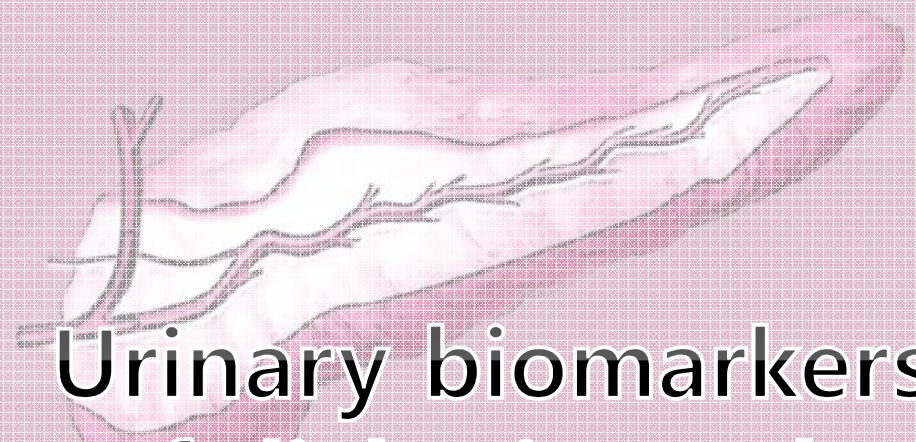
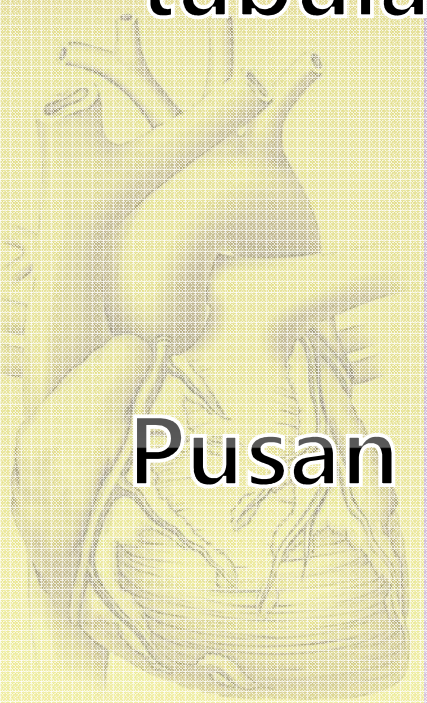


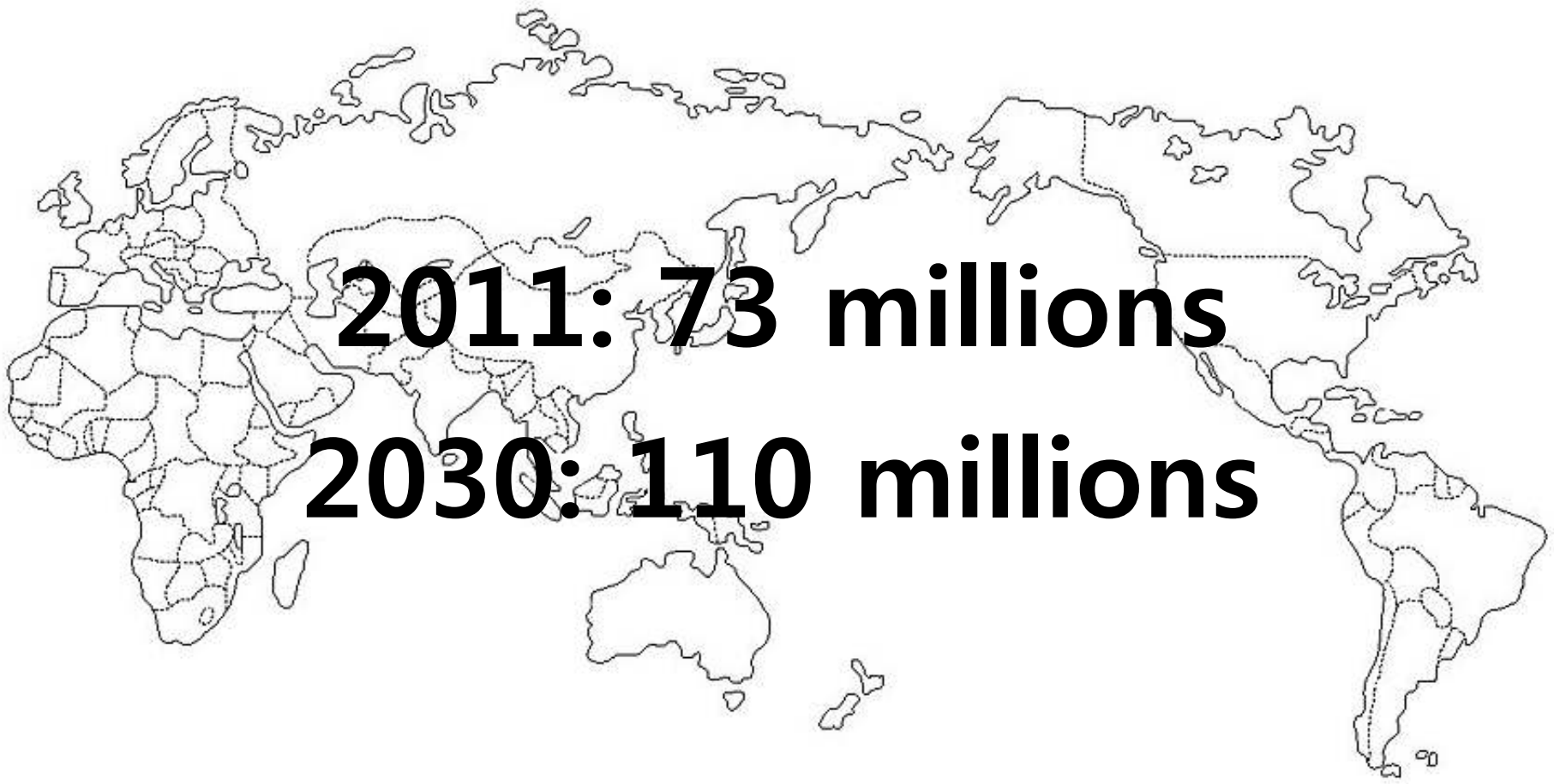
# Urinary biomarkers for early detection of diabetic nephropathy: focused on tubular and inflammatory damage



Sang Soo Kim

Pusan National University Hospital

# Overt nephropathy

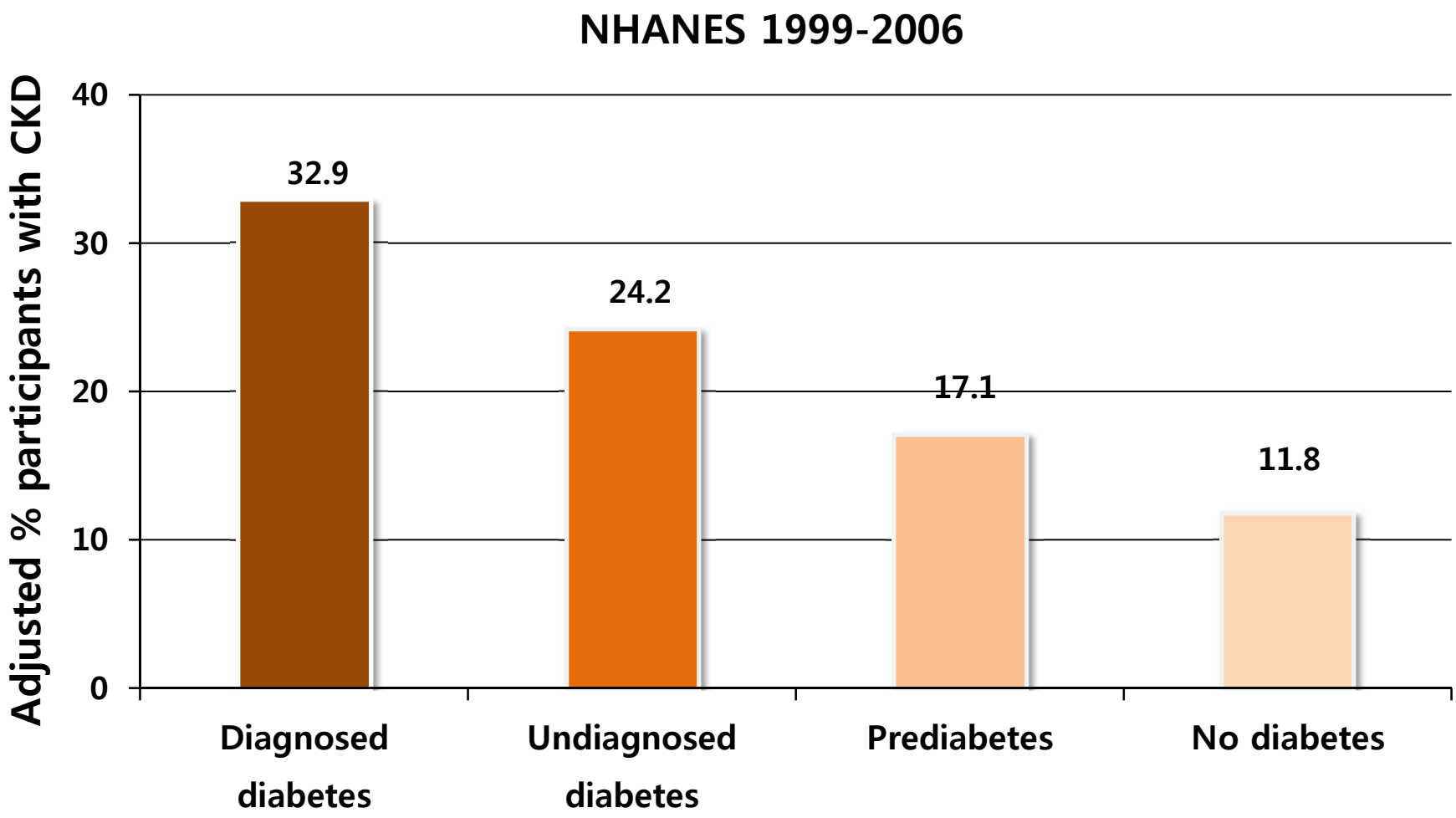


**2011: 73 millions**

**2030: 110 millions**

*IDF Diabetes Atlas 2011*
*From Dr. Remuzzi's lecture: Berlin, July 3, 2012*

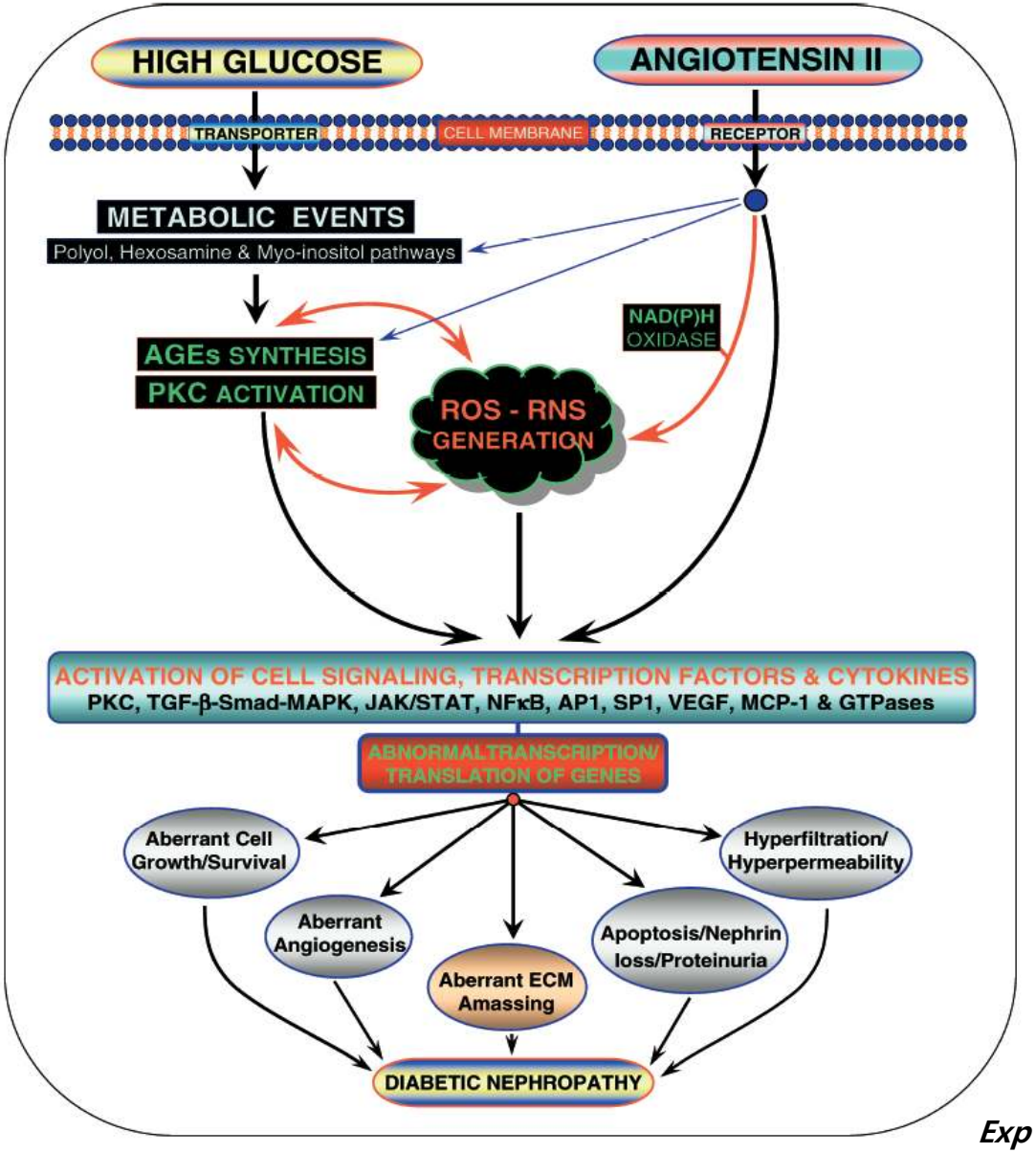
# Participants with CKD by diabetes status




* CKD, defined as MDRD-based eGFR <60mL/min/1.73m2 or ACR ≥30 mg/g

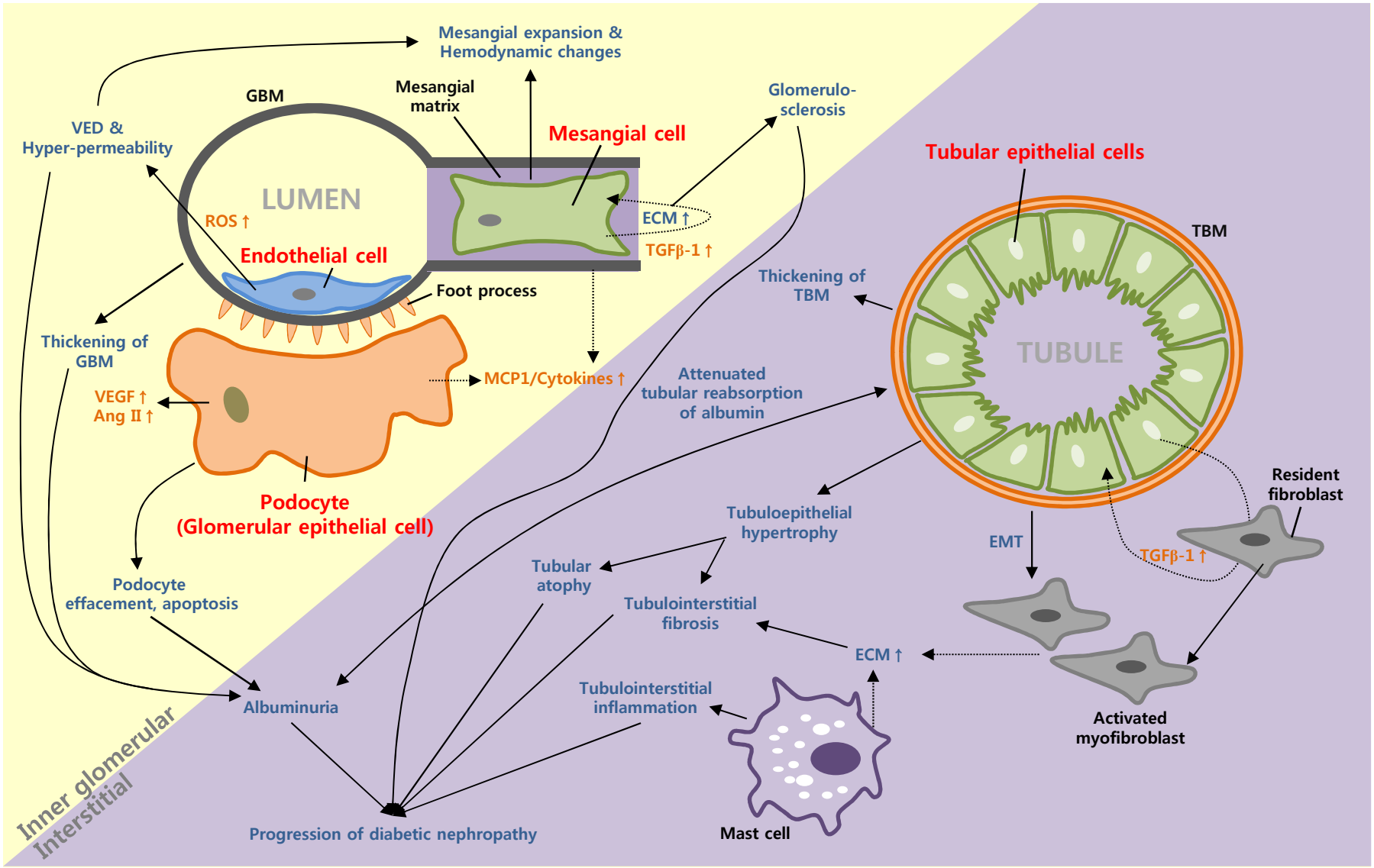
*Clin J Am Soc Nephrol 5:673-82, 2010*

# The Pathogenesis of Diabetic Nephropathy




*Exp Biol Med 233:4-11, 2008*

# Involvement of different renal cell types in pathogenesis of DN



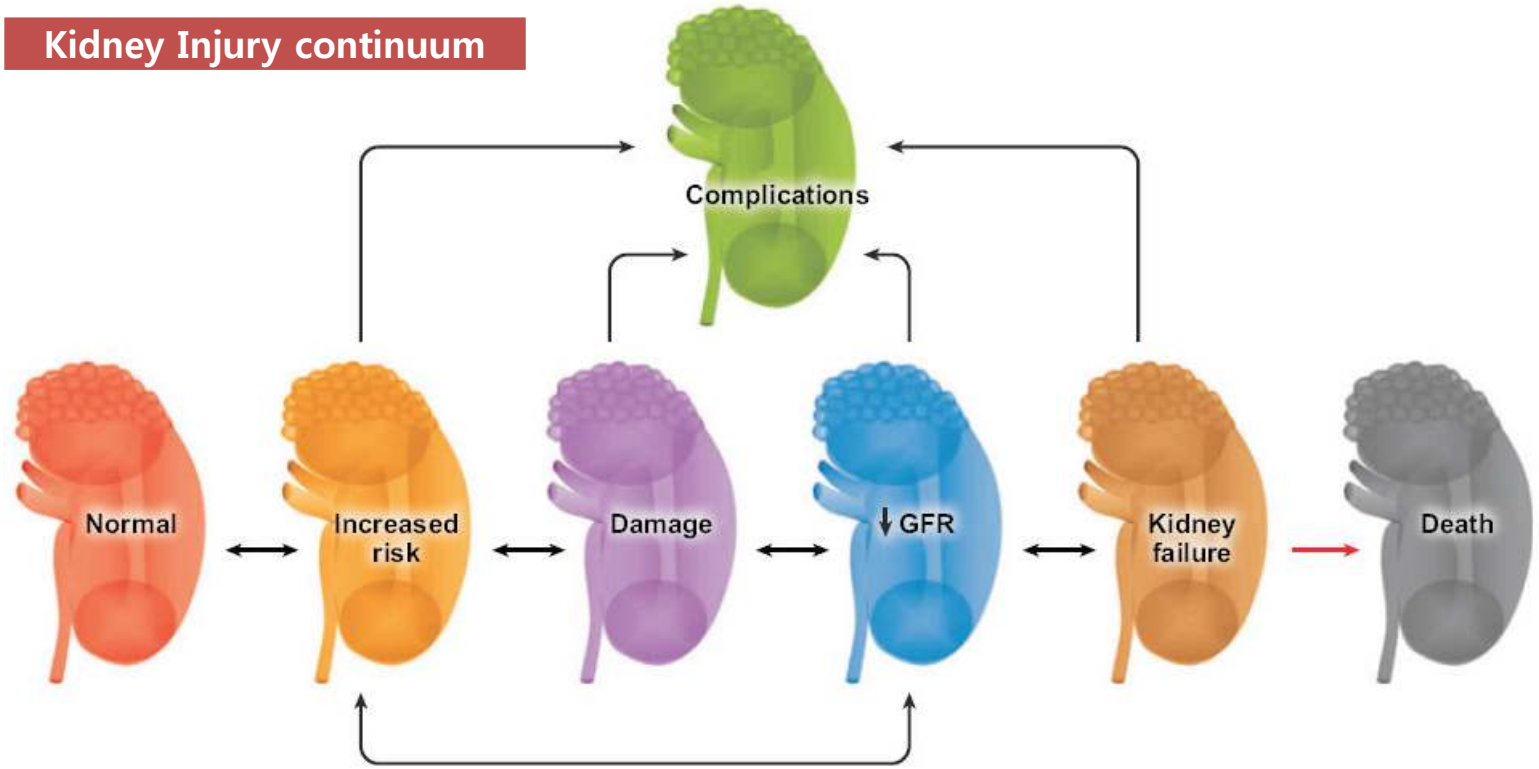

*Trends Endocrinol Metab 21:50-6, 2010*

# 5 stages 'conventional' DN (1980s)

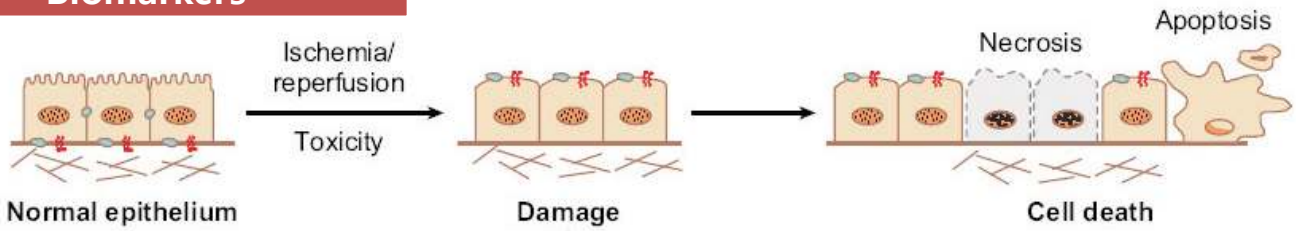
| | |
|---|---|
| Stage 1 | Reversible glomerular hyperfiltration |
| Stage 2 | Normal glomerular filtration rate (GFR) and normoalbuminuria |
| Stage 3 | Microalbuminuria and normal GFR<br>(5 to 10 years after diabetes mellitus discovery) |
| Stage 4 | Proteinuria appears and may reach nephrotic range levels (after 10 to 20 years of evolution) |
| Stage 5 | Chronic kidney disease which leads to terminal kidney disease (usual GFR slope < 10 mL/min/year) |

*Diabetes Metab 38:291-297, 2012*

## Kidney Injury continuum




## Biomarkers




| Potential Urinary Early Biomarkers | Delayed Biomarkers |
|---|---|
| ? >>> microalbuminuria | ↑ Serum creatinine (↓ GFR)<br>↑ Blood urea nitrogen |

# Urinary sample

- Easily accessible in a **non-invasive manner**,
- Presents as an ideal source of potential biomarkers for clinical diagnosis of human disease.
- Identification of **urine biomarkers** has proven to be beneficial in recent years because of **ease of handling, stability, and the ability to standardize** the various markers to creatinine or other peptides generally already present in the urine.
- Several biomarkers emerged recently that are able to **detect kidney damage earlier** than is currently possible with traditional biomarkers such as serum creatinine and proteinuria.

# Proteinuria:
# excessive urinary protein excretion

## 1. Glomerular proteinuria

- an increase in the permeability of the glomerular capillary wall to macromolecules (particularly **albumin**).
- usually results from glomerular disease.

## 2. Tubular proteinuria

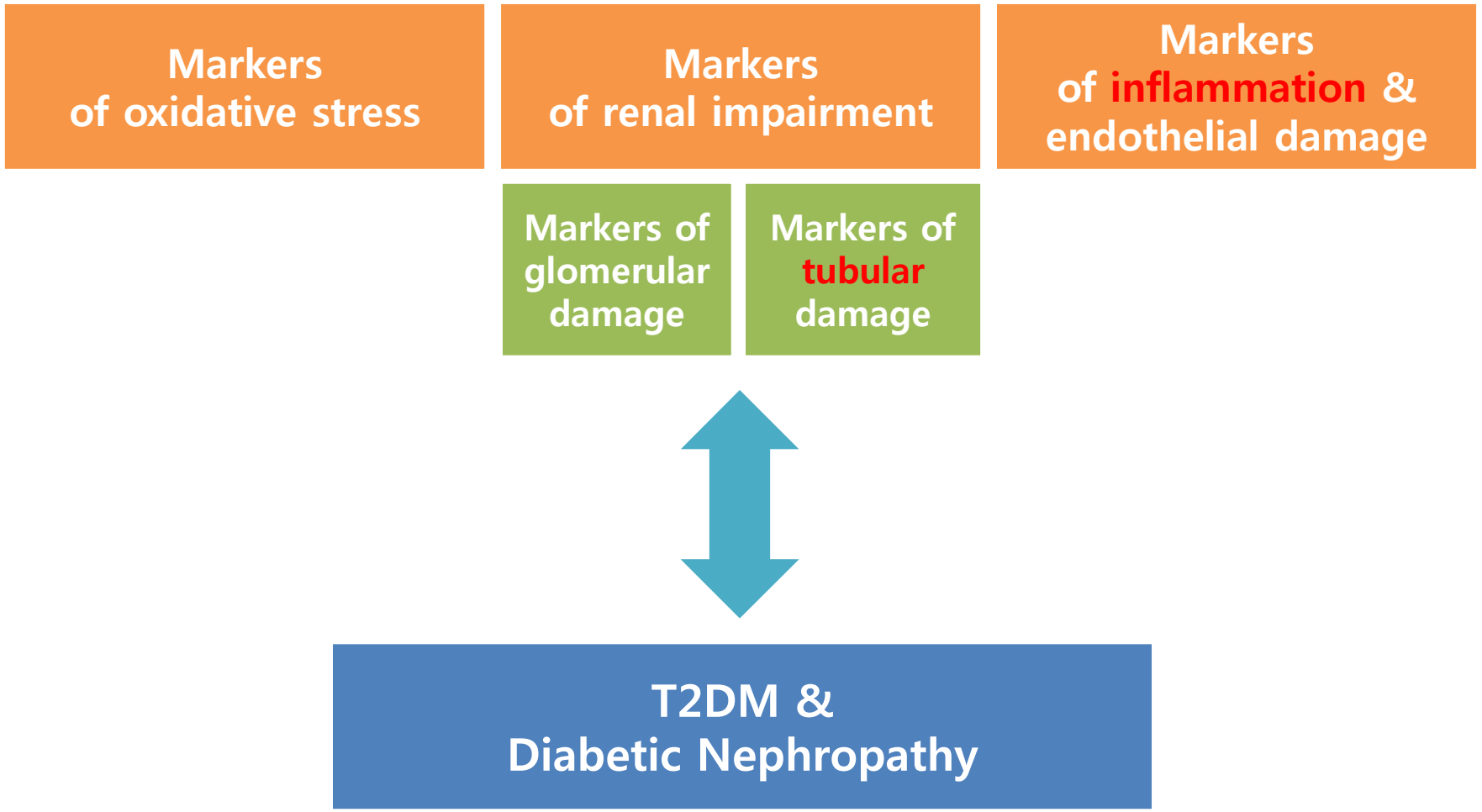
- reduced reabsorption of proteins that are normally present in the glomerular filtrate or from excretion of proteins derived from injured tubular epithelial cells.
- caused by diseases of the tubulointerstitium.

## 3. Overload proteinuria

- an excess of low-molecular-weight proteins that are normally reabsorbed by the proximal tubules.
- immunoglobulin light chains (plasma cell dyscrasias), lysozyme (myelomonocytic leukemia), myoglobin (rhabdomyolysis) or hemoglobin (intravascular hemolysis).

*Can Med Assoc J 177:361-368, 2007*

# Urinary marker of diabetic nephropathy



# Some urinary biomarkers of oxidative stress associated with type 2 diabetes

- **8-Hydroxy-2'deoxyguanosine (8-OHdG)** *
- Acrolein-lysine adducts
- **Isoprostanes** *
- Specific isoprostane: 8-iso-prostaglandin F2alpha
- Malondialdehyde
- **Pentosidine** *
- **Pyrraline** *

* investigated in the study about diabetic nephropathy

*J Lab Clin Med 144: 92–99, 2004*
*Diabetes Care 26: 1507–1512, 2003*
*J Pharm Biomed Anal 36: 101–104, 2004*
*Diabetologia 45: 877–882, 2002*
*Clin Chim Acta 339:1–9, 2004*
*Nephro 91: 327–329, 2002*
*Diabetes 54: 3274–3281, 2005*

# Markers of glomerular damage

# Studies evaluating urinary biomarkers associated with glomerular damage

| Biomarkers | Ref. | Type of DM (n) / Characteristics | Study design | Main findings |
|---|---|---|---|---|
| **Albumin** | Aldler et al. 2003 | T2DM (5,102) / Newly diagnosed DM | Observed and modeled data in UKPDS (10yr FU) | Progression to micro-: 2.0%/yr<br>Micro- → Macro-: 2.8%/yr<br>Prevalence: Micro- 24.9% / Macro- 5.3% |
| **Albumin** | Afgphahi et al. 2011 | T2DM (3,667) / Normo- & GFR>60 | Observational (5 yr FU) | 20%: developed albuminuria |
| **Type IV collagen** | Lijima et al. 1998 | T2DM (94) / Normo- + Micro- | Observational (1yr FU) | uIVC, higher in Micro- than Normo- |
| **Type IV collagen** | Kotajima et al. 2000 | T2DM (82) / Cr <1.4, Normo- | Cross-sectional | uIVC, higher in DM than control |
| **Type IV collagen** | Katavetin et al. 2010 | T2DM (30) / Proteinuria | Observational (4.2 yr FU) | uIVC, negatively correlated with annual GFR change. |
| **Transferrin** | Narita et al. 2006 | T2DM (117) / Normo- | Observational (5 yr FU) | uTf, higher in patents who progressed to micro. |
| **Transferrin** | Kazumi et al. 1999 | T2DM (77) / Normo- | Observational (2 yr FU) | uTf, lower in patents who remained normo. |

# Albuminuria

- MW **65 kDa**
- A well-established marker of **glomerular damage**.
- A marker and independent risk factor of **development** of both **renal** and CVD in both the diabetic and the general population.
- might actually play a direct role in the **progression of renal disease**
- **Microalbuminuria** - the first sign of diabetic renal impairment or incipient nephropathy
- 20 to 40% of type 2 diabetic patients develop microalbuminuria within 10–15 years of diagnosis,
- Macroalbuminuria occurs at a later stage, within 15–20 years, in 20–40% of patients

***N Engl J Med 346:1145-1151***

# Normoalbuminuric renal insufficiency

- **Prevalence of micro- and macroalbuminuria in T2DM with chronic renal insufficiency**

| | Subjects With Type 2 Diabetes Mellitus, % (95% Confidence Interval)† | Population Estimate in Millions (95% Confidence Interval) |
|---|---|---|
| Microalbuminuria (sampled n = 64) | 45 (31-59) | 0.6 (0.3-0.7) |
| Macroalbuminuria (sampled n = 47) | 19 (10-28) | 0.2 (0.1-0.3) |
| Retinopathy (sampled n = 58) | 28 (21-36) | 0.3 (0.2-0.4) |
| No retinopathy or albuminuria (sampled n = 51)‡ | 30 (21-39) | 0.3 (0.2-0.4) |

- **In Korean T2DM**

| | | Normo-albuminuria | Micro-albuminuria | Macro-albuminuria |
|---|---|---|---|---|
| All (n=562) | eGFR ≥60 | 305 (74.2) | 96 (23.4) | 10 (2.4) |
| | eGFR <60 | 44 (29.1) | 50 (33.1) | 57 (37.8) |
| Without RAS inhibitors (n=133) | eGFR ≥60 | 67 (81.7) | 15 (18.3) | 0 (0.0) |
| | eGFR < 60 | 18 (35.3) | 21 (41.2) | 12 (23.5) |

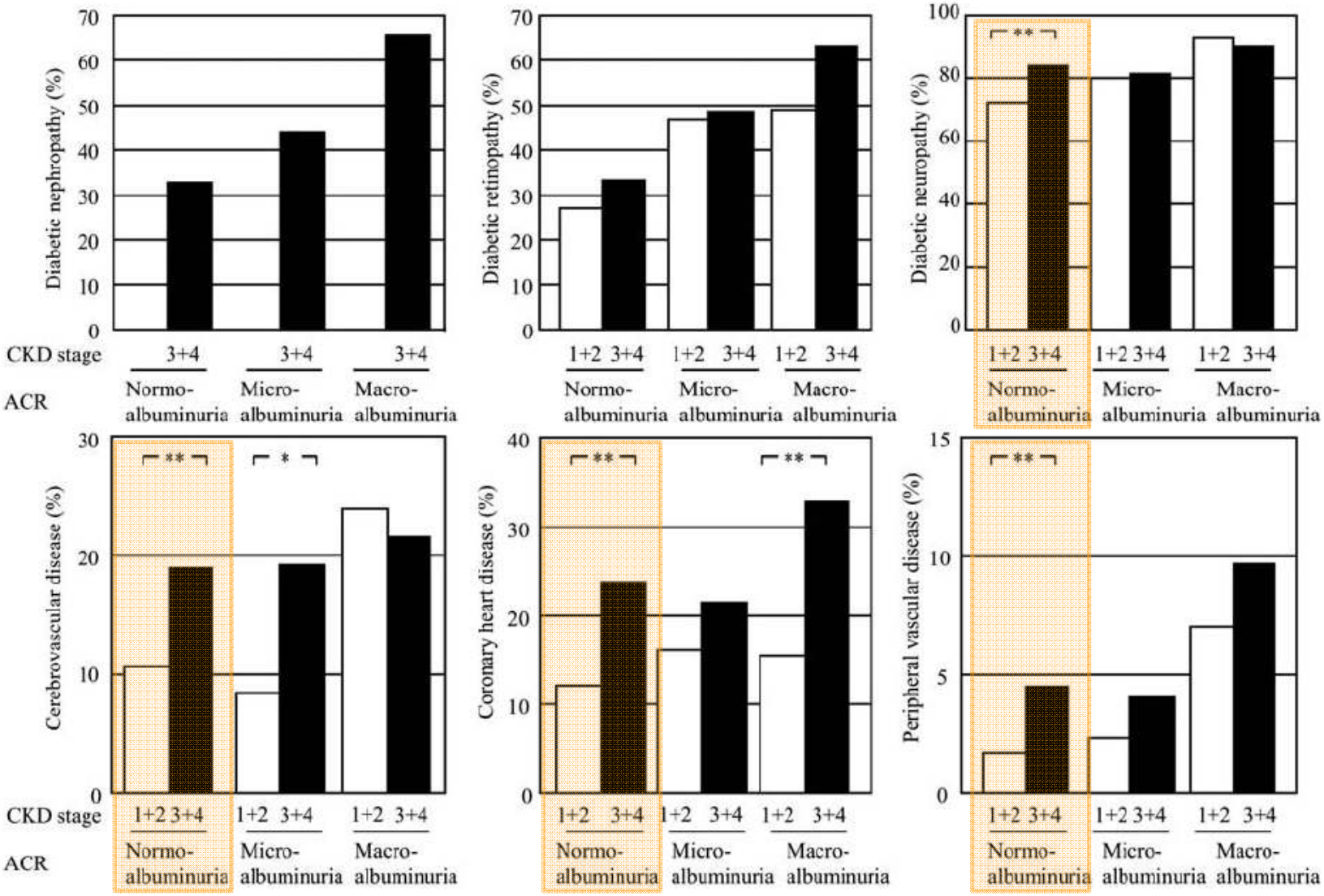
*JAMA 289:3273-3277, 2003*
*J Korean Med Sci 24:S75-81, 2009*

# Normoalbuminuric renal insufficiency

| | eGFR | | |
|---|---|---|---|
| | >60 ml/min per 1.73 $m^2$ | 15–60 ml/min per 1.73 $m^2$ | *P* |
| *n* | 576 | 84 | — |
| Female subjects | 444 (77.4) | 51 (60.2) | 0.002 |
| Age (years) | 56.8 ± 9.5 | 62.9 ± 10.3 | <0.001 |
| Diabetes duration (years) | 9.3 ± 7.1 | 10.1 ± 7.0 | 0.318 |
| BMI (kg/$m^2$) | 28.9 ± 5.2 | 29.0 ± 5.4 | 0.841 |
| Male waist circumference (m) | 0.98 ± 0.11 | 0.100 ± 0.65 | 0.449 |
| Female waist circumference (m) | 0.95 ± 0.12 | 0.98 ± 0.12 | 0.348 |
| Obesity | 518 (90.9) | 75 (89.7) | 0.375 |
| Smoking | 97 (16.4) | 9 (11.4) | 0.474 |
| Systolic blood pressure (mmHg) | 130.0 ± 22.2 | 146.0 ± 23.7 | 0.069 |
| Diastolic blood pressure (mmHg) | 86.0 ± 12.3 | 87.0 ± 16.9 | 1.000 |
| Hypertension | 334 (58.7) | 56 (67.5) | 0.140 |
| HOMA-IR* | 4.84 (0.3–45) | 11.4 (1.6–44.7) | 0.015 |
| A1C (%) | 7.07 ± 2.18 | 6.74 ± 2.35 | 0.259 |
| Fasting plasma glucose (mg/dl) | 175 ± 70.2 | 164 ± 76.7 | 0.521 |
| Cholesterol (mg/dl) | | | |
| Total | 205.3 ± 43.9 | 222.8 ± 52.0 | 0.014 |
| HDL | 46.4 ± 11.9 | 44.9 ± 10.6 | 0.288 |
| LDL | 122.1 ± 40.1 | 143.0 ± 46.0 | 0.028 |
| Triglycerides (mg/dl) | 158 (55–549) | 176 (47–842) | 0.006 |
| UAE rate (mg/l) | 4 (0.1–28.5) | 2.35 (1–17) | 0.181 |
| Creatinine (mg/dl) | 0.9 (0.5–1.2) | 1.22 (0.98–2.6) | — |
| eGFR (ml/min per 1.73 $m^2$) | 88.0 (60.7–186.5) | 54.2 (31.3–59.7) | — |
| Metabolic syndrome | 403 (70.2) | 68 (81.9) | 0.027 |

***Diabetes care 30: 1998-2000, 2007***

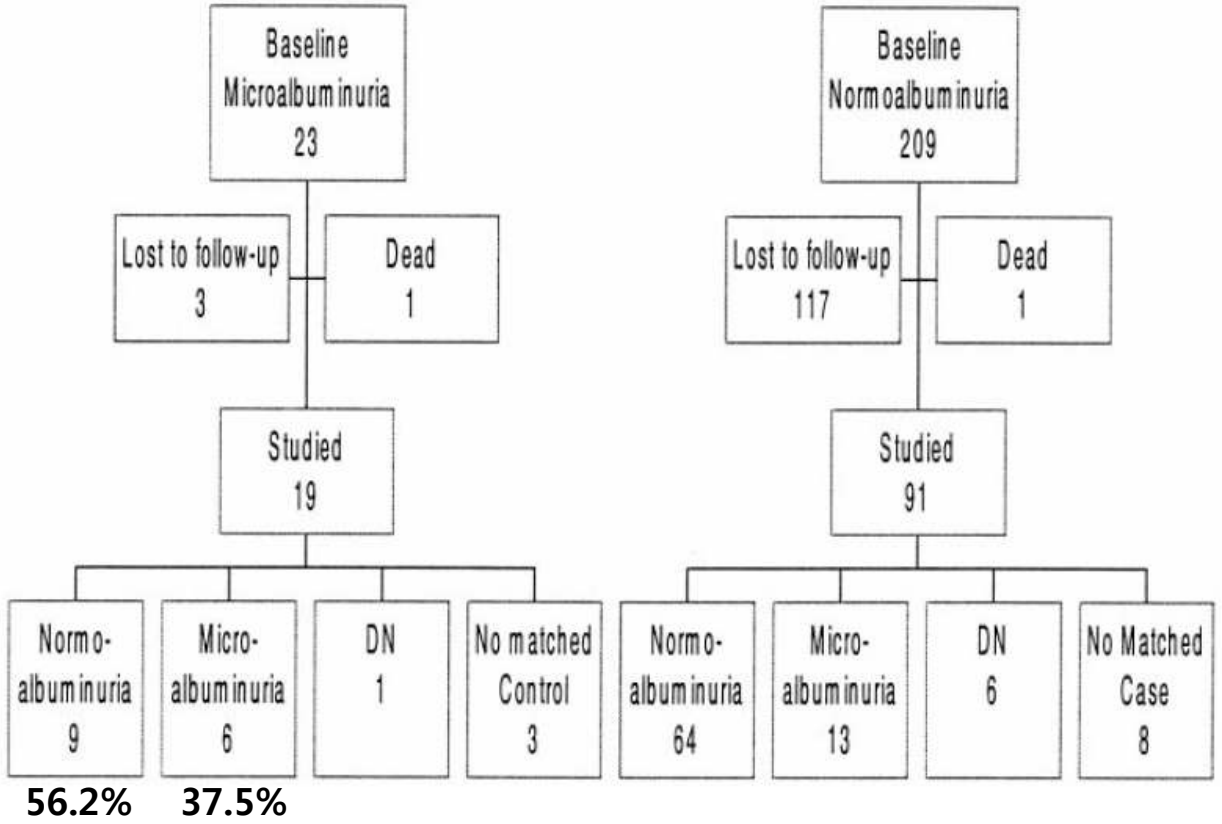
# Normoalbuminuric renal insufficiency




*Nephrol Dial Transplant 25: 1161-1167, 2010*

# Does Microalbuminuria Predict Diabetic Nephropathy?

- A prospective longitudinal study of patients with T1DM and T2DM with and without MA
- 7 year follow-up




**The negative predictive value of microalbuminuria among T2DM for DN was 77% while the positive value was only 43%,**

*Diabetes Care 24:1560-1566, 2001*

# Microalbuminuria in DN

- It has variable course !
  - The progression to macroalbuminuria is unpredictable
  - It dose **not always** lead to the development of nephropathy[1]
- Some diabetic patients
  - even if urinary albumin levels are in the normal range
    they have advanced renal pathological changes
    and progressive kidney function decline[2]

- **Need for more sensitive and specific biomarker**
  **than urinary albumin !**

*1. Diabetes 54:2164-2171, 2005*
*2. Diabetes Metab Res Rev 26:150-171, 2010*

# Urinary Transferrin

- Slightly larger than albumin (**MW 76.5** >65 kDa)
- Indicates **glomerular dysfunction** when present in urine
- With a pI one unit higher than albumin, **less anionic**
- **More readily filtered** through the glomerular barrier and excreted in urine

*Rinsho Byori 46: 277–282, 1998*
*Diabetes Care 22: 1176–1180, 1999*
*Diabetes Care 25: 1176–1181, 2004*

# Increased Urinary Excretion of Transferrin Predict Development of Microalbuminuria in T2DM

- **140 type 2 diabetic patients with normoalbuminuria from outpatients**

| | Control subjects | Progressors | Nonprogressors | *P* value (progressors vs. nonprogressors) |
|---|---|---|---|---|
| U-Alb/Cr (mg/gCr) | 5.3 (3–14.8) | 9.0 (3.7–29) | 6.5 (1.1–24) | 0.0003 |
| U-IgG/Cr (mg/gCr) | 1.9 (1.2–4.6) | 3.9 (0.96–7.7) | 2.4 (0.83–6.5) | 0.0031 |
| U-CRL/Cr (g/gCr) | 42 (12–90) | 71 (38–170) | 53 (0.36–84) | 0.0094 |
| **U-Tf/Cr (g/gCr)** | **140 (53–440)** | **290 (150–970)** | **140 (29–1300)** | **0.0001** |
| U-NAG/Cr (U/gCr) | 2.2 (0.16–5.0) | 3.7 (0.47–11) | 3.8 (0.067–11) | NS |

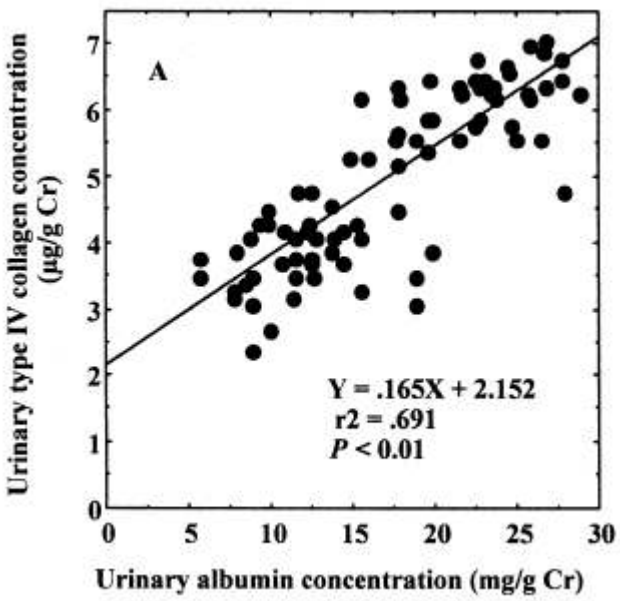
*Diabetes Care 29: 142-144, 2006*

# Extracellular matrix accumulation: Type IV collagen

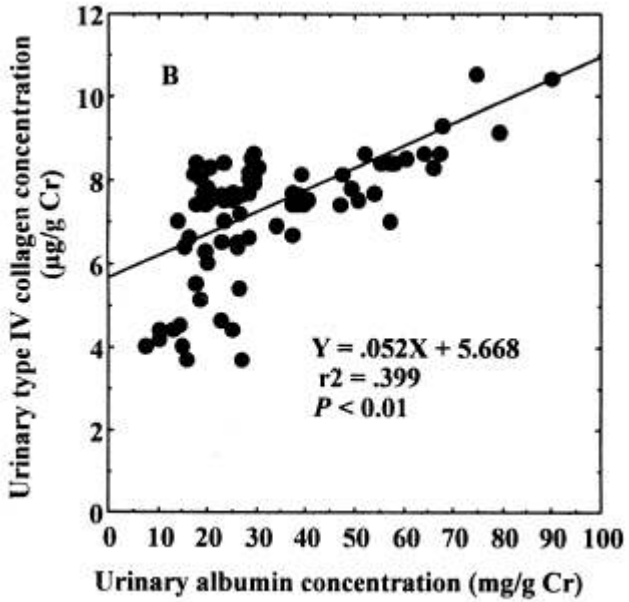
- A Major component of **basement membranes**
- Provides structural/functional support to various cell types
- Expressed in both the **glomerular/tubular basement membrane and mesangial matrix**
- **~540 kDa**, its excretion → the rate of matrix synthesis/degradation

*Rinsho Byori 46: 277–282, 1998*
*Clin Nephrol 55: 357-364, 2001*
*J Clin Lab Anal 12: 378–382, 1998*

# Type IV collagen as an early marker for diabetic nephropathy in T2DM




• At the beginning of the study




• 6 months later

- 62.2% of the patients developed an increased type IV collagen excretion within 6 months and 32.9% of these showed **an increase in urinary type IV collagen excretion but not in albuminuria.**
- uIVC may be a useful marker for early diabetic nephropathy

*J Diabetes Complications 14: 13-17, 2000*


Markers of tubular damage

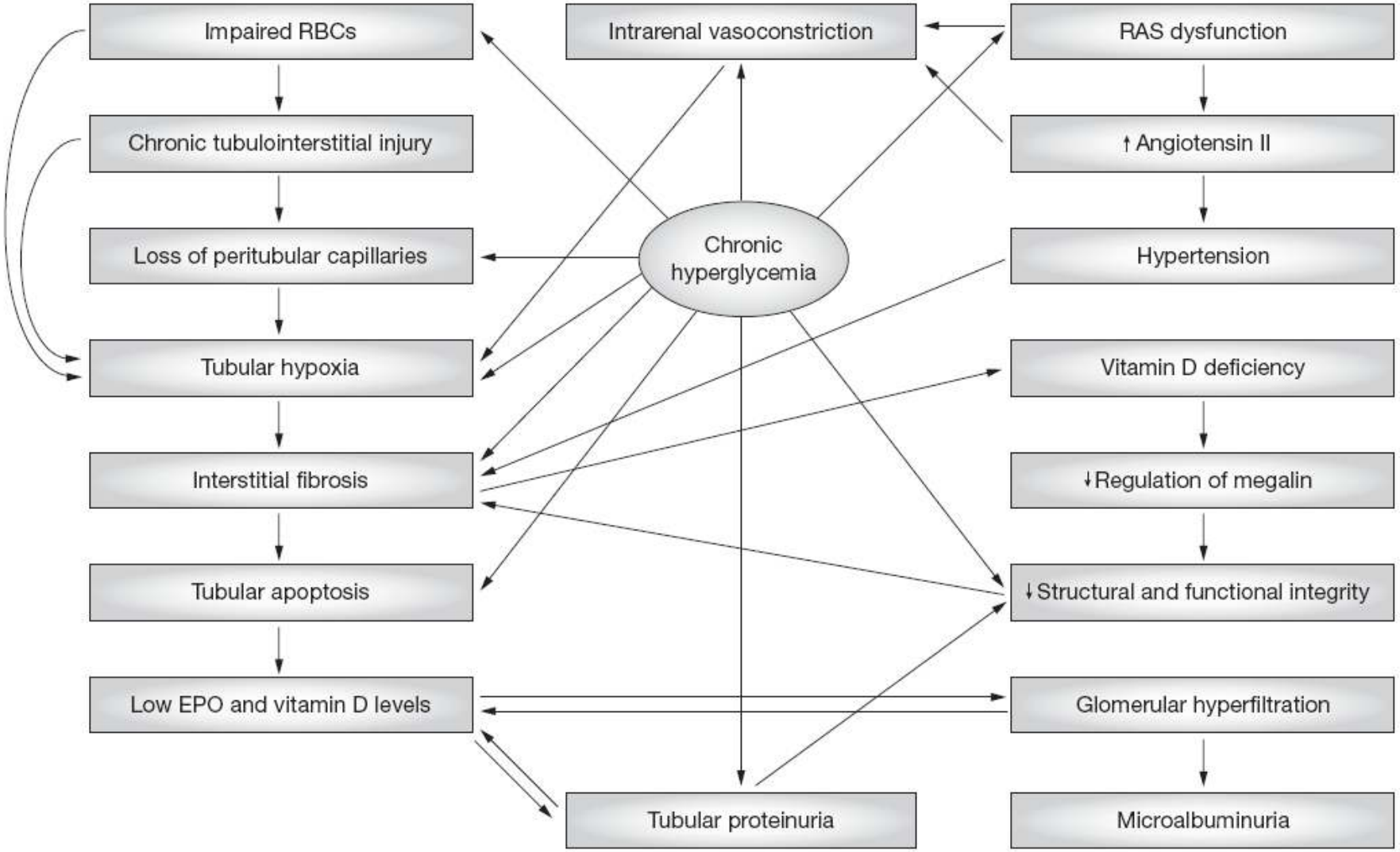
# Tubular damage markers in T2DM

- **Tubular damage markers**

  - extensively investigated in the field of predicting the occurrence of **acute kidney injury (AKI)** after various nephrotoxic insults, such as ischemia during cardiac surgery, sepsis, and administration of contrast medium.

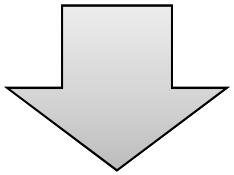
- Little research has been done in patients with **CKD including DM**.
- Recently, several studies have provided evidence for the involvement of **renal tubular dysfunction** as well as glomerulopathy in the **CKD progression in diabetic patients** with macrovascular diseases.

# The hypoxic tubular hypothesis of diabetic nephropathy




*Nat Clin Pract Nephrol 4: 216-226, 2008*

| Impaired reabsorption by the tubular cells | Increased secretion of enzymes by tubular epithelia cells |
| --- | --- |
| RBP4<br>A1MG<br>B2MG<br>Immunoglobulin light chains<br>Cystatin C<br>KIM-1<br>NGAL<br>FABP | NAG<br>AAP |



Tubular protein

# Urinary Tubular markers for diabetic nephropathy

| Urinary Tubular Markers | Ref. | Type of diabetes (n) / Characteristics | Study design | Main findings |
|---|---|---|---|---|
| U-NGAL | Yang et al. 2009 | T2DM (74) / All GFR | 1-year observational | U-NGAL was correlated with GFR at baseline and follow-up level. |
| U-NGAL | Bolignano et al. 2009 | T2DM (56) / GFR ≥ 30, RASi(+) | Cross-sectional | U-NGAL increased with increasing degree of albuminuria. |
| U-LFABP | Nielsen et al. 2009 | T1DM (148) / All GFR | Cross-sectional; Invervention (ACEi) | U-FFABP was higher in NA vs. control and associated with albuminuria. ACEi reduced U-LFABP from baseline. |
| U-LFABP | Nielsen et al. 2010 | T1DM (2010) / All GFR | 18-year Prospective observational | High u-LFABP predict the initiation and progression of DN and all-cause mortality. |
| U-KIM-1, U-NGAL | Nielsen et al. 2010 | T1DM (148) / All GFR | Cross-sectional; Invervention (ACEi) | U-NGAL and U-KIM-1 were elevated in NA of T1DM. U-NGAL increase significantly with increasing albuminuria. |
| **U-Cystatin C** | **Jeon et al. 2011** | **T2DM (332) / All GFR** | **Cross-sectional** | **U-cystatin C increased with increasing degree of albuminuria** |
| U-NGAL, U-LFABP, U-KIM-1 | Nielsen et al. 2011 | T1DM (63) / GFR ≥ 60, DN (+) anti-HT (-) | 3-year Intervention (RAS) | After adjustment, all markers had no association with a faster decline in GFR. |

# Urinary Tubular markers for diabetic nephropathy

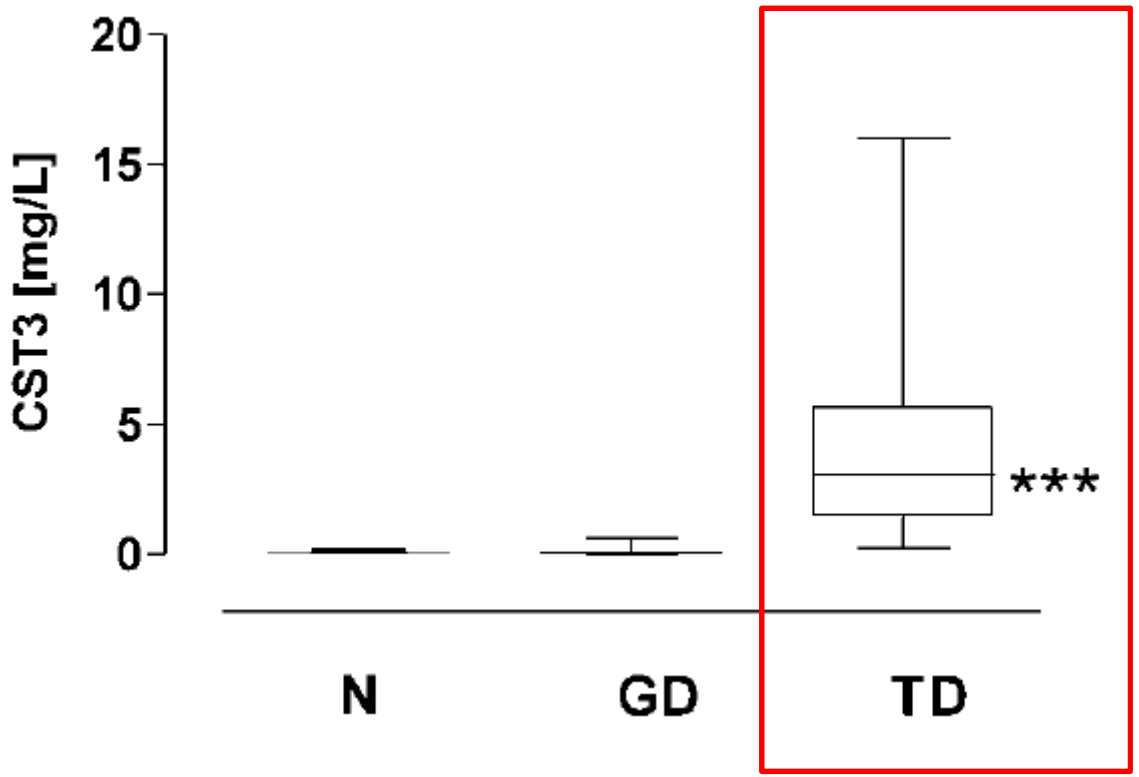
| Urinary Tubular Markers | Ref. | Type of diabetes (n) / Characteristics | Study design | Main findings |
|---|---|---|---|---|
| U-NAG, U-NGAL, U-HFABP, U-KIM-1, U-cystatin C | Nauta et al. 2011 | T1DM and T2DM (94) / All GFR | Cross-sectional | All markers, except KIM-1, were associated with albuminuria. U-HFABP was associated with GFR. |
| U-KIM-1, U-NAG | Vaidya et al. 2011 | T1DM (363) / All GFR | 2-year Prospective observational | Lower levels of u-KIM-1 and u-NAG were associated with the regression of MA |
| **U-KIM-1, U-LFABP, U-NGAL (U-IL-18, U-Ang)** | **Kim et al. 2012** | **T2DM (118) / eGFR ≥ 60 RASi (-)** | **Cross-sectional** | **Urinary tubular markers were associated with albuminuria and correlated with U-IL-18 and U-Ang.** |
| U-Cystatin C | Kim et al. 2012 | T2DM (237) / eGFR ≥ 30 | Prospective observational | U-cystatin C preidict of the decline of eGFR (even in patients with eGFR ≥ 60) |
| U-NGAL, U-NAG, U-KIM-1 | Fu et al. 2012 | T2DM (101) / All GFR Duration of diabetes : ≤ 5 years Anti-HT (-) | Cross-sectional | Only U-NGAL was correlated with eGFR |
| U-NAG, U-KIM-1, U-NGAL | Fu et al. 2012 | T2DM (88) / Hyperfiltration vs. normal GFR | Cross-sectional | Higher urinary tubular markers (NGAL and KIM-1) were found in hyperfiltration group. |

# Urinary cystatin C

- **Cystatin C**
  - a **13-kD** endogenous cysteine proteinase inhibitor
  - a stable production rate by most nucleated cells
  - its plasma half-life is short (2hr in humans)
  - low molecular weight and positive charge at physiological pH
    → it is **freely filtered by the kidney glomerulus**
  - be considered as an **endogenous marker of the GFR**
  - **reabsorbed by the proximal tubules**
- **Urinary cystatin C**
  - After glomerular filtration, CST3 is **reabsorbed by the proximal tubular cells, where it is almost completely catabolized**, with the remaining uncatabolized CST3 eliminated in the urine.

***Clin Chim Acta 323: 121-128, 2002***

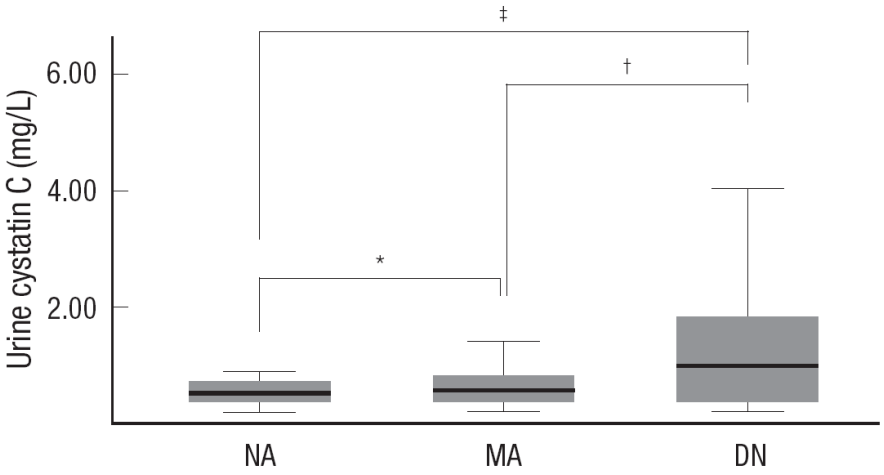
# Urinary cystatin C as a specific marker of tubular dysfunction




*Clin Chem Lab Med 44: 288-291, 2006*

# Urinary cystatin C as an early biomarker of nephropathy in type 2 diabetes

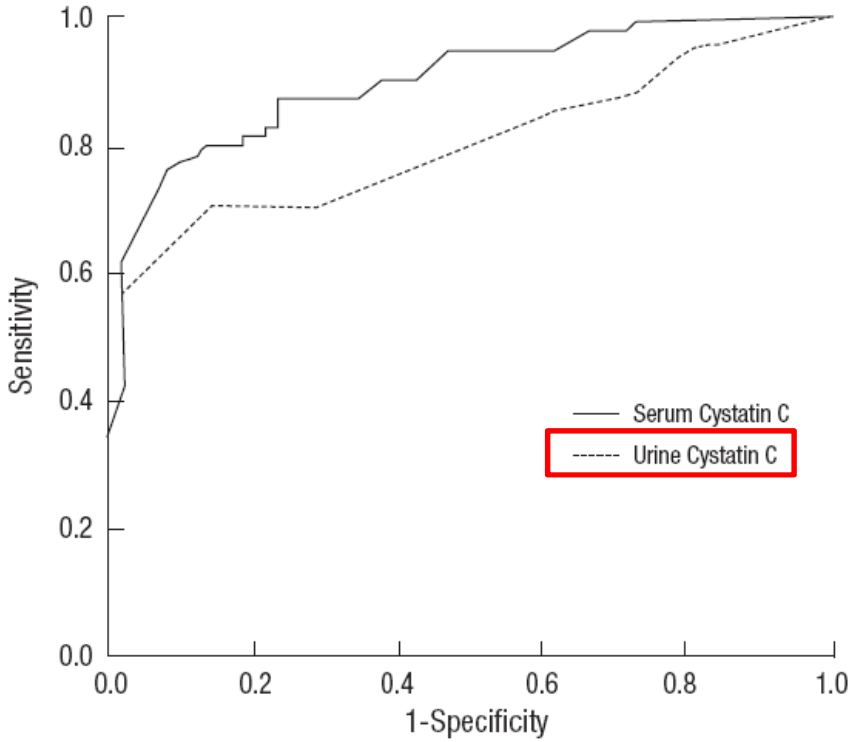
- 335 patients with type 2 diabetes who visited **PNUH (**Jan. 2008 – Oct. 2009)




**Independent factors associated with eGFR <60mL/min/1.73m² in normoalbuminuria**

| Variables | Odds ratio | 95% Confidence interval | *P* value |
|---|---|---|---|
| MDRD equation | | | |
| Serum cystatin C | 14.6 | 4.79-44.52 | < 0.001 |
| Urine cystatin C | 10.1 | 3.32-30.92 | < 0.001 |
| EPI-equation | | | |
| HDL cholesterol | 0.952 | 0.91-0.99 | 0.023 |

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; HDL, high-density lipoprotein; MDRD, Modification of Diet in Renal Disease.




**eGFR <60 mL/min/1.73 m²**
The AUC for was 0.906 for serum cystatin C
**0.807 for urine cystatin C**

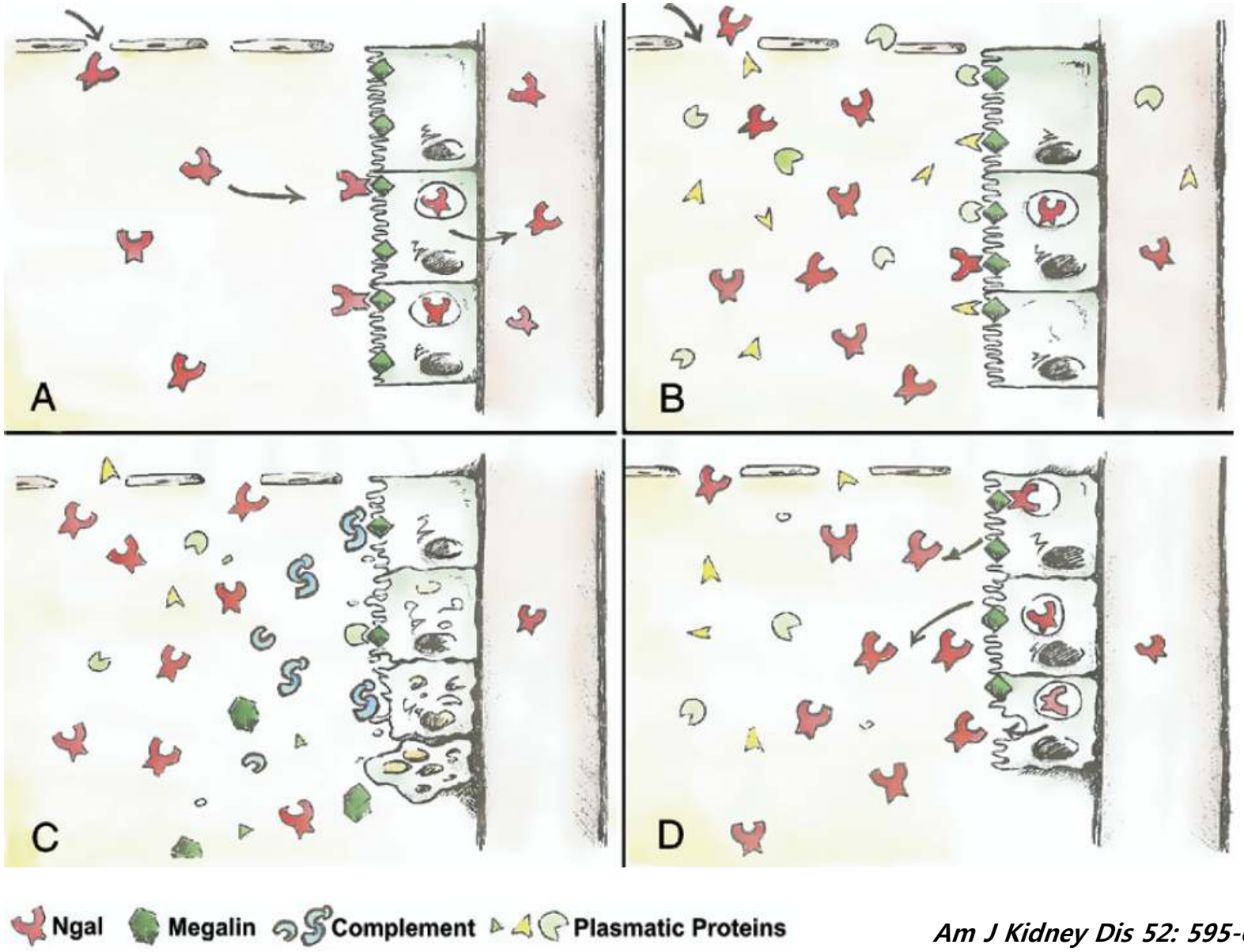
*YK Jeon & SS Kim et al. J Korean Med Sci 26:258-263, 2011*

# Urinary Neutrophil gelatinase-associated lipocalin (NGAL)

- a member of lipocalin family,
- originally identified as **25-kDa** protein covalently associated with human MMP-9 from human neutrophils.
- stored mainly in the specific granules of **neutrophils**,
- also expresses at very low-levels in several human tissues, including **kidney**, trachea, lungs, stomach, and colon.
- found to possess diverse functions such as transporting, activating MMP-9, inducing apoptosis, regulating immune response and so on.
- can **trigger nephrogenesis** by stimulating the conversion of mesenchymal cells into kidney epithelia.
- also plays a **renoprotective role** through enhancing **tubule cell proliferation in kidney injury**, especially in ischemia-reperfusion injury.
- is a marker closely associated with obesity, insulin resistance, and hyperglycemia in human beings.

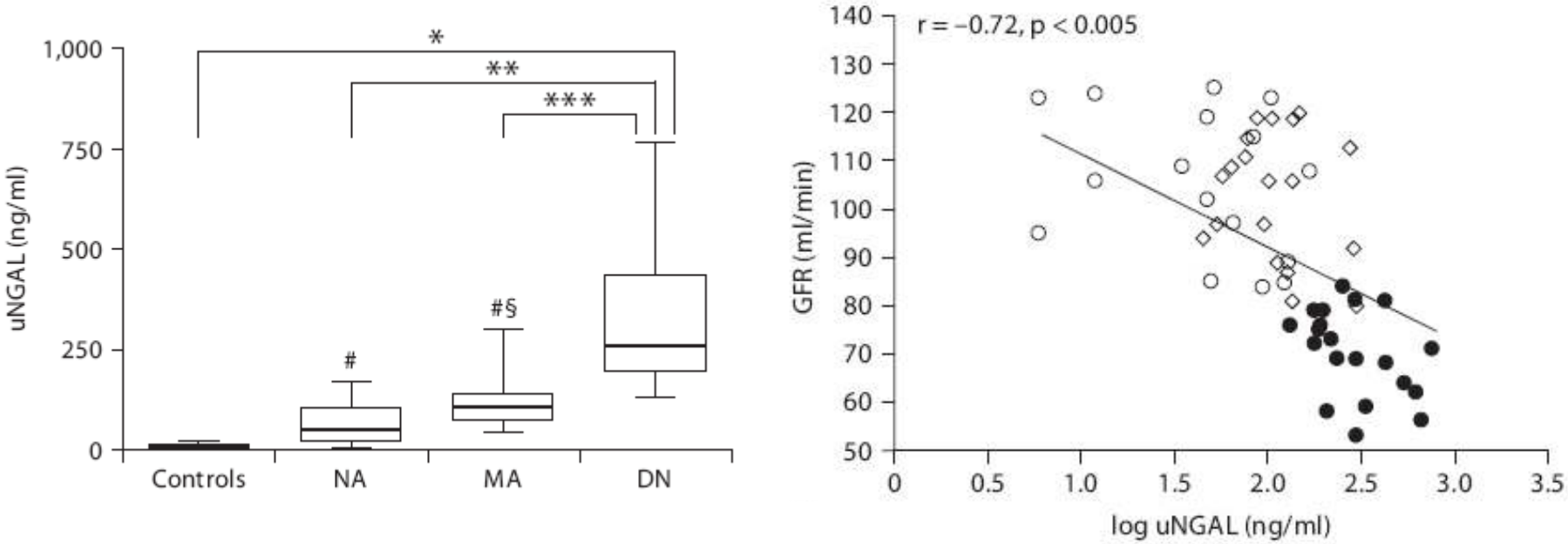
*J Clin Invest 115: 610–621, 2005*
*Clin Chem 53: 34–41, 2007*

# NGAL as a marker of kidney damage




*Am J Kidney Dis 52: 595-605, 2008*

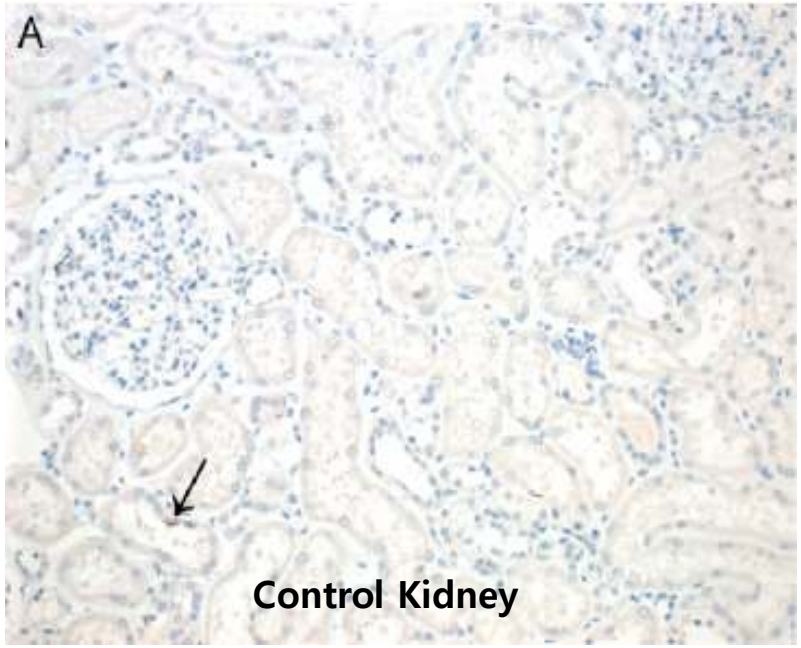
# Urinary NGAL as an early biomarker of nephropathy in T2DM

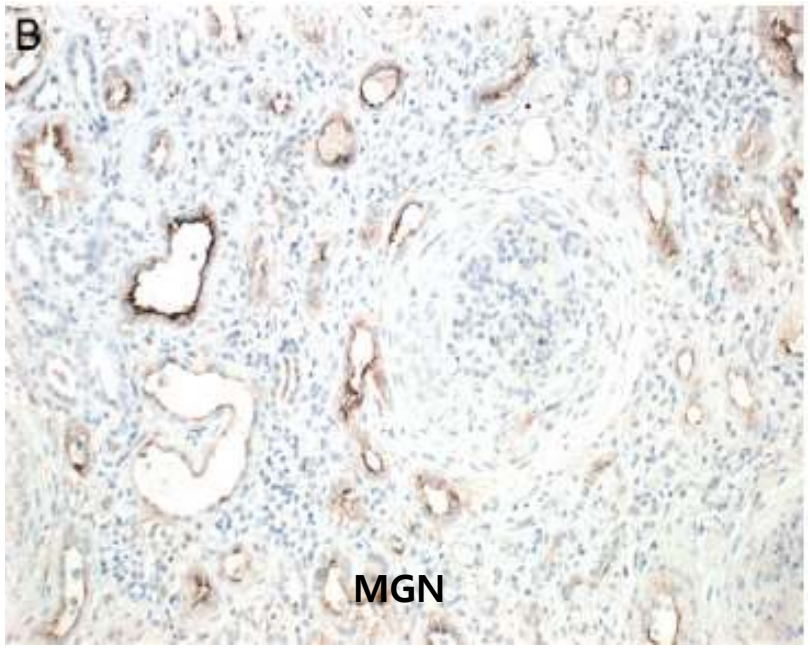



*Kidney Blood Press Res 32: 91-98, 2009*

# Kidney Injury Molecule-1 (KIM-1)

- A **transmembrane tubular protein** with unknown function.
- Undetectable in normal kidneys, **markedly induced in experimental renal injury**.
- Expressed on the apical membrane of **proximal tubule cells** and its ectodomain is cleaved and released into the lumen of the tubule ultimately appearing in the urine where it is stable.
- A highly sensitive and specific biomarker for **proximal tubule injury** because of a wide variety of pathophysiological states and toxins in animals and humans.



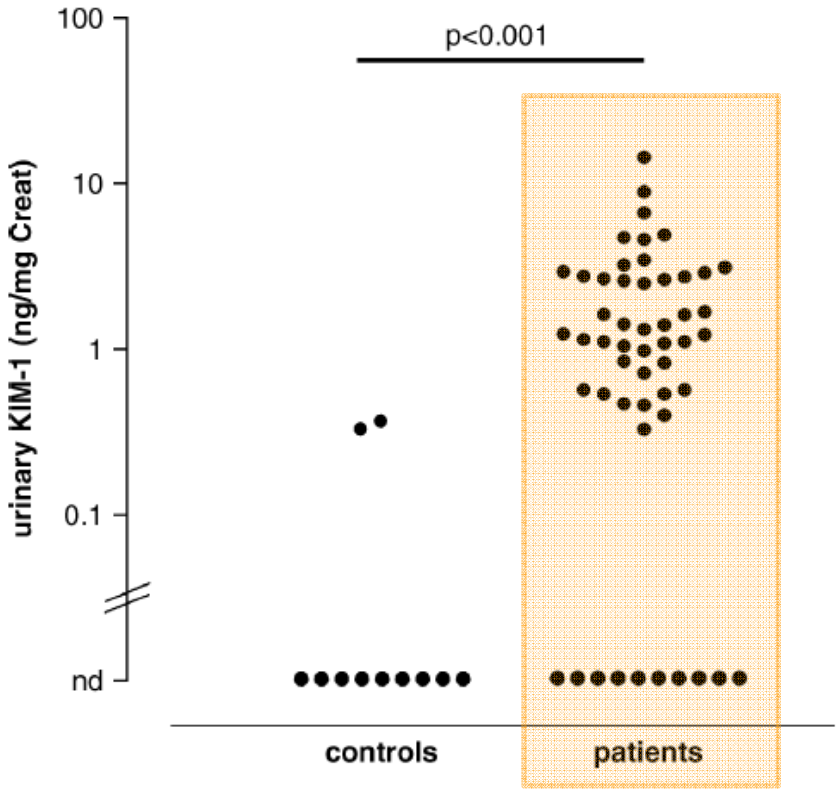




*J Patholo 212:209-217, 2007*

# Kidney Injury Molecule-1 (KIM-1)

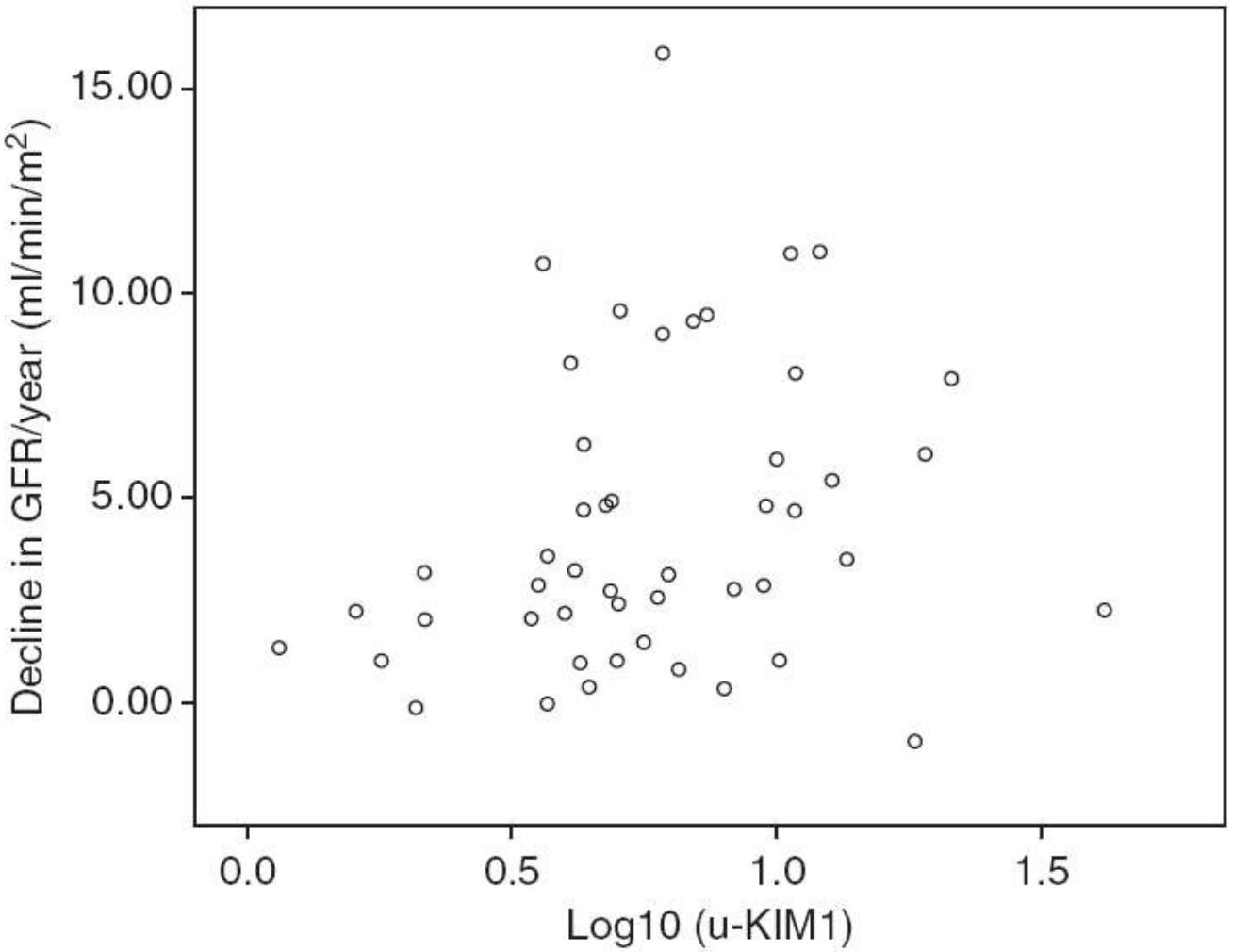
| | M/F (*n*) | Age (years) |
|---|---|---|
| Control | 4/3 | 62 (28–83) |
| Diabetic nephropathy | 4/4 | 62 (41–72) |
| Focal glomerulosclerosis | 7/4 | 39 (14–80) |
| Hypertension | 3/3 | 51 (22–79) |
| IgA nephropathy | 8/2 | 26 (4–50)** |
| Membranous GN | 12/5 | 52 (30–77) |
| Minimal change | 7/4 | 25 (2–70)* |
| Mesangial proliferative GN | 6/3 | 31 (5–59)* |
| Systemic lupus erythematosus | 2/5 | 49 (33–55)* |
| Acute allograft rejection | 4/3 | 58 (43–67) |
| Chronic allograft nephropathy | 4/4 | 51 (30–66) |
| Wegener's granulomatosis | 5/3 | 71 (39–83) |

| | Tubular KIM-1 ($n = 102$) | | Urinary KIM-1 ($n = 43$) | |
|---|---|---|---|---|
| **Parameters** | ***r*-Value** | ***p*-Value** | ***r*-Value** | ***p*-Value** |
| *Histological parameters* | | | | |
| Tubular KIM-1 expression | | | 0.47 | 0.002 |
| MME | 0.30 | 0.002 | 0.04 | NS |
| FGS | 0.22 | 0.025 | −0.17 | NS |
| IF | 0.39 | <0.001 | 0.01 | NS |
| Interstitial MØ | 0.59 | <0.001 | 0.35 | 0.025 |
| Glomerular MØ | 0.44 | <0.001 | 0.40 | 0.016 |
| *Clinical parameters* | | | | |
| Serum creatinine | 0.53 | <0.001 | 0.036 | NS |
| Creatinine clearance | −0.60 | <0.001 | −0.46 | 0.007 |
| eGFR | −0.59 | <0.001 | −0.37 | 0.016 |
| Proteinuria | 0.12 | NS | 0.14 | NS |




*J Patholo 212:209-217, 2007*

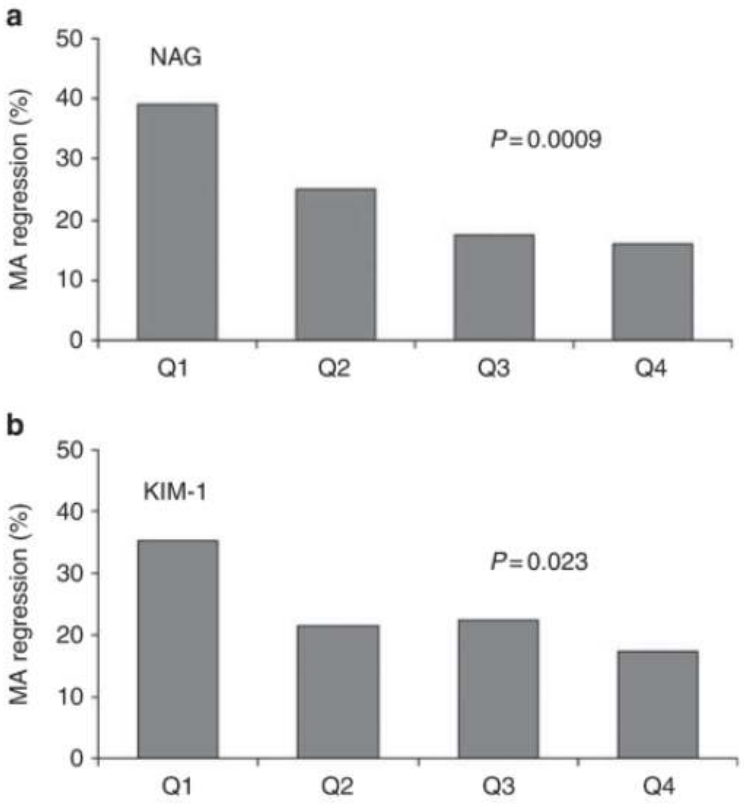
# KIM-1, not predict the decline in GFR in T1DM with overt nephropathy




***R=0.24, P=0.1***

*Kidney Int 79:1113-1118, 2011*

# Regression of microalbuminuria in T1DM is associated with lower levels of urinary KIM-1




| | Crude model | | Adjusted model | |
|---|---|---|---|---|
| **Biomarkers** | **OR (95% CI)** | ***P*-value** | **OR (95% CI)** | ***P*-value** |
| *NAG* | | | | |
| Q1 vs Q4 | 3.4 (1.5–7.6) | 0.003 | 4.8 (1.9–12.7) | 0.0013 |
| Q2 vs Q4 | 1.8 (0.7–4.1) | 0.20 | 2.3 (0.9–6.3) | 0.089 |
| Q3 vs Q4 | 1.1 (0.5–2.7) | 0.81 | 1.5 (0.5–4.2) | 0.45 |
| Q4 REF | 1.0 REF | | 1.0 REF | |
| *KIM-1* | | | | |
| Q1 vs Q4 | 2.6 (1.2–5.8) | 0.019 | 2.6 (1.1–6.1) | 0.03 |
| Q2 vs Q4 | 1.3 (0.6–3.0) | 0.55 | 1.0 (0.4–2.6) | 0.95 |
| Q3 vs Q4 | 1.4 (0.6–3.2) | 0.47 | 1.3 (0.5–3.4) | 0.52 |
| Q4 REF | 1.0 REF | | 1.0 REF | |

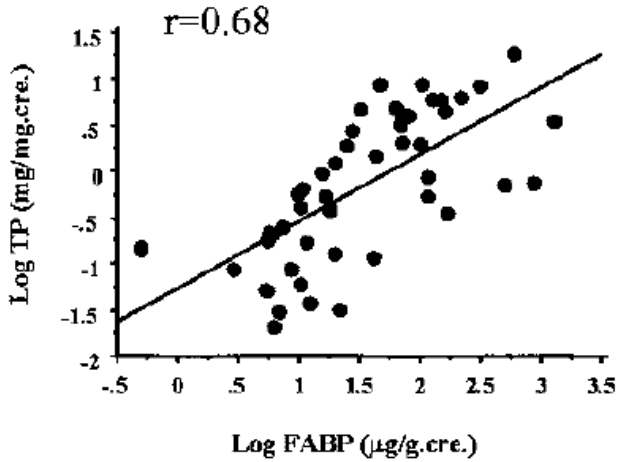
***Kidney Int 79: 464-470, 2011***

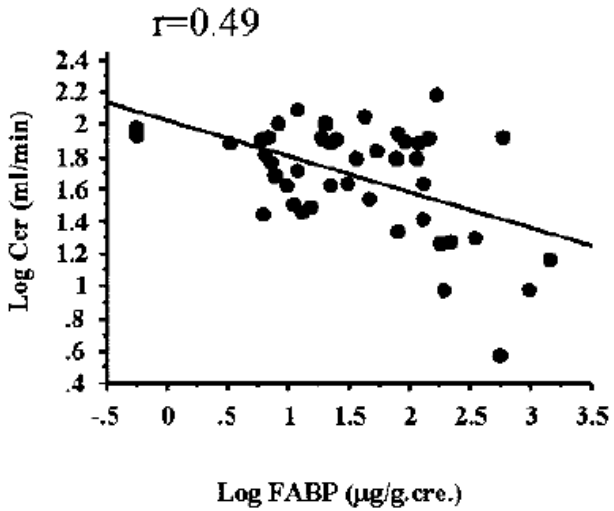
# Liver-type Fatty acid binding protein (L-FABP)

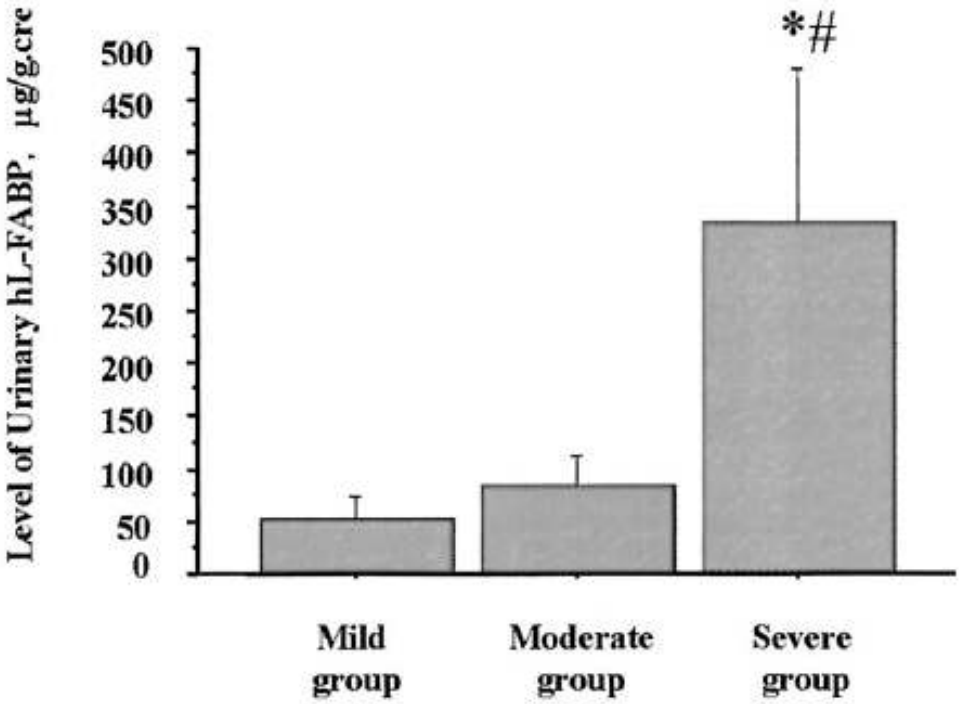
- **Free fatty acids (FFAs)** - bound to albumin, filtered through the glomeruli, and reabsorbed into the proximal tubules.
- FFAs bound to albumin
  - might play a role in the **development of tubulointerstitial damage**.
  - play some role in the **progression of tubulointerstitial disease**.[1]
- FFAs loaded on the proximal tubules are bound to cytoplasmic fatty acid-binding protein (FABP) and transported to mitochondria or peroxisomes, where they are metabolized by β-oxidization.[2]
- **In human proximal tubules**, the liver-type FABP (L-FABP) of **14 kd** is expressed.[3]
- L-FABP might be a key regulator of fatty acid homeostasis in the cytoplasm.[4]


[1] *Kidney Int 62:1628-1637, 2002*
[2] *Biochim Biophys Acta 1081:1-24, 1991*
[3] *Biochem J 288:285-290, 1992*
[4] *J Biol Chem 278:21429-21438, 2003*

# Urinary excretion of L-FABP reflects stress overload on the proximal tubules



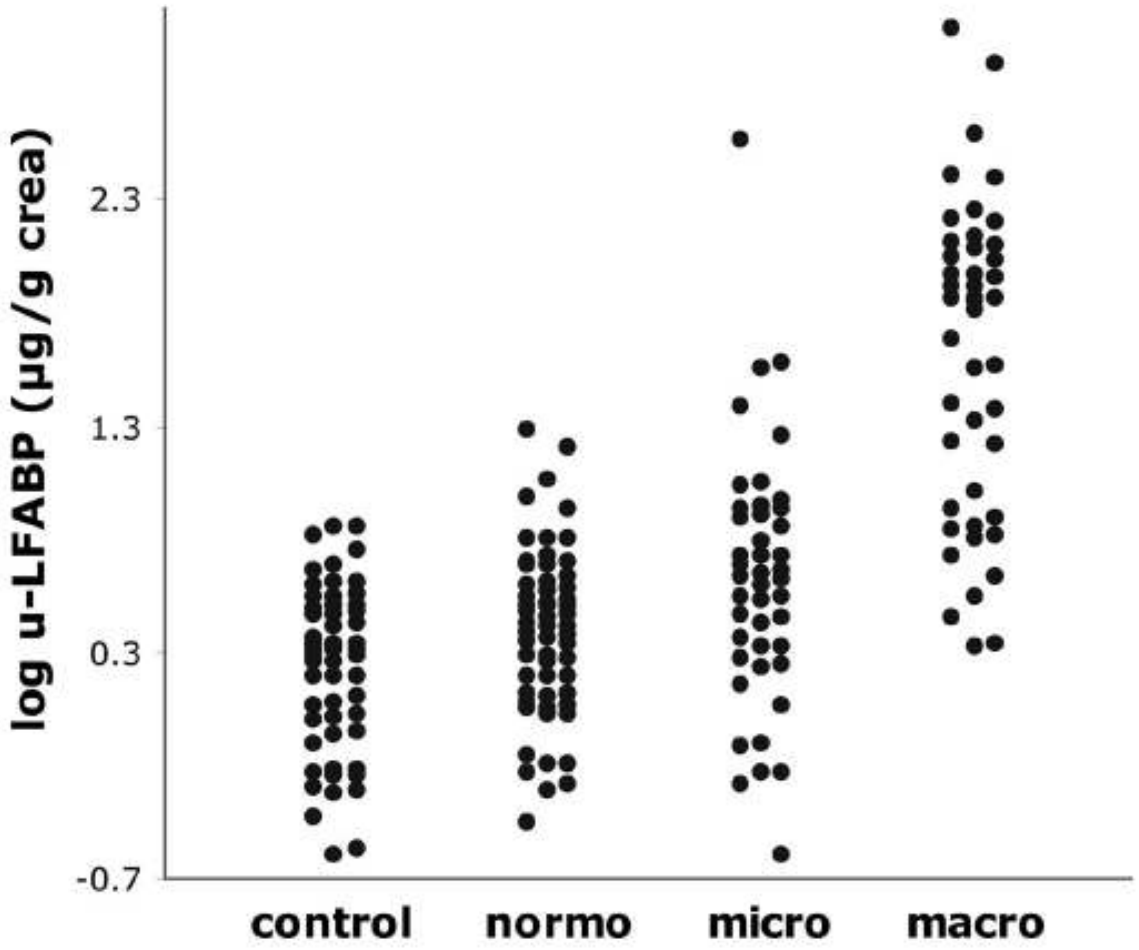




• Tubulointerstitial damage

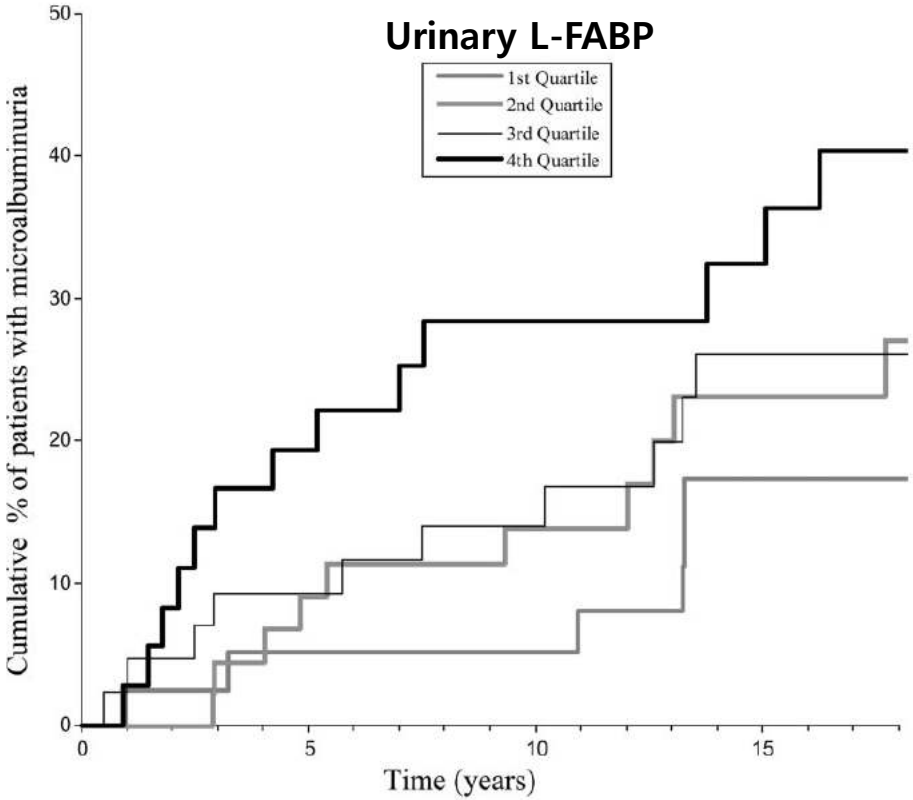
*Am J Pathol 165:1243-1255, 2004*

# Increased urinary L-FABP in T1DM




*Diabetes Care 32:1684-1688, 2009*

# Urinary L-FABP predicts progression to nephropathy in T1DM




**u-LFABP predicts the development of microalbuminuria when adjusted for known risk factors**

| | OR | P value |
|---|---|---|
| **Sex (male)** | **4.19 (1.62–10.87)** | **0.003** |
| Age (year) | 1.02 (0.99–1.05) | 0.274 |
| **SBP(mmHg)** | **2.00 (1.36–2.95)** | **0.001** |
| A1C (%) | 1.03 (0.995–1.06) | 0.100 |
| DBP (mmHg) | 0.99 (0.94–1.04) | 0.758 |
| **Log (u-albumin [mg]/24 h)** | **12.36 (2.58–59.32)** | **0.002** |
| **Log (u-LFABP [ng/ml]/u-Cr)** | **2.28 (1.14–4.58)** | **0.021** |

*Diabetes Care 33:1320-1324, 2010*

# Clinical Implication of Urinary Tubular Markers in the Early Stage of Nephropathy with T2DM

**In Type 2 diabetic patients in early course of DN**

- **To evaluate the association of several urinary tubular markers with albuminuria.**
- **To evaluate the association of urinary tubular markers with urinary IL-18 and angiotensiogen.**

*SS Kim et al. Diabetes Res Clin Pract 97:251-257; 2012*

# Patients and Methods

**Inclusion criteria**
- Age ≥ 18years
- **eGFR ≥ 60mL/min/1.73m$^2$**
- Stable renal function for at least 5months
- **No History of RAS inhibitors (withdrawn for at least 2months)**

**Exclusion criteria**
- Other renal disease except for DN
- Urinary tract infection
- Other active diseases

**Type 2 diabetic patients**
Feb. 2009 – Jan. 2010
N=118

**Non-diabetic control**
Fasting Plasma Glucose < 100mg/dL
eGFR ≥ 60mL/min/1.73m$^2$
**N = 25**

**Normoalbuminuria**
**$U_{ACR}$ < 30mg/g creatinine**
**N = 58**

**Microalbuminuria**
**30 ≤ $U_{ACR}$ < 300 mg/g creatinine**
**N = 33**

**Macroalbuminuria**
**300 ≤ $U_{ACR}$ mg/g creatinine**
**N = 27**

*SS Kim et al. Diabetes Res Clin Pract 97:251-257; 2012*

# Patients and Methods

- A random spot urine and blood sample were obtained at their clinic visit.
- The medical history and anthropometric measurements were also recorded on the same day.
- **The urinary markers** were measured by using an **ELISA kit** from in random spot urine
  - **KIM-1** (R&D systems, Minneapolis, MN, USA)
  - **NGAL** (R&D systems, Minneapolis, MN, USA)
  - **L-FABP** (CMIC Co. Ltd, Tokyo, Japan)
  - **IL-18** (MBL-Medical&Biological Laboratories, Nagoya, Japan)
  - **Angiotensinogen** (IBL, Gumma, Japan).

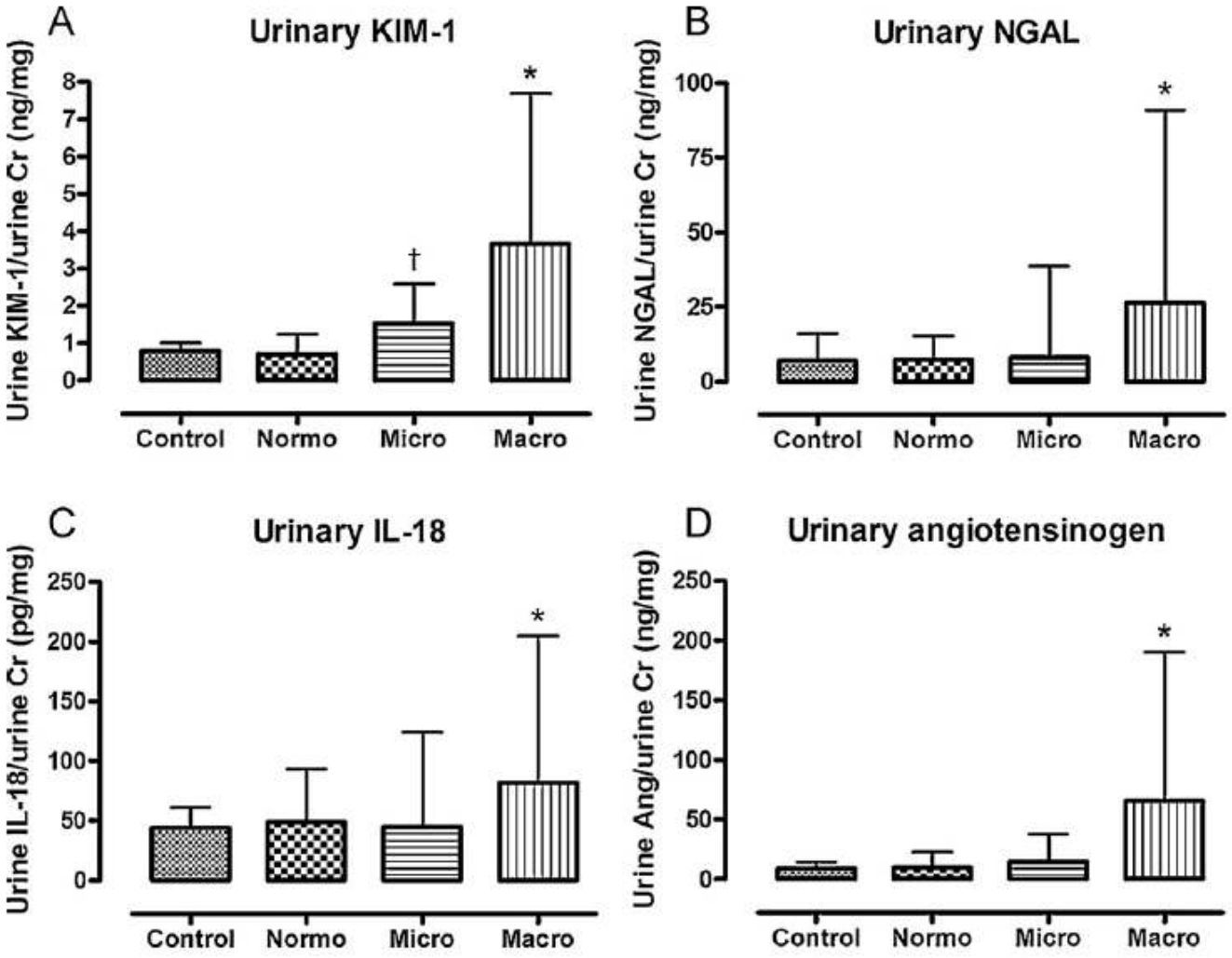
**Tubular Damage marker**

**Proinflammatory marker**

**Intrarenal RAS marker**

*SS Kim et al. Diabetes Res Clin Pract 97:251-257; 2012*

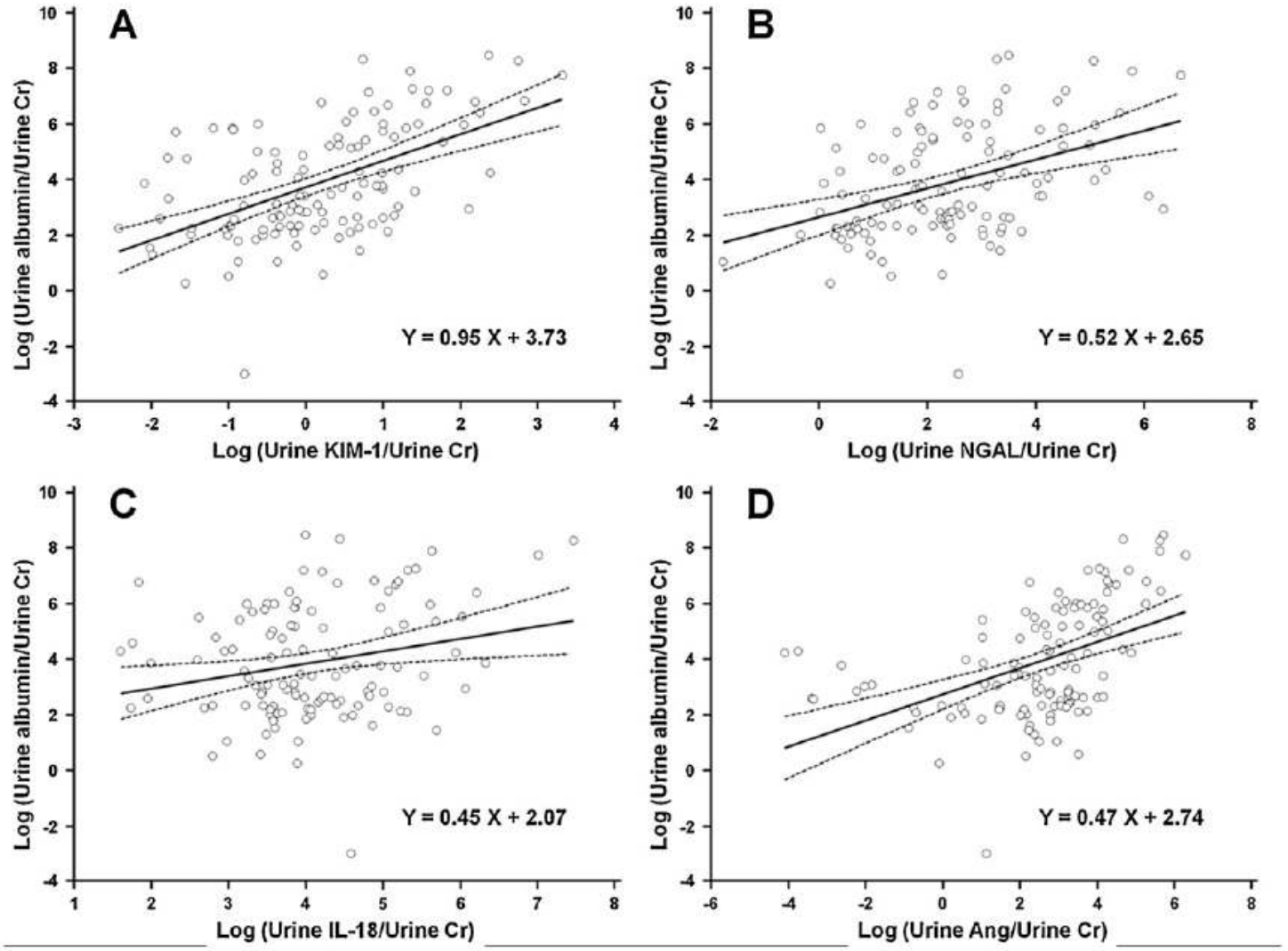
# Urinary markers according to albuminuria status




*P < 0.05, compared with nondiabetic control group and all the other groups of diabetic nephropathy.
†P < 0.05, compared with nondiabetic control group and normoalbuminuria group.
Ang, angiotensinogen; Cr, creatinine; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase-associated lipocalin.

*SS Kim et al. Diabetes Res Clin Pract 97:251-257; 2012*

# Correlations with albuminuria & urinary markers




Ang, angiotensinogen; Cr, creatinine; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase-associated lipocalin.

*SS Kim et al. Diabetes Res Clin Pract 97:251-257; 2012*

# Correlations between urinary markers and albuminuria

**Table 2 – Correlations between urinary markers in type 2 diabetic patients.**

| | Urine KIM-1/urine Cr* | Urine NGAL/urine Cr* | Urine IL-18/urine Cr* | Urine Ang/urine Cr* |
|---|---|---|---|---|
| Urine KIM-1/urine Cr* | – | 0.62† | 0.64† | 0.48† |
| Urine NGAL/urine Cr* | 0.62† | – | 0.56† | 0.31‡ |
| Urine IL-18/urine Cr* | 0.64† | 0.56† | – | 0.39† |
| Urine Ang/urine Cr* | 0.48† | 0.31‡ | 0.39† | – |

Ang, angiotensinogen; Cr, creatinine; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase-associated lipocalin.
* Logarithm-transformed values were used for analysis.
† $P < 0.001$
‡ $P < 0.01$

**Table 3 – Regression analyses of urinary ACR as a dependent variable in type 2 diabetic patients.**

| Urinary markers | Univariate | | Multivariate† | |
|---|---|---|---|---|
| | Standard β | P | Standard β | P |
| Urine KIM-1/urine Cr* | 0.54 | <0.001 | 0.48 | <0.001 |
| Urine NGAL/urine Cr* | 0.40 | <0.001 | 0.33 | <0.001 |
| Urine IL-18/urine Cr* | 0.23 | 0.011 | 0.16 | 0.072 |
| Urine Ang/urine Cr* | 0.47 | <0.001 | 0.36 | <0.001 |

ACR, albumin-to-creatinine ratio; Ang, angiotensinogen; Cr, creatinine; eGFR, estimated glomerular filtration rate; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; LDL, low-density lipoprotein; NGAL, neutrophil gelatinase-associated lipocalin; SBP, systolic blood pressure.
* Logarithm-transformed values were used for analysis.
† Adjustment for age, duration of diabetes, SBP, eGFR, LDL cholesterol and HbA1c.

*SS Kim et al. Diabetes Res Clin Pract 97:251-257; 2012*

# Conclusion

- **Urinary tubular markers** ,such as KIM-1 and NGAL, may be independent factors associated with albuminuria in patients with conserved **eGFR in the early stage of diabetic nephropathy (eGFR ≥60 mL/min/1.73 $m^2$).**

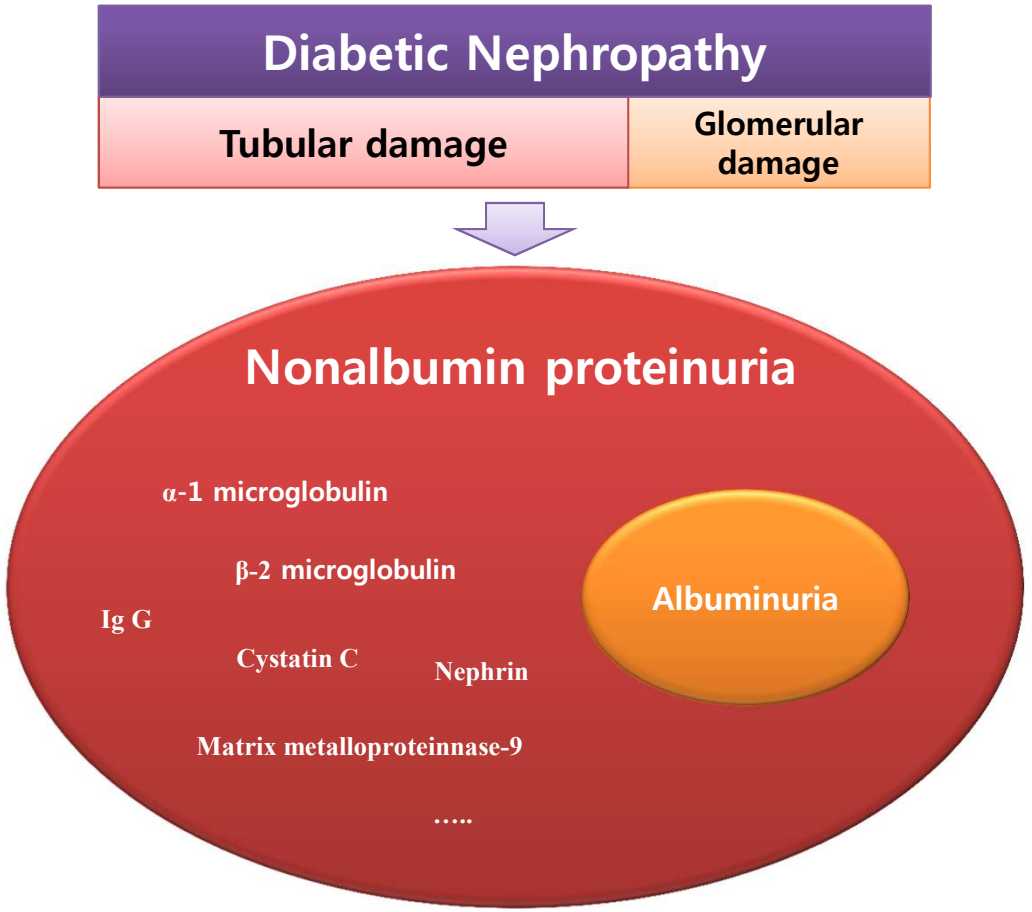
- **Urinary markers of inflammation** and **intrarenal RAS activation** were associated with the severity of diabetic nephropathy as assessed by albuminuria and significantly correlated with urinary tubular markers.

- **Close relationship between chronic inflammation/ intrarenal RAS activation and renal tubular damage in development of diabetic nephropathy.**

***SS Kim et al. Diabetes Res Clin Pract 97:251-257; 2012***

# Urinary cystatin C & Tubular Proteinuria

# Nonalbumin proteinuria




**Nonalbumin proteinuria (NAP)** = total proteinuria – albuminuria

→ are biomarkers of renal damage in various renal disease.

# Is there a consistent relationship between urine albumin & total protein?

- At normal levels of protein loss, **albumin is a minor component of total urinary protein**; as protein loss increases, albumin becomes the most significant single protein present. [1]

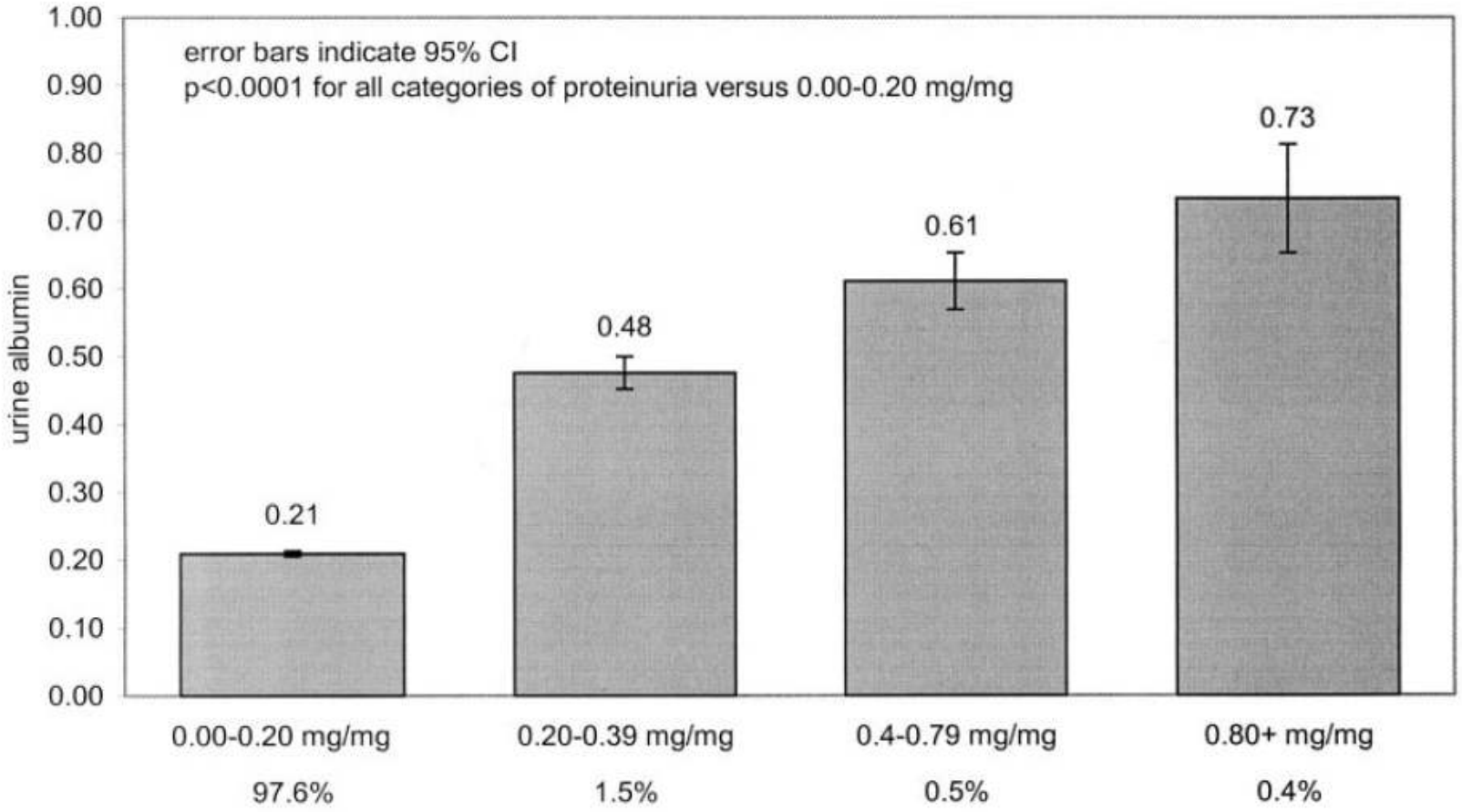
- At lower levels of proteinuria the proprotional contribution of albumin tends to be far more variable than at higher levels. [2]

- The SIGN guidelines suggest that a rough conversion of ACR to PCR [3]
  : X 2, when total protein loss is <1g/24h
  : X 1.3, while at protein losses exceeding this value.

*1. Ann Clin Biochem 46:205-217, 2009*
*2. Ann Clin Biochem 30:101-103, 1993*
*3. Scottish Intercollegiate Guideline Network. Diagnosis and Management of Chronic Kidney Disease: Guideline 103*

# Urine albumin as a proportion of total protein

Among the general Australian adult population (n=10,596)




*Nephrol Dial Tranplant 18:2170-2174, 2003*

# What about 'missed' tubular proteinuria?

Among the general Australian adult population (n=10,596)

| | | Proteinuria | | |
|---|---|---|---|---|
| | | Positive | Negative | |
| Albuminuria | Positive | 2.2% | 4.6% | 6.8% |
| | Negative | 0.2% | 93.0% | 93.2% |
| | | 2.4% | 97.6% | 100.0% |

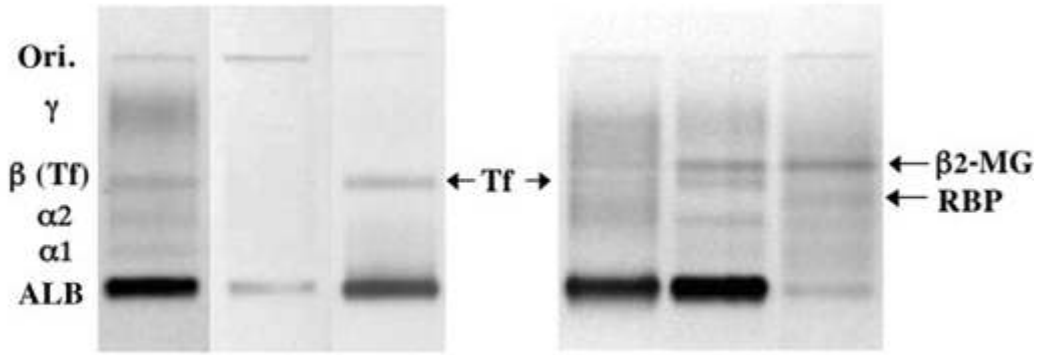
Albuminuria = urine albumin:creatinine ratio ≥30 mg/g; proteinuria = protein:creatinine ratio ≥0.20 mg/mg.
Sensitivity 91.7% (95% CI 87.7–94.5%) and specificity 95.3% (95% CI 94.9–95.7%). Positive predictive value 32.4% (95% CI 29.0–35.8%) and negative predictive value 99.8% (95% CI 99.7–99.9%).

Of those with proteinuria (2.4% of the population, defined as a PCR 23 mg/mmol)
- 92% had albuminuria (defined as an ACR 3.4 mg/mmol);
- 8% (0.2% of the general population) had an ACR within the reference range

***Nephrol Dial Tranplant 18:2170-2174, 2003***

# Urinary leakage of proteins other than albumin can indicate 'tubular' rather than glomerular damage

**The electrophoretic profiles of urinary proteins on cellulose acetate (CA) membrane**




| Renal histology | Positive | Negative | Total |
|---|---|---|---|
| *Urinary protein profile: Glomerular pattern* (Sensitivity 100, Specificity 85.7) | | | |
| Glomerular | 79 | 0 | 79 |
| Others | 3 | 18 | 21 |
| | 82 | 18 | 100 |
| *Urinary protein profile: Tubular pattern* (Sensitivity 100, Specificity 94.9) | | | |
| TI injury | 2 | 0 | 2 |
| Others | 5 | 93 | 98 |
| | 7 | 93 | 100 |
| *Urinary protein profile: Mixed pattern* (Sensitivity 57.9, Specificity 100) | | | |
| Combination† | 11 | 8 | 19 |
| Others | 0 | 81 | 81 |
| | 11 | 89 | 100 |

†Combination of glomerular and tubulointerstitial (TI) injury, which implies glomerular lesion associated with more than 25% distribution of TI injury.

| | Urinary protein profile | | |
|---|---|---|---|
| Features of renal biopsy | Glomerular | Mixed | Tubular |
| Glomerular injury | 79 | 0 | 0 |
| Combination of glomerular and TI injury | 3 | 11 | 5 |
| 25–49% TI injury | 3 | 3 | 0 |
| ≥50% TI injury | 0 | 8 | 5 |
| TI injury | 0 | 0 | 2 |
| Total | | | 100 |

The *kappa* statistic: $K = 0.76$, $Z = 9.46$, $P < 0.001$.
TI, tubulointerstitial.

*Nephrology 12:191-196, 2007*

# Predictive role of NAP on graft loss & death in renal transplant recipients

| Proteinuria (g/day) | Number of patients | Urinary albumin excretion (g/day) | Nonalbumin proteinuria (g/day) | Nonalbumin (%) |
|---|---|---|---|---|
| 0 | 219 | 19.6 ± 58.3 | 0 | 0 |
| 0.01 to 0.25 | 172 | 37 ± 32 | 124 ± 44 | 76 ± 24 |
| 0.26 to 0.50 | 107 | 116 ± 78 | 226 ± 80 | 66 ± 21 |
| 0.51 to 0.75 | 40 | 210 ± 113 | 363 ± 107 | 64 ± 19 |
| 0.76 to 1 | 15 | 376 ± 156 | 498 ± 144 | 57 ± 17 |
| >1 | 63 | 1318 ± 855 | 966 ± 572 | 44 ± 18 |

**Table 5:** Respective predictive value of urinary albumin excretion and nonalbumin proteinuria on graft loss

| | OR | 95% CI | p-Value |
|---|---|---|---|
| Univariate analyses | | | |
| Macroalbuminuria vs. microalbuminuria | 16.41 | 7.49–35.97 | <0.0001 |
| Nonalbumin proteinuria (yes vs. no) | 29.09 | 8.80–96.20 | <0.0001 |
| Multivariate analyses | | | |
| Nonalbumin proteinuria (yes vs. no) | 14.58 | 4.07–52.25 | <0.0001 |
| Macroalbuminuria vs. microalbuminuria (yes vs. no) | 6.57 | 3.05–14.16 | <0.0001 |

*Nephrology 12:191-196, 2007*

# The aims of the study

- To evaluate the impact of **urinary cystatin C** on the progression of type 2 diabetic nephropathy
- To determine whether **urinary cystatin C** has an association with the decline of GFR in type 2 diabetic patients.
- As well as whether **urinary NAP** has any correlation with urinary cystatin C or has any effect on the decline in GFR.

***SS Kim et al. Diabetes Care 36:656-661, 2013***

# SUBJECTS AND METHODS

- A prospective observational study / Department of Endocrinology at **PNUH**
- A total of **264 Korean T2DM** at the **outpatient** clinics (Finally, **237** patients F/U)
- Enrollment: May 2008 and December 2009 / Followed up until March 2012
- The median follow-up period was **29.0 months** (range, 13.0–44.0 months).
- **Inclusion criteria:**
  - Age ≥18 years and **eGFR ≥30 mL/min/1.73 $m^2$**
- **Exclusion criteria:**
  - Thyroid disorders or who had been medicated within 6 monhts prior to the study
  - Active urinary tract infection,
  - Renal disease other than diabetic nephropathy,
  - Neoplastic disorders,
  - Severe liver dysfunction,
  - Active or chronic persistent infection or inflammatory disorders (including use of corticosteroid),
  - Pregnancy,
  - A recent (within 6 months) history of AMI, stroke or occlusive peripheral vascular disease.

***SS Kim et al. Diabetes Care 36:656-661, 2013***

# SUBJECTS AND METHODS

- The **serum and urinary cystatin C levels**

  - measured by the latex agglutination test

    (Modular P800, Roche, Diagnostics, Mannhein, Germany).

  ** The interassay and intraassay coefficients of variations of cystatin C in our laboratory were as follows: <4.2% and <3.4%, respectively, for serum and <7.9% and <10.1%, respectively, for urine.*

- **Nonalbumin protein-to-creatinine ratio (NAPCR)**

  = PCR (protein-to-creatinine ratio)

  – ACR (albumin-to-creatinine ratio)

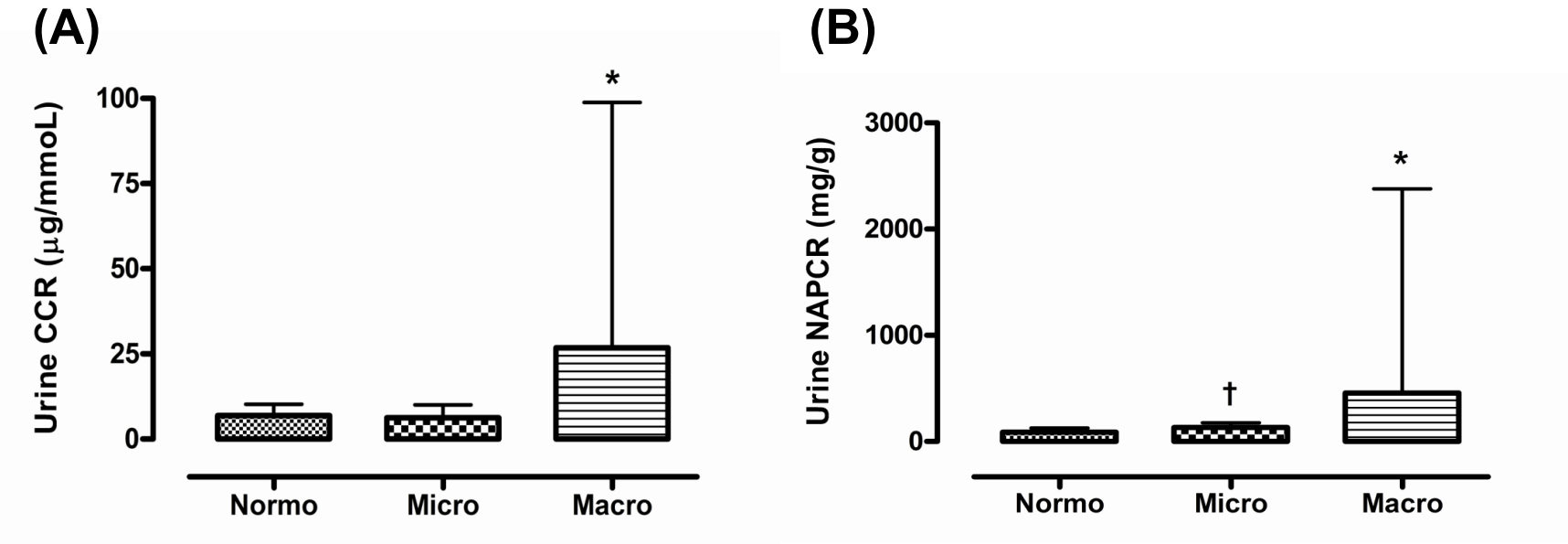
- *The lowest detetable level and the coefficient of variation in our laboratory were as follow: for total proteinuria, 0.7 mg/dL and <4.8%, respectively; for albuminuria, 0.2 mg/dL and <7.4%, respectively.*

***SS Kim et al. Diabetes Care 36:656-661, 2013***

# Baseline urinary CCR and NAPCR according to albuminuria

Table 1—*Baseline characteristics of metabolic and laboratory parameters in patients with type 2 diabetes*

| | Normoalbuminuria ($n$ = 149) | Microalbuminuria ($n$ = 58) | Macroalbuminuria ($n$ = 30) | $P$ value |
|---|---|---|---|---|
| Urine albumin, mg/dL* | 0.9 (0.6–1.5) | 5.0 (3.0–9.6)§ | 88.5 (46.2–121.3)§‖ | <0.001 |
| Urine ACR, mg/g* | 11 (7–17) | 56 (38–109)§ | 962 (502–1737)§‖ | <0.001 |
| Urine cystatin C, mg/L* | 0.05 (0.03–0.08) | 0.04 (0.02–0.09) | 0.13 (0.06–0.56)§‖ | <0.001 |
| Urine CCR, μg/mmol* | 6.9 (4.2–10.2) | 6.2 (3.3–10.0) | 26.8 (6.2–85.1)§‖ | <0.001 |
| Urine NAP, mg/dL* | 7.7 (5.3–11.4) | 10.2 (6.3–14.3) | 42.2 (26.7–133.7)§‖ | <0.001 |
| Urine NAPCR, mg/g* | 89 (66–126) | 131 (92–173)† | 456 (238–2173)§‖ | <0.001 |




*$P$ <0.001 versus the normoalbuminuria and microalbuminuria groups. †$P$ < 0.001 versus the normoalbuminuria group.
CCR, cystatin C-to-creatinine ratio; NAPCR, nonalbumin protein-to-creatinine ratio.

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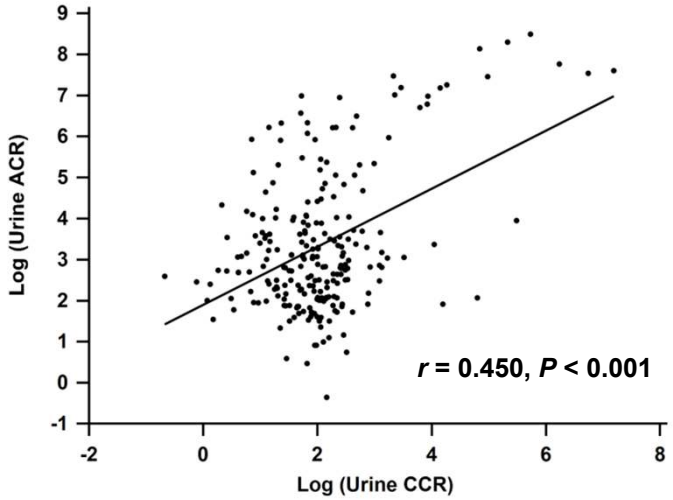
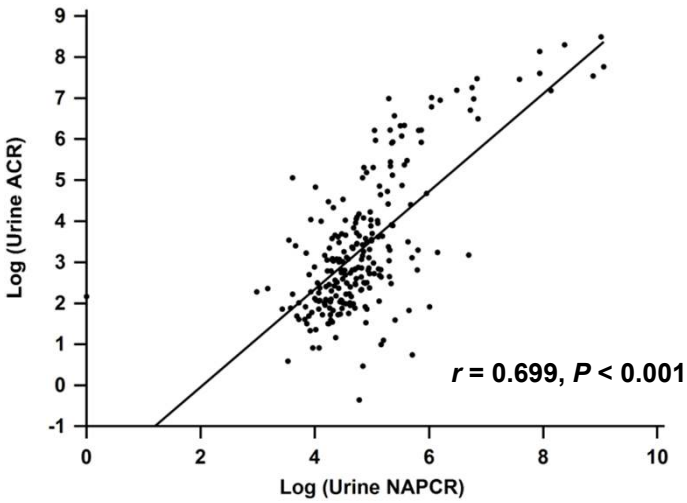
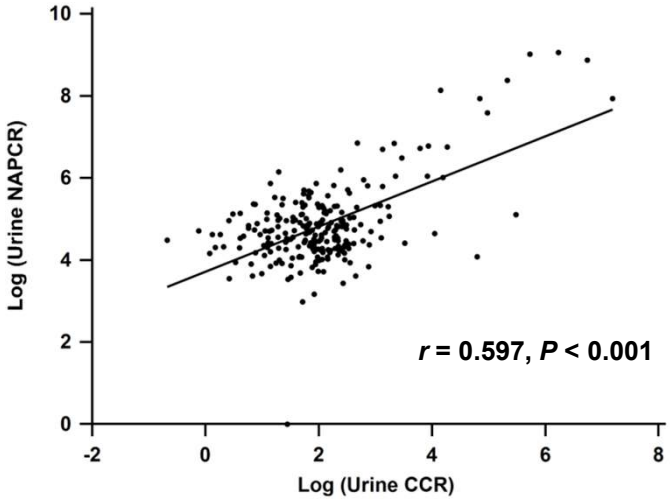
# Correlations among urinary ACR, CCR and NAPCR




Logarithm-transformed values were used for analysis.
ACR, albumin-to-creatinine ratio; CCR, cystatin C-to-creatinine ratio; PCR, NAPCR, nonalbumin protein-to-creatinine ratio.

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# Correlations between urine CCR and NAPCR with clinical variables

| | Urine CCR* | | | |
|---|---|---|---|---|
| | Univariate | | Multivariate | |
| Variables | Standard β | *P* | Standard β | *P* |
| Duration of diabetes | 0.193 | 0.003 | **0.121** | **0.041** |
| HbA1c | 0.201 | 0.002 | **0.125** | **0.033** |
| SBP | 0.188 | 0.004 | **0.117** | **0.045** |
| Urine ACR* | 0.450 | <0.001 | **0.319** | **<0.001** |
| Baseline eGFR | -0.341 | <0.001 | **-0.189** | **0.003** |
| | **Urine NAPCR** * | | | |
| | Univariate | | Multivariate | |
| Variables | Standard β | *P* | Standard β | *P* |
| Duration of diabetes | 0.254 | <0.001 | **0.132** | **0.006** |
| HbA1c | 0.251 | <0.001 | **0.127** | **0.008** |
| SBP | 0.258 | <0.001 | **0.141** | **0.003** |
| Triglyceride* | 0.142 | 0.029 | -0.031 | 0.520 |
| Urine ACR* | 0.699 | <0.001 | **0.602** | **<0.001** |
| Baseline eGFR | -0.377 | <0.001 | **-0.108** | **0.033** |

*Logarithm-tranformed values were used for analysis. ACR, albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate; NAPCR, nonalbumin protein-to-creatinine ratio; SBP, systolic blood pressure.

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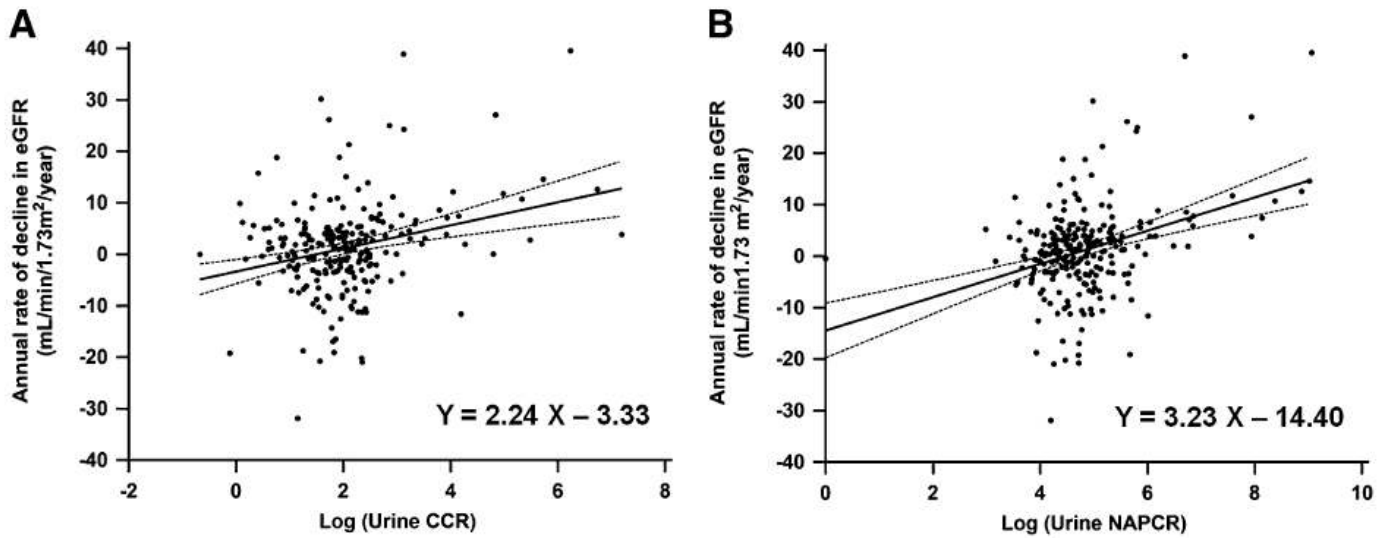


**Figure 1**—*Single regression analysis of the annual rate of the decline in eGFR by using urinary CCR (A) and NAPCR (B). Logarithm-transformed values of urinary CCR and NAPCR were used for analysis.*

Table 2—***Multiple regression analysis of the annual rate of decline in eGFR as a dependent variable***

| | Urine CCR | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | All ($n$ = 237) | | eGFR ≥60 mL/min/1.73 m$^2$ ($n$ = 218) | | Normoalbuminuria ($n$ = 149) | | eGFR ≥60 mL/min/1.73 m$^2$ and normoalbuminuria ($n$ = 144) | |
| Model | Standard β | *P* value | Standard β | *P* value | Standard β | *P* value | Standard β | *P* value |
| 1 | 0.272 | <0.001 | 0.170 | 0.012 | 0.114 | 0.166 | 0.083 | 0.321 |
| 2 | 0.254 | <0.001 | 0.169 | 0.014 | 0.137 | 0.087 | 0.083 | 0.310 |
| 3 | 0.260 | <0.001 | 0.172 | 0.013 | 0.146 | 0.072 | 0.090 | 0.272 |
| 4 | 0.160 | 0.021 | 0.144 | 0.031 | 0.097 | 0.199 | 0.084 | 0.292 |
| | Urine NAPCR | | | | | | | |
| | All ($n$ = 237) | | eGFR ≥ 60 mL/min/1.73 m$^2$ ($n$ = 218) | | Normoalbuminuria ($n$ = 149) | | eGFR ≥60 mL/min/1.73 m$^2$ and normoalbuminuria ($n$ = 144) | |
| Model | Standard β | *P* value | Standard β | *P* value | Standard β | *P* value | Standard β | *P* value |
| 1 | 0.361 | <0.001 | 0.262 | <0.001 | 0.215 | 0.009 | 0.176 | 0.035 |
| 2 | 0.413 | <0.001 | 0.286 | 0.001 | 0.261 | 0.002 | 0.202 | 0.017 |
| 3 | 0.412 | <0.001 | 0.282 | 0.001 | 0.261 | 0.002 | 0.201 | 0.018 |

Model 1, crude; model 2, adjusted for age and significant clinical parameters including $HbA_{1c}$, SBP, uric acid, urine ACR, and baseline eGFR; model 3, adjusted for use of RAS inhibitors and lipid-lowering agents; model 4, adjusted for serum cystatin C.

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Table 3—*Logistic regression of development of CKD stage 3 or greater (eGFR <60 mL/min/1.73 m$^2$) at last follow-up*

| | Univariate analysis | | Multivariate analysis* with urine CCR | | Multivariate analysis* with urine NAPCR | |
|---|---|---|---|---|---|---|
| Variable | OR | 95% CI (*P* value) | OR | 95% CI (*P* value) | OR | 95% CI (*P* value) |
| Age, years | 1.08 | 1.03–1.13 (0.002) | — | — | — | — |
| SBP, mmHg | 1.03 | 1.01–1.05 (0.012) | — | — | — | — |
| $HbA_{1c}$, % | 1.32 | 1.05–1.66 (0.018) | 1.64 | 1.09–2.46 (0.018) | — | — |
| Uric acid, mg/dL | 1.99 | 1.53–2.59 (<0.001) | 2.70 | 1.51–4.82 (0.001) | 1.69 | 1.07–2.68 (0.026) |
| Baseline eGFR, mL/min/1.73 m$^2$ | 0.86 | 0.83–0.91 (<0.001) | 0.86 | 0.84–0.93 (<0.001) | 0.87 | 0.82–0.93 (<0.001) |
| RAS inhibitor use, yes | 27.22 | 3.64–203.38 (0.001) | 15.67 | 0.75–327.42 (0.076) | 33.68 | 1.92–590.97 (0.016) |
| Lipid-lowering agent use, yes | 1.28 | 0.57–2.86 (0.548) | — | — | — | — |
| Urine ACR group | | | | | | |
| Normoalbuminuria | 1 | Ref. | 1 | Ref. | — | — |
| Microalbuminuria | 3.27 | 1.05– 10.19 (0.041) | 2.46 | 0.42–14.36 (0.316) | — | — |
| Macroalbuminuria | 55.61 | 17.97–172.15 (<0.001) | 13.81 | 2.02–94.35 (0.007) | — | — |
| Urine CCR tertile | | | | | | |
| 1st tertile | 1 | Ref. | 1 | Ref. | — | — |
| 2nd tertile | 0.58 | 0.14–2.53 (0.473) | 0.40 | 0.05–3.12 (0.380) | — | — |
| 3rd tertile | 7.26 | 2.62–20.14 (<0.001) | 6.85 | 1.19–39.40 (0.031) | — | — |
| Urine NAPCR tertile | | | | | | |
| 1st tertile | 1 | Ref. | — | — | 1 | Ref. |
| 2nd tertile | 2.00 | 0.18–22.52 (0.575) | — | — | 4.08 | 0.27–61.15 (0.309) |
| 3rd tertile | 48.71 | 6.44–368.43 (<0.001) | — | — | 29.35 | 2.93–294.14 (0.004) |

Ref., reference population group. *Multivariate models using the backward likelihood method were adjusted for age, SBP, $HbA_{1c}$, uric acid, baseline eGFR, urine ACR, RAS inhibitor use, lipid-lowering agent use, and urine CCR and NAPCR.

***SS Kim et al. Diabetes Care 36:656-661, 2013***

# Summary & Conclusion

- Urinary cystatin C and NAP–both clinical tubular damage markers–positively correlated with each other at baseline.
- Both markers were significantly associated with the **annual decline of in eGFR** in type 2 diabetic nephropathy.
- In particular, both tubular damage makers affected a decline in eGFR at the early stage of nephropathy in type 2 diabetic patients **(eGFR ≥60 mL/min/1.73 $m^2$)**.
- **Urinary NAP** affected eGFR decline in patients with both **eGFR ≥60 mL/min/1.73 $m^2$ and normoalbuminuria**, although urinary cystatin C did not reach to the statistical significance.
- In addition, the increased levels of the 2 markers were also associated with CKD stage 3 at the last follow-up.

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# Summary & Conclusion

- It is suggested that **urinary cystatin C and NAP** along with albuminuria

  - may be sensitive and specific markers for predicting renal impairment in type 2 diabetic patients

  - may help understand the role of tubular damage in pathophysiologic mechanisms of the development and progression of type 2 diabetic nephopathy.

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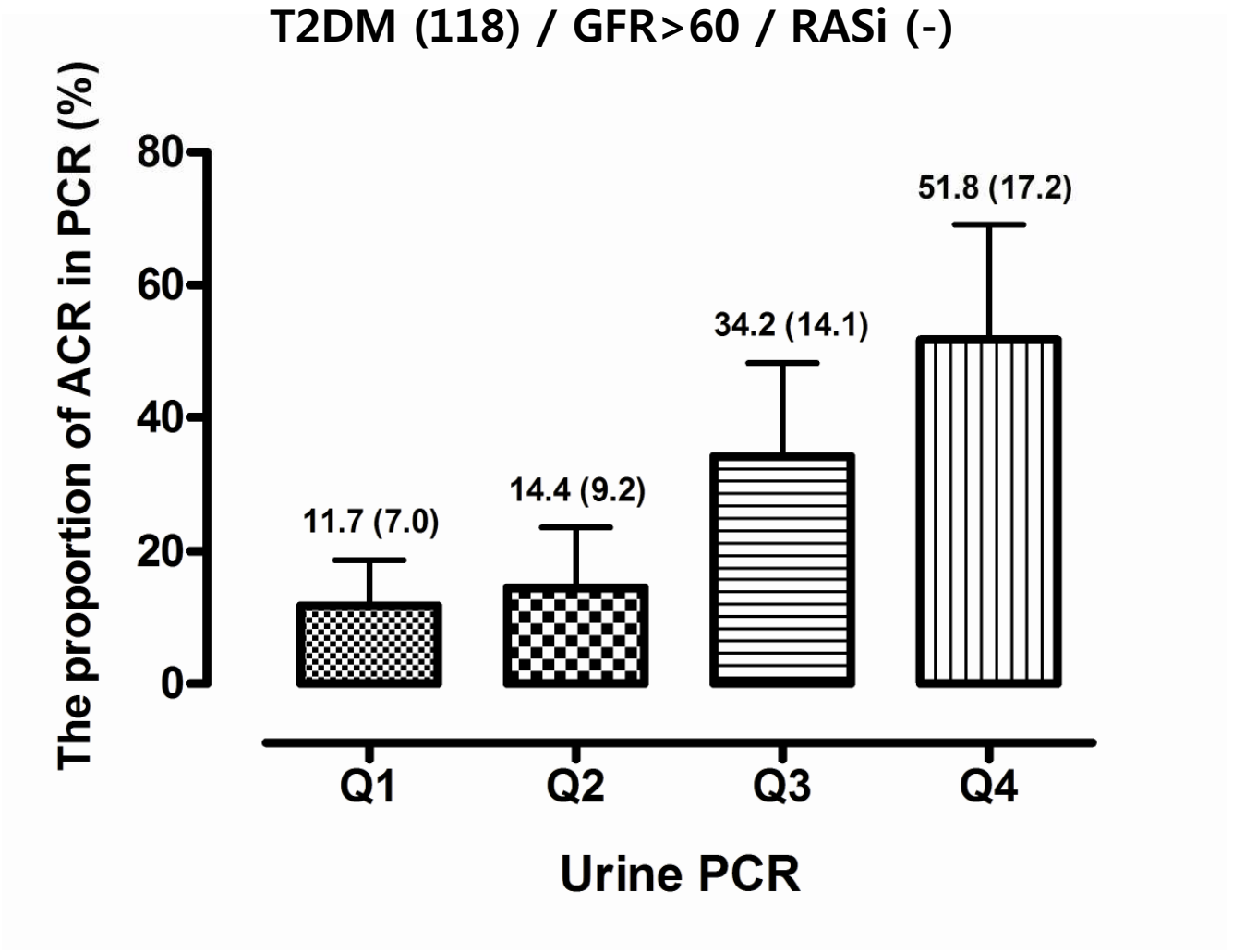
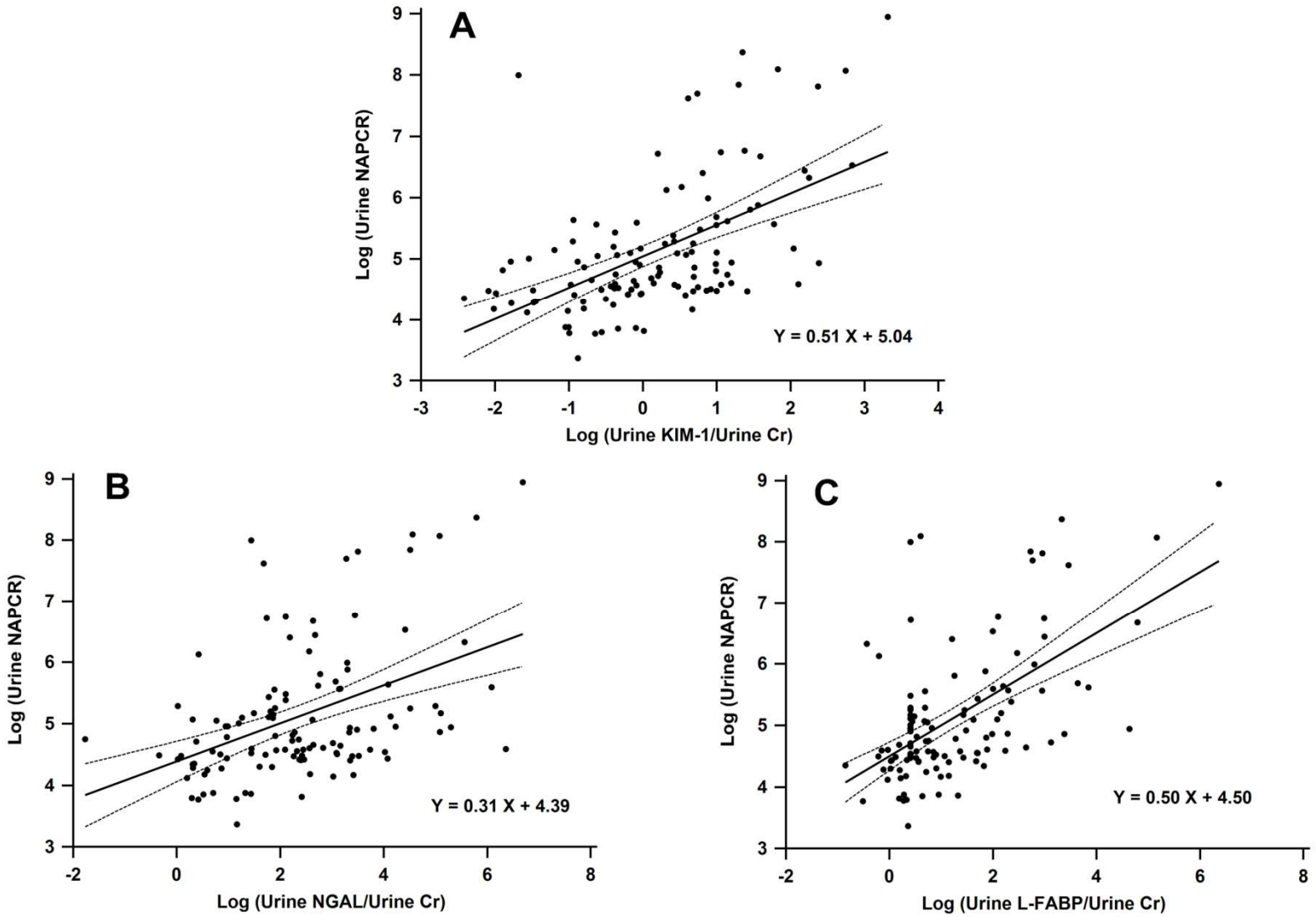
# Urinary NAP for Tubular Marker

# Urinary ACR as a proportion of PCR according quartile groups of urinary PCR




*SS Kim et al. Unpublished data 2013*

# . Single regression analyses of urinary NAPCR in relation to urinary tubular markers




*SS Kim et al. Unpublished data 2013*

# Multiple regression analysis of urinary NAPCR as a dependent variable

| | All patients (n=118) | | | | | |
|---|---|---|---|---|---|---|
| | Urine KIM-1/Cr | | Urine NGAL/Cr | | Urine L-FABP/Cr | |
| Model | Standard β | *P* | Standard β | *P* | Standard β | *P* |
| 1 | **0.543** | **<0.001** | **0.442** | **<0.001** | **0.580** | **<0.001** |
| 2 | **0.530** | **<0.001** | **0.406** | **<0.001** | **0.536** | **<0.001** |
| 3 | **0.170** | **0.010** | **0.142** | **0.015** | **0.262** | **<0.001** |
| | Normoalbuminric patients (n=58) | | | | | |
| | Urine KIM-1/Cr | | Urine NGAL/Cr | | Urine L-FABP/Cr | |
| Model | Standard β | *P* | Standard β | *P* | Standard β | *P* |
| 1 | **0.292** | **0.026** | **0.372** | **0.004** | **0.429** | **0.001** |
| 2 | 0.272 | 0.065 | **0.345** | **0.010** | **0.430** | **0.001** |
| 3 | 0.216 | 0.161 | **0.302** | **0.030** | **0.430** | **0.001** |

Model 1, Crude; Model 2, adjusted for significant variables including age, duration of diabetes, SBP, eGFR, LDL cholesterol, and HbA1c; Model 3, adjusted for significant variables and ACR. ACR, albumin-to-creatinine ratio; Cr, creatinine; eGFR, estimated glomerular filtration rate; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid-binding protein; LDL, low-density lipoprotein; NAPCR, nonalbumin protein-to-creatinine ratio; NGAL, neutrophil gelatinase-associated lipocalin; SBP, systolic blood pressure.

***SS Kim et al. Unpublished data 2013***

# Summary & conclusions

- The proportional contribution of albumin to total protein was relatively small in lower quartile group.
- Urinary NAP independently correlated with several urinary tubular markers after adjusting clinical confounding factors including albuminuria in type 2 diabetic patients with eGFR ≥60 mL/min/1.73 $m^2$.
- Urinary NAP also remained significantly associated with some tubular markers in only normoalbuminuric patients.

- **Urinary NAP** as indicator for **tubular damage**, **beyond albuminuria**, could be used as early biomarker to detect the development and progression in diabetic nephropathy based on clinical practice.

***SS Kim et al. Unpublished data 2013***

# Markers of Inflammation

# Some urinary biomarkers of inflammation and endothelial dysfunction associated with type 2 diabetes

- Adiponectin
- Angiotensin-converting enzyme
- **Angiotensinogen ?**
- **Endothelin-1**
- Fibrinopeptide A
- **Interleukin-6**
- **Monocyte chemoattractant protein-1**
- Nitric oxide
- Orosomucoid/*α-1 acid glycoprotein*
- **Transforming growth factor-*β***
- Thrombomodulin
- Thromboxane
- **Tumour necrosis factor-*α***
- Vascular endothelial growth factor

***Diabetes Metab Res Rev 26: 150-71, 2010***

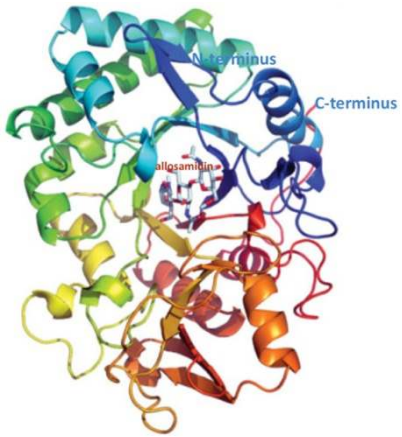
# Clinical implication of plasma and urine YKL-40, as a proinflammatory biomarker, on early stage of nephropathy in T2DM

# What is YKL-40?

**Y K L - 40**

the first three N-terminal amino acids
-tyrosine (Y)
-lysine (K)
-leucine(L)

the apparent molecular weight 40 kDa




- ***In vitro*** secreted by macrophages during late stages of differentiation & by activated macrophages

- ***In vivo*** expressed in human VSMCs in adventitial vessels & in subpopulations of macrophages and VSMCs in different tissues with inflammation and extracellular matrix remodeling as in atherosclerotic plaques

***J Bone Miner Res 1986, 1:485-486, 1986***

| In vitro secretion by |
| --- |
| Neutrophils |
| Activated macrophages |
| Macrophages in late stage of differentiation |
| Differentiated VSMCs |
| Arthritic chondrocytes |
| Fibroblast-like synovial cells |

| In vivo secretion by |
| --- |
| Macrophages and VSMCs in atherosclerotic plaques |
| Macrophages in inflamed synovial membranes |
| Macrophages in acute bacterial infections |
| VSMCs in adventitial vessels |
| CD68+ macrophages/Giant cells in Giant cell artheritis |

| Acute infectious conditions |
| --- |
| Purulent meningitis |
| Pneumonia |
| E.coli endotoxemia |

| Chronic inflammatory conditions |
| --- |
| RA |
| Osteoarthritis |
| SLE |
| IBD |
| Sarcoidosis |

| Cancers |
| --- |
| Osteosarcoma |
| Glioblastoma |
| Myeloid leukaemia cell lines |
| Breast |
| Colon/rectum |
| Ovary |
| Lung |
| Prostate |
| Kidney |
| Melanoma |

| Conditions characterized by fibrosis |
| --- |
| Alcoholic cirrhosis |
| Liver disease characterized by fibrosis |

| Conditions characterized by subclinical inflammation |
| --- |
| Atherosclerosis/atherosclerotic plaques |
| Insulin resistance/T2D |

# YKL-40 & CAD

- Serum YKL-40 levels in patients with coronary artery disease

  ***Coron Artery Dis 18:391-396, 2007***

- YKL-40 a new biomarker in patients with acute coronary syndrome or stable coronary artery disease

  ***Scand Cardiovasc J 42:295-302, 2008***

- Serum levels of YKL-40 increases in patients in patients with acute myocardial infarction

  ***Coron Artery Dis 19:257-263, 2008***

- Increased serum YKL-40 and C-reactive protein levels are associated with angiographic lesion progression in patients with coronary artery disease

  ***Atherosclerosis 210:590-595, 2010***

- Increased YKL-40 expression in patients with carotid atherosclerosis

  ***Atherosclerosis 211:589-595, 2010***

- High serum YKL-40 concentration is associated with cardiovascular and all-cause mortality in patient with stable coronary artery disease

  ***Eur Heart J 30:1066-1072, 2009***

# YKL-40 & DM

- YKL-40, a biomarker of inflammation, is elevated in patients with type 2 diabetes and is related to insulin resistance

***Inflamm Re 55"53-59, 2006***

- Plasma YKL-40; A BMI-Independent Marker of Type 2 Diabetes

***Diabetes 57:3078-3082, 2008***

- Increased Circulating and Visceral Adipose Tissue Expression Levels of YKL-40 in Obesity-Associated Type 2 Diabetes Are Related to Inflammation: Impact of Conventional Weight Loss and Gastric Bypass

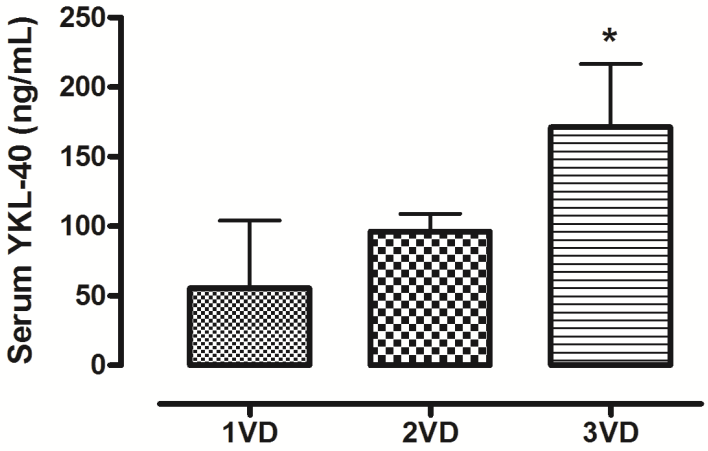
***J Clin Endocrionol Metab 96:200-209, 2011***

- YKL-40, a Marker of Inflammation and Endothelial Dysfunction, Is Elevated in Patients With Type 1 Diabetes and Increases With Levels of Albuminuria

***Diabetes Care 32:323-328, 2009***

# Increased serum YKL-40 level are related to angiographic severity and extent of CAD in patients with T2DM

- Serum YKL-40 was measured in 78 patients (including 38 patients with T2DM) with stenosis of > 50% of luminal diameter in at least one of coronary artery in coronary angiography.
- Coronary angiography from Pusan National University Hostpital between Jun 2009 and May 2011.
- The atherosclerotic burden was obtained by three independent angiographic scores: the severity, Gensini score (GS) and Extent score (ES).




| | Gensini score | | Extent score | |
|---|---|---|---|---|
| | Standard β | *P* | Standard β | *P* |
| Model 1 | **0.329** | **0.003** | **0.380** | **0.001** |
| Model 2 | **0.325** | **0.006** | **0.343** | **0.004** |
| Model 3 | **0.317** | **0.009** | **0.330** | **0.007** |
| Model 4 | **0.262** | **0.040** | **0.296** | **0.024** |

Model 1, crude; Model 2, adjusted for age and sex; model 3, adjusted for history of antihypertensive, lipid-lowering and anti-platelet agents; model 4, adjusted for clinical parameters.

***SS Kim et al. Unpublised Data 2013***

# Patients and Methods

**Inclusion criteria**
- Age ≥ 18years
- **eGFR ≥ 60mL/min/1.73m$^2$**
- Stable renal function for at least 5months
- **No History of RAS inhibitors (withdrawn for at least 2months)**

**Exclusion criteria**
- Other renal disease except for DN
- Urinary tract infection
- Other active diseases

**Type 2 diabetic patients**
Jan. 2011 – Jun. 2011
N=75

**Non-diabetic control**
Fasting Plasma Glucose < 100mg/dL
eGFR ≥ 60mL/min/1.73m$^2$
**N = 22**

**Normoalbuminuria**
**$U_{ACR}$ < 30mg/g creatinine**
**N = 25**

**Microalbuminuria**
**30 ≤ $U_{ACR}$ < 300 mg/g creatinine**
**N = 25**

**Macroalbuminuria**
**300 ≤ $U_{ACR}$ mg/g creatinine**
**N = 25**

*SS Kim et al. J Diabetes Complications 26:308-312, 2012*

# Patients and Methods

- ✓ A random spot urine and blood sample were obtained from subjects at the clinic visit.
- ✓ **Plasma and urine concentrations of YKL-40** were analyzed by a commercial **ELISA kit**. Human Chitinase 3-like 1 immunoassay kits were purchased from R&D Systems, Minneapolis, MN, USA.
- ✓ **eGFR** : calculated using the Modification of Diet in Renal Disease (MDRD) formula
  - MDRD = 186 × (serum creatinine [mg/dL])-1.154 × (age in years)-0.203
  - correction factor of 0.742 for women
- ✓ **FeYKL-40** : calculated according to the formula
  - FeYKL-40 = (urine YKL-40 [ng/mL] × serum Cr [mg/dL])/(plasma YK-40 [ng/mL] × urine Cr [mg/dL]) × 100

*SS Kim et al. J Diabetes Complications 26:308-312, 2012*

Clinical parameters of healthy control subjects and type 2 diabetic patients according to albuminuria status.

| | Nondiabetic control ($n=22$) | Type 2 diabetic patients | | | $P$ value |
|---|---|---|---|---|---|
| | | Normoalbuminuria ($n=25$) | Microalbuminuria ($n=25$) | Macroalbuminuria ($n=25$) | |
| Sex, male/female | 13/9 | 11/14 | 10/15 | 12/13 | 0.599 |
| Age, years | 52.4 ± 5.8 | 55.6 ± 11.1 | 57.0 ± 11.6 | 56.0 ± 9.8 | 0.407 |
| BMI, kg/m$^2$ | 23.5 ± 2.9 | 23.8 ± 3.5 | 23.6 ± 3.8 | 22.9 ± 2.8 | 0.790 |
| Duration of diabetes, years | – | 6.7 ± 5.9 | 8.7 ± 5.6 | 9.6 ± 7.1 | 0.254 |
| SBP, mm Hg | 120.1 ± 15.0 | 125.1 ± 13.1 | 126.4 ± 14.6 | 133.4 ± 21.0[a] | 0.049 |
| DBP, mm Hg | 74.6 ± 8.2 | 77.7 ± 11.1 | 79.4 ± 12.1 | 81.6 ± 12.2 | 0.191 |
| HbA1c, % | – | 7.4 ± 1.5 | 8.5 ± 1.5[c] | 9.0 ± 2.5[c] | 0.001 |
| eGFR*, ml/min/1.73 m$^2$ | 85.6 ± 10.0 | 94.5 ± 17.9 | 98.2 ± 22.3 | 90.7 ± 19.8 | 0.123 |
| Total cholesterol, mg/dl | 197.0 ± 32.9 | 192.5 ± 39.3 | 189.6 ± 36.3 | 206.1 ± 46.2 | 0.483 |
| LDL cholesterol, mg/dl | 130.5 ± 31.7 | 117.4 ± 31.6 | 120.2 ± 33.3 | 129.7 ± 60.7 | 0.604 |
| HDL cholesterol, mg/dl | 54.6 ± 13.9 | 45.4 ± 16.1 | 48.0 ± 11.1 | 56.2 ± 24.2 | 0.091 |
| Triglyceride, mg/dl† | 126.0 (62.0–167.8) | 147.0 (114.0–254.0) | 144.0 (88.0–220.0) | 151.0 (111.0–245.5) | 0.142 |
| CRP, mg/dl† | 0.05 (0.03–0.10) | 0.06 (0.03–0.17) | 0.07 (0.04–0.26) | 0.06 (0.03–0.23) | 0.428 |
| Plasma YKL-40, ng/ml† | 25.0 (17.0–34.1) | 45.1 (31.9–62.3)[a] | 68.9 (44.2–115.4)[b,c] | 74.0 (46.6–148.2)[b,c] | <0.001 |
| Urine YKL-40/urine Cr, ng/mg† | 0.12 (0.02–0.30) | 0.09 (0.04–0.45) | 0.12 (0.07–0.35) | 0.46 (0.24–2.03)[b,c,e] | <0.001 |
| FeYKL-40, %† | 0.004 (0.001–0.013) | 0.003 (0.001–0.015) | 0.001 (0.001–0.004) | 0.004 (0.003–0.015) | 0.055 |
| Antihypertensive medication, n (%) | – | 5 (20.0) | 5 (20.0) | 12 (52.2)[c, e] | <0.001 |
| Lipid lowering agent, n (%) | – | 11 (44.0) | 7 (28.0) | 7 (28.0) | 0.383 |
| CVD, n (%) | – | 1 (4.0) | 5 (20.0) | 4 (16.0) | 0.223 |
| Diabetic retinopathy, n (%) | – | 7 (28.0) | 11 (44.0) | 16 (64.0)[d] | <0.001 |

Data are expressed as means ± SD for parametric variables and median (interquartile range) for nonparametric variables. BMI, body mass index; CRP, C-reactive protein; Cr, creatinine; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Fe, fractional excretion; Hdl, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

* Calculated by Modification of Diet in Renal Disease equation.

† Logarithm-transformed values were used for comparison.

[a] $P<0.05$ vs. control.

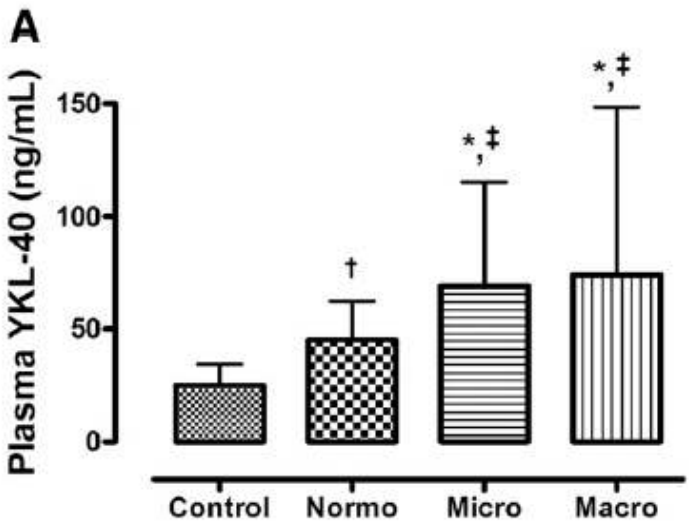
[b] $P<0.001$ vs. control.

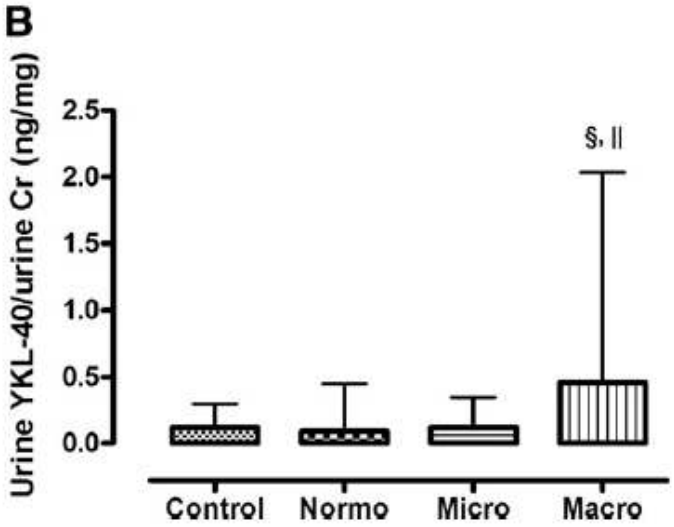
[c] $P<0.05$ vs. normoalbuminuria.

[d] $P=0.001$ vs. normoalbuminuria.

[e] $P<0.05$ vs. microalbuminuria.

*SS Kim et al. J Diabetes Complications 26:308-312, 2012*




Correlations between plasma YKL-40 level with clinical variables in type 2 diabetic patients.

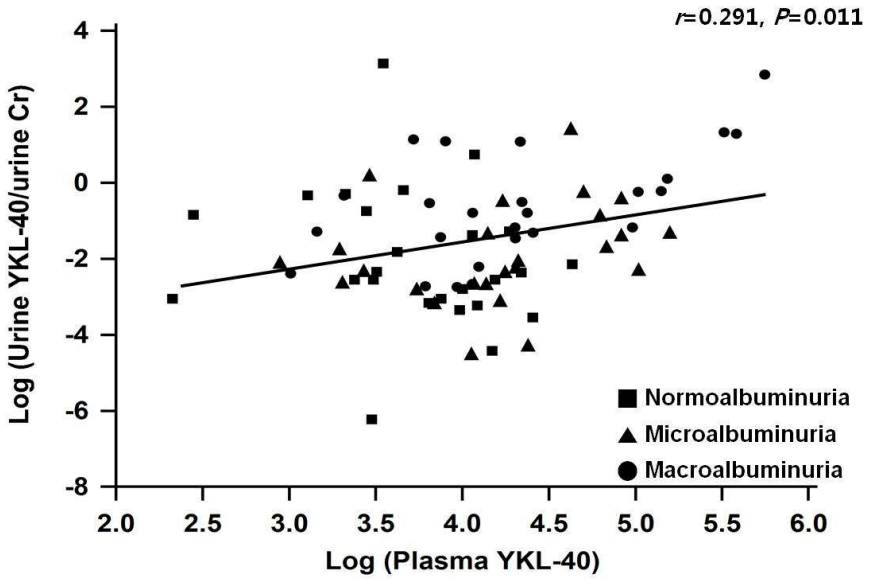
| | Univariate | | Multivariate | |
|---|---|---|---|---|
| | Standard $\beta$ | $P$ | Standard $\beta$ | $P$ |
| Age | 0.192 | 0.099 | 0.215 | 0.038 |
| ACR* | 0.309 | 0.007 | 0.273 | 0.010 |
| Triglyceride* | 0.385 | 0.001 | 0.360 | 0.001 |

* Logarithm-transformed values were used for analysis. ACR, albumin-to-creatinine ratio.

Correlations between urine YKL-40 level with clinical variables in type 2 diabetic patients.

| | Univariate | | Multivariate | |
|---|---|---|---|---|
| | Standard $\beta$ | $P$ | Standard $\beta$ | $P$ |
| HbA1c | 0.260 | 0.024 | 0.264 | 0.021 |
| ACR* | 0.362 | 0.001 | 0.229 | 0.042 |
| CRP | 0.205 | 0.080 | – | – |
| Triglyceride* | 0.309 | 0.007 | 0.331 | 0.003 |

* Logarithm-transformed values were used for analysis. ACR, albumin-to-creatinine ratio.



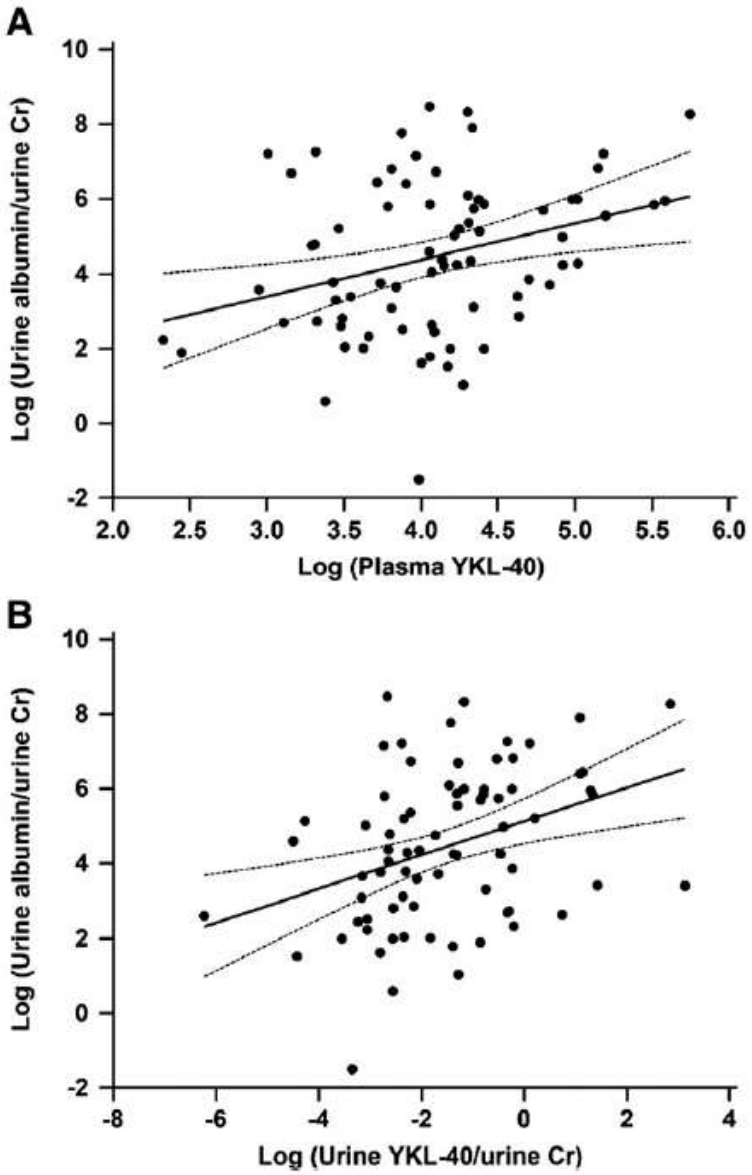




Fig. 2. Single regression analyses for urinary albumin with plasma (A) and urine (B) YKL-40. Logarithm-transformed values were used for analysis. Cr, creatinine.

*SS Kim et al. J Diabetes Complications 26:308-312, 2012*

Multiple regression analysis of urinary ACR as a dependent variable in type 2 diabetic patients.

| Model | Plasma YKL-40 | | Urine YKL-40 | |
|---|---|---|---|---|
| | Standard $\beta$ | $P$ | Standard $\beta$ | $P$ |
| Model 1 | 0.309 | 0.007 | 0.362 | 0.001 |
| Model 2 | 0.339 | 0.004 | 0.366 | 0.001 |
| Model 3 | 0.330 | 0.004 | 0.347 | 0.002 |
| Model 4 | 0.397 | <0.001 | 0.238 | 0.037 |
| Model 5 | 0.359 | 0.001 | 0.128 | 0.241 |

Model 1, crude; model 2, adjusted for age and sex; model 3, adjusted for previous cardiovascular disease and history of antihypertensive and lipid-lowering agents; model 4, adjusted for significant clinical parameters including duration of diabetes, SBP, eGFR, LDL cholesterol, HbA1c, and CRP; model 5, adjusted for corresponding plasma or urine YKL-40. ACR, albumin-to-creatinine ratio; CRP; C-reactive protein; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; SBP, systolic blood pressure.

*SS Kim et al. J Diabetes Complications 26:308-312, 2012*

# Conclusion

- ✓ **Plasma YKL-40** measurement might become a useful and noninvasive tool **for early incipient diabetic nephropathy** as well as **for the evaluation of renal involvement of type 2 diabetic patients**.

- ✓ Although **urinary YKL-40** has a limited role in early development of diabetic nephropathy in this study, **further study is need to evaluate in whole spectrum of diabetic nephropathy** including patients with moderate to severe decrease of GFR.

- ✓ **YKL-40**, as a proinflammatory marker, **might play an important role in the development of early diabetic nephropathy** with **relatively conserved eGFR**.

***SS Kim et al. J Diabetes Complications 26:308-312, 2012***

# Urine biomarker discovery: the furture

**2 principal approaches**

- the study of **candidate biomarkers** (usually tubular proteins, cytokines, growth factors and inflammatory mediators) in specific diseases where laboratory studies have suggested a **pathological link**

- biomarker discovery studies in which urine is screened for disease-associated proteins using an array of technologies, predominantly based on **mass spectrometry**

*Can Med Assoc J 177:361-368, 2007*

# The Human Kidney and Urine Proteome Project (www.hkupp.org/)

Home

HKUPP Human Kidney & Urine Proteome Project

HUPO Human Proteome Organisation

**About us** | **Meetings** | **Links**

### Standardization

Kidney
- Glomerulus
- Tubules

Urine
- Normal "**A tentative standard protocol for urine collection and storage**" NEW
  Call for public opinions:
  info@hkupp.org
- Proteinuric
- Exosome

### Proteomics

2DEGE
LC-MS/MS
CE-MS
SELDI-TOF MS

### Database

Kidney
- Glomerulus
  2DGE-LC-Based Proteomics
  2DGE-Based Proteomics
- Proximal Tubule
- Distal Tubule
- Collecting Duct
  Rat IMCD
  Rat IMCD Phosphoprotein
- Others
  Transcriptomics & Proteomics

Urine
- Normal
- Proteinuric
- Exosome

### Collaborations

Human Proteome Organisation
Human Protein Atlas
HUPO Plasma Proteome Project
PSI

### What's New

▾March 10, 2011

**HKUPP sympohsium** will be held on July 30, 2011 at Toki Messe, Niigata, JAPAN as an International Satellite symposium of **2011 Annual Meeting of Japanese Proteomics Society**.

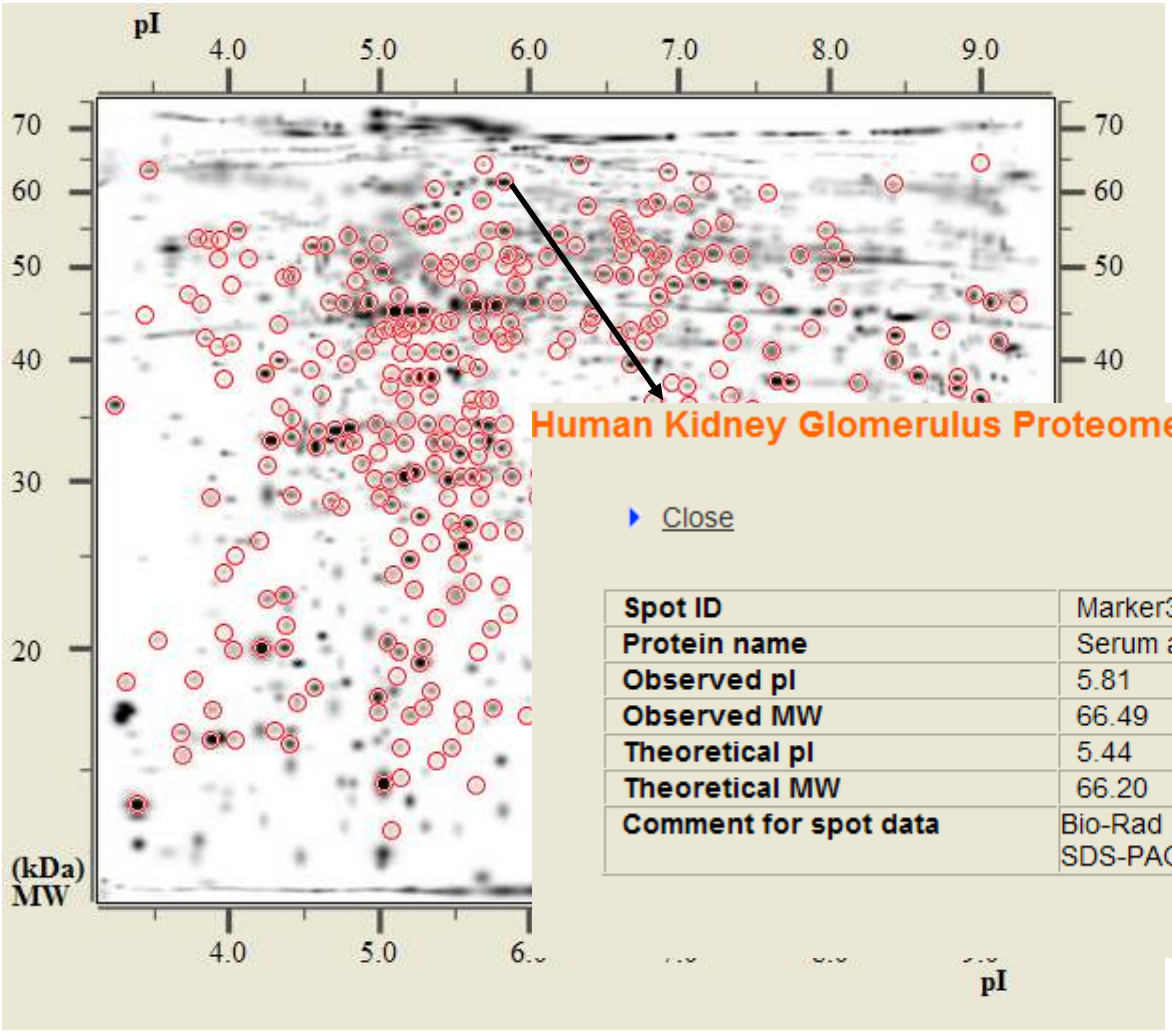
▾November 10, 2010

**The HUPO 10th Annual World Congress** will be held on September 4-7, 2011 at Geneva Palexpo, Genova, Switzerland. IMPORTANT DATES
Submissions of abstrancts from 1 December 2010 to **16 March 2011**.
A **HKUPP symposium** has been scheduled and the Symposists may be selected from Abstracts.

Past News

# The Human Kidney and Urine Proteome Project (www.hkupp.org/)

## Glomerulus – 2DGE-Based Proteomics




**Human Kidney Glomerulus Proteome Database**

Close

| | |
|---|---|
| **Spot ID** | Marker3 |
| **Protein name** | Serum albumin, bovine |
| **Observed pI** | 5.81 |
| **Observed MW** | 66.49 |
| **Theoretical pI** | 5.44 |
| **Theoretical MW** | 66.20 |
| **Comment for spot data** | Bio-Rad<br>SDS-PAGE low molecular weight mass standards |

# Overall Summary

- **Urinary markers** - easily accessible in a non-invasive manner.
- The onset and level of **albuminuria** has been considered as both a prognostic biomarker and a surrogate end point of progressive diabetic nephropathy
- **Microalbuminuria** has several limitations for biomarkers of diabetic nephropathy**. We have also need for more sensitive and specific biomarker than urinary albumin !**
- Recently numerous studies have been done to explore the biomarkers for diabetic nephropathy.
- Especially, **urinary tubular markers** have clinical meaning as biomarkers of diabetic nephropathy.
- **Urinary NAP** may reflect the **summation of tubular protein** and easily be measured in real clinical practice.
- **Urinary NAP,** along with albuminuria, may be sensitive and specific markers for predicting renal impairment in type 2 diabetic patients.


Thank you!