2nd Latin American Fabry Round Table

FABRY DISEASE AND THE KIDNEY

KIDNEY PATHOPHYSIOLOGIC PATHWAYS OF DISEASE REVISITED

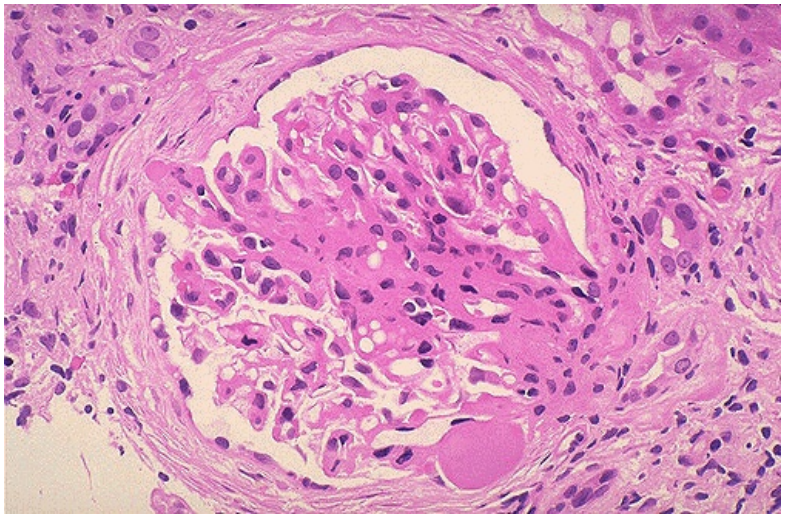
FOCUS ON THE PODOCYTE AND OTHER ISSUES

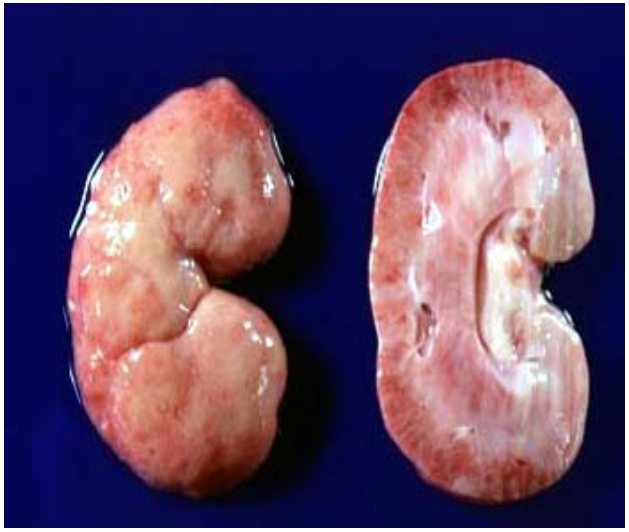
HERNÁN TRIMARCHI

CHIEF NEPHROLOGY AND RENAL TRANSPLANT SERVICES
HOSPITAL BRITÁNICO DE BUENOS AIRES
ARGENTINA

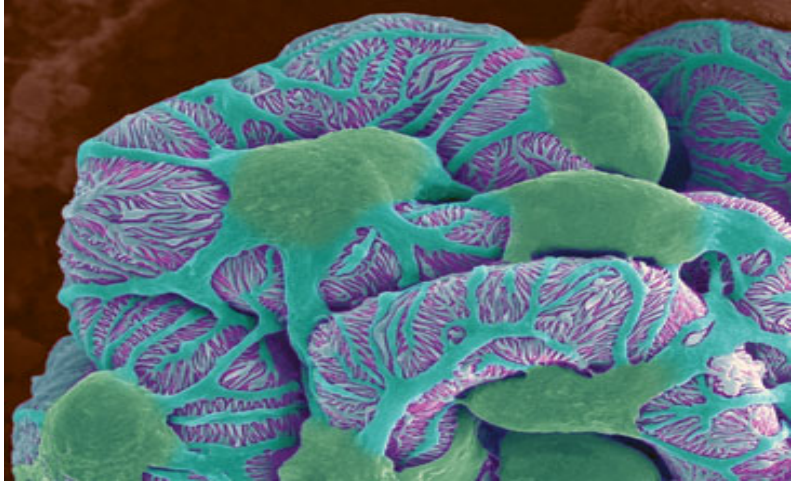
SERVICIO DE NEFROLOGÍA

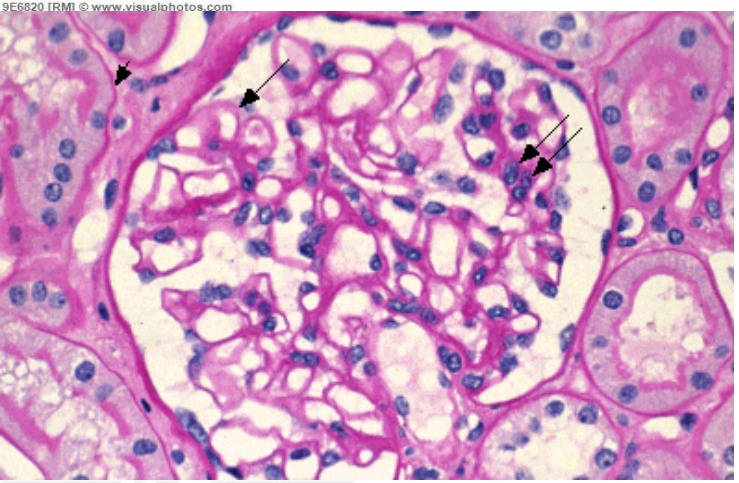
PATIENT WITH FABRY DISEASE

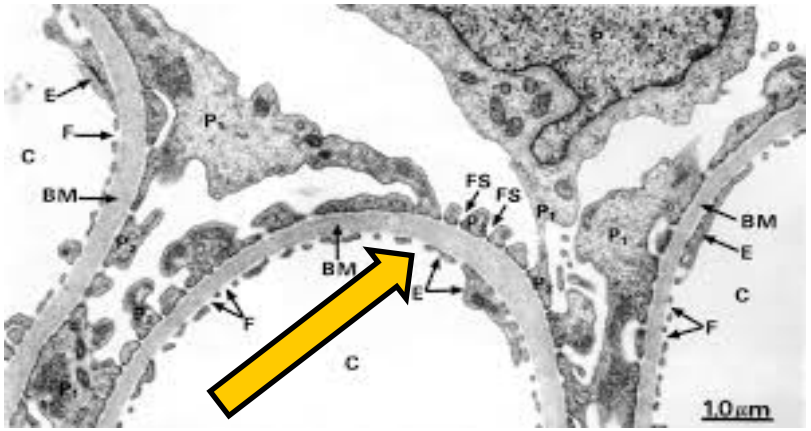





visualphotos.com
9E6820 [RM] © www.visualphotos.com



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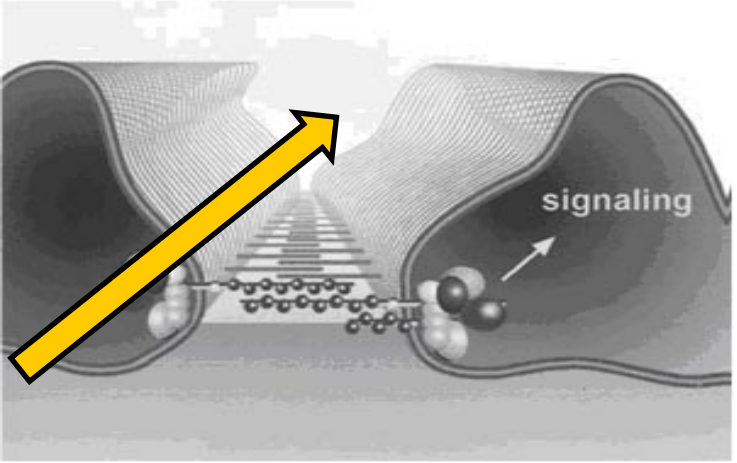
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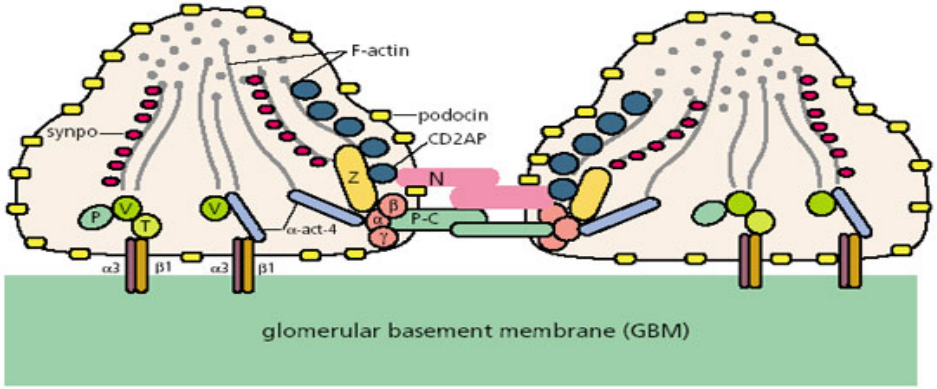
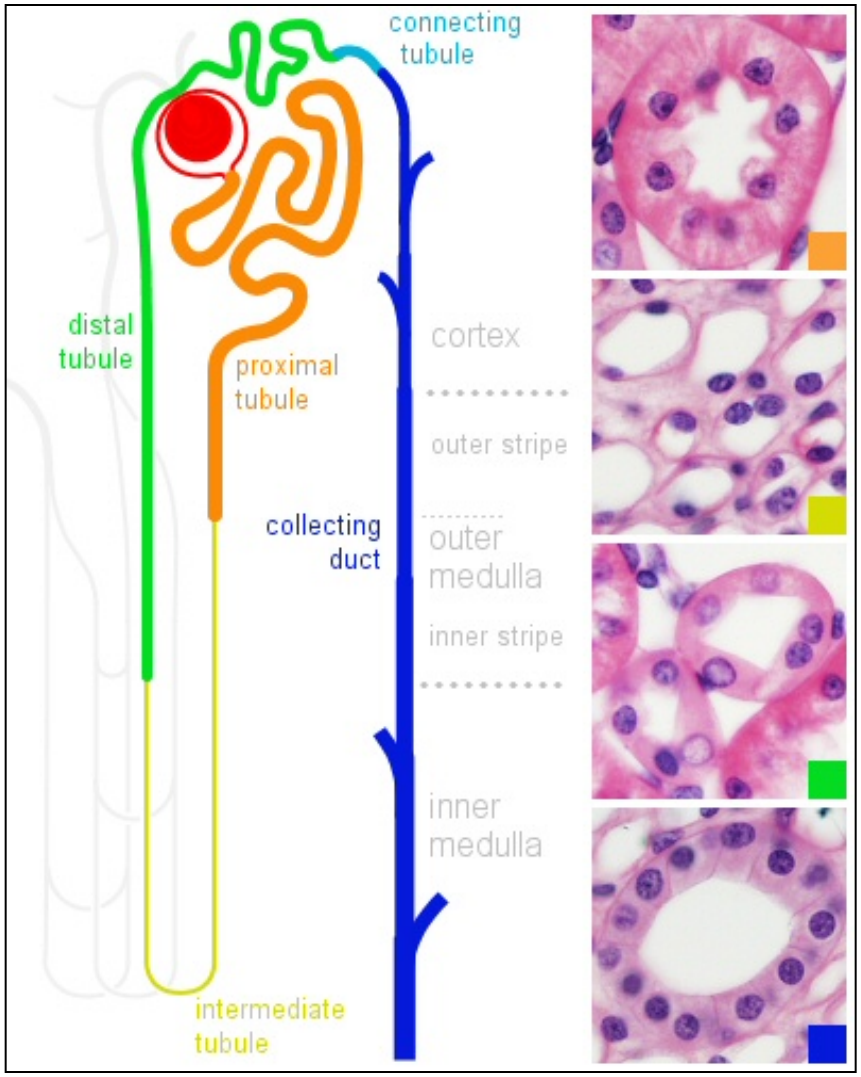

F-actin
podocin
synpo
CD2AP
Z
N
P-C
α-act-4
α3
β1
α3
β1
glomerular basement membrane (GBM)



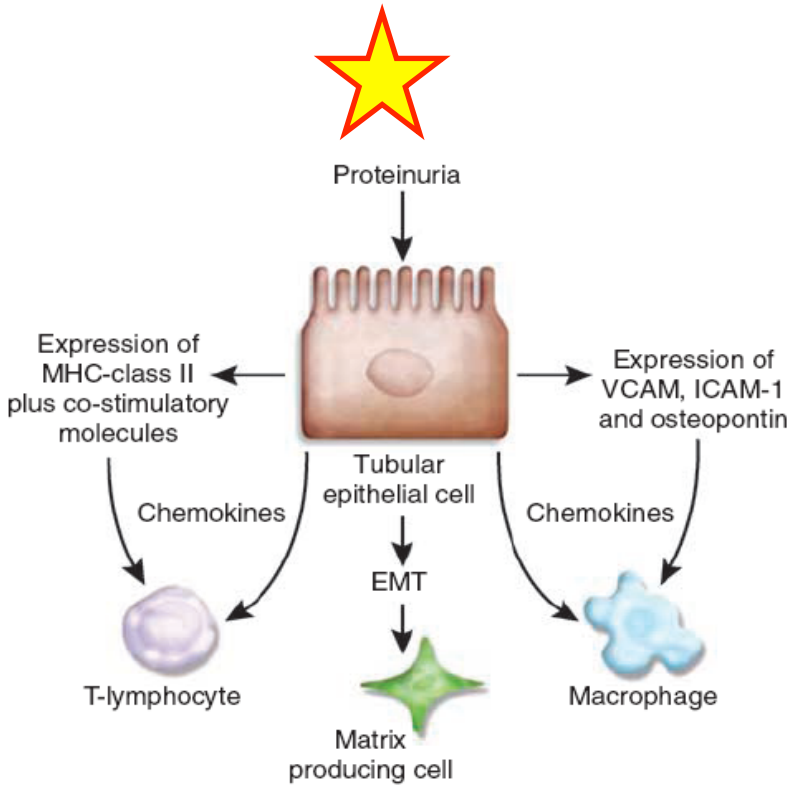


Figure 1 | Effects of proteinuria on tubular epithelial cells. Increased protein absorption by tubular cells may result in direct tubular toxicity, release of chemokines and cytokines, increased expression of adhesion and MHC class II molecules along with co-stimulatory molecules. The net effect is an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

Proteinuria – What component of the barrier is responsible?

The podocyte,

Glomerular basement membrane,

Fenestrated endothelium, or the

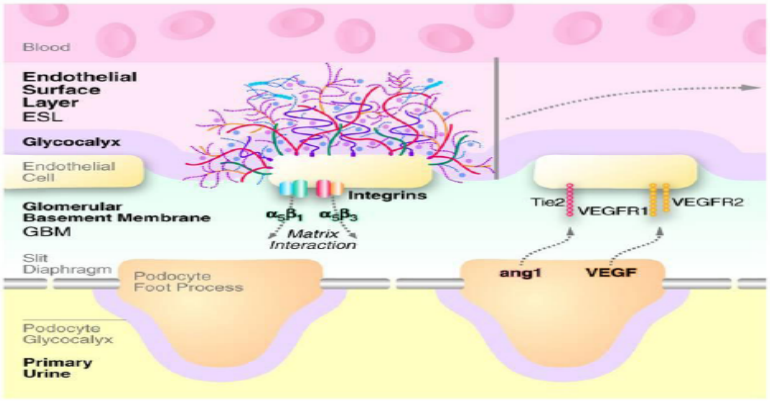
Endothelial surface layer?

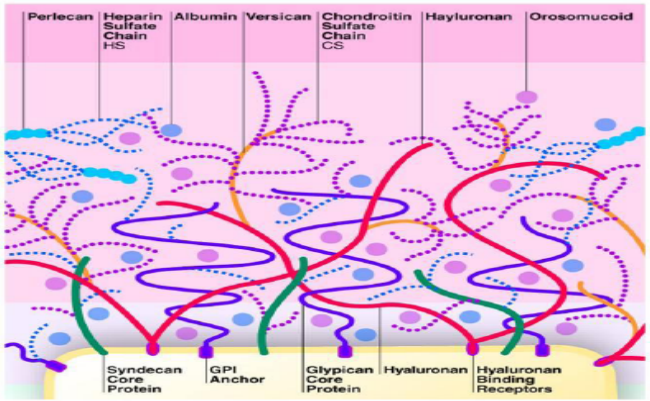
The correct answer is probably – All of the above!

The Sahlgrenska Academy

UNIVERSITY OF GOTHENBURG

Glomerular endothelial cell surface layer





Haraldsson, Nyström & Deen. Properties of the glomerular barrier and mechanisms of proteinuria. Physiol. Rev. 88: 451-487 2008;

Podocyte injury in focal segmental glomerulosclerosis: Lessons from animal models (a play in five acts)

VD D'Agati[1]

Kidney International (2008) **73,** 399–406; doi:10.1038/sj.ki.5002655;

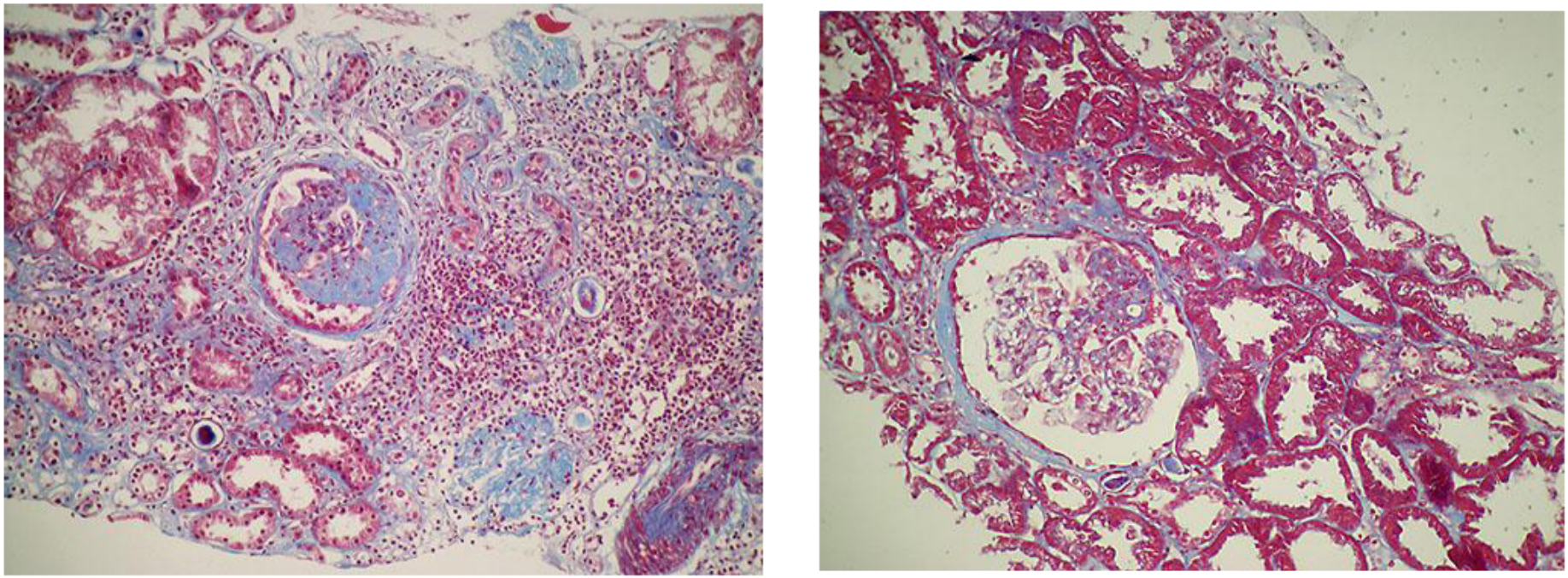


Fig. 1. Focal and segmental glomerulosclerosis and mild to moderate interstitial fibrosis. Trichrome stain. ×400.

Case Rep Nephrol Urol 2013;3:51–57

DOI: 10.1159/000351516

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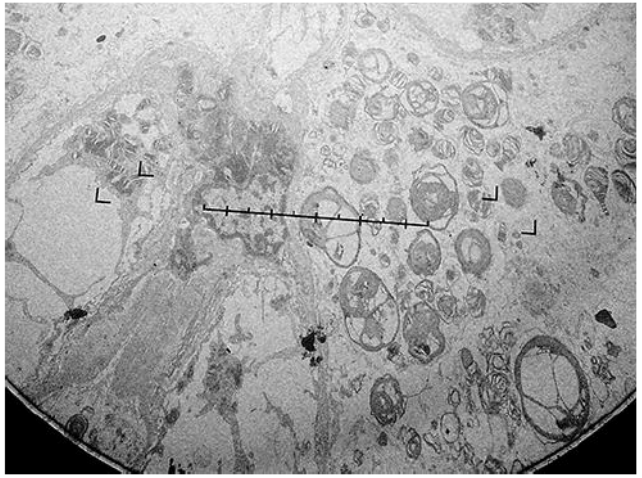
ACT 1: SEEING IS BELIEVING: ULTRASTRUCTURAL STUDIES PROVIDE MECHANISTIC INSIGHTS



Case Reports in Nephrology and Urology

Case Rep Nephrol Urol 2013;3:51–57

DOI: 10.1159/000351516
Published online: May 4, 2013

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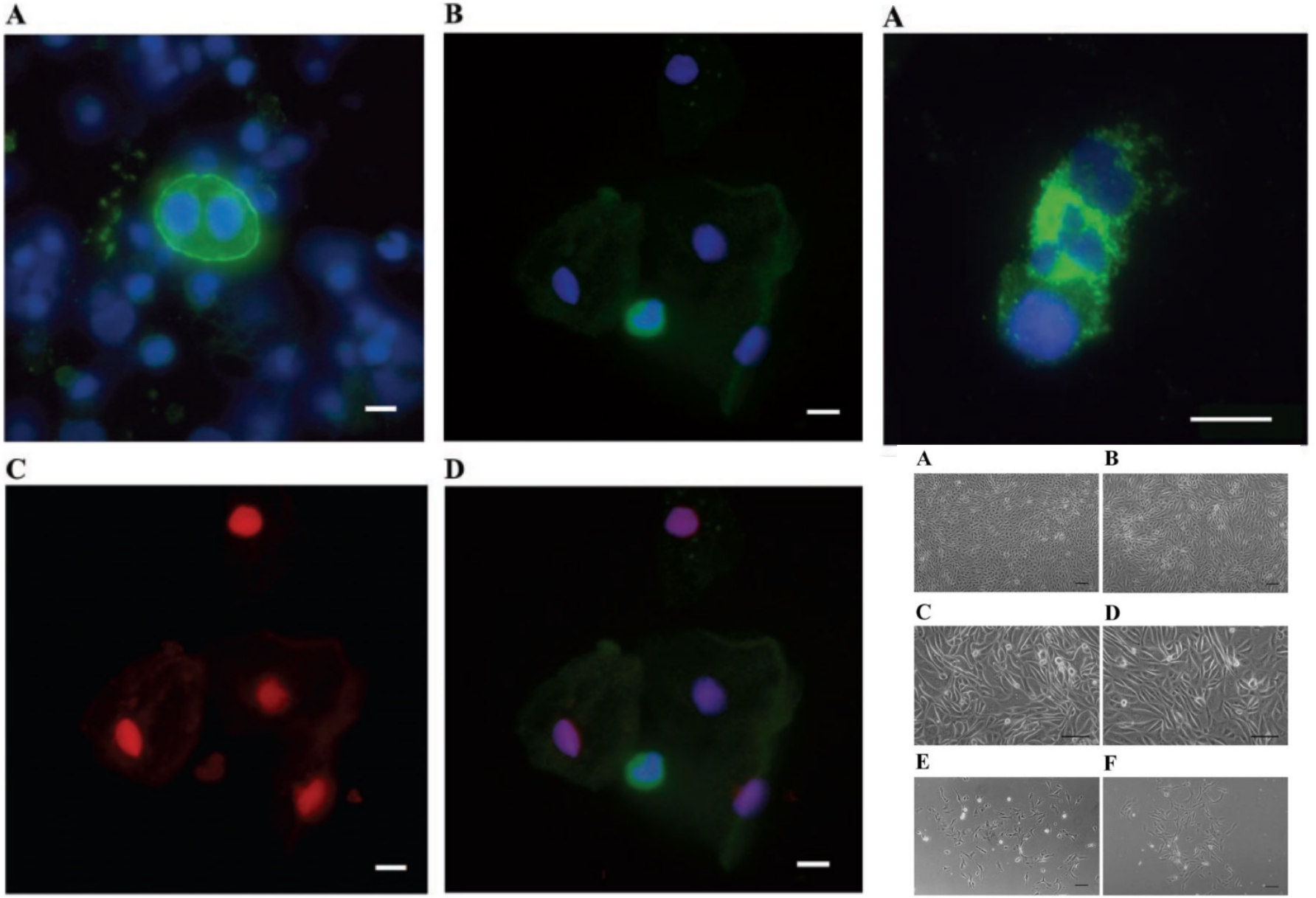
Initially Nondiagnosed Fabry's Disease when Electron Microscopy Is Lacking: The Continuing Story of Focal and Segmental Glomerulosclerosis

H. Trimarchi[a] A. Karl[a] M.S. Raña[a] M. Forrester[a] V. Pomeranz[a]
F. Lombi[a] A. Iotti[b]

[a]Nephrology Service and [b]Histopathology Service, Hospital Británico, Buenos Aires, Argentina

ACT 2: GOING, GOING, GONE… THE ROLE OF PODOCYTE DEPLETION

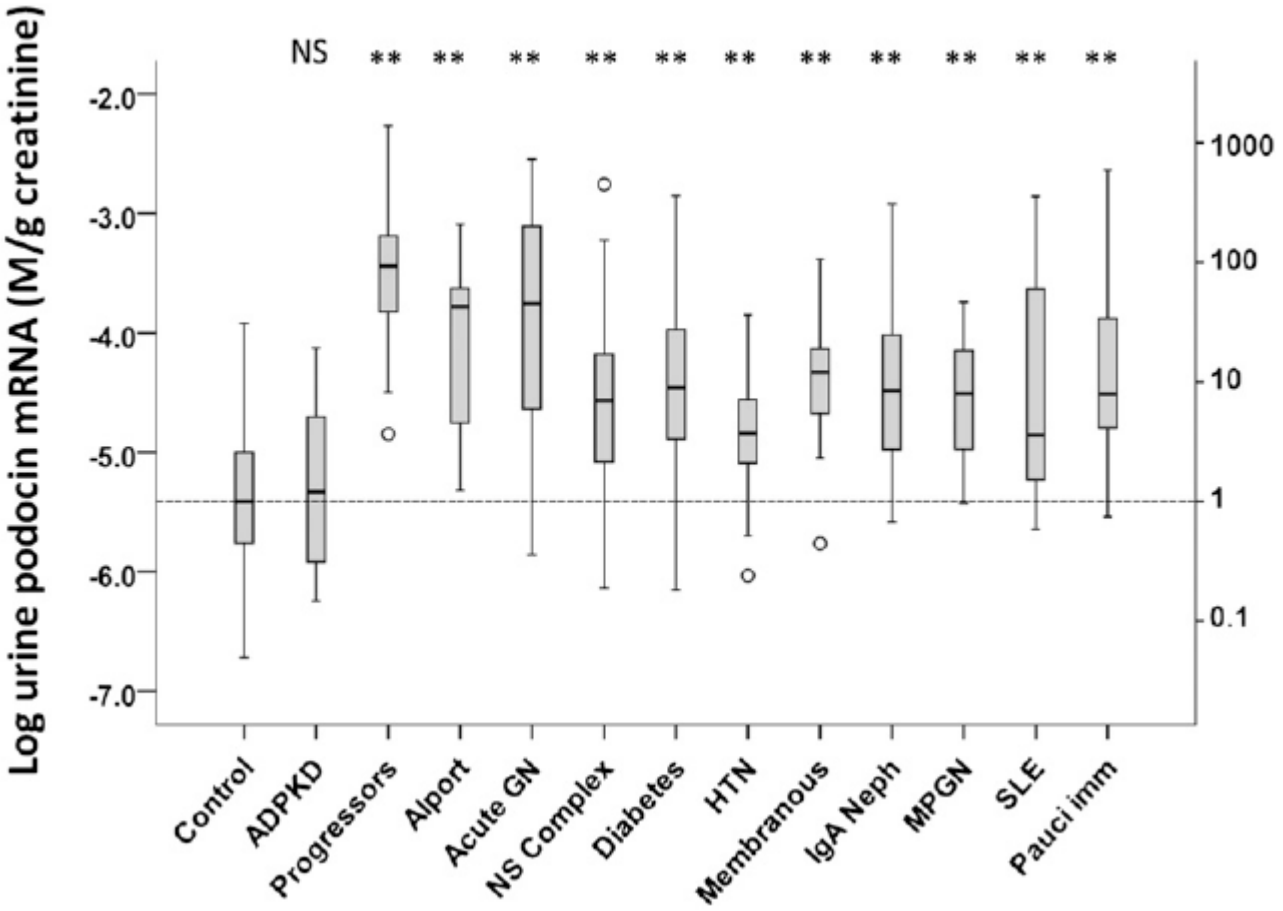


Vogelmann et al.

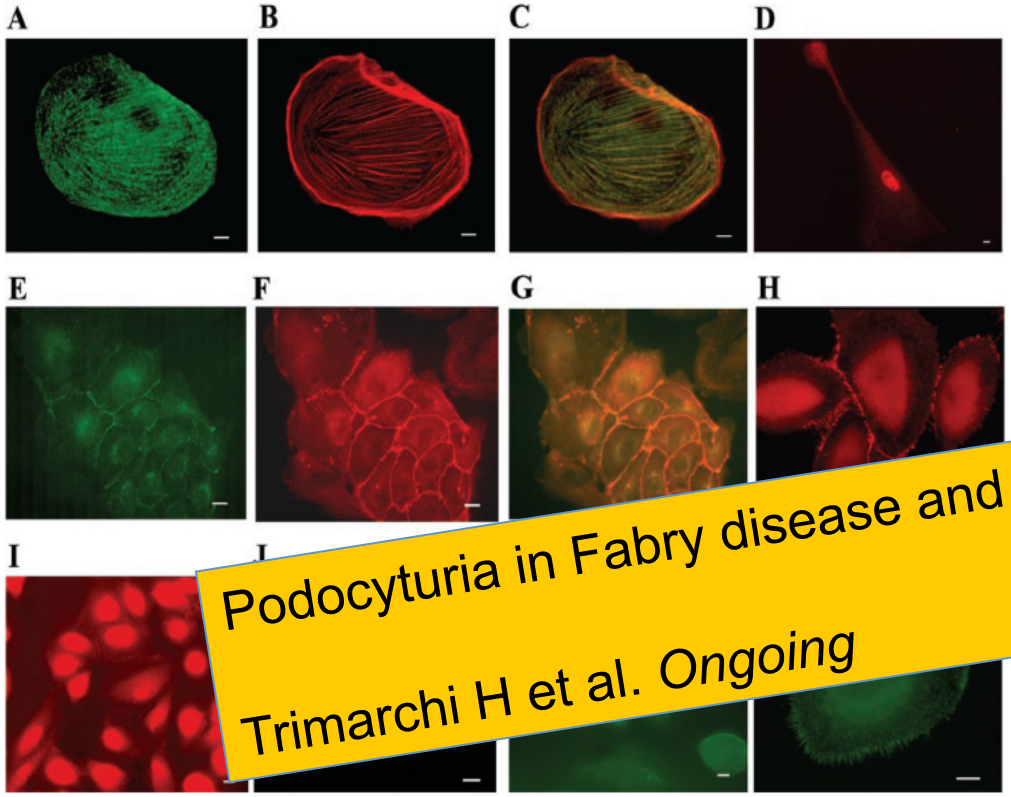
Am J Physiol Renal Physiol. 2003 July ; 285(1): F40–F48.

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†

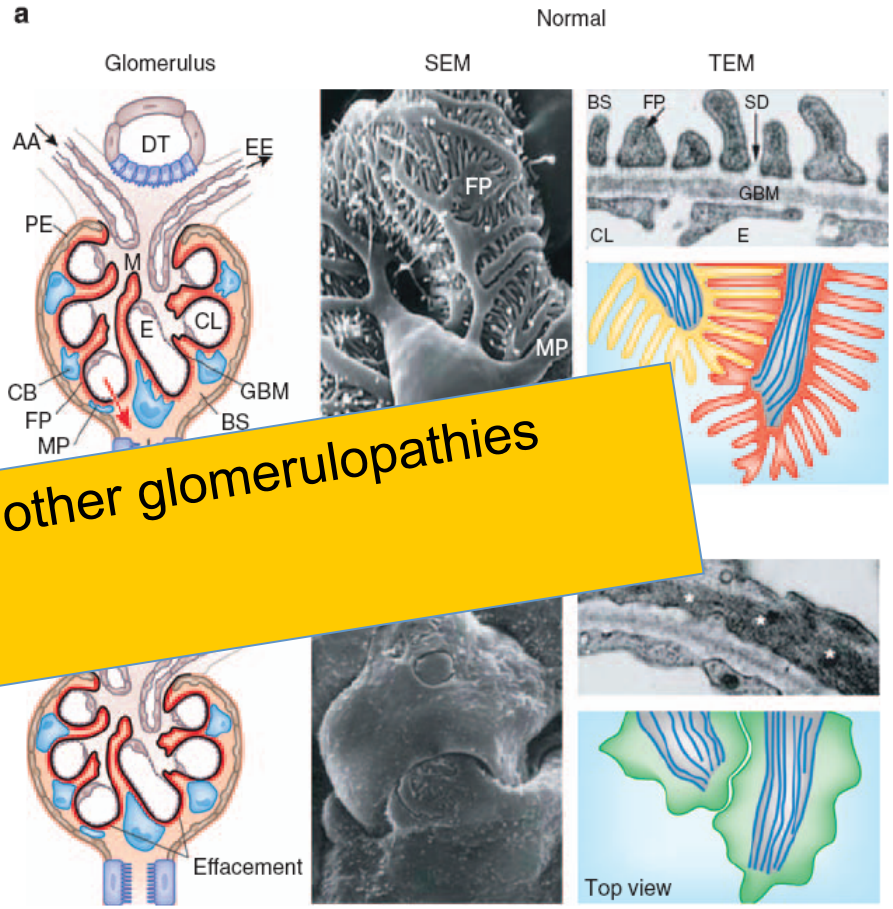


J Am Soc Nephrol **24:** 2081–2095, 2013



Vogelmann et al.

Am J Physiol Renal Physiol. 2003 July ; 285(1): F40–F48.



Podocyturia in Fabry disease and other glomerulopathies

Trimarchi H et al. *Ongoing*

Molecular Genetics and Metabolism
2013 | 108 | 2 | S76-S77

Podocyturia correlates with proteinuria in patients with Fabry disease (FD) and is a potential biomarker of Fabry nephropathy

Cecilia Ponchiardi Brent Fall Ronald Scott Stefanie Uhrich Michael Mauer
Chester Whitley Jeffrey Pippin Stuart Shankland Jonathan Jefferson Behzad Najafian

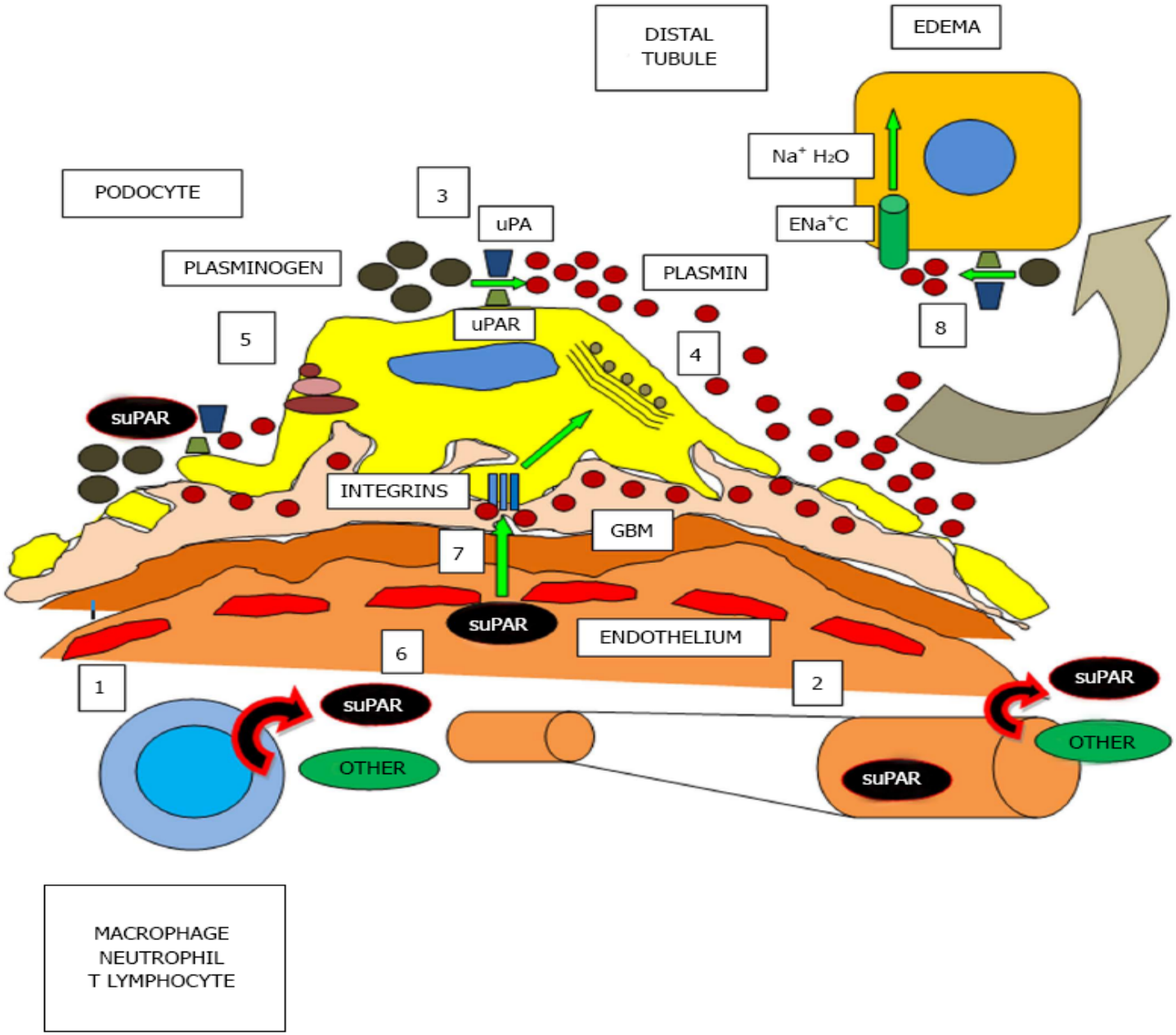
ACT 3: PROOF OF CONCEPT: ANIMAL MODELS AND THE GENETIC BASIS OF FSGS

PRIMARY: GENETIC – protein mutations
ACQUIRED – permeabilty factors

SECONDARY: Reduced renal mass
Vesicoureteral reflux
Obesity
HIV
As a result of a primary glomerular disease- FABRY

Kidney International (2008) **73,** 399–406; doi:10.1038/sj.ki.5002655;

Trimarchi H. Focal segmental glomerulosclerosis and suPAR



World J Nephrol 2013 November 6; 2(4): 103-110

(19) **United States**

(12) **Patent Application Publication**

Reiser

(10) **Pub. No.: US 2011/0212083 A1**

(43) **Pub. Date: Sep. 1, 2011**

(54) **ROLE OF SOLUBLE UPAR IN THE PATHOGENESIS OF PROTEINURIC KIDNEY DISEASE**

(75) Inventor: **Jochen Reiser**, Miami, FL (US)

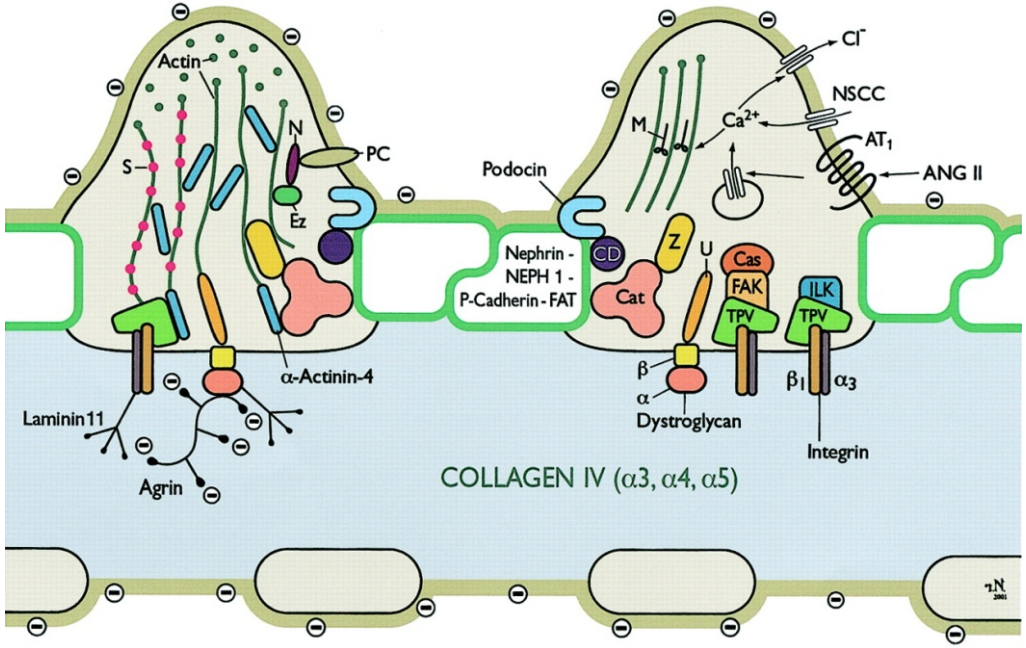
Publication Classification

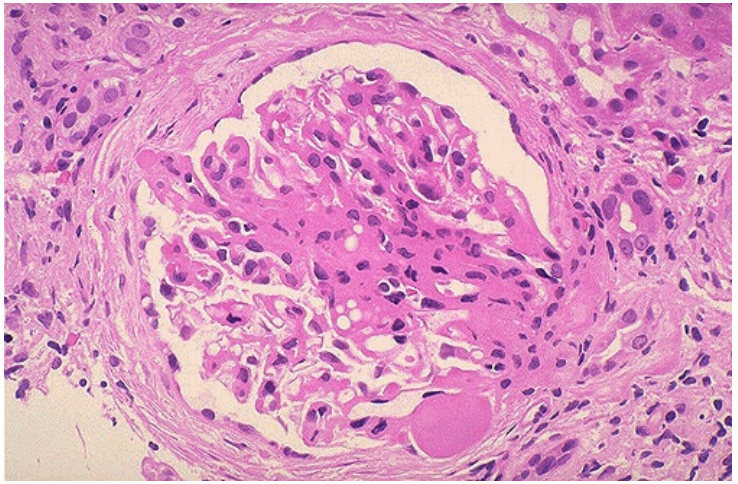
(51) **Int. Cl.**

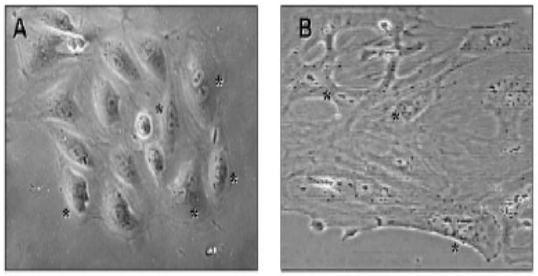
A61K 39/395	(2006.01)
A61K 31/7105	(2006.01)
A61K 38/02	(2006.01)

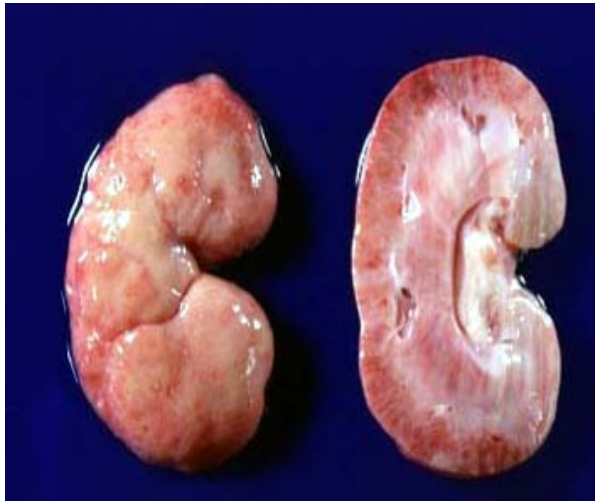
thereafter detecting the presence of uPAR. Preferred conditions include kidney disease comprises: podocyte diseases or disorders, proteinuria, glomerular diseases, membranous glomerulonephritis, focal segmental glomerulonephritis, minimal change disease, nephrotic syndromes, pre-eclampsia, eclampsia, kidney lesions, collagen vascular diseases, stress, strenuous exercise, benign orthostatic (postural) proteinuria, focal segmental glomerulosclerosis (FSGS), IgA nephropathy, IgM nephropathy, membranoproliferative glomerulonephritis, membranous nephropathy, sarcoidosis, Alport's syndrome, diabetes mellitus, kidney damage due to dr[illegible], Fabry's disease, [illegible]ctions, aminoaciduria, Fanconi

ACT 4: MORE IS NOT NECESSARILY BETTER: PODOCYTE DYSREGULATION









Iney International (2008) **73,** 399–406; doi:10.1038/sj.ki.5002655;

Biomarkers of Fabry Disease Nephropathy

Raphael Schiffmann,* Stephen Waldek,† Ariela Benigni,‡ Christiane Auray-Blais§

*Institute of Metabolic Disease, Baylor Research Institute, Dallas, Texas; †Hope Hospital, Salford Royal Hospital Trust, Manchester, United Kingdom; ‡Mario Negri Institute for Pharmacological Research, Bergamo, Italy; and §Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, Quebec, Canada

It is suggested that biomarkers of renal complications of Fabry disease are likely to be useful for diagnosis and to follow the natural disease progression or the effect of specific therapeutic interventions. Traditionally, globotriaosylceramide (Gb_3) in urine has been used to evaluate the effect of specific therapy, such as enzyme replacement therapy (ERT). Although urinary Gb_3 decreases significantly with ERT, it has not yet been shown to be a valid surrogate marker in treatment trials. We propose a detailed study of the nature and origin of Gb_3 combined with a prospective collaborative trial that combines Gb_3 changes with the effect of ERT on clinical nephrological outcome measures. Existing biomarkers such as general proteinuria/albuminuria or specific proteins such as *N*-acetyl-β-D-glucosaminidase should be evaluated along with novel proteomic or metabolomic studies for biomarker discovery using mass spectrometry or nuclear magnetic resonance. Standard scoring of all pathologic aspects of kidney biopsies may also be a promising way to assess the effect of therapy.

Clin J Am Soc Nephrol 5: 360–364, 2010. doi: 10.2215/CJN.06090809

Globotriaosylceramide (Gb_3), the main substrate of the deficient α-galactosidase A in Fabry disease, is known to be increased in patients' urine (2,3). It is consistently elevated in

One key question remains unanswered: Where does the Gb_3 biomarker come from? Urinary Gb_3 has its origin mostly in kidney tubular cells of the kidney and urinary collecting system. These assumptions are based on the presence of Gb_3 in lysosomes of renal tubular cells shed in the urine and an 80%

would indicate that the shedding of podocytes or even leakage through renal glomeruli of circulating Gb_3 may also be a significant source of this glycosphingolipid in urine (4,14).

Urinary Proteomics

Urinary and Plasma Metabolomics

Proteinuria

This is the most important biomarker in Fabry kidney disease (1,30). Protein levels should be kept as low as possible. When elevated, protein levels should be brought down with angiotensin converting enzyme inhibitors/angiotensin receptor blockers to <0.5 g/24 h (20).

ACT 5: THE MISSING LINK: HOW IRREVERSIBLE FOOT PROCESS EFFACEMENT AND PODOCYTE LOSS PROMOTE GLOMERULOSCLEROSIS

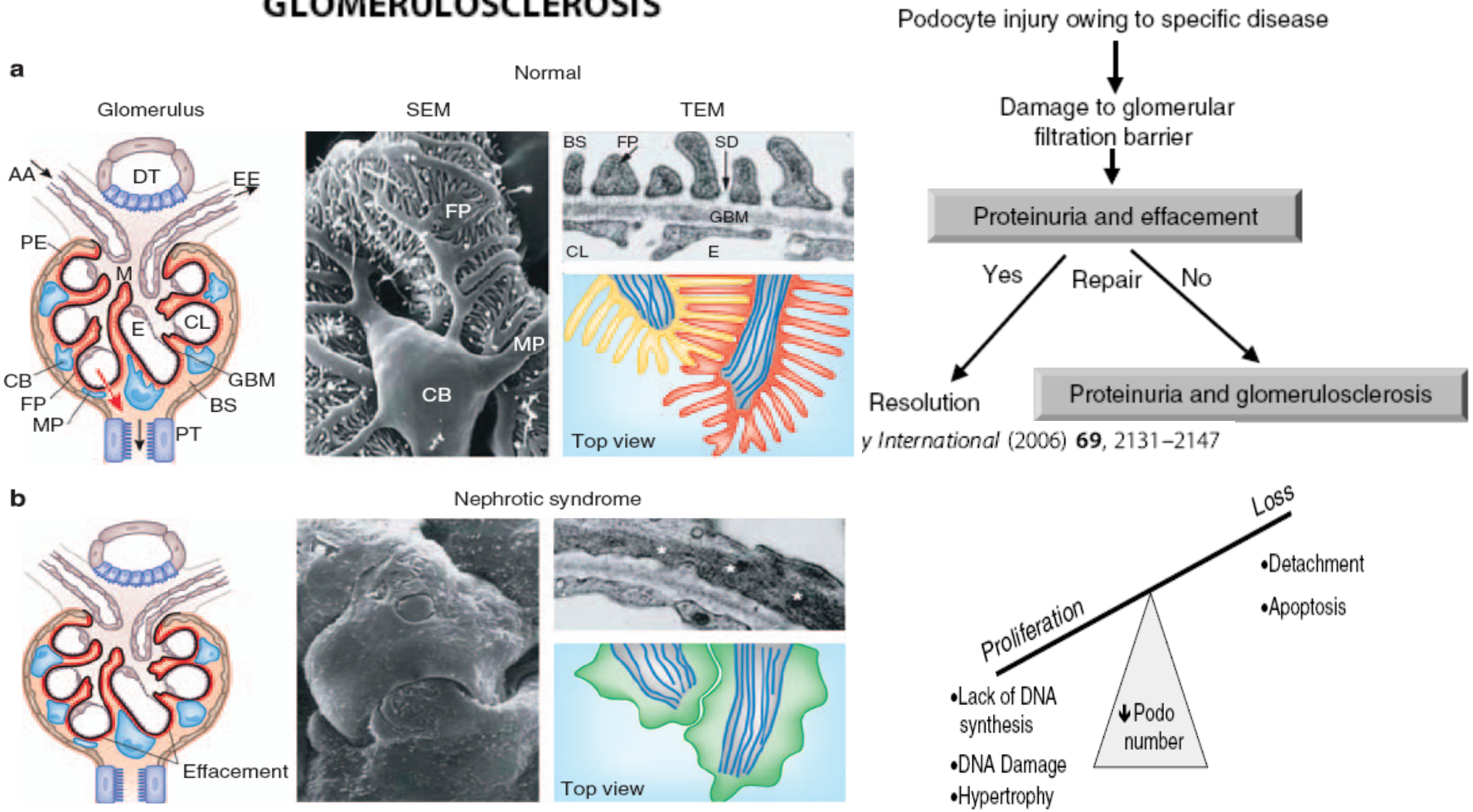


Figure 2 | Factors governning podocyte number. Total podocyte (podo) number is a balance between proliferation and loss. Podocyte number is reduced by either a decrease in proliferation owing to lack of DNA synthesis, DNA damage or hypertrophy, and/or an in crease in podocyte loss owing to detachment and apoptosis.

The podocyte's response to injury: Role in proteinuria and glomerulosclerosis

SJ Shankland[1]

Table 1 | Diseases of the podocyte

Podocyte disease	Cause of injury	Mechanism/mediator
Membranous nephropathy	Anti-podocyte antibodies	C5b-9
Minimal change disease	T cell mediated	Not well defined
Classic FSGS	Hereditary	α-Actinin-4 mutation Podocin mutation CD2AP haploinsufficiency
	Increased Pgc owing to: • Obesity • Diabetes • Hypertension • Reduced nephron number ↓Podocyte number	Podocyte stress–tension Apoptosis Detachment Lack of proliferation DNA damage Hypertrophy
	Circulating factors Sporadic disease	Permeability factor(s) α-Actinin-4 mutation Podocin mutation
Cellular/collapsing FSGS	Infections	HIV Parvo B19?
	Drugs	Pamidronate Interferon
Diabetic nephropathy	Metabolic Increased Pgc	Hyperglycemia Podocyte stress–tension
Amyloid	Amyloid protein deposition	Amyloid spicules directly injure podocyte
MPGN	Deposition of antigen–antibody complexes	Splitting of GBM Podocyte effacement

FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane.

FABRY DISEASE

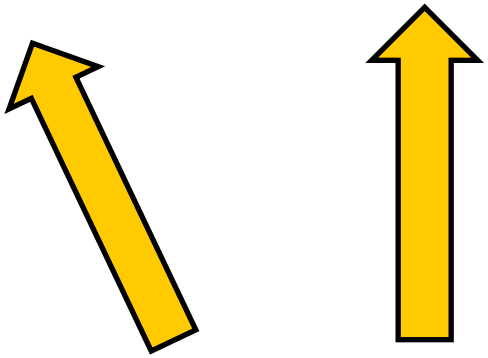
GL-3 *globotriaosylceramide*
LYSO GL-3 deposition

?

Table 5 | Podocyte number in glomerular diseases

Podocyte number normal	Podocyte number decreased	Podocyte number increased
Membranous nephropathy	Membranous nephropathy	HIV-associated nephropathy
Minimal change disease	Diabetic nephropathy	Cellular/collapsing FSGS
Classic FSGS	Classic FSGS	Crescentic glomerulonephrtitis
	Amyloid	
	Aging	

FSGS, focal segmental glomerulosclerosis.

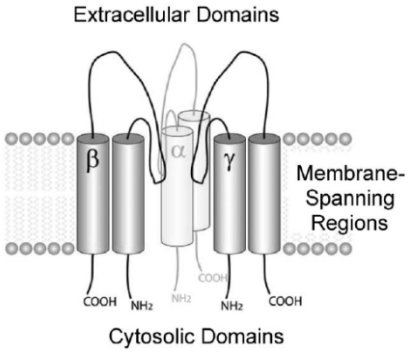


WHERE MAY FABRY DISEASE BE?

Kidney International (2006) **69**, 2131–2147

Hypertension

Journal of the American Heart Association



American Heart Association®

The Amiloride-Sensitive Endothelial Sodium Channel and Vascular Tone

David G. Warnock

Hypertension. 2013;61:952-954; originally published online March 4, 2013;

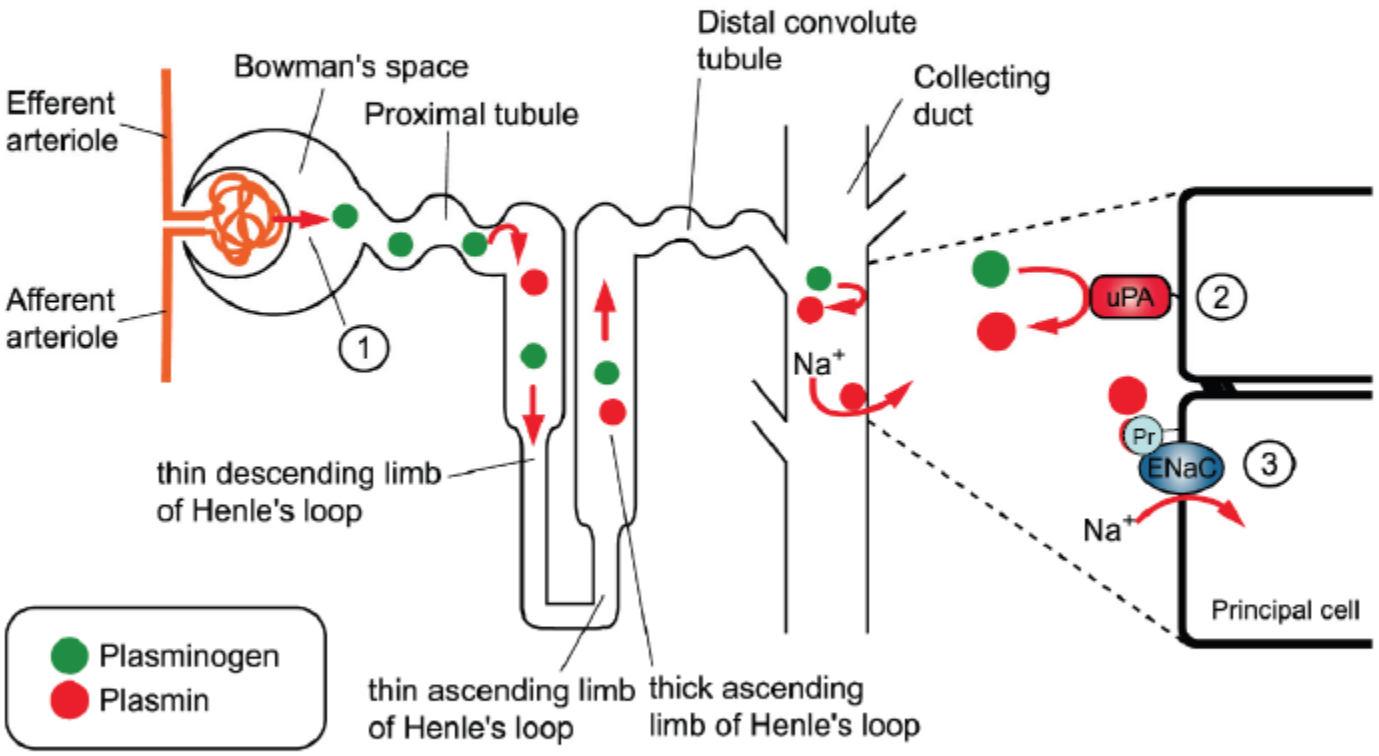
AMILORIDE AS AN ALTERNATE ADJUVANT ANTIPROTEINURIC AGENT IN FABRY DISEASE. THE POTENTIAL ROLES OF PLASMIN AND uPAR

Trimarchi H, Forrester M, Lombi F, Pomeranz V, Raña MS, Karl A, Andrews J

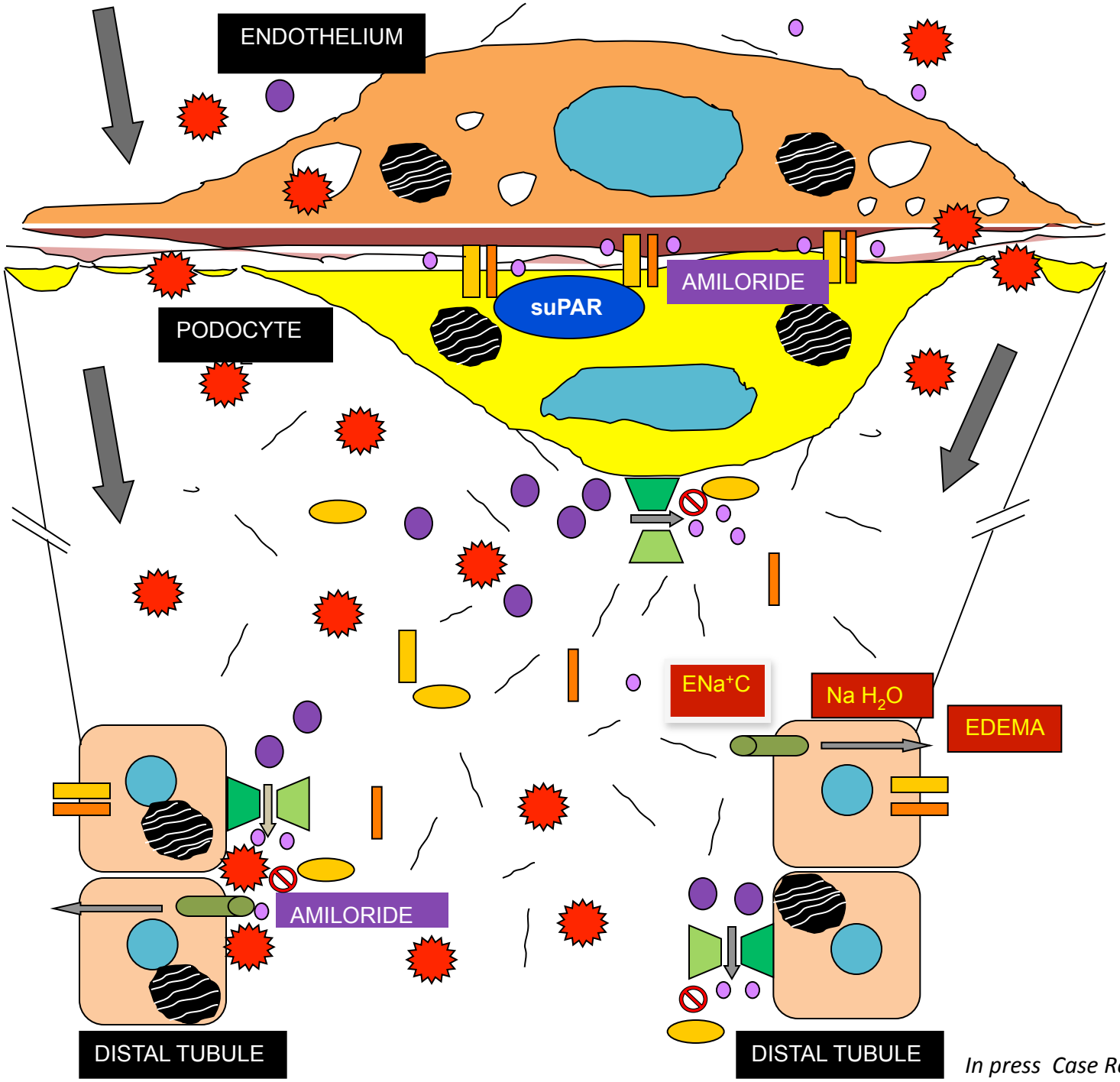
Nephrology Service, Hospital Británico de Buenos Aires, Argentina

In press Case Reports in Nephrology

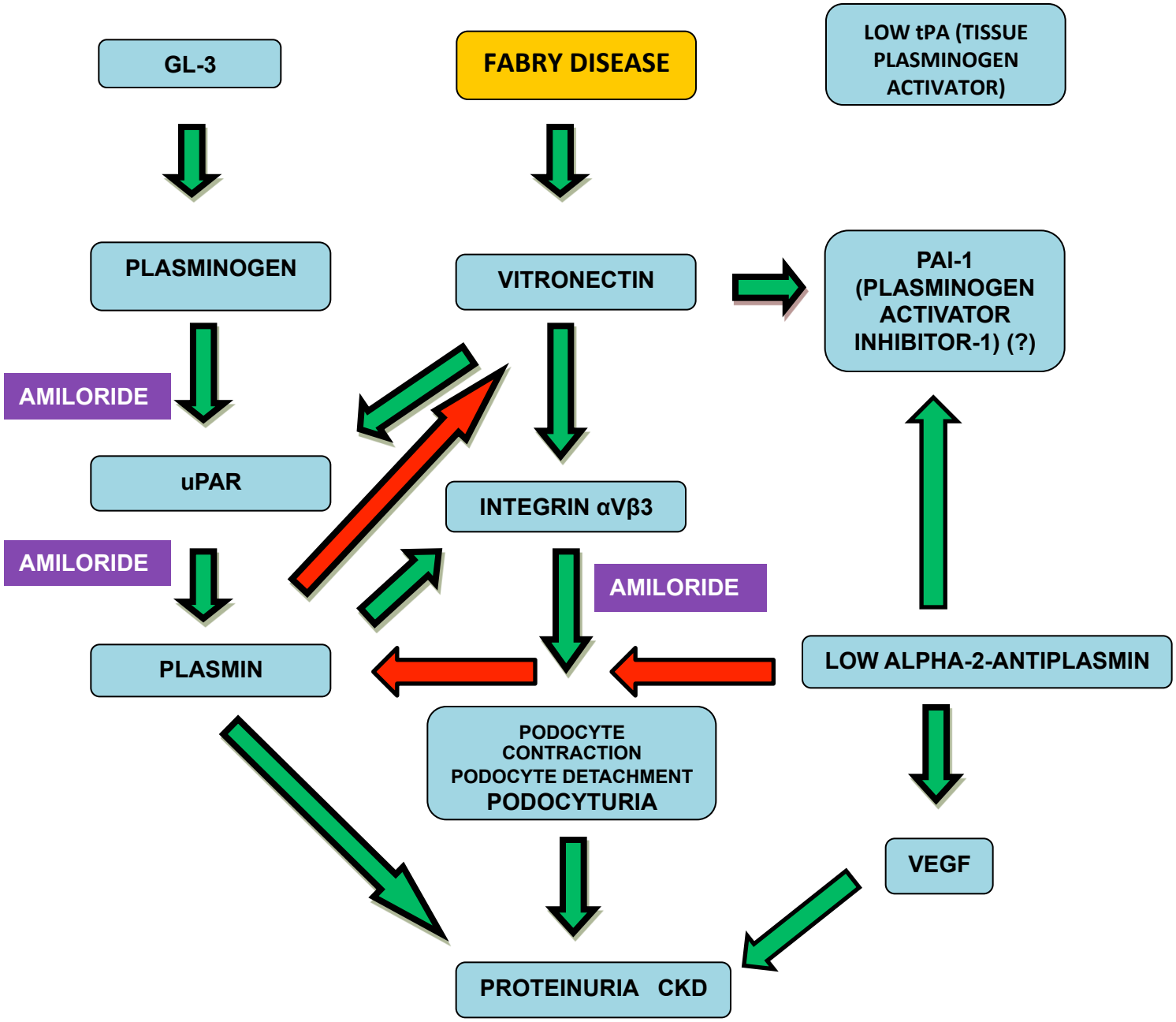
A novel model for stimulation of sodium reabsorption in nephrotic syndrome



Relevant for human pathophysiology?



In press Case Reports in Nephrology



In press Case Reports in Nephrology

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 [5].

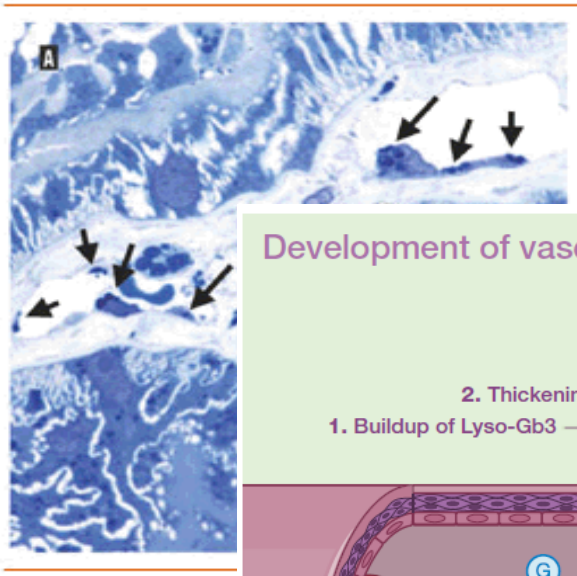
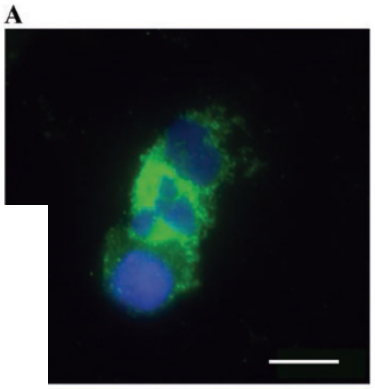
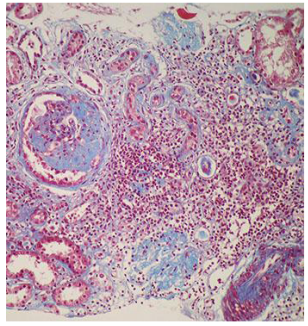
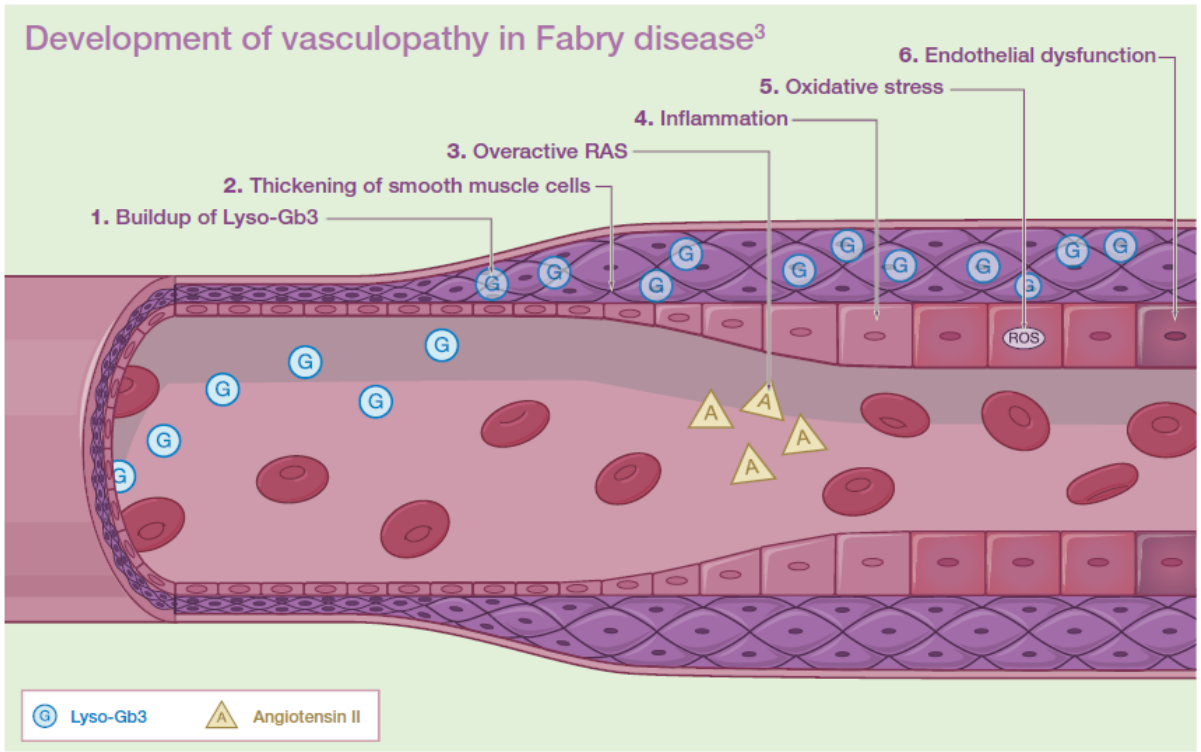


Figure 1. Renal Capillary Endothelium
Light microscopy of renal capillary endothelium in a patient with Fabry disease – arrows indicate

DECREASED NO



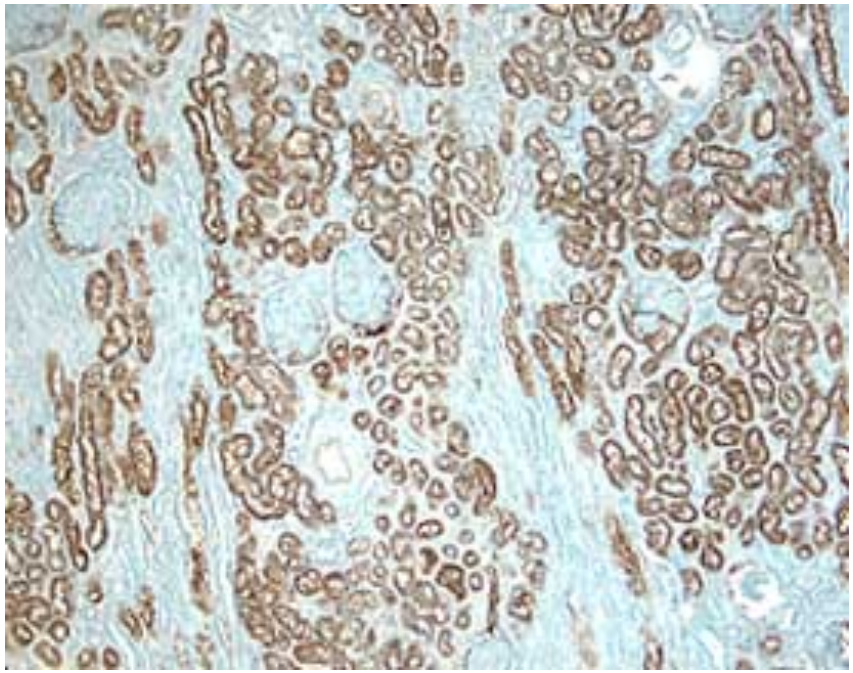


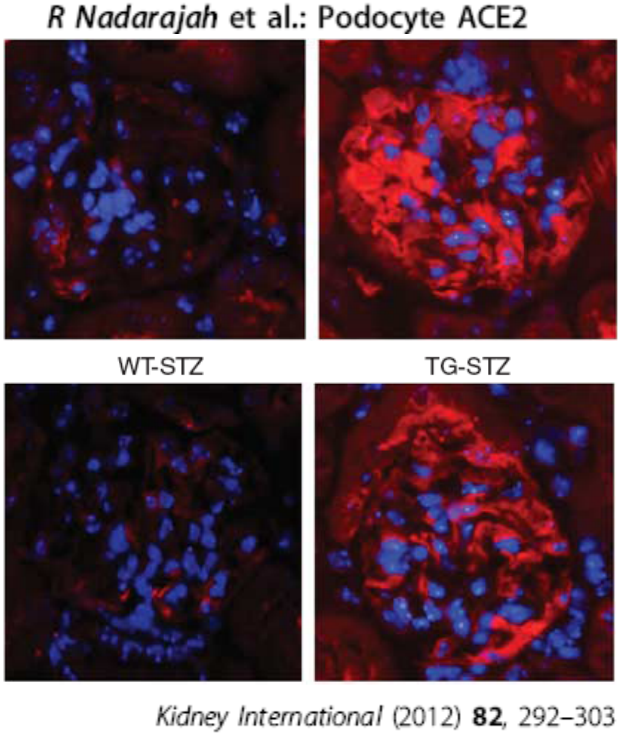


5. Najafian B, Svarstad E, Bostad L, Gubler MC, Tøndel C, Whitley, Mauer M. Progressive podocyte injury and globotriaosylceramide (GL-3) accumulation in young patients with Fabry disease. Kidney Int 2011; 79: 663–670.

Interestingly, besides the well-known **angiotensin II** roles in vasoconstriction, inflammation and fibrosis, it is also involved in the pathogenesis of Fabry's disease at the *second hit* stage.

Angiotensin converting enzyme (ACE) is expressed in the plasma membrane of vascular endothelial cells, epithelial cells of renal proximal tubules, gastrointestinal tract, heart and in various regions of the brain, the main tissues affected in Fabry disease [6].



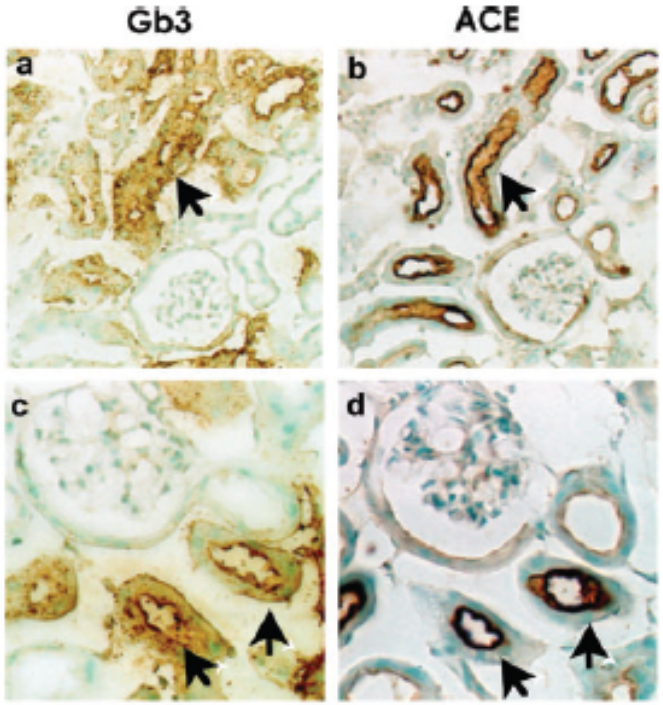


6. Carneiro Batista E, Carvalho LR, Casarini DE, Karaoglanovic Carmona A, dos Santos EL, Dias da Silva E, dos Santos RA, Ryuichi Nakaie C, Muñoz Rojas MV, Macedo de Oliveira S, Bader M, D'Almeida V, Martins AM, Picoly-Souza K, Bosco Pesquero J. ACE activity is modulated by the enzyme α-galactosidase A. J Mol Med 2011; 89:65–74.

Glycobiology vol. 15 no.12 pp. 1257–1267, 2005
doi:10.1093/glycob/cwj015
Advance Access publication on August 3, 2005

Murine glycosyltransferases responsible for the expression of globo-series glycolipids: cDNA structures, mRNA expression, and distribution of their products

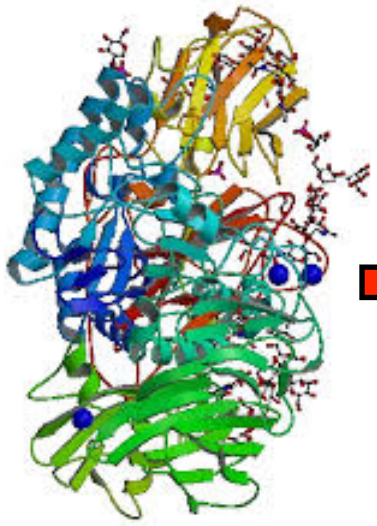
Moreover, in immunohistological analysis, GL3 is mainly expressed in the proximal tubules as revealed with coincidental expression with angiotensin-converting enzyme (ACE), suggesting that GL3 and AII may be implicated in sodium and bicarbonate homeostasis.



Yuko Fujii[2,3], Shin-ichiro Numata[2], Yoko Nakamura[2], Takashi Honda[3], Keiko Furukawa[2], Takeshi Urano[2], Joelle Wiels[4], Makoto Uchikawa[5], Noriyuki Ozaki[3], Sei-ichi Matsuo[6], Yasuo Sugiura[3], and Koichi Furukawa[1,2]

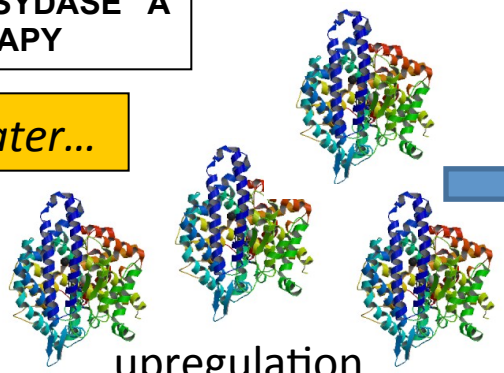
It appears that treatment with recombinant α-galactosydase A decreases ACE activity probably mediated by the release of the galactose residues from the ACE molecule.

The degree of ACE glycosylation is important for the catalytic properties of the enzyme.
In addition, glycosylation plays an important role in the release of ACE from the membrane.



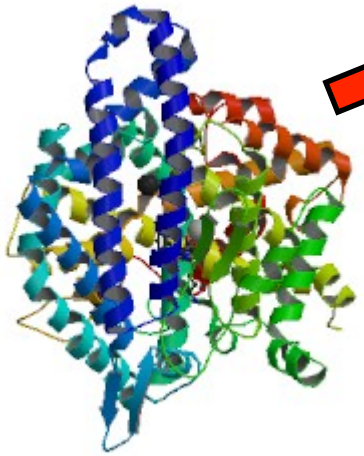
α-GALACTOSYDASE A THERAPY

2 weeks later…

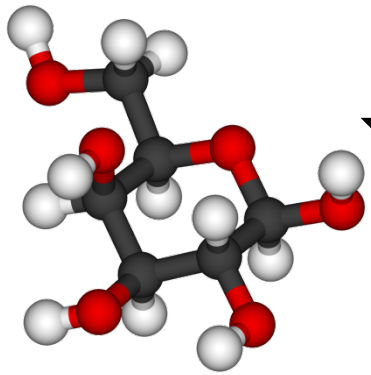


upregulation

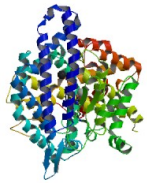
ACE



ANGIOTENSIN II



RELEASE OF GALACTOSE RESIDUES



ACTIVITY and RELEASE

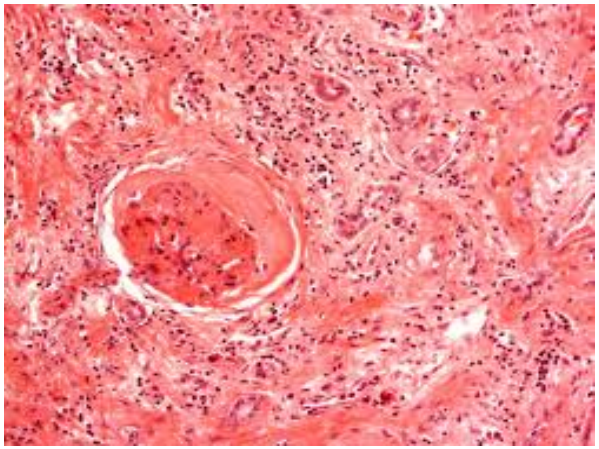
6. Carneiro Batista E, Carvalho LR, Casarini DE, Karaoglanovic Carmona A, dos Santos EL, Dias da Silva E, dos Santos RA, Ryuichi Nakaie C, Muñoz Rojas MV, Macedo de Oliveira S, Bader M, D'Almeida V, Martins AM, Picoly-Souza K, Bosco Pesquero J. ACE activity is modulated by the enzyme α-galactosidase A. J Mol Med 2011; 89:65–74.

α- Galactosidase A therapy may not be enough to protect the kidney, particularly at advanced stages of the disease and in proteinuric subjects.

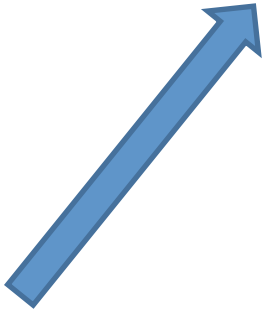
ACEi/ARB therapy as an adjunctive therapy [4,8]

4. Torra S. Renal manifestations in Fabry disease and therapeutic options. Kidney Int 2008; 74: (Suppl 111), S29–S32.

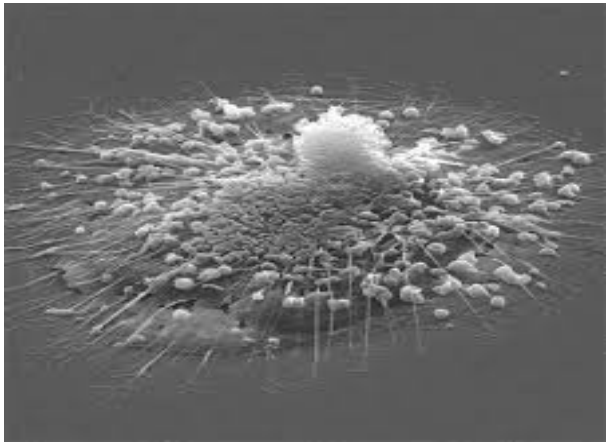
8. Tahir H, Jackson LL, Warnock DG. Antiproteinuric Therapy and Fabry Nephropathy: Sustained Reduction of Proteinuria in Patients Receiving Enzyme Replacement Therapy with Agalsidase-β. J Am Soc Nephrol 2007; 18: 2609–2617.



Thrombospondin-1 TGF-β1 VEGF FGF-2 are higher in kidneys from Fabry mice compared to wild-type mice.



caspases



PLASMINOGEN PLASMIN ALPHA-2-ANTIPLASMIN tPA PAI-1 [9,10]

9. Lee MH, Choi EN, Jeon YJ, Jung SC. Possible role of transforming growth factor-β1 and vascular endothelial growth factor in Fabry disease nephropathy. Int J Mol Med 2012; 30: 1275-1280.

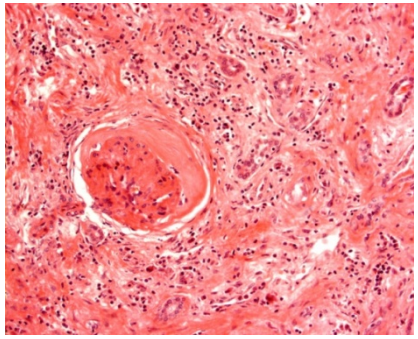
10. Moore DF, Krokhin, Beavis, Ries, Robinson, Goldin, Brady, Wilkins, Schiffmann PNAS 2007; 104: 2873-2878

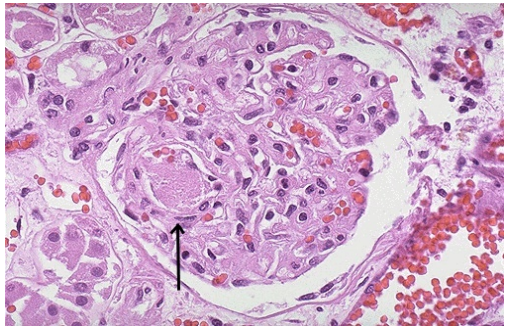
Globotriaosylsphingosine actions on human glomerular podocytes: implications for Fabry nephropathy

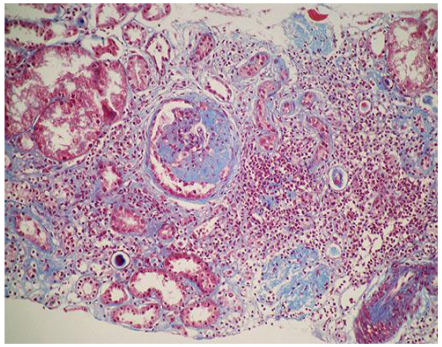
Maria D. Sanchez-Niño[1], Ana B. Sanz[2], Susana Carrasco[1], Moin A. Saleem[3], Peter W. Mathieson[3], José M. Valdivielso[4], Marta Ruiz-Ortega[1], Jesus Egido[1] and Alberto Ortiz[1]

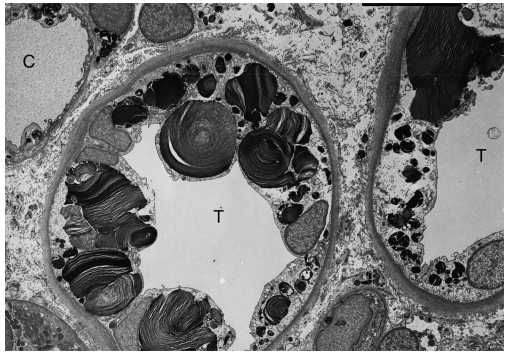
Nephrol Dial Transplant 2011; 26: 1797-1802.

Sclerotic and thrombotic events can certainly contribute to ischemia and hypoperfusion, eventually leading to renal insufficiency. All these biomarkers and cytokines have been described to be elevated in focal and segmental glomerulosclerosis [14,15] and may explain the response to low-dose steroid therapy that Fabry subjects may require as adjunctive therapy.









ISCHEMIA
CKD

14. Yang W, Wang J, Shi L, Yu L, Qian Y, Liu Y, Wang W, Cheng S. Podocyte injury and overexpression of vascular endothelial growth factor and transforming growth factor-beta 1 in adriamycin-induced nephropathy in rats. Cytokine 2012; 59:370–376.
15. Lee HS. Mechanisms and consequences of TGF-ß overexpression by podocytes in progressive podocyte disease. Cell Tissue Res 2012; 347:129–140.

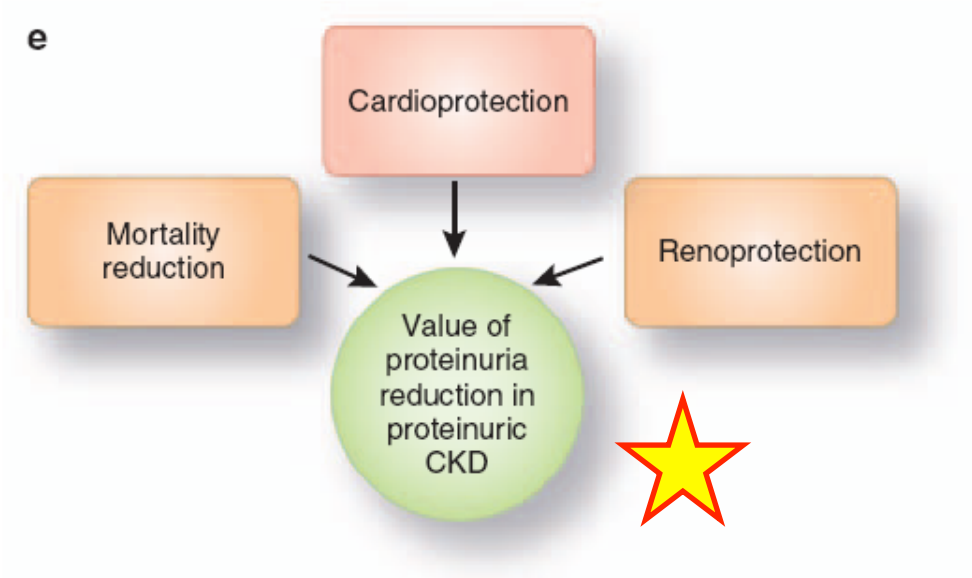
BASIC RESEARCH www.jasn.org

THE MISSING LINK

Loss of the Endothelial Glycocalyx Links Albuminuria and Vascular Dysfunction

Andrew H.J. Salmon,*†‡ Joanne K. Ferguson,* James L. Burford,‡ Haykanush Gevorgyan,‡ Daisuke Nakano,‡§ Steven J. Harper,* David O. Bates,* and Janos Peti-Peterdi‡

J Am Soc Nephrol 23: 1339–1350, 2012. doi: 10.1681/ASN.2012010017




THANK YOU