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Original Article

Down-regulation of OATP1B proteins correlates with hyperbilirubinemia in advanced cholestasis

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Abstract: Aim: Organic anion-transporting polypeptides OATP1B1 and OATP1B3 are sinusoidal membrane transporters mediating liver uptake of a wide range of substrates including conjugated and unconjugated bilirubin, xenobiotics and drugs. Absence of OATP1Bs in the liver causes Rotor syndrome. Our aim was to correlate OATP1B expression with hyperbilirubinemia in common liver diseases. Methods: Immunoreactivity of five antibodies against human OATP1Bs was tested on frozen and formalin-fixed paraffin-embedded liver tissue of mouse strains transgenic for *SLCO1B1* or *SLCO1B3* and on human specimens. The proportion of hepatocytes expressing OATP1Bs was then assessed immunohistologically in formalin-fixed paraffin-embedded liver samples obtained from patients with hepatocellular and primary biliary liver diseases. *UGT1A1* promoter TATA-box and *SLCO1B1* rs4149056 genotyping was performed to rule out individuals predisposed to hyperbilirubinemia. Results: The most specific detection of OATP1B3 was achieved with the H-52 (sc-98981) antibody. OATP1B1 was specifically recognized with the ESL (ab15441) anti-OATP1B1 antibody, but only in frozen sections. The MDQ (ab15442) anti-OATP1B1 antibody cross-reacted with both OATP1B proteins in liver tissue of the transgenic mouse strains. Expression of the OATP1B proteins was decreased in advanced liver diseases and inversely correlated with serum bilirubin levels. The reduction was more pronounced in advanced primary biliary diseases (1.9±1.1 vs. 2.7±0.6; P=0.009). Conclusions: Down-regulation of OATP1B proteins may contribute to pathogenesis of jaundice accompanying advanced cholestatic liver diseases.

Keywords: Bilirubin, cholestasis, immunohistochemistry, liver disease, organic anion transporter

Introduction

Organic anion-transporting polypeptides (OATPs in humans, Oatps in rodents) are multispecific transporters expressed in numerous epithelial cells throughout the body, transporting predominantly large and hydrophobic organic anions [1, 2]. OATP1B1 (also termed OATP-C, OATP2, SLC21A6 or LST-1, gene *SLCO1B1*) and OATP1B3 (OATP8, SLC21A8 or LST3, gene *SLCO1B3*) are highly homologous proteins with similar genomic organization into 15 exons. Both proteins are glycosylated and have similar secondary structures with 12 predicted transmembrane helices with both termini located intracellularly [3, 4]. OATP1Bs mediate the Na^+-independent uptake of conjugated and unconjugated bilirubin, unconjugated bile salts and many other organic anions in human hepatocytes [2]. Expression of *SLCO1B1* and *SLCO1B3* is restricted to human hepatocytes and the corresponding protein products are localized to the basolateral (sinusoidal) membrane [3-6]. While OATP1B1 is expressed throughout the lobule, OATP1B3 is predominantly localized to the centrilobular zone [3, 7]. Expression of OATP1B proteins is regulated at the transcriptional [2, 8, 9] and/or protein level [2].

Several polymorphisms in OATP1B1 and OATP1B3 are known to affect kinetics and disposition to transport various OATP1B substrates of either endogenous or exogenous origin [10-13]. The OATP1B1 rs2306283 polymorphism p.N130D is associated with development of severe hyperbilirubinemia in neonates [14], the OATP1B1 rs4149056 polymorphism p.V174A with higher serum bilirubin levels in healthy adults [15, 16] and two non-coding variants in *SLCO1B3* may contribute to idiopathic mild unconjugated hyperbilirubinemia [17].

Adaptive changes in expression of liver bilirubin transporters in both hereditary and acquired cholestatic liver diseases-down-regulation of the canalicular multidrug resistance-associated protein MRP2 expression and up-regulation of sinusoidal MRP3 expression-explain the impairment of liver bilirubin uptake and excretion [18-22]. Since complete absence of both OATP1B1 and OATP1B3 results in Rotor-type hereditary jaundice [23, 24], down-regulation of OATP1Bs might also contribute to conjugated hyperbilirubinemia in common hepatobiliary diseases.

Our aims were to select antibodies suitable for specific detection of both or either of the OATP1B proteins on formalin-fixed paraffin-embedded liver specimens by testing them in OATP1B1- and OATP1B3-transgenic mice and to correlate liver expression of OATP1Bs with both forms of plasma bilirubin, cholestatic markers and histological findings in various forms of biliary and parenchymal liver diseases.

Materials and methods

Mouse strain

The human OATP1B1 and OATP1B3 transgenic mice crossed back into a *Slco1a/1b*$^{-/-}$ background to obtain the corresponding humanized rescue strains [23, 25, 26] were used. All animals were between 9 and 14 weeks of age. Mice were kept in a temperature-controlled environment with a 12-h light/12-h dark cycle and received a standard diet (AM-II; Hope Farms) and acidified water *ad libitum*. Housing and handling of the animals was in line with the institutional guidelines complying with Dutch legislation.

Patients, biochemistry tests and human liver specimens

Fifty-two patients with end-stage liver disease who underwent orthotopic liver transplantation at the Institute for Clinical and Experimental Medicine between 2008 and 2013 were classified according to their underlying diagnosis. Two groups were constituted. The group of parenchymal liver diseases consisted of patients with alcoholic liver cirrhosis (ALD, n=9), cirrhosis owing to chronic hepatitis C (HCV, n=8), and autoimmune cirrhosis (AIH, n=4). Patients with primary sclerosing cholangitis (PSC, n=11), primary biliary cirrhosis (PBC, n=9), and biliary atresia (BA, n=11) were included in the group of patients suffering from primary biliary diseases. Control liver specimens were obtained from 5 patients who underwent liver resection for metastatic cancer.

Serum samples obtained the day of liver transplantation were analysed for total and conjugated bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyltransferase (GGT) and alkaline phosphatase (ALP) activity by routine clinical biochemistry methods.

Liver specimens were collected from the explanted livers. At least ten samples were obtained from the right and the left lobe and one from the *lobus caudatus*. Not less than two samples of normal liver tissue were taken from patients undergoing resection of liver metastases. All tissue blocks were formalin-fixed immediately after removal and processed for routine histological assessment.

Molecular analysis

Written informed consent was obtained from the patients before their genetic examination. Genomic DNA was extracted from peripheral leukocytes and *UGT1A1* TATA-box promoter polymorphism rs8175347 and the *SLCO1B1* c.521T>C (p.V174A) coding polymorphism rs4149056 were genotyped by direct sequencing on the Applied Biosystems ABI 3130 genetic analyzer (Life Technologies, Prague, Czech Republic).

Primary antibodies

Five antibodies directed against the amino or carboxyl terminus of OATP1B1, Oatp1b2 and

OATP1B3 were tested for immunohistochemical detection of human OATP1Bs (**Table 1**) in frozen and paraffin sections. The MDQ mouse monoclonal anti-OATP2 antibody (ab15442, Abcam, Cambridge, UK) was reported as cross-reacting with both OATP1B1 and OATP1B3 on Western blot, immunoprecipitation and immunocytochemistry [27]. The ESL mouse monoclonal anti-OATP2 antibody (ab15441, Abcam) was declared as specific for OATP1B1 on frozen sections. The third mouse monoclonal antibody Oatp2 A-2 (sc-376424, Santa Cruz Biotech nology, Dallas, TX) should cross-react with human OATP and OATK family members. The rabbit anti-human polyclonal anti-SLCO1B1/OATP2 antibodies LS-C8521 (imunoaffinity purified) and LS-C8522 (unpurified serum), both purchased from LifeSpan Biosciences (Seattle, WA), were raised against a 17 amino acid peptide with identical yet proprietary sequence located near the C-terminus of human OATP-1B1. Both LS-C8521 and LS-C8522 should specifically recognize human OATP1B1 in ELISA or on Western blot. The OATP8 H-52 rabbit polyclonal anti-human antibody (sc-98981, Santa Cruz Biotechnology, Dallas, TX) was recommended for detection of OATP1B3 and, to a lesser extent, OATP1B1.

Immunohistochemical staining

Mouse and human liver tissues were fixed in 10% neutral buffered formalin and embedded in paraffin blocks. Four μm thick paraffin sections were cut and deparaffinized. Cryostat sections (8 μm) of frozen liver tissues were fixed in cold acetone for 10 minutes, dried and rinsed in 0.2% Triton X-100 for 5 minutes and in phosphate-buffered saline (PBS). Sections of an adult liver without cholestasis were used as a positive control in human studies and liver sections processed without incubation with primary antibodies served as negative controls in both animal and human studies.

All paraffin sections were pretreated by enzymatic digestion with proteinase K (Dako, Glostrup, Denmark) or by a heat-induced epitope retrieval (HIER) technique of incubation for 30 minutes at 96°C in citrate buffer-pH 6.0 (Dako), Tris/EDTA buffer pH 8.0 (Leica, Wetzlar, Germany), Tris/EDTA buffer pH 9.0 (Dako) and High pH buffer (Dako). Sections without pretreatment were also used in parallel. Endogenous peroxidase activity was then blocked by 0.3% H_2O_2 in 70% methanol for 30 minutes. To prevent non-specific binding, the sections were incubated with the serum from the host of the secondary antibody. Subsequent incubations with primary antibodies (dilution 1:50 and 1:100) were done overnight at +4°C.

For detection of primary antibodies a two-step (Dako, Histofine) or a three-step (Vector, Laboratories, Burlingame, CA) visualization system was used. The two-step detection of primary antibodies was performed using the EnVision + System-HRP Labelled Polymer anti-rabbit or anti-mouse (Dako) or the Simple Stain MAX PO (MULTI) Universal Immuno-peroxidase Polymer anti-mouse, anti-rabbit Histofine (Nichirei Biosciences, Tokyo, Japan) for 30 minutes. Finally, the specimens were stained with the Dako Liquid DAB Substrate-Chromogen System (Dako) for 2 minutes and counterstained with Harris's haematoxylin before they were embedded in Pertex® Mounting Medium (Histolab, Gothenburg, Sweden). The three-step detection of primary antibodies was started by 30 minutes incubation with biotinylated anti-mouse or rabbit IgG (H+L) (Vector) diluted 200× in 1% bovine serum albumin. The sections were incubated with R.T.U. Vectastain Elite ABC Reagent (Vector) for another 30 minutes and then stained with 3,3'-diaminobenzidine (Dako) for 2 minutes. Counterstaining with Harris's haematoxylin was performed at the end.

To minimize the reactivity of the secondary anti-mouse antibody with endogenous immunoglobulin in the mouse tissue, frozen and paraffin sections of mouse livers were stained with the Dako ARK™ (Animal Research Kit) Peroxidase (Dako).

Immunohistological expression of OATP1Bs was evaluated independently by two histopathologists using the following scoring system: 0-no positivity detected, 1-positivity in less than 33% hepatocytes, 2-positivity in 33-66% of hepatocytes, 3-positivity in more than 66% hepatocytes. Correctness of the immunohistochemical reactions was verified by positive controls on each slide. Histological evaluation was performed in 2-6 slices with sectional area measuring 120-150 mm^2 obtained from different sites of the liver specimen in each case. If no positivity was detected (e.g. score 0), additional 2-3 sections from different sites of the

Table 1. Primary antibodies

Primary antibody	Immunogen amino acid positions	Immunogen sequence	Recommended applications	Known cross-reactivity
OATP 2 MDQ	OATP1B1, 1-24	MDQNQHLNKTAEAQPSENKKTRYC	WB, IP, IHC-Fr, ICC/IF	OATP1B3 on WB
OATP2 ESL	OATP1B1 671-691	ESLNKNKHFVPSAGADSETHC	WB, IP, IHC-Fr, ICC/IF	no
Oatp2 A-2	Oatp1b2, 611-660	ASFLPALFILILMRKFQFPGDIDSSDTDPAEMKLTAKESKCTNVHRSPTM	WB, IP, ICC/IF and ELISA	OATPs and OATKs
OATP2 LS-C8521	OATP1B1, C-term*	17 aa, sequence not provided	WB and ELISA	no
OATP2 LS-C8522	OATP1B1, C-term*	17 aa, sequence not provided	WB and ELISA	no
OATP8 H-52	OATP1B3, 651-702	FQGKDTKASDNERKKVMDEANLEFLNNGEHFVPSAGTDSKTCNLDM QDNAAAN	WB, IP, IHC-Fr, IHC-P, ICC/IF and ELISA	OATP1B1

Legend: †amino acid positions not provided; *Abbreviations:* WB-Western blot, IP-immunoprecipitation, IHC-immunohistochemistry, Fr-frozen, P-paraffin, ICC-immunocytochemistry, IF-immunofluorescence.

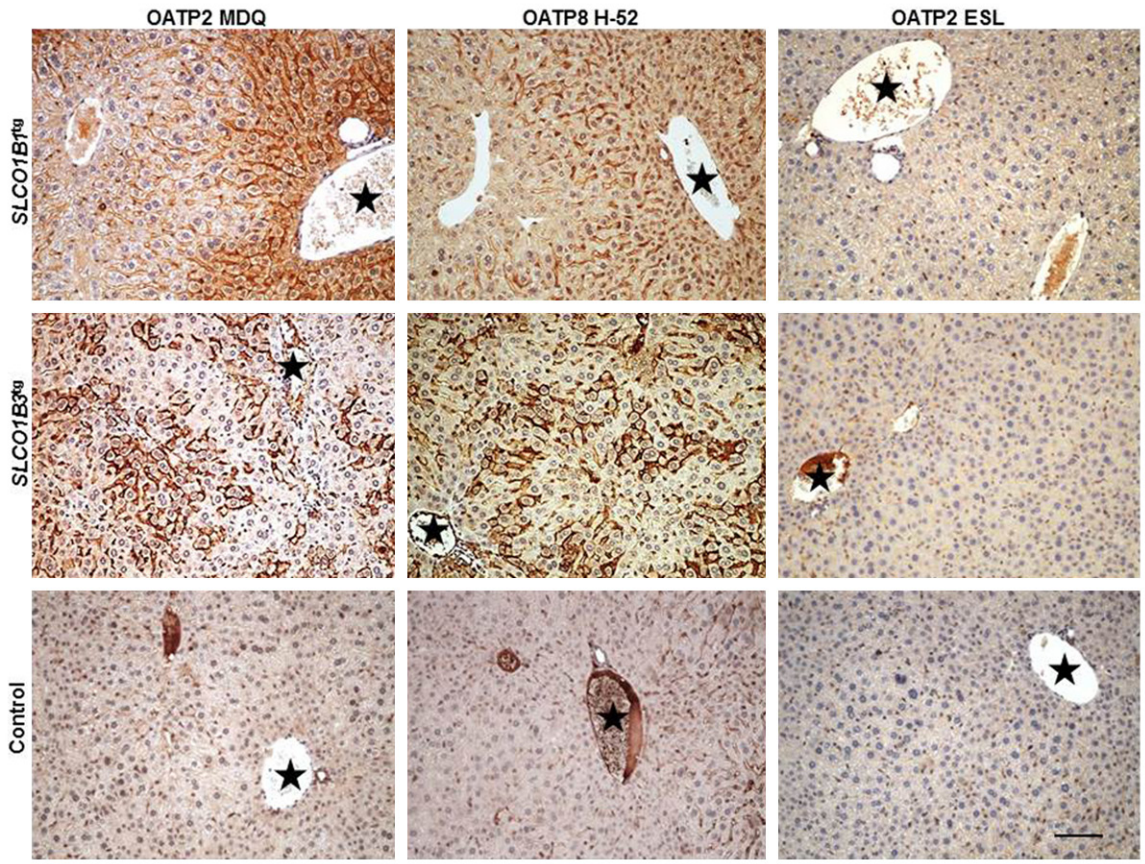

Figure 1. Immunohistological expression of OATP1B proteins in the formalin-fixed liver specimens of transgenic mice. MDQ and H-52 antibody positively stained the basolateral membrane of hepatocytes in both transgenic mouse strains. No specific positivity was observed with the ESL antibody in paraffin sections. Control: *Slco1a/1b*$^{-/-}$ [35]. Asterisks mark portal triads. Paraffin sections, HIER pretreatment at pH 6.0. Bar corresponds to 50 µm. Original magnification ×200.

explanted liver were stained. Since the architecture of the liver lobules was completely altered in the advanced stages of the liver diseases, the staining patterns of OATP1B proteins in liver parenchyma were irregular and zonal expression of the transporter proteins with centrilobular (perivenular) accentuation previously described in some studies [3, 7, 27] could not be assessed.

Statistical analysis

Results are expressed as the mean ± SD for the two patient groups. To calculate the statistical significance of the differences between the groups, the Mann-Whitney test was used. The relations between the parameters were estimated by the nonparametric Spearman's correlation coefficient. An exponential model was used for significant correlations. Two-sided $P<0.05$ was considered statistically significant.

Results

Specificity of anti-OATP1B antibodies in frozen and formalin-fixed liver tissue

The anti-OATP2 MDQ antibody detected both OATP1B1 and OATP1B3 polypeptides in frozen and, after HIER pretreatment, in formalin-fixed liver tissue of mice transgenic for either *SLCO1B1* or *SLCO1B3* (**Figure 1**). Immunopositivity for both OATP1Bs was localized to the basolateral membrane of hepatocytes. Immunostaining in the *SLCO1B1*^{tg} mouse strain was accentuated in periportal areas of the liver lobules whereas the staining pattern in *SLCO1B3*^{tg} mice was irregular with random distribution of positive hepatocytes. The staining in *SLCO1B1* transgenes was weak compared to the *SLCO1B3* animals.

In healthy human liver tissue with preserved lobular architecture the distribution of the anti-

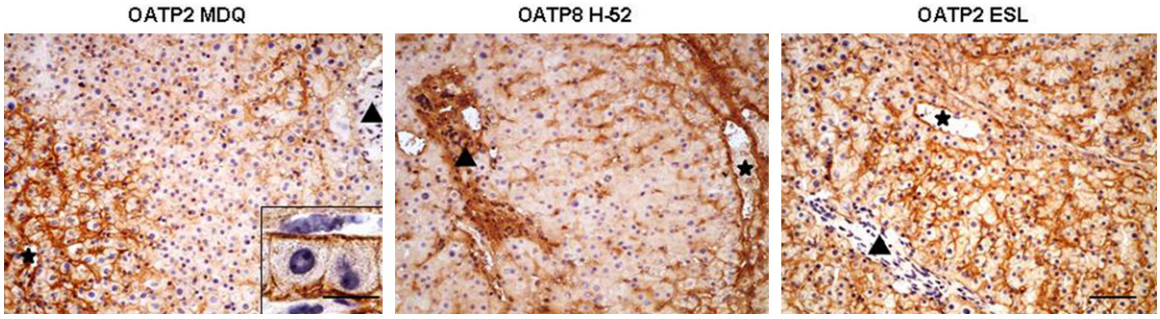

Figure 2. Immunohistological expression of OATP1B proteins in frozen human liver tissue. MDQ and H-52 immunostaining showed accentuation in centrilobular area with only weak signal around the portal triads. ESL antibody gave strong panlobular signal. Positivity was localized to the basolateral membrane of hepatocytes (inset). Asterisks mark central vein, triangles mark portal triads. Bar corresponds to 50 μm, in inset 20 μm. Original magnification ×200, inset ×600.

OATP2 MDQ immunostaining showed accentuation in centrilobular (perivenular) areas with only weak signal around the portal triads (**Figure 2**). The polarity of the cell plasma membrane staining of normal hepatocytes localized to the basolateral (sinusoidal) membrane was easily discernible (**Figure 2**, inset).

The rabbit polyclonal antibody OATP8 H-52 gave a strong positive signal in *SLCO1B3*^{tg} mice (both frozen and paraffin sections) with a pattern similar to that observed with the MDQ antibody. The cross-reactivity with OATP1B1 in *SLCO1B1*^{tg} was also present but weak (**Figure 1**).

Specific detection of OATP1B1 was obtained with the ESL anti-OATP2 antibody in frozen sections of both *SLCO1B1*^{tg} mouse and human livers with periportal accentuation of staining in mouse tissue and diffuse panlobular staining in human specimens (**Figure 2**). The antibody did not cross-react with OATP1B3 in *SLCO1B3*^{tg} mice. Unfortunately, the ESL antibody did not recognize specifically OATP1B1 protein in formalin-fixed paraffin-embedded liver tissue.

None of the other tested anti-OATP1B1 antibodies Oatp2 A-2, LS-C8521 and LS-C8522 detected OATP1B1 and/or OATP1B3 either in frozen or in formalin-fixed paraffin-embedded sections of mouse and human liver tissue.

Clinical, laboratory and molecular characteristics of the candidate patients

To rule out individuals genetically predisposed to hyperbilirubinemia, *UGT1A1* promoter TATA-box and *SLCO1B1* rs4149056 genotyping was performed in all patients considered for inclusion in the study. A homozygous genotype A $(TA)_7TAA$ typical for Gilbert syndrome was identified in one patient with PSC and in two patients with hepatitis C. Moreover, three homozygotes for the *SLCO1B1* c.521C allele were identified in the group of primary biliary diseases (BA, PSC and PBC) and one patient with alcoholic cirrhosis. All these seven patients were excluded from further statistical evaluations.

Clinical and laboratory characteristics of the remaining 45 enrolled patients are presented in **Table 2**. As expected, total and conjugated serum bilirubin levels and GGT and ALP activities were higher in the patients suffering from primary biliary disorders compared to the individuals with primary hepatocellular diseases (**Table 2**) and the differences were statistically significant (**Table 3**). However, no difference between the serum unconjugated bilirubin levels was detected (**Table 3**).

Expression of OATP1Bs in advanced liver diseases

Immunohistological expression of the OATP1B proteins detected by the MDQ antibody (ab-15442) in paraffin sections was irregular in advanced liver diseases with variable intensity of positive staining ranging from none or only small groups of positive cells to diffuse strong positivity. Moreover, polarity of the cell staining localized to the basolateral (sinusoidal) membrane of hepatocytes in the normal liver tissue with preserved lobular architecture was retained only partially in the setting of cirrhosis.

Table 2. Clinical and laboratory characteristics of the patient groups

Diag.	N	Sex M/F	Stage (fibrosis)	Child-Pugh score	Bili T (μmol/L)	Bili C (μmol/L)	ALP (μkat/L)	GGT (μkat/L)	OATP1B score
PSC	9	7/2	4	7.7±3.3	107±181	82±144	7.0±4.0	5.7±5.1	2.2±0.7
PBC	8	0/8	4	8.0±3.2	388±326	284±239	5.4±4.0	4.0±2.7	1.7±1.3
BA	10	6/4	4	9.3±1.6	282±277	183±193	5.9±5.0	1.9±1.2	1.7±1.3
ALD	8	6/2	4	8.3±1.4	48±25	21±10	3.4±2.0	1.7±1.0	2.8±0.5
HCV	6	5/1	4	8.0±1.7	46±39	27±24	2.4±1.1	2.0±1.3	2.7±0.8
AIH	4	2/2	4	7.5±2.4	66±56	26±14	2.5±1.0	2.0±1.6	2.5±0.6

Legend: Bili T-total bilirubin, Bili C-conjugated bilirubin, ALP-alkaline phosphatase, GGT-γ-glutamyltransferase, OATP1B score-immunohistological expression score of OATP1B proteins, PSC-primary sclerosing cholangitis, PBC-primary biliary cirrhosis, BA-biliary atresia, ALD-alcoholic liver disease, HCV-hepatitis C virus, AIH-autoimmune hepatitis.

Table 3. Comparison of biochemical values and immunohistological OATP1B expression in the group of primary biliary and parenchymal diseases

	Biliary (n=27)		Parenchymal (n=18)		
	mean	SD	mean	SD	p
Bili T (μmol/L)	255	280	51	36	0.005
Bili C (μmol/L)	179	203	24	16	0.001
Bili U (μmol/L)	76	85	27	24	0.093
ALP (μkat/L)	6.1	4.3	2.9	1.6	0.005
GGT (μkat/L)	4	3.9	1.9	1.2	0.019
OATP1B score	1.9	1.1	2.7	0.6	0.009

Legend: Bili U-unconjugated bilirubin, Bili T-total bilirubin, Bili C-conjugated bilirubin, ALP-alkaline phosphatase, GGT-γ-glutamyltransferase, OATP1B score-immunohistological expression score of OATP1B proteins.

The immunohistological OATP1B expression was decreased in advanced stages of both groups of patients with significantly lower values in the group of primary biliary disorders (1.9±1.1 vs. 2.7±0.6; *P*=0.009; **Table 3**).

Inverse correlations between the immunohistological OATP1Bs expression score and serum total, conjugated and unconjugated bilirubin levels were observed in the advanced stages of primary biliary diseases. By contrast, no statistically significant correlation was found between the same parameters in the group of primary hepatocellular (parenchymal) diseases (**Figure 3**). Moreover, expression of OATP1Bs did not correlate with the activity of cholestatic enzymes in both groups of diseases (data not shown).

Discussion

Despite extensive efforts, we did not achieve specific immunostaining of either OATP1B1 or OATP1B3 in formalin-fixed paraffin-embedded liver specimens with any of the 5 tested antibodies. The only two effective antibodies, MDQ and H-52, cross-reacted with both OATP1Bs whereas the anti-OATP1B1 antibodies ESL, Oatp2 A-2, LS-C8521 and LS-C8522 did not provide specific reactions with the membrane antigens on paraffin specimens. Cross-reactivity of the mouse monoclonal anti-OATP2 antibody [MDQ] (ab15442) with both human OATP-1B1 and OATP1B3 proteins in Western blot, immunoprecipitation and immunocytochemistry of transfected cells has already been described by Cui et al. [27]. In our study we proved that the same antibody also recognizes antigenic determinants of both OATP1B proteins after immunohistochemical processing of formalin-fixed paraffin-embedded tissue sections and can serve as a useful tool in the diagnosis of Rotor syndrome caused by simultaneous absence of both OATP1B transporters [23, 24]. Our results obtained with the MDQ antibody in transgenic mouse and normal human liver tissue sections are consistent with previous reports [6, 25, 27]. It should be noted though, that polarity and zonal accentuation of the OATP1B transporters distribution is substantially altered in the advanced stages of liver diseases characterized by complete parenchymal architectural disturbance and vascular reorganization.

In the second part of the study, based on the immunodetection of both OATP1Bs with the MDQ antibody, we observed lower immunohistological expression of OATP1B in end-stage liver diseases. The decrease was more marked in the group of primary biliary disorders characterized by predominantly obstructive type of cholestasis compared to the primary non-cholestatic parenchymal diseases. Our observa-

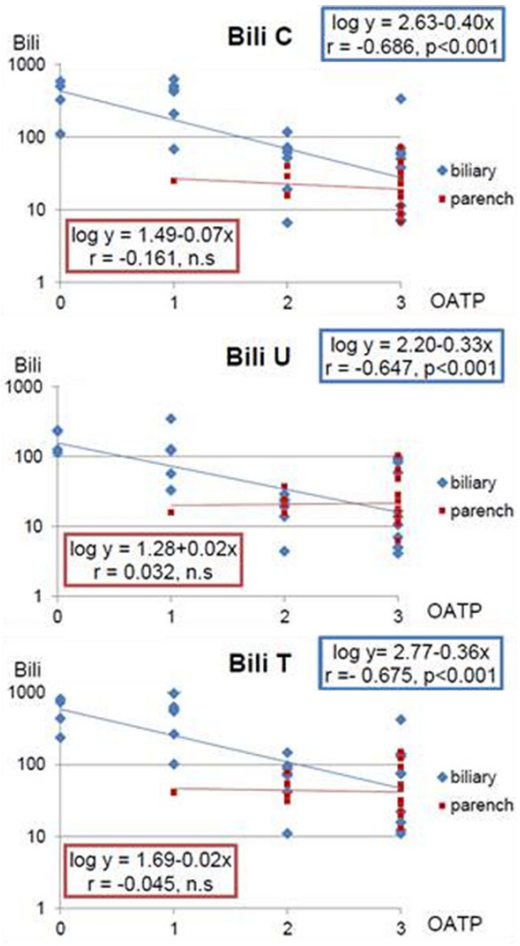

Figure 3. Correlation between the expression score of OATP1Bs and serum bilirubin levels. The OATP1Bs expression score correlates inversely with serum conjugated (C), unconjugated (U) and total (T) bilirubin level in the group of primary biliary disorders (blue), but not in primary parenchymal diseases (red). The *P* values indicate statistical significance of correlation expressed as Pearson correlation coefficient r.

tions are well in accordance with the previously published studies demonstrating down-regulation of OATP1B mRNA levels and/or protein products in patients suffering from PSC, PBC, and biliary atresia [20, 21, 28-30]. Substantial differences between biliary and parenchymal diseases at the same stage (e.g. cirrhosis) strongly indicate presence of distinct mechanisms resulting in decreased numbers of the OATP1B-expressing cells and/or reduced density of OATP1B transporters at the basolateral membrane of hepatocytes.

Alteration of hepatobiliary transporters in hereditary and acquired liver diseases explains impaired hepatic (re)uptake and excretion of both forms of bilirubin, bile salts, and other biliary constituents resulting in cholestasis and jaundice [18, 19]. Cholestasis with blockade of MRP2-mediated transport is followed by up-regulation of the basolateral homologue MRP3 at the basolateral (sinusoidal) membrane of hepatocytes and conjugated bilirubin is secreted into sinusoidal blood via MRP3 with consequent urinary excretion [31, 32]. This MRP3 induction in cholestatic conditions, mediated by transcriptional pathways associated with bile acids, is supposed to protect cholestatic hepatocytes from glucuronides [33-35]. A substantial fraction of bilirubin conjugated in the liver and splanchnic organs secreted into portal and sinusoidal blood via MRP3, is subsequently taken up by hepatocytes via OATP1B1 and OATP1B3 for final biliary excretion [23, 36]. Except for up-regulation of canalicular and basolateral efflux pumps, elevation of serum bilirubin levels in advanced stages of biliary diseases may also be, at least in part, a consequence of the decreased basolateral bilirubin uptake which is supposed to represent a part of an adaptive process protecting hepatocytes against accumulation of toxic biliary constituents during chronic cholestasis [20, 22, 36].

Since the human material has been collected retrospectively in this study, only formalin-fixed and paraffin-embedded tissue was available in most of the patients. Considering the fact that immunohistochemistry combined with calculation of the OATP1B-expressing cell rate is a semiquantitative method, quantification of OATP1B protein expression should be performed in prospectively collected fresh liver tissue.

We conclude that the MDQ antibody can serve as a tool in histopathological differential diagnosis of hyperbilirubinemia syndromes and may be helpful in identification of Rotor subjects. Down-regulation of both OATP1B proteins altering bilirubin re-uptake at the basolateral membrane of cholestatic hepatocytes may, apart from impaired MRP2 and MRP3 expression, contribute to molecular pathogenesis of predominantly conjugated hyperbilirubinemia accompanying advanced liver diseases with predominantly obstructive type of cholestasis.

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Disclosure of conflict of interest

None.

Abbreviations

AIH, autoimmune hepatitis; ALD, alcoholic liver disease; ALT, alanine transaminase; ALP, alkaline phosphatise; AST, aspartate transaminase; BA, biliary atresia; GGT, γ-glutamyltransferase; HCV, hepatitis C virus; HIER, heat-induced epitope retrieval; HNF1α, hepatocyte nuclear factor 1α; HNF3β, hepatocyte nuclear factor 3β; MRP2/ABCC2, multidrug resistance-associated protein 2/ATP-Binding Cassette Sub-Family C Member 2; MRP3/ABCC3, multidrug resistance-associated protein 3/ATP-Binding Cassette Sub-Family C Member 3; OATP, organic anion-transporting polypeptide; OATP1B1, organic anion-transporting polypeptide 1B1; OATP1B3, organic anion-transporting polypeptide 1B3; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; SLCO1B1, solute carrier organic anion transporter family member 1B1; SLCO1B3, solute carrier organic anion transporter family member 1B3; UGT1A1, uridine diphosphate glucuronosyltransferase 1A.

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