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Serum-soluble urokinase receptor levels do not distinguish focal segmental glomerulosclerosis from other causes of nephrotic syndrome in children

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In this prospective study, we measured serum levels of the soluble urokinase receptor (suPAR) in pediatric patients with nephrotic syndrome of various etiologies. Mean levels of suPAR were 3316 pg/ml in 99 patients with steroid-resistant focal segmental glomerulosclerosis and 3253 pg/ml in 117 patients with biopsy-proven minimal change disease, which were similar to that of 138 patients with steroid-sensitive nephrotic syndrome (3150 pg/ml) and 83 healthy controls (3021 pg/ml). Similar proportions of patients in each group had suPAR over 3000 pg/ml. Compared with controls, suPAR levels were significantly higher in patients with focal segmental glomerulosclerosis (FSGS) and estimated glomerular filtration rate (eGFR) under 30 ml/min per 1.73 m^2 (6365 pg/ml), congenital nephrotic syndrome (4398 pg/ml), and other proteinuric diseases with or without eGFR under 30 ml/min per 1.73 m^2 (5052 and 3875 pg/ml, respectively; both significant). There were no changes following therapy and during remission. Levels of suPAR significantly correlated in an inverse manner with eGFR ($r = -0.36$) and directly with C-reactive protein ($r = 0.20$). The urinary suPAR-to-creatinine ratio significantly correlated with proteinuria ($r = 0.25$) in 151 patients and controls. Using generalized estimating equations approach, serum suPAR significantly correlated with eGFR (coefficient = −13.75), age at sampling (2.72), and C-reactive protein (39.85). Thus, serum suPAR levels in nephrotic syndrome are similar to controls, and do not discriminate between FSGS, minimal change disease, or steroid-responsive illness.

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Focal segmental glomerulosclerosis (FSGS) is an important cause of steroid-resistant nephrotic syndrome in children. Clinical and experimental evidence suggests the pathogenic role of circulating permeability factors, including soluble urokinase plasminogen activating receptor (suPAR).[1,2] Serum suPAR levels were found to be elevated in Caucasian adults with primary FSGS[3] and in two cohorts of children with FSGS from Europe and the United States.[4] Animal models further showed that membrane-bound uPAR activates podocyte β3 integrin signaling, resulting in foot process effacement and a glomerulopathy resembling FSGS.[3,5] However, recent observations have questioned the utility of suPAR in mediating proteinuria in childhood-onset FSGS and other proteinuric kidney diseases across diverse populations.[6,7]

This prospective study examined blood levels of suPAR in a large, carefully phenotyped cohort of Indian children with steroid-resistant FSGS and minimal change disease, steroid-sensitive nephrotic syndrome, other proteinuric chronic kidney diseases (CKDs), and healthy controls. Sequential specimens were collected in a subgroup of patients to examine the relationship of serum suPAR during nephrotic-range proteinuria and remission. Urinary levels of suPAR were estimated in a proportion of patients with steroid-resistant FSGS, minimal change disease, and controls.

RESULTS

During April 2012 to May 2013, we collected 617 blood samples from 469 patients and 83 controls. Baseline characteristics of patients are shown in Table 1. Patients with FSGS were older than those with steroid-resistant minimal change disease ($P = 0.005$), similar in age to patients with steroid-sensitive nephrotic syndrome and healthy controls, and younger than other proteinuric CKD ($P < 0.0001$). The estimated glomerular filtration rate (eGFR) was below 30 ml/min per 1.73 m^2 in 21 patients with steroid-resistant FSGS, 38 with other proteinuric kidney diseases, and 2 with congenital nephrotic syndrome.

Table 1 | Characteristics of patients ($n = 469$) and healthy controls ($n = 83$)

	Steroid-resistant nephrotic syndrome		Steroid-sensitive nephrotic syndrome ($n = 138$)	Congenital nephrotic syndrome ($n = 9$)	Proteinuric chronic kidney disease ($n = 85$)	Controls ($n = 83$)
	FSGS ($n = 120$)	MCD ($n = 117$)				
Boys	83 (69.2%)^^	85 (72.7%)^^	113 (81.9%)*	7 (77.8%)	63 (74.1%)	42 (50.6%)
Age at onset, months	54.3 ± 38.7 (20.9–78.3)	41.9 ± 30.9** (20.5–54.4)	42.8 ± 30.9** (20.9–54.1)	1.1 ± 0.9** (0.5–1.3)	82.1 ± 48.9*** (46.6–124.2)	—
Age at sampling, months	113.1 ± 57.1 (62.0–152.7)	93.2 ± 51.8** (57.8–126.7)	106.8 ± 49.0 (71.3–136.8)	12.2 ± 8.0*** (8.2–20.5)	150.0 ± 46.3*** (113.9–184.8)	100.1 ± 49.7 (60.0–204)
Hypertension	72 (62.1%)	46 (39.3%)**	52 (39.1%)***	1 (11.1%)*	51 (60.0%)	5 (6.0%)
eGFR, ml/min per 1.73 m^2	84.9 ± 42.9 (61.1–109.2)	105.8 ± 32.8*** (83.4–129)	105.2 ± 29.6*** (86.4–124.6)	51.2 ± 34.9* (35.8–59.1)	59.3 ± 43.1*** (22.1–91.5)	95.6 ± 25.4 (79.5–111.5)
Serum albumin, g/dl	3.0 ± 1.2 (2.0–4.0)	3.2 ± 1.3 (2.1–4.2)	3.2 ± 1.3 (2.2–4.4)	1.5 ± 0.4** (1.3–1.7)	3.6 ± 1.0** (2.8–4.4)	4.8 ± 0.3 (4.6–5.0)

Abbreviations: eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; MCD, minimal-change disease.
Values represent proportions (%) or mean ± s.d. (interquartile range).
P versus FSGS = *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$; ^^*P* versus controls = $P < 0.01$.

Of 237 patients with steroid-resistant nephrotic syndrome secondary to histologically proven FSGS or minimal-change disease, 62 (26.2%) patients were sampled at diagnosis of corticosteroid resistance. Therapies at the time of sampling included calcineurin inhibitors ($n = 62$), mycophenolate mofetil (MMF; $n = 14$), or prednisone ($n = 78$) with or without enalapril; 21 patients with FSGS and CKD stages 4 and 5 were receiving supportive care. Of 34 patients with FSGS screened for mutations in two genes, 2 had heterozygous *WT1* mutation associated with the Frasier syndrome (IVS9 + 4C > T in intron 9 and IVS9 + 5G > A in exon 9), 3 had heterozygous variation *R229Q*, and one each had heterozygous *NPHS2* mutation at c.890C > T in exon 8 and c.557_560delTAAT in exon 5.

Of 138 patients with steroid-sensitive nephrotic syndrome, 109 were not receiving any therapy; others were receiving prednisone alone ($n = 10$), or levamisole ($n = 7$), MMF ($n = 3$), or a calcineurin inhibitor ($n = 9$). Disorders in patients with other proteinuric CKDs were glomerular diseases in 63 and nonglomerular conditions in 22 patients. The former included membranoproliferative glomerulonephritis type 1 ($n = 13$) or type 2 ($n = 8$), pauci-immune crescentic glomerulonephritis ($n = 7$), secondary FSGS ($n = 7$; associated with reflux nephropathy, crescentic glomerulonephritis, or hypertension), chronic glomerulonephritis ($n = 6$), Alport syndrome ($n = 5$), hemolytic uremic syndrome ($n = 4$), IgA nephropathy ($n = 4$), and others ($n = 9$). Nonglomerular conditions were posterior urethral valves ($n = 8$), neurogenic bladder ($n = 5$), nephronophthisis ($n = 3$), solitary kidney ($n = 2$), and unknown causes ($n = 4$).

Sixty patients had more than one blood sample in different states of illness. Table 2 shows corresponding eGFR, urine protein-to-creatinine ratio (Up/Uc), and highly sensitive C-reactive protein (CRP) levels in samples from various categories. Levels of CRP were similar in patients with steroid resistance secondary to FSGS with eGFR > 30 ml/min per 1.73 m^2, minimal change disease, and steroid-sensitive nephrotic syndrome (supplementary Figure 1; online; analysis of variance (ANOVA), $P = 0.067$). Values in healthy controls (0.9 ± 1.2 mg/l) were lower than for FSGS (2.4 ± 6.3 mg/l; $P = 0.033$). CRP values were similar within subgroups of the above categories (all ANOVA $P > 0.1$). Compared with controls, these values were significantly higher in patients with CKD stages 4 and 5 associated with FSGS (4.0 ± 7.2 mg/l; $P = 0.0003$) and other proteinuric diseases (6.6 ± 16.9 mg/l; $P = 0.003$).

suPAR levels and underlying etiology

Figure 1 shows serum suPAR in samples from various categories ($n = 617$). The mean ± s.e.m. levels were similar in patients with steroid-resistant nephrotic syndrome associated with FSGS (3316.1 ± 101.2 pg/ml) and minimal change disease (3252.9 ± 115.7 pg/ml; $P = 0.69$). Levels in patients with FSGS were comparable to steroid-sensitive nephrotic syndrome (3149.6 ± 96.8 pg/ml; $P = 0.24$) and healthy controls (3021.1 ± 155.5 pg/ml; $P = 0.11$), but lower than proteinuric CKD and eGFR > 30 ml/min per 1.73 m^2 (3875.0 ± 220.4 pg/ml; $P = 0.009$) or proteinuric CKD and eGFR < 30 ml/min per 1.73 m^2 (5051.6 ± 386.0 pg/ml; $P < 0.0001$).

Table 3 shows serum suPAR in samples from subgroups within each clinical category ($n = 617$). The levels were similar in patients with FSGS with nephrotic-range proteinuria (3320.7 ± 137.4 pg/ml) compared with remission (3309.9 ± 150.1 pg/ml, $P = 0.96$). Patients with FSGS and eGFR < 30 ml/min per 1.73 m^2 (6365.1 ± 605.4 pg/ml) showed higher levels compared with FSGS and eGFR > 30 ml/min per 1.73 m^2 (3316.1 ± 101.2 pg/ml, $P = 0.0003$), and healthy controls ($P < 0.0001$). Levels of suPAR were similar in patients with steroid-resistant minimal change disease during nephrotic-range proteinuria (3303.7 ± 186.6 pg/ml) and remission (3203.6 ± 139.6 pg/ml; $P = 0.67$). Values were significantly higher in patients with congenital nephrotic syndrome compared with controls ($P = 0.0009$). Patients with steroid-sensitive nephrotic syndrome sampled at the initial episode, remission, or relapse showed similar suPAR levels (ANOVA, $P = 0.45$) that were also similar to controls (ANOVA, $P = 0.42$).