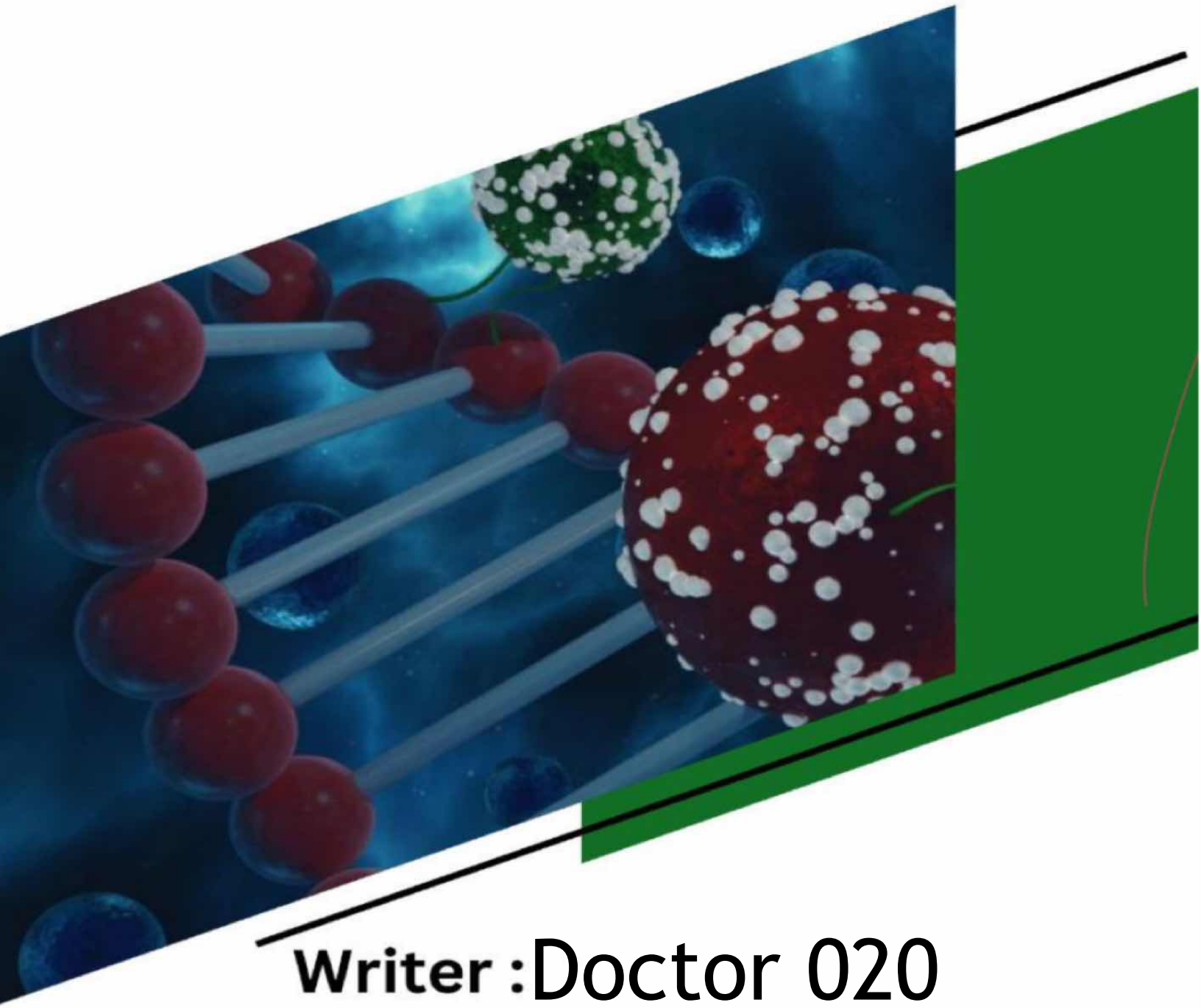
Doctor 021

METABOLISM

Sheet no. 24 A



Writer : Doctor 020

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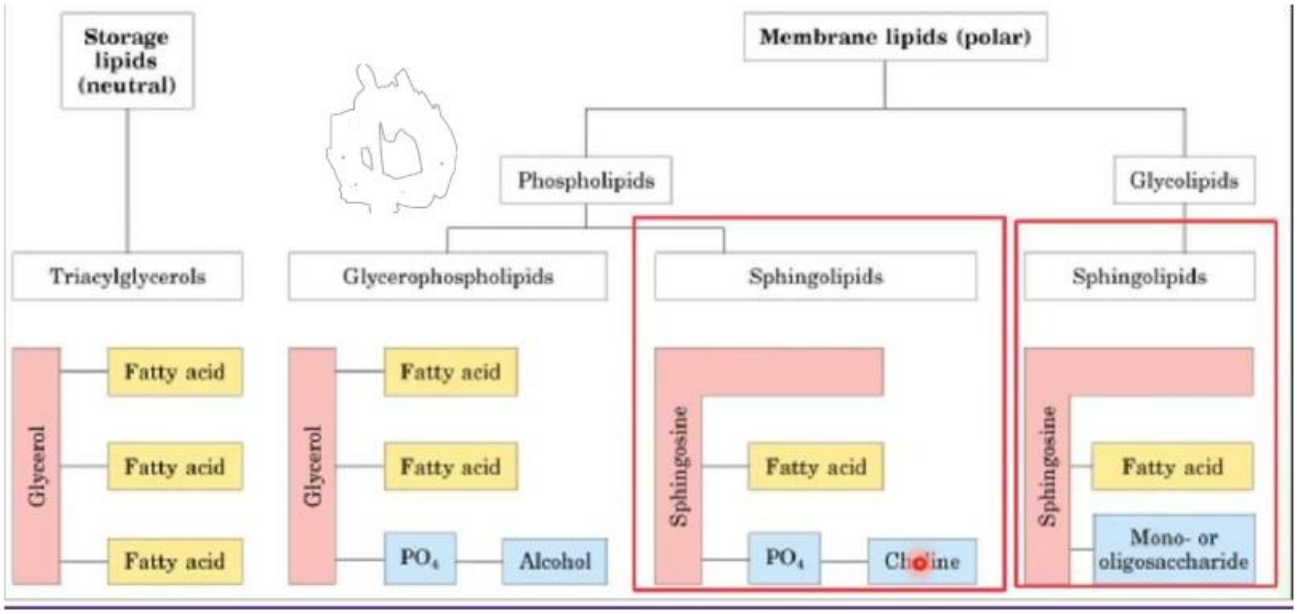
Doctor : Mamoun Ahram

Sphingolipids:

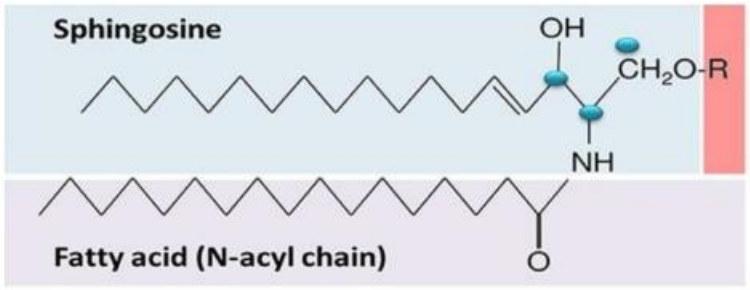


There are two types of sphingolipids**: sphingophospholipid** (Sphingomyelins) & **Sphingoglycolipids**. Both share the presence of sphingosine as their backbone and one fatty acid; however, they differ in the head group connected to sphingosine ❼

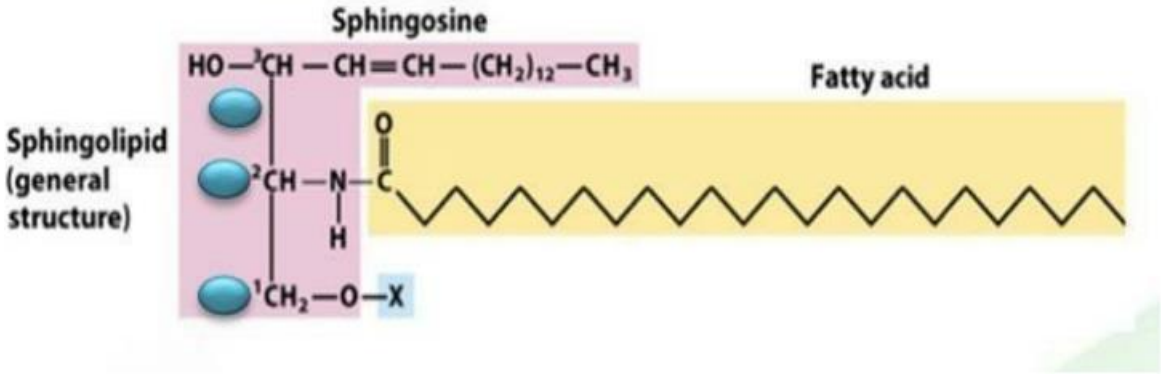
- **Sphingophospholipid** head group: **phosphocholine**
- **Sphingoglycolipids** head group: **Mono or oligosaccharide**

Structure of sphingolipids

Notice that C1 is connected to an oxygen and the head group, while C2 is associated with Nitrogen connected to a fatty acid chain. Moreover, C3 is associated with an OH group and an unsaturated alkyl group containing a C=C (double bond).



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



Ceramides are the basic sphingolipids and the precursors with H connected to sphingosine as the head group (it will be ionized at physiological pH).

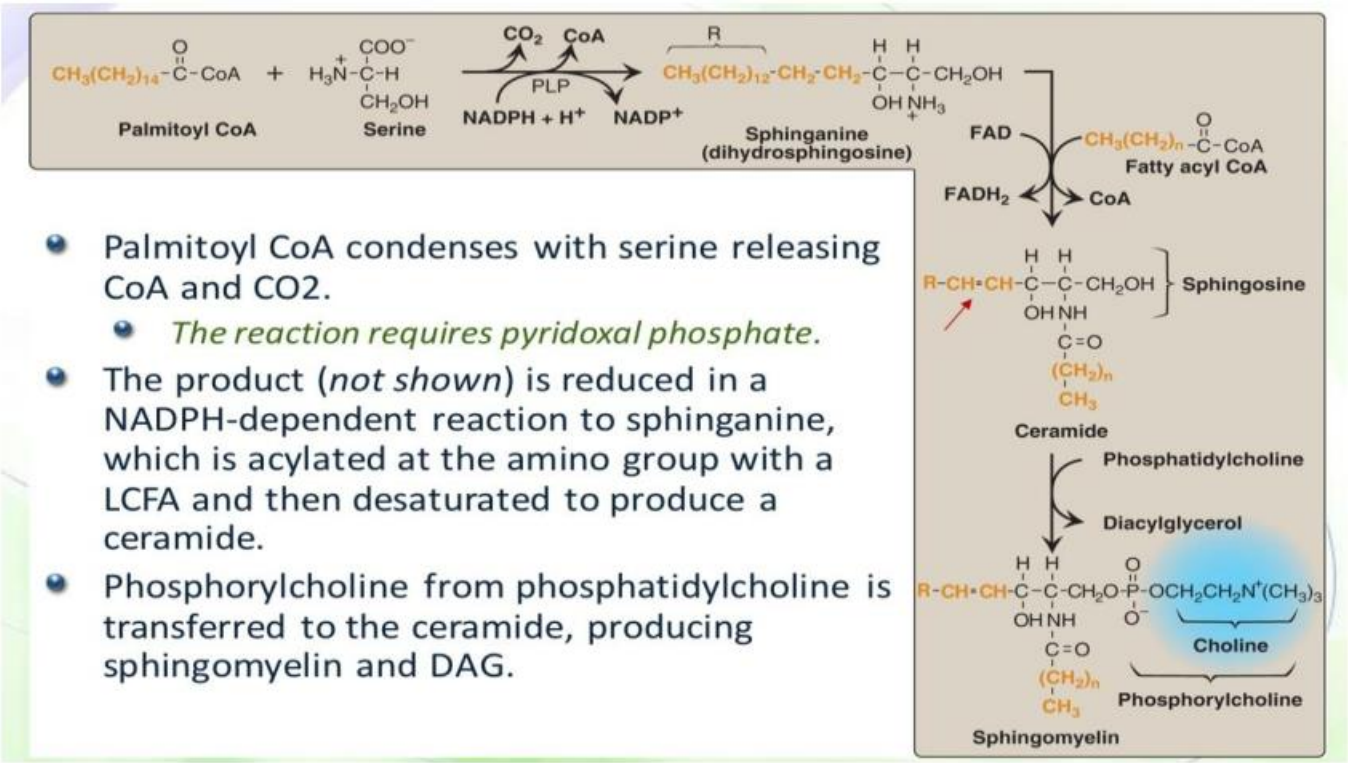
Recall: Phosphatidic acids is the basic glycerophospholipids.

Glycosphingolipids are further classified into: **Cerebroside** (the basic type),**Globosides**
(**Lactosylceramides**), **Gangliosides**, and **Sulfatides**.

Synthesis of sphingomyelin

Then another activated fatty acid in the form of Fatty acyl CoA is transferred to Sphinganine **, specifically to the serine portion**. Moreover, FAD is Reduced to FADH2 to oxidize the Palmitoyl (forming the double bond), and by this **Ceramide** is produced.

Phosphorylcholine from phosphatidylcholine is transferred to the ceramide, producing **sphingomyelin** and DAG.



Deficiency of sphingomyelinase

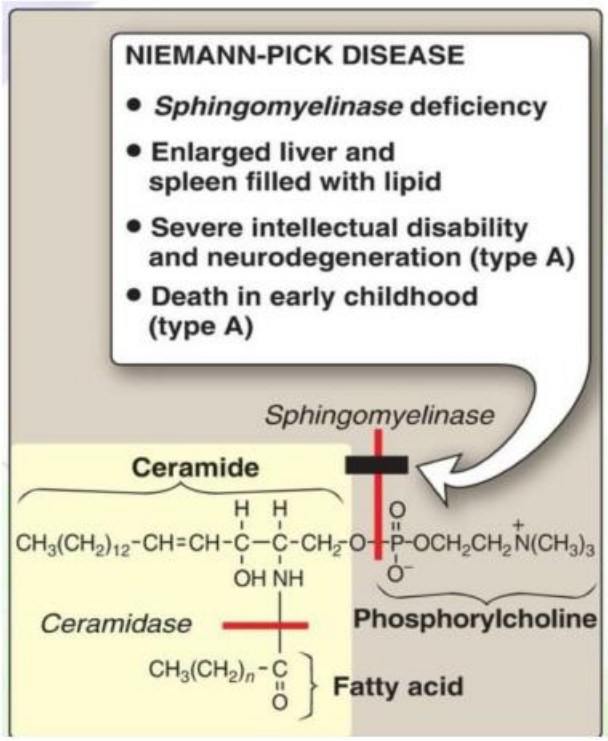
Just like any other molecules in our bodies, sphingomyelin should be degraded and renewal from time to time.

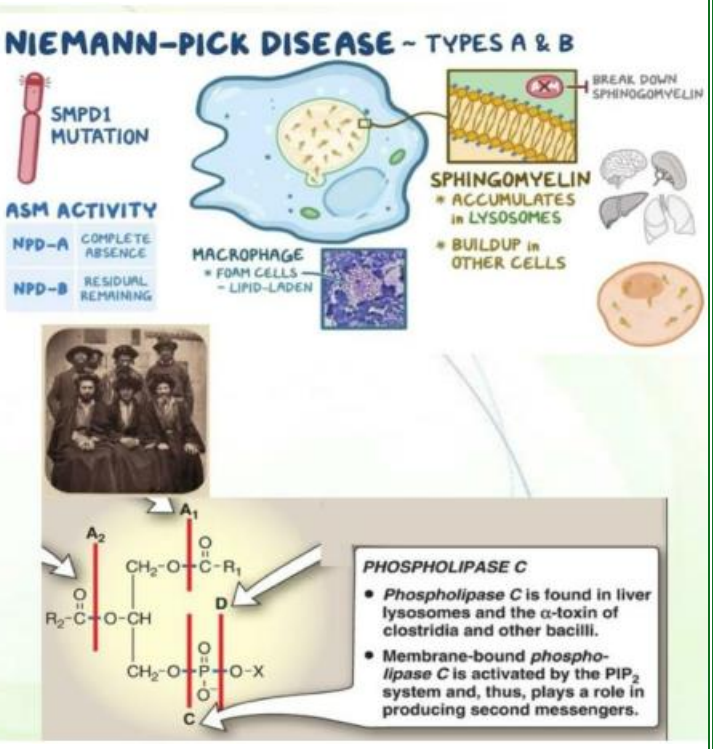
Sphingomyelin degradation is carried by the enzyme **sphingomyelinase** (similar to phospholipase C, as it cleaves the head group right before the phosphate group, releasing **phosphorylcholine** and converting Sphingomyelin into ceramide).

Any factor that will affect the degradation process will cause a disease, one of these diseases is **Niemann-Pick Disease** (one of Lysosomal storage diseases, discussed later).

- **Results from Sphingomyelinase deficiency**
- Enlarged liver and spleen with lipids
- Mental retardation in infants

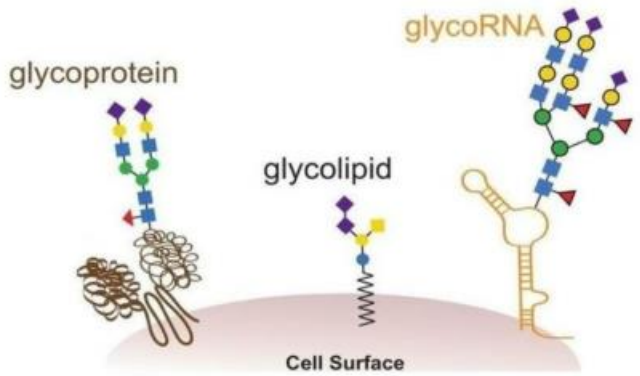
- Two types: type A and type B
- **Type A** is very severe, where there is **no enzymatic activity at all**, resulting in intellectual disabilities, neurodegeneration, and death in early childhood.
- **Type B**, gene is mutated and defective; however, **not 100% deficiency**, thus symptoms are less severe.
- Common among Ashkenazi Jews (middle eastern Jews).





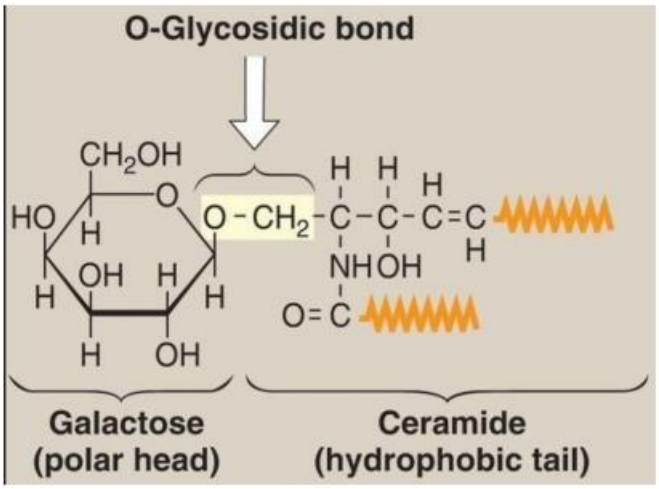
Glycosphingolipids (glycolipids)

Many molecules could be glycosylated for many purposes for example, proteins are glycosylated to form glycoproteins; moreover, RNAs can be glycosylated to form glycol RNA. However, we are concerned with **glycolipids**.



- They are made of ceramide (precursor)

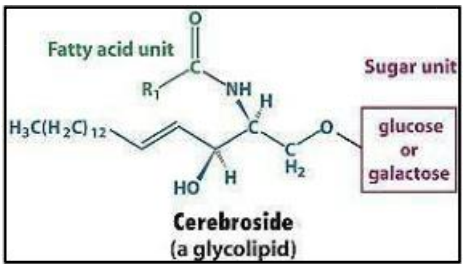
- They are located in the outer leaflet of the plasma membrane (adhesion, recognition, and signaling)
- A sugar(s) is attached to ceramide by an **O-glycosidic bond.**
- **The number and type** of carbohydrate moieties present determine the type of glycosphingolipid.



Types of glycolipids

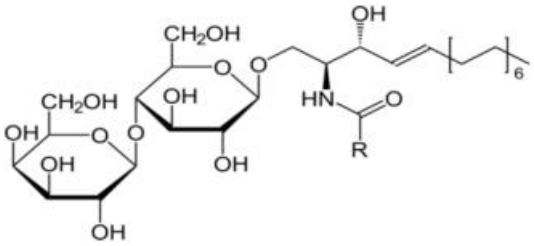
Glycolipids are either **Neutral glycosphingolipids** or **Acidic glycosphingolipids**

Neutral glycosphingolipids: (head group doesn't have a charge at physiological pH)



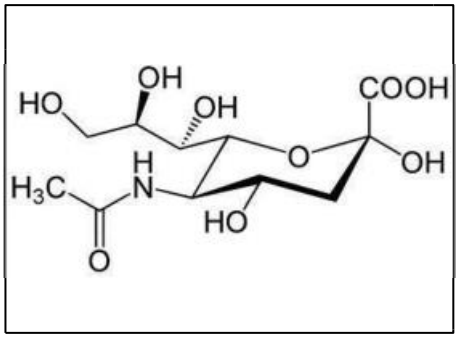
- **Cerebrosides** are the simplest; they are either glucocerebrosides or galactocerebroside

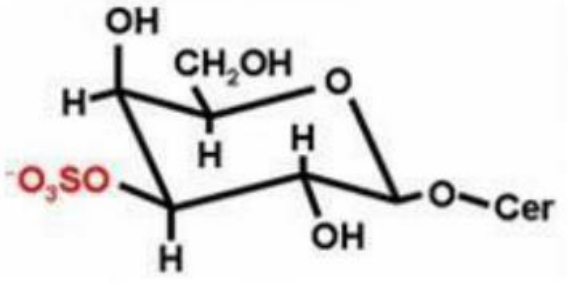
- **Globosides** (ceramide oligosaccharides), in which **neutral oligosaccharides** are added as a head group for example, **Lactosylceramide**



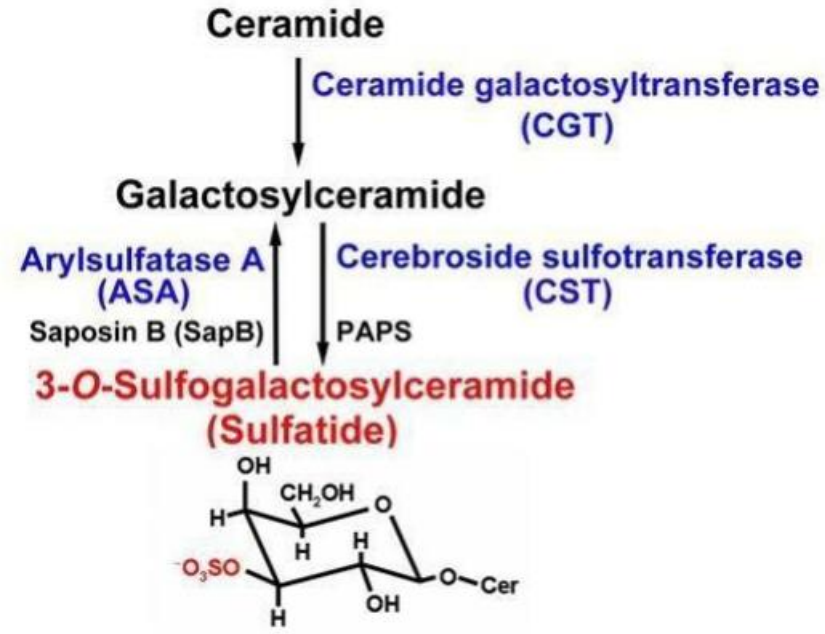
Acidic glycosphingolipids

-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**.



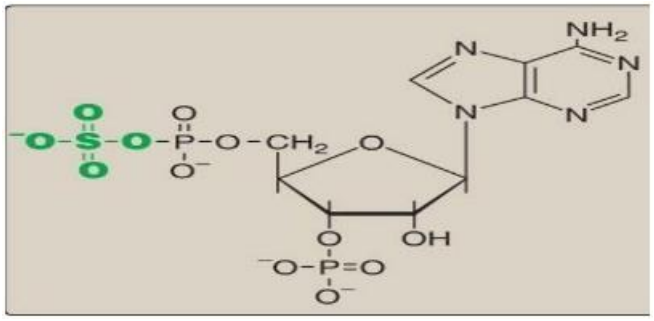


Sulfatides



Transfer sugar(galactose) to ceramide

Transfers sulfate group from PAPS to galactosyl ceramide



PAPS are the doners of sulfates groups

Gangliosides

They are designated as **G** (for ganglioside) plus a **subscript** (M, D, T, or Q) to indicate **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:

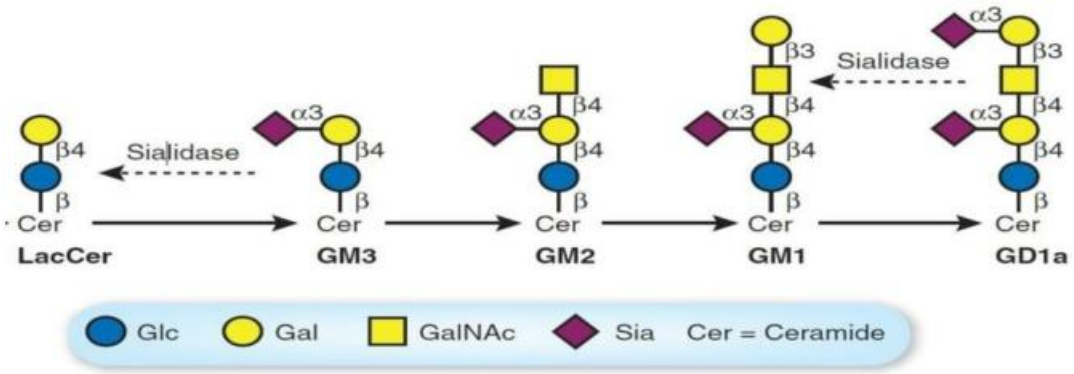
- GM1 contains 5–1 = 4 sugar residues
- GD3 contains 5–3 = 2 sugar residues

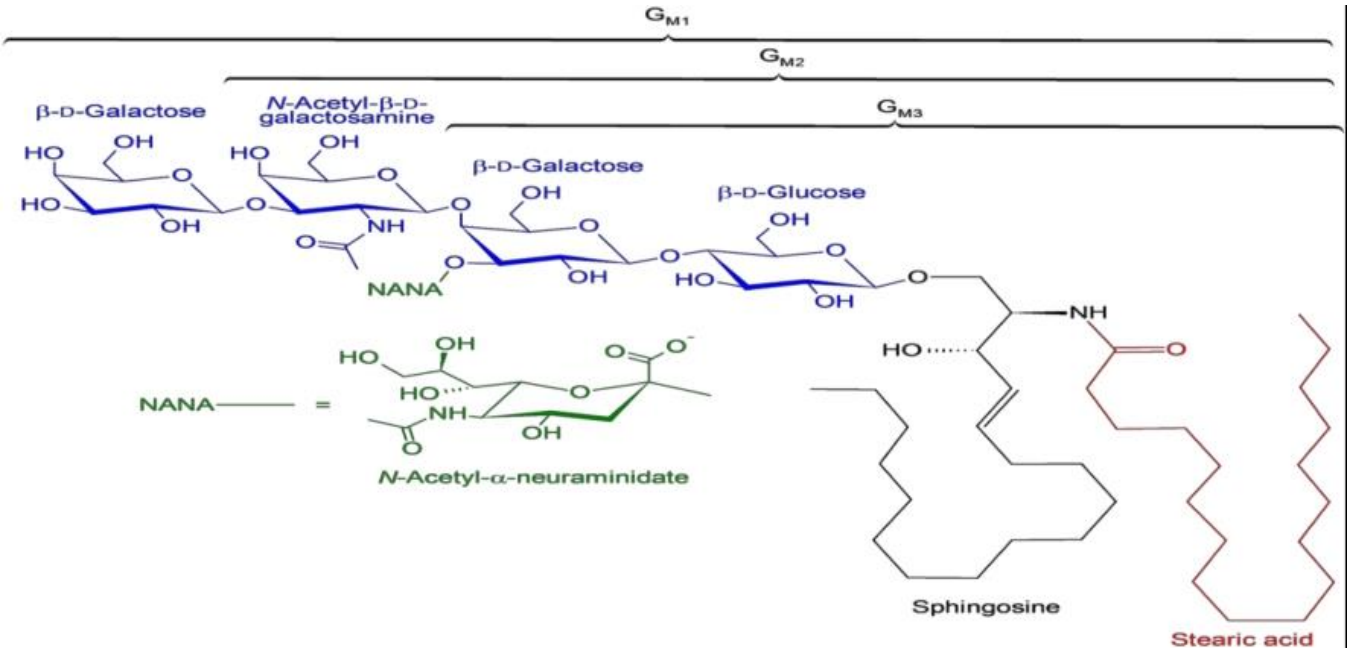
Further elaboration: **GM1**

G = Ganglioside.

M= Mono = One molecule of Sialic acid (NANA).

1 => 5-1 = 4 = 4 sugars are present in the molecule **(indirect indication)**.

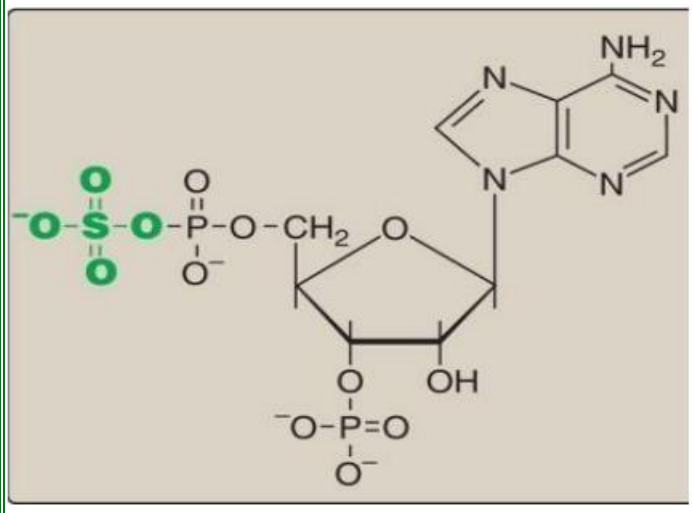




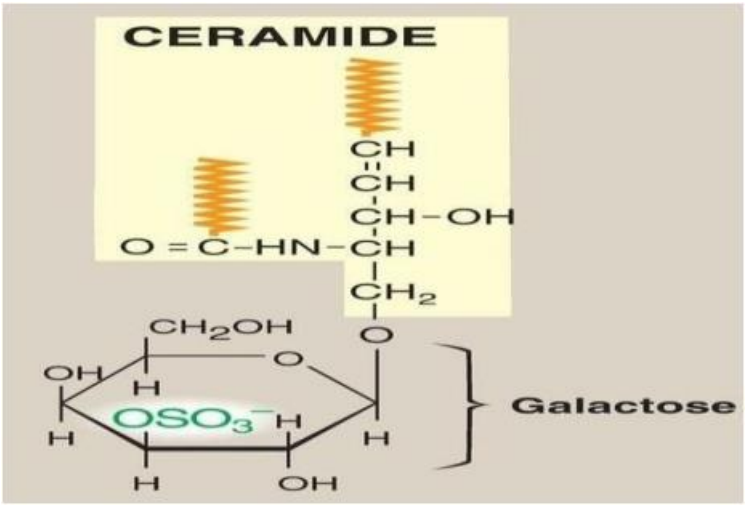
Synthesis of glycosphingolipids I

- 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 (Ex: **Ceramide glycosyl transferase**)
- **A sulfate** group from the sulfate carrier **3′-phosphoadenosine-5′-phosphosulfate ([PAPS]**, is added by a **sulfotransferase** to the 3′-hydroxyl group of the galactose in a galactocerebroside, forming the **sulfatide (galactocerebroside 3-sulfate)**.

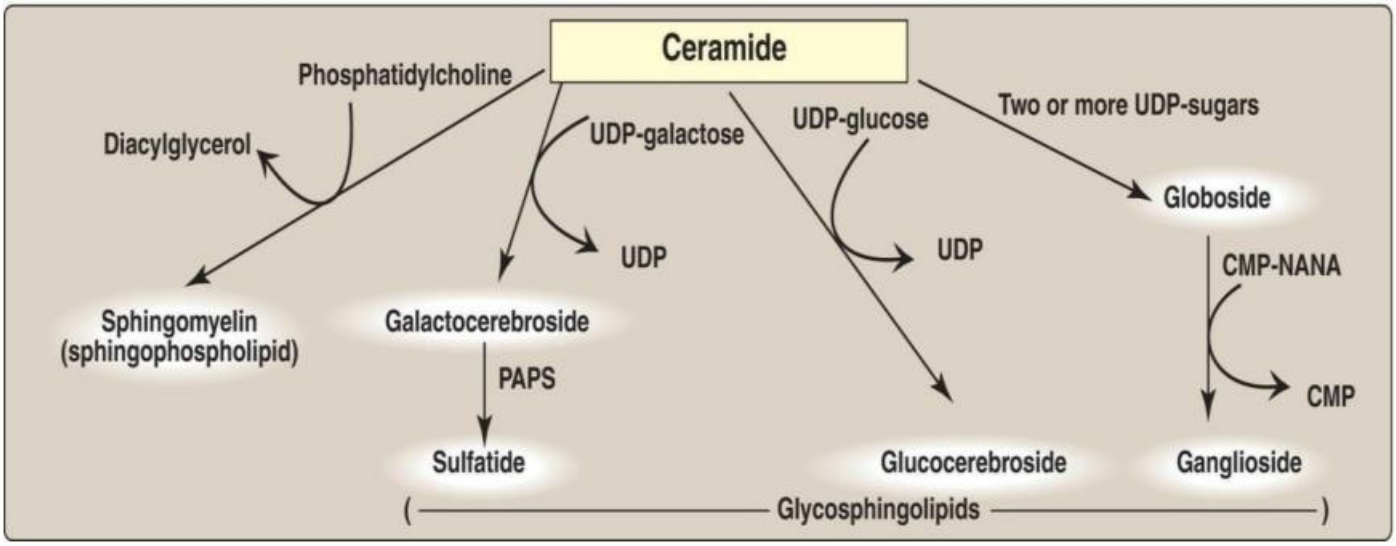
PAPS



Galactosyl-ceramide



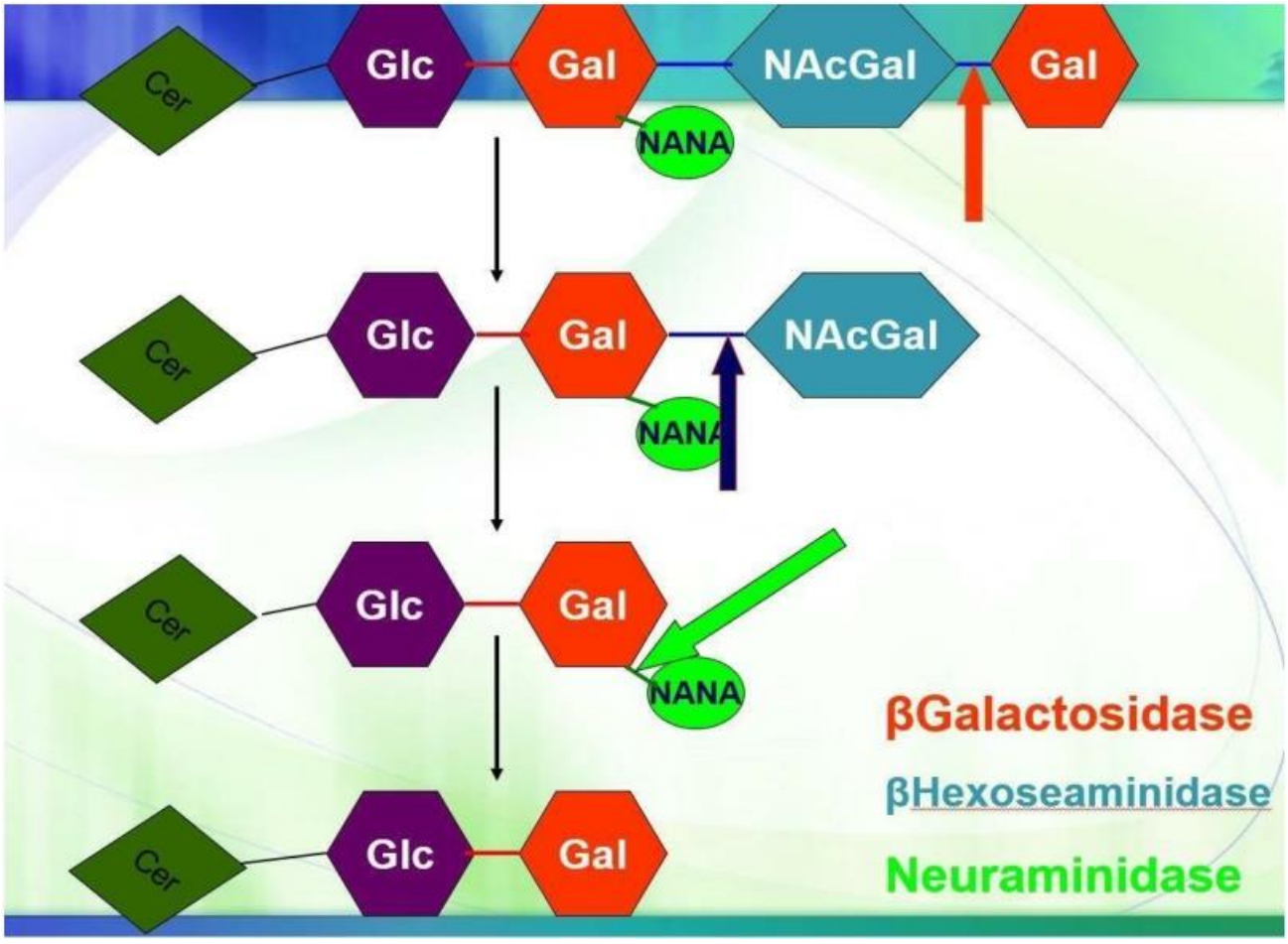
- **The following figure summarizes the synthesis of different Sphingolipids:-**



Note :- All the sugars incorporated in Glycolipids must be activated by UDP. However NANA is activated by a molecule of CMP.

Degradation of glycosphingolipids

- Degradation (Renewal) of Glycosphingolipids is achieved by internalization by phagocytosis (**Macrophages**) into phagosomes that fuse with the lysosomes thus forming Phagolysosomes that contain hydrolytic enzymes.
- The lysosomal enzymes hydrolytically and irreversibly remove the sugars **sequentially** starting with the last one added and ending with the first one added.
- Defect in the degradation **of glycosphingolipid**, as well as **glycosaminoglycans** and **glycoproteins**, causes **lysosomal storage diseases**. (Ex: **Sphingolipidoses)**



Notice for the previous figure that :

1- **Galactose** is cleaved by **Beta-galactosidase**

2- **N-acetyl-Galactosamine (NAcGal)** is cleaved by **Beta-hexosaminidase**

3- **Sialic acid (NANA)** is cleaved by **Neuraminidase**

Sphingolipidoses: Genetic disorders related to defective degradation of sphingolipids

- **The rate of biosynthesis of the accumulating lipid is normal**
- usually, **only a single sphingolipid type accumulates** (the substrate for the deficient degradative enzyme accumulates in the involved organs).
- The disorders are **progressive and can be fatal.**
- **There is extensive phenotypic variability in patients due to :-**

  The Particular defective gene that causes the disorder (**the clinical type**)

  Locus heterogeneity: the types of mutations (the degree of damage or change within the gene) within the gene that produce the defective enzyme. ***To further elaborate***: individual (A) has a certain mutation in gene (1) that leads to 10% deficiency in the degradative enzyme activity while individual (B) has a different mutation on the same gene (1) that leads to complete loss of enzymatic activity. This phenotypic variability is known as **Locus heterogeneity (Allele heterogeneity)**

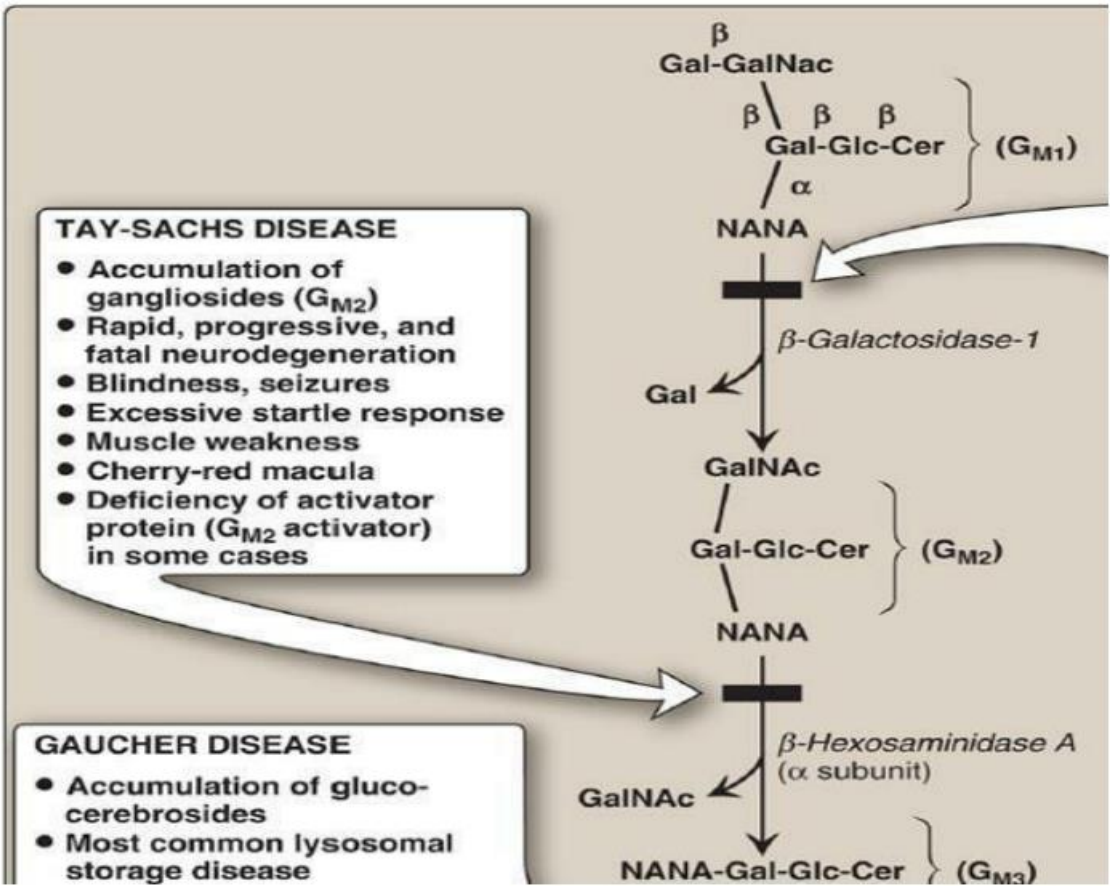
- **They are autosomal-recessive disorders (**carried on autosomal chromosomes not sex chromosomes)**, (except for Fabry disease, which is X linked)**. **The incidence of the sphingolipidoses is low in most populations**, except for **Gaucher and Tay-Sachs diseases, which, like Niemann-Pick disease**, **show a high frequency in the Ashkenazi Jewish population.**

- Bottom line : Severity depends on both the type of the enzyme deficient as well as nature of the mutation that happened in that gene.
- ***Despite being generally Autosomal recessive disorders***, in some cases **heterozygosity** (one mutated gene, one healthy gene) may be associated with some symptoms in some selective patients.

-Now let's examine some of the clinically important diseases (The doctor said that the symptoms aren't as important, so focus on what accumulates and what enzyme is being deficient. There are also other conditions that the doctor didn't explain).

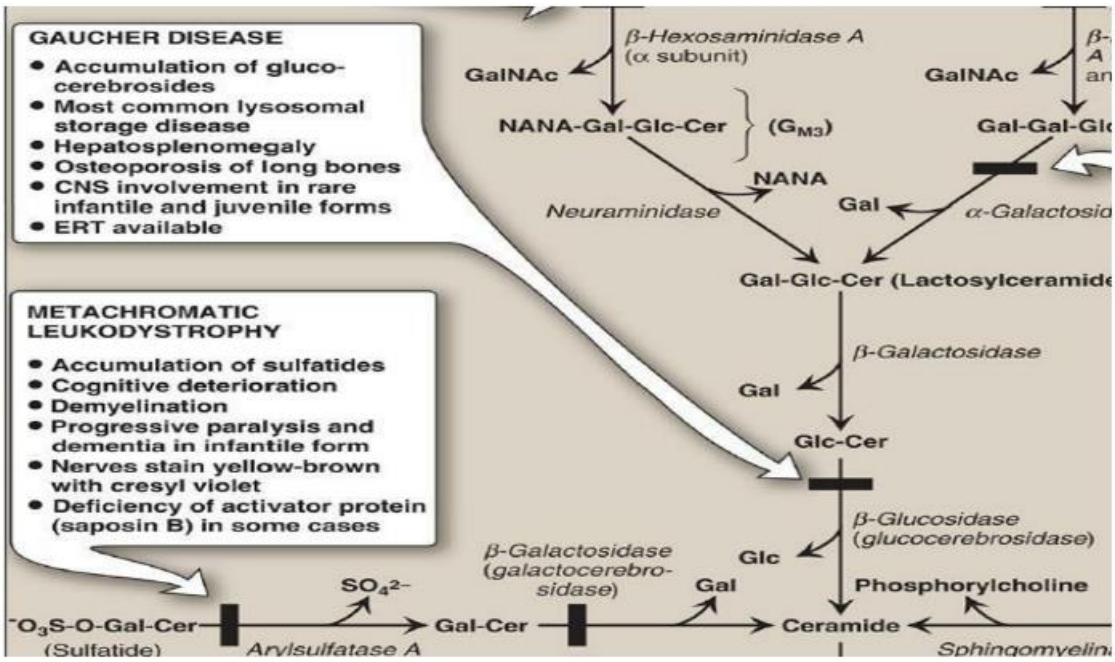
1- TAY-SACHS disease:

- **Characterized by accumulation of Gangliosides (Gm2)** due to defective enzymatic activity of **Beta-Hexosaminidase** A which leads to inability to cleave the **N-acetyl Galactosamine.**
- **Rapid ,progressive and fatal neurodegeneration. Blindness and seizures**



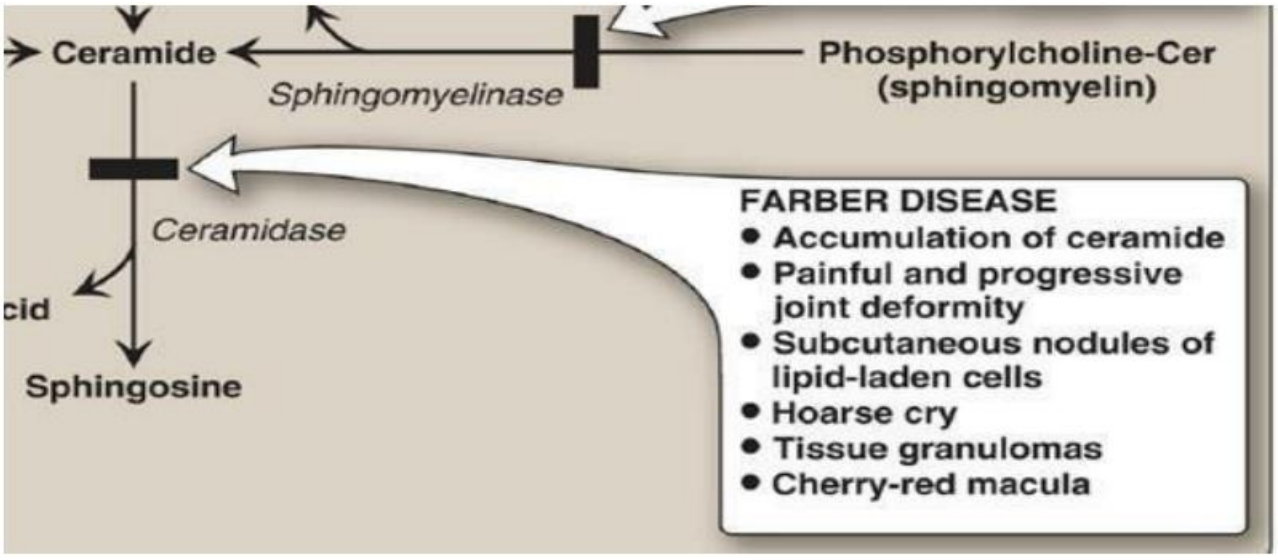
2- Gaucher's disease:

- **Characterized by accumulation of glucocerebroside** due to defective enzymatic activity **of Beta-glucosidase**, also known as **Glucocerebrosidase** which leads to inability to cleave **Glucose.**
- **Most common lysosomal storage diseases.**
- **Hepatomegaly as well as neural defects.**
- **Enzyme replacement Therapy can be used (ERT).**

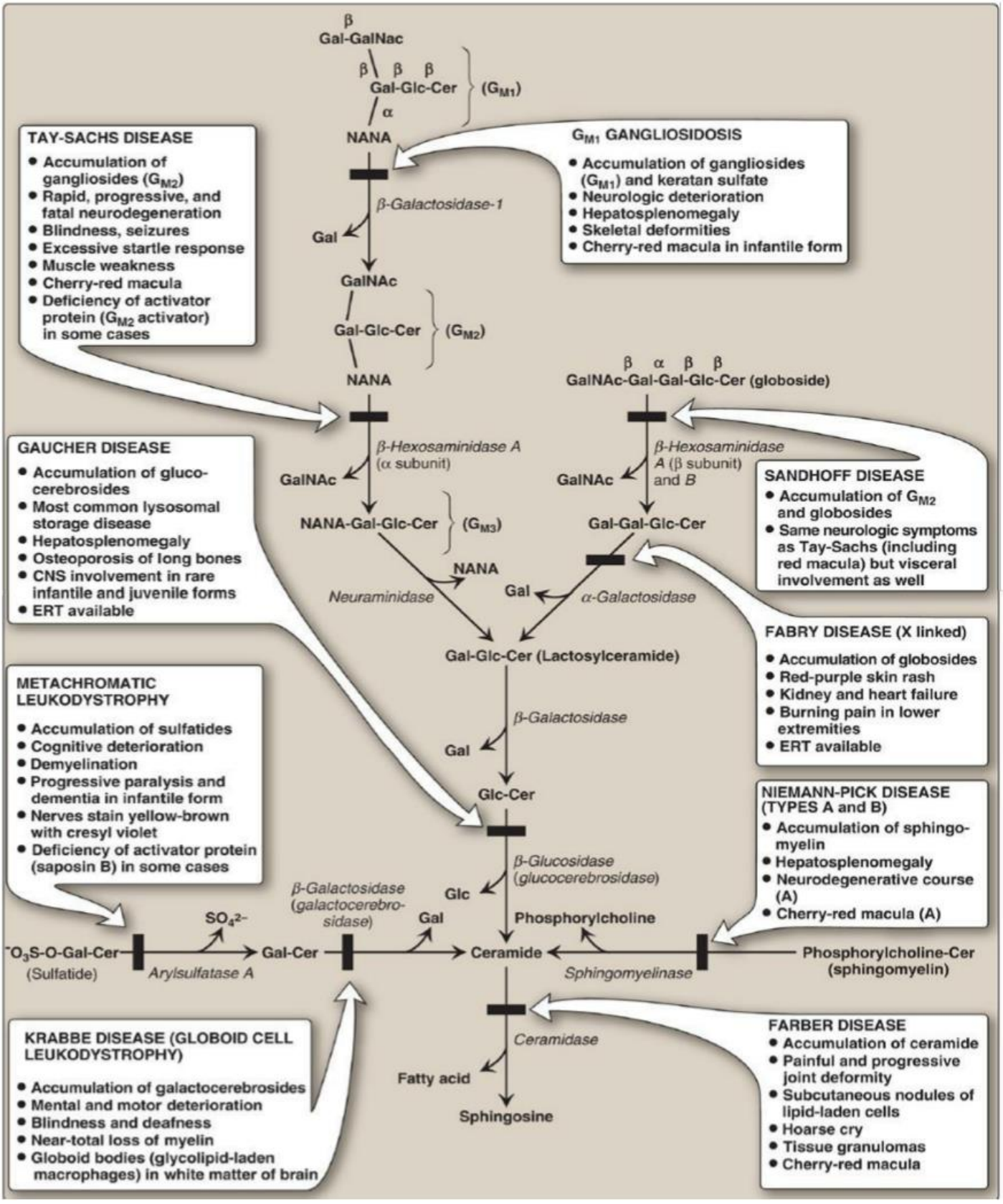


3- Farber's disease:

- **Characterized by the accumulation of ceramide** due to defective enzymatic activity of **Ceramidase** which leads to inability to convert **ceramide** back to **sphingosine.**
- **Painful and progressive joint deformity.**



Remember: All lysosomal storage diseases are Autosomal recessive Expect for Fabry's disease which is X-linked


β
Gal-GalNac
β β β
Gal-Glc-Cer
(GM1)
α
NANA
β-Galactosidase-1
Gal
GalNAc
Gal-Glc-Cer
(GM2)
NANA
β α β β
GalNAc-Gal-Gal-Glc-Cer (globoside)
β-Hexosaminidase A
(α subunit)
GalNAc
β-Hexosaminidase
A (β subunit)
and B
GalNAc
NANA-Gal-Glc-Cer
(GM3)
Gal-Gal-Glc-Cer
NANA
Neuraminidase
Gal
α-Galactosidase
Gal-Glc-Cer (Lactosylceramide)
β-Galactosidase
Gal
Glc-Cer
β-Glucosidase
(glucocerebrosidase)
Glc
β-Galactosidase
(galactocerebro-
sidase)
Gal
Phosphorylcholine
SO4 2-
3'O3S-O-Gal-Cer
(Sulfatide)
Arylsulfatase A
Gal-Cer
Ceramide
Sphingomyelinase
Phosphorylcholine-Cer
(sphingomyelin)
Ceramidase
Fatty acid
Sphingosine
TAY-SACHS DISEASE
• Accumulation of gangliosides (GM2)
• Rapid, progressive, and fatal neurodegeneration
• Blindness, seizures
• Excessive startle response
• Muscle weakness
• Cherry-red macula
• Deficiency of activator protein (GM2 activator) in some cases
GM1 GANGLIOSIDOSIS
• Accumulation of gangliosides (GM1) and keratan sulfate
• Neurologic deterioration
• Hepatosplenomegaly
• Skeletal deformities
• Cherry-red macula in infantile form
GAUCHER DISEASE
• Accumulation of gluco-cerebrosides
• Most common lysosomal storage disease
• Hepatosplenomegaly
• Osteoporosis of long bones
• CNS involvement in rare infantile and juvenile forms
• ERT available
SANDHOFF DISEASE
• Accumulation of GM2 and globosides
• Same neurologic symptoms as Tay-Sachs (including red macula) but visceral involvement as well
FABRY DISEASE (X linked)
• Accumulation of globosides
• Red-purple skin rash
• Kidney and heart failure
• Burning pain in lower extremities
• ERT available
METACHROMATIC LEUKODYSTROPHY
• Accumulation of sulfatides
• Cognitive deterioration
• Demyelination
• Progressive paralysis and dementia in infantile form
• Nerves stain yellow-brown with cresyl violet
• Deficiency of activator protein (saposin B) in some cases
NIEMANN-PICK DISEASE (TYPES A and B)
• Accumulation of sphingo-myelin
• Hepatosplenomegaly
• Neurodegenerative course (A)
• Cherry-red macula (A)
KRABBE DISEASE (GLOBOID CELL LEUKODYSTROPHY)
• Accumulation of galactocerebrosides
• Mental and motor deterioration
• Blindness and deafness
• Near-total loss of myelin
• Globoid bodies (glycolipid-laden macrophages) in white matter of brain
FARBER DISEASE
• Accumulation of ceramide
• Painful and progressive joint deformity
• Subcutaneous nodules of lipid-laden cells
• Hoarse cry
• Tissue granulomas
• Cherry-red macula

Diagnosis and treatment

Diagnosis:

✦ Measure enzyme activity in cultured fibroblasts or peripheral leukocytes

✦ Analyzing DNA using Next generation sequencing or genome analysis.

Treatment:

✦ Recombinant human enzyme replacement therapy although limited as it is hard for supplied enzymes to reach and enter the target cells. Used in Gaucher disease and Fabry disease (expensive)

✦ Bone marrow transplantation: Used in Gaucher disease as the cells that are usually affected are macrophages which are derived from the bone marrow. Therefore, we can transplant a healthy bone-marrow and in other cases , we can use **gene therapy** to edit stem cells in the bone marrow.

Substrate reduction therapy done by reducing the amount of substrate of the deficient enzymes which may help to some extent.

✦Gaucher disease: Pharmacologic reduction of glucosylceramide.

The End

V2

Page 11: locus heterogeneity = allele heterogeneity