52 weeks; a rare myocyte appears completely replaced by glycogen and remains unaffected by ERT (asterix, b). (c) Demonstrates the heavy glycogen load present at 3 weeks in patient C; glycogen is distributed at the periphery of many cells (red arrow, c). After 52 weeks of ERT (d), there has been little glycogen clearance, and many myocytes appear completely replaced by glycogen, similar to the appearance of the isolated cell in (b). (HRLM with Richardson's/PAS stain, magnification ×400).

Thurberg et al., Lab Invest. 86:1208-1220, 2006

Pompe- Autopsy of a Patient on ERT

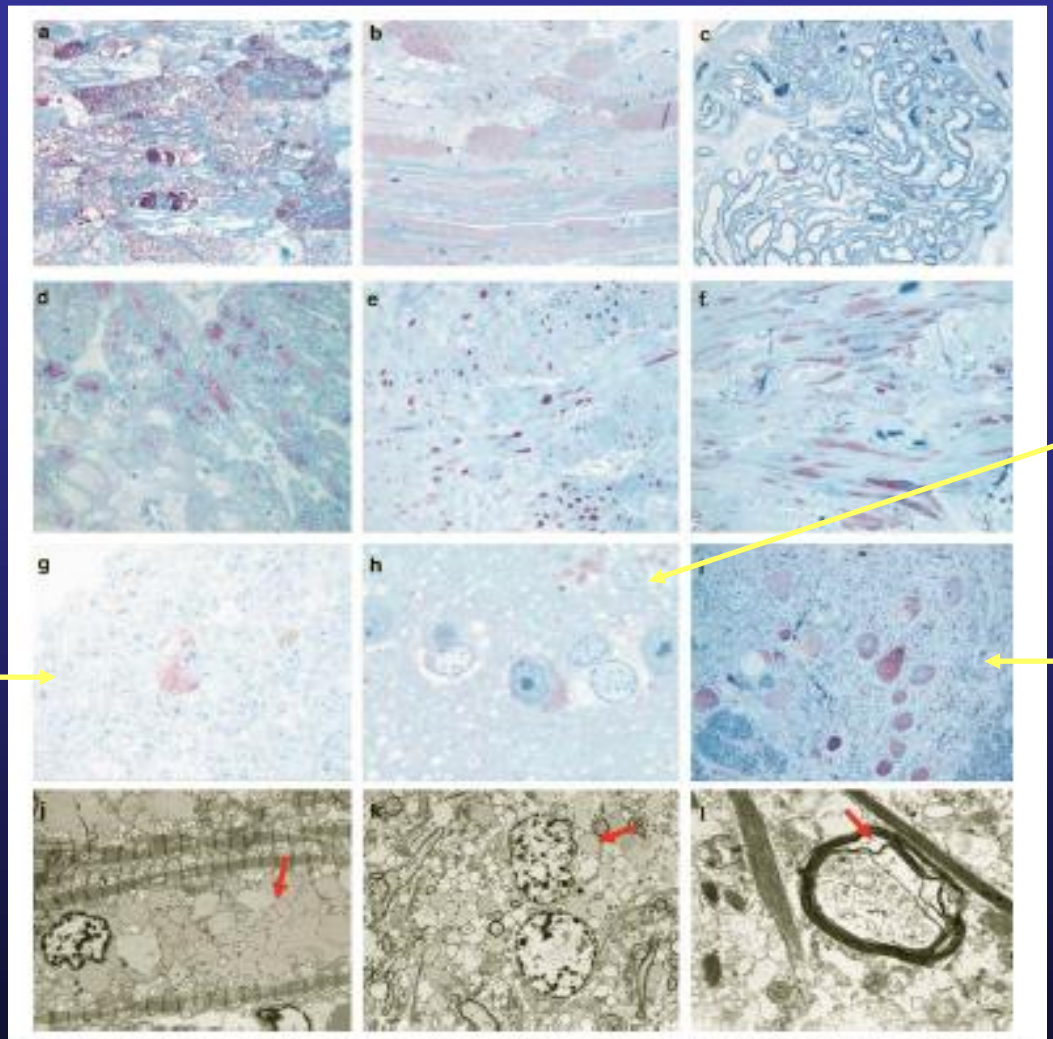


Figure 5 Examination of autopsy tissues from patient B demonstrates abnormal glycogen accumulation in multiple organs and cell types. (a) Skeletal muscle, deltoid (H&LM with Richardson's/PAS stain, ×400); (b) Skeletal muscle, diaphragm (H&LM with Richardson's/PAS stain, ×400); (c) Nerve from diaphragm: glycogen can be seen in Schwann cell cytoplasm (H&LM with Richardson's/PAS stain, ×1000); (d) Heart, interventricular septum (H&LM with Richardson's/PAS stain, ×600); (e) Bladder, smooth muscle cells of the muscularis propria (H&LM with Richardson's/PAS stain, ×400); (f) Large artery, smooth muscle cells of the media (H&LM with Richardson's/PAS stain, ×1000); (g) Cerebellum: glycogen accumulation can be seen in a Purkinje cell (H&LM with Richardson's/PAS stain, ×600); (h) Frontal lobe: glycogen accumulation in a neuron (H&LM with Richardson's/PAS stain, ×1000). (i) Spinal cord: lysosomal glycogen can be seen in motor neurons of the ventral horn (H&LM with Richardson's/PAS stain, ×400); (j) Cardiomyocyte of the interventricular septum contains multiple glycogen-filled lysosomes adjacent to the nucleus. (EM: ×20 000). (k) Clusters of membrane-bound glycogen vacuoles are present in motor neurons of the ventral horn (EM: ×6000). (l) Myelin sheaths of nerve fibers exhibit splitting (EM: ×44000).

Neuron in frontal lobe

Purkinje cell in cerebellum

Anterior horn cell in spinal cord

Thurberg et al., Lab Invest. 86:1208-1220, 2006

Pompe Disease Response to ERT
Lessons Learned?

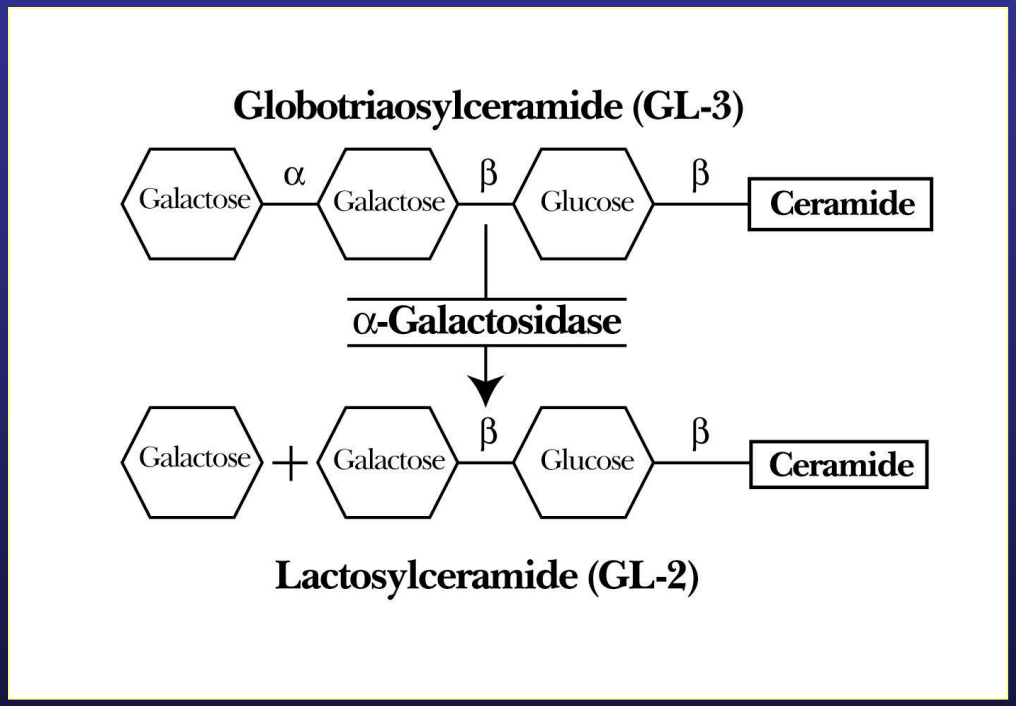
- CRIM negative do worse due to antibody production
  - Immune modulation starts when ERT begins
- High titer antibodies impair efficacy in anyone
  - May require immune modulation
- The longer you wait to start treatment, the worse the response
- Cardiac muscle responds better than skeletal muscle
- Infantile onset patients may develop a neurodegenerative disorder
- Efficacy in later-onset Pompe- mostly stabilization

Anderson-Fabry Disease

- Lysosomal storage of globotriaosylceramide (GL3, Gb3)
- α-galactosidase A deficiency
- X-linked disorder. Females can be as severe as males, have late-onset organ involvement, or be asymptomatic.
- Incidence:
  - 1:40-60,000 males, classic form
  - 1:5-10,000 males, milder variants
- Usually familial

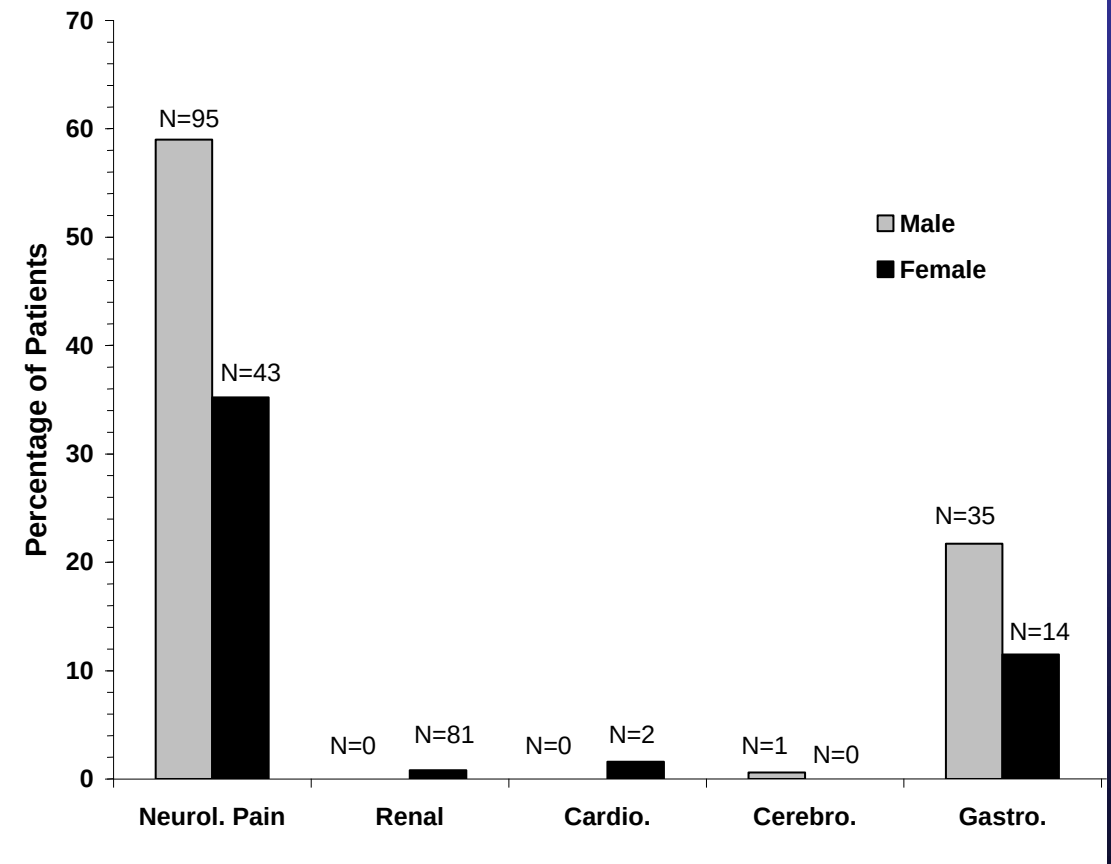
- Onset of symptoms (neuropathic pain, decreased sweating, GI, exercise intolerance) before age 10 in classic males.
- Diagnosis usually delayed at least 10 years after symptom onset.
- Disease progression varies enormously, even in families.
- Untreated- renal failure, cardiomyopathy, strokes, premature death
- Diagnosis: enzyme assay (wbc best), molecular
- Biomarkers: GL3, lysoGL3

Metabolic Defect



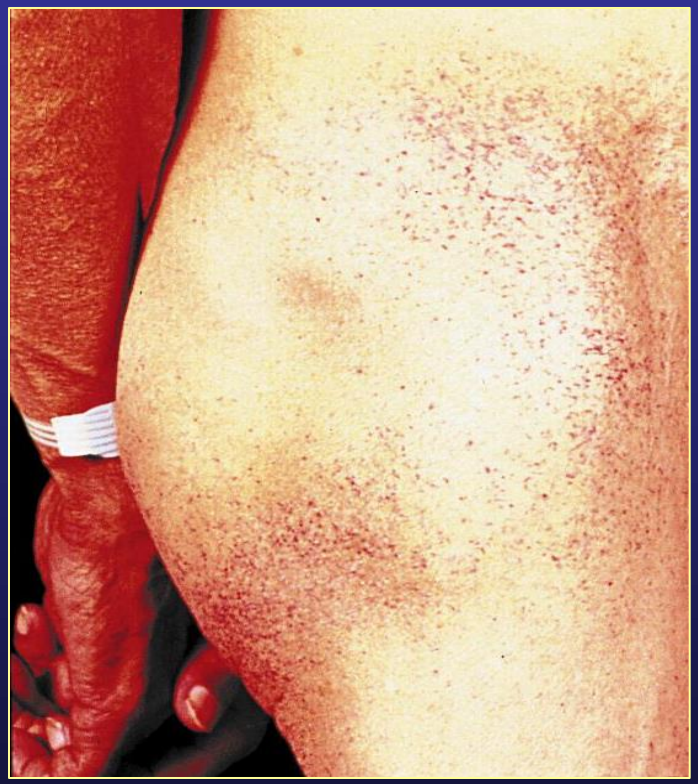
Presenting Symptoms in Children

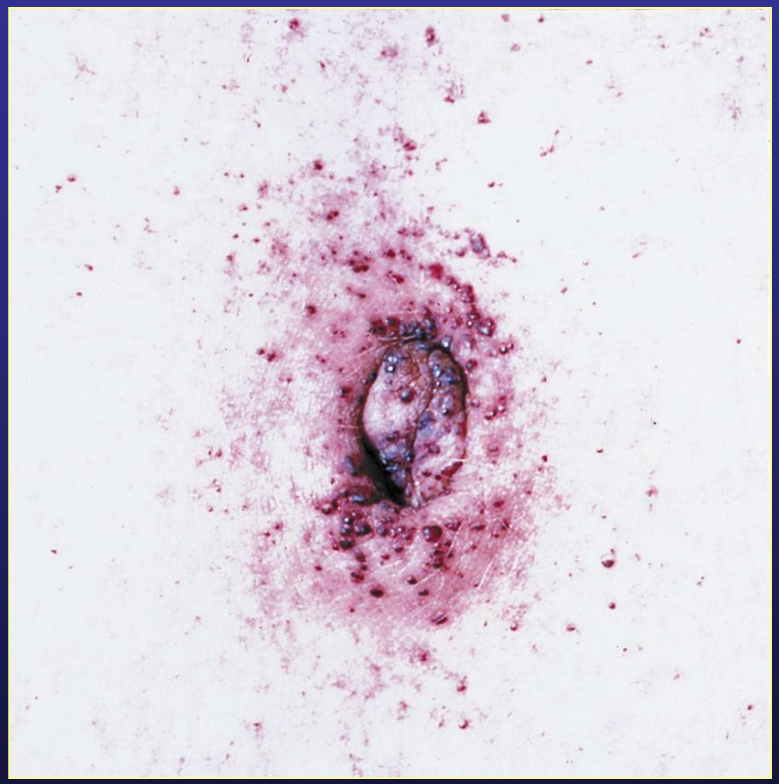
Registry Data



Hopkin et al., Pediatr Res, 2008

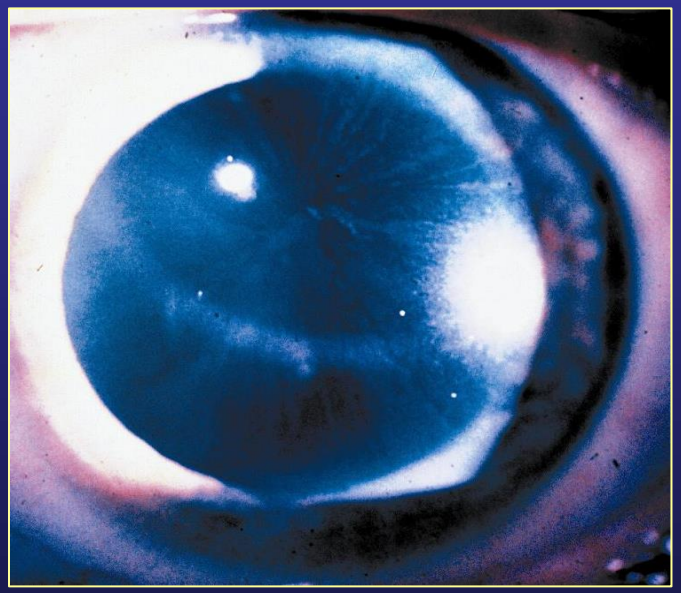
Angiokeratomas

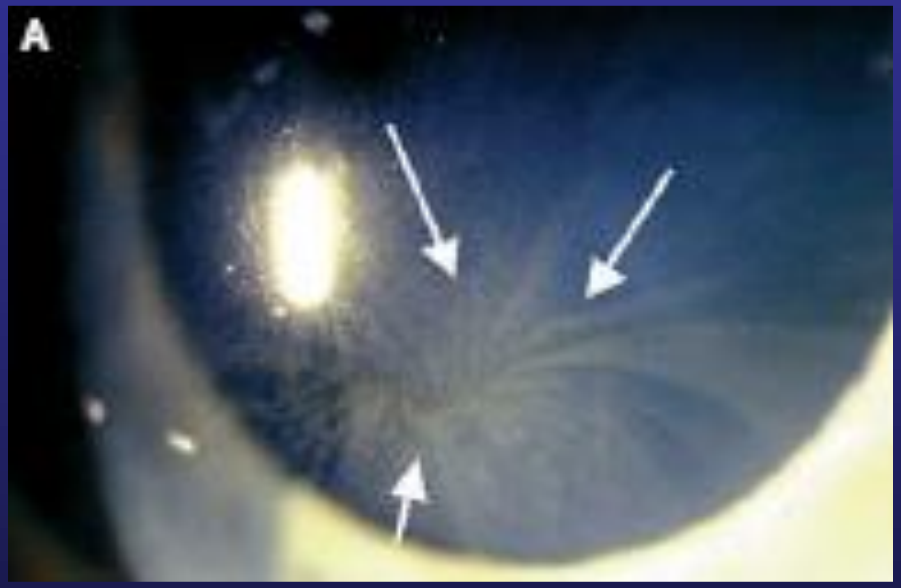




With permission, from R.J. Desnick, PhD, MD

Corneal Opacity

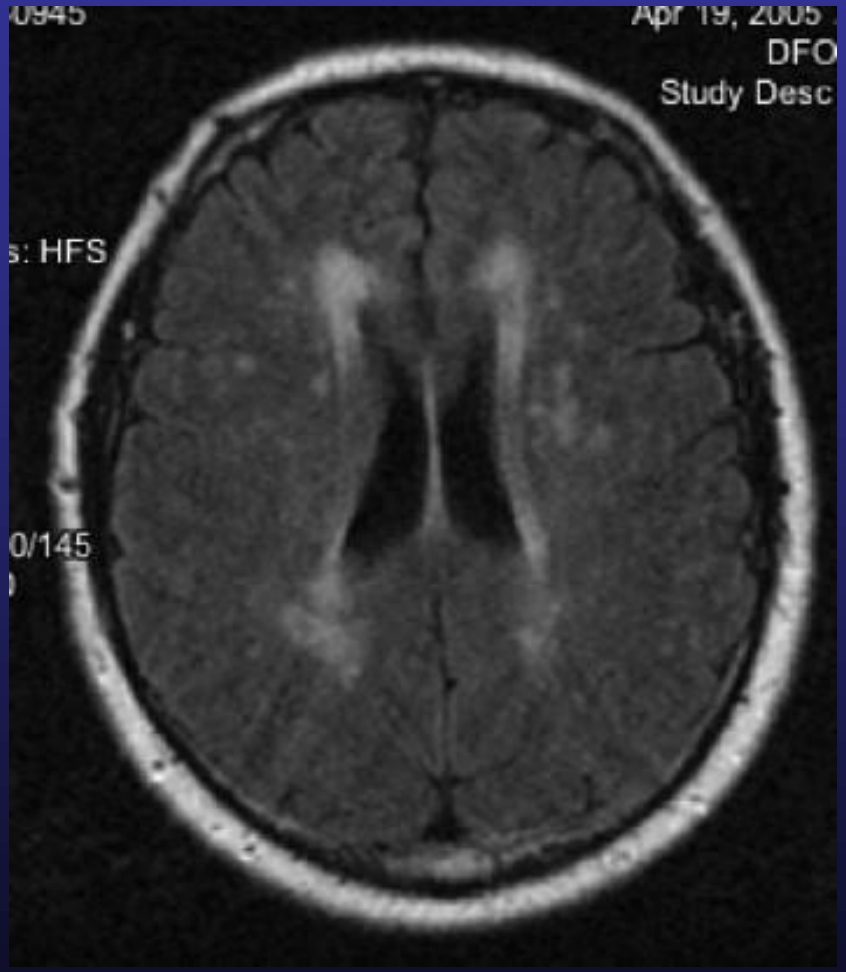




Note “spoke-like” pattern on cornea, visible through slit-lamp ophthalmoscopy

Courtesy of Genzyme

Fabry Leukoencephalopathy



Moore et al., J Neurolog Sci 257:258-263, 2007

Fabry vascular disease

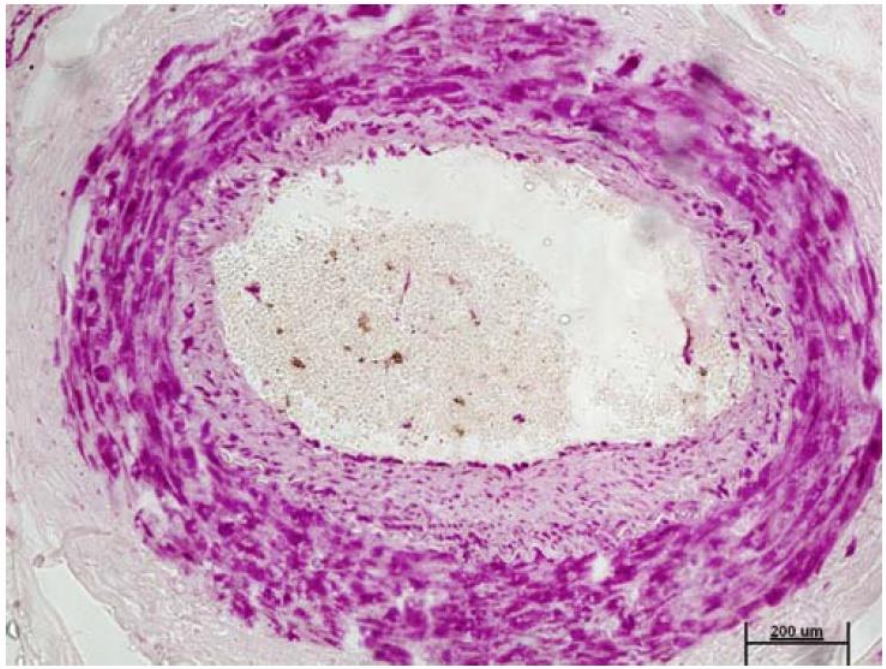


Fig. 3 FD hemizygote. Coronary artery branch; frozen section stained with PAS; massive Gb3Cer storage in the media and intima including the endothelium. Obj. ×10

J Inherit Metab Dis 31:753, 2008

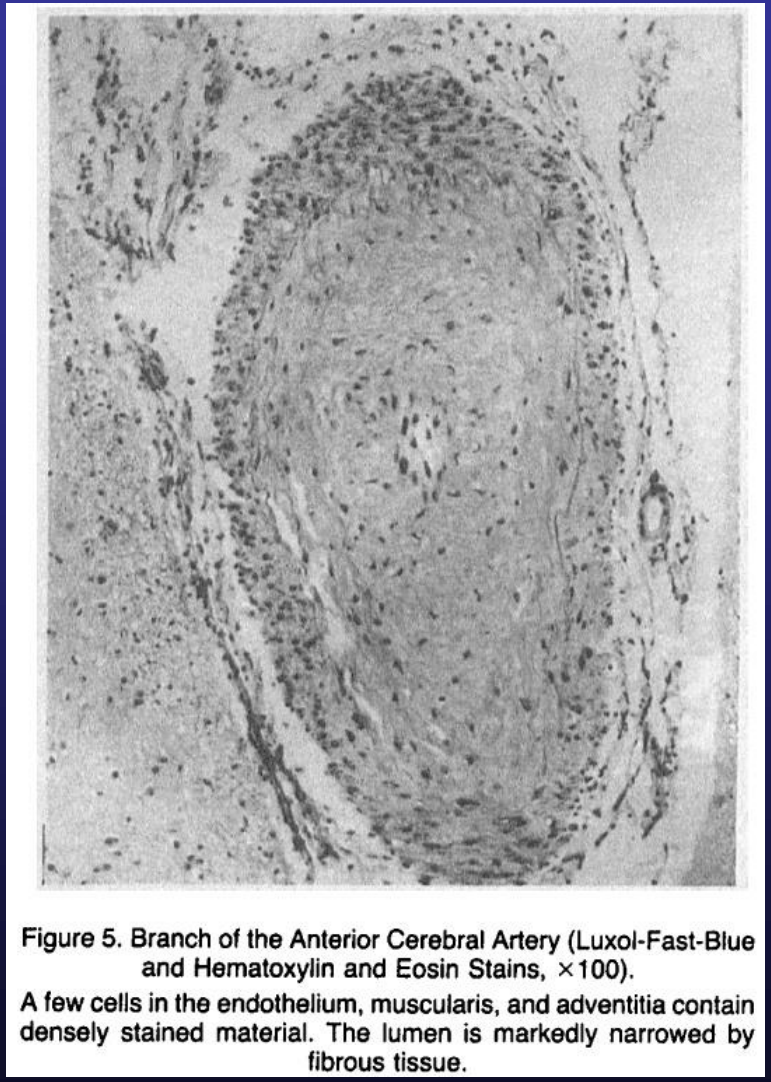


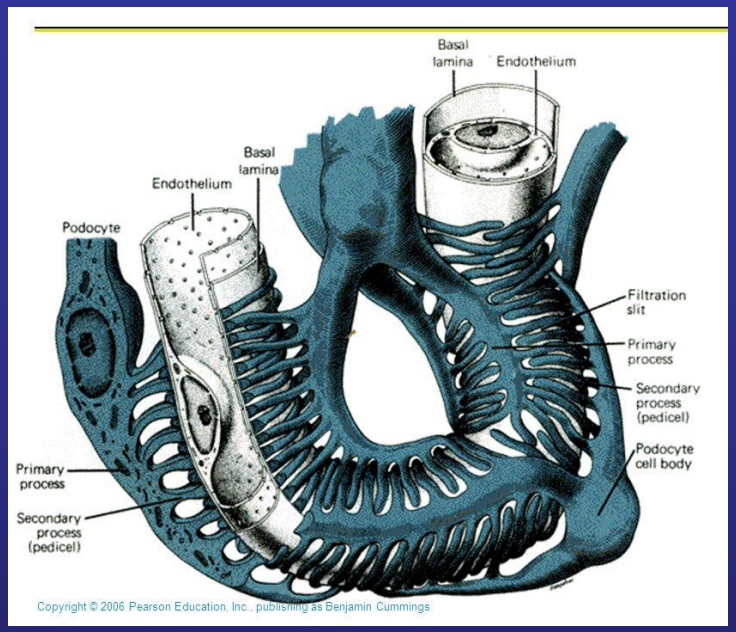
Figure 5. Branch of the Anterior Cerebral Artery (Luxol-Fast-Blue and Hematoxylin and Eosin Stains, ×100).

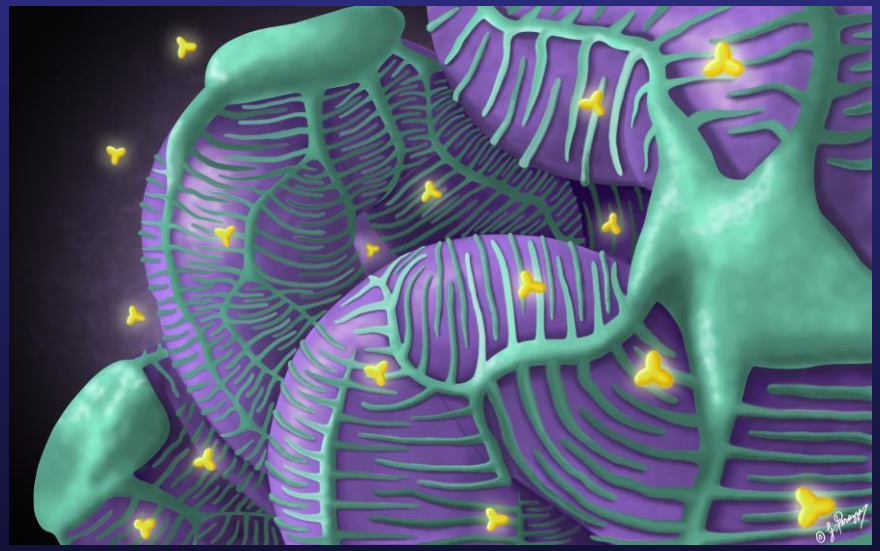
A few cells in the endothelium, muscularis, and adventitia contain densely stained material. The lumen is markedly narrowed by fibrous tissue.

NEJM 310:106, 1984

Fabry Nephropathy
Progression in Classic Males

- Before birth- Podocyte storage
- First and second decade- microalbuminuria, hyperfiltration, glomerular sclerosis begins
- Third decade on- worsening proteinuria, glomerular sclerosis → renal failure





https://indigo.uic.edu/handle/10027/8105

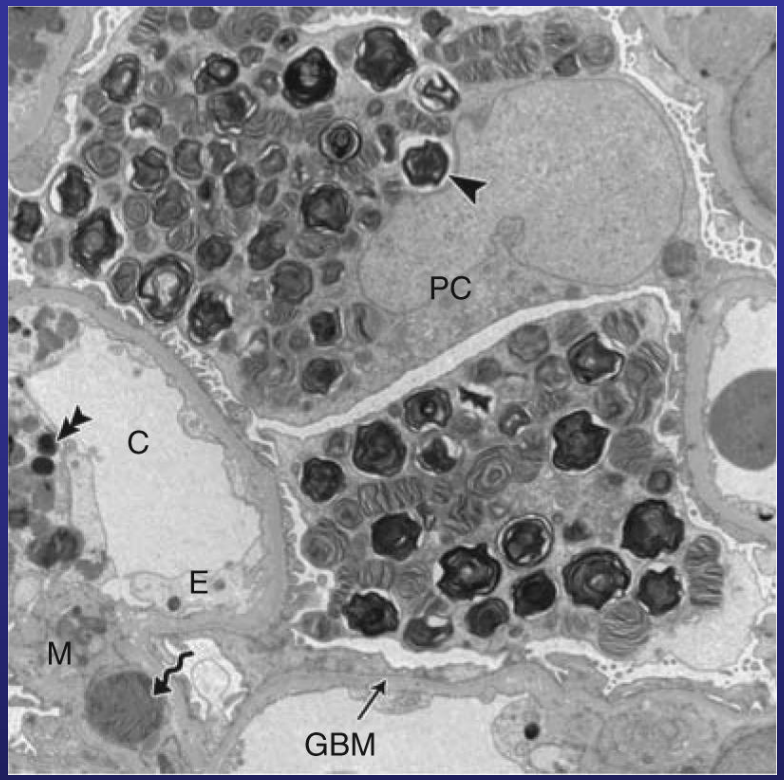
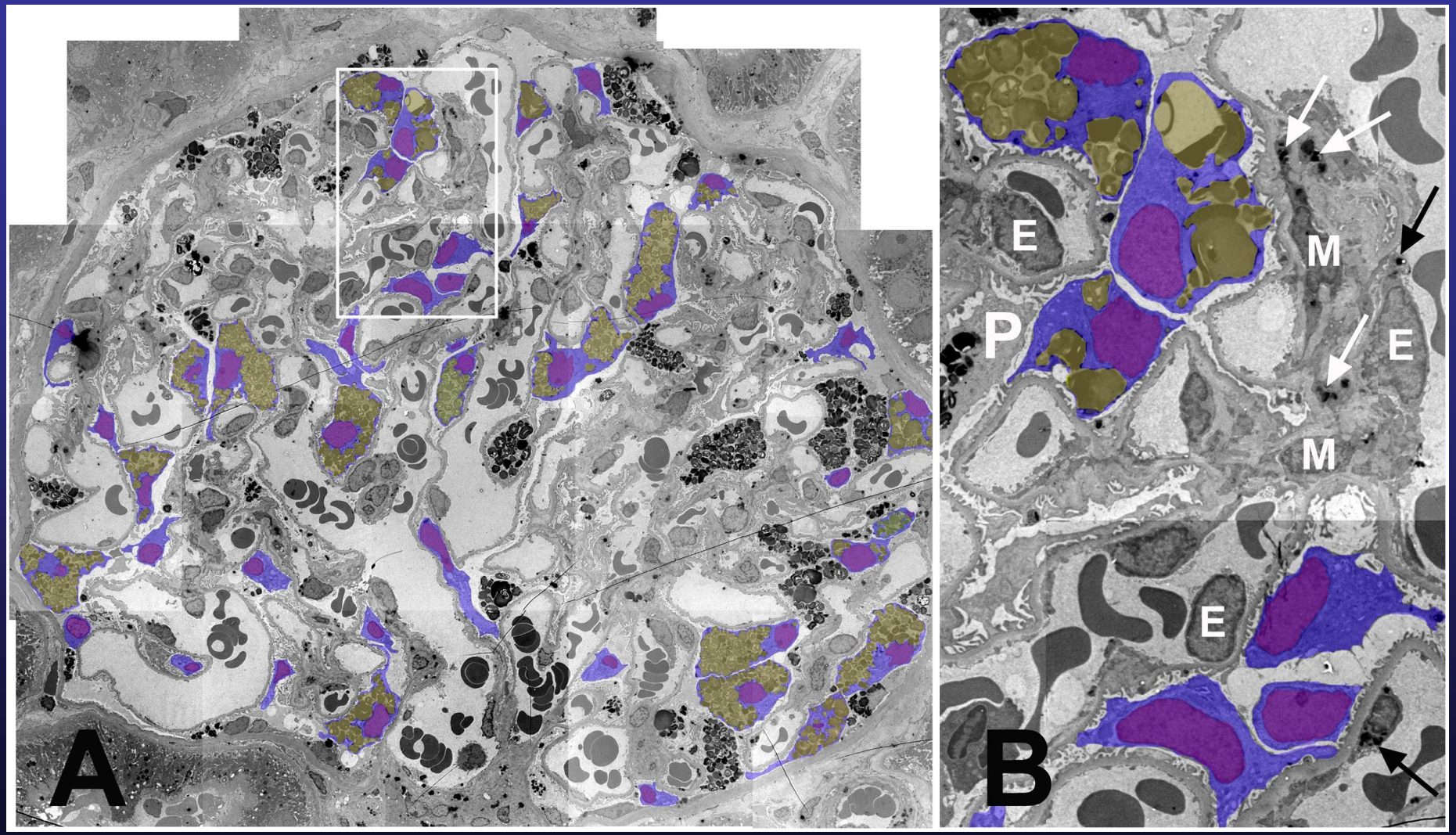


Figure 1 | Globotriaosylceramide (GL-3) inclusions in glomerular podocytes (arrowhead), endothelial cells (double arrowhead), and mesangial cells (spiral arrow) from the kidney biopsy of a Fabry patient (TEM × 11,000).
C, capillary lumen; E, endothelial cell; GBM, glomerular basement membrane; M, mesangium; PC, podocyte; TEM, transmission electron microscopy.

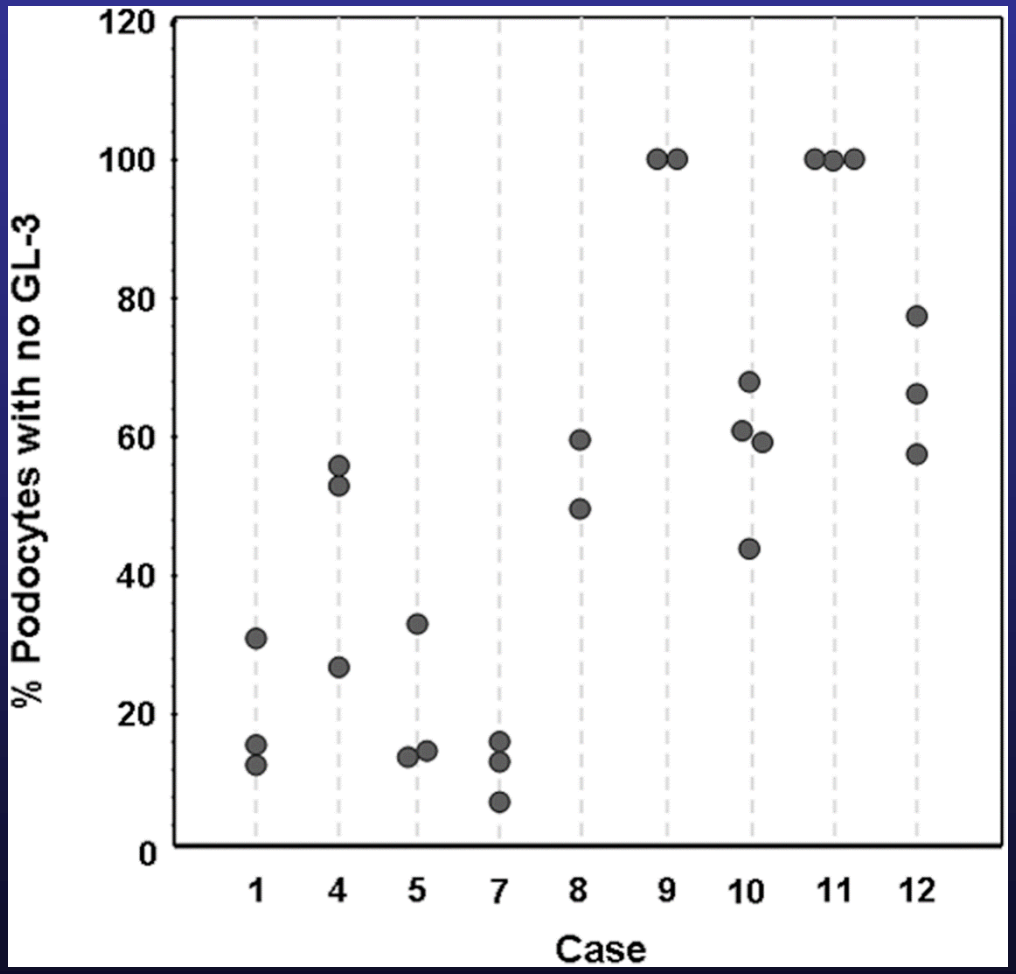
Najafian et al, Kidney Int 79:633-670, 2011

Mosaicism of Podocyte Involvement in Females



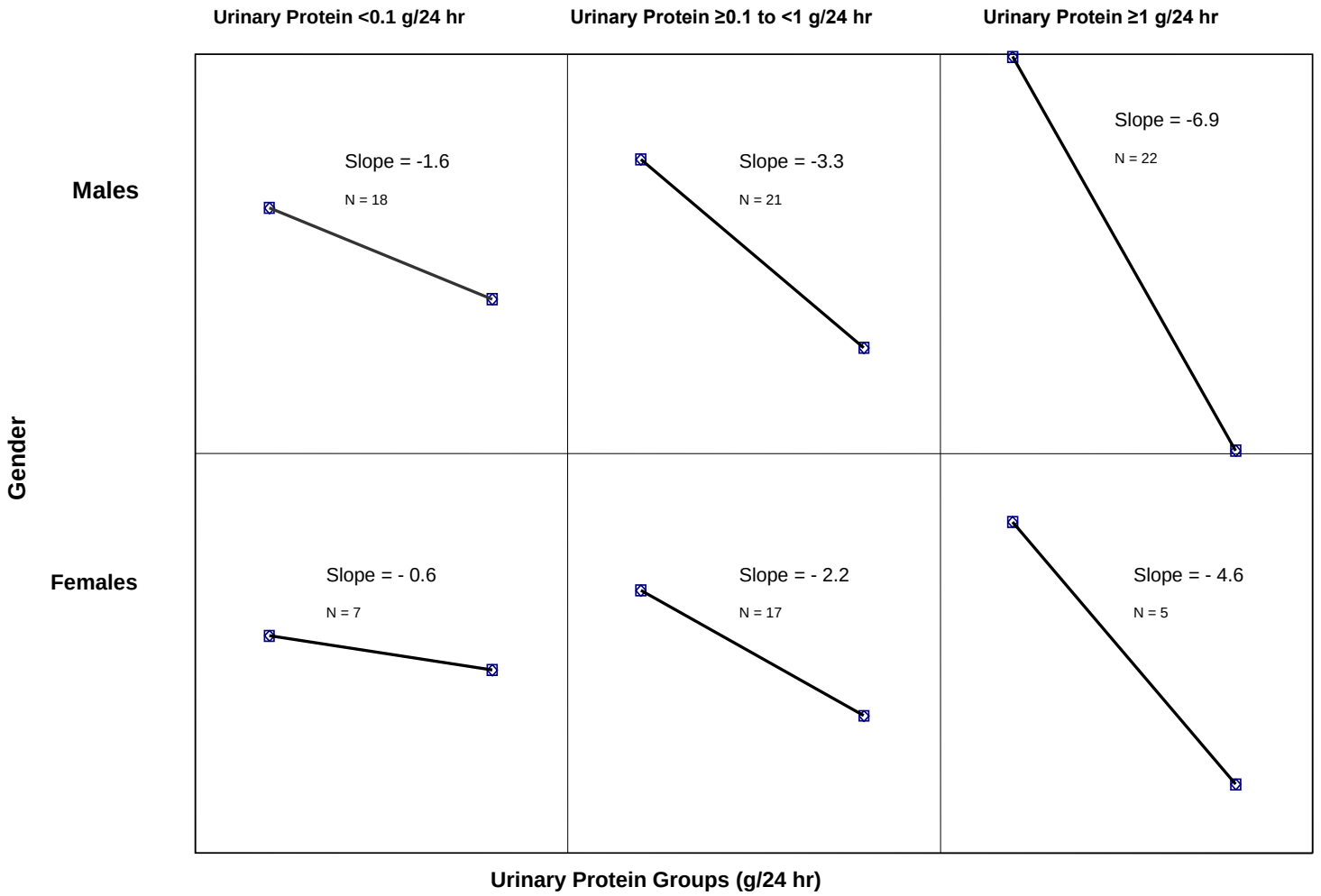
Mauer M, et al, PLoS One 9(11):e112188, 2014

Variability in Podocyte Involvement Between Females and Between Glomeruli



Mauer M, et al, PLoS One 9(11):e112188, 2014

Yearly decline based on eGFR slopes (ml/min/1.73 m^2/yr) in Fabry males and females by 24 hr urinary protein groups

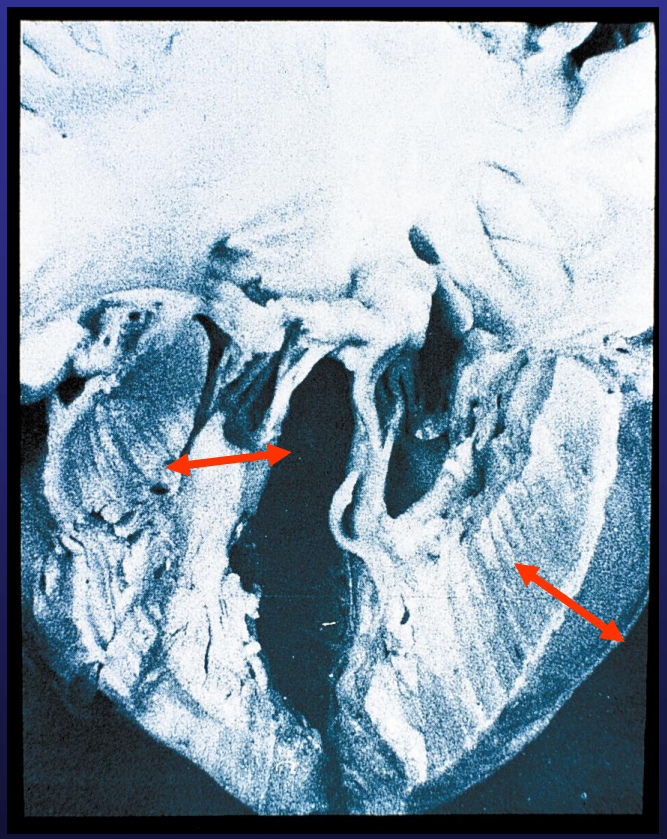


Shiffmann et al. Nephrol Dial Transplant 24:2102-11, 2009

- GL3 binds Shiga Toxin (causes hemolytic uremic syndrome)
- GL3 associates with megalin/cubulin in the proximal tubules to reabsorb protein
- SRT inhibition or KO of GL3 synthase in mice (mice don’t have glomerular GL3) preserves renal function against myoglobin or gentamicin injury
- Increased proteinuria leads to increased tubular reabsorption, tubule damage, and hyperfiltration through tubular-glomerular feedback causing increased afferent pressure, key features of Fabry nephropathy

Morace et al, Kidney International 96:327-341, 2019

Left Ventricular Hypertrophy



From R.J. Desnick, PhD, MD

- LVH in 50-year-old Fabry patient
- Note markedly thickened myocardium

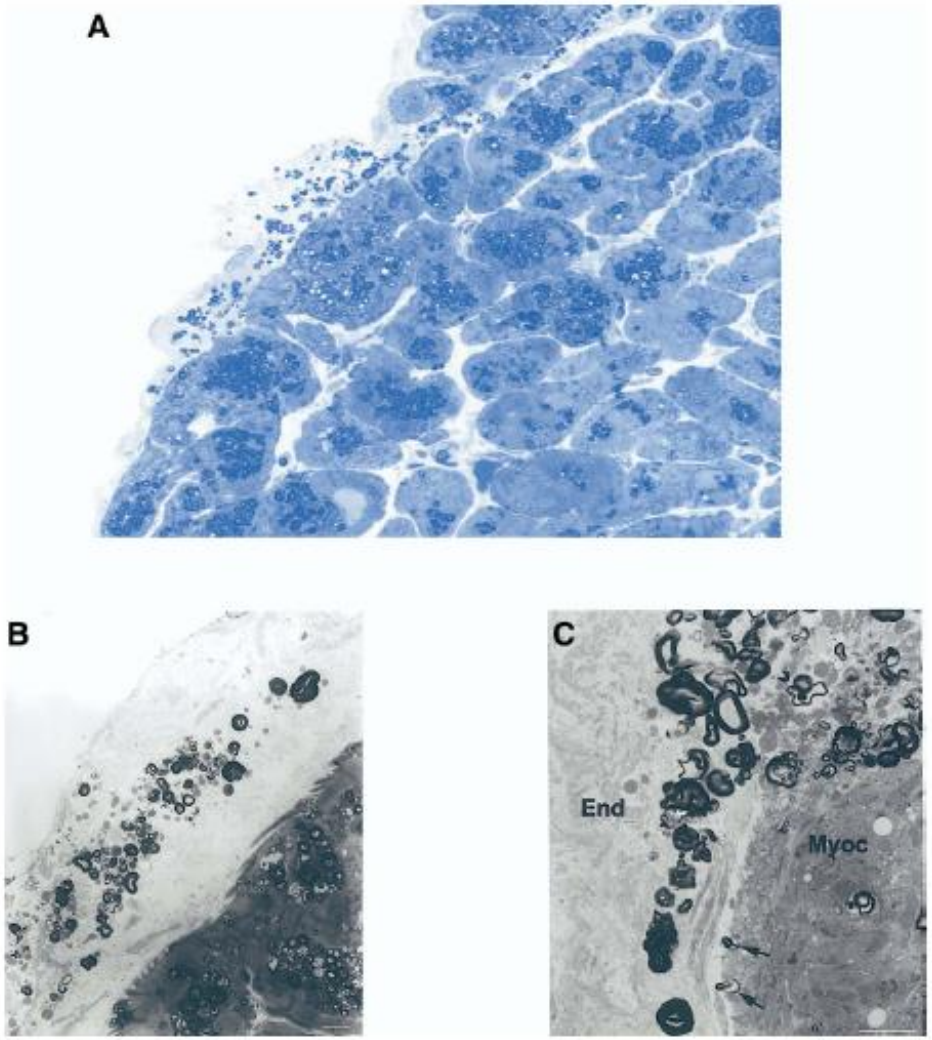


Figure 2. Semithin (A) and ultrathin (B,C) sections from left ventricular endomyocardial biopsy of the same patient of Figures 1A and 1D. In panel A, osmiophilic bodies intensely stained by Azur II are seen in the endocardium, in the subendocardial space, and in myocardium. In the subendocardial space, they are localized in the region of empty spaces seen at H and E histology sections. In the myocardial tissue, a gradient of storage material can be appreciated from the subendocardial to the inner layer. At electron microscopy (B,C) the osmiophilic bodies appear to consist of glycosphingolipids organized in membrane-bounded bodies diffusely present in the context of the endocardium (End), occupying the subendocardial space as a free storage material and inside the myocytes (Myo). Arrows indicate membrane-bounded bodies at the boundaries between a myocardiocyte and the subendocardial space, suggesting a process of release from the cell to the extracellular space. (A) Azur II original magnification ×100. (B,C) Bars = 1 μm.

Pieroni et al., JACC 47:1663-71, 2006

Females can have cardiac fibrosis without hypertrophy

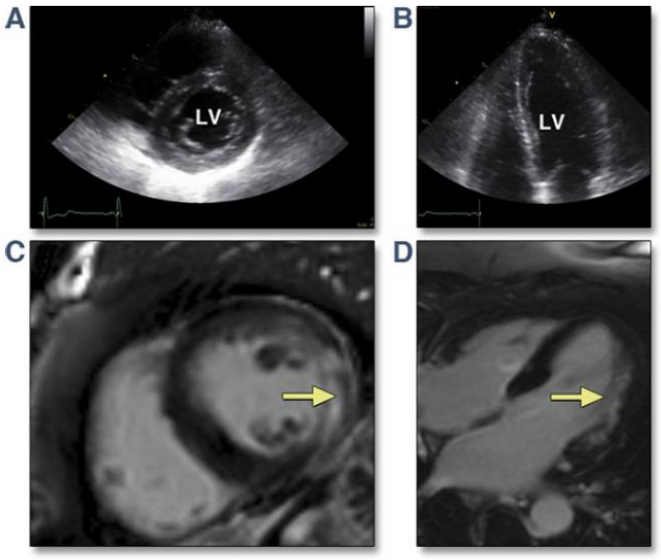


Figure 6. Echocardiographic and CMR Images of a Female Patient Without LV Hypertrophy but With LE

(**A**) Short-axis echocardiographic view. (**B**) 4-chamber echocardiographic view. The LV cavity is marked with LV. The images clearly show that no LV hypertrophy is present. (**C**) Short-axis view of the same patient using the LE technique by CMR. (**D**) 4-chamber CMR view. The **arrows** indicate LE in the lateral wall. Abbreviations as in Figures 1 and 2.

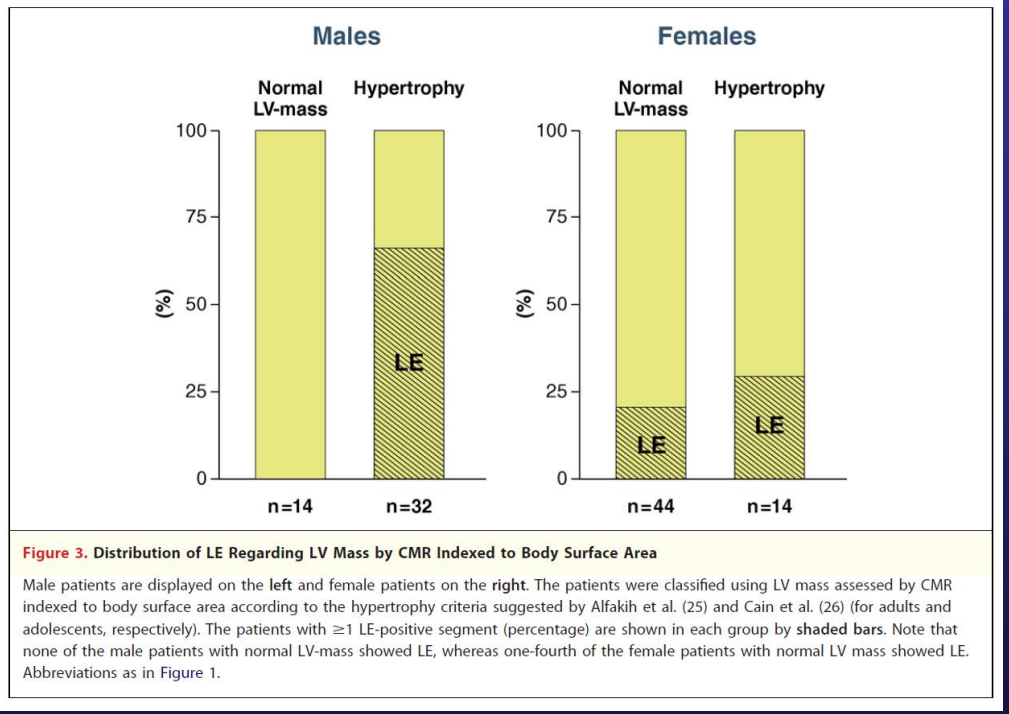
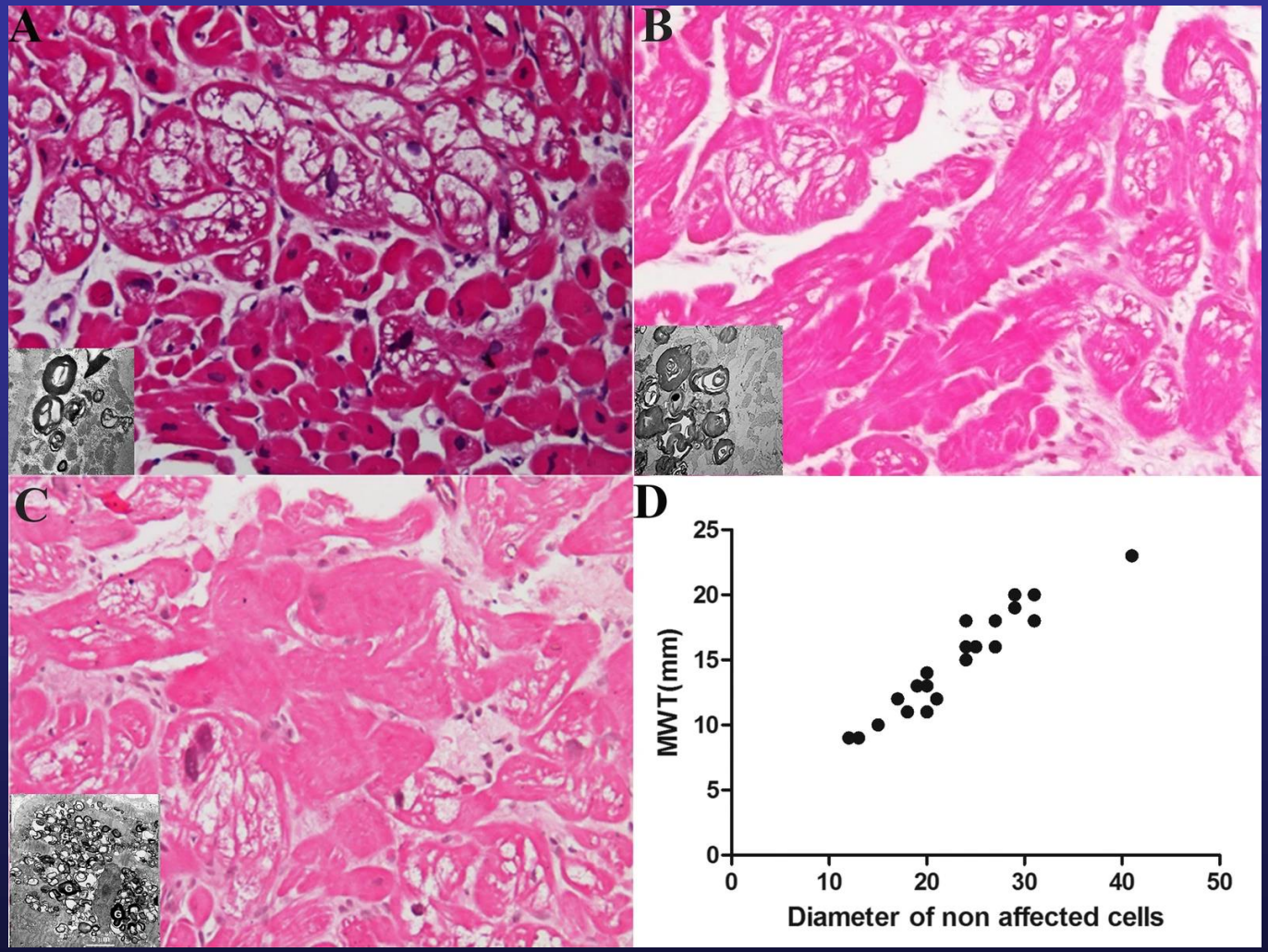


Figure 3. Distribution of LE Regarding LV Mass by CMR Indexed to Body Surface Area

Male patients are displayed on the **left** and female patients on the **right**. The patients were classified using LV mass assessed by CMR indexed to body surface area according to the hypertrophy criteria suggested by Alfakih et al. (25) and Cain et al. (26) (for adults and adolescents, respectively). The patients with ≥1 LE-positive segment (percentage) are shown in each group by **shaded bars**. Note that none of the male patients with normal LV-mass showed LE, whereas one-fourth of the female patients with normal LV mass showed LE. Abbreviations as in Figure 1.

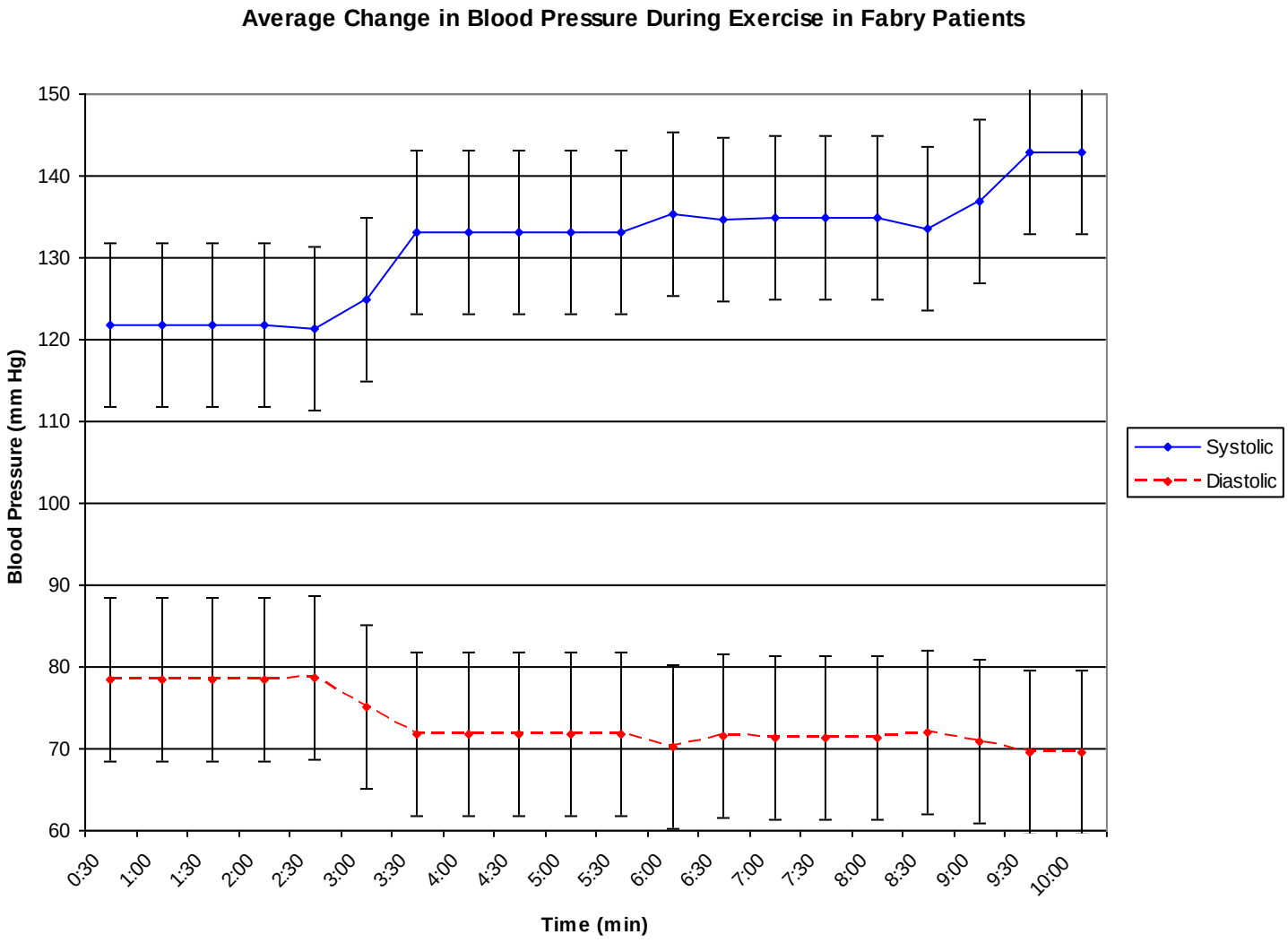
Niemann et al., J Am Coll Cardiol Img 4:592-601, 2011

Hypertrophy of unaffected cardiomyocytes correlates with severity of cardiomyopathy in female patients with Fabry disease



a Mosaic with normal and affected myocytes in a female with pre-hypertrophic Fabry Cardiomyopathy
b Moderate hypertrophy of unaffected myocytes in Fabry female with moderate LV hypertrophy.
c Severe hypertrophy with disarray of unaffected myocytes interspersed with enlarged vacuolated cells in a Fabry female with severe LV hypertrophy who died.
d correlation between MWT and diameter of non affected cells showing a linear correlation

Chimenti C, et al, Orphanet J Rare Dis 16:169, 2021


Average Change in Blood Pressure During Exercise in Fabry Patients
Blood Pressure (mm Hg)
150
140
130
120
110
100
90
80
70
60
0:30
1:00
1:30
2:00
2:30
3:00
3:30
4:00
4:30
5:00
5:30
6:00
6:30
7:00
7:30
8:00
8:30
9:00
9:30
10:00
Time (min)
Systolic
Diastolic

Small Airway Problems in the Lungs of Fabry Patients

Pulmonary Function Test	Fabry All n=39	Fabry Males n=15	Fabry Females n=24	Fabry Male v. Female *p*
$FEV_1/FVC < 70$	7	4	3	0.47
FEV_1/FVC mean (SD)	76 (10.4)	76 (8.1)	76 (11.7)	0.9
FVC mean (SD)	102% (15.9)	95% (15.5)	106% (15.1)	0.04
FVC < 80%	4	3	1	0.41
FEF_{25-75} <40%	6	2	4	0.87
FEF_{25-75} <55% but >40%	12	6	6	0.44
FEF_{25-75} <65% but >55%	4	2	2	0.8
All FEF_{25-75} <65%	**22**	**10**	**12**	0.39

Pulmonary Disease in Fabry (not much data in the Registry)

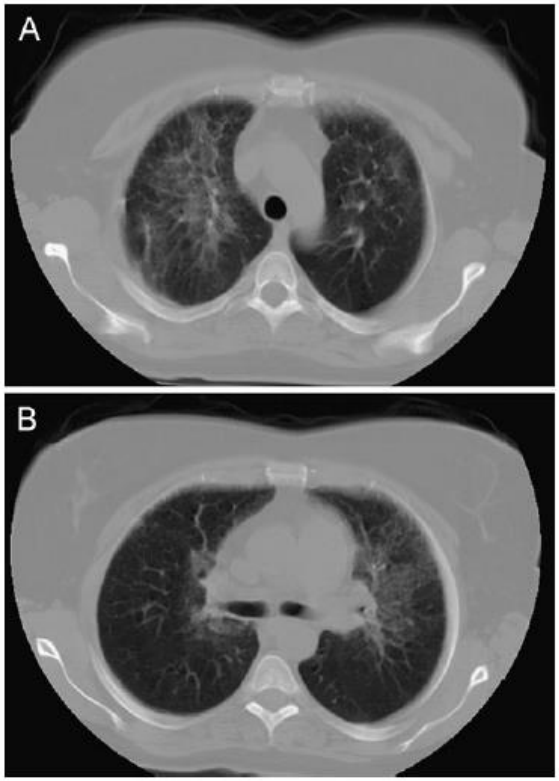


Fig. 2 Axial CT images demonstrating patchy, bilateral 'ground glass' pulmonary infiltrates in (**A**) the right middle lobe and (**B**) the left lingula

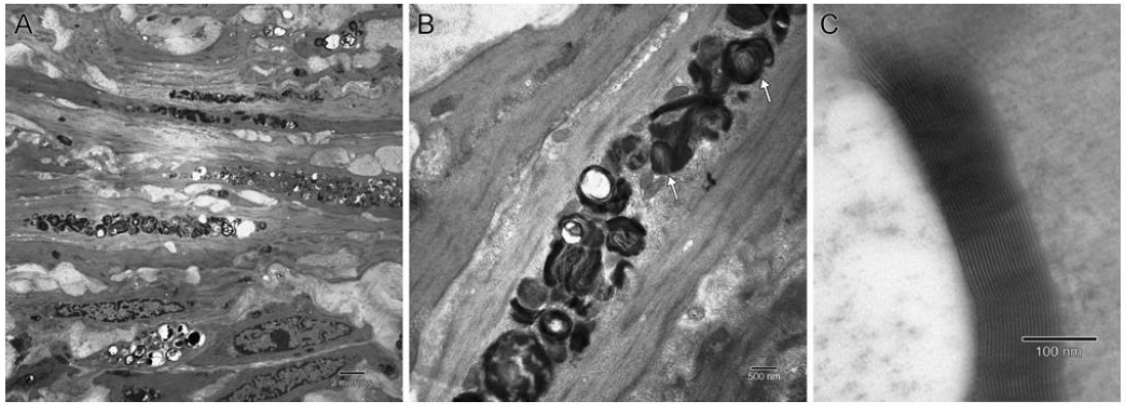


Fig. 3 Electron photomicrographs of the patient's lung biopsy specimen showing (**A**) arterial smooth-muscle cells with multiple inclusions, (**B**) higher magnification disclosing some inclusions to have a whorled appearance (arrows), and (**C**) very high magnification of a portion of an inclusion, demonstrating the periodicity of the lamellated structures

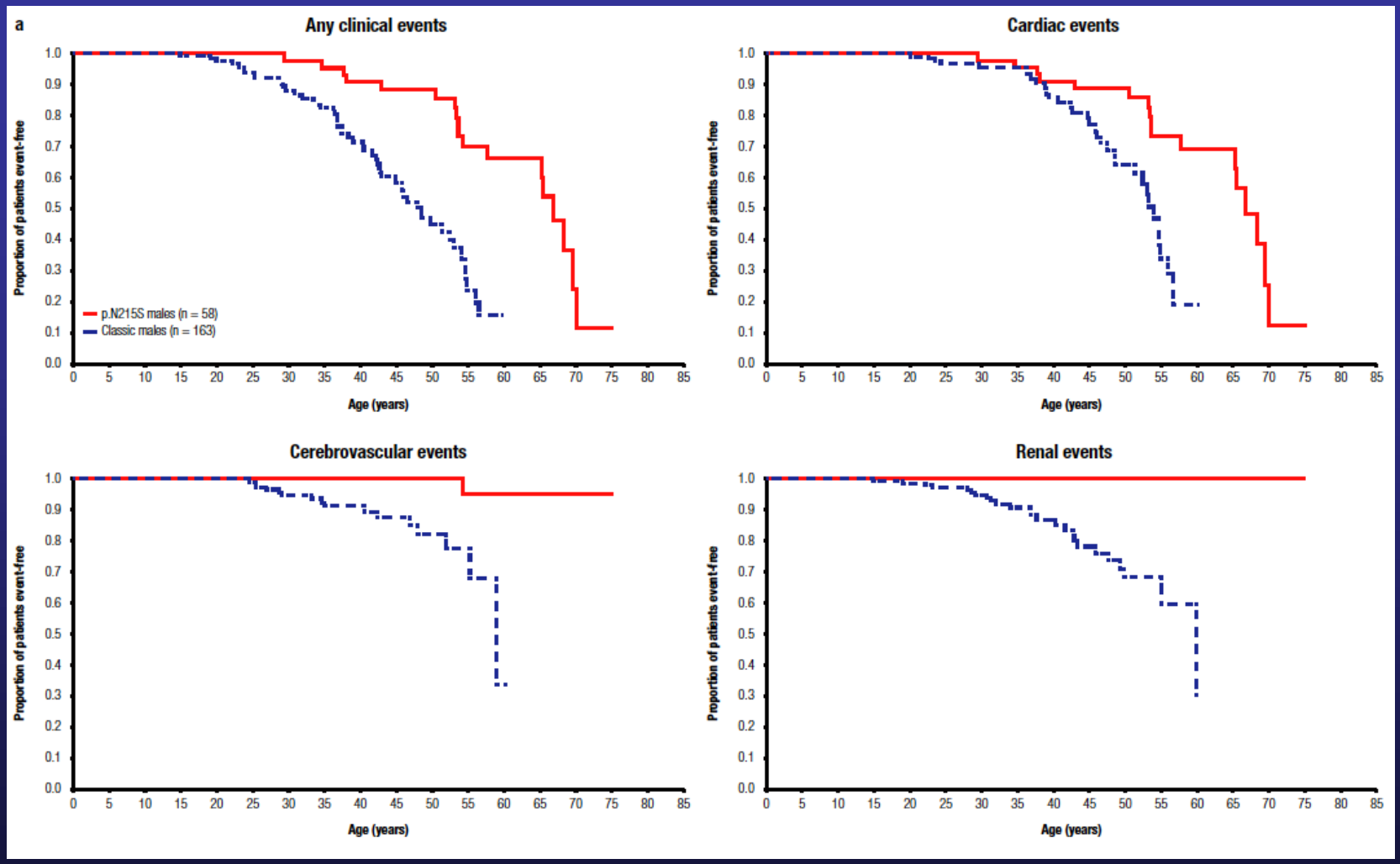
Wang et al: Enzyme replacement therapy stabilizes obstructive pulmonary Fabry disease associated with respiratory globotriaosylceramide storage. J Inherit Metab Dis (2008) 31 (Suppl 2):S369–S374

Phenotype-Genotype Correlations

- Nonsense, frameshift, deletions, splice site mutations usually cause classic, early onset disease in males, are crm-, and have higher biomarkers (GL-3 and lyso-GL3)
- Missense mutations often have some residual enzyme activity, cause more slowly progressive disease, are crm+, and have lower biomarkers

p.N215S

- Eliminates a N-glycosylation site
- Activity about 16% of normal
- Reported as a “cardiac variant”
- Most common later-onset mutation outside of Asia
- Strokes and significant renal disease are uncommon in males
  - Multicenter (Fabry Registry)- Germain et al., Mol Genet Genomic Med, 2018
  - Large single center (London)- Lavalle et al., PLOS One 13(4):e0193550



Germain et al, Mol Genet Genomic Med, 2018

GLA p.A143T

- First reported in an asymptomatic male infant with deficient enzyme activity and no family history of Fabry (Eng et al., Molec Med 3:174-182, 1997).
- The mutation in Anderson's family
- 1:1,975 alleles, 1:2,619 males in Gnomad
  - Highest in Europeans (1:1,054), rare in Asians
- 21% residual enzyme activity *in vitro*
- Classified as a VUS or benign by most labs
  - (labs usually do not report out benign variants)
- Some biopsy proven affected individuals
- Most seem unaffected

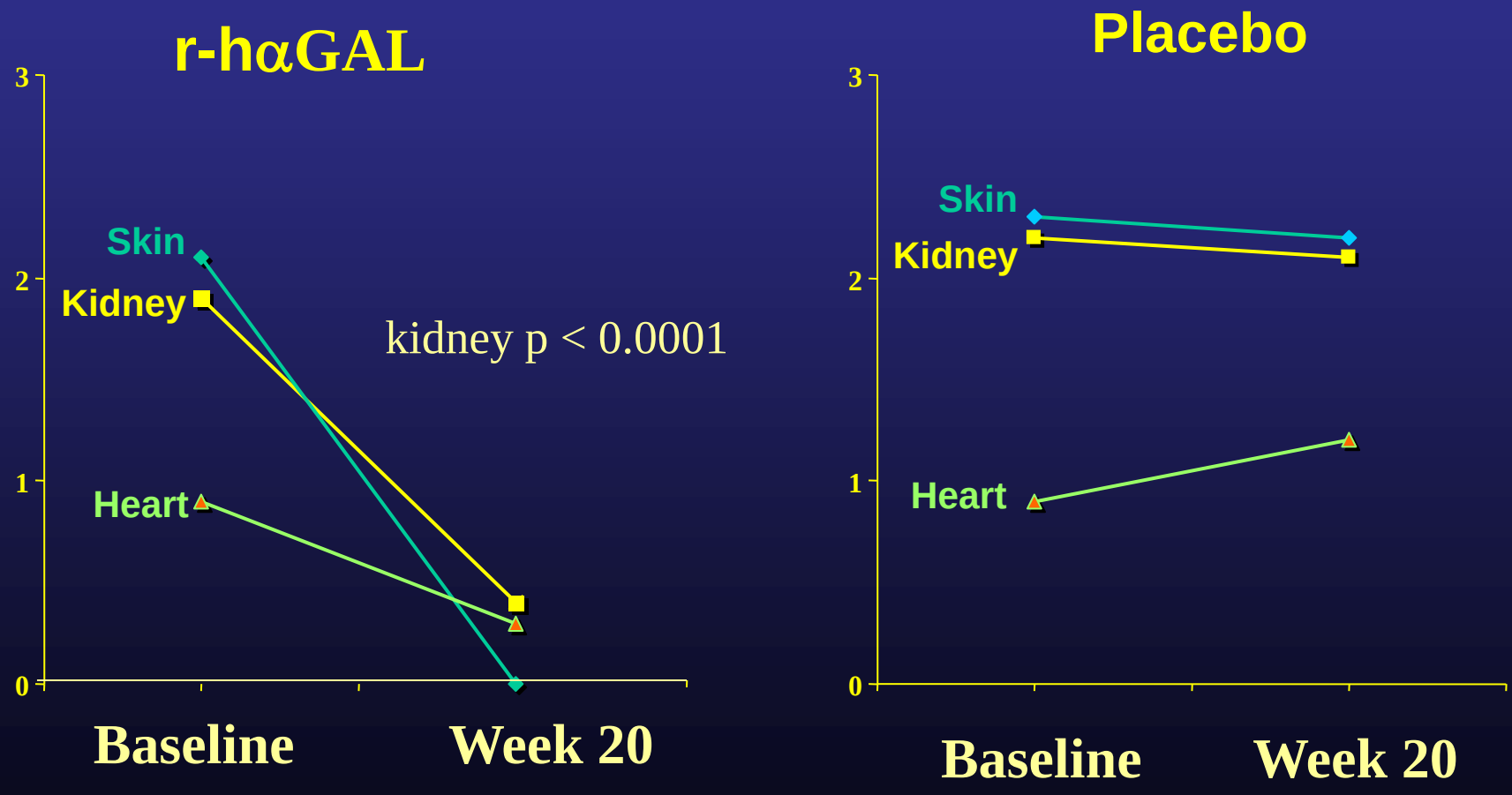
c.636+319G>A (IVS4+919G>A)

- 1:6,000 males in Taiwan, common in Asians
- Causes later onset cardiac disease (hypertrophy >40 years), but there is apparent non-penetrance in some
- Cardiac fibrosis (by MRI) common before LVH in males and females (Hsu et al., J Am Col Cardiology, 68:2254-2263, 2016)

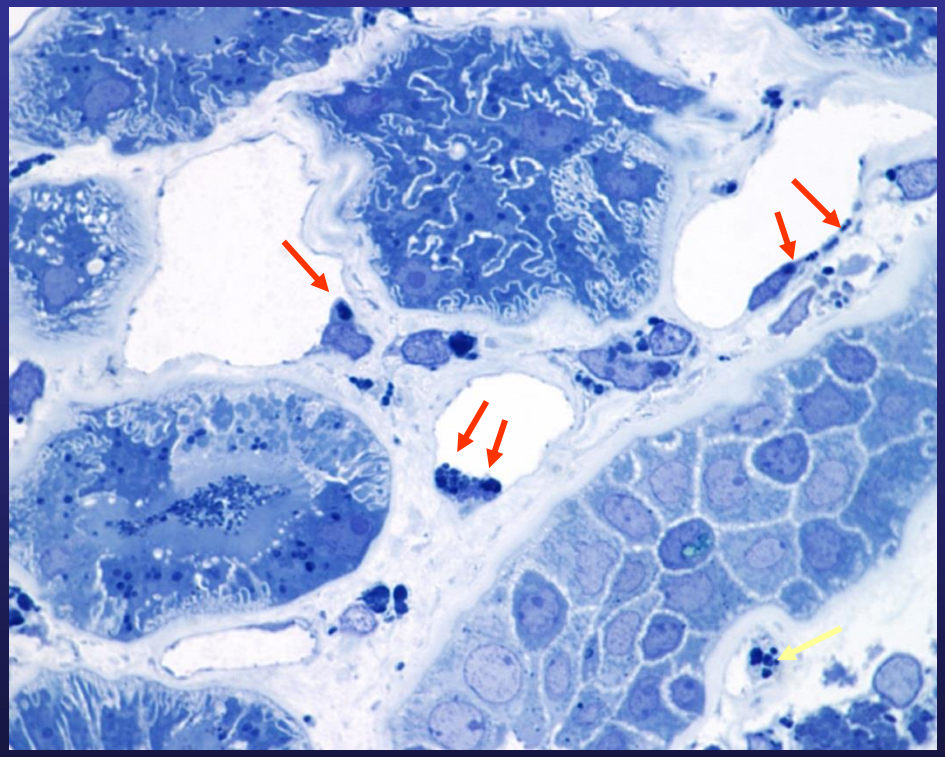
Mean Capillary Endothelium Scores

As Treated Population

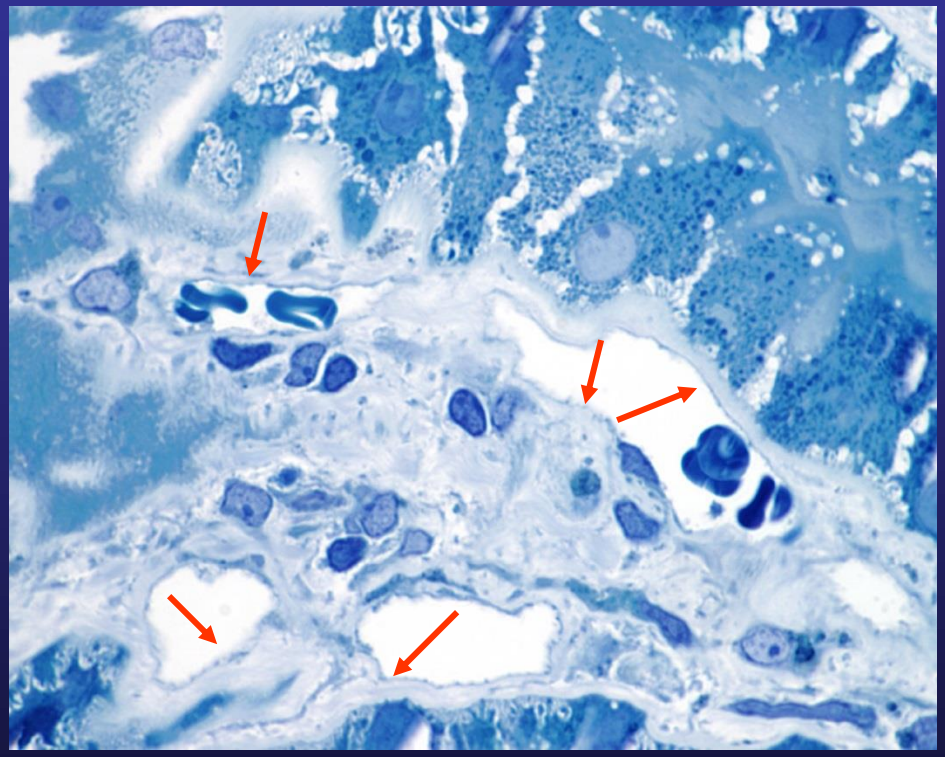
(Scale 0-3)



Primary Endpoint
GL-3 Is Cleared From Peritubular Capillary Endothelium



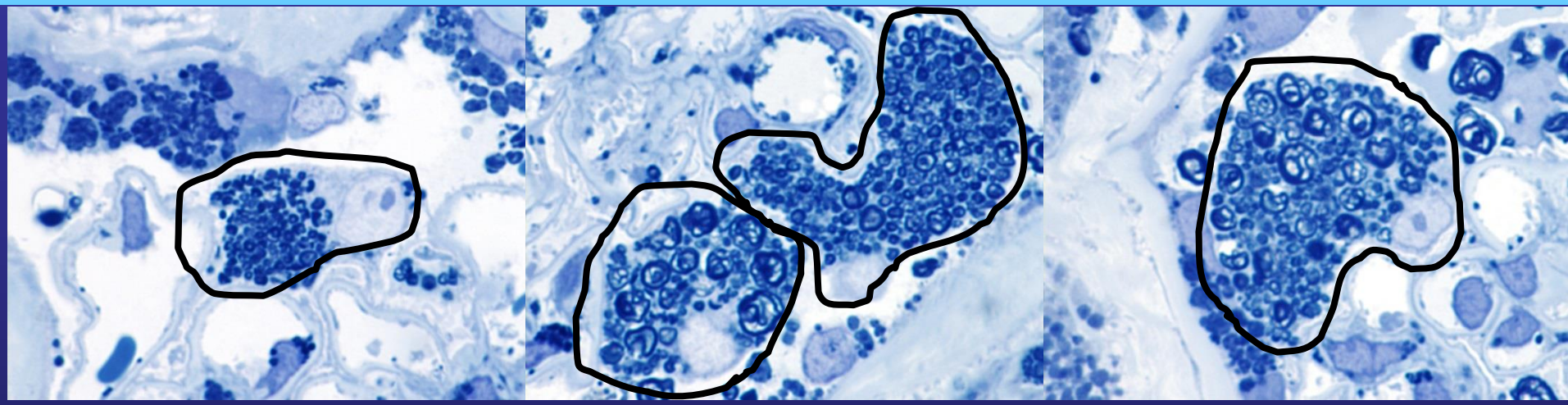
Pre-treatment



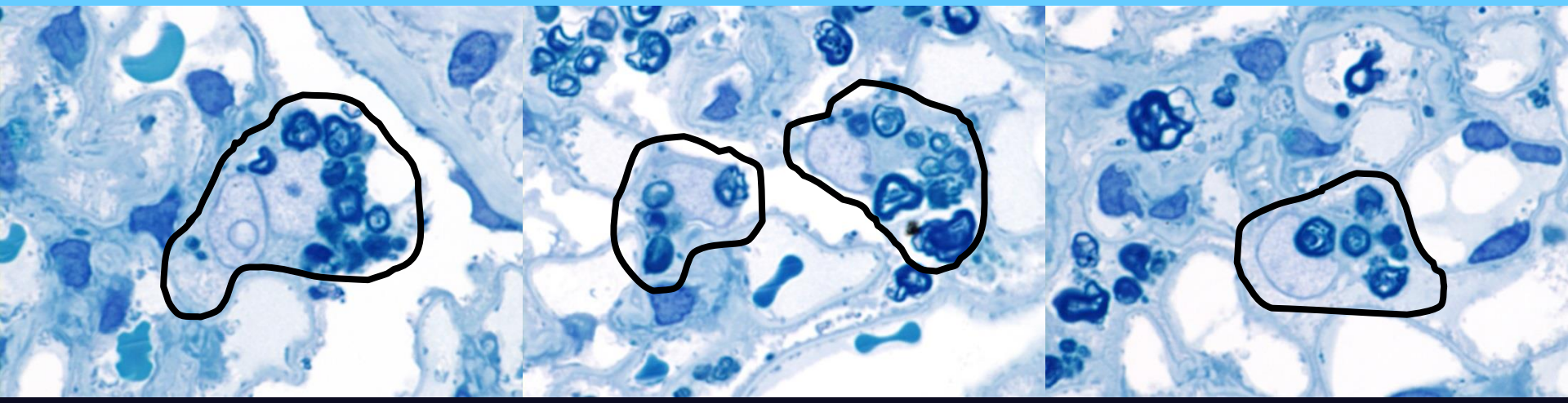
Post-treatment

GL-3 Levels Are Reduced in Podocytes

Pre-treatment

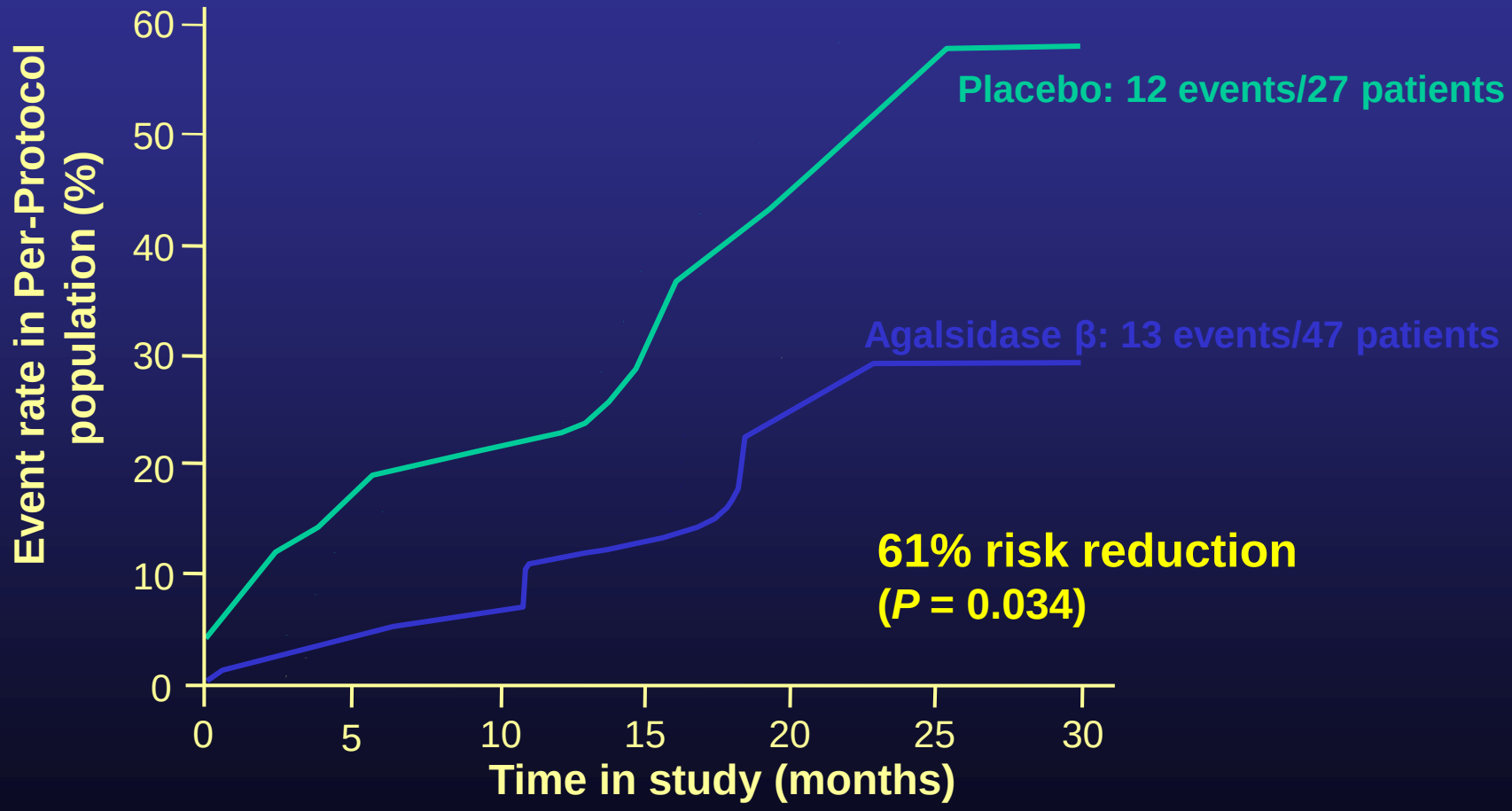


Post-treatment



Per-protocol adjusted primary endpoint

Proteinuria ratio-adjusted Kaplan-Meier predicted probability of an event



Graph is adapted from Banikazemi M, et al. *Ann Intern Med.* 2007;146:77-86.

Table 3
Recommendations for initiation of ERT in pediatric patients with FD.

	US Consensus panel recommendations
Symptomatic male or female pediatric patient	• Treatment with ERT should be considered and is appropriate if Fabry symptoms are present in boys or girls at any age • Signs and symptoms warranting treatment suggest major organ involvement – Neuropathic pain crises/Fabry neuropathy – Renal disease (decline in eGFR, pathological albuminuria or pathological proteinuria, creatinine elevation, cellular GL-3 accumulation or evidence of tissue damage such as podocyte effacement on renal biopsy) – Cardiac disease (cardiomyopathy or arrhythmia (including sinus bradycardia) attributable to FD) – Recurrent abdominal pain and diarrhea (excluding alternative causes) – Exercise intolerance and impaired sweating
Asymptomatic male patients with classical (severe) mutations	Timing of ERT depends on individual case (balancing risks and benefits of therapy) Serious discussion regarding the timing of ERT initiation is recommended by age 8–10 years for boys with classical mutations
Asymptomatic female patients and asymptomatic male patients with late-onset mutations or variants of unknown significance	Decision to defer ERT should be based on comprehensive longitudinal monitoring for the development of clinical symptoms and signs of disease, as defined above Family history of the female patients should also be considered

eGFR, estimated GFR; ERT, enzyme-replacement therapy; FD, Fabry disease; GL-3, globotriaosylceramide.

Hopkin et al, Mol Genet Metab, 117:104, 2016

Table 2
Recommendations for initiation of ERT in adult male and female patients with classic or later-onset mutations, or *GLA* VUS.

Adult patient population	Recommendation for the initiation of ERT
Classic Fabry mutation	
• Male patient, symptomatic or asymptomatic	• ERT should be considered and is appropriate in all patients at any age of presentation[a]
• Female patient, symptomatic	• Signs/symptoms suggesting major organ involvement, warranting initiation of ERT - neuropathic pain, pain crises, Fabry disease neuropathy - proteinuria/albuminuria NOT attributable to other causes, evidence of renal impairment (may require renal biopsy if isolated) - stroke or TIA - symptomatic cardiac disease not due to other causes (dyspnea, palpitations, syncope, chest pain) - recurrent diarrhea, chronic, disabling GI dysfunction (excluding alternative causes) - exercise intolerance and impaired sweating
• Female patient, asymptomatic[b]	• ERT should be considered if there is laboratory, histological, or imaging evidence of injury to the kidney, heart, or the CNS - renal disease: decreased GFR (< 90 mL/min/1.73 m^2 adjusted for age > 40 years [GFR category $\geq$ G2], persistent albuminuria > 30 mg/g [albuminuria category A2 or A3]), podocyte foot process effacement or glomerulosclerosis on renal biopsy, moderate or severe GL-3 inclusions in a range of renal cell types - silent strokes, cerebral white matter lesions (on brain MRI)[c] - asymptomatic cardiac disease (cardiomyopathy or arrhythmia, cardiac fibrosis on contrast cardiac MRI) • ERT should also be considered if a skewed X chromosome inactivation pattern with predominant expression of the mutant *GLA* allele with or without very low α-Gal A activity have been demonstrated in the presence of signs and symptoms of disease
Later-onset Fabry mutation or missense *GLA* VUS	
• Male and female patients	• ERT should be considered and is appropriate if there is laboratory, histological, or imaging evidence of injury to the kidney, heart, or the CNS, as detailed above, even in the absence of typical Fabry symptoms. The abnormalities should be attributable to Fabry disease; this may require histological assessment or biochemical evidence of GL-3 accumulation • The advice of an expert in genetics and management of Fabry disease should be sought for interpretation of the pathogenicity of any VUS • Individuals with well characterized benign *GLA* polymorphisms should not be treated with ERT • In the absence of demonstrable Fabry disease-related tissue pathology or clinical symptoms, ERT may not be appropriate, particularly in heterozygous female patients. These patients should be monitored regularly by a multidisciplinary care team

CNS, central nervous system; ERT, enzyme replacement therapy; α-Gal A, α-galactosidase A; GFR, glomerular filtration rate; GI, gastrointestinal; GL-3, globotriaosylceramide; MRI, magnetic resonance imaging; TIA, transient ischemic attack; VUS, variant of unknown significance.

[a] Treatment decisions may be influenced by advanced elderly age of the patient and severe comorbidity.

[b] Treatment decisions in female patients may be guided by the X chromosome inactivation profile, if assessed. Predominant expression of the mutant *GLA* allele is generally associated with rapid disease progression, requiring closer monitoring and early therapeutic intervention [6].

[c] See also online Appendix D.

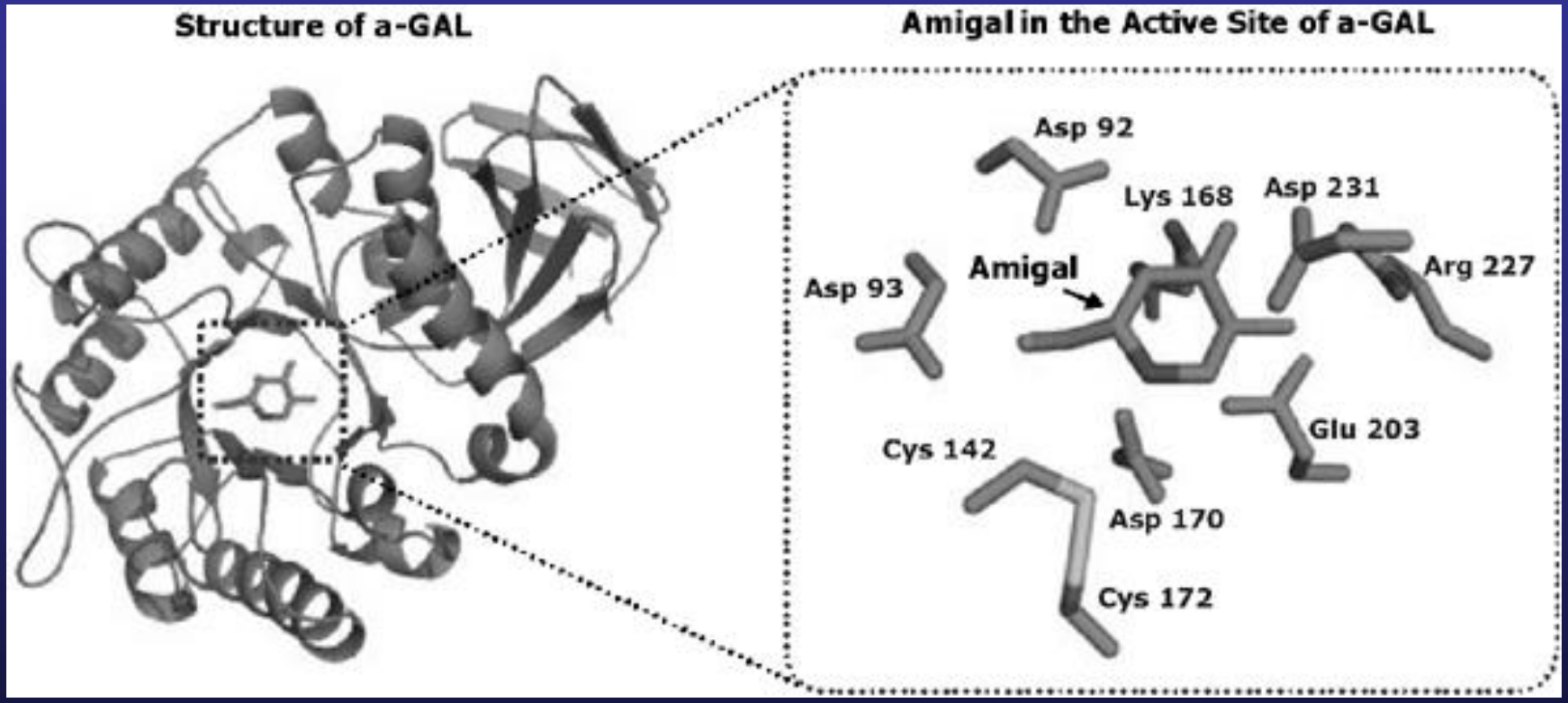
Ortiz et al, Mol Genet Metab 123:416, 2018

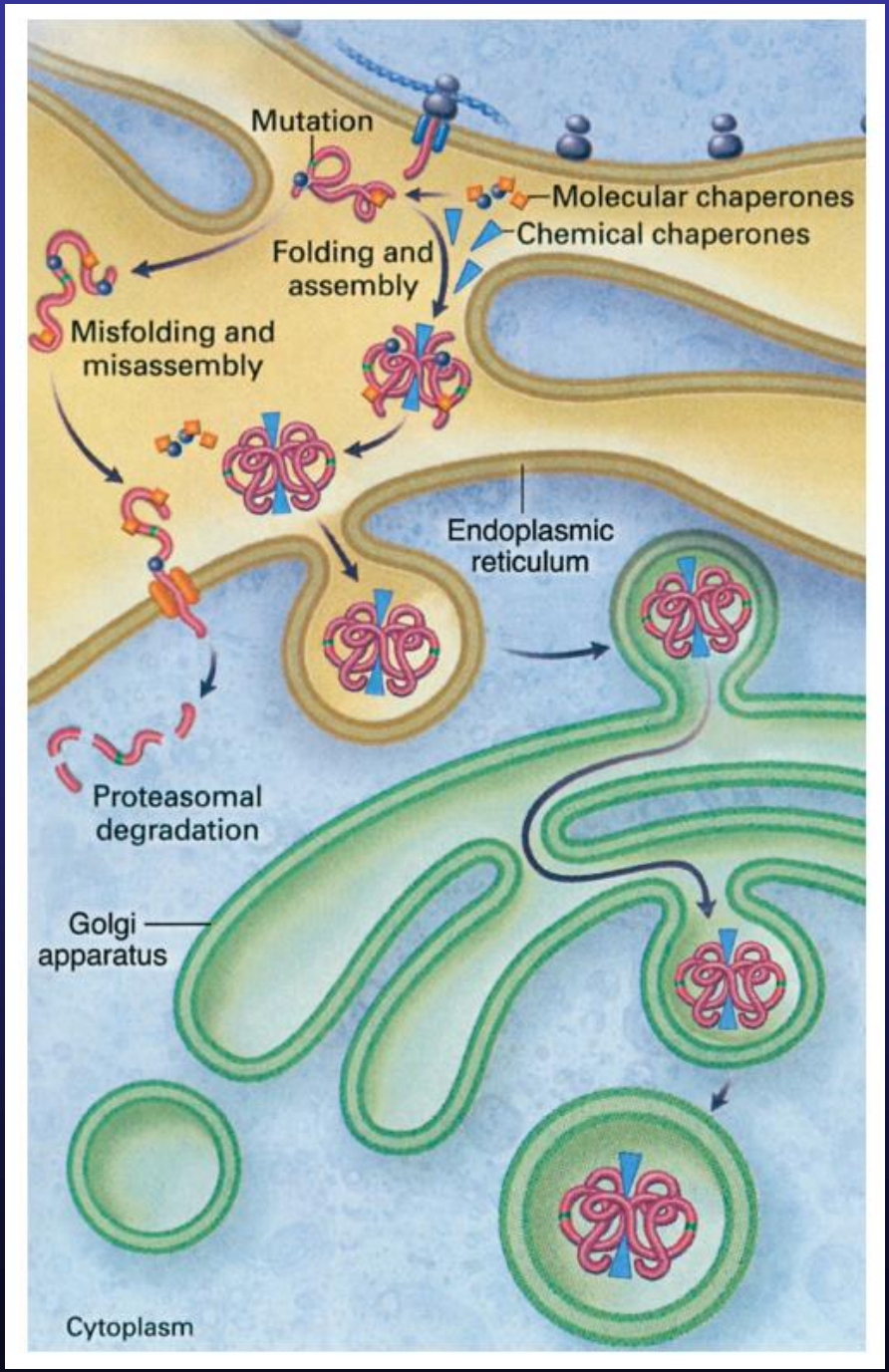
Antibody Formation

- Most males make antibodies to enzyme and many have infusion reactions early during treatment, most females do not
- Some males tolerize over time
- Neutralizing antibodies, when present, can interfere with efficacy, especially at lower doses of enzyme

Chaperone Therapy

- Migalastat (Amicus) oral therapy
  - FDA approved 2018
- Use is limited to ”amenable” mutations
- Increases endogenous functional enzyme, the amount depends on the particular mutation
- Over 18 months not inferior to ERT
  - Hughes et al, J Med Genet 54:288-296, 2017
- *Post- hoc* analyses suggests possible cardiac benefit over ERT
  - Hughes et al, Germain et al, NEJM 375:545-555, 2016


Structure of a-GAL
Amigal in the Active Site of a-GAL
Asp 92
Lys 168
Asp 231
Asp 93
Amigal
Arg 227
Glu 203
Cys 142
Asp 170
Cys 172



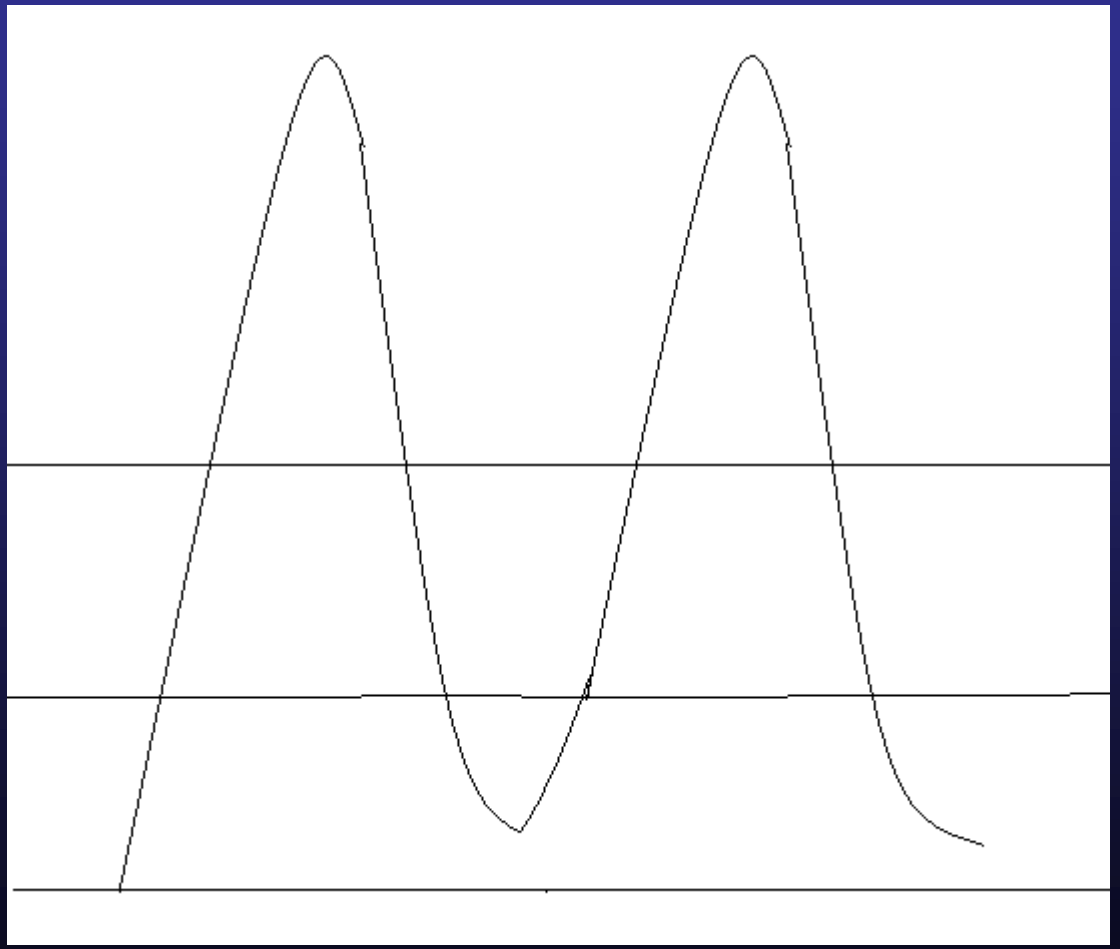
Chemical Chaperones

Perlmutter, Peds Res 52:932, 2002

Unique dosing problems with a chaperone/ competitive inhibitor

Chaperoning

Enzyme active



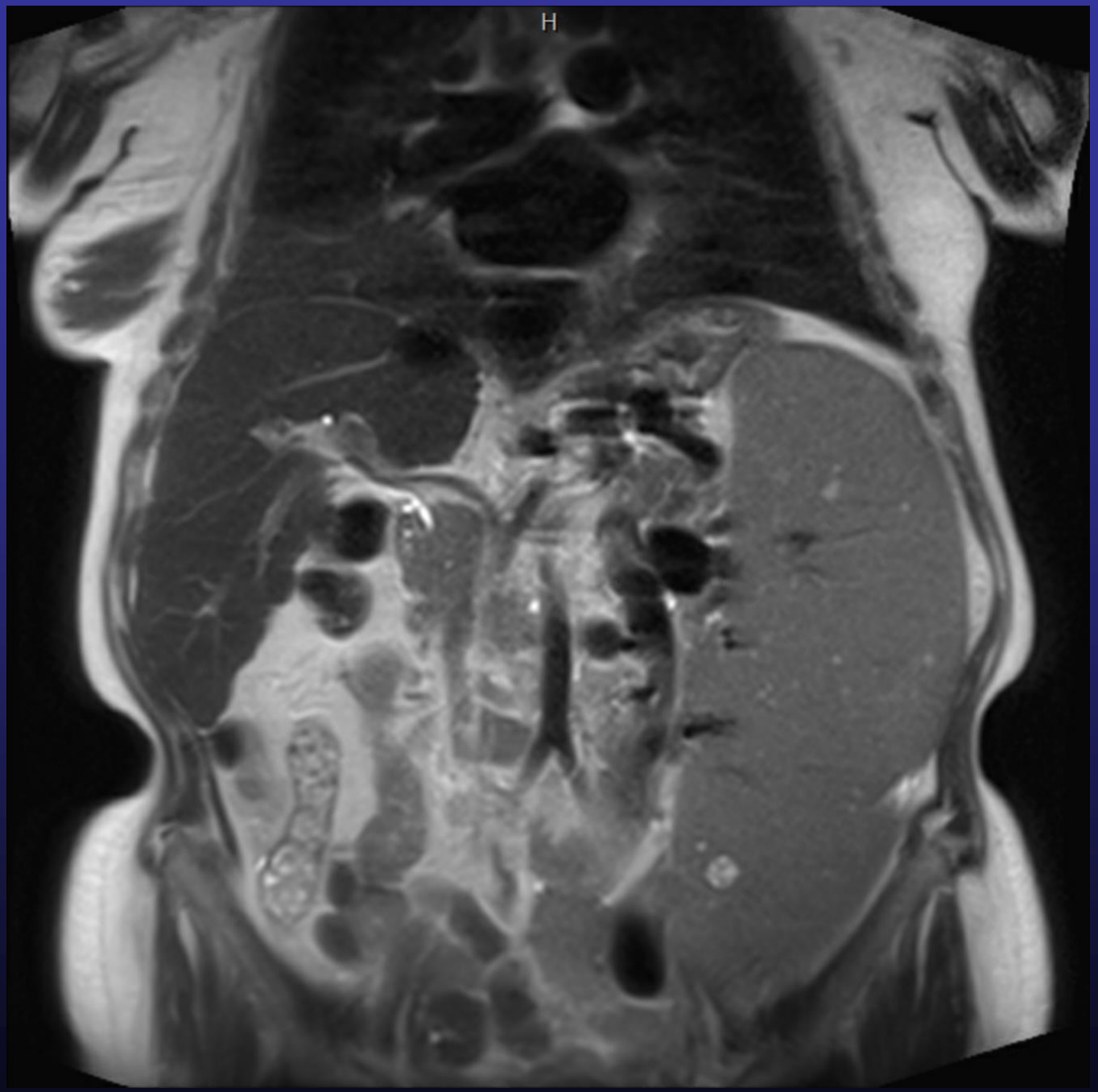
Mutation Resources

- International Fabry Disease Genotype-Phenotype Database (Mt. Sinai) dbfgp.org
- Sakuraba database fabry-database.org
- Amicus *in vitro* assay data- supplementary table, Benjamin et al Genet Med 19:430-438, 2017

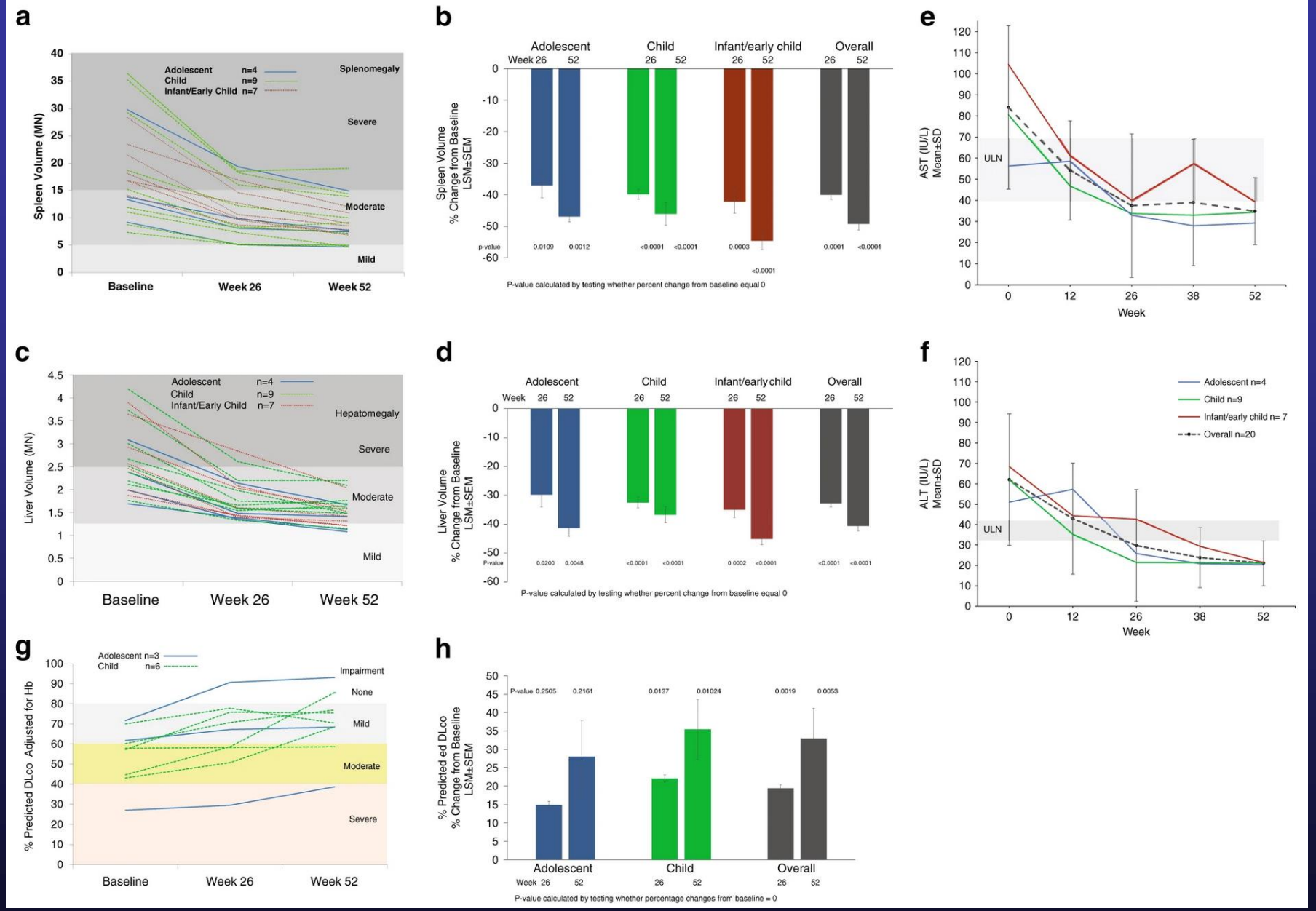
Salvador Dalí, Galacidalacidesoxiribunucleicacid

Acid sphingomyelinase Deficiency

- Niemann-Pick A (neurovisceral)
  - rapid neurodegeneration, organ enlargement, FTT, early mortality
- Niemann-Pick B (chronic neurovisceral)
  - greatest storage is in reticuloendothelial cells-spleen, liver, lungs
  - Abnormal lipids, anemia, thrombocytopenia, impaired lung diffusion capacity and fibrosis, portal hypertension
- Intermediate cases (NPA/B)



51 year old
Hepatic fibrosis
Portal hypertension
Thrombocytopenia
Interstitial lung disease



Diaz GA, Jones SA, Scarpa M, Mengel KE, Giugliani R, Guffon N, Batsu I, Fraser PA, Li J, Zhang Q, Ortemann-Renon C. One-year results of a clinical trial of olipudase alfa enzyme replacement therapy in pediatric patients with acid sphingomyelinase deficiency. Genet Med. 2021 Aug;23(8):1543-1550.

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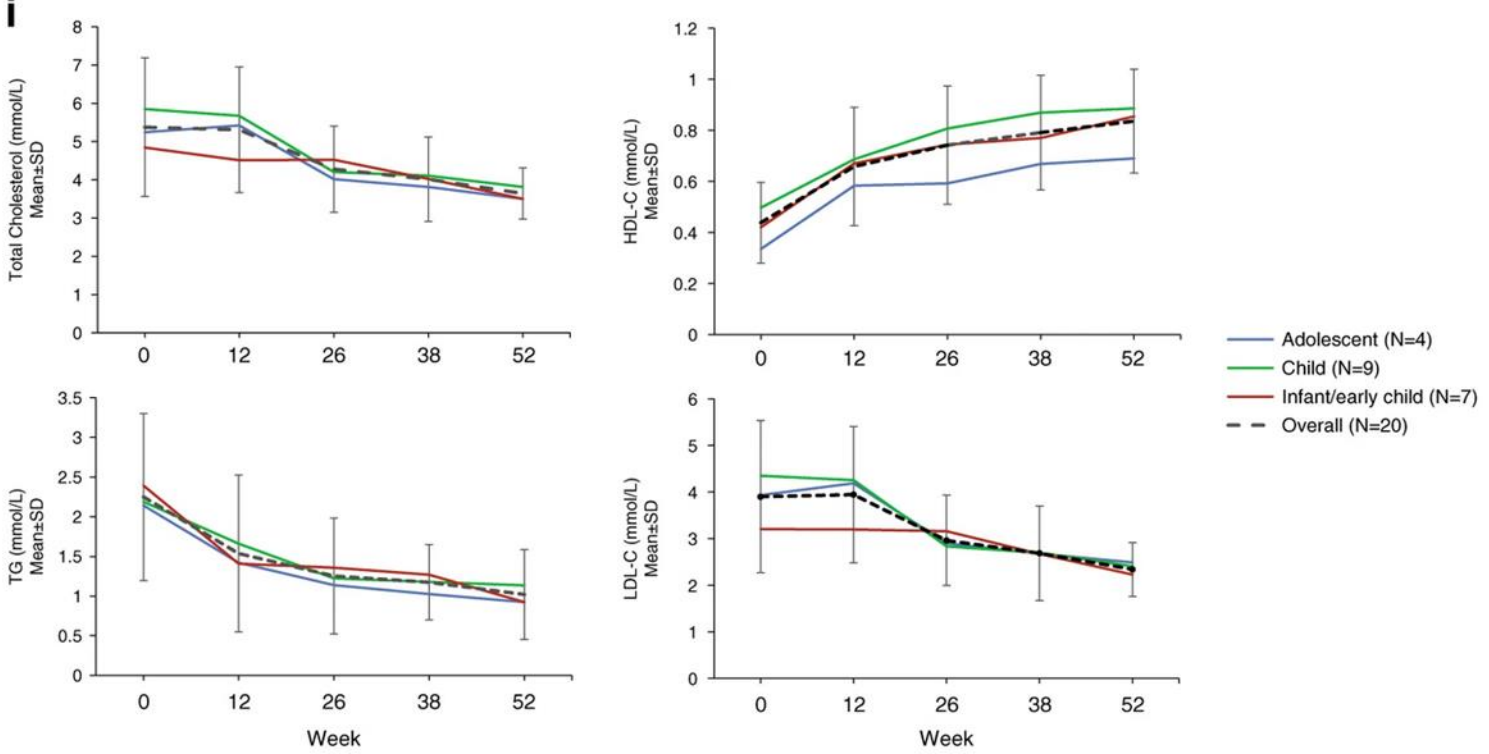

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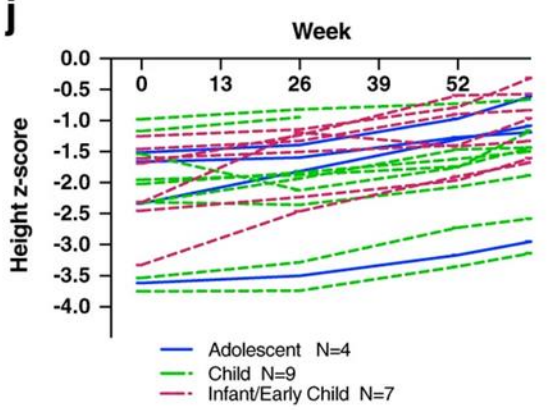
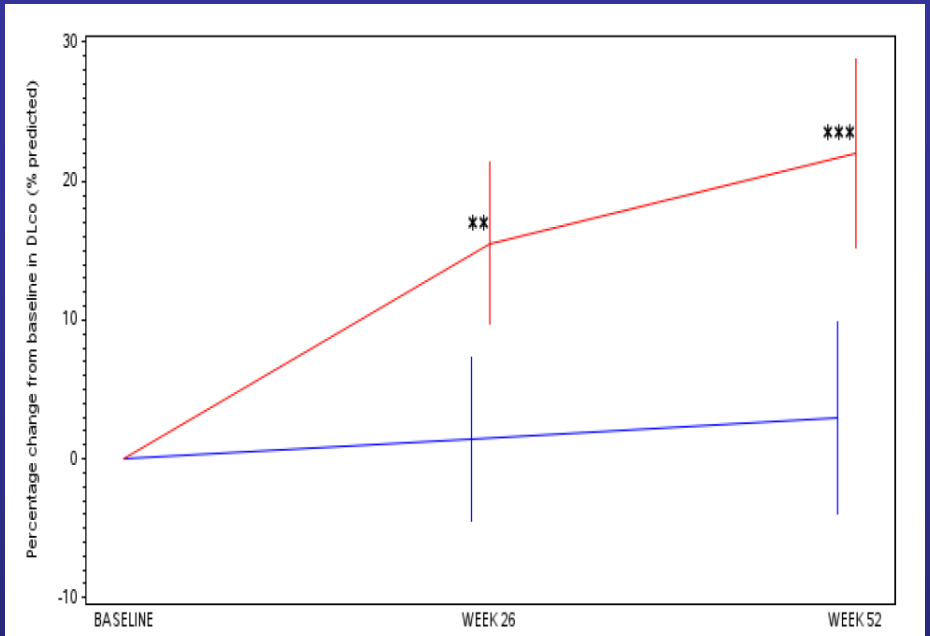
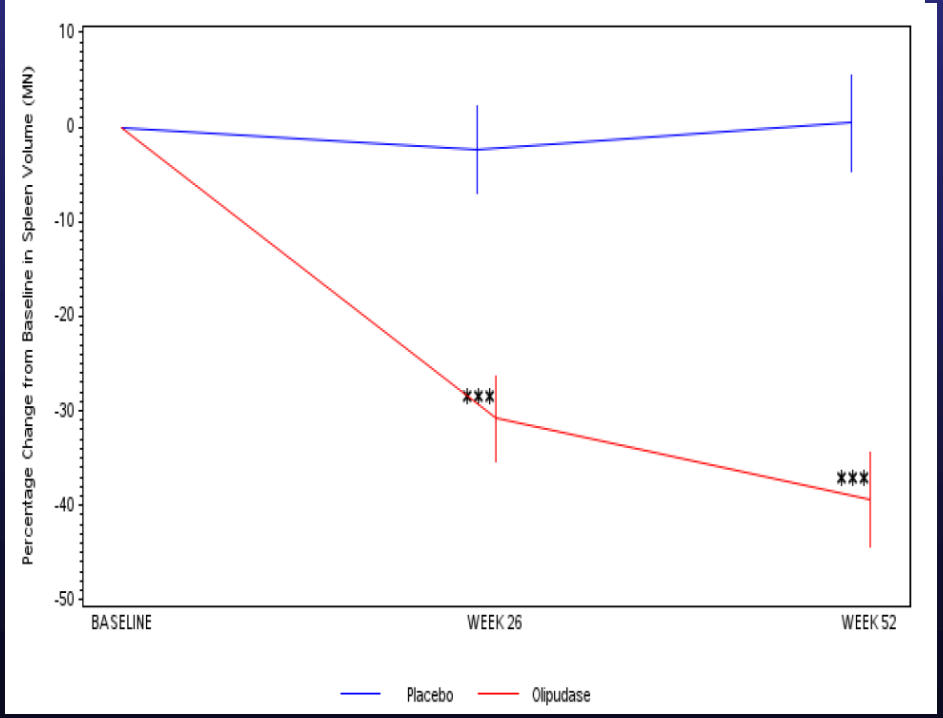


Group	Height z-score change from baseline Least Square Mean (95% CI) P value	
	Wk26	**Wk52**
Adolescent (n=4)	0.206 (-0.347,0.758) 0.2502	0.606 (-0.159,1.370) 0.0763
Child (n=9)	0.074 (-0.158,0.306) 0.4761	0.371*(0.103,0.639) **0.0148**
Infant/Early Child (n=7)	0.484 (0.077, 0.890) **0.0298**	0.736 (0.405,1.068) **0.0023**
Overall (n=20)	0.231 (0.060, 0.402) **0.0112**	0.555 (0.377,0.733) **<0.0001**

P value calculated by testing whether change from baseline=0
* based on eight patients





Double-blind, placebo controlled ERT trial

	DFI12712 ASCEND			
	Placebo (N = 18)	**Olipudase alfa (N = 18)**	**LS Mean difference**	**P-value**
% change in DLco (% predicted)	2.96 (3.38)	21.97 (3.34)	19.01 (4.76)	0.0004
% change in spleen volume (MN)	0.48 (2.50)	-39.45 (2.43)	-39.93 (3.50)	<.0001
% change in liver volume (MN)	-1.42 (4.00)	-31.67 (3.85)	-30.25 (5.55)	<.0001
% change in platelet count (pre-infusion)	2.49 (4.19)	16.82 (4.00)	14.33 (5.78)	0.0185
Change in chest X-ray interstitial improvement	0.28 (0.13)	-0.91 (0.12)	-1.19 (0.18)	<.0001
% change FVC (% predicted)	1.42 (1.53)	6.74 (1.63)	5.32 (2.24)	0.025
Change in HRCT GG	0.18 (0.16)	-0.49 (0.16)	-0.67 (0.22)	0.006
Change in HRCT ILD	0.09 (0.16)	-0.36 (0.15)	-0.45 (0.22)	0.047
% change ALT (IU/L) (pre-infusion)	-0.98 (8.68)	-36.55 (8.32)	-35.58 (12.04)	0.006

Diagnose and treat LSDs before it is too late…

Rush, Snakes and Arrows

Medical Biochemical Genetics at Emory

- 7 geneticists (5 biochemical), behavioral pediatrician
- Metabolic nutrition team with research
- Newborn screening follow up program and NIH pilot studies
- Genetic Clinical Trials Center- >30 protocols
- Large patient volume
- Adult and pediatric inpatient consultation service (CHOA and EUH)

Contact
william.wilcox@emory.edu

Time for Questions

MEDICAL BIOCHEMICAL GENETICS

CLINICAL CORE SEMINAR SERIES

Hosted by:

University of Colorado Anschutz Medical Campus

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Please leave feedback for this session using the QR code below or use this link.

Recordings of lectures (and handouts, if applicable) will be found later today at our dropbox until a permanent place is found.