
New Insights into the Pathogenesis and Therapies of FSGS
FLAVIO VINCENTI

Causes of Focal Segmental Glomerulosclerosis

Type of Disease	Cause
Primary (idiopathic) form	Mediated by circulating permeability factor(s)
Secondary forms	
Familial or genetic	Mutations of specific podocyte genes
Virus-associated	Human immunodeficiency virus, type 1, parovirus B19, simian virus 40, cytomegalovirus, Epstein-Barr virus
Drug-induced	Heroin; interferons alfa, beta, and gamma; lithium; pamidronate; sirolimus; calcinceurin-inhibitor nephrotoxicity; anabolic steroids
Adaptive*	Conditions with reduced renal mass: oligomeganephronia, unilateral renal agents, renal dysplasia, reflux nephropathy, sequelaenm to cortical necrosis, surgical renal ablation, renal allograft, aging kidney, obesity

*The adaptive form is mediated by adaptive structural-functional responses to glomerular hypertension caused by elevated glomerular capillary

FSGS

- Familial cases (up to 18% of FSGS) are due to mutations of podocyte proteins, nephrin ($NPHS_1$), podocin ($NPHS_2$), α-actinin-4 ($ACTN_4$), CD2-associated protein (CD2AP), phospholipase C-ε1 (PLCE1) and the transient receptor potential cation channel (TRPC6).

Therapeutic Interventions for FSGS

- Traditional therapeutic interventions for FSGS are confined to gross immune modulation (despite lack of evidence that the disease is immunologically driven) with high dose steroids, cyclosporine, cytotoxic and antiproliferative agents, anti-CD20 mAbs with mixed success and significant adverse side effects.

The Lancet • Saturday 19 August 1972

RECURRENCE OF IDIOPATHIC NEPHROTIC SYNDROME AFTER RENAL TRANSPLANTATION

John R. Hoyer
Robert L. Vernier
John S. Najarian
Leopoldo Raij
Richard L. Simmons
Alfred F. Michael

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Etiology of the Recurrent FSGS

- Clinical evidence of a suspected circulating permeability factor (PF)*
  1. Recurrence can occur within hours of transplantation of a normal kidney in a FSGS patient with massive proteinuria and effacement of podocytes
  2. Remission can be induced with plasmapheresis
  3. A newborn child from a mother with FSGS had transient proteinuria
  4. Prompt recovery of a kidney with recurrence of FSGS when retransplanted in a non-FSGS recipient (Gallon L, et al. N Eng J Med 2012; 366:1648-49)

*First suggested by R.J. Shalhoub (Lancet 2: 556, 1974)

USA TODAY, March 22, 2012

Kidney transplanted twice in two weeks

Organ does well in its second patient

By Janice Lloyd
USA TODAY

By Laura Brown, Northwestern Memorial Hospital

Medical first: Lorenzo Gallon, second from left, with patients Erwin Gomez, left, Ray Fearing and Cera Fearing.

In what looks like a medical first, doctors reported Wednesday that a kidney transplanted into one patient, which then started to fail, was successfully removed and transplanted into a second patient, who is doing well.

Lorenzo Gallon supervised the transplants over a two-week span in June at Northwestern Memorial Hospital in Chicago, says the report in *The New England Journal of Medicine*.

While transplanted kidneys have been removed from deceased recipients and given to someone else, this is the first time in the U.S. a living recipient has passed along a donated kidney, says Joel Newman with the United Network for Organ Sharing (UNOS).

Since the surgery, Gallon determined that although the kidney was once diseased, it is now healthy in the second patient. Never before, Gallon said, has a disease affecting a kidney in one patient been reversed in another recipient.

"Normally, when a transplant isn't successful, we have to take it out and discard it because it is doing more damage than good," said Gallon, medical director of the hospital's kidney transplant program.

The kidney belonged to Cera Fearing, 21, of Elk Grove, Ill. She first donated the organ June 16 to her brother Ray Fearing, 27, who suffers from one of the most common kinds of kidney disease, focal segmental glomerulosclerosis. It forms scar tissue in the part of the kidney that filters out waste and ultimately causes kidney failure. In patients with FSGS, disease recurrence is about 50% after a transplant.

Gallon informed Fearing that it was a life-threatening condition for him, but that the kidney could probably be reused for someone else. Gallon performed the second transplant June 30.

"Giving it to someone else seemed like the right thing to do," said Ray Fearing, who undergoes dialysis several times a week and is not currently a candidate for another kidney. "This was a gift to me, and I wanted to pass along the gift."

Erwin Gomez, 67, of Valparaiso, Ind., the second recipient, is a surgeon and father of five, and he is thriving. "I consider myself blessed," said Gomez, a diabetic who was in end-stage kidney failure.

Cera Fearing knew her donation "wasn't a sure thing for Ray, but I am happy the kidney could help someone."

Gallon said Gomez looks good: "It's almost like when you put water into a flower that hasn't seen water for a month."

Most transplanted organs, about 80%, are kidneys, said Jill Finnie of UNOS. About 92,000 patients are on the wait list for kidneys. "So many people are in need," she says. "Finding ways to reuse kidneys is important."

Etiology of the Recurrent FSGS

- Experimental evidence for a PF
  1. Rats infused with FSGS sera develop proteinuria (Zimmerman et al. Nephron. 1985; 40:241-5)
  2. In vitro bioassay of FSGS sera increase the permeability of isolated glomeruli (Savin et al. New Eng J Med 334:878-883, 1996)
  3. Experimental evidence of suPAR as the circulating PF (Wei et al. Nature Medicine 2011; 17:952-960)

Treatment of Recurrent FSGS

- High dose CNI's
  - The effect of CSA and Tacrolimus in FSGS is unrelated to their immunosuppression mechanism of action

The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine A

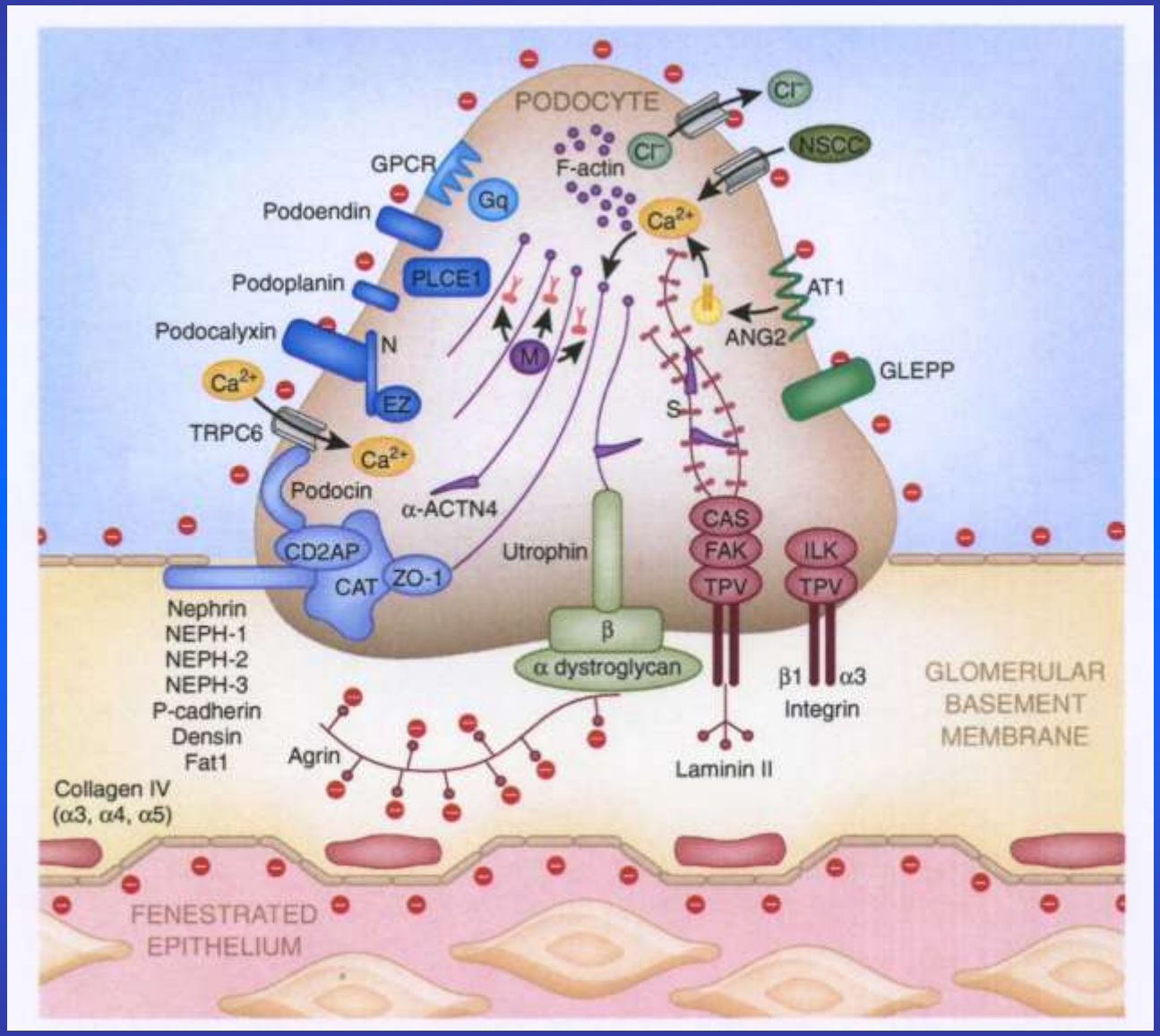
Christian Faul[1,2], Mary Donnelly[1,2], Sandra Merscher-Gomez[1,2], Yoon Hee Chang[2,5], Stefan Franz[2,5], Jacqueline Delfgaauw[2,5], Jer-Ming Chang[3], Hoon Young Choi[2], Kirk N Campbell[1,2], Kwanghee Kim[2], Jochen Reiser[1,4] & Peter Mundel[1,2]

Nature Medicine 2008;14: 931-8

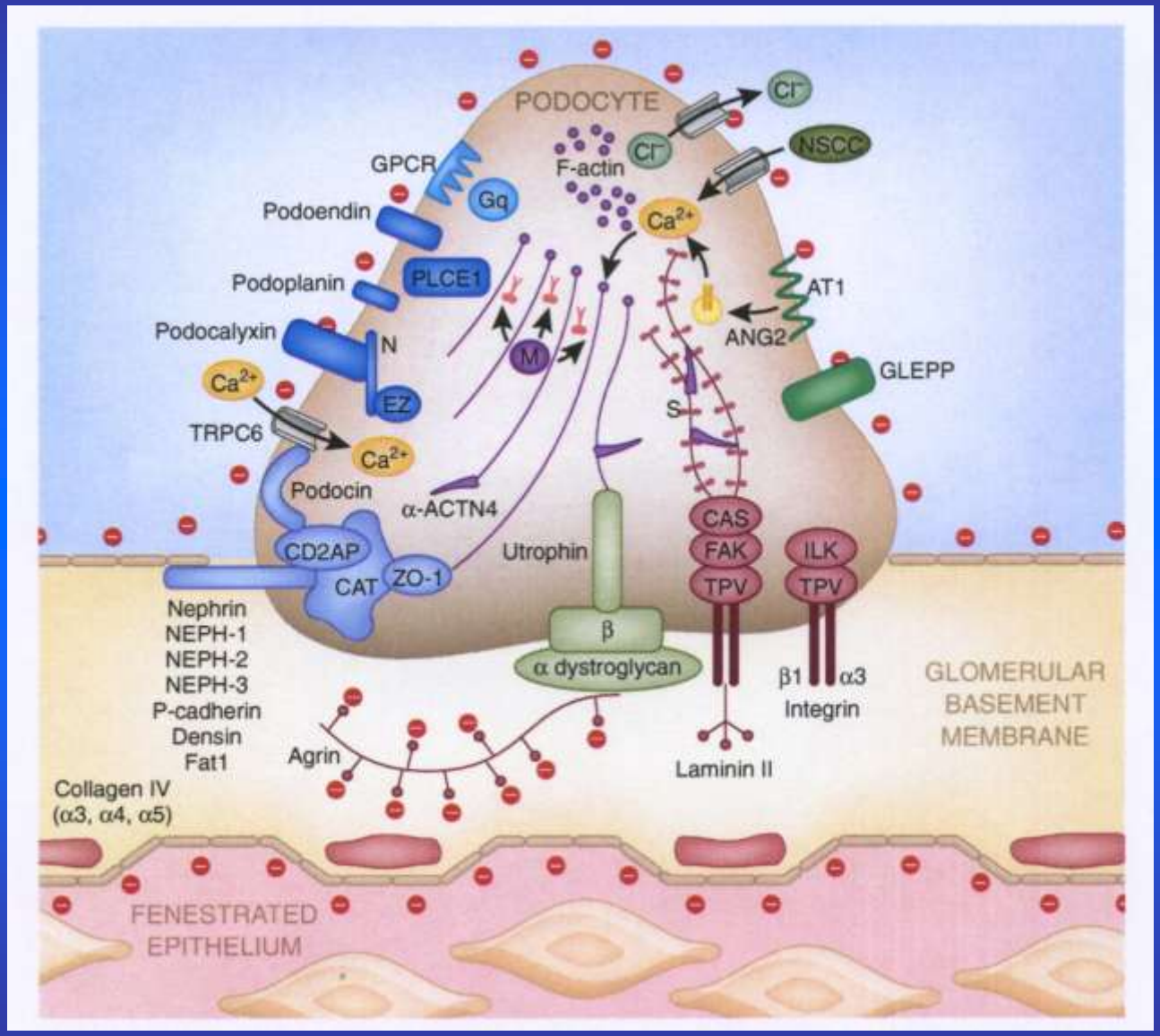
Summary of the Effect of CNIs on the Podocyte

- The data show that the beneficial effect of CsA on proteinuria is not dependent on NFAT inhibition in T cells, but rather results from the stabilization of the actin cytoskeleton in kidney podocytes.
- CsA blocks the calcineurin-mediated dephosphorylation and degradation of synaptopodin, a regulator of Rho GTPases , the master regulator of the actin/myosin cytoskeleton in podocytes.
- These results represent a new view of calcineurin signaling and shed further light on the treatment of proteinuric kidney disease.

Important Components of the Podocyte



Important Components of the Podocyte



Treatment of Recurrent FSGS

- Plasmapheresis
  - First reported by Zimmerman et al (Nephron 1985)
  - First large series of patiets with recurrent FSGS treated with plasmapheresis from UCSF (Artero et al AJM 1992)

The American Journal of Medicine 1992; Vol. 92

Recurrent focal glomerulosclerosis: Natural history and response to therapy

Mary Artero M.D.[a], Claude Biava M.D.[b], William Amend M.D.[a], Stephen Tomlanovich M.D.[a] and Flavio Vincenti M.D.[a]

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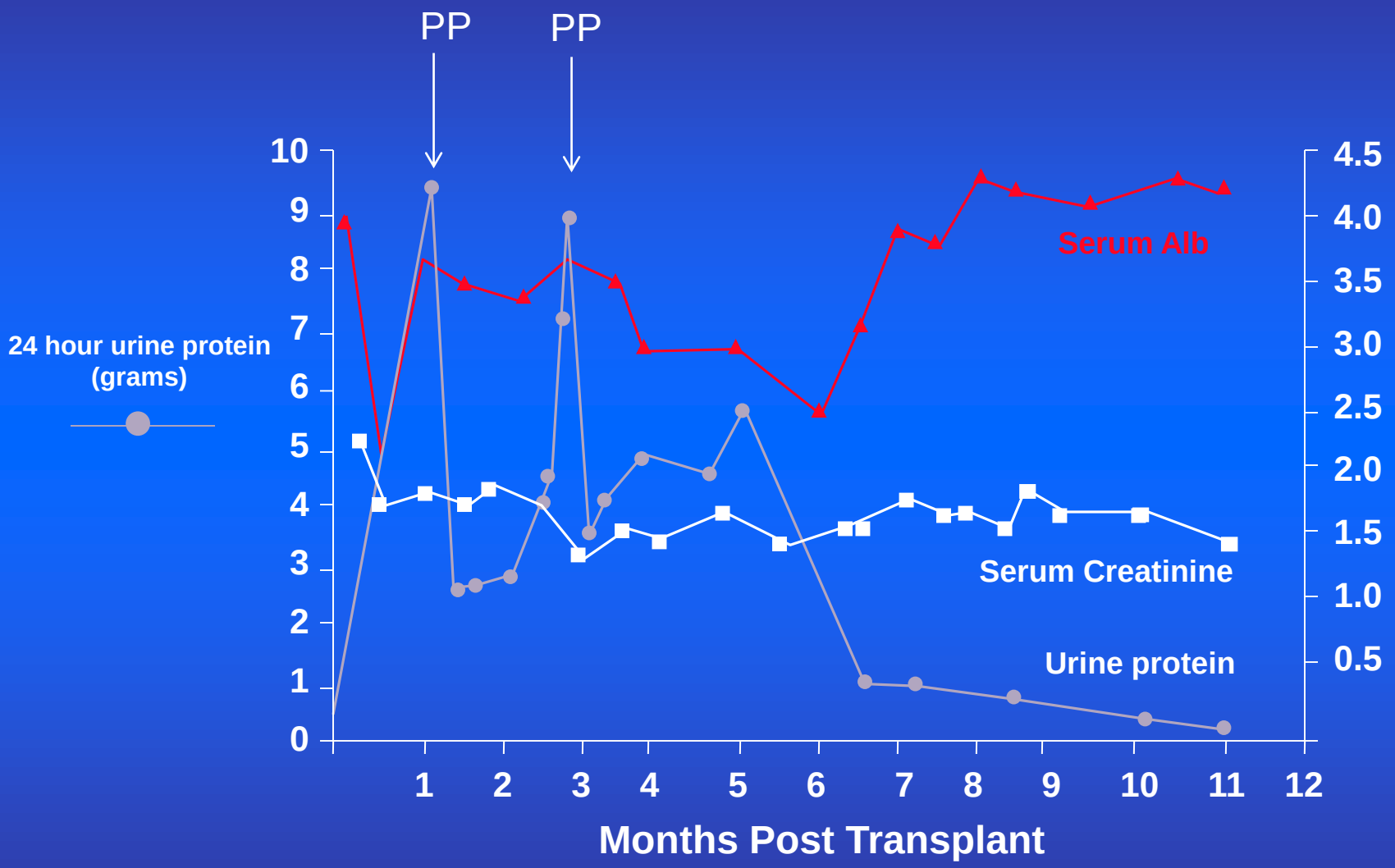
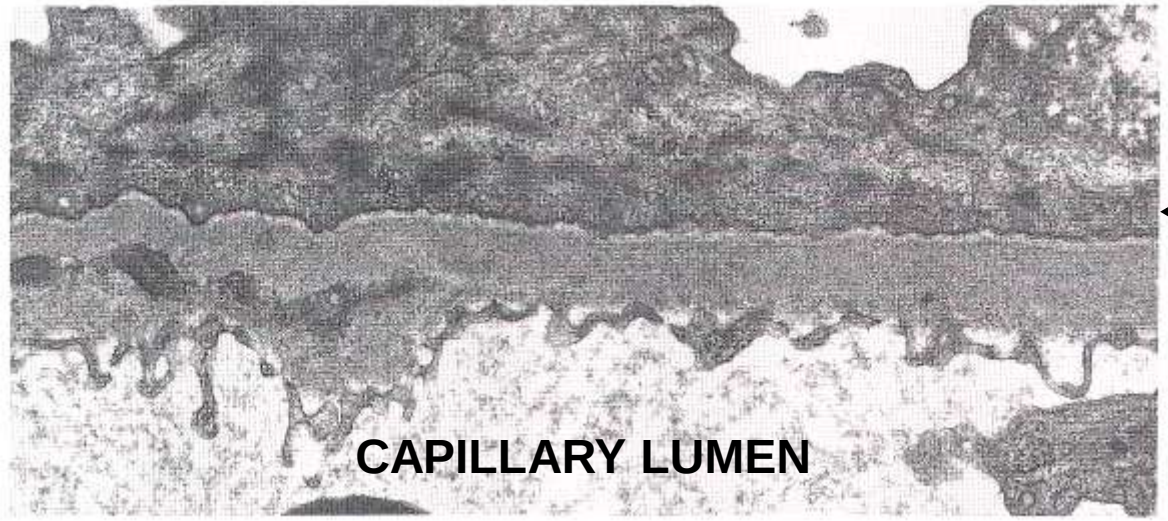
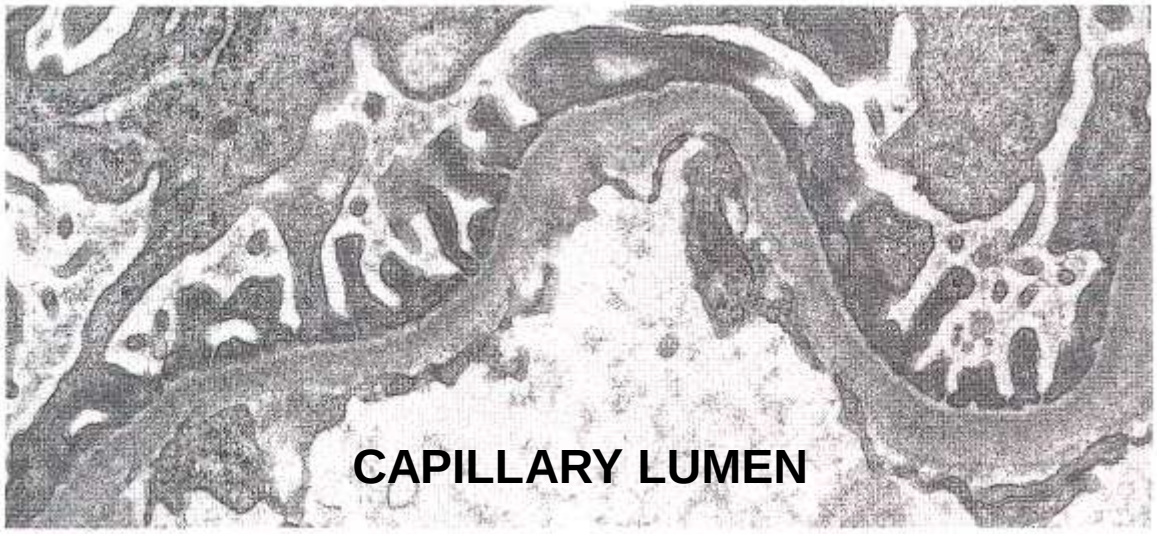

PP
PP
24 hour urine protein (grams)
10
9
8
7
6
5
4
3
2
1
0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Serum Alb
Serum Creatinine
Urine protein
1
2
3
4
5
6
7
8
9
10
11
12
Months Post Transplant

BEFORE PHERESIS





AFTER PHERESIS

Treatment of Recurrent FSGS

- Rituximab
  - Mechanism of action unrelated to CD20 and its effect on B cell depletion or antibody suppression

N ENGL J MED 354;18 WWW.NEJM.ORG MAY 4, 2006

Resolution of Recurrent Focal Segmental Glomerulosclerosis Proteinuria after Rituximab Treatment

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Benita K. Book, M.S.
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American Journal of Transplantation 2008; 8: 222–227
Blackwell Munksgaard

doi: 10.1111/j.1600-6143.2007.02021.x

Case Report

Rituximab Failed to Improve Nephrotic Syndrome in Renal Transplant Patients With Recurrent Focal Segmental Glomerulosclerosis

J. M. Yabu[a,*], B. Ho[b,c], J. D. Scandling[b] and F. Vincenti[a]

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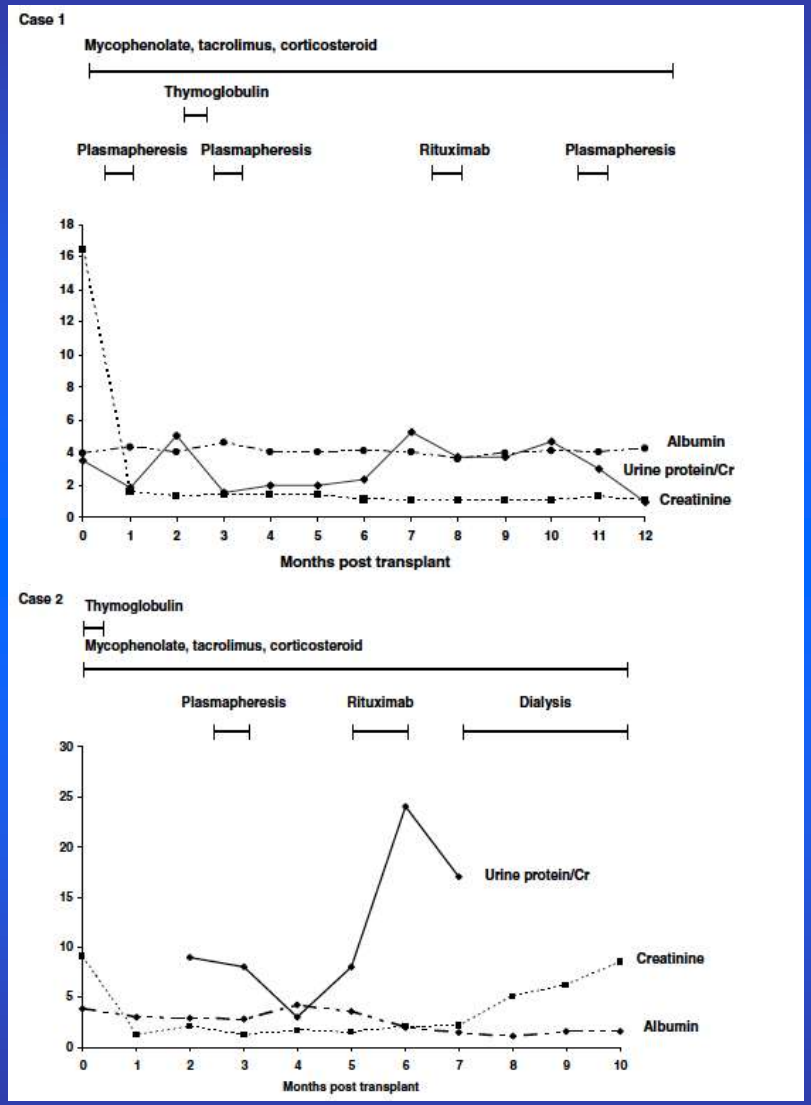
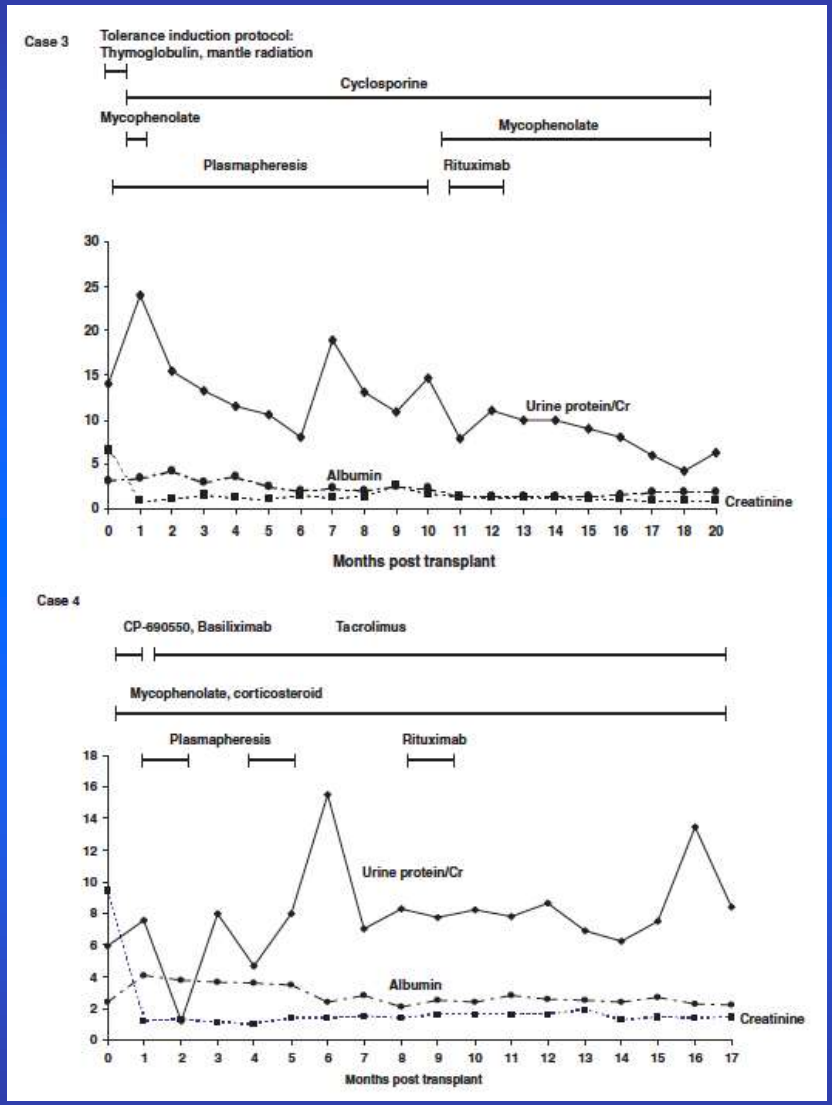
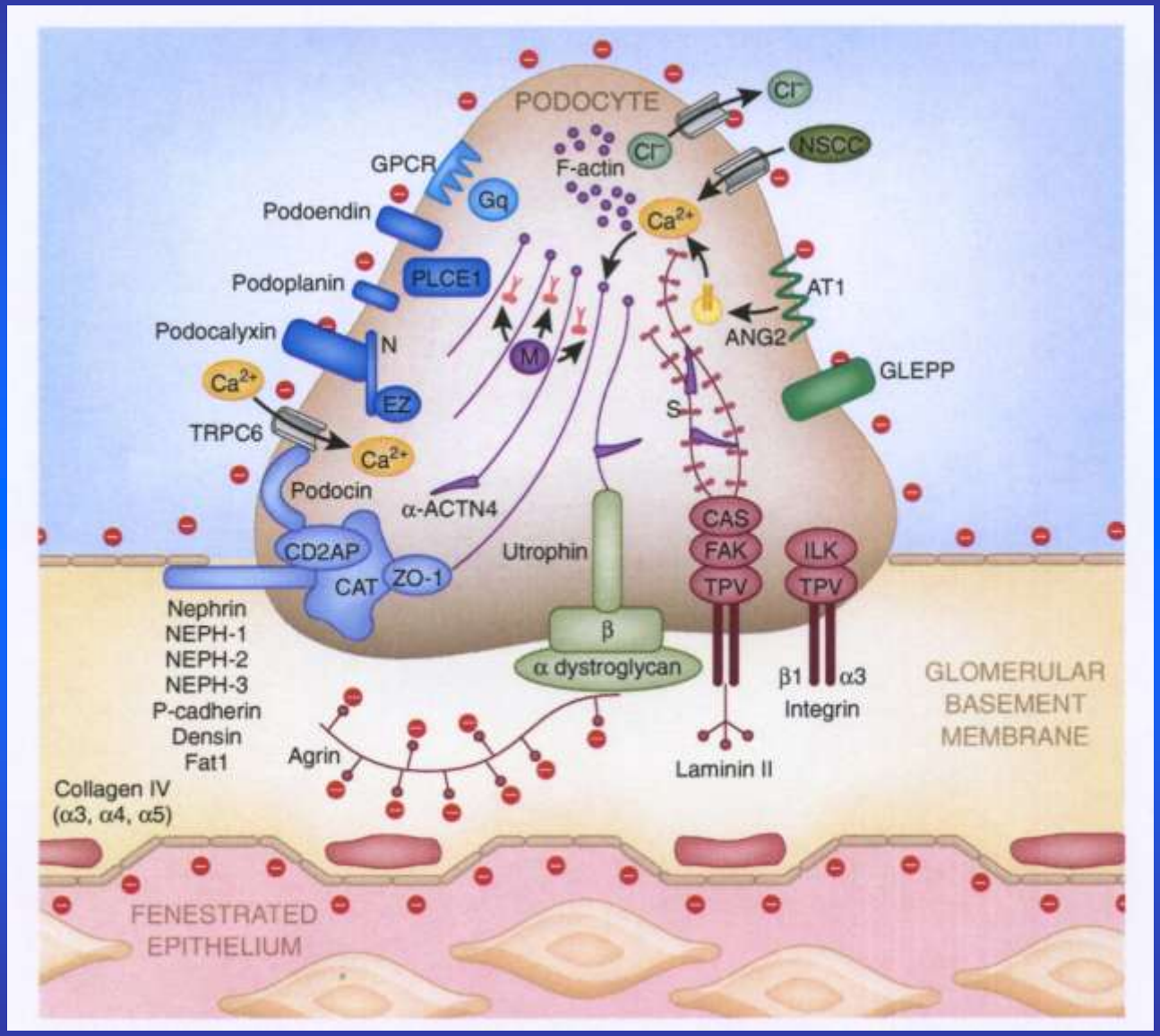
[b] *Department of Medicine, Stanford University, Palo Alto, CA*
[c] *Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA*
* *Corresponding author: Julie M. Yabu, yabuj@surgery.ucsf.edu*

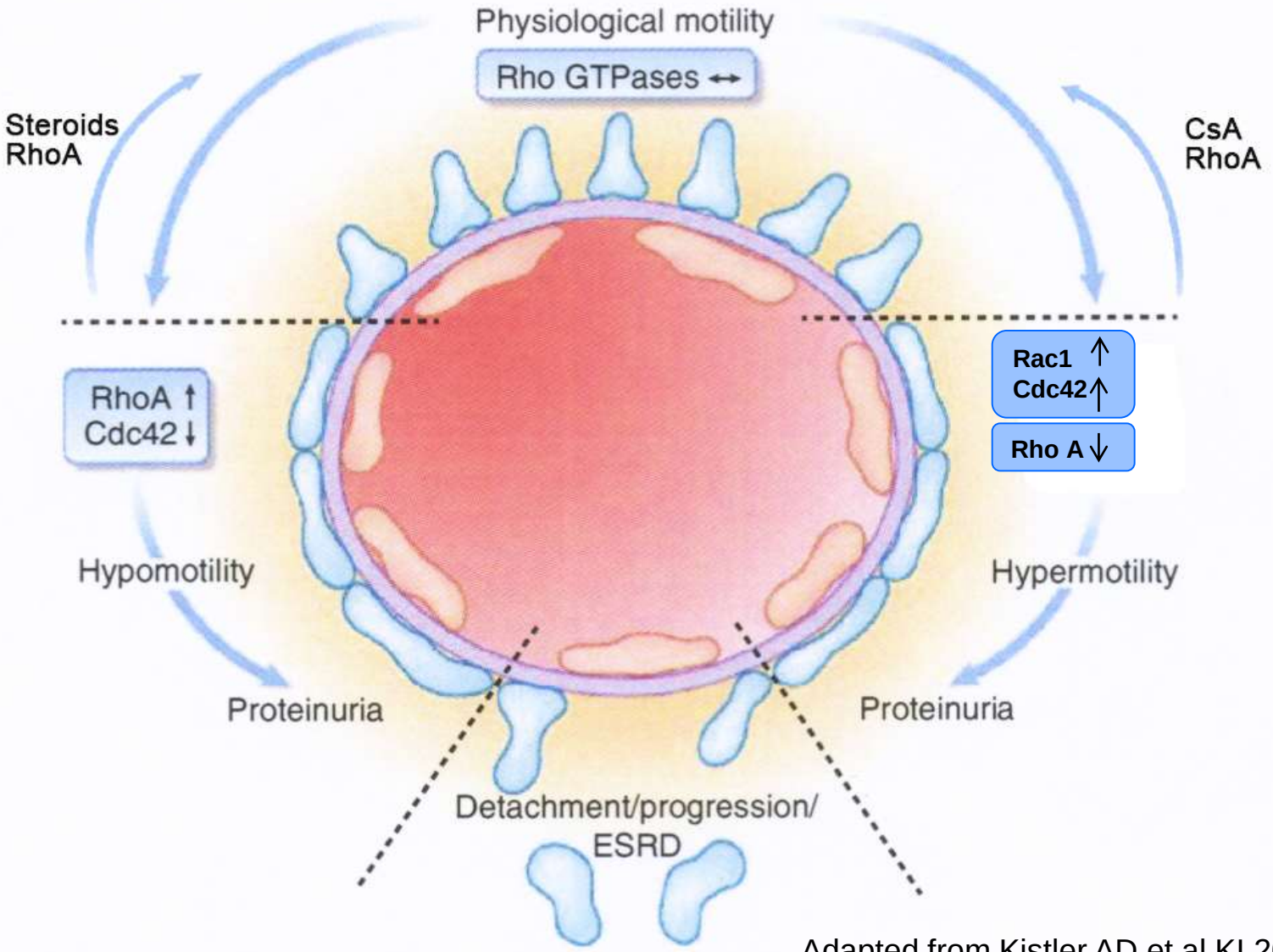

Case 1
Mycophenolate, tacrolimus, corticosteroid
Thymoglobulin
Plasmapheresis
Plasmapheresis
Rituximab
Plasmapheresis
Albumin
Urine protein/Cr
Creatinine
Months post transplant
Case 2
Thymoglobulin
Mycophenolate, tacrolimus, corticosteroid
Plasmapheresis
Rituximab
Dialysis
Urine protein/Cr
Creatinine
Albumin
Months post transplant


Case 3
Tolerance induction protocol:
Thymoglobulin, mantle radiation
Cyclosporine
Mycophenolate
Mycophenolate
Plasmapheresis
Rituximab
Urine protein/Cr
Albumin
Creatinine
Months post transplant
Case 4
CP-690550, Basiliximab
Tacrolimus
Mycophenolate, corticosteroid
Plasmapheresis
Rituximab
Urine protein/Cr
Albumin
Creatinine
Months post transplant

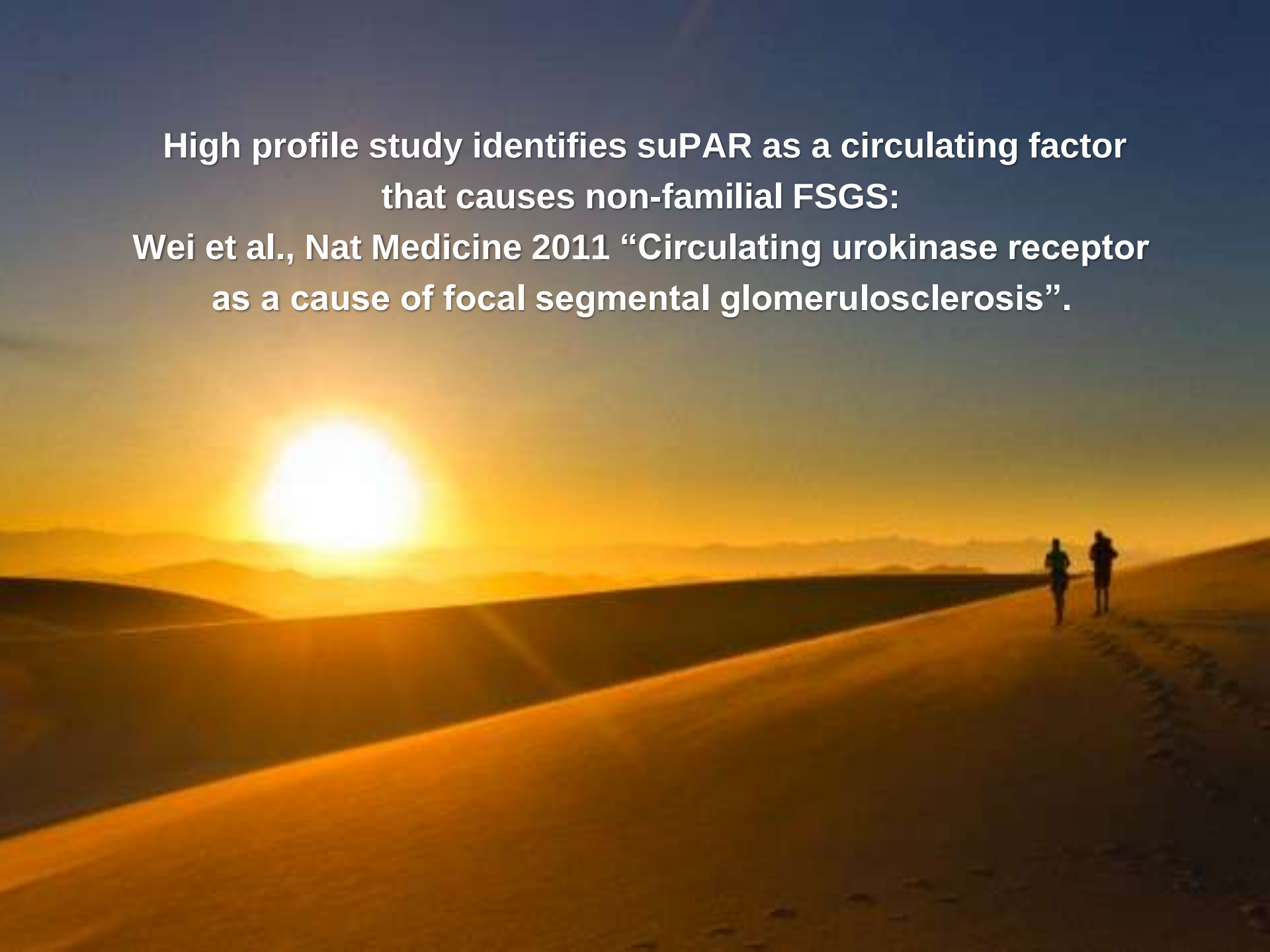
Important Components of the Podocyte



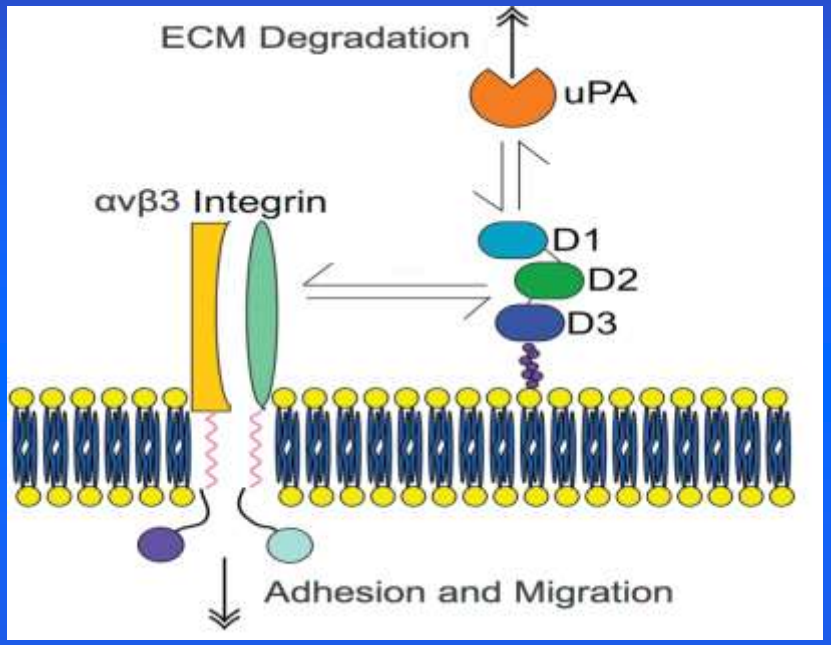
The Complex World of Podocyte Motility and Function



Adapted from Kistler AD et al KI 2012


High profile study identifies suPAR as a circulating factor
that causes non-familial FSGS:
Wei et al., Nat Medicine 2011 “Circulating urokinase receptor
as a cause of focal segmental glomerulosclerosis”.

uPAR Coordinates Signaling and Migration

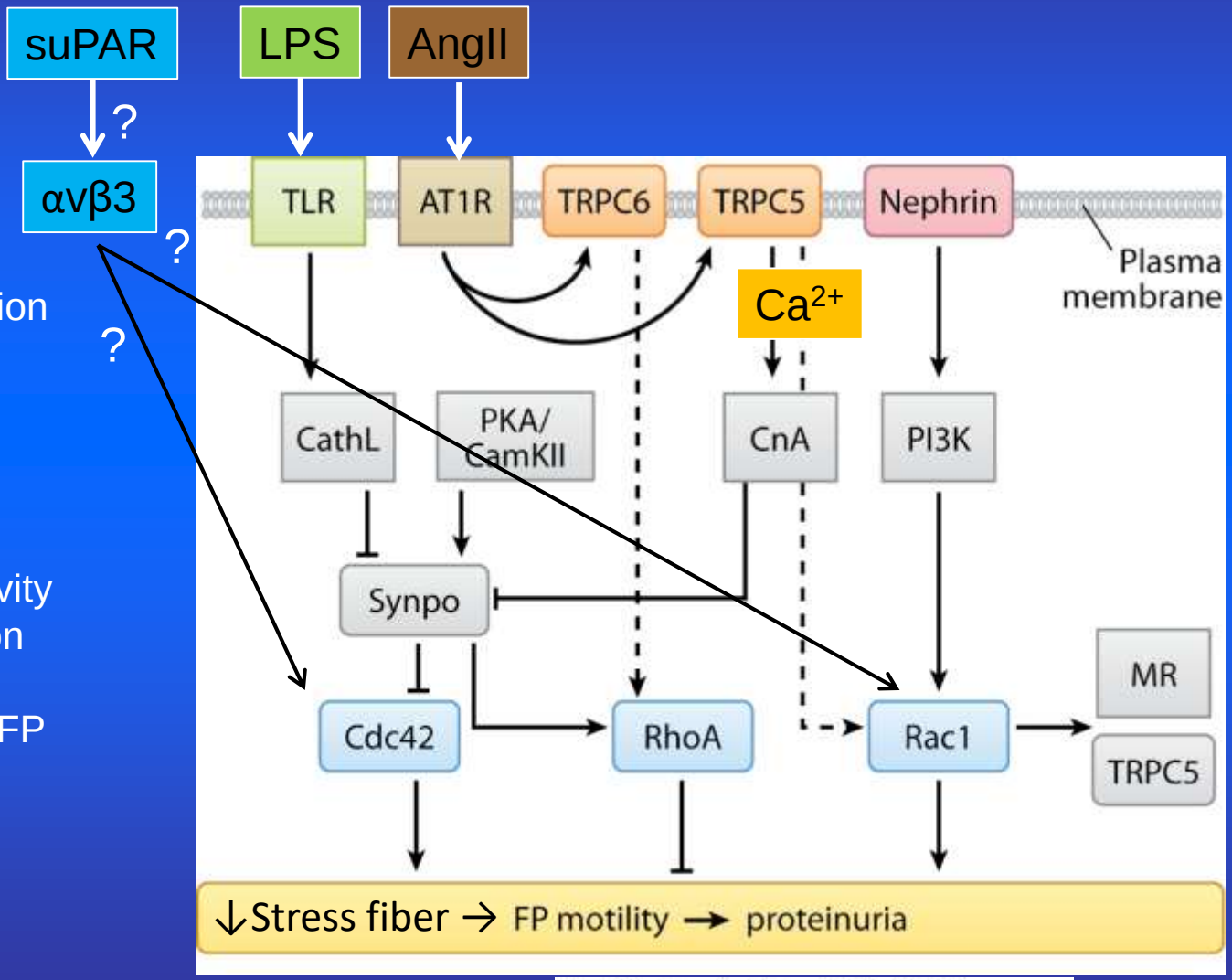


uPAR also interacts with αvβ3 integrin to effect kidney podocyte foot effacement

Hypothesis: suPAR activation of β3 stimulates Cdc42/Rac1 activation and actin remodeling, leading to FP effacement

RhoA promotes the formation of stress fibers and focal contacts, producing a stationary phenotype

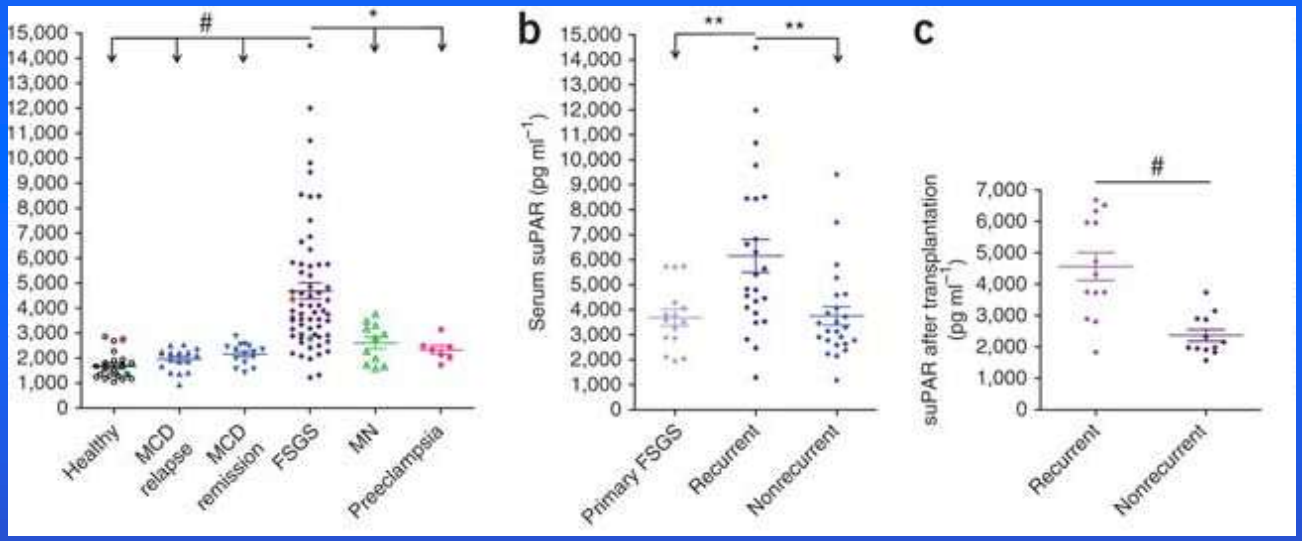
Switch to Cdc42/Rac1 activity triggers actin polymerization and membrane protrusion, leading to cell motility and FP effacement



Greka A, Mundel P. 2012. Annu. Rev. Physiol. 74:299–323

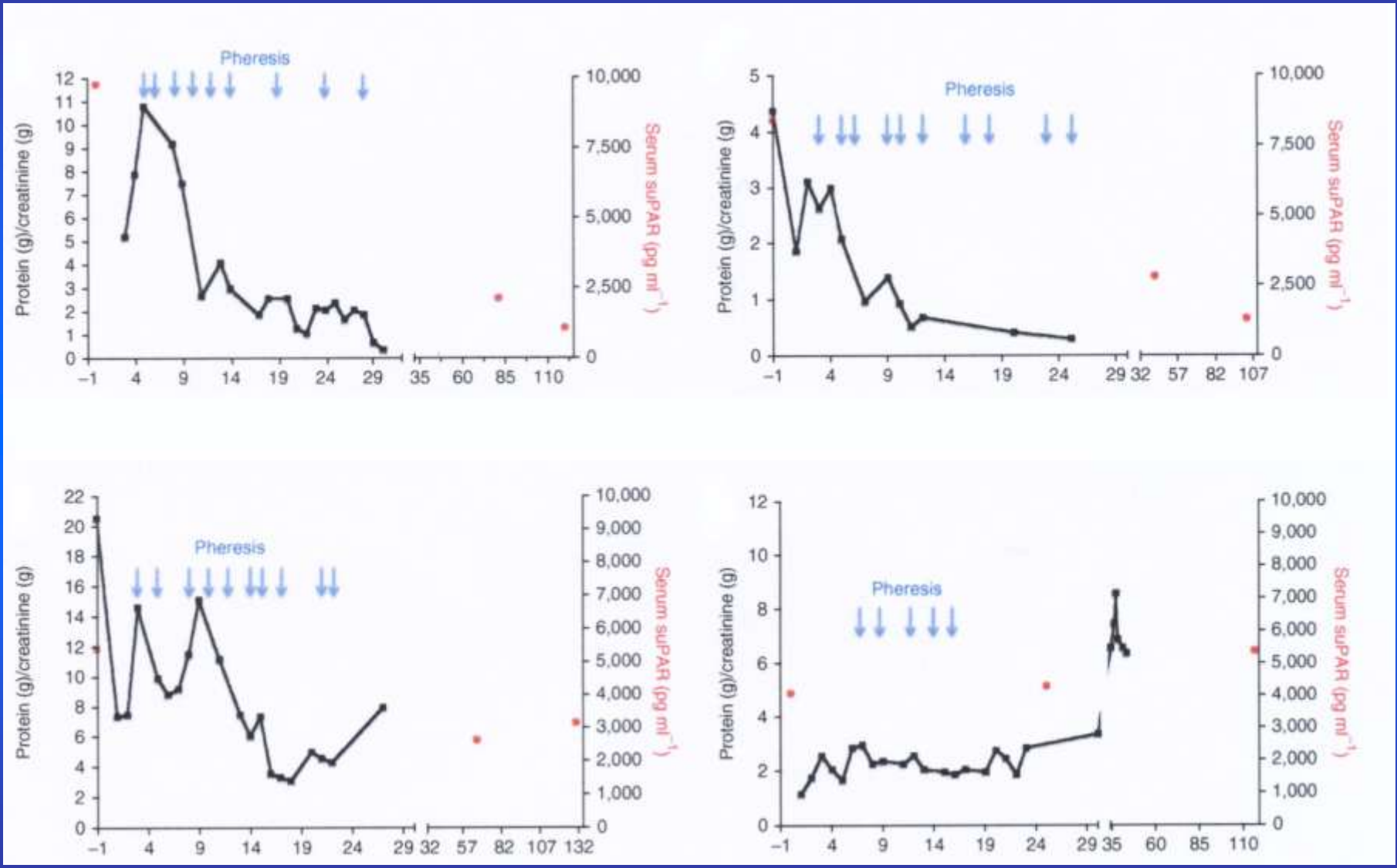
Correlation of elevated suPAR serum levels with severity of FSGS disease

suPAR measurement in the serum of human subjects with glomerular disease



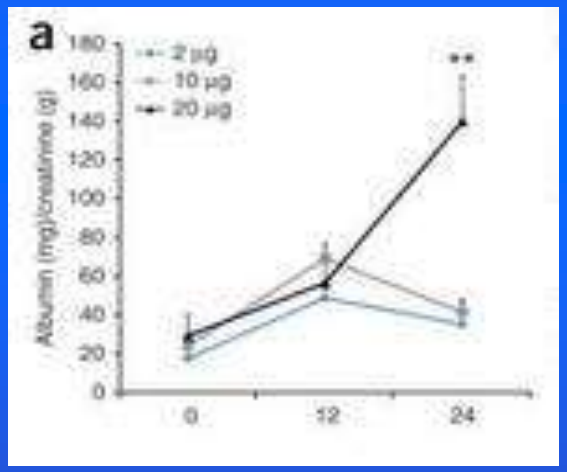
Wei, et al. Nature Medicine 2011

The Effect of Plasmapheresis on suPAR and Proteinuria

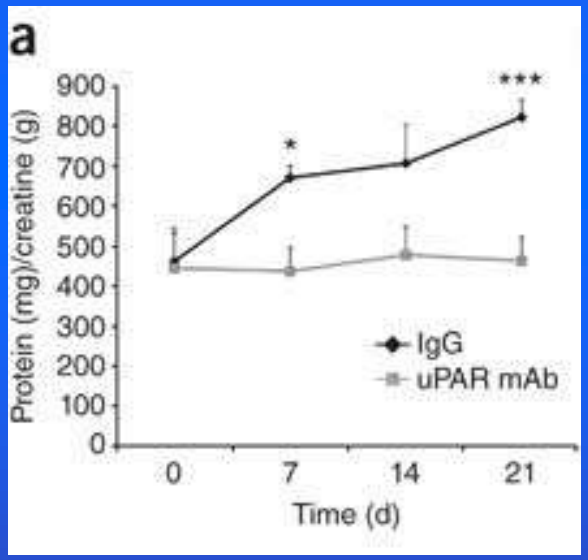


Studies in mouse models and human biopsied tissue define suPAR-beta3 integrin-driven MOA

Injection of suPAR causes proteinuria*



Injection of blocking suPAR Ab ameliorates kidney disease*



BUT

suPAR Has Not Been Confirmed To Be A Biomarker for FSGS (or Recurrent FSGS)

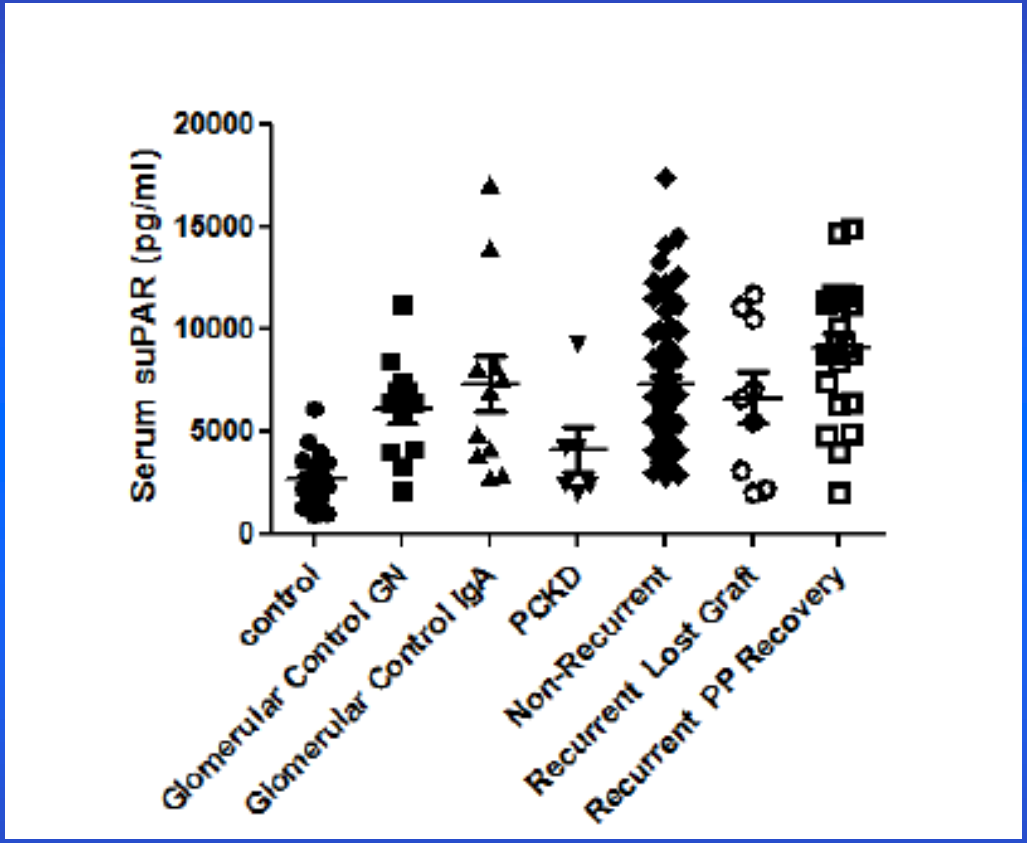
A. Meijers B, et al. The soluble urokinase receptor is not a clinical marker for focal segmental glomerulosclerosis. Kidney International 2014; 85:636-640.

B. Wada et al. A multicenter cross-sectional study of circulating soluble urokinase receptor in Japanese patients with glomerular disease. Kidney International 2014; 85: 641-648.

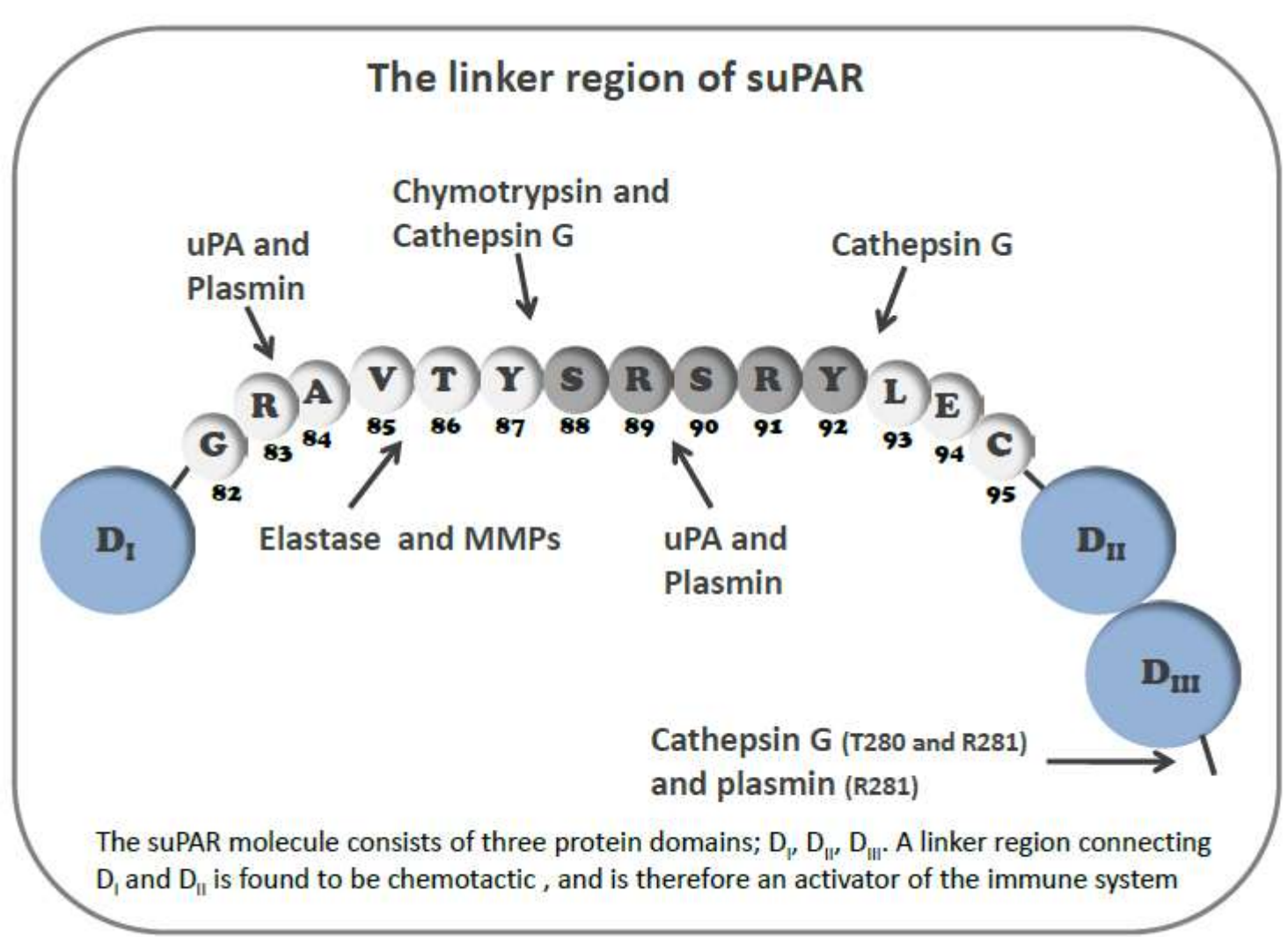
C. Bock ME, et al. Serum soluble urokinase-type plasminogen activator receptor levels and idiopathic FSGS in children: a single-center report. Clin J Am Soc Nephrol 2013; 8:1304-11.

D. Beaudreuil S, et al. Protein A immunoadsorption cannot significantly remove the soluble receptor of urokinase from sera of patients with recurrent focal segmental glomerulosclerosis. Nephrol Dial Transplant 2014; 29:458-463.

UCSF Results (R & D Systems)



suPAR also circulates in multiple fragment forms due to protease cleavage



http://www.suparnostic.com/images/stories/molecular_function_of_supar.pdf

Has the circulating permeability factor in primary FSGS been found?

J. Ashley Jefferson[1] and Stuart J. Shankland[1]

Kidney International (2013) **84,** 235–238. doi:10.1038/ki.2013.204

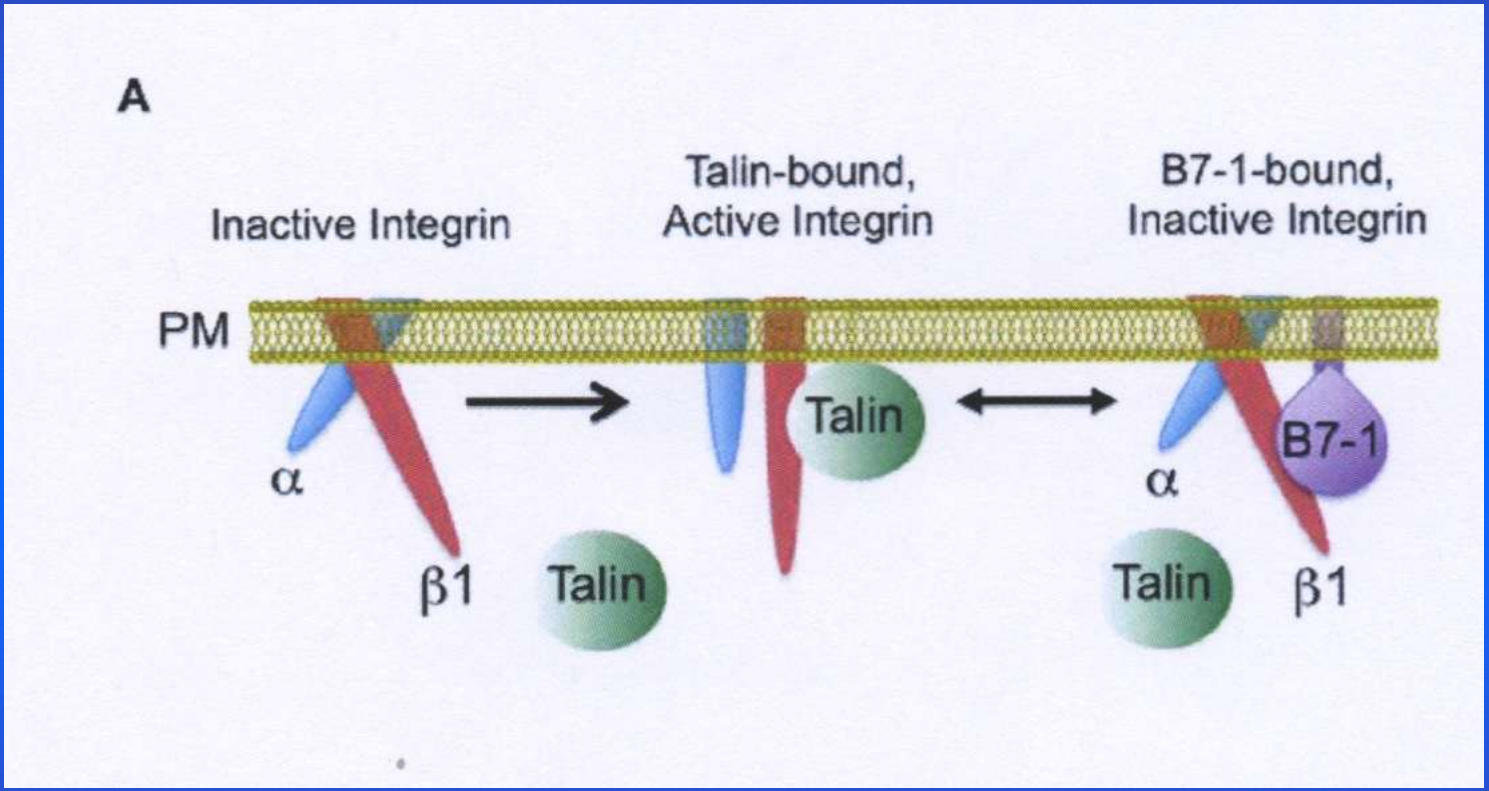
The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

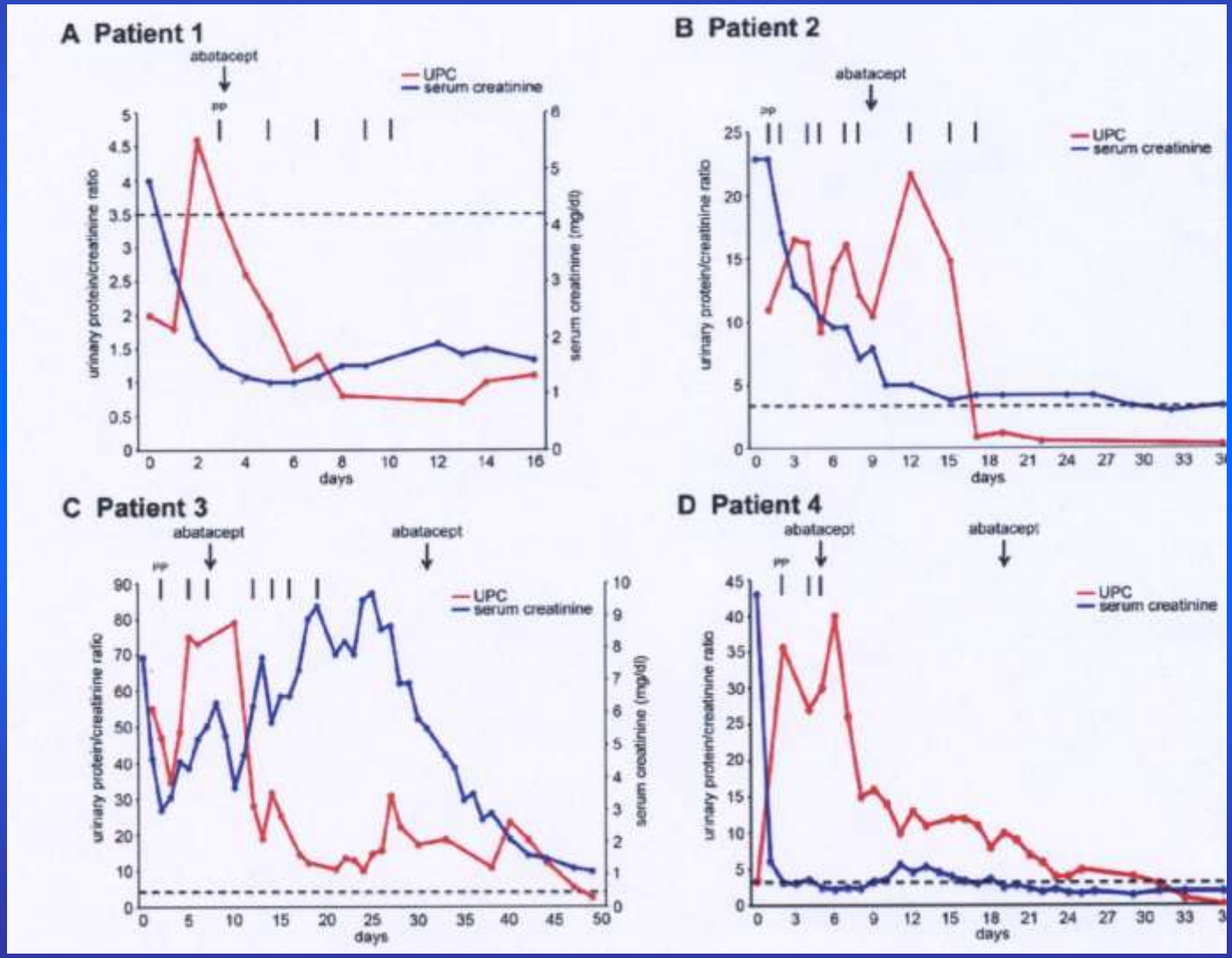
Abatacept in B7-1–Positive Proteinuric Kidney Disease

Chih-Chuan Yu, M.Sc., Alessia Fornoni, M.D., Ph.D., Astrid Weins, M.D., Ph.D., Samy Hakroush, M.D., Dony Maiguel, Ph.D., Junichiro Sageshima, M.D., Linda Chen, M.D., Gaetano Ciancio, M.D., Mohd. Hafeez Faridi, Ph.D., Daniel Behr, Kirk N. Campbell, M.D., Jer-Ming Chang, M.D., Hung-Chun Chen, M.D., Jun Oh, M.D., Christian Faul, Ph.D., M. Amin Arnaout, M.D., Paolo Fiorina, M.D., Ph.D., Vineet Gupta, Ph.D., Anna Greka, M.D., Ph.D., George W. Burke III, M.D., and Peter Mundel, M.D.

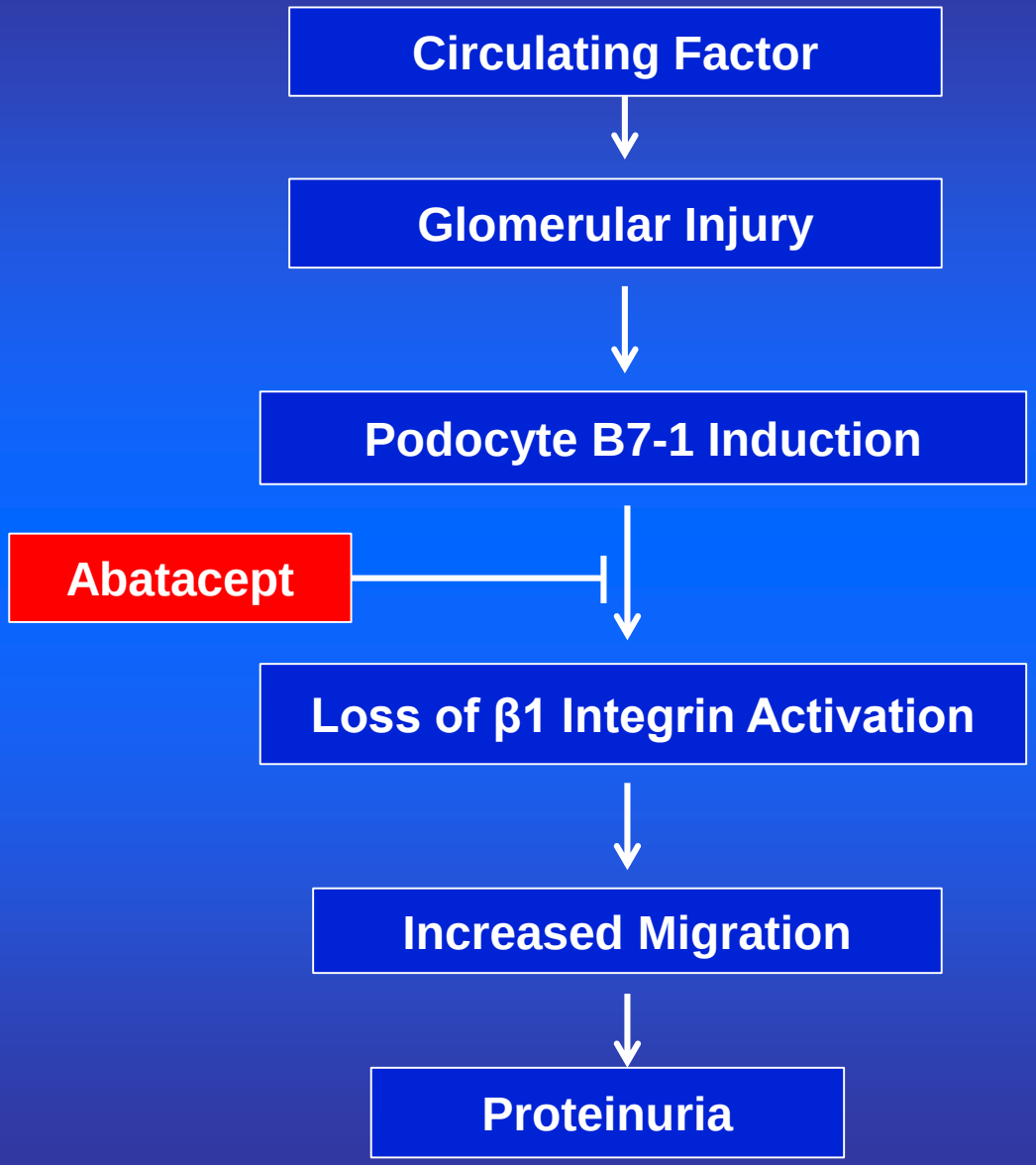
Graphical Summary of Mechanism of B7-1 and Abatacept Action in Podocytes



Treatment of Proteinuria with Abatacept in Recurrent FSGS



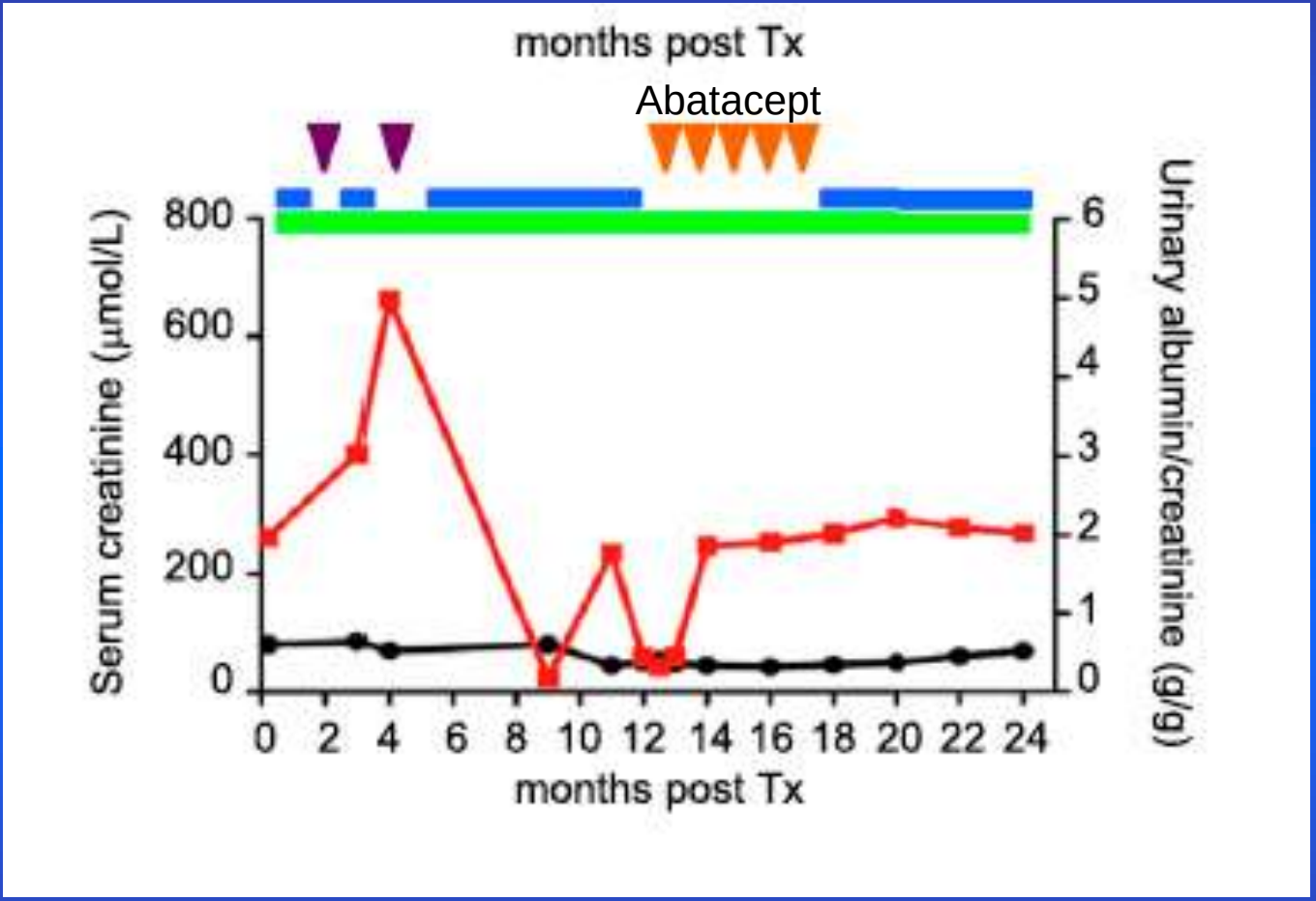
Mechanism of Injury



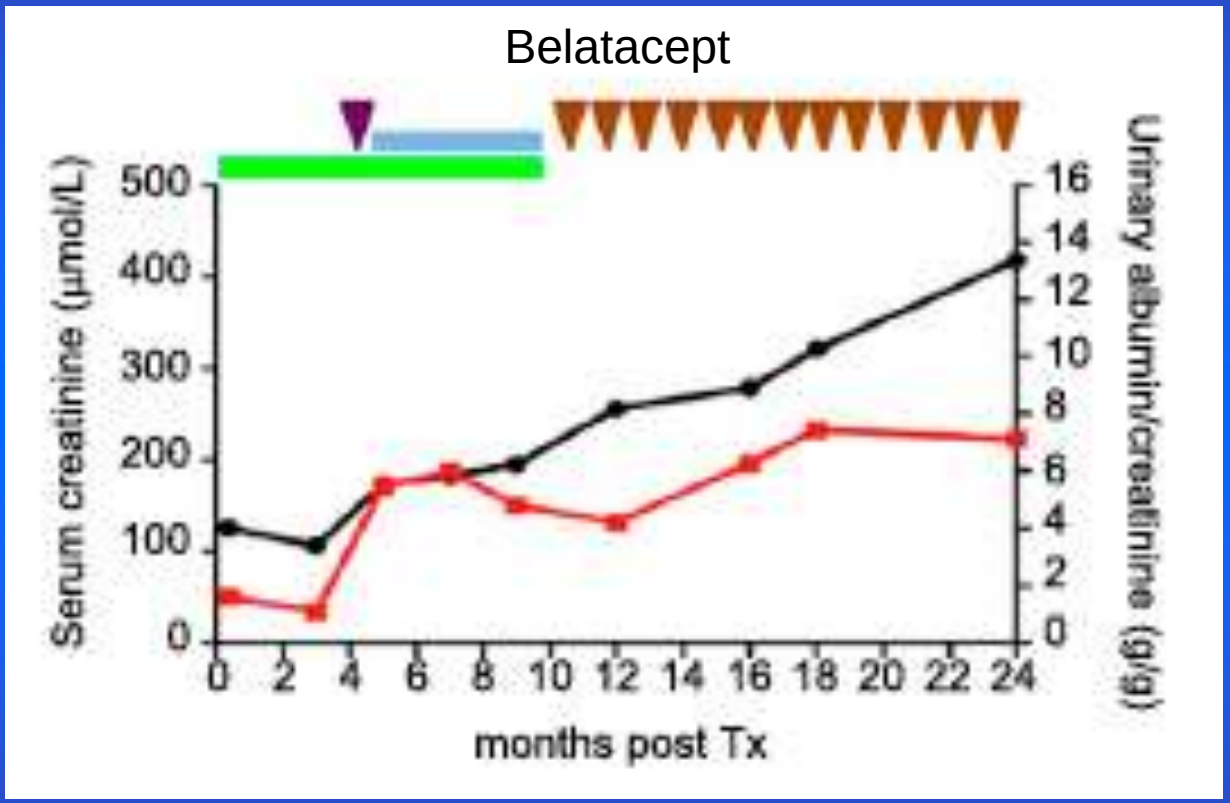
CLINICAL RESEARCH www.jasn.org

B7–1 Blockade Does Not Improve Post–Transplant Nephrotic Syndrome Caused by Recurrent FSGS

Marianne Delville,*† Emilie Baye,‡ Antoine Durrbach,§ Vincent Audard,‖ Tomek Kofman,‖ Laura Braun,¶ Jérôme Olagne,¶** Clément Nguyen,‡ Georges Deschênes,†† Bruno Moulin,¶ Michel Delahousse,‡‡ Gwenaëlle Kesler-Roussey,§§ Séverine Beaudreuil,§ Frank Martinez,‖ Marion Rabant,¶¶ Philippe Grimbert,‖ Morgan Gallazzini,*‡ Fabiola Terzi,*‡ Christophe Legendre,*‖ and Guillaume Canaud*‡‖


months post Tx
Abatacept
Serum creatinine (µmol/L)
800
600
400
200
0
Urinary albumin/creatinine (g/g)
6
5
4
3
2
1
0
0 2 4 6 8 10 12 14 16 18 20 22 24
months post Tx

Belatacept


Serum creatinine (µmol/L)
500
400
300
200
100
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Urinary albumin/creatinine (g/g)
16
14
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8
6
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2
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0 2 4 6 8 10 12 14 16 18 20 22 24
months post Tx

Science Translational Medicine Integrating Medicine and Science

Science

Science Translational Medicine Home > 1 October 2014 > Delville et al., 6:(256): 256ra136

RESEARCH ARTICLE

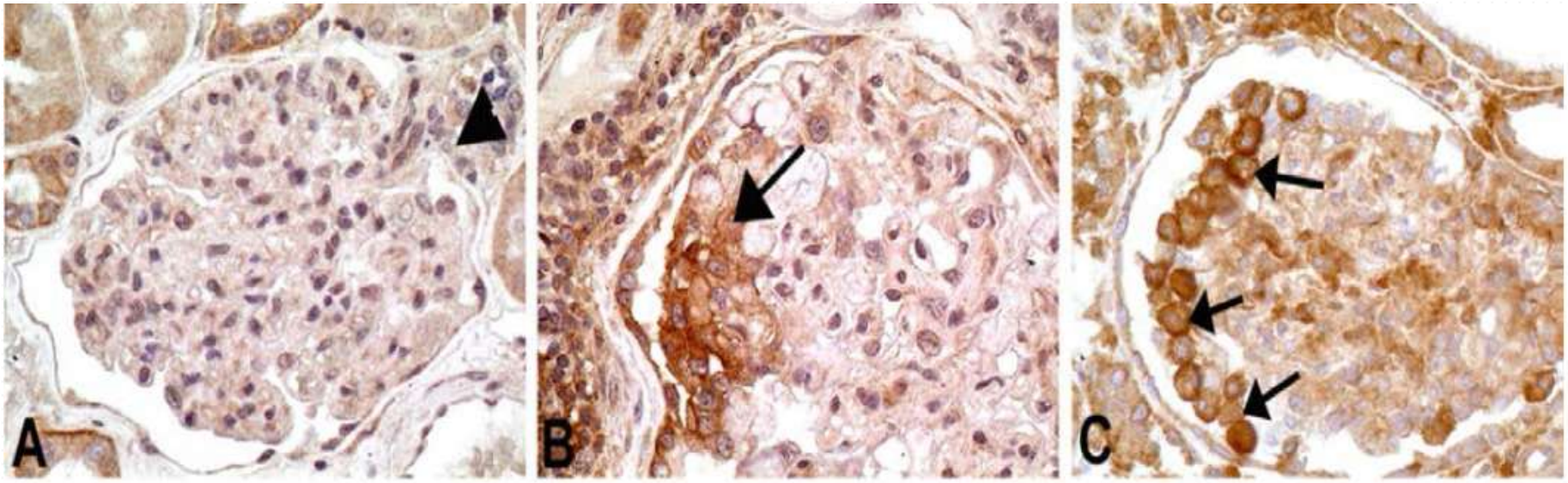
KIDNEY DISEASE

A circulating antibody panel for pretransplant prediction of FSGS recurrence after kidney transplantation

Marianne Delville,[1]* Tara K. Sigdel,[2]* Changli Wei,[3]* Jing Li,[3] Szu-Chuan Hsieh,[2] Alessia Fornoni,[4] George W. Burke,[5] Patrick Bruneval,[6] Maarten Naesens,[7] Annette Jackson,[8] Nada Alachkar,[8] Guillaume Canaud,[1] Christophe Legendre,[1] Dany Anglicheau,[1†] Jochen Reiser,[3†‡] Minnie M. Sarwal[2†‡]

Recurrence of focal segmental glomerulosclerosis (rFSGS) after kidney transplantation is a cause of accelerated graft loss. To evaluate pathogenic antibodies (Abs) in rFSGS, we processed 141 serum samples from 64 patients with and without primary rFSGS and 34 non-FSGS control patients transplanted at four hospitals. We screened about 9000 antigens in pretransplant sera and selected 10 Abs targeting glomerular antigens for enzyme-linked immunosorbent assay (ELISA) validation. A panel of seven Abs (CD40, PTPRO, CGB5, FAS, P2RY11, SNRPB2, and APOL2)

CD10 is expressed in podocytes in rFSGS



B7.1 and suPAR Fail as Potential Biomarkers to Detect Podocyte Injury and Focal Segmental Glomerulosclerosis in Kidney Biopsies

Zoltan G. Laszik and Flavio G. Vincenti

University of California San Francisco Medical Center, San Francisco, California

BACKGROUND

The mechanism of injury to podocytes in focal segmental glomerulosclerosis (FSGS) remains unclear.

Both B7.1 (CD80) and suPAR have been proposed to cause FSGS but the findings have not been validated.

To survey these markers as potential mediators of podocyte injury and as an aid to histologic diagnosis of FSGS we evaluated the expression of B7-1 and uPAR in native and transplant (Tx) kidney biopsies (Bx), and the expression of uPAR in mice injected with suPAR.

METHODS

Study groups included Bx with FSGS (n=10), early post-Tx recurrent FSGS prior to (n=15) and post-plasmapheresis (n=7). Native Bx with membranous nephropathy (MN) (n=10) and minimal change disease (MCD (n=5), and normal 6 month post-Tx protocol Bx [n=10] served as controls.

Immunostains were performed on formalin-fixed paraffin-embedded (FFPE) and frozen tissues for B7.1 and uPAR. B7.1 mRNA expression was also assessed by next generation *in situ* hybridization (ISH) on FFPE.

Signal co-localization was evaluated via co-stain with the podocyte marker synaptopodin. In addition, uPAR immunohistochemical expression was also evaluated in the kidneys of wild type and uPAR -/- mice infused with recombinant suPAR.

Electron microscopy was used in the mouse kidneys to assess foot process effacement in conjunction with renal functional studies.

RESULTS

Figure 1A. B7.1 / synaptopodin double immunofluorescence. Representative images of renal cortex from a patient with membranous nephropathy. The B7.1 signal is located in the immune deposits; the podocytes are negative are negative.

Figure 1B. uPAR IHC in mouse kidney. uPAR shows weak comparable endothelial positivity in WT control mice (1) and those WT injected with Fc (2).

RESULTS

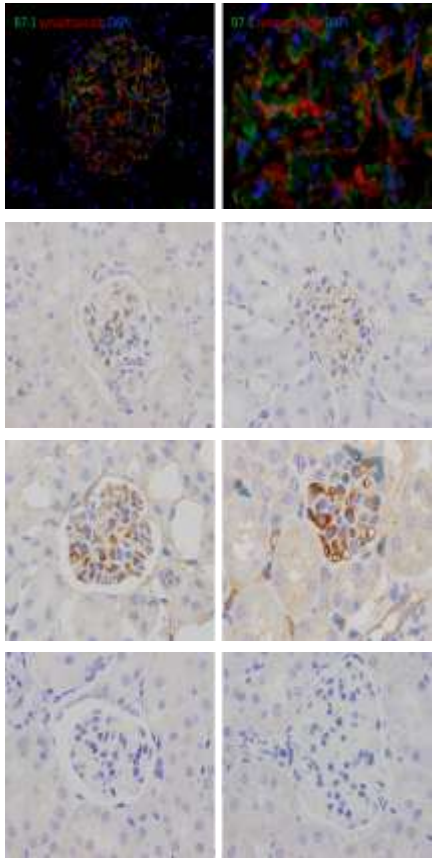
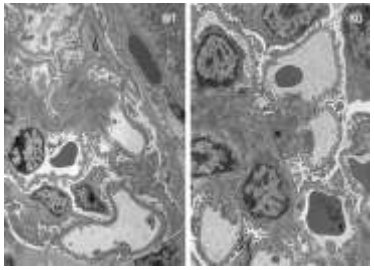


Figure 1C. uPAR IHC stain shows stronger endothelial positivity in WT mice injected with 100 µg (1) vs 20 µg of uPAR-Fc (2). Podocytes are negative.

Figure 1D. uPAR IHC stain is negative in uPAR KO mice following 100 µg of uPAR-Fc (1) or Fc (2) injection.

RESULTS



B7.1 protein and mRNA were not expressed in native kidneys with FSGS or MCD or in transplant kidneys with recurrent FSGS. In MN, B7.1 was localized only to the immune deposits.

No apparent uPAR immunoreactivity was present in native kidneys or recurrent FSGS.

suPAR infusion did not produce proteinuria or effacement of podocytes in wild type or uPAR deficient mice.

uPAR was detected along the glomerular endothelial cells but not in podocytes in wild type mice while uPAR stain remained negative in uPAR deficient mice even after suPAR injection.

CONCLUSION

The data suggest that B7.1 and suPAR may not play a significant role in podocyte injury in native and transplant kidneys with FSGS.

B7.1 and suPAR immunostains and ISH seem to have a limited value as diagnostic markers in kidney biopsies with FSGS and recurrent FSGS post-transplant.

Conclusion

- Recurrent FSGS remains a challenging disease
- The role of suPAR, B7.1 (CD80) and anti-CD40 remains to be elucidated
- Understanding of the physiology of the podocyte will ultimately elucidate the pathophysiology of this disease and lead to rational treatments