Association of Histologic Variants in FSGS Clinical Trial with Presenting Features and Outcomes


Summary
Background and objectives FSGS histologic variants have correlated with outcomes in retrospective studies. The FSGS Clinical Trial provided a unique opportunity to study the clinical impact of histologic variants in a well defined prospective cohort with steroid-resistant primary FSGS.

Design, setting, participants, & measurements Renal biopsies of 138 FSGS Clinical Trial participants aged 2–38 years enrolled from 2004 to 2008 were analyzed using the Columbia classification by core pathologists. This study assessed the distribution of histologic variants and examined their clinical and biopsy characteristics and relationships to patient outcomes.

Results The distribution of histologic variants was 68% (n=94) FSGS not otherwise specified, 12% (n=16) collapsing, 10% (n=14) tip, 7% (n=10) perihilar, and 3% (n=4) cellular. Individuals with not otherwise specified FSGS were more likely to have subnephrotic proteinuria (P=0.01); 33% of teenagers and adults had tip or collapsing variants compared with 10% of children, and subjects with these variants had greater proteinuria and hypoalbuminemia than not otherwise specified patients. Tip variant had the strongest association with white race (86%) and the lowest pathologic injury scores, baseline creatinine, and rate of progression. Collapsing variant had the strongest association with black race (63%), and the highest pathologic injury scores (P=0.003), baseline serum creatinine (P=0.003), and rate of progression. At 3 years, 47% of collapsing, 20% of not otherwise specified, and 7% of tip variant patients reached ESRD (P=0.005).

Conclusions This is the first prospective study with protocol-defined immunomodulating therapies confirming poor renal survival in collapsing variant and showing better renal survival in tip variant among steroid-resistant patients.

Introduction
FSGS is a leading cause of proteinuria and ESRD in children and adults. The National Institutes of Health-funded multicenter FSGS Clinical Trial (FSGS-CT) examined outcomes in 138 steroid-resistant children and young adults aged 2–40 years (1,2). Entry criteria included corticosteroid-resistant primary FSGS documented by renal biopsy at the referring institution and confirmed by one of the study’s expert core renal pathologists. Patients were randomized to a 12-month course of either cyclosporine (CSA) or mycophenolate mofetil and dexamethasone (MMF/DEX) (1); 46% of CSA-treated patients and 33% of MMF/DEX patients achieved at least partial remission at 1 year, whereas only 19% of CSA and 9% of MMF/DEX patients achieved complete remission (1). These outcome differences were not statistically significant.

A major working classification recognizes five histologic variants of FSGS: FSGS not otherwise specified (NOS), perihilar, cellular, tip, and collapsing (3). Often referred to as the Columbia classification, this schema was chosen for renal biopsy analysis within the FSGS-CT (3). This histologic approach has been shown to differentiate presenting clinical features and outcomes in several nonrandomized retrospective studies (4,5). Some investigators have argued that achievement of complete or partial remission of nephrotic syndrome is a better predictor of outcome in FSGS than the histologic variant (6).

The FSGS-CT provided a unique opportunity to study the clinical impact of histologic variants in a well defined prospective cohort with steroid-resistant primary FSGS. The goals of this study were to use FSGS-CT prospectively collected data to (1) assess the distribution of histologic variants in children and young adults with steroid-resistant FSGS, (2) examine clinical and biopsy characteristics of patients with each variant, and (3) assess the relationship of histologic variants to outcome, including ESRD. Our major hypotheses were that collapsing variant FSGS would...
be associated with black race, nephrotic range proteinuria, and greater risk of ESRD and that tip variant would be associated with white race, nephrotic range proteinuria, and lower risk of ESRD. A major question was whether the requirement of steroid resistance at study entry would alter these histologic paradigms.

Materials and Methods

Eligibility Criteria
Patients were eligible for study enrollment if they had primary FSGS confirmed on renal biopsy review by one of eight core renal pathologists located in California (A.H.C. and C.C.N.), Washington (C.E.A.), Texas (D.A.S.), New York (V.D.D. and J.P.), and North Carolina (J.C.J. and D.B.T.). Clinical eligibility criteria included age of proteinuria onset and age at enrollment 2–40 years, estimated GFR (eGFR) ≥40 ml/min per 1.73 m², urine protein/creatinine ratio (Up/c) >1 g/g, and corticosteroid resistance defined as persistent proteinuria after a minimum of 4 weeks of corticosteroid therapy. Patients with clinical evidence of possible secondary FSGS because of conditions such as morbid obesity (body mass index ≥40), HIV infection, etc. were excluded. Details are provided in previous publications (1,2).

Pathologic Criteria
Renal biopsy materials identified by FSGS-CT code number were reviewed by a study pathologist according to geographic origination. Reviewed biopsy materials included (1) final pathology report, including light microscopic, immunofluorescence, and electron microscopic descriptions from the referring pathologist; (2) original light microscopic slides, including hematoxylin/eosin, periodic acid–Schiff, Masson trichrome, and Jones silver stains; (3) immunofluorescence images or descriptions from the report; and (4) electron microscopic images. If required for diagnosis, unstained slides or paraffin blocks were requested for additional studies. Participants with biopsies that showed another primary disease (Alport syndrome, C1q nephropathy, etc.) were excluded from the FSGS-CT.

Before the FSGS-CT began, the panel of renal pathologists set consensus diagnostic criteria based on the literature in conference calls. They conferred on difficult cases. Pathologists graded light microscopic findings, including total number of glomeruli, number of globally sclerotic glomeruli, number of segmentally sclerotic glomeruli, percent cortical area with tubular atrophy and interstitial fibrosis evaluated visually to the nearest 10% (7), severity of arteriosclerosis (0, absent; 1, mild; 2, moderate; 3, severe) (8), and type of FSGS (1, NOS; 2, perihilar; 3, cellular; 4, tip; 5, collapsing) (3). Details of the Columbia classification have been published (3). In brief, the following definitions were used. NOS showed segmental capillary lumina with or without foam cells, hyalinosis, and karyorrhexis and without tip or collapsing lesions. Tip showed at least one glomerulus with a segmental lesion involving the tip domain (outer 25% of the tuft next to the proximal tubule origin) where the tubular pole is identified, and there is either adhesion or confluence of podocytes with parietal or tubular cells at the tubular lumen or neck, without collapsing lesions. Collapsing showed at least one glomerulus with either segmental or global glomerular capillary wall collapse with hypertrophy and hyperplasia of the overlying epithelial cells.

Definition of Nephrosis
Up/c between >1 and ≤2 g/g and serum albumin ≥2.5 g/dl defined mild nephrosis. Up/c between 2 and ≤6 g/g or serum albumin between ≥1.5 and <2.5 g/dl defined intermediate nephrosis. Up/c >6 g/g or serum albumin <1.5 g/dl defined severe nephrosis. Edema was not used in the definition, because patients may have received diuretics and diet modification to control edema before study entry.

Estimation of GFR
Participant eGFR was estimated using the Schwartz formula for age <18 years and the Cockroft–Gault formula for age ≥18 years (1). The measured weight was used in the Cockroft–Gault calculations, because it was not possible to estimate dry weight accurately in edematous patients.

Statistical Analyses
Data were summarized as means ± SDs, medians and interquartile ranges, or counts and percentages. Cross-sectional comparisons across groups at baseline used Kruskal–Wallis (rank sum) tests for continuous data and Pearson chi-squared, Fisher exact, or Cochran–Mantel–Haenszel tests for categorical data as appropriate. We related variant to serum creatinine (SCR) and Up/c ratio at baseline and week 26, and we related variant to serum albumin (SCR) and Up/c ratio as percent change to week 26, after which patients failing to achieve partial remission terminated their intervention per study protocol. Log transformations were used when comparing SCR with Up/c to reduce positive skewness, with ANOVA to control for randomized treatment assignment. All P values are from two-tailed tests.

Time from randomization to ESRD was summarized using Kaplan–Meier curves; the log-rank test was used to compare time to ESRD across variants, with stratification for treatment assignment. Because only 28 cases progressed to ESRD, only a limited number of potential ESRD predictors could be considered at one time by Cox proportional hazard modeling. Multiple models were run: first with the pathology predictor, adjusting only for treatment group, and then adjusting for baseline eGFR, proteinuria, and age. Pathology predictors included subtype (NOS, subtype collapsing, percent cortical parenchyma with tubular atrophy/interstitial fibrosis, percent glomeruli with global sclerosis, percent glomeruli with segmental sclerosis, and percent glomeruli with any sclerosis (segmental or global)). Tip subtype was not considered in this analysis, because only one tip case reached ESRD.
Results

Demographics and Clinical Parameters by Histologic Subtype

A total of 138 eligible patients enrolled in the FSGS-CT trial. On FSGS histologic subtype assessment, the majority of biopsies was classified as NOS (n=94, 68%), with fewer patients showing collapsing (n=16, 12%) and tip (n=14, 10%) variants (Figure 1). The numbers of patients diagnosed with perihilar (n=10, 7%) and cellular (n=4, 3%) variants were too few for meaningful statistical analysis.

Demographic and clinical data are summarized in Table 1. There were no significant differences in sex, birth weight, or baseline hypertension or obesity among patients with NOS, tip, and collapsing variants. NOS was the most common variant in all age groups. Ages at onset and at biopsy were significantly older for patients with collapsing variant compared with NOS variant (P=0.005 and P=0.02, respectively). Within the NOS group, more patients (47%) were children aged 2–12 years than teenagers (29%) or adults (24%, P=0.02). Patients with tip variant were most likely to be Caucasian (86%), with median age at onset of 15 years, intermediate between NOS (median=13 years) and collapsing variants (median=16.5 years). Patients with collapsing FSGS were more likely to be of black race (63%, P=0.03), have edema (69%, P=0.03), and have renal functional impairment (mean SCR=1.3 mg/dl, P=0.003; median eGFR=82 ml/min, P=0.006) at enrollment compared to patients with NOS and tip variants. Features of nephrotic syndrome were significantly more pronounced in collapsing and tip variants compared with NOS variant, including higher Up/c ratio (P=0.02), serum cholesterol (P=0.002), and LDL (P<0.001) and lower serum albumin (P=0.04) and presence of edema (P=0.03). Severe or intermediate nephrosis was present in 94% of patients with collapsing variant and 93% of patients with tip variant compared to 69% of patients with NOS (P=0.03).

Morphologic Findings

All three FSGS histologic subtypes had glomeruli with both global and segmental glomerulosclerosis; however, the degree varied by subtype (Table 2). Degree of global glomerulosclerosis differed significantly overall (P=0.05), with a greater median percentage of globally sclerotic glomeruli seen in NOS compared with tip (P=0.02). Biopsies with collapsing variant had a higher percentage of glomeruli with segmental lesions (P<0.001), particularly compared to biopsies with NOS (P<0.001). Collapsing variant also had more glomerulosclerosis of any type, indicating higher total glomerular injury (P=0.02). Degree of tubular atrophy/interstitial fibrosis was lowest in tip (5% [0%, 5%]), intermediate in NOS (10% [3%, 25%]), and highest in collapsing variant (20% [7.5%, 70%], P=0.003). There was no significant difference in arteriosclerosis severity between the variants (data not shown).

The extent of renal injury among subtypes varied across age groups (Table 3). NOS cases showed more segmental glomerulosclerosis in teenagers than children (P=0.04). The NOS group also showed differences in tubulo-interstitial scarring by age group (P<0.001). Teenagers had more tubular atrophy and interstitial fibrosis than children (P=0.006), and adults had more tubular atrophy and interstitial fibrosis than teenagers (P=0.002), despite similar clinical duration of disease. Global glomerulosclerosis was rare in tip variant biopsies (most reporting no glomeruli affected), with no demonstrable differences across age groups. In contrast to the NOS variant, adolescents and adults with tip variant had similar percentages of tubular atrophy and interstitial fibrosis (7.5% [2.5%, 10%] and 5% [5%, 5%], respectively), which tended to involve a greater area of the tubulo-interstitium in adults compared with children (0% [0%, 2.5%], P=0.08).

Patient Outcome by Histologic Subtype

ESRD was assessed at up to 5 years in a few patients (Table 4). Mean follow-up for patients with functioning kidneys was 2.9±1.1 years; median (25th, 75th percentile)
Pathologic Predictors of ESRD

Among variables considered as predictors of ESRD, collapsing variant (compared with all others), percent globally sclerotic glomeruli, percent segmentally sclerotic glomeruli, percent glomerulosclerosis of any type, and percent tubular atrophy/interstitial fibrosis were significant predictors of ESRD after adjusting for treatment group, but NOS (compared with all others) was not. After additional adjustment for baseline eGFR, Up/c, and age, only percent tubular atrophy/interstitial fibrosis emerged as a significant predictor of ESRD (hazard ratio [95% confidence interval]=1.21 [1.03, 1.42] per 10 percentage point increase, P=0.02), whereas percent glomerulosclerosis of any type was a marginally significant predictor (hazard ratio [95% confidence interval]=1.16 [0.97, 1.38] per 10 percentage point increase, P=0.13).

Discussion

Pathologists recognize the histologic diversity of FSGS, because lesions differ in location relative to the glomerular vascular and tubular poles and morphologic features of hyalinosis, capillary collapse, and endocapillary and extracapillary hypercellularity (3,4,9–12). The Columbia classification for histologic FSGS variants uses standardized
definitions that can be applied to both primary and secondary FSGS (3). These variants include the common generic NOS type, the aggressive collapsing type, the abruptly presenting tip type, the early-stage cellular type, and the perihilar type common in secondary adaptive FSGS (13–17). Several retrospective series have suggested that FSGS histologic subtypes vary in incidence and correlate with remission status and outcome. This study assessed histopathologic distribution and renal outcomes of children and young adults with steroid-resistant FSGS using prospective data.

The strength of this histopathology study is that it capitalizes on precise treatment and monitoring per the FSGS-CT protocol, histologic variant determination based on core biopsy review, and prospectively determined clinical outcomes assessing the relationship between histologic variant and clinical outcomes. This study identified NOS as the most common subtype, comprising 68% of this cohort, followed by collapsing (12%), tip (10%), perihilar (7%), and cellular variants (3%) (4,5). In comparison, FSGS subtyping at Columbia University showed 62.3% NOS or perihilar variants, 23.7% collapsing, 9.4% tip, and 4.5% cellular variants (4). Among 197 FSGS biopsies at the University of North Carolina at Chapel Hill, subtypes included 42% NOS, 26% perihilar, 11% collapsing, 17% tip, and 3% cellular (5). The low perihilar variant prevalence

<table>
<thead>
<tr>
<th>Table 2. Morphologic findings by FSGS histologic subtype</th>
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<tr>
<td>Morphologic Findings</td>
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<tr>
<td>---------------------------------------------------------</td>
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<tr>
<td>Total number of cases</td>
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<tr>
<td>Percent global glomerulosclerosis (n*)</td>
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<tr>
<td>Percent segmental glomerulosclerosis (n*)</td>
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<tr>
<td>Percent any glomerulosclerosis (n*)</td>
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<tr>
<td>Percent tubular atrophy/interstitial fibrosis</td>
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</table>

All values are expressed as median percentage of glomeruli in a biopsy or median percentage of cortical area in a biopsy with the designated histologic finding (25th, 75th percentile); n* is the available n for a given value. Where not specified, the available n is the same as the total number of cases in the top row. P values are for Kruskal–Wallis rank sum three-way comparisons and selected two-way comparisons across histologic subtypes.

<table>
<thead>
<tr>
<th>Table 3. Morphologic findings in FSGS variants by age at biopsy</th>
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<tbody>
<tr>
<td>Morphologic Findings</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Not otherwise specified</td>
</tr>
<tr>
<td>Global glomerulosclerosis (%)</td>
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<tr>
<td>Segmental glomerulosclerosis (%)</td>
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<tr>
<td>Tubular atrophy/interstitial fibrosis (percent area involved)</td>
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<tr>
<td>Tip variant</td>
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<tr>
<td>Global glomerulosclerosis (%)</td>
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<tr>
<td>Segmental glomerulosclerosis (%)</td>
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<tr>
<td>Tubular atrophy/interstitial fibrosis (percent area involved)</td>
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<tr>
<td>Collapsing variant</td>
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<tr>
<td>Global glomerulosclerosis (%)</td>
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<tr>
<td>Segmental glomerulosclerosis (%)</td>
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<td>Tubular atrophy/interstitial fibrosis (percent area involved)</td>
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</table>

All values are median (25th, 75th percentile). P values are for Kruskal–Wallis rank sum three-way comparisons and selected two-way comparisons across histologic subtypes.

*Insufficient cases for analysis.
(7%) in the FSGS-CT is not surprising, because secondary (adaptive) FSGS was excluded. Although body mass index in the range of 30–39 was not an exclusion criterion, patients with features of obesity-related glomerulopathy were not enrolled. Thus, there was no obvious relationship between the perihilar variant and obesity in this trial. The tip variant was less frequent than in the Columbia cohort. The FSGS-CT steroid resistance criterion may have selected for less steroid-responsive histopathologic variants.

The FSGS-CT findings support significant differences in demographic features between histologic subtypes. Tip and collapsing variants were more common in teenagers and adults than children (5,6,12,14–19). Only 29% of tip patients were children aged 2–12 years at onset of FSGS, whereas 43% were over 18 years. The age differences are notable considering age at entry was capped at 40 years, whereas 43% were over 18 years. The age differences are notable considering age at entry was capped at 40 years, whereas 43% were over 18 years. The age differences are notable considering age at entry was capped at 40 years, whereas 43% were over 18 years. The age differences are notable considering age at entry was capped at 40 years, whereas 43% were over 18 years.

Table 4. Outcome by FSGS histologic subtype

<table>
<thead>
<tr>
<th>Outcome Measures</th>
<th>Not Otherwise Specified</th>
<th>Tip</th>
<th>Collapsing</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>0.80 (0.40, 1.30) (n=94)</td>
<td>0.90 (0.60, 1.10) (n=13)</td>
<td>1.25 (1.00, 1.60) (n=16)</td>
<td>0.01b</td>
</tr>
<tr>
<td>Week 26</td>
<td>0.85 (0.50, 1.40) (n=86)</td>
<td>0.80 (0.40, 1.20) (n=11)</td>
<td>1.80 (1.20, 2.60) (n=15)</td>
<td>0.003b</td>
</tr>
<tr>
<td>Percent change weeks 0–26</td>
<td>15% (0%, 50%)</td>
<td>−8% (−17%, 33%)</td>
<td>50% (19%, 89%)</td>
<td>0.13b</td>
</tr>
<tr>
<td>Urine protein/creatinine ratio</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Week 0</td>
<td>3.6 (1.8, 6.7) (n=94)</td>
<td>4.7 (3.5, 8.9) (n=14)</td>
<td>7.6 (4.4, 10.7) (n=16)</td>
<td>0.11b</td>
</tr>
<tr>
<td>Week 26</td>
<td>1.2 (0.3, 2.9) (n=84)</td>
<td>1.2 (0.6, 2.4) (n=11)</td>
<td>4.2 (1.1, 6.9) (n=13)</td>
<td>0.50b</td>
</tr>
<tr>
<td>Percent change weeks 0–26</td>
<td>−67% (−83%, −36%)</td>
<td>−69% (−87%, −50%)</td>
<td>−51% (−80%, −32%)</td>
<td>0.67b</td>
</tr>
<tr>
<td>Percent ESRD at 3 yr</td>
<td>20% (16)</td>
<td>7% (1)</td>
<td>47% (6)</td>
<td>0.005d</td>
</tr>
</tbody>
</table>

All values are median (25th, 75th percentile).

*Week 0 is the onset of treatment.

bP value for overall difference among histologic subtypes obtained by applying ANOVA to log serum creatinine and log urine protein/creatinine ratio after controlling for treatment assignment.

*Percentages with ESRD at 3 years were estimated by the Kaplan–Meier method.

*Global P value from the log-rank test with stratification by treatment assignment.

Some investigators have suggested that initial achievement of complete or partial remission of nephrotic syndrome is a better predictor of outcome than the FSGS histologic variant (6). In the FSGS-CT study, all patients were steroid-resistant, allowing evaluation of FSGS morphology only in those patients with initial steroid resistance. The collapsing group experienced the greatest risk of ESRD (47% at 3 years) compared with ESRD rates of 20% for NOs and 7% for tip variant. Follow-up of 225 patients in a published retrospective study revealed that complete or partial remission was highest for tip lesion (76%), intermediate for cellular (44%) and NOs (39%), and lowest for collapsing variant (13%) (4). Renal survival was inversely related to remission status, with 6% of tip, 28% of cellular, 35% of NOs, and 65% of collapsing variant patients reaching ESRD (4). Most studies of tip variant have concluded that the majority of patients is initially steroid-responsive. In the study by Stokes et al. (12), 58.6% of unselected tip patients achieved complete remission, and 13.8% had partial remission after initial corticosteroid treatment. Therefore, despite the negative selection bias of initial steroid resistance in the FSGS-CT study, which might be expected to enrich for cases with an innately more aggressive course, the tip variant remained the most prognostically favorable subgroup. Interestingly, the striking outcome differences between tip and collapsing subtypes prevailed, despite similarly severe nephrotic syndrome at presentation and similar UP/c at 26 weeks. The trial treatment protocol eliminates the possibility that the poor ESRD outcome in the collapsing variant is caused by lack of therapy because of perceived treatment futility.

The major limitation of this pathology study is small sample size. This sample size prevented us from analyzing the

duration of disease, possibly because symptoms of nephrotic syndrome led to early clinical recognition.
perihilar \( (n=10) \) and cellular \( (n=4) \) FSGS variants. The small sample size also contributed to having only 28 patients reach ESRD, constraining our ability to obtain precise estimates of relationships between histology and renal outcomes as well as our ability to determine if the randomized treatments altered the association of outcomes with histologic factors. With a larger sample size and consequently, more occurrences of ESRD, we could have adjusted for additional baseline characteristics, possibly altering the conclusions drawn regarding the relationship of histologic factors and renal outcomes. A second limitation is that, because this pathologic study was conducted on a cohort of patients suitable for the FSGS clinical trial, it was not based on a representative sample from all children and young adults with FSGS.
Consequently, these results may not be generalizable to all children and adults with steroid-resistant FSGS.

The FSGS-CT study findings support a histologic classification distinguishing NOS, collapsing, and tip variants of primary FSGS. The emergence of tubulo-Interstitial scarring as the best predictor of outcome in multivariate analysis is not surprising, because this feature was applicable to all variants, whereas only 12% of biopsies were collapsing subtype. Based on the low prevalence of cellular variant, future iterations of the classification may consider whether the cellular form warrants differentiation from NOS after the possibility of unsampled tip lesion has been excluded by serial sectioning. In contrast, the poor outcome of the collapsing variant justifies an approach that differentiates cellular from collapsing forms. In some case series, the median time to ESRD for FSGS is 5–15 years. However, median FSGS-CT trial follow-up was only 3 years at the time of this publication. The short time course also underscores the strength of the statistically significant differences observed. Longer follow-up is needed to determine whether even greater differences in outcome will emerge.

Acknowledgments
This paper was supported by National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases Grants U01-DK63385, DK63490, DK63455, DK063549, and DK80095. The National Institutes of Health Clinical and Translational Science Awards Program supported the research facilities.

Disclosures
None.

References

Received: June 16, 2012 Accepted: October 27, 2012

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

This article contains supplemental material online at http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.06100612/-/DCSupplemental.