

# Serial Estimates of Serum Permeability Activity and Clinical Correlates in Patients with Native Kidney Focal Segmental Glomerulosclerosis

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**Abstract.** A serum or plasma factor in certain patients with focal segmental glomerulosclerosis (FSGS) has been found to increase glomerular albumin permeability ( $P_{\text{alb}}$ ) and causes proteinuria in experimental animals. High  $P_{\text{alb}}$  is associated with recurrence of FSGS after transplantation, but serial studies of  $P_{\text{alb}}$  activity in patients with native kidney FSGS have not been performed, and the relationship between  $P_{\text{alb}}$  and remission of proteinuria is not known. This study was designed to determine  $P_{\text{alb}}$  activity before, during, and after 24 wk of treatment with cyclosporine or placebo given as part of a randomized controlled trial in steroid-resistant FSGS patients with nephrotic range proteinuria. Pretreatment  $P_{\text{alb}}$  averaged  $0.36 \pm 0.22$  and was not significantly different between treat-

ment groups and was not altered during or after the test medication. There was no association between  $P_{\text{alb}}$  activity and remission or relapse in proteinuria. The average  $P_{\text{alb}}$  activity in native kidney FSGS was lower than in previously reported patients with posttransplant recurrence of the disease, and its level did not vary during the course of the study. The antiproteinuric effect of cyclosporine appeared independent of changes in  $P_{\text{alb}}$ . This finding is consistent with a direct effect of cyclosporine on glomerular barrier function and/or that within this group of patients the variations in proteinuria are not reflected in changes in  $P_{\text{alb}}$  because of its limits in terms of reproducibility and responsiveness.

Focal segmental glomerulosclerosis (FSGS) is the most common primary glomerulonephritis that leads to end-stage renal disease (1,2) It was originally reported almost half a century ago from a postmortem study of children with nephrotic syndrome (3). The pathology of the disease has been well described since then and it now includes a number of histologic variants (4–6). It appears to be a heterogeneous condition (7) that is not universally responsive to immunosuppression and is frequently characterized by steroid resistance (8–24). Hypertension and renal dysfunction are commonly found at presentation. Thirty to fifty percent of patients will respond to prolonged treatment with corticosteroids, but a high percentage of nonresponders will progress to renal failure (10,12,19,25).

Many patients who do progress to end-stage renal disease (ESRD) will subsequently receive a renal transplant. The disease process, including both the clinical manifestations and the focal and segmental lesions on pathology will recur in up to 40% of these renal allografts, especially in patients whose disease course was rapid in their native kidney (26–28). In

many cases, the proteinuria can be immediate and massive. The propensity for recurrence, reports of a positive response to plasmapheresis posttransplantation in such patients, and *in vitro* and *in vivo* studies examining the effects of infusion of patients plasma into rats have led to research focused on the identification of a circulating factor that alters glomerular permeability (29–35). Savin *et al.* have developed an *in vitro* method for assessing the permeability of glomeruli to albumin ( $P_{\text{alb}}$ ) (33,35), and they have shown that this method is useful in assessing risk of recurrence of FSGS after transplantation. However,  $P_{\text{alb}}$  activity changes over time and in relationship to treatment in native kidney FSGS have not been measured.

In the present study,  $P_{\text{alb}}$  was assessed in serum samples of adults with steroid-resistant nephrotic syndrome secondary to FSGS who were involved in a clinical trial to test the efficacy of cyclosporine. Cyclosporine has been shown to block the *in vitro* effects of FSGS on glomerular permeability (36,37), but its effect *in vitro* on  $P_{\text{alb}}$  levels over time in patients with native kidney FSGS have not been reported.

## Materials and Methods

### Study Subjects

A subset of 27 patients representing approximately 50% of those enrolled in a randomized controlled trial were studied. Additional details of the study protocol and results of the parent trial have been reported (24). All patients must have failed to achieve a remission of proteinuria after a minimum of 8 wk of  $\geq 1$  mg/kg oral prednisone treatment. Other entry criteria included continued proteinuria of  $\geq 50$  mg/kg or  $\geq 3.5$ g/d, creatinine clearance (CrCl)  $\geq 42$  ml/min per

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1.73m<sup>2</sup>, BP ≤ 135/85 mmHg and dietary protein intake ≤ 0.8 g/kg. A nephropathologist masked to patient assignment also had to confirm the pathologic diagnosis. Eighteen of the patients in the current substudy had been assigned cyclosporine treatment, and nine patients placebo. These patients were arbitrarily selected by the principle investigator of the parent trial, but treatment assignment and outcome were unknown to laboratory staff assessing the P<sub>alb</sub> activity. Twice as many cyclosporine-treated patients as placebo patients were chosen; the cyclosporine group had a higher remission rate, and we anticipated that the largest changes in P<sub>alb</sub> would be seen in the patients with the greatest changes in proteinuria.

### Randomization, Treatment, and Surveillance

Patient selection and randomization in the parent study were performed per study protocol (24). Selection and submission of specimens for P<sub>alb</sub> assay were performed after the treatment protocol had been completed. The laboratory staff, including those performing the P<sub>alb</sub> assay was blinded to treatment assignment and to clinical response. All samples for P<sub>alb</sub> were obtained at the same time as the cyclosporine/placebo trough level assessment (*i.e.*, 12 h after dose). Treatment with cyclosporine or placebo, plus low-dose prednisone (0.15 mg/kg) in all patients was continued for 26 wk and was then tapered to 0 over 4 to 8 wk. Patients on an angiotensin-converting enzyme inhibitor (ACE<sub>i</sub>) or an angiotensin receptor blocker before the trial were allowed to remain on them during the study, but these classes of drug could not be introduced during the treatment part of the trial.

Clinical and laboratory evaluations were at selected times from pretreatment up to 208 wk after study entry. At randomization and after 26 wk of treatment, blinded assessments on serum samples for P<sub>alb</sub> were made through the laboratory of one of the collaborators (VS). In 15 patients, a third sample was obtained between week 40 and the end of the observation period, after cyclosporine or placebo treatment had been discontinued for a minimum of 12 wk.

### Outcome Measures

The primary outcome of the parent study was the number of complete or partial remission in proteinuria by week 26 in the two arms of the trial. This was also assessed at weeks 52, 78, 104 and at the last follow-up. In this study, we used the same end point definitions for outcome; complete remission (CR) was defined as ≤0.3 g/d proteinuria plus stable renal function, and a partial remission (PR) was both a 50% reduction of initial proteinuria and ≤3.5 g/d with stable renal function. Stable renal function was defined as a CrCl that was within 15% of the initial value. Secondary end points included progressive disease (CRI) defined as doubling of initial creatinine, end-stage renal disease (ESRD) defined as a CrCl ≤ 12 ml/min per 1.73m<sup>2</sup>, start of dialysis, or transplantation.

### P<sub>alb</sub> Assay

The principles of the *in vitro* assay, the methodology, and preparation of patients' sera for the assay have been described previously (35). Glomeruli isolated from Sprague-Dawley rats by standard sieving techniques in an isolation medium containing an oncotic agent, bovine serum albumin (BSA, 4 g/dl), are incubated at 37°C with a diluted sample of serum (2% vol/vol) from a study participant. An oncotic gradient across the capillary wall was caused by replacing the medium with fresh medium with BSA (1 g/dl), and the resulting expansion of glomerular capillaries was measured using videomicroscopy. An increase in albumin permeability results in a decrease in effective oncotic gradient and in diminished glomerular capillary

expansion ( $\Delta V$ ). P<sub>alb</sub> was calculated as  $1 - (\sigma_{alb})$ , where  $\sigma_{alb} = (\Delta V_{experimental} / \Delta V_{control})$ . P<sub>alb</sub> values for each specimen were reported as means of values of five or more glomeruli.

### Statistical Analyses

Prior measurement of P<sub>alb</sub> values in normal people after incubation in serum or plasma had a mean value ± SD of 0.00 ± 0.13 to 0.06 ± 0.2 (35,43). In earlier articles, we had defined individual values >0.5 as abnormal, based on the value that best separated recurrent from nonrecurrent FSGS disease after renal transplant and because it represented a value of more than 2 SD above the mean of normal sera (35). This definition was not used in the current data set because we are now aware of the wider range in P<sub>alb</sub> in those with native kidney disease, including patients in the part of the FSGS disease spectrum chosen for this study (35,38) and because we were most interested in inpatient changes in activity over time. For this latter parameter we defined a value of ≥0.3 as a significant difference. This was based on previously published data that had shown agreement on replicate samples of <0.3 in 83% of cases (35). Results are reported as mean ± SD and/or ranges. Averages were compared using ANOVA. Non-parametric tests employed Fischer exact test, and a *P* < 0.05 was considered significant.

### Results

Baseline demographics and laboratory parameters of the 27 patients in this study are shown in Table 1. The average age was 36 yr, and the group was predominately Caucasian. Sixty-one percent of cyclosporine-treated and 44% of placebo-treated patients were male. Responders and nonresponders in proteinuria, their corresponding medication assignment, and P<sub>alb</sub> activity before and after treatment, as well as the absolute difference and direction of change over time in these parameters are given in Table 2. Changes before and after treatments in the laboratory parameters by medication assignment to cyclosporine or to placebo are outlined in Table 3. P<sub>alb</sub> values from study patients showed a distribution from 0 to 0.98. The average initial P<sub>alb</sub> was 0.36 ± 0.22 and did not differ between the treatment groups. The average was higher than the mean P<sub>alb</sub> of normal serum samples but was lower than the P<sub>alb</sub> in patients with recurrence of FSGS in renal allografts (35).

Changes over the experimental treatment period by medication assignment in serum creatinine, creatinine clearance and proteinuria are shown in Tables 3. Neither serum creatinine nor creatinine clearance were different in either group at the end of the treatment period but 13/18 of the cyclosporine treated patients had a remission in proteinuria *versus* only 1 of 9 placebo patients (*P* = 0.05) (Table 2). Paired analysis looking at changes in P<sub>alb</sub> from before to after treatment compared to changes in urine protein over the same time frame showed no association. Also no association was seen when the groups regardless of treatment assignment were divided into responder and nonresponders. The rates of remission in proteinuria were similar to those found in the parent study. P<sub>alb</sub> values overall did not change over time in the entire patient population, 0.36 ± 0.22 (before) *versus* 0.38 ± 0.27 (after) or by treatment assignment, (cyclosporine, 0.31 ± 0.23 [before] *versus* 0.46 ± 0.28 [after]; placebo group, 0.41 ± 0.21 [before] *versus* 0.36 ± 0.25 [after]).

Table 1. Baseline demographic and laboratory data

Characteristics	Cyclosporine	Placebo
Number	18	9
Age	36 ± 11 <sup>a</sup> [21 to 59] <sup>b</sup>	36 ± 9 [23 to 47]
Gender (% males)	61	44
Racial group, <i>n</i> (%)		
White	17 (94)	8 (89)
Black	1 (6)	1 (11)
Creatinine (mg/dl) <sup>c</sup>	1.2 ± 0.4 [0.5 to 1.2]	1.1 ± 0.6 [0.6 to 2.6]
Creatinine clearance (ml/min per 1.73 m <sup>2</sup> )	72 ± 24	66 ± 30
Proteinuria (g/d) <sup>d</sup>	7.2 [3.6 to 14.4]	9.5 [4 to 22.4]
P <sub>alb</sub>	0.31 ± 0.23 [0 to 0.75]	0.41 ± 0.21 [0.09 to 0.78]

<sup>a</sup> ± values are SD.

<sup>b</sup> Values in brackets are ranges.

<sup>c</sup> To change to SI units: ×88.4.

<sup>d</sup> Median.

In the 15 patients whose P<sub>alb</sub> was measured after the drugs had been discontinued for 12 to 40 wk, the mean P<sub>alb</sub> was 0.49 ± 0.29. In these patients, as in the entire population, there was no relationship between P<sub>alb</sub> or change in P<sub>alb</sub> and change in proteinuria or progression to chronic renal insufficiency.

## Discussion

The short time to recurrence of FSGS posttransplantation and the beneficial effects of plasmapheresis therapy on their proteinuria supports the hypothesis that a circulating factor (or factors) may be involved in the pathogenesis of FSGS. Further evidence is offered by a case report that described the transient occurrence of the nephrotic syndrome in a neonate whose mother had known FSGS (39). In addition, earlier studies have shown that injection of sera from patients with recurrent FSGS into rats increased urinary protein excretion (40). Some investigators have reported this proteinuria to be quite variable and have concluded that it is not a reliable method for assaying activity (31), but we have found the degree of proteinuria induced depends on the potency of the preparation and on the amount injected (41). Measurements of P<sub>alb</sub> *in vitro* in our laboratory are consistent with the hypothesis that a circulating factor increases glomerular permeability. It has also been shown that P<sub>alb</sub> activity is higher in patients with recurrent FSGS both before and after transplant compared with those without recurrence (35) and that P<sub>alb</sub> activity is reduced by plasmapheresis in some patients with both native kidney FSGS and with recurrent disease (38). Although the circulating factor has not been fully characterized, it appears to be non-Ig, glycosylated, low-molecular weight protein (41). We have tested the capacity of several experimental manipulations to change P<sub>alb</sub> and have found it increased or decreased after a wide range of agents analogous to those postulated to cause glomerular injury in experimental and human renal disease (42–44). These findings suggest that the P<sub>alb</sub> assay is a functional assay rather than an assay of a specific substance. This is supported by data that has suggested P<sub>alb</sub> activity is a balance between plasma factors that enhance *versus* those that

Table 2. Proteinuria

	Proteinuria (g/d)/P <sub>alb</sub>			
	Pretreatment	Posttreatment	Delta	
<b>Responders</b>				
cyclosporine group	9.9/0.30	3.2/0.28	−6.7/−0.02	
	9.5/0.75	1.1/0.98	−8.4/+0.23	
	10.2/0.00	0.2/0.21	−10/+0.21	
	3.6/0.30	0.6/0.46	−3.0/+0.16	
	4.0/0.33	0.7/0.46	−3.3/+0.13	
	5.3/0.70	0.5/0.49	−4.8/−0.21	
	4.1/0.26	1.7/0.57	−2.4/+0.31	
	4.6/0.09	0.4/0.41	−4.2/+0.32	
	7.0/0.45	1.9/0.30	−5.1/−0.15	
	5.8/0.16	1.2/0.18	−4.6/+0.02	
	7.2/0.34	3.1/0.89	−4.1/+0.55	
	5.8/0.16	0.6/0.18	−5.2/+0.02	
	10.9/0.00	2.1/0.89	−8.8/+0.89	
placebo group	4.0/0.33	1.2/0.14	−2.8/−0.19	
<b>Nonresponders</b>				
cyclosporine group	10.7/0.37	4.4/0.4	−6.3/+0.03	
	10.0/0.00	3.6/0.00	−6.4/0.0	
	10.1/0.39	12.1/0.55	+2.0/+0.16	
	5.1/0.50	14.5/0.15	+9.4/−0.35	
	14.4/0.55	12.1/0.80	−2.3/+0.25	
	placebo group	5.2/0.78	7.4/0.53	+2.2/−0.25
		9.5/0.24	20/0.34	+10.5/+0.10
		7.8/0.38	6.5/0.14	−1.3/−0.24
		17.1/0.53	13.9/0.37	−3.2/−0.16
		22.4/0.32	17.7/0.46	−4.7/+0.14
14.5/0.61	11.9/0.75	−2.6/+0.14		
14.1/0.09	5.8/0.00	−8.3/−0.09		
5.9/0.38	3.4/0.51	−2.5/+0.13		

inhibit permeability. We have previously demonstrated that certain factors present in normal sera, *e.g.*, apoprotein J and E

Table 3. Changes in laboratory parameters pretreatment and posttreatment

	Cyclosporine		Placebo	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment
Creatinine (mg/dl) <sup>a</sup>	1.2 ± 0.4 <sup>b</sup>	1.4 ± 0.6	1.1 ± 0.6	1.4 ± 0.8
Creatinine clearance (ml/min per 1.73 m <sup>2</sup> )	72 ± 24	54 ± 24	66 ± 30	60 ± 36
Proteinuria (g/d) <sup>c</sup>	7.2 [3.6 to 14.4]	3.1 [0.2 to 14.5]	9.5 [4 to 22.4]	7.4 [1.2 to 20.0]
P <sub>alb</sub>	0.31 ± 0.23	0.46 ± 0.28	0.41 ± 0.21	0.36 ± 0.25

<sup>a</sup> To change to SI: ×88.4.

<sup>b</sup> ± values are SD.

<sup>c</sup> Median [range].

inhibit permeability activity induced by FSGS serum *in vitro* (45). Others have provided data that suggests there is a urinary loss of inhibitory factors of P<sub>alb</sub> in nephrotic patients. Co-incubation of sera with P<sub>alb</sub> activity with homologous urine resulted in neutralization of the permeability abnormality *in vitro*. This was not due to urinary levels of apo J or E, suggesting other inhibitors are present (46).

Although P<sub>alb</sub> is increased by diverse agents, it has been used by us and others in studies of FSGS as a measure of circulating permeability activity that may be important in causing and perpetuating proteinuria. Serial measurements of P<sub>alb</sub> over the course of the disease in native kidney FSGS patients have not been previously reported. In our study, P<sub>alb</sub> did not change in any consistent fashion in either the cyclosporine-treated or placebo-treated patients regardless of the change in proteinuria over the course of the trial. There was no association between partial or complete remission of proteinuria during treatment with cyclosporine or placebo in either initial P<sub>alb</sub> or change in P<sub>alb</sub> during treatment (Table 2). This is similar to the lack of correlation seen in P<sub>alb</sub> activity and clinical outcome after plasmapheresis in native kidney FSGS (38). The activity levels were on average lower than earlier studies, perhaps reflecting a less severely affected population and a different part of the disease spectrum compared with earlier studies that compared P<sub>alb</sub> activity in posttransplant patients whose native kidney disease was FSGS and in all cases had induced end-stage renal failure (35). In contrast, our selection criteria for the parent study were designed to provide a relatively homogeneous sample of FSGS patients who, although they were resistant to corticosteroid therapy, had stable renal function during the 6-mo pretreatment period. This would result in the exclusion of patients at both extremes of the disease spectrum, *i.e.*, those with steroid-sensitive disease and those with rapid progression to renal failure.

One explanation of the data is that there is a direct protective effect of cyclosporine on the glomerular permeability barrier independent of P<sub>alb</sub>. This interpretation is consistent with previous *in vitro* findings that cyclosporine protects glomeruli from the increase permeability induced by FSGS sera with P<sub>alb</sub> activity (36).

An alternative or additional explanation relates to the assay in terms of its reproducibility and responsiveness. The reproducibility of the assay in this case relates to the test-retest

component. This has been established for P<sub>alb</sub> in previous studies at <0.3 in 83% of cases (35). This in turn means the responsiveness or the ability of the assay to reflect or be sensitive to change, requires a relatively large difference in values between time points in each individual tested. If we restrict our evaluation to those patients that achieved this value, only five cases were identified (Table 2) and no association with change in proteinuria was observed within this group.

In summary, the sera of patients in the study had increased P<sub>alb</sub> activity compared with normal sera, but they were lower than that seen in patients with rapidly progressive FSGS diseases and in patients with recurrent posttransplant FSGS disease. The P<sub>alb</sub> did not change significantly during the study in either of the treatment groups and did not decrease during remission or increase during worsening of proteinuria. One possible explanation of this discordance between P<sub>alb</sub> and proteinuria is that there is a direct protective effect of cyclosporine on the glomerular permeability barrier independent of other factors that may either inhibit or enhance permeability activity (45,46). An alternate or additional explanation is related to the assay's limits related to reproducibility and responsiveness. These limits as discussed would reduce the likelihood of detecting a relationship between P<sub>alb</sub> and disease activity, since the required value to be considered biologically relevant (≥0.3) is large relative to the total range of the assay.

Multiple factors influence the pathophysiology of FSGS. The current study of P<sub>alb</sub> addresses only the capacity of sera to cause immediate increase in glomerular macromolecular permeability. It was not focused on providing information about potential mechanisms of sclerosis or the role of fibrogenic and/or inflammatory agents in the disease pathogenesis (47,48). Our study in this selected group of steroid-resistant nephrotic patients with relatively well-preserved renal function found no relationship between P<sub>alb</sub> and outcome. Additional studies with larger numbers and a broader spectrum of native kidney FSGS over longer periods of time may be required to determine if there is any correlation between P<sub>alb</sub> activity and the clinical course of the disease.

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## References

1. US Renal Data System. USRDS: 1995 Annual Data Report, Bethesda, The National Institute of Health, National Institute of Diabetes and Digestive and Kidney Disease. 1995
2. US Renal Data System. USRDS: 1997 Annual Data Report, Bethesda, The National Institute of Health, National Institute of Diabetes and Digestive and Kidney Disease, 1997
3. Rich, AR: A hitherto undescribed vulnerability of the juxta-medullary glomeruli in lipid nephrosis. *Bull John Hopkins Hospital* 100: 173–187, 1957
4. Miyata J, Takebayashi S, Taguchi T, Naito S, Harada T: Evaluation and correlation of clinical and histological features of focal segmental glomerulosclerosis. *Nephron* 44: 115–120, 1986
5. Schwartz MM, Korbet SM, Rydell J, Borok R, Genchi R: Primary focal segmental glomerular sclerosis in adults: Prognostic value of histologic variants. *Am J Kidney Dis* 25: 845–852, 1995
6. Schwartz MM, Korbet SM: Primary focal segmental glomerulosclerosis: Pathology, histological variants, and pathogenesis. *Am J Kidney Dis* 22: 874–883, 1993
7. D'Agati V: The many masks of focal segmental glomerulosclerosis. *Kidney Int* 46: 1223–1241, 1994
8. Beauvils H, Alphonse JC, Guedon J, Legrain M: Focal glomerulosclerosis: Natural history and treatment. A report of 70 cases. *Nephron* 21: 75–85, 1978
9. Cameron JS, Turner DR, Ogg CS, Chantler C, Williams DG: The long-term prognosis of patients with focal segmental glomerulosclerosis. *Clin Nephrol* 10: 213–218, 1978
10. Rydel JJ, Korbet SM, Borok RZ, Schwartz MM: Focal segmental glomerular sclerosis in adults: Presentation, course, and response to treatment. *Am J Kidney Dis* 25: 534–542, 1995
11. Mongeau JG, Robitaille PO, Clermont MJ, Merouani A, Russo P: Focal segmental glomerulosclerosis (FSG) 20 years later. From toddler to grown up. *Clin Nephrol* 40: 1–6, 1993
12. Banfi G, Moriggi M, Sabadini E, Fellin G, D'Amico G, Ponticelli C: The impact of prolonged immunosuppression on the outcome of idiopathic focal-segmental glomerulosclerosis with nephrotic syndrome in adults. A collaborative retrospective study. *Clin Nephrol* 36: 53–59, 1991
13. Brodehl J, Brodehl J, Helmchen U, Hoyer PI, Burghard R, Ehrlich JH, Zimmerhackl RB, Klein W, and Wonigeit K: Cyclosporin A treatment in children with minimal change nephrotic syndrome and focal segmental glomerulosclerosis. *Klin Wochenschr* 66: 1126–1137, 1988
14. Burgess E: Management of focal segmental glomerulosclerosis: Evidence-based recommendations. *Kidney Int Suppl* 70: S26–S32, 1999
15. Ingulli E, Singh A, Baqi N, Ahmad H, Moazami S, Tejani A: Aggressive, long-term cyclosporine therapy for steroid-resistant focal segmental glomerulosclerosis. *J Am Soc Nephrol* 5:1820–1825, 1995
16. Lieberman KV, Tejani A: A randomized double-blind placebo-controlled trial of cyclosporine in steroid-resistant idiopathic focal segmental glomerulosclerosis in children. *J Am Soc Nephrol* 7: 56–63, 1996
17. Meyrier A, Condamin MC, Broneer D: Treatment of adult idiopathic nephrotic syndrome with cyclosporin A: Minimal-change disease and focal-segmental glomerulosclerosis. Collaborative Group of the French Society of Nephrology. *Clin Nephrol* 35[Suppl 1]: S37–S42, 1991
18. Nagai R, Cattran DC, Pei Y: Steroid therapy and prognosis of focal segmental glomerulosclerosis in the elderly. *Clin Nephrol* 42: 18–21, 1994
19. Pei Y, Cattran D, Delmore T, Katz A, Lang A, Rance P: Evidence suggesting under-treatment in adults with idiopathic focal segmental glomerulosclerosis. Regional Glomerulonephritis Registry Study. *Am J Med* 82: 938–944, 1987
20. Ponticelli C, Rizzoni G, Edefonti A, Altieri P, Rivolta E, Rinaldi S, Ghio L, Lusvardi E, Gusmano R, Locatelli F: A randomized trial of cyclosporine in steroid-resistant idiopathic nephrotic syndrome. *Kidney Int* 43: 1377–1384, 1993
21. Ponticelli C, Edefonti A, Ghio L, Rizzoni G, Rinaldi S, Gusmano R, Lama G, Zacchello G, Confalonieri R, Altieri P: Cyclosporin versus cyclophosphamide for patients with steroid-dependent and frequently relapsing idiopathic nephrotic syndrome: A multicentre randomized controlled trial. *Nephrol Dial Transplant* 8: 1326–1332, 1993
22. Tarshish P, Tobin JN, Bernstein J, Edelmann CM, Jr: Cyclophosphamide does not benefit patients with focal segmental glomerulosclerosis. A report of the International Study of Kidney Disease in Children. *Pediatr Nephrol* 10: 590–593, 1996
23. Walker RG, Kincaid-Smith P: The effect of treatment of corticosteroid-resistant idiopathic (primary) focal and segmental hyalinosis and sclerosis (focal glomerulosclerosis) with cyclosporin. *Nephron* 54: 117–121, 1990
24. Cattran DC, Appel GB, Hebert LA, Hunsicker LG, Pohl MA, Hoy WE, Maxwell DR, Kunis CL: A randomized trial of cyclosporine in patients with steroid-resistant focal segmental glomerulosclerosis. North America Nephrotic Syndrome Study Group. *Kidney Int* 56: 2220–2226, 1999
25. Cattran DC, Rao P: Long-term outcome in children and adults with classic focal segmental glomerulosclerosis. *Am J Kidney Dis* 32: 72–79, 1998
26. Hoyer JR, Vernier RL, Najarian JS, Raji L, Simmons RL, Michael AF: Recurrence of idiopathic nephrotic syndrome after renal transplantation. *Lancet* 2: 343–348, 1972
27. Artero M, Biava C, Amend W, Tomlanovich S, Vincenti F: Recurrent focal glomerulosclerosis: natural history and response to therapy. *Am J Med* 92: 375–383, 1992
28. Senggutuvan P, Cameron JS, Hartley RB, Rigden S, Chantler C, Haycock G, Williams DG, Ogg C, Koffman G: Recurrence of focal segmental glomerulosclerosis in transplanted kidneys: Analysis of incidence and risk factors in 59 allografts. *Pediatr Nephrol* 4: 21–28, 1990
29. Cochat P, Kassir A, Colon S, Glastre C, Tourniaire B, Parchoux B, Martin X, David L: Recurrent nephrotic syndrome after transplantation: early treatment with plasmapheresis and cyclophosphamide. *Pediatr Nephrol* 7: 50–54, 1993
30. Artero ML, Sharma R, Savin VJ, Vincenti F: Plasmapheresis reduces proteinuria and serum capacity to injure glomeruli in patients with recurrent focal glomerulosclerosis. *Am J Kidney Dis* 23: 574–581, 1994
31. Dantal J, Bigot E, Bogers W, Testa A, Kriaa F, Jacques Y, Hurault dL, Niaudet P, Charpentier B, Souillou JP: Effect of plasma protein adsorption on protein excretion in kidney-transplant recipients with recurrent nephrotic syndrome. *N Engl J Med* 330: 7–14, 1994

32. Laufer J, Ettenger RB, Ho WG, Cohen AH, Marik JL, Fine RN: Plasma exchange for recurrent nephrotic syndrome following renal transplantation. *Transplantation* 46: 540–542, 1988
33. Savin VJ, Sharma R, Lovell HB, Welling DJ: Measurement of albumin reflection coefficient with isolated rat glomeruli. *J Am Soc Nephrol* 3: 1260–1269, 1992
34. Godfrin Y, Dantal J, Perretto S, Hristea D, Legendre C, Kreis H, Souillou JP: Study of the in vitro effect on glomerular albumin permselectivity of serum before and after renal transplantation in focal segmental glomerulosclerosis. *Transplantation* 64: 1711–1715, 1997
35. Savin VJ, Sharma R, Sharma M, McCarthy ET, Swan SK, Ellis E, Lovell H, Warady B, Gunwar S, Chonko AM, Artero M, Vincenti F: Circulating factor associated with increased glomerular permeability to albumin in recurrent focal segmental glomerulosclerosis. *N Engl J Med* 334: 878–883, 1996
36. Sharma R, Sharma M, Ge X, McCarthy ET, Savin VJ: Cyclosporine protects glomeruli from FSGS factor via an increase in glomerular cAMP. *Transplantation* 62: 1916–1920, 1996
37. Sharma R, Savin VJ: Cyclosporine prevents the increase in glomerular albumin permeability caused by serum from patients with focal segmental glomerular sclerosis. *Transplantation* 61: 381–383, 1996
38. Feld SM, Figueroa P, Savin V, Nast CC, Sharma R, Sharma M, Hirschberg R, Adler SG: Plasmapheresis in the treatment of steroid-resistant focal segmental glomerulosclerosis in native kidneys. *Am J Kidney Dis* 32: 230–237, 1998
39. Kemper MJ, Wolf G, Muller-Wiefel DE: Transmission of glomerular permeability factor from a mother to her child. *N Engl J Med* 344: 386–387, 2001
40. Zimmerman SW: Increased urinary protein excretion in the rat produced by serum from a patient with recurrent focal glomerular sclerosis after renal transplantation. *Clin Nephrol* 22: 32–38, 1984
41. Sharma M, Sharma R, McCarthy ET, Savin VJ: “The FSGS factor:” enrichment and in vivo effect of activity from focal segmental glomerulosclerosis plasma. *J Am Soc Nephrol* 10: 552–561, 1999
42. Trachtman H, Futterweit S, Singhal PC, Franki N, Sharma M, Sharma R, Savin V: Circulating factor in patients with recurrent focal segmental glomerulosclerosis postrenal transplantation inhibits expression of inducible nitric oxide synthase and nitric oxide production by cultured rat mesangial cells. *J Investig Med* 47: 114–120, 1999
43. Sharma R, Khanna AK, Sharma M, Savin VJ: Transforming growth factor increases glomerular albumin permeability via hydroxyl radicals. *Kidney Int* 58: 131–136, 2000
44. McCarthy ET, Sharma M: Indomethacin protects permeability barrier from focal segmental glomerulosclerosis serum. *Kidney Int* 61: 534–541, 2002
45. Sharma RD, Sharma M, McCarthy ET, Ge XL, Savin V: Components of normal serum block the focal segmental glomerulosclerosis factor activity in vitro. *Kidney Int* 58: 1973–1079, 2000
46. Carraro M, Caridi G, Bruschi M, Artero M, Bertelli R, Zennaro C, Musante L, Candiano G, Perfumo Francesco, Ghiggeri GM: Serum glomerular permeability activity in patients with podocin mutations (NPHS2) and steroid resistant nephrotic syndrome. *J Am Soc Nephrol* 13: 1046–1052, 2002
47. Kriz W: Progressive renal failure — Inability of podocytes to replicate and the consequences for development of glomerulosclerosis. *Nephrol Dial Transplant* 11: 1738–1742, 1996
48. Diamond JR, Karnovsky MJ: Focal and segmental glomerulosclerosis: Analogies to atherosclerosis. *Kidney Int* 33: 917–924, 1988

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