METABOLISM

بسم الله الرحمن الرحيم

FINAL – Lecture 9

# Metabolism of Sphingolipids

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

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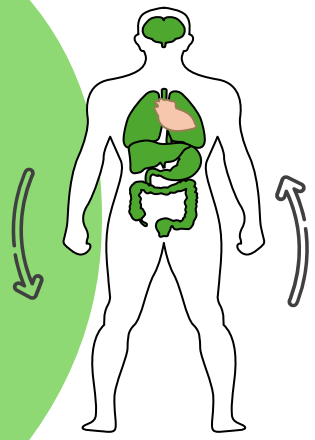
Done by:

- **Muthanna Khalil**

# Metabolism of Sphingolipids

Dr. Diala Abu-Hassan

Some relevant information mentioned at the end of the lecture:

Sphingolipids are so called because of their resemblance to Sphynx (أبو الهول) by 2 aspects:

1. Similar shape → long sphingosine backbone with a head group.
2. Mysterious function → research is currently investigating their effects in the pathogenesis of HIV and other viral infections as well as in protein accumulations such as prions.

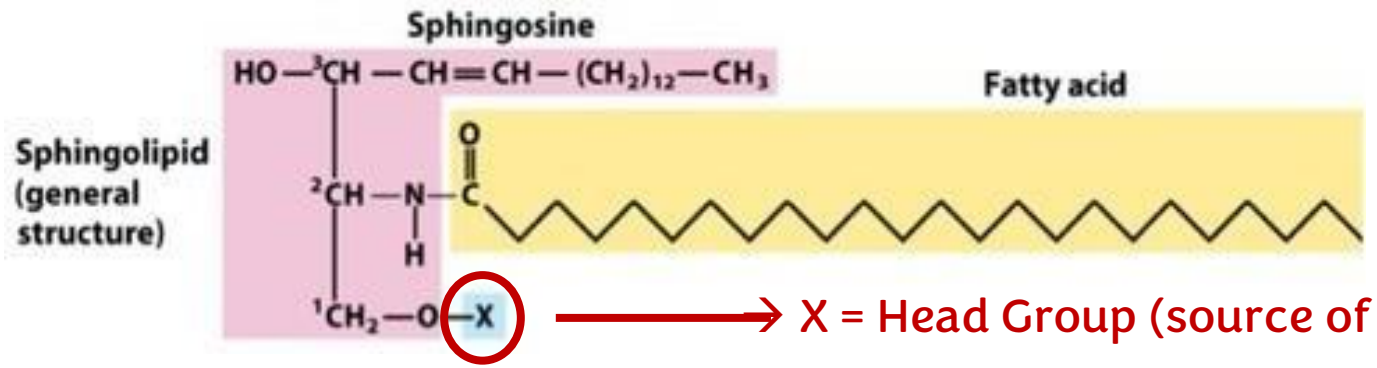
# Recall Sphingolipids

- In comparison with glycerophospholipids, sphingolipids have:
  - A sphingosine backbone (instead of glycerol)
  - 2 vacant slots for attachment (instead of 3)
    - 1 slot for a fatty acid (instead of 2 slots in glycerophospholipids)
    - 1 for the head group [same as in glycerophospholipids see next slide for details]

  - Sphingosine is a large backbone (compared with glycerol); it has:
    - Hydrocarbon chain with a double bond (first tail)
    - Amino group at C2 for fatty acid attachment (second tail)
    - Alcohol group for the attachment of the head group

    See image in the next slide

  - Sphingolipids are less eminent in plasma membrane composition when compared with glycerophospholipids, however they are known to exist in considerable amounts in lipid rafts (recall cytology L1) alongside other structures such as glycoproteins and lipids such as cholesterol.




**If X = H, It is ceramide (the father of sphingolipids)**

# Structure of sphingolipids

**Sphingomyelin**

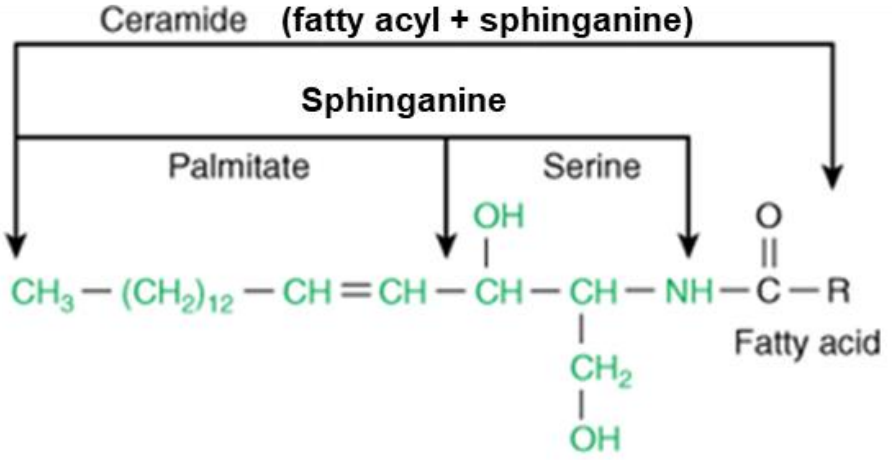
**Glycolipids**

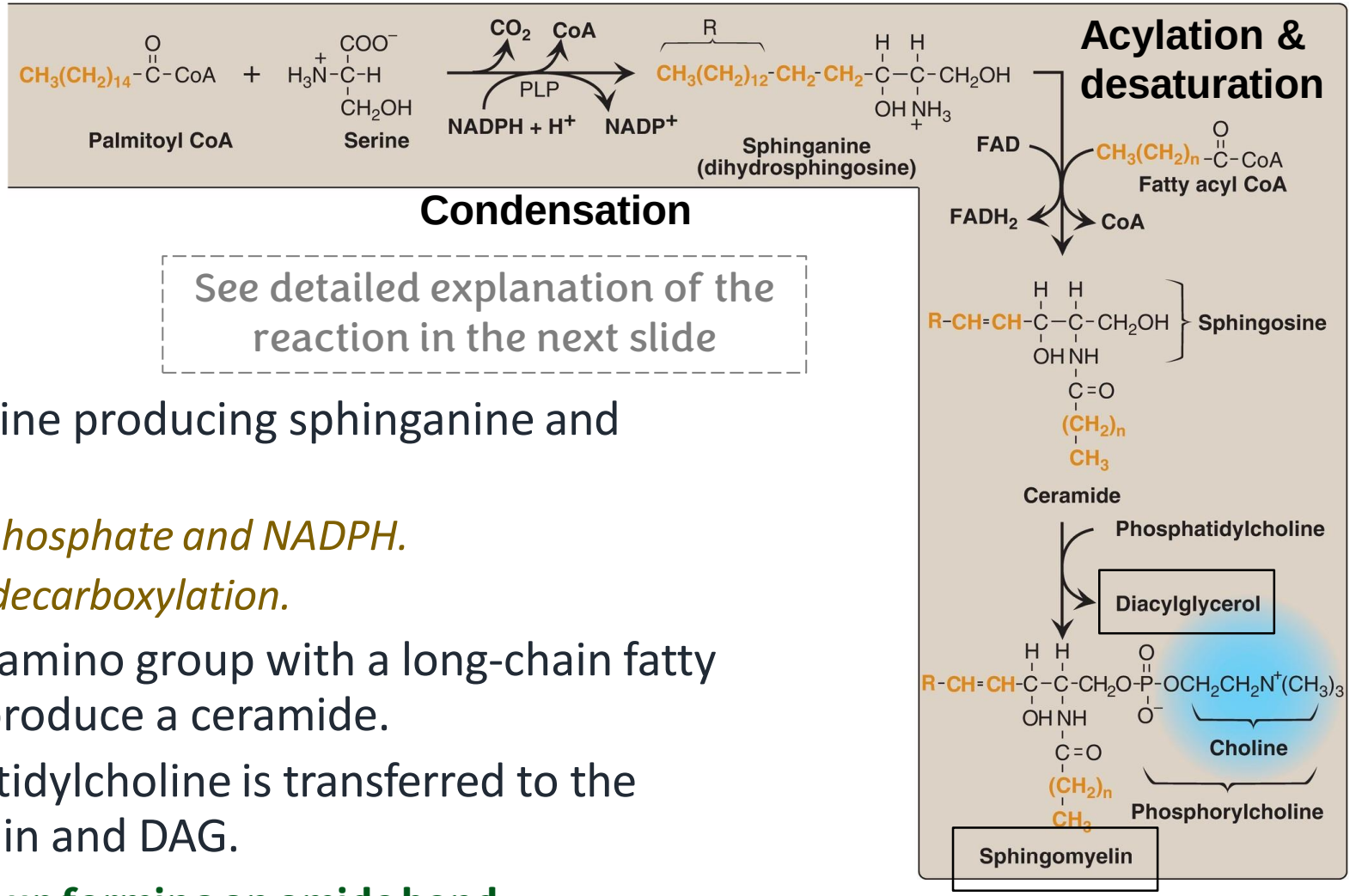
| Substituent (R) | Sphingolipid |
|---|---|
| H | Ceramides |
| Phosphocholine | Sphingomyelins |
| Sugar (s) | Glycosphingolipids |
| - Single sugar (glucose or galactose) | - Cerebrosides |
| - Lactose (disaccharide) | - Lactosylceramides |
| - Oligosaccharide | - Gangliosides |
| - Sugar + sulfate | - Sulfatides |

**More details on them later in this lecture**

# Synthesis of sphingomyelin

**Head group = phosphocholine**
Present in myelin sheath
In the outer leaflet of the plasma membrane



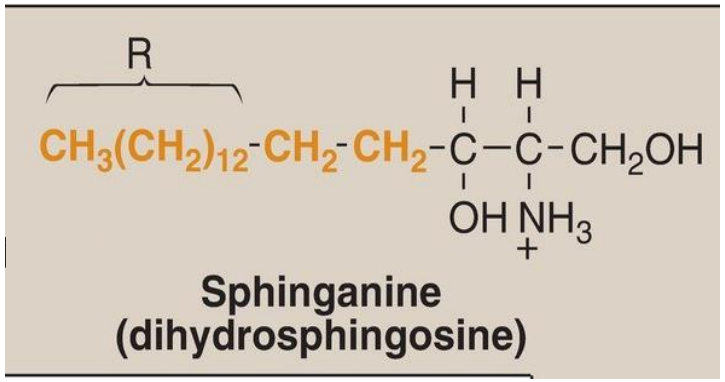




See detailed explanation of the reaction in the next slide

- Palmitoyl CoA condenses with serine producing sphinganine and releasing CoA and $CO_2$.
  - *The reaction requires pyridoxal phosphate and NADPH.*
  - *The needed energy comes from decarboxylation.*
- Sphinganine is **(1)** acylated at the amino group with a long-chain fatty acid and then **(2)** desaturated to produce a ceramide.
- Phosphorylcholine from phosphatidylcholine is transferred to the ceramide, producing sphingomyelin and DAG.

**(1): activated LCFA attacks the amino group forming an amide bond.**
**(2): palmitic acid is saturated; sphingosine is not; $2H^+$ are removed (reducing FAD).**

# Synthesis of sphingomyelin (2)




- The precursors of sphingosine are

1. **Palmitoyl-CoA** (activated palmitate)
2. The amino acid **serine**

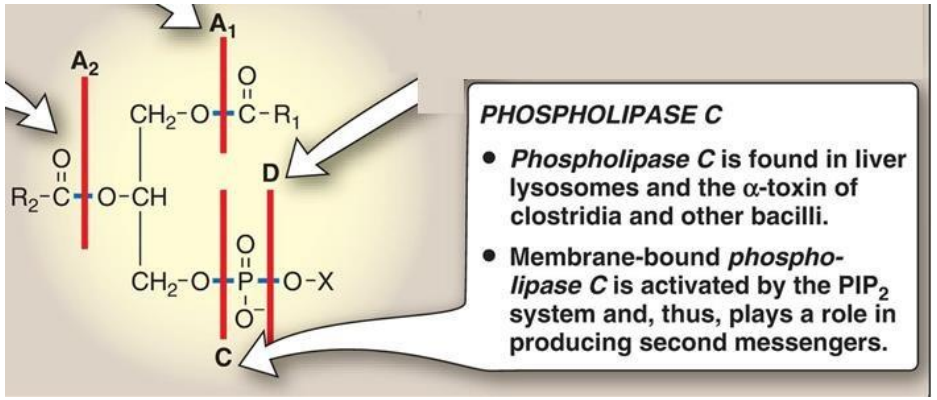
During the reaction of the 2 above:

- CoA is lost (the carbonyl group of palmitoyl-CoA is attached to the α-carbon of serine; the carbonyl is further reduced to a secondary alcohol).
- The carboxyl group of the serine is lost.
- This is a reduction (biosynthetic) reaction, so NADPH is oxidized to $NADP^+$.

The product is called sphinganine, whose characteristics:

- Palmitic (16C) chain attached to decarboxylated serine (2C) → 18C molecule.
- On carbon 2, the normal amino group of serine is still there.
- Two OH groups (reduced palmitate carbonyl on C3, and the OH of serine on C1).

# Degradation of sphingomyelin and the deficiency of sphingomyelinase




- ✓ Sphingomyelin is degraded by sphingomyelinase, a lysosomal enzyme and a type of phospholipase C.
- ✓ The ceramide **(leftover after cleavage)** is cleaved by ceramidase into sphingosine and a free fatty acid
- ✓ The ceramide and sphingosine released regulate signal transduction pathways by influencing the activity of protein kinase C and they also promote apoptosis.
- ✓ Niemann-Pick disease (Types A and B) is an autosomal recessive disease (lysosomal storage disease)
- ✓ Enlarger liver and spleen because of lipid deposits
- ✓ Type A is more severe than B
- ✓ Niemann-Pick disease occurs in all ethnic groups
- ✓ Type A is more frequent in the Ashkenazi Jewish population

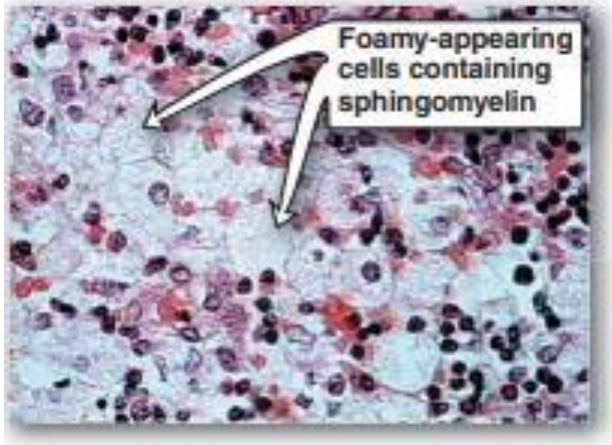
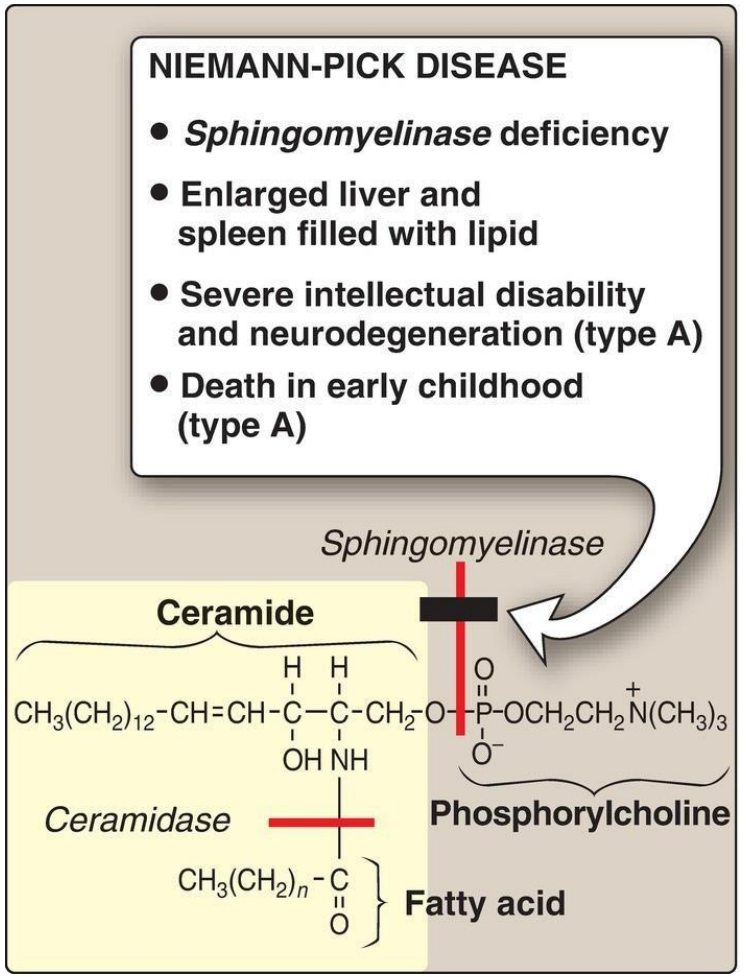



**Figure 17.13**
Accumulation of lipids in spleen cells from a patient with Niemann-Pick disease.



# Glycosphingolipids (glycolipids)

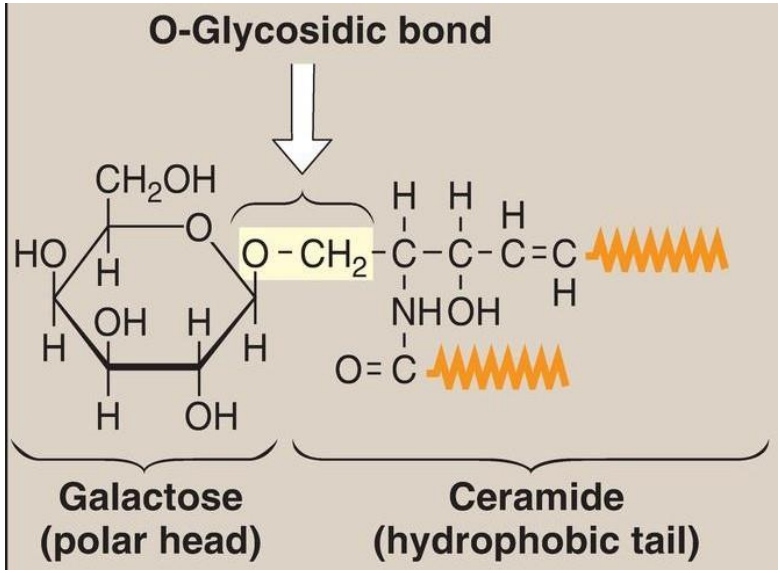
- They are made of ceramide (precursor).
- A sugar(s) is attached to ceramide by an O-glycosidic bond.
- The number and type of carbohydrate moieties determine the type of glycosphingolipid.
- They are localized in the outer leaflet of the plasma membrane and exposed extracellularly (adhesion, recognition, and signaling).
- Their hydrophobic ceramide tail inserts into the outer phospholipid leaflet, while the glycan headgroup extends outwardly.

Structure + Nomenclature

A) Cerebroside [X = **mono**saccharide]
B) Globoside [X = **di-** or **oligo**saccharide]
C) Ganglioside [X = **oligo**saccharide; with ***sialic*** acid]
D) Sulfatides [glycolipid + ***sulfate*** on the sugar]

**A & B** are neutral, while ***C & D*** are ***acidic*** (-)

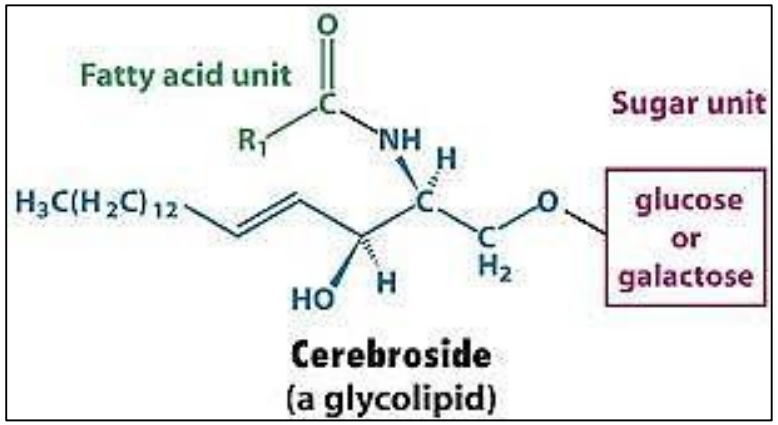
Structure of galactocerebroside



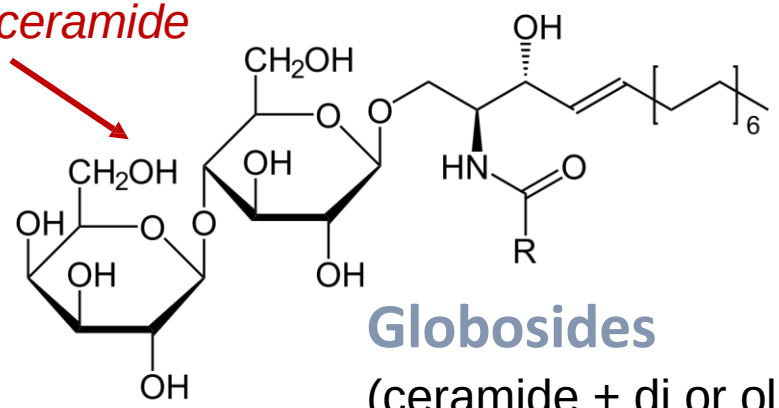
# Types of glycolipids

## Neutral glycosphingolipids

- **Cerebrosides** are the simplest.
- Gluco- or Galactocereobrosides




*Lactosylceramide*

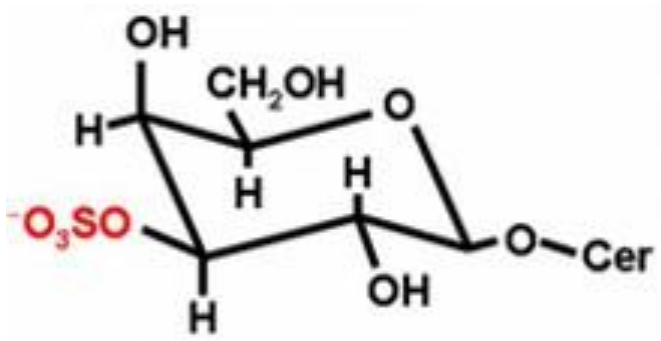


**Globosides**

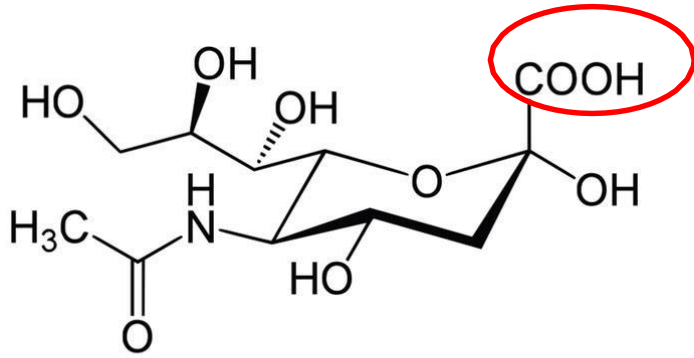
(ceramide + di or oligosaccharides)

## Acidic glycosphingolipids (gangliosides)

- They are negatively charged at physiologic pH due to attachment of N-acetylneuraminic acid ([NANA] or sialic acid, in gangliosides or by sulfate groups in sulfatides.



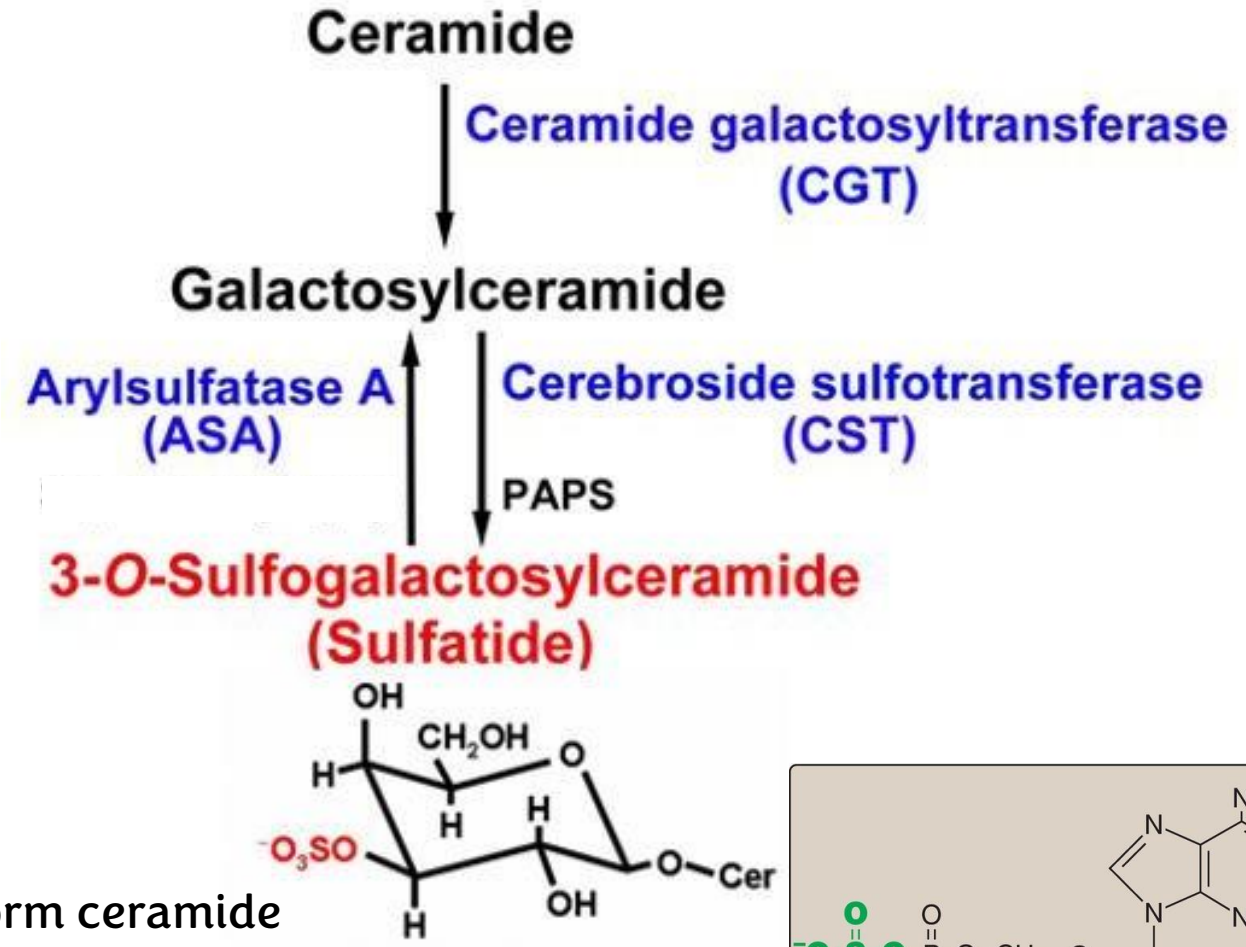
Sulfatide



***N*-acetylneuraminic acid**

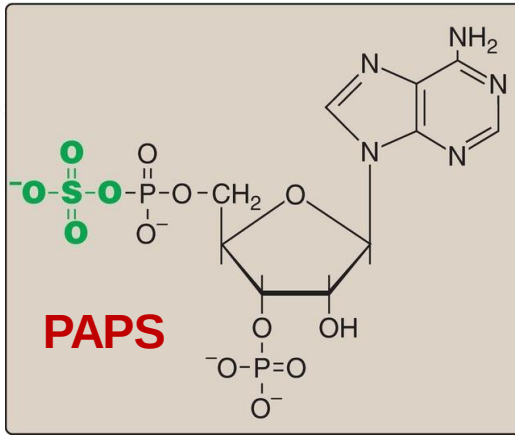
# Synthesis of Glycosphingolipids and Sulfatides

- Synthesis of glycosphingolipids occurs primarily in the Golgi apparatus by sequential addition of glycosyl monomers transferred from UDP-sugars to the acceptor molecule by glycosyltransferases.
- A sulfate group from the sulfate carrier **(donor)** 3′-phosphoadenosine-5′-phosphosulfate (PAPS), is added by a **sulfotransferase** to a galactose in a galactocerebroside, forming the sulfatide galactocerebroside sulfate.

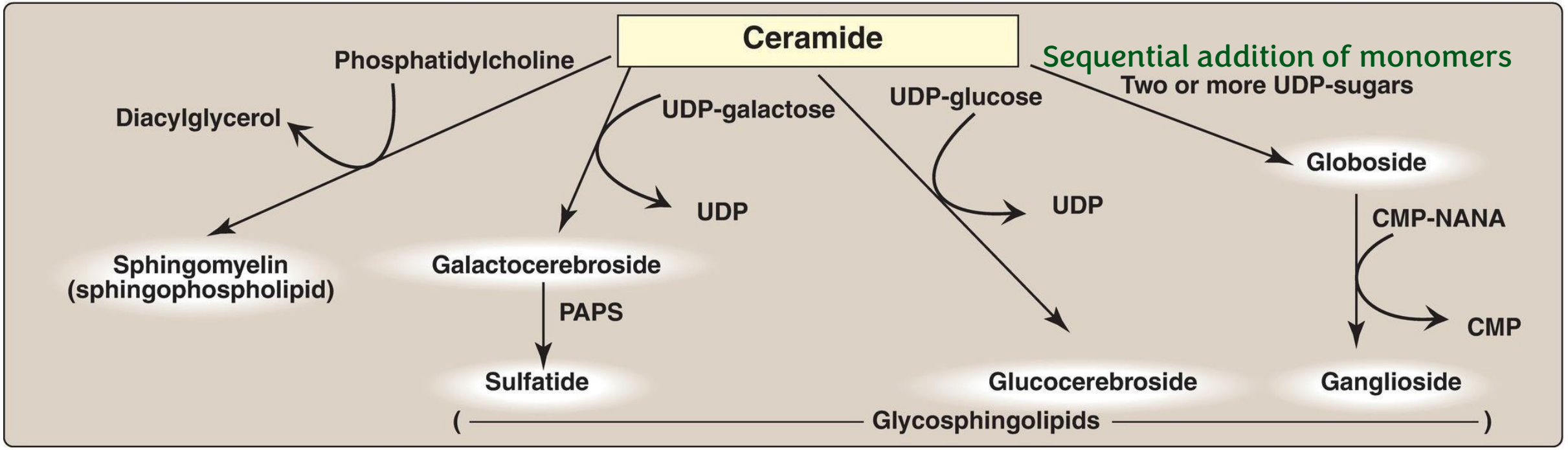



**The 2 steps of forming galactocerebroside sulfate form ceramide**

| Step | Main Substrate | Enzyme | Carrier | Product |
|---|---|---|---|---|
| 1 | Ceramide | Glycosyl transferase | UDP-galactose | Galactocerebroside |
| 2 | Galactocerebroside | Sulfotransferase | PAPS | Galactocerebroside sulfate |



# Synthesis of Glycosphingolipids and Sulfatides



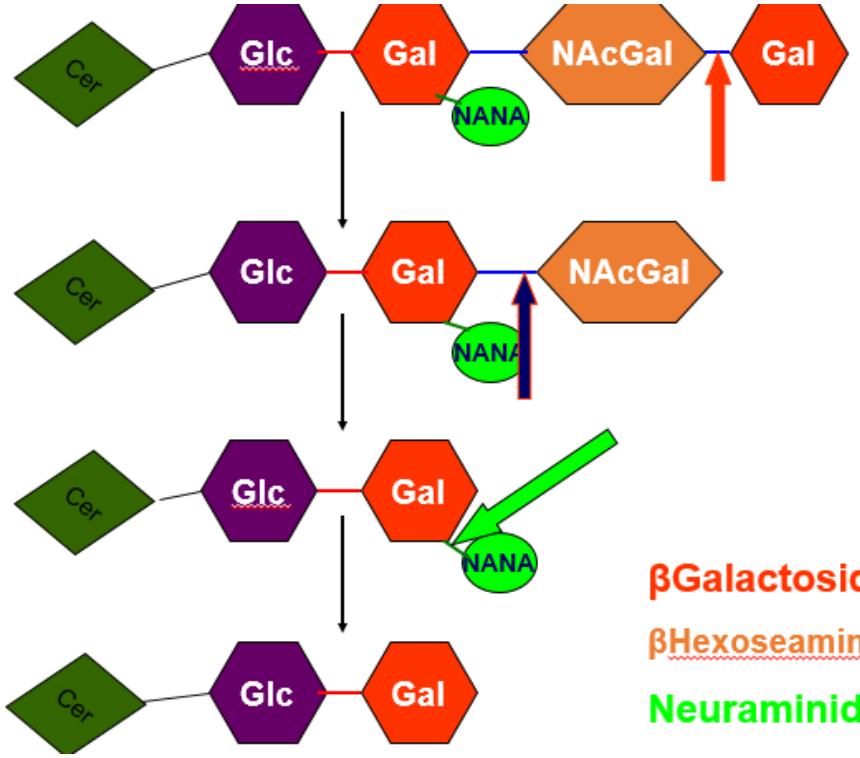

This figure summarizes the synthesis of glycolipids, so make sure you understand it very well.

Notes:

- NANA = Sialic acid is the hallmark of gangliosides
- Any sugar monomer must be carried by UDP
- PAPS is the sulfate donor

# Glycosphingolipid (GSL) degradation

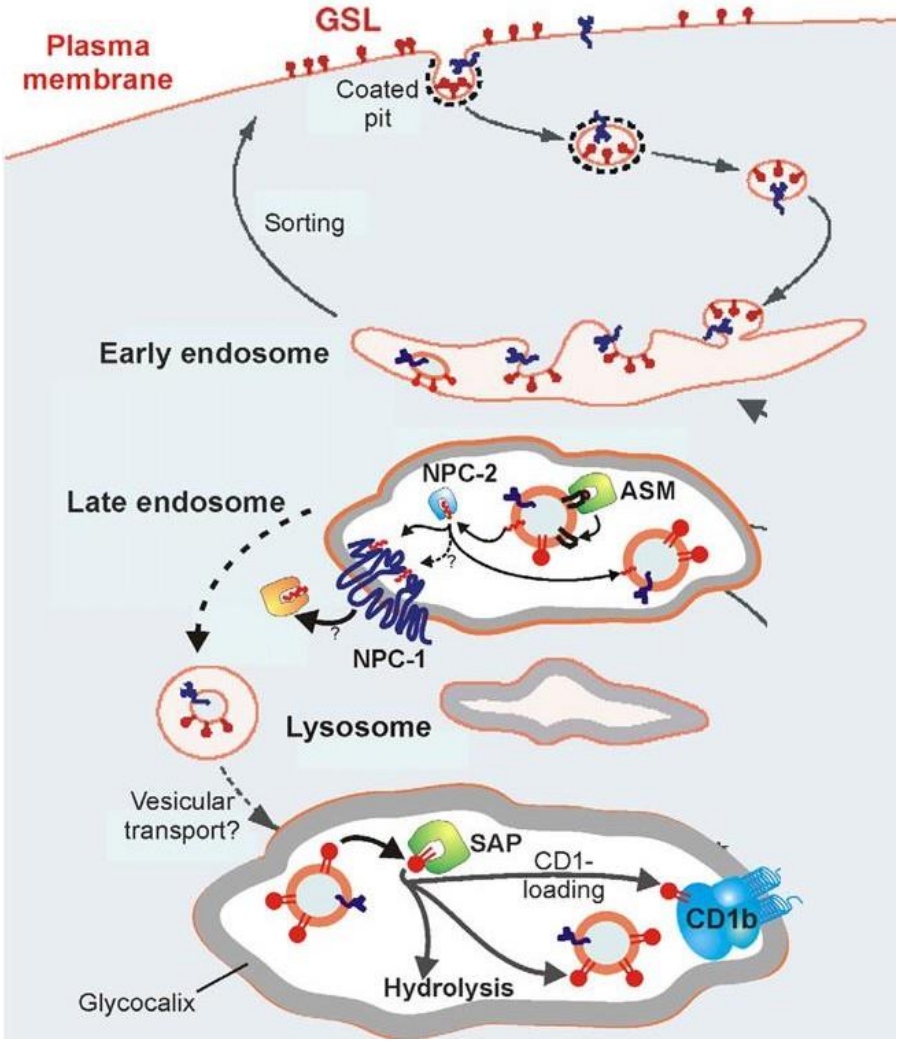
- Glycosphingolipids are phagocytosed into the endosomes that fuse with the lysosomes.
- The lysosomal hydrolases remove the sugars sequentially starting with the last one added and ending with the first one added.




βGalactosidase

βHexoseaminidase

Neuraminidase




- Defect in the degradation of glycosphingolipid, glycosaminoglycans, and glycoproteins causes “lysosomal storage diseases”.
- Sialidase (neuroaminidase) is the glycosidase that removes sialic acid during ganglioside degradation

# Degradation of Glycolipids

- They are part of the membrane, so they are internalized into early endosomes that mature into late endosomes and then fuse with lysosomes where the degradation takes place.
- The sugar part with be degraded by different enzymes depending on the nature of the glycosidic bond, and the terminal residues are cleaved first. The lipid part is then degraded as discussed *here*.

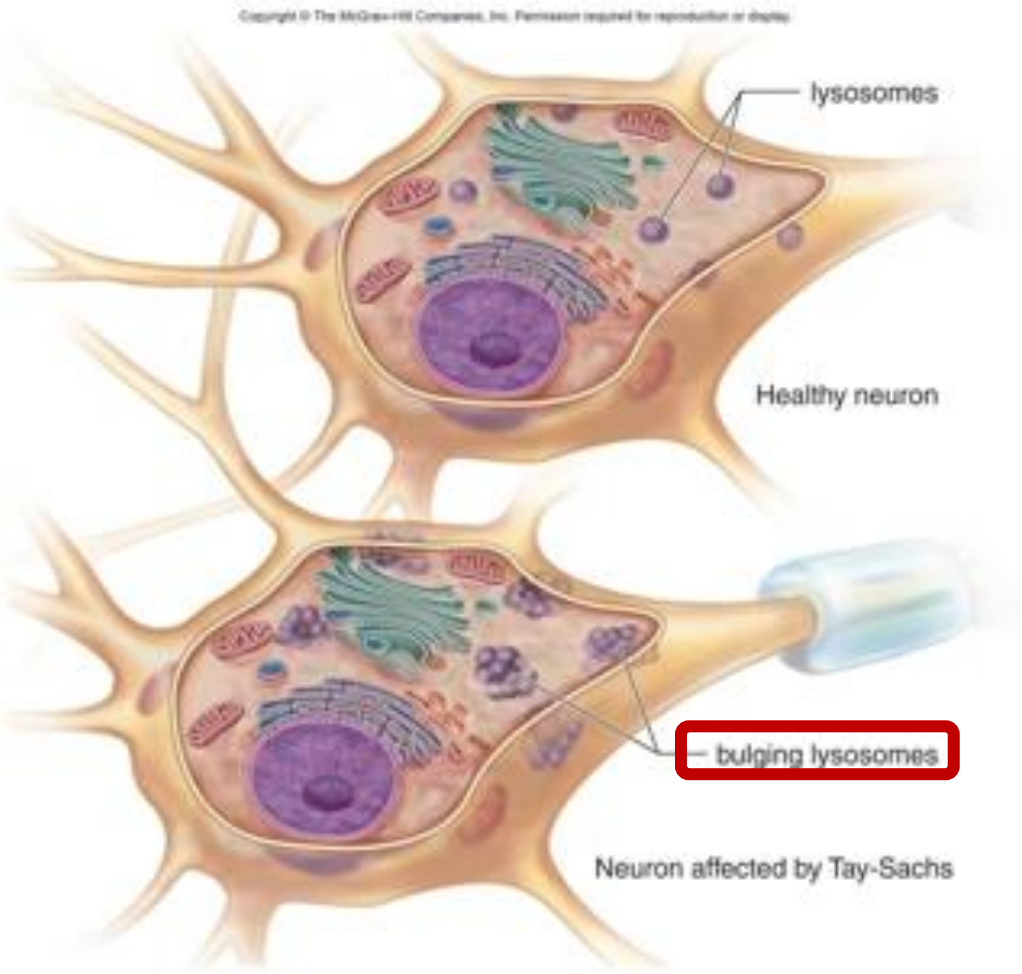
The coming slides illustrate different diseases related with sphingolipids metabolism.

The information which the DR emphasized on (in slides 15-17) are enclosed with dark red rectangles .

# Application: Sphingolipidoses (lysosomal disease)

- Sphingolipidoses: **lysosomal** diseases characterized by mutations in genes that encode lysosomal hydrolases or activator proteins engaged in the intralysosomal **degradation of sphingolipids.**

- Usually, only a single sphingolipid (the substrate for the deficient enzyme) accumulates in the involved organs. The extent of the phenotype depends on the type and number of mutations.
- The disorders are progressive becoming more severe with aging and can be fatal.
- There is **extensive phenotypic variability** due to:
  - **Allele** heterogeneity: different mutations within the **same gene** (different alleles)
  - **Locus** heterogeneity: **different genes** are defective (locus = position, location).
- They are autosomal-recessive disorders, *except* for Fabry disease, which is X linked.
- The incidence of sphingolipidoses is low in most populations, except for Gaucher and Tay-Sachs diseases, which, like Niemann-Pick disease, show a high frequency in the Ashkenazi Jewish population.




**Accumulation of $G_{M2}$ Gangliosides:**

$G_M$ naming of gangliosides:

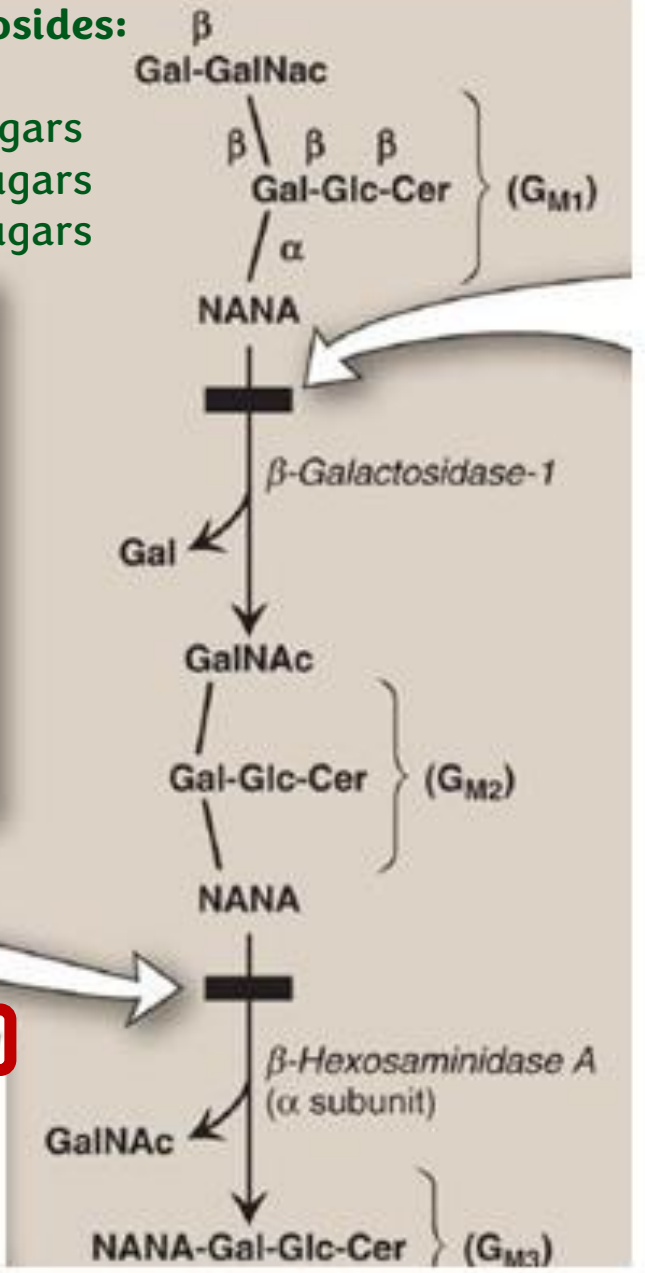
$G_{M1}$ →(5 – 1 = **4**) non-NANA sugars

$G_{M2}$ →(5 – 2 = **3**) non-NANA sugars

$G_{M3}$ →(5 – 3 = **2**) non-NANA sugars

**TAY-SACHS DISEASE**

- Accumulation of gangliosides ($G_{M2}$)
- Rapid, progressive, and fatal neurodegeneration
- Blindness, seizures
- Excessive startle response
- Muscle weakness
- Cherry-red macula
- Deficiency of activator protein ($G_{M2}$ activator) in some cases




**Hexosaminidase A deficiency**

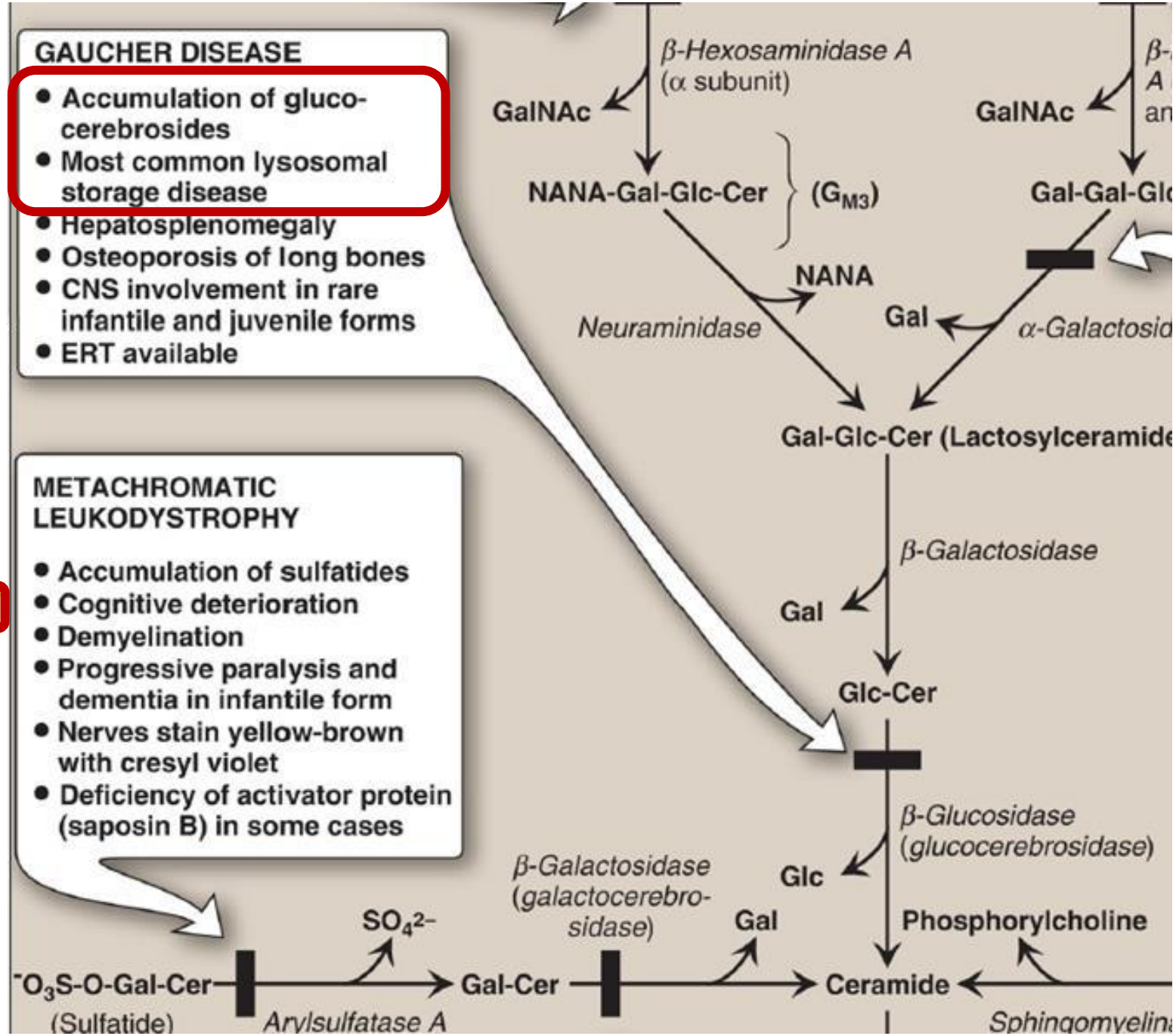
Cleaves sugar amines

# Tay-Sachs disease

(Glucosidase deficiency)
Enzyme replacement therapy ←

# Gaucher disease

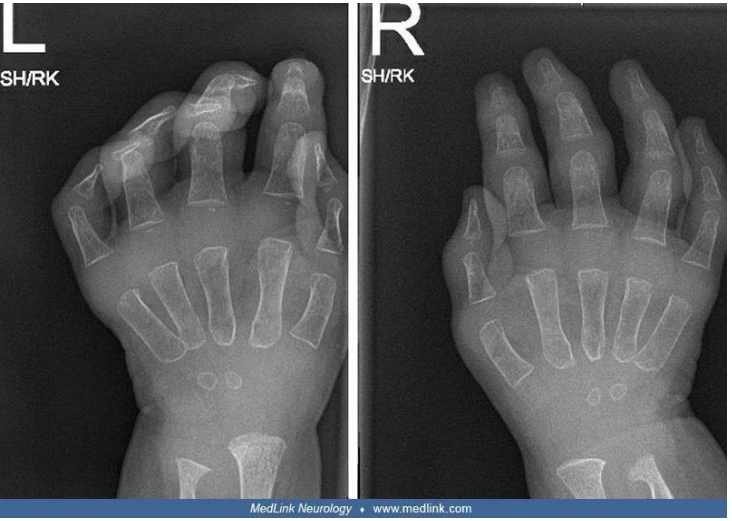
* MOST COMMON LYSOSOMAL STORAGE DISORDER

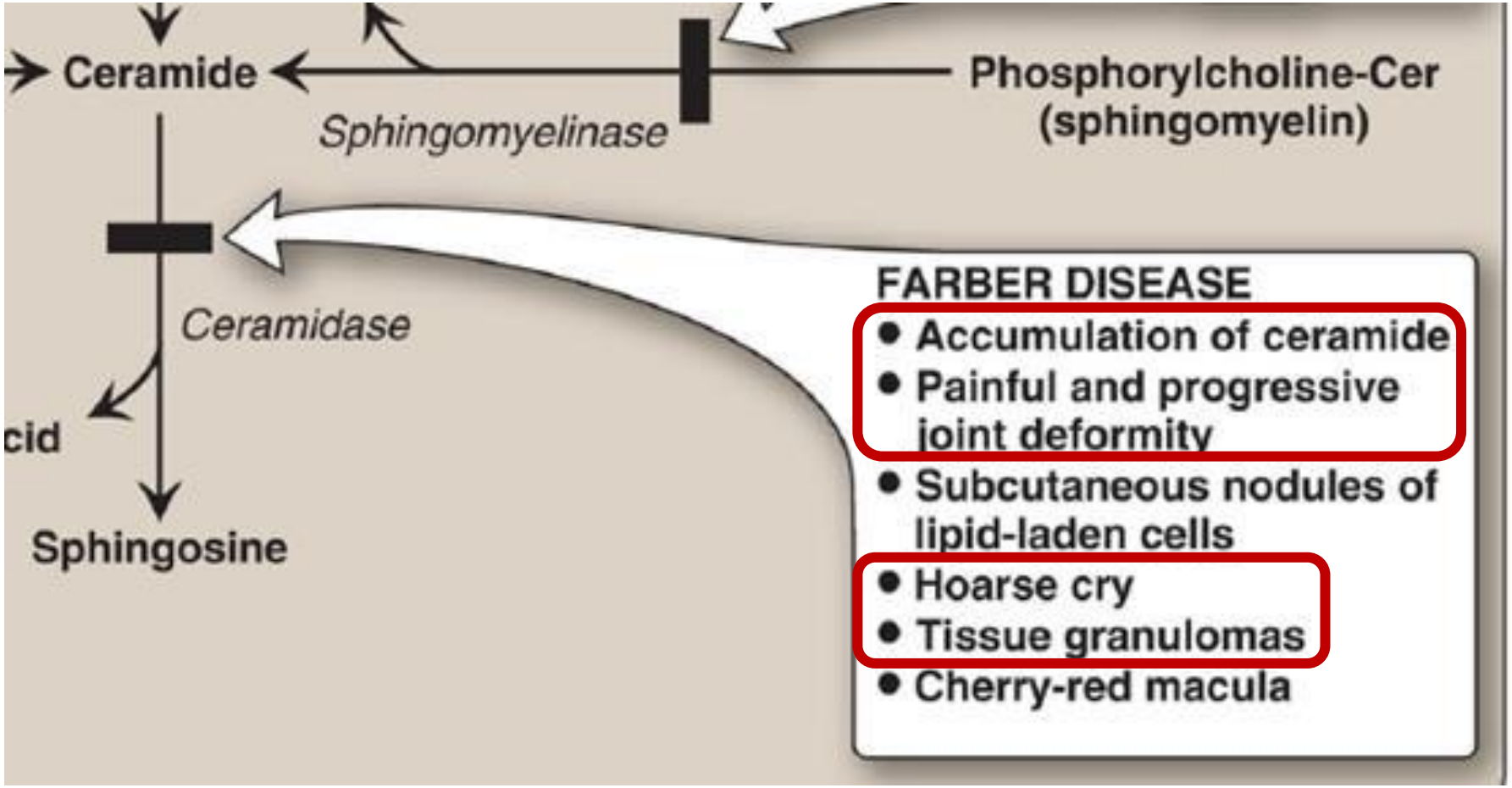


# Farber disease

*(not Fabry; careful)*


**Deformed joints**



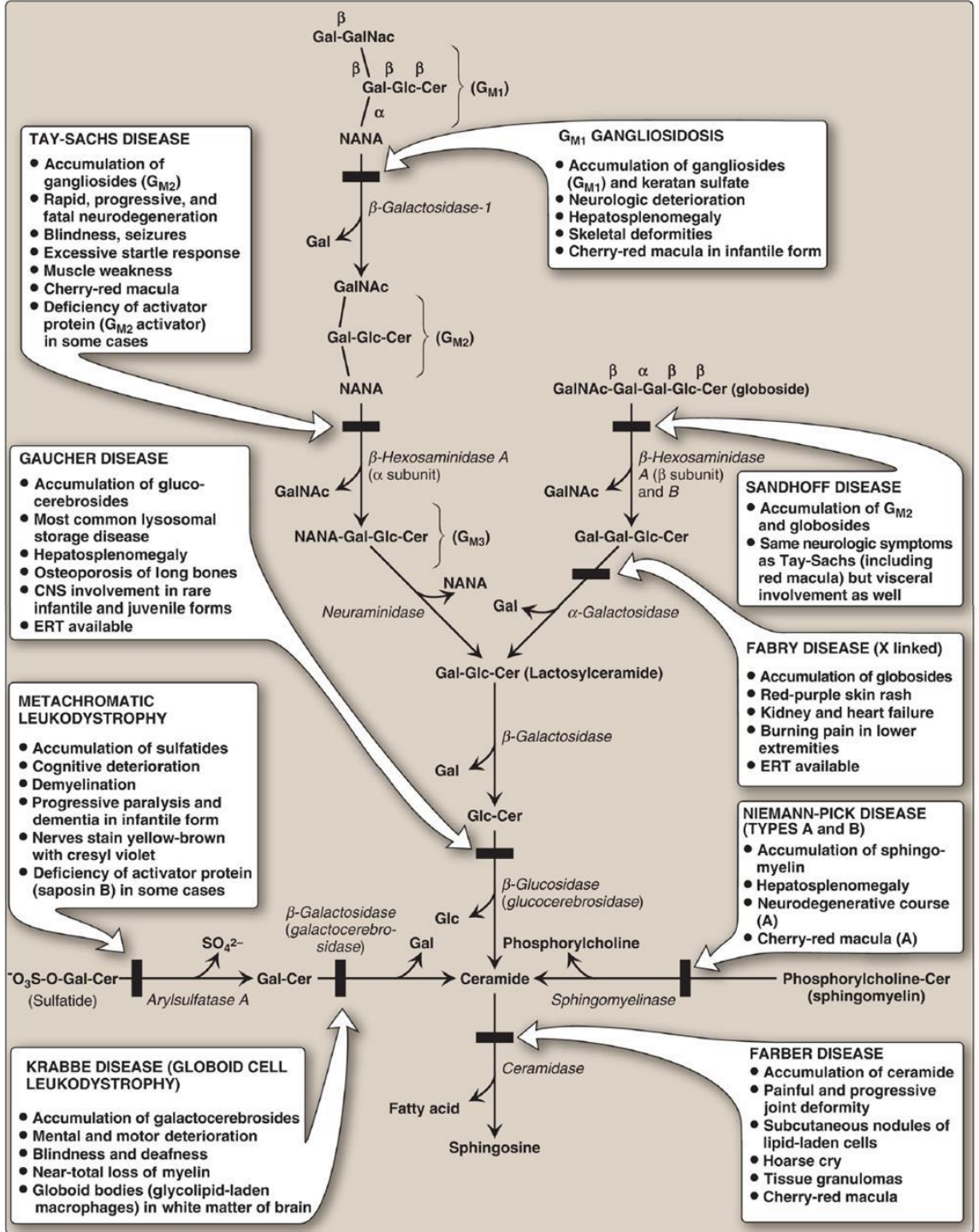
# Diagnosis and treatment

- Diagnosis:
  - Measure enzyme activity in cultured fibroblasts or peripheral leukocytes
  - Analyzing DNA ➔ Molecular approach

➔ Biochemical approach

- Treatment:
  - Recombinant human enzyme replacement therapy
    - Gaucher disease and Fabry disease (expensive)
      - **Galacto**sidase deficiency
      - X-linked
  - Bone marrow transplantation:
    - Gaucher disease
- Substrate reduction therapy
  - Gaucher disease: reducing the amount of glucocerebroside produced in the body pharmacologically

Less accumulation due to less substrate availability



# For any feedback, scan the code or click on it.

Corrections from previous versions:

| Versions | Slide # and Place of Error | Before Correction | After Correction |
|---|---|---|---|
| V0 → V1 | 3<br>6<br>11<br>14 | has<br>palmityl-CoA<br>Text overlapping<br>phonotype | have<br>palmitoyl-CoA<br>Overlap removed<br>phenotype |
| V1 → V2 | | | |

# Additional Resources:

# رسالة من الفريق العلمي:

## References used:

1. Campbell Biochemistry, 7th Ed. Chapter 21

## Extra References for the Reader to Use:

1. Lippincott Illustrated Reviews Chapter 17


من قالها من النهار موقنا بها فمات من يومه قبل أن يمسي فهو من أهل الجنة، ومن قالها من الليل وهو موقن بها فمات قبل أن يصبح فهو من أهل الجنة.