Lysosomal storage diseases

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What are Lysosomes?

Christian De Duve:
from Christmas 1949
to the Nobel price (1965)

Claude’s technique of separating the components of cells by spinning them in a centrifuge, he noticed that the cells’ release of an enzyme called acid phosphatase increased in proportion to the amount of damage done to the cells during centrifugation.

De Duve reasoned that the acid phosphatase was enclosed within the cell in some kind of membranous envelope that formed a self-contained organelle. He calculated the probable size of this organelle, christened it the lysosome, and later identified it in electron microscope pictures.

De Duve's discovery of lysosomes answered the question of how the powerful enzymes used by cells to digest nutrients are kept separate from other cell components.
Endocytosis

Exocytosis

ER

Lysosome

Autophagy

Macroautophagy

Microautophagy

Autophagosome

Autolysosome

Golgi complex

Vesicles containing acid hydrolase

Early endosome

Late endosome/MVB

Residual body

Plasma membrane
Lysosomes and LROs are involved in: cholesterol homeostasis, plasma membrane repair, bone and tissue remodelling, pathogen defense, cell death and cell signaling.
### Classification

- **Characteristic of the defective enzyme or protein**
- **Nature of the accumulated substrate**

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<th>Disease</th>
<th>Defective protein</th>
<th>Main storage materials</th>
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<tr>
<td>Sphingolipidosis</td>
<td>α-Galactosidase A</td>
<td>Globo- and globotriaosylceramide, and blood-group B substances</td>
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<td>MPS</td>
<td>GM2-activator protein</td>
<td>GM2 gangliosides and related glycolipids</td>
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<td>Mucopolysaccharidoses (MPS)</td>
<td>IDUA (Hurler, Scheie, Hurler-Scheie)</td>
<td>IDuronate-2-sulphatase</td>
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<td>MPS I (Hurler)</td>
<td>α-L-iduronidase</td>
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<td>MPS II (Hunter)</td>
<td>Iduronate-2-sulphatase</td>
<td>Dermatan sulphate and heparan sulphate</td>
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<td>MPS IIIB (Sanfilippo)</td>
<td>Heparan N-sulphatase (sulfamidase)</td>
<td>Heparan sulphate</td>
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<td>MPS IIIC (Sanfilippo)</td>
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<td>MPS IID (Sanfilippo)</td>
<td>N-Acetylα-glucosaminidase</td>
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<tr>
<td>Morquio-A disease</td>
<td>N-Acetylgalactosamine-6-sulphate-sulphatase</td>
<td>Keratan sulphate, chondroitin-6-sulphate</td>
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<td>Morquio-B disease</td>
<td>β-Galactosidase</td>
<td>Keratan sulphate</td>
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<td>MPS VI (Maroteaux-Lamy)</td>
<td>N-Acetylgalactosamin-4-sulphatase (aryl sulphatase B)</td>
<td>Dermatan sulphate</td>
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<td>MPS VII (Sly)</td>
<td>β-Glucuronidase</td>
<td>Heparan sulphate, dermatan sulphate, chondroitin-4- and -6-sulphates</td>
</tr>
</tbody>
</table>

### Oligosaccharidoses and glycoproteinosis

- Sphingolipidosis
- MPS
- Oligosaccharidoses-
  & glycoproteinosis
- Defect of integral membranes proteins
- Others

- Pompe (glycogen-storage disease type II) | α-Glucosidase | Glycogen |
- Cystinosis | Cystatin | Cystine |
- Danon disease | LAMP2 | Cytoplasmatic and glycoproteinosis |
- Infantile sialic-acid-storage disease and | Sialin | Sialic acid |
- Salla disease | | |
- Mucopolysaccharidoses (ML IV) | Mucolipin-1 | Lipids and acid mucopolysaccharides |
- Niemann-Pick C1 | NPC1 and 2 | Cholesterol and sphingolipids |

### Others

- Gaucher's disease | Cathepsin A | Sialylglycosaminoglycans |
- I-cell and pseudo-Hurler polydystrophy (ML II and ML III, respectively) | UDP-N-acetylglucosamine-l-fucosyltransferase | Oligosaccharides, mucopolysaccharides and lipids |
- Multiple sulphatase deficiency | Co-formylglycin-generating enzyme | Sulphatides |
- Neuronal ceroid lipofuscinosis (NCL) (Batten disease) | CLN1 (protein palmityltransferase-1) | Lipidated thioesters |
- NCL2 (Batten disease) | CLN2 (Tripeptidyl amino peptidase-1) | Subunit c of the mitochondrial ATP synthase |
- NCL3 (Batten disease) | Aralarine transporter | Subunit c of the mitochondrial ATP synthase |

Futerman AH et al, Nature reviews, 2004
This Classification is not always accurate

• **Lipids and sugars accumulation**
  – *Mucolipidosis II/III:*
    • Alpha-Beta subunit gene defect- Gamma subunit gene defect
  – *Mucolipidosis IV:*
    • Mucolipin membrane protein calcium channel

• **Sphingomyelin accumulation:**
  – *Niemann Pick A-B: sphingomyelinase defect*
  – *Niemann Pick C:*
    • Cholesterol transporter NPC1
    • Cholesterol binding protein NPC2
We diagnose what we know
Gaucher

1 in 5-7000 live births

Frequency 1 in 5-7,500 live births

Meikle et al. JAMA (1999)
Why is an accurate diagnosis important?

- Accurate counseling
- Recurrence risk
- Prenatal diagnosis
- Patient prognosis
- Support group
- Specific treatment
Accumulating substrate

Genes
Gene therapy

1882  1934  1950/1960  Mid-1980  1990s onwards

MD clinical description
Deficient enzyme; Enzyme replacement therapy
Cellular basis of the diseases
- Enzyme deficiency

- Substrate accumulation

- Cell damage and dysfunction
- Organ dysfunction
Medical Tools

Diagnosis

Think

Data: Ask - Listen - Examine
Population

Ashkenazi Child with hyperacusis:
Tay Sachs

Jewish Yemenite patient with neurodegeneration:
Metachromatic leukodystrophy
Screening of mutations for LSD

- Gaucher disease: Ashkenazi Jews 1/15
- Mucolipidosis IV: Ashkenazi Jews 1/100
- Niemann Pick A: Ashkenazi Jews 1/90
- MLD: Yemenite Jews: 1/50
- Krabbe Disease: Djabel el Mokaber 1/12
Ask & Listen

Family history
Pedigree
Inheritance
Family History of X-Linked disorder

- Hunter disease
- Fabry disease
- Danon disease
Family history: autosomal recessive & Population history

2/3 1/30 1/4 1/1800
You see what you look for and you look for what you know.
Examine - Look

• Age of first symptom
• Evolution of the disease
• Clinical symptoms
A difficult task

• Genetic Heterogeneity:
  – One phenotype - many genes

• Mucolipidosis III:
  – alpha-beta subunit gene
  – gamma subunit gene
Phenotype heterogeneity

• One enzyme – one gene- many phenotypes
  • Gaucher type I non neuronopathic
  • Gaucher type II neuronopathic
  • Gaucher type III neuronopathic
How to Recognize an affected patient?

A 14-year-old Hunter Syndrome Patient With a 7-year-old Height Age

Photographs courtesy of Joseph Muenzer, MD, PhD, Chapel Hill, NC.
Why Can we Easily miss the affected patient?

- Variability of the diseases
- > 50 different LSDiseases
- Multisystemic & complex diseases
- Progressive diseases
- High index of suspicion is needed
- We do not always remember these diseases
Variable diseases

Mild Form of MPS II
- Insidious onset
- Normal intelligence
- Variable life expectancy (some to 50s)

Severe Form of MPS II
- Onset by 2 to 4 years of age
- Impaired intelligence
- Life expectancy 10 to 15 years
A Progressive Disease

1 year - 3 years
Recurrent ear infections
Rhinorrhea
Respiratory infections
Inguinal hernia

4 years
Enlarged Tonsils

5 years
Tonsillectomy
Anesthesia

6 years
DIAGNOSIS

Not all the patients have the characteristic features at a young age
Multisystemic disorders

- Phenotype not usually evident at birth
- Heterogeneous presentation
- Significant eventual loss of mobility and functional independence
- Bone dysplasia
- Joint stiffness and restriction
- Short stature
- Hernia
- Hepatosplenomegaly
- And more....
The Laboratory Tools

• Identification of the chemical nature of compounds accumulating as a result of the enzyme deficiency:
  – Urine MPS - Oligosaccharides
  – Quantitative\ qualitative
Morphologic studies

– Em: fibroblasts- rectal biopsy

Membranous lamellar cytoplasmic bodies (lipids) + Granulated material (Water-soluble)
Renal pathology in Fabry disease.
Filipin: Isolated from *Streptomyces filipensis* binds to cholesterol.
• Demonstration of a specific enzyme deficiency
  – Leukocytes - serum
• BUT
  – Leukocytes + Serum: Mucolipidosis II – III
  – Fibroblasts: Niemann Pick C
• Gene Mutation analysis:
  – Carriers identification
  – Prenatal Diagnosis on fetal DNA
  – Preimplantation genetic diagnosis
Definitive diagnosis

• **Mutation analysis:**
  – Identify carrier in the family
  – Prenatal diagnosis
  – Preimplantation genetic diagnosis
The benefice of a correct diagnosis

- Accurate counseling
- Recurrence risk

PREVENTION:
- Prenatal diagnosis
- Preimplantation
  Genetic Diagnosis
The benefice of a correct diagnosis

• Stop unnecessary testing
• Patient prognosis
• Support group
• Proper follow up for complications
  preventions and symptomatic therapy
• Specific treatment as early as possible
Diagnosis as **Early** as possible

Early Therapy

6 months  5 years  9 years  30 years

PEDIATRICS Volume 121, February 2008
The enzyme replacement therapy concept (ERT era)

First proof of concept
1974: Roscoe Brady (NIH)
Large dose of mannose terminated acid beta glucosidase (Placenta, CHO):

Enzyme Replacement Therapy

- **Gaucher**: Glucocerebrosidase
- **Fabry**: alpha galactosidase
- **Pompe**: alpha glucosidase
- **Hurler**: alpha-L iduronidase
- **Hunter**: iduronate II sulfatase
- Niemann Pick type B: acid sphyngomyelinase
- **Maroteaux-Lamy (MPS VI)**: arylsulfatase
- **MPS IV**: trial
- ........
What can we expect?

- Change the disease natural history
- Increases life expectancy
- Improvement of systemic abnormalities

BUT

- Still depends on the type MPS, mutation
- Clinical severity
- Age of the patient at diagnosis
- Neurological involvement
Disadvantages

- Price
- Once a week intravenous infusion 0.5 mg/kg
- Does not cross the blood brain barrier
- Bone disease
Substrate Inhibition: Concept

Normal

Enzyme Replacement

Substrate Inhibition

synthesis degradation

synthesis degradation

synthesis degradation
Decreased efficiency of GAG production

Expression of genes Coding for enzymes involved in GAG Synthesis

Activation of tyrosine kinases

Proteins phosphorylation

Signaling pathways

Coding for enzymes involved in GAG Synthesis

Transcription

Nucleus

Proteins phosphorylation

Activation of tyrosine kinases

EGF

EGFR

EGFR-TK

Cell membrane

GENISTEIN
LSDs: rare diseases?

- Awareness is key for diagnosis
- Communication with the laboratories are essential
- Prevention of disease complications
- Specific therapies are available we need to diagnose as soon as possible
- Genetic prenatal/preimplantation diagnosis is feasible diagnosis is needed to permit the choice for the families
תודה

Mordillo