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TISSUE DISTRIBUTION AND HEPATIC AND RENAL ONTOGENY OF THE MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN (Mrp) FAMILY IN MICE

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Running Title: Mrp Family of Transporters in Mice.

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Abbreviations:
Mrp Multidrug resistance-associated protein
Cyp Cytochrome P450
ABCC ATP-Binding Cassette Transporter, subfamily C

Abstract: Analysis of the mouse genome has revealed eight Multidrug resistance-associated (Mrp) transporters, with mouse homologues for all human MRPs except MRP8. Whereas MRP expression in tissues of humans and rats has been examined, no characterization exists for mice. Furthermore, the ontogeny of mouse Mrps is unknown, and such knowledge may be helpful in understanding age-related pharmacokinetics. Therefore, the purpose of this study was to quantitatively determine: 1) expression of the Mrp family in 12 different tissues, 2) gender variations in Mrp expression in liver and kidney, and 3) whether Mrp expression is altered during development. Highest expression of the Mrp family members is as follows: Mrp1 in testes, ovary, and placenta; Mrp2 in intestine, followed by liver and kidney; Mrp3 in large intestine; Mrp4 in kidney; Mrp5 in brain, followed by lung and stomach; Mrp6 in liver; Mrp7 in testes, intestine, and kidney; and Mrp9 solely in testes. Gender differences in Mrp expression were observed: Mrp1, 3, and 4 in kidney, as well as Mrp1 and 4 in liver were female-predominant. Ontogeny of the four Mrps expressed in liver was as follows: Mrp2 and Mrp4 were expressed at adult levels at birth; Mrp3 reached adult levels at day 30, and Mrp6 was not expressed until day 10. In kidney, Mrp1 and Mrp5 were expressed at adult levels at birth, whereas Mrp2, 3, 4, and 6 generally increased over time. In conclusion, marked differences in expression of the individual Mrp family members exist in various tissues, with age, and with gender.

Introduction:

Since the discovery of MRP1 in 1992 (Cole et al., 1992), eight additional human MRPs have been cloned. Thus, the human MRP family is currently composed of nine members, MRP1-9 (Kruh and Belinsky, 2003). MRPs are members of the ATP-Binding Cassette transporter superfamily, and are grouped into the (ABC) C subfamily, a group which includes the cystic fibrosis transporter (CFTR), as well as the sulphonylurea receptors (SUR1 and SUR2) (Dean and Allikmets, 2001). Structurally, MRP1, 2, 3, 6, and 7 are composed of an amino-terminal transmembrane domain (TMD) and a P-glycoprotein-like core, consisting of two TMDs and two nucleotide-binding domains, whereas MRP4, 5, 8, and 9 lack the amino-terminal TMD found in the other MRPs (Tusnady et al., 1997). Mouse orthologs have been identified for all human MRP genes except MRP8.

As indicated by functional studies, MRPs are efflux transporters for structurally diverse amphipathic chemicals and organic anions. MRP1, 2, and 3 confer resistance to a variety of anticancer drugs including anthracyclines, vinca alkaloids, and methotrexate, and transport organic anions such as glutathione and glucuronide conjugates. Unlike MRP1, 2, and 3, MRP4 and 5 do not confer resistance to anthracyclines or vinca alkaloids. Overexpression of MRP4 and 5 is associated with increased cellular efflux of purine analogues (e.g. 6-mercaptopurine and thioguanine) and nucleoside-based antiviral drugs [e.g. Adefovir] (Schuetz et al., 1999; Wielinga et al., 2002). MRP4 and 5 also transport cyclic nucleotides, such as cAMP and cGMP (Wielinga et al., 2002). Furthermore, several compounds of physiological and pharmacological importance, such

as methotrexate, estradiol-17β-glucuronide ($E_2$17βG), bile acids, prostaglandins, and dehydroepiandrosterone-3-sulfate were recently shown to be transported by MRP4 (Chen et al., 2001; Wielinga et al., 2002; Reid et al., 2003; Zelcer et al., 2003). Mutations in human MRP6 are associated with pseudoxanthoma elasticum, a hereditary disease characterized by progressive dystrophic mineralization of elastic fibers (Bergen et al., 2000). In vitro transport studies showed that MRP6 transports the anionic cyclopentapeptide and endothelin antagonist BQ-123 (Madon et al., 2000) and glutathione conjugates, and that MRP6 expression in tumor cells can confer weak resistance to some anticancer drugs (Belinsky et al., 2002). Recently, MRP7 was shown to transport $E_2$17βG, but not cyclic nucleotides, methotrexate, or bile acids (Chen et al., 2003); whereas MRP8 was reported to transport cyclic nucleotides (Guo et al., 2003). No functional studies have been reported for MRP9.

Neonatal sensitivity to various chemicals has always been of concern to clinicians. The liver serves a critical role in the pharmacokinetics of xenobiotics. It is known that expression of some drug metabolizing enzymes is low in neonates**,** and this is often exacerbated in infants born premature. For example, development of UDP-glucuronosyltransferases (UGTs) plays a significant role in the metabolism and elimination of endogenous and exogenous chemicals, and insufficiency during development can lead to 1) hyperbilirubinemia, 2) kernicterus, and 3) gray-baby syndrome (Kawade and Onishi, 1981).

The development of transporters is not well characterized, although some data suggest that lower expression of transporters in young animals is important in the disposition of drugs and other chemicals. For example, neonatal rats are sensitive to

cardiac glycoside toxicity because expression of the uptake transporter Oatp2 is low in liver, thus delaying the elimination of cardiac glycosides, and increasing toxicity (Guo et al., 2002). Similarly, neonatal jaundice may be due in part to poor expression of the hepatic canalicular transporter Mrp2 (Johnson et al., 2002; Huang et al., 2003).

Tissue distribution and ontogeny data are an important component of understanding and extrapolating pharmacokinetic data from mice to humans. Knowledge of the expression patterns of mouse Mrps is limited. Therefore, the purpose of this study is to determine: 1) the mRNA expression of mouse Mrp transporters in twelve different tissues, 2) whether gender differences in Mrp mRNA expression exist between male and female mice in various tissues, and 3) Mrp mRNA expression in liver and kidney from prenatal day 2 to 45 days of age.

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Materials and Methods:

Animals: Male and female C57BL/6 mice (n=10 per gender) were purchased from Jackson Laboratories (Bar Harbor, Maine) and tissues were collected at approximately eight weeks of age. The following tissues were taken: liver, kidney, lung, stomach, duodenum, jejunum, ileum, large intestine, brain, testes, term placenta from pregnant dams (n=5), and pooled ovaries (n=5 samples; 1 sample=10 ovary pairs from 10 individual mice). Ovaries were purchased from Charles River laboratories from C57BL/6 mice undergoing ovariectomy for other experimental purposes. Tissues were snap-frozen in liquid nitrogen, and stored at -80°C.

Ontogeny: Mice (C57BL/6) were bred at the University of Kansas Medical Center laboratory animal facilities, and livers and kidneys were collected from male and female mice at -2, 0, 5, 10, 15, 23, 30, 35, 40, and 45 days of age (n=5/gender/age).

RNA Extraction: Total RNA was isolated using the RNA Bee reagent (Tel-test, Friendswood, TX) according to the manufacturer's instructions. RNA concentrations were determined spectrophotometrically, and quality of RNA was determined by gel electrophoresis.

Development of Specific Oligonucleotide Probe Sets for bDNA Analysis. The Mrp gene sequences were accessed from GenBank. The target sequences were analyzed by

ProbeDesigner® Software Version 1.0, and the probe design and target regions are designated in Table 2. The oligonucleotide probes were specific for only one mRNA transcript. All oligonucleotide probes were designed with a T_m of approximately 63°C, enabling optimal hybridization conditions. Each probe set was submitted to the National Center for Biotechnology Information (NCBI) for nucleotide comparison by the basic local alignment search tool (BLASTn) to ensure minimal cross-reactivity with other mouse genomic sequences and expressed sequence tags.

Branched DNA Assay. The specific Mrp oligonucleotide probes were diluted in Tris-EDTA buffer, pH 8.0, according to instructions provided with the Quantigene® bDNA Signal Amplification Kit (Bayer Diagnostics, East Walpole, MA). Total RNA (1 μg/μl; 10μl) was added to each well of a 96-well plate containing 50 μl of capture hybridization buffer and 50 μl of each diluted probe set. Total RNA was allowed to hybridize overnight at 53°C in a hybridization oven. Subsequent hybridization steps were carried out according to 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.

Statistical Analysis. Error bars represent standard errors of the mean. Data were analyzed by a two-tailed Student's t-test. Asterisks (*) represent statistical differences ($p \leq 0.05$) in mRNA levels between male and female mice.

Results:

Tissue Distribution of the Mouse Mrp Family. Mouse Mrp1 mRNA expression was quite low in most of the tissues examined, but was highly expressed in gonads and placenta (Fig 1). Expression of Mrp1 in liver was very low, yet detectable, with higher levels in livers from female than from male mice. Expression of Mrp2 was highest in small intestine, decreasing from duodenum to ileum (Fig 1). High expression of Mrp2 was also observed in liver and kidney. No gender differences in Mrp2 mRNA expression were observed for the tissues analyzed. Mrp3 was highest in the large intestine, with expression detected throughout the GI tract (Fig 1). Mrp3 mRNA was moderately expressed in liver and kidney. Marked gender-specific expression was observed in kidney, with Mrp3 expressed about 14-fold higher in female than male kidney. Mrp4 expression was highest in kidney, with moderate expression in ovary, lung, stomach, and liver (Fig 1). Female predominant expression of Mrp4 was observed in liver and kidney, where it was approximately 2-2.5 fold higher in female than in male mice.

In mice, Mrp5 mRNA expression was ubiquitous in the tissues analyzed, with highest expression detected in brain, followed by lung, stomach, kidney, and reproductive tissues, such as testes, ovaries, and placenta (Fig 2). No gender-specific expression patterns were observed for Mrp5. Mrp6 mRNA expression was much higher in liver than any other tissue, but expression was detected in small intestine and kidney (Fig 2). Gender-specific expression patterns were not observed for Mrp6. Mrp7 was expressed in most tissues, with highest expression in testes, followed by small intestine, kidney, ovary, placenta, and lung (Fig 2). Mrp9 was almost solely expressed in testes and at extremely high levels, with virtually no expression observed in any other tissue (Fig 2).

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Hepatic Ontogeny of Mrps. Of the eight mouse Mrps, only Mrp2, 3, 4, and 6 are significantly expression in liver. Therefore, expression of these Mrps was quantified from prenatal day -2 to 45 days of age. Expression of Mrp2 in mice increased markedly between two days before birth to parturition, and remained relatively constant thereafter. Mrp3 expression was low until 3 weeks of age, and reached adult levels by one month of age (Fig 3). Mrp4 expression was maximal at birth, and decreased about 70% by 2 weeks of age, but was relatively constant thereafter. A gender difference in Mrp4 expression was noted, with slightly higher levels in female than male mice. Mrp6 expression was not detectable in mouse liver until 10 days of age, at which time the mRNA levels were the highest. The expression of Mrp6 decreased about 60% by day 15, and remained relatively constant thereafter.

Renal Ontogeny of Mrps. Of the eight mouse Mrps, six Mrps (Mrp1-6) are significantly expressed in kidney (Fig 4 and 5). Mrp7 also had minor expression at 8 weeks of age (Fig 2), thus the ontogeny of Mrp7 was also examined, but the expression was very minor, and this basal expression changed little over time (data not shown). These six Mrps all were expressed at birth, and were also detectable prenatally. The expression of Mrp1 was rather constant throughout the ontogenic period, with female-predominant expression observed at days 30 and 45. In general, expression of Mrp2, 3, 4, and 6 increased with age, with Mrp2 exhibiting male-predominant expression at some timepoints (day 10 and 15), and with Mrp3 and 4 exhibiting female predominant expression at days 30 and 45. Expression of Mrp3 increased almost four-fold between 10 and 15 days of age, but then the expression in males decreased to levels seen at birth. In contrast, Mrp4 expression increased both in males and females after birth, but more so in

females than males. Mrp5 expression was highest at birth, and gradually decreased over the first month, whereas Mrp6 increased gradually with time. Mrp5 also exhibited statistically significant male-predominant expression at day 5 and 10.

Discussion:

Mrp1 is highly expressed in reproductive tissues such as testes, ovaries, and placenta in mice (Fig 1). Humans have high expression of MRP1 in lung, bladder, spleen, testes, thyroid, and adrenal glands (Zaman et al., 1994; Kruh et al., 1995), however, the data from humans (northern blotting) indicates little variation between these tissues, and it is thus difficult to compare these studies to the present data obtained in mice. Contrasting data between species exists, with modest MRP1 expression in ovary and placenta of humans, yet high expression in mice. Rats have the highest expression of Mrp1 in large intestine and stomach, but reproductive tissues were not examined (Cherrington et al., 2002). Marked expression of mouse Mrp1 in testes, ovary, and placenta is suggestive of a physiological function other than efflux of xenobiotics and chemotherapeutic drugs. The functional involvement of Mrp1 as an efflux transporter in testes has been demonstrated, and Mrp1 serves to protect the testes from chemotherapeutic agents, such as etopside (Wijnholds et al., 1997). MRP1 has been shown to transport steroid conjugates such as 17β-estradiol-glucuronide with high affinity (Jedlitschky et al., 1996), however, high expression of Mrp1 in ovary/testes could suggest involvement in unconjugated steroid transport**.**

Mrp2 expression in mice is highest in small intestine, followed by liver and kidney. This parallels data from humans and rats, in which expression of MRP2 is also high in liver and intestine (Kool et al., 1997; Cherrington et al., 2002). In liver, MRP2 serves as the canalicular efflux pump for many organic anions that are transported across the canalicular membrane into bile. Mutations in the MRP2 gene in humans (Dubin-Johnson Syndrome) and rats (TR-, EHBR) results in conjugated hyperbilirubinemia, due

to a decreased ability to excrete bilirubin-glucuronides (Ito et al., 1997; Toh et al., 1999). Prominent expression of Mrp2 in the small intestine suggests that Mrp2 is involved in excretion of xenobiotics into the intestinal lumen. Deficiencies in intestinal Mrp2 expression have been linked to alterations in intestinal transport (Dietrich et al., 2001).

MRP3 expression is highest in large intestine in humans, mice, and rats. In mice, Mrp3 is highly expressed in colon, with significant expression throughout the digestive tract, liver, and kidney (Fig 1). Studies using isolated basolateral membrane vesicles from rat intestine suggest that Mrp3 is involved in intestinal transport (Shoji et al., 2004). Mrp3 is a highly inducible, retrograde transporter that can efflux organic anions from hepatocytes into blood for eventual excretion into urine (Slitt et al., 2003; Trauner and Boyer, 2003). Mrp3 is moderately expressed in livers of mice (Fig1) and humans (Kool et al., 1997; Cherrington et al., 2002), but in rat is barely detectable.

In mice, Mrp4 is predominantly expressed in kidney, with moderate expression in stomach and ovary (Fig 1). In rats and humans, Mrp4 is most highly expressed in kidney and lung (Kool et al., 1997; Chen and Klaassen, 2004). In rat kidney, Mrp4 is expressed in the apical membrane of proximal tubules, and has been functionally described as important in urinary efflux of cAMP and cGMP, while also playing an important role in the blood-brain barrier (van Aubel et al., 2002; Leggas et al., 2004). High expression in kidney and low expression in liver suggest that the role of Mrp4 in hepatic transport is minor, under naïve conditions.

Mrp5 in mice is predominantly expressed in brain with significant expression in gonads, placenta, lung, and stomach. In humans, MRP5 is ubiquitously expressed, with highest expression in skeletal muscle, followed by brain (Kool et al., 1997). Rat Mrp5

expression has not been fully characterized, however, efflux of adefovir, an anti-viral compound, from rat brain has been correlated with Mrp5 expression (Dallas et al., 2004).

In mouse, highest expression of Mrp6 is in liver, with minor expression in proximal portions of the intestine as well (Fig 2). Similarly, humans and rats have high MRP6 expression in liver, as well as kidney and intestine (Kool et al., 1999; Madon et al., 2000). Whereas expression of MRP6 is consistently high in liver of all three species, the functional significance of this expression is not understood. Furthermore, the significance of MRP6 expression in intestine and kidney has not been revealed, yet mutations in MRP6 are known to be associated with pseudoxanthoma elasticum (Bergen et al., 2000). MRP6 is functionally quite different from other Mrps, with poor affinity for other Mrp substrates (Madon et al., 2000).

Mrp7 expression in mice was high in testes, with significant intestinal expression, and moderate expression in ovary and placenta. Humans also have highest expression of MRP7 in testes, with moderate expression in skin (Hopper et al., 2001). MRP7 was only detectable in these two human tissues by RT-PCR, suggesting minimal overall expression (Hopper et al., 2001; Chen et al., 2003). Much like other MRPs, MRP7 transports anticancer compounds, estrogen-glucuronides, and leukotrienes (Hopper et al., 2001; Chen et al., 2003).

MRP9 expression is highly expressed in mouse and human testes, yet expression is unknown for rats. Tissue distribution of mouse Mrp9 indicates sole expression in testes, with virtually no mRNA expression observed in other tissues (Fig 2). This is in agreement with previous work showing Mrp9 to be highly expressed in seminiferous tubules in mice (Shimizu et al., 2003). Functional aspects of MRP9 have not been

elucidated, but expression of MRP9 was shown to be high in human testes and in breast tissue (Bera et al., 2002).

Several genes involved in drug disposition exihibit gender-predominant expression patterns. For example, a one-fold higher expression of Mdr1b in female, as compared to male, kidney was observed previously (Schinkel et al., 1994). Furthermore, gender-specific Mrp expression patterns and alterations in drug disposition have not been reported. However, examples of gender differences in renal excretion of organic anions or cations are numerous, including para-aminohippuric acid and furosemide (Cerrutti et al., 2002). The potential involvement of gender specific Mrp expression could lead to altered efflux into urine.

Female-predominant expression of some Mrp transporters was observed in mice. Most notable were female-predominant expression of Mrp4 in liver and kidney, as well as marked differences in Mrp3 expression in liver, where expression was 14-fold higher in females than males (Fig 1). Although higher hepatic expression of Mrp1 was noted in females, the functional significance is questionable because of its low expression in liver.

The ontogeny study illustrates expression patterns in liver as a function of developmental age (Fig 3). In liver, Mrp2 was expressed at adult levels at birth, however, Mrp3 and Mrp6 expression increased during the first few weeks of life. In contrast, rat Mrp2 and Mrp4 mRNA and protein increase gradually over time, with rat Mrp4 exhibiting male-predominant expression in liver (Johnson et al., 2002; Chen and Klaassen, 2004). Mouse Mrp4 also has a unique pattern of development in liver, with maximal expression at birth, and decreasing during the first 10 days of age to adult levels.

Mrp6 is not expressed until 10 days of age, when the mRNA levels are almost three-fold higher than in adult mice.

There are several Mrps expressed in kidney, including Mrps 1-6. The renal ontogeny of these transporters can be divided into three expression patterns: 1) Mrp1 expression remains relatively constant from birth to adulthood, 2) expression of Mrp2, 3, and 4 increase during the first few weeks of age, and 3) highest expression of Mrp5 is seen at birth, and expression decreased during the first few weeks (Fig 4 and 5). The majority of Mrps were not expressed at adult levels in newborn animals, thus suggesting that Mrp substrates would not be readily excreted by the kidney.

Several gender-predominant patterns of expression were observed in kidney. Mrp1, 3, and 4 all showed female-predominant expression by 6 weeks of age (Fig 4 and 5). Male expression of renal Mrp1 and Mrp3 was similar to adult female levels at 3 weeks of age, but then expression in males decreased markedly (Fig 5). In contrast, Mrp4 expression increased in female kidneys after 30 days of age, whereas in males, it remained relatively constant.

In conclusion, highest expression of the Mrp family members was observed as follows: Mrp1 in testes, ovary, and placenta; Mrp2 in intestine, followed by liver and kidney; Mrp3 in large intestine; Mrp4 in kidney; Mrp5 in brain, followed by lung and stomach; Mrp6 in liver; Mrp7 in testes, intestine, and kidney; and Mrp9 solely in testes. Expression of several Mrps in reproductive tissues was high, suggesting a role in transport of hormones or other endogenous substrates. Furthermore, significant gender differences in Mrp1, Mrp3 and Mrp4 expression may lead to altered disposition of chemicals in kidney. The ontogeny data demonstrate that several Mrps do not exhibit

mature expression until one month of age or later, suggesting slower xenobiotic elimination during postnatal development. Taken together, these data create a foundation that describes tissue distribution, ontogeny, and gender specific expression of Mrps in mouse, which will be useful in understanding pharmacokinetic data in mice, and in extrapolation of data from mice to humans.

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Footnotes:

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Figure 1. Tissue distribution of Mrp1-4 mRNA in twelve mouse tissues: liver, kidney, lung, stomach, duodenum, jejunum, ileum, large intestine, brain, testes, ovary, and placenta. Data are quantified by the bDNA assay and expressed as mean relative light units ± S.E.M. Asterisks (*) represent statistically significant differences between male and female mice ($P<0.05$).

Figure 2. Tissue distribution of Mrp5, 6, 7 and 9 mRNA in twelve mouse tissues: liver, kidney, lung stomach, duodenum, jejunum, ileum, large intestine, brain, testes, ovary, and placenta. Data are quantified by the bDNA assay and expressed as mean relative light units ± S.E.M. Asterisks (*) represent statistically significant differences between male and female mice ($P<0.05$).

Figure 3. Ontogeny of Mrp2, 3, 4, and 6 mRNA expression in male and female mouse liver. Livers from male and female mice were removed on prenatal day -2, and postpartum day 0, 5, 10, 15, 23, 30, 35, 40, and 45. All data expressed as mean relative light units ± S.EM. for a minimum of five male and five female mice in each age group. Asterisks (*) denote a statistical significance between male and female mice ($P<0.05$).

Figure 4. Ontogeny of Mrp1, 2, and 3 expression in male and female mouse kidney. Kidneys from male and female mice were removed on prenatal day -2, and postpartum day 0, 5, 10, 15, 22, 30, and 45. All data expressed as mean relative light units ± S.EM. for a minimum of five male and five female mice in each age group. Asterisks (*) denote a statistical significance between male and female mice ($P<0.05$).

Figure 5. Ontogeny of Mrp4, 5, and 6 expression in male and female mouse kidney. Kidneys from male and female mice were removed on prenatal day -2, and postpartum day 0, 5, 10, 15, 22, 30, and 45. All data expressed as mean relative light units ± S.EM. for a minimum of five male and five female mice in each age group. Asterisks (*) denote a statistical significance between male and female mice ($P<0.05$).

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Table 1. Nomenclature and Accession Numbers for Mouse Mrps.

Mrp nomenclature	ABC nomenclature	Accession Number	bDNA Target Sequence
Mrp1	Abcc1	NM_008576	2554-2898 bp
Mrp2	Abcc2	NM_013806	2497-2921 bp
Mrp3	Abcc3	NM_029600	2204-2610 bp
Mrp4	Abcc4	XM_139262	22-368 bp
Mrp5	Abcc5	NM_013790	686-1219 bp
Mrp6	Abcc6	NM_018795	4023-4397 bp
Mrp7	Abcc10	AF406642	4582-4956 bp
Mrp9	Abcc12	NM_172912	287-702 bp

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Table 2. Branched DNA Probesets for the Mouse Mrp Family. Capture extenders (CE), label extenders (LE) , and blockers (BL) serve in the branched DNA assay to 1) bind the mRNA to the well, 2) create a tree of alkaline phosphatase molecules to produce luminescence when substrate is added, and 3) block non-specific interactions between the probes and the mRNA transcript, respectively.

Mrp1	Target Sequence	Function	Sequence
	2554-2574	CE	ccggtctagcagctcctgataTTTTTctcttggaaagaaagt
	2611-2629	CE	ccaggtcctgctcagcgttTTTTTctcttggaaagaaagt
	2712-2731	CE	gatgcctctgcaggtgctttTTTTTctcttggaaagaaagt
	2772-2792	CE	agttcggctatgctgctgtgtTTTTTctcttggaaagaaagt
	2793-2812	CE	ccttagctccagccttctgcTTTTTctcttggaaagaaagt
	2575-2592	LE	ctcagcgaaggccccatcTTTTTaggcataggacccgtgtct
	2593-2610	LE	ggcataggtgcgcaggaaTTTTTaggcataggacccgtgtct
	2630-2650	LE	tgacactgtcatcctccgaggTTTTTaggcataggacccgtgtct
	2651-2672	LE	tttgactccttccctgaaccacTTTTTaggcataggacccgtgtct
	2673-2690	LE	atcccattttccaccggcTTTTTaggcataggacccgtgtct
	2691-2711	LE	cctacggtgtctgtcaccagcTTTTTaggcataggacccgtgtct
	2753-2771	LE	tgctggctggtatccccacTTTTTaggcataggacccgtgtct
	2813-2834	LE	tccattagcttccacgtctcctTTTTTaggcataggacccgtgtct
	2835-2854	LE	ctgtctgggccttgtctgctTTTTTaggcataggacccgtgtct
	2855-2874	LE	cactgacagctgcacctgccTTTTTaggcataggacccgtgtct
	2875-2898	LE	aatggccttcatgtagttccagtaTTTTTaggcataggacccgtgtct
	2732-2752	BL	tgtgggaagacgagttgctga
Mrp2	**Target Sequence**	**Function**	**Sequence**
	2497-2518	CE	tctctaagatggtgcctttcccTTTTTctcttggaaagaaagt
	2543-2566	CE	tcttagcgaacactcctttcttgtTTTTTctcttggaaagaaagt
	2591-2609	CE	gcctctccttcaggtcccgTTTTTctcttggaaagaaagt
	2697-2718	CE	gctgttctcccttctcatggtcTTTTTctcttggaaagaaagt
	2738-2756	CE	ctgccggacctagagctgcTTTTTctcttggaaagaaagt
	2875-2900	CE	ttcaggtagatggagaactttaccttTTTTTctcttggaaagaaagt
	2519-2542	LE	ccatcaggtcactataggatccctTTTTTaggcataggacccgtgtct
	2567-2590	LE	aatgcttcatgaatgtcttccagtTTTTTaggcataggacccgtgtct
	2610-2633	LE	tcctcactgtcgttatcgactgtaTTTTTaggcataggacccgtgtct

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	2634-2654	LE	agcccacagtcaccatcctctTTTTTaggcataggacccgtgtct
	2655-2676	LE	aatttcctccacagttgggatcTTTTTaggcataggacccgtgtct
	2757-2774	LE	agggacttcccacgcctgTTTTTaggcataggacccgtgtct
	2775-2799	LE	acttttaattttcaaggagcttttgTTTTTaggcataggacccgtgtct
	2800-2823	LE	ctccttcttattcaaggcattcacTTTTTaggcataggacccgtgtct
	2824-2852	LE	ttaattagtttttgtcctttcactacttcTTTTTaggcataggacccgtgtct
	2853-2874	LE	cccagtttccacaaattccttcTTTTTaggcataggacccgtgtct
	2901-2921	LE	catcctaccgcctgcagatatTTTTTaggcataggacccgtgtct
	2677-2696	BL	aaggaagctgcatcgtcagg
Mrp3	**Target Sequence**	**Function**	**Sequence**
	2246-2265	CE	tctcatgaactgcttgcggaTTTTTctcttggaaagaaagt
	2290-2307	CE	ccggttctggacctccccTTTTTctcttggaaagaaagt
	2379-2405	CE	gtctctgctatctcctctttgattaatTTTTTctcttggaaagaaagt
	2496-2513	CE	ccgatagcagctgcgcttTTTTTctcttggaaagaaagt
	2552-2571	CE	gttctgctggccatgttcctTTTTTctcttggaaagaaagt
	2204-2225	LE	ttgtctgtcaggtctgtgtgggTTTTTaggcataggacccgtgtct
	2226-2245	LE	cctcgtagatggctggctcaTTTTTaggcataggacccgtgtct
	2338-2356	LE	tcaccagcgcctccttctcTTTTTaggcataggacccgtgtct
	2357-2378	LE	gcgccagtctccttagtctttgTTTTTaggcataggacccgtgtct
	2406-2427	LE	cacactcagcttcacattgcctTTTTTaggcataggacccgtgtct
	2514-2533	LE	cgctgagccacacattggctTTTTTaggcataggacccgtgtct
	2572-2591	LE	ccgagccttacggaggtcttTTTTTaggcataggacccgtgtct
	2592-2610	LE	ccctagggcggcgtagacaTTTTTaggcataggacccgtgtct
	2266-2289	BL	ttcagaagacaaggagctcatctc
	2308-233	BL	tttgtgtgtttcttgggcatagt
	2428-2451	BL	catagacttggcataatcccagta
	2452-2476	BL	agatagatagcgtggtacagagtcc
	2477-2495	BL	tggccaccatacaggaggc
	3361-3379	BL	tctccaccacccaggggc
Mrp4	**Target Sequence**	**Function**	**Sequence**
	65-82	CE	cccagctccggttctgccTTTTTctcttggaaagaaagt

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	83-102	CE	gccgagatgagggagctttTTTTTctcttggaaagaaagt
	125-145	CE	atcgatccagattttcccctcTTTTTctcttggaaagaaagt
	217-238	CE	cctcatggttccagtgaacagaTTTTTctcttggaaagaaagt
	321-344	CE	tatccatttttccaggaagatcttTTTTTctcttggaaagaaagt
	22-42	LE	gacttgatgagcgcaatcaggTTTTTaggcataggacccgtgtct
	43-64	LE	cacaattccaaccttttccctgTTTTTaggcataggacccgtgtct
	146-168	LE	ccaatttcggttgtcaagatcttTTTTTaggcataggacccgtgtct
	193-216	LE	acaggttcctgtggtatgattgacTTTTTaggcataggacccgtgtct
	260-278	LE	gctcctcgtccgtgtgctcTTTTTaggcataggacccgtgtct
	279-296	LE	cctccaaggccctccacaTTTTTaggcataggacccgtgtct
	345-368	LE	tggatccagattctgctaattcagTTTTTaggcataggacccgtgtct
	103-124	BL	gggttctgacagcctgaagagg
	169-192	BL	attttcttccttaagtcgtgaagc
	239-259	BL	attgaaggggtccaggttttt
Mrp5	**Target Sequences**	**Function**	**Sequence**
	686-704	CE	ggccaggccctgaatgctaTTTTTctcttggaaagaaagt
	874-894	CE	aagggatctgtccatgcatgaTTTTTctcttggaaagaaagt
	1026-1046	CE	ggcaggtgcttccaaagagagTTTTTctcttggaaagaaagt
	1047-1069	CE	ggaggagccttgttcttgattctTTTTTctcttggaaagaaagt
	1112-1134	CE	gattttcccggtatctcatttctTTTTTctcttggaaagaaagt
	1158-1177	CE	ttgggcttgatggtgaaggaTTTTTctcttggaaagaaagt
	1199-1219	CE	gacttccctgaccctgttcgtTTTTTctcttggaaagaaagt
	705-726	LE	ttttgttgtaggcatggatggtTTTTTaggcataggacccgtgtct
	727-75	LE	gatacctgtgtaaaaactcctgccTTTTTaggcataggacccgtgtct
	751-771	LE	ggttgtcatccaggagctcctTTTTTaggcataggacccgtgtct
	793-812	LE	cagccacctcattgcacaggTTTTTaggcataggacccgtgtct
	813-83	LE	gaggtccagccgcactgcTTTTTaggcataggacccgtgtct
	831-851	LE	ggtaatcagggcaatgctgatTTTTTaggcataggacccgtgtct
	895-912	LE	caagccccgcataggctgTTTTTaggcataggacccgtgtct
	913-933	LE	actgcacagcgtaggagatggTTTTTaggcataggacccgtgtct
	958-978	LE	ctgtctccgatgccagtctgaTTTTTaggcataggacccgtgtct
	1088-1111	LE	gcattctcaaaggttacttctcccTTTTTaggcataggacccgtgtct

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	1135-1157	LE	cactttcttaaggaccagagggaTTTTTaggcataggacccgtgtct
	1178-1198	LE	cccacaatgcctatcttttccTTTTTaggcataggacccgtgtct
	772-792	BL	tgaacaggaaaaagggagcct
	852-873	BL	gaacaatcatcaggccagtggt
	934-957	BL	cggtgaactggaatagtccagtta
	979-998	BL	cacggaagtgaaccgtgctt
	999-1025	BL	agtcttgatatagtggttgatcctctc
Mrp6	**Target Sequence**	**Function**	**Sequence**
	4104-4119	CE	gcgctgcccagatgccTTTTTctcttggaaagaaagt
	4160-418	CE	cactcatattgcagctggccaTTTTTctcttggaaagaaagt
	4238-4256	CE	ggttttccggagaagggctTTTTTctcttggaaagaaagt
	4257-4278	CE	cctcgtccaggatgaggatctgTTTTTctcttggaaagaaagt
	4318-4333	CE	cgctccagggccgcctTTTTTctcttggaaagaaagt
	4360-4377	CE	gcaggcggtgagcgataaTTTTTctcttggaaagaaagt
	4023-4042	LE	gggtcctgagggatgatggtTTTTTaggcataggacccgtgtct
	4043-4062	LE	gagagcctgggaacaggacaTTTTTaggcataggacccgtgtct
	4063-4081	LE	aggtccaggttcatccgcaTTTTTaggcataggacccgtgtct
	4120-414	LE	ccttgagctgcactgtctccaTTTTTaggcataggacccgtgtct
	4141-4159	LE	ggcaggctggtcacgaaggTTTTTaggcataggacccgtgtct
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	4279-4297	LE	gggtccacagaggcagtcgTTTTTaggcataggacccgtgtct
	4298-4317	LE	gcatctgcatctccgtccctTTTTTaggcataggacccgtgtct
	4334-4359	LE	gcagtaaggtacactgtgtaaaccagTTTTTaggcataggacccgtgtct
	4378-4397	LE	ggcacagtccatcacggagcTTTTTaggcataggacccgtgtct
Mrp7	**Target**	**Function**	**Sequence**
	4603-4619	CE	cgttgggctccagcagcTTTTTctcttggaaagaaagt
	4659-4676	CE	tgagctcggccagctccaTTTTTctcttggaaagaaagt
	4697-4717	CE	gaacaggaaaggctcctgaggTTTTTctcttggaaagaaagt
	4892-4912	CE	tgtaagaagagcccttgccagTTTTTctcttggaaagaaagt

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	4677-4696	LE	gatgacagccagctgggatcTTTTTaggcataggacccgtgtct
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	4937-4956	LE	tccacacttgctgtggcctcTTTTTaggcataggacccgtgtct
	4582-4602	BL	cggaagagcaccagaaacagg
	4638-4658	BL	gctggctggtgtccacattgt
Mrp9	**Target**	**Function**	**Sequence**
	306-327	CE	ttcccaaaggatctggaatctcTTTTTctcttggaaagaaagt
	351-368	CE	cccagggaggccttctcaTTTTTctcttggaaagaaagt
	471-492	CE	ggtaatgtgctggaggatttggTTTTTctcttggaaagaaagt
	593-613	CE	ggatcgctgtgcggtaatttaTTTTTctcttggaaagaaagt
	634-655	CE	gcaggttttcgaagattagcgtTTTTTctcttggaaagaaagt
	287-305	LE	ttggcgttgatgtccgatgTTTTTaggcataggacccgtgtct
	369-387	LE	gaatttccagaccacgcggTTTTTaggcataggacccgtgtct
	410-431	LE	cacaggatgttggctacaacgtTTTTTaggcataggacccgtgtct
	432-450	LE	caaggctgccatgacgatgTTTTTaggcataggacccgtgtct
	451-470	LE	tgaatgagaactgtcggcccTTTTTaggcataggacccgtgtct
	493-513	LE	gatgtgtccggaggagatgctTTTTTaggcataggacccgtgtct
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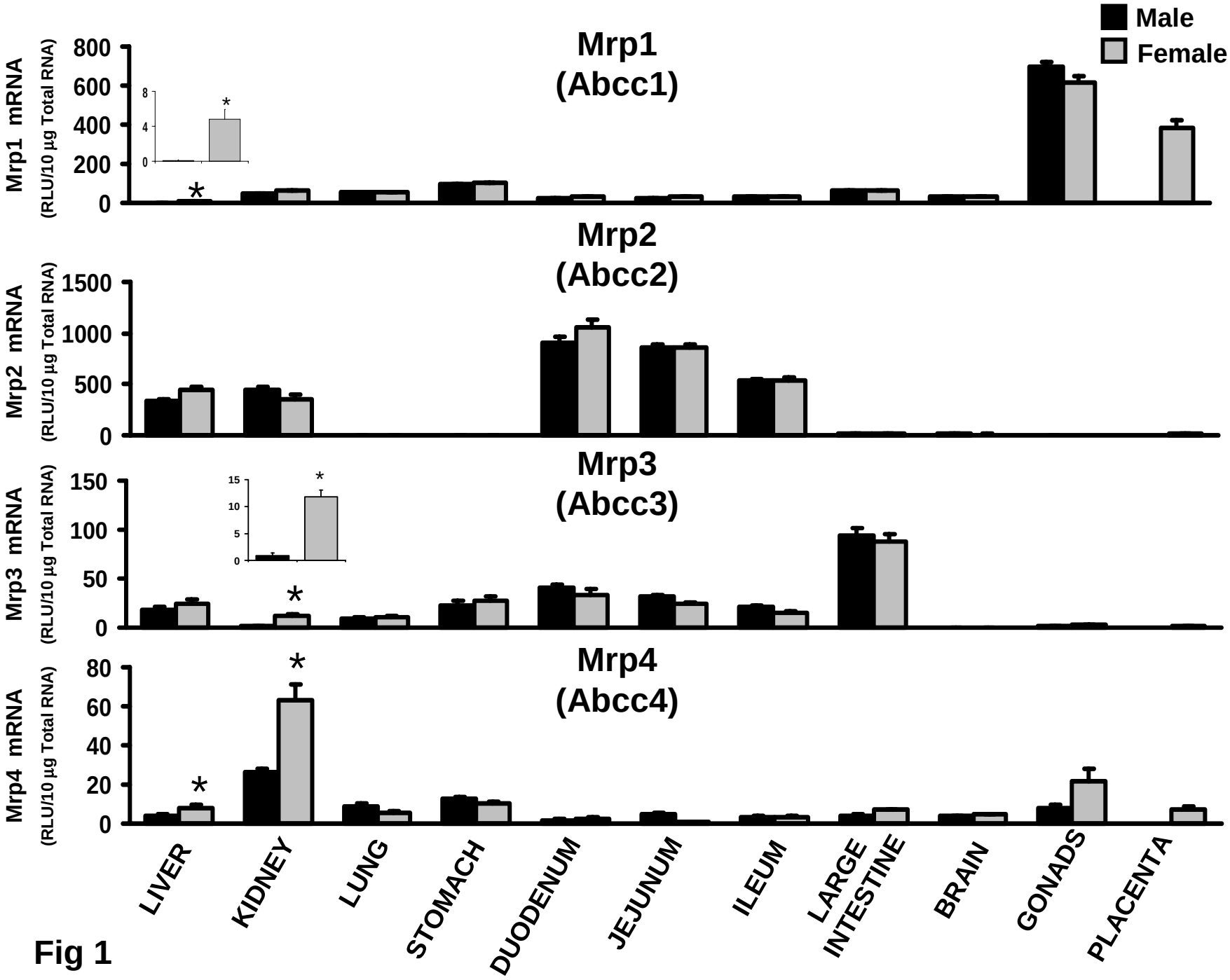

Fig 1

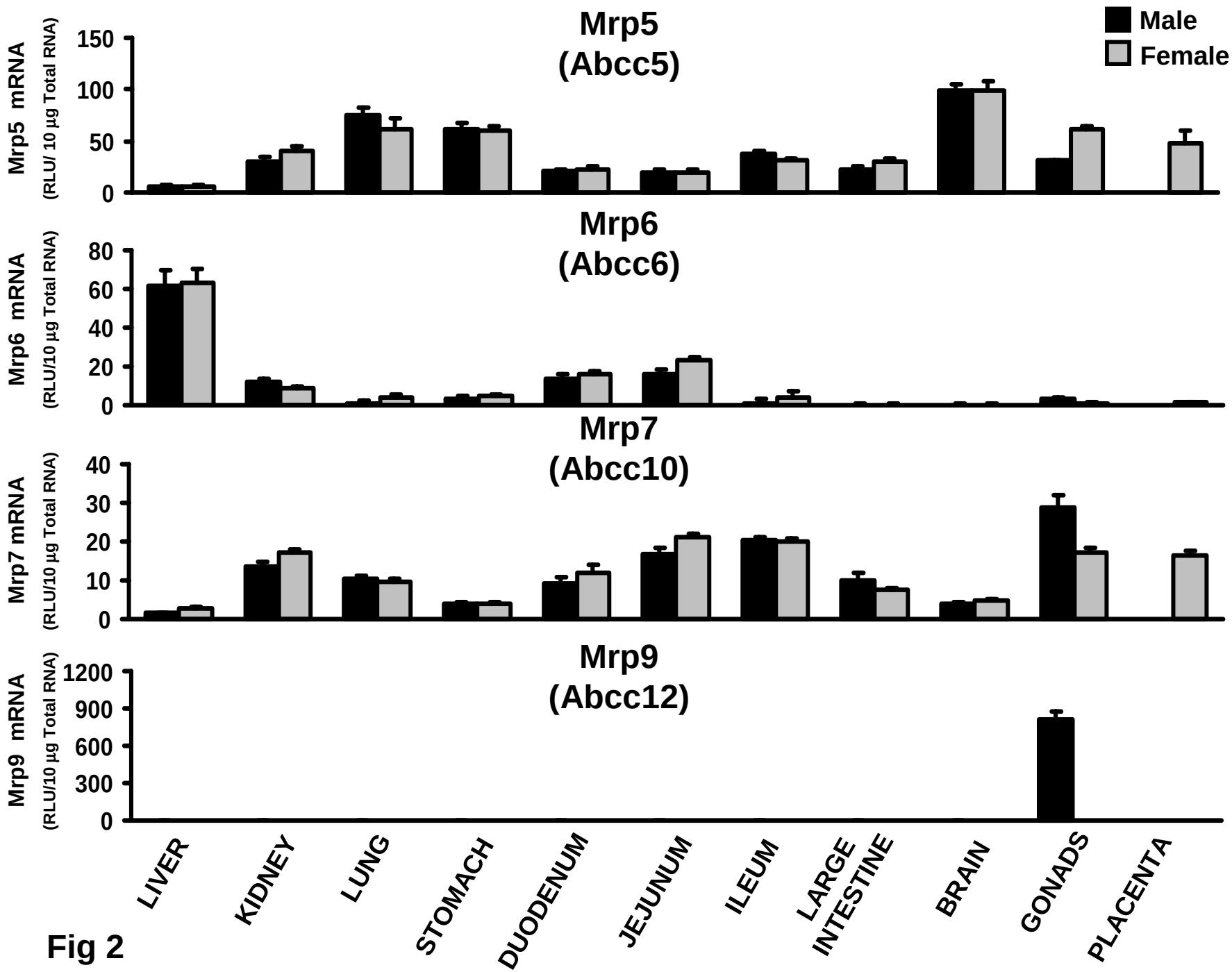

Fig 2

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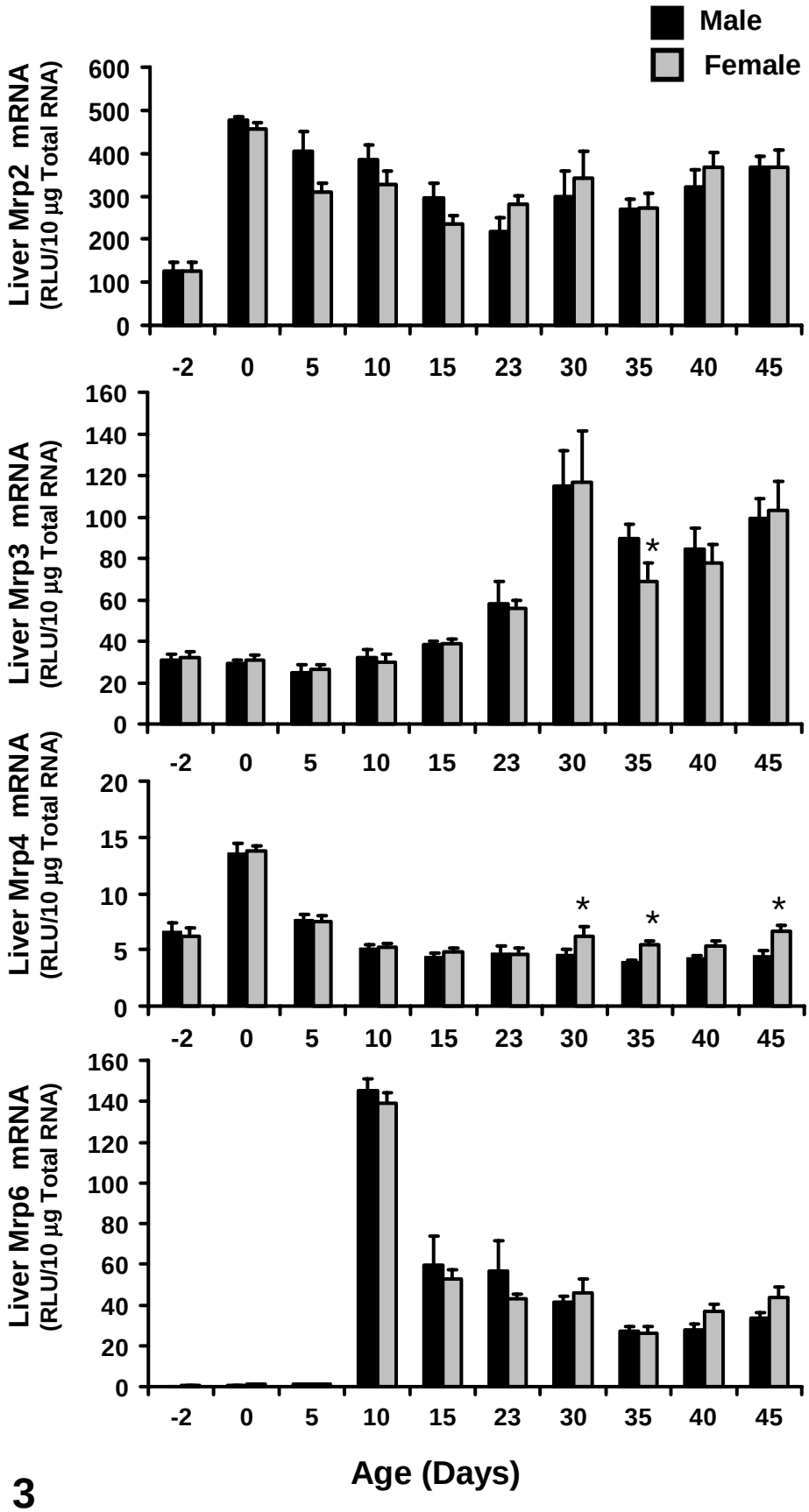

Fig 3

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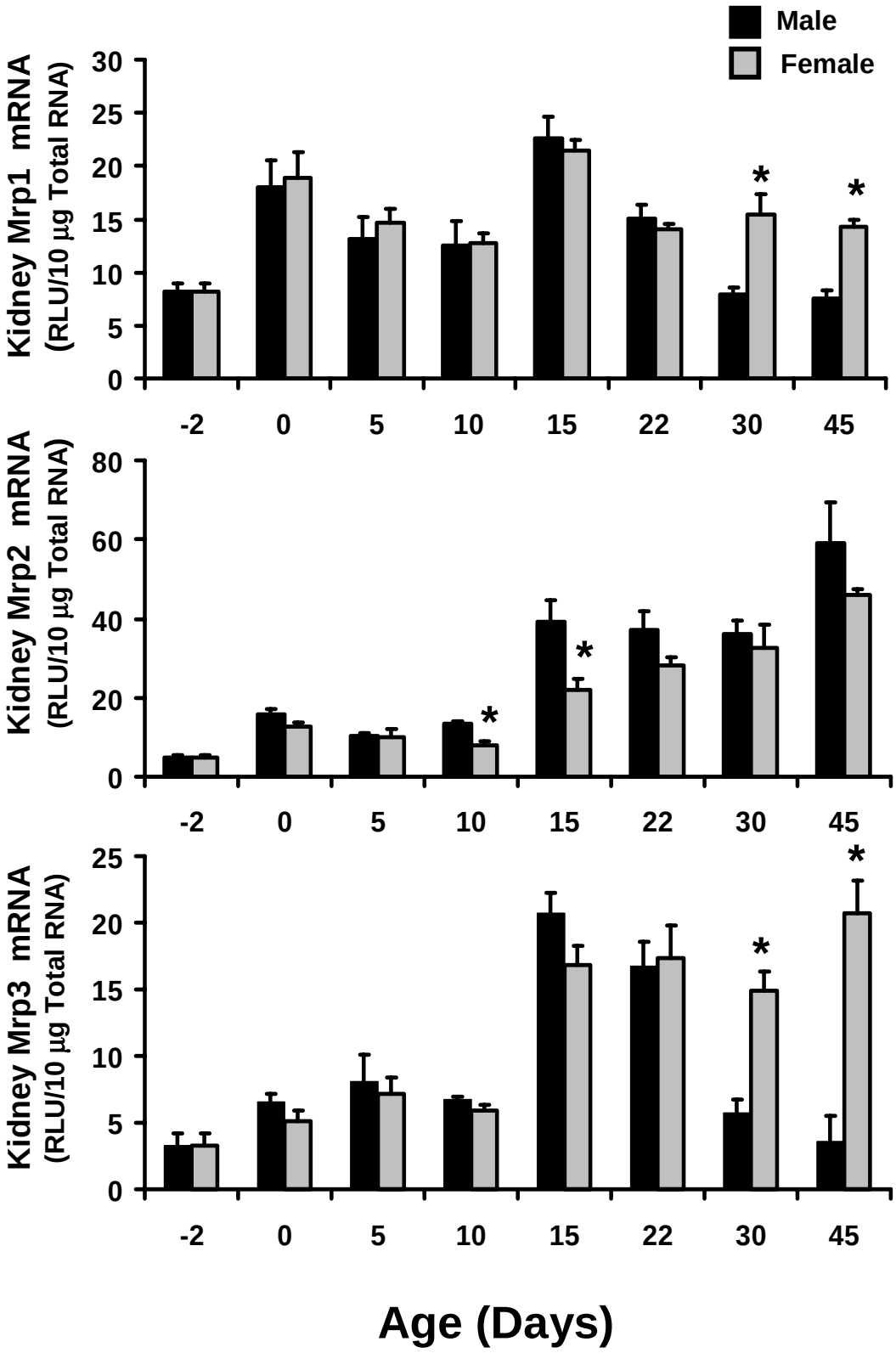

Fig 4

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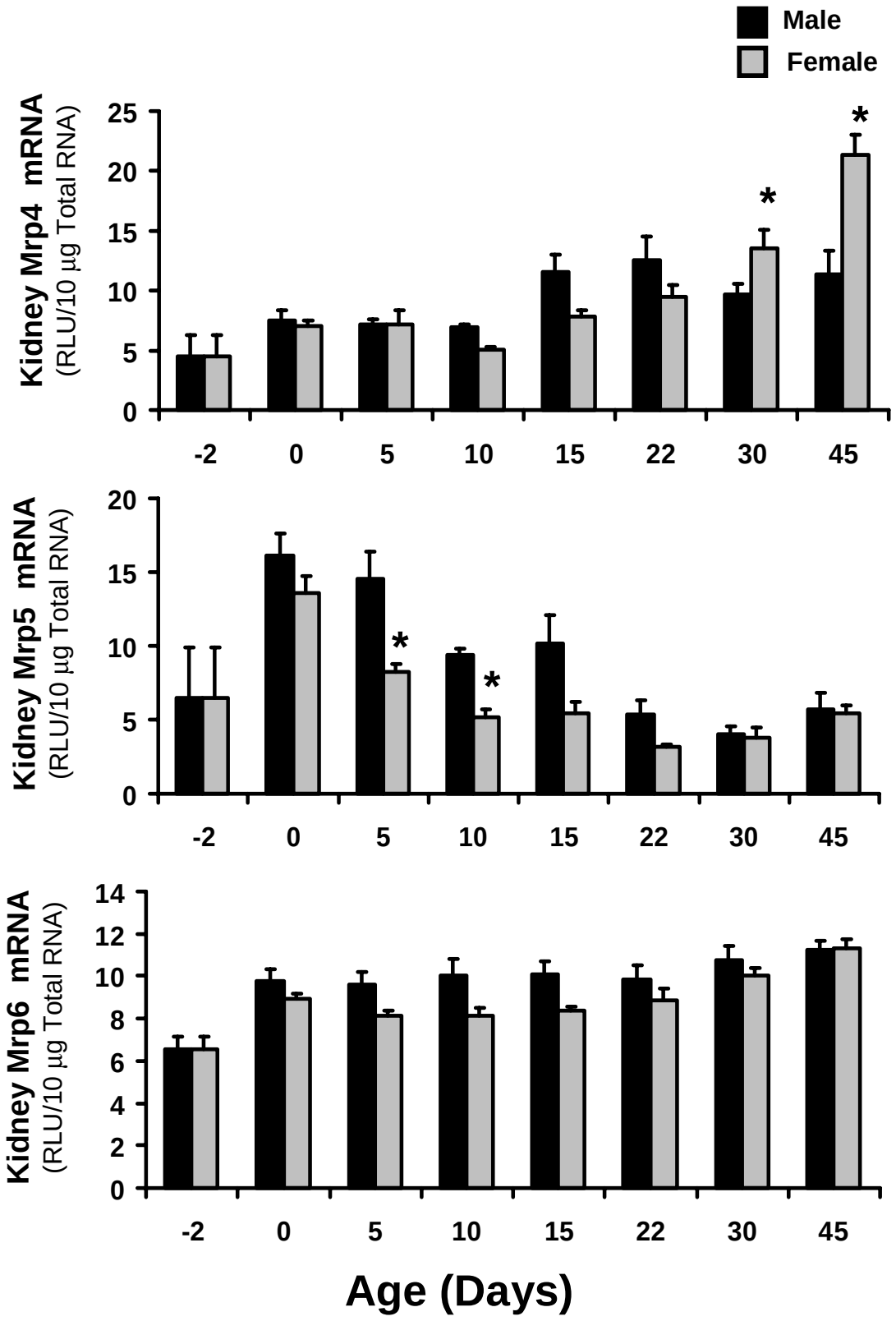

Fig 5