Primary and Recurrent Focal Segmental Glomerulosclerosis Closely Link to Serum Soluble Urokinase-type Plasminogen Activator Receptor Levels

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ABSTRACT

Background. Serum soluble urokinase-type plasminogen activator receptor (suPAR) is implicated in the pathogenesis of native and recurrent focal segmental glomerulosclerosis (FSGS). It is elevated in two-thirds of subjects with primary FSGS, but not in people with other glomerular diseases that can differentiate FSGS and other glomerular diseases.

Methods. We measured the serum soluble urokinase receptor levels and determined their association with clinical and pathologic data in 86 patients with primary FSGS, 5 repeat renal biopsy FSGS, and 6 recurrent FSGS post-transplantation. Healthy controls and patients with minimal change disease and membranous nephropathy were used as controls. The suPAR levels were measured by commercial enzyme-linked immunosorbent assay kits.

Results. Patients with primary FSGS (median: 4232, interquartile range 1299–9714 pg/mL) had significantly higher levels of suPAR than those of patients with minimal change disease (median: 2784 pg/mL), membranous nephropathy (median: 3478 pg/mL), and healthy individuals (median: 1994 pg/mL). There was no significant difference in suPAR levels between the 65 patients with minimal change disease and 85 patients with membranous nephropathy. The suPAR levels increased in the 5 repeated renal biopsy FSGS and 6 recurrent FSGS post-transplantation.

Conclusions. The suPAR levels were significantly but positively correlated with FSGS, not only primary FSGS but also recurrent FSGS post-transplantation, but negatively correlated with other glomerular diseases. Thus, suPAR levels can differentiate primary FSGS and other glomerular diseases.

FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS) is a major cause of steroid-resistant nephrotic syndrome in patients and lead to end-stage renal disease [1]. It affects both native and allograft transplanted kidneys [2–4]; the recurrence rate after transplantation is approximately 30% in children and adult FSGS patients [5]. The pathophysiology and etiology of primary FSGS is still unknown. The podocyte may have a key role in the early stages and progression of the lesion observed in FSGS and the primary FSGS is regarded as a podocytopathy [6,7]. These podocytes and their foot processes constitute the renal filtration barrier [8]. Loss of podocyte structural integrity and function is thought to be the central abnormality leading to FSGS [9]. The diagnosis of FSGS is commonly based on histopathologic findings on kidney biopsy results. However, the histopathologic findings are sometimes inconsistent with the clinical impression because of the focal and segmental characteristic of FSGS. Biopsy site and time affect these findings. The development of

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Table 1. The Demographic and Clinical Parameters of Patients With Primary FSGS

Parameter	N = 86
Age (y, median, range)	32, 16–78
Gender (male/female)	48/38
Nephrotic syndrome, n (%)	84 (97.7)
24-hour urine protein (g per 24 h, median, IQR)	7.8, 5.8–14.3
Albumin (g/l, mean ± SD)	20.9 ± 8.2
Microscopic hematuria, n (%)	65 (75.6)
Serum creatinine at presentation (μmol/L; median IQR)	97.1, 68.5–160.7
Percentage of sclerosis in glomeruli (%; mean ± SD)	20.1 ± 17.8
Percentage of global sclerosis in glomeruli (%; mean ± SD)	2.5 ± 5.8
Percentage of segmental sclerosis in glomeruli (%; mean ± SD)	13.0 ± 12.5
Arteries hyaline degeneration, n (%)	70 (81.4)
Acute kidney injury, n (%)	26 (30)

Abbreviations: FSGS, focal segmental glomerulosclerosis; IQR, interquartile range.

adjunctive tests might provide additional insight into useful features of FSGS.

Recently, some studies confirmed that circulating permeability factors likely play a major role in the pathogenesis of primary and recurrent FSGS [10]. Furthermore, compelling evidence suggests that the soluble urokinase-type plasminogen activator receptor (suPAR) is one of these causative permeability factors [11]. In two studies by Wei et al [11,12], the majority of participants with primary FSGS had significantly higher levels of suPAR compared with those with other glomerular diseases, such as minimal change disease (MCD), membranous nephropathy (MN), et cetera. Furthermore, patients with recurrent FSGS had higher levels of suPAR before transplantation and during the course of FSGS recurrence post-transplantation [11]. In our study, we measured serum suPAR levels in a variety of primary glomerular diseases including primary FSGS, MCD, and MN to differentiate FSGS with other primary glomerular diseases, especially MCD.

MATERIALS AND METHODS

Patients

According to the definition of primary FSGS in the Columbia classification, 86 patients with primary FSGS with complete clinical and pathologic data, diagnosed in the Kidney Disease Center, The First Affiliated Hospital, College of Medicine, Zhejiang University, between January 2004 and January 2012, were enrolled in this study. FSGS secondary to other primary glomerular diseases, such as lupus nephritis, MN, pauci-immune glomerulonephritis, diabetic nephropathy, and immunoglobulin A (IgA) nephropathy, were excluded. The clinical and pathologic data were collected at the time of presentation. Of 86 patients, for 5 patients the second biopsy confirmed FSGS but the first biopsy had diagnosed MCD.

Moreover, 65 patients with MCD, 85 patients with MN, 5 patients with repeated renal biopsy, 6 patients with recurrent FSGS post-transplantation, and 69 age- and gender-matched physical examination subjects were used as disease and healthy controls.

Histopathology

Renal biopsy was performed at the time of diagnosis. Biopsy specimens were evaluated with light, direct immunofluorescence, and electron microscopy, and were diagnosed by two pathologists. Both pathologists examined the biopsy results separately, being blinded to each other as well as patients' clinical data. Differences in diagnosis between the two pathologists were resolved by re-reviewing the biopsy results and coming to a consensus.

For light microscopy, all biopsy specimens were formalin fixed (10%, pH 7.2) and paraffin embedded according to standard procedures. For histopathologic analysis, 2-μm sections were stained with hematoxylin-eosin, periodic acid-Schiff, Masson's tri-chrome (Masson), and periodic acid-silver methenamine. For direct immunofluorescence, IgG, IgM, IgA, C3c, C1q, fibrinogen, and albumin were detected by fluorescein isothiocyanate–conjugated antibodies (Dako, Copenhagen, Denmark) on frozen tissues. The fluorescence intensity was determined using a semiquantitative scale of 0 to 4+. For electron microscopy, in brief, the tissue was fixed in 2.5% glutaraldehyde and 1% osmium tetroxide, then dehydrated in graded acetone, and embedded in Epon 812. Ultrathin sections were cut by ultrathin microtome (Leica UTR, Leica, Germany) at a thickness of 80 nm and placed on nickel grids. Then, ultrathin sections were stained with uranyl acetate and examined by a transmission electron microscope (Tecnai 10, Philips, Holland).

The renal biopsy findings were categorized according to the Columbia FSGS classification system. Eighty-six patients with any of the structural manifestations of FSGS were entered into the registry.

Sample Collection

Serum samples of patients were collected at the day of renal biopsy. The serum samples from 69 age- and gender-matched physical examination healthy donors were collected as normal controls. Serum was separated within 30 min of blood draw and stored at −80°C in multiple aliquots until analyzed. Repeated freeze/thaw cycles were avoided.

Quantification of suPAR

We detected the concentration of serum suPAR in human subjects using Quantikine Human uPAR Immunoassay (R&D Systems Minneapolis, MN, USA), following the manufacturers protocol. In brief, the principle of the assay was a five-step procedure: 1) 96-well polystyrene microplates were pre-coated with a mouse monoclonal

Table 2. The Demographic Data and Serum suPAR Levels of Patients and Healthy Controls

	Primary FSGS	Minimal Change Disease	Membranous Nephropathy	Healthy Controls
Number of subjects	86	65	85	69
Age (y: median, range)	32, 16–78	39, 18–69	51, 34–75	35, 20–46
Gender (male/female)	48/38	34/31	50/35	39/30
Serum suPAR (pg/mL) (median, IQR)	4232, 1299–9714	2784, 1190–5529	3478, 1008–8460	1994, 1241–3327

Abbreviations: suPAR, soluble urokinase-type plasminogen activator receptor; FSGS, focal segmental glomerulosclerosis; IQR, interquartile range.