Gender Differences in mRNA Expression of ATP-Binding Cassette Efflux and Bile Acid Transporters in Kidney, Liver, and Intestine of 5/6 Nephrectomized Rats

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ABSTRACT:

ATP-binding cassette (ABC) transporters including multidrug resistance proteins (Mdr), multidrug resistance-associated proteins (Mrp), and breast cancer resistance protein (Bcrp/Abcg2) play major roles in tissue defense. Abcg5/g8 is essential in cholesterol efflux. The present study was aimed at elucidating alteration in expression of these transporters and bile-acid transporters during chronic kidney disease (CKD) and underlying molecular mechanisms. Seven weeks after 5/6 nephrectomy (Nx), mRNA expression of 16 aforementioned transporters in kidney, liver, jejunum, and large intestine of male and female Nx rats was quantified with the branched DNA signal amplification assay. In Nx males, intestinal expression of all the transporters remained unchanged; hepatic expression of most transporters was not altered, except increases in Mdr1a, Mrp3, and Abcg8. In male remnant kidneys, kidney-predominant transporter Abcg2 decreased and correlated with CKD severity, whereas Mdr1b, Mrp3, and ileal bile-acid transporter increased and correlated with CKD severity. Such changes were largely absent inNx females. Renal alterations of these transporters correlated with increases of cytokines and/or decreases of nuclear receptors such as estrogen receptor α and glucocorticoid receptor. Renal protein expression of Mrp2 increased, whereas that of Mrp4 remained unchanged in both genders of Nx rats. Treatment of rat proximal tubule NRK-52E cells with interleukin (IL)-1β and IL-6 increased Mrp3 mRNA expression. In conclusion, during CKD, renal expression of many ABC transporters was altered at the transcriptional level, whereas hepatic mRNA expression of most ABC transporters remained unchanged. Down-regulation of steroid hormone receptors and increase of inflammatory cytokines may contribute to alteration of transporter gene expression in kidney during CKD.

The incidence of chronic kidney disease (CKD) is increasing steadily because of increases in the etiological factors of CKD, namely, aging, diabetes, hypertension, and renal disease. Kidneys are vital in maintaining metabolic homeostasis through transporter-mediated active secretion and/or reabsorption of numerous endobiotic and xenobiotics. CKD causes a loss of metabolic homeostasis, affecting not only kidney but also liver and intestine (Guevin et al., 2002; Leblond et al., 2002; Nolin et al., 2003). Studies indicate that CKD patients need on average more than seven drugs to control their kidney and comorbid diseases. Excessive and/or inappropriate uses of drugs increase the risk of adverse effects and health care cost (Owen, 2003). Therefore, it is important to understand alteration of transporters during CKD and its underlying molecular mechanisms.

Members of efflux transporters, including multidrug resistance protein (MDR), multidrug resistance-associated protein (MRP), and breast cancer resistance protein (BCRP/ABCG2), play key roles in tissue defense by transporting metabolic waste and toxic chemicals out of cells (Bodo et al., 2003; Leonard et al., 2003; Leslie et al., 2005). MDR1 (P-glycoprotein) and ABCG2 preferentially efflux large, hydrophobic positively charged molecules, whereas MRP pump out both hydrophobic uncharged molecules and water-soluble anionic compounds (Bodo et al., 2003).

The human MRP family has nine members, MRP1 through MRP9 (Kruh and Belinsky, 2003); rat orthologs have been identified for all the human MRP genes except MRP7 and MRP8. MRP1 through MRP3 confer resistance to various anticancer drugs and transport organic anions, such as glutathione and glucuronide conjugates of xenobiotics and endobiotics (Kruh and Belinsky, 2003). MRP4 and MRP5 efflux purine analogs (e.g., 6-mercaptopurine and thioguanine) and nucleoside-based antiviral drugs [e.g., 9-(2-phosphonylmethoxyethyl) adenine] (Reid et al., 2003). MRP4 and MRP5 also transport cyclic nucleotides cAMP and cGMP (Jedlitschky et al., 2000). Despite the importance of MRP, only the expression of MRP2 during CKD has been investigated (Laouri et al., 2001).

ABCG2/BCRP is critical in preventing cytotoxicity induced by dietary phototoxin or hypoxia via preventing cellular accumulation of phototoxin or heme (Jonker et al., 2002; Krishnamurthy et al., 2004).
ABCG2 also transports glucuronide and sulfate conjugates (Mizuno et al., 2004; Zamek-Gliszczynski et al., 2006). ABCG2/BCRP expression is high in rat kidney and intestine (Tanaka et al., 2005). Nothing is known regarding ABCG2/BCRP expression during CKD. Dysfunction of lipid metabolism is a characteristic of CKD (Li and Vaziri, 2002). In liver and intestine, ABCG5 and ABCG8 efflux is known regarding ABCG2/BCRP expression during CKD. ABCG2 also transports glucuronide and sulfate conjugates (Mizuno et al., 2004; Zamek-Gliszczynski et al., 2006). ABCG2/BCRP expression is high in rat kidney and intestine (Tanaka et al., 2005). Nothing is known about alteration of these bile acid transporters during CKD, although marked alteration in blood and urine profiles of bile acids has been reported (Jimenez et al., 2002).

CKD has profound effects on expression of certain xenobiotic metabolizing enzymes and transporters. Some specific isoforms of cytochromes P450 are selectively changed in liver of CKD rats (Leblond et al., 2000, 2001). Sulfation appears normal, whereas glucuronidation and acetylation are decreased in CKD patients. Interestingly, renal expression of the peptide transporter, Pep2, increased at 2 weeks but decreased at 16 weeks after 5/6 nephrectomy (Nx) (Inui et al., 2000; Takahashi et al., 2001), suggesting that renal transporter expression is altered with CKD progression.

There is a gender difference in CKD progression; female CKD patients and animals progress slower to end-stage CKD (Ishikawa et al., 2000; Takahashi et al., 2001). Sulfation appears normal, whereas glucuronidation and acetylation are decreased in CKD patients. Interestingly, renal expression of the peptide transporter, Pep2, increased at 2 weeks but decreased at 16 weeks after 5/6 nephrectomy (Nx) (Inui et al., 2000; Takahashi et al., 2001), suggesting that renal transporter expression is altered with CKD progression.

The mRNA expression of these transporters was first determined in pooled samples of total RNA from kidneys, livers, and intestines by the branched DNA (bDNA) signal amplification assay. When apparent gender differences or differences between sham control and Nx group were observed in the pooled samples, individual RNA samples were analyzed for statistical analysis. Renal mRNA expression of these transporters was correlated with CKD markers and various transcription factors, cytokines, and hormones reported previously (Lu et al., 2006) to delineate the association of altered expression of each transporter with CKD severity, and to elucidate the potential regulatory mechanism of gene expression of each transporter.

### Materials and Methods

#### Animals

The Nx and sham-operated adult male and female Sprague-Dawley rats were directly purchased from Charles River Laboratories, Inc. (Wilmington, MA) (Lu et al., 2006). Rats (175–225 g) were randomly allotted to Nx or sham operation groups.

#### RNA Extraction

Total tissue RNA was extracted using RNA-Bea reagent (Tel-Test, Inc., Friendswood, TX) according to the manufacturer’s protocol. Each RNA pellet was redissolved in 0.1 to 0.2 ml of diethyl pyrocarbonate-treated water. RNA concentrations were quantified by UV absorption at 260 nm. RNA integrity was confirmed by agarose gel electrophoresis of 22°C with alternating 12-h light/dark cycles and were allowed water and rat chow ad libitum (Teklad, Harlan, Indianapolis, IN). Seven weeks after Nx or sham operation, the remnant kidney, liver, and intestine were collected, weighed, rinsed with cold 0.9% saline, and snap-frozen in liquid nitrogen for storage at −80°C.

#### bDNA Signal Amplification Assay

The bDNA assay is a high-throughput and quantitative method for mRNA quantification (Canales et al., 2006) that has been used extensively in our laboratory (Leazer and Klaassen, 2003; Maher et al., 2005a; Lu et al., 2006). The mRNA of genes examined was quantified using Quantigene bDNA signal amplification kit (Bayer Diagnostics, East Walpole, MA) with modifications (Leazer and Klaassen, 2003). The probes for all the rat genes determined herein except Mrp9 have been reported previously (Leazer and Klaassen, 2003). The probes for rat Mrp9 are shown in Table 1. Total RNA (4–10 µg/well) was added to each well of a 96-well plate containing capture hybridization buffer (50 µl) and each diluted probe set (50 µl) and was allowed to hybridize to the probe set overnight at 53°C. Subsequent washing and hybridization steps were carried out following the manufacturer’s protocol. Luminescence of the 96-well plate was quantified with a Quantiplex 320 bDNA luminometer interfaced with Quantiplex Data Management software version 5.02 for data analysis. The luminescence for each well is reported as relative light units (RLU) per 4 or 10 µg of total RNA.

#### Western Blot Analysis of Renal Protein Expression of Mrp2 and Mrp4

Kidney tissues were homogenized on ice in a buffer (25 mM HEPES, pH 7.6, 1.5 mM EDTA, 10% glycerol, 1 mM DTT, 0.1 mg/ml phenylmethylsulfonyl fluoride, 0.5 M KCl, and 1% protease inhibitor mixture) in a Dounce homogenizer (Contes, Vineland, NJ) for 20 strokes. The homogenates were centrifuged at 1500g for 10 min at 4°C to remove cell debris, and the supernatant was further centrifuged at 100,000g for 30 min at 4°C. The resultant pellet, crude membrane, was resuspended in the same buffer and stored at −80°C. Protein concentrations were determined with Bradford reagent. Protein expression of Mrp2 and Mrp4 was determined by Western blot (Chen et al., 2005).
Briefly, protein samples (50 µg) were loaded to 10% SDS-polyacrylamide gel electrophoresis gels. After electrophoresis, proteins were transferred to polyvinylidene difluoride membranes, and filters were probed with a polyclonal antibody against rat Mrp2 (Buchler et al., 1996) or human MRP4 (Alexis Biochemicals, Lausen, Switzerland) (Chen et al., 2005). Signals were visualized with horseradish peroxidase-conjugated secondary antibody.

Effects of Cytokines on Mrp3 mRNA Expression in Rat Proximal Tubule Epithelial NRK-52E Cells. NRK-52E cells were maintained in Dulbecco’s modified Eagle’s medium (American Type Culture Collection, Manassas, VA) supplemented with 10% fetal bovine serum (American Type Culture Collection). Cells were seeded into a 24-well plate at 6000 cells/well and were treated with interleukin (IL)-1β or IL-6 at 0.1, 1.0, or 10 ng/ml for 48 h. Cell lysates (50 µg) were analyzed for determining mRNA expression of Mrp3 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using Quantigene bDNA signal amplification kit as described above. Cellular Mrp3 mRNA expression was normalized with GAPDH mRNA.

Statistics. Data are presented as mean ± S.E. of six or seven kidneys. Data without error bar represent mean of duplicate samples pooled from six or seven tissue samples and normalized with GAPDH mRNA. Differences between groups were determined by analysis of variance, followed by Duncan’s multiple-range test with significance set at p < 0.05. The correlation of renal mRNA expression of transporters with disease parameters of CKD, as well as renal mRNA expression with transcription factors, cytokines, and blood levels of hormones was analyzed with Correlation Matrices within Statistica for Windows release 4.5 (StatSoft, Inc., Tulsa, OK). Renal mRNA expression of transporters, cytokines, blood levels of hormones, and renal caspase-3 activity in Nx male and female rats have been reported previously (Lu et al., 2006). The disease parameters of CKD used for correlation analysis are blood urea nitrogen (BUN), urinary excretion of urea, creatinine clearance (CrCl), urinary excretion of creatinine, glomerular filtration rate, proteinuria, albuminuria, blood hemoglobin, renal caspase-3 activity, and renal protein concentration (Lu et al., 2006).

Results

Expression of Mrp in Kidney, Liver, Jejunum, and Large Intestine of Male and Female Nx Rats. Mrp1 mRNA had low expression in liver but high expression in kidney, jejunum, and large intestine. Mrp1 mRNA expression was not different in these tissues in Nx rats compared with the sham-operated control rats (Fig. 1A). However, in Nx males, renal expression of Mrp1 correlated positively with BUN (Fig. 1B) and proteinuria and negatively with blood hemoglobin (r = 0.81, 0.81, and −0.82, respectively). The functional significance of such correlation is currently unknown. Additionally, in Nx males, renal Mrp1 mRNA correlated highly with death receptor Fas mRNA (Fig. 1C).

Mrp2 mRNA was expressed high in liver but low in kidney and intestine (Fig. 1D). In both sham-operated and Nx rats, females expressed higher levels of Mrp2 mRNA in liver than males (Fig. 1D). In Nx rats, Mrp2 mRNA tended to increase in kidney, liver, and jejunum of both genders but only reached significance in kidney of Nx females (Fig. 1D). In Nx rats, renal and hepatic mRNA expression of Mrp2 did not correlate with severity of CKD.

Mrp3 mRNA was expressed the highest in large intestine and at low levels in liver and kidney with female predominance (Fig. 1E). However, in Nx rats, Mrp3 mRNA increased in kidney (+43%) and liver (+4.9-fold) of males but decreased in kidney (−26%) and remained unchanged in liver of females, resulting in abolishment of the gender difference (Fig. 1E). In Nx males, renal and hepatic Mrp3 mRNA expression correlated highly (Fig. 1F), and they correlated positively with proteinuria (Fig. 1G) (r = 0.93 and 0.87, respectively), indicating a positive association of renal and hepatic Mrp3 expression with severity of CKD. In Nx males, both renal and hepatic Mrp3 mRNA correlated positively with renal mRNA expression of IL-1β, IL-6 (Fig. 1H), and IL-10 (r = 0.80, 0.93, and 0.81 for renal Mrp3 and r = 0.72, 0.93, and 0.79 for hepatic Mrp3, respectively). In contrast, in Nx females, renal Mrp3 mRNA did not correlate with any CKD parameter but correlated positively with renal mRNA expression of NF-E2-related nuclear factors 2 (Nrf2) (Fig. 1I).

Mrp4 mRNA was expressed most abundantly in kidney and remained unchanged in Nx rats (Fig. 2A). Mrp4 mRNA expression in large intestine was slightly more in females than males (Fig. 2A). Renal Mrp4 expression did not correlate with any parameters of CKD.
Large intestine had the highest levels of Mrp5 mRNA, and Nx females had higher Mrp5 mRNA in large intestine than Nx males (Fig. 2B). In kidney, Mrp5 mRNA in sham-operated females was 90% higher than males and tended to increase in Nx males but decrease in Nx females, resulting in abolishment of gender difference in Nx rats (Fig. 2B).

Renal expression of Mrp6 mRNA correlated positively with IL-10 in both Nx males and females (r = 0.80 and 0.76, respectively) but did not correlate with any parameters of CKD in Nx rats.

Mrp6 mRNA was expressed highest in liver and remained unchanged in the four tissues of Nx rats (Fig. 2C). Moreover, renal Mrp6 mRNA did not correlate with any parameters of CKD in Nx rats.

The tissue distribution of rat Mrp9 mRNA expression has not been reported. Our search of GenBank identified rat Abcc12 mRNA (GenBank ID 40786452). Rat Mrp9 mRNA was predominantly expressed in testes and at low levels in kidney, liver, and intestine (Fig. 3A), an expression pattern similar to that in mice (Maher et al., 2005b). In Nx rats, renal Mrp9 mRNA tended to increase in males but remained unchanged in females. Data without error bars represent mean of duplicate samples pooled from six or seven tissue samples and normalized by GAPDH mRNA. * p < 0.05 compared with the same gender sham-operated group; #, p < 0.05 compared with male group with the same operation.

Expression of Mdr in Kidney, Liver, Jejunum, and Large Intestine of Male and Female Nx Rats. In sham-operated rats, Abcg5 and Abcg8 were expressed predominantly in small intestine (Fig. 5, A and B). In Nx rats, Abcg5 mRNA increased 1.4-fold and 94% in liver of males (p = 0.06) and females (p < 0.05), respectively. In Nx rats, Abcg8 mRNA increased 1.6- and 1.1-fold in liver of males and females, respectively. However, hepatic expression of Abcg5 and Abcg8 did not correlate with CKD severity. Abcg5 and Abcg8 mRNA remained unchanged in small intestine of Nx rats (Fig. 5, A and B). Similar to Mrp2 and Mdr2, there was large interindividual variation in renal expression of Mdr2 mRNA (Fig. 4C).

Abcg2 was highly expressed in kidney and intestine but was low in liver, with male predominance in kidney (Fig. 5C). In Nx rats, renal Abcg2 mRNA decreased in both genders, with the decrease more prominent in males (by 39%) than females (by 22%), resulting in abolishment of the gender difference in Nx rats (Fig. 5C). In Nx males, renal expression of Abcg2 correlated with CrCl (Fig. 5D) and BUN (r = −0.81). Additionally, renal Abcg2 mRNA correlates with estrogen receptor α (ERα) mRNA (Fig. 5E) in Nx males, whereas it correlated highly with Nrf2 mRNA (r = 0.93) in Nx females.
Expression of Bile Acid Transporters in Kidney, Liver, Jejunum, and Large Intestine of Male and Female Nx Rats. In sham-operated rats, Ntcp (Fig. 6A) and Bsep mRNA (Fig. 6B) were exclusively expressed in liver. However, in Nx rats, there was a large interindividual variation in renal expression of Ntcp and Bsep mRNA; increases of renal Ntcp and Bsep mRNA were only statistically significant in Nx females (Fig. 6, A and B). Hepatic mRNA expression of Ntcp and Bsep remained unchanged in Nx rats.

The Ibat mRNA was predominantly expressed in ileum (data not shown) and moderately expressed in kidney and jejunum (Fig. 6C). Renal basal expression of Ibat mRNA was higher in females than males (Fig. 6C); however, in Nx rats, renal Ibat mRNA decreased in females but increased in males, resulting in a reversed higher renal Ibat mRNA in Nx males than Nx females (Fig. 6C). In Nx rats, Ibat mRNA remained unchanged in jejunum (Fig. 6C) and ileum (data not shown). In Nx males, renal Ibat mRNA correlated positively with BUN and proteinuria \( r = 0.83 \) and 0.93, respectively, positively with renal mRNA expression of IL-6 and IL-10 \( r = 0.83 \) and 0.98, respectively), but negatively with ER\( \alpha \) \( r = -0.94 \).

**Protein Expression of Mrp2 and Mrp4 in Remnant Kidneys of Male and Female Nx Rats.** Renal protein expression of Mrp2 and Mrp4 was analyzed by Western blot. Renal basal protein expression of Mrp2 was very low in sham-operated males but tended to be higher in sham-operated females; Mrp2 protein expression increased in remnant kidneys of both Nx males and Nx females (Fig. 7). In contrast, renal protein expression of Mrp4 remained unchanged in both genders of Nx rats (data not shown), consistent with the lack of change in renal Mrp4 mRNA expression (Fig. 2). Protein expression of Mrp3 was not analyzed because of the lack of specific antibody for rat Mrp3.

**Effects of Cytokines on Mrp3 mRNA Expression in Rat Proximal Tubule NRK-52E Cells.** To investigate the putative inductive effects of cytokines on renal expression of Mrp3 (Fig. 3), rat proximal tubule NRK-52E cells were treated with cytokines IL-1\( \beta \) or IL-6 for 48 h. As shown in Fig. 8, the basal Mrp3 mRNA expression was very low in NRK-52E cells; both IL-1\( \beta \) and IL-6 induced Mrp3 mRNA expression at doses of 1.0 and 10 ng/ml, whereas only IL-6...
induced Mrp3 expression at 0.1 ng/ml. The inducing effect of IL-6 on Mrp3 expression was stronger than IL-1β.

**Discussion**

The present study shows that in the CKD model of Nx rats, renal mRNA expression of ABC transporters is altered with large interindividual variations; hepatic mRNA expression of most ABC transporters remains unchanged, except that there are increases in Mrp3, Mdr1a, and Abcg5/g8 mRNA expression. Intestinal mRNA expression of all the transporters examined remains unchanged.

The Mrp family transports structurally diverse substrates. The increase of renal and hepatic Mrp2 expression during CKD is consistent with a previous report (Laouari et al., 2001). Mrp2 is localized on the canalicular membrane of hepatocytes and apical membrane of renal tubule cells and intestinal epithelial cells, responsible for excretion of a large variety of compounds. The higher hepatic Mrp2 expression in Nx females may contribute to renoprotection in females during CKD.

Mrp3 transports a wide range of chemicals, such as anticancer drugs methotrexate and etoposide, bile acids, as well as glucuronide and sulfate conjugates (Hirohashi et al., 2000; Zeng et al., 2000; Zelcer et al., 2001). In Nx males, renal induction of Mrp3 correlates positively with CKD severity. Very interestingly, hepatic Mrp3 mRNA is also markedly induced and correlates highly with renal Mrp3 and severity of CKD. In liver, Mrp3 is localized on the basolateral membrane of hepatocytes and transports substrates back into blood (retrotransport). Chemical induction of hepatic Mrp3 in rats results in a marked increase in blood levels and urinary excretion of acetaminophen glucuronides, whereas biliary excretion of acetaminophen glucuronide decreases, even though the canalicular efflux transporter Mrp2 is induced (Slitt et al., 2003). Recent studies in Mrp3-null mice show essential roles of Mrp3 in hepatic retrotransport of glucuronides of acetaminophen, morphine, and bilirubin (Borst et al., 2007). These studies strongly indicate a critical role of Mrp3 in determining biliary versus urinary excretion of conjugates. Thus, during CKD, the marked hepatic up-regulation of Mrp3 may result in less biliary excretion of metabolites, aggravating the workload of the kidneys. Moreover, Mrp3 is expressed on the basolateral membrane of proximal tubules, mediating renal efflux of Mrp3 substrates. During CKD, renal induction of Mrp3 will decrease urinary excretion of these metabolites, which is detrimental to the body. Therefore, hepatic and renal induction of Mrp3 may be an important pathogenic factor in CKD progression and contributes to drug accumulation in CKD patients.

Inflammatory cytokines play important roles in regulating transporter expression. The present study shows that in Nx males, both renal and hepatic Mrp3 mRNA correlate positively with renal mRNA expression of IL-1β, IL-6, and IL-10. Moreover, our in vitro study showed that Mrp3 mRNA expression was induced by IL-1β and IL-6 in rat proximal tubule cells. Interestingly, inflammatory cytokines, such as IL-6, increase Mrp3 expression in human hepatoma cells (Lee and Piquette-Miller, 2001, 2003) but decrease Mrp3 expression in mouse liver and hepatoma cells (Siewert et al., 2004; Geier et al., 2005). Additionally, Mrp3 is induced in rat liver by endotoxin lipopolysaccharide (Donner et al., 2004), which increases inflammatory cytokines. Circulating levels of IL-1β and IL-6 significantly increase.
in male Nx rats (Sener et al., 2007). In human CKD patients, IL-6 activity is often markedly up-regulated and has been shown to predict worse outcome (Stenvinck et al., 2002). Further across-species comparative studies on the role of inflammatory cytokines in hepatic and renal Mrp3 expression and drug disposition are warranted. In Nx females, renal Mrp3 mRNA correlates positively with transcription factor Nrf2, an up-regulator of Mrp3 (Maher et al., 2007). Thus, a decrease of renal expression of Nrf2 (Lu and Klaassen, unpublished results) may be responsible for renal down-regulation of Mrp3 in Nx females.

Renal expression of Mdr1b, but not Mdr1a, increases in Nx males. Renal expression of Mdr1b remains unchanged 3 weeks post-Nx; however, renal protein expression of P-glycoprotein, which represents both Mdr1a and Mdr1b, remains unchanged after Nx (Lauoauri et al., 2001). The present data of unchanged Mdr1a mRNA may explain the discrepancy between P-gp mRNA and protein expression in their study (Lauoauri et al., 2001). In contrast, hepatic expression of Mdr1a, but not Mdr1b, increases in Nx males, which may explain the insignificant change in hepatic expression of total P-glycoprotein in Nx males (Lauoauri et al., 2001). In rats, Mdr1a and Mdr1b appear to have different mechanisms of gene regulation, although they share similar tissue distribution (Brady et al., 2002). The higher expression of Mdr1b in female kidneys may help urinary excretion of hydrophobic compounds, contributing to less severity of CKD in Nx females.

Abcg2/Bcrp transports a wide variety of chemicals, such as heme, phototoxins, and anticancer drugs (Staud and Pavek, 2005). Abcg2 protects cells from hypoxia-induced injury through preventing intracellular accumulation of heme (Krishnamurthy et al., 2004). In Nx males, renal expression of Abcg2 correlates negatively with CKD severity, suggesting a protective role of Abcg2 in rat kidney. Nevertheless, Abcg2 expression is low in human kidney (Maliepaard et al., 2002); therefore, caution is needed when extrapolating the significance of renal down-regulation of Abcg2 during CKD from rats to humans. ERα is a potent activator of the Abcg2 gene (Ee et al., 2004). Thus, the marked decrease of renal ERα expression (Lu et al., 2006) may contribute to decreased renal expression of Abcg2 in Nx males.

Abcg5 and Abcg8 form heterodimers with each other to promote efflux of plant sterols and cholesterol in liver and intestine (Graf et al., 2003). The current novel finding of hepatic induction of Abcg5/g8 may explain the lack of hypercholesterolemia during CKD, although hypertriglyceridemia is common in CKD patients (Kayser, 1994). Biliary hypersecretion of cholesterol via Abcg5/g8 is a risk factor of cholesterol cholelithiasis (Wittenburg et al., 2003). Hepatic induction of Abcg5/g8 may be the underlying mechanism of increased incidence of gallstones observed in CKD patients (Li Vecchi et al., 2003).

The apical transporter Ibat plays a key role in uptake of bile acids into renal proximal tubules. Renal induction of Ibat during CKD will increase renal reabsorption and thus decrease urinary excretion of bile acids (Jimenez et al., 2002). In Nx males, renal Ibat mRNA correlates positively with IL-6, suggesting that down-regulation of ERα and/or up-regulation of inflammatory cytokines (Lu et al., 2006) may be responsible for renal induction of Ibat in Nx males.

In Nx rats, hepatic expression of bile-acid transporters Ntcp and Bsep remains unchanged; however, renal expression of bile acid transporters is altered markedly. Renal Ibat and Mrp3 mRNA increase and correlate positively with CKD severity. In normal kidneys, the filtered bile acids are reabsorbed via the apical Ibat (St-Pierre et al., 2001). Mrp3 transports bile acids (Zelcer et al., 2003) and is localized on the basolateral membrane of proximal tubules (Kuroda et al., 2004). Thus, Mrp3 may play an essential role in effluxing bile acids from the proximal tubules back into the blood after they are reabsorbed from the filtrate by Ibat. Renal expression of Mrp3 and Ibat increases in Nx males but decreases in Nx females, suggesting tubular reabsorption of dihydroxy and trihydroxy bile acids increases in Nx males but decreases in Nx females. Moreover, ectopic induction of Ntcp and Bsep occurs in certain remnant kidneys, which may alter renal reabsorption of bile acids. The maintenance of hepatic expression but aberrant alteration of renal bile acid transporters may explain decreased renal excretion of bile acids and marked alteration of blood and urine profiles of bile acids in CKD patients with normal liver function (Yamaga et al., 1986; Jimenez et al., 2002).

It is noteworthy that protein expression of certain transporters was not investigated in this study because of the lack of high quality antibodies and/or technical issues. Protein expression and/or activities of these transporters may differ from their mRNA expression. For example, Naud et al. (2007) recently reported down-regulation of protein expression of Mrp2 and P-glycoprotein without changes in their mRNA expression in the intestine of Nx rats.

In conclusion, both renal and hepatic mRNA expression of certain ABC efflux transporters are altered during CKD. Hepatic induction of Mrp3 will increase retrotransport of metabolites back into blood, decreasing their biliary excretion and augmenting renal workload; whereas renal induction of Mrp3 will increase renal reabsorption and thus decrease urinary excretion. Thus, renal and hepatic induction of Mrp3 may be an important pathogenic factor in CKD progression, contributing to accumulation of endogenous chemicals and drugs in CKD patients. During CKD, renal down-regulation of ERα/glucocorticoid receptor and increase of local and systemic inflammation (Stenvinck et al., 2002; Lu et al., 2006) may cause aberrant alteration of renal/hepatic transporter expression.

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