Serum Soluble Urokinase-Type Plasminogen Activator Receptor Levels and Idiopathic FSGS in Children: A Single-Center Report

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Summary

Background and objectives FSGS is the primary cause of childhood nephrotic syndrome leading to ESRD. Permeability factors, including circulating serum soluble urokinase-type plasminogen activator receptor (suPAR), have been postulated as putative causes in adults with primary FSGS. Similar results have yet to be proven in children.

Design, setting, participants, & measurements This cross-sectional single-center study assessed the association of serum suPAR in children with FSGS or other glomerular and nonglomerular kidney diseases.

Results This study examined 110 samples retrieved from 99 individuals (between January 2011 and April 2012), aged 1–21 years; of these individuals, 20 had primary FSGS, 24 had non-FSGS glomerular disease, 26 had non-glomerular kidney disease, and 29 were healthy controls. suPAR levels were not significantly different in children with FSGS, non-FSGS glomerular disease, and healthy controls (P > 0.05). However, suPAR levels (median [25%–75%]) were higher in children with nonglomerular kidney disease (3385 pg/ml [2695–4392]) versus FSGS (2487 pg/ml [2191–3351]; P < 0.05). Female patients with nephrotic-range proteinuria (U-Pr/Cr > 2) had lower suPAR levels than those without proteinuria (2380 pg/ml [2116–2571] versus 3125 pg/ml [2516–4198], respectively; P < 0.001). This trend was not seen among male participants; suPAR levels in all female participants were lower than in male participants (P = 0.03). Thirty-four patients studied were kidney transplant recipients; transplant status was not associated with suPAR levels in patients with FSGS or non-FSGS diagnoses, independent of proteinuria, race, or sex (P > 0.05).

Conclusions On the basis of these results, circulating suPAR is unlikely the leading cause for childhood idiopathic FSGS.

Introduction

Primary FSGS is the most common histologic form of nephrotic syndrome resulting in ESRD in children and adults. Although the glomerular insult in FSGS was once thought to be secondary to immunologic dysregulation, numbers of identified disease-causing mutations resulting in defects in the structure and function of podocytes and anchoring elements continue to expand (1), supplanting an immunologic basis for disease. Nonetheless, evidence that FSGS may be caused by a circulating factor continues to accumulate since its first proposition in the early 1970s (2). Evidence for a circulating factor is supported by both clinical studies and animal models. Clinical investigations have shown that FSGS may recur after transplantation (3,4), and can be treated with therapeutic plasmapheresis, inducing disease remission through removal of a possible circulating factor (5,6). Moreover, resolution of recurrent FSGS after allograft removal and graft re-transplantation into a second recipient without disease recurrence has recently been reported, further emphasizing a possible role for a soluble, inherent disease-causing factor (7). Similarly in animal models, rapid induction of proteinuria and foot-process effacement has been demonstrated after infusion of human FSGS serum into wild-type rats, providing additional support for a serum permeability factor as a causative agent in FSGS (8,9).

Recent studies suggest the soluble form of the urokinase-type plasminogen activator receptor (suPAR), a protein derived from cleavage and release from the cell membrane-bound urokinase plasminogen activator receptor, as the responsible causative serum factor in adult patients with primary FSGS (10).

To determine the potential clinical role of suPAR in pediatric practice, we measured serum suPAR concentration in children with primary FSGS, non-FSGS glomerular disease, or nonglomerular kidney disease, and in healthy controls.
Materials and Methods

We studied 99 individuals, aged 1–21 years. Twenty patients had primary FSGS, 24 patients had non-FSGS glomerular disease, 26 patients had nonglomerular kidney disease, and 29 were healthy controls. Patients were identified by searching the electronic medical record, in which diagnosis codes for nephrotic syndromes, FSGS, CKD, and obstructive uropathy linked to encounters from January 2011 through April 2012 were queried. Diagnoses were confirmed by review of the medical record (radiologic, laboratory, and pathology reports). De-identified study samples were retrieved from blood obtained during testing performed for other purposes but in which remaining blood volume was sufficient for our purposes; timing of specimen collection was not uniform between patients and thus occurred at varying time points of disease course. In patients in whom repeat samples were available, each was at least 14 days apart; samples were analyzed as unique specimens. Repeat samples were analyzed in patients only as their nephrotic syndrome status changed. The study was approved by our institutional review board; requirement to obtain informed consent was waived as there was no direct patient contact or study-specific blood sampling.

Clinical parameters recorded included clinical diagnosis, sex, age (years), height (centimeters), kidney transplant status, immunosuppressant and angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) therapy status, obese versus nonobese status, spot urine protein/creatinine ratio (U-Pr/Cr), serum creatinine (milligrams per deciliter) and serum albumin (grams per deciliter) corresponding in time to when the suPAR specimen was retrieved. Patients were classified as having nephrotic-range proteinuria if U-Pr/Cr at time of suPAR sample collection was ≥2 (11,12). Patients were grouped into four cohorts: FSGS and non-FSGS glomerular disease based on kidney biopsy findings, nonglomerular kidney disease based on clinical diagnosis (hypoplasia/dysplasia, obstructive uropathy, or cortical necrosis), or healthy controls. Estimated GFR (eGFR) was calculated using the Schwartz formula (13). Obesity was defined as body mass index >95th percentile for sex/age (14).

Serum suPAR was measured with the Quantikine Human suPAR Immunoassay (R&D Systems). The intra-assay and inter-assay coefficients of variation were 4.6% and 5.5%, respectively. Peripheral venous blood was drawn by standard venipuncture into a Vacutainer (Becton Dickinson, Franklin Lakes, NJ) containing EDTA or heparin, inverted to mix, and centrifuged at 1000×g, 4°C. Serum was aliquoted into tubes and stored at −80°C. Samples were thawed once to eliminate the effects of multiple freeze/thaw cycles.

The primary outcome measure was defined as measured serum suPAR level (picograms per milliliter). Data were analyzed using comparative statistics: The t test was used for comparison of two groups of parametric data. Mann-Whitney U and ANOVA on ranks were performed, as appropriate, on nonparametric data and results were presented as medians with interquartile ranges (IQRs). Further analysis of suPAR levels between cohorts was completed (ANOVA with Tukey post hoc comparisons: general linear model) accounting for sex, ethnicity, and presence or absence of nephrotic-range proteinuria. All analyses were performed using PASW Statistics software (version 18.0; SPSS, Chicago, IL). α was set at <0.05 for significance.

Given our number of healthy controls (n=29), we were able to demonstrate 82.5% power (β=0.83) to detect a difference of 400 pg/ml in serum suPAR level compared with the FSGS patient cohort.

Results

We retrieved 110 samples from 99 patients. In patients in whom repeat samples were analyzed, each was, at minimum, 14 days apart; samples were analyzed independently. Of the 99 participants, 8 patients had repeat samples analyzed, 7 patients had two samples analyzed, and 1 patient had the remaining four samples analyzed, evenly distributed over 1 year. Repeat samples were retrieved in patients only as their nephrotic syndrome status changed. Age, sex, and race distributions were similar between patients with FSGS, non-FSGS glomerular disease, and nonglomerular kidney disease (Table 1). Of the patients with FSGS, one had an identified genetic mutation, WT1, Denys-Drash syndrome. There were no obese patients at the time of sample retrieval. Patients in our study population received various immunosuppressants (tacrolimus, mycophenolate mofetil, sirolimus, azathioprine, and corticosteroids) as well as ACEi/ARB therapies (Table 2). There was no significant difference in serum suPAR levels when comparing patients receiving immunosuppressant therapy with those patients who were not (median 3190 pg/ml [2515–4677 pg/ml] versus 2915 pg/ml [2410–3115 pg/ml]; P=0.33), and when comparing patients receiving ACEi/ARB therapy with those who were not (2897 pg/ml [2482–3792 pg/ml] versus 2525 pg/ml [2335–3075 pg/ml]; P=0.13).

Serum suPAR and Kidney Disease Diagnoses

Serum suPAR levels did not differ significantly in patients with FSGS compared with non-FSGS glomerular disease or healthy controls (P>0.05). Specifically, suPAR levels among patients with minimal change disease (MCD), membranoproliferative GN, IgA nephropathy, and lupus nephritis were similar to those of patients with FSGS. Conversely, when comparing children with nonglomerular CKD (median 3385 pg/ml [2695–4392 pg/ml]) with FSGS (median 2487 pg/ml [2191–3351 pg/ml]), suPAR serum levels were higher in the former cohort (P<0.05) (Figure 1 and Table 3).

Serum suPAR Levels and Proteinuria

Patients with nephrotic-range proteinuria, independent of diagnosis, did not have significantly higher suPAR levels than patients without proteinuria (median 3190 pg/ml [2437–3787 pg/ml] and 2577 pg/ml [2282–3225 pg/ml], respectively; P=0.73). The degree of proteinuria, as measured by the U-Pr/Cr, did not correlate with suPAR levels in the overall patient cohort (r=0.19, P=0.15), or in the FSGS patient cohort alone (r=0.13, P=0.95) (Figure 2).

Serum suPAR Levels and Filtering Function

Serum suPAR was found to inversely correlate with eGFR in our overall cohort (r=0.26, P=0.02). A similar
association was found among patients with FSGS ($r=0.53$, $P=0.003$) (Figure 3). Conversely, in patients with non-FSGS glomerular disease or nonglomerular kidney disease, an association between serum suPAR level and eGFR was not found ($P>0.05$ for both).

**Table 1. Demographic data of the study population**

<table>
<thead>
<tr>
<th>Demographic</th>
<th>FSGS; $n=20$</th>
<th>Non-FSGS Glomerular Disease; $n=24$</th>
<th>Nonglomerular CKD; $n=26$</th>
<th>Healthy Controls; $n=29$</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>12.1±5.0</td>
<td>10.9±6.7</td>
<td>14.0±5.1</td>
<td>n/a</td>
<td>0.14</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 12 (60)</td>
<td>13 (54)</td>
<td>18 (69)</td>
<td>n/a</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>Female 8 (40)</td>
<td>11 (46)</td>
<td>8 (31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td>Black 6 (30)</td>
<td>7 (29)</td>
<td>3 (11)</td>
<td>n/a</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Caucasian 8 (40)</td>
<td>8 (33)</td>
<td>15 (58)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Latino 6 (30)</td>
<td>9 (38)</td>
<td>7 (27)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD or n (%). N=110 samples in 99 patients. In repetitive studies for a given patient, there was a minimum of a 14-day interval. Because we performed a retrospective, de-identified analysis for the healthy controls, we could not ascertain ethnicity, age, and sex. $P$ values represent comparisons between groups: FSGS, Non-FSGS Glomerular Disease, Nonglomerular CKD. n/a, not available.

**Table 2. Clinical descriptors of our study population**

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>FSGS; $n=20$</th>
<th>Non-FSGS Glomerular Disease; $n=24$</th>
<th>Nonglomerular CKD; $n=26$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunosuppression</td>
<td>5 (25)</td>
<td>8 (33)</td>
<td>11 (42)</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>2 (10)</td>
<td>1 (0.4)</td>
<td>0</td>
</tr>
<tr>
<td>Tacrolimus/mycophenolate mofetil</td>
<td>9 (45)</td>
<td>5 (21)</td>
<td>14 (54)</td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>1 (0.50)</td>
<td>7 (29)</td>
<td>0</td>
</tr>
<tr>
<td>Mycophenolate mofetil/sirolimus</td>
<td>1 (0.5)</td>
<td>0</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>1 (0.5)</td>
<td>1 (0.4)</td>
<td>0</td>
</tr>
<tr>
<td>Corticosteroid</td>
<td>0</td>
<td>2 (0.8)</td>
<td>0</td>
</tr>
<tr>
<td>Angiotensin converting enzyme inhibitor</td>
<td>7 (35)</td>
<td>10 (42)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Angiotensin inhibitor/angiotensin receptor blocker</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transplant recipients ($n=34$)</td>
<td>9 (45)</td>
<td>5 (21)</td>
<td>16 (62)</td>
</tr>
<tr>
<td>Obese</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Estimated GFR (ml/min per 1.73 m$^2$)</td>
<td>81.9±47.3</td>
<td>92.4±43.2</td>
<td>84.4±49.1</td>
</tr>
<tr>
<td>Urine protein/creatinine ratio</td>
<td>2.14 (0.31–9.90)</td>
<td>0.92 (0.28–3.61)</td>
<td>0.65 (0.25–2.72)</td>
</tr>
</tbody>
</table>

Data are presented as $n$ (%), mean ± SD, or median (interquartile range). $P=0.60$ for estimated GFR; $P=0.39$ for urine protein/creatinine ratio.

Serum suPAR Levels and Sex

suPAR levels in all female children with kidney disease, irrespective of diagnosis, were lower than in male patients (2577 pg/ml [2302–3332 pg/ml] versus 3230 pg/ml [2570–3827], respectively; $P=0.02$). Female patients with nephrotic-range proteinuria (U-Pr/Cr >2) had lower suPAR levels than those without proteinuria (2380 pg/ml [2116–2571 pg/ml] versus 3125 pg/ml [2516–4196 pg/ml], respectively; $P<0.001$).

Serum suPAR Levels and Age

Age at time of blood suPAR measurement was inversely associated with suPAR concentration for the overall patient cohort ($r=0.30$, $P=0.01$). This association was maintained when controlling for transplant status, proteinuria, and eGFR ($P<0.001$). Among patients with FSGS, an association between age and serum suPAR was not demonstrated ($P=0.66$); conversely, among patients with non-FSGS diagnoses, age was inversely related to suPAR level, even when controlling for transplant status, proteinuria, and eGFR ($P=0.01$).

Serum suPAR Levels and Ethnicity

Serum suPAR levels did not differ significantly between patients of different ethnic backgrounds when comparing our patient cohorts. Notably, only in African-American children were suPAR levels higher numerically in patients with FSGS versus non-FSGS CKD diagnoses (3557 pg/ml [2705–4170 pg/ml] versus 3280 pg/ml [2787–3752 pg/ml], respectively; $P=0.69$). The converse was true in both Latino and Caucasian patients. The difference between FSGS and non-FSGS suPAR levels was greater and significant in Caucasian children (2165 pg/ml [2017–2362 pg/ml] versus 3145 pg/ml [2550–4460 pg/ml], respectively; $P<0.001$).
Serum suPAR Levels and Transplantation

Transplant status was not associated with suPAR in patients with FSGS or non-FSGS diagnoses, independent of proteinuria, race, or sex (P=0.05). Serum suPAR levels in patients with FSGS did not differ significantly from those in healthy controls. suPAR, soluble urokinase receptor-type plasminogen activator. The median for each group is represented by the horizontal line.

Discussion

The findings of this study underscore the complexity of putative mechanisms of idiopathic childhood FSGS. With
our study results, we were unable to support recently published data that identified suPAR as a circulating, causative FSGS factor (7). Rather, we showed that suPAR concentrations did not differ when comparing children with FSGS, non-FSGS glomerular disease, and healthy controls. Furthermore, suPAR levels did not correlate with the degree of proteinuria. Given the absence of a clear association between suPAR and clinical disease, it appears that clinical measurement of serum suPAR has low value as a diagnostic tool in pediatric patients with primary nephrotic syndromes.

Our analysis of human serum samples demonstrated that median suPAR levels were elevated above the diagnostic threshold for FSGS posited by Wei and colleagues (3000 pg/ml) (10) in all patients with CKD, except those with FSGS in which suPAR was considerably lower. Our finding that suPAR levels in healthy controls were similar to those reported in comparable children (15–18) imparts validity to the suPAR levels we measured in our patients with forms of CKD. Numerous studies have shown serum suPAR levels to be increased with nonspecific inflammation or immune activation (15–23). In fact, urokinase plasminogen activator receptor is expressed on various cell types (neutrophils, lymphocytes, monocytes, and endothelial and tumor cells). After cleavage from the cell surface, suPAR is found in organic fluids (blood, saliva, cerebrospinal fluid, and urine) and takes part in various immunologic functions, including cell adhesion, migration, proteolysis, and tissue remodeling, among others (10,24,25). Our results of suPAR elevation in our patient cohorts with non-FSGS diagnoses might be explained by low-grade inflammation that is activated in children with certain forms of CKD (26). Our findings might be explained by results recently described by Wei and colleagues (27), which showed that C-reactive protein levels were low and did not correlate with serum suPAR levels in two large cohorts of patients with FSGS, thereby supporting the classification of FSGS as a noninflammatory disease. Serum suPAR levels were not elevated in our FSGS cohort, but were similar to those in control patients (both groups in which inflammation likely plays a lesser role), and were higher in non-FSGS diagnoses of CKD, where inflammation may have a greater effect.

Although we were unable to support the role of suPAR as a potential causative circulating factor of FSGS in children, we found differences in serum levels between

Figure 2. Correlation analysis of suPAR with proteinuria in pediatric CKD and FSGS patients. (A) Correlation between serum suPAR levels and proteinuria in the overall patient study cohort ($r=0.19$, $P=0.15$). (B) Correlation between serum suPAR levels in patients with FSGS diagnoses when studied alone ($r=0.13$, $P=0.95$). There was no significant correlation between levels of serum suPAR and proteinuria in the overall patient cohort, or in patients with FSGS when studied alone. suPAR, soluble urokinase-type plasminogen activator receptor; U-Pr/Cr, urine protein/creatinine ratio.
sexes as well as trends toward differing patterns of suPAR among patients of different ethnic backgrounds. These findings warrant further consideration of mechanistic possibilities. Certain ethnicities may have an exaggerated suPAR response. Prior literature has established that suPAR levels are elevated in adults of African origin compared with Caucasians, and may be an indicator of cardiovascular disease (28). Although we did not find a consistent elevation in suPAR levels among African-American patients compared with other ethnicities, our data did show a trend toward higher serum suPAR levels in African-American patients with FSGS as compared with African-American patients with FSGS diagnoses when studied alone ($r=0.53$, $P=0.003$). In the overall study cohort, and in FSGS patients when studied alone, there was a significant inverse correlation between serum suPAR levels and eGFR.

Literature on variation by sex in serum suPAR levels is limited mostly to homogenous northern European populations, and contrary to our findings, demonstrated that suPAR levels are higher in female patients than in male patients (27,30,31). Again, the discrepancy in our findings may be explained by the age ranges of our cohort. Interestingly, in the recently published more racially diverse North American FSGS Clinical Trial (CT) cohort (27), female participants were not shown to have higher suPAR levels than male participants; it is possible that if the age distribution of these patients had been more similar to ours (not 2–40 years of age, but rather 1–21), the results of our study and those found in the North American FSGS CT cohort might have been more similar. The mechanism by which suPAR is elevated in adult women may not pertain to prepubertal/pubertal patients. Further investigation of this finding is warranted to understand the possible role of sex hormones on serum suPAR levels. We realize that differences in serum suPAR levels between sexes may, in part, be affected by the actively nephrotic state, which may alter the differing hormonal effects of stimulated liver protein synthesis. Unfortunately, serum lipid profiles were not available for our patients, adding a note of uncertainty as to the mechanism of the sex-based differences.

Figure 3. | Correlation analysis of suPAR with eGFR in pediatric CKD and FSGS patients. (A) Correlation between serum suPAR levels with eGFR (Schwartz equation) in the overall patient study cohort ($r=0.26$, $P=0.02$). (B) Correlation between serum suPAR levels with eGFR in patients with FSGS diagnoses when studied alone ($r=0.53$, $P=0.003$). In the overall study cohort, and in FSGS patients when studied alone, there was a significant inverse correlation between serum suPAR levels and eGFR.
As recently described by Wei and colleagues (27), we too found that age was inversely related to serum suPAR. Our trend may indicate that as children age, serum suPAR norms may decrease and approach levels similar to those reported in adults (10). Our findings emphasize the need for further development of serum suPAR norms for children.

Our observation of a negative correlation between serum suPAR and eGFR reflects similar results recently reported both in a small adult cohort (32) and the North American FSGS CT/PodoNet study (27). These data underscore the importance of further investigation of the effect of differing stages of CKD on serum suPAR levels. The finding of this negative trend is intriguing because it implies that serum suPAR in patients with CKD is influenced by other, yet unidentified factors, beyond the inflammatory process present in CKD (24). A larger study, including patients of many ages, at all stages of CKD would be necessary to clarify these issues.

One limitation of our study is that as a result of the sample de-identification process, demographic data were not available for our control cohort. Future study of healthy pediatric cohorts will help address the question of whether suPAR levels do indeed vary in children based on sex, age, and ethnic backgrounds. A further limitation of our study is that biopsy data were not available for all patients with non-FSGS glomerular disease (specifically MCD). Because biopsy is only appropriate in school-aged children after a full course of corticosteroids fails to induce remission, only three of our five patients with MCD had biopsy-confirmed diagnoses; the remaining two responded to steroid therapy. It is possible that these two patients were misdiagnosed and may have steroid responsive FSGS. However, it is in the minority of patients with FSGS that have complete corticosteroid-induced remissions. We therefore add only a mild note of caution to our interpretations. Finally, the limitations of univariate analysis for data interpretation warrant consideration; univariate models are less comprehensive compared with multivariate models and do not allow modeling of correlations or inversions between many factors. Given our single-center pediatric experience, and resultant reduced sample size, univariate analysis was the most appropriate method for our data. Nonetheless, these limitations should be given consideration on interpretation of our study findings.

Our data suggest that serum suPAR levels were not affected by transplantation. We included kidney transplant recipients in our study to determine whether transplantation moderates suPAR levels in patients with FSGS. By comparing nonproteinuric and proteinuric patients with FSGS before and after transplantation, we were able to demonstrate that the absence of an association between suPAR and recurrence of nephrotic syndrome was not influenced by transplant status. Suggestions for use of serum suPAR levels for prediction of FSGS recurrence after transplantation should therefore be utilized with caution. We recognize the limitation that we were unable to study suPAR levels longitudinally across transplantation. Given the conflicting results of our data compared with those published by Wei and colleagues (10), we therefore plan to study the role of change in suPAR levels through transplantation in pediatric patients with FSGS.

On the basis of our study of nearly 100 children, circulating suPAR is unlikely to be a leading cause of childhood idiopathic FSGS. Given treatment success in recurrent FSGS after transplantation with therapeutic plasmapheresis through indiscriminate removal of relatively large-size circulating plasma molecules, it appears that suPAR is merely one of many putative “circulating factors” involved. Identification of possible co-mediators of disease pathogenesis and their interplay in patients with idiopathic FSGS warrants further investigation.

Acknowledgments

M.E.B. is supported in part by a grant from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (K12 HD058844). C.B.L and H.E.P. are supported, in part, by grants from the National Institute of Diabetes and Digestive and Kidney Diseases.

Disclosures

None.

References


Received: July 30, 2012 Accepted: March 11, 2013

Published online ahead of print. Publication date available at www.cjasn.org.