Glomerular Permeability Is Altered by Loss of P0, a Myelin Protein Expressed in Glomerular Epithelial Cells

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The myelin protein 0 (MPZ or P0) is a transmembrane glycoprotein that represents the most abundant myelin component. Mutations in the *P0* gene are associated with one form of autosomal dominant demyelinating peripheral neuropathy, Charcot-Marie-Tooth disease type 1B (CMT1B). Because CMT1 may be associated with renal involvement, mostly focal segmental glomerulosclerosis, we hypothesized that P0 could be expressed in the kidney. P0 mRNA was detected by reverse transcriptase–PCR in the human and mouse renal cortex. P0 transcripts were identified by *in situ* hybridization at different stages of the mouse kidney development, especially in embryonic structures that give rise to the glomerulus. P0 protein was also detected by Western blot in human and rat glomerular extracts and in a human podocyte cell line using a monoclonal anti-P0 antibody. Immunofluorescence studies on human kidney sections showed that the podocytes were intensely labeled. Immunogold electron microscopy disclosed a predominant staining of the membranes of intracellular vesicles in podocytes. P0 was also detected in the podocyte cell membrane, including at the foot processes. $P0^{-/-}$ mice exhibited mild growth retardation and demyelinating neuropathy similar to the one observed in patients with CMT1B. They also presented mild albuminuria, without significant ultrastructural change of the glomerular basement membrane or the podocytes. These results demonstrate that P0, the major myelin protein, is also expressed during nephrogenesis and in mature kidney, mostly in podocytes. They suggest that *P0* gene mutations might be involved in renal diseases.

J Am Soc Nephrol 16: 3350–3364, 2005. doi: 10.1681/ASN.2005050509

Glomerular epithelial cells, podocytes, are highly specialized cells that participate in the glomerular filtration barrier through the filtration slits bounded by the interdigitated foot processes and the synthesis of glomerular basement membrane (GBM) components. In the past few years, genetic studies of familial forms of nephrotic syndrome in human have expanded our knowledge on the cell biology of podocytes. They led to the identification of proteins that are functionally important in maintaining permselectivity of the glomerulus. These proteins include nephrin and podocin located at the slit diaphragm and the cytoskeleton-associated α-actinin 4 protein (1–3). *In vitro* experimental studies and knockout mouse models have further identified additional podocyte proteins, interacting mostly with nephrin and podocin for the maintenance of the slit diaphragm integrity. It is interesting that some of the podocyte proteins are electively coexpressed in the central nervous system. These include the cytoskeletal component synaptopodin, nephrin, densin, glomerular epithelial protein 1, the synaptic vesicle molecule Rab3A and its effector Rabphilin-3a, and the neuronal amino acid transporters CAT3 and EAAT2 (4–9). However, none of these proteins has been found in the myelinated peripheral nervous system, although focal segmental glomerulosclerosis (FSGS) has been reported in the most common familial form of demyelinating neuropathy, Charcot-Marie-Tooth (CMT) disease (10–12).

In this study, we investigated the renal expression and role of the myelin protein 0 (MPZ or P0). P0 is the most abundant protein of the peripheral nervous system (PNS), produced by Schwann cells. P0 is a small, integral membrane glycoprotein that has a single Ig-like extracellular domain and is responsible for membrane adhesion and compaction of the myelin membranes (13,14). Mutations in the *P0* gene have been associated with hereditary peripheral demyelinating neuropathy, including CMT1B, Dejerine-Sottas disease, and congenital hypomyelination neuropathy (15). Expression of P0 outside the PNS has not been reported so far, except for thymic stromal cells (16). Here, we demonstrate that P0 is synthesized in the kidney and mostly localized in the podocytes from earlier stages of glomerulogenesis. Furthermore, knockout mice, whose neurologic phenotype is closely similar to the human disease (17), show increased glomerular permeability to albumin, suggesting a role for P0 in the control of glomerular permselectivity and its possible implication in CMT-associated renal disease.

Materials and Methods

Reverse Transcriptase–PCR

Total RNA was extracted from human adult kidney cortex (normal pole of cancer kidney) and mouse kidney using RNAwiz (Ambion Inc.,

Received May 16, 2005. Accepted July 20, 2005.

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

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ISSN: 1046-6673/1611-3350

Austin, TX). After a 1-h incubation at 37°C with DNAse (Ambion Inc.), cDNA synthesis was performed using Superscript First Strand synthesis System for reverse transcriptase–PCR (RT-PCR; Invitrogen, Carlsbad, CA). PCR then was carried out using different sets of specific primers (Table 1). Amplification products were subcloned using the pGEM-T Easy vector system II (Promega, Madison, WI), and the corresponding clones were sequenced.

Western Blot

Proteins from human and rat isolated glomeruli were extracted by lysis using a detergent-based buffer and protease inhibitors (Complete Mini; Roche Diagnostic Corp., Indianapolis, IN). Protein extracts were also prepared according to the same procedure, from the human podocyte cell line established in our laboratory (18). Total protein extracts were run on a 12% SDS-PAGE gel and transferred to a nitrocellulose membrane (Immobilon-P; Millipore, Bedford, MA) by electroblotting. After blocking unspecific binding in 10% skimmed milk PBS, membranes were incubated overnight with anti-P0 mAb (1:1000 in 5% skimmed milk TBS/0.5% Tween) (19) at 4°C, washed in 0.5% Tween/TBS, and then incubated for 2 h with alkaline phophatase–conjugated goat anti-mouse Ig (Chemicon International, Temecula, CA). The anti-P0 mAb was omitted in controls. Blots were developed using nitro blue tetrazolium and 5-bromo-4-chloro-indolyl phosphate reagents (Sigma-Aldrich, Steinhiem, Germany).

Immunofluorescence Staining

Immunofluorescence was performed on 3-μm frozen sections from human adult kidney fixed 3 min in acetone. After washing three times in PBS, sections were incubated for 1 h with 10% FCS-PBS and incubated overnight with anti-P0 mAb (1:100 diluted in 10% FCS-PBS) at 4°C. After washes in PBS, sections then were incubated with FITC-conjugated goat anti-mouse Ig antibody (1:200 diluted in 10% FCS-PBS; Biovalley, Marne La Vallée, France). Microscopy was performed with a Zeiss Axiophot2 microscope (Carl Zeiss Jena Gmbh, Jena, Germany).

Double Immunofluorescence Labeling and Confocal Microscopy

For dual fluorochrome labeling, the slides were incubated simultaneously with anti-P0 mAb (1:100 diluted in 10% FCS-PBS) and rabbit anti-podocin antibody (1:1000 in 10% FCS/PBS; a gift from C. Antignac; INSERM Unit 574, Paris, France) (20) or rabbit anti-nephrin antibody (1:50 in 10% FCS/PBS; a gift from F. Shimizu, Niigata, Japan) (21). After washing in PBS, the slides were incubated with FITC-conjugated goat anti-mouse Ig antibody (1:200 diluted in 10% FCS-PBS; Sigma) and TRITC-conjugated goat anti-rabbit IgG (1:200; Sigma). Sections were examined with a Zeiss confocal microscope (Carl Zeiss Microscopy).

Immunogold Electron Microscopy

Immunogold electron microscopy was performed on ultrathin frozen sections of kidney biopsies from healthy subjects. Briefly, pieces of biopsies were fixed in 4% freshly prepared formaldehyde (by depolymerization of paraformaldehyde) and 0.1% distilled glutaraldehyde (Merk, Darmstadt, Germany) in 100 mM phosphate buffer (pH 7.2) for 6 to 12 h at 4°C, soaked in sucrose, and frozen and stored in liquid nitrogen. Ultrathin frozen sections were processed for indirect immunogold labeling, as described (22). A control experiment in which the first antibody was omitted was performed.

In Situ *Hybridization*

Mouse kidneys sampled at 17.5 dpc and in the neonate period were fixed in paraformaldehyde 4%, paraffin-embedded, and sectioned (5 μm). The mouse P0 riboprobes were synthesized from a 497-bp PCR product (spanning from 5 to 492 bp in mouse P0 cDNA), then subcloned into the pGEM-Teasy vector (Promega). The antisense probe was synthesized after digestion with PstI using the T7 polymerase, and the sense probe was synthesized after digestion with SphI using the Sp6 polymerase. The riboprobes were labeled with digoxigenin-11-UTP (Roche) according to the manufacturer's instructions. *In situ* hybridization was carried out as described previously (23).

Knockout Mice Studies

The generation and neurologic phenotyping of $P0^{-/-}$ mice have been described previously (17). These mice are on a C57Bl/6 background. All experiments were performed according to the European recommendations for the care and use of laboratory animals.

Mouse Genotyping. DNA was isolated from mouse tail, and mice were genotyped by PCR amplification with the following primers: mP0-Ex1(F) CCCCGCTCTCTCCACCCCACAGACA and mP0-Ex1(R) GGGCAGCCAGGATAGGGCTGGGGC. A 1250-bp fragment was amplified in $P0^{-/-}$ mice.

Serum and Urine Analysis. Individual mice were housed overnight in a diuresis cage, and urine was collected after 24 h. Individual urine samples were analyzed by 10% SDS-PAGE, and gels were stained with Coomassie blue. Samples that were applied to the gel were normalized for creatinine content. Urine albumin and creatinine concentrations were assayed by ELISA and automated Jaffe method, respectively. Blood was collected from anesthetized mice by cardiac puncture,

Table 1. Primer sequences and predicted product size

Molecule	ID Number	Primer Sequences	Position	Product Size
Human protein myelin 0 (exons 2 to 3)	NM000530	CCGGCCCAGGCCATCGTGGT	125 to 143	356 bp
		CAGCGTGACCTGAGAGGTCTTGCCCA	456 to 481	
Human protein myelin 0 (exons 4 to 6)		CTGGCTACGCAGGCAGGCGGCCCT	595 to 618	616 bp
		CCCCCGGGGCAGGTGAGGGGTAGG	1187 to 1211	
Human protein myelin 0 (exons 1 to 6)		GGAATTCATGCTCCGGGCCCCTGCCCCTG	20 to 42	801 bp
		GCTCTAGATCCCCCGCCCGGCCCGCTAA	800 to 821	
Mouse protein myelin 0 (exons 1 to 4)	NM008623	CGGGGCTCCCTCCTCCAGCCCCA	9 to 23	497 bp
		CCACCCCGAGGATGCCCCCGA	484 to 505	
Mouse protein myelin 0 (exons 1 to 6)		GGGCTAGCCTACCCCAGCTATGGCTCCCG	1 to 11	750 bp
		GCTCTAGAGCCCGGCCGCTAACCGCTAT	741 to 750	

and serum samples were analyzed for creatinine by automated Jaffe method.

Histologic Analysis. Kidneys were removed from killed mice, fixed in Dubosq-Brazil, dehydrated, and paraffin embedded. Sections were stained with Trichrome, silver stain, or periodic acid-Schiff. For electron microscopy, tissue samples from male $P0^{+/+}$ and $P0^{-/-}$ mice were processed as described previously (8). For immunofluorescence, frozen kidney sections (3 μm) were incubated as above with the anti-podocin antibody.

Results

Expression of P0 in mouse, rat, and human kidney was demonstrated by combined approaches including RT-PCR, Western blot, immunochemistry, immunoelectron microscopy, and *in situ* hybridization.

RT-PCR

RT-PCR analysis used different sets of primers (Table 1). The full-length P0 cDNA could be amplified from normal human and mouse kidney cortex, together with shorter cDNA, which encode different domains of P0 (Figure 1). Sequencing of the amplified PCR products revealed 100% identity with the peripheral nerve P0 cDNA from human and mouse, respectively.

Immunoblotting

The glomerular expression of P0 protein was detected by immunoblot using monoclonal mouse anti-P0 antibody raised against the extracellular part of the rat P0 protein (18). Blotting of extracts from isolated human glomeruli and from a human podocyte cell line both revealed a single 30-kD band as described earlier in human peripheral nerve lysates (Figure 2A) (24). A doublet at approximately 28 kD was detected in rat glomerulus protein extracts, similar to the one observed with the nerve rat protein (Figure 2B) (19).

Immunofluorescence

Immunofluorescence of normal adult human kidney sections showed a glomerular positivity along the capillary wall with a pattern highly suggestive of podocyte staining (Figure 3). Extraglomerular staining was present also in vessel, specifically in endothelial cells. No tubular and interstitium labeling was present. Double staining was performed with anti-P0 antibody (green) and anti-nephrin and anti-podocin antibodies (red; Figure 4). P0 tightly co-localized with nephrin. Dual labeling with podocin also showed a co-localization along with an extension of P0 positivity at the periphery of podocin staining.

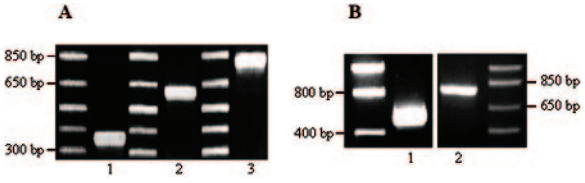

Figure 1. Myelin protein 0 (P0) mRNA expression in the human and mouse kidney cortex. Total RNA from the human (A) and the mouse (B) kidney cortex was used as a template in reverse transcriptase–PCR. (A) Three different combinations of primers were used to amplify the extracellular (lane 1) and intracellular/3′UTR (lane 2) domains of human P0 cDNA as well as the full-length stretch (lane 3). (B) Two combinations of primers were used to amplify the extracellular and transmembrane domains (lane 1) and the full-length stretch of the mouse P0 cDNA (lane 2), respectively.

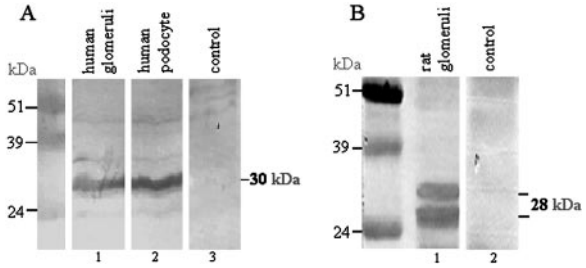

Figure 2. (A) Immunoblot of human glomerular lysate (lane 1) and human podocyte cell line (lane 2) with mAb to the extracellular domain of P0 showed a reactive protein band at 30 kD. (B) mAb P0 immunoblotted a doublet at approximately 28 kD in rat glomerular lysate. Controls including omission of the anti-P0 mAb were negative. Molecular weight markers are shown in the left lane.

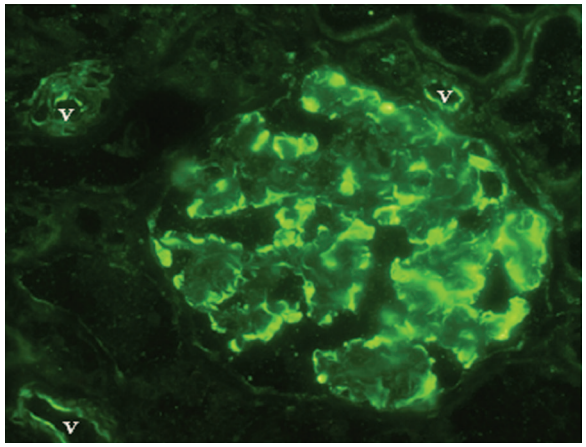

Figure 3. Normal adult human kidney. Indirect immunofluorescence on frozen 3-μm section with monoclonal anti-P0 antibody. A podocyte labeling is observed in glomeruli, with a comma-like pattern staining on the external side of glomerular capillary wall. Staining was also observed outside the glomeruli in the endothelium of vessels (v). Magnification, ×400.

Immunoelectron Microscopy

The glomerular localization of P0 protein was determined precisely by electron microscopy. Gold particles were distributed in the podocyte cell body, especially in cytoplasmic vesicles, as well as in the nearby actin cytoskeleton (Figure 5A). Podocyte synthesis of P0 protein was attested by the dense labeling of endoplasmic reticulum (Figure 5B). Particles were also found in foot processes, being occasionally close to the slit diaphragm (Figure 5C). Sparse reactivity of the glomerular capillary endothelia was present, but no significant labeling was observed in GBM. Control experiment, in which the first antibody was omitted, was completely negative.

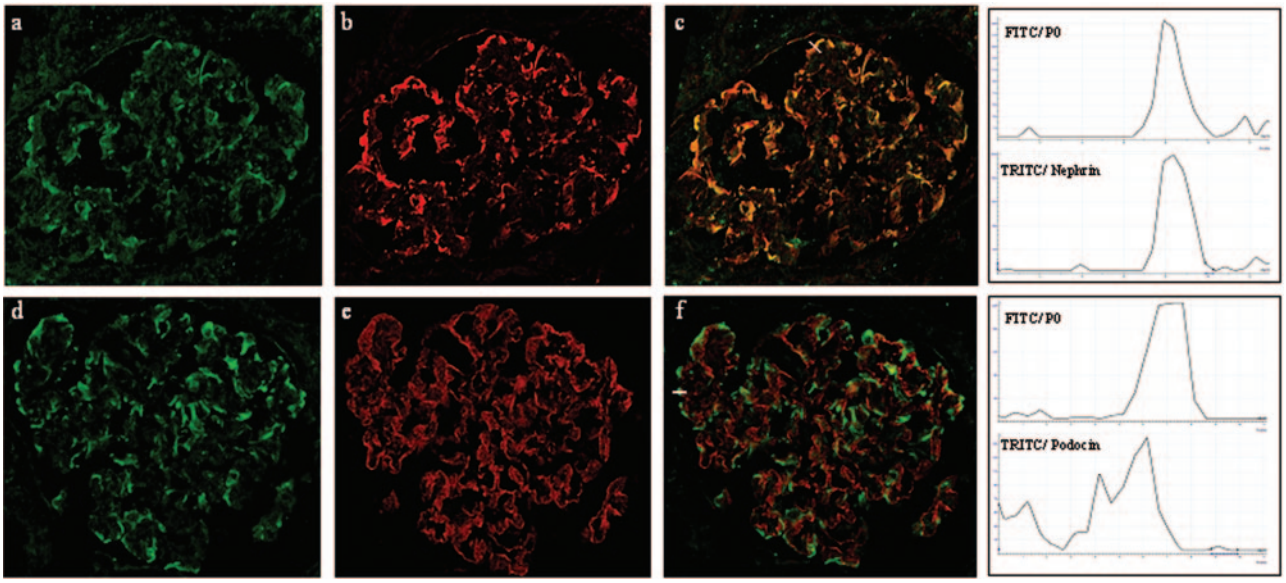

Figure 4. Normal adult human kidney. Dual immunofluorescence and confocal microscopy with monoclonal anti-P0 antibody (green; a and d) and polyclonal antibodies (red) against nephrin (b) and podocin (e). The respective merge images are shown in c and f. Quantitative analysis of the signals recorded at the level of the bars shown in c and f is presented on the right-hand side of the figure. Note the superimposition of P0 and nephrin signals, whereas P0 and podocin show only partial overlap. Magnification: ×400.

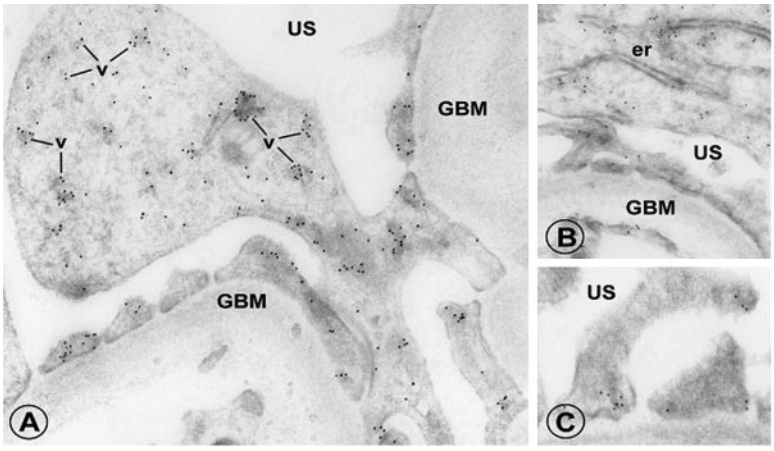

Figure 5. Normal adult human kidney. Immunoelectron microscopy with anti-P0 antibody. (A) Gold particles are localized in podocyte cell body, mostly in vesicles, sometimes in close contact with actin network. (B) Staining of the endoplasmic reticulum. (C) Staining in foot processes close to the slit diaphragm. Note the absence of particle in the slit diaphragm. GBM, glomerular basement membrane; US, urinary space; er, endoplasmic reticulum; V, vesicle.

In Situ *Hybridization*

To analyze developmental expression of P0/MPZ during nephrogenesis, we conducted *in situ* hybridization in the 17.5-dpc and neonate mouse kidney. In the developing metanephric kidney, P0 transcript was detected in the peripheral cortex (Figure 6A) and in primitive nephronic structures that give rise to mature glomeruli including vesicles and S-shaped bodies (Figure 6B and insert). Antisense riboprobe also labeled the ureteric bud and the collecting ducts, although theses structures were not stained by immunofluorescence in the adult kidney. No specific signal was observed with the sense probe (Figure 6C). Similar results were obtained in the neonate kidney (data not shown).

Renal Phenotyping of P0$^{-/-}$ Mice

To establish the potential role of P0 in glomerular permselectivity, we analyzed P0$^{-/-}$ mice that were previously established by one of us (17). These mice present with demyelinating neuropathy closely resembling the neurologic phenotype of patients with CMT1B and heterozygous *P0* gene mutation. In addition to neurologic signs that appear during the first month of life, we observed a moderate growth retardation in P0$^{-/-}$ mice (22.4 ± 0.78 g; $n = 7$) compared with P0$^{+/-}$ (27.4 ± 1.42 g; $n = 9$; $P < 0.05$) and P0$^{+/+}$ mice (27.8 ± 1.48; $n = 5$; $P < 0.05$). As shown in Figure 7A, P0$^{-/-}$ mice exhibited significantly higher albuminuria/creatininuria ratio than P0$^{+/-}$ and P0$^{+/+}$

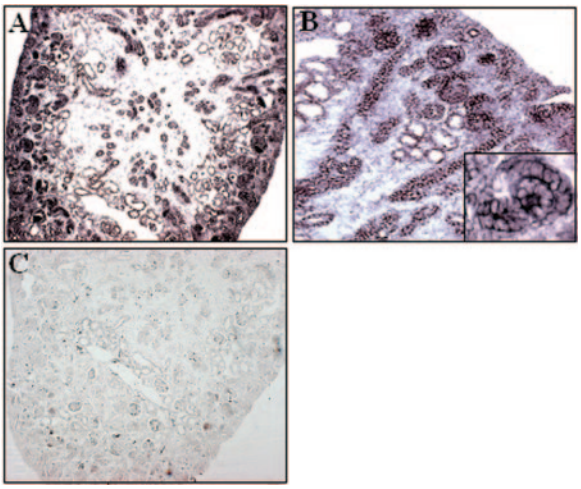

Figure 6. A 17.5-d postcoitum mouse kidney. *In situ* hybridization with P0 antisense riboprobe labeled with digoxigenin. (A) A strong signal is seen in the peripheral cortex and collecting ducts. (B) Expression of P0 transcript in primitive nephronic structure including vesicles, S-shape body (insert) and in ureteric bud and collecting ducts. (C) Absence of signal with sense probe. Magnification: ×100 in A and C; ×200 in B; ×400 in B insert.

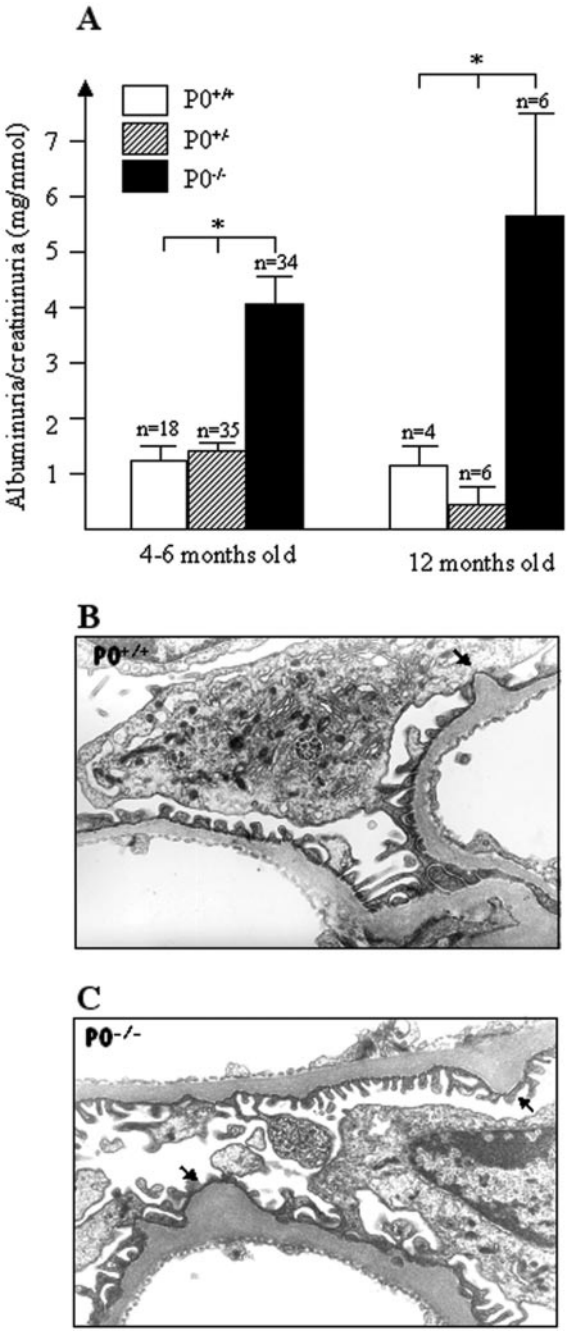

Figure 7. Renal phenotyping of $P0^{-/-}$ mice. (A) Albuminuria/urine creatinine ratio in $P0^{+/+}$, $P0^{+/-}$, and $P0^{-/-}$ mice at 4 to 6 mo and at 12 mo. Values are the mean ± SEM of *n* mice. *$P < 0.05$ $P0^{-/-}$ *versus* $P0^{+/-}$ and $P0^{-/-}$ *versus* $P0^{+/+}$. (B and C) Electron microscopy study of 12-mo male $P0^{+/+}$ and $P0^{-/-}$ mice from the same litter. Focal areas of GBM thickening (→) are observed both in $P0^{-/-}$ and in control mice without alteration of foot processes.

mice, at both 4 to 6 mo and 12 mo of age. Urinary albumin excretion did not increase significantly at 12 mo, compared with earlier age. Electrophoresis of urinary proteins confirmed that albumin was predominant (data not shown).

For analyzing histologic lesions underlying increased urinary albumin excretion in $P0^{-/-}$ mice, mice were killed at 6 and 12 mo of age. Serum creatinine/body weight ratio was not significantly different between $P0^{-/-}$ (1.33 ± 0.18 μmol/L per g body wt; $n = 7$), $P0^{+/-}$ (1.05 ± 0.39 μmol/L per g body wt; $n = 4$), and $P0^{+/+}$ mice (1.41 ± 0.17 μmol/L per g body wt; $n = 7$) at 6 mo. Kidney weight/body weight ratio was similar in $P0^{-/-}$, $P0^{+/-}$, and $P0^{+/+}$ mice (0.0191, 0.0195, and 0.0183, respectively). No significant glomerular, vascular, or interstitial abnormality was noted by histologic examination of $P0^{-/-}$ kidney sections that were stained with hematoxylin eosin and Trichrome (data not shown). Immunofluorescence examination with anti-podocin antibodies of kidneys from $P0^{-/-}$ mice and age- and gender-matched $P0^{+/+}$ littermates showed normal expression of the protein (data not shown). Electron microscopy that was performed at 4 and 12 mo of age showed no significant abnormality, including no foot process effacement, both in $P0^{-/-}$ mice and in their controls (Figure 7, B and C). These results are compatible with the entity referred to as "no change disease" previously described in experimental models and in cases of human nephrotic syndrome (25–27).

Discussion

In this work, we demonstrate the presence of P0 protein in human, rat, and mouse podocytes and increased urinary albumin excretion in mice that are deficient in this protein. These results were unexpected because P0 protein expression was thought to be limited to the peripheral myelinating Schwann cells (13). In the peripheral nerves, this transmembrane glycoprotein induces and maintains myelin compaction around nerve fibers through homophilic interactions of its Ig-like extracellular domains between opposed P0 proteins. Heterozygous *P0* gene mutations in human define CMT1B. This demy-

elinating peripheral neuropathy is a consequence of uncompacted myelin sheaths, responsible for slowed nerve conduction velocity. In addition, P0 was described recently in thymic stroma cells, where its role remains elusive (16). Thymic expression may account for P0/MPZ central tolerance induction, as described for other tissue-specific antigens.

In addition to Schwann cells and thymic stroma cells, we show that full-length P0 mRNA transcripts are expressed in mouse and human kidney cortex and that expression of intact P0 protein can be detected by Western blot in human and rat glomeruli and in human cultured podocytes. Immunofluorescence study identified a glomerular staining with a pattern highly suggestive of podocyte labeling, as attested by partial and complete co-localization with podocin and nephrin, respectively. Extraglomerular labeling was also present in vessel endothelium but absent in tubule segments. Our immunogold results confirmed P0 synthesis in the endoplasmic reticulum and further highlighted P0 subcellular distribution within the podocyte cell body, mainly in vesicular structures and in foot processes, sometimes close to slit diaphragm. *In situ* hybridization in 17.5-dpc and neonate mouse kidneys disclosed P0 transcript in embryonic structures that give rise to mature glomerulus, including vesicles and S-shaped bodies, and also in the collecting duct. These results indicate that P0 is synthesized from the early stages of glomerular development and also transiently in ureteric bud–derived structures.

The identification of the myelin protein P0 in podocyte extends the range of proteins whose expression is shared by nervous cells and the glomerular epithelial cell. However, P0 to date is the only podocyte protein to be expressed in the PNS.

To investigate the role of P0 in glomerular epithelial cells and filtration barrier, we determined the renal functional and morphologic phenotype of $P0^{-/-}$ mice. Although their neurologic phenotype close to the human disease has been investigated extensively, there were no data on their renal function. Whereas heterozygous mice were normal, mice that lacked the P0 protein showed urinary albumin loss in the absence of significant ultrastructural alterations of podocyte foot processes or GBM. Focal areas of thickening of the GBM were detected in $P0^{-/-}$ mice but also in control. The renal phenotype of $P0^{-/-}$ mice seems to be closely similar to the one reported in $DDR1^{-/-}$ mice, in which the loss of discoidin domain receptor-1 in podocytes is associated with moderate high molecular weight proteinuria. Ultrastructural alterations of $DDR1^{-/-}$ glomeruli remain focal, with thickening affecting <4% of the GBM and adjacent loss of slit diaphragm (28). In contrast with $P0^{-/-}$ mice, synaptopodin-deficient mice do not show renal abnormalities as judged by electron microscopy and urinary protein excretion analysis, although podocytes normally express a large amount of synaptopodin (29). Albumin excretion rate was neither increased in $Ptpro^{-/-}$ mice that lack the glomerular epithelial protein 1, even after uninephrectomy, although scanning electron microscopy disclosed morphologic podocyte alterations (30). Overall, these data suggest that P0 plays a significant role in glomerular permselectivity. The expression of P0 mostly in vesicles in the podocyte cell body is compatible with a role in cell transport as suggested for Rab3 (8). Because the $P0^{-/-}$ mice are on C57Bl/6 background, which is known to confer resistance to glomerulosclerosis after nephron reduction or toxic injury (31), we are currently backcrossing the C57Bl/6–$P0^{-/-}$ mice to other backgrounds that are more prone to the development of renal diseases.

$P0^{-/-}$ mice may represent a new model of albuminuria without detectable morphologic alteration of the glomerular filtration barrier, a condition that is more common than expected and now referred as "no change disease." Even if most proteinuric renal diseases are associated with massive loss of podocyte foot processes, there are indeed human and experimental models of nephrotic-range proteinuria in which this podocyte abnormality is absent. In human, a familial form of nephrotic syndrome without podocyte alteration has been previously reported (25), and a sporadic case of massive proteinuria with only segmental effacement of foot processes affecting 20 to 30% of capillary loops has been observed (26). In the rat, injection of mAb 5-1-6 directed against nephrin induces massive proteinuria without any foot process damage (27). Altogether, these observations suggest that albuminuria of glomerular origin can occur in the absence of slit diaphragm alterations even at the electron microscopy level.

Almost 20 cases of glomerular disease, mainly focal segmental glomerulosclerosis, have been reported in the course of familial demyelinating neuropathy. Genetic analysis, available in only two cases of CMT with FSGS, did not show mutations in *PMP22*, *P0*, and *Connexin-32* genes, which are the three genes most commonly altered in CMT (12). Our results prompt the search for *P0* mutation in cases of CMT with kidney diseases, as well as in autosomal familial forms of glomerulopathies that are not linked to most commonly mutated genes, including *ACTN4*, *NPHS1*, and *NPHS2*.

In conclusion, we have first identified in the murine and human glomerulus, P0, the major myelin protein of the PNS. Results that were obtained in $P0^{-/-}$ mice suggest that P0 plays some role in glomerular permselectivity, although this role remains to be defined.

Acknowledgments

This work was supported by grants from INSERM, University Paris 6, and AURA (Association pour l'Utilisation du Rein Artificiel and by EU contract QLG1-CT-2002-01215).

We thank Professor D. Vallat (University of Limoges, France) for helpful discussion and support and Philippe Fontanges for technical assistance.

This work was presented at the 38th Annual Meeting of the American Society of Nephrology, St. Louis, MO, November 2004.

References

1. Kestila M, Lenkkeri U, Mannikko M, Lamerdin J, McCready P, Putaala H, Ruotsalainen V, Morita T, Nissinen M, Herva R, Kashtan CE, Peltonen L, Holmberg C, Olsen A, Tryggvason K: Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. *Mol Cell* 1: 575–582, 1998
2. Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fuchshuber A, Dahan K, Gubler MC, Niaudet P, Antignac C: NPHS2, encoding the glomerular protein podocin, is mu-

tated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet* 24: 349–354, 2000
3. Kaplan JM, Kim SH, North KN, Rennke H, Correia LA, Tong HQ, Mathis BJ, Rodriguez-Perez JC, Allen PG, Beggs AH, Pollak MR: Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. *Nat Genet* 24: 251–256, 2000
4. Mundel P, Heid HW, Mundel TM, Kruger M, Reiser J, Kriz W: Synaptopodin: An actin-associated protein in telencephalic dendrites and renal podocytes. *J Cell Biol* 139: 193–204, 1997
5. Putaala H, Soininen R, Kilpelainen P, Wartiovaara J, Tryggvason K: The murine nephrin gene is specifically expressed in kidney, brain and pancreas: Inactivation of the gene leads to massive proteinuria and neonatal death. *Hum Mol Genet* 10: 1–8, 2001
6. Ahola H, Heikkila E, Astrom E, Inagaki M, Izawa I, Pavenstadt H, Kerjaschki D, Holthofer H: A novel protein, densin, expressed by glomerular podocytes. *J Am Soc Nephrol* 14: 1731–1737, 2003
7. Thomas PE, Wharram BL, Goyal M, Wiggins JE, Holzman LB, Wiggins RC: GLEPP1, a renal glomerular epithelial cell (podocyte) membrane protein-tyrosine phosphatase. Identification, molecular cloning, and characterization in rabbit. *J Biol Chem* 269: 19953–19962, 1994
8. Rastaldi MP, Armelloni S, Berra S, Li M, Pesaresi M, Poczewski H, Langer B, Kerjaschki D, Henger A, Blattner SM, Kretzler M, Wanke R, D'Amico G: Glomerular podocytes possess the synaptic vesicle molecule Rab3A and its specific effector rabphilin-3a. *Am J Pathol* 163: 889–899, 2003
9. Gloy J, Reitinger S, Fischer KG, Schriber R, Boucherot A, Kunzelmann K, Mundel P, Pavenstädt H: Amino acid transport in podocytes. *Am J Physiol* 278: F999–F1005, 2000
10. Lemieux G, Neemeh JA: Charcot-Marie-Tooth disease and nephritis. *Can Med Assoc J* 97: 1193–1198, 1967
11. Paul MD, Fernandez D, Pryse-Phillips W, Gault MH: Charcot-Marie-Tooth disease and nephropathy in a mother and daughter with a review of the literature. *Nephron* 54: 80–85, 1990
12. Mache CJ, Auer-Grumbach M, Soleiman A, Windpassinger C, Wagner K, Ring E: Charcot-Marie-Tooth disease and focal and segmental glomerulosclerosis [Abstract]. *Nephrol Dial Transplant* 18: 259, 2003
13. Lemke G, Axel R: Isolation and sequence of a cDNA encoding the major structural protein of peripheral myelin. *Cell* 40: 501–508, 1985
14. Sakamoto Y, Kitamura K, Yoshimura K, Nishijima T, Uyemura K: Complete amino acid sequence of PO protein in bovine peripheral nerve myelin. *J Biol Chem* 262: 4208–4214, 1987
15. Hayasaka K, Himoro M, Sato W, Takada G, Uyemura K, Shimizu N, Bird T, Conneally PM, Chance PF: Charcot-Marie-Tooth neuropathy type 1B is associated with mutations of the myelin P0 gene. *Nat Genet* 5: 31–34, 1993
16. Visan L, Visan IA, Weishaupt A, Hofstetter HH, Toyka KV, Hunig T, Gold R: Tolerance induction by intrathymic expression of P0. *J Immunol* 172: 1364–1370, 2004
17. Martini R, Zielasek J, Toyka KV, Giese KP, Schachner M: Protein zero (P0)-deficient mice show myelin degeneration in peripheral nerves characteristic of inherited human neuropathies. *Nat Genet* 11: 281–286, 1993
18. Delarue F, Virone A, Hagege J, Lacave R, Peraldi MN, Adida C, Rondeau E, Feunteun J, Sraer JD: Stable cell line of T-SV40 immortalized human glomerular visceral epithelial cells. *Kidney Int* 40: 906–912, 1991
19. Archelos JJ, Roggenbuck K, Schneider-Schaulies J, Toyka KV, Hartung HP: Detection and quantification of antibodies to the extracellular domain of P0 during experimental allergic neuritis. *J Neurol Sci* 117: 197–205, 1993
20. Roselli S, Gribouval O, Boute N, Sich M, Benessy F, Attie T, Gubler MC, Antignac C: Podocin localizes in the kidney to the slit diaphragm area. *Am J Pathol* 160: 131–139, 2002
21. Kawachi H, Abrahamson DR, St John PL, Goldstein DJ, Shia MA, Matsui K, Shimizu F, Salant DJ: Developmental expression of the nephritogenic antigen of monoclonal antibody 5-1-6. *Am J Pathol* 147: 823–833, 1995
22. Regele HM, Fillipovic E, Langer B, Poczewki H, Kraxberger I, Bittner RE, Kerjaschki D: Glomerular expression of dystroglycans is reduced in minimal change nephrosis but not in focal segmental glomerulosclerosis. *J Am Soc Nephrol* 11: 403–412, 2000
23. Allory Y, Matsuoka Y, Bazille C, Christensen EI, Ronco P, Debiec H: The L1 cell adhesion molecule is induced in renal cancer cells and correlates with metastasis in clear cell carcinomas. *Clin Cancer Res* 11: 1190–1197, 2005
24. Tachi N, Kozuka N, Ohya K, Chiba S, Sasaki K, Uyemura K: Expression of peripheral myelin protein zero in sural nerve of patients with Charcot-Marie-Tooth disease 1B. *Pediatr Neurol* 24: 33–35, 2001
25. Branten AJ, van den Born J, Jansen JL, Assmann KJ, Wetzels JF: Familial nephropathy differing from minimal change nephropathy and focal glomerulosclerosis. *Kidney Int* 59: 693–701, 2001
26. Good KS, O'Brien K, Schulman G, Kerjaschki D, Fogo AB: Unexplained nephrotic-range proteinuria in a 38-year-old man: A case of "no change disease." *Am J Kidney Dis* 43: 933–938, 2004
27. Kawachi H, Kurihara H, Topham PS, Brown D, Shia MA, Orikasa M, Shimizu F, Salant DJ: Slit diaphragm-reactive nephritogenic MAb 5-1-6 alters expression of ZO-1 in rat podocytes. *Am J Physiol* 273: F984–F993, 1997
28. Gross O, Beirowski B, Harvey SJ, McFadden C, Chen D, Tam S, Thorner PS, Smyth N, Addicks K, Bloch W, Ninomiya Y, Sado Y, Weber M, Vogel WF: DDR1-deficient mice show localized subepithelial GBM thickening with focal loss of slit diaphragms and proteinuria. *Kidney Int* 66: 102–111, 2004
29. Deller T, Korte M, Chabanis S, Drakew A, Schwegler H, Stefani GG, Zuniga A, Schwarz K, Bonhoeffer T, Zeller R, Frotscher M, Mundel P: Synaptopodin-deficient mice lack a spine apparatus and show deficits in synaptic plasticity. *Proc Natl Acad Sci U S A* 100: 10494–10499, 2003
30. Wharram BL, Goyal M, Gillespie PJ, Wiggins JE, Kershaw DB, Holzman LB, Dysko RC, Saunders TL, Samuelson LC, Wiggins RC: Altered podocyte structure in GLEPP1 (Ptpro)-deficient mice associated with hypertension and low glomerular filtration rate. *J Clin Invest* 106: 1281–1290, 2000
31. Li-Jun Ma, Fogo AB: Model of robust induction of glomerulosclerosis in mice: Importance of genetic background. *Kidney Int* 64: 350–355, 2003