0026-895X/10/7806-1079–1087$20.00
MOLECULAR PHARMACOLOGY
Copyright © 2010 The American Society for Pharmacology and Experimental Therapeutics
Mol Pharmacol 78:1079–1087, 2010

Vol. 78, No. 6
65201/3642600
Printed in U.S.A.

The Human Organic Anion Transporter Genes *OAT5* and *OAT7* Are Transactivated by Hepatocyte Nuclear Factor-1α (HNF-1α)

Kerstin Klein, Christoph Jüngst,[1] Jessica Mwinyi, Bruno Stieger, Franz Krempler, Wolfgang Patsch, Jyrki J. Eloranta, and Gerd A. Kullak-Ublick

Department of Clinical Pharmacology and Toxicology, University Hospital Zurich, Switzerland (K.K., C.J., J.M., B.S., J.J.E., G.A.K.-U.); Zurich University Research Priority Programme "Integrative Human Physiology" (ZIHP), Switzerland (B.S., J.J.E., G.A.K.-U.); Department of Internal Medicine, Krankenhaus Hallein, Austria (F.K.); and Department of Laboratory Medicine, Paracelsus Medical University, Salzburg, Austria (W.P.)

Received March 31, 2010; accepted September 9, 2010

ABSTRACT

Organic anion transporters (OATs) are anion exchangers that transport small hydrophilic anions and diuretics, antibiotics, nonsteroidal anti-inflammatory drugs, antiviral nucleoside analogs, and antitumor drugs across membrane barriers of epithelia of diverse organs. Three OATs are present in human liver: OAT2, OAT5, and OAT7. Given that hepatocyte nuclear factor-1α (HNF-1α) has previously been shown to regulate the expression of several hepatocellular transporter genes, we investigated whether the liver-specific human *OAT* genes are also regulated by HNF-1α. Short interfering RNAs targeting HNF-1α reduced endogenous expression of OAT5 and OAT7, but not OAT2, in human liver-derived Huh7 cells. Luciferase reporter gene constructs containing the *OAT5 (SLC22A10*) and *OAT7 (SLC22A9*) promoter regions were transactivated by HNF-1α in HepG2 cells. Two putative HNF-1α binding elements in the proximal *OAT5* promoter, located at nucleotides $-68/-56$ and $-173/-160$, and one element in the *OAT7* promoter, located at nucleotides $-14/-2$ relative to the transcription start site, were shown to bind HNF-1α in electromobility shift assays, and these promoter regions also interacted with HNF-1α in chromatin immunoprecipitation assays. A correlation between HNF-1α and OAT5 ($r = 0.134$, $P < 0.05$) or OAT7 ($r = 0.461$, $P < 0.001$) mRNA expression levels in surgical liver biopsies from 75 patients further supported an important role of HNF-1α in the regulation of *OAT* gene expression.

Introduction

Members of the solute carrier family *SLC22* play a pivotal role in drug absorption and excretion. They share a predicted membrane topology consisting of twelve α-helical transmembrane domains (TMDs), a large glycosylated extracellular loop between TMDs 1 and 2, and a large intracellular loop between TMDs 6 and 7 with consensus sequences for phosphorylation (Burckhardt and Wolff, 2000; Koepsell and Endou, 2004). The SLC22 protein family can be divided into subgroups according to transport mechanisms and substrates: the organic cation transporters, the organic carnitine transporters, and the organic anion transporters (OATs) (Koepsell and Endou, 2004). Most members of the *SLC22* family are polyspecific and transport multiple different substrates. OATs are anion exchangers that efficiently transport small hydrophilic anions. Furthermore, the transport substrates for OATs include anionic drugs, such as diuretics, antibiotics, nonsteroidal anti-inflammatory drugs, antiviral nucleoside analogs, and antitumor drugs (Sweet, 2005).

Three OATs have been detected in human liver: OAT2 (gene symbol *SLC22A7*), OAT5 (*SLC22A10*), and OAT7 (*SLC22A9*) (Sun et al., 2001; Eraly and Nigam, 2002; Shin et al., 2007). The rat Oat2 (Simonson et al., 1994) and the human OAT7 proteins (Shin et al., 2007) have been shown to be localized to the sinusoidal, but not bile canalicular, membranes of hepatocytes. Human OAT5 has so far been detected only by Northern blotting (Sun et al., 2001; Eraly and Nigam, 2002), hence the subcellular localization of OAT5 remains

This study was supported by the Swiss National Science Foundation [Grant 320030_120463/1]; the Novartis Foundation for Biomedical Research, the Center of Clinical Research at the University Hospital Zurich; the Austrian Science Fund (Fonds zur Förderung der wissenschaftlichen Forschung) [Grant P19893-B05]; the Land Salzburg; and the Verein für Medizinische Forschung Salzburg.

K.K. and C.J. contributed equally to this work.

[1] Current affiliation: Department of Medicine II, Saarland University Hospital, Saarland University, Homburg, Germany.

Article, publication date, and citation information can be found at http://molpharm.aspetjournals.org.
doi:10.1124/mol.110.065201.

ABBREVIATIONS: TMD, transmembrane domain; OAT, organic anion transporter; SLC, solute carrier; HNF hepatocyte nuclear factor; EMSA, electromobility shift assay; ChIP, chromatin immunoprecipitation; siRNA, small interfering RNA; PCR, polymerase chain reaction; PBS-T, phosphate-buffered saline–Tween 20; bp, base pair(s).

elusive. The human *OAT7* has been mapped to chromosomal region 11q13.1 (Shin et al., 2007). The human *OAT5* has been identified by a tBLASTn search of the expressed sequence tag database, and the OAT5 cDNA has been cloned by two groups (Sun et al., 2001; Eraly and Nigam, 2002). An updated sequence record for *OAT5* (NM_001039752) was published by Taylor et al. (2006). Human *OAT5* has been mapped to chromosomal region 11q12.3. The mouse and rat Oat5 (*Slc22a19*) proteins have been shown to be exclusively expressed in kidneys (Youngblood and Sweet, 2004; Anzai et al., 2005) and are unlikely to be homologs of the human OAT5 protein (Koepsell and Endou, 2004). Phylogenetic analysis on the entire *SLC22* family in human, mouse, and rat has revealed no homologs for human *OAT5* and *OAT7* (Jacobsson et al., 2007). Whereas the transport mechanisms for human OAT2 (Sun et al., 2001; Babu et al., 2002; Khamdang et al., 2003; Kobayashi et al., 2005) and OAT7 (Shin et al., 2007) have been characterized previously, transport substrates for human OAT5 are still under investigation.

Changes in the expression levels of OATs could influence the hepatic clearance of substrates from sinusoidal blood. Therefore, we addressed the question of whether transcriptional regulatory mechanisms that have been described previously for other OAT/Oat members (Popowski et al., 2005; Ahn and Nigam, 2009) are also involved in the regulation of human *OAT2*, *OAT5*, and *OAT7* gene expression. A role for HNF-1α in regulating *OAT1*/*Oat1* (Saji et al., 2008) and *OAT3* (Kikuchi et al., 2006) gene expression has been reported previously. In this study, we show that HNF-1α is capable of increasing human *OAT5* and *OAT7* promoter activities, whereas endogenous knockdown of HNF-1α decreases OAT5 and OAT7 mRNA expression. In contrast, human OAT2 mRNA expression is unaffected by modulation of HNF-1α expression levels in hepatic cell lines. Consistent with this, we show in electromobility shift assays (EMSAs) and chromatin immunoprecipitation assays (ChIPs) that HNF-1α is capable of binding DNA elements in the proximal *OAT5* and *OAT7* promoters. Further support for the role of HNF-1α in regulation of *OAT* gene expression in human liver stems from the correlation between HNF-1α and OAT mRNA expression levels in human liver biopsies.

Materials and Methods

Chemicals. Deoxyadenosine 5′-[α-^{32}P]-triphosphate (6000 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Schwerzenbach, Switzerland). Restriction enzymes were from Roche Diagnostics (Rotkreuz, Switzerland), PuRe *Taq* Ready-To-Go PCR beads from GE Healthcare (Glattbrugg, Switzerland), and T4 DNA Ligase from Promega (Dübendorf, Switzerland). The oligonucleotides were synthesized by Microsynth (Balgach, Switzerland). Other chemicals were purchased from Sigma-Aldrich (Buchs, Switzerland) unless stated otherwise.

Cells. The human hepatoma cell lines HepG2 (LGC Promochem, Wesel, Germany) and Huh7 (JCRB Cell Bank, Japan) were cultured in RPMI-1640 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Sigma-Aldrich), 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen, Basel, Switzerland). Cells were cultured at +37°C in a humidified atmosphere containing 5% CO_2. Fresh primary human hepatocytes were obtained from Lonza Verviers SPRL (Verviers, Belgium).

Transfection of Cells with siRNA. Huh7 cells were transfected on six-well plates at 70% confluence. Per transfection, 15 μl of TransIT-TKO reagent (Mirus Bio LLC, LabForce) was mixed with 250 μl of serum-free OptiMEM (Invitrogen), followed by addition of either the SMARTpool siRNAs targeting HNF-1α (Dharmacon, Lafayette, CO) or siCONTROL nontargeting siRNA 2 (Dharmacon) to a final concentration of 100 nM. After 15-min incubation at room temperature, the siRNA mixtures were added to the 1250 μl of RPMI-1640 medium supplemented with 10% fetal bovine serum contained in each well. To knock down HNF-4α expression, Huh7 cells were transfected with siRNAs as described by Popowski et al. (2005). Cells were harvested 24 h after transfection, and total cellular RNA and protein were extracted as described previously (Popowski et al., 2005).

Isolation of RNA, Reverse Transcription, and Quantitative Real-Time PCR. Total RNAs from Huh7 cells or PHHs were isolated using the TRIzol reagent (Invitrogen). Total RNAs from 10 human tissues were derived from the Human Total RNA Master Panel (Clontech, Saint-Germain-en-Laye, France). RNAs were reverse-transcribed as described previously (Klein et al., 2009). Real-time PCR was performed on an 7900HT Fast Real-Time PCR system (Applied Biosystems, Rotkreuz, Switzerland) by using the TaqMan Gene Expression Assays for human HNF-1α, HNF-4α, OAT1, OAT2, OAT5, OAT7, OATP1B1, and OATP1B3 (Applied Biosystems). Constitutively expressed human β-actin was measured as an internal standard for sample normalization (Applied Biosystems). Isolation of total RNA from human liver biopsies and reverse transcription was described previously (Hahne et al., 2008). Levels of HNF-1α, OAT5, OAT7, and OAT2 mRNAs in liver biopsy material were quantified using TaqMan Gene Expression Assays as mentioned above and an iCycler iQ Multicolour Real-Time PCR Detector (Bio-Rad Laboratories, Hercules, CA). Constitutively expressed acidic ribosomal protein p0 mRNA was used as an internal standard for normalization of mRNA abundance. The relative mRNA levels were calculated by the comparative threshold cycle method.

Immunoblot Analysis. From siRNA-transfected Huh7 cells, 15 μg of whole protein extracts were separated on 10% SDS polyacrylamide gels and electroblotted onto Hybond ECL nitrocellulose membranes (GE Healthcare). Membranes were blocked for 1 h in 5% (w/v) nonfat milk in PBS-T [0.1% (v/v) Tween 20 in phosphate-buffered saline]. After blocking, the membranes were probed with an antibody against HNF-1α (Santa Cruz Biotechnology Inc., Lab Force, Nunningen, Switzerland) or HNF-4α (Santa Cruz Biotechnology Inc.) at concentrations of 1 μg/ml After three washes with nonfat milk/PBS-T, the horseradish peroxidase-conjugated secondary goat anti-rabbit antibody (Pierce, Lausanne, Switzerland) was added at a concentration of 10 ng/ml in 5% (w/v) nonfat milk/PBS-T for 1 h. Blots were washed three times in PBS-T, followed by detection with the SuperSignal West Femto Maximum Sensitivity Substrate (Pierce) and exposure on Hyperfilm ECL (GE Healthcare). To verify equal loading of the protein samples, the blots were stripped with Restore Western blot stripping solution (Pierce), reblocked, and reprobed for constitutively expressed Ku-70 antigen. The Ku-70 antibody (Santa Cruz Biotechnology Inc.) was used at a concentration of 50 ng/ml, and a horseradish peroxidase-conjugated rabbit anti-goat antibody (Dako Schweiz AG, Baar, Switzerland) was used as secondary antibody at a concentration of 167 ng/ml.

Reporter Gene Constructs and Expression Vectors. Based on the genomic sequence (NC_000011) available in the U.S. National Center for Biotechnology Information database, fragments corresponding to the promoter regions of the human *OAT5* and human *OAT7* genes were PCR-amplified using the oligonucleotide primers shown in Table 1 and human genomic DNA (Clontech) as a template. PCR products were cloned into the pGEM-T vector and subcloned into the luciferase vector pGL3basic (Promega) to generate the *OAT5* and *OAT7* promoter reporter constructs. Point mutations were introduced into the *OAT5*(−265/+187) construct and the *OAT7*(−73/+249) construct using the QuikChange site-directed mutagenesis kit (Stratagene, Basel, Switzerland) and oligonucleotides listed in Table 1. The coding region of the human *HNF-1α* gene (NM_000545) was obtained through PCR amplification using oligonucleotide primers

listed in Table 1 and human universal cDNA (Clontech) as a template. The PCR product was cloned into the pGEM-T vector and subcloned into the pcDNA3.1(−) vector (Invitrogen). The sequences of all constructs were verified by DNA sequencing (Microsynth).

Transient Transfections and Reporter Assays. HepG2 cells were seeded on 48-well plates at a density of 10^5 cells/well and cotransfected with 400 ng of the luciferase reporter constructs and 200 ng of the expression plasmids, using FuGENE HD reagent (Roche Diagnostics), at a ratio of 3 μl of FuGENE HD per 1 μg of DNA. To normalize the amount of DNA transfected, the pcDNA3.1(−) vector was added where appropriate. To control for transfection efficiency, 100 ng of the *Renilla reniformis* luciferase (phRG-TK) reporter plasmid (Promega) were cotransfected. Cells were harvested 36 h after transfection, and luciferase activities were determined using the Dual Luciferase Assay System (Promega) by using a Luminoskan Ascent microplate luminometer (Thermo Fisher Scientific, Wohlen, Switzerland). Reporter activities obtained for the empty pGL3basic corresponding to each test condition, as well as for the test construct containing the test promoter in the control conditions, were set to 1 and fold activities are shown relative to this.

Electrophoretic Mobility Shift Assays. EMSA binding reactions were performed as described previously (Klein et al., 2009). Oligonucleotides used in EMSAs (Table 1) were designed to have a 5′-AGCT overhang in the top strand and a 5′-GATC overhang in the bottom strand when annealed, allowing radioactive labeling by fill-in reactions. Two microliters of in vitro-translated HNF-1α (Tnt-HNF-1α) or 10 μg of nuclear extract extracted from HepG2 cells using the NE-PER kit (Pierce) were used for DNA-binding reactions. In supershift experiments, 1 μg of HNF-1α antibody (Santa Cruz Biotechnology Inc.) was added to the extracts 1 h before the radioactive probe and incubated at + 4°C. Immediately after the binding reactions, the samples were loaded onto pre-electrophoresed 5% (acrylamide/bis 30:1) native acrylamide gels and run at 200 V in 0.5× Tris borate-EDTA for 1.5 h. The gels were fixed in 10% (v/v) acetic acid for 10 min, dried onto DEAE cellulose paper (DE81; Whatman GmbH, Dassel, Germany) under vacuum, and exposed to Kodak BioMax MR-1 films (Sigma-Aldrich) at −80°C.

Chromatin Immunoprecipitation Assays. HepG2 cells were grown on 10-cm plates to 80% confluence, harvested, and processed through chromatin immunoprecipitation using the ChIP-IT Express kit (Active Motif, Rixensart, Belgium). Shearing of chromatin was achieved by 10 pulses of sonication with 30-s pauses on ice between each pulse, using the Branson Digital Sonifier (Branson Ultrasonics, Danbury, CT) at power setting 25%. Aliquots of sheared chromatin were incubated either without antibody, with 1 μg of negative control mouse IgG1 (Dako Denmark A/S, Glostrup, Denmark), or with 1 μg of anti-HNF-1α antibody (Santa Cruz Biotechnology). Two amplicons were assayed for immunoprecipitation test, by using PuRe *Taq* Ready-To-Go PCR beads (GE Healthcare) and the oligonucleotide primers listed in Table 1, comprising HNF-1α target regions in *OAT5*, *OAT7*, and *OATP1B1* promoters as well as nontarget regions located within intron 1 of each gene. After 40 cycles, 10 μl of each PCR product were resolved on 2% agarose gels and detected with SYBR Safe DNA Gel Stain (Invitrogen). Sizes of PCR products were as follows: *OAT5* promoter, 253 bp; *OAT5* intron, 311 bp; *OAT7* promoter, 208 bp; *OAT7* intron, 272 bp; *OATP1B1* promoter, 301 bp; *OATP1B1* intron, 264 bp.

Study Subjects. Our study subjects included 75 obese patients without type 2 diabetes who underwent weight-reducing surgery. Tissue biopsies obtained from liver during surgery were collected in RNAlater (Ambion, Austin, TX). All study subjects provided informed consent, and study protocols were approved by the local ethics committee.

Statistical Analysis. All quantitative data are reported as means ± S.D. Differences between experimental groups were analyzed by unpaired *t* test (siRNA experiments) and one-way analysis

TABLE 1

Sequences of oligonucleotides used for cloning, for mutagenesis, as EMSA probes, and in ChIP assays

Only the top strands are shown for oligonucleotides used in EMSA assays. Where applicable, restriction sites introduced are underlined and corresponding enzymes used are given in parentheses. Nucleotides mutated for experiments in figures 4 and 5 are shown in bold.

Oligonucleotide	Sequence (5′→3′)	Purpose
hOAT5 −764 forward	ACGCGTGTTATTTCTTGCAACTGCATGTAAATCTATAATAAT (MluI)	*hOAT5* (−764/+187) cloning
hOAT5 + 187 reverse	CTCGAGGAGGCACAATGGCTTTACGGAGA (XhoI)	*hOAT5* (−764/+187) cloning
hOAT5 −471 forward	ACGCGTGAGCTGAACACTGCCTTTCTCTTCTATTT (MluI)	*hOAT5* (−471/+187) cloning
hOAT5 −265 forward	ACGCGTAAACCCTGACTTTCTGCTCTTTTCATGG (MluI)	*hOAT5* (−265/+187) cloning
hOAT5 −41 forward	ACGCGTGAAGTATCTCTATGAAATAATGATCTCTATGACTAC (MluI)	*hOAT5* (−41/+187) cloning
hOAT7 −848 forward	ACGCGTGCTAAGTGGGATGTGTGAATAAAAATGGAGAGTTAC (MluI)	*hOAT7* (−848/+249) cloning
hOAT7 +249 reverse	CTCGAGTAGAGGCAAAATGACTGTATCCAGAGAGG (XhoI)	*hOAT7* (−848/+249) cloning
hOAT7 −73 forward	ACGCGTCAGAAAAGTGTAGACCAAAGTCCAGTG (MluI)	*hOAT7* (−73/+249) cloning
hOAT7 +16 forward	ACGCGTCGACTGGGAGTATCTGAGC (MluI)	*hOAT7* (+16/+249) cloning
hHNF-1α forward	GAATTCGAGCCATGGTTTCTAAACTGAGCCAG (EcoRI)	cloning *hHNF-1α*
hHNF-1α reverse	GGATCCGTGGTTACTGGGAGGAAGAGG (BamHI)	cloning *hHNF-1α*
hOAT5-HNF-1α mut1 (−173/−160)	CCTAGAAATAACAACACTGTTCCCCCGATTTCTTTTAGC	site-directed mutagenesis
hOAT5-HNF-1α mut2 (−68/−56)	GGAGAGAGTAAACCTT**CCC**ACGGATTTTATTGTCAACAGC	site-directed mutagenesis
hOAT7-HNF-1α mut (−14/−2)	GACATGAAAAAGTAGAATTT**CCC**ACACATTTCATTGTAAACGACTG	site-directed mutagenesis
hOAT5 WT (−173/−160)	AGCTATAACAACACTGTTTAACCGATTTCTTTTA	EMSA
hOAT5 mut 1 (−173/−160)	AGCTATAACAACACTGTT**CCC**CCGATTTCTTTTA	EMSA
hOAT5 WT (−68/−56)	AGCTAGAGAGTAAACCTTTAAACGGATTTTATTG	EMSA
hOAT5 mut 2 (−68/−56)	AGCTAGAGAGTAAACCTT**CCC**ACGGATTTTATTG	EMSA
hOAT7 WT (−14/−2)	AGCTAAGTAGAATTTTAAACACATTTCATTGTAAACGACTGGG	EMSA
hOAT7 mut (−14/−2)	AGCTAAGTAGAATTT**CCC**ACACATTTCATTGTAAACGACTGGG	EMSA
HNF-1α consensus	AGCTGGTTAATCATTAACG	EMSA
hOAT5 −256 forward	AACCCTGACTTTCTGCTCTTTTCATGGAA	ChIP
hOAT5 −3 reverse	CATAGAGATCATTATTTCATAGAGATACTTCTAGC	ChIP
hOAT5 intron forward	CCAGACAGATCTCAAACTCCTGACC	ChIP
hOAT5 intron reverse	GAAATCCACTCTGATTTGCTTCTGTAACATG	ChIP
hOAT7 −120 forward	CCTGGAATGACCGCATACCATGTAG	ChIP
hOAT7 + *89* reverse	CGTTTTGGGGTCAGATAGGTAGCC	ChIP
hOAT7 intron forward	GTATGTGACCTGGGTGTTTAGAATAACAC	ChIP
hOAT7 intron reverse	CAATGACAGGCTGAGCATCACTGAC	ChIP
hOATP1B1 −132 forward	TGGCAACTGGAGTGAACTCTTAAAACT	ChIP
hOATP1B1 +169 reverse	GGGCTCAGAATGTAAGCGTGTGGA	ChIP
hOATP1B1 intron forward	CCCTTCTCCAATTCAGAAAGTTGTCTGCC	ChIP
hOATP1B1 intron reverse	AGGAGTGGGGTGCAGGACAGAATC	ChIP

of variance with Tukey's post hoc test (luciferase transfections) by using Prism (GraphPad Software, San Diego, CA). P values <0.05 were considered significant. To estimate associations between hepatic transcript levels, we used multivariate linear regression to adjust for age, sex, body mass index, and homeostasis model assessment-insulin resistance. Logarithmic transformation on transcript variables was used to fulfill general linear model assumptions. The correlation statistics were performed using the software package SPSS 17 (SPSS Inc., Chicago, IL).

TABLE 2
Expression levels of four SLC22A mRNAs in a range of human tissues

Tissue	Gene			
	hOAT1 (hSLC22A6)	*hOAT2 (hSLC22A7)*	*hOAT7 (hSLC22A9)*	*hOAT5 (hSLC22A10)*
Liver	−	+++	++	++
PHH	−	+++	++	+
Kidney	++	++	−	−
Small intestine	−	−	−	−
Colon	−	−	−	−
Brain	−	−	−	−
Lung	−	−	−	−
Placenta	−	−	−	−
Bone marrow	−	−	−	−
Testis	−	−	−	−
Skeletal muscle	−	−	−	−

TaqMan real-time PCR threshold cycle values: +++, 20–25; ++, 25–30; +, 30–35; −, over 35.
PHH, primary human hepatocytes.

Results

Expression Profiling of OAT mRNAs in Human Tissues. The presence of OAT2, OAT5, and OAT7 mRNA in the human liver has previously been demonstrated by Northern blotting (Sun et al., 2001; Eraly and Nigam, 2002; Shin et al., 2007). We first verified this more quantitatively by performing TaqMan real-time PCR analysis on their expression, as well as that of OAT1, on RNA samples derived from 10 human tissues and primary human hepatocytes (Table 2). We confirmed that OAT2, OAT5, and OAT7 mRNAs, but not OAT1 mRNA, are abundantly expressed in the liver and primary human hepatocytes. As reported previously (Sun et al., 2001), OAT2, together with OAT1, was also detected in the kidney. None of the other nine tissues examined expressed quantifiable levels of the four OAT mRNAs.

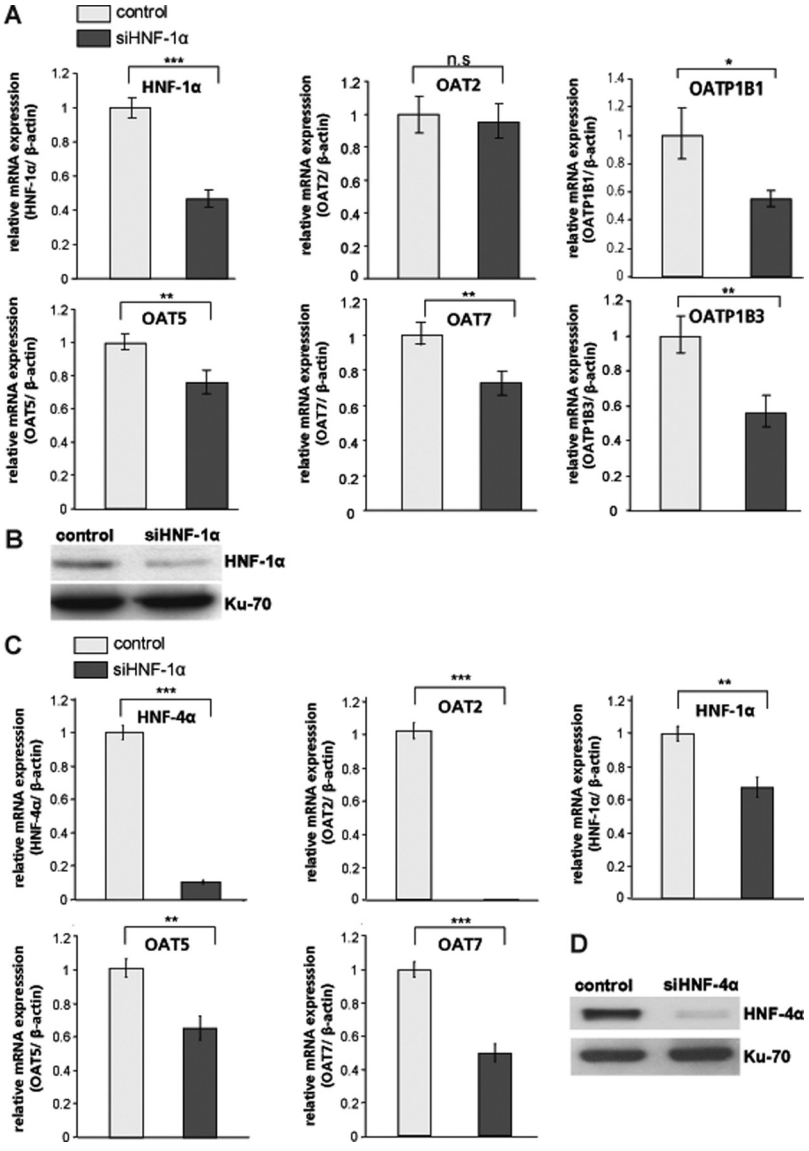


Fig. 1. Short interfering RNA-mediated knockdowns of endogenous HNF-1α and HNF-4α levels lead to reduced mRNA expression of human OATs and OATPs. A, transfection of siRNA targeting HNF-1α reduces the levels of HNF-1α, OAT5, OAT7, OATP1B1, and OATP1B3, but not of OAT2, mRNAs in Huh7 cells, compared with cells transfected with an equal amount of control siRNA. B, an immunoblot of protein samples isolated from siRNA-transfected Huh7 cells. siRNAs targeting HNF-1α reduce the levels of HNF-1α protein, but not protein levels of the housekeeping gene Ku-70. C, transfection of siRNA targeting HNF-4α strongly reduces the levels of HNF-4α and OAT2 mRNAs in Huh7 cells compared with cells transfected with an equal amount of control siRNA. The mRNA levels of HNF-1α, OAT5, and OAT7 are more modestly, yet significantly, decreased by suppression of HNF-4α expression. D, an immunoblot of protein samples isolated from siRNA-transfected Huh7 cells. SiRNAs targeting HNF-4α reduce the levels of HNF-4α protein, but not protein levels of the housekeeping gene Ku-70. n.s., not significant. **, $p < 0.01$; ***, $p < 0.001$.

Short Interfering RNA-Mediated Knockdown of Endogenous HNF-1α Expression Results in Reduced mRNA Expression of Human OAT5 and OAT7. We next studied whether the three human liver *OAT* genes are regulated by similar mechanisms. A role for HNF-1α in regulating *OAT/Oat* gene expression was previously suggested (Kikuchi et al., 2006; Saji et al., 2008). The liver-specific transporter genes *OATP1B1* (*SLCO1B1*) and *OATP1B3* (*SLCO1B3*) have been shown to be regulated by HNF-1α (Jung et al., 2001). To study the role of HNF-1α in the regulation of OATs, we reduced the endogenous HNF-1α expression by transfecting Huh7 cells with specific siRNAs against HNF-1α. To control for any nonspecific effects, we transfected an equal amount of control siRNAs not known to target any human genes. As shown in Fig. 1, by transfecting HNF-1α-specific siRNA, levels of both HNF-1α mRNA (Fig. 1A) and HNF-1α protein (Fig. 1B) were significantly reduced. This reduction was accompanied by decreased mRNA levels for both OAT5 and OAT7. In contrast, OAT2 mRNA expression was not affected by reduced HNF-1α expression (Fig. 1A). In support of the role for HNF-1α in the regulation of OAT5 and OAT7 expression, the mRNA expression levels of the known target genes for HNF-1α, OATP1B1 and OATP1B3 (Jung et al., 2001), were similarly suppressed by the HNF-1α knockdown (Fig. 1A). These data suggest that HNF-1α is involved in maintaining the baseline expression of OAT5 and OAT7, but not OAT2, in human liver-derived cells. Because the gene encoding HNF-1α itself is known to be regulated by HNF-4α (Jung and Kullak-Ublick, 2003), we studied the effects of HNF-4α siRNA on HNF-1α expression and on the expression of its newly identified target genes *OAT5* and *OAT7* (Fig. 1, C and D). Levels of both HNF-4α mRNA and protein were efficiently reduced by the siRNAs, as was the mRNA expression level of the direct HNF-4α target gene, *OAT2* (Popowski et al., 2005). As expected, there was also a more modest, yet significant, suppression of mRNA expression of HNF-1α, as well as of OAT5 and OAT7, upon suppression of HNF-4α expression. In promoter luciferase studies, we observed no activating effect by HNF-4α on *OAT5* and *OAT7* promoters (data not shown), and we thus propose that the HNF-4α siRNAs lead to down-regulation of OAT5 and OAT7 via a suppressive effect on their direct transactivator HNF-1α.

HNF-1α Transactivates the *OAT5* and *OAT7* Promoters. To further study the effects of HNF-1α on *OAT5* and *OAT7* promoter activities HepG2 cells were cotransfected with reporter-linked *OAT5*(−764/+187) and *OAT7*(−848/+249) promoter constructs, either with or without an expression plasmid for HNF-1α. As shown in Fig. 2, overexpression of HNF-1α significantly transactivated both the *OAT5* and *OAT7* promoters. Next, a series of 5′ deletions of the *OAT5*(−764/+187) and the *OAT7*(−848/+249) promoter constructs were constructed to identify the promoter regions that account for the transactivation by HNF-1α. Deletion of the region upstream of −265 bp had no effect on HNF-1α-mediated induction of *OAT5* promoter activity, whereas a further deletion of the promoter region to position −41 bp resulted in the loss of HNF-1α-mediated transactivation (Fig. 2A). Whereas the *OAT7*(−848/+249) promoter construct showed approximately 2-fold induction upon cotransfection of the HNF-1α expression plasmid, deletion of the genomic region upstream of −73 resulted in an increase in this effect (Fig. 2B), suggesting that there are promoter elements between −848 and −73 that are capable of attenuating the effect of HNF-1α on the *OAT7* promoter. Further deletion from −73 to +16 of the *OAT7* promoter led to a loss of HNF-1α-mediated transactivation. In summary, the HNF-1α-responsive regions were located between −265 and −41 bp in the *OAT5* promoter and between −73 and +16 bp in the *OAT7* promoter, leading us to focus our subsequent experiments on these promoter regions.

Identification of Putative HNF-1α Response Elements in the *OAT5* and *OAT7* Promoters. An in silico analysis was carried out on the *OAT5* promoter sequence between nucleotides −265 and −41 and on the *OAT7* promoter sequence between nucleotides −73 and +16 in an attempt to identify potential HNF-1α binding sites. Web-based software tools for locating possible transcription factor binding sites, such as TESS (Schug, 2008), MatInspector Software (Cartharius et al., 2005), and PROMO (Messeguer et al., 2002) could not identify any putative HNF-1α binding sites within these promoter regions. Visual inspection revealed the existence of two potential HNF-1α binding sites located between nucleotides −173 and −160 and nucleotides −68 and −56 in the *OAT5* promoter sequence. The site −173/−160 differs from the HNF-1α consensus sequence (Mendel and Crabtree, 1991) by 5 bp, whereas the −68/−56 site contains four divergences from the consensus sequence (Fig. 3A). One potential HNF-1α binding site in the *OAT7* promoter was identified by aligning the *OAT5*(−265/−41) promoter sequence with the *OAT7*(−73/+81) promoter sequence (Fig. 3B) using the EMBOSS pairwise alignment algorithms (http://www.ebi.ac.uk/Tools/emboss/align/index.html). The potential HNF-1α binding site in the proximal *OAT7* promoter is located between nucleotides −14/−2 and bears a high degree of similarity to the *OAT5* −68/−56 binding site. The *OAT7* −14/−2 site differs from the HNF-1α consensus sequence by 5 bp (Fig. 3A).

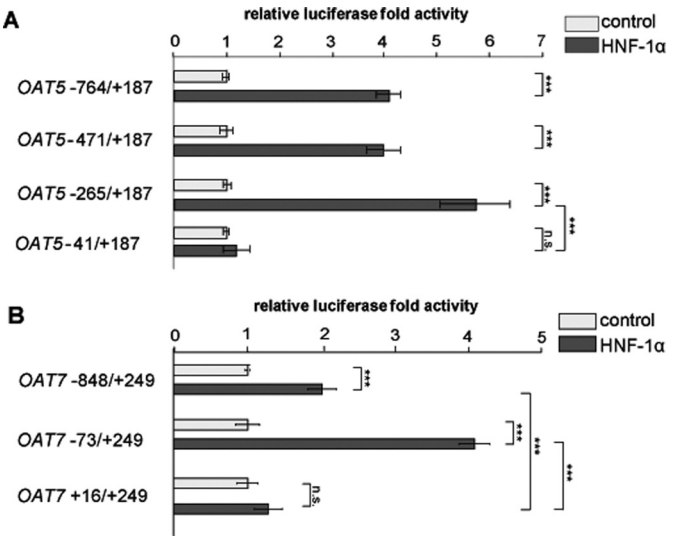


Fig. 2. HNF-1α transactivates the human *OAT5* and the human *OAT7* promoters. HepG2 cells were cotransfected with reporter-linked *OAT5* (A) and *OAT7* (B) promoter deletion constructs (nucleotide numbering relative to the transcription start site). With each construct, either a cytomegalovirus promoter-driven expression plasmid for HNF-1α or an empty pcDNA3.1(−) vector (control) was cotransfected. Relative luciferase activities obtained for pcDNA3.1(−)-transfected cells are set to 1, and the fold activities in other test conditions are shown relative to these. n.s., not significant. ***, $p < 0.001$.

Two HNF-1α Binding Sites within the *OAT5* Promoter and One Site in the *OAT7* Promoter Are Functional. To investigate the role of the identified putative HNF-1α binding sites in HNF-1α-mediated transactivation of the *OAT5* and *OAT7* promoters, these DNA elements were mutated by site-directed mutagenesis, and cotransfection experiments were performed. The transactivation by HNF-1α was significantly reduced in cotransfections upon mutating the *OAT5*(−173/−160) and *OAT5*(−68/−56) motifs, compared with the wild-type *OAT5*(−265/+187) reporter construct (Fig. 4A). Simultaneous disruption of both elements resulted in a complete loss of HNF-1α-mediated induction of *OAT5* promoter activity. Mutagenesis of the single binding site (−14/−2) in the *OAT7*(−73/+249) promoter construct was sufficient to completely disrupt HNF-1α-mediated induction of *OAT7* promoter activity (Fig. 4B). These results demonstrate that all putative HNF-1α response elements identified by in silico

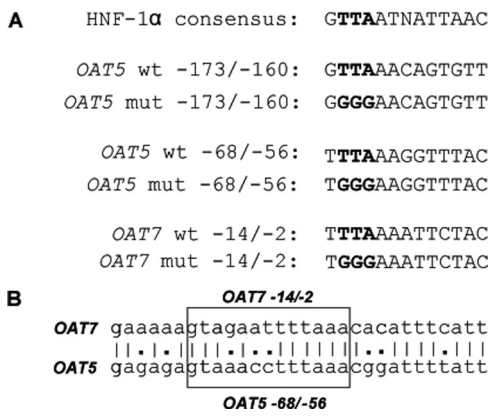


Fig. 3. Identification of putative HNF-1α elements within the human *OAT5* and *OAT7* promoters. A, in the top row, the consensus sequence of a HNF-1α response element is shown. Sequence and location relative to the transcriptional start site of the potential HNF-1α response elements of the *OAT5* and *OAT7* promoters are shown. Nucleotides mutated for experiments in Figs. 4 and 5 are shown in bold. B, proximal promoter sequences of *OAT5* and *OAT7* were analyzed pairwise. The *OAT5*(−68/−56) and the *OAT7*(−14/−2) HNF-1α elements (box) show a high degree (76.9%) of similarity.

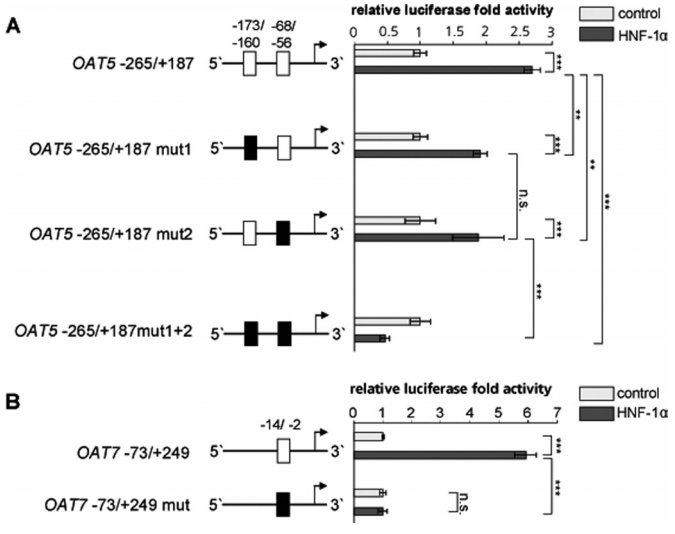


Fig. 4. Two HNF-1α elements within the human *OAT5* promoter and one element in the human *OAT7* promoter are functional. HepG2 cells were cotransfected with reporter-linked *OAT5* promoter construct −265/+187 (A) and *OAT7* promoter construct −73/+249 (B), as well as with the promoterless reporter vector pGL3basic. HNF-1α elements in the promoter constructs were mutated (black boxes) by site-directed mutagenesis. With each construct, either a cytomegalovirus promoter-driven expression plasmid for HNF-1α or an empty pcDNA3.1(−) vector (control) was cotransfected. Relative luciferase activities obtained for pcDNA3.1(−)-transfected cells are set to 1, and the fold activities in other test conditions are shown relative to these. n.s., not significant. **, $p < 0.01$. *** $p < 0.001$.

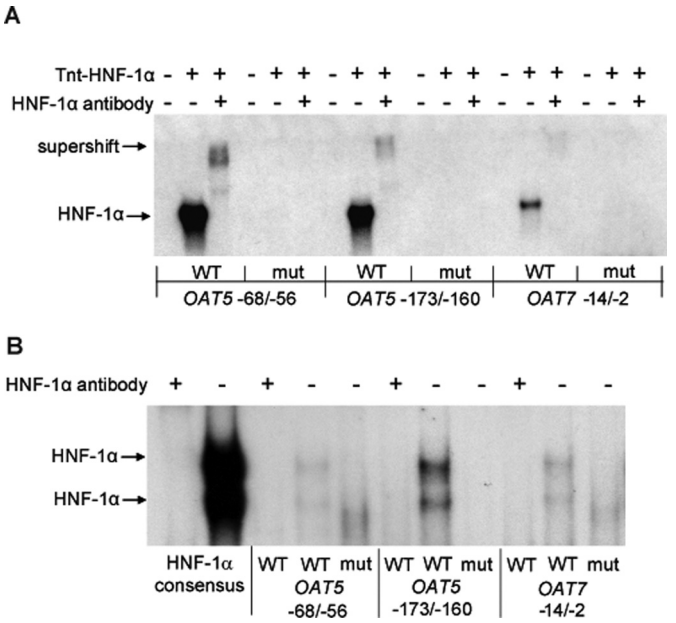


Fig. 5. HNF-1α specifically binds to two elements within the *OAT5* promoter and one element in the *OAT7* promoter. EMSAs were performed using in vitro translated HNF-1α (Tnt-HNF-1α) (A) or HepG2 nuclear extracts (B) and the *OAT5* and *OAT7* sequences containing the wild-type (WT) or mutated (mut) HNF-1α elements, or HNF-1α consensus binding motif, as radiolabeled probes. Numbers indicate the position of the HNF-1α elements within oligonucleotide probes. To identify protein-DNA complexes specifically formed between HNF-1α and the radiolabeled probes, samples preincubated with HNF-1α antibodies were included as indicated.

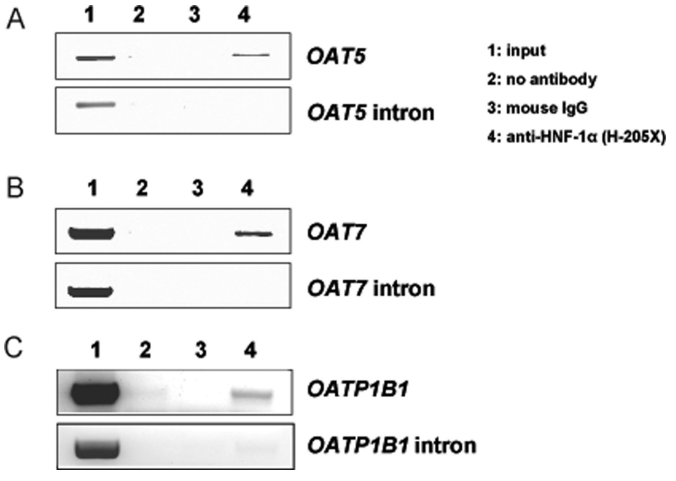


Fig. 6. HNF-1α interacts with the HNF-1α-responsive regions of the *OAT5*, *OAT7*, and *OATP1B1* promoters within HepG2 cells. ChIP samples were incubated either without antibodies, with negative control mouse IgG, or with anti-HNF-1α antibodies. Two amplicons for each gene were assayed for using primers flanking the HNF-1α target regions in the *OAT5*, *OAT7*, and *OATP1B1* promoters as well as primers derived from nontarget regions located within intron 1 of each gene. HNF-1α-specific antibody was efficient in precipitating the promoter regions of *OAT5* (A), *OAT7* (B), and *OATP1B1* (C), whereas no significant signal was obtained for the intronic regions of the three genes. 1, input; 2, no antibody; 3, mouse IgG; 4, anti-HNF-1α antibody.

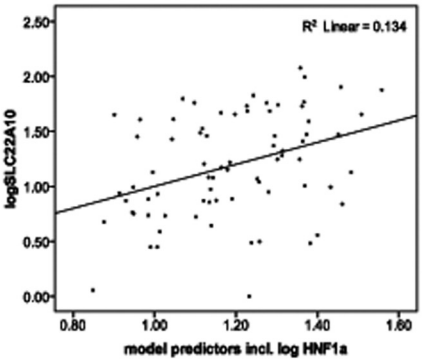
analysis within the *OAT5* and *OAT7* promoter regions are functional.

HNF-1α Binds to Two Elements within the Proximal *OAT5* Promoter and One Element in the *OAT7* Promoter. To confirm that HNF-1α is capable of directly binding to the elements in the *OAT5* and *OAT7* promoters, EMSAs were performed using wild-type and mutated double-stranded oligonucleotides comprising the *OAT5*(−68/−56), the *OAT5*(−173/−160), and the *OAT7*(−14/−2) HNF-1α binding sites and in vitro translated HNF-1α protein (Fig. 5A). HNF-1α was able to form complexes with all three wild-type probes. The complexes were disrupted after addition of HNF-1α antibodies. No complex formation was found with mutated *OAT5* and *OAT7* oligonucleotides. Likewise, two complexes of similar mobility to those DNA-protein complexes forming on the HNF-1α consensus motif could form on all three wild-type, but not mutant, *OAT* probes, when these were incubated with nuclear extracts derived from HepG2 cells (Fig. 5B). These endogenous complexes did not form on the mutant *OAT* probes, and the addition of the HNF-1α antibody disrupted the formation of these complexes. The two complexes are likely to be two differentially modified (e.g., phosphorylation) isoforms of HNF-1α. These data indicate that HNF-1α is capable of binding to both HNF-1α-responsive elements in the proximal *OAT5* and the single element in the proximal *OAT7* promoter sequences in vitro.

HNF-1α Interacts with Its Responsive Regions in the *OAT5* and *OAT7* Promoters within Living Cells. To investigate whether there is a direct interaction between HNF-1α and the *OAT5* and the *OAT7* promoter regions within living cells, we performed ChIP analysis in HepG2 cells. Proteins were cross-linked to DNA in vivo using formaldehyde, the cells were lysed and the chromatin was sheared into fragments of 300 to 600 bp. Next, immunoprecipitations were performed using an anti-HNF-1α antibody. The anti-HNF-1α antibody efficiently precipitated *OAT5*

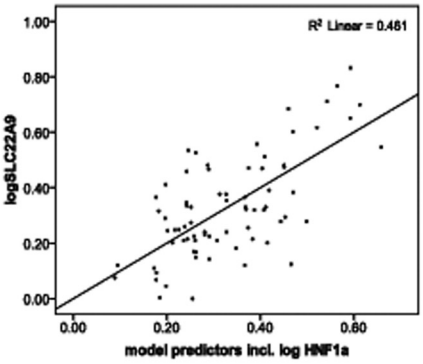
A

Variable	Regression coefficient (SE)	P
Sex, male/female (25/50)	-0.077 (0.118)	0.517
Age, years ± SD (38.5±10.4)	-0.012 (0.005)	0.019
BMI, kg/m^2 ± SD (44.05±6.63)	0.002 (0.009)	0.861
HOMA-IR ± SD (4.57±3.58)	0.024 (0.017)	0.154
Hepatic HNF-1α transcripts, AU	0.694 (0.311)	0.029



B

Variable	Regression coefficient (SE)	P
Sex, male/female (25/50)	0.036 (0.036)	0.331
Age, years±SD (38.5±10.4)	0.001 (0.002)	0.696
BMI, kg/m^2 (44.05±6.63)	0.003 (0.003)	0.238
HOMA-IR±SD (4.57±3.58)	0.008 (0.005)	0.129
Hepatic HNF-1α transcripts, AU	0.658 (0.096)	< 0.001



C

Variable	Regression coefficient (SE)	P
Sex, male/female (25/50)	0.102 (0.039)	0.012
Age, years±SD (38.5±10.4)	-0.002 (0.002)	0.279
BMI, kg/m^2 (44.05±6.63)	0.004 (0.003)	0.233
HOMA-IR±SD (4.57±3.58)	-0.001 (0.006)	0.870
Hepatic HNF-1α transcripts, AU	0.775 (0.104)	< 0.001

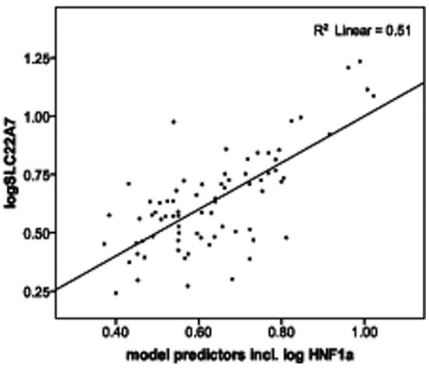


Fig. 7. Correlation analysis of OAT5 (A), OAT7 (B) and OAT2 (C) mRNA levels in surgically resected human liver tissue from 75 obese patients without diabetes in relation to HNF-1α mRNA using multivariate linear regression analysis, including important clinical variables as additional regression model predictors. Clinical variables are given as mean ± S.D. Log transformed values of OAT5, OAT7, OAT2, and HNF-1α mRNA levels were used. R^2, coefficient of determination (adjusted R^2 values and P values: A, $R^2 = 0.071$, $P = 0.029$; B, $R^2 = 0.422$, $P < 0.001$; C, $R^2 = 0.475$, $P < 0.001$). AU, arbitrary units.

(Fig. 6A) and *OAT7* (Fig. 6B) promoter regions containing the HNF-1α response elements, whereas the nonspecific mouse IgG antibody did not yield a precipitate. As a positive control, we performed chromatin immunoprecipitations on the known direct target for HNF-1α (i.e., *OATP1B1*) using the same samples. Like *OAT5* and *OAT7*, the HNF-1α antibody, but not the negative control antibody, precipitated the HNF-1α-responsive region of the *OATP1B1* gene (Fig. 6C). As an additional specificity control, we amplified short regions located in the first introns of the *OAT5*, *OAT7*, and *OATP1B1* genes, using the same ChIP samples as PCR templates. The anti-HNF-1α antibody was not able to efficiently precipitate the intronic regions from any of the three genes (Fig. 6, A–C). These results confirm that HNF-1α specifically interacts with the regions of the *OAT5* and *OAT7* promoters containing the identified HNF-1α response elements within living cells.

HNF-1α mRNA Expression Correlates with OAT mRNA Levels in Human Liver Samples. To study the relevance of HNF-1α in the regulation of hepatic OAT5 and OAT7 expression in vivo, we measured HNF-1α, OAT5, and OAT7 mRNA levels in 75 surgically resected human liver samples by quantitative TaqMan PCR. Using multiple regression models, we found a borderline association of HNF-1α and OAT5 mRNA expression (Fig. 7A) and a strong correlation between HNF-1α and OAT7 mRNA levels (Fig. 7B). Furthermore, we measured the OAT2 mRNA levels in the same samples. Surprisingly, the OAT2 mRNA levels were also strongly correlated with HNF-1α expression levels (Fig. 7C), even if we have found no evidence that the *OAT2* promoter is a direct target for HNF-1α. Significant associations between OAT mRNA expression levels were also found after adjusting the results for sex, age, body mass index, and homeostasis model assessment-insulin resistance (Table 3).

Discussion

Hepatic drug uptake from the sinusoidal blood and excretion into bile are important functions of the liver. OATs are located at the barrier epithelia of various tissues, where they mediate absorption and excretion of a range of metabolites, signaling molecules, and drugs (Ahn and Nigam, 2009). Three members of the *OAT* family have been detected at the mRNA level, in human liver: OAT2 (*SLC22A7*), OAT5 (*SLC22A10*), and OAT7 (*SLC22A9*) (Sun et al., 2001; Eraly and Nigam, 2002; Shin et al., 2007). Transport properties of OAT2, which is expressed at high levels in the liver and to a lesser extent in kidneys, have been studied in detail (Sun et al., 2001; Babu et al., 2002; Khamdang et al., 2003; Kobayashi et al., 2005). Human OAT7 exhibits a narrow substrate selectivity compared with other OAT family members and was shown to transport estrone sulfate and dehydroepiandrosterone sulfate in exchange for butyrate (Shin et al., 2007). Human OAT5 mRNA is detectable only in liver (Sun et al., 2001; Eraly and Nigam, 2002). OAT5 transport substrates are still under investigation. Changes in the expression levels of OATs could influence the hepatic clearance of transport substrates from sinusoidal blood.

TABLE 3

Multivariate linear regression analysis correlating OAT mRNA levels in human liver tissue

Model predictors: Sex, BMI, Age, HOMA-IR, respective log OAT mRNA level. Log OAT mRNA level values were used in the calculations.

	OAT5	OAT7	OAT2
Model predictors incl. OAT5		$P = 0.025$ $R^2 = 0.167$	$P = 0.019$ $R^2 = 0.174$
Model predictors incl. OAT7	$P = 0.049$ $R^2 = 0.146$		$P < 0.001$ $R^2 = 0.529$
Model predictors incl. OAT2	$P = 0.078$ $R^2 = 0.131$	$P < 0.001$ $R^2 = 0.517$	

P, model significance; R^2, coefficient of determination.

A role for HNF-1α in regulating *SLC22* gene expression was suggested by studies in HNF-1α knockout mice that showed altered expression of several transporter genes in liver, kidney, and duodenum compared with wild-type animals (Shih et al., 2001; Maher et al., 2006). HNF-1α plays a prominent role in the regulation of a wide variety of hepatocyte-specific genes, although it is also expressed in other tissues. The HNF-1α consensus DNA-binding site is an inverted palindrome with which HNF-1α interacts as a homodimer (Mendel and Crabtree, 1991). Human and mouse *OAT1*/*Oat1* (Saji et al., 2008), *OAT3* (Kikuchi et al., 2006), and *URAT1*/*Urat1* (Kikuchi et al., 2007) promoter activities have been shown to be regulated by HNF-1α and HNF-1β. In addition to certain members of the *SLC22* family, promoter regions of other human *SLC* transporter genes, such as *SLCO1B1*, *SLCO1B3* (Jung et al., 2001), *AE2* anion exchanger (*SLC4A2*) (Malumbres et al., 2003), Na^+-glucose cotransporter gene *SGLT1* (*SLC5A1*) (Martín et al., 2000), type I Na^+-phosphate cotransporter (*NPT1*; *SLC17A1*) (Soumounou et al., 2001), concentrative nucleoside transporter 2 (*CNT2*; *SLC28A2*) (Yee et al., 2009), and sodium-dependent vitamin C transporter protein 1 (*SVCT1*; *SLC23A1*) (Michels and Hagen, 2009) have been shown to be regulated by HNF-1α. HNF-1α variants containing common polymorphisms that are associated with increased type 2 diabetes risk exhibit decreased transactivation potential on glucose transporter 2 (*SLC2A2*) promoter activity (Holmkvist et al., 2006). The human *OAT1* (Ogasawara et al., 2007) and *OAT2* (Popowski et al., 2005) promoter activities are increased by another liver-enriched transcription factor: HNF-4α. We have not been able to detect a role of HNF-4α as a direct regulator of the human *OAT5* and *OAT7* promoters (data not shown), although we note that siRNA-mediated knockdown of HNF-4α in liver-derived cells resulted in a modest, but significant, suppression of OAT5 and OAT7 mRNA levels, probably via suppression of HNF-1α expression.

In this study we showed that the human *OAT5* and human *OAT7* transporter genes are also regulated by HNF-1α. We identified two functional binding elements in the proximal *OAT5* promoter and one element in the *OAT7* promoter that are essential for the HNF-1α-mediated increase in promoter activities in liver-derived cells. Mutagenesis of both elements in the *OAT5* promoter resulted in a complete loss of HNF-1α-mediated transactivation, whereas disruption of either single element led to slightly decreased transactivation of *OAT5* promoter activity. Mutagenesis of the single binding element in the *OAT7* promoter was sufficient to disrupt HNF-1α-mediated transactivation of the *OAT7* promoter. We showed in EMSAs and ChIPs that HNF-1α directly binds to the identified promoter elements both in vitro and within living cells. A correlation between HNF-1α and OAT5 or OAT7 mRNA expression levels in human liver tissue further supports the important role of HNF-1α in the regulation of *OAT* gene expression. We also observed a correlation be-

tween HNF-1α levels and OAT2 mRNA levels in human liver tissue, although no evidence that HNF-1α regulates the *OAT2* promoter via direct interactions has been obtained from HNF-1α siRNA experiments (Fig. 1A) or promoter-luciferase studies. This suggests further complexity of the regulation of the *OAT* transporter genes and indicates that HNF-1α may be an important determinant of hepatic OAT expression by both direct and indirect mechanisms. Significant correlations between OAT5, OAT7, and OAT2 mRNA levels were also found, suggesting common regulatory mechanisms between the *OAT* genes in human liver in vivo. We note that the patient cohort employed here is highly obese, which may be considered a potential confounding factor.

In summary, these data emphasize the central role of HNF-1α in regulating human *SLC22A* transporter gene expression. It will be of interest to further investigate whether *OAT5* and *OAT7* gene expression is altered via disturbed HNF-1α signaling in disease states, such as diabetes.

Acknowledgments

We thank Christian Hiller, Lia Hofstetter, and Penelope Hahne for excellent technical assistance, and members of our teams for fruitful discussions.

References

Ahn SY and Nigam SK (2009) Toward a systems level understanding of organic anion and other multispecific drug transporters: a remote sensing and signaling hypothesis. *Mol Pharmacol* **76:**481–490.

Anzai N, Jutabha P, Enomoto A, Yokoyama H, Nonoguchi H, Hirata T, Shiraya K, He X, Cha SH, Takeda M, et al. (2005) Functional characterization of rat organic anion transporter 5 (Slc22a19) at the apical membrane of renal proximal tubules. *J Pharmacol Exp Ther* **315:**534–544.

Babu E, Takeda M, Narikawa S, Kobayashi Y, Enomoto A, Tojo A, Cha SH, Sekine T, Sakthisekaran D, and Endou H (2002) Role of human organic anion transporter 4 in the transport of ochratoxin A. *Biochim Biophys Acta* **1590:**64–75.

Burckhardt G and Wolff NA (2000) Structure of renal organic anion and cation transporters. *Am J Physiol Renal Physiol* **278:**F853–F866.

Cartharius K, Frech K, Grote K, Klocke B, Haltmeier M, Klingenhoff A, Frisch M, Bayerlein M, and Werner T (2005) MatInspector and beyond: promoter analysis based on transcription factor binding sites. *Bioinformatics* **21(13):**2933–2942.

Eraly SA and Nigam SK (2002) Novel human cDNAs homologous to Drosophila Orct and mammalian carnitine transporters. *Biochem Biophys Res Commun* **297:**1159–1166.

Hahne P, Krempler F, Schaap FG, Soyal SM, Höffinger H, Miller K, Oberkofler H, Strobl W, and Patsch W (2008) Determinants of plasma apolipoprotein A-V and APOA5 gene transcripts in humans. *J Intern Med* **264:**452–462.

Holmkvist J, Cervin C, Lyssenko V, Winckler W, Anevski D, Cilio C, Almgren P, Berglund G, Nilsson P, Tuomi T, et al. (2006) Common variants in HNF-1 alpha and risk of type 2 diabetes. *Diabetologia* **49:**2882–2891.

Jacobsson JA, Haitina T, Lindblom J, and Fredriksson R (2007) Identification of six putative human transporters with structural similarity to the drug transporter SLC22 family. *Genomics* **90:**595–609.

Jung D and Kullak-Ublick GA (2003) Hepatocyte nuclear factor 1 alpha: a key mediator of the effect of bile acids on gene expression. *Hepatology* **37:**622–631.

Jung D, Hagenbuch B, Gresh L, Pontoglio M, Meier PJ, and Kullak-Ublick GA(2001) Characterization of the human OATP-C (SLC21A6) gene promoter and regulation of liver-specific OATP genes by hepatocyte nuclear factor 1 alpha. *J Biol Chem* **276:**37206–37214.

Khamdang S, Takeda M, Babu E, Noshiro R, Onozato ML, Tojo A, Enomoto A, Huang XL, Narikawa S, Anzai N, et al. (2003) Interaction of human and rat organic anion transporter 2 with various cephalosporin antibiotics. *Eur J Pharmacol* **465:**1–7.

Kikuchi R, Kusuhara H, Hattori N, Kim I, Shiota K, Gonzalez FJ, and Sugiyama Y (2007) Regulation of tissue-specific expression of the human and mouse urate transporter 1 gene by hepatocyte nuclear factor 1 alpha/beta and DNA methylation. *Mol Pharmacol* **72:**1619–1625.

Kikuchi R, Kusuhara H, Hattori N, Shiota K, Kim I, Gonzalez FJ, and Sugiyama Y (2006) Regulation of the expression of human organic anion transporter 3 by hepatocyte nuclear factor 1alpha/beta and DNA methylation. *Mol Pharmacol* **70:**887–896.

Klein K, Kullak-Ublick GA, Wagner M, Trauner M, and Eloranta JJ (2009) Hepatocyte nuclear factor-4alpha and bile acids regulate human concentrative nucleoside transporter-1 gene expression. *Am J Physiol Gastrointest Liver Physiol* **296:**G936–G947.

Kobayashi Y, Ohshiro N, Sakai R, Ohbayashi M, Kohyama N, and Yamamoto T (2005) Transport mechanism and substrate specificity of human organic anion transporter 2 (hOat2 [SLC22A7]). *J Pharm Pharmacol* **57:**573–578.

Koepsell H and Endou H (2004) The SLC22 drug transporter family. *Pflugers Arch* **447:**666–676.

Maher JM, Slitt AL, Callaghan TN, Cheng X, Cheung C, Gonzalez FJ, and Klaassen CD (2006) Alterations in transporter expression in liver, kidney, and duodenum after targeted disruption of the transcription factor HNF1alpha. *Biochem Pharmacol* **72:**512–522.

Malumbres R, Lecanda J, Melero S, Ciesielczyk P, Prieto J, and Medina JF (2003) HNF1alpha upregulates the human AE2 anion exchanger gene (SLC4A2) from an alternate promoter. *Biochem Biophys Res Commun* **311:**233–240.

Martín MG, Wang J, Solorzano-Vargas RS, Lam JT, Turk E, and Wright EM (2000) Regulation of the human Na(+)-glucose cotransporter gene, SGLT1, by HNF-1 and Sp1. *Am J Physiol Gastrointest Liver Physiol* **278:**G591–G603.

Mendel DB and Crabtree GR (1991) HNF-1, a member of a novel class of dimerizing homeodomain proteins. *J Biol Chem* **266:**677–680.

Messeguer X, Escudero R, Farré D, Núñez O, Martínez J, and Albà MM (2002) PROMO: detection of known transcription regulatory elements using species-tailored searches. *Bioinformatics* **18:**333–334.

Michels AJ and Hagen TM (2009) Hepatocyte nuclear factor 1 is essential for transcription of sodium-dependent vitamin C transporter protein 1. *Am J Physiol Cell Physiol* **297:**C1220–C1227.

Ogasawara K, Terada T, Asaka J, Katsura T, and Inui K (2007) Hepatocyte nuclear factor-4{alpha} regulates the human organic anion transporter 1 gene in the kidney. *Am J Physiol Renal Physiol* **292:**F1819–F1826.

Popowski K, Eloranta JJ, Saborowski M, Fried M, Meier PJ, and Kullak-Ublick GA (2005) The human organic anion transporter 2 gene is transactivated by hepatocyte nuclear factor-4 alpha and suppressed by bile acids. *Mol Pharmacol* **67:**1629–1638.

Saji T, Kikuchi R, Kusuhara H, Kim I, Gonzalez FJ, and Sugiyama Y (2008) Transcriptional regulation of human and mouse organic anion transporter 1 by hepatocyte nuclear factor 1 alpha/beta. *J Pharmacol Exp Ther* **324:**784–790.

Schug J (2008) Using TESS to predict transcription factor binding sites in DNA sequence. *Curr Protoc Bioinform* **21:**2.6.1–2.6.15.

Shih DQ, Bussen M, Sehayek E, Ananthanarayanan M, Shneider BL, Suchy FJ, Shefer S, Bollileni JS, Gonzalez FJ, Breslow JL, et al. (2001) Hepatocyte nuclear factor-1alpha is an essential regulator of bile acid and plasma cholesterol metabolism. *Nat Genet* **27:**375–382.

Shin HJ, Anzai N, Enomoto A, He X, Kim do K, Endou H, and Kanai Y (2007) Novel liver-specific organic anion transporter OAT7 that operates the exchange of sulfate conjugates for short chain fatty acid butyrate. *Hepatology* **45:**1046–1055.

Simonson GD, Vincent AC, Roberg KJ, Huang Y, and Iwanij V (1994) Molecular cloning and characterization of a novel liver-specific transport protein. *J Cell Sci* **107:**1065–1072.

Soumounou Y, Gauthier C, and Tenenhouse HS (2001) Murine and human type I Na-phosphate cotransporter genes: structure and promoter activity. *Am J Physiol Renal Physiol* **281:**F1082–F1091.

Sun W, Wu RR, van Poelje PD, and Erion MD (2001) Isolation of a family of organic anion transporters from human liver and kidney. *Biochem Biophys Res Commun* **283:**417–422.

Sweet DH (2005) Organic anion transporter (Slc22a) family members as mediators of toxicity. *Toxicol Appl Pharmacol* **204:**198–215.

Taylor TD, Noguchi H, Totoki Y, Toyoda A, Kuroki Y, Dewar K, Lloyd C, Itoh T, Takeda T, Kim DW, et al. (2006) Human chromosome 11 DNA sequence and analysis including novel gene identification. *Nature* **440:**497–500.

Yee SW, Shima JE, Hesselson S, Nguyen L, De Val S, Lafond RJ, Kawamoto M, Johns SJ, Stryke D, Kwok PY, et al. (2009) Identification and characterization of proximal promoter polymorphisms in the human concentrative nucleoside transporter 2 (SLC28A2). *J Pharmacol Exp Ther* **328:**699–707.

Youngblood GL and Sweet DH (2004) Identification and functional assessment of the novel murine organic anion transporter Oat5 (Slc22a19) expressed in kidney. *Am J Physiol Renal Physiol* **287:**F236–F244.

Address correspondence to: Gerd Kullak-Ublick, Department of Clinical Pharmacology and Toxicology, University Hospital Zurich, Rämistrasse 100, CH-8091 Zurich, Switzerland. E-mail: gerd.kullak@usz.ch