Disorders of Sphingolipidosis
THE PRINCIPLE CLASSES OF MEMBRANE LIPIDS

- Membrane lipids (polar)
  - Phospholipids
    - Glycerophospholipids
      - Fatty acid
      - PO₄
      - Alcohol
    - Sphingolipids
      - Fatty acid
      - PO₄
      - Choline
  - Glycolipids
    - Sphingolipids
      - Fatty acid
      - Mono- or oligosaccharide
The common moiety of all sphingolipids is ceramide. Ceramide consists of sphingosine (amino alcohol) attached by an amide linkage to a long-chain or very long-chain fatty acid. Sphingosine is a long chain aliphatic amino alcohol. Sphingomyelin has ceramide + phosphoric acid and choline. Glycosphingolipids are composed of amino alcohol sphingosine + long chain fatty acid + monosaccharide units. Neuraminic acid-containing glycosphingolipids are named gangliosides. Synthesis and degradation of sphingolipids take place in different cellular compartments.
Glycosphingolipids (glycolipids) are essential components of all membranes in the body, but they are found in greatest amounts in nerve tissue.

- They are located in the outer leaflet of the plasma membrane, where they interact with the extracellular environment.
- As such, they play a role in the regulation of cellular interactions, growth, and development.
- When cells are transformed (that is, when they lose control of cell division and growth), there is a dramatic change in the glycosphingolipid composition of the membrane.
- Glycosphingolipids are antigenic, and they have been identified as a source of blood group antigens.
- Various embryonic antigens specific for particular stages of fetal development, and some tumor antigens. [The carbohydrate portion of a glycolipid is the antigenic determinant.]
In humans at least 60 different sphingolipids have been identified.

Sphingolipids at cell surfaces are sites of biological recognition.

Very prominent in neuronal plasma membranes.

Carbohydrate moieties of sphingolipids define the human blood groups.

The kinds and amounts of gangliosides vary dramatically during development.
Ceramide is the parent compound. Other polar head groups can be attached at position X.
SPHINGOLIPIDS

The 3-carbon backbone is analogous to the 3-carbons of glycerol. At C3 there is the long chain amino alcohol sphingosine.
At C2 there is a fatty acid which is usually saturated or monounsaturated, and can be either 16, 18, 22, or 24 carbons long.
SPHINGOLIPIDS

Glycosphingolipids are a sub-group of sphingolipids that contain sachharide headgroups.

<table>
<thead>
<tr>
<th>Name of sphingolipid</th>
<th>Name of X</th>
<th>Formula of X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceramide</td>
<td>—</td>
<td>$\text{H}$</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>Phosphocholine</td>
<td>$\text{PO}_4\text{OCH}_2\text{CH}_2\text{N(CH}_3\text{)}_3^+$</td>
</tr>
<tr>
<td>Neutral glycolipids</td>
<td></td>
<td></td>
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<tr>
<td>Glucosylcerebroside</td>
<td>Glucose</td>
<td></td>
</tr>
<tr>
<td>Lactosyleceramide</td>
<td>Di-, tri-, or tetra-</td>
<td></td>
</tr>
<tr>
<td>(a globoside)</td>
<td>saccharide</td>
<td></td>
</tr>
<tr>
<td>Ganglioside GM2</td>
<td>Complex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>oligosaccharide</td>
<td></td>
</tr>
</tbody>
</table>
Glycosphingolipids

• **Cerebrosides**
  • One sugar molecule
    – Galactocerebroside – in neuronal membranes
    – Glucocerebrosides
      – cerebrosides are found predominantly in the brain and peripheral nervous tissue, with high concentrations in the myelin sheath.

• **Sulfatides** or sulfogalactocerebrosides
  • Are cerebrosides that contain sulfated galactosyl residues.
  • Are found predominantly in nerve tissue and kidney.
Glycosphingolipids

- **Globosides**: ceramide oligosaccharides
  - are produced by attaching additional monosaccharides (including GalNAc) to a glucocerebroside.

- **Gangliosides**: have a more complex oligosaccharide. They are derivatives of ceramide oligosaccharides, and contain one or more molecules of NANA (N-acetylneuraminic acid).
  - Biological functions: cell-cell recognition; receptors for hormones.
  - Are found primarily in the ganglion cells of the central nervous system, particularly at the nerve endings.
  - Common gangliosides: $G_{M1}$, $G_{M2}$, $G_{M3}$, $G_{D1a}$, $G_{D1b}$, $G_{T1a}$, $G_{T1b}$, $G_{q1b}$
Sphingolipids

• The concentration of individual sphingolipids varies from tissue to another, e.g., the neutral sphingolipid and glucosylceramide are common constituents of liver and spleen, hence the principal symptoms of glucosylceramidosis involve these organs.

• Two main types of sphingolipids are predominante in CNS.
  1- Galactosylceramide and sulphatide in white matter.
  2- Gangliosides in grey matter.
Degradation of Glycosphingolipids

- Glycosphingolipids are internalized by endocytosis.
- All of the enzymes required for the degradative process are present in lysosomes, which fuse with the endocytotic vesicles.

- The lysosomal enzymes hydrolytically cleave specific bonds in the glycosphingolipid.

- Degradation is a sequential process following the rule “last on, first off,” in which the last group added during synthesis is the first group removed in degradation.

- If a specific hydrolase required for the degradation process is partially or totally missing, a sphingolipid accumulates in the lysosomes.

- Lipid storage diseases caused by these deficiencies are called sphingolipidoses.

- A specific lysosomal hydrolytic enzyme is deficient in each disorder. Therefore, usually only a single sphingolipid (the substrate for the deficient enzyme) accumulates in the involved organs in each disease.
These enzymes catalyze the stepwise removal of sugar units, finally yielding a ceramide.
Sphingolipidosis

- Sphingolipids are membranous elements of cells and tissues throughout the body with high concentrations in brain and nervous organs.
- Sphingolipidoses are lysosomal storage disorders in which sphingolipids accumulate in one or several organs as a result of primary deficiency in enzymes or activator proteins involved in their degradative pathway.
- They are characterized by excessive accumulation of lipids in affected organs and functional impairment of the organs.
## Sphingolipid Storage Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Enzyme Deficiency</th>
<th>Principal Storage Substance</th>
<th>Major Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G_{M_1}$ Gangliosidosis</td>
<td>$G_{M_1}$ β-galactosidase</td>
<td>Ganglioside $G_{M_1}$</td>
<td>Mental retardation, liver enlargement, skeletal involvement, death by age 2</td>
</tr>
<tr>
<td>Tay–Sachs disease</td>
<td>Hexosaminidase A</td>
<td>Ganglioside $G_{M_2}$</td>
<td>Mental retardation, blindness, death by age 3</td>
</tr>
<tr>
<td>Fabry’s disease</td>
<td>α-Galactosidase A</td>
<td>Trihexosylceramide</td>
<td>Skin rash, kidney failure, pain in lower extremities</td>
</tr>
<tr>
<td>Sandhoff’s disease</td>
<td>Hexosaminidases A and B</td>
<td>Ganglioside $G_{M_2}$ and globoside</td>
<td>Similar to Tay–Sachs disease but more rapidly progressing</td>
</tr>
<tr>
<td>Gaucher’s disease</td>
<td>Glucocerebrosidase</td>
<td>Glucocerebroside</td>
<td>Liver and spleen enlargement, erosion of long bones, mental retardation in infantile form only</td>
</tr>
<tr>
<td>Niemann–Pick disease</td>
<td>Sphingomyelinase</td>
<td>Sphingomyelin</td>
<td>Liver and spleen enlargement, mental retardation</td>
</tr>
<tr>
<td>Farber’s lipogranulomatosis</td>
<td>Ceramidase</td>
<td>Ceramide</td>
<td>Painful and progressively deformed joints, skin nodules, death within a few years</td>
</tr>
<tr>
<td>Krabbe’s disease</td>
<td>Galactocerebrosidase</td>
<td>Deacylated galactocerebroside</td>
<td>Loss of myelin, mental retardation, death by age 2</td>
</tr>
<tr>
<td>Metachromatic leukodystrophy</td>
<td>Arylsulfatase A</td>
<td>Sulfatide</td>
<td>Mental retardation, death in first decade</td>
</tr>
<tr>
<td>(Sulfatide lipidosis)</td>
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</tbody>
</table>
Sphingolipidoses

- Subgroups of sphingolipidoses are grouped together such as:
  - Disorders with prominent hepatosplenomegaly (Niemann–Pick and Gaucher diseases)
  - Disorders with central and peripheral demyelination (metachromatic leukodystrophy and Krabbe diseases)
  - Disorders with prominent neuronal storage (gangliosidoses)
  - All sphingolipidoses are autosomal recessive inherited except Fabry disease; X-linked
Diagnosis

- By the clinical presentation, the course of the classical forms of the various diseases are often characteristic.
  - Measuring enzyme activity in cultured fibroblasts or peripheral leukocytes.
  - Histologic examination of the affected tissue [shell-like inclusion bodies are seen in Tay-Sachs, and a wrinkled tissue paper appearance of the cytosol is seen in Gaucher disease].
  - Direct detection of mutation by DNA.
  - Prenatal diagnosis, using cultured amniocytes or chorionic villi.
Treatment

- Effective treatment is unavailable for sphingolipidoses especially those with neurologic forms.
- Organ transplantation is effectively used in non-neuronopathic forms. e.g., Gaucher has been treated by bone marrow transplantation.
- Recombinant human enzyme replacement therapy in some disorders, but the monetary cost is extremely high.
Tay-Sachs disease

- A fatal disease which is due to the deficiency of hexosaminidase A activity
- Autosomal recessive. Gene on chr 15, more than 100 mutations.
- East – European JEWS = 1 in 30 carrier rate
- Accumulation of ganglioside $G_{M2}$ in the brain.
- Mental retardation, blindness, inability to swallow
- A “cherry red“ spot develops on the macula (back of the eyes)
SYMPTOMS

• Cherry red spot at the back of the retina
  – In all patients with TSD
  – Caused by gangliosides in eye cells
SYMPTOMS

• Three forms; classified by time of onset of symptoms
  – Infantile
  – Juvenile
  – Adult/Late Onset
SYMPTOMS

• Infantile
  – Develop normally for first 6 months
  – Nerve cells become distended with gangliosides
    • Mental and physical abilities deteriorate
  – Become blind, deaf and unable to swallow
  – Muscles atrophy
  – Paralysis
  – Usually die by age 4 or 5
SYMPTOMS

• Juvenile
  – 2 to 10 years old
  – Difficulties with speech, thought and movement
    • Unsteady walking
    • Uncontrollable movement
  – Trouble swallowing
  – Die between 5 and 15 years old
SYMPTOMS

• Adult/Late onset
  – Rarest form
  – Begins in patients 20 to 30 years old
  – Unsteady walk
  – Psychiatric illness
    • Schizophrenia
    • Psychosis
  – Most full time wheelchair users
  – Usually non-fatal
Morphological Change in Brain in Tay-Sachs Disease

- **Brain** → Ballooning of Neurons & Cytoplasmic vacuoles (fat stain positive material)
- **Electronic Microscopy** = Onion skin layers of Cytoplasmic membrane
- **Later** → destruction of neurons
Diagnostic Tests

- In Tay-Sachs (and Sandhoff disease) confirmation of the clinical diagnosis is easy by appropriate enzyme testing on leukocytes or cultured fibroblasts.

- *Electron microscopic examination* of a skin or conjunctival biopsy may provide strong evidence in diagnosis by demonstrating concentric lamellated bodies in nerve endings.

- The definitive diagnosis requires gene sequencing.
Treatment

- No effective curative treatment is currently available.
- Only supportive treatment
  - Antiseizure medications
  - Feeding tubes
  - Nutrition/hydration techniques
  - Airway management
- Gene therapy is still at an early experimental stage in mouse models.
Prevention

- **Prenatal diagnosis**
  - To determine if fetus has gene from both parents

- **Mate Selection**
  - Can avoid marriage between two carriers

- **Pre-implantation genetic diagnosis**
  - Only healthy zygote are implanted
Gaucher Disease
Gaucher's disease is the most common of the lysosomal storage diseases. It is caused by a hereditary deficiency of the enzyme glucocerebrosidase (also known as acid β-glucosidase).

- The enzyme acts on glucocerebroside (glucosylceramide).
- When the enzyme is defective, the sphingolipids accumulate in the spleen, liver, kidneys, lungs, brain, and bone marrow.
- It is an autosomal recessive disorder. The gene is on chromosome 1 q21.
- About 1 in 100 people in the United States are carriers of the most common type of Gaucher disease, while the carrier rate among Ashkenazi Jews is 1 in 15.
Gaucher Disease

• Three recognized types:
  – Type I (Noncerebral juvenile)
    • Most common in Ashkenazi Jew (1:450)
  – Type II (Infantile cerebral)
    • 1 in 100,000 live births
    • Death usually occurs w/in 1 year
  – Type III (Chronic neuropathic)
    • 1 in 50,000 live births
**Signs and symptoms**

- Painless hepatomegaly and splenomegaly.
- Hypersplenism: the rapid and premature destruction of blood cells, leading to anemia, neutropenia and thrombocytopenia (with an increased risk of infection and bleeding).
- Neurological symptoms occur only in:
  - Type II: serious convulsions, hypertonia, mental retardation, apnea.
  - Type III: muscle twitches known as myoclonus, convulsions, dementia, ocular muscle apraxia.
- Osteoporosis: 75% develop visible bony abnormalities due to the accumulated glucosylceramide. A deformity of the distal femur in the shape of an flask is commonly described (necrosis of the femur joint).
- Yellowish-brown skin pigmentation
Gaucher Disease

- **Type I**: (chronic non-neuropathic), 99%
  - Decreased enzyme activity
  - Without CNS involvement
  - Predominantly spleen & skeleton
  - Thrombocytopenia
  - Bone pain
  - Progressive but compatible with long life
  - European Jews
Gaucher Disease

• **Type II**: (acute neuropathic)
  • No residual enzyme activity
  • Infantile
  • Progressive involvement of CNS & early death
  • Hepatosplenomegaly
• **Type III** (the chronic neuropathic form)
  - Can begin at any time in childhood or even in adulthood, and occurs in approximately 1 in 100,000 live births.
  - It is characterized by slowly progressive but milder neurologic symptoms compared to the acute or type II.
• Major symptoms:
  -- Enlarged spleen and/or liver
  -- Seizures, poor coordination
  -- Skeletal irregularities
  -- Eye movement disorders
  -- Blood disorders including anemia and respiratory problems.
  -- Patients often live into their early teen years and adulthood.
Metabolic Derangement

- The enzyme catalyses the breakdown of glucocerebroside, a cell membrane constituent of red and white blood cells.
- The macrophages that clear these cells are unable to eliminate the waste product, which accumulates, and turn into Gaucher cells, which appear on light microscopy to resemble crumpled-up paper.
- In the brain (type II and III), glucocerebroside accumulates due to the turnover of complex lipids during brain development and the formation of the myelin sheath of nerves.
- Different mutations in the beta-glucosidase determine the remaining activity of the enzyme, and, to a large extent, the phenotype.
Aspirated bone marrow cells from patient with Gaucher disease

The "crumpled tissue paper" appearance of the cytoplasm of Gaucher cells is caused by enlarged, elongated lysosomes filled with glucocerebroside.
The disease is caused by mutations of the GBA (acid β-glucosidase) gene (1q21).
- N370S, the most common mutation in Ashkenazim.
- The second most frequent mutation, L444P is more frequently associated with types II and III.
- Complex alleles due to genetic rearrangements are more often associated with severe forms, including perinatal lethal forms.
Diagnosis

- Bone marrow examination for Gaucher cells.
- Demonstration of a deficient enzyme activity in lymphocytes or leukocytes using artificial fluorogenic substrate.
- A definitive diagnosis is made with genetic testing. As there are numerous different mutations, sequencing of the beta-glucosidase gene is sometimes necessary to confirm the diagnosis.
- Prenatal diagnosis is available, and is useful when there is a known genetic risk factor.
Treatment

• Splenectomy (rarely cures)
• Enzyme replacement treatment:
  – Recombinant enzyme: extremely expensive
  – Placental glucocerebrosidase (Ceredase)

• Bone marrow transplantation
  - Treatment of choice in advanced disease

• Gene therapy: future