

# Ανάπτυξη Βιοτράπεζας για την Ανίχνευση Πρώιμων Βιοδεικτών σε Ασθενείς με Χρόνια Νεφρική Νόσο



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***Νεφρολογικό και Μεταμοσχευτικό Κέντρο***

***Πανεπιστημιακό Νοσοκομείο Πατρών***

# Causes of chronic kidney disease in humans

## Idiopathic Glomerular diseases

- § IgA nephropathy
- § Membranous nephropathy
- § Focal segmental glomerulosclerosis

## Multisystem diseases

- § SLE, Vasculitides (Wegener dis, microscopic polyangitis)
- § Thrombotic microangiopathy (HUS/TTP)
- § Multiple myeloma

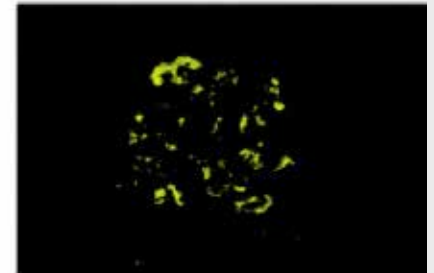
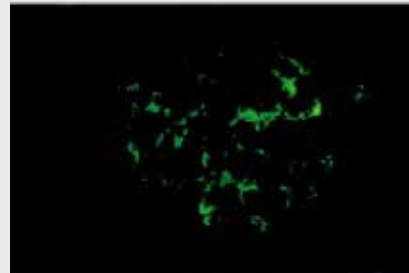
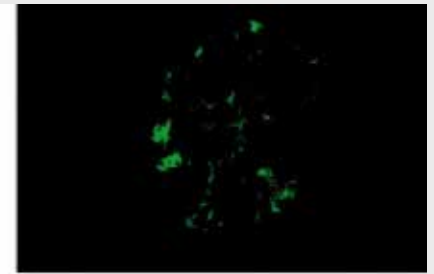
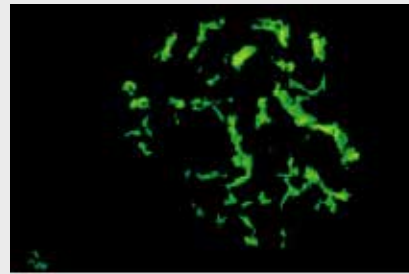
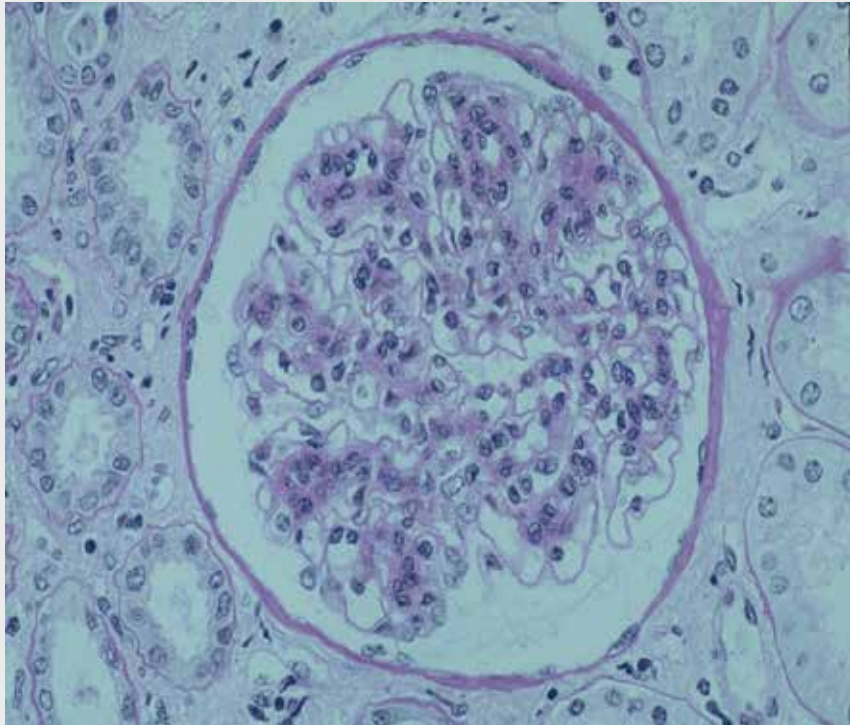
## Metabolic diseases

- § Diabetes mellitus

## Hemodynamic causes

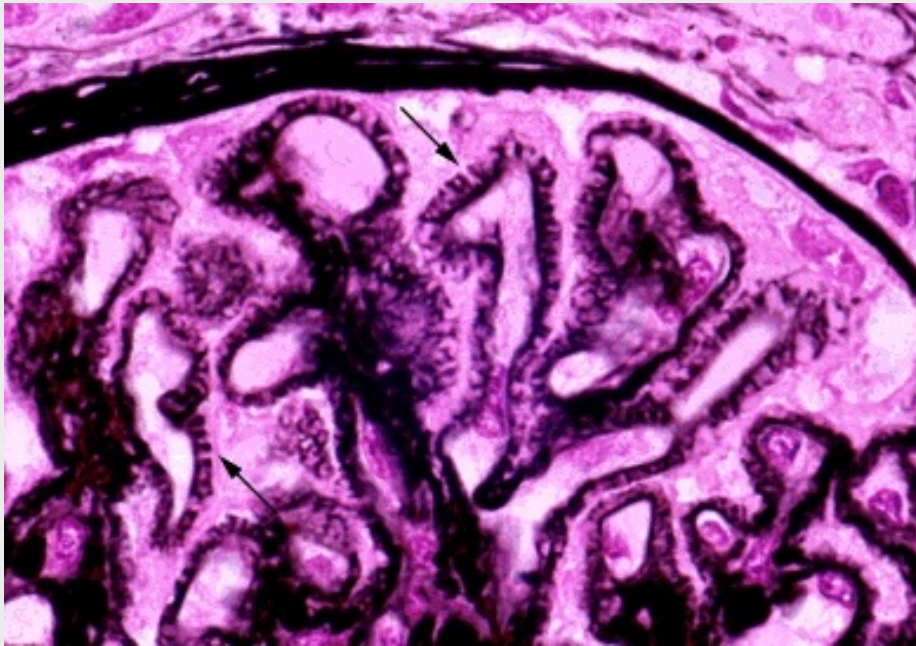
- § Arterial hypertension

# IgA nephropathy

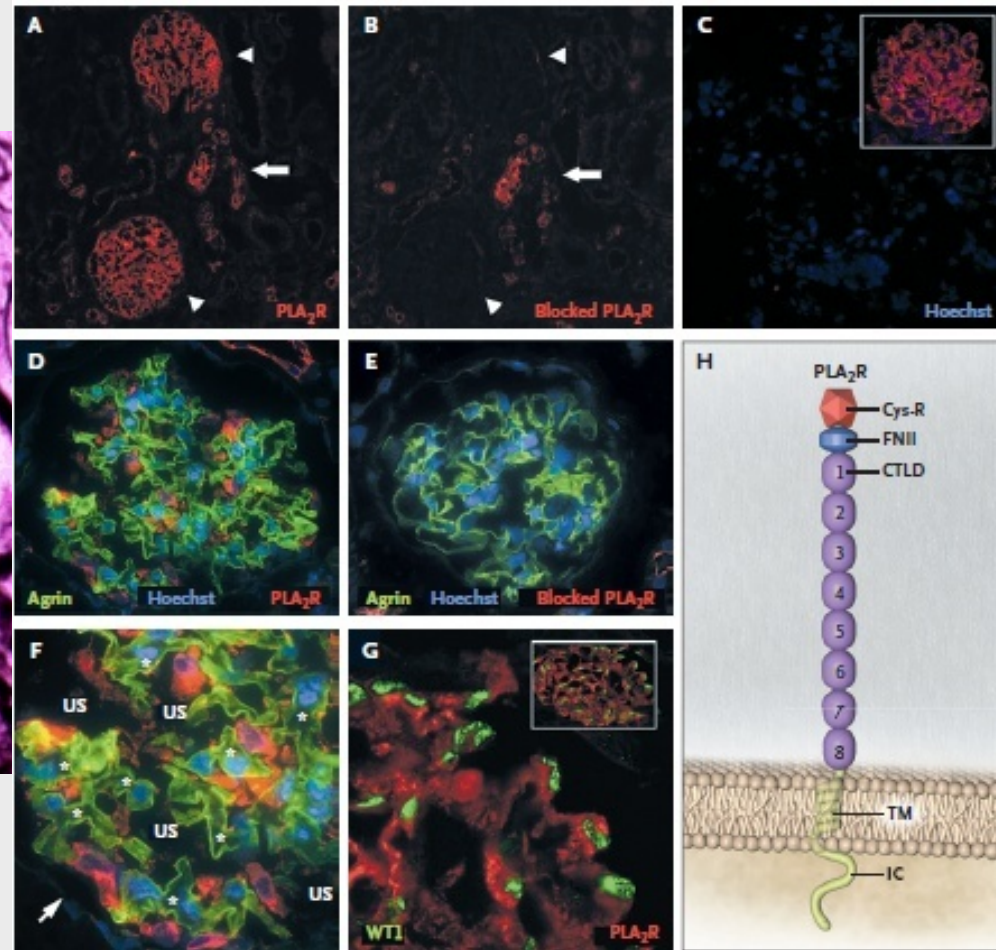




# Membranous nephropathy



**Silver stain: spikes**  
**Increased thickness the**  
**of glomerular basement**  
**membrane**



**M-type phospholipase A2 receptor**

# Pathogenesis of glomerulonephritis

Deposition of immune complexes within the glomeruli



Activation of complement components



Chemo-attraction of monocytes/mononuclear cells, platelets



Local production of chemokines, cytokines, growth factors (PDGF, TGF- $\beta_1$ )



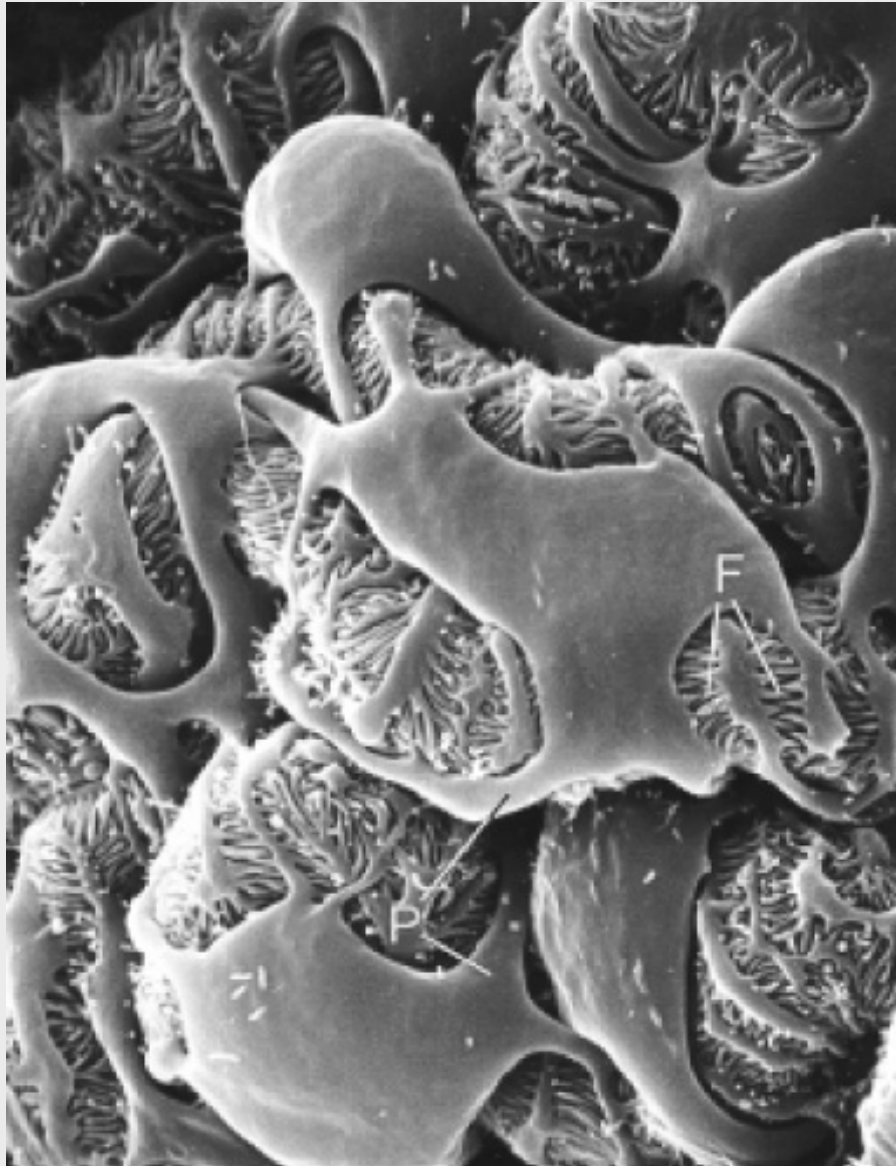
Activation of intrinsic renal cells  
(mesangial, tubular epithelial cells, interstitial cells)



**RENAL FIBROSIS AND SCARRING**

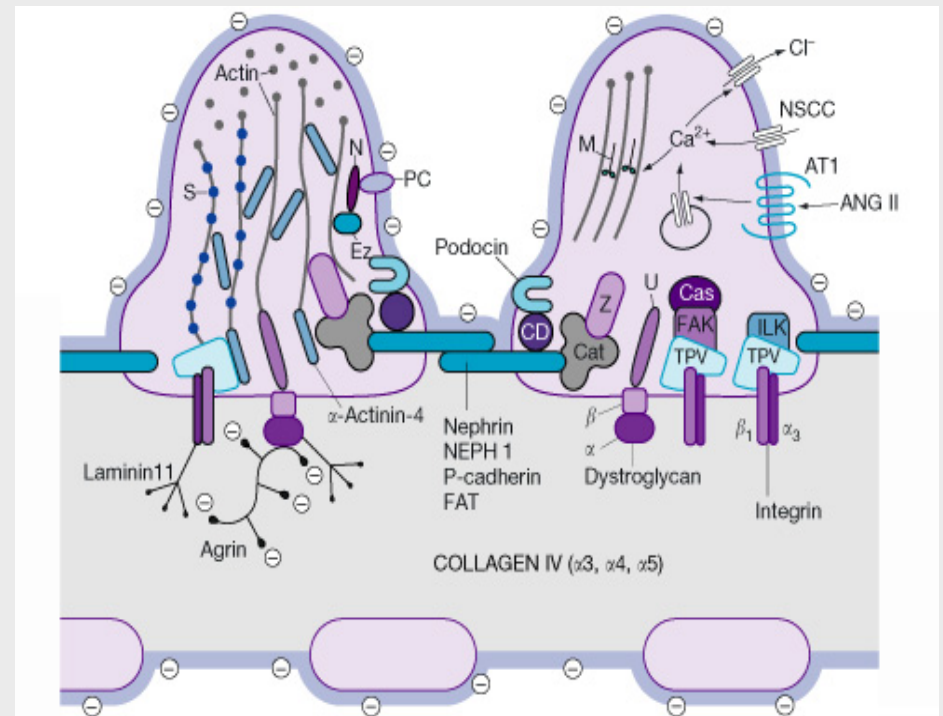
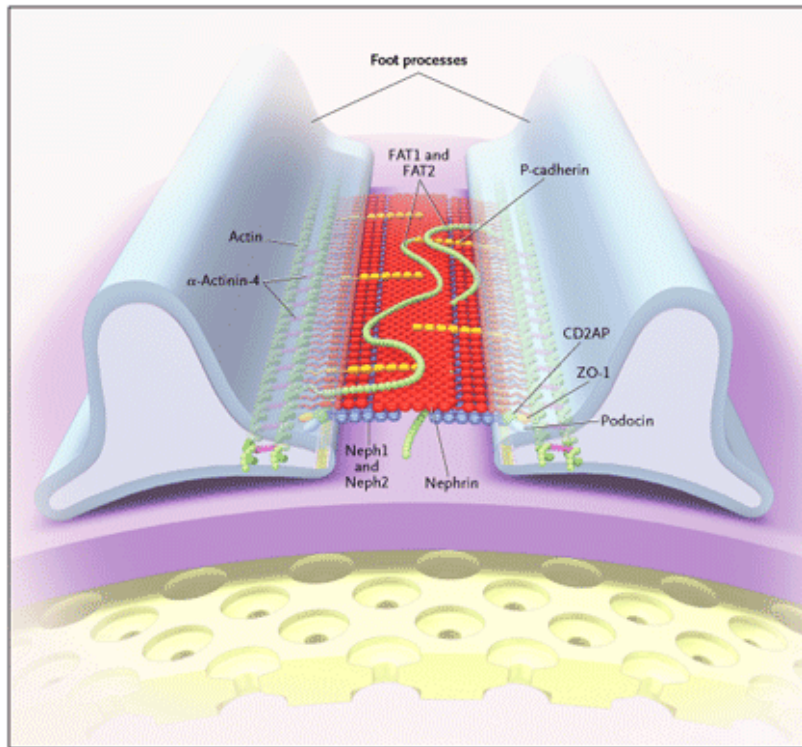
# Podocytes

(electron scanning micrograph)



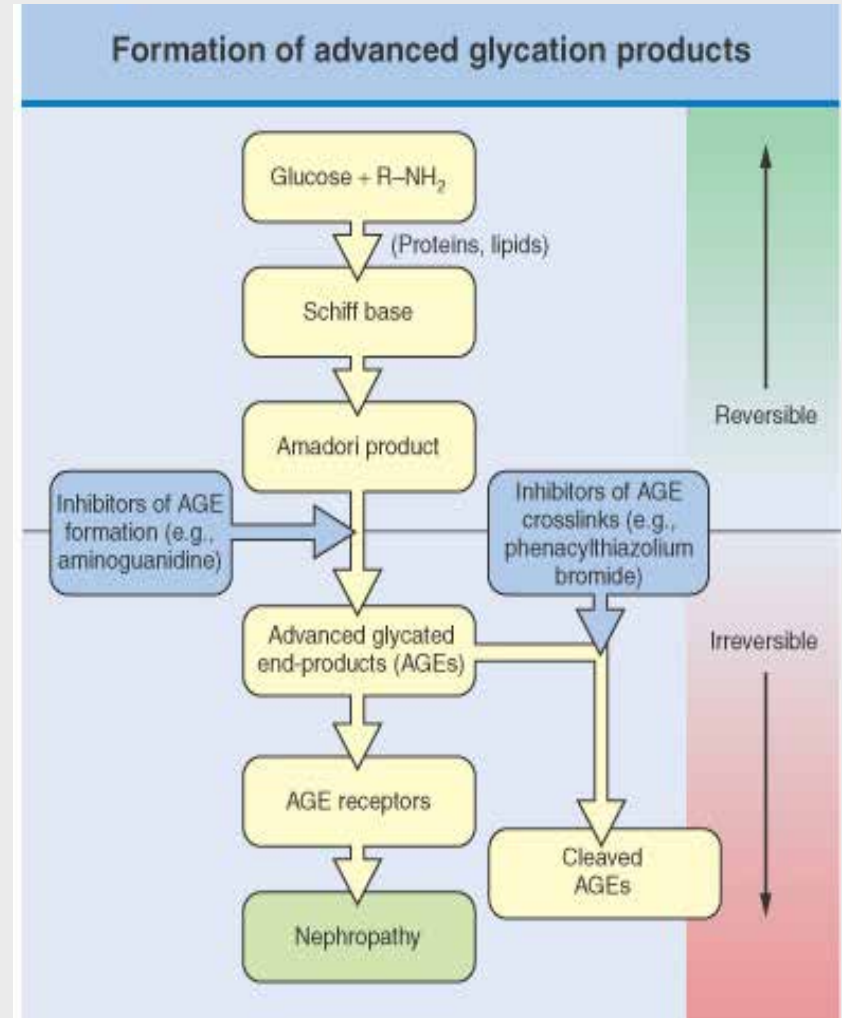
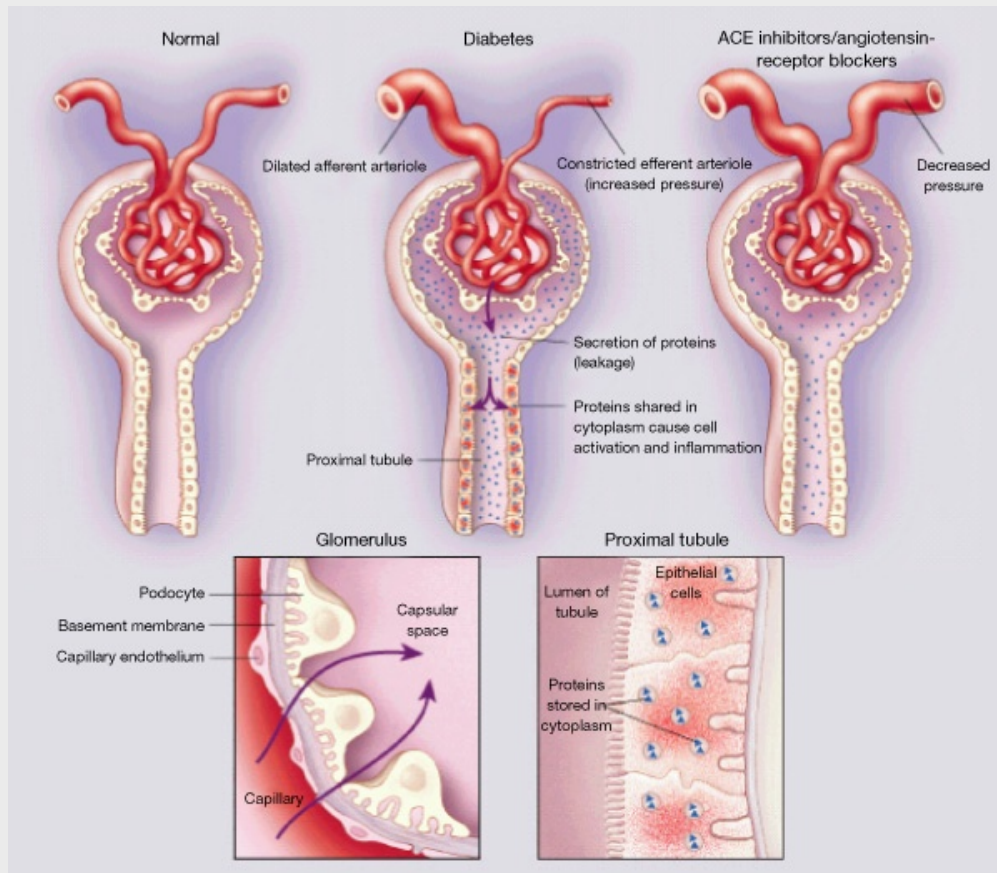


# Components of the Slit-Diaphragm Protein Complex that form a Porous Slit-Diaphragm Filter



*Tryggvason K et al New Engl J Med 2006*

# Diabetic nephropathy

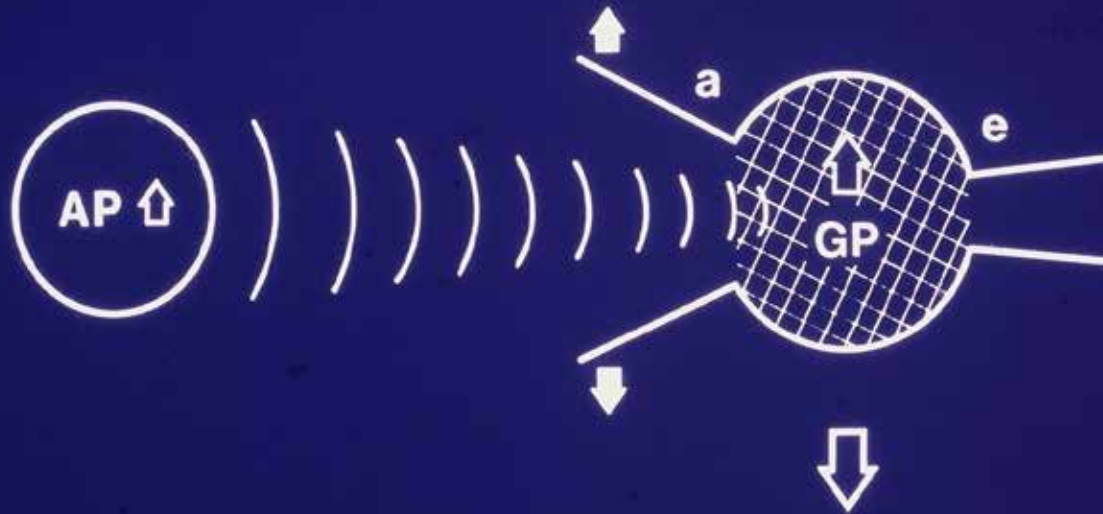




# Glomerular changes in arterial hypertension

## GLOMERULOSCLEROSIS

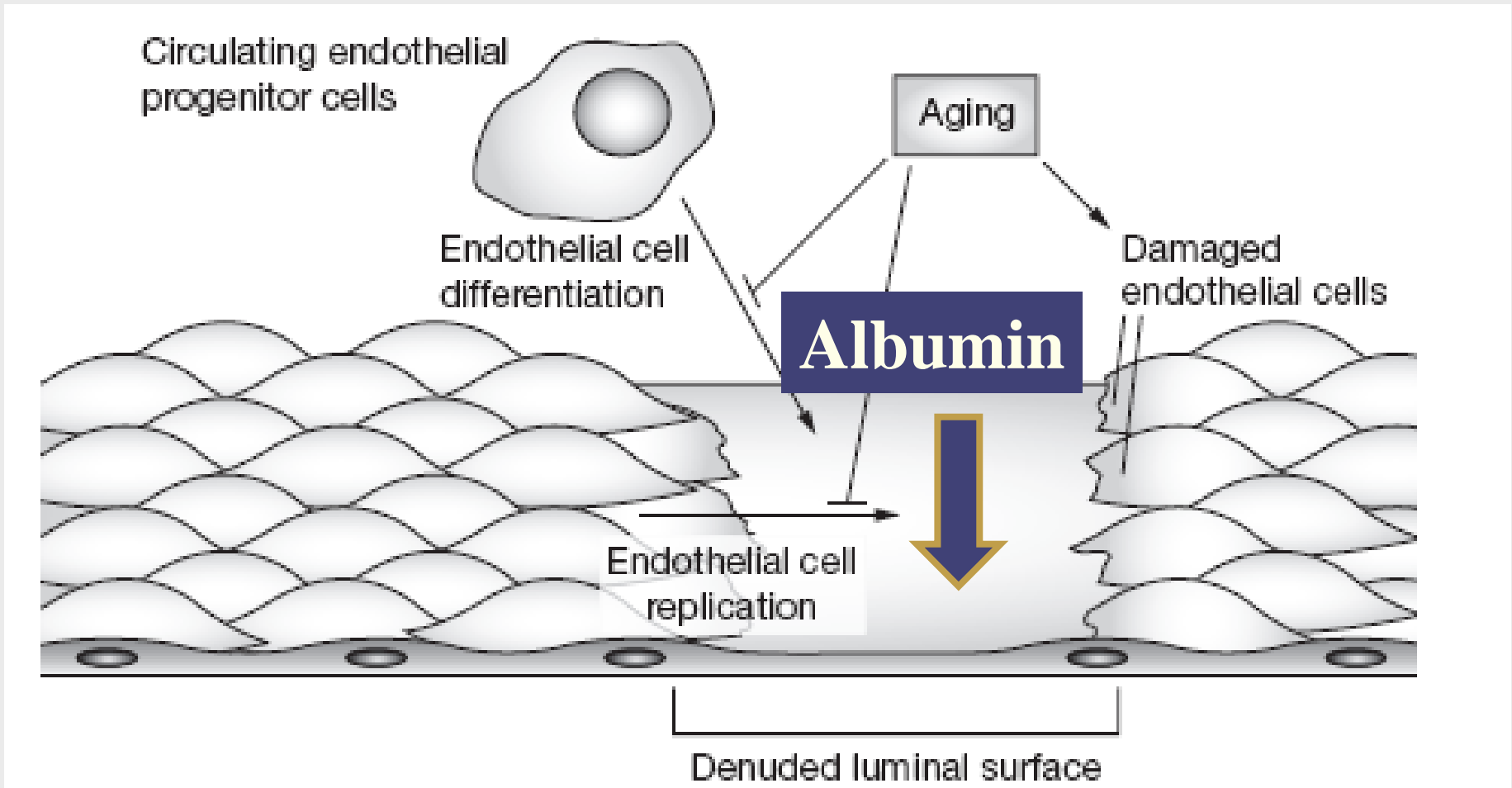
### HYPERTENSION



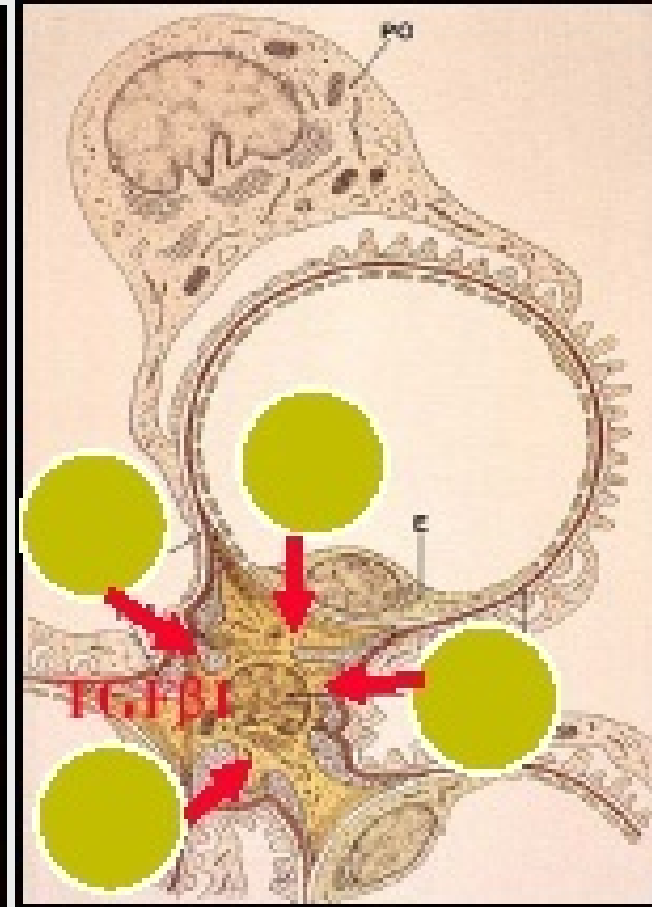
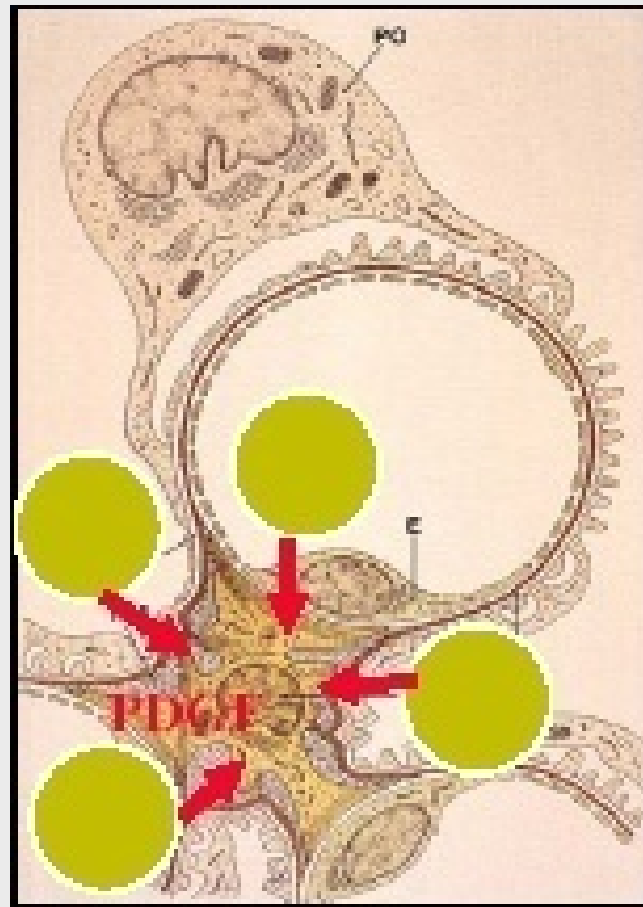
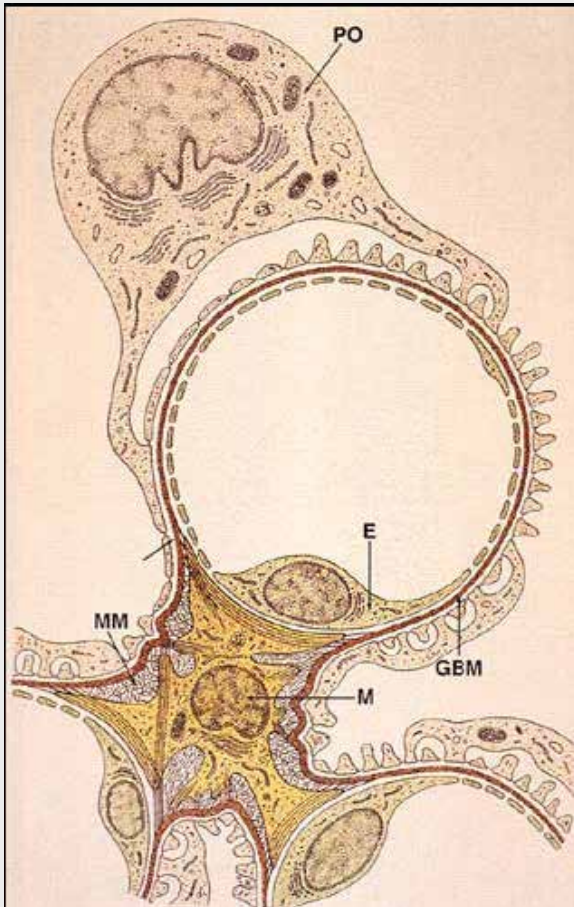
## GLOMERULOSCLEROSIS

# Albuminuria is a non-specific sign of ill health

## The Urine ESR/CRP!



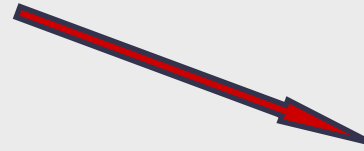
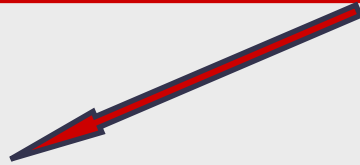
# Action of growth factors in the mesangium



**Glomerular Injury**



**Phenotypic Alteration of Mesangial Cells**



**Alteration of  
Extracellular Matrix**

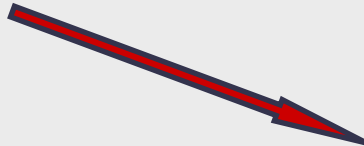
**Increased Susceptibility to  
Apoptotic Stimuli**



**Pro-apoptotic Stimuli (ROS etc) →**

**Accumulation of Extracellular Matrix**

**Cell Deletion by Apoptosis**



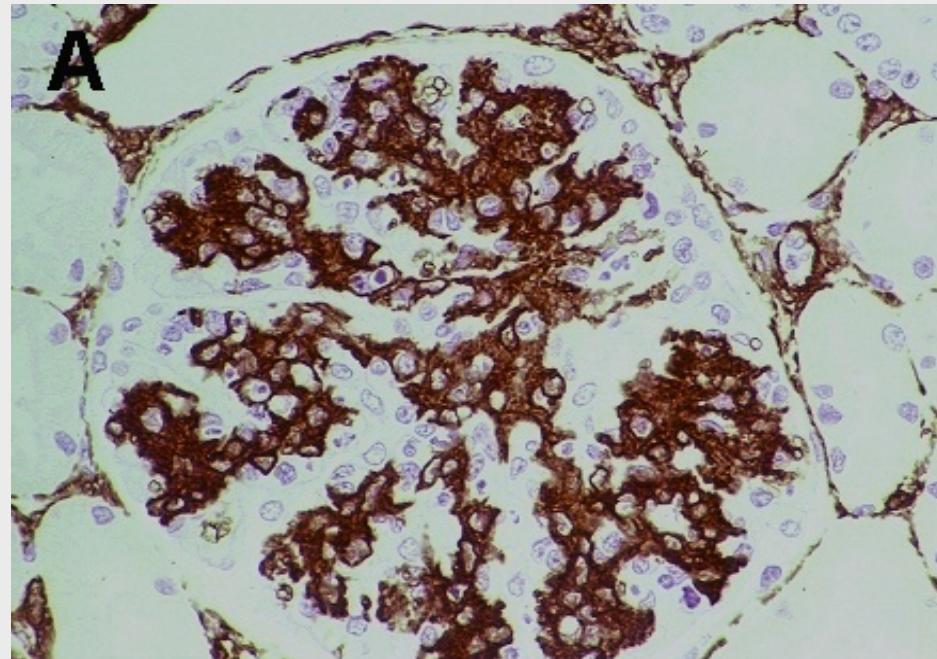
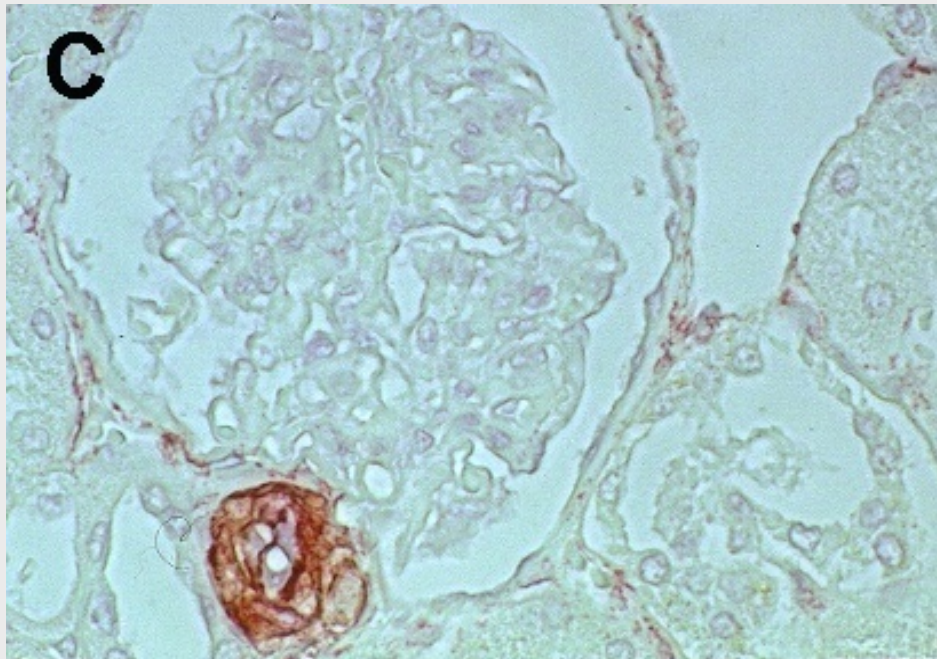
**Progression of Glomerulosclerosis**



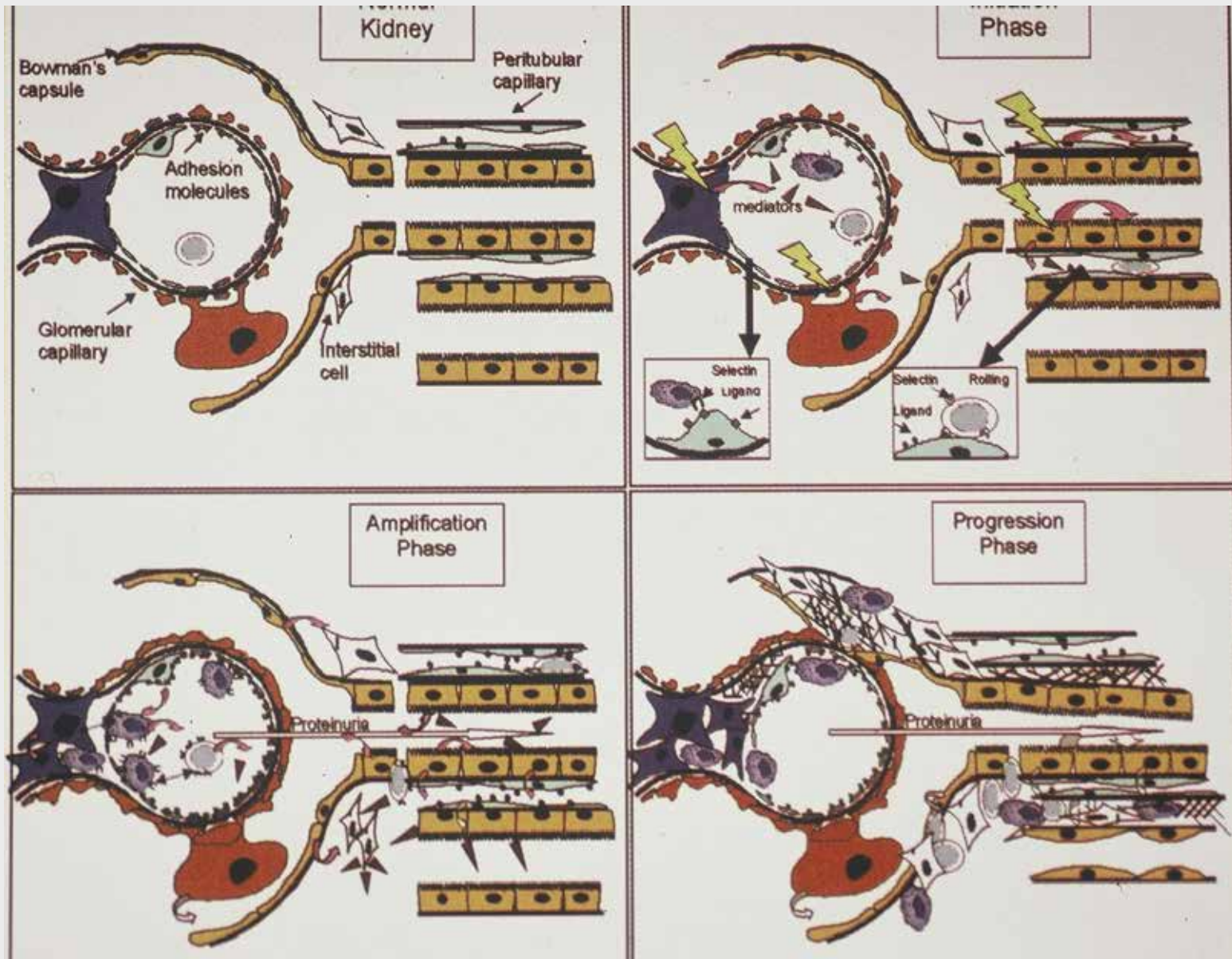
# $\alpha$ -SMA (smooth muscle actin) expression in human kidney

Normal glomerulus

Mesangialproliferative GN

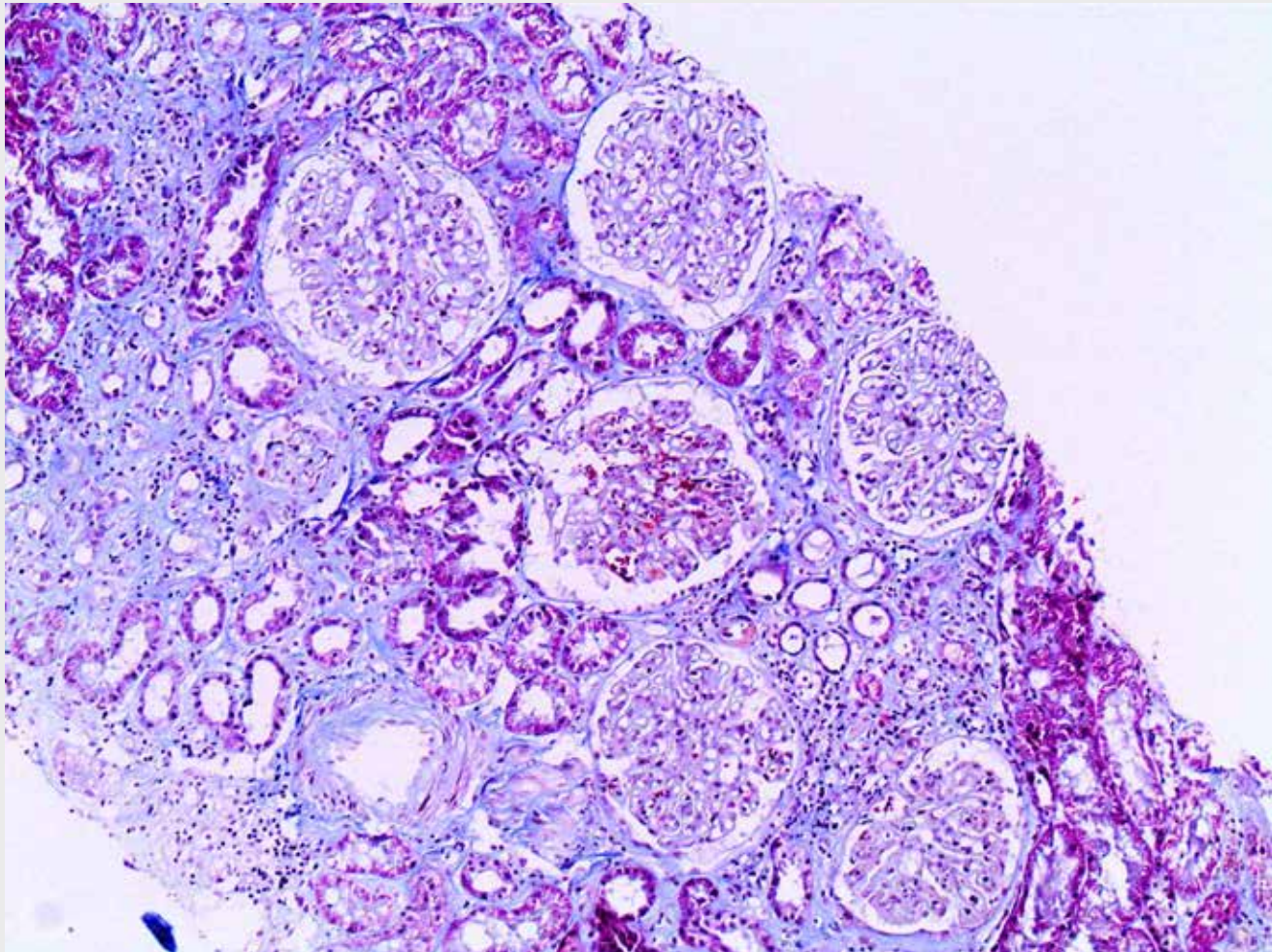


# Mechanisms of kidney injury and progression

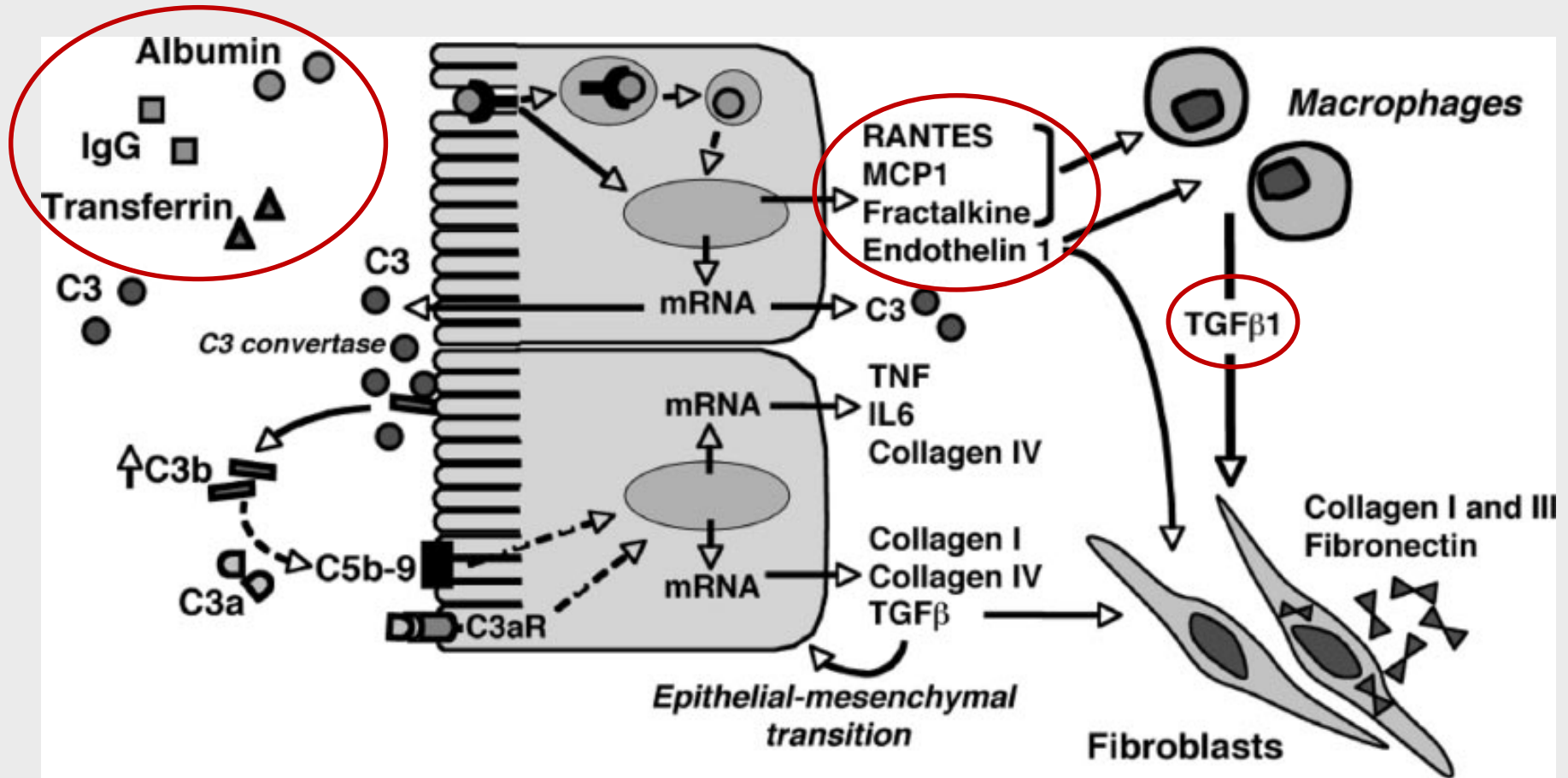




# IgA nephropathy with interstitial inflammation and fibrosis



# Induction of transcription factors



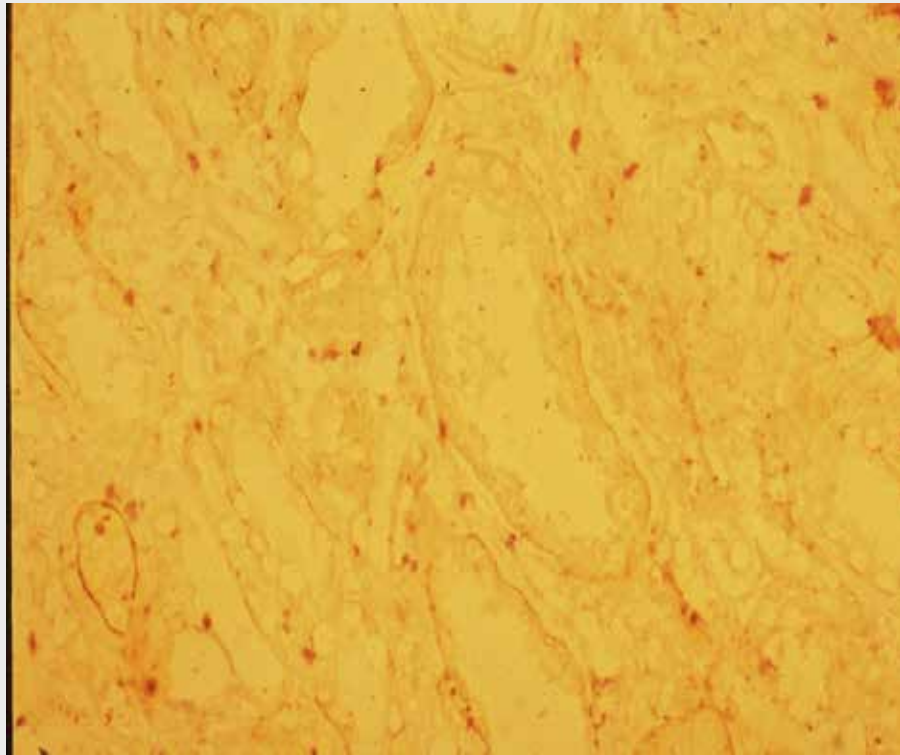
- § Non selective proteinuria induces NF-κB
- § Chemokine production (MCP – 1, RANTES)
- § Inflammatory interstitial infiltration

*Abbate M et al. J Am Soc Nephrol 2006*  
*Eardley KS et al. Kidney Int 2006*

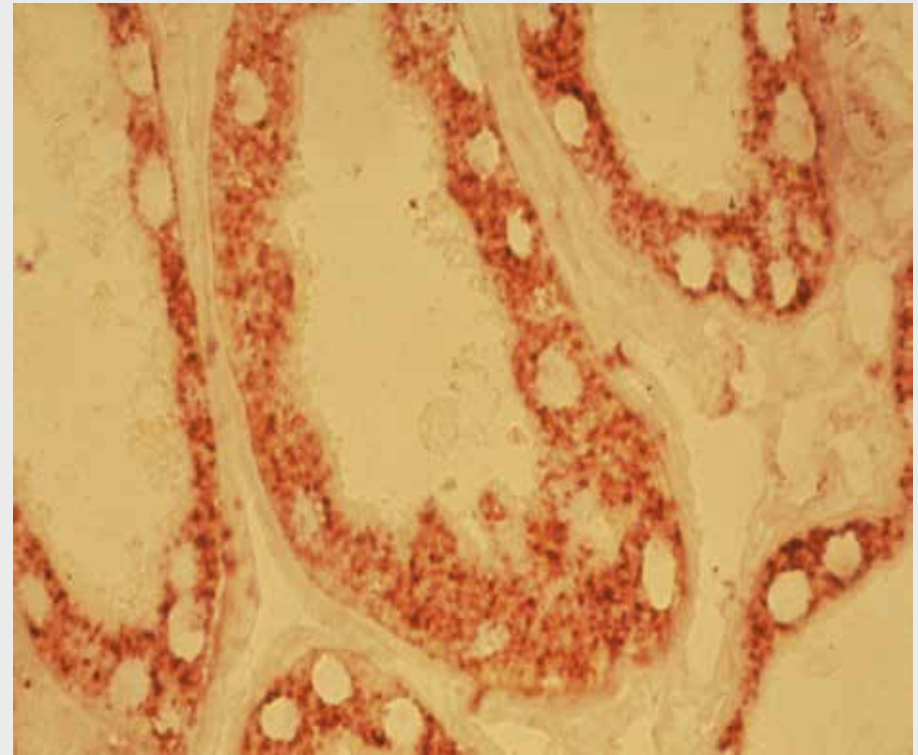


# TGF-beta m-RNA in the kidney and urine of patients with glomerular disease and proteinuria

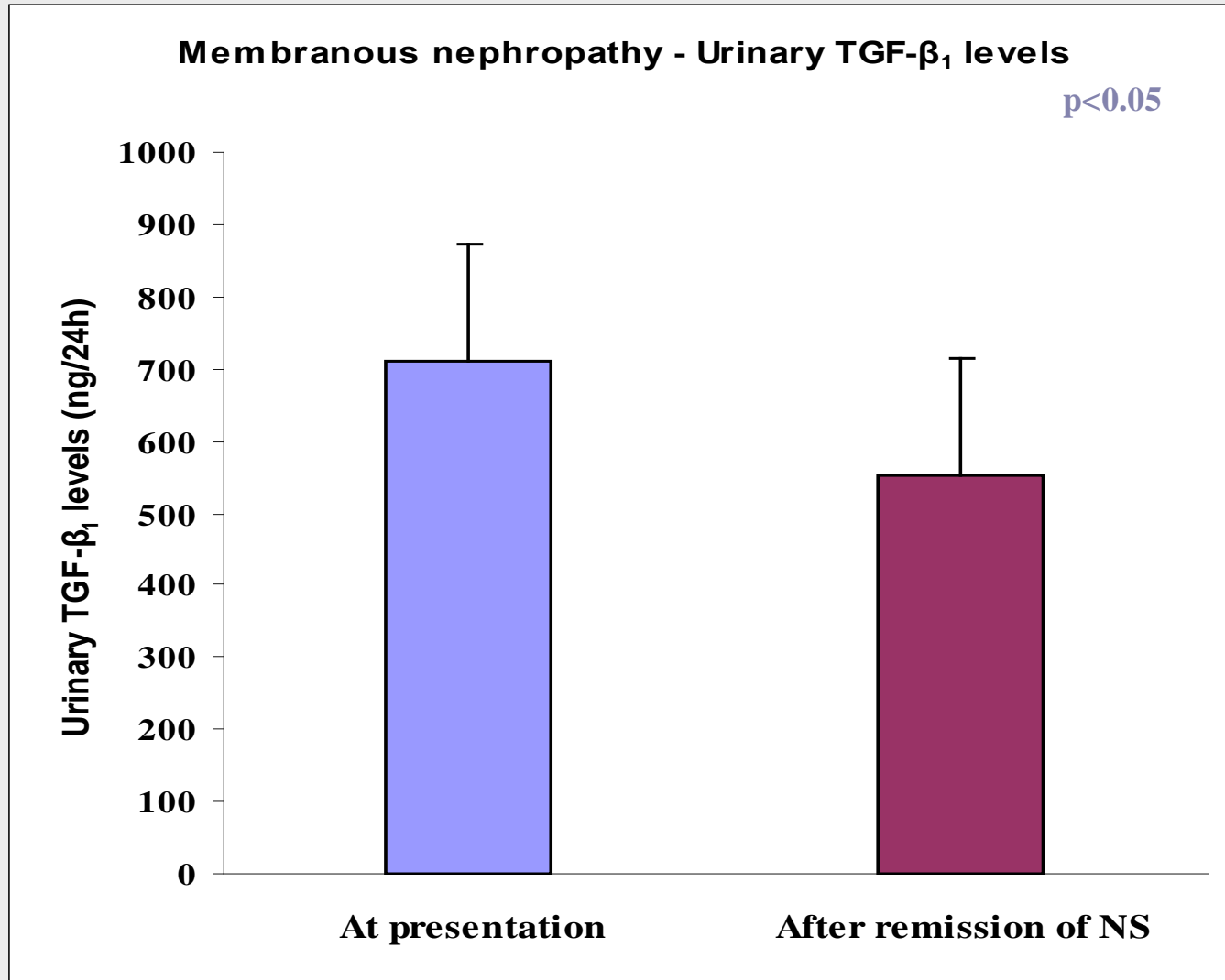
**Control biopsy**



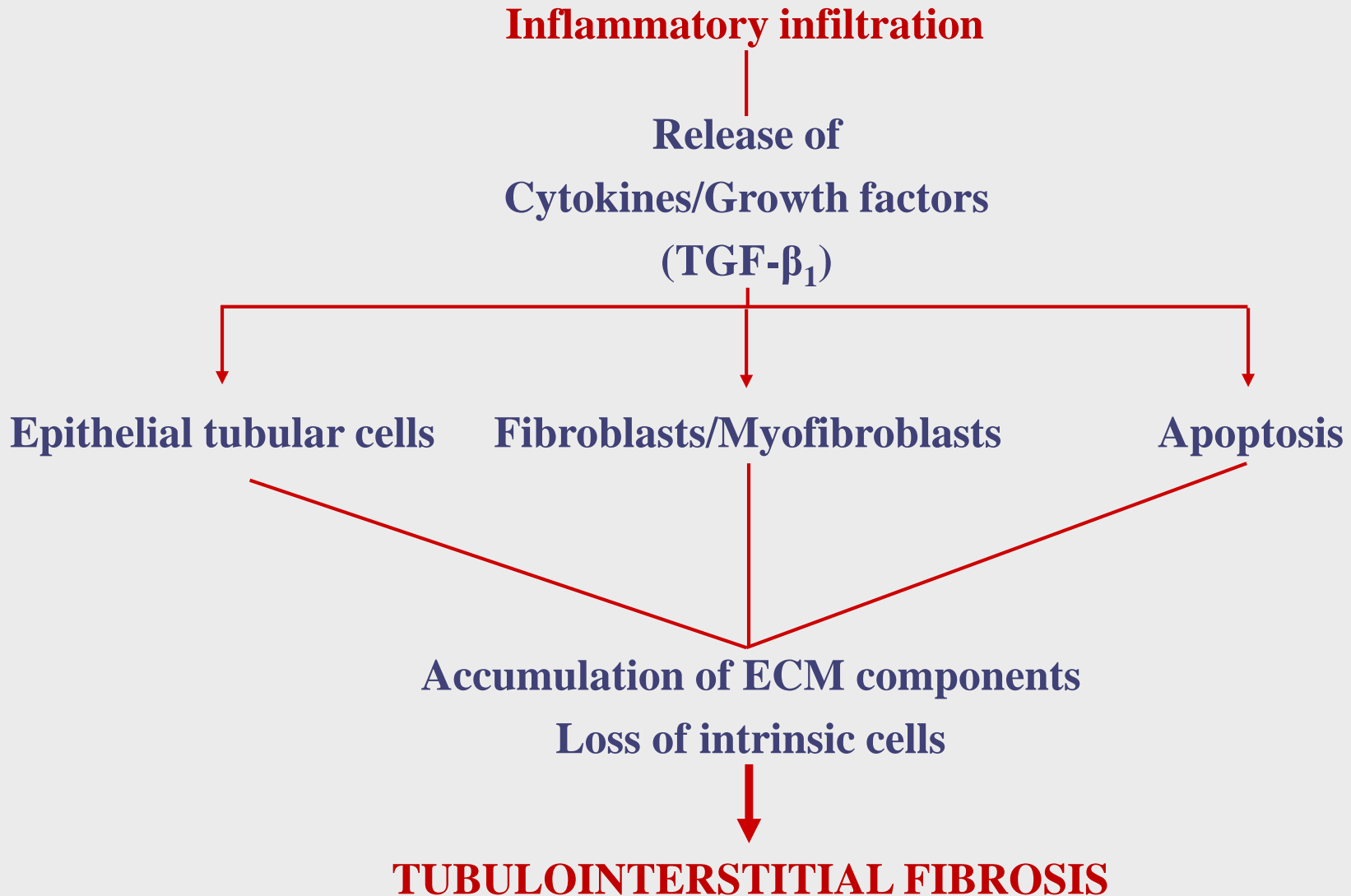
**Membranous nephropathy with heavy proteinuria**



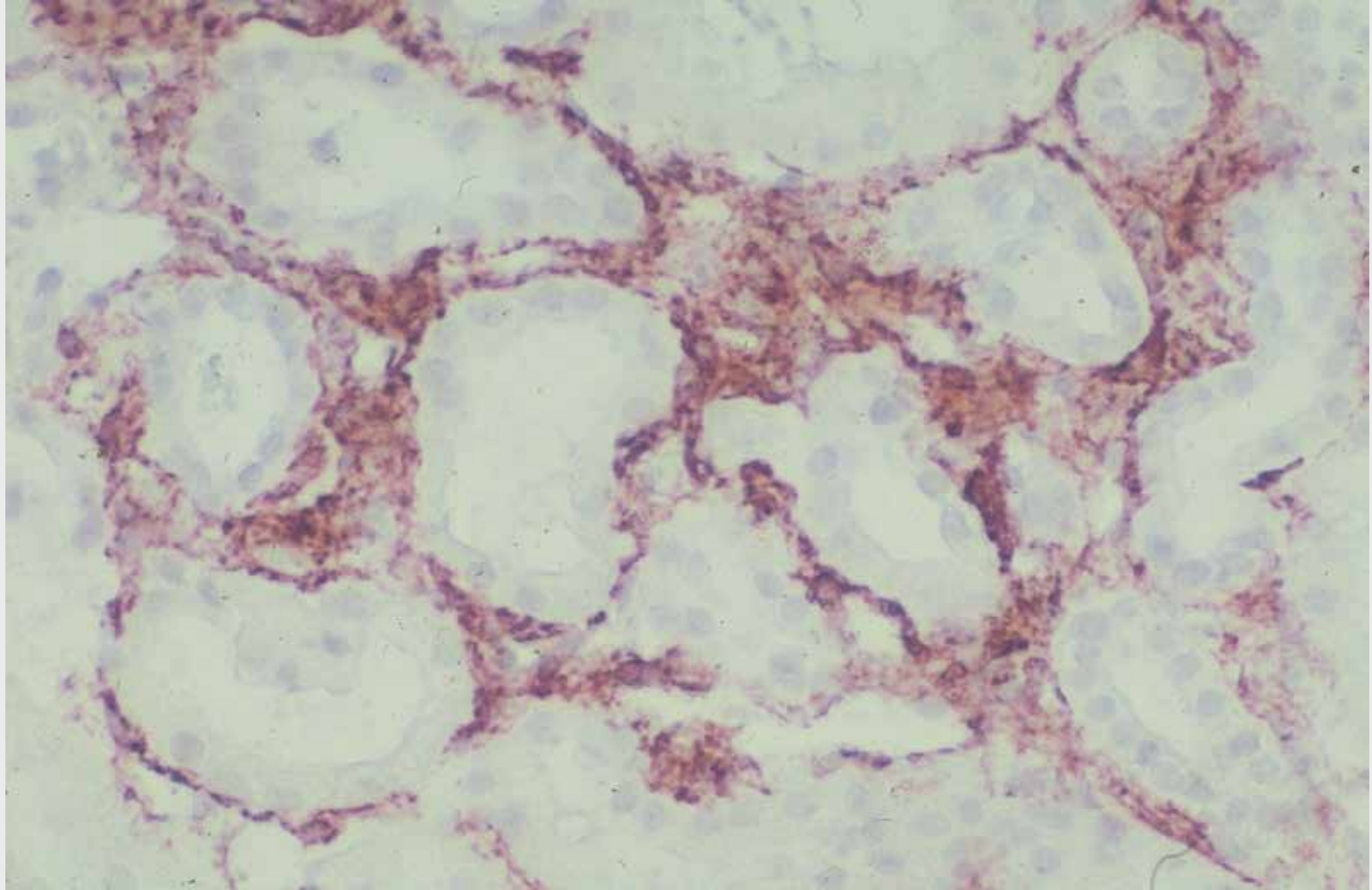
# TGF-beta levels in the urine of patients with membranous GN



# Tubulointerstitial injury in glomerulonephritis



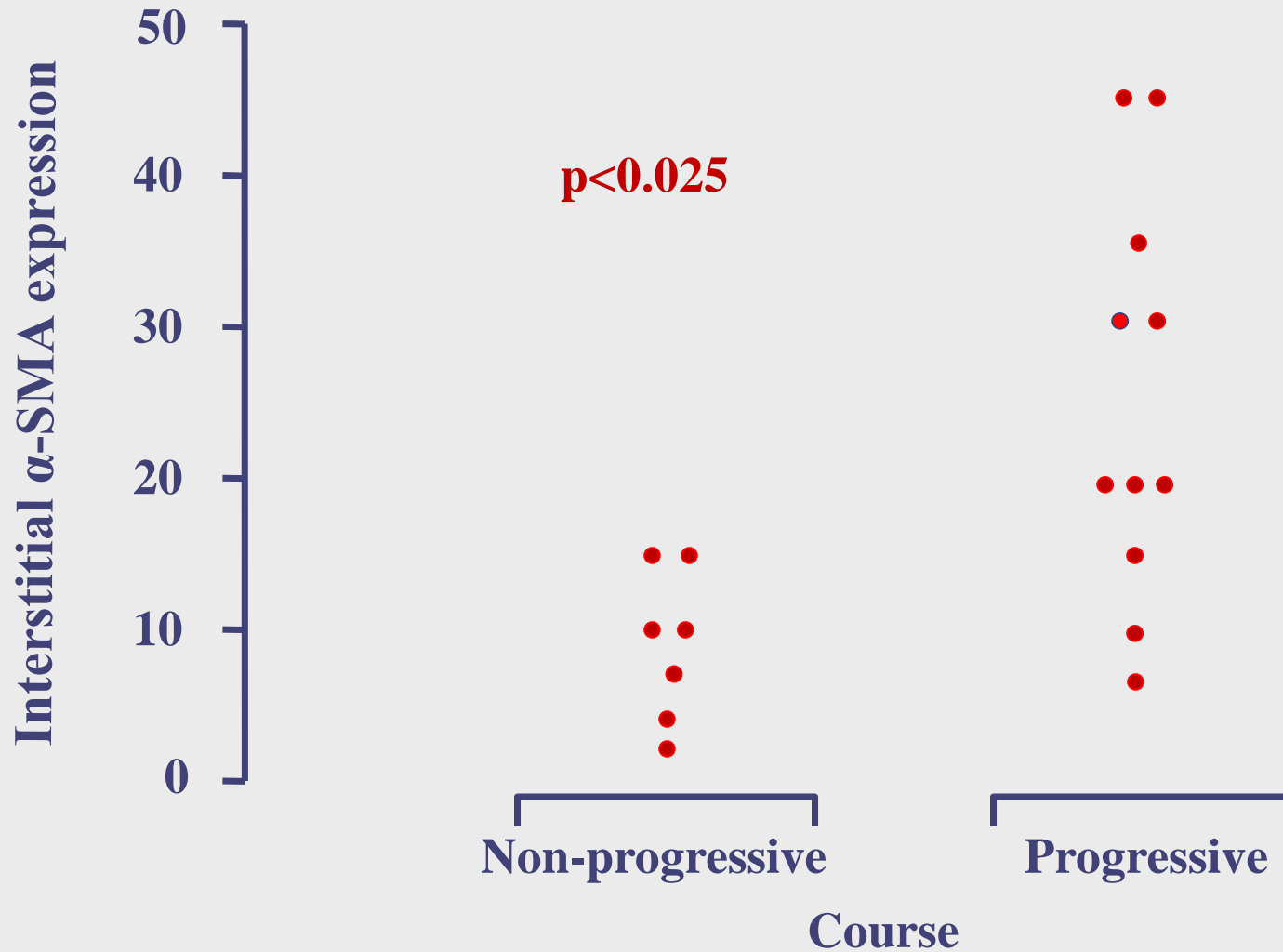
# Myofibroblasts predictors of progression of mesangial IgA nephropathy?



*Goumenos D, Brown CB, El Nahas AM. Nephrol Dial Transpl 1994*



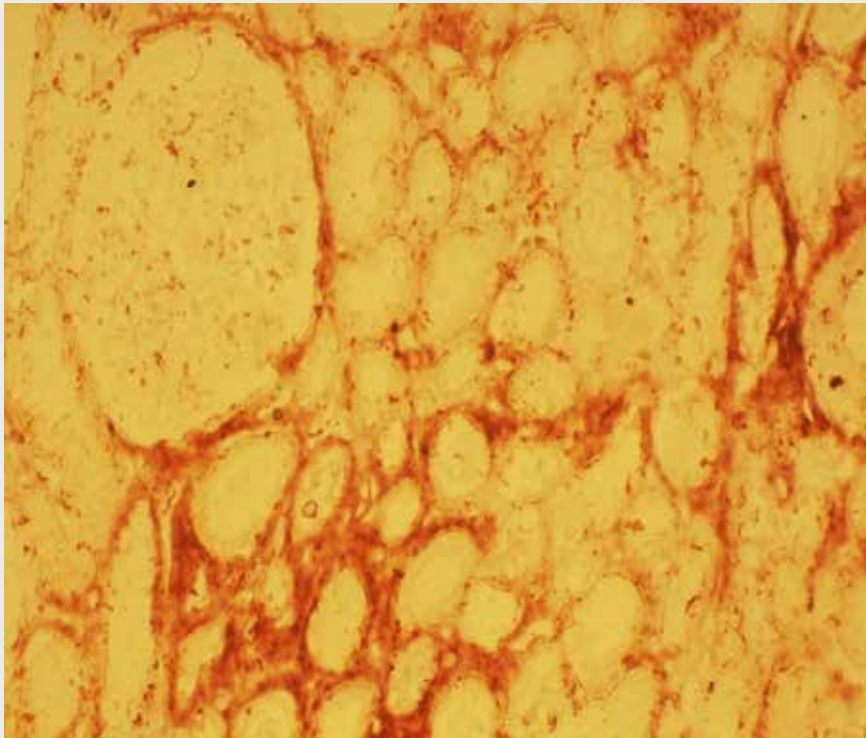
# Myofibroblasts and IgA nephropathy



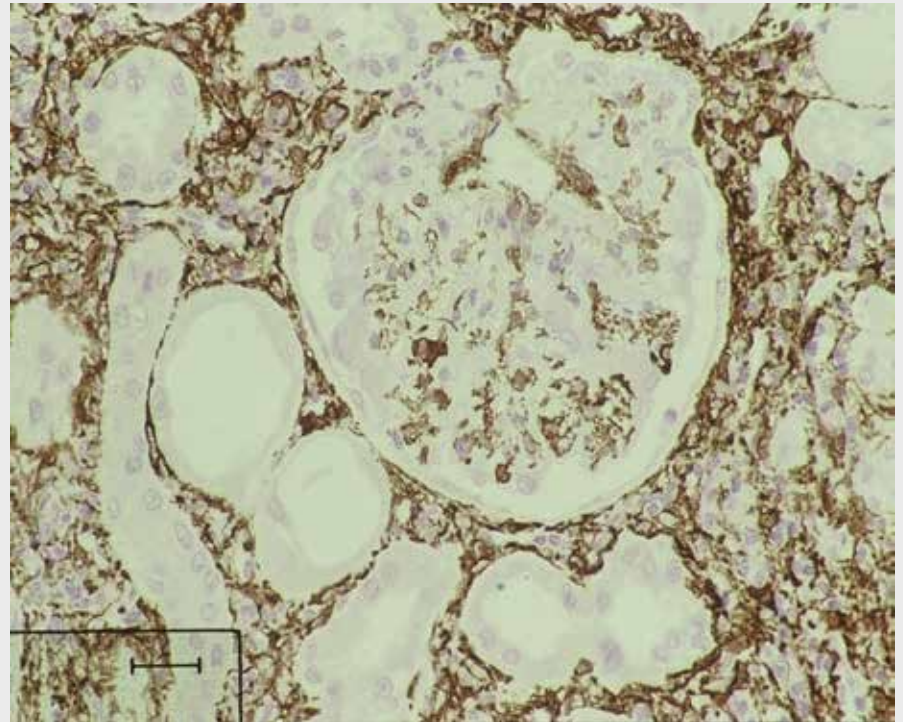
*Goumenos D, Brown CB, El Nahas AM. Nephrol Dial Transpl 1994*

# TGF-beta and myofibroblasts: A potential pathway towards renal scarring in human glomerular disease

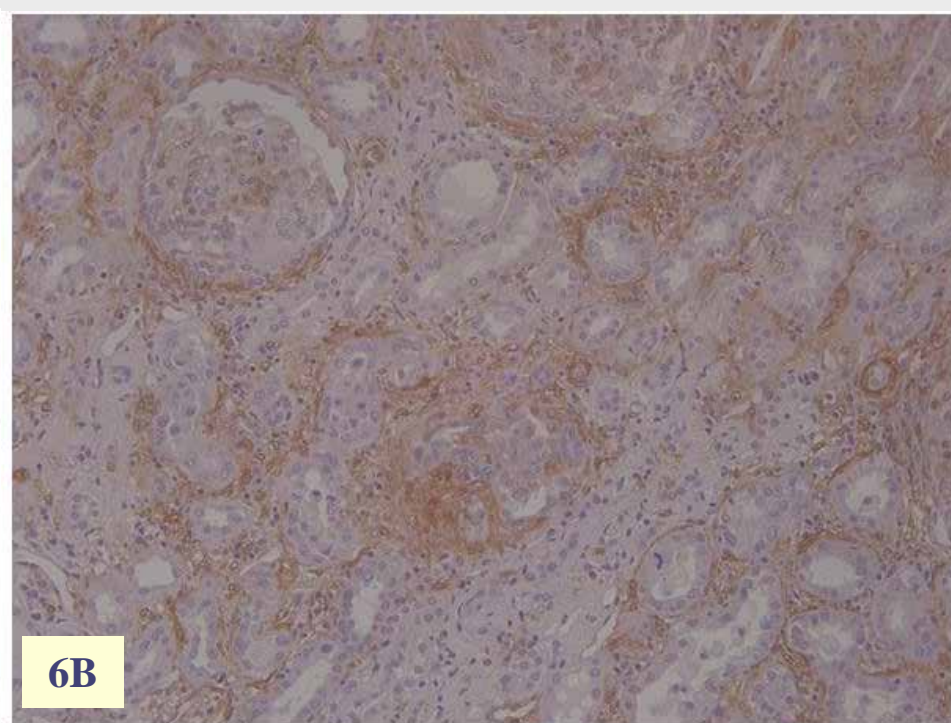
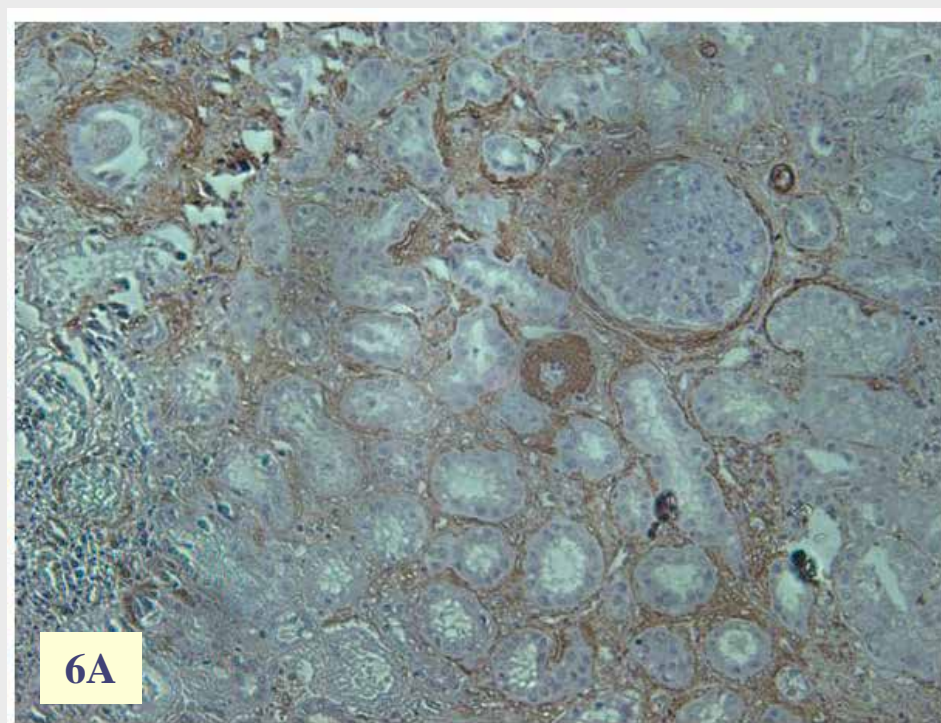
**TGF- $\beta_1$  m-RNA**



**$\alpha$ -SMA expression**



# The expression of Transgelin in human glomerulonephritis of various etiology



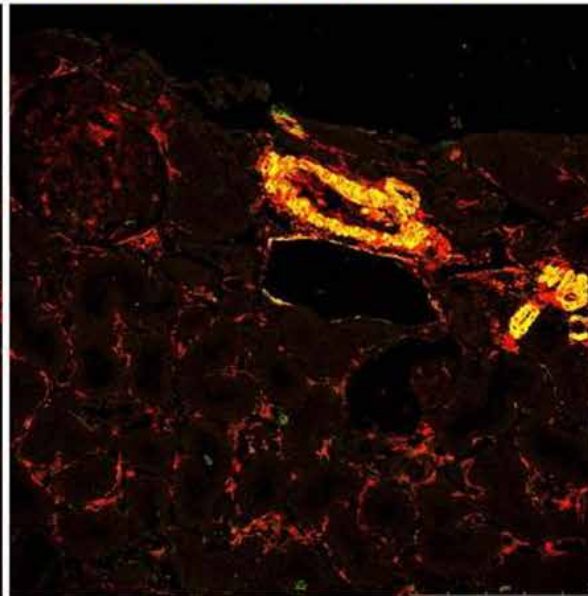
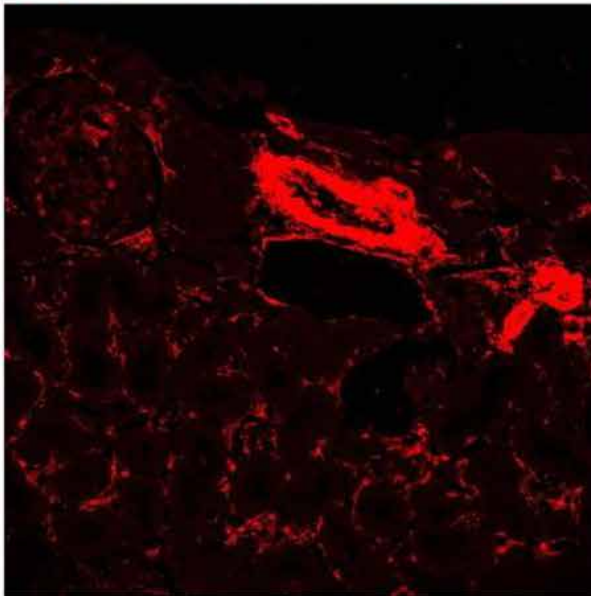
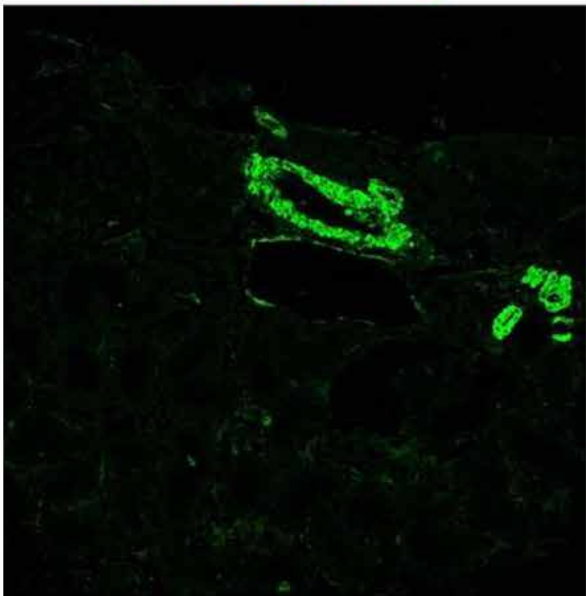
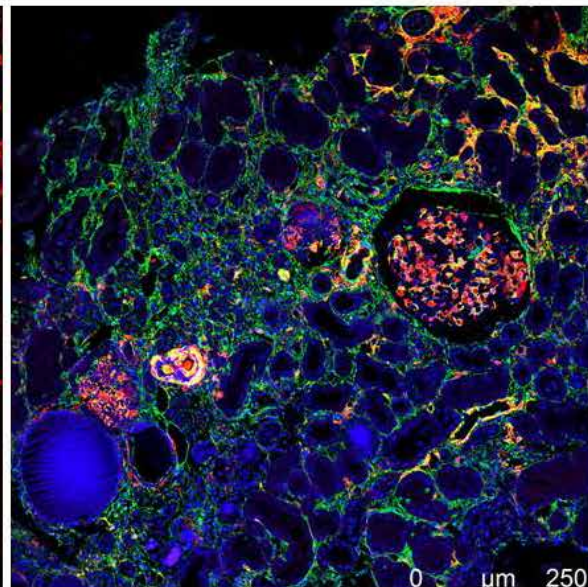
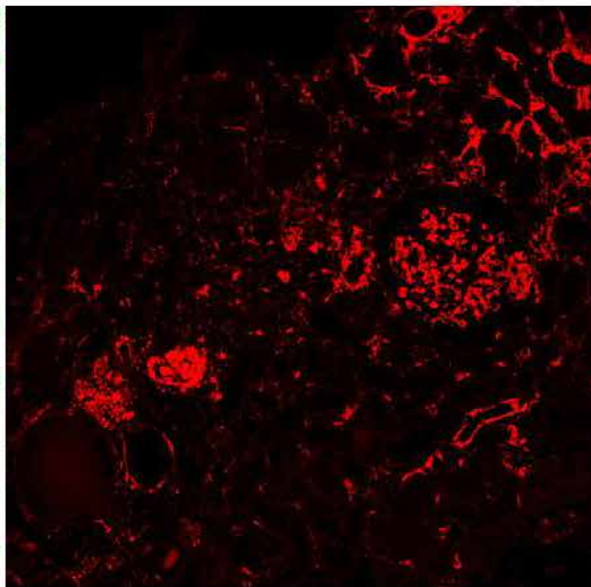
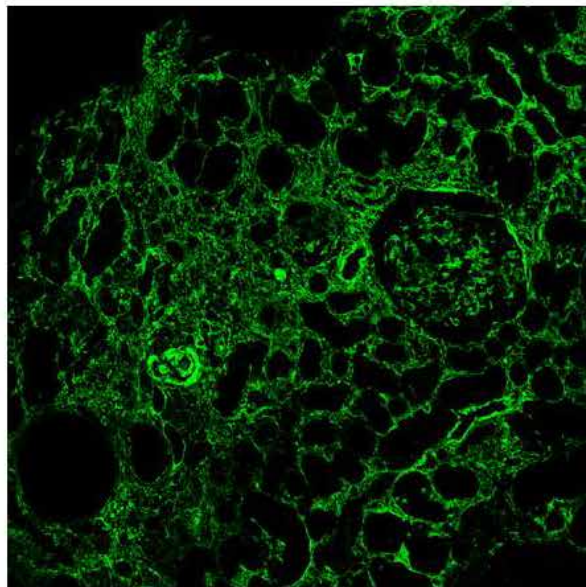
*Gerolymos M et al Nephron Clinical Practice 2011*



$\alpha$ SMA

Transgelin  
(SM22)

$\alpha$ SMA/  
Transgelin



# The expression of Transgelin in human glomerulonephritis of various etiology

## Conclusions

Intense transgelin expression was observed in renal tissue of patients with glomerulonephritis.

The observed differences in the pattern of transgelin and  $\alpha$ -SMA expression suggest that either different subpopulations of myofibroblasts exist, or these proteins are activated at different stages of renal injury/scarring.

*Gerolymos M et al Nephron Clinical Practice 2011*



# Tissue transglutaminase [tTg] in Kidney Scarring: A wound response enzyme

## ECM Accumulation

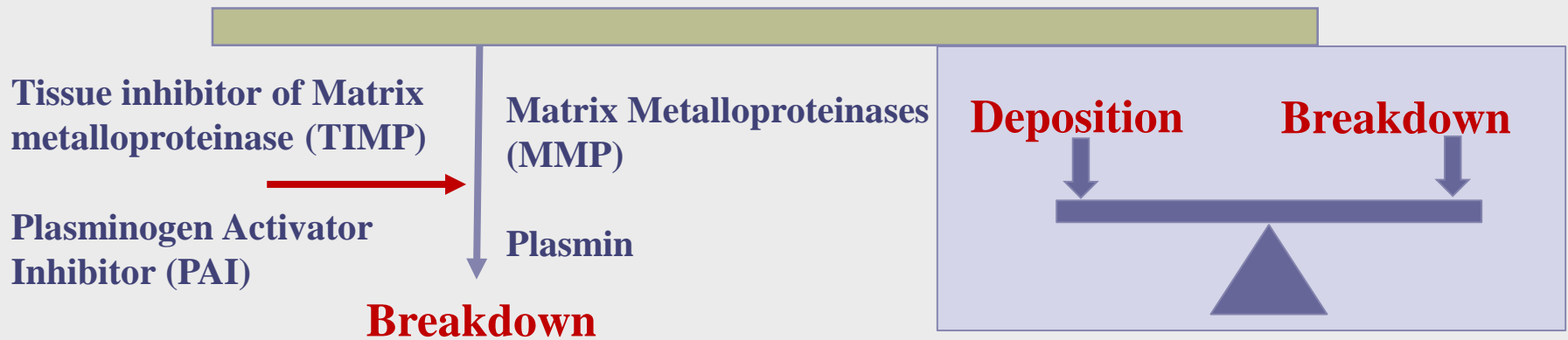
- ü Accelerated Deposition of ECM proteins
- ü Alters ECM to make it less susceptible to MMP breakdown

## Cell death

- ü Crosslinks intracellular proteins into an insoluble matrix
- ü Death if cell becomes compromised with no release of cell components

## TGF $\beta$ 1 activation

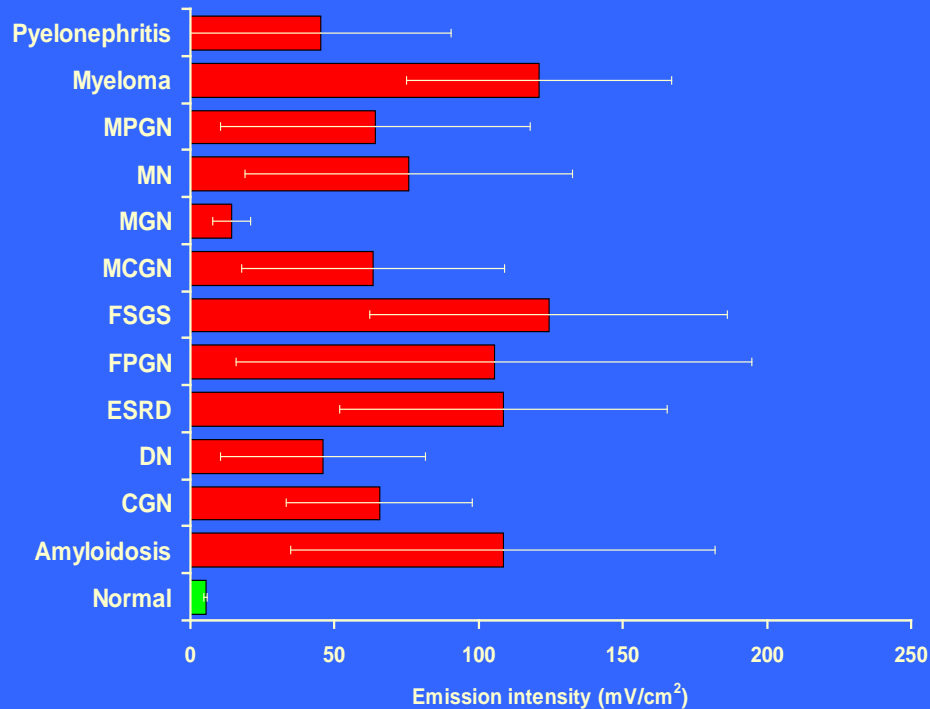
- ü Recruitment of large Latent complex by crosslinking to the ECM
- ü Arrested latent complex subject to activation



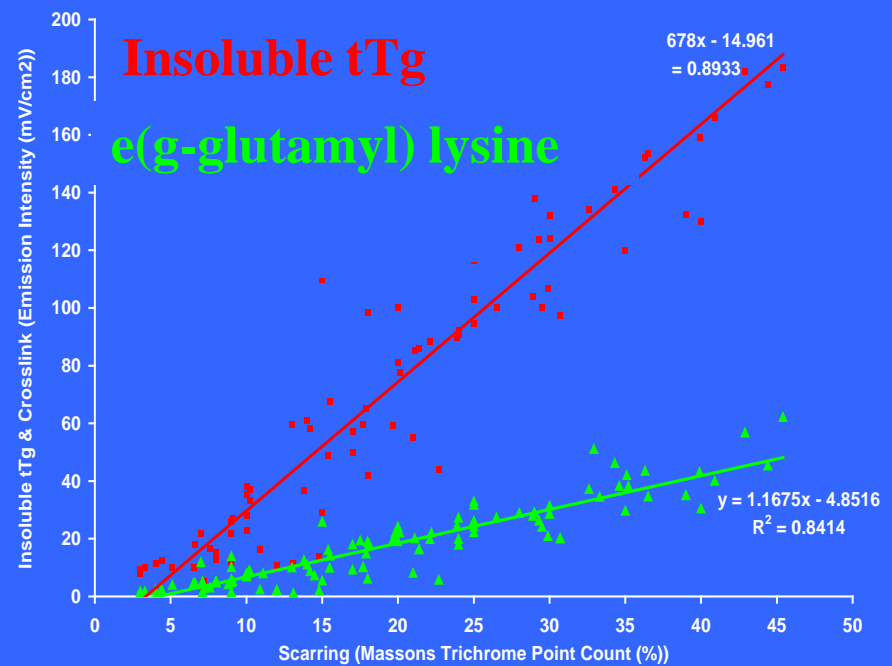
# tTg levels in kidney Scarring

## tTg levels by Diagnosis

n=136



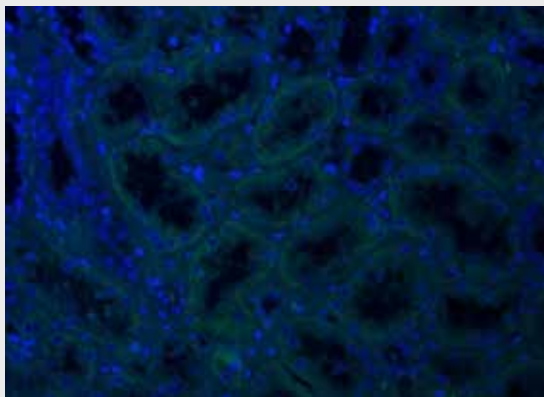
## Correlation of tTg and Crosslink with Renal Scarring



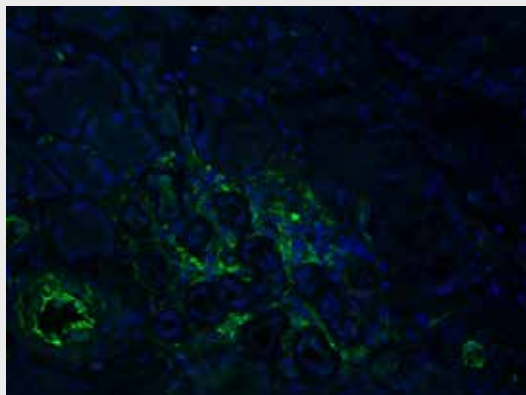
# Transglutaminase type 2 expression in the renal tissue of patients with membranous nephropathy treated by pred + CsA

32 patients with MGN, 14 with repeated biopsy after 2 years  
**indirect immunofluorescence on paraffin embedded sections**

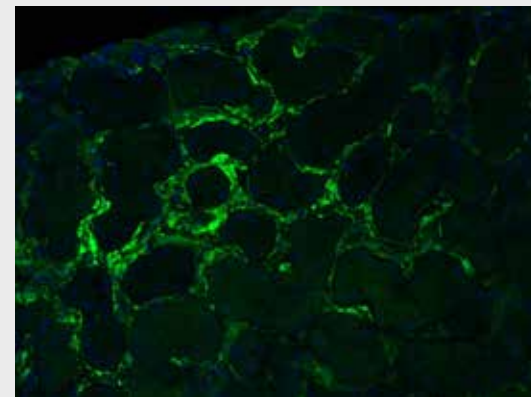
**Controls**



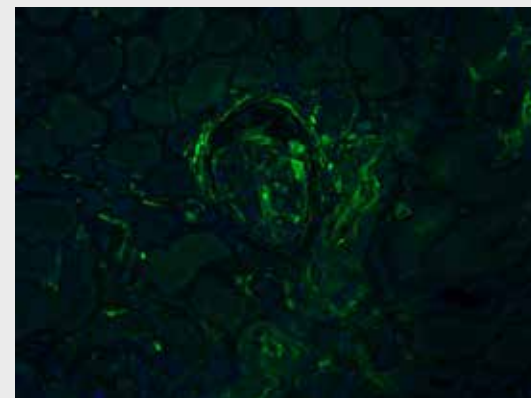
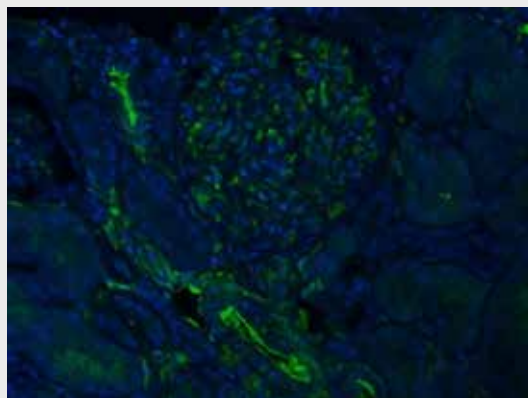
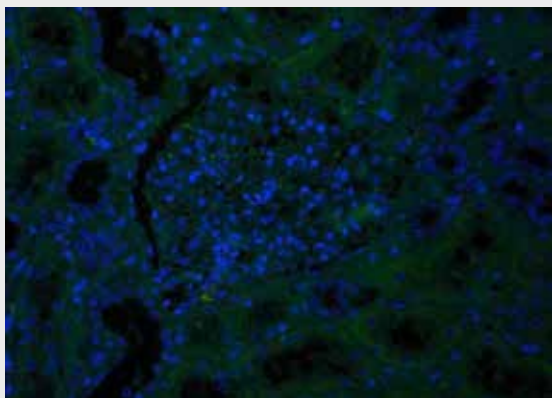
**Patients before treatment**



**Patients after treatment**



**TG2 in the kidney increased to 35 fold from baseline**



# Conclusions

The expression of TG2 in the biopsy sections of patients was greater in those with more severe interstitial fibrosis and/or more advanced glomerular sclerosis at presentation

Most patients with increased TG2 expression in the 1<sup>st</sup> biopsy showed **deterioration of interstitial fibrosis** in the repeat biopsy

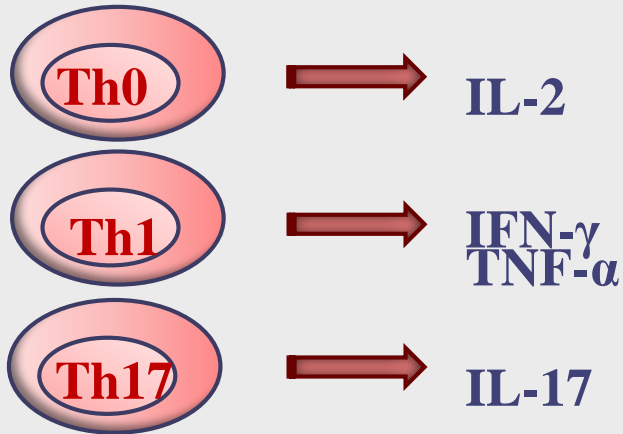


**TG2 expression an early marker (?)**



# T helper (Th) - cytokines in the urine of patients with primary GN treated with immunosuppressive drugs: can they predict outcome?

## Pre-inflammatory



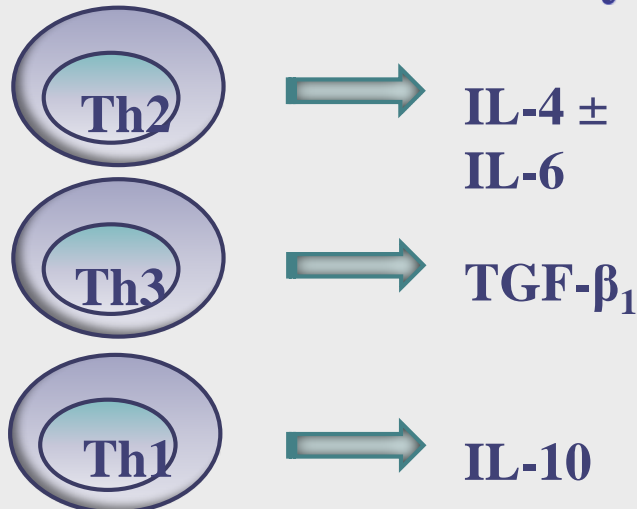
§ 97 patients with primary GN and 17 healthy subjects

ü MGN: n=36

ü IgAN: n=31

ü MCD/FSGS: n=30

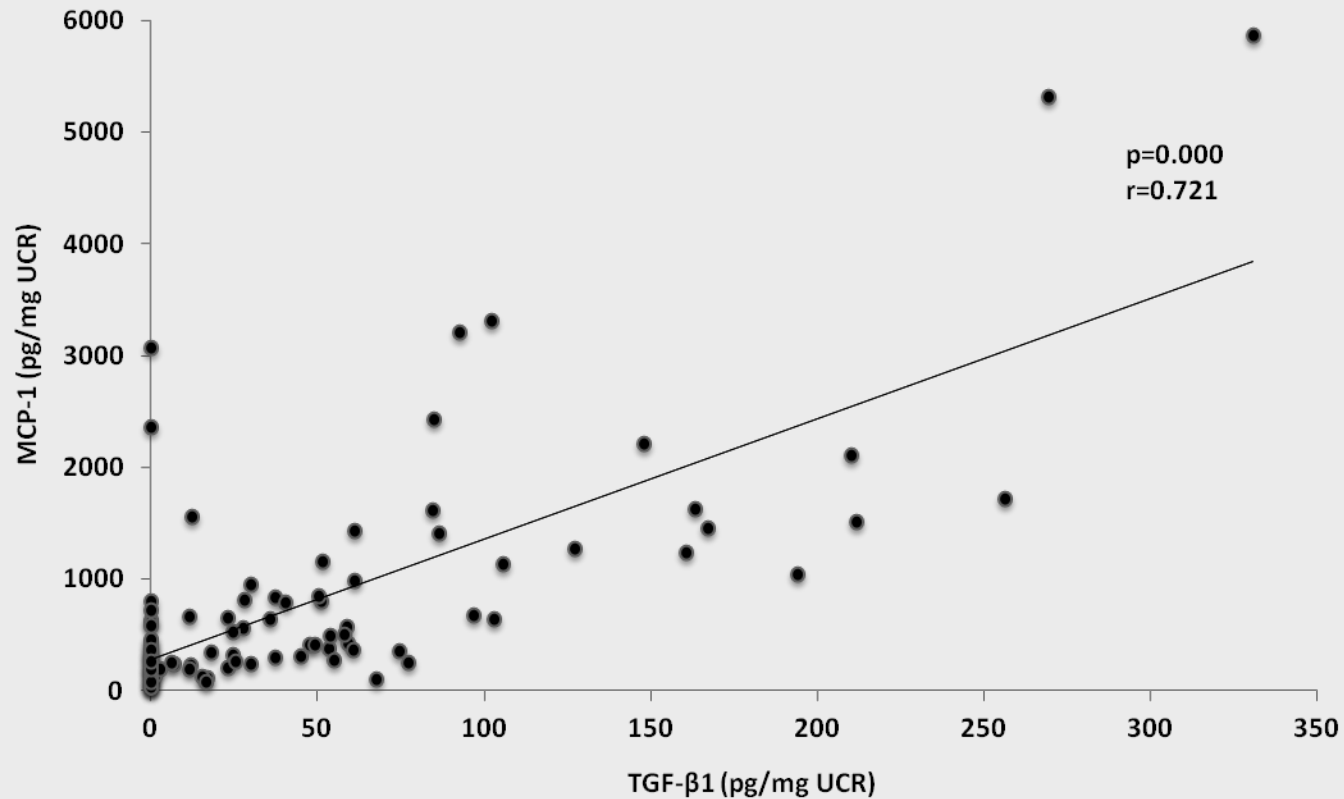
## Anti-inflammatory



§ All patients treated by immunosuppressive drugs and at the time of measurement were either in clinical remission or still had active disease with proteinuria

§ Follow up 5 years from measurement

# Correlation between the concentration of excreted TGF- $\beta$ 1 and MCP-1 in all patients



# T helper (Th) - cytokines in the urine of patients with primary glomerulonephritis treated with immunosuppressive drugs; can they predict outcome?

- § Patients had higher urinary levels of most cytokines in comparison to controls
- § Patients in clinical remission had higher urinary excretion of IL-2, IL-6, TGF- $\beta$ 1 and MCP-1
- § The main difference between patients with proteinuria and those in clinical remission was MCP-1 urinary excretion
- § The urinary excretion of MCP-1 and TGF- $\beta$ 1 was significantly higher in patients with MN who showed deterioration of renal function over a follow up period of 5 years, **a finding suggestive of a potential prognostic value of these two cytokines.**

## New Biomarkers for human kidney disease

- ∅ The identification of more specific markers of the scarring process in the renal tissue and urine might contribute to the development of more effective therapeutic measures towards the delay of renal disease progression

*Anti - cytokines*

*Anti - inflammatory*

*Anti - fibrotic*





# UCB Proposal

## Hellenic Chronic Kidney Disease Biorepository

§ Aim: To build a renal disease biorepository with longitudinal sample collection (every 3 months) of urine, plasma and serum over a minimum 3 year collection period with ideally paraffin embedded and frozen (cryostat) biopsy sections taken at some point during the 3 year collection period.



**400,000 pounds for 3 years with potential extension of 2 more years**