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A multicenter cross-sectional study of circulating soluble urokinase receptor in Japanese patients with glomerular disease

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Elevated serum-soluble urokinase receptor (suPAR) levels have been described in patients with focal segmental glomerulosclerosis (FSGS) in several different cohorts. However, it remains unclear whether this is the case for Japanese patients and whether circulating suPAR can be clinically useful as a diagnostic marker. To determine this, we measured serum suPAR levels in 69 Japanese patients with biopsy-proven glomerular diseases in a cross-sectional manner. The serum suPAR levels showed a significant inverse correlation with renal function by univariate (R^2 of 0.242) and multivariate ($\beta = 0.226$) analyses. Even after excluding patients with renal dysfunction, no significant difference in the suPAR levels was detected among the groups. Receiver operating characteristic analysis and measures of the diagnostic test performance showed that suPAR was not a useful parameter for differentiating FSGS from the other glomerular diseases (AUC-ROC: 0.621), although a small subgroup analysis showed that patients with FSGS, treated with steroids and/or immunosuppressants, had significantly lower suPAR levels. Patients with ANCA–associated glomerulonephritis had significantly higher levels of suPAR compared with the other disease groups, which may be owing to their lower renal function and systemic inflammation. Thus, suPAR levels are significantly affected by renal function and have little diagnostic value even in patients with normal renal function.

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Focal segmental glomerulosclerosis (FSGS) is one of the leading causes of steroid-resistant nephrotic syndrome. Research on the pathogenesis of primary FSGS has been intensified because of identification of the podocyte as the major cellular target;[1] however, the disease mechanism has not been fully elucidated. One of the best-recognized notions is that some FSGS cases may be associated with a circulating factor. This concept is supported by the recurrence of FSGS soon after renal transplantation,[2,3] by the response of proteinuria to plasmapheresis[4,5] or immunoabsorption,[6,7] and by a case of nephrotic syndrome in a newborn whose mother had FSGS.[8] Recently, Gallon *et al.*[9] reported that reimplantation of a kidney allograft with FSGS recurrence into another patient resulted in proteinuria remission, which strongly suggested involvement of a circulating factor.

Wei *et al.*[10] have recently reported that soluble urokinase receptor (suPAR) was a promising candidate for circulating permeability factor by evaluating the circulating suPAR levels in sera from primary and recurrent FSGS patients. They proposed that suPAR has a causal role in the development of FSGS based on their finding that urokinase receptor (uPAR) can cause foot process effacement through the activation of β3-integrin signaling.[11] The researchers also evaluated the suPAR levels in two large cohorts of children and adults with biopsy-proven primary FSGS.[12] Although they demonstrated that the suPAR levels were significantly elevated in primary FSGS patients from both cohorts, there has been an intense

debate over suPAR as a diagnostic marker.[13–16] Recently, Huang *et al.*[17] reported that the suPAR levels in primary FSGS patients have a considerable overlap with other glomerular diseases such as minimal change disease (MCD) and membranous nephropathy (MN), although these differences are significant. In a study of pediatric patients, Bock *et al.*[18] demonstrated that the suPAR levels in FSGS patients are even lower than those in non-glomerular kidney diseases.[18] In Japan, it is estimated that approximately 3800–4500 adults develop nephrotic syndrome annually, and primary FSGS accounts for approximately 10% of the new-onset nephrotic syndrome cases.[19] However, no data on the serum suPAR levels in Japanese FSGS patients are available yet.

In the present investigation, we performed a multicenter cross-sectional study using sera from patients with primary glomerular diseases (including FSGS) to determine whether the serum suPAR levels in Japanese patients are useful as a diagnostic marker.

RESULTS

Demographic and clinical characteristics of patients with glomerular diseases

We studied 69 serum samples from patients with biopsy-proven glomerular diseases and 17 serum samples from healthy volunteers. The subjects' demographic and clinical data are listed in Table 1. The median age of the patients was 54 years, ranging from 17 to 82 years. The patients were categorized according to the histopathological diagnoses (FSGS, MCD, IgA nephropathy (IgAN), and MN) performed by pathologists in each facility, and no overlaps were reported.

In our cohort, the MN patients (67.9 ± 10.3 years of age) were significantly older than the MCD patients (41.2 ± 18.1 years of age; $P = 0.005$), the IgAN patients (42.2 ± 20.8 years of age; $P = 0.007$), and the healthy control subjects (45.3 ± 15.5 years of age; $P = 0.0115$). All of the disease groups showed significantly lower serum albumin levels compared with healthy control group; however, no significant difference was observed between any two disease groups. As for serum total cholesterol levels, we found that the MCD patients (422.1 ± 139.3 mg/dl) had significantly higher levels compared with the FSGS patients (324.3 ± 102.0 mg/dl; $P = 0.0461$), the IgAN patients (265.5 ± 101.0 mg/dl; $P = 0.0029$), MN patients (284.3 ± 95.0 mg/dl; $P = 0.0192$), and control subjects (206.1 ± 35.7 mg/dl; $P < 0.0001$). The FSGS patients' serum total cholesterol levels were significantly higher than those of control subjects ($P = 0.0016$). We measured C-reactive protein (CRP) in the patients with these renal diseases, because it has been reported that the serum suPAR concentration rises with nonspecific inflammation.[20–22] No significant difference in CRP was detected among the disease groups and the control group. The renal function represented by the estimated glomerular filtration rate (eGFR) was significantly higher in the control group (79.9 ± 15.8 ml/min per 1.73 m^2) compared with that in the FSGS group (54.4 ± 25.6 ml/min per 1.73 m^2; $P = 0.0066$). The urinary protein excretion was compared within the disease groups. The MCD patients (9138.1 ± 3874.7 mg per day or mg/gCre) excreted significantly more urinary protein than did the IgAN patients (3874.4 ± 2476.2 mg per day or mg/gCre; $P = 0.02$) or the FSGS patients (5753.2 ± 4772.3 mg per day or mg/gCre; $P = 0.04$). The amount of proteinuria in MN patients (7538.3 ± 2711.9 mg per day or mg/gCre) was also larger than in IgAN patients ($P = 0.048$).

Serum suPAR levels in primary glomerular diseases

We analyzed the unadjusted data on the serum suPAR levels according to the histological diagnosis of the glomerular diseases (Table 1). The serum suPAR levels significantly differed among the five groups, including the control group (one-way analysis of variance (ANOVA), $P < 0.0001$, effect size $f = 0.716$, power $(1 - \beta) = 0.999$). In our cohort, however, the suPAR levels in the FSGS group (3119.0 ± 1036.6 pg/ml) did not significantly differ from any other disease groups. Moreover, no significant difference was observed between any disease groups. The serum suPAR concentrations in the

Table 1 | Demographic/clinical characteristics

	All patients, $n = 69$	FSGS, $n = 38$	MCD, $n = 11$	IgAN, $n = 11$	MN, $n = 9$	Control, $n = 17$	*P*-value
Age (years)	52.8 ± 18.5	55.6 ± 16.3	41.2 ± 18.1	42.2 ± 20.8	67.9 ± 10.3^{A,B,C}	45.3 ± 15.5	0.0007
Gender (male)	41 (59.4%)	26 (68.4%)	6 (54.5%)	5 (45.5%)	4 (44.4%)	9 (52.9%)	0.5060
Alb (mg/dl)	2.58 ± 1.00	2.56 ± 0.98	2.04 ± 0.96	3.33 ± 1.11	2.41 ± 0.37	4.63 ± 0.33^{D,E,F,G}	<0.0001
TC (mg/dl)	325.5 ± 117.9	324.3 ± 102.0^{H}	422.1 ± 139.3^{I,J,K,L}	265.5 ± 101.0	284.3 ± 95.0	206.1 ± 35.7	<0.0001
CRP (mg/dl)	0.29 ± 0.43	0.30 ± 0.47	0.23 ± 0.34	0.37 ± 0.58	0.26 ± 0.27	0.09 ± 0.13	0.4187
Steroids/immunosuppressants (yes)	19 (27.9%)	11 (29.7%)	5/6 (45.5%)	1/10 (9.1%)	2/7 (22.2%)	0 (0%)	0.0321
eGFR (ml/min per 1.73 m^2)	61.9 ± 27.6	54.4 ± 25.6	76.5 ± 29.6	68.1 ± 22.0	68.4 ± 33.1	79.9 ± 15.8^{M}	0.0062
suPAR (μg/ml)	2896.8 ± 961.7	3119.0 ± 1036.6^{N}	2374.9 ± 588.8	2311.3 ± 777.1	3311.9 ± 655.3^{O}	1745.1 ± 395.4	<0.0001
UP (mg per day or mg/g Cre)	6226.2 ± 4357.3	5753.2 ± 4772.3	9138.1 ± 3874.7^{P,Q}	3874.4 ± 2476.2	7538.3 ± 2711.9^{R}	N/A	0.0211[a]

Abbreviations: Alb, albumin; ANOVA, analysis of variance; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; HSD, honest significant difference; IgAN, IgA nephropathy; MCD, minimal change disease; MN, membranous nephropathy; NA, not available; suPAR, soluble urokinase receptor; TC, total cholesterol; UP, urinary protein.

The data are presented as the mean ± s.d. Differences among the groups were analyzed by a one-way ANOVA. The multiple comparisons for age, TC, and eGFR were performed by Tukey's HSD mean separation tests. A nonparametric Steel-Dwass test was used for Alb and suPAR. Differences between the disease groups in gender and steroids/immunosuppressants administration were determined by χ^2 tests. A: $P = 0.0048$ vs. MCD; B: $P = 0.0074$ vs. IgAN; C: $P = 0.0115$ vs. control; D: $P < 0.0001$ vs. FSGS; E: $P = 0.0002$ vs. MCD; F: $P = 0.0146$ vs. IgAN; G: $P = 0.0004$ vs. MN; H: $P = 0.0016$ vs. control; I: $P = 0.0461$ vs. FSGS; J: $P = 0.0029$ vs. IgAN; K: $P = 0.0192$ vs. MN; L: $P < 0.0001$ vs. control; M: $P = 0.0066$ vs. FSGS; N: $P < 0.0001$ vs. control; O: $P = 0.0006$ vs. control; P: $P = 0.04$ vs. FSGS; Q: $P = 0.02$ vs. IgAN; R: $P = 0.048$ vs. IgAN.

[a]Because quantitative data of urinary protein for healthy controls are not available, UP in the only disease groups were analyzed by one-way ANOVA.