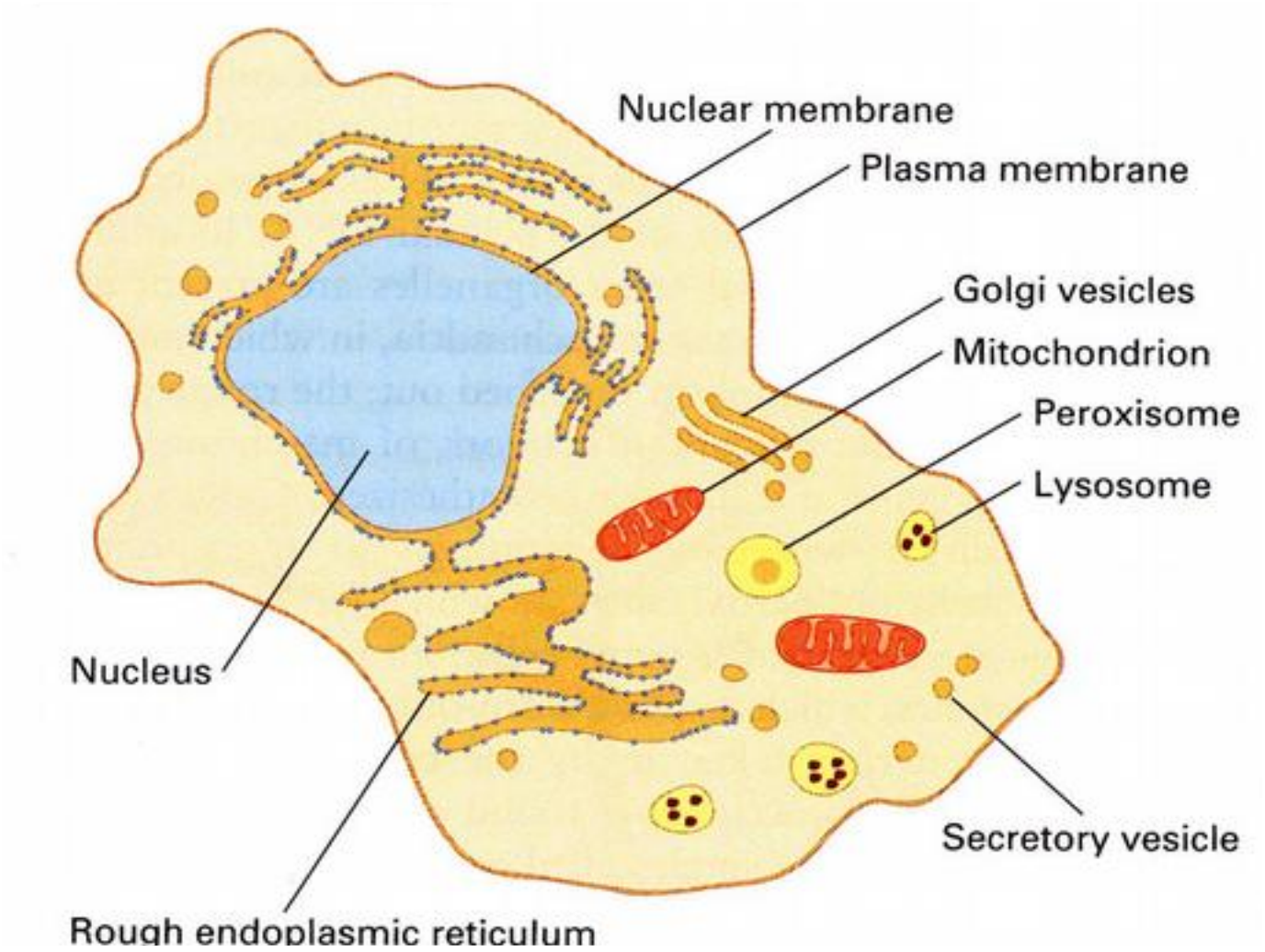
Lysosomes and lysosomal disorders

Eukaryotic cell



Alberts *et al :* Molecular biology of the cell 6 edition

Lysosomes

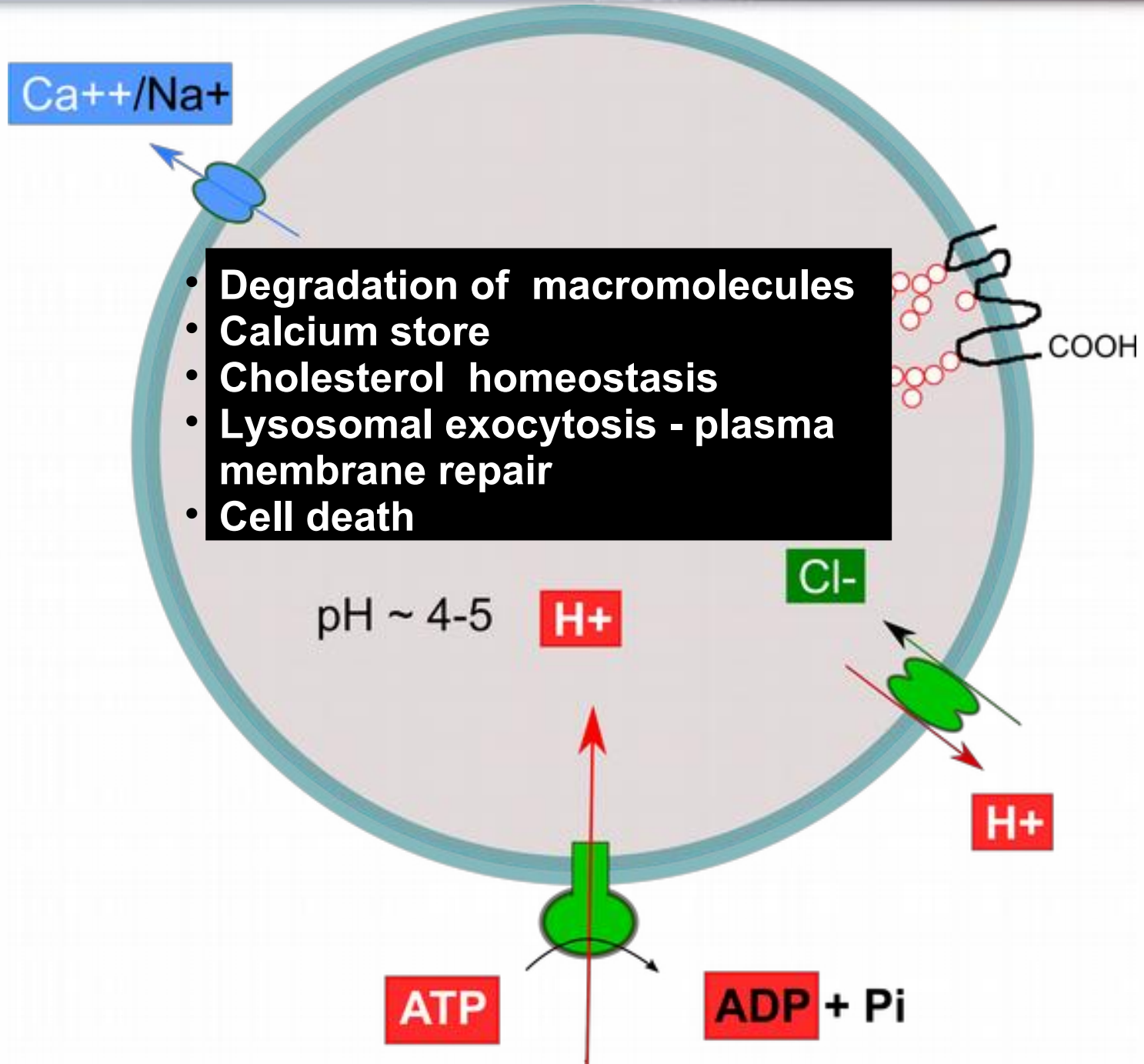


Image M.H.

Lysosomes

Lysosomes

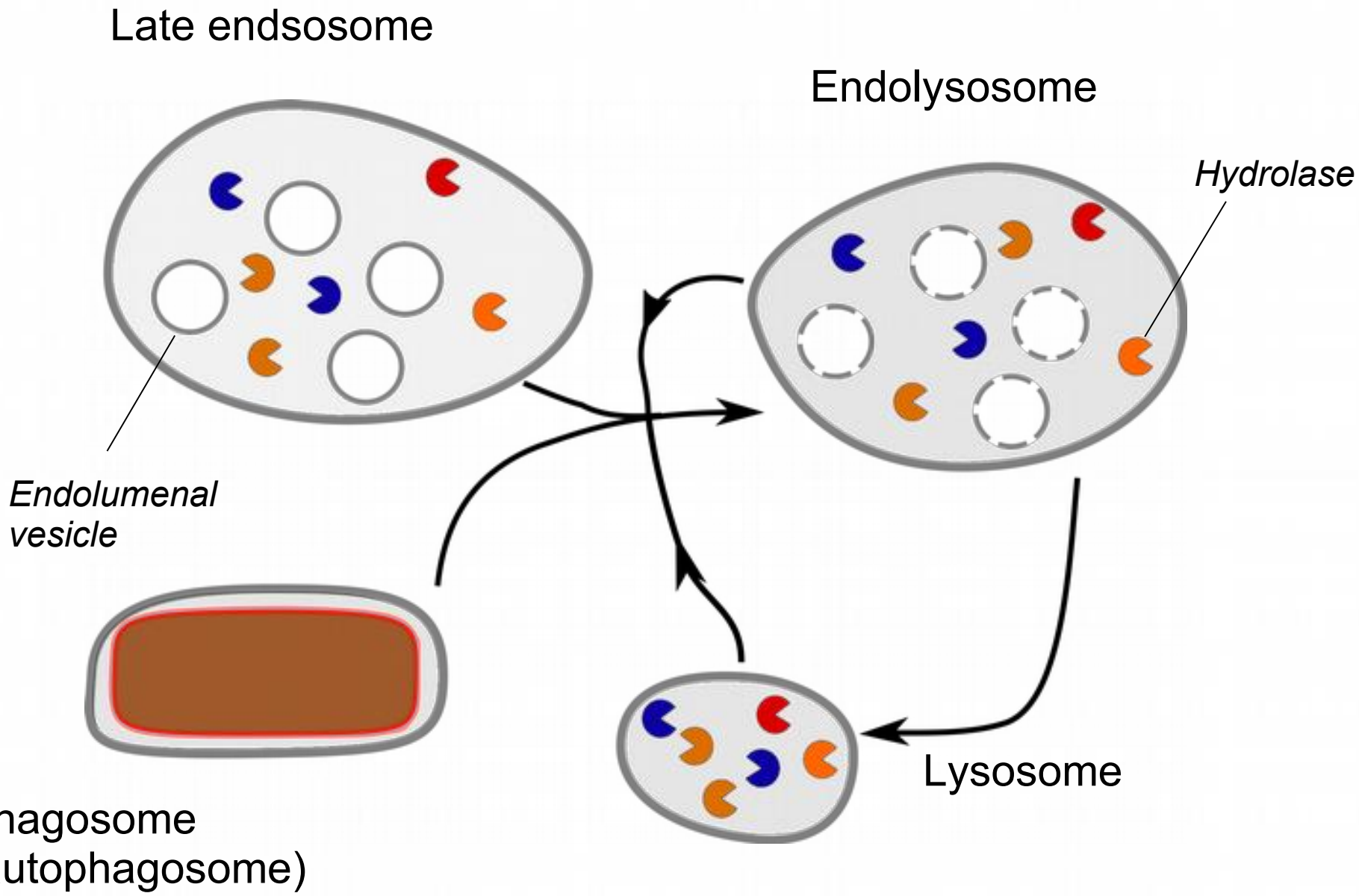
Lysosomes are the principal sites of intracellular degradation of macromolecules

about 40 types of **acid hydrolases** -
proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases, and sulfatases.
acidic pH optimum – protection of cytosol (neutral pH)

acidic environment – (pH 4.5 -5) – maintained by **vacuolar H^+ ATPase**
H+ gradient drives transport of small molecules across the membrane

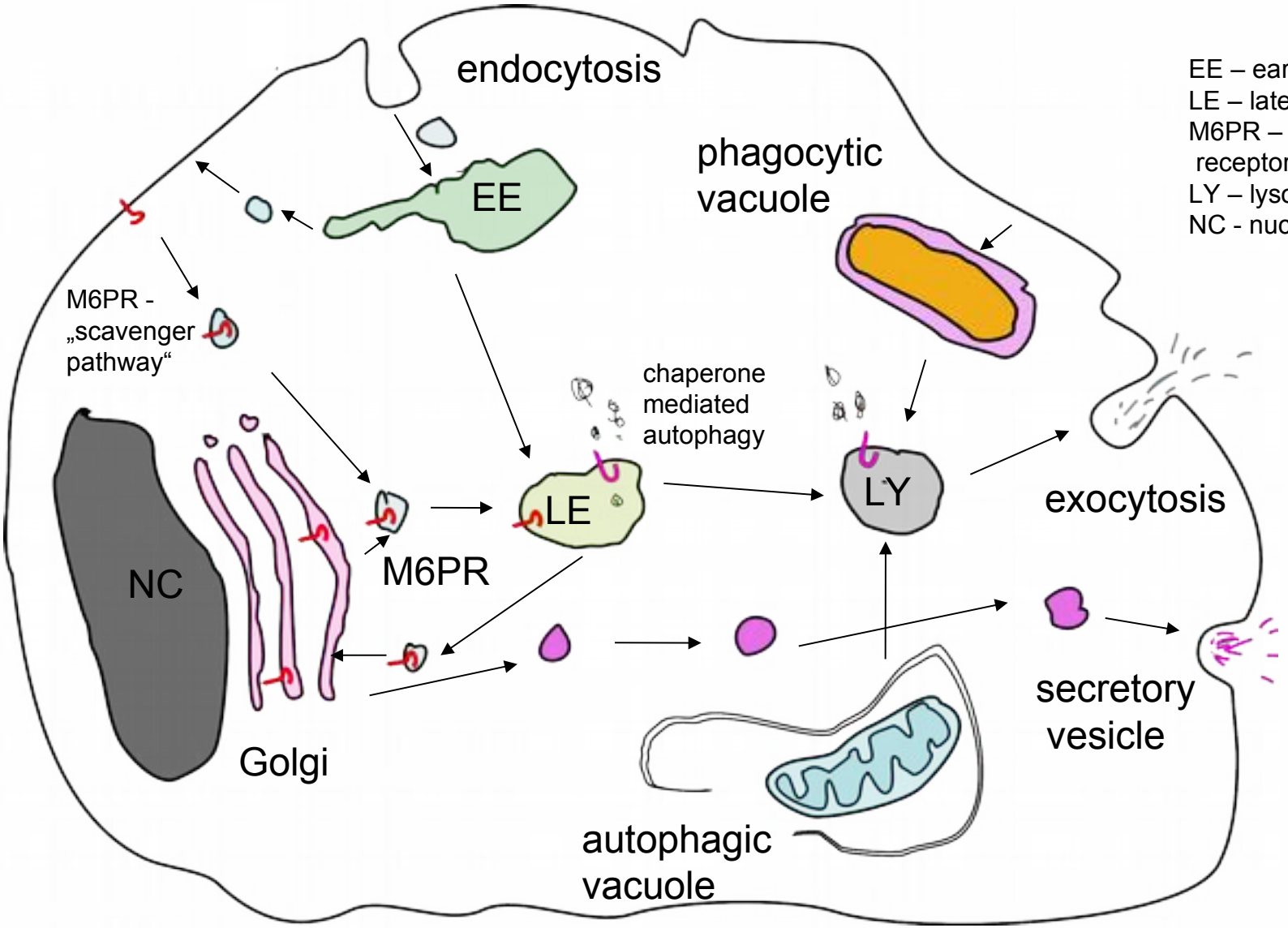
lysosomal membrane proteins are **highly glycosylated** –
protection from proteolytic attack
provide interface for various lysosomal functions

Maturation of lysosomes



adapted from Alberts et al. Molecular cell biology

Lysosomes and vacuolar transport



EE – early endosome
LE – late endosome
M6PR – mannosa-6-phosphate receptor
LY – lysosome
NC - nucleus

Image M.H.

“Unusual” lysosomes

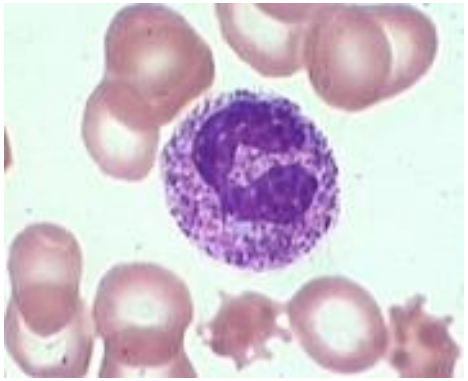
Secretory lysosomes /Lysosome-related organelles

In some cells (often of haematopoietic origin) there are organelles that have properties of <u>both lysosomes and secretory granules</u>

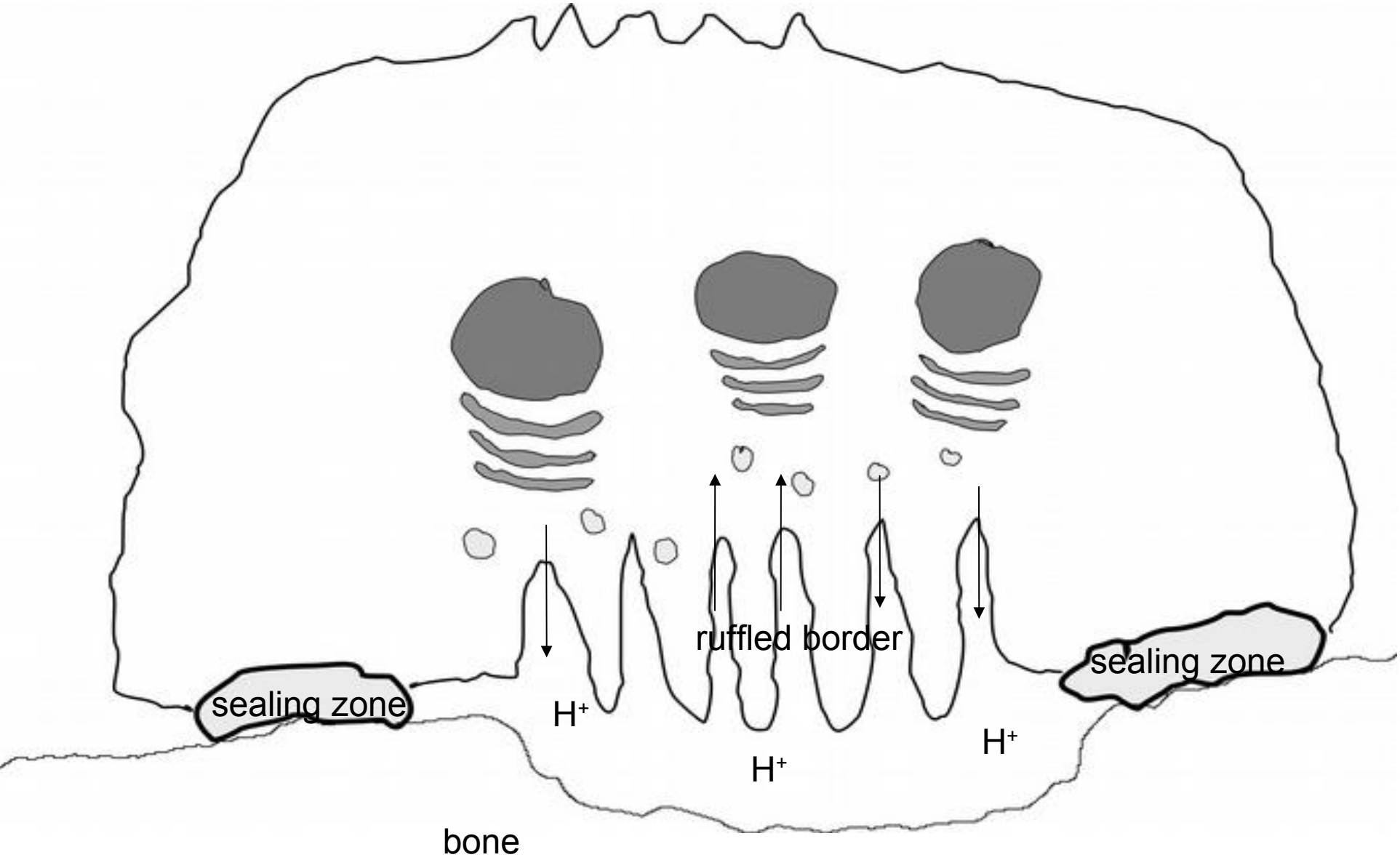
- acidic pH
- lysosomal membrane and lumenal proteins
- exocytosis in response to a stimulus

Lysosome-related organelles (LRO)

-lytic granules (NK cells and cytotoxic T-lymphocytes)
-azurophilic granules →
-melanosomes
-"external" lysosomes of osteoclasts
- delta-granules in platelets

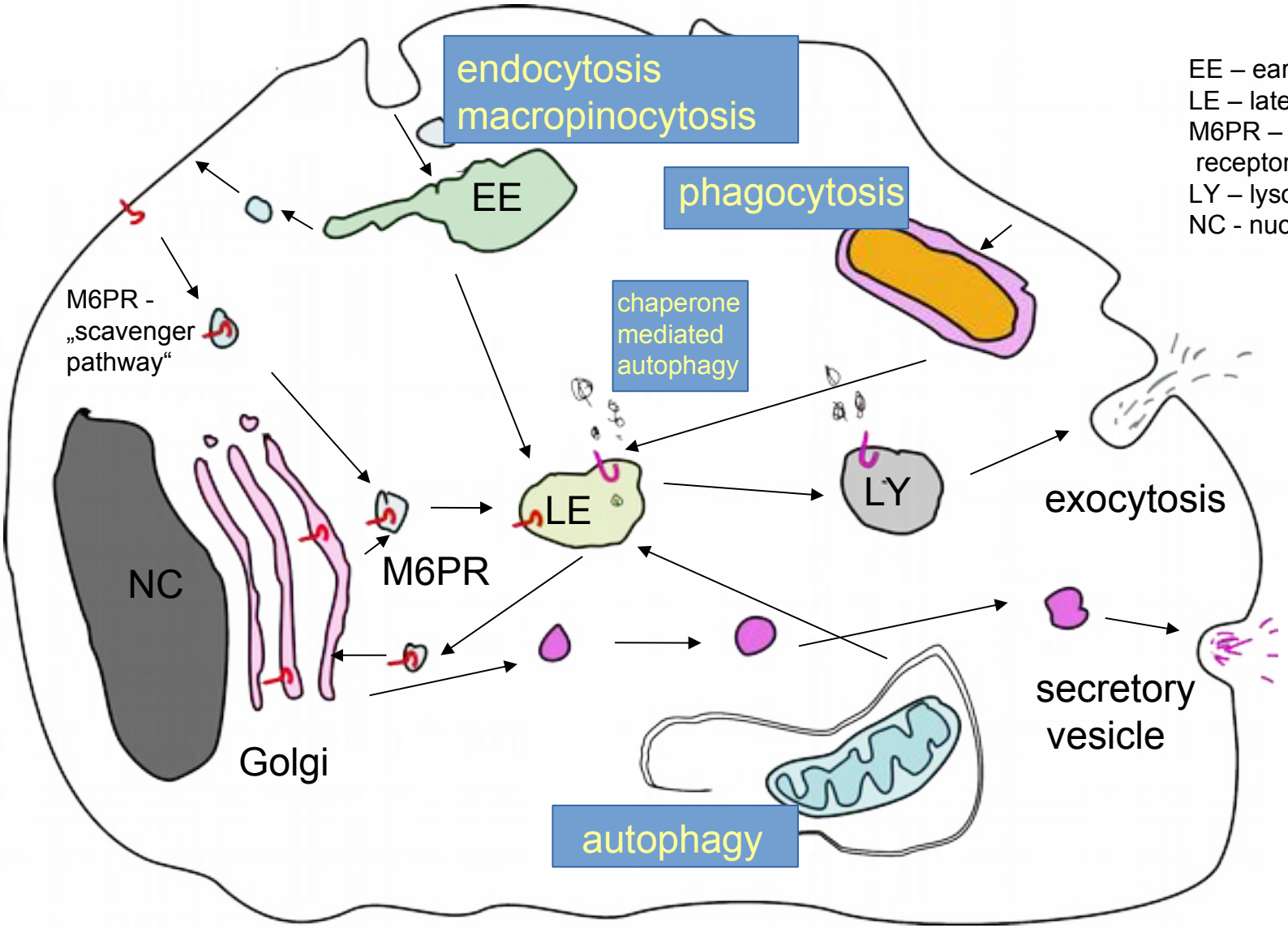


Lysosome-related organelles - osteoclast



Transport of proteins and material for degradation to lysosomes

Multiple pathways deliver material to lysosomes



EE – early endosome
LE – late endosome
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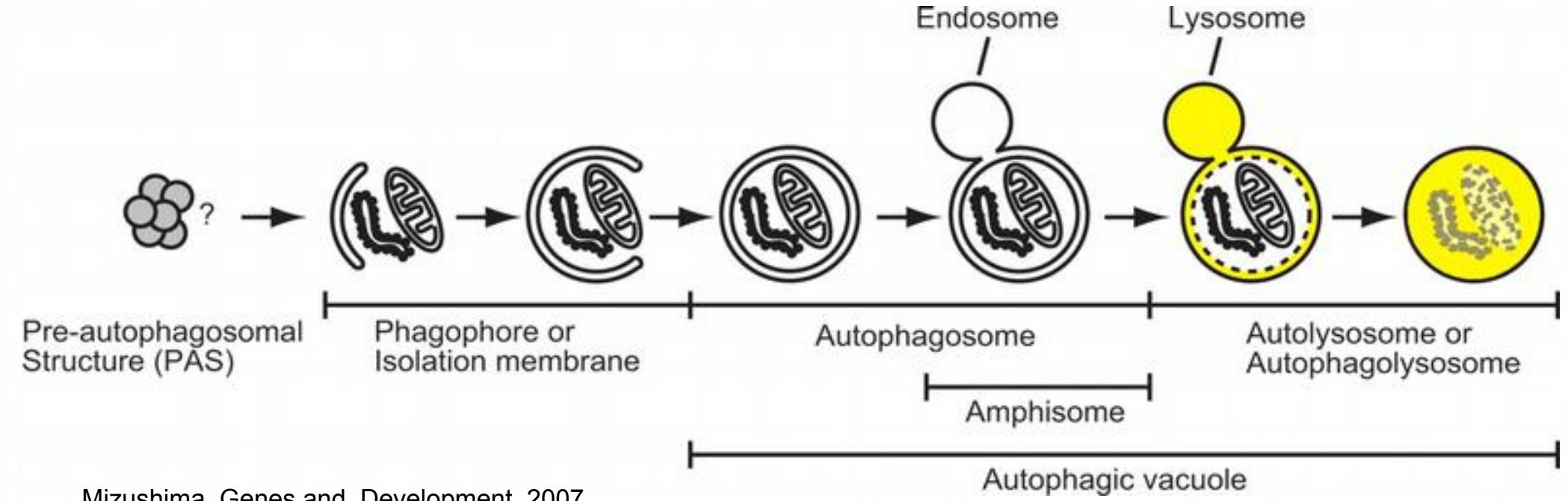
Image M.H.

Autophagy is a process of self-degradation of cellular components

Double-membrane **autophagosomes** sequester organelles or portions of cytosol and fuse with lysosomes

Autophagy is upregulated in response to signals such as:

- starvation
- growth factor deprivation
- ER stress
- pathogen infection.



Mizushima, Genes and Development, 2007

Autophagy

Macroautophagy

Microautophagy

Chaperone-mediated autophagy

proteins containing specific signal sequence
translocation of proteins driven by binding of chaperones
internalization via lamp2a receptor in the lysosomal membrane

Lysosomal membrane protein **LAMP2** is a receptor involved in fusion of autophagic vacuoles with lysosomes

Import of lysosomal proteins into lysosome

Soluble lysosomal proteins :

– mannose-6 phosphate receptor

Lysosomal membrane proteins:

- signals in short C-terminal “tail”)
- signals are recognised by adaptor proteins (AP3..)

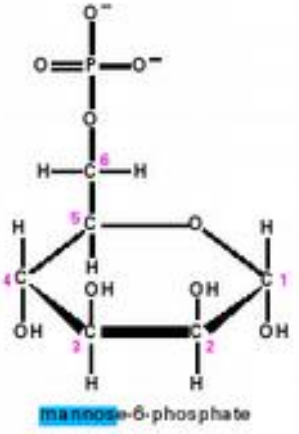
Other

- glucocerebrosidase, lysosomal acid phosphatase
- prosaposin
- sortilin, LIMPII

Transport of soluble lysosomal proteins by mannose-6-phosphate receptors

Sorting of proteins containing MP6 signal

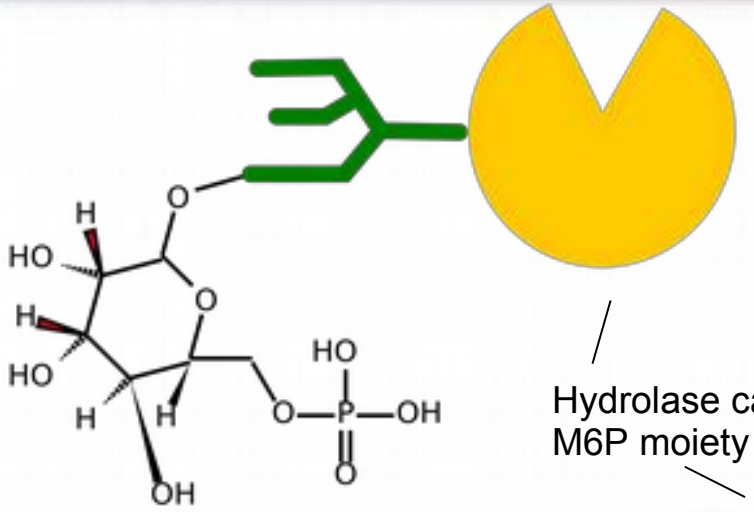
The majority of soluble (luminal) lysosomal proteins is transported into lysosome via mannose-6-phosphate receptor



M6P signal is built on N-linked oligosaccharides of hydrolases by Glc Nac phosphotransferase in *cis*-Golgi

N-acetylglucosamine phosphotransferase (**GlcNac phosphotransferase**) **recognises a 3-D pattern on lysosomal enzymes**

Protective GlcNac group is enzymatically removed in *trans*-Golgi, leaving M6P exposed



Hydrolase carrying M6P moiety

GlcNac

GlcNac phosphotransferase

GlcNac di-phospho uridine

Hydrolase with N-linked oligosaccharide

UMP

Image M.H.

Sorting of proteins containing MP6 signal

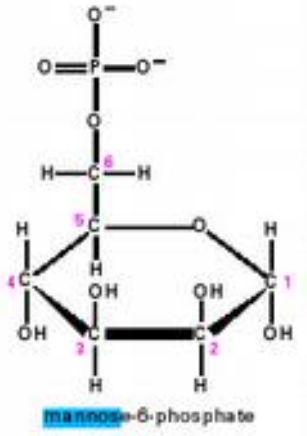
protein-M6P-M6PR → **lysosome**

protein-M6P protein

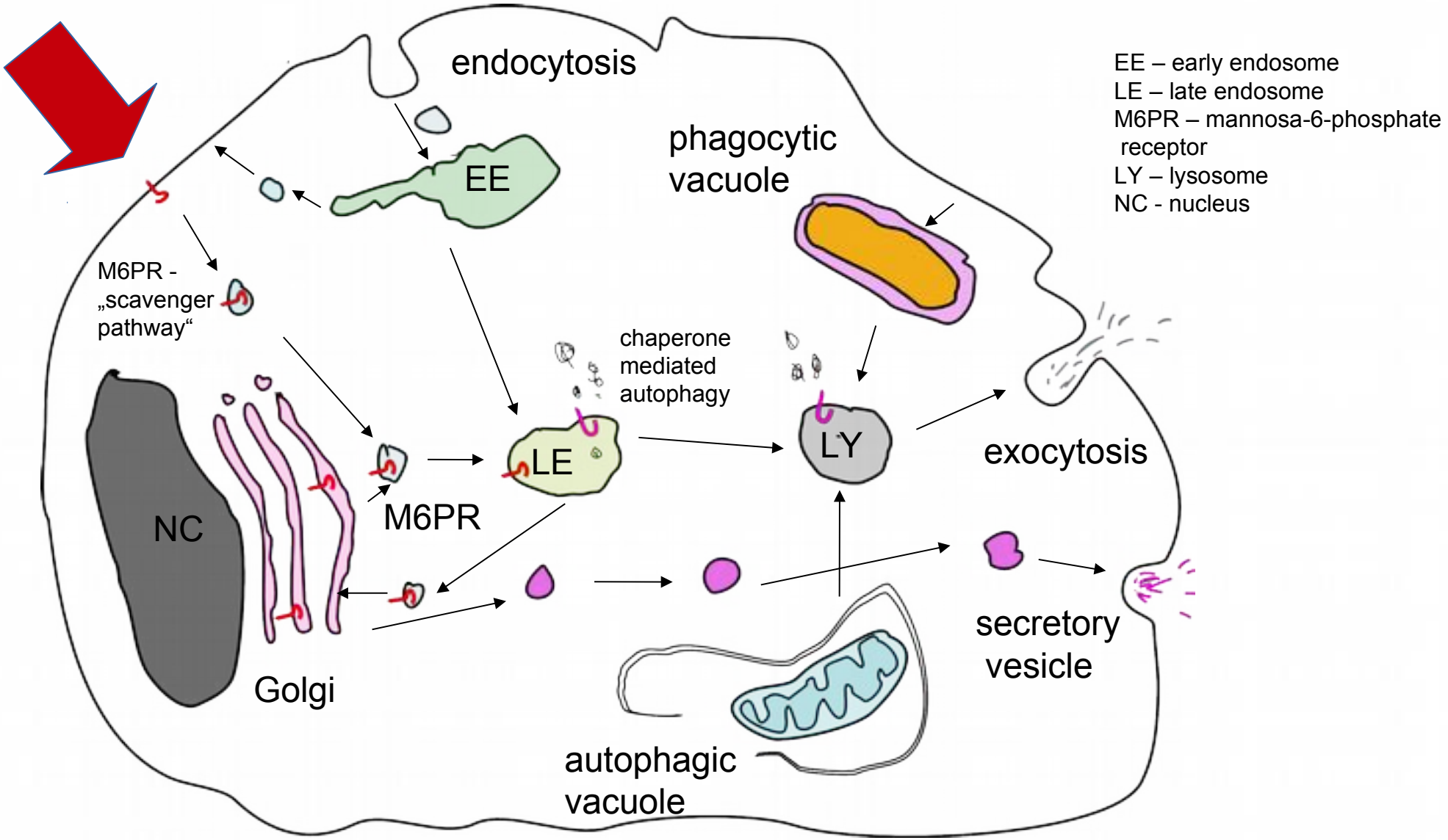
protein → **Secretion pathway**

cis-Golgi

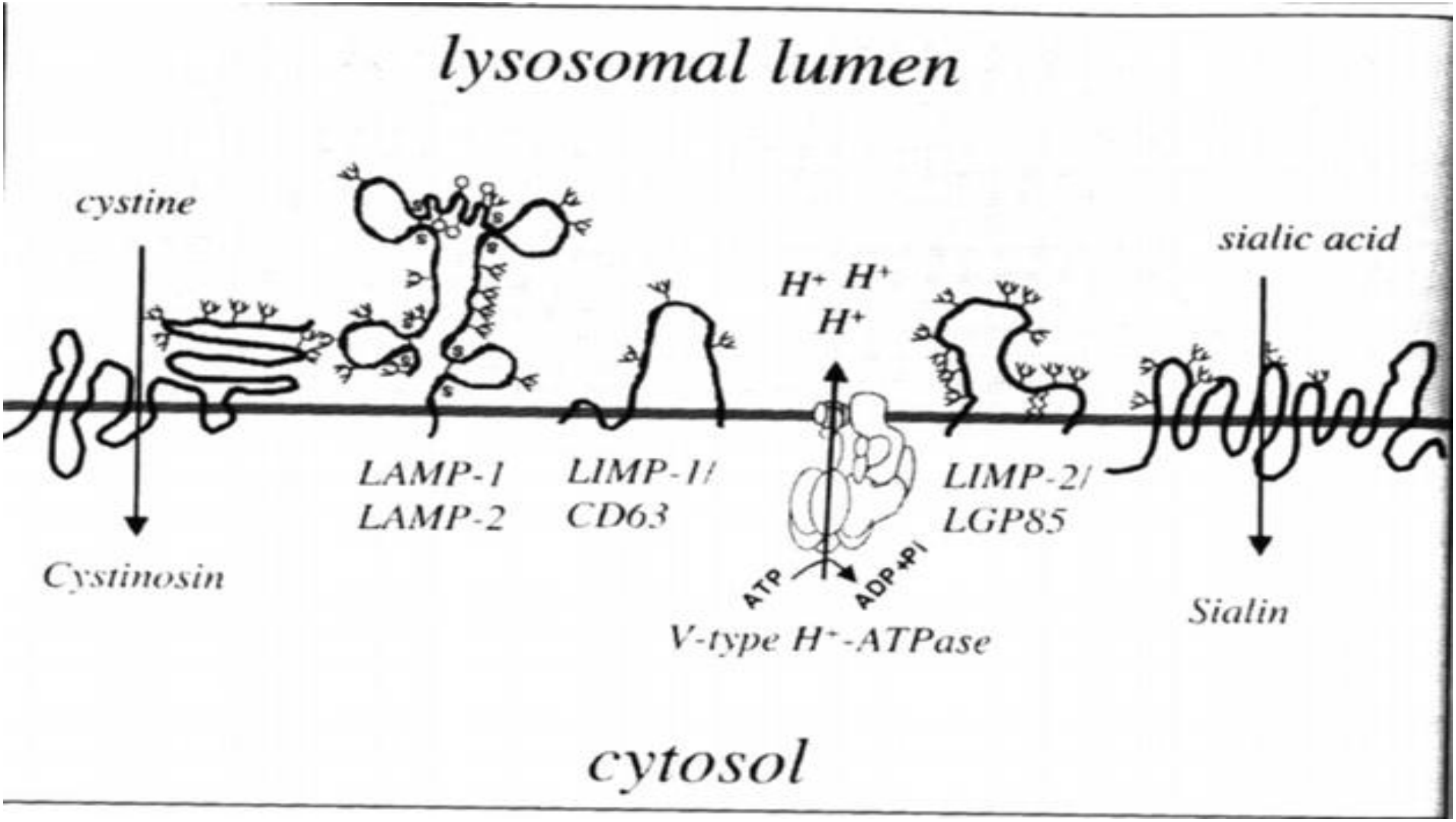
trans-Golgi



MP6 receptors capture lysosomal enzymes by receptor-mediated endocytosis at plasma membrane



Lysosomal membrane proteins

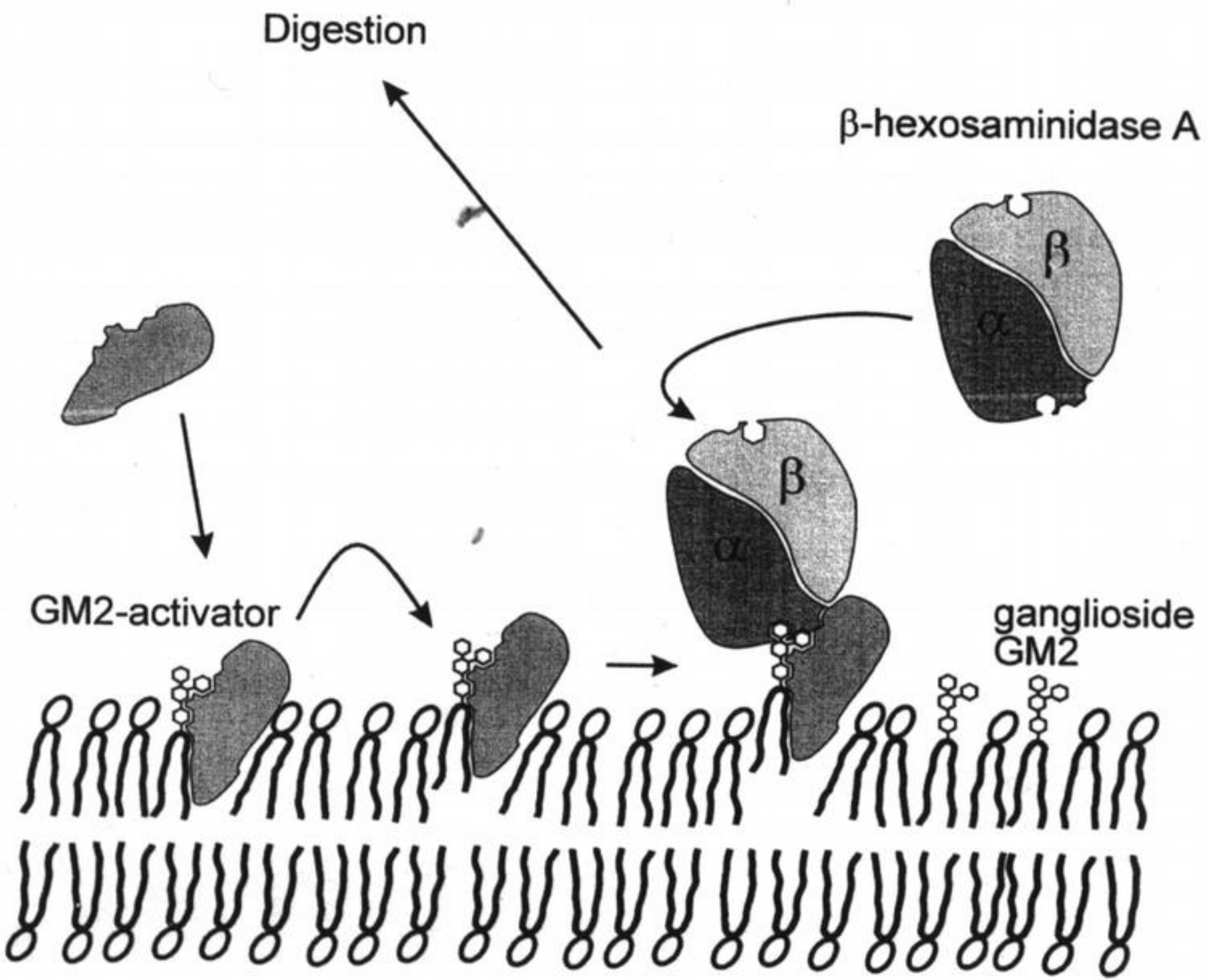


Lysosomal membrane contains more than 100 proteins, majority of which have unknown function. Proteins with known function include receptors, molecules participating in vesicular transport, transporters of small molecules, vacuolar ATPase etc.

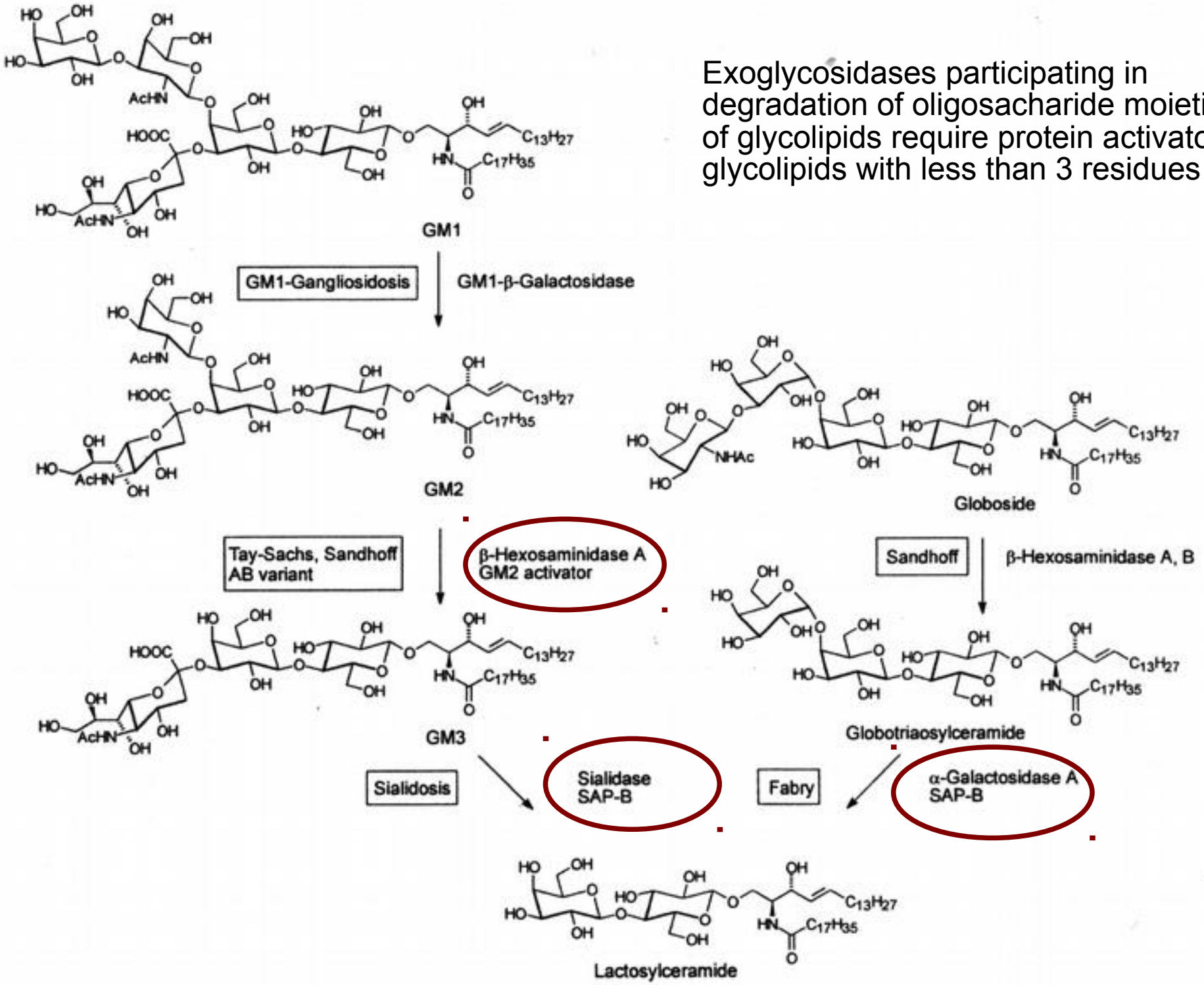
Oligosaccharide chains at the inner face of lysosomal mebrane for a glycocalix protecting the membrane from the attack of hydrolases

LAMP 2 (lysosomal associated membrane protein 2) is a receptor for autophagic vacuoles

Activators of lysosomal hydrolases


Digestion
β-hexosaminidase A
β
α
β
α
GM2-activator
ganglioside
GM2

Exoglycosidases participating in degradation of oligosacharide moieties of glycolipids require protein activators for glycolipids with less than 3 residues

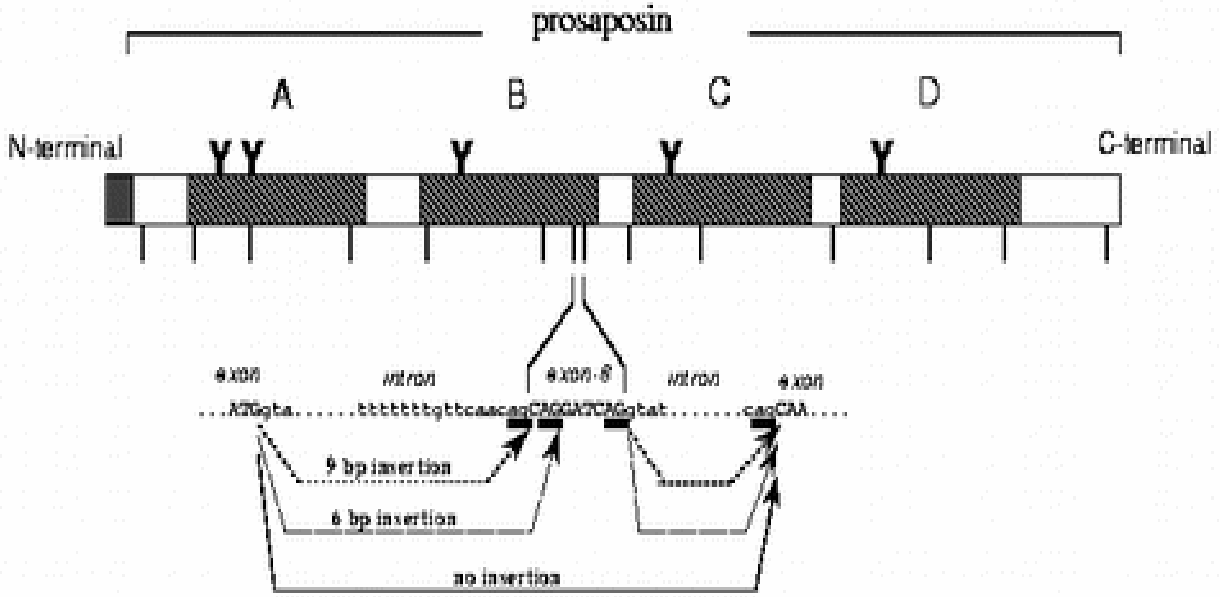


Activators of lysosomal hydrolases

Saposins A,B,C,D

deficits of saposins lead to variant forms of disorders caused by deficiencies of enzymes they activate

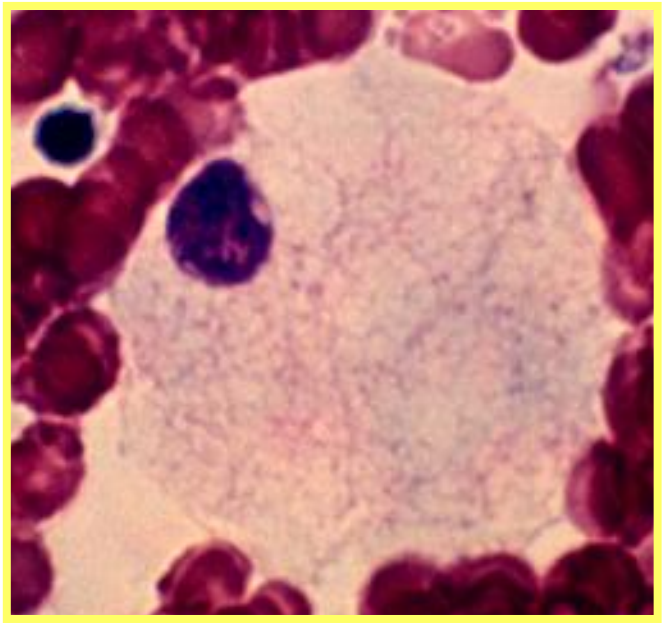
GM2 activator
activates
hexosaminidase A

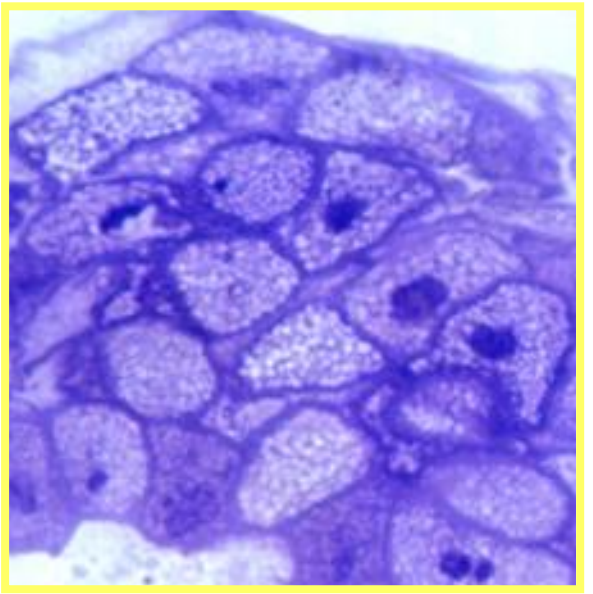


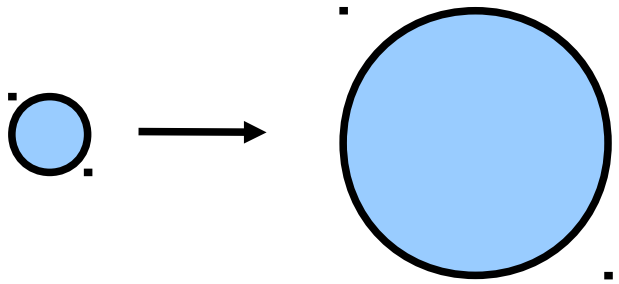
General features of lysosomal disorders

Lysosomal („storage“) diseases

Deficiencies of proteins from the lysosomal system lead to storage of material in lysosomes

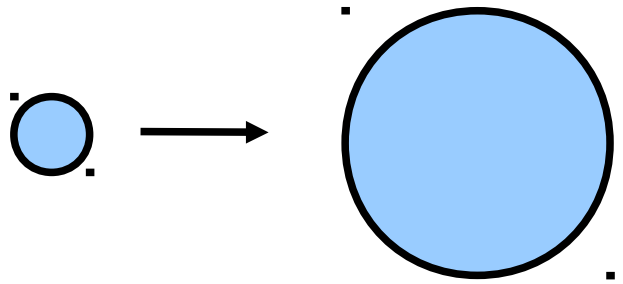






Lysosomal („storage“) diseases

<u>Disorders of transport</u> of enzymes into lysosome or disorders of substrate transport (e.g. due to a disruption of vesicular transport inside the cell) can also lead to lysosomal storage



LSD: Common phenotypical features and affected organs

Central nervous system: neurodegeneration, ...
Spleen, liver : hepato and splenomegaly, hepatopathy ...
Skeleton: Facial dysmorphy, dysostosis multiplex, ...
Peripheral nervous system: peripheral neuropathy, ..
Heart – cardiomyopathy, valve disease, …
Kidney : renal failure, nefrolithiasis ...
Skin : agiokeratomas, ...
Eye: cataracts, corneal clouding, cherry-red spot, retinal degeneration, …
Ear: Sensorineural deafness, …
Bone marrow: anemia
Lungs:
...

MPS VI, http://www.ojrd.com/content/figures/1750-1172-5-5-4-l.jpg

Lysosomal disorders

Hereditary disorders associated with storage of material within the lysosomes

1. Disorders of glycan degradation - mucopolysaccharidoses and glycoproteinoses
2. Lipidoses
3. Proteinoses
4. Disorders of lysosomal transport of metabolites
5. Disorders of transport of proteins into lysosomes

Alteration of metabolic, signalling, and transport pathways in lysosomal disorders

- Accumulation of secondary metabolites
- Alterations of calcium homeostasis
- Free radicals and oxidative stress
- Neuroinflammation
- Abnormal autofagy

-

Alteration of metabolic, signalling, and transport pathways in lysosomal disorders

- **Neuroinflammation**

- Signs of neuroinflammation is present essentially in all lysosomal disorders with CNS involvement
- Activation of immune system – microglia and astrocytes
- Similar findings are present in „classic“ neurodegenerative disordrders
- Chronic glial activation in lysosomal disorders apparently contributes to neuronal damage

Overview of lysosomal disorders

Lysosomal enzymes

30 enzymes – hereditary deficiencies of which cause human diseases

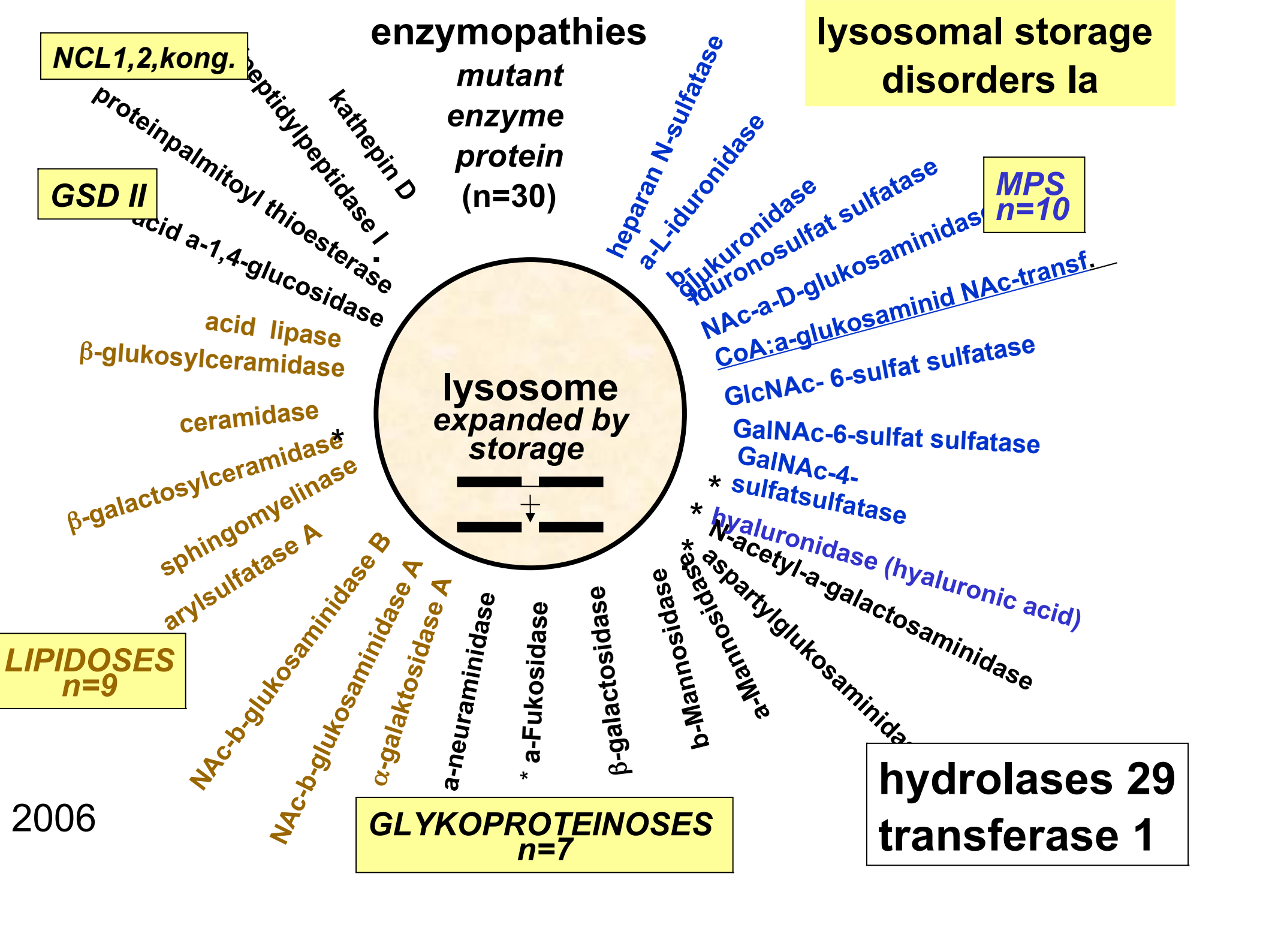
lipids – lipidoses, including sphingolipidoses

glykosaminoglycans – mucopolysaccharidoses

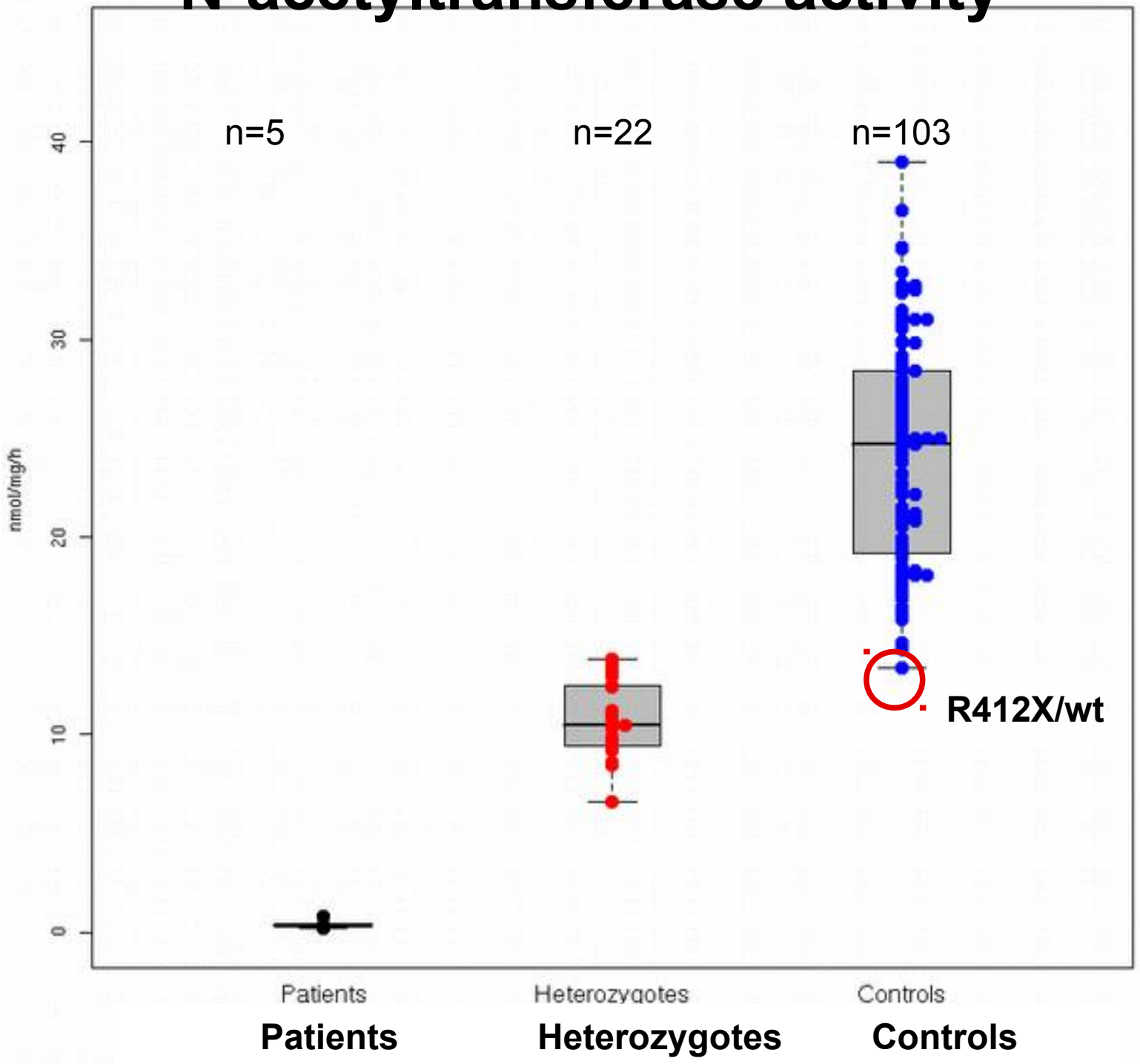
N-glycans, oligosacharides – glycoproteinoses

glycogen – glycogenosis type II (Pompe)

proteins – proteinoses


enzymopathies
mutant
enzyme
protein
(n=30)
lysosomal storage disorders la
NCL1,2,kong.
GSD II
proteinpalmitoyl thioesterase
kathepin D
heparan N-sulfatase
a-L-iduronidase
b-glukuronidase
iduronosulfat sulfatase
NAc-a-D-glukosaminidase
CoA:a-glukosaminid NAc-transf.
MPS
n=10
GlcNAc- 6-sulfat sulfatase
GalNAc-6-sulfat sulfatase
GalNAc-4-
* sulfatsulfatase
* hyaluronidase (hyaluronic acid)
N-acetyl-a-galactosaminidase
aspartylglukosaminida
*aseMannosidase
b-Mannosidase
β-galactosidase
* a-Fukosidase
a-neuraminidase
α-galaktosidase A
NAc-b-glukosaminidase A
NAc-b-glukosaminidase B
arylsulfatase A
sphingomyelinase
β-galactosylceramidase
*
ceramidase
β-glukosylceramidase
acid lipase
acid a-1,4-glucosidase
peptidylpeptidase I
lysosome
expanded by
storage
LIPIDOSES
n=9
GLYKOPROTEINOSES
n=7
hydrolases 29
transferase 1
2006

N-acetyltransferase activity



Lipidoses – 9 types

Gaucher disease – glucocerebrosidase deficiency

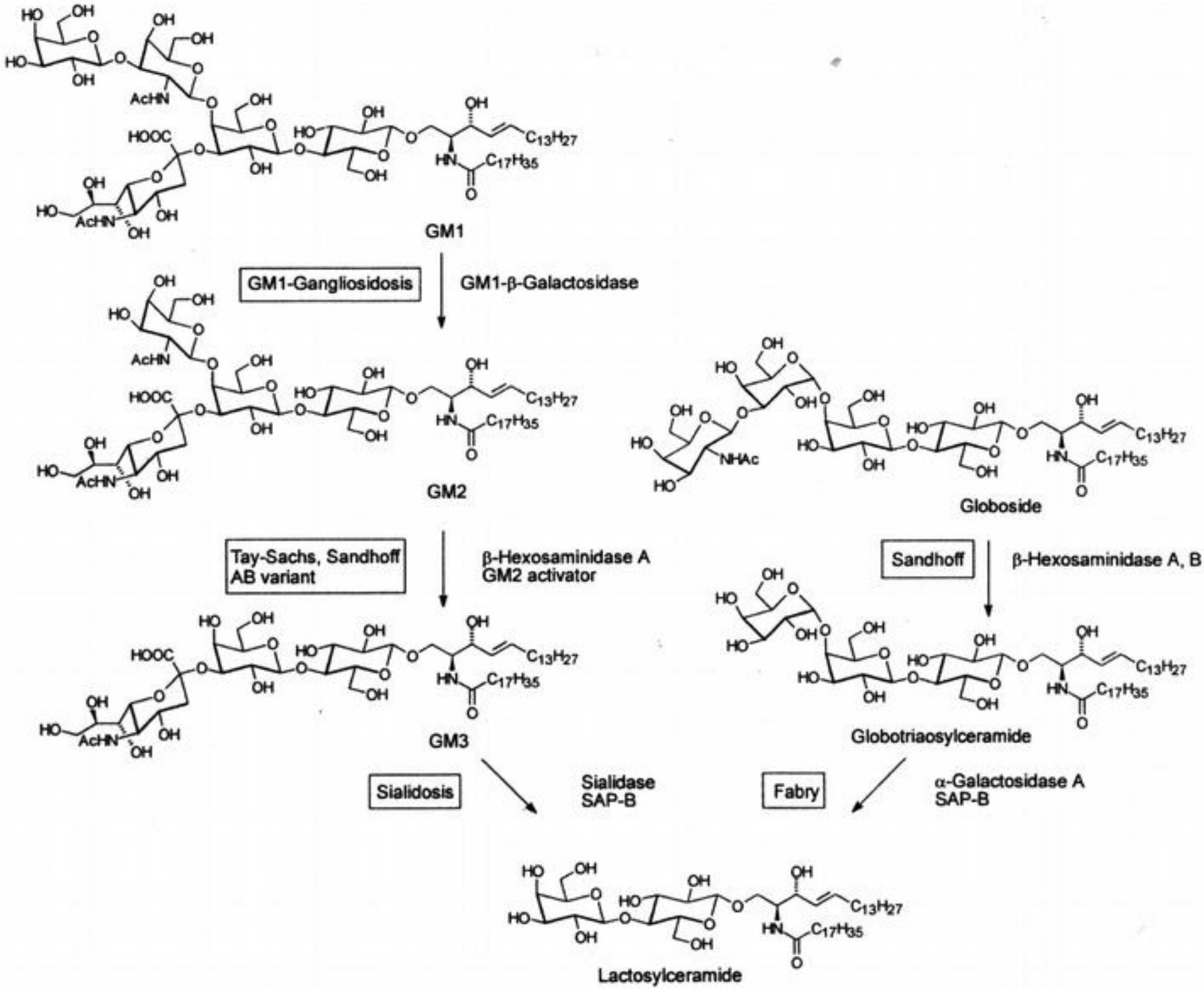
Fabryho disease – alpha-galactosidase A deficiency

Niemann-Pick disease type A/B – acid sphingomyelinase deficiency

Niemann-Pick disease type C - deficit of proteins involved in intracellular transport of unesterified cholesterol

Krabbe disease - beta-galactosylceramidase deficiency

Metachromatic leukodystrophy – arylsulfatase A deficiency

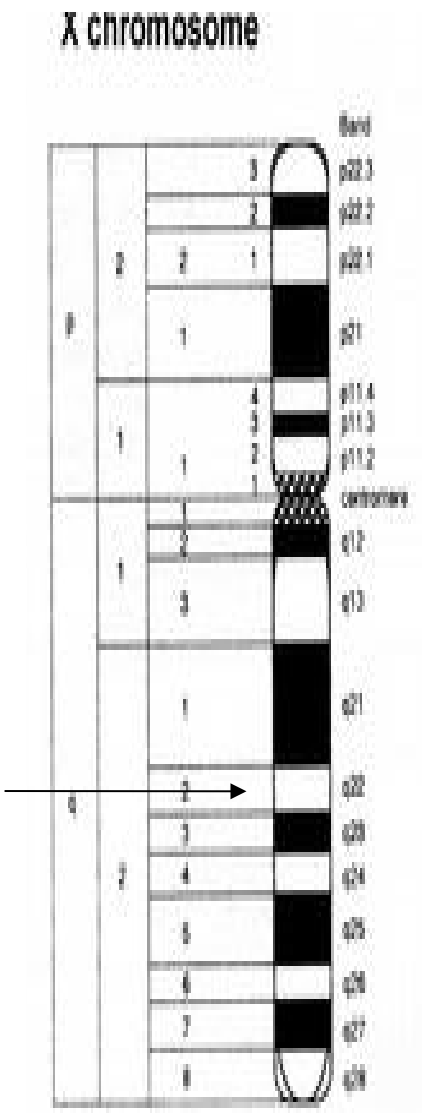

GM1
GM1-Gangliosidosis
GM1-β-Galactosidase
GM2
Globoside
Tay-Sachs, Sandhoff
AB variant
β-Hexosaminidase A
GM2 activator
Sandhoff
β-Hexosaminidase A, B
GM3
Globotriaosylceramide
Sialidosis
Sialidase
SAP-B
Fabry
α-Galactosidase A
SAP-B
Lactosylceramide

Fabry disease – alpha-galactosidase A deficiency

X-linked disease

lysosomal storage of glycolipids with terminal alpha-galactose, predominantly globotriaosylceramide

storage in vessel endothel, smooth muscle of the vessels, cardiomyocytes, glomerules and tubules and other cell types



Fabry disease – symptoms

hypertrophic cardiomyopathy, arythmias

chronic progressive renal disease leading to renal failure

TIA, parestesias

angiokeratomas , cornea verticilata

X-linked disease

In females the severity of phenotype depends on X-inactivation

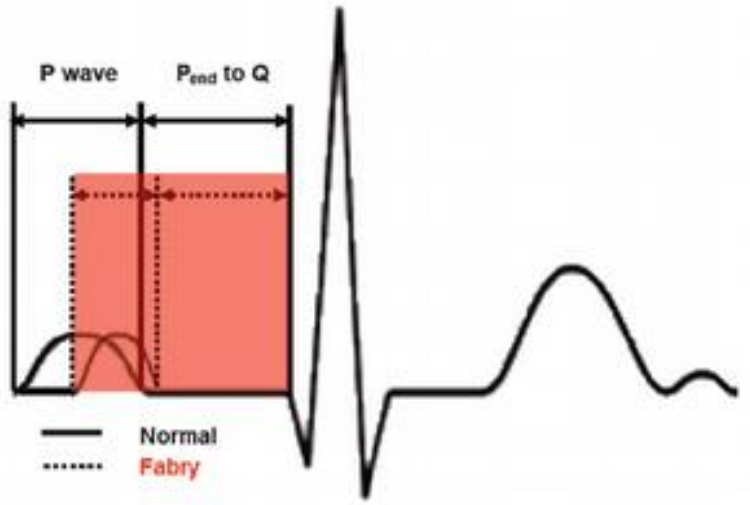


Figure 3 Schematic illustration of the changes in PQ-interval depending on P-wave duration in Fabry disease (FD) compared with normal controls. Shortening of the PQ-interval was predominantly caused by a shorter P-wave duration in patients with FD. Dashed lines, arrows and red zone indicates changes in FD.

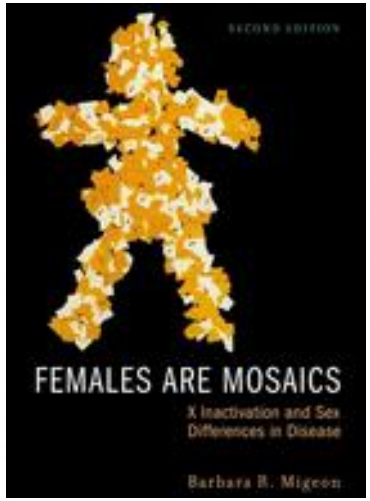
Females are mosaics

The size of X-inactivation patches differs between tissues

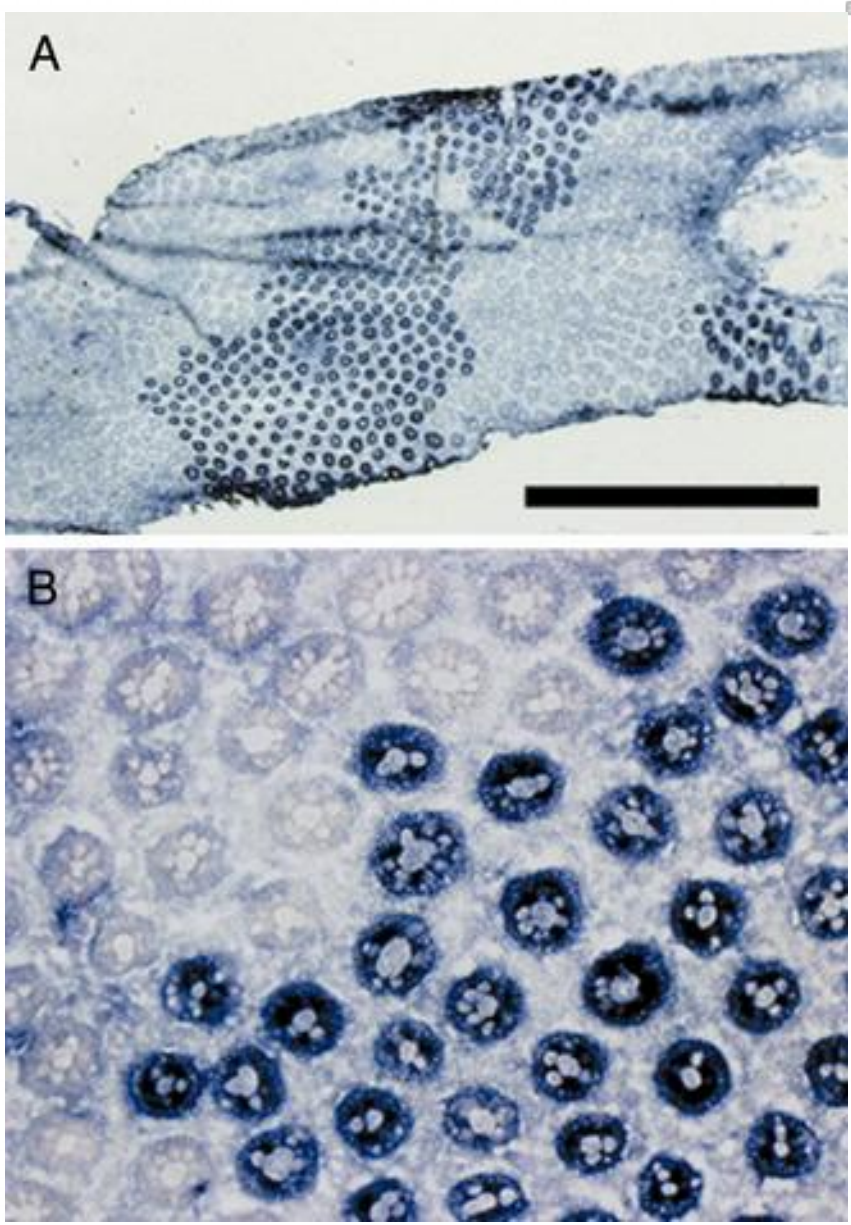
Skewing of X-inactivation may influence phenotype

Patch size may confound testing of clonality, enzyme activity etc. in tissues

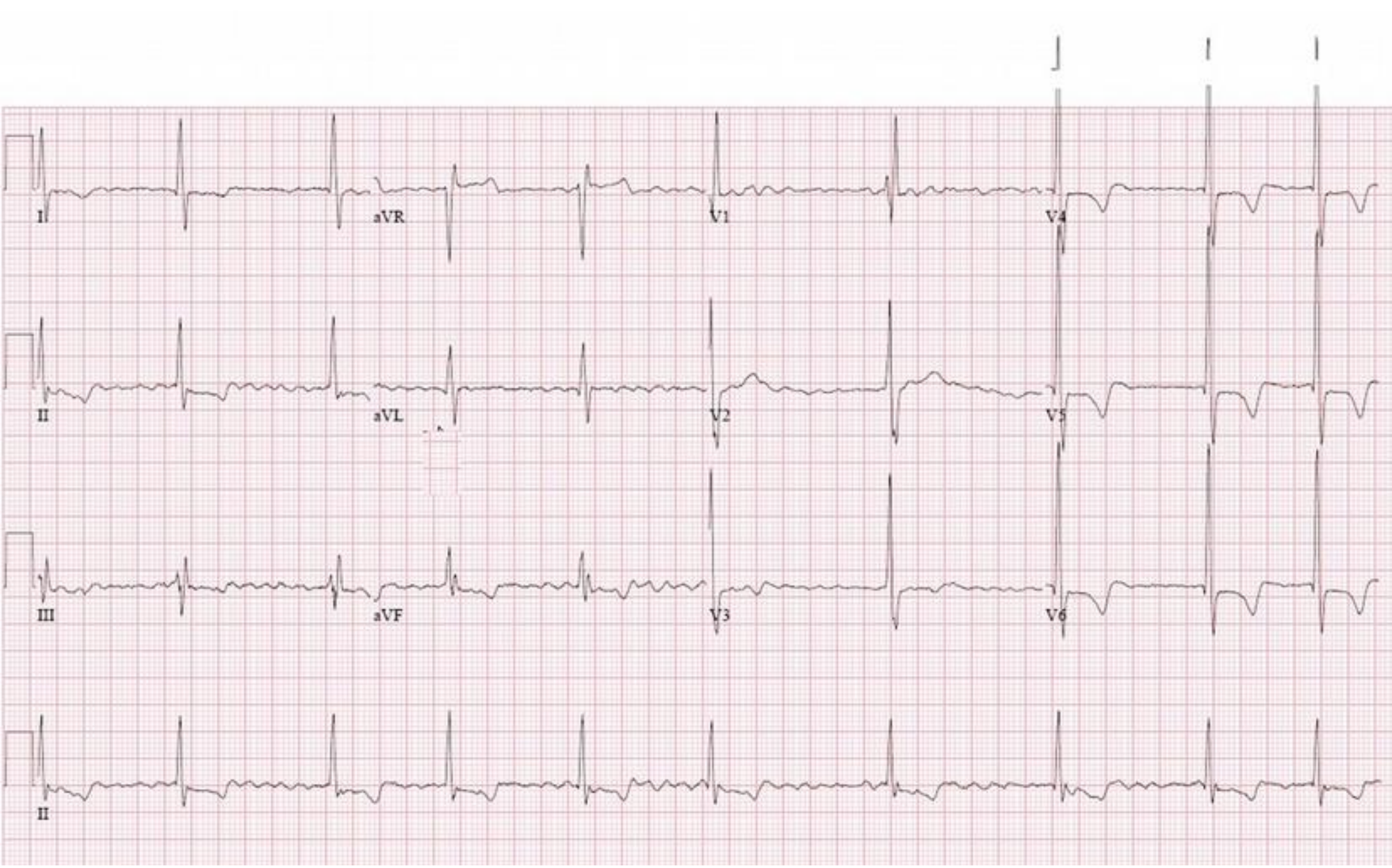
Patch size may influence cross-correction of the defect by endocytosis of enzyme from cells expressing wild-type allele (in heterozygotes)



Oxford University Press



Marco Novelli et al. PNAS 2003;100:3311-3314
G6PD staining in the intestine in G6PD carriers



Example of arrythmia in Fabry disease :Atrial fibrillation with slow ventricular response and a heart rate of 56 bpm. Criteria for LVH with diffuse abnormal repolarization.

http://www.lysosomalstorageresearch.ca/Fabry_eClinic/electrocardiography-ecg.html

Gaucher disease

Lysosomal storage disorder

Deficiency of glucocerebrosidase (acid beta glucosidase)

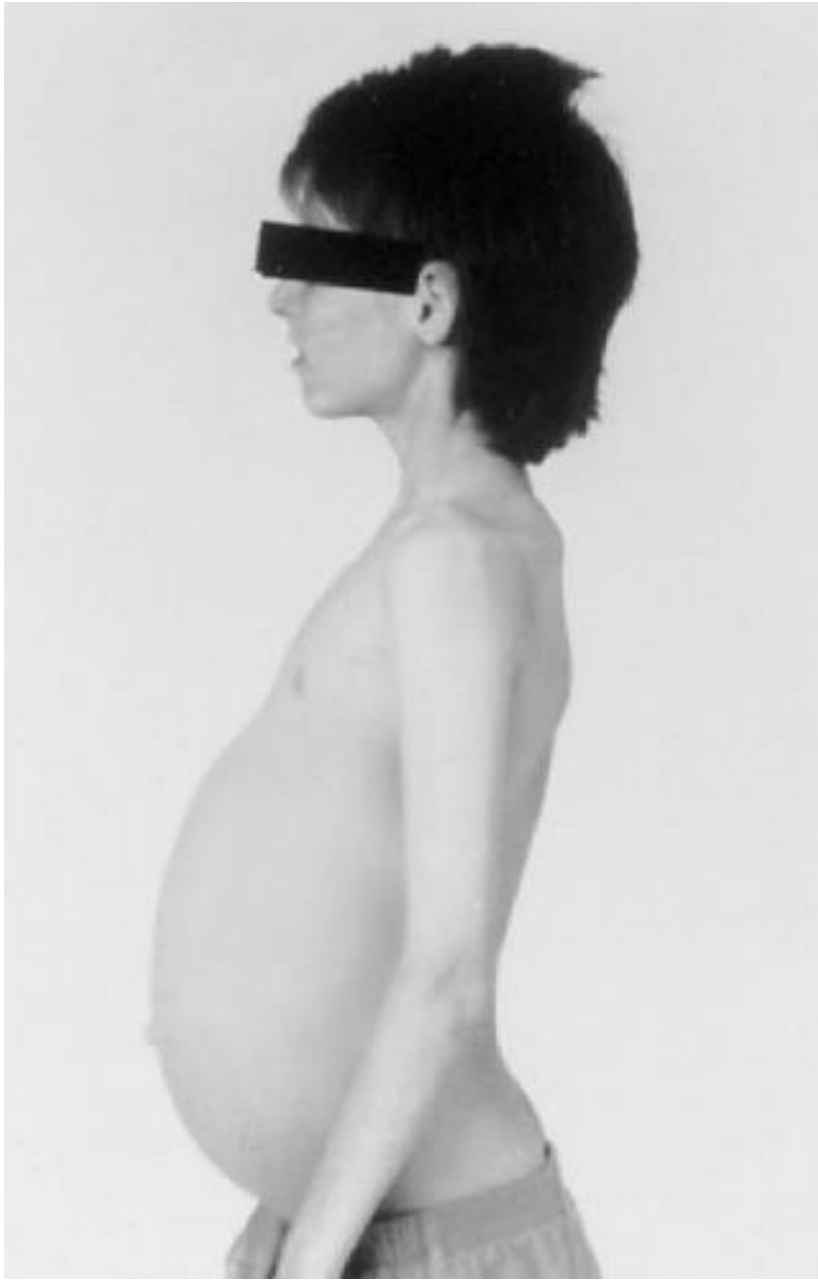
Accumulation of glucosylceramide preferentially in cells of macrophage origin (Gaucher cells)

Multisystem disorder

Hepatomegaly, splenomegaly, bone disease, trombocytopenia, anemia, lung infiltration

In type 2 and 3 Gaucher disease: CNS disease

Clinical variability, chronic progresion
Type 1: chronic non-neuronopathic
Type 2: acute neuronopathic
Type 3: chronic neuronopathic



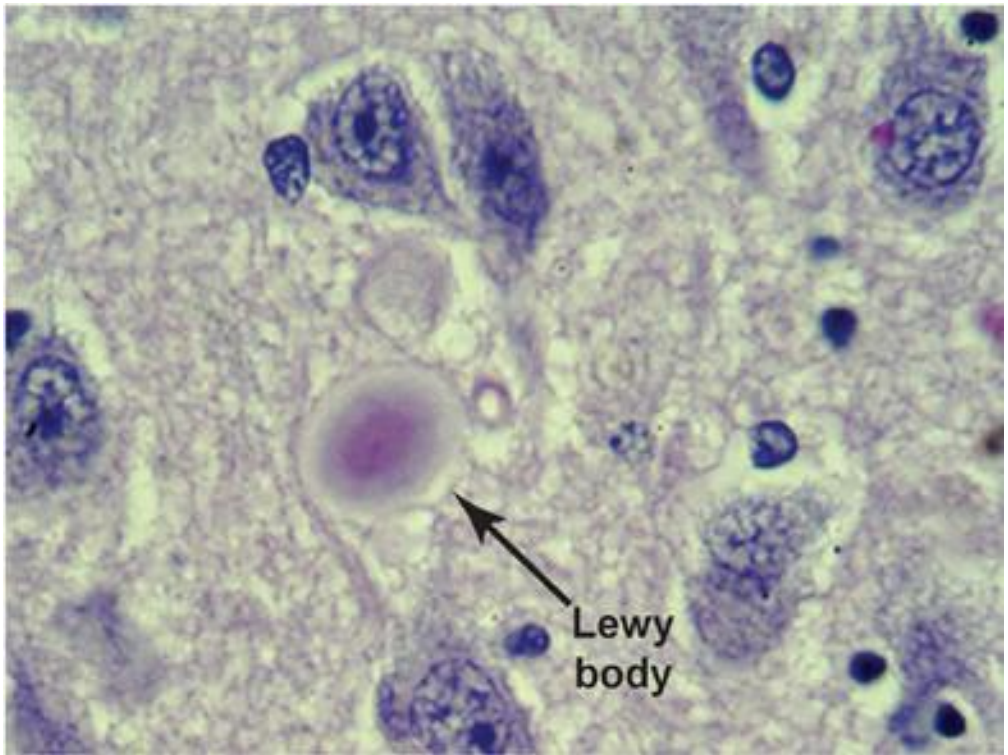
Heterozygosity or homozygosity for a mutation in the glucocerebrosidase gene(GBA) is a susceptibility factor for Parkinsons disease (PD)

Molecular mechanism is not clear , ? tau protein or α-synuclein transport disorder ?

Strong epidemiologic evidence for the association,
5%-10% of PD patients carry GBA mutations,
Odds-ratio 16-28

Mutant glucocerebrosidase is present in Lewy bodies in Gaucher patients with Parkinson disease

Association with GBA mutations also shown in dmentia with Lewy bodies



Niemann-Pick disease type C

- Disorder of **intracellular lipid trafficking**

- Neurovisceral disorder : highly variable clinical picture
- Prolonged neonatal jaundice of cholestasis, hepatosplenomegaly or isolated splenomegaly
- Later **progresssive neurological disease** – ataxia , clumsiness, falls, spasticity, seizures, dysarthia or dysphagia
- tyúical signs : vertical gaze palsy, gelastic cataplexy
- **psychiatric signs:** presenile cognitive decline, dementia, paranoia (hallucinations, ...)

Niemann-Pick type C disease

- Disorder of intracellular lipid trafficking, especially of cholesterol
- accumulation of unesterified cholesterol and glycolipids in late endosomes/lysosomes
- Disorder of LDL-derived cholesterolu
- abnormal fusion of late endosomes and lysosomes, abnormal filling of lysosomes with Ca^{++}

Mutations in two cholesterol-transporting proteins : NPC1 and NPC2

NPC1 is more frequent (about 95% of NPC)

- (Note: Niemann-Pick type A and B are caused by the deficiency of acid sphingomyelinase)

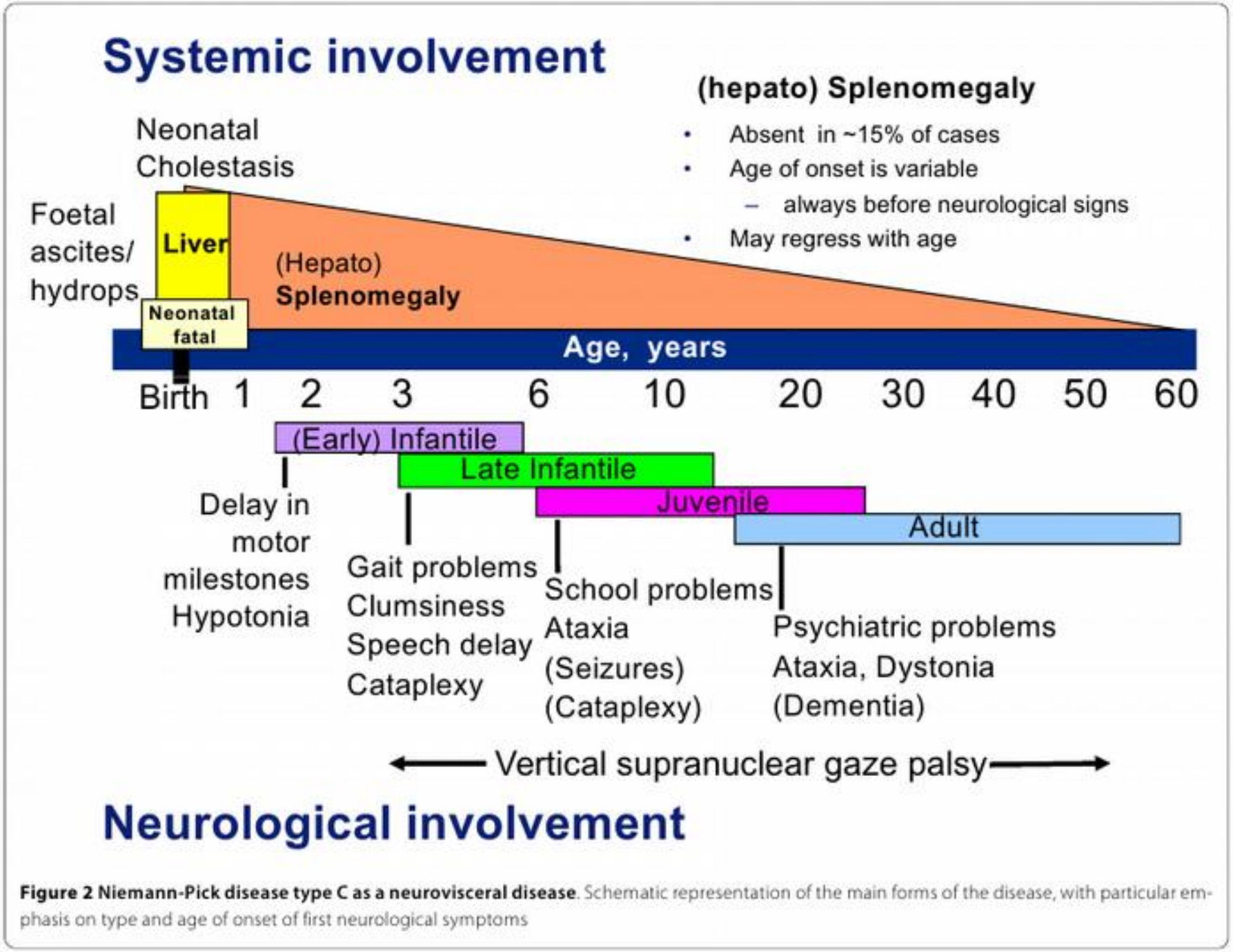
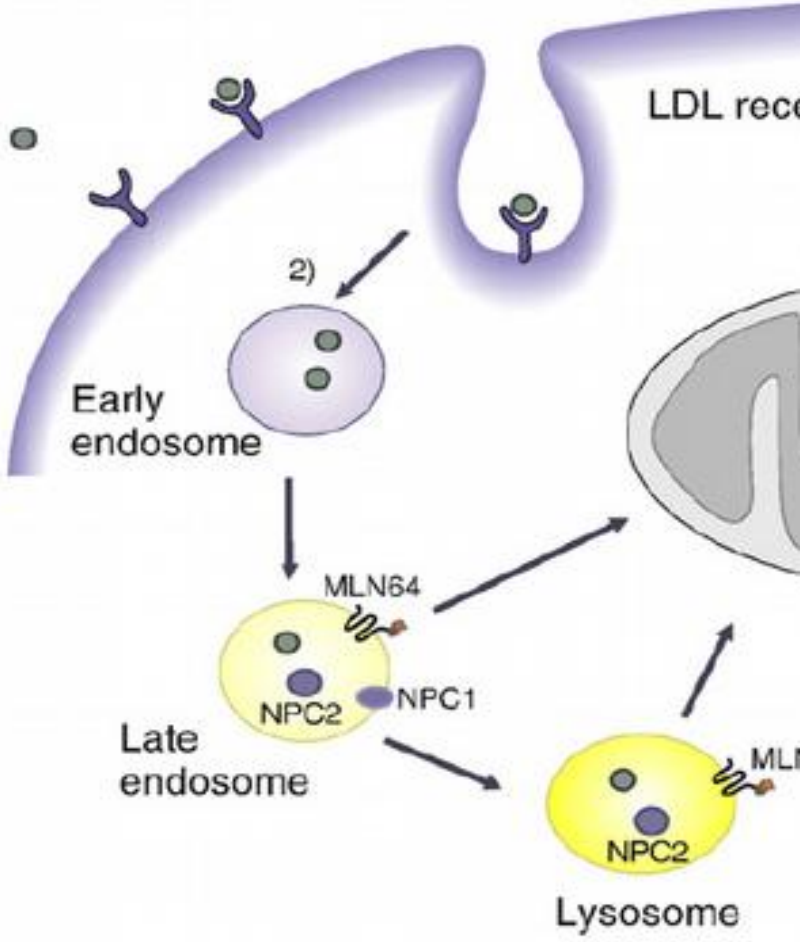


Figure 2 Niemann-Pick disease type C as a neurovisceral disease. Schematic representation of the main forms of the disease, with particular emphasis on type and age of onset of first neurological symptoms

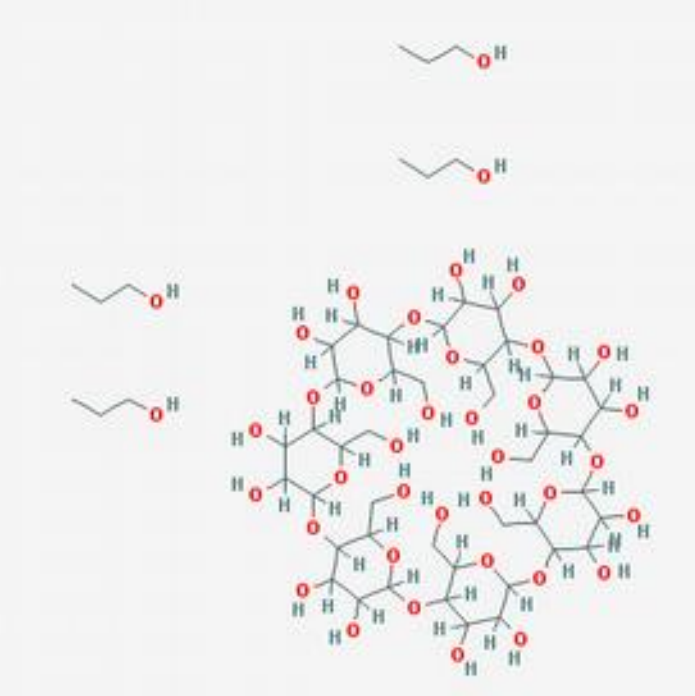
Vanier 2010

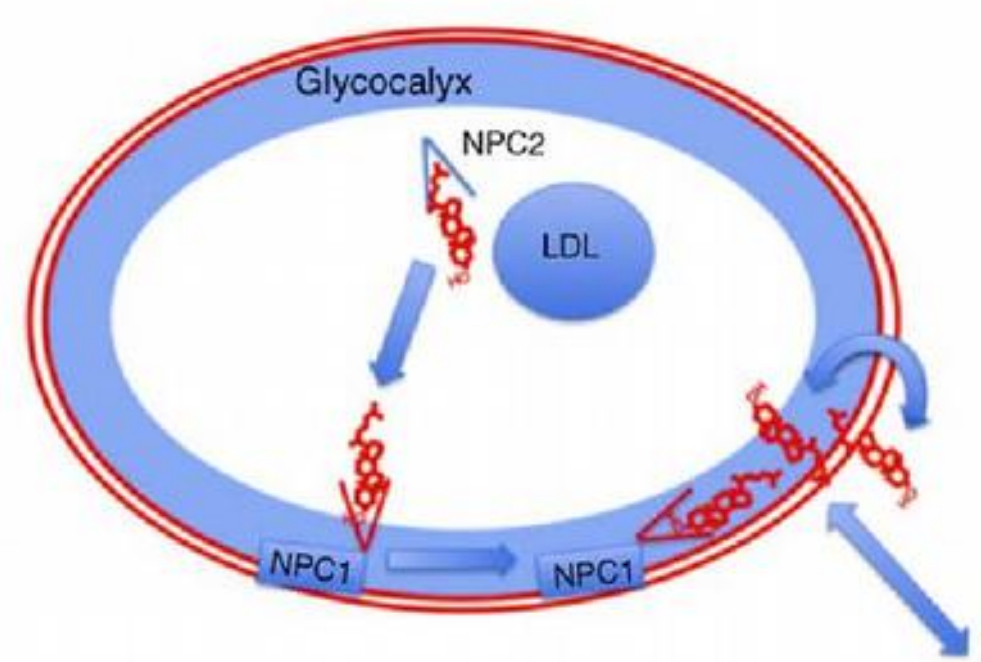
Intracellular transport of LDL cholesterol



Function of NPC1 and NPC2

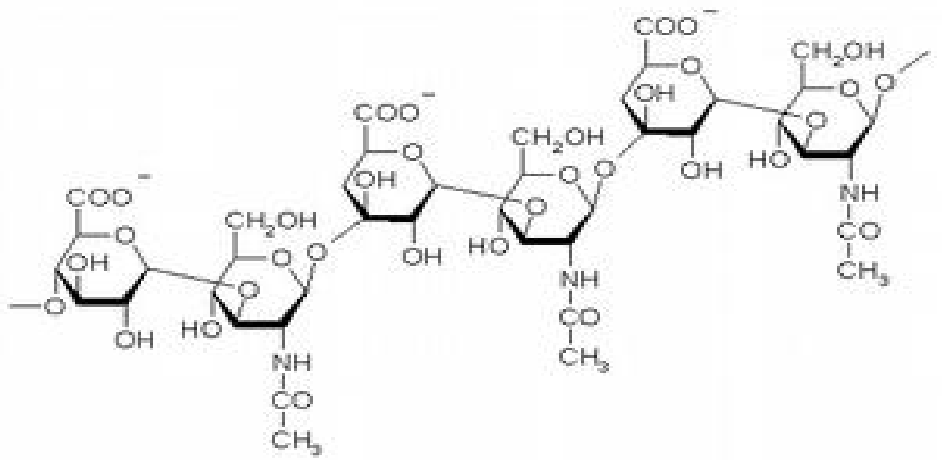
- Soluble NPC2 binds LDL-derived cholesterol and transfers it to NPC1
- NPC1 transfers cholesterol molecules across glycocalix at the lumenal face of the lysosome
- Treatment in trials: propyl beta cyclodextrin intrathecally





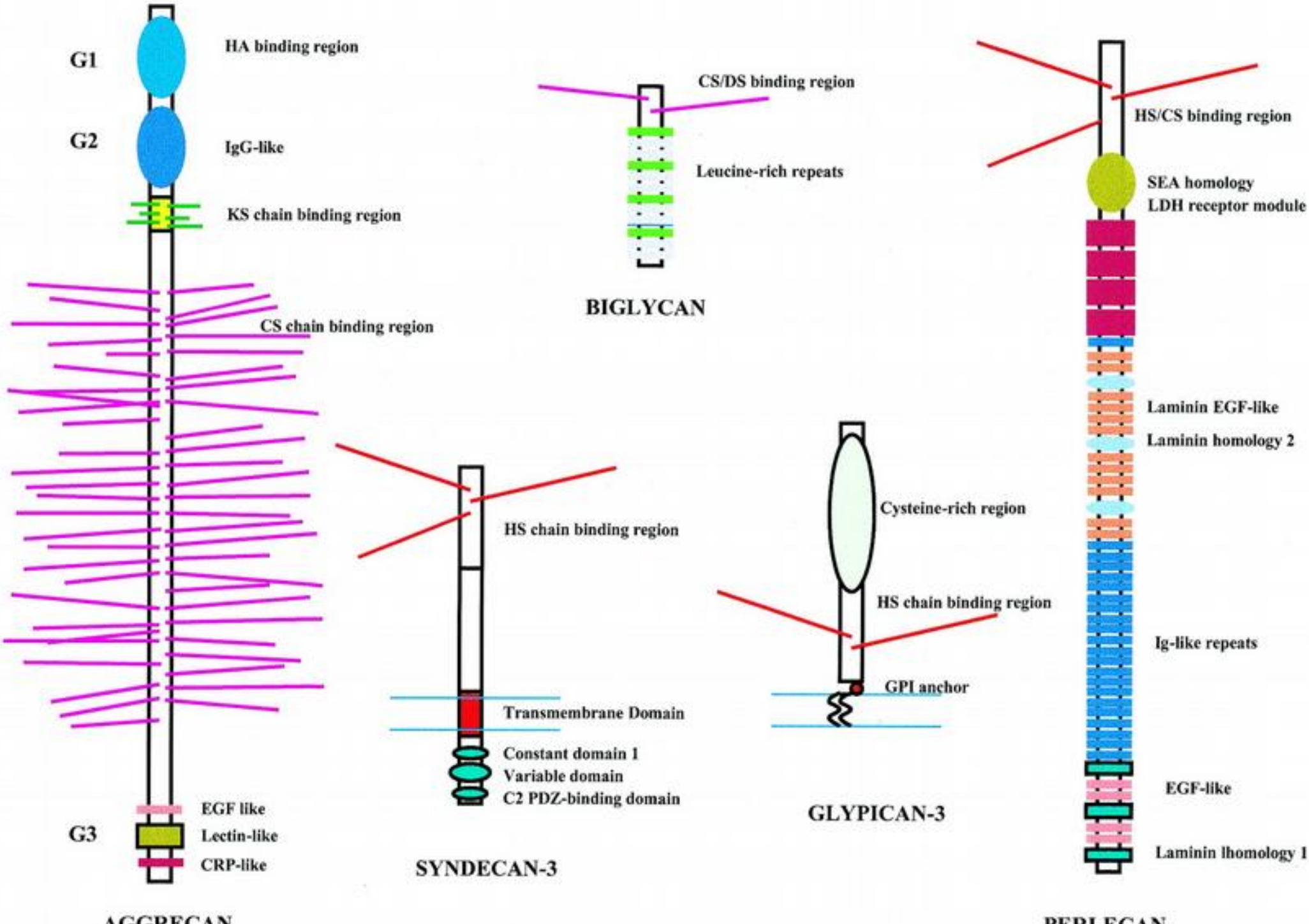
Mucopolysaccharides

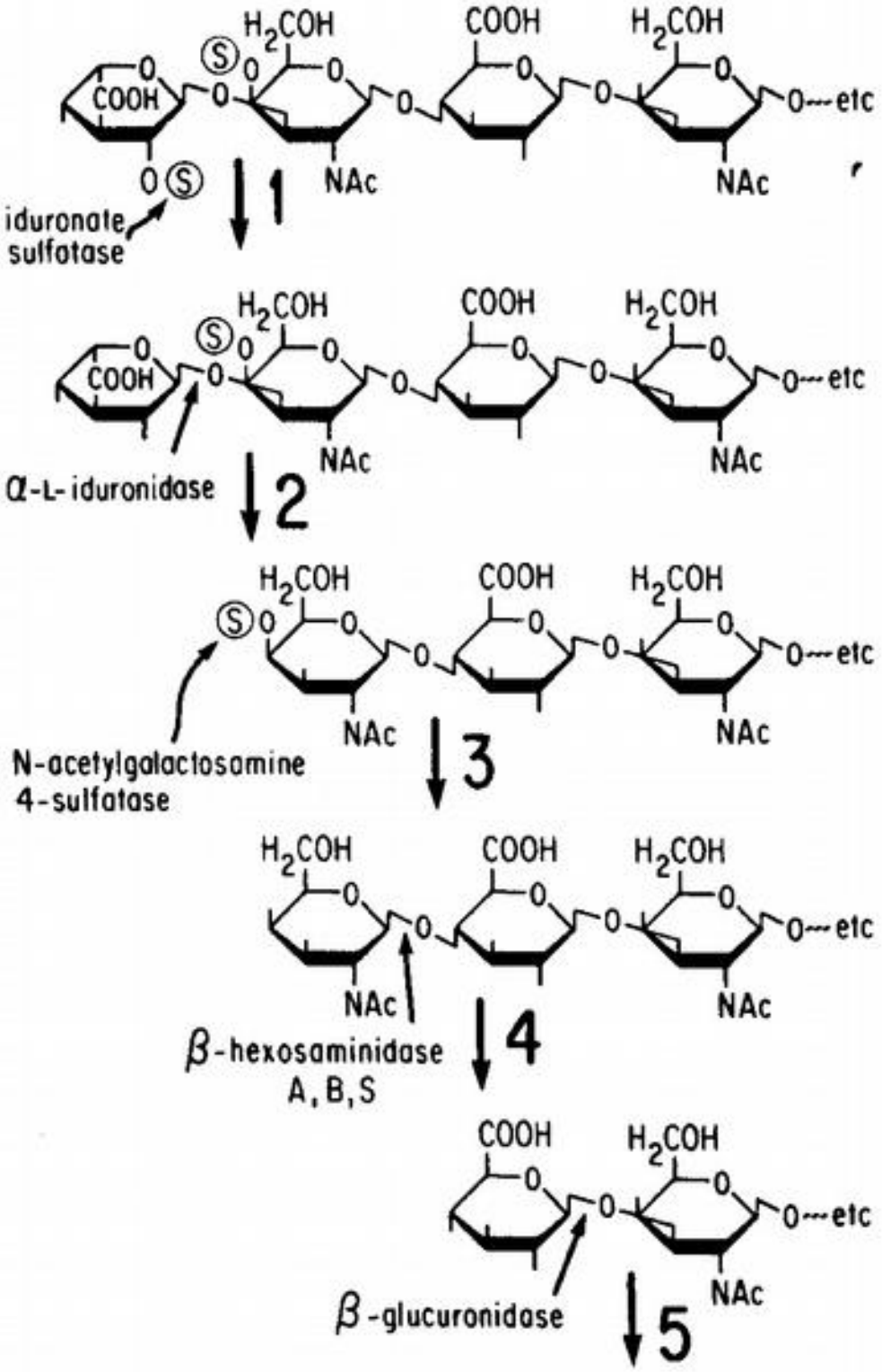
Polysaccharides



Heparan sulfate
Dermatan sulfate
Keratan sulfate
Chondroitin sulfate

Families of proteoglycans expressed in cartilage: representative members





Glycosaminoglycans are degraded by sequential action of glycosidases

Mucopolysaccharidoses

11 disorders

Most common :
MPS I Hurler disease - deficiency of alpha-iduronidase, AR-inheritance
MPS II - Hunter disease - deficiency of iduronate sulfatase , X-linked

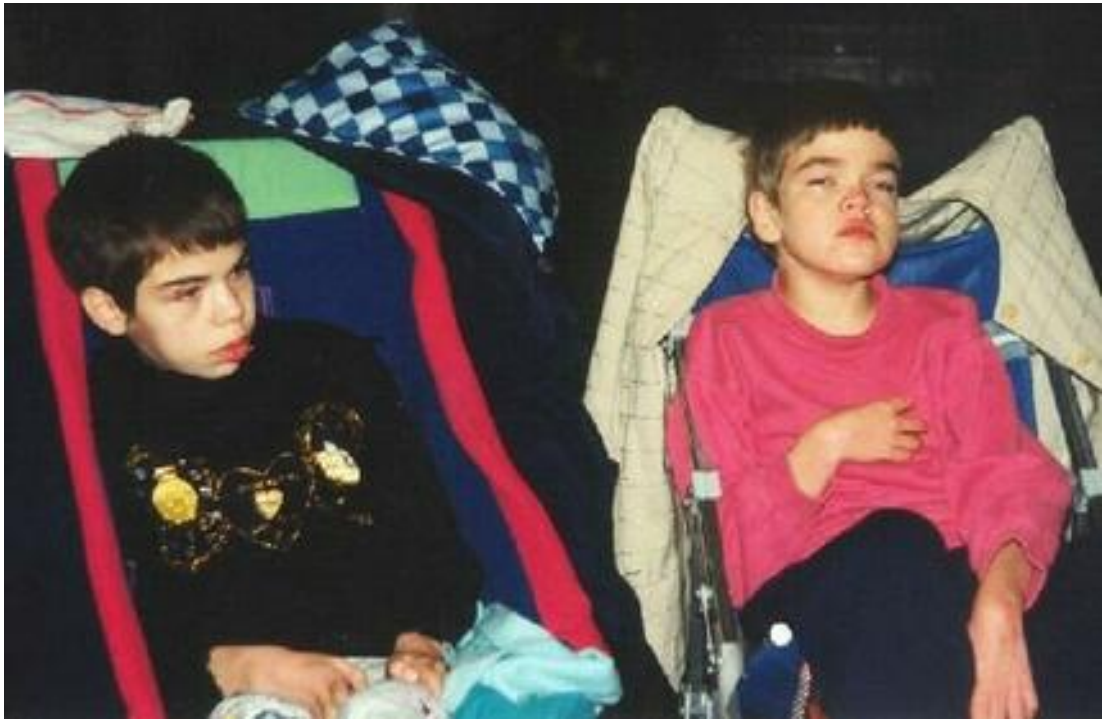
Common symptoms
Progressive dementia, hepatosplenomegaly, coarse features (gargoylism), bone disease (dysostosis multiplex),
corneal opacities, cardiac disease

Mukopolysacharidosis III, MPS III
Sanfilippo disease

In the first years of life normal development
At 2 – 6 years of age prominent hyperactivity, sleep disorders, slowly progressive dementia

Coarse facies, coarse hair
drsné vlasy, small
hepatosplenomegaly

Spasticity, dementia,
death usually
between 15 - 25 years
of age



Glycoproteinoses: Hereditary deficits of enzymes degrading sugar moieties of glycoproteins
Clinically similar to mucopolysaccharidoses

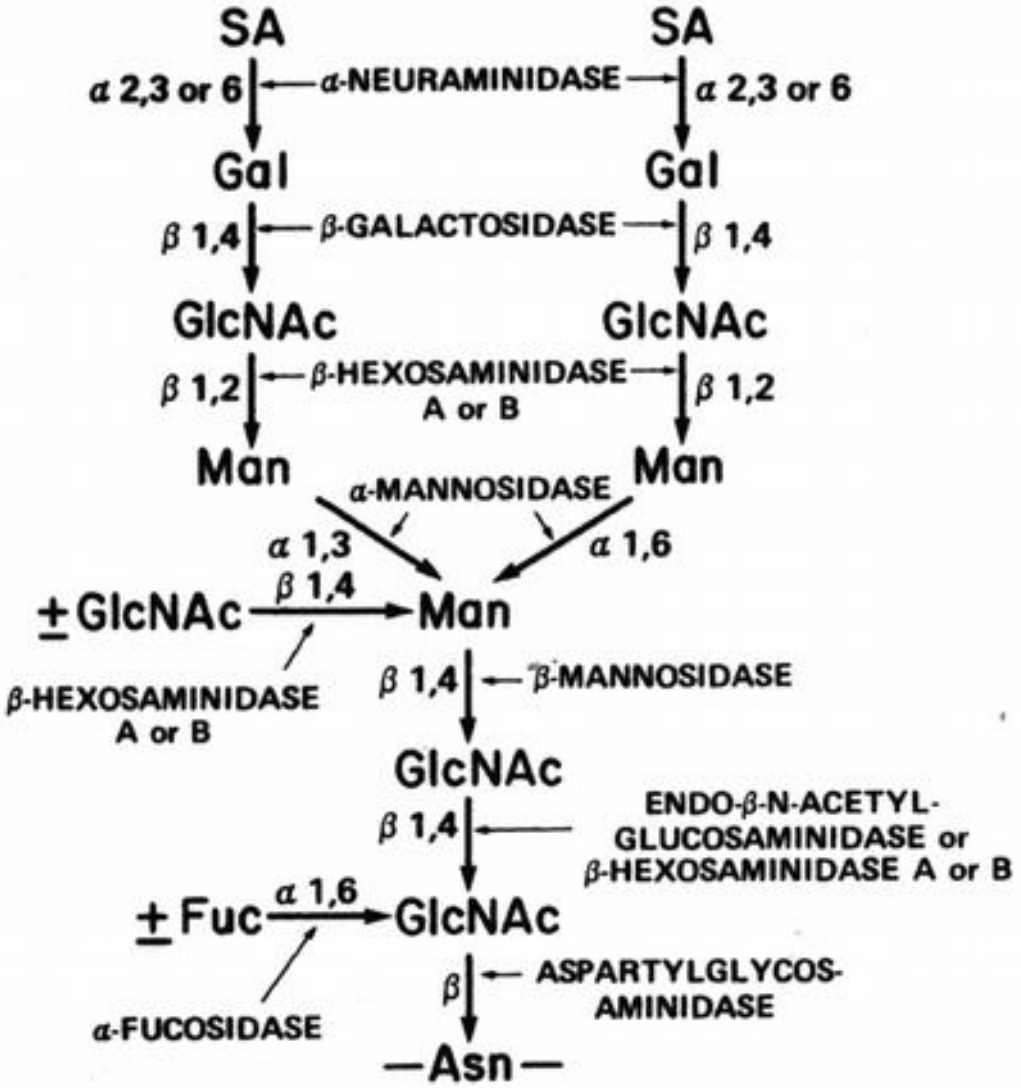


Fig. 140-4 Probable steps in degradation of complex oligosaccharide structure.

I-cell disease (mucolipidosis II)

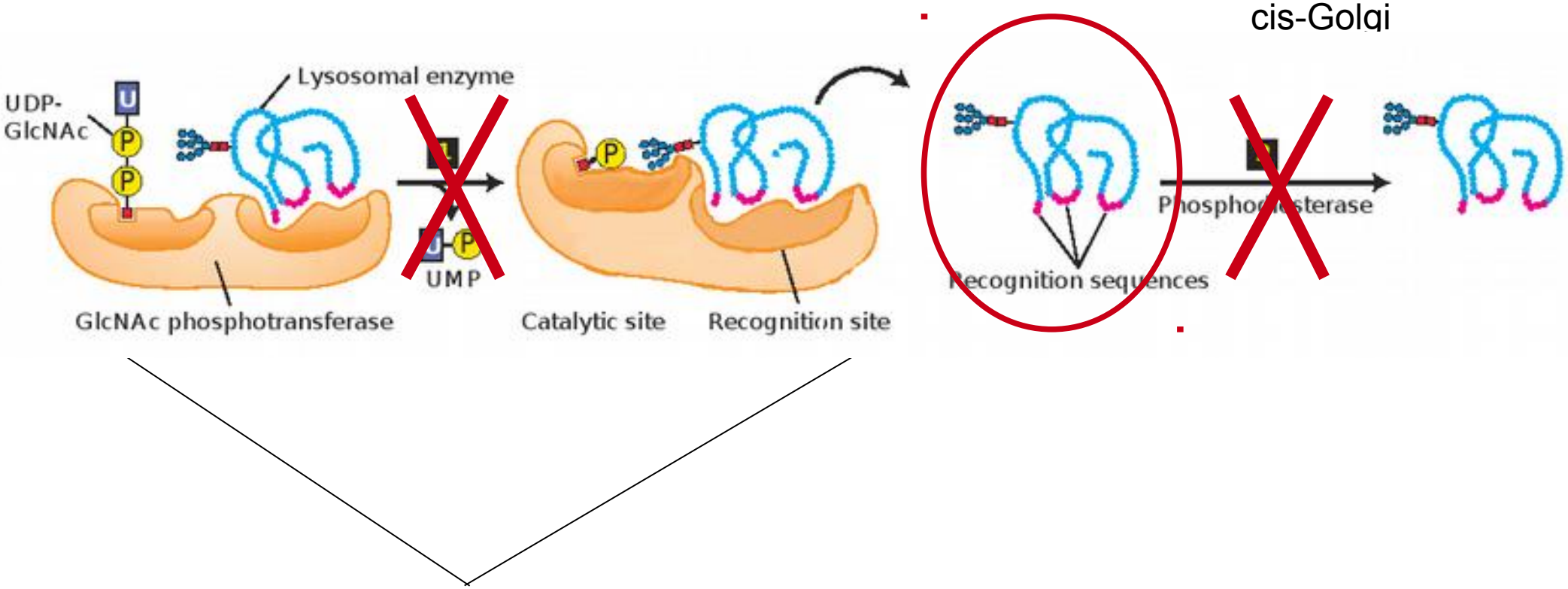
Disorder of transport M6P-tagged lysosomal proteins due to mutations in GlcNAC phosphotransferase

increased activities of lysosomal proteins in extracellular fluid

decreased activities of multiple lysosomal enzymes in lysosomes

enlarged lysosomes

Mutations in GlcNAc transferase gene



Mutations in GlcNAc transferase gene

protein-M6P-M6PR

lysosome

protein

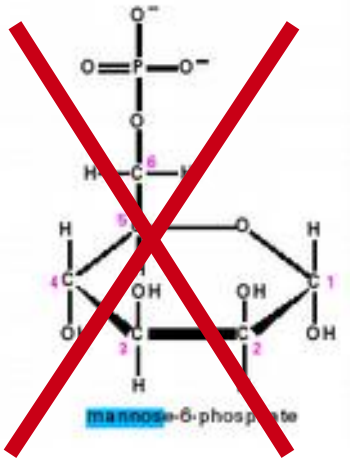
Proteins transported normally by M6PR are not targeted to lysosomes

protein

secretion

trans-Golgi

... instead, they are secreted out of the cell.



I-cell disease (Mucolipidosis II)

Deficiency of GLCNac-phosphotransferase
Coarse facies, thickening of gums, small hepatomegally and splenomegally, dysostosis multiplex
psychomotor delay, mental deficit
elevated activities of lysosomal hydrolases in plasma, low activities in tissues
Vacuolization of lymphocytes („Inclusion cell“) = storage lysosomes

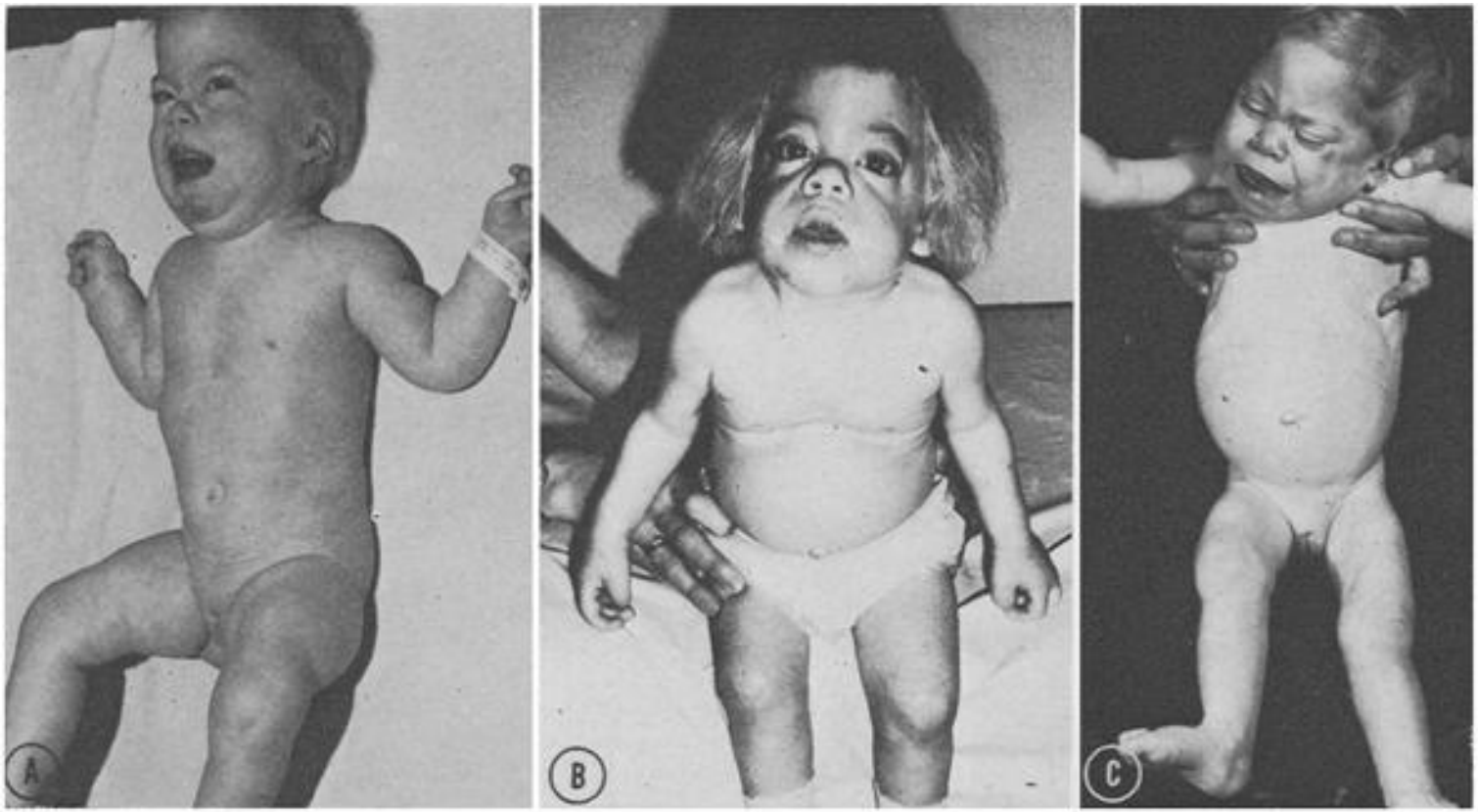
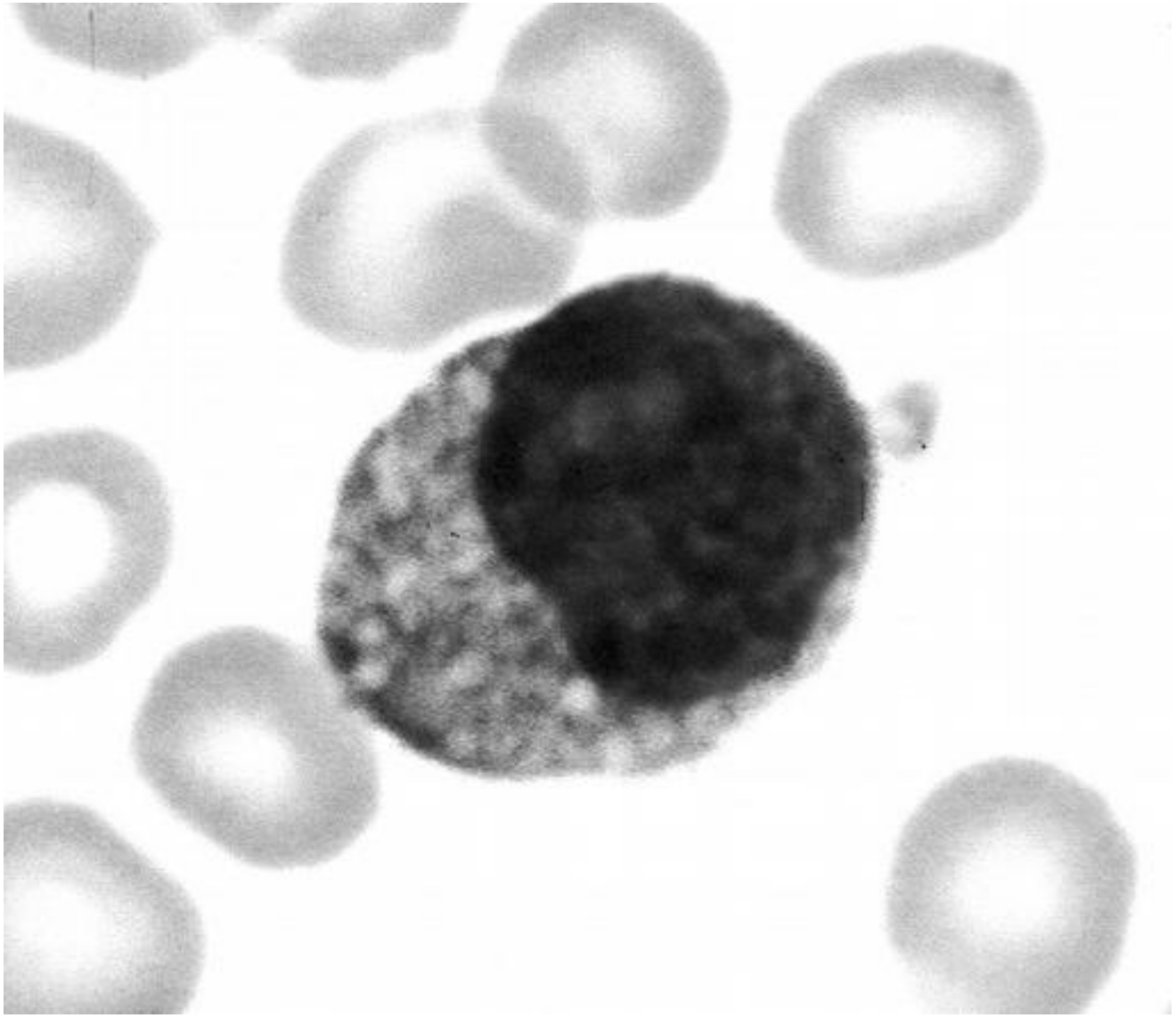
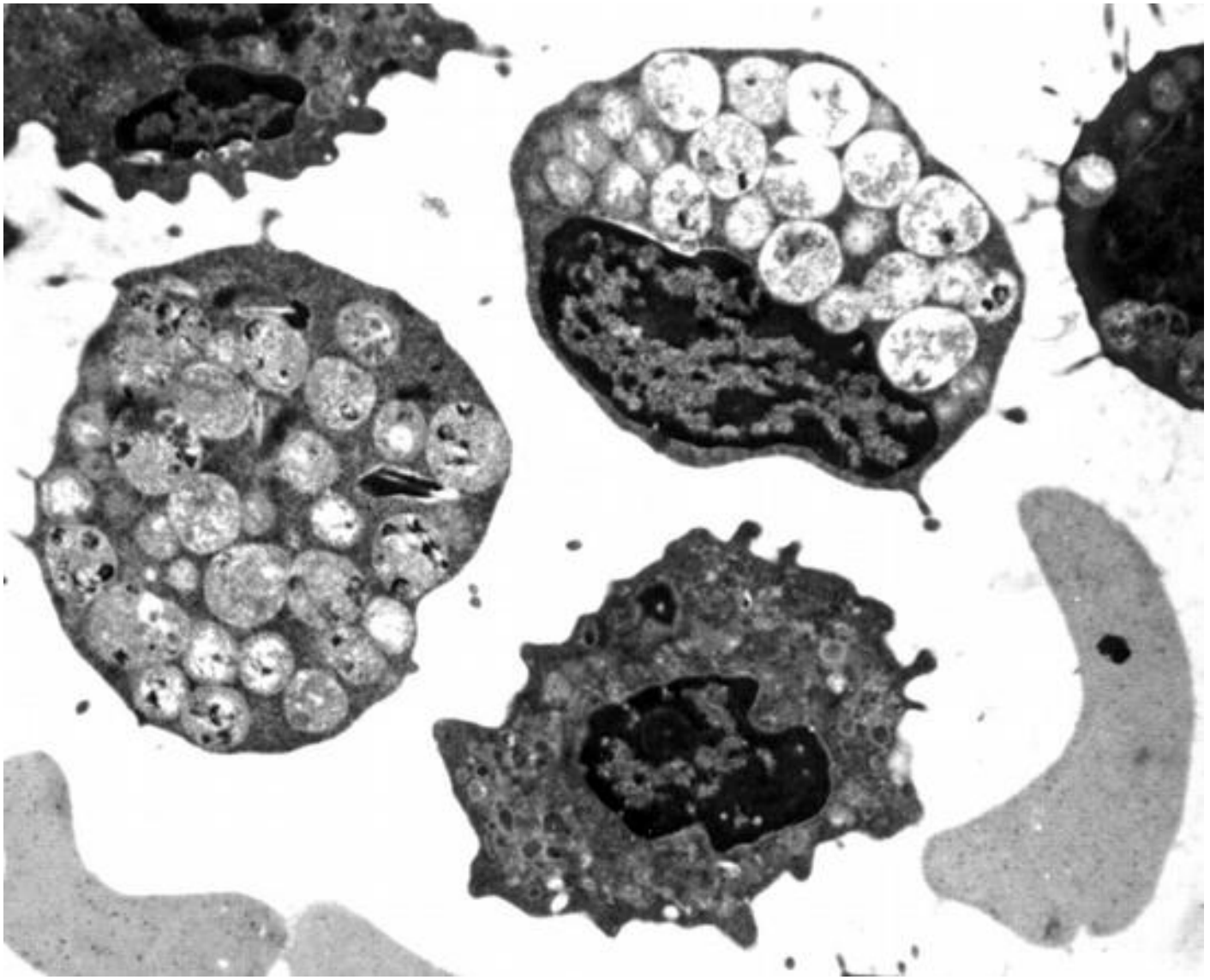


Figure 1 A lymphocyte with many vacuole-like inclusions (original magnification, x900).



van der Meer, W et al. J Clin Pathol 2001;54:724-726

Figure 3 Electron microscopic image of lymphocytic vacuoles containing round osmiophilic structures (original magnification, x15 000).



van der Meer, W et al. J Clin Pathol 2001;54:724-726

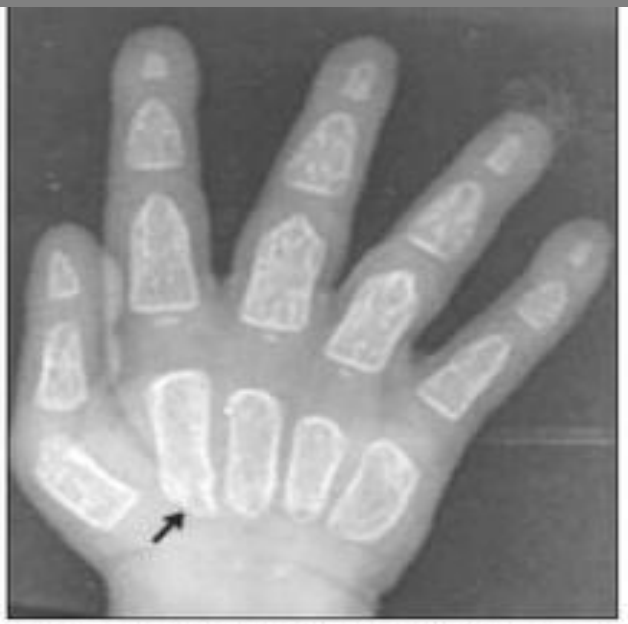


Figure 2 a: X-ray of hand showing shortening of tubular bones and proximal tapering of 2nd to 5th metacarpals

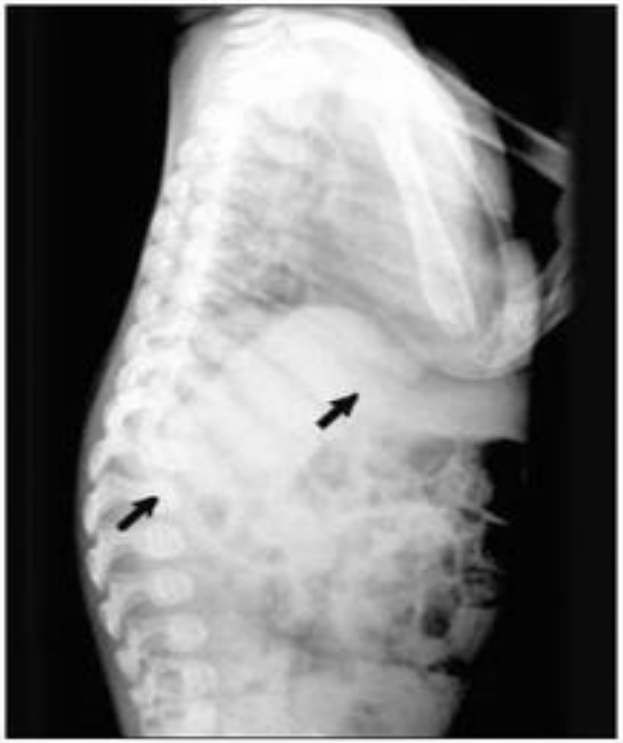


Figure 2b: Lateral X-ray of the spine showing ovoid vertebral bodies and "hammer shaped" vertebrae. The ribs are widened and "oar shaped"

Dysostosis multiplex in I-Cell disease

Kumar et al, J Postgrad Med. 2005 Jul-Sep;51(3):232-3.

Danon disease – LAMP2 deficiency

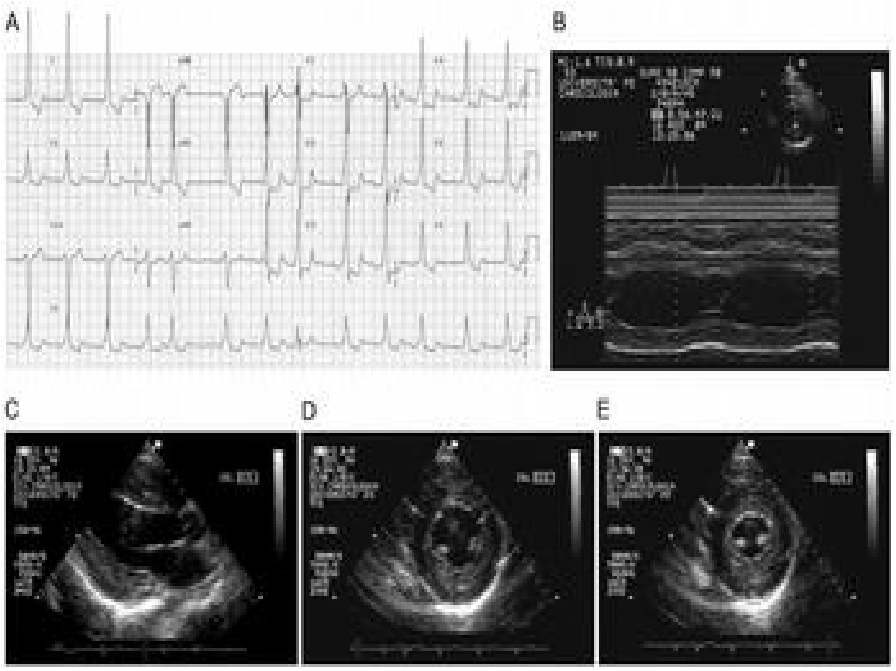
Lamp 2 participates in fusion of lysosomes with autophagic vacuoles

ırdiomyopathy - usually hypertrophic
rythmia - typically preexcitation syndrome - WPW

Intelectual disability in some patients

Other symptoms:
myopathy
sudden death

X-linked disease -
females have usually milder phenotype



Accumulation of autophagic vacuoles
predominantly in cardiac and skeletal muscle

Danon disease

- Cardiomyopathy : hypertrophic, dilated, Wolf-Parkinson-White syndome
- Skeletal myopathy: proximal muscle weakness
- Intelectual disability

Earlier onset in males: typically after the first decade of life

Females : diagnosis typically in third decade of life

X-linked disorder

Treatment : no causal therapy, heart transplantation, defibrilators

Differential diagnosis : Pompe disease, vacuolar myopathies

Deficiencies of lysosomal permeases lead to lysosomal accumulation of small molecules

- Cystinosis : Cystinosin deficiency

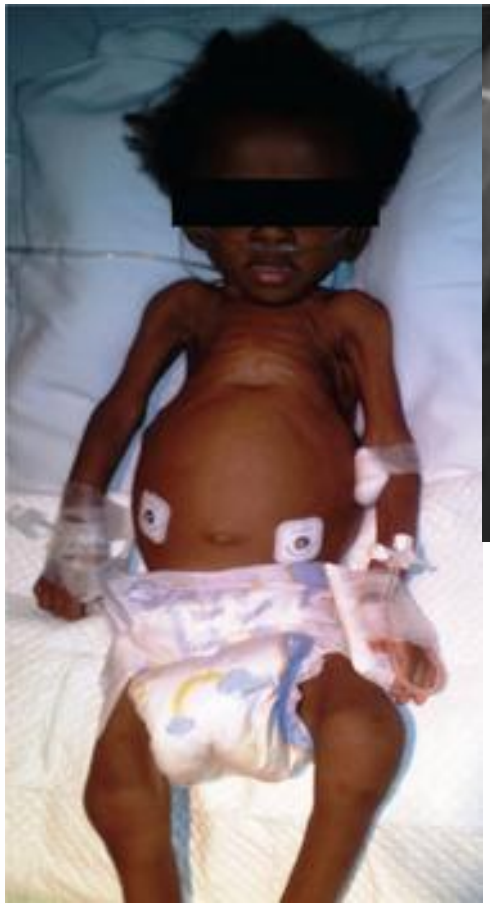
renal disease with Fanconi syndrome
corneal crystals, photophobia, growth retardation
hypothyroidism

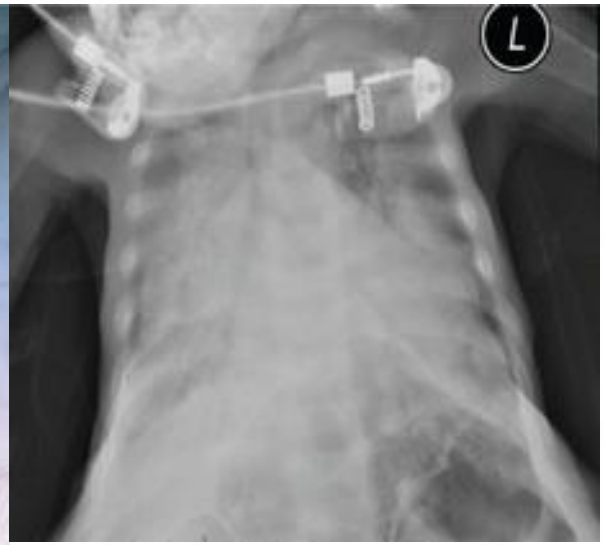
- normal inteligence
- lysosomal acumulation of cystine

- Isolated ocular form

- mixed disulfide with cysteamine is transported

by permease for lysine

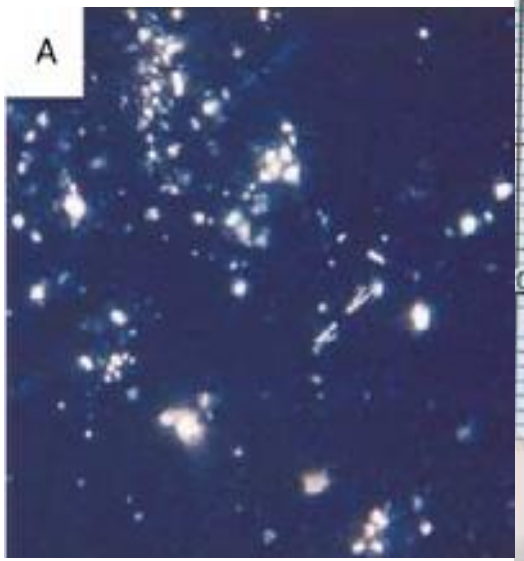


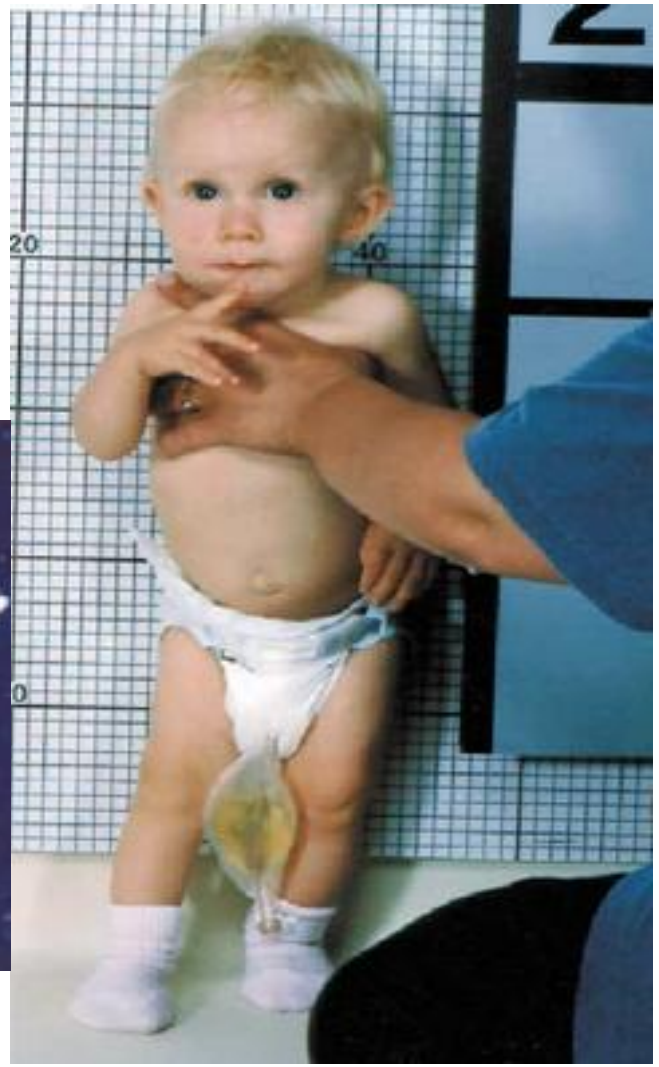


El Naggari et al.Sultan Qaboos Univ Med J. 2014 May; 14(2): e245–e248.

Lysosomal transporters deficiencies

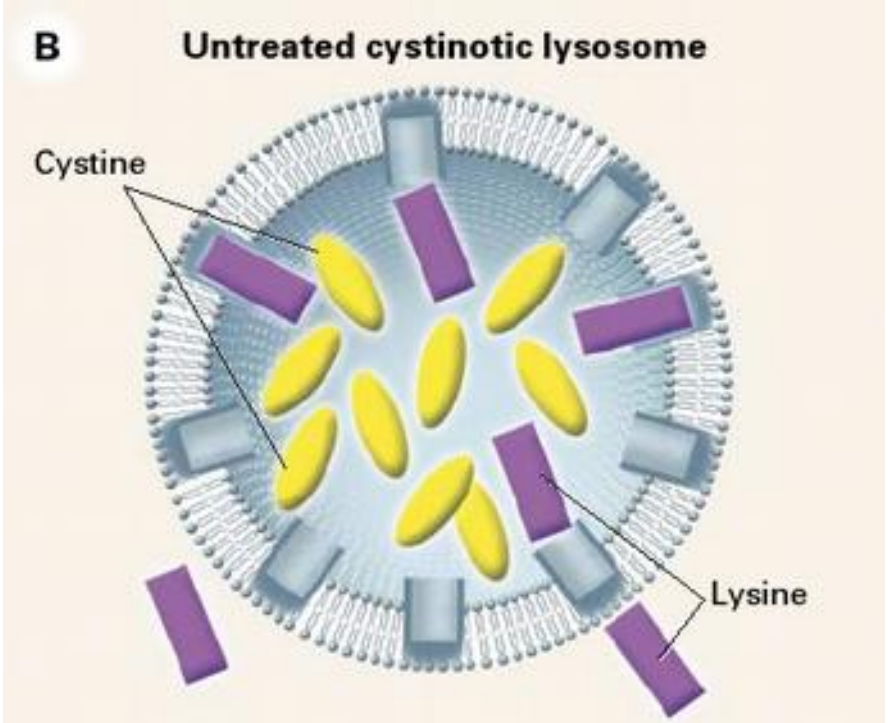
<u>Cystinosis – cystinosin deficiency</u>
renal disease with Fanconi syndrome
renal failure – renal transplantation
corneal crystals , photophobia
growth retardation
hypothyroidism
normal inteligence

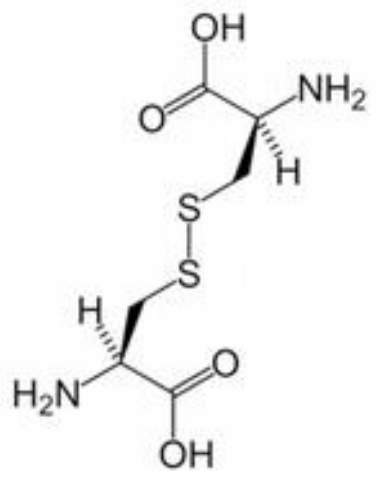




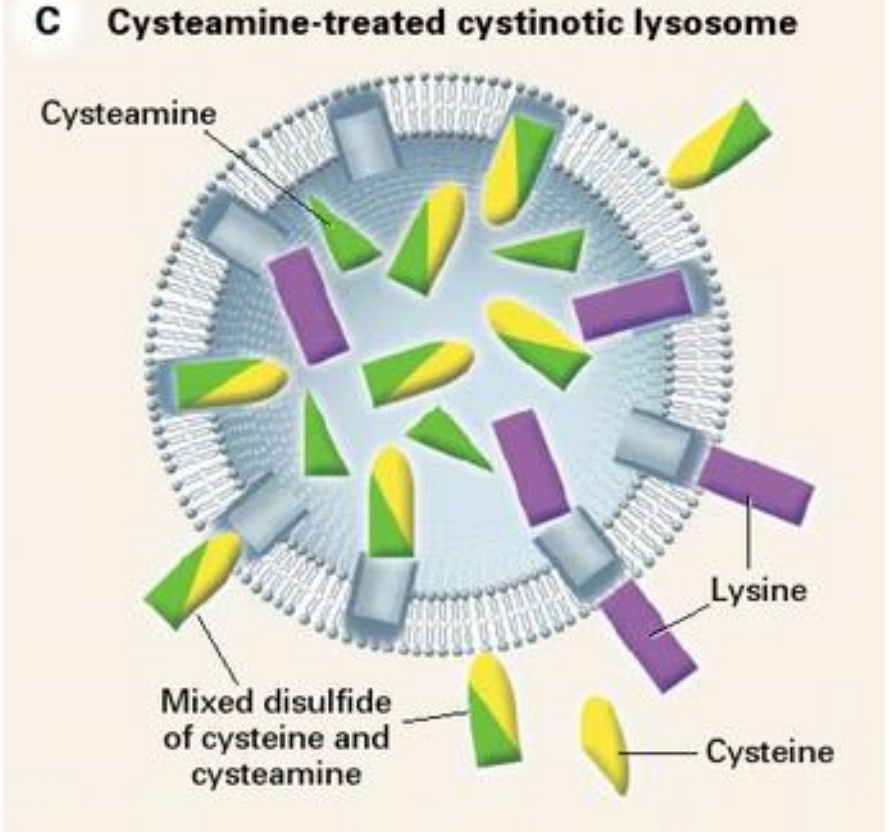
ocular form

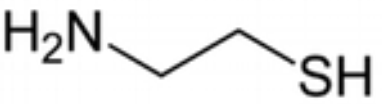
<u>Sialuria – sialin deficiency</u>





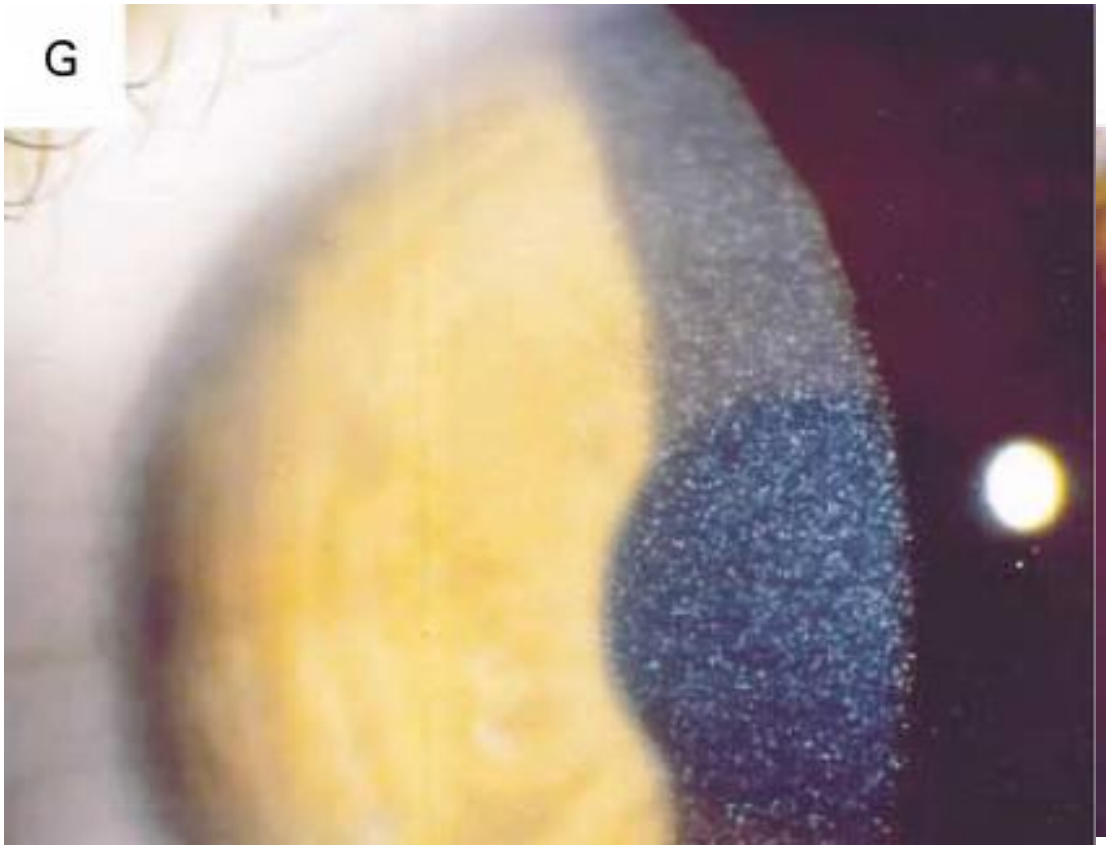
cystine

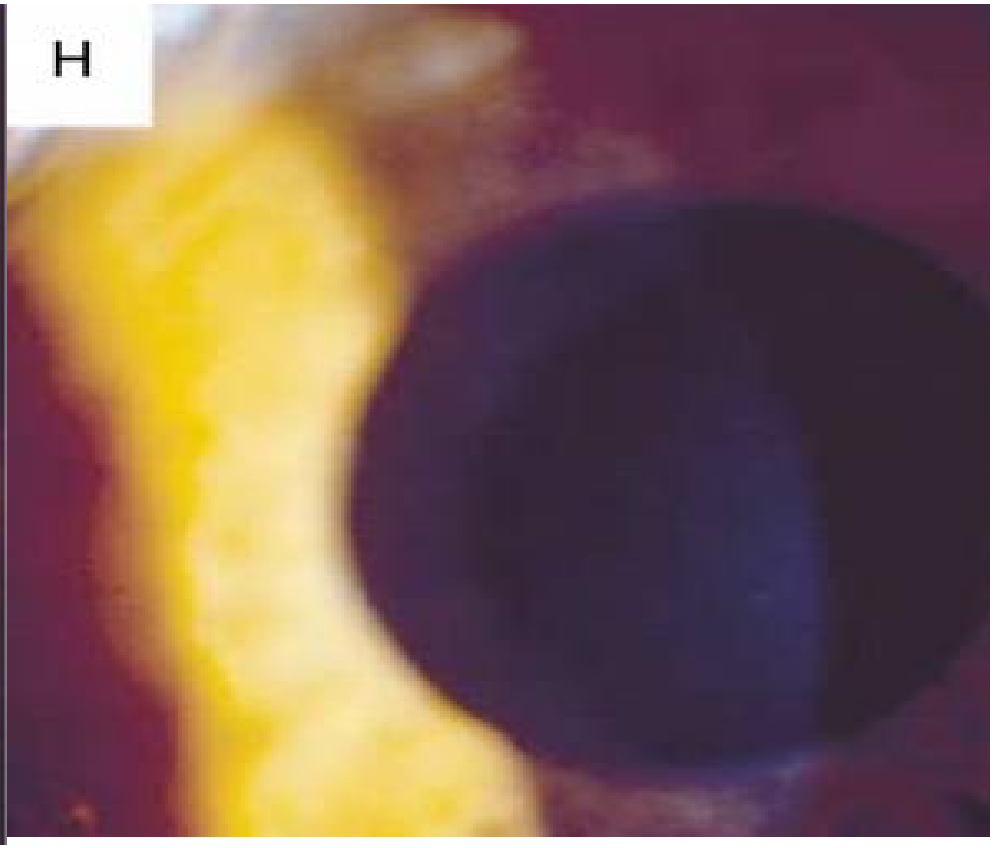


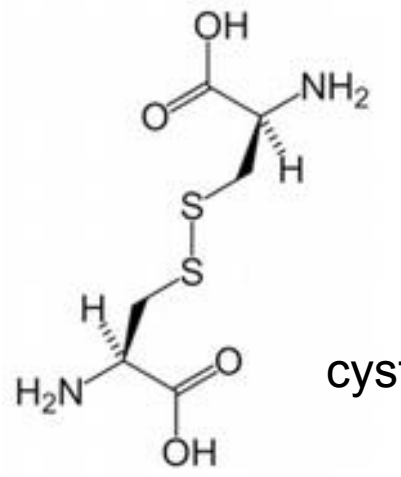


cysteamine

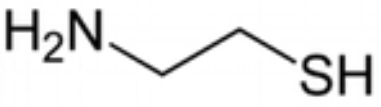
Cystinosis





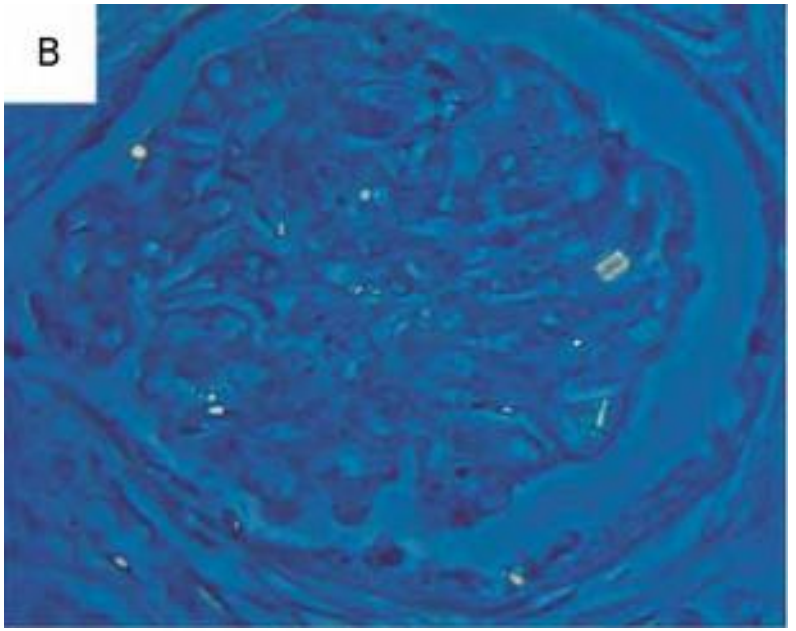


cystin



cysteamin

Cystinosis



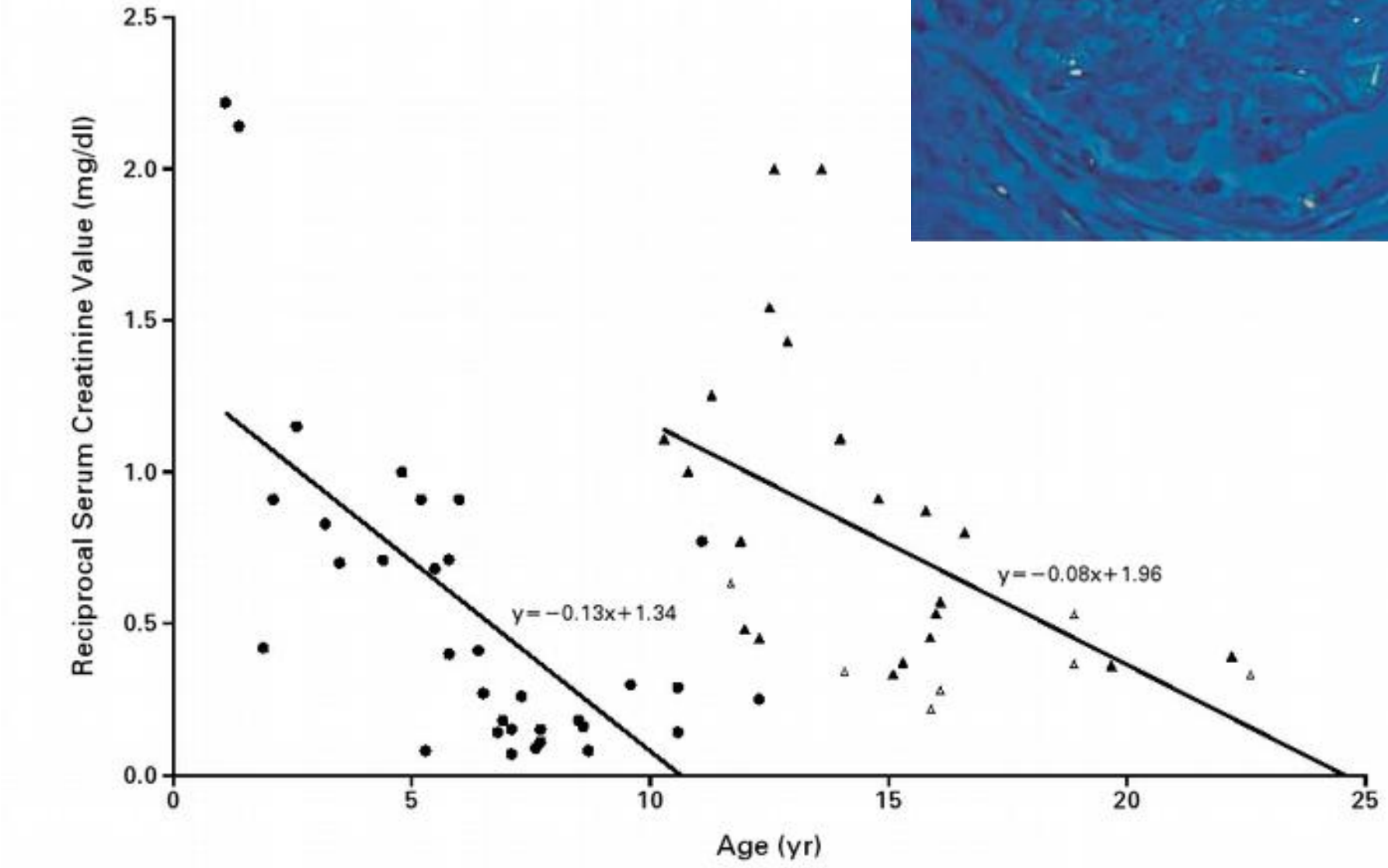


Figure 4. Renal Function in Patients with Cystinosis Treated with Cysteamine and in Untreated Patients, According to Age.

Disorders of lysosome-related organelle biogenesis and function

A group of hereditary disorders often associated with
- albinism (melanosome dysfunction)
- visual impairment
- bleeding tendency(platelet dysfunction)
- inflammatory bowel disease
- lung fibrosis
- immunodeficiency
- “huge lysosomes” in tissues

Heřmanský-Pudlák,Griscelli,
Chediak-Higashi syndromes

heatherkirkwood.blogspot.cz

Diagnostics and treatment of lysosomal disorders

Treatment

Supplementation of deficient protein

Bone marrow transplantation_

Enzyme replacement therapy

Reduction of stored substrate

substrate inhibition therapy

Bone marrow transplantation

Haematopoietic stem cell transfer

Pro:
In contrast to enzyme replacement therapy can influence CNS disease

Con:
High morbidity and mortality

Lysosomal disorders
Mucopolysacharidosis I
- Modifies natural course of the disease
- Early treatment can prevent neurological disease
- Residual disease

Other MPS disorders
MPS III – no improvement of neurological progression
Other lysosomal disorders

Peroxisomal disorders
X-ALD

http://www.bmtinfonet.org/bmt/bmt.book/chapter.1.html#p13

Enzyme supplementation therapy

Supplementation of deficient enzyme in regular infusions

Gaucher disease (glucocerebrosidase)
Fabry disease (alpha galactosidase A)
Pompe disease (acid alpha glucosidase)
MPS I (alpha iduronidase)
MPS II (alpha iduronate sulfatase)
MPS VI, Maroteaux-Lamy (arylsulfatase B)
Niemann-Picko disease B (acid sphingomyelinase)
MPS IVA, Morquio A, ...

Production of recombinant enzymes

Genzyme, TKT, Biomarin, Shire, Inotech, ...

Enzyme supplementation therapy in Gaucher disease

Receptor-mediated endocytosis

Macrophage targeted glucocerebrosidase
- treatment with exoglycosidases

Mannose receptor
(macrophages, endothelia, liver)

Regular infusions

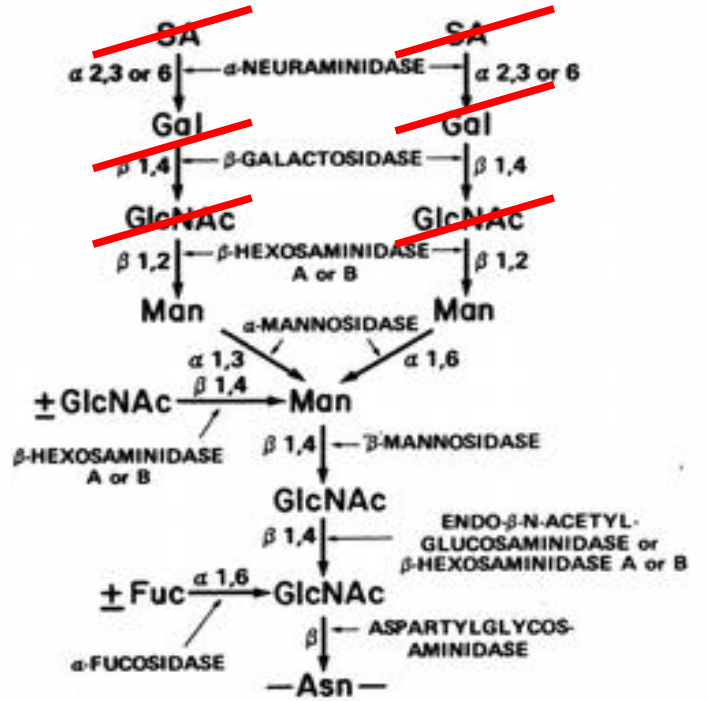


Fig. 140-4 Probable steps in degradation of complex oligosaccharide structure.

Originally glucocerebrosidase isolated from human placentas (Ceredase, Genzyme)

Recombinant enzyme

Cerezyme (Genzyme) – Cho cells

Does not cross haematoencephalic barrier

High costs

Enzyme supplementation therapy

Supplementation of deficient enzyme in regular infusions

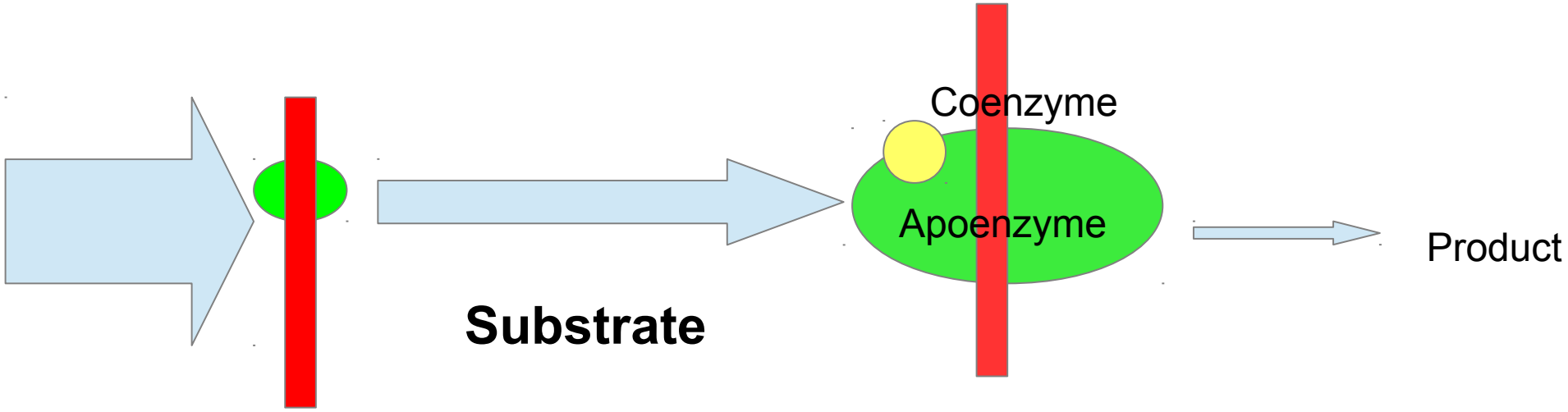
Gaucher disease (glucocerebrosidase)
Fabry disease (alpha galactosidase A)
Pompe disease (acid alpha glucosidase)
MPS I (alpha iduronidase)
MPS II (alpha iduronate sulfatase)
MPS VI, Maroteaux-Lamy (arylsulfatase B)
Niemann-Pick disease B (acid sphingomyelinase)
MPS IVA, Morquio A, ...

Production of recombinant enzymes

Genzyme, TKT, Biomarin, Shire, Inotech, ...

b) Inhibition of enzymes in the metabolic pathway proximal to the metabolic block

„ Substrate inhibition (reduction) therapy“



Substrate inhibition therapy

Mutant enzymes have residual activities

N-butyldeoxyjirinomycin (Zavesca)

Inhibitor of glucosylceramide synthase

Gaucher disease, GM1 gangliosidosis

Diagnostics

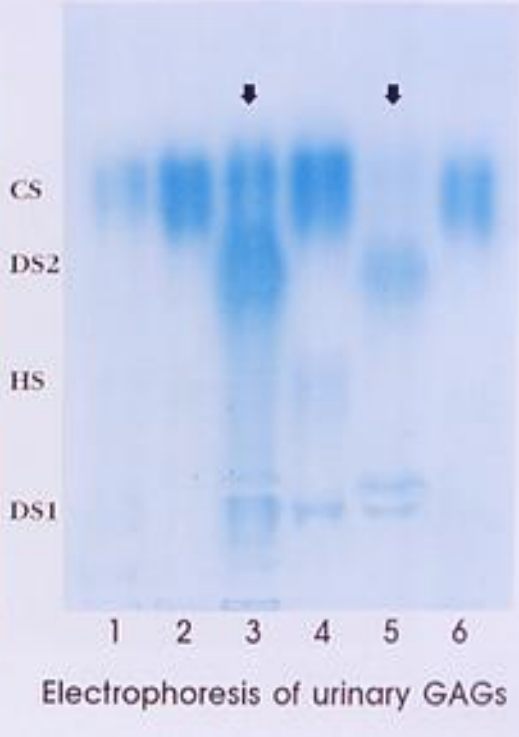
Measurement of metabolites

Enzyme activity measurement

Mutation analysis

Morphological diagnostics

MPS I in a 6-year-old girl



Electrophoresis of urinary GAGs