Cloning of Rat Homologue of Podocin: Expression in Proteinuric States and in Developing Glomeruli

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Abstract. Podocin is identified as a product of the gene mutated in a patient with autosomal recessive steroid-resistant nephrotic syndrome. Although podocin is reported to be located at the slit diaphragm area, the precise role of podocin for maintaining the barrier function of the slit diaphragm has not been clearly elucidated. A rat homologue of podocin was cloned, and the expression of podocin was investigated and then compared with the nephrin and the ZO-1 expressions in rat experimental proteinuric models and in developing glomeruli. Amino acid sequences of rat and human podocin are highly homologous (84.3% identity). The domain structure of podocin is also highly conserved between rat and human. The mRNA expression for podocin was detected in glomeruli and the nerve tissues. The localization of podocin has close proximity to that of nephrin in normal adult rat glomeruli. Podocin staining was restricted to the basal side of the podocyte of the early developing stage, whereas nephrin staining was detected on the basolateral surface of podocyte. The redistribution of podocin was observed in the anti-nephrin antibody (ANA)-induced nephropathy and puromycin aminonucleoside (PAN) nephropathy. The redistribution of podocin paralleled with nephrin in ANA nephropathy but not in PAN nephropathy. Podocin is observed at the site of tight junction newly formed in proteinuric state in PAN nephropathy. It is postulated that podocin is one of the critical components of a slit diaphragm for maintaining the barrier function of the glomerular capillary wall.

Materials and Methods
Cloning of Rat Podocin

To clone a rat homologue of podocin, we employed a PCR with the primers designed from a human podocin sequence. PCR cloning was performed basically according to the method described previously (14). Total RNA was prepared from normal Wistar rat glomeruli with TRI ZOL (Life Technologies BRL, Gaithersburg, MD), which was utilized to synthesize cDNA with SuperScript II (Life Technologies BRL) accord-
ing to the manufacturer’s protocol. Synthesized cDNA was used as a template. Templates were ampliﬁed in a DNA with PROGRAM TEMP CONTROL SYSTEM PC-800 (ASTEC, Fukuoka, Japan) for 35 to 50 cycles at 48°C to 55°C of annealing temperature. The PCR products that were close to an expected size were cloned into a Topo Vector (Invitrogen, Carlsbad, CA), and DNA sequences were determined by an automated DNA sequencer (ABI 310; Perkin-Elmer Japan, Urayasu, Japan). From a partial clone of rat podocin, gene-speciﬁc primers were designated and PCR was performed again. To obtain the sequences containing the initiation codon of rat podocin, we performed a 5′-RACE with a nated and PCR was performed again. To obtain the sequences containing the initiation codon of rat podocin, we performed a 5′-RACE with a

**Antibodies**

To produce a speciﬁc antibody for rat podocin, we chose a peptide of 21 amino acids, RGNRGRAPDGVERQSAGRM, as immuno-
gen. Two rabbits were immunized with 1.0 mg of peptide conjugated with the carrier protein KLH and boosted twice with 0.5 mg of antigen. The rabbits were bled 2 wk after the last immunization. The speciﬁcity of the anti-podocin antibody was conﬁrmed by the Western blot analysis. Mouse monoclonal antibody to rat nephrin (mAb 5-1-6), and rabbit polyclonal anti-rat nephrin antibody were prepared as described previously (6,14). Mouse monoclonal anti-ZO-1 antibody, anti-glial ﬁbrillary acidic protein (GFAP), and anti-rat endothelial cell antigen 1 (RECA1) antibodies were purchased from Zymed Laboratory Inc. (San Francisco, CA), Chemicon International (Temecula, CA), and Serotec Ltd (Oxford, UK), respectively.

**Western Blot Analysis**

Normal rat glomeruli are isolated with PBS-protease inhibitors (PI) and sequentially solubilized with 1% Triton X-100, RIPA-buffer (0.1% SDS, 1% DOC, 1% Triton X-100, 150 mM NaCl, 10 mM EDTA in 25 mM Tris-HCl, pH 7.2) with PI, and separated into Triton X-100–soluble (TXS), RIPA-soluble (RIPAS), and RIPA-insoluble (RIPAI) fractions. RIPAI fraction was solubilized with SDS-PAGE sample buffer (2% SDS, 10% glycerol, 5% 2-mercaptoethanol in 62.5 mM Tris-HCl, pH 6.8). Solubilized material was subjected to SDS-PAGE with 10% or 7.5% acrylamide gel according to the method of Laemmli et al. (21) and transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA) by electrophoretic transblotting for 30 min using Trans-Blot SB (Bio-Rad). After blocking with BSA, the strips of membranes were exposed to one of the following: anti-rat podocin, anti-rat podocin pre-absorbed with rat podocin peptide used for immu-

**Immunostaining**

Normal and proteinuric rats were perfused with 1.0% paraformal-dehyde ﬁxative buffered with 0.1 mol/L sodium phosphate buffer (PB; pH 7.4) and immersed in the same ﬁxative for 1 h at 4°C. The samples were rinsed three times with 10% sucrose for 20 min. The tissue samples were then inﬁltrated with 40% polyvinylpyrrolidone (Sigma)/2.3 mol/L sucrose buffered with 0.1 mol/L PB, embedded, and frozen quickly. Ultrathin cryosections of the samples were mounted on Formvar/carbon grid. After quenching free aldehyde with phosphate buffered saline (PBS)–0.01 mol/L glycine, sections were incubated with anti-podocin antibody. They were then incubated with 10 nm of gold-conjugated anti-rabbit IgG (British BioCell, Cardiff, UK). After immunostaining, they were ﬁxed with 2.5% glutaral-de-
hyde in 0.1 mol/L PB (pH 7.4). The sections were then contrasted with 2% uranyl acetate for 20 min, and adsorption stained with 3% polyvinyl alcohol containing 0.2% uranyl acetate for 10 min.

**Semiquantitative Reverse Transcription (RT)-PCR**

Five micrograms of total RNA prepared from glomeruli were utilized to synthesize cDNA. Three microliters of the reverse transcription mixture were utilized for the subsequent PCR. Primers used for rat podocin and GAPDH were described above. The PCR product corresponds to 235 bp, and the target sequence is located in the putative extracellular domain close to the membrane-spanning region. The PCR was performed for 20 to 30 cycles of denaturation at 94°C for 30 s, annealed at 58°C for 30 s, and extended at 72°C for 1 min. The optimal cycle number was determined in a preliminary trial to be in the linear phase of amplification. The PCR products were subjected to electrophoresis with 1.5% agarose gel and stained with ethidium bromide. The band intensity was determined by an image analysis using a Macintosh computer and densitometry software (Densitograph; ATTO, Tokyo, Japan). All results were corrected for the amount of mRNA in the sample by dividing by the intensity of the internal control, GAPDH.

**Real-Time RT-PCR**

Real-time PCR was performed basically according to the manufacturer’s manual. cDNA was synthesized as described above. cDNA, specific primers, and SYBR Green (Takara, Otsu, Japan) were mixed with a Takara Ex Taq R-PCR Version For Real Time PCR kit (Takara). PCR reactions were run on a Smart Cycler System (Takara). The sequences of the primers of podocin and GAPDH were described above. The reactions and run for all samples were performed in duplicate.

**Induction of Proteinuric State**

**ANA Nephropathy.** A total of 20 rats were intravenously injected with 6 mg of anti-rat nephrin mAb 5-1-6, and five rats each were sacrificed just before injection, 1 h, 24 h, or 5 d after injection. Small pieces of the rat’s kidney tissues were snap-frozen and used for immunofluorescence study. Glomerular RNA was prepared from the remaining kidney tissues pooled from five rats for semiquantitative RT-PCR and real-time PCR analysis. Twenty-four-hour urine samples were collected, and their protein concentrations were measured by colorimetric assay with a Bio-Rad Protein Assay Reagent (Bio-Rad) using BSA as a standard. For Western blot analysis, five rats were intravenously injected with 6 mg of anti-nephrin mAb 5-1-6 and sacrificed on day 5. Glomeruli were isolated from pooled kidneys from five rats of each group and solubilized directly with SDS-PAGE sample buffer or sequentially solubilized. For immunoelectron microscopic study, three rats were treated with anti-nephrin mAb 5-1-6 and sacrificed on day 5.

**Puromycin Aminonucleoside (PAN) Nephropathy.** A total of 20 rats were intravenously injected with 10 mg/100 g body weight of PAN, and five rats each were sacrificed just before injection and 1 h, 24 h, or 9 d after injection. The cryostat sections for the immunofluorescence study and total RNA for RT-PCR were prepared as described above. The amount of urinary protein excretion/24 h was determined as described above. For Western blot analysis, five rats were intravenously injected with 10 mg/100 g body wt of PAN and sacrificed on day 9 as described above. For immunoelectron microscopic study, three rats were treated with PAN and sacrificed on day 9.

**Results**

**Cloning of Rat Podocin cDNA**

The complete nucleotide sequences of rat homologue of podocin and its deduced amino acid sequences are shown in Figure 1. The nucleotide sequencing analysis revealed an open reading frame of 1149 nucleotides coding for a predicted protein of 383 amino acids, which is the same as human podocin (deduced molecular weight, 42 kd). The deduced amino acid sequence of rat podocin showed 84.3% identity and 97.1% similarity to human podocin (Figure 2). N-terminal beyond the stomatin-like sequences of rat podocin (97 amino acids) has 63.9% identity to that of human podocin.

![Figure 1. Nucleotide sequences of rat podocin cDNA and its deduced amino acid sequence. Reading frame of this sequence starts at nucleotide 62 and extends until nucleotide 1213, thereby coding protein of 383 amino acids. The sequence data are available from GenBank under accession number (AY039651).](image-url)
ExPASy search showed that the rat podocin has a single transmembrane site, which is the same as human podocin. The sequence of rat podocin showed 92.7% identity and 98.9% similarity to mouse podocin. The sequence of rat podocin for N-terminal 97 amino acids showed 82.8% identity to that of mouse podocin.

**Western Blot Finding of Podocin in Normal Glomerular Protein**

An antibody to the peptide of 21 amino acids of the rat podocin sequence was detected to bind to a single band with molecular weight of approximately 42 kd in an extract of normal rat glomeruli. The calculated molecular weight of a 383-amino acid protein of rat podocin is 42 kd. The ExPASy search showed that rat podocin has only one putative N-glycosylation site. The 42-kd band is compatible for the specific band of rat podocin. A weak band of podocin was detected in the Triton X-100–solubilized fraction, and the stronger bands were detected in the RIPA-solubilized and RIPA-insolubilized fractions (Figure 3A). In contrast, a clearer band of nephrin was detected in the Triton X-100–solubilized fraction. No positive band was detected with the anti-podocin antibody pre-absorbed with the peptide used for immunization or pre-immune rabbit serum.

**Organ-Specific Expression of Rat Podocin**

The mRNA expression for podocin was detected in the kidney and in the nervous tissues (Figure 4). A clear specific band of PCR product of podocin was detected in the glomerulus and cortex, whereas a faint signal was detected in the whole kidney. A positive band was also detected in the cerebrum, cerebellum, and the medulla oblongata.

**Expression of Podocin in Glomeruli of Normal and Proteinuric Rats**

**Dual-Labeling Immunofluorescent Study.** Podocin staining is detected as the very fine linear-like pattern along the capillary loop in a normal adult section of a rat (Figure 5A). Dual-labeled immunofluorescent study of podocin and nephrin with a normal adult section of a rat showed the close proximity of these molecules (Figure 5, A through C), although some non-overlapping staining is detected. In ANA nephropathy, the staining pattern of podocin shifted to a coarse granular pattern on day 5, when severe proteinuria was detected (89.1 ± 11.5 mg/24 h) (Figure 5D). In PAN nephropathy, the staining pattern of podocin also shifted to a coarse granular pattern, and its intensity decreased on day 9, when massive proteinuria was detected (374.4 ± 57.9 mg/24 h) (Figure 5G). The shift of podocin staining was paralleled with nephrin staining in ANA
nephropathy (Figure 5, D through F) but not in PAN nephropathy (Figure 5, G through I). Dual-labeling study with podocin and ZO-1 showed that these molecules were closely localized (Figure 5J). In ANA nephropathy, ZO-1 staining (red) was shifted similarly to podocin (Figure 5K). In PAN nephropathy, although the intensity of podocin staining (green) was dramatically decreased, that of ZO-1 (red) was preserved (Figure 5L).

**Immunoelectron Microscopic Study.** In normal rat glomeruli, gold particles indicating the localization of podocin are detected on the cytoplasmic face close to the point of slit diaphragm (Figure 6A and B). In ANA nephropathy, structural integrity of slit diaphragm is largely unaffected, although foot process effacement is partially observed. Gold particles for podocin are detected on the cytoplasmic face at the preserved slit diaphragm (Figure 6C). The finding in the preserved glomeruli in ANA nephropathy is very similar to that in normal rat glomeruli. In ANA nephropathy, gold particles are also observed diffusely in the cytoplasm at the area of foot process effacement (Figure 6D). In PAN nephropathy, effacement of foot processes was broadly observed. Gold particles are observed at the site where tight junction is newly formed in proteinuric states in PAN nephropathy (Figure 6E, F and G).

**Semiquantitative Western Blot Analysis.** To determine whether the reduced staining of podocin in proteinuric states represents a loss of immunoreactive protein or simply redistribution, Western blot analysis was performed using whole glomerular lysate solubilized with SDS-PAGE sample buffer. As shown in Figure 7A, intensity of podocin bands was significantly reduced in PAN nephropathy (6.35 ± 4.60%), but that was unchanged in ANA nephropathy. To further analyze whether the pattern of distribution of podocin is changed in proteinuric states, Western blot analysis with sequentially solubilized materials was performed (Figure 7B). The pattern of distribution of podocin in three fragments is unchanged between normal and ANA nephropathy.

**Semiquantitative RT-PCR and Real-Time PCR Analyses.** Semi-quantitative RT-PCR study showed that decreased expression of podocin mRNA was observed in the glomeruli of a rat 24 h after induction of ANA nephropathy (Figure 8A). In contrast, decreased expression of podocin was not observed in PAN nephropathy at any point in time during the examination (Figure 8B). Real-time RT-PCR finding also indicated that the mRNA expression for podocin was decreased in rats 24 h after the induction of ANA nephropathy (Figure 9).

**Developmental Expression of Rat Podocin**
Podocin staining was first detected at the early capillary loop stage. The staining of podocin was restricted in the basal surface of the podocyte. No podocin signal was detected in the lateral surface of the podocyte, whereas nephrin staining was detected as a basolateral surface of podocyte in the early developing stage (Figure 10, A through C). Dual-labeling study of podocin with ZO-1 (Figure 10, D through F) also showed that podocin staining was restricted in the basal surface of the podocyte.

**Podocin Staining in Nerve Tissues**
Positive immunostaining of podocin was detected in the cerebrum, cerebellum, and the medulla oblongata. Staining of podocin in the cerebrum is shown in Figures 10G through 10I). Dual labeling study with anti-GFAP antibody showed that podocin staining was observed at the GFAP-positive cells, but podocin staining is restricted more than GFAP staining. Clear podocin staining was observed at a side of GFAP-positive cells (Figure 10G). Dual-labeling study with RECA1 showed that podocin was localized just in the outer area of vessels (Figure 10H). Close proximity of podocin and nephrin was also observed in cerebrum sections (Figure 10I), although some nephrin staining that was not accompanied with podocin was observed.

**Discussion**
In this study, we cloned the rat homologue of podocin to investigate the expression of podocin in proteinuric models of rat. We compared the expression of podocin with that of nephrin and ZO-1 in normal matured and developing glomeruli and the glomeruli of proteinuric states using experimental models of rat. The sequencing of rat podocin revealed that the deduced amino acid sequence of rat nephrin shows 84.3% identity to human podocin. Recently, Schwarz et al. (20) cloned the mouse homologue of podocin. The sequence of rat podocin has 92.7% identity to mouse podocin. The domain structure of podocin is also highly conserved between rat, mouse, and human. Podocin presents similarities with proteins of the stomatin family and is considered to have a similar molecular function (17). It is suggested that MEC-2, a member of the stomatin protein family plays a role in linking ion channels to the cytoskeleton in the primary sensory neuron. Although the recent reports showed that podocin has interaction with nephrin and CD2AP (19,20), the precise function of podocin has not been clarified. Western blot analysis with sequentially solubilized material showed that the fractions of RIPA-soluble and RIPA-insoluble materials were rich in podocin, although the Triton X-100 solubilized fraction was rich in nephrin. It was suggested that podocin is resistant to solubilization with mild detergent (20). These findings might suggest that podocin has a connection with the cytoskeletal component.

The podocin staining was observed as a linear-like pattern with fine granule along the capillary loop in normal adult rat
Figure 5. Localization of podocin, nephrin, and ZO-1 in glomeruli of proteinuric states. For dual-labeling immunofluorescence, cryostat sections were incubated with rabbit anti-rat podocin, FITC-conjugated anti-rabbit IgG, anti-rat nephrin mAb 5-1-6, and then TRITC-conjugated anti-mouse IgG1, respectively. The localization of podocin (green staining: A, D, G), nephrin (red staining: C, F, I), and double exposure findings (B, E, H) were shown. Close proximity of podocin and nephrin localization is observed in the normal rat glomeruli, although some nonoverlapping staining is detected. The staining pattern of rat podocin and nephrin in normal rat glomeruli was the linear-like pattern of the continuous fine granules (A through C). Podocin staining shifted a discontinuous coarse granular pattern in the proteinuric state caused by ANA (D). Nephrin staining shifted concomitantly with podocin (E and F). In PAN nephropathy (G through I), podocin and nephrin staining shifted in a discontinuous pattern and intensity of staining was dramatically decreased. It should be noted that nephrin staining was not always coincided with podocin as indicated by the arrows (H). Double-exposure findings of podocin (green staining) and ZO-1 (red staining) are shown (J through L). Close localization of podocin and ZO-1 was observed in normal rat glomeruli (J). In ANA nephropathy, the localization of podocin shifted concomitantly with ZO-1 (K). In PAN nephropathy, although the intensity of podocin staining (green staining) was dramatically decreased, that of ZO-1 (red staining) was preserved (L).
glomeruli. The localization of podocin is very close to that of nephrin (Figure 5, A through C) and ZO-1 (Figure 5G). Immunoelectron microscopic study showed that podocin is localized on the cytoplasmic face close to the point of slit diaphragm (Figure 6, A through B). These findings indicate that podocin is a slit diaphragm associated protein. Anti-podocin antibody used in this study recognizes a peptide of 21 amino acids located at N-terminal region of rat podocin. Immunoelectron microscopic findings in this study indicated that N-terminal region of rat podocin locates at cytoplasm as same as human podocin (18).

In this study, we analyzed the expression of podocin in proteinuric states. In ANA nephropathy, the staining intensity of podocin clearly decreased and its staining pattern shifted to the coarse granular pattern (Figure 5, D through F). In ANA nephropathy, structural integrity of slit diaphragm is largely unaffected, although foot process effacement is partially observed. Immunogold particles for podocin are observed at the cytoplasm at the preserved slit diaphragm and also observed diffusely in the cytoplasm at the site where foot process effacement was observed (Figure 6, C and D). We could not detect the reduced intensity of podocin band by Western blot analysis using glomerular lysate from rats of ANA nephropathy. Although the decreased expression of mRNA of podocin was detected at the early phase of ANA nephropathy, mRNA expression of podocin recovered on day 5, when the amount of proteinuria peaked. Although the precise mechanism of proteinuria caused by ANA remains unclear, we have previously reported that ANA injected intravenously binds the extracellular site of nephrin and alters the molecular composition of the slit diaphragm, thereby causing proteinuria (7,23). We showed here that podocin is redistributed concomitantly with nephrin in ANA nephropathy. All these findings suggest that altered localization of podocin is concerned with the development of proteinuria in ANA nephropathy. In PAN nephropathy, the shift of the localization of podocin and the decreased intensity of podocin band in Western blot analysis were observed, although the decreased mRNA expression of podocin was not detected at any time points examined. The findings suggest that podocin is actively degraded and/or secreted into urine in proteinuric state. We have previously reported that nephrin staining shifted to a discontinuous granular pattern in PAN nephropathy (14). It should be noted that the redistribution of podocin was not concomitant with nephrin in PAN nephropathy (Figure 5, G through I). The dual-labeling immunofluorescent findings clearly showed that podocin was localized apart from nephrin. Immunoelectron microscopic findings showed that immunogold particles for podocin lay at the site where nephrin-labeled membranes were not present.
tight junction was formed in proteinuric state of PAN nephropathy (Figure 6, E and F). Although PAN nephropathy is widely used as a model of minimal change nephrotic syndrome, the pathogenesis of proteinuria is not clearly explained. It is reported that oxygen radicals that are produced during the metabolism of PAN contribute to the podocyte injury (24). In PAN nephropathy, some pathogenic factors might cause the disassembling of nephrin with podocin. The disconnection of nephrin and podocin, as well as the decreased expression of these proteins, might result in the dysfunction of the slit diaphragm.

In this study, we also analyzed the expression of podocin in developing glomeruli. Immunohistochemical studies with a neonatal rat kidney section showed that podocin first appeared in the early capillary loop stage, later than the nephrin expression. Podocin expression was restricted to the basal side of the podocyte of the developing glomeruli. No positive staining of podocin was observed on the lateral side of the podocyte, whereas the nephrin expression was detected not only in the basal surface but also in the lateral surface of podocyte. The findings showed that nephrin was not accompanied with podocin at the early developing stage. Previously, we reported that nephrin was first expressed as the membrane protein and became to be concentrated at the slit pore (25). The findings obtained here suggest that nephrin and podocin originate from a different part of the cell and are assembled at the slit pore. It is conceivable that the connected localization of nephrin and

Figure 8. Semiquantitative RT-PCR analysis of rat podocin in ANA-induced nephropathy (A) and PAN nephropathy (B). The characteristic agarose gel electrophoretic pattern from one of three independent experiments is shown (top). Ratios of the densitometric signal of podocin to that of the internal control (GAPDH) were analyzed (bottom). The data are shown as ratios relative to normal findings and expressed as mean ± SD of three independent experiments. The mRNA expression for podocin was decreased 24 h after ANA injection (A). In PAN nephropathy, a decreased expression for podocin was not observed at any point in time of examination (B).

Figure 9. Real-time RT-PCR analysis of podocin in rats 24 h after ANA injection. The number of PCR cycles was shown on the x-axis, and the difference between measured fluorescence and the baseline was shown on the y-axis. A decreased expression of podocin was detected in rats of ANA nephropathy.
podocin might be necessary for the maturation of the slit diaphragm.

We show here that the expression of podocin is restricted in glomerular podocyte in the kidney. However, RT-PCR analysis indicates that mRNA of podocin was expressed not only in the kidney but also in the nerve tissues such as the cerebrum, cerebellum, and the medulla oblongata. The expression of some podocyte-associated molecules, such as synaptopodin and nephrin, was also restricted to podocyte and nerve tissues (26,27). These findings suggest that podocyte shared its specific function with the nerve tissues. Galloway and Mowat reported the syndrome that was characterized by nephrotic syndrome associated with central nerve system anomalies (28). Some reports postulated that the mutation of a molecule that is expressed both in the podocyte and in nerve tissue is involved in this syndrome (29,30). The dual-labeling immunofluorescence study of podocin with anti-GFAP antibody, a marker of astrocyte (31), showed that podocin staining was detected at

Figure 10. Localization of podocin in developing glomeruli (A through F) and cerebrum (G through I). Dual-labeling immunofluorescence findings of podocin (green staining) with nephrin (red staining) in early capillary loop stage were shown (A, podocin; B, merge; C, nephrin). Clear staining of rat podocin was detected in the basal side of podocyte of developing glomeruli (A). Nephrin staining was detected not only in the basal side but also in the lateral side of the podocyte (arrow) (C). Dual-labeling immunofluorescence findings of podocin (green staining) with ZO-1 (red staining) in late capillary loop stage are shown (D, podocin; E, merge; F, ZO-1). Podocin staining is restricted in the basal side of podocyte (D). Close localization of podocin and ZO-1 in glomeruli was observed as yellow (E). ZO-1 staining is observed not only in glomeruli but also in junctions between tubular epithelial cells. Double-exposure finding of podocin (green staining) with GFAP (G), RECA1 (H), and nephrin (I) in cerebrum is shown. Podocin staining was observed at the GFAP-positive cells (G). Clear podocin staining was observed at a side of GFAP-positive cells (arrow). Dual-labeling study with RECA1 showed that podocin was localized outer area of vessels (H, arrow). Close proximity of podocin and nephrin was also observed in cerebrum sections (I), although some nephrin staining that was not accompanied with podocin was observed.
GFAP-positive cells. Podocin staining was observed at the end foot processes and at a side of astrocyte facing vessels (Figure 10G). The dual-labeling study with RECA1, a marker of endothelial cell (32), shows that podocin is localized just outer area of vessels (Figure 10H). It is conceivable that clear podocin staining is at the side of astrocyte-facing vessels. We also observed here that podocin and nephrin are closely localized in nerve tissues as well as glomerular podocyte (Figure 10I).

Astrocyte surrounds neuron or vessels. Recently, astrocyte is reported to play a critical role for maintaining the barrier function of blood-brain barrier (33,34). These findings might suggest that podocin and nephrin play an important role for maintaining the barrier function not only in glomerular capillary wall but also in blood-brain barrier.

In conclusion, the deduced amino acid sequence of rat podocin showed 84.3% identity to human podocin. Podocin expression was detected not only in the kidney but also in the nerve tissues. Podocin expression was first detected in the early capillary loop stage. Podocin expression was restricted at the basal surface of podocyte of the developing glomeruli. Podocin localization was very closed in nephrin in normal glomeruli. Podocin was redistributed in both proteinuric states caused by PAN and ANA. It is postulated that podocin is one of critical components of the slit diaphragm for maintaining the barrier function of the glomerular capillary wall.

Acknowledgments
This work was supported by Grant-Aids for Scientific Research (B) (13557084 to H. Kawachi), Grant-Aids for Scientific Research (B) (14370317 to H. Kawachi), and Grant-Aids for Scientific Research (B) (08457286 to F. Shimizu) from Ministry of Education, Science, Culture and Sports of Japan. We wish to thank Ms. M. Kayaba and Ms. C. Nagasawa for their excellent technical assistance. We wish to thank Dr. Hitoshi Takahashi (Department of Pathology, Brain Research Institute, Niigata University) for his helpful advice. A portion of this study was presented at the ASN/ISN World Congress, San Francisco, California, October 2001, and was published in abstract form (J Am Soc Nephrol 12: 680A, 2001).

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