THE PODOCYTE AND FABRY DISEASE

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2015
The spectrum of podocyte diseases

- Congenital nephrotic syndrome of the Finnish type
- Diffuse mesangial sclerosis
- DMS
- CNSF
- Alport +
- MCD
- FSGS
- Collapsing GN
- Imm/Infl GN
- HTN
- Diab GN
- Aging

Genetic

Environmental

Loss of some podocytes (20%) is associated with mesangial expansion possibly as an attempt to reduce the filtration surface area.

Loss of podocytes beyond a critical level results in a fibrotic glomerular response in that part of the glomerulus.

Loss of podocytes resulting in appearance of bare areas of filtration surface results in adhesion of the bare surface to Bowman’s capsule (synechia).

Loss of podocytes beyond a critical level results in widespread scarring of that glomerulus.
### Table 1 | Sphingolipid accumulation in glomerular diseases of genetic and non-genetic origin.

<table>
<thead>
<tr>
<th>Disease</th>
<th>OMIM</th>
<th>Mutated gene</th>
<th>Chromosomal location</th>
<th>Sphingolipid accumulating</th>
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</thead>
<tbody>
<tr>
<td><strong>Sphingolipid accumulation in glomerular disease of genetic origin</strong></td>
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<tr>
<td>Gaucher</td>
<td>230800</td>
<td>Acid beta-glucosidase 1 (GBA1)</td>
<td>1q22</td>
<td>GlcCer</td>
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<td>Tay–Sachs</td>
<td>272800</td>
<td>Hexoseaminidase A (HEXA)</td>
<td>15q23</td>
<td>GM2</td>
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<td>Sandhoff</td>
<td>268800</td>
<td>Hexoseaminidase B (HEXB)</td>
<td>5q13</td>
<td>GM2</td>
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<td>Fabry</td>
<td>301500</td>
<td>Alpha-galactosidase A (GLA)</td>
<td>Xq22</td>
<td>Gb3, Lyso-Gb3</td>
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<td>Hereditary inclusion body myopathy 2</td>
<td>600737</td>
<td>UDP-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE)</td>
<td>9p13</td>
<td>Hyposialylation of glycoproteins such as podocalyxin?</td>
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<tr>
<td>Niemann–Pick</td>
<td>257220</td>
<td><em>NPC1 NPC2 SMPD1</em></td>
<td>18q11 14q24 11p15</td>
<td>Sphingomyelin</td>
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<td>Nephrotic syndrome of the Finnish type</td>
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<td>NPHS1</td>
<td>19q13</td>
<td>O-acetyl-GD3</td>
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<td><strong>Sphingolipid accumulation in glomerular disease of non-genetic origin</strong></td>
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<tr>
<td>Diabetic kidney disease</td>
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<td>GlcCer, GM3, S1P, sphingosine?</td>
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<td>Puromycin aminonucleoside (PAN)-induced nephropathy</td>
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<td>GD3, O-acetyl-GD3</td>
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<td>HIV-associated nephropathy (HIVAN)</td>
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<td>Gb3</td>
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<td>Focal segmental glomerulosclerosis (FSGS)</td>
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<td>Sphingomyelin</td>
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<td>Acute ischemia reperfusion injury</td>
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<td></td>
<td>Ceramide</td>
</tr>
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<td>Pathway</td>
<td>Morphology and size</td>
<td>Coat</td>
<td>Small GTPase</td>
<td>Cargo</td>
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<td>Clathrin-mediated</td>
<td>Vesicular</td>
<td>Clathrin</td>
<td>Rab5</td>
<td>RTKs, GPCR, TGF-βR, Notch, Tfr, LDLR, β-arrestin, Wnt/β-catenin</td>
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<tr>
<td>Caveolae-mediated</td>
<td>Flask-shaped</td>
<td>Caveolin 1 and 2</td>
<td>Unclear</td>
<td>GPI-APs, TGF-βR, CTxB, viruses, folic acid, IGF-1R, Wnt/β-catenin</td>
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<td>CLIC/GEEC</td>
<td>Tubular</td>
<td>None</td>
<td>Cdc42, Arf1</td>
<td>GPI-APs, glycosphingolipids, cholera toxin</td>
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<td>Arf6-mediated</td>
<td>Tubular</td>
<td>None</td>
<td>Arf6</td>
<td>β-arrestins, MHC I-II</td>
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<td>Flotillin-mediated</td>
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<td>Flotillin 1 and 2</td>
<td>None</td>
<td>CTxB, GPI-AP, proteoglycans</td>
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<td>IL-2R&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Vesicular</td>
<td>None</td>
<td>RhoA, Rac1</td>
<td>IL-2Rβ, yc cytokine receptor</td>
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<td>Macropinocytosis</td>
<td>Ruffled</td>
<td>None</td>
<td>Rac1, Cdc42, Arf6, Rab5</td>
<td>Fluid, RTKs, bacteria</td>
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<tr>
<td>Phagocytosis</td>
<td>Cargo shaped</td>
<td>None</td>
<td>Rac1, RhoA, Cdc42</td>
<td>Nutrients, pathogens, dead cells, and cellular debris</td>
</tr>
</tbody>
</table>
In Fabry disease, reduced activity of the lysosomal enzyme α-galactosidase A leads to lysosomal accumulation of Gb3, resulting in characteristic inclusions called zebra bodies in various organs and cell types.

Another substrate of α-galactosidase A, globotriaosylsphingosine (known as lysoglobotriaosylceramide-LysoGb3) is an active metabolite that acts as a profibrotic agent in cultured human podocytes.

Analysis of kidney biopsy samples from 14 patients (median age 12 years) with Fabry disease demonstrated age-dependent accumulation of its substrate Gb3 in podocytes.

Enzyme replacement therapy with recombinant α-galactosidase A in patients with Fabry disease successfully cleared glycolipid accumulation from the kidney and vasculature.
Gb₃ accumulates in lysosomes

Lysosomal function reduced

Secondary effects on cellular function – autophagy, reduced mitochondrial energy production and apoptosis

Proteinuria

Vascular disease

Renal failure

Cellular proliferation

Accumulation of Gb₃ and lyso-Gb₃

Progressive intracellular accumulation of Gb₃ leads to cellular changes and histological damage

Rupture of the lysosome and damage due to local inflammation

Deposition of Gb₃ can promote vascular smooth muscle cell proliferation and the release of mediators involved in other nephropathies

Addition of Gb₃ to cultured endothelial cells has been found to increase oxidative stress and upregulation of the expression of cell adhesion molecules

Vasculopathy: local up-regulation of the RAS
The suggested mechanisms of renal injury in Fabry disease include vascular compromise secondary to deposition of GL3 within the arterial wall, which should be considered as the first hit, with a concomitant decrease in nitric oxide synthesis and a tendency to microthrombotic events, podocyte injury and detachment with secondary glomerulosclerosis, and tubular atrophy and interstitial fibrosis.

However, the mechanisms leading to podocyte damage in Fabry disease have not been deeply studied yet.

As mice differ from humans in their glomerular lipid metabolism this question cannot be addressed in the Fabry mouse model.

The accumulation of glycosphingolipids in podocytes could have various implications.

Lipid-protein interactions in podocytes play an important role in intracellular signal transduction of podocytes.

Besides the established role of slit diaphragm signaling for podocyte cell survival and polarity, recent data show the significance of **AUTOPHAGY** associated signaling in the development of glomerulopathies.
Accumulation of Gb₃ is accompanied by an increase in autophagosomes, suggesting that deregulated autophagy pathways have some involvement in the pathogenesis of glomerular damage in Fabry disease.
Autophagy enables the cell to have access to nutrients in situations of stress or starvation. Intracellular material is degraded in a lysosome dependent mechanism, and autophagy serves as an intracellular recycling system.

**AUTOPHAGY**

An isolation membrane engulfs intracellular targets to become an autophagosome containing the LC3-II isoform of the essential autophagy protein LC3. The autophagosome then fuses with a lysosome to form a so called autophagolysosome, which contains damaged and dysfunctional organelles like mitochondria.
a Macroautophagy

Expansion → Phagophore/isolation membrane → Completion → Autophagosome → Fusion → Lysosome/vacuole → Degradation

b Chaperone-mediated autophagy

Translocation → Sequestration

LAMP-2A, Hsp70 chaperone, Protein, KFERQ motif, Metabolite transporter
Macroautophagy is characterized by the sequestration of structures targeted for destruction into double-membrane vesicles called autophagosomes. Complete autophagosomes first fuse with endosomes before finally exposing their content to the hydrolytic interior of lysosomes. The resulting metabolites are transported into the cytoplasm and used either for the synthesis of new macromolecules or as a source of energy.
As mice differ from humans in their glomerular lipid metabolism this question cannot be addressed in the Fabry mouse model
Interestingly, these changes were accompanied by an increase in autophagosomes as indicated by an increased abundance of LC3-II and a loss of mTOR kinase activity, a negative regulator of autophagy.

Dysregulated autophagy in α-galactosidase A-deficient podocytes may be the result of deficient mTOR kinase activity.
mTOR negatively regulates the formation of autophagy vesicles and promotes the recovery of autophagosomes (AV) and lysosomes (Lys) from autophagolysosomes (ALV) in podocytes (continuous lines).

In Fabry disease a-Gal A dysfunction leads to an accumulation of Gb3 in lysosomes, an increase in autophagosomes and furthermore dysregulates autophagy signaling (dashed lines) by an inhibition of mTOR and its upstream regulator AKT (continuous line).
Glomerular injury

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

- Podocyte loss (necrosis, apoptosis, detachment)

- Glomerular enlargement

- Podocyte phenotype switch
  - Effective podocyte depletion
    - Glomerulosclerosis
      - Progression to ESKD
PODOCYTURIA IN FABRY DISEASE IS ELEVATED IN UNTREATED VS TREATED ADULT PATIENTS AND DOES NOT CORRELATE WITH PROTEINURIA OR RENAL FUNCTION

Trimarchi H et al. To be presented at ERA-EDTA London 2015
Each Podocyte Counts!

Each glomerulus has 500-600 podocytes.

Podocytes do not efficiently proliferate.

Podocyte loss is cumulative in time

Once a glomerulus loses more than ~20% of its podocytes, it scars down. This injury is irreversible.

1,500,000 – 2,000,000 glomeruli

1,000,000,000 podocytes

100 podocytes/glomerulus loss of 1 nephron

200,000,000 of podocyte losses leads to ESRD

PODOCYTES 2% Tryggvason 2011
Urine Podocyte mRNAs, Proteinuria, and Progression in Human Glomerular Diseases

Larysa Wickman,* Farsad Afshinnia,† Su Q. Wang,† Yan Yang,† Fei Wang,† Mahboob Chowdhury,† Delia Graham,* Jennifer Hawkins,† Ryuzoh Nishizono,† Marie Tanzer,* Jocelyn Wiggins,† Guillermo A. Escobar,§ Bradley Rovin,‖ Peter Song,‡ Debbie Gipson,* David Kershaw,* and Roger C. Wiggins†
Why do podocytes detach in Fabry disease?

The αvβ3 integrin (also known as the vitronectin receptor) anchors the podocyte to the glomerular basement membrane; when activated, it causes podocyte contraction and eventually contributes to the detachment of the cell from the glomerulus and its appearance in the urine.

The urinary excretion of $\alpha v\beta 3$ integrin is elevated in subjects with Fabry disease.

Increased expression of the $\beta 3$ component is observed in glomerular epithelial cells and in Bowman’s capsular epithelial layer and tubular cells, and the amount of vitronectin (a molecule involved in adhesion and fibrinolysis) is moderately increased in the kidney from Fabry patients.

Therefore, the expression of the integrin $\alpha v\beta 3$ may be involved in podocyte contraction and eventual detachment from the glomerular basement membrane and could be another pathophysiological cause of proteinuria, finally contributing to the progression of renal injury in Fabry disease.
In Fabry disease, the decline in renal function over time is related to the degree of proteinuria and, in untreated patients, is more rapid when the eGFR is below 60 ml/min/1.73 m².

Male sex and hypertension are also significant risk factors for development of renal failure.

Protein overload may cause an increase in the levels of inflammatory mediators, and interstitial accumulation of these mediators may lead to renal scarring.

In patients with undiagnosed Fabry renal disease, a significant number of glomeruli may already be sclerotic. Reduced nephron mass thus increases the risk of further renal damage from hyperfiltration, proteinuria, and activation of angiotensin II.

![Image of diagram](image)

*Figure 1: Effects of proteinuria on tubular epithelial cells.* Increased protein absorption by tubular cells may result in direct tubular toxicity, release of chemokines and cytokines, increased expression of adhesion and MHC class II molecules along with co-stimulatory molecules. The net effect is an increased influx of mononuclear inflammatory cells. The evidence for direct proteinuria-induced EMT is weak.
It is an early sign of Fabry nephropathy

Often the most frequent clinical manifestation

Proteinuria is an independent risk factor affecting the extent of renal decline in treated and untreated patients, and in determining the success of ERT.

Data from 1,262 adult patients (585 males, 677 females) in the Fabry Registry demonstrated overt proteinuria (>300 mg/day) in 43% and 26% of males and females with CKD stage 1, respectively, with higher proportions in patients with more advanced kidney involvement.

Proteinuria should be monitored regularly and treated appropriately.