CASE 6
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Joint Session Nephropathology / Electron Microscopy:
Electron microscopy differential diagnosis of glomerular deposits slide seminar, 9.9.2019
CASE HISTORY

• 13-Year-old girl
• **Complaints:** Abdominal pain, diarrhea, vomiting
• **Family history:** History of chronic kidney disease and long term haemodialysis in her mother.
• **Physical examination:**
  - Bilateral corneal abnormalities
  - Body weight and height were below 3rd percentile according to her age group
• **Blood pressure:** 120 /80 mmHg
• **Lab:**
  - Hb: 10.8 g/dL
  - MCV: 75fl
  - Ferritin:4,9 ng/ml
  - Total protein: 5,3 gr/dl
  - Albumin:2,7 gr/dl
  - AST: 60 IU/l [N:0-50 IU/l]
  - ALT: 58 IU/l [N:0-40 IU/l]
  - Kidney function tests were within normal limits

• **Urine protein (24h):** 2200 mg/day.
• **Urine microalbumin (24 h):** 1290 mg/day [N: 0-30 mg/day].
• No hematuria.
Kidney biopsy:

Cortex and medulla.
31 glomeruli were available for evaluation.
2 glomeruli were globally sclerosed.
ENLARGED PODOCYTES WITH FOAMY CYTOPLASM
CYTOPLASMIC BALLONING IN PODOCYTES

PAS (-)  Trichrome (-)  Methanamine silver (-)  Colloidal iron (-)
Immunofluorescence

- IgG: negative
- IgM: negative
- IgA: negative
- C3: negative
- C1q: negative
- Kappa: negative
- Lambda: negative

Semi-thin sections
Electron microscopy

Myelin – like inclusions in podocyte cytoplasms characterized by lamellation of dark and pale layers.

Focal foot process effacement
DIAGNOSIS

• FABRY DISEASE (?)
However!

- Fabry disease is a genetic disease with X-linked recessive transmission.
- The disease primarily affects males.

Facial features in two brothers with AFD and their unaffected sister, showing thickening of the lips and nasolabial folds in the affected males.

FABRY DISEASE- ETIOLOGY AND PATHOGENESIS

• A Mutation in α-galactosidase A gene (GLA) on long arm of X chromosome (Xq22.1) causes deficiency or decreased activity of α-galactosidase A enzyme.

[α-Galactosidase A enzyme cleaves glycosphingolipids and blood group B substance.]

• Glycosphingolipids (mostly in the form of GL3) accumulates in endothelial and smooth muscle cells, podocytes, distal tubular cells, collecting ducts, sweat glands, cardiac myocytes, macrophages, dorsal root ganglia, perineurium, cornea stroma, and other cells.

• Endothelial involvement leads to microvascular obstruction
  - Pro-inflammatory and procoagulant effects
FABRY DISEASE - PRESENTATION: Varies according to the severity of enzyme deficiency

• Males (classical presentation);
  ✓ Acroparesthesia
  ✓ Hypohidrosis
  ✓ Angiokeratomas
  ✓ Corneal dystrophy
  ✓ Progressive renal, cardiac and cerebrovascular disease

• Female heterozygotes show variable manifestations

• Renal involvement
  ✓ Urinary concentration defects
  ✓ Proteinuria
  ✓ CRF
  ✓ ESRF
FABRY DISEASE- MACROSCOPY


BIOPSY APPEARANCE

• Diagnosis may be suspected on a renal biopsy under a stereomicroscope.
• Glomeruli are paler and whiter compared to non-Fabry cases.
FABRY DISEASE-MICROSCOPY

- **Glomeruli**
  
  - Swollen podocytes with vacuolated cytoplasm (Podocyte cytoplasm appear expanded, foamy, pale, and lacy due to lipid deposits dissolved in processing)

  - Mesangial expansion
  - Mesangial hypercellularity
  - Segmental glomerular sclerosis
  - Obsolescent glomeruli
  - Endothelial cells appear normal in routine sections although they also contain lipid
FABRY DISEASE - MICROSCOPY

- **Tubules and interstitium**
  - Distal tubules have abundant lipid deposits, which make them pale and vacuolated in routine sections
  - Tubular atrophy
  - Interstitial inflammation
  - Interstitial fibrosis
  - Lipid containing interstitial fibroblasts
  - Granulomatous interstitial nephritis (rare)
• Vessels
  ✓ Endothelium of all vessels accumulate lipid
    - Not visible in capillary endothelium in routine sections
      - Sometimes appreciated in arteries as lacy, vacuolated cytoplasm
    - Intimal fibrosis can be prominent
  ✓ Lipid in arterial smooth muscle makes them appear pale in routine sections
  ✓ Arteriolar hyalinosis may present as peripheral nodular replacement of smooth muscle, indistinguishable from calcineurin inhibitor toxicity
FABRY DISEASE - IMMUNOFLUORESCENCE

- Generally negative, except IgM and C3 in segmental scars
- Incidental IgA deposition observed in renal biopsies


Fabry-like laminated myelin body associated with IgA nephropathy.
Yoshida A, Morozumi K, Takeda A, Koyama K, Oikawa T.


[Fabry nephropathy in a female with superposed IgA glomerulonephritis].
[Article in Italian]
Pisani A, Sessa A, Sabbatini M, Andreucci MV, Fusco C, Balletta M, Cianciaruso B.


The coincidence of IgA nephropathy and Fabry disease.


Clinical and pathological characteristics of Fabry disease combined with IgA nephropathy in Chinese patients.
FABRY DISEASE-
ELECTRON MICROSCOPY

HISTOLOGIC FEATURES ON PLASTIC EMBEDDED SECTIONS

✓ Lipid granules most conspicuous as dense granules filling podocytes and distal tubular epithelium
✓ High power (100Xoil) useful to detect lipid droplets in endothelium
  - Endothelial lipid varies from rare granules to large aggregates that bulge into lumen
✓ Podocyte lipid decreased in areas of segmental sclerosis, adhesion, or collapse
FABRY DISEASE - ELECTRON MICROSCOPY

Most typical feature

DEPOSITION OF LYSOSOMAL, LAMINATED MYELIN-LIKE INCLUSION BODIES

- Podocytes
  - Endothelial cells
  - Mesangial cells
- Arteriolar smooth muscle and endothelial cells
- PTC endothelial cells
- Tubular epithelium
Fischer EG, Moore MJ, Lager DJ. Membrane-like, large subendothelial deposits arranged in geographic layers, resembling contour lines on a map.
ACCORDINGLY!

- Our patient has;
  - A history of chronic kidney disease and long term haemodialysis in her mother.
  - Bilateral corneal abnormalities
  - Abdominal pain, diarrhea and vomiting
  - Anemia
  - Proteinuria

CONSISTENT FINDINGS WITH FABRY DISEASE
Do the presence of myelinoid bodies always confirm the diagnosis of Fabry disease?
Chloroquine-induced lipidosis


How can we make a distinction from Fabry Disease?

- Curvilinear bodies
- KP-1 (+) cells with finely vacuolated cytoplasm filling the glomerular capillary lumina
- Mesangial cell with small dense round granular inclusions within irregular clear vacuoles (lysosomes) (Human Pathology 2003;34(3):285-289)

Remember!
- Neuronal ceroid lipofuscinosis

Cellular debris in the swollen endothelial cell (Modern Pathology 2005;18:733-738)
Do the presence of myelinoid bodies always confirm the diagnosis of Fabry disease?

- Chloroquine treatment
- Amiadarone treatment
- Aminoglycoside treatment
- Exposure to silica

should be sought after even if the patient has features thought to be diagnostic for Fabry disease.
Table 1: Medical diseases affecting the kidney parenchyma, with histological vacuolization of cells [8–10]

<table>
<thead>
<tr>
<th>Disease</th>
<th>Podocytes</th>
<th>Mesangial cells</th>
<th>Glomerular capillary endothelium</th>
<th>Proximal tubular cells</th>
<th>Distal tubular cells</th>
<th>Interstitial cells</th>
<th>Vascular smooth muscle cells</th>
<th>Vascular endothelium</th>
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<tr>
<td>Aspartylglycosaminuria</td>
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<td>Fabry disease</td>
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<td>Fucosidosis</td>
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<td>Gaucher disease</td>
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<td>Glycogen storage disease type I</td>
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<td>GM1 gangliosidosis</td>
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<td>Hurler’s disease</td>
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<td>L-cell disease</td>
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<td>LCAT deficiency</td>
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<td>Lipoprotein glomerulopathy</td>
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<td>Mannosidosis</td>
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<td>Metachromatic leukodystrophy</td>
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<td>Neuronal ceroid lipofuscinosis</td>
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<td>Niemann–Pick disease</td>
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<td>Sandhoff disease</td>
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<td>Type III hyperlipoproteinemia</td>
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<td>Alport disease</td>
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<td>Nephrotic range proteinuria</td>
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<td>Minimal change disease</td>
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*GM1 monosialotetrahexosylganglioside, LCAT lecithin/cholesterol acyltransferase, + vacuolization present*
STORAGE DISEASES PRIMARILY AFFECTING PODOCYTES

**FABRY DISEASE**
- Storage material extracted by solvents during preparation cannot be stained
- Podocyte cytoplasm stains dark blues with toluidine blue in plastic embedded sections for EM
- Ultrastructural examination reveals vacuoles filled with whirled lamellar myeloid bodies

**I-CELL DISEASE and GM1 GANGLIOSIDOSIS**
- No clinical evidence of renal disease
- EM reveals vacuoles that contain small amount of membranous or lamellar material

**NEPHROSIALIDOSIS**
- Storage material causes vacuolated podocytes, parietal epithelium, endothelium, tubules, and interstitial cells
- Storage material is Hale’s colloidal iron positive
DIFFERENTIAL DIAGNOSIS

• I-CELL DISEASE (Mucolipidosis type II)

Podocyte lipid not removed by routine processing

Podocyte deposits positive for colloidal iron

Empty podocyte vacuoles which contain strands of membranous to lamellar material of unknown composition

Strands of membranous to lamellar material more irregular in structure and distribution in I-cell disease
DIFFERENTIAL DIAGNOSIS

- Nephrosialidosis
  - Impressive podocyte deposits identical to Fabry disease
  - Podocyte deposits positive for colloidal iron (identical to I-cell disease)
  - **EM:**
    - Membrane bound vacuoles in podocytes, endothelium, mesangial cells and parietal epithelium
      - Round
      - Almost equal in size
      - Contain electron-dense bodies lining the inner surface

DIFFERENTIAL DIAGNOSIS – ADVANCED CASES

FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS)
- Fabry patients commonly develop FSGS due to either direct lipid effect on podocyte or decreased nephron mass
- Lipid may be inconspicuous at this stage and can be overlooked in light microscopy
- EM is the best method to detect lipid (Readily detected in podocytes)

ARTERIOSCLEROSIS
- Vascular disease in Fabry disease similar to usual arteriosclerosis in hypertension
- If lipid in podocytes (or other sites) not appreciated, diagnosis may be missed
OUR PATIENT’S CLINICAL HISTORY LEARNED AFTER REPORTING THE KIDNEY BIOPSY

• **Clinical history:** She had been suffering from anhidrosis and acroparesthesia for about 1 year.

• **The patient’s family history was noteworthy:**
  - Her mother was a known Fabry patient with an end-stage kidney disease.
  - Her 8-year old sister also had acroparesthesia and anhidrosis.

• **The clinical suspicion of Fabry disease had already been confirmed** by markedly reduced plasma and leucocyte alpha-gal A levels of 4.7 nmol/h/ml and 5.2 nmol/mg/h, respectively.

• **Genetic analysis:** Heterozygote mutation

• The patient had been following closely for any signs of renal involvement on a regular basis.

• 4 Years after, with the appearance of proteinuria, she underwent a renal biopsy.
OUR CASE – CLINICAL FOLLOW-UP

✓ Immediately after confirmation of diagnosis by renal biopsy, enzyme replacement therapy (ERT) was started and she was clinically followed for 14 years with a period of partial remission.

<table>
<thead>
<tr>
<th>Year</th>
<th>Urinary Protein (mg/day)</th>
<th>Urinary albumin (mg/day)</th>
<th>2001</th>
<th>2001</th>
<th>2002</th>
<th>2008</th>
<th>2009</th>
<th>2015</th>
<th>2015</th>
<th>PREGNANCY</th>
<th>2015</th>
<th>2017</th>
<th>2019</th>
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<tr>
<td>2001</td>
<td>2200</td>
<td>1290</td>
<td>1290</td>
<td>1290</td>
<td>566</td>
<td>600</td>
<td>604</td>
<td>1003</td>
<td>1129</td>
<td>PREGNANCY</td>
<td>1334</td>
<td>1100</td>
<td>1450</td>
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<tr>
<td>2009</td>
<td>10</td>
<td>8</td>
<td>45</td>
<td>99</td>
<td>12</td>
<td>8</td>
<td>12</td>
<td>45</td>
<td>99</td>
<td>ERT WAS INTERRUPTED</td>
<td>1299</td>
<td>987</td>
<td>432</td>
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<tr>
<td>2015</td>
<td>1003</td>
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**Key Events:**
- BIOPSY
- ERT INITIATION
- RESUMPTION OF ERT AT 22th WEEK OF GESTATION
- PREGNANCY
- ERT WAS INTERRUPTED

Kidney functions are normal

June 2019
# Systemic evaluation

<table>
<thead>
<tr>
<th></th>
<th>2001</th>
<th>2019</th>
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<tr>
<td><strong>Ophtalmological</strong></td>
<td>Cornea verticiliata</td>
<td>Cornea verticiliata</td>
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<tr>
<td><strong>ENT</strong></td>
<td>Normal</td>
<td><strong>Tinnitus (Intermittent)</strong></td>
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<tr>
<td><strong>Dermatological</strong></td>
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<td>Normal</td>
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<tr>
<td><strong>Gastrointestinal system</strong></td>
<td>Abdominal pain, vomiting, diarrhea, abdominal distension</td>
<td>Abdominal pain, vomiting, diarrhea, abdominal distension (Intermittent)</td>
</tr>
<tr>
<td><strong>Cardiological</strong></td>
<td>Normal</td>
<td>Normal</td>
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<tr>
<td><strong>Cranial MRI</strong></td>
<td>Normal</td>
<td><strong>Several ischemic gliotic foci in the left frontal white matter</strong></td>
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</table>
Take home messages

• Light microscopic features of Fabry disease is not specific to Fabry disease.

• Ultrastructural examination is necessary for the correct diagnosis. However, an absence of complete history or unawareness of a possible drug therapy may result in misdiagnosis of Fabry disease.

• Fabry disease may result in significant renal involvement during childhood period even in female patients.

• Early initiation of ERT may stabilize renal function and delay renal replacement therapy.