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Podocyte Foot Process Effacement in Post-reperfusion Allograft Biopsies Correlates with Early Recurrence of Proteinuria in Focal Segmental Glomerulosclerosis

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Abstract

Background—Focal segmental glomerulosclerosis (FSGS) is a relatively prevalent glomerular disorder that often progresses to end-stage renal disease. 30 to 80% of kidney transplant (KT) recipients with FSGS will experience recurrence characterized by proteinuria and podocyte damage. We hypothesized the degree of podocyte foot process (FP) effacement in post-reperfusion transplant biopsies can be used to predict the development of clinical recurrence of FSGS.

Method—Nineteen pairs of pre- and post-reperfusion biopsy specimens were studied. We evaluated the degree of FP effacement in post-reperfusion KT biopsies by counting the number of widened FP per capillary loop. Early recurrence of FSGS was defined as development of nephrotic range proteinuria between day 3 and 30 post-transplant.

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Jei-wen Chang performed clinical data collection and analysis and generated the initial and final drafts of the manuscript; Junichiro Sageshima performed clinical data collection and analysis and edited the drafts of the manuscript. Junichiro Sageshima, Linda Chen and Gaetano Ciancio performed patient care, tissue biopsy, clinical data collection and editing the drafts of the biopsies; Victoriano Pardo performed pathologic scoring on graft biopsies, antibodies; Hsin-Lin Tsai, Jochen Reiser, Changli Wei, George W Burke III and Alessia Fornoni participated in research design and edited the drafts and the final version of the manuscript; and all the authors have critically reviewed the manuscript.

Results—Early recurrence occurred in seven of 19 grafts (36.8%) at a mean of 4.29 ± 1.89 days. The mean score of FP effacement in post-reperfusion allograft biopsies was 0.72 ± 0.31 and 1.35 ± 0.63 in the non-recurrent and recurrent group, respectively ($p=0.039$). There was an association between FP effacement and proteinuria ($p=0.04$). The FP effacement score predicts early recurrence with a sensitivity of 71.4% and specificity of 91.7%.

Conclusion—FP effacement can be observed within minutes after reperfusion in renal transplantation of FSGS recipients that will ultimately develop recurrent FSGS. This suggests a key role for the podocyte injury in the pathogenesis of recurrent FSGS and further supports the presence of circulating factors causing FP effacement. The FP effacement score in the post reperfusion KT biopsy may become a useful predictive test if validated in larger studies.

Keywords

foot process; post-reperfusion; recurrence; focal segmental glomerulosclerosis

Focal segmental glomerulosclerosis (FSGS) is one of the leading glomerular diseases that progress to end-stage renal disease (ESRD). Overall, nearly 30 to 50% of patients progress to ESRD over 5 to 20 years. However, in some patients with the collapsing variant, a more rapid progression to ESRD within a 2–3 year period has been reported. After transplantation, recurrence of FSGS occurs in 30% to 40% of adults (1–2) and up to 80% in high risk children (progression to ESRD < 3 years, onset <15 years old, mesangial proliferation on native kidney biopsy) (3–5) and those with graft loss due to previous recurrent FSGS. In addition, recurrence of FSGS increases the risk of renal dysfunction and early graft loss (5, 6). Recurrence of FSGS can occur very soon after transplantation, sometimes within hours to days and is relatively rare after 2 years. The pathogenesis of recurrent FSGS remains uncertain. Several observations support a circulating permeability plasma factor (between 30 and 50 kD) in patients' serum responsible for increased glomerular permeability (7,8) and this possibility is supported by the observation that plasmapheresis (PP) can induce remission of proteinuria (3).

Podocytes are highly specialized epithelial cells which possess numerous interdigitating foot processes (FP) that cover the outer layer of the glomerular basement membrane (9). The integrity of the FP is crucial for the glomerular filtration barrier. FP effacement is an early structural change occurring in many glomerulopathies, where the degree of FP effacement correlates with the amount of proteinuria (10,11). Growing evidence supports a central role for podocyte injury in the pathogenesis of FSGS. In most clinical settings, FP effacement was the only early change seen in transplant biopsies after clinical recurrent FSGS before the occurrence of the characteristic pathologic changes of FSGS. To our knowledge, there has been no study about the degree of FP effacement in post-reperfusion biopsies before the appearance of clinical features of recurrent FSGS. Here, we analyzed if the quantitative evaluation of podocyte FP effacement in post-reperfusion kidney transplant (KT) biopsies obtained intraoperatively from recipients with FSGS may predict the recurrence of FSGS. Our findings may provide new insight into the almost immediate change of podocyte FP after the reperfusion of the KT, which may provide indirect evidence lending further support to the hypothesis that a circulating factor is responsible for the early podocyte malfunction observed in recurrent FSGS.

Results

Characteristics of the Study Population

In total, 19 pairs of pre- and post-reperfusion biopsy specimens were included in the study, 12 from living-related donors and 7 from deceased donor transplants. Three of the patients

were studied at the time of second KT; causes of the first graft loss were recurrent FSGS in 2 cases and recurrent FSGS combined with chronic rejection in the other case. Basic demographics and clinical data are shown in Table 1. Six patients had undergone nephrectomy of his or her native kidneys. Five patients received preemptive transplants. Recurrence of FSGS was defined as urinary PCR > 3 between day 3 and 30 after transplantation in the absence of acute rejection or urinary tract infection, and was compared to cases that did not experience early recurrence (Non-recurrent). Recurrence of FSGS occurred in 7 of 19 grafts (36.8%) and was observed at a mean of 4.29 ± 1.89 days (range 3–7 days).

Clinical Comparisons between Non-recurrent and Recurrent Groups

The mean age at onset of FSGS was 11.77 ± 10.46 years and 11.14 ± 10.98 years for patients in the non-recurrent group and in the recurrent group, respectively. Although not statistically significant, the time from diagnosis to first dialysis was shorter in the early recurrent group (4.72 ± 3.73 years) when compared to the Non-recurrent group (6.66 ± 4.17 years), and the percentage of patients with ESRD within three years of diagnosis was 57.1% in recurrent versus 25.0% in the non-recurrent. Univariate analysis showed that gender, age at onset of disease, time delay to ESRD, duration of dialysis before transplantation, native bilateral nephrectomy, and delayed graft function were not significantly different between groups (Table 1). Electron micrographs of post-reperfusion KT biopsies are shown on Figure 1. In the case of the recurrent group, the post-reperfusion KT biopsy showed diffuse FP effacement (Figure 1C–I). The score of FP effacement in post-reperfusion KT biopsy was 0.72 ± 0.31 and 1.35 ± 0.63 in the non-recurrent and recurrent group, respectively. The score of FP effacement was significantly associated with recurrence of nephrotic range proteinuria ($p = 0.039$). There was also an association between degree of FP effacement and degree of proteinuria ($p = 0.04$, Figure 2). The FP effacement score predicted the development of early recurrence of nephrotic range proteinuria with sensitivity of 71.4% and specificity of 91.7% (Figure 3). In this cohort of patients, the positive predictive value (PPV) and the negative predictive value (NPV) were calculated as 83.3% and 84.6% respectively. Although this could change in different patient cohorts with different recurrence rate, this test has a potential to predict proteinuria and should be validated in larger studies.

Characteristics of the seven patients with recurrence are listed in Table 2. All seven patients received rituximab and PP was carried out in five of seven patients who had recurrence. Of the seven patients, complete remission was noted in three, partial remission also in three, and no response in one. On analyzing the long-term outcomes, biopsy-proven late recurrent FSGS occurred in 1 allograft in the non-recurrent group and 1 case in the recurrent group. Both of them lost allograft function and returned to dialysis after 373 and 257 days of transplantation, respectively. One additional allograft was lost in the recurrent group because of rejection. For the non-recurrent group, the overall graft survival was 88.9% at 1 year. The 1-year survival rate in recurrent group was 85.7%. Although the graft survival was better among patients without early recurrence, there was no significant difference between the two groups at 1 year ($p = 0.226$). Among the children with non-recurrent disease, two had mild deterioration of allograft function with a serum creatinine of 1.9 and 1.7 mg/d, respectively, and mild proteinuria associated with chronic rejection during a follow-up of 2.33 ± 1.66 years. The remaining patients in the early recurrent group have normal renal function with mild asymptomatic proteinuria in one case and nephrotic range proteinuria in one case after a mean follow-up of 1.77 ± 1.16 years.

Discussion

In the immediate post-KT period, proteinuria has been reported to occur between 9 and 30% (12–14). Heavy proteinuria in a transplant kidney is associated with poor graft survival

(15,16). The possible etiology of post-transplant proteinuria involves many factors, including ischemia-reperfusion injury, acute rejection, delayed graft function, calcineurin-inhibitor toxicity, residual native kidney proteinuria, de novo or recurrent glomerulonephritis and use of drugs such as mTOR inhibitors. The ischemia-reperfusion injury in living donor transplantation may cause proteinuria during the first 24 hours, which rarely exceeds 1 g per day (17). For cadaveric kidney donation, the glomerular proteinuria usually disappears within 48 hours (18), a condition that limits the ability to utilize urine PCR in the first two post-operative days to diagnose FSGS recurrence. A few weeks post-KT, proteinuria of native kidney origin generally resolves in KT recipients with immediate graft function (19). Previous studies showed that persistent post-transplant proteinuria greater than 1500 mg/day and /or increase in proteinuria by >500 mg/day early after successful kidney transplantation indicate the presence of allograft nephropathy (20,21). In this study, we used the 3rd day to 1 month post operative day urine PCR>3 and we placed a stent in the newly transplanted kidney in recipients with significant residual urine output from native or previously transplanted kidneys to differentiate clinical recurrent and non-recurrent groups.

Since Hoyer et al. first described recurrent FSGS in 1972 (22), many articles about the clinical course and outcome of recurrent FSGS in renal transplant recipients have been published. Post-transplant recurrence occurs in 35–40% of adults with FSGS and in more than 80% of subsequent renal grafts after post-transplant recurrence and graft loss. Recurrence is an important cause of allograft dysfunction and loss. Briganti et al. estimated the hazard ratio for allograft loss secondary to recurrent FSGS was about 2 (23). In our own experience, the early recurrence rate of FSGS was 64% in relatively high risk patients (historical control) and 26% in those that were treated with one dose of rituximab (11). Risk factors reported to be associated with recurrence include a rapid progression of the primary disease to ESRD within 3 years, children less than 15 years of age, mesangial proliferation in native kidney, living donation, early onset of nephrotic range proteinuria in allograft, and a previous allograft due to recurrent disease (24,25). As some of these traditional risk factors did not strongly correlate with increased risk of recurrence in our patient population, we have investigated if histological findings that can be quantitatively assessed in the immediate post-transplant period may offer a stronger predictor of recurrent disease after transplantation.

The most frequent recurrence characterized by massive proteinuria developed a few hours to days after renal transplantation. Nephrotic range proteinuria usually precedes the development of characteristic histological lesions. Diffuse FP effacement by electron microscopy is usually the only initial finding in early graft biopsies performed at 2–7 days after the onset of recurrence (26). However, all prior studies about recurrent FSGS examined biopsies obtained in the initial phases of clinically evident recurrence rather than before the onset of proteinuria. The immediate appearance of proteinuria after transplantation suggests that some podocyte changes indeed occur immediately after reperfusion of the transplanted kidney and may indicate a susceptibility of the transplanted organ to experience recurrent FSGS. To the best of our knowledge, our study is the first to demonstrate that podocyte FP effacement in post-reperfusion allograft biopsy correlates with early recurrence of FSGS. It is likely that FP effacement observed in post-reperfusion biopsies is a reversible process, and that in those patients with high levels of circulating factor, podocyte injury may persist and contribute to the development of an irreversible lesion. The podocyte injury eventually leads to the detachment of podocytes, podocytopenia and progressive glomerulosclerosis. According to our data, routine intraoperative allograft biopsies could be introduced as a useful tool to predict recurrence of disease in FSGS patients by evaluating the degree of FP effacement which then provides the opportunity for early intervention. Our semi-quantitative analysis of FP effacement also demonstrated that the degree of podocyte FP

effacement significantly correlates with severity of proteinuria. It is likely that FP effacement in post-reperfusion KT biopsies is a reversible phenomenon that simply reflects the susceptibility to recurrent disease, similar to what we have recently demonstrated for the down-regulation of sphingomyelin phosphodiesterase-acid-like 3b (SMPDL-3b) in a similar collection of post-reperfusion KT biopsies (11). As all these patients had received rituximab after the post-reperfusion biopsy was obtained, and we have demonstrated a significant reduction in post-transplant proteinuria in patients receiving rituximab (11), it is likely that the use of rituximab reduced the predictive value of FP effacement in our study. One may argue that analysis of FP effacement in post-reperfusion KT biopsies may help to guide decisions on prevention strategies once validated in larger studies.

The pathophysiology of primary FSGS remains uncertain (27,28). Recurrent FSGS in renal transplants provides a unique model to study the pathogenesis of FSGS. The rapid appearance of proteinuria post renal transplantation implicates the presence of a circulating factor associated with increased glomerular permeability (29,30). Injection of serum from FSGS patients into rat causes proteinuria (31,32) providing additional evidence for the existence of a circulating factor in FSGS patients. The use of PP and immunoadsorption with protein A for the treatment and prophylaxis in the kidney transplant recipients at high-risk of recurrent FSGS is also based on this premise. Our study showed immediate effacement of FP in the post-reperfusion biopsies which indirectly supports the presence of a circulating factor in some patients' serum that may induce disruption of FP integrity and be responsible for immediate recurrence. Although Savin et al. (30,33–35) showed that the permeability factor is a low-molecular-weight protein (30–50 kD), the identity of this protein has remained elusive for many years. Recent insights into podocyte biology have identified a urokinase receptor (uPAR) integral to the maintenance of the slit diaphragm through its ability to form signalling complexes with other transmembrane proteins including lipid-dependent activation of $\alpha v \beta 3$ integrin (8). Activation of this receptor and its downstream pathways result in podocyte effacement, proteinuria, glomerular damage and loss of renal function. More recently, we have demonstrated that suPAR is elevated in patients with FSGS and is highest in patients with post-transplantation recurrent disease. Further, this 20–50 kDa circulating protein can be partially removed by PP, and beneficial responses can be observed in cases where suPAR levels drop below the threshold of sufficiently activating podocyte $\beta 3$ integrin, which would otherwise lead to hypermotility of podocyte foot processes with foot process effacement and proteinuria. As many of these 19 patients did not have available sera for suPAR determination, it will remain to be established with future studies whether circulating level of suPAR correlates with the degree of FP effacement and/or if a composite assay inclusive of pre-transplant suPAR and the analysis of FP effacement in post-reperfusion biopsies may represent a more powerful predictor of clinical outcome.

Poor response to various immunosuppressive therapeutic trials suggests that a mechanism other than immunological pathophysiology is involved in the progression and in the recurrence of FSGS. Rituximab is an anti-CD20 monoclonal chimeric antibody that is used to treat rheumatoid arthritis, B-cell non-Hodgkin's lymphoma and post-transplant-lymphoproliferative disorder. Recently, there are several reports showing rituximab may be an alternative treatment or adjuvant therapy in plasmapheresis-resistant recurrent FSGS (36,37). As we have recently demonstrated that the effect of rituximab in recurrent FSGS may result from a direct action of rituximab to preserve SMPDL-3b and acid sphingomyelinase activity in podocytes (11), it may become important to understand the link between FP effacement and SMPDL-3b expression in biopsies. However, data from this study as well as from our reported cohort demonstrated that approximately 30% of the patients who received one dose of rituximab (375 mg/m^2) perioperatively, still developed recurrence, supporting the contribution of more than one factor in the pathogenesis of recurrent FSGS. Recently,

the costimulatory molecule B7-1 in podocytes has been shown to increase the glomerular permselectivity in animal models (38). It would be interesting to determine if B7-1 is upregulated in post reperfusion kidney biopsies in patients with recurrent FSGS and to determine if abatacept may protect podocyte function under those circumstances. Our study supports experimental models of FSGS suggesting that podocyte injury plays a central role in the pathogenesis of recurrent FSGS (39,40) and allows for the development of targeted podocyte therapy.

Our series is the first to report biopsy data in the post-reperfusion KT in recipients with FSGS. In this study, we demonstrated that FP effacement can be observed within 1–2 hours of reperfusion in renal transplantation of FSGS recipients. The observed correlation between effacement of podocyte FP in post-reperfusion biopsies and early recurrence of nephrotic range proteinuria suggests a key role for the podocyte injury in the pathogenesis of recurrent FSGS, a condition that negatively affects long-term graft survival. The limitations of this study include the small size and the retrospective study design. Further larger multicenter prospective studies will be required to determine if FP effacement in the peri-operative setting can be utilized as a predictor of recurrent FSGS and/or can be utilized as a guide for therapeutic decision-making.

Materials and Methods

Study Design

Between January 2004 through June 2010, patients with a biopsy-proven diagnosis of idiopathic FSGS that received a KT allograft at the Division of Kidney and Pancreas Transplantation at the University of Miami School of Medicine were enrolled in this study. Secondary FSGS and non-FSGS causes of ESRD were excluded. All patients received a single dose of rituximab following post-reperfusion kidney biopsy and within 24 hours of transplant (41). This study was approved by the University of Miami Miller School of Medicine institutional review board.

Immunosuppressive therapy consisted of induction with thymoglobulin and anti-CD25 antibody (basiliximab or daclizumab) (42) followed by maintenance treatment with tacrolimus and mycophenolate mofetil or mycophenolic acid. Tacrolimus typically was initiated when renal function was stable during the immediate post-operative period. The doses were adjusted to maintain trough drug levels of 5–7 ng/mL.

Histology and Electron Microscopy

To examine immediate post-transplant events on the allograft after reperfusion, wedge KT biopsies were obtained in the operating room pre-reperfusion and 1–2 hours post-reperfusion of the kidney just prior to wound closure. In the case of living donors, the kidney was flushed with lactated ringer's solution (with added heparin, solu-medrol, sodium bicarbonate and lidocaine). For deceased donor kidneys, the pre-reperfusion biopsy was obtained when the kidney was taken off the pulsatile perfusion pump (43). All renal specimens were examined for light microscopy, immunofluorescence, and electron microscopy. All the post-reperfusion biopsies were immediately preserved in Karnovsky's solution for EM, dehydrated with ethanol and embedded in Epon 812. Only living donor related pre-reperfusion biopsies were placed immediately in Karnovsky's, while those from deceased donors were processed from the formalin-fixed tissue blocks. The ultrathin sections were stained with 4% uranyl acetate and lead citrate and examined with a JEOL electron microscope. The renal pathologist was blinded to the patient data. Photos of glomerular capillaries were taken at random, avoiding areas adjacent to the vascular pole, mesangium or tangential capillary sections.

We printed digital negatives of electron micrographs at 12000 $\times$ from 2 glomeruli per biopsy that included a mean of 17 capillaries per glomerulus. From each photograph, measurements included the number of capillary loops; and the number of FP revealing a width that exceeded 5 times that of the estimated adjacent capillary basal lamina. We used a modified method to analyze the score of FP effacement by calculating total effaced FPs divided by total number of available capillaries (44).

Early and Long-term Renal Outcome Evaluation

An external ureteral stent was placed in the newly transplanted kidney to differentiate urine from the transplanted organ from that of the native kidneys or of previously transplanted kidneys. The early graft function was assessed by serum creatinine, and blood urea nitrogen. Immediate graft function (IGF) was defined as creatinine < 3 mg/dL by post-operative day 5. Slow graft function (SGF) was defined as persistence of serum creatinine concentration more than 3 mg/dL but not requiring dialysis on day 5 post-transplantation. Delayed graft function (DGF) was defined as the requirement for dialysis within the first week after renal transplantation. The presence of proteinuria was also monitored closely by urine protein creatinine ratio (PCR) on daily spot morning specimens. The maximum value of all the urine PCR obtained during 3rd postoperative day to the 1st post-transplant month was used for analysis. The diagnosis of early recurrence was made on the basis of the clinical recurrence of heavy proteinuria with urinary PCR > 3 in the absence of acute rejection or urinary tract infection. PP was performed with a single volume per session with 5% albumin replacement 3 cycles/week in those with persistent high urine PCR. The response to treatment is defined as complete response, with PCR less than 0.3, 0.3–3 as partial response, while greater than 3 classified as non-response.

Patients underwent regular post-kidney transplant out-patient clinic follow-up for lab tests and checkups. Additional KT biopsies were performed when there was persistent, unexplained impairment of renal function or new-onset proteinuria. Late recurrences were defined as recurrences after one month of the transplant with biopsy-proven FSGS. Graft loss was defined as permanent return to dialysis or estimated glomerular filtration rate <15 mL/min/1.73 m². Clinical data included age, gender, age at onset of FSGS, time to first dialysis, duration of dialysis, bilateral native nephrectomy, time of early recurrence, peak urine PCR, and long-term allograft outcomes. We also categorized age at onset of nephrotic level as <15 years old and progression to ESRD within 3 years or longer. These patients were divided into two groups as early recurrent and non-early recurrent group. The clinical data, foot process effacement score and long-term outcomes between these two groups were analyzed.

Statistical analysis

Univariate analysis was performed on clinical variables for recurrent and non-recurrent group to identify potential risk factors for recurrence. Categorical variables were tested with chi-square and Fisher's exact tests. Continuous variables of patients are reported as the mean $\pm$ SD and tested with Student's test. Linear regression analysis was performed between score of FP effacement and logarithmic peak urine PCR. Graft survival of the transplant in patients with and without recurrent FSGS was compared by the Kaplan-Meier method, using the log rank test. Data were analyzed with SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as $p < 0.05$. Sensitivity and specificity of the FP effacement score were evaluated by generating a receiver operating characteristic (ROC) curve.

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References

1. Newstead CG. Recurrent disease in renal transplants. *Nephrol Dial Transplant*. 2003; 18(suppl 6): 68.
2. Choy BY, Chan TM, Lai KN. Recurrent glomerulonephritis after kidney transplantation. *Am J Transplant*. 2006; 6(11):2535. [PubMed: 16939521]
3. Ponticelli C. Recurrence of focal segmental glomerular sclerosis (FSGS) after renal transplantation. *Nephrol Dial Transplant*. 2010; 25(1):25. [PubMed: 19875378]
4. Maizel SE, Sibley RK, Horstman JP, Kjellstrand CM, Simmons RL. Incidence and significance of recurrent focal segmental glomerulosclerosis in renal allograft recipients. *Transplantation*. 1981; 32(6):512. [PubMed: 7041354]
5. Vinai M, Waber P, Seikaly MG. Recurrence of focal segmental glomerulosclerosis in renal allograft: an in-depth review. *Pediatr Transplant*. 2010; 14(3):314. [PubMed: 20519016]
6. Abbott KC, Sawyers ES, Oliver JD 3rd, et al. Graft loss due to recurrent focal segmental glomerulosclerosis in renal transplant recipients in the United States. *Am J Kidney Dis*. 2001; 37:366. [PubMed: 11157379]
7. Savin VJ, Sharma R, Sharma M, et al. Circulating factor associated with increased glomerular permeability to albumin in recurrent focal segmental glomerulosclerosis. *N Engl J Med*. 1996; 334:873. [PubMed: 8596569]
8. Wei C, El Hindi S, Li J, Fornoni A, et al. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nat Med*. 2011; 17(8):952. [PubMed: 21804539]
9. Somlo S, Mundel P. Getting a foothold in nephrotic syndrome. *Nat Genet*. 2000; 24(4):333. [PubMed: 10742089]
10. Powell HR. Relationship between proteinuria and epithelial cell changes in minimal lesion glomerulopathy. *Nephron*. 1976; 16(4):310. [PubMed: 765875]
11. Koop K, Eikmans M, Baelde HJ, et al. Expression of podocyte-associated molecules in acquired human kidney diseases. *J Am Soc Nephrol*. 2003; 14:2063. [PubMed: 12874460]
12. Cheigh JS, Mouradian J, Susin M, et al. Kidney transplant nephrotic syndrome: relationship between allograft histopathology and natural course. *Kidney Int*. 1980; 18(3):358. [PubMed: 7007710]
13. Hohage H, Kleyer U, Bruckner D, August C, Zidek W, Spieker C. Influence of proteinuria on long-term transplant survival in kidney transplant recipients. *Nephron*. 1997; 75:160. [PubMed: 9041535]
14. Peddi VR, Dean DE, Hariharan S, Cavallo T, Schroeder TJ, First MR. Proteinuria following renal transplantation: correlation with histopathology and outcome. *Transplant Proc*. 1997; 29(1–2):101. [PubMed: 9122914]
15. Halimi JM, Laouad I, Buchler M, et al. Early low-grade proteinuria: Causes, short-term evolution and long-term consequences in renal transplantation. *Am J Transplant*. 2005; 5:2281. [PubMed: 16095510]
16. Amer H, Fidler ME, Myslak M, Morales P, Kremers WK, Larson TS, Stegall MD, Cosio FG. Proteinuria after kidney transplantation, relationship to allograft histology and survival. *Am J Transplant*. 2007; 7(12):2748. [PubMed: 17941956]
17. Artz MA, Dooper PM, Meuleman EJ, van der Vliet JA, Wetzels JF. Time course of proteinuria after living-donor kidney transplantation. *Transplantation*. 2003; 27(2):421. [PubMed: 12883203]
18. Stefanidis I, Heintz B, Stöcker G, Mrowka C, Sieberth HG, Haubeck HD. Association between heparan sulfate proteoglycan excretion and proteinuria after renal transplantation. *J Am Soc Nephrol*. 1996; 7(12):2670. [PubMed: 8989747]

19. D'Cunha PT, Parasuraman R, Venkat KK. Rapid resolution of proteinuria of native kidney origin following live donor renal transplantation. *Am J Transplant*. 2005; 5:351–355. [PubMed: 15643995]
20. Myslak M, Amer H, Morales P, et al. Interpreting post-transplant proteinuria in patients with proteinuria pre-transplant. *Am J Transplant*. 2006; 6(7):1660. [PubMed: 16827868]
21. Morath C, Zeier M. When should post-transplantation proteinuria be attributed to the renal allograft rather than to the native kidney? *Nat Clin Pract Nephrol*. 2007; 3(1):18. [PubMed: 17183258]
22. Hoyer JR, Vernier RL, Najarian JS, Raij L, Simmons RL, Michael RF. Recurrence of idiopathic nephrotic syndrome after renal transplantation. *Lancet*. 1972; 2(7773):343. [PubMed: 4114718]
23. Briganti EM, Russ GR, McNeil JJ, Atkins RC, Chadban SJ. Risk of renal allograft loss from recurrent glomerulonephritis. *N Engl J Med*. 2002; 347(2):103. [PubMed: 12110738]
24. Senggutuvan P, Cameron JS, Hartley RB, et al. Recurrence of focal segmental glomerulosclerosis in transplanted kidneys: analysis and risk factors in 59 allografts. *Pediatr Nephrol*. 1990; 4:21. [PubMed: 2206875]
25. Baum MA, Stablein DM, Panzarino VM, Tejani A, Harmon WE, Alexander SR. Loss of living donor renal allograft survival advantage in children with focal segmental glomerulosclerosis. *Kidney Int*. 2001; 59:328. [PubMed: 11135087]
26. Cheong HI, Han HW, Park HW, et al. Early recurrent nephrotic syndrome after renal transplantation in children with focal segmental glomerulosclerosis. *Nephrol Dial Transplant*. 2000; 15(1):78. [PubMed: 10607771]
27. Meyrier AY. Treatment of focal segmental glomerulosclerosis with immunophilin modulation: when did we stop thinking about pathogenesis? *Kidney Int*. 2009; 76(5):487. [PubMed: 19494796]
28. Marszal J, Saleem MA. The Bioactivity of Plasma Factors in Focal Segmental Glomerulosclerosis. *Nephron Exp Nephrol*. 2006; 104(1):e1. [PubMed: 16707909]
29. Shalhoub RJ. Pathogenesis of lipoid nephrosis: a disorder of T-cell function. *Lancet*. 1974; 2:556. [PubMed: 4140273]
30. Savin VJ, Sharma R, Sharma M, et al. Circulating factor associated with increased glomerular permeability to albumin in recurrent focal segmental glomerulosclerosis. *N Engl J Med*. 1996; 334:878. [PubMed: 8596570]
31. Le Berre L, Godfrin Y, Lafond-Puyet L, et al. Effect of plasma fractions from patients with focal and segmental glomerulosclerosis on rat proteinuria. *Kidney Int*. 2000; 58:2502. [PubMed: 11115084]
32. Sharma M, Sharma R, Reddy SR, McCarthy ET, Savin VJ. Proteinuria after injection of human focal segmental glomerulosclerosis factor. *Transplantation*. 2002; 73(3):366. [PubMed: 11884932]
33. Sharma M, Sharma R, McCarthy ET, Savin VJ. The focal segmental glomerulosclerosis permeability factor: biochemical characteristics and biological effects. *Exp Biol Med*. 2004; 229:85.
34. Savin VJ, McCarthy ET, Sharma M. Permeability factors in focal segmental glomerulosclerosis. *Semin Nephrol*. 2003; 23(2):147. [PubMed: 12704575]
35. Savin VJ, McCarthy ET, Sharma R, Charba D, Sharma M. Galactose binds to focal segmental glomerulosclerosis permeability factor and inhibits its activity. *Transl Res*. 2008; 151:288. [PubMed: 18514139]
36. Dello Strologo L, Guzzo I, Laurenzi C, et al. Use of rituximab in focal glomerulosclerosis relapses after renal transplantation. *Transplantation*. 2009; 88(3):417. [PubMed: 19667947]
37. Tsagalis G, Psimenou E, Nakopoulou L, Laggouranis A. Combination treatment with plasmapheresis and rituximab for recurrent focal segmental glomerulosclerosis after renal transplantation. *Artif Organs*. 2010; 35(4):420. [PubMed: 20637013]
38. Reiser J, von Gersdorff G, Loos M, et al. Induction of B7-1 in podocytes is associated with nephrotic syndrome. *J Clin Invest*. 2004; 113(10):1390. [PubMed: 15146236]
39. Kriz W. The pathogenesis of 'classic' focal segmental glomerulosclerosis-lessons from rat models. *Nephrol Dial Transplant*. 2003; 18:39.
40. Hara M, Yanagihara T, Kihara I. Urinary Podocytes in Primary Focal Segmental Glomerulosclerosis. *Nephron*. 2001; 89(3):342. [PubMed: 11598401]

41. Fornoni A, Sageshima J, Wei C, et al. Rituximab targets podocytes in recurrent focal segmental glomerulosclerosis. *Sci Transl Med*. 2011; 3(85) 85ra46.
42. Sageshima J, Ciancio G, Gaynor JJ, et al. Addition of anti-CD25 to thymoglobulin for induction therapy: delayed return of peripheral blood CD25-positive population. *Clin Transplant*. 2011; 25(2):E132. [PubMed: 21083765]
43. Ciancio G, Gaynor JJ, Sageshima J, et al. Favorable outcomes with machine perfusion and longer pump times in kidney transplantation: a single-center, observational study. *Transplantation*. 2010; 90(8):882. [PubMed: 20703178]
44. van den Berg JG, van den Bergh Weerman MA, Assmann KJ, Weening JJ, Florquin S. Podocyte foot process effacement is not correlated with the level of proteinuria in human glomerulopathies. *Kidney Int*. 2004; 66(5):1901. [PubMed: 15496161]

Figure 1.

Electron micrographs of representative pre-reperfusion and post-reperfusion KT biopsies. (A) Representative ultrastructure of the pre-reperfusion KT biopsy. Post-reperfusion KT biopsies were examined in non-recurrent (B) and recurrent (C–I) patients. These images suggest an increased effacement of the podocyte foot process in the post-reperfusion KT biopsies along with enlargement of the podocytes in the recurrent group.

Figure 2.

Correlation of logarithmic peak urine protein creatinine ratio and foot process effacement score. There was an association between degree of foot process effacement and proteinuria ($r^2 = 0.475$, $p = 0.04$).

Figure 3.

A receiver operating characteristic curve of foot process effacement score and FSGS recurrence. Using a cut-off value of 1.22, the score predicted the development of early FSGS recurrence with sensitivity of 71.4% and specificity of 91.7%.

Table 1

Comparison of patient demographics and clinical data between non-recurrent and recurrent group

	Non-recurrent group (n=12)	Recurrent group (n=7)	p value
Sex M/F	6/6	3/4	1.000
Race (Black/non-black)	5/7	0/7	0.106
Age at onset of disease (years)	11.77 ± 10.46	11.14 ± 10.98	0.903
Age less than 15 years at onset of disease	66.7%	57.1%	1.000
Time to first time of dialysis	6.66 ± 4.17	4.72 ± 3.73	0.325
ESRD within 3 years of diagnosis	25.0%	57.1%	0.326
Duration of dialysis before transplantation (years)	2.86 ± 1.78	4.25 ± 3.40	0.338
Native nephrectomy	25.0%	42.9%	0.617
Age at transplantation (years)	20.78 ± 8.61	18.21 ± 10.86	0.576
Preemptive transplantation	33.3%	14.3%	0.603
Second transplantation (%)	16.7%	14.3%	1.000
Donor type (living, %)	66.7%	57.1%	1.000
Delayed graft function	8.3%	14.3%	1.000
Score of FP effacement	0.72 ± 0.31	1.35 ± 0.63	*0.039

*
p < 0.05.

Table 2

Recurrent patient characteristics at time of recurrence and response to treatment

Number	Onset of proteinuria (POD)	Peak urine PCR	Graft function	PP	Total number of PP sessions	Rituximab	Response to treatment
1	4	3.89	IGF	N	0	Y	CR
2	3	3.85	SGF	Y	3	Y	No response
3	3	5.20	IGF	Y	5	Y	CR
4	3	4.62	IGF	Y	5	Y	PR
5	3	61.0	IGF	Y	10	Y	PR
6	7	3.4	IGF	Y	5	Y	PR
7	7	3.38	IGF	N	0	Y	CR

POD: post-operative day; PCR: protein creatinine ratio; IGF: immediate graft function; SGF: slow graft function; CR: complete response; PR: partial response; PP: plasmapheresis