Podocyte foot process effacement as a diagnostic tool in focal segmental glomerulosclerosis

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Podocyte foot process effacement is characteristic of proteinuric renal diseases. In minimal change nephrotic syndrome (MCNS) foot processes are diffusely effaced whereas the extent of effacement varies in focal segmental glomerulosclerosis (FSGS). Here we measured foot process effacement in FSGS and compared it to that in MCNS and in normal kidneys. A clinical diagnosis was used to differentiate idiopathic FSGS from secondary FSGS. Median foot process width, determined morphometrically by electron microscopy, was 3236 nm in 17 patients with idiopathic FSGS, 1098 nm in 7 patients with secondary FSGS, and 1725 nm in 15 patients with MCNS, as compared to 562 nm in 12 control patients. Multivariate analysis showed that foot process width did not correlate with proteinuria or serum albumin levels but was significantly associated as an independent factor with the type of disease. Foot process width over 1500 nm differentiated idiopathic from secondary FSGS. Median foot process width, determined morphometrically by electron microscopy, was 3236 nm in 17 patients with idiopathic FSGS, 1098 nm in 7 patients with secondary FSGS, and 1725 nm in 15 patients with MCNS, as compared to 562 nm in 12 control patients. Multivariate analysis showed that foot process width did not correlate with proteinuria or serum albumin levels but was significantly associated as an independent factor with the type of disease. Foot process width over 1500 nm differentiated idiopathic from secondary FSGS. Multivariate analysis showed that foot process width did not correlate with proteinuria or serum albumin levels but was significantly associated as an independent factor with the type of disease. Foot process width over 1500 nm differentiated idiopathic from secondary FSGS.


KEYWORDS: focal segmental glomerulosclerosis; foot process; podocyte; proteinuria; renal morphology; pathology

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Received 27 August 2007; revised 8 June 2008; accepted 25 June 2008; published online 27 August 2008
study, we have performed a morphometric analysis of podocyte foot processes in patients diagnosed with idiopathic FSGS and FSGS secondary to maladaptive responses. The degree of foot process effacement in FSGS and FSGS variants was compared to the degree of foot process effacement in MCNS and normal kidneys.

RESULTS
Baseline characteristics of adult patients with FSGS are shown in Table 1. By clinical criteria, idiopathic FSGS was diagnosed in 17 patients and FSGS secondary to maladaptive responses was diagnosed in 12 patients. A secondary cause was identified in seven patients: reflux nephropathy (n = 2), atrophic kidney (n = 2), unilateral agenesis of the kidney (n = 1), nephrectomy after hydronephrosis (n = 1), and obesity (n = 1; BMI of 33.2 kg/m²). Five patients had nephrotic range proteinuria with a normal serum albumin, compatible with a diagnosis of FSGS secondary to maladaptive responses.12 The baseline characteristics of these five patients were not different from patients with an identifiable secondary cause (Table 1). However, we cannot exclude idiopathic FSGS in these patients with absolute certainty. Therefore, to generate unbiased data, results of these patients with possible secondary FSGS are reported but not included in the statistical analysis.

Four patients with idiopathic FSGS and two patients with secondary FSGS were treated with an ACE inhibitor (ACEI) at the time of renal biopsy. Perihilar FSGS was more common in patients diagnosed with secondary FSGS, whereas the tip lesion was seen more often in idiopathic FSGS (Table 1). As expected proteinuria and serum cholesterol were lower in patients with secondary FSGS. These patients also had a higher serum creatinine concentration compared to idiopathic FSGS. This is probably related to the more indolent course of FSGS secondary to maladaptive responses. As a consequence a renal biopsy is often not performed until renal function deteriorates. Another reason for the higher serum creatinine in patients with secondary FSGS may be that many have a loss of functioning nephrons as a stimulus for the maladaptive response.

Eight patients with idiopathic FSGS, one patient with secondary FSGS and one patients with possible secondary FSGS received immunosuppressive therapy with prednisone (n = 4) or prednisone and cyclophosphamide/cyclosporine (n = 6) after renal biopsy. Remission rate at 5 years was significantly higher in patients with idiopathic FSGS (87%) compared to patients with secondary FSGS (14%; P < 0.01). Remission rate at 5 years was 0% in patients with possible secondary FSGS.

Patients with MCNS presented with a mean proteinuria of 9.2 ± 4.1 g per day and a mean serum albumin of 21 ± 5 g/l (P = NS and P < 0.05, respectively, for the difference with FSGS). Mean age at biopsy was 38 ± 19 years (P < 0.01 for the difference with FSGS). A renal biopsy was performed within 1 month after presentation in 63% of patients with MCNS. Use of ACEi at the time of biopsy in these patients was not recorded; however, such therapy was unlikely to be started in patients with a sudden onset of a nephrotic syndrome. The controls had no proteinuria with a mean serum albumin of 39 ± 7 g/l. Mean age at biopsy was 51 ± 16 years.

Morphometric analysis and determinants of foot process width
Median FPW was 2290 nm (range, 626–8632 nm) in patients with FSGS irrespective of the underlying cause (idiopathic or secondary) and 1725 nm (range, 1216–2685 nm) in MCNS (Figure 1; P < 0.05). FPW in normal kidneys was significantly lower compared to FSGS and MCNS with a median FPW of 562 nm (range, 508–827 nm; P < 0.001).

Foot process width correlated with type of disease (MCNS, idiopathic or secondary FSGS; r = 0.61; P < 0.001). FPW also correlated with age at biopsy (r = 0.39; P < 0.05).

Table 1 | Characteristics of patients with FSGS at biopsy

<table>
<thead>
<tr>
<th></th>
<th>Idiopathic FSGS (n=17)</th>
<th>FSGS secondary to maladaptive responses (n=12)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>12/5</td>
<td>4/3</td>
<td>0.68</td>
</tr>
<tr>
<td>Age at biopsy (years)</td>
<td>52 ± 13</td>
<td>54 ± 14</td>
<td>0.57</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>112 ± 56</td>
<td>262 ± 18</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum albumin (g/l)</td>
<td>21 ± 5</td>
<td>37 ± 5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proteinuria (g per day)</td>
<td>9.4 ± 3.8</td>
<td>5.0 ± 1.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>11.1 ± 2.3</td>
<td>6.8 ± 1.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>110 ± 14</td>
<td>120 ± 17</td>
<td>0.21</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>72%</td>
<td>86%</td>
<td>0.28</td>
</tr>
<tr>
<td>Presentation to biopsy (months)</td>
<td>2.4 (0.5–16.1)</td>
<td>63.7 (0.3–135)</td>
<td>&lt;0.01</td>
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<tr>
<th></th>
<th>FSGS variant</th>
<th>With identifiable secondary cause (n=7)</th>
<th>Without identifiable secondary cause (possible secondary FSGS; n=5)*</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presentation to biopsy (months)</td>
<td>2.4 (0.5–16.1)</td>
<td>63.7 (0.3–135)</td>
<td>120 (60.1–477)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

FSGS, focal segmental glomerulosclerosis; F, female; M, male; NOS, not otherwise specified.

*These patients were not included in the statistical analysis.
but not with serum albumin ($r = -0.13; P = \text{NS}$), serum creatinine ($r = -0.11; P = \text{NS}; n = 24$) or proteinuria ($r = 0.24; P = \text{NS};$ Figure 2). On multivariate analysis, type of disease (MCNS, idiopathic or secondary FSGS) was the only determinant of FPW ($P < 0.001$).

We further analyzed the differences in FPW between idiopathic and secondary FSGS in comparison with MCNS (Figure 3). Foot process effacement was most severe in idiopathic FSGS and intermediate in MCNS, as reflected by a FPW of 3236 nm (range, 1606–8632 nm) and 1725 nm (range, 1216–2685 nm) respectively ($P = 0.001$ for idiopathic FSGS vs MCNS). Foot processes were relatively preserved in FSGS secondary to maladaptive responses, with a FPW of 1098 nm (range, 626–1800 nm). Statistical significance was $P < 0.001$ for idiopathic vs secondary FSGS and $P = 0.001$ for MCNS vs secondary FSGS. FPW in patients with possible secondary FSGS was 701 nm (range, 664–1258 nm).

The degree of overlap in FPW between idiopathic and secondary FSGS was low. Patients with idiopathic FSGS were characterized by a FPW above 1500 nm (Table 2). Receiver operating characteristics curve analysis showed that this cutoff value differentiated patients with idiopathic FSGS from secondary FSGS with a high sensitivity (100%) and specificity (72%). The degree of overlap between MCNS and idiopathic or secondary FSGS was high, and FPW could not accurately differentiate between these diseases.

**Foot process width and FSGS variants**

Including both patients with idiopathic and secondary FSGS, the degree of foot process effacement was not significantly different between FSGS variants (Figure 4). Median FPW was 3848 nm (range, 957–8631 nm) for FSGS not otherwise specified (NOS), 2551 nm (range, 1606–4414 nm) for the tip variant and 1570 nm (range, 626–6486 nm) for perihilar FSGS. FPW was significantly lower in patients with MCNS (1725 nm; range, 1217–2685 nm) compared to patients with the tip variant ($P < 0.05$).

On multivariate analysis, type of disease (idiopathic or secondary FSGS) but not FSGS variant was the only determinant of FPW in patients with FSGS. Within the group of patients with idiopathic FSGS, the degree of foot process effacement was most severe in FSGS NOS (5015 nm; range, 1875–8632 nm), intermediate in the tip variant (2551 nm; range, 1606–4414 nm), and least severe in MCNS (1725 nm; range, 1217–2685 nm). The differences in foot process effacement were statistically significant, with $P < 0.05$ for FSGS NOS vs FSGS tip variant, $P = 0.001$ for FSGS NOS vs MCNS, and $P < 0.05$ for FSGS tip variant vs MCNS.

**DISCUSSION**

Podocyte foot process effacement is present in most proteinuric diseases, such as MCNS, FSGS, membranous
nephropathy, and IgA nephropathy. It is considered to be a stereotypical reaction of podocytes to injury or damage. The exact mechanism resulting in foot process effacement remains unknown.\(^{13,14}\) Our current study confirms previous findings suggesting that the degree of foot process effacement is primarily dependent on the nature of the underlying disease and not a consequence of proteinuria.\(^2\) In multivariate analysis, type of disease was the only determinant of FPW. This finding correlates with more recent insights into podocyte biology, indicating that both proteinuria and morphological alterations in podocytes or slit pores are consequences of podocyte injury.\(^{13,15,16}\) In an experimental model of acutely induced proteinuria, we observed widespread effacement of foot processes before the onset of proteinuria.\(^{16}\) Also from human studies, there is evidence that proteinuria and podocyte alterations are not necessarily interdependent.\(^ {17}\) We have previously described a familial nephropathy, characterized by marked long-standing proteinuria but with normal podocytic foot processes.\(^ {18}\) These observations strengthen our conclusion that it is the type of disease (MCNS, idiopathic, or secondary FSGS), rather than the amount of proteinuria that determines foot process effacement.

Admittedly, counting of the foot processes and the necessary assessment of the distinctive separations between individual foot processes can be somewhat subjective as illustrated by Figures 5–8. However, in our study this had no major impact on the conclusions of the paper. First, in >95% of all counted separations the decision was unambiguous; second, counting was performed in a blinded fashion, without awareness of patients characteristics; third, adding the debatable separations would have increased the absolute number of foot processes by a few percent, however, without affecting the differences between the groups.

In our patient group, FPW also correlated with age, due to a significant lower age in patients with MCNS compared to patients with FSGS. However, it seems unlikely that the difference in age explains the difference in FPW. On multivariate analysis type of disease, not age, was the only determinant of FPW. Furthermore, there was also no correlation between FPW and age in our control patients (data not shown). If age is an important determinant, FPW should have increased with age in these patients with ages ranging from 30 to 70 years.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Predicting disease type by foot process width or disease type</th>
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<tr>
<td>Foot process width</td>
<td>FSGS secondary to maladaptive responses (n=7)</td>
</tr>
<tr>
<td>&gt;1500 nm</td>
<td>17</td>
</tr>
<tr>
<td>&lt;1500 nm</td>
<td>0</td>
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</tbody>
</table>

FSGS, focal segmental glomerulosclerosis; MCNS, minimal change nephrotic syndrome.

P<0.001 for difference between idiopathic FSGS and FSGS secondary to maladaptive responses.
P=not significant for difference between MCNS and FSGS secondary to maladaptive responses.
P<0.001 for difference between idiopathic FSGS and MCNS.
The expression of angiotensin II receptors in podocytes has been associated with the development of FSGS and could affect FPW through foot process contractility and cytoskeleton dynamics. Therefore, treatment with an ACEi may reduce the extent of foot process effacement. In our study, only a small number of patients with FSGS were treated with ACEi at the time of renal biopsy and treatment showed no correlation with FPW. Admittedly, data on ACEi use in patients with MCNS were not available. However, even the highest possible correlation between ACEi use and FPW in patients with MCNS would not have changed the results (data not shown). Type of disease still remained the only determinant of FPW.

Over the last years several causes of podocyte injury have been identified that can lead to foot process effacement. Important causes are interference with structural components of the slit diaphragm complex and its lipid rafts, direct interference with the actin-cytoskeleton and interference with podocyte-GBM interaction. In addition, at least in some patients with idiopathic FSGS and MCNS there is evidence that podocyte injury is the direct result of a circulating factor. Thus far, identification of this factor has been unsuccessful. The difference in foot process effacement in idiopathic FSGS and MCNS in our study suggests that the underlying cause of podocyte injury differs between the two disorders. This is in agreement with the notion that different plasma factors appear to be involved in idiopathic FSGS and MCNS.

Admittedly, the difference may not be associated with the underlying cause, but merely represent a difference in time to biopsy. Patients with MCNS were biopsied earlier after onset of proteinuria and foot process effacement may not have reached the maximum extent. To our knowledge there are no studies in humans showing a correlation between time of biopsy and the extent of foot process effacement in MCNS. Some data are available from animal studies. A Japanese group developed an animal model similar to MCNS. Injection of monoclonal antibody 5-1-6, an antigen directed at the extracellular domain of the rat homolog of nephrin, causes massive proteinuria without histologic abnormalities on light microscopy. Even after repeated doses, light microscopy showed minimal glomerular lesions. At 8 days after injection of monoclonal antibody 5-1-6 partial retraction of foot processes was seen on electron microscopic examination. In this model, proteinuria preceded the effacement of foot processes. Thus, if a biopsy is performed shortly after the onset of proteinuria foot process effacement may be less severe. However, it is questionable whether this model applies to humans with MCNS. Even after repeated doses for 25 weeks, only partial foot process effacement was found. This is clearly different from humans with MCNS, who show complete foot process effacement on renal biopsy.

The difference in time interval is notable. Our results do not exclude a single underlying cause of podocyte injury in idiopathic FSGS and MCNS. In patients with MCNS, idiopathic FSGS tip variant and idiopathic FSGS NOS, we observed a gradual, but significant, increase in the severity of foot process effacement. This finding could also indicate that idiopathic FSGS variants and MCNS reflect different stages of the same underlying disease. Injury to the podocytes results in foot process effacement and proteinuria, initially without light microscopic lesions (MCNS). As the disease progresses, podocytic foot processes become more effaced and light microscopic lesions appear, first the tip variant and as damage to the foot processes continues, sclerotic lesions (FSGS NOS) develop. This concept is supported by recent data from the literature. Howie et al. reported on patients...
with an initial diagnosis of MCNS. Tip lesions developed in subsequent biopsies from these patients. In one patient, tip lesions progressed to FSGS NOS lesions. We observed a similar pattern in a transplant kidney from a patient with recurrent FSGS after renal transplantation (Smeets et al. Morphological variants of FSGS reflect differences in developmental stage of the lesion. *J Am Soc Nephrol* 18:216A, 2007). Some glomeruli only showed FSGS tip variant, whereas FSGS NOS was present in other glomeruli, suggesting that a single cause can result in different FSGS variants. Obviously, FSGS and MCNS are complex diseases that are manifestations of many underlying causes and this sequence of events will not apply to all patients with FSGS or MCNS. It is unlikely that inclusion of patients with genetic mutations in GBM or podocyte proteins (dystroglycans, nephrin, podocin, α-actinin-4) contributed to the difference in foot process effacement. Familial forms were excluded after examination of the patient charts. Sporadic genetic mutations are also unlikely, as these mutations are rare in adult patients and if present they are characterized by therapy resistance.

Foot processes in patients with FSGS secondary to maladaptive responses were more conserved than in
idiopathic FSGS and MCNS. In fact, the difference in foot process effacement with idiopathic FSGS was large enough to define a cutoff value for FPW that can predict idiopathic or secondary FSGS with a high sensitivity and specificity. Our results are in agreement with studies in a specific group of patients with secondary FSGS due to obesity.\(^{10,30}\) These latter studies demonstrated that obesity-related FSGS was characterized by relatively mild foot process fusion, indicating that idiopathic FSGS and FSGS secondary to maladaptive responses can be distinguished by different morphologic features. Admittedly measurement of FPW is quite laborious. To reach the discriminative threshold of 1500 nm, we needed to count an average of 2.6 and 3.8glomeruli for patients with idiopathic and secondary FSGS, respectively. However, less time consuming techniques are not reliable. In agreement with data from D’Agati\(^{11}\), estimation of the mean percentage of the glomerular surface area affected by foot process fusion did not allow us to differentiate between idiopathic and secondary FSGS (data not shown). Similarly, a manual count of the number of foot processes per individual capillary loop, without measurement of the GBM, was far less predictive compared to measurement of FPW. Alternatively, clinical parameters such as serum albumin are often sufficient to distinguish between idiopathic FSGS and FSGS secondary to maladaptive responses. In a study of 37 patients with nephrotic range proteinuria due to biopsy proven FSGS, Praga et al.\(^{12,31}\) showed that serum albumin was significantly lower in patients with presumed idiopathic FSGS (serum albumin <30 g/l) than compared to FSGS secondary to maladaptive responses (serum albumin >35 g/l). However, in patients with a serum albumin between 30 and 35 g/l without an apparent secondary cause, the distinction between idiopathic and secondary forms of FSGS often poses a challenge to the nephrologist caring for patients with FSGS.\(^{32}\) In these patients, foot process measurement could be helpful in guiding diagnosis and prevent inappropriate treatment with steroids and cytotoxic agents that are not effective in secondary forms of FSGS.\(^{33}\) Our study did not include patients with a serum albumin between 30 and 35 g/l without a secondary cause, therefore future studies in this patient group are needed to determine whether morphometric analysis of podocyte foot processes can guide diagnosis and treatment of FSGS.

In conclusion, our study demonstrates that FPW correlates very well with idiopathic FSGS, MCNS, and FSGS secondary to maladaptive responses, independent of the degree of proteinuria. Foot processes are more effaced in idiopathic FSGS NOS and FSGS tip variant than in MCNS. Most severe foot process effacement is found in patients with idiopathic FSGS NOS. These findings suggest that some forms of idiopathic FSGS and MCNS may have a single underlying cause. Additional studies are necessary to test this hypothesis. In selected cases measurement of FPW can be useful to distinguish between idiopathic FSGS and FSGS secondary to maladaptive responses.

**MATERIALS AND METHODS**

**Patients and controls**

In total, 24 patients with biopsy proven FSGS were included in the study. Light microscopic assessment of glomeruli for FSGS lesions was performed in accordance with the Columbia classification system described by D’Agati et al.\(^{34}\) This classification defines five light microscopic patterns of FSGS: FSGS not otherwise specified (NOS), perihilar variant, cellular variant, tip variant, and collapsing variant. Adult patients with one of the above light microscopic variants of FSGS and either negative immunofluorescence or only segmental IgM and/or C3 were considered for the study.

For comparison we have used renal biopsy material of patients with MCNS and control patients. Data on FPW of 12 patients with MCNS and six patients after renal transplantation (used as control) were available from a previous study.\(^{35}\) We have added three additional patients with MCNS and six control renal tissues, consisting of the apparently unaffected part of kidneys removed because of a malignancy.

**Light microscopy and electron microscopy**

For light microscopy pieces of kidneys were fixed in Bouin’s solution overnight at room temperature, dehydrated, and embedded in paraplast (Amstelstad, Amsterdam, Netherlands). Thick sections (Two µm) were stained with periodic acid Schiff and methenamine silver.

For electron microscopy, small pieces of kidneys were fixed in 2.5% glutaraldehyde dissolved in 0.1 M sodium cacodylate buffer (pH 7.4) overnight at 4 °C and washed in the same buffer. The tissue fragments were postfixed in cacodylate-buffered 1% OsO\(_4\) for 2 h, dehydrated, and embedded in Epon 812 (Merck, Darmstadt, Germany). Ultrathin sections were cut on an ultratome (Leica, Reichert Ultracuts, Wien, Austria), and contrasted with 4% uranyl acetate for 45 min and subsequently with lead citrate for 4 min at room temperature. Sections were examined in a Jeol 1200 EX2 electron microscope (JEOL, Tokyo, Japan).

**Immunofluorescence microscopy**

For immunofluorescence, kidney fragments were snap frozen in liquid nitrogen, and 2 µm cryostat sections were incubated with fluorescein-labeled antisera directed to human IgG, IgM, IgA, C1q, C3, κ, λ, and fibrinogen. The sections were examined with a fluorescence microscope (Leica microsystems GmbH, Heidelberg, Germany).

**Measurements of foot processes and glomerular basement membrane**

Negatives of electron micrographs (magnification ×6000) were scanned at 600 d.p.i. resolution using a flatbed scanner (Epson Perfection 1200 Photo, Epson Europe, Amsterdam), resulting in a specimen-level pixel size of \( 7 \times 7 \) nm\(^2\). Measurement of the resulting images was performed using Zeiss KS400 (Carl Zeiss Imaging Systems, Germany). The system was calibrated using the marker bar on the electron micrographs. The magnification data were verified by a grating replica with parallel lines (2160 lines/mm; EMS, Washington, USA). For five open random capillary loops in each of five randomly selected glomeruli per specimen, the GBM was indicated interactively using a graphic tablet. The total circumference of the capillary loop was included in the image in >75% of cases. Only the free filtering surface of the capillary loop was studied. The image analysis software was used to measure the length of the GBM for each loop. Also, for each loop the number of

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podocytic foot processes was manually counted and expressed as the number of foot processes per μm GBM length, resulting in 25 measurement points for each specimen. A foot process was defined as any connected epithelial segment butting on the basement membrane, separated from the cytoplasmic extensions of the adjacent foot processes by lateral membranes. A new foot process was counted only if the lateral membranes of both foot processes were clearly identifiable over the entire length, that is, from the apical side till the attachment on the GBM (Figure 5). The presence of a clear slit, with or without a diaphragm was thus not a prerequisite.

To illustrate how the foot processes were counted, representative electron microscopic images of the three main study groups (idiopathic FSGS, FSGS secondary to maladaptive responses, and MCNS) are shown in Figures 6–8. The separations between the foot processes are marked with arrows.

For each patient, the average FPW was calculated by dividing the total number of foot processes by the total length of the GBM. A correction factor of π/4 was used to correct for presumed random variation in the angle of the section relative to the long axis of the podocyte. The measurements were performed without knowledge of the clinical data. As indicated above, we have used data of 18 patients that were previously reported. To evaluate the influence of interobserver variation and differences in equipment, we have randomly measured 32 negatives of electron micrographs from these patients. FPW measured by our method was 1584 ± 740 nm fitting very well with the results from the previous study, which measured a FPW of 1432 ± 805 nm (P = NS). The correlation between the results obtained in both centers was high (r = 0.97; P < 0.001), although there was a systematic bias, measurements in our center (RUNMC) being 11% (confidence interval: 6–16%) lower.

Clinical data
Medical records were reviewed for clinical and laboratory data at renal biopsy. Data collected were: age, sex, blood pressure, level of protein excretion, serum creatinine, serum albumin, serum cholesterol, use of immunosuppressive therapy, and antihypertensive medication, initiation of dialysis and death. In addition, the medical records were reviewed for diseases associated with secondary FSGS: obesity (BMI > 30 kg/m²), renal atrophy, unilateral renal agenesis, reflux nephropathy, infections (human immunodeficiency virus, parvovirus B19), medication (pamidronate, lithium, interferon-α), intravenous drug abuse, family history of renal disease, sickle cell anemia, or malignancies.

Definitions
Presentation was defined as the time when proteinuria was first detected. Nephrotic syndrome was defined as proteinuria of ≥ 3 g per day in association with serum albumin concentration of ≤ 30 g/l. Patients treated with antihypertensive drugs or with a blood pressure > 140/90 mm Hg were considered hypertensive. A complete remission was defined as proteinuria < 0.3 g per 24 h with a stable serum creatinine concentration (< 50% increase from baseline) and a partial remission was defined as proteinuria between 0.3 and 2.0 g per 24 h with ≥ 50% reduction in proteinuria from baseline and a stable serum creatinine concentration.

Idiopathic FSGS was defined as a serum albumin ≤ 30 g/l in two measurements in the 3-month period before and after renal biopsy, with a normal renal size and anatomy (observed by intravenous urogram or renal ultrasound), a body mass index < 30 kg/m² and no other discernible cause of FSGS. A clinical diagnosis of FSGS secondary to maladaptive responses was made in patients with an identifiable cause and nephrotic range proteinuria (≥ 3 g per day) with a serum albumin > 35 g/l in two measurements in the 3-month period before and after renal biopsy. 12 A clinical diagnosis of possible FSGS secondary to maladaptive responses was made in patients without an identifiable cause and nephrotic range proteinuria (≥ 3 g per day) with a serum albumin > 35 g/l in two measurements in the 3-month period before and after renal biopsy.

Statistical analysis
Values are given as means ± s.d. or median (range) when appropriate. Differences in continuous data were analyzed with use of the Wilcoxon summed rank test or Kruskal–Wallis test in case of more than two groups. If the result of the Kruskal–Wallis test was significant (P < 0.05), then pairwise comparisons were performed with the Wilcoxon rank-sum test. Fisher’s exact test was used for categorical data. Spearman’s rank correlation coefficients were calculated to assess the relation of FPW to age, proteinuria, serum albumin, serum creatinine, use of ACEi/angiotensin receptor blockers (all at biopsy), and disease type (MCNS, idiopathic or secondary FSGS). Multiple regression analysis was performed in a forward stepwise fashion to determine the relationship between FPW and variables that were significant in univariate analysis, with P < 0.05 for inclusion of variables. As for strongly skewed variables the high values may have a disproportionate influence on the outcome of the analysis, the natural log transformation was used to reduce their impact.

Receiver operating characteristics curves were used to determine the most discriminative threshold for FPW in predicting MCNS, idiopathic or secondary FSGS. A two-sided P-value < 0.05 was considered as the level of statistical significance. The analysis was performed using SPSS 14.0 for windows (SPSS Inc., Cambridge, MA, USA).

DISCLOSURE
All the authors declared no competing interests.

ACKNOWLEDGMENTS
J. K. J. Deegens was supported by a grant from the Dutch Kidney Foundation (PV 02). This work was presented in part at the 2004 (St Louis, MO, USA, 27 October– 1 November 2004) American Society of Nephrology annual meetings and published in abstract form (J Am Soc Nephrol 15: 557A, 2004). We thank J. van der Laak from the Department of Pathology for his expert technical assistance.

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