ORIGINAL ARTICLE

Decreased glomerular filtration as the primary factor of elevated circulating suPAR levels in focal segmental glomerulosclerosis

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Abstract

Background Circulating factor(s) has been thought to be the underlying cause of focal segmental glomerulosclerosis (FSGS), and recent studies foster this idea by demonstrating increased soluble urokinase receptor (suPAR) levels in the serum of FSGS patients.

Methods To explore the possible contribution of suPAR in FSGS pathogenesis, we analyzed serum suPAR levels in 17 patients with FSGS and compared them with those in patients with steroid-sensitive nephrotic syndrome, chronic glomerulonephritis, or non-glomerular kidney diseases.

Results Serum suPAR levels in patients with FSGS were higher than those in patients with steroid-sensitive nephrotic syndrome or chronic glomerulonephritis, but not higher than those in patients with non-glomerular kidney diseases. suPAR levels negatively correlate with estimated glomerular filtration rate and were decreased after renal transplantation in patients with FSGS as well as in those with non-glomerular kidney diseases. Furthermore, 6 FSGS patients with post-transplant recurrence demonstrated that suPAR levels were not high during the recurrence.

Conclusions Based on our results, elevated suPAR levels in FSGS patients were attributed mainly to decreased glomerular filtration. These data warrant further analysis for involvement of possible circulating factor(s) in FSGS pathogenesis.

Keywords Soluble urokinase receptor · Focal segmental glomerulosclerosis · Post-transplant recurrence

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Introduction

Focal segmental glomerulosclerosis (FSGS) is a common pattern of histopathological findings seen in children with nephrotic syndrome, and it is one of the leading causes of end-stage renal disease (ESRD) [1]. The pathogenesis of primary FSGS remains largely unclear. Intrinsic defects, which affect the structure and function of glomerular epithelial cells called podocytes, have been found in some FSGS patients [2]. In most patients with sporadic FSGS, however, serological dysregulation has been thought to be the cause because FSGS recurs in 40 % patients after transplantation and can often be treated with therapeutic post-transplant plasmapheresis [1, 3]. Post-transplant recurrence is of great clinical importance because the recurrence of proteinuria after renal transplantation has a negative impact on graft survival [4]. Recently, a case of recovery from FSGS after retransplantation of an allograft that was failing in the first recipient because of recurrent primary FSGS was reported [5], further emphasizing clinical evidence of specific disease-causing circulating factor(s) in FSGS patients.

A recent study demonstrated increased soluble urokinase receptor (suPAR) levels in the serum of FSGS patients [6, 7]. uPAR is a glycosylphosphatidylinositol (GPI)-anchored three-domain protein, which has been identified as being a cellular receptor for urokinase [8]. suPAR, its soluble form, is released into the circulation by cleavage of the GPI anchor [9]. suPAR is present under physiological conditions in the human blood, and elevated suPAR levels have also been reported in some malignant neoplasms or infectious diseases [10].

However, subsequent studies have provided conflicting results with regard to the association of serum suPAR levels with proteinuria and specific elevation of suPAR levels in FSGS patients [11–13]. Serum suPAR levels can also be affected by kidney function, complicating the issue [7, 12]. While suPAR levels at the time of post-transplant recurrence of FSGS are crucial for elucidating the role of suPAR in the pathogenesis of FSGS, serial suPAR levels across kidney transplantation have not been reported. Further, changes in suPAR levels by kidney transplantation in FSGS and other kidney diseases have also been unclear.

To determine the correlation of suPAR levels with FSGS activity, we measured suPAR levels in patients with primary FSGS and other kidney diseases across renal transplantation. In some cases, we analyzed circulating suPAR levels in the recurrent state after transplantation. The results demonstrated that circulating suPAR levels depend largely on renal function, and there was no disease-specific elevation of suPAR in FSGS compared with those in other kidney diseases with comparable estimated glomerular filtration rate (eGFR). Notably, suPAR levels were not elevated in the recurrent state after renal transplantation in the 6 FSGS cases analyzed. These data warrant further analysis for possible circulating factor(s) in FSGS pathogenesis.

Materials and methods

Samples

The study was approved by the ethics committee of the Tokyo Women's Medical University School of Medicine, the Tokyo University School of Medicine, and the Japanese Red Cross Nagoya Daini Hospital. Samples were retrieved from blood collected for tests performed for other medical purposes, but in which remaining blood volume was sufficient for our purposes. The samples were assigned codes to render them anonymous.

The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul, as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism."

Patient characteristics

We analyzed serum suPAR in 20 samples from 17 biopsy-proven FSGS patients (Table 1). Four patients were analyzed for NPHS2 mutations, and none had a causative mutation [14]. These 20 samples were obtained at the time when patients had not received kidney transplantation. Paired samples before and after kidney transplantation from 4 patients were also obtained. Paired samples before transplantation and during post-transplant recurrence were obtained from 2 FSGS patients (Table 2). From 4 other FSGS patients with post-transplant recurrence, a total of 6 heparinized samples obtained during the post-transplant recurrence were also analyzed (Table 2). Plasma suPAR levels or serum samples obtained after renal transplantation were not included in the statistical analysis in Tables 1 and 3 and Fig. 1, and are only shown in Fig. 2 and Table 2.

To compare suPAR levels of FSGS with those of other kidney diseases, suPAR levels in 26 serum samples from 20 patients with steroid-sensitive nephrotic syndrome (SSNS), 24 samples from 24 chronic glomerulonephritis (CGN) patients, and 24 samples from 24 patients with non-glomerular kidney diseases were also examined (Table 1).

Patients with steroid-sensitive nephrotic syndrome (SSNS group) exhibited normal kidney function, and none of them underwent kidney transplantation. Among 20 patients with SSNS, 7 had steroid-dependent NS and 5 had frequently relapsing NS. Seven samples were obtained when the patients had massive proteinuria.

Diagnoses of 24 patients with chronic glomerulonephritis (CGN group) included IgA nephropathy (n=12), Henoch–Schönlein purpura nephritis (n=3), membranoproliferative glomerulonephritis (n=3), and other diseases (n=6). None of this group underwent kidney transplantation.

In the non-glomerular kidney disease group, 11 patients exhibited hypo-/dysplastic kidney disease, and 2 patients were diagnosed with autosomal recessive polycystic kidney disease. The remainder of the patients had other diseases (autosomal dominant polycystic kidney disease, Joubert syndrome, Bardet–Biedel syndrome, interstitial nephritis, or another). Among them, 3 underwent analysis of paired samples before and after kidney transplantation (Fig. 2). Samples obtained after renal transplantation were not included in statistical analysis in the Tables and Fig. 1, and are only shown in Fig. 2.

Demographic data of the study population are shown in Table 1. The mean age was 13.9±8.8 years. Age, sex distributions, and serum C-reactive protein (CRP) levels were not statistically different among the four groups.

Repeat samples from patients were analyzed only when the clinical status of patients, including degree of proteinuria and renal function (change in eGFR by 25 %), changed.

Table 1 Characteristics of the study cohort. Data are presented as mean ± standard deviation

	FSGS	SSNS	CGN	Non-glomerular kidney disease
n (sample)	20	26	24	24
Sex (male/female)	8/12	15/11	12/12	16/8
Age (years)	13.1±5.2	13.7±10.2	16.4±9.1	12.4±9.3
Urinary protein (g/gCre)	5.5±6.4	2.5±4.5	1.3±3.8	0.6±1.5
CRP (mg/dL)	0.02±0.05	0.04±0.13	0.12±0.39	0.06±0.17
eGFR (mL/min/1.73 m^2)	66.3±60.1	123.7±21.5	104.7±42.3	42.4±36.8
(95%CI)	(38.2–94.5)	(115.0–132.4)	(86.9–122.6)	(26.9–58.0)
Serum suPAR (pg/mL)	2,837±1,266	1,824±859	1,686±950	3,483±1,777
(95%CI)	(2,245–3,430)	(1,477–2,171)	(1,284–2,087)	(2,732–4,233)

CRP C-reactive protein, *eGFR* estimated glomerular filtration rate, *CI* confidence interval, *suPAR* soluble urokinase receptor, *FSGS* focal segmental glomerular sclerosis, *SSNS* steroid-sensitive nephrotic syndrome, *CGN* chronic glomerulonephritis

Definition and clinical assessment

A diagnosis of post-transplant recurrence of FSGS was based on the presence of clinical recurrence of NS (massive proteinuria [more than 1 g protein in a 24-h collected urine]) after renal transplantation. Renal biopsy of recurrent cases showed diffuse effacement of podocyte foot processes by electron microscopy or FSGS region by light microscopy in the absence of transplant glomerulopathy.
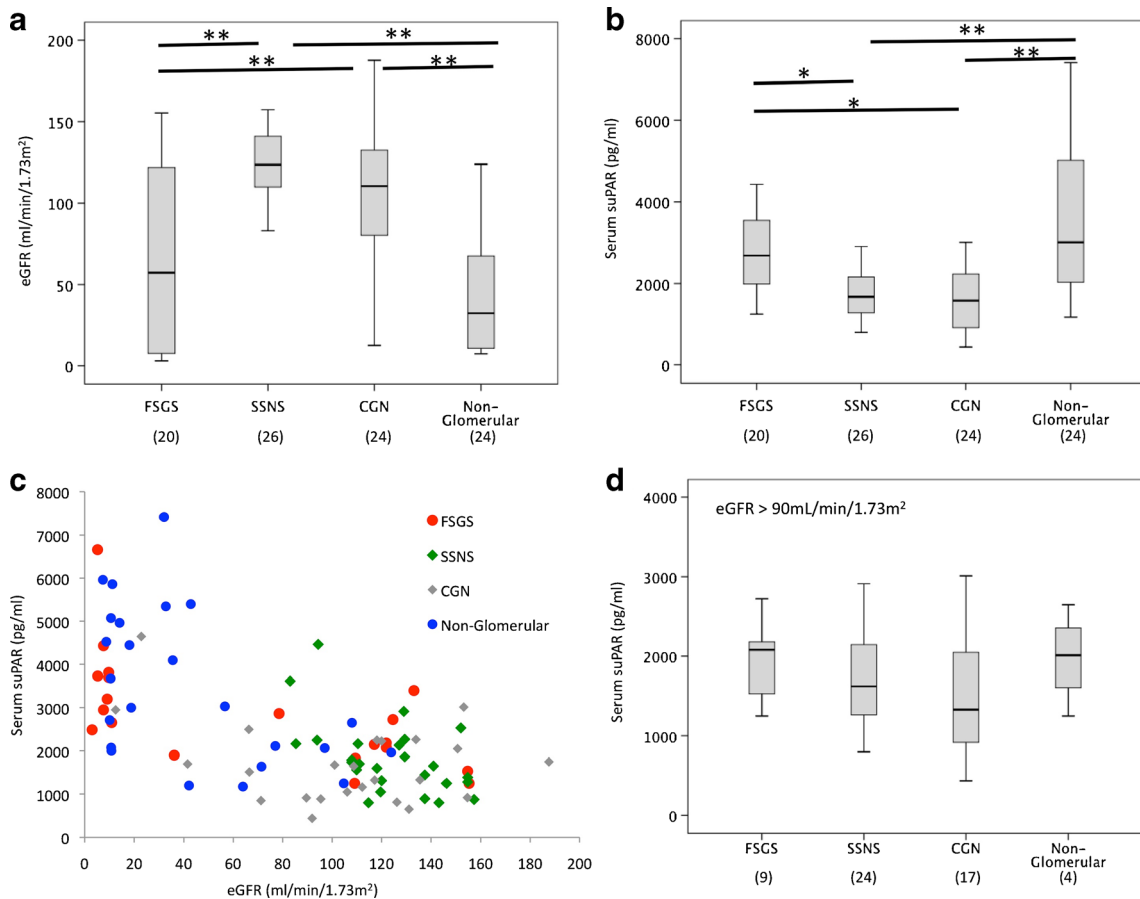

Fig. 1 **a** Estimated glomerular filtration rate (eGFR) and **b** serum suPAR levels in the four groups with numbers of the samples in each group. *Single and double asterisk* denote statistical significance *p*<0.05 and *p*<0.01 respectively. **c** Serum soluble urokinase receptor (suPAR) levels and eGFR in patients with focal segmental glomerular sclerosis (FSGS; *red*), steroid-sensitive nephrotic syndrome (SSNS; *green*), CGN (*grey*), and non-glomerular kidney disease (*blue*). **d** Serum suPAR levels in patients with normal kidney function (eGFR>90 mL/min/1.73 m^2). The differences between groups did not reach statistical significance

Table 2 Circulating suPAR levels before and during post-transplant recurrence of FSGS

Patient	Age	Sex	POD	Sample	suPAR (pg/mL)	eGFR	Urinary protein	Response to PEX
1	17.5	Female	−7	Plasma	3,010	4.7	N.D.	CR
	17.5		7	Plasma	*1,560*	39.6	27.2	
2	8.1	Male	24	Plasma	*1,854*	68.4	5.2	CR
3	6.6	Male	43	Plasma	*2,223*	23.3	5.7	CR
4	17.7	Female	−2	Serum	4,430	7.6	N.D.	PR
	17.8		37	Serum	*2,497*	42.4	282 mg/dL*	
5	17.2	Female	−7	Plasma	3,698	7.7	N.D.	Failure
	17.3		21	Plasma	*1,941*	83.6	9.5	
6	12.9	Male	−5	Serum	2,652	10.9	N.D.	Failure
	13		3	Serum	*1,568*	150	49.1	

These samples were obtained as plasma or serum

suPAR levels during post-transplant recurrence are marked in *italics*

Urinary protein levels were indicated by concentration (*asterisk*) or urinary protein creatinine ratio (g/gCre)

N.D. not determined, *POD* post-operative day, *PEX* plasma exchange, *CR* complete response, *PR* partial response, *eGFR* estimated glomerular filtration rate

Recorded clinical parameters included clinical diagnosis, sex, age, height, kidney transplant status, immunosuppressant therapy, spot urine protein/creatinine ratio, serum creatinine (milligram per deciliter) and serum CRP corresponding in time to when the suPAR specimen was retrieved. Patients with apparent infection were excluded. FSGS was diagnosed by means of renal biopsy. The eGFR in children was estimated using the Schwartz formula [15] (younger than 2 and older than 11 years of age) and the formula of the Japanese Society of Pediatric Nephrology (JSPN) [16] (2–11 years old). In patients older than 22 years, the Japanese equation for eGFR [17] was used.

Measurement of suPAR

Serum suPAR levels were measured using a Human uPAR Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA). Whole blood samples were collected in blood-collecting tubes without adding ethylenediaminetetraacetic acid (EDTA) or heparin. After clot removal by centrifugation, the supernatant was designated as serum. Heparinized plasma samples were obtained from four FSGS patients (Table 2). Serum or plasma samples were thawed once or twice to eliminate the effects of multiple freeze/thaw cycles.

Statistical analyses

Normally distributed variables were expressed as mean ± standard deviation and compared using Student's t test or one-way analysis of variance (ANOVA). Further analysis between cohorts was performed using Bonferroni's test. The Pearson product–moment correlation coefficient was measured to evaluate the association between circulating suPAR and the variables of interest, while controlling for age, proteinuria, and eGFR. p values of less than 0.05 were considered to be statistically significant. Analyses were performed using SPSS software (version 20; SPSS, Chicago, IL, USA).

Table 3 Correlation coefficients between serum suPAR levels and the indicated parameters

	FSGS	SSNS	CGN	Non-glomerular	Total
eGFR	−0.626*	−0.530*	–	−0.535*	−0.636*
Age	–	–	–	−0.545*	−0.269*
Urinary protein	–	–	–	–	–

eGFR estimated glomerular filtration rate, *FSGS* focal segmental glomerular sclerosis, *SSNS* steroid sensitive nephrotic syndrome, *CGN* chronic glomerulonephritis

Correlation coefficients are filled in the table if they are statistically significant ($p<0.05$)

*Statistical significance ($p<0.01$)

Results

Serum suPAR in FSGS and other kidney diseases

Characteristics of the patients in the four groups were shown in Table 1. Among the four groups, eGFR in the FSGS and non-glomerular kidney disease groups was lower than that in the SSNS and CGN groups (Fig. 1a). On the contrary, serum suPAR levels were higher in the FSGS and non-glomerular kidney disease groups than those in the SSNS and CGN groups (Fig. 1b). The difference in suPAR between the FSGS
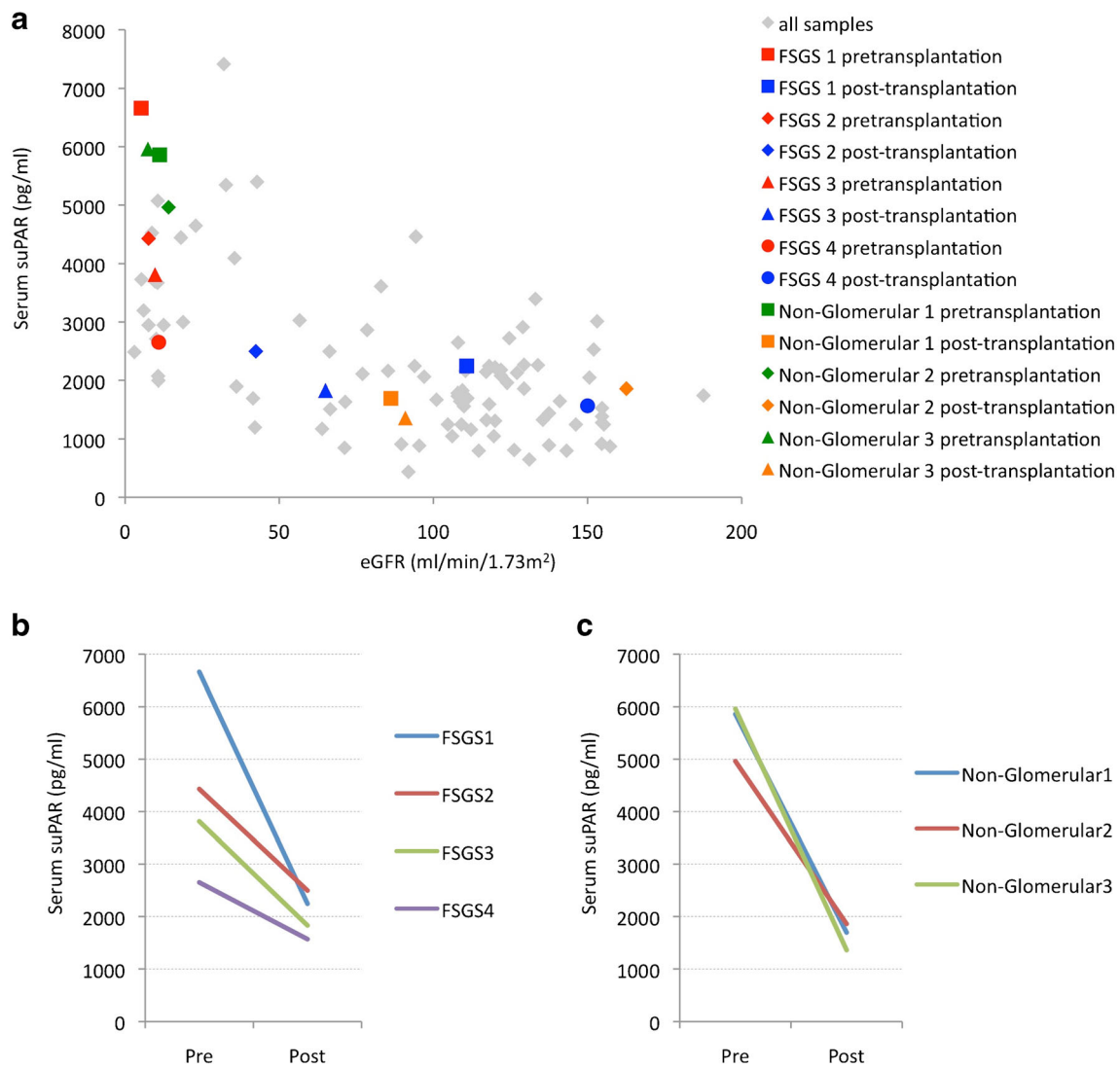
Fig. 2 Serum suPAR levels and eGFR of 7 patients who underwent kidney transplantation. Results of the analysis of paired samples obtained before and after transplantation in 4 patients with FSGS **a**, **b** and 3 patients with non-glomerular kidney disease (1 patient with Joubert syndrome and 2 patients with hypoplastic kidney) (**a**, **c**) are shown. suPAR soluble urokinase receptor, eGFR estimated glomerular filtration rate

group and non-glomerular kidney group did not reach statistical significance ($p=0.56$).

Using a data set of 94 serum samples from patients with FSGS and other diseases, we analyzed the association of serum suPAR levels with proteinuria, age, and kidney function (Table 3). Serum suPAR inversely correlated with eGFR in the overall patient cohort and in the FSGS, SSNS, and non-glomerular kidney group (Fig. 1c, Table 3). Serum suPAR levels showed a weak inverse correlation with age in the overall patient cohort and in the non-glomerular kidney disease group (Table 3). Of note, serum suPAR levels did not correlate with levels of proteinuria in the overall patient cohort or in any of the four groups (Table 3).

Because suPAR correlates negatively with eGFR, suPAR with normal eGFR (>90 mL/min/1.73 m^2) in each group was

compared (Fig. 1d). Serum suPAR from the patients with normal kidney function was $2,123\pm703$ pg/mL, and the difference among the four groups did not reach statistical significance (Fig. 1d). These results suggest that higher levels of suPAR in the FSGS and non-glomerular kidney disease groups might be attributed to lower glomerular filtration in these two groups.

Effects of renal transplantation on circulating suPAR levels

To analyze the effect of glomerular filtration on serum suPAR in FSGS patients, suPAR levels in paired serum samples obtained before and after transplantation were analyzed (Fig. 2). Four FSGS patients underwent living donor kidney transplantation because of end-stage renal failure. In all 4 of these FSGS patients, eGFR was

successfully increased after transplantation (8.4±2.5 to 92.1±48.0 ml/min/1.73 m^2, Fig. 2). Compared with suPAR levels before transplantation (4,389±1,685 pg/ml), suPAR after transplantation was significantly decreased in these FSGS patients (2,035±415 pg/ml, Fig. 2).

Paired samples from 3 patients in the non-glomerular kidney disease group who had hypo-/dysplastic kidney disease or Joubert syndrome and underwent living donor kidney transplantation were also analyzed. Before transplantation, all 3 patients exhibited elevated suPAR levels (5,594±550 pg/ml). After successful transplantation with recovery of kidney function, suPAR levels significantly decreased (1,638±255 pg/ml, Fig. 2). These results clearly indicate that high suPAR levels before transplantation in FSGS are not disease-specific, and are due to decreased glomerular filtration.

suPAR levels during post-transplant recurrence of FSGS

In 6 FSGS patients, serum or plasma samples were obtained during early post-transplant recurrence. Circulating suPAR levels before or during post-transplant recurrence in these patients are shown in Table 2. Some of these patients responded well to plasma exchange therapy, potentially supporting the pathogenic role of circulating factor(s). However, even in these patients, serum or plasma suPAR levels at recurrence in the acute phase were not significantly high (less than 2,500 pg/ml). These results indicate that post-transplant recurrence was not caused by elevated suPAR levels in these patients.

Discussion

In the present study, we analyzed circulating suPAR levels in patients with FSGS and compared them with those in patients with other kidney diseases. suPAR levels of FSGS patients were higher than those of SSNS or CGN patients, but not higher than those in patients with non-glomerular kidney diseases. Serum suPAR levels correlated inversely with eGFR, and suPAR levels were decreased after renal transplantation in ESRD patients with FSGS and hypo-/dysplastic kidney diseases. Furthermore, suPAR levels during post-transplantation recurrence were not high compared with those of patients with other kidney diseases.

Inverse correlation of glomerular filtration rate and serum suPAR levels has been verified in several previous reports [11–13, 18–20]. Our results demonstrated that suPAR levels in FSGS patients were not higher than those of other kidney diseases with comparable renal function, and that suPAR levels of patients with various kinds of diseases with decreased glomerular filtration often exceed 4,000 pg/mL (Fig. 1c). While the estimated molecular mass of uPAR is

55–60 kDa [21], circulating suPAR is produced and modified by intramolecular cleavage and variable glycosylation [21], resulting in the 22-kDa fragment in serum [6]. As proteins of this size are known to be sieved through the glomerular filter, decreased glomerular filtration can result in increased serum suPAR levels. Our results suggest that loss of functional nephrons, whether caused by FSGS or by other kidney diseases, results in high circulating suPAR levels, and recovery of kidney function by renal transplantation restores normal suPAR levels. Because overexpression of suPAR in podocytes or the administration of suPAR in mice results in proteinuria by activating podocyte β-integrin [6], elevated suPAR levels in patients with decreased glomerular filtration can lead to a vicious cycle of renal damage. Renal transplantation and recovery of glomerular filtration could contribute to amelioration of the damage caused by uremic toxins and suPAR. Removal or neutralization strategies for circulating suPAR levels [22, 23] will provide evidence for the role of suPAR in patients with kidney disease.

The correlativity of suPAR levels with proteinuria has been controversial. In some FSGS patients, the decrease in proteinuria after immunosuppressant therapy accompanies the relative decline in suPAR levels [6]. In another cohort, a weak correlation was reported between serum suPAR levels and proteinuria [13]. In the event of post-transplant FSGS recurrence, resolution of effacement of podocyte foot processes after plasmapheresis or immunosuppression has been reportedly linked to the decline in serum suPAR levels [24]. However, other groups demonstrated that the degree of proteinuria did not correlate with suPAR levels in the kidney disease patient cohort, or in the FSGS patient cohort [12, 20]. In the present study, serum suPAR levels did not correlate with proteinuria in all four groups. Moreover, increased suPAR levels during post-transplant recurrence were not observed in FSGS patients with relapsed massive proteinuria within several weeks of transplantation. Some of these patients were at least partially responsive to plasmapheresis, indicating that circulating factor(s) underlie the pathogenesis of proteinuria in these patients. These results suggest that circulating factor(s) other than suPAR, or a specific suPAR fragment that is not detected by ELISA analysis, can cause proteinuria and post-transplant recurrence in these patients.

This study has some limitations. First, our results are based on samples from small and mono-racial patients from a few institutions. Owing to the small number of patients in this study, it is still unclear whether the negative data for the specific role of suPAR in FSGS are generally applicable to FSGS with a broad range of clinical scenarios. Regarding this point, the results of a cohort study of Asian [25, 26] and European [27] FSGS patients were published during the revision process of this manuscript. These data have also shown that suPAR levels are significantly affected by renal function and have little diagnostic value. Together with our results that

suPAR levels in post-transplant recurrence were not elevated, these results question the validity of the pathogenic role of suPAR in FSGS. Second, although FSGS was confirmed by renal biopsy in all patients, secondary FSGS may not be excluded in the FSGS cohort, as genetic analysis of mutations was not performed in the majority of FSGS patients. This could have resulted in the inclusion of FSGS patients with podocyte dysfunction rather than circulating factor(s) in our study, although NPHS2 mutations are not a major cause of chronic renal insufficiency caused by sporadic steroid-resistant nephrotic syndrome or heavy proteinuria in Japanese children [14, 28]. Third, all samples were obtained during on-treatment visits and the timing of sampling during the disease course varies greatly. Therefore, the effects of therapy or other factors may have interfered with intrinsic serum factor levels [7]. Prospective studies on the measurement of suPAR levels before any treatment or at specific time points in the disease course may be required to define the genuine association between serum factors and FSGS pathogenesis.

This study examined suPAR levels before and after renal transplantation in FSGS as well as other kidney diseases. Our study revealed that elevated suPAR levels in FSGS patients are primarily attributed to low glomerular filtration. We also demonstrated that, although the number of patients is small, elevated suPAR levels are not common in the post-transplant recurrence of FSGS, even in those in whom circulating factor(s) seem to play a role in FSGS pathogenesis. These data warrant further analysis of the possible humoral mediators of FSGS pathogenesis.

Disclosure The authors declare that they have no competing interests.

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