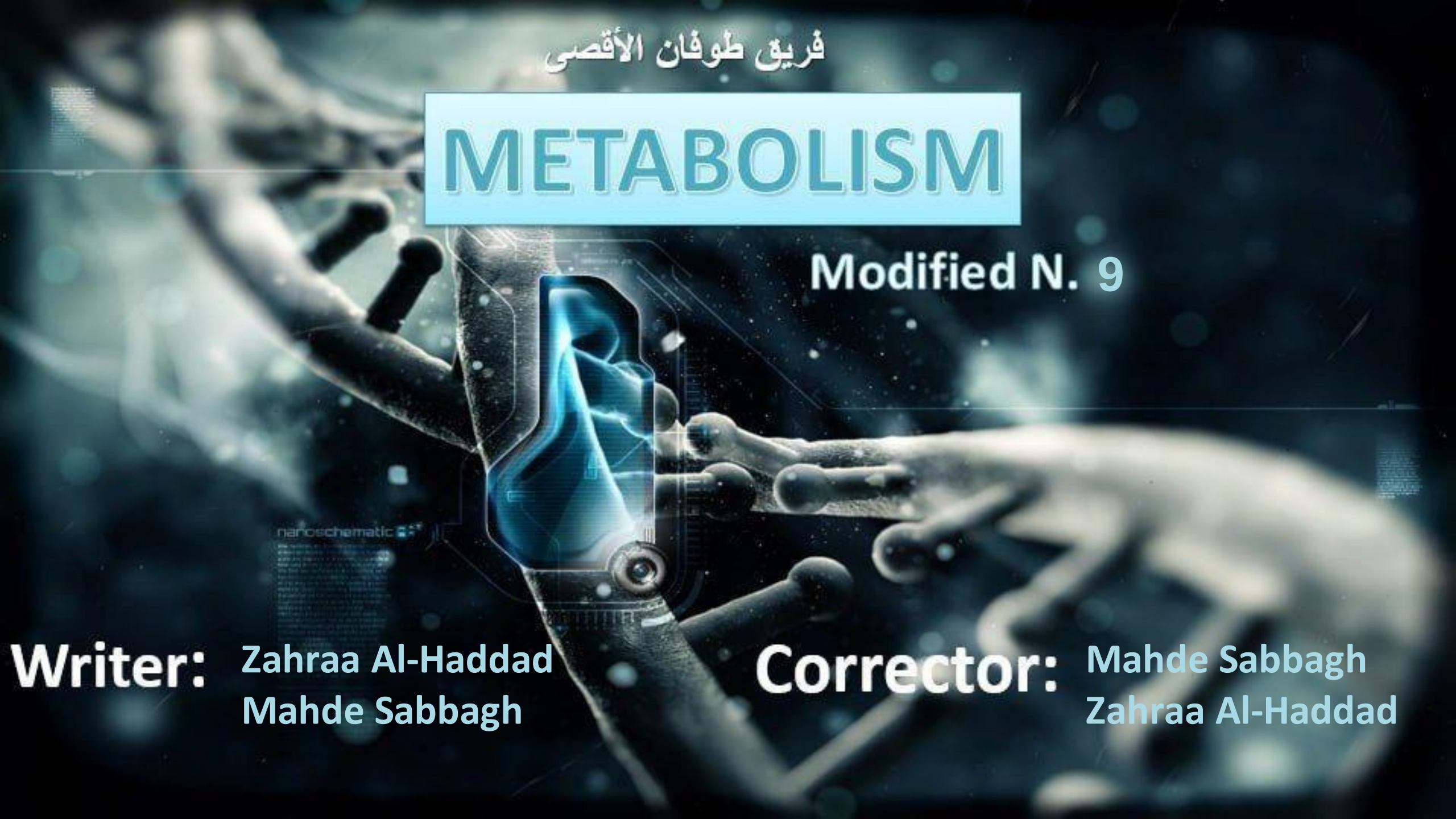
فريق طوفان الأقصى

# METABOLISM

Modified N. 9

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# Metabolism of lipids VI:

## *Sphingolipids*

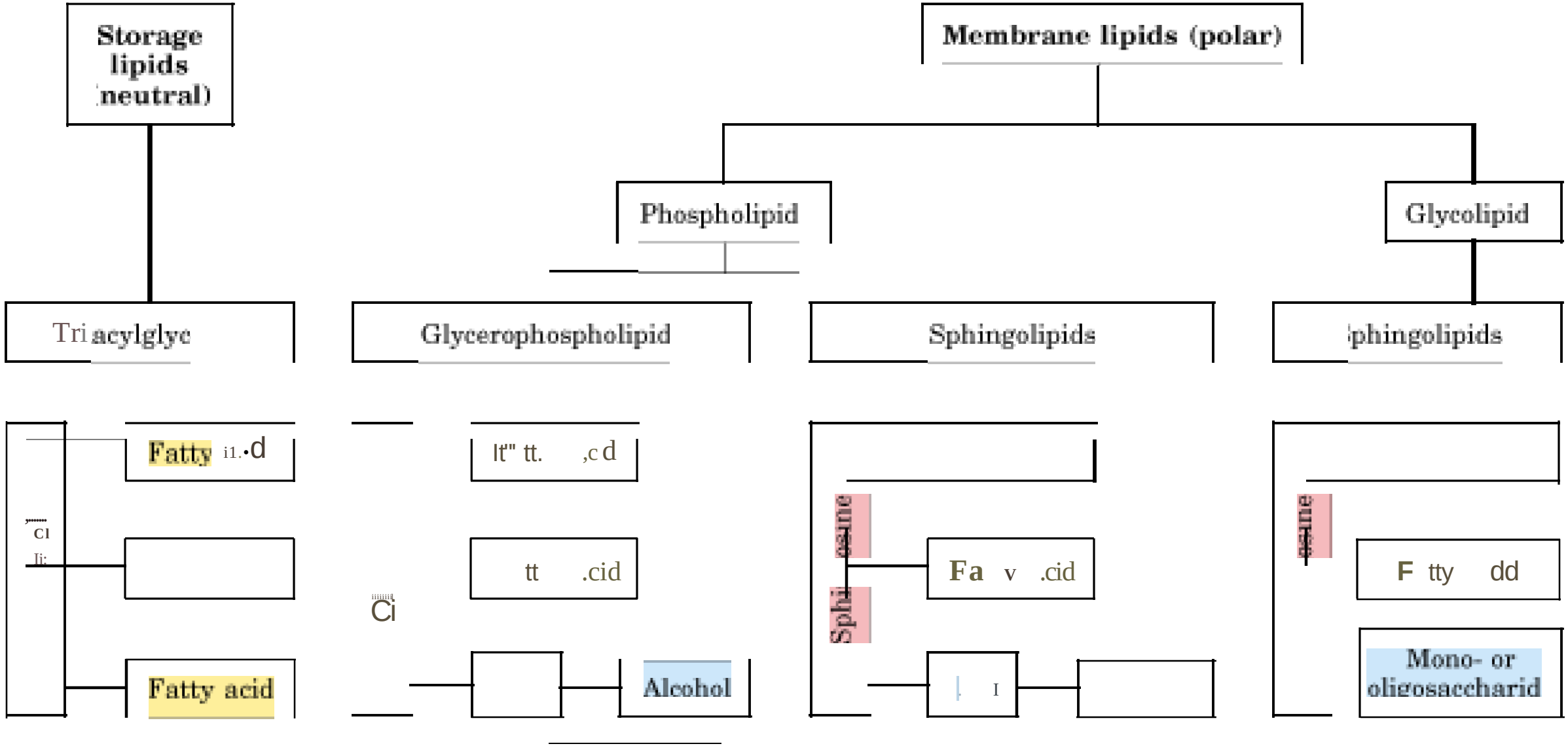
Prof. Mamoun Ahram

**NOTE:**

**Psst.. Dr. Mamoun suggested many "nice" exam questions in this lecture, so make sure you collect them all. Enjoy hunting!!**

# Resources

- This lecture
- Lippincott's Biochemistry, Ch. 17


Storage lipids neutral)
Membrane lipids (polar)
Phospholipid
Glycolipid
Tri acylglyc
Glycerophospholipid
Sphingolipids
phingolipids
Fatty
Fatty acid
Alcohol
Fa v .cid
F tty dd
Mono- or oligosaccharid

- **The complement in this slide:**

There are **two types** of sphingolipids

1 One molecule has phosphate (sphingomyelin)

2 Glycolipids

They share two things **in common:**

A- Sphingosine backbone

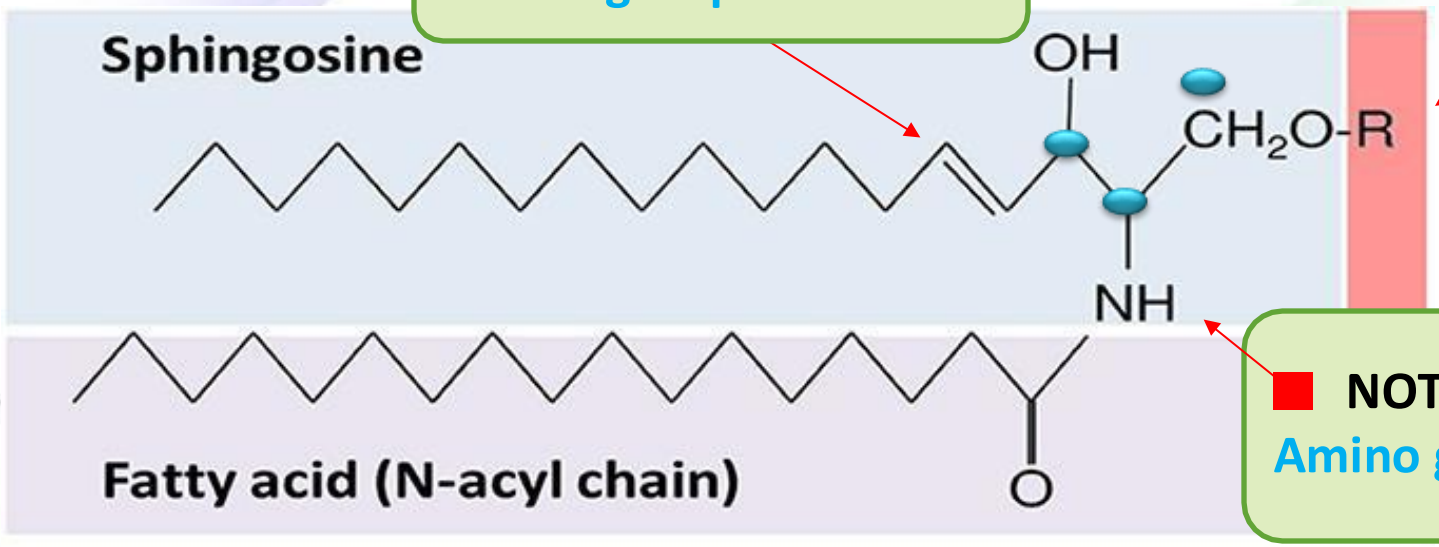
B- fatty acid linked to C-2

They differ in their **head group**; for

- Sphingomyelin, it's $PO_4$ + Choline
- Glycolipids, it's a sugar molecule

# Structure of sphingolipids

**NOTE #1:**
Alkene group

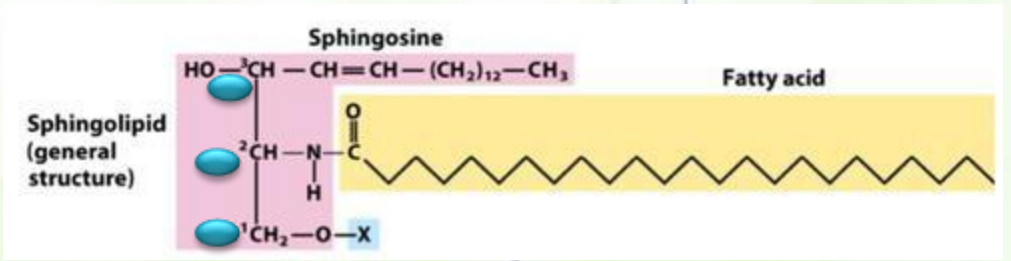



**NOTE #3:**
R-group can be either a small one or a large hydrocarbon chain (fatty acid).

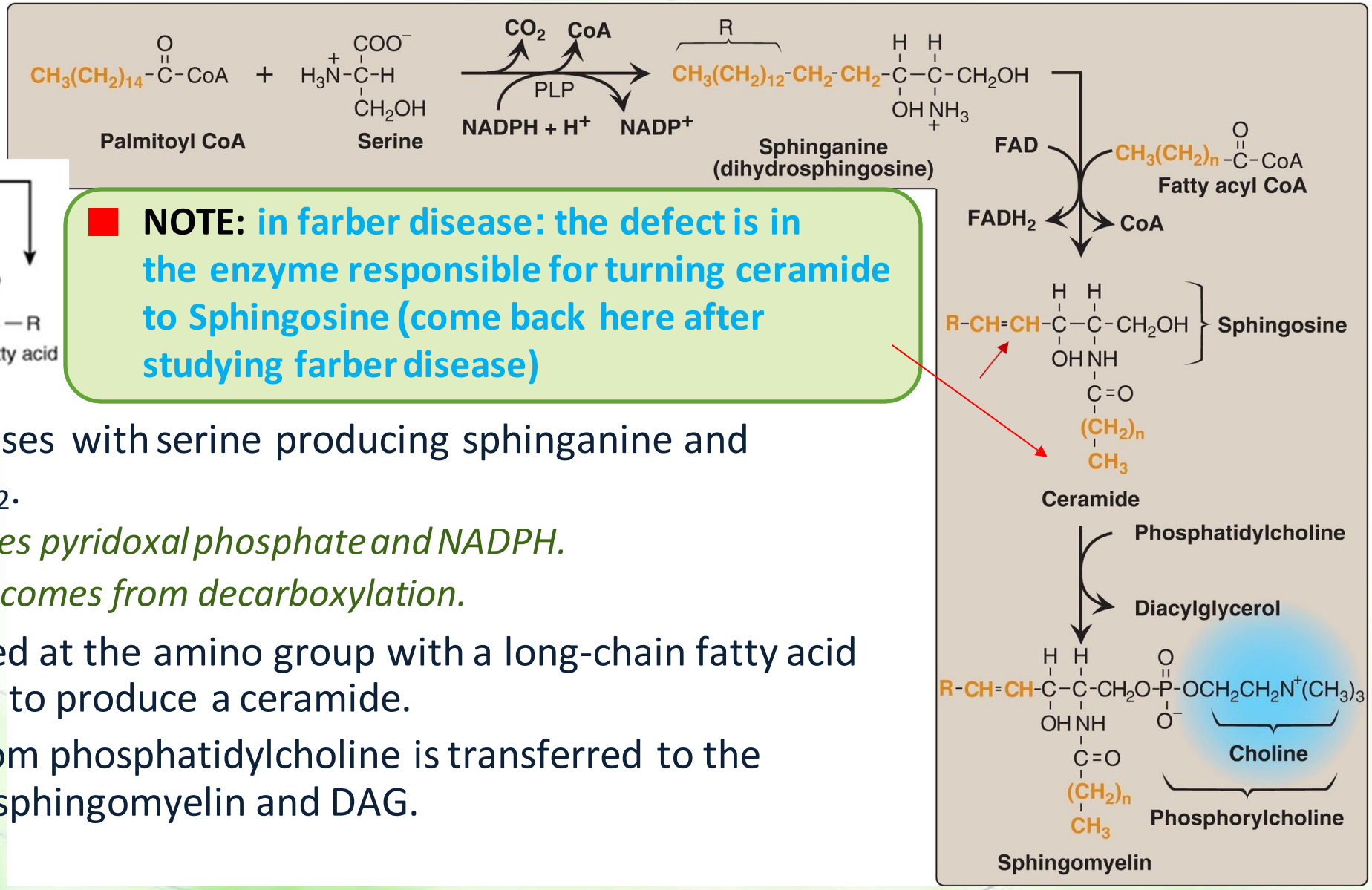
**NOTE #2:**
Amino group

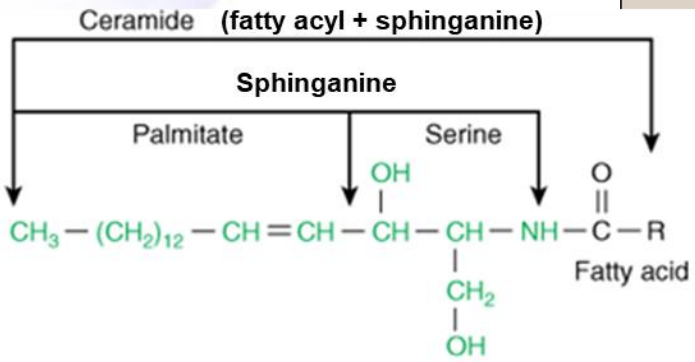
| Substituent (R) | Sphingolipid |
|---|---|
| H | Ceramides |
| Phosphocholine | Sphingomyelins |
| Sugar (s)<br>- Single sugar (glucose or galactose)<br>- Lactose (disaccharide)<br>- Oligosaccharide<br>- Sugar + sulfate | Glycosphingolipids<br>- Cerebrosides<br>- Lactosylceramides<br>- Gangliosides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

**Watch out ⚠ These 3 notes represent how you can tell that this is a sphingolipid.**



# Synthesis of sphingomyelin




**NOTE:** in farber disease: the defect is in the enzyme responsible for turning ceramide to Sphingosine (come back here after studying farber disease)

- 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 acylated at the amino group with a long-chain fatty acid and then desaturated to produce a ceramide.
- Phosphorylcholine from phosphatidylcholine is transferred to the ceramide, producing sphingomyelin and DAG.

*The complement in this slide:

Synthesis starts with the following:

1 A palmitate that has to be **activated.** And a fatty acid when activated, has a **CoA.** Palmitate is the chain of sphingosine.

2 A Serine amino acid. And Ser is the source of the nitrogen that is present in sphingosine.

3 Both together generate an intermediate called "sphinganine"

This rxn requires **NADPH** AND **pyridoxal phosphate** (PLP, as a cofactor)

PLP is like the opposite of biotin;

PLP is for decarboxlation rxns

Meanwhile biotin is for carboxylation rxns

Notice the structure of sphinganine… in orange: palmitate, then we have the Ser part in black (notice amino group from Ser)

This rxn, because it's a condensation rxn, it requires energy. Energy comes from two sources:

- The release of CoA.
- Decarboxylation of the molecule.

Generating the energy that drives the condensation rxn

***The complement in this slide:**

By the way, these can be exam questions!!

- What is the amino acid that is involved in the synthesis of sphingolipids?

**Ans: Serine**

- How is the reaction of condensing palmitoyl CoA and Serine driven?

**Ans: by release of CoA & decarboxylation**

- What is the product of the condensation of palmitoyl CoA & Serine?

**Ans: sphinganine (intermediate)**

As you can see, it's very simple ;)

Anyways, let's continue with the steps...

- **The complement in this slide:**

4- Oxidation of the molecule (palmityl chain specifically).

The condensation drives the creation or introduction of the **double bond** that we see in the sphingolipid (notice: FAD --> $FADH_2$)

Also happens an integration or introduction of a fatty acid molecule, regardless of what that fatty acid molecule is. It doesn't have a particular identity, could be any fatty acid (palmitate, saturated, unsaturated... Whatever!)

Generating **Ceramide,** which the precursor of all sphingolipids, whether glycolipids or sphingomyelin.

(Remember: meanwhile phosphatidate is the precursor of glycerophospholipids)

*The complement in this slide:

5- Now, in order to introduce choline that makes sphingomyelin, it has to be in the form of **phosphatidylcoline.** So the result is: **Sphingomyelin** 

Notice the structure of choline $-CH_2CH_2N(CH_3)_3$

Sphingomyelin is present in the nerve tissue.

This can be another *really nice* exam question:

- When choline is introduced to ceramide to create sphingomyelin, how is it introduced? Or in what form?

A- phosphocholine

B- choline with CoA

C- phosphatidylcholine

**Ans: C**

- **The complement in this slide:**

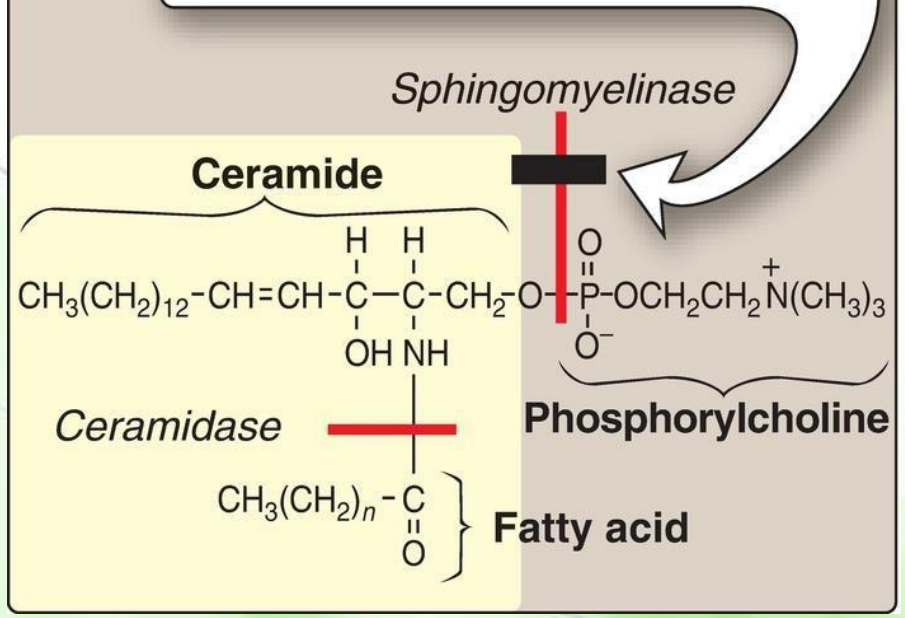
-Student: "what is the difference between phosphocholine and phosphatidylcholine?"

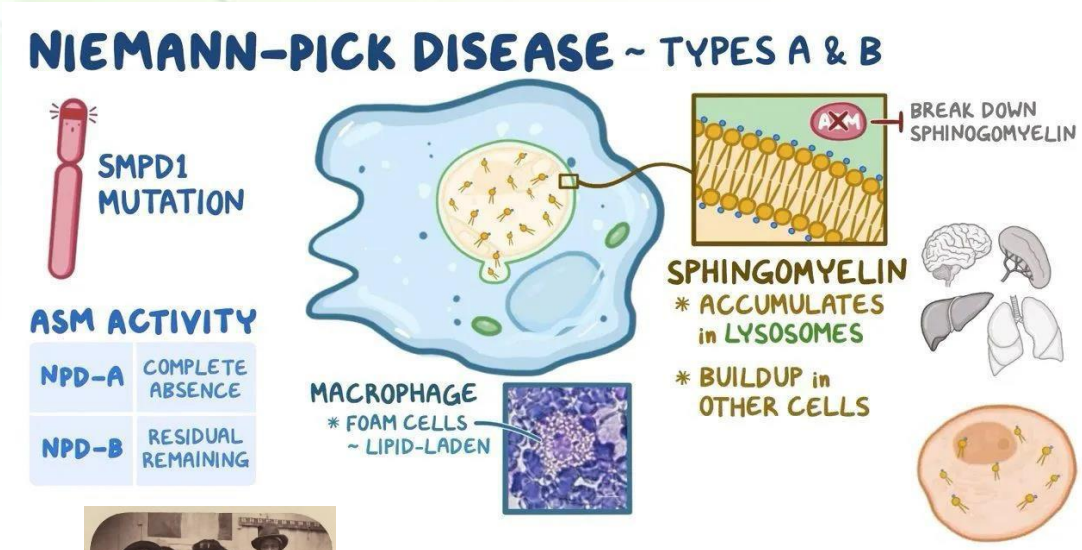
=Dr. Mamoun: "phosphocholine is a phosphorylated choline without glycerol, phosphatidylcholine has glycerol backbone + phosphate + choline"

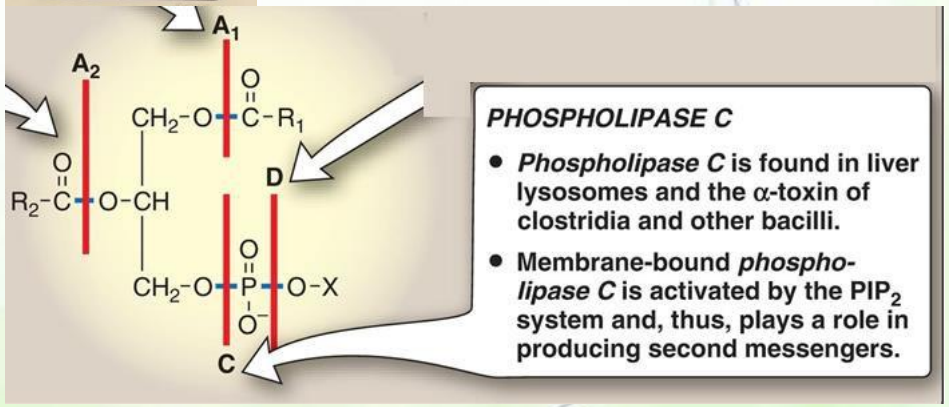
# Deficiency of sphingomyelinase

## NIEMANN-PICK DISEASE

- *Sphingomyelinase* deficiency
- Enlarged liver and spleen filled with lipid
- Severe intellectual disability and neurodegeneration (type A)
- Death in early childhood (type A)



⑩ **The complement in this slide:**

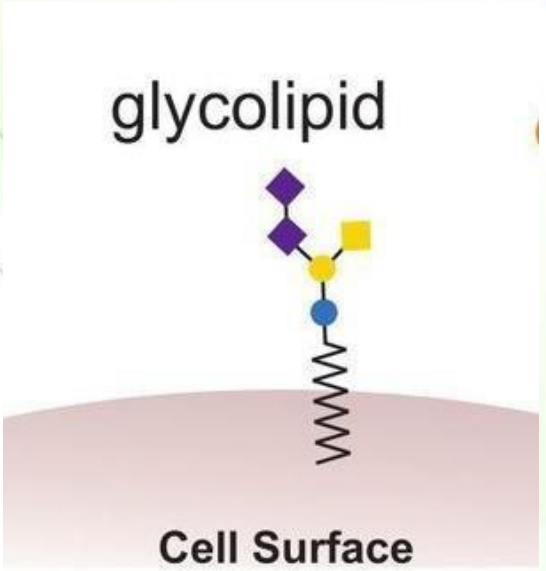
Sphingolipids have to go through degradation by certain enzymes. For sphingomyelin, the enzyme is known as **sphingomyelinase.** So, if we have a defect in this enzyme, there will be accumulation of sphingomyelin, and that can destroy or damage tissues. As you already know, sphingomyelin is abundant in the nervous system. So, in case of accumulation, a mental retardation will mainly occur.

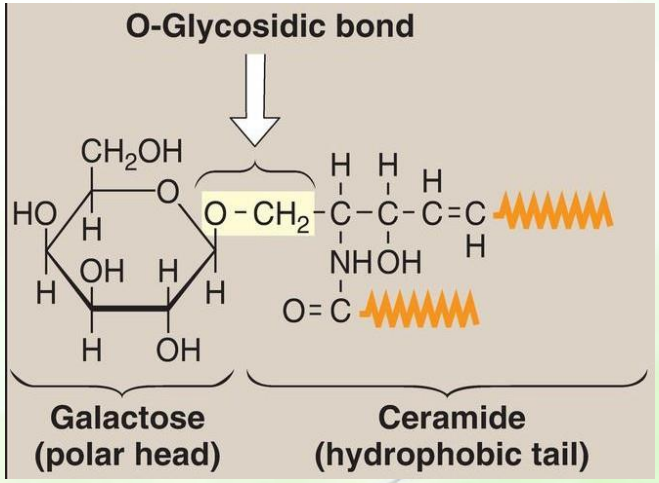
**Neimann-Pick disease** (common among jews) results from the deficiency of sphingomyelinase. **Another nice exam question.**

There are different types of Neimann-Pick disease depending on the severity (type A, type B and so on).

# 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.



***The complement in this slide:**

- One or several sugar molecule(s) can be attached by an O-glycosidic bond (O is up). This bond is present on the anomeric carbon.

"Anomeric carbon ةرمو افلا ةرطيق يه ةتويركلا يلا بتضلا حولت"

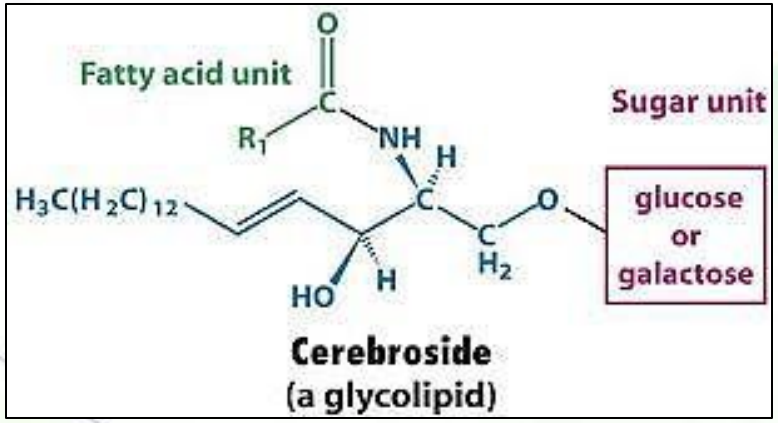
Sugars are usually localized extracellularly, important for cell identity and signaling.

- The two hydrocarbon chains (palmitate & fatty acid) are inserted into the plasma membrane.

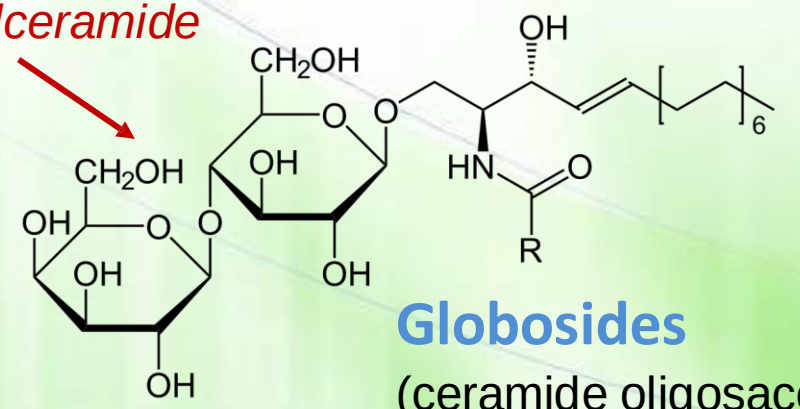
# Types of glycolipids

## Neutral glycosphingolipids

- **Cerebrosides** are the simplest.




*Lactosylceramide*

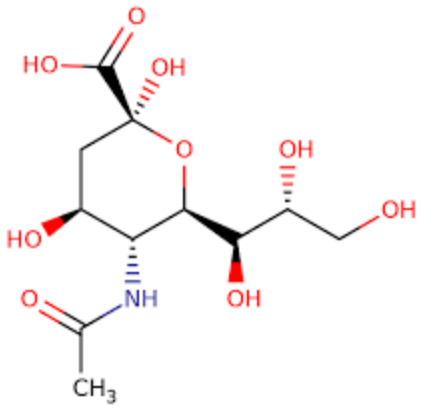


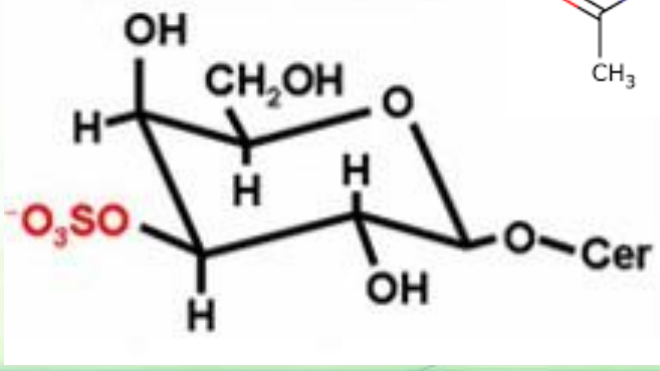
**Globosides**

(ceramide oligosaccharides)

## Acidic glycosphingolipids (gangliosides)

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





**The complement in this slide:**

1 Cerebrosides, the simplest, carry just a single sugar molecule . Have two types:

A- glucocerebroside

B- galactocerebroside

2 Globosides, more complex, carry more than one monosaccharide (di/trisaccharide, etc.)

Eg: Lactosylceramide. Has Lactose which consists of two sugar molecules: glucose and galactose

3 Gangliosides, these are acidic. Why? Because in addition to carrying more than a monosaccharide (usually trisaccharide), one of them has to be sialic acid (Nacetylneuraminic acid). Sialic acid is a modified sugar and it's acidic of course.

- Some Sugar molecules (galactose molecules) can be modified by a sulfate group, and carried by ceramide, now we call them sulfatides..

So, **Sulfatides** are galactocerobrosides that have a sulfate group on galactose molecule.

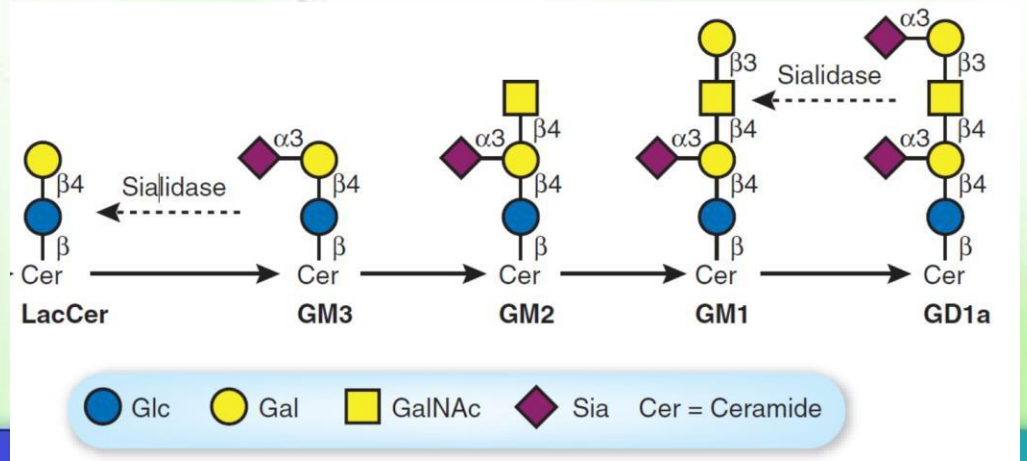
* Look at a picture in slide 22, to see its structure.

# More on gangliosides and sulfatides

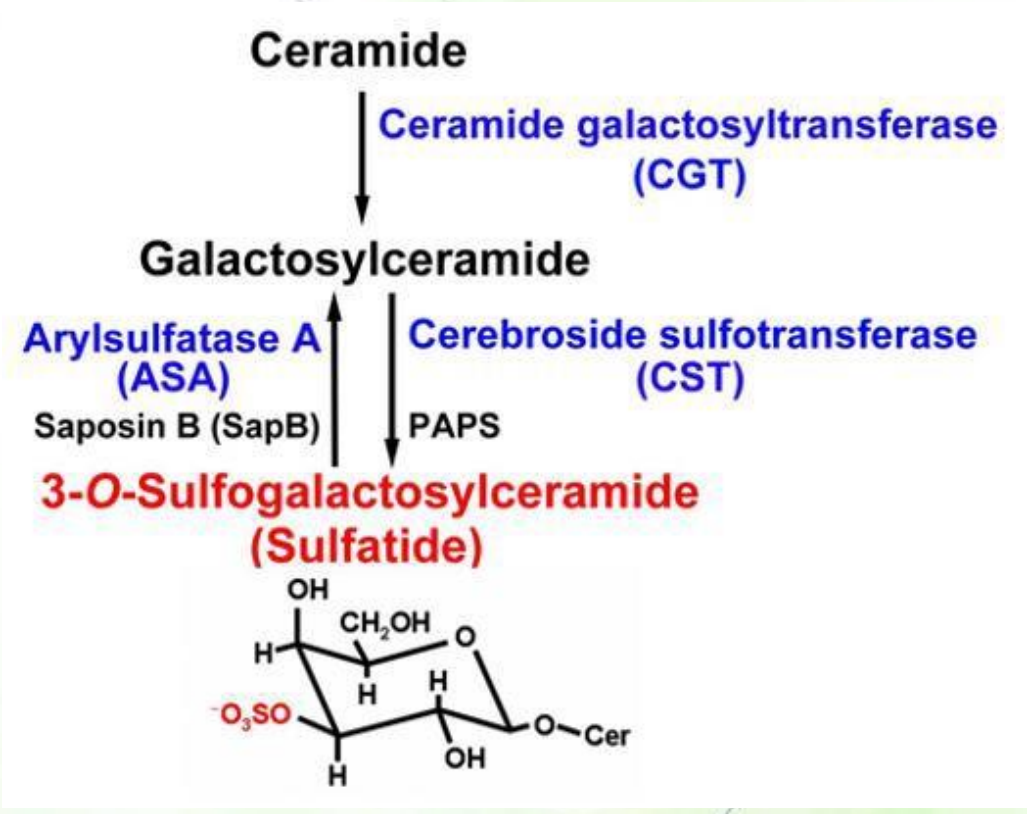
https://chat.openai.com/share/b7c23fa3-71d4-4d6e-bb09-7c90df1d1876

## Gangliosides

- They are designated as G (for ganglioside) plus a subscript (M, D, T, or Q) to indicate the number of sialic acid molecules: 1 (mono), 2 (di), 3 (tri), or 4 (quatro), and then numbers to indicate **indirectly** the number of sugar residues subtracted from 5:
  - GM1 contains 5–1 = 4 sugar residues
  - GD3 contains 5–3 = 2 sugar residues




## Sulfatides



***The complement in this slide:**

Let's talk a liiittle bit more about gangliosides...

The designation/naming of the gangliosides is a little off. but don't worry, we'll simplify it for you.

- ✓ Designated by letters, G is for ganglioside
- ✓ Followed by a transcript (M, D, T or Q) indicating the no. of sialic acid molecules.

M= mono Di= two T= tri Q= quadra

- ✓ Then the no. of sugar molecules

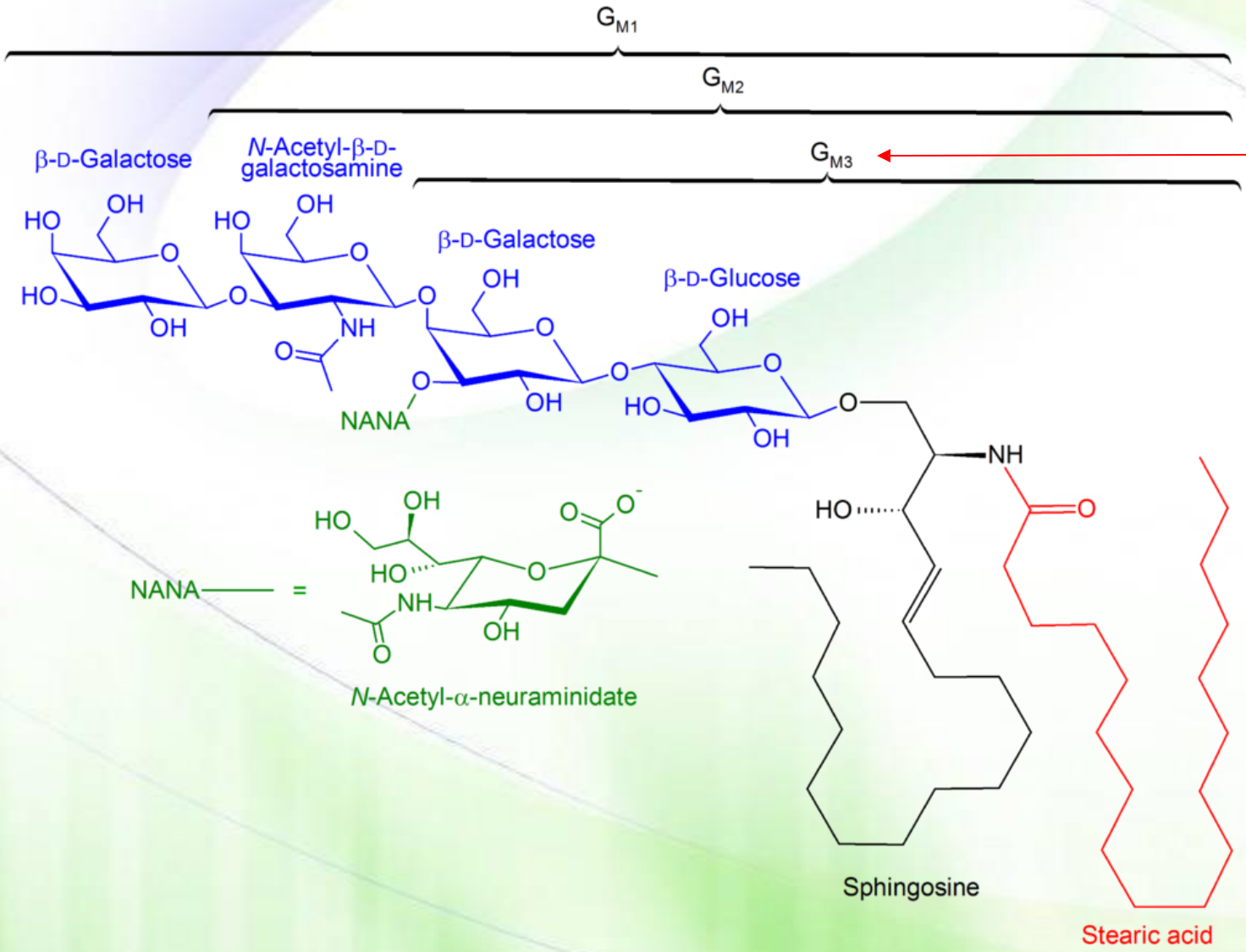
*GM1 → 5 - 1 = 4 sugar molecules

*GM3 → 5 - 3 = 2 sugar molecules

فرعن بولطم شحم5، شيل5نم مقرلا حوطب ينعي؟

Don't waste your time.

# An example



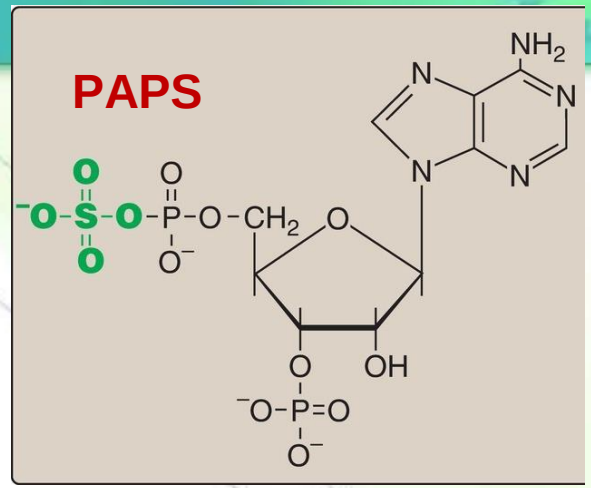

**NOTE:**

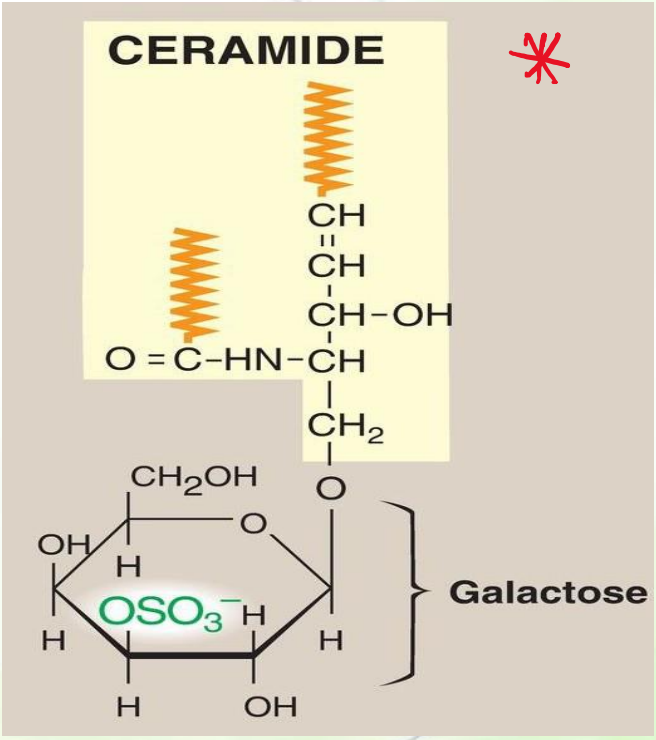
**5 – 3 = 2 sugar molecules.**

**You're halfway through.. Remember to drink water and fix your posture, Dr! :))**

# Synthesis of glycosphingolipids I

- 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 3′-phosphoadenosine-5′-phosphosulfate (PAPS), is added by a sulfotransferase to a galactose in a galactocerebroside, forming the sulfatide galactocerebroside sulfate.



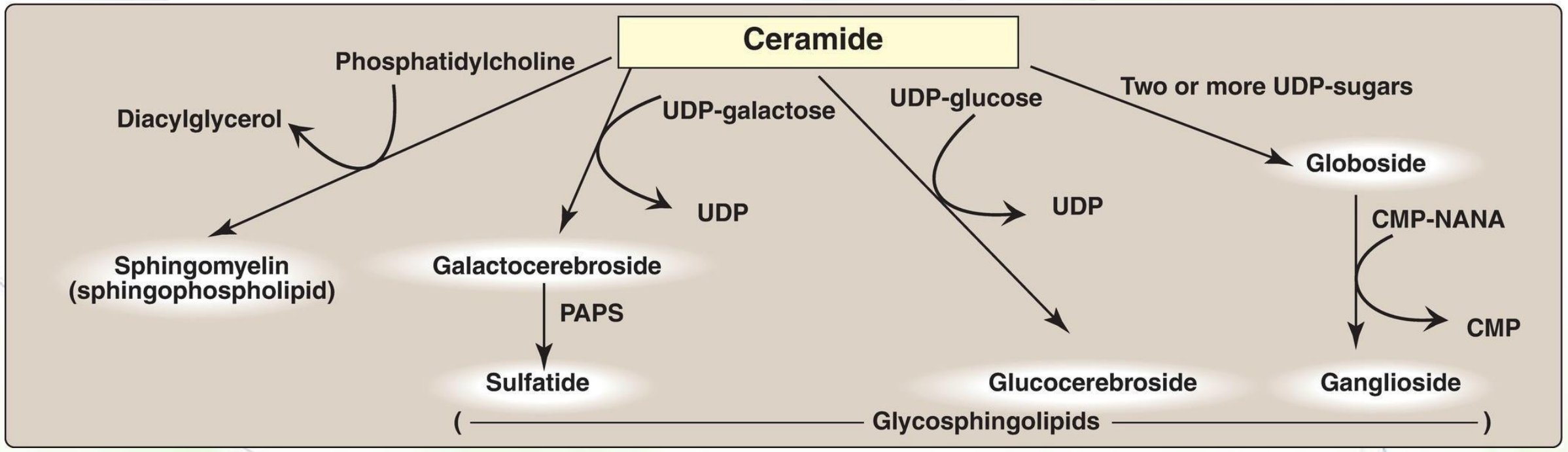



- **The complement in this slide:**

*Synthesis occurs in the golgi apparatus.
*Sugars have to be activated by attaching UDP to the sugar molecules.
(remember: meanwhile phospholipids -in the previous lecture- are activated by CDP)
*the addition of sugar molecules is done enzymatically by glycosyltransferases
*if we want to add a sulfate group, we have to have a donor of it. That donor is PAPS.

Again, a nice exam question: **what is the donor of the sulfate group? PAPS**

# Synthesis of glycosphingolipids II



**The complement in this slide:**

so basically, we start with Ceramide, if we add Phosphatidylcholine we get Sphingomyelin.

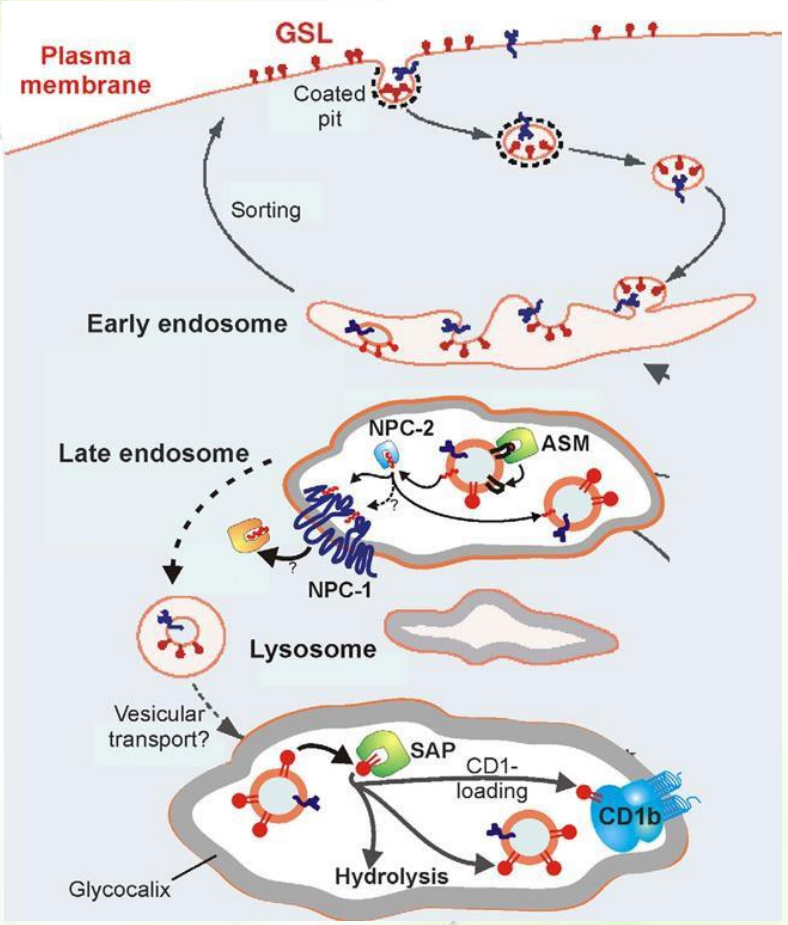
we can add a single sugar, for ex: the addition of UDP-galactose gives us Galactocerebroside which can be further converted to Sulfatide by the addition of a sulfate group (by PAPS), or we can add UDP-glucose which gives us Glucocerebroside.

It is also possible to add two or more UDP-sugars resulting in Globoside which can be converted to Ganglioside by the addition of sialic acid.

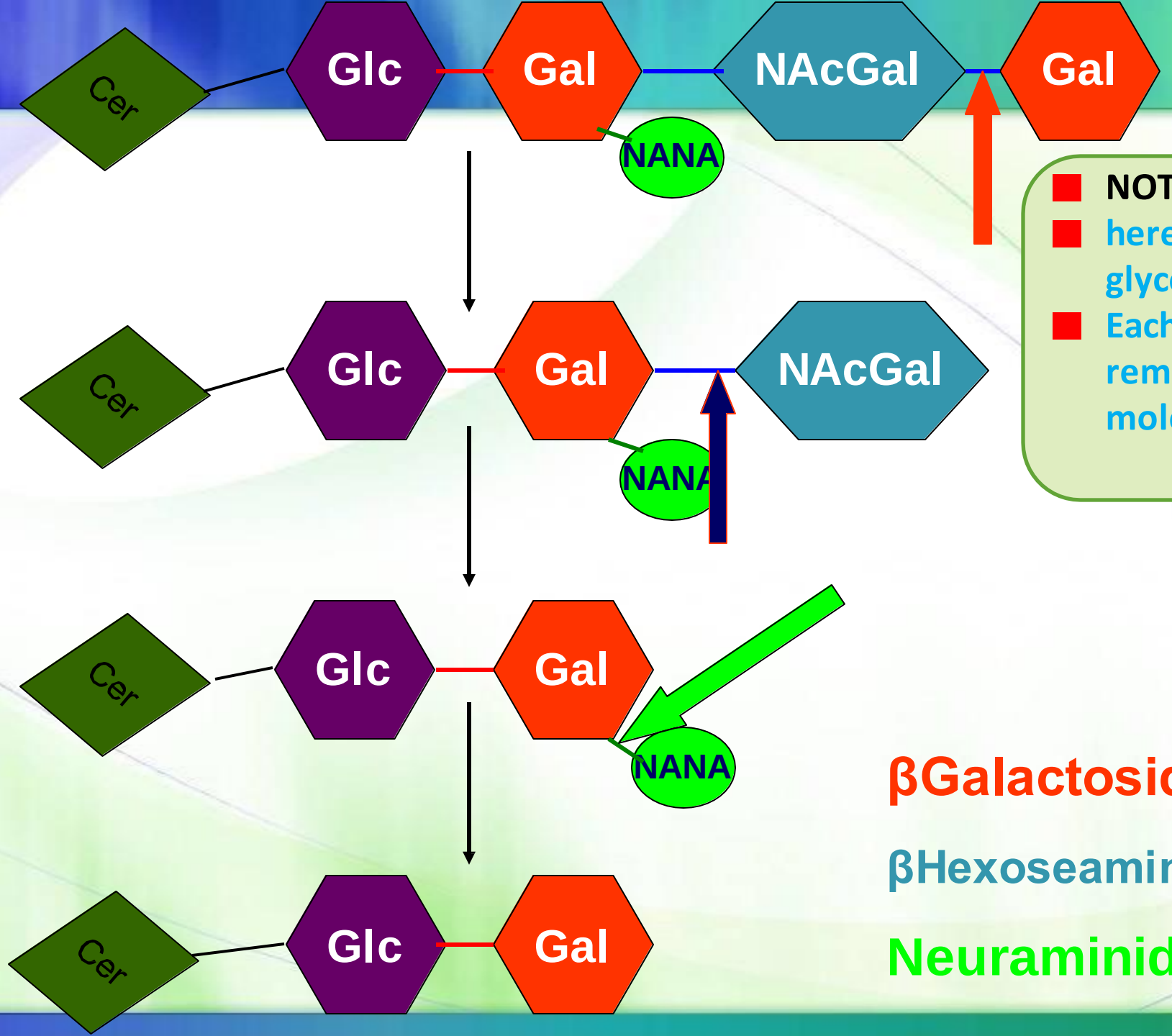
**Note:** all sugars to be added must be activated by UDP (UDP-galactose, Etc..)

# Glycosphingolipid degradation

- Glycosphingolipids are phagocytosed into the lysosomes that fuse with the phagosomes.
- The lysosomal hydrolases remove the sugars sequentially starting with the last one added and ending with the first one added.
- Defect in the degradation of glycosphingolipid, glycosaminoglycans, and glycoproteins causes "lysosomal storage diseases".



- **The complement in this slide:**
- There should be continues turnover (renewal) of sphingolipids, this degradation happens in the lysosomes, it is achieved by internalization (phagocytosis) which results in the formation of vesicles, those vesicles fuse with the endosome, then they undergo maturation followed by condensation with the lysosomes where they undergo degradation by hydrolytic enzymes
- What about the removal of sugar molecules? Are they removed all at once from the ceramide molecule?
  The answer is no, they are removes sequentially (further explained in the upcoming slides).




**NOTE:**

- here you can see the glycosphingolipid.
- Each enzyme will remove a specific sugar molecule at a time

βGalactosidase

βHexoseaminidase

Neuraminidase

- **The complement in this slide:**
- Galactosidase= for the removal of Galactose (there is also a gluco sidase for the removal of glucose).
- Hexoseaminidase= for the removal of N-acetylgalactoseamine.
- Neuroamineidase= for the removal of sialic acid.
- So, they are Removed one after another sequentially.

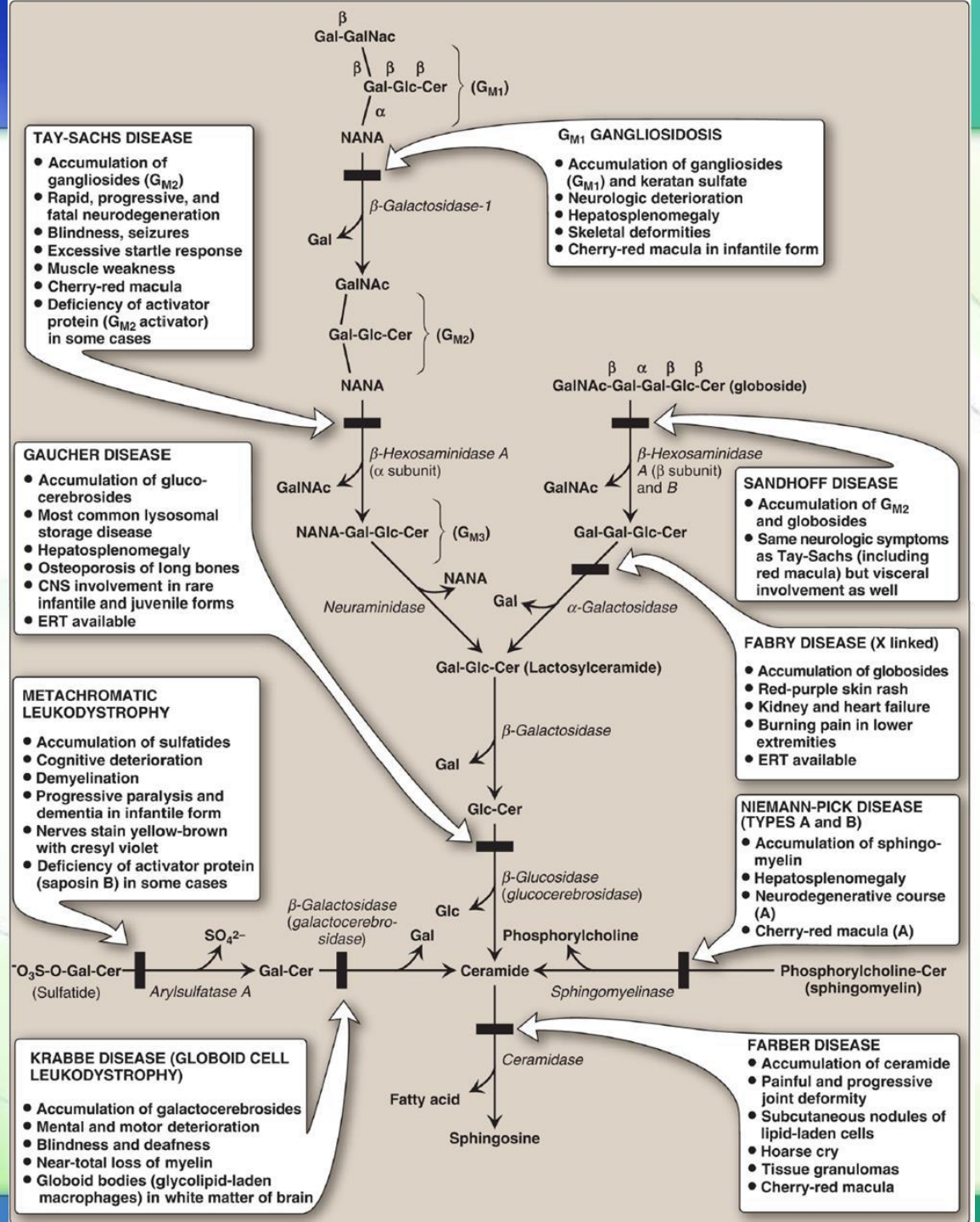
# Sphingolipidoses

- Sphingolipidoses: disorders related to defective degradation of sphingolipids
- Usually, only a single sphingolipid (the substrate for the deficient enzyme) accumulates in the involved organs.
- 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.

- **The complement in this slide:**
- Generally, any defect in an enzyme will result in an accumulation of its substrate.
- So, Sphingolipidoses (a defect in one or multiple enzymes responsible for the degradation of a specific sphingolipid) will result in accumulation of its substrate leading to neuro damage resulting in mental retardation.
- The severity of this disorder depends on the level of defectivity of this enzyme and on the level of expression (activity) of the defected enzyme.
- There will be phenotypic variability depending on the genetic compositions.
- An allele is the type of gene (for example eye color, some people have blue eyes, others have green eyes etc..) that someone carries, for most of our genetic information we have two alleles one is maternal, and the other is paternal, when both alleles are the same, we call it homogeneous, when they are different, we call it heterogenous.
- Allele heterogeneity = what gene was defected (for what enzyme).
- Locus heterogeneity = the location of the defect on the enzyme (is it on the regulatory site, the active site or an insignificant site etc..).
- Active site mutation is the most severe!

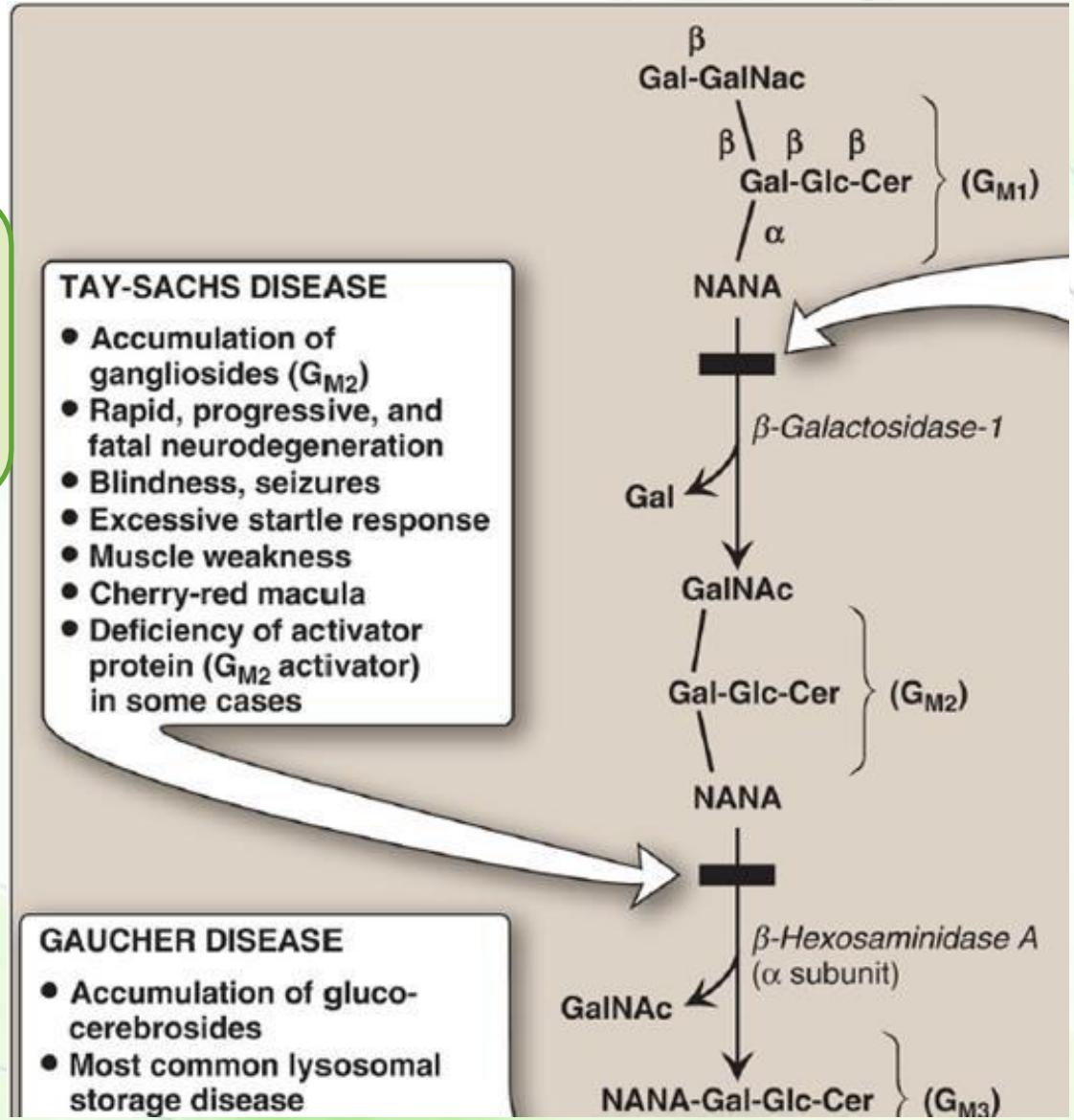
- **The complement in this slide:**
- X linked= the gene is found on the X chromosome.
- Gaucher disease (a type of Sphingolipidoses) is the most common, with an incidence rate of (1/70000).

- **Note:**
- This is a picture that describes the pathways responsible for the degradation of different sphingolipids and the diseases associated with them.
- We are only going to focus in 3 of them.



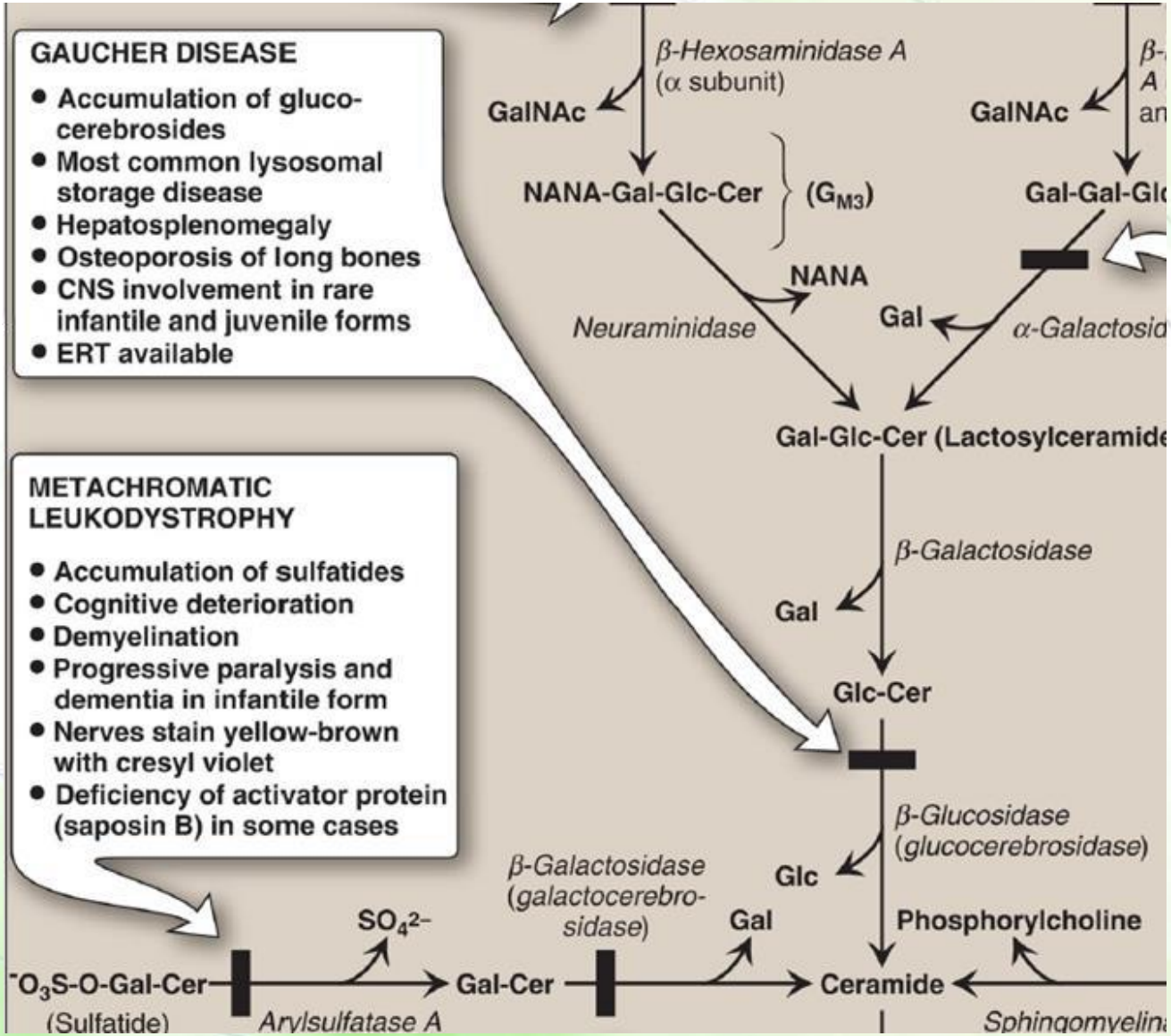
# Tay-Sachs disease

- **Note:**
- **This defect is common among jews.**



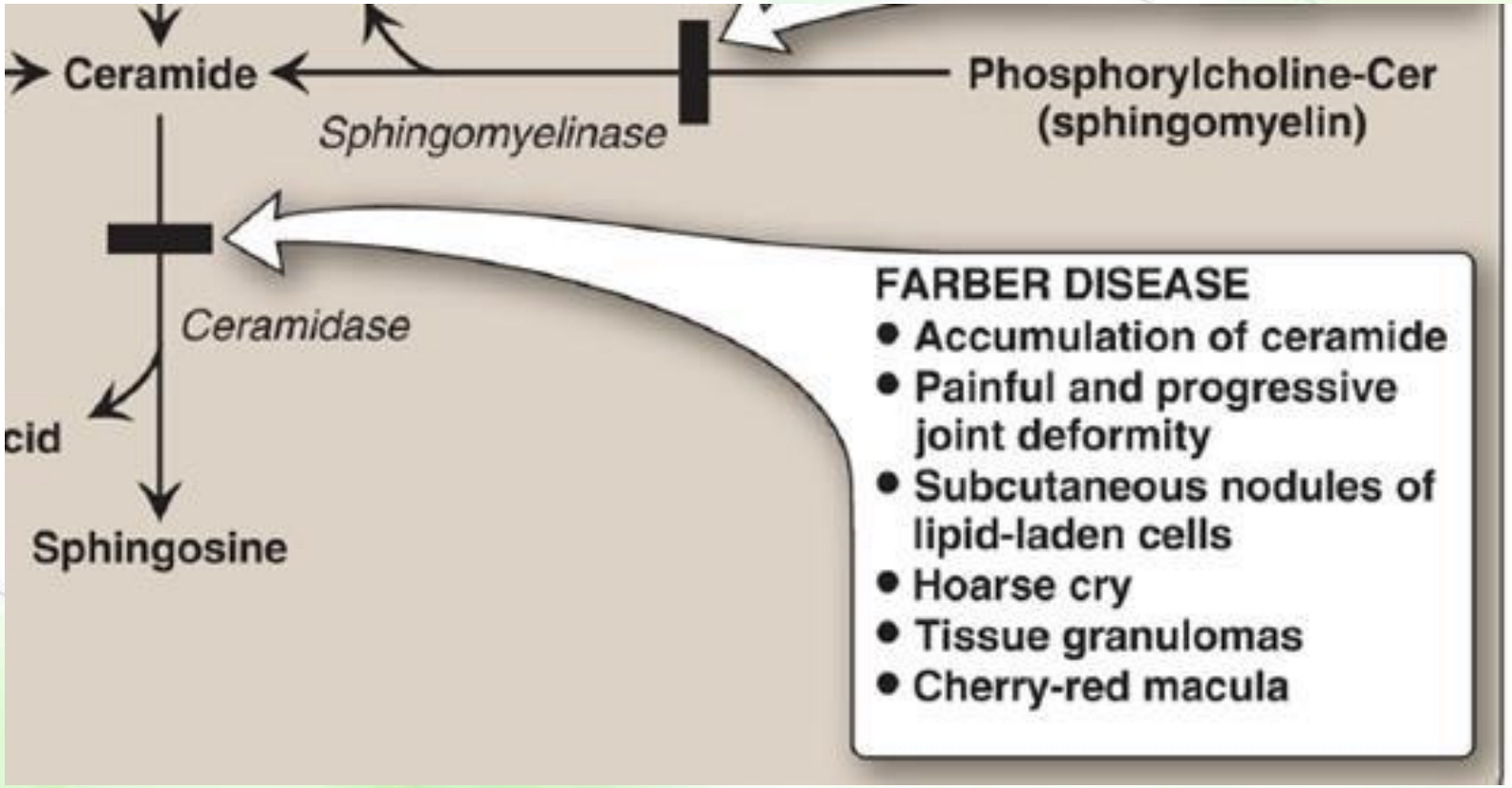
- **The complement in this slide:**
- **The defective enzyme is β-Hexoseaminidase (hexo because it is responsible for the removal of hexoses, aminidase because it removes the N-acetyl group)**
- **There are different types of Hexosaminidases (a/b etc..).**

# Gaucher disease



- **The complement in this slide:**
- The defective enzyme is glucosidase, which is responsible for the removal of glucose.
- So, this defect will result in accumulation of glucocerebrosides (ceramide + glucose).
- Again, they all lead to some type of mental retardation.

# Farber disease



- **The complement in this slide:**
- The defective enzyme is the enzyme that is responsible for the degradation of ceramide (the parent molecule of sphingolipids), which in normal conditions turns ceramide to sphingosine (removes the fatty acid which was added at the last steps of the synthesis of sphingomyelin, go back to slide 7).
- So, it will result in accumulation of ceramide.

# Diagnosis and treatment

- Diagnosis:
  - Measure enzyme activity in cultured fibroblasts or peripheral leukocytes
  - Analyzing DNA
- Treatment:
  - Recombinant human enzyme replacement therapy
    - Gaucher disease and Fabry disease (expensive)
  - Bone marrow transplantation:
    - Gaucher disease
- Substrate reduction therapy
  - Gaucher disease: Pharmacologic reduction of glucosylceramide

**Note:** we basically measure the activity of the enzyme suspected to carry the defect.

**Note:** more cost effective

**Note:** displace the defective gene with a normal one, but it's expensive and doesn't work well.

- **V1 :**_Sugar molecules (acetylneuraminic acid for example) can be modified by a sulfate group, and carried by gangliosides, now we call them sulfatides.So, Sulfatides are the gangliosides that have a sulfate group.

**Slide 18**

**V2** • Some Sugar molecules (galactose molecules) can be modified by a sulfate group, and carried by ceramide, now we call them sulfatides..
So, Sulfatides are galactocerobrosides that have a sulfate group on galactose molecule.