# Urinary biomarkers of chronic kidney disease

Lin-Li Lv M.D., PhD
Prof. Bi-Cheng Liu M.D., PhD
Institute of Nephrology, Zhong Da Hospital,
Southeast University School of Medicine

东南大学肾脏病研究所
Institute of Nephrology Southeast University

# Novel biomarker is needed for Precise Medicine of kidney disease

- China's adult prevalence rate of CKD is 10.8%
- The biomarkers for early detection and dynamic monitor of kidney disease is still missing
- Novel biomarkers are needed for precise diagnosis

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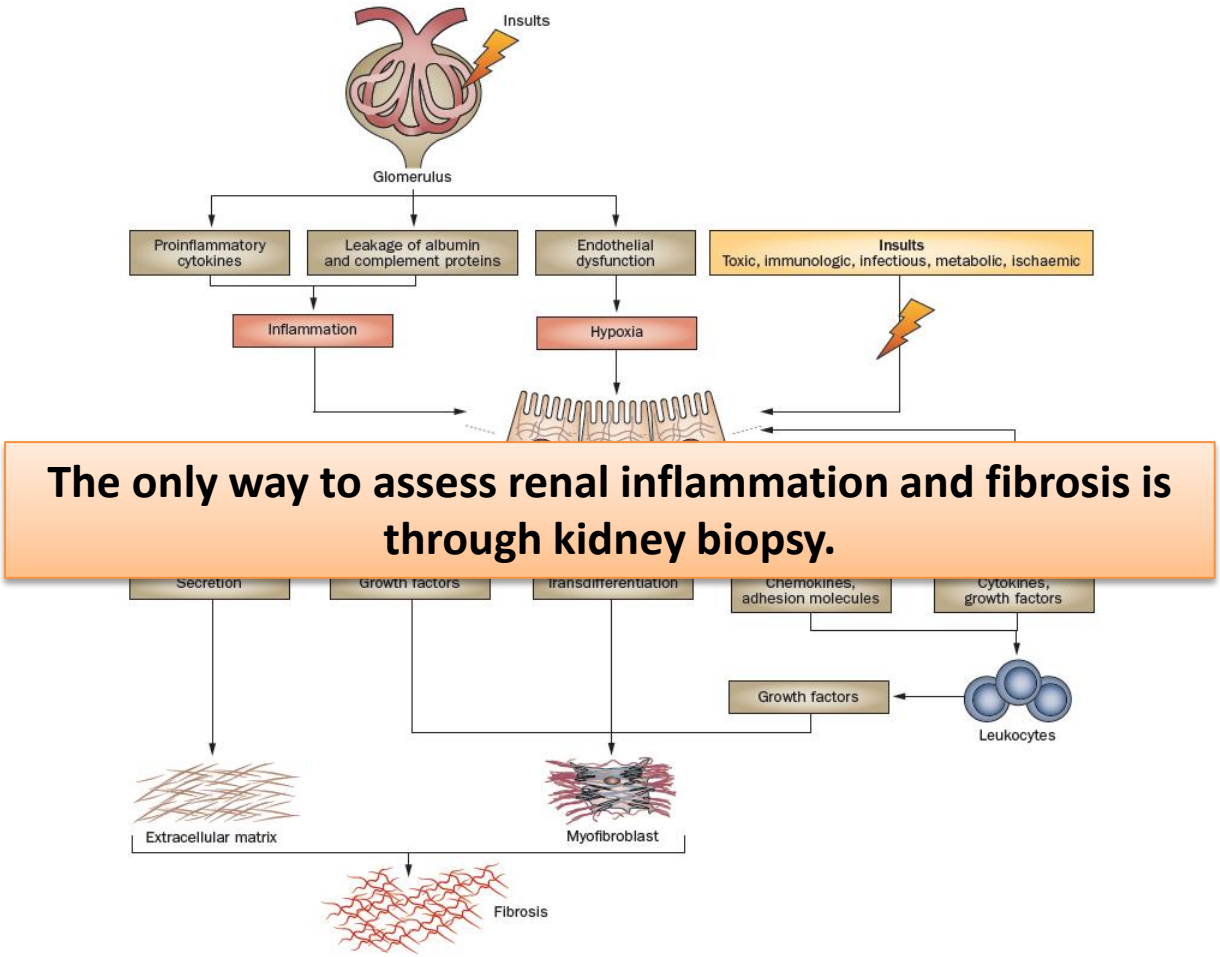
Zhang L, et al. Lancet. 2012 Mar 3;379(9818):815-22.

# CKD is a progressive disease from inflammation to fibrosis




**The only way to assess renal inflammation and fibrosis is through kidney biopsy.**

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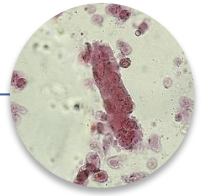
Meng et al. Nat Rev Nephrol. 2014 Sep;10(9):493-503

# Noninvasive diagnostic biomarkers are needed to complement kidney biopsy

- Kidney biopsy is an invasive procedure and cannot be performed repeatedly
- It is associated with discomfort and a risk of major complications
- Cannot be employed in the early detection and screening test

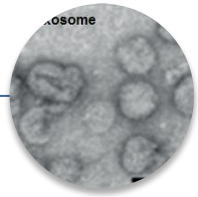
# Urine contains rich biological information:
# An ideal source for non-invasive biomarker

Differential centrifugation



**Sediment**

**Supernatant**



**Extracelluar vesicle**

**Cell (debris), extracelluar vesicle**
**Protein**
**RNA (mRNA, non-coding RNA)**

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# Urine sediment examination is a well-established test in the evaluation of patients with kidney disease

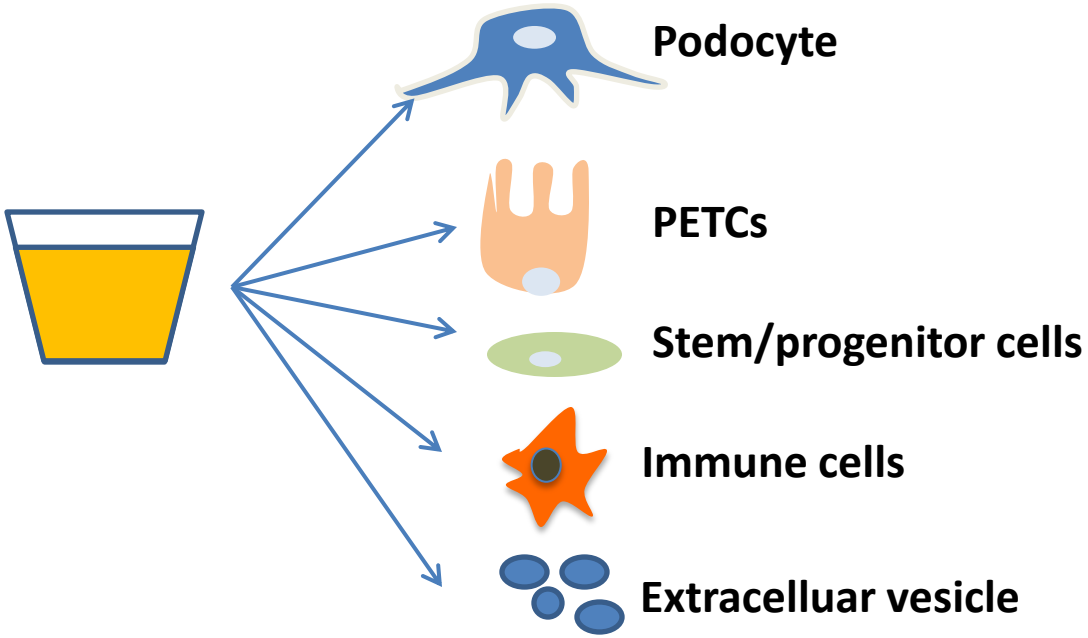

**More information to be explored in Urine sediment!**

The Physician
Gerrit DOU
Leiden(1631-1675)

东南大学肾脏病研究所
Institute of Nephrology Southeast University

Perazella MA. Am J Kidney Dis. 2015 Nov;66(5):748-55

# Human Urine as a Noninvasive Source of Kidney Cells

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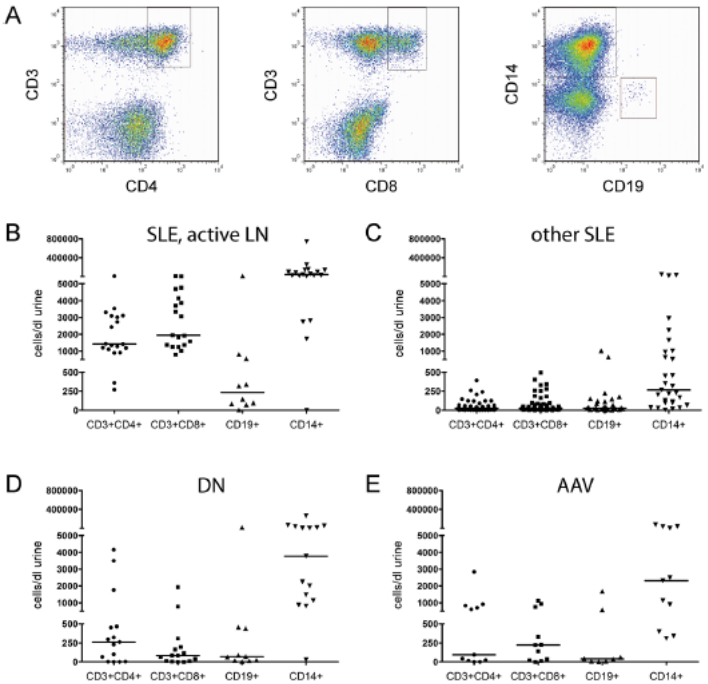
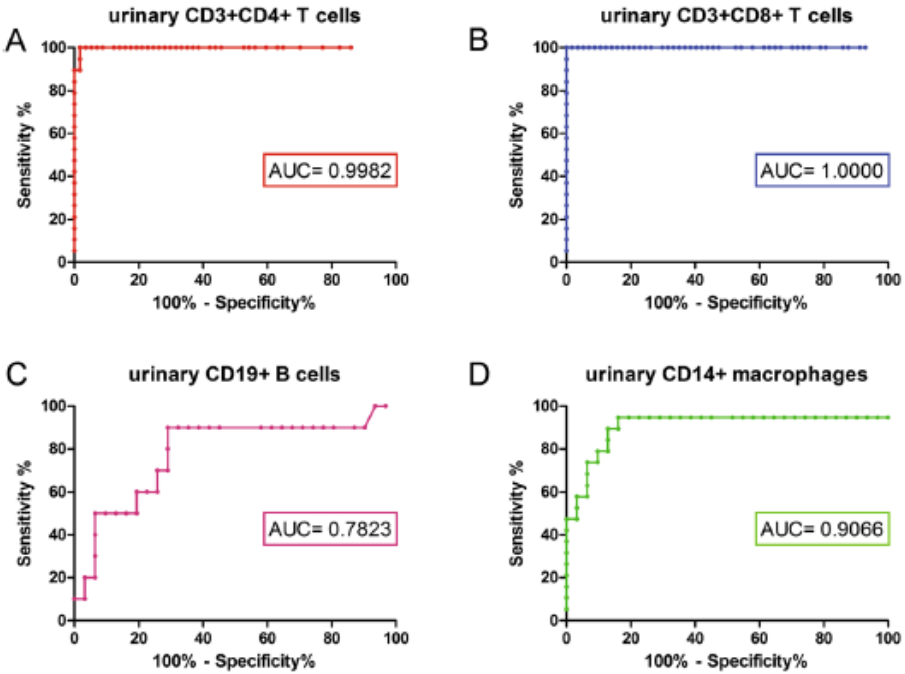
Arcolino F et al. Stem Cells Int. 2015;2015:362562

# Urinary immune cell biomarker for Lupus nephritis

**Urinary cells in SLE patients and in patients with different nephropathies.**




**Diagnosing proliferative lupus nephritis (LN) among SLE patients**

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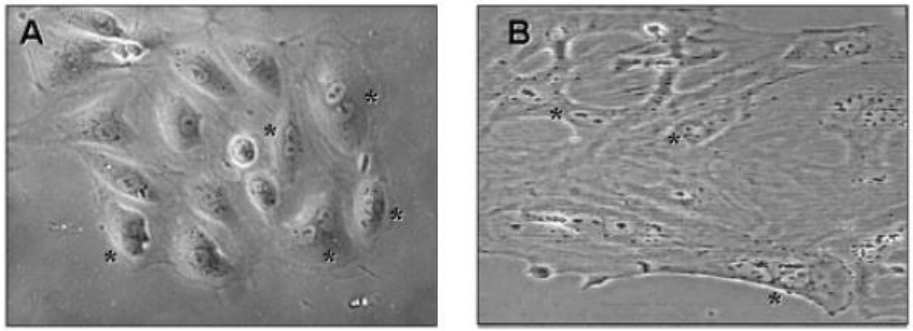
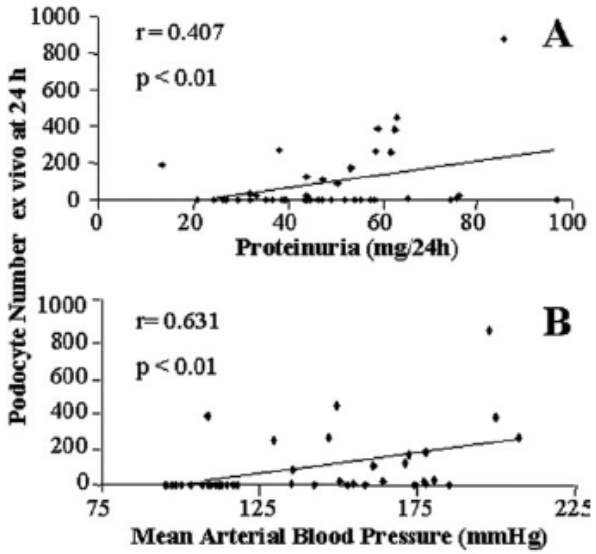
Institute of Nephrology Southeast University

Kopetschke et al. Arthritis Research & Therapy (2015) 17:94

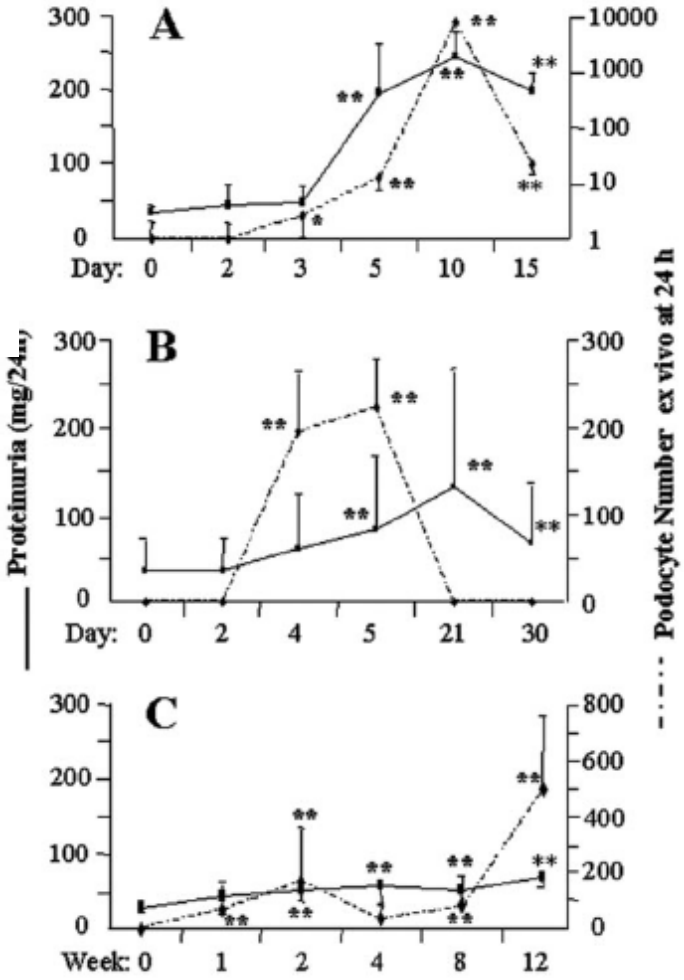
# Urinary Podocyte Loss Is a Specific Marker of Ongoing Glomerular Damage

**Viable Podocytes from the Urine**




**Time course of podocyturia and proteinuria in the different disease models**




PAN

Anti-Thy 1.1 nephritis

5/6 nephrectomy

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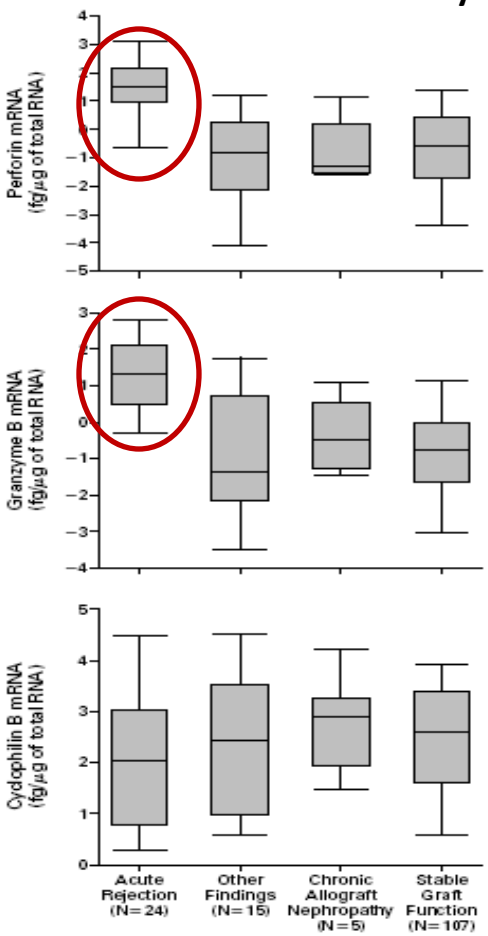
Yu D et al. J Am Soc Nephrol 16: 1733–1741, 2005

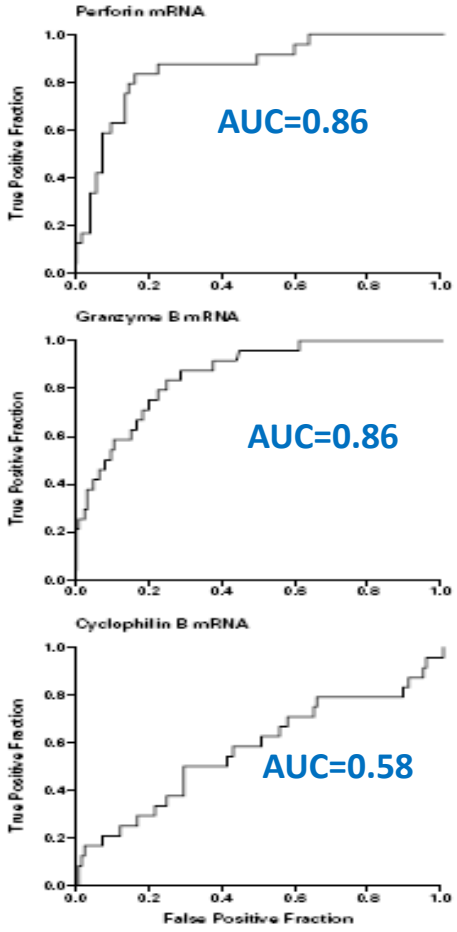
# Urinary sediment RNA—Novel CKD Biomarkers

- Urinary sediments contains shedding cells originated from the kidney and urinary tract.
- Real-time PCR can make rapid detection with low cost.
- Genetic markers are more stable compared with protein markers.

# Urinary Perforin, Granzyme B mRNA in Diagnosing Acute Rejection of Renal Allografts

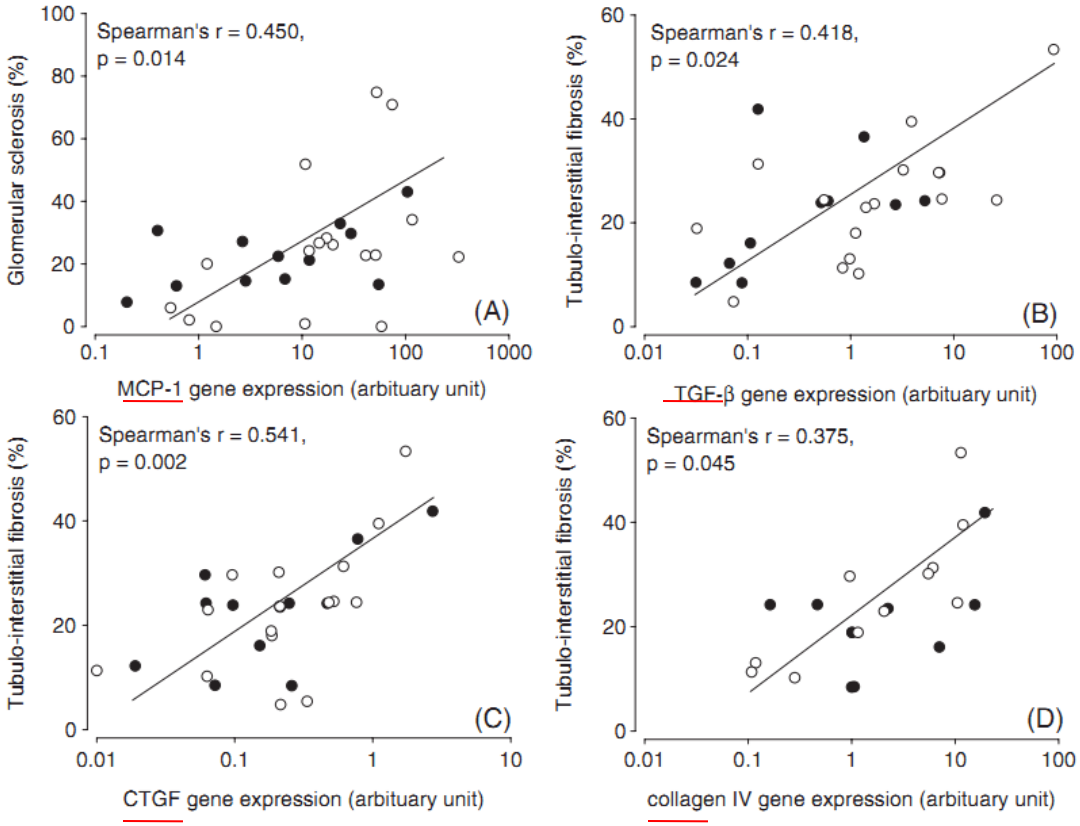
**Levels of mRNA in Urinary Cells.**




Li B et al. **N Engl J Med. 2001 Mar 29;344(13):947-54.**

# Relation Between Cytokine mRNA Expression in Urinary Sediment and Histological Damage




Szeto CC, et al. Nephrol Dial Transplant. 2005 Dec;20(12):2793-6.

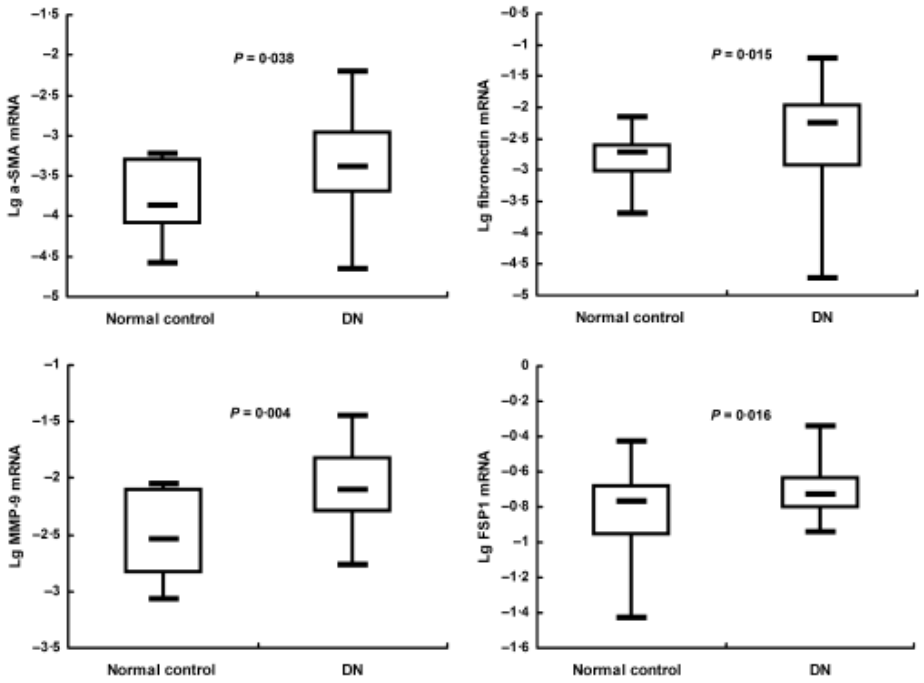
# Urinary HGF mRNA may be a useful tool for predicting CKD progression

| | Hazard Ratio | 95% Confidence Interval | *P* |
|---|---|---|---|
| Sex* | 2.203 | 1.137-4.265 | 0.019 |
| Age (y) | 1.044 | 1.021-1.067 | <0.001 |
| Proteinuria (g/d) | 1.076 | 1.032-1.121 | <0.001 |
| Estimated GFR (mL/min/1.73 $m^2$) | 0.951 | 0.934-0.969 | <0.001 |
| Renal diagnosis | | | <0.001† |
| Diabetic nephropathy | 5.222 | 1.748-15.604 | 0.003‡ |
| Tubulointerstitial scarring (%) | 1.042 | 1.029-1.055 | <0.001 |
| Glomerulosclerosis (%) | 1.021 | 1.011-1.032 | <0.001 |
| Urinary mRNA expression§ | | | |
| CTGF | 1.453 | 0.946-2.232 | 0.088 |
| HGF | 1.035 | 1.003-1.067 | 0.031 |
| Vascular endothelial growth factor | 0.998 | 0.989-1.006 | 0.607 |
| TGF-β1 | 0.977 | 0.920-1.036 | 0.436 |
| MCP-1 | 2.536 | 0.633-10.167 | 0.189 |
| Collagen I | 1.367 | 0.730-2.561 | 0.329 |
| Collagen III | 1.003 | 0.995-1.012 | 0.419 |
| Collagen IV | 1.027 | 0.943-1.118 | 0.543 |
| Fibronectin | 1.022 | 0.933-1.119 | 0.643 |
| Caspase 3 | 0.952 | 0.736-1.231 | 0.705 |
| α-Smooth muscle actin | 0.958 | 0.870-1.056 | 0.392 |

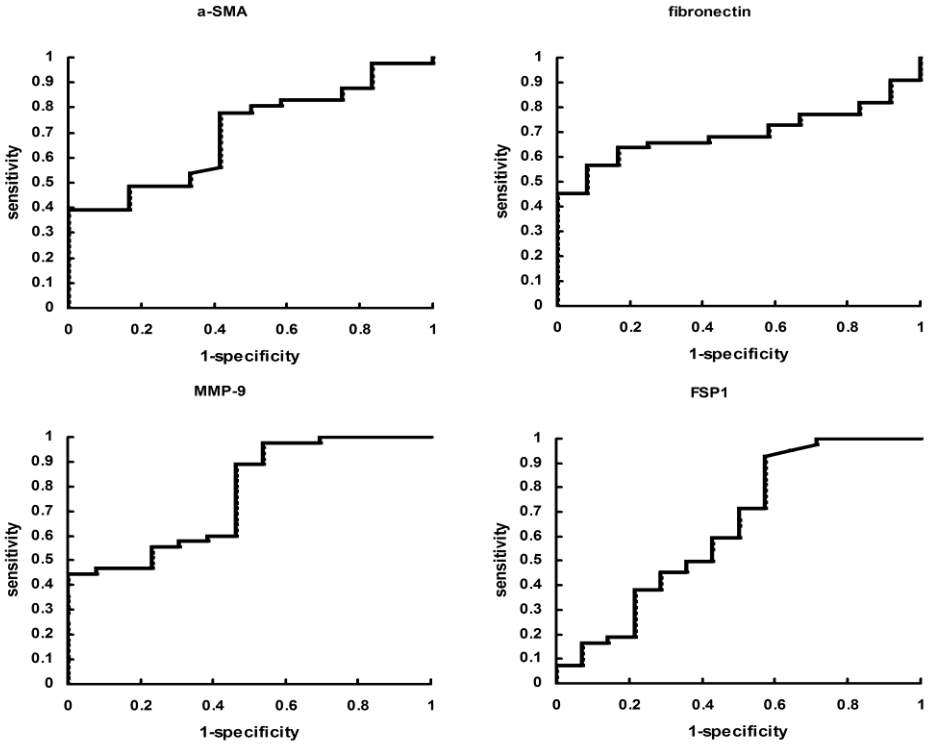
Szeto CC et al, Am J Kidney Dis. 2006 Apr;47(4):578-86.

# Urinary sediment α-SMA, fibronectin, MMP-9 and FSP1 mRNA increased significantly in DN patients

## Urine mRNA level




## ROC of urine mRNA for identifying DN

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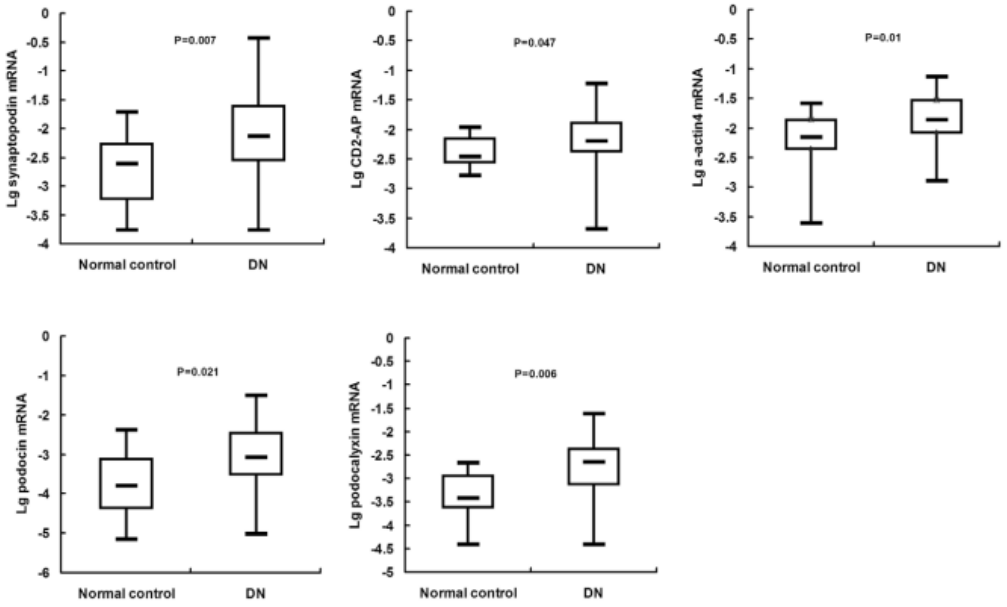
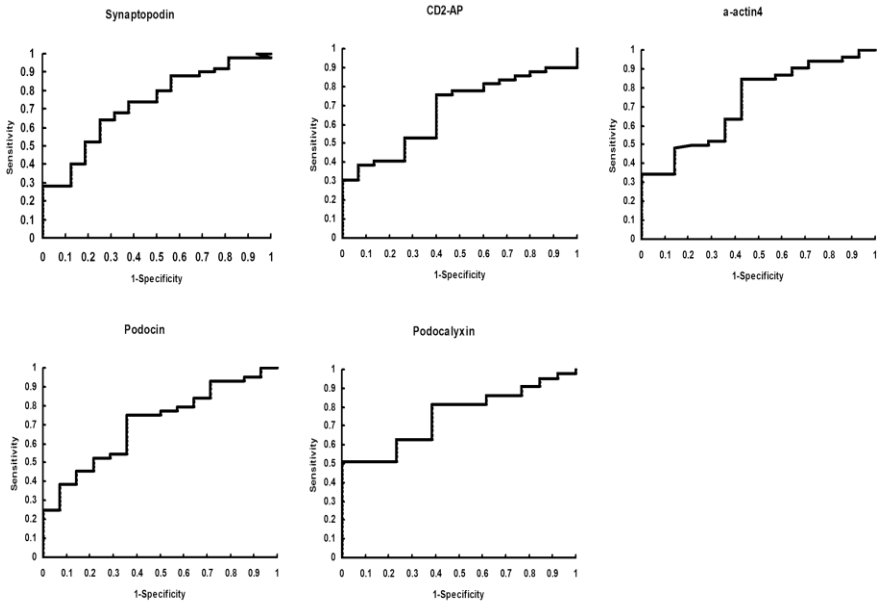
Zheng M, Clin Endocrinol (Oxf). 2012 May;76(5):657-64.

# Urinary sediment Podocyte mRNA expression in diagnosing DN

## Urine mRNA level




## ROC of urine mRNA for identifying DN

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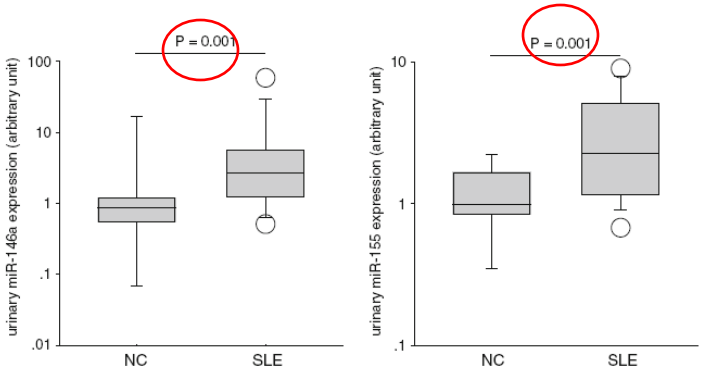
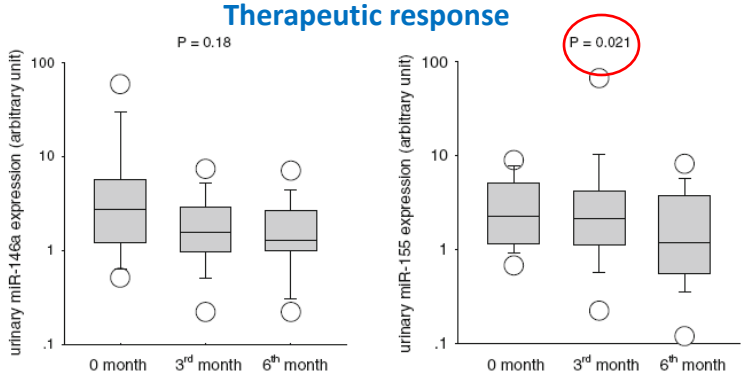
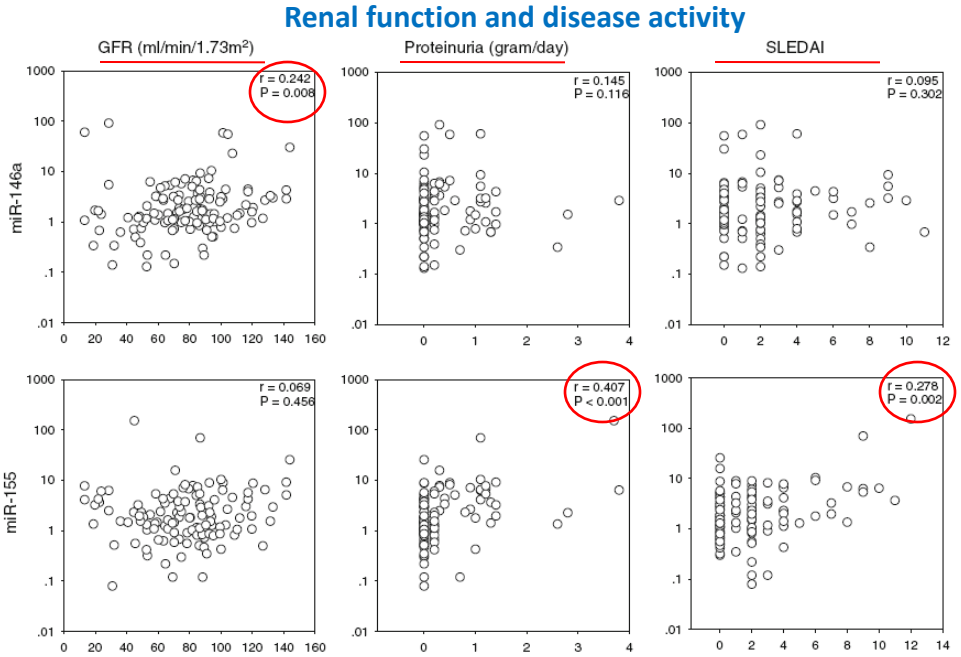
Zheng M et al PLoS One. 2011; 6(5): e20431.

# miR-146a and miR-155 from urine sediment could be used as potential markers of lupus nephritis

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Wang G et al. Clin Rheumatol (2012) 31:435–440

# PCR array for Urinary sediment mRNA Detection

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *COL5A2* | *AGT* | *NFKB1* | *MAPK14* | *transferrin* | *RBP4* | *cytokeratin18* | *FABP-1* | *Fn* | *PAI-1* | *α-SMA* | *H* |
| *wt-1* | *IFN-γ* | *MCP-1* | *TNF-α* | *nephrin* | *TGF β* | *MAPK8* | *JAG2* | *JAG1* | *NOTCH4* | *NOTCH3* | *G* |
| *NOTCH2* | *NOTCH1* | *NGAL* | *KIM-1* | *ACE2* | *ACE* | *REN* | *RAGE* | *PPARG* | *UMOD* | *B2M* | *F* |
| *AMBP* | *CP* | *RANTES* | *LAMA5* | *LAMC2* | *BMP7* | *SMAD7* | *SMAD4* | *SMAD3* | *SMAD2* | *SMAD1* | *E* |
| *ILK* | *ETS1* | *LEF1* | *SNAI2* | *SNAI1* | *TWIST1* | *CDH2* | *DES* | *FSP1* | *VIM* | *CDH1* | *D* |
| *COL4A1* | *COL3A1* | *TIMP1* | *TIMP2* | *MMP9* | *MMP2* | *PODXL2* | *PODXL* | *ACTN4* | *SYNPO* | *FAT1* | *C* |
| *CDH3* | *ZO-1* | *ITGB1* | *ITGA3* | *CD2AP* | *NPHS2* | *CRP* | *VCAM1* | *ICAM1* | *IL18* | *IL8* | *B* |
| *IL6* | *IL1B* | *IL1A* | *EGF* | *FGF23* | *PDGF-B* | *VEGF-B* | *VEGF-C* | *CTGF* | *IGF1* | *HGF* | *A* |

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Zheng M Lv LL et al. PLoS One. 2012; 7(5): e34824.

证书号第1611525号

# 发明专利证书

发明名称：基于实时荧光PCR的尿沉渣细胞肾脏纤维化检测芯片

发 明 人：刘必成;曹玉溪;雷向东

专 利 号：ZL 2013 1 0535181.3

专利申请日：2013年11月01日

专 利 权 人：东南大学

授权公告日：2015年03月25日

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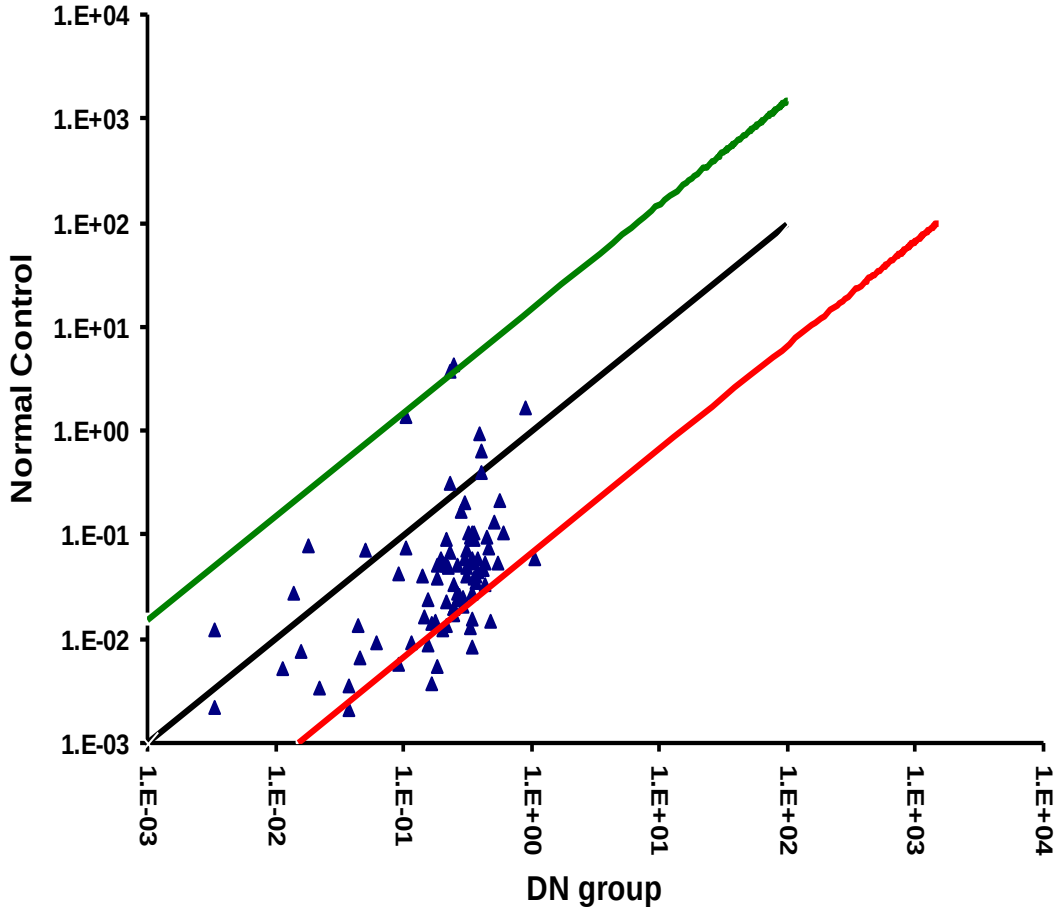
2015年03月25日

# Fold Change Expressions of multiple mRNAs Between DN Patients and Controls



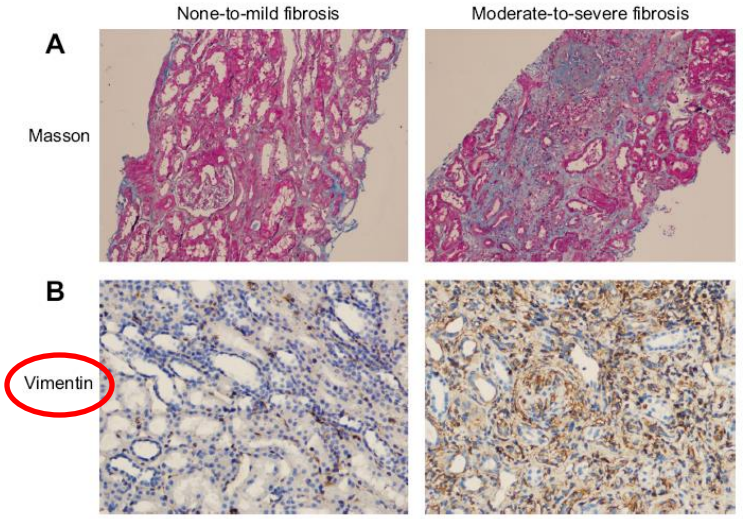

Fold change expressions of mRNAs between DN patients and controls. Red and green lines represent fold change above or below 15 respectively. Those mRNAs with 15 fold increased levels when DN patients were compared with normal controls included 8 ones as followings: NOTCH3, ACTN4, CDH2, ACE, FAT1, COL4A1, SYNPO, TWIST1. And TIMP-1 was found with 15 fold decreased levels in DN group compared with normal controls.

Zheng M, Lv LL et al. PLoS One. 2012; 7(5): e34824.

# Vimentin mRNA of urine sediment was identified from the screening study of CKD

Table 3. *Functional categories and fold changes of 21 differentially regulated mRNAs*

| Gene Name | Functional Category | Fold Change | *P* Value |
|---|---|---|---|
| VIM | Mesenchymal cell marker | 9.99 | <0.001 |
| HGF | Growth factor | 8.50 | 0.014 |
| FGF2 | Growth factor | 8.39 | 0.036 |
| MMP7 | Matrix metalloproteinase family | 7.13 | 0.007 |
| PPARG | Type II nuclear receptor | 5.26 | 0.005 |
| SMAD7 | TGFβ family | 5.02 | 0.016 |
| MCP-1(CCL2) | Chemotactic cytokine | 4.71 | <0.001 |
| SMAD2 | TGFβ family | 4.66 | 0.014 |
| RANTES(CCL5) | Chemotactic cytokine | 4.57 | 0.046 |
| PODXL | Podocyte marker | 4.43 | 0.013 |
| TGFβ1-R | TGFβ family | 4.27 | 0.038 |
| FSP-1 | S100 family | 3.39 | <0.001 |
| CCL4 | Chemotactic cytokine | 3.37 | 0.008 |
| P53 | Tumor suppressor gene | 3.25 | 0.012 |
| TLR2 | Toll-like receptor | 3.08 | 0.036 |
| PAI-1 | Serine protease inhibitor | 3.06 | 0.021 |
| FN1 | Extracellular matrix component | 2.92 | 0.040 |
| TIMP1 | Tissue inhibitor of metalloproteinases | 2.74 | <0.001 |
| TNF | TNF family | 2.63 | 0.006 |
| MMP9 | Matrix metalloproteinase family | 2.55 | 0.024 |
| TGFβ1 | TGFβ family | 2.23 | 0.006 |

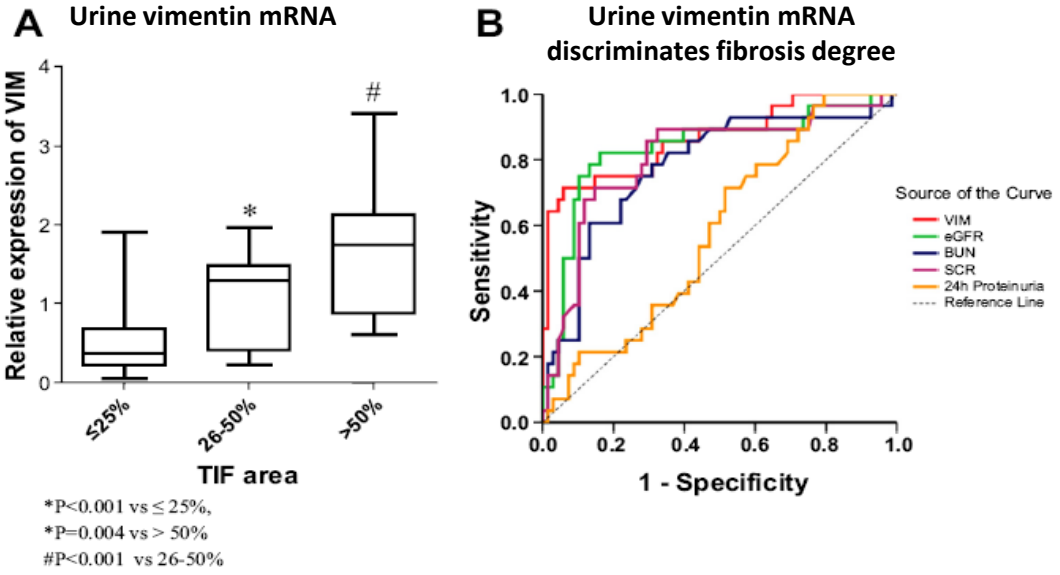



Cao Y, Lv LL et al. Am J Physiol Renal Physiol 309: F514–F522, 2015.

# vimentin mRNA was validated in an independent set of study

Table 2. *Characteristics of the study subjects*

| | Control ($n$ = 31) | | CKD ($n$ = 135) | | $P$ Value | |
|---|---|---|---|---|---|---|
| | Training ($n$ = 11) | Validation ($n$ = 20) | Training ($n$ = 39) | Validation ($n$ = 96) | Training | Validation |
| Age, yr | 36 ± 10 | 34 ± 10 | 42 ± 15[a] | 42 ± 15[a] | 0.253[c] | 0.056[c] |
| Gender (male/female) | 4/7 | 8/12 | 19/20 | 40/56 | 0.468[d] | 0.170[d] |
| SBP, mmHg | 119 ± 10 | 126 ± 8 | 136 ± 15[a] | 133 ± 20[a] | 0.061[c] | 0.084[c] |
| DBP, mmHg | 80 ± 8 | 82 ± 6 | 86 ± 19[a] | 83 ± 14[a] | 0.279[c] | 0.271[c] |
| Scr, mmol/l | 63 ± 8 | 61 ± 9 | 116 ± 90[a] | 93 ± 57[a] | 0.001[c] | <0.001[c] |
| eGFR,[e] $ml^{-1} \cdot min^{-1} \cdot 1.73\ m^{-2}$ | 116 ± 10 | 117 ± 11 | 82 ± 36[a] | 90 ± 35[a] | <0.001[c] | <0.001[c] |
| BUN, mmol/l | 4.71 ± 1.07 | 3.80 ± 0.97 | 7.27 ± 3.91[a] | 6.80 ± 4.62[a] | 0.001[c] | <0.001[c] |
| Proteinuria, g/day | / | / | 2.77 (0.11,18.32)[b] | 2.89 (0.29,12.80)[b] | / | / |
| Score of glomerular sclerosis | / | / | 0.80 (0.00,4.00)[b] | 0.52 (0.00,4.00)[b] | / | / |
| Score of TIF, % | / | / | 18 (0.5,80)[b] | 10 (0.5,90)[b] | / | / |




*P<0.001 vs ≤ 25%,
*P=0.004 vs > 50%
#P<0.001 vs 26-50%

Cao Y, Lv LL et al. Am J Physiol Renal Physiol 309: F514–F522, 2015.

# Urine supernatant inflammatory biomarkers for LN

- LN are often treated without the benefit of kidney pathology after initial treatment
- A continuous read-out of kidney pathology would be helpful in following therapy for LN
- Urine inflammatory cytokines might be biomarker of histological activity.

# Urine MCP-1 and Lupus Nephritis Disease Activity

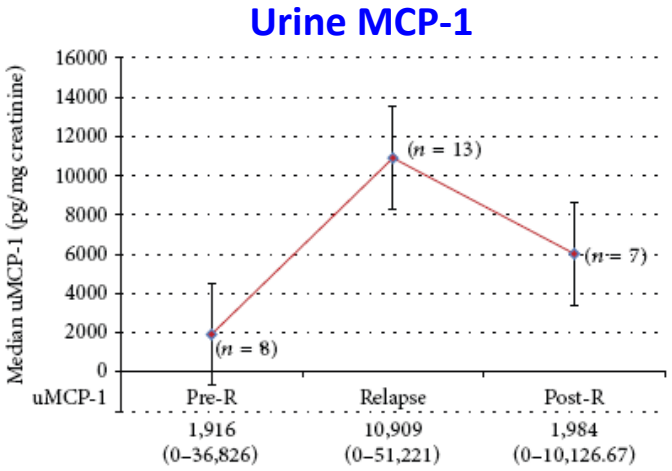



Table 4: Association of uMCP-1 with parameters of LN activity on follow-up.

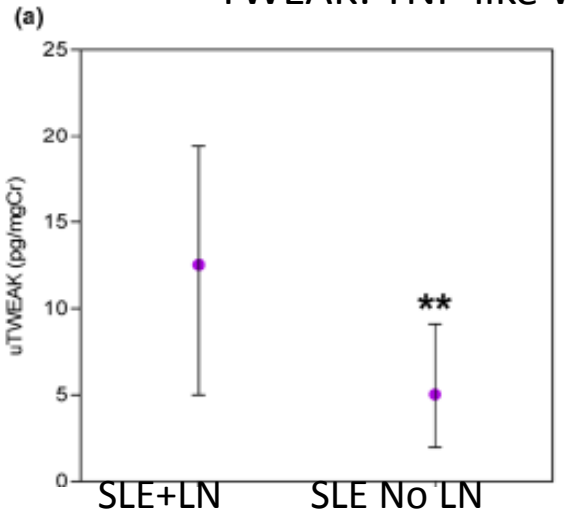
| Spearman's rho variable (active : inactive LN) | Baseline (47 : 53) | | 2 months (29 : 71) | | 4 months (22 : 78) | |
|---|---|---|---|---|---|---|
| | $r_{sp}$ | $p$ value | $r_{sp}$ | $p$ value | $r_{sp}$ | $p$ value |
| Serum albumin | −0.35 | **0.001** | −0.32 | **0.001** | −0.22 | **0.03** |
| Serum creatinine | 0.09 | 0.38 | 0.14 | 0.15 | 0.24 | **0.01** |
| eGFR | −0.10 | 0.30 | −0.15 | 0.12 | −0.24 | **0.01** |
| Anti-dsDNA Ab titers (IU) | −0.04 | 0.64 | −0.19 | 0.06 | 0.01 | 0.89 |
| C3 (mg/dL) | −0.09 | 0.34 | −0.29 | **0.003** | −0.04 | 0.70 |
| C4 (mg/dL) | 0.02 | 0.80 | −0.23 | **0.02** | −0.01 | 0.86 |
| Proteinuria (uPCI) | 0.39 | **0.001** | 0.48 | **<0.001** | 0.41 | **<0.001** |
| Leukocyturia | 0.26 | **0.008** | 0.21 | **0.03** | 0.19 | 0.06 |
| Haematuria | 0.13 | 0.18 | 0.09 | 0.38 | 0.11 | 0.24 |
| SLEDAI-2K global score | 0.27 | **0.006** | 0.42 | **<0.001** | 0.29 | **0.004** |
| SLEDAI-2K renal score | 0.39 | **0.001** | 0.43 | **<0.001** | 0.35 | **0.001** |
| SLEDAI-2K-extrarenal score | −0.08 | 0.42 | −0.18 | 0.74 | −0.11 | 0.27 |

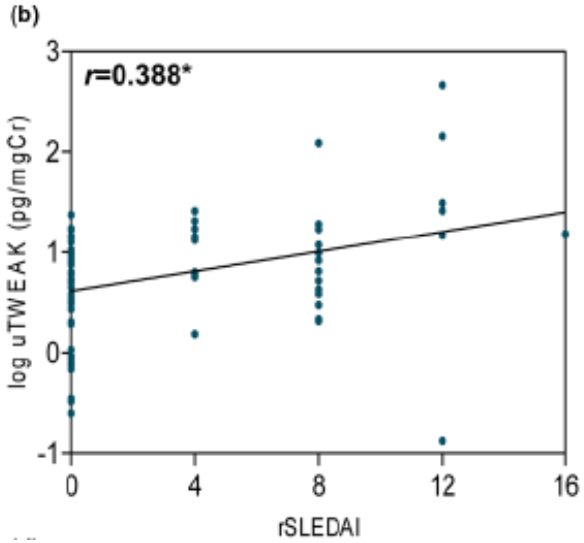


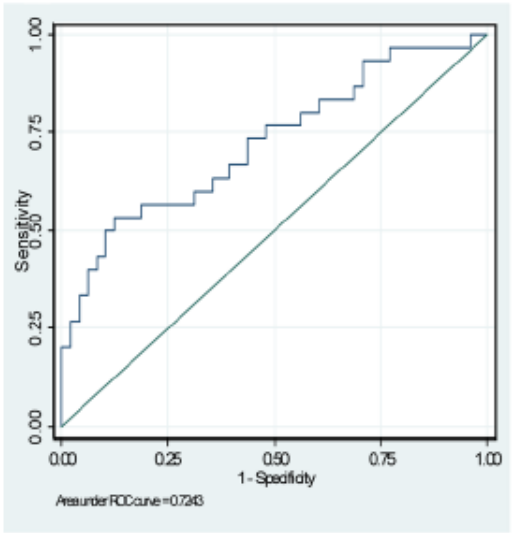
Alharazy S et al. Autoimmune Dis. 2015; 2015: 962046.

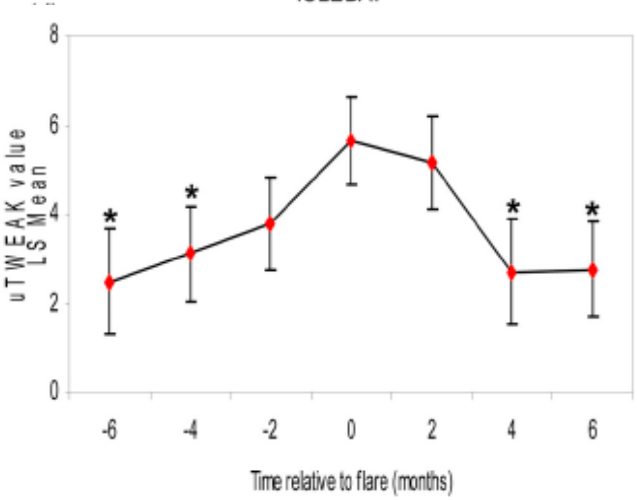
# Urinary TWEAK as a biomarker of lupus nephritis

TWEAK: TNF-like weak inducer of apoptosis



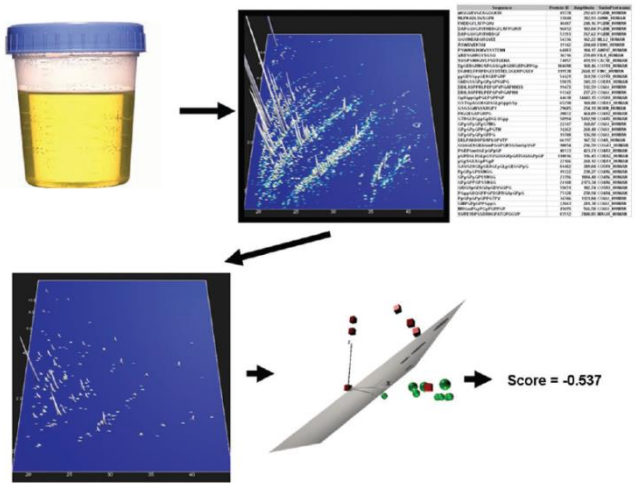




Schwartz N et al. Arthritis Res Ther. 2009;11(5):R143.

# High-throughput analysis of urine protein: Urine proteomics

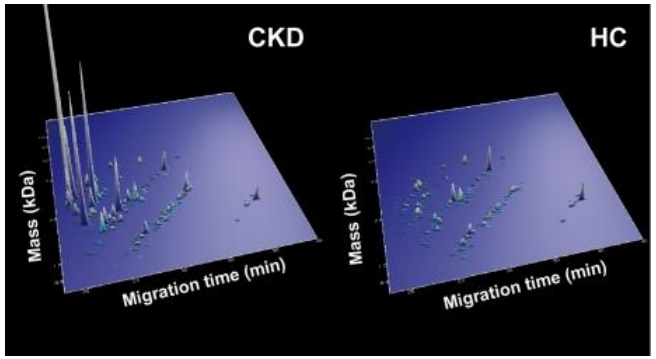



- Abundance of molecular information obtained from urinary proteome analysis.
- It is  at the transition towards clinical implementation.



Mischak. Nephrol Dial Transplant (2015) 30: 532–537

# Urinary peptides (CKD273) is well suited for the early detection of CKD and for prognosis of progression




J Am Soc Nephrol. 2015 Aug;26(8):1999-2010. doi: 10.1681/ASN.2014050423. Epub 2015 Jan 14.

**Diagnosis and Prediction of CKD Progression by Assessment of Urinary Peptides.**

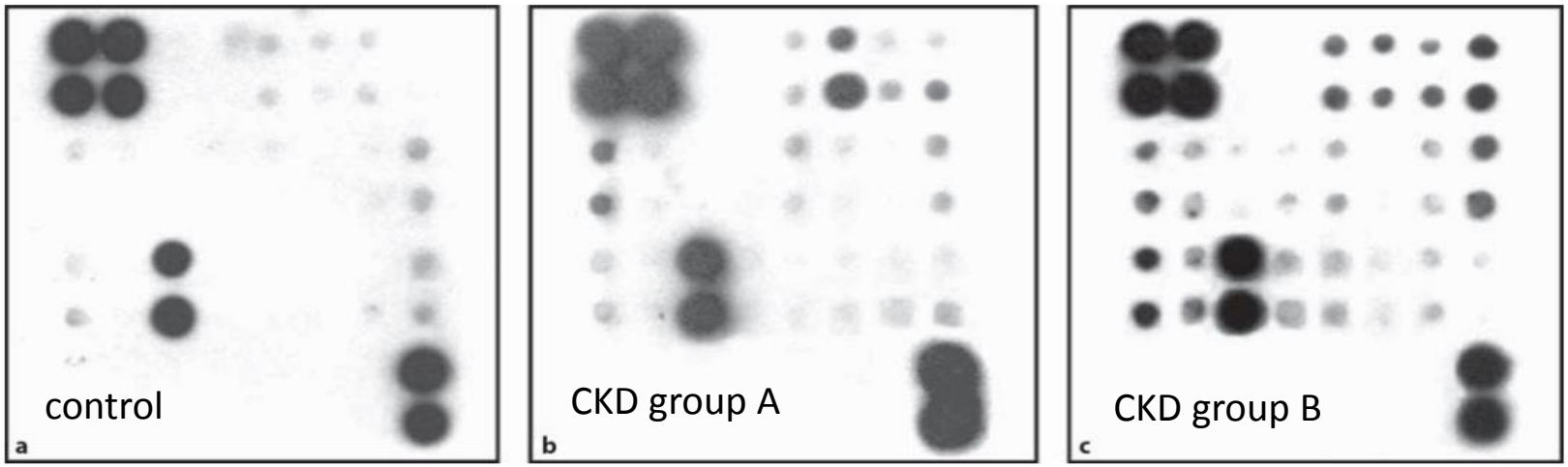
Nephrol Dial Transplant (2014) 29: 1563–1570
doi: 10.1093/ndt/gfu039
Advance Access publication 2 March 2014

Multicentre prospective validation of a urinary peptidome-based classifier for the diagnosis of type 2 diabetic nephropathy

**PRIORITY, a multicentric intervention study (3280 diabetic patients)**

Siwy J et al. Nephrol Dial Transplant. 2014 Aug;29(8):1563-70.
Schanstra et al. J Am Soc Nephrol 2015; doi: 10.1681/ASN.2014050423

# Urine cytokine increased in CKD patients



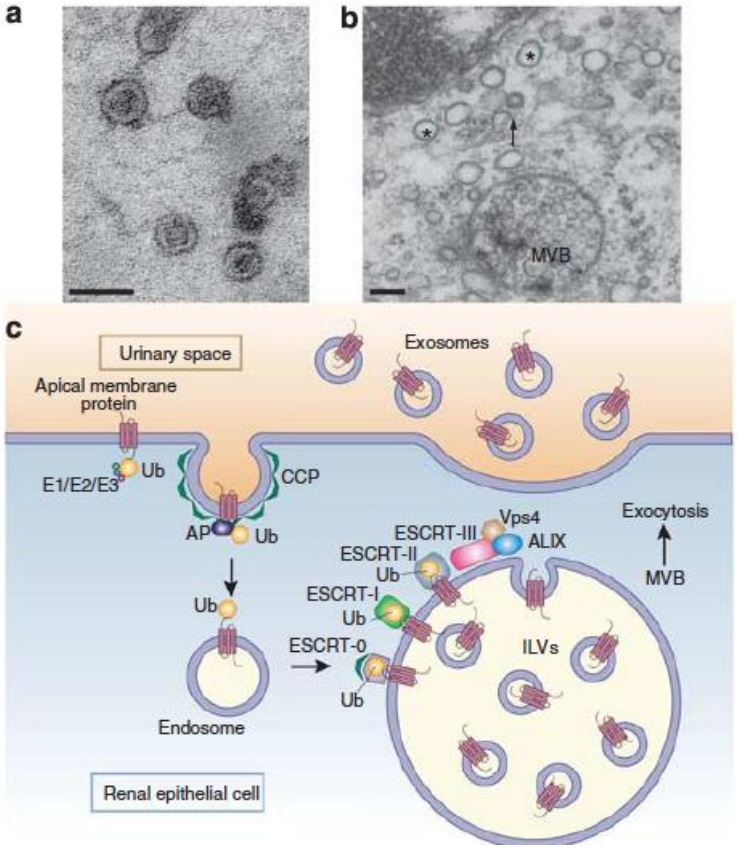

| | Control group | CKD group A | CKD group B |
|---|---|---|---|
| E-selectin | 1.0 | 4.4 ± 2.4[1] | 3.4 ± 2.1[1] |
| Fas | 1.0 | 4.3 ± 2.4[1] | 3.7 ± 3.7[1] |
| ICAM-1 | 1.0 | 4.3 ± 1.7[1] | 1.5 ± 0.8 |
| IL-2 | 1.0 | 4.9 ± 4.7[1] | 1.3 ± 0.8 |
| MCP-1 | 1.0 | 4.9 ± 4.3[1] | 8.7 ± 5.1[1] |
| MMP-2 | 1.0 | 2.3 ± 1.2[1] | 1.9 ± 1.5 |
| MMP-9 | 1.0 | 492.8 ± 129.5[1] | 198.7 ± 82.2[1] |
| PDGF-BB | 1.0 | 0.2 ± 0.3[1] | 0.6 ± 0.4 |
| RANTES | 1.0 | 3.7 ± 1.0[1] | 6.1 ± 8.1[1] |
| TGF-β | 1.0 | 2.3 ± 1.4[1] | 0.8 ± 0.1 |
| TIMP-1 | 1.0 | 3.2 ± 1.3[1] | 6.9 ± 0.6[1] |
| TNF-α | 1.0 | 13.5 ± 11.5[1] | 27.4 ± 45.5[1] |
| VCAM-1 | 1.0 | 0.5 ± 0.7[1] | 1.0 ± 1.2 |
| VE-cadherin | 1.0 | 1.4 ± 0.7 | 7.3 ± 12.4[1] |
| VEGF | 1.0 | 3.6 ± 2.4[1] | 6.6 ± 5.6[1] |

The relative value of cytokine in each group was converted into the n-fold change, which was expressed as means ± SD. For explanation of abbreviations, see table 3.

**Liu BC et al. Am J Nephrol. 2006;26(5):483-90.**

# Urinary Extracellular Vesicles (EVs)-fluid biopsy of kidney

- Urine EVs are small particles originating from cells of different nephron segments or of the urinary tract
- Released with cytoplasmic proteins, lipids, nucleic acids
- Urinary EVs represent a unique source of information for diagnostic purposes

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Musante et al. Front Endocrinol (Lausanne) 2014; 5: 149.
van Balkom et al. Kidney International (2011) 80, 1138–1145

# Subsets of Extracellular Vesicles

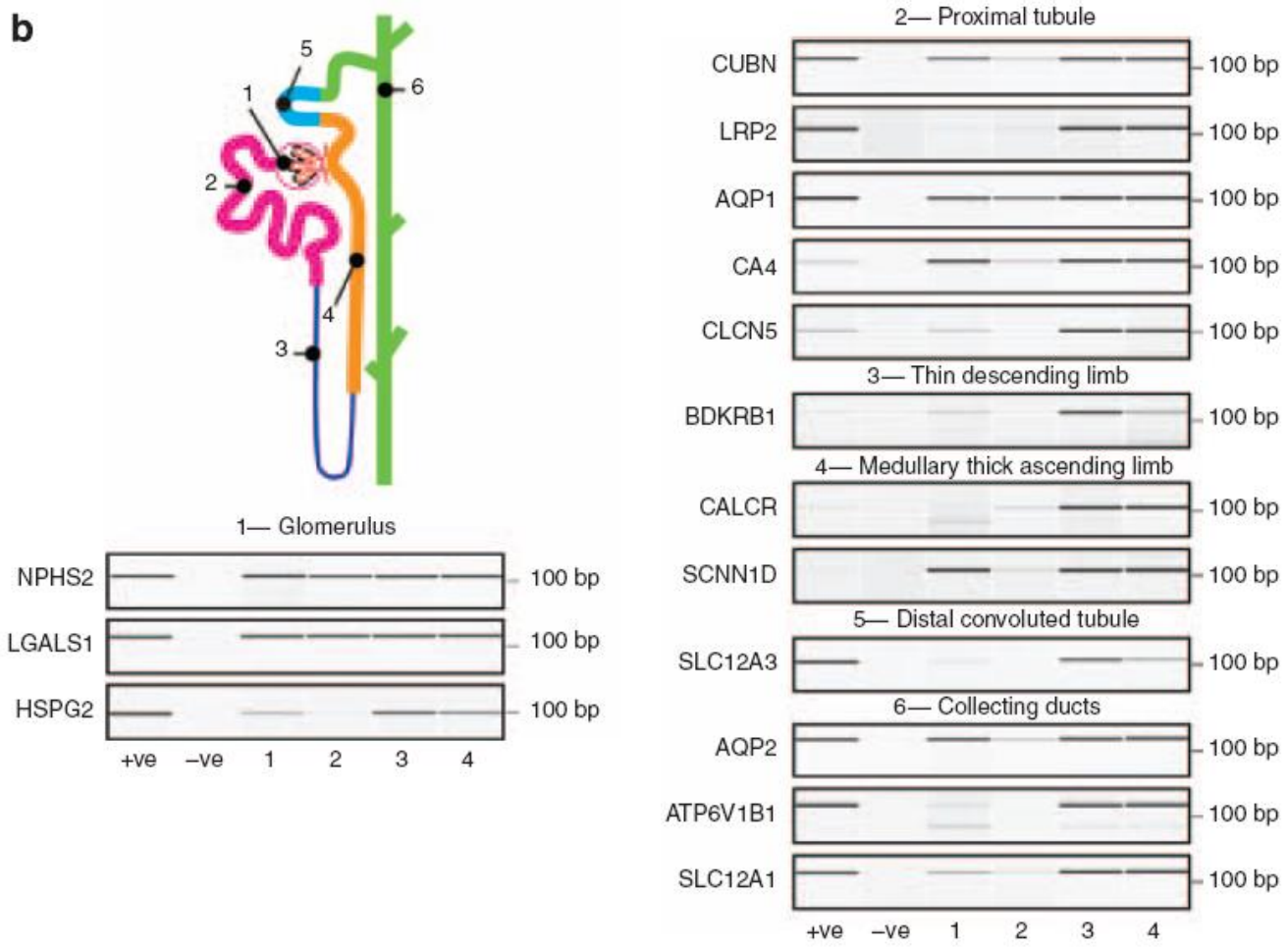
**Table 1** Characteristics of exosomes, microvesicles and apoptotic bodies

| | Exosomes | Microvesicles | Apoptotic bodies |
|---|---|---|---|
| Size | 30–100 nm | 100–1000 nm | Up to 4000 nm |
| Formation and release | Formed intracellularly within multivesicular bodies | Shed from plasma membrane surface | Cellular breakdown<br>Release from cellular blebs during apoptosis |
| Isolation and detection | Ultracentrifugation, electron microscopy, western blotting, mass spectrometry, nanoparticle tracking analysis | Differential centrifugation, flow cytometry, electron microscopy, western blotting, mass spectrometry, nanoparticle tracking analysis | Flow cytometry using e.g. FITC-conjugated annexin V antibody, electron microscopy |
| Markers | Alix, TSG101 and the tetraspanin proteins CD81 and CD9 | Integrins, selectins, markers of parental cells | Genomic DNA and intact organelles, externalized phophatidylserine |
| References | 1–4 | 1–5 | 2–4 |



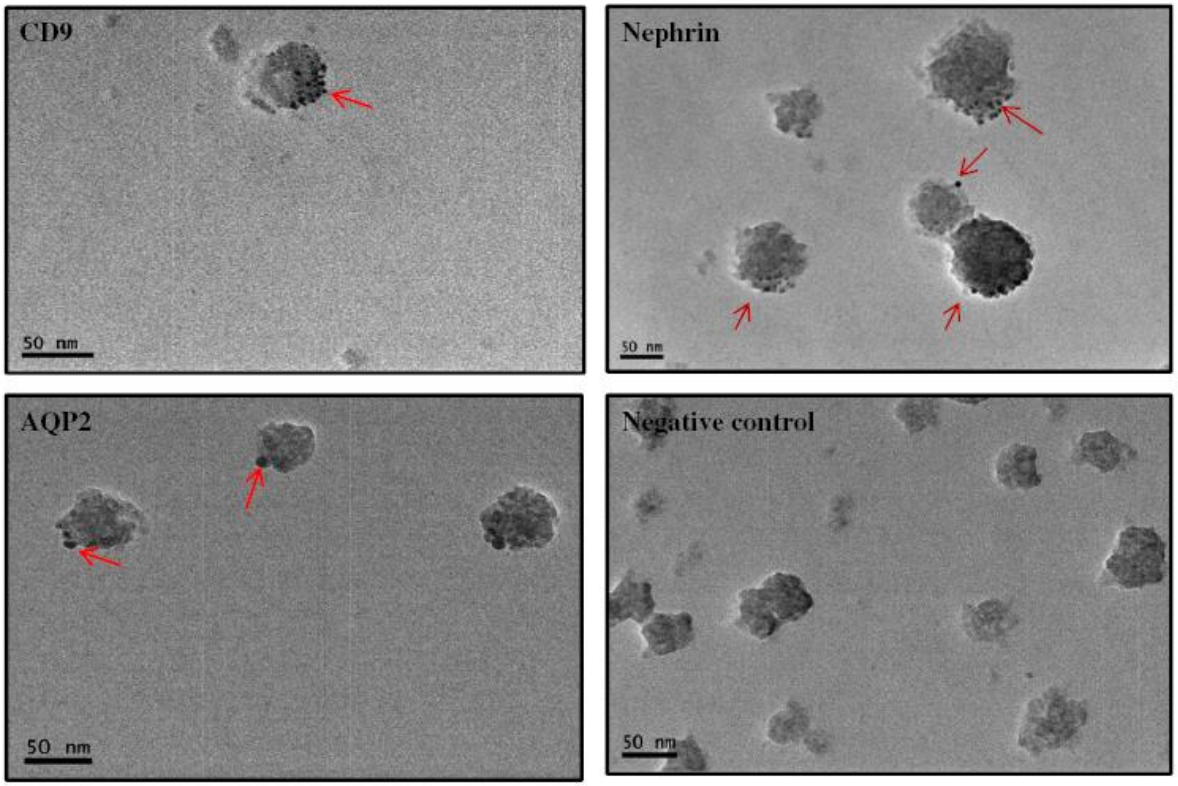
Fang et al. Nephrology 18 (2013) 1–10

# Urinary exosome contain mRNA transcripts encoding specific genes from various regions of kidney



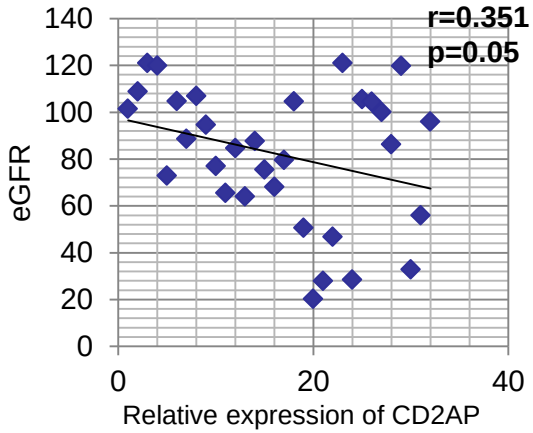

Miranda KC et al. Kidney Int. 2010 Jul;78(2):191-9.

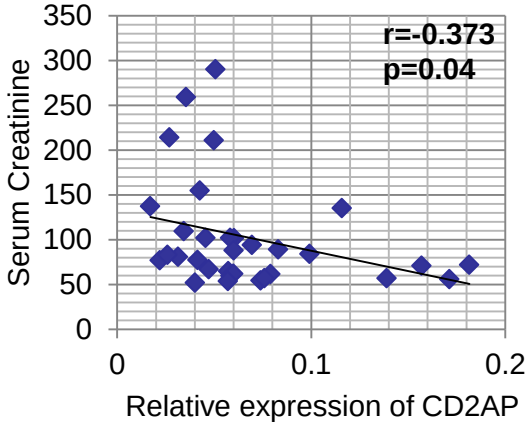
# Exosome from podocyte was isolated from urine

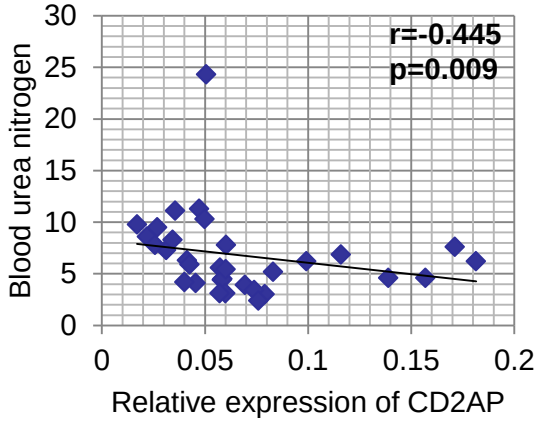


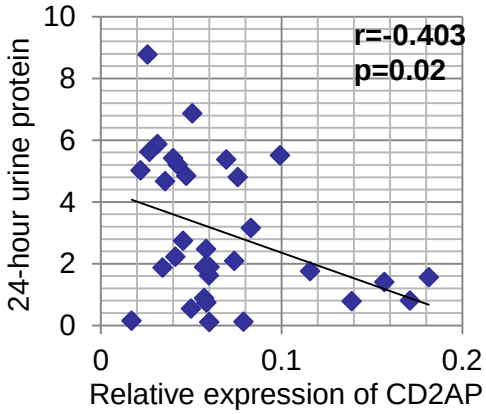

Lv LL, Liu BC et al. Clin Chim Acta. 2014 Jan 20;428:26-31

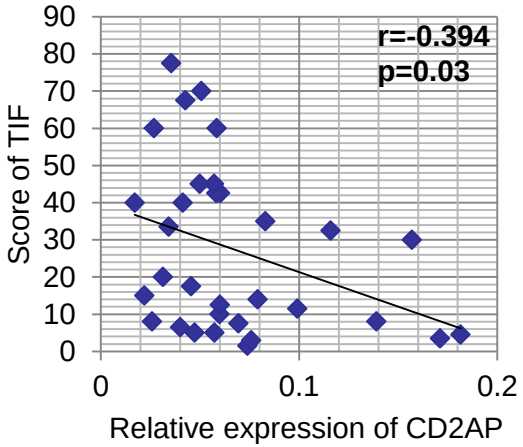
# Urinary exosome CD2AP mRNA correlated with kidney function and histological change

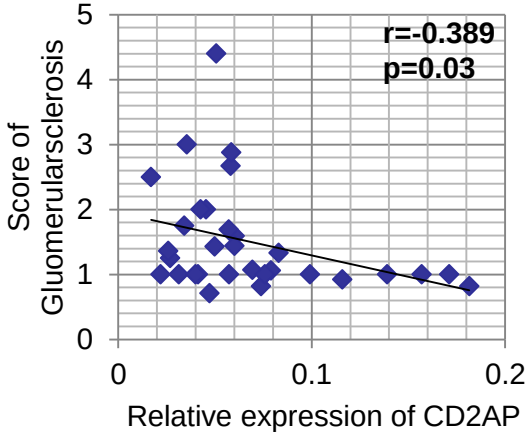
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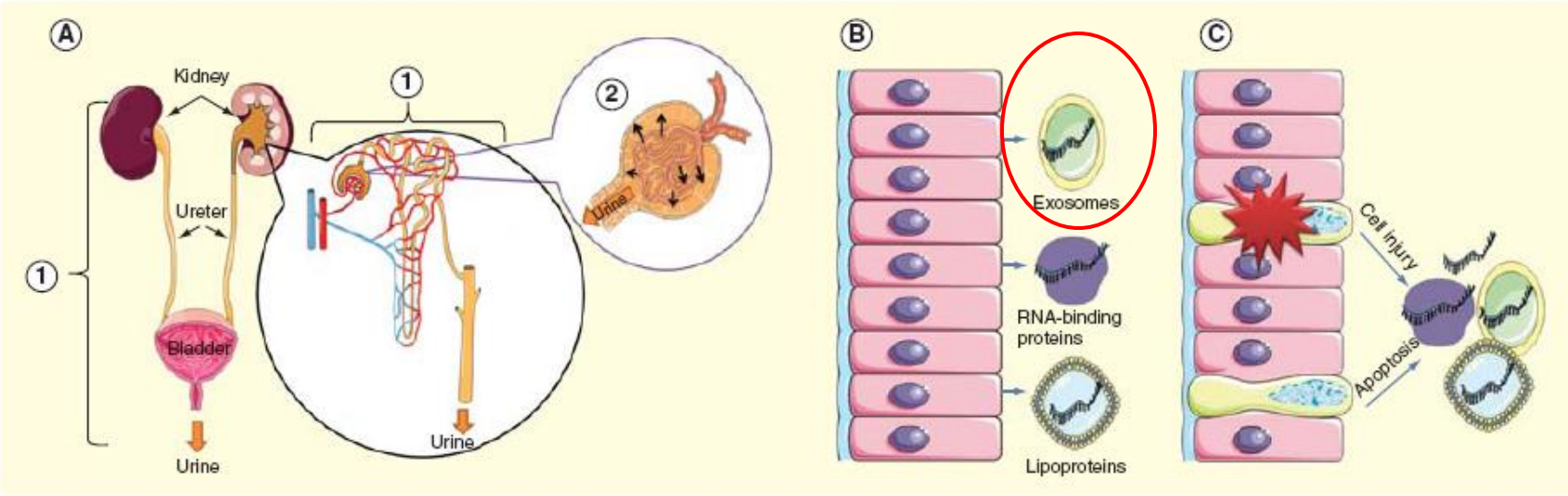
Lv LL, Liu BC et al. Clin Chim Acta. 2014 Jan 20;428:26-31

# miRNAs was mainly packed in urinary exosome

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Papadopoulos T et al. Expert Rev Mol Diagn. 2015 Mar;15(3):361-74.

**Table 1. Concentration of RNA isolated from different body fluids.[a]**

| Sample | Median total RNA concentration, μg/L (interquartile range) | Number of detectable miRNAs |
|---|---|---|
| Amniotic fluid | 570 (354) | 359 |
| Breast milk | 47 240 (73 180) | 429 |
| Bronchial lavage | 1128 (886) | 260 |
| Cerebrospinal fluid | 111 (66) | 212 |
| Colostrum | 585 (NA) | 386 |
| Peritoneal fluid | 775 (345) | 397 |
| Plasma | 308 (104) | 349 |
| Pleural fluid | 470 (190) | 210 |
| Saliva | 1945 (2495) | 458 |
| Seminal fluid | 17 770 (7673) | 436 |
| Tears | 564 (631) | 320 |
| Urine | 94 (129) | 204 |

[a] As estimated by the Agilent 2100 Bioanalyzer using the RNA 6000 Pico Total RNA chip, median concentration across all 5 samples except colostrum. The number of detected miRNAs in each body fluid is based on the number of miRNA species with a level of >80% of the global mean.

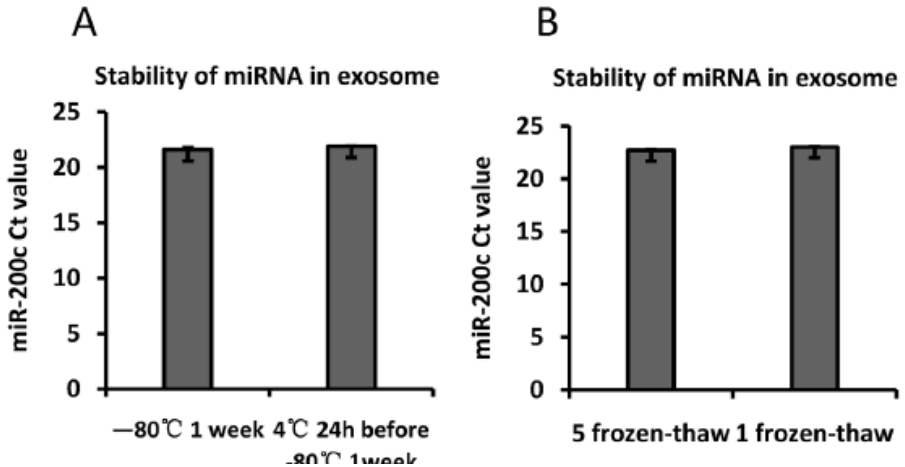
**A variety of miRNA can be detected in urine**

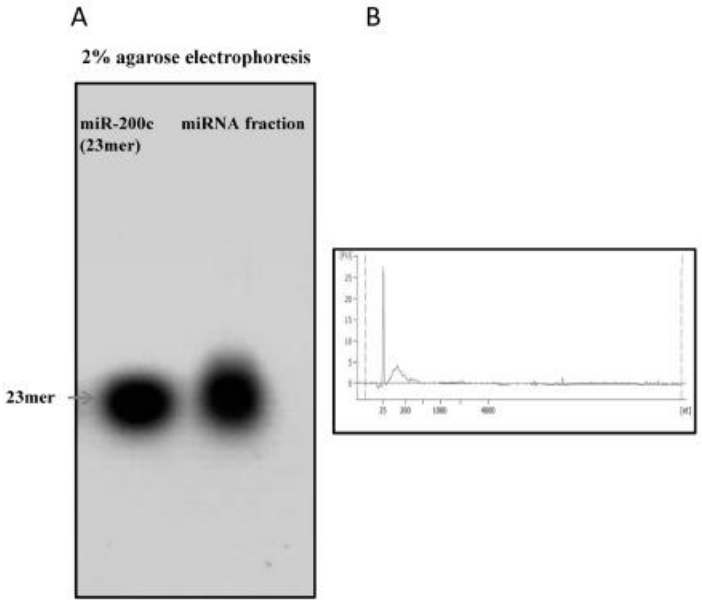
Weber et al. Clinical Chemistry 2010

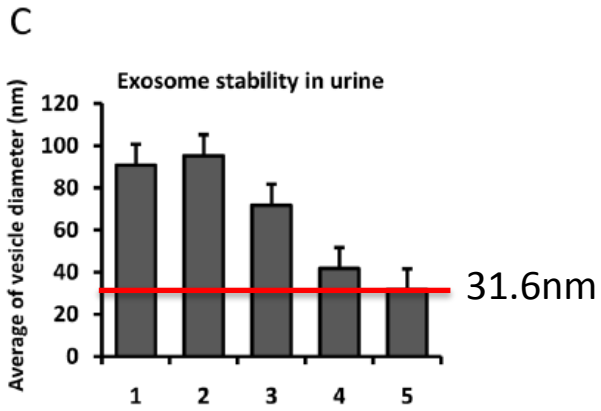
# Advantages of urine exosome miRNA as biomarker

- Exosome may protect RNA during urine passage
- More stable than RNA extracted from whole urine
- Derived from functioning cells
- miRNA was enriched in exosome

# Stability of exosome miRNA




1. 4℃, 24hours
2. 2, 37℃, 24hours
3. 3. -80℃, 24hours
4. 4. -80℃, 6months
5. 5. -80℃C, 12months

Lv LL, Liu BC et al. Int J Biol Sci. 2013; 9(10): 1021–1031.

# Detection of miRNA

**Real-time PCR assays**

**Simple and accurate**

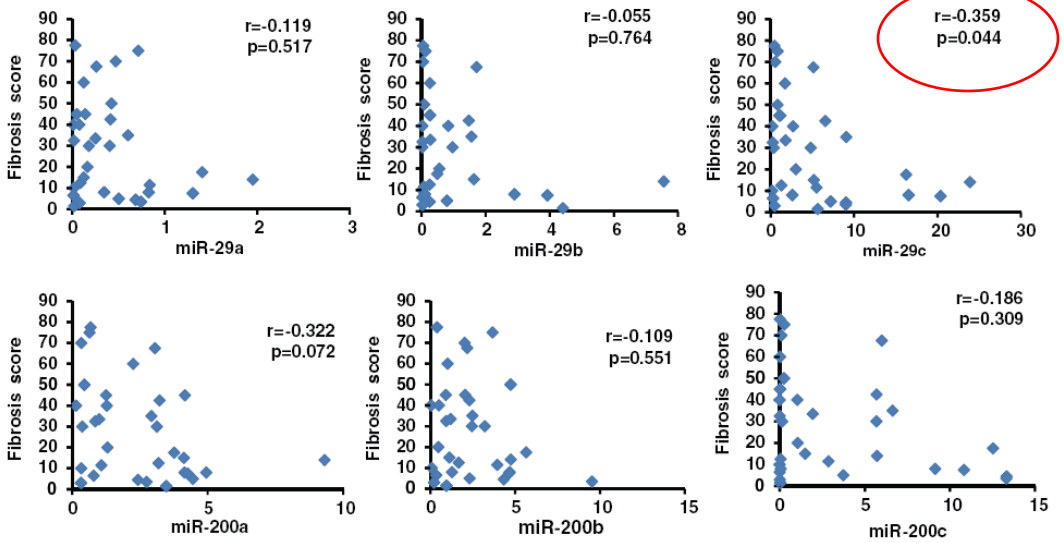
**PCR-Array**

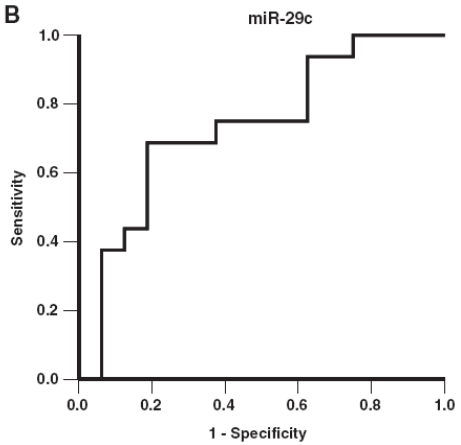

**High-throughput**

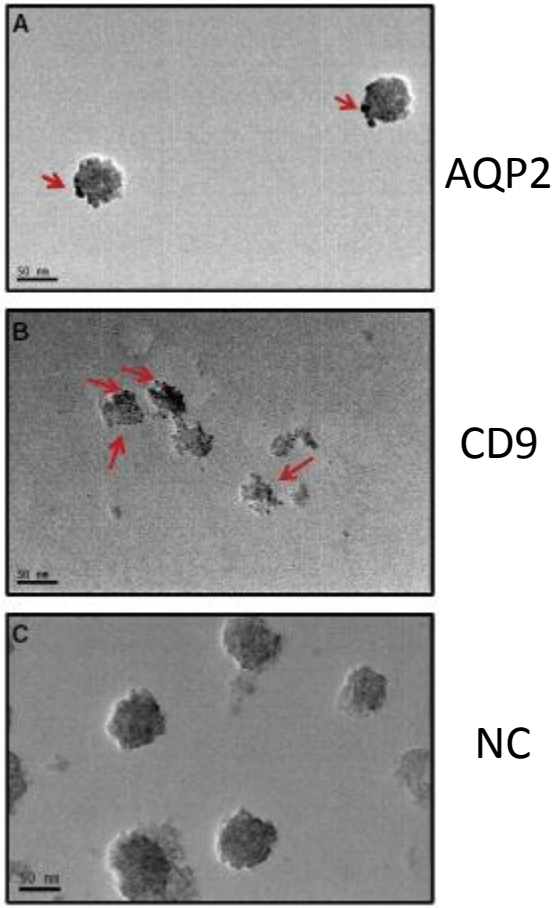
**Next-generation sequencing**

**Identify novel miRNA, high sensitivity**

# miR-29c from urinary exosome is potential biomarkers of renal fibrosis




*Lv LL, Liu BC et al. Am J Physiol Renal Physiol 305: F1220–F1227, 2013.*

# Challenges in kidney disease Biomarker Study

- Large cohort validation studies is lacked for most new biomarkers and very few novel biomarkers have been translated to clinic
- Urine (exosome) samples are often collected, processed and stored under different protocols
- Sensitivity and specificity not comparable to traditional clinic parameters

# Phases of diagnostic or prognostic biomarker studies

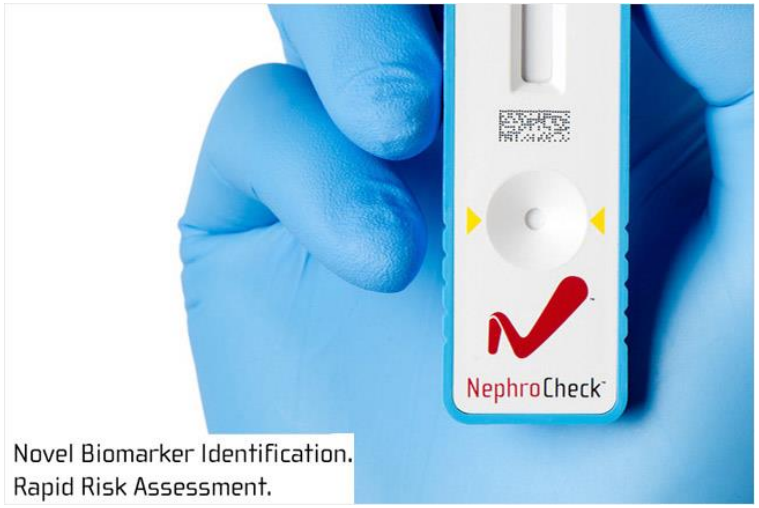
| | Description | Aim of study | sample sizes |
|---|---|---|---|
| Ia | Discovery | Identification of promising biomarkers | 10–100 |
| Ib | Assay development, assay validation | Define and optimize analytical process into robust, reproducible, and valid device | 10–100 |
| Ic | Retrospective validation | Clinical assay detects disease; development of first algorithm for combination test | 50–500 |
| II | Retrospective refinement | Validation of early detection properties of biomarker (set); development and/or refinement of algorithm(s) for combination tests | 100–1,000 |
| III | Prospective investigation | Determination of diagnostic accuracy (sensitivity, specificity) in the situation of clinical routine | 200–1,000 |
| IVa | Randomized controlled trial | Quantification of effect of making the biomarker information available to the doctor to reduce disease burden | 200–1,000 |
| IVb | Health economics study | Quantification of cost-effectiveness | |

U.S. Department of Health and Human Services

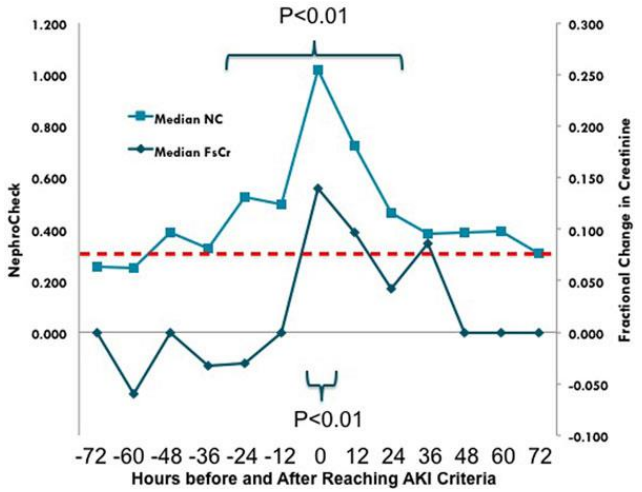
U.S. Food and Drug Administration
Protecting and Promoting *Your* Health

FDA News Release

# FDA allows marketing of the first test to assess risk of developing acute kidney injury




**Urine TIMP2 × IGFBP7 increases 24 hours before severe AKI**




M Ostermann et al. Crit Care. 2014; 18(Suppl 1): P380.
**Koyner et al.** JASN July 2015 26: 1485-1488

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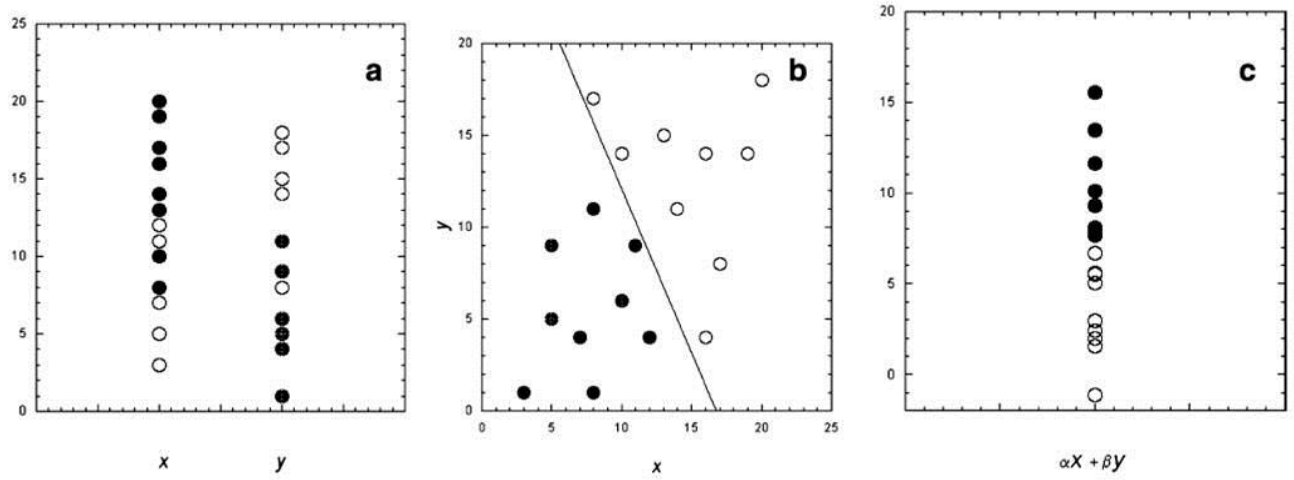
# Multivariate biomarker to improve diagnostic performance

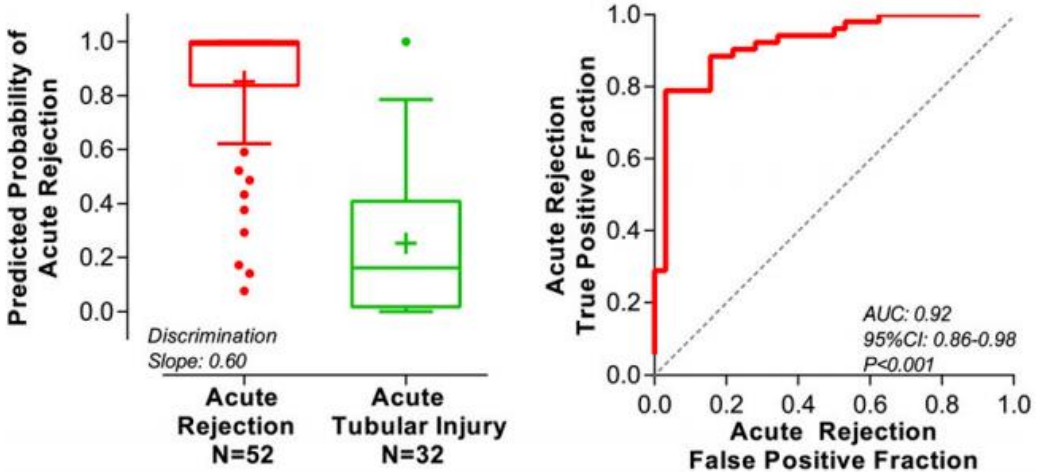
- Novel biomarker combined with traditional clinic parameters
- Multiple novel biomarker combined






Lemley et al, Pediatr Nephrol, 2007

# Composite biomarker with multiple novel biomarkers




Development of a six-gene urinary cell diagnostic signature to differentiate acute rejection (AR) from acute tubular injury( ATI) :

(0.523lnCD3)+(1.023lnCD105)+(0.813lnTLR4)+(21.163lnCD14)
+(0.283 lnComplement Factor B)+(20.793lnVimentin)

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Matignon M et al. J Am Soc Nephrol. 2014 July; 25(7): 1586–1597.

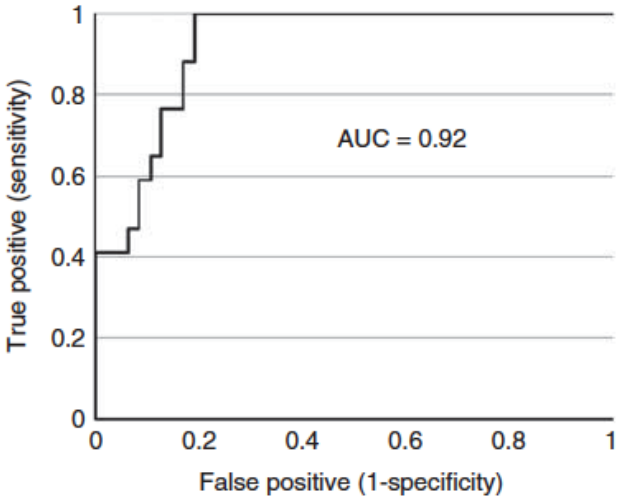
# Combination of novel biomarker with traditional clinic parameters

**Table 3 | Performance characteristics of biomarkers of interstitial inflammation for all biopsies[a]**

| Biomarker | Threshold value[b] | Misclassifications (%) | Sens. (%) | Spec. (%) | PPV (%) | NPV (%) | AUC under ROC |
|---|---|---|---|---|---|---|---|
| uMCP-1 | 2.2 | 10/64 (16) | 83 | 85 | 67 | 93 | 0.87 |
| uLFABP | 118 | 14/64 (22) | 65 | 83 | 58 | 87 | 0.75 |
| uHepcidin | 136.5 | 22/64 (34) | 83 | 60 | 42 | 90 | 0.70 |
| uPCR | 3.7 | 18/61 (30) | 56 | 76 | 45 | 83 | 0.65 |
| SCr | 1.43 | 13/64 (20) | 83 | 79 | 58 | 93 | 0.86 |
| Equation (1) $Y_1$ (applied to all biopsies) | 1 | 9/64 (14) | 100 | 81 | 67 | 100 | 0.92 |
| Equation (1) $Y_1$ (applied to 49 biopsies) | 1 | 6/49 (12) | 100 | 83 | 68 | 100 | 0.91 |

$$Y_1 = 0.992 * \ln(uMCP - 1) + 2.213 * \ln(Scr)$$

$$Y_2 = 4.177 * \ln(uPCR) - 1.425 * \ln(uHEP)$$

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Zhang et al. Kidney Int. 2012 February; 81(4): 401–406

# Summary

- Different component of urine contain rich information for biomarker discovery, among which urine (exosome) mRNA , miRNA were interesting target for further study.
- Large cohort validation study is needed for translating novel biomarkers to clinic.

**We are expecting more potential urine biomarker translated to clinic!**

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Supported by:
- The key project of the national natural scientific foundation
- The national natural scientific foundation
- “973” grant（key member)

# Thank you!

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