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Expression of OAT1 and OAT3 in differentiating proximal tubules of the mouse kidney

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Summary. Organic anion transporter 1 (OAT1) and OAT3 in the proximal tubules (PT) of the kidney play important roles in the elimination of harmful endogenous compounds and xenobiotics from the body. We investigated the temporal and spatial expression of OAT1 and OAT3 in the differentiating PT in mouse kidney. Ontogenic expression of OAT1 and OAT3 was investigated by immunohistochemical analysis. The S1, S2, and S3 segments of the PT were identified using antibodies to aquaporin 1 (AQP1), Na^+-HCO_3^- cotransporter 1 (kNBC1), and AQP4. OAT1 immunoreactivity was first detected at PT in the inner cortex of 15-day-old fetuses (F15) and in the outer cortex of 7-day old pups. OAT3 was first observed in the distal tubule of F14 and in S2 segment of the PT of F16 and in S1 and S3 segments around the time of birth; expression increased through postpartum day 21. The ontogenic pattern of expression of OAT1 and OAT3 in the differentiating PT suggests that both transporters may function in the S2 segment in the fetus, but not until after birth in S1 and S3 segments.

Key words: OAT1, OAT3, Proximal tubule, Mouse kidney, Immunohistochemistry

Introduction

In mammals, organic anion transporters (OATs) play an important role in the elimination of endogenous organic anions and clinically important xenobiotics, including antibiotics, antitumor drugs, nonsteroidal anti-inflammatory drugs, and environmental toxins (Klaassen and Lu, 2008). Several OAT isoforms, such as OAT1 (SLC22A6), OAT2 (SLC22A7), OAT3 (SLC22A8), OAT4 (SLC22A11), Oat5 (Slc22a19), and URAT1 (SLCA12) (Buist et al., 2002; Leazer and Klaassen, 2003; Youngblood and Sweet, 2004), are expressed in the kidney (Eraly et al., 2003; Anazai et al., 2006; Zhou and You, 2007). The most abundant OATs in the kidney are OAT1 and OAT3, which are both localized to the basolateral membrane of the proximal tubule (PT) (Sekine et al., 1997; Burckhardt and Burckhardt, 2003; Dantzler and Wright, 2003; Wright and Dantzler, 2004; Bahn et al., 2005). Secretion of organic anionic compounds through PT cells is achieved via unidirectional transcellular transport, which involves the uptake of organic anion compounds from the blood across the basolateral membrane, and subsequent exit across the brush-border membrane into the luminal fluid (Wolff et al., 1997; Ullrich, 1997; Burckhardt and Wolff, 2000; Sweet et al., 2003).

OATs likely play important roles in the maintenance of fetal homeostasis and the transition from fetal to neonatal life (St-Pierre et al., 2004). Before birth, the major mechanism of excretion is transplacental transfer from fetal to maternal blood, followed by excretion into the maternal urine. After birth, the major route of excretion of potentially toxic organic anions is through the urine (Wood et al., 2005).

The ability of the kidney to secrete organic acids before birth is low, and increases gradually after birth (Horster and Lewy, 1970; Kleinman and Lubbe, 1972; Stopp et al., 1978; Elbourne et al., 1990). Investigations of the expression of mouse OAT1 and OAT3 in the developing kidney have been limited to analysis of mRNA levels using Northern blot and *in situ* hybridization (Brady et al., 1999; Pavlova et al., 2000; Sweet et al., 2001, 2006; Eraly et al., 2003). In the developing rat kidney, OAT1 and OAT3 mRNA expression in the renal cortex is low before birth and increases dramatically after birth (Nakajima et al., 2000; Buist et al., 2002). Although embryonic expression of mouse OAT mRNA is well documented (Brady et al., 1999; Pavlova et al., 2000; Sweet et al., 2001, 2006), no

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data exist regarding the expression of OAT1 and OAT3 proteins in the developing mouse kidney. Elucidation of the ontogeny of organic anion secretion is central to understanding how anionic drugs are handled by the fetus and neonate compared with adults.

The aim of this study was to determine precise spatial and temporal expression of OAT1 and OAT3 proteins in the differentiating PT of mouse kidney using immunohistochemistry with segment-specific antibodies for the PT.

Materials and methods

Animals and tissue preparation

C57BL/6 mice were used in all experiments. Animal care and experimental procedures were performed under approval of the Animal Care Committees of The Catholic University of Korea. Prenatal kidneys were obtained from 13-, 14-, 15-, 16- to 18-day-old fetuses (F13-F18), and postnatal kidneys were obtained from 1-, 4-, 7-, 14- to 21-day-old pups (P1-P21) and male adults (8-10 weeks). The kidneys of prenatal animals were preserved by immersion fixation and those of postnatal animals were preserved by *in vivo* perfusion through the heart. The kidneys were briefly perfused with phosphate-buffered saline (PBS) at an osmolality of 298 mOsm/kg H_2O (pH 7.4) to remove all blood, followed by perfusion with a periodate-lysine-2% paraformaldehyde (PLP) solution for 10 min. After perfusion, the kidneys were removed and sliced into sections (1-2 mm thick), which were further fixed by immersion in the same fixative overnight at 4°C.

Primary antibodies

OAT1 and OAT3 expression was detected using affinity-purified rabbit polyclonal OAT1 and OAT3 antibodies (kindly provided by Dr. H. Endou, Kyorin University, Japan), which have been characterized in detail in previous studies (Nakajima et al., 2000). Rabbit polyclonal antibody directed against AQP1 (Chemicon Inc., Temecula, CA, USA) was used as a marker for S1, S2 and S3 segments of the proximal tubule (Sabolić et al., 1992; Kim et al., 1999). A rabbit polyclonal antibody

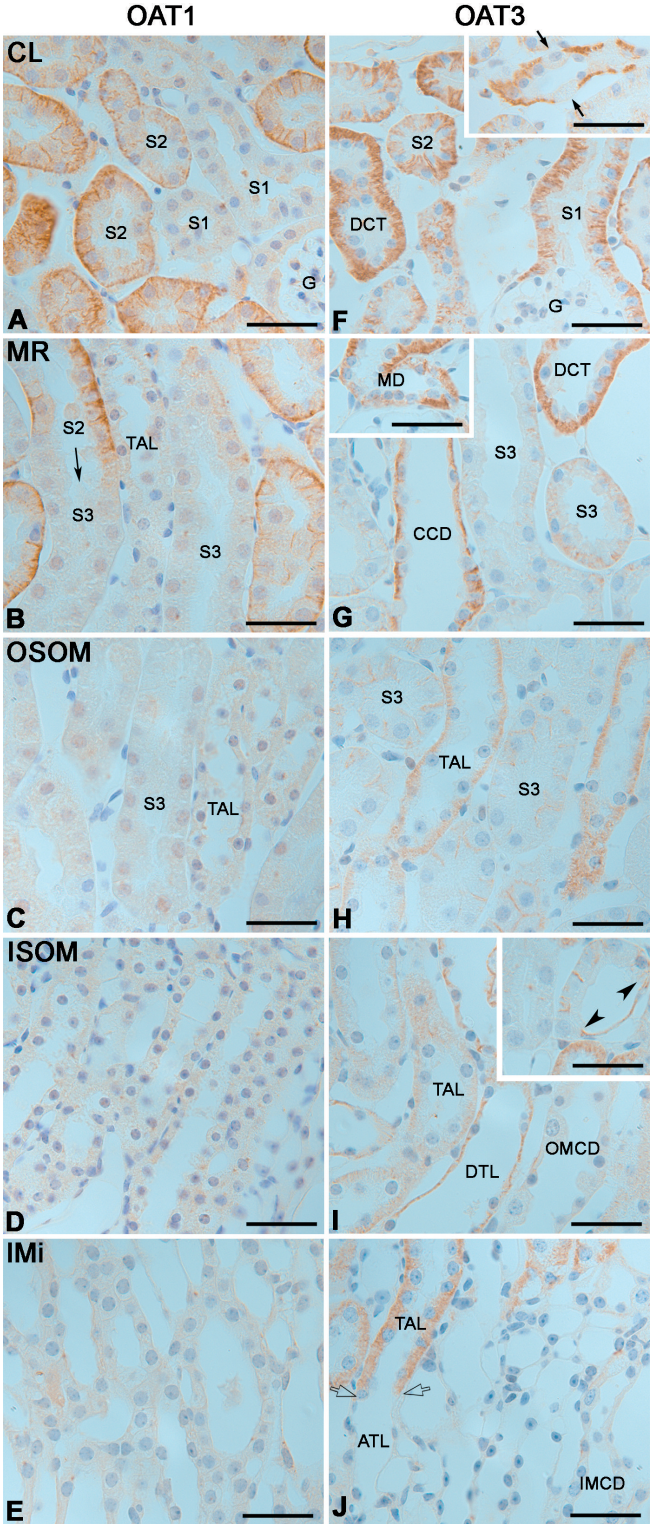

Fig. 1. Light micrographs illustrating immunostaining for OAT1 **(A-E)** and OAT3 **(F-J)** in 4 µm thick wax sections of adult mouse kidney. **A-E.** OAT1 immunoreactivity was observed at the basolateral membrane of the S2 segment but not in the S1 and S3 segments. **F-J.** OAT3 was expressed in the S1 and S2 segments, and weakly in the S3 segment of the proximal tubule. OAT3 was also expressed in the several parts of nephron. Arrows in the inset of F indicate the OAT3-negative intercalated cells. Arrowheads in the inset of I indicate the transitional zone of the S3 segment and the descending thin limb (DTL). Open arrows in J indicate the junction between the thick ascending limb (TAL) and ascending thin limb (ATL). CL, cortical labyrinth; MR, medullary ray; OSOM, outer stripe of the outer medulla; ISOM, inner stripe of the outer medulla; IMi, initial part of inner medulla; DCT, distal convoluted tubule; CCD, cortical collecting duct; OMCD, outer medullary collecting duct; MD, macula densa; IMCD, inner medullary collecting duct. Scale bars: 30 µm.

directed against AQP4 (Chemicon Inc) was used as a marker for S3 segments of the proximal tubule (Kim et al., 2001), a rabbit polyclonal antibody directed against kNBC1 (Chemicon Inc) was used as a marker for S1 and S2 segments of the proximal tubule (Roussa et al., 2004; Endo et al., 2006). An antibody against Na^+-K^+-ATPase α1 (Upstate Biotechnology, Lake Placid, NY, USA) was used to identify the distal tubule (Sabolić et al., 1999).

Post-embedding immunoperoxidase methods for single immunolabeling

After fixation, kidney was embedded in wax and cut transversely at a thickness of 4 µm using a microtome. Tissue sections were hydrated with graded ethanol and rinsed in tap water. The sections were rinsed with PBS, incubated in normal donkey serum for 1 hour, and subsequently incubated overnight with antibody against OAT1 and OAT3 (1:3,000) at 4°C. The sections were rinsed with PBS and incubated for 2 hours in peroxidase-conjugated donkey anti-rabbit IgG, Fab fragment, and washed again with 0.05M Tris buffer (pH 7.6). For detection of OAT1 and OAT3, the sections were treated with 0.05% 3,3'-diaminobenzidine and 0.01% H_2O_2 mixture. The sections were washed with distilled water, dehydrated with graded ethanol and xylene, mounted in Canada balsam, and examined by light microscopy.

Pre-embedding immunoperoxidase methods for single immunolabeling

After fixation, tissue sections were cut transversely through the entire kidney at a thickness of 50 µm using a vibratome (Pelco 102, Series 1000; Technical Products International, St. Louis, MO, USA). Sections were processed by immunohistochemistry using an indirect immunoperoxidase method. All sections were washed three times in PBS containing 50 mM NH_4Cl for 15 min. Prior to incubation with the primary antibodies, the sections were pretreated with a graded series of ethanol, and then incubated for 4 h with solution A (PBS containing 1% bovine serum albumin (BSA), 0.05% saponin, and 0.2% gelatin). The tissue sections were incubated overnight at 4°C with antibodies directed against OAT1 and OAT3 (1:3000), AQP4 (1:100) and kNBC1 (1:50) diluted in solution A. After several washes in solution B (PBS containing 0.1% BSA, 0.05% saponin, and 0.2% gelatin), the tissue sections were incubated for 2 h in horseradish peroxidase-conjugated donkey anti-rabbit IgG Fab fragment (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) diluted 1:100 in solution C (PBS containing 1% BSA). The samples were then rinsed in solution B, and then in 0.05 M Tris buffer (pH 7.6). To detect horseradish peroxidase, the sections were incubated in 0.1% 3,3'-diaminobenzidine (DAB) in 0.05 M Tris buffer for 5 min; H_2O_2 was then added to a final concentration of 0.01% and the incubation was continued for 10 min. The sections were washed three times with 0.05 M Tris buffer, dehydrated with a graded series of ethanol, and embedded in Poly/Bed 812 resin (Polysciences, Warrington, CA, USA). All samples were examined with a light microscope.

Immunofluorescence double labeling analysis

The 50 µm vibratome sections were washed three

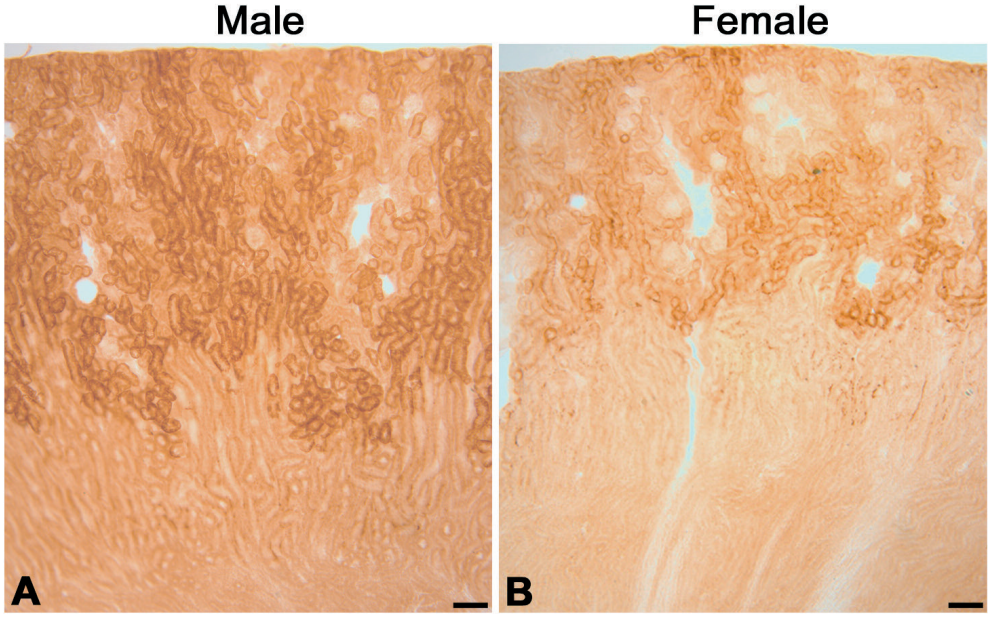

Fig. 2. Light micrographs illustrating immunostaining of OAT1 in 50 µm thick vibratome sections of the male **(A)** and female **(B)** adult kidney. OAT1 immunoreactivity in the male adult kidney is stronger than in the female adult kidney. Scale bars: 10 µm.

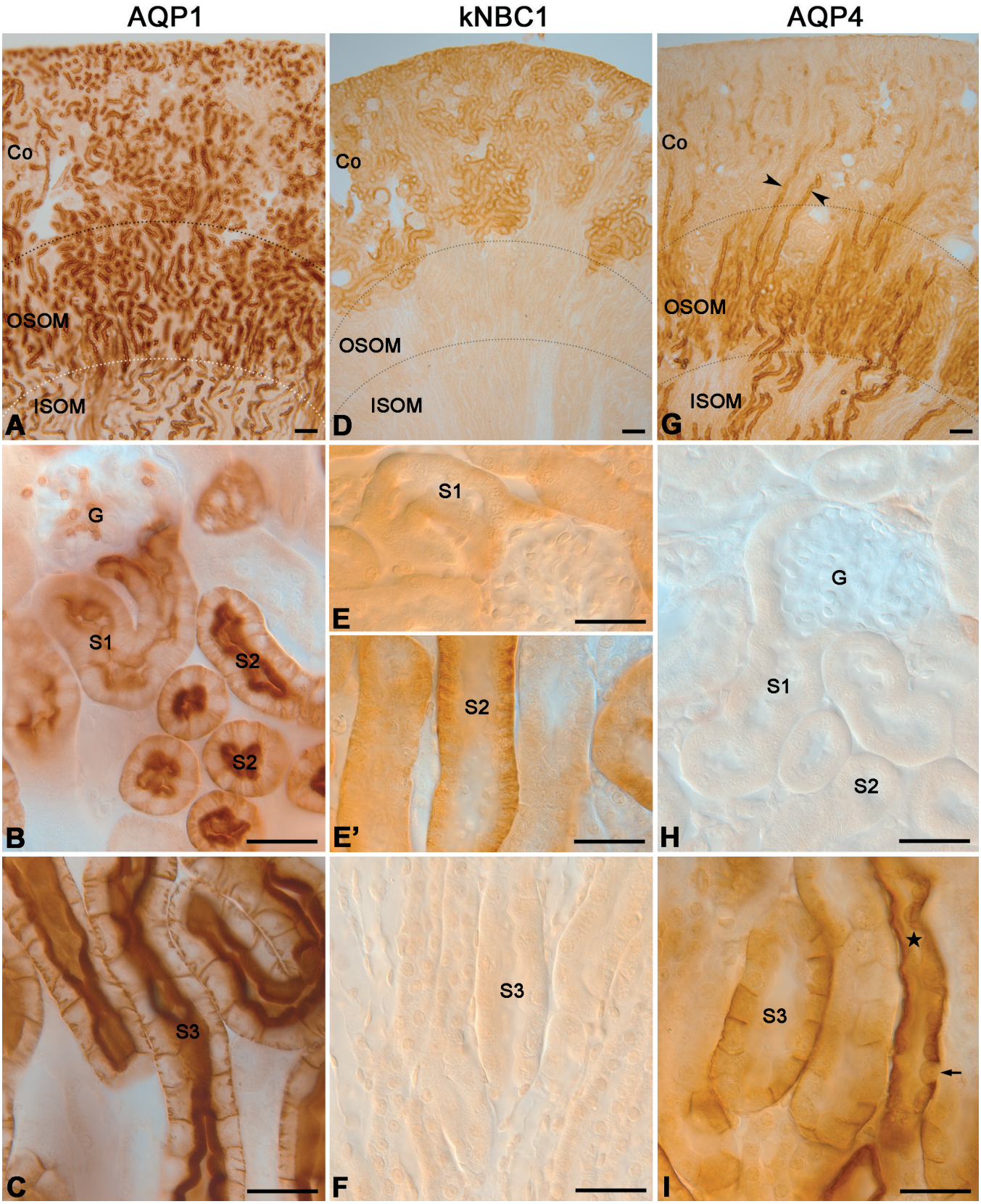

Fig. 3. Light micrographs illustrating immunostaining for AQP1 (**A-C**), kNBC1 (**D-F**), and AQP4 (**G-I**) in 50 µm thick vibratome sections of adult male kidney. **A-C.** AQP1 was expressed on the apical and basolateral membrane in all segments of the proximal tubule. **D-F.** kNBC1 was expressed on the basolateral membrane in the S1 and S2 segments but not in the S3 segment. **G-I.** AQP4 was expressed in the S3 segment but not in the S1 and S2 segments of the proximal tubule. Arrowheads in G indicate the AQP4-positive cortical collecting duct. The star in I indicates the AQP4-positive outer medullary collecting duct. The arrow indicates an AQP4-negative intercalated cell. Co, cortex; OSOM, outer stripe of the outer medulla; ISOM, inner stripe of the outer medulla; G, glomerulus. Scale bars: A, D, G, 0.1 mm; B, C, E, E', F, H, I, 30 µm.

times for 5 min each in phosphate buffer (PB), followed by 10 min incubation with MeOH Triton X (MeOH 10%, 10% TrX 10%, PB 71%, H_2O_2 9%). The sections were incubated in 5% normal donkey serum and 1% Triton X-100 in PBS (PBST) for 1 h at room temperature, and incubated overnight with rabbit polyclonal antibodies to OAT1 and OAT3 diluted in PBS containing 0.5% Triton X-100 (PBST-0.5%) at 4°C. The sections were then rinsed for 30 min with PBST and incubated with appropriate antibodies conjugated with Cy3 (1:700; Jackson ImmunoResearch) diluted in PBST-0.5% at 4°C for 2 h. For double labeling, the sections were then rinsed for 30 min and incubated in 10% normal donkey serum for 1 h at room temperature, followed by overnight incubation with rabbit polyclonal antibodies to AQP1, kNBC1 and AQP4 diluted in PBST-0.5% at 4°C. The sections were then rinsed for 30 min with PBST and incubated with appropriate secondary antibodies conjugated with Alexa Fluor 488 (1:800; Jackson ImmunoResearch) diluted in PBST-0.5% at 4°C for 2 h. Sections were viewed using a confocal microscope (Axioimager M1, ZEISS, Germany); captured images were converted to JPG format, and the image contrast levels were adjusted using Adobe Photoshop v. 7.0 (Adobe Systems, Inc., San Jose, CA).

Results

Expression of OAT1 and OAT3 in adult mouse kidney

OAT1

Light microscopic immunohistochemistry showed intense expression of OAT1 on the basolateral plasma membrane in the S2 segment of the PT (Fig. 1A,B). However, no OAT1 immunostaining was observed in the S1 (Fig. 1A) or S3 (Fig. 1B,C) segment of the PT. Furthermore, no OAT1 immunoreactivity was observed in the outer and inner medulla (Fig. 1D,E).

OAT3

OAT3 expression was detected in the basolateral plasma membrane in the PT cells (Fig. 1F-H). Immunoreactivity was also seen on the basolateral plasma membrane in distal convoluted tubule cells (Fig. 1F), connecting tubule cells (Fig. 1F), principal cells of the cortical collecting duct (Fig. 1G), thick ascending limb cells (Fig. 1H-J), descending thin limb cells (Fig. 1I), and outer medullary collecting duct (Fig. 1I). OAT3 was not expressed in the intercalated cells (Fig. 1F inset), the macula densa (Fig. 1G inset), the ascending thin limb cells (Fig. 1J) and inner medullary collecting ducts (Fig. 1J). In the PT, the intensity of OAT3 immunoreactivity was strongest in the S2 segment and weakest in the S3 segment (Fig. 1F-H). However, immunoreactivity for OAT3 was weaker in the PT than in the distal convoluted tubule, thick ascending limb and connecting tubule.

We observed gender-specific differences in expression of OAT1 in the adult kidney (Fig. 2). As in previous reports, the immunoreactivity of adult male was stronger than that of female (Ljubojevic et al., 2004).

AQP1, kNBC1 and AQP4

In the PT of adult male mice, AQP1 was expressed on the apical and basolateral membrane of all segments of the proximal tubule at various levels (S1 < S2 < S3) (Fig. 3A-C) as previously reported (Sabolić et al., 1992; Kim et al., 1999). kNBC1 was expressed in the cortex but not in the medullary ray, the outer stripe of the outer medulla, or the inner stripe of the outer medulla. In the cortex, kNBC1 was expressed on the basolateral membrane of the S1 and S2 segments but not in the S3 segment (Fig. 3D-F) as previously reported (Roussa et al., 2004; Endo et al., 2006), whereas AQP4 was expressed only in the S3 segment (Fig. 3G-I) as previously reported (Kim et al., 2001).

The localization patterns of OAT1, OAT3, AQP1, kNBC1, and AQP4 in the adult mouse kidney, as found in this study, are summarized in Figure 4.

Expression of OAT1 and OAT3 in developing mouse kidney

OAT1

We did not detect any expression of OAT1 in the fetal kidneys of embryonic day 13 (F13) and F14 mice (data not shown). On gestational day 15, OAT1 staining first appeared on the basolateral membrane of the PT in the inner cortex (Fig. 5A). The level of OAT1 expression increased continuously during development (Fig. 5B-E). OAT1 staining was not detected in the outer cortex until postnatal day 7 (P7) (Fig. 5D). On P21, the localization of OAT1 was nearly identical to that in the adult kidney (Fig. 5E).

OAT3

No immunoreactivity for OAT3 was observed in the kidneys of F13 mice (data not shown). OAT3 staining appeared first in the DT on F14 (Fig. 5F) and in the PT

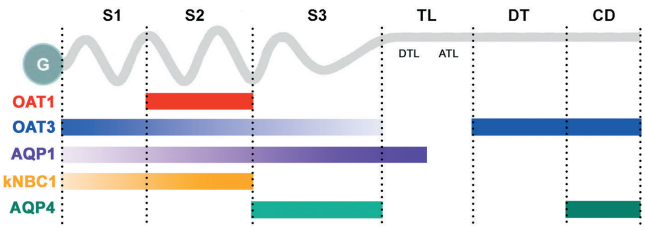

Fig. 4. A diagram summarizing the distribution of various transporters expressed in proximal tubule in adult mouse kidneys based on our results in this study. S1, S1 part of proximal tubule; S2, S2 part of proximal tubule; S3, S3 part of proximal tubule; TL, thin limb; DT, distal tubule; CD, collecting duct.

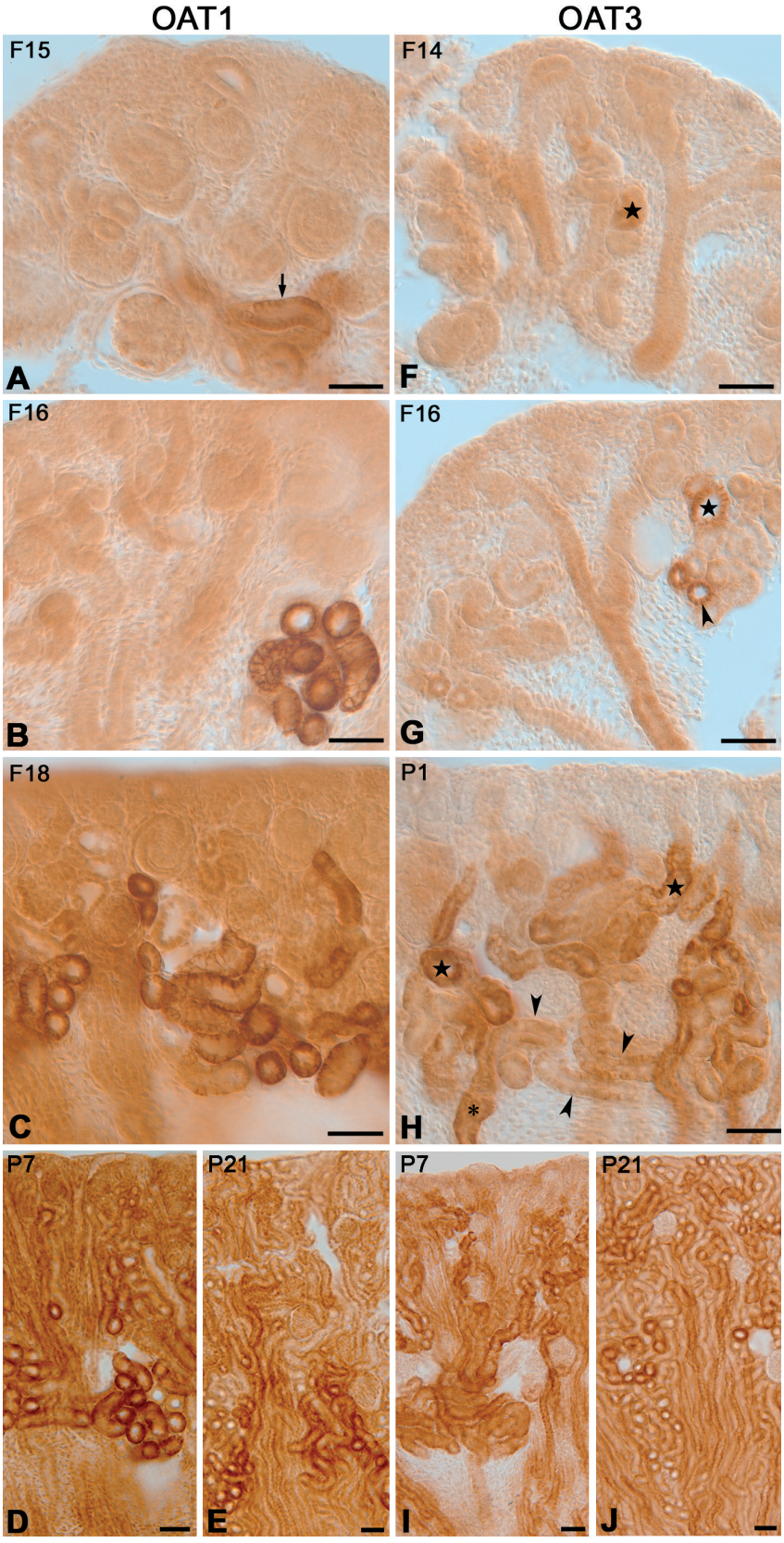

Fig. 5. Light micrographs illustrating immunostaining of OAT1 **(A-E)** and OAT3 **(F-J)** in 50 µm thick vibratome sections of the developing kidney. **A.** OAT1 immunoreactivity was first detected in the middle part of the proximal tubule on gestational day 15 (arrow). **B-E.** OAT1 immunolabeling increased during development. **F.** OAT3 appeared first in the distal anlage (star) on gestational day 14. **G.** OAT3-positive proximal tubules are also observed on gestational day 16 (arrowhead). **H.** After birth, OAT3 was expressed in all segments of the proximal tubule in the inner cortex (arrowheads) and distal tubule (stars) and thick ascending limb (asterisk). **I-J.** OAT3 immunolabeling increased during development. Scale bars: 50 µm.

on F16 (Fig. 5G). OAT3 first appeared distinctly on the basolateral plasma membrane of the PT cells in the S2 segment on F16, and in S1 and S3 segments around the time of birth (Fig. 5H). OAT3 staining was not detected in the outer cortex until P7 (Fig. 5I). On P14 and P21 (Fig. 5J), the localization of OAT3 was nearly identical to that in the adult kidney.

In postnatal kidney (on P7), we could not observe any gender-specific differences in expression of OAT1 (Fig. 6).

Multiple immunofluorescence analysis for localization of OAT1 and OAT3

Immunofluorescence staining with anti-AQP1, anti-kNBC1, and anti-AQP4 antibodies of kidney sections was also performed to confirm the localization of OAT1 and OAT3 in tubule segments in developing mouse kidneys.

The expressions of OAT1 and OAT3 in the developing mouse kidney are shown in figures 7-9. In the F14 fetal kidney, no OAT1 was expressed (Figs. 7A-C, 8A-C). OAT1 was present in the S2 segment, but not in the S1 (Fig. 7D-F) and S3 segments (Fig. 7G-I) of the PT in the fetal kidney. OAT1 immunoreactivity was clearly detected on the kNBC1-positive PTs in the fetal kidney (Fig. 8D-F). OAT3 was not expressed on F14 (Fig. 9A-C). In the fetal kidney on F16 and F18, OAT3 was expressed on the basolateral plasma membrane of both the AQP1-positive PT and the AQP1-negative distal tubule (Fig. 9D-I). However, the fluorescence intensity for OAT3 was higher in the distal tubule than in the PT.

Discussion

Our result is the first study to identify the temporal and spatial expression patterns of OAT1 and OAT3 proteins in the differentiating PT of the mouse kidney, including during fetal stages.

Our results showed that in the PT of the adult mouse kidney, OAT1 is localized only on the basolateral plasma membrane of the S2 segment, whereas OAT3 is distributed in all segments but with variable intensity (S2 > S1 > S3) (Shimomura et al., 1981). In the differentiating PT, OAT1 was first detected on F15, whereas OAT3 immunoreactivity was observed in the S2 segment on F16 and in the S1 and S3 segments around the time of birth. The expression level increased through P21.

Endou and colleagues cloned this OAT1 and later used diaminobenzidine staining of the rat kidney to show OAT1 expression only in the S2 segment (Tojo et al., 1999). However, the use of immunofluorescence revealed OAT1 expression in two or all three segments (Kojima et al., 2002; Bahn et al., 2005). This difference was attributed to the greater sensitivity of the immunofluorescence technique compared with diaminobenzidine staining. We observed OAT1 expression only in the S2 segment but not in the S1 and S3 segments in the adult and developing mouse kidney using three different immunohistochemical techniques: pre- and post-embedding immunoperoxidase, and confocal immunofluorescence methods. Taken together, these results suggest that OAT1 is expressed only on the basolateral membrane of the S2 segment of the PT cells in the mouse kidney.

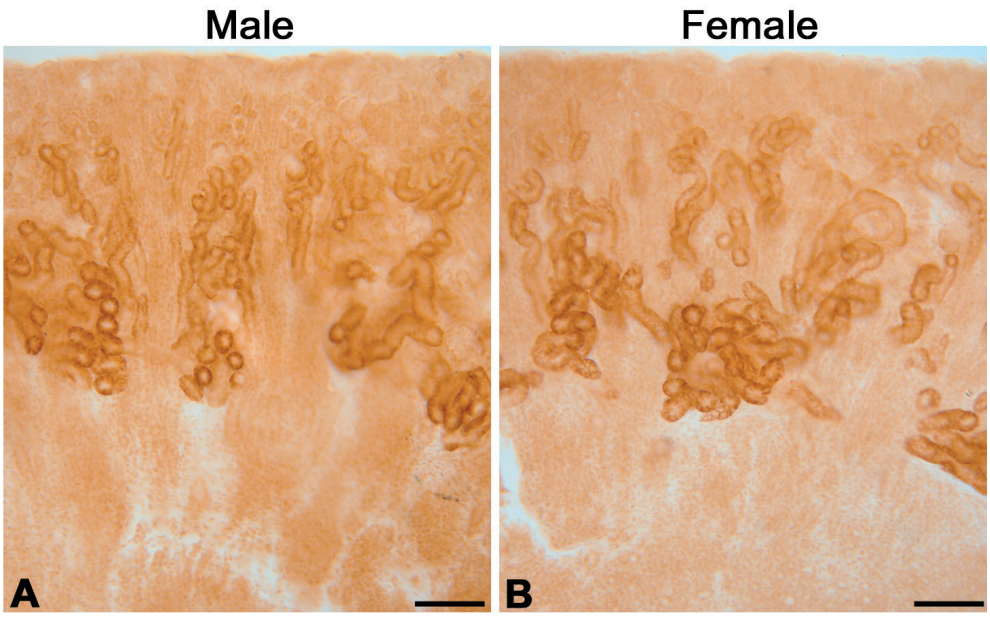

Fig. 6. Light micrographs illustrating immunostaining of OAT1 in 50 µm thick vibratome sections of the male **(A)** and female **(B)** from 7-day pups. OAT1 immunoreactivity was similar between male and female kidneys from postnatal 7-day pups. Scale bars: 10 µm.

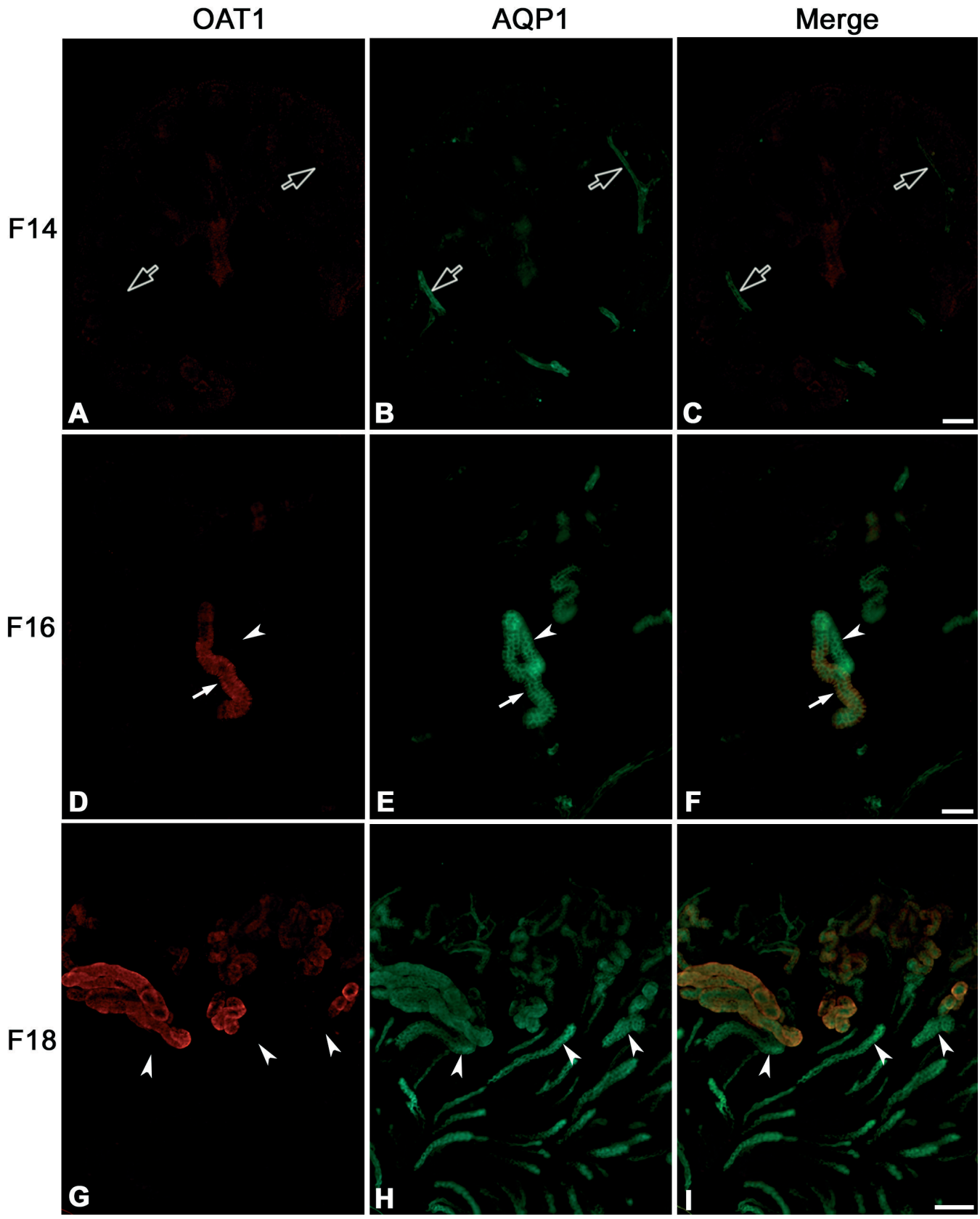

Fig. 7. Double immunofluorescence staining of OAT1 (red) and AQP1 (green) in 50 µm thick vibratome sections of the developing mouse kidneys. **A-C.** On gestational day 14, no OAT1 immunoreactivity was observed, but AQP1 was detected in the arcuate artery (open arrows). **D-F.** On gestational day 16, OAT1 was expressed in the differentiating AQP1-positive proximal tubules (arrows). Arrowheads indicate OAT1-negative and AQP1-positive S1 segment. **G-I.** No OAT1 was observed in the AQP1-positive differentiating descending limb including the S3 segment of the proximal tubule (arrowheads). Scale bars: 0.1 mm.

OAT3 was isolated from the rat (Burckhardt and Burckhardt, 2003) and first detected in the mouse kidney by Brady et al. (1999). Consistent with the results of Kojima et al. (2002) and Bahn et al. (2005), our results show localization of OAT3 on the basolateral plasma membrane of the S1, S2, and S3 segments of the PT and expression in the distal tubule, including the thick ascending limb and the cortical collecting duct.

We found a difference in the segmental localization of OAT1 and OAT3 in the PT in the adult mouse kidney; this finding suggests that OAT1 and OAT3 differ in functional contributions to the basolateral uptake of organic anions. Both OAT1 and OAT3 are the organic anion-dicarboxylate exchangers (Sweet et al., 2003). However, the transport modes of OAT1 and OAT3 are distinct. OAT1 mediates high-affinity transport of para-aminohippurate (PAH) (Sekine et al., 1997; Race et al, 1999), whereas OAT3 mediates low-affinity transport (Race et al., 1999). OAT3 shows high affinity for many substances, such as ß-actin antibiotics and sulfate and glucuronide conjugates (Ohtsuki et al., 2004).

OAT1 mRNA was first detected on F14 in the mouse kidney (Brady et al., 1999; Sweet et al., 2006) and on F18 in the rat kidney (Nakajima et al., 2000). The expression of OAT1 protein was detected on F20 by Western blot analysis but was not observed by immunohistochemistry in the fetal rat kidney (Nakajima et al., 2000). A faint signal for OAT1 protein was detected on P0, and the expression level increased through adulthood. In contrast, in the developing mouse kidney, we distinctly detected OAT1 protein on the basolateral plasma membrane of the S2 segment of the PT cells on F15.

Previous reports showed gender-specific differences in OAT1 mRNA and protein levels in the rat and mouse kidney (Buist et al., 2002; Buist and Klaassen, 2004; Ljubojevic et al., 2004). Consistent with this result, we observed that OAT1 immunoreactivity in the adult male kidney was stronger than that of the adult female kidney. In the developing kidney, however, OAT1 expression in male P7 was similar to that of female P7. This finding is consistent with a previous report (Buist and Klaassen, 2004), in which a significant gender-specific difference in OAT1 mRNA levels was observed in postnatal 30-days. In earlier ages, the difference of gender was not significant. We confirmed that the protein level was consistent with the mRNA level.

We observed OAT3 protein expression in the inner cortex on F14, which is consistent with previous findings of OAT3 mRNA in kidney on F14 using *in situ* hybridization and Northern blot analysis (Sweet et al., 2001, 2006). Interestingly, however, we confirmed that OAT3-positive tubules are AQP1-negative distal tubules until F15. After F16, OAT3 was detected in both the distal tubule and the AQP1-positive PT, especially in the S2 segment, and in the S1 and S3 segments after birth.

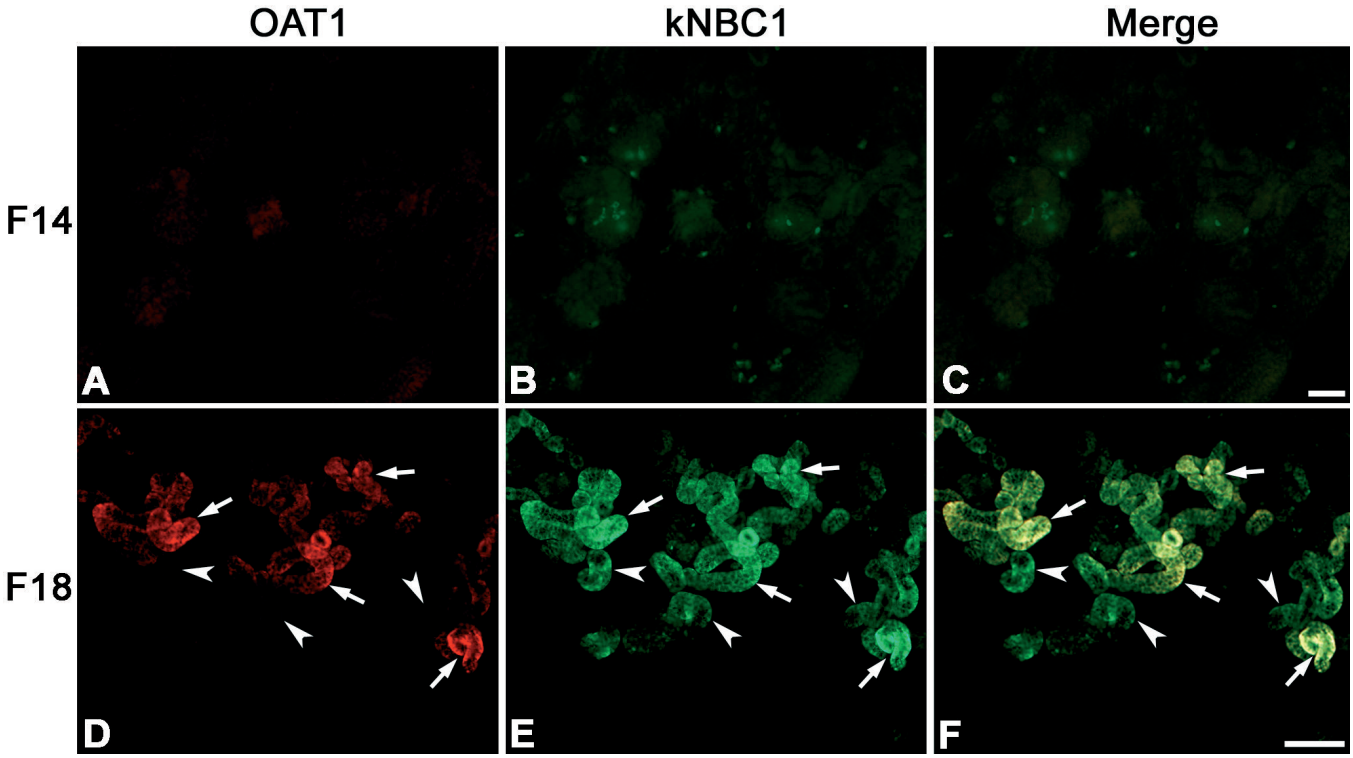

Fig. 8. Double immunofluorescence staining of OAT1 (red) and kNBC1 (green) in 50 µm thick vibratome sections of the developing mouse kidney. **A-C.** On gestational day 14, no immunoreactivity was observed. **D-F.** OAT1 was detected on gestational day 18 and colocalized with kNBC1 in the proximal tubules (arrows). Arrowheads indicate OAT1-negative and kNBC1-positive S1 segment. Scale bars: 0.1 mm.

Our data are consistent in demonstrating a marked increase in the expression of OAT1 and OAT3 after birth. These data suggest that the immature excretory capacity of the PTs of the neonatal kidney can be attributed, at least in part, to the low expression level of OAT1 and OAT3.

Organic anion transporters likely function in the maintenance of fetal homeostasis and the transition from fetal to neonatal life. After birth, the major route of excretion of potentially toxic organic anions is through the urine (Carnegie and Robertson, 1978). These functions are associated with the maturation of the capacity to transport organic anions after birth in humans (Hook and Hewitt, 1977; Hosoyamada et al., 1999) and in experimental animals such as the rabbit (Hirsch and Hook, 1970), rat (Braunlich, 1988; Buist and Klaassen,

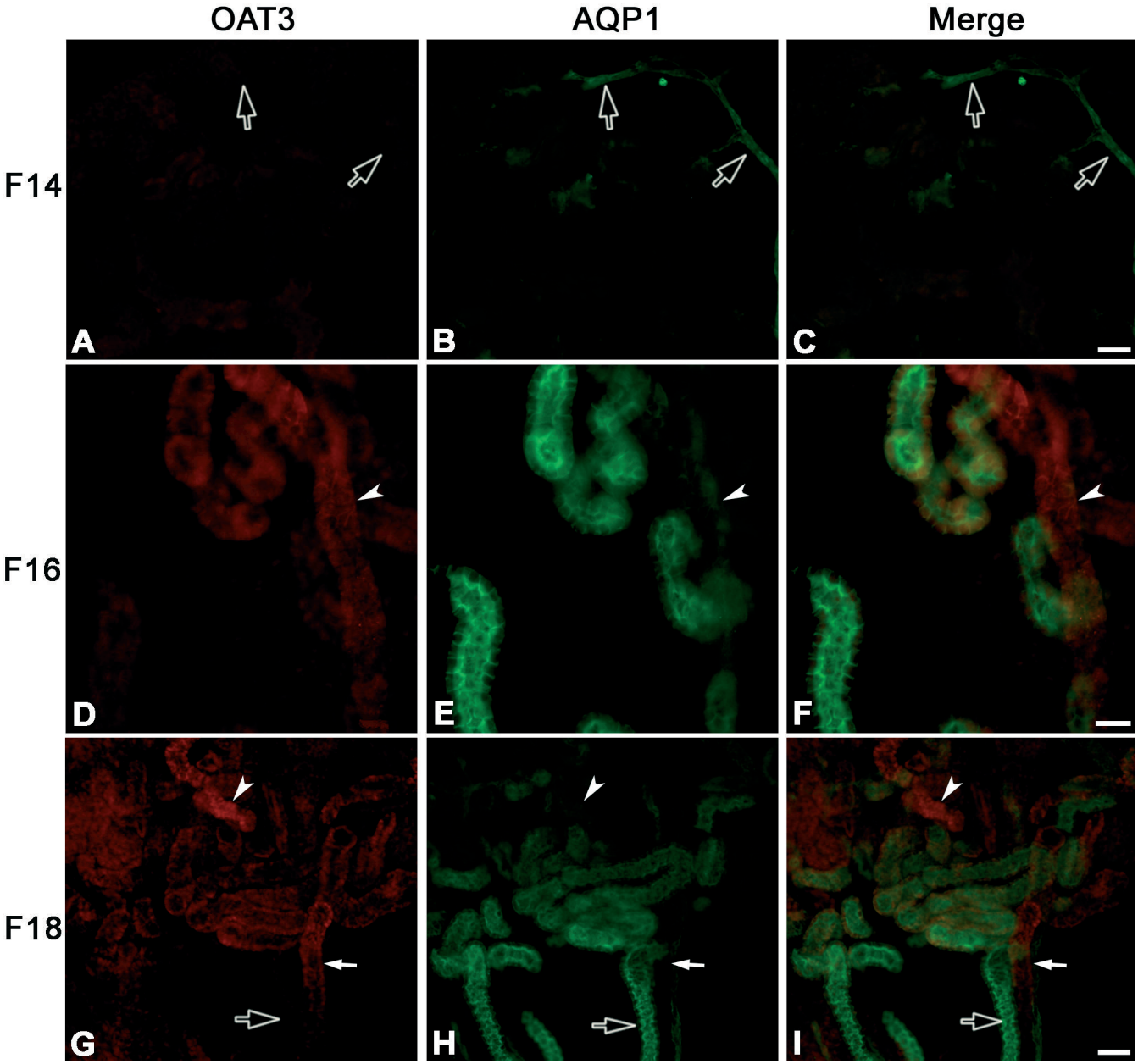

Fig. 9. Double immunofluorescence staining of OAT3 (red) and AQP1 (green) in 50 μm thick vibratome sections of the developing mouse kidney. **A-C.** On gestational day 14, there was no labeling except for the AQP1-positive developing arcuate artery (open arrows). **D-I.** OAT3 labeling was detected in the AQP1-positive proximal tubules, AQP1-negative distal convoluted tubules (arrowheads), and thick ascending limbs (arrows) on gestational day 16 and gestational day 18. Open arrows indicate the OAT3-negative/AQP1-positive differentiating descending limb. Scale bars: 0.1 mm.

2004), mouse (Pavlova et al., 2000; Buist and Klaassen 2004), and sheep (Alexandet and Nixon, 1962; Wood et al., 2005). The maturation may reflect the observation that the PT length is not fully mature at birth (Fetterman et al., 1965).

Although the PT is the major site of renal organic anion transport, little is known about its development and maturation. We observed the differentiation of PTs using antibodies to AQP1 (a marker for the S1, S2 and S3 segments), kNBC1 (a marker for the S1 and S2 segments), and AQP4 (a marker for the S3 segment). Interestingly, AQP1 and kNBC1 immunoreactivity was intense in the S2 segment on F15 but was weak in the S1 segment right after birth. In contrast, AQP4, a specific marker for the S3 segment, was faintly expressed around the time of birth and distinctly on P14. These results show the ontogenic pattern of the expression of OAT1 and OAT3, and raise the possibility that the PT matures first in the S2 segment and later in the S1 and S3 segments.

Knockout mouse models of OAT1 (Eraly et al., 2006) and OAT3 (Sweet et al., 2002) have been recently generated, and although neither knockout mouse model shows morphological changes, both exhibited a profound loss of organic anion transport. Isolated renal slices from OAT1 knockout mice demonstrate substantial loss of PAH transport and in urinary secretion. In contrast, isolated kidney slices from OAT3 knockout mice exhibit a significant loss of transport of taurocholate, estrone sulfate, and PAH, and the intact brain choroid plexus showed significant loss of transport of fluorescein. These studies provide experimental evidence for the crucial involvement of OAT1 and OAT3 in the disposition of these organic anions. Because anion transporters, including OAT1 and OAT3, are vital to the elimination of a wide variety of xenobiotics, elucidation of the specific mechanisms involved in the regulation of renal OAT expression may provide insight into the mechanisms controlling the development and maturation of the drug-handling capacity in the kidney. This information could also explain clinical differences in renal drug handling by premature infants, neonates, children, and adults.

In conclusion, the expression of both OAT1 and OAT3 proteins appears distinctly on the basolateral plasma membrane of the PT at gestational day 15, and the expression level increases around the time of birth. These results suggest that the fetal expression of both transporters coincides with the increased importance of the PT as the primary route for excretion of organic anions even in fetal mouse kidney.

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