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Tissue distribution and ontogeny of mouse organic anion transporting polypeptides (Oatps)

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List of nonstandard abbreviations: Oatps, organic anion transporting polypeptides; Pgt, prostaglandin transporter; Gst, gonad-specific transporter; PCN, pregnenalone-16α-carbonitrile; bDNA assay, branched DNA signal amplification assay.

Abstract

Organic anion transporting polypeptides (Oatps) are Na^+-independent solute carriers for cellular uptake of organic compounds. The purpose of this study is to determine: 1) the constitutive mRNA expression of the 15 mouse Oatp genes in 12 tissues, 2) whether there are gender differences in Oatp expression, and 3) the ontogenic expression of Oatps in liver and kidney. The mRNA expression of the 15 mouse Oatps was quantified using the branched DNA technique. Oatp1a1, 1a4, 1b2, and 2b1 are expressed in liver at relatively high levels, with Oatp1b2 being exclusively expressed in liver. Oatp1a1, 1a6, 3a1, and 4c1 are highly expressed in kidney. Oatp1a4 and 1c1 are highly expressed in brain. Oatp1a5, 6b1, 6c1, and 6d1 are predominant in testes. Oatp2a1, 4a1, and 5a1 are predominantly expressed in placenta. In liver, expression of Oatp1a1 was male-predominant, whereas expression of Oatp1a4 and 1a6 was female-predominant. In kidney, expression of Oatp1a1, 3a1, and 4c1 was higher in males than females. Hepatic expression of Oatp1a1, 1a4, 1a6, 1b2, and 2b1 gradually increased after birth and reached adult levels by 6 weeks of age. Only Oatp2a1 was expressed at adult levels at birth. In kidney, expression of mouse Oatp1a1, 1a6, and 3a1 was lower at birth than at 6 weeks of age, whereas, expression of mouse Oatp1a4, 2a1, and 2b1 was similar at birth and at 6 weeks of age. These data on the tissue distribution and ontogenic expression of mouse Oatps, will aid in understanding the pharmacokinetics and toxicokinetics of drugs and other chemicals.

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Introduction

Organic anion transporting polypeptides (rodents: Oatps; human: OATPs) mediate sodium-independent transport of various amphipathic organic solutes, such as bile acids, dyes (e.g. sulfobromophthalein), drugs (e.g. rifampicin, digoxin), toxins (e.g. microcystin), steroid conjugates, eicosanoids, and thyroid hormones into cells. Currently, 9 human *OATP*s, 13 rat, and 15 mouse *Oatp*s have been identified. Hydropathy analysis predicts that all OATPs/Oatps contain 12-transmembrane domains. Additionally, all OATPs/Oatps contain the consensus sequence D-X-RW-(I,V)-GAWW-X-G-(F,L)-L at the border of extracellular loop 3 and transmembrane domain 6, termed the "OATP superfamily signature" (Hagenbuch and Meier, 2003).

Only a few of the OATPs/Oatps have been characterized at the functional, structural, and regulatory levels. Rat Oatp1a1, the first cloned member of the OATP/Oatp gene family, is expressed in liver and kidney, with broad substrate specificity (Jacquemin et al, 1994). Rodent Oatp1b2 and human OATP1B1 and 1B3 are selectively expressed in liver, where they are involved in hepatic uptake of albumin-bound compounds (Abe et al, 1999; Konig et al, 2000; Li et al, 2002). Oatp1a6, 1c1, and 4a1 are expressed mainly in kidney, brain, and placenta, respectively, and their physiological roles remain unclear. The recently cloned human and rat OATP4C1/Oatp4c1 transports digoxin across the basolateral membrane of renal proximal tubules (Mikkaichi et al, 2004). Recently, rat Oatp6b1, 6d1, and human OATP6A1 were cloned (Suzuki *et al.*, 2003). These three transporters are predominantly expressed in testes and transport dehydroepiandrosterone (DHEA) and DHEA sulfate into testes (Suzuki *et al.*, 2003).

Neonates are more sensitive to the toxicity of some chemicals than adults. This is an important factor to consider in drug therapy, and in chemical exposure. The increased

susceptibility of immature rodents to some chemical exposures was first investigated in the late 1950s, and reduced hepatic biotransformation of some chemicals was shown to be responsible for the increased susceptibility (Jondorf *et al*., 1958; Fouts and Adamson, 1959). However, for a number of chemicals, it has been shown that newborns have a decreased ability to clear chemicals from blood. For example, ouabain is transported into hepatocytes by active transport and is excreted into bile largely without being biotransformed (Eaton and Klaassen, 1978). Newborn rodents are more sensitive to cardiac glycoside toxicity because of their low capacity to remove ouabain from the blood (Klaassen, 1972). In addition, Stacey and Klaassen (1979) showed that ouabain uptake into hepatocytes in newborns is low, and increases with age. Liver and kidney are two major detoxication organs that eliminate chemicals from the body. Developmental changes of transporters in both organs can significantly influence the disposition of endo- and exogenous compounds.

Mice are becoming a more commonly used experimental animal model because of the availability of knockout mice. However, information on transporter systems, including Oatps, in mice is poorly characterized. Therefore, the purpose of this study is to determine: 1) the constitutive expression of the 15 mouse Oatp genes by measuring their mRNA levels in 12 tissues, 2) whether there are gender differences in mouse Oatp expression, and 3) the ontogeny of mouse Oatps in mouse liver and kidney.

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Materials and Methods

Tissue Distribution. Eight-week-old adult male and female C57BL/6 mice (*n* = 10/gender) were purchased from Jackson Laboratories (Bar Harbor, Maine), and housed according to the American Animal Association Laboratory Animal Care guidance. Eleven tissues (liver, kidney, lung, stomach, duodenum, jejunum, ileum, large intestine, brain, testis, and ovary) were collected. Placenta was removed from pregnant mice on gestation day 17. The tissues were snap-frozen in liquid nitrogen. The intestine was longitudinally dissected, rinsed in saline, and divided into three equal-length sections (referred to as duodenum, jejunum, and ileum), before being snap-frozen in liquid nitrogen. All tissues were stored at -80°C.

Ontogeny. Mice were bred in the animal facilities at the University of Kansas Medical Center. Liver and kidney from male and female C57BL/6 mice were collected at -2, 0, 5, 10, 15, 22, 30, 35, 40, and 45 days of age (*n* = 5/gender/age).

RNA Isolation. Total RNA was isolated using RNAzol Bee reagent (TelTest Inc., Friendswood, TX) as per the manufacturer's protocol. The concentration of total RNA in each sample was quantified spectrophotometrically at 260 nm. The integrity of each RNA sample was evaluated by formaldehyde-agarose gel electrophoresis before analysis.

Branched DNA (bDNA) Signal Amplification Assay. The bDNA technique is a robust, high-throughput method used for characterizing mRNA expression. The mRNA for Oatp1a1, 1a4, 1a5, 1a6, 1b2, 1c1, 2a1, 2b1, 3a1, 4a1, 4c1, 5a1, 6b1, 6c1, and 6d1 was measured using the bDNA assay (Quantigene® bDNA signal amplification kit; Bayer Diagnostics, East Walpole, MA), with modifications according to Hartley and Klaassen (2000). The gene sequences of mouse Oatps were accessed from GenBank (Table 1). Multiple oligonucleotide probe sets (containing capture probes, label probes, and blocker probes) specific to a single mRNA

transcript were designed using ProbeDesigner® software, version 1.0 (Bayer Corp., Emeryville, CA). Probe sets for each Oatp are shown in Table 2. Each probe developed in ProbeDesigner was submitted to the National Center for Biotechnology Information for nucleotide comparison by the basic local alignment search tool (BLASTn; NCBI, Bethesda, MD) to ensure minimal cross-reactivity with other known mouse sequences and expressed sequence tags. Oligonucleotides with a high degree of similarity (>80%) to other mouse gene transcripts were eliminated from the design. Probes were designed with a melting temperature of approximately 63°C, enabling hybridization conditions to be held constant (i.e., 53°C) during each hybridization step, and for each probe set. All probes were synthesized (i.e., 50 nmol synthesis scale) by Operon Technologies (Palo Alto, CA), and obtained desalted and lyophilized. Total RNA (1 µg/µl) was added to each well (10 µl/well) of a 96-well plate containing 50 µl of capture hybridization buffer and 50 µl of diluted probe set. For each gene, total RNA was allowed to hybridize to the probe set overnight at 53°C. Subsequent hybridization steps were carried out per the manufacturer's protocol, and luminescence was measured with a Quantiplex® 320 bDNA luminometer interfaced with Quantiplex® data management software (version 5.02), for analysis of luminescence from 96-well plates. The luminescence for each well is reported as relative light units per 10 µg of total RNA.

Statistics. Differences between genders were determined by student's T test. Statistical significance was considered at $p < 0.05$.

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Results

Tissue Distribution of mouse Oatps. mRNA expression of 15 mouse Oatps was quantified in 12 major tissues. Data are shown in Fig. 1 to 4. Expression of Oatp1a1 mRNA (Fig. 1) was highest in liver and kidney of male mice, but its expression in other tissues was much lower. A gender difference in Oatp1a1 mRNA expression was observed in both liver and kidney, with higher levels in males. Expression of Oatp1a4 mRNA (Fig. 1) was highest in liver and brain, followed by testes and ovaries. A gender difference in Oatp1a4 expression in liver was observed, with higher levels being expressed in females. Expression of Oatp1a5 mRNA (Fig. 1) was highest in testes, moderate in ovary, and low in the other tissues. Oatp1a6 mRNA (Fig. 1) was almost exclusively expressed in kidney among the collected tissues. Oatp1a6 mRNA was expressed much less in liver than kidney. However, a gender difference of Oatp1a6 was noted in liver, with higher levels in females.

Expression of Oatp1b2 mRNA (Fig. 2) was predominant in liver, with negligible expression in the other 11 tissues. Oatp1c1 (Fig. 2) was the only Oatp primarily expressed in brain. Oatp2a1, a prostaglandin transporter, was highest in placenta, moderate in lung and stomach, and lower in other tissues (Fig. 2). Oatp2b1 (Fig. 2) appeared to be ubiquitously expressed, with highest levels in liver and small intestine.

Oatp3a1 (Fig. 3) expression was highest in kidney and lung, with moderate expression in testes and ovaries. In kidney, Oatp3a1 mRNA was 3.3-fold higher in males than that in females, as previously noted (Melia et al, 1998). Also shown in Fig. 3, mouse Oatp4a1, like rat Oatp4a1, was abundantly expressed only in placenta (Leazer and Klaassen, 2003). Oatp4c1 (Fig. 3) was mainly found in lung and kidney. A gender difference in renal expression of Oatp4c1 was observed, with higher levels in males than females. Oatp5a1 (Fig. 3) expression was

predominant in placenta, with lower levels in testes and lung. Oatp6b1, 6c1, and 6d1 (Fig. 4), three mouse homologues of the human gonad-specific transporter (GST), were exclusively expressed in testes.

Ontogeny of Oatps in male and female mouse liver. The neonatal patterns of Oatp mRNA expression in male and female mouse liver are shown in Figs. 5 and 6. In adult mice, Oatp1a1 expression was mainly detected in liver and kidney, with higher levels in males than females. As shown in Fig. 5, there was minimal expression of Oatp1a1 in mouse liver before 15 days of age. Thereafter, hepatic Oatp1a1 levels reached detectable levels at day 23, and reached adult levels by 30 days of age. Male-predominant expression was observed at all ages when Oatp1a1 was detectable (day 23 and thereafter). Fig. 5 illustrates that the expression of Oatp1a4 was also low at birth, but gradually increased to adult levels by approximately 23 days of age. The levels of Oatp1a4 mRNA in males decreased by 30 days of age, resulting in females expressing more Oatp1a4 than males. The ontogeny of Oatp1a6 expression is also shown in Fig. 5. Expression of Oatp1a6 mRNA was detected in liver of mice of all ages. Between day 5 and 10 there was an increase of Oatp1a6 mRNA. Similar to that observed for Oatp1a4, Oatp1a6 decreased in males between 15 and 45 days of age, resulting in higher Oatp1a6 expression in liver of adult females than males.

Oatp1b2 is a relatively liver-specific transporter, as indicated in Fig. 2. The expression of Oatp1b2 was minimal 2 days before birth, but its expression between birth and 10 days of age was about half that seen in adult mice (Fig. 6). Adult levels of Oatp1b2 were attained by 23 days of age. No gender difference in expression of Oatp1b2 was observed at any age. Oatp2a1 (Pgt) expression in liver was similar at all ages. Oatp2b1 expression at different ages is also shown in

Fig. 6. Before 15 days of age, Oatp2b1 expression was minimal, increased moderately at day 15, and reached adult levels by 23 days of age.

Ontogeny of Oatps in male and female mouse kidney. The postnatal developmental patterns of Oatp mRNA expression in male and female mouse kidney are shown in Figs. 7 and 8. As shown in Fig. 7, there was minimal expression of Oatp1a1 in mouse kidney at 22 days of age, followed by detectable levels at day 30, and reaching adult levels by 45 days of age. The male-predominant expression was observed at day 30 and 45. Fig. 7 illustrates that the expression of Oatp1a4 was similar at all ages. In contrast to Oatp1a4 expression in liver, Oatp1a4 shows no gender difference in kidney. The expression of Oatp1a6 was low at birth (Fig. 7), and gradually increased until 15 days of age, when Oatp1a6 reached adult levels.

Oatp2a1 mRNA expression in both male and female mouse kidney was similar at all ages after birth, but was lower before birth (Fig. 8). Ontogenic expression of Oatp2b1 in kidney was similar to that of Oatp2a1 expression, being expressed at a low level 2 days before birth, but increasing to adult levels at birth. Oatp3a1 expression in mouse kidney was low before 30 days of age, but at 30 days of age, the expression of Oatp3a1 in males increased, whereas it remained low in the females.

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Discussion

The expression of mouse Oatps vary among tissues. The Oatps that have relatively high expression in liver are Oatp1a1, 1a4, 1b2, and 2b1, among which Oatp1b2 is almost exclusively expressed in liver. The Oatps that have relatively high mRNA expression in kidney are Oatp1a1, 1a6, 3a1, and 4c1. Furthermore, Oatp1a4 and 1c1 are highly expressed in brain. Oatp1a5, 6b1, 6c1, and 6d1 are predominantly expressed in testes, and Oatp2a1, 4a1, and 5a1 are predominantly expressed in placenta.

The tissue distribution of Oatp homologues in rats and mice is similar, but not identical. In both rats and mice, Oatp1a1 is highly expressed in liver and kidney (Li et al., 2002); Oatp1a4 in liver and brain (Noé et al., 1997; Guo et al., 2002a); Oatp1a6 in kidney (Ogura et al., 2000; Choudhuri et al., 2001); Oatp1b2 in liver (Li et al., 2002); Oatp1c1 in brain (Sugiyama et al., 2003); Oatp4a1 in placenta (Leazer and Klaassen, 2003); and Oatp6b1 and 6c1 in testes (Suzuki et al., 2003). The high expression of Oatp1a5 in mouse testes has not been reported previously, whereas in rats, it has been shown that Oatp1a5 is highly expressed in choroid plexus (Choudhuri et al., 1998), moderate in the retina (Abe et al., 1998), and slight in liver, kidney, ileum, and testes (Choudhuri et al., 2003; Augustine et al., 2005). Also, there is no mouse or human homologue of rat Oatp1a3 (Oat-K1), which is highly expressed in rat kidney (Saito et al., 1996). It should also be noted that humans do not have any rodent Oatp1a subfamily members (Oatp1a1, 1a4, 1a5, and 1a6), however, OATP1A2, a specific human OATP1A subfamily member, does not have rodent homologues. Also, other Oatp subfamily members have comparable tissue distribution in humans and mice. For example, OATP1B/Oatp1b members (mouse Oatp1b2, human OATP1B1, 1B3) are predominantly expressed in liver of both humans and mice (Abe et al., 1999; Li et al., 2002). OATP1C1/Oatp1c1 is detected in brain (Sugiyama et al., 2003).

OATP2A1/Oatp2a1 and 2B1/2b1 is expressed ubiquitously (Lu et al., 1996a; Nishio et al., 2000). OATP6/Oatp6 subfamily members (mouse 6b1, 6c1, and 6d1, human OATP6A1) are exclusively expressed in testes of humans and mice (Suzuki et al., 2003).

Organ-specific expression of Oatps contribute to tissue-specific distribution of drugs and to tissue-specific toxicity. For example, pravastatin must enter the liver in order to exert its anti-lipidemic effect. Human OATP1B1 has been shown to transport pravastatin with high affinity (Hsiang et al., 1999). Phalloidin and microcystin both selectively accumulate in liver leading to hepatotoxicity. Rat Oatp1b2, human OATP1B1, and OATP1B3 are known to transport phalloidin and microcystin (reviewed by Hagenbuch and Meier, 2003). Conversely, poor extraction of a drug by liver (due to a deficiency in uptake transporters) can cause elevated plasma levels of the drug, leading to toxicities in organs other than liver. A well-defined example is the uptake of cardiac glycosides into neonatal rat liver, which is immature with respect to adult liver, resulting in a LD50 for ouabain in newborns about $1/100^{th}$ of that in adult rats (Klaassen, 1972). This observation corresponds well with the low expression of Oatp1a4 in liver of young rats (Guo et al., 2002a), and the finding that Oatp1a4 transports cardiac glycosides with high affinity (Noé et al., 1997). Therefore, understanding tissue distribution and substrate specificity of various Oatps will help predict tissue distribution of Oatp substrates, and provide information concerning which Oatp should be targeted or avoided when developing a new drug.

The present study indicates that there is relatively poor expression of mouse Oatps in the gastrointestinal tract, namely the stomach, duodenum, jejunum, ileum, and large intestine. Of the Oatps, only Oatp2b1 has relatively high expression in intestine. The relatively poor expression of Oatps in the intestine suggests that Oatps probably play a relatively minor role in intestinal absorption of chemicals. Actually, it is generally believed that most xenobiotics are

mainly absorbed from the intestine by simple diffusion (Schwenk, 1987). Instead, Oatps appear to play an important role in distribution and elimination of chemicals, as suggested by high expression of Oatps in liver, kidney, lung, brain, testes, and placenta. As previously stated, Oatp1b2 is responsible for transport of microcystin and phalloidin into liver to produce hepatotoxicity, as well as uptake of sulfobromothelein (BSP) into liver for subsequent excretion into bile.

Gender differences in Oatp1a1 expression were observed in mouse liver and kidney, with higher expression in males than females. It has been shown that in rat and mouse kidney, male-predominant expression of Oatp1a1 is androgen-dependent (Isern et al., 2001; Lu et al., 1996). This gender specific pattern may explain some physiological/toxicological phenomena. For instance, Oatp1a1 is localized to the apical membrane domain of proximal tubules in kidney, where it reabsorbs organic anions from the lumen. The male-predominant expression of Oatp1a1 may be responsible for the 250-fold higher rate of urinary excretion of exogenously administered radioactive estradiol-17β-D-glucuronide in females as compared with male rats (Gotoh et al., 2002).

In contrast to the lack of gender differences in the expression of Oatp1a4 and 1a6 in rats, gender differences in the expression of these two transporters were observed in mouse liver, with higher expression in females than males, the opposite of Oatp1a1 (Li et al., 2002). Oatp3a1 and 4c1 were expressed at higher levels in female kidney than in males. These gender differences might be due to sex hormones, or due to gender-related secretion patterns of growth hormone (Waxman et al., 1991; Noshiro and Negishi, 1986; Aida and Negishi, 1993; Buist et al., 2003). Further, investigation is needed to elucidate the regulatory mechanisms of these gender-related differences in Oatp expression.

The ontogenic expression of the various Oatps in mouse liver exhibited different developmental patterns of expression. The low expression of Oatp1a4 in development may result in a higher toxicity of ouabain in newborn mice. In newborn rats, low expression of Oatp1a4 results in less uptake of ouabain into liver, and thus high amounts of ouabain remain in blood and other tissues, causing toxicity (Klaassen, 1972; Guo et al, 2002a). The importance of Oatp1a4 has been further illustrated by the finding that pregnenalone-16α-carbonitrile (PCN), a prototypical rodent pregnane-X-receptor (PXR) ligand, stimulates hepatic clearance of cardiac glycosides in newborn rats, resulting in decreased toxicity of cardiac glycosides (Klaassen, 1974a,b). Oatp1a4 is a sinusoidal hepatic uptake transporter, with high affinity for cardiac glycosides (Noé et al., 1997). PCN treatment dramatically accelerates the maturation of hepatic Oatp1a4 mRNA and protein levels in neonatal rats (Guo et al., 2002a). Thus, newborn rats are more sensitive to cardiac glycoside toxicity because of their low capacity to remove ouabain from the blood, and PCN protects newborns from cardiac glycoside toxicity because it induces Oatp1a4 and the uptake of ouabain into liver.

Kidney is another important organ for chemical disposition. The kidney in newborns is immature and renal functions are limited. The present data indicate that renal expression of Oatps is minimal at or before birth, and then gradually increases to adult levels. Low renal expression of Oatps in newborns may protect the kidney from toxicity of Oatp substrates.

Oatp6b1, 6c1, and 6d1 are mouse homologues of human and rat gonad-specific transporters, and are thought to be responsible for testicular uptake of dehydroepiandrostane (DHEA) and DHEA sulfate, precursors of in vivo androgen and thus estrogen biosynthesis (Suzuki et al, 2003). In this study, Oatp6b1, 6c1, and 6d1 were found to be exclusively expressed in testes.

In conclusion, the present study shows that mouse Oatp expression is highly variable among tissues. There are also gender- and age-related differences in the expression of these uptake transporters. These variances most likely result in tissue-, gender-, and age-related differences in the pharmaco- and toxicokinetic profiles of xenobiotics. Furthermore, the similarities and differences in Oatp expression between rat, mouse, and human will aid in extrapolation of rodent pharmacokinetic data to humans.

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Footnotes:

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Figure Legends

Fig. 1. Tissue distribution of Oatp1a1, 1a4, 1a5, and 1a6 mRNA.

Total RNA from both male and female C57BL/6 mouse tissues (n = 10/gender) was analyzed by the bDNA assay for expression of each Oatp mRNA. Data are presented as mean ± S.E.M. Asterisks indicate statistically significant differences between male and female mice (p<0.05).

Fig. 2. Tissue distribution of Oatp1b2, 1c1, 2a1, and 2b1 mRNA.

Total RNA from both male and female C57BL/6 mouse tissues (n = 10/gender) was analyzed by the bDNA assay for expression of each Oatp mRNA. Data are presented as mean ± S.E.M. Asterisks indicate statistically significant differences between male and female mice (p<0.05).

Fig. 3. Tissue distribution of Oatp3a1, 4a1, 4c1, and 5a1 mRNA.

Total RNA from both male and female C57BL/6 mouse tissues (n = 10/gender) was analyzed by the bDNA assay for expression of each Oatp mRNA. Data are presented as mean ± S.E.M. Asterisks indicate statistically significant differences between male and female mice (p<0.05).

Fig. 4. Tissue distribution of Oatp6b1, 6c1, and 6d1 mRNA.

Total RNA from both male and female C57BL/6 mouse tissues (n = 10/gender) was analyzed by the bDNA assay for expression of each Oatp mRNA. Data are presented as mean ± S.E.M. Asterisks indicate statistically significant differences between male and female mice (p<0.05).

Fig. 5. Ontogenic expression of mouse Oatp1a1, 1a4, and 1a6 mRNA in mouse liver.

Total RNA from C57BL/6 mice at each age (n = 5/gender) was analyzed by the bDNA assay. Data are presented as mean ± S.E.M. Asterisks indicate statistically significant differences between male and female mice (p<0.05).

Fig. 6. Ontogenic expression of mouse Oatp1b2, 2a1, and 2b1 mRNA in mouse liver.

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Total RNA from C57BL/6 mice at each age (n = 5/gender) was analyzed by the bDNA assay. Data are presented as mean ± S.E.M. Asterisks indicate statistically significant differences between male and female mice (p<0.05).

Fig. 7. Ontogenic expression of mouse Oatp1a1, 1a4, and 1a6 mRNA in mouse kidney.

Total RNA from C57BL/6 mice at each age (n = 5/gender) was analyzed by the bDNA assay. Data are presented as mean ± S.E.M. Asterisks indicate statistically significant differences between male and female mice (p<0.05).

Fig. 8. Ontogenic expression of mouse Oatp2a1, 2b1, and 3a1 mRNA in mouse kidney.

Total RNA from C57BL/6 mice at each age (n = 5/gender) was analyzed by the bDNA assay. Data are presented as mean ± S.E.M. Asterisks indicate statistically significant differences between male and female mice (p<0.05).

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Table 1. Nomenclatures and Genebank Accession Numbers for Mouse Oatps.

Current nomenclature	Slc nomenclature	Accession #
Oatp1a1	Slc21a1	AB031813
Oatp1a4	Slc21a5	AB031814
Oatp1a5	Slc21a7	NM_130861
Oatp1a6	Slc21a13	AF213260
Oatp1b2	Slc21a10	AB031959
Oatp1c1	Slc21a14	NM_021471
Oatp2a1	Slc21a2	NM_033314
Oatp2b1	Slc21a9	BC019209
Oatp3a1	Slc21a11	NM_023908
Oatp4a1	Slc21a12	BC030720
Oatp4c1	-	NM_172658
Oatp5a1	Slc21a15	XM_129381
Oatp6b1	Slc21a16	AK006249
Oatp6c1	Slc21a18	AK016647
Oatp6d1	Slc21a17	AK014872

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Table 2. Oligonucleotide probes generated for analysis of mouse Oatp mRNAs expression by Quantigene branched DNA signal amplification assay

Name	Target region	Function	Sequence
Oatp1a1	1279-1307	CE	agtgatcttaaacttcttcataataaagcTTTTTctcttggaaagaaagt
	1308-1331	CE	tgctatgtatgcagctttcttgacTTTTTctcttggaaagaaagt
	1431-1452	CE	ccccatatagagggtgctgaacTTTTTctcttggaaagaaagt
	1476-1497	CE	ttaagcagctgcaccttgtgttTTTTTctcttggaaagaaagt
	1603-1622	CE	cccaatgcagctgcaattttTTTTTctcttggaaagaaagt
	1623-1646	CE	gactgcagatgagtttcctgatgaTTTTTctcttggaaagaaagt
	1228-1252	LE	ggaggcaagctataaacacctatgaTTTTTaggcataggacccgtgtct
	1253-1278	LE	cactgattaaatatccaaggcatactTTTTTaggcataggacccgtgtct
	1332-1354	LE	tattcagataaggacaggccaaaTTTTTaggcataggacccgtgtct
	1406-1430	LE	tcctttataagaggtggttaatccaTTTTTaggcataggacccgtgtct
	1453-1475	LE	gcagtcagcaaggacatttttctTTTTTaggcataggacccgtgtct
	1498-1517	LE	cactggatcccatgtgtccgTTTTTaggcataggacccgtgtct
	1518-1537	LE	gctaggccattgtccccacaTTTTTaggcataggacccgtgtct
	1538-1557	LE	cgaggcaggctgacatgtaaTTTTTaggcataggacccgtgtct
	1558-1579	LE	ccaacagacttctcacagcctgTTTTTaggcataggacccgtgtct
	1647-1667	LE	gcctttcttacacagccccagTTTTTaggcataggacccgtgtct
	1668-1689	LE	gcagcttgttgtcacactcaggTTTTTaggcataggacccgtgtct
	1355-1383	BL	tcaacaaatagttacagagaaaaataaaa
	1384-1405	BL	gcaactgggaaattatcacagg
	1580-1602	BL	gaaacaccatgttggttccagtt
Oatp1a4	526-545	CE	gcctgtgcagttttgttccgTTTTTctcttggaaagaaagt
	591-616	CE	gaggaaatgaggtatcgatattaagaTTTTTctcttggaaagaaagt
	715-735	CE	cactctgttgggtcttgcgttTTTTTctcttggaaagaaagt
	452-474	LE	ttgataagcccaactacagacgtTTTTTaggcataggacccgtgtct
	546-570	LE	gcacatcctacaccaatcatgatagTTTTTaggcataggacccgtgtct
	667-690	LE	tccgtacacacaaagctatttgagTTTTTaggcataggacccgtgtct
	793-812	LE	tgggagtttcacccattccaTTTTTaggcataggacccgtgtct
	833-858	LE	tcagattttgcaaaatcttctatgtaTTTTTaggcataggacccgtgtct
	886-907	LE	gccaatggtcattcctgtttctTTTTTaggcataggacccgtgtct
	859-885	BL	aaaatcccaatatataaaggagagttt
	475-497	BL	gatttcctatctcaaagctccca
	498-525	BL	aagtaactcacgaatataatcaacagaa
	571-590	BL	aacaccccaggcccataact
	617-640	BL	tgtttcatattcatatctgcccat
	641-666	BL	gacaagttgcttgtaggtaaaattgt
	691-714	BL	ggctttaaggtctgtgttctgttt

	736-762	BL	cacattaatgatttcatttctttcaca
	763-792	BL	cgtataatgtttcctaccagtacatatatc
	813-832	BL	ggaaatacccaagggcatga
Oatp1a5	95-118	CE	aatccttttctctgtttctcccatTTTTTctcttggaaagaaagt
	119-137	CE	atctgaccccatgggttgcTTTTTctcttggaaagaaagt
	188-213	CE	atataaattcctgagagtgatttggaTTTTTctcttggaaagaaagt
	214-239	CE	tctctatttgtgtaagcatggaattcTTTTTctcttggaaagaaagt
	412-430	CE	tcggcccatgaggaaatgaTTTTTctcttggaaagaaagt
	506-526	CE	ttgcgttggctttaaggtctgTTTTTctcttggaaagaaagt
	41-69	LE	gactattctttaaacagtcttaccactgaTTTTTaggcataggacccgtgtct
	70-94	LE	gttgttcttctgattgtctccaaatTTTTTaggcataggacccgtgtct
	164-187	LE	tacatatgcacatgttaatgccaaTTTTTaggcataggacccgtgtct
	263-286	LE	gctcccattgataagtccaactatTTTTTaggcataggacccgtgtct
	338-362	LE	caatcatgataggtctgtgcaatttTTTTTaggcataggacccgtgtct
	363-385	LE	gcccataatcacacatccaatacTTTTTaggcataggacccgtgtct
	527-548	LE	ctttcacacactctgcagggtcTTTTTaggcataggacccgtgtct
	549-576	LE	acatatatccacattaatgatttcatttTTTTTaggcataggacccgtgtct
	577-600	LE	ccacgtatgatgtttcctaccagtTTTTTaggcataggacccgtgtct
	601-622	LE	catgatgggagtttcaccaattTTTTTaggcataggacccgtgtct
	138-163	BL	cagaaacatcttgatcttagaaaagc
	240-262	BL	agatgtggggatatcgaattgtc
	287-310	BL	caacaaaagatttccaatctcaaa
	311-337	BL	tgttccaaagtaactcacgagtataat
	386-411	BL	ggtaaggacattaagaaacaccctag
	431-456	BL	ggtgaaatcgttgtttcatattcata
	457-480	BL	ctgtttgaggacaagttgcttgta
	481-505	BL	tgttctgttttccatacacaagaag
Oatp1a6	471-497	CE	gaggtagtgatattatgaaacaccctaTTTTTctcttggaaagaaagt
	498-518	CE	cgtatctgcccatgaggaaatTTTTTctcttggaaagaaagt
	742-768	CE	ccaatgtataaaggagaattttctgatTTTTTctcttggaaagaaagt
	973-994	CE	ctttgggagtgtttttggaaagTTTTTctcttggaaagaaagt
	995-1017	CE	tccccattatcctgtaatccttcTTTTTctcttggaaagaaagt
	425-449	LE	caccaatcatgataggtctatgcagTTTTTaggcataggacccgtgtct
	450-470	LE	ggcccataactgcacaaccaaTTTTTaggcataggacccgtgtct
	546-567	LE	aagctgtttgaggacaagttgcTTTTTaggcataggacccgtgtct
	568-590	LE	gggatctgttttccacacacaaaTTTTTaggcataggacccgtgtct
	640-668	LE	ctaccagtacatatatccacattaatgatTTTTTaggcataggacccgtgtct
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	693-714	LE	cctaaaggcatgatgggagtttTTTTTaggcataggacccgtgtct
	769-794	LE	caatcatcttcccaacttctaaaattTTTTTaggcataggacccgtgtct
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	866-888	LE	gtgggagttatggtcaggtcatcTTTTTaggcataggacccgtgtct
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	930-951	LE	aggacattcactcctgcacagaTTTTTaggcataggacccgtgtct
	519-545	BL	ttgtaggtgaaattgttgtttcatatt
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	615-639	BL	ttaatttctttcacacactctgctg
	715-741	BL	ttggcaaagtcttctatataggaaata
	795-817	BL	catcaaatatccaagtattgggc
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	909-929	BL	ccaaaaagccaatccaccaag
	952-972	BL	aagaaaaaaggggatgctggtc
Oatp1b2	497-517	CE	atactcccaatgcccatgatgTTTTTctcttggaaagaaagt
	538-562	CE	gcatacctgtaatatcccatgaagaTTTTTctcttggaaagaaagt
	615-640	CE	gttccagtgagtgatgtagtttgattTTTTTctcttggaaagaaagt
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	563-589	LE	tgtagagaactgatgtcattttctgttTTTTTaggcataggacccgtgtct
	590-614	LE	gactaaacaggtcaacgtggagttaTTTTTaggcataggacccgtgtct
	641-664	LE	cctttctccattatctcaggtgagTTTTTaggcataggacccgtgtct
	686-711	LE	ccatcaagacataaatccaggtgtatTTTTTaggcataggacccgtgtct
	712-732	LE	ctatcccacgaagcatgttccTTTTTaggcataggacccgtgtct
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	795-817	BL	cgcatccacatagatcttggtac
Oatp1c1	1989-2013	CE	actgctaaggtgtagatacccagagTTTTTctcttggaaagaaagt
	2103-2120	CE	gcctgcaggagcctctgcTTTTTctcttggaaagaaagt
	2371-2395	CE	cccatatttgtttgtttccatgattTTTTTctcttggaaagaaagt
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	1934-1962	LE	cacctcaagagtaatatatatccaggtatTTTTTaggcataggacccgtgtct
	2080-2102	LE	ttccacatttcttaaatccccatTTTTTaggcataggacccgtgtct
	2143-2167	LE	cgtggttaatcccaggtatatgtgtTTTTTaggcataggacccgtgtct
	2168-2186	LE	cagacaccgtgcccaggagTTTTTaggcataggacccgtgtct
	2211-2237	LE	tcgagacatatttttttctttaaaacaaTTTTTaggcataggacccgtgtct
	2238-2261	LE	ttgtggttattaagctgctgtgttTTTTTaggcataggacccgtgtct
	2287-2307	LE	gcgcatgtctccttttttgatgTTTTTaggcataggacccgtgtct
	2308-2326	LE	caatccacgatcccttgcaTTTTTaggcataggacccgtgtct
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	2014-2035	BL	tgggattcctgcaagaactctt
	2036-2056	BL	aacaccaaagtacacaggggc
	2057-2079	BL	ttgaggcatgaagtgtcaatcaa
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	2187-2210	BL	aaagtacagccatgcttaggaaga
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	2327-2346	BL	cctggccagtacttgggctg
	2347-2370	BL	ttttttaaagtcgtgtctccttg
	2396-2423	BL	ttcatttacatgtattggataaaatgac
	2448-2469	BL	aagcagaaaggaattgcacatt
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	2203-2221	LE	ttggcaaacgctcagaggaTTTTTaggcataggacccgtgtct
	2242-2261	LE	ggtggtgcttccgataccgtTTTTTaggcataggacccgtgtct
	2262-2281	LE	aggaccctcaggcactggacTTTTTaggcataggacccgtgtct
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	2167-2183	BL	ggggctgggaccgtcaa
Oatp2a1	481-502	CE	tgctggtctccttctgggtatcTTTTTctcttggaaagaaagt
	557-576	CE	gatcccaaatggctgaatggTTTTTctcttggaaagaaagt
	679-700	CE	cgaagatcctcagcatgactgaTTTTTctcttggaaagaaagt
	779-798	CE	gcctgaggagatgagcaggcTTTTTctcttggaaagaaagt
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	541-556	LE	gcaccgtcccaacgccTTTTTaggcataggacccgtgtct
	599-618	LE	cagaggcgagttggtaggctTTTTTaggcataggacccgtgtct
	661-678	LE	gcccagcaggtacccgaaTTTTTaggcataggacccgtgtct
	701-721	LE	tgtccactctgccgtagtccaTTTTTaggcataggacccgtgtct
	722-742	LE	ggctcaggttcactgtagccgTTTTTaggcataggacccgtgtct
	743-761	LE	atccaccgagggtcacctgTTTTTaggcataggacccgtgtct
	762-778	LE	ccagccaccaggctccgTTTTTaggcataggacccgtgtct
	577-598	BL	ctgcaaagtcgtccacatagga
	619-644	BL	gcgatagcaaataagatggagatata
	645-660	BL	agccggcccaaacacg
Oatp2b1	1991-2013	CE	gaagaactggaggcctatgaatcTTTTTctcttggaaagaaagt
	2097-2120	CE	tgttgtagttccgagctctttacaTTTTTctcttggaaagaaagt
	2145-2166	CE	cctcctggaatccttaggcttcTTTTTctcttggaaagaaagt
	2222-2241	CE	gggtgctgatttgcccattgTTTTTctcttggaaagaaagt
	1932-1949	LE	ctccgcccacaggtcaggTTTTTaggcataggacccgtgtct
	1950-1970	LE	tcgtagtagcggcagacagctTTTTTaggcataggacccgtgtct
	1971-1990	LE	ggtttcggagcaggtcatggTTTTTaggcataggacccgtgtct

	2014-2034	LE	caccagggatccacttttgaaTTTTTaggcataggacccgtgtct
	2035-2056	LE	tggccaaaactaaggtaaagcaTTTTTaggcataggacccgtgtct
	2079-2096	LE	gtggtcctggtgctggccTTTTTaggcataggacccgtgtct
	2121-2144	LE	ttctctggtcctgatgcttatagcTTTTTaggcataggacccgtgtct
	2184-2202	LE	tgccactcctggtcagggtTTTTTaggcataggacccgtgtct
	2203-2221	LE	ttggcaaacgctcagaggaTTTTTaggcataggacccgtgtct
	2242-2261	LE	ggtggtgcttccgataccgtTTTTTaggcataggacccgtgtct
	2262-2281	LE	aggaccctcaggcactggacTTTTTaggcataggacccgtgtct
	2057-2078	BL	tctctgctctgttgcctcaaga
	2167-2183	BL	ggggctgggaccgtcaa
Oatp3a1	511-529	CE	ggtgccttctgcctcccatTTTTTctcttggaaagaaagt
	571-589	CE	gcagattaggtccgggtcgTTTTTctcttggaaagaaagt
	726-750	CE	atcgtgaacaggattcctatgtagaTTTTTctcttggaaagaaagt
	772-794	CE	agaaagagcccagaataaatccaTTTTTctcttggaaagaaagt
	491-510	LE	cggatctcgacagcctcgtaTTTTTaggcataggacccgtgtct
	530-550	LE	cccattggtggcacagacatcTTTTTaggcataggacccgtgtct
	551-570	LE	ggcctgtcatcactgctggaTTTTTaggcataggacccgtgtct
	590-607	LE	cgtggctgtccggttacgTTTTTaggcataggacccgtgtct
	608-632	LE	caataagcagcatgtacatcatgttTTTTTaggcataggacccgtgtct
	684-706	LE	cacgtggtcgtcaatataggagaTTTTTaggcataggacccgtgtct
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Fig. 1

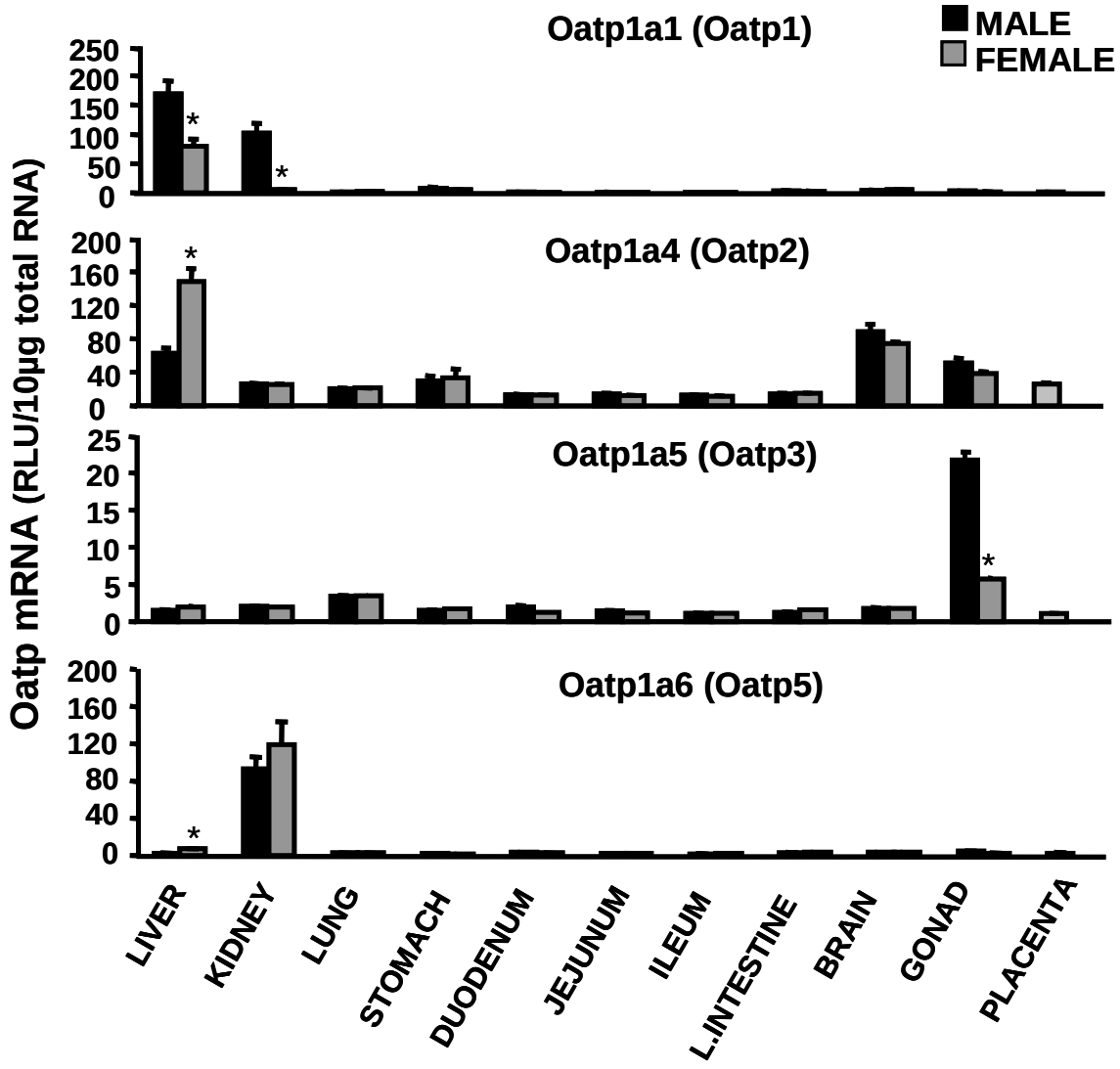

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Fig. 2

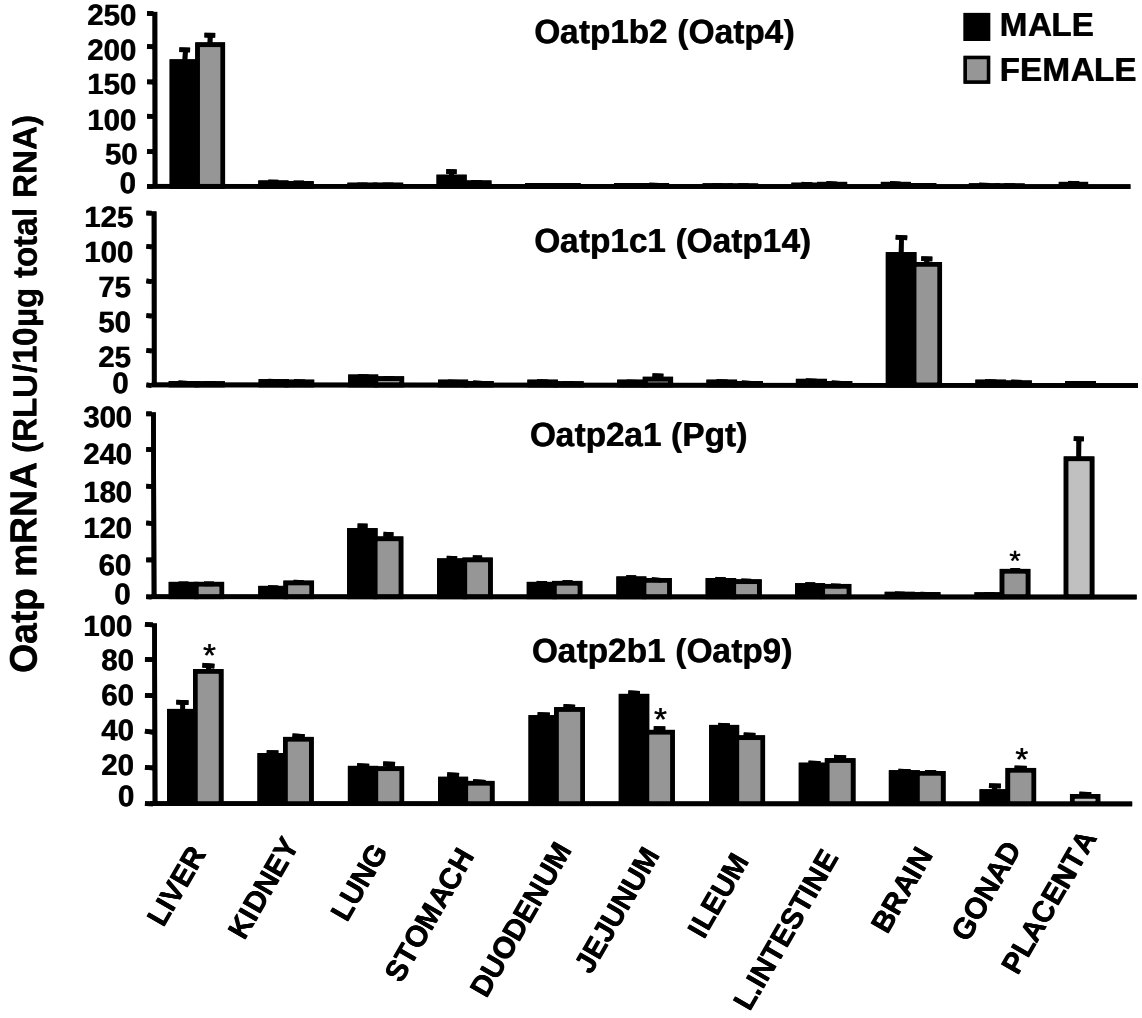

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Fig. 3

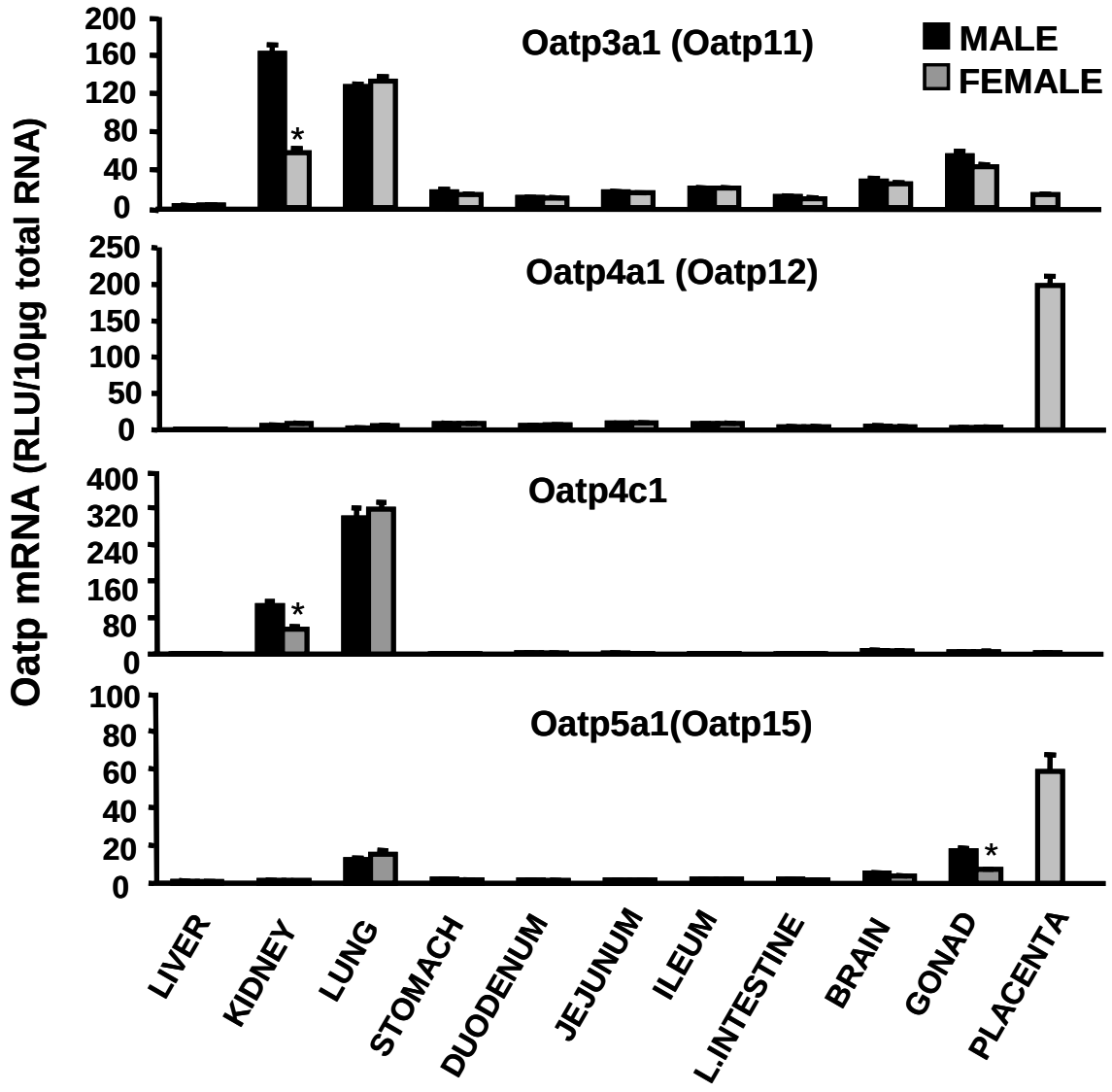

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Fig. 4

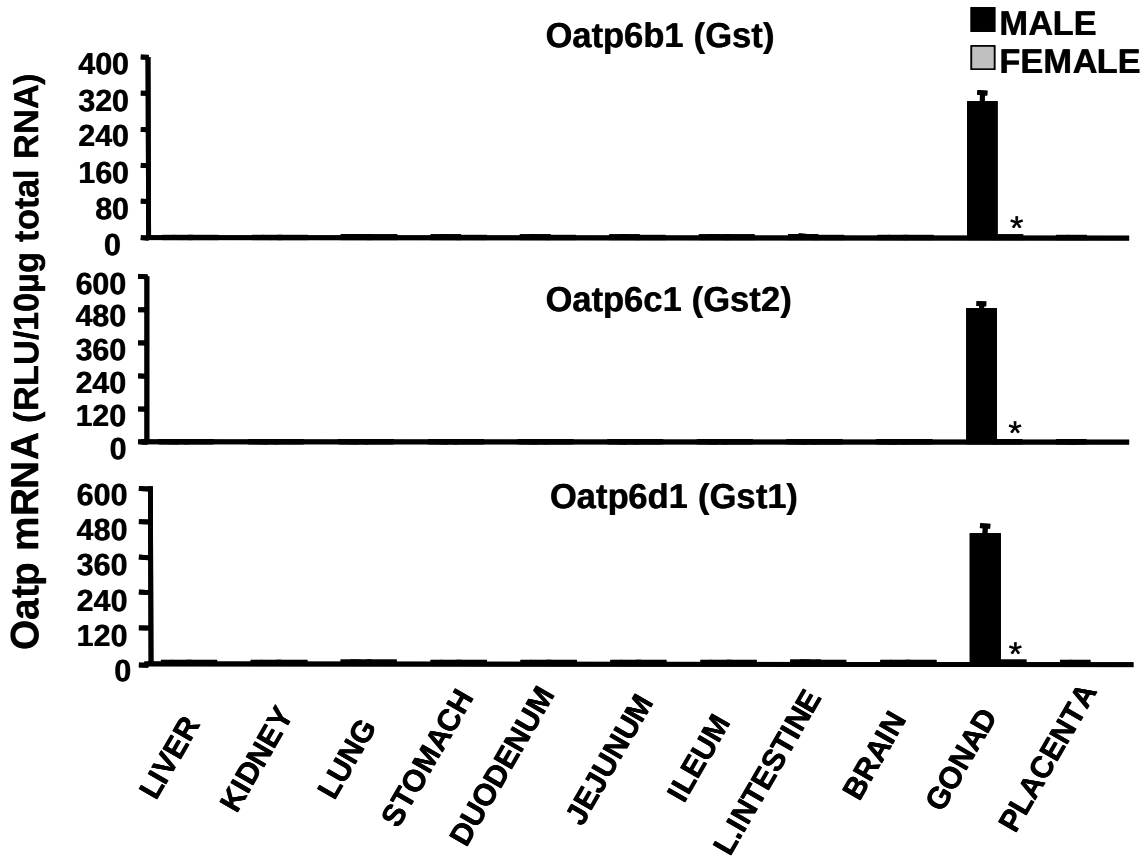

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Fig. 5

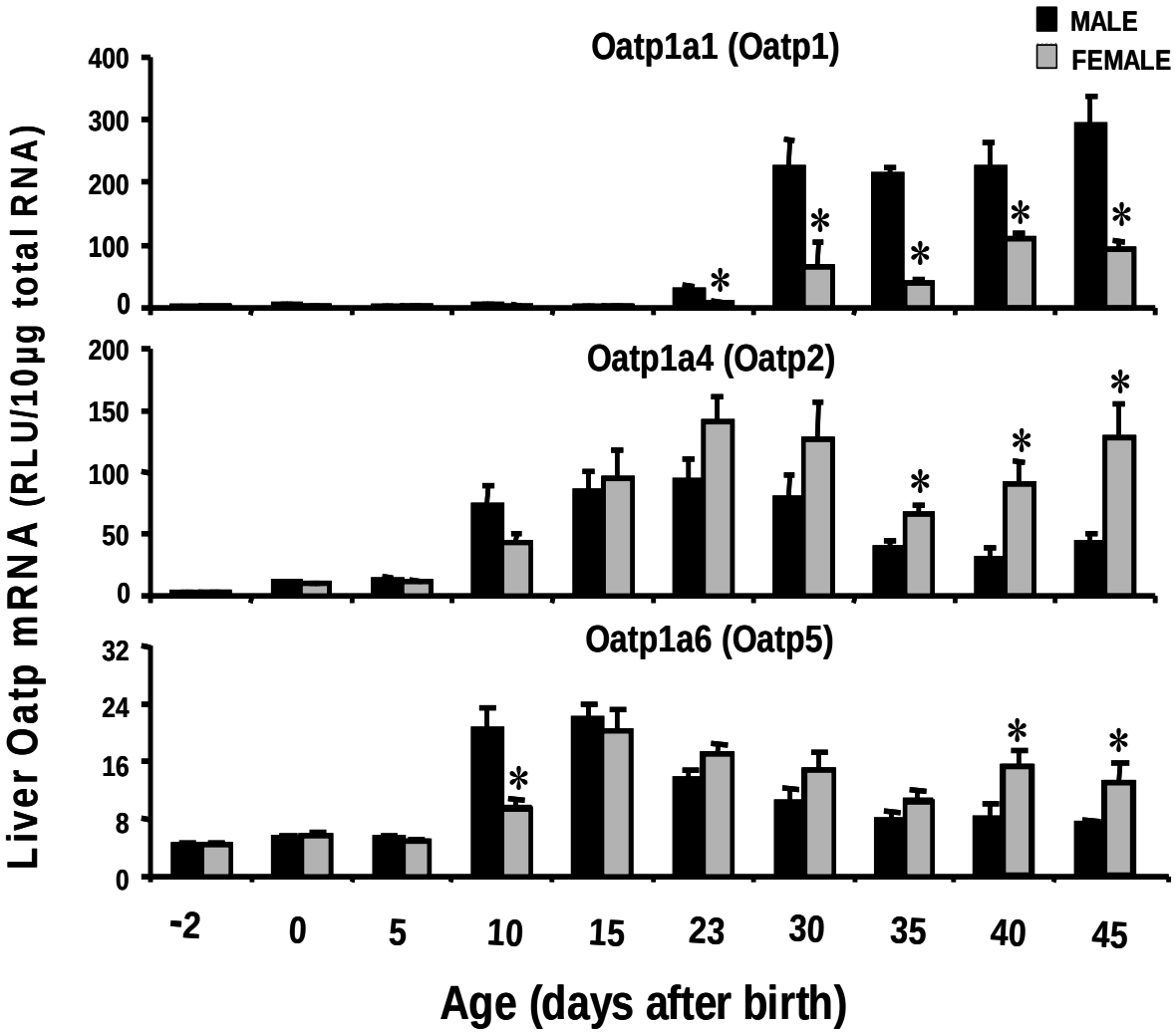

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Fig. 6

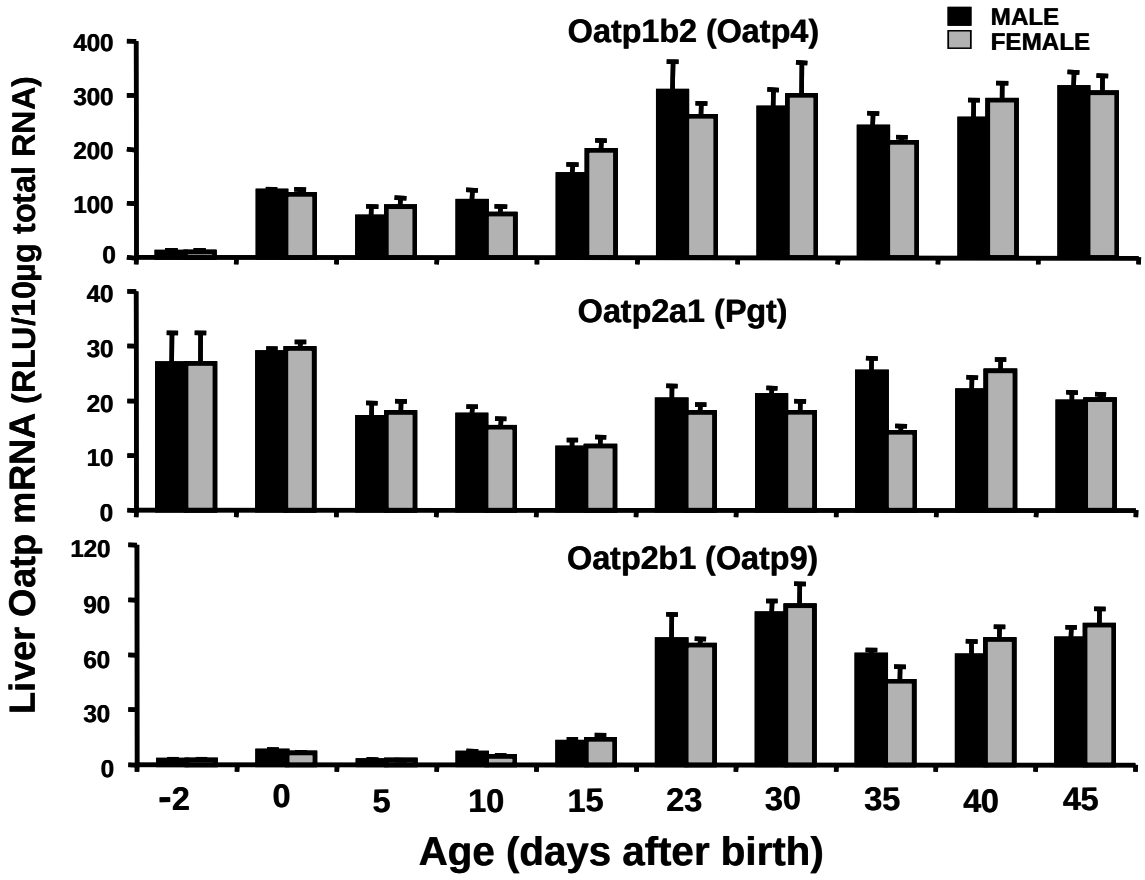

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Fig. 7

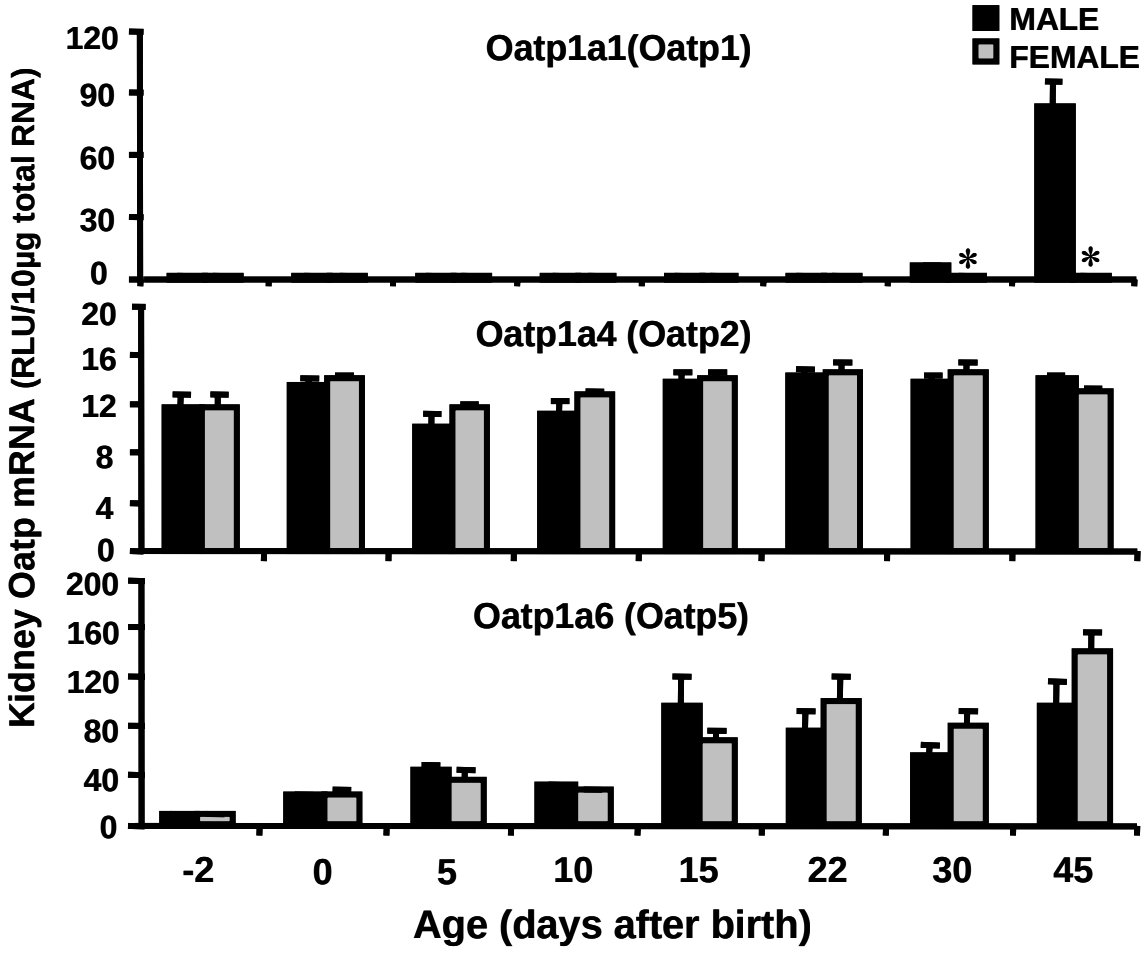

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Fig. 8