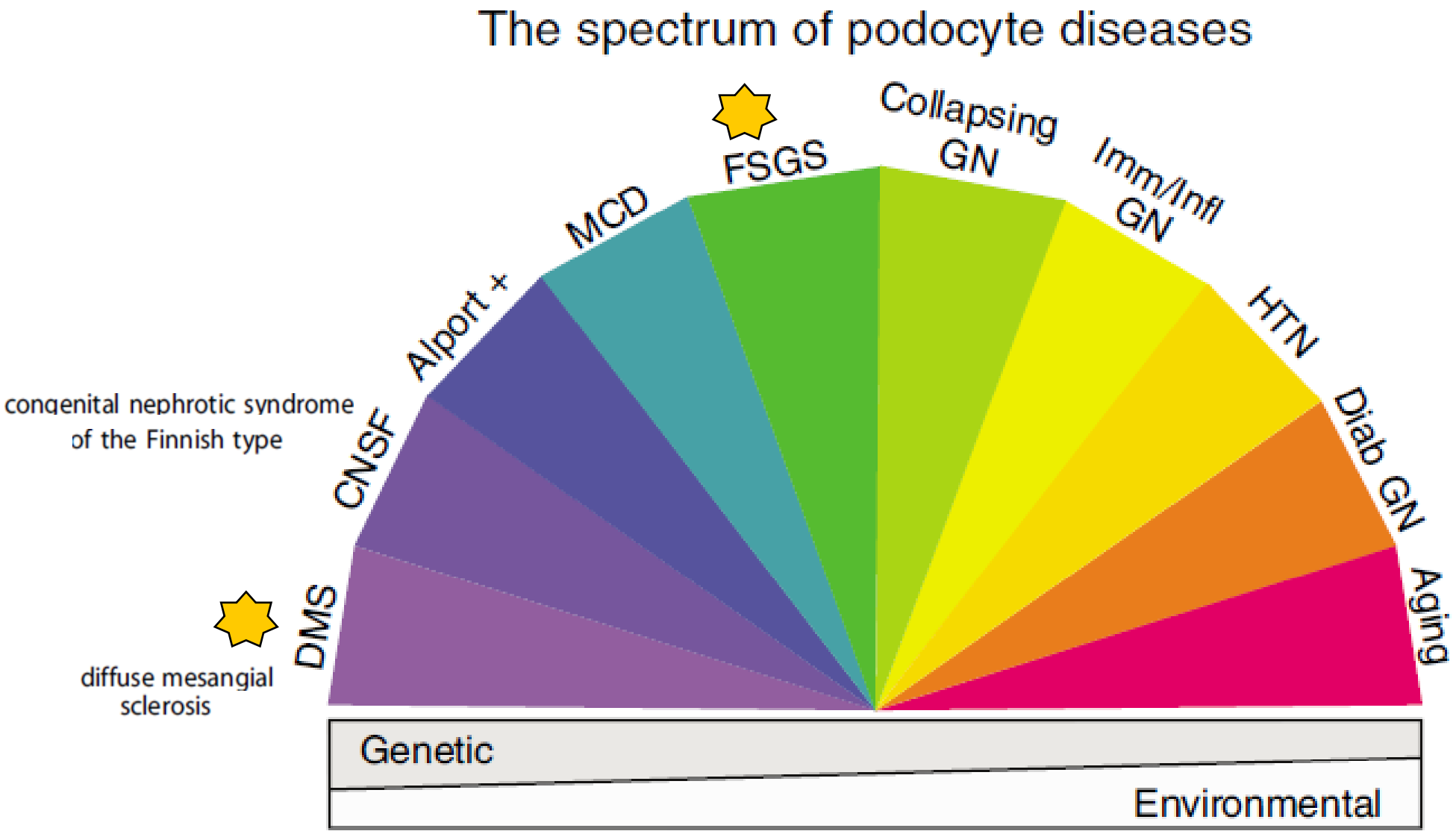
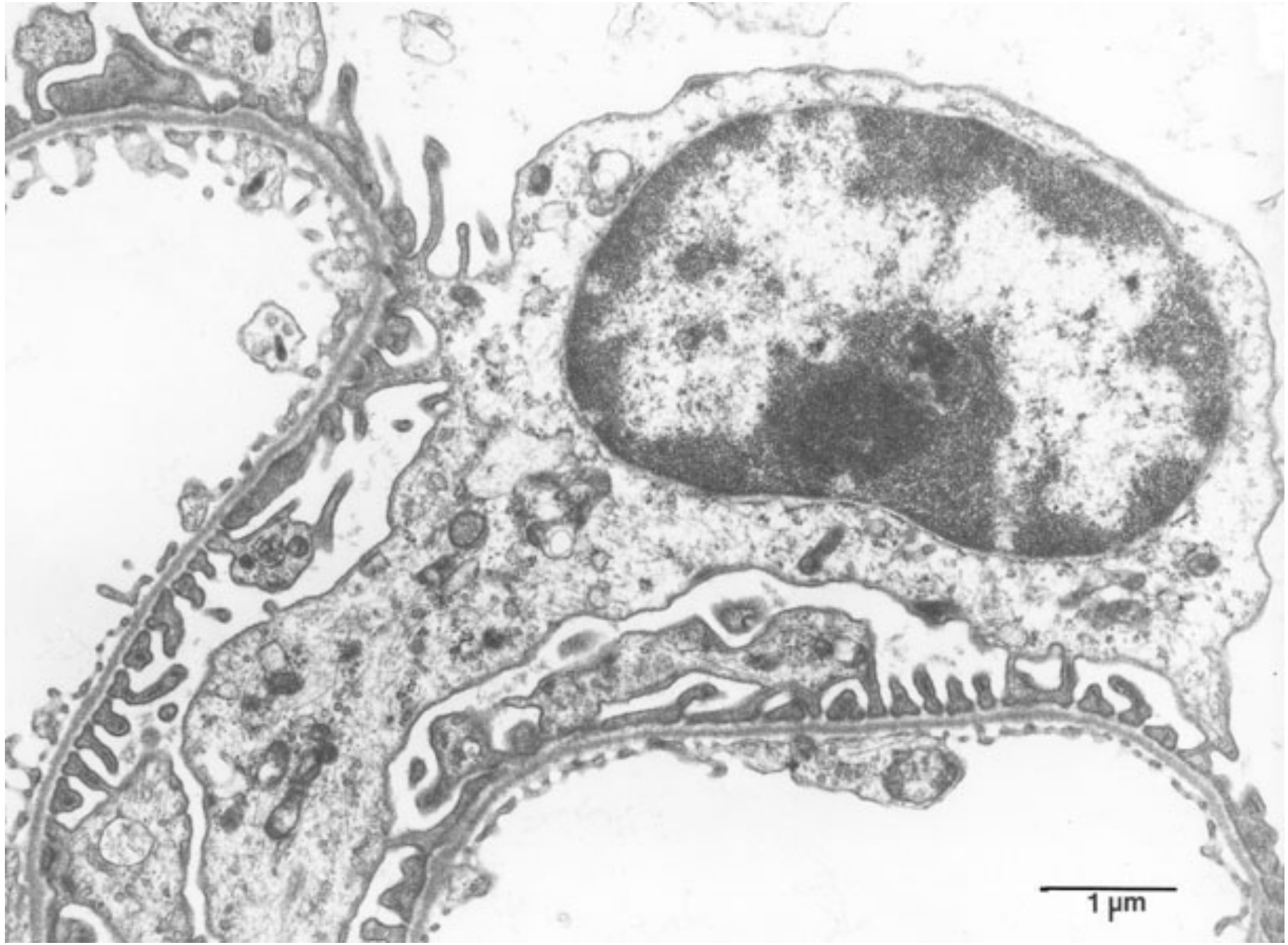
# THE PODOCYTE AND FABRY DISEASE

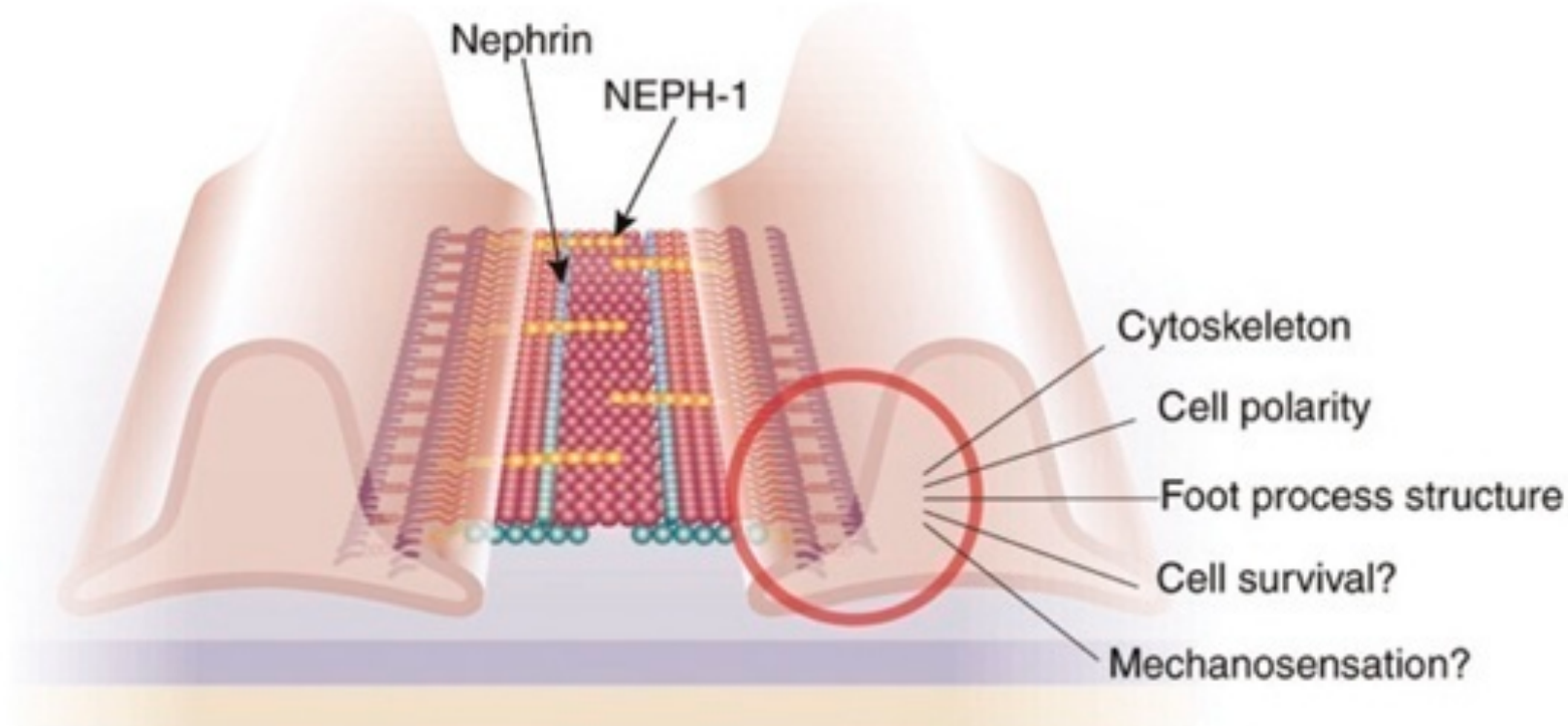
HERNÁN TRIMARCHI

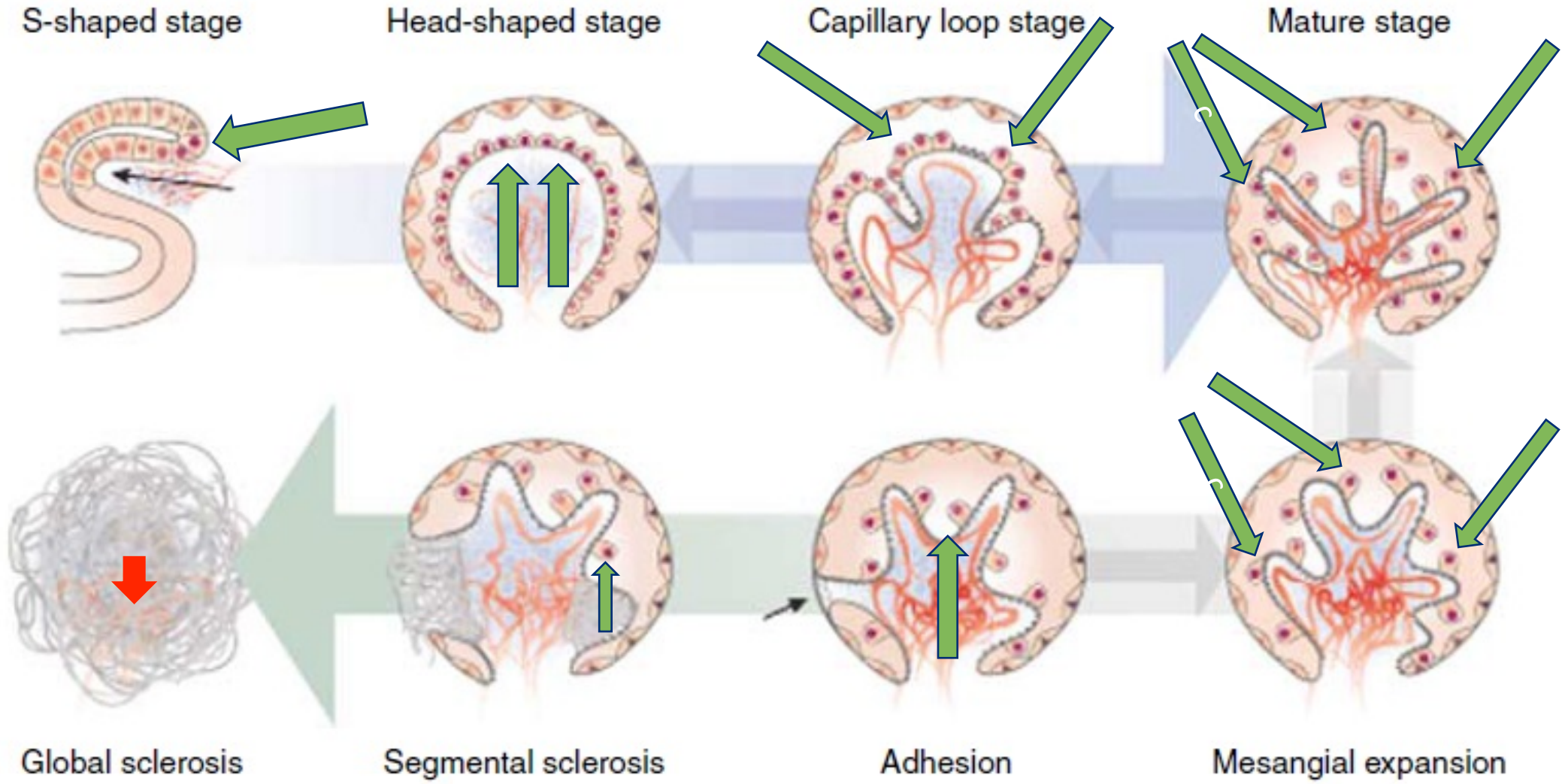
**HOSPITAL BRITÁNICO DE BUENOS AIRES**
**ARGENTINA**

**2015**


The spectrum of podocyte diseases
FSGS
Collapsing GN
Imm/Infl GN
HTN
Diab GN
Aging
MCD
Alport +
CNSF
congenital nephrotic syndrome of the Finnish type
DMS
diffuse mesangial sclerosis
Genetic
Environmental


1 µm


Nephrin
NEPH-1
Cytoskeleton
Cell polarity
Foot process structure
Cell survival?
Mechanosensation?




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

**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 widespread scarring of that 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)

*RC Wiggins*: Spectrum of podocytopathies *Kidney International* (2007) **71**, 1205–1214

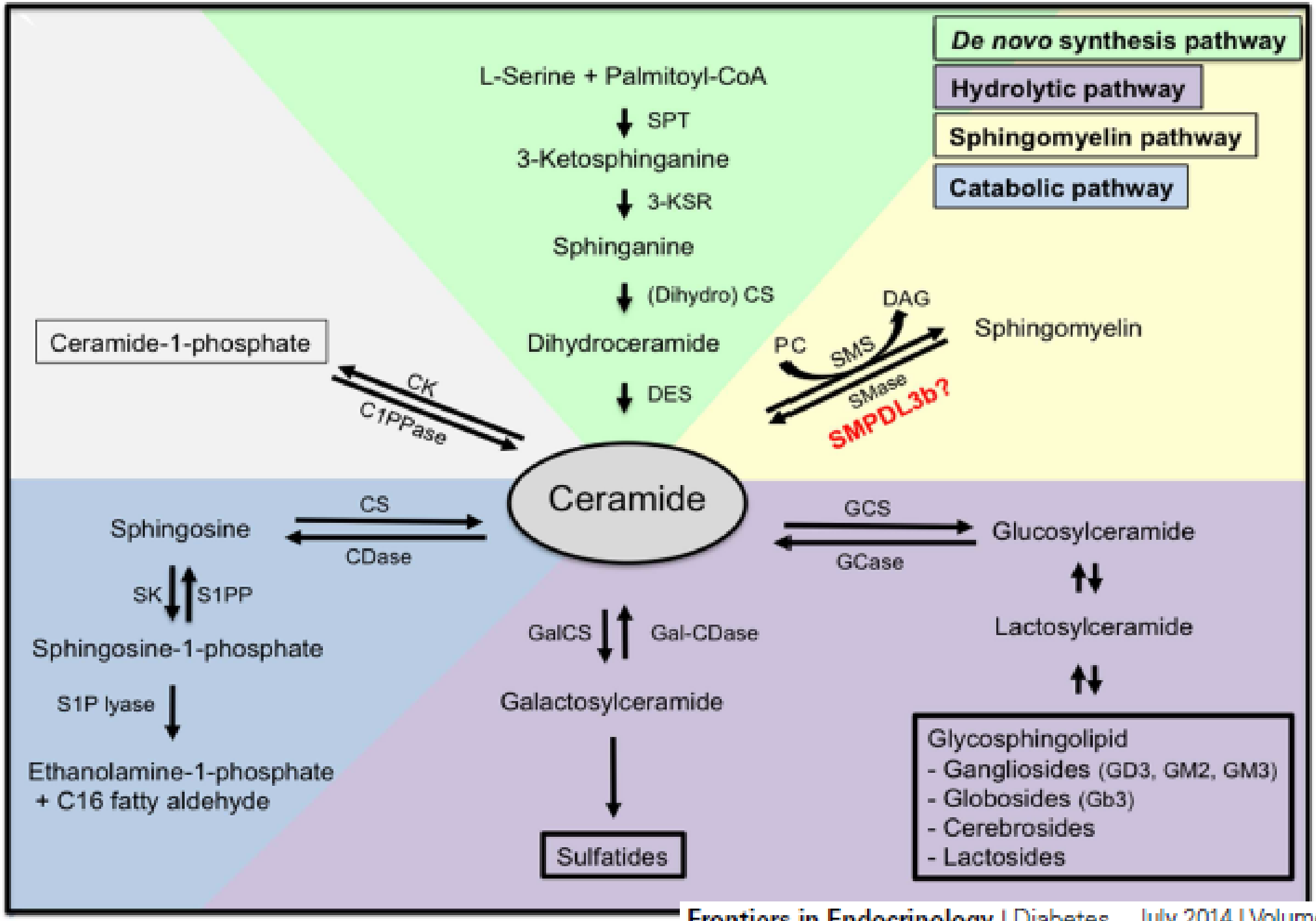
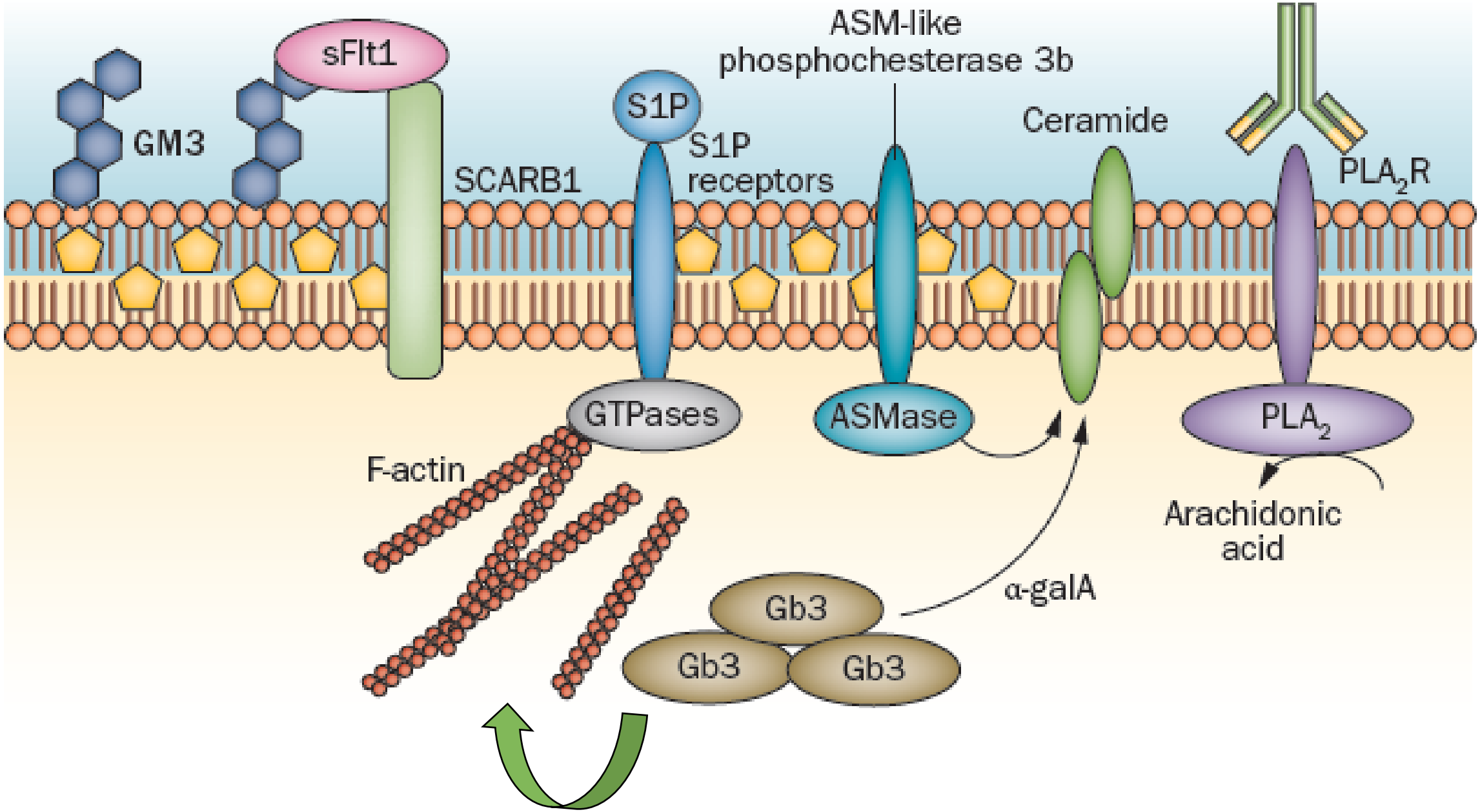

De novo synthesis pathway
Hydrolytic pathway
Sphingomyelin pathway
Catabolic pathway
L-Serine + Palmitoyl-CoA
SPT
3-Ketosphinganine
3-KSR
Sphinganine
(Dihydro) CS
Dihydroceramide
DES
Ceramide-1-phosphate
CK
C1PPase
DAG
PC
SMS
SMase
SMPDL3b?
Sphingomyelin
Ceramide
CS
CDase
Sphingosine
SK
S1PP
Sphingosine-1-phosphate
S1P lyase
Ethanolamine-1-phosphate
+ C16 fatty aldehyde
GalCS
Gal-CDase
Galactosylceramide
Sulfatides
GCS
GCase
Glucosylceramide
Lactosylceramide
Glycosphingolipid
- Gangliosides (GD3, GM2, GM3)
- Globosides (Gb3)
- Cerebrosides
- Lactosides

**Table 1 | Sphingolipid accumulation in glomerular diseases of genetic and non-genetic origin.**

| Disease | OMIM | Mutated gene | Chromosomal location | Sphingolipid accumulating |
|---|---|---|---|---|
| **SPHINGOLIPID ACCUMULATION IN GLOMERULAR DISEASE OF GENETIC ORIGIN** | | | | |
| Gaucher | 230800 | Acid beta-glucosidase 1 (GBA1) | 1q22 | GlcCer |
| Tay–Sachs | 272800 | Hexoseaminidase A (HEXA) | 15q23 | GM2 |
| Sandhoff | 268800 | Hexoseaminidase B (HEXB) | 5q13 | GM2 |
| Fabry | 301500 | Alpha-galactosidase A (GLA) | Xq22 | Gb3, Lyso-Gb3 |
| Hereditary inclusion body myopathy 2 | 600737 | UDP-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (GNE) | 9p13 | Hyposialylation of glycoproteins such as podocalyxin? |
| Niemann–Pick | 257220<br>607616<br>257200 | *NPC1 NPC2 SMPD1* | 18q11 14q24 11p15 | Sphingomyelin |
| Nephrotic syndrome of the Finnish type | 256300 | NPHS1 | 19q13 | *O*-actetyl-GD3 |
| **SPHINGOLIPID ACCUMULATION IN GLOMERULAR DISEASE OF NON-GENETIC ORIGIN** | | | | |
| Diabetic kidney disease | | | | GlcCer, GM3, S1P, sphingosine? |
| Puromycin aminonucleoside (PAN)-induced nephropathy | | | | GD3, *O*-actetyl-GD3 |
| HIV-associated nephropathy (HIVAN) | | | | Gb3 |
| Focal segmental glomerulosclerosis (FSGS) | | | | Sphingomyelin |
| Acute ischemia reperfusion injury | | | | Ceramide |

**Table 1** Mechanisms of endocytosis

| Pathway | Morphology and size | Coat | Small GTPase | Cargo | Function |
|---|---|---|---|---|---|
| Clathrin-mediated[a] | Vesicular 150–200 nm | Clathrin | Rab5 | RTKs, GPCR, TGF-ßR, Notch, TfR, LDLR, ß-arrestin, Wnt/ß-catenin | Cell signaling, vesicular transport |
| Caveolae-mediated[a] | Flask-shaped 50–120 nm | Caveolin 1 and 2 | Unclear | GPI-APs, TGF-ßR, CTxB, viruses, folic acid, IGF-1R, Wnt/ß-catenin | Cell signaling, lipid regulation, vesicular transport, transcytosis |
| CLIC/GEEC | Tubular | None | Cdc42 Arf1 | GPI-APs, glycosphingolipids, cholera toxin | Actin dynamics and cellular stress pathways, differentiation and apoptosis, focal adhesion, fluid-phase uptake, oncogenesis |
| Arf6-mediated | Tubular | None | Arf6 | ß-arrestins, MHC I-II | Membrane curvature |
| Flotillin-mediated | Vesicular | Flotillin 1 and 2 | None | CTxB, GPI-AP, proteoglycans | Lipid raft-mediated endocytosis, |
| IL-2R[a] | Vesicular 50–100 nm | None | RhoA, Rac1 | IL-2Rß, γc cytokine receptor | IL-2R endocytosis and signaling |
| Macropinocytosis[a] | Ruffled | None | Rac1, Cdc42, Arf6, Rab5 | Fluid, RTKs, bacteria | Extracellular fluid uptake, actin dynamics |
| Phagocytosis | Cargo shaped | None | Rac1, RhoA, Cdc42 | Nutrients, pathogens, dead cells, and cellular debris | Uptake of nutrients, pathogens, and cellular debris by professional phagocytic cells; opsonization of foreign particles followed by actin rearrangements |




Fornoni, A. *et al. Nat. Rev. Nephrol.* **10**, 379–388 (2014)

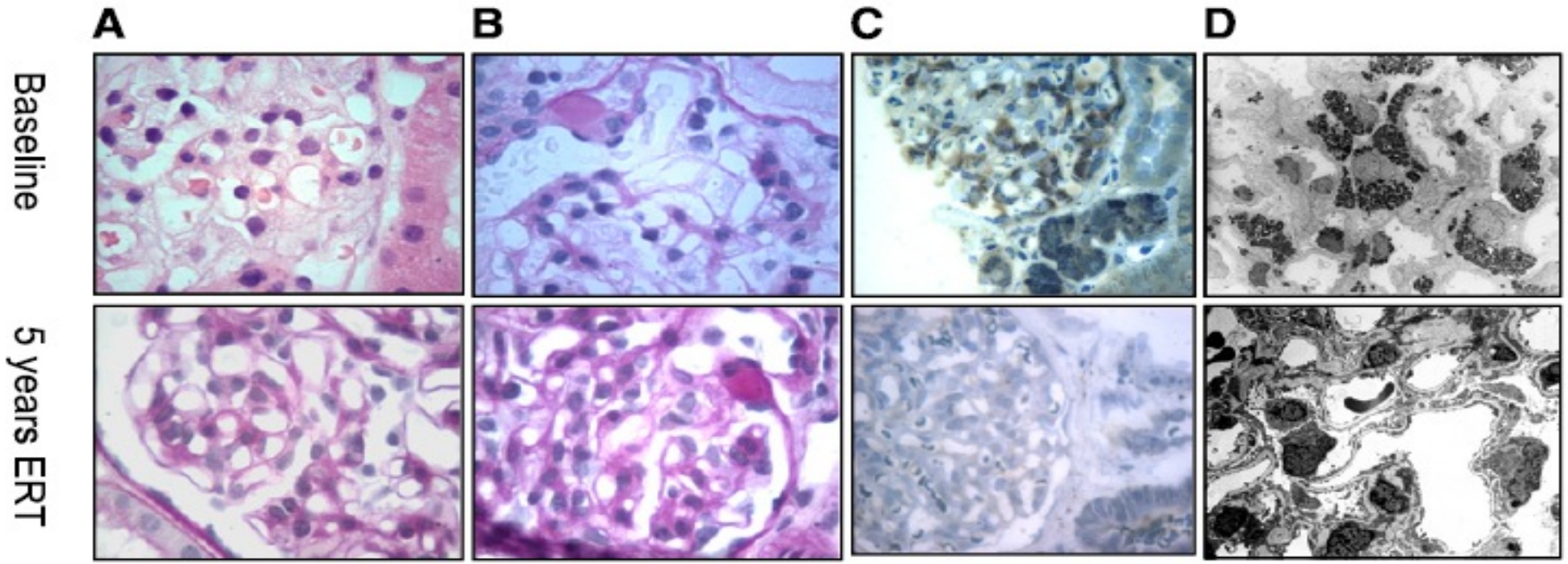
In Fabry disease, reduced activity of the lysosomal enzyme α-galactosidase A leads to lysosomal accumulation of Gb3, resulting in characteristic inclusions called zebra bodies in various organs and cell types.

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.

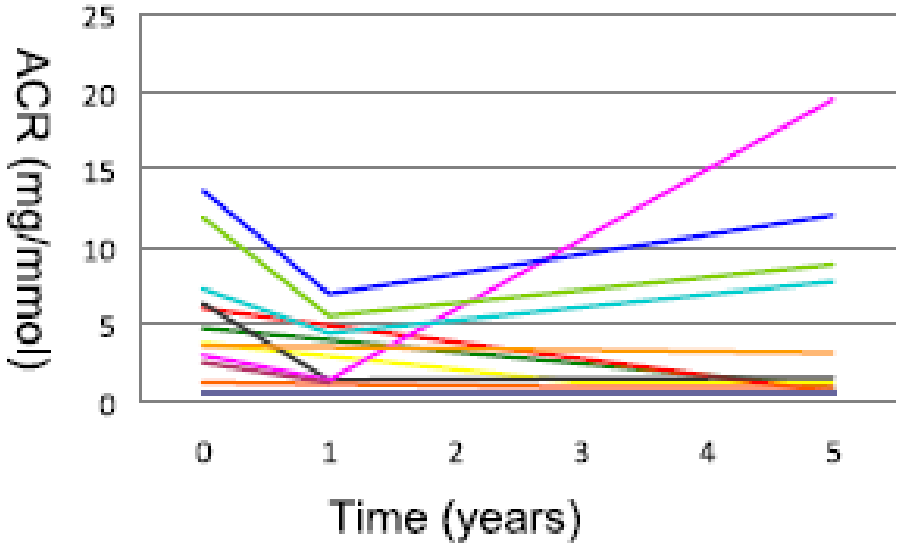
Analysis of kidney biopsy samples from 14 patients (median age 12 years) with Fabry disease demonstrated age-dependent accumulation of its substrate Gb3 in podocytes.

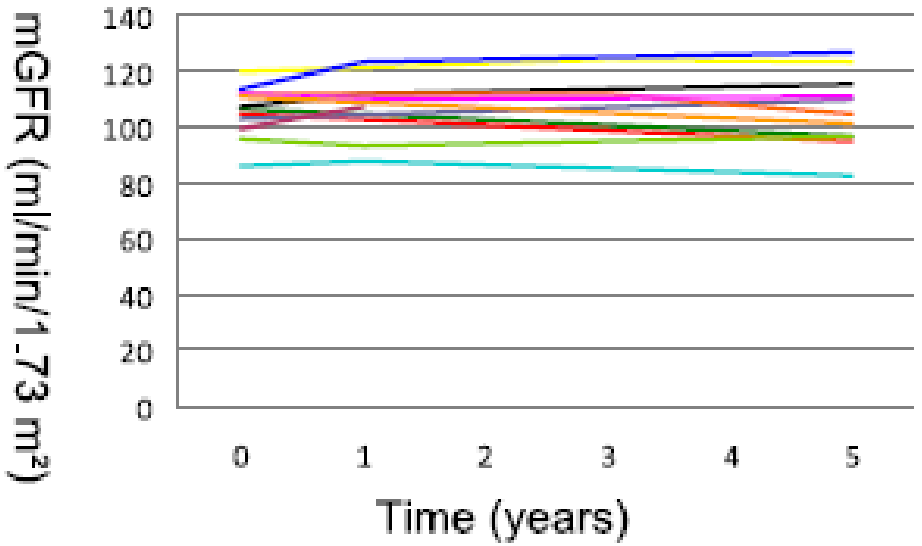
Enzyme replacement therapy with recombinant α-galactosidase A in patients with Fabry disease successfully cleared glycolipid accumulation from the kidney and vasculature.

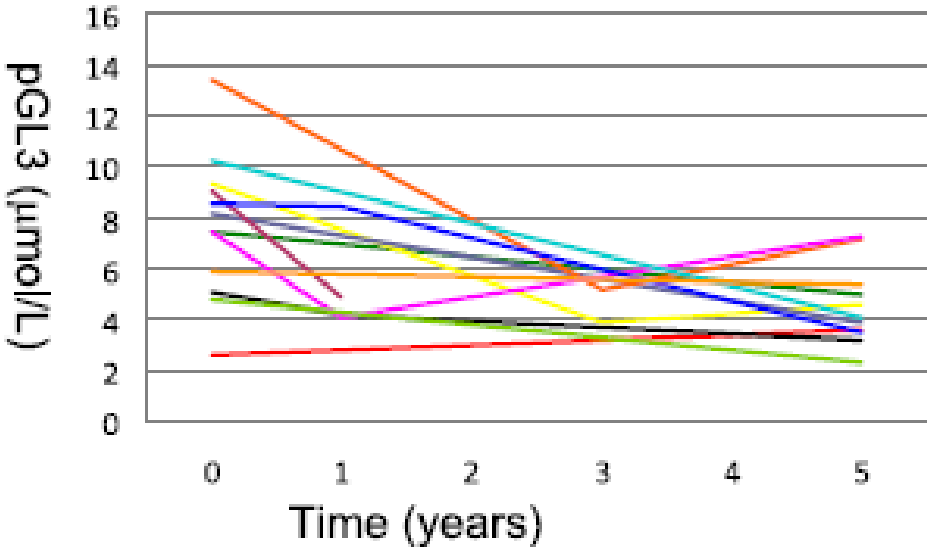
Fornoni, A. *et al. Nat. Rev. Nephrol.* **10**, 379–388 (2014)

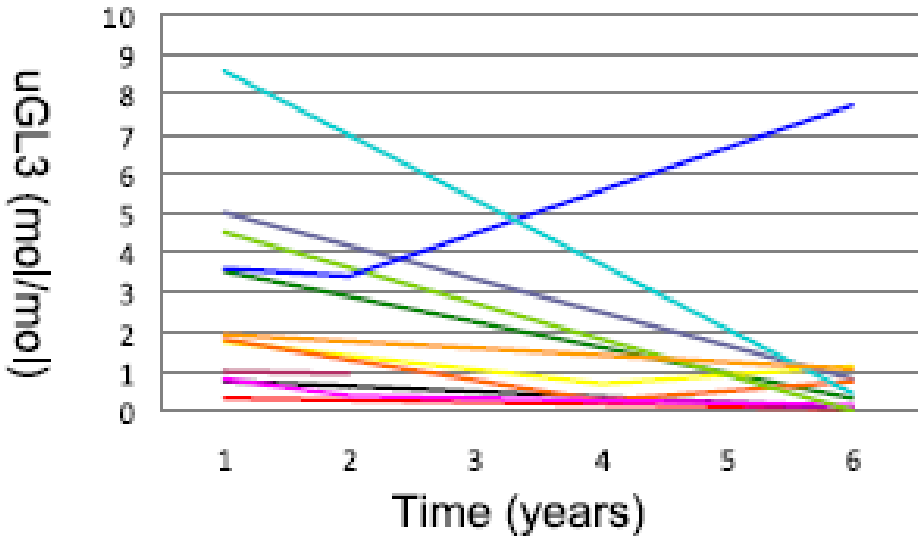


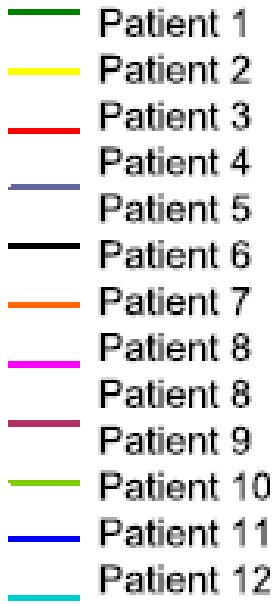

Tøndel Renal Biopsies in Fabry Disease J Am Soc Nephrol **24**: 137–148, 2013

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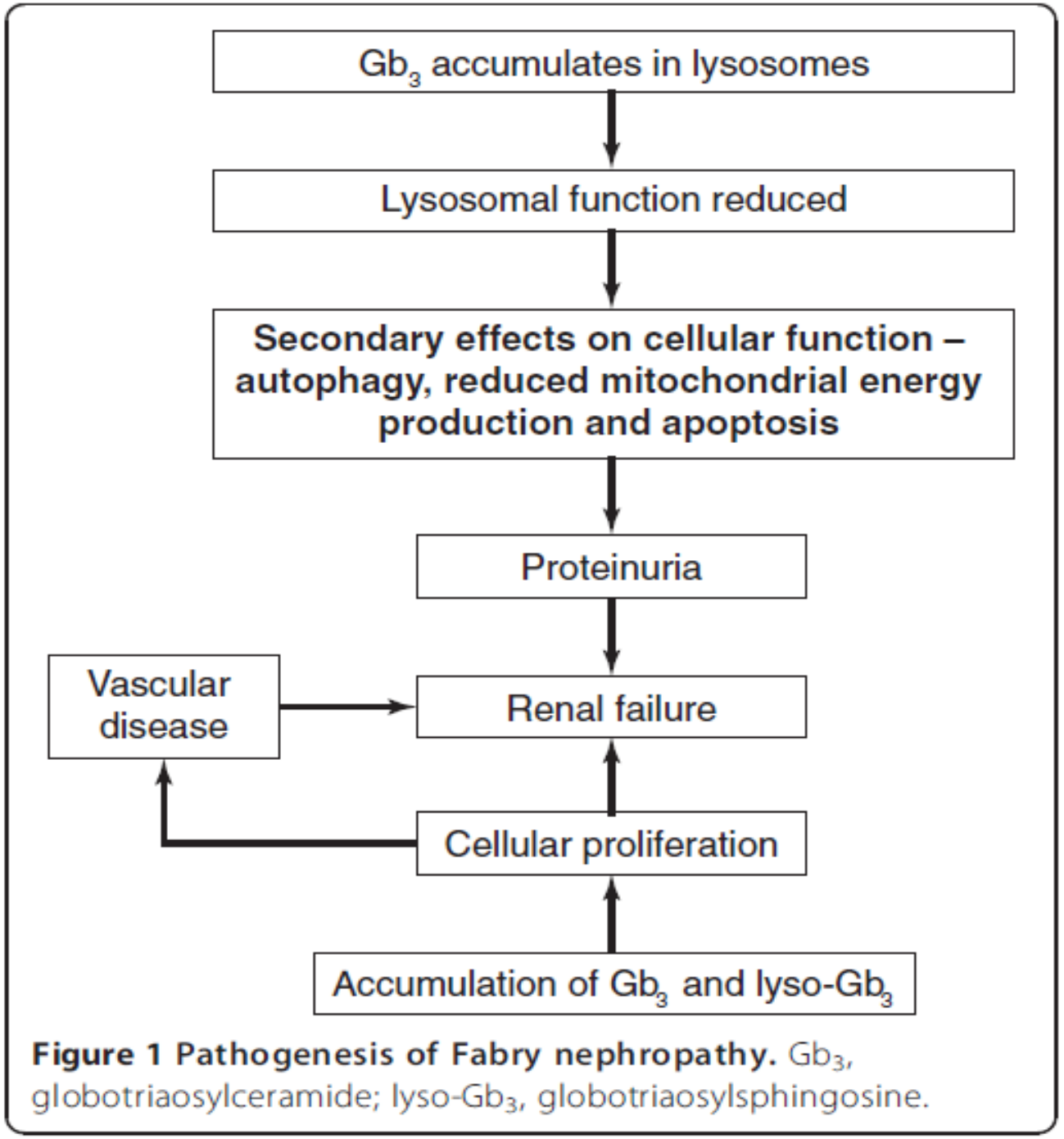



**Figure 1 Pathogenesis of Fabry nephropathy.** $Gb_3$, globotriaosylceramide; lyso-$Gb_3$, globotriaosylsphingosine.

Progressive intracellular accumulation of $Gb_3$ leads to cellular changes and histological damage

Rupture of the lysosome and damage due to local inflammation

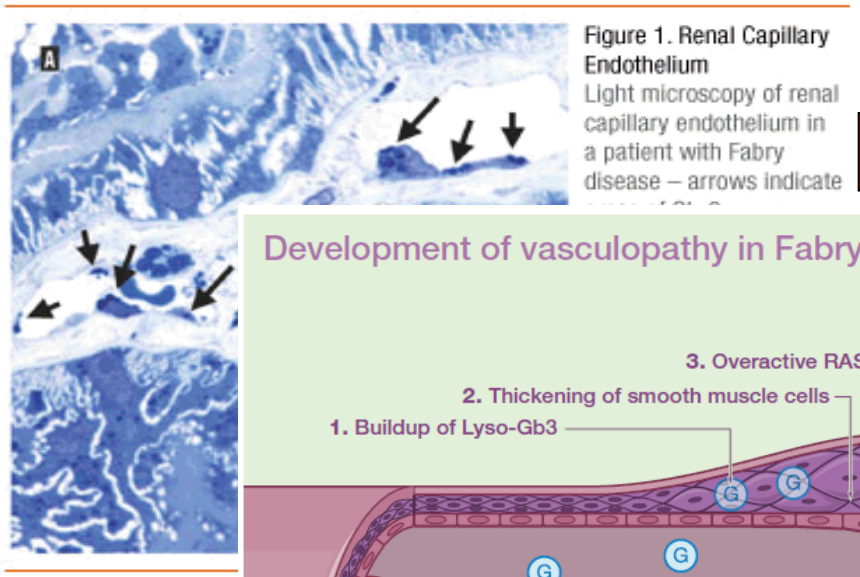
Deposition of $Gb_3$ can promote vascular smooth muscle cell proliferation and the release of mediators involved in other nephropathies

Addition of $Gb_3$ to cultured endothelial cells has been found to increase oxidative stress and upregulation of the expression of cell adhesion molecules

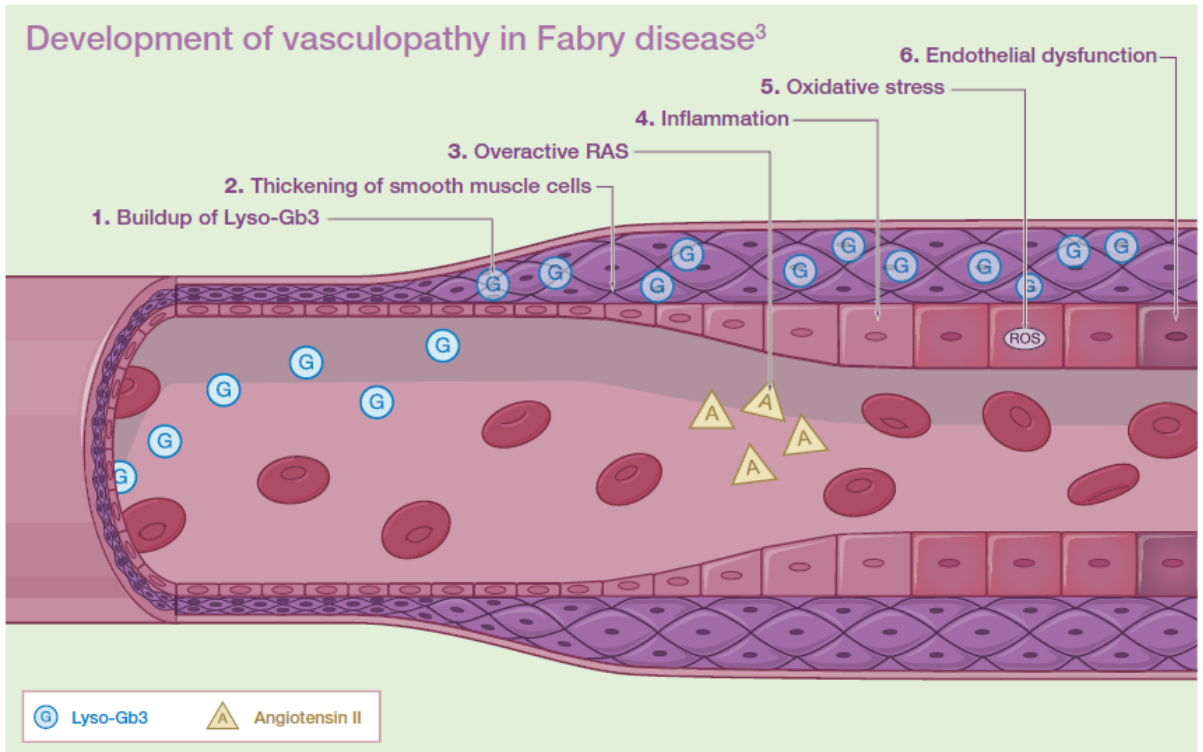
Vasculopathy: local up-regulation of the RAS

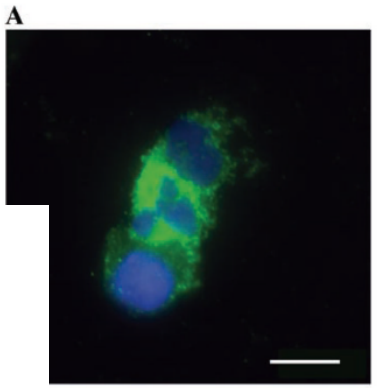
Waldek and Feriozzi *BMC Nephrology* 2014, **15**:72

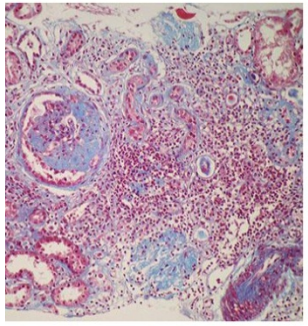
The suggested mechanisms of renal injury in Fabry disease include vascular compromise secondary to deposition of GL3 within the arterial wall, which should be considered as the *first hit*, with a concomitant decrease in nitric oxide synthesis and a tendency to microthrombotic events, podocyte injury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




DECREASED NO



Najafian B, Svarstad E, Bostad L, Gubler MC, Tøndel C, Whitley, Mauer M. Progressive podocyte injury and globotriaosylceramide (GL-3) accumulation in young patients with Fabry disease. Kidney Int 2011; 79: 663–670.

**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

Waldek and Feriozzi *BMC Nephrology* 2014, **15**:72

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

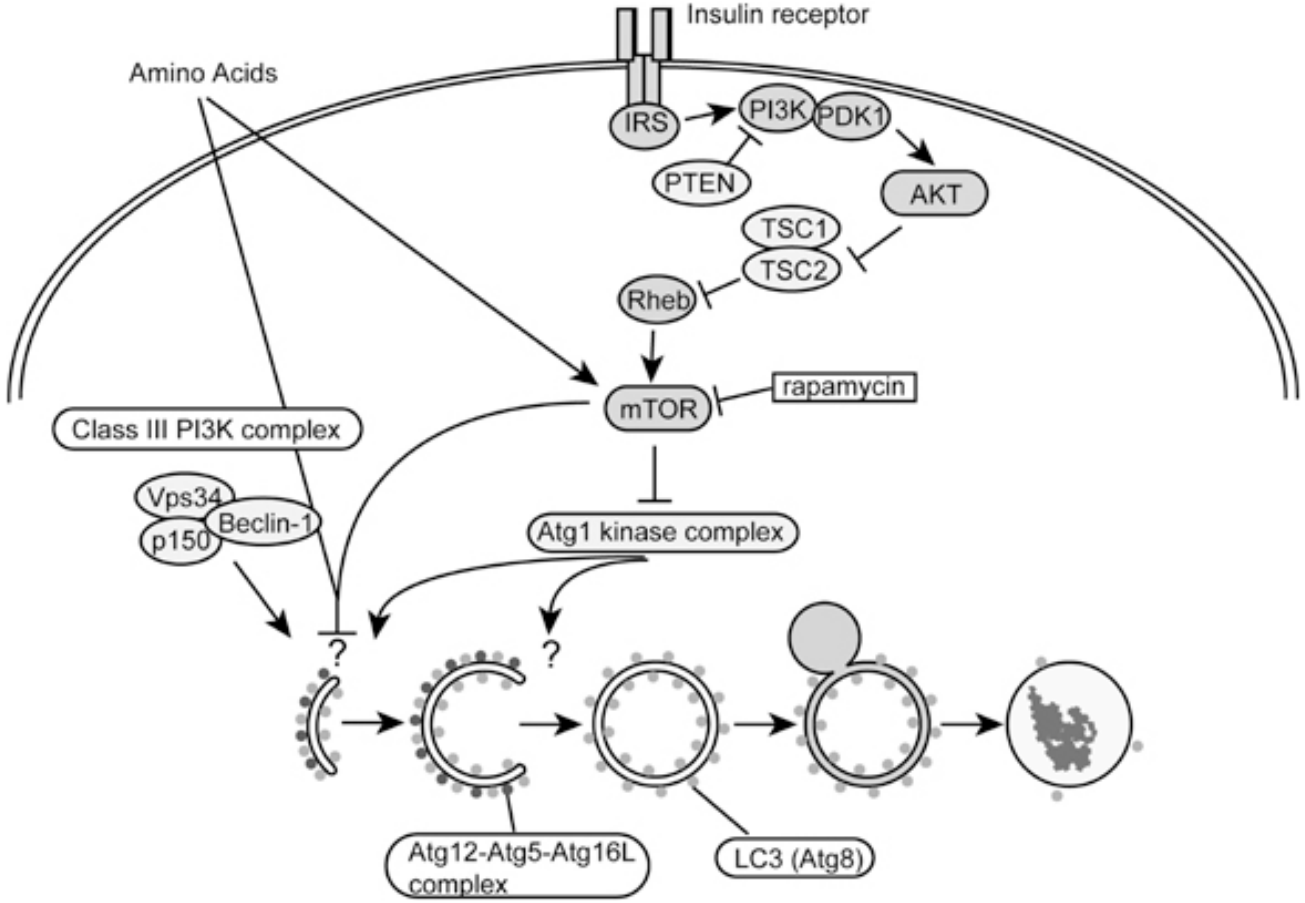
Besides the established role of slit diaphragm signaling for podoycte cell survival and polarity,
recent data show the significance of **AUTOPHAGY** associated signaling in the development of glomerulopathies

Liebau PLOS ONE May 2013 | Volume 8 | Issue 5 | e63506

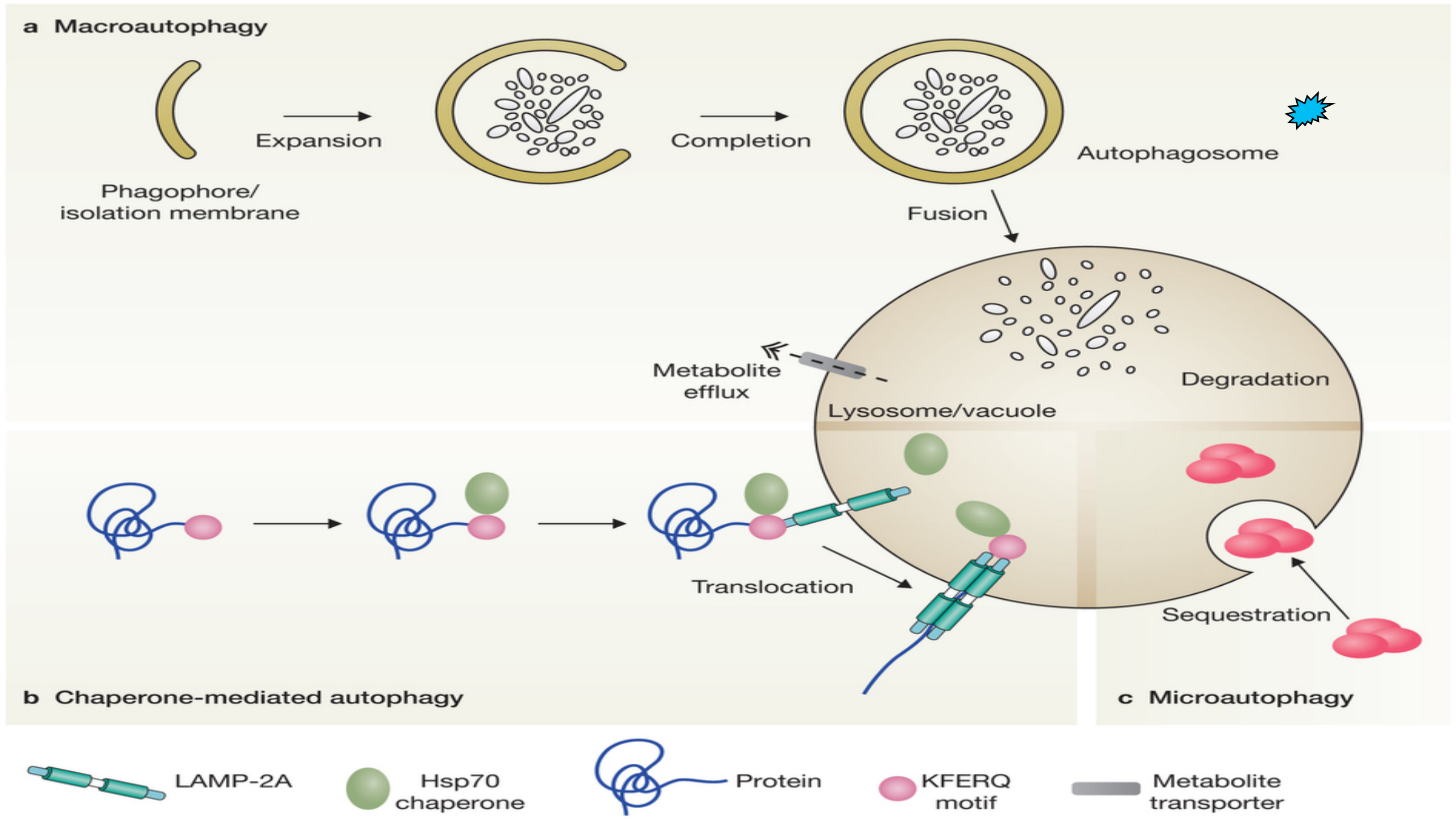
Accumulation of $Gb_3$ is accompanied by an increase in autophagosomes, suggesting that deregulated autophagy pathways have some involvement in the pathogenesis of glomerular damage in Fabry disease.

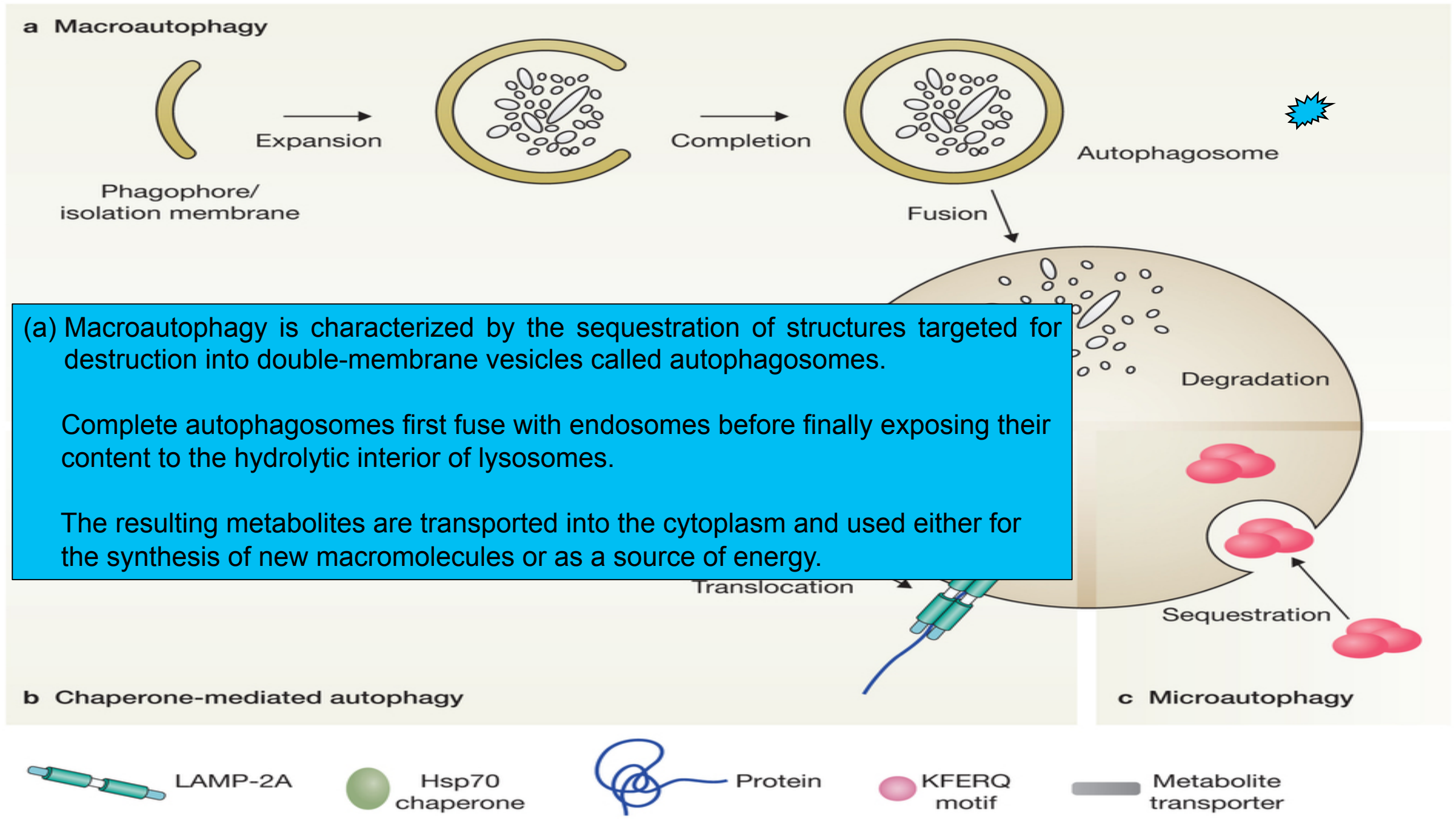
# AUTOPHAGY

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.




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
Phagophore/
isolation membrane
Expansion
Completion
Autophagosome
Fusion
Metabolite
efflux
Degradation
Lysosome/vacuole
Translocation
Sequestration
b Chaperone-mediated autophagy
c Microautophagy
LAMP-2A
Hsp70
chaperone
Protein
KFERQ
motif
Metabolite
transporter




(a) 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.

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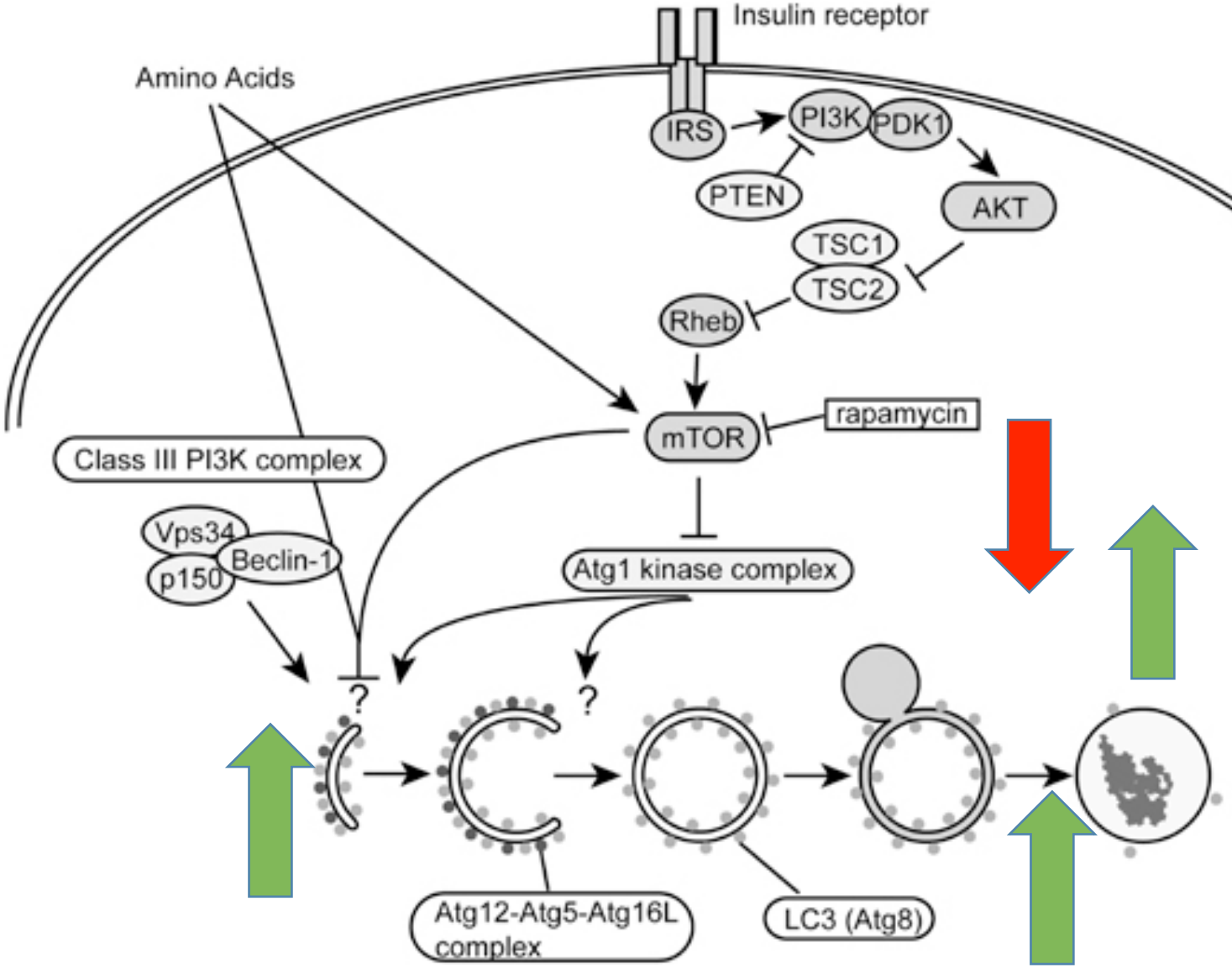
PLOS | ONE

# Dysregulated Autophagy Contributes to Podocyte Damage in Fabry's Disease

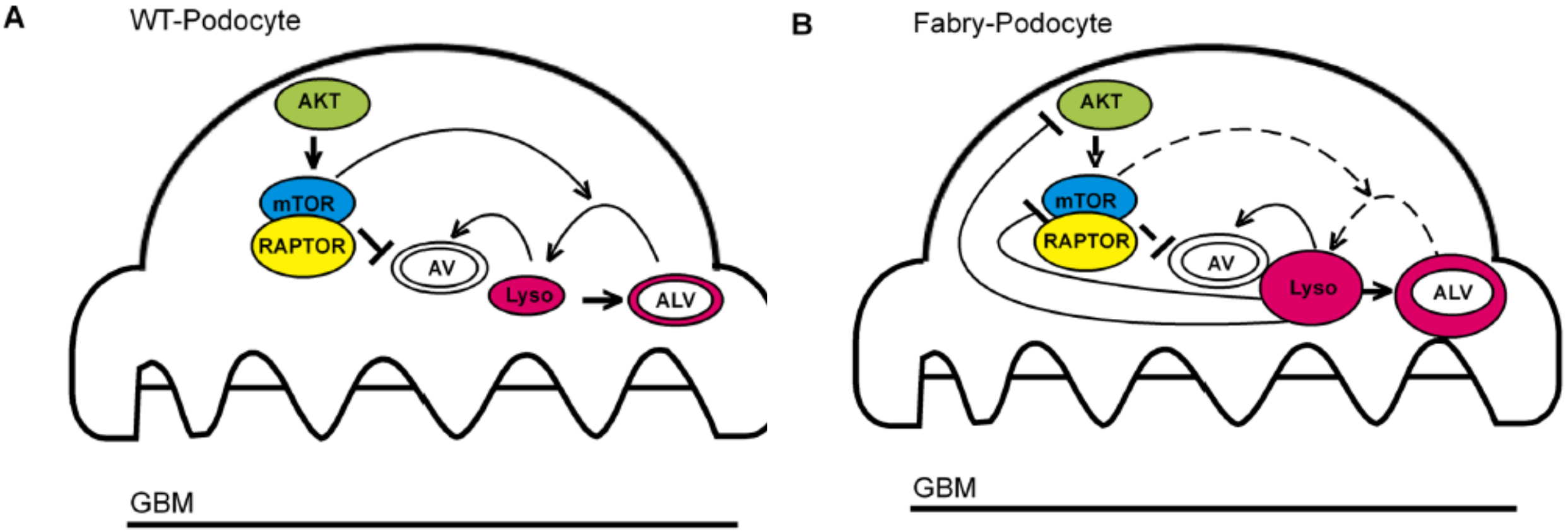
Max C. Liebau[1,2☯], Fabian Braun[1☯], Katja Höpker[1], Claudia Weitbrecht[1], Valerie Bartels[1], Roman-Ulrich Müller[1,4,5], Susanne Brodesser[3,4], Moin A. Saleem[6], Thomas Benzing[1,4,5], Bernhard Schermer[1,4,5], Markus Cybulla[7¶], Christine E. Kurschat[1,4*¶]

**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.



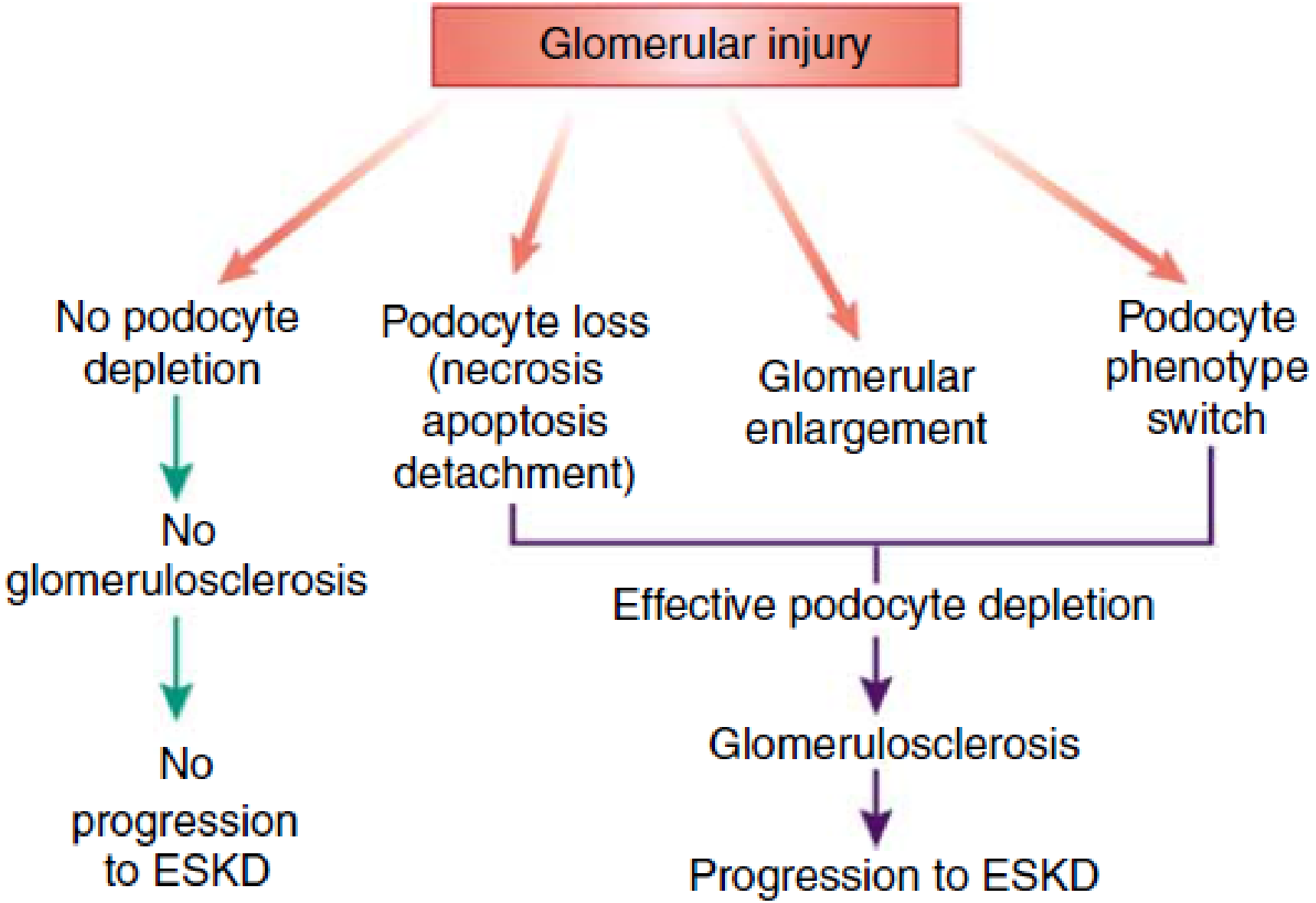

Dysregulated autophagy in a-galactosidase A-deficient podocytes may be the result of deficient mTOR kinase activity.




Model on the effect of a-Gal depletion in podocytes

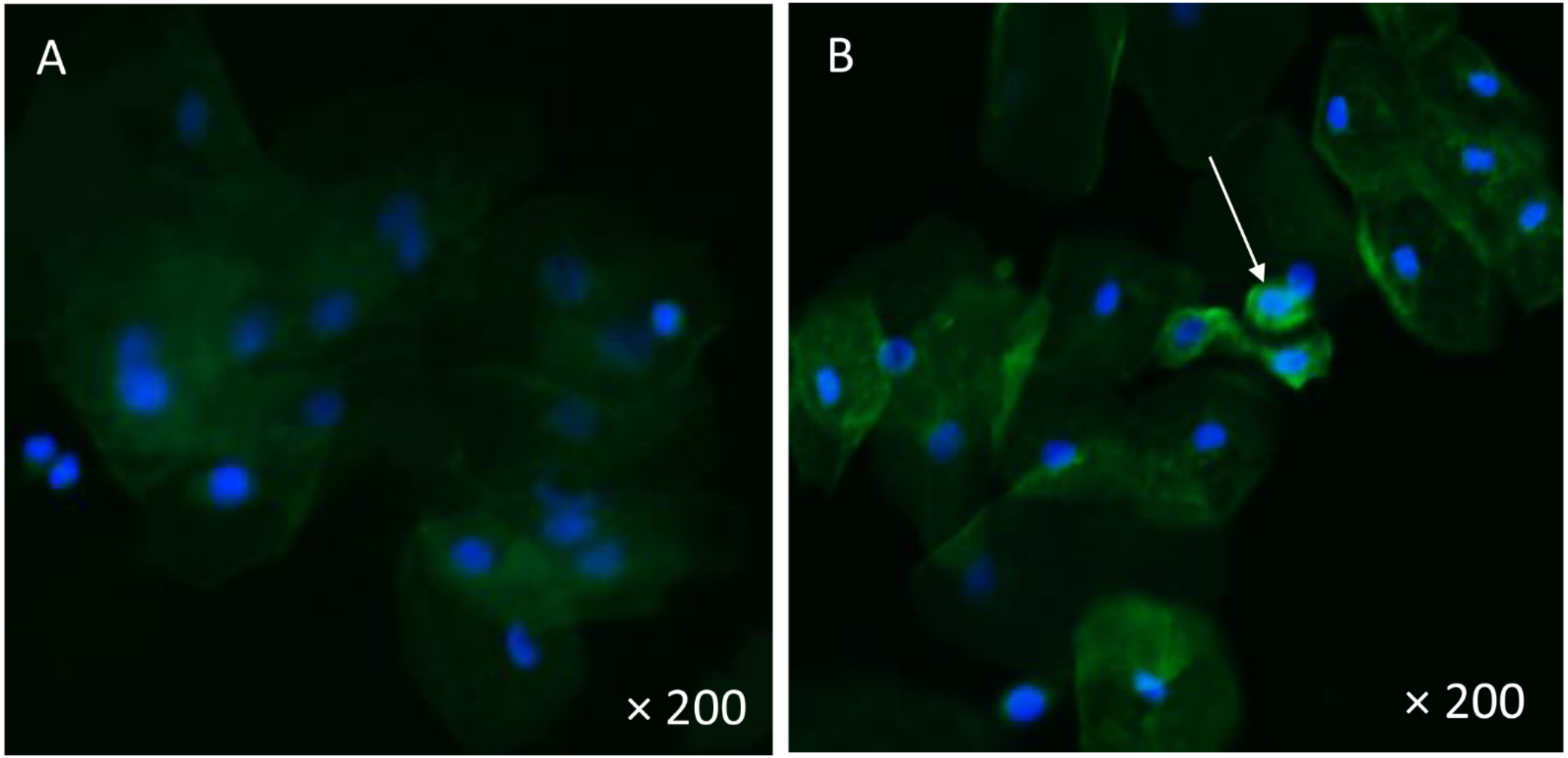
mTOR negatively regulates the formation of autophagy vesicles and promotes the recovery of autophagosomes (AV) and lysosomes (Lys) from autophagolysosomes (ALV) in podocytes (continuous lines).

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).




*RC Wiggins*: Spectrum of podocytopathies *Kidney International* (2007) **71,** 1205–1214;

# PODOCYTURIA IN FABRY DISEASE IS ELEVATED IN UNTREATED VS TREATED ADULT PATIENTS AND DOES NOT CORRELATE WITH PROTEINURIA OR RENAL FUNCTION

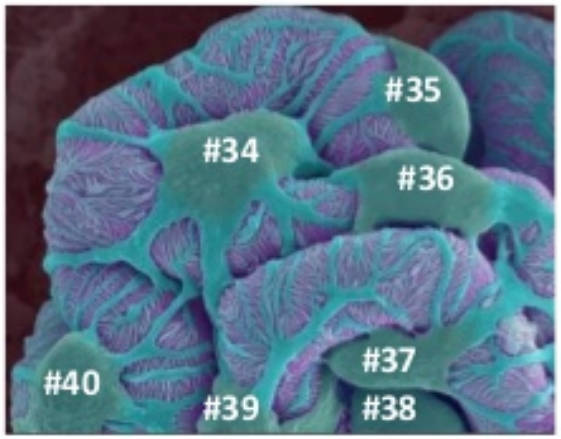



*Trimarchi H et al. To be presented at ERA-EDTA London 2015*

# Each Podocyte Counts!

**PODOCYTES 2%**
**Tryggvason 2011**

1,500,000 **– 2,000,000 glomeruli**




Each glomerulus has 500-600 podocytes.

Podocytes do not efficiently proliferate.

Podocyte loss is cumulative in time

**1,000,000,000 podocytes**

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

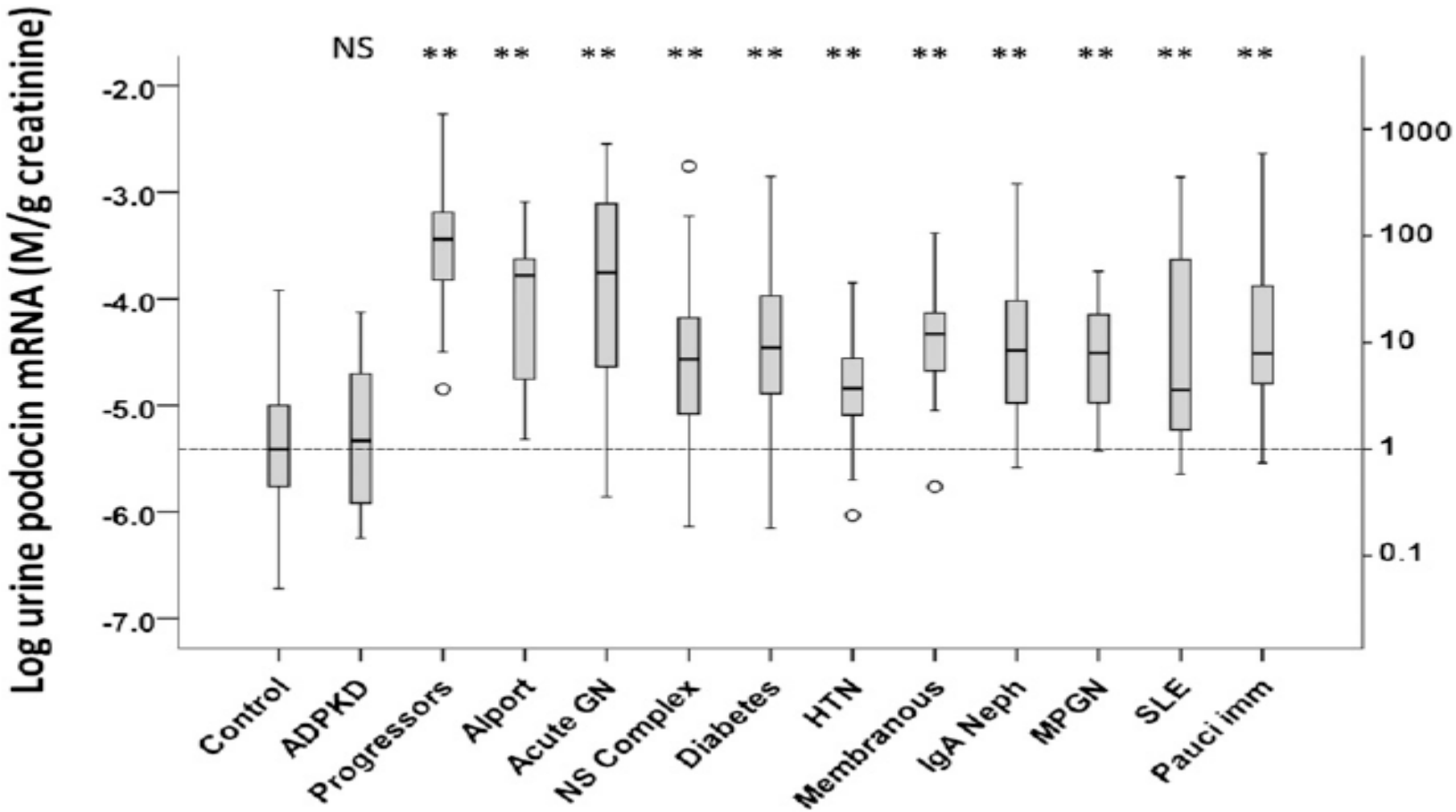
**100 podocytes/glomerulus loss of 1 nephron**

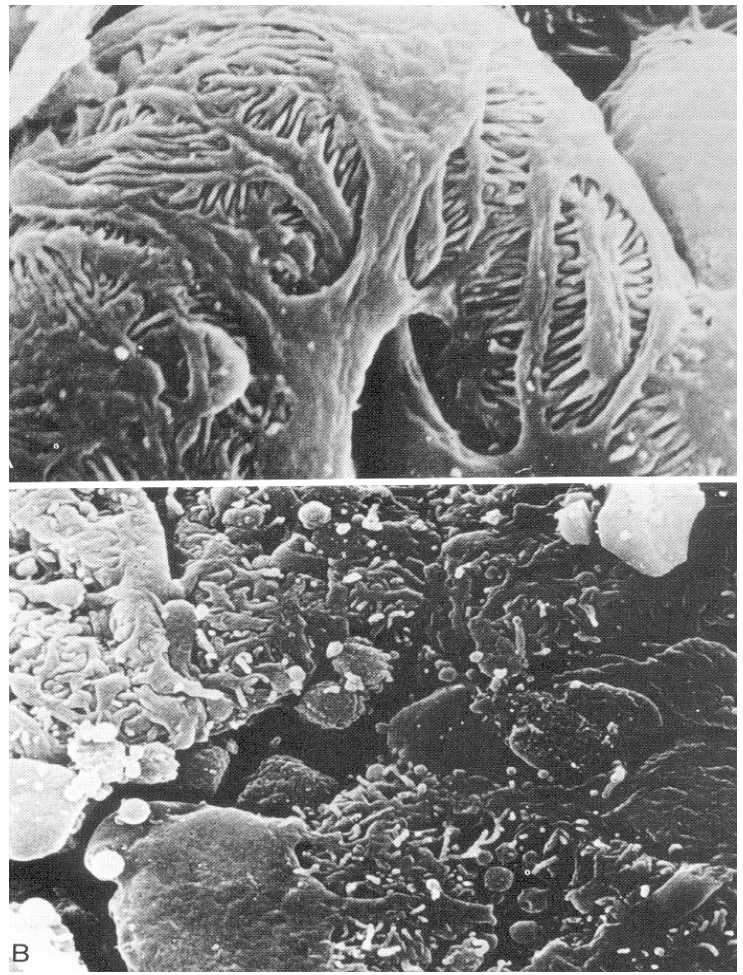
**200,000,000 of podocyte losses leads to ESRD**

*With permission B Najafian FSIG Expert Fabry Conference San Diego 2014*

# 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†



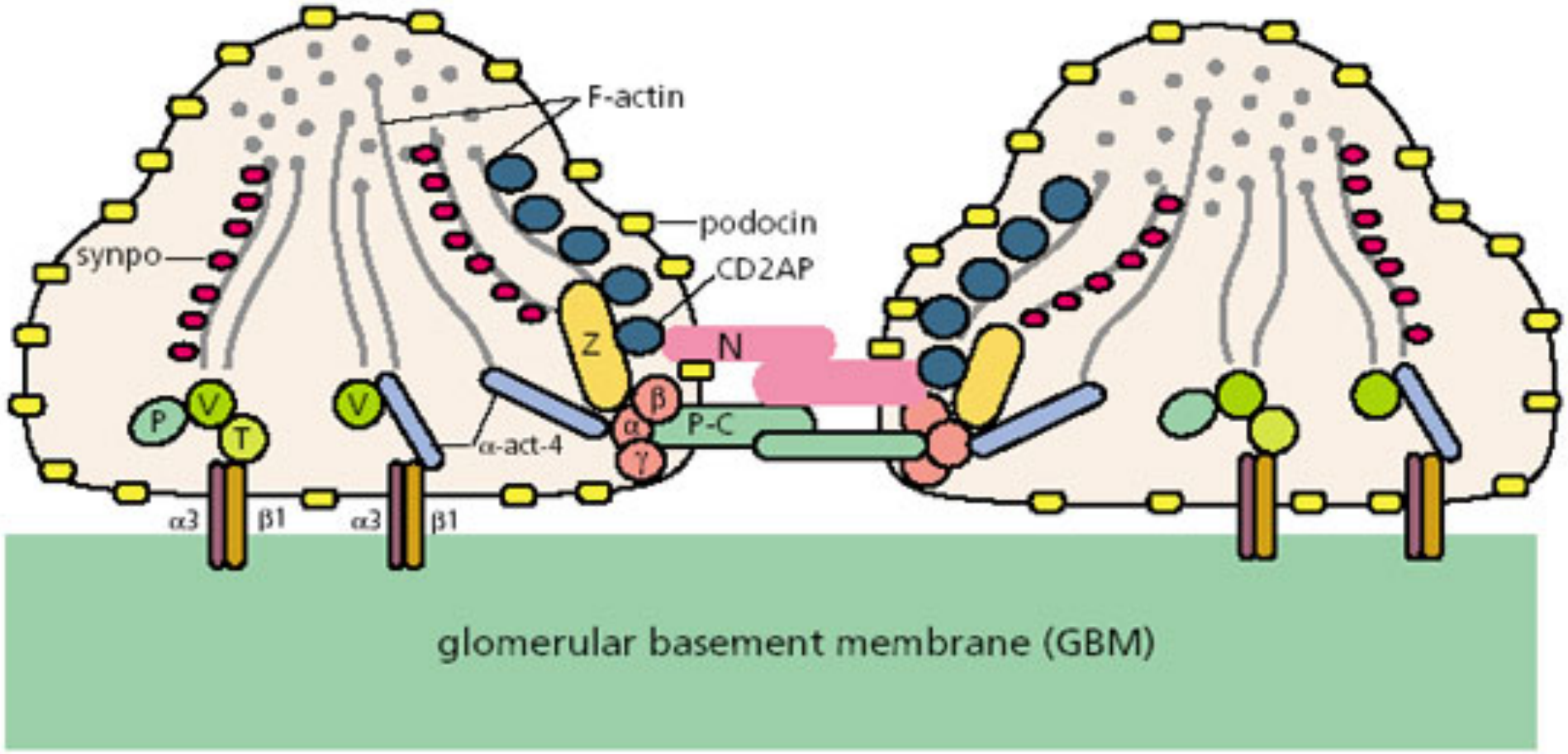




J Am Soc Nephrol **24:** 2081–2095, 2013

# Why do podocytes detach in Fabry disease?

The $\alpha v\beta 3$ 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.

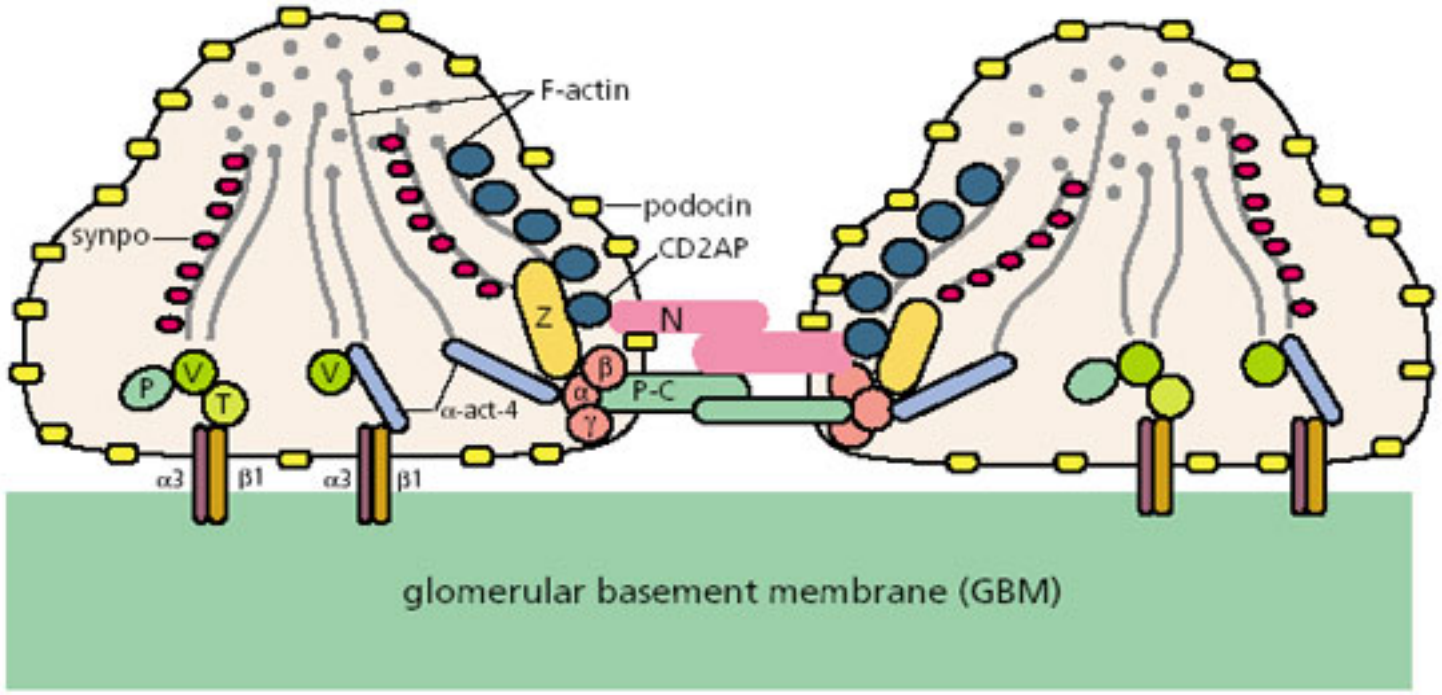



*K. Utsumi, K. Itoh, R. Kase et al., "Urinary excretion of the vitronectin receptor (integrin α (V) β3) in patients with Fabry disease," Clinica Chimica Acta, vol. 279, no. 1-2, pp. 55-68, 1999.*

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.

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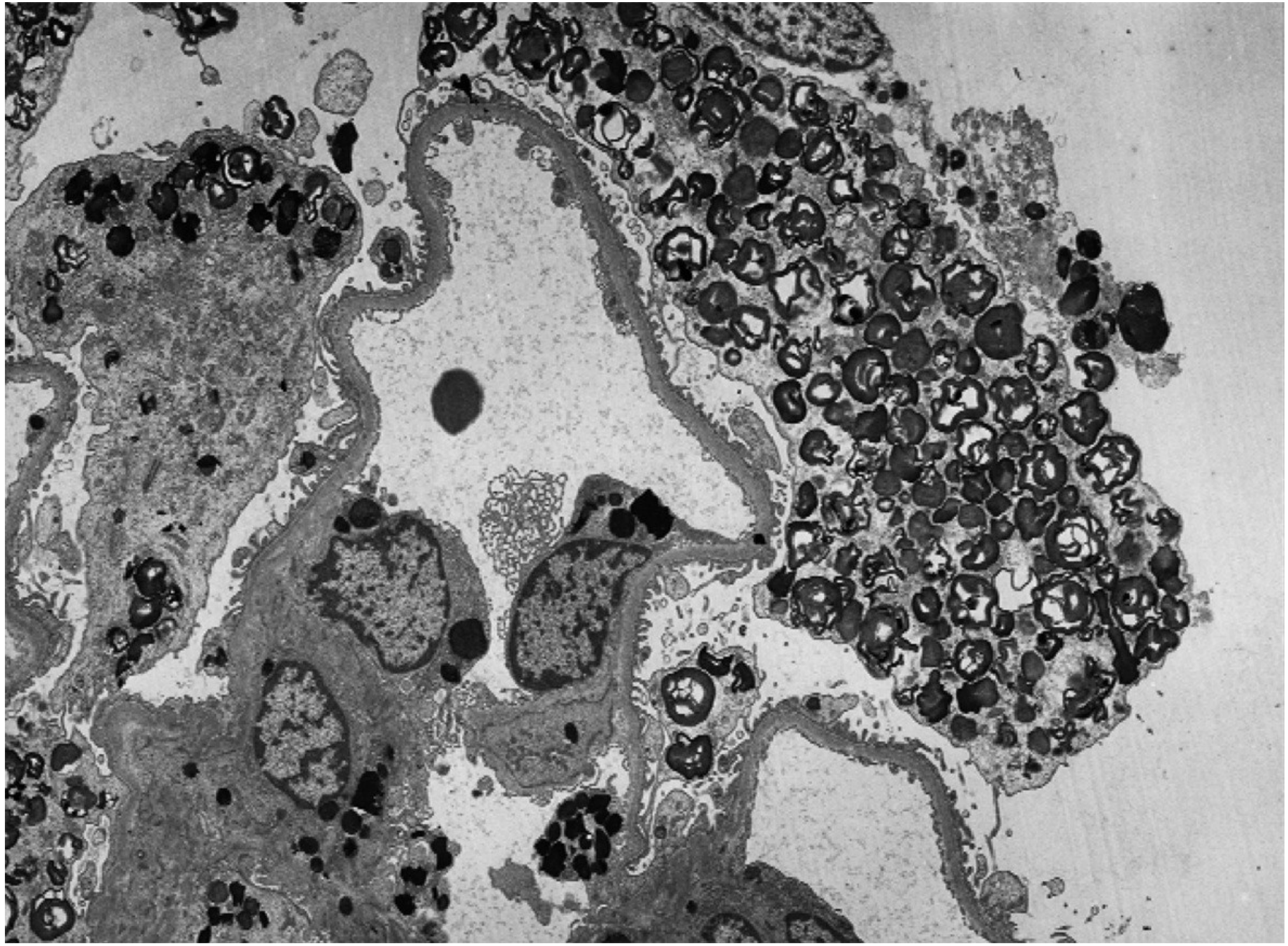


In Fabry disease, the decline in renal function over time is related to the degree of proteinuria and, in untreated patients, is more rapid when the eGFR is below 60 ml/min/1.73 $m^2$.

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.

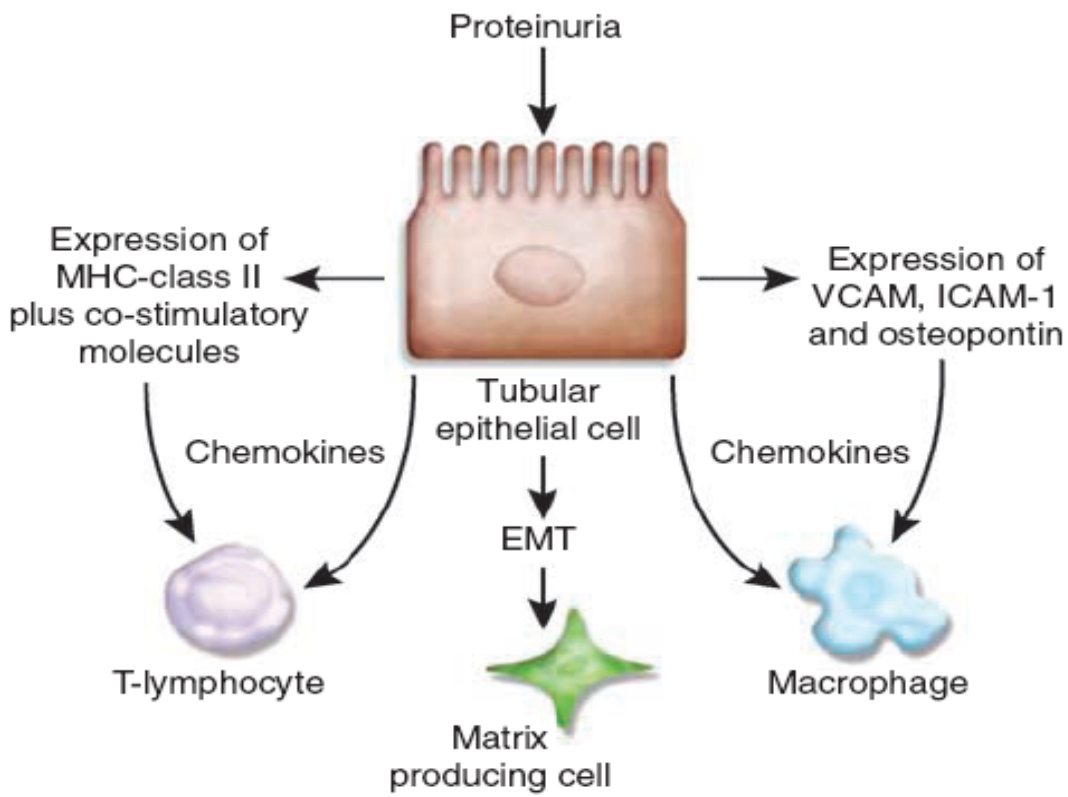



**Figure 1 | Effects of proteinuria on tubular epithelial cells.** Increased protein absorption by tubular cells may result in direct tubular toxicity, release of chemokines and cytokines, increased expression of adhesion and MHC class II molecules along with co-stimulatory molecules. The net effect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

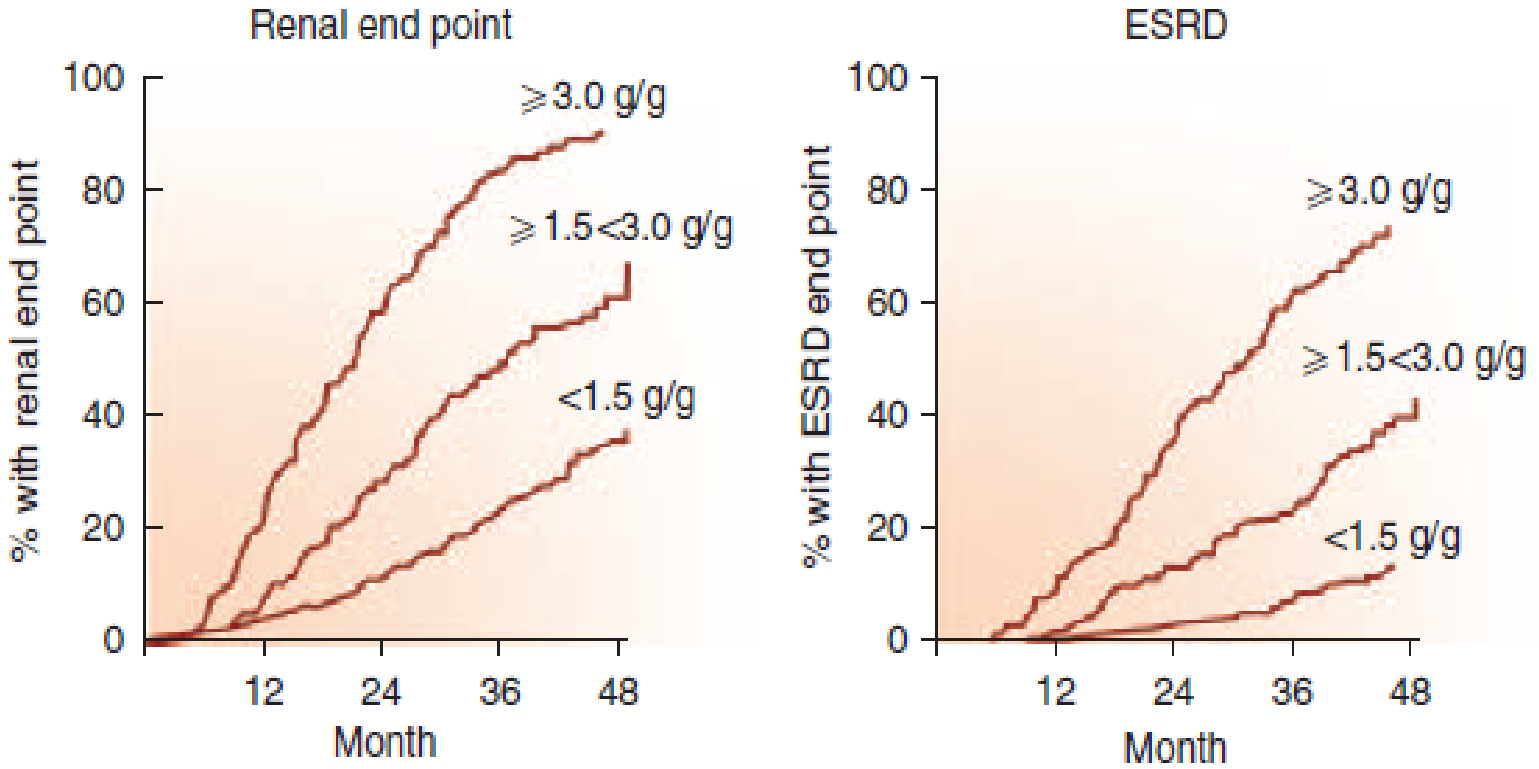
Waldek and Feriozzi *BMC Nephrology* 2014, **15**:72

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.

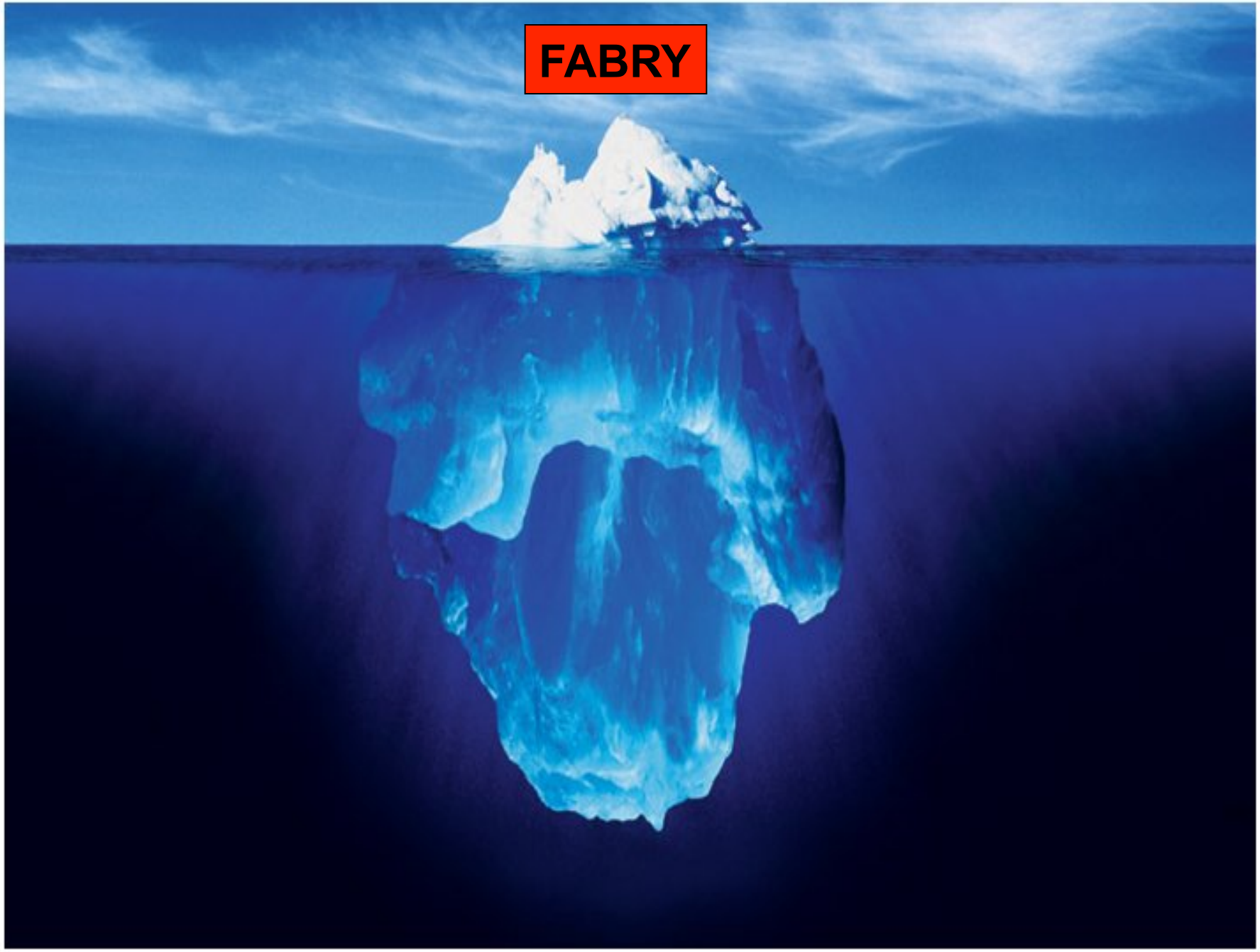
Proteinuria




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.

Waldek and Feriozzi *BMC Nephrology* 2014, **15**:72


FABRY


Glaciar Perito Moreno Santa Cruz Argentina