Messenger RNA Expression of Podocyte-Associated Molecules in Urinary Sediment of Patients with Lupus Nephritis

GANG WANG, FERNAND MAC-MOUNE LAI, LAI-SHAN TAM, KWOK-MING LI, KA-BIK LAI, KAI-MING CHOW, KAM-TAO PHILIP LI, and CHEUK-CHUN SZETO

ABSTRACT. ***Objective.*** To examine urinary expression of podocyte-associated molecules in patients with lupus nephritis (LN).

Methods. We studied 32 patients with active LN (Active group) and 17 patients with inactive lupus (Silent group). Messenger RNA expression of nephrin, podocin, and synaptopodin in urinary sediment was quantified by real-time polymerase chain reaction and compared to other clinical measures.

Results. The urinary concentrations of nephrin, podocin, and synaptopodin were significantly higher in the Active than the Silent group ($p < 0.05$ for all comparisons). There was no relation between urinary gene expression and the histological class of LN, but urinary nephrin expression correlated with proteinuria ($r = 0.480$, $p < 0.01$) and the score of the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI; $r = 0.578$, $p < 0.01$). Urinary podocin expression also correlated with SLEDAI score ($r = 0.389$, $p = 0.006$). After initiation of immunosuppressive treatment, all patients were followed for an average of 13.7 ± 2.4 months. The decline of the glomerular filtration rate (GFR) correlated with urinary expression of podocin ($r = 0.406$, $p = 0.005$) and synaptopodin ($r = 0.337$, $p = 0.021$). In a multiple linear regression model, urinary podocin expression and baseline GFR were independent predictors of GFR decline.

Conclusion. The concentration of podocyte-associated molecules in urinary sediment correlated with lupus activity and GFR decline. The clinical utility of quantifying urinary expression of podocyte-associated molecules for risk stratification of patients with LN deserves further study. (First Release Nov 1 2007; J Rheumatol 2007;34:2358–64)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS
URINALYSIS
NEPHRIN
PODOCYTE-ASSOCIATED MOLECULES

Lupus nephritis (LN) is one of the most serious clinical manifestations of systemic lupus erythematosus (SLE). It is generally believed that development of renal injury in SLE originates from the complex immune responses triggered by interactions between autoantibodies and renal antigens[1]. However, the exact immunopathogenesis of LN is still elusive. Recent studies show that podocyte injury occurs early in LN after deposition of immune complex[2-4], preceding the subsequent glomerular damage[5]. Other research also shows that podocyte loss is a marker of disease activity in LN, which can be reversed by immunosuppressive therapy[6,7]. In addition to podocyte loss, the expression pattern of podocyte-associated molecules, such as nephrin, podocin, and podoplanin, in patients with LN is significantly different from that of healthy controls[8]. Therefore, detection of podocyte and associated molecular changes may be a useful way to assess the severity of LN.

Traditionally, renal biopsy is the standard method to study podocyte changes. Recently, urinary podocyte counting has been used as a noninvasive method in some diseases[6,9]. However, these studies used sophisticated immunofluorescence methods for podocyte examination and had limited clinical applicability. We hypothesize that quantification of messenger RNA expression of podocyte-specific genes in urine sediment by reverse transcription and real-time quantitative polymerase chain reaction (PCR) may be a valuable noninvasive method for studying LN and other kidney diseases[10].

From the Department of Medicine and Therapeutics and Department of Anatomical and Cellular Pathology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong.

Supported in part by the CUHK research accounts 6901031 and 8500008.

G. Wang, MB, Research Fellow; L-S. Tam, MBChB, MRCP(UK), Associate Professor; K-M. Li, MD, FRCPC, Professor; K-B. Lai, MPhil, Research Assistant; K-M. Chow, MBChB, MRCP(UK), Associate Consultant; K-T.P. Li, MD, FRCP, FACP, Consultant; C-C. Szeto, MD, FRCP (Edin), Professor, Department of Medicine and Therapeutics; F.M-M. Lai, MD, FRCPA, Professor, Department of Anatomical and Cellular Pathology, Prince of Wales Hospital, The Chinese University of Hong Kong.

Address reprint requests to Dr. C-C. Szeto, Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China. E-mail: ccszeto@cuhk.edu.hk

Accepted for publication August 7, 2007.

MATERIALS AND METHODS

Patients. We recruited 32 consecutive patients with active LN who required renal biopsy (the Active group) and 17 patients with silent lupus (the Silent group). All patients were diagnosed according to the American College of Rheumatology diagnostic criteria[11]. Active LN was determined by individual physicians according to criteria as described[12]. A whole-stream early morning urine specimen was collected by all patients (on biopsy day for the Active group) for gene expression study. Clinical data including SLE Disease Activity Index (SLEDAI), serum creatinine, urea, glomerular filtration rate (GFR), and 24 h urine protein were recorded. GFR was estimated by a standard equation[13]. Seven healthy volunteers were also studied (the Healthy Group), and their target gene expression levels were used as calibrator.

Messenger RNA extraction and reverse transcription. The method of urinary messenger RNA extraction has been described[10]. In brief, urine samples were centrifuged at 3000 rpm for 30 min and at 13,000 rpm for 5 min at 4°C. Supernatant was then discarded and the urinary cell pellet was lysed by RNA lysis buffer (Qiagen Inc., Mississauga, Ontario, Canada). Specimens were stored at –80°C until used. RNeasy mini kits (Qiagen) were used to extract total RNA according to manufacturer's protocol. The purity of RNA was confirmed by the relative absorbance at 260/280 nm ratio using a spectrometer. The integrity of RNA isolated from urinary sediment by this method was demonstrated in our previous study by running total RNA in denaturing 1.5% agarose gel, which revealed clear 28S and 18S rRNA bands suitable for real-time quantitative PCR[14]. For reverse transcription, 5 μl (around 2 μg) total RNA was mixed with 1 μl random primers (150 ng), 1 μl dNTP mix (10 mM each), 4 μl 5× first-strand buffer, 2 μl DTT (0.1 M), 1 μl Superscript II RNase H reverse transcriptase (all from Invitrogen™, Life Technologies, Philadelphia, PA, USA) and made up to 20 μl with H_2O. Reverse transcription was performed at 65°C for 5 min, 25°C for 10 min, 42°C for 50 min, and then inactivation reaction at 70°C for 10 min. The resulting cDNA was stored at –80°C until used.

Real-time quantitative PCR. We quantified urinary gene expression of nephrin, podocin, and synaptopodin using the ABI Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA, USA). Commercially available Taqman primers and probes, including 2 unlabeled PCR primers and 1 FAM™ dye-labeled TaqMan® MGB probe were used for all the target genes (all from Applied Biosystems). The primer and probe sequences were as described[15]; they were all deliberately designed across the intron-exon boundary so as not to detect possible genomic DNA. For real-time quantitative PCR, 10 μl universal master mix, 1 μl primer and probe set, 2 μl cDNA, and 7 μl H_2O were mixed to make a 20 μl reaction volume. Each sample was run in triplicate. Real-time quantitative PCR was performed at 50°C for 2 min, 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. 18S rRNA (Applied Biosystems) was used as a housekeeping gene to normalize mRNA expression of each target gene. Results were analyzed with Sequence Detection Software version 1.7 (Applied Biosystems). In order to calculate the differences of expression level for each target gene among samples, the $\Delta\Delta C_T$ method for relative quantitation was used according to the manufacturer's manual. For amplification, efficiency of all TaqMan gene expression assays is equivalent to any other target assay; it is not necessary to validate the PCR efficiencies of the target and endogenous control(s). As noted above, average expression level of healthy volunteers was used as calibrator and the expression level of target genes was expressed as a ratio relative to that of the healthy controls. As described by Schmid, *et al*[16], nephrin and podocin were also expressed as a ratio to synaptopodin expression.

Histological analysis of kidney biopsy. Renal pathology was classified according to the revised International Society of Nephrology/Renal Pathology Society system[17]. The histological activity and chronicity indices were also scored by standard means[18]. Briefly, activity index is the sum of semiquantitative scores of 6 lesions including hypercellularity, leukocyte infiltration, subendothelial hyaline deposits, interstitial inflammation, necrosis, and cellular crescents. Each lesion is scored 0 to 3 and the last 2 items are scored twice. The maximum of the activity index is 24 points. The chronicity index is the sum of 4 semiquantitative scores comprising glomerular sclerosis, fibrous crescents, tubular atrophy, and interstitial fibrosis. Each lesion is scored 0 to 3 and the maximum is 12 points. Evaluation of biopsy specimens was performed by a single pathologist (FML).

Morphometric study of kidney biopsy. Jones' silver staining was performed on 5-μm thick sections of renal biopsy specimen. As described[19], we used a computerized image analysis method to semiquantify nephrosclerosis. Briefy, a Leica Twin Pro image analysis system (Leica Microsystems, Wetzlar, Germany) was connected to a Leica DC500 digital camera on a Leica DMRXA2 microscope working with a 40× objective (final calibration: 0.258 mm/pixel) and to a microcomputer for storage of the morphometric measurements and to perform image analysis by using image-analyzing software (MetaMorph 4.0; Universal Imaging Corp., Downingtown, PA, USA). Ten glomeruli and 10 randomly selected areas were assessed in each patient and the average percentages of scarred glomerular and tubulointerstitial areas, as represented by the percentage of the area with positive staining, were computed for each patient.

Clinical followup. After the initial assessment, all except one patient were followed for at least 12 months. The clinical management was decided by the individual nephrologist and not affected by the study. In general, patients of the Active group were treated with corticosteroid, together with cyclophosphamide or mycophenolate. Therapeutic response was classified into complete remission, partial remission, and no response as defined[20]. Renal function test, including serum creatinine, urea and proteinuria levels, was assessed at least every 4 months. As noted above, estimated GFR was calculated by a standard equation[13]. The rate of GFR change, which reflects the severity of chronic progressive renal damage, was calculated by the least-square regression method.

Statistical analysis. Statistical analysis was performed by SPSS for Windows software, version 11.0 (SPSS Inc., Chicago, IL, USA). All the results are presented as mean ± SD unless otherwise specified. Since data of target gene expression levels were highly skewed, either log-transformation or nonparametric statistical method was used. We used Mann-Whitney U-test to compare gene expression levels between groups and Spearman's rank-order correlations to test associations between untransformed gene expression levels and clinical measures. Since the distribution of gene expression data of the Active and Silent groups became normal after log-transformation, Student's t-test was used to compare the 2 groups. A p value < 0.05 was considered statistically significant. All probabilities were 2-tailed. In this series, we used 40 real-time quantitative PCR cycles as the detection limit of any target gene expression. To allow log-transformation of expression data, an expression value equal to half the detection limit (i.e., 41 cycles) was assigned when no detectable level of a transcript was found at 40 cycles, as recommended by Helsel[21].

RESULTS

The demographic and baseline clinical data of the study subjects are summarized in Table 1. Renal biopsy of the Active group showed pure mesangial disease (class II, 2 cases), pure

Table 1. Demographic and baseline clinical data.

Characteristic	Active Group, n = 32	Silent Group, n = 17	p*
Sex (F:M)	26:6	16:1	—
Age, yrs	37.0 ± 10.9	40.2 ± 8.0	p = 0.19
Serum creatinine, μmol/l	116.6 ± 103.8	103.9 ± 72.7	p = 1.00
Urine protein, g/day	2.94 ± 2.06	0.64 ± 1.23	p < 0.01
Estimated GFR, ml/min	67.9 ± 39.6	70.7 ± 26.0	p = 0.60
SLEDAI	12.6 ± 5.5	0.6 ± 0.9	p < 0.01

GFR: glomerular filtration rate; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index. * Data compared by Mann-Whitney U-test.

membranous disease (class V, 9 cases), pure proliferative glomerulonephritis (class III or IV, 8 cases), and mixed proliferative and membranous disease (11 cases); 2 cases had insufficient material for histological classification. Histological activity and chronicity indices of the Active group were 7.0 ± 4.8 and 2.5 ± 2.0, respectively. Morphometric study showed that percentages of scarred glomerular and tubulointerstitial area were 13.56 ± 9.22% and 10.26 ± 3.83%, respectively.

Comparison of gene expression between groups. The concentrations of nephrin, podocin, and synaptopodin in the Active and Silent groups are summarized in Figure 1A. Urinary mRNA expression of nephrin, podocin, and synaptopodin was significantly higher in the Active than the Silent Group (Student's t test, $p < 0.01$ for nephrin and podocin, $p = 0.024$ for synaptopodin). After adjustment for synaptopodin, gene expression of nephrin and podocin was still significantly higher in the Active group ($p < 0.01$ for both) (details not shown). There was no difference in the expression levels of urinary nephrin, podocin, and synaptopodin between patients with membranous (class V) and proliferative (class III or IV) nephritis (Figure 1B).

Relation with baseline clinical measures. The relation between urinary mRNA expression of podocyte-associated molecules and baseline clinical measures is displayed in Figure 2. Urinary nephrin expression correlated significantly with SLEDAI ($r = 0.578$, $p < 0.01$) and proteinuria ($r = 0.480$, $p < 0.01$), but not with GFR ($r = 0.013$, $p = 0.9$). Urinary podocin expression correlated significantly with SLEDAI ($r = 0.389$, $p = 0.006$), but not with proteinuria ($r = 0.203$, $p = 0.162$) or GFR ($r = 0.014$, $p = 0.9$).

Relation with renal histology. The correlation between urinary mRNA expression of podocyte-associated molecules and the degree of histological damage as quantified by morphometric study is summarized in Table 2. There was no significant correlation between urinary gene expression and the degree of glomerular and tubulointerstitial scarring. In addition, there was no correlation between urinary mRNA expression of podocyte-associated molecules and histological and chronicity index.

Relation with treatment response and renal function decline. The patients were followed for an average of 13.7 ± 2.4 months. After the initial induction treatment, 12 patients in the Active group had complete remission, 8 had partial remission, and 7 showed no response. Therapeutic response could not be determined in 5 patients because of inactive class II disease at diagnosis (2 cases), insufficient material for histologic diagnosis (2 cases), and loss to followup (1 case). There was no significant difference in urinary gene expression levels of target genes among patients with complete remission, partial remission, and no response.

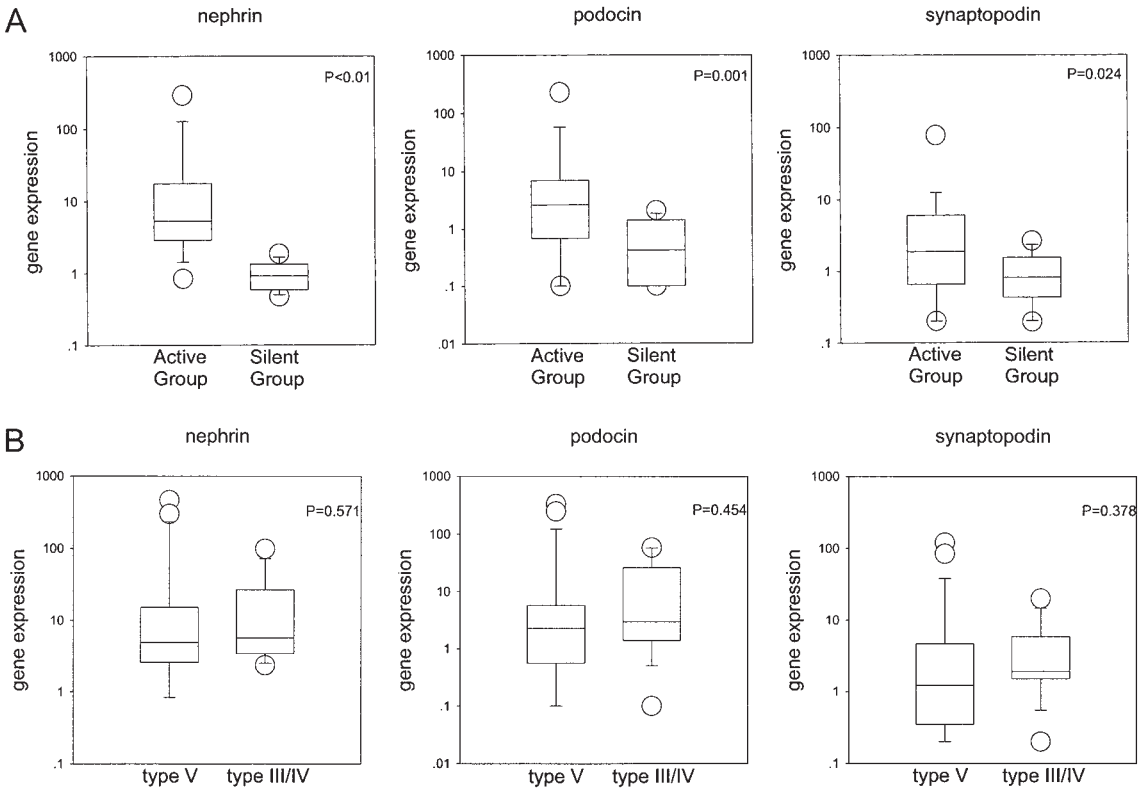

Figure 1. Comparison of mRNA expression of podocyte-associated molecules in urinary sediment between (A) Active and Silent groups, (B) type V lupus nephritis and type III/IV lupus nephritis. Boxes indicate median, 25th and 75th percentile; whisker caps indicate 5th and 95th percentile; circles indicate outliers. Data compared by Mann-Whitney U-test.

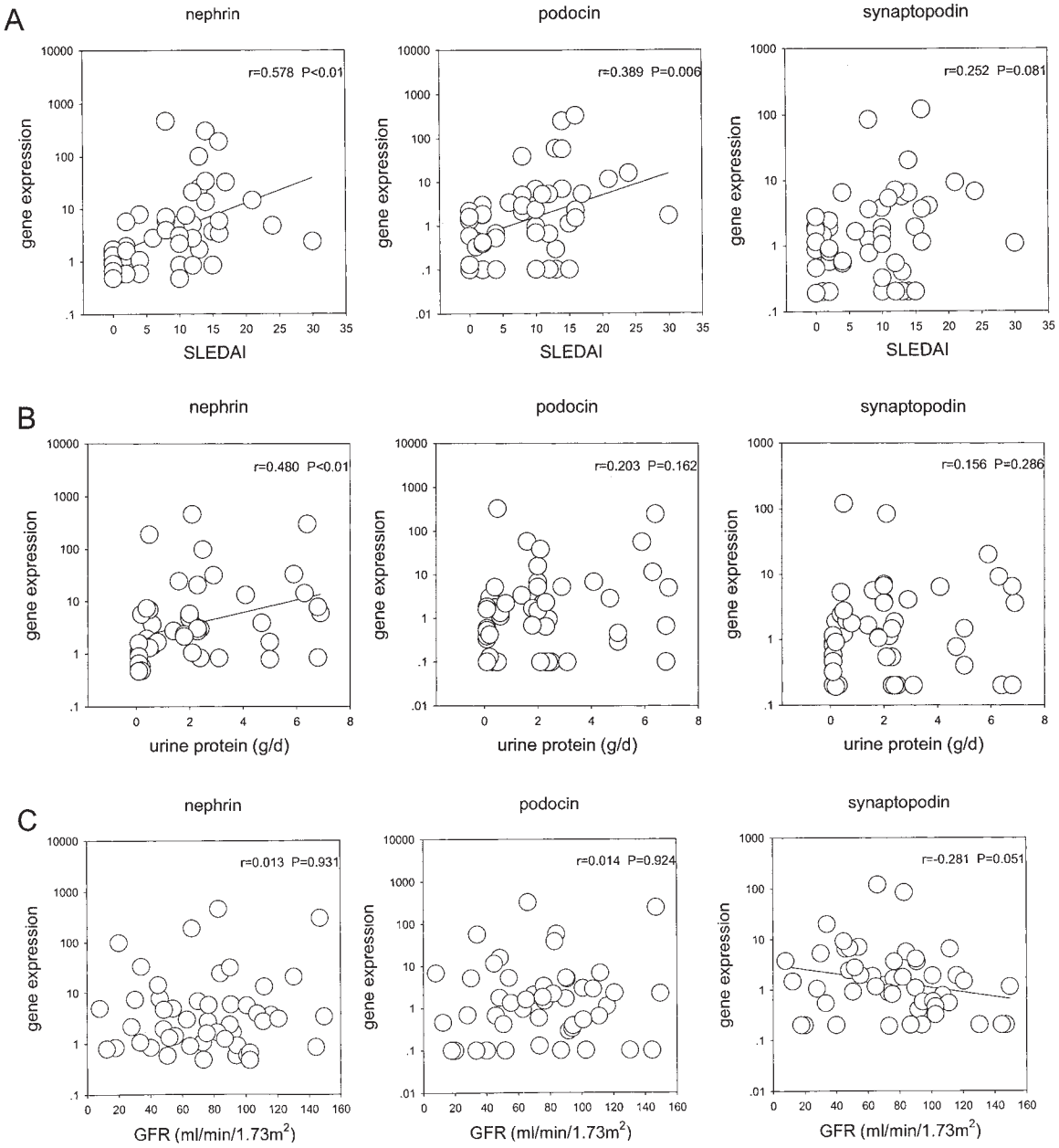

Figure 2. Relations between mRNA expression of podocyte-associated molecules in urinary sediment and (A) SLEDAI scores, (B) proteinuria, and (C) estimated glomerular filtration rate (GFR). Data compared by Spearman's correlation coefficient.

The average rate of GFR decline after the initial induction phase of immunosuppressive therapy was –0.54 ± 2.19 ml/min/month. The rate of GFR decline correlated with the urine expression of podocin ($r = 0.406$, $p = 0.005$) and synaptopodin ($r = 0.337$, $p = 0.021$) (Figure 3). With a multiple linear regression model to adjust for the baseline SLEDAI, GFR, proteinuria, and histological measures, we found that urinary podocin concentration and baseline GFR were independent predictors of GFR decline (Table 3).

DISCUSSION

LN remains an important cause of mortality and morbidity in patients with SLE[22,23]. Ideal markers for risk stratification and disease monitoring are still lacking[24]. Recent studies showed that urinary mRNA expression of cytokines and growth factors may be a valuable noninvasive method for assessment of activity and severity of LN[12,25,26]. However, quantification of urinary cytokine gene expression is not always reliable because it is prone to contamination with inflammatory cells

Table 2. Correlation between urinary mRNA expression of podocyte-associated molecules and the degree of histological damage.

Manifestation	Nephrin	Podocin	Synaptopodin
Activity index	r = 0.112	r = –0.014	r = 0.233
	p = 0.577	p = 0.946	p = 0.241
Chronicity index	r = –0.039	r = 0.014	r = 0.173
	p = 0.847	p = 0.946	p = 0.388
Glomerular scarring	r = –0.019	r = –0.053	r = 0.162
	p = 0.916	p = 0.772	p = 0.376
Tubulointerstitial scarring	r = 0.027	r = 0.134	r = 0.194
	p = 0.884	p = 0.465	p = 0.287

Data are correlated by Spearman's correlation.

Table 3. Multiple linear regression model of the rate of change of GFR.

	Unstandardized B	95% CI	p
Constant	1.806	–0.542 to 4.154	0.125
Podocin	0.016	0.004 to 0.027	0.011
GFR	–0.033	–0.008 to –0.058	0.012

from bladder origin, as well as gene expression by noninflammatory cells. We measured the mRNA of podocyte-associated molecules in urinary sediment because these target genes are specifically expressed in podocytes. In addition, podocyte injury is now recognized as a key step in the pathogenesis of LN as well as other primary glomerulonephritis.

In our study, all patients from both the Active and Silent groups had detectable urinary mRNA expression of podocyte associated molecules. Indeed, all healthy controls also had detectable urinary expression of these molecules, albeit at a much lower level. We found higher levels of podocyte-associated molecules in the urinary sediment of the Active Group than in that of the Silent Group. In theory, the increase in gene expression may reflect either more podocytes are present in the urine or an upregulation of podocyte-associated molecule expression per podocyte. Besides the absolute expression level, which reflects the overall quantity of gene expression in urine sediment, we also normalize nephrin and podocin expression by that of synaptopodin, a method that is often used to control for the expression of other podocyte-associated molecules[15,16]. Since the urinary expressions of both nephrin and podocin of the Active group remained higher than those in the Silent group after adjusting for synaptopodin expression, our result suggests an increase in urinary podocyte loss as well as an upregulation of the corresponding mRNA levels per podocyte in patients with active LN. The reason for nephrin and podocin upregulation in urinary podocytes is unknown. Clement, *et al*[27] recently reported a downregulation of nephrin in intraglomerular podocytes in experimental nephrotic syndromes, suggesting that variation in podocyte gene expression profile is related to its destination — loss in urine or retention in the diseased glomeruli. It is, however, important to note that synaptopodin mRNA and protein levels do change in proteinuric diseases[16,28,29], and therefore the gene expression of synaptopodin cannot be regarded as a simple "housekeeping gene."

Significant correlations are observed between urinary expression of podocyte associated molecules and SLEDAI, the degree of proteinuria, and baseline GFR. The correlation coefficients remain similar when the calculations are done with log-transformed values (details not shown). Of these relations, the correlation with SLEDAI is intriguing because this index is an assessment of overall disease activity rather than limited to kidney involvement. Our results suggest that measurement of urinary expression of podocyte-associated molecules in LN reflects lupus activity.

In our study, all active patients received standard immunosuppressive treatment. However, baseline gene expression level of urinary podocyte-associated molecules apparently did not predict therapy response. When patients with proliferative and membranous lesions were analyzed separately, there was no difference in baseline urinary gene expression for patients

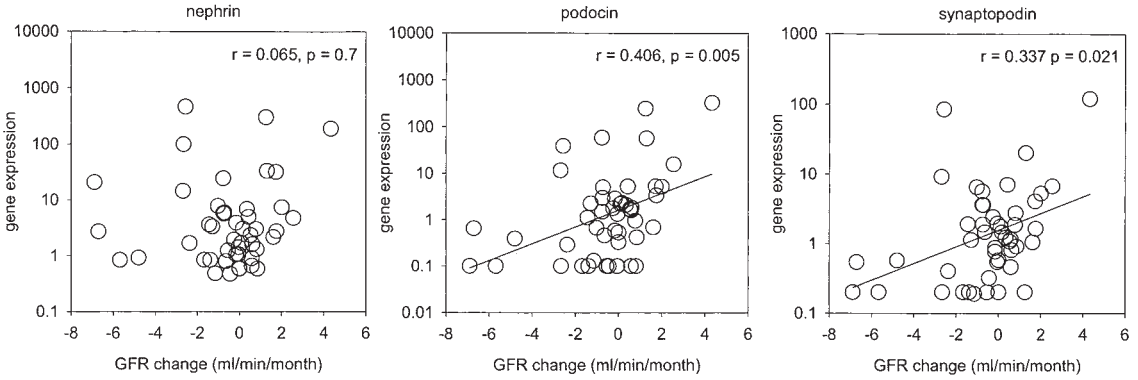

Figure 3. Relations between mRNA expression of podocyte-associated molecules in urinary sediment and the rate of change of estimated glomerular filtration rate (GFR), which reflects the severity of chronic progressive renal damage. A positive value of GFR change indicates improvement in renal function. Data compared by Spearman's correlation coefficient.

with and without response to treatment in either group (details not shown), although the number of cases in each group was small. The urinary expression of podocyte-associated molecules correlates with the rate of decline of renal function, but not the histological chronicity index or glomerular or tubulointerstitial scarring. This phenomenon can possibly be explained because patients with mild disease would have minimal podocyte loss, while patients with grossly scarred kidneys would not have much podocyte remaining for urinary loss. Our study does not have any data on the delayed change in urinary gene expression profile after immunosuppressive therapy. Previous studies, however, have shown that the number of urinary podocytes decreases with immunosuppressive therapy[30,31]. Further studies are needed to clarify the role of serial measurement of these markers, and the rate of urinary podocyte loss at different disease stages.

A considerable proportion of the patients in our study had membranous nephropathy (class V). The distribution of the histological type of LN appears to be different from that in other studies, including our previous reports[32,33]. The reason for this discrepancy remains unknown, but is unlikely to be selection bias because consecutive cases with renal biopsy were recruited in our study. We found that patients with type V nephritis showed the same expression level of the target genes as those with other types of nephritis, probably because of the small sample size. This phenomenon could also be explained because urinary podocyte loss is also present in patients with proliferative LN[34]. In addition, occult type V LN may coexist with proliferative LN histologically[17].

We found that urinary expression of podocyte-associated molecules was elevated in patients with active LN. The degree of upregulation of urinary nephrin expression correlated with SLEDAI, proteinuria, and renal function, while urinary podocin expression correlated with the rate of decline of GFR after therapy. The clinical utility of quantifying urinary expression of podocyte-associated molecules for risk stratification of LN deserves further study.

REFERENCES

1. Tang S, Lui SL, Lai KN. Pathogenesis of lupus nephritis: an update. Nephrology (Carlton) 2005;10:174-9.
2. Kraft SW, Schwartz MM, Korbet SM, Lewis EJ. Glomerular podocytopathy in patients with systemic lupus erythematosus. J Am Soc Nephrol 2005;16:175-9.
3. Han TS, Schwartz MM, Lewis EJ. Association of glomerular podocytopathy and nephrotic proteinuria in mesangial lupus nephritis. Lupus 2006;15:71-5.
4. Zhao Z, Weinstein E, Tuzova M, et al. Cross-reactivity of human lupus anti-DNA antibodies with alpha-actinin and nephritogenic potential. Arthritis Rheum 2005;52:522-30.
5. Mathieson PW. What has the immune system got against the glomerular podocyte? Clin Exp Immunol 2003;134:1-5.
6. Nakamura T, Ushiyama C, Suzuki S, et al. Urinary podocytes for the assessment of disease activity in lupus nephritis. Am J Med Sci 2000;320:112-6.
7. Nakamura T, Ushiyama C, Shimada N, et al. Effect of cyclophosphamide or azathioprine on urinary podocytes in patients with diffuse proliferative lupus nephritis. Nephron 2001;87:192-3.
8. Koop K, Eikmans M, Baelde HJ, et al. Expression of podocyte-associated molecules in acquired human kidney diseases. J Am Soc Nephrol 2003;14:2063-71.
9. Nakamura T, Ushiyama C, Suzuki S, et al. Urinary excretion of podocytes in patients with diabetic nephropathy. Nephrol Dial Transplant 2000;15:1379-83.
10. Li B, Hartono C, Ding R, et al. Noninvasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. N Engl J Med 2001;344:947-54.
11. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997;40:1725.
12. Chan RW-Y, Lai FM-M, Li EK-M, et al. The effect of immunosuppressive therapy on the messenger RNA expression of target genes in the urinary sediment of patients with active lupus nephritis. Nephrol Dial Transplant 2006;21:1534-40.
13. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 1999;130:461-70.
14. Szeto CC, Chan RW, Lai KB, et al. Messenger RNA expression of target genes in the urinary sediment of patients with chronic kidney diseases. Nephrol Dial Transplant 2005;20:105-13.
15. Szeto CC, Lai KB, Chow KM, et al. Messenger RNA expression of glomerular podocyte markers in the urinary sediment of acquired proteinuric diseases. Clin Chim Acta 2005;361:182-90.
16. Schmid H, Henger A, Cohen CD, et al. Gene expression profiles of podocyte-associated molecules as diagnostic markers in acquired proteinuric diseases. J Am Soc Nephrol 2003;14:2958-66.
17. Weening JJ, D'Agati VD, Schwartz MM, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. Kidney Int 2004;65:521-30.
18. Grande JP, Balow JE. Renal biopsy in lupus nephritis. Lupus 1998;7:611-7.
19. Bruneval P, Bariety J, Belair MF, Mandet C, Heudes D, Nicoletti A. Mesangial expansion associated with glomerular endothelial cell activation and macrophage recruitment is developing in hyperlipidaemic apoE null mice. Nephrol Dial Transplant 2002;17:2099-107.
20. Chan TM, Li FK, Tang CS, et al. Efficacy of mycophenolate mofetil in patients with diffuse proliferative lupus nephritis. Hong Kong-Guangzhou Nephrology Study Group. N Engl J Med 2000;343:1156-62.
21. Helsel DR. Less than obvious: statistical treatment of data below the detection limit. Environ Sci Technol 1990;24:1766-74.
22. Cervera R, Khamashta MA, Font J, et al. Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. Medicine 2003;82:299-308.
23. Boumpas DT, Sidiropoulos P, Bertsias G. Optimum therapeutic approaches for lupus nephritis: what therapy and for whom? Nat Clin Prac Rheumatol 2005;1:22-30.
24. Houssiau FA. Management of lupus nephritis: an update. J Am Soc Nephrol 2004;15:2694-704.
25. Avihingsanon Y, Phumesin P, Benjachat T, et al. Measurement of urinary chemokine and growth factor messenger RNAs: a noninvasive monitoring in lupus nephritis. Kidney Int 2006;69:747-53.
26. Chan RW-Y, Lai FM-M, Li EK-M, et al. Messenger RNA expression of RANTES in the urinary sediment of patients with lupus nephritis. Nephrology (Carlton) 2006;11:219-25.
27. Clement LC, Liu G, Perez-Torres I, Kanwar YS, Avila-Casado C, Chugh SS. Early changes in gene expression that influence the course of primary glomerular disease. Kidney Int 2007;72:337-47.

28. Srivastava T, Garola RE, Whiting JM, Alon US. Synaptopodin expression in idiopathic nephrotic syndrome of childhood. Kidney Int 2001;59:118-25.
29. Hirakawa M, Tsuruya K, Yotsueda H, et al. Expression of synaptopodin and GLEPP1 as markers of steroid responsiveness in primary focal segmental glomerulosclerosis. Life Sci 2006;79:757-63.
30. Nakamura T, Ushiyama C, Suzuki S, et al. Urinary podocytes for the assessment of disease activity in lupus nephritis. Am J Med Sci 2000;320:112-6.
31. Nakamura T, Ushiyama C, Hara M, et al. Comparative effects of plasmapheresis and intravenous cyclophosphamide on urinary podocyte excretion in patients with proliferative lupus nephritis. Clin Nephrol 2002;57:108-13.
32. Chan RW, Tam LS, Li EK, et al. Inflammatory cytokine gene expression in the urinary sediment of lupus nephritis patients. Arthritis Rheum 2003;48:1326-31.
33. Chan RW, Lai FM, Li EK, et al. Expression of chemokine and fibrosing factor messenger RNA in the urinary sediment of patients with lupus nephritis. Arthritis Rheum 2004;50:2882-90.
34. Nakamura T, Ushiyama C, Shimada N, et al. Effect of cyclophosphamide or azathioprine on urinary podocytes in patients with diffuse proliferative lupus nephritis. Nephron 2001;87:192-3.