Gaucher Disease
From Lysosomal Storage to Immunopathology

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Personal background in research on Gaucher disease
• PhD biochemistry: biochemical properties of glucocerebrosidase in relation to Gaucher disease: promotor - Joseph Tager & John Barranger
• Remained active in research on Gaucher disease from 1983 - now:
  - Over 150 publications on the topic in peer-reviewed journals
  - Co-founder of European Working Group on Gaucher Disease:
    - EWGGD chairman 1994-2010 and present honorary board member
• Academic position: professor in medical biochemistry and chairman of department of biochemistry, Academic Medical Center, University of Amsterdam

Heterogeneous clinical expression of Gaucher disease
Manifestations ranging from neonatal to asymptomatic

Characteristic disease manifestation
• Visceral:
  - Hematological symptoms
  - Hepatosplenomegaly
  - Skeletal deterioration
• Others:
  - [Neurological symptoms – types 2&3]
  - [Skin defects – colloid type]
Molecular basis of Gaucher disease
Glucocerebrosidase gene and mutations

- **Locus of GBA gene**
  - Chromosome 1q21
- **Recessive transmission**
- **Reported mutations**

  - GBA gene at locus 1q21 encodes for a 497 AA glycoprotein

Enzyme deficiency → lysosomal lipid accumulation

- Glucocerebrosidase (GBA)
  - GaCer: glucosylceramide

Type 1 Gaucher disease, a macrophage disorder
Accumulation of lipid-laden macrophages in tissues

- Hematological abnormalities
- Organomegaly
- Skeletal deterioration
- Metabolic abnormalities

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Genetic heterogeneity → phenotypic heterogeneity

Marked phenotypic heterogeneity, marked genetic heterogeneity

- A lower residual enzyme activity is associated with a more severe disease
- We cannot accurately predict disease severity based solely on GBA genotype
- Even monozygotic twins may differ in phenotype

Discordant monozygotic Gaucher twins

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Patient A</th>
<th>Patient B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (mM)</td>
<td>4.5</td>
<td>7.3</td>
</tr>
<tr>
<td>Trombocytes (E10^9/l)</td>
<td>72</td>
<td>488</td>
</tr>
<tr>
<td>Leukocytes (E10^9/l)</td>
<td>4.8</td>
<td>7.2</td>
</tr>
<tr>
<td>Liver volume (ml)</td>
<td>1712</td>
<td>1540</td>
</tr>
<tr>
<td>Spleen volume (ml)</td>
<td>915</td>
<td>148</td>
</tr>
<tr>
<td>b-glucosidase (U)</td>
<td>1.5</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Type 1 Gaucher disease: a macrophage disorder

- Sources of GlcCer substrate:
  - Recycling of endogenous membrane
  - Lipoprotein endocytosis
  - Phagocytosis of senescent cells
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Gaucher cells driving visceral pathology

Pathological cascade

Is type 1 Gaucher disease only a disorder of macrophages?

Evidence in favor:

• BMT has been an effective treatment for type 1 GD patients
• Selective k.o. of GBA in blood cells offers a decent type 1 GD mouse model (Karlsson, Métry)
• BMT using as donor a type 1 GD patient caused classic type 1 GD in the recipient

However:

Did we really look carefully enough? Poor follow-up of BMT-treated patients;
Are we absolutely sure that GBA deficiency in other cells than macrophages does not cause some problems?

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Disease → storage cells → therapeutic correction

• Without Gaucher cells, no disease manifestation
• Therapy aims to remove/prevent Gaucher cells

Therapy
Therapies for type 1 Gaucher disease

- Correction of enzyme deficiency in macrophages:
  - ERT - enzyme replacement therapy
- Reduction of GlcCer and higher glycosphingolipids:
  - SRT - substrate reduction therapy
- Other (considered) interventions:
  - BMT, gene therapy, chaperone therapy

Enzyme replacement therapy:
variable individual responses to enzyme dose

Disease → storage cells → biomarkers

- Can we biochemically monitor in plasma the presence of Gaucher cells in tissues?

Plasma protein and lipid biomarkers of Gaucher cells
Protein biomarkers

- Numerous abnormalities in proteins have been observed in plasma of symptomatic Gaucher patients
- Only some of these abnormalities are directly linked to the presence of pathological Gaucher cells:
  - Chitotriosidase
  - CCL18/PARC

Chitotriosidase: a marker for Gaucher disease
Elevated serum chitotriosidase in symptomatic Gaucher patients

- Thousand-fold increased activity in serum of symptomatic patients
- Pitfall: one in every 20 individuals is deficient

Gaucher cells as source of chitotriosidase

- Evidence by:
  1. Histochemistry
  2. In situ hybridisation
  3. Correlation tissue [GlcCer] (Gaucher cells) with [chitotriosidase]

- Correlations of plasma chitotriosidase levels with platelet count, spleen and liver volumes
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Chitotriosidase, the human macrophage chitinase

Plasma chitotriosidase changes
upon enzyme therapy in two sisters (L444P homozygotes)

Use in clinical management of type 1 GD patients

- Monitoring of plasma chitotriosidase
  used in clinical management of type 1 Gaucher patients:
  - Confirmation of diagnosis
  - Decision making on initiation of therapy
  - Optimal individualised ERT dosing regimen
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Chitotriosidase

- Serial plasma chitotriosidase assessment is commonly used to assist clinical management of type 1 GD
- Plasma chitotriosidase does not reflect neurological complications in type 2/3 GD and poorly reflects (ongoing) skeletal disease

Chitotriosidase (2)

- Pitfalls with chitotriosidase measurement using conventional substrate:
  A. ‘Substrate inhibition’ at saturating substrate concentration
  B. Common G102S CHIT1 polymorphism affecting specific activity with commercial 4MU-chitotrioside substrate

Superior substrate for chitotriosidase assay

- More sensitive, reproducible and convenient assay
- Michaelis-Menten kinetics
  - use of saturating substrate:
    - Linear in time
    - Proportional to enzyme input
    - CHIT-G102 and CHIT-S102 show same kinetics

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Chitotriosidase deficiency

- Common occurrence of 24 BP duplication causing enzyme absence
- Carriers show half normal plasma chitotriosidase activity (gene dosis effect)

24 bp dupl. carrier frequency

<table>
<thead>
<tr>
<th>Region</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td>35%</td>
</tr>
<tr>
<td>Japan</td>
<td>38%</td>
</tr>
<tr>
<td>Indonesia</td>
<td>33%</td>
</tr>
<tr>
<td>Ashkenazim</td>
<td>37%</td>
</tr>
<tr>
<td>African Americans</td>
<td>19%</td>
</tr>
<tr>
<td>Central Africa</td>
<td>10%</td>
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</tbody>
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Need for alternative marker for Gaucher cells: PARC/CCL18

Chemokine CCL18/PARC

  - Increased expression of mRNA in Gaucher spleen
- Boot RG et al., Blood 2004; 103: 33-9
  - Increased concentrations in plasma of GD patients detected by ELISA
  - Source of CCL18 are also Gaucher cells (histochem, hybridisation)
  - Correlations with disease manifestations

Elevated CCL18 levels in Gaucher serum

- About 20-50 fold elevated
- Also produced by Gaucher cells
Analysis of protein composition of laser-dissected Gaucher cells using LC-MS³ proteomics

Example of newly discovered protein abnormality: Galectin-3 (LEG3)
- LC-MS³ analysis: Galectin-3 abundantly present in Gaucher cells
- Galectin-3 is also elevated in plasma of type 1 Gaucher patients

Markers of skeletal disease in Gaucher patients
- Conventional markers for osteoclasts and osteoblasts are not very helpful to assess skeletal disease in Gaucher patients
- Plasma chitotriosidase or CCL18 do not reflect skeletal disease well, since these markers originate from Gaucher cells in various body locations
MIP-1a and MIP-1b are elevated in GD plasma

MIP-1b or MIP-1a are not produced by Gaucher cells

Plasma MIP-1b and (persistent) skeletal disease


Relative corrections in plasma chitotriosidase and MIP's differ: distinct cellular sources of circulating chitotriosidase and MIP's

Lack of sufficient MIP-1b response to ERT, <85 pg/ml, seems predictive for ongoing skeletal disease

Larger series should confirm this
Potential lipid biomarkers

- Elevated plasma GlcCer and other lipids, as detected with HPLC and MS methods

Increased plasma GlcCer & GM3 in type 1 GD patients

<table>
<thead>
<tr>
<th>GlcSph (nM)</th>
<th>GlcCer (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.3</td>
</tr>
<tr>
<td>Gaucher</td>
<td>257.2</td>
</tr>
<tr>
<td>Sap C-/-</td>
<td>256.295</td>
</tr>
<tr>
<td>Increase</td>
<td>187 x</td>
</tr>
</tbody>
</table>

Increased plasma GlcSph in type 1 Gaucher patients

GlcSph: glucosylsphingosine
GlcCer: glucosylceramide

Mass spectrometric GlcSph quantification
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Plasma GlcSph correction by ERT: similarity to CCL18 and CHIT

Plasma GlcSph and disease manifestations

Glucosylsphingosine: a cytotoxic compound

- Reported to:
  - Cause hemolysis of red blood cells
  - Damage cholinergic neurons
  - Interfere with IGF-1 signalling (growth retardation)
  - Activate phospholipase A2, increasing arachidonic acid (inflammation)
  - Reduce AMP activated kinase, favoring energy consumption
  - Inhibit protein kinase C (osteoblastogenesis)
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Plasma GlcSph: a Gaucher biomarker

- Extent of GlcSph abnormality correlates with degree of primary deficiency:
  - Relationship with GBA genotype
- Extent of GlcSph abnormality correlates with a disease manifestation:
  - Relationship with hepatomegaly
- Extent of GlcSph abnormality correlates with markers of storage cells:
  - Correlation with chitotriosidase and CCL18
- Extent of GlcSph abnormality associated with worsening/correction disease
- Specificity: so far only observed in GBA and saposin C deficient patients
- Context:
  A. GlcSph substrate for primary deficient enzyme GBA
  B. GlcSph accumulating in (induced) storage cells
  C. GlcSph known as toxic substance

Skin pathophysiology
Crucial role glucosylceramide:ceramide in barrier function of stratum corneum

- Pathology in skin, brain and bone
- Metabolic abnormalities: insulin resistance
- Growth retardation
- Increased incidence of some cancers
Brain pathophysiology: different hypotheses

Causes of neurological complications?
- Pathological neuronal cytosolic Ca2+ increase?
- Cytotoxic glucosylsphingosine?
- Role for activated microglia?
- Problems with autophagy?

Bone disease:
The equilibrium between osteoblasts and osteoclasts
Bone modelling by ongoing synthesis (osteoblasts) and degradation (osteoclasts)

Osteoporosis in Gaucher disease

Excessive osteoclast activity  Deficient osteoblast activity

- Opponents for both possibilities, but can we exclude that local Gaucher cells are a detrimental third player?
  - i.e. is osteoporosis in Gaucher patients intrinsically comparable to that in normal individuals?
- Gaucher cells, or surrounding macrophages, do secrete potential relevant factors: cathepsin K, TRAP, MIP1-alpha, MIP1-beta

Our lack of understanding the underlying biology of skeletal disease in Gaucher patients hampers a rational intervention for the problem

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**Metabolic abnormalities**

- Increased hepatic glucose production
- Insulin resistance
- Low adiponectin levels

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**Impaired insulin sensitivity due to excessive ganglioside in lipid rafts**

- Elevated ganglioside GM3 in Gaucher patients

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**Growth retardation**

- High incidence of growth retardation in type 1 Gaucher disease patients
- Pediatric type 1 Gaucher disease is associated with low levels of IGF-1
- ERT results in a significant increase of IGF-1, nevertheless some of the patients continue to present growth failure
- IGF-1 receptor is located in lipid rafts; is there an IGF-1 insensitivity due to excessive gangliosides, comparable to the insulin resistance?
- If so, compounds like AMP-DNM or eliglustat, that inhibit ganglioside biosynthesis, might have a beneficial effect in patients not responding to ERT

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### Increased risk for cancer

**Increased incidence of cancer in adult Gaucher disease in Western Europe**


Blood Cells, Molecules, and Diseases 36 (2002) 33–38

- Indications for increased incidences of multiple myeloma and hepatocellular carcinoma in absence of preexisting cirrhosis
- Since we do not understand the underlying biology, it is impossible to predict whether ERT will reduce this risk

### Increased risk for parkinsonism → research

- Gaucher carrierness imposes an increased risk for parkinsonism
- The underlying mechanism has not yet been elucidated
- This intriguing finding will undoubtedly increase the fundamental interest in function of glucocerebrosidase and the biology of Gaucher disease

### Immune system: the missing link?

- To which extent does the immune system act as missing link to the various pathophysiological processes in Gaucher patients?
- Considerations:
  - Abnormalities in macrophages
  - Secondary changes in other cell types, e.g. caused by increased gangliosides
  - Circulating factors influencing immune cells, e.g. glucosylphosphoinosine
Acknowledgements

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