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Alterations in transporter expression in liver, kidney, and duodenum after targeted disruption of the transcription factor HNF1α☆

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ARTICLE INFO

Article history:
Received 16 January 2006
Accepted 20 March 2006

Keywords:
Hepatocyte nuclear factor 1 alpha
Gene expression
Transcription factor
Transporter
ABC
SLC
Gene regulation

Abbreviations:
Cyp, cytochrome P450
HNF, hepatocyte nuclear factor
MODY-3, maturity onset diabetes of the young type 3
Mrp, multidrug resistance-associated protein
Mdr, multidrug resistance protein
Oat, organic anion transporter
Oct, organic cation transporter
NIDDM, non-insulin dependent diabetes mellitus

ABSTRACT

The transcription factor hepatocyte nuclear factor 1α (HNF1α) is involved in regulation of glucose metabolism and transport, and in the expression of several drug and bile acid metabolizing enzymes. Targeted disruption of the *HNF1α* gene results in decreased Cyp1a2, and Cyp2e1 expression, and increased Cyp4a1 and Cyp7a1 expression, suggesting these enzymes are HNF1α target genes. Since hepatic metabolism can be coordinately linked with drug and metabolite transport, this study aims to demonstrate whether HNF1α regulates expression of a variety of organic anion and cation transporters through utilization of an *HNF1α*-null mouse model. Expression of 32 transporters, including members of the Oat, Oatp, Oct, Mrp, Mdr, bile acid and sterolin families, was quantified in three different tissues: liver, kidney, and duodenum. The expression of 17 of 32 transporters was altered in liver, 21 of 32 in kidney, and 6 of 32 in duodenum of *HNF1α*-null mice. This includes many novel observations, including marked downregulation of Oats in kidney, as well as upregulation of many Mrp and Mdr family members in all three tissues. These data indicate that disruption of *HNF1α* causes a marked attenuation of several Oat and Oatp uptake transporters in liver and kidney, and increased expression of efflux transporters such as Mdrs and Mrps, thus suggesting that HNF1α is a central mediator in regulating hepatic, renal, and intestinal transporters.

☆ National Institute of Health grants ES-09716 and ES-07079.
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0006-2952/$ – see front matter
doi:10.1016/j.bcp.2006.03.016

1. Introduction

Transporters serve a multitude of functions, including import and export of critical endogenous substrates, as well as aiding in absorption, distribution, and elimination of many xenobiotics. Transporters characteristically move water-soluble compounds and hydrophilic metabolites into and out of cells because these molecules do not pass readily through membranes. Due to a wide range of compounds that need passage through membranes, a host of transporters have evolved to manage the cellular environment. In this regard, several transporter families exist to aid in disposition of chemicals, including the organic anion transporters (Oat), organic anion transporting polypeptide transporters (Oatp), organic cation transporters (Oct), multidrug resistance protein transporters (Mdr), multidrug resistance-associated protein transporters (Mrp), the bile acid and sterolin transporter families, along with Bcrp (breast cancer resistance protein; Abcg2). Whereas this is only a fraction of the total transporters that exist, these transporter families have been implicated as being important in the uptake and elimination of xenobiotics in liver, kidney, and intestine—all organs which play a vital role in drug disposition.

HNF1α is a transcription factor that is known to regulate the basal expression of many genes, and has been referred to as a "master" transcription factor due to its control over other known nuclear receptors and transcription factors [1]. HNF1α is expressed in liver, kidney, intestine, stomach, and pancreas [2,3], and has been implicated in the regulation of bile acid, fatty acid, and drug metabolism in vivo [4–6]. Disruption of HNF1α results in decreased expression of several cytochrome P450s including Cyp1a2, Cyp2c29, Cyp2d9, Cyp2e1, and Cyp7b1, and increased expression of Cyp2b10, Cyp3a11, Cyp4a1, Cyp7a1, Cyp8b1, and Cyp39a1 [6]. Similarly, HNF1α is believed to be critical for regulation of insulin-like growth factor 1 (IGF-1), and the resulting loss of IGF-1 expression in the absence of HNF1α gives a phenotype resembling Laron dwarfism, and non-insulin-dependent diabetes mellitus (NIDDM) [7] suggesting the possibility of compensation by other transcription factors or altered interactions with co-activator and co-repressor proteins that are central to normal expression of HNF1α target genes [8,9].

The development of *HNF1α*-null mice allows for an in vivo model for examining the physiological role of HNF1α. Mutations in the HNF1α gene exist in humans and lead to maturity-onset diabetes of the young type 3 (MODY-3), and the resulting physiological consequences seem to be modeled well by the null mouse [7].

HNF1α has also been implicated in the downregulation of only a few transporters, including human organic-anion-uptake transporters OATP-C and OATP8, the human sodium-glucose transporter (SGLT1), as well as mouse Oatp4 and the sodium-phosphate transporters Npt1, 2, and 4 in mouse [10–12]. However, this probably represents only a handful of transporters that might be regulated by HNF1α, whether direct or indirect. Decreased binding of HNF1α to 5′ flanking regions of transporters such as Ntcp and Oatps may be a critical event in bile-acid homeostasis, as this may lead to impaired hepatic uptake during cholestasis [13,14]. However, very little work has focused on whether HNF1α regulates other solute carrier transporter families (i.e. Oats, Octs, Oatps), as well as its effects on the efflux transporters (i.e. Mdrs, Mrps). Similarly, the tissue distribution of HNF1α suggests that this transcription factor may be critical in other organs besides liver, especially in kidney and in intestine. To identify HNF1α target genes in these three tissues the branched DNA assay (Genospectra; Fremont, CA) was utilized to examine expression of a large battery of transporters in both wildtype (WT) and *HNF1α*-null mice. This study aims to determine whether HNF1α is critical for endo- and xenobiotic transporter expression in a variety of tissues.

2. Materials and methods

2.1. Mice

HNF1α-null mice were engineered using Cre-*loxp* recombination as described previously [7]. Because *HNF1α*-null mice are infertile, null mice were derived by cross-breeding heterozygote male and female mice. $HNF1\alpha^{(+/+)}$ littermates were used as wildtype (WT) controls. Tissues from eight male *HNF1α* WT and eight *HNF1α*-null adult mice under 4 months of age were collected and snap frozen in liquid nitrogen. Tissues were stored at −80 °C until further use.

Table 1 – Nomenclature of transporters involved in liver, kidney and duodenum disposition of endogenous and exogenous substrates

Common name	Nomenclature	Accession number
Oat1	Slc22a6	NM_008766
Oat2	Slc22a7	NM_144856
Oat3	Slc22a8	NM_031194
Oatp1a1 (Oatp1)	Slc21a1	NM_013797
Oatp1a4 (Oatp2)	Slc21a5	NM_030687
Oatp1a5 (Oatp3)	Slc21a7	NM_130861
Oatp1a6 (Oatp5)	Slc21a13	NM_023718
Oatp1b2 (Oatp4)	Slc21a10	NM_020495
Oatp2a1 (Pgt)	Slc21a2	NM_033314
Oatp2b1 (Oatp9)	Slc21a9	NM_175316
Oatp3a1 (Oatp11)	Slc21a11	NM_023908
Oatp4c1 (Oatp19)	Not determined	NM_172658
Oct1	Slc22a1	NM_009202
Oct2	Slc22a2	NM_013667
Oct3	Slc22a3	NM_011395
Octn1	Slc22a4	NM_019687
Octn2	Slc22a5	NM_011396
Octn3	Slc22a9	NM_019723
Mrp1	Abcc1	NM_008576
Mrp2	Abcc2	NM_013806
Mrp3	Abcc3	XM_358306
Mrp4	Abcc4	XM_139262
Mrp5	Abcc5	NM_013790
Mrp6	Abcc6	NM_023732
Mdr1a	Abcb1a	NM_011076
Mdr1b	Abcb1b	NM_011075
Mdr2	Abcb4	NM_008830
Ntcp	Slc10a1	NM_011387
Bsep	Abcb11	NM_021022
Ibat	Slc10a2	NM_011388
Bcrp	Abcg2	NM_011920
Abcg5	Abcg5	NM_031884
Abcg8	Abcg8	NM_026180

2.2. RNA isolation

Total RNA was isolated using RNAzol Bee reagent (Tel Test, 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. Total mRNA was diluted to 1 μg/μl for the bDNA assay.

2.3. Development of specific oligonucleotide probe sets for bDNA analysis

Gene sequences were accessed from GenBank, and the target sequences were analyzed by ProbeDesigner® Software Version 1.0 (Table 1). Each oligonucleotide probe is 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 logarithmic alignment search tool (BLASTn) to ensure minimal cross-reactivity with other known mouse genomic sequences and expressed sequence tags.

2.4. Branched DNA assay

The branched DNA assay uses 96-well format plates and a specific probe to capture the transcript of interest, and is similar to enzyme-linked immunosorbent assays (ELISA). Using signal amplification by amplifier and label probes, the luminescence produced is in proportion to the relative number of transcripts present. Gene-specific oligonucleotide probes were diluted in lysis buffer according to instructions provided by the Quantigene® bDNA Signal Amplification Kit (Bayer Diagnostics, East Walpole, MA). Total RNA (1 μg/μl; 10 μl) was added to each well of a 96-well plate containing 50 μl 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.

2.5. Statistical analysis

Data were analyzed by Student's *t*-test. Error bars represent standard error of the mean. Asterisks (*) represent statistical differences ($p \leq 0.05$) between wildtype and *HNF1α*-null mice.

3. Results

3.1. Hnf1α disruption alters expression of the organic anion transporter (Oat) family in liver, kidney, and duodenum

Oats are solute uptake transporters known to play an important role in kidney. Oat2, which has the highest expression of Oats in liver, had markedly lower hepatic mRNA expression in *Hnf1α*-null mice as compared to WT mice (Fig. 1),

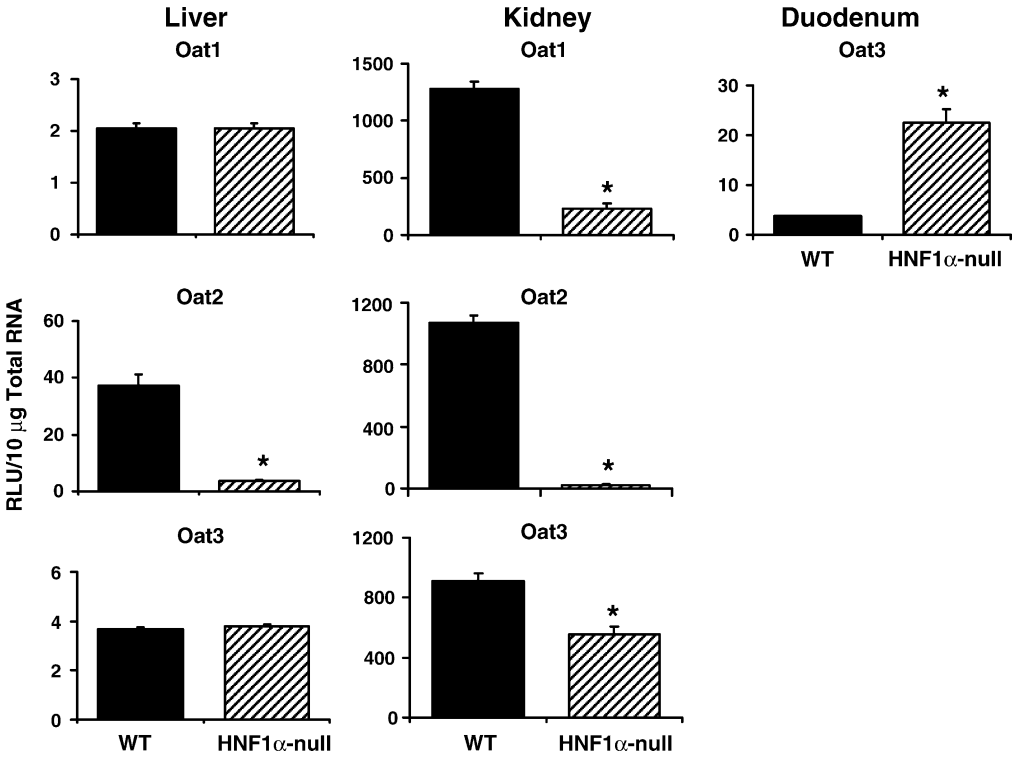

Fig. 1 – Expression of Oat1, 2, and 3 in liver (left panel), kidney (middle panel), and duodenum (right panel) in WT and HNF1α-null mice. Relative light units are expressed as the mean ± S.E. Asterisks represent a statistical difference between WT and HNF1α-null mice. Data are summarized in Table 2.

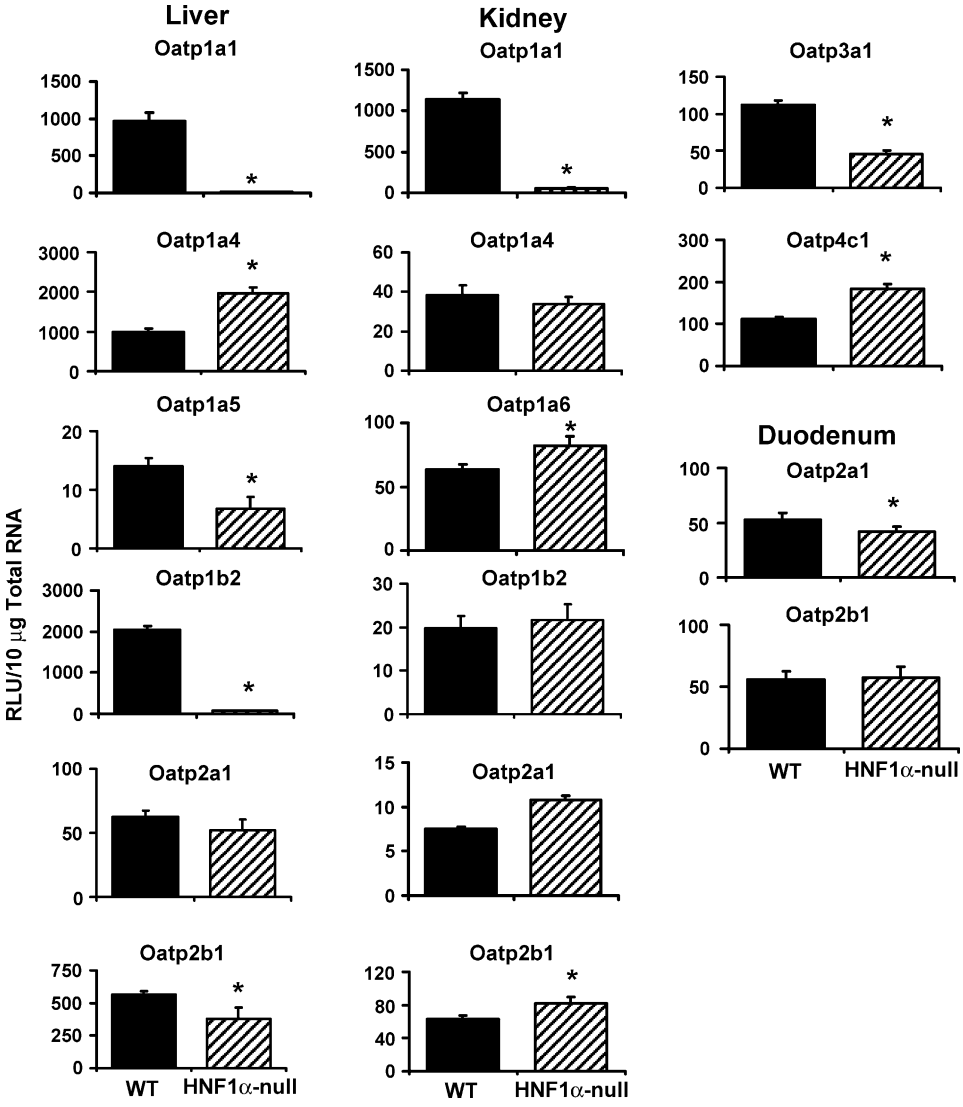

Fig. 2 – Expression of Oatp1a1, 1a4, 1a5, 1a6, 1b2, 2b1, 3a1, 4c1, and 2a1 in liver (left panel), kidney (middle panel), and duodenum (right panel) in WT and HNF1α-null mice. Relative light units are expressed as the mean ± S.E. Asterisks represent a statistical difference between WT and HNF1α-null mice. Data are summarized in Table 2.

whereas Oat1 and Oat3 remained unchanged (Table 2). Oat1, 2 and 3 expression in kidney was lower in null than WT mice; expression of Oat1 and 2 in *HNF1α*-null kidney was <20% than in WT mice. In duodenum, Oat3 expression was five-fold higher in *Hnf1α*-null mice than in WT mice, whereas the expression of Oat1 and 2 in duodenum was below the limits of detection.

3.2. *Hnf1α disruption alters expression of the organic anion transporting polypeptide (Oatp) family in liver, kidney, and duodenum*

Oatps are solute uptake transporters that are important in a variety of tissues, including liver and kidney [15]. The mRNA expression of Oatp1a1, 1b2, and 2b1 in liver of *Hnf1α*-null mice was markedly lower than in WT mice (Fig. 2). Oatp1a1 and 1b2 expression was virtually absent in null mice, with more moderate decreases in Oatp1a5 and 2b1. In contrast, Oatp1a4 was higher in *HNF1α*-null liver, with roughly two-fold higher expression, respectively than WT controls.

Whereas the *HNF1α*-null mouse data in liver correlates with previous data for Oatp1a1 and 1b2, the data varies for Oatp1a4 expression, with downregulation of Oatp1a4 seen earlier by gene microarray [16]. It is unclear if the mice used for the array were in fact male or female, so what might be occurring is a notable gender difference in Oatp1a4 expression. Such a gender difference is known occur for Oatps, with markedly higher expression in males than females (Cheng et al., in review). While the data seems contradictory, it is interesting to note that decreased expression of Oatp1a4 was observed in female *HNF1α*-null mice, with expression being about 30% lower in female *HNF1α*-null mice than WT (data not shown). The marked decrease in liver of Oatp1b2, the main hepatic uptake transporter in rodents, has been reported previously in

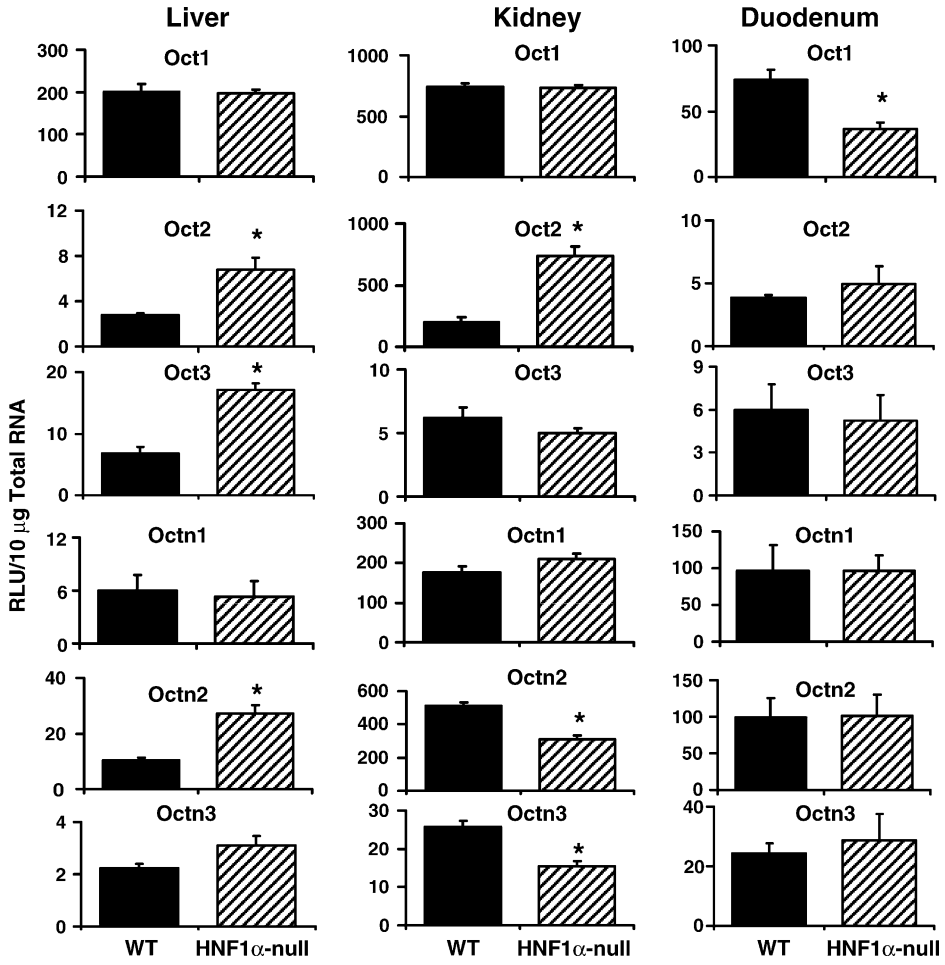

Fig. 3 – Expression of Oct 1–3, and Octn1–3 in liver (left panel), kidney (middle panel), and duodenum (right panel) in WT and HNF1α-null mice. Relative light units are expressed as the mean ± S.E. Asterisks represent a statistical difference between WT and HNF1α-null mice. Data are summarized in Table 2.

HNF1α-null mice, as has the decrease in Oatp 1a1 and 1a4 [17,18].

In kidney, markedly lower levels of Oatp1a1 were observed in *HNF1α*-null mice, with slightly lower expression of Oatp1a6 and 3a1 than in WT mice. Oatp2b1 and 4c1 were modestly higher in *HNF1α*-null kidneys, compared to WT kidneys. Oatp1a4, 1b2 and 2a1 mRNA expression was essentially unchanged in kidney, and Oatp1a5 expression was not detectable. In duodenum, only Oatp2a1 and Oatp2b1 were expressed, but no difference between the two genotypes was observed.

The two Oatps with duodenal expression had minor differences between WT and HNF1α-null mice, with Oatp2b1 mRNA levels being unchanged, and Oatp2a1 expression was slightly less in HNF1α-null mice.

3.3. *Hnf1α disruption alters expression of the organic cation transporter (Oct) family in liver, kidney, and duodenum*

There are six Oct transporters, Oct1-3 and Octn1-3. The Oct with the highest expression in liver is Oct 1 [19]. Its expression, as well as Octn1 and Octn3, was not altered in HNF1α-null mice (Fig. 3). However, Oct 2, 3, and n2 expression was higher in HNF1α-null mice. Kidney is the main tissue for Oct1 and 2 expression, as well as for Octn1 and Octn2 (Alnouti et al., in preparation). In kidney, Oct2 was increased more than three-fold in HNF1α-null mice, whereas Octn2 and Octn3 were decreased about 50%. Oct1, 3 and n1 levels remained unchanged between the two genotypes in kidney. In duodenum, the lack of HNF1α decreased only Oct1, and had no effect on the other Octs and Octns.

3.4. *Hnf1α disruption alters expression of the multidrug resistance-associated protein (Mrp) family in liver, kidney, and in duodenum*

Mrps are a family of efflux transporters that serve to export various conjugated anionic compounds out of various tissues, most notably in liver, kidney and intestine. In liver, Mrp2 transports chemicals into bile, whereas the other Mrps transport chemicals from liver back into blood. The absence

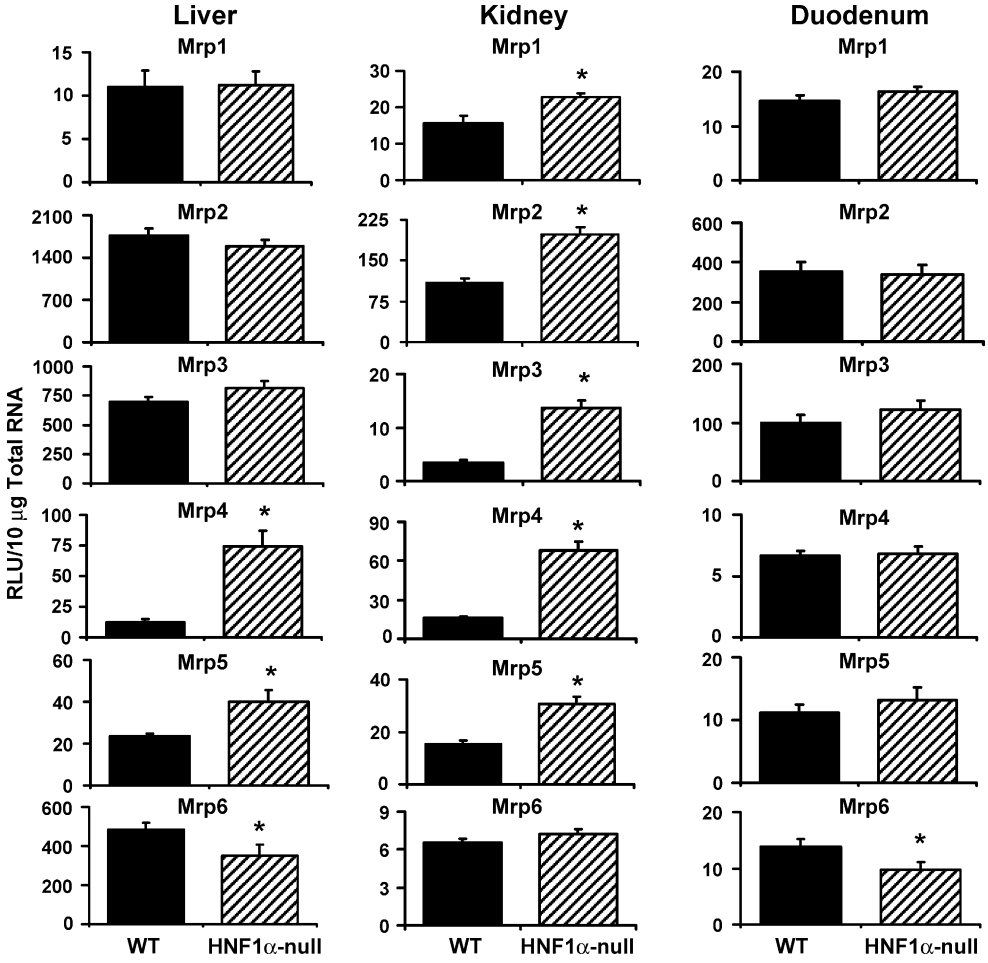

Fig. 4 – Expression of Mrp1–6 in liver (left panel), kidney (middle panel), and duodenum (right panel) in WT and HNF1α-null mice. Relative light units are expressed as the mean ± S.E. Asterisks represent a statistical difference between WT and HNF1α-null mice. Data are summarized in Table 2.

of HNF1α did not have an effect on the expression of Mrp1, 2, and 3 in liver (Fig. 4). However, the hepatic expression of Mrp4, 5, and 6 was higher in *HNF1α*-null mice. Similarly, the renal expression of Mrp1–5 was increased in the *HNF1α*-null mice, compared to WT mice. In duodenum, only Mrp6 showed any alterations in expression, with a 30% decrease in the *HNF1α*-null mice.

3.5. *Hnf1α disruption alters expression of the multidrug resistance protein (Mdr) family in liver, kidney, and duodenum*

Much like Mrps, Mdrs are efflux transporters that serve to export various conjugated and unconjugated compounds out of various tissues, most notably in intestine in brain. Mdr1a and Mdr2 expression in liver was approximately tripled in *HNF1α*-null mice as compared to WT mice, whereas Mdr1b expression was not altered (Fig. 5). Similar changes were observed in kidney, with increased expression in *HNF1α*-null mice for Mdr1a, 1b, and 2. Mdr1a, the only Mdr with significant expression in duodenum, has higher expression in *HNF1α*-null mice over WT mice.

3.6. *Hnf1α disruption alters expression of bile acid transporters, as well as members of the Abcg family in liver, kidney, and duodenum*

Ntcp is responsible for the hepatic uptake of bile acids, and Bsep exports bile acids from the liver into bile. The expression of Ntcp and Bsep transporter was relatively unaltered by targeted disruption. In both liver and kidney, Ibat expression was markedly lower in the absence of *HNF1α* (Fig. 6). Expression of Ibat has been previously shown to be dependent on *HNF1α* in ileum [16]. Ibat in duodenum has also been observed previously and in this experiment to be poorly expressed, and *HNF1α* disruption did not alter duodenal expression. Abcg2 (Bcrp), is a half-transporter with high expression in kidney, intestine and liver that can transport several chemotherapeutic drugs, such as mitoxantrone [20,21]. Abcg2 expression was not altered in any of the tissues examined. Abcg5 and Abcg8 are half-transporters that heterodimerize to form an efflux transporter of sterols, specifically in the duodenum and liver. Abcg5 expression was higher in *HNF1α*-null mice in both kidney and intestine (Fig. 6). However, a lower expression of Abcg8

Table 2 – mRNA expression in HNF1α-null mice vs. WT mice

	Liver	Kidney	Duodenum
Oat1	Not detected	↓ 82%	Not detected
Oat2	↓ 87%	↓ 98%	Not detected
Oat3	Not detected	↓ 39%	↑ 513%
Oatp1a1 (Oatp1)	↓ 99%	↓ 95%	Not detected
Oatp1a4 (Oatp2)	↑198%	No change	Not detected
Oatp1a5 (Oatp3)	↓ 47%	Not detected	Not detected
Oatp1b2 (Oatp4)	↓ 97%	No change	Not detected
Oatp1a6 (Oatp5)	Not detected	↓ 33%	Not detected
Oatp2b1 (Oatp9)	↓ 33%	↑ 30%	No change
Oatp3a1 (Oatp11)	Not detected	↓ 31%	Not detected
Oatp4c1 (Oatp19)	Not detected	↑ 64%	Not detected
Oatp2a1 (Pgt)	No change	↑ 44%	No change
Oct1	No change	No change	↓ 50%
Oct2	↑ 144%	↑ 261%	No change
Oct3	↑ 152%	No change	No change
Octn1	No change	No change	No change
Octn2	↑ 160%	↓ 39%	No change
Octn3	No change	↓ 41%	No change
Mrp1	No change	↑ 47%	No change
Mrp2	No change	↑ 80%	No change
Mrp3	No change	↑ 303%	No change
Mrp4	↑ 518%	↑ 330%	No change
Mrp5	↑ 68%	↑ 102%	No change
Mrp6	↓ 28%	No change	↓ 30%
Mdr1a	↑ 207%	↑ 257%	↑ 181%
Mdr1b	No change	↑ 298%	Not detected
Mdr2	↑ 196%	↑ 97%	Not detected
Ntcp	No change	No change	Not detected
Bsep	↓ 16%	No change	Not detected
Ibat	↓ 86%	↓ 82%	No change
Bcrp	No change	No change	No change
Abcg5	No change	↑ 342%	↑ 58%
Abcg8	No change	No change	↓ 47%

Arrows (↑, ↓) represent higher or lower expression (% change) in HNF1α-null as compared to WT mice. Expression that was below the limits of detection or absent is marked as "Not detected".

in duodenum was detected in *HNF1α*-null mice as compared to WT mice.

4. Discussion

The results of the present study demonstrate that Hnf1α controls a multitude of transporters involved in the disposition of a wide variety of substrates. These data support the concept that Hnf1α plays a role not only in the regulation of drug and bile acid metabolism, but also in the uptake and export of substrates for these enzymes.

Hnf1α serves as a central mediator for many other transcription factors, and while much of this regulation is not understood, speculation of possible methods of regulation exist. Using the chromatin immunoprecipitation assay (ChIP) for Hnf1α binding, Hnf1α was shown to influence expression of other critical mediators of transcription, including LRH-1, SHP, RAMP, CREBL2, SREBF2 [22], and decreased expression of FXR and SHP were noted by microarray in *HNF1α*-null mice [18]. Some transporters have already been identified to be downstream targets of HNF1α regulated-transcription factors, including regulation of Mrp3 and Ibat by SHP and LRH-1 [23,24]. Thus, the scope of other transcription factors that are altered in *HNF1α* deficiency may be vast, and may lead to significant changes in both drug metabolism and in disposition of chemicals.

Many physiological parameters also change in the absence of HNF1α. The normal homeostasis and transport of endogenous substrates is markedly altered, and some of these changes may be attributed to alterations in transporter levels. Glucose levels are quite high in serum of HNF1α-null mice, and glucose in urine was about 330-fold higher in urine of the null mice than the WT mice [7]. Cholesterol levels were also increased, with roughly three-fold higher cholesterol levels in HNF1α-null mice at 12 weeks of age. Liver damage may also be prevalent in HNF1α-null mice, with increased alanine aminotransferase (ALT) values (roughly 10-fold) and triglyceride serum levels [5]. Because the expression of so many transporters change in these null mice, several alterations in transporter expression may be occurring at once, leading to multiple simultaneous changes in both uptake and efflux of endogenous compounds.

In cholestatic and hepatic injury models, HNF1α binding in transient regulation of genes that control disposition of bile acids has been established. However, although HNF1α may a significant transcription factor involved in cholestasis, it does not seem to be the "master regulator" of this entire process. Already many transporters have been linked with bile-acid uptake, including Ntcp, Oatps and Ibat [13,14,24], and with bile-acid export, including Mrp3, 4, and Bsep [24–27]. Some of these genes, such as Ntcp and Oatp4 in rat, have been shown to have decreased HNF1α binding activity in endotoxin-induced cholestasis [14,17]. However, in *HNF1α*-null mice, no such changes in Ntcp expression were observed. This was further confirmed and corroborated as a species difference; Ntcp is regulated by HNF1α binding in rats, whereas in mice and humans, Ntcp is HNF1α-independent [28].

However, other transporters seem to be highly dependent on HNF1α for expression. Human Oatp-C, and mouse Oatp1 and Oatp2 are dependent on HNF1α for basal expression [10,18], suggesting that these genes may also be temporally downregulated during cholestasis. Furthermore, dramatic increases in serum, urine, and fecal bile acid content were observed in *HNF1α*-null mice, further strengthening this concept [7]. In general, the liver may serve to protect itself during cholestasis by downregulating uptake transporters via HNF1α, such as the Oatps, while upregulating basolateral transporters such as Mrp4, 5, and 6 (Fig. 4) in liver to relieve accumulation of bile acids and biliary constituents. Furthermore, the upregulation of Mrp2 and Mrp4 (Fig. 4) in kidneys of *HNF1α*-null mice could mimic the decrease in HNF1α binding that occurs during cholestasis, and these transporters, which are localized to the apical membrane in kidneys, could serve to remove these excess bile acids and biliary constituents from the blood into urine, [29,30]. In addition, other efflux transporters regulated by HNF1α, such as Mdrs, may be involved in this process as well.

Humans with non-insulin dependent diabetes (NIDDM) are often treated with glibenclamide, a member of the

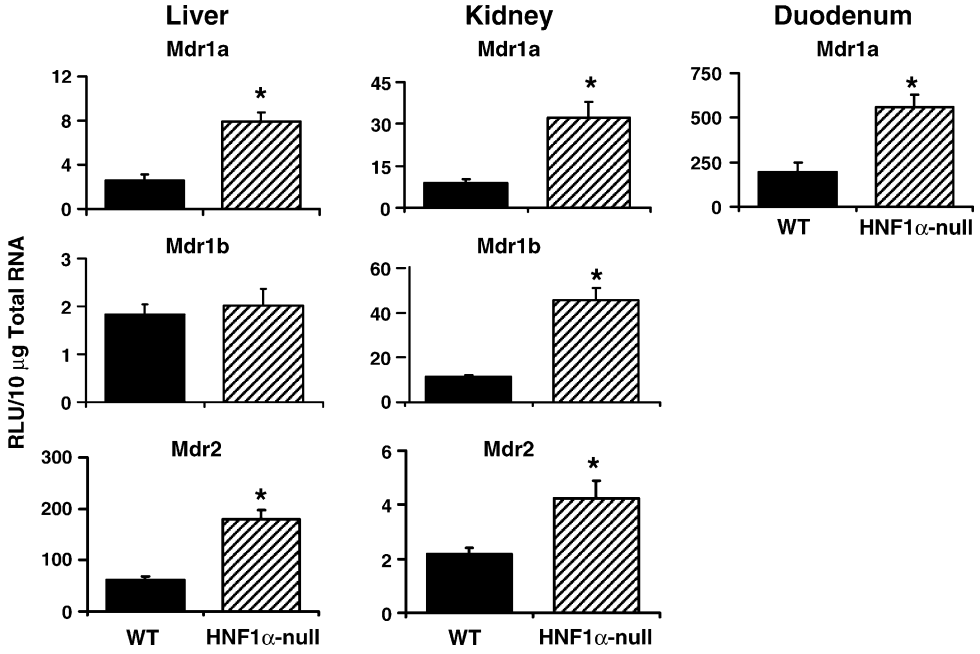

Fig. 5 – Expression of Mdr1a, 1b, and 2 in liver (left panel), kidney (middle panel), and duodenum (right panel) in WT and HNF1α-null mice. Relative light units are expressed as the mean ± S.E. Asterisks represent a statistical difference between WT and HNF1α-null mice. Data are summarized in Table 2.

sulfonylurea class of antidiabetic drugs. Interestingly, the pharmacokinetics of glibenclamide are markedly altered, with its uptake into hepatocytes greatly decreased in patients with MODY-3 [31]. This phenomenon might be due to the marked decrease in basolateral uptake transporter expression observed in *Hnf1α*-null mice, which exhibit the NIDDM phenotype. The Oatps, which are responsible for a large share of uptake of xenobiotics, have markedly decreased expression in these null mice. The most likely candidate for this poor uptake in the null mice may be Oatp1b2, which is the most similar in homology to the human Oatp-C and 8 [32]. This decreased uptake into liver may suggest that the dosage of glibenclamide should be carefully monitored in MODY-3 patients, otherwise increased serum levels could lead to hypoglycemia.

Changes in transporter expression may markedly alter the disposition of many pharmaceuticals, as well as toxicants. Patients with decreased or abolished HNF1α expression may have increased or decreased half-lives of drugs, depending on the substrate specificity for transporters. With the marked alterations in Oat/Oct expression, changes in disposition of non-steroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, antibiotics, and many other drug compounds may have diminished excretion in MODY3 patients [33]. Such changes could lead to diminished urinary excretion and higher serum levels of circulating drug. Similar effects may occur with increased Mdr and Mrp expression, and an *HNF1α* mutation may lead to greatly increased transport of compounds such as antivirals, nucleotide analogs, steroids, and other pharmaceuticals [34–36]. With increased Mdr/Mrp expression, increases in intestinal and uptake into the brain could be quite profound for good substrates, and could lead to increased sensitivity and toxicity. Although these changes in mRNA expression may not fully translate to functionally expressed protein, the changes in mRNA observed suggest good candidate compounds that might have altered disposition in *HNF1α*-null mice.

Large changes in drug and steroid metabolism are also known to occur when HNF1α expression is attenuated. Cyp1a2, Cyp2e1, Cyp3a11, and Cyp4a1 all have altered expression, and drug metabolism is most likely altered in null mice [6,37]. Similarly, phase-II metabolism is attenuated, with the basal transcription of UGT1a1, 1a7, 1A8, 1A9, and 1A10 being dependent on HNF1α in a rat and human models [38–40]. Initial regulation by HNF1α has also been suggested for GSTs [41], and a general regulation of phase-I and phase-II metabolism is suggested by the various changes in these multiple genes. Alterations in metabolism of chemicals may lead to alternate conjugation pathways, and altered substrate specificity for transporters. For example, the preferred substrates for Mrp1 and 2 are glutathione conjugates [42], whereas Mrp3 may have higher affinity for sulfate conjugates [35]. Alterations in metabolism may lead to differences in vectoral excretion, shifting normal disposition of a drug in vivo, and possibly leading to changes in clearance.

Interestingly, the term ''liver-enriched'' transcription factor might have allowed the critical regulation of transporters in kidney by HNF1α to go largely unnoticed. Some of the most dramatic changes in expression observed in the Hnf1α-null mice were with the Oat family members in kidney, where expression of Oat1 and 2 was almost completely absent, and the expression of Oat3 much lower than in WT mice (Fig. 1). Conversely, the expression in kidney of many of the MDR and Mrp family members (Fig. 4) was higher in HNF1α-null mice, with higher expression of Mdr1a/1b and Mdr2, as well as Mrp1,

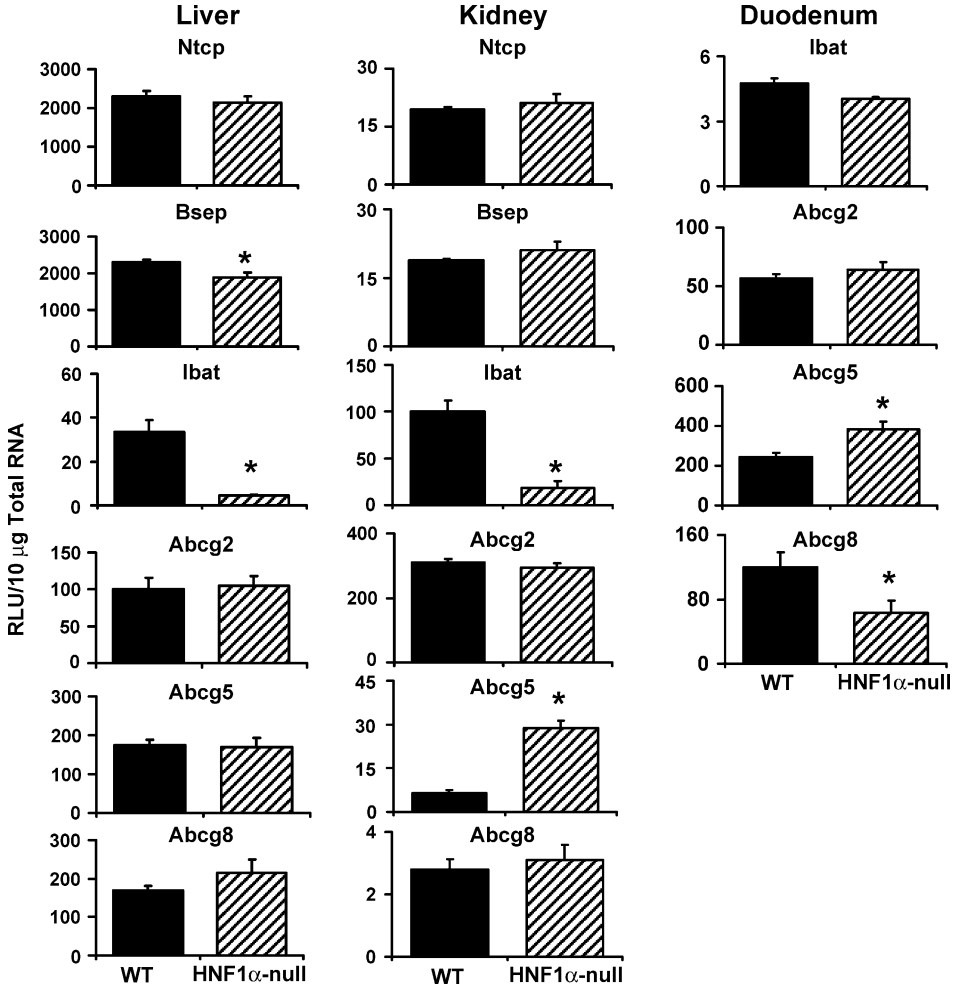

Fig. 6 – Expression of Ntcp, Bsep, Ibat, Bcrp, Abcg5, and Abcg8 in liver (left panel), kidney (middle panel), and duodenum (right panel) in WT and HNF1α-null mice. Relative light units are expressed as the mean ± S.E. Asterisks represent a statistical difference between WT and HNF1α-null mice. Data are summarized in Table 2.

2, 3, 4, and 5 than controls. Thus, decreased HNF1α expression would seem to be renal-protective, and the upregulation of these transporters would serve to help rid the kidney of excess toxicants by increasing transport into urine.

The present study examines the expression of 32 different transporters in liver, kidney, and duodenum using bDNA analysis. Transporters in liver showing at least a two-fold increase in *HNF1α*-null mice include Mrp4, Mdr1a, Oct2, Oct3, Octn2, and Oatp2. In kidney, two-fold higher or greater expression was observed for Mrp3, Mrp4, Mrp5, Mdr1a, Mdr1b, Oct2, Abcg5, Oatp9, and Oatp19, whereas in intestine, only Mdr1a and Oat3 were elevated two-fold over WT mice. Because HNF1α is critical in basal expression of target genes, large decreases in transporter expression were observed. Transporters that had less than 20% expression in HNF1α-null mice compared to WT mice in liver were: Ibat, Oatp1, Oatp5, and Oat2. In kidney, Oat1, Oat2, Ibat, and Oatp1 were all expressed in the *HNF1α*-null mice at less than 20% of normal. No marked decreases were observed in duodenum.

In conclusion, general trends on how HNF1α affects the various families of transporters could be made from this experiment. For example, disruption of HNF1α generally caused augmented expression of efflux Mrp and Mdr family members in liver and kidney. Alternatively, loss of HNF1α caused marked attenuation of many uptake Oat and Oatp family members. The response for the Octs was rather mixed, suggesting a diverse family of transporters with a broad range of functions. Similarly, the bile acid transporters were rather unresponsive to loss of HNF1α, with the exception of Ibat, which was markedly attenuated in liver and kidney. In summary, HNF1α, probably in combination with other coactivators and repressors, serves to control expression of hepatic, renal and duodenal transporters in combination with drug and bile acid metabolizing enzymes.

Acknowledgements

The authors would like to thank Katy Allen and Drs. Matt Dieter, Hong Lu, Chuan Chen, and Susan Buist for input and discussion, as well as the Laboratory Animal Resource Center at KUMC for their excellent contributions in helping with the mice.

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