Dendrin expression in glomerulogenesis and in human minimal change nephrotic syndrome

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Abstract
Background. Dendrin is an 81-kD cytosolic protein hitherto described in the brain, where it is associated with the actin cytoskeleton. Recently, we found dendrin in foot processes of mouse glomerular podocytes. Here we describe its expression both during mouse glomerulogenesis and in the normal and diseased human kidney for the first time.

Methods. Dendrin expression was characterized using RT–PCR and immunohistochemistry and semi-quantified using immunoelectron microscopy.

Results. In glomerulogenesis, dendrin mRNA and protein appeared first at the early capillary loop stage. It was concentrated to the pre-podocytes on the basal side of podocalyxin, an apical cell membrane marker. In human tissue, dendrin transcripts were detected in the brain and kidney. In the mature kidney dendrin localized solely in the podocytes, close to the filtration slit diaphragms. A comparison with the slit-associated protein zonula occludens-1 (ZO-1) was done in minimal change nephrotic syndrome (MCNS). Dendrin and ZO-1 were re-distributed from slit regions to the podocyte cytoplasm in areas with foot process effacement (FPE). In areas without FPE, dendrin and ZO-1 distributions were unchanged compared to controls. The total amounts of dendrin or ZO-1 markers were unchanged. This differs from nephrin that, according to our previous results, is also decreased in non-effaced areas.

Conclusions. The expression of dendrin during glomerulogenesis and in the normal human kidney is similar to that previously shown for nephrin, which suggests that dendrin associates with the slit diaphragm complex. In MCNS patients, dendrin and ZO-1 are re-distributed within the podocytes. Whether this is a cause or a consequence of FPE remains unclear.

Keywords: dendrin; glomerulogenesis; immunoelectron microscopy; nephrotic syndrome

Introduction

The kidney glomeruli filter enormous amounts of plasma to form primary urine but still only trace amounts of protein normally reach the urine. The structure forming this size-, shape- and charge-selective barrier consists of three components: the fenestrated capillary endothelium, the glomerular basement membrane (GBM) and the epithelial podocyte foot processes with their interposed slit diaphragm (SD). Diseases affecting the glomerular podocytes are characterized by proteinuria, in itself considered an independent promoter of renal failure [1]. The SD is regarded as the main barrier against proteinuria. It was first described by Rodewald and Karnovsky in 1974 [2], but the molecular composition of the SD remained unknown until the discovery of NPHS1 and its gene product nephrin in 1998 [3]. After nephrin, many proteins crucial for the barrier function of the SD have been described, such as podocin, Neph 1, FAT, TRPC6 [4–8] and, most recently, filtrin [9]. The SD is attached to the actin cytoskeleton through intracellular adaptor proteins including zonula occludens-1 (ZO-1) [10,11], CD2AP [12,13], Nck-1 and Nck-2 [14,15]. The role of ZO-1 in proteinuria is not entirely clear. Its relation to nephrin could not initially be confirmed using immunoprecipitation [16] but there may at least be an indirect association since injection of the anti-nephrin antibody in rats results in a decrease in ZO-1 [17]. Recently, mutations in the encoding gene of the intracellular enzyme PLε1 have been shown to cause proteinuria [18].

Minimal change nephrotic syndrome (MCNS) is the most common (>85%) cause of nephrotic syndrome in children, and also accounts for ~20% of apparently idiopathic nephrotic syndromes in adults [19]. Renal biopsies from MCNS patients show no pathology except for the typical ultrastructural feature of proteinuric disease, that is...
Dendrin expression in glomerulogenesis and in human minimal change nephrotic syndrome

Table 1. Clinical and laboratory findings at the time of biopsy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Proteinuria (g/24 h)</th>
<th>S-albumin (g/l)</th>
<th>Time since onset of proteinuria</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>&gt;3</td>
<td>15</td>
<td>1 week</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>11.4</td>
<td>22</td>
<td>4 months</td>
<td>Oral steroid</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>0.3</td>
<td>32</td>
<td>3 weeks</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1.0</td>
<td>17</td>
<td>2 months</td>
<td>Oral steroid</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>2.2</td>
<td>36</td>
<td>15 months</td>
<td>Oral steroid</td>
</tr>
</tbody>
</table>

Podocyte foot process effacement (FPE) [20]. The pathogenesis of MCNS and the mechanisms leading to FPE are poorly understood. Studies of the expression of SD proteins in MCNS and other forms of proteinuric disease show divergent results. In our electron microscopic (EM) study on MCNS patients, we found reduced amounts as well as a re-distribution of nephrin from slits in the cytoplasm [21].

As a part of a large-scale project aimed at identifying novel transcripts and gene products in podocytes [22], we recently described the expression and subcellular distribution of dendrin protein in the glomerulus [23]. In that study we showed that in the mouse kidney dendrin is expressed solely by podocytes, and at the ultrastructural level dendrin protein is localized to the foot processes close to the insertion of the SD. Furthermore, Asanuma et al. recently showed that dendrin can directly interact with nephrin and CD2AP [24]. Dendrin was originally identified in rat brain [25,26], where it seems to interact with α-actinin in postsynaptic dendritic spines [27], possibly modulating the structure of the actin cytoskeleton. Regulation of the actin cytoskeleton architecture in the podocyte foot processes is of considerable interest as proteinuric diseases are almost invariably associated with extensive reorganization of the cytoskeleton (observed as FPE), as seen for example in MCNS and focal segmental glomerulosclerosis [28]. α-actinin-4 is known to play an important role in organizing the cytoskeleton in the podocyte as mutations in the encoding gene ACTN4 cause familial focal glomerulosclerosis [29]. As dendrin can interact with the actin-bundling protein α-actinin in neurons, and loss of α-actinin-4 function in podocytes can cause proteinuric disease, it is interesting to study the expression of dendrin when podocyte morphology is disturbed, for instance in proteinuric disease.

In the present work, we studied the expression of dendrin in mouse glomerulogenesis, in the normal human kidney and in kidney biopsies obtained from patients with MCNS. We also studied the subcellular expression of ZO-1 in patients with MCNS.

Subjects and methods

Patients

Patients with MCNS were chosen for the study. Under the light microscope the kidney histology appeared normal without any immune deposits whereas EM studies revealed FPE.

Clinically, the adult patients had proteinuria of at least 3 g/24 h. The children were generally treated with steroids and biopsied due to poor response and all but one of them had proteinuria >1 g/m² body surface area. The clinical data are presented in Table 1. As controls, we used tissue obtained from normal portions of kidneys removed due to localized neoplasms. All procedures were approved by the local ethics committee.

RT–PCR

The expression of dendrin transcripts in a variety of human tissues was studied using RT–PCR. Dendrin-specific oligonucleotides for PCR analysis (left 5′-CTGGATGGCC CACTGTCTCT-3′, right 5′-CGGATCCAGACACGA GA-3′) were designed to amplify 1719 bp products according to the predicted cDNA sequences (www.ensembl.org). As a template for PCR analysis we used cDNA libraries generated from different adult human tissues (Human Multiple Tissue cDNA Panel I, Clontech Laboratories, Palo Alto, CA, USA). As a positive control, we used primers amplifying glyceraldehyde-3-phosphate dehydrogenase, GAPDH (Clontech Laboratories, Palo Alto, CA, USA). PCR amplification was carried out with Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) and the amplified fragments were analysed on a 1.5% agarose gel.

In situ hybridization

The probes for in situ hybridization were synthesized by subcloning the PCR products obtained from RT–PCR analysis (see above) into the PCR II-TOPO Dual Promoter Vector (Invitrogen, Carlsbad, CA, USA). Anti-sense and sense mRNAs were prepared by using T7 or SP6 RNA polymerases. In situ hybridization experiments with 35S-labelled probes were performed on snap-frozen tissue sections collected from newborn mouse kidneys as previously described [30].

Primary antibodies

The generation of rabbit antiserum directed against mouse dendrin protein has been described recently [23]. Rabbit anti-ZO-1 antibodies for immuno-EM were purchased from Zymed (San Francisco, CA, USA). Anti-podocalyxin antibodies were purchased from R&D systems (Minneapolis, MN, USA) and the anti-nephrin antibody was a mouse monoclonal antibody against the extracellular domain of human nephrin (50A9) [31].

Western blotting

We compared the human extracts of isolated glomeruli and kidney tissue lacking glomeruli. The glomeruli were isolated from cadaver kidneys unsuitable for transplantation (from the IV Department of Surgery of Helsinki, Finland)
as described earlier [32]. The western blotting followed standard procedures using the polyvinyl difluoride membrane and HRP-conjugated secondary antibody (Amersham Biosciences, Buckinghamshire, UK). As a positive loading control, we used the anti-\(\beta\)-actin antibody (Abcam, Cambridge, UK).

**Immunofluorescence**

The samples from newborn mouse kidneys or adult human cadaver kidneys (see western blotting above) were snap-frozen, and the cryosections (10 \(\mu\)m) were postfixed with -20°C acetone followed by blocking in 5% normal goat serum. The primary antibodies were incubated overnight at 4°C, followed by 1-h incubation with the secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). For double-labeling stains, the incubations were performed sequentially. Microscopy was performed with a Leica confocal laser-scanning microscope.

**Immunoperoxidase staining**

From paraffin-embedded renal biopsies, 2.5-\(\mu\)m-thick sections were pre-treated with tris-EDTA (Dako, Glostrup, Denmark) followed by 3% \(\text{H}_2\text{O}_2\) in methanol. Blocking was performed with 10% milk for 30 min. The rabbit anti-dendrin antibody (1:250) was incubated overnight and normal rabbit IgG was served as a negative control. The HRP-conjugated secondary antibody (Envision\textsuperscript{TM}, Dako, Glostrup, Denmark) was added for 30 min at room temperature and visualized by the DAB/H\(\text{H}_2\text{O}_2\) substrate. Nuclei were stained with haematoxylin.

**Immunoelectron microscopy**

The preparation and examination were carried out as previously described [21]. Gold-conjugated protein A was used to detect primary antibodies.

**Semi-quantification of gold markers**

From each specimen (cases and controls), three individual glomeruli were examined. Ten photomicrographs were taken at random locations in the peripheral capillary loops from each glomerulus and copies were printed at a final magnification of \(\times 29\) 700. To delimit the foot processes in the micrographs, a parallel line was drawn 3 cm (corresponding to 1 \(\mu\)m) from the basement membrane on the epithelial side. The area of the foot processes within this zone was estimated as described in detail earlier [33]. Briefly, a transparent grid with \(1 \times 1\) cm squares was placed randomly over the micrograph so that each interception corresponded to an area of 0.113 \(\mu\)m\(^2\). The GBM length on each micrograph was measured with a semi-automatic interactive image analyser (Videoplan\textsuperscript{TM}, Carl Zeiss, Germany) and the number of slits was counted. The following calculations were made (Table 2):

1. Slits/\(\mu\)m GBM: the number of slits was expressed as slits per micrometre of GBM length. This was used to distinguish diseased from normal-looking areas, and <1 slit/\(\mu\)m was considered as FPE.
2. \(\text{Au}/\mu\text{m}^2\): the total number of gold markers in the foot processes and slit membranes were counted and expressed as the number of gold particles per square micrometre (\(\text{Au}/\mu\text{m}^2\))
3. \(\text{Au}/\text{slit}\): to quantify the amount of dendrin in and around the slits, a \(1 \times 1\) cm square on a transparent film was placed centrally on each slit. Gold markers within this defined square (corresponding to 0.1 \(\mu\)m\(^2\)) were counted and expressed as \(\text{Au}/\text{slit}\).
4. Percentage of \(\text{Au}\) on slits: to determine whether there is redistribution of dendrin within the podocytes, the number of gold markers located in the vicinity of the slits ('\(\text{Au}/\text{slit}\)) was divided by the total number of gold particles in the foot process area as defined above.

**Table 2. Semi-quantitative immunoelectron microscopic findings**

<table>
<thead>
<tr>
<th>Glomeruli</th>
<th>Slits/(\mu)m</th>
<th>(\text{Au}/\mu\text{m}^2)</th>
<th>(\text{Au}/\text{slit})</th>
<th>Percentage of (\text{Au}) on slits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dendrin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls ((n = 5))</td>
<td>1.75 ± 0.26</td>
<td>1.24 ± 0.39</td>
<td>0.10 ± 0.03</td>
<td>36 ± 12</td>
</tr>
<tr>
<td>MCNS, areas with FPE</td>
<td>0.52 ± 0.11</td>
<td>0.94 ± 0.31</td>
<td>0.13 ± 0.09</td>
<td>17 ± 9*</td>
</tr>
<tr>
<td>MCNS, areas without FPE ((n = 5))</td>
<td>1.64 ± 0.27</td>
<td>1.51 ± 0.96</td>
<td>0.18 ± 0.11</td>
<td>32 ± 4</td>
</tr>
<tr>
<td>ZO-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls ((n = 5))</td>
<td>1.48 ± 0.30</td>
<td>2.05 ± 0.51</td>
<td>0.27 ± 0.05</td>
<td>43 ± 9</td>
</tr>
<tr>
<td>MCNS, areas with FPE</td>
<td>0.51 ± 0.14</td>
<td>1.53 ± 0.50</td>
<td>0.27 ± 0.10</td>
<td>19 ± 5*</td>
</tr>
<tr>
<td>MCNS, areas without FPE ((n = 5))</td>
<td>1.29 ± 0.16</td>
<td>2.23 ± 0.36</td>
<td>0.29 ± 0.03</td>
<td>45 ± 11</td>
</tr>
</tbody>
</table>

Values are mean ± SD; \(*P < 0.05\) versus control.

FPE = foot process effacement, defined as areas with <1 slit/\(\mu\)m GBM; Controls = normal tissue from kidneys removed due to localized neoplasms; \(\text{Au}\) = immunogold labels used in immunoelectron microscopy.

**Statistical analyses**

Data are presented as mean ± SD. Groups were compared with the Mann–Whitney \(U\)-test. All analyses were performed using Statistica 7 software (Statsoft, Tulsa, OK, USA).

**Results**

**Expression of dendrin in various human tissues**

RT–PCR analysis demonstrated human dendrin transcripts in brain and kidney tissue (Figure 1). No PCR products
Dendrin expression in glomerulogenesis and in human minimal change nephrotic syndrome

Expression of dendrin in the mature human kidney

The anti-dendrin antiserum was raised against the recombinant mouse dendrin protein, and was therefore first tested to determine whether it cross-reacted with the human dendrin. In the western blotting of human kidney fractions, anti-dendrin antiserum reacted with a protein sized ~80 kDa in the human kidney glomerular lysate, whereas the lysate obtained from the rest of the kidney showed no significant reactivity (Figure 2A). The size of the human dendrin protein was in line with previously published data on rat and mouse dendrin proteins [23,26]. Antibody directed against β-actin, used as a positive loading control, detected an expected 42 kDa protein in both lanes (Figure 2A). Pre-immune serum gave no reactivity in the glomerular lysate (data not shown).

Immunofluorescence and immunoperoxidase staining of adult human kidneys showed dendrin-specific immunoreactivity in a linear pattern along the glomerular capillary loops. Double labelling with nephrin demonstrated nearly complete co-localization of the two proteins in the human kidney at the light-microscopic level (Figure 2B).

Expression of dendrin in mouse glomerulogenesis

During mouse glomerulogenesis, dendrin mRNA was first detected at the early capillary loop stage by using in situ hybridization (Figure 3). Grains for dendrin mRNA were located to the developing podocytes (Figure 3A, red arrows). The signal for dendrin mRNA seemed to be strongest at the late capillary loop stage when the formation of the foot processes begins (Figures 3B–E). No signal was observed outside glomerular capillary tufts in newborn mouse kidneys and sense probes gave only background signal (Figure 3A, inset).

Expression of dendrin in minimal change disease

In immunohistochemical studies we observed linear staining for dendrin along the capillary loops in glomeruli from patients with MCNS. There was no significant
Fig. 3. Expression of dendrin during mouse glomerulogenesis as studied using in situ hybridization. Dendrin mRNA is detected exclusively in developing glomerular tufts (A, red arrows). Sense control gives no specific signal (A, inset). No signal for dendrin mRNA is observed in the vesicle (B) and the S-shaped (C) stage glomeruli. Dendrin mRNA is first detectable at the capillary loop stage glomerulus (D). Grains labelling dendrin mRNA are located in the developing podocytes. In maturing stage glomerulus (E), the signal is scattered around the periphery of the developing glomerulus suggesting localization to the pre-podocytes. Magnification ×600.

difference in the staining patterns between the samples from MCNS and controls. No other structures stained positively (Figure 5).

Immunoelectron microscopy

Despite the clinical heterogeneity between the MCNS patients (Table 1), there was no apparent difference in the extent of FPE. The ultrastructural distribution of dendrin and ZO-1 in normal and MCNS kidneys using semi-quantitative immuno-EM is shown in Table 2. Dendrin was localized to the podocyte foot processes, and ~40% of dendrin immuno-gold label was filtration slit-associated in normal human kidneys (Figure 6). Interestingly, dendrin seemed to be located slightly more apically than ZO-1. There was negligible labelling in other parts of the glomeruli and in non-glomerular compartments of the kidneys.

Fig. 4. Expression of dendrin during mouse glomerulogenesis as studied by IFL staining. In newborn mouse kidney, immunoreactivity for dendrin (green) is detected exclusively in developing glomerular tufts (overview). In S-shaped stage, no immunoreactivity for dendrin is detected, whereas staining for podocalyxin (red) is observed on the apical membranes of pre-podocytes. In capillary loop stage glomerulus, the staining for dendrin is observed on the basal side of podocalyxin, and occasionally, the staining is detected as dots between the developing podocytes suggesting localization to the cell junctions of pre-podocytes. In maturing stage glomerulus, the staining for dendrin is found as a line around the capillary loops on the basal side of podocalyxin. Red arrows indicate the apical side of pre-podocytes in merged figures. Magnifications: ×100 (overview), ×600 (single glomeruli), ×1200 (insets).

Fig. 5. Immunohistochemical staining for dendrin in normal (A) and MCNS (B) kidneys. Dendrin forms a linear pattern on the epithelial side of the glomerular capillary loops, corresponding to the podocytes (arrow). No obvious differences can be found between MCNS patients and controls. Size bar = 50 µm.
In MCNS, there was no significant change in the overall amount of dendrin or ZO-1, neither in areas with nor without FPE. The proportion of gold markers that was found in close proximity to the remaining slits was significantly reduced in areas with FPE, for ZO-1 19% versus 43% in controls, \( P = 0.008 \) and for dendrin 17% versus 36%, \( P = 0.03 \). However, in individual slits, the amounts of dendrin and ZO-1 (Au/slit) were comparable in the control and MCNS specimens.

### Discussion

During mouse glomerulogenesis, dendrin mRNA and protein were first detected in early capillary loop glomeruli, and the protein was localized to the basal side of podocytin, which is a marker for the apical plasma membrane of podocytes [34,35]. Interestingly, immunoreactivity for dendrin was observed as dots between the developing podocytes suggesting localization to the premature podocyte–podocyte cell junctions. Previously, the slit diaphragm (SD) proteins ZO-1 and nephrin have been found in these premature junctional complexes [36]. This supports the idea that dendrin is associated with the SD as these premature junctions are believed to develop into mature Sds.

This is the first study of the expression of the dendrin protein in the normal and diseased human kidney. RT–PCR demonstrated dendrin transcripts exclusively in brain and kidney tissue, which is in agreement with our previous expression profiling in mice [23]. In immunofluorescence studies of dendrin in normal human kidneys, double staining with nephrin, the core component of the SD, showed overlapping of these two proteins. Ultrastructurally, dendrin was localized intracellularly in the foot process of the podocyte. Dendrin seemed to associate with the SD, and our impression was that it resided slightly apically in relation to ZO-1. These findings in humans validate the idea that dendrin is associated with the SD as these pre-mature junctions are believed to develop into mature Sds.

Dendrin expression to resemble that of ZO-1 [37,38]: there was no apparent change in the nephrotic kidney. This differs from recent results in experimental glomerulonephritis in mouse, where dendrin relocates to the nucleus and promotes podocyte apoptosis [24]. However, in that study glomeruli developed crescent formation and sclerosis, which is quite different from human MCNS. Our aim was to elucidate whether we could find an altered expression of these proteins ultrastructurally in MCNS and get some clues about their possible role in FPE. In MCNS, the degree of FPE often varies considerably, even within one single glomerulus. Immuno-EM offers the opportunity to separately study diseased and normal areas in the same glomerulus. This is an advantage compared to light microscopy where areas with and without FPE cannot be distinguished. Therefore we compared areas with and without FPE in the same patients semi-quantitatively. There was no significant change in the absolute amounts of dendrin or ZO-1, neither in areas with nor in areas without FPE. We therefore believe that our data indicate a redistribution of these proteins from the SD area into the podocyte cytoplasm. If dendrin, or ZO-1, was primarily responsible for the maintenance of the SD, the total amount of gold (per square unit) would be decreased in both effaced and non-effaced areas.

In the previous ultrastructural study of the expression of nephrin in MCNS [21] we found that nephrin was similarly re-distributed but the overall amount of gold-labelled nephrin (\( \text{Au/µm}^2 \)) was lower in the podocytes of the MCNS cases, both in areas with and without FPE. With dendrin and ZO-1, no such significant decrease was observed, which might imply a more central role for nephrin in MCNS.

Proteinuria, a hallmark for many glomerular diseases, is often accompanied by FPE. Results regarding SD-associated molecules in human proteinuric states are conflicting [21,38–41], most likely due to the variability in methods used and diseases studied. In puromycin aminonucleoside nephrosis of the rat, a common model for nephrotic syndrome and FPE [42], the expression of podocyte-associated molecules seems to vary with the course of the disease, i.e. with the time elapsed since the puromycin injection [43–47]. Most likely, MCNS and other human nephrotic syndromes have a ‘natural course’, where the expression of structural proteins and corresponding mRNAs varies with time.

The exact roles of the glomerular basement membrane (GBM) and the SD in the glomerular filtration barrier are still unclear. Noakes et al. [48] found in 1995 that knocking out the GBM component laminin β2 in mice led to proteinuria. Jarad et al. [49] demonstrated that in this scenario proteinuria appeared before any epithelial FPE or change in the expression of the SD-associated molecules nephrin and podocin. The authors conclude that these phenomena are secondary to ‘disorganization’ of the GBM [49], β2-laminin deficiency in humans also causes proteinuria [50]. Neutralizing circulating vascular endothelial growth factor (VEGF) can also produce proteinuria without FPE, as can deletion of the GBM collagen α3 chain [51]. Interestingly, glomerular proteinuria without correlation to the extent of FPE has also been shown in MCNS [52]. Lahdenkari et al. found FPE not only in proteinuric glomeruli but also in non-proteinuric.
MCNS kidneys [53]. If one expands this view, FPE can result from multiple forms of insult to the podocyte, as summarized by Mundel and Shankland [54]. Clearly, several pathogenic mechanisms can lead to proteinuria.

Dendrin is a novel protein first identified in neurons of the rat [25,26]. It was thought not to be expressed outside the brain. However, we and others have found dendrin also in the podocytes of the glomerulus [23,24]. Podocytes represent only a very small portion of renal cells, and therefore dendrin expression is hard to detect in whole kidney fractions. Dendrin is hydrophilic but devoid of putative membrane-spanning regions. In neurons, it interacts with the cytoskeletal protein α-actinin [27], which might imply an importance for cell shape. One of the four isoforms of α-actinin is expressed in the glomerular podocyte, to bundle and possibly organize its actin cytoskeleton. The function of dendrin, however, in the normal glomerulus and its potential role in disease is unknown. It was presumed that dendrin could be another important player in such a dramatic change of cell shape as FPE, and hence in proteinuria. We therefore conducted this study to elucidate its possible role in the pathogenesis of MCNS. If dendrin were crucial in the pathogenesis of MCNS, we would have expected a significant loss of dendrin gold markers. However, our results do not support such a dramatic effect. In preserved slits, the amounts of dendrin are unchanged. Also, in areas without FPE, there was no change in dendrin expression compared to controls. We believe that one might therefore look upon the re-distribution of dendrin and ZO-1 as phenomena secondary to FPE for some other reason not yet identified. Further studies are needed to clarify the role of dendrin in human proteinuric diseases.

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Conflict of interest statement. K.T. is co-founder of and has stock ownership in NephroGenex, Inc. but no consultant fees at the moment.

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Dendrin expression in glomerulogenesis and in human minimal change nephrotic syndrome


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