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Urinary Podocyte Biomarkers and Glomerular Histologic Change

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The glomerular diseases, including diabetic kidney disease, hypertensive glomerular disease, and chronic glomerular nephritis, comprise approximately 80% of the causes of end stage kidney disease (ESKD). Urinary protein concentration is frequently measured as a noninvasive assessment of glomerular diseases because it is characteristic and the most important predictor of outcome in glomerular diseases (1–3). However, proteinuria can be caused not only by glomerular diseases but also, by the other conditions, such as increased quantity of protein in serum and low reabsorption of protein at proximal tubules. As proteinuria is a common feature in many glomerular diseases, renal biopsy, an invasive procedure for renal pathologic examination, is required for establishment of the exact diagnosis and the decision of the therapeutic strategy in patients with proteinuric kidney diseases.

Glomerular visceral epithelial cells (podocytes) are terminally differentiated cells that cover the outer layer of the glomerular basement membrane. They play a key role in the maintenance of the blood-urine barrier, which prevents proteins and large molecules from passing into the urinary space and ensures the selective permeability of the glomerular capillary. Previous studies have revealed that the development of human glomerular diseases resulted in the reduction of podocyte number and density in the glomerulus, and podocyte loss *per se* causes glomerulosclerosis in diphtheria toxin–treated transgenic rats, in which the human diphtheria toxin receptors were expressed only in podocytes (4). The detachment of viable podocytes is the major mechanism for podocyte loss. Hara *et al.* (5) showed that podocytes and fragments of podocytes in urine were detected in patients with a variety of glomerular diseases using podocalyxin antibody. Podocyte detachment and podocyte loss into urine have been demonstrated in human glomerular diseases as well as animals with experimental kidney diseases (6). Urinary mRNA expression levels of podocyte-associated genes, including *NPHS2* (podocin) and *PODXL* (podocalyxin), increase with progression of diabetic nephropathy (7). The potential clinical importance of urinary podocytes is demonstrated by the observations that urinary podocytes are a more specific marker of glomerular injury than proteinuria and that they decreased in patients with glomerular diseases following treatment of cerivastatin, renin-angiotensin system inhibitors, or pioglitazone (8–11). These results suggest that measuring urinary podocytes might be a useful clinical test for monitoring of glomerular diseases.

In *Kidney360*, Fukuda *et al.* (12) reveal the association of urinary podocyte marker mRNA expression levels with renal histologic change in patients with glomerulonephritis (GN). They collected urine samples from 184 patients with glomerular diseases and 12 healthy volunteers during a 3-year period. The cases consisted of 15 patients with minor glomerular abnormality with mild proteinuria and/or microscopic hematuria, 15 patients with minimal change disease, 60 patients with immunoglobulin A (IgA) nephropathy, 15 patients with membranous nephropathy, 19 patients with crescentic GN, ten patients with lupus nephritis, and 38 patients with the other glomerular diseases, such as membranoproliferative GN, IgA vasculitis, and focal segmental glomerulosclerosis (FSGS). The mean age in this cohort ranged from 30s to 60s depending on the glomerular disease (65 years in patients with membranous diseases, 40 years in patients with lupus nephritis, and 34 years in healthy controls). Urinary samples were centrifuged, and RNA was purified from the pellet. Podocin mRNA expression was quantified through reverse transcription-polymerase chain reaction (RT-P CR). Urinary supernatant podocalyxin was quantified using a sandwich ELISA. Both urinary sediment podocin mRNA expression and urinary supernatant podocalyxin were correlated with proteinuria. Interestingly, urinary podocin mRNA expression levels were significantly elevated in patients with IgA nephropathy and patients with crescentic GN compared with patients with other glomerular diseases and massive proteinuria. Moreover, urinary podocin mRNA expression was associated with the rate of crescentic formation in the two glomerular diseases. Urinary supernatant podocalyxin significantly increased in membranous nephropathy and subepithelial dense deposit–type lupus nephritis. The investigators examined the relationship between glomerular histologic classification and those two urinary markers in patients with IgA nephropathy, patients with crescentic GN, patients with membranous nephropathy, and patients with lupus nephritis, indicating that urinary podocin mRNA expression levels were elevated in patients

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whose glomerular histology displayed extracapillary proliferative type and that urinary supernatant podocalyxin levels increased in patients whose glomerular histology showed subepithelial dense deposit type.

Although renal biopsy is the standard procedure for diagnosis and evaluation for treatment, it is an invasive procedure and has several complications. Therefore, we usually measure urinary protein levels for disease activity after diagnosis of glomerular diseases through renal biopsy. Urine biomarkers are promising as they are noninvasive, are easily accessible, and can be collected repeatedly. This study provides a novel insight into the association between urinary podocyte biomarkers and glomerular histologic findings in patients with various kinds of glomerular diseases, showing that crescent formation and subepithelial dense deposit were related to increased urinary *NPHS2* mRNA expression and urinary podocalyxin protein, respectively. However, the molecular mechanisms for these relations were unknown. Fukuda *et al*. (13) previously showed that urinary podocyte mRNA (*NPHS2* mRNA) was correlated with severe glomerular lesions, such as segmental glomerular sclerosis and acute extracapillary proliferation (or cellular crescent), in patients with IgA nephropathy. As they also displayed that urinary *NPHS2* mRNA expression was not associated with podocyte depletion demonstrated by density and average number per tuft using transducing-like enhancer of split 4 immunostaining (13,14), there may be other molecular mechanisms than podocyte loss for increased urinary *NPHS2* mRNA expression in patients with crescentic formation.

Asao *et al*. (15) have displayed the relationship between urinary podocalyxin levels and severity of acute extracapillary abnormality evaluated by Shigematsu classification in patients with IgA nephropathy (16). Fukuda *et al.* (17) have also demonstrated that urinary *NPHS2* mRNA expression increased in type 2 diabetic nephropathy. More specific urinary biomarkers for each glomerular histologic finding will be needed for the clinical setting. To find more specific urinary biomarkers in glomerular diseases, we should clarify the molecular mechanism for glomerular histologic changes, including crescent formation and subepithelial dense deposit. The one way to examine the association between transcriptional profile and glomerular histologic change is spatial transcriptomics, a quantitative analysis of transcriptome in the histologic sections (18). This spatial transcriptomics analysis is commercially available and has applications for human kidney sections (19). Recently, this technology has been developed for high-resolution spatial transcriptomics, in which each bead captures one to two cells (Slide-seq) (20). Further studies using the special transcriptomics may promote our understanding of transcriptional profiles for glomerular histologic lesions, which may lead to finding a new specific biomarker.

In summary, Fukuda *et al*. (12) show the association between glomerular histologic lesions and urinary podocyte markers. This study provides an intriguing clue for future studies to find molecular mechanisms for renal histologic changes and urinary-specific biomarkers in patients with glomerular diseases.

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Author Contributions

K. Inoue conceptualized the study; wrote the original draft; and reviewed and edited the manuscript.

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