HOW DOES ALPHA GALACTOSIDASE DEFICIENCY LEAD TO CELL DAMAGE & HOW CAN IT BE REPAIRED?

Alberto Ortiz
IIS-Fundacion Jimenez Diaz and REDINREN
Madrid, Spain
Disclosure of Interests

- Genzyme, a Sanofi company: consultancy, honoraria
- Shire, honoraria
A rational therapeutic approach requires a good grasp of the pathogenesis

The problems in Fabry nephropathy:

a) No satisfactory animal model
b) Rare disease
c) Very long natural history

Compromised ability to
• Generate hypothesis
• Test hypothesis in adequately powered RCT
Pathogenesis of Fabry nephropathy: 3 sequential problems, each requiring a specific therapeutic approach

**Problem 1:** glycolipid accumulation
- Enzymatic defect
- Glycolipid accumulation

**Problem 2:** tissue injury
- Black box

**Problem 3:** organ dysfunction

**Clinical:**
- Albuminuria → Proteinuria
- Decreased eGFR → RRT

**Subclinical:**
- Podocyte injury: foot process effacement
- Podocyte loss: glomerulosclerosis

Fabry nephropathy is a **progressive proteinuric chronic kidney disease** of **metabolic origin**.

RRT Mean age 40 years

Natural history: **40 years**: implications for clinical trials assessing **hard end-points**

Ortiz A et al. NDT 2008
What basic concepts did we learn from chronic kidney disease?

Current KDIGO CGA classification of CKD

**Albuminuria** (not exactly proteinuria)

GFR

Albuminuria and CKD progression

Unless proven otherwise, these general concepts apply to Fabry nephropathy

**Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012**

<table>
<thead>
<tr>
<th>Persistent albuminuria categories</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description and range</td>
<td>Normal to mildly increased</td>
<td>Moderately increased</td>
<td>Severely increased</td>
</tr>
<tr>
<td>GFR categories (ml/min/1.73 m²)</td>
<td>&lt;30 mg/g &lt;3 mg/mmol</td>
<td>30-300 mg/g 3-30 mg/mmol</td>
<td>&gt;300 mg/g &gt;30 mg/mmol</td>
</tr>
<tr>
<td>G1 Normal or high</td>
<td>≥90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2 Mildly decreased</td>
<td>60-89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3a Mildly to moderately decreased</td>
<td>45-69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3b Moderately to severely decreased</td>
<td>30-44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G4 Severely decreased</td>
<td>15-29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G5 Kidney failure</td>
<td>&lt;15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

no other markers of kidney disease, no CKD; Yellow: moderately increased risk; Orange: high risk; Red,
What basic concepts did we learn from chronic kidney disease?

Current KDIGO CGA classification of CKD

**Albuminuria** (not exactly proteinuria)

<table>
<thead>
<tr>
<th>GFR</th>
<th>ACR &lt;10</th>
<th>ACR 10–29</th>
<th>ACR 30–299</th>
<th>ACR ≥300</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR &gt;105</td>
<td>1.1</td>
<td>1.5</td>
<td>2.2</td>
<td>5.0</td>
</tr>
<tr>
<td>eGFR 90–105</td>
<td>Ref</td>
<td>1.4</td>
<td>1.5</td>
<td>3.1</td>
</tr>
<tr>
<td>eGFR 75–90</td>
<td>1.0</td>
<td>1.3</td>
<td>1.7</td>
<td>2.3</td>
</tr>
<tr>
<td>eGFR 60–75</td>
<td>1.0</td>
<td>1.4</td>
<td>1.8</td>
<td>2.7</td>
</tr>
<tr>
<td>eGFR 45–60</td>
<td>1.3</td>
<td>1.7</td>
<td>2.2</td>
<td>3.6</td>
</tr>
<tr>
<td>eGFR 30–45</td>
<td>1.9</td>
<td>2.3</td>
<td>3.3</td>
<td>4.9</td>
</tr>
<tr>
<td>eGFR 15–30</td>
<td>5.3</td>
<td>3.6</td>
<td>4.7</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Albuminuria and survival
- All cause
- CV

Unless proven otherwise, these general concepts apply to Fabry nephropathy.
Pathogenesis of Fabry nephropathy: 3 sequential problems, each requiring a specific therapeutic approach

A Fabry patient with UACR 40 mg/day with normal GFR, That is, having problem 1 + problem 2
May have cell and tissue injury resulting from both
a) Glycolipid accumulation
b) CKD itself
And requires therapeutic intervention on both problems

Problem 1: glycolipid accumulation
Problem 2: tissue injury
Problem 3: organ dysfunction
Pathogenesis of Fabry nephropathy: 3 sequential problems, each requiring a specific therapeutic approach

What are the expectations for ERT alone when started at this stage?

- Problem 1: glycolipid accumulation
- Problem 2: tissue injury
- Problem 3: organ dysfunction
Pathogenesis of Fabry nephropathy: 3 sequential problems, each requiring a specific therapeutic approach

What conclusions can be drawn from studies of ERT alone enrolling patients at different stages of the disease? And even with different severity of the underlying mutation?

- **Problem 1:** glycolipid accumulation
- **Problem 2:** tissue injury
- **Problem 3:** organ dysfunction

What CKD guidelines apply to Fabry patients?

- In adults aged $\geq 50$ years with CKD, we recommend treatment with a statin (1B).

- In adults aged 18–49 years with CKD, we suggest statin treatment in people with DM (2A).

Fabry not mentioned..... Should it be considered “general” CKD or “DM-equivalent” CKD?

SHARP key RCT
Randomized nearly 10,000 patients, 5 year follow-up
Events placebo 13.4%, statins 11.3% (17% decrease)

These recommendations do not refer to patients treated with chronic dialysis or kidney transplantation.
What basic concepts did we learn from diabetic nephropathy?

1. May lead to ESRD despite treating the metabolic defect

2. Nephroprotection slows the loss of GFR

Reference: Adapted from Friedman, 1999
What basic concepts did we learn from diabetic nephropathy?

Irbesartan Diabetic Nephropathy Trial (IDNT): 1715 T2DM hypertensive patients with DN (mean sCr 1.7 mg/dL)

3. But RCTs showing the beneficial effects of RAS blockade enrolled thousands of patients!

At 2.6 years, irbesartan was associated with a 20% lower risk of the primary end point (doubling of sCr, ESRD or death than placebo.)
Therapy for Fabry nephropathy: 3 sequential approaches

Problem 1: clear glycolipids

Decrease synthesis, increase clearance ERT

Problem 2: provide add-on tissue protection (nephroprotection)

Borrow concepts from diabetic nephropathy
Unravel the black box in Fabry nephropathy

Problem 3: replace organ function
Therapy for Fabry nephropathy: 3 sequential approaches

**Problem 1:** glycolipid accumulation

**Q1.** What are the key cell targets in Fabry nephropathy?

**Q2:** What ERT dose is required to clear these key targets?

Different cell types may require different ERT doses

**Q3:** How long it takes to clear these key targets?

Clearance may take longer in some cell types

What is the role of albuminuria in Fabry nephropathy?

- Albuminuria (proteinuria ≈ albuminuria x 2) is a major risk factor for progression of CKD in Fabry disease

Warnock NDT 2012, Wanner CJASN 2010
What is the meaning of albuminuria in Fabry nephropathy?

Happy, healthy podocyte

Not-so-happy Fabry podocyte full of deposits
Albuminuria usually indicates podocyte injury.
Podocyte inclusions and albuminuria in Fabry

Podocyte inclusions vs proteinuria

No relationship between \(v(\text{Inc}/\text{Endo})\) and proteinuria

Age 2-19 years

Relationship between age and podocyte (\(Vv(\text{Inc/PC})\)), and endothelial cell (\(Vv(\text{Inc/Endo})\)) GL-3 fractional volume of inclusions per cytoplasm

Segmental foot process effacement in all glomeruli

Tondel et al. AKD 2009. Najafian et al. KI 2010
The podocyte depletion hypothesis

Glomerular injury

No podocyte depletion

No glomerulosclerosis

No progression to ESKD
The podocyte depletion hypothesis

Glomerular injury

- No podocyte depletion
  - No glomerulosclerosis
  - No progression to ESKD

- Podocyte loss (necrosis, apoptosis, detachment)

- Podocyte enlargement

- Podocyte phenotype switch
  - Effective podocyte depletion
    - Glomerulosclerosis
    - Progression to ESKD

Wiggins 2007
Podocyte loss is known to result in glomerulosclerosis (glomerular fibrosis)
The two problems with podocyte clearance in Fabry nephropathy

1. They are **outside** the vessels

2. They are very **long-lived** cells, with little if any turnover
ERT response of podocytes and albuminuria
renal biopsy before and after 5 years of ERT in young patients on agalsidase alpha or beta

The higher the cumulative dose, the better the podocyte clearance

The better the podocyte clearance, the more reduction in albuminuria

highly significant correlation between podocyte clearance and cumulative agalsidase dose (r=0.804; P=0.002)
While it is relatively easy to clear endothelial cells, it may take years and high doses of ERT to clear podocytes.

Clearance of podocytes was associated with improved albuminuria.

Since albuminuria is a marker for disease progression, podocytes appear to be key target cells in the kidney.

Let's try to fill the black box for podocytes!

Glycolipid accumulation → Black box → Tissue injury
The molecule: Lyso-Gb3

Elevated globotriaosylsphingosine is a hallmark of Fabry disease

Johannes M. Aerts*, Johanna E. Groener*, Sijmen Kuiper*, Wilma E. Donker-Koopman*

VSMC proliferation

Fabry disease circulating lyso-Gb3 concentration range

PNAS 2008
The cell type: cultured human podocytes

The results: At concentrations found in the circulation of Fabry patients, lyso-Gb3 reproduces some of the effects of high glucose in podocytes

- Secretion of TGF-β1 leading to autocrine stimulation of extracellular matrix secretion
- Activation of Notch1 leading to inflammatory and profibrotic responses

Vitamin D receptor activators downregulate fibrosis mediators induced by lyso-Gb3 in cultured podocytes

100 nM Lyso-Gb3

Control

Fibronectin

Type-IV collagen

α-tubulin

Sanchez-Niño et al, NDT 2011
Vitamin D receptor activators downregulate fibrosis mediators induced by lyso-Gb3 in cultured podocytes

100 nM Lyso-Gb3

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Paricalcitol</th>
<th>Calcitriol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibronectin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type-IV collagen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-tubulin</td>
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Sanchez-Niño et al, NDT 2011
May this help Fabry patients?
Paricalcitol antiproteinuric in Fabry nephropathy

25-OH-Vitamin D (ng/mL) 22.17 ± 11.2
RAS targeting: 0.7/patient
Paricalcitol 1 μg/d

Pisani et al. NDT 2014
Evolving paradigm of Fabry disease

The clean-up the pipes paradigm

Problem

Glycolipids obstruct pipes (arteries)

Solution

ERT cleans pipes

Result

Problem solved

The leaking pipe paradigm

Problem

Glycolipids lead to obstructed and leaking pipes (albuminuria)

Solution

ERT cleans pipes

Result

Pipes clean but still broken!!!
Take home message

• The issue in Fabry disease is **not just glycolipid accumulation**... At least for the majority of patients currently on ERT throughout the world (mean age at start of ERT 40 years in Fabry Registry)

• While clinical trials showed that ERT efficiently clears endothelial cells, clearance of additional cell types, such as podocytes may be required for organ protection... And this may require higher ERT doses

• Different forms of tissue injury may require different add-on therapeutic approaches

• The fact that **lyso-Gb3**, which is usually not normalized by ERT, elicits adverse cellular responses in podocytes suggests that normalization of lyso-Gb3 may be a **therapeutic target** and that as long as that is not achieved, patients may require add-on tissue protective therapy
The bathtub paradigm of glycolipid accumulation
Lyso-Gb3 and other cell types
• lysoFb3
• Complete replacement: insulin vs pancreas tx
• Ckd: dialysis and uremia
• Open the drainage
  – Replace the enzyme
  – “repair” the enzyme
• Close the tap